International Journal of

# JAEFS

Agriculture, Environment and Food Sciences

Volume 6 • Issue 4 • December 2022

e-ISSN: 2618-5946

PUBLISHING

www.jaefs.com

International Journal of
JAEFS
Agriculture, Environment and Food Sciences

edit PUBLISHING

International Journal of
JAEFS
Agriculture, Environment and Food Sciences

edit PUBLISHING

## EDITORIAL BOARD

International Journal of
JAEFS
Agriculture, Environment and Food Sciences

edit PUBLISHING

## REVIEWER BOARD

International Journal of
JAEFS
Agriculture, Environment and Food Sciences

edit PUBLISHING

## JOURNAL INFORMATION


| | |
|---|---|
| **Journal Name** | International Journal of Agriculture, Environment and Food Sciences |
| **Abbreviation** | Int. J. Agric. Environ. Food. Sci. |
| **Subjects** | Agriculture, Environment and Food Sciences |
| **e-ISSN** | 2618-5946 |
| **Publisher** | Edit Publishing; Edit Teknoloji Ar-Ge San. Tic.Ltd. Şti. Diyarbakır, Türkiye |
| **Owner** | Gultekin Ozdemir |
| **Language** | English |
| **Frequency** | Quarterly (March, June, September, December) |
| **Type of Publication** | International, Scientific, Open Access, Double blinded peer review, Widely distributed periodical |
| **Manuscript Submission and Tracking System** | JAEFS uses the submission system of TUBITAK ULAKBIM JournalPark Open Journal Systems - http://dergipark.gov.tr/jaefs |
| **Licence** | Journal is licensed under a Creative Commons Attribution 4.0 International License |
| **Legal Responsibility** | Authors are responsible for content of articles that were published in Journal. |
| **Indexed and Abstracted in** | TÜBİTAK ULAKBİM TR Dizin, Crossref, Directory of Open Access Journal (DOAJ), AGORA (Access to Global Online Research in Agriculture), AGRIS (Agricultural Science and Technology Information), WorldCat, Google Scholar, SOBIAD, Scilit, ROAD (Directory of Open Access Scholarly Resources), Neliti, International Citation Index, ROOT Indexing, ResearchBib, Index Copernicus International, ESJI, JournalTOCs, TEELS, ResearchGate, Microsoft Academic |
| **Address** | International Journal of Agriculture, Environment and Food Sciences<br>Edit Teknoloji Arge Sanayi ve Ticaret Limited Şirketi Dicle Teknokent Dicle Üniversitesi Kampüsü Yiğit Çavuş Mah. Silvan Yolu Üzeri Kat:2 No:26, Diyarbakır, Türkiye |
| **Contact** | Prof. Dr. Gultekin Ozdemir<br>Phone: +90 532 545 07 20<br>E-Mail: editor@jaefs.com - jaefseditor@gmail.com<br>Web: www.jaefs.com - dergipark.gov.tr/jaefs |

International Journal of
JAEFS
Agriculture, Environment and Food Sciences

edit
PUBLISHING

## AIM AND SCOPE

"International Journal of Agriculture, Environment and Food Sciences" (JAEFS) is an international

journal, which publishes original research and review articles dealing with Agriculture, Environment and Food Sciences.

JAEFS Journal is an international, scientific, open access periodical published in accordance with independent, unbiased, and doubleblinded peerreview principles.

Journal publishes Quarterly (March, June, September, December).

The publication language of the journal is English and continues publication since December 2017.

A Digital Object Identifier (DOI) number has been assigned for each article accepted to be published in JAEFS, starting from December 2017.

International Journal of Agriculture, Environment and Food Sciences are as of interest include, but are not limited to: Agricultural Engineering and Technology, Environmental Engineering and Technology, Food Engineering and Technology, Agriculture, Environment, Food, Agriculture History, Agricultural Economics, Agronomy, Animal Sciences, Aquaculture, Biochemistry, Biotechnology, Bioinformatics and Data Science, Crop Science, Dairy Science, Extension Science and Education, Entomology, Environmental Science, Fish and Fisheries, Food Processing, Food Chemistry, Food Culture, Food Health and Nutrition, Food History, Food Industry Development, Food Marketing, Food Policy and Practices, Food Safety, Forestry, Horticulture, Information Technologies and Systems, Irrigation, Molecular Biology, Organic Agriculture, Plant Physiology, Plant Breeding, Plant Protection, Plant Sciences, Precision Agriculture, Rural Development and Policy, Sustainable Agriculture, Soil Science, Plant Nutrition, Energy Crops, Veterinary, Water Resources.

Open Access Statement

International Journal of Agriculture, Environment and Food Sciences (JAEFS) publishes fully open access journal, which means that all articles are available on the internet to all users immediately upon publication.

All the original articles and review papers published in JAEFS journal are free to access immediately from the date of publication.

International Journal of Agriculture, Environment and Food Sciences (JAEFS) don't charge any fees for any reader to download articles and reviews for their own scholarly use.

Users are allowed to read, download, copy, distribute, print, search, or link to the full texts of the articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author. This is in accordance with the BOAI definition of open access.



International Journal of Agriculture, Environment and Food Sciences also operates under the Creative Commons Licence CC-BY-NC.

All authors publishing with the JAEFS accept these as the terms of publication.

Benefits of open access for Authors include:

Free access for all users worldwide

Authors retain copyright to their work

Increased visibility and readership

Rapid publication

No spatial constraints

Copyright Policy

Authors who publish with International Journal of Agriculture, Environment and Food Sciences (JAEFS) agree to the following terms:

Authors transfer the copyright to International Journal of Agriculture, Environment and Food Sciences (JAEFS) . Authors, however, upon the publication of their work, can republish it in an another journal or book provided that they obtain the permission of the Journal. Moreover, they are to include the note indicating that their work has first been published in International Journal of Agriculture, Environment and Food Sciences (JAEFS) and the link to their work in the journal.

Authors, upon the publication of their work, can publish it in online environments and/or social networks (personal webpages, course content websites, Facebook, etc.). However, they also provide the link to the work as published in the journal.

International Journal of
JAEFS
Agriculture, Environment and Food Sciences

edit PUBLISHING

# INSTRUCTIONS TO AUTHORS

## MANUSCRIPT PREPARATION

Please read all of the following manuscript preparation instructions carefully and in their entirety. The manuscript must be in good scientific English; this is the author's responsibility.

All authors must have read and approved the most recent version of the manuscript.

Manuscripts can only be submitted through the International Journal of Agriculture, Environment and Food Sciences

online manuscript submission and evaluation system

Manuscripts submitted via any other medium will not be processed.

Manuscripts submitted to the International Journal of Agriculture, Environment and Food Sciences will first go through a technical evaluation process where the editorial office staff will ensure that the manuscript has been prepared and submitted in accordance with the journal's guidelines.

Submissions that do not conform to the journal's guidelines will be returned to the submitting author with technical correction requests.

The journal management requires that all authors register to ORCID at http://orcid.org/register and get a free ORCID ID.

All submissions are screened by a similarity detection software.

We are using iThenticate plagiarism solution and our maximum allowed score, for the document in which the Materials and Methods and References sections truncated, is 20%. Higher scores are not allowed and the author will be given the plagiarism report and the manuscript will be returned to the authors.

You may download the International Journal of Agriculture, Environment and Food Sciences

Manuscript Template here.

## MANUSCRIPT STRUCTURE

### Font

Manuscripts must be prepared as a Microsoft Word file.
Word document, Times New Roman, 10 point, single line space.
Page margins are 2.5 for all sides.

### Length

Maximum length for articles is 20 pages.
Articles over 20 pages in length can only be considered on an exceptional basis.

### Title

A concise title of the paper.
Use bold 14-point Times Roman font.
Use title lowercase.
Make title in centered.

### Name(s) and surname(s) of author(s)

Use centered, bold 10-point Times Roman font.

### Affiliation(s) of author(s)

Use 8-point Times Roman font.
E-mail address of the corresponding author
Use 8-point Times Roman font.

### ORCID(s) of author(s)

Use 8-point Times Roman font.

The journal management requires that all authors register to ORCID at https://orcid.org/register and get a free ORCID ID.

### Abstract

Each article is to be preceded by a succinct abstract, of up to 200 words, that highlights the objectives, methods, results, and conclusions of the paper. The abstract should state briefly the purpose of the research, the principal results and major conclusions.

The abstract body is typed in Times New Roman, 10 pt.

### Keywords

About four Keyword or phrases in order of importance, separated by commas and typed in Times New Roman, 10 pt.

### Headings

Use bold, lowercase, 10 Times New Roman font for headings.

### Introduction

This should define the problem and, if possible, the frame of existing knowledge.

Please ensure that people not working in that particular field will be able to understand the intention.

The word length of the introduction should be 150 to 300 words.

### Materials and Methods

Materials and methods should be clearly presented to allow the reproduction of the experiments.

### Results and Discussion

Results should be clear and concise and give the significance of the results of the work. Data must not be repeated in figures and tables.

Discussion should interpret the results in reference to the problem outlined in the introduction and of related observations by the author/s or others.

Implications for further studies or application may be discussed.

### Conclusion

A conclusion should be added if results and discussion are combined.

### Tables and Figures

Tables should have a short descriptive title.
The unit of measurement used in a table should be stated.
Tables should be numbered consecutively.
Figures should be prepared in GIF, TIFF, JPEG or PowerPoint.
Tables and Figures should be appropriately cited in the manuscript.

### Acknowledgments

Acknowledgments of financial support, advice, or other kinds of assistance should be given at the end of the text under the heading "Acknowledgements". The names of funding organizations should be written in full.

### References

They should be grouped at the end of the paper in surname order of appearance.

Use lowercase, 10 Times New Roman font for References.

The references must be written in alphabetical order by authors' names,

with the following elements:

Original Papers

Surname, N.N., Surname, N.N. (Year). The full title of the article. Journal Name, volume, and issue, first and last page. DOI number.

Book or Proceeding

Surname, N.N., Surname, N.N. (Year). Title of the contribution. In: Title of the book or proceeding. Volume (Publisher, City, first and last page

Thesis

Surname, N.N. (Year). Title of the thesis, University and Faculty, City. pages.

International Journal of
JAEFS
Agriculture, Environment and Food Sciences

edit PUBLISHING

CONTENTS

International Journal of
JAEFS
Agriculture, Environment and Food Sciences

edit PUBLISHING

CONTENTS

International Journal of
JAEFS
Agriculture, Environment and Food Sciences

edit PUBLISHING

## CONTENTS

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 502-506
DOI: https://doi.org/10.31015/jaefs.2022.4.1

# Acceptability of *Pennisetum purpureum* by West African dwarf rams as influenced by manure application rates

**Toyese Williams**[1] **and Victoria Ojo**[1]

[1] Federal University of Agriculture, Abeokuta, Department of Pasture and Range Management, Ogun-State. Nigeria

## Abstract

The present study evaluated the chemical composition and acceptability of Pennisetum purpureum by West African dwarf (WAD) rams as influenced by manure application rates. The experiment was a completely randomized design replicated three times. Grasses that have been fertilized with 5 and 10 t ha-1 of swine manure and unfertilized (control (0 t ha-1)), were harvested from the experimental site 9 weeks after planting from 15 cm above ground level and evaluated for proximate and fibre composition. The harvested grasses were also offered to nine WAD rams on cafeteria basis, to assess the acceptability indices of the grass based on treatments. Results showed that the grass with manures consistently had higher ($p<0.05$) crude protein (CP) contents than the control (unfertilized plants) while all the grasses with different manure rates had similar ($p>0.05$) neutral detergent fibre (NDF). Consumption, co-efficient of preference and relative palatability of P. purpureum by WAD rams were improved by manure application, where highest values of acceptability indices was recorded in grass with manure rate of 5 t ha-1 over 10 t ha-1 manured grass. Percentage consumption, co-efficient preference and relative palatability index were in the order 5 t ha-1 > 10 t ha-1 > 0 t ha-1 (unfertilized). From the result, it can be concluded that P. purpureum with manure had a better chemical composition profile (higher CP) and better acceptability than unfertilized grass. Enhancing P. purpureum with manure at 5 t ha-1 had higher ash content and improved acceptability by WAD rams than grass with 10 t ha-1 manure application rate.

**Keywords:** Acceptability, Nutritive value, Pennisetum purpureum, Swine manure

**Citation:** Williams, T., Ojo, V., (2022). Acceptability of *Pennisetum purpureum* by West African dwarf rams as influenced by manure application rates. International Journal of Agriculture, Environment and Food Sciences, 6 (4), 502-506.

**Received:** 21 September 2021
**Revised:** 14 September 2022
**Accepted:** 22 September 2022
**Published Online:** 18 October 2022

**Correspondence:** Toyese Williams
**E-mail:** aiextoyese521@gmail.com



## INTRODUCTION

Inadequate nutrition is one of the factors that generally affect livestock productivity. Despite the naturally endowed vegetation, there are still inadequate feeds and feedstuffs for livestock in Nigeria. Akinlade et al. (2005) reported that a major problem facing small ruminant animal producers is how to feed the animals adequately all year round. Consequently, the issue of palatability and nutritive quality changes has become a matter of interest and great concern to researchers (Huston et al., 1993). Ruminants in the tropics are raised predominantly on grasses which are inherently poor in digestibility, nutritive value and unavailable in the off-season (Babayemi, 2009). Forage plants depend on soil for their supply of nutrients while ruminants obtain the majority of their nutrients from plants growing on such soil. Fertilizers are needed to improve soil chemical and bio-

logical properties and this reflects on the phytonutrient contents and palatability of herbage plants (Alalade, et al., 2013). However, inorganic fertilizers are expensive and this has made researchers to have shifted attention to examining the performance of crops with organic manure considering that organic manure is cheap and readily available (Ojo et al., 2013). Manure has a longer-lasting effect than the equivalent nutrient levels to chemical fertilizer. This is because a large proportion of the mineral nutrients are combined with organic substances which are released gradually as they decay, hence improved yield may continue years after the addition of manure to the soil (Plaster, 1992).

Elephant grass (Pennisetum purpureum Schumach.) is a major high yielding tropical grass, which is very versatile and can be grown under a wide range of conditions and systems. Elephant grass requires high level of fertilizer and a regular water supply (Mannetje, 1992). With no or inadequate fertilizers, yields are in the range of 2-10 t DM/ha/year (Bogdan, 1977) lower than 20 to 80 t DM/ha/year for normal yield.

Free choice intake and acceptability study is a quick assessment of the physical quality of a feed. Coefficient of Preference (CoP) is a direct measure of acceptability and nutritional capability of feedstuff or forage. In recent times cafeteria techniques have been used to assess the acceptability of some forages (Babayemi et al., 2006). The feed intake or the palatability of forage is regulated by many factors: harvesting, physical and metabolic feedback and secondary metabolites. This study evaluated the effect of swine manure rates on the chemical composition and acceptability of Pennisetum purpureum by West African dwarf rams.

## MATERIALS AND METHODS

The experiment was conducted at the Pasture Unit of Federal University of Agriculture, Abeokuta (FUNAAB) Farm, Ogun State, Nigeria (7°58′ N, 3°20′ E; 75 masl). The site is situated in the derived savannah agro-ecological zone of Southwest Nigeria with average annual rainfall of 1,037 mm. Mean monthly temperature ranges from 25.7 °C in July to 30.2 °C in February (earth.google. com/).

The experimental land area was ploughed twice and allowed to rest for a period of two weeks before harrowing. The land area was divided into 3 equal blocks with a buffer zone of 1 m between blocks, while each plot measured 4 × 5 m, with a buffer zone of 1 m between plots. Analysis of the soil from the site indicated that it contained 0.12 % total nitrogen, 1.41 % organic carbon and 30.40 mg kg-1 phosphorus. Swine manure was collected from the Piggery Section of the Directorate of University Farms FUNAAB, 14 days before application in bi-axially oriented polypropylene bags. Following collection of the manure, it was air dried under a barn for a period of 11 days after which it was analyzed to determine its nutrient content. The swine manure was collected from pigs that had been fed a standard "Pigs finisher diet" and chemical analysis of the manure revealed that it contained: Ca: 4.13%, P: 1.21%, Mg: 2.42% and K: 1.91% on DM basis. The manure was raked into the soil of individual plots according to application rates (0, 5 and 10 t ha-1) for the grass in a single application 2 weeks before planting the grass. Stem cuttings of 30 cm long of P. purpureus was planted at 1m x 1m per plot, according to treatment. The plots were kept weed-free as much as possible throughout the experimental period. Nine WAD rams (three treatment groups of three animals each) with weights ranging between 10-15 kg were used for the study and they were tagged for easy identification. The animals were from Sheep unit of Universiy farms with regular health care. The environment was regularly disinfected. The grass were harvested based on the treatment from the experimental site 9 weeks after planting from 15 cm above ground level. Grass of 1 kg based on treatments were introduced on a cafeteria basis in feeding troughs to the animals and the experiment was repeated for five consecutive days. Nine feeding troughs containing the harvested grass based on the manure treatments with two troughs being empty to avoid border bias, were set before the animals, thus each animal has free access to each of the diets in the trough. The positioning of the feeding troughs containing the different treatment of grass were changed daily to prevent bias by the animals taking a particular part of the pen as the position for a particular type of grass. The grasses were offered at 08.00hr and withdrawn by 08.30hr and the left-over were weighed and recorded. Grasses were offered before the animals were allowed to graze or offered any feed for the day. The experiment was a completely randomised design replicated three times. The preference for the grass based on manure rates were calculated as the percentage of the grass consumed relative to grass offered for five days

$$\% \text{ Consumption} = \frac{\text{Grass offered} - \text{Grass remnant}}{\text{Grass offered}} \times 100$$

The preferred grass was assessed from the coefficient of preference (CoP) value, calculated from the ratio between the intakes for the individual grass, divided by the average intake of the grass (Babayemi et al., 2006). The grass were therefore said to be relatively acceptable if the CoP is greater than unit.

The CoP was expressed as:

$$\text{CoP} = \frac{\text{Intake for individual grass offered}}{\text{Mean intake of all the grass offered}}$$

**Table 1.** Effect of manure application rates on proximate and fibre composition (%) of Pennisetum purpureum

| Factors | DM | CP | EE | Ash | NDF | ADF | ADL | Hemicellulose | Cellulose |
|---|---|---|---|---|---|---|---|---|---|
| Manure (t ha-1 ) | | | | | | | | | |
| 0 | 96.18 | 8.12b | 12.61 ab | 10.06a | 59.50 | 39.25a | 7.88a | 20.25c | 31.37a |
| 5 | 96.22 | 9.80 a | 12.20 b | 10.10 a | 59.21 | 36.43 b | 5.12 b | 22.78 b | 31.31 a |
| 10 | 96.52 | 10.12a | 12.77 a | 8.82b | 60.33 | 35.73c | 4.71c | 24.60a | 31.02b |
| SEM | 0.43 | 0.32 | 0.11 | 0.22 | 0.24 | 0.54 | 0.50 | 0.65 | 0.07 |
| P – value | 0.000 | 0.000 | 0.053 | 0.000 | .148 | 0.000 | 0.000 | .000 | 0.050 |

Means on the same column with different superscript differ significantly ($p<0.05$)
DM: Dry matter, CP: Crude protein, EE: Ether extract, NDF: Neutral detergent fibre, ADF: Acid detergent fibre, ADL: Acid detergent lignin
SEM: Standard error of mean

Daily relative palatability index (RPI) was calculated for the grass by dividing the consumption values for each treatment by that of the highest value and multiplied by 100 (Larbi et al., 1993).

The RPI is expressed as follows:

$$\text{RPI (\%)} = \frac{\text{Consumption values for each grass} \times 100}{\text{Grass consumption highest value}}$$

Subsamples (300 g) of harvested grass based on manure treatments were oven-dried at 65 $^{o}$C to constant weight, following which they were milled and allowed to pass through a 1 mm sieve screen. The dry matter, ether extract, crude protein and ash contents were determined according to the standard methods of AOAC (2000). Neutral detergent fibre (NDF), acid detergent fibre (ADF), and acid detergent lignin (ADL) were determined according to Van Soest et al. (1991). Cellulose concentration was estimated as the difference between ADF and ADL concentrations, while hemicellulose concentration was estimated as the difference between NDF and ADF concentrations.

### Statistical Analysis

Data collected was subjected to one way analysis of variance using the general linear hypothesis testing (GLHT) package of the R Statistical software (R Core Team, 2020). Mean values were separated using Tukey's HSD test.

## RESULT AND DISCUSSION

In Table 1, the manured grass consistently has higher ($p<0.05$) CP contents than the control (unfertilized grass). Higher significance of ash content was recorded in grass fertilized with 5 t ha -1 of swine manure and in the unfertilized above the one fertilized with 10 t ha -1 of swine manure. Significantly ($p<0.05$) highest ADF and ADL contents were recorded for unfertilized grass above the grass with manures. The increase in CP contents with grass with manure may have been due to the fact that manuring makes more nutrient available to the grass, such that the soil nutrient resource pool in the manured plots was larger than the control. This findings emphasizes the importance of fertilization for grasses to get high quality forages, and is consistent with the reports of some previous reports in the literature which also reported improved CP content with manuring (McRoberts et al., 2016; Utamy et al., 2018). The significantly ($p<0.05$) lower ash content recorded at the highest manure application rate for P. Purpureum suggests that higher nutrient availability may have a possible antagonist effect on ash accumulation of P. purpureum. It is possible that luxury consumption of an abundantly rich soil nutrient pool brought about by manure application caused a passive absorption of ash accumulation in P. purpureum. In the literature, McRoberts et al. (2018) reported that this passive response is possibly curtailed when soil nutrient levels decline such that higher accumulation of ash (inorganic nutrients) is favoured by the plants as seen in the unfertilized P. purpureum plant. More so, higher ash content in grass fertilized with 5 t ha -1 of swine manure above the one with 10 t ha -1 of swine manure, might be that 5 t ha -1 of swine manure meet the nutrient needs of the grass. This findings reflect the different nutrient absorption strategy employed by the grass given the levels of nutrient resource available. The NDF recorded is within the range of 600-650 g kg-1 suggested as the critical limit above which efficiency of utilization of tropical forages by ruminants would be impaired (Muia, 2000). The moderate fibre levels of the grass depending on different manure rates will help to facilitate the colonization of ingesta by rumen microorganism which in turn might induce higher fermentation rates, that will help in improving digestibility, intake and animal performance.

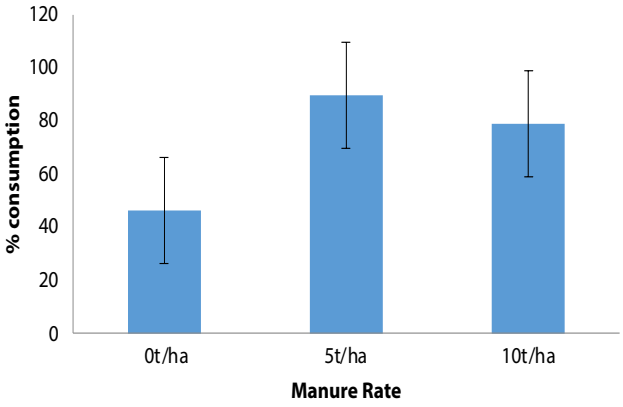



**Figure 1.** Influence of manure application rates on Percentage consumption of P. purpureum by WAD rams

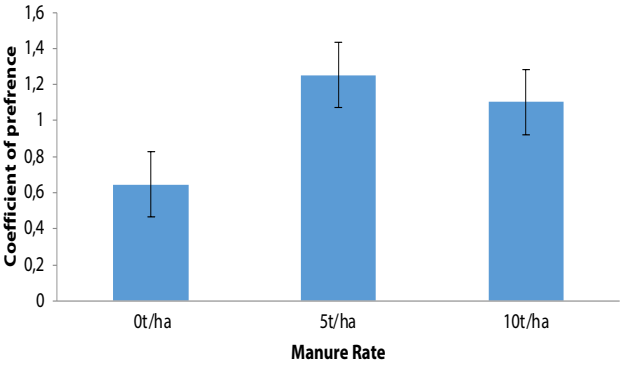



**Figure 2.** Influence of manure application rates on Co-efficient of preference of P. purpureum by WAD rams

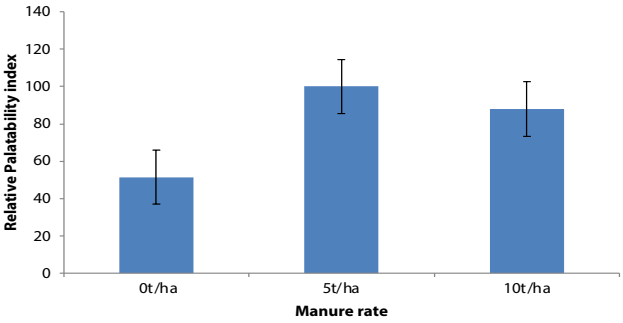



**Figure 3.** Influence of manure application rates on Relative palatability index of P. purpureum by WAD rams

Percentage consumption of P. purpureum, co-efficient of preference and relative palatability index by WAD rams were improved by manure application while unfertilized grass had lower acceptability (Figures 1, 2 and 3). This could be as a result of higher CP contents in fertilized grass since CP has been reported to enhance higher intake (Ergon et al., 2017) as well as moderate fibre contents. Moreover, Milne (1991) reported that diet selection in ruminants is complex. The relative palatability index (RPI) was a bit higher than the range of 0 to 70 % reported by Goatcher md Church (1970) as the preference range for ruminant animals. Palatability is a complex phenomenon determined by dietary type and environmental variables (Molyneux and Ralph, 1992). The differences reported could have been as a result of variations in environmental variables.

## CONCLUSION

From the study, it can be concluded that P. purpureum with manure application rates had higher protein contents and moderate fibre constituents and were more accepted by animals than the unfertilized grass. However, the acceptability indices investigated demonstrated that the P. purpureum manured with 5 t ha -1 was highly acceptable by rams compared to the one with manure rate of 10 t ha -1. In addition, the application of manure is recommended because of its beneficial effects on the chemical composition and acceptability by animals.

However, the coefficient of preference demonstrated that the P. purpureum fertilised with 5 t ha -1 is highly acceptable compared to unfertilized grass.

## COMPLIANCE WITH ETHICAL STANDARDS

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

The contributions of the authors to the present study is equal. The authors read and approved the final manuscript. The authors verify that the Text, Figures and Tables are original and that they have not been published before.

### Ethical approval

This research was in compliance with the guideline and animal experimentation protocol of the College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, Nigeria.

### Data availability

All data associated with this research were indicated and used in the manuscript submitted.

### Consent for publication

All authors consented to the publication of this manuscript.

## REFERENCES

Akinlade, J. A., Farinu, G. O., Olujide, A. M., Ojebiyi, O.O., Aderinola, O. A and Togun, V. A. (2005). "Evaluation of shrubby legumes (Stylosanthes guianensis and Stylosanthes scabra) and cotton seed cake on the performance of West African Dwarf Sheep", Proceeding of 10th Annual Conference of Animal Science Association of Nigeria (ASAN). University of Ado-Ekiti, Nigeria. Sept 12-15,2005 Pp 184-186.

Alalade, J. A., Akanbi, W. B., Akingbade, A. A., Akinlade, J. A., Gbadamosi, J. and Emiola, C. B. (2013). Effects of Manure Types on Herbage Yield and Nutritive Value of Panicum maximum. International Journal of Applied Research and Technology, 2(11): 77 – 81.

AOAC. (2010). Official method of analysis. The Association of Analytical Chemists. 15th Ed. Arlington, USA.

Babayemi, O.J. (2009). Silage quality, dry matter intake and digestibility by African dwarf Sheep of Guinea grass (Panicum maximum cv Ntchisi) harvested at 4 and 12 week regrowths. Afr. J. Biotechnol., 8: 3988-3989.

Babayemi, O.J., Bamikole, M.A. and Omojola, A.B. (2006). Evaluation of the nutritive value and free choice intake of two aquatic weed (Neophrolepis biserrata and spirodelaplyrhiza) by west African dwarf goats. Trop. Subtropical Agro Ecosys., 6: 15-22

Bogdan, A. V. (1977). Tropical pasture and fodder plants. Longman, 475 pp.

Ergon, Kirwan, L., Fystro, G., Bleken, M. A., Collins, R. P., & Rognli, O. A. (2017). Species interactions in a grassland mixture under low nitrogen fertilization and two cutting frequencies. II. Nutritional quality. Grass and Forage Science, 72(2), 333–342. [CrossRef]

Goatcher, W. D. and Church, D. C. (1970). Taste responses in ruminants. IV. Reactions of pygmy goats, normal goats, sheep and cattle to acetic acid and quinine hydrochloride. Journal of Animal Science. 31:373.

Huston, J. E. (1993). Symposium. Dairy goats. Forage utilization and nutrient requirements of the goats. Journal of Dairy Science 61: 988-993.

Larbi, A., Osakwe, I. I. and Lambourne, J. W. (1993). Variation in relative palatability to sheep among Gliricidia sepium provenances. Agroforestry Systems 22:221 –224.

Mannetje, L.'t. (1992). Pennisetum purpureum Schumach. Record from Proseabase. Mannetje, L.'t and Jones, R.M. (Editors). PROSEA (Plant Resources of South-East Asia) Foundation, Bogor, Indonesia.

McRoberts, K. C., Ketterings, Q. M., Parsons, D., Hai, T. T., Quan, N. H., Ba, N. X., Nicholson, C. F. and Cherney, D. J. R. (2016). Impact of Forage Fertilization with Urea and Composted Cattle Manure on Soil Fertility in Sandy Soils of South-Central Vietnam. International Journal of Agronomy, 4709024. [CrossRef]

McRoberts, K. C., Parsons, D., Ketterings, Q. M., Hai, T. T., Quan, N. H., Ba, N. X., Nicholson, C. F. and Cherney, D. J. R. (2018). Urea and composted cattle manure affect forage yield and nutritive value in sandy soils of south-central Vietnam. Grass and Forage Science, 73(1), 132–145. [CrossRef]

Milne, J. A. (1991). Diet selection by grazing animals. Proc. Nutr. Soc 50:77.

Molyneux, R. J. and Ralph, M. N. (1985). Plant toxins and palatability to herbivores. Journal of Range Management. 45:1-18.

Muia, J. M. K. (2000). Use of Napier grass to improve smallholder milk production in Kenya PhD Thesis. Wageningen Agricultural University. The Netherlands.

Ojo, V. O. A., Dele, P. A., Olanite, J. A., Adeoye, S. A., Ajayi, S. O. and Idowu, O. M. O. (2013). Influence of manure type on the productivity of Pennisetum purpureum under different planting method and age. Journal of Organic Agriculture and Environment 1 (1):71 - 77.

Plaster, E.J. (1992). Soil science and management (2nd Edition) Delmer publisher Inc. pp 321.

Core Team, R. (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. [URL]

Utamy, R. F., Ishii, Y., Idota, S. and Khairani, L. (2018). Effect of repeated application of manure on herbage yield, quality and wintering ability during cropping of dwarf napiergrass with Italian ryegrass in Hilly Southern Kyushu, Japan. Agronomy, 8(3). [CrossRef]

Van soest, P. J, Robertson, J.B and Lewis, B.A. (1991). Methods for directory neutral detergent fibre and nonstarch polysaccharides in relation to animal nutrition. J. dairy Sci., 74:3583-3597.

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 507-515
DOI: https://doi.org/10.31015/jaefs.2022.4.2

# Resource use efficiency and factors influencing maize production in Kuje Area Council, Federal Capital Territory, Nigeria

**Funso Omolayo Alabuja[1], Luka Anthony[2], Elizabeth Samuel Ebukiba[3]**

[1] Department of Agricultural Economics, University of Abuja, PMB 117, Gwagwalada, Federal Capital Territory, Nigeria
[2] Department of Agricultural Economics, University of Abuja, PMB 117, Gwagwalada, Federal Capital Territory, Nigeria
[3] Department of Agricultural Economics, University of Abuja, PMB 117, Gwagwalada, Federal Capital Territory, Nigeria

**Citation:** Alabuja, F.O., Anthony, L., Ebukiba, E.S. (2022). Resource use efficiency and factors influencing maize production in Kuje Area Council, Federal Capital Territory, Nigeria. International Journal of Agriculture, Environment and Food Sciences, 6 (4), 507-515.

**Received:** 29 March 2022
**Revised:** 05 August 2022
**Accepted:** 18 August 2022
**Published Online:** 19 October 2022

**Correspondence:** Luka Anthony
**E-mail:** gqluka11@gmail.com



## Abstract

This study estimated resource-use efficiency and factors influencing maize production in Kuje Area Council, Federal Capital Territory, Nigeria. The specific objectives were to; determine the socioeconomic characteristics of maize farmers; analyse cost and returns associated with maize production; evaluate factors influencing the output of maize production; determine the resource-use efficiency of maize production and identify the constraints faced by farmers in maize production in the study area. Multistage sampling technique was employed to select 60 sampled maize farmers in the study area. The following tools of analysis were used to achieve the specific objectives of the study. Descriptive statistics; Gross margin analysis; resource-use efficiency and Cobb Douglass production function. The results showed that majority, 75 percent, of the sampled respondents were male while 73.3 percent were married. From the results it can further be deduced that 48.3 percent of the sampled respondents attended secondary school and 31.7 percent attended tertiary institution while the rest stopped at primary school level or did not have any formal education. The results also indicated that about 55 percent of the sampled maize farmers had household size ranges within 6-10. 100 percent of the sampled respondents had no access to credit and 83.4 percent had no access to extension services. From the analysis of cost and returns associated with maize production in the study area, the total revenue (TR) realized on average was N1,269,152.69 and the average total variable cost (TVC) was N188,462.69, the gross margin obtained was N1,080,690. With this result we can say that maize production is profitable in the study area. The results of the resource-use-efficiency revealed that farm size, seed input and labour input were underutilized while fertilizer input and chemical input were over utilized by maize farmers. The results of the Cobb Douglass production function model revealed that the factors influencing total output of maize production in the Study area were farm size ($P<0.1$), labour ($P<0.01$), chemical ($P<0.01$) and Fertilizer $P<0.05$). The major constraints faced by maize farmers in the study area include; inadequate capital, lack of fertilizer and lack of extension agent. Therefore, the study recommends that maize farmers should be encouraged to join the farmers' association, and supported with credit facilities. Government should supply inputs like agrochemical, fertilizer and improved seed varieties to maize farmers at a subsidized rate and at appropriate time and extension agents are to guide the farmers in the usage of these inputs while mechanize farming system should be encouraged by providing tractors to replace local farm implements. Good roads are essential in linking maize production areas with available markets around the study area.

**Keywords:** Resource-use, Efficiency, Maize, Production, Nigeria

## INTRODUCTION

The level of agricultural development in Nigeria has not met the required demand of its teaming population, despite the abundant endowment of the country's different types of natural resources and vast land mass available for agri-

cultural cultivation. Although agricultural production is practiced by both small and large-scale farmers in nearly all parts of the country, majority of farmers especially the small-scale farmers still live in abject poverty (UNDP, 2009). These category of farmers are characterized by low level of productivity, low income level, large family size, lack of formal education, credit facilities, inefficiency in the use of resources available to them, continuous use of crude implements, and low savings and investment (Panwal et al.,2006). The term resource is used in reference to available means for producing goods and services. These goods in return are used to satisfy needs. Major resources used in agriculture are; land, labour, capital and management. Other agricultural inputs include seeds, fertilizers and chemicals. Resource management is the effective and efficient deployment of organization or farm resources at the right time. The importance of management cannot be over-emphasized because its effects could either be positive or negative if properly or carelessly employed respectively.

Maize (Zea mayz) is among most of the widely planted and cultivated cereal crops in the world. It is the fourth most consumed cereal ranked below sorghum, millet, and rice (FAOSTAT, 2008). In Nigeria, there is an increasing demand for maize on daily basis (Sadiq et al., 2013). This is because maize grains are jointly utilized for feeding poultry livestock and as food by many families (Ogunniyi, 2011). Nigeria is reported as the Africa's second largest producer of maize only after South Africa (FAOSTAT, 2018; PWC, 2021). As earlier mentioned maize is one of the best ingredients used in preparation of animal feeds. The cereal serves as industrial raw material in developed countries for different purposes. Maize has not only served as a source of food for man and livestock for years, but it has also served as a means and source of income generation and foreign exchange earnings for the country (Alabi and Abdulazeez, 2018). In addition, it is as an essential raw material for the industrial production of fuel, starch, medicines and food sweeteners. Levulinic acid, a chemical derived from maize is equally used as an anti-freeze ingredient, and can replace toxic petroleum-based ingredients. Ethanol produced from maize is used as a biomass fuel. maize straw serves as a cheap source of energy in homes for heating furnaces (Egwuma et al, 2019; Amaza, et al, 2021). Nigeria's maize production stood at about 11 million metric tons in 2021 (Amaza, et al, 2021). However, due to low productivity, current yields are unable to meet domestic demand which is estimated at about 12 – 15 MMT. That is, a supply gap of nearly 4 MMT exists per annum. This gap necessitated the export ban on maize in Nigeria to encourage domestic production and supply of the commodity (PWC, 2021).

Smallholder farmers in rural areas continue to face poor economic conditions which affect their living standards and maize production situation. The returns to land in terms of output have been on the decrease especially where increased population and non-agricultural use compete for land use (Babatunde et al.,2007). To achieve optimum production level, resources must be used efficiently. Successful planning and result-oriented policies require the technical knowledge of the productivity of farm resources to know the necessary adjustment to achieve a correct input mix (Assa et al, 2020). Despite its importance, maize production in Nigeria is predominated by traditional smallholders who rely on traditional methods of production. Resources are underutilized in addition to use of low amenities, which gives rise to low output and hence, low farm income. The supply of maize has also not been able to meet its demand despite the adoption of improved packages for maize production (Babatunde et al., 2008). For example, improved variety, recommended planting date, recommended fertilizer rate, recommended planting depth and spacing have not significantly increased productivity. Despite the introduction of hybrid maize, sufficient production has not been achieved as there is still a significant drop in the output of maize (Ayindea et al., 2011). According to Assa et al, (2020), low yield variety, lack of incentives, high cost of inputs, price fluctuation, diseases and pest, lack of storage facilities are the causes of low maize production in Nigeria. Given that the rate of population growth in Nigeria is increasing rapidly, there must be an increase in maize production to meet the growing demand (Ike and Amusa, 2004). Since the present maize output has not measured up to its potential yield of 5-8 tonnes per hectare, it is pertinent to ascertain if the resources available for the farmers are efficiently utilized to increase their present level of maize production. This is required to make Nigeria self-sufficient in maize production to the extent of having large surpluses for export and foreign exchange earnings.

## Research Questions

This study intended to provide answers to the following research questions: -

(i) What are the socio-economic characteristics of maize farmers in the study area?
(ii) What are the costs and returns associated with maize production in the study area?
(iii) What are factors influencing output of maize production in the study area?
(iv) What are the resource-use efficiencies of maize production in the study area?
(v) What are the constraints of maize production faced by farmers in the study area?

## Objectives of the Study

The broad objective was to evaluate resource-use effi-

ciency and factors influencing maize production in Kuje Area Council, Federal Capital Territory, Nigeria. The specific objectives were to;

(i) Determine the socio-economic characteristics of maize farmers,
(ii) Analyse the costs and returns associated with maize production,
(iii) Evaluate factors influencing output of maize production,
(iv) Determine the resource-use efficiency of maize production,
(v) Identify the constraints faced by farmers in maize production in the study area

## MATERIALS AND METHODS

### The Study Area

The study was conducted in the Federal Capital Territory (FCT), in Kuje Area Council. Kuje is located within Latitudes $8^0$ $53^I$ $47^{II}$ North and Longitudes 70 $14^I$ $35^{II}$ East. The council has a total land area of about 1,644 Km2. It is located 40 Km towards the south-west part of Abuja and is bounded by Nasarawa State. The temperature of the area is generally high especially around February and March. The climate of the area is that of tropical wet and dry with wet season lasting for about five to seven months (5-7 months). Total annual rainfall ranges from 1000 – 1500 mm. Potential evapotranspiration rates is slightly high, value reaching up to 3.5mm/day. The north-east trade wind sweeps the zone between October to March bringing dryness to the area. The land in the Area Council is sloppy-plane topography which is about 410 mm above mean sea level. The Area Council also has a population of 97,367 people (NPC, 2006) predominantly made up of farmers hey cultivate crops such as; maize, sorghum, rice, beans, groundnut, millet, yam, vegetables.

### Sampling Techniques and Sample Size

Multi-stage sampling techniques was used to select respondents for the study area. First stage involved simple random selection using ballot box raffle draw method to select Kuje Area Council. Stage two, involved random sampling procedure using ballot box raffle draw method to select two wards in the study area Kuje central and Gaube. Stage three involved using a random sampling ballot box raffle draw method to select 60 maize farmers in the selected areas (Kango and Chukuku villages) for the administration of questionnaire. The total sample size of 60 questionnaires were administered to respondents in the area

### Method of Data Collection

Primary data were used for the study. These data were collected by interview method, using structured questionnaires. The questionnaires covered: (a) demographic information such as age, farming experience, marital status, educational level, household size, extension contact, membership of associations, and farm size, (b) production information on maize such as inputs used (land, seeds, labour, fertilizer and agro-chemicals) and output obtained, (c) market information like price of input and quantity sold, and (d) constraints to maize production.

### Methods of Data Analysis

### Descriptive Statistics

This is the act of summarizing and giving a descriptive amount of numerical information in form of report, charts, and diagrams. The goal of descriptive statistics is to gain information from collected data. The descriptive statistics involved the use of percentages, means, and frequency distribution tables. This was use to achieve specific objectives one (i) and five (v).

### Gross Margin Analysis

This analysis was used to estimate the costs and returns analysis of maize production. It is a very important planning tool in situations where fixed capital is a negligible portion of the farming enterprises as is the case of subsistence agriculture (Olukosi and Erhabor,2001). It is used to evaluate the profitability of an individual enterprise and is given as;

GM = TR – TVC .....(1)

Where;

GM = Gross Margin (₦ /Hectare)

TR = Total Revenue (₦/Hectare)

TVC = Total Variable Cost (₦/Hectare)

This was used to achieve specific objective two (ii)

### Cobb-Douglas Production Model

The Cobb-Douglas production function represents the relationships between two or more inputs typically physical and labour and the number of output that can be produced.

Mathematically;

$$Y = b_0 X_1^{b_1} X_2^{b_2} X_3^{b_3} X_4^{b_4} X_5^{b_5} U_i \quad \text{.....(2)}$$

When linearized, the Cobb-Douglas production model becomes:

$L_n = b_0 + b_1 L_n X_1 + b_2 L_n X_2 + b_3 L_n X_3 + b_4 L_n X_4 + b_5 L_n X_5 + U_i$.....(3)

Where, Ln= Natural Logarithm
Y= Output of Maize (Kg)
$b_0$ Constant Term (Intercept)
$X_1$= Farm Size (in Hectares)
$X_2$= Seed Input (Kg)
$X_3$= Labour Input(Mandays)
$X_4$= Fertilizer Input (kg)
$X_5$= Agrochemicals Input (Litres)

$b_1$-$b_5$ = Coefficient of Parameters Estimated
$U_i$= Error Term
This was used to achieve specific objective three (iii)

### Resource-use Efficiency of Maize Production

To measure the resource-use efficiency of maize production in the study area, the Marginal Value Products (MVP) of the resources used were estimated by multiplying the Marginal Physical Product (MPP) of the inputs with the price of the output. The values were then compared with the cost of the resources Marginal Factor Cost (MFC) in order to make inference on the efficiency of resource-use. The following was estimated to determine the resource-use efficiency of maize production:

$$r = \frac{MVP}{MFC} \quad \text{----------(4)}$$

Where;
r = Efficiency Ratio (Units)
r = 1, Resources employed by the Farmer were Efficiently Utilized,
r > 1 Resources employed by the Farmers Were Under Utilized, and
r1, Resources employed by the Farmers Were Over Utilized.
The MPPs and, MVPs were derived as follows:
Linear:

$$MPP = \frac{dy}{dx} = b_i; \quad MVP = b_i.P_y \quad \text{.....(5)}$$

Semi – Log:

$$P = \frac{bi}{x}; \quad MVP = \frac{bi}{x}.P_y \quad \text{.....(6)}$$

Double – Log:

$$MPP = \frac{bi}{x}; \quad MVP = \frac{bi}{x}.P_y \quad \text{.....(7)}$$

The Elasticity of Production ($E_p$) is the regression coefficients. Return to Scale (RTS) was estimated as:

$$RTS = \sum_{i=1}^{n} E_P \quad \text{.....(8)}$$

This was used to achieve specific objective four (iv)

## RESULTS AND DISCUSSION

### Socio-Economic Characteristics of the Sampled Respondents in the Study Area

Table1 presents the analysis of the socioeconomic characteristics of the sampled respondents, the results show that majority of the sampled respondents were male while 25% were female, this result indicates that maize production was dominated by male farmers in the study area. This could be as a result of the labour required for maize production. Majority (73%) of the sampled respondents were married. Married families have an advantage of more supply of labour for maize cultivation which could lead to increase in total output of maize. Also table 1 depicts that 55% of the sampled respondents had 6-10 members per household and 36% had 1-5 members per household while others have more than ten members. This suggests that most of the sampled respondents had at least 6 members per household which shows that they have a significant labour supply for maize production and this could improve the total output of maize in the study area. Furthermore, the results revealed that 33.40% of the sampled respondents had 1-5 years farming experience while 26.70% had 6-10 years of farming experience in the study area. Experience they say is the best teacher. Therefore, there is tendency of increased output due to the fact that majority of the farmers were not newbies in the business since they were familiar with the management practices involved in the maize production cycle. About 48.30% of the sampled maize farmers had secondary education while 31.70% attained tertiary level of education and 1.7% and 18.3% had no formal education and primary education respectively. The implication of this result shows that most of the sampled maize farmers were educated and this could aid them in accessing information about improved methods of maize production which in turn will improve their chances of adopting new technologies and innovations in agriculture. Education level of a farmer could determine how efficient the farmer would be, especially in the area of resource utilization which could lead to output and profit maximization. This result is in line with (Alabi et al, 2020 and Ebukiba et al, 2020) who reported that education is an important factor that can influence small-scale farmers to adopt new innovations and research findings related to their area of production. When a farmer is educated, there is high probability that he will take advantages of innovations and new technologies easily which would eventually lead to improved yield and increase in output. Analysis also shows that 100% of the sampled respondents could not access credit facilities. This implies that there is no provision of credit facilities to help farmers with fund to increase their scale of production in the study area thereby limiting their ability to expand. Majority (83.40%) of the sampled maize farmers could not have access to extension services while only 16.7% had access to extension services. Extension services help farmers to access price information, methods of inputs utilization like chemical and fertilizer application and access to improved seeds which could influence their resource-use efficiency. Most of the sampled farmers were not opportune to these privileges. The result also indicated that only 16.7% of the sampled maize farmers were visited by extension agents. This percentage is too low to inspire other farmers positively. Many of the farmers (66.3%) were members of farmers' association. This could aid group marketing and access to financial facilities among farmers in the study area.

**Table 1.** Socioeconomic Characteristics of the Sampled Maize Farmers in the Study Area

| Variables | Frequency | Percentage | Mean |
|---|---|---|---|
| Gender | | | |
| Male | 45 | 75.00 | |
| Female | 15 | 25.00 | |
| Marital Status | | | |
| Single | 16 | 26.70 | |
| Married | 44 | 73.30 | |
| Household Size (Units) | | | 7 |
| 1-5 | 22 | 36.70 | |
| 6-10 | 33 | 55.00 | |
| 11-15 | 5 | 23.80 | |
| Farming Experience (Years) | | | 6 |
| 1-5 | 20 | 33.40 | |
| 6-10 | 16 | 26.70 | |
| 11-15 | 6 | 9.90 | |
| 16-20 | 7 | 11.70 | |
| 21-25 | 6 | 10.00 | |
| 26-30 | 3 | 5.10 | |
| 31-35 | 2 | 3.40 | |
| Educational Level | | | |
| No formal education | 1 | 1.70 | |
| Primary education | 11 | 18.30 | |
| Secondary education | 29 | 48.30 | |
| Tertiary education | 19 | 31.70 | |

**Table 1.** Socioeconomic Characteristics of the Sampled Maize Farmers in the Study Area (continuous)

| Variables | Frequency | Percentage | Mean |
|---|---|---|---|
| Access to Credit | | | |
| Yes | 0 | 0 | |
| No | 60 | 100 | |
| Access to Extension | | | |
| Services | | | |
| Yes | 10 | 16.70 | |
| No | 50 | 83.40 | |
| Number of Visit (Days) | | | |
| 0 | 51 | 85.00 | |
| 1-2 | 9 | 15.00 | |
| Farmers Association | | | |
| Yes | 40 | 66.30 | |
| No | 20 | 33.30 | |
| Farm Size (Hecters) | | | 2.1 |
| 1-2 | 59 | 98.40 | |
| 3-5 | 1 | 1.70 | |
| Cropping System | | | |
| Sole | 49 | 81.70 | |
| Mixed | 11 | 18.40 | |
| Total | 60 | 100.00 | |

Source: Field Survey (2021)

## Cost and Returns Analysis of Maize Production in the Study Area

Table 2 shows the analysis of cost and returns associated with maize production in the study area. The result shows that the total revenue(TR) was N1,269,152.69 while the total variable cost (TVC) was N188,462.69 respectively. The cost of agrochemicals was N15,611.86 while the average cost of fertilizer was N 25,478.33. The cost of labour was N 142,211.67 and was the highest of the total variable cost. The Gross Margin obtained was N1,080,690. These results showed that maize production is profitable in the study area. The result is consistent with the findings of Ebukiba et al, (2020 who asserted that maize production is a profitable venture.

**Table 2.** Cost incurred and Return Obtained from Maize Production in the Study Area

| Variables | Average Value(Naira) | Percentage |
|---|---|---|
| A. Variable Cost | | |
| a. Seed Cos | 5,160.83 | 2.74 |
| b. Labor Cost | 142,211.67 | 75.46 |
| c. Fertilizer Cost | 25,478.33 | 13.52 |
| d. Agrochemical Cost | 15,611.86 | 8.28 |
| B. Total Variable Cost | 188,462.69 | |
| C. Total Revenue | 1,269,152.69 | |
| D. GM= TR-TVC | 1,080,690.00 | |

Source: Field Survey (2021)

## Resource-use Efficiency of Maize Production in the Study Area

Table 3 shows the resource-use efficiency of maize production in the study area. r=1 shows that resources employed by the farmers were efficiently utilized; r>1 shows that resources employed by the farmers were underutilized while r <1 shows that the resources employed by the farmers were over utilized. The farm size ratio was -2.24 which shows that the land resource used by maize farmers in the study area were over utilized. The seed input ratio was 3261.62 which revealed that the resource was underutilized by maize farmers in the study area. The labour input ratio was 5467.57 which depicts that the labour resource was underutilized by maize farmers in the study area this is in comformity with Assa et al, (2020). Also the fertilizer input ratio was 0.57 which indicates that fertilizer was over utilized by sampled maize farmers. The agrochemical ratio was 0.16 which shows that agrochemicals were over utilized by maize farmers. This result is in agreement with (Ume et al, 2016) who reported in a research on the impact of resource utilization on output that the efficient utilization of available resources determines the rate of output that will be obtained. The results are also in line with Ume et al, (2018) who reported that the over-utilization of resources implied that less profit was maximized. The possible reasons for the over utilization of the resources, could be because of the inability of farmers to allocate their resources efficiently as a result of lack of technical-know-how.

**Table 3.** Resource-use Efficiency of Maize Production in the Study Area

| Value Factor Unit | MVP | MFC | MVP/ MFC | Remarks |
|---|---|---|---|---|
| Farm Size | 170.93 | 76.22 | 2.24 | Under utilized |
| Seed | 8134477.58 | 24.94 | 3261.62 | Under utilized |
| Labour | 4417850.31 | 808.01 | 5467.57 | Under utilized |
| Fertilizer | 3094.84 | 5455.42 | 0.57 | Over utilized |
| Chemical | 271.82 | 1781.54 | 0.16 | Over utilized |

Source: Field Survey (2021)

## Factors Influencing Total Output of Maize Production in the Study Area

Table 4 presents the results of the analysis of Cobb Douglass production functional model to determine factors influencing total output of maize production in the study area. The results show that there are four statistically significant factors influencing maize production in the study area. These include farm size, labour input, chemical input and fertilizer. Farm size influenced the total output of maize positively and it was statistically significant at (P<0.1) probability level. The coefficient of farm size was 0.092 which implies that a unit change in farm size will result in 9.2% increase in the total output of maize in the study area. As a result of the expansion of farm size by maize farmers will results in the increase in total output of maize due to increase in farm size. This result is in agreement with Erabor (2001) who reported that large farm size leads to positive increase in total output. Labour input influenced total output of maize positively and it was statistically significant at (P<0.01). The magnitude of the coefficient of labour input (0.974) implies that a unit increase in labor supply in maize production will result in 97.4% percent increase in the total output of maize in the study area holding other variables constant. More supply of labour leads to significant increase in the total output due to the high number of labour involved in the farm operation. This result agrees with (Assa et al, 2020) who reported that the level of output can be measured by the level of labor input involved in the cause of production cycle. Also chemical input influenced the total output of maize negatively in the study area and it was statistically significant at (P<0.01) probability level. The magnitude of chemical input is -0.0353 implying that a unit increase in the chemical applied to the maize farm by farmers will result in the decrease in the total output of maize in the study area. The implication of this

result is that the application of more chemicals to maize farms will lead to 3.53% decrease in the total output of maize in the study area. This could be as a result of wrong usage of chemical (herbicide) on the farmland and it may affect the yield of maize output. This might be as a result of lack of knowledge on method of application by farmers considering the fact that majority did not have access to extension services. This result is contrary to Anthony et al, (2021) who reported that chemical inputs influence total output or yield of crop positively in their study but is in conformity with the results of Assa et al, (2020). Fertilizer influenced the output of maize positively and it was statistically significant at (P<0.05) probability level. The magnitude of the coefficient of fertilizer was 0.098, which implies that a unit change in fertilizer will result in 9.8% increase in the total output of maize in the study area This is in line with Assa et al, (2020). The value of the coefficient of multiple determination (R2) was 0.59 which implies that 59% of the variation in the total output of maize is explained by explanatory variable included in the model, the value of the F-statistic which is the joint contribution of the all explanatory variables was 3.008 and statistical significant at (P<0.01) probability level.

**Table 4.** Results of the Cobb Douglass Production Functional model for Factors Influencing Total Output of Maize Production in the Study Area

| Variables | Coefficients | Standard Error | T- Value | Significant |
|---|---|---|---|---|
| (Constant) | 2.695* | 0.675 | 3.990 | 0.000 |
| Farm Size Input | 0.092*** | 0.049 | 1.877 | 0.650 |
| Seed Input | -0.011 | 0.037 | -0.211 | 0.990 |
| Labour Input | 0.974* | 0.336 | 2.898 | 0.00 |
| Chemical Input | -0.353* | 0.030 | -11.767 | 0.001 |
| Fertilizer | 0.098** | 0.044 | 2.227 | 0.029 |
| R- Square | 0.59 | | | |
| Adjusted R2 | 0.473 | | | |
| F-Value | 3.008 | | | |

Source; Field Survey (2021) *, **, ***, Statistically Significant at (P<0.01) (P<0.05) (P<0.1) Respectively.

## Constraint Encountered in Maize Production by Sampled Farmers in the Study Area

Table 5 shows the analysis of constraints of maize production in the study area. The results show that majority 53.3% of the sampled respondents were faced with inadequate capital while 18.3%of the sampled respondents depicts lack of fertilizer as the major constraint in maize production. The result further revealed that 16.7% of the sampled respondents had no access to extension agents who are supposed to teach advanced methods of maize production to farmers in the study area. The result also indicated that about 10% of the sampled maize farmers identified bad roads as one of the major barrier to effective maize production in the study area, and 1.7% of the sampled respondents encountered government policy on land as a challenge to efficient maize production in the study area.

**Table 5.** Constraints of Maize Production in the Study Area

| Variables | Frequency | Percentage Rank |
|---|---|---|
| Inadequate capital | 32 | 53.3 1st |
| Government policy | 1 | 1.7 |
| High cost of input | 0 | 0 |
| Lack of fertilizer | 11 | 18.3 2nd |
| Lack of extension agent | 10 | 16.7 3rd |
| Bad roads | 6 | 10 |
| Total | 60 | 100 |

Source, Field Survey, (2021)

## CONCLUSION

According to the findings emanating from this research work, the study revealed that maize production is profitable in the study area with the prospect of increase in production and increase in the income of the maize farmers. The study also holds future prospects of improving the well-being of the farming family in the study area. However, despite the profitability of maize production in the study area, maize farmers encountered the problem of inadequate capital which was ranked first while lack of fertilizer ranked second and lack of extension agent was ranked third. Poor road network was also identified as a constraint of maize production in the study area. Therefore, the study recommends that;

1. Maize farmers should be encouraged to join the farmers' association so they can pool their productive resources for large scale farming. Also, farmers should be encouraged by providing credit facilities to them as a motivating factor to encourage their involvement in maize production for earning income to improve their family wellbeing.
2. Government should supply inputs like agrochemicals to maize farmers at a subsidized rate and at appropriate time. They should also encourage mechanized farming systems because mechanization leads to lower cost of production thereby causing an increase in the proper utilization of resources in order to bring about increase in output.
3. Government should provide good roads linking maize production areas to ease farmers in evacuating their produce and provide adequate market for maize farmers' products.
4. Government and private sector should make available and affordable soft micro credit loan at good times for production activities to farmers.

## COMPLIANCE WITH ETHICAL STANDARDS

### Conflict of interest

The authors declared no conflict of interest.

### Author contribution

The contribution of the authors to the present study is equal. The authours contributed in designing the research, data collection, data analysis, interpretation of results and copy editing All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Ethics committee approval is not required.

### Funding

No financial support was received for this study.

### Data availability

Not applicable.

### Consent for publication

Not applicable.

## REFERENCES

Alabi, O. O, and Abdulazeez, I. (2018). Economics of Maize (Zea mays) Production in Igabi Local Government Area, Kaduna State, Nigeria Journal of Agricultural Faculty of Gaziosmanpasa University 35 (3), 248-257

Alabi, O.O., Oladele, A.O., and Aladele N.O. (2020). Economic Market Decisions Among Marginal Maize Farmers in Abuja, Nigeria: Applications of Double Hurdle Model and Factor Analysis. Russian Journal of Agriculture and Socio-economic Sciences, 8(104), PP 114-125

Amaza, P.; Mailumo, S. and Silong, A. (2021). The Political Economy of the Maize Value Chain in Nigeria, Agricultural Policy Research in Africa (APRA) Working Paper 60, Brighton: Future Agricultures Consortium 1-56

Anthony, L., Alabi, O.O., Ebukiba, E.S., and Gamba, V. (2021). Factors Influencing Output of Rice Produced and Choice of Marketing Outlets Among Smallholder Farming Households, Abuja, Nigeria. Sarhad Journal of Agriculture, 37(1): 262-277

Assa, O. S., Suleiman, R. Emeghara, U. U. Yahaya, U. F. Olagunju, O. E. Onwuegbunam, N. E. Akanni-John, R. Ganiyu, L, Omodona, S. and Olukotun, O. (2020). Resource Use Efficiency among Maize Farmers in Lere Local Government Area of Kaduna State, Nigeria. Asian Journal of Advanced Research and Reports 14(1): 1-9

Babatunde , R.O. Omotesho, O.A. and Sholotan, O.S. (2007). Factors Influencing Food Security Status of Rural Farming Households in North Central Nigeria. Agricultural Journal, 2: 351-357

Ayindea, E. Muchiea, O.M. and Olatunjib, G. B. (2011). Effect of Climate Change on Agricultural Productivity in Nigeria: A Co-integration Model Approach J Hum Ecol, 35(3): 189-194

Ebukiba, E.S., Anthony, L and. Adamu, S. M. (2020). Economics and Technical Efficiency of Maize Production Among Small Scale Farmers in Abuja, Nigeria: Stochastic Frontier Model Approach European Journal of Agriculture and Food Sciences Vol. 2, No. 6, November 2020

Egwuma, H., Dutse, F. Oladimeji, Y.U.,Ojeleye, O.A., Ugbabe, O.O. and Ahmed, M.A. (2019). Demand and Supply Estimation of Maize in Niger State, Nigeria. Journal of Agriculture and Agricultural Technology Vol. 5 No. 2, December 2019: Pp 12-20

FAOSTAT (2018). Food and Agriculture Organization of the United Nations. [URL]

FAOSTAT (2008). "FAO Statistics, Food and Agriculture Organization of the United Nations,"Rome,2012. [URL]

Ike, D. Amusa, U. (2004). Yield of Maize (Zea Mays L.) Under rainfed Conditions. Indian J. Soil Conservation. 24(2):625-129.

NPC (2006). National Population Commission of Nigeria, Sample Survey.

Ogunniyi L. T. (2011). Profit Efficiency Among Maize Producers in Oyo State, Nigeria ARPN Journal of Agricultural and Biological Science VOL. 6, NO. 11, 1-17

Olukosi, J.O. and Erhabor, P.O. (2005). Introduction to Farm Management Economics: Principles and Applications. Agitab Publishers Limited, Zaria, Kaduna, Nigeria. pp.77-83.

Panwal, E.F., Noble, N.1. and Joseph, B. (2006). Resource-use and productivity among rain fed and irrigated Irish potato producers in plateau state Nigeria. Paper presented at the proceedings of the Farm Management Association of Nigeria (FAMAN) zo" annual conference held at forestry research institute pf Nigeria. Federal college of forestry. Jos. Plateau state. September 2006.

PWC, (2021). Positioning Nigeria as Africa's Leader in Maize Production for AfCFTA

Sadiq, M.S. Yakasai, M.T. Ahmad, M.M. Lapkene, T.Y. and Abubakar, M. (2013). Profitability and Production Efficiency of Small-Scale Maize Production in Niger State, Nigeria Volume 3, Issue 4 (Mar.-Apr.2013), PP 19-23 www.iosrjournals.org

Ume, S.I, Onuh N.C, Jiwuba FO, Onunka BN. (2016). Technical Efficiency Among Women Cassava Small Holder Farmers in Ivo Local Government Area of Ebonyi State. 1-12, Asian Journal of Agricultural Economics, Extension and Rural Sociology. 2016; 5(4) :12 -19

Ume, S., Ezeano, C.I., Chukwuigwe, O., Gbughemobi, B.O (2018). Resource-use and Technical Efficiency of Okra Production Among Female Headed Household: Implication for Poverty Alleviation in The Rural Areas of South East, Nigeria Volume 3; Issue 2; March 2018; International Journal of Advanced Research and Development. Page No. 1028-1040

UNDP (2001). Human Development Report 2001 Making new technologies work for human development New York Oxford Oxford University Press

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 516-521
DOI: https://doi.org/10.31015/jaefs.2022.4.3

# Automation of liquid fertilizer application aystem in a direct drill machine

**Behice Boran Aktepe[1], Yılmaz Bayhan[2], Eray Önler[3]**

[1] Tekirdağ Namık Kemal University, Institue of Natural and Applied Sciences, Biosystem Engineering Main Science Division, Tekirdağ, Türkiye

[2] Tekirdağ Namık Kemal University, Agricultural Faculty, Biosystem Engineering Department, Tekirdağ, Türkiye

[3] Tekirdağ Namık Kemal University, Agricultural Faculty, Biosystem Engineering Department, Tekirdağ, Türkiye

**Citation:** Aktepe, B.B., Bayhan, Y., Onler, E. (2022). Automation of liquid fertilizer application aystem in a direct drill machine. International Journal of Agriculture, Environment and Food Sciences, 6 (4), 516-521.

**Received:** 29 June 2022
**Revised:** 11 August 2022
**Accepted:** 18 August 2022
**Published Online:** 21 October 2022

**Correspondence:** Yılmaz Bayhan
**E-mail:** ybayhan@nku.edu.tr



## Abstract

An automation system was designed to measure and monitor the amount of liquid fertilizer sprayed on a direct drill machine with the ability to apply liquid fertilizer. The clogging of spray nozzles is a common problem in liquid fertilizer machines. The objective is to detect clogged spray nozzles during the application of liquid fertilizer and to allow the tractor driver to monitor the amount of liquid fertilizer discharged with this designed automation system. It gives a visual warning to the driver when the flow is zero. Flow sensors are mounted on the spray nozzles of the machine. The machine was operated at 540 rpm PTO speed. A total of 30 measurements were taken from each flow sensor with 3 replications. The actual volume was found from the amount of liquid collected in the scaled vessels that are placed under the spraying nozzles and the measured volume obtained from the flow sensors. The calibration curve of the liquid fertilizer system was created by performing a regression analysis with measurements taken from the sensors and scaled vessels. At the same time, the real volume and the volume measured by the sensor were compared with the t-test and analyzed whether there was a statistically $p< 0.05$ difference between the measurements. As a result of the measurements, the volume measured by the sensor was slightly higher than the actual volume. The mean difference was calculated as 0.17 liters and the standard deviation as 0.08 liters. The regression curve between the actual and the measured volume shows that there is a high linear relationship and the regression coefficient was calculated as $R2= 0.947$. At the same time, since $p=0.012$ ($p<0.05$) was found as a result of the independent two-way t-test, it was determined that there was no statistical difference between the actual volume and the measured volume.

**Keywords:** Direct drill, Liquid fertilizer, Automation

## INTRODUCTION

The products grown by agricultural production constantly use nutrients from the soil. The source of the nutrients used is mineral building materials and various organic materials. Although some soils are rich in nutrients, they may have a poor amount of nutrients available to the plant. During the transition from primitive agriculture to advanced methods for feeding the increasing population, fertilization was the first adopted method when productivity-enhancing methods were investigated (Ülger et al., 1996).

Fertilizers are substances that increase the productivity of the soil by regaining plant nutrients that are reduced in the soil as a result of agricultural production. Fertilizers are a powerful tool used to increase food quality while increasing agricultural production (Shojaei, et al, 2022). Compared to other agricultural inputs, fertilizers alone provide a yield increase of more than 40%, making very important contributions to world food security, raising living standards, and fighting

hunger. The growing number of people in the world is causing an increase in our food needs. The decrease in arable land per person requires more plant production per unit of area. It is already understood that fertilizers will be at an important point in sustainable agriculture in the future as they are today. (Eraslan et al., 2010, Rahman and Zhang, 2018).

Materials such as farm manure, plant residues, animal waste, artificial fertilizers, and green manure are used to increase soil fertility (Gökçebay, 1986; Ülger, 1982, Tekin, 2002)

30-40% of the fertilizer consumed in our country is met through imports (Eraslan et al., 2010). One of the biggest problems for our agricultural producers is the high input costs in production and the fluctuations in prices. Fertilizer is one of the most important cost items in agricultural production and, for these reasons, sufficient fertilizer cannot be used in agriculture. Yield and quality losses are experienced due to insufficient fertilization (Kaplan et al., 2000).

When the fertilizer type suitable for the grown product is used in the right amount, yield and quality increase. For this reason, it is necessary to produce new forms and different types of fertilizers, taking into account the crop and soil to be grown (Taban et al., 2016).

Due to increasing fertilizer prices and increasing environmental awareness, more people are using approaches to reduce the use of chemical inputs in agriculture are used by more people (Şahin, 2016). Liquid fertilization is the subject of attention in this respect (Çelik and Bayhan, 2020).

Liquid fertilization is the delivery of nutrients to the plant dissolved in water. Liquid fertilizer has important benefits in plant nutrition (Halil et al., 2017; Gökçen, 2019):

• prevents toxic effects on the seed;

• Protects the seed against harmful insects and diseases in the seed bed,

• It increases the level of microbiological activity by increasing the density of microorganisms in the soil. Increasing the microbiological activity has a positive effect on the yield.

• Since the fertilizer is in liquid form, it allows plants to absorb plant nutrients more easily

The most important problem encountered in the liquid fertilization machines used is the clogging of the fertilization nozzles. Additionally, monitoring how much fertilizer is sprayed during the fertilization process is important to the success of the application.

In this study, an automation system was developed to measure and monitor the amount of liquid fertilizer sprayed on a direct drill machine used in grain planting after sunflower harvest in the Thrace Region.

## MATERIALS AND METHODS

The research was carried out in the agricultural machinery workshop, Tekirdag Agricultural Faculty, 40°59 '30"N latitude and 27°34'55"E longitude, in the years 2021. The altitude is 10 m above sea level. The climate of Tekirdag is characterized by a Mediterranean type with mild and rainy winters and hot and dry summers at the coast, while a continental type prevails inside.

### Direct Drill Machine and Nozzles

In the study, a direct drill machine with a liquid fertilizer system manufactured by Altayoglu Agricultural Machinery Food Agriculture and Farmer Ind.Trade.Co.Ltd. was used. General dimensions of the direct drill machine are given in the Table 1.

**Table 1.** General dimensions of the direct drill machine.

| General Dimension | Road Position | Working Position |
|---|---|---|
| Length (mm) | 2400 | 2400 |
| Width (mm) | 3250 | 5650 |
| Height (mm) | 1750 | 1500 |
| Weight (kg) | 880 | |
| Working width (mm) | 2375 | |
| Operation Speed (km/h) | 5-7 | |
| Power Requirement | >95 | |
| 3 Point Linkage Category | Category-II | |
| Number of opener seed bed | 19 | |
| Opener type | Spring type | |
| Distance between the seed coulters (mm) | 125 | |
| Grain tank capacity (lt) | 373 | |
| Fertilizer tank capacity (lt) | 393 | |

TeeJet TG-3 Full Cone Spray Tip nozzles (Figure 1, TEEJET TG-3 FULL CONE SPRAY TIP BRASS. (n.d.)) are used in the liquid fertilization system. The spray nozzles are made of brass material and have a 50 degree spray angle.

**Figure 1.** TeeJet TG-3 Spray Nozzle

### Tractor

New Holland L95 model tractor was used in this experiment. The pump of the liquid fertilizer machine takes its rotation movement from the PTO connection. 540 rpm PTO speed was used in the study.

### Arduino Mega

In the developed system, Arduino Mega microcontroller unit was used in order to perform the calculation process by processing the data received from the sensor and to display the results on the user screen. Table 2 shows the technical specifications of Arduino Mega.

**Table 2.** Technical Specifications of Arduino Mega

| | |
|---|---|
| Length | 101.52 mm |
| Width | 53.3 mm |
| Weight | 37 g |
| Microcontroller | ATmega2560 |
| Input Voltage | 7-12V |
| Digital I/O Pins | 54 |
| Number of Analog Pins | 16 |
| DC Current per I/O Pin | 20 mA |
| Flash Memory | 256 KB (ATmega2560) 8 KB bootloader |
| EEPROM | 4 KB (ATmega2560) |
| Clock Speed | 16 MHz |

### Nextion Touch Screen

Nextion is a visualization interface used for ease of use between device/application and human. Because of its own special editor, human-machine interfaces can be created easily. It can be controlled with a microcontroller by UART (Universal Asynchronous Receiver Transmitter). It has been preferred in the study because it is easy to use and works in harmony with Arduino. The technical specifications of the Nextion HMI smart 2.8 inch touch TFT LCD screen are given in Table 3.

**Table 3.** Touch Screen Specifications

| | |
|---|---|
| Resolution | 320x240 |
| Color Space | RGB 65 K |
| Screen Size | 57.6 mm (Length) x 43.2 mm (Width) |
| Internal Memory | 4 Mb Flash Memory |

### Liquid Flow Sensor

A water flow sensor with a plastic body, rotor, and hall effect sensor is used. The measurement is made when the water flowing through the sensor rotates the rotor, and the hall sensor generates a signal depending on the rotor rotation speed. It produces 4.5 pulses for every liter of liquid that passes through it. In this way, the flow rate of the fluid passing through it or the total volumetric flow amount is measured. The technical specifications of the YF-S201 liquid flow sensor are given in Table 4.

**Table 4.** Liquid Flow Sensor Specifications

| | |
|---|---|
| Operating Voltage | 5V-24V DC |
| Operating Current | 15 mA |
| Output Type | Digital Pulse |
| Measuring Flow Range | 1-30 L/dk |
| Maximum Operating Pressure | ≤1.75MPa |

### Experiment Setup

Flow sensors are mounted on the spray nozzles of the liquid fertilizer. The experiments were carried out with the tractor at a standstill, at 540 rpm PTO speed. A total of 30 measurements were taken from each flow sensor with 3 replications (Figure 2). To determine the actual amount of liquid fertilizer sprayed by the measured spray nozzles, scaled vessels were placed under each spray nozzle. The calibration curve of the system was created by regression analysis of the measurements taken from the sensors and scaled vessels.

**Figure 2.** Calibration measurements taken for the designed system

In addition, the real volume and the volumes measured by the sensor were compared with the t-test and it was analyzed whether there was a statistically $p<0.05$ difference between the measurements.


## RESULTS AND DISCUSSION

The flowmeter sensor used in the designed automation system produces 4.5 pulses per lt/min. In the software, the flow rate of the liquid flowing through the sensor and the total volume of liquid sprayed are calculated according to these pulse numbers received from the sensors. These calculated values are shown to the user on a screen. When the flow is interrupted, the indicator corresponding to that spray nozzle turns red, visually showing the blockage in the spray nozzle to the user (Figure 3.).

This study showed automation system is enhancing the ergonomy and simplfying the application as the other researchers also point out (Sharma and Sonwane, 2017).

A total of 30 measurements were taken from the spray nozzles, and the volume data measured from the sensor and the actual volume data measured from the scaled vessels are given in Figure 4. Figure 5 shows the regression curve for these measurements. The volume measured by the sensor appears to be slightly higher than the actual volume. The minimum difference between the actual volume and the measured volume was found to be 0, and the maximum difference was 0.31 lt. The mean difference was calculated as 0.17 lt and the standard deviation as 0.08 lt.

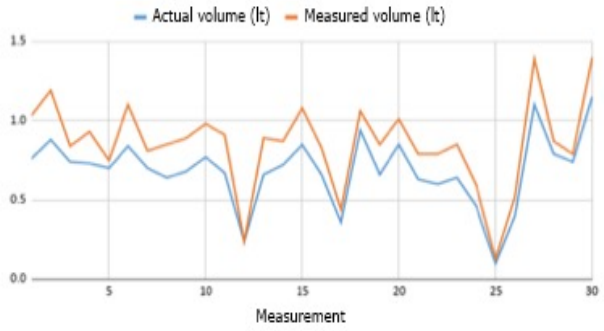



**Figure 4.** Graph of measured volume versus actual volume

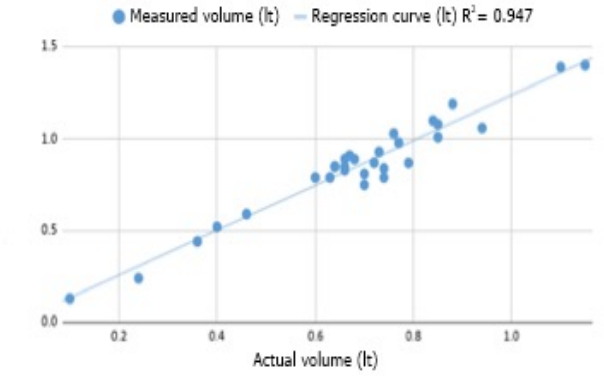



**Figure 5.** Regression curve between actual volume and measured volume

The actual volume taken from the collection vessels and the volumes measured by the sensor were compared with the t-test and it was analyzed whether there was a statistically $p<0.05$ difference between the measurements. Since $p = 0.02$ ($p<0.05$) was found as a result of the independent two-way t-test, it was revealed that there was no statistical difference between the two measurements.

## CONCLUSION

In the Thrace Region, an automation system has been developed to measure and monitor the amount of liquid fertilizer sprayed in a direct drill machine, which has the feature of both direct drill and liquid fertilizer application in grain planting after the sunflower harvest. It has been determined that this developed automation system works within reliable limits, that the clogging of the nozzles gives a visual warning and that the tractor driver can easily monitor the amount of fertilizer sprayed during the fertilization and that this system can be used in practice. The most important limitation of deploying automation systems in agriculture is the cost (Rehman et al., 2022). This low-cost system addresses the need of a low-cost but useful automation solution.

We can summarize the results of the study as follows:

It detects clogged fertilizing nozzles during liquid fertilization. (Visual warning when flow is zero at the spray nozzle)

It allows the tractor driver to monitor the amount of fertilizer sprayed during fertilization.

There is a strong linear relationship of R2=0.947 between the values measured by the designed system and the amount of fertilizer actually sprayed.

The independent two-way t-test performed reveals that there is no statistically significant ($p<0.05$ significance level) difference between the actual measurements and the measurements made by the sensor.

Suggestions on what can be done in future studies on the designed system can be grouped under the following headings:

The flow measurement sensors used in the study are plastic. Liquid fertilizer has a high corrosion effect. For long-term operation, sensors made of corrosion-resistant materials can be preferred.

The sensors used in the study are in turbine structure. Solid material in liquid fertilizer can cause clogging in the sensors. For this reason, in future studies, a comparison can be made by testing flow meters that measure by magnetic method.

In order to test the mechanical durability of the designed system, longer field use and testing is needed.

## COMPLIANCE WITH ETHICAL STANDARDS

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Ethics committee approval is not required.

### Funding

No financial support was received for this study.

### Data availability

Not applicable.

### Consent for publication

Not applicable.

## REFERENCES

Çelik, Y., Bayhan, Y. (2020). A research on investigation of the application possibilities of direct drill machine with liquid fertilizer Assembly for grain planting stubble. International Journal of Innovation Engineering and Science Research 4(6), 1-10.

Eraslan, F., İnal, A., Güneş, A., Erdal, İ., & Coşkan, A. (2010). Türkiye'de kimyasal gübre üretim ve tüketim durumu, sorunlar, çözüm önerileri ve yenilikler. TMMOB ziraat mühendisleri odası, ziraat mühendisliği vii. Teknik kongresi, 11, 15. (in Turkish)

Gökçebay, A. (1986). Tarım Makinaları-I. Ankara: Ankara Üniversitesi Ziraat Fakültesi Yayınları, No: 979. (in Turkish)

Gökçen, M. Y. (2019). Farklı sıvı gübrelerin ekmeklik buğdayda (*Triticum aestivum* L.) verim ve kalite üzerine etkileri (Master's thesis, Namık Kemal Üniversitesi). (in Turkish)

Halil, Ü. N. A. L., Erdoğan, H., Gürcan, S., Satioğlu, S., & Özgür, F. (2017). Sıvı gübre dağıtma makinasının farklı çalışma hızlarındaki işletme özelliklerinin belirlenmesi. Uludağ Üniversitesi Ziraat Fakültesi Dergisi, 31(1), 49-60. (in Turkish)

Kaplan, M., Aktaş, M., Güneş, A., Alpaslan, M., & Sönmez, S. (2000). Türkiye Gübre Üretim ve Tüketiminin Değerlendirilmesi. V. Türkiye Ziraat Mühendisliği Teknik Kongresi, 881, 900. (in Turkish)

Rahman, K.M.A., Zhang, D. (2018). Effects of fertilizer broadcasting on the excessive use of inorganic fertilizers and environmental sustainability. Sustainability, 10(3), 759

Rehman A., Saba, T., Kashif, M., Fati, S.M., Bahaj, S.A., Chaudry, H., (2022). A Revisit of internet of things technologies for monitoring and control strategies in smart agriculture. Agronomy, 12(1), 127.

Sharma, M.O., Sonwane, P.M., (2017). 2017 International Conference on Computation of Power, Energy, Information and Communication. 516-521

Sındır, K.O. Tekin, A.B., Economics of variable rate fertilizer application, EE & AE '2002 – International Scientific Conference Rousse, Bulgaria. 2002

Shojaei, M.J., Or, D., Shokri, N. (2022). Localized Delivery of Liquid Fertilizer in Coarse-Textured Soils Foam as Carrier. Transport in Porous Media 143, 787-795.

Şahin, G. (2016). Türkiye'de Gübre Kullanım Durumu ve Gübreleme Konusunda Yaşanan Problemler. Turkish Jour-

nal of Agricultural Economics, 22(1). (in Turkish)

Ülger, P., Güzel, E., Kayışoğlu, B., Eker, B., Akdemir, B., Pınar, Y. ve Bayhan, Y. (1996). Tarım Makinaları İlkeleri. İstanbul: Fakülteler Matbaası. (in Turkish)

Ülger, P. (1982). Tarımsal Makinaların İlkeleri ve Projeleme Esasları. Erzurum: Ankara Üniversitesi Ziraat Fakültesi Yayınları, No: 280. (in Turkish)

Taban, S., İbrikçi, H., Ortaş, İ., Karaman, M.R., Orhan, Y. ve Güneri, A. (2016). Türkiye'de gübre üretimi ve kullanımı. (in Turkish)

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 522-529
DOI: https://doi.org/10.31015/jaefs.2022.4.4

# Synthesis, characterization and evaluation of antimicrobial activities of silver nanoparticles obtained from *Rumex acetosella* L. (Sorrel) plant

**Necmettin Aktepe[1], Hafize Bütüner[2], Ayşe Baran[2], M. Fırat Baran[3], Cumali Keskin[3]**

[1] Mardin Artuklu University Faculty of Health, Department of Nursing, Mardin, Türkiye
[2] Mardin Artuklu University, Department of Biology, Institute of graduate, Mardin, Türkiye
[3] Mardin Artuklu University, Vocational School of Health Services, Department of Medical Services and Techniques, Mardin, Türkiye

**Citation:** Aktepe, B.B., Bayhan, Y., Onler, E. (2022). Automation of liquid fertilizer application aystem in a direct drill machine. International Journal of Agriculture, Environment and Food Sciences, 6 (4), 522-529.

## Abstract

*Rumex acetosella* L. (sorrel) is a plant belonging to the Polygonaceous family and is a species that grows naturally across Turkey. In this study, the characterization of silver nanoparticles (AgNPs) obtained from the Rumex acetocella L. (RA) plant using the green synthesis method was performed and their antimicrobial activities were investigated. AgNPs were successfully synthesized in the first stage of the study using plant extract taken from plant samples collected from the natural growing environment. Characterization of synthesized AgNPs was performed using appropriate analytical methods (UV-vis, FT-IR, XRD, SEM-EDX, TEM, Zeta Potential and Zeta Sizer). According to the analysis results, it was determined that AgNPs had a maximum absorbance at 476 nm wavelength, a pentagonal, hexagonal, and spherical appearance, a size of 29.16 nm, and a zeta potential of -9.88 mV. The antimicrobial activities of AgNPs were tested using the microdilution technique, in which Minimum Inhibition Concentration (MIC) values were determined on gram-positive Staphylococcus aureus, Bacillus subtilis and gram-negative Pseudomonas aeruginosa, Escherichia coli bacteria and Candida albicans fungus. It showed a very strong antimicrobial effect on C. albicans, S. aureus and P. aeruginosa. Consequently, AgNPs had stronger antimicrobial activity at low concentrations and when compared to commercial antibiotics.

**Keywords:** AgNP, Antimicrobial activity, Characterization, Green synthesis. Rumex acetosella L.

**Received:** 10 July 2022
**Revised:** 10 August 2022
**Accepted:** 18 August 2022
**Published Online:** 25 October 2022

**Correspondence:** Necmettin Aktepe
**E-mail:** necmettinaktepe@gmail.com



## INTRODUCTION

Rumex acetosella L. is a plant belonging to the Polygonaceous family, popularly known as kuzukulağı (sorrel), ebemekşisi, ekşilik, ekşimik, and turşuotu. Studies have reported that Rumex acetosella L. is rich in phenolic compounds that are produced by secondary metabolism in plants. Phenolic compounds, which are synthesized in the cell wall to cope with the ever-changing stress conditions of plants, play a crucial role in reproduction, growth, and metabolism of plants. They function as defense mechanisms against pathological viral and fungal infections, parasites, predators, and environmental factors that may be harmful to the plant (Liu, 2013). Plants accumulate phenolic compounds in their tissues as an adaptive response to adverse environmental conditions, and they have a vital role in regulating various environmental stresses such as excessive light, low temperatures, pathogen infection, herbivores, and nutritional deficiencies. Phenolic compounds are a rich source of antioxidants for the plant due to their binding properties such as the active aromatic core and the hydroxyl groups of the aromatic ring, and the protein-phenol complex, and bring the plant with anti-inflammatory, antitumor, antibacterial, antiviral, and antifungal properties (Naikoo et al., 2019). The Rumex acetosella L. plant is also rich in phenolic compounds, which

in turn suggests that it may have antioxidant, anticancer, and antimicrobial activities(Isbilir & Sagiroglu, 2013).

Nanotechnology is a developing science that uses the ability of particles to expand at nanosizes and become more sensitive when compared to its unique counterparts. Nanotechnology has effective application areas ranging from traditional chemical techniques to biological, medical, and environmental technologies. The "green synthesis", namely the synthesis of nanoparticles produced by biological systems refers to the process by which plants or their metabolites combine nanoparticles (Parveen et al., 2016). This new method reduces the toxic effects of NPs produced by conventional (physical and chemical) methods. In addition, green synthesis is preferred as a better alternative to physical and chemical methods in the production of nanoparticles due to its numerous advantages such as simplicity, rapid synthesis, biocompatibility, environmental friendliness and low cost (Atalar et al., 2021; Gour and Jain, 2019). The green synthesis method is commonly used to synthesize metallic nanoparticles such as silver, gold, cadmium, copper, zinc, and platinum. AgNPs stand out among metallic nanoparticles due to their strong, light, catalytic, and antimicrobial properties (Baran et al., 2019, Hatipoğlu, 2022, Hatipoğlu, 2021).

It has been reported that multi-drug resistant bacteria have become one of the most serious public health issues in recent years, causing over 670,000 infections and over 33,000 fatalities per year in the European Union alone. A few species are responsible for the vast majority of these infections and fatalities (Cassini et al., 2019). These species are multi-drug resistant (MDR) Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa bacteria (Kakoullis et al., 2021). Candida albicans, a yeast fungus that causes the majority of infections in humans and is a difficult-to-treat microorganism. In recent years, there has been an upsurge in antibiotic-resistant C. albicans strains, posing a threat to public health (Volkova et al., 2021). To fight against multi-drug resistance, researchers have intensified their studies on next-generation drug design alternatives to traditional antibiotics. Because nanoparticles target several cellular pathways simultaneously, it is extremely difficult for microorganisms to develop resistance to nanoparticles. Therefore, nanoparticles could be an excellent alternative to conventional antibiotics to treat antibiotic-resistant microbial infections(Singh et al., 2020). The aim of the present study is to produce AgNPs by green synthesis method using Rumex acetocella L. plant, to characterize these nanoparticles with appropriate methods and to evaluate their antimicrobial activities against pathogenic microorganisms.

## MATERIAL AND METHOD

### Plant Specimen

The Rumex acetosella L. (sheep's sorrel) plant was used to synthesize AgNPs. The plant specimen was collected in May 2021 from natural growing zones in the Kızık village of Sandıklı district, Afyonkarahisar province, during its blooming period. The R. acetosella L. plant we collected was cleansed of dust and dirt and allowed to dry in an appropriate room temperature and humidity setting before being used in experimental tests.

### Preparation of Plant Extract

After grinding the dried plant parts, 100 g was weighed and placed in a beaker containing 800 mL of distilled water and allowed to boil for 2 hours at 85 °C. The resulting extract after boiling was filtered using Whatman No.1 filter paper, the pulp was discarded, and the resultant plant extract was stored in the refrigerator at +4 °C for use in the next phases.

### Preparation of Silver Nitrate (AgNO3) Solution

Silver nitrate (AgNO3) solution (1 mM), which will be used to prepare silver nanoparticles, was prepared using Alpha-aesar brand AgNO3 having an analytical purity of 99.8 %.

### Production of AgNPs through Green Synthesis

To synthesize silver nanoparticles, 100 mL of 1mM AgNO3 solution was poured into a beaker, and 20 mL of the previously prepared plant extract was added and left to react at room temperature under constant conditions. The color of the solution darkened after around 150 minutes as a consequence of the transformation of silver ions to AgNPs (Figure 1). The dark solution generated by the reduction of silver ions was centrifuged for 30 minutes at 14000 rpm. Following centrifugation, the liquid portion that had accumulated on the top of the falcon tubes was discarded, and the solid portion at the bottom was washed 10 times with distilled water and centrifuged again until the color of the solution turned clear at the end of the washing procedure. The resultant silver nanoparticles were left to dry at 85°C for 48 hours. After being ground with a glass stirrer, the dried nanoparticles were stored in a dark environment to be used in characterization procedures (Aktepe et al., 2021; Baran and Acay, 2019).

### Ultraviolet-visible (UV-vis) Spectroscopy

After mixing the previously prepared AgNO3 solution with the plant extract at a certain rate for the production of AgNPs, measurements were taken at various time intervals in the range of 400 to 800 nm using a UV-1601 220V Shimadzu® model UV-vis Spectrophotometer.

### Fourier Transform Infrared (FT-IR) Spectroscopy

In order to determine the functional groups involved in the synthesis of AgNPs and responsible for the decrease in the plant extract, FT-IR analyzes were performed in the range of 4000-400 cm-1 using the Perkin Elmer Spectrum One® model device.

**Figure 1.** a) Plant extract b) Color change observed during the formation of silver nanoparticles.

### X-ray Diffraction (XRD)

The RadB-DMAX II® model computer-controlled X-ray Diffractometer was used to analyze the crystal structure and size of silver nanoparticles at the range of 3°≤ 2θ ≤80°. The average crystal particle sizes of the nanoparticles were calculated using the Debye-Scherrer (D = Kλ/ (β cosθ) formula (Hatipoğlu, 2021).

### Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX) Spectroscopy

The EVO 40 LEQ model Scanning Electron Microscope (SEM) was used to identify the morphological characterization of the synthesized silver nanoparticles. Energy Dispersive X-ray (EDX) Spectroscopy was utilized to identify the presence of silver in the synthesized nanoparticles and to detect its proportion in the elemental composition.

### Transmission Electron Microscopy (TEM)

Jeol Jem 1010® model Transmission Electron Microscope (TEM) was used to determine the morphology and size distribution of nanoparticles.

### Zeta Potential and Zeta Sizing

The Malvern® model Zeta Potential device was used to analyze the surface charge distribution and estimated size of AgNPs.

### Determination of Antimicrobial Activities of Synthesized AgNPs

The antimicrobial activities of AgNPs produced from the Rumex acetosella L. plant using the green synthesis method were assessed using the microdilution method, which indicated the lowest concentration (MIC) required for prevention of microorganism growth. The MIC values of AgNPs produced from the plant extract, 1 mM silver nitrate solution (AgNO3), and commercial antibiotics were analyzed to make these assessments.

### Microorganisms tested for antimicrobial activity

To determine the antimicrobial activities of AgNPs, cultures of gram-positive bacteria S. aureus (ATCC 29213) and B. subtilis (ATCC 11774), gram-negative bacteria P. aeruginosa (ATCC 27853) and E. coli (ATCC 25922) and the fungus C. albicans (ATCC 10231) were selected, all of which were incubated overnight. The plates were then incubated. 24 hours at 36°C in an incubator. antibiotics and AgNO3 MIC values were determined(Keskin and Güvensen 2022).

## RESULTS AND DISCUSSION

### Characterization of Silver Nanoparticles

### Analysis of UV-vis Spectroscopy

Identical optical properties of metallic nanoparticles interact with light, resulting in the appearance of the surface plasmon resonance (SPR) band. AgNP production is indicated by the reduction of Ag+ ions to Ag0 by oxidized plant components and the color of the solution turning from yellow to brown as a consequence of vibrations (SPR) on the plasma surface. Nanoparticle stability is determined using UV-vis spectroscopy. Due to the plasmon resonance of AgNPs, the absorption spectra generating on the surface of AgNPs have an absorption capacity of 425 to 475 nm (Banerjee et al., 2014).

In the present study, the addition of 10 mM AgNO3 solution at a 3:7 ratio to the plant extract, which was originally yellow, caused a color shift from yellow to brown in a short period of time. Following that, UV-vis measurements at 30th, 60th, and 90th minutes determined that AgNPs produced by vibrations on the plasma surface exhibited maximum absorbance at 476 nm (Figure 2). This finding corresponds to the data in previous studies. A similar study by Baran, M. F. et al., (2019) reported that AgNPs produced from olive leaf extract had a maximum absorbance value of 468 nm (Baran et al., 2019).

### Analysis of Fourier Transform Infrared Spectroscopy

FT-IR analyses were conducted (Figure 3a, 3b) to identify possible functional groups in plant phytochemicals that play a role as reducing and stabilizing agents in AgNPs synthesis, and the FT-IR spectrum of R. acetocella L. extract and the synthesized AgNPs were compared. It represents the peak -OH (hydroxyl) functional group at 3338 cm-1, the peak -C≡N functional group at 2114 cm-1, and the peak -C=O (carbonyl) functional group at 1635

cm-1 seen in the spectra. It is possible to assert that the reaction took place in these groups due to the frequency shifts in these peaks. Acay et al., reported similar functional groups in the characterization of AgNPs produced by using Vitis vinifera leaf extract (Acay & Baran, M. F., 2019), which is compatible with the present study. Baran A., et al. (2022) also reported the existence of -C=O and -OH groups in their study on the leaves of the Cicer arietinum plant.

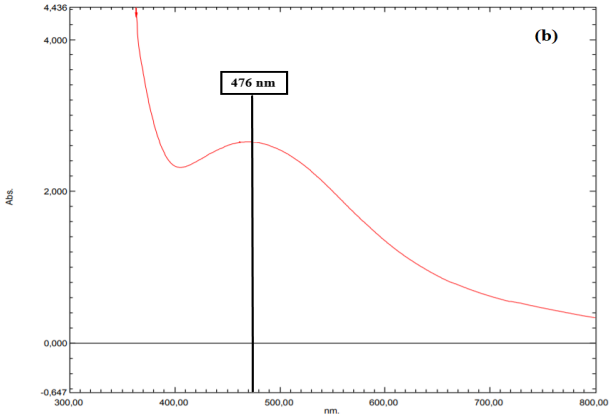



**Figure 2.** Maximum absorbance value of AgNPs synthesized by Rumex acetosella L. plant in UV-vis spectroscopy

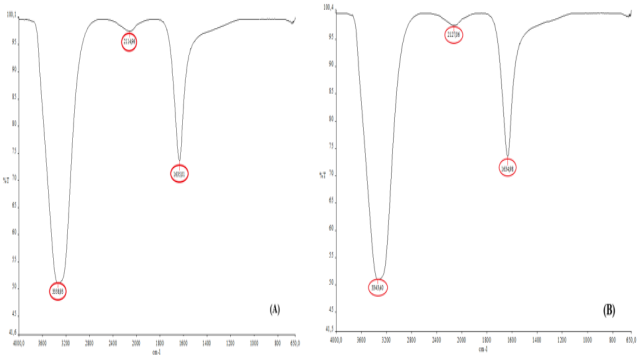



**Figure 3.** Functional groups involved in reduction by FT-IR analysis: a) FT-IR spectrum of Rumex acetosella L. plant extract, b) FT-IR spectrum of synthesized AgNPs

## Analysis of X-ray Diffraction

XRD analysis was done to examine the crystal structure and size of AgNPs produced from the Rumex acetosella L. plant by the green synthesis method. Figure 4 shows the X-ray Diffraction pattern of AgNPs. The peak reflections on Bragg diffraction plans (111), (200), (220) and (311) were calculated with values corresponding to 2θ (38.18°, 46.31°, 64.47° and 77.46°) in the XRD spectrum of the synthesized nanoparticles. The average crystal size of AgNPs was determined to be around 29.16 nm by using the Debye-Scherrer formula ($D = K\lambda / (\beta \cos\theta)$). Similarly, Adil et al., (2019) reported a crystal size of 22.48 nm for AgNPs produced from the Hypericum triquetrifolium Turra plant.

## Analysis of Scanning Electron Microscopy and Energy Dispersive X-ray Spectroscopy

SEM is a powerful electron microscope and a popular surface imaging technique. SEM produces a high-resolution, magnified three-dimensional image and provides information on the purity of the sample (Atalar et al., 2021). It magnifies images using electrons rather than light, as conventional microscopes do (Sharma et al., 2019). This microscobe was used to analyze the surface morphology of AgNPs produced from the R. acetosella L. plant. When the SEM images were examined, the silver nanoparticles exhibited a spherical structure, as seen in Figure 5.

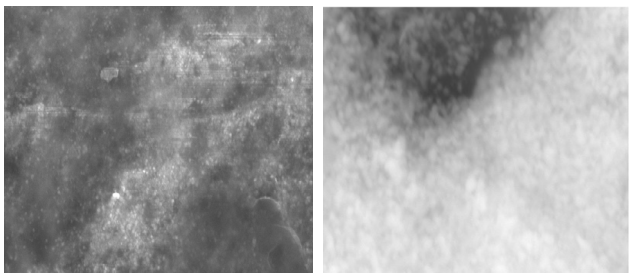


**Figure 5.** SEM images of AgNPs synthesized by Rumex acetosella L. plant

EDX Spectroscopy was used to validate the presence of silver (Ag) in the elemental composition and to identify other elemental compositions. According to the EDX analysis, Ag appeared to emit a strong signal in the silver area, indicating that there was a significant quantity of Ag in the element composition and therefore verifying the production of AgNPs (Ma et al., 2016). Furthermore, the presence of compounds in Rumex acetosella L. extract may cause weak signals from Chlorine (Cl), Carbon (C), and Oxygen (O) determined by EDX data (Figure 6). Consistent with the findings of the present study, Azhdari et al. (2020) reported that silver nanoparticles synthesized from the Stachys lavandulifolia plant exhibited a spherical morphology as verified by SEM and they had a high proportion of silver in elemental composition as determined by EDX.

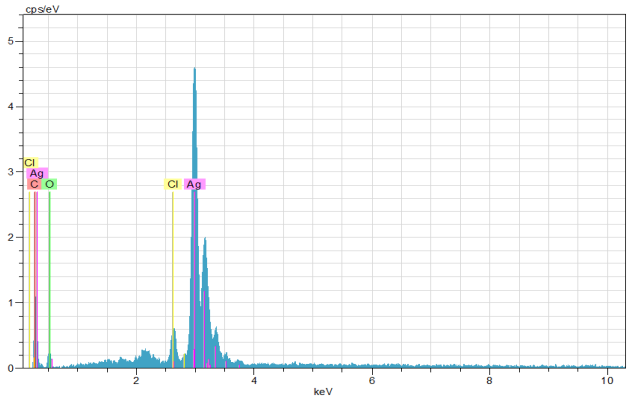



**Figure 6.** Elemental composition of AgNPs synthesized by Rumex acetosella L. plant by EDX

## TEM Analysis

The morphological appearance and size of AgNPs synthesized from R. acetosella L. plant were analyzed using TEM. TEM analysis revealed that the nanoparticles were of numerous morphologies, including pentagonal, hexagonal, and spherical (Figure 7). The findings of this study are consistent with the research of Banerjee et al. (2014), who reported triangular, pentagonal, hexagonal and spherical silver nanoparticles produced from Musa balbisiana (banana), Azadirachta indica (neem) and Ocimum tenuiflorum (black tulip).

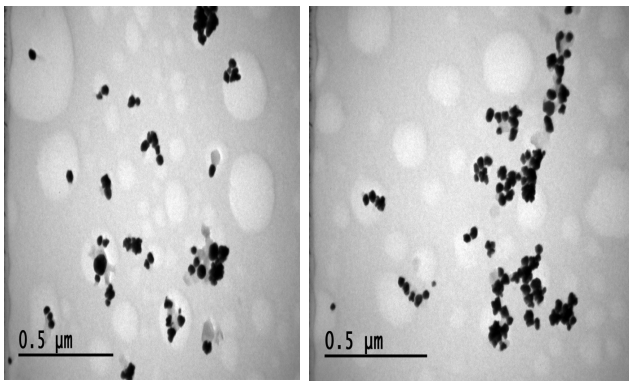



**Figure 7.** TEM images of AgNPs synthesized by Rumex acetosella L. plant

## Analysis of Zeta Potential and Zeta Sizing

The zeta potential, also known as the electrokinetic potential, is a measure of charge stability that indicates the stability of colloidal nanoparticles. It controls every interaction between particles in a suspension. The zeta potential has values ranging between +100 mV and -100 mV. When the zeta potential is more than +30 mV or less than -30 mV, it indicates that a suspension is significantly stable. The zeta potential depends on the velocity of the particle moving under the influence of the electric field and the viscosity of the dispersion medium (Naser et al., 2020). Zeta potential and Zeta sizing analyses were used to determine the surface charge distribution and approximate size of AgNPs synthesized using the green synthesis method. According to the results of the zeta analysis, the electrical charge of the nanoparticles was determined as 9.88 mV (Figure 8a). The negative electrical charge of AgNPs indicates that there is no aggregation in the suspension and the nanoparticles have a stable structure. As shown in Figure 8b, the size of the resultant silver nanoparticles was determined to be in the range of 25.59-124 nm. Likewise, Varadavenkatesan et al. (2017) reported that the electrical charge of AgNPs synthesized from Vigna mungo was negative and the average size of these silver nanoparticles was in the range of 28.21-91.28 nm..

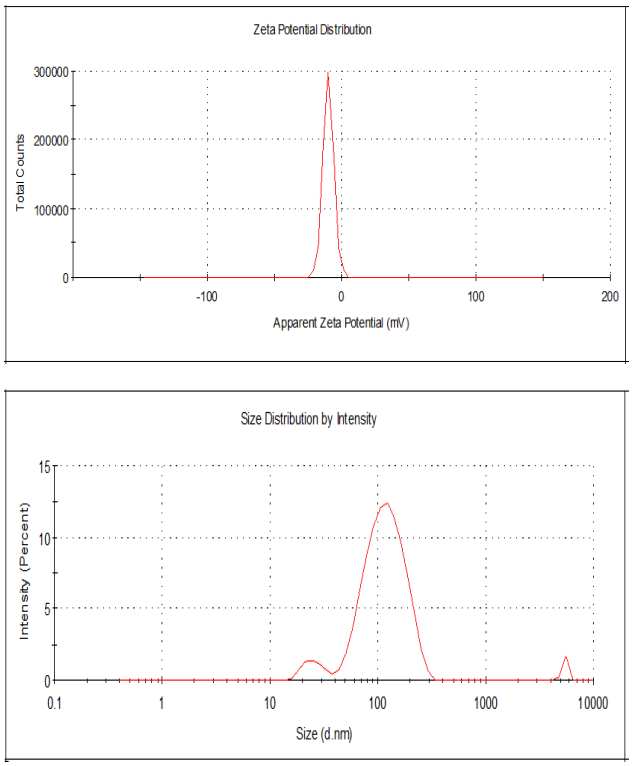



**Figure 8.** a: Zeta potential plot of AgNPs synthesized by Rumex acetocella L. plant b: Zeta size plot of AgNPs synthesized by Rumex acetocella L. plant

## Assessment of Antimicrobial Activities of Silver Nanoparticles

In the present study, the possible antimicrobial effects of AgNPs produced by the green synthesis method from the Rumex acetosella L. plant on gram-positive (S. aureus ATCC 29213, B. subtilis ATCC 11774) and gram-negative (P. aeruginosa ATCC 27853, E. coli ATCC 25922) bacteria and C. albicans ATCC 10231 fungus, which have pathogenic effects on humans, were evaluated. The MIC values were measured using a 1 mM silver nitrate solution (AgNO3) and commercial antibiotics for positive control in order to make comparison with AgNPs. The findings suggested that AgNPs had antimicrobial activity even at low concentrations, and it was more effective than AgNO3 and antibiotics.

When the data in Table 2 and Figure 9 were examined in the present study, AgNPs demonstrated an antibacterial effect on Gr+ bacteria at concentrations of 0.125 µg mL-1 for S. aureus (ATCC 29213) and 0.250 µg mL-1 for B. subtilis (ATCC 11774). According to the MIC values, AgNPs were found to be roughly twenty times more effective on S. aureus and five times more effective on B. subtilis than silver nitrate and vancomycin antibiotic employed as a positive control. These data indicated that S. aureus bacteria were more sensitive to AgNPs than B. subtilis bacteria.

**Table 2.** MIC values (µg mL1) showing the antimicrobial activities of AgNPs, silver nitrate and antibiotics

| | Microrganisms | AgNPs µg mL-1 | AgNO3 µg mL-1 | Antibiotic µg mL-1 |
|---|---|---|---|---|
| Gram (+) Bacteria | *S. aureus* | 0.125 | 2.65 | 2 |
| | ATCC 29213 | | | |
| | *B. subtilis* | 0.250 | 1.32 | 1 |
| | ATCC 11774 | | | |
| Gram (-)Bacteria | *E. coli* | 1.00 | 0.66 | 2 |
| | ATCC 25922 | | | |
| | *P. aeruginosa* | 0.50 | 0.66 | 2 |
| | ATCC 27853 | | | |
| Yeast | *C. albicans* | 0.012 | 0.66 | 2 |
| | ATCC 10231 | | | |

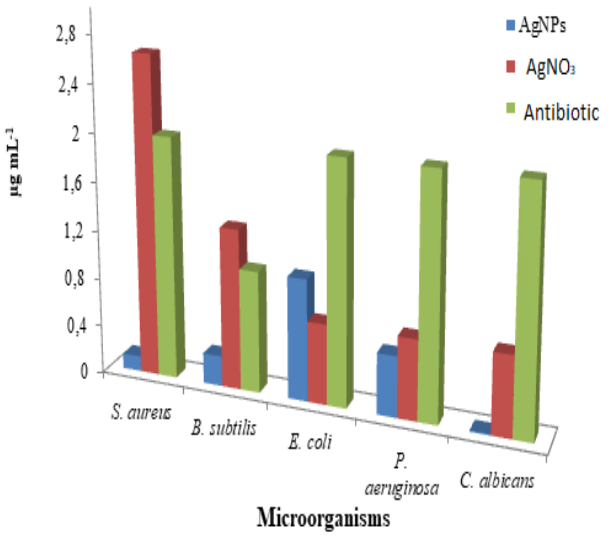



**Figure 9.** MIC values (µg mL-1) of AgNPs, antibiotics and silver nitrate against the microorganisms

MIC values of Gr- bacteria indicated that AgNPs exhibited an antibacterial effect at concentrations of 1.00 µg mL-1 for E. coli (ATCC 25922) and 0.50 µg mL-1 for P. aeruginosa (ATCC 27853). Based on these findings, it was determined that AgNPs were more efficient against E. coli and P. aeruginosa than the colistin antibiotic employed as a positive control and exhibited almost the same effect as silver nitrate solution. P. aeruginosa bacteria are also observed to be more sensitive to AgNPs than E. coli bacteria.

These results indicated that AgNPs exhibited more antimicrobial activities against gram-positive bacteria than gram-negative bacteria, and that gram-positive bacteria were more sensitive to AgNPs than gram-negative bacteria. Gram-negative bacteria become more negatively charged than gram-positive bacteria due to a lipopolysaccharide layer on the outer surface of their cell wall. Therefore, we think that the electrostatic repulsive force between negatively charged AgNPs and gram-negative bacteria inhibited partially AgNPs from penetrating the bacterial cell.

AgNPs were observed to have a considerable antifungal effect on C. albicans (ATCC 10231), one of the pathogenic fungal species, at a concentration of 0.012 g mL-1. Based on this MIC value, AgNPs were found to be fifty-five times more effective than silver nitrate and one hundred sixty-six times more effective than the fluconazole antibiotic employed as a positive control on C. albicans. At this point, it was concluded that the phytochemicals in the content of the examined plant were much more effective in terms of antifungal activity.

A similar study reported that AgNPs produced from Cynara scolymus L. (artichoke) plant by green synthesis method were effective on S. aureus, B. subtilis, P. aeruginosa, E. coli and C. albicans at concentrations of 0.12, 0.25, 0.07, 0.13, 0.03 µg mL-1, respectively. In the same study, AgNPs were reported to be effective at lower concentrations than silver nitrate and antibiotics (Baran et al., 2021). In another similar study, it was reported that AgNPs synthesized from Punica granatum (pomegranate) leaves showed antibacterial activity at 0.050-0.125 mg mL-1 concentrations on E. coli, P. aeruginosa, S. aureus, B. subtilis and Proteus vulgaris bacteria (Singhal et al., 2021). AgNPs synthesized from the Zea mays L. (corn) plant were reported to exhibit antimicrobial effects on E. coli, S. aureus bacteria, and C. albicans fungus at concentrations of 0.084, 0.337, and 0.021 µg mL-1, respectively (Eren & Baran, 2019). MIC values of AgNPs synthesized

from Hypericum triquetrifolium Turra (hypcricum) plant against E. coli and S. aureus bacteria and C. albicans fungus were reported as 0.041, 0.662 and 0.020 µg mL-1, respectively (Adil et al., 2019). The effective concentrations of AgNPs synthesized using Euphorbia hirta (asthma plant) on S. aureus and E. coli bacteria were reported to be 0.82 and 0.67 µg mL-1, respectively (Kumar et al., 2016). All of these studies suggest that AgNPs produced from various plants using the green synthesis method may have antimicrobial effects against the same pathogenic microorganisms at different concentrations.

## CONCLUSION

Antibiotic resistance to commonly used pharmaceuticals poses a threat to human health. Bioactive compounds and nanoparticles synthesized by plants are a goldmine in the fight against multidrug-resistant microorganisms and may be used as alternatives to traditional medications. The fact that the strong inhibitory effect of AgNPs synthesized by R. acetosella on these pathogenic microorganisms was higher than similar studies suggests that it would contribute to the development of antibiotics against the agents of infections caused by resistant microorganisms. Further studies are required in this field.

### Compliance with Ethical Standards

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Ethics committee approval is not required.

### Funding

No financial support was received for this study.

### Data availability

Not applicable.

### Consent for publication

Not applicable.

## REFERENCES

Acay, H., Baran, M. F., & Eren A. (2019). Investigation of Antimicrobial Activity of Silver Nanoparticles Produced by Green Synthesis Using Common Grape (*Vitis vinifera*) Leaf Extract. [CrossRef]

Adil, U., Adil, K., Baran, M. F., Keskin, C., & Atalar, M. N. (2019). Investigation of Antimicrobial Activity and Characterization, Synthesis of Silver Nanoparticles from Hypericum triquetrifolium Turra Plant. Journal of the Institute of Science and Technology, 9(3), 1467-1475. [CrossRef]

Aktepe, N., Baran, A., Nuri, A. M., Baran, M. F., Keskin, C., Düz, M. Z., . . . Kavak, D. E. (2021). Biosynthesis of Black Mulberry Leaf Extract and Silver NanoParticles (AgNPs): Characterization, Antimicrobial and Cytotoxic Activity Applications. MAS Journal of Applied Sciences, 6(3), 685-700. [CrossRef]

Atalar, M. N., Baran, A., Baran, M. F., Keskin, C., Aktepe, N., Yavuz, Ö., & İrtegun Kandemir, S. (2021). Economic fast synthesis of olive leaf extract and silver nanoparticles and biomedical applications. Particulate Science and Technology, 1-9. [CrossRef]

Azhdari, S., Sarabi, R. E., Rezaeizade, N., Mosazade, F., Heidari, M., Borhani, F., . . . Khatami, M. (2020). Metallic SPIONP/AgNP synthesis using a novel natural source and their antifungal activities. RSC Advances, 10(50), 29737-29744. [CrossRef]

Banerjee, P., Satapathy, M., Mukhopahayay, A., & Das, P. (2014). Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: synthesis, characterization, antimicrobial property and toxicity analysis. Bioresources and Bioprocessing, 1(1), 1-10.

Baran, A., Baran, M. F., Keskin, C., Kandemir, S. I., Valiyeva, M., Mehraliyeva, S., . . . Eftekhari, A. (2021). Ecofriendly/rapid synthesis of silver nanoparticles using extract of waste parts of artichoke (*Cynara scolymus* L.) and evaluation of their cytotoxic and antibacterial activities. Journal of Nanomaterials, 2021. [CrossRef]

Baran M. F. , Acay H. Antimicrobial Activity Of Silver Nanoparticles Synthesized with Extract of Tomato plant Against Bacterial and Fungal Pathogens. Middle Black Sea Journal of Health Science. 2019; 5(2): 67-73.

Baran, M. F., Saydut, A., & Adil, U. (2019). Gümüş nanomalzeme sentezi ve antimikrobiyal uygulamaları. Dicle Üniversitesi Mühendislik Fakültesi Mühendislik Dergisi, 10(2), 689-695, (in Turkish). [CrossRef]

Baran, A., Baran, M. F., Keskin, C., Hatipoğlu, A., Yavuz, Ö., İrtegün Kandemir, S., Adican, M. T., Khalilov, R., Mammadova, A., Ahmadian, E., Rosić, G., Selakovic, D., & Eftekhari, A. (2022). Investigation of antimicrobial and cytotoxic properties and specification of silver nanoparticles (AgNPs) derived from Cicer arietinum L. green leaf extract. Frontiers in Bioengineering and Biotechnology, 10, 855136. [CrossRef]

Cassini, A., Högberg, L. D., Plachouras, D., Quattrocchi, A., Hoxha, A., Simonsen, G. S., . . . Cecchini, M. (2019). Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. The Lancet infectious diseases, 19(1), 56-66.

Eren, A., & Baran, M. (2019). Green synthesis, characterization and antimicrobial activity of silver nanoparticles (AgNPs) from maize (*Zea mays* L.). Applied Ecology and Environmental Research, 17(2), 4097-4105. [CrossRef]

Gour, A., & Jain, N. K. (2019). Advances in green synthesis of nanoparticles. Artificial cells, nanomedicine, and biotechnology, 47(1), 844-851.

Hatipoğlu, A. (2021). Green synthesis of silver nanoparticles using Abelmoschus esculentus leaf and antimicrobial effects on some food pathogens. Artvin Coruh University Journal

of Forestry Faculty, 22(2), 239-246. [CrossRef]

Hatipoğlu, A. (2021). Green synthesis of gold nanoparticles from Prunus cerasifera pissardii nigra leaf and their antimicrobial activities on some food pathogens. Progress in Nutrition, 23(3), e2021241.

Hatipoğlu, A. (2022). Green Synthesis of Silver Nanoparticles and Their Antimicrobial Effects on Some Food Pathogens. Süleyman Demirel University Journal of Natural and Applied Sciences, 26(1), 106-114. [CrossRef]

Isbilir, S. S., & Sagiroglu, A. (2013). Total phenolic content, antiradical and antioxidant activities of wild and cultivated Rumex acetosella L. extracts. Biological agriculture & horticulture, 29(4), 219-226. [CrossRef]

Kakoullis, L., Papachristodoulou, E., Chra, P., & Panos, G. (2021). Mechanisms of antibiotic resistance in important gram-positive and gram-negative pathogens and novel antibiotic solutions. Antibiotics, 10(4), 415. [CrossRef]

Keskin, D. & Güvensen, N. (2022). Investigation of antimicrobial properties and chemical composition of different extracts of Sweet gum leaves (Liquidambar orientalis) . International Journal of Agriculture Environment and Food Sciences , 6 (1) , 13-18 . [CrossRef]

Kumar, V., Gundampati, R. K., Singh, D. K., Bano, D., Jagannadham, M. V., & Hasan, S. H. (2016). Photoinduced green synthesis of silver nanoparticles with highly effective antibacterial and hydrogen peroxide sensing properties. Journal of Photochemistry and Photobiology B: Biology, 162, 374-385. [CrossRef]

Liu, R. H. (2013). Health-promoting components of fruits and vegetables in the diet. Advances in nutrition, 4(3), 384S-392S. [CrossRef]

Ma, X., Zhou, B., Budai, A., Jeng, A., Hao, X., Wei, D., . . . Rasse, D. (2016). Study of biochar properties by scanning electron microscope–energy dispersive X-ray spectroscopy (SEM-EDX). Communications in Soil Science and Plant Analysis, 47(5), 593-601. [CrossRef]

Naikoo, M. I., Dar, M. I., Raghib, F., Jaleel, H., Ahmad, B., Raina, A., . . . Naushin, F. (2019). Role and regulation of plants phenolics in abiotic stress tolerance: An overview. Plant signaling molecules, 157-168. [CrossRef]

Naser, D. K., Abbas, A. K., & Aadim, K. A. (2020). Zeta potential of Ag, Cu, ZnO, CdO and Sn nanoparticles prepared by pulse laser ablation in liquid environment. Iraqi Journal of Science, 2570-2581. [CrossRef]

Parveen, K., Banse, V., & Ledwani, L. (2016). Green synthesis of nanoparticles: their advantages and disadvantages. AIP conference proceedings,

Sharma, S., Jaiswal, S., Duffy, B., & Jaiswal, A. K. (2019). Nanostructured materials for food applications: spectroscopy, microscopy and physical properties. Bioengineering, 6(1), 26. [CrossRef]

Singh, A., Gautam, P. K., Verma, A., Singh, V., Shivapriya, P. M., Shivalkar, S., . . . Samanta, S. K. (2020). Green synthesis of metallic nanoparticles as effective alternatives to treat antibiotics resistant bacterial infections: A review. Biotechnology Reports, 25, e00427. [CrossRef]

Singhal, M., Chatterjee, S., Kumar, A., Syed, A., Bahkali, A. H., Gupta, N., & Nimesh, S. (2021). Exploring the Antibacterial and Antibiofilm Efficacy of Silver Nanoparticles Biosynthesized Using Punica granatum Leaves. Molecules, 26(19), 5762. [CrossRef]

Varadavenkatesan, T., Vinayagam, R., & Selvaraj, R. (2017). Structural characterization of silver nanoparticles phyto-mediated by a plant waste, seed hull of Vigna mungo and their biological applications. Journal of Molecular Structure, 1147, 629-635. [CrossRef]

Volkova, M., Atamas, A., Tsarenko, A., Rogachev, A., & Guskov, A. (2021). Cation Transporters of Candida albicans—New Targets to Fight Candidiasis? Biomolecules, 11(4), 584. [CrossRef]

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 530-536
DOI: https://doi.org/10.31015/jaefs.2022.4.5

# Socio-economic determinants of smallholder rice (*Oryza sativa*) farmer's access to Loan facilities, Abuja, Nigeria

**Olugbenga Omotayo Alabi**[1] , **Chinwe Edith Anekwe**[1]

[1] Department of Agricultural Economics, University of Abuja, PMB 117, Gwagwalada-Abuja, Abuja, Nigeria

**Citation:** Alabi, O.O., Anekwe, C.E., (2022). Socio-economic determinants of smallholder rice (Oryza sativa) farmer's access to Loan facilities, Abuja, Nigeria. International Journal of Agriculture, Environment and Food Sciences, 6 (4), 530-536.

**Received:** 28 August 2022
**Revised:** 15 September 2022
**Accepted:** 30 September 2022
**Published Online:** 05 November 2022

**Correspondence:** Olugbenga Alabi
**E-mail:** omotayoalabi@yahoo.com



## Abstract

This research study focused on socio-economic determinants of smallholder rice farmer's access to loan facilities, Abuja, Nigeria. Multi-stage sampling technique was adopted to select 100 smallholder rice farmers. Primary sources of data were used. Data were collected from smallholder rice farmers with the help of well-designed and well-structured questionnaire. Statistical and econometric tools were used for data analysis. The results show that 82% of smallholder rice farmers were between 31 to 50 years of age. About 67% of smallholder rice farmers had access to loan facilities. Sources of loan obtained by smallholder rice farmers are formal (68.66%) and informal sources (31.34%). Averagely, smallholder farmers obtained 200, 754.72 Naira and 129,558.82 Naira from formal and informal sources respectively. Age ($X_1$), farm size ($X_2$), household size ($X_3$), marital status ($X_5$), access to extension services ($X_7$) and membership of cooperative organizations ($X_8$) were statistically and significant socio-economic factors influencing smallholder rice farmers access to loan facilities at ($P<0.05$). In addition, level of education was statistically and significant socio-economic factors influencing smallholder rice farmers access to loan facilities at ($P<0.01$).The constraints encountered by smallholder rice farmers in accessing loan facilities were: lack of collaterals for formal sources, cumbersome administrative procedures, high interest rate for formal sources, late disbursement of loan, long distant to financial institutions for formal sources, small amount of loan, and short re-payment period. The study recommends that loan with single digit interest rate should be provided by the government; cumbersome administrative procedures involved in accessing loan should be addressed, micro finance banks should be located in rural areas to meet the need of smallholder rice farmers loan demand; smallholder rice farmers should be encouraged to join cooperative organizations in order for them to have easy access to loan facilities.

**Keywords:** Smallholder Rice Farmers, Loan Facilities, Socio-Economic Factors, Nigeria

## INTRODUCTION

Rice (*Oryza sativa*) is an important and major staple food security crop in the world. Rice ranks third coming after wheat and maize in terms of worldwide production (Imolehin and Wada, 2000). Nigeria is the largest producer of rice in West Africa, (Falola, Ayinde and Ojehomon, 2013). Rice accounted for 12% of total cereals produced in Nigeria (Ademiluyi, Okeke-Agulu, and Folorunso, 2021). In 2021, rice paddy production for Nigeria stands at 9 million metric tonnes

(CBN, 2022). In 2020, rice paddy production in Nigeria stands at 8.17 million metric tonnes. The production of milled rice in 2021 and 2020 stands at 5.0 million metric tonnes and 4.89 million metric tonnes respectively. In Nigeria, production of milled rice is 5.0 million metric tonnes, inspite of this, 6.7 million metric tonnes is consumed in Nigeria resulting in a deficit of 2 million metric tonnes which is left to be imported (USDA, 2020). Nigeria ranks third-ties with Iraq after Philippines and China in the group of major rice importing countries in the world (Awe, 2006). Local rice production cannot meet the demand for its teeming population, this has led to demand-supply gap (Oloyede, Muhammad-Lawal, Amolegbe, Olaghere and Joseph (2020). The demand and supply gap in rice production is widening, resulting in huge import bill on rice. Rice imports have affected the domestic production of Nigeria's local rice (Ademiluyi, Okeke-Agulu, and Folorunso, 2021). In, 2014, Thailand alone exported 1.3 million metric tonnes of rice to Nigeria but due to Anchors Borrowers Programme, by 2016 and 2021, rice import from Thailand had fallen to 58, 000 metric tonnes and 2,160 metric tonnes respectively (CBN, 2022). The annual consumption of rice per capital has grown significantly from 18Kg in 1980s, to 22 Kg in 1990s, to 29 Kg in 2008 and 32Kg in 2021 (Akande, 2003; USAID, 2008; USDA, 2020). Nigerian rice has a lot of potentials for increased rice productivity as the country is blessed with abundant rice growing environment (Nwaobiala and Adesope, 2013). Nigeria has estimated 4.6 million hectares of land suitable for rice production, but only 1.8 million hectares of land representing 39% is under rice cultivation (Danbata et al, 2013). Five major rice production systems have been identified, these are: upland rainfed, inland shallow swamp, deep water, floating lowland, and irrigated production system. Rice is used in the preparation of local dishes that are eaten in every homes especially during festivals and ceremonies (Ekeleme et al, 2008). Rice is used in making wine, beer, spirit, and vinegar. Rice wine contains 10 – 15 % alcohol and is usually made from glutinous rice. About 90% of domestic rice production in Nigeria comes from weakly organized, peasant, resource-poor smallholder farmers (USAID, 2009; Cadoni and Angelucci, 2013). Smallholder rice farmers use low-input strategy to rice production, minimum input requirements and low productivity (IFAD, 2012). Smallholder rice farmers are faced with many challenges such as: limited access to productive inputs and assets, low productivity, inadequate market and rural infrastructures, inadequate support extension and research services, constrained enabling environment post-harvest losses and paucity of opportunity for agricultural value addition (IFAD, 2012).

A loan is property, money, or other materials goods given to another party in exchange for future repayment of the loan value or principal amount along with interest or finance charges. A loan may be specific, one-time amount, or it can be made available up to a specific limit (Kagan, 2019). Access to loan could increase the willingness of smallholder farmers to adopt new farming technologies which can increase production as well as income (Li and Zhu, 2015). Agricultural loan reduces poverty, enhances productivity and promotes standard of living of smallholder farmers. Availability and accessibility to loan by smallholder farmers can alleviate capital constraints on agricultural households, farm loan remain one of the major means of improving farm capital investment. Lack of access to adequate loan can have significant and negative effects on various aggregate and household level outcomes including agricultural productivity, technology adoption, nutrition, health, food security, and household welfare. Smallholder farmers are faced with inadequate and restricted access to capital and limited access to loan facilities.

Without loan, smallholder farmers have little chance of substantially increasing their production.

### Objectives of the Study

This research study focused on socio-economic determinants of smallholder rice farmer's access to loan facilities, Abuja, Nigeria. Specifically, the objectives were:

(i) determine the socio-economic characteristics of smallholder rice farmers,

(ii) identify sources and amount of loan accessed by smallholder rice farmers,

(iii) evaluate socio-economic factors influencing smallholder rice farmer's access to formal loan facilities, and

(iv) determine the constraints encountered by smallholder rice farmers in accessing loan facilities in the study area.

## METHODOLOGY

This research study was conducted in Federal Capital Territory, Abuja, Nigeria. Abuja lies between Latitudes $9^0\,4^{l}\,20^{ll}$ North and Longitudes $7^0\,29^{l}\,28^{ll}$ East. The population of Abuja is about 3,464, 000 people in 2021 and 3,652,000 people in 2022. Abuja experiences three weather conditions. The weather conditions are: harmattan period, wet season, and dry season. The harmattan period is brief and comes in between the dry and wet seasons. Abuja is located in the savannah zone vegetation. The vegetation in the area is classifies into three savannah types: shrub savannah, savannah woodland, and grassy savannah. The people are predominantly farmers, they are involved in growing crops and rearing of animals. Crops grown include: rice, maize, sorghum, yam, millet, soyabean, garden egg, beans, and groundnut. Animals kept include: sheeps, poultry, goats, cattle, turkey, and rabbit. Multi-stage random sampling technique was

employed. One hundred (100) smallholder rice farmers were sampled. Data were of primary sources. Data were obtained from smallholder rice farmers with the help of well-designed and well-structured questionnaire. The questionnaire was designed considering the objectives of the study stated and analytical tools employed. Analytical tools employed for of data analysis include:

### Descriptive Statistics

This include the use of measures of central tendency and it involves the use of mean, percentages, and frequency distributions to summarize the socio-economic characteristics of smallholder rice farmers as stated in specific objective one (i). Descriptive statistics was also used to summarize the sources and amount of loan accessed by smallholder rice farmers as stated in specific objective two (ii).

### Probit Dichotomous Regression Model

The dichotomous response model is defined as follows:

$$Y = \alpha_0 + \alpha_1 X_1 + \alpha_2 X_2 + \alpha_3 X_3 + \alpha_4 X_4 + \alpha_5 X_5 + \alpha_6 X_6 + \alpha_7 X_7 + \alpha_8 X_8 + U_i$$

Y=Dichotomous Response Model (1, Access to Formal Loan;0, Otherwise),

$X_1$=Age of Smallholder Rice Farmers (Years),

$X_2$=Farm Size (Hectares),

$X_3$=Household Size (Units)

$X_4$=Gender (1, Male;0,Otherwise)

$X_5$=Marital Status (1, Married;0, Otherwise)

$X_6$=Level of Education(0, Non–Formal;1, Primary;2, Secondary;3, Tertiary)

$X_7$=Access to Extension Services (1, Access;0,Otherwise)

$X_8$=Membership of Cooperative Organizations (1, Member;0, Otherwise)

$U_i$=Error Term,

$\alpha_1$-$\alpha_8$ =Regression Coefficients,

$\alpha_0$ =Constant Term,

This was used to achieve specific objective three (iii).

### Principal Component Model

This is a statistical technique that can transform many interrelated variables into few uncorrelated ones. This was used to determine the constraints encountered by smallholder rice farmers in accessing loan facilities as stated in specific objective four (iv).

## RESULTS AND DISCUSSION

Socio-Economic Characteristics of Smallholder Rice Farmers in the Study Area

The socio-economic characteristics of smallholder rice farmers under consideration were: age, gender, marital status, household size, level of education, farming experience, farm size, cooperative memberships, and access to loan facilities as presented in Table 1. The results show that 82% of smallholder rice farmers were between the age of 31 to 50 years. The smallholder farmers are within the active, productive age, and they are young, energetic and resourceful. This is in line with findings of Alabi et al (2020b). This is also similar to findings of Girei et al (2018) who reported that age has significant influence on the way smallholder farmers make farm decisions with respect to production decisions, risk aversion, and on adoption of improved agricultural technologies. Gender of smallholder rice farmers was classified into male and female. Smallholder rice production in the area was male dominated (78%), while female has 22%. This is also in line with findings of Alabi et al (2021b). Agronomic practices involved in rice production is labour intensive, so it is male dominated, while female take part in processing and other livelihood activities that can earn income for the household. About 39% of smallholder rice farmers were single, 56% were married, and 05% were divorced. Kuye and Ogiri (2009) indicated that married respondents among smallholder rice farmers are likely to incur more expenditures on family upkeeps. According to Saliu et al (2017), household size is the total number of individuals who live within and feed in the same house. About 58% of smallholder rice farmers had between 1 to 5 people per household. The average number of people per smallholder rice farming household was 6 people. This is similar to the findings of Alabi et al (2020b) who reported that smallholder rural rice farmers had on the average of 7 people per household. Furthermore, 87% of smallholder rice farmers had formal education, while 13% had non-formal education. This is in agreement with results of Alabi et al (2020a) who reported that education of smallholder farmers is an important and significant factor that can influence smallholder farmer's adoption of new innovations and research findings. Educated farmers adopt new innovations and research findings quickly, and also understand the guidelines involved in accessing formal loan. Averagely, smallholder rice farmers had 7 year's experiences in rice production. About 75% of smallholder rice farmers had less than 11 year's experiences in rice production. This finding is in line with Maurice et al (2015). Most (67%) of the sampled smallholder rice farmers had between 1 to 2 hectares of planted farm land. The average farm size was 2.28 hectares of planted farm land. The results confirmed that they are smallholder, smallscale, resource poor rice farmers. Also, 67% of smallholder rice farmers had access to formal and informal sources of loan facilities, while 33% do not have access to any form of loan facilities. About 78% of smallholder rice farmers belong to one form of cooperative organizations or the other, through memberships of coop-

erative organizations they could as groups access farm inputs, fertilizer inputs, seeds inputs, credit facilities such as loan facilities and they can participate in bulk purchase their farm inputs and bulk sold their farm produce.

## Sources and Amount of Loan Facilities Accessed by Smallholder Rice Farmers in the Study Area

The sources of loan facilities obtained by smallholder rice farmers was presented in Table 2. The amount of loan accessed by smallholder rice farmers was presented in Table 3. The major sources of loan to smallholder rice farmers was through formal (68.66%) and informal sources (31.34%). The average loan accessed from formal sources by smallholder rice farmers was 200,754.2 Naira with the maximum interest rate of 36% charged per annum. The minimum and maximum amount of loan accessed from formal sources by smallholder rice farmers were 30,000 Naira and 500,000 Naira respectively. Averagely, the amount of loan accessed from informal sources by smallholder rice farmers was 129, 558.82 Naira with maximum interest rate of 20%. The minimum and maximum amount of loan accessed by smallholder rice farmers were 25,000 Naira and 400,000 Naira respectively. This result is in line with findings of Alabi et al (2016). According to Kuye and Ogiri (2009) the average value of loan applied and received was 169, 583.33 Naira in Cross River State, Nigeria. Formal sources provide loan to smallholder rice farmers at higher interest rate, which makes it difficult and unaffordable.

## Socio-Economic Factors Influencing Smallholder Rice Farmers Accessed to Formal Loan Facilities in the Study Area

The socio-economic factors influencing smallholder rice farmers access to formal loan facilities was examined using Probit dichotomous response model as presented in Table 4. The socio-economic factors under considerations were age, farm size, household size, gender, marital status, level of education, access to extension services, and membership of cooperative organizations. All explanatory variables included in the Probit dichotomous response model had positive coefficients.

Level of education ($X_6$) was statistically significant in influencing smallholder rice farmers access to formal loan facilities at ($P<0.01$). Age ($X_1$), farm size ($X_2$), household size($X_3$), marital status($X_5$) and access to extension services ($X_7$) were statistically and significant factors influencing smallholder rice farmers access to formal loan facilities at ($P<0.05$). The positive marginal effects of the explanatory variables implies that a unit increase in farm size, level of education and being membership of cooperative organizations will by 19.77%, 35.65% and 20.72% increase the likelihood and probability of the smallholder rice farmers having access to formal loan fa

**Table 1.** Socio-Economic Characteristics of Smallholder Rice Farmers

| Socio-Economic Characteristics | Frequency | Percentage | Mean |
|---|---|---|---|
| Age of Farmers (Years) | | | |
| 31 – 40 | 54 | 54.00 | 42.0 |
| 41 – 50 | 28 | 28.00 | |
| 51 – 60 | 18 | 18.00 | |
| Gender | | | |
| Male | 78 | 78.00 | |
| Female | 22 | 22.00 | |
| Marital Status | | | |
| Single | 39 | 39.00 | |
| Married | 56 | 56.00 | |
| Divorced | 05 | 05.00 | |
| Household Size (Units) | | | |
| 1 – 5 | 58 | 58.00 | |
| 6 – 10 | 26 | 26.00 | 6.0 |
| 11 – 15 | 16 | 16.00 | |
| Level of Education (Years) | | | |
| Primary | 28 | 28.00 | |
| Secondary | 52 | 52.00 | |
| Tertiary | 07 | 07.00 | |
| Non-Formal | 13 | 13.00 | |
| Farming Experience (Years) | | | |
| 1 – 5 | 52 | 52.00 | |
| 6 – 10 | 23 | 23.00 | |
| 11 – 15 | 19 | 19.00 | 7.0 |
| 16 – 20 | 06 | 06.00 | |
| Farm Size (Hectares) | | | |
| 1 – 2 | 67 | 67.00 | 2.28 |
| 3 – 4 | 27 | 27.00 | |
| 5 – 6 | 06 | 06.00 | |
| Cooperative Memberships | | | |
| Yes | 78 | 78.00 | |
| No | 22 | 22.00 | |
| Access to Loan Facilities | | | |
| Yes | 67 | 67.00 | |
| No | 33 | 33.00 | |
| | | | |
| Total | 100.00 | 100.00 | |

Source: Field Survey (2021)

**Table 2.** Sources of Loan Obtained by Smallholder Rice Farmers

| Sources of Loan | Frequency | Percentage |
|---|---|---|
| Informal Sources | 21 | 31.34 |
| Formal Sources | 46 | 68.66 |
| Total | 67 | 100.00 |

Source: Field Survey (2021)

cilities respectively. This is in line with findings of Alabi et al (2016) and Ameh & Iheanacho (2017) who reported that educated farmers has courage, boldness and technical know-how required to approach financial institutions for loan facilities. According to Asogwa, Abu and Ochoche (2014) who stated that level of education raises smallholder rice farmers' knowledge and level of awareness about the needs for loan for increased agricultural output. The maximum likelihood estimates revealed that the Log Likelihood value was -44.89681, the Chi square value was 15.79 which was statistically significant at (P<0.01). The Pseudo R square was 0.6498, this means that 64.98% of variations in access to formal loan facilities were explained by the explanatory variables included in the Probit dichotomous response model.

## Constraints Encountered by Smallholder Rice Farmers in Accessing Loan Facilities in the Study Area

The constraints encountered by smallholder rice farmers in accessing loan facilities were subjected to principal component analysis and presented in Table 5. Principal component model is widely used statistical technique that can reduce many interrelated variables into few uncorrelated ones. Seven constraints with Eigen-values greater than one (1) were retained by the model. Constraints with Eigen-values less than one (1) were discarded by the principal component model. Lack of collaterals in obtaining formal loan was ranked 1st with Eigen-value of 3.28907 based on the perceptions of the smallholder rice farmers and this explained 11.61% of all constraints retained by the principal component model. Cumbersome administrative procedures in obtaining formal loan was ranked 2nd with Eigen-value of 3.1001 based on the perceptions of smallholder rice farmers and this explained 10.23% of all constraints retained by the principal component model. Other constraints were high interest rate in obtaining formal loan (3rd), late disbursement of loan (4th), long distant to financial institutions (5th), small amount of loan (6th), and short re-payment period (7th). This is in line with findings of Alabi et al (2021a). All constraints retained by the principal component model by smallholder rice farmers in accessing loan facilities explained 71.65% of all constraints encountered by the smallholder rice farmers. The Chi-square value was 3702.21 and was statistically significant at (P<0.01).

**Table 3.** Amount of Loan Accessed by Smallholder Rice Farmers

| Sources of Loan | Mean (Naira) | Minimum Amount (Naira) | Maximum Amount (Naira) |
|---|---|---|---|
| Formal Sources | 200,754.72 | 30,000 | 500,000 |
| Informal Sources | 129,558.82 | 25,000 | 400,000 |

Source: Field Survey (2021)

**Table 4.** Maximum Likelihood Estimates of the Probit Dichotomous Regression Model

| Variables | Coefficients | Standard Error | Z-Score | Marginal Effects |
|---|---|---|---|---|
| Age ($X_1$) | 0.00639** | 0.00256 | 2.50 | 0.01625 |
| Farm Size ($X_2$) | 0.07872** | 0.02894 | 2.72 | 0.19770 |
| Household Size ($X_3$) | 0.08264** | 0.02839 | 2.91 | 0.21022 |
| Gender ($X_4$) | 0.31760* | 0.16715 | 1.90 | 0.09232 |
| Marital Status ($X_5$) | 0.04593** | 0.02274 | 2.02 | 0.01168 |
| Level of Education($X_6$) Access to Extension Services ($X_7$) | 0.21879*** | 0.06128 | 3.57 | 0.35653 |
| Membership of Cooperative Organizations | 0.49172** | 0.21194 | 2.32 | 0.12508 |
| (X8) | 0.31462* | 0.15889 | 1.98 | 0.20721 |
| Constant | | | | |
| Log Likelihood | 3.1021 | 1.3371 | 2.32 | |
| LR Chi2 | -44.89681 | | | |
| Prob > Chi2 | 15.79 | | | |
| Pseudo $R^2$ | 0.00027 | | | |

Source: Field Survey (2021)
*-Significant at 10% probability level
**-Significant at 5% probability level
***-Significant at 1% probability level

**Table 5.** Principal Component Analysis of Constraints Encountered by Smallholder Rice Farmers in Accessing Loan Facilities in the Study Area

| Constraints | Eigen-Value | Difference | Proportion | Cumulative |
|---|---|---|---|---|
| Lack of Collateral | 3.28907 | 0.2709 | 0.11615 | 0.11615 |
| Cumbersome Administrative Procedures | 3.1001 | 0.2686 | 0.10231 | 0.21846 |
| High Interest Rate | 2.9043 | 0.2601 | 0.15062 | 0.36908 |
| Late Disbursement of Loan | 2.3003 | 0.2590 | 0.09016 | 0.45924 |
| Long Distant to Financial Institutions | 1.9110 | 0.2501 | 0.08173 | 0.54097 |
| Small Amount of Loan | 1.8076 | 0.2209 | 0.09932 | 0.64029 |
| Short Re-Payment Period | 1.5001 | 0.2009 | 0.07621 | 0.71650 |
| Bartlett Test of Sphericity | | | | |
| KMO | 0.7176 | | | |
| Chi Square | 3702.21*** | | | |
| Rho | 1.00000 | | | |

Source: Computed from Data Analysis (2021)

## CONCLUSION AND RECOMMENDATIONS

This research study has established that smallholder rice farmers are young, energetic, resourceful, and their productive age. Some smallholder rice farmers do not belong to any members of cooperative organizations, this make it difficult for them to access loan facilities, farm inputs, fertilizer inputs, and seeds inputs. In addition, they could not participate in bulk purchase of farm inputs or bulk sold farm produce as done by members of cooperative organizations. The smallholder rice farmers obtained loan facilities through formal and informal sources. Loan obtained by smallholder rice farmers from formal sources are with high interest rate. Averagely, smallholder rice farmers obtained 200, 754.72 Naira and 129,558.82 Naira from formal and informal sources respectively. The socio-economic factors statistically and significantly influencing smallholder rice farmers access to formal loan facilities were age, farm size, household size, marital status, level of education, access to extension services and members of cooperative organizations. Seven constraints with Eigen-values greater than one (1) retained by the principal component model and encountered by smallholder rice farmers in accessing loan facilities were: lack of collaterals in obtaining formal loan, cumbersome administrative procedures, high interest rate of formal loan, late disbursement of loan, long distant to financial institutions, small amount of loan, and short re-payment period. The following recommendations were made based on the findings of this research study:

(i) Loans should be made available at affordable interest rate, with single digit, to smallholder rice farmers.

(ii) The cumbersome administrative procedures involved in accessing loan facilities by smallholder rice farmers should be addressed

(iii) Government should make a provision of special microfinance banks that should be located in rural arears to meet the need of farmers' loan demand.

(iv) Training, education and workshops should be organized by extension agents for smallholder rice farmers on guidelines involved in accessing formal loan facilities.

(v) Smallholder rice farmers should be encouraged to join cooperative organizations in order for them to easily access loan facilities.

(vi) Farm inputs, seeds inputs, tractors, fertilizers, credit facilities should be provided for smallholder rice farmers at subsidized price.

## COMPLIANCE WITH ETHICAL STANDARDS

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Ethics committee approval is not required.

### Funding

No financial support was received for this study.

### Data availability

Not applicable.

### Consent for publication

Not applicable.

## REFERENCES

Ademiluyi, I.O., Okeke-Agulu,K.I and Folorunso, S.T (2021). Economics of Rice Production in Wase Local Government Area of Plateau State, Nigeria. International Journal of Food Science and Agriculture 5 (2): 214 – 218. [CrossRef]

Akande, T. (2003). The rice sector in Nigeria. United Nation Crop Project (UNCP) Country Agricultural Project on trade liberalization in Agricultural sector and the environment, Geneva. Pp 10.

Alabi, O.O.; Lawal,A.F and Chiogor, H.O (2016). Access to Formal Credit Facilities among Smallscale Crop Farmers in Gwagwalada Area Council, Abuja, Nigeria. Russian Journal of Agricultural and Socio-Economic Sciences (RJOAS)1(49):57-66. [CrossRef]

Alabi,O.O., Oladele, A.O and Oladele, N.O (2020a). Economic Market Decisions among Marginal Maize Farmers in Abuja, Nigeria: Applications of Double Hurdle Model and Factor Analysis. Russian Journal of Agricultural and Socio-Economic Sciences 8(104): 114 – 125. [CrossRef]

Alabi,O.O, Oladele, A.O,,and Oladele,N.O (2020b).Socio-Economic Factors Influencing Perceptions and Adaptation of Rural Rice Farmers to Climate Change, Abuja, Nigeria: Applications of Heckman Two-Stage Model. Russian Journal of Agricultural and Socio-Economic Sciences (RJOAS) 8 (104): 45 – 56. [CrossRef]

Alabi,O. O, Oladele, A.O, and Usman, M.B (2021a). Determinants of Agricultural Loan Decision Making Process for Rice (Oryza sativa) Farmers in Abuja, Nigeria: Applications of Heckman Two-Stage Model and Factor Analysis. Journal of Agribusiness and Rural Development, University of Life Sciences, Poznan, Poland 1 (59): 29 -38. [CrossRef]

Alabi, O.O., Sunday,A.G., Waziri-Ogwu, P.R,Umar, S.A, Osundiya, O.N, Olumuyiwa, S.A, Emeghara, U.U, Sanusi, S.O, Omole, E.B and Shaba, M.G (2021b). Smallholder Rural Women Rice (Oryza sativa) Farmers' Decisions Making Process, Ag-

ricultural Intensification and Poverty Status, Abuja, Nigeria. Australian Journal of Science and Technology 5 (1):448-459. [URL]

Ameh, M and Iheanacho, A.C (2017). Socio-Economic Factors Influencing Agricultural Loan Acquisition among Smallscale Rice Farmers in Benue State. International Journal of Innovative Agriculture and Biology Research 5 (4): 8 – 17. [URL]

Asogwa, B. C., Abu, O., and Ochoche, G.E (2014). Analysis of Peasant Farmers Access to Agricultural Credit in Benue State, Nigeria. European Journal of Economics, Management and Trade 4 (10): 1525 - 1543. [CrossRef]

Awe, O. (2006). Ban on Rice Importation Depresses Global Trade. Punch 2006, May 20.

Cadoni P.,and Angelucci F (2013) Analysis of Incentives and Disincentives for Rice in Nigeria. Technical Notes Series, MAFAP, FAO, Rome, 2013. [URL]

CBN (2022). Central Bank of Nigeria, Annual Report. [URL]

Danbata, N., Anounye, J.C., Gana, A.S, and Abo, M.E (2013). Grain Physiochemical and Milling Qualities of Rice (Oryza sativa L) Cultivated in South East, Nigeria. J. Applic. Agric. Res 5: 61 - 71. [URL]

Ekeleme, F.,Kamara, A.Y., O moigiu,L.O., Tegbaru, A., Mshelia,J and Onyibe, J.E (2008). Guide to Rice Production in Borno State, Nigeria, IITA, Ibadan, Nigeria. [URL]

Falola, A., Ayinde, O.E., and Ojehomon, V.T (2013). Economic Analysis of Rice Production among the Youths in Kwara State, Nigeria. Albanian Journal of Agricultural Science 12 (3): 503 – 510. [URL]

Girei, A.A., Ohen, S.B., and Filli, F.B (2018). Econometrics Analysis of Soyabean Production in Doma Local Government Area of Nasarawa State, Nigeria. Journal of Agricultural Science and Practice 3(4): 90 – 96. [CrossRef]

IFAD (2012). International Fund for Agricultural Development, Federal Republic of Nigeria: Value Chain Development Programme (VCDP), Programme Design Report: Volume 1-Main Report. IFAD, West and Central Africa Division.

Imolehin, E. D. and Wada, A. C. (2000). Meeting the rice production and consumption demands of Nigeria with improved technologies. National Cereals Research Institute Badeggi Niger State, Nigeria. 12p. [URL]

Kagan, J (2019). Banking: Loan Basics. Investopedia.

Kuye, O.O and Ogiri, O.M (2009). Analysis of Factors Influencing Access to Formal Loan among Smallscale Swamp Rice Farmers in Obubra Local Government Area, Cross Rivers State, Nigeria. International Journal of Agricultural Economics 4 (6): 307 – 313. [CrossRef]

Li, Z., and Zhu, X (2015). Analysis of Credit Using and Rationing under Farmer's Risk Attitude Difference Asian Agricultural Research 7(4): 24 – 32. [CrossRef]

Maurice, C., Adamu, Y and Joseph, M (2015). Analysis of Cost Efficiency in Food Crop Production among Smallscale Farmers in Adamawa State, Nigeria. Global Journal of Agricultural Sciences 14: 17 – 25.

Nwaobiala, C.U and Adesope, O.M (2013). Economic Analysis of Smallholder Rice Production Systems in Ebonyi State, South East, Nigeria. Russia Journal of Agricultural and Socio-Economic Sciences 11 (23): 3 – 10. [CrossRef]

Saliu, L.A., Abdulrazaq, S.A and Yette, F.E (2017). Socio-Economic Analysis of Soyabean Production among Smallscale Farmers in Kaduna State, Nigeria. Proceedings of the 18th Annual Conference of the Nigerian Association of Agricultural Economists held at Federal University of Agriculture, Abeokuta, Nigeria, 16th – 19th October, 2017. [CrossRef]

Oloyede, W.O., Muhammad – Lawal, A., Amolegbe, K.B., Olaghere, I.L., and Joseph, I.A (2020). Comparative Analysis of the Profitability of Rice Production Systems in Kwara State, Nigeria. Agrosearch 20 (2): 82 - 101. [CrossRef]

USDA (2020). United States Department of Agriculture.

USAID (2008). Rice Fortification in Developing Countries: A Central Review of the Technical and Economic Feasibility. United States Agency for International Development. [URL]

USAID (2009). United State Agency for International Development, Global food security response Nigeria (rice study). Attachment IV to the Global Food Security Response West Africa (Rice Value Chain Analysis). [URL]

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 537-544
DOI: https://doi.org/10.31015/jaefs.2022.4.6

# Effects of different agricultural wastes on yield and quality in *Pholiota nameko* cultivation

**Osman Daşdelen[1], Flavien Shimira[1], Ecem Kara[2], Gökhan Baktemur[2], Hatıra Taşkın[1]**

[1] Department of Horticulture, Faculty of Agriculture, Cukurova University, 01330 Adana, Türkiye
[2] Department of Plant Production and Technologies, Faculty of Agricultural Sciences and Technologies, Sivas University of Science and Technology, Sivas, Türkiye

## Abstract

*Pholiota nameko* is commonly consumed in the Far East but not yet known or grown in Türkiye. Therefore, in this study, determination of the cultivation conditions of *P. nameko* in different substrate mixtures and the effect of these substrates on yield and quality were investigated. In this research, six different substrate mixtures were used: 2 oak sawdust + 1 wheat bran (C), 2 peanut waste + 1 wheat bran (O1), 1 oak sawdust + 1 peanut waste + 1 wheat bran (O2), 2 almond shell + 1 wheat bran (O3), 1 oak sawdust + 1 almond shell + 1 wheat bran (O4), 2 wheat straw + 1 wheat bran (O5) and 1 oak sawdust + 1 wheat stalk + 1 wheat bran (O6). During the study, mycelia development time, biological efficiency rate (BE), total yield, mushroom weight, cap diameter, cap thickness, stipe diameter, stipe length, dry matter, pH and moisture content (in growing mixtures) were determined. pH and moisture content were recorded in three different periods; after sterilization, after mycelia development time and after harvest. As a result of the study, the fastest mycelial growth was obtained from the control group, with 14.25 days. The highest total yield and biological efficiency were obtained from O1 medium with 216.26 g $kg^{-1}$ and 49.11%, respectively. The highest amount of dry matter was recorded in O4 with 12.23%.

**Keywords:** *Pholiota nameko*, Mushroom, Agricultural wastes, Cultivation, Türkiye

**Citation:** Daşdelen, O., Shimira, F., Kara, E., Baktemur, G., Taşkın, H. (2022). Effects of different agricultural wastes on yield and quality in *Pholiota nameko* cultivation. International Journal of Agriculture, Environment and Food Sciences, 6 (4), 537-544.

**Received:** 09 August 2022
**Revised:** 16 September 2022
**Accepted:** 20 September 2022
**Published Online:** 09 November 2022

**Correspondence:** Hatıra Taşkın
**E-mail:** hatirataskin1@gmail.com



## INTRODUCTION

Mushrooms have been known and used for nutritional and medicinal purposes from the past to the present. In addition to their high water content (88-91%), they contain many amino acids, vitamins and minerals (Matilla et al., 2002). Mushroom protein is identified as high quality and digestible. Proteins consist of different amino acids and nine of the amino acids are known as essential amino acids (lysine, methionine, tryptophan, threonine, valine, leucine, isoleucine, histidine, phenylalanine) (Chang and Miles, 2004; Taşkın and Büyükalaca, 2019). In addition, extracts obtained from medicinal mushrooms have begun to be used in multivitamin pills. Today, as the value of the mushroom is better understood, the curiosity and interest in its cultivation have increased rapidly. Mushroom cultivation does not require land and does not depend on climate. Also, all family members including children can work in mushroom production. All these make mushroom cultivation advantageous compared to other agricultural activities (Taşkın ve Büyükalaca, 2019).

Mushroom production in the world has increased continuously over the years.

While cultivated mushroom production in Türkiye was 7 thousand tons in 2000, it has reached 61 thousand tons today (TÜİK, 2021). The most cultivated mushroom species in the world is *Agaricus bisporus*. This is followed by *Pleurotus* species with 27% production and *Lentinula edodes* with 17% production. These three species constitute 74% of the total cultivated mushroom production in the world (Eren and Pekşen, 2016). Other species, such as *Grifola frondosa*, *Ganoderma lucidum*, and *Pholiota*, are also produced in small quantities around the world. *P. nameko* (T. Itô) S. Ito & S. Imai (*P. microspora*) has a slippery and smooth mucus layer on its surface. This mucus is thought to be useful for energizing human brain's cognitive functions (Li et al., 2010). *P. nameko*, besides being delicious, contains crude protein, fat and carbohydrates, crude fibre, calcium, phosphorus, iron, multivitamins, and various amino acids (Meng et al. 2019). Cultivation of *P. nameko*, also known as *P. microspora*, was first performed in Japan (Zhu et al, 2016). In Japan, freshly cut wood logs were used to grow *P. nameko* in 1921. While most fungal species cannot effectively use freshly cut wood, *P. nameko* can use logs containing living cells. Sawdust spawn was first used in 1931, and in 1960, wheat bran, rice bran and sawdust were used in the commercial culture of P. *nameko* (Neidleman, 2004; Gizaw, 2015). It is one of the most produced and popular mushrooms speciesin Japan and China, and its production quantity is very close to *L. edodes* and *F. velutipes* (Gizaw, 2015).

The huge volume of different agricultural wastes produced after the harvesting and processing of agricultural products has emerged as an important environmental problem. These agricultural wastes are usually burned or thrown into the environment. A total of 600 million tons of waste (100 million tons of forest industry waste and 500 million tons of agricultural waste) are generated every year and regarded as environmental pollution. With such a high amount of waste, approximately 360 thousand tons of mushrooms can be produced. Cultured mushroom species are saprotrophic, and wood logs have been used in their cultivation as sawdust. However, new environmental protection policies and their implementations safeguard and limit the use of forest products. This situation pushes mushroom producers to find alternative substrate materials to wood (Meng et al. 2019).

In this study, it was aimed to culture P. nameko, which is not yet produced in Türkiye, in different agricultural wastes generated in Türkiye in order to determine the substrate mixtures that provide the best yield and quality, as well as to detect the ideal cultivation conditions of P. nameko, aiming to establish a new income source for local mushroom producers.

## MATERIALS AND METHODS

This study was carried out at Prof. Dr. Saadet BÜYÜKALACA tissue culture laboratory and in the mushroom growing rooms of Cukurova University (Adana, Türkiye) between 2020 and 2021. *P. nameko* spawn was obtained from a private company. In the experiments, oak sawdust, peanut waste, almond shell and wheat stalk were used as agricultural wastes. In addition, wheat bran and soy flour were added (Table 1).

**Table 1.** Growing mixtures content used in *Pholiota nameko* production

| Substrates | Short names |
|---|---|
| 2 oak sawdust + 1 wheat bran | C |
| 2 peanut waste + 1 wheat bran | O1 |
| 1 oak sawdust + 1 peanut waste + 1 wheat bran | O2 |
| 2 almond shell + 1 wheat bran | O3 |
| 1 oak sawdust + 1 almond shell + 1 wheat bran | O4 |
| 2 wheat stalk + 1 wheat bran | O5 |
| 1 oak sawdust + 1 wheat stalk + 1 wheat bran | O6 |

All of the substrates were crushed and were soaked in the tap water filled containers until obtaining suitable moisture (70%). After this step, pH adjustment of substrates was performed with a pH meter. If the pH was not appropriate, lime was added. Substrates were filled into 1 kg high temperature resistant polypropylene bags. The bags were sterilized in the autoclave for 90 minutes at 1.2 atm pressure at 121°C. Following the cooling of the bags, spawn inoculation was carried out in the sterile bench using 25- 30 g spawn per bag.

After the inoculation process, the bags were transferred to the mushroom growing rooms, which had 22±1°C temperature and 70-80% humidity. With the development of mycelia, the humidity was reduced to 18 ±1°C. With the observation of mushrooms' development, the ventilation of the room and the humidity level were kept between 90-95% to keep the moisture safeguard the moisture of substrates. Since *P. nameko* does not require light for the development of mycelia, room lighting was not performed at this stage. After observing the first fruit bodies, room lighting was carried out for 12 hours at 200 lux. With the completion of the mycelia development, the bags were cut into 5 cm small openings with the sterile scalpel to encourage the formation of mushrooms. During experiments, biological efficiency rate, total yield, mushroom weight, cap diameter and thickness, stipe diameter and length, as well as dry matter content were measured. In addition, pH and moisture analyzes of substrates were carried out at three different periods: after sterilization, after mycelia development and after harvest. Cap diameter-thickness, stipe diameter and length were measured (in mm) with a caliper in five samples.

Total yield and mushroom weight were determined (in g) on a scale. For dry matter amount, fresh samples were weighed and dried at adjusted temperatures of 65°C until their weight became constant. Then, the dried samples were weighed and the dry matter of all samples was detected (in %). The biological efficiency rate was calculated according to Royse (1985).

This study was carried out according to the randomized complete block design, with three repetitions and three bags in each repetition. The collected data were analyzed in the JMP statistical program. LSD test was performed on the data where the difference is statistically significant. In addition, JMP correlation analysis was applied to the treatments thought to have a relationship.

## RESULTS AND DISCUSSION

### Mycelia development time, biological efficiency rate, total yield and mushroom weight of *Pholiota nameko* grown in different growing mixtures

Mycelia development was observed in all growing mixtures in this study and differences in mycelia development times were found to be statistically significant (Table 2). Mycelia development times ranged between 14.25 and 29.00 days. The fastest mycelia development was observed in the control with 14.25 days and in O6 with 15.75 days. The slowest mycelia development was recorded in O2 with 28 days and in O1 with 29 days (Table 2). While oak sawdust in the growing mixtures positively affected mycelia development, peanut waste and almond shell caused a delay. Mycelia development time may vary depending on mushroom strains, spawn quality, environmental conditions of the mushroom growing rooms and substrate characteristics (Sánchez, 2004). In a study conducted by Rong et al. (2016), five different *P. nameko* isolates were cultured in a growing mixture including 60% cotton seed shell, 18% sawdust, 15% wheat bran, 5% corn flour, 1% gypsum and 1% lime. As a result of their study, while the highest mycelial growth rate was obtained from the JZB2116005 isolate with 2.56±0.03 mm/g, the lowest mycelial growth rate was recorded in the control JZB2116001 isolate with 2.34±0.01 mm/g. *Pholiota microspora* was cultured in five different growing mixtures including poplar sawdust, corn stalk, wheat bran, corn flour, soybean, gypsum and lime by Meng et al. (2019). The highest mycelial development was observed in T2 (38% poplar sawdust, 38% corn stalk, 15% wheat bran, 5% corn flour, 2% soy flour, 1% gypsum-lime) and T3 (19% poplar sawdust, 57% corn stalk, 15% wheat bran, 5% corn flour, 2% soy flour, 1% gypsum-lime) mixtures. In a study by Hal et al. (2021), the effects of different growing mixtures on the yield and quality of G. lucidum were tested. The spawn of *G. lucidum* was inoculated into eight different growing mixtures and the mycelia development time varied between 28.00 and 44.67 days. The shortest time was observed in mixtures of oak sawdust-peanut shell-wheat bran and vine pruning waste-wheat bran with 28.00 days. Kara et al. (2021) investigated the effects of six different growing mixtures on the yield and quality of *Grifola frondosa*. Mycelia development time varied between 35.00 and 41.67 days. The fastest mycelia development was determined in the mixture of oak sawdust and wheat straw, with 35.00 days.

As mycelia development time, the biological efficiency rate varies depending on the substrate mixtures, mushroom strains and environmental conditions (Barreto et al. 2008). In this study, the biological efficiency rate varied between 20.02 (O4) and 49.11 (O1) and it was found to be statistically significant (Table 2). The highest biological efficiency rate was observed in O1 and was followed by C and O4. Although the mycelia development completed in O2, O3, O5 and O6, fructification could not occur. Therefore, the biological efficiency rate could not be determined. Gizaw (2015) tested six different growing mixtures for the cultivation of *P. nameko*. The highest yield (797.33 g) and biological efficiency (53.27%) were obtained from the mixture of eucalyptus sawdust and wheat bran. This was followed by a mixture of 30% wheat bran and cotton seed (732.33 g). The lowest average yield (550.8 g) and biological activity rate (48.98%) were obtained from the mixture of 10% wheat bran and *Cordia africana* sawdust. Rong et al. (2016) inoculated five different *Pholiota* isolates into a substrate containing 60% cottonseed hull, 18% sawdust, 15% wheat bran, 5% corn flour, 1% gypsum and 1% lime. The highest biological efficiency was obtained from JZB2116005 with 67.88%, while the control JZB2116001 isolate had the lowest rate with 41.35%. When some studies performed with different mushroom species were examined in terms of biological efficiency, it was found to be between 5.31% and 16.37% in *G. lucidum* (Hal et al. 2021), between 93.65% in *L. edodes* (Baktemur et al., 2022), between 17.34% and 44.86% in *Pleurotus eryngii* (Baştuğ, 2021), between 22.83% and 29.29% in *G. frondosa* (Kara et al. 2021).

The total yield of *P. nameko* cultivated on different substrate mixtures was given in Table 2 (Figure 1). Yield values varied according to the substrate materials used. The highest yield value was obtained from O1 with 216.26 g $kg^{-1}$, followed by the control with 163.71 g $kg^{-1}$ and O4 with 88.96 g $kg^{-1}$. Fructification did not occur in O2, O3, O5 and O6 mixtures. The highest yield was obtained from the peanut waste and wheat bran mixture. It is observed that the use of peanut waste, which is an important agricultural product in the Çukurova region of Türkiye, has a positive effect on the yield of *P. nameko*. In the mixtures prepared using the wheat stalk, which is widely cultivated in the region, mycelia development has been fast, but mushroom formation has not been realized. Meng et al. (2019) determined differences between the yield

values of *P. microspora* in five growing mixtures, including poplar sawdust, corn stalk, wheat bran, corn flour, soy flour and gypsum at different ratios. When the total yield values were compared, the highest yield values were obtained from T2 (38% poplar sawdust, 38% corn stalk, 15% wheat bran, 5% corn flour, 2% soy flour, 1% gypsum-lime) with 275.66 g and T3 (19% poplar sawdust, 57% corn stalk, 15% wheat bran, 5% corn flour, 2% soy flour, 1% gypsum-lime) with 255.3 g. The yield was found to be between 25.00 and 68.44 g $kg^{-1}$ in nine different growing mixtures, including oak-poplar-beech sawdust, wheat, bran and paddy at different ratios in *G. lucidum* by Erkel (2009). In another study, the average yield of *P. ostreatus* and *P. florida* was reported to be between 27.0 and 42.0 g 100 $g^{-1}$ and 28.3 and 34.0 g 100 $g^{-1}$, respectively (Kırbağ and Korkmaz, 2013). In a study carried out by Baktemur et al. (2022), the average yield values varied between 55.99 and 299.59 g kg-1. Among the substrates, the highest yield was found in the mixture of oak sawdust, wheat stalk and wheat bran with 299.59 g $kg^{-1}$. Hal et al. (2021) found the highest and lowest yield values in corncob-bran mixture and control oak sawdust with 66.58 g $kg^{-1}$ and 25.32 g $kg^{-1}$ respectively, in *G. lucidum*. In a study performed by Kara et al. (2021), it was reported that the average yield of *G. frondosa* varied between 124.82 and 55.02 g $kg^{-1}$.

**Figure 1**. Pholiota nameko obtained from this study

In Table 2, the mushroom weight of *P. nameko* cultivated on different growing mixtures is presented. Differences between mixtures were found to be statistically significant. There was no statistical difference between the weight of mushrooms obtained from O1 (16.30 g) and O4 (16.66 g) and the highest weight was obtained from these two mixtures. It was followed by the control group with 11.93 g. Yen (2008) reported that the average mushroom weight of *G. lucidum* isolates in different sawdust mixtures was between 11.38 g and 15.16 g. Yakupoğlu and Pekşen (2011) stated that the average mushroom weight of *G. lucidum* varies between 7.99 g and 31.19 g. The average mushroom weight was 21.21 g in *P. ostreatus* and 16.73 g in *P. sajor-caju* (Kurt, 2008) and was between 10.14 and 39.47 g in *L. edodes* (Sözbir, 2014). In a study conducted by Ranjbar et al. (2017), the highest mushroom weight of L. edodes was detected in the mixture with rice bran as 33.51 g. Baktemur et al. (2022) reported that the mushroom weight in *L. edodes* varied between 33.52 and 14.98. Kara et al. (2021) determined the maximum and minimum mushroom weight in E5 and E4 mixtures as 33.92 g and 17.26 g respectively, in *G. frondosa*.

**Table 2.** Mycelia development time (days), biological efficiency rate (%), total yield (g $kg^{-1}$) and mushroom weight (g) of *Pholiota nameko* cultivated in different growing mixtures

| Growing mixtures | Mycelia development time | Biological efficiency rate | Total yield | Mushroom weight |
|---|---|---|---|---|
| C | 14.25 D | 38.31 B | 163.71 B | 11.93 B |
| O1 | 28.00 A | 49.11 A | 216.26 A | 16.30 A |
| O2 | 29.00 A | - | - | - |
| O3 | 24.50 B | - | - | - |
| O4 | 24.75 B | 20.02 C | 88.96 C | 16.66 A |
| O5 | 17.75 C | - | - | |
| O6 | 15.75 CD | - | - | |
| | LSD***= 2.62 | LSD***= 8.12 | LSD***= 35.47 | LSD***= 2.71 |

1. The statistical differences between the averages shown in separate letters in the same column were found to be significant.
2. N.S. Not Significant; *. P<0.05. **p ≤ 0.01. ***p ≤ 0.001

## Dry matter amount, cap diameter-thickness and stipe diameter-lenght of *Pholiota nameko* grown in different growing mixtures

The amount of dry matter of the *P. nameko* cultivated in different mixtures is given in Table 3. Differences between the mixtures in terms of the amount of dry matter were found to be statistically significant. The highest amount of dry matter was obtained from the O4 (12.23%), followed by C (10.31%) and O1 (10.67%) and there was no statistical difference between these two mixtures. In a study carried out by Baktemur et al. (2021), it was found that the average amount of dry matter varies between 8.59% and 11.65 % in *L. edodes*. The highest amount of

dry matter was obtained from the mixture of peanut shell and wheat bran with 11.65%. This was followed by the mixture of oak sawdust, corncob and wheat bran (11.21%) and the mixture of oak sawdust, vine pruning waste and wheat bran (10.99%). Kara et al. (2021) reported that the amount of dry matter of *G. frondosa* ranged from 13.57% to 14.79%.

The cap diameter-thickness, stipe diameter and length of the P. nameko samples which were cultivated in growing mixtures prepared by mixing different substrates at different ratios, were measured with a caliper and recorded in mm. It was determined that there was a statistically significant difference between these values (Table 3). It was observed that the cap diameter of the mushroom samples (68.04 mm) obtained from the O1 was larger. The cap diameter value of the mushrooms obtained from C (41.22 mm) and O4 (41.47 mm) was statistically in the same group. Yakupoğlu and Pekşen (2011) reported that the cap diameter of *G. lucidum* was between 42.8 mm and 84.5 mm. Atila (2020) stated that the cap diameter of *G. lucidum* was between 58.0 and 92.4 mm. The average cap diameter value of *L. edodes* ranged from 45.36 to 61.33 mm and the highest cap diameter value was obtained from the mixture of vine pruning waste and wheat bran as 61.33 mm (Baktemur et al. 2022).

There was no statistically significant difference between O1 (68.04 mm) and O4 (10.21 mm) for the cap thickness parameter, and the highest values were obtained from these mixtures. The cap thickness of the mushrooms obtained from the control group was lower. Veena and Pandey (2011) reported that the average cap thickness of *G. lucidum* varies between 6.9 and 8.1 mm. Hal et al. (2021) found that average cap thickness ranged from 8.68 to 10.24 in *G. lucidum* and the highest result was obtained from a mixture of corncob and wheat bran.

The highest stipe diameter was obtained from C (23.18 mm). This was followed by O1 (13.74 mm) and O4 (12.68 mm), respectively. It has been reported that the average stipe diameter value varies between 8.89 and 27.24 mm (Baktemur et al., 2022). For the stipe length, C (48.89) and O1 (51.67) were statistically in the same group. Average stipe length value changed between 21.31 and 49.11 mm in *L. edodes* cultured different growing mixtures (Baktemur et al., 2022). While the highest stipe length was obtained from a mixture of wheat stalk and wheat bran with 63.28 mm, the shortest value was recorded in the mixture of oak sawdust and wheat bran with 40.49 mm in *P. eryngii* by Baştuğ (2021).

**Table 3.** Dry matter amount (%), cap diameter-thickness (mm) and stipe diameter-lenght (mm) of Pholiota nameko cultivated in different growing mixtures

| Growing mixtures | Dry matter amount | Cap diameter | Cap thickness | Stipe diameter | Stipe lenght |
|---|---|---|---|---|---|
| K | 10.31 B | 41.22 B | 8.97 B | 23.18 A | 48.89 AB |
| O1 | 10.67 B | 68.04 A | 10.61 A | 13.74 B | 51.67 A |
| O4 | 12.23 A | 41.47 B | 10.21 A | 12.68 B | 37.07 B |
| | LSD*** = 0.52 | LSD*** = 11.94 | LSD*** = 1.06 | LSD*** = 4.26 | LSD*** = 14.37 |

## pH values of *Pholiota nameko* grown in different growing mixtures

The pH values of the growing mixtures used in *P. nameko* cultivation at three different periods are given in Table 4. The differences between the period average and the mixture x period interaction were found to be statistically significant, however, the mixture average was not statistically significant. Since the mushroom formation was not be provided from O3, O5 and O6 mixtures and infection problems in A4 after harvest, pH analysis could not performed in these mixtures.

The relationship between the mixture x period interaction was found to be important and the highest pH value was recorded in O1 and O2 as 8.33 and 8.44, and 8.44, respectively, at the after harvest period. The lowest pH value was determined in the control group at the after harvest period as 5.97. The average pH value of the mixtures has been statistically in the same group. In the analyses performed at different periods (after sterilization, after mycelia development and after harvest), there were statistical differences between the periods. There were no significant differences between the periods after sterilization and after mycelia development. Yakupoğlu and Pekşen (2011) reported that the pH values of the mixtures prepared using wood chips in *G. lucidum* were reported between 5.80 and 7.35, and they ranged from 5.70 to 7.05 in the mixture prepared using wood-chip. Atila (2020) found that the pH value of *G. lucidum* was between 4.43 and 6.42. Zadrazil (1978) stated that when pH value was higher than 8 and less than 4, the development of *Pleurotus* species is prevented and development of mycelia is slow in acidic mixtures (pH = 4) is slow. Sun and Yu (1989) determined that the mycelia of *P. sapidus* developed well in mixtures having pH 5.4-6.0 (Küçükomuzlu and Pekşen, 2005). Özçelik and Pekşen (2006) reported that pH of *L. edodes* ranged between 6.65 and 7.08. Adenipekun and Oklelade (2012) determined that the change in pH value may be associated with the presence of metabolic waste products in the mixture and the increase in amino nitrogen content. Kara et al. (2021) reported the most appropriate pH range for the cultivation of *G. frondosa* was between 5.20 and 5.45.

**Table 4.** pH values of Pholiota nameko grown in different growing mixtures at different periods

| Growing mixtures | Periods | | | |
|---|---|---|---|---|
| | After sterilization | After mycelia development | After harvest | Mean |
| C | 6.12 hı | 5.97 ı | 6.57 f | 6.22 |
| O1 | 7.50 b | 7.15 c | 8.33 a | 7.66 |
| O2 | 6.98 cd | 7.22 c | 8.44 a | 7.55 |
| O3 | 6.62 ef | 7.72 b | - | 4.78 |
| O4 | 6.29 gh | 7.64 b | - | 4.64 |
| O5 | 6.44 fg | 7.14 cd | - | 4.53 |
| O6 | 6.87 de | 7.08 cd | - | 4.65 |
| Mean | 6.67 A | 7.13 A | 3.33 B | |

LSDperiod*=0.11, LSDmixture=Ö.D., LSDperiodxmixture***=0.02

**Table 5.** Moisture content of *Pholiota nameko* grown in different growing mixtures at different periods (%)

| Growing mixtures | Periods | | | |
|---|---|---|---|---|
| | After sterilization | After mycelia development | After harvest | Mean |
| C | 64.50 de | 64.84 de | 59.99 fg | 63.11 |
| O1 | 68.21 bcd | 72.46 a | 62.94 ef | 67.87 |
| O2 | 63.43 ef | 65.76 cde | 70.46 ab | 66.55 |
| O3 | 48.28 h | 47.20 h | - | 31.83 |
| O4 | 57.17 g | 57.92 g | - | 38.36 |
| O5 | 70.80 ab | 70.25 ab | - | 47.02 |
| O6 | 70.9 ab | 69.36 abc | - | 46.77 |
| Mean | 63.33 A | 63.97 A | 27.63 B | |

LSDperiod**= 1.62, LSDmixtures= Ö.D., LSDperiodxmixture***= 4.28

### Moisture content of *Pholiota nameko* grown in different growing mixtures at different periods

Variance analysis was performed at three different periods in terms of the moisture content of the mixtures. Statistically significant differences have been determined in the mixture, period and their interaction. The highest moisture was recorded in O1 at after mycelia development period with 72.46 %. The lowest moisture content was obtained from O3 at after sterilization period (48.28%) and at after mycelia development period (47.20%). There was no statistically significant difference between after the sterilization period (63.33 %) and after mycelia development time (63.97%).

In a study conducted on *L. edodes*, the amount of moisture of the growing mixtures during the cultivation was examined. During the development, it was determined that the amount of moisture in the mixtures was maintained. The moisture amount was detected between 92.00 % and 93.85 % at the beginning of the study. It ranged from 90.90% to 95.44% at the fructification period (Morais et al. 2000). In *P. eryngii*, the highest and lowest moisture levels were determined at after sterilization period with 67.27% and after harvest period with 65.03%, respectively (Baştuğ et al. 2021). The highest moisture was recorded in the mixture of poplar sawdust and wheat bran with 72.10% and it was followed by wheat stalk and wheat bran mixture (71.12%) and oak sawdust, wheat stalk and wheat bran mixture (69.22%) (Baktemur et al. 2022). In *G. frondosa*, mixture x time interaction was found to be important and the highest amount of moisture was determined in E4 with 82.22% at after harvest period. The lowest value detected in E5 (67.12%) after sterilization period.

## CONCLUSION

During the study, many parameters were investigated to reveal the effects of the different substrate mixtures on the yield and quality of *P. nameko* mushroom. Although mycelia development was completed in all mixtures used, fructufication was observed in C, O1 and O4 mixtures. Since peanut is one of the main products of the Cukurova Region of Türkiye, it is very important that peanut waste can be used in mushroom cultivation in the region. It is recommended to try new substrates with peanut waste at different ratios. The wastes of wheat, which is intensively cultivated in the region, played an important role in shortening the period of mycelial development. It is also recommended to try new mixtures experiments based on wheat straw with different materials.

## COMPLIANCE WITH ETHICAL STANDARDS

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Ethics committee approval is not required.

### Funding

This study was financially supported by Çukurova University Scientific Research Projects Coordinating Office (Project code: FYL-2020-12798).

### Data availability

Not applicable.

### Consent for publication

Not applicable.

## Acknowledgements

Authors are thankful to to Çukurova University Scientific Research Projects Coordinating Office (Project code: FYL-2020-12798) for supporting this study financially and Sylvan Cultivating Excellence for P. nameko spawn.

## REFERENCES

Adenipekun, C.O., Okunlade, O.A. (2012). Biodegradation of rattan wood and maize stovers by *Pleurotus ostreatus*. Nature and Science, 10(5), 49–57. [Google Scholar]

Atila, F. (2020). Comparative study on the mycelial growth and yield of *Ganoderma lucidum* (Curt.: Fr.) Karst. on different lignocellulosic wastes. Acta Ecologica Sinica, 40,153–157. [CrossRef]

Baktemur, G., Kara, E., Yarar, M., Yılmaz, N., Ağçam, E., Akyıldız, A., Taşkın, H. (2022). Yield, quality and enzyme activity of shiitake mushroom (*Lentinula edodes*) grown on different agricultural wastes. Notulae Botanicae Horti Agrobotanici Cluj- Napoca, 50 (1), 12553. [CrossRef]

Barreto, S.M., Lopez, M.V., Levin, L. (2008). Effect of culture parameters on the production of the edible mushroom *Grifola frondosa* (maitake) in tropical weathers. World Journal of Microbiology and Biotechnology, 24, 1361–1366. [CrossRef]

Baştuğ, G. (2021). Effect of Different Agricultural Wastes on Yield and Quality in Cultivation of *Pleurotus eryngii*. Msc Thesis, Cukurova University, Faculty of Agriculture, Adana, Türkiye, 69 p.

Chang, S.T., Miles P.G. (2004). Mushrooms. Cultivation, nutritional value, medicinal effect, and environmental impact. CRC Press, Boca Raton. [CrossRef]

Eren, E., Pekşen, A. (2016). Status and Future Outlook of Cultivated Mushroom Sector in Turkey. Turkish Journal of Agriculture-Food Science And Technology, 4(3), 189–196. [CrossRef]

Erkel, E.I. (2009). The effect of different substrate mediums on yield of *Ganoderma lucidum* (Fr.) Karst. Journal of Food Agriculture and Environment, 77(3), 841–844. [Google Scholar]

Gizaw, B. (2015). Cultivation and yield performance of *Pholiota nameko* on different agro industrial wastes. Academia Journal of Food Research, 3(3), 032–042. [Google Scholar]

Hal, Y.B., Yarar, M., Kara, E., Baktemur, G., Taşkın, H. (2021). Farklı Tarımsal Atıkların *Ganoderma lucidum* Yetiştiriciliğinde Verim ve Kalite Üzerine Etkisi. Çukurova Tarım ve Gıda Bilimleri Dergisi, 36(2), 275–288. (in Turkish) [Google Scholar]

Kacar, B. (1972). Bitki ve Toprağın Kimyasal Analizleri, II. Bitki Analizleri. Ankara Üniversitesi Ziraat Fakültesi Yayınları, No: 453, Ankara. (in Turkish)

Kara, E., Baktemur, Yarar, M., Taşkın, H. (2021). Effect of different growing substrates on yield and quality in cultivation of maitake (*Grifola frondosa*) mushroom. Akademik Ziraat Dergisi, 10(2), 201–218. [CrossRef]

Kırbağ, S., Korkmaz, V. (2013). The Effect of Cellulosic Wastes on the Growth and Yield Performance of *Pleurotus spp.* Artvin Coruh University Journal of Forestry Faculty, 14(2), 239–244. [Google Scholar]

Kurt, Ş. (2008). The Possibility of Using Different Agricultural Wastes in Oyster Mushroom (*Pleurotus ostreatus*, *Pleurotus sajor-caju*) Cultivation. PhD Thesis, Cukurova Üniversity, Adana, Türkiye, 212 p.

Küçükomuzlu, B., Pekşen, A. (2005). Effects of Substrate Weights on Yield and Quality of *Pleurotus* Mushroom Species. Journal of Faculty of Agriculture OMU, 20(3), 64–71.

Li, H., Zhang, M., Ma, G. (2010). Hypolipidemic effect of the polysaccharide from *Pholiota nameko*. Nutrition, 26(5), 556–562. [CrossRef]

Matilla, P., Salo-Väänänen, P., Könkö, K., Aro, H., and Jalava, T. (2002). Basic Composition and Amino Acid Contents of Mushrooms Cultivated in Finland. J. Agric. Food Chem., 50(22): 6419–6422. [CrossRef]

Meng, L., Fu, Y., Li, D., Sun, X., Chen, Y., Li, X., Shuai X, Xiao L, Li C, Song B, Li, Y. (2019). Effects of corn stalk cultivation substrate on the growth of the slippery mushroom (*Pholiota microspora*). RSC Advances, 9(10): 5347–5353. [CrossRef] doi: 10.1039/c8ra10627d

Morais, M.H., Ramos, A.C., Matos, N., Oliveira, E.J.S. (2000). Note. Production of shiitake mushroom (*Lentinus edodes*) on lignocellulosic residues. Food Science And Technology International, 6(2): 123–128. [CrossRef]

Özçelik, E., Pekşen, A. (2006). The effects of different substrates prepared by hazelnut husk on yield and some mushroom properties in *Lentinus edodes*. Journal of Faculty of Agriculture OMU, 21(1), 65–70.

Ranjbar, M.E., Olfati, J.A., Amani, M. (2017). Influence of enriched soaking water on shiitake (*Lentinus edodes* (Berk.) Singer) mushroom yield and properties. Acta Agriculturae Slovenica, 109(3), 555–560. [Google Scholar]

Rong, C.B., Song, S., Niu, Y.R., Xu, F., Liu, Y., Zhao, S., Wang, S.X. (2016). Selection of a highly productive strain of *Pholiota adiposa*. Mycosphere, 7(2), 226–235.

Royse, D.J. (1985). Effect of spawn run time and substrate nutrition on yield and size of the shiitake mushroom. Mycologia, 77 (5), 756–762. [CrossRef]

Sánchez, C. (2004). Modern aspects of mushroom culture technology. Applied Microbiology and Biotechnology, 64(6), 756–62. [CrossRef]

Sözbir, G.D. (2014). The effects of different additive substance on yield, lentinan and chemical composition of *Lentinus edodes* (shiitake). PhD Thesis, Kahramanmaraş Sütçü İmam University, Kahramanmaraş, Türkiye, 175 p.

Sun, P.J., Yu, J.J. (1989). The cultivation of *Pleurotus* mushrooms on unsterilized substrates in the feeld. Mushroom Science. Part II. Proceedings of the twelfth international congress on the science and cultivation of edible fungi. September 1987, Brauncshweig, Germany. International Society for Mushroom Science, 219–228.

Taşkın, H., Büyükalaca, S. (2017). Minimally Processed Mushrooms. In: Minimally Processed Refrigerated Fruits and

Vegetables (Yildiz, F., Wiley, R., editors). Food Engineering Series. Springer, Boston, MA. [Google Scholar]

TÜİK, (2021). Turkish Statistical Instiyute. [URL]

Veena, S.S., Pandey, M. (2011) Paddy straw as a substrate for the cultivation of Lingzhi or Reishi medicinal mushroom, *Ganoderma lucidum* (W.Curt.:Fr.) P. Karst. in India. International Journal of Medicinal Mushrooms, 13(4):397–400. [Google Scholar]

Yakupoğlu, G., Pekşen, A. (2011). Influence of Particle Size and Different Substrates Containing Tea Waste on Yield and Some Morphological Characters of *Ganoderma lucidum* Mushroom. Ekoloji, 20(78), 41–47. [Google Scholar]

Yen, F. (2008) Comparision of mycelia growth, yield and some quality properties of different *Ganoderma lucisum* sp. Strain grown in liquid and solid growth medium. Msc Thesis, Çukurova University, Adana, Türkiye, 51 p.

Zadrazil, F. (1978). Cultivation of Pleurotus. In: The biology and cultivation of edible mushrooms (Chang, S.T., Hayes, W.A., editors), Academic Press, New York, pp. 521–557.

Zhu, G., Hayashi, M., Shimomura, N., Yamaguchi, T., Aimi, T. (2016). Expression of αglucosidase duringmorphological differentiation in the basidiomycetous fungus *Pholiota microspora*. Journal of Basic Microbiology, 56, 1036–1045. [CrossRef]

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 545-556
DOI: https://doi.org/10.31015/jaefs.2022.4.7

# Impact of COVID-19 on the productivity of ornamental fish farmers: A case of ornamental fish farms in Colombo district in Sri Lanka

**W.A.R.N. Weerasinghe**[1] , **S.H.P. Malkanthi**[1]

[1] Department of Agribusiness Management, Faculty of Agricultural Sciences, Sabaragamuwa University of Sri Lanka, 70140, Sri Lanka

**Citation:** Weerasinghe, W.A.R.N., Malkanthi, S.H.P. (2022). Impact of COVID-19 on the productivity of ornamental fish farmers: A case of ornamental fish farms in Colombo district in Sri Lanka. International Journal of Agriculture, Environment and Food Sciences, 6 (4), 545-556.

**Received:** 07 September 2022
**Revised:** 25 September 2022
**Accepted:** 20 October 2022
**Published Online:** 12 November 2022

**Correspondence:** W.A.R.N.Weerasinghe
**E-mail:** ruwaniweerasinghe1994@gmail.com



## Abstract

Ornamental fish production in Sri Lanka has become a valuable foreign exchange earner during recent past years. However, COVID-19 pandemic has been explored worldwide and it has affected the world trade and economy from different perspectives. This study was conducted to explore the impact of the COVID-19 pandemic on ornamental fish production. , Moreover, study on present status of ornamental fish farmers' major constraints and suggestions to overcome those constraints of ornamental fish farmers were also conducted. In Colombo district, four important Divisional Secretariat (DS) divisions were purposely selected for the study, as they are the main ornamental fish production areas of the district. The data was collected from a randomly selected 60 farmers using a field survey from July to October 2020. Descriptive statistics, two sample Wilcoxon signed-rank test and chi-square test were used to analyses the data. Results revealed that, there are both negative and positive impacts on ornamental fish production due to the COVID-19 pandemic. Ornamental fish farmers have to face mainly production and marketing problems. Some of them have engaged in coping strategies and resilience methods during COVID-19 period. Coping strategies have shown a significant association with the income and the production capacity of the farms. However, the majority of ornamental fish farmers haven't engaged in resilience methods during the COVID-19 pandemic. Other than the COVID-19 pandemic, the major constraints of ornamental fish production were lack of market information about the changes in the overseas markets and demand, the low price given for the product and lack of communication between farmers and exporters. Ideal "ORNAFISBIZ" model is suggested for overcome those constraints in order to protect the ornamental fish farmers in this area.

**Keywords:** Colombo district, COVID-19 pandemic, Negative impacts, Ornamental fish, Present status

## INTRODUCTION

Ornamental fish production is a vital part of the aquaculture industry in many countries. Ornamental fish are the most popular pets in the world, and ornamental fish keeping is the second most popular hobby after the photography because of their amazing shapes, dazzling colors, and behavior. The decorative fish is known as the "Living Jewels" fish. Ornamental fish industry in Sri Lanka has a lot of promise, thanks to the country's vast geography, diverse species, and substantial research and development efforts previously made by the relevant

organizations. Due to the great economic opportunities and prospects, it is quickly gaining attraction. Ornamental fish farming and their propagation have an attractive activity for many people in the world, which provides not only aesthetic beauty and pleasure, but also financial benefits. As a result, ornamental fish industry has become a multibillion-dollar industry in the world. Initially, ornamental fish keeping was mostly a hobby practiced in rich countries. But it has recently gained popularity in developing countries as well.

Sri Lanka has a tropical climate country in the world. As a result, the aquarium sector is well-suited to various parts of the country (Perera, 2009). Freshwater, marine, and brackish water species are the main parts of Sri Lanka's ornamental fish industry. About 50 years ago, a few entrepreneurs pioneered ornamental fish farming. Since then, it has grown into a booming sector with an export market, providing profit and employment to a large number of people. As a non-food fishery industry, the aquaculture sector, ornamental fish breeding, culture, and trade offer fantastic job and income opportunities. Furthermore, it is both environmentally and socially acceptable industry. (Wijesekara & Yakupitiyage, 2001).

The ornamental fish production in Sri Lanka has originated with a household-based small-scale industry which has a long history. In 1930s, there were several small-scale importers, breeders, and hobbyists in the country. Commercial aquariums have been established at first in 1952 in Colombo. It has now developed into a thriving industry with an export market, affording profit and employment opportunities to many people (Kuruppu, 1998). The large-scale growers have started to move in to rural areas of the dry zone of the country due to overcrowding, high labor costs, and lack of land for fish outgrowing facilities in urban areas. Currently, the ornamental fish outgrowing systems have expanded to the Northcentral, North-western, and Central provinces of the country (Weerakoon, 1997). Ornamental fisheries in Sri Lanka have a good potential due to an enormous geographical conditions, extensive species diversity, and intensive research and development efforts that are already put in by the associated institutions.

The aquarium fish sector in Sri Lanka has become a valuable foreign exchange earner during recent years (Heenatigala, 2007). Ornamental fish export contribution of Sri Lanka was 2% for the world market (SLEDB, 2019). Foreign exchange earning value of ornamental fish in Sri Lanka was grown noticeably since 2018 (Table 1).

**Table1.** Foreign exchange earning values of ornamental fish in (US$Mn)

| Year | Value(US$Mn) |
|---|---|
| 2016 | 13 |
| 2017 | 15 |
| 2018 | 16 |
| 2019 | 16 |

Source: Sri Lanka Export Development Board, 2019

## Production process of ornamental fish in Sri Lanka

Wijesekara & Yakupitiyage, (2001) have mentioned that, the ornamental fish production sector comprises of different categories as; fish breeders, fish growers, out growers, middlemen, collectors and exporters.

However, in Sri Lankan context, it is difficult to find out fish breeders and growers separately, because there are performing both activities together in their farms. Therefore, generally both of two categories are known as ornamental fish farmers. They are vital part of the ornamental fish industry value chain because of supplying fish either direct or indirect to the market. In Sri Lanka, in the year 2020, the National Aquaculture Development Authority (NAQDA) of Sri Lanka has categorized the ornamental fish farmers based on their production capacity per month. Those categories are;

- III group -small scale farms (Monthly production< 833 fish)
- V group (Monthly production 833-8333 fish)
- IX group (Monthly production 8333-83333)
- VI group (Monthly production > 83333)

Production capacity was estimated by considering the number and sizes of the tanks and also mud ponds availability in the farm. There are two major types of fish cultural systems in ornamental fish farms. They are large outdoor mud ponds and indoor/outdoor cement tanks and also use the glass tanks for the breeding purpose. The method of culture is depend on the variety of fish. Usually varieties such as Angel fish, Carp and Gold fish are raised in mud ponds and Guppies, Mollies, Swordtails and platys in cement tanks (Agri Farming, 2020).

When consider the Corona Virus, the first instance of this virus was reported on December 31, 2019, in Wuhan City, Hubei Province, China. It has linked to Wuhan's Huanan seafood wholesale market. On March 11, 2020, the World Health Organization (WHO) announced the outbreak COVID-19 (Demirci et al., 2020). At present, the virus has spread to almost all countries, leading to millions of cases and thousands of deaths. Most of the countries have implemented social distancing measures and more stringent lockdowns, in order to slow the spread of the virus (Bennett,et al., 2020). Senten,et al (2020) has reported that the corona virus (COVID-19 pandemic) has disrupted the lives and livelihoods of everyone on our planet.

Coping and adaptive measures are represented early

responses during the first five months of the COVID-19 pandemic. While short-term coping will remain important as the pandemic spreads and possibly re-emerges in countries, actors, and institutions relevant sector can carry adaptive responses forward and engage in a process of learning and building strength to prevent future shocks (Love, et al., 2020).Coping strategies mean that the ability to build the capacity for learning and adaptation. Adaptive management is a method of managing complex socio-ecological systems based on incremental, iterative, experiential learning and decision making, backed up by active monitoring of decision outcomes and feedback from their consequences (Milestad et al., 2008).According to Mensah and Merkuryev (2014), "resilience" is defined as a substance's ability to return to its former state after being deformed. Following COVID-19, efforts to promote resilience should evaluate resilience against what, for whom, and for what purpose. And also be aware of the potential for these decisions to have a cascading effect (Love, et al., 2020).

As the pandemic shifts and possibly re-emerges in countries, there is a continuing need for coping responses to maintain the sector's core functions and protect vulnerable populations working in or dependent. And also coping responses contribute the adapting the difficult situations. A key transition point for actors will be knowing when to shift from short-term coping strategies to the development and implementation of longer-term adaptation strategies and resilience-building .it is necessary to prevent future tremors and respond to ongoing stressors. These shifts will be staggered in time as the pandemic progresses through countries and regions of the world. An additional consideration is how specialized adaptations should be, because increasing resilience to future pandemics may reduce general resilience to an unknown.

According to that, the COVID-19 pandemic has influenced by different perspectives on the different kind of sectors because their impacts are varied. When ornamental fish production is concerned, it is not the same as other sectors, because it is dealing with live beings. Therefore, the overall objective of this research was to study the impact of COVID-19 pandemic on ornamental fish production from the farmers' viewpoint. Moreover, some specific objectives of the study were to find the present situation of ornamental fish production in Colombo district, including coping strategies and resilience methods that farmers have being using for adapting pandemic situation, and also to highlight the farmers' constraints during the COVID-19 pandemic and suggest the solutions for those main constraints of the ornamental fish farmers during the COVID -19 pandemic period.

## MATERIALS AND METHODS

Colombo district was purposely selected for the study, due to most of the ornamental fish farms are there. Main ornamental fish farming activities such as breeding, grow-out, and export activities are conducting around the Colombo district (Wijesekara & Yakupitiyage, 2001). Then, four DS divisions were selected based on the records of the National Aquaculture Development Authority (NAQDA),that were having higher numbers of ornamental fish farmers. They were Padukka, Seethawaka, Kesbewa, and Kaduwela DS divisions. As there were only 60 ornamental fish farmers in these four DS divisions, data was collected from all of them. Primary data were obtained using a researcher-administered questionnaire survey from July to October 2020.Closed-ended and open-ended questions were included in the questionnaire and it was pre-tested before using.

The descriptive statistics were used to identify the socio-economic factors of ornamental fish farmers and a two-sample Wilcoxon signed-rank test was used the examined the impact differences before and during the COVID-19 pandemic. Also, the chi-square test was used to identify the association between socio-economic characteristics and coping strategies.


**Figure 1.** Study area of the research

In this study, ten hypotheses were tested as five hypotheses related to impacts of COVID-19 and the other five hypotheses were related to the association between socio-economic characteristics and coping strategies during COVID-19 pandemic.

### Research hypothesis

There was five sets of hypotheses to assess the impact differences before and during COVID-19 pandemic

$H_{01}$ - There is no significant difference between production capacity of farmers before and during COVID-19 pandemic.

$H_{02}$ - There is no significant difference between selling amount of farmers before and during COVID-19 pandemic.

$H_{03}$ - There is no significant difference between cost of production of farmers before and during COVID-19 pandemic.

$H_{04}$ - There is no significant difference between number of fish varieties before and during COVID-19 pandemic
$H_{05}$ - There is no significant difference between number of market opportunities of farmers before and during COVID-19 pandemic.
The other five sets of hypotheses were used to analyze the association between socio-economic characteristics and coping strategies during COVID-19 pandemic.
$H_{01}$-There is no association between monthly income from ornamental fish farming and coping strategies
$H_{02}$-There is no association between production capacities of the farms and coping strategies
$H_{03}$-There is no association between training of farmers and coping strategies
$H_{04}$-There is no association between experiences of farmers and coping strategies
$H_{05}$-There is no association between educational levels of farmers and coping strategies
Data Analysis was done by SPSS version 21 and Microsoft Excel Software 2013 package.

## RESULTS AND DISCUSSION

General characteristics of the ornamental fish farms

General characteristic of the ornamental fish farms such as cultural activities, size of the farm, nature of production facility, water source, type of labors use in ornamental fish farming and production capacity on the farm were studied in details. The results are shown in table 2.

As per the results in table 2, majority of the cultural activities in the farms were breeding and rearing activities. Wijesekara & Yakupitiyage, (2001) also indicated similar results in their study on "Ornamental fish industry in Sri Lanka: Present status and constraints", the dominant categories were breeding and rearing. The reason behind this result is that, the farmers can obtain financial benefits when conducting both activities in the farm. But it can be varied based on the varieties of ornamental fish. Furthermore, results revealed that, the majority of farm size was less than 20 perches and also a considerable amount of farms have belonged to 20-119 perches category. A few farms have more than 320 perches (2 acres) of land area. These large size farms were mainly situated away from the urban areas. But most of the farms situated close to urban areas are small size farms, due to limited land sizes in urban areas.

While most of farms (30%) have both cement tanks and glass tanks as their production facilities, 27% of farms having only cement tanks. For the rest of the farms (18%) have used all kinds of production facilities. However, Wijesekara & Yakupitiyage, (2001) mentioned that, main farms have only cement tanks.

But based on this result compare to past , more glass tanks are using at present, In the study on "farming ornamental fish" Wills in 2020 has mentioned that, traditionally, most of the Asian production of ornamental fish has been undertaken extensively, using static outdoor ponds or tank systems. However, it varies based on the fish varieties, farmers' capabilities, and size of the farms.

**Table 2.** General characteristics of ornamental fish farms (n=60)

| General characteristics | | Percentage (%) |
|---|---|---|
| Cultural activities on the farms | Breeding | 06.7 |
| | Rearing | 08.3 |
| | Out-growing | 03.3 |
| | Breeding and Rearing | 78.3 |
| Size of the ornamental fish farms | < 20 perches | 40.0 |
| | 20-119 perches | 28.3 |
| | 120-219 perches | 16.7 |
| | 220-319 perches | 06.7 |
| | >320 perches | 08.3 |
| Nature of production facility on ornamental fish farms | Cement tank | 27.0 |
| | Mud ponds | 03.0 |
| | Glass tanks | 05.0 |
| | Cement tanks and Mud ponds | 15.0 |
| | Cement tanks and Glass tanks | 30.0 |
| | Mud ponds and Glass tanks | 02.0 |
| | All | 18.0 |
| Water sources on ornamental fish farms | Well | 43.0 |
| | Tap water | 27.0 |
| | River water | 24.0 |
| | Other | 06.0 |
| Type of labor of farms | Family labor | 65.0 |
| | Hired | 32.0 |
| | Both | 03.0 |
| Production capacity on the farm | <833 | 06.6 |
| | 833-8333 | 46.7 |
| | 8333-83333 | 30.0 |
| | >83333 | 10.0 |

When consider the water sources, most of the ornamental fish farms (43%) used wells as the water source. While

27% of farms used tap water, 6% of farms used other water sources such as canals under the natural water channels. The reason behind this result is that, water quality (water PH, Temperature, dissolved gases (Oxygen and Carbon-dioxide), salinity, etc) is a vital factor for production of ornamental fish. It was also mentioned by Usha, Sharath, & Prashant, (2013) on their study of "Study on ornamental fish diversity and water quality of Adda Hole stream, Kabbinale forest range, Western Ghats". Also, most of the farmers have mentioned that, when use tap water as the water source, it is badly affected for the fish production. There were some water quality problems such as chlorine content, etc. But some farmers used tap water under special management practices. 24% of the farms used river water as the water source, especially for the mud ponds.

With regard to type of labour, 65% of the farms in this area used family laborers. Also, 32% of farms used hired laborers and only 3% of farms use both labour sources. Hired labour is very costly and it increases the cost of production. Production capacity is determined based on the NAQDA production capacity categorization procedure. According to that, majority (46.7%) of the farms belonged to the 833-8333 per month production category or V group. While 30% of the farms were belonging to the 8333-83333 category or IX group), 6.6% of farms belonged to less than 833 production capacity per month. However, there was not the high production capacity as these farmers didn't have facilities to reach to that level. .

## Demographic characteristics of the farmers

Demographic characteristics of the farmers were studies and the results are presented in table 3.

As shown in Table 2, out of the respondents, the majority of the ornamental fish farmers were male and a minimum of them represented female. In Sri Lanka, there were more men involved in ornamental fish production. But a small number of females were also engaged in farming because they are looking forward to an additional income generation path at the household level. The age distribution of the sample highlighted that majority of the ornamental fish farmers belonged to the 28-47 years category. The second largest group accounted for the 48-67 age category in the study area and there were not a considerable amount of farmers under the less than 28 age category.

The result revealed that most of the younger generation was not engaged in ornamental fish production. Dominantly mid-age category people had involved for farming and they have more experience regarding this field. According to the educational background of the sample, the majority (53%) of the respondents have studied up to the advanced level (A/L) and around 22 % had studied up to the ordinary level (O/L).

**Table 3.** Demographic characteristics of the ornamental farmers (n=60)

| Demographic characteristics | | Percentage (%) |
|---|---|---|
| Gender | Male | 81.7 |
| | Female | 18.3 |
| Age | <28 years | 06.7 |
| | 28-47 years | 28.0 |
| | 48-67 years | 21.0 |
| | >68 years | 07.0 |
| Education level | No formal education | 20.0 |
| | Below grade 5 | 03.0 |
| | Up to O/L | 22.0 |
| | Up to A/L | 53.0 |
| | Degree and above | 20.0 |
| Income of ornamental fish farmers (LKR) | <25000 | 14.0 |
| | 25000-50000 | 14.0 |
| | 50000-75000 | 14.0 |
| | 75000-100000 | 18.0 |
| | >100000 | 40.0 |
| Experience of ornamental fish farmers | Less than 1 year | 05.0 |
| | 1-5 years | 33.3 |
| | 5-10 years | 23.3 |
| | More than 10 years | 38.3 |
| Training on ornamental fish farmers | Not training | 49.0 |
| | NARA | 08.0 |
| | NAQDA | 25.0 |
| | Other institute | 18.0 |
| Farming status on ornamental fish farmers | Full time | 60.0 |
| | Part time | 40.0 |

Only 2% of the farmers were degrees and above. 3% of farmers didn't have formal education. Wijesekara & Yakupitiyage, (2001) also indicated similar results in their study "A study of the constraints affecting ornamental fish production in Sri Lanka". Most of the ornamental fish farmers belonged to the more than 100000 LKR category and the second majority of respondents were in the 75000-100000LKR category. 14% of farmers belonged to the less than 25000LKR category. The result revealed that ornamental fish farming is a productive field for in-

come generation if it is conducting the correct manner. Farmers' income varies according to their production of fish varieties and their target market. While the majority of farmers had more than 10 years of experience in the field, there was a noticeable amount of farmers having 1 to 5 years of experience. Only a few farmers were less than 1-year of experience and they were newcomers to the field. According to the result, a considerable amount of farmers had functioned on their farms for more than 10 years and now they have in a stable position. While 49% of the ornamental fish farmers didn't have formal training on ornamental fish, 51 % had undergone training at different institutes including the National Aquatic Resources Research and Development Agency (NARA), National Aquaculture Development Authority (NAQDA), and other institutes. Of these, only 25% of the studied population had been trained at Rambadagalla's three days training program which is conducted by National Aquaculture Development Authority (NAQDA). Wijesekara & Yakupitiyage, (2001) mentioned that the majority of farmers didn't have formal training on ornamental fish production but in the present, it varied because most of the farmers have interested in the training programs to develop their knowledge and skills for conducting farm activities in the right manner.

The majority of ornamental fish farmers conducted farms full-time. Less amount of farmers conducted their farms part-time. The majority of farmers have identified that ornamental fish farming is the best path for generating income therefore most of the farmers have engaged to work full time with ornamental fish farming activities.

## Positive and Negative impact during COVID-19 pandemic

Positive and negative impacts were analyzed from the identification of the difference between selling amount, production capacity, cost of production, number of fish varieties, and number of market opportunities of ornamental fish farmers before and during the COVID-19 pandemic. The data was not normal distribution which was named as non-parametric data. Therefore two-sample Wilcoxon signed-rank test was used for comparison of those factors' median values before and during the COVID-19 pandemic.

The selling amount was measured by the number of fish that they were selling per month before and during the COVID-19 period. Results indicated (Table 3) that the sum of rank (1501) was a high value regarding the positive rank. According to that during the COVID-19 period selling amount had increased more than before COVID-19. And also test significant value was 0.000 based on the negative rank. Therefore null hypothesis was rejected so that there was a significant difference between before and during the COVID-19 period. There was a noticeably high sales amount when compared with before COVID-19 sales amount per month. According to that, there was good market demand for ornamental fish during the COVID-19 period than before the COVID-19 pandemic. But the main problem of farmers was that couldn't have the ability to fulfill the market requirement because of their low production capacity.

The cost of production per month was measured by using the following equation (Gray, 2001).

Cost of production= fixed cost + variable cost

Under the fixed cost, the cost is calculated basically brood stocks cost, labor cost, and water and electricity cost. The variable cost calculated basically feed cost, Packaging & delivery, medication, traveling overseas, and filter repairs cost. Results revealed that positive rank value was the high sum of rank value; during the COVID-19 period, the cost of production increased more than the earlier cost of production. The significant value was 0.000. It was less than 0.05 value according to the median value was a significant difference. Hence during the COVID-19 period, the cost of production was a significant difference.

Production capacity was measured by the number of fish rearing before and during the COVID-19 pandemic. Negative ranks represented the high sum of rank value (950) which means was during COVID-19 production capacity less than before COVID-19 production capacity per month. Table 3 shows the variation of the positive and negative rank and significant value based on the positive rank. The significant value was 0.000 which was less than the 0.05 value. Therefore median value was significantly different. According to that production capacity was significantly different before and during the COVID-19 period. The reason behind the result, most of the farmers have suspended breeding activities because of feed problems and also a considerable amount of fish died because of disease infection. Disease infections have been noticeably high during the COVID-19 pandemic situation because some farmers have functioned breeding process continuously then they had kept the fish in the tank more than the recommended amount of fish. Hence disease infection and death of fish problems had increased during the pandemic situations.

## Current ornamental fish production status

There weren't changes in fish varieties produced by the farmers before and during the COVID-19 period. The negative rank sum of rank value and the positive rank sum of rank value wasn't high differences between them because in both situations farmers' production fish varieties were the same and also significant value was greater than 0.05 values in order to that the null hypothesis was accepted (table 03). Hence median value was not significantly different before and during the COVID-19 period.

**Table 4.** Positive and negative impact on ornamental fish farmers

| Factors | | | Sum of Rank | Asymp. Sig. (2-tailed) |
|---|---|---|---|---|
| Selling amount of ornamental fish before and during COVID-19 pandemic | Negative Ranks[a] | 269.0 | a. During COVID-19 Selling amount per month < Before COVID-19 Selling amount per month | 0.000 |
| | Positive Ranks[b] | 1501.0 | b. During COVID-19 Selling amount per month > Before COVID-19 Selling amount per month | |
| Production capacity of ornamental fish before and during COVID-19 pandemic | Negative Ranks[a] | 950.0 | a. During COVID-19 Production capacity per month < Before COVID-19 Production capacity per month | 0.000 |
| | Positive Ranks[b] | 131.0 | b. During COVID-19 Production capacity per month > Before COVID-19 Production capacity per month | |
| Cost of production before and during COVID-19 pandemic | Negative Ranks[a] | 147.50 | a. During COVID-19 cost of production per month < Before COVID-19 cost of production pre month | 0.000 |
| | Positive Ranks[b] | 842.50 | b. During COVID-19 cost of production per month > Before COVID-19 cost of production pre month | |
| Number of fish varieties before and during COVID-19 pandemic | Negative Ranks[a] | 80.0 | a. During COVID-19 number of fish varieties < before COVID-19 number of fish varieties | 0.867 |
| | Positive Ranks[b] | 73.0 | b. During COVID-19 number of fish varieties > Before COVID-19 number of fish varieties | |
| Number of Market opportunities for ornamental fish before and during COVID-19 pandemic | Negative Ranks[a] | 24.0 | a. During COVID-19 Market opportunity < Before COVID-19 Market opportunity | 0.171 |
| | Positive Ranks[b] | 12.0 | b. During COVID-19 Market opportunity > Before COVID-19 Market opportunity | |

**Table 5.** Current ornamental fish production status

| Fish variety | | Percentage (%) |
|---|---|---|
| Angle | *Pterephylum scalare* | 15.91 |
| Swordtail | *Poecilia reticulate* | 10.61 |
| Carp | *Cyprinus carpio* | 10.61 |
| Guppy | *Poecilia reticulate* | 9.85 |
| Fighter | *Betta splendens* | 9.09 |
| Zibra | *Branch ydanio rerio* | 6.82 |
| Discuss | *Svmphpsodon aequjfasciatus* | 5.3 |
| Plety | *Xiphophorus maculates* | 5.3 |
| Goldfish | *Carassius auratus* | 5.3 |
| Molly | *Poecilia sphenops* | 5.3 |
| Tetra | *Paracheirodon innesi* | 4.55 |
| Oscar | *Astronotus ocellatus* | 4.55 |
| Barbe | *Puntigrus tetrazona* | 2.27 |
| Gourami | *Trichogaster lalius* | 2.27 |
| Flowerhorne | *Paraneetroplus synspilus* | 0.76 |

The ornamental fish farmers didn't have to engage in changing their production of fish varieties during the COVID-19 period because they didn't have market problems for selling their products. The current status of production of ornamental fish varieties in the study area was shown in table 04. According to the results, in the study area Angle varieties were dominantly produced on the farms. And also sword-tail, Carp, Guppy, and Fighters varieties have been produced in considerable amounts. Those five varieties have high production capacity within the studied area. Malawi, Catfish, and Flower hone varieties have a low production capacity in the studied area.

## Current market opportunities of ornamental fish farmers

The market opportunity was measured by the number of the target market of farmers that was going to sell their fish. Table 3 represented the sum of rank values before and during the COVID-19 period. The result revealed that the sum of rank variance was not considerably changed during the COVID-19 period. And also test statistic's significant value was greater than the 0.05 value. Hence farmers' market opportunities were not significantly different before and during the COVID-19 period. Based on the study, farmers had sold their production for supply exporters, local market, both local market and direct exporters, both local market and supply exporters at the present. In fact, Wijesekara & Yakupitiyage, (2001) mentioned that most of the farmers sold their products to the supply exporters in his study. Based on that majority of farmers (35%) had sold products to the supply exporters and 31.7% of farmers sold for the local market (table 5)

**Table 6.** Current market opportunities of ornamental fish farmers

| Market opportunity | Percentage(%) |
|---|---|
| Local market | 31.7 |
| Supply Exporters | 35 |
| Local and Supply exporters | 21.7 |
| Local and Direct export | 6.7 |

The reason behind the results, the export market has a considerably high demand than the local market. Therefore most of the farmers have been engaged in selling their products to the supply exporters, and also supply exporters have paid high prices than the local market. But the supply exporters were mainly concerned about the fish quality when purchasing the farmers' products. Hence most of the time farmers' products have been rejected due to quality problems. According to that, the COVID-19 pandemic didn't have an effect on changing the market opportunity of the farmers.

## Problems of ornamental fish farmers during the COVID-19 pandemic

Problems of ornamental fish farmers were categorized into three parts as production problems, market problems, and other problems that they had to face during the COVID-19 pandemic and presented in three sections in below.

The result revealed (Table 6) that 87.7% of farmers were faced with the problem of receiving fish feed and additives. As most of the fish feed imports to Sri Lanka, farmers have difficulty accessing the feed during COVID- 19 period due to COVID-19 adversely affected for the import and export market. Therefore it is directly affected maintaining the fish quality. The second major production problem is the death of fish. The main reason was that most of the farms were situated in a limited area and farm activities function during the lockdown period with the continued breeding process. Hence they had to face space problems on the farm. As a result, they had stocked exceeded fish amount rather than recommended level which adversely affected the fish died. The percentage of other production problems was shown (table 6) based on the priority of the problems. The cleaning problem within the farms was low percentage because most of the farms didn't hire the laborers as they function their work with family members.

Those production problems have varied based on the farm category (table.7). Based on the V group (833-8333) and the IX group (8333-83333) category farms dominantly have to face production problems during the COVID-19 period. As they have maintained their activities within a limited land area, haven't the adequate land area to function for farm activities. Hence they had to face production problems rather than other groups of III group (833 )and VI group (> 83333) of farms.

The III group of farms had a minor influence on the production problems due to their production capacity being low level. Their production problems influence had a minimum level when compared with other groups. VI group category farms also had a minor influence on the production problems because they have sufficient income levels rather to other groups of farms.

**Table 7.** Problems of ornamental fish farmers

| Problems of ornamental fish farmers | | Percentage (%) |
|---|---|---|
| Production problems | Problem of receiving feed and additives | 87.7 |
| | Death of fish | 80.7 |
| | Problem of maintain quality | 78.9 |
| | Difficult to function natural and artificial breeding activity properly | 77.2 |
| | Difficult to find quality breeding fish | 61.4 |
| | Problem of receiving medicine | 35.1 |
| | Disease infection | 31.6 |
| | Cleaning problems in farm | 22.8 |
| Market problems | Problem of transport facility | 98.2 |
| | No stable price | 34.5 |
| | Lack of buyers | 34.5 |
| | Reject the stock due to the weight problem | 32.7 |
| | Lack of collecting by sellers | 27.3 |
| Other problems | Lack of involvement of government and other responsible organization | 96.6 |
| | Lack of loan facilities | 86.2 |
| | Lack of insurance facilities | 72.4 |
| | Labor problem | 25.9 |
| | Difficult to recover the loan | 25.9 |

Within the marketing problems, the majority 98.2% of farmers had problems with transport facilities. During the COVID-19 pandemic, the government had put travel restrictions between the districts. In Sri Lanka police issued the travel pass for the essential services. But ornamental fish farmers couldn't have access to the pass due to the responsible persons had less awareness about the ornamental fish industry. Therefore the majority of

the farmers couldn't obtain the feed and other facilities during the COVD-19 period due to the problem of transport facilities. Table 6 shows, other market problems based on prioritization. The 34.5% percentage of farmers had the problem of stable prices and lack of buyers. 32.7% of farmers had faced the problem of rejecting the stock due to the weight problem. When exporting the products, flight charges are calculated based on the weight of the packing box. Weigh of the ornamental fish packages have low when compared with the other packages' weight. Hence most of the packages were rejected during the COVID-19 period due to the limited flight facilities. Table 7 indicated the farmers' market problems impact based on the production capacity on the farm. According to that V group (833-8333) and the IX group (8333-83333) category farms dominantly faced market problems rather than other farms' categories.

**Table 8.** The problems percentage with the farm groups

| | | <833 | 833-8333 | 8333-83333 | 83333< |
|---|---|---|---|---|---|
| Production problems | Frequency | 4.0 | 25.0 | 18.0 | 6.0 |
| | Percentage (%) | 7.5 | 47.2 | 34.0 | 11.3 |
| Market problems | Frequency | 4.0 | 22.0 | 18.0 | 6.0 |
| | Percentage (%) | 8.0 | 44.0 | 36.0 | 12.0 |

Table 6 indicated under the other problems category that 96.6% of farmers had a problem of lack of involvement of government and other responsible organizations. And also 86.2% of farmers couldn't access loan facilities due to the lack of loan facilities allocating ornamental fish industry. There was a low percentage of the problem of labor and recovery of the loan facilities. The majority of the farms worked with family labor and some farms' laborers stayed in the house. Therefore there was a noticeably low level of labor problems during the COVID-19 period.

## Coping strategies and Resilience methods of ornamental fish farmers during COVID-19 pandemic

Most of the farmers (58.3%) had used special coping strategies during the COVID-19 pandemic for responding to the COVID-19 outbreak. However, 41.7% of farmers were not engaged to use coping strategies during the COVID-19 pandemic. As indicated (Figure 2) 44.4% of farmers had engaged to reduce the frequency of fish feeding due to the feed problems during the COVID-19 pandemic. And also 34.9% of farmers had used the method of suspending the breeding activities due to the problem of receiving feed and the problem of space in the tanks. Based on the results, farmers had used the same feed for each stage, they didn't function sorting process and used alternative feed instead of fish feed during the COVID-19 pandemic situation as the adaptation strategies. SAARC Agriculture Centre (SAC), (2017) mentioned that mainly farmers should be concerned about the feed management process. feed process is differed based on the fish stages such as nursery stage, and growth stage, and also feed should be supplied at the right time and required amount. In fact, the farmers had shifted from the actual management practices to engaging the coping strategies.

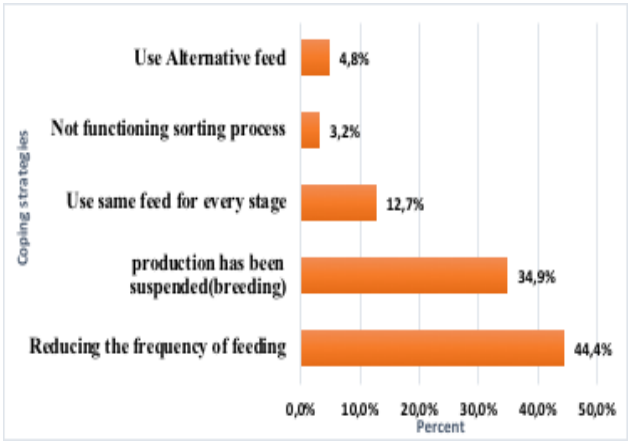



**Figure 2.** Coping strategies of ornamental fish farmers

Within the study area,46.7% of farmers have used resilience methods for getting back to their original state. But 53.3% of farmers didn't use resilience methods. The reason for the result, the market demand for ornamental fish had noticeably increased during the COVID-19 pandemic, therefore, most farmers had the ability to continue their farming activities without using resilient methods. Among the farmers engaging in using resilience methods, 36.4% of farmers have hoped to gain financial support for getting back to the original stage. 29.5% of the farmers wished to engage in the changing of fish varieties based on the market demand because market demand varies according to the fish varieties.

## Analyze the association between socio-economic characteristics and coping strategies

The education level of the farmers, experience of farmers, training on farmers, monthly income of the farmers, and production capacity of the farms was used as the socio-economic characteristics. The relationships were measured using the chi-square test. The coping strategies were used as the dependent variable. According to the results of table 8, there was a significant association between the monthly income of farmers and using coping strategies,$x2$ (4, N=57)=33.03,p=0.000. and also, there was a significant association between the production capacity per month and use of coping strategies,$x2$ (3, N=56)=16.686,p=0.001, Socio-economic characteristics of the education level of farmers, the experience of farmers and training of the farmers were not a significant association with the using of coping strategies because there was significant value was greater than 0.05 value.

Table 08 explained that the association between the coping strategies and production capacity on the farm.

Low production capacity farms (<833) were dominantly engaged in using coping strategies during the COVID-19 period. Large production capacity farms (>83333) were not engaged in using coping strategies. Low production capacity farms had more problems rather than large production capacity farms because of they didn't have sufficient income levels to maintain the farming activities. As a result, they had to use coping strategies for adapting to the pandemic situation. And also more than 100000 LKR income category farms were not engaged in using coping strategies during the COVID-19 period because they had adequate facilities to maintain their farms. But other income category farms had to follow coping strategies for adapting to the critical situation.

**Table 9.** Relationship between coping strategies and socio-economic characteristics

| socio-economic characteristics | df | Asymp. Sig. (2-sided) |
|---|---|---|
| Education level of farmers | 4 | 0.771 |
| Experience of farmers | 3 | 0.201 |
| Training of farmers | 3 | 0.768 |
| Monthly income from ornamental fish production | 4 | 0.000 |
| Production capacity of the farm | 3 | 0.001 |

## The current main constraints of ornamental fish farmers besides the impact of the COVID- 19 pandemic

Ornamental fish farmers already have constraints besides the impact of the COVID-19 pandemic. The current main constraints of ornamental fish farmers besides the COVID- 19 impact were assessed using 10 statements along 5 points Likert scale as Strongly Agree, Agree, Neutral, Disagree, and Strongly Disagree. Based on table 9, there were very high mean values (>4) for five statements. Those high mean values indicated that farmers strongly agree with those five statements namely; Lack of market information about the changes in the overseas demand, Low price given for the product, and lack of communication between farmers.

Farmers have mainly ridden with this constraint besides the COVID-19 pandemic due to the poor information linkage between the farmer and the buyers. Therefore farmers don't have updated knowledge about the market demand of the fish varieties. Hence they produced the same fish varieties without any idea of the market demand then they couldn't sell their products due to the surplus of the market regarding the same fish varieties. As a result, they have engaged to suspend their production process and give up the farming process. This study has suggested the "Ideal "ORNAFISBIZ" Model (figure 3) for avoiding the major constraint of the farmers (Devi,et al., 2017). The ideal model is shown below which is also beneficial for developing the farmers' production. And also the model helps to avoid ornamental fish farmers' barriers as well.

This model is only suggested for the information linkage between the farmers and buyers as a solution for avoiding the major constraints. At the initial stage, farmers' production process should be prepared in a productive way because farmers when linked with the market could supply quality fish for both domestic and export markets. If they couldn't supply quality fish, buyers could be refused the purchasing their products. This model is vital to both markets because they could determine and select the farmers based on their product quality and their supply capacity.

In this model, at the beginning farmers' production category is divided into three parts. There are pre-production, production, and post-production. The pre-production stage should be facilitated mainly by training and knowledge to keep the production process and facilitate the infrastructure and loan facilities to enhance their production capacity. During the production process, supply the diagnostic services, access to modern improved technology facilities, and monitoring the process. At the post-production, stage farmers should be graded based on the fish quality and quantity and issued the certificate to them for further identification. It is a pivot part of the information linkage model because the buyers can easily identify the nature of the farmers' production before purchasing. And also farmers could be selected the buyers based on their production capacity, and quality, and they could be functioned and manage their production process based on the market demand.

The second step was all the farmers' linkage together as the district wise through the district society of ornamental fish farmers. It helps to follow the information toward the farmers because there isn't a high population of ornamental fish farmers therefore should be built up the relationship among them to follow the information. If most of the farmers have engaged to produce the same varieties without interconnection with them, it could be caused to deduct the market demand regarding those varieties.

In the last stage district society of ornamental fish, farmers should be connected with the district collector centers which is the place to coordinate both parties by providing information to both sides. The collector center has linked both parties forwardly and backwardly. This is the

**Table 10:** Constraints of ornamental fish farmers

| Serial No | Statement | Mean | Std. Deviation | Remark |
|---|---|---|---|---|
| 01 | Lack of market information about the changes in the overseas demand | 4.60 | 0.764 | Strongly Agree |
| 02 | Low price given for the product | 4.53 | 0.769 | Strongly Agree |
| 03 | Lack of communication between farmers and buyers | 4.28 | 0.804 | Strongly Agree |
| 04 | Delays and non-payments for products | 2.92 | 0.809 | Disagree |
| 05 | High investment cost | 4.53 | 0.623 | Strongly Agree |
| 06 | Difficulties in entering export market | 2.95 | 1.141 | Disagree |
| 07 | Difficulties in finding markets for products | 3.05 | 2.740 | Agree |
| 08 | Difficulties in maintaining the enterprise in off-season | 3.53 | 0.911 | Agree |
| 09 | High maintain cost | 3.53 | 0.623 | Strongly Agree |
| 10 | Export in fish in same cases for prices | 3.67 | 0.752 | Agree |

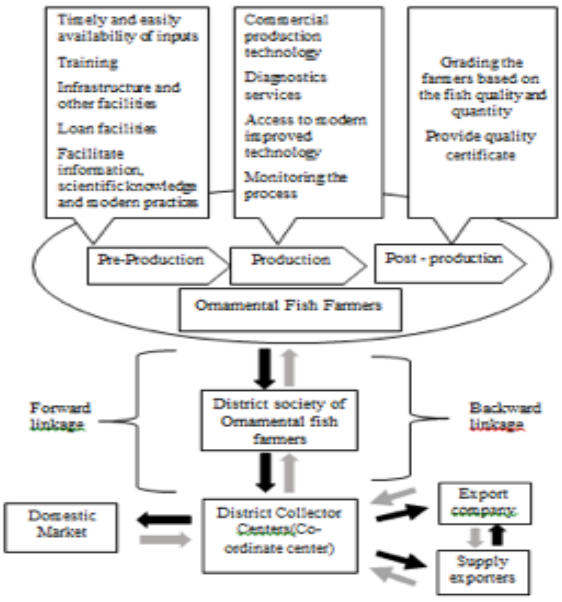



**Figure 3.** Ideal "ORNAFISBIZ" Model for Sri Lanka to information link between the farmers and Exporters

place where is kept all information about farmers and market information. This is the ideal model to avoid the farmers' major constraints.

## CONCLUSION

According to the findings, Most of the ornamental fish farms dominantly conducted breeding and rearing activities together as the cultural activities and the majority of ornamental fish farms belonged to the 8333-83333 per month production category. Male farmers are mainly participating in ornamental fish production and also a higher number of farmers have education levels up to A/L within the study area. The farmers have more than 10 years of experience in the field. So, farmers have a good background on engage in ornamental fish farming. However, most of the farmers didn't have to get any formal training regarding ornamental fish production. Apart from that majority of farmers have conducted their farming activities as a full-time occupation.

The COVID-19 pandemic has rapidly spread around the world. It has caused negative and positive impacts regarding ornamental fish production in the country. As the major positive impact during the COVID-19 period was, the selling amount of ornamental fish had unexpectedly increased but farmers couldn't have the supply market requirements due to the low production capacity. Major negative impacts during the COVID-19 period, farmers' ornamental fish production capacity decreased during the COVID-19 period due to the main production problems. The cost of production for farmers' had increased during the COVID-19 period compared with the before COVID-19 period due to the increase in feed price (mainly Brine shrimp price). Fish verities produced by the Farmers didn't change during the COVID-19 period. Most of the farmers produce the same fish varieties before and during the COVID-19 period. In the study area, most of the farmers had dominantly produced Angle, Swordtail, Carp, Guppy, and Fighters. Farmers' market opportunities hadn't changed during the COVID-19 period because they sold their production same target market during the COVID-19 period. Dominantly farmers sell products to the supply exporters who are the person connected with the direct exporters.

Farmers suffered from production, market, and other problems during the COVID-19 period. Among them, the problem of receiving feed and additives and died of ornamental fish were the main production problems of the farmers. Under the marketing problems were difficulties in transport facilities and unavailability of a stable price, lack of buyers, reject the stock due to the weight problem, and lack of collecting by sellers. Lack of involvement of government and other responsible organizations and lack of loan facilities were the main problems categorized under the other problems of the farmers.

During the COVID-19 period, most of the farmers have engaged in using coping strategies for responding to the outbreak. Mostly they followed reducing the frequency of feeding strategies, suspended breeding activities, using the same feed for every stage, and used alternative feed respectively during the COVID-19 period. Those coping strategies had been influenced by the reduction of farmers' production capacity and quality of the fish.

There was an association between the income level of the farmers and the production capacity of the farms with the use of coping strategies. The education level of the farmers and experience and training of the farmers didn't show an association with the use of coping strategies. While high-income farmers were not engaged in using coping strategies, low-income farmers had been mostly engaged to follow the coping strategies. Also, high production capacity farms were not engaged to use coping strategies. Although most of the farmers are not hoping to use resilience methods for re-building their production process, few farmers had involved to use resilience methods among them dominantly hoping to obtain financial support for enhancing the production capacity.

## COMPLIANCE WITH ETHICAL STANDARDS

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original.and that they have not been published before.

### Ethical approval

Not applicable.

### Funding

This research received no external funding

### Data availability

Not applicable.

### Consent for publication

Not applicable.

### Acknowledgements

We would like to thank to the ornamantal fish farmers who are supported us from prviding the valuable information.

## REFERENCES

Agri Farming. (2020). Retrieved from Fish Farming in Sri Lanka- A full guide. [URL]

Bennett, N. J., Finkbeiner, E. M., Ban, N. C., Belhabib, D., Jupiter, S. D., Kittinger, J. N., Mangubhai, S., Scholtens, J., Gill, D., & Christie, P. (2020). The COVID-19 Pandemic, Small-Scale Fisheries and Coastal Fishing Communities. Coastal Management, 48(4), 336–347. [CrossRef]

Demirci, A., Şimşek, E., Can, M. F., Akar, Ö., & Demirci, S. (2020). Has the pandemic (COVID-19) affected the fishery sector in regional scale? A case study on the fishery sector in Hatay province from Turkey. Marine and Life Sciences, 2(1), 13-17. [URL]

Devi, B., Krishnan, M., Ananthan, P., & Pawar, N. (2017). A producer company - An ideal value chain model for ornamental fish trade. International Journal of Fisheries and Aquatic Studies, 5(6), 115-120. [Google Scholar]

Gray, S.-A. (2001). An economic & production assessment model for ornamental fish production in jamaica. [URL]

Kaiser, H., Britz, P., Endemann, F., Haschick, R., Jones, C. L. W., Koranteng, B., Kruger, D. P., Lockyear, J. F., Oellermann, L. K., Olivier, A. P., Rouhani, Q., & Hecht, T. (1997). Development of technology for ornamental fish aquaculture in South Africa. South African Journal of Science, 93(8), 351–354. [Google Scholar]

Love, D.C., Allison, E.H., Asche, F., Belton, B., Cottrell, R.S., Froehlich, H.E., . . . Zhang , W. (2020). Emerging COVID-19 impacts, responses, and lessons for building resilience in the seafood system. [CrossRef]

Mandal, S., Mahapatra, B. K., Tripathi, K., Verma, M. R., Datta, K. K., & Ngachan, S. V. (2007). Agribusiness Opportunities of Ornamental Fisheries in North-Eastern Region of India. Agricultural Economics Research, 20, 471–488.

Milestad , R. (2003). Building Farm Resilience Challenges and Prospects for Organic Farming. [URL]

National Aquaculture Development Authority in Sri Lanka . (2020). [URL]

Perera, P. (2019, May). Export oriented Ornamental Fish Industry in Sri Lanka. [Google Scholar]

SAARC Agriculture Centre (SAC). (2017). Best Management Practices in Aquaculture Capacity Building and Policy Development. (S. S. Giri, Ed.)

Senten, J., Smith, M. A., & Engle, C. R. (2020). Impacts of COVID-19 on U.S. aquaculture, aquaponics, and allied businesses. Journal of the World Aquaculture Society, 51(3), 574–577. [Google Scholar]

Sri Lanka Export Development Board. (2021). Aquarium fish. [URL]

Usha, A., Sharath, Y., & Prashanth, R. (2013). Study of ornamental fish diversity and water quality of Adda Hole stream, Kabbinale forest range, Western Ghats. Advances in Applied Science Research, 4(5), 158-164. [URL]

Wijesekara, R., & Yakupitiyage, A. (2001). Ornamental fish industry in Sri Lanka: present status and future trends. Aquarium Sciences and Conservation. [CrossRef]

Yue, G. H. (2019). The ornamental fish industry in SIngapore. Journal of Fisheries Of China, 43(1). [CrossRef]

Zhang, S., Wang, S., Yuan, L., Liu, X., & Gong, B. (2020). The impact of epidemics on agricultural production and forecast of COVID-19. China Agricultural Economic Review, 202001. [CrossRef]

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 557-567
DOI: https://doi.org/10.31015/jaefs.2022.4.8

# Seroprevalence of *Salmonella spp.* infection in different types of poultry and biosecurity measures associated with Salmonellosis

**Mirza Mienur Meher[1], Md. Arman Sharif[2], Abdullah Al Bayazid[3]**

[1] Bangabandhu Sheikh Mujibur Rahman Agricultural University, Faculty of Veterinary Medicine and Animal Science, Department of Microbiology and Public Health, Gazipur-1706, Bangladesh

[2] Bangabandhu Sheikh Mujibur Rahman Agricultural University, Faculty of Veterinary Medicine and Animal Science, Department of Anatomy and Histology, Gazipur-1706, Bangladesh

[3] Bangabandhu Sheikh Mujibur Rahman Agricultural University, Faculty of Veterinary Medicine and Animal Science, Gazipur-1706, Bangladesh

**Citation:** Meher, M.M., Sharif, M.A., Bayazid, A.A. (2022). Seroprevalence of *Salmonella spp.* Infection in Different Types of Poultry and Biosecurity Measures Associated with Salmonellosis. International Journal of Agriculture, Environment and Food Sciences, 6 (4), 557-567.

**Received:** 07 March 2022
**Revised:** 01 October 2022
**Accepted:** 05 October 2022
**Published Online:** 19 November 2022

**Correspondence:** Mirza Mienur Meher
**E-mail:** mienur@bsmrau.edu.bd

## Abstract

*Salmonella spp.* infection is considered a crucial trouble of the poultry industry in Bangladesh. Hence, this study was aimed to estimate the seroprevalence of *Salmonella spp.* in poultry along with biosecurity practices that are associated with this *Salmonella spp.* infection. The study was conducted during the period from January to September, 2021 in Mymensingh and Gazipur district of Bangladesh. A total of 314 samples were considered to determine the seroprevalence. Seroprevalence was determined by performing the rapid serum plate agglutination test. The result revealed that the overall *Salmonella spp.* seroprevalence was 47.77% in the study area. The higher seroprevalence was in Mymensingh (51.59%) than Gazipur (45.21%) without significant ($p>0.05$) difference. The highest seroprevalence was in broiler (51.33%) where in layer and sonali was 32.67% and 16% respectively. Seroprevalence was significantly ($p<0.05$) higher in summer for layer (56.45%), broiler (60.64%) and sonali (51.22%) than the winter seasons. In layer farms, the flock size of >2000 to <2500 had significantly ($p<0.05$) higher (71.43%) seroprevalence. In broiler farms, 15 to 30 days old birds had significantly ($p<0.01$) higher (77.05%) seroprevalence than other age. Among the different categorical level of biosecurity practices, the poultry farms that used surface water (OR=0.182, 95% CI=0.106-0.314); disinfectant regularly (OR=0.296, 95% CI=0.171-0.511); having density of 8-10 birds/meter2 (OR=0.379, 95% CI=0.219-0.654); cleaned waterer and feeder regularly (OR=0.503, 95% CI=0.294-0.862); and having visitor restriction (OR=0.375, 95% CI=0.219-0.643) showed lower tendency ($p<0.001$) to seroprevalence. In brief, Strict farm hygienic practice and biosecurity measures are significantly linked to decrease the *Salmonella spp.* seroprevalence.

**Keywords:** *Salmonella spp.*, Seroprevalence, Biosecurity, Broiler, Layer, Sonali



## INTRODUCTION

Poultry farming in Bangladesh is a fast-growing sub-sector which takes an important place in socio-profitable development especially pastoral livelihood development by generating employment prospects (Islam & Nishibori, 2009). This subsector plays an important part in narrowing the gap between demand and force of protein of animal origin (Islam & Nishibori, 2009). Particularly, this subsector contributes nearly 40% of the total meat supply and more than 25% of Bangladesh's total human protein demands (Abdullah et al., 2019; Hamid et al.,

2016). However, several factors reduce the growth rate of this sector. Among them, poultry diseases are the major constraints (Karim, 2003). About 30% of poultry birds die annually in Bangladesh due to outbreaks of several infectious diseases. The major infectious and contagious diseases are Avian influenza, Gumboro disease, Newcastle disease, Mycoplasmosis, Colibacillosis, Salmonellosis, and Fowl cholera frequently affecting marketable Poultry farm (Chanie et al., 2009). Among the bacterial diseases *Salmonella spp.* infection is one of the major problems for poultry in Bangladesh, which is considered a crucial trouble of the poultry assiduity (Rahman et al., 2016). In Bangladesh, the circumstance of *Salmonella spp.* infection is about 21-30% in layer and about 15% in broiler which is measured as the loftiest frequency among different types of poultry disease (Rahman et al., 2017), among which a variety of acute and habitual diseases in poultry are included (Laxman Bahadur et al., 2016). Salmonellosis in poultry causes significant profitable loss due to mortality and reduced product (Rahman et al., 2016). Salmonellosis in chickens caused by *Salmonella pullorum* and *Salmonella gallinarum* and is appertained to as pullorum complaint and fowl typhoid, independently. Pullorum disease occurs in chicks during their first many days of life and causes severe enteritis and bacteremia (Rahman et al., 2016). Whereas, fowl typhoid is a disease of mature chicken and causes either acute enteritis with greenish diarrhea or a habitual complaint of the genital tract that reduces egg product (Rahman et al., 2016). Chicks can be infected with *Salmonella spp.* by vertical transmission through infected parents or by horizontal transmission through hatcheries, sexing in defiled hatcheries, cloacal infection, and transportation of outfit and feed (Kabir, 2010). Motile Salmonella spp. (paratyphoid group) infection causes salmonellosis in chickens with zoonotic significance (Kabir, 2010). Basically, *Salmonella spp.* are short bacilli, 0.7-1.5×2.5 µm, Gram-negative, aerobic or facultative anaerobic, positive catalase, negative oxidase; they raise sugars with gas product, produce H2S, are non sporogenic, and are typically motile with peritrichous flagella, except for *Salmonella pullorum* and *Salmonella gallinarum*, which are immotile (Gantois et al., 2009; Rahman et al., 2016). The diseases frequencies in a particular area depends on several factors like geographical condition, immunization status of the ranch, quality and condition of the chicks, bio-security status of farm etc. Biosecurity measure commonly may be the implementation of policies, practices, and essential actions that enhance preparedness and prevent the introduction and rapid spread of diseases within the country and across national borders (Fathelrahman et al., 2020). The increasing preparedness against biosecurity threats has a tendency of reduction to disease outbreaks and also the poultry production systems need an increase drive for improved biosecurity practices (Maduka et al., 2016). In Bangladesh is particularly at risk of transmission of infectious diseases because of its high population density and widespread contact between people and animals. Hence the most important measure for sustainable and profitable product on a poultry point must be to have in place forward defenses similar as a biosecurity Program. Though, the several studies were conducted on seroprevalence of *Salmonella spp.* in poultry in different area of Bangladesh (Hossain et al., 2010; Jalil & Islam, 2012; Sabuj et al., 2019; Sikder et al., 2005). But the effect of different biosecurity practices in preventing the seroprevalence of *Salmonella spp.* in poultry farm has not been studied before. Moreover, update information on the seroprevalence of *Salmonella spp.* in different types of poultry (Layer, Broiler and Sonali) is essential to design a prevention and control strategies. In fact, the hygienic property of the poultry products depends on the management and health status of poultry and predominantly on the conception and the biosecurity grade of the poultry houses. Hence, this study was aimed to estimate the seroprevalence of *Salmonella spp.* infection in commonly farming types of poultry (Layer, Broiler and Sonali) along with biosecurity practices that are associated with this infection in Mymensingh and Gazipur district of Bangladesh.

## MATERIALS AND METHODS

### Study area and period

This study was designed to collect the samples from two districts namely Mymensingh and Gazipur of Bangladesh, lie between the latitudes of 24.30°N to 24.88°N and longitudes of 90°16′4″E to 90.73°E (Figure 1). The software ArcGIS-ArcMap version 10.8 (ESRI, USA) was applied to show the study area. The study was conducted during the period from January to September, 2021.

### Sample Size Estimation

The sample size in the study area was determined by the formula of Daniel (1999).

$$\mathbf{n} = \frac{\boldsymbol{Z}^2\boldsymbol{P}(1-\boldsymbol{P})}{\boldsymbol{d}^2}; \mathbf{n} = \frac{(1.96)^2 \times 0.31(1-0.31)}{(0.05)^2};$$

$$\mathbf{n} = 328.69 \cong 329$$

Where,

n = sample size,

Z = 1.96 (95% confidence level),

P = expected prevalence or proportion (in proportion of one; 31.25%, P = 0.31), and

d = precision (in proportion of one; whereas P=0.31, therefore d = 0.05).

In the previous study, the authors (Mridha et al., 2020) found the 31.25% overall prevalence of *Salmonella spp.* in

Gazipur, Tangail, and Dhaka districts of Bangladesh. So, the expected prevalence was considered as 31.25%. The estimated sample size was 329. A total of 330 samples was collected and finally, 314 samples were considered to test for determining the seroprevalence.

## Sample collection

The blood samples were collected aseptically from wing vein of the selected birds without any anticoagulant. All the selected birds were from small-scale poultry farm, and the farms having less than 2500 birds were considered as small-scale farming. A total of 314 samples were for detection of seroprevalence of *Salmonella spp.* infection. After collection of blood using sterilized syringe and needle, the syringes having the blood sample were placed in a standing position in a cool box and kept for 6 hours to separate the serum. After separation of serum, the serum samples were transferred to 1.5 ml micro centrifuge tubes and stored in refrigerator until perform the Serum Plate Agglutination (SPA) test.

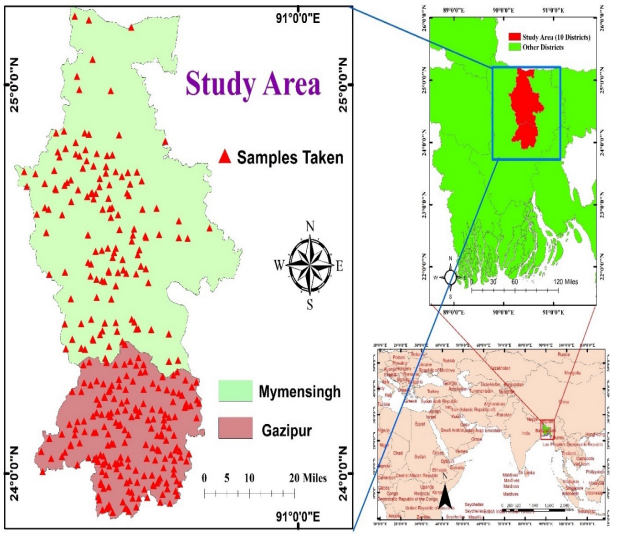



Figure 1. Spatial location of the study area (Mymensingh and Gazipur District) in Bangladesh.

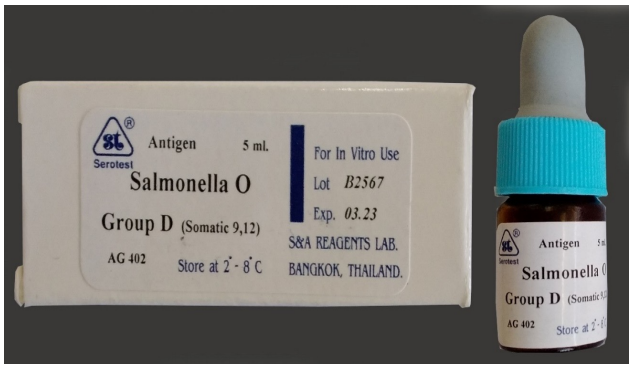



Figure 2. *Salmonella spp.* antigen for serum plate agglutination (SPA) test to detect seroprevalence.

All the blood samples were collected from non-vaccinated birds. At the same time, data on poultry farm were collected from the farmer. The questionnaire to collect the data on biosecurity practices was prepared according to the previous study (Meher et al., 2020).

## *Salmonella spp.* antigen

*Salmonella spp.* antigen (Serotest® SP, S& A Reagent Lab, Thailand) was used for rapid serum plate agglutination (SPA) test to detect antibodies due to infections caused by both standard and variant strains of *Salmonella pullorum* and *Salmonella gallinarum* in the sera samples. The antigen was killed and colored *Salmonella O* group D (Somatic 9, 12) (Figure 2).

## Detection of *Salmonella spp.* infection by serum plate agglutination (SPA) test

The SPA test was performed according to the methods described by Sikde et al. (2005). Briefly, equal amount of antigen and serum (0.02 ml of antigen and 0.02 ml of bird's serum) were positioned on a glass plate side by side with micropipettes. Afterward, antigen and serum sample were mixed methodically by mixing with a small tooth pick. To observe the reaction, the glass plate was brightened from below for avoiding unnecessary heat from the light source. In case of positive reaction, the definite clumps were formed within 2 minutes just after mixing the serum and antigen. The clumps usually began to appear and became condensed at the periphery of the mixture. The absence of agglutination reaction was characterized as negative reaction. Precaution was taken to avoid the false positive result due to natural granulation of the antigen.

## Level of Infection

The level of infection was determined by the strength of the clamps (Figure 3). In general, the clumps begun and concentrated from the periphery of the mixture. The strength of the agglutination reaction was measured according to the methods followed by Hossain et al. (2007). In brief,

No infection (–) = No clumps with no background clearing.

Low infection (+) = Small clumps with no background clearing.

Medium infection (++) = Medium sized clumps with almost complete background clearing.

Heavy infection (+++) = Large to very large clumps (mostly in the periphery) with complete background clearing.

## Gross Pathological Lesions

Some of dead birds were randomly selected from the poultry farms where the blood samples were taken for SPA test. After a systemic dissection of the organs of dead

birds, the changes in the organs were recorded and the variations were compared with the results of Kumari et al. (2013) to confirm suspect clinically the *Salmonella spp.* infection cases. The sterilized distinct set of apparatuses were used for each case to complete the post-mortem examination and the methods applied which followed by the authors Hossain et al. (2017).

## Statistical analysis

All the data were arranged in Microsoft excel (Microsoft corp. 2019) and then transferred to "Statistical Package for Social Sciences (SPSS)" version 25.0 to perform the statistical test. The association between the categorical explanatory variable with outcome were estimated by Pearson's Chi-square. On the other hand, when more than 20% of cells of 2×2 contingency table had expected count less than 5, the P value of continuity correction was considered but when the table more than the 2×2 contingency then P value of Fisher exact tests was accounted. Moreover, if the table is 1×3, the one sample Chi-square test was performed. A regression model was used to determine the significant associations of *Salmonella spp.* seropositive case with the common biosecurity practices of poultry farm. The Binary logistic regression analysis was performed using the enter methods. Before performing all the statistical test, the assumptions were checked found suitable. The p value ≤ 0.05 was considered as significant result.

## Results

This study revealed that the seroprevalence of *Salmonella spp.* infection was 47.77% in the study area (Table 1). Among the two districts, the seroprevalence of *Salmonella spp.* infection was higher in Mymensingh (51.59%) than Gazipur (45.21%), though there was no significant (p>0.05) difference. Among the 150 positive cases, highest proportion (51.33%) was detected in broiler species, where in layer and sonali was 32.67% and 16% respectively.

In case of layer birds, seroprevalence was significantly (p<0.05) higher in summer seasons (56.45%) than the winter seasons (Table 2). In winter season the moderate level of infection was significantly (p<0.05) higher (64.29%). The Salmonella spp. seroprevalence of the farms that flock size was >2000 to <2500 birds had significantly (p<0.05) higher (71.43%) seroprevalence. Although, the 30-39 weeks old birds were more seroprevalent (70.00%) but had no any significant (p>0.05) differences with the other ages. similarly, the seroprevalence was higher (56.76%) in Mymensingh without any significant (p>0.05) difference with Gazipur district for the layer birds. However, in Gazipur the significant amount of layer birds (57.14%) was moderately infected.

The table 3 shows the seroprevalence in broiler birds, where the seroprevalence was significantly (p<0.05) higher in summer season (60.64%) and 15 to 30 days old birds (77.05%). The flocks having the birds of >1500 to ≤2000 was higher (59.09%) seroprevalent. The moderate level of infection was significantly (p<0.05) higher (61.11%) in the birds of the flock size about >2000 to <2500. Among the infected broiler birds, highest proportion (44.16%) had the moderate level of infection.

The seroprevalence of Salmonella spp. Infection in sonali chickens is presented in table 4. The result shows the significantly (p<0.05) higher seroprevalence in summer seasons of 51.22%. Moreover, the higher seroprevalence was observed in the sonali birds of flock size ≤500 (54.55%), 61 to ≥90 days of age (52.17%) and in Mymensingh district (40.91%). Among the infected sonali birds, highest proportion (41.67%) had the low level of infection.

The Figure 4 shows the gross pathological lesions which indicated that the *Salmonella spp.* infection. The pathological lesions including the black color enlarged liver with congestion and necrotic foci, splenomegaly, pericarditis, misshapen and congested ova had been found in all dead birds of the farms that had the SPA test positive samples.

In Table 5, considering the biosecurity parameters, the significant (p<0.05) number of infected birds were in the farms used surface water (67.74%), used disinfectant irregularly (59.78%), had the density of >10 birds/meter2 (55.70%), cleaned water and feeder irregularly (55.56%), and had no any visitors' restrictions (59.35%). However, among the different types of poultry (Layer, Broiler and Sonali), there was no any significant differences in term of different categorical levels of biosecurity parameters that commonly practiced.

**Table 1.** Prevalence of Salmonella spp. infection in different poultry farms of Gazipur and Mymensingh district of Bangladesh.

| Categories | | No. of Sample Tested | Positive Case (Prevalence) | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Overall | | Farm Species | | | |
| Variable | Level | | n (%) | P value | Layer (%) | Broiler (%) | Sonali (%) | P valueOSC |
| Area | Gazipur | 188 | 85 (45.21) | 0.321 | 28(32.94) | 42(49.41) | 15(17.65) | 0.323 |
| | Mymensingh | 126 | 65 (51.59) | | 21(32.31) | 35(53.85) | 9(13.85) | 0.494 |
| | Total | 314 | 150 (47.77) | | 49(32.67) | 77(51.33) | 24(16) | 0.429 |

"OSC" = p value of one sample Chi-square test. Significant at 1% (P<0.01); Significant at 5% (P<0.05).

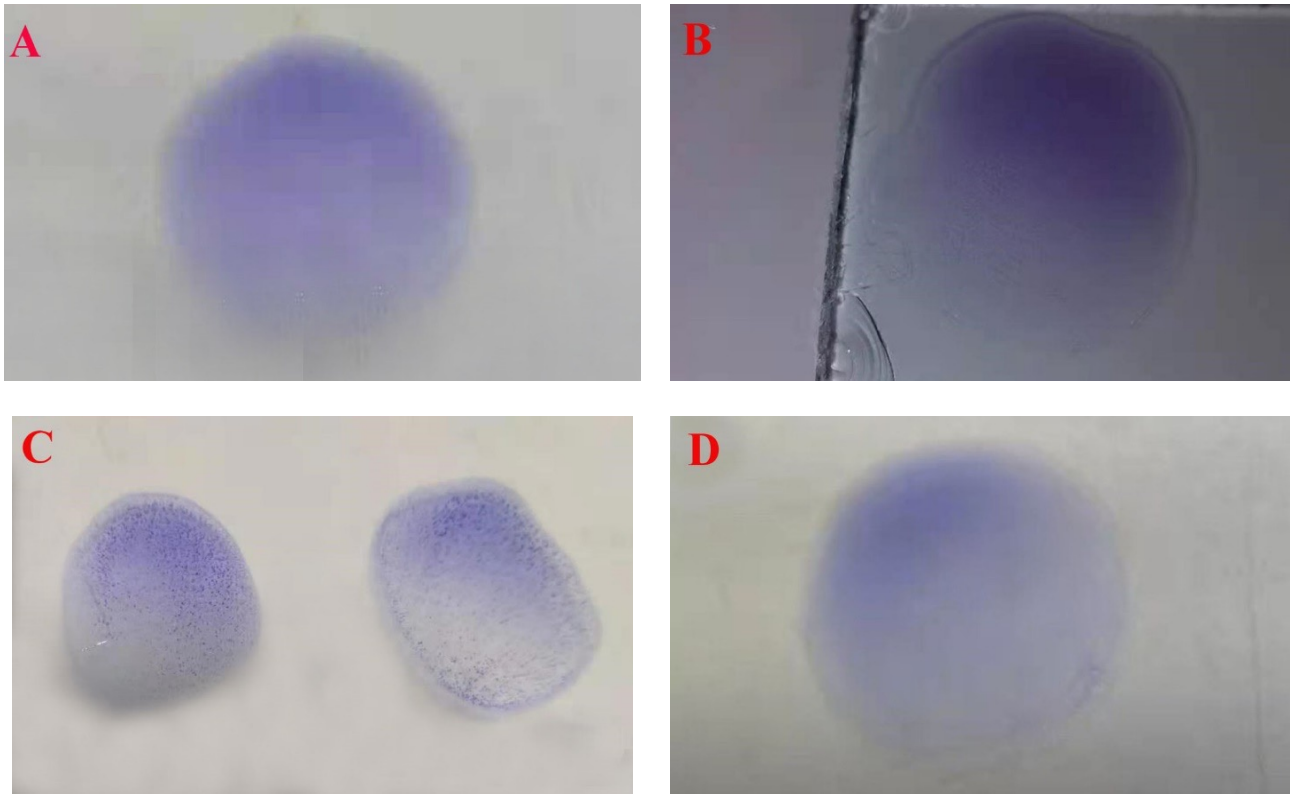



**Figure 3.** The strength of the agglutination reaction showed (A) Low level of infection, (B) Moderate level of infection, (C) High level of infection and (D) No infection

**Table 2.** Prevalence of *Salmonella spp.* infection in commercial Layer birds with respect to different parameters

| Categories | | No. of Sample Tested | Positive Case | | | Level of Infection | | | |
|---|---|---|---|---|---|---|---|---|---|
| Variable | Level | | (N) | Prevalence (%) | P value | Low | Moderate | High | P value [osc] |
| Season | Winter | 39 | 14 | 35.90 | 0.044 | 2(14.29) | 9(64.29) | 3(21.43) | 0.046 |
| | Summer | 62 | 35 | 56.45 | | 9(25.71) | 15(42.86) | 11(31.43) | 0.449 |
| Flock Size | ≤500 | 15 | 5 | 33.33 | 0.015 | 1(20) | 4(80) | 0(0) | 0.074 |
| | >500 to ≤1000 | 22 | 6 | 27.27 | | 2(33.33) | 1(16.67) | 3(50) | 0.607 |
| | >1000 to ≤1500 | 19 | 11 | 57.89 | | 4(36.36) | 5(45.45) | 2(18.18) | 0.529 |
| | >1500 to ≤2000 | 17 | 7 | 41.18 | | 1(14.29) | 4(57.14) | 2(28.57) | 0.368 |
| | >2000 to <2500 | 28 | 20 | 71.43 | | 3(15) | 10(50) | 7(35) | 0.157 |
| Age (Weeks) of the Birds | <10 | 13 | 6 | 46.15 | 0.160 | 1(16.67) | 3(50) | 2(33.33) | 0.607 |
| | 10-19 | 15 | 8 | 53.33 | | 2(25) | 6(75) | 0(0) | 0.030 |
| | 20-29 | 17 | 10 | 58.82 | | 1(10) | 4(40) | 5(50) | 0.273 |
| | 30-39 | 10 | 7 | 70.00 | | 3(42.86) | 2(28.57) | 2(28.57) | 0.867 |
| | 40-49 | 26 | 7 | 26.92 | | 1(14.29) | 5(71.43) | 1(14.29) | 0.102 |
| | Above 50 | 20 | 11 | 55.00 | | 3(27.27) | 4(36.36) | 4(36.36) | 0.913 |
| Area | Gazipur | 64 | 28 | 43.75 | 0.208 | 4(14.29) | 16(57.14) | 8(28.57) | 0.018 |
| | Mymensingh | 37 | 21 | 56.76 | | 7(33.33) | 8(38.1) | 6(28.57) | 0.867 |
| | Total | 101 | 49 | 48.51 | | 11(22.45) | 24(48.98) | 14(28.57) | 0.059 |

"OSC"= p value of one sample Chi-square test. Significant at 1% (P<0.01); Significant at 5% (P<0.05)

**Table 3.** Prevalence of Salmonella spp. infection in commercial Broiler birds with respect to different parameters.

| Categories | | No. of Sample Tested | Positive Case | | | Level of Infection | | | |
|---|---|---|---|---|---|---|---|---|---|
| Variable | Level | | (N) | Prevalence (%) | P value | Low | Moderate | High | P Value[OSC] |
| Season | Winter | 56 | 20 | 35.71 | 0.003 | 5(25) | 8(40) | 7(35) | 0.705 |
| | Summer | 94 | 57 | 60.64 | | 13(22.81) | 26(45.61) | 18(31.58) | 0.104 |
| Flock Size | ≤500 | 23 | 12 | 52.17 | 0.699 | 2(16.67) | 6(50) | 4(33.33) | 0.368 |
| | >500 to ≤1000 | 36 | 20 | 55.56 | | 5(25) | 7(35) | 8(40) | 0.705 |
| | >1000 to ≤1500 | 34 | 14 | 41.18 | | 4(28.57) | 5(35.71) | 5(35.71) | 0.931 |
| | >1500 to ≤2000 | 22 | 13 | 59.09 | | 2(15.38) | 5(38.46) | 6(46.15) | 0.368 |
| | >2000 to <2500 | 35 | 18 | 51.43 | | 5(27.78) | 11(61.11) | 2(11.11) | 0.030 |
| Age (Days) of the Birds | <15 | 49 | 16 | 32.65 | 0.001 | 4(25) | 7(43.75) | 5(31.25) | 0.646 |
| | 15-30 | 61 | 47 | 77.05 | | 10(21.28) | 20(42.55) | 17(36.17) | 0.186 |
| | 31- 45 and above | 40 | 14 | 35.00 | | 4(28.57) | 7(50) | 3(21.43) | 0.395 |
| Area | Gazipur | 83 | 42 | 50.60 | 0.842 | 5(11.9) | 22(52.38) | 15(35.71) | 0.005 |
| | Mymensingh | 67 | 35 | 52.24 | | 13(37.14) | 12(34.29) | 10(28.57) | 0.819 |
| | Total | 150 | 77 | 51.33 | | 18(23.38) | 34(44.16) | 25(32.47) | 0.082 |

"OSC" = p value of one sample Chi-square test. Significant at 1% (P<0.01); Significant at 5% (P<0.05)

**Table 4.** Prevalence of Salmonella spp. infection in commercial Sonali birds in respect to different parameters.

| Categories | | No. of Sample Tested | Positive Case | | | Level of Infection | | | |
|---|---|---|---|---|---|---|---|---|---|
| Variable | Level | | (N) | Prevalence (%) | P value | Low | Moderate | High | P Value[OSC] |
| Season | Winter | 22 | 3 | 13.64 | 0.003 | 1(33.33) | 1(33.33) | 1(33.33) | 1.000 |
| | Summer | 41 | 21 | 51.22 | | 9(42.86) | 7(33.33) | 5(23.81) | 0.565 |
| Flock Size | ≤500 | 11 | 6 | 54.55 | 0.305 | 2(33.33) | 2(33.33) | 2(33.33) | 1.000 |
| | >500 to ≤1000 | 10 | 5 | 50.00 | | 2(40) | 3(60) | 0(0) | 0.247 |
| | >1000 to ≤1500 | 13 | 2 | 15.38 | | 0(0) | 0(0) | 2(100) | 0.135 |
| | >1500 to ≤2000 | 12 | 5 | 41.67 | | 2(40) | 2(40) | 1(20) | 0.819 |
| | >2000 to <2500 | 17 | 6 | 35.29 | | 4(66.67) | 1(16.67) | 1(16.67) | 0.223 |
| Age (Days) of the Birds | <30 | 27 | 9 | 33.33 | 0.179 | 4(44.44) | 2(22.22) | 3(33.33) | 0.717 |
| | 31-60 | 13 | 3 | 23.08 | | 1(33.33) | 1(33.33) | 1(33.33) | 1.000 |
| | 61- 90 and above | 23 | 12 | 52.17 | | 5(41.67) | 5(41.67) | 2(16.67) | 0.472 |
| Area | Gazipur | 41 | 15 | 36.59 | 0.736 | 8(53.33) | 7(46.67) | 0(0) | 0.022 |
| | Mymensingh | 22 | 9 | 40.91 | | 2(22.22) | 1(11.11) | 6(66.67) | 0.097 |
| | Total | 63 | 24 | 38.10 | | 10(41.67) | 8(33.33) | 6(25) | 0.607 |

"OSC" = p value of one sample Chi-square test. Significant at 1% (P<0.01); Significant at 5% (P<0.05)

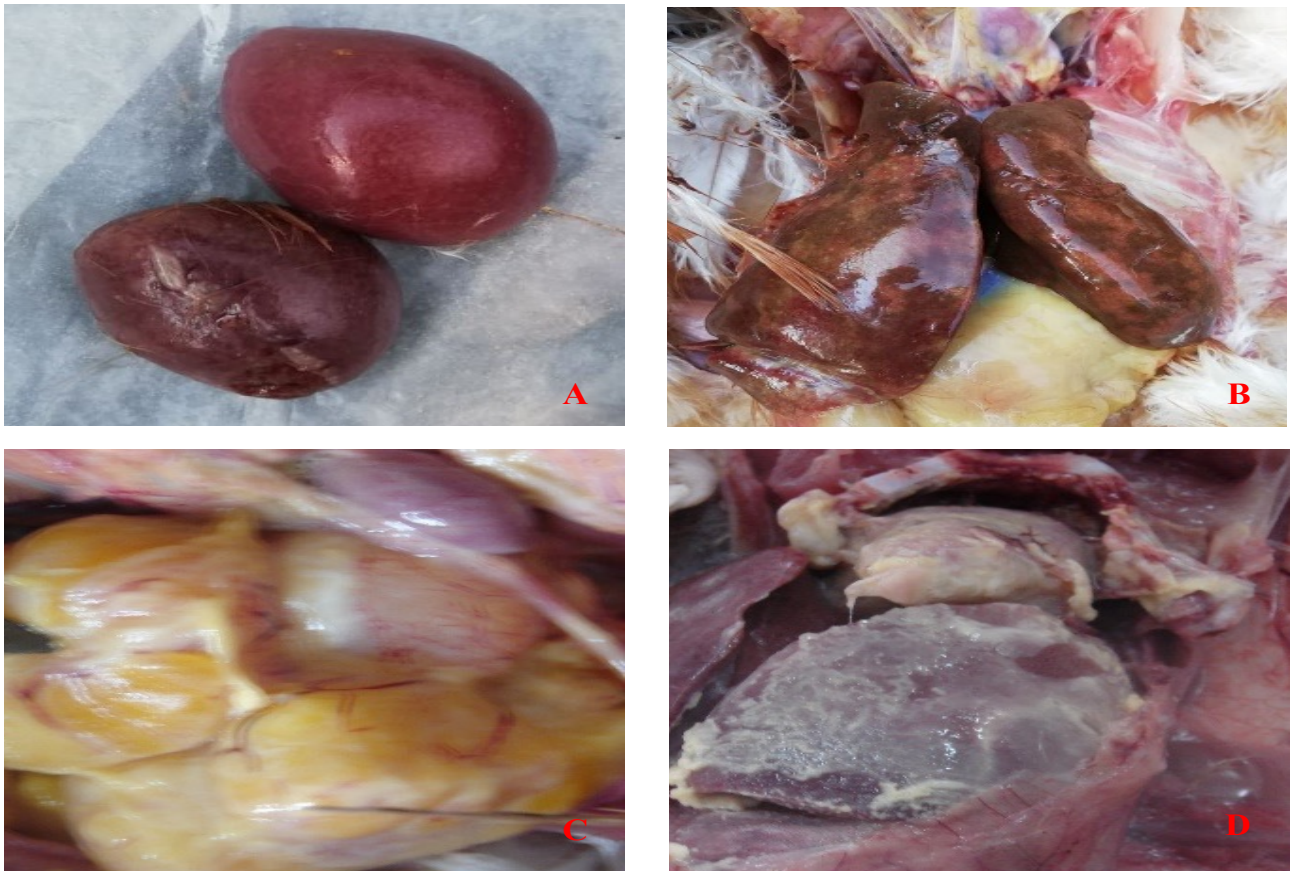



**Figure 4.** The gross pathological leshions. (A) Spleen larger than normal size with blackish discoloration. (B) Enlaged congested liver with bronze discoloration. (C) Congested and misshapened egg follicles. (D) Pericarditis conjugation with Escherichia coli infection

**Table 5.** Prevalence of *Salmonella spp.* infection in commercial birds in respect to biosecurity parameters.

| Categories | | N | Positive Case | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | Overall | | | Farm Species | | | |
| Variable | Level | | (n) | Prevalence (%) | P value | Layer | Broiler | Sonali | P value |
| Source of water in farm | Underground | 159 | 45 | 28.30 | 0.00 | 13(28.89) | 21(46.67) | 11(24.44) | 0.197 |
| | Surface | 155 | 105 | 67.74 | | 36(34.29) | 56(53.33) | 13(12.38) | |
| Use of disinfectants | Regularly | 130 | 40 | 30.77 | 0.001 | 16(40) | 17(42.5) | 7(17.5) | 0.406 |
| | Irregularly | 184 | 110 | 59.78 | | 33(30) | 60(54.55) | 17(15.45) | |
| Density of birds (birds/meter2) | 8 to 10 | 156 | 62 | 39.74 | 0.007 | 23(37.1) | 34(54.84) | 5(8.06) | 0.061 |
| | >10 | 158 | 88 | 55.70 | | 26(29.55) | 43(48.86) | 19(21.59) | |
| Disposal of dead birds | Burned/buried | 148 | 65 | 43.92 | 0.239 | 18(27.69) | 32(49.23) | 15(23.08) | 0.111 |
| | Thrown away | 166 | 85 | 51.20 | | 31(36.47) | 45(52.94) | 9(10.59) | |
| Well ventilation | Yes | 140 | 61 | 43.57 | 0.221 | 19(31.15) | 33(54.1) | 9(14.75) | 0.876 |
| | No | 174 | 89 | 51.15 | | 30(33.71) | 44(49.44) | 15(16.85) | |
| Cleaning of waterer and feeder | Regularly | 152 | 60 | 39.47 | 0.006 | 19(31.67) | 30(50) | 11(18.33) | 0.831 |
| | Irregularly | 162 | 90 | 55.56 | | 30(33.33) | 47(52.22) | 13(14.44) | |
| Visitors restricted | Yes | 159 | 58 | 36.48 | 0.001 | 22(37.93) | 28(48.28) | 8(13.79) | 0.554 |
| | No | 155 | 92 | 59.35 | | 27(29.35) | 49(53.26) | 16(17.39) | |
| Total | | 314 | 150 | 47.77 | | 49(32.67) | 77(51.33) | 24(16) | 0.429 |

Significant at 1% (P<0.01); Significant at 5% (P<0.05)

**Table 6.** Logistic regression analysis of common biosecurity practices in poultry farm associated to *Salmonella spp.* seroprevalence.

| Categories | | Wald | Odd Ratio | P value | 95% C.I. for O. R. | |
|---|---|---|---|---|---|---|
| **Variable** | **Level** | | | | **Lower** | **Upper** |
| Source of water in farm | Underground | 37.663 | 0.182 | 0.000 | 0.106 | 0.314 |
| | Surface | | | Reference | | |
| Use of disinfectants | Regularly | 18.983 | 0.296 | 0.000 | 0.171 | 0.511 |
| | Irregularly | | | Reference | | |
| Density of birds (birds/meter2) | 8 to 10 | 12.121 | 0.379 | 0.000 | 0.219 | 0.654 |
| | >10 | | | Reference | | |
| Disposal of dead birds | Burned/ buried | 2.625 | 0.641 | 0.105 | 0.375 | 1.098 |
| | Thrown away | | | Reference | | |
| Well ventilation | Yes | 0.628 | 0.805 | 0.428 | 0.47 | 1.378 |
| | No | | | Reference | | |
| Cleaning of waterer and feeder | Regularly | 6.266 | 0.503 | 0.012 | 0.294 | 0.862 |
| | Irregularly | | | Reference | | |
| Visitors restricted | Yes | 12.747 | 0.375 | 0.000 | 0.219 | 0.643 |
| | No | | | Reference | | |
| Constant | | 47.818 | 17.446 | 0.000 | | |
| $R^2$= 0.284 (Cox & Snell R Square) , 0.379 (Nagelkerke R Square) | | | | | | |
| Hosmer and Lemeshow test p value: 0.400; Significant at 1% (P<0.01); Significant at 5% (P<0.05); C.I.= Confidence Interval; O.R. = Odd Ratio. | | | | | | |

The Table 6 shows the binary logistic analysis to determine the effect of common biosecurity practices in poultry farm on the likelihood that the farm had the seroprevalence of *Salmonella spp.* The result revealed that the several variables of common biosecurity practices on the probability had the influence on *Salmonella spp.* seroprevalence. The logistic model contained seven independent variables (Source of water, Use of disinfectants, Density of birds, Disposal of dead birds, Ventilation, Cleaning of waterer and feeder, and Visitors restricted). The regression model was statistically significant, $\chi 2$ (7, N = 314) = 105.02, p < .001, representing that the model was able to distinguish between *Salmonella spp.* seropositive and seronegative farm in terms of biosecurity practices. This model as a whole, could clarify between 28.4% (Cox and Snell R square) and 37.9% (Nagelkerke R squared) of the variance in *Salmonella spp.* seroprevalence status. The model was also correctly classified 73.9% of cases. Hence, the goodness of fit for this model was determined by the Hosmer and Lemeshow test, in which the p value of 0.400 (p>0.05) indicates that final model is fit. The seroprevalence of *Salmonella spp.* had a lower (OR=0.182; 95% CI: 0.106-0.314) tendency (p<0.001) in the farm used underground water compared with the farm used surface water. The other categorical level of biosecurity practices, regular use of disinfectant, density of 8-10 birds/meter2, disposal of dead birds by buried or burned, Ventilation, Cleaning of waterer and feeder regularly, and restriction of visitor access ensued the odd ratio (OR) of 0.182 (p=0.00, 95% CI=0.106-0.314), 0.296 (p=0.00, 95% CI=0.171-0.511), 0.379 (p=0.00, 95% CI=0.219-0.654), 0.641 (p=0.105, 95% CI=0.375-1.098), 0.805 (p=0.428, 95% CI=0.47-1.378), 0.503 (p=0.012, 95% CI=0.294-0.862), and 0.375 (p=0.00, 95% CI=0.219-0.643) respectively. All these values of odd ratio indicate that the less likelihood to *Salmonella spp. seroprevalence*.

## DISCUSSION

The present study revealed that the seroprevalence of *Salmonella spp*. in Mymensingh was higher than the Gazipur district in Bangladesh. The overall seroprevalence is very close to the findings of another research (Sabuj et al., 2019) where report was 42% seroprevalence in layer birds in Cox's Bazar district of Bangladesh. But the overall seroprevalence of this study was higher than the findings of Hossain et al. (2010), Sikder et al. (2005), Barua et al. (2012) where they reported that 14.1% in Rajshahi and surrounding districts, 23.46% in Patuakhali district, 18% in Chittagong district of Bangladesh respectively. On the other hand, the authors Jalil & Islam, (2012) reported 65.9% seroprevalence in chicken in Khulna district of Bangladesh. Among the two study districts, in Mymensingh the seroprevalence was higher than the Gazipur district. This might be due to variation in the farming strategies and number of farms in these two different study areas.

In the study area, mainly three types of poultry (Layer, Broiler and Sonali) are commercially reared. Among them, the higher seroprevalence was observed in broiler. Similarly, Naurin et al. (2013) also observed higher prevalence in broiler than the indigenous chicken. The highest seroprevalence of *Salmonella spp.* in broiler might be due to overcrowding along with inadequate hygienic measures in the farms. In addition, the higher seroprevalence in broiler indicated that broilers could be an important

reservoir of *Salmonella spp.* Naurin et al. (2013). Among the variation of seroprevalence of *Salmonella spp* in different types of poultry, the author Naurin et al. (2013) found significantly higher prevalence about 70% in broiler than layer and sonali birds. This result is in line with our findings where the seroprevalence was higher in broiler.

There was a significant impact of seasonal variation on the seroprevalence of *Salmonella spp.* infection in all types of poultry (layer, broiler and sonali). In accordance with the present findings, Jalil & Islam (2012) also reported the seroprevalence of *Salmonella spp.* infection was significantly higher in summer (82%) than winter (50%) seasons. Similarly, a study conducted by Naurin et al. (2013) reported that the significantly higher prevalence of *Salmonella spp.* in poultry in the Mymensingh area during summer as compared to rainy season. The increasing bacterial growth in summer season along with the hot humid weather that could decrease the immunity of birds against infection, ultimately increase the *Salmonella spp. seroprevalence* (Hossain et al., 2010). Moreover, the warm temperature may be a major factor to provide the suitable environment for the growth and proliferation of *Salmonella spp.* (Guthrie, 1992). Similarly, a study conducted in Nepal recorded that the highest prevalence of *Salmonella spp.* during summer in chicken raw meat (Maharjan et al., 2006). Though the seroprevalence in broiler and sonali birds did not significantly influenced by the flock size, but layer birds had significant differences. Among the categorized flock size, the higher flock size led to higher seroprevalence. Similarly, the author Hossain et al., (2010) and Jalil & Islam (2012) reported the higher seroprevalence of 17.8% and 81.4% in the farm of flock size 4501 to 5000 and 5000 to above respectively.

In different study, the seroprevalence of *Salmonella spp.* in different ages layer birds was variable. The authors, Jalil & Islam (2012), Hossain et al. (2010) and Sabuj et al. (2019) found the highest seroprevalence of *Salmonella spp.* infection was 76.6% at 56 weeks of age, 27.2% at ≥64 weeks of age and 68% at >55 weeks of age respectively. These findings contradicted our result. However, our study was in line with the authors Sikder et al. (2005) who reported the highest seroprevalence was 30.8% at 39 weeks of age. In different study, there was variation of seroprevalence according to age which might be due to dissimilarities in study area and management practices. In case of broiler the *Salmonella spp.* infection was higher at the age of 15 to 30 days. This observation is supported by the Djeffal et al. (2018) who reported that the farms having the broiler at the age of 15-30 days were more infected than the birds at the age of 45-60 days.

On the other hand, the gross pathological changes of the organs of dead birds from SPA test positive farms indicated the salmonellosis which supported the findings of Kumari et al. (2013).

In this study seven common practices for farm biosecurity were considered as parameters to determine the association with *Salmonella spp.* seroprevalence. The *Salmonella spp.* seroprevalence had significant association with all the parameters except the parameters of dead bird's disposal system and ventilation system. The samples taken from the farms using surface water were significantly ($p<0.01$) more seroprevalent to *Salmonella spp.* than the farm using the ground water. Because the surface waters including those waters used for irrigation and refreshment or as a drinking water which are the potential source for *Salmonella spp.* contamination (Levantesi et al., 2012). However, when the surface water is treated mainly by the organic acids, it may reduce the number of *Salmonella spp.* (Argüello et al., 2013). The use of disinfectant, especially when the regularly is also a significant biosecurity practice to minimize the *Salmonella spp.* seroprevalence (OR=0.296). Irregular and insufficient cleaning and disinfection may lead to persistence of *Salmonella spp.* in poultry houses (Trampel et al., 2014). The density of birds (birds/meter$^2$) also had the significant ($p<0.01$) role, where the farms had 8 to 10 birds/meter$^2$ had lower prevalence (39.74%) and less likelihood (OR=0.379) to seroprevalence than the farms had >10 birds/meter$^2$. Though this observation contraindicates the finding of Djeffal et al. (2018) who reported that, <10 birds/meter$^2$ is a risk factor (OR=2.25) without any significant association. But in another study, Elgroud et al. (2009) noted that there was significant($p<0.01$) effect (OR=7.7). Moreover, our findings are in agreement with another literature relating that poultry house having the high density of birds, is a risk factor favoring the infection by *Salmonella spp.* (Heyndrickx et al., 2002). The irregular cleaning of feeder and waterer in the poultry farm contributed to significantly ($p<0.01$) higher seroprevalence and the regular cleaning practices (OR=0.503) farms were less like to seroprevalence. This finding is supported by another research mentioning that the poultry can be infected by the horizontal transmission of *Salmonella spp.* during the rearing period through feeding drinking water, and contaminated equipment (Tabo et al., 2013). Another important biosecurity practices, the restricted access of visitors in poultry farms that had less tendency (OR=0.375) to *Salmonella spp.* seroprevalence than the farms having free access. Though these findings differ from the study conducted by Hamilton & Dornan (1992), they found that culture of *Salmonella spp.* from floor litter or drinking water has no any significant association with restrictions on visitors. But the restriction of visitors is one of the important steps for poultry biosecurity to minimize the contamination.

## Limitations

The present study has some limitations. First, only the

sample were subjected to test rapid serum plate agglutination (SPA). Second, only eight farm practices were considered for biosecurity parameters. Lastly, presence of data collector in the working situation may had some influence on responses to a questionnaire.

## CONCLUSIONS

The seroprevalence of *Salmonella spp.* in different types of poultry was detected both in Mymensingh and Gazipur district. The seroprevalence was comparatively higher in Mymensingh and in broiler farm. Summer is the more prevalent season than winter for salmonellosis in poultry. Strict farm hygienic practice and biosecurity measures are significantly linked to decrease the *Salmonella* infection. Therefore, the control of *Salmonella spp.* seroprevalence depends on limiting the sources of contam¬ination and transmission by implementing the biosecurity measures. However, further study could be the serotyping and molecular identification of salmonellosis in poultry maintained in different grade of biosecurity measures.

## COMPLIANCE WITH ETHICAL STANDARDS

### Conflict of interest

There is no conflict of interest among the authors

### Author contribution

MMM involved in conception and design of the experiments, questionnaire development, implementation of research, statistical analysis and manuscript writing. MAS contributed to revise the manuscript. ALB collected the questionnaire data and experimentation. All authors read and approved the manuscript and also contributed it critically for important intellectual content.

### Ethical approval

The ethics of this study was in accordance to research ethics followed by the Department of Microbiology and Public Health, Faculty of Veterinary Medicine and Animal Science, Bangabandhu Sheikh Mujibur Rahman Agricultural University. The ethical consent number was BSMRAU/FVMAS/MPH/20(Ethical Approval)/2020/03. Moreover, the verbal consent was also obtained from the poultry farmer.

### Funding

Ministry of Science and Technology of Bangladesh (R & D Project)

### Acknowledgements

All the poultry farmers, from where the samples were taken are thanked for their assistance in performing the experiment. The author, Mirza Mienur Meher is gratefully acknowledged to the funding of the Ministry of Science and Technology of Bangladesh.

### Data availability

Not applicable.

### Consent for publication

Not applicable.

## REFERENCES

Abdullah, A. M. S., Tamim, M., Nanda, B., Md., A. R., Md., S. I., & Md., A. B. (2019). Passive surveillance of clinical poultry diseases in an Upazila Government Veterinary Hospital of Bangladesh. African Journal of Microbiology Research, 13(29), 632–639. [CrossRef]

Argüello, H., Carvajal, A., Costillas, S., & Rubio, P. (2013). Effect of the Addition of Organic Acids in Drinking Water or Feed During Part of the Finishing Period on the Prevalence of *Salmonella* in Finishing Pigs. Foodborne Pathogens and Disease, 10(10), 842–849. [CrossRef]

Barua, H., Biswas, P. K., Olsen, K. E. P., & Christensen, J. P. (2012). Prevalence and Characterization of Motile *Salmonella* in Commercial Layer Poultry Farms in Bangladesh. PLoS ONE, 7(4), e35914. [CrossRef]

Chanie, M., Negash, T., & Tilahun, S. B. (2009). Occurrence of concurrent infectious diseases in broiler chickens is a threat to commercial poultry farms in Central Ethiopia. Tropical Animal Health and Production, 41(7), 1309–1317. [CrossRef]

Daniel, W. W. (1999). Biostatistics: A Foundation for Analysis in the Health Sciences (7th ed.). John Wiley & Sons, Ltd.

Djeffal, S., Mamache, B., Elgroud, R., Hireche, S., & Bouaziz, O. (2018). Prevalence and risk factors for *Salmonella spp.* contamination in broiler chicken farms and slaughterhouses in the northeast of Algeria. Veterinary World, 11(8), 1102–1108. [CrossRef]

Elgroud, R., Zerdoumi, F., Benazzouz, M., Bouzitouna-Bentchouala, C., Granier, S. A., Frémy, S., Brisabois, A., Dufour, B., & Millemann, Y. (2009). Characteristics of *Salmonella* Contamination of Broilers and Slaughterhouses in the Region of Constantine (Algeria). Zoonoses and Public Health, 56(2), 84–93. [CrossRef]

Fathelrahman, E. M., El Awad, A. I., Yousif Mohamed, A. M., Eltahir, Y. M., Hassanin, H. H., Elfatih Mohamed, M., & L. K. Hoag, D. (2020). Biosecurity Preparedness Analysis for Poultry Large and Small Farms in the United Arab Emirates. Agriculture, 10(10), 426. [CrossRef]

Gantois, I., Ducatelle, R., Pasmans, F., Haesebrouck, F., Gast, R., Humphrey, T. J., & Van Immerseel, F. (2009). Mechanisms of egg contamination by *Salmonella* Enteritidis. FEMS Microbiology Reviews, 33(4), 718–738. [CrossRef]

Guthrie, R. K. (1992). Salmonella. CRC Press, Boca Raton, Ann Arbor, London.

Hamid, M. A., Rahman, M. A., Ahmed, S., & Hossain, K. M. (2016). Status of Poultry Industry in Bangladesh and the Role of Private Sector for its Development. Asian Journal of Poultry Science, 11(1), 1–13. [CrossRef]

Hamilton, R. A., & Dornan, J. C. (1992). Neural tube defects--prenatal diagnosis and management. The Ulster Medical Journal, 61(2), 127–133. [URL]

Heyndrickx, M., Vandekerchove, D., Herman, L., Rollier, I., Grijspeerdt, K., & De Zutter, L. (2002). Routes for *salmonella* contamination of poultry meat: epidemiological study from hatchery to slaughterhouse. Epidemiology and Infection, 129(2), 253–265. [CrossRef]

Hossain, K. M. M., Ali, M. Y., & Haque, M. I. (2007). Seroprevalence of Mycoplasma Gallisepticum Infection in Chicken in the Greater Rajshahi District of Bangladesh. Bangladesh Journal of Veterinary Medicine, 5(1 & 2), 09–14.

Hossain, K. M. M., Hossain, M. T., & Yamato, I. (2010). Seroprevalence of *Salmonella* and Mycoplasma gallisepticum infection in chickens in Rajshahi and surrounding districts of Bangladesh. International Journal of Biology, 2(2), 74–80. [CrossRef]

Hossain, M. R., Meher, M. M., & Afrin, M. (2017). Epidemiological Investigation of Pasteurella multocida Infection in Poultry in Gazipur District of Bangladesh. Bangladesh Journal of Veterinary Medicine, 15(2), 91–95.

Islam, M. A., & Nishibori, M. (2009). Indigenous naked neck chicken: a valuable genetic resource for Bangladesh. World's Poultry Science Journal, 65(1), 125–138. [CrossRef]

Jalil, M., & Islam, M. (2012). Serological Survey of *Salmonella* Infection in Non-Vaccinated Commercial Layer Birds in Khulna District of Bangladesh. Bangladesh Journal of Veterinary Medicine, 9(1), 27–31. [CrossRef]

Kabir, S. M. L. (2010). Avian Colibacillosis and Salmonellosis: A Closer Look at Epidemiology, Pathogenesis, Diagnosis, Control and Public Health Concerns. International Journal of Environmental Research and Public Health, 7(1), 89–114. [CrossRef] https://doi.org/10.3390/ijerph7010089

Karim, M. J. (2003). Current disease pattern in poultry with special emphasis on parasites and their methods of control. Proceeding of the 3rd International Poultry Show and Seminar of World Poultry Science Association. Bangladesh, 119–123.

Kumari, D., Mishra, S. K., & Lather, D. (2013). Pathomicrobial studies on *Salmonella* Gallinarum infection in broiler chickens. Veterinary World, 6(10), 725–729. [CrossRef]

Laxman Bahadur, D., Ishwari Prasad, D., Saroj Kumar, Y., Md., A., & Md. Zohorul, I. (2016). Prevalence and antibiotic resistance profile of *Salmonella* from livestock and poultry raw meat, Nepal. International Journal of Molecular Veterinary Research, 6(1), 1–22. [CrossRef]

Levantesi, C., Bonadonna, L., Briancesco, R., Grohmann, E., Toze, S., & Tandoi, V. (2012). *Salmonella* in surface and drinking water: Occurrence and water-mediated transmission. Food Research International, 45(2), 587–602. [CrossRef]

Maduka, C. V., Igbokwe, I. O., & Atsanda, N. N. (2016). Appraisal of Chicken Production with Associated Biosecurity Practices in Commercial Poultry Farms Located in Jos, Nigeria. Scientifica, 2016, 1–9. [CrossRef]

Meher, M. M., Islam, J., & Afrin, M. (2020). Investigation of Risk Factors and Biosecurity Measures Associated with Prevalence of Newcastle Disease Virus in Broiler Farms. Turkish Journal of Agriculture - Food Science and Technology, 8(11), 2426–2432. [CrossRef]

Mridha, D., Uddin, M. N., Alam, B., Akhter, A. H. M. T., Islam, S. S., Islam, M. S., Khan, M. S. R., & Kabir, S. M. L. (2020). Identification and characterization of *Salmonella spp.* from samples of broiler farms in selected districts of Bangladesh. Veterinary World, 13(2), 275–283. [CrossRef]

Naurin, S., Islam, M. A., & Khatun, M. M. (2013). Prevalence of Salmonella in Apparently Healthy Chickens in Mymensingh, Bangladesh. Microbes and Health, 1(1), 30–33. [CrossRef]

Rahman, M. A., Rahman, M. M., Moonmoon, M., Alam, K. J., & Islam, M. Z. (2017). Prevalence of common diseases of broiler and layer at Gazipur district in Bangladesh. Asian Journal of Medical and Biological Research, 3(2), 290–293. [CrossRef]

Rahman, M., Rahman, M., Meher, M., Khan, M., & Anower, A. (2016). Isolation and antibiogram of *Salmonella spp.* from duck and pigeon in Dinajpur, Bangladesh. Journal of Advanced Veterinary and Animal Research, 3(4), 386. [CrossRef]

Sabuj, A. Al, Rahman, M., Barua, N., Zobayda Farzana Hoque, A. P., Islam, K., & Mohammad Mahmudul, H. (2019). Seroprevalence of Salmonella Infection in Commercial Layer Chickens in Cox ' s Bazar District , Bangladesh. American Journal of Microbiological Research, 7(1), 18–23. [CrossRef]

Sikde, A. J., Islam, M. A., Rahman, M. M., & M B Rahman. (2005). Seroprevalence of Salmonella and Mycoplasma gallisepticum Infection in the Six Model Breeder Poultry Farms at Patuakhali District in Bangladesh. International Journal of Poultry Science, 4(11), 905–910. [CrossRef]

Sikder, A. J., Islam, M. A., Rahman, M. M., & Rahman, M. B. (2005). Seroprevalence of Salmonella and Mycoplasma gallisepticum infection in the six model breeder poultry farms at Patuakhali district in Bangladesh. International Journal of Poultry Science, 4(11), 905–910. [CrossRef]

Tabo, D., Diguimbaye, C. D., Granier, S. A., Moury, F., Brisabois, A., Elgroud, R., & Millemann, Y. (2013). Prevalence and antimicrobial resistance of non-typhoidal Salmonella serotypes isolated from laying hens and broiler chicken farms in N'Djamena, Chad. Veterinary Microbiology, 166(1–2), 293–298. [CrossRef]

Trampel, D. W., Holder, T. G., & Gast, R. K. (2014). Integrated farm management to prevent Salmonella Enteritidis contamination of eggs. Journal of Applied Poultry Research, 23(2), 353–365. [CrossRef]

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 568-578
DOI: https://doi.org/10.31015/jaefs.2022.4.9

# Public health risk assessments associated with heavy metal levels in panga fish fillets imported from Vietnam

**Serdar Kilercioglu**[1,2] • **Ali Riza Kosker**[2] • **Ece Evliyaoglu**[3]

[1] Cukurova University Biotechnology Research and Application Center, Adana, Türkiye
[2] Cukurova University Fisheries Faculty, Adana, Türkiye

**Citation:** Kilercioglu, S., Kosker, A.R., Evliyaoglu, E. (2022). Public health risk assessments associated with heavy metal levels in panga fish fillets imported from Vietnam. International Journal of Agriculture, Environment and Food Sciences, 6 (4), 568-578..

**Received:** 09 October 2022
**Revised:** 04 November 2022
**Accepted:** 07 November 2022
**Published Online:** 24 November 2022

**Correspondence:** Serdar Kilercioglu
**E-mail:** skilercioglu@cu.edu.tr



## Abstract

*Pangasius hypophthalmus* (panga fish) is farmed in the Mekong River (Vietnam), which is known as a polluted river, and exported to many countries. The present study aimed to determine heavy metal levels in frozen panga fillets imported from Vietnam as well as the risks of heavy metals to human health. Panga fillets belonging to four brands were bought from three supermarkets in Adana city, Turkey, and heavy metals (As, Cd, Hg, Pb, Ni, Cu, Mn and Co) were analyzed. To analyze the potential risks to human health, EWI (estimated weekly intake), THQ (target hazard quotient), and CR (lifetime cancer risk) values were calculated to assess the potential risks to consumer health of the metal content in panga fillets. The health risk assessment values were calculated for children and adults according to the frequency of consumption once, three and seven times a week. The results revealed that the presence of heavy metals in the studied panga fillets was below permissible limits indicated by WHO (World Health Organization), EPA (United States Environmental Protection Agency) and TKB (Turkish Fisheries Laws and Regulations). The EWI, THQ or ∑THQ and CR values were below PTWI (provisional tolerable weekly intake), 1 and $10^{-5}$, respectively. Remarkably, the highest values of the EWI/PTWI ratio and THQs were found for children.

**Keywords:** Metal toxicity, *Pangasius hypophthalmus*, Consumer health risks, Cancer risks

## INTRODUCTION

Human health is firmly related to diet quality (Korkmaz et al., 2019). Fish meat, which has high protein levels and low calories (Copat et al., 2013), is one of the most important food sources for the human diet. Moreover, fish meat has important specialities, such as reducing cardiovascular disease risks and promoting the nervous system, thanks to ingredients such as polyunsaturated fatty acids and particularly omega-3 fatty acids (Herrera-Herrera et al., 2019). A healthy diet that contains amino acids, vitamins, fatty acids, etc. must not contain deleterious substances, such as heavy metals (Fair et al. 2018; Korkmaz et al., 2019).

Metals are elements that exist in low concentration ranges and are found in a balance in nature (Simionov et al., 2019). Their balance in nature changes with the degree of human intervention, and their amounts may reach a dangerous level for living. Heavy metals are specifically spread to the environment through industrial production, iron and steel production and mining(Jayanthi et al., 2019); thus, their concentrations in the environment and concentration levels depend on in-

creased risks. Toxic substances that are released into the water columns affect aquatic organisms and accumulate in their bodies (Maanan, 2008). This accumulation reaches humans with the consumption of aquatic organisms and could adversely affect human health.

Cadmium (Cd) is known for its mobility and high toxicity among metals. One feature of cadmium is its ability to replace and behave similarly to calcium. This ability has been accepted as a reason to enter the human body and accumulate to a high level in several organs (Kubier et al., 2019). Lead (Pb) is an element that is used in various fields and exists naturally on Earth's crust. Lead is classified as cancerogenic; moreover, it affects organs and systems in humans (Başaran, 2022). Mercury (Hg) is a metal found in nature in various forms, such as inorganic mercury and metallic mercury. It has toxic effects and enters the body easily through mucous membranes and the lung. Mercury damages the gut and kidney. Acute exposure to elemental mercury vapor can lead to fatal pneumonitis (Bernhoft, 2012). Arsenic (As) is one of the most toxic compounds exposed to the natural environment. It is used to manufacture pesticides, insecticides and various products. The main concern of being exposed to arsenic is its potential for cancerogenic effects (Ratnaike, 2003). Nickel (Ni) is an element that exists in nature. It is used in various industrial fields and thus spreads to the environment. Nickel can accumulate in different forms in the body, but the way that it is metabolized is not clear (Denkhaus & Salnikow, 2002). Copper (Cu) exists in nature and is needed in mammalian nutrition. The important part of the copper intake is supplied from drinking water. Copper, although it is an essential metal, is thought to be associated with various neurological disorders in the event of high intake. (Stern et al., 2007). Manganese (Mn) is one of the most abundant elements on Earth (Chen et al., 2018).

It is an essential metal that plays vital roles in nervous system function, energy metabolism, immunological functions and hormone functions (Santamaria, 2008). Cobalt (Co) is a trace element that exists on Earth and shows high similarity with iron and nickel in the case of chemical properties. Cobalt is necessary for physiological function in the body and plays a vital role (Leyssens et al., 2017).

Studies particularly show that fish are quite sensitive to toxic substances in the water (Alibabić et al., 2007; Copat et al., 2013). Pollutants may accumulate in fish bodies because fish are the last step of the aquatic food chain. Thus, fish are a very important bioindicator for metal pollution in the aquatic environment (Authman, 2015).

Panga fish (P. hypophthalmus, Sauvage 1878) live and are cultured in Southeast Asia, particularly in the Mekong River, Vietnam. Fillets of this species have the increasing demand by consumers due to their taste and lower price (Ruiz-de-Cenzano et al., 2013). The aquaculture of panga fish has evolved from rural activity to commercial business over time(Orban et al., 2008). Moreover, the Mekong delta and river were stated to be polluted by pesticides, sewage treatment plants, and human-made pollutants. As a downside to these, panga was indicated to affect these pollutants (Rodríguez et al., 2018).

Frozen fillets of panga have a growing demand for consumption in Turkey; thus, imported panga fillets are sold in markets countrywide. In the present study, the levels of arsenic, cadmium, mercury, lead, nickel, copper, manganese, and cobalt in panga fillets were examined. Furthermore, to predict possible risks to consumer health, estimated weekly intake (EWI), target hazard quotient (THQ), and lifetime cancer risk (CR) were calculated for one, three and seven days/week consumption in adults and children. This study is important to analyze the potential health risks of imported panga fillets that are distributed from Vietnam to the world.

## MATERIALS AND METHODS

### Sample Collection

Samples of frozen panga fillet were selected in supermarkets (three supermarkets, four brands) located in Adana, a city located in the Eastern Mediterranean region of Turkey. Samples were grouped as G1, G2, G3 and G4. Each group represents one brand. Four packs were purchased for each brand. Three fish fillets for each pack were analysed (n=12 for one brand). As a result, the present study was carried out with 48 fillets in total. Fish samples were weighed, homogenized and then stored at -20 °C until analyses.

### Metal Analysis

The weights of fillets belonging to groups are given in Table 1. The method of (Agah et al., 2009) was modified and implemented as follows. Sample extraction was performed by taking 0.5 g from homogenized muscle tissues. Then, 2 ml H2O2 (Merck) and 5 ml HNO3 (Merck) were added to the weighed (0.5 g) samples in a tube, and the samples were burned with a microwave oven (CEM Mars 5) for 1 h at 180-190 °C. After acid digestion, the final volume of the samples was brought up to 20 ml with distilled water and then filtered with a 0.2 µm glass microfibre (Whatman). Samples were analyzed without extra dilution. The obtained data were multiplied by the dilution factor coefficient (20 / 0.5 = 40). The metal contents of the samples were given as µg / g wet weight.

### Device Conditions

All analyses were carried out by ICP-MS (Perkin Elmer Nexion 2000P) in Cukurova University Central Research Laboratory. The recoveries of metals were in the range of

94 - 100.1 %. The operating conditions of the device were as follows: RF power 1600 W, plasma gas flow rate 15 L min -1, auxiliary gas flow1.2 L min -1, nebulizer gas flow 0.97, and carrier gas flow 5.3 min -1.

## Health risk estimation

To determine potential risks related to the consumption of panga fillets, EWI (estimated weekly intake), THQ (target hazard quotient) and CR (lifetime cancer risk) values were calculated according to consumption frequencies of one, three and seven times a week. Potential risk calculations were made separately for adults and children and based on data provided by (US EPA, 2000) and (US EPA, 2019), respectively. The average body weight and lifetimes were assumed to be 70 kg and 70 years for adults and 32 kg and 7 years for children. The calculation was performed using the formulae below.

In contrast to other metals, the majority of arsenic consists of the nontoxic organic form (Arsenobetaine) (Castro-González & Méndez-Armenta 2008). Thus, for calculating the risk factors of As, its inorganic toxic form was assumed to be 3 % of the total concentration (Andaloro et al., 2012; EFSA, 2009; Kosker, 2020).

EWI, THQ and CR were calculated with the formulas [(CM × IR) / BW] (US EPA 2000),[(EF × ED × IR × CM) / (RfD × BW × AT)]× $10^{-3}$ (US EPA, 2019) and [(EF × ED × IR × CM × CsF) / (BW × AT)] × $10^{-3}$ (US EPA, 2019), respectively. In EWI calculations, CM defines the yearly average concentration of metal (µg / g) in muscle tissue, IR defines 1-, 3- and 5-day / person / week consumption rates (FAO, 2017) and BW defines consumer body weight.

In Turkey, fish consumption is 16.82 g / person / day (Kosker, 2020). The consumption frequencies (one, three and seven times a week) were compared to PTWI (provisional tolerable weekly intake). PTWI is based on body weight (mg / kg BW), and its mean describes the estimated amount of a substance that can be taken weekly for a lifetime without health risks.

THQ (target hazard quotient) indicates the ratio between the reference dose (RfD) of metals and the exposure rate to them. The THQ value demonstrates the noncarcinogenic risks of metals. In THQ calculations, EF demonstrates the frequency of exposure (one, three and seven times a week; 52, 156 and 365 days per year), and ED shows the exposure period (70 years was used for adults, while seven years was used for children). IR indicates the amount of consumption, and CM indicates the yearly average concentration of metal (µg/g) in the tissues. RfD states the oral reference doses for metals, and RfD values for Cd, Pb, Hg, As, Ni, Cu, Mn and Co are given as 1× $10^{-3}$, 4×$10^{-3}$, 1×$10^{-4}$, 3×$10^{-4}$, 2×$10^{-3}$, 0.04, 0.14, and 3×$10^{-4}$, respectively (Mwakalapa et al., 2019; US EPA, 2019). BW indicates body weight (70 kg for adults and 32 kg for children). AT represents the noncarcinogenic average period (356 days / year × ED). THQ and TTHQ values (total THQ values of all elements) > 1 indicate possible risks of health issues in addition to cancer in consumers.

CR (lifelong cancer risk) gives the individual cancer risk exposed to metal pollution through consumption. Its calculation was performed according to (US EPA, 2019). High carcinogenic risk is mentioned in the case of CR values above $10^{-5}$. Another value that is used in CR calculation is CsF (cancer slope factor). The CsF values for Cd, Pb, Hg and As are 6.3, 8.5 × $10^{-3}$, 1.5 and 1.5, respectively.

## Statistical Analyses

All experiments were carried out in triplicate, and the results are reported as the mean ± standard deviation. SPSS 20.0 software was used to perform all statistical analyses. The experimental data were checked with Levene's test to assess the equality of variances. Intergroup differences in data were evaluated using one-way ANO-

**Table 1.** Fillet weights (g) and mean and standart deviation of metal levels in edible muscle fillet of Panga (mg/kg)

| | G1 | G2 | G3 | G4 |
|---|---|---|---|---|
| | The average weights of fillets (mean ± std. deviation) | | | |
| | 256.50±25.82 | 191±28.85 | 188.25±14.58 | 254.12±31.14 |
| | **Metal levels (mg / kg)** | | | |
| **Cd** | 4.6×$10^{-3}$±7.1×$10^{-4a}$ | 6×$10^{-3}$±5.4×$10^{-4a}$ | 4.4×$10^{-3}$±1.6×$10^{-3a}$ | 4.9×10-3±2×$10^{-3a}$ |
| **Pb** | 0.17±0.05$^{a}$ | 0.13±0.03$^{a}$ | 0.14±0.04$^{a}$ | 0.12±0.04$^{a}$ |
| **Hg** | 1.7×$10^{-2}$±1.1×$10^{-3a}$ | 2.9×$10^{-3}$±1.2×$10^{-3b}$ | 1.8×$10^{-2}$±1.7×$10^{-3a}$ | 1.6×$10^{-2}$±2.5×$10^{-3a}$ |
| **As** | 1.3×$10^{-2}$±3×$10^{-3ab}$ | 1.2×$10^{-2}$±2.4×10-$^{3b}$ | 1.6×$10^{-2}$±3.8×$10^{-3a}$ | 1.3×$10^{-2}$±2.3×$10^{-3ab}$ |
| **Ni** | 0.17±0.03$^{a}$ | 0.10±0.02$^{b}$ | 0.10±0.03$^{b}$ | 0.11±0.03$^{b}$ |
| **Cu** | 0.23±0.05$^{a}$ | 0.16±0.01$^{a}$ | 0.20±0.08$^{a}$ | 0.22±0.09$^{a}$ |
| **Mn** | 0.18±0.04$^{ab}$ | 0.17±0.03$^{ab}$ | 0.20±0.04$^{a}$ | 0.14±0.02$^{b}$ |
| **Co** | 6.3×$10^{-3a}$ | 5.2×$10^{-3}$±1.3×$10^{-3a}$ | 5.2×$10^{-3}$±2.1×$10^{-3a}$ | 5×$10^{-3}$±7×$10^{-4a}$ |

Values in the same line with different superscripts are statistically different (P < 0.05).

VA with Duncan's and Games-Howell posthoc tests. The level of significance was determined as $P < 0.05$.

## RESULTS AND DISCUSSION

Fillet weights and metal levels (mg/kg) in panga fillets are listed in Table 1. The group variances of metals excluding Cu were homogenous. The total accumulations of heavy metals were found to be Cu > Mn > Pb > Ni > Hg ≃ As > Co > Cd. The intergroup differences in Cd, Pb, Co and Cu were not significant ($P > 0.05$). Hg levels in G2 were significantly lower than those in the other groups ($P < 0.05$). TheMn and As levels of G3 were significantly higher than those of G4 and G2, respectively. Ni levels in G1 were significantly higher than those in the other groups. Differences other than those mentioned above were not statistically significant.

### Elementel Composition

### Cadmium

Cadmium is nonessential and very toxic to organisms and the environment (Stancheva et al., 2010). Cadmium is implicated in carcinogenic diseases (Rakib et al., 2021). Permissible amounts of cadmium in fish are 0.05, 1.4 and 0.5 mg/kg according to Turkish Fisheries Laws and Regulations (TKB) (Köker et al., 2021), EPA (EPA, 1989) and WHO (WHO, 1989), respectively. In the present study, the cadmium levels of the groups were found to be close to each other, and moreover, their levels were found tobe quite low and below the indicated limits (Table 1). Similar to the present study, quite low cadmium amounts from varied fish species' muscles were shown in different countries, such as Bangladesh, Italy, Turkey and Spain (Töre et al., 2021; Varol et al., 2019). In another study carried out with panga fillets, the Cd amount was found to be in the range of 0.01–0.03 µg / g ww (Dambrosio et al., 2016). Mologni et al. (2016) reported that the amounts of cadmium in panga fillets imported from different regions of Vietnam (Dong Thap & Can Tho) differ, and the results from the Can Tho region were found to be closer to the results we obtained.

### Lead

Lead, as an important contaminant, naturally occurs in the environment. It is also considered a neurotoxic agent that affects the rates of survival and growth in vertebrates (Rakib et al., 2021). It moves in nature due to atmospheric convection (Bosch et al., 2016). Permissible amounts of lead in fish are 0.3 and 0.5 mg / kg according to TKB (Köker et al., 2021) and WHO (WHO, 1989), respectively. In the present study, the differences in lead levels of the groups were not significant ($P > 0.05$), and all of them were found to be below the indicated limits. Hajrić et al. (2022) reported that the average Pb levels were 0.004 mg / kg in trout and 0.007 mg / kg in carp in Bosna-Herzegovina. Pb levels were found to be in the range of 0.0025 - 0.0156 mg / kg in different parts of China (Li et al., 2022). Heavy metal pollution was reported to vary according to seasons from different fish species and regions of India. Furthermore, Pb levels were reported in the average range of 0.088 and 0.135 mg / kg (Pandion et al., 2022). In our study, although it was not significant, the results of G1 were found to be higher than those of the others. This case may be based on regional differences.

### Mercury

Mercury is a heavy metal that does not naturally exist in living organisms (Köker et al., 2021), and it is mentioned to affect the human neurological system (Rakib et al., 2021). Permissible amounts of mercury in fish are0.5 and 0.5 mg / kg according to TKB (Köker et al., 2021) and WHO (WHO, 1989), respectively. In the present study, the results were below the indicated limits, but the G2 data were significantly lower than those in the other groups ($P < 0.05$). The region where the fish in group 2 are cultured may be further away from the region that is contaminated with mercury. Botwe (2021) indicated that Hg levels in different fish were found to be at the average of 0.11 ± 0.01 mg / kg, and Hajrić et al. (2022) pointed out that the average Hg levels were 0.081 and 0.052 mg / kg in trout and carp, respectively. These values are much higher than those of G1, G2, G3 and G4 in the present study. In a similar study, the average levels of mercury were found to be 0.20 ± 0.20, 0.27 ± 0.19, 0.19 ± 0.16, 0.18 ± 0.22 and 0.16 ± 0.18 mg / kg in muscles of P. Hypophthalmus (Rodríguez et al., 2018). These results are quite above the results of the present study but below limits. This difference may be based on environmental pollution of aquaculture regions or from the way the fish is obtained: fishing or aquaculture.

### Arsenic

The inorganic forms of As show the highest toxicity, and various organisms have been shown to be affected by As toxicity (Kumari et al., 2017). Absorption of As is quite high, and its distribution affects the whole body, including the placenta (EFSA, 2009). The European Food Safety Authority (EFSA) reported that limits of As should be reevaluated because of its harmful effects on health (EFSA, 2009); moreover, TKB defined its limit as 1 mg/kg for consumable fish meat (Köker et al., 2021). In the present study, the average As levels were $1.3 \times 10^{-2} \pm 3 \times 10^{-3}$, $1.2 \times 10^{-2} \pm 2.4 \times 10^{-3}$, $1.6 \times 10^{-2} \pm 3.8 \times 10^{-3}$ and $1.3 \times 10^{-2} \pm 2.3 \times 10^{-3}$ mg / kg in G1, G2, G3 and G4, respectively. The difference between G3 and G2 was significant ($P < 0.05$). Although there are some significant differences, all results were below the legal limits. Töre et al. (2021) reported similar results for As from Capoeta trutta and Carassius gibelio species in the Tigris River in Turkey. Furthermore, heavy metal pollution was reported to change by season, and in a study, the highest As levels in pike-

perch were found in spring (Dehghani et al., 2022).

### Nickel

Chronic exposure to nickel compounds has negative effects on human health. Nickel is thought to be one of the causes of contact dermatitis, allergic reactions and cancer (Duda-Chodak & Blaszczyk, 2008). Furthermore, nickel is linked to the potential to lead to lung fibrosis and heart and kidney diseases (Denkhaus & Salnikow, 2002). In the present study, the average nickel level of G1 was 0.17 ± 0.03 mg / kg, which was significantly higher than those in the other groups. The permissible daily dose of nickel is 1.46 mg / kg according to EPA (Alibabić et al., 2007; EPA, 1980). The results obtained are below the permissible limits. Alibabić et al. (2007) reported that the average concentration of nickel was 0.15 mg / kg in Salmonidae species. This value is higher than those in G2, G3 and G4 when compared to the present study.Łuszczek-Trojnar et al. (2015) stated the high nickel levels (the average level of 5.2 ± 1.2 mg / kg) from frozen pangasius fillets in Poland. This is quite higher than those from our study. Another study conducted with panga fillets imported from Vietnam indicated that the nickel level was below the detectable limit (Duarte et al., 2019). Aquaculture regions and exposure times to metals might be thought to be important in this difference.

### Copper

Copper status in the body, both deficiency and excess, can affect health cases. Copper is associated with neurological disorders, including Alzheimer's disease and prion diseases (Stern et al., 2007). The permissible limits of copper are higher than those of many metals; its limits are 54 and 30 mg / kg for EPA (1989) and WHO (1989), respectively (Köker et al., 2021). Copper was the only metal whose results were not homogeneous, and the difference among groups was not significant ($P > 0.05$). G1 had the highest average level of 0.23 ± 0.05 mg / kg, which was well below the specified limits. Many studies have shown that copper levels found in fish muscle are below permissible limits (Molognoni et al., 2016; Mortuza & Al-Misned, 2015; Pragnya et al., 2020). Copper deficiency and excess can lead to health problems (Stern et al., 2007). Its permissible limits are quite high compared to many other metals since humans also need copper. Studies on the accumulation of copper in fish meat show that its accumulation is mostly far from posing a threat to humans.

### Manganese

The brain is accepted to be the main target of manganese toxicity because manganese-related toxicity leads to neurological disorders. Moreover, manganese has important roles in immune and antioxidative responses, development, reproduction and energy metabolism in humans (Chen et al., 2018). The WHO reported that the daily requirement of manganese was suggested to be in the range of 2 to 9 mg in the human diet (Łuszczek-Trojnar et al., 2015), and its permissible limit is 1 µg / g (Töre et al., 2021). In the present study, the results of all groups were found to be below permissible limits; furthermore, the manganese levels of G3 were found to be significantly higher than those of G4 ($P < 0.05$). Łuszczek-Trojnar et al. (2015) stated that manganese levels were reported to have an average level of 0.3 ± 0.02 mg / kg in pangasius fillets that came from Asia to Poland markets. This level was lower than those found in Alaska pollock, rainbow trout and tilapia. Elnimr (2011) indicated quite low levels of manganese (0.08 µg / g) in P. hypophthalmus when compared to the present study.

### Cobalt

Cobalt, as an essential metal, plays a vital role in biochemical processes and shows toxic effects in the case of intake above a certain concentration (Zaynab et al., 2022). Moreover, cobalt is a necessary metal for B12 vitamin function (Leyssens et al., 2017). Cobalt shows a tendency to accumulate in the liver, and it is kept at a minimum in muscle tissue (Jayanthi et al., 2019). In the present study, there were no significant differences among groups, and the highest level was found in G1, with an average level of $6.3\times 10^{-3}$ mg / kg, while the levels of cobalt in the other groups were very close to each other. Pragnya et al. (2020) stated that cobalt was below the detectable limits in P. hypophthalmus, and they interpreted that fish came from fishing rather than aquaculture. The maximum permissible limit of cobalt is defined as 1.5mg / kg by the WHO (Jayanthi et al., 2019). The results we found were quite below the limit explained.

### Health risk assessment of metals

Metal and metalloid accumulation in the marine environment can affect marine species as well as humans who consume these species. Although muscle tissue in marine species contains lower metal concentrations than other tissues (Solgi & Beigzadeh-Shahraki, 2019), it may pose a risk depending on the amount consumed. Therefore, EWI (Table 2), THQ (Table 3) and CR (Table 4) were calculated to assess health risks based on panga fillet consumption. To understand the risk effects of metals and metalloids on children and adult consumers, all calculations were made for the consumption of 1, 3 and 7 days per week. PTWI values were used as reference values (safe levels) of heavy metals for making a comparison with EWI levels. In the present study, the EWI values of all samples were much lower than the PTWI values (Table 2).

For the samples studied, the consumption of panga fillets does not pose a risk to consumers regarding the heavy metals studied. Because the limit value is not determined for cobalt (Solgi & Beigzadeh-Shahraki, 2019),

its PTWI has not been set, but its value of maximum tolerable daily intake (MTDI) was indicated as 100 µg / kg body weight (Kukusamude et al., 2021). According to the data above, the MTDI of cobalt was calculated as 7 mg / kg and 3.2 mg / kg for adults and children, respectively. The cobalt data in the present study were found tobe quite below the MTDI calculated.

The highest values of the EWI / PTWI ratio excluding Mn were found for children and in G2. They were found tobe 0,002857, 0,0256, 0,0175, 0,004, 0,018, and 0,007 for Cd, Pb, Hg, As, Ni and Cu, respectively. The highest EWI / PTWI ratios of Mn were found for children and in G4 at 0,004151 and 0,003882 for males and females, respectively. Soegianto et al. (2020) indicated that in the tissues of Anadara granosa (L., 1758), the EWIs of Pb and Cu were found to be below the PTWI values, but the EWI of Cr was

**Table 2.** Estimated weekly intake (EWI; µg / kg BW) for each analyzed metals

| EWI | | Day | Cd | Pb | Hg | As | Ni | Cu | Mn | Co |
|---|---|---|---|---|---|---|---|---|---|---|
| G1 | Adult | 7 | 0,01 | 0,29 | 0,03 | 0,02 | 0,29 | 0,40 | 0,31 | 0,01 |
| | | 3 | 0,00 | 0,13 | 0,01 | 0,01 | 0,12 | 0,17 | 0,13 | 0,00 |
| | | 1 | 0,00 | 0,04 | 0,00 | 0,00 | 0,04 | 0,06 | 0,04 | 0,00 |
| | Children | 7 | 0,02 | 0,64 | 0,07 | 0,05 | 0,63 | 0,88 | 0,69 | 0,02 |
| | | 3 | 0,01 | 0,28 | 0,03 | 0,02 | 0,27 | 0,38 | 0,29 | 0,01 |
| | | 1 | 0,00 | 0,09 | 0,01 | 0,01 | 0,09 | 0,13 | 0,10 | 0,00 |
| G2 | Adult | 7 | 0,01 | 0,23 | 0,00 | 0,02 | 0,17 | 0,28 | 0,29 | 0,01 |
| | | 3 | 0,00 | 0,10 | 0,00 | 0,01 | 0,07 | 0,12 | 0,13 | 0,00 |
| | | 1 | 0,00 | 0,03 | 0,00 | 0,00 | 0,02 | 0,04 | 0,04 | 0,00 |
| | Children | 7 | 0,02 | 0,50 | 0,01 | 0,04 | 0,37 | 0,62 | 0,64 | 0,02 |
| | | 3 | 0,01 | 0,21 | 0,00 | 0,02 | 0,16 | 0,27 | 0,28 | 0,01 |
| | | 1 | 0,00 | 0,07 | 0,00 | 0,01 | 0,05 | 0,09 | 0,09 | 0,00 |
| G3 | Adult | 7 | 0,01 | 0,24 | 0,03 | 0,03 | 0,17 | 0,35 | 0,34 | 0,01 |
| | | 3 | 0,00 | 0,10 | 0,01 | 0,01 | 0,07 | 0,15 | 0,15 | 0,00 |
| | | 1 | 0,00 | 0,03 | 0,00 | 0,00 | 0,02 | 0,05 | 0,05 | 0,00 |
| | Children | 7 | 0,02 | 0,53 | 0,07 | 0,06 | 0,37 | 0,77 | 0,75 | 0,02 |
| | | 3 | 0,01 | 0,23 | 0,03 | 0,03 | 0,16 | 0,33 | 0,32 | 0,01 |
| | | 1 | 0,00 | 0,08 | 0,01 | 0,01 | 0,05 | 0,11 | 0,11 | 0,00 |
| G4 | Adult | 7 | 0,01 | 0,21 | 0,03 | 0,02 | 0,20 | 0,39 | 0,23 | 0,01 |
| | | 3 | 0,00 | 0,09 | 0,01 | 0,01 | 0,08 | 0,17 | 0,10 | 0,00 |
| | | 1 | 0,00 | 0,03 | 0,00 | 0,00 | 0,03 | 0,06 | 0,03 | 0,00 |
| | Children | 7 | 0,02 | 0,46 | 0,06 | 0,05 | 0,43 | 0,85 | 0,51 | 0,02 |
| | | 3 | 0,01 | 0,20 | 0,03 | 0,02 | 0,18 | 0,36 | 0,22 | 0,01 |
| | | 1 | 0,00 | 0,07 | 0,01 | 0,01 | 0,06 | 0,12 | 0,07 | 0,00 |
| PTWI values (µ / kg) | | | 7 | 25 | 4 | 15 | 35 | 125 | 180.7 (male)<br>193.2 (female) | |
| | | | (FAO/WHO)* (Arvay et al., 2015) | (Solgi et al., 2019) | (Solgi et al., 2019) | (Kosker, 2020) | (Kukusamude et al., 2021) | (Kosker, 2020) | (Kukusamude et al., 2021) | |

* The Joint FAO/WHO Expert Committee on Food Additives, FAO: The Food and Agriculture Organization of the United Nations

found to be higher than its PTWI. Similarly, the EWIs of Cd, Pb, Hg and As were below recommended PTWI values (Özden & Erkan, 2016).

THQ is accepted as an indicator to determine potential risks to consumer health (Kosker, 2020). THQ or total THQ values of metals > 1 indicate that the metal intake level is ≥ RfD. THQ or ∑THQ indicates that the metal content in food may cause various health problems for consumers (Kosker, 2020). In the present study, THQ and ∑THQ values were below reference value 1. The calculated THQ values for children were higher than those for adults in G1, G3 and G4. These results show that the panga fillets studied do not pose a health risk for either adult or children consumers in terms of heavy metals. Özden and Erkan (2016) reported THQ values of < 1 in fish studied but also a total THQ value of > 1 in female fish. Similarly, muscle samples of five fish species were reported to have no potential health risk in terms of the intake of toxic metals (Varol & Sünbül, 2018).

The CR value is used to calculate the cancer risk of metals in food for consumers. The CR value must be above $10^{-5}$ to mention a health risk. CR calculations were made based on (US EPA, 2019). In the present study, all results were found to be below the reference value of $10^{-5}$. Among the CR values calculated for children, CRs of Hg in the G1, G3 and G4 groups and CRs of Cd in all groups were detected at closer levels to $10^{-5}$ when compared to others.

## CONCLUSIONS

In this study, panga fish fillets of four different brands were examined in terms of toxic metal accumulation and risks to human health. According to the results, fillets were suitable for heavy metal consumption, but other pollutants, such as organochlorine pesticides or polychlorinated biphenyls, may also be risk factors for consumers, and these kinds of pollutants must be researched. For food safety, all potential pollutants must be researched considering legal limits. However, regular

**Table 3.** Target hazard quotient (THQ), total target hazard quotient (TTHQ) for metals

| THQ | | Day | Cd | Pb | Hg | As | Ni | Mn | ∑THQ |
|---|---|---|---|---|---|---|---|---|---|
| G1 | Adult | 7 | 0,00 | 0,01 | 0,01 | 0,01 | 0,01 | 0,00 | 0,04 |
| | | 3 | 0,00 | 0,00 | 0,01 | 0,00 | 0,01 | 0,00 | 0,02 |
| | | 1 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,01 |
| | Children | 7 | 0,00 | 0,02 | 0,02 | 0,02 | 0,03 | 0,00 | 0,08 |
| | | 3 | 0,00 | 0,01 | 0,01 | 0,01 | 0,02 | 0,00 | 0,05 |
| | | 1 | 0,00 | 0,00 | 0,00 | 0,11 | 0,01 | 0,00 | 0,12 |
| G2 | Adult | 7 | 0,00 | 0,01 | 0,00 | 0,01 | 0,01 | 0,00 | 0,02 |
| | | 3 | 0,00 | 0,00 | 0,00 | 0,00 | 0,01 | 0,00 | 0,01 |
| | | 1 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 |
| | Children | 7 | 0,00 | 0,01 | 0,00 | 0,01 | 0,02 | 0,00 | 0,05 |
| | | 3 | 0,00 | 0,01 | 0,00 | 0,01 | 0,01 | 0,00 | 0,03 |
| | | 1 | 0,00 | 0,00 | 0,00 | 0,09 | 0,00 | 0,00 | 0,10 |
| G3 | Adult | 7 | 0,00 | 0,01 | 0,01 | 0,01 | 0,01 | 0,00 | 0,03 |
| | | 3 | 0,00 | 0,00 | 0,01 | 0,00 | 0,01 | 0,00 | 0,01 |
| | | 1 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,01 |
| | Children | 7 | 0,00 | 0,01 | 0,02 | 0,02 | 0,02 | 0,00 | 0,07 |
| | | 3 | 0,00 | 0,01 | 0,01 | 0,01 | 0,01 | 0,00 | 0,04 |
| | | 1 | 0,00 | 0,00 | 0,00 | 0,13 | 0,00 | 0,00 | 0,14 |
| G4 | Adult | 7 | 0,00 | 0,01 | 0,01 | 0,01 | 0,01 | 0,00 | 0,03 |
| | | 3 | 0,00 | 0,00 | 0,01 | 0,00 | 0,01 | 0,00 | 0,01 |
| | | 1 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,00 | 0,01 |
| | Children | 7 | 0,00 | 0,01 | 0,02 | 0,02 | 0,02 | 0,00 | 0,07 |
| | | 3 | 0,00 | 0,01 | 0,01 | 0,01 | 0,01 | 0,00 | 0,04 |
| | | 1 | 0,00 | 0,00 | 0,00 | 0,11 | 0,00 | 0,00 | 0,12 |

**Table 4.** Target carcinogenic risk (CR) for Cd, Pb, Hg and As

| CR | | Day | Cd | Pb | Hg | As |
|---|---|---|---|---|---|---|
| G1 | Adult | 7 | 5.07E-06 | 2.55E-07 | 4.55E-06 | 3.39E-06 |
| | | 3 | 3.04E-06 | 1.53E-07 | 2.73E-06 | 2.03E-06 |
| | | 1 | 1.01E-06 | 5.1E-08 | 9.11E-07 | 6.78E-07 |
| | Children | 7 | 1.11E-05 | 5.57E-07 | 4.55E-05 | 7.42E-06 |
| | | 3 | 6.65E-06 | 3.34E-07 | 5.98E-06 | 4.45E-06 |
| | | 1 | 2.22E-06 | 1.11E-07 | 1.99E-06 | 1.48E-06 |
| G2 | Adult | 7 | 6.61E-06 | 1.96E-07 | 7.56E-07 | 3.01E-06 |
| | | 3 | 3.96E-06 | 1.18E-07 | 4.54E-07 | 1.8E-06 |
| | | 1 | 1.32E-06 | 3.93E-08 | 1.51E-07 | 6.01E-07 |
| | Children | 7 | 1.45E-05 | 4.29E-07 | 7.56E-06 | 6.58E-06 |
| | | 3 | 8.67E-06 | 2.58E-07 | 9.92E-07 | 3.95E-06 |
| | | 1 | 2.89E-06 | 8.59E-08 | 3.31E-07 | 1.32E-06 |
| G3 | Adult | 7 | 4.94E-06 | 2.1E-07 | 4.73E-06 | 4.32E-06 |
| | | 3 | 2.96E-06 | 1.26E-07 | 2.84E-06 | 2.59E-06 |
| | | 1 | 9.87E-07 | 4.19E-08 | 9.45E-07 | 8.64E-07 |
| | Children | 7 | 1.08E-05 | 4.58E-07 | 4.73E-05 | 9.45E-06 |
| | | 3 | 6.48E-06 | 2.75E-07 | 6.2E-06 | 5.67E-06 |
| | | 1 | 2.16E-06 | 9.17E-08 | 2.07E-06 | 1.89E-06 |
| G4 | Adult | 7 | 5.4E-06 | 1.81E-07 | 4.09E-06 | 3.4E-06 |
| | | 3 | 3.24E-06 | 1.09E-07 | 2.46E-06 | 2.04E-06 |
| | | 1 | 1.08E-06 | 3.62E-08 | 8.19E-07 | 6.8E-07 |
| | Children | 7 | 1.18E-05 | 3.96E-07 | 4.09E-05 | 7.43E-06 |
| | | 3 | 7.09E-06 | 2.37E-07 | 5.37E-06 | 4.46E-06 |
| | | 1 | 2.36E-06 | 7.92E-08 | 1.79E-06 | 1.49E-06 |

monitoring of the metal content of seafood products exported to many countries is important for consumer health.

## COMPLIANCE WITH ETHICAL STANDARTS

### Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Author Contributions

All authors contributed to the study's conception and design. Sample collection and burning process were carried out by Serdar Kilercioglu and Ece Evliyaoglu, also the health risk calculations were made by Ali Riza Kosker. Moreover, all authors have contributed to the writing phase.

### Ethical approval

Ethics committee approval is not required.

### Funding

No financial support was received for this study.

### Data availability

All data generated or analysed during this study are included in this published article.

### Consent to Publish

All authors exist in the present study approve to publish this manuscript. Furthermore, there is no other one who has rights to this manuscript.

## REFERENCES

Agah, H., Leermakers, M., Elskens, M., Fatemi, S. M. R. & Baeyens, W. (2009). Accumulation of trace metals in the muscle and liver tissues of five fish species from the Persian Gulf. Environmental Monitoring and Assessment, 157(1–4), 499–514. [CrossRef]

Alibabić, V., Vahčić, N. & Bajramović, M. (2007). Bioaccumulation of Metals in Fish of Salmonidae Family and the Impact on Fish Meat Quality. Environmental Monitoring and Assessment, 131(1–3), 349–364. [CrossRef]

Andaloro, F., Romeo, T., Renzi, M., Guerranti, C., Perra, G., Consoli, P., Perzia, P. & Focardi, S. E. (2012). Alteration of potential harmful elements levels in sediments and biota from the central Mediterranean Sea (Aeolian Archipelago) following an episode of intense volcanic activity. Environmental

Monitoring and Assessment, 184(7), 4035–4047. [CrossRef]
Árvay, J., Tomáš, J., Hauptvogl, M., Massányi, P., Harangozo, Ľ., Tóth, T., Stanovic, R., Bryndzova, S. & Bumbalová, M. (2015). Human exposure to heavy metals and possible public health risks via consumption of wild edible mushrooms from Slovak Paradise National Park, Slovakia. Journal of Environmental Science and Health, Part B, 50(11), 833-843.
Authman, M. M. (2015). Use of Fish as Bio-indicator of the Effects of Heavy Metals Pollution. Journal of Aquaculture Research & Development, 6(4), 1–13. [CrossRef]
Başaran, B. (2022). An assessment of heavy metal level in infant formula on the market in Turkey and the hazard index. Journal of Food Composition and Analysis, 105, 104258. [CrossRef]
Bernhoft, R. A. (2012). Mercury Toxicity and Treatment: A Review of the Literature. Journal of Environmental and Public Health, 2012, 1–10. [CrossRef] DOI: https://doi.org/10.1155/2012/460508
Bosch, A. C., O'Neill, B., Sigge, G. O., Kerwath, S. E. & Hoffman, L. C. (2016). Heavy metals in marine fish meat and consumer health: a review. Journal of the Science of Food and Agriculture, 96(1), 32–48. [CrossRef]
Botwe, B. O. (2021). Heavy metal concentrations in five fish species from the Gulf of Guinea and their human health implications. Regional Studies in Marine Science, 44, 101763. [CrossRef]
Castro-González, M. I. & Méndez-Armenta, M. (2008). Heavy metals: Implications associated to fish consumption. Environmental Toxicology and Pharmacology, 26(3), 263–271.
Chen, P., Bornhorst, J. & Aschner, M. A. (2018). Manganese metabolism in humans. Frontiers in Bioscience, 1655–1679.
Copat, C., Arena, G., Fiore, M., Ledda, C., Fallico, R., Sciacca, S. & Ferrante, M. (2013). Heavy metals concentrations in fish and shellfish from eastern Mediterranean Sea: Consumption advisories. Food and Chemical Toxicology, 53, 33–37. [CrossRef]
Dambrosio, A., Normanno, G., Storelli, A., Barone, G., Ioanna, F., Errico, L., Centoducati, G. & Storelli, M. M. (2016). Aspects of Vietnamese Sutchi Catfish (Pangasius Hypophthalmus) Frozen Fillet Quality: Microbiological Profile and Chemical Residues. Journal of Food Safety, 36(4), 532–536. [CrossRef]
Dehghani, A., Roohi, A. A. & Dehghani, A. (2022). Trophic transfer, bioaccumulation, and health risk assessment of heavy metals in Aras River: case study—Amphipoda- zander – human. Environmental Science and Pollution Research, 1–10. [CrossRef]
Denkhaus, E. & Salnikow, K. (2002). Nickel essentiality, toxicity, and carcinogenicity. Critical Reviews in Oncology/Hematology, 42(1), 35–56. [CrossRef]
Duarte, G. S. C., Takemoto, R. M., Yamaguchi, M. U., Matos, L. S. & Pavanelli, G. C. (2019). Evaluation of the concentration of heavy metals in fillets of Pangasius hypophthalmus (Sauvage, 1878), Panga, imported from Vietnam. Int J Dev Res, 9(10), 30181–30186.
Duda-Chodak, A. & Blaszczyk, U. (2008). The impact of nickel on human health. Journal of Elementology, 13(4), 685–693.
EFSA (2009). Scientific opinion on arsenic in food, CONTAM (EFSA Panel on Contaminants in the Food Chain). EFSA Journal, 7(10), 1351.
Elnimr, T. (2011). Evaluation of some heavy metals in Pangasius hypothalmus and Tilapia nilotica and the role of acetic acid in lowering their levels. International Journal of Fisheries and Aquaculture, 3(8), 151–157.
EPA (US Environmental Protection Agency) (1980). Ambient water quality criteria document for nickel EPA-440/4-80-060, NTIS PB 81-117715. Cincinnati: OH-US Environmental Criteria and Assessment Office (ECAO).
EPA (US Environmental Protection Agency) (1989). Assessing Human Health Risks from Chemically Contaminated Fish and Shellfish: A Guidance Manual.
Fair, P. A., White, N. D., Wolf, B., Arnott, S. A., Kannan, K., Karthikraj, R. & Vena, J. E. (2018). Persistent organic pollutants in fish from Charleston Harbor and tributaries, South Carolina, United States: A risk assessment. Environmental Research, 167, 598–613. [CrossRef]
FAO (Food Administration Organization) (2017). Food Balance Sheets.
Hajrić, D., Smajlović, M., Antunović, B., Smajlović, A., Alagić, D., Tahirović, D., Brenjo, D., Clanjak-Kudra, E., Djedjibegovic, J., Porobic, A. & Poljak, V. (2022). Risk assessment of heavy metal exposure via consumption of fish and fish products from the retail market in Bosnia and Herzegovina. Food Control, 133, 108631. [CrossRef]
Herrera-Herrera, C., Fuentes-Gandara, F., Zambrano-Arévalo, A., Higuita, F. B., Hernández, J. P. & Marrugo-Negrete, J. (2019). Health Risks Associated with Heavy Metals in Imported Fish in a Coastal City in Colombia. Biological Trace Element Research, 190(2), 526–534. [CrossRef]
Jayanthi, R., Rao, V. A., Babu, R. N., Sivakumar, T., Sriram, P. & Abraham, R. J. J. 2019. Analysis of essential heavy metals in ready-to-eat chicken meat products of Chennai city. Journal of Entomology and Zoology Studies, 7(3), 427–432.
Köker, L., Aydın, F., Gaygusuz, Ö., Akçaalan, R., Çamur, D., İlter, H., Ayoğlu, N. F., Topbaş, M. & Albay, M. (2021). Heavy Metal Concentrations in Trachurus Mediterraneus and Merlangius Merlangus Captured from Marmara Sea, Turkey and Associated Health Risks. Environmental Management, 67(3), 522–531. [CrossRef]
Korkmaz, C., Ay, Ö., Ersoysal, Y., Köroğlu, M. A. & Erdem, C. (2019). Heavy metal levels in muscle tissues of some fish species caught from north-east Mediterranean: Evaluation of their effects on human health. Journal of Food Composition and Analysis, 81, 1–9. [CrossRef]
Kosker, A. R. (2020). Metal and fatty acid levels of some commercially important marine species from the northeastern Mediterranean: benefits and health risk estimation. Environmental Monitoring and Assessment, 192(6), 1–16.
Kubier, A., Wilkin, R. T. & Pichler, T. (2019). Cadmium in soils and groundwater: A review. Applied Geochemistry, 108,

104388. [CrossRef]

Kukusamude, C., Sricharoen, P., Limchoowong, N. & Kongsri, S. (2021). Heavy metals and probabilistic risk assessment via rice consumption in Thailand. Food Chemistry, 334, 127402. [CrossRef]

Kumari, B., Kumar, V., Sinha, A. K., Ahsan, J., Ghosh, A. K., Wang, H. & DeBoeck, G. (2017). Toxicology of arsenic in fish and aquatic systems. Environmental Chemistry Letters, 15(1), 43–64. [CrossRef]

Leyssens, L., Vinck, B., Van Der Straeten, C., Wuyts, F. & Maes, L. (2017). Cobalt toxicity in humans—A review of the potential sources and systemic health effects. Toxicology, 387, 43–56. [CrossRef]

Li, Y., Wang, X., Du, H, Xiao, G. & Guo, L. (2022). Heavy metal accumulation and health risk assessment of crayfish in the middle and lower reaches of Yangtze River during 2015–2017. Environmental Monitoring and Assessment, 194(1), 24. [CrossRef]

Łuszczek-Trojnar, E., Błoniarz, P., Winiarski, B., Drąg-Kozak, E. & Popek, W. (2015). Comparison of cadmium, zinc, manganese and nickel concentrations in fillets of selected species of food fish. Scientific Annals of the Polish Society of Animal Production, 11, 75–84.

Maanan, M. (2008). Heavy metal concentrations in marine molluscs from the Moroccan coastal region. Environmental Pollution, 153(1), 176–183. [CrossRef]

Molognoni, L., Vitali, L., Ploêncio, L. A., Santos, J. N. & Daguer, H. (2016). Determining the arsenic, cadmium, lead, copper and chromium contents by atomic absorption spectrometry in Pangasius fillets from Vietnam. Journal of the Science of Food and Agriculture, 96(9), 3109–3113. [CrossRef]

Mortuza, M. G. & Al-Misned, F. A. (2015). Trace elements and heavy metals in five cultured and captured fishes from Rajshahi City, Bangladesh. Biomedical Sciences Today, 1, 1–9.

Mwakalapa, E. B., Simukoko, C. K., Mmochi, A. J., Mdegela, R. H., Berg, V., Bjorge Müller, M. H., Lyche, J. L. & Polder, A. (2019). Heavy metals in farmed and wild milkfish (Chanos chanos) and wild mullet (Mugil cephalus) along the coasts of Tanzania and associated health risk for humans and fish. Chemosphere, 224, 176–186. [CrossRef]

Orban, E., Nevigato, T., Lena, G. D., Masci, M., Casini, I., Gambelli, L. & Caproni, R. (2008). New trends in the seafood market. Sutchi catfish (Pangasius hypophthalmus) fillets from Vietnam: Nutritional quality and safety aspects. Food Chemistry, 110(2), 383–389. [CrossRef]

Özden, Ö. & Erkan, N. (2016). Evaluation of Risk Characterization for Mercury, Cadmium, Lead and Arsenic Associated with Seafood Consumption in Turkey. Exposure and Health, 8(1), 43–52. [CrossRef]

Pandion, K., Khalith, S. B. M., Ravindran, B., Chandrasekaran, M., Rajagopal, R., Alfarhan, A., Chang, S. W., Ayyamperumal, R., Mukherjee, A. & Arunachalam, K. D. (2022). Potential health risk caused by heavy metal associated with seafood consumption around coastal area. Environmental Pollution, 294, 118553. [CrossRef]

Pragnya, M., Dinesh, K. S., Solomon Raju, A. J. & Murthy, L. N. (2020). Bioaccumulation of heavy metals in different organs of Labeo rohita, Pangasius hypophthalmus, and Katsuwonus pelamis from Visakhapatnam, India. Marine Pollution Bulletin, 157, 111326. [CrossRef]

Rakib, M. R. J., Jolly, Y. N., Enyoh, C. E., Khandaker, M. U., Hossain, M. B., Akther, S., Alsubaie, A., Almalki, A. S. A., Bradley, D. A. (2021). Levels and health risk assessment of heavy metals in dried fish consumed in Bangladesh. Scientific Reports, 11(1), 14642. [CrossRef]

Ratnaike, R. N. (2003). Acute and chronic arsenic toxicity. Postgraduate Medical Journal, 79(933), 391–396. [CrossRef]

Rodríguez, M., Gutiérrez, Á. J., Rodríguez, N., Rubio, C., Paz, S., Martín, V., Revert, C. & Hardisson, A. (2018). Assessment of mercury content in Panga (Pangasius hypophthalmus). Chemosphere, 196, 53–57. [CrossRef]

Ruiz-de-Cenzano, M., Beser, U., Cervera, M. L., & de la Guardia, M. 2013. Fast determination of fish mineral profile. Application to Vietnamese panga fish. Ecotoxicology and Environmental Safety, 95, 195–201. [CrossRef]

Santamaria, A. B. (2008). Manganese exposure, essentiality & toxicity. Indian Journal of Medical Research, 128(4), 484–500.

Simionov, I. A., Cristea, V., Petrea, S. M. & Sirbu, E. B. (2019). Evaluation Of Heavy Metals Concentration Dynamics in Fish From The Black Sea Coastal Area: An Overview. Environmental Engineering and Management Journal, 18(5), 1097–1110. [CrossRef]

Soegianto, A., Putranto, T. W. C., Lutfi, W., Almirani, F. N., Hidayat, A. R., Muhammad, A., Firdaus, R. A., Rahmadhani, Y. S., Fadila, D. A. N. & Hidayati, D. (2020). Concentrations of Metals in Tissues of Cockle Anadara granosa (Linnaeus, 1758) from East Java Coast, Indonesia, and Potential Risks to Human Health. International Journal of Food Science, 2020, 1–9. [CrossRef]

Solgi, E. & Beigzadeh-Shahraki, F. (2019). Accumulation and Human Health Risk of Heavy Metals in Cultured Rainbow Trout (Oncorhynchus mykiss) Form Different Fish Farms of Eight Cities of Chaharmahal and Bakhtiari Province, Iran. Thalassas: An International Journal of Marine Sciences, 35(1), 305–317. [CrossRef]

Stancheva, M., Peycheva, K. & Makedonski, L. (2010). Assessment of heavy metal distribution in muscle, skin and gills of two fish species from the Black Sea, Bulgaria. Scientific Works of the University of Plovdiv, LVII(part 2), 41.

Stern, B. R., Solioz, M., Krewski, D., Aggett, P., Aw T-C, Baker, S., Crump, K., Dourson, M., Haber, L., Hertzberg, R., Keen, C., Meek, B., Rudenko, L., Schoeny, R., Slob, W. & Starr, T. (2007). Copper and Human Health: Biochemistry, Genetics, and Strategies for Modeling Dose-response Relationships. Journal of Toxicology and Environmental Health, Part B, 10(3), 157–222. [CrossRef]

Töre, Y., Ustaoğlu, F., Tepe, Y. & Kalipci, E. (2021). Levels of toxic metals in edible fish species of the Tigris River (Turkey); Threat to public health. Ecological Indicators, 123, 107361.

[CrossRef]

US EPA (2000). Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume II. Risk Assessment and Fish Consumption Limits.

US EPA (2019). Regional screening levels (RSLs) – equations. Retrieved from https://www.epa.gov/risk/regional-screening-levels-rsls-equations

Varol, M., Kaya, G. K. & Sünbül, M. R. (2019). Evaluation of health risks from exposure to arsenic and heavy metals through consumption of ten fish species. Environmental Science and Pollution Research, 26(32), 33311–33320. [CrossRef]

Varol, M. & Sünbül, M. R. (2018). Multiple approaches to assess human health risks from carcinogenic and non-carcinogenic metals via consumption of five fish species from a large reservoir in Turkey. Science of The Total Environment, 633, 684–694. [CrossRef]

WHO (1989). Evaluation of certain food additives and the contaminants mercury, lead and cadmium.

Zaynab, M., Al-Yahyai, R., Ameen, A., Sharif, Y., Ali, L., Fatima, M., Khan, K. A. & Li, S. (2022). Health and environmental effects of heavy metals. Journal of King Saud University - Science, 34(1), 101653. [CrossRef]

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 579-584
DOI: https://doi.org/10.31015/jaefs.2022.4.10

# Modeling wastewater treatment plant (WWTP) performance using artificial neural networks: Case of Adana (Seyhan)

**Metin Dağtekin**[1] • **Bekir Yelmen**[2]

[1] Ceyhan Vocational School, Cukurova University, Ceyhan-Adana, Türkiye
[2] Department of Environmental Protection Control Adana Metropolitan Municipality, Adana, Türkiye

**Citation:** Dagtekin, M., Yelmen, B. (2022). Modeling wastewater treatment plant (WWTP) performance using artificial neural networks: Case of Adana (Seyhan). International Journal of Agriculture, Environment and Food Sciences, 6 (4), 579-584..

**Received:** 25 September 2022
**Revised:** 24 October 2022
**Accepted:** 26 October 2022
**Published Online:** 26 November 2022

**Correspondence:** Metin Dagtekin
**E-mail:** mmdagtekin@gmail.com



## Abstract

In this study, performance estimation of biological wastewater treatment plants (WWTP) was made by applying Artificial Neural Network (ANN) techniques. As material, 355-day data from Adana Metropolitan Municipality Seyhan wastewater treatment plant for 2021 were used. Of the data used, 240 were evaluated as training data and 115 as test data. In the establishment of the ANN model, the daily chemical oxygen demand (COD), daily water flow (Qw) and daily suspended solids (SS) parameters at the entrance of the WWTP were used as input parameters. The daily biological oxygen demand (BOD) parameter was determined as the output parameter. In the study, feed forward back propagation ANN model (FFBPANN) was used to estimate the daily BOD amounts at the entrance of the WWTP. In the statistical analysis, the correlation ($R^2$) values of the input parameters with BOD were found to be 0.906 for COD, 0.294 for Qw and 0.605 for SS. The $R^2$ value was determined as 0.891, the MAE value was 10.32% and the RMSE value was 722.21 in the network structures where the best results were obtained for the test and training data (in the 4-4-1 ANN model). As a result of the study, it was concluded that the ANN model was successful in estimating the BODs of the WWTPs in obtaining reliable and realistic results, and that effective analyzes with the simulation of their nonlinear behavior could be used as a good performance evaluation tool in terms of reducing operating costs.

**Keywords:** Artificial neural network, Biological oxygen demand, Modeling, Waste water treatment plant

## INTRODUCTION

Artificial neural networks collect information about examples, make generalizations, and then make decisions about new examples using the information they have learned when compared to examples they have never seen. Due to these learning and generalization features, artificial neural networks find wide application in many fields of science today and reveal the ability to successfully solve complex problems (Ergezer et al., 2003). In other words, they are computer programs that imitate biological neural networks, which are parallel and distributed information processing structures that are inspired by the human brain, connected to each other through weighted connections, and composed of processing elements, each of which has its own memory (Elmas, 2003).

In addition to factors such as global warming and seasonal changes, the damage to the environment is increasing rapidly due to unsustainable resource consumption. The water used for various vital activities is sent to wastewater treatment

plants through different methods. The estimation of the operating parameters of the plant in the treatment of water with conventional methods takes a long time and constitutes a significant obstacle in terms of efficiency. In addition to the difficulty of the treatment process in the wastewater treatment plants established to purify the wastewater and deliver it to the receiving environments, various models are needed for the efficient operation of the treatment plants (Khatae, 2009). Recently, computer aided methods have found wide application in environmental issues. These methods can be defined as a complex system formed as a result of the interconnection of processors, such as many neurons in the human brain, by various methods. Artificial neural networks have a high approximation capability and have the advantage of solving problems in a short time (Kologirou, 1999; Bechtler et al., 2001). Especially in fields such as agriculture and industry, expressing physical systems with equations and solving mathematical models with computer aid is one of the problems encountered (Hanbay et al., 2006). In a study conducted in Canada, two ANN models, back propagation network and radial basis function, were developed to estimate the nitrogen content in sewage waters. In this developed model, a simulation of nitrogen concentration in wastewater was applied. In this simulation applied, it has been shown that wastewater has fertilizer potential (Sharma et al., 2003). Biological oxygen demand (BOD) is of great importance in terms of water quality in treatment plants. However, it is important to measure and correctly estimate this parameter from an environmental point of view (Hamed et al., 2004; Aguilera et al., 2001). The fact that the BOD measurement process takes up to five days naturally increases the cost. Regression analysis used in the measurement is an important parameter in defining water quality. However, due to non-linear relationships, obtaining effective results does not provide a good modeling opportunity compared to traditional methods. Various methods that can be used for nonlinear cases are widely used today (ANN, BM, ASBS). With the back propagation algorithm, which is one of these methods, it is possible to adjust the weights in order to bring the margin of error to the desired value. This process can be repeated until the optimum solution is reached (Yurtoglu, 2005).

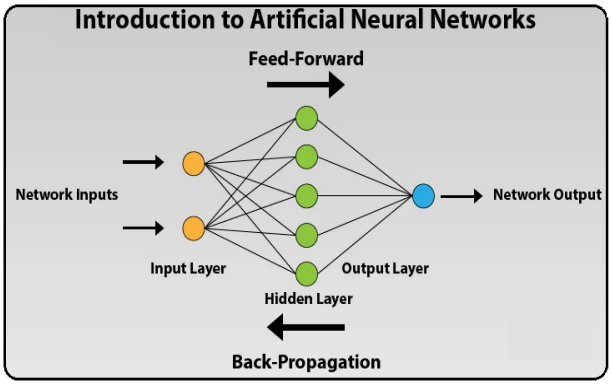
In recent years, ANN-based models with one or more inputs and one or two outputs have been used to predict WWTP performance and ensure plant efficiency. The learning process takes place by training the samples and processing the input and output data. In other words, learning takes place by repeating the training algorithm until a convergence is achieved by using these data (Keskin et al., 2007). In this study, with the help of Artificial Neural Network (ANN) techniques, the performance of Adana Seyhan biological wastewater treatment plant (WWTP) was estimated. In addition, in this estimation, alternative methods have been tried to be determined to reduce BOD measurement costs.

## Artificial Neural Networks (ANNs)

ANNs mimic the working functions of the human brain. They are logical software developed to produce new information by generalizing. They are also artificial systems that model the functions of the human brain (Öztemel, 2012). ANNs can establish connections between memorization and information, together with learning as self-learning mechanisms (Elmas, 1994) [14]. The success of modeling various systems by training existing data with ANN has increased the usability of ANN (Haykin, 1994; Özcalık et al., 2003).

According to the algorithm of ANN, nerve cells are arranged in multiple layers to correlate between inputs and outputs. In an ANN model, there are three layers as input, hidden and output layers, and a network can have more than one hidden layer (Yaldız, 2006). A typical ANN model is given in Figure 1 (URL, 2019).

**Figure 1.** Artificial Neural Network Model




## Feed Forward Back Propagation ANN Model (FFBPANN)

Feedforward neural networks allow for one-way signal flow, and most are organized in layers (Weatherford et al.,2003). The outputs of cells in one layer are given as inputs to the next layer over weights. The input layer transmits the information it receives from the external environment to the hidden layer without making any changes. The network output is determined by processing the information in the hidden and output layer (Öztemel, 2012). In Feedback Artificial Neural Networks (ANNs), at least one cell's output is given as an input to itself or to other cells, and usually the feedback is done through a delay element (Kebalcı, 2014).

## MATERIALS AND METHODS

### Collection of Data

In the study, 355-day data from Adana Metropolitan Municipality Seyhan wastewater treatment plant for 2021 were used as material. Of the data used, 240 were evaluated as training data and 115 as test data. In the establishment of the ANN model, the daily chemical oxygen demand (COD), daily water flow ($Q_w$) and daily suspended

solids (SS) parameters at the entrance of the WWTP were used as input parameters. The daily BOD parameter was determined as the output parameter. Biological oxygen demand (BOD) is shown as one of the most important parameters in the management and planning of water quality. The flow chart of Adana Metropolitan Municipality Seyhan Wastewater Treatment Plant is given in Figure 2 (URL, 2019).

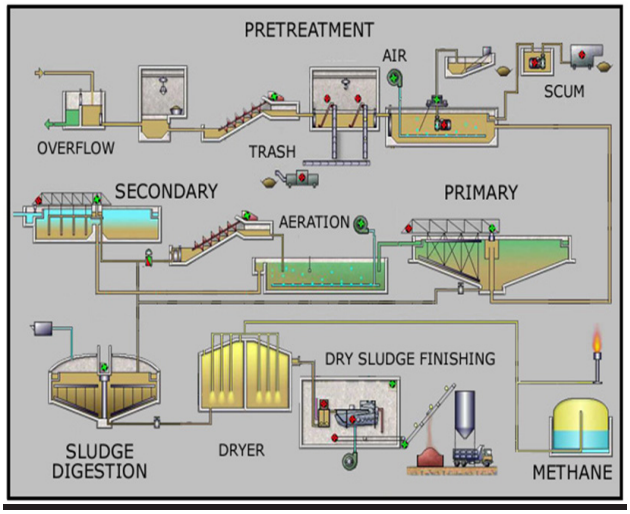



**Figure 2.** Wastewater Treatment Process Step by Step

The statistical analysis results of the data obtained from the treatment plant used as the material in the study are given in Table 1. In the table, the Xave, Sx, Cv, Csx, xmin, and xmax parameters show the mean, standard deviation, variance, skewness, minimum and maximum values of each data, respectively. As seen in Table 1, while the most variable data was seen in SS, the relationship between COD and BOD parameters changed linearly. It is seen that the water flow rate (Qw) is inversely proportional to the BOD ($R^2$=-0.294).

## Application of the FFBP ANN Model

**Table 1.** Data Used in the Study and Statistical Analysis

| Data | BOD (mg/l) | COD (mg/l) | SS (mg/l) | Qw ($m^3$/day) |
|---|---|---|---|---|
| Xaverage | 228.978 | 384.299 | 209.041 | 154494.274 |
| Sx | 74.197 | 136.920 | 104.294 | 7625.813 |
| Cv(Sx/Xaverage) | 0.324 | 0,356 | 0.499 | 0.049 |
| CSX | -0.120 | -0.576 | 0.433 | -0.777 |
| Xmin | 63 | 131.000 | 58 | 106999 |
| Xmax | 474 | 880 | 766 | 178279 |
| Correlation with BOD (R) | 1.000 | 0.906 | 0.605 | -0.294 |

Analysis of 355 data consisting of COD, SS and Qw and BOD from an output vector was considered in the study. The data used did not give very good results in predicting the ANN model. For this reason, the data were normalized and divided into two groups to form training and test sets between 0 and 1. In the study, 240 of the two groups formed from 355 data sets were used as training sets and 115 as test sets. The estimated network structure of the model applied in the study is given in Figure 3.

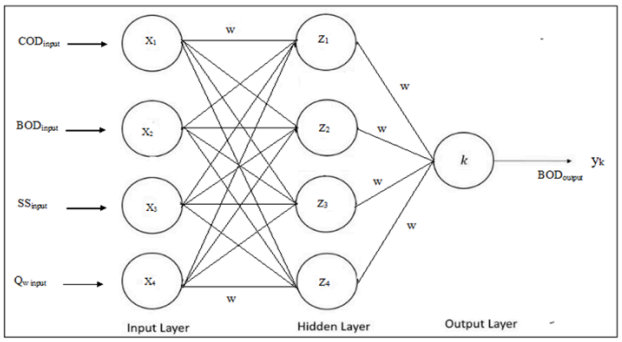



**Figure 3.** Network Structure Estimated in Artificial Neural Networks Model for WWTP Process Control

In the study, statistical functions of correlation coefficient ($R^2$), mean square error (RMSE) and mean absolute error (MAE) were used to evaluate the error levels of the data used (Landeras et al., 2008; Traore et al., 2010; Trejo-Perea et al., 2009; Yılmaz et al., 2008). The equations used in the calculation of the correlation coefficient ($R^2$), mean square error (RMSE) and mean absolute error (MAE) statistical functions are given below;

$$R^2 = \frac{\left[\sum_{i}^{m}(yi-\bar{y})(Oi-\bar{O})\right]}{\sum_{i=1}^{m}(yi-\bar{y})^2\sum(Oi-\bar{O})^2}$$

In the equation;
m, the number of data tested,
Oi, predicted data in neural network,
yi, the calculated amount of data.

$$X_n = \frac{\left(E_{gerçek} - X_{\min}\right)}{X_{\max} - X_{\min}}$$

In the equation;
Xn , Normalized data value,
Xmin, the min value of the data to be normalized,
Xmax, the max value of the data to be normalized.

$$MAE = \frac{1}{N}\sum_{i=1}^{N}\left|y_i - y_i^{'}\right|$$

In this equation;

$\bar{y}$ , the average of the calculated amount of data (yi).

$y = \alpha_1 x + \alpha_0$

In the equation;

x, the argument (amount of data),

α0, intersection,

α1, denotes slope.

$$RMSE = \sqrt{\frac{\sum_{i=1}^{N}\left(yi - yi^{'}\right)^2}{N}}$$

RMSE, the Root of Mean Squared Errors

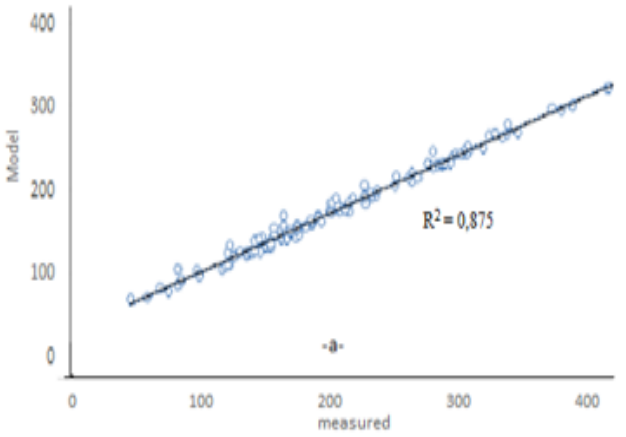
## CONCLUSION

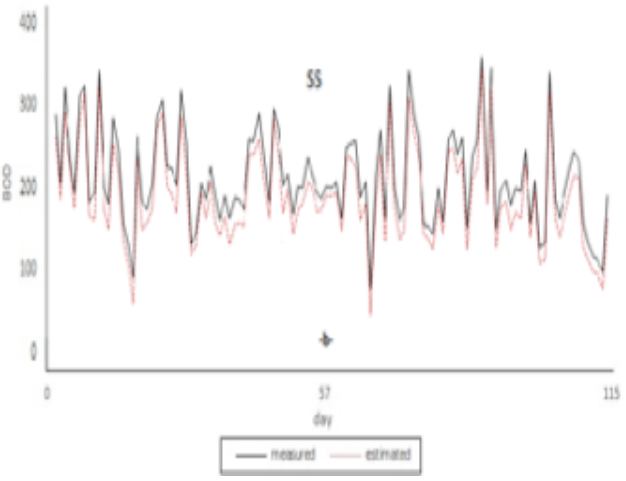
### Performing Sensitivity Analysis Using FFBP ANN Model

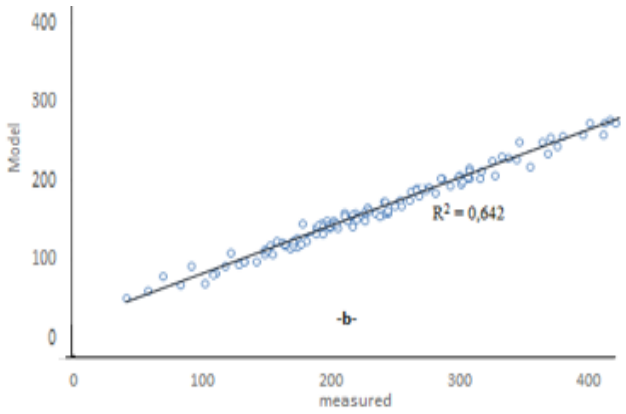
The selection of the input parameters to be used in the FFBP ANN model is important for the performance of the model (Elmas, 2007). Sensitivity analysis, which gives the BOD estimation and the most effective input combinations, was found using the IBGYYSA model (Table 2). In the BOD estimation, almost most of the input parameters were determined to be important compared to the stability analysis. As can be seen in Figures 4 a-b-c, as a result of the calculation of the efficiency degrees in the BOD estimation, it was determined that the most effective parameter was COD, while the least effective parameter was Qw.

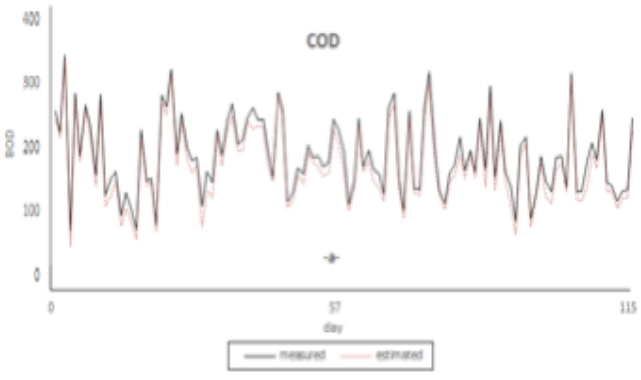
**Table 2.** Determining the Most Effective FFBP ANN Model Using Sensitivity Analysis

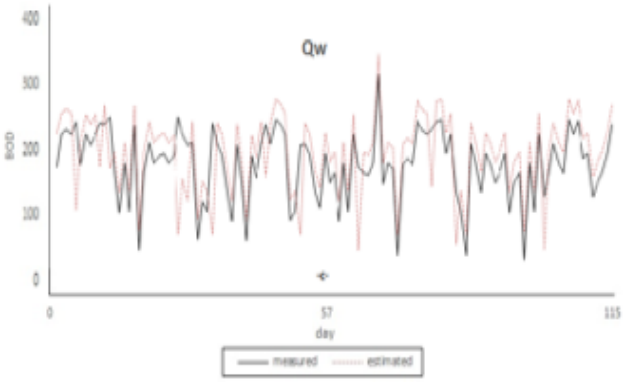
| | MAE (%) | RMSE | $R^2$ |
|---|---|---|---|
| COD+SS+Qw | 10.32 | 722.21 | 0.891 |

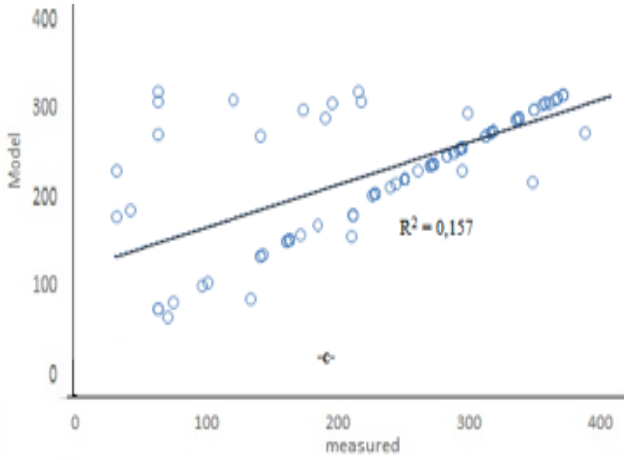





**Figures 4 a-b-c.** Determining the Performance of Input Parameters in Estimating BOD with FFBPANN

## Determining the Most Appropriate FFBP ANN Model

RMSE and $R^2$ performance functions are used to determine the active FFBP ANN model. The number of hidden layer neurons was determined as 4 from the test set performance values as a result of various trials, as shown in Table 3. The ANN (4-4-1) model with the highest performance was determined.

**Table 3.** Determining the Most Effective FFBP ANN Model

| ANN (Number of neurons in layers) | Number of Iterations | $R^2$ | RMSE |
|---|---|---|---|
| ANN(4, 2, 1) | 1000 | 0.887 | 743.12 |
| ANN (4, 3,1) | 1000 | 0.886 | 759.23 |
| ANN (4, 4, 1) | 1000 | 0.891 | 722.21 |
| ANN (4, 5, 1) | 1000 | 0.883 | 764.17 |
| ANN (4, 2, 1) | 2000 | 0.867 | 781.16 |
| ANN (4, 3, 1) | 2000 | 0.869 | 779.22 |
| ANN (4, 4, 1) | 2000 | 0.857 | 792.23 |
| ANN (4, 5, 1) | 2000 | 0.860 | 786.51 |

In the study, the model was tested after the FFBPANN model was trained. It can also be seen from the trend graphs that the estimated values of FFBPANN as a result of the tests performed with BOD are very close to the observed values (Figure 5).

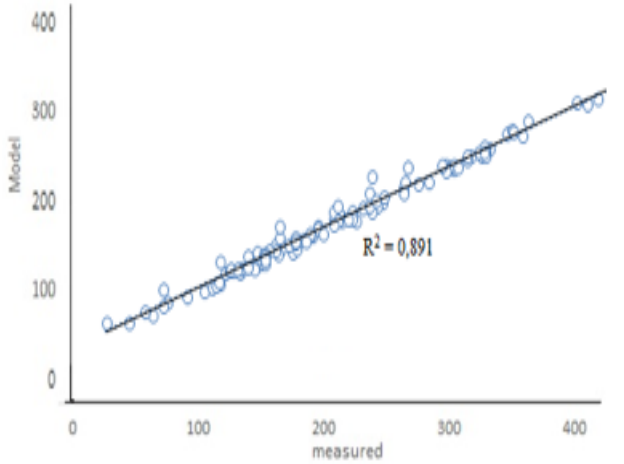




**Figure 5.** Comparison of BOD Estimates with Measured BODs in the FFBP ANN Model

## RESULTS

In this study, using the FFBPANN model, the values of 355 daily BOD amounts (R2) for 2021 at the entrance of Adana-Seyhan wastewater treatment plant were found to be 0.906 for COD, -0.294 for Qw and 0.605 for SS. The $R^2$ value was determined as 0.891, the MAE value was 10.32%, and the RMSE value was 722.21 in the network structures where the best results were obtained for the test and training data (in the 4-4-1 ANN model). These values show that the ANN model used in the study gives very successful results. As a result of calculating the efficiency degrees in BOD estimation by using all of the inputs used for all models, it was determined that the most effective parameter was COD, while the least effective parameter was Qw. In addition, it was determined that the use of ANNs in BOD estimation of all input parameters (COD, SS and Qw) in the sensitivity analysis performed for the determination of the most effective model, because an effective FFBPANN model depends on the input parameters, gives much better results than the conventional models. Well-trained ANN parameters are important for the wastewater treatment processes used in WWTPs to give reliable estimates. In this study, it has been concluded that the ANN model is successful in estimating the BODs of WWTPs in terms of reliable and realistic results, and that effective analyzes with simulation of nonlinear behavior can be used as a good performance evaluation tool in terms of reducing operating costs.

## COMPLIANCE WITH ETHICAL STANDARDS

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

## Ethical approval
Ethics committee approval is not required.

## Funding
No financial support was received for this study.

## Data availability
Not applicable.

## Consent for publication
Not applicable.

## REFERENCES

Aguilera, P.A., Frenich, J.A., Torres Castro, H., Vidal, J.L.M., Canton, M., (2001). Application of the Kohonen Neural Network in Coastal Water Management: Methodological Development for the Assessment and Prediction of Water Quality. Water Research 35 (17), 4053-4062. [Google Scholar]

Bechtler, H., Browne, M.W., Bansal P.K., Kecman, V., (2001). New approach to dynamic modeling of vapour compression liquid chillers: artificial neural networks, Applied Thermal Engineering, 21, 941-953 [Google Scholar]

Ergezer, H., Dikmen, M., Özdemir, E., (2003). Artificial Neural Networks and Recognition Systems. Pivolka, 2(6), 14-17.

Elmas, C., (2007). Artificial Intelligence applications, Seçkin Publications, 425 p.

Elmas, C., (2003). Artificial Neural Networks: Theory, Architecture, Education, Practice. Seçkin Publishing, Ankara.

Hamed, M., Khalafallah, M.G., Hassanein, E.A., (2004). Prediction of wastewater treatment plant performance using artificial neural network. Environmental Modeling and Software 19;919-928 [Google Scholar]

Hanbay, D., Türkoğlu, İ., Demir, Y., (2006). Modeling of Varikap Diode with Adaptive Network Based Fuzzy Inference System, ASYU-Intelligent Systems, Innovations and Application Symposium, İstanbul, 19-21.

Haykin, S., (1994). Neural Networks, A Comprehensive Foundation, Macmi.

Khataee, A.R., (2009). Photocatalyticremoval of C.I. Basic Red 46 on immobilized TiO2 nanoparticles: Artificialneural network modeling. Environ. Technol. 30; 1155-1168. [CrossRef]

Kologirou, S., (1999). Applications of artificial neural networks in energy systems: a review, Energy Conversion and Managemenet, 40 (3), 1073-1087 [Google Scholar]

Landeras, G., Ortiz-Barredo, A., Lopez, J.J., (2008). Comparison of Artificial Neural Network Models and Empirical and Semi-Empirical Equations For Daily Reference Evapotranspiration Estimation in the Basque Country (Northem Spain), Agricultural Water Management, 95:553-565 [Google Scholar]

Sharma, V., Negi, S. C., Rudra, R. P., Yang, S., (2003). Neural networks for predicting nitrate-nitrogen in drainage water, Agricultural Water Management, 63(3), 169-183. [CrossRef]

Yurtoğlu, H., (2005). Predictive Modeling with Artificial Neural Networks Methodology: The Case of Turkey for Some Macroeconomic Variables. General Directorate of Economic Models and Strategic Studies, Specialization Thesis, 104 p., Ankara.

Keskin, M. E., Taylan, E. D., (2007). Stochastic Modeling of Flows in the Central Black Sea Basin. IMO Technical Journal, 4271-4291.

Öztemel E, (2012). Artificial Neural Networks, Papatya Publications 3rd Edition.

Özçalık, H. R., Küçüktüfekçi, A., (2003). Flat and Inverse Modeling of Dynamic Systems with Artificial Neural Networks. KSU Journal of Science and Engineering, 6(1), 26-35.

Kabalcı, E., (2014). Artificial neural networks. Lecture Notes, Retrieved from https://ekblc.files.wordpress.com/2013/09/ysa.pdf

URL1, (2019). Artificial Neural Network Model, Retrieved from https://data-flair.training/blogs/wp-content/uploads/sites/2/2019/07/Introduction-to-Artificial-Neural-Networks.jpg

URL2, (2019). Wastewater Treatment Process Step by Step (https://www.gustawater.com/blog/wastewater-treatment-process.html)

Traore, S., Wang, Y.M., Kerh, T., (2010). Artificial Neural Network for Modeling Reference Evapotranspiration Complex Process in Sudano-Sahelian Zone, Agricultural Water Management, AGWAT-2943; Page 8. [Google Scholar]

Trejo-Perea, M., Herrera-Ruiz, G., Rıos- Moreno, J., Miranda, R. C., Rivas-Arazia, E., (2009). Greenhouse Energy Consumption Prediction using Neural Networks Models. Int. J. Agric. Biol., Mexico, 11(1).

Weatherford, L.R., Gentry, T. W., Wilamowski, B., (2003). Neural network forecasting for airlines: A comparative analysis. Journal of Revenue and Pricing Management. 1(4):319-331. [CrossRef]

Yilmaz, E.C., Dogan, E., (2008). Modelling of Wastewater Treatment Plant Performance Using Adaptive Neuro Fuzzy Inference System, Elect Left Sci Eng, 4(1),1-9.

Yıldız, Ö., (2006). Use of Artificial Neural Networks in Exchange Rate Estimation, Osmangazi University Institute of Social Sciences Master's Thesis, Eskişehir.

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 585-591
DOI: https://doi.org/10.31015/jaefs.2022.4.11

# The effect of rice milling time and feed rate on head rice yield and color properties

**A. Konuralp Eliçin**[1] • **Reşat Esgici**[2] • **Abdullah Sessiz**[1]

[1] Dicle University, Faculty of Agriculture, Department of Agricultural Machinery and Technologies Engineering, Diyarbakır/Türkiye

[2] Dicle University, Bismil Vocational High School, Programs of Agricultural Machinery, Diyarbakır/Türkiye

**Citation:** Elicin, A.K., Esgici, R., Sessiz, A. (2022). The effect of rice milling time and feed rate on head rice yield and color properties. International Journal of Agriculture, Environment and Food Sciences, 6 (4), 585-591

**Received:** 01 August 2022
**Revised:** 10 September 2022
**Accepted:** 15 September 2022
**Published Online:** 29 November 2022

**Correspondence:** A. Konuralp Elicin
**E-mail:** konuralp.elicin@dicle.edu.tr



## Abstract

This study has been carried out to evaluate the head rice yield, kernel broken and the chance of color depend on milling time and feed rate. In this study, threshed rice kernels by combine-harvester at different cylinder speeds, rice harvesting season in 2018, were used for experiment test. According to results, head rice yield decreased slightly as the cylinder speed increased, with yields varying between 71.40 % and 70.28 %. Processing time was found to have a highly significant effect ($p<0.01$) on the quantities of unbroken kernel, broken kernel, bran, yield and husk. The highest rate of broken kernel was obtained at a processing time of 25 seconds, and the lowest values were obtained at a processing time of 10 seconds. With a processing time of 10 seconds, the quantities of unbroken kernel, broken kernel, bran, yield and husk were 70.74, 3.260, 0.810, 74.37 and 24.82 %, respectively. When the processing time was 25 seconds, the quantity of broken kernel decreased from 70.74 percent to 62.86 percent, and yield decreased from 74.37 % to 67.58 %. The broken kernel ratio increased from 3.26 percent to 4.633 percent and bran ratio increased from 0.8642 percent to 1.822 percent. Husk ratio, on the other hand, increased from 24.82 % to 30.60 %. In other words, as the processing time increased, so did the bran ratio and husk ratio. The highest whiteness value of 70.92 was obtained at a processing time of 25 seconds; while the lowest whiteness value of 63.81 was obtained at a processing time of 10 seconds. There were declines in a and b values as processing time increased, although the differences were not statistically significant. The highest a and b values were obtained at a processing time of 10 seconds, with -0.690 and 15.01, respectively. In conclusion, when processing paddy to rice, processing time needs to be increased to obtain a whiter rice; to have fewer broken kernels and a higher head rice yield, on the other hand, the processing time needs to be short.

**Keywords:** Rice processing, Head rice yield, Rice breaking, Milling, Husking

## INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereal crops, being an important source of food, energy and protein worldwide, and the main staple food of more than half the world's population (Roy et al., 2011; Kim and Lee, 2012). According to the FAO, global rice production was 759.6 million tons (503.9 million tons, milled basis) in 2018. Nearly 90 percent of the world's rice is grown and consumed in Asia by 60 percent of the world's population on about 11 percent of the world's cultivated land (Mejía, D.J. 2003; Buggenhout et al., 2013; FAO, 2018). The

rice grain consists of the hull or husk (18–28%) and the caryopsis or brown rice (72–82%). Brown rice consists of an outer layer (pericarp, tegmen and aleurone layers) called bran (6–7%); the germen or embryo (2–3%); and the edible portion (endosperm 89–94%) (Mejía, D.J. 2003; Champagne et al., 2004; Delcour and Hoseney, 2010; Buggenhout et al., 2013; IRRI, 2019). Rice is also the most widely grown staple food, and is a basic source of livelihood for rural households around the globe, given its high economic value. The economic value of harvested rice is based the milled rice yield and the percentage of head rice (Kim and Lee, 2012). Consumers usually prefer milled rice. Before rice is consumed, the husk, bran and germ are removed from the rough rice kernel, and this "milling" operation is a crucial step in the post-production of rice (IRRI, 2019; Zhou et al., 2015). The processing of rice involves several operations. The rice is milled to separate the husk (dehusking) and the bran (polishing), leaving behind the edible portion (endosperm) for consumption. The removal of the rice bran layers in the milling process improves the appearance, cooking quality and palatability of rice, though a major loss of nutrients and high percentage of brokenness results from mechanical milling. For this reason, producing brown rice with minimum breakage in which the maximum possible nutrients are retained, and with preferable cooking attributes, has become the primary goal of the rice processing sector (Mithy et al., 2008; Razavi and Farahmandfar., 2008; Kim and Lee, 2012; Kumar and Kalita, 2017).

The processing of rice is carried out in factories or using a rice milling machine. Rice dehusking involves the removal of the husk and bran from the paddy rice to produce head white rice grains that are sufficiently milled, free from impurities and containing a minimum number of broken grains. The basic objective in a rice milling system is to remove the husk and the bran layers, and to produce an edible, white rice kernel that is sufficiently milled and free of impurities. Depending on the requirements of the customer, the rice should have a minimum number of broken kernels (IRRI, 2019), although during such processing, rice kernels are subjected to mechanical forces during several operations, such as harvesting, threshing, drying, dehulling and milling. When these forces exceed the strength of the rice grain, the individual rice grain breaks and the quantity of broken rice kernels increases depending on the intensity of the processing (Buggenhout et al., 2013). The tendency of rice kernels to break is primarily determined by the fissures, chalkiness, immaturity and dimensions of the rice kernel. Fissured kernels usually break during milling, leading to a reduction in head rice yield, which in turn results in a poor cooking quality and a lower market value (Zhang et al., 2005).

A main challenge faced by the rice sector is minimizing the quantity of broken rice (Buggenhout et al., 2013). If the rice milling machine and operations are not properly designed, rice kernel cracking and breakages increase, resulting in a low market price. The marketing of rice as an agricultural product depends on its physical qualities after processing. The percentage of whole grain is the most important parameter in the rice processing sector. The head rice yield in milling depends on the type of mill, milling cylinder speed, degree of milling, moisture content, milling procedure and the head rice separation method (Correˆa et al., 2007; Schluterman and Siebenmorgen, 2007; Kim and Lee, 2012).

Turkey is an important producer of rice, given the suitability of its climate, soil and environmental conditions. The southeastern part of Turkey, in particular, is an important rice-producing region, and is noted particularly for two rice varieties: Karacadağ Karakılçık and Karacadağ Beyaz, with 95 percent of the cultivation area and production being in the Karacadağ region. The main problems in rice production are the high rate of loss of broken rice kernels during the harvesting, threshing and post-harvesting processing stages (dehulling and milling) (Esgici et al., 2019).

In this study, we focus on the impact of dehulling (husking) and milling on kernel breakage during the processing of rice from the paddy. The study was carried out to identify means of reducing mechanical losses during the processing of rice. To this end, it examines the effect of processing time and the rice feed rate on head rice yield, the quantity of broken grain, bran and husk, and powdered rice, and the changes of color in rice threshed at different cylinder speeds by the combine harvester.

## MATERIALS AND METHODS

### Head Rice Yield, Broken Kernels, Bran Rate and Husking

The milling tests in the study were carried out in the Laboratory of the Department of Agricultural Machinery and Technologies Engineering, Diyarbakır, TURKEY. The commonly grown rice variety Karacadağ Karakılçık (dark) was used for the experiment (Figure 1). The moisture content of the rice kernel samples was measured according in accordance with ASABE standards (ASABE, 2008). Prior to testing, four samples of 25 g rice stems were weighed and dried in an oven at 103°C for 24 h, and were then reweighed to measure the average moisture content of the rice kernels, during which an average moisture content of 10.40 percent w.b was recorded. A moisture content of 14 percent MC is considered ideal for milling (IRRI, 2019). The rice milling machine used in the experiments is shown in Figure 1.

The milling machine comprises two rubber roll dehullers that rotate at different speeds in opposite directions for the husking – being the separation of the hull from the rough rice to obtain brown rice – milling and bran removal operations.

**Figure 1.** Rice Milling Machine

The experiments were carried on rice samples subjected to five different threshing cylinder speeds (650, 750, 850, 950, and 1050 rpm) in the combine harvester. The samples were taken from the threshed paddy for each cylinder speed, while four milling times (10, 15, 20 and 25 second) and four feed rates (20, 40, 60 and 80 gr) were used as independent parameters. The rice yield, broken kernel, bran rate, husking rate and change of color were measured for the different parameters.

The ratio (%) of broken kernels in milled rice was measured by way of a manual analysis using a naked eye identification method. A sub-sample of milled rice was divided visually into broken and whole grain groups. The quantity of broken kernels was calculated by dividing the weight of the broken kernels by the whole kernels, which was then expressed in the form of a percentage. The quantities of broken and unbroken kernels, bran, husk and powdered rice were calculated using the same method. The following equation was used to calculate rice yield (Pınar and Beyhan, 1992).

$$RY = \frac{Mk}{Tk} x100$$

RY= Head rice yield, %
MK: Weight of unbroken and broken kernels and bran after milling, gr
TK: Weight of total milled rice, gr.

### Rice color and whiteness

Food color is the first parameter of quality evaluated by consumers (Markovic et al., 2013). The color of the milled rice seeds was measured on the basis of the CIE color scales L*, a* and b* using a CM11P digital colorimeter (Figure 2). L* indicates the degree of lightness or darkness of the sample on a scale of 0 (black) to 100 (white), where a low number (0–50) indicates dark and a high number (51–100) indicates light; a* indicates the degree of redness (+a) to greenness (-a), where a positive number indicates red and a negative number indicates green; and b* indicates the degree of yellowness (+b) to blueness (-b), where a positive number indicates yellow and a negative number indicates blue, respectively. The samples were placed in the holder of colorimeter, ensuring that the holder was completely filled with seeds. The measurements were made directly from the top surface of the bulk of the seeds, and the color parameters were measured in 15 replications (Hunter at al.,1987; Zielinska et al., 2012; Markovic et al., 2013; Mir et al., 2013) for each test.

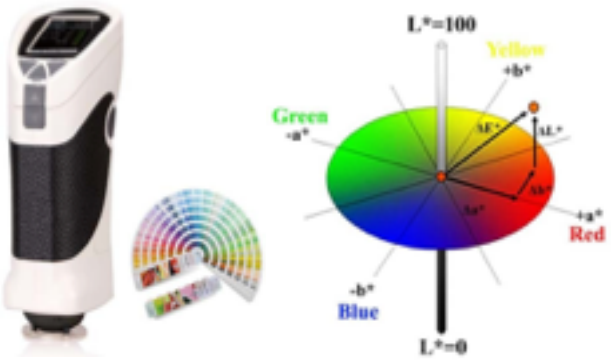



**Figure 2.** CM11P digital colorimeter

### Statistical analysis

The experiment was planned as a complete randomized plot design and data was examined using an analysis of variance (ANOVA) method. Mean separations were made for significant effects with LSD, and the means were compared at 1 % and 5 % levels of significance with Duncan multiple range tests using MSTAT-C software.

## RESULTS AND DISCUSSION

### Broken kernels, yield and husk quantities

Table 1 reports the changes in the quantities of intact kernel, broken kernel, bran, head rice yield and husk of the paddy threshed at different speeds in the combine harvester, depending on threshing speed, processing time and feed quantity. As the table shows, the cylinder speed used during harvesting had a significant effect on all of the measured parameters ($p<0.01$). Cylinder speed affected the quantity of intact (unbroken) kernels after 850 revolutions per minute. No significant difference was found between 650 and 750 rpm, whereas the quantities of intact kernel obtained at 850, 950 and 1050 rpm were significantly different. The ratio of intact kernels was the highest at 650 rpm, with 67.10 %, and the lowest ratio of 65.75 % was obtained at 1050 rpm, as the highest speed tested. This can be attributed to the strength of the collision impact during threshing. A similar situation was observed in the case of broken kernels. The quantity of broken kernels increased as cylinder speed increased. The ratio of broken kernels varied between 3.633 % and 4.485 %. The quantity of bran, similar to broken kernels, was also higher at higher speeds. The lowest bran ratio was obtained at 650 rpm with 0.680 %, and the highest bran ratio of 1.865 % was obtained from the paddy threshed at a cylinder speed of 1050 rpm. Yield, on the other hand, decreased slightly as the cylinder speed increased, with yields varying between 71.40 % and 70.28 %. This

decline can be attributed to the increase in the quantity of broken kernels and the decrease in the quantity of intact kernels at higher cylinder speeds. The same can be said for the quantity of husk, the ration of which was 29.039 % at the lowest cylinder speed of 650 rpm, and decreasing slightly at higher speeds to reach 27.665 % at the highest tested speed (Table 1). The decrease in the quantity of husk was directly related to the increase in the quantity of broken kernels and bran.

Table 1 shows the effects of processing time on the quantities of intact kernel, broken kernel, bran, yield and husk. As the processing time increased, the quantities of broken kernel and bran also increased, while the quantities of intact kernel and yield decreased. The highest proportion of broken kernel was obtained at a processing time of 25 seconds, and the lowest values were obtained at a processing time of 10 seconds. With a processing time of 10 seconds, the quantities of unbroken kernel, broken kernel, bran, yield and husk were 70.74, 3.260, 0.810, 74.37 and 24.82 %, respectively. When the processing time was 25 seconds, the quantity of broken kernel decreased from 70.74 % to 62.86 %, and yield decreased from 74.37 % to 67.58 %. The broken kernel ratio increased from 3.26 % to 4.633 % and bran ratio increased from 0.8642 % to 1.822 %. Husk ratio, on the other hand, increased from 24.82 % to 30.60 %. In other words, as the processing time increased, so did the bran ratio and husk ratio. Kim and Lee (2012) report that the quantity of loss during rice processing varies between 0.58–5.61 %, concurring with the values observed in the present study. After milling, the broken rice grains are often separated from the whole rice grains. Depending on the acceptable level of broken grain allowed by the customer, these broken kernels may then be blended in a precise ratio with the unbroken kernels to maximize the profitability of the mill (Bond, 2004; Buggenhout et al. 2013).

Changes in the quantities of unbroken kernel, broken kernel, bran, yield and husk by feed quantity shows that feed quantity had a significant effect on these values (Table 1). As the feed quantity increased the quantity of unbroken kernels and yield ratio decreased. On the other hand, an increase in feed quantity resulted in statistically significant increases in the quantities of broken kernel and bran. No significant changes were observed in the husk ratio. The lowest unbroken kernel ratio and yield were obtained at a feed quantity of 80 gr, whereas the differences between the other feed quantities were not significant. On the other hand, the highest broken kernel ratio and bran ratio were obtained at this feed quantity, with 4.233 % and 1.721 %, respectively. At feed quantities of 20, 40 and 60 grams, yield did not change, remaining at 71.08 percent, with the lowest yield being 69.87 %. This value is higher than the maximum yield values obtained by Evgi and Ülger (2006) for Baldo and Osmancık paddies, which were 59.9 % and 56.3 %, respectively. Similar findings were also reported by Singha (2012). In a study conducted of 442 paddy processing plants in five states of India, Singha (2013) found that yield varied between 58.6 % and 64 %, depending on the use of either modern or traditional processing methods. Kumar and Kalita (2017) reported theoretical average milling yield of rice for Asian countries of around 71–73 %. The main parameter used to quantify rice dehulling and milling efficiency is the head rice yield (Buggenhout et al. (2013). Andrews et al. (1992), Schluterman and Siebenmorgen, (2007), and Buggenhout et al. (2013) define

**Table 1.** Variation in intact kernel ratio, broken kernel ratio, bran ratio, rice yield and husk ratio by cylinder speed, processing time and feed quantity.

| Parameters | Intact kernel ratio, % | Broken kernel ratio, % | Bran ratio, % | (Yield), % | Husk ratio, % |
|---|---|---|---|---|---|
| Cylinder speed, (rpm) | | | | | |
| 650 | 67.10 a | 3.633 d | 0.680 d | 71.40 b | 29.039 a |
| 750 | 66.87a | 3.886c | 1.011 c | 70.95 a | 27.769 b |
| 850 | 66.82 a | 4.063 bc | 1.454 b | 70.94 a | 27.596 b |
| 950 | 66.08b | 4.155 b | 1.520 b | 70.47 a | 27.540 b |
| 1050 | 65.75 b | 4.485a | 1.865a | 70.28 a | 27.665 b |
| LSD | 0.5166 | 0.2261 | 0.1134 | 0.611 | 0.2228 |
| Processing time, (s) | | | | | |
| 10 | 70.74 a | 3.260 a | 0.810 d | 74.37 a | 24.82 d |
| 15 | 67.35 b | 4.091 b | 1.103 c | 71.64 b | 27.25 c |
| 20 | 65.15 c | 4.193 c | 1.489 b | 69.49 c | 29.02 b |
| 25 | 62.86 d | 4.633 d | 1.822a | 67.58 d | 30.60 a |
| LSD | 0.5166 | 0.2022 | 0.10149 | 0.547 | 0.2228 |
| Feed quantity, (g) | | | | | |
| 20 | 67.00 b | 3.929 b | 0.864 d | 71.08 a | 28.056 b |
| 40 | 67.07 b | 3.850 b | 1.200 c | 71.08 a | 27.720 b |
| 60 | 66.66 a | 4.165 a | 1.439 b | 71.06 a | 27.500 b |
| 80 | 65.379 a | 4.233 a | 1.721 a | 69.87 b | 28.409 a |
| LSD | 0.5166 | 0.2022 | 0.10149 | 0.547 | 0.2228 |

yield as the quantity of kernels that remain after milling. Depending on the type of processing, yield is reported to vary between 0 % and 75 %. In a study conducted by Pınar and Beyhan (1992), the paddy to rice conversion ratio was on average 60.90 percent for rice yield, 8.8 percent for broken rice, 0.8 % for powder (fine) rice, 4.9 % for bran and 24.6 % for husk.

### Rice color and whiteness

The whiteness of the threshed paddy was not affected by the increase in cylinder speed, and the differences between the values from different cylinder rotation speeds were found to be insignificant. This was true also for values a and b. No differences were identified at speeds higher than 650 rpm. On the other hand, processing time was found to have a statistically significant effect on the L value, and insignificant effects on the a and b values. As the processing time increased, the whiteness value (L) also increased (Table 2 and Figure 3). The highest whiteness value (L) was obtained at the processing time of 25 seconds with 70.92, and the lowest L value was obtained at the processing time of 10 seconds with 63.81. The effect of feed quantity on color changes, on the other hand, was insignificant for the L, a and b values. In conclusion, of the independent parameters, only processing time had a significant and noticeable effect on color changes, as can be seen in Table 2 and Figure 3.

## CONCLUSION

Processing time was found to have a highly significant effect ($p<0.01$) on the quantities of intact kernel, broken kernel, bran, yield and husk. As the processing time increased, so did the bran ratio and husk ratio, whereas yield declined. Yield was 74.37 percent at a processing time of 10 seconds, and the lowest yield of 67.58 percent was obtained at a processing time of 25 seconds. The feed quantity had a lesser effect.

Cylinder speed had an insignificant effect on the color of rice threshed and processed at different speeds, whereas processing time had a highly significant effect on the color parameters. The highest whiteness value of 70.92 was obtained at a processing time of 25 seconds; while the lowest whiteness value of 63.81 was obtained at a processing time of 10 seconds. There were declines in a

**Table 2.** L, a and b values

| Parameters | L: lightness or darkness | a: degree of redness (+a) to greenness (-a) | b: degree of yellowness 5 (+b) to blueness (-b), |
|---|---|---|---|
| Cylinder speed, (rpm) | | | |
| 650 | 67.87±0.236483 a | -1.020±0.09385 a | 13.11±0.14708 a |
| 750 | 67.86±0.236483 a | -1.474±0.09385 b | 12.38±0.14708 bc |
| 850 | 67.57±0.236483 a | -1.383±0.09385 b | 12.53±0.14708 b |
| 950 | 67.73±0.236483 a | -1.457±0.09385 b | 12.09±0.14708 c |
| 1050 | 67.46±0.236483 a | -1.368±0.09385 b | 12.14±0.14708 bc |
| LSD | 0.6606 | 0.2621 | 0.4109 |
| Milling time, (s) | | | |
| 10 | 63.81±0.21152 d | -0.690±0.083948 ns | 15.01±0.13155 ns |
| 15 | 67.15±0.21152 c | -1.279±0.083948 ns | 13.00±0.13155 ns |
| 20 | 68.92±0.21152 b | -1.660±0.083948 ns | 11.50±0.13155 ns |
| 25 | 70.92±0.21152 a | -1.732±0.083948 ns | 10.31±0.13155 ns |
| LSD | 0.5906 | 0.2345 | 0.3675 |
| Feed quantity, (g) | | | |
| 20 | 67.68±0.21152 b | -1.375±0.083948 ns | 12.186±0.13155 ns |
| 40 | 67.22±0.21152 b | -1.390±0.083948 ns | 12.523±0.13155 ns |
| 60 | 68.38±0.21152 a | -1.319±0.083948 ns | 12.441±0.13155 ns |
| 80 | 67.52±0.21152 b | -1.275±0.083948 ns | 12.660±0.13155 ns |
| LSD | 0.5906 | 0.2345 | 0.3675 |

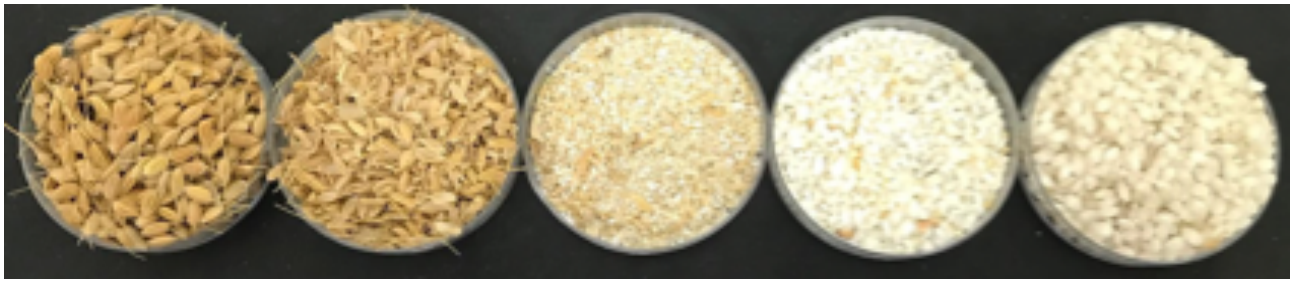


**Figure 3.** Rice color changes at different milling times.

and b values as processing time increased, although the differences were not statistically significant. The highest a and b values were obtained at a processing time of 10 seconds, with -0.690 and 15.01, respectively. In conclusion, when processing paddy to rice, processing time needs to be increased to obtain a whiter rice; to have fewer broken kernels and a higher yield, on the other hand, the processing time needs to be short.

## COMPLIANCE WITH ETHICAL STANDARDS

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Ethics committee approval is not required.

### Funding

No financial support was received for this study.

### Data availability

Not applicable.

### Consent for publication

Not applicable.

## REFERENCES

Asabe Standarts. (2008). Measurement forages. 52nd edn. American Society of Agricultural Engineers, St Joseph MI.

Bond, N. (2004). Rice milling. In: Champagne, E.T. (Ed.), Rice: Chemistry and Technology, third ed. American Association of Cereal Chemists, St. Paul, MN, USA, pp. 283–300. [URL]

Buggenhout, J., Brijs, K., Celus, I., Delcour, J.A. (2013). The breakage susceptibility of raw and parboiled rice: A review. Journal of Food Engineering, 117, 304–315. [CrossRef]

Champagne, E.T., Wood, D.F., Juliano, B.O., Bechtel, D.B. (2004). The rice grains and its gross composition. In: Champagne, E.T. (Ed.), Rice: Chemistry and Technology, third ed. American Association of Cereal Chemists, St. Paul, MN, USA, pp. 77–107. [Google Scholar]

Corre^a, P.C., Schwanz da Silva F., Jaren, C., Afonso Junnior P.C., Arana, I. (2007). Physical and mechanical properties in rice processing. Journal of Food Engineering 79 (2007) 137–142. [Google Scholar]

Delcour, J.A., Hoseney, R.C. (2010). Structure of cereals. Principles of Cereal Science and Technology, third ed. AACC International, St. Paul, MN, USA, pp. 1–22. ISBN: 1891127632 9781891127632

Esgici, R., Pekitkan, F.G., Sessiz, A. (2019). Correlation between rice stem cutting resistance and cracking force of rice kernel. Fresenius Environmental Bulletin. Vol. 28 – No. 4A/2019, p. 3014-3021. ISSN: 1018-4619. [Google Scholar]

FAO. (2018). FAO Rice market monitor. [URL]

Evcim, Ü., Ülger, P. (2006). Determination of the Relationship Between Harvest Moisture and Maximum Yield in order to Get Maximum Efficiency as a result of Fast Drying of Rice. Journal of Tekirdag Faculty of Agriculture, 3(3). [AGRIS]

Hunter, R.S., and Harold, R.W. (1987). The Measurement of Appearance, 2nd ed., John Wiley and Sons, Inc. New York, NY USA, 1987. CIE International Commission on Illumination, Recommendations on Uniform Color Spaces, Color-Difference Equations, Psychometric Color Terms, Supplement No. 2 to CIE Publication No. 15. ISBN: 978-0-471-83006-1

IRRI. (2018). IRRI Rice Knowledge bank. [URL]

Razavi, S.M.A., Farahmandfar, R. (2008) Effect of Hulling and Milling on the Physical Properties of Rice Grains. International Agrophysics. 22: 353-359. [Google Scholar]

Markovic, I., Ilic, J., Markovic, D., Simonovic, V., Kosanic, N. (2013). Color measurement of food products using CIE L* a* b* and RGB color space. Journal of Hygienic Engineering and Design, VOL: 4, p. 50–53. [Google Scholar]

Kim, S.Y, Lee, H. (2012). Effects of Quality Characteristics on Milled Rice Produced under Different Milling Conditions. J Korean Soc Appl Biol Chem., Vol: 55, 643–649, 10.1007/s13765-012-2082-9. [Google Scholar]

Kumar, D., Kalita, P. (2017). Reducing Postharvest Losses during Storage of Grain Crops to Strengthen Food Security in Developing Countries. Foods 2017, 6, 8. [Google Scholar]

Mejía, D.J. (2003). An overview of rice post-harvest technology: use of small metallic silos for minimizing losses. Proceedings of the 20th session of the International Rice Commission. Bangkok, Thailand.

Mir, S.A., Bosco, S.J.D., Sunooj, K.V. (2013). Evaluation of physical properties of 6 rice cultivars grown in the temperate region of India. International Food Research Journal, 20(4): 1521-1527. [Google Scholar]

Mithy, D., Suneel, G., Vishal, K., Rintu, B., and Satish, B. (2008) Enzymatic polishing of rice- A new processing technology. LWT-Food Sci and Technol 41, 2079–84. [Google Scholar]

Pınar, Y., Beyhan, M.A. (1992). Milling Quality and rice losses at paddy factories. 14th National Agricultural Mechanization Congres. Samsun, Turkey.

Roy, P., Orikasa, T., Okadome, H., Nakamura, N., Shiina, T. (2011) Processing conditions, rice properties, health and environment. Int J Environ Res Public Health 8: 1957-1976. [Google Scholar]

Schluterman, D.A., Siebenmorgen T.J. (2007) Relating rough rice moisture content reduction and temper-

ing duration to head rice yield reduction. Trans ASAE 50, 137–142. [AGRIS]

Singha, K. (2012). Economics of Paddy Processing Industry in India: A Case of Karnataka. Scientific & Academic Publishing, USA. ISBN: 978-1-938681-02-8. [Google Scholar]

Siebenmorgen, T.J., Counce, P.A., Lu, R., Kocher, M.F. (1992). Correlation of head rice yield with individual kernel moisture content distribution at harvest. Transactions of the Asae 35 (6), 1879–1884. [Google Scholar]

Singha, K. (2013). Paddy Processing Mills in India. An Analysis. 2013.Journal of Rice Research: Open Access. 1:2. [Google Scholar]

Zhang, Q., Yang, W., Sun, Z. (2005) Mechanical properties of sound and fissured rice kernels and their implications for rice breakage. J Food Eng. Vol:68, p.65–72. [AGRIS]

Zhou, L., Shanshan L., Kimberley P., Severino M., Guoyou, Ye., Xiangqian, Z. (2015). Factors affecting head rice yield and chalkiness in indica rice. Field Crops Research 172 (2015) 1–10. [Google Scholar]

Zielinska, M., Zapotoczny, P., Białobrzewski, I., Zuk-Golaszewska, K., Markowski, M. (2012). Engineering properties of red clover (Trifolium pratense L.) seeds. Industrial Crops and Products, 37: 69– 75. [Google Scholar]

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 592-597
DOI: https://doi.org/10.31015/jaefs.2022.4.12

# Greenhouse gases induced climate change in Turkey and Bodrum district (Mugla province)

**Bahar Ikizoglu**[1]

[1] Environmental Engineering, Engineering Faculty, Süleyman Demirel University, Isparta, Türkiye

## Abstract

Since the industrial revolution, the modes of production and consumption that were incompatible with the environment has led to several environmental problems due to global population growth and welfare demands. In this process, the composition of the atmosphere has changed due to the gradual increase in greenhouse gas emissions by human activities, leading to global climate change due to the greenhouse effect and global warming. Greenhouse gases induced by human activities (transportation, power generation, water consumption, heating, fuel consumption, etc.) are generally measured in $CO_2$ emissions and carbon footprint. Global climate change, a significant global problem, entails the changes in climate due to the greenhouse effect induced by the gases released into the atmosphere by human activities, and this impact is expressed as carbon footprint ($CO_2$ equivalent). The present study aims to investigate the current status of greenhouse gases in terms of carbon footprint and the climate change due to global warming in Turkey, and monthly precipitation, soil temperature, sea water temperature and changes in air temperature were investigated in Muğla province, Bodrum district in Turkey, which is a significant tourist destination. The results showed that global climate change has started to occur in Bodrum district.

**Keywords:** Greenhouse gases, Global warming, Climate change, Bodrum, Muğla

**Citation:** Ikizoglu, B. (2022). Greenhouse gases induced climate change in Turkey and Bodrum district (Mugla province). International Journal of Agriculture, Environment and Food Sciences, 6 (4), 592-597

**Received:** 24 August 2022
**Revised:** 05 October 2022
**Accepted:** 12 October 2022
**Published Online:** 05 December 2022

**Correspondence:** Bahar Ikizoglu
**E-mail:** baharikizoglu@sdu.edu.tr



## INTRODUCTION

Although the earth is inhabitable due to the natural greenhouse effect of the atmosphere, human race faces global warming due to the increase in greenhouse gas volume induced by anthropogenic activities that increased with population growth as a result of the industrial revolution and subsequent developments in science and technology. The climate change due to global warming is associated with the increase in average surface temperature and climatic changes as a result of the rapid increase in the greenhouse gas accumulation in the atmosphere due to human activities such as burning fossil fuels, changes in land use, deforestation and industrialization. Significant variations in extreme mean surface temperatures, ice and snow cover, precipitation, ocean temperature and salinity, wind types, drought, heavy precipitation and heat waves have been considered as the causes of climate change (Binboğa and Unal 2018; İkizoğlu 2019).

Certain greenhouse gases could be released naturally (forest fires, volcanic activities and some biological activities etc.), atmospheric emissions and concentra-

tions could increase due to human activities (agriculture, consumption of fossil fuels, etc.). Greenhouse gases include carbon dioxide, water vapor, ozone, nitrous oxides and methane. The main source of water vapor is the evaporation in the oceans. Ozone is another greenhouse gas formed by atmospheric reactions. The greenhouse gases released only by human activities include chlorofluorocarbons (CFCs), perfluorocarbons (PFCs), and sulfur hexafluoride (SF6) (Argun et al., 2019; İkizoğlu, 2020).

Ecological footprint is the size of the biologically productive land or water resources in global hectares (kha) required to reproduce the resources consumed by an individual, community or activity with current technologies and the resources required dispose of the waste generated by these activities and management of these resources (Erden-Özsoy 2015; Mızık and Yiğit 2020). Ecological footprint includes six categories of productive surface areas: carbon demand on land, forest area, cropland, built-up land, grazing land, and fishing grounds (Apaydın, 2020). Among the components of ecological footprint of Turkey in 2007, the largest share was that of the carbon footprint with 46% Carbon footprint is a measure of the environmental damage induced by human activities based on greenhouse gas production, measured in units of carbon dioxide. Carbon footprint includes of two main categories, primary carbon footprint and secondary carbon footprint. Primary carbon footprint is associated with the direct $CO_2$ emissions due to the combustion of fossil fuels, domestic energy consumption and transportation. Secondary carbon footprint is the $CO_2$ emissions during the life cycle of commodities from production to degradation. The secondary carbon footprint includes the primary footprint. Imported foods or products should be transported by land, air, sea and rail until they reach the consumer. Since the waste generated during the deterioration or consumption of these products are stored and disposed of using the same transportation routes, the secondary carbon footprint is quite important. It is known that an individual's secondary carbon footprint is equal to 54% of the total carbon footprint of that individual. However, it is not possible to exactly calculate the secondary carbon footprint. (Argun et al., 2019; Şahin and Onurbaş 2016; Gökçek et al., 2019; Mutlu, 2021 ).

The aim of this study is to concretize the increasing greenhouse gas emission data in Turkey on the basis of existing data in order to observe and determine the situation in Muğla Bodrum district.

## MATERIALS AND METHODS

The determination and analysis of the current status in Turkey was based on TUIK data and the graphs plotted based on these data.

Daily data were obtained from the Meteorology Directorate in Muğla province Bodrum district, a significant tourist destination in the Aegean Region, and monthly precipitation, monthly average soil temperature at 5 cm, monthly average air temperature, monthly average sea water temperature figures were calculated for 5 years, and the possible effects of global warming were analyzed based on the resulting graphs.


**Figure 1.** Muğla province and Bodrum district on the map of Turkey

## RESULTS AND DISCUSSION

### The distribution of greenhouse gas emissions in Turkey

In Turkey, the total greenhouse gas emission was 220 (Mt) $CO_2$ equivalent in 1990, and it increased to 508 million tons (Mt) $CO_2$ equivalent in 2019. It was 523.9 million tons (Mt) $CO_2$ eq. in 2020, an increase of 3.1%. The increase was 238.14% during the 30 years between 1990 and 2020. The total greenhouse gas emission per capita was 4 tons $CO_2$ eq. in 1990, 6.2 tons $CO_2$ eq. in 2019. and 6.3 tons $CO_2$ eq. Thus, the increase was 1.575 fold in the 30 years between 1990 and 2020 (Figure 2). Figure 3 demonstrates total greenhouse gas emissions by industry in 2020. The energy industry led the emission ranking with 70.2%, followed by agriculture (14%), industrial manufacturing and consumption (12.7%), and waste industry (3.1%). Energy industry emissions increased by 163.1% in 2020 when compared to 1990 and by 0.6% when compared to the previous year, reaching 367.6 Mt $CO_2$ eq. Industrial manufacturing and consumption emissions increased by 190.5% when compared to 1990 and by 14% when compared to the previous year, reaching 66.8 Mt $CO_2$ eq. (TUIK, 2020).

The Kyoto Protocol was based on 6 greenhouse gases. These greenhouse gases and their $CO_2$ equivalents are presented in Table 1 (GWP Global Warming Potential). $CO_2$ equivalent reveals the heat-trapping coefficient of the greenhouse gas when compared to the same volume of $CO_2$. Thus, the impact of all greenhouse gases could be standardized, facilitating the comprehension of emission calculations. Based on this chart, the most dangerous greenhouse gas is sulfur hexafluoride (SF6), and the least dangerous greenhouse gas is $CO_2$. However, the reality is the opposite since $CO_2$ emissions are quite higher when compared to other greenhouse gases (TUIK, 2020).

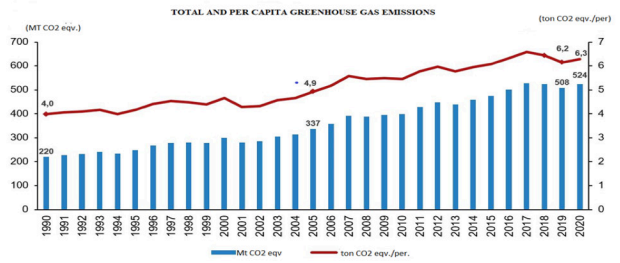



**Figure 2.** Total and per capita greenhouse gas emissions between 1990 and 2020 in Turkey ($CO_2$ eq.) (TUIK, 2020)

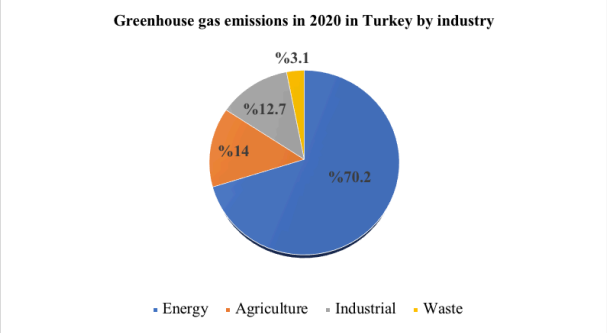



**Figure 3.** Greenhouse gas emissions in 2020 in Turkey by industry (TUIK, 2020)

Conversion of million tons of greenhouse gas (GHG) emission into million tons $CO_2$ equivalent;

Million tons (GHG) * GWP = Million tons $CO_2$ equivalent

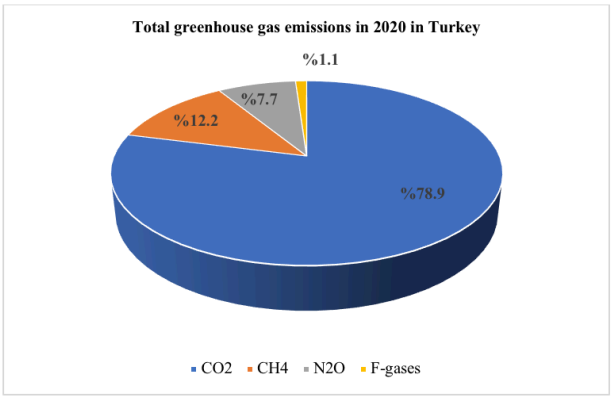
**Table 1.** The principal greenhouse gases in the Kyoto Protocol and $CO^2$ equivalents

| Gases | Global Warming Potential (GWP) |
|---|---|
| $CO_2$ | 1 |
| $CH_4$ | 25 |
| $N_2O$ | 298 |
| HFC | 124 -14800 |
| PFC | 7390-17700 |
| SF6 | 22800 |

In Figure 4, the total greenhouse gas emissions in 2020 in Turkey are presented based on the Kyoto protocol. It could be observed that 78.9% of total emissions (523.9 million tons (Mt) $CO_2$ equivalent) was $CO_2$, 12.2% was $CH_4$, and 1.1% was fluoride gases (HFCs, PFCs, SF6).

In Turkey, forestation activities should be improved to reduce greenhouse gas emissions that increase every year. Furthermore, carbon footprint should be calculated for each province [Tier 1, Tier 2, Tier 3 Approaches (from simple to specialized approach)], the current status should be determined and further measures should be investigated based on population growth predictions.

**Figure 4.** Greenhouse gas emissions in 2020 in Turkey by gas type (TUIK, 2020)




## Interpretation of the current status of climate change based on Bodrum meteorological data

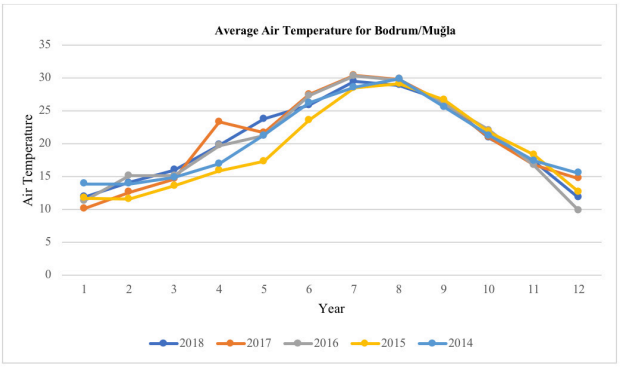



**Figure 5.** The monthly average temperatures in Bodrum in the 5-year period

The analysis of the Bodrum meteorological data revealed that the temperature differences between the seasons gradually decreased during the 5-year period as seen in the monthly average air temperature graph presented in

Figure 5. Especially during the summer and winter, the decrease in the temperature differences is accepted as a result of climate change.

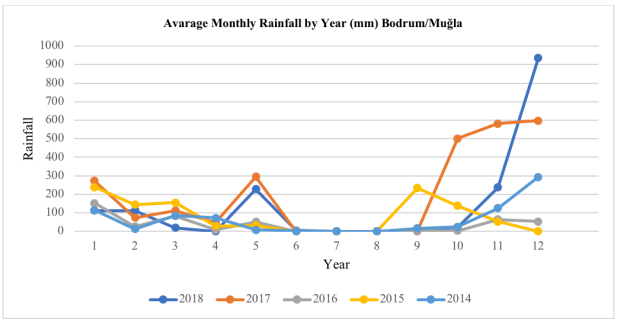



**Figure 6.** Mean monthly precipitation in Bodrum in the 5-year period

Bodrum meteorological data revealed that monthly precipitation varied during these 5 years. The precipitation regime was more irregular when compared to previous years. The summers were drier, and precipitation was irregular in the winters. In recent years, precipitation increased significantly in spring and autumn, demonstrating that Bodrum district was directly affected by global warming or climate change (Figure 6).

Global model simulations based on multiple scenarios predicted that global mean water vapor accumulation and precipitation will increase during the 21st century. In the areas where this increase was predicted, precipitation variations could be higher across the years. Models demonstrated that in warmer climates, evaporation will increase due to the increase in global average precipitation and the frequency of heavy rainfall events. On the other hand, it was predicted that precipitation will increase in certain areas, while decreasing in others, and there could be decreases in ground flow and soil moisture due to increased evaporation in areas where precipitation would increase. Seasonal and latitudinal shifts are predicted in precipitation after certain arid and semi-arid areas will get more arid. In general, precipitation may increase in summers and winters at high latitudes. Precipitation in winter may increase in mid-latitudes, tropical Africa and Antarctica, while it may increase in south and east Asia during summer. A sustained reduction in winter precipitation is expected in Australia, Central America and southern Africa. According to Hadley Center climate models and others, significant reductions in precipitation, water resources and flows are expected during the century, especially in the Eastern Mediterranean basin and the Middle East (Turkes, 2008, Atreya and Kaphle, 2020).

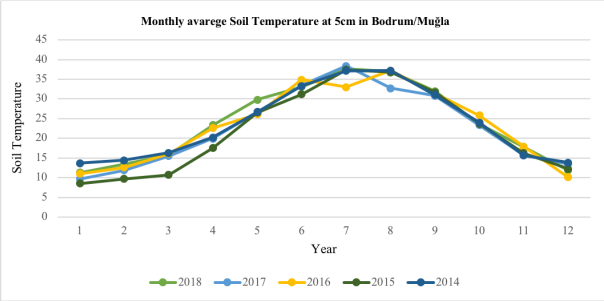



**Figure 7.** Monthly mean soil temperature at 5 cm in Bodrum by year

The average monthly soil temperature at 5 cm increased in the spring of 2018 (in April-May) when compared to the same period in the previous years in Bodrum district in Muğla. Soil temperature depends on the capture and reflection of solar energy. A portion of solar radiation is captured by the earth's surface and the remaining is reflected back to the atmosphere. As the air temperature increases, the soil temperature increases as well. The impact of global warming on the increase in soil temperature is significant (Figure 7).

The fluctuation in soil temperature is associated with changes in minimum and maximum air temperatures and solar radiation (Rathore et al., 1998). Nakadai et al., 2002 reported that soil $CO_2$ emissions are significantly associated with the temperature at 5 cm above the surface and at 0 cm, but not with the temperature below 10 cm. On the other hand, Coşkan et al. 2017 stated that there was a temperature change in the lower layers of the soil, albeit a quite slow one. Furthermore, Nakadai et al. 2002 reported that environmental $CO_2$ levels generally increase after sunset and gradually decrease after sunrise. Kim et al. (2015) reported that higher temperatures (12 and 22 °C) significantly increased $CO_2$ production in peat soil (Akbolat and Coşkan, 2020).

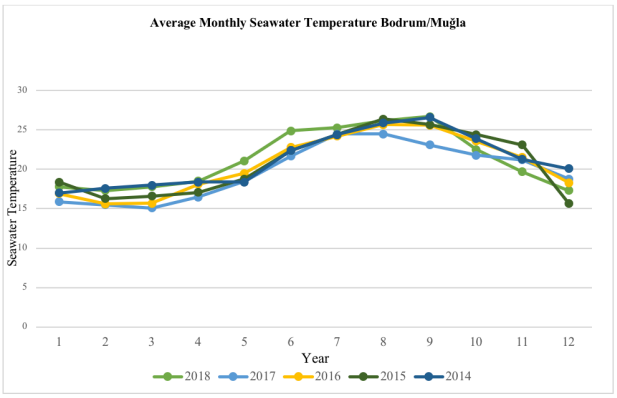



**Figure 8.** Monthly mean seawater temperatures by year in Bodrum district

The average monthly seawater temperature data for Bodrum district in Muğla province are presented for the 5-year period in Figure 8. In April-September 2018, an increase was observed in seawater temperatures when compared to previous years, which continued until October (around 27°C). This could be a direct reflection of seasonal climate change. If the increase in seawater temperatures continue, species in the habitat could change, certain species could become extinct and harmful new species could develop in the marine flora and fauna.

The rise in air and seawater temperatures is estimated to continue in the new millennium. Furthermore, the rise in seawater temperatures due to the melting of glaciers will lead to floods, erosion and increased transportation of sediment. Also, as the glaciers melt, houses, roads, airports, pipelines will be destroyed by landslides in these regions (Erdoğan et al., 2008). In addition, optimum temperature, light, air humidity and air movements, nutrients, water, air and microorganisms in the soil positively affect life and forest growth. Extreme severity in these parameters could lead to damages due to frost, fire, drought, topple, and those caused by plants and animals (fungus and insects). However, as long as there is no human intervention, all ecological factors are balanced within "natural cycles" (Muğla Province Environmental Status Report, (2021).

## CONCLUSION

The annual increase in greenhouse gas emissions leads to the depletion of the ozone layer, global warming and climate change. Without preventive measures, events such as droughts, desertification and erosion could accelerate, agricultural production could decrease in the following years, and the existing water resource problems could exacerbate in Turkey, leading to scarcity of drinking and utility water, flooding in coastal areas, deterioration of the flora and fauna diversity, harmful species (viruses and bacteria) and epidemics may increase. Therefore, today, smart homes, sustainable waste management, forestation, production of some chemicals reduction and/or prohibition etc. has become a priority worldwide.To prevent these problems, greenhouse gas emissions should be investigated, and measures should be adopted for a more liveable and self-sufficient world by countries, regions, and provinces. For this purpose, future greenhouse gas emissions should be predicted.

In the current study, the increasing greenhouse gas emissions in Turkey were investigated based on Bodrum district in Muğla province, and the increases in air, seawater, and soil temperatures at 5 cm, and the precipitation irregularities were identified.

Dissemination of renewable energy sources for heating and power generation, employment of electrical energy, expansion of green buildings and new generation hybrid motor vehicles, and reduction of unnecessary energy consumption are quite important for the sustainability of the world. Further researches should be continued to have clear data about climate change.

## COMPLIANCE WITH ETHICAL STANDARDS

### Conflict of interest

The author declare that they have no conflict of interest.

### Author contribution

The author read and approved the final manuscript. The author verify that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

There are no ethical issues with the publication of this manuscript.

### Funding

The author declared that this study has received no financial support.

### Data availability

All graphs and data obtained or generated during the investigation appear in the published article.

Consent for publication

### Acknowledgements

I would like to thank Bodrum Meteorology Station Directorate for their contributions.

## REFERENCES

Akbolat, D., Çoşkan, A. (2020). Effects of Different Soil Temperature and Reducing Soil Moisture Content on $CO_2$ Production. Journal of the Faculty of Agriculture, 15 (2), 192-198. (in Turkish). [Google Scholar]

Apaydın, Ş. (2020). Effects of Globalization on Ecological Footprint: The Case of Turkey. Journal of Research in Economics, Politics & Finance, 5(1), 23-42. (in Turkish). [Google Scholar]

Argun, M.E., Ergüç, R., Sarı, Y. (2019). Carbon Footprint Investigation of Konya/Selçuklu District. Selcuk Univ. J. Eng. Sci. Tech., 7 (2):287-297. (in Turkish) [Google Scholar]

Atreya, P. N., Kaphle, M. (2020). Visible evidence of climate change and its impact on fruit production in Nepal. International Journal of Agriculture, Environment and Food Sciences. 4 (2): 200-208. [Google Scholar]

Binboga, G., Unal, A. (2018). A Research on the Calculation of Carbon Footprint of Manisa Celal Bayar University at Sustainability Axis. International Journal of Economic and Administrative Studies, 21, 187-202. (in Turkish) [Google Scholar]

Coşkan, A., Erdal, İ., Öz, H. (2017). The Potential of Heat Source of Deeper Soil Layers on Earliness. Academia Journal of Engineering and Applied Sciences, 1, 318-324. (in Turkish) [Google Scholar]

Erden Özsoy C. (2015). Low-Carbon Economy and Carbon Footprint of Turkey. Hak İş International Journal of Labor and Society, 4 (7), 198-215. (in Turkish).

Erdoğan, N., Düzgüneş, E., Balık İ. (2008). Global Warming and Climate Change. Journal of Fisheries & Aquatic Sciences, 1, 89-94. (in Turkish). [Google Scholar]

Gökçek, Ö. B., Bozdağ, A., Demirbağ, H. (2019). Determination of the Carbon Footprint of Nigde Omer Halisdemir University Example. Omer Halis Demir University Journal of Engineering Sciences, Volume 8 (2), 721-730. [Google Scholar]

İkizoğlu, B. (2019). Renewable Energy: An Essential Resource due to the Environmental Effects of Fossil Fuels. Proceedings of the International Conference on Technology and Science, 2(4), 156-163.

İkizoğlu, B. (2020). Climate Change in Turkey. Proceedings of II. International Agricultural, Biological & Life Science Conference, 2(4), 212-216.

Kim, Y., Ullah, S., Roulet, N.T., Moore, T.R. (2015). Effect of inundation, oxygen and temperature on carbon mineralization in boreal ecosystems. Science of the Total Environment, 511, 381-392. [Google Scholar]

Mızık, E.T., Yiğit-Avdan, Z. (2020). The Cornerstone of Sustainability: Ecological Footprint. Journal of Natural Hazards and Environment, 6 (2), 451-457.

Muğla Province Environmental Status Report, (2021). Web Page, Retrieved May 2020. (in Turkish). [Google Scholar]

Mutlu, N. (2021). A research on the determination of energy consumption and carbon dioxide emissions in corn production in Harran plain. International Journal of Agriculture, Environment and Food Sciences. 5(1):23-32. [Google Scholar]

Nakadai, T., Yokozawa, M., Ikeda, H., Koizumi, H. (2002). Diurnal changes of carbon dioxide flux from bare soil in agricultural field in Japan. Applied Soil Ecology, 19 (2), 161-171. [Google Scholar]

Rathore, A.L., Pal, A.R., Sahu, K.K. (1998). Tillage and mulching effects on water use, root growth and yield of rainfed mus-

tard and chickpea grown after lowland rice. Journal of the Science of Food and Agriculture, 78, 149-161. [Google Scholar]

TUIK. (2020). Web page, Greenhouse Gas Emission Statistics 1990-2020. Retrieved July 2022 (in Turkish). [Google Scholar]

Türkeş, M. (2008). What is Climate Change? Basic Definition, Causes, Observed and Predicted Results of Climate Change. Climate Change and Environment, 1,26-37. (in Turkish).

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 598-604
DOI: https://doi.org/10.31015/jaefs.2022.4.13

# Effects of different combinations of growth regulators on the *in vitro* growth parameters of *Poncirus trifoliata* L. (Raf.)

**Elif Subaşı Sevinç**[1] **Bekir Erol Ak**[1] **İbrahim Halil Hatipoğlu**[1] **Heydem Ekinci**[1]

[1] Department of Horticulture, Faculty of Agriculture, Harran University, Şanlıurfa, Türkiye

## Abstract

In this study, an in vitro propagation protocol of *Poncirus trifoliata* L. (Raf.) rootstock, which is one of the main rootstocks used in citrus, was created. Monocular node parts of the plant, which is the source of explant, were cultured in MS nutrient media containing BAP at dissimilar concentrations (0.5-1.0-2.0 mg/l). Afterward, it was observed that shoot appearance and tillering were achieved in the node explants cultured in MS nutrient medium comprising 1.0 mg/l BAP. The shoots were removed to a new culture in MS nutrient media containing NAA and IBA for rooting. While 30% rooting was achieved in the rooting medium, 62% of the rooted plantlets were acclimated to external conditions. 2 mg/l BAP and 2 mg/l silver nitrate ($AgNO_3$) doses were supplied to the MS medium. The results of this study were obtained and a compound was found to the yellowing and leaf fall problem. As a result, in vitro propagation of *Poncirus trifoliata*, which is among the important citrus rootstocks, was tried, and its rooting ability was investigated in different nutrient media concentrations after shoots were obtained from different explants.

**Keywords:** *Poncirus trifoliata* L., *In vitro*, Silver nitrate, Propagation, Tissue culture

**Citation:** Sevinc, E.S., Ak, B.E., Hatipoglu, I.H., Ekinci, H. (2022). Effects of different combinations of growth regulators on the in vitro growth parameters of Poncirus trifoliata L. (Raf.). International Journal of Agriculture, Environment and Food Sciences, 6 (4), 598-604

**Received:** 25 September 2022
**Revised:** 24 October 2022
**Accepted:** 27 October 2022
**Published Online:** 05 December 2022

**Correspondence:** İbrahim Halil Hatipoğlu
**E-mail:** ibrahimhhatipoglu@gmail.com



## INTRODUCTION

Micropropagation is a tissue culture technique used to produce a large number of genetically similar plants from plant parts taken from a plant that has the potential to form a whole plant, in artificial nutrient media and under sterile conditions (Kozai et al. 1997; Hatipoğlu 1999; Mansuroğlu and Gürel 2001; Ak et al. 2021).

In this study, the *in vitro* propagation of the genus *Poncirus trifoliata* L., which is used as a rootstock for citrus fruits and preferred as an indoor ornamental plant in landscaping in our country, was tried. Different concentrations have been developed for rooting conditions. It is aimed to be a reference source for *in vitro* researches on this rootstock.

## MATERIALS AND METHODS

### Material

This study was carried out in the laboratory of the Department of Horticulture, Faculty of Agriculture, Harran University. In the study, 5-year-old *Poncirus trifoliata* cuttings were used as material.

## Methods

After cutting the leaves of the new cuttings taken from the plant and separating the leaves, approximately 15-20 mm green cuttings were procured. The cuttings, which were washed under water for 30 minutes in the laboratory environment, were kept at 70% ethyl alcohol concentration for 1 more minute and then rinsed with sterilized distilled water. After the rinsing phase, the materials are dipped in 5%, 10% and 20% commercial-gradely used bleach (0.525% NaOCl) to which 1-2 drops of Tween 20 are added for sterilization, and the surface is cleaned for 15 minutes, and then 3 times for 5 minutes. It was washed with sterile distilled water and sterilization was concluded. Shoot growth was observed in equal number of materials (10 each) sterilized at these 3 different bleach concentrations, and no contamination was observed at 10%.

After sterilization was concluded, the explants were transferred to the culture medium. The pH value of the culture medium containing 30 g/l sucrose, 7 g/l agar, 4.4 g/l MS and 1 mg/l BAP to support the growth and development of the plant was brought to 5.8 with added NaOH and HCl. After the culture medium was boiled, 0.3 g of $GA_3$ hormone was added to support the longitudinal growth of the plant. Then, every 7 days, the percentages of explants showing signs of contamination in the culture medium and growing healthy were affected as percentages.

Equipment such as magenta cups, blotting papers, scalpels and forceps to be used in the experiment are wrapped in aluminum foil in sufficient amounts (to be completely covered) and heated at 1 atm for 15 minutes. It was sterilized in an autoclave at 121°C under pressure. The forceps and scalpels used at every stage of the study were sterilized continuously by immersing them in 96% ethyl alcohol concentration. 15 minutes before starting work in the cabinet, the cleaning of the sterile cabinet was carried out with 70% ethyl alcohol concentration. Afterward, the sterilization of the cabin was concluded by keeping the UV lights on for 15 minutes, and then work was started in laminar flow cabinet. The nutrient media were sterilized in an autoclave at 121°C for 15 minutes under 1 atm pressure.

For the experiment, a climate cabin with a light intensity of 2500 - 3000 lux and a light intensity of 10000 lux was used. The interior of the cabin is programmed to have a photoperiod of 16 hours, with 16 hours of light and 8 hours of darkness. The temperature of the environment inside the cabinet was kept constant at 24±1°C.

After superficially sterilized cuttings were cut with at least one eye on them, they were planted in containers containing 10 ml of initial culture medium. The development of the explants was followed every 7 days until leaves and shoots were obtained in the initial culture medium. In order to reproduce the shoots, the shoot clusters with tillering were separated into individual shoots, the tillering numbers were settled and the transfer to the new culture medium was ensured. The pH value of the MS culture medium, which is preferred as the initial culture medium, containing 6 g/l agar, 1 mg/l BAP, and 20 g/l sucrose, was adjusted to 5.8 with the support of HCl and NaOH before autoclaving. After this process, the culture medium was sterilized at 121°C for 15 minutes. After sterilization, 2-4 cm long explants were taken and transferred to *in vitro* shoot culture medium without damaging the eyes on the shoots. These explants were transferred to subculture in the same shoot culture medium at intervals of 4 weeks until leaf appearance was observed in the shoot culture medium and until new shoot appearance was observed.

Each shoot of 2-5 cm long explants that formed callus was separated and transferred into different containers. Three different shoot rooting media were created.

The development of the produced materials in 3 different mediums was examined for 4 weeks and no signs of rooting were observed. Yellowing and shedding of the leaves of the plants have been observed, and the reason for this is; It has been concluded that there is ethylene production, which is called "Maturation or Aging Hormone" due to the effect of accelerating maturity and color appearance, causing fruit and leaf fall on plants (Walsh, 2003).Later, in the literature studies on this subject, studies proving that silver nitrate is a very effective inhibitor of ethylene effect were encountered. And 2 mg/l of BAP and 2 mg/l of silver nitrate ($AgNO_3$) were added to the MS medium that in order to reduce the amount of phenolic substance in the medium. The results of this study were obtained and a compound was found for the yellowing and leaf fall problem. In the rooting medium, the materials formed callus, but no rooting was observed.

In order to find an infusion to the rooting problem, a new rooting medium has been produced. 500 mL of growth hormone NAA to support rooting and growth hormone widely used in rooting of cuttings to support rooting in a medium consisting of 20 g/l sucrose, 6 g/l agarose and 1/2 MS. hormone, 500 mL of IBA was added. The pH of the rooting nutrient medium was adjusted to 5.8 with NaOH or HCl before sterilization. The environmental conditions of this rooting medium were adjusted to be compatible with the environmental conditions of the previous experiments. Results were obtained from this experiment and rooting was observed in the materials.

It was observed that the explants in the rooting medium formed a sufficient amount of roots under micropropagation conditions after about 30-35 days. After this stage, after the explants were removed from the nutrient medium. Then a mixture of 1/2 peat and ½ perlite was made provision for, this mixture was sterilized at 121°C for 15 minutes. And the explants were staggered into a sterile soil medium, which was evenly distributed in plastic containers. In order to facilitate the adaptation of the plants to the external environment, the explants were covered

with perforated transparent containers and the humidity rate was tried to be kept in balance. The pots containing the plants, which were tried to be acclimatized to the outside conditions, were opened every day more (5-10 minutes) after about a week, and the preparation of the plantlets for the external environment was supported.

Root measurements were made after the rooted plants were removed from the nutrient media and cleaned to be surprised in the soil. And the root length of each plant material was measured and the average root length was calculated. Then, they were transferred to medium containing 50% peat and 50% perlite.

The statistical evaluation of the data obtained as a result of the research in the *in vitro* technique trial for *Poncirus trifoliata* L. rootstock was taken randomly from different parts of the plant, the stages of the research were repeated several times without being dependent on each other, and each independent trial consisted of a different number of shoots. When the statistically notable transactions were detected, the differences between the mean data were subjected to the LSD test at the $P<0.05$ level. In order to settle the effects of different cytokinin, gibberellic acid and auxin densities on the parameters, PCA analysis was performed with the support of the PAST package program.

## RESULTS AND DISCUSSIONS

### Determination of Sterilization Protocol

The most basic issue in *in vitro* (tissue culture) studies is the effectual sterilization of plant materials. In the literature review, which is the first step of the research, our priority has been to investigate research on sterilization in detail. Again, the most important step *in vitro* studies is the surface sterilization of theplantmaterials to be used in these studies and the selection of the most appropriate sterilization method for the material to be used. Sterilization methods differ according to the media in which the plant material is grown and the parts of the plant material from which the explant is taken. The most commonly used disinfectants in the sterilization phase of explants are; sodium hypochlorite, ethanol, silver nitrate, calcium, mercury, and hydrogen peroxide chloride can be counted as (Babaoglu et al., 2002).

In general, the sterilization process of the explants at the beginning of the *in vitro* experiments is used to break the superficial resistance of these explants. rinsing in sterilized distilled water (Ainsley et al., 2001a). In addition, some researchers perform plant material sterilization in some stages. Some researchers (Gurel and Gulsen 1998; Ainsley et al. 2001b; Pruski et al. 2005) rinsed the explants under running water for different times 'tween 5 minutes and 2 hours before sterilizing the plant material to ensure rough cleaning. Muna et al. (1999), on the other hand, did not find rinsing the plant materials under running water sufficient to provide rough cleaning and washed them with one or two drops of Tween-20 for 10-15 minutes and then scourged to surface sterilization. In Jain and Babbar (2003), they first washed the plant materials that they would use in their studies with 10% Teepol and then rinsed them under running water for 30 minutes and passed to the surface sterilization stage. Espinosa et al. (2006) kept the plant materials in ethanol at 50-70% immersion for a short time between 30 seconds and 5 minutes and then sterilized the plant material with commercial-grade bleach.

In this study, no contamination was observed in the initial cultures of plant materials that had been superficially sterilized in 10% NaOCl. *In vitro* shoots did not undergo any morphological changes in the observed process and continued to develop healtihily. In this trial, 10% NaOCl disinfectant solution was settled to be the most suitable concentration for the surface sterilization of plant materials, and this concentration was continued to be used during the sterilization of the plant material throughout the research process.

### Shoot Development of Explants and Proliferation

In this study, the tip of the shoots and semi-woody parts of the plant material were used as explants.

There is MS nutrient medium containing 6 g/l agar and 20 g/l sucrose in the nutrient medium primed for the shoot development of the explants taken from the shoot tips of *Poncirus trifoliata* L. rootstock. In this environment, shoot growth was observed in plants. Approximately 4 weeks after the beginning of the culture, the ratios of explants that developed enough to be subcultured and explants that did not show sufficient growth were settled.

As it was applied on the shoot tip explants, plant parts formed by separating the tilled explants after the shooting medium were also grown in the same medium. By adding 0.5, 1.0 and 2.0 mg/l BAP to the same combination, the effects of node explants taken from the plant on shoot growth were investigated and the number of leaves, shoot length and shoot number were measured in the following process. (Table 1.) Approximately 3 weeks after the start of culture, the ratio of explants that developed enough to be subcultured and explants that did not show growth were settled.

In the study, firstly, the explants taken from the plant were taken into the shooting medium at different BAP doses (0.5 mg/L, 1 mg/L and 2 mg/L) and their development was examined. The growth rates of explants were calculated in the number of leaves, shoot number and shoot length measurements made in shoot appearance media. As a result of these calculations, the explants in the medium containing 1 mg/L BAP showed more growth than the explants in the other media. The explants in this medium were 11.1% in terms of the number of leaves compared to the explants in the medium containing 0.5 mg/L BAP; It increased 20.48% more than the explants in the medium containing 2 mg/L BAP. In terms of shoot number, 5% compared to explants in medium

**Table 1.** Explant growth chart at different BAP concentrations

| | | 1st Medium (0.5 mg/L BAP) | 2nd Medium (1 mg/L BAP) | 3rd Medium (2 mg/L BAP) | LSD (%5) |
|---|---|---|---|---|---|
| 1st Measurement | Number of Leaves | 1.50±1.14b | 3.00±0,57a | 1.68±1,52b | 0.943 |
| | Number of Shoots | 1.20±0.91b | 2.10±0,57a | 1.30±0,57b | 0.518 |
| | Length of Shoots | 1.16±0,30b | 1.63±0,15a | 1.84±0,35b | 0.468 |
| 2nd Measurement | Number of Leaves | 2.10±0,57b | 4.00±1,53a | 2.40±1,15b | 1.079 |
| | Number of Shoots | 1.70±1,00b | 2.70±0,82a | 1.70±0,57b | 0.668 |
| | Length of Shoots | 1.49±0,21b | 2.19±0,72a | 1.27±0,37b | 0.469 |
| Average | Number of Leaves | 1.80±0.30b | 3.50±0.50a | 2.04±0.36b | 1.011 |
| | Number of Shoots | 1.45±0.25b | 2.40±0.30a | 1.50±0,20b | 0.593 |
| | Length of Shoots | 1.32±0.16b | 1.91±0.28a | 1.55±0.28b | 0.468 |

The parameters that LSD values were determined and given letters were found to be statistically significant.

containing 0.5 mg/L BAP; It increased 20% more than explants in medium containing 2 mg/L BAP. In terms of shoot length, 69% compared to explants in medium containing 0.5 mg/L BAP; It showed 40% more growth than explants in medium containing 2 mg/L BAP. Thus, shoot medium containing 1 mg/L was settled as the most appropriate immersion.

In order to probe the effect of $GA_3$ on shoot growth at the proliferation stage, 2 different media were tried and the results are given in Table 2.

Then, to transfer the explants in MS culture medium containing 1 mg/l BAP, 30 g/l sucrose, 7 g/l agar, which was settled as the initial culture medium, transfer to 2 different mediums was tried. Half of the explants were given to 1st medium containing 20 g/l sucrose, 1/2 MS, 6gr/l agar, 1 mg/l BAP, 0.5 mg GA; the other half was taken into the second medium containing 20 gr/l sucrose, 2.2 mg/l MS, 6gr/l agar. The mean values of the explants, whose growth was observed for 2 weeks, in the media after 2 weeks were calculated. While the average shoot length of the explants in the 1st medium was 1.97 cm and the average number of leaves was 11.11, the average shoot length of the explants in the 2nd medium was 2.18 cm and the average number of leaves was 15.12.

Then, the development of the explants at 3 different Rooting concentrations prime for the explants that concluded their development in the BAP medium settled at the appropriate doses was observed and their measurements were made (Table 3). In these measurements, the number of leaves, shoot length and callus appearance of the explants were examined.

Callus appearance was observed in the explants, but rooting was not observed. Then, 500 mL of NAA and 500 mL of IBA were added as rooting hormone to support rooting in the medium consisting of 20 g/l sucrose, 6 g/l agar and 2.2 g/l MS. The pH of the rooting nutrient medium

**Table 2.** Effects of GA3 on shoot development

| | | 1st Medium (20 g/l sucrose, 2.2 mg/l MS, 6gr/l agar, 1 mg/L BAP) | 2nd Medium (20 g/l sucrose, 2.2 mg/l MS, 6gr/l agar, 1 mg/l BAP, 0.5 mg $GA_3$) | LSD (%5) |
|---|---|---|---|---|
| 1st Measurement | Number of Leaves | 12.44±2.51a | 8.37±9.33b | 3.816 |
| | Length of Shoots | 1.80±0.30a | 1.43±0.62a | n.s. |
| 2nd Measurement | Number of Leaves | 14.56±3.05a | 11.11±3.05a | n.s. |
| | Length of Shoots | 2.10±0.43a | 1.87±0.43a | n.s. |
| Average | Number of Leaves | 13.50±1.06a | 9.74±1.37b | 4.236 |
| | Length of Shoots | 1.95±0.15a | 1.65±0.22b | 0.620 |

The parameters that LSD values were determined and given letters were found to be statistically significant.

**Table 3.** Explant growth at different rooting concentrations

| | | 1st Medium (0,25 mL/l NAA) | 2nd Medium (0,15 mL/l NAA+ 0,15 mL/l IBA) | 3rd Medium (0,15 mL/l IBA) | LSD (%5) |
|---|---|---|---|---|---|
| 1st Measurement | Number of Leaves | 2.50±0.57a | 2.70±1.00a | 1.90±1.00a | n.s. |
| | Length of Shoots | 9.43±2.08a | 10.00±0.68a | 8.80±6.08a | n.s. |
| 2nd Measurement | Number of Leaves | 2.75±0.57a | 4.20±2.33a | 2.60±0.57a | n.s. |
| | Length of Shoots | 6.00±2.08a | 11.00±8.18a | 11.89±4.35a | n.s. |
| Average | Number of Leaves | 2.62±0.12a | 3.45±0.75a | 2.25±0.35a | n.s. |
| | Length of Shoots | 7.71±0.71b | 10.50±0.50a | 10.34±1.54a | 7.506 |

The parameters that LSD values were determined and given letters were found to be statistically significant.

was first adjusted to 5.8. Results were obtained from this experiment and the rooting phase of the explants was realized. The rooted explants were stunned with 50% peat and 50% perlite and their roots were expected to develop well in order to acclimate them to the external environment, and after they had developed sufficiently, they were transferred to the media and continued to be observed. 62% of these plants healthily continued theiır lives (Figure 1.).


**Figure 1.** Images of explant development at different stages (1.Shoot-forming explant, 2. Explants in proliferation medium, 3 Explants in rooting medium, 4. Rooted explant)

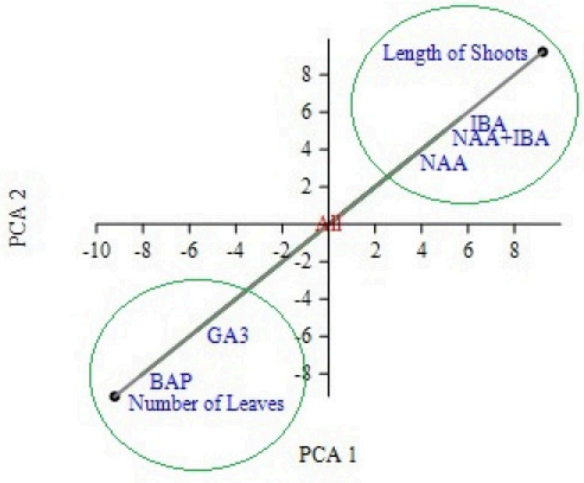



**Figure 2.** Effects of hormones according to PCA

In the statistical evaluation (PCA) made as a result of these measurements, it was concluded that cytokinin and gibberellin were effectual in the number of leaves and auxins were effectual in the length of shoots (Figure 2.).

Ali and Mirza (2006) have experienced that the appropriate medium to ensure callus appearance in propagation experiments from Citrus explants with tissue culture tack is MS medium with 1.5 mg/l 2,4 D added, and MS medium with 3 mg/l BA added for rooting. Amgai et al. (2016) investigated the effects of 10 different hormone levels including BAP and IAA in MS medium and 3 different culture periods of 4, 8 and 12 weeks on the reproduction of C. reticulata Blanco (mandarin) plant *in vitro*. The most successful shoot appearance was obtained in nutrient media containing 0.2 mg/l IAA and 0.5 mg/l BAP. Cengiz and Kacar (2019) carried out micropropagation and rooting experiments of citrus rootstocks named 'C-35 citrange' and 'Tuzcu 31-31 orange' under the influence of the traditionally known solid culture and temporary immersion principle in tissue culture, in comparison with the Plantform bioreactor system. In solid culture micropropagation experiments on citrus rootstocks, Murashige, Woody Plant (WPM) and Skoog (MS) were also used as a plant growth regulator with nutrient media Kinetin (KIN) (0; 0.5; 1.0 mg L-1), Isopentenyladenine (2IP) (three different concentrations: 0; 1.0; 2.0 mg L-1) and Benzylaminopurine (BAP) (0; 1.0; 2.0 mg L-1). In rooting trials; MS, ½ MS, WPM to nutrient media Indole-3-butyric acid (IBA) (0; 0.5; 1.0; 2.0 mg L-1) and Naphthaleneaceticacid (NAA) (0; 0.5; 1, 0; 2.0 mg L-1) by including different concentrations of plant growth regulators added. The more suitable micropropagation results betwitx these two genotypes were MS nutrient medium containing 2.0 mg L-1 BAP and the more suitable rooting results were ½ MS nutrient medium containing 0.5 mg L-1 NAA. As a result of these trials in terms of Plantform system plant quality, good results were obtained in the tillering medium in these two different genotypes. In the medium for rooting, the Plantform system was observed to be more valuable than the solid culture nutrient medium. In plants propagated and rooted in the plantform system; It was settled that there was no improvement as a result of scanning with SSR markers.

Chamandoosti(2017) conducted research on the shoot appearance of *C. latifolia* plant under *in vitro* conditions. In his study, he observed that the most successful shoot development was in the nutrient medium containing 0.053 µM NAA and 4.44 µM BA. The most successful medium in terms of plant height was the nutrient medium with 0.049 µM IBA and 4.44 µM BA. Again in this study, the effect of 1 mg/l NAA and 1 mg/l IBA on rooting of 9 different genotypes was examined. In rooting studies, the data of root length, root number and plant height were evaluated. In rooting studies, IBA was settled as the most effectual growth regulator for the height and length of the roots of the plant, and NAA as the most effectual growth regulator for the number of roots.

## CONCLUSION

In the results of the study, 10% NaOCl disinfectant solving was settled as the most suitable assiduity for the sterilization of the plant material used in the experiment, and this concentration was continued to be used in the surface sterilization of the plant material throughout the research process. For the starting medium, the medium containing 1.0 mg/l BAP, where the plants developed best, was settled as the best engrossment and the process was continued with this concentration. While the study was going on, the problem of yellowing and shedding of leaves was encountered. The reason for this problem was thought to be ethylene production in the environment, and ethylene in the environment was tried to be evacuated, but the result was unsuccessful. Later, studies were found proving that silver nitrate is a very effective inhibitor of ethylene effect. 2 mL/l BAP, 2 mL/l and silver nitrate (AgNO3), which is thought to reduce the amount of phenolic substance in the medium, were added to the MS medium. The results of this study were obtained and a solving was found to the yellowing and leaf fall problem. And finally, various attempts were made for rooting and the result was obtained by adding 500 mL/l NAA and 500 mL/l IBA to the medium consisting of 2.2 g/l MS containing 20 g/l sucrose, 6 g/l agar.

As a result, *in vitro* propagation of *Poncirus trifoliata* L., which is one of the important citrus rootstocks, a widely known hedge plant and also a very preferred indoor landscape plant, was tried, and its rooting ability was investigated in different nutrient media concentrations after shoots were obtained from different explants. Thus, different denseness has been developed for *in vitro* rooting of three-leaf rootstock. With plant tissue culture experiments, it is possible to scan and detect different characteristics of plants more quickly and to reproduce them at the same time. In the study, it is seen that *Poncirus trifoliata* L. It can be a very suitable material for propagation by rootstock tissue culture. However, it is recommended that the appearance obtained as a result of plant tissue culture experiments be tested again under field conditions. Today, citrus cultivation is a very important issue. The fact that no citrus is grown in some countries in the world is proof of how important citrus imports and exports are. For this reason, today, due to both commercial-grade and scientific studies, breeding studies of citrus rootstocks and varieties continue without slowing down in the world.

## COMPLIANCE WITH ETHICAL STANDARDS

### Conflict of interest

The authors declared that there is no actual, potential or perceived conflict of interest in this research article.

### Author contribution

The contribution of the authors to the present study is equal. All the authors verify that the text, figures, and tables are original. The authors read and approved the final manuscript.

### Funding

This study was supported financially by the Harran University Scientific Research Projects Coordination Unit (HUBAK) with project number 21096.

### Ethical approval

Ethics committee approval is not required.

### Data availability

Not applicable.

### Consent for publication

Not applicable.

## REFERENCES

Ainsley, P.J., Collins, G.G., Sedgley, M.(2001a). In vitro rooting of almond (*Prunus dulcis* Mill.). In vitro Cellular and Development BiologyPlant, 37:778-785. [Google Scholar]

Ainsley, P.J., Hammerschlag, F.A., Bertozzi, T., Collins, G.G., Sedgley, M.(2001b). Regeneration of almond from immature seed cotyledons. Plant Cell, Tissue and Organ Culture, 67: 221-226. [Google Scholar]

Ali, S., Mirza, B. (2006). Micropropagation of rough lemon (Citrus jambhiri Lush.): Effect of explants type and hormone concentration. Acta Botanica Croatica 65(2), 137-146. [Google Scholar]

Ak, B.E., Karahan Kiyar, P., Hatipoglu, I.H., Dikmetas, B.(2021). Effects of different BA and IBA concentrations on proliferation and rooting of 'Garnem' rootstock in-vitro propagation. International Journal of Agriculture Environment and Food Sciences, 5(4):470-476, [CrossRef]

Amgai, R.B., Prasai, H.K., Pandey, Y.R. (2016). Hormonal effect on mandarin orange (Citrus reticulata Blanco) micropropagation. Nepal Journal of Biotechnology, 4(1), 33-36. [CrossRef]

Babaoglu, M., Gurel, E. Ozcan, S.(2002). Bitki biyoteknolojisi. Selçuk Üniversitesi Vakfı Yayınları, 374s. (In Turkish)

Cengiz, M., Aka Kacar, Y. (2019). Micropropagation of some citrus rootstocks with classical and new generation tissue culture techniques. Turkish Journal of Agriculture - Food Science and Technology 7(9):1469. [CrossRef]

Chamandoosti, F. (2017). The utilities of Citrus tissue culture. International Journal of Environmental and Agriculture Research 3: 36-46. [Google Scholar]

Espinosa, A.C., Pijut, P.M., Michler, C.H. (2006). Adventitious shoot regeneration and rooting of Prunus serotina in vitro cultures. Hortscience, 41(1): 193-201. [CrossRef]

Gurel, S., Gulsen, Y.(1998). The effects of IBA and BAP on in vitro shoot production of almond (Amygdalus communis L.).Tr.J. of Botany, 22: 375-379. [Google Scholar]

Hatipoglu, R. (1999). Bitki biyoteknolojisi. Çukurova Üniv. Zir. Fak. Genel Yayın No:190, Ders Kitabı Yayın No: A-58, Adana,176s. (In Turkish)

Iqbal, N., Khan, N. A., Ferrante, A., Trivellini, A., Francini,

A., Khan, M.I.R. (2017). Ethylene role in plant growth, development and senescence: interaction with other phytohormones. Frontiers in Plant Science, v.8, p.475, 2017. [CrossRef]

Jain, N., Babbar, S.B. (2003). Regeneration of "juvenile" plants of black plum, (Syzygium cuminii Skeels) from nodal exp of mature trees. Plant Cell, Tissue and Organ Culture, 73: 257-263. [CrossRef]

Kozai, T., Kubota, C., Jeong, B.R. (1997). Environmental control forthelarge-scaleproduction of plants through in vitro techniques. Plant Cell Tiss. Organ Culture, 51: 49-56. [CrossRef]

Mansuroglu, S., Gurel, E.(2001). Bitki biyoteknolojisi-I Doku kültürü ve uygulamaları. Selçuk Üniversitesi Basımevi, Konya, 281s. (In Turkish)

Muna, A.S., Ahmad, A.K., Mahmoud , K., Abdulrahman, K.(1999). In vitro propagation of a semi-drawting cherry rootstock. Plant Cell and Tissue And Organ Culture, 59: 203- 208. [CrossRef]

Pruski, K., Astatkie, T., Nowak, J.(2005). Tissue culture propagation of Mongolian cherry (Prunus fruticosa) and nanking cherry (Prunus tomentosa). Plant Cell, Tissue and Organ Culture, 82: 207-211. [CrossRef]

Simsek, O. , Donmez, D., Aka Kaçar, Y. (2018). Bazı turunçgil anaçlarının in vitro kuraklık stresi koşullarında performanslarının araştırılması. Yuzuncu Yıl University Journal of Agricultural Sciences, 28 (3) , 305-310 . [CrossRef] (In Turkish)

Walsh, C.S. (2003). Plant growth regulation - Concise encyclopedia of temperate tree fruit. Edited by Baugher T.A. and Singha, 245-250, ISBN 1560229411, Haworth Press.

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 605-613
DOI: https://doi.org/10.31015/jaefs.2022.4.14

# Energy balance and greenhouse gas (GHG) emissions of Sauceboat Pepper (*Capsicum annuum* L.) production in Türkiye

**Mehmet Fırat Baran**[1] • **A. Konuralp Eliçin**[2] • **Korkmaz Bellitürk**[3] • **Ahmet Çelik**[4]

[1] Department of Biosystem Engineering, Faculty of Agriculture, Siirt University, Siirt, Türkiye
[2] Department of Agricultural Machinery and Technologies Engineering, Faculty of Agriculture, Dicle University, Diyarbakır, Türkiye
[3] Department of Soil Science and Plant Nutrition, Faculty of Agriculture, Tekirdag Namik Kemal University, Tekirdag, Türkiye
[4] Agricultural Engineering Program, Faculty of Agriculture, Adıyaman University, Adıyaman, Türkiye

**Citation:** Baran, M.F., Elicin, A.K., Bellitürk, K., Celik, A. (2022). Energy balance and greenhouse gas (GHG) emissions of Sauceboat Pepper (*Capsicum annuum* L.) production in Türkiye. International Journal of Agriculture, Environment and Food Sciences, 6 (4), 605-613

**Received:** 04 September 2022
**Revised:** 01 October 2022
**Accepted:** 09 October 2022
**Published Online:** 05 December 2022

**Correspondence:** Ahmet Konuralp Eliçin
**E-mail:** konuralp.elicin@dicle.edu.tr



## Abstract

In this study, the efficiency of energy consumption and the amount of greenhouse gas emissions from the cultivation of sauceboat pepper were determined. The experiments and research data are based on the 2020 growing season and were conducted in the Karaisali district of Adana province, Turkey. The primary data used in this study, such as the financial system, labor efficiency, fuel consumption levels, weights of tools and machinery used in sauceboat pepper production, fertilizer, and seedling quantities, were obtained from existing calculations, previous studies, and various sources. The energy ratio, specific energy, energy productivity, and net energy in sauceboat pepper were calculated as 0.82, 0.98 MJ $kg^{-1}$, 1.02 kg $MJ^{-1}$, and -6845.51 MJ $ha^{-1}$, respectively. In the case of the sauceboat peppers, the energy of the fuel oil had the highest share of the total energy input, 31.65%. It was followed by energy for planting seedlings, energy for fertilizer, water energy for irrigation, energy for human labour, energy for spraying, and energy for machinery, with 21.55%, 19.64%, 12.55%, 8.59%, 4.45%, and 1.87%, respectively. Total GHG emissions were estimated as 3703.54 $kgCO_{2\text{-eq}}$ $ha^{-1}$ for sauceboat pepper highest-quality production portion in human labour (31.18%). Human labour was followed by diesel fuel consumption (25.79%), machine (0.08%), seedling planting (15.90%), nitrogen fertilizer (15.88%), phosphate fertilizer (4.09%), herbicides (3.68%), fungicides (1.93%), calcium consumption (0.09%), magnesium application (0.08%) and iron (0.52%). In addition, the GHG value for the production of sauceboat peppers was calculated to be 0.096 $kgCO_{2\text{-eq}}$ $kg^{-1}$.

**Keywords:** Energy ratio, Sauceboat pepper, Greenhouse gas ratio, Greenhouse gas emissions

## INTRODUCTION

Pepper is an indispensable vegetable consumed in many ways all over the world, and its consumption in the form of pastes, spices, pickled vegetables, or fresh vegetables is increasing day by day in parallel with the population. It is also used to make pickles, sauces, and dishes by roasting. Pepper has a very high nutritional value. Fresh green pepper has 29 calories per 100 g, 4.2 g carbohydrates, 1.1 g protein, 0.2 g fat, 93 g water and 1.4 g cellulose. Green peppers are rich in vitamins A, B1, B2 and C and also contain vitamins P and K and alkaloids. The oil content of pepper seeds is 25-28% (Keleş, 2007).

The motherland of pepper is South America, it belongs to the Solanaceae family and the most common variety is called *Capsicum annuum* L. (Keleş 2007). Capia

pepper (*Capsicum annuum* L.) has a long, conical shape and is consumed as soon as it turns red. It is also called "sauceboat" or "oil pepper" (Azder et al., 2020).

12,000,000 tons of pepper is produced worldwide, of which about 23% are produced in China, 10% in Turkey, and 9% in Nigeria. Pepper is grown to varying degrees throughout Turkey. According to Turkstat data from 2021, 3.09 million tons of pepper was produced in Turkey. 46.75% of these are capia pepper for pastes, 34.44% are pointed pepper, 13.62% are bell pepper, and 5.19% are Charleston pepper (Turkstat, 2022). In recent years, Turkey's total pepper export was reported to be 97.31 thousand tons and import was 251 tons (Güvenç, 2020).

Although produced and consumed in large quantities, it suffers from yield and quality losses due to plant and soil nutrient deficiencies (Ortaş, 2012). Vegetative nutrient deficiencies are directly related to the plant and soil properties in which it grows (Sabbağ et al., 2015). Pepper (2013) suggested that soil health is equivalent to human health. Therefore, soil quality parameters should be determined in detail and sensitively in agricultural production.

Energy analysis of crop output is critical for describing and categorizing agricultural systems based on energy consumption. To increase efficiency and minimize inputs in production, inputs and outputs should be thoroughly studied (Sabah, 2010). Among agricultural inputs, it is emphasized that the highest price increase in terms of producers is in chemical fertilizers. However, agricultural production with fertilizer programs based on planning and soil analysis in agriculture prevents producers from being adversely affected by rising fertilizer prices (Bellitürk, 2019; Çelik et al., 2020; Kılbacak et al., 2021).

On the other hand, more intensive energy use leads to significant environmental problems such as greenhouse gas (GHG) emissions that affect human health, so the efficiency of productive use of inputs becomes very important for sustainable agricultural production. Greenhouse gas emissions in agricultural production arise from the use of machinery, diesel fuel consumption, use of chemical fertilizers, and electricity consumption, and of course, an increase in energy use leads to an increase in greenhouse gas emissions.

To increase the energy-optimal strategy, either efficiency or input must be increased. In particular, the use of fuel, fertilizers, pesticides, machinery and tractors, which account for a large part of the total energy input, should be reduced. Within certain limits, it is possible to increase efficiency. But energy usage productivity value can be reduced by proper use of inputs (spraying, mechanization and fertilizers) (Çelen, 2016).

A number of studies have been conducted on peppers and some greenhouse products to determine the energy use efficiency, and these studies have evaluated the energy use efficiency of peppers (El-Helepi, 1997; Çanakçı and Akinci, 2006; Farani et al., 2012; Naderi et al., 2019); some greenhouse products (Özkan et al., 2004; Hedau et al., 2014; Nourani and Bencheikh,2017); onion and tomato (İbrahim and İbrahim, 2013); and greenhouse peppers (Çebi et al., 2017), lavender (Gokdogan 2016), plum (Baran et al.,2017), vetch (Baran, 2017), tobacco (Baran and Gokdogan,2015), mulberry (Gokdogan et al., 2017) and canola (Baran et al.,2014). Some other studies have also been conducted on agricultural products to determine the GHG emissions from the production of organic and conventional wheat (Meisterling et al., 2009), organic and diminished vegetable and fruit input practices (Clark et al., 2016), some vegetables (Maraseni et al., 2010), various fruits (Eren et al., 2019a), various field crop varieties (Eren et al., 2019b), and various medicinal aromatic plant varieties (Eren et al., 2019c), onion (Ozbek et al.,2021).

The aim of this study is to investigate the efficiency of energy consumption and greenhouse gas emissions in the production of sauceboat pepper in the Karaisali district of Adana in 2020.

## MATERIALS AND METHODS

### Explanation of the field of research

This research has been conducted in a farmer's field, 10 decares, that is located 4 km away from Karaisalı district of Adana. The dominant climate in Adana is Mediterranean and the annual average precipitation extending to long years is 644.6 mm and the average temperature is 19,1 °C (Anonymous, 2021b). In the study, the various input amounts used in the production of sauceboat pepper and the output values obtained were taken from different sources (Turkish Statistical Institute as well as previous related or similar studies), and the technical data of agricultural tools and machinery were taken from applications and catalogues in the region. The cultural and care practises employed in pepper production in Adana province are as follows.

### Inputs Used in the Study

Pepper seedling planting norm ranges between 2500-5000 pcs/da depending on the type of pepper and the distances between rows. The seedling planting norm in this current study was 4000 pcs/da, the average weight of a seedling was 7.4 gr and yield were 3850 kg/da. The following have been used as fertiliser in the trial area; 10 kg/da pure nitrogen, 15 kg/da pure phosphor, 5 kg/da pure potassium, 3 kg/da pure calcium, 1 kg/da pure iron and 1kg/da pure magnesium. 2 fungicide and 7 insecticide applications have been performed against pest and

diseases. In the experimental area, irrigation was done every 4-7 days, depending on the air temperature, sandiness of soil and water demand of the plant both before and after planting seedlings and first irrigation. Although it has been reported in the studies that the average annual water consumption of the pepper plant is between 600-900 mm (Şen, 2015), the average water consumption was calculated as 750 mm/ha. In pepper production, 1 driver was employed in soil cultivation, 1 driver and 1 assistant in bottom fertilization, and 1 worker was employed during irrigation operations. In the harvesting process, 16 workers were employed for an average of 1 ha field for picking peppers.

$$\text{Energy use efficiency} = \frac{\text{Energy output } \left(\frac{\text{MJ}}{\text{ha}}\right)}{\text{Energy input } \left(\frac{\text{MJ}}{\text{ha}}\right)} \quad \text{(Eq.1)}$$

$$\text{Energy productivity} = \frac{\text{Pepper output } \left(\frac{\text{kg}}{\text{ha}}\right)}{\text{Energy input } \left(\frac{\text{MJ}}{\text{ha}}\right)} \quad \text{(Eq.2)}$$

$$\text{Specific energy} = \frac{\text{Energy input } \left(\frac{\text{MJ}}{\text{ha}}\right)}{\text{Pepper output } \left(\frac{\text{kg}}{\text{ha}}\right)} \quad \text{(Eq.3)}$$

Net energy = Energy output (MJ $ha^{-1}$) - Energy input (MJ $ha^{-1}$) (Eq.4)

**Table 1.** Energy equivalents in agricultural production

| Inputs | Energy Equivalent (MJ $unit^{-1}$) | References |
|---|---|---|
| Human labour (h) | 1.96 | Davoodi and Houshyar, 2009 Mousavi Avval et al., 2011 |
| Machine production energy (kg) | | |
| Tractor | 64.8 | Sing, 2002; Özkan et al., 2004 |
| Tillage equipment | 62.7 | Canakci and Akinci, 2006 |
| Fuel (L) | | |
| Diesel | 35.69 | Eren, 2011 |
| Oil | 6.51 | Sabah, 2010 |
| Fertilizers (kg) | | |
| Nitrogen fertilizer | 47.10 | Kaltcshmitt and Reinhardt, 1997 |
| Phosphate fertilizer | 11.1 | Hedau et al., 2014 |
| Potassium fertilizer | 6.7 | Hedau et al., 2014 |
| Calcium | 8.8 | Naderi et al., 2019 |
| Magnesium | 8.8 | Naderi et al., 2019 |
| Iron | 33.00 | Medina et al., 2006 |
| Irrigation water | 0.63 | Özkan et al., 2004<br>Hedau et al., 2014 |
| Spraying (kg) | | |
| Insecticides | 101.2 | Erdal et al., 2007 |
| Fungicides | 216 | Erdal et al., 2007 |
| Plant material | | |
| Pepper Seedlings | 0.20 | Bojacá et al., 2012 |
| Output | | |
| Pepper | 0.8 | Kaltcshmitt and Reinhardt, 1997 |

**Table 2.** GHG emission equivalents in agricultural production

| Inputs | Unit | GHG emission equivalents (kg$CO_2$-eş $unit^{-1}$) | References |
|---|---|---|---|
| Human labour | h | 0.700 | Nguyen et al., 2012 |
| Machinery | MJ | 0.071 | Pishgar-Komleh et al., 2012 |
| Diesel fuel | L | 2.760 | Clark et al., 2016 |
| Nitrogen (N) | kg | 5.88 | Clark et al., 2016 |
| Phosphorus (P2O5) | kg | 1.010 | Clark et al., 2016 |
| Potassium(K2O) | kg | 0.580 | Clark et al., 2016 |
| Calcium | kg | 0.11 | Clark et al., 2016 |
| Magnesium | kg | 0.30 | Anonymous, 2021 b |
| Iron | kg | 1.910 | Anonymous, 2021 b |
| Herbicides | kg | 23.100 | Maraseni et al., 2010 |
| Fungicides | kg | 14.300 | Maraseni et al., 2010 |
| Pepper Seedling | kg | 1.99 | Clark et al., 2016 |

In this study, the inputs were identified to be human labour energy, equipment energy, fertilizer energy, diesel fuel energy, spraying energy, irrigation water energy, and pepper seedlings. The output was judged to be sauceboat pepper yield. The values of the pepper production inputs were calculated using the units indicated in Table 1. The energy equivalent coefficients were calculated using previous energy analysis research. By summing the energy equivalents of all inputs in MJ units, the total energy equivalent was calculated. "Energy use efficiency equation (1), energy productivity equation (2), specific energy equation (3), and net energy equation (4) were calculated by using the following formulates (Mandal et al., 2002; Mohammadi et al., 2008; Suha et al., 2019)" to determine the energy usage efficiency in sauceboat pepper production.

$$GHG_{ha} = \sum_{i=1}^{n} R(i) \times EF(i) \quad \text{(Eq.5)}$$

Here;
∑R(i) : The application rate of input, i ($unit_{input} ha^{-1}$),
EF (i) : The GHG emission coefficient of input i ($kg\text{-}CO_{2\text{-}eq} unit_{input}^{-1}$).

The coefficients of GHG emissions of agricultural inputs are shown in Table 2. However, as adopted by Houshyar et al. (2015) & Khoshnevisan et al. (2014), an index is calculated to measure the quantity of released kg $C_{O2\text{-}eq}$ per kg yield.

$$I_{GHG} = \frac{GHG_{ha}}{Y} \quad \text{(Eq.6)}$$

Here;
$I_{GHG}$ : GHG ratio,
Y : The yield (kg per ha). as kg per ha.

**Table 3.** Energy Balance in Sauceboat Pepper Production

| Inputs | Units | Input used per hectare (unit $ha^{-1}$) | Energy values (MJ/ha) | Ratio (%) |
|---|---|---|---|---|
| Human Labour | (h) | 1649.69 | 3233.40 | 8.59 |
| Tillage | | 11.42 | 22.39 | |
| Planting seedlings | | 358.27 | 702.21 | |
| Harvest | | 1280.00 | 2508.80 | |
| Machinery power | (h) | 41.36 | 702.28 | 1.87 |
| Tractor | | 20.68 | 267.37 | |
| Tillage | | 11.42 | 189.64 | |
| Planting seedlings | | 9.26 | 245.26 | |
| Fuel + Oil | (l) | 346.05 | 11915.75 | 31.65 |
| Tillage | | 124.88 | 4299.96 | |
| Planting seedlings | | 221.17 | 7615.79 | |
| Fertilizers | (kg) | 250.00 | 7392.00 | 19.64 |
| Nitrogen | | 100.00 | 4710.00 | |
| Phosphorus | | 150.00 | 1665.00 | |
| Potassium | | 50.00 | 335.00 | |
| Calcium | | 30.00 | 264.00 | |
| Magnesium | | 10.00 | 88.00 | |
| Iron | | 10.00 | 330.00 | |
| Spraying | (kg) | 10.90 | 1677.08 | 4.45 |
| Fungicide | | 5.00 | 1080.00 | |
| Insecticide | | 5.90 | 597.08 | |
| Planting seedling | (piece) | 40000.00 | 8000.00 | 21.25 |
| Irrigation water | (m3) | 7500.00 | 4725.00 | 12.55 |
| Total Input | (MJ ha-1) | | 37645.51 | |
| Output | (kg / ha) | | | |
| Sauceboat Pepper Yield | | 38500 | 30800.00 | 100 |
| Total Output | (MJ ha-1) | | 30800.00 | |

per production.

The units shown in Table 2 represent the inputs for pepper production. When determining the energy equivalent and Greenhouse Gas Emissions coefficients, previous energy balance and greenhouse gas emissions research were considered.

The following equation adopted from Hughes et al. (2011) has been used to determine GHG emission:

Table 3 contains the energy balance results and related computations. Table 4 shows measures of energy balance in sauceboat pepper production. Each parcel's total fuel usage is calculated as l ha-1. The quantity of fuel utilized was calculated using the full tank approach (Göktürk, 1999; El Saleh, 2000; Sonmete, 2006). The total time spent in the trial area determines the labor yield of the area (ha $h^{-1}$). Sonmete (2006), Güzel (1986), and Özcan (1986) are examples of this. Chronometers are used to track the amount of time spent on various agricultural

tasks.

In addition, the direct and indirect energy inputs in the manufacturing of sauceboat pepper were calculated separately. The energy value of gasoline and oil in the manufacturing of tomato paste consumed by agricultural tools and machinery are considered as direct energy input, and the energy values consumed for human labour, agricultural tools and machinery, fertilizer, pesticides and seeds are considered as indirect energy inputs Koçtürk et al., (2009). The pepper production level in Turkey in 2020 was 2 636 905 ton and pepper cultivation in Adana during the same year was 139 793 tons (Anonymous 2021a).

## RESULTS AND DISCUSSION

### Energy Use Efficiency

The energy balance of sauceboat pepper production in Adana is shown in Table 3 and energy use efficiency values in sauceboat pepper cultivation are listed in Table 4. As shown that in Table 3, 11915.75 MJ ha$^{-1}$ of fuel-oil energy has been consumed and the ratio of this value to the total energy input was 31,65%, making it the highest input. Per 1 ha area, this was followed by seedling planting energy by 8000 MJ ha$^{-1}$ and 21,25%, fertiliser energy input by 7392.00 MJ ha$^{-1}$ and 19,64%, irrigation energy by 4725.00 MJ ha$^{-1}$ and 12,55%, human labour by 3233,40 MJ ha$^{-1}$ and 8.59%, pesticide energy input by 1677.08 MJ ha$^{-1}$ and 4,45%, tool/machine energy by 702,284 MJ ha$^{-1}$ and 1,87%. In sauceboat pepper production, agricultural energy input was calculated as 37645,51 MJ ha$^{-1}$ and agricultural energy output has been calculated as 46400.00 MJ ha$^{-1}$.

As indicated in Table 4, the energy ratio in sauceboat pepper production in Adana has been calculated as 0.82. While the energy ratio found in this study was 0.82, Naderi et al., (2019) reported the energy rate in red pepper production as 0.004, Çanakçı et al., (2006) determined the energy rate in pepper production as 0.19 and Özkan et al. (2004) found the energy rate in pepper production as 0.99. In other studies, Nourani et al., (2017) reported that the energy ratio of tomato, eggplant, cucumber and pepper was 0.82 in their study in Algeria, and İbrahim and İbrahim found the energy ratio of tomato as 0.20 in their study in Nigeria and Çebi et al., (2017) found the energy ratio in greenhouse head salad as 2.29.

**Table 4.** Energy use efficiency values in sauceboat pepper cultivation

| Indicators | Unit | Values |
|---|---|---|
| Energy Ratio | - | 0.82 |
| Specific Energy | MJ kg-1 | 0.98 |
| Energy Productivity | kg MJ-1 | 1.02 |
| Net Energy Efficiency | MJ ha-1 | -6845.51 |

Net energy efficiency (MJ ha$^{-1}$) is defined as the difference between the total amount of energy obtained after production and the total amount of energy utilized in production operations (Baran et al., 2016). Energy efficiency, expressing the amount of product yield per energy use per unit area, was found to be 1.02 kg / MJ, while specific energy, expressing the amount of energy used per product, was found to be 0.98 MJ / kg (Table 4). In other similar studies; Kuswardhani et al., (2013) determined the energy efficiency in open lettuce production as 0.69 kg / MJ and specific energy as 1.45 MJ / kg, Çebi et al., (2017) figured out energy efficiency as 2,86 kg/MJ and specific energy as 0.35 MJ/kg in greenhouse lettuce cultivation, while Razavinia et al., (2015) determined energy efficiency as 1.67 kg/MJ and specific energy as 0.595 MJ/kg in lettuce cultivation. With regards to sauceboat pepper production under Adana conditions, net energy efficiency has been estimated as 8754.49 MJ ha$^{-1}$, when only number of seeds taken from unit cultivation area (ha) was taken into consideration.

**Table 5.** Energy inputs for sauceboat pepper production

| Indicators | Energy input (MJ ha$^{-1}$) | Ratio (%) |
|---|---|---|
| Direct energy[a] | 15149.15 | 40.24 |
| Indirect energy[b] | 22496.36 | 59.76 |
| Total | 37645.51 | 100.00 |
| Renewable energy c[c] | 11233.40 | 29.84 |
| Non-renewable energy [d] | 26412.11 | 70.16 |
| Total | 37645.51 | 100.00 |

[a] Human labour energy, fuel-oil energy;

[b] Seed energy, chemical fertilizer energy, pesticide energy, machine energy;

[c] Human labour energy, seed energy;

[d] Fuel-oil energy, agricultural insecticide energy, chemical fertiliser energy, machine energy

Table 5 depicts the distribution of inputs utilized in the manufacturing of sauceboat pepper according to direct, indirect, renewable, and non-renewable energy categories. The distribution of direct energy in total energy was found to be 40.24%, the indirect energy component of total energy was determined to be 59.76 percent. Renewable energy sources are inexhaustible energy sources and their most important feature is that they are energy sources that do not harm the nature. Non-renewable energy sources, on the other hand, are limited, energy resources that can be exhausted, and the vast majority of them harm the environment (Çebi et al., 2017). In the research area, renewable energy accounted for 29.84 percent of total energy used in pepper production, whereas non-renewable energy accounted for 70.16 percent of total energy. Due to the limited usage of machinery in

**Table 6.** Total GHG emissions in sauceboat pepper cultivation

| Inputs | Unit | The input used per area (unit ha$^{-1}$) | GHG Emissions (kg $CO_2$-eş ha$^{-1}$) | Ratio (%) |
|---|---|---|---|---|
| Human Workforce | h | 1649.69 | 1154.78 | 31.18 |
| Machine | MJ | 41.36 | 2.94 | 0.08 |
| Diesel Fuel | l | 346.05 | 955.09 | 25.79 |
| Nitrogen (N) | kg | 100.00 | 588.00 | 15.88 |
| Phosphorus ($P_2O_5$) | kg | 150.00 | 151.50 | 4.09 |
| Potassium | kg | 50.00 | 29.00 | 0.78 |
| Calcium | kg | 30.00 | 3.30 | 0.09 |
| Magnesium | kg | 10.00 | 3.00 | 0.08 |
| Iron | kg | 10.00 | 19.01 | 0.52 |
| Herbicide | kg | 5.90 | 136.29 | 3.68 |
| Fungicide | kg | 5.00 | 71.50 | 1.93 |
| Seedling | kg | 296 | 589.04 | 15.90 |
| TOTAL | - | - | 3703.54 | 100.00 |
| GHG Ratio (per kg) | - | - | 0.096 | |

pepper production and low energy consumption from non-renewable energy sources, the renewable energy ratio in total energy is low.

## Greenhouse Gas (GHG) Emission

The outcomes of GHG emissions of regarding the productions of sauceboat pepper are given in Table 6. Total greenhouse gas emission is calculated as 3703.51 kg-$CO_{2\text{-eq}}$ ha$^{-1}$. The highest share of total GHG emissions belongs to human workforce (31.18%). Human workforce is followed by diesel fuel consumption (25.79%), seedling planting (15.90), nitrogen fertilizer (15.88%), phosphate fertilizer (4.09%), herbicide (3.68%), fungicide (1.93%), potassium (0.78%), calcium (0.09%) and magnesium (0.08%). The GHG ratio (per kg yield) is determined as 0.096 kg$CO_{2\text{-eq}}$ kg$^{-1}$. In other similar studies, Yousefi et al. (2013) has reported the total greenhouse gas emission in pepper cultivation as 14390.85 kg$CO_{2\text{-eq}}$ ha$^{-1}$, Tongwane et al. (2016) reported the total greenhouse gas emission in tomato cultivation as 34.251 kg$CO_{2\text{-eq}}$ ha$^{-1}$ and the ve GHG ratio as 1.65 kg$CO_{2\text{-eq}}$ kg$^{-1}$, and they reported it as 165.368 kg$CO_{2\text{-eq}}$ ha$^{-1}$ in potato cultivation and GHG ratio as 1.48 kg$CO_{2\text{-eq}}$ kg$^{-1}$, Elhami et al. (2016) have reported the total greenhouse gas emission in chickpea as 6884.14 kg$CO_{2\text{-eq}}$ ha$^{-1}$ and GHG ratio as 3.03 kg$CO_{2\text{-eq}}$ kg$^{-1}$, while Eren et al. (2019) reported the following GHG emission values in different types of fruits; GHG emission in organic grape 1452.75 kg$CO_{2\text{-eq}}$ ha$^{-1}$ and GHG ratio 105 g$CO_{2\text{-eq}}$ kg$^{-1}$, GHG emission in apple as 3722.33 kg$CO_{2\text{-eq}}$ ha$^{-1}$ and GHG ratio as 0.092 kg$CO_{2\text{-eq}}$ kg$^{-1}$, GHG emission in watermelon as 1402.01 kg$CO_{2\text{-eq}}$ ha$^{-1}$ and GHG ratio as 0.077 kg$CO_{2\text{-eq}}$ kg$^{-1}$, GHG emission in cantaloupe as 1141.24 kg$CO_{2\text{-eq}}$ ha$^{-1}$ and GHG ratio as 0.041 kg$CO_{2\text{-eq}}$ kg$^{-1}$, GHG emission in plump as 930.20 kg$CO_{2\text{-eq}}$ ha$^{-1}$ and GHG ratio as 0.146 kg$CO_{2\text{-eq}}$ kg$^{-1}$, GHG emission in pomegranate as 438.33 kg$CO_{2\text{-eq}}$ ha$^{-1}$ and GHG ratio as 0.146 kg$CO_{2\text{-eq}}$ kg$^{-1}$ and GHG emission in organic strawberry as 8226.35 kg-$CO_{2\text{-eq}}$ ha$^{-1}$ and GHG ratio as 0.783 kg$CO_{2\text{-eq}}$ kg$^{-1}$.

## CONCLUSION

In this research, sauceboat pepper production energy usage efficiency and GHG emission were calculated in the 2020 production season in Karaisalı district of Adana province. The energy ratio in enterprises engaged in the production of sauceboat pepper production was found to be 0.82. As a result of the calculations, it is seen that fuel-oil energy has the highest share among the production inputs, followed by seedling planting, irrigation, fertilizer, human, medicine and machine labour energies, respectively. Total GHG has been defined. The highest energy consumption in fuel-oil input is seen in soil tillage, cultivation and other processes. In addition, seedling planting took the second place and fertilizer energy took the third place in energy consumption. For this reason, it is thought that different and alternative tillage methods and fertilization methods should be investigated for the reduce of the fuel oil input and fertilizer energy in sauceboat pepper production. Another important conclusion to be drawn from this study is that fertilization programs must be applied according to soil analysis in order to make the correct fertilization. Such research discloses crucial results in terms of both the scientific world and agricultural production in terms of protecting farmers from being negatively affected by price rises in production inputs, which are mostly caused by oil prices.

## COMPLIANCE WITH ETHICAL STANDARDS

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Ethics committee approval is not required.

### Funding

No financial support was received for this study.

### Data availability

Not applicable.

### Consent for publication

Not applicable.

## REFERENCES


Anonymous, (2021a). Crop Production Statistics. (Access date: 11 May 2021). [FAO]

Anonymous, (2021b). Adana ili tarımsal yatırım rehberi. (in Turkish).

Azder, G., Göçmen, E., İstanbulluoğlu, A. (2020). Tekirdağ Koşullarında Farklı Sulama Seviyelerinin Kapya Biberin (Capsicum Annum Cv. Kapija) Verim ve Verim Bileşenleri Üzerine Etkileri. Tekirdağ Ziraat Fakültesi Dergisi, 17 (3), 422-431. (In Turkish) [CorssRef]

Baran, M.F., Gokdoğan, O., Karaağaç, H.A. (2014). Kanola Üretiminde Enerji Kullanım Etkinliğinin Belirlenmesi (Kırklareli İli Örneği), Türk Tarım ve Doğa Bilimleri Dergisi, ISSN: 2148-3647, Cilt:1 (3), Sayfa: 331-337, 2014.

Baran, M. F, Gökdoğan, O. (2015). Determination of Energy Input-Output of Tobacco Production in Turkey, American-Eurasian J. Agric. & Environ. Sci., 15 (7): 1346-1350, ISSN 1818-6769, IDOSI Publications, DOI: 10.5829/idosi.aejaes.2015.15.7.12671

Baran, M.F., Gökdoğan, O. (2016). Determination Of Energy Balance of Sugar Beet Production in Turkey: A Case Study For Kirklareli Province, Springer Energy Efficiency (2015) 9: (2) 487-494, ISSN: 1570-6478; DOI 10.1007/s12053-015-9375-x

Baran, M.F, Gökdoğan, O, Oğuz, H.İ. (2017). Determining of Energy Input-Output Analysis in Plum (Prunus domestica L.) Production, Springer, Erwerbs-Obstbau, ISSN: 0014-0309, DOI: 10.1007/s10341-017-0332-z, Volume 59, Issue 4, pp 331–335

Baran, M.F. (2017). Energy and Economic Analysis of Vetch Production in Turkey: A Case Study From Thrace Region Feb - Fresenıus Environmental Bulletin, Volume 26-No:3/2017 , pages:1996-1972, ISSN 1018-4619

Bellitürk, K. (2019). Changes in Plant Nutrition for a Sustainable Agriculture: Past, Present and Future. ISPEC International Conference on Agriculture and Rural Development-III, 20-22 December 2019, 590-600, (Oral Presentation, Full Paper), Van, Türkiye. ISBN: 978-605-7811-43-1

Bojacá, C.R., Casilimas H. A., Gil R., Schrevens E. (2012). Extending the input-output energy balance methodology in agriculture through cluster analysis, Energy, vol.47, pp. 465-470, Ed. Elsevier, London. DOI: 10.1016/j.energy.2012.09.051

Clark, S., Khoshnevisan, B., Sefeedpari, P. (2016). Energy efficiency and greenhouse gas emissions during transition to organic and reduced-input practices: Student farm case study. Ecological Engineering, 88; 186-194. http://dx.doi.org/10.1016/j.ecoleng.2015.12.036

Canakci, M., Akinci, I. 2006. Energy use pattern analyses of greenhouse vegetable production. Energy 31 (8), 1243–1256. DOI: 10.1016/j.energy.2005.05.021

Çebi, U.K, Aydın, B., Çakır, R., Altıntaş, S. (2017). Örtü Altı Baş Salata (Lactuca sativa cv Salinas) Üretiminin Enerji Kullanım Etkinliği ve Ekonomik Analizi, Türk Tarım ve Doğa Bilimleri Dergisi 4(4): 426–433, https://dergipark.org.tr/tr/pub/turkjans/issue/31628/346678

Çelen, İ.H. (2016). Tarımsal Uygulamalarda Enerji Kullanımı Üzerine Bir Değerlendirme, Electronic Journal of Vocational Colleges- S: 18-29 Aralık 2016, https://dergipark.org.tr/tr/pub/ejovoc/issue/36634/417036

Çelik, A., Bellitürk, K., Sakin, E. (2020). New Approaches and Applications in Agriculture. Editor: M. Fırat Baran, IKSAD Publishing House. Chapter 14: Agriculture Friendly Bio-Fertilisers in Waste Management: Vermicompost and Biochar. pp: 302-327, Ankara. ISBN: 978-625-7279-66-6

Davoodi, M.J.Ş., Housyar, E. (2009). Energy Consumption of Canola and Sunflower Production İn Iran. American-Eurasian J. Agric. & Environ. Sci., 6(4): 381-384. ISSN 1818-6769, IDOSI Publications.

Elhami, B., Akram, A., Khanali, M. (2016). Optimization of energy consumption and environmental impacts of chickpea production using data envelopment analysis (DEA) and multi objective genetic algorithm (MOGA) approaches. Information Processing in Agriculture, 3 (3); 190-205. https://doi.org/10.1016/j.inpa.2016.07.002

El-Helepi, M.M. (1997). Energy and Economic Analyses of Pepper Production under Plasticulture and Conventional Systems, Department of Agicultural Economics McGill University, Montreal. 0-612-37117-4

El Saleh, Y. (2000). Syria and Turkey lentil and chickpea harvest mechanization research on the identification of opportunities. Ph.D. Thesis, Cukurova University, Institute of Science and Technology (Unpublished), Adana, Turkey (In Turkish) (Ulusal Tez Merkezi, Tez No: 95507)

Erdal, G., Esengun, K., Erdal, H., Gunduz, O. (2007). Energy use and economical analysis of sugar beet production in Tokat province of Turkey. Energy, 32: 35-41. https://doi.org/10.1016/j.energy.2006.01.007

Eren, Ö. (2011). Çukurova Bölgesinde Tatlı Sorgum (Sorghum Bıcolor (L.) Moench) Üretiminde Yaşam Döngüsü Enerji ve Çevresel Etki Analizi. Çukurova Üniversitesi Fen Bilimleri Enstitüsü Tarım Makinaları Anabilim Dalı Doktora Tezi. Adana 2011. https://doi.org/10.31590/ejosat.852286

Eren, Ö., Baran, M.F., Gökdogan, O. (2019a). Determination of Greenhouse Gas Emissions (Ghg) in The Production of Different Fruits in Turkey, Feb - Fresenıus Envıronmental Bulletın, ISSN 1018-4619, Volume 27-No:1/2019 , pages: 464-472, ISSN 1018-4619

Eren Ö., Gökdoğan, O., Baran, M.F. (2019b). Determination of Greenhouse Gas Emissions (Ghg) in The Production of Different Plants in Turkey, Feb - Fresenıus Environmental Bulletın, ISSN 1018-4619, Volume 27-No:2/2019 , pages: 1158-1166, ISSN 1018-4619

Eren Ö., Gökdoğan, O., Baran, M.F. (2019c). Determination of Greenhouse Gas Emissions (GHG) in the Production of Different Aromatic Plants in Turkey, Türk Tarım ve Doğa Bilimleri Dergisi, 6(1): 90-96, 2019, https://doi.org/10.30910/turkjans

Farani, S.M., Bahrami, H. (2012). Efficiency Evaluation in Bell Pepper Production of Greenhouses in Iran, J. Life Sci. Biomed. 3(1): 69-74, ISSN 2251-9939

Gökdoğan, O. (2016). Determination of Input-Output Energy and Economic Analysis of Lavender Production in Turkey, Int J Agric & Biol Eng, IJABE, May, 2016 9(3): 154 161, Open Access at http://www.ijabe.org

Gökdoğan, O, Baran, M.F. (2017). Determination of Energy Use Efficiency of Some Apple (Malus Communis L) Production In Turkey:A Case Study of Eğirdir Region, Springer, Erwerbs-Obstbau, ISSN: 0014-0309, Volume 59, pp 13–18, DOI 10.1007/s10341-016-0290-x.

Gökdoğan, O, Oğuz, H.İ, Baran, M.F. (2017). Energy Input-Output Analysis in Organic Mulberry (Morus spp.) Production in Turkey: A Case Study Adiyaman-Tut Region, Springer, Erwerbs-Obstbau, ISSN: 0014-0309, DOI: 10.1007/s10341-017-0333-y, Volume 59, Issue 4, pp 325-330

Göktürk, B. (1999). A study on the Determination of some properties of dry onion for harvesting, development of the excavator knife type harvesting machine, and comparison with other harvesting methods. Ph.D. Thesis, Trakya University, Institute of Science and Technology, Tekirdağ, Turkey (Ulusal Tez Merkezi, Tez no: 84760)

Güzel, E. (1986). A study on the mechanization of the dismantling and blending of peanuts in Çukurova Region and the Determination of the properties of the plant for mechanization. Turkey Agricultural Equipment Association Professional Publications, Publication Number: 47, Ankara, Türkiye.

Güvenç, İ. (2020). The Production, Foreign Trade and Comparative Advantages of Peppers in Turkey. KSU J. Agric. Nat. 23 (2): 441-445. DOI:10.18016/ksutarimdoga.vi.595512

Hedau, N.K., Tuti, M.D., Stanley, J., Mina, B.L., Agrawal, P.K., Bisht, J.K., Bhatt, J.C. (2014). Energy-use efficiency and economic analysis of vegetable cropping sequences under greenhouse condition, Energy Efficiency (2014) 7:507–515, DOI:10.1007/s12053-013-9239-1

Houshyar, E., Dalgaard, T., Tarazgar, M.H., Jorgensen, U. (2015). Energy input for tomato production what economy says, and what is good for the environment. Journal of Cleaner Production, 89; 99-109, DOI: 10.1016/j.jclepro.2014.11.022

Hughes, D.J., West, J.S., Atkins, S.D., Gladders, P., Jeger, M.J., Fitt, B.D. (2011). Effects of disease control by fungicides on greenhouse gas emissions by U.K. arable crop production. Pest Manag Sci, 67; 1082-1092, https://doi.org/10.1002/ps.2151

İbrahim, H.Y., İbrahim, H.I. (2013). Energy Inputs and Climate Change: A Case Study of vegetable Production under Fadama In North Central Nigeria, Trakia Journal of Sciences, No3, pp 343-347, ISSN 1313-3551 (online)

Kaltcshmitt, M., Reinhardt, G.A. (1997). Ecological energy balance sheets in renewable energy source. Friedr. Vieweg, Sohn Verlagsgesellschaft mbH, Braunschweig, Weisbaden, Germany.

Keleş, D. (2007). Farklı Biber Genotiplerinin Karakterizasyonu ve Düşük Sıcaklığa Tolerans, Çukurova Üniversitesi Fen Bilimleri Enstitüsü, Doktora Tezi, (In Turkish) (Ulusal Tez Merkezi, Tez no: 212528)

Khoshnevisan, B., Shariati, H.M., Rafiee, S., Mousazadeh, H. (2014). Comparison of energy consumption and GHG emissions of open field and greenhouse strawberry production. Renewable and Sustainable Energy Reviews, 29; 316-324, DOI: 10.1016/j.rser.2013.08.098

Kılbacak, H., Bellitürk, K., Çelik, A. (2021). Bitkisel ve Hayvansal Atıklardan Vermikompost Üretilmesi: Yeşil Badem Kabuğu ve Koyun Gübresi Karışımı Örneği. Akademik Perspektiften Tarıma Bakış (Editör: Gülşah Bengisu). IKSAD Publishing House. ISBN: 978-605-70345-3-3. Bölüm 2, s: 19-44, Ankara, ISBN: 978-605-70345-3-3

Koçtürk, O.M., Engindeniz, S. (2009). Energy and Cost Analysis of Sultana Grape Growing: A case study of Manisa, west Turkey. African Journal of Agricultural Research, 4(10):938-943, ISSN 1991-637X

Kuswardhani, N., Soni, P., Shivakoti, G.P. (2013). Comparative energy input-output and financial analyses of greenhouse and open field vegetables production in West Java, Indonesia. Energy, 53(2013): 83-92, DOI: 10.1016/j.energy.2013.02.032

Mandal, K.G., Saha, K.P., Ghosh, P.K., Hati, K.M., Bandyopadhyay, K.K. (2002). Bioenergy and economic analysis of soybean-based crop production systems in central India. Biomass and Bioenergy 23: 337-345, https://doi.org/10.1016/S0961-9534(02)00058-2

Maraseni, T.N., Cockfield, G., Maroulis, J., Chen, G. (2010). An assessment of greenhouse gas emissions from the Australian vegetables industry. Journal of Environmental Science and Health, Part B, 45:6, 578-588, https://doi.org/10.1080/03601234.2010.493497

Medina, A., Cooman, A., Parrado, C.A., Schrevens, E. (2006). Evaluation of Energy Use and Some Environmental Impacts for Greenhouse Tomato Production in the High Altitude Tropics, Proc. IIIrd IS on HORTIMODEL2006, Acta Horticulturae, 718, ISHS 2006, pp. 415-422, International Society for Horticultural Science, Leuven/Belgium, https://doi.org/10.17660/ActaHortic.2006.718.48

Meisterling, K., Samaras, C., Schweizer, V. (2009). Decisions to reduce greenhouse gases from agriculture and product transport: LCA case study of organic and conventional wheat, Journal of Cleaner Production 17, 222–230, DOI: 10.1016/j.jclepro.2008.04.009

Mousavi-Avval, S.H., Rafiee, S., Jafari, A., Mohammadi, A. (2011). Energy Flow Modeling and Sensitivity Analysis of Inputs For Canola Production In İran. Journal of Cleaner Production, 19 -1464-1470, (www.elsevier.com/locate/biombioe, USA). DOI : 10.1016/j.jclepro.2011.04.013

Mohammadi, A., Tabatabaeefar, A., Shahin, S., Rafiee, S., Keyhani, A. (2008). Energy use and economical analysis of potato production in Iran a case study: Ardabil province. Energy Convers Manag 49: 3566-3570, https://doi.org/10.1016/j.enconman.2008.07.003

Naderi, S.A, Dehkordi, A.L, Taki, M. (2019). Energy and environmental evaluation of greenhouse bell pepper production with life cycle assessment approach, Elsevier, Environmental and Sustainability Indicators 3-4, DOI: 10.1016/j.indic.2019.100011

Nguyen, T.L.T., Hermansen, J.E. (2012). System expansion for handling co-products in LCA of sugar cane bio-energy systems: GHG consequences of using molasses for ethanol production. Applied Energy, 89; 254-261, DOI:10.1016/j.apenergy.2011.07.023

Nourani, A., Bencheikh, A. (2017). Energy Balance Analysis And

Mechanization Indexfor Greenhouse Vegetable Production In Southern of Algeria. An Overview of Biskra Province, INMATEH-Agricultural Engineering, Vol. 51, No.1, https://www.researchgate.net/publication/316580256

Ortaş, İ. (2012). Do Maize and Pepper Plants Depend on Mycorrhizae in Terms of Phosphorus and Zinc Uptake. Journal of Plant Nutrition, 35:11: 1639-1656, DOI: 10.1080/01904167.2012.698346

Özbek, O., Gokdogan, O., Baran, M.F., 2021, Investıgatıon on Energy use Effıcıency and Greenhouse Gas Emıssıons (Ghg) of Onıon Cultıvatıon , Feb - Fresenıus Environmental Bulletın, ISSN 1018-4619, Volume 30 -No:02/2021 pages:1125-1133

Özcan, M.T. (1986). Comparison of lentil harvesting and blending methods in terms of work efficiency, quality, energy consumption and cost, and research on the development of an appropriate harvesting machine. Turkey Agricultural Equipment Corporation Press, Release No. 46. Ankara, Turkey (In Turkish)

Özkan, B., Kürklü, A., Akçaöz, H. (2004). An input-output energy analysis in greenhouse vegetable production: a case study for Antalya region of Turkey. Biomass Bioenergy, 26(1): 189-195, https://doi.org/10.1016/S0961-9534(03)00080-1

Pepper, I.L. (2013). The Soil Health: Human Health Nexus. Critical Reviews in Environmental Science and Technology, DOI:10,1080/10643389,2012,694330.

Pishgar-Komleh, S.H., Sefeedpari, P., Ghahderijani, M. (2012). Exploring energy consumption and $CO_2$ emission of cotton production in Iran. Journal of Renewable and Sustainable Energy, 4: 033114-033115, https://doi.org/10.1063/1.4727906

Razavinia, B., Fallah, H., Niknejad, Y. (2015). Energy efficiency and economic analysis of winter caltivation (lettuce, bersim clover, broad bean) in Mazandaran province of Iran. Biological Forum, 7(1): 1452-1460, ISSN No. (Print): 0975-1130

Sabbağ, Ç., Akça, E. (2015). Adıyaman Yerel Biberinin Besin ve Yetiştiği Toprak Özellikleri, GAP VII. Tarım Kongresi Bildiri Kitabı, 1-4. 28 Nisan – 01 Mayıs 2015 Şanlıurfa, ISBN: 978-975-7113-45-4

Sabah, M. (2010). Söke Ovasında İkinci Ürün Yağlık Ayçiçeği Üretiminde Enerji Kullanımı. Çukurova Üniversitesi Fen Bilimleri Enstitüsü Tarım Makinaları Anabilim Dalı. Yüksek Lisans Tezi. Adana, (Ulusal Tez Merkezi, Tez no: 276913)

Singh, J.M. (2002). On farm use pattern in different cropping systems in Haryana, India. Master Thesis (unpublished). International Institute of Management, University of Flensburg, Germany.

Sonmete, M.H. (2006). Harvest-threshing mechanization and development possibilities of beans. Ph.D. Thesis, Selcuk University Institute of Science and Technology (Unpublished), Konya, Türkiye, (Ulusal Tez Merkezi, Tez no: 183162)

Suha, E., Azmi, Y., Muhammad R.M., Nazmi M.N., Modather, M. (2019). Energy utilization in major crop cultivation, Energy, Vol: 173, Pages 1285-1303, ISSN 0360-5442,

Şen, E. (2015). Sanayi Biberinde (Capsicum Annuum L.) Damla Sulama Uygulamalarının Bazı Verim Ve Kalite Özellikleri Üzerine Etkileri. Adnan Menderes Üniversitesi Fen Bilimleri Enstitüsü Tarımsal Yapılar Ve Sulama Anabilim Dalı Yüksek Lisans Tezi, (Ulusal Tez Merkezi, Tez no: 382419)

Tongwane, M., Mdlambuzi, T., Mokhele Moeletsi, M., Tsubo, M.,Vuyo, M., Lunga Grootboom L. (2016). Greenhouse gas emissions from different crop production and management practices in South Africa, Elsevier, Environmental Development, Vol: 19, 23–35, https://doi.org/10.1016/j.envdev.2016.06.004

Turkstat, (2022). https://www.tuik.gov.tr/ (Access date: 13 September 2022)

Yousefi, M., Omid, M., Rafiee, S.H., Khoshnevisan, B. (2013). Modeling GHG emission and energy consumption in selected greenhouses in Iran, International Journal of Energy and Environment Vol: 4, Issue 3, 2013 pp.511-518, ISSN 2076-2895 (Print)

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 614-621
DOI: https://doi.org/10.31015/jaefs.2022.4.15

# The effect of sorbitol applications on total phenolic, flavonoid amount, and antioxidant activity in Safflower (*Carthamus tinctorius* L.)

**Alper Dilek**[1] • **Ebru Batı Ay**[2] • **Muhammed Akif Açikgöz**[3] • **Beril Kocaman**[2]

[1] Amasya University, Institute of Science, Department of Biotechnology, 05500, Amasya, Türkiye
[2] Amasya University, Suluova Vocational School, Department of Plant and Animal Production, 05500, Amasya, Türkiye
[3] Ordu University, Faculty of Agriculture, Department of Field Crops, 52200, Ordu, Türkiye

**Citation:** Dilek, A., Batı Ay, E., Açıkgöz, M.A., Kocaman, B. (2022). The effect of sorbitol applications on total phenolic, flavonoid amount, and antioxidant activity in Safflower (*Carthamus tinctorius* L.). International Journal of Agriculture, Environment and Food Sciences, 6 (4), 614-621

**Received:** 25 September 2022
**Revised:** 26 October 2022
**Accepted:** 31 October 2022
**Published Online:** 06 December 2022

**Correspondence:** Ebru Batı Ay
**E-mail:** ziraatciebru@hotmail.com



## Abstract

It is already known that secondary metabolites in plants are affected by many parameters such as biotic and abiotic stress conditions and can vary in quantity. Sorbitol, which is used to increase osmolality in cells, is an important source of abiotic stress. The present study was conducted to determine the effects of sorbitol applications at different doses by foliar spraying on total phenolic, total flavonoid, and antioxidant activity in Safflower (*Carthamus tinctorius* L.) at different times. Olein variety was used as the material in the study and different sorbitol doses (0.5 g/L, 25 g/L, 50 g/L and 100 g/L) were applied by foliar spraying. Sorbitol doses were applied in three different growth periods (before, during, and after blooming). The total amount of phenolic and flavonoid substances and DPPH free radical scavenging activity of the drugs obtained from all samples were examined after the harvest was completed. Although it was determined that the effect of increasing sorbitol doses applied in different growth periods on the total amount of phenolic and flavonoid substances and on the DPPH free radical scavenging activity was statistically not significant, the interaction of sorbitol doses x different growth periods in the total phenolic substance content was significant. The highest total phenolic content was determined as 133.00 mg GAE/g in the before blooming period of the plant in safflower. The total amount of flavonoid substance was determined as 24.46 mg QE/g in the before blooming period of the highest plant. The highest DPPH free radical scavenging activity was found to be 92.86% in the after blooming period. As a result, when the study outcomes were evaluated according to different development periods in safflower, it is possible to rank the development periods of the total phenolic substance content and total flavonoid substance amounts as before blooming > blooming period > after blooming. We can list the antioxidant activity as after blooming > blooming period > before blooming.

**Keywords:** Asteraceae,Plant growth and development periods, Bioactive compounds, Osmotic stress

## INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is an important industrial plant of the Asteraceae or Compositaea family. Although safflower has been produced for different purposes in many countries in the world since ancient times, the most important among these purposes is medicinal production and use. Safflower (*Carthamus tinctorius* L), which is a member of the Compositaea family among the natural an-

tioxidant sources, is an important medicinal and aromatic plant. Safflower seeds are rich in unsaturated fatty acids and its flowers are rich in flavonoids, which are very beneficial for human health. Hydroxysafflor Yellow A (HYSA) is among the most important flavonoids in the petals of safflower flowers and plays important roles in the pharmacological effects of flavonoids (Feng et al., 2013; Wang et al., 2013). Flavonoids are important in the processes related to flowering and especially color change of the safflower plant (Tanaka et al., 2010). Flavonoids, which give yellow and red pigments, are the main active components of the safflower plant. The content and component of flavonoids affect the quality of safflower directly. For this reason, the number of studies conducted on the regulation of the biosynthesis of flavonoids is increasing with each passing day (Ren et al., 2019).

Stress is a condition limiting or hindering the regularly functioning biological and physiological systems and normal functions of plants (Mahajan and Tuteja, 2005). In plants, stress is divided into two groups, biotic and abiotic. Biotic stress is caused by polysaccharides (dextran, chitin, pectin and cellulose) and micro-organisms (yeast extract, fungi and bacteria) consisting of plant cell walls of biological origin. Abiotic stress, on the other hand, is physical, chemical and hormonal factors that do not have biological origin. Physical stress factors are light, ultraviolet light (UV, UV A, UV B and UV C), osmotic (sorbitol, proline and polyethylene glycol) stress, drought, salinity and thermal stress (Açıkgöz, 2017; Açıkgöz, 2021). Osmotics such as mannitol or sorbitol inhibit mineral uptake from cells. For this reason, the growth and development of plants slows down and is affected negatively (Dodds and Roberts, 1985; Thompson et al., 1986; Açıkgöz et al., 2019; Açıkgöz, 2020). There are many studies employing Polyethylene Glycol (PEG) or sorbitol to create artificial drought stress. Sorbitol solution is not metabolized by plants. Also, it reduces the osmotic potential of the nutrient medium and creates water stress that is not metabolized by plants (Rai et al., 2011; Bidabadi et al., 2012; Placide et al., 2012; Vanhove et al., 2012). Although the resistance of the plant to drought stress changes according to the duration of exposure to stress, it also changes according to the period of exposure to stress.

Secondary substances in the safflower plant are affected by many parameters such as variety, sowing time and frequency, fertilization, irrigation, development periods, harvest time, and environmental stress conditions. It was reported by previous studies that these parameters cause quantitative variations in secondary substances (Kizil et al., 2008; Mohammadi and Tavakoli., 2015; Caliskan and Caliskan., 2018). Also, there are studies reporting that different harvest times of safflower significantly change the secondary metabolite content of the plant (Salem et al., 2011). However, there are not many studies conducted on the effects of the stress factors created by foliar spraying on the chemical composition of the plant.

The present study was conducted to determine the total phenolic, total flavonoid substance amounts and DPPH free radical scavenging activity of sorbitol applications at different growth stages (before blooming, during blooming, after blooming) and by foliar spraying in Safflower (*C. tinctorius*).

## MATERIALS AND METHODS

### Material

The present study was conducted in the greenhouse area of the Plant and Animal Production Department of Amasya University Suluova Vocational School Campus in 2021 production period. As the study material, Olein variety obtained from a commercial company was used.

### Equipment Used

The Thermo Scientific-Evolution 201 spectrophotometer was the main equipment used in the study. Also, Lab Companion-BS 06 water bath, Rotary Evaporator RE300, DENVER-S1234 analytical scale, IKA-KS 501 shaker, Memmert Inc. 153 med $CO_2$ oven, and ISOLAB micropipettes were the main equipment used in the analysis.

### Method

### Trial Design

The trial was performed according to the randomized plots trial design in different growth periods (pre-bloom, flowering period, post-bloom) in six repetitions and different sorbitol doses (0.5 g/L, 25 g/L, 50 g/L, 100 g/L) on 19.03.2021. Safflower seeds were sown in sterilized plastic pots (22.5 cm depth, 25 cm diameter) after filling them with a mixture of sand, field soil and burnt barn manure. Seeds were sown at a depth of 3-5 cm, 10 in each pot. It was determined on 06.04.2021 that seeds started to grow in all pots. The dilution process was made so that 5 plants with similar appearance remained after emergence. During the study, when the soil surface was dry in all of the pots, irrigation was performed to keep the soil moist. Before the flowering period of the plants, a calculation of 20 kg/da was made and ammonium sulphate fertilizer was given to the pots. Before flowering, sorbitol applications were made by foliar spraying method on 23.04.2021. The sorbitol applications were done as foliar spraying on 18.06.2021 and 02.07.2021, respectively during the flowering period and after the flowering. When the plants reached harvest maturity, they were harvested on 19.07.2021 before the seeding period.

### Extraction method

The samples were crushed in a hand mill after the harvest was completed. The fragmented plant samples were taken into 50 mL falcons and stored at 25°C. In the extraction of plant samples in falcon tubes, 5 g of plants were weighed on analytical scale. The plant samples were then placed in lidded glass jars and 200 ml of methanol was added to and mixed with the help of a shaker.

The resulting mixtures were extracted by ultrasonic-assisted extraction method for 2 hours. Ultrasonic-assisted extraction method was preferred because it is an effective method for preserving the structural and molecular properties of bioactive compounds by preventing damage to the extracts because it is applied at high efficiency, fast and low temperatures. The supernatant obtained at the end of the extraction was then filtered. The final product (the supernatant) was passed through the Rotary Evaporator and the solvent was removed from the medium.

### Total phenolic content method

The Folin-Ciocalteu Method was used to determine the total amount of phenolic substances (Folin and Ciocalteu, 1927). The extracts that were prepared at a concentration of 1mg/mL were taken into 0.1 mL tubes and 4.5 mL of distilled water was added. After adding 0.1 mL Folin-Ciocalteu reagent and 0.3 mL of 2% sodium carbonate solution, the tubes were mixed and kept in the dark for 2 hours. It was measured against blank (distilled water) at 760 nm (Slinkard and Singleton, 1997). The amount of phenolic substance was expressed as gallic acid. The comparison with gallic acid was made in the calibration curve. The results are given as mg gallic acid/g (mg GAE/g) in the dried sample.

### Total flavonoid substance amount method

The total flavonoid substance content was determined with quercetin standard solution according to the method that was developed by Park et al. (2008). The plant extract was placed in 1 mL test tubes and 2 mL distilled water, 0.15 mL of 0.5 M $NaNO_2$ and 0.15 mL of 0.3 M $AlCl_3$ reagent were added. After waiting for 5 minutes, 1 mL NaOH was added and the absorption was measured at 510 nm with a spectrophotometer. The calibration curve was compared with quercetin. The total amount of flavonoid substance was defined as mg equivalents of quercetin (mg QE/g) per g dried sample.

### DPPH free radical scavenging activity method

In the present study, antioxidant activity was determined by using the DPPH free radical scavenging method. Herbal solutions of different concentrations (25-400 µg/mL) were prepared and 0.5 mL was taken from these solutions and 3 mL ethanol and 300 mL 20 mg/L DPPH solution were added. After adding butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and trolox (100-500 µg/mL) to this solution, the mixture was shaken vigorously and absorbance was measured at 517 nm and 0.75 mL water was used as a control instead of the sample. The percent inhibition activity was calculated with the equation given below.

Free radical scavenging activity %= [(A0 - A1) / A0] x 100.

(A0 = control absorbance and A1 = sample solution absorbance)

### Statiscal analysis

The experiments were done in six replications and the results are presented as mean ± SD. The data were subjected to analysis of variance (ANOVA) using statistical analysis system software (SPSS) version 22. Significant differences were calculated using the smallest significant difference (Tukey), with differences considered statistically significant at $P < 0.05$.

### Total Phenolic Substance Amount

It was found in the present study that the highest total phenolic substance content was observed in the pre-flowering period with 133 mg GAE/g and 5 g/L sorbitol application. The lowest amount of total phenolic substance was obtained from 79.21 mg GAE/g and 50 g/L sorbitol applications in the post-flowering period. There were no statistically significant effects of increasing sorbitol doses and different growth periods on the total amount of phenolic substances separately (Table 1). The effect of different growth periods on the total phenolic content of the plant is very important. When the previous studies were evaluated, it was found that the amounts of total flavonoid, phenolic and antioxidant activities in plants vary according to different development periods and harvest times. It was understood that the reasons for these differences were factors such as the genetic difference of the cultivars or populations used, the development stage of the plant, the harvest hours, the organ from which the sample was taken, the location and environmental stress conditions. Yolci et al. (2021) harvested the safflower plant at different harvest periods and examined the changes in the total phenolic substance content of the plant during these periods. The highest total phenolic content (132.30 mg GA/100g) was obtained from the 2nd harvest time, which was two weeks after the beginning of flowering. In their study, Kuşoğlu (2015) reported that the total phenolic content of safflower plant extracts was in the range of 35.33-276 mg/ml as gallic acid equivalent. The total amount of phenolic substances in the leaves of the echinops (*Echinops orientalis* Trautv.) plant from the Asteraceae family was found to be 45 µg GAE/mg (Yılmaz, 2012). In a study that was conducted on *Vaccinium vitisidaea* L. plant, root, leaf and fruit were harvested at different times, and decreases were detected in total phenol and total antioxidant activity in leaf samples depending on the prolongation of the harvest time. However, it was also reported that there was no change in root and fruit (Bujor, Ginies, Popa, and Dufour, 2018). Again, in another study that was conducted on *Lycopus lucidus* plant, it was reported that total phenolic substance and antioxidant activity decreased depending on the delay in harvesting, but some values increased in terms of phenolic contents (Lu et al., 2015). It was reported in some studies that total phenol, flavonoid and antioxidant activity did not change with the prolongation or shortening of the harvest time, but they gave fluctuating results (Nemeth-Zamborine, Seidler-Łożyko-

wska and Szabo, 2019; Ribeiro et al., 2020). As seen in the present study, although the highest amount of phenolic substance was detected during the flowering period, it was found to be the lowest in the post-flowering period, which is the maturation period of the plant.

periods were effective on the amount of flavonoid substances. Although the highest amount of flavonoid substance was observed in the before blooming period, it was found that the amount of flavonoid substance decreased 1.5 times in the after blooming period. When

**Table 1.** The Total Phenolic Substance Amount (mg GAE/g) in Safflower (*C. tinctorius* L.) that Underwent Sorbitol Application at Different Periods and at Different Doses

| Growth Periods | Sorbitol Doses (g/L) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 5 | 25 | 50 | 100 | Mean |
| **Belofe blooming** | 123.69± 1.75abc | 133.00 ±2.20a | 92.42± 1.55de | 127.39± 3.10ab | 107.64± 0.95bd | 116.82± 1.00 |
| **Blooming** | 92.03± 0.90de | 96.79± 2.15de | 100.31± 1.30cde | 96.50± 1.80de | 106.68± 1.08bd | 98.46± 2.20 |
| **After blooming** | 108.00± 2.00bcd | 98.06± 1.00de | 102.41± 1.65cde | 79.21± 0.78e | 103.17± 1.70cd | 98.17± 1.72 |
| **Mean** | 107.90± 2.34 | 109.28± 1.74 | 98.38± 0.85 | 101.03± 1.44 | 105.83± 1.60 | |

*There were no statistically significant differences between the mean values shown with the same letter.

## Total Flavonoid Substance Amount

It was found that the highest total flavonoid substance amount in Safflower that underwent different growth stages (before blooming, flowering period, after blooming) and different doses was 24.46 mg QE/g and 5 g/L sorbitol application in the before blooming period (Table 2). However, the lowest total flavonoid substance content was obtained from 50 g/L sorbitol with 16.09 QE/g in the post-flowering period. It was seen in the study that the effect of different growth periods on the total amount of flavonoid substances of the plant was very important. In their study, Yolci et al. (2021) reported that the total amount of flavonoid substances in the safflower plant harvested at different periods was 19.15 mg QE/100g in the period one week after the beginning of flowering. Kuşoğlu (2015) reported that the total amount of flavonoid substance in safflower plant leaf extracts was 97.41 ± 2.13 µg catechin/mg). It was determined as 62.2 ± 1.9 mg QE/g (Yu et al., 2013) in a study investigating the effect of flower harvesting at different weeks on the flavonoid cartamidine substance in safflower and the amount of cartamidine increased gradually in the harvests made from the beginning of flowering until the third week, and gradually decreased in the harvests after the third week (Steberl, Hartung, Munz and Graeff-Hönninger, 2020). As seen in the literature, the amount of flavonoid substances in plants varied according to the harvest time. In the present study, it was found that different development compared with previous studies, it was found that the study was in agreement with the literature in terms of decreasing the amount of flavonoid substance as the development period progressed.

## DPPH Free Radical Scavenging Activity

It was seen that the highest DPPH free radical scavenging activity in safflower with different doses of sorbitol applied in different growth periods to increase the antioxidant activity was 92.86% in the after-blooming period with 5 g/L sorbitol application (Table 3). However, the period with the lowest DPPH activity was obtained from 100 g/L sorbitol applications with 64.49% during the blooming period. Although the highest DPPH was seen in the after blooming period, this was 1.5 times less in the before blooming period. Kuşoğlu (2015) reported the highest activity in the flower extract as 96% as a result of the DPPH radical removal activity of the safflower plant. The DPPH activity of echinops (*Echinops orientalis* Trautv.) from the Asteraceae family was 65%, 70% and 20% in seeds, leaves and stems, respectively (Yılmaz, 2012). When these values weare compared with safflower, it was higher than the safflower plant (Ay et al., 2018). In a study conducted on Galanthus elwesii, the harvest was made from flower, leaf, root and bulb parts at the beginning of flowering and fruit ripening periods and determined that the highest antioxidant activity was in the leaf and fruit ripening period. It was also reported that growth periods were effective on antioxidant activity.

**Table 2.** The Amount of Total Flavonoid Substance (mg QE/g) in Safflower (*C. tinctorius* L.) that Underwent Sorbitol Application at Different Periods and at Different Doses

| Growth periods | Sorbitol Doses (g/L) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 5 | 25 | 50 | 100 | Mean |
| **Before blooming** | 17.48± 0.30 | 24.46± 0.30 | 17.55± 1.00 | 18.02± 0.45 | 22.92± 0.32 | 20.08± 0.78ab |
| **Blooming period** | 20.06± 0.72 | 24.02± 0.62 | 21.63± 0.50 | 23.35± 1.12 | 23.73± 1.00 | 22.55± 1.10a |
| **After blooming** | 18.33± 0.16 | 17.74± 0.78 | 20.87± 0.90 | 16.09± 0.48 | 19.16± 0.10 | 18.43± 0.60b |
| **Mean** | 18.62± 0.20 | 22.07± 0.70 | 20.01± 0.30 | 19.15± 0.88 | 21.93± 0.15 | |

*There were no statistically significant differences between the mean values shown with the same letter.

**Table 3.** The DPPH Free Radical Scaveng,ng Activity (% inhibition) in Safflower (*C. tinctorius* L.) that Underwent Sorbitol Application at Different Periods and at Different Doses

| Growth periods | Sorbitol Doses (g/L) | | | | | |
|---|---|---|---|---|---|---|
| | 0 | 5 | 25 | 50 | 100 | Mean |
| **Before blooming** | 69.12± 2.00 | 72.00± 0.80 | 77.47± 1.30 | 78.09± 1.28 | 64.49± 0.87 | 72.23± 0.56b* |
| **Blooming period** | 78.90± 0.90 | 71.93± 1.38 | 71.57± 2.35 | 86.56± 0.82 | 86.65± 2.00 | 79.12± 1.20b |
| **After blooming** | 88.47± 1.75 | 92.86± 1.36 | 89.50± 1.42 | 85.01± 1.40 | 82.21± 1.16 | 87.61± 0.75a |
| **Mean** | 78.83± 0.80 | 78.93± 0.96 | 79.51± 0.90 | 83.22± 1.10 | 77.78± 0.64 | |

*There were no statistically significant differences between the mean values shown with the same letter.

These studies and our results show that the highest antioxidant activity was detected during maturation periods, which is in agreement with the literature data. DPPH free radical scavenging activities in different plants were 75% in sorrel, 82% in dill, 40% in arugula, 23% in cress and 67.5% in the leaves of turnp radish (Isbilir, 2008; Akagun, 2009). When all these data were compared, it was seen that the DPPH radical scavenging activity of the safflower plant was considerably higher than the antioxidant activity of other plants.

## CONCLUSION

Safflower, which is one of the natural antioxidant sources, is from the Asteraceae family, and its secondary metabolite components, amount and antioxidant activity change in different growth periods (before blooming, blooming period, after blooming). For this reason, significant variations may occur in plant samples to be collected at different times in plants. In this research, the effects of sorbitol applications at different periods (before blooming, blooming period, after blooming) and foliar spraying on total phenolic, total flavonoid and DPPH free radical scavenging activities were investigated in safflower.

When evaluated according to different growth periods in safflower, it was found that the amount of total flavonoid substance and total phenolic substance were the highest before the blooming period. It is possible to list the growth periods as before blooming> blooming period> after blooming for total flavonoid amount and total phenolic substance amount. Contrary to what is seen in total flavonoid and total phenolic substance amounts in DPPH free radical scavenging activity, the period when DPPH free radical scavenging activity is highest is after blooming period. It is possible to list the growth periods of DPPH free radical removal activity as after blooming> blooming period> before blooming.

As a conclusion, it was found that the antioxidant activity of safflower varies significantly according to the growth period of the plant. For this reason, it was understood that the development period of the plant can be optimized depending on the expected targets and stress factors can be applied in the periods where the highest yield and quality antioxidant activity will be obtained, considering variabilities.

## COMPLIANCE WITH ETHICAL STANDARDS

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

The contribution of the authors to the present study is equal.

All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Ethics committee approval is not required.

### Funding

We are thankful to Scientific Research Projects Unit (BAP) of Amasya University for providing support to this research, with the number of FMB-BAP 21-0496 BAP Project.

### Data availability

Not applicable.

### Consent for publication

Not applicable.

### Acknowledgements

This study is a part of Aper Dilek's master's thesis.

## REFERENCES

Açıkgöz, M.A. (2017). *Achillea gypsicola* türünde kallus kültürü ile sekonder metabolit üretim potansiyelinin belirlenmesi. Ordu University, PhD Thesis (Printed), Ordu.

Açıkgöz, M.A., Kara, Ş.M., Aygün, A., Özcan, M.M., Ay, E.B. (2019). Effects of methyl jasmonate and salicylic acid on the production of camphor and phenolic compounds in cell suspension culture of endemic Turkish yarrow (*Achillea gypsicola*) species. Turk J Agric For 43: 351–359. [CrossRef]

Açıkgöz, M.A. (2020). Establishment of cell suspension cultures of *Ocimum basilicum* L. and enhanced production of pharmaceutical active ingredients. Ind Crops Prod 148:112278. [CrossRef]

Açıkgöz, M.A. (2021). Effects of sorbitol on the production of phenolic compounds and terpenoids in the cell suspension cultures of *Ocimum basilicum* L. Bio-

logia 76, 395–409. [CrossRef]
Abu-Romman, S. (2010). Responses of Cucumber Callus to Sorbitol-Induced Osmotic Stress. Journal of Genetic Engineering and Biotechnology. 8(2): 45-50.
Akman, Y., Ketenoğlu, O., Kurt, L., Güney, K., Hamzaoğlu, E. ve Tuğ, N. (2007). "Angiospermae (Kapalı Tohumlular)", Palme Yayınları, İstanbul. (in Turkish)
Albiski, F., Najla, S., Sanoubar, R., Alkabani, N. and Murshed, R. (2012). In vitro screening of potato lines for drought tolerance. Physiology and Molecular Biology of Plants. 18(4), 315-321.
Ay, B. E., Gül, M., Açikgöz, M.A., Yarilgaç, T., and Kara, Ş. M. (2018). Assessment of Antioxidant Activity of Giant Snowdrop (*Galanthus elwesii* Hook) Extracts with Their Total Phenol and Flavonoid Contents. Indian Journal of Pharmaceutical Education and Research, 52(Issue 4 (Suppl), 128-132.
Babaoğlu, M. (2006). "Dünya ve Türkiye'de aspir bitkisinin tarihi, kullanım alanları ve önemi", Trakya Tarımsal Araştırmalar Enstitüsü, Edirne. (in Turkish)
Baydar, H. (2000). Effects of Gibberellic Acid on Male Sterility, Seed Yield and Oil and Fatty Acid Syntheses of Safflower (*Carthamus tinctorius* L.). Turkish Journal of Biology. 24: 159-168.
Bidabadi, SS., Meon S., Wahab, Z., Subramaniam, S. and Mahmood, M. (2012). In vitro selection and characterization of water stress tolerant lines among ethyl methanesulphonate (EMS) induced variants of banana (*Musa* spp., with AAA genome). Australian Journal of Crop Science, 6(3), 567-575.
Brito, G., Costa, A., Fonseca, H. M., and Santos, C. V. (2003). Response of *Olea europaea* ssp. maderensis in vitro shoots exposed to osmotic stress. Scientia Horticulturae, 97(3-4), 411-417.
Bujor, O. C., Ginies, C., Popa, V. I., and Dufour, C. (2018). Phenolic compounds and antioxidant activity of lingonberry (*Vaccinium vitis-idaea* L.) leaf, stem and fruit at different harvest periods. Food chemistry, 252, 356-365.
Bündig, C., Vu, T. H., Meise, P., Seddig, S., Schum, A., and Winkelmann, T. (2017). Variability in osmotic stress tolerance of starch potato genotypes (*Solanum tuberosum* L.) as revealed by an in vitro screening: role of proline, osmotic adjustment and drought response in pot trials. Journal of Agronomy and Crop Science, 203(3), 206-218.
Caliskan, S. and Caliskan. M.E. (2018). Row and plant spacing e_ects on the yield and yield components of saffower in mediterrranean-type environment. Turkish Journal of Field Crops, p. 85–92.
Coşge Şenkal, B., Kıralan, M., and Ramadan, M.F. (2015). Impact of harvest times on the quality characteristics of oils recovered from different safflower (*Carthamus tinctorius*) cultivars sown in spring and autumn. Eurpean Food Research and Technology, 2015.
Corleto, A., Alba, E., Polignano, G. B., and Vonghia, G. (1997). Safflower: A Multipurpose Species with Unexploited Potential and World Adaptability. The Research in Italy. IV th International Safflower Conference, (2-7 June), 23-31 Bari, Italy.
Dodds, J. H., and Roberts, L. W. (1985). Experiments in plant tissue culture. International Potato Center.
Eryiğit, T. (1998). Farklı hasat zamanlarının aspir (*Carthamus tinctorius* L.)'in verim ve kalite özelliklerine etkileri üzerinde bir araştırma. Yüzüncü Yıl Üniversitesi Tarla Bitkileri Anabilim Dalı Yüksek Lisans Tezi. Sayfa, 57. (in Turkish)
Fan, L., and Guo, M. (2013). Progress of Safflower (*Carthamus tinctorius* L.) Regeneration through Tissue Culture, Journal of Medical Colleges of Pl. 289-301.
Faostat (2018) http://www.fao.org/faostat/en/#data. Accessed 8 Mar 2018.
Farran M, Barbour GW, Usayran NN, Kayouli C. (2010). Metabolizable energy and amino acid digestibility of decorticated extruded safflower meal. Poultry Science, 89:1962–1966. [CrossRef]
Feng, Z. M., He, J., Jiang, J. S., Chen, Z., Yang, Y. N., and Zhang, P. C. (2013). NMR solution structure study of the representative component hydroxysafflor yellow A and other quinochalcone C-glycosides from *Carthamus tinctorius*. Journal of natural products, 76(2), 270-274.
Gautam, S., Anjani, K., and Srivastava, N. (2016). In vitro evaluation of excess copper affecting seedlings and their biochemical characteristics in *Carthamus tinctorius* L. (variety PBNS-12). Physiology and Molecular Biology of Plants, 22(1), 121-129.
Geçgel, Ü., Demirci, M., Esendal, E., Taşan, M. (2007). Fatty Acid Composition of the Oil from Developing Seeds of Different Varieties of Safflower (*Carthamus tinctorius* L.). J Amer Oil Chem Soc (2007) 84:47–54.
Gopal, J. and Iwama, K. (2007). In vitro screening of potato against water-stress mediated through sorbitol and polyethylene glycol. Plant cell reports, 26(5), 693-700.
Hall, C.A., and Cuppett, S. L. (1997). Structure-activities of natural antioxidants. In Antioxidant Methodology In Vivo and In Vitro Concepts Aruoma, O. I., Cuppett, S. L., Eds.; AOCS Press: Champaign, IL, pp 2-29.
Harun-Or-Rashid, M., Miah, MB., and Islam, SS. (2021). In vitro morphophysiological screening of drought-tolerant potato genotypes. In Vitro Cellular & Developmental Biology-Plant, 57(3), 519-528.
Hassan, NA., and Bekheet, SA. (2008). Mid-term storage and genetic stability of strawberry tissue cultures. Egyptian Journal of Genetics And Cytology, 37(2).
Hudson, B. J. F., Ed. (1990). Food Antioxidants; Elsevier Applied Science: London
İşbilir, Ş. S. (2008). Yaprakları salata-baharat olarak tüketilen bazı bitkilerin antioksidan aktivitelerinin incelenmesi. Trakya Üniversitesi Kimya Ana Bilim Dalı Doktora Tezi Sayfa, 132. (in Turkish)
Keleş, R., Öztürk, Ö. (2012). Farklı Ekim Zamanlarının Bazı Aspir Çeşitlerinde Verim ve Kalite Üzerine Etkileri. Tarım Bilimleri Araştırma Dergisi, 5(1), 112-117. (in Turkish)

Kırıcı, S. (1998). İki Aspir Çeşidinde Gibberellik Asidin (GA3) Agronomik Özellikler ve Çiçek Verimi ile Boyar Madde Oranına Etkileri. Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi, 7 (1): 10-30. (in Turkish)

Kielkowska, A., Adamus, A., and Oleksyk, A. (2012). In vitro selection of Allium cepa for water stress. In VI International Symposium on Edible Alliaceae, 21-12 May, Fukuoka (Japan).

Kizil, S., Çakmak, Ö., Kirici, S. A. L. İ. H. A., and İnan, M. (2008). A comprehensive study on safflower (*Carthamus tinctorius* L.) in semi-arid conditions. Biotechnology & Biotechnological Equipment, 22(4), 947-953.

Kuşoğlu, E. (2015). Aspir (*Carthamus tinctorius* L.) bitkisinin fenolik bileşiklerinin ve antioksidan aktivitesinin tayini (Master's thesis, Fen Bilimleri Enstitüsü).

Larson, R. A., (1988). The antioxidants of higher plants. Phytochemistry, 27(4), 969- 978.

Li, B., Xu, X., Zhang, L., Han, J., Bian, C., Li, G., ... & Jin, L. (2020). Above-ground biomass estimation and yield prediction in potato by using UAV-based RGB and hyperspectral imaging. ISPRS Journal of Photogrammetry and Remote Sensing, 162, 161-172.

Lim, S. Y., Hwang, J. Y., Yoon, H. R. and Paık, Y. S. (2007). Antioxidative Phenolics from the Petals of *Carthamus tinctorius*. Journal of the Korean Society for Applied Biological Chemistry. Cilt 50, sayı 4, Sf.304-307.

Lu, Y. H., Huang, J. H., Li, Y. C., Ma, T. T., Sang, P., Wang, W. J., and Gao, C. Y. (2015). Variation in nutritional compositions, antioxidant activity and microstructure of Lycopus lucidus Turcz. root at different harvest times. Food chemistry, 183, 91-100.

Mahajan, S., & Tuteja, N. (2005). Cold, salinity and drought stresses: an overview. Archives of biochemistry and biophysics, 444(2), 139-158.

Marssaro, AL., Lucymeire, SM., Jailson, LC., Silva Ledo, CA. Santos-Serejo, JA. (2017). Simulation of in vitro water deficit for selecting drought-tolerant banana genotypes. Pesquisa Agropecuária Brasileira, 52 (12): 1301-1304.

Mohammadi, M., and Tavakoli, A. (2015). Effect of harvest time of spring safflower (*Carthamus tinctorius* L.) florets on the production of red and yellow pigments. Quality Assurance Safety of Crops and Foods. p. 581–588.

Nagaraj, G. (2009). Oilseeds Properties, Processing, Products and Procedures. 2nd Ed. New India pub., New Delhi, India.

Nemeth-Zamborine, E., Seidler-Łożykowska, K., and Szabo, K. (2019). Effect of harvest date on yield and secondary compounds of lemon balm (*Melissa officinalis* L.). Journal of Applied Botany and Food Quality.

Okcu, M., Tozlu, E., Dizikısa, T., Kumluay, A.M., Pehluvan, M., Kaya, C. (2010). Erzurum Sulu Koşullarında Bazı Aspir (*Carthamus tinctorius* L.) Çeşitlerinin Tarımsal Özelliklerinin Belirlenmesi. Atatürk Üniversitesi Ziraat Fakültesi Dergisi, 41 (1), 1-6. (in Turkish)

Özdemir, F. A., Aymelek F. ve Karataş, F. (2011). Aspir Bitkisinde Redükte, Okside Glutatyon ile A, C, E Vitamini ve β-karoten Miktarlarının Araştırılması. Fırat Üniversitesi Fen Bilimleri Dergisi, sayı 23, Sf.65-71. (in Turkish)

Özel, A., Demirbilek, T., Çopur, O., Gür, A. (2004). Harran ovası kuru koşullarında farklı ekim zamanları ve sıra üzeri mesafelerinin aspir (*Carthamus tinctorius* L.)'in taç yaprak verimi ve bazı bitkisel özelliklerine etkisi. Harran Ün. Z.F. Dergisi, 8(3/4):1-7. (in Turkish)

Patil, J.G., Ahire, M.L., Nitnaware, K.M., Panda, S., Bhatt, V.P., Kishor Kavi, P.B., Nikam, T.D. (2013). In vitro propagation and production of cardiotonic glycosides in shoot cultures of Digitalis purpurea L. by elicitation and precursor feeding. Applied microbiology and biotechnology, 97(6): 2379-2393.

Placide, R., Carpentier, S., and Swennen, R. (2012). Development of in vitro technique to screen for drought tolerant banana varieties by sorbitol induced osmotic stress. African Journal of Plant Science, 6(15), 416-425.

Ren, S., Wu, Y., Liu, S., Zhou, M., and Ma, S. (2019). Explicit cross-lingual pre-training for unsupervised machine translation. arXiv preprint arXiv:1909.00180.

Rai, MK., Kalia RK., Singh, R., Gangola, MP., and Dhawan, AK. (2011). Developing stress tolerant plants through in vitro selection - an overview of the recent progress. Environmental and Experimental Botany, 71(1), 89-98.

Ribeiro, D. A., Camilo, C. J., Nonato, C. D. F. A., Rodrigues, F. F. G., Menezes, I. R. A., Ribeiro-Filho, J., and da Costa, J. G. M. (2020). Influence of seasonal variation on phenolic content and in vitro antioxidant activity of Secondatia floribunda A. DC. (Apocynaceae). Food Chemistry, 315, 126277.

Salem, N., Msaada, K., Hamdaoui, G., Limam, F., and Marzouk, B. (2011). Variation in phenolic composition and antioxidant activity during flower development of safflower (*Carthamus tinctorius* L.). Journal of Agricultural and Food Chemistry, 59(9), 4455-4463.

Shi, M., Kwok, K.W., Wu, J.Y. (2007). Enhancement of tanshinone production in *Salvia miltiorrhiza* Bunge (red or Chinese sage) hairy-root culture by hyperosmotic stress and yeast elicitor. Biotechnology and applied biochemistry, 46(4): 191-196.

Singh, V., Nimbkar, N. (2006). Safflower (*Carthamus tinctorius* L.). Chapter, 6, 167-194.

Singh, B., Sahu, P.M., Sharma, R.A. (2017). Effect of elicitors on the production of pyrroloquinazoline alkaloids by stimulating anthranilate synthase activity in Adhatoda vasica Nees cell cultures. Planta, 1-13.

Sorkheh, K., Shiran, B., Khodambshi, M., Rouhi, V., and Ercisli, S. (2010). In vitro assay of native Iranian almond species (Prunus L. spp.) for drought tolerance. Plant Cell Tiss Organ Cult. 105(3), 395-404.

Steberl, K., Hartung, J., Munz, S., and Graeff-Hönninger, S. (2020). Effect of row spacing, sowing density, and harvest time on floret yield and yield components of

two safflower cultivars grown in southwestern Germany. Agronomy, 10(5), 664.

Tanaka, T., Godin, B., Bhavane, R., Nieves-Alicea, R., Gu, J., Liu, X., and Ferrari, M. (2010). In vivo evaluation of safety of nanoporous silicon carriers following single and multiple dose intravenous administrations in mice. International journal of pharmaceutics, 402(1-2), 190-197.

Thompson, MR., Douglas, TJ., Obata-Sasamoto, H., and Thorpe, TA. (1986). Mannitol metabolism in cultured plant cells. Physiologia Plantarum, 67(3), 365-369.

Wang, C. Y., Liu, Q., Huang, Q. X., Liu, J. T., He, Y. H., Lu, J. J., and Bai, X. Y. (2013). Activation of PPARγ is required for hydroxysafflor yellow A of *Carthamus tinctorius* to attenuate hepatic fibrosis induced by oxidative stress. Phytomedicine, 20(7), 592-599.

Vanhove, AC., Vermaelen, W., Panis, B., Swennen, R., and Carpentier, SC. (2012). Screening the banana biodiversity for drought tolerance: can an in vitro growth model and proteomics be used as a tool to discover tolerant varieties and understand homeostasis. Frontiers in Plant Science, 3, 176.

Yang, Y., Pu, X., Qu, X., Chen, F., Zhang, G., Luo, Y. (2017). Enhanced production of camptothecin and biological preparation of N1-acetylkynuramine in *Camptotheca acuminata* cell suspension cultures. Applied microbiology and biotechnology, 101(10): 4053-4062.

Yılmaz, S. (2012). Echinops orientalis Trautv. Bitkisindeki Sekonder Metabolitlerin İzolasyonu, Yapı Tayini, Antioksidan Aktivitelerinin İncelenmesi, Yüksek Lisans Tezi, Gaziosmanpaşa Üniversitesi Fen Bilimleri Enstitüsü, Tokat. (in Turkish)

Yolci, M. S., Tunçtürk, R., ve Tunçtürk, M. (2022). Farklı Ekstraksiyon Çözücüleri ve Hasat Zamanlarının Aspir (*Carthamus tinctorius* L.) Çiçeklerinin Toplam Fenol ve Flavonoid Miktarları ile Antioksidan Aktivitesi Üzerine Etkileri. International Journal of Life Sciences and Biotechnology, 5(1), 97-109. (in Turkish)

Yu, S. Y., Lee, Y. J., Kım, J. D., Kang, S. N., Lee, S. K., Jang, J. Y., Lee, H. K, Lım, J. H. and Lee, O. H. (2013). Phenolic Composition, Antioxidant Activity and Anti-Adipogenic Effect of Hot Water Extract from Safflower (*Carthamus tinctorius* L.) Seed. Nutrients. Cilt 5, sayı. 12, Sf. 4894-4907.

Zaker, A., Sykora, C., Gössnitzer, F., Abrishamchi, P., Asili, J., Mousavi, S.H., and Wawrosch, C. (2015). Effects of some elicitors on tanshinone production in adventitious root cultures of *Perovskia abrotanoides* Karel. Industrial Crops and Products, 67: 97-102.

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 622-631
DOI: https://doi.org/10.31015/jaefs.2022.4.16

# The effects of foliar zinc application on grain antioxidant traits in some winter durum wheat cultivars at different growth stages

**Gizem Coşkun**[1] • **Fevzi Topal**[2] • **Bilge Bahar**[1,2,3]

[1] Department of Field Crops, Institute of Graduate Education, University of Gümüşhane, Gümüşhane, Türkiye
[2] Department of Food Engineering, Faculty of Engineering and Natural Sciences, University of Gümüşhane, Gümüşhane, Türkiye
[3] Şiran Mustafa Beyaz Vocational High School, University of Gümüşhane, Gümüşhane, Türkiye

**Citation:** Coskun, G., Topal, F., Bahar, B. (2022). The effects of foliar zinc application on grain antioxidant traits in some winter durum wheat cultivars at different growth stages. International Journal of Agriculture, Environment and Food Sciences, 6 (4), 622-631

**Received:** 02 October 2022
**Revised:** 01 November 2022
**Accepted:** 06 November 2022
**Published Online:** 06 December 2022

**Correspondence:** Bilge Bahar
**E-mail:** bilgebahar74@gmail.com



## Abstract

This study was aimed to investigate the grain antioxidant activity (DPPH· and $ABTS^{\cdot+}$ radical scavenging activities and cuprac reducing capacity), contents of total phenolic compounds, flavonoid and total antioxidant capacity of five winter durum wheat cultivars under the foliar application of 0.2% of zinc ($ZnSO_4.7H_2O$) at different growth stages of grain filling (milky or dough ripeness). The study was carried out in randomized blocks according to the split plot design with three replications in the cultivars of Ç.1252, Eminbey, Kızıltan-91, Meram-2002 and Selçuklu-97.

In the study, it was determined that some of the antioxidant traits ($ABTS^{\cdot+}$, total flavonoid and zinc content of grain) were not statistically different between foliar zinc application stages of the milky and dough ripeness, but zinc application in one of these stages showed significantly higher values in terms of these traits compared to the untreated of zinc (control). Also, cultivars and zinc×cultivar interaction for DPPH· radical scavenging activity, total phenolic compounds and total flavonoids showed significant variations. Within the frame of these results, it was found that foliar application of zinc at different stages of grain filling in durum wheat had statistically significant effects on some antioxidant traits; however, in subsequent studies, it was advised that it would be more beneficial to expand the study by increasing the dose and the number of growth stages.

**Keywords:** Antioxidant traits, Durum wheat, Flavonoids, Phenolics, Zinc

## INTRODUCTION

Among the widely grown field crops, wheat plays an important role in daily energy intake, especially in developing countries; it meets about 50% of the daily energy intake in many Central Asian and Middle Eastern countries, and this rate can exceed 70% in rural areas (Çakmak, 2008).

Today, 40% of the world's population, especially in developing countries, is faced with insufficient microelement intake. In these regions, durum wheat is often grown under harsh, drought-prone and even marginal conditions. These vulnerable environments often cause production variability due to variation in annual precipitation. Generally, poverty maps and micronutrient deficiency maps can be used with information on durum wheat production area and per capita wheat intake to identify target regions where biological yield is cost-effective (Çakmak, 2008).

In daily life, irreversible destructions occur in the human body due to various factors such as smoking, alcohol, environmental pollution and stress. Today, phenolic compounds and antioxidant-rich functional foods come to the fore against these damages. Structures such as phenolic substances, carotenoids and vitamin E in wheat are natural powerful antioxidants and are important in preventing some diseases (Menteş-Yılmaz, 2011).

Phenolic substances are aromatic compounds that have hydroxyl groups and can esterify with carbohydrates (Shahidi and Naczk, 1995; Duthie and Crozier, 2000). Phenolic acids, flavonoids, stilbenes, coumarins and tannins are among these compounds (Dinelli et al., 2009). Herbal phenolics are products of normal metabolism, and their amount varies according to plant variety, growing conditions and maturation level (Adom et al., 2005; Kim et al., 2006). Phenolic compounds are important compounds that increase the natural resistance of metabolism against oxidative damage and prevent lipid peroxidation (Gülçin, 2007; 2012). The interest in phenolic acids is increasing due to the protective potential of phenolic acids against oxidative damage such as cancer, stroke and coronary heart disease thanks to a diet rich in fruits and vegetables (Annakkaya, 2012).

Oxygen creates reactive oxygen species (ROS) such as superoxide, singlet oxygen and hydroxyl radical in the respiratory sequence in humans (Prado et al., 2020). If the accumulation of reactive oxygens in the human body is not eliminated with antioxidants, adverse effects such as aging, coronary diseases, wear and tear of cells, cancer, and collapse of the immune system occur under the resulting "oxidative stress". Antioxidants are natural substances that remove these negative effects of free radicals (Liu et al., 2018). Free radicals are reactive structures that contain unstable electrons in their outer orbitals (Fang et al., 2002). In addition, these structures are usually small molecules and can easily pass through cell membranes (Jensen, 2003). Oxidative stress occurs when pro-oxidants, which play a role in accelerating oxidation in tissues, overtake antioxidants. For this reason, the survival of cells under oxygenated conditions is made possible by the introduction of enzymatic and non-enzymatic antioxidants into the system (Sies, 1991).

Due to the phytochemicals (vitamin E, phenolic compounds and carotenoids), wheat is considered as a natural antioxidant source (Menteş-Yılmaz, 2011). Researchers focusing on this subject focused on wheat bran and stated that there is not only a load fiber ratio in the bran; they also reported that it increases the total antioxidant activity with the phenolic acids it contains (Kim et al., 2006). The phenolic acids do not result in a uniform distribution in the layers of the grain; it is stated that it is mostly found in aleurone, fruit peel and germ, and a small amount is located in the starchy endosperm layer (Menteş-Yılmaz, 2011).

In this study, it was aimed to examine the effects of foliar application of zinc on the antioxidant properties at two different growth periods, such as milky or dough ripeness, in winter durum wheat cultivars, and to determine the effects of application period×cultivar interactions in terms of these properties to be examined.

## MATERIALS AND METHODS

### Materials

Grains of five durum wheat cultivars (Meram-2002, Selçuklu-97, Kızıltan-91, Eminbey and Ç-1252) grown under field conditions of Şiran/Gümüşhane/TÜRKİYE, at the 2013-2014 growth season and stored under storage conditions at a temperature of around 18 °C and a relative humidity of 50-60% were used as material. Foliar application of zinc (0.2% of $ZnSO_4.7H_2O$) at two different growth stages, such as milky or dough ripeness, in these winter durum wheat cultivars were done.

### Methods

#### Determination of DPPH· free radicals removal activity

DPPH· free radical scavenging activity was determined using the method of Blois (1958). 1 mM solution of DPPH· was used as a free radical. Stock solutions prepared previously at a concentration of 1 mg $mL^{-1}$ were used as samples. Stock solutions were transferred to test tubes to form solutions at concentrations of 20 µg $µL^{-1}$, respectively, and the total volume was made up with ethanol to reach 2000 µL. Then, 500 µL of the stock DPPH· solution was added to each sample tube, followed by incubation for 30 minutes at room temperature and in the dark, and the absorbance at 517 nm was measured against an ethanol blank. As controls, 2000 µL of ethanol and 500 µL of DPPH· solution was used. The decreased absorbance compared to the control gave the remaining amount of DPPH· solution, that is, the free radical scavenging activity.

#### $ABTS^{\cdot+}$ radical removal activity

$ABTS^{\cdot+}$ radical scavenging activity was determined according to the method of Re et al. (1999). First, 7 mM $ABTS^{\cdot+}$ solution was prepared. $ABTS^{\cdot+}$ radicals were produced by adding 2.45 nM persulfate solution to this solution. Before using the $ABTS^{\cdot+}$ radical solution, the control solution was adjusted to 0.700±0.025 nm with a phosphate buffer with an absorbance of 0.1 M at 734 nm and a pH of 7.4. The 20 µg $mL^{-1}$ concentrations of the extracts whose $ABTS^{\cdot+}$ radical scavenging activity will be examined were completed to 1500 µL with ethanol. Then, 500 µL of ABTS.+ radical solution was added and incubated for 30 minutes at room temperature. Absorbances were recorded at 734 nm against the blank consisting of ethanol.

#### $Cu^{2+}$-$Cu^{+}$ reduction capacity

In the prepared extracts, the $Cu^{2+}$ reduction activities were performed with a slight modification (Ak and Gülçin, 2008) of the copper ion reduction method (Apak et

al., 2006). 125 µL of $CuCl_2$ solution (0.01 M), 125 µL of ethanolic neocuprine solution ($7.5x10^{-3}$ M) and 125 µL of $CH_3COONH_4$ buffer solution (1 M) were added to the tubes containing the extracts prepared at a single concentration (20 µg $µL^{-1}$), respectively. Final volumes were made up to 1 mL with distilled water; after 30 minutes, absorbance values were measured against the blank at 450 nm. Pure water was used as the blank.

### Determination of total antioxidant amount

500 µL of flour was taken from the extract sample and 2500 µL of distilled water was added; 1000 µL of molybdate reagent was added to the resulting mixture; after vortexing the mixture, it was incubated for 90 minutes in a 95 °C water bath with the mouths closed. It was taken from the water bath and waited for 20-30 minutes to come to room temperature and 500 µL of distilled water was used instead of the sample as a blank. The absorbance of the obtained reaction mixtures was read in the spectrophotometer at 695 nm (Kasangana et al., 2015). The total antioxidant amount was calculated from the unit of ascorbic acid equivalent (AAE) by using the regression equation in the standard ascorbic acid graph (Figure 1).

### Determination of total phenolic compound

The amount of phenolic compounds in the prepared extracts was determined by Singleton et al. (1999) and gallic acid was used as the standard phenolic compound. For this, firstly, a standard graph of gallic acid was created (Figure 2). In order to determine the amount of phenolic compounds in durum flour extracts, the prepared stock solution was used. 750 µg of the extract was taken from the stock solution and placed in a metric cup and the volume was made up to 23 mL with distilled water. 500 µL of Folin-Ciocalteu reagent and 1500 µL of 2% $Na_2CO_3$ were added to the mixture after 3 minutes. The samples were mixed for 2 hours at room temperature. Then, the absorbance of the samples at 760 nm was read against a blank consisting of pure water. The amount of gallic acid equivalent (GAE) corresponding to the absorbance values of the samples was determined with the help of the equation obtained from the standard graph (Figure 2). The results are given as gallic acid equivalents (Köksal and Gülçin, 2008).

### Determination of total flavonoids

In the prepared extracts; total amount of flavonoids were made according to the method of Park et al. (1997). 750 µg of extract was added to a vezin cup. The extract, which was then transferred to the test tube, was diluted with 4300 µL of ethanol solution containing 100 µL (1 M) of $CH_3COOK$ and 100 µL (10%) $Al(NO_3)_3$ solutions and mixed in a vortex. After incubation at room temperature for 40 minutes, absorbance at 415 nm was recorded. Quercetin was used as a standard for the determination of total flavonoid concentration and the total flavonoid concentration was determined as microgram quercetin equivalent (QE) from the equation obtained from the standard quercetin graph (Figure 3).

### Determination of grain zinc content

It has been determined according to the EPA 6020 method; 0.5 grams of each flour sample was weighed, 4 mL of 65% $HNO_3$ and 6 mL of $H_2O_2$ were added to it, burned in the microwave (Milestone Start D) and diluted with 50 mL of ultrapure water. The elemental content of the samples was analyzed by inductively coupled plasma-mass spectrometry (inductively coupled plasma mass spectrometry: ICP-MS; Agilent brand ICP-MS 7700e series) technique. Agilent mix 2a standard was used in the analyses.

### Evaluation of Data

The data obtained from the laboratory studies, according to the randomized blocks split plot design, with three replications; control and zinc application periods were arranged by placing in the main plots and the cultivars in the sub plots. Statistical analyzes of the data (F test and EGF test) were performed with the JMP statistical package program.

## RESULTS AND DISCUSSION

The mean squares obtained from the variance analyzes of the antioxidant traits of the grain that zinc applied from the leaves during the stage of milky or dough ripeness of some winter durum wheat varieties were given in Table 1; the mean values for these traits were given in Table 2-8.

### DPPH· Free Radical Scavenging Activity

DPPH· free radical scavenging activity was determined as the inhibition rate (%) by comparing the absorbance data of each sample in 20 µg $mL^{-1}$. This value was determined according to the following formula:

DPPH· free radical scavenging activity (%) = $[(\lambda_{517(C)} - \lambda_{517(S)})/ \lambda_{517(C)}] \times 100$

In the above formula, $\lambda_{517(s)}$ is the absorbance value determined after the sample is added to the DPPH· free radical solution; $\lambda_{517(C)}$ shows the absorbance value of the control containing only DPPH· free radical solution. BHA, BHT, α-tocopherol and trolox were used as positive controls in the studies.

There are no statistically significant differences in foliar zinc application stages in durum wheat cultivars (Table 1); however, the lowest DPPH· inhibition rate was obtained from the milky ripeness stage (3.28%) while the highest value was obtained from the zinc application stage of dough ripeness (3.54%) (Table 2). Cultivars showed significant changes at $p<0.05$ level (Table 1); the highest DPPH· inhibition rate was obtained from Ç.1252 cultivar (3.76%), and the lowest value was obtained from Selçuklu-97 cultivar (3.06%) (Table 2). Zilic et al. (2013) reported that the antioxidant capacity measured as DPPH· radical scavenging activity was similar in bread and durum whe

ats, but there were significant differences between genotypes within the species; it supports our study showing that statistically significant changes in DPPH· radical scavenging activity were observed among the cultivars.

In addition, Zinc×Cultivar interaction for DPPH· radical scavenging activity showed significant differences ($p$<0.01, Table 1); this interaction is due to the fact that the cultivars other than Kızıltan-91 are in statistically different groups in terms of high DPPH· radical scavenging activity in the control, milky and dough ripeness (Table 2).

**Table 1.** Mean squares and coefficients of variation related to the effects of zinc application at the stages of milky or dough ripeness on the antioxidant traits in durum wheat cultivars.

| Variation Source | Df | Mean Squares | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | DPPH | ABTS | Kuprac | Total Phenolic | Total Flavonoid | Total Antioxidant | Zinc |
| **Replicate (R)** | 2 | 0.704 | 17.215 | 0.00207 | 6.910 | 0.062 | 95.92 | 18.432 |
| **Zinc (Zn)** | 2 | 0.265 | 35.271* | 0.00001 | 31.027 | 2.184** | 47.49 | 655.62** |
| **R*Zn&Random (Error 1)** | 4 | 0.207 | 3.144 | 0.00559 | 5.520 | 0.047 | 56.31 | 33.999 |
| **Cultivar (C)** | 4 | 0.595* | 11.875 | 0.00094 | 16.652* | 0.336** | 92.87 | 150.564** |
| **Zn×C** | 8 | 0.783** | 13.565 | 0.00201 | 14.744* | 0.305** | 49.93 | 22.822 |
| **Error 2** | 24 | 0.184 | 5.854 | 0.00114 | 5.405 | 0.069 | 46.10 | 23.412 |
| **CV (%)** | | 12.52 | 8.13 | 12.43 | 4.22 | 20.24 | 7.85 | 4.69 |

Df, degree of freedom; CV, coefficient of variation; *, ** show the probability levels of p<0.05, p<0.01, respectively.

## DPPH· Free Radical Scavenging Activity

DPPH· free radical scavenging activity was determined as the inhibition rate (%) by comparing the absorbance data of each sample in 20 µg $mL^{-1}$. This value was determined according to the following formula:

DPPH· free radical scavenging activity (%) = $[(\lambda_{517(C)} - \lambda_{517(S)})/ \lambda_{517(C)}] \times 100$

In the above formula, $\lambda_{517(s)}$ is the absorbance value determined after the sample is added to the DPPH· free radical solution; $\lambda_{517(C)}$ shows the absorbance value of the control containing only DPPH· free radical solution. BHA, BHT, α-tocopherol and trolox were used as positive controls in the studies.

There are no statistically significant differences in foliar zinc application stages in durum wheat cultivars (Table 1); however, the lowest DPPH· inhibition rate was obtained from the milky ripeness stage (3.28%) while the highest value was obtained from the zinc application stage of dough ripeness (3.54%) (Table 2). Cultivars showed significant changes at $p$<0.05 level (Table 1); the highest DPPH· inhibition rate was obtained from Ç.1252 cultivar (3.76%), and the lowest value was obtained from Selçuklu-97 cultivar (3.06%) (Table 2). Zilic et al. (2013) reported that the antioxidant capacity measured as DPPH· radical scavenging activity was similar in bread and durum wheats, but there were significant differences between genotypes within the species; it supports our study showing that statistically significant changes in DPPH· radical scavenging activity were observed among the cultivars.

In addition, Zinc×Cultivar interaction for DPPH· radical scavenging activity showed significant differences ($p$<0.01, Table 1); this interaction is due to the fact that the cultivars other than Kızıltan-91 are in statistically different groups in terms of high DPPH· radical scavenging activity in the control, milky and dough ripeness (Table 2).

**Table 2.** Mean values of DPPH. inhibition rates of grain in zinc applications at the stage of milky or dough ripeness in durum wheat cultivars (%)

| Cultivars (C) | Zinc Application Stages (Zn) | | | |
|---|---|---|---|---|
| | Control | Milky ripeness | Dough ripeness | Mean |
| **Ç.1252** | 3.66 a-d | 3.31 c-e | 4.30 a | 3.76 a |
| **Eminbey** | 2.87 ef | 4.07 ab | 3.45 b-e | 3.46 a-c |
| **Kızıltan-91** | 3.49 b-e | 3.49 b-e | 3.02 d-f | 3.33 bc |
| **Meram-2002** | 3.96 a-c | 2.97 d-f | 3.65 a-d | 3.53 ab |
| **Selçuklu-97** | 3.35 b-e | 2.55 f | 3.27 c-f | 3.06 c |
| **Mean** | 3.47 | 3.28 | 3.54 | 3.43 |
| **$LSD_{Zn}$** | | ns | | |
| **$LSD_{C}$** | | 0.42 | | |
| **$LSD_{Zn×C}$** | | 0.72 | | |

*There is no difference at the 0.05 probability level between the mean values with the same letter groups. LSD shows the least significant difference between the mean values. ns means not important.

### $ABTS^{\cdot+}$ Radical Scavenging Activity

Similar to $DPPH^{\cdot}$ free radical scavenging activity; $ABTS^{\cdot+}$ radical scavenging activity was also determined as the inhibition rate (%) by comparing the absorbance data of each sample at 20 µg $mL^{-1}$. This value was calculated according to the following formula:

$ABTS^{\cdot+}$ radical scavenging activity (%) = $[(\lambda_{734(C)} - \lambda_{734(S)})/\lambda_{734(C)}] \times 100$

In the above formula, $\lambda_{734(S)}$ is the absorbance value determined after adding the sample to the $ABTS^{\cdot+}$ free radical solution; $\lambda_{734(C)}$ shows the absorbance value of the control containing only $ABTS^{\cdot+}$ free radical solution. BHA, BHT, α-tocopherol and trolox were used as positive controls in the studies.

Statistically significant differences ($p<0.05$) were found in durum wheat cultivars in terms of foliar zinc application stages (Table 1); while the lowest $ABTS^{\cdot+}$ inhibition rate was obtained from the control application without zinc application (28.10%), the highest value was obtained from the zinc application stage of dough ripeness (31.17%) (Table 3). Although there are no statistically significant differences in $ABTS^{\cdot+}$ inhibition rate in durum wheat cultivars (Table 1); it varied between 28.82% (Kızıltan-91) and 31.56% (Eminbey) (Table 3). While data are not for durum wheat, Ragaee et al. (2006) are in agreement with the findings. The Zinc×Cultivar interaction did not show statistically significant differences in $ABTS^{\cdot+}$ radical scavenging activity (Table 1).

**Table 3.** Mean values of ABTS.+ inhibition rates of grain in zinc applications at the stage of milky or dough ripeness in durum wheat cultivars (%)

| Cultivars (C) | Zinc Application Stages (Zn) | | | |
|---|---|---|---|---|
| | Control | Milky ripeness | Dough ripeness | Mean |
| **Ç.1252** | 23.67 | 31.43 | 31.70 | 28.93 |
| **Eminbey** | 30.22 | 32.18 | 32.28 | 31.56 |
| **Kızıltan-91** | 29.47 | 28.81 | 28.20 | 28.82 |
| **Meram-2002** | 28.88 | 29.68 | 32.01 | 30.19 |
| **Selçuklu-97** | 28.29 | 27.62 | 31.67 | 29.19 |
| **Mean** | 28.10 b* | 29.94 a | 31.17 a | 29.74 |
| **$LSD_{Zn}$** | | 1.80 | | |
| **$LSD_{C}$** | | ns | | |
| **$LSD_{Zn×C}$** | | ns | | |

*: There is no difference at the 0.05 probability level between the mean values with the same letter groups. LSD shows the least significant difference between the mean values. ns means not important.

### $Cu^{2+}$-$Cu^{+}$ (Cuprac) Reducing Capacity

In durum wheat, the cupric ion ($Cu^{2+}$) reducing capacity of the flour sample extracts taken from the grains that were ground with their bran was determined as the absorbance of the solutions at 20 µg $mL^{-1}$ concentration at 450 nm, and these absorbance values are given in Table 4. In durum wheat cultivars, cuprac reducing capacity did not show a statistically significant difference in terms of foliar zinc application stages (milky and dough ripeness) (Table 1 and Table 4). Although there is no statistically significant difference in cuprac reducing capacity of durum wheat cultivars (Table 1); mean absorbance value varied between 0.262 (Eminbey) and 0.287 (Meram-2002) (Table 4). Zinc×Cultivar interaction did not show significant differences in terms of cuprac reduction capacity (Table 1).

### Total Antioxidant Content

Ascorbic acid was used as a standard in the determination of total antioxidant amounts in flour samples taken from grains ground with bran in durum wheat cultivars. Using the regression equation in the standard graph given in Figure 1, the total antioxidant amount was calculated from the unit of ascorbic acid equivalent (AAE) according to the formula below, and the mean values are given in Table 5.

C = [((Absorbance + 0.1917) / 0.0052) ×10]

C: Concentration (mg AAE 100 $g^{-1}$ DM)

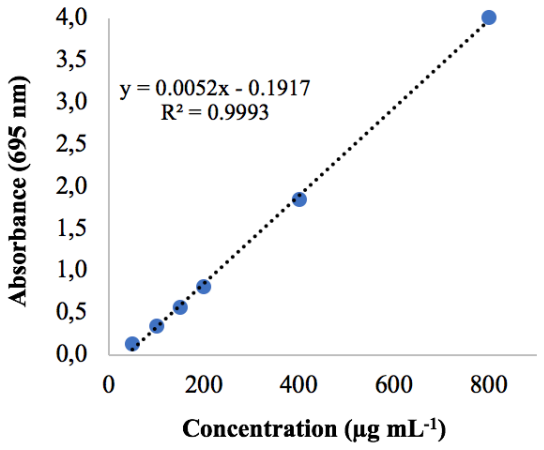



**Figure 1.** Standard graph of total antioxidant content

**Table 4.** Mean values of cuprac reduction capacity of grain in zinc applications at the stage of milky or dough ripeness in durum wheat cultivars (absorbance value)

| Cultivars (C) | Zinc Application Stages (Zn) | | | |
|---|---|---|---|---|
| | Control | Milky ripeness | Dough ripeness | Mean |
| **Ç.1252** | 0.257 | 0.267 | 0.269 | 0.265 |
| **Eminbey** | 0.234 | 0.306 | 0.245 | 0.262 |
| **Kızıltan-91** | 0.296 | 0.248 | 0.279 | 0.274 |
| **Meram-2002** | 0.287 | 0.292 | 0.283 | 0.287 |
| **Selçuklu-97** | 0.287 | 0.242 | 0.274 | 0.268 |
| **Mean** | 0.272 | 0.271 | 0.270 | 0.271 |
| **$LSD_{Zn}$** | | | ns | |
| **$LSD_{C}$** | | | ns | |
| **$LSD_{Zn\times C}$** | | | ns | |

LSD shows the least significant difference between the mean values. ns means not important.

There was no statistically significant difference between foliar zinc application stages (milky and dough ripeness) in terms of total antioxidant content in durum wheat cultivars (Tables 1 and 5). However, the highest value was obtained from zinc application stage of dough ripeness (89.01 mg AAE 100 $g^{-1}$ DM).

Although there are no statistically significant differences in total antioxidant content of durum wheat cultivars (Table 1); it varied between 81.76 mg AAE 100 $g^{-1}$ DM (Meram-2002) and 90.01 mg AAE 100 $g^{-1}$ DM (Selçuklu-97) (Table 5). Menteş-Yılmaz (2011) also reports that the total amount of antioxidants significantly varies according to the cultivars. Zinc×Cultivars interaction did not differ in terms of total antioxidant content (Table 1).

## Total Phenolic Compound Content

Gallic acid was used as a standard in the determination of the total phenolic content in the evaporated ethanol extracts of the flour samples taken from the grains ground with the bran in durum wheat, and the total phenolic compound content was calculated as gallic acid equivalent (GAE) unit from the regression equation in the standard graph given in Figure 2.

Absorbance = 0.0019×[GAE]

The total phenolic compound content of durum wheat cultivars were obtained by using the above formula is given in Table 6.

Although the total phenolic content of durum wheat cultivars did not show statistically significant differences in terms of foliar zinc application stages (Table 1); while the lowest total phenolic content was obtained from the milky ripeness stage (34.5 µg GAE $mg^{-1}$ extract), the highest value was obtained from dough ripeness (37.1 µg GAE $mg^{-1}$ extract) (Table 6). Cultivars showed significant changes at the $p<0.05$ level (Table 1); the highest total phenolic compound content was obtained from the cultivars of Ç.1252 (37.6 µg GAE $mg^{-1}$ extract) and Eminbey (37.2 µg GAE $mg^{-1}$ extract), while the lowest value was obtained from Kızıltan-91 (34.2 µg GAE $mg^{-1}$ extract) (Table 6). Mpofu et al. (2006) also reported that the total phenolic compound content showed significant changes according to the genotypes. Our thesis findings showed values close to the lower limit (37.1 µg GAE $mg^{-1}$) stated by Sedej et al. (2010) for total phenolic content in whole wheat flour. This may be due to the fact that the plant material in our study was grown under minimum input conditions.

In addition, Zinc×Cultivar interaction for total phenolic content shows significant differences ($p<0.05$) (Table 1); this interaction is due to the fact that the Ç.1252 cultivar showed high total phenolic compound values in the control and dough ripeness stage, while low values obtained in the milky ripeness stage (Table 6).

## Total Flavonoid Content

Quercetin was used as a standard for the determination of the total flavonoid content in the evaporated ethanol extracts of the flour samples taken from the grains ground with the bran in durum wheat cultivars. The total flavonoid content was calculated from the quercetin equivalent (QE) unit according to the formula below from the regression equation in the standard graph given in Figure 3, and the mean values were given in Table 7.

Absorbance = 0.0103×[QE]

Total flavonoid content in durum wheat cultivars showed statistically significant differences ($p<0.01$) in terms of foliar zinc application stages (Table 1); the lowest total flavonoid content was obtained from the control without zinc application (0.857 µg QE $g^{-1}$ extract), while the

highest values were from the stage of milky ripeness (1.504 µg QE $g^{-1}$ extract) and dough ripeness (1.532 µg QE $g^{-1}$ extract) (Table 7). The cultivars showed a significant change at the $p<0.01$ level in terms of total flavonoid content (Table 1); the highest flavonoid content was obtained from Ç.1252 cultivar (1,590 µg QE $g^{-1}$ extract), while the lowest value was obtained from Kızıltan-91 cultivar (1,061 µg QE $g^{-1}$ extract) (Table 7). These findings are under the findings of Murathan and Özdinç (2018), who reported that the total flavonid content was in the range of 99.67-302.1 mg $100g^{-1}$.

In addition, Zinc×Cultivar interaction showed significant differences ($p<0.01$) for flavonoid content (Table 1); because of this interaction, cultivars other than Ç.1252 showed low values in terms of total flavonoid content in the control group that did not receive zinc; in other words, it is understood that it is due to being in the lower group statistically. Indeed, it is observed that other cultivars, except Ç.1252, show higher values for total flavonoid content at the stages of milky and dough ripeness compared to the control (Table 7).

## Zinc Content

Statistically significant differences ($p<0.01$) were found in zinc content in durum wheat cultivars in terms of foliar application stages (Table 1); as expected, the lowest zinc content was obtained from the control application without zinc (39.39 mg $kg^{-1}$), while the highest values were from zinc application at the milky ripeness (51.85 mg $kg^{-1}$) and dough ripenes (49.46 mg $kg^{-1}$) stages (Table 8). Cultivars showed significant changes at the $p<0.01$ level (Table 1); this change was determined between 39.68 mg $kg^{-1}$ (Ç.1252) and 49.61 mg $kg^{-1}$ (Selçuklu-97) (Table 8). Zinc×Cultivar interaction did not differ in terms of grain zinc content.

**Table 5.** Mean values of total antioxidant content of grain in zinc applications at the stage of milky or dough ripeness in durum wheat cultivars (mg AAE 100 g-1 DM)

| Cultivars (C) | Zinc Application Stages (Zn) | | | |
|---|---|---|---|---|
| | Control | Milky ripeness | Dough ripeness | Mean |
| **Ç.1252** | 84.42 | 89.49 | 85.76 | 86.56 |
| **Eminbey** | 85.82 | 84.46 | 92.80 | 87.69 |
| **Kızıltan-91** | 93.39 | 88.07 | 85.60 | 89.02 |
| **Meram-2002** | 78.05 | 82.38 | 84.84 | 81.76 |
| **Selçuklu-97** | 86.42 | 87.54 | 96.06 | 90.01 |
| **Mean** | 85.62 | 86.39 | 89.01 | 87.01 |
| **$LSD_{Zn}$** | | | ns | |
| **$LSD_{C}$** | | | ns | |
| **$LSD_{Zn×C}$** | | | ns | |

LSD shows the least significant difference between the mean values. ns means not important.

**Table 6.** Mean values of total phenolic compound content of grain in zinc applications at the stage of milky or dough ripeness in durum wheat cultivars (µg GAE mg-1 extract)

| Cultivars (C) | Zinc Application Stages (Zn) | | | |
|---|---|---|---|---|
| | Control | Milky ripeness | Dough ripeness | Mean |
| **Ç.1252** | 38.1 a-c* | 33.1 e | 41.6 a | 37.6 a |
| **Eminbey** | 35.7 c-e | 36.1 b-e | 39.7 ab | 37.2 a |
| **Kızıltan-91** | 34.6 c-e | 34.6 c-e | 33.5 e | 34.2 b |
| **Meram-2002** | 37.4 b-d | 33.9 de | 34.7 c-e | 35.4 ab |
| **Selçuklu-97** | 38.1 a-c | 34.5 c-e | 36.0 b-e | 36.2 ab |
| **Mean** | 36.8 | 34.5 | 37.1 | 36.1 |
| **$LSD_{Zn}$** | | | ns | |
| **$LSD_{C}$** | | | 2.3 | |
| **$LSD_{Zn×C}$** | | | 3.9 | |

*There is no difference at the 0.05 probability level between the mean values with the same letter groups. LSD shows the least significant difference between the mean values. ns means not important.

**Table 7.** Mean values of total flavonoid content of grain in zinc applications at the stage of milky or dough ripeness in durum wheat cultivars (µg QE $g^{-1}$ extract)

| Cultivars (C) | Zinc Application Stages (Zn) | | | |
|---|---|---|---|---|
| | Control | Milky ripeness | Dough ripeness | Mean |
| **Ç.1252** | 1.694 a* | 1.597 a | 1.481 a | 1.590 a |
| **Eminbey** | 0.522 cd | 1.748 a | 1.375 ab | 1.215 bc |
| **Kızıltan-91** | 0.388 d | 1.359 ab | 1.437 a | 1.061 c |
| **Meram-2002** | 0.728 cd | 1.392 ab | 1.764 a | 1.294 bc |
| **Selçuklu-97** | 0.955 bc | 1.424 a | 1.602 a | 1.327 b |
| **Mean** | 0.857 b | 1.504 a | 1.532 a | 1.298 |
| **$LSD_{Zn}$** | 0.221 | | | |
| **$LSD_{C}$** | 0.256 | | | |
| **$LSD_{Zn×C}$** | 0.443 | | | |

*There is no difference at the 0.05 probability level between the mean values with the same letter groups. LSD shows the least significant difference between the mean values. ns means not important.

**Table 8.** Mean values of zinc content of grain in zinc applications at the stage of milky or dough ripeness in durum wheat cultivars (mg kg-1)

| Cultivars (C) | Zinc Application Stages (Zn) | | | |
|---|---|---|---|---|
| | Control | Milky ripeness | Dough ripeness | Mean |
| **Ç.1252** | 35.12 | 43.72 | 40.20 | 39.68 b* |
| **Eminbey** | 41.08 | 55.10 | 49.67 | 48.61 a |
| **Kızıltan-91** | 38.23 | 54.03 | 51.03 | 47.76 a |
| **Meram-2002** | 42.78 | 49.68 | 54.05 | 48.84 a |
| **Selçuklu-97** | 39.76 | 56.71 | 52.35 | 49.61 a |
| **Mean** | 39.39 b | 51.85 a | 49.46 a | 46.90 |
| **$LSD_{Zn}$** | | 5.91 | | |
| **$LSD_{C}$** | | 4.71 | | |
| **$LSD_{Zn×C}$** | | ns | | |

*There is no difference at the 0.05 probability level between the mean values with the same letter groups. LSD shows the least significant difference between the mean values. ns means not important.

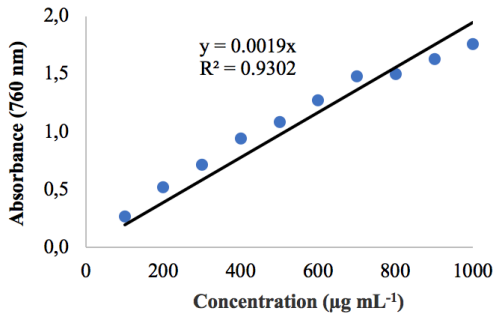



**Figure 2.** Standard graph for total phenolic compound content

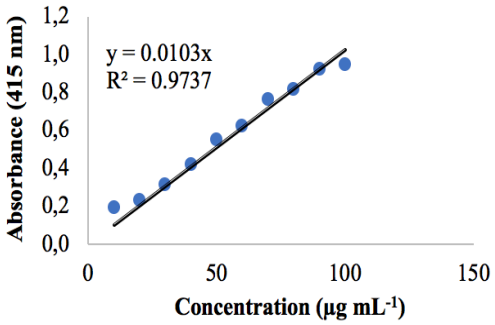



**Figure 3.** Standard graph of total flavonoid content

## Conclusion and Recommendations

With this study, it is concluded that some antioxidant properties of grain such as flavonoid content, $ABTS^{+}$ radical scavenging activity and zinc content can be further increased by foliar zinc application at the stage of dough ripeness of grain. In addition, it is understood that it would be more beneficial to carry out this study on larger plots, with more genotypes, and even by increasing the application periods. Moreover, including the pasta properties in the scope of the study in addition to the physical and chemical quality criteria, it will provide new contributions to the literature and pasta industry.

## COMPLIANCE WITH ETHICAL STANDARDS

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

The contribution of the authors to the present study is equal. However, Aut. G. COŞKUN done his MSc thesis; Aut. F. TOPAL made much supports for the laboratory works; and Aut. B. BAHAR made statistical analysis and the paper writing together with the coordination.
All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Ethics committee approval is not required.

### Funding

This study, which was the MSc thesis project of the author (Gizem COŞKUN), was supported by the GÜBAP2907 (Graduate Student Support Program) with the project number of 20.E3109.07.01 in the Gümüşhane University.

### Data availability

Not applicable.

### Consent for publication

Not applicable.

### Acknowledgements

Authors are thankful to support of the GÜBAP2907 in Gümüşhane University. They also thanks to graduate students who are Ebubekir ÖZLER and Selahattin KOCABAŞ because of their laboratory conribution.

## REFERENCES

Adom, K.K., Sorrells, E.M., Liu. R.H. (2005). Phytochemicals and Antioxidant Activity of Milled Fractions of Different Wheat Varieties. J. Agric. Food Chemistry, 53, 2297-2306 [Google Scholar]

Ak, T., Gülçin. İ. (2008). Antioxidant and Radical Scavenging Properties of Curcumin, Chemico-Biological Interaction, 174, 27-37. [Google Scholar]

Annakkaya, P. (2012). Turna Yemişi (*Vaccinium macrocarpon*) ve Mersin (*Myrtus communis*)'in Liyofilize Edilmiş Su Ekstrelerinin Antioksidan Kapasitelerinin Belirlenmesi ve Fenolik İçeriklerinin Aydınlatılması. Yüksek Lisans Tezi, Atatürk Üniversitesi Fen Bilimleri Enstitüsü, Erzurum, 48s. (in Turkish)

Apak, R., Güçlü, K., Özyürek, M., Karademir, S.E., Erça, E. (2006). The Cupric Ion Reducing Antioxidant Capacity and Polyphenolic Content of Some Herbal Teas. International Journal of Food Science and Nutrition, 57, 292-304. [Google Scholar]

Blois, M.S. (1958). Antioxidant Deteminations by the Use of A Stable Free Radical. Nature, 26, 1199-1200. [Google Scholar]

Çakmak, I. (2008). Enrichment of Cereal Grains With Zinc: Agronomic or Genetic Biofortification, Plant Soil, 302, 1-17. [Google Scholar]

Dinelli, G., Carretero, A.S., Di Silvestro, R., Marotti, I., Fu, S., Benedettelli, S., Ghiselli, L., Gutierrez, A.F. (2009). Determination of Phenolic Compounds in Modern and Old Varieties of Durum Wheat Using Liquid Chromatography Coupled With Time-of-Flight Mass Spectrometry. Journal of Chromatography A, 1216, 7229-7240. [Google Scholar]

Duthie, G., Crozier, A. (2000). Plant-Derived Phenolic Antioxidants. Current Opinion in Clinical Nutrition and Metabolic Care, 3(6), 447-451.

Fang, Y. Z., Yang, S., WU, G. (2002). Free Radicals, Antioxidants and Nutrition. Elsevier Applied Science, 18, 872-879. [Google Scholar]

Gülçin, İ. (2007). Comparison of in Vitro Antioxidant and Antiradical Activities of Ltyrosine and L-Dopa. Amino Acids, 32, 431-438. [Google Scholar]

Gülçin, İ. (2012). Antioxidant Activity of Food Constituents and Overview. Archives of Toxicology, 86, 345-391. [Google Scholar]

Jensen, S.J.K. (2003). Oxidative Stress and Free Radicals. Journal of Molecular Structure: Theochem, 666-667, 387-392. [Google Scholar]

Kasangana, P.B., Haddad P.S., Stevanovic T. (2015). Study of Polyphenol Content and Antioxidant Capacity of Myrianthus arboreus (Cecropiaceae) Root Bark Extracts. Antioxidants, 4(2), 410-426. [Google Scholar]

Kim, K.H., Tsa, R., Yang, R., Cui, S.W. (2006). Phenolic Acid Profiles and Antioxidant Activities of Wheat Bran Extracts and Effect of Hydrolysis Conditions. Food Chemistry, 95, 466-473. [Google Scholar]

Köksal, E. ve Gülçin, İ., 2008. Antioxidant Activity of Cauliflower (*Brassica oleracea* L.) Turkish Journal of Agriculture and Forestry, 32, 65-78. [Google Scholar]

Liu, Z., Ren, Z., Zhang, J., Chuang, C.C., Kandaswamy, E., Zhou, T., Zuo, L. (2018). Role of ROS and Nutritional Antioxidants in Human Diseases. Frontiers in Physiology, 9, Article 477, 1-14. [Google Scholar]

Menteş-Yılmaz, Ö. (2011). Türkiye'de Yetiştirilen Başlıca Buğday Çeşitlerinin Antioksidan Aktivitelerinin ve Fenolik Asit Dağılımlarının Belirlenmesi ve Ekmeğin Nar Kabuğu Ekstraktı ile Zenginleştirilmesi. Doktora Tezi, Ankara Üniversitesi Fen Bilimleri Enstitüsü, Ankara, 89s. (in Turkish)

Mpofu, A., Sapirstein, H.D. ve Beta, T. (2006). Genotype and Environmental Variation in Phenolic Content, Phenolic Acid Composition and Antioxidant Activity of Hard Spring Wheat. Journal of Agricultural and Food Chemistry, 54, 1265-1270. [Google Scholar]

Murathan, T. Z., Özdinç, M. (2018). Ardahan ve Elâzığ illerinde Yetişen *Anchusa azurea* Miller var. *azurea* Bitkisinin Biyoaktif Bileşenleri ve Antioksidan Kapasitesi Üzerine Bir Araştırma. Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 21(4), 529-534. [Google Scholar]

Park. Y.K., Koo, M.H., Ikegaki, M., Contado, J.L. (1997). Comparison of the Flavonoid Aglycone Contents of Apis mellifera Propolis From Various Regions of Brazil. Arquivos De Biologia Technologia, 40, 97-106.

[Google Scholar]

Prado, F.M., Scalfo, A.C., Miyamoto, S., Medeiros, M.H.G., Di Mascio, P. (2020). Generation of Singlet Molecular Oxygen by LipidHydroperoxides and Nitronium Ion. Photochemistry and Photobiology, 96, 560-569. [Google Scholar]

Ragaee, S., Abdel-Aal, E.M., Noaman, M. (2006). Antioxidant Activity and Nutrient Composition of Selected Cereals for Food Use. Food Chemistry, 98, 32-38. [Google Scholar]

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. (1999). Antioxidant Activity Applying An Improved ABTS Radical Cation Decolorization Assay. Free Radical Bioology and Medicine, 26, 1231-1237. [Google Scholar]

Sedej, I.J., Sakač, M.B., Mišan, A. Č., Mandić, A. I. (2010). Antioxidant Activity of Wheat and Buckwheat Flours. Proceedings for Natural Sciences Matica Srpska Novi Sad, 118, 59-68. [Google Scholar]

Shahidi, F., Naczk, M. (1995). Food Phenolics: Sources. Chemistry. Effects. Applications. Technomic Publishing Company Inc. ISBN 1-56676-279-0, USA. 331s.

Sies, H. (1991). Oxidative Stress: From Basic Research to Clinical Application. The American of Journal Medicine, 91, 31-38. [Google Scholar]

Singleton, V.L., Orthofer, R., Lamuela-Raventos, R.M. (1999). Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants By Means of Folin-Ciocalteu Reagent. Methods in Enzymology, 299, 152-178. [Google Scholar]

Zilic, S., Hadži-Taškovic Šukalovic, V., Dodig, D., Maksimovic, V., Kandic, V. (2013). Free Soluble Phenolic Compound Contents and Antioxidant Capacity of Bread and Durum Wheat Genotypes. Genetika, 45(1), 87-100. [Google Scholar]

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 632-636
DOI: https://doi.org/10.31015/jaefs.2022.4.17

# Insecticidal Effects of Some *Bacillus thuringiensis* Commercial Biopreparats on the Larvae of the Tomato Leaf Miner, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae)

**Alime Bayındır Erol**[1] • **Oktay Erdoğan**[1]

[1] Faculty of Applied Sciences, Organic Farming Business Management Department, Pamukkale University, 20600, Çivril/Denizli, Türkiye

## Abstract

Effects of 5 commercial biopreparats named; Dacron®, Florbac®, Dipel®, Delfin® and Rebound® containing different *Bacillus thuringiensis* (*Bt*) strains on the 3rd instar larvae of the tomato leaf miner, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) were determined under laboratory conditions. *T. absoluta*, tomato (Gusto F1) variety and some commercial biopreparats were used as materials in the study. Production of *T. absoluta* larvae were performed under the conditions of the growth chambers where the tomato variety was grown (25±1°C, 60±5% RH and 16:8 photoperiod). Under laboratory conditions, 1, 3 and 5% concentrations of commercial biopreparats were applied on the 3rd instar larvae of the pest, and the experiment was carried out with 5 replications according to a randomized parcels design. As a result of the application of 1% IU/mg concentrations of Dacron® and Delfin® commercial biopreparats, 100% mortality rate was determined in the 3rd instar larvae of *T. absoluta* on the 7th day counts. When 3 and 5% IU/mg concentrations of all commercial biopreparats were applied, a mortality rate of 100% was recorded on the 3rd instar larvae of *T. absoluta* on the 5th and 7th day counts. *In-vitro*, as the concentration of commercial biopreparats increased, mortality rate of the 3rd instar larvae of *T. absoluta* increased. However, it would be appropriate to conduct field trials with the same commercial biopreparats to recommend their use of them commercially.

**Keywords:** *Bacillus thuringiensis*, *Tuta absoluta*, Mortality rate, Biopesticide

**Citation:** Bayındır Erol, A., Erdoğan, O. Insecticidal Effects of Some Bacillus thuringiensis Commercial Biopreparats on the Larvae of the Tomato Leaf Miner, Tuta absoluta (Meyrick, 1917) (Lepidoptera: Gelechiidae). International Journal of Agriculture, Environment and Food Sciences, 6 (4), 632-636.

**Received:** 24 August 2022
**Revised:** 25 September 2022
**Accepted:** 27 September 2022
**Published Online:** 13 December 2022

**Correspondence:** Alime Bayındır Erol
**E-mail:** abayindir@pau.edu.tr



## INTRODUCTION

Tomato (*Solanum lycopersicum* L.) is an annual vegetable crop in the Solanaceae family. It is one of the important sources of income in the regions where it is grown. Various problems are encountered in tomato production, such as plant nutrition, irrigation, diseases, pests and weeds' control (Duman, 2016). The tomato leaf miner, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae), is among the economic pests that cause quality and quantity losses in tomato crop (Anonymous, 2016). The first record of *T. absoluta* in Turkey was in Izmir in 2009 and later it spreaded to other regions (Kılıc, 2010). Although different control methods such as: cultural, biological, biotechnical and chemical control are used for pest control, chemicals are preferred due to their easy application and high effect in a short time. But in this context, many chemical insecticides have been reported creating resistance in the pests (Siqueira et al., 2000). Besides, use of these chemicals, resulted in negative effects on the environment and human health (Durmusoglu et al., 2010). Biological control is among the safe alternative cont-

rol methods applied (Zappala et al., 2013). Among biological control agents, the entomopathogens cause the death of the insects effectively (Karabörklü et al., 2018). *Bacillus thuringiensis* (*Bt*), the most widely used bacterial bioinsecticide among entomopathogens, is a gram positive bacteria in the Bacillaceae family (Feitelson et al., 1992). It is reported that *Bt* is used for the control of pests from orders Lepidoptera, Coleoptera, Diptera, Hymenoptera, Homoptera, Orthoptera and Mallophaga. This bacteria produces specific crystal protein inclusions (δ endotoxin) with toxic insecticidal effects for the control of pest species (Pazos and Salamanca, 2007). Because of this feature, *Bt* has become the most important biopesticide in the world market. It has been reported that *Bt* preparations applied in powder form on tomato plants grown in greenhous and fields are effective (Youssef and Hassan, 2013). The present study aimed to evaluate the effects of commercial biopreparats containing different strains of *Bt* on the 3rd instar larvae of *T. absoluta* under laboratory conditions.

## MATERIALS AND METHODS

### Tomato plants

Tomato seedlings of Gusto F1 variety (10-15 cm), obtained from a private seedling company, were used in the experiments. Tomato seedlings were transplanted into (20-25 cm diameter) plastic pots containing a 1:1 mixture of soil and peat. The planted tomato seedlings were irrigated periodically and maintenance procedures were carried out. During the growing period, no fertilization or chemical control was used in case of diseases and/or pests.

### Producing of Tuta absoluta

The leaves infested with *T. absoluta* from tomato fields in Çivril district of Denizli province were brought to the laboratory in plastic storage containers with a paper bag. Infestation was ensured by leaving the larvae in the galleries from the leaves and leaving them on clean tomato plants in a climate room. Thus, the production of the tomato lifeminer was continued with the transfer of clean tomato plants to the growth chamber at regular intervals. The production of tomato plants and tomato lifeminer was carried out in the growth chambers of Pamukkale University Faculty of Applied Sciences, Department of Organic Farming Business Management, at 25±1°C, 65±5% RH and 16:8 hrs photoperiod.

### Commercial biopreparats used

Commercial biopreparats containing different strains of *Bt* used in the experiments were obtained from companies. Information of the commercial biopreparats used in the experiment are given in Table (1).

### Efficacy of the commercial biopreparats

In order to determine the effects of the commercial biopreparats on the tomato leaves, the experiment was carried out with 5 replications according to randomized parcels design. For this purpose, a layer of blotter paper was placed inside the plastic Petri dish (10 cm diameter) and a compound tomato leaf was placed on it. Five individuals of the 3rd instar *T. absoluta* larvae were placed on tomato leaf. 1, 3 and 5% IU/mg concentrations of each biopreparat were sprayed 3 times, from a distance of 15-20 cm, with the help of a hand sprayer. The experiments were carried out in growth chambers with 25±1°C, 65±5% RH and 16 hrs photoperiod.

### Statistical analysis

Data obtained were analyzed one-way ANOVA and to calculate the differences between groups, the Tukey multiple comparison test ($P < 0.05$) was used. Using the IBM SPSS® (Version 20.0, August 2011, SPSS Inc., Chicago, IL, USA) statistics program version. The mortality rates of treated *T. absoluta* larvae were corrected by (Abbott, 1925).

## RESULTS

Effects of 1, 3 and 5% IU/mg concentrations of commercial bioprepares containing different strains of *Bt* on the 3rd instar larvae of *T. absoluta* are given in Table (2). As a result of 1% dose applications of commercial bioprepares, the highest mortality rates were determined in Rebound® commercial biopreparat with 40.00% on the 1st day counts, in Delfin® commercial biopreparat with 96.00% on the 3rd day counts, and in Dacron® and Delfin® commercial biopreparats with 100% on the 7th day counts. As a result of 3% dose applications of commercial bioprepares, the highest mortality rates were detected in Dacron® and Dipel® commercial biopreparats with %44 on the 1st day counts, and 100% on the 3rd day counts in Dipel® commercial biopreparats. On the 5th and 7th day counting results of the trial, 100% mortality rate was recorded in all commercial biopreparats. As a result

**Table 1.** Content and active ingredient ratio of commercial biopreparats used in the experiment

| Trade name | Active ingredient | Active ingredient ratio |
|---|---|---|
| Dacron® WP | *Bacillus thuringiensis berliner var. kurstaki* | 32000 IU/mg |
| Florbac® WG | *B. thuringiensis var. aizawai* strain ABTS-1857 | 35000 DBM/mg |
| Dipel® DF | *B. thuringiensis subsp. kurstaki* ABTS-351 | 32000 CLU/mg |
| Delfin® WG | *B. thuringiensis berliner var.* kurstaki | 32000 IU/mg |
| Rebound® WP | *B. thuringiensis var. kurstaki* | 16000 IU/mg |

of 5% dose applications of commercial bioprepares, the highest mortality rates on the 3rd day counts were determined in the commercial biopreparats Delfin® (100%), Dacron® (96%), Rebound® (96%) and Dipel® (91%). These commercial biopreparats were statistically at the same group. On the 5th and 7th day counts, 100% mortality rate was determined in all commercial biopreparats (Table 2).

**Table 2.** Effects of commercial biopreparats on the 3rd instar larvae of *Tuta absoluta*

| Biopreparats | Time Intervals (Days) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 1st | | 3rd | | 5th | | 7th | |
| | NLI | Effect% | NLI | Effect% | NLI | Effect% | NLI | Effect% |
| | 1% IU/mg Concentration | | | | | | | |
| Dacron® | 4.40a | 12.00 | 0.60bc | 88.00 | 0.00b | 100.00 | 0.00c | 100.00 |
| Florbac® | 5.00a | 0.00 | 2.40bc | 52.00 | 0.80b | 84.00 | 0.20bc | 96.00 |
| Dipel® | 4.80a | 4.00 | 0.80bc | 84.00 | 0.20b | 96.00 | 0.20bc | 96.00 |
| Delfin® | 4.80a | 4.00 | 0.20c | 96.00 | 0.20b | 96.00 | 0.00c | 100.00 |
| Rebound® | 3.00b | 40.00 | 1.60bc | 68.00 | 1.60b | 68.00 | 1.00bc | 80.00 |
| Control | 5.00a | - | 5.00a | - | 5.00a | - | 5.00a | - |
| | 3% IU/mg Concentration | | | | | | | |
| Dacron® | 2.80bc | 44.00 | 0.40bc | 92.00 | 0.00b | 100.00 | 0.00b | 100.00 |
| Florbac® | 4.80a | 4.00 | 0.20c | 96.00 | 0.00b | 100.00 | 0.00b | 100.00 |
| Dipel® | 2.80bc | 44.00 | 0.00c | 100.00 | 0.00b | 100.00 | 0.20b | 100.00 |
| Delfin® | 4.60a | 8.00 | 1.20b | 76.00 | 0.00b | 100.00 | 0.00b | 100.00 |
| Rebound® | 4.20a | 16.00 | 0.40bc | 92.00 | 0.00b | 100.00 | 0.00b | 100.00 |
| Control | 5.00a | - | 5.00a | - | 5.00a | - | 4.40a | - |
| | 5% IU/mg Concentration | | | | | | | |
| Dacron® | 3.20a | 36.00 | 0.20c | 96.00 | 0.00b | 100.00 | 0.00b | 100.00 |
| Florbac® | 3.60a | 28.00 | 2.20b | 52.00 | 1.00b | 100.00 | 0.20b | 100.00 |
| Dipel® | 3.80a | 24.00 | 0.40c | 91.00 | 0.00b | 100.00 | 0.20b | 100.00 |
| Delfin® | 4.00a | 20.00 | 0.00c | 100.00 | 0.00b | 100.00 | 0.00b | 100.00 |
| Rebound® | 4.60a | 8.00 | 0.20c | 96.00 | 0.20b | 100.00 | 0.00b | 100.00 |
| Control | 5.00a | - | 4.80a | - | 4.40a | - | 4.40a | - |

*The means followed by the same letters within columns are not significantly different from each other according to Tukey's HSD (P< 0.05). NLI: Number of living individuals.

## DISCUSSIONS

Studies have shown that *B. thuringiensis* var. *kurstaki* (*Btk*) isolates were found to be effective on all larval instars of *T. absoluta* (Giustolin et al., 2001; Cabello et al., 2009). In another study conducted with B1, B2, B3 and B4 isolates of *Btk*, the highest mortality rates were determined were 93.3, 90, 86.7 and 80% for 4th instar larvae, respectively (Youssef and Hassan, 2013). It was determined that *T. absoluta* damage was reduced by 98% as a result of the application of Turex®, Dipel DF® and Costar® commercial biopreparats containing *Bt* to tomato leaves under laboratory conditions (Gonzalez-Cabrera et al., 2011). As a result of the application of *Bt* (0.5 g/l) isolate under laboratory and greenhouse conditions, a mortality rate of 55-65% was recorded in the 2nd instar larvae of *T. absoluta* (Jallow et al., 2018).

One day after the application of (*Bt*) isolate at a concentration of $10^6$ cells/ml to *T. absoluta* larvae using the spray method, the highest mortality rates were recorded in the 2nd and 3rd instar larvae (Rezaei and Talaei-Hassanlous, 2016). Alsaedi et al. (2017) applied $10^6$ cells/ml concentration of *Btk* isolate to the 1st, 2nd, 3rd and 4th instar larvae

of *T. absoluta* and they have reported that the highest mortality rates were 20, 22.66, 18.66 and 23.33%, respectively.

It was reported that the damage of *T. absoluta* decreased by 91.92% as a result of the application of *Btk* (*Bt*, Delfin® WG, 32000 IU/ mg) -(100 g/hl) + granulated sugar (150 g/hl) in greenhouse conditions (Doganlar et al., 2015). In a study conducted by Satıs (2013) under field conditions, the rate of *T. absoluta* contamination was recorded as 14.8% in the plot where Rebound® (*Btk*, 150g+100g sugar/100 l water concentration) was applied, and as 28.2% in the control plots. In another study conducted under field conditions, a mortality rate of 75.9% was determined with Dipel®2X (*Btk*, 6.4%, 100 g/100 l concentration) (Moussa et al., 2013). As a result of the highest rate of application of Costar®, one of the commercial preparats, under field conditions, reported that the 3rd instar larvae showed higher sensitivity than the 2nd instar larvae (Tsoulnara and Port, 2016). In another study, the commercial preparats were 70% effective for Delfin® and 59% for Rebound at the licensed concentration (Catalbudak et al., 2018).

## CONCLUSIONS

It was concluded that the 1% dose applications of commercial biopreparats named: Dacron®, Florbac®, Dipel®, Delfin® and Rebound® applied against the 3rd instar *T. absoluta* larvae, under laboratory conditions, where caused 100% mortality by Dacron® biopreparat on the 5th day counts and Delfin® commercial biopreparats on the 7th day counts. 3 and 5% dose applications of all commercial biopreparats used against tomato lifeminer were recorded 100% mortality on the 5th and 7th day counts. The resulted percentage of mortality increased by increasing the concentration of the commercial biopreparats tested. Evaluation of the tested commercial biopreparats under field conditions is needed.

## COMPLIANCE WITH ETHICAL STANDARDS

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Ethics committee approval is not required.

### Funding

No financial support was received for this study.

### Data availability

Not applicable.

### Consent for publication

Not applicable.

## REFERENCES

Abbott, W. S. (1925). A method of computing the effectiveness of an insecticide. J Econ Entomol 18(2): 265–267. [Google Scholar]

Alsaedi, G., Ashouri, A., Talaei-Hassanloui, R. (2017). Evaluation of *Bacillus thuringiensis* to control *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) under laboratory conditions. Agric Sciences 8: 591-599. [CrossRef]

Anonymous, (2016). Combating tomato diseases and pests. Ministry of Food, Agriculture and Livestock, 64p.

Cabello, T., Gallego, J.R., Vila, E., Soler, A., del Pino, M., Carnero, A., Hernandez, S., Polaszek, A. (2009). Biological control of the South American tomato pinworm, *Tuta absoluta* (Lepidoptera: Gelechiidae), with releases of *Trichogramma achaeae* (Hymenoptera: Trichogrammatidae) on tomato greenhouse of Spain. IOBC-WPRS Bulletin 49: 225-230. [Google Scholar]

Catalbudak, E. S., Aydın, A., Durmusoglu, E. (2018), Determination of efficacy of some biopreparats on *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) larvae. Turk Bull Entomology 8(4): 113-122 (in Turkish). [CrossRef]

Duman, İ. (2016). Industrial tomato cultivation. Journal of Turkish Seed Association 17: 18-21.

Durmusoglu, E., Tiryaki, O., Canhilal, R. (2010). Pesticide use, residue and durability problems in Turkey. Turkey Agricultural Engineering 7th Technical Congress, Ankara, Proceedings 2, 589-607 (in Turkish).

Doganlar, M, Yıldırım, A. E., Yigit, A. (2015). Efficacy of *Bacillus thuringiensis* var. *kurstaki* and some environmentally friendly pesticides on the Tomato moth, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Turk J Biol Control, 6(1): 13-24. [Google Scholar]

Feitelson, J. S., Payne, J., Kim, L. (1992). *Bacillus thuringiensis*: insects and beyond. BioTechnol 10: 271–275. [Google Scholar]

Giustolin, T. A., Vendramim, J. D., Alves, S. B., Vieira, S. A., Pereira, R. M. (2001). Susceptibility of *Tuta absoluta* (Meyrick) (Lep., Gelechiidae) reared on two species of Lycopersicon to *Bacillus thuringiensis* var. *kurstaki*. J Appl Entomology 125: 551-556. [CrossRef]

Gonzalez-Cabrera, J., Mollà, O., Montan, H., Urbaneja, A. (2011). Efficacy of *Bacillus thuringiensis* (Berliner) in controlling the tomato borer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). BioControl 56: 71–80. [Google Scholar]

Jallow, M. F. A., Dahab, A. A., Albaho, M. S., Devi, V. Y. (2018). Efficacy of some biorational insecticides against *Tuta absoluta* (Meyrick) (Lepidoptera Gelechiidae) under laboratory and greenhouse conditions in Kuwait. J. Appl. Entomology 143: 187–195. [CrossRef]

Karabörklü, S., Azizoğlu, U., Azizoglu, Z. B. (2018). Recombinant entomopathogenic agents: a review of biotechnological approaches to pest insect control. W J Microbiol Biotech 34(1): 14. [CrossRef]

Kılıc, T. (2010). First record of *Tuta absoluta* in Turkey. Phy-

toparasitica 38(3): 243-244. [CrossRef]

Moussa, S., Baiomy, F., Sharma, A., El-Adl, F. E. (2013). The status of tomato leafminer *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Egypt and potential effective pesticides. Academic J Entomol 6(3): 110-115. [CrossRef]

Pazos, L., Salamanca, C. (2007). Three-dimensional structure of *Bacillus thuringiensis* toxins. Acta Biol Colomb 12: 19-32.

Rezaei, A. A., Talaei-Hassanlous, R. (2016). The use of *Bacillus thuringiensis*-based products in biocontrol of tomato leaf miner, *Tuta absoluta* (Lepidoptera Gelechiidae). Int. J. Agric. Innov. Research 4: 814–817.

Siqueira, H. A., Guedes, R. N., Picanco, M. C. (2000). Insecticide resistance in populations of *Tuta absoluta* (Lepidoptera: Gelechiidae). Agricultural and Forest Entomology 2: 147-153. [CrossRef]

Satıs, Ü. (2013). Effect of *Bacillus thuringiensis* var. *kurstaki* and Chlorantraniliprole+Abamectin on larve of the tomato leafminer *Tuta absoluta* (Meyrick, 1917) (Lepidoptera:Gelechiidae) on tomato (*Lycopersicon esculentum* Mill.). Harran University Institute of Science and Technology, Master Thesis, 46 p (in Turkish).

Tsoulnara, D., Port, G. (2016). Efficacy of a *Beauveria bassiana* strain, *Bacillus thuringiensis* and their combination against the tomato leafminer *Tuta absoluta*. Entomol Hellenica 25: 23-30. [CrossRef]

Youssef, N. A., Hassan, G. M. (2013). Bioinsecticide activity of *Bacillus thuringiensis* isolates on tomato borer, *Tuta absoluta* (Meyrick) and their molecular identification. African J Biotech 12: 3699-3709. [Google Scholar]

Zappala, L., Biondi, A., Alma, A., Al-Jboory, I. J., Arno, J., Bayram, A., Chailleux, A., El-Arnaouty, A., Gerling, D., Guenaoui, Y., Shaltiel-Harpaz, L., Siscaro, G., Stavrinides, M., Tavella, L., Aznar., R. V., Urbaneja, A., Desneux, N. (2013). Natural enemies of the South American moth, *Tuta absoluta*, in Europe, North Africa and Middle East, and their potential use in pest control strategies. J Pest Sciences 86: 635–647.

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 637-643
DOI: https://doi.org/10.31015/jaefs.2022.4.18

# Effects of different doses of ammonium nitrate applications on nutrient content in some types of grass: nutritional support

**Yavuz Alkan**[1] • **Tolga Sarıyer**[2] • **Cafer Türkmen**[3] • **Abdullah Kelkit**[4]

[1] Landscape and Ornamental Plants, Vocational Instıtute of Lapseki, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye
[2] Horticulture, Agriculture, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye
[3] Soil Science and Plant Nutrition, Agriculture, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye
[4] Landscape Design, Faculty of Architecture and Design, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye

**Citation:** Alkan, Y., Sarıyer, T., Turkmen, C., Kelkit, A. (2022). Effects of different doses of ammonium nitrate applications on nutrient content in some types of grass: nutritional support. International Journal of Agriculture, Environment and Food Sciences, 6 (4), 637-643.

**Received:** 18 September 2022
**Revised:** 01 October 2022
**Accepted:** 06 October 2022
**Published Online:** 10 December 2022

**Correspondence:** Yavuz Alkan
**E-mail:** yalkan58@comu.edu.tr



## Abstract

This study was conducted under ecological conditions of Çanakkale (Turkiye) province to investigate the effects of different ammonium nitrate (33% N) dose (0, 50 and 100 g $m^{-2}$) on nitrogen, protein, macro-micro nutrient analyses of 3 different commonly cultivated grass varieties (*Lolium perenne* TOPGUN, *Festuca rubra* SERGEİ and *Poa pratensis* AVALANCHE). Plant nitrogen-protein analyses (nitrogen, protein) were determined besides plant macro-micro nutrient analyses (phosphorus, potassium, calcium, magnesium, ıron, manganese) of grass plants were determined. In the study, in addition to having information about the mineral content of grass species removed from the soil, the potential of the cuttings, which are formed when the grass plants are mowed, when used as green manure or compost has been determined.

Fertilizer treatments increased nitrogen, phosphorus, potassium and protein contents of grass clippings and decreased calcium, iron, magnesium and manganese contents. In general, it was determined that the application of ammonium nitrate (33% N) at a dose of 100 g $m^{-2}$ increased the nitrogen, protein, phosphorus and potassium amounts of plants compared to the application of ammonium nitrate (33% N) at a dose of 50 g $m^{-2}$. Especially nitrogen phosphorus potassium fertilizers are among the fertilizers applied in intensive amounts in the agricultural sector. It is seen that nitrogen-containing ammonium nitrate fertilizer applied to grass plants increases the nitrogen content of grass plants. When the results of the study were examined, important information was obtained about how the fertilization density affects the nutrient content of the grass plants according to the species.

**Keywords:** Grass species, Grass clippings, Fertilization, Mineral composition, Aesthetic and functional effects

## INTRODUCTION

World consumption of major mineral fertilizer elements such as nitrogen, phosphorus and potassium has increased steadily from 112 million metric tons in 1980 to 143 million metric tons in 1990 to meet the increasing food demands and has remained stable over the last ten years. Unused minerals can intrude into surface waters, hold on to soil particles or cause air pollution (Taiz and Zeiger, 2008). According to the data of 115 countries in terms of nitrogen, it is reported that 161 metric tons of nitrogen was consumed for products up taking 73-86 metric tons of nitrogen and nitrogen use efficiency was calculated as 46%

(Zhang et al., 2021). Since a significant part of nitrogenous fertilizers is used to provide continuous green cover in sportive and recreational areas, the nitrogen balance between the nitrogen applied to green areas and the nitrogen removed through mowing is an important issue. It is thought that the data obtained from this study, which was designed to determine the effects of different nitrogen fertilization applications on mineral composition of grass, will have great contributions to scientific literature (Atar, 2020). The grass clippings that emerge during mowing can be used in the form of green manure, mulch and compost (Anonymous, 2021a). Organic materials obtained from grass clippings can help in reaching the ideal form (management) when the soil is sandy, heavy clayey or poor in organic matter.

Composting is a process of inoculating grass clippings or the other plant materials with a small amount of soil, including microorganisms that decompose organic matter, until reaching odorless, dark-colored and slightly moist form through aeration and maturation stages. Grass clippings with high nitrogen contents may offer great contributions to compost piles. Grass clippings should not solely be a compost material, but should be mixed with dry materials such as leaves or straw (Anonymous, 2021b). When grass clippings are used as mulch, they help maintain soil moisture and prevent weeds on soil surface. Clippings can also be used as green manure (Anonymous, 2021c). Agustina and Sriharti (2020) reported that they obtained compost by mixing grass clippings with goat manure, rice bran and three different commercial microbial activators.

Fernando et al. (2015) conducted a study to determine whether an approach focused on producing perennial herbs as a source for bio-based (bioenergy) crops in marginal Mediterranean soils (without consuming soil nutrients, water resources or adversely affecting biological and landscape diversity) could diminish greenhouse gas (GHG) emissions. In their study, they mentioned that the demand for more new, various and sustainable agricultural production systems has increased due to reasons such as the decrease in supply and safety of petroleum based materials, climate change, and increase in environmental concerns. They concluded in their study that it is possible to grow perennial herbs in marginal Mediterranean soils and would have relatively few environmental side effects if properly managed.

Among the purposes of fertilization applications is to determine the degree of effect of high-dose fertilization on plants. Williams and Silcock (1997) conducted an ammonium nitrate fertilization study on *Sphagnum magellanicum* plant. It was reported that 1 g N m-2 year-1 ammonium nitrate treatments promoted plant growth and development; 3 g and 10 g N m-2 year-1 treatments did not have any favorable effects on plant growth and development. It was stated that increase in N without an increase in carbon quantity of the plant caused a decrease in C:N ratio in plant tissues.

Some plant species may remove more nutrients from soil than other species. Singh et al. (2015) conducted a study on mineral composition and nutrient removal of 6 perennial grass species *[(sugarcane (Saccharum spp.* hybrid), energy cane (*Saccharum spp*. hybrid), sweet cane *(S. arundinaceum (Retz.)* Jesw.), elephant grass *(P. purpureum (Schum.)),* giant miscanthus (Miscanthus × giganteus (Greef and Deuter ex Hodkinson and Renvoize), giant reed (*A. donax L.*)]. The greatest N, P and K removals were observed in sugarcane, sweet cane, energy cane and elephant grass varieties and the lowest in giant miscanthus variety. Significant variations were observed in nutritional composition of the varieties.

The amount of nutrients that plants remove from the soil can also vary according to the dose of fertilizer used (Balcı and Taban, 2018). Kleiber and Komosa, (2011) applied 0, 50, 100, 150, 200 mg N dm-3 ammonium nitrate doses to soils in which mixture of different grass species [(perennial ryegrass (*L. perenne L.*) var. Grasslands Nui (45%), tall fescue (*F. arundinacea Schreb*) Finelawn (25%), red fescue (*F. rubra*)] Hack.) Olivia (10%), red fescue (*F. rubra Hack*.) Boreal (15%) and Kentucky bluegrass (*P. pratensis L.*) Balin (5%)] were sown. It was reported that Fe content of above-ground parts of the plants decreased in 150 and 200 mg N dm-3 treatments and there was no significant difference in the other treatments. It was also reported that Mn contents increased in 50, 100, 150 mg N $dm^{-3}$ treatments and there was no significant difference in the other treatments.

In the light of above-specified studies, objectives of the present study were set as:

- To determine the effects of ammonium nitrate fertilizer doses on mineral composition of the plants,
- To determine the changes in nutritional composition of grass species when the clippings were used in compost or mulch production,
- To determine optimum fertilization practice for classical grass applications.

## Grass Plants In General

Grass plant; predominantly aesthetic and functional use and marked by different shades of green, can grow up to 10-15 cm from the surface, form superficial roots and can be walked on ground cover plants. Within the scope of design and planning principles and elements, it is essential for landscaping or open and green areas. Especially in urban areas, the richness of aesthetic and functional use is the determinant of the quality of life of the individual today. In other words, it is an indispensable landscape dimension that offers the opportunity for the individual to renew himself or to use recreationally in terms of physical and spiritual. Grass species, which play an important role in the landscape as a green surface cover, are members of the Graminae family. With the spread of outdoor

sports such as football and golf, the role of grass areas in planning has also started to increase (Anonymous, 2008).

Definition and Importance Grass plants are horizontal elements of space. It gives vitality and beauty to the environment in which they are located. Grass plants; It has many functional effects in parks and gardens, playgrounds, promenades. Functional Effects • Absorbs the sun's rays in large areas. • Eliminates dust problem. • Prevents erosion in inclined places. • Provides a place of play and rest for people. Aesthetic Effects • Provides a beautiful appearance. • Beautifies the urban appearance. • Creates color harmony with other plants used in the park and garden. Lawn plants are the cornerstones of grass cover. Grass plants should tend to form a densely formal structure, especially spread out with leaf shoots. Grass plants have some characteristics that they are desirable to have. These are; Main Features • Resistant to form • Strength ability • Regeneration strength • Competitiveness • Rooting intensity Auxiliary features • Resistance to diseases • Pressability • Minimal tangling • Suitable color • We can list it as drought resistance. In order to grow grass plants in the best way, it is necessary to know the botanical features of plants in this group, such as leaves, roots, stems, rhizomes, stolon (Anonymous, 2008).

The general definitions of the 3 grass seeds evaluated within the scope of this study are mentioned below.

**Lolium perenne (English grass);** It is a type of perennial grass that is not very resistant to being pressed and in hot climate, developing quickly, preferring more flat areas. It stands out for its plump structure and fringe root, which loves fertilization (Anonymous, 2008).

**Festuca L. (Yumak);** It has a thin and hard texture and is resistant to printing. It is a type of grass that is close to blue or green in color, resistant to arid and unproductive soils, loves perennial, shaded environments.

**Poa pratensis L. (Meadow panicle grass):** This species, which is resistant to diseases, contraction and drought, is perennial and attracts attention with its fine texture and dark green color. It is preferred in repairing deteriorating places in grass areas with good development in shaded and calcareous environments (Anonymous, 2008).

## MATERIALS AND METHODS

The study was carried out under the ecological conditions of Çanakkale province. This study, which provides a different approach to the lawn area facility within the scope of landscape design and planning, focuses on the inclusion of clippings obtained from mowing the lawn in the fertilizer support program. In this context, in the study, the reactions of 3 different grass species suitable for the ecology of the region to the application of Ammonium Nitrate in different doses and the determination of nutrients within the scope of the content were investigated. Therefore, a significant development in cultivation will be achieved by using the clippings obtained due to mowing in the fertilizer support program within the scope of the nutrients it contains. A map of the location of the workspace is given in Figure 1.

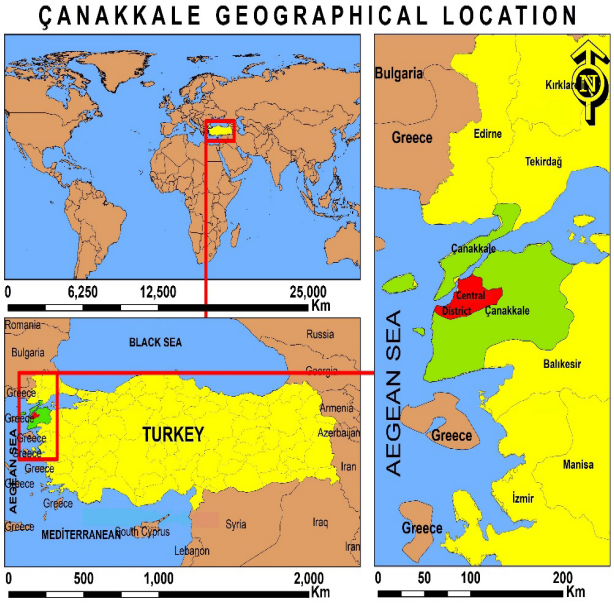



**Figure 1.** The location map of the study area

The *L. perenne* TOPGUN (British Grass), *F. rubra* SERGEI (Red Festuca) and *P. pratensis* AVALANCHE (Blue Grass) were used as the plant material of the present study. Ammonium nitrate (33% N) fertilizer was applied to grass species at 0, 50 100 g m-2 doses (Table 1). Seeds were manually sown into equal-size boxes (40x60x10 cm) filled with equal quantity of soil. Boxes of grass varieties irrigated daily with a watering can until the sand filled with water and boxes irrigated until excess water seeps out of the holes in the bottom of the boxes. A plastic sheet was kept ready next to the trial in case the newly planted grass seeds would come out of the container in case of rain. In order to protect the grass seeds from excessive heat and sunlight until germination, the seeds were covered with a net shading material with 40% light transmission until germination was completed.

It is known that all cultivars included in the study are drought resistant cultivars. It is known that the FR variety is a shade-tolerant variety. In addition, it is known that LP and FR varieties are easily germinated and tilled varieties. It is known that the PP variety spreads easily with the help of root stems.

Experiments were built in randomized plots design with 5 replications on 36 plots (3 grass species x 3 treatments including control x 4 replicates). Statistical analyses were conducted on measurement, observation and analysis data.

### Analysis-based parameters of grass plants

Plant nitrogen-protein analyses; green parts of dried grass plants were ground in a plant mill with steel blades. Ground samples were wet-digested (Müftüoğlu et

al., 2014) and nitrogen contents were determined with the use of Kjeldahl device (Bremner, 1965). Resultant nitrogen contents were multiplied by a coefficient of 6.25 to get protein contents of the samples (Kacar and İnal, 2008).

Plant macro-micro nutrient (K, P, Mg, Ca, Fe, Mn) analyses; previously dried and ground (Kacar and İnal, 2008; Müftüoğlu et al., 2014) samples were wet-digested (Jones et al., 1991) and resultant extracts were subjected to macro-micro nutrient analyses in an ICP-OES (Perkin Elmer Optima 8000) device. A verification reading was performed in the device with the control sample in every 20 readings.

**Table 1.** Grass varieties and fertilizer doses

| Grass Varieties | Fertilizer Doses ($g.m^{-2}$) | Code | Replicate |
|---|---|---|---|
| Lolium perenne TOPGUN (British Grass) | 0 | LP0 | 4 |
| | 50 | LP50 | 4 |
| | 100 | LP100 | 4 |
| Festuca rubra SERGEI (Red Festuca) | 0 | FR0 | 4 |
| | 50 | FR50 | 4 |
| | 100 | FR100 | 4 |
| Poa pratensis AVALANCHE (Blue Grass) | 0 | PP0 | 4 |
| | 50 | PP50 | 4 |
| | 100 | PP100 | 4 |

Experimental soil properties; medium-textured field soil taken from Lapseki (Çanakkale/Turkey) location was used in present experiments. Soil samples were initially dried at shade, then fragmented with a wooden hammer and pass through 2 mm sieve. Samples were made ready for analyses as indicated by Müftüoğlu et al. (2014). Soil samples were analyzed for the relevant parameters in accordance with the relevant methods and results are provided in Table 2.

Statistical analysis: Experimental data were subjected to variance analysis in accordance with randomized plots design with the use of SAS.9 software. Significant means were compared with the use of LSD test (P<0.05) (Yurtsever, 1984). Bi-plot analysis was used for interpretation of data on morphological and chemical parameters.

## RESULTS AND DISCUSSION

In all varieties, nitrogen, protein, potassium contents were increased with increasing dose of fertilization treatments (Table 3). Nakano et al. (2008), reported that increasing the dose of applied N at active tillering and anthesis periods generally increased grain protein content in bread wheat cultivar 'Minaminokaori'. Godebo et al. (2021), applied N (0, 23, 46, 69 kg Nha-1) and $K_2O$ (0, 30, 60 kg Nha-1) on bread wheat and increased N doses resulted as increasion of K uptake in bread wheat (Triticum aestivum L.) at 0 doses of $K_2O$ treatments.

**Table 2.** Experimental soil properties

| Soil properties | Analysis value | Unit | Method |
|---|---|---|---|
| Soil reaction (pH) 1:2.5 aqueous mixture | 6,44 (Slight acidic) | -- | (Richards, 1954) |
| Soil salinity (EC) 1:2.5 aqueous mixture | 0,23 (Unsaline) | dS $m^{-1}$ | (Richards, 1954) |
| Soil organic matter (OM) | 1,82 (Low) | % | (Jackson, 1958) |
| Lime ($CaCO_3$) | 3,86 (Low) | % | (Allison ve Moodie, 1965) |
| Texture (Sand-Silt-Clay) | 35-29-36 (Clay-Loam-CL) | % | Bouyoucos (1951) |
| Total nitrogen | 0,12 | % | Bremner (1965) |
| Available phosphorus | 10 | ppm | Olsen et al. (1954) |

In LP variety, manganese content was not change with both fertilization treatments and there were not important statistical change (P<0,05) in ıron content with 100 g m-2 fertilization. Sharp increase was detected in phosphorus content with 100 g m-2 fertilization. Calcium and magnesium contents statistically (P<0,05) decreased with both fertilization treatments while ıron content decreased with 50 g m-2 fertilization.

Iron contents did not change with both fertilization treatments in FR variety. In PP variety, iron contents decreased with both fertilization treatments. Kleiber and Komosa (2011) reported decreasing iron contents of grass species with 150 and 200 mg N dm-3 ammonium nitrate treatments.

Manganese contents, which did not change in LP variety with fertilization treatments; decreased in FR variety with fertilization treatments. In PP variety it did not change with 50 g.m-2 fertilization treatment and increased with 100 g.m-2 fertilization treatment. Kleiber and Komosa (2011) reported increasing manganese contents of grass species with 50, 100 and 150 mg N dm-3 ammonium nitrate treatments.

In FR variety, phosphorus changes in plots without and with 100 g m-2 fertilization were placed into the same group statistically (P<0,05). Calcium and magnesium contents decreased statistically (P<0,05) with fertilization treatments and more decrease occurred with more fertilization doses.

In PP variety there was a visible increase in phosphorus content with both fertilization treatments. Decreasing calcium contents were seen in all varieties with fertilization treatment. PP variety didn't show same results with other varieties about decreasion of magnesium content with fertilization treatments.

In non-fertilized plots, significant differences were not observed in potassium, nitrogen and protein contents. As compared to the other varieties, LP variety had gre-

ater phosphorus contents and PP variety had greater iron contents. FR variety had lower magnesium content than the other varieties. In terms of phosphorus, iron and magnesium contents, afore-mentioned varieties were placed into the same statistical group (P<0,05). In terms of calcium content, grass species were ordered as LP > FR > PP and manganese contents as FR > LP > PP.

PC1 (1st Component) and PC2 (2nd Component) are the two main components used to achieve the biplot. In study, PC1 and PC2 scores describe the interaction of 3 grass varieties and 2 fertilization applications for 8 mineral components by 78,5%.

Biplot graph for mineral contents of grass plants (Figure 2) revealed that the plots without fertilization (L0, F0, P0) and iron, magnesium, calcium and manganese parameters were placed in the same direction (PC1<0). The fertilized plots (L50, L100, F50, F100, P50, P100) were placed in the same direction (PC1<0) with nitrogen, phosphorus, potassium, protein parameters.

Such a case revealed that a decrease was seen in iron, magnesium, calcium and manganese contents of grass varieties and an increase was seen in nitrogen, phosphorus protein and potassium contents of grass varieties with fertilization treatments.

When the issues other than fertilization applications were evaluated, it was seen that the FR variety had almost twice the manganese content of the other varieties. It has been observed that the manganese content of a compost to be produced using the clippings obtained from this variety will be much higher than the compost produced from other varieties. The same is true for the iron content of the PP variety. From here, it is seen that the elements in some soils rich in the mentioned elements can be transferred to the soils that need these elements with the help of grass plant compost by using specific grass varieties.

It was observed that fertilizer treatments at different doses resulted in different mineral contents in different grass varieties. In addition, it was determined that ammonium nitrate fertilization significantly increased the phosphorus and potassium contents of grass plants. This showed that only as a result of nitrogen-containing fertilization, grass plants also absorb the phosphorus and potassium contents of the soil. It is seen that the use of clippings fertilized with ammonium nitrate in a compost application to be made using grass clippings will contribute to indirectly benefiting from the ammonium nitrate fertilizer applied to grass plants. This contribution may also be in the direction of benefiting more from the phosphorus and potassium content in the soil where grass plants are grown. At the same time, it should not be forgotten that if the grass plant provides the desired parameters such as surface coating, texture, color, plant height as a result of fertilization applied at a low rate, fertilizing at high doses can be avoided both in terms of environmental health and economically. In addition, as seen in the study, a decrease was observed in the micro element content of grass with ammonium nitrate fertilization. This shows that the micro element content of the compost material, which produced using grass clippings with ammonium nitrate fertilizer applied, will be lower than the subject without fertilizer application. If the compost produced from grass clippings is planned to be applied to the farm, it is thought that it may be beneficial to consider this situation.

Further research is recommended to be conducted under micro-scale controlled conditions with different grass mixtures, different fertilizers and different soil types for longer durations.

**Table 3.** Change in some nutrients of the seedlings based on grass varieties and fertilization treatments

| Varieties | Nitrogen dose (g m$^{-2}$) | Nitrogen (%) | Protein (%) | Phosphorus | Potassium | Calcium | Magnesium | Iron | Manganese |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | *(ppm)* | | | |
| *Lolium perenne* Topgun (LP) | 0 | 1.69 F* | 10.57 F | 1942 CD | 13789 D | 11079 A | 6553 AB | 1011 BC | 125.2 BC |
| | 50 | 2.41 D | 15.09 D | 1991 BCD | 22706 B | 7291 CDE | 5684 C | 737.7 C | 119.5 BC |
| | 100 | 2.77 A | 17.29 A | 2607 ABC | 27157 A | 7617 CD | 5223 C | 906.3 BC | 118.6 BC |
| *Festuca rubra* Sergeı (FR) | 0 | 1.69 F | 10.54 F | 1577.71 D | 13560.3 D | 10057.6 B | 6305.92 B | 932.3 BC | 218.3 A |
| | 50 | 2.26 E | 14.1 E | 2791.62 A | 21414.6 B | 8072.54 C | 5727.07 C | 844 BC | 149.6 B |
| | 100 | 2.76 A | 17.28 A | 1583.39 D | 27543.2 A | 6503.42 E | 4564.28 D | 924.6 BC | 133.4 BC |
| *Poa pratensis* Avalanche (PP) | 0 | 1.68 F | 10.49 F | 1432 D | 10900 D | 8108 C | 6530 AB | 1625 A | 105.3 C |
| | 50 | 2.53 C | 15.81 C | 2655 ABC | 17384 C | 6747 DE | 6961 A | 1081 BC | 102.5 C |
| | 100 | 2.63 B | 16.43 B | 2720 AB | 21043 B | 6741 DE | 6428 B | 1313 AB | 121.7 CB |
| LSD | | 0.0935 | 0.5846 | 735.86 | 3276.2 | 1021.2 | 504.24 | 478.45 | 38.844 |

*: Means indicated with different capital letters in the same column are significantly different.

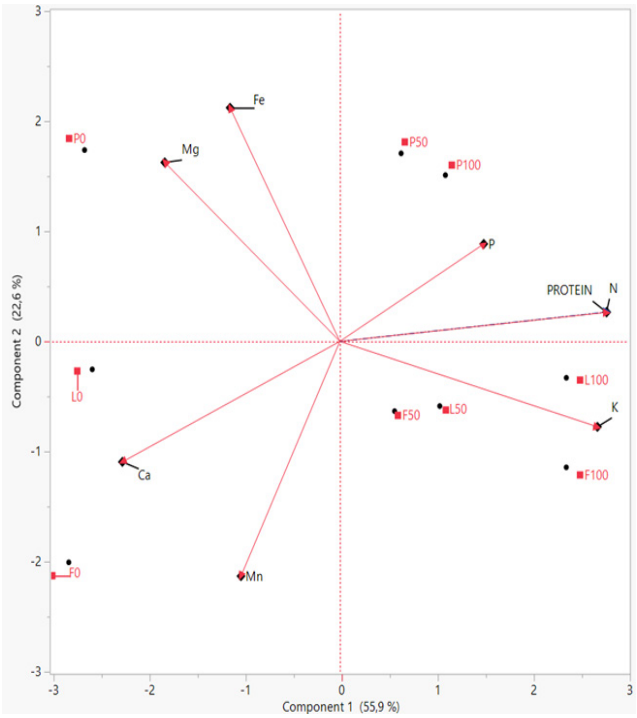



**Figure 2.** Biplot graph for changes in mineral contents of grass plants based on grass varieties and fertilization treatments

## COMPLIANCE WITH ETHICAL STANDARDS

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

Associate Professor Dr. Yavuz Alkan took part in determining the subject and scope of the experiment. It also provided the identification and supply of tools and equipment required for the Test. Contributed to the establishment of the experiment. Contributed to the writing of the article.

Prof. Dr. Cafer Türkmen: Made some analyzes and contributed to some analyzes. Contributed to the wri-ting of the article.

Dr. Tolga Sarıyer: Contributed to the establishment of the experiment. Involved in the inclusion of some analyzes in the study. Made some analyzes and contributed to all analyzes. Contributed to the writing of the article.

Prof. Dr. Abdullah Kelkit: Contributed to the writing of the article. Made the final check of the article.

All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Ethics committee approval is not required.

### Funding

This study was supported by Çanakkale Onsekiz Mart University Scientific Research Project Unit. Proje No: FBA-2020-3129.

### Data availability

Not applicable.

### Consent for publication

Not applicable.

### Acknowledgements

We would like to thank Çanakkale Onsekiz Mart University Scientific Research Projects Coordination Unit for their support to the project numbered FBA-2020-3129.

## REFERENCES

Agustina, W., Sriharti T. (2020). Composting of grass clippings using different commercial microbial activators. IOP Conference Series: Earth and Environmental Science. IOP Conf. Series: Earth and Environmental Science 462 (2020) 012002. [Google Scholar]

Allison, L. E., C.D. Moodie. (1965). Carbonate. In: Methods of Soil Analysis, Part II (eds. C.A. Black et al.). American Society of Agronomy Inc., No: 9, Madison, Wisconsin, pp. 1379-1400. [Google Scholar]

Anonymous, (2008). Milli Eğitim Bakanlığı, MEGEP (Mesleki Eğitim ve Öğretim Sisteminin Güçlendirilmesi Projesi). (in Turkish) [URL]

Anonymous, (2021a). Çim kupürleri ile ne yapmalı? (in Turkish) [URL]

Anonymous, (2021b). What to do with lawn clippings. [URL]

Anonymous, (2021c). Grass Clippings, Mulch, Green Manure. [URL]

Atar, B. (2020). Effects of extra sulfur fertilization on soil pH and grain mineral concentration in wheat. International Journal of Agriculture Environment and Food Sciences, 4 (1), 101-106. DOI: 10.31015/jaefs.2020.1.11

Balcı, M., Taban, S. (2018). Effect of boron treatments on boron distribution and fresh leaf yield of tea plant. International Journal of Agriculture Environment and Food Sciences, 2 (3), 74-81. [Google Scholar]

Bouyoucos, G. J. (1951). A Recalibration of Hydrometer Method for Making Mechanical Analysis of Soils. Agronomy Journal, 43:434-438. [Google Scholar]

Bremner, J. M. (1965). İnorganic forms of nitrogen In: C.A. Black et al(ed). Methods of Soil Analysis, Part 2. Agronomy 9:1179-1237. Am. Soc. of Agron., Inc. Madison, Wisconsin, USA.

Fernando, A., L., Boleo, S., Barbosa, B., Costa, J., Duarte, M. P., Monti, A. (2015). Perennial Grass Production Opportunities on Marginal Mediterranean Land. Bioenerg. Res. (2015) 8:1523–1537. [Google Scholar]

Godebo, T., Laekemariam, F., Loha, G. (2021). Nutrient uptake, use efficiency and productivity of bread wheat (Triticum aestivum L.) as afected by nitrogen and potassium fertilizer in Keddida Gamela Woreda, Southern Ethiopia. Environmental Systems Research. (2021) 10 (12): 1-16. [CrossRef]

Jackson, M. L. (1958). Soil Chemical Analysis. Prentice-Hall, Inc. Englewood Cliffs, New Jersey, USA.

Jones, Jr, J. B., Wolf, B., Mills, H. A. (1991). Plant analysis handbook. A practical sampling, preparation, analysis, and interpretation guide. Micro-Macro Publishing, Inc.. [Google Scholar]

Kacar, B., İnal, A. (2008). Bitki Analizleri. Nobel Akademik Yayıncılık, Ankara (in Turkish)

Kleiber, T., Komosa, A. (2011). Inlfuence Of Increasing Nitrogen Fertilization On Content Of Microelements In Grasses Cultivated On Ornamental Lawns. J. Elem. s. 195–203. [CrossRef]

Müftüoğlu, N. M., Türkmen, C., Çıkılı, Y. (2014). Toprak ve Bitkide Verimlilik Analizleri (2. Basım). Nobel Akademik Yayıncılık Eğitim Danışmanlık Tic. Ltd. Şti., Ankara Dağıtım Kültür Mah. Mithatpaşa Cad. No: 74 B01/02 Kızılay Ankara, ISBN: 978-605-133-895-8, 218 s. (in Turkish)

Nakano, H., Morita, S., Kusuda, O. (2008). Effect of Nitrogen Application Rate and Timing on Grain Yield and Protein Content of the Bread Wheat Cultivar 'Minaminokaori' in Southwestern Japan. Plant Prod. Sci., 11(1): 151-157. [CrossRef]

Olsen, S. R., Cole, C. V., Watanabe, F. S., Dean, L. A. (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular 939. U.S. Government Printing Office, Washington D.C.

Richards, L. A. (1954). Diagnosis and improvement of saline and alkali soils (Vol. 78, No. 2, p. 154). LWW.

Singh, M. P., Erickson, J. E., Sollenberger, L. E., Woodard, K. R., Vendramini, J. M. B., Gilbert, R. A. (2015). Mineral Composition and Removal of Six Perennial Grasses Grown for Bioenergy. Agronomy Journal. Volume 107, Issue 2. 466-474. [CrossRef]

Taiz, L., Zeiger, E. (2008). Plant Physiology. Sinauer Associates, Inc., Publishers. Orijinal ISBN: 0-87893-823-0. p. 67.

Williams, B. L., Silcock, D. J. (1997). Nutrient and microbial changes in the peat profile beneath Sphagnum magellanicum in response to additions of ammonium nitrate. Journal of Applied Ecology, Vol. 34, No. 4 (Aug., 1997), 961-970. [CrossRef]

Yurtsever, N. (1984). Deneysel İstatistik Metotları. Toprak Gübre ve Araştırma Enstitüsü Yayınları. Genel Yayın No:121, Teknik Yayın No:56, Ankara. (in Turkish)

Zhang, X., Zou, T., Lassaletta, L., Mueller, N. D., Tubiello, F. N., Lisk, M. D., Lu, C., Conant, R. T., Dorich, C. D., Gerber, J., Tian, H., Bruulsema, T., Maaz, T. M., Nishina, K., Bodirsky, B. L., Popp, A., Bouwman, L., Beusen, A., Chang, J., Havlik, P., Leclere, D., Canadell, J. G., Jackson, R. B., Heffer, P., Wanner, N., Zhang, W., Davidson, E. A. (2021). Quantification of global and national nitrogen budgets for crop production. Nature Food, 2(7), 529-540. [CrossRef]

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 644-647
DOI: https://doi.org/10.31015/jaefs.2022.4.19

# Nutritional value of *Juncus acutus* in the wetland of Kızılırmak delta

**Sebahattin Albayrak**[1]

[1] Ondokuz Mayıs University, Vocational School of Bafra, Samsun, Türkiye

## Abstract

*Juncus acutus*, a wetland plant, helps improve habitats for both terrestrial and aquatic creatures and spawning grounds for fish. A variety of wetland birds use between the trunks of the *juncus* as a shelter. They also help prevent soil erosion due to their extensive root systems. On the other hand, it can be said that it has a negative effect on the feeding of grassland animals due to the high density in wetlands.

Plant materials from Juncus acutus, which is intensively located in the Kızılırmak Delta, were taken in two different periods, in April and July. The changes in the nutrient content of the plant during the spring and summer periods were investigated and the quality features were divulged. According to the research results; It was observed that the water buffaloes in the Kızılırmak Delta were not fed with *Juncus acutus* for feeding purposes, the forage quality was low, but the livestock grazed a little on *Juncus acutus* when they first went to the wetland in early spring.

**Keywords:** Kızılırmak Delta, *Juncus*, Quality, Buffalo

**Citation:** Albayrak, S. (2022). Nutritional value of *Juncus acutus* in the wetland of Kızılırmak delta. International Journal of Agriculture, Environment and Food Sciences, 6 (4), 644-647.

**Received:** 20 October 2022
**Revised:** 01 November 2022
**Accepted:** 04 November 2022
**Published Online:** 12 December 2022

**Correspondence:** Sebahattin Albayrak
**E-mail:** sebahattinalbayrak@omu.edu.tr



## INTRODUCTION

When the main habitat types and related habitats are examined in the Kızılırmak Delta, it has been determined that there are 12 different species groups (plant associations, plant communities). These groups of species have come together in different parts of the delta according to their relations and contacts with fresh and salt water, changes in soil structure and formed plant communities with different floristic compositions (Şahin et al. 2013). One of these plant communities is salt marshes.

Salt marshes are distinguished from dunes and forests in the Kızılırmak Delta, especially in the vicinity of lakes, in that they grow on heavier textured alluvial soil at the base. The soil structure is relatively salty and is well salted in places. Most of these swamps are submerged in winter by the growth of lakes. Therefore, it consists of water and salt resistant species. The dominant species of these communities are *Juncus* species (Şahin et al. 2013).

*Juncus* species are generally considered beneficial in wild ecosystems. *Juncus* is used as food and habitat by a wide variety of mammal and bird species (Hoag and Zierke 1998). *Juncus* can be said to have positive effects on wetlands in erosion control, sediment deposition and stabilization, nutrient uptake and conversion, wildlife food and cover, restoration and creation of wetland ecosystems, and wastewater treatment applications (Stevens et al. 2012).

It is known that *Juncus* is soft during the fresh sprout period when they give new

shoots from the soil and are eaten fondly by buffaloes. In this way, the spread of the pods, which are eaten and atrophied during the sprouting period, is taken under control. However, due to the fluctuation of the buffalo population in the delta in the last 20-30 years, the pressure on grazing of cows has not followed a regular course. Buffaloes in the delta are not affected by this plant with their thick skin, and they spend a lot of time here as they like to lie in swampy soil. Therefore, the plants between the buffaloes are overgrazed, and pits are formed in the places where the buffaloes tread heavily on the soft soil. (Anonymous, 2018). The reason why buffaloes live in wetlands is that they have problems withstanding high temperatures. This is because they have skin that is about six times thicker than that of cattle and they have about one-sixth as many sweat glands as cattle (Sambraus and Spannl-Flor 2005).

In this study; The quality characteristics of *Juncus acutus*, which is densely located in the Kızılırmak Delta, were examined in two different periods, March and July.

## MATERIALS AND METHODS

This research was carried out in the Kızılırmak Delta in March and July 2022. The study area, its northern end, is at 41° 4323´ N, 35° 5813´ E; Its southern end is located between 41° 3359´ N, 35° 5903´ E. The altitude of the area from the sea is in the range of 0-5 m. Plant samples were taken from *Juncus acutus*, which is densely located in the Kızılırmak Delta, in 2 different periods (March and July). The plant samples taken were dried in a drying cabinet at 70°C for 48 hours (Albayrak and Oten, 2020). For crude protein content, 1 g of each grinded sample was weighed and crude protein ratios were determined as % by applying the Kjeldahl method with the help of previously prepared solutions. ADF and NDF analyzes were made with the help of ANKOM 220 Fiber Analyzer (Ankom Technology, Magedon, NY, USA) according to the principles reported by ANKOM technology (Albayrak and Öten, 2020). Samples were analyzed in 3 replications.

Total digestible nutrients (TDN), Dry matter intake (DMI), Digestible dry matter (DDM), Metabolic energy (ME) and Relative feed value (RFV) were calculated according to the equation specified by (Albayrak et al. 2012).

TDN = (-1.291 x ADF) + 101.35

DMI = 120% NDF % dry matter basis

DDM = 88.9-(0.779 x ADF % dry matter basis)

ME = 0.15 x ADF (MJ/kg KM)

RFV = DDM x DMI x 0.775

## RESULTS AND DISCUSSION

The values of the quality parameters of *Juncus acutus* are given in Table 1. In their studies on *Juncus acutus*, it was reported that the crude protein ratio was in the range of 4.85-7.10% (Erdem et al. 2015; Genç et al. 2017; Joshi et al. 2018). In order to maintain its weight in various ruminant animals, the crude protein ratio of the forage consumed should be at least 6 -8% CP (Esmaeli and Ebrahimi, 2003; Moinuddin et al. 2012). In our study, HP ratio of *Juncus acutus* decreased from 5.27% in April to 4.12% in July. Therefore, it can be said that the water buffaloes released to the delta in the Kızılırmak Delta in April ate the *Juncus acutus*, although partially, and the crude protein ratio of the plant remained within the quality limits during this period. The stem ends of *Juncus acutus* are pointed and stinging. Therefore, they do not graze in the late period. In the absence of grazing pressure, they develop and spread easily. However, it is known that *Juncus acutus* is soft during the fresh sprouting period when it gives new shoots from the soil, and it is fondly eaten by buffaloes taken to the delta with spring (Anonymous, 2018).

**Table 1.** Average Crude protein ratio (CP), Fiber insoluble in acid solvents ADF), Insoluble fiber in neutral solvents (NDF), Total digestible nutrients (TDN), Dry matter intake (DMI), Digestible dry matter (DDM), Metabolic energy ( ME) and Relative Feed Value (RFV)

| | CP (%) | ADF (%) | NDF (%) | TDN (%) | ME (MJ/kg) | RFV |
|---|---|---|---|---|---|---|
| April | 5.27 | 45.82 | 72.58 | 42.20 | 6.87 | 68.18 |
| July | 4.12 | 48.94 | 77.23 | 38.17 | 7.34 | 61.14 |

The ADF rate of *Juncus acutus* was determined as 45.82% and the NDF rate as 72.58% in April, these values increased to 48.94% and 77.23%, respectively, in July. While the total digestible nutritional value (TDN) was 42.20% in April, it was 38.17% in July.

Fibers contain the least digestible parts of the herb. NDF ratio is always higher than ADF ratio (Cash and Bowman, 1993). In studies on the determination of ADF and NDF ratios of Juncus acutus plant; Young et al. (2017) found the ADF rate to be 48.81% and the NDF rate to be 74.83%. Çetinkaya and Erdem (2015) determined the ADF rate of *Juncus acutus* as 45.84% and the NDF rate as 73.14%. Like non-halophytic roughages, most halophytic roughages have high nutritional and palatability early in development, whereas the reverse is true late in growth (El Shaer, 2010). As a matter of fact, in our study, while the ADF and NDF ratios of *Juncus acutus* were lower at the beginning of spring, it was observed that the fiber content of the plant increased in the middle of summer. The chemical composition of halophyte roughages affects their flavor. For example, if the percentage of crude fiber is high in roughage, it will play an important role in the selection by the livestock. Forages with high fiber content are generally preferred by cattle more than sheep and goats (Attia-Ismail, 2018). Considering that the study area is grazed by the buffalo population, it should be considered that *Juncus acutus*, which lives in a salty environment, is preferred by this livestock.

The metabolic energy values of *Juncus acutus* were mea-

**Figure 1.** Grazing of *Juncus acutus* by water buffaloes

**Figure 2.** *Juncus acutus*. The measuring stick is 1 m long

**Figure 3.** Water buffaloes resting in mud in area surrounded by *Juncus acutus.*

sured as 6.87-7.34 MJ/kg DM. Nutritional value parameters differed from species to species, and it has been reported in many studies that the nutritional values of various species may differ from each other. As a matter of fact, Erdem et al. (2015) The metabolic energy value of *Juncus acutus* was 6.44, Genç et al. (2017) 6.89, Çetinkaya and Erdem (2015) determined 8.65 MJ/kg DM.

The Relative feed value (RFV) of the plant decreased from 68.18 in April to 61.14 in July. It is reported that if the RFV value of fodder is greater than 180, it is in the highest quality class, in the range of 150-180 superior, 125-150 good, 100-120 medium. If the RFV of the herb is below 100, it has low quality values (Albayrak and Öten, 2020). In the present study, relative feed value of *Juncus acutus* has low-quality class.

## CONCLUSION

It was concluded that the quality values of *Juncus acutus*, which is densely located in the Kızılırmak Delta, are low. Although it is seen that the buffalo population grazing in the delta grazes the plant at the beginning of spring; *Juncus acutus* should not be seen as the main food source for livestock.

## COMPLIANCE WITH ETHICAL STANDARDS

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Ethics committee approval is not required.

### Funding

No support received

### Data availability

Not applicable.

### Consent for publication

Not applicable.

## REFERENCES

Albayrak, S., Öten., M. (2020). Döl kontrolü parsellerindeki yonca (Medicago sativa L.) genotiplerinin verim ve kalite özellikleri ile genel kombinasyon yeteneklerinin belirlenmesi. Anadolu Tarım Bilim. Dergisi. 20(3): 353-360. (in Turkish) [Google Scholar]

Albayrak, S., M. Türk., O. Yüksel., Yılmaz., M. (2012). Forage Yield and the Quality of Perennial Legume-Grass Mixtures under Rainfed Conditions. Not Bot Hort Agrobot Cluj. 39(1):114-118. (in Turkish) [CrossRef]

Anonymous. (2018). Kızılırmak deltası su ayak izinin belirlenmesi projesi. Çevre ve Şehircilik Bakanlığı. Ankara. 528s. (in Turkish)

Attia-İsmail, S.A. (2018). Halophytes as Forages. New Perspectives in Forage Crops. [Google Scholar]

Cash, D., Bowman., H.F. (1993). Alfalfa hay quality testing, Montguide, Agriculture, MT-9302. [Google Scholar]

Çetinkaya, N., Erdem, F. (2015). Effects of Different Juncus

acutus: Maize Silage Ratios on Digestibility and Rumen Cellulolytic Bacteria. Kafkas Üniversitesi, Veteriner Fakültesi Dergisi. 21(4): 499-505. [CrossRef]

El Shaer, H.M. (2010). Halophytes and salt-tolerant plants as potential forage for ruminants in the Near East region. Small Ruminant Research 91 : 3–12. [CrossRef]

Erdem, F., Çetinkaya, N. Nispet, C., Altın, E. (2015). Estimation of organic matter digestibility, metabolizable energy, phenolic compounds and antioxidant activity of stems and seeds of the *Juncus acutus* plant in ruminant nutrition. South African Journal of Animal Science 45 ( 5): 502-509. [CrossRef]

Esmaeli, N., Ebrahimi., A. (2003). Necessity of determining animal unit requirement based on the quality of forage. *Iranian J. Nat. Resourc*., 55: 579-596. [Google Scholar]

Genç, B., Çetinkaya, N, Selçuk, Z., Salman, M. (2017). Nutritive values of common plant species on natural grassland in Kızılırmak Delta. Vet Hekim Der Derg 88(1): 21-30. [Google Scholar]

Hoag, J.C., Zierke, M. (1998). A reference guide for the collection and use of ten common wetland plants of the Great Basin and Intermountain West. Riparian/ Wetland Project Information Series No. 13 (February 1998). USDA, NRCS, Plant Materials Center, Aberdeen, Idaho. 13 pp. [Google Scholar]

Joshi, A., Kanthaliya, B., Arora, J. (2018). Halophytes of Thar Desert: Potential source of nutrition and feedstuff. International Journal of Bioassays 8.1 5674-5683. [CrossRef]

Moinuddin, M., Gulzar, S., Aziz, I., Alatar, A.R.A., Hegazy, A K., Khan, M. (2012). Evaluatıon Of Forage Qualıty Among Coastal And Inland Grasses From Karachı. Pak. J. Bot., 44(2): 573-577. [Google Scholar]

Filho, C.V.S., Rodrigues, L.R. de A. (2001). Evaluatıon Of Ten Tropıcal Grasses In The Northwest Regıon Of The State Of São Paulo-Brazıl. The XIX International Grassland Congress took place in São Pedro, São Paulo, Brazil from February 11 through February 21, 2001. [Google Scholar]

Sambraus, H.H., Spannl-Flor, M. (2005). Artgemäße Haltung von Wasserbüffeln. Tierärztliche Vereinigung für Tierschutz e. V., Merkblatt Nr. 102. [Google Scholar]

Stevens, M., Hoag, C., Tilley, D., John, L. St. (2012). Plant Guide for mountain rush (*Juncus arcticus* ssp. *littoralis)*. USDA-Natural Resources Conservation Service, Aberdeen Plant Materials Center. Aberdeen, Idaho 83210.

Şahin, B., Aslan, S., Ayyıldız, G., Vural., M. (2013). Kızılırmak Deltasında görülen habitat tipleri. III. Ulusal Sulak alanlar Kongresi. 271-276. 23-25 Ekim 2013. Samsun. (in Turkish)

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 648-659
DOI: https://doi.org/10.31015/jaefs.2022.4.20

# Rootstock potential of auto and Allotetraploid Citron [*Citrullus lanatus* var. *citroides* (L. H. Bailey) Mansf.] for Watermelon [*Citrullus lanatus* var *lanatus* (Thunb.) Matsum. & Nakai] under hydroponic conditions: plant growth and some physiological characteristics

**Alim Aydın**[1] • **Halit Yetişir**[2] • **Hakan Başak**[3] • **Metin Turan**[4] • **Metin Tuna**[5]

[1] Agriculture and Geothermal Project Coordinator, Kırşehir Ahi Evran University, Kırşehir, Turkiye
[2] Horticulture, Agriculture, Erciyes University, Kayseri, Turkiye
[3] Horticulture, Agriculture, Kırşehir Ahi Evran University, Kırşehir, Turkiye
[4] Engineering and Architecture, Genetics and Bioengineering, Yeditepe University, Istanbul Turkiye
[5] Field Crops, Agriculture, Tekirdag Namik Kemal University, Tekirdag, Turkiye

**Citation:** Aydin, A., Yetisir, H., Basak, H., Turan, M., Tuna, M. (2022). Rootstock potential of auto and Allotetraploid Citron [*Citrullus lanatus* var. *citroides* (L. H. Bailey) Mansf.] for Watermelon [*Citrullus lanatus* var l*anatus* (Thunb.) Matsum. & Nakai] under hydroponic conditions: plant growth and some physiological characteristics. International Journal of Agriculture, Environment and Food Sciences, 6 (4), 648-659.

**Received:** 16 October 2022
**Revised:** 04 November 2022
**Accepted:** 08 November 2022
**Published Online:** 14 December 2022

**Correspondence:** Alim Aydın
**E-mail:** alim.aydin@ahievran.edu.tr



## Abstract

The emergence of some physiological and fruit quality problems due to the common squash rootstocks used in watermelon has led researchers to search for alternative rootstocks sources. Exploitation of novel Citrullus germplasm such as citronmelon (*Citrullus lanatus* var. *citroides*) is an alternative to avoid these problems. In this study, rootstocks potential of auto and allotetraploid citrullus genotypes for watermelon were investigated as regard to plant growth and some physiological parameters under hyroponic conditions. Plant length was significantly affected by rootstock genotype and the longest plant stem was measured in watermelon plants grafted on N7-4T tetraploid rootstock (62.67 cm) while the shortest stem was measured in grafted plants onto autotetraploid Calhounn Gray with 14.33 cm. Among the graft combinations, N7-4T/CT (93.33 g) and CN7-5T/CT 95.00 g) graft combination produced the highest shoot fresh and dry weight. As in shoot fresh weight, the exploitationted on to tetraploid rootstock produced higher root fresh and dry weight than the plants grafted on diploid rootstocks and commercial rootstock. The highest root fresh and dry weight were determined in the plants grafted on to autotetraploid N5-4T and allotetraploid CN7-5T. Root characteristics were significantly affected by rootstock genotypes. The N, P, K and Ca contents of the leaves of the CT watermelon cultivar grafted on different rootstocks were significantly affected by the rootstocks. This study showed that citrullus tetraploid genotypes (auto and allo) to be produced by polyploidy method can be an important alternative rootstock source for watermelon.

**Keywords:** Hydroponic culture, Rootstock, Scion, Tetraploid, Citron and Watermelon

## INTRODUCTION

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nakai], a member of the Cucurbitaceae family and a fruit-bearing vegetable, was the second most-produced vegetable after tomato worldwide with a production of 101620420 tons in an area of 3053258 ha in 2020. Watermelon, grown in many parts of the world, covers 7% of the fruit and vegetable production areas. However, watermelon production is concentrated in China, Middle East countries, India, USA, Africa, Japan and some European countri (Faostat, 2022). As in other vegetable species, watermelon is also susceptible to many abiotic and biotic stress factors. The primary factor in coping with stress factors is genotypic resistance/tolerance. The development of cultivars with multiple resistance to both biotic and abiotic stress fac-

tors takes a very long time or is sometimes not possible due to genetic barriers between species. For this reason, one of the approaches used to overcome with stress conditions and to support plant growth is to graft sensitive commercial genotypes onto the stress-resistant genotypes (rootstocks) (Amaro et al., 2014; Rouphael, Cardarelli, Rea, & Colla, 2012; Zhong & Bie, 2007) . The primary starting point for grafting in vegetable crops is to prevent damage caused by soil-borne pests and pathogens (Oda, 2002; H Yetisir & Sari, 2003). However, in the last few decades, it has also been reported that grafting of vegetable crops onto suitable rootstock improves tolerance to abiotic stresses such as drought, low soil temperature and salinity, to increase water and nutrient use efficiency, and fruit yield and quality (Edelstein, Plaut, & Ben-Hur, 2011; Lee & Oda, 2010; Nisini et al., 2002; Oda, 2002; Rivero, Ruiz, & Romero, 2003; Romero, Belakbir, Ragala, & Ruiz, 1997; Shimada & Nakamura, 1977). Although utilization rates varies, the most common commercial rootstocks for watermelons are Cucurbita interspecific hybrids (*C. moschata* Duch. × *C. maxima* Duch.) and bottle gourd (*Lagenaria siceraria* Standl.) hybrids. Grafting can affect the plant performance positively or negatively depending on the rootstock/scion interaction. There is consensus among researchers that grafting watermelon on Cucurbita or Lagenaria rootstocks tends to increase plant vigor, fruit weight, and yield (Alexopoulos, Kondylis, & Passam, 2007; Cushman & Huan, 2008; Yetişir, Kurt, Sari, & Tok, 2007). Rootstock/scion incompatibility is the most obvious cause of the adverse effect on the grafted watermelons. However, different effects on pant growth, yield and fruit quality were commonly reported even in compatible graft combinations. These effects are due to rootstock/scion interaction, which can alter nutrient and water uptake, hormone synthesis, photosynthesis, and other metabolic processes (Aloni, Cohen, Karni, Aktas, & Edelstein, 2010). Grafted plants onto vigorous roostocks absorb more water and ions than ungrafted plants and carry these water and ions to the above ground graft. Ion uptake is regulated by a complex communication mechanism between the scion and rootstock and vigorous rootstocks with higher root volume increase plant nutrient uptake (Albacete et al., 2009; Gregory et al., 2013; Huang et al., 2013; Nawaz et al., 2016; Schwarz, Öztekin, Tüzel, Brückner, & Krumbein, 2013; Uygur & Yetisir, 2009).

It has been recently reported that grafting on different rootstocks can play an important role in the differentiation of physiological processes of watermelons and induce different gene expressions in the scion (Aslam et al., 2020; Liu et al., 2016). Therefore, there are conflicting reports in the literature about the effect of grafting on pumpkin rootstocks on the fruit quality of watermelon. One way to solve the quality problems in grafted watermelon production mentioned above may be to use rootstocks developed from the Citrullus genus (Edelstein et al., 2014). The most important disadvantage of the genotypes that can be used as rootstock for watermelon in the Citrullus germplasm is that their plant vigor is weaker than the existing commercial gourd rootstocks. With polyploidy breeding method, it is possible to develop polyploid genotypes with higher plant vigor. An important promising alternative rootstock candidate for watermelon is Citrullus lantus var. citroides (LH Bailey) Mansf. ex Greb., also known as citron melon (Fredes et al., 2016). Levi et al., (2014), reported that seedless (triploid) watermelon cultivars produced higher yield when grafted onto autotetraploid *Citrullus lanatus* var. *citroides* rootstocks compared with those grafted onto commercial Cucurbita or Lagenaria rootstocks. According to available literature, studies on the rootstock potential of autotetraploid genotypes developed from the Citrullus genera are quite limited and there are no studies on the rootstock potentials of allotetraploid Citrullus genotypes.

Therefore, in this the rootstock potential of auto and allotetraploids of *Citrullus lanatus* var. *citroides* for watermelon was investigated under hydroponic conditions and it has been determined that there are promising tetraploids producing vegetative growth results close to commercial rootstocks.

## MATERIALS AND METHODS

### Plant materials

Plant materials used in hydroponic testing are auto and allotetraploids, original diploid parent lines, commercial rootstocks (RS841 and Argentario), and the Crimson Tide watermelon variety. In 2019 growing season, hybrids were produced between Calhoun Gray watermelon cultivar and N3, N5 and N7 citron genotypes. Auto and allotetraploid genotypes were produced at Erciyes University, Faculty of Agriculture, Department of Horticulture within the framework of a Ph.D. study. Chromosome number was duplicated according to (Ra et al., 1995) by %0.5 colchicine application. Argentario Commercial *Cucurbita* and *Lagenaria* rootstocks and Crimson Tide watermelon cultivar were used for comparison. The list of plant materials used in the study is given in Table 1.

### Seedling Production and Grafting

One hundred seeds from each rootstock and a sufficient number of seeds from Crimson Tide (scion) were sown in multipots filled witha mixture of peat (pH: 6.0–6.5) and perlite in a 2:1 ratio and then the appropriate seedlings were selected for the grafting process at the first true leaf stage. The seedlings were grafted "Splice Grafting" (single cotyledon) following the procedure described by Lee & Oda (2010), while non-grafted Crimson Tide seedlings were used as control plants Thirty seedlings from each rootstock were grafted. Grafted seedlings were kept in a post-graft care unit in 90-95% humidity, 22-25 °C, and semi-shaded for one week. The graft success rate was evaluated 14 days after grafting and the survival rate was expressed as a percentage of the total number of the grafted plants. Plants with severely wilted scion and root-

**Table 1.** Rootstocks code, name and ploidy level

| Rootstock Code | Rootstock Ploidy Level |
|---|---|
| Calhoun GrayT (*Citrullus lanatus*) | Autotetraploid |
| N3T (*Citrullus lanatus* var. *citroides*) | Autotetraploid |
| N5-3T (*Citrullus lanatus* var. *citroides*) | Autotetraploid |
| N5-4T (*Citrullus lanatus* var. *citroides*) | Autotetraploid |
| N7-3T (*Citrullus lanatus* var. *citroides*) | Autotetraploid |
| N7-4T (*Citrullus lanatus* var. *citroides*) | Autotetraploid |
| CN3-2T (*Citrullus lanatus* var. *citroides)* | Allotetraploid |
| CN3-3T (*Citrullus lanatus x C. lanatus* var. *citroides*) | Allotetraploid |
| CN5T (*Citrullus lanatus x C. lanatus* var. *citroides*) | Allotetraploid |
| CN7-4T (*Citrullus lanatus x C. lanatus* var. *citroides*) | Allotetraploid |
| CN7-5T (*Citrullus lanatus x C. lanatus* var. *citroides*) | AlloTetraploid |
| Calhoun GrayD (*Citrullus lanatus)* | Diploid |
| N3D (*Citrullus lanatus* var. *citroides*) | Diploid |
| N5D (*Citrullus lanatus* var. *citroides*) | Diploid |
| N7D (*Citrullus lanatus* var. *citroides*) | Diploid |
| CN3D (*Citrullus lanatus x C. lanatus* var. *citroides*) | Diploid |
| CN5D (*Citrullus lanatus x C. lanatus* var. *citroides*) | Diploid |
| CN7D (*Citrullus lanatus x C. lanatus* var. *citroides*) | Diploid |
| RS841 (*C. maxima* x *C. moschata*) | Diploid |
| Argentario (*Lagenaria siceraria*) | Diploid |
| Crimson Tide (Scion) (*Citrullus lanatus*) | Diploid |

stock were considered dead. The graft succes rate was calculated with the formula = (Number of live plants/total grafted plants) × 100%.

## Establishment of Experiment in Hydroponic Culture

A hydroponic culture test was carried out in the fully automated venlo type glass R&D greenhouse of Kırşehir Ahi Evran University. The grafted plants acclimated to greenhouse conditions were transferred to 136 L plastic containers after roots were washed from growth media, each container was filled with nutrient solution and continuously aerated by an air pump. The upper surface of the containers is covered with styrofoam and the plants are placed in the holes. The nutrient solution contained 1.5 mM calcium nitrate ($Ca(NO3)_2$ 250 µM monopotassium phosphate ($KH_2PO_4$), 500 µM potassium sulfate ($K_2SO_4$), 325 µM magnesium sulfate ($MgSO_4.7H_2O$), 50 µM sodium chloride (NaCl). Micronutrients were 80 µM iron (Fe) (III) ethylenediaminetetraacetic acid (EDTA) sodium (Na), 0.4µM manganese sulfate ($MnSO_4$), 0.4µM zinc sulfate ($ZnSO_4$), 0.4µM copper sulfate ($CuSO_4$), 8µM boric acid ($H_3BO_3$), 0.4µM sodium molybdate ($Na_2MoO_4$). The electrical conductivity of the growing solution was maintained at 1.50 dS/m and the pH was between 6.5-7 (Hoagland & Arnon, 1950). The study was carried out under controlled greenhouse conditions (22-24 °C day / 16-18C night and 60% relative humidity) for 21 days. The experiment was set up according to a completely randomized block design with three replications and six plants in each replication.

## Plant Growth Measurements

After three weeks of growing, plants were harvested and separated into shoot and roots. Stem length (cm), diameter (mm) and number of leaf per plant were determined. The fresh and dry weights (g $plant^{-1}$) of stem and root were determined. In order to determine shoot and root dry weight, plant materials were dried in a air-forced oven for 48 h at 70 °C. The root length (cm $plant^{-1}$), volume ($cm^3$) and diameter (mm) of the plants was determined by using the special software program WinRHIZO (Win/Mac RHIZO Pro V. 2002c Regent Instruments Inc. Canada) (Ulas, Doganci, Ulas, & Yetisir, 2019).

## Chlorophyll Meter Measurements

Leaf chlorophyll index was determined with the CM 1000 Chlorophyll Meter. During the growth period, leaf chlorophyll index in fully developed leaves in all graft combinations was determined by two measurements with the CM 1000 chlorophyll meter.

## Plant Nutrient Analysis

After harvest fresh plant material was divided into two parts. One part was frozen in liquid nitrogen and stored-for later use. The remaining fresh plant material was dried at 70°C for 24 hours. For the determination of N, Ca and K concentrations, 100 mg dried plant material was extracted by boiling in 5 ml MilliQ for one hour. The solution was filtered through 0.2 mm filters (Whatman, England) and N, Ca, P and K contents in the filtrate were analyzed using high-performance liquid chromatography (HPLC, Shimadzu Japan). The HPLC system was equipped with a ø 4.6 mm 6125 mm Shodex IC YS-50 column (Showa Denko). As an eluent, 4.0 mM methane sulfonic acid was used in HPLC graded $H_2O$ (J.T. Baker, The Netherlands) with a flow rate of 1 ml $min^{-1}$. Final ion concentrations in the filtrate were calculated according to a calibration curve.

## Statistical Analysis

Data from the hydroponic culture study was sunjected to one-way analysis of variance (ANOVA) at 5% significance level (IBM, Chicago, IL, USA) using SPSS version 18.0 and

means were compared using the Duncan test. Correlation analysis was performed root characteristics and other measured parameters in grafted watermelon onto different rootstock grown under hydroponic conditions using SPSS software (version 22.0, Chicago, IL, USA).

## RESULTS AND DISCUSSION

Grafting success in Citrullus rootstocks was 8% to 17% higher than in commercial rootstocks. While the graft success rate was 98% in grafted plants onto Citrullus rootstocks, it was 83.33% in grafted plants onto Argentrio (*Lagenaria siceraria)* rootstock and 90.00% in grafted plants onto RS841 (*C.maxima* x *C. moschata*) rootstock (Table 2). Since the cost of grafted watermelon seedlings is higher than that of ungrafted seedlings, the survival rate after grafting should be high. The grafting techniques used, rootstock/scion genotype, pre- and post-grafting care conditions affect the success of grafting in watermelon. The single cotyledon grafting method is preferred in cases where the scion has a thin as in watermelon, cucumber and melon (Sakata, Ohara, & Sugiyama, 2007). This method is done when the rootstock and scion are of similar size and the first true leaf of the rootstock begins to develop (approximately 7 to 10 days after planting) (Oda, 2002). In our study, the high success rate of grafting on Citrullus rootstocks may be due to the fact that the rootstock and the scion belong to the same genera, or the diameter of the Citrullus rootstocks is proportional to the diameter of the scion. Similarly, a higher graft success in Citrullus rootstocks was reported in previous studies (Aydın et al., 2022; Kaseb et al., 2020; Levi et al., 2014).

The results of stem diameter, length and number of leaves per plant are given in Table 3. Plants grafted on tetraploid (auto-allo) Citrullus rootstocks had thicker stems than those grafted on diploid rootstocks. The highest stem diameter was measured in N7-3T/CT (6.45 mm), CN3-3T/CT (6.44 mm) and CN7-5T/CT (6.18 mm) respecti-

**Table 2.** Number of grafted plants, number of grafted plants, and graft success (%)

| Rootstock Ploidy Level | Graft Combinations (Rootstock/Scion) | Number of Grafted Plants | Number of Surviving Plants | Graft Success Rate (%) |
|---|---|---|---|---|
| Autotetraploid | Calhon GrayT/Crimson Tide | 30 | 30 | 100.00 |
| Autotetraploid | N3T/CT | 30 | 29 | 96.67 |
| Autotetraploid | N5-3T/CT | 30 | 30 | 100.00 |
| Autotetraploid | N5-4T/CT | 30 | 30 | 100.00 |
| Autotetraploid | N7-3T/CT | 30 | 30 | 100.00 |
| Autotetraploid | N7-4T/CT | 30 | 29 | 96.67 |
| Allotetraploid | CN3-2T/CT | 30 | 30 | 100.00 |
| Allotetraploid | CN3-3T/CT | 30 | 29 | 96.67 |
| Allotetraploid | CN5T/CT | 30 | 29 | 96,67 |
| Allotetraploid | CN7-4T/CT | 30 | 29 | 96.67 |
| Allotetraploid | CN7-5T/CT | 30 | 29 | 96.67 |
| Average | | | | 98.18 |
| Diploid | Calhon GrayD/CT | 30 | 30 | 100.00 |
| Diploid | N3D/CT | 30 | 29 | 96.67 |
| Diploid | N5D/CT | 30 | 30 | 100.00 |
| Diploid | N7D/CT | 30 | 29 | 96.67 |
| Diploid | CN3D/CT | 30 | 30 | 100.00 |
| Diploid | CN5D/CT | 30 | 29 | 96.67 |
| Diploid | CN7D/CT | 30 | 29 | 96.67 |
| Average | | 30 | | 98.10 |
| Diploid | RS841/CT | 30 | 27 | 90.00 |
| Diploid | Argentario/CT | 30 | 25 | 83.33 |

vely. The lowest stem diameter was measured in ungrafted Crimson Tide plants with 3.94 mm. Plant main stem length length was significantly affected by rootstock genotypes. The longest main stem length was 62.67 cm in plants grafted on tetraploid rootstock N7-4T, while the shortest main stem length was determined in Calhoun Gray T/ CT (14.33 cm), CN7D/CT (19.33 cm) and N7D/CT (20.33 cm) graft combination. The number of leaves per plant in plants grafted on tetraploid rootstocks ($\bar{x}$=10.75) is higher than the number of leaves in diploid plants grafted onto diploid rootstocks ($\bar{x}$= 9.22). In the study, the highest number of leaves per plant was recorded in the CN3-3T/CT (15.33 leaves plant-1) graft combination, while the lowest number of leaves was determined in the ungrafted Crimson Tide (5.33 leaves plant$^{-1}$) (Table 3). It was reported that polyploid plants have notable differences from diploids in their external morphological characteristics, mainly the shape and size of roots, stems, leaves, flowers and fruits due to chromosome duplication, and an increase in DNA content usually results in increased cell and organ size in plants (Corneillie et al., 2019).

The shoot and root fresh and dry weights of grafted plants onto rootstocks with diploid and tetraploid ploidy levels were presented in Table 4. The plants grafted on diploid rootstocks had less biomass than plants grafted on tetraploid rootstocks. Plants grafted on tetraploid plants produced more shoot fresh weight than plants grafted on diploid rootstocks, except plants grafted on auto-tetraploid Calhoun Gray. At the same time, the plants grafted on tetraploid rootstocks produced higher shoot fresh weight than ungrafted control plants (CT) and grafted plants on commercial rootstock RS841.The highest shoot fresh and dry weights were obtained in N7-4T/CT graft combination with 93.33 g plant$^{-1}$, and 10.25 g plant$^{-1}$, respectively while the lowest shoot fresh and dry weights were determined in the plants grafted onto diploid Calhoun with 11.00 g plant$^{-1}$ and 1.38 g plant$^{-1}$, respectively. Plants grafted on both diploid and tetraploid Calhoun Gary had the lowest shoot fresh and dry weights. As in shoot biomass, the root biomass was also significantly affected by rootstock genotype and ploidy level. Auto and allotetraploid produced higher root fresh weight than diploid corresponding rootstocks, ungrafted control plants and commercial rootstocks. The average root fresh weight of plants grafted on tetraploid rootstocks was 30.45 g plant$^{-1}$, while the average root fresh weight of plants grafted on diploid rootstocks was 11.46 g plant$^{-1}$. The graft combination with the highest root fresh weight was N5-4T/CT with 61.67 g plant$^{-1}$ while the lowest root fresh weight was measured in the Calhon Gray D/CT graft combination with 5.20 g plant$^{-1}$. Similar to root fresh weight, tertaploid rootstocks had higher root dry weight. The highest root dry weight was measured in the CN7-5T/CT (1.43 g plant$^{-1}$) and N7-4T/CT (1.40 g plant$^{-1}$) graft combinations, while the lowest root dry weight was measured in the Calhoun Gray D/CT (0.23 g plant$^{-1}$) and N3D/CT (0.24 g plant$^{-1}$) graft combinations

**Table 3.** Plant stem diameter, plant lengtht and number of leaves per plant in watermelon grafted on to different rootstocks grown under hydroponic conditions

| Rootstock Ploidy Level | Graft Combinations (Rootstock/ Scion) | Plant Stem Diameter (mm) | Plant Height (cm plant$^{-1}$) | Number of Leaves per Plant |
|---|---|---|---|---|
| Autotetraploid | Calhon GrayT/CT | 4.83c-f* | 14.33i | 7.67gh |
| Autotetraploid | N3T/CT | 5.53a-e | 30.67d-h | 9.33e-h |
| Autotetraploid | N5-3T/CT | 5.14b-f | 34.67cg | 10.00c-f |
| Autotetraploid | N5-4T/CT | 5.85ab | 39.33b-e | 12.00bc |
| Autotetraploid | N7-3T/CT | 6.45a | 27.67e-h | 9.40e-h |
| Autotetraploid | N7-4T/CT | 5.36a-e | 62.67a | 9.67d-g |
| Allotetraploid | CN3-2T/CT | 5.73a-d | 41.33b-d | 9.70e-g |
| Allotetraploid | CN3-3T/CT | 6.44a | 37.67b-f | 15.33a |
| Allotetraploid | CN5T/CT | 5.58a-d | 45.33bc | 13.67ab |
| Allotetraploid | CN7-4T/CT | 5.40a-e | 26.67f-i | 11.67b-d |
| AlloTetraploid | CN7-5T/CT | 6.18ab | 48.00b | 9.77d-g |
| Average | | 5.68 | 37.12 | 10.75 |
| Diploid | Calhon GrayD/CT | 4.24d-f | 18.67 hi | 9.33ce |
| Diploid | N3D/CT | 5.62a-d | 23.33g-i | 9.00e-h |
| Diploid | N5D/CT | 3.98f | 30.00d-h | 9.57c-g |
| Diploid | N7D/CT | 4.26d-e | 20.33hi | 8.00f-h |
| Diploid | CN3D/CT | 3.89f | 26.67f-i | 13.33b |
| Diploid | CN5D/CT | 3.96f | 20.33 hi | 7.33hi |
| Diploid | CN7D/CT | 4.51c-e | 19.33 hi | 8.00f-h |
| Average | | 4.24 | 22.67 | 9.22 |
| Diploid | RS841/CT | 4.63c-f | 20.33 hi | 7.35hi |
| Diploid | Argentario/ CT | 5.50a-e | 29.33d-h | 7.43hi |
| Diploid | Crimson Tide (ungrafted) | 3.94f | 30.67d-h | 5.33i |

*Values denoted by different letters are significantly different between genotypes within columns at p< 0.05

(Table 4). In agreement with the current study, a significant increase in shoot dry weight in tetraploid and hexaploid compared to diploids was reported in Arabidopsis (Zhang et al., 2019). The general belief of many researchers (del Pozo & Ramirez-Parra, 2014; Dudits et al., 2016; Głowacka, Jeżowski, & Kaczmarek, 2010; Li et al., 2012) that ploiploidization increases biomass production is consistent with our results.

The genotypic difference in leaf chlorophyll index was significant. The leaf chlorophyll index varied from 325 (CT) to 510 (CN3-3T/CT). The highest chlorophyll index was recorded in CN3-3T/CT, N7-4T/CT and CN3-2T/CT graft combinations, while ungrafted control plants and the grafted plants onto Argentario and tetraploid Calhoun Gray had the lowest chlorophyll index (Table 5). When shoot/root ratio were compared, it was determined that there was a significant difference between rootstock/scion combinations and the ratios of diploid rootstocks were higher than tetraploids. This shows that since tetraploid plants promote shoot growth, the shoot/root ratio of tetraploid plants is less than that of plants grafted on diploid plants. In a way that confirms our findings. Fredes et al., (2016) and Levi et al., (2014), reported that the use of tetraploid (allo and auto) watermelon rootstocks with a strong root system can provide high graft compatibility and a high survival rate, while the increased chlorophyll content and high antioxidant activities of tetraploid watermelon rootstocks can promote plant growth and stress tolerance without negatively af-

**Table 4.** Shoot fresh weight, shoot dry weight, root fresh weight and root dry weight of watermelon grafted on different rootstocks grown under hydroponic conditions

| Rootstock Ploidy Level | Graft Combinations (Rootstock/Scion) | Shoot Fresh Weight (g plant$^{-1}$) | Shoot Dry Weight (g plant$^{-1}$) | Root Fresh Weight (g plant$^{-1}$) | Root Dry Weight (g plant$^{-1}$) |
|---|---|---|---|---|---|
| Autotetraploid | Calhon GrayT/CT | 16.67jk* | 2.34h-j | 6.63i | 0.35gh |
| Autotetraploid | N3T/CT | 33.33f-h | 4.09e-g | 20.00ef | 0.69c-g |
| Autotetraploid | N5-3T/CT | 40.00f-h | 4.71c-f | 18.33e-g | 0.77c-f |
| Autotetraploid | N5-4T/CT | 75.00b | 8.16b | 61.67a | 1.28ab |
| Autotetraploid | N7-3T/CT | 50.00ce | 5.58c-e | 15.00e-i | 0.55d-h |
| Autotetraploid | N7-4T/CT | 93.33a | 10.25a | 43.33c | 1.40a |
| Allotetraploid | CN3-2T/CT | 56.67c | 6.05c | 30.00d | 0.87cd |
| Allotetraploid | CN3-3T/CT | 60.00c | 5.93cd | 31.67d | 0.80c-e |
| Allotetraploid | CN5T/CT | 35.00f-h | 3.97e-h | 21.67e | 0.52d-h |
| Allotetraploid | CN7-4T/CT | 55.00cd | 6.12c | 33.33d | 0.96bc |
| AlloTetraploid | CN7-5T/CT | 95.00a | 9.50ab | 53.33b | 1.43a |
| Average | | 55.45 | 6.06 | 30.45 | 0.88 |
| Diploid | Calhon GrayD/CT | 11.00k | 1.38j | 5.20i | 0.23h |
| Diploid | N3D/CT | 20.00i-k | 2.38h-j | 6.70i | 0.24h |
| Diploid | N5D/CT | 33.33f-h | 4.34d-g | 18.33e-g | 0.64c-h |
| Diploid | N7D/CT | 35.00f-h | 3.35f-I | 11.67f-i | 0.37f-h |
| Diploid | CN3D/CT | 43.33e-g | 4.32d-g | 10.00g-i | 0.35gh |
| Diploid | CN5D/CT | 18.33i-k | 1.92ij | 11.67f-i | 0.39f-h |
| Diploid | CN7D/CT | 30.00g-i | 2.87g-i | 16.67e-h | 0.57d-h |
| Average | | 27.29 | 2.94 | 11.46 | 0.44 |
| Diploid | RS841/CT | 26.67h-j | 2.67g-j | 8.93hi | 0.28h |
| Diploid | Argentario/CT | 40.00e-g | 3.67f-h | 15.00e-i | 0.39f-h |
| Diploid | Crimson Tide (ungrafted) | 25.00h-j | 2.96g-i | 15.00e-i | 0.46e-h |

*Values denoted by different letters are significantly different between genotypes within columns at $p<0.05$

**Table 5.** Leaf chlorophyll content and shoot/root ratio of grafted watermelon grown under hydroponic conditions

| Rootstock Ploidy Level | Graft Combinations (Rootstock/ Scion) | Leaf Chlorophyll Content (CM 1000 Chlorophyll Meter) | Shoot/Root Ratio |
|---|---|---|---|
| Autotetraploid | Calhon GrayT/CT | 335.00gh | 1.13f |
| Autotetraploid | N3T/CT | 413.67ef | 1.67c-f |
| Autotetraploid | N5-3T/CT | 396.67f | 2.22b-d |
| Autotetraploid | N5-4T/CT | 442.00cd | 1.22ef |
| Autotetraploid | N7-3T/CT | 510.00a | 3.33a |
| Autotetraploid | N7-4T/CT | 506.67a | 2.22b-d |
| Allotetraploid | CN3-2T/CT | 500.00ab | 1.94e-f |
| Allotetraploid | CN3-3T/CT | 521.00a | 1.94e-f |
| Allotetraploid | CN5T/CT | 483.33b | 1.67c-f |
| Allotetraploid | CN7-4T/CT | 348.33cd | 1.65c-f |
| AlloTetraploid | CN7-5T/CT | 430.00de | 1.77c-f |
| Average | | 444.24 | 1.89 |
| Diploid | Calhon GrayD/CT | 436.67df | 2.17c-e |
| Diploid | N3D/CT | 430.00de | 3.05ab |
| Diploid | N5D/CT | 426.67de | 1.83e-f |
| Diploid | N7D/CT | 431.67de | 3.00ab |
| Diploid | CN3D/CT | 420.00de | 3.50a |
| Diploid | CN5D/CT | 455.00b | 1.67c-f |
| Diploid | CN7D/CT | 440.00cd | 2.00e-f |
| Average | | 434.29 | 2.46 |
| Diploid | RS841/CT | 355.00g | 1.59c-f |
| Diploid | Argentario/CT | 340.00gh | 2.67a-c |
| Diploid | Crimson Tide (ungrafted) | 325.00h | 1.22ef |

*Values denoted by different letters are significantly different between genotypes within columns at $p<0.05$

fecting fruit quality in watermelon.

The average root length of the grafted plants onto tetraploid rootstocks grown under hydroponic conditions is approximately 57% longer than the average root length of the plants grafted onto diploid rootstocks. In general, all grafted plants produced longer roots than the non-grafted control plants. The graft combination with the highest root length was CN5T/CT 7906.86 cm and the while the lowest root length was measured in plants grafted on diploid Calhoun Gray rootstock (272.69 cm) and non-grafted Crimson Tide (187.13 cm) respectively. In similar manner, the CN5T/CT graft combination producing the highest root length also had the highest root volume with 11.48 $cm^3$ $plant^{-1}$. The lowest root volume was measured in the CN3D/CT graft combination (0.16 $cm^3$) and non-grafted Crimson Tide plants (0.41 $cm^3$). The mean root diameter affected by rootstock genotype ranged from 0.34 mm to 0.58 mm. Plants grafted on tetraploid rootstocks ($\bar{x}$=0.45) had thicker roots than those grafted on diploid rootstocks ($\bar{x}$=0.41). CN5T/CT (0.58 mm) and CN3-3T/CT (0.52 mm) graft combinations had the highest root diameter, while the lowest root diameter was measured in plants grafted on Argentario (0.34 mm) rootstock. The mean root diameter of commercial rootstocks was calculated as 0.37 mm. (Table 6). Similarly, increases in plant biomass due to polyploidization have been reported in different species in previous studies. It has been reported that polyploid orchids significantly increased in various growth parameters, including fresh weight, dry weight, shoot length, root length and

leaf width, compared to diploid orchids (Chung, Kuo, & Wu, 2017). Polyploid plants showed higher leaf and root growth compared to diploid plants in *Artemisia cina* (Kasmiyati, Kristiani, & Herawati, 2020), Kim et al., (2004), reported that the number of adventitious roots in polyploid ginseng plants is higher than in diploid plants. Similar to the results of the present study the tetraploid watermelon line USVL-360 (citron) showed vegetative growth as much as commercially available cucurbit rootstocks and provided resistance against root-knot nematode (Levi et al., 2014).

Grafted plants onto different rootstocks grown under hydroponic conditions were compared in terms of leaf N, P, K, and Ca content (Table 7). All plant nutrients showed significant differences based on rootstocks. Plants grafted on tetraploid rootstocks had higher leaf nitrogen content than those grafted on diploid rootstocks, and the leaf nitrogen content of watermelons grafted onto tetraploid and diploid rootstocks was 2% and 1.7% respectively. The highest N content was determined in the CN3-3T/CT (2.57%) and N7-4T/CT graft combination, respectively, while the lowest N content was measured in the CN7D/CT (1.19%) graft combination. Leaf P content showed variation from 0.10% to 0.27% between graft combinations. In general, higher content of P was recorded in the plants grafted onto tetraploid rootstocks compared to control and diploid rootstocks. While the plants grafted onto tetraploid Calhoun Gray, N3T, N5-3T, N5-4T, N7-3T, and N7-4T had the highest leaf P content,

**Table 6.** Root length, root volume, and root diameter of grafted watermelon grown under hydroponic conditions

| Rootstock Ploidy Level | Graft Combinations (Rootstock/ Scion) | Root Length (cm plant$^{-1}$) | Root Volume (cm$^3$/plant$^{-1}$) | Root Diameter (mm plant$^{-1}$) |
|---|---|---|---|---|
| Autotetraploid | Calhoun Gray T/ CT | 1199.55ef | 2.98df | 0.43e |
| Autotetraploid | N3T/CT | 1756.36e | 1.88f | 0.37gh |
| Autotetraploid | N5-3T/CT | 5646.29b | 6.49c | 0.39fg |
| Autotetraploid | N5-4T/CT | 4086.59cd | 6.18c | 0.44de |
| Autotetraploid | N7-3T/CT | 3870.89d | 5.56c | 0.44de |
| Autotetraploid | N7-4T/CT | 5277.32 | 2.46ef | 0.37gh |
| Allotetraploid | CN3-2T/CT | 3972.10cd | 6.07c | 0.45de |
| Allotetraploid | CN3-3T/CT | 3333.54d | 6.54c | 0.52b |
| Allotetraploid | CN5T/CT | 7906.86a | 11.48a | 0.58a |
| Allotetraploid | CN7-4T/CT | 4847.44bc | 8.81b | 0.48c |
| AlloTetraploid | CN7-5T/CT | 5871.39b | 8.36b | 0.43e |
| Average | | 4342.58 | 6.07 | 0.45 |
| Diploid | Calhoun Gray D/CT | 272.69fg | 0.61gh | 0.36hi |
| Diploid | N3D/CT | 1506.58e | 1.88f | 0.40f |
| Diploid | N5D/CT | 3147.09d | 3.78de | 0.40f |
| Diploid | N7D/CT | 1532.92d | 2.17f | 0.43e |
| Diploid | CN3D/CT | 1506.86e | 0.16h | 0.46d |
| Diploid | CN5D/CT | 3370.60d | 3.94d | 0.39fg |
| Diploid | CN7D/CT | 3067.14e | 5.35c | 0.40f |
| Average | | 1857.75 | 2.56 | 0.41f |
| Diploid | RS841/CT | 3333.54d | 5.67c | 0.39fg |
| Diploid | Argentario/CT | 1506.58d | 5.33c | 0.34i |
| Diploid | Crimson Tide (non-grafted ) | 187.13g | 0.41h | 0.40f |

Values denoted by different letters are significantly different between genotypes within columns at $p<0.05$

non-grafted control plants had the lowest P content with 0.01% (Table 6). Leaf potassium content was significantly affected by rootstocks and an increase in leaf K content was observed with polyploidization. The highest K content was determined in the CN3-3T/CT (4.59%) graft combination, while the lowest K content was obtained in the plants grafted onto commercial rootstocks (Argentario and RS841) and non-grafted control plants. (Table 7). When the graft combinations were evaluated in terms of leaf Ca content, the average leaf Ca content of the plants grafted on tetraploid rootstocks was 1.34%, while the average leaf Ca content of the plants grafted on diploid rootstocks was 1.02%. The appropriate rootstock/scion combination in vegetable grafting increased the nutrient uptake and use efficiency in many vegetable species (Nawaz et al., 2016; Schwarz, Rouphael, Colla, & Venema, 2010). In agreement with current study, it has been reported in previous studies that grafting onto rootstocks with strong root system increases the plant nutrient uptake and use efficiency (Schwarz et al., 2013; Uygur & Yetisir, 2009). Yetisir et al., (2013), that the N, Ca and K contents of watermelon leaves grafted on different gourd rootstocks (Cucurbita and Lagenaria) with strog root system were higher than the non-grafted plant. Huang et al., (2016), reported that grafting watermelon on squash rootstocks with a strong root system increased plant growth and grafted plants had twice the P content than non-grafted watermelon plants. Similarly, previous studies have reported improved water and nutrient uptake, nutrient utilization, and plant biomass growth by selecting appropriate rootstocks for spesific scion cultivars (Albacete et al., 2009; Gregory et al., 2013;

**Table 7.** N, P, K and Ca content of watermelon leaves grafted on different rootstocks grown under hydroponic conditions

| Rootstock Ploidy Level | Graft Combinations (Rootstock/ Scion) | N% | P% | K% | Ca% |
|---|---|---|---|---|---|
| Autotetraploid | Calhoun Gray T/ CT | 2.23bc* | 0.26ab | 2.16b | 1.46a |
| Autotetraploid | N3T/CT | 2.28bc | 0.27a | 2.09c | 1.45a |
| Autotetraploid | N5-3T/CT | 2.13ce | 0.25a-d | 2.12bc | 1.45a |
| Autotetraploid | N5-4T/CT | 2.16cd | 0.25a-c | 2.16b | 1.48a |
| Autotetraploid | N7-3T/CT | 1.93d-h | 0.25a-d | 1.77d | 1.41ab |
| Autotetraploid | N7-4T/CT | 2.57a | 0.25a-d | 1.77d | 1.45ab |
| Allotetraploid | CN3-2T/CT | 2.07c-f | 0.24b-d | 1.92d | 1.33bc |
| Allotetraploid | CN3-3T/CT | 2.57a | 0.18hi | 4.59a | 1.19d |
| Allotetraploid | CN5T/CT | 1.91d-h | 0.22d-g | 1.91d | 1.30c |
| Allotetraploid | CN7-4T/CT | 1.75g-j | 0.20f-h | 1.61d | 1.29c |
| AlloTetraploid | CN7-5T/CT | 1.78g-i | 0.23c-e | 1.65d | 1.32c |
| Average | | 2.00 | 0.23 | 2.16 | 1.34 |
| Diploid | Calhon Gray D/CT | 1.84f-i | 0.18hi | 1.09de | 0.82i |
| Diploid | N3D/CT | 1.85e-i | 0.22d-g | 1.72d | 0.81i |
| Diploid | N5D/CT | 1.65h-j | 0.20e-h | 1.65d | 1.12de |
| Diploid | N7D/CT | 1.50j | 0.18hi | 1.38d | 1.10ef |
| Diploid | CN3D/CT | 1.61i-j | 0.20f-h | 1.50d | 1.04ef |
| Diploid | CN5D/CT | 1.48j | 0.17hi | 1.48d | 1.01 |
| Diploid | CN7D/CT | 1.19k | 0.15i | 1.10d | 0.87hi |
| Average | | 1.70 | 0.19 | 1.42 | 1.02 |
| Diploid | RS841/CT | 1.86d-i | 0.19gh | 1.02de | 0.87hi |
| Diploid | Argentario/CT | 2.46ab | 0.16i | 0.78e | 0.94gh |
| Diploid | Crimson Tide (non-grafted ) | 1.94d-h | 0.10j | 1.06de | 0.85hi |

Values denoted by different letters are significantly different between genotypes within columns at $p<0.05$

Yetisir et al., 2013).

### Correlation Between Root Characteristics and Plant Growth

The correlation between root growth parameters and shoot growth parameters, chlorophyll index, leaf N %, % P, % K and % Ca parameters are presented in Table 8. A significant positive correlation was found between root fresh weight and stem diameter (0.476), plant height (0.699), number of leaves per plant (329), shoot fresh weight (873), shoot dry weight (841), and N% (413) at $p<0.01$ level and a significant positive correlation was found at $p<0.05$ level with chlorophyll index (292). There was a positive correlation at $p<0.01$ level between root dry weight and stem diameter (0.366), plant height (0.591), shoot fresh weight (0.764), shoot dry weight (0.728), and leaf N content (0.436) and there was a positive correlation between chlorophyll index (0.314) and P% (0.305) at $p<0.05$ level. Significant positive correlations between root length and stem diameter (0.364), plant height (0.561), shoot fresh weight (0.515), shoot dry weight (0.522), chlorophyll index (0.353) and leaf N (0.436) at $p<0.001$ level was determined and correlation between root length and number of leaves per plant was significant (0.271) at $p<0.05$. The root volume was positively correlated with stem diameter (0.415; $p<0,01$), plant length (0.319; $p<0.01$) shoot fresh weight (0.369; $p<0.01$), shoot dry weight (0.330; $p<0.01$) and leaf nitrogen content (0.248; $p<0.01$). A positive correlation was determined between root diameter and number of leaves per plant (0.382) and %K (0.347) at $p<0.01$ significance level. Significant positive correlations between root characteristics of rootstocks and vegetative growth parameters of scions have also been reported in several previous studies (Albacete et al., 2009; Gregory et al., 2013; Schwarz et al., 2010; Uygur & Yetisir, 2009; Yetisir et al., 2013)

**Tablo 8.** Pearson's correlation coefficients (r values) between root characteristics and other measured parameters in grafted watermelon onto different rootstock grown under hydroponic conditions

| Parametetrs | Stem Diameter | Plant Length | Leaves Number Per Plant | Shoot Fresh Weight | Shoot Dry Weight | Chlorophyll Content | N | P | K | Ca |
|---|---|---|---|---|---|---|---|---|---|---|
| Root Fresh Weight | 0.476** | 0.699** | 0.329** | 0.873** | 0.841** | 0.292* | 0.413** | 0.195 | 0.147ns | -0.031ns |
| Root Dry Weight | 0.366** | 0.591** | 0.244ns | 0.764** | 0.728** | 0.314* | 0.436** | 0.305* | 0.190ns | 0.025ns |
| Root Length | 0.364** | 0.561** | 0.271* | 0.515** | 0.522** | 0.353** | 0.462** | 0.240ns | 0.151ns | -0.229ns |
| Root Volume | 0.415** | 0.319* | 0.243ns | 0.369** | 0.330** | 0.140ns | 0.248* | 0.014ns | 0.156ns | -0.188ns |
| Root Diameter | 0.165ns | -0.121 | 0.382** | 0.094ns | 0.079ns | 0.021ns | 0.217ns | 0.029ns | 0.347** | -0.136ns |

* and ** denote P ≤ 0.05 and 0.01, respectively, and ns as not significant.

## CONCLUSION

In this study, it has been shown that tetraploid (auto and allo) genotypes of the Citrullus genus can increase plant growth and plant nutrient uptake when used as rootstock. According to the results of our study, plant biomass development and leaf nutrient content of plants grafted on tetraploid rootstocks are higher than the plants grafted on diploid rootstocks. Some auto (N7-3T and N7-4T) and allo (CN3-2T and CN3-3T) tetraploid genotypes that we developed in our previous studies and used as rootstock in this study presented superior performance to commercial rootstocks (RS841 and Argentaio) in terms of plant growth parameters. Therefore, in terms of vegetative growth and nutrient uptake, tetraploid (allo and auto) watermelon rootstocks developed from Citrullus genus are expected to become commercially available for different horticultural crops in the near future. More detailed studies are needed to determine the effect of tetraploid rootstocks on the fruit yield and quality characteristics, plant nutrient metabolism and some stress tolerance of the scion when they are used as rootstocks for cultivated watermelons.

## COMPLIANCE WITH ETHICAL STANDARDS

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Ethics committee approval is not required.

### Funding

No financial support was received for this study.

## Data availability

Not applicable.

## Consent for publication

Not applicable.

## REFERENCES


*Faostat*. (2022). Agricultural Statistics. Retrieved 05/31/2022 from https://www.fao.org/faostat/en/#data/QCL

Albacete, A., MartÍnez-AndÚjar, C., Ghanem, M. E., Acosta, M., SÁnchez-Bravo, J., Asins, M. J., … PÉrez-Alfocea, F. (2009). Rootstock-mediated changes in xylem ionic and hormonal status are correlated with delayed leaf senescence, and increased leaf area and crop productivity in salinized tomato. *Plant, Cell & Environment*, *32*(7), 928–938. [CrossRef]

Alexopoulos, A. A., Kondylis, Angelos., & Passam, H. C. (2007). Fruit yield and quality of watermelon in relation to grafting. *International Journal of Food, Agriculture and Environment*, *5*, 178–179. [Google Scholar]

Aloni, B., Cohen, R., Karni, L., Aktas, H., & Edelstein, M. (2010). Hormonal signaling in rootstock-scion interactions. *Scientia Horticulturae*, *127*(2), 119–126. [CrossRef]

Amaro, A. C. E., Macedo, A. C., Ramos, A. R. P., Goto, R., Ono, E. O., & Rodrigues, J. D. (2014). The use of grafting to improve the net photosynthesis of cucumber. *Theoretical and Experimental Plant Physiology*, *26*(3–4), 241–249. [CrossRef]

Aslam, A., Zhao, S., Azam, M., Lu, X., He, N., Li, B., … Liu, W. (2020). Comparative analysis of primary metabolites and transcriptome changes between ungrafted and pumpkin-grafted watermelon during fruit development. *PeerJ*, *8*, e8259. [Google Scholar]

Aydın, A., Yetişir, H., Başak, H., Güngör, R., Şengöz, S., & Çetin, A. N. (2022). Investigation of appropriate grafting method and plant applications to increase grafting success in cucumber. *International Journal of Agriculture, Environment and Food Sciences*, *6*(2), 275–284. [CrossRef]

Chung, Y.-L., Kuo, Y.-T., & Wu, W.-L. (2017). Development of SSR markers in Phalaenopsis orchids, their characterization, cross-transferability and application for identification. In *Orchid biotechnology III* (pp. 91–107). World Scientific. [Google Scholar]

Corneillie, S., de Storme, N., van Acker, R., Fangel, J. U., de Bruyne, M., de Rycke, R., … Boerjan, W. (2019). Polyploidy affects plant growth and alters cell wall composition. *Plant Physiology*, *179*(1), 74–87. [Google Scholar]

Cushman, K. E., & Huan, J. (2008). Performance of four triploid watermelon cultivars grafted onto five rootstock genotypes: Yield and fruit quality under commercial growing conditions. *Acta Horticulturae*, *782*, 335–341. [CrossRef]

del Pozo, J. C., & Ramirez-Parra, E. (2014). Deciphering the molecular bases for drought tolerance in A rabidopsis autotetraploids. *Plant, Cell & Environment*, *37*(12), 2722–2737. [Google Scholar]

Dudits, D., Török, K., Cseri, A., Paul, K., Nagy, A. v, Nagy, B., … Dobrev, P. (2016). Response of organ structure and physiology to autotetraploidization in early development of energy willow Salix viminalis. *Plant Physiology*, *170*(3), 1504–1523. [Google Scholar]

Edelstein, M., Plaut, Z., & Ben-Hur, M. (2011). Sodium and chloride exclusion and retention by non-grafted and grafted melon and Cucurbita plants. *Journal of Experimental Botany*, *62*(1), 177–184. [CrossRef]

Edelstein, M., Tyutyunik, J., Fallik, E., Meir, A., Tadmor, Y., & Cohen, R. (2014). Horticultural evaluation of exotic watermelon germplasm as potential rootstocks. *Scientia Horticulturae*, *165*, 196–202. [CrossRef]

Fredes, A., Roselló, S., Beltrán, J., Cebolla-Cornejo, J., Pérez-De-Castro, A., Gisbert, C., & Belén Picó, M. (2016). Fruit quality assessment of watermelons grafted onto citron melon rootstock. *Journal of the Science of Food and Agriculture*, *97*(5), 1646–1655. [CrossRef]

Głowacka, K., Jeżowski, S., & Kaczmarek, Z. (2010). In vitro induction of polyploidy by colchicine treatment of shoots and preliminary characterisation of induced polyploids in two Miscanthus species. *Industrial Crops and Products*, *32*(2), 88–96. [Google Scholar]

Gregory, P. J., Atkinson, C. J., Bengough, A. G., Else, M. A., Fernández-Fernández, F., Harrison, R. J., & Schmidt, S. (2013). Contributions of roots and rootstocks to sustainable, intensified crop production. *Journal of Experimental Botany*, *64*(5), 1209–1222. [CrossRef]

Hoagland, D. R., & Arnon, D. I. (1950). The water-culture method for growing plants without soil. *Circular. California Agricultural Experiment Station*, *347*(2nd edit). [Google Scholar]

Huang, Y., Li, J., Hua, B., Liu, Z., Fan, M., & Bie, Z. (2013). Grafting onto different rootstocks as a means to improve watermelon tolerance to low potassium stress. *Scientia Horticulturae*, *149*, 80–85. [CrossRef]

Huang, Y., Zhao, L., Kong, Q., Cheng, F., Niu, M., Xie, J., … Bie, Z. (2016). Comprehensive Mineral Nutrition Analysis of Watermelon Grafted onto Two Different Rootstocks. *Horticultural Plant Journal*, *2*(2), 105–113. [CrossRef]

Kaseb, M., Umer, M. J., Gebremeskel, H., Mahmud, E., Diao, W., Yuan, P., … He, N. (2020). Comparative Physiological and Biochemical Mechanisms in Di, Tri, and Tetraploid Watermelon (Citrullus lanatus L.) Grafted by Branches. *Preprints (Www.Preprints.Org)*. [CrossRef]

Kasmiyati, S., Kristiani, E. B. E., & Herawati, M. M. (2020). Effect of induced polyploidy on plant growth, chlorophyll and flavonoid content of Artemisia cina. *Biosaintifika: Journal of Biology & Biology Education*, *12*(1), 90–96. [Google Scholar]

Kim, Y.-S., Hahn, E.-J., Murthy, H. N., & Paek, K.-Y. (2004). Effect of polyploidy induction on biomass and ginsenoside accumulations in adventitious roots of ginseng. *Journal of Plant Biology*, *47*(4), 356–360. [Google Scholar]

Lee, J.-M., & Oda, M. (2010). Grafting of Herbaceous Vegetable and Ornamental Crops. *Horticultural Reviews*, 61–124. [CrossRef]

Levi, A., Thies, J. A., Wechter, P. W., Farnham, M., Weng, Y., & Hassell, R. (2014). USVL-360, a Novel Watermelon Tetraploid Germplasm Line. *HortScience*, *49*(3), 354–357. [CrossRef]

Liu, N., Yang, J., Fu, X., Zhang, L., Tang, K., Malangisha Guy, K., … Zhang, M. (2016). Genome-wide identification and comparative analysis of grafting-responsive mRNA in watermelon grafted onto bottle gourd and squash rootstocks by high-throughput sequencing. *Molecular Genetics and Genomics*, *291*, 621–633. [CrossRef]

Li, X., Yu, E., Fan, C., Zhang, C., Fu, T., & Zhou, Y. (2012). Developmental, cytological and transcriptional analysis of autotetraploid Arabidopsis. *Planta*, *236*(2), 579–596. [Google Scholar]

Nawaz, M. A., Imtiaz, M., Kong, Q., Cheng, F., Ahmed, W., Huang, Y., & Bie, Z. (2016). Grafting: A technique to modify ion ac-

cumulation in horticultural crops. *Frontiers in Plant Science*, *7*(OCTOBER2016), 1457. [CrossRef]

Nisini, P. T., Colla, G., Granati, E., Temperini, O., Crino, P., & Saccardo, F. (2002). Rootstock resistance to fusarium wilt and effect on fruit yield and quality of two muskmelon cultivars. *Scientia Horticulturae*, *93*(3–4), 281–288. [Google Scholar]

Oda, M. (2002). Grafting of herbaceous vegetable and ornamental crops. *Horticultural Reviews, Volume 28*, *28*, 61. [Google Scholar]

Ra, S. W., Yang, J. S., Ham, I. K., Moon, C. S., Woo, I. S., Hong, Y. K., & Roh, T. H. (1995). Effect of remaining potato stems on yield in grafting plants between mini-tomato and potato. *RDA Journal of Agricultural Science (Korea Republic)*, *37*(2), 390–393. [Google Scholar]

Rivero, R. M., Ruiz, J. M., & Romero, L. (2003). Role of grafting in horticultural plants under stress conditions. *Journal of Food Agriculture and Environment*, *1*, 70–74. [Google Scholar]

Romero, L., Belakbir, A., Ragala, L., & Ruiz, J. M. (1997). Response of plant yield and leaf pigments to saline conditions: Effectiveness of different rootstocks in melon plants (Cucumis melo L.). *Soil Science and Plant Nutrition*, *43*(4), 855–862. [CrossRef]

Rouphael, Y., Cardarelli, M., Rea, E., & Colla, G. (2012). Improving melon and cucumber photosynthetic activity, mineral composition, and growth performance under salinity stress by grafting onto Cucurbita hybrid rootstocks, *50*(2), 180–188. [CrossRef]

Sakata, Y., Ohara, T., & Sugiyama, M. (2007). The history and present state of the grafting of cucurbitaceous vegetables in Japan. *Acta Horticulturae*, *731*, 159–170. [CrossRef]

Schwarz, D., Öztekin, G. B., Tüzel, Y., Brückner, B., & Krumbein, A. (2013). Rootstocks can enhance tomato growth and quality characteristics at low potassium supply. *Scientia Horticulturae*, *149*, 70–79. [CrossRef]

Schwarz, D., Rouphael, Y., Colla, G., & Venema, J. H. (2010). Grafting as a tool to improve tolerance of vegetables to abiotic stresses: Thermal stress, water stress and organic pollutants. *Scientia Horticulturae*, *127*(2), 162–171. [CrossRef]

Shimada, N., & Nakamura, K. (1977). Plant nutritional studies on the grafting of the garden crops. i. nutrient absorption by the grafted plants (watermelon, bottle gourd) from the culture solutions of several nutrient levels. *Nippon Dojohiryogaku Zasshi. Journal of the Science of Soil and Manure, Japan*. [Google Scholar]

Ulas, A., Doganci, E., Ulas, F., & Yetisir, H. (2019). Root-growth Characteristics Contributing to Genotypic Variation in Nitrogen Efficiency of Bottle Gourd and Rootstock Potential for Watermelon. *PLANTS-BASEL*, *8*(3). [CrossRef]

Uygur, V., & Yetisir, H. (2009). Effects of rootstocks on some growth parameters, phosphorous and nitrogen uptake watermelon under salt stress. *Journal of Plant Nutrition*, *32*(4), 629–643. [CrossRef]

Yetisir, Halit, Özdemir, A. E., Aras, V., Candır, E., & Aslan, Ö. (2013a). Rootstocks effect on plant nutrition concentration in different organ of grafted watermelon. *Agricultural Sciences*, *2013*(05), 230–237. [CrossRef]

Yetisir, Halit, Özdemir, A. E., Aras, V., Candır, E., & Aslan, Ö. (2013b). Rootstocks effect on plant nutrition concentration in different organ of grafted watermelon. *Agricultural Sciences*, *2013*(05), 230–237. [CrossRef]

Yetişir, H., Kurt, fiener, Sari, N., & Tok, F. M. (2007). Rootstock Potential of Turkish Lagenaria siceraria Germplasm for Watermelon: Plant Growth, Graft Compatibility, and Resistance to Fusarium. *Turk J Agric For*, *31*, 381–388. [Google Scholar]

Yetisir, H, & Sari, N. (2003). Effect of different rootstock on plant growth, yield and quality of watermelon. *Australian Journal of Experimental Agriculture*, *43*(10), 1269–1274. [Google Scholar]

Zhang, N., Bao, Y., Xie, Z., Huang, X., Sun, Y., Feng, G., … Xiong, J. (2019). Efficient characterization of tetraploid watermelon. *Plants*, *8*(10), 419. [Google Scholar]

Zhong, Y. Q., & Bie, Z. L. (2007). Effects of grafting on the growth and quality of cucumber fruits. *Acta Horticulturae*, *761*, 341–347. [CrossRef]

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 660-666
DOI: https://doi.org/10.31015/jaefs.2022.4.21

# Evaluation of the heavy metal content of Sweetgum tree (*Liquidambar orientalis* Mill.) distributed in Mugla province

**Fatma Alptekin**[1] • **Mahmut Yıldıztekin**[2]

[1] Department of Environmental Sciences, Graduate School of Natural and Applied Sciences, Muğla Sıtkı Kocman University, Mugla, Türkiye

[2] Department of Herbal and Animal Production, Koycegiz Vocational School, Muğla Sıtkı Kocman University, Mugla, Türkiye

## Abstract

This study, it was aimed to investigate the nutritional status and heavy metal contents of *Liquidambar orientalis* Mill., an endemic species naturally distributed in Marmaris, Koycegiz, and Fethiye districts of Mugla province. When the soil heavy metal contents are examined, it has been determined that the Cr and Ni values are quite high in the Koycegiz and Marmaris samples. Cd, Co, Cr, Ni and Pb contents in the leaves, respectively; it was determined to be in the range of 0.04-0.09 ppm, 0.4-1.80 ppm, 2.85-6.87 ppm, 9.28-40.49 ppm and 0.52-2.15 ppm. Although some heavy metals were found to be above the limit values, no physical evidence was found that the plants in the study were adversely affected by these high values metabolically.

**Keywords:** Sweetgum (*Liguidambar orientalis* Mill.), Soil, Heavy Metal, Koycegiz, Mugla

**Citation:** Alptekin, F., Yildiztekin, M. (2022). Evaluation of the heavy metal content of Sweetgum tree (*Liquidambar orientalis* Mill.) distributed in Mugla province. International Journal of Agriculture, Environment and Food Sciences, 6 (4), 660-666.

**Received:** 25 September 2022
**Revised:** 15 October 2022
**Accepted:** 19 October 2022
**Published Online:** 20 December 2022

**Correspondence:** Mahmut Yıldıztekin
**E-mail:** mahmutyildiztekin@mu.edu.tr



## INTRODUCTION

The lands of Mugla province, which have suitable climatic conditions, are located within the borders of the Büyük Menderes Basin and the Western Mediterranean Basin, and the summers are hot and the winters are mild. In addition to having 1124 kilometers of coastline, our province is also significant in terms of agricultural practices (Anonim, 2004). Mugla province and its districts have fertile soil and a temperate climate, which support a diverse range of medicinal and aromatic plants that spread naturally. Despite the fact that the sweetgum plant (*Liquidambar orientalis* Mill.) is an endemic species, it grows in the areas between Koycegiz, Fethiye, Datca, and Cine Stream. The research, however, has determined that the only place where the sweetgum tree is seen as a forest is the Lake of Koycegiz and its surroundings (Kurt et al., 2008). Sweetgum (*Liquidambar orientalis* Mill.) tree is a member of the *Hamamelidaceae* family that can grow up to 35 meters tall and blooms in March-April (Yaltırık et al., 2000). The leaf widths range from 5-7 cm, and the number of lobes on the leaves ranges from 3 to 5. The sweetgum tree, which sheds its leaves in November and December, is a species that opens and sheds its seeds depending on its environment (Öztürk et al., 2008).

The population has grown rapidly within the limits of industrialization and urbanization, and the environment has been polluted by various pollutants. Exhaust gases emitted by vehicles used for transportation, for example, pollute the environment and release harmful substances. There are significant increases in the number of vehicles in certain periods, particularly in regions where new roads are being built or tourism is high. Exhaust gases, the subject of the study, pollute the

environment by being toxic to animals, plants, and all other living things, particularly humans (Aksoy et al., 2000). Although exhaust gases have different effects on the atmosphere, soil, plant, and animal health, they should be evaluated as a whole due to the negative effects caused by all factors such as polluting the atmosphere, deteriorating soil structure, reducing plant yield, endangering plant nutrient content, and endangering animal health (Kıl and Paksoy, 2014). It has been proposed that increased heavy metals cause stress in plants, resulting in physiological and genetic disorders, a decrease in yield, and crop losses (Munzuroğlu et al., 2004).

Toxic gases from motor vehicle exhausts cause dust on the roads as a result of the rapid movement of vehicles on highways, polluting our air, water, and soil resources (Tunçer, 2020). On the other hand, Sweetgum Tree (*Liquidambar orientalis* Mill.), which grows along highways and has a high medicinal value, has recently been used in many fields, raising the question of how reliable it is in terms of health.

Based on these findings, we focused our research on the sweetgum tree (*Liquidamber orientalis* Mill.) plant, which grows particularly along roadsides and has high medicinal value. The plant *Liquidambar orientalis* is also known as the Anatolian sweetgum tree or the daily tree (Velioğlu et al., 2008). The Anatolian sweetgum tree (*Liquidambar orientalis* Mill.), which is found in the southwestern part of Turkey and partially in Rhodes Island, is an endemic species that is almost non-existent elsewhere in the world. It has been reported that it grows in areas with high groundwater and along streams (Kurt et al., 2008). In this study, leaf and soil samples of the sweetgum plant were collected from the districts of Marmaris, Koycegiz, and Fethiye in Mugla province, along heavily trafficked highways, and the heavy metal contents of both the plant and the soil were investigated. Sweetgum is a plant species that is used in industries such as pharmaceuticals and perfumery. The purpose of this research is to determine the heavy metal content of the sweetgum tree, which is a medicinal and aromatic plant.

## MATERIALS AND METHODS

### Field Studies

The sweetgum tree (*Liquidambar orientalis* Mill.) plant was used in this study, and leaf and soil samples were collected from Koycegiz, Marmaris, and Fethiye districts of Mugla province. Soil samples were collected from 0-30 cm depth at five different points along the highway and 50 meters away. Leaf samples were collected separately from healthy parts of the tree located north, south, east, and west of the road, as well as 50 meters inland from the road. Soil and leaf samples were transported to the laboratory with labels indicating the region from which they were collected and stored under proper conditions.

The sample stations and their coordinates are as follows:

**Table 1.** Coordinates of Sampling Areas

| Location | Road distance (m) | Latitude | Longitude |
|---|---|---|---|
| FETHIYE | 0 | 36°71′54.90″N | 29°02′09.40″E |
| | 50 | | |
| KOYCEGIZ | 0 | 36°99′20.70″N | 28°64′69.60″E |
| | 50 | | |
| MARMARIS | 0 | 36°85′09.90″N | 28°28′48.90″E |
| | 50 | | |

### Analysis Studies

The soil samples were sent to Mulga Sıtkı Koçman University Research Laboratory after the preliminary stages were completed in our laboratory in exchange for service procurement. At the analysis stage of soil samples; texture (sand, clay, spindle ratio) by hydrometer method, lime; calcimetrically, the amount of organic matter according to the wet burning method (the soil samples taken are dried in an oven at 105 °C, weighed during the process, 0.5 g is weighed in microwave tubes and 2 ml of nitric acid and 6 ml of hydrochloric acid are added) (Walkley and Black, 1934), pH and EC were determined with a combined pH-EC meter. The total heavy metal values determined in the soil samples were read in the $HNO_3$-HCl mixture (1:3 v/v ratio) by wet burning and ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometer) device (Kacar, 1995).

After the preliminary stages were completed in the laboratory, samples were collected from healthy leaves from all over the tree by measuring 50 meters from the highway and road from the sweetgum (*Liquidambar orientalis* Mill.) plant and placed in 1.5-2.0 kg bags and taken to Muğla Sıtkı Koçman University Research Laboratory. The ash obtained from the burning of the leaves was extracted using 2 N warm HCl and distilled water. Total heavy metal values were determined in plant samples by wet burning in an HNO3-HCl mixture (1:3 v/v ratio) and reading in an ICP-OES (Inductively Coupled Plasma-Optical Emission Spectrometer) device (Kacar, 1995).

## RESULTS AND DISCUSSION

### Analysis Results of Soils

The soils where sweetgum plants grow are generally clay textured, and samples taken from the roadsides of Fethiye and Marmaris were determined to be in the clay-loam structure class (Table 2). In general, sweetgum trees grow well in moist, alluvial, clay, and loam soils and river beds (Acar et al., 1993). According to a 2016 study, the Oak (*Quercus robur* L.) tree grows well in deep, sandy, and clay textured soils (Bektaş et al., 2016).

The pH values of the soil of the sweetgum plant were determined to be the lowest in the area 50 meters away from the roadside of Marmaris (7.46), and the highest in the sample taken from the far area of the Fethiye district

**Table 2.** Soil Properties in the Study Area

| Location | Road distance (m) | EC (dS/m) | pH | Kireç (%) | Organic matter (%) |
|---|---|---|---|---|---|
| FETHIYE | 0 | 0,44 | 8.00 | 6.20 | 1.74 |
| | 50 | 1.05 | 8.82 | 15.42 | 0.80 |
| KOYCEGIZ | 0 | 0.58 | 7.78 | 0.45 | 2.93 |
| | 50 | 0.50 | 7.75 | 0.49 | 6.41 |
| MARMARIS | 0 | 0.35 | 8.23 | 1.36 | 0.75 |
| | 50 | 0.38 | 7.46 | 0.45 | 4.72 |

(8.82). The average pH of all analyzed soils was found to be 8. (Table 2). In other words, all soils have been determined to be slightly or strongly alkaline, with the exception of the sample taken from the study area, which is located far from the road in the Marmaris district. According to Acar et al. (1993), the soil pH value of the sweetgum plant is between 7 and 8, and they prefer basic soils. The electrical conductivity (EC) values of the soils where the sweetgum plant grows were determined to be the lowest in the Marmaris roadside (0.35 dS/m) and the highest in the Fethiye district (1.05 dS/m). The average electrical conductivity (EC) of the soils in the study area was calculated to be 0.55 dS/m (Table 2). According to Waters et al. (1972), the limit values for electrical conductivity (EC) in soils should be between 1.51-2.25 mS/cm. Considering the limit values, it is clear that all of the soils in the studied region are salt-free and have no salinity issues.

When the lime contents of the soils belonging to the research plant were examined, the soils of the Koycegiz Sweetgum forest and the Marmaris district 50 m away from the road had the lowest 0.45%, and the soil samples from the Fethiye district had the highest (14.8%). The average lime rate of all soils in the study area is 4.06%. (Table 2). According to Allison and Moodie (1965), the adequacy of lime content in soils should be between 5.1 and 15.0%. Given the amount of lime in the study area's samples, 50% was determined at very low rates. The organic matter content of the soils in the area where the sweetgum plant grows has been determined to be 0.75%, with the lowest in Marmaris roadside soils and the highest in the inner part of Koycegiz (6.41%). The amount of organic matter ranged between 0.60-2.74% (Sinik, 2011). When the lowest and highest values in our study were compared, it was discovered that soils where sweetgum plants were grown had higher levels of organic matter.

## Heavy metal concentrations in soil samples

Heavy metals accumulated in soil not only have an impact on productivity and ecosystem activities, but they also have an impact on plant health because they affect plant metabolic processes (such as photosynthesis, respiration, growth, and development), as well as animal and human health due to the disrupted food chain (Asri and Sonmez, 2006). This study was conducted to determine whether there is heavy metal pollution of the sweetgum plant, which is primarily used for medicinal purposes but has a variety of other applications depending on its proximity and distance to highways. The samples were taken 50 meters from the highway and road for this purpose, and the results are shown in Table 3.

Heavy metals in the soil are known to have negative effects on soil fertility, living things in the soil, and other living things throughout the food chain at various concentrations. The highest Cd value of all soil samples tested was 1.14 ppm, and the lowest was 0.40 ppm (Table 3). Soil Cd limit levels have been reported to be 2.5 mg $kg^{-1}$ (Saatci et al., 1988). Our results were found to be lower than the reference values, indicating that no cadmium-based pollution exists. According to a study conducted to determine the toxic effect levels of heavy metals in the soil and the toxic doses in the plant, 7.2 and 6.9 mg $kg^{-1}$ Cd in clay and clay loam textured soils (Gedikolu et al., 1997).

**Table 3.** Heavy Metal Concentrations of Soil Samples Taken from Different Distances of Sweetgum Tree

| Location | Road distance (m) | ppm | | | | | |
|---|---|---|---|---|---|---|---|
| | | Cadmium (Cd) | Cobalt (Co) | Chromium (Cr) | Molybdenum (Mo) | Nickel (Ni) | Lead (Pb) |
| FETHIYE | 0 | 0.40 | 18.07 | 545.67 | 0.01 | 701.45 | 4.12 |
| | 50 | 0.51 | 9.74 | 1128.82 | 0.07 | 477.78 | 1.64 |
| KOYCEGIZ | 0 | 1.14 | 104.89 | 565.14 | 0.04 | 2883.51 | 3.53 |
| | 50 | 0.86 | 82.07 | 499.70 | 0.40 | 2426.99 | 5.00 |
| MARMARIS | 0 | 0.75 | 23.41 | 420.55 | 0.04 | 880.14 | 6.90 |
| | 50 | 0.95 | 83.29 | 445.14 | 0.09 | 2467.19 | 4.60 |

The Co value of sweetgum plant soil samples was determined to be 104.89 ppm at the highest and 9.74 ppm at the lowest. The Co pollution value is 50 ppm, according to Kabata-Pendias (1979). There is a high level of Co pollution in Koycegiz soil samples and in areas far from the road where the Marmaris Sweetgum plant grows (Table 3). Co pollution should not be blamed solely on automobiles or industrial activity. That is, it is possible to develop the interpretation that fertilizers and pesticides used in agricultural activities near the Koycegiz Sweetgum forest contribute to heavy metal pollution. Yan et al. (2013) examined soil samples taken from the Tibetan Plateau's roadside and discovered that the Co (cobalt) concen-

tration in the soils decreased as they moved away from the traffic density. In the Fethiye and Koycegiz samples, the Co value decreases as you move away from the road, whereas the opposite is true in the Marmaris sample. In another study, samples taken from roads and park areas were examined, and the Co (cobalt) content was found to be 102.48 ppm in the sample taken from the campus's roadside (Keleş, 2007).

The Cr concentrations in the soils have been determined to range between 420.55 and 1128.82 ppm. The range of Cr reference values in soils is 5.00-1500.00 pmm (Alloway, 1990). The Cr content of the soils we examined was found to be between the reference value. In a 2011 study, Yaylali-Abanuz determined the Cr value to be 10-1161 ppm (Yaylali-Abanuz, 2011). It corresponds to the findings of our study. The adequacy levels of Cr element content in soil, however, are generally used as 100 mg/kg (Saatci et al., 1988; Hakerlerler et al., 1994).

When the Ni values of the soils in the study area are examined, the lowest and highest are 477.78 ppm (soil taken from an area far from the Fethiye road) and 2883.51 ppm (soil from the Koycegiz roadside). According to the data collected, the nickel (Ni) content of the soils varies depending on their proximity and distance to the road. The allowable limit value for determining our country's soil heavy metal pollution levels has been reported as 50 mg/kg (Saatci et al., 1988; Hakerlerler et al., 1994; Elmaci, 1995). The results of samples taken from the roadside in Fethiye and Koycegiz revealed that the element Ni concentration is extremely high, indicating pollution. Keleş (2007) collected soil samples from roads and park areas in Konya and determined the concentration of Ni (nickel) element in the roadside samples to be 1832 ppm. The highest value was determined in our study in the sample taken from the roadside in Koycegiz, and it shows parallelism with our study.

The total Pb content of the soil samples analyzed ranged between 1.64 and 6.90 ppm. The reference range for soil Pb has been reported to be 2.00-300.00 ppm (Alloway, 1990). The Pb concentration in the soil samples examined in this study was within the acceptable range. According to Koljonen (1992), the Pb concentration in the soil was 17 ppm. The Pb value in the soils studied in this study was lower. Heavy metal contents of soil samples collected from Konya's roads and park areas in 2007 were investigated, with the highest Pb value being 60 ppm (Keles, 2007). According to the findings of the study, it is the inverse of ours.

Heavy metal content was determined in soil samples collected from Denizli's urban, industrial, and semi-urban roadsides. Heavy metal concentrations have been reported to be higher in industrial areas than on urban roads (Celik et al., 2005). This situation served as a model for our research, and the discovery of high concentrations of heavy metals near the road supports the theory that it was caused by vehicle traffic.

## Leaf Samples' Heavy Metal Contents

The heavy metal content of the leaf parts of the sweetgum plant, which grows naturally in Mugla province's Fethiye, Koycegiz, and Marmaris districts, was investigated. Table 4 depicts the changes in the heavy metal content of the plant leaves under investigation.

The Cd values of the leaf samples collected from all areas studied were 0.09 ppm at the highest and 0.04 ppm at the lowest. In plants, the Cd reference range has been reported as 0.005-0.03 ppm (O'neill, 1993). When the results are compared to these standards, the Cd average of 0.007 ppm, which is within the recommended range, demonstrates that no cadmium-based pollution exists (Table 4). Kratz investigated the accumulation of Pb and Cd on the leaves of the *Pinus sylvestris* (Scotch pine) plant in 1996. It has been suggested that the amount of Pb and Cd in samples taken from cities and rural areas with heavy traffic is higher (Kratz, 1996). The study's findings are similar to ours.

**Table 4.** Concentrations of Heavy Metals in Sweetgum Leaf Samples

| Location | Road distance (m) | ppm | | | | | |
|---|---|---|---|---|---|---|---|
| | | Cd | Co | Cr | Mo | Ni | Pb |
| FETHIYE | 0 | 0.06 | 0.83 | 2.85 | 0.22 | 13.33 | 1.13 |
| | 50 | 0.09 | 0.59 | 3.96 | 0.63 | 9.28 | 0.97 |
| KOYCEGIZ | 0 | 0.09 | 1.00 | 5.58 | 1.07 | 40.49 | 1.15 |
| | 50 | 0.04 | 0.40 | 4.42 | 0.14 | 15.06 | 0.52 |
| MARMARIS | 0 | 0.07 | 1.80 | 3.76 | 0.49 | 30.61 | 0.93 |
| | 50 | 0.05 | 0.71 | 6.87 | 0.15 | 16.54 | 0.73 |

The highest Co level determined in the leaves of Sweetgum plant samples taken from Fethiye, Koycegiz, and Marmaris districts was 1.80 ppm in the sample taken from the Marmaris roadside, and the lowest level was 0.40 ppm in the plant sample taken from the Koycegiz district's far area. The Co limit value in plants was stated to be 0.05-0.5 ppm (Kabata-Pendias and Mukherjee (2007), and the Co-related pollution was higher than the limit value in all three locations in the samples taken near the road. According to a 2015 study on the heavy metal ra-

tios of the leaves of the Tea (*Camellia sinensis*) plant along the Eastern Black Sea Coast, the amount of Co in traffic areas was highest on the Rize coastal road and lowest on the Kemalpasa coastal road (Uzuner, 2015). While the Co value was high in the leaves of the sweetgum plant taken from the roadside of Marmaris in our study, it was determined at the lowest level in the samples taken from the area 50 m away from the road in Koycegiz district, which coincides with the result that heavy metal accumulations decrease as we move away from the main road, which we emphasized in this study.

The highest Cr value was 6.87 ppm in samples 50 m away from the road in the Marmaris district, and the lowest value was 2.85 ppm in the Fethiye roadside plant sample. The toxic value of Cr in plants is stated to be 2 ppm (Kloke et al., 1984). Our values are significantly higher than toxic values. This situation can be explained by the fact that this region has a lot of agricultural activities, a mineral deposit, and some social activities like picnics. The Cr concentration in the leaf of *Salix fragilis* L. (crisp willow) was measured in 2018, and Cr values ranged from 0.83-65.94 ppm (Yavuzer et al., 2018). The highest Mo value was found in the sample taken from the roadside in Koycegiz (1.07 ppm), and the lowest value was found in the samples taken from the remote area of the Koycegiz road (0.014 ppm). Mo breakpoints are typically in the 0.1-0.5 ppm range (Kabata-Pendias and Mukherjee) (2007). Except for the Fethiye roadside sample, the study's results were above the reference values, and the Mo concentration was found to be quite high on the roadsides of Marmaris and Koycegiz.

The highest Ni level in the leaves of the plant samples was found in the Koycegiz district roadside area (40.49 ppm), and the lowest Ni level in the samples far from the Koycegiz road (97.13 ppm). The toxic value of Ni has been reported to be 30 ppm (Kastori et al., 1997). When the data was examined, it was discovered that the Ni element was above the toxic value in samples taken from the roadside in Koycegiz and Marmaris, implying that traffic pollution was present. The heavy metal content of leaf samples of the *Camellia sinensis* (tea) plant from the Black Sea coastal road and where there is no traffic was examined in 2015. The Ni element was found to be 7.10-0.37 ppm in the analysis results, indicating that there was traffic-related pollution (Uzuner, 2015).

Finally, when the leaf Pb level of the sweetgum plant was measured, the lowest was 0.52 ppm in samples taken far from the Koycegiz road, while the highest was 1.15 ppm in a sample taken near the road in the same district. The leaf Pb element reference range was reported to be 1.0-5.0 ppm (Kloke et al., 1984). Heavy metal content was determined in the leaves of *Platanus orientalis* L. (sycamore) trees located on both sides of Silahtar Street in Ankara. Pb accumulation has been determined to be more intense, particularly in trees located in the median in the middle of the road (Topa, 1995). It followed the same pattern as our study. Çavuşoğlu et al. (2008) discovered that traffic density and pollution increased in direct proportion to the amount of Pb in larch (*Pinus nigra* var. *caramanica*) tree leaves on the roadsides in Kırıkkale. The high amount of Pb in leaf samples taken near the road can be attributed to the absorption of gases emitted by the leaves as a result of exhaust gases and tire wear on the road.

## CONCLUSION

The sweetgum tree (*Liquidambar orientalis* Mill.) is a valuable endemic with numerous applications. There has been a great loss in the number of sweetgum plants over the years due to human intervention and natural reasons. The increased number of vehicles and widespread use of transportation, as a result of the rapidly increasing population and developing technology, harm agricultural and forest products grown along roadsides and cause heavy metal pollution in soils and plants. In light of this information, this study, which is critical for the long-term viability of the medicinal sweetgum plant, examined the levels of some heavy metals originating from the exhaust gases of soil and leaf samples collected from the roadside of Fethiye, Koycegiz, and Marmaris, as well as the inner region 50 meters away from the road. However, our research revealed that some heavy metal contents were above the established limit values. As the study area, it is predicted that tourism activities will be intense and the population will increase significantly, especially during the season, and there may be significant increases in the level of pollution caused by exhaust gases since there are many vehicle entrances and exits on this basis. The pollution levels of plants growing along roadsides were assessed using normal and toxic values reported by various researchers. Metal concentrations in soil and plant structures have been found to decrease as one moves away from the highway. It is hoped that the parts of the sweetgum plant that comprise the study material, especially given its high medicinal value, are below the critical levels of heavy metals established by health institutions. With this study, it is hoped that, in addition to the periodic evaluation of heavy metal concentrations in some edible and medicinal plants growing along roadsides, the plants under study will provide an opportunity to shed light on the continuity of trust and quality.

## COMPLIANCE WITH ETHICAL STANDARDS

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Ethics committee approval is not required.

**Funding**
No financial support was received for this study.
**Data availability**
Not applicable.
**Consent for publication**
Not applicable.
**Acknowledgements**
This article is extracted from Fatma Alptekin's thesis for MS degree in Muğla Sıtkı Koçman University

## REFERENCES

Acar, M. G., Acar, M.İ., Gemici, Y., Genç, A., Özel, N. (1993) Anadolu Sığla (*Liquidambar orientalis* Mill.) Ormanlarının ve Günümüzdeki Durumu, *2. Uluslararası Ekoloji ve Çevre Sorunları Sempozyumu,* Türk-Alman Kültür İşleri Kurulu Yayın Dizisi No: 3 Ankara. (in Turkish).

Aksoy, A., Duman, F., Demirezen, D. (2000) Atmosferdeki Ağır Metallerin Tutulmasında Bitki Tüylerinin Rolü ve Ağır Metal Dağılımında Rüzgarın Etkisi, *Kayseri. Erciyes Üniversitesi Fen Bilimleri Enstitü Dergisi,* 16 (1-2):31 – 37. (in Turkish). [Google Scholar]

Allison, L. E., Moodie, C.D. (1965) *Cörbonate. In C. A. 'Black (Ed.) Methods of Part 2*, sorı Analy is.Agronomy 9; 1379- ı 396, Aıner, Agron Madison, Wisconsin", SOI: U.S.A.

Alloway, B. (1990) *Heavy Metals in Soils,* Blackie and Sou Ltd., Glasgow and London.

Anonim, *Muğla İli Bahçe Bitkileri Meyve Üretimi,* Muğla Valiliği İl Çevre ve Orman Müdürlüğü, Çevre Durum Raporu, 2004. (in Turkish).

Asri, F. Ö., Sönmez, S. (2006). Ağır Metal Toksisitesinin Bitki Metabolizması Üzerine Etkileri. Derim, 23(2), 36-45. (in Turkish). [Google Scholar]

Bektaş, İ., Oruç, S., Bal, C.B., Ak, K.A. (2016) Saplı Meşe (*Quercus robur* L. ) Odununun Fiziksel Özelliklerinin Toprak Değişkenleriyle İlişkisi*, Ormancılık Dergisi,* 61-71. (in Turkish). [Google Scholar]

Çavusoğlu, K.,Çakır, Ş., Kırındı, T. (2008) Kırıkkale ilinin çeşitli bölgelerinde yol kenarlarından toplanan *Pinus nigra* (j.f. arnold) subsp. *Nigra var. Caramanica* (loudon) rehder türündeki kurşun (Pb) kirliliğin araştırılması**,** *Kütahya Fen Bilimleri Ensititüsü Dergisi,* Sayı: 11 Eylül 2006, ISSN-1302-3055. (in Turkish).

Çelik, A., Kartal, A.A.,Akdoğan, A., Kaksa, Y. (2005) Determining the heavy metal pollution in Denizli (Turkey) by using *Robinio pseudo-acacia* L.*, Environment International*, 31(1): 105-112. [Google Scholar]

Elmacı, Ö. L. (1995). *Güney Marmara Bölgesi Sanayi Domates Alanlarındaki Toprak, Sulama Suyu ve Domates (Lycopersicum Esculentum) Meyvelerinde Ağır Metal İçeriklerinin Belirlenmesi*, Doktora Tezi, E.Ü. Fen Bilimleri Enstitüsü, İzmir. (in Turkish).

Gedikoğlu, İ., Kalınbacak, K., Yalçıklı, A., Yurdakul, İ. (1998) "*Bazı Ağır Metallerin Topraktan Ekstraksiyon Yöntemlerinin Karşılaştırılması ve Buğday Yetiştirilerek Kalibrasyonu",* Toprak ve Su Kaynakları Araştırma Yıllığı 1997. (in Turkish).

Hakerlerler, H., Anaç, D., Okur, B., Saatçi N. (1994). Gümüldür ve Balçova'daki Satsuma Mandarin Bahçelerinde Ağır Metal Kirliliğinin Araştırılması, E.Ü. Araştırma Fonu Proje No: 92-ZRF-47, Bornova-İzmir. (in Turkish).

Kabata-Pendias, A., Pendias, A. (1979) Current problems in chemical degration of soils, paper presented at the Conf On Soil and Plant Analyses in Environment Protection, Falenty/Warsaw, October 29,7.

Kabata-Pendias A & Mukherjee A.B. (2007) Trace Elements from Soil to Human, Springer-Verlag, Berlin, Heidelberg.

Kacar, B. (1995) Toprak Analizleri, Bitki ve Toprağin Kimyasal Analizleri: III, Ankara Üniversitesi Ziraat Fakültesi Eğitim Araştirma ve Geliştirme Vakfi Yayinlari, No: 3, Ankara, s:705. (in Turkish).

Kastori, R., Petrovic, N., Arsenijevic-Maksimovic, I. (1997) Heavy metals and plants, In: R Kastori (Ed.), *Heavy Metals in the Environment, Institute of Field and Vegetable Crops*, Novi Sad, pp. 196-257.

Keleş, C. (2007) *Konya Şehir Merkezi Yol ve Parklarında Ağır Metal Kirliliği*, Yüksek Lisans Tezi, Selçuk Üniversitesi, Fen Bilimleri Enstitüsü, s.73. (in Turkish).

Kıl, R., Paksoy, M. (2014) Organik ve inorganik gübrelerin karnabaharda bitki gelişimi ve verime etkisi, *10. Sebze Tarımı Sempozyumu,* 2-4 Eylül, Tekirdağ. (in Turkish).

Kloke, A., Sauerbeck, D. R., Vetter, H. (1984) *The contamination of plants and soils with heavy metals and the transport of metals in terrestrial food chains, In Changing metal cycles and human health* , Springer, Berlin, Heidelberg, pp:113-141. [Google Scholar]

Koljonen, T. (1992) *Geochemical Atlas of Finland, Part 2: Till*, Geological Survey of Finland, Espoo, Finland, 218 pp.

Kratz, W. (1996) Heavy metals, PAH and PCB in scots pine needles, *Unweltwiss, Schadst-Forsch.*,8(3), 130. (CA 125:121950). [Google Scholar]

Kurt, L. (2008) *Köyceğiz_Dalyan Ö.Ç.K. BölgesindeBulunan Anadolu Sığla Ağacı'nın (Liquidamar orientalis* Mill.*) Doğal Ortamında Korunması ve* İzlenmesi, Özel Çevre Koruma Kurumu. (in Turkish).

Munzuroğlu, F.K., Zengin, Ö. (2004) Effects of lead ($Pb^{+2}$) and copper ($Cu^{+2}$) on the growth of root, shoot and leaf of bean (*Phaseolus vulgaris* L.) seedlings, *Gazi Üniversitesi Fen Bilimleri Dergisi,*17: 1-10. (in Turkish). [Google Scholar]

O'neill, P. (1993) *Environmental Chemistry,* Second edition, Chapman&Hall, London, s:268.

Öztürk M, Çelik A, Güvensen A., Hamzaoğlu E. (2008) Ecology of tertiary relict endemic *Liquidambar orientalis* Mill. forests, F*orest Ecology and Management* Volume 256, Issue 4, Pages 510-518. [Google Scholar]

Saatçi, F., Hakerlerler, H., Tuncay, H., & Okur, B. (1988). İzmir İli ve Civarındaki Bazı Önemli Endüstri Kuruluşlarının Tarım Arazileri ve Sulama Sularında Oluşturdukları Çevre Kirliliği Sorunu Üzerinde Bir Araştırma. *EÜ*

*Araştırma Fonu Proje*, (127). (in Turkish).

Şinik, E. (2011) E*dirne İlinde Bulunan Asit Karakterli Toprakların Bitki Besin Elementleri ve Bazı Ağır Metal İçeriklerinin Belirlenmesi Üzerine Bir Araştırma,* Yüksek Lisans Tezi, Namık Kemal Üniversitesi, Toprak Bilim ve Bitki Besleme Anabilim Dalı, Tekirdağ, s. 60. (in Turkish).

Topa, S.(1995 ) *Ankara Cadde Ağaçlarından Platonus orientelis L. da Ağır Metal Birikimi,* Yüksek Lisans Tezi, Gazi Üniversitesi, Fen Bilimleri Enstitüsü, Ankara. (in Turkish).

Tunçer, B. (2020). *Bazı Tarım Bitkilerinde Ağır Metal Birikiminin Trafik Yoğunluğuna Bağlı Değişimi*, Yüksek Lisans Tezi, Kastamonu Üniversitesi, Fen Bilimleri Enstitüsü, Kastamonu, s. 87. (in Turkish).

Uzuner, P. (2015) *Doğu Karadeniz Sahil Boyunca Camellia sinensis* var. *sinensis* (L.*) Kuntze (Çay)' de Ağır Metal Kirliliğinin Araştırılması,* Yüksek Lisans Tezi, Recep Tayip Erdoğan Üniversitesi, Rize, s.104. (in Turkish).

Velioğlu, E., Kandemir, G., Tayanç, Y., Çengel, B., Alan, M., Kaya, Z. (2008) *Türkiye' de Sığla (Liquidambar orientalis* Mill.*) Populasyonlarının Genetik Yapısının Moleküler Belirteçlerle Belirlenmesi ve Koruma Stratejileri Geliştirilmes*i, Çevre ve Orman Bakanlığı Orman Ağaçlarını ve Tohumları Islah Araştırma Müdürlüğü, Teknik Bülten No: 20, Ankara. (in Turkish).

Walkley, A., Black, I.A. (1934) An Examination of Degtjareff Method for Determining Soil Organic Matter and a Proposed Modification of the Chromic Acid Titration Method, *Soil Sci.* 37:29-37. [Google Scholar]

Waters, W.E., Nesmith, J., Geraldson, C.M., Woltz, S.S. (1972) The Interpretation of Soluble Salt Tests and Soil Analysis by Different Produces, *Florida Flower Grower*, 1972, 9 (4): 1-10.

Yaltırık, F., Efe, A. (2000) *Dendroloji-Ders Kitabı*, İstanbul Üniversitesi Yayınları, Yayın No: 4265, İstanbul. (in Turkish).

Yan, X., Gao, D., Zhang, F., Zeng, C., Xiang, W., Zhang, M. (2013) Relationships Between Heavy Metal Concentrations in Roadside Topsoil and Distance to Road Edge Based on Field Observations in the Qinghai-Tibet Plateau, *China. Int. J. Environ. Res. Public Health*, 10:762- 775. [Google Scholar]

Yavuzer, H., Osma, E. (2018) *Salix fragilis* L. (Gevrek Söğüt)'in Ağır Metal Kirlenmesinde Biomonitör Olarak Değerlendirilmesi, *Anadolu Üniversitesi Bilim ve Teknoloji Dergisi C- Yaşam Bilimleri ve Biyoteknoloji,* 7(2): 122-129. (in Turkish). [Google Scholar]

Yaylalı-Abanuz, G. (2011) Heavy Metal Contamination of Surface Soil around Gebze Industrial Area, *Microchemical Journal, Turkey* 99, 82-92. [Google Scholar]

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 667-675
DOI: https://doi.org/10.31015/jaefs.2022.4.22

# Investigation of yield and yield components in main crop soybean genotypes in Adana conditions

**Mustafa Yaşar**[1] • **Mehmet Sezgin**[2]

[1] Department of Plant Production and Technologies, Faculty of Applied Sciences, Muş Alparslan University, Muş, Türkiye
[2] Variety Registration and Seed Certification Center, Ankara, Türkiye

## Abstract

The research was carried out in Adana conditions to determine the relati-onships between seed yield and yield components of some soybean ge-notypes depending on the years. In this research, which was carried out in 2015 and 2016 years, it was established with 5 soybean varieties and one variety candidate and according to Randomized Complete Block De-sign with 4 replications. In the combined analysis of variance, the year*genotype interaction was insignificant in terms of yield and yield components, while other sources of variation (year and genotype) were found to be statistically significant compared to P <0.01. Seed yield showed changed between 5782-6788 kg ha-1 in 2015 and 3597-4359 kg ha-1 in 2016. In the correlation between the examined traits; While there was a significant and positive relationship between seed yield and day to flowering, days to maturity, first pod height and oil rate, there was a significant and negative relationship between 1000 seed weight and the number of pods per plant. The varyans analysis showed that the inter-actions of year, genotype and year*genotype were significant in terms of seed yield and yield components of genotypes. As a result, Cinsoy, variety candidate 1 (Mona) and Blaze of varieties showed high performance in Adana conditions, and it was determined that the effect of the environment was higher than the effect of genotype and interaction in terms of seed yield and other traits. As a result of this study, the variety candidate 1 was registered under the name Mona. In addition, it was concluded that Cinsoy, Blaze and newly registered Mona varieties can be recommended for main crop soybean cultivation in Adana conditions.

**Keywords:** Soybean, Main product, Seed yield and yield components, Interaction

**Citation:** Yasar, M., Sezgin, M. (2022). Investigation of yield and yield components in main crop soybean genotypes in Adana conditions. International Journal of Agriculture, Environment and Food Sciences, 6 (4), 667-675.

**Received:** 21 October 2022
**Revised:** 20 November 2022
**Accepted:** 22 November 2022
**Published Online:** 20 December 2022

**Correspondence:** Mustafa Yaşar
**E-mail:** mustafa.yasar@alparslan.edu.tr



## INTRODUCTION

Soybean (*Glycine max* L.) is an important industrial plant in terms of oil, protein and carbohydrates. The origin of soy is known as Korea, Japan, China and Far East countries. Soybean has found a wide production area in South American countries, especially Argentina, Brazil and Paraguay, with the expansion of land in parallel with the increase in yield, especially thanks to the technological innovations in recent years (OECD/FAO, 2019). Soybean is one of the plants with the most (61%) production among oilseed plants and is among the 5-6 most important plants in the world in terms of plant food source. (Lopes da Silva et all, 2017). It is the world's leading source of high-quality protein and edible oil for both human food and animal feed. In addition, thanks to the ability of soybean as a legume

plant to benefit from nitrogen in the air, it can increase soil fertility for the plants to be planted after it by adding nitrogen to the soil (Morsy et al., 2015) and it is known to save fertilizer. Therefore, soybean can be described as one of the most suitable plants for crop rotation. One of the biggest reasons for the increase in world consumption of soy is that it is the raw material of many industrial industries and is used in the production of biodiesel (Kinney and Clemente, 2004).

According to SoyStats data, 367.8 million tons of soybean production is made in an area of 136.8 million hectares in the world in 2021. The countries that produce the most soy in the world are Brazil, USA, Argentina, China, India, Paraguay and Canada. Turkey is at the bottom of the world ranking with 182 thousand tons of soybean production in an area of 44 thousand hectares in the 2021 production season. Soybean consumption in Turkey reached 2.9 million tons in 2021. However, only 6% of consumption is met by local production and the remaining part is met by imports, approximately 94% (TUIK, 2022). More importance should be given to soybean studies both in Turkey and in the world, in order to combat the drought that emerged with global warming and to eliminate the vegetable oil deficit, which has increased in importance in the food crisis.

Soybean plant has generally adapted to different climatic regions and can be grown in many parts of Turkey. The provinces with the highest soybean production in Turkey are Adana, Mersin, Kahramanmaraş, Osmaniye, Samsun and Hatay. Approximately 63% of soybean production in Turkey is produced in Adana (TÜİK, 2022). Varieties that adapt more easily to climatic conditions, show high seed yield and oil performance are more preferred by producers. The fact that both yield and other characteristics are affected by different environmental conditions increases the importance of environment genotype interaction. The G x E (Genotype x environment) interaction is defined by the variation in performance of varieties according to changing environmental conditions. However, if this interaction does not change the yield order of genotypes in different environments, there is no problem in terms of cultivar recommendation (Kaya and Atakisi, 2002). The main purpose of yield studies is to predict the performance of the best variety in the future using available data. However, the GE effect is the biggest obstacle in determining the effectiveness of a genotype in different environments and choosing stable genotypes, affecting yield and production (Khomari et al., 2017; Ansarifard et al., 2020). In addition, many different studies are carried out to determine stable varieties in terms of seed yield and to reveal the effect of GE interaction. Whingham and Minor, (1978), Karaslan et al., (1998), Eswari and Rao (2006), Sudaric et al., (2006), Çopur et al., (2009), Karasu et al., (2009), Karaaslan, (2011), Hu and Wiatrak, (2012), Wheeler and Von Braun, (2013), Verma and İzhar (2017), İlker et al., (2018), Cubukcu et al., (2020) reported that genotype, year and year*genotype interactions are important in terms of seed yield in their studies.

The aim of this study is to analyze the effect of year, genotype, year*genotype interaction for yield and yield components obtained from studies conducted in different environmental conditions in main product soybean cultivation, to determine stable varieties and to suggest suitable varieties for the region.

## MATERIALS AND METHODS

### Materials

The study was carried out with 5 soybean variety and one variety candidate in Adana conditions in 2015-2016. Information on the genotypes used in the study is pre-

**Table 1.** Some Information About the Varieties

| Varietys | Variety Owner Organization | Registration Year | Reclamation place | 1000 Seed Weight (g) | Plant Height (cm) | First Pod Height (cm) | Days to maturity | Protein Ratio (%) | Oil Ratio (%) |
|---|---|---|---|---|---|---|---|---|---|
| SA 88 | Agrova Agri. Ind. Ltd. Co. | 1996 | Türkiye | 126-177 | 78-130 | 9-15 | 112-143 | 38 | 19 |
| Cinsoy | Aegean Agricultural Res. Ins. | 2010 | Türkiye | 137-184 | 89-117 | 13-19 | 126-150 | 31 | 21 |
| Ataem 7 | West Mediterranean Agricultural Res. Ins. | 2006 | Türkiye | 148-166 | 108-145 | 14-27 | 120-181 | 32 | 24 |
| Atakişi | Çukurova Uni. Fac. of Agriculture | 2006 | Türkiye | 142-213 | 88-124 | 10-19 | 84-127 | 27 | 23 |
| Vary 1 (Mona) | Polen Seeds Ltd. Co. | 2017 | Argentina | 118-266 | 99-115 | 10-16 | 117-149 | 39 | 21 |
| Blaze | May Agro Seeds Inc. | 2009 | Türkiye | 149-212 | 75-98 | 11-14 | 138-156 | 34 | 21 |

***Source:*** *Variety Registration and Seed Certification Center, Ankara,-2022.*

sented in Table 1, information on the location in Table 2, and the climatic data of the location in detail in Table 3. The variety candidate (Candidate 1) used in the study was registered under the name as Mona in 2017.

**Table 2.** Information about the Location

| Location Coordinates | | | |
|---|---|---|---|
| **Location** | **Altitude (m)** | **Latitude** | **Longitude** |
| Adana/Yüregir/ Doğankent | 11 | 36°51'13.31"K | 35°20'46.21"D |

kage program, and the factors that were found to be important were determined according to their importance levels, and the traits that were found to be important were evaluated and grouped according to the LSD test.

## RESULTS AND DISCUSSION

The variance analysis values of the traits examined in the study were given in Table 4; the averages of flowering days and days to maturity and the resulting groups in Table 5, the averages of 1000 seed weight and plant height and the resulting groups in Table 6, the averages of the

**Table 3.** Climate Data of Locations

| | Climate Factors | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Location** | **Total Precipitation (mm)** | | | **Average temperature (°C)** | | | **Average Humidity (%)** | | |
| **Adana/Yüregir/Doğankent** | **Years** | | | **Years** | | | **Years** | | |
| **Months** | **2000-2022 (Uzun Yıllar)** | **2015** | **2016** | **2000-2022 (Uzun Yıllar)** | **2015** | **2016** | **2000-2022 (Uzun Yıllar)** | **2015** | **2016** |
| April | 39.9 | 15.5 | 5.6 | 18.2 | 15.5 | 19.1 | 70.3 | 69.9 | 64.0 |
| May | 43.4 | 58.9 | 75.0 | 22.3 | 21.0 | 20.6 | 65.9 | 73.7 | 74.3 |
| June | 22.4 | 25.8 | 6.2 | 26.1 | 24.0 | 25.8 | 69.9 | 77.2 | 72.8 |
| July | 3.2 | 1.2 | 0.7 | 28.9 | 27.2 | 27.7 | 75.3 | 76.3 | 77.4 |
| August | 3.5 | 0 | 4.4 | 29.5 | 28.6 | 28.0 | 75.0 | 69.9 | 77.1 |
| September | 23.4 | 9.3 | 28.5 | 26.9 | 26.8 | 24.6 | 71.3 | 71.8 | 68.7 |
| October | 27.3 | 56 | 1.5 | 22.6 | 21.8 | 21.4 | 65.0 | 72.9 | 61.5 |

***Source:*** *General Directorate of Meteorology-Ankara*

### Methods

This study was carried out according to the Randomized Complete Block Design with 4 replications. Trial sowing was done on 28 April 2015 and 01 May 2016. In the trials, planting depth was determined as 3-5 cm, spacing between rows 60 cm, spacing between rows 3-4 cm, plot length 5 m and 4 rows and only the middle 2 rows were harvested in the trials. The seeds used in the trial were 25 cc for 8 kg of seeds. Treated with 1x109 *Bradyrhizobium japonicum* nitrogen bacteria. In the trials, 36 kg $ha^{-1}$ N and 92 kg $ha^{-1}$ $P_2O_5$ fertilizer were used. During the growing period, 2 hand hoes and 6 irrigations were made, and a total of 700 mm of water need was met with irrigation. The first irrigation was done before flowering, and irrigation was done at 15-20 day intervals during the pod formation and seed filling periods.

In the research; flowering days (days), days to maturity (days), plant height (cm), first pod height (cm), number of pods per plant (plant.$number^{-1}$), 1000 seed weight (g) and oil ratios (%) examines were taken according to the directive of Ankara Variety Registration and Certification Center to measure soybean agricultural values.

### Statistical Analysis

The combined variance analysis of the data obtained from the research was made using the JMP Pro 13 pac-

first pod height and the number of pods per plant and the resulting groups Table 7, the averages of seed yield and oil ratio characteristics and the resulting groups in Table 8, and the correlation values of the bilateral relations between the examined properties in Table 9. It was determined that there were statistically significant differences at the level of 1% and 5% between the year, genotype and year*genotype interaction in terms of all the traits examined (Table 1).

### Days to maturity (day)

In terms of days to maturity of the genotypes; it was indicated that there were statistically significant differences at the level of 1% between year, genotype and year*genotype interaction (Table 4). Days to maturity varied between 135.7 and 145.7 days depending on the years. A longer day to maturity time was calculated in the first year (2015) in which the study was conducted compared to the second year (2016). It is thought that factors such as precipitation and low temperatures were effective in the longer duration of the days to maturity period in the first year. Days to maturity of the genotypes varied between 130.0 and 147.8 days. Among the longest days to maturity variety candidate (Mona) - the shortest days to maturity was determined in SA 88 variety. In the year*genotype interaction, days to maurity varied between 130 days (SA

**Table 4.** Variance Analysis Table For The Examined Characteristics

| Variation Sources | DF | Seed yield | Flowering days | Days to maturity | Plant Height | First Pod Height | number of pods per plant | 1000 seed weight | Oil ratio |
|---|---|---|---|---|---|---|---|---|---|
| Model | 17 | 42737.6 | 6.62745 | 242.971 | 415.856 | 46.0999 | 104.678 | 7323.71 | 1.05613 |
| Year | 1 | 646607** | 16.3333** | 1200** | 1371.74** | 682.521** | 387.603* | 115591** | 7.0227** |
| Genotype | 5 | 9948.13** | 10.5333** | 379.483** | 1034.2** | 13.5448** | 78.73 | 1032.19** | 1.56645** |
| Year* Genotype | 5 | 2043.28 | 8.53333** | 206.15** | 83.3988** | 4.78483** | 166.403* | 634.718* | 0.48311** |
| Error 1 | 6 | 3329.01 | 0.16667 | 0.38889 | 18.3042 | 1.58806 | 27.71 | 96.2135 | 0.11396 |
| Error 2 | 30 | 969.2 | 0.23333 | 0.139 | 7.557 | 0.6054 | 34.852 | 156.65 | 0.02749 |
| CV (%) | | 6.03 | 1.82 | 0.26 | 2.28 | 6.09 | 10.55 | 5.93 | 0.73 |

***, p<0.01; *0.01<P<0.05; **:% 1; *:%5 level is statistically significant.DF: degrees of freedom*

88 in 2016, Cinsoy and Ataem 7), and 160 days (Atakisi in 2016) (Table 5). Obtaining the shortest and longest day to maturity time values of the year*genotype interaction from the same year (2016), shows that the physiological death period is mostly under the influence of the genetic characteristics of the genotypes. For this reason, it shows that years are not as effective as genetic characteristics of genotypes on days to maturity.

## Flowering days (day)

In terms of the number of flowering days of the genotypes; it was examined that there were statistically significant differences at the level of 1% between year, genotype and year*genotype interaction (Table 4). Flowering days of the genotypes varied between 25.8 days (in 2015) and 27.0 days (in 2015) depending on the years (Table 5). Flowering days of the genotypes varied between 25.4-28.1 days. The longest flowering days was determined in variety candidate 1 (Mona), and the shortest flowering days was determined in Atakisi variety. In the year*genotype interaction, it varied between 24.3 days (Atakisi 2016) and 30.3 days (variety candidate 1 in 2016) (Table 5). The fact that flowering days changes in the same year shows that this feature is affected by the environment but mostly due to the genotype.

**Table 5.** Means and Groups of Days to Maturity and Flowering Days Characteristics

| Genotypes | Days to Maturity (day) | | | Flowering days (day) | | |
|---|---|---|---|---|---|---|
| | 2015 | 2016 | Average | 2015 | 2016 | Average |
| SA 88 | 130.0 g | 130.0 g | 130.0 D | 25.8 d | 25.0 e | 25.4 E |
| Cinsoy | 145.3 d | 130.0 g | 137.6 C | 26.0 d | 26.0 d | 26.0 D |
| Ataem 7 | 145.3 d | 130.0 g | 137.6 C | 27.3 c | 26.0 d | 26.6 C |
| Atakişi | 160.0 a | 135.0 f | 147.5 A | 24.3 f | 26.0 d | 25.1 E |
| Vary candidate 1 (Mona) | 147.5 b | 148.0 b | 147.8 A | 30.3 a | 26.0 d | 28.1 A |
| Blaze | 146.0 c | 141.0 e | 143.5 B | 28.5 b | 26.0 d | 27.3 B |
| **Average** | 145.7 A | 135.7B | 140.7 | 27.0 a | 25.8 b | 26.4 |
| LSD 0.05 Year | | 0.44 | | | 0.28 | |
| LSDF 0.05 Genotype | | 0.38 | | | 0.49 | |
| LSD 0.05 Year* Genotype | | 0.53 | | | 0.69 | |

## 1000 Seed weight (g)

In terms of 1000 seed weight (g) of genotypes; it was examined that there were statistically significant differences at the level of 1% between year and genotypes, and at the level of 5% on interaction (Table 4). The 1000 seed weight varied between 162.0 and 260.1 over the years. Genotypes varied between 195.5 and 221.4 g Maximum 1000 seed weight was taken from Ataem 7 variety, at least from SA 88 variety. In the year*genotype interaction, 1000 seed weight; it has been examined that it varies between 144.5 g (SA 88 in 2015) and 277.6 g (Ataem 7 in 2016). Blaze, Candidate 1, Ataem 7 and Cinsoy genotypes had the highest values in terms of 1000 see weight in 2016. The fact that 1000 seed weights are in different years indicates that this characteristic is mostly caused by the environment (Table 6).

## Plant height (cm)

In terms of plant height of genotypes; it was examined that there were statistically significant differences at the level of 1% between year, genotype and year*genotype interaction (Table 4). Depending on the years, the plant height varied between 115.2 and 125.9. Plant heights of genotypes; It varied between 106.1-135.0 cm (Table 6). In the year*genotype interaction, the plant height was 102.1 cm (Candidate 1) in 2016 and 140.0 cm in 2015. The change in all genotypes on the basis of years shows that the effect of the environment is very dominant.

## First pod height (cm)

**Table 6.** Averages and Groups of 1000 Seed Weight and Plant Height Characteristics

| Genotypes | 1000 Seed Weight (g) | | | Plant Height (cm) | | |
|---|---|---|---|---|---|---|
| | 2015 | 2016 | Average | 2015 | 2016 | Average |
| SA 88 | 144.5 d | 246.0 b | 195.2 B | 127.5 c | 116.4 f | 122.0 B |
| Cinsoy | 161.0 cd | 272.6 a | 216.8 A | 120.0 ef | 111.9 g | 116.0 C |
| Ataem 7 | 165.3 c | 277.6 a | 221.4 A | 135.8 b | 134.2b | 135.0 A |
| Atakişi | 166.0 c | 229.7 b | 197.8 B | 140.0 a | 124.4 cd | 132.2 A |
| Vary candidate 1 (Mona) | 166.8 c | 266.6 a | 216.7 A | 122.0 de | 102.1 h | 112.0 D |
| Blaze | 168.0 c | 268.0 a | 218.0 A | 110.0 g | 102.3 h | 106.1 E |
| **Average** | 162.0 b | 260.1 a | 211.1 | 125.9 a | 115.2 b | 120.6 |
| LSD 0.05 Year | | 6.92 | | | 3.02 | |
| LSDF 0.05 Genotype | | 12.78 | | | 2.8 | |
| LSD 0.05 Year* Genotype | | | | | 3.96 | |

In terms of first pod height of genotypes; it was examined that there were statistically significant differences at the level of 1% between year, genotype and year*genotype interaction (Table 4). Depending on the years, the height of the first pod varied between 9.0-16.5. First pod height of genotypes; it varied between 11.0 – 15.0 cm. In terms of first pod height of genotypes, the first pod height was taken from the shortest variety SA 88 and the longest from Atakisi variety. In the year*genotype interaction, it was taken from SA 88 cultivar with 6.5 cm and Atakisi variety with 20.0 cm in 2016. (Table 7). The examined change in all genotypes in 2015 and 2016 shows that this trait is more affected by the environmental conditions.

## Seed yield (kg ha$^{-1}$)

In terms of seed yield of genotypes; It was found to be statistically significant at the 1% level between years and genotypes and insignificant in terms of year*genotype interaction, however, it varied between 48.0 (variety candidate 1 in 2015) and 67.7 (Cinsoy in 2015). Number of pods per plant in genotypes; varied between 48.0 plant number$^{-1}$ variety candidate 1 in 2015 and Cinsoy in the same group) and 67.7 plant number$^{-1}$ (Cinsoy in 2016) (Table 7). Examine of changes on the basis of years in all genotypes shows that this trait is more affected by the environment.

**Table 7.** Averages and Groups of The First Pod Height and the Number of Pods Per Plant

| Genotypes | First Pod Height (cm) | | | Number of Pods per Plant (number) | | |
|---|---|---|---|---|---|---|
| | 2015 | 2016 | Average | 2015 | 2016 | Average |
| SA 88 | 15.3 b | 6.5 d | 11.0 C | 56.1 b-e | 63.5 ab | 59.9 A |
| Cinsoy | 16.0 b | 9.1 c | 12.5 B | 48.5 e | 67.7 a | 58.1 A |
| Ataem 7 | 16.0 b | 9.2 c | 12.6 B | 55.8 b-e | 52.9 de | 57.2 A |
| Atakişi | 20.0 a | 9.9 c | 15.0 A | 52.0 de | 62.5 a-c | 55.0 AB |
| Vary candidate 1 (Mona) | 16.0 b | 9.6 c | 12.8 B | 48.0 e | 54.1 c-e | 54.3 AB |
| Blaze | 16.0 b | 9.9 c | 13.0 B | 58 b-d | 52.1 de | 51.0 B |
| **Average** | 16.5 a | 9.0 b | 12.8 | 53.1 b | 58.8 a | 56.0 |
| LSD 0.05 Year | | 0.89 | | | 3.71 | |
| LSDF 0.05 Genotype | | 0.79 | | | | |
| LSD 0.05 Year* Genotype | | 1.12 | | | 8.52 | |

## Number of pods per plant (plant number $^{-1}$)

In terms of the number of pods per plant of genotypes; was examined that there were statistically significant differences at the level of 5% in terms of year and year*genotype interaction, and it was statistically insignificant between genotypes (Table 4). Depending on the years, the number of pods per plant varied between 53.01 and 58.8. The maximum number of pods per plant was obtained in 2016. It varied between 51.0 and 59.9. The lowest number of pods per plant was taken from Blaze variety and the highest from SA 88 variety. In the year*genotype interaction (Table 4). Depending on the years yield (kg ha$^{-1}$) varied between 3994-6316 kg ha$^{-1}$. It is thought that the total precipitation in the first development period of the plant was higher in 2015 than in 2016. Seed yields of the genotypes varied between 4781 kg ha$^{-1}$ (Atakisi) and 5460 kg ha$^{-1}$ (Blaze). In the year*genotype interaction, it varied between 3597 kg ha$^{-1}$ and 6788 kg ha$^{-1}$. In terms of seed yield, Cinsoy, variety candidate 1 and Blaze genotypes had the highest values in 2015, while Ataem 7 and Atakisi genotypes formed the lowest seed yield group in 2016 (Table 8). While all genotypes gave the highest seed

yield in 2015, the lowest seed yield was obtained in 2016. This situation strengthens the opinion that the effects of environmental changes over the years on genotypes are very strong.

### Oil ratio (%)

In terms of oil ratio of genotypes; year was found to be statistically significant at the 1% level between genotype and year*genotype interaction (Table 4). Depending on the years, oil ratio (%) varied between 22.3-23.0. Oil ratios of the genotypes ranged from 22.1 to 23.3. In terms of oil ratio of genotypes, the lowest oil ratio was taken from Atakisi variety, and the highest oil ratio was taken from variety candidate 1 genotype. In the year*genotype interaction, it varied between 22.0 (SA 88 in 2016, Ataem 7, Atakisi) to 24.0% (variety candidate 1 in 2015). While the highest value in terms of oil ratio was obtained from variety candidate 1 in 2015, the lowest oil ratios were obtained from SA 88, Ataem 7 and Atakisi genotypes in the same group in 2016 (Table 8).

According to the analysis from Table 9, yield and flowering days (r=0.4889**), days to maturity (r=0.5393**), first pod height (r=0.822**) and oil ratio (r=0.7114**) statistically significant and positive correlation at the level of 1%; It was determined that there was a statistically significant and negative relationship at the level of 1% between 1000 seed weight (r=-0.8592**) and 5% between the number of pods per plant (r=-0.4102*).

It was examined that there was a significant and positive relationship at the 1% level between the number of flowering days and the oil ratio (r=0.703**).

While there was a significant and positive correlation at the 1% level between days to maturity and the height of the first pod (r=0.701**); it was examined that there was a 5% and negative correlation between the number of pods per plant (r=-0.4479*) and 1000 seed weight (r=-0.4414*).

While there is a significant and positive relationship at the level of 1% between plant height and first pod height (0.4739**); It was determined that 1000 seed weight (r=-0.439*) had a negative relationship at the level of 5%.

While there was a significant and negative relationship at the 5% level between the height of the first pod and the number of pods per plant (r=-0.427*), there was a significant and positive relationship between the oil ratio (r=0.4618**) at the 1% level.

It was examined that there was a significant and positive correlation at the 1% level between the number of pods per plant and 1000 seed weight (r=0.3043**).

It was determined that there was a significant and negative relationship at the 1% level between 1000 seed weight and oil ratio (r=-0.5506**).

While the total precipitation (15.5-58.9-25.8) in April, May and June in Adana conditions in 2015 is close to the long-term average (39.9-43.4-22.4); however, total precipitation in April, May and June in 2016 (5.6-75.0-6.2) remained below the long-term average (Table 3). Therefore, while the average seed yield of the variyeties was 6316 kg ha$^{-1}$ in 2015, the average of the varieties remained at 3994 kg ha$^{-1}$ in 2016. In terms of seed yields; In 2015 and 2016, the most negative change occurred in Ataem 7 cultivars, while the least change occurred in SA 88 and Blaze cultivars. This situation strengthened the judgment that the performance of varieties is mostly affected by the environment. Many researchers have also state that seed yield is most affected by the environment. Many researchers, in their studies in different genotypes and locations, Çalışkan and Arıoğlu (2004) 2410-2628 kg ha$^{-1}$, Arıoğlu et al., (2012) 2752-3674 kg ha$^{-1}$, Arıoğlu et al., (2015) seed yields vary between 4288-5377 kg ha$^{-1}$ and Ozkan et al., (2019) 1330-4010 kg ha$^{-1}$ and Ahmadi and Arien (2022) 1046-1212 kg ha$^{-1}$ and they reported that the seed yield was more affected by the environment and agricultural practices. Mebrahtu and Elmi (1997), Carvalho et al. (2002), Hossain et al. (2003), Oliveira et al. (2012), El-Refaey et al. (2013), and Morsy et al. (2015) reported that some varieties have high yields in suitable environments, while others are adapted to bad environments. In addition, Yothasiri and Somwang (2000), Primomo et al.,

**Table 8.** Averages and Groups of Seed Yield and Oil Ratio Characteristics

| Genotypes | Seed Yield (kg ha$^{-1}$) | | | Oil Ratio (%) | | |
|---|---|---|---|---|---|---|
| | 2015 | 2016 | Average | 2015 | 2016 | Average |
| SA 88 | 5782 c | 3847 ed | 4814 B | 23.2 b | 22.0 e | 22.6 C |
| Cinsoy | 6788 a | 4317 d | 5552 A | 23.1 b | 22.7 c | 22.9 B |
| Ataem 7 | 6245 b | 3597 f | 4921 B | 22.6 c | 22.0 e | 22.3 D |
| Atakişi | 5785 c | 3778 ef | 4781 B | 22.1 e | 22.0 e | 22.1 E |
| Vary candidate 1 (Mona) | 6735 a | 4069 de | 5402 A | 24.0 a | 22.6 cd | 23.3 A |
| Blaze | 6559 a | 4359 d | 5460 A | 23.1 b | 22.4 d | 22.7 C |
| **Average** | 6316 a | 3994 b | 5155 | 23.0 a | 22.3 b | 22.7 |
| LSD 0.05 Year | | 40.75 | | | 0.23 | |
| LSDF 0.05 Genotype | | 31.79 | | | 0.16 | |
| LSD 0.05 Year* Genotype | | | | | 0.23 | |

**Table 9.** Correlation Values of the Bilateral Relations Between the Examined Traits

| Examined Characteristics | Seed Yield | Flowering days | Days to maturity | Plant Height | First Pod Height | Number of Pods per Plant | 1000 Seed Weight |
|---|---|---|---|---|---|---|---|
| Seed Yield | | | | | | | |
| Flowering days | 0.4889** | | | | | | |
| Days to maturity | 0.5393** | 0.1602 | | | | | |
| Plant Height | 0.2102 | -0.1415 | 0.1101 | | | | |
| First Pod Height | 0.822** | 0.244 | 0.701** | 0.4739** | | | |
| Number of Pods per Plant | -0.4102* | -0.2121 | -0.4479* | -0.1241 | -0.427* | | |
| 1000 Seed Weight | -0.8592** | -0.2964 | -0.4414* | -0.439* | -0.8502 | 0.3043** | |
| Oil Ratio | 0.7114** | 0.703** | 0.2016 | -0.1271 | 0.4618** | -0.2735 | -0.5506** |

*** % 1; *:%5 level is statistically significant.*

(2002) and Olievira et al. (2012) reported that genotypes with higher stability or good adaptability in a wide range of environments for seed yield. Sudaric et al., (2006), in their study on soybean, reported that the interaction of environment, genotype, and GE is important in terms of seed yield. In many studies, it has been reported that adverse environmental conditions have a negative effect on the growth and seed yield of soybean plants (Whingham ve Minor, 1978; Hu ve Wiatrak, 2012; Wheeler ve Von Braun, 2013). Çubukçu et al., 2020, determined that the GE interaction was statistically significant ($P<0.01$) in terms of seed yield. Karasu et al., (2009) reported that genotype, year, location effects and genotypexyr x location interactions are important. While the data obtained in this study were in agreement with some studies, some were different. The main reasons for these differences are thought to be due to different location, climate and soil characteristics or differences in the genetic structures of the cultivars used.

Yield components affecting seed yield in soybean have a strong positive relationship with flowering days, days to maturity and first pod height. Aremu and Ojo (2005), in their study in different environments, reported that the year*genotype interaction is important for days to maturity and the number of pods per plant.

In the studies of environment*genotype interaction in soybean cultivation, varieties that adapt more easily to climatic conditions and show higher seed and oil yield performance are more preferred by producers in different regions. For this reason, it is extremely important that the seed yield of the desired genotypes does not fluctuate much under different environmental conditions. However, if this interaction does not change the yield order of the genotypes in different environments, there is no problem in terms of cultivar proposal (Kaya and Atakisi, 2002).

In terms of the characters examined in the study; yield, flowering days, days to maturity, plant height, first pod height, 1000 seed weight and oil ratios were found to be statistically significant at the 1% level between year and genotype. In the relations between the examined characters; It was concluded that the yield was significant and positive at the level of 1% between the number of days of flowering, days to maturity and the height of the first pod.

## CONCLUSION

In this study; It was conducted in 2015 and 2016 to test the performance of 5 varieties and one variety candidate (Mona) in main product soybean cultivation in Adana conditions in terms of yield and yield components. According to the results of the analysis, SA 88, Cinsoy, Atakisi and Blaze varieties, which did not show much change depending on the years in terms of yield and yield elements, came to the fore. However, the best results in terms of seed yield and yield components were obtained from Cinsoy, Candidate 1 (Mona) and Blaze varieties. As a result, year, genotype and year*genotype interactions were examined in terms of yield and yield elements of the main crop soybean cultivars and it was concluded that Cinsoy, Candidate 1 (Mona) and Blaze cultivars showed high performance in Adana conditions and the effect of the environment was higher than the genotype effect. In line with the data obtained as a result of this study, the variety candidate 1 was registered with the name Mona with its high performance. In addition, it was concluded that Cinsoy, Mona and Blaze varieties can be recommended for the main product soybean cultivation in Adana conditions.

## COMPLIANCE WITH ETHICAL STANDARDS

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Ethics committee approval is not required.

### Funding

No financial support was received for this study.

## Data availability

Not applicable.

## Consent for publication

Not applicable.

## Acknowledgements

Thanks for their support in this study, Republic of Türkiye Ministry of Agriculture and Forestry, Ankara Variety Registration and Seed Certification Center.

## REFERENCES

Ahmadi, A. Y., Arien, M. (2022). Poultry manure effects on yield and some agronomic components of soybean (*Glycine max* L.) under Khost Agro-Ecological Conditions, Afghanistan. International Journal of Agriculture Environment and Food Sciences, 6(1), 1-6. [Google Scholar]

Ansarifard, I., Mostafavi, K., Khosroshahli, M., Reza Bihamta, M., Ramshini, H. (2020). A study on genotype–environment interaction based on GGE biplot graphical method in sunflower genotypes (*Helianthus annuus* L.). Food Science and Nutrition, 8 (7), 3327-3334. [CrossRef]

Aremu, C.O., Ojo, D.K. (2005). Genotype x environment interaction and selection for yield and related traits in soybean. Moor Journal of Agricultural Research, 6(1 & 2):81-86. [Google Scholar]

Arioglu, H., Bakal, H., Gulluoglu, L., Kurt, C., Sinan, S., Onat, B. (2015). The Determination of some important argonomical and quality properties of soybean (*Glycine max* L. Merr) varieties in double cropped condations Türkiye 11. Field Crops Congress 7-10 September 2015, Çanakkale, 358-362.

Arioglu, H., Ozyurtseven, S., Gulluoglu, L. (2012). The Determination of crude oil yield and fatty acids composition of some soybean (*Glycine max* L. Merr) varieties in double cropped conditions-II. Çukurova Üniversitesi Ziraat Fakültesi Dergisi, 27(2), 1-10. [Google Scholar]

Carvalho, C.G.P., Arias, C.A.A., Toledo, J.F.F., Almeida, L.A., Kiihl, R.A., Oliveira, M.F. (2002). Adaptability and stability study of soybean lines developed for high yield in Parana State using four methodologies. Crop Breeding and Applied Biotechnology 2(2),247-256. [Google Scholar]

Calıskan, S., Arioglu, H. (2004). Determination of soybean cultivars and lines for the second crop produciton under amik plain conditions Mustafa Kemal Üniversitesi Ziraat Fakültesi Dergisi, 1(2), 23-32. [Google Scholar]

Copur, O., Gur, M. A., Demirel, U., Karakus, M. (2009). Performance of some soybean (*Glycine max* L. Merr.) genotypes double cropped in semi-arid conditions. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 37(2), 145-151. [Google Scholar]

Cubukçu, P., Karakus, M., Vurarak, Y., Şahar, A.K., Yıldırım, U.A. (2020). Determining of performance some advanced soybean lines in Adana and Sanlıurfa locations. International Journal of Eastern Mediterranean Agricultural Research, 3(1), 1-16. [Google Scholar]

EL-Refaey, EL-Seidy, R.A., El-Borai, M.A., Abu Sein, T.M. (2013). Phenotypic stability parameters of yield for some soybean genotypes. Egypt Journal of Plant Breed. 17(2), 455-466.

Eswari, K.B., Rao, M.V.B. (2006). Analysis of stability for some characters in soybean (*Glycine max* L. Merr.). International Journal of Agricultural Sciences, 2(2):559-561.

Hossain, M.A., Rahman, L., Shamsuddin, A.K.M. (2003). Genotype-environment interaction and stability analysis in soybean. Journal of Biological Sciences, 3(11), 1026-1031. [Google Scholar]

Hu, M., Wiatrak, P. (2012). Effect of planting date on soybean growth yield and grain quality: Review. Agronomy Journal, 140(3), 785-790. [Google Scholar]

Ilker, E., Kocatürk, M., Kadiroğlu, A., Altınbaş, M., Yıldırım, A., Öztürk, G. Yıldız, H. (2018). Stability analyses for double cropping in soybean (*Glycine max* L. Merr.). Turkish Journal of Field Crops, 23(2), 80-84.

Karaaslan, D. (2011). Determınation of some soyabean lines yield and quality components grown as second crop in Diyarbakır conditions. Harran Tarım ve Gıda Bilimleri Dergisi, 15(3), 37-44. [Google Scholar]

Karaaslan, D., Boydak, E., Gür, M. A. (1998). Effecet of different sowing dates of soybean (*glycine max* l.) varieties on yield and yield components. Harran Üniversitesi Ziraat Fakültesi Dergisi, 2(4):55-64. [Google Scholar]

Karasu, A., Öz, M., Göksoy, A. T., Turan, Z. M. (2009). Genotype by environment interactions, stability, and heritability of seed yield and certain agronomical traits in soybean. African Journal of Biotechnology, 8(4), 580-590. [Google Scholar]

Kaya, Y., Atakisi, İ.K. (2002). Stability analysis in different yield characters in sunflower (*Helianthus Annuus* L.) Anadolu Journal of Aegean Agricultural Research Institute, 12 (2), 1 – 20. MARA. [Google Scholar]

Khomari, A., Mostafavi, K., Mohammadi, A. (2017). Stability study of yield in sunflower (*Helianthus annuus* L.) cultivars using AMMI method. Journal of Crop Breeding, 9(23), 117–124. [Google Scholar]

Kinney, A.J., & Clemente, T.E. (2004). Modifying soybean oil for enhanced performance in biyodisel blends. Fuel Processing Technology, 86(10), 1137-1147. [Google Scholar]

Lopes da Silva, Borem, F., Sediyama, A. Ludke, T. (2017). Soybean Breeding. Springer, USA. [Google Scholar]

Mebrahtu, T., Elmi, A. (1997). Stability analysis of vegetable soybean. Soybean Genetics Newsletter 24, 214-216. [Google Scholar]

Morsy, A.R., Fares, W.M., Ragheb, S.B., Ibrahim, M.A. (2015). Stability analysis of some soybean genotypes using a simplified statistical model. Journal of Plant Production, 6(12), 1975-1990. [Google Scholar]

OECD-FAO, (2019). OECD-FAO Agricultural Outlook 2019-2028. Chapter 2. Latin American Agriculture: Prospects and Challenges.

Oliveira, L.G., Hamawaki, O.T., Simon, G.A., Sousa, L.B., Nogueira, A.P.O., Rezende, D.F., Hamawaki, C.D.L. (2012). Adaptability and stability of soybean yield in two soybean producing regions. Bioscience Journal, 28(6), 852-861. [Google Scholar]

Ozkan, R., Bayhan, M., Albayrak, O., Karaaslan, D., Yildirim, M. (2019). Second crop potential of soybean lines for Diyarbakır location on the yield and quality. International Journal

of Agriculture Environment and Food Sciences, 3(3), 121-126.

Primomo, V.S., Falk, D.E., Ablett, G.R., Taner, J.W., Rajcan, I. (2002). Genotype x environment interactions, stability, and agronomic performance of soybean with altered fatty acid profiles. Crop Sciences 42, 37-44. [Google Scholar]

SoyStats, (2022). World Oilseed Production. [URL]

Sudaric, A., Simic, D., Vrataric M. (2006). Characterization of genotype by environment interactions in soybean breeding programmes of southeast europe. Plant Breeding, 125, 191-194. [Google Scholar]

TÜİK, (2022). Soybean Production and Fields in Turkey. [URL]

Verma, N., Izhar, T. (2017). Seasonal variations in soybean genotypes under different environments. Journal of Pharmacognosy and Phytochemistry, 6(5):1326-1329. [Google Scholar]

Yothasiri, A., Somwang, T. (2000). Stability of soybean genotypes in Central Plain Thailand. Agriculture and Natural Resources, 34(3), 315-322. [Google Scholar]

Wigham, D.K., Minor, H.C. (1978). Agronomic characteristics and enviromental stres. Soybean Physiology, Agronomy and Utilization (Ed. by AG Norman). Academic Pres. London. 249p.

Wheeler, T., Braun J. V. (2013). Climate change impacts on global food security. Science, 341, 508-513. [Google Scholar]

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 676-682
DOI: https://doi.org/10.31015/jaefs.2022.4.23

# Phenolic composition of common produced raisins in Türkiye

**Ali Güler**[1]

[1] Viticulture Research Institute, Manisa, Türkiye

## Abstract

This study aimed to determine individual phenolic compounds of common produced Turkish raisins. As material, Sultan 7, Antep Karasi and Razaki raisins were used. The HPLC method was performed for the analysis of 11 phenolic compounds. The major phenolic acid was *trans*-caftaric acid and the major flavan-3-ol was also (+)-catechin. The *trans*-caftaric acid varied between 21.56 and 46.84 µg/g in the samples and (+)-catechin between 2.21 and 74.12 µg/g. Caffeic acid was the second most abundant phenolic acid with 11.06-21.54 µg/g. The highest gallic acid, (+)-catechin, (-)-epicatechin, caffeic acid and quercetin hydrate concentrations were found in Razaki, and *trans*-caftaric acid, p-coumaric acid and *trans*-resveratrol in Antep Karasi. The *trans*-resveratrol was only detected in Antep Karasi raisin with 0.84 µg/g. The strong significant correlations were observed between investigated phenolic compounds. As result, the phenolic profiles of three Turkish raisins were revealed, and the correlations among these compounds were investigated. The findings on raisins indicated that Turkish raisins are a good source of polyphenols.

**Keywords:** Raisin, Polyphenol, Caftaric acid, Resveratrol, Correlation

**Citation:** Guler, A. (2022). Phenolic composition of common produced raisins in Türkiye. International Journal of Agriculture, Environment and Food Sciences, 6 (4), 676-682.

**Received:** 06 November 2022
**Revised:** 15 December 2022
**Accepted:** 19 December 2022
**Published Online:** 20 December 2022

**Correspondence:** Ali Güler
**E-mail:** aligguler@gmail.com



## INTRODUCTION

Türkiye has suitable ecological conditions for viticulture and a considerable genetic grapevine diversity. Raisins are an important dried product with 290,000 tons produced on average in the last five years in Türkiye (TMO, 2021). In addition, Türkiye is one of the largest raisin (dried grape) producer and exporter in the world. Although both seeded and seedless grapes are dried, mostly seedless raisins are exported. Seeded raisins are generally traded in the domestic market. The grapes are dried by dipping to a solution or spraying them with a solution under the sun in our country. The used dipping solution includes 5% potassium carbonate and 1% dipping oil. This pre-treatment accelerates drying by resolving and removing the wax layer on the grape surface. This process provide increment in the colour lightness depending on the reduction in drying period.

In recent years, consumer demand and interest in healthy and reliable dried fruits have increased. In particular, raisins are one of the most often preferred because of their high nutritional characteristics. Raisins are a rich source of phenolic compounds. They contain remarkable concentration of flavonol glycosides and phenolic acids (Karadeniz et al., 2000). Previous studies have showed that raisins contain gallic, *trans*-caftaric, *trans*-coutaric, coumaric, protocatechuic and ferulic

acids, *trans*-resveratrol, catechin, epicatechin, rutin, myricetin, quercetin, kaempferol, malvidin-3-O-glucosides and its acylated esters (Karadeniz et al., 2000; Breksa, et al., 2010; Kelebek et al., 2013). Polyphenols are generally classified into two groups as flavonoids and non-flavonoids. In grape pulp, phenolic acids form a group of non-flavonoids. Grape-based products can be unstable due to phenolic acids, which affect the constitution of colour pigments such as yellow or brown. This is one of the most prominent problems associated with the drying of white grapes. (Kelebek et al., 2013). Flavonoid group consist of flavan-3-ols, flavonols, and anthocyanin. Flavonoids having high antioxidant power, have several functional properties for human health (Kelebek et al., 2013; Guler et al., 2022). After the drying process, many of the grape polyphenols have high bioavailability. According to Schuster et al. (2017), consumption of the raisin in a daily diet reduces blood sugar and pressure, and cholesterol (low density lipoprotein) compared with snacks having equal caloric carbohydrates. In addition, raisin consumption is associated with the reduction of cardiovascular diseases and also positively affects intestinal flora. Beside, raisin play role in the prevention of many chronic diseases such as cardiovascular diseases, type 2 diabetes, intestinal diseases and dental caries (Schuster et al., 2017). As similar, Williamson and Carughi (2010) reported that raisins can reduce the postprandial insulin response, control glycemic index, affect certain oxidative biomarkers, and promote satiety.

There are limited studies of raisin phenolic composition although Türkiye is one of the most important raisin producer and exporter in the world. The current study aims to determine individual phenolic compounds of Sultan 7 that is registered a few years ago, Antep Karasi and Razaki raisins.

## MATERIALS AND METHODS

### Materials

The main materials of this study are Sultan 7, Razaki and Antep Karasi raisins that were dried under the sun. The fresh grapes were supplied from the vineyards of Viticulture Research Institute, Manisa. After being dried, raisins were picked up from drying area and stored at +4 °C until used for analysis. During the drying process, the weather conditions were at seasonal normals and no rain. Drying times were 7 days for Sultan 7 and 10 days for Razaki and Antep Karasi. Figure 1 shows the images of the analysed raisins. Sultan 7 a seedless grape variety and registered by Viticulture Research Institute, Manisa in 2011. This grape variety is commonly used for drying because of high drying efficiency and raisin quality. Razaki is a seed grape variety and it is used for table and drying. Its berries are green-pinky yellow, long ellipsoidal, large (6-7 g), 2-4 seed and neutral flavor. The clusters of Razaki are winged conical-cylindirical, large (400-500 g) and loose (Çelik, 2002). Antep Karasi is synonyms of Kilis karasi and Horoz karasi grape varieties. Its berries are blue-black, long ellipsoidal, very large (8-9 g), 2-3 seed and tanninous, and clusters are winged conical, large (700-800 g) and well-filled (Çelik, 2002). It is mostly grown for table and drying purposes.

**Figure 1.** Raisin pictures (Sultan 7, Razaki and Antep Karasi)

### Sample preparation and extraction

Extraction of the polyphenols from raisin samples was performed using the procedure described Kelebek et al. (2013) with slightly modifications. Raisin samples were powdered in liquid nitrogen by using a homogenizer (Ultra Turrax T25 D, Ika, Germany) at 10,000 rpm. Obtained raisin powder mixed with 1 volume of acetone and then homogenised by using Ultra Turrax for 3 min at 10,000 rpm. Homogenised samples were filtered using a Buhner funnel Whatmann no.1. The extraction procedure was repeated with aqueous methanol (30:70 v/v) to till a clear solution was achieved. The obtained filtrate was centrifuged for 10 min at 4000 rpm (Nuve NF 400, Türkiye) and the clear part was separated. The methanol was evaporated using by a rotary evaporator (Ika RV 10, Germany) under vacuum conditions at 40 °C. The solid phase extraction (SPE) was used for the purification of obtained extract. Before the analysis, the preconditioning of the SPE cartridge C18 (Bond Elut C18, 100mg 3 mL, Agilent Technologies, US) was conducted with 5 mL ethyl acetate, 5 mL methanol (0.01% HCl) and 2 mL distilled water (0.01% HCl), respectively. The 1 mL extract was added to the preconditioned cartridge followed by 2 mL distilled water. Then, the cartridge was dried by using a block heater (TAB 24-2, Türkiye) under nitrogen gases at 35 °C. The phenolic compounds were loaded with ethyl acetate (5 mL) and evaporated under reduced pressure at 40°C in the rotary evaporator. The purified extract was dissolved

at methanol.

### Analysis of phenolic compounds by HPLC

The method reported by Özkan and Göktürk Baydar (2006) with some modifications were performed to quantify the individual polyphenols. The HPLC (high performance liquid chromatography) system was an Agilent Technologies 1260 Infinity equipped with a quaternary pump, on-line degasser, column heater, auto-sampler, and UV-diode array detector (Agilent, Waldborn, Germany). The analytical separation was performed using by C18 ODS 250×4.6 mm, 5 µm (Agilent) column. The following phenolic compounds were detected: Gallic acid, (+)-catechin, caffeic acid and p-coumaric acid at 280 nm, *trans*-caftaric acid, vanillic acid, (-)-epicatechin, ferulic acid, sinapic acid and *trans*-resveratrol at 320 nm, and quercetin hydrate at 360 nm wavelength. The samples were filtered by using a syringe filter (PTFE, 0.45 µm, Sartorius) before injection. The flow rate was set to 1 ml/min and the column temperature to 30 °C. The filtered sample was injected into the system as 10 µl volume. The ultrapure water: formic acid (99.8:0.2 v/v) (A) and methanol (B) were mobile phases. The gradient program started with 100%A and changed to 5% B along 3 min, and 20% B along min 18, held for 20% B 18 min (isocratic step). Followed by elution program was 75% A and 25% B at min 30, 70% A and 30% B at min 40, 60% A and 40% B at min 50, 50% A and 50% B at min 55, and 100% B at min 65. 100% A elution was performed for 5 min to return to the initial condition. The obtained data were analysed using by a software program (Agilent ChemStation OpenLAB). Phenolic compounds were identified according to their retention times and spectra in comparison with analytical standards. The concentration of individual polyphenols was calculated using by calibration curves. The results were expressed as µg in g raisin. The retention times, wavelength, linear range, equation and correlation coefficient of investigated phenolic compounds were presented in Table 1.

### Statistical analysis

All analysis were performed in triplicate (n=6), and obtained means are reported with the standard deviations. Results were analysed by one-way ANOVA test, Duncan's multiple range test was used to compare the significant differences of the mean values at 0.05 level. In addition, the correlation coefficients of Pearson were calculated to examine the relationships among polyphenols.

## RESULTS AND DISCUSSION

The results for polyphenol compounds quantified in raisin samples are presented in Table 2. The statistical differences were found between raisin samples for gallic acid, *trans*-caftaric acid, (-)-epicatechin, *p*-coumaric acid, ferulic acid, sinapic acid, *trans*-resveratrol and quercetin hydrate at level 0.05. However, there were no significant statistical differences among raisins for vanillic and caffeic acid means ($p>0.05$).

The most abundant phenolic compounds were phenolic acids in raisin samples. The major phenolic acid was *trans*-caftaric acid with amounts of 21.56-46.84 µg/g in the samples. The highest *trans*-caftaric acid content was in Antep Karasi, and the lowest in Sultan 7. The finding of *trans*-caftaric acid concentration in Sultan 7 raisin was higher than previously reported Sultaniye raisin data by Kelebek et al. (2013), but lower than Thompson seedless raisin values obtained by other authors (Karadeniz et al., 2000; Parker et al., 2007; Breksa et al., 2010; Fabani et al., 2017). In a previous study, the *trans*-caftaric acid concentration of Antep Karasi was found as 92.99 mg/kg, and between 20.48 and 114 mg/kg in other four raisin samples (Kelebek et al. 2013). This study *trans*-caftaric acid finding in Antep Karasi was lower than previous reported data. In addition, Fabani et al. (2017) studied on alterations of phenolic compounds in grapes during the drying processing and expressed that *trans*-caftaric acid values in the raisins varied from 3.26 to 19.0 mg/100 g DW. Breksa et al. (2010) found *trans*-caftaric acid concentration ranging from 153.5 to 598.7 mg/kg DW in 16 raisin cultivars. In another study, Karadeniz et al. (2000) reported that *trans*-caftaric acid was 39.6 mg/kg in sun dried raisins, 45.2 mg/kg in dipped raisins and 84.3 mg/kg golden raisins. As similar, Parker et al. (2007) also ex-

**Table 1.** Linearity parameters of investigated phenolic compounds

| Compounds | Retention time, (min) | Wavelength, (λ) | Linear range (µg/g) | Equation | Correlation Coefficient ($R^2$) |
|---|---|---|---|---|---|
| Gallic acid | 12.8 | 280 | 1-50 | y=22.4601x-10.567 | 0.9999 |
| *trans*-caftaric acid | 25.4 | 320 | 1-50 | y=25.3824x-16.225 | 0.9999 |
| Vanillic acid | 34.0 | 280 | 1-50 | y=53.2441x-21.866 | 0.9998 |
| Caffeic acid | 35.4 | 320 | 5-50 | y=6.2370x-13.5031 | 0.9979 |
| *p*-coumaric acid | 46.9 | 280 | 1-50 | y=12.2091x-8.7214 | 0.9996 |
| Ferulic acid | 48.9 | 320 | 0.5-25 | y=57.2881x-9.1455 | 0.9999 |
| Sinapic acid | 50.1 | 320 | 1-50 | y=46.1044x-10.5248 | 0.9999 |
| (+)-Catechin | 27.0 | 280 | 1-50 | y=7.1567x-2.0443 | 0.9996 |
| (-)-Epicatechin | 45.5 | 320 | 0.5-25 | y=68.0424x-10.1415 | 0.9999 |
| *trans*-resveratrol | 58.3 | 320 | 0.5-25 | y=61.0464x-14.638 | 0.9999 |
| Quercetin hydrate | 62.4 | 360 | 5-50 | y=25.1789x-66.5442 | 0.9964 |

**Table 2.** Phenolic compositions of raisin samples

| Phenolic compound (µg/g) | Sultan 7 | Antep Karasi | Razaki |
|---|---|---|---|
| Gallic acid | 2.09±0.49$^{c}$ | 4.42±0.93$^{b}$ | 7.11±1.28$^{a}$ |
| *trans*-caftaric acid | 24.75±0.01$^{ab}$ | 46.84±9.84$^{a}$ | 21.56±3.99$^{b}$ |
| Vanillic acid | 1.83±0.01 | 4.67±1.23 | 2.85±0.36 |
| Caffeic acid | 11.06±0.01 | 11.85±2.39 | 21.54±7.19 |
| *p*-coumaric acid | 2.80±0.65$^{b}$ | 18.76±4.38$^{a}$ | 10.12±0.44$^{ab}$ |
| Ferulic acid | 0.93±0.37 | 0.68±0.15 | 2.51±1.40 |
| Sinapic acid | 1.74±0.30$^{b}$ | 1.36±0.24$^{b}$ | 3.97±0.02$^{a}$ |
| (+)-Catechin | 2.21±0.01$^{c}$ | 17.91±2.86$^{b}$ | 74.12±8.72$^{a}$ |
| (-)-Epicatechin | 0.67±0.02$^{b}$ | 0.60±0.07$^{b}$ | 1.23±0.26$^{a}$ |
| *trans*-resveratrol | nd | 0.84±0.09 | nd |
| Quercetin hydrate | 7.01±0.33$^{b}$ | 7.03±0.89$^{b}$ | 15.82±1.02$^{a}$ |

The values indicated by different letters within each row are significantly different at 0.05 level.
*nd. Not detected

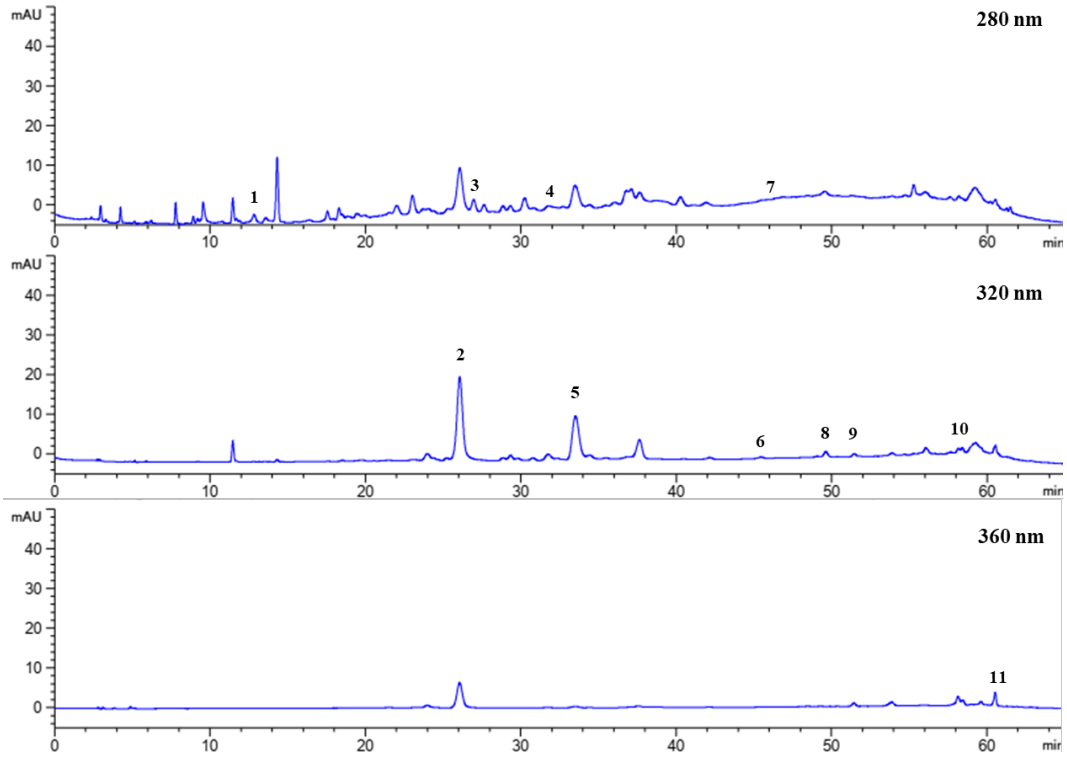



**Figure 2.** HPLC chromatogram of Antep Karasi phenolic compounds. 1: gallic acid, 2: *trans*-caftaric acid, 3: (+)-catechin, 4: vanillic acid, 5: caffeic acid, 6: (-)-epicatechin, 7: *p*-coumaric acid, 8: ferulic acid, 9: sinapic acid, 10: *trans*-resveratrol, 11: quercetin hydrate

pressed that golden raisin *trans*-caftaric acid (130.4 mg/kg) concentration was higher than sun-dried raisin (41.4 mg/kg).

Gallic acid concentrations were 2.09 µg/g in Sultan 7, 4.42 µg/g in Antep Karasi and 7.11 µg/g in Razaki. In a comparison with the literature, current study findings were lower than results of Kelebek et al. (2013) and Meng et al. (2011), and higher than Fabani et al. (2017) datas. Caffeic acid was the second most abundant phenolic acid in raisin samples with 11.06-21.54 µg/g. These caffeic acid findings are compatible with literature (Meng et al., 2011). Regarding investigated raisins, *p*-coumaric acid concentrations varied between 2.80 and 18.76 µg/g. Meng et al. (2011) studied on Chinese raisin phenolic contents and found 2.38-23.45 µg/g DW *p*-coumaric acid in the 10 raisin samples. The current study *p*-coumaric acid findings were in agreement mentioned research. Vanillic acid concentrations were 1.83 µg/g in Sultan 7, 2.85 µg/g in Razaki and 4.67 µg/g in Antep Karasi raisins. Kelebek et al. (2013) reported that vanillic acid means varied from 0.27 to 0.98 µg/g DW in five raisin samples and the highest value was in Antep Karasi. The vanillic acid results were higher than the last mentioned study findings. The highest ferulic acid was in Razaki with 2.51 µg/g,

followed by Sultan 7 with 0.93 µg/g and Antep Karasi with 0.68 µg/g. In literature, Kelebek et al. (2013) reported considerably low vanillic acid concentration (0.40-1.49 mg/kg) in five raisins while Meng et al. (2011) found notably high results in Chinese 10 raisins. In a comparison with the literature, our findings closer to Kelebek et al. (2011) datas. Sinapic acid amounts were determined 1.74 µg/g in Sultan 7, 1.36 µg/g in Antep Karasi and 3.97 µg/g in Razaki raisins.

The Antep Karasi HPLC chromatogram is indicated on Figure 2. Considering investigated Sultan 7, Antep Karasi and Razaki raisins, it is revealed that they contains of considerable individual phenolic acids. In particular, *trans*-caftraric, p-coumaric and caffeic acids were abundant in Turkish raisins.

(+)-Catechin and (-)-epicatechin compounds and their polymers are very powerful antioxidants and they have more antioxidant activity than vitamin E (Rice Evans et al., 1997). Their concentration in grape products is considerably significant for this reason. In the present study, (+)-catechin and (-)-epicatechin from flavan-3-ols were investigated in raisin samples. The highest (+)-catechin concentration was found in the Razaki raisin with 74.12 µg/g, followed by Antep Karasi with 17.91 µg/g and Sultan 7 with 2.21 µg/g. In a study, (+)-catechin concentrations of five Turkish sun-dried raisins were detected between 56.31 and 419 mg/kg (Kelebek et al. 2013). Meng et al. (2011) found that (+)-catechin ranged from 10.4 to 66.47 µg/g DW in 10 Chinese raisins. In another study, (+)-catechin concentrations in Argentinean sun-dried raisins were determined 15-158 mg/100 g DW (Fabani et al., 2017). In this respect, our current findings were low compared with reported results by Kelebek et al. (2013) and Fabani et al. (2017), and in accordance with expressed findings by Meng et al. (2011). (-)-Epicatechin contents varied from 0.60 to 1.23 µg/g in raisin samples. In previous studies, (-)-epicatechin concentrations in raisins were reported ranging from 19.18 to 117 mg/kg by Kelebek et al. (2013) and 15-27 mg/100 mg DW by Fabani et al. (2017). In addition, Fabani et al. (2017) could not detected (-)-epicatechin in sun-dried Sultaniye and Superior raisins. Moreover, it is claimed that the drying process under the sun could be completely degraded the flavan-3-ols in raisin because of probably enzymatic oxidation (Karadeniz et al., 2000; Fabani et al., 2017). In particular, our (-)-epicatechin findings in Sultan 7 and Antep Karasi were low, but they were not consistent with results reported by these literatures. It is thought that these differences may be affected by cultivar and drying conditions as well as variety differences. USDA data related to the flavan-3-ols in raisins support our findings (Haytowitz et al., 2018).

Resveratrol is a polyphenol compound classified in stilbenes and has possible benefficial effects on human health with high antioxidant properties. *Trans*-resveratrol was only detected in Antep Karasi raisin (0.84 µg/g). Breksa et al. (2010) detected *trans*-resveratrol in the justly one raisin sample (B53-122) with 0.8 µg/g DW in investigation of 16 raisins. Karadeniz et al. (2000) could no detected the *trans*-resveratrol in Thompson Seedless raisin, and explained this situation by the fact that the capability of grapes to produce resveratrol is lost during the ripening period. On the other hand, Roychev et al. (2020) reported that *trans*-resveratrol concentrations were ranged from 1.97 to 18.62 mg/kg in 26 raisins of seedless hybrids, Corinthian Black raisin and Gamay Freaux and Sangiovese grapes. This current study result of *trans*-resveratrol was in agreement with the literature, since its concentration could change depending on variety and fungal infections (Karadeniz et al., 2000; Fabani et al., 2017; Roychev et al., 2020).

**Table 3.** The correlations among phenolic compounds in raisins

| Correlation | Gallic acid | trans-caftaric acid | (+)-Catechin | Vanillic acid | Caffeic acid | (-)-Epicatechin | p-coumaric acid | Ferulic acid | Sinapic acid | Quercetin hydrate |
|---|---|---|---|---|---|---|---|---|---|---|
| Gallic acid | 1 | 0.088 | 0.901** | -0.043 | 0.584 | 0.784** | 0.462 | 0.458 | 0.715* | 0.788** |
| *trans*-caftaric acid | 0.088 | 1 | -0.271 | -0.006 | -0.409 | -0.318 | 0.821* | -0.360 | -0.349 | -0.595 |
| (+)-Catechin | .901** | -0.271 | 1 | 0.145 | 0.713 | 0.874** | 0.127 | 0.629 | 0.856* | 0.939** |
| Vanillic acid | -0.043 | -0.006 | 0.145 | 1 | 0.156 | -0.254 | 0.099 | -0.129 | -0.407 | 0.108 |
| Caffeic acid | 0.584 | -0.409 | 0.713 | 0.156 | 1 | 0.657* | -0.414 | 0.673 | 0.532 | 0.714* |
| (-)-Epicatechin | 0.784** | -0.318 | 0.874** | -0.254 | 0.657* | 1 | -0.069 | 0.852** | 0.852** | 0.883** |
| *p*-coumaric acid | 0.462 | 0.821* | 0.127 | 0.099 | -0.414 | -0.069 | 1 | -0.203 | 0.002 | -0.129 |
| Ferulic acid | 0.458 | -0.360 | 0.629 | -0.129 | 0.673 | 0.852** | -0.203 | 1 | 0.730* | 0.673 |
| Sinapic acid | 0.715* | -0.349 | 0.856* | -0.407 | 0.532 | 0.852** | 0.002 | 0.730* | 1 | 0.912** |
| Quercetin hydrate | 0.788** | -0.595 | 0.939** | 0.108 | 0.714* | 0.883** | -0.129 | 0.673 | 0.912** | 1 |

** Correlation is significant at the 0.01 level.
* Correlation is significant at the 0.05 level.

Quercetin is a polyphenol that belongs to the flavonol group. Potential benefits of quercetin are protection from oxidative stress, cardiovascular disease, cancer, anti-inflammatory and age-related neurological degeneration (Carughi et al., 2008). Quercetin hydrate varied between 7.01 and 15.82 µg/g in raisin samples. The highest quercetin concentration was in Razaki, and the lowest in Sultan 7 raisin. Breksa et al. (2010) reported that quercetin 3-*o*-glucoside contents changed from 7.4 to 69.3 µg/g DW in raisin samples. Chinese raisin quercetin concentrations varied between 17.95 and 326.7 µg/g DW (Meng et al., 2011). The other study showed that the quercetin 3-*o*-glucoside concentrations of Turkish raisins were detected from 2.79 to 12.83 mg/kg (Kelebek et al., 2013). Karadeniz et al. (2000) found that quercetin glycoside were 7.3-41.5 mg/kg in different processed raisins. The highest quercetin concentration was observed in golden raisins followed by dipped and sun-dried raisins. Findings related to the quercetin were in agreement with mentioned previous studies. It has been revealed that sun exposure to grapes affects the quercetin concentrations (Price et al., 1995). According to Soleas et al. (1997), the grape variety and water status could affect the quercetin concentration.

Pearson correlations between investigated polyphenols are presented in Table 3. The linear correlation coefficients among analysed polyphenols were significant at 0.01 and 0.05 levels. There were strong correlations between gallic acid and (+)-catechin, (-)-epicatechin and quercetin hydrate. Similarly, positive strong correlations were observed between (+)-catechin and (-)-epicatechin and quercetin hydrate. Congruently, significant correlations were also observed between (-)-epicatechin and (+)-catechin, ferulic acid, sinapic acid and quercetin hydrate. In addition quercetin hydrate strongly correlated with gallic acid, (+)-catechin, (-)-epicatechin and sinapic acid at 0.01 level.

## CONCLUSION

In this study, the polyphenol profiles of three raisins from Türkiye, which are commonly produced and marketed, were evaluated. The phenolic content findings in raisins indicated that Turkish raisins are a good source of polyphenols. It was determined that Antep Karasi and Razaki being seeded grapes have rather phenolic content than Sultan 7. The most abundant phenolic compounds were phenolic acids in investigated samples**.** The major phenolic acid *was trans*-caftaric acid with 21.56-46.84 µg/g, and the major flava-3-ol was (+)-catechin with 2.21-74.12 µg/g. *Trans*-resveratrol could be only detected in Antep Karasi raisin. The highest gallic acid, (+)-catechin, (-)-epicatechin, caffeic acid and quercetin hydrate concentrations were detected in Razaki, and *trans*-caftaric acid, *p*-coumaric acid and *trans*-resveratrol in Antep Karasi. Further research is needed to determine the phenolic profile of Turkish raisin genotypes and the effects of the drying process.

## COMPLIANCE WITH ETHICAL STANDARDS

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Ethics committee approval is not required.

### Funding

No financial support was received for this study.

### Data availability

Not applicable.

### Consent for publication

Not applicable.

### Acknowledgements

Author is thankful to colleagues Ahmet Candemir, K.Emre Özaltın and F. Belgin Aşıklar for supports.

## REFERENCES

Breksa III, A. P., Takeoka, G. R., Hidalgo, M. B., Vilches, A., Vasse, J., & Ramming, D. W. (2010). Antioxidant activity and phenolic content of 16 raisin grape (Vitis vinifera L.) cultivars and selections. Food Chemistry, 121(3), 740-745. [CrossRef]

Carughi, A., Lamkinv, T., & Perelman, D. (2008). Health benefits of sun-dried raisins. Health Research and Studies Centre, 30, 511-9.

Çelik, H. (2002). Grape cultivar catalog. Sunfidan A.Ş. Eğitim Kitapları Serisi:2, Üner Yayıncılık, Evren Ofset A.Ş., Ankara. (in Turkish).

Fabani, M. P., Baroni, M. V., Luna, L., Lingua, M. S., Monferran, M. V., Panos, H., ... & Feresin, G. E. (2017). Changes in the phenolic profile of Argentinean fresh grapes during production of sun-dried raisins. Journal of Food Composition and Analysis, 58, 23-32. [CrossRef]

Guler, A., Candemir, A., Ozaltin, K. E., Asiklar, F. B., & Saygac, S. (2022). Determination of Biochemical Characteristics, Antioxidant Activities, and Individual Phenolic Compounds of 13 Native Turkish Grape Juices. Erwerbs-Obstbau, 1-11. [CrossRef]

Haytowitz, D. B., Wu, X., & Bhagwat, S. (2018). USDA Database for the Flavonoid Content of Selected Foods, Release 3.3. US Department of Agriculture, 173. [Google Scholar]

Karadeniz, F., Durst, R. W., & Wrolstad, R. E. (2000). Polyphenolic composition of raisins. Journal of Agricultural and Food Chemistry, 48(11), 5343-5350. [CrossRef]

Kelebek, H., Jourdes, M., Selli, S., & Teissedre, P. L. (2013). Comparative evaluation of the phenolic content and antioxidant capacity of sun-dried raisins. Journal of the Science of Food and Agriculture, 93(12), 2963-2972. [CrossRef]

Meng, J., Fang, Y., Zhang, A., Chen, S., Xu, T., Ren, Z., ... & Wang, H. (2011). Phenolic content and antioxidant capacity of Chinese raisins produced in Xinjiang Province. Food Research International, 44(9), 2830-2836. [CrossRef]

Özkan, G, Göktürk Baydar N. (2006). A direct RP-HPLC

determination of phenolic compounds in Turkish red wines. Mediterr Agric Sci 19:229–234. [Google Scholar]

Parker, T. L., Wang, X. H., Pazmiño, J., & Engeseth, N. J. (2007). Antioxidant capacity and phenolic content of grapes, sun-dried raisins, and golden raisins and their effect on ex vivo serum antioxidant capacity. Journal of agricultural and food chemistry, 55(21), 8472-8477. [CrossRef]

Price, S. F., Breen, P. J., Valladao, M., & Watson, B. T. (1995). Cluster sun exposure and quercetin in Pinot noir grapes and wine. American Journal of Enology and Viticulture, 46(2), 187-194. [Google Scholar]

Rice Evans, C.A., Miller, N.J., Paganga, G., 1997. Antioxidant properties of phenolic compounds. Trends Plant Sci. 4, 152–159. [CrossRef]

Roychev, V., Tzanova, M., Keranova, N., & Peeva, P. (2020). Antioxidant content and antioxidant activity in raisins from seedless hybrid vine varieties with coloured grape juice. Czech Journal of Food Sciences, 38(6), 410-416. [CrossRef]

Schuster, M. J., Wang, X., Hawkins, T., & Painter, J. E. (2017). A Comprehensive review of raisins and raisin components and their relationship to human health. Journal of Nutrition and Health, 50(3), 203-216. [CrossRef]

Soleas, G. J., Dam, J., Carey, M., & Goldberg, D. M. (1997). Toward the fingerprinting of wines: cultivar-related patterns of polyphenolic constituents in Ontario wines. Journal of Agricultural and Food Chemistry, 45(10), 3871-3880. [CrossRef]

TMO, (2021). 2020 Yılı Kuru Üzüm Sektör Raporu. Toprak Mahsülleri Ofisi Genel Müdürlüğü. (in Turkish). [URL]

Williamson, G., & Carughi, A. (2010). Polyphenol content and health benefits of raisins. Nutrition Research, 30(8), 511-519. [CrossRef]

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 683-693
DOI: https://doi.org/10.31015/jaefs.2022.4.24

# Effects of Cucurbita, Lagenaria and Citrullus rootstocks on pollen and fruit characters, seed yield and quality of $F_1$ hybrid watermelon

**Veysel Aras**[1] • **Nebahat Sarı**[2] • **İlknur Solmaz**[3]

[1] Alata Horticultural Research Institute, Mersin, Türkiye
[2] Çukurova University, Faculty of Agriculture, Department of Horticulture, Adana, Türkiye (Retired)
[3] Çukurova University, Faculty of Agriculture, Department of Horticulture, Adana, Türkiye

**Citation:** Aras, V., Sai, N., Solmaz, I. (2022). Effects of Cucurbita, Lagenaria and Citrullus rootstocks on pollen and fruit characters, seed yield and quality of $F_1$ hybrid watermelon. International Journal of Agriculture, Environment and Food Sciences, 6 (4), 683-693

**Received:** 30 October 2022
**Revised:** 25 November 2022
**Accepted:** 28 November 2022
**Published Online:** 22 December 2022

**Correspondence:** Veysel Aras
**E-mail:** varas2001@yahoo.com



## Abstract

In the study, two commercial *Cucurbita maxima*×*Cucurbita moschata* hybrid (Nun9075 and TZ148), *Lagenaria* spp. hybrid (Argentario) and one local *Lagenaria* spp. genotype (3335) and one *Citrullus amarus* genotype (PI 296341) were used as rootstocks. The male and female parents of two watermelon hybrids (187×125 and 11×162) were used as scions. Ungrafted plants formed the control group and also each parent was grafted on itself. Male parents were used for pollen measurements. Fruit weight, fruit height, fruit diameter, fruit rind thickness, total soluble solid content, total number of seeds, total seed weight and 1000 seeds' weight and seed vigor tests were carried out in fruits of female parents crossed with male parents grafted onto the same rootstock. According to results of this study, the highest pollen production was obtained from Argentario while in terms of pollen germination and pollen viability there was no difference between applications in general. It was determined that grafting on Argentario and 3335 rootstocks increased fruit height, fruit diameter, fruit rind thickness, and the amount of total soluble solid, and the best performing rootstocks in terms of fruit weight were found to be Argentario, 3335, TZ148 and NUN9075. The highest seed number, seed weight and 1000 seeds weight were obtained from those grafted on TZ148 and NUN9075 rootstocks. Although variable results were obtained in terms of early germination, there was no rootstock that came to the fore in general. There was no difference between the rootstocks regarding accelerated aging test and controlled deterioration test.

**Keywords:** Watermelon, Grafting, Pollen Tests, Seed Yield And Quality

## INTRODUCTION

Waterrmelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) is one of the most economically important species of the Cucurbitaceae family. The world watermelon production is about 101.620.420 million tonnes and Turkey ranks as 2nd most producer country after China (Faostat 2020). Grafting has been used on fruit trees and vines for thousands of years (Kombo & Sari 2019). Grafting in vegetables began in the late 1920s, first by grafting watermelon on pumpkin (*Cucurbita moschata*) and later on bottle gourd (*Lagenaria siceraria*) in Korea and Japan (Lee 1994). Grafting was originally adopted in China, Japan and Korea, where land use was intensive and crop rotation was difficult. Subsequently, grafting became widespread in countries such as Australia (Tran-Nguyen et al. 2012). It became widespread in western countries in the early 1990s and is now widely used around the world. The usage of grafted seedlings in watermelon has expand-

ed fast due to their resistance to Fusarium wilt, earliness, yield, fruit quality, and their tolerance to biotic and abiotic stress conditions. *C. maxima x C. moschata* interspecific hybrids and *Lagenaria siceraria* hybrids are the most extensively employed commercial rootstocks for watermelon (Qin et al. 2014; Edelstein et al. 2017; Fallik et al. 2019). Splice grafting of watermelon, in which both cotyledons are excised from the rootstock, has recently advanced to achieve all these goals (Devi et al. 2020).

Grafting is a process that first includes the selection of the appropriate rootstock and scion, then combining the rootstock and scion with the appropriate grafting method, and acclimatizing the grafted plant until it heals. Grafting watermelon (*Citrullus lanatus*) onto resistant rootstocks is an efficient biotic and abiotic stress control approach (Devi et al. 2021). Since the first report of watermelon grafting for resistance to disease in 1927, adoption has progressively increased in Greece, Japan, Israel, Korea, and Turkey reaching 95% (Devi et al. 2020).

The use of rootstocks in watermelon can rapidly transfer nutrients from the soil to the pen, avoid soil diseases and increase plant tolerance in soil salinity, low soil temperatures and moist soil conditions (Salam et al. 2002; Claudio et al. 2019; Tripodi et al. 2019). The type of rootstock affects plant growth, yield and fruit quality (Yetişir et al. 2003; El-Kersh et al. 2016; Fallik et al. 2016; Fredes et al. 2016; Huang et al. 2016; Maršić et al. 2016; Özdemir et al. 2016; Zaaroor et al. 2016; Liu et al. 2017; Ceylan et al. 2018; Gaion et al. 2018; Zhong et al. 2018; Meng et al. 2019; Siamak & Paolo 2019; Zhang et al. 2019) and also seed yield and qualities (Kombo & Sari 2019; Hussein & Sari 2020).

According to our knowledge so far, no study has been found on the effect of rootstocks on hybrid watermelon seed production. In this study, the effects of Cucurbita, Lagenaria and Citrullus rootstocks on the seed yield and quality of two hybrid watermelons were tried to be determined. In addition, the effects of grafting on pollen productivity in male parents and fruit characters in female parents were also investigated.

## MATERIALS AND METHODS

This study was carried out at the greenhouses and laboratories of the Alata Horticultural Research Institute, Mersin-Turkey during the spring/summer seasons of 2019 and 2020.

### Plant Materials

Nun9075 (Nunhems Seed Company, Holland) and TZ148 (Syngenta seed Company, Holland) from commercial rootstocks of *C. maxima*×*C. moschata* group; Argentario (HM Clause Seed Company, France) from commercial rootstock and 3335 from Turkish landraces of *Lagenaria* spp. group; PI 296341 from *Citrullus amarus* were used as rootstocks. Nun9075 and TZ148 are hybrid rootstocks widely grown in Türkiye. Gourd group rootstocks are not widely used in Türkiye lately. The hybrid Argentario, which was used in Turkey for a period, was selected from the gourd group. In addition, from the gourd group, Yetişir et al. (2007) and Karaca et al. (2012) developed 3335 lines were used. PI296341 is from the *Citrullus amarus* group and is a genotype resistant to races 0, 1, 2 of fusarium in watermelon. Each parent was also self-grafted. 187×125 and 11×162 hybrids developed by Alata Horticultural Research Institute, Türkiye were used as scions. The 187×125 hybrid is a hybrid with a striped rind pattern and a fruit weight of 8-9 kg. The 11×162 hybrid is a hybrid with a dark green rind pattern and a weight of 9-10 kg. Both watermelon hybrids were transferred to a private sector company. Two hybrids' parents were grafted onto each rootstock. Non-grafted watermelon plants of parents were considered as controls. The experimental design was the factorial experiment in randomized complete block design with three replicates with thirty plants in each replicate.

### Seed sowing, grafting, and transplanting

In this study, seed sowing for all scions and rootstocks was started on January, 3rd, 2019 for the first year of the experiment and on December, 30th 2019 for the second year (2020). The grafting was performed with the splice/one cotyledon grafting method in Antalya Seedling Company.

Inoculation is done when the rootstock, cotyledons and the first true leaf begin to develop. A cotyledon and growth tip is cut. The seedling is cut obliquely from the base of one cotyledon to 0.8-1.0 cm below the other cotyledon; one cotyledon and its growth tip are removed. The length of the cut on the hypocotyl of the pen should be the same as that of the rootstock and should be at an angle of 35° to 45° (Davis et al., 2008; Bie et al., 2017). The pen is attached to the rootstock and fixed with the grafting clip. The grafted plants were kept in a dark environment at 25 °C and 100% humidity for three days before being moved to a greenhouse maintained at 21 °C to 30 °C or until the joint had healed. All seedlings were planted as follow: for 2019, seedlings were planted on 22 March 2019 at 36°37'47.91"N latitude, 34°20'38"E longitude, and 4 m above sea level; for the year 2020, on 06 March 2020, at 36°37'50.73"N latitude, 34°20'43.68"E longitude and 4 m above sea level, different E.P.E. covered greenhouses in Alata Horticultural Research Institute with a 1.5 m between and 0.4 m within plant distances. Plants were grown in a single stem by hanging them on a rope and removing their secondary axes.

Climatic data were taken in both years by hobo devices placed in greenhouses. Monthly minimum, maximum and average temperature and relative humidity values are given in Table 1. In 2019, the highest temperatures were seen in April (51.19 °C) and May (50.01 °C), after which shading dust was applied on the plastic greenhouse. In terms of relative humidity, the highest value was taken in June (100.00%) and the lowest value was

taken in March (91.36%). In 2020, the highest (46.4 °C) and the lowest (4.2 °C) temperatures were seen in March. In terms of relative humidity, the highest (97.5%) relative humidity was recorded in March, and the lowest (23.1%) relative humidity was recorded in May (Table 1).

The irrigation and fertilization were performed with a drip irrigation system. The irrigation system started with the planting seedlings of all accessions used in this study and were given when necessary depending on the climate observed in the greenhouse. The analysis of soil of used greenhouses was carried out during the experiment. The first year of the study (2019) was sandy, calcareous, slightly salty, weak inorganic substances, slightly alkali, and sufficient in phosphorus and potassium; and in another hand for the second year (2020) was loamy, very calcareous, normal in salinity, weak inorganic substances, alkali, poor in potassium sufficient for phosphorus quantity (Table 2). According to the results of soil analysis, fertilizers were given as pure substance as 140-160 kg N/ha, 80-100 kg $P_2O_5$/ha, 60-80 kg $K_2O$/ha (Güçdemir 2012). Fertilizers were given by drip irrigation. Nitrogen, phosphorus and potassium are given by dividing the watermelon into three according to the three growing stages of the watermelon. The first stage is the period until the first female flower. The second period is the period from the stage when the first female flower is seen until the fruits reach the size of an apple. The third stage is the period from when the fruits reach the size of an apple until harvest. Pesticides were applied for observed diseases and pests regularly, and weeding and pruning were mechanically performed.

Crossing started on 15 April, in 2019 and on 06 April in 2020, and was completed on 17 May in 2019 and on 15 May in 2020. The hybridizations were carried out by closing the male and female flowers with clips one day before the anthesis and crossing female flowers by the males, in the next morning. 187 female parent with 125 male parent and 11 female parent with 162 male parent were pollinated. Three male flowers were used in each pollination. Female parents and male parents grafted on the same rootstocks were crossed. One fruit was set on each plant.

To determine the effect of grafting on pollen viability, germination and amount, male flowers at anthesis stage were picked from each replication and brought to the laboratory. Nine male flowers were used for each replication. For pollen viability TTC test (Eti 1991; Gök et al. 2007; Kombo & Sari 2019), for pollen germination agar in petri dishes test (Gök et al. 2007), and for the amount of pollen per flower hemacytometer test (Eti 1990) were performed.

Fruit harvest was carried out on 18 July in 2019 and on 08 July in 2020. Then, fruit weight, fruit height, fruit diame-

**Table 1.** Climate values in greenhouses during the trial months in 2019 and 2020

| Year | Climate Factor | | March | April | May | June | July |
|---|---|---|---|---|---|---|---|
| 2019 | Temperature (°C) | Minimum | 10.42 | 7.40 | 10.94 | 15.11 | 16.63 |
| | | Maximum | 45.17 | 51.19 | 50.01 | 47.07 | 48.82 |
| | | Average | 22.91 | 23.01 | 27.00 | 29.23 | 32.39 |
| | Relative humidity (%) | Minimum | 33.74 | 25.86 | 22.71 | 34.21 | 29.45 |
| | | Maximum | 91.36 | 95.87 | 96.81 | 100.00 | 92.60 |
| | | Average | 70.87 | 72.35 | 72.21 | 76.83 | 63.57 |
| 2020 | Temperature (°C) | Minimum | 4.2 | 7.5 | 10.5 | 12.9 | 22.3 |
| | | Maximum | 46.4 | 33.4 | 44.6 | 41.2 | 40.5 |
| | | Average | 19.9 | 19.4 | 23.9 | 26.4 | 29.9 |
| | Relative humidity (%) | Minimum | 25.5 | 24.0 | 23.1 | 33.3 | 41.9 |
| | | Maximum | 97.5 | 72.4 | 97.2 | 97.0 | 89.6 |
| | | Average | 71.4 | 72.4 | 67.0 | 71.0 | 70.8 |

**Table 2.** Soil analysis of used greenhouses

| Analyzes | Limit Values | Analysis Results (0-30 cm) | |
|---|---|---|---|
| | | 2019 | 2020 |
| Texture (100 g/ml) | 30-50 | 28.00 (sandy) | 34.00 (loamy) |
| Total Calcitic ($CaCO_3$ %) | 5-15 | 24.00 (calcareous) | 27.20 (high calcareou) |
| Salinity E.C. ds/m (25 °C) | 0-0.8 | 1.55 (slightly salty) | 0.85 (optimum) |
| Organic matter (%) | 3-4 | 2.76 (defficient) | 2.20 (defficient) |
| pH 1: 2,5 | 6.0-7.0 | 7.72 (slightly alkaline) | 7.66 (alkaline) |
| Available potassium (mg/kg) | 244-300 | 250.60 (sufficient) | 54.70 (very low) |
| Receivable phosphorus (mg/kg) | 20-40 | 25.10 (sufficient) | 34.10 (optimum) |

ter, fruit rind thickness, and total soluble solid content of grafted and ungrafted female parents (187 and 11) were determined. Fruit measurements were made on 5 fruits for each application. Seeds were manually extracted, and thoroughly washed and put on fine wire mesh to dry.

Total number of seeds, total seed weight and 1000 seeds weight, germination at 52nd, 60th and 68th hours after sowing on germination paper, total germination rate in accelerated aging test and total germination rate in controlled deterioration test were determined for grafted and ungrafted female parental lines (187 and 11) crossed by male parents (125 and 162). The standard germination test (International Seed Testing Association, 2018) was conducted using 400 seeds (100 seeds per replication) for each treatment for germination at 52nd, 60th and 68th hour after seed sowing, which were incubated at 25°C for 14 days on moisturized paper towels. Hundred seeds (25 seeds per replication) for each treatment were used for germination, accelerated aging test and controlled deterioration test. For the early germination (early count) test, the seeds of the controls and all hybrids were germinated in the dark using the paper method. Germinated seeds at the 52nd, 60th and 68th hours were counted and rates were determined (Mavi and Demir, 2010; Ermis et al. 2022). Accelerated aging test, aging pots consisting of two parts were used. The aging pot consists of an outer box of 15 × 9 × 6.5 cm and an inner chamber made of 15 × 7 × 7 cm sieve wire placed inside it. In order to provide a high relative humidity, 40 mL of pure water was placed in the outer box. The seeds were laid on the sieve wire in a single layer and placed inside the sieve wire outer box. Before all hybrid seeds were placed on the sieves. The seeds of each hybrid placed on the sieves in the aging boxes were kept at 45 °C for 144 hours ("Memmert 854 Schwabach W-Germany" trademark incubator) (Bhering et al. 2004). Then standard germination trials were established. Seed moisture was initially increased to 24% for controlled deterioration test. In order to ensure the moisture balance of the seeds whose seed moisture was increased, the seeds were kept at 5 °C for 3 days. Then it was packed hermetically by putting it in airtight packages with aluminum mixture. For the controlled deterioration test of seeds, 45 °C temperature and 48 hours aging times were used. At the end of the specified period, the seeds were taken out of their packages and standard germination tests were established (Bhering et al. 2004).

### Statistical analysis

Statistical analyses were performed using the Tukey Test in the JMP 7.0 statistics software (v7.00, SAS Institute Inc., NC 27513-2414, USA) at a significance level of P≤0.05. Statistical analyses were performed after applying angle transformation to percentage values.

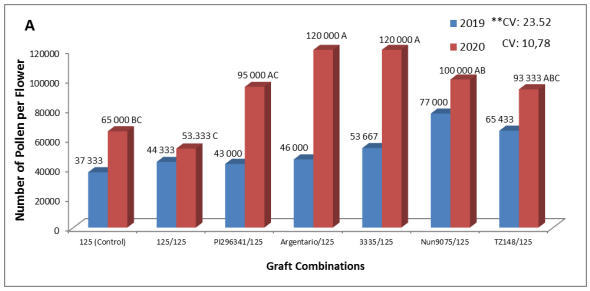
## RESULTS AND DISCUSSION

### Pollen development

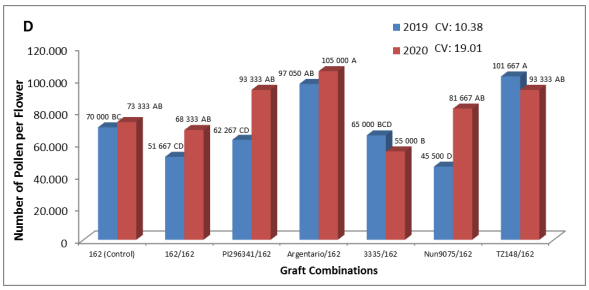
Pollen viability, pollen germination, and pollen production per male flower in male parents were examined (Figure 1). The results showed that there were no statistically significant differences in male line 125 combinations in 2019, while the highest values were obtained in Argentario and 3335 rootstocks in 2020.TZ148 had the highest value in male line 162 in 2019, while Argentario gave the highest value in 2020. In terms of pollen germination, there was no statistically significant difference between 125 and 162 male lines combinations in 2019. In 2020, the highest results were obtained from the 125/125 combination for the 125 male line and the Argentario/162 combination for the 162 male line. In terms of pollen viability, there was no statistically significant difference between the combinations in 125 male line in both years and in 162 male line in 2020. However, in 2019, the highest values in 162 male lines were obtained in combinations of 162 (control) and Nun9075/162. The reason for the high difference between the data of the two years is that it was made in different greenhouses and different soil conditions every year. The results obtained in terms of the number of pollen per flower were found to be higher than the results obtained by Stanghellini & Schultheis (2005), their average is 32 000. Regarding pollen germination results, Gök et al. (2007) had similar results, their results being 70% on average. Pollen viability results per flower are higher than those of McGregor & Waters (2013), their average being 80%, but Gök et al. (2007) and Freeman et al. (2008) got similar results comparing our study (average 90% and 97%).

### Fruit development

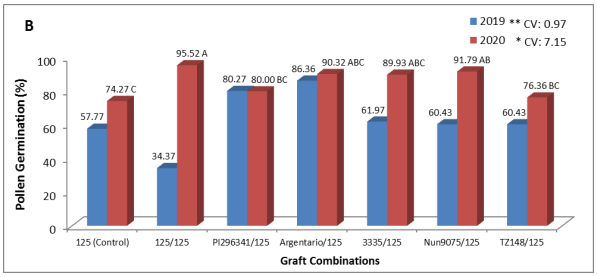
Fruit yield and quality results are presented in Figure 2 for the 2019 and 2020 growing seasons. Regarding fruit weight, Argentario and Nun9075 rootstocks came to the fore in both hybrid female parents and in both years. 3335 and Argentario rootstock came to the fore in terms of fruit height, fruit diameter, fruit rind thickness and total soluble solid content. Since the roots of *Cucurbita maxima*×*Cucurbita moschata* and gourd (*Lagenaria* spp.) rootstocks are much stronger than ungrafted watermelons, more brix accumulates and watermelon fruit weights and fruit shell thicknesses increase. The reason for the high difference between the data of the two years is that it was made in different greenhouses and different soil conditions every year. The difference in fruit weight between the data of the two years is high, because the research was carried out in different greenhouses and different soil conditions every year. Compatible with our study, Turhan et al. (2012), Abd Alla et al. (2012), Öztekin et al. (2012), Petropoulos et al. (2012), Özmen et al. (2015), El-Kersh et al. (2016), Maršić et al. (2016), Hussein & Sari (2020), Ozbahce et al. (2021a), Ozbahce et al. (2021b), Ulas (2021) and Suárez-Hernández et al. (2022) reported



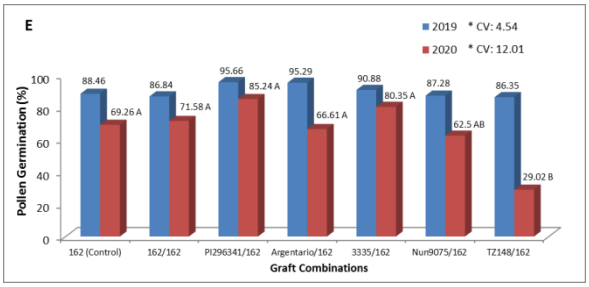

Prob>f (2019): 0.3868, Prob>f (2020): 0.0015



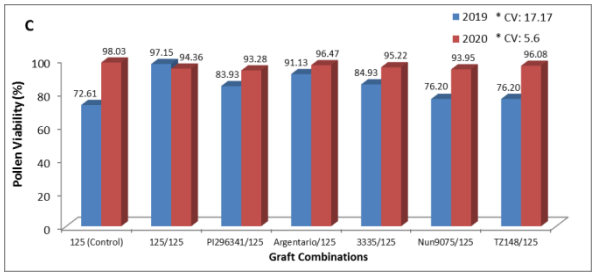

Prob>f (2019): <.0001, Prob>f (2020): 0.0256



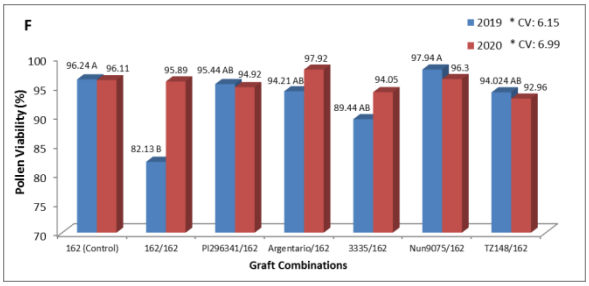

Prob>f (2019): 0,6174, Prob>f (2020): 0.0013




Prob>f (2019): 0.0196, Prob>f (2020): 0.0051




Prob>f (2019): 0.3984, Prob>f (2020): 0.3984




Prob>f (2019): 0.0237, Prob>f (2020): 0.4342

**Figure 1.** Effect of rootstocks on (**A)** number of pollen per flower of 125, (**B)** pollen germination of 125, (**C)** pollen viability of 125, (**D)** number of pollen per male flower of 162, (**E)** pollen germination of 162, (**F)** pollen viability of male parent 162 in watermelon hybrids. *Angle transformation was applied to the percent values, **Square root transformation was applied. No letters on bars means that there is no significant difference between graft combinations

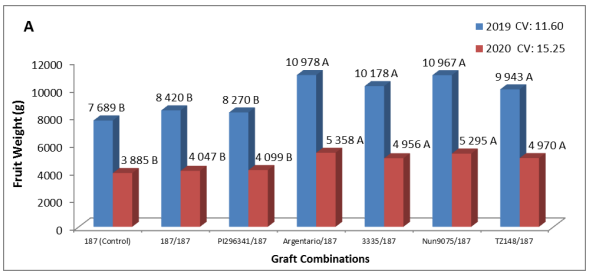
that grafted watermelons result in higher yield and quality. Eventough some studies (El-Sayed et al. 2015; Zhong et al. 2018) revealed that grafting had a negative impact on the TTS content it was also, reported that there was no difference in the content of TTS by Mohamed et al. (2012), Liu et al. (2017), Meng et al. (2019).

## Seed yield

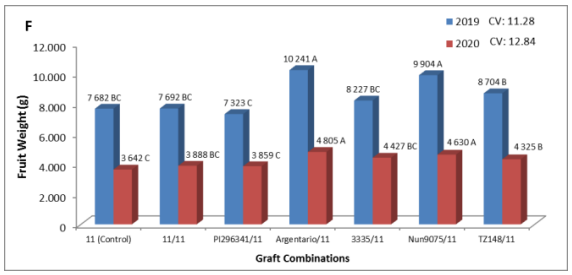
There was no statistically significant difference between the combinations in terms of total number of seeds, total seed weight and 1000 seeds weight of female line 11 in 2019. However, TZ148 and Nun9075 rootstocks stand out among the combinations in terms of total number of seed, total seed weight and 1000 seeds weight of female line 187 in both years and female line 11 in 2019 (Figure 3). TZ148 and Nun9075 rootstocks are very strong rootstocks, their roots can go very deep and the root grafted on them is minimally affected by abiotic conditions. As a result, the scion is well fed and produces healthy male and female flowers. More pollen is produced in male flowers formed by male parents grafted on these rootstocks than in ungrafted ones. As a result of these, it is not a coincidence that the seed yields of the scions grafted on TZ148 and Nun9075 rootstocks are high seed yield. Grafting was found to be important regarding to seed yield, and Abd Alla et al. (2012), Öztekin et al., (2012), Yetişir & Sari (2018), Kombo & Sari (2019), Hussein & Sari (2020) and stated that grafting increased seed yield, but Claudio et al. (2019) reported that grafting did not make any difference on seed yield. Ulas (2021), in her study on eggplant, reported that Pala grafted on Köksal $F_1$ rootstock had a 72.03% increase in total seed yield compared to ungrafted plants.

## Seed quality

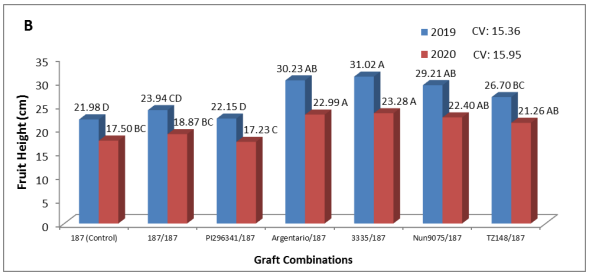
There was no statistically significant difference between the combinations in both years and in both lines for total germination at 52$^{nd}$ hour. Regarding total germination at the 60$^{th}$ hour, there was no difference in the combinations of 187 lines for 2019, while 3335 (11.3%) rootstocks stood out in 2020; while TZ148 (14.1%) stood out in the combinations of 11 lines in 2019, no statistical difference was found between the combinations in 2020. In terms of total germination at the 68$^{th}$ hour, there was no differ-



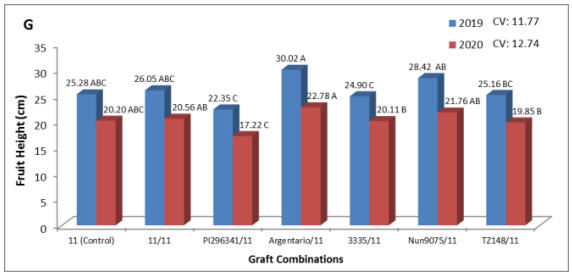

Prob>f (2019): <.0001, Prob>f (2020): <.0001



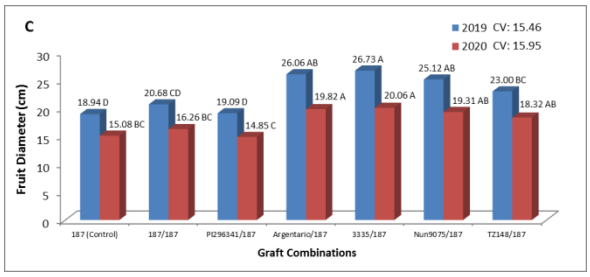

Prob>f (2019): <.0001, Prob>f (2020): <.0001



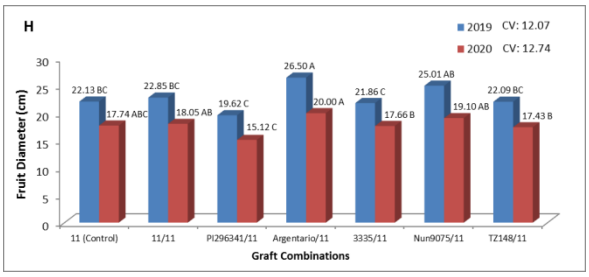

Prob>f (2019): <.0001, Prob>f (2020): <.0001



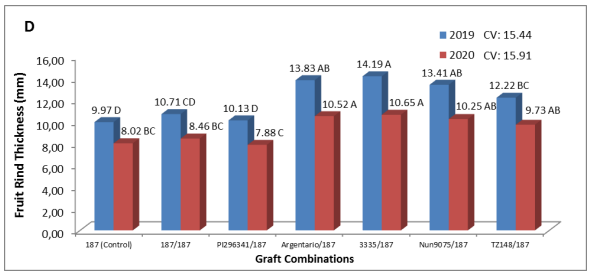

Prob>f (2019): <.0001, Prob>f (2020): <.0001



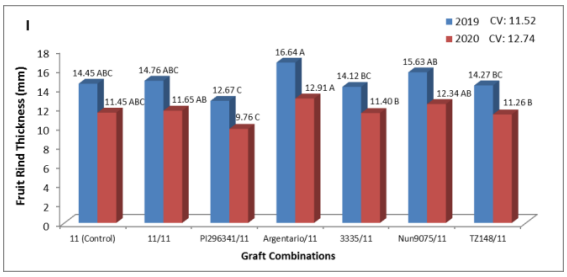

Prob>f (2019): <.0001, Prob>f (2020): <.0001



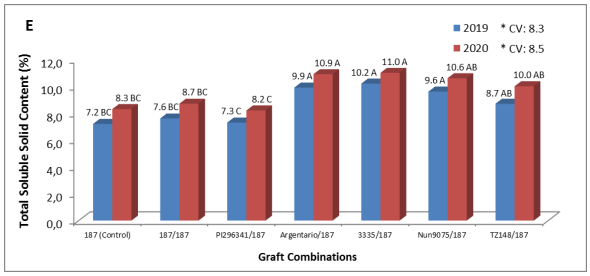

Prob>f (2019): <.0001, Prob>f (2020): <.0001



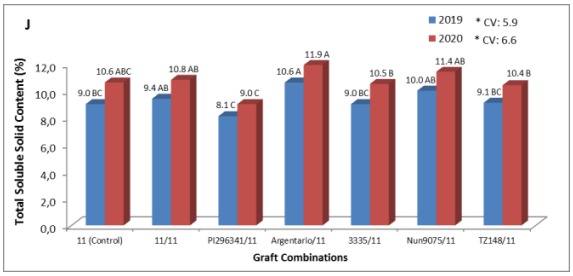

Prob>f (2019): <.0001, Prob>f (2020): <.0001




Prob>f (2019): <.0001, Prob>f (2020): <.0001

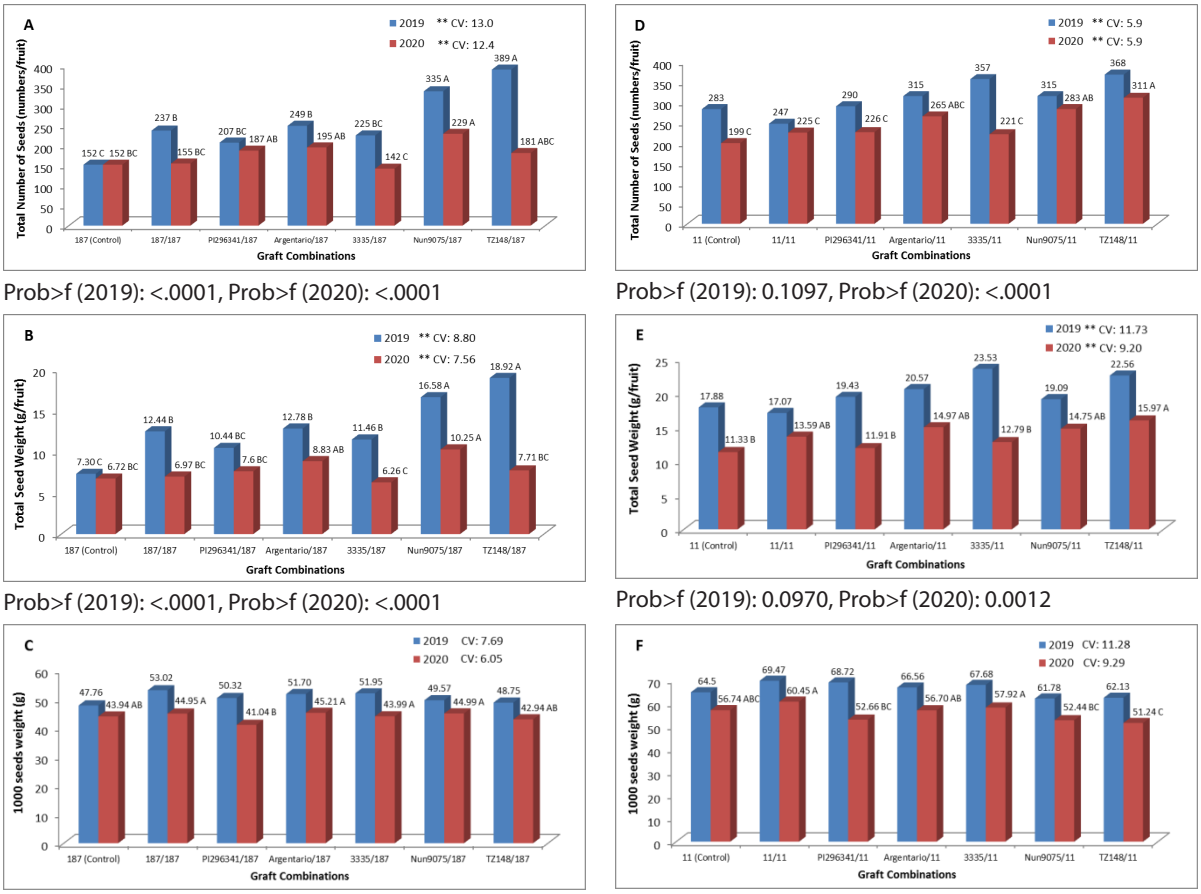



Prob>f (2019): <.0001, Prob>f (2020): <.0001




Prob>f (2019): <.0001, Prob>f (2020): <.0001

**Figure 2.** Effect of rootstocks on (**A**) fruit weight of 187, (**B**) fruit height of 187, (**C**) fruit diameter of 187, (**D**) fruit rind thickness of 187, (**E**) total soluble solid content of 187, (**F**) fruit weight of 11, (**G**) fruit height of 11, (**H**) fruit diameter of 187, (**I**) fruit rind thickness of 11, (**J**) total soluble solid content of 11 female parents in watermelon hybrids. *Angle transformation was applied to the percent values. No letters on bars means that there is no significant difference between graft combinations




Prob>f (2019): <.0001, Prob>f (2020): <.0001

Prob>f (2019): 0.1097, Prob>f (2020): <.0001

Prob>f (2019): <.0001, Prob>f (2020): <.0001

Prob>f (2019): 0.0970, Prob>f (2020): 0.0012

Prob>f (2019): 0.2836, Prob>f (2020): <.0001

Prob>f (2019): 0.0606, Prob>f (2020): <.0001

**Figure 3.** Effect of rootstocks on (**A**) total number of seeds of 187, (**B**) total seeds weight of 187, (**C**) 1000 seeds weight of 187, (**D**) total number of seeds of 11, (**E**) total seeds weight of 11, (**F**) 1000 seeds weight of female parent 11 in watermelon hybrids. *Angle transformation was applied to the percent values, **Square root transformation was applied. No letters on bars means that there is no significant difference between graft combinations

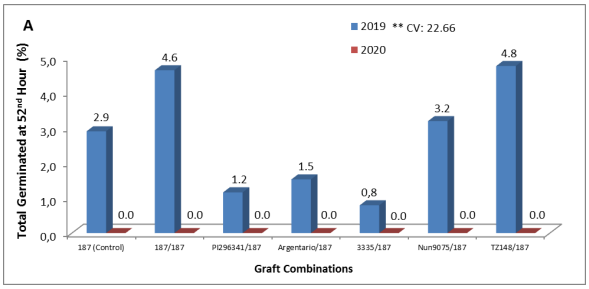
ence between the applications in both lines in 2019, but TZ148 and PI296341 rootstocks stand out in general in 2020 (Figure 4). Seed viability and quality of watermelon seeds obtained from ungrafted plants are very high in the first years. For this reason, the results obtained from both grafted and ungrafted watermelons were almost close to each other. The grafted plants feed the fruits better and as a result, better quality seeds will be obtained. In such studies to be carried out in the future, the difference between the preservation of seeds in cold storage and the quality of seeds obtained from ungrafted and grafted plants will be revealed more clearly.

There was no significant difference between grafted combinations for total germinated at accelerated aging test and total germinated at controlled deterioration test in both years and in both lines overall (Figure 5).

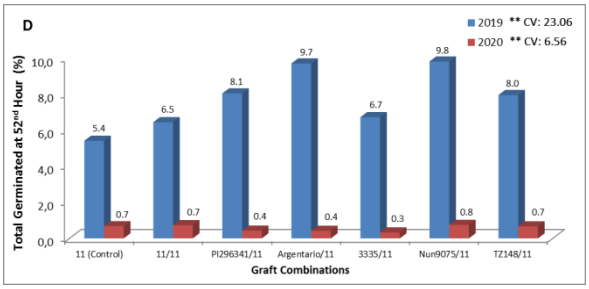
The rapid aging test shows that it is suitable for assessing the physiological quality of watermelon seeds and thus becomes a promising test for the evaluation of vigor expression (Mavi & Demir 2010; Mavi, 2011; Radke et al. 2017). Bhering et al. (2003) and Mavi (2011) found differences between applications in accelerated aging tests in their studies on watermelon.

## CONCLUSION

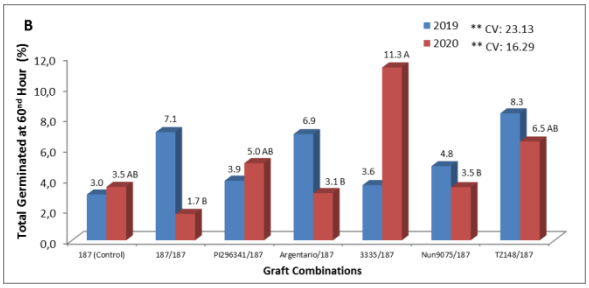
According to the results of this study, the best results in terms of pollen production was obtained from Argentario rootstock, however regarding pollen germination and pollen viability, there was no difference between rootstocks in general. It was determined that grafting on Argentario and 3335 rootstocks increased fruit height, fruit diameter, fruit rind thickness, and the amount of total soluble solid, and the best performing rootstocks in terms of fruit weight were Argentario, 3335, TZ148 and NUN9075. The highest values for seed number, seed weight and 1000 seeds weight were obtained from those grafted onto TZ148 and NUN9075 rootstocks. Although different results were obtained in terms of early germination, there was no rootstock that came to the fore in general. No difference was found between the applications in terms of accelerated aging test and controlled deterioration test. According to the results of this study, Argentario, NUN-9075 and TZ148 rootstocks performed



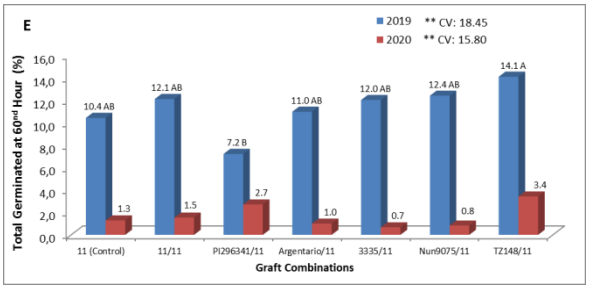

Prob>f (2019): 0.0902, Prob>f (2020):



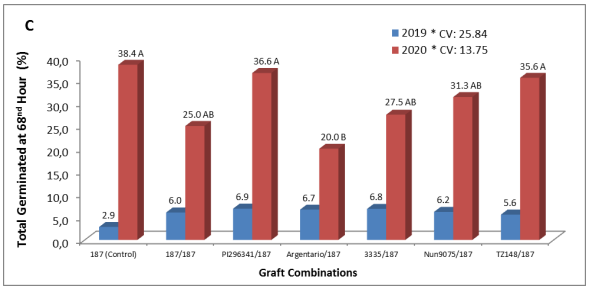

Prob>f (2019): 0.7633, Prob>f (2020): 0.7496



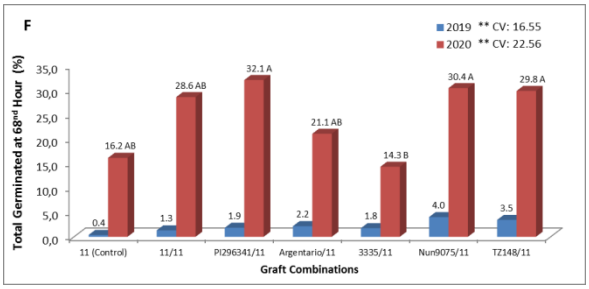

Prob>f (2019): 0.3258, Prob>f (2020): 0.0017




Prob>f (2019): 0.1061, Prob>f (2020): 0.0334

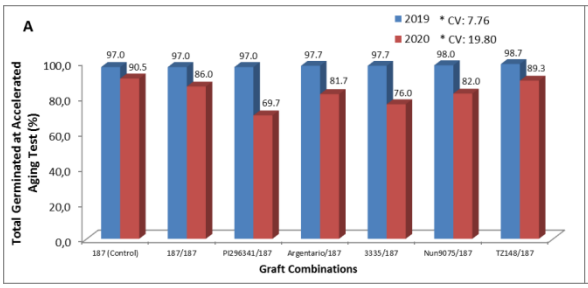



Prob>f (2019): 0.6881, Prob>f (2020): 0.0019

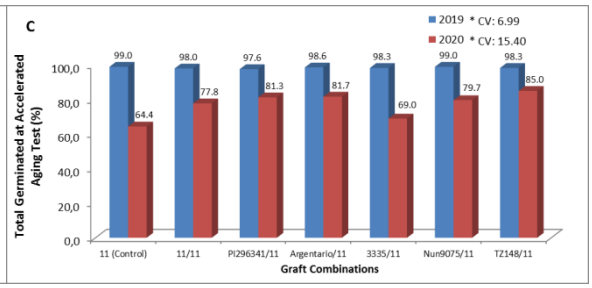



Prob>f (2019): 0.3220, Prob>f (2020): 0.0061

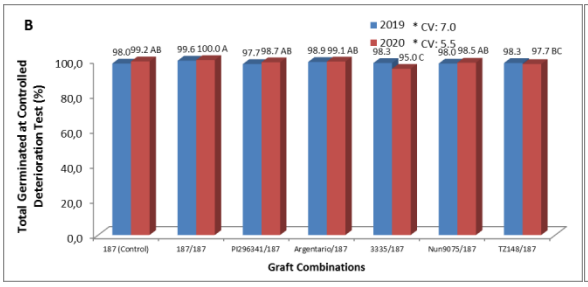
**Figure 4.** Effect of rootstocks on (**A**) total germinated at 52nd hour of 187, (**B**) total germinated at 60th hour of 187, (**C**) total germinated at 68th hour of 187, (**D**) total germinated at 52nd hour of 11, (**E**) total germinated at 60th hour of 11, (**F**) total germinated at 68th hour of 11 female parents in watermelon hybrids. *Angle transformation was applied to the percent values, **Square root transformation was applied. No letters on bars means that there is no significant difference between graft combinations



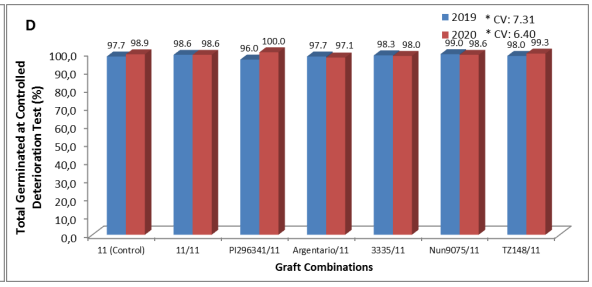

Prob>f (2019): 0.8433, Prob>f (2020): 0.1171




Prob>f (2019): 0.7970, Prob>f (2020): 0.0389




Prob>f (2019): 0.6838, Prob>f (2020): <.0001




Prob>f (2019): 0.1797, Prob>f (2020): 0.0960

**Figure 5.** Effect of rootstocks on (**A**) total germinated at accelerated aging test of 187, (**B**) total germinated at controlled deterioration test of 187, (**C**) total germinated at accelerated aging test of 11, (**D**) total germinated at controlled deterioration test of 11 female parents in watermelon hybrids. *Angle transformation was applied to the percent values. No letters on bars means that there is no significant difference between graft combinations

better than the other rootstocks and are therefore recommended as the best rootstock. In addition to the results we have obtained, we recommend the use of rootstock in $F_1$ hybrid watermelon seed production to companies due to the biotic and abiotic resistance characteristics of rootstocks.

## COMPLIANCE WITH ETHICAL STANDARDS

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Ethics committee approval is not required.

### Funding

This study was financially supported by Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies (Project No. TAGEM/BBAD/A/19/A1/P1/1545) of Türkiye.

### Data availability

Not applicable.

### Consent for publication

Not applicable.

### Acknowledgements

This study was a part of a PhD Thesis of Veysel Aras (Çukurova University, Department of Horticulture, Türkiye). The authors are thanks to the Ministry of Agriculture and Forestry, General Directorate of Agricultural Research and Policies (Project No. TAGEM/BBAD/A/19/A1/P1/1545) of Türkiye for supporting this study. And, we also like to thank Antalya Seedling Company for providing us grafted and ungrafted seedlings on different rootstocks.

## REFERENCES

Abd Alla, M.A., Mohsen, A.A. & Tanbashawy, H.A. (2012). Effect of grafting nubian watermelon on its growth, flowering, yield and fruit quality. Proc. 7th Int. Con. Biol. Sci. (Bot.), pp. 7-13. [Google Scholar]

Bhering, M.C., Fernandes, S., Dias, D.C., Barros, D. I., Santos Dias, L.U. & Tokuhisa, D. (2003). Vigor evaluation of watermelon seeds by accelerated aging test. revista brasileira de sementes, 25(2): 1-6. [Google Scholar]

Bhering, M.C., Dias, D.C.F.S, Tokuhisa, D. & Dias, L.A.D.S. (2004). Vigor evaluation of melon seeds by controlled deterioration test. Revista Brasileira de Sementes, 26(1): 125-129. [Google Scholar]

Bie, Z., Nawaz, M.A., Huang, Y., Lee, J.M., Colla, G. (2017). Introduction of vegetable grafting. In: Vegetable Grafting, Principles and Practices. Colla, G., F.P. Alfocea and D. Schwarz (Editors.). CABI Publishing, UK.

Ceylan, Ş., Alan, Ö. & Elmacı, Ö.L. (2018). Effects of grafting on nutrient element content and yield in watermelon. Ege Üniv. Ziraat Fak. Derg., 55 (1): 67-74. [Google Scholar]

Claudio, M.T.R., Nakada, P.G., Tavares, A.E.B., Magro, F.O. & Cardoso, A.I.I. (2019). Seed production and quality in grafted cucumber. International Journal of Vegetable Science, 1-6. [CrossRef]

Davis, A.R., Perkins-Veazie, P., Hassell, R., Levi, A., King, S.R. & Zhang, X. (2008). Grafting effects on vegetable quality. HortScience, 43(6), 1670–1672. [CrossRef]

Devi, P., Lukas, S. & Miles, C. (2020). Advances in watermelon grafting to increase efficiency and automation. Horticulturae, 6(4), 88. [CrossRef]

Devi, P., Tymon, L., Keinath, A. & Miles, C. (2021). Progress in grafting watermelon to manage Verticillium wilt. Plant Pathology, 70(4), 767-777. [CrossRef]

Edelstein, M., Cohen, R., Gur, A., Elkabetz, M., Pivonia, S., Grosch, R., Forster, P. & Schwarz, D. (2017). Performance of interspecific cucurbita rootstocks compared to their parental lines. Scientia Hortic., 216: 45-50. [Google Scholar]

El-Kersh, M.A.A., El-Meniawy, S.M & Abd El-Hady, S.A. (2016). Grafting can modulate watermelon growth and productivity under Egyptian conditions. 7(9): 915-922. [CrossRef]

El-Sayed, S.F., Haassan, H.A. & Gaara, M.A. (2015). Effect of different rootstocks on plant growth, yield and quality of watermelon. Annals. of Agric. Sci., 53(1): 165-175. [Google Scholar]

Eti, S. (1990). A practical method used in determining the amount of pollen. J. Agric. Faculty Cukurova Univ., 5(1): 49-58.

Eti, S. (1991). Determination of pollen viability and germination capability of some fruit species and cultivars by different *in vitro* tests. J. Agric. Faculty Cukurova Univ., 6: 69-80.

Fallik, E., Alkalai-Tuvia, S., Chalupowicz, D., Zutahy, Y., Zaaroor, M., Beniches, M. & Gamliel, A. (2016). Effects of rootstock and soil disinfection on quality of grafted watermelon fruit (*Citrullus lanatus* L.): a two-year study. Israel Journal of Plant Sciences, 63(1): 3844. [CrossRef]

Fallik, E., Alkalai-Tuvia, S., Chalupowicz, D., Popovsky-Sarid, S. & Zaaroor-Presman, M. (2019). Relationships between rootstock-scion combinations and growing regions on watermelon fruit quality. Agronomy, 9: 536. [CrossRef]

Faostat (2020). Agricultural statistics. (Date: 10 April 2022) [URL]

Freeman, J.H., Olson, S.M. & Kabelka, E.A. (2008). Pollen viability of selected diploid watermelon pollenizer cultivars. HortScience, 43(1):274-275. [CrossRef]

Fredes, A., Rosello, S., Beltrán, J., Cebolla-Cornejo, J., Pérez-de-Castro, A., Gisbert, C. & Picó, M.B. (2016). Fruit quality assessment of watermelons grafted onto citron melon rootstock. J. Sci. Food Agric., 97: 1646-1655. [Google Scholar]

Gaion, L.A, Braz, L.T. & Carvalho, R.F. (2018). Grafting in vegetable crops: a great technique for agriculture. International Journal of Vegetable Science, 24(1), 85-102. [CrossRef]

Güçdemir, İ.H. (2012). Plant Nutrition recipe preparation technique based on soil analysis and practical examples. In Plant Nutrition (Ed. Karaman M R) pp. 961-1066.

Gök, P., Yetisir, H., Solmaz, I., Sari, N. & Eti, S. (2007). Pollen Viability and germination rates of 45 watermelon genotypes. Acta Horticulturae, (731), 99–102. [CrossRef]

Huang, Y., Jiao, Y., Nawaz, M.A., Chen, C., Liu, L., Lu, Z., Kong, Q., Cheng, F. & Zhilong Bie, Z. (2016). Improving magnesium uptake, photosynthesis and antioxidant enzyme activities of watermelon by grafting onto pumpkin rootstock under low magnesium. Plant and Soil, 409: 229-246. [CrossRef]

Hussein, S. & Sarı, N. (2020). Effects of different rootstocks on seed yield and quality of triploid watermelon grown in greenhouse. Acta Hortic., 1282: 67-74. [CrossRef]

Karaca, F., Yetişir, H., Solmaz, İ., Candır, E., Kurt, S., Sari, N. & Güler, Z. (2012). Rootstock potential of Turkish *Lagenaria siceraria* germplasm for watermelon: plant growth, yield, and quality. Turkish Journal of Agriculture and Forestry, 36: 167-177. [Google Scholar]

Kombo, M.D. & Sarı, N. (2019). Rootstock effects on seed yield and quality in watermelon. Horticulture, Environment and Biotechnology, 60: 303-312. [CrossRef]

Lee, J.M. (1994). Cultivation of grafted vegetables. I. Current status, grafting methods, and benefits. HortScience 29(4):235-239. [CrossRef]

Liu, Q., Zhao, X., Brecht, J.K., Sims, C.A., Sanchez, T. & Dufault, N.S. (2017). Fruit quality of seedless watermelon grafted onto squash rootstocks under different production systems. J. Sci. Food Agric., 97: 4704-4711. [Google Scholar]

Maršić, K.N., Znidarčič, D. & Jakše, M. (2016). Diferent scion/rootstock combinations influence the yield and quality of grafted watermelon fruits. Acta Hortic., 1142: 19-24. [CrossRef]

Mavi, K. & Demir, İ. (2010). Determination of use of cold tests in watermelon seed lots as vigour test. Selçuk University Selçuk Journal of Agricultural and Food Sciences, 24 (2): 1-5. ISSN:1309-0550. [Google Scholar]

Mavi, K. (2011). Effect of different priming techniques on germination and seedling emergence and naturaly aged watermelon seed. Türkiye VI. National Horticultural Congress, Şanlıurfa. P. 496-499.

McGregor, C.E. & Waters, V. (2013). Pollen viability of $F_1$ hybrids between watermelon cultivars and disease-resistant, infraspecifc crop wild relatives. HortScience, 48: 1428-1432. [CrossRef]

Meng, J., Wu, S., Wang, X. & Yu, X., Jiang, R. (2019). Effects of different rootstocks on plant growth and fruit quality of watermelon. Agricultural Biotechnology, Cranston, 8(1): 64-68.

Mohamed, F.H., Abd El-Hamed, K.E., Elwan, M.W.M. & Hussien, M.A.N.E. (2012). Impact of grafting on watermelon growth, fruit yield and quality. Vegetable Crops Research Bulletin, 76: 99-118. [CrossRef]

Ozbahce, A., Kosker, Y., Gultekin, R., Gorgisen, C., Avag, K., Demir, Y., Yucel, S. (2021a). Impact of different rootstocks and limited water on yield and fruit quality of melon grown in a field naturally infested with Fusarium wilt. Scientia Horticulturae, 289, 110482. [CrossRef]

Ozbahce, A., Gorgisen, C., Gultekin, R., Kosker, Y., Demirci, Ş., Tari, A.F., Gonülal, E. (2021b). Influence of different rootstocks on some quality parameters of grafted melon seedling. BAHÇE, Volume 50, Issue 2, 111-117, [CrossRef]

Özdemir, A.E., Çandır, E., Yetişir, H., Aras, V., Arslan, Ö., Baltaer, Ö., Üstün, D. & Ünlü, M. (2016). Effects of rootstocks on storage and shelf life of grafted watermelons. J. Appl. Bot. Food Qual., 89: 191-201. [Google Scholar]

Özmen, S., Kanber, R., Sarı, N, & Ünlü, M. (2015). The effects of deficit irrigation on nitrogen consumption, yield, and quality in drip irrigated grafted and ungrafted watermelon. Journal of Integrative Agriculture, 14(5), 966–976. [CrossRef]

Öztekin, G.B., Tüzel, Y. & Uysal, N. (2012). Effects of different rootstocks on plant growth, yield and quality of watermelon grown in greenhouse. Acta Horticulturae, 952: 855-862.

Qin, Y., Yang, C., Xia, J., He, J., Ma, X., Yang, C., Zheng, Y., Lin, X., He, Z., Huang, Z. & Yan, Z. (2014). Effects of dual/threefold rootstock grafting on the plant growth, yield and quality of watermelon. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 42(2), 495–500. [CrossRef]

Petropoulos, S.A., Khah, E.M., & Passam, H.C. (2012). Evaluation of rootstocks for watermelon grafting with reference to plant development, yield and fruit quality. International Journal of Plant Production, 6(4): 481-492.

Radke, A.K., Soares, V.N., Xavier, F., Da, M., Rocha, P.E.E., Martins, A.B.N., Villela, F.A. (2017). Evaluation of physiological potencial of watermelon seeds by accelerated aging test. Revista Verde de Agroecologia e Desenvolvimento Sustentável, v. 12, n. 4, p. 634 - 640.

Salam, M.A., Masum, A.S.M.H., Chowdhury, S.S., Dhar, M., Saddeque, A., & Islam, M.R. (2002). Growth and yield of watermelon as influenced by grafting. J. Biol. Sci., 2: 298-299.

Siamak, S.B. & Paolo, S. (2019). Responses of grafted watermelon onto *Cucurbita pepo* Tiana $F_1$ hybrid to boron nutritional disorders. Horticultural Plant Journal, 5 (5): 213-220. [CrossRef]

Stanghellini, M.S. & Schultheis, J.R. (2005) Genotypic variability in staminate fower and pollen grain pro-

duction of diploid watermelons. HortScience 40:752-755. [CrossRef]

Suárez-Hernández, A.M., Grimaldo-Juárez, O., Ceceña-Durán, C., Vázquez-Angulo, J.C., Carrazco-Peña, L.D., Avendaño-Reyes, L., Ail-Catzim, C.E., Basilio-Cortes, U.A., Angulo-Castro, A. (2022). Influence of Seed and Fruit Characteristics of *Lagenaria siceraria* on Production and Quality of Grafted Watermelon. Horticulturae. 8(3):242. [CrossRef]

Tran-Nguyen, L.T.T., Condé, B.D., Smith, S.H. & Ulyatt, L.I. (2012). Outbreak of Fusarium wilt in seedless watermelon seedlings in the Northern Territory, Australia. Australasian Plant Disease Notes, 8(1), 5–8. [CrossRef]

Tripodi, G., Condurso, C., Cincotta, F., Merlino, M. & Verzera, A. (2019). Aroma compounds in mini-watermelon fruits from different grafting combinations. Journal of Science of Food and Agriculture. [CrossRef]

Turhan, A., Ozmen, N., Kuscu, H., Serbeci, M.S. & Seniz, V. (2012). Infuence of rootstocks on yield and fruit characteristics and quality of watermelon. Hortic. Environ. Biotechnol., 53: 336-341. [Google Scholar]

Ulas, F. (2021). Response of different rootstocks on vegetative growth, fruit and seed yield of eggplant (*Solanum melongena* L.). Genetika, 53(2), 593-608. [CrossRef]

Yetişir, H., Sarı, N. & Yücel, S. (2003). Rootstock Resistance to Fusarium wilt and effect on watermelon fruit yield and quality. Phytoparsitica, 31(2): 163-169. [Google Scholar]

Yetişir, H., Kurt, S., Sarı, N. & Tok, M.F. (2007). Rootstock potential of Turkish *Lagenaria siceraria* germplasm for watermelon: plant growth, graft compatibility, and resistance to fusarium. Turkish Journal of Agriculture and Forestry, 31: 381-388. [Google Scholar]

Yetişir, H. & Sarı, N. (2018). Fruit and seed yields of watermelon [*Citrullus lanatus* (Thunb.) Matsum. and Nakai] grafted onto different bottle gourd (*Lagenaria siceraria* Molina Standl.) rootstocks. Asian Journal of Research in Agriculture and Forestry, 1(2): 1-9. [Google Scholar]

Zaaroor, M., Alkalai-Tuvia, S., Chalupowıcz, D., Zutahy, Y., Beniches, M., Gamliel, A. & Fallik, E, (2016). Fruit Quality of Grafted Watermelon (*Citrullus lanatus*): Relationship between rootstock, soil disinfection and plant stand. Agriculturae Conspectus Scientificus, 81(2): 81-86. [Google Scholar]

Zhang, J., Wang, P., Tian, H., Wang, Y. & Jiang, H. (2019). Using a new hybrid rootstock significantly increases the grafted plant rate and watermelon yield. Int. Agrophys., 33: 97-106. [CrossRef]

Zhong, Y., Chen, C., Nawaz, M.A., Jiao, Y., Zheng, Z., Shi, X., Xie, W., Yu, Y., Guo, J., Zhu, S., Xie, M., Kong, Q., Cheng, F., Bie, F. & Huang, Y. (2018). Using rootstock to increase watermelon fruit yield and quality at low potassium supply: a comprehensive analysis from agronomic, physiological and transcriptional perspective. Scientia Horticulturae, 241: 144–151. [CrossRef]

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 694-703
DOI: https://doi.org/10.31015/jaefs.2022.4.25

# The effect of temperature and relative humidity on milk yield of holstein dairy cattle raised in agricultural enterprises under different climatic conditions

**Gökhan Gelir**[1] • **Ali Murat Tatar**[2]

[1] Dicle University, Natural and Applied Science Institute, Animal Science, Diyarbakır, Türkiye
[2] Dicle University, Agriculture Faculty, Animal Science Department, Diyarbakır, Türkiye

**Citation:** Gelir, G., Tatar, A.M. (2022). The effect of temperature and relative humidity on milk yield of holstein dairy cattle raised in agricultural enterprises under different climatic conditions. International Journal of Agriculture, Environment and Food Sciences, 6 (4), 694-703

**Received:** 01 November 2022
**Revised:** 12 December 2022
**Accepted:** 14 December 2022
**Published Online:** 24 December 2022

**Correspondence:** Gökhan Gelir
**E-mail:** g.gelir.17@gmail.com



## Abstract

This study was conducted to investigate the effect of air temperature and relative humidity on milk yield of the Holstein dairy cattle raised in Agricultural Enterprises under different climatic conditions in Turkey (Çukurova, Polatlı and Türkgeldi) and to examine the differences between the enterprises. Records of milk yield on the test day for the years 2014-2020 obtained from the General Directorate of Agricultural Enterprises (TİGEM) were utilised in the study. The data set related to milk yields included the number of Holstein cows, data on different lactation orders, control records of milk yield and information of the animals for some environmental factors. In the study, values of four temperature-humidity indices (THI) consisting of different combinations of daily maximum and minimum temperature and humidity values were examined in order to assess the effect of temperature stress on milk yield. Consequently, the difference between the enterprises in terms of the combinations of the temperature-humidity index was found to be statistically significant ($P<0.05$). The THI value calculated for the agricultural enterprises varied between 10 and 102. It was determined that dairy cattle were exposed to heat stress between May and November in Türkgeldi and Polatlı Agricultural Enterprises in the cold climate zone and between April and July in Çukurova Agricultural Enterprises in the temperate climate zone. While the THI value with low humidity weight was the optimal indicator in a humid climate, the THI value with high-temperature weight was the best indicator of heat stress in a semi-arid climate.

**Keywords:** Holstein, Heat stress, Temperature-humidity index, Milk yield, Different climatic condition

## INTRODUCTION

Turkey is geographically located between the temperate zone and the subtropical zone, surrounded by seas on three sides, the extension of the mountains and the diversity of landforms allow the formation of climate types with different characteristics. While milder climate characteristics are observed in the coastal regions due to the effect of the seas, the Northern Anatolian Mountains and the Taurus Mountains do not allow the effects of the sea to flow inland and cause continental climate characteristics to be observed in the interior of Turkey. In Turkey, continental climate, Black Sea climate, Mediterranean climate and Marmara (transitional) climate are seen. More than 900 cattle breeds exist which live in almost everywhere in the world, except for the poles, and can produce meat

and milk at various levels. However, a limited number of breeds meet most of the world's milk and meat production (Anonymous, 2015). According to 2020 data, there are 1.526 million cattle worldwide. Brazil has the highest number of cattle with 218 million heads, followed by India with 194 million heads. The USA, Ethiopia, and China follow these countries respectively, and Turkey ranks 19th with approximately 19 million cattle. In addition to these data, approximately 887 million tonnes of milk were produced in the world in 2020, India ranked first with 184 million tonnes, followed by the USA with 101 million tonnes and Pakistan with 61 million tonnes. Turkey ranked 10th with 22 million tonnes (FAO, 2021). The number of cattle in Turkey was determined as 18 million 124 thousand heads according to TSI (Turkish Statistical Institute)'s 2021 data. Turkey's share of cow's milk in total milk production is 92%. Although all regions of Turkey with different climatic characteristics have areas suitable for animal husbandry, the data indicate that while the number of bovine and ovine animals is sufficient, milk production is sufficient for the domestic market. The existence of an informal economy in milk and dairy products and the postponement of milk and dairy products in consumption habits cause disadvantages in this sector. Moreover, the rise in meat prices has increased the number of animals going to slaughter (TSI, 2021). Despite the postponement of milk and dairy products that have high nutritional value and appeal to all age groups, the rise in their prices is a bitter reality. As with all living beings, the effects of environmental conditions on dairy cattle show a diverse and complex structure. In the simplest sense, care, feeding and climatic factors can be listed among those. Temperature, humidity, air movements and cleanliness of the air come to the forefront among climatic factors (Mutaf and Sönmez, 1984). Although the temperature is a climatic factor that negatively affects the performance of dairy cattle, heat stress is induced by environmental factors such as solar radiation, high air temperature and relative humidity. This becomes more intense with the cow's own body temperature. In general, the higher the milk production, the higher the heat released after digestion and metabolism of nutrients (West, 2003). Therefore, animals with high milk yields generate more heat and are more susceptible to environmental factors that produce heat stress than animals with low milk yields. In other words, such animals are at a greater risk. The way to indicate the presence of heat stress in cattle is the use of the temperature humidity index (THI). Table 1 shows the THI values for different combinations of temperature and relative humidity. THI values that exceed 72 in the table are considered as the onset of heat stress. Higher values result in a decrease in feed consumption in cattle. A value above 77 causes a sudden and sharp drop in feed intake. Some environmental modifications should be made and different feeding methods should be adopted in order to prevent this condition (West, 1995; Johnson, 1987). More recently, Zimbelman et al. (2009) documented that productivity of high-producing cows begins to be negatively affected by heat stress at temperatures of 23 °C and 35% relative humidity, with corresponds to a THI value as low as 68. Moreover, Cook et al. (2007), has documented that as the THI increases from 56 to 74, behavioral adaptations are observed. Lying time decreases from 10.9 to 7.9 h per day, standing increases from 2.6 to 4.5 h per day, and drinking increases from 0.3 to 0.5 h per day. Collectively these studies indicate that a THI of 68 is the threshold for initiation of negative outcomes on milk production, behavior, and physiology due to heat stress. Milk yield reduction and behavioural changes are the first indicators of heat stress in dairy cattle. When heat stress is moderate, dairy cattle breathe rapidly, and sweat, and their feed consumption and milk yield decrease by approximately 10% (Kadzere et al., 2002; West, 2003; Shebab-El-Deen, 2010). When the stress becomes severe, the decrease in feed consumption and milk yield is more than 25% (Yavuz and Biricik, 2009). The temperature for the comfort zone in dairy cattle ranges between -15 °C and 25 °C (NADIS, 2022). As the air temperature rises above 25 °C, heat stress emerges in animals and consequently, while dry matter consumption decreases by 2-12%, milk yield is lost by 20-30% and this loss can reach 5-12 litres per day when the air temperature exceeds 30 °C (West, 2003). It has been reported that milk yield decreases in Holstein cows when the critical temperature exceeds 25-25 °C and the THI value exceeds 72, beyond the comfort level (Johnson, 1980; Berman et al., 1985).

This study was conducted to investigate the effect of air temperature and relative humidity on the milk yield of the Holstein dairy cattle reared in TİGEM Agricultural Enterprises (Polatlı,Türkgeldi,Çukurova) and to examine the differences between the enterprises.

## MATERIALS AND METHODS

The material of the study consisted of the control records of milk yield kept in Türkgeldi, Çukurova and Polatlı Agricultural Enterprises of the General Directorate of Agricultural Enterprises and meteorological records including minimum and maximum temperature values as well as minimum and maximum daily humidity values obtained from the General Directorate of Meteorology of these enterprises.

The control records of milk yield obtained from agricultural enterprises belong to Holstein cows in various lactation stages and cover the period between 01/01/2014 - 31/12/2020. A total of 17899 of milk yields on the control from 914 cows were assessed in Çukurova, 55794 from 2785 cows in Türkgeldi and 29918 from 1593 cows in Polatlı between the given dates.

### Some information on the Agricultural Enterprises where the study was carried out

**Table 1.** Temperature-humidity index (SNI) at varying temperature and relative humidity

| Temperature °C | Relative humidity% | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | 25 | 30 | 35 | 40 | 45 | 50 | 55 | 60 | 65 | 70 | 75 | 80 | 85 | 90 | 95 | 100 | |
| 10 | 55 | 54 | 54 | 54 | 54 | 53 | 53 | 53 | 53 | 52 | 52 | 52 | 52 | 51 | 51 | 51 | 51 | 50 | 50 | 50 | No Heat Stress |
| 11 | 55 | 55 | 55 | 55 | 55 | 54 | 54 | 54 | 54 | 54 | 54 | 53 | 53 | 53 | 53 | 53 | 52 | 52 | 52 | 52 | |
| 12 | 56 | 56 | 56 | 56 | 56 | 56 | 55 | 55 | 55 | 55 | 55 | 55 | 55 | 54 | 54 | 54 | 54 | 54 | 54 | 54 | |
| 13 | 57 | 57 | 57 | 57 | 57 | 57 | 57 | 57 | 56 | 56 | 56 | 56 | 56 | 56 | 56 | 56 | 56 | 56 | 55 | 55 | |
| 14 | 58 | 58 | 58 | 58 | 58 | 58 | 58 | 58 | 58 | 58 | 58 | 58 | 58 | 57 | 57 | 57 | 57 | 57 | 57 | 57 | |
| 15 | 59 | 59 | 59 | 59 | 59 | 59 | 59 | 59 | 59 | 59 | 59 | 59 | 59 | 59 | 59 | 59 | 59 | 59 | 59 | 59 | |
| 16 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 60 | 61 | 61 | 61 | 61 | 61 | 61 | |
| 17 | 61 | 61 | 61 | 61 | 61 | 61 | 61 | 61 | 61 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 62 | 63 | |
| 18 | 61 | 62 | 62 | 62 | 62 | 62 | 62 | 63 | 63 | 63 | 63 | 63 | 63 | 63 | 64 | 64 | 64 | 64 | 64 | 64 | |
| 19 | 62 | 63 | 63 | 63 | 63 | 63 | 64 | 64 | 64 | 64 | 64 | 65 | 65 | 65 | 65 | 65 | 66 | 66 | 66 | 66 | |
| 20 | 63 | 63 | 64 | 64 | 64 | 64 | 65 | 65 | 65 | 65 | 66 | 66 | 66 | 66 | 67 | 67 | 67 | 67 | 68 | 68 | |
| 21 | 64 | 64 | 65 | 65 | 65 | 66 | 66 | 66 | 66 | 67 | 67 | 67 | 68 | 68 | 68 | 69 | 69 | 69 | 69 | 70 | |
| 22 | 65 | 65 | 66 | 66 | 66 | 67 | 67 | 67 | 68 | 68 | 68 | 69 | 69 | 69 | 70 | 70 | 71 | 71 | 71 | 72 | Modarete Heat Stress |
| 23 | 66 | 66 | 67 | 67 | 67 | 68 | 68 | 69 | 69 | 69 | 70 | 70 | 71 | 71 | 71 | 72 | 72 | 73 | 73 | 73 | |
| 24 | 67 | 67 | 68 | 68 | 68 | 69 | 69 | 70 | 70 | 71 | 71 | 72 | 72 | 72 | 73 | 73 | 74 | 74 | 75 | 75 | |
| 25 | 67 | 68 | 68 | 69 | 69 | 70 | 70 | 71 | 71 | 72 | 72 | 73 | 73 | 74 | 74 | 75 | 75 | 76 | 76 | 77 | Severe Heat Stress |
| 26 | 68 | 69 | 69 | 70 | 71 | 71 | 72 | 72 | 73 | 73 | 74 | 74 | 75 | 76 | 76 | 77 | 77 | 78 | 78 | 79 | |
| 27 | 69 | 70 | 70 | 71 | 72 | 72 | 73 | 73 | 74 | 75 | 75 | 76 | 76 | 77 | 78 | 78 | 79 | 79 | 80 | 81 | |
| 28 | 70 | 71 | 71 | 72 | 73 | 73 | 74 | 75 | 75 | 76 | 77 | 77 | 78 | 79 | 79 | 80 | 80 | 81 | 82 | 82 | |
| 29 | 71 | 72 | 72 | 73 | 74 | 74 | 75 | 76 | 77 | 77 | 78 | 79 | 79 | 80 | 81 | 81 | 82 | 83 | 84 | 84 | |
| 30 | 72 | 73 | 73 | 74 | 75 | 76 | 76 | 77 | 78 | 79 | 79 | 80 | 81 | 82 | 82 | 83 | 84 | 85 | 85 | 86 | |
| 31 | 73 | 73 | 74 | 75 | 76 | 77 | 77 | 78 | 79 | 80 | 81 | 81 | 82 | 83 | 84 | 85 | 85 | 86 | 87 | 88 | |
| 32 | 74 | 74 | 75 | 76 | 77 | 78 | 79 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 85 | 86 | 87 | 88 | 89 | 90 | Dead Cows |
| 33 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 91 | |
| 34 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 88 | 89 | 90 | 91 | 92 | 93 | |
| 35 | | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | |
| 36 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 91 | 92 | 93 | 94 | 95 | 96 | 97 | |
| 37 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 | 98 | 99 | |
| 38 | 79 | 80 | 81 | 82 | 83 | 84 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 94 | 95 | 96 | 97 | 98 | 99 | 100 | |
| 39 | 80 | 81 | 82 | 83 | 84 | 85 | 87 | 88 | 89 | 90 | 91 | 93 | 94 | 95 | 96 | 97 | 99 | 100 | 101 | 102 | |
| 40 | 80 | 82 | 83 | 84 | 85 | 87 | 88 | 89 | 90 | 92 | 93 | 94 | 95 | 97 | 98 | 99 | 100 | 102 | 103 | 104 | |
| 41 | 81 | 83 | 84 | 85 | 86 | 88 | 89 | 90 | 92 | 93 | 94 | 95 | 97 | 98 | 99 | 101 | 102 | 103 | 105 | 106 | |
| 42 | 82 | 83 | 85 | 86 | 87 | 89 | 90 | 91 | 93 | 94 | 96 | 97 | 98 | 100 | 101 | 102 | 104 | 105 | 106 | 108 | |
| 43 | 83 | 84 | 86 | 87 | 89 | 90 | 91 | 93 | 94 | 95 | 97 | 98 | 100 | 101 | 102 | 104 | 105 | 107 | 108 | 109 | |
| 44 | 84 | 85 | 87 | 88 | 90 | 91 | 92 | 94 | 95 | 97 | 98 | 100 | 101 | 103 | 104 | 105 | 107 | 108 | 110 | 111 | |
| 45 | 85 | 86 | 88 | 89 | 91 | 92 | 94 | 95 | 97 | 98 | 100 | 101 | 103 | 104 | 106 | 107 | 109 | 110 | 112 | 113 | |
| 46 | 86 | 87 | 89 | 90 | 92 | 93 | 95 | 96 | 98 | 99 | 101 | 102 | 104 | 106 | 107 | 109 | 110 | 112 | 113 | 115 | |
| 47 | 86 | 88 | 90 | 91 | 93 | 94 | 96 | 98 | 99 | 101 | 102 | 104 | 105 | 107 | 109 | 110 | 112 | 113 | 115 | 117 | |
| 48 | 87 | 89 | 91 | 92 | 94 | 95 | 97 | 99 | 100 | 102 | 104 | 105 | 107 | 109 | 110 | 112 | 113 | 115 | 117 | 118 | |
| 49 | 88 | 90 | 91 | 93 | 95 | 97 | 98 | 100 | 102 | 103 | 105 | 107 | 108 | 110 | 112 | 113 | 115 | 117 | 119 | 120 | |
| 50 | 89 | 91 | 92 | 94 | 96 | 98 | 99 | 101 | 103 | 105 | 106 | 108 | 110 | 112 | 113 | 115 | 117 | 119 | 120 | 122 | |

SNI=(1,8 x T + 32 - [(0,55 – 0,0055 x N) x (1,8 x T – 26,8)] (Ravagnolo et al., 2000)

### Çukurova Agricultural Enterprise

The Enterprise was established in 1927 as Lentil Stallion Warehouse under the Agricultural Enterprises has been operating under TİGEM since 1984. The enterprise is located in the Kadirli-Kozan Highway Adana Province. When the long-term temperature means of Çukurova Agricultural Enterprise were analysed, the highest temperature mean was 30 $^0$C, the mean highest temperature was 34.8 $^0$C, the average sunshine duration per day was 12.6 hours, and the highest temperature was 46.8 $^0$C in July. The 20-year mean precipitation on cultivated crops was 612.2 mm. The cattle-raising activities are carried out with Holstein which are adapted to the region. In 2021, 2.897 tonnes of milk were produced from 1.01 cattle.

### Türkgeldi Agricultural Enterprise

The enterprise was established in 1938 under the name of State Seed and Sample Farm, carried out activities as State Production Farm in 1950 and has been operating under TİGEM since 1984. The enterprise is located within the borders of Lüleburgaz in Kırklareli Province, 9 km away from the district and 65 km away from the provincial centre. The 20-year mean precipitation on cultivated crops is 383.3 mm. The cattle-raising activities are carried out with Holstein and Simmental breeds which are adapted to the region. In 2021, 6,675 tonnes of milk were produced from 2.192 cattle.

### Polatlı Agricultural Enterprise

The enterprise was established in 1937 under the name of Polatlı Group, carried out activities as State Production Farm in 1950 and has been operating under TİGEM since 1984. The enterprise is located in the Upper Sakarya section of the Central Anatolia Region, within the borders of Polatlı District and 54 km south of the district. The geographical location is at GPS coordinates 39° 8′ 57″ North and 32° 7′ 20″ East. The mean annual rainfall is 360 mm. The mean highest temperature is 29.6 °C (July) and the mean lowest temperature is -3.6 °C (January).

In the study, the maximum and minimum temperature and humidity values were used to calculate the THI value in four combinations. Maximum temperature and maximum humidity (THIa), minimum temperature and minimum humidity (THIb), maximum temperature and minimum humidity (THIc) and minimum temperature and maximum humidity (THId) values were calculated by using the following equation (NRC, 1971).

SNI=(1,8 x T + 32( - [(0,55 – 0,0055 x N) x (1,8 x T – 26,8)]

SNI : Temperature humidity index,

T : Air temperature measured with a dry thermometer (°C),

N : It expresses the relative (relative) humidity in the air (%).

In the study, the model created for the data set consisting of the values related to THI types of the day that precedes the control day (Ravagnolo et al., 2000) and calculated by the equation above and the information related to fixed effects such as milk yield value, lactation order, month and year for each control day are given below to calculate the effect that would be caused by the temperature and relative humidity conditions one day before the control day of the milk yield of the cows in the enterprises.

$Y_{ijklm}=\mu+\alpha_i+\beta_j+\gamma_k+SNI_i+e_{ijklm}$

$Y_{ijklm}$= I. during lactation, j. per year, k. per month, l. m at the SNI level, milk yield on the control day,

μ = Control milk yield average,

$\alpha_i$= I. the effect of lactation order,

$B_j$ = Effect of the year,

$\gamma_k$= k. Effect of the month,

[THI] _l= l. Effect of SNI level (SNIa, SNIb, SNIc, SNId)

eijklm= i. during lactation, j. year, n. month, l. m at the SNI level. refers to the effect of random environmental factors on the control day.

Milk loss =0.0695 (SNImak-SNIthreshold)2 x D

D: It represents the ratio of the total stress duration to 24 hours during the day (at SNImax > SNIthreshold). For dairy cattle in this equation, the SNI threshold value was taken as 72.

Statistical analyses were done as GLM using the IBM SPSS Statistics for Windows, Version 25.0. Tukey's test was used to compare the means.

## RESULT AND DISCUSSIONS

Table 2 shows the maximum and minimum temperature and humidity values determined in the agricultural enterprises throughout the study.

Table 3 presents the descriptive statistics of the control

**Table 2.** Maximum temperature and humidity values determined during the working period of agricultural enterprises

| Enterprisees | Max. Temprature | Min. Temprature | Max. Humidity | Min. Humidity |
|---|---|---|---|---|
| Çukurova Agricultural Enterprise | 47,4 (19.07.2019) | -7,8 (3.01.2016) | 100 (9-15.04.2018) | 0 (19.04.2018) |
| Polatlı Agricultural Enterprise | 38,6 (3.07.2017) | -19,1 (9.01.2015) | 100 (January– July 2014, 2017-2019) | 5 (23.09.2018) |
| Türkgeldi Agricultural Enterprise | 39,8 (1.07.2017) | -16,7 (13.01.2017) | 100 (January 2014-Agust 2018) | 0 (13.09.2018) |

**Table 3.** Descriptive statistics on records kept in agricultural holdings

| | Çukurova | | | Türkgeldi | | | Polatlı | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **Lactation Order** | **N** | **Milk Yield (kg)** | **Std. Err** | **N** | **Milk Yield (kg)** | **Std. Err** | **N** | **Milk Yield (kg)** | **Std. Err** | **Average Milk Yield (kg)** |
| 1 | 8551 | 23,15 | 0,086 | 23360 | 22,19 | 0,049 | 13030 | 24,14 | 0,060 | 22,94±0,035$^{b}$ |
| 2 | 5036 | 26,92 | 0,157 | 17615 | 24,60 | 0,074 | 8911 | 26,48 | 0,093 | 25,50±0,055$^{a}$ |
| 3 | 2750 | 25,82 | 0,205 | 9100 | 24,86 | 0,114 | 4748 | 26,92 | 0,138 | 25,61±0,082$^{a}$ |
| 4 | 1154 | 24,44 | 0,341 | 3793 | 24,41 | 0,183 | 2068 | 26,49 | 0,200 | 25,03±0,128$^{a}$ |
| 5 | 306 | 23,19 | 0,450 | 1440 | 22,29 | 0,286 | 819 | 25,02 | 0,324 | 23,27±0,200$^{b}$ |
| 6 | 84 | 21,35 | 0,911 | 417 | 20,49 | 0,460 | 247 | 24,54 | 0,557 | 21,92±0,338$^{c}$ |
| 7 | 18 | 25,51 | 1,433 | 69 | 19,47 | 0,972 | 95 | 22,79 | 1,015 | 21,80±0,674$^{c}$ |
| **Year** | | | | | | | | | | |
| 2014 | 1610 | 19,76 | 0,155 | 5488 | 22,96 | 0,112 | 2388 | 22,24 | 0,148 | 22,24±0,080$^{f}$ |
| 2015 | 2481 | 24,38 | 0,143 | 6128 | 24,03 | 0,110 | 3115 | 26,51 | 0,129 | 24,76±0,074$^{c}$ |
| 2016 | 2429 | 29,14 | 0,167 | 5793 | 27,50 | 0,132 | 3653 | 25,98 | 0,121 | 27,36±0,082$^{a}$ |
| 2017 | 2860 | 29,59 | 0,197 | 7662 | 25,44 | 0,108 | 4358 | 24,03 | 0,118 | 25,83±0,077$^{b}$ |
| 2018 | 2452 | 28,03 | 0,257 | 10021 | 23,01 | 0,091 | 4959 | 25,68 | 0,114 | 24,48±0,073$^{d}$ |
| 2019 | 2746 | 20,79 | 0,158 | 10504 | 22,42 | 0,092 | 5079 | 25,62 | 0,125 | 23,06±0,068$^{e}$ |
| 2020 | 3321 | 20,63 | 0,131 | 10198 | 21,47 | 0,090 | 6366 | 26,55 | 0,114 | 22,96±0,065$^{e}$ |
| **Month** | | | | | | | | | | |
| January | 1511 | 26,31 | 0,261 | 4650 | 22,41 | 0,138 | 2351 | 25,48 | 0,165 | 23,95±0,101 |
| February | 1585 | 26,25 | 0,264 | 4576 | 23,08 | 0,139 | 2210 | 25,94 | 0,168 | 24,44±0,102$^{c}$ |
| March | 1609 | 26,35 | 0,271 | 4053 | 23,85 | 0,153 | 2455 | 26,17 | 0,169 | 25,05±0,107$^{b}$ |
| April | 1663 | 27,26 | 0,254 | 4496 | 24,27 | 0,141 | 2311 | 26,40 | 0,167 | 25,44±0,102$^{a}$ |
| May | 1597 | 26,62 | 0,251 | 5220 | 24,88 | 0,130 | 2279 | 25,28 | 0,178 | 25,28±0,097$^{ab}$ |
| June | 1533 | 25,94 | 0,240 | 4396 | 25,05 | 0,138 | 2376 | 25,41 | 0,170 | 25,32±0,098$^{ab}$ |
| July | 1424 | 23,48 | 0,211 | 4823 | 23,97 | 0,127 | 2458 | 25,08 | 0,166 | 24,20±0,091$^{cd}$ |
| August | 1293 | 21,46 | 0,213 | 4873 | 22,91 | 0,128 | 2674 | 24,95 | 0,163 | 23,32±0,093$^{gh}$ |
| September | 1332 | 21,43 | 0,242 | 4783 | 22,69 | 0,129 | 2781 | 24,73 | 0,164 | 23,14±0,094$^{h}$ |
| October | 1255 | 22,23 | 0,252 | 3817 | 23,22 | 0,151 | 2809 | 26,21 | 0,164 | 24,13±0,103$^{d}$ |
| November | 1506 | 22,27 | 0,239 | 5147 | 23,05 | 0,129 | 2744 | 25,32 | 0,146 | 23,59±0,092$^{fg}$ |
| December | 1591 | 24,82 | 0,243 | 4960 | 22,96 | 0,143 | 2470 | 24,75 | 0,160 | 23,78±0,100$^{ef}$ |
| **General** | 17899 | 24,695±0,073$^{b}$ | | 55794 | 23,525±0,040$^{c}$ | | 29918 | 25,463±0,048$^{a}$ | | |

records of the milk yields that were kept in the agricultural enterprises by lactation order, control month and year.

In table 3, the difference between the agricultural enterprises in terms of milk yield on the control day was significant (P<0.01). Accordingly, the highest mean was found in Polatlı Agricultural Enterprise (25.4630.048), followed by Çukurova (24.6950.073) and Türkgeldi Agricultural Enterprises (23.525).

The difference between the enterprises in terms of lactation order, control month and the year was found to be statistically significant (P<0.01). In terms of lactation order, the highest mean values were found in Polatlı (over 26 litres) and Türkgeldi (over 24 litres) in the 2nd-4th lactation, while Çukurova (over 25 litres) was found in the 2-3th lactation.

As for the year, the highest milk yield means on the control day were determined in Çukurova (29.5910.52) in 2017, in Türkgeldi (27.5010.04) in 2016 and in Polatlı (26.559.06) in 2020.

As for the month in which the study was conducted, the highest mean milk yield on the control day was determined in April in Çukurova (27.2610.35), in June in Türkgeldi (25.059.12) and in April in Polatlı (26.408.02).

Table 4 shows the least mean squares and standard deviation values calculated for the milk yield on the control day according to different THI (a, b, c, d) values of the enterprises. The tables indicated that although they varied in terms of enterprises, the THIa values obtained by using

**Table 4.** The least squares averages and standard errors of control milk yields according to different types of THI values in Çukurova Agricultural Enterprise

| THIa | Controls Num | Controls MY | STD Err | THIb | Controls Num | Controls MY | STD Err | THIc | Controls Num | Controls MY | STD Err | THId | Controls Num | Controls MY | STD Err |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 48 | 11183 | 25,31 | 0,098 | 40 | 176 | 23,31 | 0,532 | 50 | 11359 | 25,28 | 0,097 | 35 | 166 | 19,12 | 0,480 |
| 50 | 176 | 23,31 | 0,532 | 41 | 166 | 19,12 | 0,480 | 55 | 175 | 21,77 | 0,500 | 38 | 3685 | 24,51 | 0,162 |
| 55 | 270 | 21,34 | 0,377 | 43 | 11183 | 25,31 | 0,098 | 56 | 143 | 21,34 | 0,674 | 40 | 11359 | 25,28 | 0,097 |
| 56 | 143 | 21,34 | 0,674 | 45 | 107 | 19,22 | 0,623 | 57 | 95 | 20,54 | 0,542 | 42 | 95 | 20,54 | 0,542 |
| 62 | 3744 | 24,42 | 0,160 | 46 | 3578 | 24,66 | 0,165 | 61 | 3851 | 24,27 | 0,157 | 45 | 100 | 22,12 | 0,694 |
| 63 | 107 | 19,22 | 0,623 | 47 | 143 | 21,34 | 0,674 | 63 | 100 | 22,12 | 0,694 | 46 | 186 | 22,64 | 0,506 |
| 66 | 299 | 22,54 | 0,416 | 49 | 469 | 21,93 | 0,311 | 64 | 199 | 22,75 | 0,519 | 47 | 545 | 23,81 | 0,323 |
| 68 | 186 | 22,64 | 0,506 | 50 | 286 | 22,46 | 0,408 | 65 | 186 | 22,64 | 0,506 | 49 | 175 | 21,77 | 0,500 |
| 70 | 190 | 24,96 | 0,408 | 51 | 203 | 26,60 | 0,451 | 68 | 190 | 24,96 | 0,408 | 51 | 110 | 21,03 | 0,468 |
| 73 | 50 | 17,98 | 1,052 | 54 | 110 | 21,03 | 0,468 | 70 | 50 | 17,98 | 1,052 | 53 | 137 | 22,31 | 0,654 |
| 77 | 367 | 23,46 | 0,377 | 55 | 187 | 21,16 | 0,572 | 71 | 504 | 23,15 | 0,328 | 55 | 50 | 17,98 | 1,052 |
| 78 | 137 | 22,31 | 0,654 | 57 | 244 | 23,11 | 0,448 | 76 | 183 | 24,36 | 0,333 | 56 | 244 | 23,11 | 0,448 |
| 82 | 136 | 25,33 | 0,767 | 61 | 309 | 25,80 | 0,323 | 77 | 136 | 25,33 | 0,767 | 62 | 309 | 25,80 | 0,323 |
| 83 | 183 | 24,36 | 0,333 | 64 | 250 | 22,83 | 0,520 | 78 | 240 | 24,07 | 0,482 | 65 | 136 | 25,33 | 0,767 |
| 87 | 114 | 19,85 | 0,569 | 66 | 132 | 19,79 | 0,414 | 82 | 132 | 19,79 | 0,414 | 66 | 114 | 19,85 | 0,569 |
| 92 | 258 | 23,74 | 0,434 | 68 | 51 | 17,09 | 0,874 | 84 | 135 | 18,19 | 0,429 | 69 | 132 | 19,79 | 0,414 |
| 93 | 305 | 23,54 | 0,384 | 72 | 135 | 18,19 | 0,429 | 85 | 221 | 25,32 | 0,450 | 71 | 51 | 17,09 | 0,874 |
| 95 | 51 | 17,09 | 0,874 | 74 | 170 | 27,78 | 0,345 | | | | | 77 | 135 | 18,19 | 0,429 |
| | | | | | | | | | | | | 79 | 170 | 27,78 | 0,345 |

the maximum temperature and maximum relative humidity values ranged between 30-102; however, fluctuations in the values related to milk yield ranged between 37-75. When the THIa value exceeds 75, the milk yield began to decrease, but it tends to raise slightly above 87. In this range, milk yield dropped from 29.65±0.535 kg to 22.84±0.154 kg with a loss of 6.81 kg milk yield ($P<0.01$). Also, the point where the drop in milk yield began was 75 instead of 72, the critical value.

## Decreases in milk yield due to heat stress

It was determined that the milk yield decreased at Çukurova in the range of 70-82 when THIa was used and the number of days within this range was 123-183 days, the THIa value decreased at Türkgeldi in the range of 75-84. The number of days within this range was 61-214 days although it varied over the years. The THIa value decreased at Polatlı in the range of 71-82 and the number of days within this range was 122-183 days although it varied over the years.

If the mean THIa value in the range of 70-82 at Çukurova Agricultural Enterprise is accepted as 76, the difference between this value and 70, the critical value, at which the milk yield begins to decrease is 6 units, and since this value was exceeded for 123-183 days, the animals were exposed to heat stress. When the THIa value was 70, the mean control milk yield was 24.96±5.62 kg and the mean control milk yield which corresponds to the mean THIa value was 23.46±7.23 kg and the difference was 1.50 kg. Based on this, it is possible to conclude that the mean

**Table 5.** The least squares averages and standard deviations of milk yields according to different types of THI values in Türkgeldi Agricultural Enterprise

| THIa | Controls Num | Controls MY | STD Err | THIb | Controls Num | Controls MY | STD Err | THIc | Controls Num | Controls MY | STD Err | THId | Controls Num | Controls MY | STD Err |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 30 | 362 | 24,46 | 0,543 | 15 | 362 | 24,46 | 0,543 | 33 | 362 | 24,46 | 0,543 | 10 | 362 | 24,46 | 0,543 |
| 35 | 899 | 25,36 | 0,331 | 27 | 512 | 25,90 | 0,432 | 39 | 899 | 25,36 | 0,331 | 15 | 452 | 25,76 | 0,466 |
| 37 | 452 | 25,76 | 0,466 | 28 | 400 | 25,95 | 0,528 | 43 | 1003 | 24,15 | 0,266 | 17 | 400 | 25,95 | 0,528 |
| 38 | 671 | 24,56 | 0,332 | 30 | 452 | 25,76 | 0,466 | 44 | 452 | 25,76 | 0,466 | 22 | 512 | 25,90 | 0,432 |
| 42 | 1017 | 21,57 | 0,269 | 31 | 685 | 20,71 | 0,332 | 46 | 1021 | 21,41 | 0,267 | 24 | 671 | 24,56 | 0,332 |
| 43 | 400 | 25,95 | 0,528 | 32 | 387 | 24,63 | 0,514 | 47 | 400 | 25,95 | 0,528 | 25 | 685 | 20,71 | 0,332 |
| 45 | 336 | 22,83 | 0,438 | 33 | 671 | 24,56 | 0,332 | 48 | 979 | 22,41 | 0,295 | 27 | 387 | 24,63 | 0,514 |
| 47 | 979 | 22,41 | 0,295 | 34 | 687 | 22,20 | 0,329 | 49 | 1505 | 20,72 | 0,237 | 28 | 136 | 26,90 | 1,065 |
| 48 | 2282 | 20,42 | 0,190 | 35 | 1016 | 24,37 | 0,267 | 50 | 794 | 20,30 | 0,339 | 29 | 1164 | 26,12 | 0,277 |
| 50 | 794 | 20,30 | 0,339 | 37 | 1802 | 21,00 | 0,223 | 51 | 777 | 19,83 | 0,317 | 30 | 684 | 24,87 | 0,333 |
| 52 | 384 | 23,43 | 0,475 | 38 | 633 | 20,64 | 0,342 | 53 | 741 | 24,02 | 0,320 | 31 | 2770 | 21,55 | 0,173 |
| 53 | 357 | 24,65 | 0,423 | 39 | 2275 | 22,76 | 0,199 | 55 | 302 | 26,80 | 0,553 | 32 | 332 | 23,33 | 0,441 |
| 54 | 994 | 25,90 | 0,295 | 40 | 2079 | 24,21 | 0,215 | 56 | 1379 | 23,86 | 0,243 | 33 | 1709 | 21,97 | 0,233 |
| 56 | 1377 | 21,97 | 0,241 | 42 | 396 | 23,49 | 0,382 | 57 | 690 | 21,73 | 0,353 | 34 | 396 | 23,49 | 0,382 |
| 57 | 633 | 20,64 | 0,342 | 43 | 1594 | 21,27 | 0,223 | 58 | 633 | 20,64 | 0,342 | 36 | 783 | 20,73 | 0,329 |
| 58 | 472 | 27,00 | 0,454 | 44 | 883 | 27,67 | 0,330 | 59 | 2670 | 24,28 | 0,184 | 37 | 809 | 19,81 | 0,298 |
| 59 | 1603 | 22,95 | 0,230 | 45 | 1918 | 24,00 | 0,215 | 60 | 1785 | 23,32 | 0,221 | 38 | 11978 | 23,72 | 0,086 |
| 60 | 1427 | 23,53 | 0,241 | 46 | 13095 | 23,36 | 0,082 | 61 | 11970 | 23,28 | 0,086 | 39 | 1410 | 22,22 | 0,237 |
| 61 | 953 | 24,52 | 0,320 | 47 | 1421 | 24,59 | 0,247 | 62 | 684 | 22,11 | 0,358 | 40 | 1376 | 23,32 | 0,258 |
| 62 | 11970 | 23,28 | 0,086 | 48 | 1008 | 24,93 | 0,314 | 63 | 981 | 25,35 | 0,326 | 41 | 481 | 28,68 | 0,481 |
| 63 | 684 | 22,11 | 0,358 | 49 | 669 | 20,62 | 0,321 | 64 | 876 | 24,70 | 0,319 | 42 | 1896 | 24,08 | 0,210 |
| 65 | 1857 | 25,05 | 0,229 | 50 | 2300 | 25,22 | 0,183 | 65 | 1408 | 24,32 | 0,247 | 43 | 1316 | 25,87 | 0,279 |
| 67 | 1619 | 23,91 | 0,244 | 51 | 2162 | 24,49 | 0,208 | 66 | 1088 | 25,37 | 0,307 | 44 | 1026 | 22,02 | 0,263 |
| 68 | 481 | 28,77 | 0,442 | 52 | 1133 | 24,74 | 0,251 | 67 | 2729 | 24,79 | 0,181 | 45 | 1497 | 21,81 | 0,235 |
| 69 | 1707 | 23,37 | 0,211 | 53 | 1473 | 23,96 | 0,241 | 68 | 681 | 23,44 | 0,324 | 46 | 928 | 25,63 | 0,280 |
| 70 | 396 | 23,49 | 0,382 | 55 | 1085 | 24,71 | 0,250 | 69 | 1368 | 24,35 | 0,251 | 47 | 641 | 27,08 | 0,402 |
| 71 | 1712 | 25,58 | 0,248 | 56 | 1798 | 24,22 | 0,238 | 70 | 412 | 24,47 | 0,386 | 48 | 2920 | 25,05 | 0,163 |
| 72 | 681 | 23,44 | 0,324 | 57 | 1803 | 23,34 | 0,221 | 71 | 2164 | 25,09 | 0,214 | 49 | 819 | 21,09 | 0,318 |
| 74 | 1090 | 25,11 | 0,248 | 58 | 2713 | 22,97 | 0,171 | 72 | 287 | 25,86 | 0,477 | 50 | 634 | 25,77 | 0,376 |
| 75 | 381 | 29,65 | 0,535 | 59 | 766 | 24,13 | 0,342 | 73 | 974 | 21,17 | 0,296 | 51 | 839 | 22,60 | 0,306 |
| 76 | 1783 | 24,11 | 0,227 | 60 | 2419 | 22,34 | 0,189 | 75 | 898 | 24,28 | 0,294 | 53 | 1085 | 24,71 | 0,250 |
| 77 | 664 | 20,15 | 0,327 | 61 | 1659 | 23,58 | 0,228 | 76 | 2786 | 23,68 | 0,167 | 55 | 1798 | 24,22 | 0,238 |
| 79 | 287 | 25,86 | 0,477 | 62 | 691 | 25,51 | 0,336 | 78 | 2383 | 23,74 | 0,188 | 56 | 1113 | 23,42 | 0,269 |
| 80 | 1011 | 21,79 | 0,294 | 63 | 1363 | 20,08 | 0,222 | 79 | 999 | 24,09 | 0,300 | 57 | 1671 | 24,08 | 0,235 |
| 81 | 656 | 23,20 | 0,361 | 64 | 1484 | 24,26 | 0,215 | 80 | 713 | 25,26 | 0,346 | 58 | 1732 | 22,00 | 0,206 |
| 84 | 568 | 23,20 | 0,361 | | | | | 81 | 2872 | 21,34 | 0,154 | 59 | 432 | 22,31 | 0,364 |
| 85 | 982 | 24,60 | 0,307 | | | | | 82 | 1771 | 24,05 | 0,223 | 60 | 1702 | 22,92 | 0,231 |
| 86 | 1796 | 23,99 | 0,196 | | | | | 83 | 667 | 23,04 | 0,346 | 61 | 1051 | 22,72 | 0,302 |
| 87 | 2111 | 22,47 | 0,197 | | | | | 85 | 691 | 25,51 | 0,336 | 62 | 1011 | 23,54 | 0,304 |
| 88 | 776 | 21,87 | 0,243 | | | | | | | | | 63 | 1339 | 24,60 | 0,241 |
| 89 | 634 | 25,77 | 0,376 | | | | | | | | | 64 | 707 | 20,96 | 0,326 |
| 90 | 708 | 26,88 | 0,337 | | | | | | | | | 65 | 1106 | 19,91 | 0,217 |
| 91 | 656 | 19,13 | 0,296 | | | | | | | | | 66 | 1034 | 25,67 | 0,267 |
| 92 | 1361 | 23,10 | 0,241 | | | | | | | | | | | | |
| 93 | 667 | 23,04 | 0,346 | | | | | | | | | | | | |
| 94 | 1473 | 24,49 | 0,253 | | | | | | | | | | | | |
| 102 | 691 | 25,51 | 0,336 | | | | | | | | | | | | |

**Table 6.** The least squares averages and standard deviations of milk yields according to different types of SNI values in Polatlı Agricultural Enterprise

| THIa | Controls Num | Controls MY | STD Err | THIb | Controls Num | Controls MY | STD Err | THIc | Controls Num | Controls MY | STD Err | THId | Controls Num | Controls MY | STD Err |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 38 | 262 | 23,42 | 0,394 | 30 | 258 | 24,68 | 0,476 | 40 | 446 | 25,58 | 0,292 | 21 | 132 | 23,09 | 0,602 |
| 39 | 184 | 28,65 | 0,317 | 31 | 609 | 27,03 | 0,278 | 41 | 425 | 26,33 | 0,370 | 23 | 292 | 27,31 | 0,418 |
| 40 | 1166 | 25,67 | 0,251 | 32 | 262 | 23,42 | 0,394 | 43 | 440 | 23,68 | 0,466 | 24 | 301 | 27,63 | 0,423 |
| 41 | 474 | 23,71 | 0,451 | 33 | 132 | 23,09 | 0,602 | 44 | 258 | 24,68 | 0,476 | 26 | 258 | 24,68 | 0,476 |
| 42 | 258 | 24,68 | 0,476 | 34 | 1192 | 25,23 | 0,253 | 45 | 1192 | 25,23 | 0,253 | 28 | 474 | 23,71 | 0,451 |
| 44 | 248 | 20,75 | 0,507 | 36 | 732 | 25,13 | 0,332 | 46 | 248 | 20,75 | 0,507 | 29 | 793 | 23,62 | 0,263 |
| 45 | 417 | 25,21 | 0,388 | 37 | 248 | 20,75 | 0,507 | 48 | 370 | 29,55 | 0,357 | 30 | 425 | 26,33 | 0,370 |
| 46 | 560 | 23,83 | 0,320 | 39 | 767 | 26,13 | 0,292 | 49 | 428 | 24,06 | 0,375 | 31 | 692 | 23,00 | 0,339 |
| 47 | 370 | 29,55 | 0,357 | 40 | 370 | 29,55 | 0,357 | 50 | 132 | 23,09 | 0,602 | 33 | 1454 | 25,98 | 0,213 |
| 50 | 623 | 27,53 | 0,296 | 41 | 750 | 23,09 | 0,276 | 51 | 734 | 26,11 | 0,266 | 35 | 248 | 20,75 | 0,507 |
| 51 | 403 | 24,80 | 0,327 | 42 | 853 | 25,66 | 0,277 | 53 | 292 | 27,31 | 0,418 | 36 | 998 | 27,51 | 0,245 |
| 53 | 777 | 26,52 | 0,293 | 43 | 667 | 27,40 | 0,302 | 54 | 364 | 28,03 | 0,434 | 37 | 1721 | 26,00 | 0,193 |
| 55 | 919 | 24,62 | 0,278 | 44 | 916 | 24,79 | 0,247 | 55 | 413 | 25,19 | 0,387 | 40 | 1578 | 25,01 | 0,191 |
| 56 | 642 | 24,58 | 0,308 | 45 | 1076 | 24,34 | 0,233 | 56 | 1214 | 25,61 | 0,227 | 41 | 726 | 28,49 | 0,250 |
| 57 | 1562 | 26,47 | 0,203 | 46 | 1182 | 25,62 | 0,243 | 57 | 897 | 23,11 | 0,249 | 42 | 1189 | 26,61 | 0,216 |
| 58 | 293 | 22,98 | 0,338 | 47 | 739 | 27,86 | 0,287 | 58 | 1305 | 26,57 | 0,225 | 43 | 452 | 26,11 | 0,317 |
| 59 | 910 | 25,49 | 0,262 | 48 | 2154 | 26,56 | 0,155 | 59 | 910 | 25,49 | 0,262 | 44 | 601 | 24,97 | 0,321 |
| 60 | 628 | 25,30 | 0,325 | 49 | 509 | 27,86 | 0,436 | 60 | 1047 | 25,47 | 0,253 | 45 | 691 | 24,94 | 0,268 |
| 61 | 419 | 25,72 | 0,404 | 50 | 580 | 25,40 | 0,307 | 61 | 562 | 25,42 | 0,299 | 46 | 1204 | 25,83 | 0,264 |
| 63 | 1760 | 26,30 | 0,198 | 51 | 1043 | 23,63 | 0,244 | 62 | 1633 | 26,40 | 0,219 | 48 | 182 | 21,32 | 0,486 |
| 64 | 435 | 25,54 | 0,432 | 52 | 1188 | 26,45 | 0,256 | 63 | 599 | 24,99 | 0,303 | 49 | 471 | 27,23 | 0,398 |
| 65 | 599 | 24,99 | 0,303 | 53 | 557 | 27,10 | 0,293 | 64 | 505 | 26,12 | 0,415 | 50 | 1345 | 25,63 | 0,233 |
| 66 | 505 | 26,12 | 0,415 | 54 | 1655 | 24,98 | 0,183 | 65 | 576 | 23,46 | 0,301 | 51 | 972 | 25,81 | 0,214 |
| 67 | 394 | 23,56 | 0,379 | 55 | 376 | 23,63 | 0,475 | 66 | 1391 | 26,78 | 0,191 | 52 | 598 | 26,02 | 0,322 |
| 68 | 341 | 26,66 | 0,356 | 56 | 2301 | 24,87 | 0,173 | 68 | 1370 | 27,02 | 0,264 | 53 | 376 | 23,63 | 0,475 |
| 69 | 634 | 25,29 | 0,270 | 57 | 813 | 25,07 | 0,312 | 69 | 1199 | 25,20 | 0,266 | 54 | 1629 | 24,92 | 0,194 |
| 70 | 200 | 29,27 | 0,536 | 58 | 1871 | 26,26 | 0,199 | 70 | 666 | 24,44 | 0,280 | 55 | 961 | 24,40 | 0,283 |
| 71 | 1078 | 28,49 | 0,263 | 59 | 2467 | 25,34 | 0,176 | 71 | 1898 | 24,95 | 0,188 | 56 | 780 | 25,57 | 0,322 |
| 72 | 477 | 26,65 | 0,480 | 60 | 1719 | 25,19 | 0,219 | 72 | 1242 | 25,96 | 0,252 | 57 | 386 | 24,35 | 0,397 |
| 73 | 1156 | 25,53 | 0,259 | 61 | 503 | 23,08 | 0,336 | 73 | 1677 | 26,50 | 0,203 | 58 | 2153 | 26,15 | 0,181 |
| 74 | 609 | 22,60 | 0,344 | 62 | 607 | 27,45 | 0,357 | 74 | 886 | 23,40 | 0,270 | 59 | 1054 | 24,78 | 0,255 |
| 76 | 784 | 25,69 | 0,283 | 63 | 822 | 23,89 | 0,275 | 75 | 1538 | 24,48 | 0,203 | 60 | 2302 | 25,43 | 0,193 |
| 77 | 1074 | 25,56 | 0,231 | | | | | 76 | 1167 | 25,18 | 0,241 | 61 | 548 | 25,54 | 0,401 |
| 78 | 1334 | 24,79 | 0,235 | | | | | 77 | 1894 | 24,92 | 0,187 | 62 | 503 | 23,08 | 0,336 |
| 79 | 794 | 25,59 | 0,320 | | | | | | | | | 64 | 607 | 27,45 | 0,357 |
| 81 | 711 | 23,11 | 0,331 | | | | | | | | | 65 | 496 | 23,56 | 0,393 |
| 82 | 2304 | 25,31 | 0,169 | | | | | | | | | 66 | 326 | 24,39 | 0,348 |
| 85 | 427 | 25,72 | 0,471 | | | | | | | | | | | | |
| 86 | 326 | 24,39 | 0,348 | | | | | | | | | | | | |
| 87 | 967 | 23,98 | 0,261 | | | | | | | | | | | | |
| 88 | 1127 | 26,35 | 0,232 | | | | | | | | | | | | |
| 89 | 264 | 27,16 | 0,457 | | | | | | | | | | | | |
| 92 | 503 | 23,08 | 0,336 | | | | | | | | | | | | |

milk yield loss per cow may range between 1884-3353 kg per year. In other words, as each unit increases from 73, which is the critical value, to 81 it means 1.67 kg milk loss per animal. Similarly, the mean milk yield loss per cow in Türkgeldi and Polatlı Agricultural enterprises ranges between 1030-1601 kg and 476-1408 kg, per year, respectively.

Many studies done around the world support us; investigated the effect of heat stress on milk yield on the daily milk test records (33600) of Holstein cows in Egyptian conditions, in a study where the THI value reached the critical threshold of 79 and a daily milk yield of approximately 14.20%. found a decrease (Nasr and El-Tarabany, 2017). In another study found that the negative effect of heat stress on milk yield in Holstein cows raised in the Marmara region could be noticed at THI 65, but after THI 70, milk yield decreased irreversibly (Duru et al., 2018). In their study with 23,296 thousand brown Swiss cows in Italy between 2009 and 2018, determined that THI values greater than 74 were accepted as the beginning of heat stress in milk yield. In addition, they stated that for a unit change in the THI, 0.39 kg decrease occurred in the milk yield of each cow (Maggiolino et al., 2020)

It was determined that milk yield losses per animal were 712, 735, and 740 kg for THIb, 592, 861, and 1848 kg for THIc and 363, 340, and 441 kg for THId in the same order in Çukurova, Polatlı, Türkgeldi, respectively.

## CONCLUSION

Milk losses that may result from heat stress in enterprises are highly significant. When the annual milk yield loss per animal in all enterprises was considered, the lowest loss was observed at the THId value.

The results of the research enable dairy cattle breeders to take the necessary precautions by keeping in mind the critical periods that the study considered. It is important for the breeders to pay attention to the steps from the shelter type to the content of the ration (energy, protein) and the use of showers and fans to cool the animals.

As global warming, which has recently attracted attention and made its effects stronger each passing day, will increase in the future, the importance of developing genotypes resistant to heat stress in livestock as well as in plant products in agriculture and considering them as selection criterion raises.

Therefore, the inclusion of THI as a selection criterion in a selection index is recommended, especially for dairy cattle raised in hot environments in which profitability may continue to deteriorate over time.

## COMPLIANCE WITH ETHICAL STANDARDS

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Ethics committee approval is not required.

### Funding

No financial support was received for this study.

### Data availability

Not applicable.

### Consent for publication

Not applicable.

## REFERENCES

Anonymous, (2015). TMMO Ziraat Mühendisleri Odası. (in Turkish). [URL]

Berman A., Folman Y., Kaim M., Mamen M., Herz Z., Wolfenson D., ArieliA., Graber Y. (1985). Upper critical temperatures and forced ventilation effects for high yielding dairy cows in a subtropical climate, J. Dairy Sci. 68 1488–1495. [CrossRef]

Cook, N., Mentink, R., Bennett, T. ve Burgi, K. (2007). Isı stresi ve topallığın emziren süt ineklerinin zaman bütçeleri üzerindeki etkisi, J. Dairy Sci., 90, 1674–1682, 2007. (in Turkish) [CrossRef]

Duru,S. (2018). Bursa İli Holştayn İneklerde Günlük Süt Veriminde Isı Stresinin Başlangıç Düzeyinin Belirlenmesi , 65 , Ankara Üniv Vet Fak Derg, s. 193 – 198. (İn Turkish) [CrossRef]

FAO, (2021). Food and Agriculture Organization of the United Nations. [URL]

Johnson H.D. (1980). Environmental management of cattle to minimize the stress of climate changes, Int. J. Biometeor. 24 (Suppl. 7, Part 2) 65–78.

Johnson, H.D. (1987). Bioclimatology and the Adaptation of Livesstock, World Animal Science B5: 279

Kadzare, C. T., Murphy, M. R., Silanikove, N., Maltz, E. (2002). Heat stress in lactating dairy cows: a review.Livestock Production Sci. 77:59-91. [CrossRef]

Maggiolino A, Dahl GE, Bartolomeo N, Bernabucci U, Vitali A, Serio G, Cassandro M, Centoducati G, Santus E, De Palo P. (2020). Estimation of maximum thermo-hygrometric index thresholds affecting milk production in Italian Brown Swiss cattle. Journal of Dairy Science 103:8541-8553. [CrossRef]

Mutaf, S., Sonmez, R. (1984). Climatic environment and control in animal shelters. Aegean. Univ. Faculty of Agriculture Publications. Publication No: 438. Izmir

NADIS, (2022). National Animal Disease Information Service. Managing Heat Stres İn Dairy Cows. [URL]

Nasr, M.A.F. and El-Tarabany, M. S. (2017). Impact of three thı

levels on somatic cell count, mik yield and composition of multiparous holstein cows in a subtropical region. Journal of Thermal Biology, 64: 73-77. [CrossRef]

Ravagnolo O, Misztal I (2000) Genetic component of heat stress in dairy cattle, parameter estimation. Journal of Dairy Science 83: 2126-2130. [CrossRef]

Shehab-El-Deen, M.A.M.M., Leroy, J.L.M.R., Fadel, M.S., Saleh, S.Y.A., Maes, D., Van Soom, A. (2010). Biochemical changes in th follicular fluid of the dominant follicle of high producing dairy cows exposed to heat stress early post-partum. Anim Reprod Sci. 117: 189-200. [CrossRef]

SPSS, (2017). SPSS for Windows, Version 25.0, SPSS Inc. Chicago.

TUİK, (2021). Turkish Statistical Enstitute. [URL]

West, J.W. (2003). Effect of heat-stress on production in dairy cattle. J.Dairy Sci. 86: 2131-2144. [CrossRef]

Yavuz, H.M., Biricik, H. (2009). Süt sığırlarının sıcak stresinde beslenmesi. Uludağ Univ. J.Fac. Vet. Med.28 :1-7. (in Turkish). [Google Scholar]

Zimbleman, RB, Rhoads, RP, Rhoads, ML, Duff, GC, Baumgard, LH, Collier, R.J. (2009). A re-valuation of the impact of temperature humidity index (THI) and black globe humidity index (BGHI) on milk production in high producing dairy cows. Proceedings of the 24th Annual Southwest Nutrition and Management Conference, pp. 158–168.

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 704-710
DOI: https://doi.org/10.31015/jaefs.2022.4.26

# Comparison on flower, fruit and seed characteristics of tetraploid and diploid watermelons (*Citrullus lanatus* Thunb. Matsum. and Nakai)

**Pınar Adıgüzel**[1] • **İlknur Solmaz**[1] • **Şenay Karabıyık**[1] • **Nebahat Sarı**[2]

[1] Horticulture Department, Faculty of Agriculture, Cukurova University, Adana, Türkiye
[2] Department of Chemical and Biological Engineering, Princeton University, Princeton, New Jersey, USA

## Abstract

In this study, the characteristics of tetraploid and diploid watermelon's flowers, fruits, and seeds were examined, and their differences were investigated. As plant material, tetraploid ST101 and ST82; diploid WL92, WL124, WL134, WL216, WL259-B and WL235 were used. Flower (pollen viability, pollen germination, number of anthers and number of pollen per anther and flower); fruit (weight, length, diameter, rind thickness and TSS) and seed (weight of 1000 seed, length, width, thickness, full seed number, seed yield, embryo/seed ratio, germination and emergence) parameters were investigated. In terms of pollen parameters, diploid watermelon has a higher value than tetraploid watermelon. According to research results, average pollen germination was found to be in diploid watermelons at 74.48% and tetraploid watermelons at 71.62%. Pollen viability was determined highest in WL 235 (95.53%). In fruit parameters, tetraploid watermelons had higher values, but fruit length decreased (diploid 19.32 cm; tetraploid 15.33 cm) significantly. Considering the average values of tetraploid watermelons, a significant decrease occurred in terms of seed germination, full seed number, seed yield and embryo/seed ratio (57.22%, 225.48 seed, 6.33 g/fruit, 57.51% respectively in diploids; 37.31%, 57.67 seed, 4.90 g/fruit, 46.42% in tetraploid), and no difference observed in other seed parameters.

**Keywords:** Tetraploid, Diploid, Pollen, Watermelon, Seed Germination

**Citation:** Adiguzel, P., Solmaz, I., Karabiyik, S., Sari, N. (2022). Comparison on flower, fruit and seed characteristics of tetraploid and diploid watermelons (*Citrullus lanatus* Thunb. Matsum. and Nakai). International Journal of Agriculture, Environment and Food Sciences, 6 (4), 704-710

**Received:** 01 November 2022
**Revised:** 12 December 2022
**Accepted:** 15 December 2022
**Published Online:** 24 December 2022

**Correspondence:** Pınar Adıgüzel
**E-mail:** pinaradiguzel63@gmail.com



## INTRODUCTION

Watermelon (*Citrullus lanatus* L. Matsum. and Nakai) is an economically important vegetable in the *Cucurbitaceae* family (Zhang et al., 2019). Although watermelons are generally diploid (2n=2x=22), they can also be found in triploid (3n=3x=33) and tetraploid (4n=4x=44) structures as autopolyploids (Blakeslee and Avery, 1937). Autopolyploids differ from diploids in that they have different DNA content, contain high levels of secondary metabolite organisms, have large tissues and organs, have a high yield, and provide tolerance to biotic and abiotic stress factors (Soltis et al., 2016; Godfree et al., 2017). However, apart from these benefits, their low frequency and undetermined time of occurrence are also disadvantages (Zhang et al., 2019). To eliminate this situation, colchicine, which was applied first to the Datura plant by Blakeslee and Avery in 1937, has been the most successful method for obtaining tetraploids today (Blakeslee and Avery, 1937; Kihara, 1951; Andrus, 1971; Suying et al., 1993). Tetraploid lines are obtained by applying chemicals such as colchicine or oryzalin to diploid lines (Kihara, 1951; Lower and Johnson, 1969; Koh, 2002; Li et al., 2002; Jaskani et al.,

2004; Inan, 2007). As a result of this application, a limited number of tetraploid seedlings, a high rate of chimera seedlings, and diploid seedlings with unchanged structures have emerged (Compton and Gray, 1994; Jaskani et al., 2004). Inbreeding and tissue culture can be used to reproduce tetraploid lines (Compton and Gray, 1991; Krug et al., 2005; Zhang, 2010). Tetraploid lines can be identified through morphological observations of differences in leaf, ovary, and flower sizes as well as through chromosome counting (Sari et al., 1999; Jaskani and Khan, 2000) and flow cytometry (FCM) methods (Compton et al., 1996; Norrmann et al., 1997; Rhodes and Zhang, 2000). Finally, the qPCR method was found to be another method that allows for determining the plant ploidy level (Zhang et al., 2019). Because of the small and deeply embedded chromosomes in watermelon, the chromosome counting technique is not widely used (Fahleson et al., 1988; Şimşek et al., 2013). The flow cytometry (FCM) technique is the most preferred method because of its fast and reliable nature (Arumuganathan and Earle, 1991; Dolezel, 1998; Koh, 2002; Jaskani et al., 2004; Jaskani et al., 2005). Tetraploids are the primary parents in the triploid breeding program. The triploid plants are obtained by the cross-pollination of tetraploids and diploids (Kihara, 1951; Jaskani and Khan, 2000). Tetraploids are also valuable germplasm and cultivars (Jaskani et al., 2005). The objective of this study was to compare the flower, fruit, and seed properties of tetraploid and diploid watermelon lines.

## MATERIALS AND METHODS

This study was conducted in 2018 growing seasons in the plastic greenhouse at the Research Application Area of Horticulture Department (latitude 37°1′48.63″N, longitude 35°22′3.74″E, altitude 56 m), Cukurova University, Adana, Turkey.

### Plant Material

In this study two tetraploid (4n) lines (ST 101 and ST 82) were used as female parents, and six diploid (2n) lines (WL 124, WL 92, WL 235, WL 134, WL 259-B and WL 216) were used as male parents. Tetraploid female and diploid male parental lines were grafted onto Nun9075 interspecific hybrid (*C. maxima* x *C. moschata*) rootstock. The grafted combination seedlings were planted on 04.05.2018 in a plastic greenhouse of double row with (100-50) x 50 cm spacing distances within three repetitions. Twenty healthy plants of both tetraploid and diploid seedlings were planted. A total of 120 plants were used (20 plants x 3 replications, both tetraploid and diploid watermelon). Both female flowers and male flowers were isolated one day before anthesis. The following day morning selfing was performed. Fruit analysis was carried out on 4 plants from each plot (4 plants x 3 replicates). In the study, the number of anthers in a flower, pollen number in an anther, pollen viability rate (%) and germination rate (%); fruit characteristics [(fruit weight (g), length (cm), diameter (cm), rind thickness (mm), TSS (%)] and seed characteristics [yield (g/fruit), number of full seeds (number/fruit), 1000 seed weight (g), embryo/seed ratio (%), length (mm), width (mm) and thickness (mm), seed germination rate (%) time (day), emergence rate (%) and time (day)] parameters were examined. Seed length, width and thickness, were measured using a digital caliper (Mitutoyo), while seed weight was measured using a digital scale.

### Flower Analysis

Pollen viability and germination tests were carried out with the pollen collected from the male flowers in the anthesis stage. The %1, 2,3,5 Triphenyltetrazolium Chloride (TTC) was used to determine pollen viability rates (Norton, 1966). The TTC indicator was used to assess seed viability. The red color variation, ranging from a light to a dark red hue, was shown to result from the reaction between the dehydrogenase enzyme and the reddish component known as formazan in this indicator. The viability of the seed was analyzed based on these colors (Shivanna and Rangaswamy, 1992; Sensoy et al., 2003). The empty pollen slides when it comes into contact with the fluid dye, so it is preferable to count the pollen in-between the preparations during counting (Elçi, 1982). The TTC test results demonstrated that viable pollen turned red, semi-viable pollen pink, and non-viable pollen was white (Eti, 1991). During the pollen counting stage, pollen in the center of the lamella should be counted because the pollen at the edge of the lamella contains more oxygen, this may cause a difference in the pollen staining rate (Shivanna and Rangaswamy, 1992). Pollen germination rates were identified by using the medium containing boric acid (250 ppm), agar (1%) and sucrose (10%) in petri dishes at 25°C (Eti,1991; Karabıyık et al., 2017).

### Fruit and Seed Analysis

Fruits were weighed (g), cut in the middle and measured with a ruler to determine fruit length (cm) and diameter (cm). Rind thickness was determined using a digital caliper (Mitutoyo) total soluble solids (TSS, %) using a digital refractometer (Atago). Three fruits of each replication were used in fruit analysis. The seeds were fermented after being removed from the fruit for 3 days, then washed, and dried. Seed analyses were performed with 4 replications of 100 seeds in each repetition. Seed length, width, seed thickness, and fruit diameter were measured using a digital caliper (Mitutoyo), while seed weight was measured using a digital scale. Seeds have been germinated in an incubator (Memmert) at 25°C according to ISTA rules (ISTA, 2018) Seed germination is determined by dividing the germinated seeds by the total number of planted seeds.

The experiment was set up according to the randomized plot design. The data were analyzed using the JMP program (v8.00, SAS Institute Inc., NC 27513–2414, USA).

## RESULTS AND DISCUSSION

In terms of the average number of anthers in male flowers, there was no statistical difference between used watermelon lines. The study resulted in 5.20 anthers per flower in tetraploids and 5.04 anthers per flower in diploids (Table 1). In previous reports, it was stated that the average number of anthers (3.00) in a flower of tetraploid-diploid gourd and diploid watermelon (Kombo, 2017; Hassan et al., 2020) and (3.40) of triploid watermelon (Hussein, 2017). Normally, the number of anthers in a watermelon flower is 5, however, most of the time 2 anthers appear to be attached, so they can be counted as a single anther.

The highest numbers of pollen in the anther (22112.82) and in the flower (112604.95) were obtained from WL 134 (Table 1). According to the mean values of evaluated parameters, the diploid watermelon lines had higher values (19.735.34; 99640.4, respectively) than tetraploid lines (17128.01; 89074.29, respectively). The highest pollen number in anther (124486.70) was found from the diploid Crimson Sweet grafted onto Argentario whereas the lowest pollen number (60917.76 ) was determined in ungrafted group (Kombo 2017).

In terms of pollen viability, the highest rate (95.53%) was found from WL 235 line while the lowest value (87.60%) was obtained from ST 82 line (Table 1). According to mean values of pollen viability rate among tetraploid and diploid, in diploids (92.23%) were higher than in tetraploids (87.76%) lines. There was no statistical difference between diploid and tetraploid lines in terms of pollen germination rates. The average values of pollen germination rates were 71.62% in tetraploids and 74.48% in diploids (Table 1). The pollen germination rate was affected by the pollen collection time, incubation conditions, environment, and pollen density (La Porta and Roselli, 1991). Moreover, various factors influence pollen viability and germination rates, such as environmental factors and the pollen viability period of each plant (Nepi and Pacini, 1993). In the study of Gok et al. (2005), 45 watermelon genotypes were evaluated and the highest pollen viability rates were determined as 97.40% and 97.36% while the lowest were 49.65% and 61.08% and between Furthermore, 89.43%–88.23%, pollen germination rates were found to be the highest, and the lowest were ranged between 19.62%–20.22%. Freeman et al. (2008), reported that pollen germination rates in diploid watermelons was in range of 97% and 99.2%.

**Table 1.** The number of anthers in a flower, number of pollen in an anther, number of pollen in a flower, pollen viability rate (%) and pollen germination rate (%) in tetraploid and diploid watermelon.

| Genotype Name | The number of anthers in a flower | Number of pollen in an anther | Number of pollen in a flower | Pollen viability rate (%) | Pollen germination rate (%) |
|---|---|---|---|---|---|
| ST 82 | 5.20 | 14740.39 D | 76650.02 D | 87.60 D (70.01) | 72.75 (64.31) |
| ST 101 | 5.20 | 19515.62 ABC | 101498.57 ABC | 87.92 CD (71.36) | 70.50 (59.93) |
| Tetraploid Average | 5.20 | 17128.01 | 89074.29 | 87.76 | 71.62 |
| WL 134 | 5.08 | 22112.82 A | 112604.95 A | 92.11 BCD (73.15) | 75.88 (63.36) |
| WL 216 | 5.00 | 16737.17 CD | 83685.83 CD | 93.59 AB (76.87) | 74.00 (58.00) |
| WL 259-B | 5.17 | 21395.42 AB | 110718.46 AB | 89.50 CD (71.26) | 68.75 (54.71) |
| WL 92 | 5.00 | 19066.85 ABC | 95334.25 A-D | 93.16 ABC (74.45) | 73.75 (59.48) |
| WL 124 | 5.00 | 18497.18 BC | 92485.92 BCD | 89.50 CD (69.01) | 79.50 (61.23) |
| WL 235 | 5.00 | 20602.61 AB | 103013.07 ABC | 95.53 A (77.94) | 75.00 (59.02) |
| Diploid Average | 5.04 | 19735.34 | 99640.4 | 92.23 | 74.48 |

LSD (the number of anthers in a flower): NS; LSD (number of pollen in an anther): 3528.82***; LSD (number of pollen in a flower): 19460.16***; LSD (pollen viability, %): 4.40***; LSD (pollen germination, %): NS

NS: Not Significant; *: ***: P ≤ 0.001; **: P ≤ 0.01; *: P ≤ 0.05: shows difference according to LSD comparison. Transform values are given in parentheses.

Fruit weight (g), length (cm) diameter (cm), rind thickness (mm) and TSS (%) values are presented in Table 2. Fruit weight was found to be higher in WL92 (2846.67 g), followed by ST82 (2593.33 g) and WL 124 (2111.11 g). However, in terms of average weight, tetraploids' fruit (2193.33 g) was heavier than diploids' (2135.44 g). According to the fruit length (cm), the longest fruit (22.26 cm) was obtained from WL 134 line. The widest fruit (16.48 cm) was found from ST 82 line. Due to fruit rind thickness, the thickest fruit rind (14.61 mm) was in ST 82. The TSS was high (12.23%) in WL 124 line. Diploid watermelon lines showed the highest values in fruit length, while tetraploids were greater in terms of fruit diameter, rind thickness, and TSS.

Generally, tetraploid plants start to flower later than diploids. The diploid-triploid-tetraploid flowers sizes increase proportionally to the number of chromosomes

**Table 2.** Fruit weight (g), fruits length (cm), fruit diameter (cm), rind thickness (mm) and TSS (%) in tetraploid and diploid watermelon.

| Genotype Name | Fruit Weight (g) | Fruit Length (cm) | Fruit Diameter (cm) | Rind Thickness (mm) | TSS (%) |
|---|---|---|---|---|---|
| ST 82 | 2593.33 AB | 16.10 CD | 16.48 A | 14.61 A | 9.66 C |
| ST 101 | 1793.33 C | 14.57 DE | 15.60 ABC | 10.48 B | 11.33 AB |
| Tetraploid Average | 2193.33 | 15.33 | 16.04 | 12.55 | 10.50 |
| WL 134 | 2104.00 BC | 22.26 A | 13.93 BCD | 10.84 B | 10.13 BC |
| WL 216 | 1780.00 C | 13.92 E | 14.89 A-D | 9.45 BC | 9.60 C |
| WL 259-B | 2062.00 BC | 21.59 A | 13.85 CD | 9.49 BC | 9.43 C |
| WL 92 | 2846.67 A | 21.97 A | 16.03 A | 13.22 A | 11.43 AB |
| WL 124 | 2311.11 ABC | 17.89 BC | 15.70 AB | 7.98 C | 12.23 A |
| WL 235 | 1708.89 C | 18.26 B | 13.42 D | 11.01 B | 8.90 C |
| Diploid Average | 2135.44 | 19.32 | 14.64 | 10.33 | 10.28 |

LSD (Fruit Weight): 691.14*; LSD (Fruits Length): 1.93***; LSD (Fruit Diameter): 1.80*; LSD (Rind Thickness): 1.75*** LSD (TSS): 1.40**

NS: Not Significant; *: ***: P ≤ 0.001; **: P ≤ 0.01; *: P ≤ 0.05: shows difference according to LSD comparison. Transform values are given in parentheses.

(Kihara, 1951). The rate of TSS was higher in tetraploid watermelons than in diploid watermelons, it was between 12% -14% in tetraploid watermelons (Zhang, 2010). Şimşek et al., (2013) reported the TSS rate ranged between 8% - 10% in diploid watermelons. The fruit weights of tetraploid and diploid watermelons are found to be similar (Kihara 1951; Jaskani et al., 2005), however, Henderson (1977) stated that tetraploids form smaller fruits. The fruit weight varied between 1.5 and 2 kg in tetraploid watermelons (Zhang, 2010). In our study, the average fruit weight of diploid was 2135.44 g and 2193.33 g in tetraploid watermelon lines which are found to be similar. Tetraploid watermelons had lower values than diploids in terms of fruit length and diameter. Zhang et al. (2019), evaluated the fruit length and diameter, and found higher values in diploids than in tetraploids. It has been determined by Jaskani et al. (2005) that the rind thickness was 12.7 mm in diploid and 17.2 mm in tetraploid watermelons similarly.

The seed analysis results were presented in Table 3. The highest values were obtained in seed yield from WL 134 (12.98 g/fruit); in full seed number from WL 92 line (451 seed/fruit); in 1000 seed weight from line ST 101 (165.1

**Table 3.** Seed yield (g/fruit), number of full seed (seed/fruit), 1000 seed weight (g), embryo/seed ratio (%), seed length (mm), seed width (mm) and seed thickness (mm) in teraploid and diploid watermelon

| Genotype Name | Seed Yield (g/fruit) | Number of Full Seed (seed/fruit) | 1000 Seed Weight (g) | Embryo/Seed Ratio (%) | Seed Lenght (mm) | Seed Width (mm) | Seed Thickness (mm) |
|---|---|---|---|---|---|---|---|
| ST 82 | 4.69 DE | 70.67 EF | 66.51 B | 50.00 (45.02) CD | 9.63 A | 6.45 A | 2.65 A |
| ST 101 | 5.11 CD | 30.67 F | 165.15 A | 42.83 (40.89) D | 9.82 A | 6.06 B | 2.38 B |
| Tetraploid Average | 4.90 | 50.67 | 115.83 | 46.42 | 9.73 | 6.26 | 2.52 |
| WL 134 | 12.98 A | 339.27 B | 38.25 B | 62.14 (52.05) AB | 8.81 B | 5.55 C | 1.68 D |
| WL 216 | 5.77 CD | 166.44 D | 34.63 B | 57.07 (49.09) BC | 8.33 C | 5.06 E | 1.95 C |
| WL 259-B | 9.08 B | 261.583 C | 34.77 B | 66.46 (54.70) A | 8.64 B | 5.28 D | 1.54 E |
| WL 92 | 7.38 BC | 451.00 A | 16.37 B | 54.29 (47.50) BC | 6.46 D | 3.91 F | 1.59 DE |
| WL 124 | 0.59 F | 47.89 EF | 14.33 B | 55.12 (47.99) BC | 4.99 E | 3.15 G | 1.05 F |
| WL 235 | 2.16 EF | 86.72 E | 27.66 B | 49.99 (45.02) CD | 8.63 B | 5.16 DE | 1.49 E |
| Diploid Average | 6.33 | 225.48 | 27.67 B | 57.51 | 7.64 | 4.69 | 1.55 |

LSD (Seed Yield): 2.55***; LSD (Number of Full Seed): 48.93***; LSD (1000 Seed Weight): 56.69**; LSD (Embryo/Seed Ratio): 6.03*** LSD (Seed Lenght): 0.28*** LSD (Seed Width ): 0.16***; LSD (Seed Thickness): 0.13***;

NS: Not Significant; *: ***: P ≤ 0.001; **: P ≤ 0.01; *: P ≤ 0.05: shows difference according to LSD comparison. Transform values are given in parentheses.

**Table 4.** Seed germination (%), germination time (day), seed emergence (%) and emergence time (day) in tetraploid and diploid watermelon

| Genotype Name | Seed Germination Rate (%) | Germination Time (day) | Seed Emergence Rate (%) | Emergence Time (day) |
|---|---|---|---|---|
| ST 82 | 61.12 (51.48) A | 2.70 | 100.00 (90.04) | 1.80 |
| ST 101 | 13.50 (21.56) B | 1.50 | 60.00 (51.20) | 2.00 |
| Tetraploid Average | 37.31 | 2.10 | 80.00 | 1.90 |
| WL 134 | 65.00 (53.75) A | 0.27 | 86.67 (72.32) | 1.70 |
| WL 216 | 65.00 (53.75) A | 0.56 | 93.33 (81.21) | 3.20 |
| WL 259-B | 63.33 (52.76) A | 2.60 | 93.33 (81.20) | 1.25 |
| WL 92 | 55.00 (47.93) A | 0.79 | 80.00 (68.10) | 1.38 |
| WL 124 | 44.44 (41.77) A | 1.15 | 73.33 (63.88) | 2.84 |
| WL 235 | 50.56 (45.32) A | 0.56 | 73.33 (59.24) | 1.55 |
| Diploid Average | 57.22 | 0.98 | 83.33 | 1.99 |
| LSD (Germination %): 22.11**; LSD (Germination Time): NS; LSD (Emergence %): NS; LSD (Emergence Time): NS | | | | |

NS: Not Significant; *: ***: P ≤ 0.001; **: P ≤ 0.01; *: P ≤ 0.05: shows difference according to LSD comparison. Transform values are given in parentheses

g). In seed length, tetraploid lines ST 101 (9.82 mm) and ST 82 (9.63 mm) were in the same statistical group and had superior values. The highest seed width and thickness were determined in tetraploid ST 82 (6.45 mm and 2.65 mm respectively). The embryo/seed ratio was highest in WL 259-B (66.46%) and WL 134 (62.14%), which are in the same statistical group. In terms of seed parameters on average; diploids had the highest values in full seed number, seed yield and embryo/seed ratio; Tetraploids were superior in 1000 seed weight, seed length, seed width and seed thickness.

The seeds of tetraploid plants are thicker and broader than diploid seeds (Kihara, 1951) and more oblong-round shaped than diploids (Jaskani et al., 2005). Also, as a single layer of enlarged palisade cells forms the epidermis, there is an indented seed coat (Chopra and Swaminathan, 1960) with cracking of the palisade layer (Kihara 1951; Jaskani et al., 2005). It has been determined that while the embryo fills the seed coat in diploids, it does not fill the seed coat due to the space on the chalazion side in the seeds of tetraploids (Jaskani et al., 2005). Seed length, width and thickness in tetraploid watermelons was found 9 mm; 6.3 mm, and 2.8 mm respectively, the shape of the seed was observed as rectangular round in tetraploids by Jaskani et al. (2005). Zhang et al. (2019) reported that the seeds of tetraploid watermelons are larger than diploid watermelons. Chopra and Swaminathan (1960) examined the number of seeds per fruit and found very few in selfed tetraploid watermelons compared to open-pollinated tetraploids and diploids. Due to the number of seeds, a total of 681 seed in diploids and 446 seed in tetraploids were obtained in the study of Compton et al. (1996), 20.6-75.3 in tetraploids and 182.8-733 in diploid watermelon; Jaskani et al. (2005) determined that there were 323.5 seeds in diploid and 37.9 seeds in tetraploid watermelons As stated in other studies, a decrease in the number of seeds was observed in tetraploids. This situation is thought to be caused by the embryos of tetraploids not filling the seed coat, thus affecting the number of filled seeds.

The seed germination rate was found to be high in all lines except ST 101 tetraploid line (Table 4). Seed germination time, seed emergence rate, and emergence time values were found to be non-significant. Due to the seed emergence time (day) and rate (%), the results indicated that the earliest seed emergence time (0.27 day) was found from WL 134 line. While the highest seed emergence rate (100%) was obtained from ST 82 line. In terms of averages of seed germination and emergence in tetraploids and diploids, diploids were found to have higher values than tetraploids. Seed germination was inhibited by the seed coat formation mechanism. Based on the hard seed coat of tetrapoid seed, the water and gases passage is limited. The germination rate (%) is limited due to the seed coat thickness and the excess of air space between the embryo and rind (Jaskani et al., 2006). The germination rate is reduced not only by the thickness of seed coat but also due to the high moisture content in seed. The moisture content increases in the immature embryo, and the germination rate decreases according to the large air gap between the embryo and the seed coat (Grange et al., 2000; Grange et al., 2003). In addition, Jaskani et al. (2006), reported that the germination rates in tetraploid watermelons were 76.6% and 93.3% in diploids. The researchers have also demonstrated that the germination rates increased in diploids (98%) and tetraploids (78%), after the seeds were cracked (Jaskani et al., 2005).

## CONCLUSION

In this study, a comparison of the characteristics of tetraploid and diploid watermelons was performed. The results indicated that the increase in the number of chromosomes in watermelon plants affected the flower, fruit, and seed characteristics. Furthermore, seed yield, full seed weight, and embryo/seed ratio were decreased in tetraploid watermelon. On the other hand, the length,

width, thickness, and 1000 seed weight of the tetraploid seeds were increased. Tetraploid watermelons developed seeds larger with thicker rinds than diploid watermelons. This had a negative impact on seed germination rate/time, and emergence rate/time. The thickness of the fruit rind and seed coats in tetraploid plants has been proven to be a result of colchicine application which duplicates the chromosome number. Further studies are required to eliminate the effect of the colchicine chemical product on seed coat thickening in order to increase germination and emergence rates.

## COMPLIANCE WITH ETHICAL STANDARDS

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

N. S: Conceptualization and design of the research; P. A and Ş. K: Study data analysis; P. A: Wrote the original draft; and İ. S: Editing and preparation of the manuscript. All authors have read and approved the manuscript after N. S and I. S revised. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Ethics committee approval is not required.

### Funding

This study was funded by Cukurova University, Unit of Scientific Research Projects with project number FLY-2018-10957.

### Data availability

Not applicable.

### Consent for publication

Not applicable.

### Acknowledgements

The authors are thankful to Cukurova University, Unit of Scientific Research Projects. The authors also thank Antalya Tarım Productive, Consultant and Marketing Co., and Ismail SİMSEK for their collaboration and for providing grafted seedlings used in this work.

## REFERENCES

Andrus, C. F. (1971). Production of Seedless Watermelons, 1424. US Department of Agriculture.

Arumuganathan, K., Earle, E. D. (1991). Estimation of Nuclear DNA Content of Plants by Flow Cytometry. Plant Molecular Biology Reporter, 9(3): 229-241. [CrossRef]

Blakeslee, A. F., Avery, A. G. (1937). Methods of Inducing Doubling of Chromosomes in Plants: by Treatment With Colchicine. Journal of Heredity, 28 (12): 393-411. [CrossRef]

Chopra, V. L., Swamınathan, M. S. (1960). Induction of Polyploidy in Watermelon. Proceedings of the Indian Academy of Sciences-Section B. Springer India, 51(2), p. 57-65. [CrossRef]

Compton, M. E., Gray, D. J. (1991). Shoot Organogenesis on Cotyledons of Watermelon. Horticultural Science, 26: 772.

Compton, M .E., Gray, D .J . (1994). Adventitious Shoot Organogenesis and Plant Regeneration from Cotyledons of Tetraploid Watermelon. HortScience, 29: 211-213. [CrossRef]

Compton, M. E., Gray, D. J., Elmstrom, G. W. (1996). Identification of Tetraploid Regenerants from Cotyledons of Diploid Watermelon Cultured *in vitro*. Euphytica, 87(3): 165-172. [CrossRef]

Dolezel, J. (1998). Flow Cytometry, its Application and Potential for Plant Breeding. Current Topics in Plant Cytogenetics Related to Plant Improvement (Ed.): T. Lelley. Universitätsverlag, Vienna, 80-90.

Elçi, Ş. (1982). Observations and Research Methods in Cytogenetics. Fırat University Faculty of Arts and Sciences Publications, Biology:3, Malatya. 159.

Eti, S. (1991). Determination of Pollen Viability and Germination Capability of Some Fruit Species and Cultivars by Different *in vitro* Tests. J. Agric. Fac. Cukurova. Univ. 6:69–80.

Fahleson, J., Dixelius, J., Sundberg, E., Glimelius, K. (1988). Correlation Between Flow Cytometric Determination of Nuclear DNA Content and Chromosome Number in Somatic Hybrids within *Brassicaceae*. Plant Cell Reports, 7(1): 74-77. [CrossRef]

Freeman, J. H., Olson, S. M. and Kabelka, E. A. (2008). Pollen Viability of Selected Diploid Watermelon Pollenizer Cultivars. HortScience, 43(1), 274-275.

Godfree, R. C., Marshall, D. J., Young, A. G., Miller, C. H., Mathews, S. (2017). Empirical Evidence of Fixed and Homeostatic Patterns of Polyploid Advantage in a Keystone Grass Exposed to Drought and Heat Stress. Royal Society Open Science, 4(11): 170934. [CrossRef]

Gok, P., Yetisir, H., Solmaz, I., Sari, N., Eti, S. (2005), September. Pollen Viability and Germination Rates of 45 Watermelon Genotypes. In III International Symposium on Cucurbits 731, pp. 99-102. [CrossRef]

Grange, S. L., Leskovar, D. I., Pike, L. M., Cobb, B. G. (2000). Excess Moisture and Seedcoat Nicking İnfluence Germination of Triploid Watermelon. HortScience, 35(7): 1355-1356. [CrossRef]

Grange, S., Leskovar, D. I., Pike, L. M., Cobb, B. G. (2003). Seedcoat Structure and Oxygen-Enhanced Environments Affect Germination of Triploid Watermelon. Journal of the American Society for Horticultural Science, 128(2): 253-259. [CrossRef]

Hassan, J., Miyajima, I., Ozaki, Y., Mizunoe, Y., Sakai, K., Zaland, W. (2020). Tetraploid Induction by Colchicine Treatment and Crossing with a Diploid Reveals Less-Seeded Fruit Production in Pointed Gourd (*Trichosanthes dioica* Roxb.). Plants, 9(3): 370. [CrossRef]

Henderson, W. R. (1977). Effect of Cultivar, Polyploidy and Reciprocal Hybridization on Characters İmportant in Breeding Triploid Seedless Watermelon Hybrids. Journal of The American Society For Horticultural Science, 102(3): 293-297.

Hussein, S. (2017). The Effects of Different Rootstocks on Seed Yield and Quality in Seedless Watermelon Seed Production in Greenhouse. Çukurova University Institute of Science, Department of Horticulture, Master Thesis, Adana,65 s.

Inan, S. (2007). Obtaining Tetraploid Plants from Watermelon (*Citrullus Lanatus* (Thunb.) Matsum and Nakai) by *in vivo* and *in vitro* Methods. Çukurova University, Institute of Science, Department of Horticulture, Master Thesis, Adana, 80 p.

ISTA (International Rules for Seed Testing), Full Issue. (2018). i–19-8 (298) https://doi.org/10.15258/istarules.

Jaskani, M.J., Khan, I.A. (2000). Characterization of Interploid Hybrids of Kinnow Mandarin. In Proceedings of International Society of Citriculture, 1: 165-166.

Jaskani, M. J., Kwon, S. W., Koh, G. C., Huh, Y. C., Ko, B. R. (2004). Induction and Characterization of Tetraploid Watermelon. Horticulture Envıronment and Bıotechnology, 45(2): 60-65.

Jaskani, M. J., Kwon, S. W., Kim, D. H. (2005). Comparative Study on Vegetative, Reproductive and Qualitative Traits of Seven Diploid and Tetraploid Watermelon Lines. Euphytica, 145(3): 259-268. [CrossRef]

Jaskani, M. J., Kwon, S. W., Kin, D. H. (2005). Flow Cytometry of DNA Contents of Colchicine Treated Watermelon as a Ploidy Screening Method at MI Stage. Pakistan Journal of Botany, 37(3): 685.

Jaskani, M. J., Kwon, S. W., Kim, D. H., Abbas, H. (2006). Seed Treatments and Orientation Affects Germination and Seedling Emergence in Tetraploid Watermelon. Pakistan Journal of Botany, 38(1): 89.

Karabıyık, Ş., M.A. Sarıdaş, S. Eti, and S. Paydaş Kargı. (2017). The Effects of Boron and Calcium Applications on Pollen Characteristics and Distorted Fruit Formation in Sweet ann Strawberry Varieties. Bahçe 46(1):271–279.

Kihara, H. (1951). Triploid Watermelons. Proc. Amer. Soc. Hort. Sci.,58: 217-230.

Koh, G. C. (2002). Tetraploid Production of Moodeungsan Watermelon. Journal-Korean Socıety for Hortıcultural Scıence, 43(6): 671-676.

Kombo, M. D., (2017). Rootstock Effects on Seed Yield and Quality in Watermelon. Cukurova University Institute of Natural and Applied Sciences Deparment of Horticulture PhD Thesis, 97 p.

Krug, M.G.Z., Stipp, L.C.L., Rodriguez, A. P. M., Mendes, B.M.J. (2005). *In vitro* Organogenesis in Watermelon Cotyledons. Pesquisa Agropecuaria Brasileira, 40(9): 861-865.

La Porta, N., Roselli, G. (1991). Relationship Between Pollen Germination *in vitro* and Fluorochromatic Reaction in Cherry Clone F12/1 (*Prunus avium* L.) and Some of its Mutants. Journal of horticultural science, 66(2): 171-175. [CrossRef]

Li, L., He, Y., Lu, K. (2002). Chemical Induction Mutation in Yellow Peel Watermelon (*Citrullus Lanatus*) and Its Application to Tetraploid Watermelon Breeding. Chine Vegetables, 3: 8-11.

Lower, R. L., Johnson, K. W. (1969). Observations on Sterility of İnduced Autotetraploid Watermelons. Proc Amer Soc Hort Sci., 94:367-369

Nepi, M., Pacini, E. (1993). Pollination, Pollen Viability and Pistil Receptivity in *Cucurbita pepo*. Annals of Botany, 72(6):527-536. [CrossRef]

Norrmann, G. A., Quarín, C. L., Keeler, K. H. (1997). Evolutionary İmplications of Meiotic Chromosome Behavior, Reproductive Biology, and Hybridization in 6x and 9x Cytotypes of Andropogon Gerardii (Poaceae). American Journal of Botany, 84(2): 201-207. [CrossRef]

Norton, J.D. (1966). Testing of Plum Pollen Viability with Tetrazolium Salts. Proc. J. Am. Soc. Hortic. 89:132–134.

Rhodes, B., Zhang, X. 2000. Hybrid Seed Production in Watermelon. Journal of new seeds, 1(3-4): 69-88. [CrossRef]

Sari, N., Abak, K., Pitrat, M. (1999). Comparison of Ploidy Level Screening Methods in Watermelon: *Citrullus Lanatus* (Thunb.) Matsum. and Nakai. Scientia Horticulturae, 82(3-4): 265-277. [CrossRef]

Shivanna, K.R. and Rangaswamy, N.S. (1992). Pollen Biology. Springer-Verlag Berlin Heidelberg, 119 sayfa.

Soltis, D. E., Misra, B. B., Shan, S., Chen, S., Soltis, P. S. (2016). Polyploidy and The Proteome. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics, 1864 (8): 896-907. [CrossRef]

Suying, T., Xiuqiang, H., Jiwei, L., Wenge, L. (1993). Raising The Frequency of İnducing Tetraploid Watermelon by Treating of Colchicine. In International Symposium on Cultivar Improvement of Horticultural Crops. Part 1: Vegetable Crops 402: 18-22. Doi: 10.17660/ActaHortic.1995.402.2

Sensoy, A. S., Ercan, N., Ayar, F., Temirkaynak, M. (2003). Determination of Some Morphological Characteristics and Viability of Pollens in Some Vegetable Species in *Cucurbitaceae* Family. Journal of Akdeniz University Faculty of Agriculture, 16(1): 1-6.

Simşek, I., Göcmen, M., Sarı, N. (2013). Determination of Morphological and Cytological Differences in Diploid and Tetraploid Watermelon Plants., 30(1): 1-14. ISSN : 2149-2182

Zhang, X. (2010). Tetraploid Watermelon Producing Small Fruits. U.S. Patent No 7,652,193.

Zhang, N., Bao, Y., Xie, Z., Huang, X., Sun, Y., Feng, G., Chen, W. (2019). Efficient Characterization of Tetraploid Watermelon. Plants, 8(10): 419. [CrossRef]

RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2022; 6(4): 711-719
DOI: https://doi.org/10.31015/jaefs.2022.4.27

# Determination of awareness levels of walnut producers in plant protection applications (a case study of Bitlis province)

**Mehmet Kaplan**[1] • **Cihan Demir**[2]

[1] Faculty of Agriculture, Department of Plant Protection, Siirt University, 56100, Siirt, Türkiye
[2] Department of Mechanical and Metal Technologies, Vocational School of Technical Sciences, Kırklareli University, 39100, Kırklareli, Türkiye

**Citation:** Kaplan, M., Demir, C. (2022). Determination of awareness levels of walnut producers in plant protection applications (a case study of Bitlis province). International Journal of Agriculture, Environment and Food Sciences, 6 (4), 711-719

**Received:** 14 October 2022
**Revised:** 12 December 2022
**Accepted:** 14 December 2022
**Published Online:** 25 December 2022

**Correspondence:** Mehmet Kaplan
**E-mail:** mehmetkaplan1971@hotmail.com



## Abstract

The survey was conducted in 2020 to determine the awareness level of walnut producers regarding crop protection practices in Adilcevaz and Hizan districts of Bitlis province, where walnut production is widespread. With this in mind, a face-to-face survey was conducted using a simple random sampling method among 87 producers in a total of 10 randomly selected villages from two districts. Within the scope of the research, walnut growers were found to be highly educated and most had non-agricultural income. The manufacturers in question take into account the recommendations of sellers and Agriculture and Forestry department when choosing pesticides and deciding on the usage dose rates, that the brand recognition and active ingredients are important to pesticide preferences, that they don't use the same pesticides in the fight against the same diseases and pests, They start spraying without seeing the signs of disease and harmful factors in the plant, they apply the recommended dose in drug applications, pesticides used leave residue on product. They observe the waiting time between spraying and harvesting and wear protective clothing and masks during spraying, they do not simply throw-away empty pesticide box and packages used after the application into the agricultural fields or their surroundings, they clean the sprayer, but they use the sprayer without calibration, they mix pesticides and prefer cultural methods over chemical methods, and they do not have any knowledge about the word biopesticide. It is known that producers generally choose chemical control in the solution of plant protection issues, and the indiscriminate use of these pesticides has many adverse effects on human and environmental health.

**Keywords:** Plant Preservation Issues, Questionnaire, Walnut, Bitlis

## INTRODUCTION

Due to the vitamins it contains, walnut (*Juglans regia* L.) is a highly beneficial fruit for human nutrition and maintenance of health. Walnut has a wide range of use. As well as being consumed as snack, its leaves are used for various purposes and its trees and green bark are used for furniture manufacturing. This makes the fruit even more significant globally. Walnuts are widely grown in Turkey and constitute one of the most important livelihoods of people living in Anatolia (Keskin, 2012).

Among the hard-shelled fruits, walnut ranks first in the world with a share of 9.3% and in the first place with a production amount of 25.8% (Anonymous, 2020). According to 2019 data, People's Republic of China ranks first in global walnut

production with an annual production of 1.586.367 ton. United States of America ranks second with 613.260 ton, Iran ranks third with 409.562 ton and Turkey ranks fourth with 286.000 ton walnut production (Anonymous, 2020).

In 2020, 286 thousand tons of walnut production was realized in Turkey. The highest production was realized in Hakkari with approximately 12 thousand tons. Hakkari was followed by Kahramanmaraş with 11.4 thousand tons and Mersin with 11 thousand tons. In Bitlis, 4919 tons of walnuts were produced (Anonymous, 2021)

Walnuts have an important place in both domestic consumption and exports. In walnut orchards, there are many diseases, pests and weed species that harm alone or together. The most important of these are apple worm *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) *Callaphis juglandis* (Goeze) and *Panaphis juglandis* (Goeze) (Hemiptera: Aphididae) aphids as the main pest of walnut, Tree yellowweed *Zeuzera pyrina* (L.) (Lepidoptera: Cossidae), American white butterfly *Hyphantria cunea* (Drury) (Lepidoptera: Erebidae), apple leafhopper *Archips rosanus* (L.), common leaf bender *A. xylosteanus* (L.), Lepidoptera: Tortricidae), Pear lace bug *Stephanitis pyri* (Fabr.) (Hemiptera: Tingidae), *Lepidosaphes ulmi* (L.) (Hemiptera: Diaspididae), *Aceria erinea* (Nalepa) and Acarina: Eriophyidae, among the main diseases, there are *Gnomonia leptostyla* (Fr.: Fr) Ces & De Not., *Microstroma juglandis* (Berenger) Sacc., *Phytophthora* spp., Cystospore cancer *Leucostoma cincta* (Fr.: Fr.) Höln.. Tul. *Xanthomonas arboricola* pv. juglandis (Pierce) Vauterin et al. These are some of the common pests and diseases causing economic damage in walnut (Anonymous. 2017). Depending on periods and climate conditions, these factors can cause yield and quality losses. As such, it has been reported that at certain times, climate conditions lead to increased pest and disease population in agricultural production areas. The indiscriminate use of these pesticides has many adverse effects on human and environmental health.

The need for food increases in direct proportion to the increasing world population (Avan and Kotan, 2021). In Turkey, which has a very rich flora, it is very important to grow these plant materials in a healthy and high quality and to protect these products (Avan, 2021). It is also very important to transfer the plant protection methods to the producer correctly.

This study was conducted to identify crop protection issues in walnut fields in Bitlis due to the fact that plant protection has an important place in agricultural production and the intensive use of pesticides in walnut fields. As a result of the study, it has been determined what the producers do when they encounter plant protection problems, whom they consult, what they pay attention to in the selection and use of agricultural pesticides and spraying machines, and what they think about the effects of agricultural pesticides on the environment. Based on the results obtained, the problems in the use of plant protection products in walnut areas were determined and attention was drawn to solution-oriented processes and solutions.

## MATERIALS AND METHODS

The main material of the study was a questionnaire consisting of 20 questions made face-to-face with enterprises producing conventional walnuts in Adilcevaz and Hizan districts of Bitlis province. Obtained answers were given in terms of number and ratio. In Adilcevaz and Hizan district, which was determined as the research area of the study, 874 walnut production businesses registered with the Farmer Registration System (FRS) in 2019, and the enterprises were calculated in the 95% confidence interval (Anonymous, 2020). The above producers make up 9.52% of the 21,548 producers activity in Bitlis. It was impossible to conduct a survey with all the enterprises operating in Adilcevaz and Hizan districts, therefore, The number of producers surveyed was determined using a simple random sampling method with the following formula (Çiçek and Erkan, 1996).

n= N (pq)/ (N-1) D2+ (pq)

$$n=N\times S2\times t2 /(N-1)d2 + (S2 \times t2) \quad (1)$$

In the equation;

n: number of samples

S: population variance

N: Number of producers that make up the population

t: standard normal distribution value

d: population error term

The study was carried out error within 5%, confidence limit within 95% in determining the sample size of the study. Then, using the formula, calculated number of producers to interview as 87. The data obtained from walnut producers were evaluated by giving tables.

## RESULT AND DISCUSSION

### Socio-Economic Traits of Walnut Producers

The demographic structure of the participant enterprises in Bitlis (Adilcevaz and Hizan district) has been studied. The study indicated that all walnut producers in the districts are male, 4% are illiterate, 7% are literate, 39% are primary school graduate, 11% are secondary school graduate, 18% are high school graduate and 21% are University/College graduate (Table 1). In other similar studies, 60% of stone fruit producers in Korkuteli district of Antalya province graduated from primary school and 12% graduated from college; 62.41% - 87.50% of the farmers producing carrots in Konya were primary school graduates; 63.3% of Antalya's apple growers have a primary school degree and 14.4% of them have a university degree. 64.6% of potato growers in Nevsehir have completed primary school and 1.1% have a university degree. Almond farmers in Adiyaman are 91.4% male and 8.6% female, of which 14 have a primary school degree, 24.7%

have a secondary school degree, 40.9% have a university degree, and 20.4% have a university degree. increase; all of the vineyard producers in Mardin were male, 20% were illiterate, 64% were primary school graduates, 12% were secondary school graduates, and 4.4% university graduates. (Ay et al., 2006; Çelik and Direk, 2008; Kızılay and Akçaöz, 2009; Erdoğan and Gökdoğan, 2017; Erdoğan et al., 2017; Kaplan and Baran, 2021).

It has been observed that 77% of the walnut producers in Bitlis state has social insurance and 65.6% have non-agricultural income. (Table 1). It was also observed that 53.6% of farmers in Seyhan and Yüreğir districts of Adana state have social safety and 70.5% have no non-farm income.; In Manisa, 64% of farmers have social safety and 66% have no income other than farming; In Nevsehir province, 73.5% of farmers have social safety, 26.5% do not, of which 31.2% have non-farm income and 68.8% have non-farm income; 83.9% of almond farmers in Adıyaman have social safety and 65.6% have non-farm income; 73.9% of the viticulturists in Mardin have social safety and 55.6% have non-farm income (Emeli, 2006; Karataş and Alaoğlu, 2011; Erdoğan and Gökdoğan, 2017; Kaplan and Baran, 2021).

**Table 1.** Demographic details of the participating walnut producers in Bitlis province

| (%) Traits | Rate (%) |
|---|---|
| **Gender** | |
| Male | 100 |
| Female | 0 |
| **Education Status** | |
| Illiterate | 4 |
| Literate | 7 |
| Primary School | 39 |
| Secondary School | 11 |
| High School | 18 |
| College/University | 21 |
| **Employment Status** | |
| Farmer | 86 |
| Tradesman | 6 |
| Worker | 4 |
| Civil Servant | 2 |
| Retired | 3 |
| **Social Security** | |
| Yes | 77 |
| No | 23 |
| **Non-Agricultural Income** | |
| Yes | 65.6 |
| No | 34.4 |

## Knowledge, attitudes and behaviors of producers on plant protection products

When choosing pesticides, 61.75% of walnut producers in Bitlis (Adilcevaz and Hizan) get information from the Provincial and District Directorate of Agriculture and Forestry, 28.50% get information from vendors, and 11.75% rely on their own experience. and their neighbour (Table 2). In line with the current results, Tücer et al. (2004) reorted that 65% of Manisa winegrowers based their choice of pesticides on their provincial/district agricultural directorates, 16% based on their own experience, 11% based on their suppliers, 8 % reports that it's based on neighbors. Kalkışım et al.(2011), 43.08% of fruit producers in Gümüşhane choose their pesticides based on technical staff recommendations and 3.08% on recommendations by vendors. Erdoğan and Gökdoğan (2017) 88.9% of the producers in Nevşehir base their pesticide selections on vendors, 7.9% on their own experiences, 2.1% on agriculture agencies, 0.5% on their neighbour and 0.5% on consultant Agricultural Engineer. (Erdoğan et al. (2017) stated that 44.1% of the almond growersers in Adıyaman base their pesticide selection on Provincial Directorate of Agriculture, 32.3% on vendors, 19.4% on consultant Agricultural Engineer, 3.2% on their neighbour and 1.1% on their own experience. Kaplan and Baran (2021) reported that 34% of Mardin winegrowers base their pesticide selection on state/district agriculture and forestry offices, 52.50% on vendors and 2% on consulting agronomists, 12.5% on their own experience and neighbour. On the other hand, Özkan et al. (2003) stated that 49.7% of citrus producers in Antalya rely on their own experiences for selecting a particular pesticide, 42.8% rely on their vendors, 4% on Provincial/District Directorate of Agriculture and 3.4% on their neighbour. Dilmen et al. (2019) reported that 33% of farmers rely on vendors for agricultural pest control, 22% rely on Provincial/District Directorate of Agriculture and Forestry, 17% rely on their own experiences, 10% rely on their family, 9% rely on agricultural engineers and 1% rely on Chambers of Agriculture for technical knowledge while 6% do not seek any technical assistance.

**Table 2.** Awareness level of producers about who they get their pesticide advice from

| Where do you get pesticide (fungus, herbicide and insecticide) advice? | Rate (%) |
|---|---|
| Vendor | 25.50 |
| District Directorate of Agriculture and Forestry | 61.75 |
| My experience and My neighbors | 11.75 |
| Consulting Agricultural Technician | 1 |

When choosing (purchasing) pesticides (fungicides, herbicides and insecticides) used by walnut producers against diseases and pests, 10% considers their previous use, 40.50% considers the recommended active substance, 17% considers the brand and 32.50% take into

account the price (Table 3). İnan and Boyraz (2002) stated that 62.8% of the growers choose pesticides by taking into account the disease severity, 21.5% the spraying expenses and 15.7% the price; Boyraz et al. (2005) started that 78% of apple growers consider the severity of disease and pest, 11% price and 6% consider the spraying expenses as the main factors of selecting a particular pesticide; Erdoğan and Gökdoğan (2017) stated that 43.9% of producers choose pesticides based on trademark, 36.5% on price, 19% on effective ingredient and 0.6'% on expiration date; It was determined that 10% of the vineyard producers in Mardin chose the pesticide used against diseases and pests according to their previous use, 20.6% for the recommended effective ingredients, 28% for the trademark and 41.4% for price (Kaplan and Baran, 2021).

**Table 3.** Considerations when purchasing agricultural chemicals (insecticide, fungus and herbicide)

| What to look for when buying a crop protection product (fungus, herbicide, insecticide)? | Rate (%) |
|---|---|
| Previous use | 10 |
| Recommended active ingredient | 40.50 |
| Brand | 17 |
| Price | 32.50 |

It has been determined that 75% of the producers in Bitlis, which has a significant walnut production potential, do now no longer use the identical pesticide for the identical disease and pest, and 25% of them use the same pesticide all the time (Table 4. Erdoğan and Gökdoğan (2017) determined that 93.1% of the producers do not always use the same drug for the same disease and pest, while 6.9% of them use the same drug constantly. Kaplan and Baran (2021) reported that 63.5% of wine producers do not repeatedly use the same pesticide for the same disease and same pest, while 26.5% of them used same insecticide continuously.

**Table 4.** The knowledge levels of the producers about using the same pesticide for the same disease and pest

| Do you always use the same pesticides for the same diseases and pests? | Rate (%) |
|---|---|
| Yes | 25 |
| No | 75 |

## Opinions of Producers on Determining the Time of Spraying

Walnut producers were asked how they decided on the time of spraying against pests and diseases in the walnut fields. 18% of the producers sprayed the diseases and pests at first sight, 44.4% according to the recommendations of the District Directorate of Agriculture and Forestry, 21.85% by asking their vendors, 12.25% before seeing the diseases and pests and 3.50% by following other producers (Table 5). Effective chemical management against diseases and pests are possible only by spraying at the right time. When spraying is done in this way, both the highest effect is obtained and it is economical. The answers given by the manufacturers on this subject are remarkable. Because 39% of them say that they apply pesticides when they first detect diseases and pests or in relation to their intensity. Accordingly, it can be considered that the producers know the diseases and pests and have the necessary technical knowledge, even if it is not sufficient. As a general information, it can be accepted that the spraying times made by considering the phenology of the plant are correct. However, when it is necessary to determine the spraying time and considering the biology of the disease and pest, it is not possible for the manufacturers to determine the spraying time correctly. This topic requires specialized knowledge and experience and can only be achieved through the training of dedicated staff. in studies similar to this, Yücel et al. (1995) stated that 42.15% of the producers rely on their own experiences, 9.80% are inspired by the people around them, 34.31% consult agricultural agencies and 13.72% consult their vendors for determining the spraying time. Üremiş et al. (1996), based on their study in Cilician plain, reported that 38.64% of producers rely on their own experiences and vendor recommendations, 35% rely on experiences, 19.09% rely on vendor experiences, 5.45% rely on technical agency recommendations and 1.82% rely on pesticide tag for determining the dosage and timing of the pesticide. Zeren and Kumbur (1998) reported that 40.18% of the producers rely on vendor recommendations, 29.92% rely on their own experiences and 16.23% rely on pesticide tag for determining the dosage and timing of the pesticide. İnan and Boyraz (2002) reported that 44.20% of the farmers in Konya rely on their own experience, 24.20% rely on vendor recommendations, 20% ask other producers and 11.60% consult agricultural agencies to determine the time of pesticide-use. After conducting a study in Tokat province, Kadıoğlu (2003) reported that 58.74% of the producers consulted the technical staff in deciding to spray, 29.14% decided on their own, 6.20% decided on the recommendation of their vendors and 81% consult other farmers. Boyraz et al. (2005) stated that 35% of apple growers decide on timing upon the first sighting of disease and pests, 22% based on early warning system; Kaplan and Baran (2021) reported that 17% of vineyard producers in Mardin decide on the timing upon first time seeing diseases and pests, 25% based on recommendations by the District Directorate of Agriculture and Forestry, 36% ask their vendor, 12% before any sighting of disease and pests and 10% follow other producers. In contrast to these findings, Karaçayır (2010) stated that 43.2% of apple producers in Karaman used pesticides before each pest sighting and 56.8% used pesticides after the first pest sighting.

It has been determined that 48.75% of the producers choose the pesticide dose in the chemical control of

**Table 5.** Walnut producers' knowledge levels about determining the time of disease and pest control in their orchards

| How do you decide on the timing to spray for diseases and pests in walnut orchards? | Rate (%) |
|---|---|
| Vendor | 21.85 |
| District Directorate of Agriculture and Forestry | 44.40 |
| First sighting of pests and pests disease | 18 |
| Before sighting of any disease and pests | 12.25 |
| Follow other producers | 3.50 |

pests in walnut production, according to their vendor, 41.75% according to the Provincial/District Directorate of Agriculture and 9.50% according to their own experience (Table 6). Özkan et al. (2003) expressed that 41.71% of citrus manufactures adjust their dosage according to the label and 27.81% adjusted dosage based on experience. Kalıpcı et al. (2011) found that 8.3% of Konya producers adjusted their pesticide doses according to the label, 26.6% adjusted it according to their own experience, 11.6% according to their neighbours, 33.3% according to the recommendations of their vendors, 10.8% said they adjusted according to District Directorate of Agriculture, 3.3% to Chambers of Agriculture and 5.8% to consultant Agricultural Engineer; Gedikli (2012) started that 33.33% of manufacturers advise with their vendors and Agricultural Engineers for dosage adjustment; Erdoğan and Gökdoğan (2017) expressed that 86.2% of the farmers consulted the vendor in the selection of pesticide dosage, 11.1% did it according to their own experience, 1.1% consulted the Provincial/District Directorate of Agriculture, 1.1% according to the private advisor and 0.5% according to their neighbour; Gözener et al. (2017) reported that manufacturers based their decisions on the recommendations of the pesticide-fertiliser vendors (90.28%), according to the label of the pesticide (59.72%), according to their own experience (40.28%), state/District Administration Advice on Agriculture, according to the recommendations of Forestry and Husbandry technical staff (1.39%) and according to disease intensity (1.39%) for adjusting the pesticide dose; Kaplan and Baran (2021) reported that in the chemical control of pests in the vineyards of Mardin, 68% of the producers consulted the vendor when adjusting the pesticide dose, 20.8% of them were told by the Provincial Directorate of Agriculture, 7.2% did it by their own experience and 5% followed their neighbours.

**Table 6.** The knowledge levels of the producers regarding the dosage adjustment of the pesticides they use

| How do you adjust the dosage of pesticides (fungi, herbicides, insecticides)? | Rate (%) |
|---|---|
| Vendor | 48.75 |
| District Directorate of Agriculture and Forestry | 41.75 |
| Own Experience | 9.5 |
| Neighbour | - |

The knowledge levels of the producers regarding the recommended pesticide dose were examined. It has been determined that 82.15% of walnut growers apply exactly the recommended dosage, and 17.85% apply a dose above the recommended dose (Table 7). The vast majority of manufacturers follow the recommended dosage. This should increase the likelihood of successful chemical control. In line with the results of this study, Tücer et al (2004) found that 72% of vineyard producers applied the recommended dose, 26% increased the recommended dose, and 2% applied it on visual basis; Peker (2012) reported that 88% of Konya growers used the recommended dose, 8% increased the dose, and 4% decreased the dose; Erdoğan and Gökdoğan (2017) reported that 50.7% of manufacturers used the recommended dose and 50.3% used a higher dose. It was determined that 87.8% of Mardin vineyard producers applied the recommended dose exactly, and 12.2% applied a dose above the recommended dose. Contrary to the results of this study, Boz et al. (1998) determined that 64.47% of the producers in Aydın used higher doses.

**Table 7.** Level of knowledge on recommended doses of pesticides

| Are pesticides (fungicides, herbicides, insecticides) used at recommended doses? | Rate (%) |
|---|---|
| Recommended dose | 82.15 |
| Over the recommended dose | 17.85 |

In Bitlis (Adilcevaz and Hizan), 57.14% of walnut growers reported that pesticides leave residues in their products, 39.28% left little residue and 3.58% stated that they did not leave any residue in the product (Table 8). In Antalya, 70.4% of manufacturers said pesticide residues remained in their products, 10.4% said the pesticide residues disappeared when washed, and 19.2% did not know; 34.3% of manufacturers indicated that pesticides may leave residues in their products, 23.8% of them indicated that each pesticide leaves residues, and 18.1% of manufacturers use recommended doses. 13.4% of them said that the residual effect disappeared after cleaning and 10.4% said that no pesticide remained at all but 28.3% of Konya growers said pesticides could remain in their products, 18.3% said they did not leave residues, 7.5% said they did not know and 45.8% said pesticides were washed away by rain. said to be; 38.7% of almond growers in Adıyaman say they have pesticide residues in their products, 32.3% stated little residue is left and 29% stated that they did not leave any residue on the product. In Tokat Kazova, 44.44% of tomato growers reported that pesticides left residues in their products, 15.63% reported that pesticides were used above doses left residues, and 15.63% said all pesticides, they reported leaving a residue, and 9.38% reported having a residue. No residue when used at recommended dose (Özkan et al., 2003; Karaçayır, 2010; Kalıpcı et al., 2011; Gözener et al., 2017, Erdoğan et al., 2017). Contrary to the results of this study, exports

that 80% of the manufacturers have not reported pesticides, and 20% have observed the residue. Erdoğan and Gökdoğan (2017) is 74.1% of producers stated no residue from pesticides, 23.8% stated minimal residue while 2,1% stated significant amounts of residue.

**Table 8.** Knowledge level of producers about the pesticides leaving remain on the products

| Did you know that pesticides remain in products? | Rate (%) |
|---|---|
| They do leave residue | 57.14 |
| Minimal residue | 39.28 |
| No residue | 3.58 |

53.58% of the producers using pesticides in Bitlis (Adilcevaz and Hizan) reported that they observe the latency of pesticides, 32.14% do not observe the waiting times and 14.28% observe it from time to time (Table 9). It is an effective component that the extensive majority of manufacturers pay attention to the waiting time. Boyraz et al (2005) found that 71% of apple growers respect waiting times between spraying and harvesting, while 29% do not, Erdoğan and Gökdoğan (2017) found that 80% of potato farmers respect the waiting time between spraying and harvesting, 20% do not respect the waiting time. Contrary to the results of this study, Emeli (2006) stated that 76.6% of the producers do not comply with the waiting period after spraying, while 23.4% of them comply with the waiting period; According to Karaçayır (2010), 43.2% of producers respect waiting time, 32.8% know waiting time but do not respect waiting time, and 24% do not know waiting time. Gözener et al (2017) stated that 91.67% of the growers do not know the time required between the last spraying and harvest, 6.94% know but do not apply, and 1.39% know and apply the waiting time; Kaplan and Baran (2021) reported that 88.6% of vineyard producers using pesticides in Mardin pay attention to the waiting time in pesticides, and 11.4% do not.

**Table 9.** Knowledge level of growers about the time between spraying and harvest

| Do you comply with the waiting period for pesticides? | Rate (%) |
|---|---|
| Yes | 53.58 |
| No | 32.14 |
| Sometimes | 14.28 |

60.73% of walnut producers in Bitlis (Adilcevaz and Hizan) stated that they use protective clothing and masks while applying pesticides, 21.42% sometimes use them and 17.85% never use them. It has been determined that more than half of the walnut producers comply with the protection measures during spraying and show sufficient care (Table 10). Similar to the current findings, Özkan et al. (2003) reported that 68.8% of producers use protective clothing and mask when applying pesticides while 31.2% never use any; Bayhan et al. (2015) reported that he 76% of producers had protective measures in place and 24% did not. Contrary to the results of this study, Tücer et al. (2004) sarted that 57.82% of farmers used protective clothing and masks when applying pesticides, and 42.18% did not use them at all; Erdoğan and Gökdoğan (2017) stated that 84.7% of producers use protective clothing and mask when applying pesticides while 15.3% never use any. Kaplan and Baran (2021) reported that 48% of vineyard producers in Mardin use protective clothing and mask when applying pesticides, 16% rarely use them while 36% never use any.

**Table 10.** The knowledge level of the producers about the precautions to be taken when applying pesticides

| How do you protect your health when applying pesticides? (Using protective clothing, mask, goggles and gloves during treatment) | Rate (%) |
|---|---|
| I always use | 60.73 |
| I sometimes use | 21.42 |
| I never use | 17.85 |

Walnut producers were asked what they do with empty pesticide boxes after use. It was determined that 2% of the participants wash and reuse, 3.25% bury them in the ground, 38% throw them away, 27.15% burn them and 29.6% randomly throw them into the environment (Table 11). In studies conducted on the subject, it has been determined that producers exhibit different behaviours about empty pesticide container. Özkan et al. (2003) stated that 70.45% of manufacturers burn empty boxes, 21.81% throw them away, 14.36% bury them in the ground and 7.45% throw them into the environment; Tücer et al. (2004) reported that 60.54% of manufacturers indiscriminately throw away empty boxes, 4.98% use them for other purposes, 19% bury them, and 15.48% burn them; Ertürk et al (2012) stated that 35.6% of growers bury empty boxes in the ground, 34.6% throw them in the orchard, and 29.8% throw them in the garbage. Akbaba (2010) stated that 61.1% of Çukurova producers store their empty boxes in a designated place before burning them; Karataş and Alaoğlu (2011) stated that 65.3% of wine producers burned empty boxes, 24% threw them at random, and 10.7% buried them in the earth.; With respect to Erdoğan and Gökdoğan (2017), 68.3% of producers burn empty boxes, 16.4% bury them, 13.8% indiscriminately discard them, and 1.5% wash and reuse them. Gözener et al (2017) stated that 59.72% of manufacturers burn empty boxes, 29.17% bury them in the ground, 5.56% dispose of them in the same bins as household waste, and 5.56% discriminately dispose of them in the environment. In Mardin, 2% of vineyard producers are reported to wash and reuse empty pesticide boxes after usage, 15.6% bury them in the ground, 20% throw them away, 24.3% burn them, and 38.1% randomly throw them away (Kaplan and Baran, 2021),

**Table 11.** Knowledge level of producers on empty pesticide boxes

| What about pesticides (fungi, herbicides, pesticide boxes)? | Rate (%) |
|---|---|
| Wash & re-use | 2 |
| Bury | 3.25 |
| Thrash | 38 |
| Burn | 27.15 |
| Throw Away Randomly | 29.6 |

It has been determined that 85.75% of walnut producers clean their tools after pesticide, 10.75% sometimes clean, 3.5% never clean (Table 12). It was determined that 85.42% of the grape growers in Manisa wash their insecticides after spraying with pesticides, while 14.58% do not wash the pesticide machine; 69.2% of the producers in Iğdır are reported to clean the pesticide machine after usage, %27.9 sometimes clean it and 2.9% never clean it; 95.8% of potato producers are reported to clean their pesticide tool after usage while 4.2% do not clean; 90.3% of almond producers clean the tool after usage, 6.5% do not clean it while 3.2% sometimes clean it; 78.3% of vineyard producers clean the tool after usage, 14% sometimes clean it and 7.7% do not clean it (Tücer et al. 2004; Ertürk et al. 2012; Erdoğan and Gökdoğan, 2017; Erdoğan et al., 2017; Kaplan and Baran, 2021).

**Table 12.** Knowledge levels of producers about cleaning pesticides after use

| Do you clean the sprayer after pesticide? | Rate (%) |
|---|---|
| Yes | 85.75 |
| Sometimes | 10.75 |
| No | 3.5 |

32.5% of walnut producers reported that they use pesticides in a mixture, 22.85% use it sometimes by mixing, and 25% use it without mixing at all (Table 13). Similar to those results, Boyraz et al. (2005) 83% of apple growers make a mixture of pesticides, while 17% do not mix them; Reported by Peker (2012) that 56% of producers use mixed pesticides, 24% of producers do not use mixtures, and 20% sometimes mix them; Erdoğan and Gökdoğan (2017) stated that 56.1% of farmers used mixed pesticides and 43.9% used pesticides unmixed. Erdogan et al. (2017) expressed that he 78.5% of almond growers use mixed pesticides., 19.4% sometimes mix them and 2.1% use them without mixing at all; Kaplan and Baran (2021) reported that 56.5% of those engaged in vineyard production use pesticides in a mixture, 16.5% use it sometimes with mixing, and 28% use it without mixing at all.

57.2% of the producers apply chemical control, 18.3% cultural control, 6.7% mechanical control, 2% physical control, 12.8% biotechnical control methods and 2.62%

**Table13.** Knowledge level of producers about mixing pesticides

| Do you mix pesticides? | Rate (%) |
|---|---|
| I mix them | 32.15 |
| I mix them sometimes | 42.85 |
| I do not mix them | 25 |

of them stated that they do not apply any control methods (Table14). Similar to these findings, it is stated that 88.4% of the producers apply cultural control, 10.5% mechanical control and 1.1% physical control methods other than chemical control (Erdoğan and Gökdoğan, 2017). It has been stated that 71% of vineyard producers apply chemical control, 12.9% apply cultural control, 8% apply mechanical control, 5% apply physical control and 3.1% apply biotechnical control methods (Kaplan and Baran, 2021). Contrary to these results have been reported that 43.58% of the producers in Tokat prefer cultural control, 33.33% prefer mechanic control and 23.07% prefer physical control in addition to chemical control (Kadıoğlu, 2003).

**Table 14.** Knowledge level of producers about disease, pest and weed control methods

| Methods of controlling pests, diseases and weeds | Rate (%) |
|---|---|
| Chemical control | 57.2 |
| Cultural control | 18.3 |
| Mechanic control | 6.7 |
| Physical control | 2 |
| Biotechnical control | 12.18 |
| Biologic control | - |
| None | 2.62 |

92.85% of walnut growers say they are unfamiliar with the term biopesticide, and 7.15% stated that they know the concept of biopesticide (Table 15). In parallel with our research results, Erdoğan et al (2017) stated that 78.5% of almond farmers do not know the concept of biopesticides, and 21.5% of them know the concept of biopesticides in agricultural control. In another study conducted by Erdoğan and Gökdoğan (2017), reportedly, 97.4% of manufacturers are unfamiliar with the term biopesticide, while 2.6% are familiar with the term biopesticide. Kaplan and Baran (2021) reported that 96.5% of the vineyard producers did not know the term biopesticide, while 3.5% of them stated that they knew the concept of biopesticide. Contrary to these results, 52% of GAP manufacturers (South-eastern Anatolia Project) region already heard about the term biopesticide while %48 never heard of it (Bayhan et al., 2015). Most walnut growers in Bitlis are owned by state/provincial agricultural departments and their vendors, farming organizations and and their vendors, the agricultural organization and

**Table 15.** Knowledge levels about the term biopesticide

| Do you know biopesticides? | Rate (%) |
|---|---|
| I do not know | 92.85 |
| I do know | 7.15 |

its vendors have important duties in biopesticides and biological control.

## CONCLUSION

Plant protection has an important place in agricultural production. Due to the intensive use of pesticides in walnut production areas, this survey study was conducted to determine plant protection problems in the walnut areas of Bitlis province. As a result of the study, the precautions taken by the producers when faced with plant protection problems, the people they consulted were determined and it was determined what they paid attention to in the selection and use of agricultural pesticides and spraying machines. Within the scope of the study, the problems in the use of plant protection products in walnut production areas were determined, and attention was drawn to the work on the solution of the problems and the solutions.

At the end of the study, it was determined that the producers in Bitlis (Adilcevaz and Hizan), which has a significant walnut production potential, prioritize chemical control in the fight against existing diseases and pests in order to obtain efficient and quality products. As a result of the random and unconscious use of pesticides, both human and environmental health are affected, and it is possible for diseases and pests to develop resistance to pesticides over time. For this reason, an effective, economical and environmentally friendly control method can be employed by using more suitable pesticides in order not to be exposed to undesirable side effects.

In conclusion, it will be beneficial to give importance to integrated control against main diseases and pests in walnut fields, and to use selective pesticides that are relatively safe for human and environmental health in cases where chemical control is inevitable. In this way, pesticide residues, if practices that reduce the use of chemical pesticides are preferred and pests will be prevented from gaining resistance to pesticides, and there will be a certain level of reduction in production costs. For these reasons, it is very important to make sustainable agricultural production in a way that will reduce the possible negative effects of agricultural control activities on the agroecosystem and biological balance.

## COMPLIANCE WITH ETHICAL STANDARDS

### Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

### Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Ethics committee approval is not required.

### Funding

No financial support was received for this study.

### Data availability

Not applicable.

### Consent for publication

Not applicable.

## REFERENCES

Akbaba, B.Z. (2010). Evaluation of citrus cultivation and insecticide use in Adana province. Master Thesis, Çukurova University Institute of Science and Technology, Department of Plant Protection, 89 p, Adana.

Anonymous, (2017). Walnut Integrated Control Technical Instruction, Ministry of Food, Agriculture and Livestock, General Directorate of Agricultural Research and Policies, Department of Plant Health Research , Ankara.

Anonymous, (2020). Trade statistics for international business development (TRADE MAP) [URL]

Anonymous, (2021). Turkish Statistical Institute Database. [URL]

Avan, M. (2021). Researches on Important Medicinal and Aromatic Plants, Their Properties and Diseases in Turkey and in the World, International Eastern Anatolian Journal of Science, Engineering and Design , 3(1), 129-156. [Google Scholar]

Avan, M., Kotan, R. (2021). Use of Fungi in Agriculture as Microbial Fertilizer or Biopesticide, International Eastern Anatolia Journal of Science, Engineering and Design , 3(1), 167-191. [Google Scholar]

Ay, R, Yalçın Ş, Sökeli, E, Karaca İ. (2006). Investigation of stone fruit producer profile in Antalya province Korkuteli district in terms of plant protection practices. Journal of SDU Graduate School of Natural and Applied Sciences , 10(1):52-55.

Bayhan, E. Sağır, A. Uygur, F.N. Bayhan, S.Ö, Eren, S. Bayram, Y. (2015). Determination of plant protection problems in cotton fields of GAP Region. Turkish Entomology Bulletin, 5(3):135-146.

Boz, Ö. Erol, T. Benlioğlu, S. Öncüer, C. (1998). Socio-economic evaluation of agricultural control practices in Aydın. Turkish Journal of Entomology, 22(2):123-136.

Boyraz, N. Kaymak, S. Yiğit, F. (2005). General evaluation of chemical control practices of apple producers in Eğirdir district. Journal of Selcuk University Faculty of Agriculture, 19(36): 37-51.

Çelik, Y. Direk, M. (2008). Classification of agricultural enterpris-

es producing carrots in Konya according to the European Union agricultural accounting data network system and comparison of business success criteria. TÜBİTAK TOVAG Project 107O714, 134 s, Konya.

Çiçek, A. Erkan, O. (1996). Research and Sampling Methods in Agricultural Economics. Gaziosmanpaşa University Faculty of Agriculture Publications No: 12, Lecture Notes Series No: 6, Tokat.

Dilmen, H. Pala, F., Özer, Dilmen, M. (2019). Determining the Knowledge Level of Pistachio (*Pistacia vera L.*) Producers on Agricultural Control: The Case of Siirt Province, Turkey. Turkish Journal of Agricultural Research - Turkish Journal of Agricultural Research 7( 1): 1-8.

Emeli, M. (2006). Research on the practical problems of plant protection methods in the Seyhan and Yüreğir basins. Master's Thesis, Çukurova University Institute of Science and Technology, Department of Plant Protection, 123 s, Adana.

Erdoğan, O. Gökdoğan, O. (2017). Plant protection practices of potato producers in Nevşehir province. West Mediterranean Agricultural Research Institute Derim Journal, 34(1): 51-60.

Erdoğan, O. Tohumcu, E. Baran, M.F. Gökdoğan, O. (2017). Evaluation of Agricultural Protection Practices of Almond Producers in Adıyaman Province. Turkish Journal of Agriculture – Food Science and Technology, 5(11): 1414-1421. [Google Scholar]

Ertürk, Y.E. Bulak, Y. Uludağ, A. (2012). Environmental sensitivity of agricultural enterprises in Iğdır province in agricultural control practices. Journal of History, Culture and Art Studies, 1(4): 393-401.

Gedikli, O. (2012). Evaluation of the problems faced by the producers of spruce, Bafra and Terme districts of Samsun in terms of plant protection and the factors affecting the use of pesticides. Master's Thesis, Atatürk University, Institute of Science and Technology, Department of Plant Protection, 92 s, Erzurum.

Gözener, B. Sayılı, M. Çağlar, A. (2017). The use of pesticides in tomato cultivation in Kazova Region of Tokat province. Turkish Journal of Agriculture-Food Science and Technology, 5(5): 451-458. [Google Scholar]

İnan, H. Boyraz, N. (2002). General evaluation of pesticide use of Konya farmers. Journal of Selcuk University Faculty of Agriculture, 16(30): 88-101.

Kadıoğlu, İ. (2003). Research on the agricultural control activities of the producers in the province of Tokat. Journal of Gaziosmanpaşa University Faculty of Agriculture, 20(1): 7-15.

Kalıpcı, E. Özdemir, C. Öztaş, H. (2011). Investigation of farmers' level of education and knowledge about pesticide use and environmental sensitivity. TUBAV Journal of Science, 4(3):179-187.

Kalkışım, Ö. Onaran, A. Azeri, F.N. Turan, A. (2011). Research on the general situation of fruit growing and farmer practices in Gümüşhane province and its districts. Gumushane University Journal of Science Institute, 1(2): 123-134.

Kaplan, M. Baran, M.F. (2021). Determining the Awareness Levels of Vine Growers in Mardin Province with Regards to Crop-Protection Practises. *Erwerbs-Obstbau* 63 (1), 131–140 (Octoberber-2021). [CrossRef]

Karaçayır, H.F. (2010). Extension approaches in the use of pesticides in agricultural enterprises producing apples: The case of Karaman province. Master Thesis, Selcuk University, Institute of Science and Technology, Department of Agricultural Economics, 158 s, Konya.

Karataş, E. Alaoğlu, Ö. (2011). Plant protection practices of producers in Manisa province. Journal of Ege University Faculty of Agriculture, 48(3): 183-189.

Keskin, A.H. (2012). Konya, Walnut cultivation in Konya, Karaman Region. 10. National Agricultural Economics Congress, 5-7 September 2012, 529-534 s., Konya

Kızılay, H. Akçaöz, H. (2009). Investigation of economic loss in the use of pesticides and fertilizers in apple growing: The Case of Antalya Province. Journal of Agricultural Sciences Research, 2(1): 113-119. [Google Scholar]

Özkan, B. Vuruş, Akçaöz, H. Karadeniz, C.F. (2003). Producer attitudes and behaviors towards the use of pesticides in citrus production in Antalya province. Anadolu, J. of AARI, 13(2):103-116.

Peker, A.E. (2012). Environmental sensitivity analysis for the use of pesticides in tomato production in Konya. Journal of Iğdır University Institute of Science and Technology, 2(1): 47-54. [Google Scholar]

Tücer, A. Polat, İ. Küçüker, M. Özercan, A. (2004). Determination of the problems in pesticide applications in Manisa - Saruhanlı vineyard areas. Anadolu, J of AARI, 14(1): 128-141. [Google Scholar]

Üremiş, İ., Karaat, Ş., Gönen, O., Canıhoş, E., Kütük, H., Ekmekçi, U., Çetin, V., Aytaş, M., Kadıoğlu, İ. (1996). General Evaluation of Agrochemical Use in Çukurova Region. II National Pesticides Symposium, 18-20 November 1996, Ankara, s: 73-79

Yücel, A., Çıkman, E., Yücel, M. (1995). Farmer's View of Agricultural Struggle in the Harran Plain Before the Southeastern Anatolia Region (GAP) was put into practice. GAP Region Plant Protection Problems and Solution Proposals Symposium, 27-29 April 1995, Şanlıurfa

Zeren, O., Kumbur, H. (1998). Research on Agricultural Pharmaceutical Marketing, Usage Technique and Efficiency in İçel Province. Türk- Koop Ekin 2: 5,s:62-68