INTERNATIONAL JOURNAL OF AGRICULTURE, FORESTRY AND LIFE SCIENCES

Int J Agric For Life Sci

e-ISSN: 2602-4381

dergipark.gov.tr/ijafls

December 2023
Vol. 7, No.2

International Journal of Agriculture, Forestry and Life Sciences

Int J Agri For Life Sci

e-ISSN: 2602-4381

https://dergipark.org.tr/ijafls

Volume: 7 **Issue: 2** **Year: 2023**

IJAFLS
e-ISSN: 2602-4381

International Journal of
Agriculture, Forestry and Life Sciences

www.ijafls.org Int J Agric For Life Sci 7(2): (2023)

Editorial Advisory Board

Production Information

Journal Name	International Journal of Agriculture Forestry and Life Sciences
Abbreviation	Int J Agric For Life Sci
Subjects	Agriculture Forestry and Life Sciences
e-ISSN	2602-4381
Publisher	Volkan Okatan
Owner	Volkan Okatan
Language	English
Frequency	Biannually
Type of Publication	International Scientific Open Access Double-Blinded peer review Widely distributed periodical
Manuscript Submission and Tracking System	IJAFLS uses the submission system of TUBITAK Journalpark open journal system. http://dergipark.org.tr/ijafls
License	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	Google Scholar, Index Copernicus, ROAD, CiteSeerX, Scientific Indexing Services, ResearchBib, Eurasian Scientific Journal Index, Rootindexing, Microsoft Academic, JournalTOCs, J-Gate,
Address	International Journal of Agriculture Forestry and Life Sciences Volkan Okatan Department of Horticulture, Faculty of Agriculture and Natural Sciences, Uşak University, Uşak, Turkey
Contact	Phone + 90 506 951 28 03 Mail: ijaflsjournal@gmail.com, okatan.volkan@gmail.com Web: http://dergipark.org.tr/ijafls

Aim and Scope

The original scientific double blind peer-reviewed papers published in IJAFLS journal cover main aspects of agriculture, forestry and life sciences.

A. Agriculture
1. Agronomy
2. Horticulture
3. Plant Protection
4. Animal Science
5. Veterinary Medicine
6. Land Reclamation, Earth Observation & Surveying, Environmental Engineering
7. Biotechnology
8. Management and Economics in Rural Areas
9. Food Engineering
10. Landscape Architecture
11. Ornamental Plants
12. Integration of Agriculture and Tourism

B. Forestry (If it is about Agriculture)

C. Life Sciences (If it is about Agriculture)
1. All departments of **BIOLOGY** (If it is about Agriculture and Forestry)
2. All departments of **CHEMISTRY** (If it is about Agriculture and Forestry)

PLAGIARISM POLICY

PLAGIARISM POLICY

An initiative to help editors verify the originality of submitted manuscripts. As part of this process, selected submitted manuscripts are scanned and compared with the CrossCheck database.

Plagiarism is when an author attempts to pass off someone else work as his or her own. Duplicate publication, sometimes called self-plagiarism, occurs when an author reuses substantial parts of his or her own published work without providing the appropriate references. This can range from getting an identical paper published in multiple journals, to salami-slicing, where authors add small amounts of new data to a previous paper.

PLAGIARISM PRIOR PUBLISHING:

IJAFLS will judge any case of plagiarism on its limits. If plagiarism is detected by the editorial board member, reviewer, editor etc., in any stage of article process- before or after acceptance then we will alert the same to the author(s) and will ask them to rewrite the content or the to cite the references from where the content has been taken. If more than 30% of the paper is plagiarized- the article may be rejected and the same is notified to the author.

WHEN DOES THE PLAGIARISM CHECK DONE?

All the submitted manuscripts for publication are checked for plagiarism after submission and before starting review.

HOW IS PLAGIARISM HANDLED?

The manuscripts in which the plagiarism is detected are handled based on the extent of the plagiarism. >5%

Plagiarism: The manuscript will be given an ID and the manuscript is sent to author for content revision.

5- 30% Plagiarism: The manuscript will not be given an ID and the manuscript is sent back to author for content revision.

>30% Plagiarism: The manuscript will be rejected without the review. The authors are advised to revise the manuscript and resubmit the manuscript.

WHY MANUSCRIPTS WITH >30% PLAGIARISM IS REJECTED:

If the plagiarism is detected more than 30%, it is found that the authors are very unlikely to revise the manuscript and submit the revised version. However, authors are welcome to do the required revisions and submit the manuscript as a new submission.

WHAT CAN YOU DO IF YOU COME ACROSS A CASE OF PLAGIARISM IN ANY JOURNAL FROM ANY PUBLISHER?

If you come across a case of plagiarism in any journal from any publisher, please do inform the editorial office(s) of all the involved journals, giving them the journal names, title of manuscripts, name of authors, volume number, issue number, year of publication and any other information that you have. The editorial offices will handle the cases as per their policy.

WHAT IF PLAGIARISM DETECTED AFTER PUBLICATION

If a case of plagiarism comes to light after a paper is published in a Scholarena journal, the journal will conduct a preliminary investigation. If plagiarism is found, the journal will contact the author's institute and funding agencies. A determination of misconduct will lead the Scholarena journal to run a statement, bidirectionally linked online to and from the original paper, to note the plagiarism and to provide a reference to the plagiarised material. The paper containing the plagiarism will also be obviously marked on each page of the PDF. Depending on the extent of the plagiarism, the paper may also be formally retracted.

Publishers Ethic Rules

" International Journal of Agriculture, Forestry and Life Sciences" (IJAFLS) is an international journal, which publishes at the highest scientific level on original research and review articles dealing with Agriculture, Forestry and Life Sciences.

Originality, high scientific quality, and citation potential are the most important criteria for a manuscript to be accepted for publication. Manuscripts submitted for evaluation should not have been previously presented or already published in an electronic or printed medium. The journal should be informed of manuscripts that have been submitted to another journal for evaluation and rejected for publication. The submission of previous reviewer reports will expedite the evaluation process. Manuscripts that have been presented in a meeting should be submitted with detailed information on the organization, including the name, date, and location of the organization.

Publishers Ethic Rules

All authors submitting their works to The International Journal of Agriculture, Forestry and Life Sciences for publication as original articles attest that the submitted works represent their authors' contributions and have not been copied or plagiarized in whole or in part from other works.

It is necessary to agree upon standards of expected ethical behavior for all parties involved in the act of publishing: the author, the journal editor, the peer reviewer and the publisher.

International Journal of Agriculture, Forestry and Life Sciences ethic statements are based on COPE's Best Practice Guidelines for Journal Editors.

Publication Decisions

The editor is responsible for deciding which of the articles submitted to the International Journal of Agriculture, Forestry and Life Sciences should be published. The editor may be guided by the policies of the IJAFLS's editorial board and constrained by such legal requirements as shall then be in force regarding libel, copyright infringement and plagiarism. The editor may confer with other editors or reviewers in making this decision.

Fair Play

An editor at any time evaluate manuscripts for their intellectual content without regard to race, gender, sexual orientation, religious belief, ethnic origin, citizenship, or political philosophy of the authors.

Confidentiality
The editor and any editorial staff must not disclose any information about a submitted manuscript to anyone other than the corresponding author, reviewers, potential reviewers, other editorial advisers, and the publisher, as appropriate.

Disclosure and Conflicts of Interest
Unpublished materials disclosed in a submitted manuscript must not be used in an editor's own research without the express written consent of the author.

Duties of Reviewers

Contribution to Editorial Decisions
Peer review assists the editor in making editorial decisions and through the editorial communications with the author may also assist the author in improving the paper.

Promptness
Any selected referee who feels unqualified to review the research reported in a manuscript or knows that its prompt review will be impossible should notify the editor and excuse himself from the review process.

Confidentiality
Any manuscripts received for review must be treated as confidential documents. They must not be shown to or discussed with others except as authorized by the editor.

Standards of Objectivity
Reviews should be conducted objectively. Personal criticism of the author is inappropriate. Referees should express their views clearly with supporting arguments.

Acknowledgement of Sources
Reviewers should identify relevant published work that has not been cited by the authors. Any statement that an observation, derivation, or argument had been previously reported should be accompanied by the relevant citation. A reviewer should also call to the editor's attention any substantial similarity or overlap between the manuscript under consideration and any other published paper of which they have personal knowledge.

Disclosure and Conflict of Interest
Privileged information or ideas obtained through peer review must be kept confidential and not used for personal advantage. Reviewers should not consider manuscripts in which they have conflicts of interest resulting from competitive, collaborative, or other relationships or

connections with any of the authors, companies, or institutions connected to the papers.

Duties of Authors

Reporting Standards

Authors of reports of original research should present an accurate account of the work performed as well as an objective discussion of its significance. Underlying data should be represented accurately in the paper. A paper should contain sufficient detail and references to permit others to replicate the work. Fraudulent or knowingly inaccurate statements constitute unethical behavior and are unacceptable.

Data Access and Retention

Authors are asked to provide the raw data in connection with a paper for editorial review, and should be prepared to provide public access to such data (consistent with the ALPSP-STM Statement on Data and Databases), if practicable, and should in any event be prepared to retain such data for a reasonable time after publication.

Originality and Plagiarism

The authors should ensure that they have written entirely original works, and if the authors have used the work and/or words of others that this has been appropriately cited or quoted.

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.

Multiple, Redundant or Concurrent Publication

An author should not in general publish manuscripts describing essentially the same research in more than one journal or primary publication. Submitting the same manuscript to more than one journal concurrently constitutes unethical publishing behaviour and is unacceptable.

Acknowledgement of Sources

Proper acknowledgment of the work of others must always be given. Authors should cite publications that have been influential in determining the nature of the reported work.

Authorship of the Paper

Authorship should be limited to those who have made a significant contribution to the conception, design, execution, or interpretation of the reported study. All those who have made significant contributions

should be listed as co-authors. Where there are others who have participated in certain substantive aspects of the research project, they should be acknowledged or listed as contributors.

The corresponding author should ensure that all appropriate co-authors and no inappropriate co-authors are included on the paper, and that all co-authors have seen and approved the final version of the paper and have agreed to its submission for publication.

Hazards and Human or Animal Subjects
If the work involves chemicals, procedures or equipment that have any unusual hazards inherent in their use, the author must clearly identify these in the manuscript.

Disclosure and Conflicts of Interest
All authors should disclose in their manuscript any financial or other substantive conflict of interest that might be construed to influence the results or interpretation of their manuscript. All sources of financial support for the project should be disclosed.

Fundamental Errors in Published Works
When an author discovers a significant error or inaccuracy in his/her own published work, it is the author's obligation to promptly notify the journal editor or publisher and cooperate with the editor to retract or correct the paper.

Peer Review Process

Peer Reviewing Instructions for the "International Journal of Agriculture, Forestry and Life Sciences" Journal

Introductions
The primary aims of peer review are to decide whether or not an article should be published (based on quality and relevance to the journal), and to improve the article before publication. All submissions first go through an internal peer review process: an assigned editor makes an initial decision to accept or to reject the manuscript (e.g. topic is outside the scope of the Journal, important flaws in scientific validity, etc). If the editor believes the article may be of interest, it is sent out for external peer review. The reviewers are selected by area of expertise (reviewers who grant high quality reviews within the requested time are preferred). The editorial board is frequently consulted. Once reviews are obtained, the editor makes a judgment considering the critiques and recommendations from reviewers, and other factors such as relevance to the Journal's aims and usefulness to clinicians or researchers.

Peer Reviewer Selection
Reviewers are selected according to their background and experience in some aspect of the subject. The most desirable reviewers identify the strengths and weaknesses of the submitted paper, and analyze it from different viewpoints. The peer reviewers are asked to read and analyze the assigned manuscript and provide a written opinion of its quality, novelty, relevance and suitability for publication in The " International Journal of Agriculture, Forestry and Life Sciences " Journal. Peer reviewers also make suggestions to assist the authors in improving the article. Reviewers must not only analyze and comment on the paper, but also provide opinions about general concerns such as clarity and quality

of the writing, validity of scientific approach, and whether the article provides new information.

Ethical Guidelines for Journal Peer Reviewers

When a selected individual accepts a peer reviewing assignment, the reviewer implicitly agrees to the ethical standards that are commonly accepted in biomedical publishing. Ethical guidelines for reviewers, authors, and editors are reported by the International Committee of Medical Journal Editors in the 'Uniform Requirements for Manuscripts Submitted to Biomedical Journals' available from: www.icmje.org.

Reviewers for The " International Journal of Agriculture, Forestry and Life Sciences " Journal must agree to:

Produce as careful and objective a review as possible Respect the editor's deadline. Consider with an open mind innovations or approaches different from those of one's own.

Provide a balanced critique targeted not only to identify the strengths and weaknesses of the paper, but also to provide useful feedback to the authors to improve their manuscript, without being overly critical of minor points.

- Avoid scientific misconduct such as the misappropriation of intellectual property.
- Each manuscript should be treated as an extremely confidential document.
- The privacy of the authors' ideas must always be guaranteed.
- Direct comments about ethical concerns confidentially to the editors.
- Contacting an author with questions about the manuscript is not allowed.
- All critiques, including the latter, must be reported in the written critique.
- Declare any conflict of interest (real or perceived) identified to the editor before the end of review. Not every potential conflict necessitates a rejection.
- Reviewers are encouraged to discuss potential conflicts with the editors if they believe they can provide a fair review.
- Reject an assignment if the following conflicts are present: Financial interests (e.g. paid consultancies, stock holdings), significant professional or personal relationships or rivalries, antipathy toward study question/approach, political or special interest affiliations (e.g. religious or deep convictions that conflict with the manuscript topic).

Reviewer Guidelines

Potential reviewers are contacted by e-mail, which contains the manuscript title, abstract, and assignment deadline. The selected reviewer accepts or declines the assignment within 7 days. Failure to reply within the prescribed time will be treated as an implicit rejection. It is acceptable to propose an extended deadline when the given deadline (usually 4 weeks from the task acceptance date) cannot be met. The selected reviewers usually have extensive experience as faculty members, researchers, and published authors. Sometimes reviewers from other specific areas are selected. This selection is always well thought-out, and we encourage such potential reviewers to consider the

assignment if they can make a contribution to some aspect of the work. The following points must be provided by the reviewers in the written response:

- General Overview
- Organized Critique
- Assessment of Strengths and Weaknesses: the following should be evaluated: Literature review is up-to-date; Methods align with study purpose or research questions; Methods described in sufficient and appropriate detail; Research design or study approach is adequate; Approach to data analysis is appropriate; Thoughtful consideration given to the study limitations; Manuscript provides new information that is likely to be of interest to our readers.
- Possible improvements
- Commonly Overlooked Areas: Reviewers should carefully note: title, abstract, tables and figures, references.

Editor's Final Decision

After the peer review process has ended and an adequate number of reviews has been received, the assigned editor makes the final decision about the manuscript (accept, invite a revision, or reject) based on a consideration of all the reviewer comments, general critique, and other external factors (e.g. the article is consistent with the Journal purpose, similar articles recently published, number of accepted articles awaiting publication, potential impact of the article, etc.). Editors may consult with each other when making the decision. A decision summarizing the opinions of editors and reviewers will be sent to the corresponding author.

IJAFLS
e-ISSN: 2602-4381

International Journal of Agriculture, Forestry and Life Sciences

Open access Int J Agric For Life Sci (2023) 7(2)

Original Papers:

IJAFLS
e-ISSN: 2602-4381
International Journal of Agriculture, Forestry and Life Sciences
Original Article | Open access | Int J Agric For Life Sci (2023) 7(2): 25-29

Technical Efficiency of Honey and Beeswax Production in Kaduna State, Nigeria: Implications for Climate and Food Security Sustainability

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

[1]Department of Agricultural-Economics, Faculty of Agriculture, University of Abuja, PMB 117 Gwagwalada-Abuja, Federal Capital Territory, NIGERIA.
***Correspondence:** omotayoalabi@yahoo.com

Citation
Alabi, O.O, Anekwe, C.E, (2023). Technical Efficiency of Honey and Beeswax Production in Kaduna State, Nigeria: Implications for Climate and Food Security Sustainability. Int J Agric For Life Sci (2023) 7(2): 25-29.

Received :6 April 2023
Accepted :29 September 2023
Published Online :22 December 2023
Year: :2023
Volume :7
Issue :2 (December)
Pages :25-29

Available online https://dergipark.org.tr/ijafls

Abstract
This study evaluated the technical efficiency of honey and beeswax production in Kaduna State, Nigeria. Multi-stage sampling technique was adopted. A total sample size of 120 honey and beeswax producers was used. Primary data were collected with the aid of a structured and well-designed questionnaire. The gross margin and net farm income of honey and beeswax production per cycle were calculated at 924, 235.00 Naira and 891, 850.00 Naira respectively. This shows that honey and beeswax production was profitable in the study area. The significant predictors influencing the technical efficiency of honey and beeswax production were labour input, bee feed and sugar syrup, land size, number of beehives, quantities of antibiotics and vaccines, and cost incurred in honeybee pest, diseases, and predators control. The socio-economic predictors influencing negatively the technical inefficiency of honey and beeswax production were age, gender, household size, educational level, experience in beekeeping, and membership of cooperatives. The average technical efficiency score for honey and beeswax producers was 56.3% leaving a gap of 43.7% for improvement. The constraints faced by honey and beeswax producers were a lack of modern equipment, lack of credit facilities, inadequate extension services, inadequate training and capacity building, transportation problems, and disease, pest and predator attacks. The study recommended that modern beekeeping equipment should be provided for honey and beeswax producers for increased productivity, training and capacity building should be organized for honey and beeswax producers for increased efficiency and productivity

Key words
Technical Efficiency, Honey and Beeswax Production, Climate and Food Security, Sustainability, Nigeria.

Introduction
Beekeeping or Apiculture or Apiary is the act, business or science of managing honey bees to produce honey, beeswax, bee pollen, propolis, royal jelly, apitoxin, and other bee products for personal consumption and industrial use (Masuku, 2013). Apiary offers an enormous opportunity to ameliorate poverty and meet nutritional requirements. The demand for bees' products is expanding in both international and local markets in Nigeria. Honey production in Nigeria is still at its developmental stage, this can be attributed to inefficient use of available resources, and inadequate information on the beekeeping enterprise. Beekeeping is an activity (business) that requires little land, the quality of land is less important (Tijani et al., 2011), and it serves as a means of empowering smallholder farmers who have low capital investments (Farinde et al., 2005). Beekeeping for honey production has been identified as one the lucrative business in many parts of the world (Ahmad et al., 2016). Beekeeping is an activity (business) that provide benefits in terms of pollination of crops, employment, and conservation of biodiversity (Didas 2005). Beekeeping is an economically sustainable occupation that offers attractive opportunity for self-employment with multiple benefits. Beekeeping requires a shorter duration, and promise high returns compared to other income generating activities (Sadia et al., 2021). Beekeeping is an activity (business) with lower risk and the skills required can be acquired more easily than any other agricultural activity (Alropy et al., 2019). The beekeeping value chain is rich in employment opportunities from equipment manufacture, processing, value addition, packaging, and marketing has vast opportunities. Employment offered by beekeeping enterprise enhances household income thereby improving food security for the household.

Beekeeping practice needs to be adapted to the changing climate situations, the impact of disappearing natural habitats, dwindling floral biodiversity, emergent pests and diseases on bee populations is unprecedented. There is therefore the need for a concerted effort toward the conservation of the bee colonies and the establishment of a healthy environment with abundant bee floral resources. The use of technology in climate smart beekeeping also makes it possible to exploit all the primary bee products, this approach therefore yields ecosystem benefits and enhances farmers' income. Honeybees are pollinators and their activities in pollination promotes production in forestry, agriculture, and keep the natural resources and biodiversity stable. Nigeria consumes about 440,000 tonnes of honey annually and we produce just 10%. The global demand for honey was projected to exceed 2.8 million tonnes by 2024. Nigeria produces about 15,000 tonnes of honey annually, this is less than 3% of its potential of about 800,000 tonnes (FMARD, 2017). In the United States of America, about 109, 799, 366.60 Kg of honey worth $ 24,200,000.00 is produced each year. In 2021, the United States imported 651 million USD in honey, becoming the 1st largest importer of honey in the world. At the same year was the 45th most imported product in the United States, the United States import honey primarily from: Argentina (141 million USD), Brazil (115 million USD), India (114 million USD), New Zealand (95.3 million USD), and Vietnam (85.8 million USD). Australia produces 18, 375,000.51 Kg of honey, and Tanzania about 750, 000 pounds' worth of honey is produced annually (Canadian Statistics, 2003). Ethiopia which is the largest producer of honey in Africa and 10th largest producer in the world produces about 45,000 tonnes which accounted for about 27% and 3% of African and world honey production respectively (FAOSTAT, 2015). Honey which is one the products of honeybee contain plant sugars, fat, protein, carbohydrates, ash, phosphorous, calcium, sodium, potassium, iron, thiamine, Vitamin A, Vitamin C (ascorbic acid), and riboflavin (Olarinde et al., 2008). Honey provides a valuable food when it is consumed in its unprocessed state such as liquid, crystallized or in the comb, honey is largely used on a small as food and medicine for healing many ailments (Shuaib et al., 2009), as well as at an industrial level in baked products, candy, confectionary, jams, marmalades, breakfast cereals, milk products, beverages, and many processed products (Ahmad et al., 2016). However, the bees are exposed to several threats such as reduced biodiversity, climate change, and invasive species, predators, parasites, diseases that reduce their honey production, quality of health and longevity (UNEP, 2010). Beeswax is a secondary product from the apiary farmers, it is used in both industrial and handcrafted products (Gao et al., 2021).

Beeswax is a valuable product that can provide a worthwhile income in addition to honey. Industries use beeswax as a hydrophobic and insulating component of numerous products for example in electronic circuits, electric cables to isolate copper from moisture, to protect leather, in the preparation of inks, varnishes, protective waxes from cuttings and matches (Hepburn, 2012). Beeswax is one of the natural waxes that have been used as a support ingredient in pharmaceutics and cosmetics formulations. Beeswax goes into the composition of creams and ointments as a thickener and fat base. Beeswax is used for candle manufacture, making models for pieces in jewelry and sculpture modeling due to its malleability (Mladenoska, 2012), for shoe polishes and creams to protect can from acidic attack from fruit juices and other corrosive agents. Sterols present in beeswax are therapeutically

beneficial compounds effective in lowering cholesterol levels (Mellema, 2009). Beeswax is used for delicate skin care in cosmetology especially when it is dry, it cleans the epidermis and nourishes and softens the dermis thus preventing skin aging. The average composition of beeswax includes: - hydrocarbons (14%), monosters (35%), diesters (14%), hydroxyl monoesters (4%), triesters (3%), hydroxyl polyester (8%), monoesters acids (1%), polyester acids (2%), free fatty acids (12%). Nigeria produces about 2,500 tonnes of beeswax annually; this is less than 3% of its potential of 70,000 tonnes (FMARD 2017). In 2020, world production of beeswax was 62, 116 tonnes, led by India with 38% (23,716 tonnes), followed by Ethiopia with 5, 339 tonnes, and Argentina with 4,970 tonnes (FAOSTAT, 2022).

Technical efficiency is the capacity of honey and beeswax producers to maximize output from a stated input given available technology. The source of concern is the lack of technical know-how, and very little or nothing is known about the level of technical efficiency of honey and beeswax production. This means that if research is not strengthened, the technical efficiency and the sustainability of beekeeping for honey, beeswax and the production of other products may not be ascertained. Beekeepers encountered challenges of low yields of beekeeping products such as beeswax, honey, propolis and other products, this may be due to lack of training, and insufficient management practices. In addition, honey production is also affected by bad weather, bee diseases, predators, pests, low quality, and limited supply of honey in the value chain this may be caused by limited availability of bee forage, shortage of honeybee colonies, poor pre and post-harvest management, and backward technology (Vaziritabar and Esmaeilzade, 2016). Benefits of beekeeping also include the availability of necessary inputs locally, availability of technologies in rural localities, readily available markets both locally and internationally, and pollination of flowers for food production increases. In the USA, beekeepers are paid by farmers for providing a four-week pollination service with their bees. Beekeeping is an activity (business) that can reduce poverty and malnutrition.

Objectives of the Study

The broad objective of this study is to the evaluate technical efficiency of honey and beeswax production in Kaduna State, Nigeria. The specific objectives were to:

(i) determine the socio-economic profiles of honey and beeswax producers,
(ii) analyse the profitability of honey and beeswax production,
(iii) evaluate factors influencing the technical efficiency of honey and beeswax production,
(iv) evaluate socio-economic factors influencing technical inefficiency of honey and beeswax production,
(v) determine the technical efficiency scores of honey and beeswax producers,
(vi) determine the constraints faced by honey and beeswax producers in the study area.

Methodology

This research study was conducted in Kaduna State, Nigeria. Kaduna State occupies between Longitudes 06° 15⎪ and 08° 50⎪ East and Latitudes 09° 02⎪ and 09° 02⎪North of the equator. The State has a land area totalling 4.5 million hectares. They are involved in agricultural activities. The people are involved in honey and beeswax production. Crops grown include: okra, pepper, maize, ginger, sorghum, rice, yam, cassava, millet, and tomatoes. Animal reared include: cattle, goats, sheep, rabbit, and poultry.

Research Design

A descriptive cross-sectional research design was employed in this study to describe the socio-economic profiles or characteristics of honey and beeswax producers, and to evaluate factors influencing technical efficiency and socio-economic factors influencing technical inefficiency of honey and beeswax production.

Sampling Techniques and Sample Size

A multi-stage sampling technique was adopted for this study. In the first stage, a purposive sampling procedure was used to select Kaduna State based on the numerous numbers and concentration of honey and beeswax producers in the area. The second stage involved a random selection of four (4) local government areas using the ballot box method. In the third stage, three (3) villages were selected randomly from each local government area based on the intensity of honey and beeswax producers. In the fourth stage, from a sampling frame of 171 honey and beeswax producers, a proportionate and simple random sampling technique was used to select the desired sample size of 120 honey and beeswax producers. This study employed the formula advanced by Yamane (1967) in the determination or estimation of the sample size. The formula is stated thus:

$$n = \frac{N}{1+N(e^2)} = 120 \ldots\ldots\ldots\ldots\ldots (1)$$ Where,

n = Desired Sample Size N = Finite Size of the Population
e =Maximum Acceptable Margin of Error as Determined by the Researcher

Methods of Data Collection

The primary data for this study was collected from the honey and beeswax producers through structured questionnaire. The data involved information on socio-economic profiles of farmers and technical production of honey and beeswax data.

Methods of Data Analysis

Data were analyzed using the following descriptive and inferential statistics:

Farm Budgetary Technique: Gross margin and net farm income analysis of honey and beeswax production was estimated using the following models:

$$GM = TR - TVC \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

$$NFI = \sum_{i=1}^{n} P_i Q_i - \left[\sum_{j=1}^{m} P_j X_j + \sum_{k=1}^{k} GK\right] \ldots\ldots\ldots (3)$$ Where

P_i = Price of Honey and Beeswax ($\frac{N}{Kg}$),
Q_i = Quantity of Honey and Beeswax (Kg),
P_j = Price of Variable Inputs ($\frac{N}{Unit}$),
X_j = Quantity of Variable Inputs (Units),
$TR =$ Total Revenue obtained from Sales from Honey and Beeswax (N),
TVC = Total Variable Cost (N),
GK = Cost of all Fixed Inputs (Naira)
NFI = Net Farm Income (Naira)

The farm budgetary technique was used to analyze the profitability of honey and beeswax production as stated in specific objective two (ii).

Financial Analysis: According to Alabi *et al*. (2020), gross margin ratio is defined as:

$$Gross\ Margin\ Ratio = \frac{Gross\ Margin}{Total\ Revenue} \ldots\ldots\ldots\ldots (4)$$

According to Olukosi and Erhabor (2015), operating ratio (OR) is defined as:

$$Operating\ Ratio = \frac{TVC}{GI} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$ Where,

TVC = Total Variable Cost (Naira), GI = Gross Income (Naira),

The financial analysis was used to analyze the profitability of honey and beeswax production as stated in specific objective two (ii).

Stochastic Production Frontier Model

According to Alabi *et al*. (2022), the stochastic production frontier model is stated thus:

$Y_i = f(X_i, \beta_i)e^{v_i - u_i}$……..(6)

$l_n Y = \beta_0 + \beta_1 l_n X_1 + \beta_2 l_n X_2 + \beta_3 l_n X_3 + \beta_4 l_n X_4 + \beta_5 l_n X_5 + \beta_6 l_n X_6 + V_i - U_i$ ……..(7) where,

Y_i = Output of Honey and Beeswax Production (HBW) (kg)
X_i = Vectors of Factor Inputs β_i = Vectors of Parameters
V_i = Random Variations in Honey and Beeswax Output
U_i= Error Term due to Technical Inefficiency
X_1 = Labour Input in Mandays, this is expected to be positively related to (HBW) $X_1 > 0$
X_2 = Bee Feed and Sugar Syrup (Kg), $X_2 > 0$
X_3 = Land Size (Ha), $X_3 > 0$
X_4 = Number of Beehives (Units), $X_4 > 0$
X_5 = Quantities of Antibiotics and Vaccines (grams), $X_5 > 0$
X_6 = Cost Incurred in Honeybee Pests, Diseases, and Predators Control (Naira), $X_6 < 0$

$U_i = \alpha_0 + \alpha_1 Z_1 + \alpha_2 Z_2 + \alpha_3 Z_3 + \alpha_4 Z_4 + \alpha_5 Z_5 + \alpha_6 Z_6 \ldots. (8)$ where,

Z_1 = Age (Years), it is expected to be positively or negatively related to Technical Inefficiency, $Z_1 > 0$ $Z_1 < 0$
Z_2 = Gender (1, Male; 0, Otherwise), $Z_2 > 0$ $Z_2 < 0$
Z_3 = Household Size (Units), $Z_3 < 0$
Z_4 = Educational Level (Years), $Z_4 < 0$
Z_5 = Experience in Beekeeping (Years), $Z_5 < 0$
Z_6 = Members of Cooperative Organizations, $Z_6 < 0$
α_0 = Constant Term $\alpha_1 - \alpha_6$ = Parameters to be Estimated
U_i= Error Term due to Technical Inefficiency

Cost Saving Formula: The cost saving formula for average technical efficient (ATE) honey and beeswax producers and least technical efficient (LTE) honey and beeswax producers is stated as:

$$\text{Cost Savings} = \left[\left[1 - \frac{ATES\ or\ LTES}{MaxTES}\right] \times 100\right] \ldots.(9)$$ Where,

$ATES$ = Average Technical Efficiency Score (Units)
LTES = Least Technical Efficiency Score (Units)
MaxTES = Maximum Technical Efficiency Score (Units)

This was used specifically to achieve objective three (iii), which is to evaluate factors influencing the technical efficiency of honey and beeswax production, and objective four (iv) which is to evaluate socio-economic factors influencing the technical inefficiency of honey and beeswax production, and objective five (v), which is to determine the technical efficiency scores of honey and beeswax producers in the study area.

Principal Component Analysis: The constraints facing honey and beeswax producers and militating against honey and beeswax production were subjected to principal component analysis. This was used to achieve specific objective six (vi).

Results and Discussion

Socio-Economic Profiles of Honey and Beeswax Producers

The socio-economic profiles of honey and beeswax producers under consideration were gender, marital status, age level of education, household size, farming experience, extension contact, membership of cooperatives, and land size (Table 1).

Table 1: Socio-Economic Profiles of Honey and Beeswax Producers

Variables	Frequency	Percentage	Mean
Gender			
Male	109	90.83	
Female	11	09.17	
Marital Status			
Single	21	17.50	
Divorced	17	14.17	
Married	82	68.33	
Age (Years)			
31 – 40	24	20.00	
41 – 50	67	55.83	**45.92**
51 – 60	29	24.17	
Level of Education			
Non-Formal	09	07.50	
Tertiary	35	29.17	
Secondary	47	39.17	
Primary	29	24.16	
Household Size (Units)			
1 – 5	47	39.17	**7.00**
6 – 10	37	30.83	
11 – 15	36	30.00	
Farming Experience			
(Years)	32	26.67	**9.54**
1 – 5	47	39.17	
6 – 10	13	10.83	
11 – 15	28	23.33	
16 – 20			
Extension Contact	87	72.50	
Yes	33	27.50	
No			
Memberships of	92	76.67	
Cooperative	28	23.33	
Yes			
No	79	65.83	**1.10**
Land Size (Hectares)	21	17.50	
Less than 1.0	11	09.17	
1.1 – 2.0	09	07.50	
2.1 – 3.0	**120.00**	**100.00**	
3.1 – 4.0			
Total			

Source: Field Survey (2022) Profitability Analysis of Honey and Beeswax Production per Cycle

The gender distributions categorize honey and beeswax producers into male and female. About 90.83% (109) of honey and beeswax producers were male, while 09.17% (11) were female. The marital status distributions show that 17.50% (21) of honey and beeswax producers were single, 14.17% (17) were divorced, and 68.33% (82) were married. This finding is in line with similar results of Ahmad *et al*. (2016) who reported in their study that 90% of honey producers were male, and 78% of the respondents were married. About 75.83% of honey and beeswax producers were less than 50 years of age, the mean age was 45 years. This implies that the respondents were young, active, and resourceful in their youthful age. Also, 92.5% of honey and beeswax producers had formal education, while 07.50% of the respondents had non-formal education. The formal education attained by honey and beeswax producers includes: - tertiary (29.17%), secondary (39.17%), and primary (24.16%). According to Amanza and Maurice (2005), the level of education attained by honey and beeswax producers will determine to a large extent the producer's level of adoption of innovations, this will make them efficient in resource use which in turn will increase the output of honey and beeswax production, and hence subsequently increase profit obtained by producers. The household sizes were large with an average of 7 members per household. About 70.00% of honey and beeswax producers had less than 10 members per household. Also, 65.84% of honey and beeswax producers had less than 11 years of experience in beekeeping. According to Iheanacho (2000), the higher the number of years spent in beekeeping business, the more the apiarist becomes aware of new production techniques that can increase the level of productivity. Furthermore, 72. 50% of honey and beeswax producers had extension contact, while 27. 50% do not have extension contact. Mulatu *et al.* (2021) reported that extension activities increase the honey and beeswax producers' likelihood of adopting new technology by increasing the store of information about the current production technique. Timely contact with extension officers is important to ensure the efficient use of beekeeping technology. This extension contact helps beekeepers manage his/her productivity as well as promotes proper exploitation of honey products. About 76.67% of honey and beeswax producers belong to membership of cooperatives, while 23.33% do not belong to any cooperative associations. Memberships of cooperatives allow the honey and beeswax producers to exchange ideas skills and experiences about new production and marketing techniques. The average land size was 1.10 hectares, and about 65.83% of honey and beeswax producers had less than 1.0 hectares of land size.

Table 2 shows the profitability of honey and beeswax production per cycle. The revenue obtained from honey and beeswax production and the cost incurred were based on the prevailing market price at the time of the field survey. The total cost of honey and beeswax production was 68 150.00 Naira, this comprises of a total variable cost of 35,765.00 Naira (52.47%) and total fixed cost of 32,385.00 Naira (47.53%). The total variable cost consists of marketing cost (06.00%), bee feed cost (08.31%), transportation cost (05.47%), labour cost (05.68%), insecticide cost (04.76%), tools and equipment cost (13.57%), and honey extraction cost (08.27%). The gross margin and net farm income of honey and beeswax production were 924, 235.00 Naira and 891, 850.00 Naira respectively. This shows that the beekeeping business was profitable in the study area. This result is in line with studies conducted by Ahmad *et al*. (2016), Tijani *et al*. (2011), and Kuboja *et al*. (2016). The gross margin ratio of 0.962 implies that for every one naira invested in honey and beeswax production about 96 kobo covered profits, expenses, taxes, and depreciation. The operating ratio of honey and beeswax production was estimated at 0.0357, this means that 3% of honey and beeswax produce sales revenue was used to the cover cost of honey and beeswax sold and other operating expenses. The operating ratio is used to measure the operating efficiency and profitability of honey and beeswax production, a low operating ratio is preferable and it's reported to be a positive sign.

Table 2: Average Profitability Analysis of Honey and Beeswax Production per Cycle

Items	Kg	Amount (Naira)	% of Total Cost
Price of Honey per Kg = 0.7	……..	3,500.18	
Litre	……..	3,000.07	
Price of Beeswax per Kg	162.84	…….	
Mean Quantity of Honey (Kg)	129.99	…….	
Mean Quantity of Beeswax (Kg)		570,000	
Total Revenue of Honey		390,000	
Total Revenue of Beeswax		600,000	
Gross Income of Honey		400,000	
Gross Income of Beeswax			
Variable Cost		4,350.00	06.00
Marketing Cost		5,670.00	08.31
Bee Feed Cost		3,730.00	05.47
Transportation Cost		3,875.00	05.68
Labour Cost		3,250.00	04.76
Insecticide Cost		9,250.00	13.57
Tools and Equipment Cost		5,640.00	08.27
Honey Extraction Cost		**35,765.00**	**52.47**
Total Variable Cost			
Fixed Cost		3,870.00	05.00
Beehives		2,450.00	03.59
Rent on Land		1,250.00	01.83
Interest on Operating Capital		2,275.00	03.33
Colony Cost		1,250.00	01.83
Bucket		5,600.00	08.21
Touch Light		1,750.00	02.56
Rain Boot		1,230.00	01.80
Cutlass		1,050.00	01.54
Gloves		1,150.00	01.68
Knife		3,570.00	05.23
Bee Suites		3,790.00	05.56
Extractor		1,670.00	02.45
Hat		1,480.00	02.17
Ropes		**32,385.00**	**47.53**
Total Fixed Cost		**68,150.00**	**100.00**
Total Cost		**924,**	
Gross Margin (Honey +		**235.00**	
Beeswax)		**0.962**	
GMR		**891,850.00**	
NFI		**0.0357**	
OR			

Source: Field Survey (2022), 1 USD = 760 Naira GMR = Gross Margin Ratio, NFI = Net Farm Income, OR = Operating Ratio

Factors Influencing Technical Efficiency of Honey and Beeswax Production

The maximum likelihood estimates of factors influencing the technical efficiency of honey and beeswax production was presented in Table 3. All the predictors included in the technical efficiency component had positive coefficients. All the signs of the predictors included in the technical efficiency component were in line with apriori expectations. The significant predictors included in the technical efficiency component of the stochastic frontier production model were labour input ($P < 0.10$), bee feed and sugar syrup ($P < 0.05$), land size ($P < 0.05$), number of beehives ($P < 0.01$), quantities of

antibiotics and vaccines (P < 0.10), cost incurred in honeybee pests, diseases and predators control (P < 0.05) respectively. The coefficient of number of beehives was 0.2107, this implies that a 1% increase in a number of beehives keeping other predictors constant will lead to 21.07% increase in honey and beeswax production. The calculated return to scale (RTS) was 1.4608, this implies an increasing return to scale. The increased return to scale signifies that an increase in all the predictor inputs included in the technical efficiency components will lead to more than proportionate increase in the output of honey and beeswax produced. The coefficient of variance ratio(γ) was 0.7138, this implies that 71.38% of variations in the output of honey and beeswax production were due to differences in technical efficiency. The coefficient of total variance (σ^2) was 1.7209, which was statistically significant at ($P < 0.01$). This signifies a good fit for the model. The Log-Likelihood function was 331.21. This finding is in line with earlier results of Olarinde *et al.* (2008), and Shiferaw and Gebremedhin (2016).

Socio-Economic Factors Influencing Technical Inefficiency of Honey and Beeswax Production

Table 3 also shows the maximum likelihood results of socio-economic factors influencing technical inefficiency of honey and beeswax production. All the socio-economic factors included in the technical inefficiency component had negative coefficients. All the signs of the socio-economic factors included in the technical inefficiency component were in line with a priori expectations. The significant socio-economic factors negatively influencing technical inefficiency includes: - age (P < 0.10), gender (P < 0.05), household size (P < 0.05), educational level (P < 0.01), experience in beekeeping (P < 0.05), member of cooperatives (P < 0.05). The coefficient of educational level is -0.2453, this implies a 1% increase in experience in beekeeping will lead to a 24.53% decrease in technical inefficiency of honey and beeswax production. This result is in line with earlier findings of Walle (2020).

Table 3: Maximum Likelihood Results of the Stochastic Frontier Production Model

Variables	Parameters	Coefficient	StandardError	t-Value
Constant	β_0	2.0134*	1.0220	1.97
Labour Input	β_1	0.3450*	0.1568	2.20
Bee Feed and Sugar Syrup	β_2	0.4201**	0.1428	2.94
Land Size	β_3	0.1932**	0.0673	2.87
Number of Beehives	β_4	0.2107***	0.0544	3.87
Quantities of Antibiotics and Vaccines	β_5	0.1602*	0.0793	2.02
Cost Incurred in Honeybee Pests, Diseases and Predators Control	β_6	0.1308**	0.0440	2.97
RTS		**1.4608**		
Inefficiency Component				
Constant	α_0	1.910**	0.3906	2.56
Age	α_1	-0.1227*	0.0504	-2.43
Gender	α_2	- 0.1607**	0.0640	-2.51
Household Size	α_3	- 0.1302**	0.0487	-2.67
Educational Level	α_4	-0.2453***	0.0687	-3.57
Experience in Beekeeping	α_5	-0.2108**	0.0709	-2.97
Member of Cooperatives	α_6	-0.1708**	0.0595	-2.87
Diagnostic Statistics				
Total Variance	σ^2	1.7209***		
Variance Ratio	γ	0.7138		
Log-Likelihood		-306.12		
Likelihood Ratio Test		331.21		

Source: Data Analysis (2022), RTS = Return to Scale

*Significant at ($P < 0.10$)., **Significant at ($P < 0.05$).***Significant at ($P < 0.01$).

Technical Efficiency Scores of Honey and Beeswax Producers in the Study Area

Table 4 shows the summary statistics of technical efficiency scores of honey and beeswax producers. The majority (86.6%) of honey and beeswax producers were between 21 to 80 % efficiency levels. The mean technical efficiency was 56.30 % leaving a gap of 43.70 % for improvement. This implies that most producers were average technically efficient. In addition, the least technical efficiency score was 11.0 %, while the best performing honey and beeswax farm had the maximum technical efficiency of 92.0%. If the average honey and beeswax producers were to achieve the level of technical efficiency like most of its efficient counterparts, then the average honey and beeswax producers could make 38.81 % cost savings calculated as $\left[\left[1 - \frac{56.30}{92.00}\right] \times 100\right]$. The calculated value for the most technically inefficient honey and beeswax producers reveal a cost savings of 88.05 % calculated as $\left[\left[1 - \frac{11.0}{92.00}\right] \times 100\right]$.

Constraints Faced by Honey and Beeswax Producers

The constraints faced by honey and beeswax producers were subjected to principal component analysis (Table 5). Five (5) constraints with Eigen-value greater than one (1) were retained by the principal component model. Lack of modern beekeeping equipment's was ranked 1st with an Eigen-value of 1.9207, and this explained 16.04% of all constrained retained by the model. Lack of credit facilities was ranked 2nd with an Eigen-value of 1.8705, and this explained 15.09% of all constraints retained by the principal component model. Inadequate extension service was ranked 3rd with an Eigen-value of 1.6724, and this explained 17.23% of all constraints retained by the model. All constraints retained by the principal component model jointly explained 80.55% of all constraints included in the analysis. The Kaiser-Meyer-Olkin measures of sampling adequacy (KMO) of 0.71 and Bartlett test of sphericity of 793.01 and were statistically significant at 1 % probability level which demonstrated that the variables were feasible for principal component analysis. This result is in line with the findings of Alabi and Anekwe (2023), Alabi and Chiogor (2023), Olarinde *et al.* (2008), and Shiferaw and Gebremedhin (2016).

Table 4: Summary Statistics of Technical Efficiency Scores

Efficiency Score	Frequency	Percentage
0.00 – 0.20	08	06.67
0.21 – 0.40	12	10.00
0.41 – 0.60	45	37.50
0.61 – 0.80	47	39.17
0.81 – 1.00	08	06.67
Mean	0.5630	
Standard Deviation	0.1955	
Minimum	0.11	
Maximum	0.92	

Source: Field Survey (2022)

Table 5: Principal Component Model of Constraints Encountered by Honey and Beeswax Producers

Constraints	Eigen-Value	Difference	Proportion	Cumulative
Lack of Modern Beekeeping Equipments	1.9207	0.3207	0.1604	0.1604
Lack of Credit Facilities	1.8705	0.2621	0.1509	0.3113
Inadequate Extension Services	1.6724	0.1749	0.1723	0.4836
Inadequate Training or Capacity Building	1.6602	0.3607	0.1803	0.6639
Transportation Problem	1.4504	0.2816	0.1209	0.7848
Diseases Pest and Predator Attack	1.4005	0.2104	0.0207	0.8055
Bartlett Test of Sphericity				
Chi Square	793.01***			
KMO	0.7107			
Rho	1.00000			

Source: Field Survey (2022), KMO – Kaiser-Meyer-Olken

Conclusion

This study has established that beekeeping activity (business) is a profitable in the area. Honey and Beeswax producers were middle aged farmers, and the enterprise is dominated by male. The gross margin and net farm income were calculated at 924, 235.00 Naira and 891, 850.00 Naira respectively. Labour input, bee feed and sugar syrup, land size, number of beehives, the quantity of

antibiotics and vaccines, cost incurred in honeybee pests, diseases and predator control were the significant predictors influencing technical efficiency or output of honey and beeswax production. The significant socio-economic factors influencing negatively the technical inefficiency of honey and beeswax production include: age, gender, household size, educational level, experience in beekeeping, and member of cooperatives. The mean technical efficiency scores for honey and beeswax producers were 56.30% leaving a gap of 43.70% for improvement. The constraints faced by honey and beeswax producers by ranking include: lack of modern beekeeping equipment (1st), lack of credit facilities (2nd), inadequate extension services (3rd), inadequate training or capacity buildings (4th), transportation problem (5th), diseases and predators attack (6th).

Recommendations

The following recommendations were made based on the research findings:

(i) Modern beekeeping technologies should be provided for honey and beeswax producers for increase productivity, climate and food security sustainability.

(ii) Extension officers should be employed to disseminate research findings, innovations and new technologies to honey and beeswax producers.

(iii) Credit facilities should be made accessible and affordable by the government for honey and beeswax producers. This will enable them to access new beekeeping technologies.

(iv) Training and capacity building should be provided for honey and beeswax producers for increase productivity.

Statement of Conflict of Interest

The author(s) declare no conflict of interest for this study.

Author's Contributions

The contribution of the authors is equal

References

Ahmad, O.S., Alabi, O.O., Daniel, P.O (2016). Resource-Use Efficiency of Honey Production in Kachia Local Government Area, Kaduna State, Nigeria. *Journal of Agricultural Studies*, 4(1): 117 – 125. DOI: https://doi.org/ 10.5296/jas.v4i1.8790

Alabi, O.O Chiogor, O.H (2023). Technical Efficiency of Tiger Nut (*Cyperus esculentus*) Production in Katsina State: Socio-Economic Drivers and Implications for Consumers Health Benefits. *Australian Journal of Science and Technology,* 7 (1): 46 – 53

Alabi, O.O., Anekwe C.E (2023). Economics of Climate Smart Agricultural Practices (CSAPs) Used by Smallholder Sorghum Producers in Nigeria. *Australian Journal of Science and Technology,* 7(1); 65 – 71.

Alabi, O.O., Oladele, A.O Oladele, N.O (2020). 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.DOI: https://doi.org/ 10.18551/rjoas.2020-08.14.

Alabi, O.O., Oladele, A.O., Maharazu, I (2022). Economies of Scale and Technical Efficiency of Smallholder Pepper (Capsicum species) Production in Abuja, Nigeria. *Journal of Agricultural Sciences* (Belgrade), 67 (1): 63 – 82. DOI: https://doi.org/10.2298/JAS2201063A

Altropy, E.T., Desouki, N.E., Alnafissa, M.A (2019). Economics of Technical Efficiency in White Honey Production: Using Stochastic Frontier Production Function. *Saudi Journal of Biological Sciences,* 26: 1478 – 1484.

Amanza, P.S Maurice, D.C (2005). Identification of Factors that Influence Technical Efficiency in Rice-Based Production Systems in Nigeria. Paper Presented at Workshop on Policies and Strategies for Promoting Rice Production and Food Security in Sub-Saharan Africa, November 7 – 9, 2005, Cotonou (Benin). Pp 1-9.

Didas, R (2005). Beekeeping Project in South Western Uganda. *Bee World* 86, 69 – 76. Farinde, A.J., Soyebo, K.O., and Oyedokan, M.O (2005). Improving Farmers' Attitude towards Honey Production Experience in Oyo State, Nigeria. *Journal of Human Ecology*, 18 (1): 21 -33.

FAOSTAT (2022). Beeswax Production in 2020, Crops/Regions/ World List/Production Quantity. UN Food and Agriculture Organization, Corporate Statistical Database Retrieved 23 July, 2022.

FMARD (2017). Federal Ministry of Agriculture and Rural Development of Nigeria, Annual Report

Gao, Y., Lei, Y., Wu Y., Liang, H., Li, J., Pei, Y., Li, Y., Li, B., Luo, X., Liu, S (2021). Beeswax: A Potentials Self Emulsifying Agent for the Construction of Thermal-Sensitive Food W/O Emulsion. *Food Chemistry,* 2, 021, 349, 129203. DOI: https://doi.org/10.1016/j.foodchem.2021.129203

Hepburn, H.R (2012). Honeybees and Wax: An Experimental Natural History. Springer Science & Business Media.

Iheanacho, A.C (2000). Pattern and Technical Efficiency of Resource Use in Millet Based Crop Mixtures in Borno State of Nigeria. *Research Journal of Science,* 6 (1 and 2): 97 – 103

Kuboja, N.M. Isinika, A.C., Kilima, F.T.M (2016). Comparative Economic Analysis of Beekeeping Using Traditional and Improved Beehives in the Miombo Woodlands of Tabora and Katavi Regions, Tanzania. *Huria Journal*, Vol 22: 109 – 123 DOI: https://doi.org/10.4314/huria.v2211

Mellema, M (2009). Co-Crystals of Beeswax and Various Vegetable Waxes with Sterois Studied by X Ray Diffraction and Diferentials Scanning Calorimetry. *Journal Am. Oil* Chem. *Soc.* 86(6): 499 – 505. DOI: https://doi.org/10.1007/s11746.009-1385-4

Mladenoska,I (2012). The Potential Application of Novel Beeswax Edible Coatings Containing Coconut Oil in the Minimal Processing of Fruits 1 (2): 26 – 34

Mulatu, A., Marisennayya., S., Bojago, E (2021). Adoption of Modern Hive Beekeeping Technology: The Case of Kacha-Birra Woreda, Kembata Tembaro Zone, Southern Ethiopia. *Advances in Agriculture*, 1 – 20.DOI: https://doi.org/10.1155/2021/4214020

Olukosi, J.O and Erhabor, P.O (2015). *Introduction to Farm Management Economics:Principles and Applications*. Agitab Publishers Limited, Zaria, Kaduna, Nigeria pp77 – 83

Olarinde, I.O., Ajao, A.O., Okunola, S.O (2008). Determinants of Technical Efficiency in Beekeeping Farms in Oyo State, Nigeria: A Stochastic Production Frontier Approach.

Research Journal of Agriculture and Biological Sciences, 4(1): 65 – 69.

Sadia, F.T., Hossain, M.S., Begum, R Sujan, M.H.K (2021). Comparative Profitability Analysis and Resource Use Efficiency of Beekeeping Using Wooden and Poly Hive in Some Selected Areas of Bangladesh. *International Journal of Agricultural Research Innovations and Technology,* 11(1): 84 – 91.

Masuku, M.B (2013). Socio-Economic Analysis of Beekeeping in Swaziland; A Case Study of the Manzani Region, Swaziland. *Journal of Development and Agricultural Economics,* (6): 236 – 241. DOI:

Shiferaw, K Gebremedhin, B (2016). Technical Efficiency of Smallscale Honey Producers in Ethiopia: A Stochastic Frontier Analysis. LIVES Working Paper 20, Nairobi, Kenya: International Livestock Research Institute (ILRI) 1 – 21.

Tijani, B.A., Ala, A.L., Maikasuwa, M.A., Ganawa, N (2011). Economic Analysis of Beekeeping in Chibok Local Government Area of Borno State, Nigeria. *Nigerian Journal of Basic and Applied Science*, 19(2): 285 – 292.

UNEP (2010). Environment for Development: Indigenous Knowledge in Africa.http:// www.unep.org/ik/Pagos.asp? Id=Swaziland accessed 27-10-2010.

Vaziritabar, S, Esmaeilzade, S.M (2016). Profitability and Socio-Economic Analysis of Beekeeping and Honey Production in Karaj State, Iran. *Journal of Entomology and Zoology Studies*, 4(4): 1341 – 1350.

Walle, D (2020). Technical Efficiency and its Determinants of Honey Production: The Case of Bibugu District of Amhara Region, Ethiopia. *Academic Journal of Research and Scientific* Publishing, 2 (18): 141 – 172.

Yamane, T (1967). *Elementary Sampling Theory*, Volume 1, 371 – 390, Englewood Cliff:Prentice Inc.

IJAFLS
e-ISSN: 2602-4381
International Journal of Agriculture, Forestry and Life Sciences
Original Article | Open access | Int J Agric For Life Sci (2023) 7(2): 30-32

Aroma compounds and chemical content of four selected mulberry genotypes using gas chromatography (gc-ms) in Turkey

Aysegül Burğut[1] **Mesut ADA**[1] **Ömer Faruk BİLGİN**[1]* **Nesibe Ebru KAFKAS**[1]

[1]University of Çukurova, Faculty of Agriculture, Department of Horticulture, Adana, Türkiye.
***Correspondence:** ofarukbilgin35@gmail.com

Citation
Burgut, A., Ada, M., Bilgin, Ö.F., Kafkas, N.E., (2023). Aroma compounds and chemical content of four selected mulberry genotypes using gas chromatography (gc-ms) in Turkey. Int J Agric For Life Sci (2023) 7(1): 30-32.

Received :20 May 2023
Accepted :13 September 2023
Published Online :22 December 2023
Year: :2023
Volume :7
Issue :2 (December)
Pages :30-32

Available online https://dergipark.org.tr/ijafls

Abstract
The objective of this work was to identify and quantify the volatile compounds, pH, and soluable solid content (SSC) in four mulberry genotypes. The volatile compounds were extracted by solid phase microextraction (SPME). The pH results of the four mulberry genotypes were changed from 5.99 to 6.47. Soluable solid matter (SSM) of A5 genotype (Morus alba L.) had the highest rate of 14.6%, followed by S7 (Molus rubra L.) with %14.04, P1 genotype (M. alba var. laevigata) with %13.24, and S6 (Morus rubra L.) with %12. Identification and quantification of aroma components in the four mulberry genotypes were performed by gas chromatography using a mass selective detector (GS-MS). We detetermined 21 aroma compounds (2 esters, 3 alcoholes, 1 aldehydes, 4 ketones, 11 acids) in P1 genotype, 20 aroma compound (4 alcoholes, 1 aldehydes, 2 ketones, 12 acids, 1 other compound) in S7 genotype, 14 compound (1 esters, 1 alcohole, 0 aldehydes, 2 ketones, 10 acids) in A5 genotype, and 16 aroma compound (1 esters, 3 alcoholes , 0 aldehydes, 1 ketones, 11 acids) in S6 genotype. Esters, terpenes, aldehyde, and ketones were identified to be the small classes of aroma compounds in the mulberry fruit samples. Alcohols were the most considerable group of volatile compounds in mulberry. Among the alcohols, 2-(2-ethoxy ethoxy) ethanol was the most abundant component in fruits of four mulberry genotypes. In addition to alcohols, one of the most extensive aroma compounds, which are acids, account for one of the most significant proportions of the total aroma composition in terms of the number of aroma components. This study provides valuable information about the volatile compounds in mulberry fruits. This information can be used to develop new cultivars of mulberry fruits with improved aroma.

Key words
Mulberry, SPME, Morus alba L., Morus rubra L., Morus alba var. laevigata, SSM, pH.

Introduction
Mulberry, which belongs to the genus Molus in the family Moraceae, is cultivated in the wild or cultivated in many countries for its foliage. It is an important food source for silkworms (Bomby x mori). This plant includes twenty-four species . Generally, there are three types of mulberries, such as white (Morus alba), black (M. nigra), and red (M. rubra). But, among them, Morus alba L. is prevalent (Butt et al., 2008; S, Ercisli et al., 2007). However, the colour of mulberry fruit cannot be used to determine mulberry species. Mulberries are widely distributed in diverse regions changing from tropical to temperate , which shows their high adaptability to diverse environmental conditions (S, Ercisli et al., 2007). Turkey is one of the most important centres for mulberry cultivation and has a long history of cultivation. The most commonly cultivated mulberry species with edible fruits in Turkey are Morus nigra, Morus rubra, Morus alba and Morus laevigata (Özgen et al., 2009; Yıldız, 2013). In Turkey, mulberry fruit is often eaten fresh, dried, or made into molasses and jam because of its delicious taste, pleasant colour, low calorie content, and high nutritional value. For centuries, mulberry fruit has also been used in folk medicine for its pharmacological effects, including reducing fever, treating sore throat, protecting the liver and kidneys, improving vision, and lowering blood pressure in Turkey(Sezai Ercisli, 2004; Yang et al., 2014). The rich gene pool of mulberry in Turkey remains crucial as farmers and rural communities, especially in developing countries, have contributed significantly to the creation, conservation and availability of wild mulberry genetic resources in the past and in the present (Choudhary et al., 2013; Sezai Ercisli, 2004; Yang et al., 2014). Because of its nutritional value, mulberry fruit is consumed in fresh and processed forms. Mulberry fruit can be used in various forms, such as jam , syrup, pulp, ice cream, vinegar, and concentrate alcohol. In addition, mulberry leaves and organs are used pharmacologically throughout the world, particularly in China (Koyuncu, 2004). According to Chinese medicine, they are effective in monitoring diseases due to their sedative effect. They act by relieving the symptoms of fever, sore throat, and cough, and by protecting the liver, improving vision, facilitating urine flow, and lowering blood pressure (Li et al., 2009). Recent studies have shown that mulberry has significant effects on human nutrition and health through its constituents, such as organic acids, phenols, and sugars (S, Ercisli et al., 2007; Koyuncu, 2004; Özgen et al., 2009; Zhang et al., 2008). Some mulberry species' chemical composition and nutritional potential have been reported in studies worldwide (Darias-Martín, 2003; S, Ercisli et al., 2007; Gerasopoulos, 1997; Mohammad Imran et al., 2010; Xing et al., 2018). Only a few mulberry species have been studied for their edible fruits (Morus alba, Morus indica, Morus nigra, and Morus laevigata) and wood (Imran et al., 2010). In the research on mulberries, properties of chemical and pomological were usually studied. (Gundogdu et al., 2011) studied the biochemical content (the content of organic acids, phenolic compounds, sugars, vitamin C (ascorbic acid), and antioxidant capacity) of fruits of white mulberry (Morus alba L.), black mulberry (Morus nigra L.), and red mulberry (Morus rubra L.) fruits cultivated in the east of Turkey. Some researchers determined some biochemical parameters (dry matter, total sugars, total acidity, ascorbic acid, and pH) of black mulberries from different regions of Turkey (Elmaci et al., 2005; S, Ercisli et al., 2007; Koyuncu, 2004; Lale et al., 1996). In the other detailed study, the polyphenolic profile of 11 Morus alba fruits grown in the Vojvodina region of northern Serbia was investigated using ultra-high performance liquid chromatography (UHPLC) to identify and quantify polyphenols (Natić et al., 2015). In addition, some researchers found numerous flavonoids in the genus Morus (Chu et al., 2006; Ercisli et al., 2008; Özgen et al., 2009). In the earlier study, the flavour characteristics of three cultivars of black mulberry (Morus nigra) from the Aegean region of Turkey were determined a total of 18 flavour compounds in the three cultivars using GC/MS (Elmaci et al., 2005). Zhu et al., (2018) investigated the volatile compounds of mulberries taken from three cultivars (M. nigra, Y1, M. Macroura, Y2 and M. Alba, Y3) using fiffirent techniques, found that 41, 37 and 41 compounds, respectively. In a comparative study of aromatic compounds in fruit wines from raspberries, strawberries, and mulberries in the central region of Shaanxi, The authors found that 27, 30, and 31 odorants were detected, respectively. In addition, They found that alcohols formed the most abundant group, followed by esters and acids(Feng et al., 2015) .
However, the aroma content in mulberries has yet to be adequately studied. Moreover, to our knowledge, there are no comparative studies on the aroma composition using GC-MS in the mulberries. The aim of this study is to investigate the aroma compounds of 4 different mulberry genotypes selected from the Mediterranean region and Eastern Anatolia, which occupy an important place in mulberry cultivation in Turkey.

Material
Fruit samples were collected at harvest time from one genotype of white mulberry (M. alba L.), one genotype of purple mulberry (M. nigra L.), and two genotypes of red mulberry (M. rubra L.) grown in the experimental plot at the Horticultural Application and Research Farm of Cukurova University in Adana. This cultivation area was established with the support of Tubitak

projects on an area of two decares. The projects were carried out in 2021 and aimed to select promising mulberry genotypes from the Mediterranean region and Eastern Anatolia. Approximately one gramme of fruit samples from each genotypes were stored at -20 ◦C before analysis.

Method

Total soluble solids (%)

The juices of mulberry fruits belonging to the selected types were squeezed, and the amounts of total soluble solids were determined in three replicates using a hand refractometer

pH meter measurement

The juices of mulberry fruits belonging to the selected types was measured with a glass electrode pH meter with 3 replications.

Sample preparation

About 1 g of the fruit samples were weighed, immediately into a 20 ml head-space vial, including 1 mL NaCl saturated solution to hamper any enzyme reaction.

Identification of aroma compounds

The volatile compounds of the mulberry samples were extracted by solid-phase microextraction (SPME: polydimethylsiloxane–divinylbenzene DVB/CAR/PDMS 50/30 µm were supplied by Supelco (Bellefonte, PA, USA). One gram of the homogenized mulberry samples was weighed, and 1 mL of CaCl2 was added to a headspace vial and incubated at 40°C for 30 minutes. Identification and quantification of aroma compounds were performed using a gas chromatography-mass spectrometer (GC/MS, Shımadzu GC-2010 Plus) equipped with a HP -An Innowax Agilent column (30 m × 0.25 mm i.d., 0.25 µm thickness), and helium was used as the carrier gas at a linear rate of 1.0 mL/min. The temperature of the GC oven was maintained at 40 °C and programmed to 260 °C at a rate of 5 °C/min and then held constant at 260 °C for 40 min. Head Space teqnique SPME The temperature of the injector was set at 250 °C. The MS was recorded in electron impact ionization (EI) at 70 eV. The mass range was m/z 30-400. A library search was performed using the commercial libraries of Wiley, NIST, and Flavor GC-MS. Mass spectra were also compared with those of reference compounds and confirmed with retention indices from published sources. Relative percentages of separated compounds were calculated from total ion chromatograms.

Statistical analysis

All the result data were processed with the JMP (v8.00, SAS Institute Inc., USA) package program. All results were given as the mean ± standard error (SE) and Least Significant Diffrence test (LSD test) and 5% significance level were used to determine different groups.

Result and Discussion

Total soluble content (%) and ph

The results of the four mulberry genotypes of the total soluable content amounts are given in fig 1. A5 genotype (*Morus alba* L.) had the highest rate of 14.6%, followed by S7 (*Molus rubra* L.) with %14.04, P1 genotype (M. alba var. laevigata) with %13.24, and S6 (Morus rubra L.) with %12. The pH values of the four mulberry genotypes were changed from 5.99 to 6.47. Considering the results, the fruits of A5 (*M. alba* L.) and S7 (*Molus rubra* L.) can be suggested for processing due to their higher TSS. In contrast, genotype P1 (M. alba var. laevigata) can be suggested for fresh fruit production because it bears attractive, larger fruit. In previous studies, some authors determined 15.27-30.80% in TSS and 3.60-5.65 in pH of fruits of M. alba, M. rubra, and M. nigra from different regions of Turkey (Aslan, 1988; Burğut et al., 2006; Cam, 2000; S, Ercisli et al., 2008; Erkaleli et al., 2015). TSS and pH results of our study were parallel to the results of these studies.

Aroma composition of four mulberry genotypes with using GC-MS

The results of the aroma component analysis of four mulberry genotypes are summarized as percent (%) in Table 1. The results of our study have been studied that the four mulberry genotypes had various volatile profiles. Method of SPME fibre coupled with GC-MC on four mulberry genotypes revealed 21, 20,14 and 16 volatile compounds in P1, S7, A5, and S6 genotypes, respectively (Table 1). The chemical groups of volatile compounds found in all fruits were: Aldehydes, alcohols, ketones, esters, terpenes and other compounds (Fig. 1). We detetermined 21 aroma compounds (2 esters, 3 alcoholes, 1 aldehydes, 4 ketones, 11 acids) in P1 genotype, 20 aroma compound (4 alcoholes, 1 aldehydes, 2 ketones, 12 acids, 1 other compound) in S7 genotype, 14 compound (1 esters, 1 alcohole, 0 aldehydes, 2 ketones, 10 acids) in A5 genotype, and 16 aroma compound (1 esters, 3 alcoholes, 0 aldehydes, 1 ketones, 11 acids) in S6 genotype (Table 1). Table 1 shows that 2-(2-ethoxyethoxy)-ethanol was highest in all mulberry samples. 2-(2-Ethoxyethoxy)-ethanol, diphenyl-methanone, hexadecanoic acid, hexanoic acid, and 2,4-diisocyanato-1-methyl-benzene had higher abundances than other compounds.

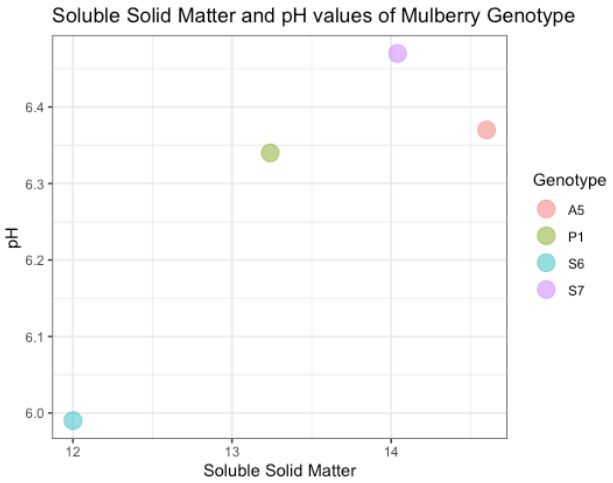

Fig 1. Some juice characteristics of four mulberry genotypes

Esters, terpenes, aldehydes, and ketones were shown to be the small classes of aroma compounds in the mulberry fruit samples. These groups were stated, which are given in Table 1. Esters included 1,2-Benzenedicarboxylic acid, bis(2-methyl propyl) ester, and Nonyl acetate. GC-MC analysis revealed that these compounds provide fruity, apple and red berry notes that form the essential aroma of mulberry. These results are consistent with the literature (Feng et al., 2015). Terpenes include 2,4-diisocyanate-1-methylbenzene, the major aroma components in all mulberry genotypes based on the combination of SPME (Fig 2). Only one aldehyde in four genotypes, which is 2,5-dimethyl-Benzaldehyde. Aldehydes are common and have been determined in earlier mulberry studies (Calín-Sánchez et al., 2013; Feng et al., 2015; Jiang et al., 2015). As shown in Table 1, alcohols were mulberry's most large group of volatile compounds. Among the alcohols, 2-(2-ethoxy ethoxy) ethanol was the most abundant component in mulberry fruit. Alcohols are formed by breaking amino acids, carbohydrates, and lipids (Antonelli et al., 1999; X. Li et al., 2014). Feng et al. (2015) noted alcohols were the largest group of aromatic compounds identified, with 25 compounds in mulberry. These results are consistent with our study. In addition to alcohols, the most abundant aroma compounds, acids account for one of the most significant proportions in the total aroma composition in terms of the number of aroma components (Fig 2). A total of 12 acids are 9,12-Octadecadienoic acid (Z, Z), Acetic acid, Decanoic acid, Dodecanoic acid, Formic acid, Heptadecene-(8)-Carbonıc Acıd-(1), Hexadecanoic acid, Hexanoic acid, Nonanoic acid, Octadecanoic acid, Octanoic acid and Tetradecanoic acid. There are few studies on the aroma composition of Morus species. (Zhang et al., 2011) determined thirty compounds in the fruits of Morus alba, but few of them matched those found in our study. The same situation is found in a study of (Göğüş et al., 2011). They reported 45 volatile compounds, but only a few were identified in the current study. These significant discrepancies in the results of studies could be because of a variesty of factors, which are extraction methods, climate, soil and cultivation methods.

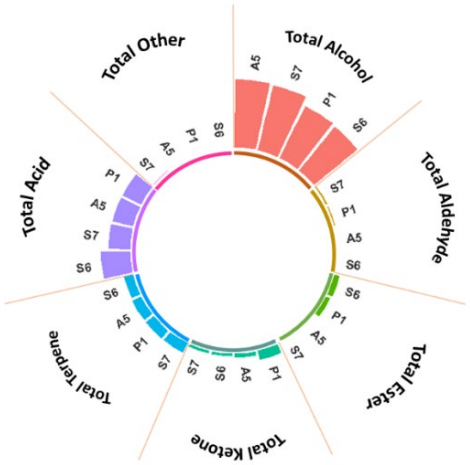

Fig 2. Circos graph plotting of main chemical groups in four mulberry genotypes

Table 1. Aroma compunds of four mulberry genotypes selected from Mediterranean region and Eastern Anatolia

	P1	S7	A5	S6
Esters				
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	0,46		0,35	
Nonyl acetate	3,86			5,23
Total Ester	**4,32**	**0**	**0,35**	**5,23**
Alcoholes				
1,4-Butanediol	0,45	0,4		0,73
2-(2-ethoxyethoxy)- Ethanol	**54,14**	**60,76**	**65,44**	**53,5**
Heptadecyl alcohol		1,99		0,44
Phytol	1,36	1,37		
Toplam Alcoholes	**55,95**	**64,52**	**65,44**	**54,67**
Aldehydes				
2,5-dimethyl- Benzaldehyde	0,79	1,14		
Total Aldehydes	**0,79**	**1,14**	**0**	**0**
Ketones				
Diphenyl- Methanone	0,46	1,87	3,3	2,69
Butyrolactone	0,65	0,58		
Civetone	6,11			
diphenyl- Methanone	1,83		0,59	
Total ketones	**9,05**	**2,45**	**3,89**	**2,69**
Acids				
9,12-Octadecadienoic acid (Z,Z)-	0,58	0,5	0,84	1,92
Acetic acid	2,99	2,11		0,41
Decanoic acid	1	0,57	0,89	0,91
Dodecanoic acid	0,89	0,49	0,91	0,86
Formic acid	2	1,13		
Heptadecene-(8)-Carbonıc Acıd-(1)		4,99	6,12	8,27
Hexadecanoic acid	6,22	5,32	6,83	10,83
Hexanoic acid	1,86	1,57	1,41	0,7
Nonanoic acid	1,82	0,55	0,97	0,44
Octadecanoic acid	0,91	1,02	1,2	2,12
Octanoic acid	1,1	0,99	0,93	1
Tetradecanoic acid	0,57	1,92	0,5	0,92
Total Acids	**19,94**	**21,16**	**20,6**	**28,38**
Terpenes				
2,4-diisocyanato-1-methyl-Benzene	9,97	10,21	9,72	9,01
Total Terpenes	**9,97**	**10,21**	**9,72**	**9,01**
Other compounds				
2,6-bis(1,1-dimethylethyl)-4-methyl-Phenol		0,52		
Total other compounds	**0**	**0,52**	**0**	**0**

Conclusion

In this study, SPME was used in conjunction with GC-MS to analyse the volatiles of four mulberry genotypes. It is a feasible technique to determine the quality and quantity of aroma components in mulberries. The results show that the four genotypes have different volatile profiles. The chemical groups of volatile compounds found in all fruits were: Aldehydes, alcohols, ketones, esters, terpenes and other compounds. The mulberries have been determined various aromatic substances Alcohols were the most considerable group of volatile compounds in mulberry. Among the alcohols, 2-(2-ethoxy ethoxy) ethanol was the most abundant component in mulberry fruit. In addition to alcohols, the most abundant aroma compounds, acids accounts for one of the most significant proportion of the total aroma. This study provides valuable information about the volatile compounds in mulberry fruits.

Statement of Conflict of Interest

The authors are declared that they have no conflict with this research article.

Author Contribution

M.A., and A.B: laboratory work, and field work, N.E.K., ÖFB; article writing

References

Aslan, M. M. (1988). *Selection of promising mulberry genotypes from Malatya*. (Doctoral Dissertation, MSc Thesis).

Burğut, A., N. F. Türemiş. (2006). *Adana ili ve çevre ilçelerinde yetişen sofralık ve sanayiye uygun dutların seleksiyonu. II.* Ulusal Üzümsü Meyveler Sempozyumu.

Butt, M., Nazir, A., Sultan, M. (2008). Morus alba L. nature's functional tonic. *Trends in Food Science & Technology*, *19*, 505–512. doi: 10.1016/j.tifs.2008.06.002

Cam, I. (2000). *Selection of promising mulberry genotypes from Edremit and Gevas district of Turkey (Doctoral dissertation, MSc Thesis.* Yuzuncu Yil University, Department of Horticulture, Van, p 53).

Choudhary, R., Chaudhury, R., SK Malik. (2013). Genetic stability of mulberry germplasm after cryopreservation by two-step freezing technique. *African Journal of Biotechnology*, *12*(41). Retrieved from https://academicjournals.org/journal/AJB/article-full-text-pdf/5397E4E30548/

Chu, Q., Lin, M., Tian, X., A, J. Y. (2006). Study on capillary electrophoresis–amperometric detection profiles of different parts of Morus alba L. *Journal of Chromatography* , *1116*, 286–290.

Darias-Martín, J. , L.-R. G. , H.-C. J. , D.-D. E. , D.-R. C. (2003). Alcoholic beverages obtained from black mulberry. *Food Technology and Biotechnology*, *41*(2), 173–176.

Elmaci, Y., Altug, T. (2005). *Gc / Ms and Flavor Profile Analysis Techniques*. *28*, 163–170.

Ercisli, S, Orhan E. (2007). Chemical composition of white (Morus alba), red (Morus rubra) and black (Morus nigra) mulberry fruits. *Fruit Chemistry*, 243–251. doi: 10.1007/978-3-540-34533-6_12

Ercisli, S, Orhan, E. (2008). Some physico-chemical characteristics of black mulberry (Morus nigra L.) genotypes from Northeast Anatolia region of Turkey. *Scientia Horticulturae*, *116*, 41–46.

Ercisli, Sezai. (2004). A short review of the fruit germplasm resources of Turkey. *Genetic Resources and Crop Evolution*, *51*(4), 419–435. doi: 10.1023/B:GRES.0000023458.60138.79

Erkaleli, Z. , Dalkiliç, z. (2015). Uşak ili ulubey ilçesinde yetişen karadutlarin (morus nigra l.)* morfolojik, fenolojik ve pomolojik özelliklerinin belirlenmesi. *Adnan Menderes Üniversitesi Ziraat Fakültesi Dergisi*, *13(1)*, 89–106. Retrieved from https://dergipark.org.tr/en/pub/aduziraat/article/278377

Feng, Y., Liu, M., Ouyang, Y., Zhao, X., Ju, Y., Fang, Y. (2015). Comparative study of aromatic compounds in fruit wines from raspberry, strawberry, and mulberry in central Shaanxi area. *Food & Nutrition Research*, *59*(1), 29290. doi: 10.3402/fnr.v59.29290

Gerasopoulos, D., S. G. (1997). Quality characteristics of four mulberry (Morus sp) cultivars in the area of Chania, Greece. *Journal of the Science of Food and Agriculture*, *73*(2), 261–264.

Gundogdu, M., Muradoglu, F., Sensoy, R. (2011). Determination of fruit chemical properties of Morus nigra L., Morus alba L. and Morus rubra L. by HPLC. *Scientia Horticulturae* , *132*, 37–41.

Imran, M, Khan, H., Shah, M., Khan, R. (2010). Chemical composition and antioxidant activity of certain Morus species. *Journal of Zhejiang University Science*, 973–980.

Imran, Mohammad, Khan, H., Shah, M., Khan, R., Khan, F. (2010). Chemical composition and antioxidant activity of certain Morus species. *Journal of Zhejiang University: Science B*, *11*(12), 973–980. doi: 10.1631/JZUS.B1000173

Koyuncu, F. (2004). Organic acid composition of native black mulberry fruit. *Chemistry of Natural Compounds*, *40*(4), 367–369. doi: 10.1023/B:CONC.0000048249.44206.E2

Lale, H., Ozcagiran, R. (1996). *A study on pomological, phenologic and fruit quality characteristics of Mulberry (Morus sp.) species*. Derim.

Li, W., Li, T., Agricultural, K. T.-A. J. of, 2009, undefined. (2009). Flavonoids from mulberry leaves by microwave-assisted extract and anti-fatigue activity. *Academicjournals.Org*, *4*(9), 898–902. Retrieved from https://academicjournals.org/journal/AJAR/article-full-text-pdf/877CD9538934.pdf

Natić, M. M., Dabić, D. Č., Papetti, A, Akšić, M. M. F., Ognjanov, V, Ljubojević, M, Tešić, Ž. L. (2015). Analysis and characterisation o phytochemicals in mulberry (Morus alba L.) fruits grown in Vojvodina, North Serbia. *Food Chemistry*, *171*, 128–136.

Özgen, M., Serce, S., Kaya, C., Zgen, M. O. ", Serçe, S. (2009). Phytochemical and antioxidant properties of anthocyanin-rich Morus nigra and Morus rubra fruits. *Elsevier*. doi: 10.1016/j.scienta.2008.08.007

Xing, L, Liu, R, G., X., Z. J., Wang, C, Zhou, G., Zhang, W. (2018). The proteomics homology of antioxidant peptides extracted from dry-cured Xuanwei and Jinhua ham. *Food Chemistry*, *266*, 420–426.

Yang, S., Wang, B., Sinica, Y. L. (2014). Advances in the pharmacological study of Morus alba L. *Yao Xue Xue Bao= Acta Pharmaceutica Sinica,* *49*(6), 824–831. Retrieved from https://europepmc.org/article/med/25212027

Yıldız, O. (2013). Physicochemical and sensory properties of mulberry products: Gümüşhane pestil and köme. *Turkish Journal Of Agriculture And Forestry*, *37*(6), 762–771. doi: 10.3906/tar-1301-41

Zhang, W., Han, F., He, J., Duan, C. (2008). HPLC-DAD-ESI-MS/MS analysis and antioxidant activities of nonanthocyanin phenolics in mulberry (Morus alba L.). *Journal of Food Science*, *73*(6), 512–528.

Zhu, J., Wang, L., Xiao, Z., Niu, Y. (2018). Characterization of the key aroma compounds in mulberry fruits by application of gas chromatography–olfactometry (GC-O), odor activity value (OAV), gas. *Food Chemistry*, *245*, 775–785. Retrieved from https://www.sciencedirect.com/science/article/pii/S0308814617319325

IJAFLS
e-ISSN: 2602-4381
International Journal of Agriculture, Forestry and Life Sciences
Original Article | Open access | Int J Agric For Life Sci (2023) 7(2): 33-35

An Eco-Friendly Approach for The Management of Root-Knot Nematodes in Tomato

Shraddha Timsina[1]*

[1]G.P. Koirala college of Agriculture and Research Centre(GPCAR),Gothgaun,Morang,Nepal.
***Correspondence:** shraddhatimsina999@gmail.com

Citation
Timsina, S. (2023). An Eco-Friendly Approach for The Management of Root-Knot Nematodes in Tomato. Int J Agric For Life Sci (2023) 7(2): 33-35.
Received :12 June 2023
Accepted :26 September 2023
Published Online :22 December 2023
Year: :2023
Volume :7
Issue :2 (December)
Pages :33-35

Available online https://dergipark.org.tr/ijafls

Abstract
Root-knot nematodes (RKNs) are microscopic soil-borne pests that pose a significant threat by attacking the roots of numerous plant species. This causes a lot of damage and can reduce the amount of crops produced. RKNs have a unique ability to survive in the soil for extended periods, even in the absence of a host plant which has increased its threat by making it more devastating. An experiment was done on a tomato field at the Lamjung campus that had the root-knot nematode problem to see how well different treatments worked on these nematodes in tomato plants. Treatments like Neem cake, chicken manure, Lantana camara, Trichoderma, and Fosthiozate, were arranged in Randomized Complete Block Design and replicated four times. The disease parameter i.e., the root-knot index was recorded along with the growth parameter yield at the time of harvest. Chicken manure worked the best in reducing the root-knot problem, with a score of (0.87), and it helped produce the highest yield (12.38 t/ha). Neem cake and Lantana camara also did well. Also, the more root-knot issues there were, the fewer tomatoes were produced. This connection was very strong and significant

Key words
Root-knot nematodes, Tomato, Meloidogyne incognita, botanicals, management.

Introduction
The tomato (Lycopersicon esculentum Mill.) belongs to the family Solanaceae and is a widely cultivated, nutritious fruit (Haque et al., 1999). It originated from the highlands of South America where it was originally called "tomato". It is globally recognized that tomato holds a prominent position in vegetable production and consumption, closely following potatoes and sweet potatoes (Hossain et al., 2010). Tomatoes are good for health as they provide minerals, essential amino acids, sugars, dietary fibers, and vitamins (Sesso et al., 2003). Tomato cultivation is experiencing increasing popularity in Nepal, with districts such as Kavre, Dhankuta, Sarlahi, Dhading, Dang, Kathmandu, Rupandehi, etc (K. Singh et al., 2015). On a global scale, the leading countries in tomato production include the United States, China, India, and subsequently Turkey (Villanueva Gutierrez et al., 2018). The tomato alone covers 22,566 hectares of land with an average productivity of 18.01 mt/ha (MoALD, 2018/2019). In the Lamjung district, tomatoes are among the major vegetables, following cauliflower and cabbage, occupying an area of 177 hectares with average productivity of 14.4 mt/ha (MoALD, 2018/2019).

Root-knot, caused by Meloidogyne spp., stands out as one of the most widespread and devastating biotic stresses that tomatoes face throughout their growing season (Hunt et al., 2009). Meloidogyne incognita is extensively prevalent and is regarded as a highly significant pest in terms of economic impact (Hussain et al., 2011). Among cultivated vegetables, tomatoes, eggplants, okra, peppers, gourds, and melons are highly susceptible to root-knot nematodes. With a host range encompassing 44 plant families, this disease is prevalent across tropical, subtropical, and some warm temperate regions worldwide (Hayward, 1991; Ji et al., 2005). The typical symptoms of infestation include the formation of galls of various sizes on the roots of plants. Since RKNs extract nutrients and minerals from the plants, symptoms such as stunting, chlorosis (yellowing), premature wilting, malformed fruits, and reduced plant growth become evident (Williamson, 1998). Plant growth and productivity can be negatively impacted by reduced nutrient and water uptake due to the presence of M. incognita infection, which leads to the deformation of roots (Kepenekci et al., 2016; McCarter, 2008). Nematode control is primarily achieved through various methods, including plant resistance, crop rotation (Chitwood, 2002), and the use of biocontrol agents (Mukhtar et al., 2003; Rahoo et al., 2011). Due to the economic and hazardous consequences associated with chemical nematicides, researchers have shifted their focus towards implementing biological methods to control Meloidogyne spp (Randhawa et al., 2001). Bio-control presents a viable and environmentally friendly alternative for safeguarding plants, offering significant potential for advancing sustainable agriculture. The effectiveness of bio-control is contingent upon factors such as the specific nematode species, the host plant, the root exudates it produces, and the other crops involved in the rotation (S. Singh et al., 2010). Root-knot nematode infestation leads to an average loss of 20.6% in tomato yield (Sasser, 1989).

Once a field becomes infested with RKN nematodes, complete eradication becomes a challenging task for farmers. However, it is possible to manage their population below the threshold level. In the study area, there has been limited assessment and research conducted on the spread and eco-friendly management of root-knot diseases. Hence, this research aimed to explore alternative management strategies and sustainable approaches by utilizing various biological agents and plant extracts to mitigate the impact of these diseases.

Materials And Methods
The experiment was done at IAAS Lamjung Campus in Nepal between March and July 2022. This place is 700 masl and known to have a worm problem affecting plants. The test was done on a tomato variety called Srijana. The experiment followed a Complete Randomized Block Design (RCBD) using six different treatments like Neem cake, Chicken Manure, Lantana camara, Trichoderma, and Fosthiozate each replicated four times. Each plot measured 3m*2m with 16 plants, spaced at 50 cm*50 cm apart, with rows set 75 cm*75cm apart. There was a 1-meter gap between each trial and 50 cm between plots. A 20-ton FYM was used as the base, and the advised fertilizer mix of N: P: K @ 200:120:100 kg/ha was added. Other agronomical practices such as irrigation, weeding, pruning, staking, and plant protection were carried out as required.

Table 1. Treatment Details

S.N	Treatments	Application dose	Application time
T1	Neem Cake	3t/ha of water	2 weeks before transplanting
T2	Chicken Manure	10t/ha	2 weeks before transplanting
T3	Lantana Camara	0.5%(W/w) or 10t/ha	2 weeks before transplanting
T4	Trichoderma	200 gram/6kg of manure	7 days before transplanting
T5	Fosthiozate	2ml/l of water	7 days before transplanting
T6	Control	Use of recommended dose of fertilizers only	

Root-Knot index

The root-knot index, a disease parameter, was recorded, along with growth

parameter yield at the time of harvesting of tomato The tagged plant was carefully uprooted after 90 days of transplanting and the roots were washed gently with tap water and root-knot was observed. The degree of root-knot nematode infection was recorded according to the rating degree given by (Thies et al., 1997) as under:

Yield

The fruits harvested from the selected plants were taken and weighed an average yield was calculated

Statistical Analysis

The data of the experiments were statistically analyzed, using RStudio for analysis of variances. All the values were presented as the mean which was compared according to Critical Difference (C.D.) at P = 0.05 level. Duncan's Multiple Range Test was employed to test for significant differences between the treatments.

Results

Root-Knot index

Six treatments were tested to see how well they tackled the Root-knot Nematodes problem in tomato plants using environmentally friendly methods. The results in (Figure 1) show all the treatments helped reduce the Gall index compared to the group that wasn't treated. Each one worked in different ways against the worms. The data showed clear differences ($p \leq 0.001$) in how effective they were. Chicken manure exhibited the highest reduction in the root-knot index with a score of (0.87), which stood out from the others. Neem cake was next best with (2.62), then came Lantana camara (2.91), Trichoderma (3.31), Fosthiozate (3.5), and the group that wasn't treated was last with (4.75). It is important to highlight that the control group displayed the highest root-knot index, indicating its ineffectiveness in managing the nematodes. In the end, the data showed chicken manure worked the best, followed by neem cake, Lantana camara, and Fosthiozate. The group that wasn't treated did the worst against the root-knot nematodes.

Table 2. Gall Index value to score Root-knot nematodes on tomato

S. N	Score	Index value
1	1	No galls or egg mass present
2	2	1-3% roots galled or covered with egg mass
3	3	4-10% roots galled
4	4	11-25% roots galled
5	5	26-35% roots galled
6	6	36-50% roots galled
7	7	51-65% roots galled
8	8	66-80% roots galled
9	9	>80% roots galled

Table2: Effects of different treatments on Gall index and Yield of tomato

Treatment	Shoot Length	Root Length	Shoot diameter	Root weight	Yield (t/ha)	Gall index
Neem Cake	141.32	24.32	4.27	38.18	9.59^{a}	2.9100^{b}
Chicken Manure	143.81	25.12	4.28	37.81	12.38^{b}	0.8750^{c}
Lantana Camara	143.31	28	4.72	42.50	9.33^{b}	2.6250^{b}
Trichoderma	138.31	26.87	4.61	44.18	8.13^{b}	3.3125^{b}
Fosthiozate	134.06	29.18	4.19	38.18	5.66^{c}	3.5000^{ab}
Control	136.44	27	4.46	39.46	4.59^{c}	4.7500^{a}
LSD(0.05)	18.89	6.43	0.57	9.50	1.87	1.29
SEM(+-)	2.55	0.88	0.078	1.286	0.39	0.35
F-Proab	ns	ns	ns	ns	***	***
CV%	8.98	15.95	8.67	15.73	15.03	28.625
Grand Mean	139.54	26.75	4.425	40.057	8.28	2.99

*Note: SEM±, Standard Error of mean; C.V, Coefficient of variation; LSD, Least significant difference. Means in the column with same letter (s) in superscript indicate no significant difference between treatments at 0.05 level of significance; '***' Significant at 0.001 level of Significance; '**' Significant at 0.01 level of Significance; '*' Significant at 0.05 level of Significance. Value in parenthesis indicates the original mean value.*

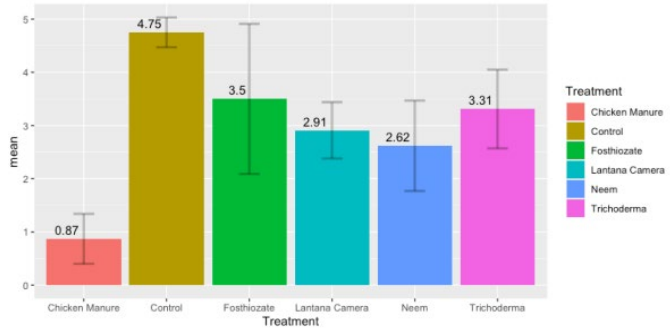

Figure 1: Effects of different treatments on the Gall index of Tomato

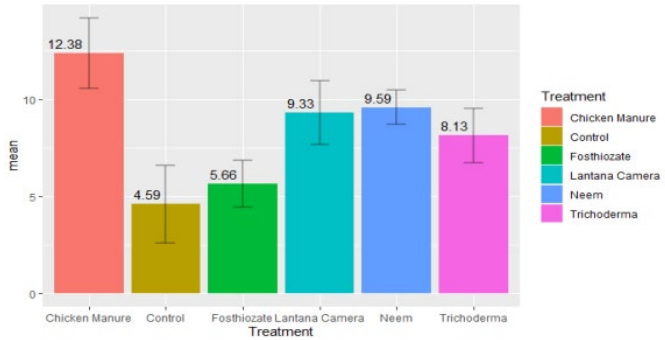

Figure 2: Effects of different treatments on Yield of tomato

Yield

Six treatments were tested to see how they could safely help tomato plants grow more and deal with the Root-knot Nematodes issue. The results in (Figure 2) showed that every treatment helped the plants produce more than the group that wasn't treated. Each method had different impacts on the plant's growth and health. The data showed clear differences ($p \leq 0.001$) in how much each group produced. Chicken manure made plants grow the most, giving (12.38 t/ha), which was notably more than the rest. Neem cake came next with (9.59 t/ha), then Lantana camara with (9.33 t/ha), and Trichoderma with (8.13 t/ha). The group that wasn't treated grew the least, with (4.59 t/ha). Summing up, chicken manure worked the best for growth, followed by neem cake, Lantana camara, and Fosthiozate. The group that got no treatment grew the least.

Correlation between Yield and Root Knot index

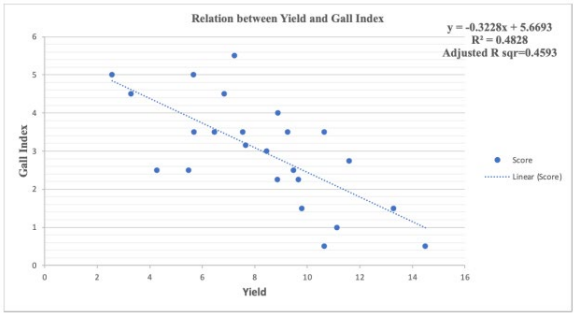

Figure 3: Correlation between Yield and Gall index

The presented graph illustrates a significant negative correlation between the Yield and Gall index, with a significance level of 0.001%. This indicates that as the severity of the disease increases, there is a corresponding decrease in crop yield, resulting in significant losses. The adjusted R-square value of 0.45 implies that 45% of the changes in the dependent variable can be attributed to the factors included in this model.

Discussion

This finding aligns with the research conducted by (Abdel-Dayem et al., 2012) which also highlighted the nematicidal and fertilizing properties of chicken manure. The elevated levels of Nitrogen and Phosphorous present in chicken manure have positively impacted plant growth, resulting in increased resistance against nematode attacks. It is because of the addition of nutrients to the soil as a result of organic matter decomposition, the direct killing or inhibiting effects of decomposed materials on nematodes, and the root`s capacity to absorb water and nutrients required for photosynthesis. Similar results were also documented by (Pérez et al., 2005) who observed that chicken manure exhibited effectiveness in reducing the gall index, particularly under elevated temperatures. Specifically, at a temperature of 30°C, the efficacy of chicken manure against the gall index was notably high. This result was supported by (Chindo et al., 1990) who reported an increase in yield attributed to various factors. This can be attributed to several factors, including enhanced nutrient availability for the plants, improved soil conditions leading to increased root growth, and subsequently, better utilization of soil nutrients. These factors collectively minimize nematode damage. Additionally, the application of poultry manure brings about changes in both the biotic and abiotic environment surrounding the plants, ultimately altering the relationships between hosts and parasites.

(Sumbul et al., 2015) also reported that oil-cakes serve as a highly suitable and readily accessible substrate for promoting the robust growth of beneficial microorganisms, thereby benefiting plants in numerous ways. It has been observed that these microorganisms play a crucial role in accelerating the decomposition of oil-cakes. Consequently, this decomposition process aids in reducing the nematode population, offering a valuable benefit to the plants. (Bhattacharya et al., 1987) also reported similar findings regarding the decomposition of the Neem cake. They discovered that among the various chemical constituents found in neem kernels, the nematotoxic properties are solely attributed to a group of compounds called limonoids, which belong to B-furano-triterpenoids. Neem cake is rich in nitrogen content, making it a valuable source of organic manure in agricultural fields, leading to an increase in crop yield. Furthermore, the roots of plants grown in soil amended with neem cake undergo physiological changes that make them unsuitable for nematode penetration and development. This contributes to a certain level of resistance in plants against nematode infections.

(Ghimire et al., 2015) also reported a similar finding of larvicidal activity of lantana camara due to the presence of camaric acid and olenolic acids. (Qamar et al., 2005) also found similar findings which explain the different metabolites like lantanoides acid, camaric acid, and oleanolic acid possessing nematicidal activity. A similar finding was found by (Sahebani et al., 2008) who explore the different defense enzymes leading to systematic resistance in plants. The presence of *Trichoderma harzianum* in the roots resulted in an increase in the activity of defense-related plant enzymes, such as peroxidase, chitinase, B-1,3-glucanases, lipoxigenase, and phenylalanine ammonia-lyase. Since nematode eggshells contain proteins and chitin, which are targets for *T. harzianum* and other nematophagous fungi, these fungi produce extracellular chitinase, protease, and other lytic enzymes that aid in egg penetration. In the untreated control group, the crop yield was significantly lower due to the severe infestation of disease and the development of root-knot, which hampers the efficient uptake of nutrients.

Conclusion

The treatments showed noticeable differences in both disease parameters and yield. The results showed chicken manure worked the best at fighting the root-knot nematode disease and increasing tomato yield, with neem cake and Lantana camara coming next. Different organic amendments have diverse effects on nematode suppression, which depend on various factors such as the types of compounds released, dosage, soil characteristics, and nematode population levels. So, it's recommended to keep using eco-friendly treatments that are available and affordable for the best long-term results. This method can be an alternative to chemical solutions and can help keep the environment clean. It's best to use this technique to prevent issues and be a part of global efforts to keep soil good for growing in the future.

Acknowledgement

We extend our sincere gratitude and heartfelt appreciation to IAAS, Lamjung, for providing us with the opportunity to conduct this research. We are deeply grateful to Mr. Rajan Paudel and Mr. Sanjay Mahato, Assistant Professors, for their invaluable guidance and support throughout the study period. We would also like to thank all the people directly or indirectly involved in the study process wholeheartedly.

Statement of Conflict of Interest

The author(s) declare no conflict of interest for this study.

Author's Contributions

The contribution of the authors is equal

References:

Abdel-Dayem, E., Erriquens, F., Verrastro, V., Sasanelli, N., Mondelli, D., Cocozza, C. J. H. (2012). Nematicidal and fertilizing effects of chicken manure, fresh and composted olive mill wastes on organic melon. 49, 259-269.

Bhattacharya, D., Goswami, B. K. J. R. N. (1987). Comparative efficacy of neem and groundnut oil-cakes with aldicarb against MeZuidugyne incognita in tomato. 10(4), 467-470.

Chindo, P., Khan, F. J. I. J. o. P. M. (1990). Control of root-knot nematodes, Meloidogyne spp., on tomato, Lycopersicon esculentum Mill., with poultry manure. 36(4), 332-335.

Chitwood, D. J. J. A. r. o. p. (2002). Phytochemical based strategies for nematode control. 40(1), 221-249.

Ghimire, G., Gupta, R., Keshari, A. K. J. N. J. o. Z. (2015). Nematicidal activity of Lantana camara L. for control of Root-knot nematodes. 3(1), 1-5.

Haque, M., Islam, M., Rahman, M. J. B. J. A. S. (1999). Studies on the presentation of semi-concentrated tomato juice. 26(1), 37-43.

Hayward, A. J. A. r. o. p. (1991). Biology and epidemiology of bacterial wilt caused by Pseudomonas solanacearum. 29(1), 65-87.

Hossain, M. E., Alam, M. J., Hakim, M., Amanullah, A., Ahsanullah, A. J. B. R. P. J. (2010). An assessment of physicochemical properties of some tomato genotypes and varieties grown at Rangpur. 4(3), 135-243.

Hunt, D. J., Handoo, Z. A. (2009). Taxonomy, identification and principal species. In Root-knot nematodes (pp. 55-97): CABI Wallingford UK.

Hussain, M., Mukhtar, T., Kayani, M. J. J., Journal of Animal, & Sciences, P. (2011). Assessment of the damage caused by Meloidogyne incognita on okra (Abelmoschus esculentus). 21(4), 857-861.

Ji, P., Momol, M., Olson, S., Pradhanang, P., Jones, J. J. P. d. (2005). Evaluation of thymol as biofumigant for control of bacterial wilt of tomato under field conditions. 89(5), 497-500.

Kepenekci, I., Hazir, S., Lewis, E. E. J. P. m. s. (2016). Evaluation of entomopathogenic nematodes and the supernatants of the in vitro culture medium of their mutualistic bacteria for the control of the root-knot nematodes Meloidogyne incognita and M. arenaria. 72(2), 327-334.

McCarter, J. P. J. N. b. (2008). Nematology: terra incognita no more. 26(8), 882-884.

MoALD. (2018/2019). Statistical Information of Nepalese Agriculture. Kathmandu, Nepal: Agri-Business Promotion and Statistics, Ministry of Agriculture and Livestock Development (MoALD).

Mukhtar, T., Pervaz, I. J. I. J. o. A., Biology. (2003). In vitro evaluation of ovicidal and larvicidal effects of culture filtrate of Verticillium chlamydosporium against Meloidogyne javanica. 5(4), 576-579.

Pérez, P. E. Z., López, C. C., Zárate, P. M. J. A. e. d. a. (2005). El español Antonio Romera y la historiografía artística chilena: una propuesta de organización fundacional. 78(310), 133-144.

Qamar, F., Begum, S., Raza, S., Wahab, A., Siddiqui, B. J. N. p. r. (2005). Nematicidal natural products from the aerial parts of Lantana camara Linn. 19(6), 609-613.

Rahoo, A. M., Mukhtar, T., Gowen, S. R., Pembroke, B. J. P. J. o. Z. (2011). Virulence of entomopathogenic bacteria Xenorhabdus bovienii and Photorhabdus luminescens against Galleria mellonella larvae. 43(3), 543-548.

Randhawa, N., Sakhuja, P., Singh, I. J. P. D. R.-L.-. (2001). Management of root knot nematode Meloidogyne incognita in tomato with organic amendments. 16(2), 274-276.

Sahebani, N., Hadavi, N. J. S. (2008). Biological control of the root-knot nematode Meloidogyne javanica by Trichoderma harzianum. 40(8), 2016-2020.

Sasser, J. N. J. P.-p. n. t. f. s. h. e. (1989). Plant-parasitic nematodes: the farmer's hidden enemy.

Sesso, H. D., Liu, S., Gaziano, J. M., Buring, J. E. J. T. J. o. n. (2003). Dietary lycopene, tomato-based food products and cardiovascular disease in women. 133(7), 2336-2341.

Singh, K., Bhandari, R. J. S. P., Kathmandu. (2015). Vegetable crops production technology. 103-107.

Singh, S., Mathur, N. J. B. S., & Technology. (2010). In vitro studies of antagonistic fungi against the root-knot nematode, Meloidogyne incognita. 20(3), 275-282.

Sumbul, A., Rizvi, R., Mahmood, I., Ansari, R. A. J. A. J. P. P. (2015). Oil-cake amendments: Useful tools for the management of phytonematodes. 9(3), 91-111.

Thies, J., Mueller, J., Fery, R. J. J. o. t. A. S. f. H. S. (1997). Effectiveness of Resistance to Southern Root-knot Nematode in Carolina Cayenne 'Pepper in Greenhouse, Microplot, and Field Tests. 122(2), 200-204.

Villanueva Gutierrez, E. E. J. I. P. a. t. F. o. L. A., Horticulture, & Science, C. P. (2018). An overview of recent studies of tomato (*Solanum lycopersicum spp*) from a social, biochemical and genetic perspective on quality parameters. (2018: 3).

Williamson, V. M. J. A. R. o. P. (1998). Root-knot nematode resistance genes in tomato and their potential for future use. 36(1), 277-293.

IJAFLS
e-ISSN: 2602-4381
International Journal of Agriculture, Forestry and Life Sciences
Original Article | Open access | Int J Agric For Life Sci (2023) 7(2): 36-39

White ginger (*Zingiber officinale*) powder as feed additive in the diet of broiler chicks

Taiwo Ayobami Sunmola[1]* **Comfort Dooshima TULEUN**[1]

[1]Department of Animal Nutrition, Joseph Sarwuan Tarka University Makurdi, Makurdi, Benue State, Nigeria
Department of Animal Breeding and Physiology, Joseph Sarwuan Tarka University Makurdi, Makurdi, Benue State, Nigeria
***Correspondence:** ta.sunmola@gmail.com

Citation
Sunmola T.A., Tuleun, C.D., (2023). White ginger (Zingiber officinale) powder as feed additive in the diet of broiler chicks. Int J Agric For Life Sci (2023) 7(2): 36-39.
Received :25 June 2023
Accepted :28 September 2023
Published Online :22 December 2023
Year: :2023
Volume :7
Issue :2 (December)
Pages :36-39

Available online https://dergipark.org.tr/ijafls

Abstract
White ginger (Zingiber officinale) powder as feed additive in the diet of broiler chicks was investigated. Feeding trial was conducted using one hundred and forty-four (144) day-old Abor acre plus strains broiler chicks. The birds were randomly assigned to four (4) dietary treatments with thirty six (36) birds per treatment divided into three (3) replicates of twelve (12) birds each in a completely randomized designed. The trials lasted for 28 days. Four experimental diets were formulated for the broiler chicks as T1, T2, T3 and T4 of 0, 0.20, 0.25 and 0.30 % white ginger powder respectively. Data were collected on growth performance, nutrient digestibility and production cost. Data collected were subjected to analysis of variance and the means were separated using Duncan's Multiple Range Test. The results shown that white ginger powder contains 92.70% dry matter, 8.20% ash, 13.90% crude fibre, 1.89 % fats, 12.60 % crude protein and 55.41 % carbohydrate. There were significant ($p<0.05$) improvements in final weight, weight gain, feed intake and feed conversion as the level of white ginger powder increased above T2 (0.20 %). Nutrient digestibility was not significantly differs across the treatment. Production cost was better at inclusion level 0.20 %. Therefore, use of white ginger powder as feed additive in broiler chicks up to 0.30 % is recommended while considering growth performance and nutrient digestibility and 0.20 % when production cost is considered.

Key words
Feed additives, Growth, Nutrients digestibility, Cost.

Introduction
Increase in regulations regarding the use of antibiotic growth promoters and the rise in consumer demand for poultry products, the quest for alternative products or approaches has intensified in recent years (Gadde et al., 2017). In the last decade, herbs and phytogenic compounds have attracted a lot of attention due to their potential role as an alternative to antibiotic growth promoters in monogastric animals (Khan and Naz, 2013). The interest to use the medicinal plants is associated to its safety, healthy for human and less cost compared with synthetic chemical drugs. Some decrease the level of serum lipids which lead to improve immune function in animals (Yadgar and Yavuz, 2015). Several studies conducted on the use of herbs and phytogenic compounds as natural alternative to synthetic antibiotics in broilers have revealed its potential benefits on the health of broiler birds as well as functions as enhancing digestion by stimulating endogenous enzymes (Brugali 2003), improve synthesis of digestive enzymes, increase in body weight and better feed conversion ratio (Greathead, 2003).

Ginger, one of such comparable natural alternatives, is a perennial herb belonging to the family *Zingiberaceae*. Ginger is rich in beneficial biologically active compounds (Ogbuewu et al., 2017). Study conducted by Zhao et al. (2011) revealed that ginger is advantageous for the greater productivity of poultry by improving the quality of feed and birds appetite, enhance the nutrient absorption and facilitates gastric enzymes flow. Onu (2010) reported that addition of ginger at 0.25 % in the basal diet of broiler chicks resulted in improved weight gain and feed conversion ratio. Al-Homidan (2005) observed reduced growth rate in starter broilers (1 to 4 wk) when ginger was fed at the rate of 6 g/kg diet and at 6th week of age (Moorthy et al., 2009).

FAO (2008) rated Nigeria as the fifth world producer of ginger with an estimated annual output of 138,000 tons. Ginger can be considered as one of the best options to fill the gap in preference to antibiotics. In Nigeria, white and yellow gingers are some of the varieties that are cheaply available whereas, yellow ginger had only been extensively researched on in broiler chicken production FAO (2008); information is lacking on the use of white ginger as feed additive in broiler chickens. Therefore, this study investigated the potential effect of white ginger powder as feed additives on growth performance, nutrient digestibility and production cost on broiler chicks.

Materials and Methods
Experimental Site
The study was conducted at the Poultry unit of the Livestock, Teaching and Research Farm, Joseph Sarwuan Tarka University Makurdi, (JOSTUM) Benue State, Nigeria. Makurdi is located between latitude 7^{0}44′ 1.50" N and longitude 8^0 31′ 17.00" E in the Guinea Savanna Zone of Nigeria. The area has an annual rainfall season of between 6 - 8 months (March - October) ranging from 508 to 1016 mm with minimum and maximum temperatures of 22.8^{0}C and maximum temperature of 40.03 ^{0}C respectively. The relative humidity ranges between 37.3 % and 59.2 % (TAC, 2021).

Collection and Processing of White Ginger
Fresh white ginger rhizomes were procured from the local market within Makurdi town, Benue State, Nigeria. The rhizomes were thoroughly washed in clean water to remove the adhering soil and chopped into smaller pieces using sharp knives. The chopped fresh white ginger rhizomes were sun-dried on a flat and clean concrete floor to a saved moisture content (>12 %). Dried white ginger was ground using a hammer mill of 2mm to obtain white ginger powder. The sample was airtight and properly stored for subsequent laboratory analysis and feed additive usage.

Experimental Birds and Management
One hundred and forty four day old broilers chicks were used for this study. The birds were procured from a reputable hatchery in Ibadan, Oyo state Nigeria. Before the arrival of the birds, all sanitary procedures such as cleaning, washing and disinfection of the pen and other equipment were observed. The chicks were individually weighed at the commencement of the study to ensure no bias was introduced in weight among the treatment groups. The broiler chicks were randomly assigned to four dietary treatments replicated three times containing 12 birds per replicate in a completely randomized design. The birds were raised in a deep litter system; feed and water were provided *ad libitum* throughout the period of the experiment which lasted for 4 weeks.

Experimental diets
Four experimental diets were formulated to meet the minimum nutrient requirements of the experimental birds (Table 1). T_1 served as control contained 0 % white ginger while T_2, T_3 and T_4 had 0.20, 0.25 and 0.30 % inclusion levels respectively. Four experimental diets were formulated to meet the minimum nutrient requirements of the experimental birds (Table 1). T_1 served as control contained 0 % white ginger while T_2, T_3 and T_4 had 0.20, 0.25 and 0.30 % inclusion levels respectively. The mixed feed using the formula in Table 1 were divided into four places as T_1, T_2, T_3 and T_4 of the same quantity. The test ingredient (white ginger powder) was weighed separately using a sensitive scale of 2 kg (Metler scale) for each treatment. Each treatment was pre-mix with 1 kg of the total mixed diet to ensure uniformity before mixing with each of the compounded feed. After mixing, each group was replicated into three and separately packed in a saved bag, well labeled and kept for subsequent feeding trial

Data collection
Initial weights of the birds were taken at the beginning of the study and weekly thereafter. Average final weights were taken at the end of the experiment by the ratio of total final weight to the number of birds in a group. Average daily feed intake was measured as the average feed given minus the left over feed divided by the number of the experimental days.

Table 1. Gross composition of the experimental starter broiler diets (kg)

Treatments	T_1	T_2	T_3	T_4
Ingredients				
Yellow maize	53.00	53.00	53.00	53.00
Soya bean meal	30.50	30.50	30.50	30.50
Groundnut cake	4.00	4.00	4.00	4.00
BDG	2.60	2.50	2.50	2.50
Rice bran	2.00	2.00	2.00	2.00
Bone meal	3.00	3.00	3.00	3.00
Blood meal	3.00	3.00	3.00	3.00
Palm oil	1.00	1.00	1.00	1.00
L-Lysine	0.15	0.15	0.15	0.15
Herbo-Methionine	0.20	0.20	0.20	0.20
Vit./min. premix*	0.25	0.25	0.25	0.25
Common salt	0.25	0.25	0.25	0.25
White ginger powder	0.00	0.20	0.25	0.30
Total	100	100	100	100
Calculated analysis				
ME (Kcal/kg)	2941	2941	2941	2941
Crude protein (%)	23.20	23.20	23.20	23.20
Crude fibre (%)	4.03	4.03	4.03	4.03
Ether extract (%)	4.75	4.75	4.75	4.75
Lysine (%)	1.49	1.48	1.48	1.48
Methionine (%)	0.54	0.54	0.54	0.54
Calcium (%)	1.29	1.29	1.29	1.29
Available Ph (%)	0.71	0.71	0.71	0.71

**To provide the following per kg of diet vitamin A – 15,000.00IU, Vitamin D3 - 3, 000,000IU, Vitamin E- 30,000IU, Vitamin K3,000mg, Vitamin B1 3000,mg Vitamin B2-6000mg, Vitamin B6- 5,000mg, Vitamin B12-40mg, Biotin 200mg, Niacin-40,000mg, Pantothenic acid 15,000mg, Folic acid 2,000mg, choline 300,000mg, Iron 60,000mg, manganese 80,000mg, copper 25,000mg, Zinc 80,000mg cobalt 150mg, Iodine 500mg. (feed formulation was done using the feedwin software application); Ctrl = control, ME – metabolizable energy, BDG - Brewer dried grain, Ph – Phosphorus, Vit./min. – vitamin/mineral*

The feed conversion ratio is the quantity of the daily feed consumed per bird divided by daily weight gain per bird. The daily weight gain per bird was computed by dividing the difference between the average final weights minus the average initial weight divided by number of experimental days.

Nutrient digestibility

Nutrient digestibility evaluation was done at the end of week three (3) and terminated at the end of week four. Two birds per replicate group were selected and transferred into metabolic cages. A 3-days acclimatization period was allowed for the birds, and the respective diets were offered to the birds. Daily feed intake and daily faecal output were recorded for 4 days. The droppings were collected per replicate once daily at 8:00 am, weighed and dried in an oven at 70° C to constant weight. Dried excreta were bulked and ground, experimental diets and feacal samples were used to determine their respective proximate constituent according to AOAC (2006)

Production cost

The cost of each experimental diet was calculated according to the prices of ingredients, based on quotes obtained in July 2022 when the study was carried out. The prices of ingredients/kg used to establish feed costs were: yellow maize, ₦250.00k; soybean meal, ₦325.00k; groundnut cake, ₦250.00k; brewer dried grain, ₦170.00k; rice bran, ₦100.00k; bone meal, ₦100.00k; blood meal, ₦180.00k; palm oil ₦650.00k; L-lysine, ₦1000.00k; vitamin and mineral supplements, ₦1800.00k and white ginger powder, ₦2300.00k. Feeding cost was determined based on total feed intake per animal multiplied by the cost of the diet used. For initial bird value, the unit price per day-old chick (₦220.00k) was used. The final value received for each bird was obtained by dividing the final total weight of the bird by the average price per kg of live broiler (₦1200.00k), as practiced in Makurdi, Benue state, North-central Nigeria in October 2022. Cost of feed was calculated from the cost of ingredients used in feed preparation. Feed cost per weight gain was calculated by multiplying the feed cost per kg by total feed intake divided by total weight gain. Feed cost/chick was calculated by multiplying feed intake per day by the number of days multiplied by the feed cost per kilogram. Operational cost per bird was calculated by adding all other expenses except expenses on feed and purchasing price of chicks. Total cost of production was calculated by adding cost of day-old chick, feed cost per chick and operational cost. Cost saving due to addition of white ginger powder was calculated by subtracting the respective total cost of production from control. Feed cost as a percentage of total production cost was calculated by dividing cost of feed per kg with total cost of production multiplied by hundred.

Statistical analysis

Data collected from the study were subjected to analysis of variance (ANOVA), where significant differences occurred; the means were separated using Duncan Multiple Range Test. The results were considered significant at 5 % level of probability.

Results and Discussions

The result in Table 2 shows that white ginger powder contains 92.70% dry matter, 8.20% ash, 13.90% crude fibre, 1.89 % fats, 12.60 % crude protein and 55.41 % carbohydrate. The dry matter content of a material determined it shelf-life, it revealed stable duration of the feed material during storage. The value obtained is within the range values of 89 % to 95 % reported by Edmond et al. (2018). The crude protein 12.60 % observed is higher than 7.70% reported by Nuhu et al. (2018) but lower compared to 14.00 % reported by Oshomoh et al. (2016). The sample contains protein below 20 %; this may imply that the relative dietary importance of this spice is to improve the nutritive value of the feed material (Hashemi and Davoodi, 2010). The ether extract is lower than those reported by Nuhu *et al.* (2018); Oshomoh et al. (2016) and Ikpeama et al. (2014) which ranged from 3.30 % to 12.00 %. The differences observed may probably reflect the varietal difference of the samples. The crude fibre obtained from the sample may pose no threat since they are not usually fed in isolation but as additives with other feedstuff. Hence, the fibre contents may also serve as a boost to the total dietary fibre of the diet. Minerals are important elements of the diet because of their physiological and metabolic function in the body. Percentage ash content recorded for white ginger implies that it contains more minerals which may have dietary usefulness such as copper, zinc, iron, selenium which can make it a dietary antioxidant enzyme activator. Proximate analysis revealed that white ginger (*Zingiber officinale*) can be ranked as carbohydrate rich due to their high calorie content 55.41 %. The sample was found to be relatively good dietary component of carbohydrate.

Effect of white ginger powder on growth performance of broiler chicks is presented in Table 3. There were significant ($p<0.05$) improvements in final weight, weight gain, feed intake and feed conversion as the level of white ginger powder increased above T_2 (0.20 %).

Table 2. Proximate composition of white ginger powder

Nutrients (%)	Compositions
Dry matter	92.70
Crude protein	12.60
Crude fibre	13.90
Ether extract	1.89
Ash	8.20
Nitrogen free extract	55.41

Table 3. Effect of White Ginger Powder on Growth Performance of Starter Broiler Chicks

Experimental Diets						
Parameter	T_1	T_2	T_3	T_4	**SEM**	**P-value**
AIW (g)	39.00	39.00	39.00	39.00	0.00	0.00
AFW (g)	817.00^{b}	815.00^{b}	830.00^{ab}	849.00^{a}	14.10	0.74
ADWG (g)	27.80^{b}	27.20^{b}	28.3^{a0b}	28.90^{a}	0.50	0.74
ADFI (g)	46.40^{a}	43.60^{b}	44.90^{ab}	45.80^{ab}	0.97	0.82
FCR	1.66^{b}	1.60^{ab}	1.58^{a}	1.58^{a}	0.04	0.72

AIW = average initial weight; AFW = average final weight; ADWG = average daily weight gain; ADFI = average daily feed intake; FCR = feed conversion ratio; SEM = standard error of mean. T_1 = Control diet; T_2 = 0.20 % white ginger powder; T_3 = 0.25 % white ginger powder; T_4 = 0.30 % white ginger powder

Improvement with increased in inclusion levels of white ginger in body weight, weight gain and feed conversion due to supplementation of ginger powder may be attributed to the beneficial effect of phytogenic substances found in ginger such as flavonoids, saponin Ikpeama et al. (2014) that possess antimicrobial, antifungal and antioxidant activities in broiler chicks; thereby improve the utilization of dietary nutrients (Kumari et al., 2007). On the other hand, Platel and Srinivasan (2000) reported that ginger had the ability to stimulate the digestive system, such as stimulation of intestinal lipase, sucrose and maltase activities as well as the secretion of pancreatic lipase, amylase, trypsin and chymotrypsin enzymes which enhances the feed utilisation. The improvement observed is in agreement with the report of several researcher Thejanuo et al (2019); Karangiya et al. (2016); Oleforuh et al. (2014) and Zomrawi et al. (2012) whom reported significant $p<0.05$ differences between the treatment means of birds on final weight, weight gain, feed intake and feed conversion ratio when fed phytogenic materials. There was an increase in average final weight that ranged from 815 g – 849 g. However, treatments T_4 (0.30 %) and T_2 (0.20 %) recorded the highest and least values of 849 g and 815 kg respectively. Feed intake and feed conversion ratio recorded ranged

from 43.6 g – 46.4 g and 1.58 – 1.66 respectively. It was observed that control recorded the highest mean values of feed intake and feed conversion ratio respectively compare to other treatments fed white ginger based diets. This result aligned with the reports of (Rebh et al., 2014 and Talukder et al., 2017) which stated that addition of ginger and its extract in the diet of broiler chick significantly improved feed conversion ratio.

Effect of white ginger powder on apparent digestibility coefficient of broiler chicks is presented in Table 4. There were no significant ($p>0.05$) differences for all the parameters observed across the dietary treatments. This could be attributed to the stimulation effect of digestive enzymes by bioactive compounds of ginger. Ginger was reported to enhance pancreatic lipase activity, intestinal lipase, disaccharides, sucrose and maltase activities which has favourable influence on gut function; which is the primary mode of action for growth in promoting feed additives (Windisch et al 2008). Zhao et al. (2011) reported that ginger enhances animal's nutrient digestion and absorption because of their positive effect on gastric secretion and digestive enzyme activities.

Table 4. Effect of white ginger powder on apparent digestibility coefficient apparent digestibility coefficient of starter broiler chicks

Experimental Diets						
Parameter (%)	**T_1**	**T_2**	**T_3**	**T_4**	**SEM**	**P-value**
DM	70.5	72.8	69.6	72.6	1.43	0.52
CP	73.0	70.1	67.9	69.0	1.19	0.53
CF	69.2	65.8	65.3	70.2	1.11	0.35
EE	72.3	65.4	65.1	69.5	1.38	0.19
NFE	68.2	69.7	64.4	64.6	1.37	0.48

DM = Dry Matter; CP = Crude protein; CF = Crude Fibre; EE = Ether Extract; NFE = Nitrogen Free Extract; SEM = standard error of mean; T_1 = Control diet; T_2 = 0.20 % white ginger powder; T_3 = 0.25 % white ginger powder; T_4 = 0.30 % white ginger powder

Non-significant differences observed across the treatments is in line with that of Kafi et al., 2017 who reported non-significant ($p>0.05$) differences on nutrient utilization of broiler chickens when included ginger powder in their diet. In contrary, Duwa (2020) observed significant differences when fed ginger at the inclusion levels between 0 - 6 %. Different results by difference authors may be attributed to difference in inclusion levels, botanical composition of the test ingredients, climatic variation and the general management of the birds.

Effect of white ginger powder on production cost of broiler chicks is presented in Table 5. The marginal increased in the amount of feed cost per kg diet recorded for broiler chicks fed diets containing white ginger powder was a function of the additional cost of test ingredients. Higher feed cost per chick recorded for birds fed the control diet resulted from the higher feed intake compared to other dietary groups fed white ginger powder. Higher feed cost per weight gain observed on birds fed T_2 (0.20 % white ginger) showed that the birds in the group less efficiently utilized the feed consumed than those in other group. The major concern of the farmer is how well the animals utilised or convert the feed consumed into the body flesh. Therefore, the lower the feed cost per weight gain, the better it is in terms of profit maximization. Total production cost was lower in the groups fed white ginger powder relative to control resulted from the higher feeding cost. Cost saving observed across the treatments with the best observed in T_2 (0.20 % white ginger) indicated that white ginger powder is economically beneficial in poultry feed as an additive. This finding confirmed the report of Duwa et al. (2020) who observed that the profits were made on all the birds fed diet containing turmeric powder. The higher feed cost as a percentage of the total cost of production observed for control is attributed to higher feed consumed lead to higher feed cost per kg by the group. The result of production cost obtained in this study confirm the report of Gerson et al. (2009) who stated that the use of the phytogenic feed additive in broiler chicken diets had an economic advantage when feed cost is considered. Minh et al. (2010) also reported that supplementation of dried ginger to broiler diets reduced feed costs.

Table 5. Effect of white ginger powder on production cost of starter broiler chicks

Experimental Diets				
Economic Indices	**T_1**	**T_2**	**T_3**	**T_4**
C of DOC (₦/chick)	220.00	220.00	220.00	220.00
FC (₦/kg)	273.00	277.00	278.00	280.00
FC (₦/chick)	357.00	343.73	345.16	349.38
FC/WG (₦/kg)	420.14	429.67	415.85	411.04
OPC (₦/chick)	80.00	80.00	80.00	80.00
TCP (₦/chick)	657.11	643.73	645.16	649.38
CS due to spices (₦/chick)	-	13.37	11.95	7.73
FC (% TCP)	54.34	53.39	53.50	53.80

FC = feed cost; CS = Cost savings; DOC = Day old chicks; C = Cost; TCP = Total cost of production; OPC = Operational cost; T_1 = Control diet; T_2 = 0.20 % white ginger powder; T_3 = 0.25 % white ginger powder; T_4 = 0.30 % white ginger powder;

Conclusion

From the findings of this study, it showed that white ginger powder possesses some nutrients adequately; which qualified it to be used as a nutritive feed additive in a broiler chick's diet. Supplementation of white ginger powder up to 0.30 % in broiler chicks' diet had beneficial effect on growth performance, nutrient utilization was not significantly affected by the test ingredient. Production cost was better at inclusion level of 0.30 % considering the feed cost per weight gain, the major concern of the poultry farmer is how well the birds utilised the feed consumed. Therefore, use of white ginger powder as feed additive in broiler chicks up to 0.30 % is recommended while considering growth performance, nutrient digestibility as well as production cost when feed utilisation is put into consideration at the starter phase. While 0.20 % white ginger powder is recommended when considering feed cost, saving cost due to the white ginger powder and total production cost

Conflict of Interest

The authors declare that they have no conflict of interest.

Author's Contributions

The authors contributed equally to this manuscript.

References

AL-Homidan, A. A. (2005). Efficacy of using different sources and levels of Allium sativum and Zingiber officinale on broiler chicks' performance. *Saudi Journal of Biological Sciences,* 12: 96–102.

AOAC (2006). Official method of analysis, method 991.36. Fat (crude) in meat and meat products. 18th Ed. AOAC International, Gaithersburg, MD.

Brugalli, I. (2003). Alimentacaoalternativa: An utilizacao de fitoterapicosounutraceuticoscomomoduladores da imunidade e desempenho animal. Anais do SimposiosobreManejo e Nutricao de Aves e Suinos; Campinas, Sao Paulo.Brasil. Campainas: (BNA,), pp. 167-182.

Duwa, H., Amaza, I. B., Dikko, M. I., Raymond, J. B. Paullyne, U. O. (2020). Effect of ginger (*Zingiber officinale*) on the growth performance and nutrient digestibility of finisher broiler chickens in semi-arid zone of Nigeria. *Nigerian Journal of Animal Science,* 22 (3): 279-286

Edmond, A. D., Yolande, D. D., Martin, K. D., Lucien, P. K. Vanessa, W. T. B. (2018). Proximate and Mineral Composition and Some Anti-Nutritional Factors of Flour from Five Improved Sweet Potato (*Ipomoea Batatas* (L.) Lam) Varieties Roots. *European Journal of Pharmaceutical and Medical Research, 5(11), 126-137*

FAO (2008). Food and Agricultural Organization of United Nations: Economic and Social Department: The Statistical Division. Top ten Ginger Producers.

Gadde, U., Kim, W. H., Oh, S. T. Hyun, S. L. (2017). Alternatives to antibiotics for maximizing growth performance and feed efficiency in poultry: *A review. Animal Health Research Reviews*, 18(1); 26–45

Gerson, N. S., Anildo C. J., Lucas C. Alexandre, M. G. (2009): Phytogenic additive as an alternative to growth promoters in broiler chickens. *Ciência Rural, Santa Maria*, 39(2): 522-527

Greathead, H. (2003). Plants and plant extracts for improving animal productivity. *Proceedings of Nutrition Society*, 62: 279-290.

Hashemi, S. R, Davoodi, H. (2010). Phytogenics as new class of feed additive in poultry industry. *Journal of Animal and Veterinary Advances,* 9, 2295-2304.

Ikpeama, A., Onwuka, G. I. and Nwankwo, C. (2014). Nutritional Composition of Tumeric (*Curcuma longa*) and its Antimicrobial Properties. *International Journal of Scientific and Engineering Research,* 5 (10) 1087 – 1089.

Kafi, A., Uddin, M. N., Uddin, M. J., Khan, M. M. H. Haque, M. E. (2017). Effect of Dietary Supplementation of Turmeric (*Curcuma longa*), Ginger (*Zingiber officinale*) and their Combination as Feed Additives

on Feed Intake, Growth Performance and Economics of Broiler. *International Journal of Poultry Science,* 16: 257-265.

Karangiya, V. K., Savsani, H. H., Patil, S. S., Gadariya, M. R., Marandi, S. Chavda, M. R. (2016). Effect of Using Ginger and Garlic Powder as Natural Feed Additives on Performance and Carcass Quality of Broiler Chick. *Indian Veterinary Journal,* 93 (10) : 11 – 13

Khan, R. U. Naz, S. (2013) The applications of probiotics in poultry production. *World's Poultry Science Journal*, 69: 453-461

Kumari, P., Gupta, M.K., Ranjan, R., Singh, K.K. Yadava, R. (2007). *Curcuma longa* as seed additive in broiler birds and its patho-physiological effects. *Indian Journal of Experimental Biology,* 45:272-277.

Minh, D. V., Huyen, L. V., Thieu, P., Tuan, T. Q., Nga, N. T. Khiem, N. Q. (2010). Effect of supplementation of Ginger (Zingiber officinale) and Garlic (Allium sativum) Extracts (Phyto-Antibiotics) on Digestibility and Performance of Broilers Chicken. MEKARN Conference. Livestock Production, Climate Change and Resource Depletion. http://www.mekarn.org/workshops/pakse/abstracts/minh_nias.htm

Moorthy, M., Ravi, S., Ravikumar, M., Viswanathan, K. Edwin, S. C. (2009). Ginger, Pepper and Curry Leaf Powder as Feed Additives in Broiler Diet. *International Journal of Poultry Science*, 8: 779-782.

Nuhu, B., Abba, A. Amina, L. (2018). The profitability of broiler chickens raised on graded levels of maize offal and wheat offal – based diets. *Journal of Poultry Science,* 67(25): 298 – 302.

Ogbuewu, I. P., Mbajiorgu, C. A. Okoli, V. (2017). Antioxidant activity of ginger and its effect on blood chemistryand production physiology of poultry. *Comparative Clinical Pathology,* 28:655–660

Oleforuh, V. U. O., Chukwu, G. C. Adeolu, A. I. (2014). Effect of Ground Ginger and Garlic on the Growth Performance, Carcass Quality and Economics of Production of Broiler Chickens. *Global Journal of Bio-Science and Technology*, 3 (3) 225-229

Onu, P. N. (2010). Evaluation of two herbal spices as feed additives for finisher broilers. *Biotechnology in Animal Husbandry*, 26: 383-392.

Oshomoh, E. O., Udinyiwe, O. C. Idu, M. (2016). Nutritional compositions and antimicrobial sensitivities of Aqueous extracts of *Zingiber officinale* ROSC and *Allium sativum* L on oral microorganisms. *African Scientist,* 17 (1)81 – 91

Platel, K. Srinivasan, K. (2000). Influence of dietary spices and their active principles on pancreatic digestive enzymes in albino rats. *Food/Nahtung*, 44:42-46.

Rebh, A. Y., Kumar, P. Neeraj, R. (2014). Effect of supplementation of ginger root powder in ration on performance of broilers. *European Academic Research*, 2(3): 4204-4213.

TAC (2021). Home page (http://www.nist.gov/tac/2021). Retrieved August 13, 2021.

Talukder, S., Hasan, M. M., Noman, Z. A., Sarker, Y. A., Paul, T. K. Sikder, M. H. (2017). Effect of dietary supplementation of ginger extract on growth, carcass characteristics and haematological parameters in broilers. *Asian Journal of Medical and Biological Research*, 3(2): 211-215.

Thejanuo, R. V., Vidyarth, K. Zuyie, R. (2019). Effect of Dietary Supplementation of Ginger Powder (*Zingiber officinale*) on Performance of Broiler Chicken. *Livestock Research International*, 7 (2) 125-131.

Windisch, W., Schedle, K., Plitzer, C. Kroismayr, A. (2008). Use of phytogenic products as feed additives for swine and poultry. *Journal of Animal Science,* 86, 140-148

Yadgar, G. S. Yavuz, G. (2015). Sumac (RhusCoriaria L.) and Ginger (ZingiberOfficinale) as Feed Additive in Poultry Nutrition. *KSU Journal of Natural Science*,18 (3), 44-48

Zhao, X., Yang, Z. B., Yang, W. R., Wang, Y., Jiang, S. Z. Zhang, G. G. (2011). Effects of ginger root (Zingiberofficinale) on laying performance and antioxidant status of laying hens and on dietary oxidation stability. *Poultry Science,* 90: 1720-1727

Zomrawi, W. B., Abdel, K. A., Dousa, B. M. Mahala, A. G. (2012). The effect of ginger root powder (zingiber officinale) supplementation on broiler chicks performance, blood and serum constituents, *Online Journal of Animal and Feed Research,* 6: 457-460

IJAFLS
e-ISSN: 2602-4381
International Journal of Agriculture, Forestry and Life Sciences
Original Article | Open access | Int J Agric For Life Sci (2023) 7(2): 40-42

A Study on the Differences of Yield and Yield Components Among Some Winter Cereal Species in Kahramanmaraş Condition

Mustafa Yıldırım [1] **Songül Çiftçi Sakin**[1] **Ömer Süha Uslu** [1*]

[1]Kahramanmaraş Sütçü İmam University, Faculty of Agriculture, Depatment of Field Crops, Kahramanmaraş, Türkiye
***Correspondence:** suhauslu@ksu.edu.tr

Citation
Yıldırım, M., Çiftçi Sakin, S., Uslu, Ö.S. (2023). A Study on the Differences of Yield and Yield Components Among Some Winter Cereal Species in Kahramanmaraş Condition. Int J Agric For Life Sci (2023) 7(2): 40-42.

Received :7 August 2023
Accepted :20 September 2023
Published Online :22 December 2023
Year: :2023
Volume :7
Issue :2 (December)
Pages :40-42

Available online https://dergipark.org.tr/ijafls

Abstract
This study was carried out in Kahramanmaraş ecological conditions in the 2020-2021 growing season in order to determine the differences among yield and yield components in some cereal species (triticale, wheat, barley and oat). The fertile tiller number (number), hectolitre weight (kg), bunch/spike grain number (number), bunch/spike grain weight (g), thousand seed weight (g) and grain yield (kg da-1) characteristics of cereal species were taken into consideration. Except for the number of fertile tiller, significant differences were found among the winter cereals in other characters. Wheat (68.24 kg) and triticale (67.45 kg) gave the highest values in terms of hectolitre weight. The number and weight of bunch/spikes gave the highest triticale values (25.27 number and 1.16 g, respectively). Oats (28.35 g) and triticale (26.86 g) took the first place in terms of thousand kernel weight. Wheat (702.96 kg da-1) had the highest grain yield, followed by barley, triticale and oats. According to the results, it has been seen that there are differences among the cereal species considered in terms of yield and yield components in Kahramanmaraş conditions and the order of the species in terms of characters has changed.

Key words
Barley, Wheat, Yield, Yield components, Triticale, Oat.

Introduction
Cereals are among the most consumed foods in the world, which are still agriculturally produced in the world (Sarwar et al., 2013). The most important reason for this is that it constitutes an important source of basic foods for the global population. One of the reasons why it is an important source of basic foods is that it is rich in nutritional content. For example, in the grain of winter cereals (although it varies depending on the grown variety, cultivation technique and harvest period), carbohydrate is 79.50%, protein 13.60%, fat 2.30%, cellulose 2.50% and ash 2.10% (Geçit, 2016).

According to TUIK 2021 statistical data in Türkiye, while the cultivation area of wheat in winter cereals is 6.759.751 ha in Türkiye, 137.438 ha in Kahramanmaraş. Production is 17.936.270 tons in Türkiye, 553.924 tons in Kahramanmaraş and the yield is 24.3 t ha^{-1} in Türkiye, 8 t ha^{-1} in Kahramanmaraş. While the cultivation area of barley is 3.197.043 ha in Türkiye, 43.582 ha in Kahramanmaraş, while the production is 6.193.553 tons in Türkiye, 108.482 tons in Kahramanmaraş and while the yield is 19.8 t ha^{-1} in Türkiye, 2.5 t ha^{-1} in Kahramanmaraş. While the cultivation area in oats is 511.007 ha in Türkiye, 24.5 ha in Kahramanmaraş, while the production is 4.028.850 tons in Türkiye, in Kahramanmaraş it is 94 tons, while the yield is 12.2 t ha^{-1} in Türkiye, in Kahramanmaraş it is 9 t ha^{-1}. In triticale, while the cultivation area is 140.874 ha, it is 712 ha in Kahramanmaraş, while the production is 901.112 tons in Türkiye, in Kahramanmaraş it is 2.144 tons, the yield is 16.9 t ha^{-1}, while it is observed as 10 t ha^{-1} in Kahramanmaraş (TUIK, 2022). This research aimed to compare the yield and yield factors based on the demand for increase in production due to the use of winter cereals in human and animal nutrition in Turkey and in the world, as well as in value-added products in the industry recently. Comparison of winter cereals (wheat, triticale, oat and barley) in terms of yield and yield elements in Kahramanmaraş ecological conditions is discussed.

Material and Methods
The research was carried out in Kahramanmaraş ecological conditions in the 2020-2021 growing season in Kahramanmaraş Sütçü İmam University, Faculty of Agriculture, Field Crops Department Research and Application area. Coordinates of the experiment area are given in Figure 1. In the study, triticale, wheat, barley and oat species, which are among the winter cereal types, were selected as material. In the study, Ayşehanım variety in triticale, Balkoni variety in wheat, Ibaiona variety in barley and Kahraman variety in oats were used. The experiment was established in a randomized complete block design (RCBD) with 3 replications. Seed species were sown on 18.11.2020 with a row length of 6 m, 6 rows and 500 m^{-2} seeds with experiment drill. Calculated over a total of 16 kg da^{-1} pure nitrogen, half of the base fertilizer 20.20.0 compound fertilizer was applied. The remaining amount of nitrogen was used as top fertilizer with urea fertilizer. The Mediterranean climate is observed in Kahramanmaraş province where the study was conducted. The climate data of the months and long years in which the experiment was carried out are given in Table 1 (Anonymous, 2022a).

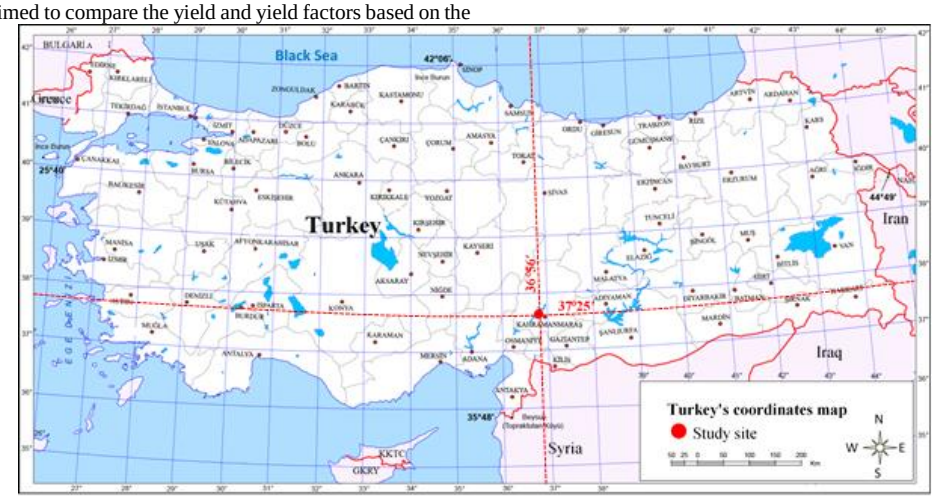

Figure 1. The coordinates of experimental area

Table 1. Climatic data of Kahramanmaraş province where the experiment was conducted

Months	Total Precipitation (mm)		Average Temperature (°C)	
	2020-2021	Long Years (1930-2021)	2020-2021	Long Years (1930-2021)
November	27.2	78.0	12.4	11.8
December	23.6	130.6	8.2	6.6
January	41.6	124.0	7.0	4.8
February	25.8	112.2	9.3	6.2
March	35.4	95.1	10.4	10.4
April	10.0	73.0	16.6	15.1
May	12.8	38.8	23.5	20.1
June	0.0	8.6	25.5	24.9
Total	176.40	660.30	--	--
Mean.	--	--	14.11	12.49

The soil analysis result of the field where the experiment was carried out is given in Table 2. According to Table 2, the soil of the study area; clay loam (52.8%), pH close to neutral 6.92, salt-free (0.11%), slightly calcareous (0.20%), organic matter at medium level (2.61%), potassium content sufficient (218.90 mg kg^{-1}), In terms of phosphorus content, it was found to be at a moderate level (15.14 mg kg^{-1}) (Anonymous, 2021b).

Table 2. Some physical and chemical properties of the soils of the trial area

Characteristics	Saturation (%)	Salt (%)	pH	Lime $CaCO_3$ (%)	Fhosphorus P_2O_5 (mg kg^{-1})	Potassium K_2O (mg kg^{-1})	Organic matter (%)
Results	52.8	0.11	6.92	0.20	15.14	218.90	2.61

In the experiment, among the yield factors, the number of fertile tiller (number), spike seed number (pieces), spike seed weight (g), thousand kernel weight (g) and hectolitre weight (kg), and grain yield (kg da^{-1}) properties were examined. It was harvested with a parcel harvester on June 7th, 2021, when the time of full maturity of the seeds.

Examined features in 10 plants in each plot; for the number of kernel in bunch and spike, by counting the kernels in the bunch; weighing the seeds in the bunch or spike on a sensitive scale with 0.001 g sensitivity for the weight of the bunch and the seed, for the number of fertile tiller, counting the number of bunch in a 1 meter row for the number of fertile tiller, and counting the seeds obtained from all plots four times for a thousand kernel weight, and it was measured in grams after taking the average and multiplying by 10, and for hectolitre weight by calculating in a 1/l hectolitre device. Seed yield was found by harvesting 6 rows of all plots with a plot harvester and converting the obtained seed yield to decare yield. Statistical analysis of the data was made in the SAS (2013) statistical package program.

Results and Discussion

In this study, in which different grain types (triticale, wheat, barley and wheat) were compared, the average values of their effects on the investigated properties are given in Table 3.

Table 3. Bunch length, number of fertile tillers, hectolitre weight, spike seed number, spike seed weight, thousand kernel weight and average values of seed yield.

Cereal Species	Fertil Tiller Number (number)	Hektolitre Weight (kg)		Spike Seed Number (number)		Spike Seed Weight (g)		Thousand Kernel Weight (g)		Seed Yield (kg da^{-1})	
Triticale	3.27	67.45	A	25.27	a	1.16	a	26.86	b	552.22	ab
Wheat	3.60	68.24	A	17.27	c	0.79	b	22.99	c	702.96	a
Barley	4.33	55.56	Ab	10.93	d	0.49	c	26.94	b	688.70	a
Oat	3.53	49.92	B	20.07	b	0.82	b	28.35	a	465.37	b
LSD (%5)	0.87^{ns}	14.40*		1.38**		0.29**		1.396**		218.55^{ns}	
CV (%)	11.90	11.96		3.76		18.05		2.66		18.16	

According to the results of the analysis of variance in the study, the statistical difference between the number of fertile siblings was found to be insignificant (Table 4). Atak and Çiftçi (2005) determined that the number of fertile tillers of triticale according to varieties was between 3.63 $plant^{-1}$ and 4.55 $plant^{-1}$ in the first year, between 3.30 $plant^{-1}$ and 3.7 $plant^{-1}$ in the second year, as a result of two years of research. They reported that the number of fertile tillers varies depending on climate change, stating that the reason for the difference in the number of fertile tillers according to years is due to the amount of precipitation over the years.

According to the variance analysis results of the study, the difference between the cereal species of hectoliter weight was found to be statistically significant ($P<0.05$) (Table 4). In previous studies, Mut et al. (2006), in their research to determine some characteristics of the triticale plant, the hectolitre weight of triticale was 67.30 kg and 71.80 kg in the first year of Amasya location, 63.30 kg and 73.30 kg in the second location of Amasya location, 65.80 kg and 73.00 kg in Samsun location, according to the average of three locations. they reported that it varies between 65.90 kg and 71.90 kg. Yanbeyi and Sezer (2006), in their research with triticale lines in Samsun ecological conditions, determined that the hectolitre weight of triticale was between 57.80 kg and 76.30 kg, according to the two-year average. Doğan and Kendal (2012), in their study conducted in Diyarbakır ecological conditions, reported that the hectolitre weight of bread wheat genotypes varied between 77.60 kg and 82.40 kg, according to the two-year average. Chaudhary et al. (2017) observed that the hectolitre weight of four different barley varieties varied between 56.60 kg and 62.70 kg. Jokinen et al. (2021), in their study to estimate the quality of oat flour, they determined the average hectolitre weight of oat seed to be 59.30 kg. Kahraman et al. (2021), in their study on 14 oat genotypes in Edirne and Kırklareli ecological conditions, they determined the hectolitre weight between 47.00 kg and 59.80 kg according to the average of both locations.

According to the results of the analysis of variance in the study, the difference between the cereal species ($P<0.01$) was found to be statistically significant in the number of seed per bunch and spike. It was observed that the number of seed per bunch and spike in cereal species varied between 10.93 and 25.27. The highest number of seed per spike was found in triticale with 25.27, and the least number of seed per spike was determined in barley with 10.93 (Table 4). In previous studies, Giunta and Motzo (2005) reported that triticale had a higher number of seed than wheat when they compared triticale (Antares variety) and wheat (Duilio and Creso varieties) species in Italian ecological conditions in terms of the number of seed per spike. In his study, Monouchehr (2006) reported that the number of seed per spike was the most important factor with a direct effect on seed yield in the correlation analysis in barley. Duğan (2010), in his study, compared the triticale plant with the winter cereals in terms of yield, reported that the number of seed per spike of triticale varied between 28.20 and 118.78 pieces among the varieties, and among the winter cereals, the oat variety had more seed per spike than the triticale varieties, and triticale stated that it was higher than bread wheat and barley in terms of the number of seed in the spike. Mendez-Espinoza et al. (2019), as a result of researching the agricultural characteristics between triticale and bread wheat in Mediterranean conditions in their studies, found that despite the lower spike number per square meter, triticale was 35% higher than the seed number per spike, which is in line with our findings. Mut et al. (2021), in their research on 255 oat genotypes in the Central and Western Black Sea Region, emphasized that the number of seed in a bunch of oat genotypes varied between 51.54 and 155.00, and that the number of seed in a bunch was an important feature in terms of seed yield. Aydoğan and Yağdı (2022), in their study in Bursa ecological conditions, reported that the number of seed per spike in 41 wheat varieties varied between 40.83 and 71.93.

According to the results of the analysis of variance in the study, the statistically significant difference ($P<0.01$) between the spike seed weight was found. The spike seed weight in cereal species varied between 0.49 g and 1.16 g. The highest spike seed weight was found in triticale with 1.16 g, and the lowest spike seed weight was found in barley with 0.49 g (Table 4). In previous studies, Giunta and Motzo (2005) reported that when triticale (Antares variety) and wheat (Duilio and Creso varieties) species, which are included in the Italian ecological conditions, were compared in terms of spike seed weight, triticale had a higher seed number than wheat and their findings were consistent with our findings. Duğan (2010), in his study, compared the triticale plant with winter cereals in terms of yield, reported that the spike seed weight of triticale varied between 1.32 g and 3.72 g among varieties, and found that triticale had a higher spike seed weight than bread wheat, barley and oats in

winter cereals. In addition, he reported that the spike seed weight was directly related to the photosynthesis capacity of the plants and changed depending on the genotype, climate and growing conditions. Mut et al. (2021), in their research on 255 oat genotypes in the Central and Western Black Sea Region, they determined that the seed weight of the oat genotypes varies between 1.44 g and 4.85 g, and also the bunch seed weight differs according to the genotypes. Aydoğan and Yağdı (2022), in their study in Bursa ecological conditions, stated that the spike seed weight in 41 wheat cultivars varied between 3.33 g and 1.61 g.

According to the results of the analysis of variance in the research, the difference between the cereal species ($P<0.01$) was found to be statistically significant in thousand kernel weight. The thousand kernel weight in cereal species varied between 28.353 g and 22.987 g. Among the winter cereal species, the highest thousand kernel weight was found in oats with 28.353 g, and the lowest thousand kernel weight was found in wheat with 22.987 g (Table 4). Duğan (2010), reported that it had a lower thousand kernel weight in his study, compared the triticale plant with the winter cereals in terms of yield, reported that the thousand kernel weight of triticale varied between 26.62 g and 44.91 g among the varieties, and that among the winter cereals, triticale had a higher thousand kernel weight than oat varieties, but it was higher than wheat and barley varieties. Kahraman et al. (2017), in their research to determine the agricultural characteristics of 16 different oat genotypes, stated that the thousand kernel weight varied between 18.70 g and 45.00 g. Mendez-Espinoza et al. (2019), as a result of researching the agricultural characteristics between triticale and bread wheat in Mediterranean conditions, they found that triticale was 16% higher in terms of thousand kernel weight. Kaziu et al. (2019) stated that the thousand kernel weight varies between 21.60 g and 30.30 g in their study with 10 oat genotypes. Kahraman et al. (2021), in their study on 14 oat genotypes in Edirne and Kırklareli ecological conditions, they recorded that the thousand kernel weight was between 21.10 g and 41.30 g according to the average of both locations. Aydoğan and Yağdı (2022), in their study in Bursa ecological conditions, reported that the thousand kernel weight in 41 wheat varieties varied between 32.67 g and 57.28 g.

According to the results of the analysis of variance in the study, the statistical difference in seed yield between cereal species was found to be insignificant (Table 4). In previous studies, Dugan (2010), in his study, compared triticale plant with winter cereals in terms of seed yield, reported that the seed yield of triticale varied between 255.00 kg da^{-1} and 646.08 kg da^{-1} among varieties, and 100 kg da^{-1} of bread wheat among winter cereals. He stated that it has a higher seed yield than barley at 200 kg da^{-1} and 400 kg da^{-1} than oat. Karahan and Sabancı (2010), in their research, observed that the average seed yield of 9 different barley varieties in Diyarbakır and Ceylanpınar ecological conditions was 540 kg da^{-1} in Diyarbakır, 36 kg da^{-1} in Ceylanpınar, and the overall average seed yield was 428 kg da^{-1}. Yıldırım and Çakmak (2013) observed that the average seed yield of the locations was between 2764 kg ha^{-1} and 5125 kg ha^{-1} in hundred wheat materials in Eskişehir and Mahmudiye locations. Kahraman et al. (2017), in their research to determine the agricultural characteristics of 16 different oat genotypes, reported that the seed weight data ranged from 372.10 kg da^{-1} to 734.80 kg da^{-1}. Mendez-Espinoza et al. (2019) determined the highest seed yield in the triticale type with 200 g m^{-2} as a result of their research on the agricultural characteristics between triticale and bread wheat in Mediterranean conditions. Karaman et al. (2020) stated that the seed yield of different bread wheat genotypes was between 354.51 kg da^{-1} and 810.77 kg da^{-1} in Diyarbakir ecological conditions. In the study of Yürürdurmaz et al. (2021), as a result of their research in Kahramanmaraş ecological conditions, reported that the average seed yield of 10 different barley genotypes was 572.90 kg da^{-1} in the first year, 461.00 kg da^{-1} in the second year and the average seed yield of the years was 517.00 kg da^{-1}. Aydoğan and Yağdı (2022), in their study in Bursa ecological conditions, observed that the seed yield of 41 wheat varieties varied between 294.00 kg da^{-1} and 656.23 kg da^{-1}.

Conclusion

According to the results of the research, it was observed that there were statistically significant differences among the winter cereals in other characters except the number of fertile tillers. In terms of hectolitre weight, oats had a very low value compared to other types. Triticale had the highest number of grains per spike and number of grains per spike. Oat and triticale took the first place in terms of thousand kernel weight. Wheat had the highest grain yield, followed by barley, triticale and oats. As a result, it has been observed that there are differences in yield and yield elements among the cereal species considered in Kahramanmaraş conditions, and the order of the species in terms of characters has changed. It is seen that conducting such studies using more varieties for each species in different locations and years will yield more accurate

Statement of Conflict of Interest

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

Author's 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 results.

References

Anonymous (2022a). Kahramanmaraş Meteorology Provincial Directorate.

Anonymous (2022b). Soil analysis, KSU Faculty of Agriculture, Soil Laboratory.

Atak, M., Çiftçi, C.Y. (2005). The effects of different planting frequencies on yield and some yield elements in Triticale (*xTriticosecale* Wittmack). Journal of Agricultural Sciences, 11(1), 98-104.

Chaudhary, A., Sewhag, M., Hooda, V.S., Singh, B., Kumar, P. (2017). Effect of different dates of sowing on yield attributes, yield and quality of Barley (*Hordeum vulgare* L.) cultivars. Journal of Applied and Natural Science, 9 (1): 129-132.

Doğan, Y., Kendal, E. (2012). Determination of grain yield and some quality characteristics of bread wheat (*Triticum aestivum* L.) genotypes. Journal of GOU Faculty of Agriculture, 29(1), 113-121.

Duğan, S. (2010). Determination of adaptability of triticale to different soil conditions and comparison with other winter cereals in terms of yield and quality. Master Thesis. Field Crops Department. Namık Kemal University, Institute of Science and Technology. p. 159.

Geçit, H.H. (2016). Winter cereals (wheat, barley, oats, rye, triticale). Ankara University Press. Faculty of Agriculture.

Giunta, F., Motzo, R. (2005). Grain yield, dry matter, and nitrogen accumulation in the grains of durum wheat and spring triticale cultivars grown in a Mediterranean environment. Australian J. Of Agric. Research, (56), 25-32.

Jokinen, L., Pihlava, J.M., Puganen, A., Sontag-Strohm, T., Linderborg, K.M., Holopainen-Mantila, U., Hietaniemi, V., Norflund, E. (2021). Predicting the properties of ındustrially produced oat flours bythe characteristics of native oat grains or non-heat-treated groats. Foods, (10),3-21.

Kahraman, T., Avcı, R., Yıldırım, M. (2021). Determination of grain yield, yield components and quality characteristics of oat (*Avena sativa* L.) genotypes. KSU Journal of Agriculture and Nature, 24(5), 1003-1010.

Kahraman,T., Avcı, R., Kurt, C. (2017). Determination of grain yield, quality and agricultural characteristics of some oat (*Avena sativa* L.) genotypes. Journal of Field Crops Central Research Institute, 26 (Special Issue),

Karahan, T., Sabanci, C.O. (2010). Determination of yield and yield components of some barley (*Hordeum vulgare* L.) cultivars in South-eastern Anatolian ecological conditions. West Mediterranean Agricultural Research Institute Derim Journal, 27(1), 1-11.

Karaman, M., Seydoşoğlu, S., Çam, B. (2020). Investigation of bread wheat (*Triticum aestivum* L.) genotypes in terms of agricultural characteristics in augmented trial design in Diyarbakir province conditions. Eurasia Journal of Mathematics, Engineering, Natural & Medical Sciences, 7(9), 195-205.

Kaziu, I., Kashta, F., Selami, A. (2019). Estimation of grain yield, grain components and correlations between them in some oat cultivars. Albanian j. Agric. Sci.,18 (1), 13-19.

Mendez-Espinoza, A.M., Romero-Bravo, S., Estrada, F., Garriga, M., Lobos, G.A., Castillo, D., Matus, I., Aranjuelo, Í., del Pozo, A. (2019). Exploring agronomic and physiological traits associated with the differences in productivity between triticale and bread wheat in Mediterranean environments. Frontiers in Plant Science, (10), 404.

Monouchehr, A. (2006). Path analysis of barley (*Hordeum vulgare* L.). yield. Journal of Agricultural Science, 12(3), 227-232.

Mut, Z., Albayrak, S., Töngel, Ö. (2006). Determination of grain yield and some properties of Triticale (*xTriticosecale* Wittmack) lines. Journal of Agricultural Sciences, 12 (1), 56-64.

Mut, Z., Erbaş Köse, Ö., Akay, H., Sezer, İ. (2021). Evaluation of some characteristics of local oat genotypes collected from Central and Western Black Sea Region. Journal of Iğdır University Institute of Science and Technology, 11(2), 1582-1594.

Sarwar, M.H., Sarwar, M.F., Sarwar, M., Qadri, N.A., Moghal, S. (2013). The importance of cereals (Poaceae: Gramineae) nutrition in human health: A review. Journal of Cereals and Oilseeds, 4(3), 32-35.

TUIK, (2022). Turkish Statistical Institute, www.tuik.gov.tr, (Access Date: 01.09.2022).

Yanbeyi, S., Sezer, I. (2006). Research on yield and yield components of some triticale lines in Samsun conditions. OMU Agr. Fac. Journal, 21(1), 33-39.

Yıldırım, M. Cakmak, M. (2013). Effects of sister line mixtures on grain yield and some quality parameters in wheat. Journal of Food Agriculture and Environment, 11(3), 915-923.

Yururdurmaz, C., Kurt, A., Kara, R., Akkaya, A. (2021). Investigation of some physiological properties of barley during flowering and maturation period in Kahramanmaraş conditions. KSU Journal of Agriculture and Nature, 24 (6): 1304-1314.

IJAFLS
e-ISSN: 2602-4381
International Journal of Agriculture, Forestry and Life Sciences
Original Article | Open access | Int J Agric For Life Sci (2023) 7(2): 44-46

Impact of Seed Priming Treatments to Enhance Germination of Black Mustard Against Dormancy

Fatma Kayaçetin[1]*

[1]Kalecik Vocational School, Medicinal and Aromatic Plants Program, Ankara University, Ankara, Türkiye
***Correspondence:** fatmakayacetin@gmail.com

Citation
Kayaçetin, F., (2023). Impact of Seed Priming Treatments to Enhance Germination of Black Mustard Against Dormancy. Int J Agric For Life Sci (2023) 7(2): 44-46.
Received :29 August 2023
Accepted :19 December 2023
Published Online :22 December 2023
Year: :2023
Volume :7
Issue :2 (December)
Pages :44-46

Available online https://dergipark.org.tr/ijafls

Abstract
Black mustard (Brassica nigra L. Brassicaceae) represents an important source of raw materials for many agro-industry branches in Turkey and elsewhere in the world. Black mustard is a highly significant plant for its aromatic, medicinal, and therapeutic uses, as well as its potential as an alternative energy plant. In our country, cultivar development studies are carried out on wild genotypes of this species. One of the major challenges to black mustard growth and yield is its homogeneous, simultaneous, and fast emergence. Presoaking seeds in water (hydroprimed) and polyethylene glycol (PEG) (osmoprimed), has been demonstrated to enhance the germination of seeds of different species. The seeds of pure line black mustard originated in the Ankara, Turkey, and were used in this study. The purpose of the study was to measure the effects of durations (12, 24, 36, and 48 h); unprimed, hydroprimed, and five osmoprimed doses (-0.1, -0.2, -0.4, 0.6, and -0.8 MPa of PEG) treatments on seed germi-nation and seedling establishment characteristics of black mustard for 14 days. The present findings demonstrate that priming durations and treatments of black mustard seeds significantly affected final germination percentage, mean germination time, root and seedling length, and seedling fresh and dry weight among different treatments compared to the control. This positive effect on germination especially shortened the mean germination time. In terms of the properties studied, the optimal priming time is 12 and 24 h, for hyropriming and osmopriming doses (-0.1, -0.2, and -0.4 Mpa of PEG). The study indicated that the implemented priming treatments can be useful in improving the capability of black mustard on germination treatments.

Key words
Hydropriming; Osmopriming; Priming duration; Germination; *Brassica nigra*.

Introduction
Black mustard (*Brassica nigra* L. *Brassicaceae*) is an annual growth habit plant, is widely cultivated for its blackish brown-red seeds that are slightly more pungent than the other mustard seeds, such as white or brown mustard (Palle-Reisch et al., 2013; Kayacetin, 2020; Lietzow, 2021). It is important for its aromatic and medicinal uses (Darwesh, 2017; Nisar et al., 2018; Asaduzzaman et al., 2021; Mayekar et al., 2021) as well as its potential as an alternative energy plant (Kinay and Kayacetin, 2023).

In the entire life cycle of the plant, seed germination is the initial step for growth and development, which result in the emergence of a radicle to form a primary root (Kayacetin et al., 2018). Quantitative parameters like final germination percentage, mean germination time, root and seedling length, seedling fresh and dry weight, and synchronization of the whole germination process are thought to be highly significant. According to Kayacetin (2019), there are germination obstacles in the black mustard seeds, and thus, there are difficulties in homogeneous, rapid, and simultaneous emergence.

Dormancy is an internal state of the seed that inhibits its germination. It is examined in two groups as primary and secondary dormancy. Primary dormancy refers to innate dormancy, and secondary dormancy refers to a dormant state induced in the nondormant seed by conditions inconvenient for germination. These reasons may be the cause of dormancy in the seeds of many weed species (Benech-Arnold et al., 2000). Dormancy and germination are vital phenomena in the life cycle of all species. Although the seeds vary considerably according to the species, they have different germination and dormancy characteristics according to their genetic structure (Gupta, 2016). Plant growth regulators such as PEG have been recommended to break seed dormancy and enhance germination (Bao et al., 2010; Luo et al., 2022; Hassan et al., 2023)

Seed priming is a simple and effective technology to help ensure homogeneous, simultaneous, and rapid emergence, thus leading to better crop yields (Finch-Savage and Bassel, 2016; Raj and Raj, 2019). Recently published studies showed that priming treatment can positively affect seed germination and seedling growth in the *Brassicaceae* family (Guragain et al., 2023; Kayacetin, 2023). As primed seeds are physiologically close to the germination stage, they show an improved germination percentage, early and uniform germination, improved growth characteristics, and faster and more homogeneous emergence (Fu et al., 2022; Okello et al., 2022). The Priming technique is one of the most effective options to shorten germination time and advence germination percentage to increase yield (Kayacetin, 2021; Thakur et al., 2022).

For the uniform emergence of the seed, osmopriming and hydropriming are the most effective methods (Singh et al., 2017; Pandey et al., 2022). The positive contributions of osmopriming on germination in sorghum (Zhang et al., 2015), in savory (Vidak et al., 2022), in wheat (Farooq et al., 2022), in sesame (Biswas and Dutta, 2021) and in black cumin (Kayacetin, 2022); whereas hydropriming on germination in mustard (Thapa et al., 2022), in sorghum (Demb´el´e et al., 2021), and in lemon balm (Hatami et al., 2021) have been previously reported.

Current and future work while focusing on the different uses of mustard plants and seeds, the negative effects on germination and emergence will be tried to be eliminated both in terms of breeding and agriculture. Information on enhancing the germination of black mustard against dormancy is still limited. Therefore, it was thought that the purpose of the research was to identify the effects of priming durations (12, 24, 36, and 48 h); unpriming, hydropriming, and five osmopriming doses (-0.1, -0.2, -0.4, 0.6, and -0.8 MPa of PEG) on seed germination and seedling establishment characteristics of black mustard (*Brassica nigra* L.) for 14 days on the dormancy-breaking and germination indices.

Material and Method
This study was done using pure line seeds of the *Brassica nigra*, obtained from the Research Institute in Ankara, Turkey (Table 1). The experimental design consisted of two factors (priming duration × seed treatment) regulated in a completely randomized design with three replicates. The osmotic potential of PEG-6000 was arranged at-0.2,-0.4,-0.6, and-0.8 MPa according to Michel and Kaufmann (1973). The priming durations (12, 24, 36, and 48 hours) were the main factors, seed treatments [unprimed (control), hydroprimed, and five osmoprimed doses (-0.1,-0.2,-0.4, 0.6, and-0.8 MPa of PEG)] was the subfactors. Samples of 150 seeds (three replicates of 50 seeds each) were placed in 9-cm Petri dishes containing two layers of filter paper.

Black mustard seeds were treated with immersed solution of -0.2, -0.4, -0.6, and -0.8 MPa polyethylene glycol for 12, 24, 36, and 48 hours and in distilled water (hydroprimed) for 12, 24, 36, and 48 hours. The unprimed seeds were used as control (unprimed). The seeds were carefully dried after priming to the first moisture level at 25 ± 3 °C and were and used 24 h after priming.

Seeds were kept at 22 ± 1 °C in the dark for germination for 14 days (Fallah-Toosi and Baki, 2013). Black mustard seed was considered to have germinated when >1 mm radicle emerged. Germination percentage was recorded every 24 h for 7 days. The mean germination time was numbered according to ISTA (2003). Shoot length, root length, seedling freshness and dry weight were

evaluated in five seedlings selected randomly from each replicate after the 14th day. Dry weight was evaluated after drying samples in an oven at 70 °C for 48 h (Böhm, 1979). The final germination percentage (FGP) and mean germination time (MGT) were determined according to the method of Kader (2005).

$$\text{FGP (\%)} = \frac{\text{Total number of germinated seeds}}{\text{Total number of observed seeds}} \times 100$$

$$\text{MGT (day)} = \frac{n1 \times d1 + n2 \times d2 + n3 \times d3 + \cdots}{\text{Total number of days}}$$

n= number of seeds newly germinated at day;
d= days counted from the start of the germination test

Data statistical analysis

Analysis of variance of the experimental data was computed using the JMP 13 Statistical Software. The differences among the means were performed by LSD Test ($p < 0.05$).

Results and Discussion

The interaction effect of seed priming durations and seed priming treatment on final germination percentage (FGP), mean germination time (MGT), root length (RL), seedling length (SL), seedling fresh weight (SFW), and seedling dry weight (SDW) was significant (Table 2). While the maximum GP (97.33%) was obtained with the treatment of 12 and 24 h durations at -0.1 and -0.2 MPa of PEG priming, the minimum GP (64.67%) was determined with the treatment of 48 h durations at -0.8 MPa of PEG priming (Table 2). The maximum MGT (2.28 d) was obtained with unprimed, while the minimum MGT (1.17 d) was determined at -0.6 MPa of PEG for 48 h durations. The maximum RL (3.23 cm) and SL (6.21 cm) were obtained at -0.6 MPa and 0.4 MPa of PEG for 12 h durations, respectively, whereas the minimum RL (0.74 cm) and SL (2.36 cm) were obtained at -0.8 MPa of PEG for 48 h durations. The maximum SFW (8.28 mg) and SDW (0.71 mg) were obtained at -0.4 MPa for 12 h durations. The minimum SFW (0.83 mg) was determined at -0.8 MPa of PEG for 48 h durations. 48 h × -0.2 MPa of PEG priming and 48 h × -0.4 MPa PEG priming were detected at the minimum SDW (0.25 mg), and no significant differences were observed (Table 2). At the beginning of germination, rapid water uptake slows down after seed-based metabolic activities, resulting in the emergence of radicles leading to germination (Kayacetin, 2022). These results may explain that priming durations and treatments could improve theemergence performance of black mustard seeds, which is approved by earlier studies on some cultured species like black cumin (Kayacetin, 2022), sunflower (Bourioug et al., 2020), caraway (Mirmazloum et al., 2020), and kenaf (Lee et al., 2018). All priming processes used in this research made it possible to break dormancy in black mustard seeds considered dormant under appropriate conditions. Kayacetin (2023) demonstrated that osmo-priming treatments of black cumin using −0.2 or −0.4 MPa PEG for 24 or 36 hours demonstrated improved germination ability. Also, Trisnawaty et al. (2021) reported that different seed priming treatments resulted in significant seedling growth and reduced MGT in capers. In stevia, Shahverdi et al. (2017) demonstrated that PEG positively affected many aspects of plant growth, including seed germination and seedling growth. The rise in seedling growth traits with priming treatment could play a vital role in regulating plants's primary seedling growth. The major influence of the priming treatments was improved germination; therefore, post-germination progress could also be improved by priming seedling treatments. Considering seed priming treatments, it was noted that priming treatments improved seedling growth, which ended up with the maximum RL and SL. Kayacetin (2023) in black cumin noted that the maximum RL and SL were determined with the treatment of 36 h priming time at -0.4 MPa osmoprimed. This supports the idea that with priming treatments, the seed performs faster water uptake than normal germination. Previous studies showed that osmo and hydropriming achieved faster emergence and germination compared to control for germination (Neamatollahi et al., 2009; Kartika et al., 2021; Kayacetin, 2022; Bahreininejad, 2023).

A significant difference was detected among the priming durations for all the investigated characteristics (Table 3). While the maximum GP (91.24%) was obtained with the application of 24 h durations, the minimum GP (75.62%) was detected with the application of 48 h durations (Table 3). The minimum MGT (1.40 d) was determined with the application of 48 h durations, while the maximum MGT (1.61 d) was obtained within a 12 h duration. The maximum RL (2.23 cm) and SL (4.96 cm) were obtained for 12 and 36 h durations, respectively, whereas the minimum RL (1.19 cm) and SL (3.49 cm) were obtained for 48 h durations, respectively. The maximum SFW (5.41 mg) and SDW (0.46 mg) were obtained for 24 and 12 h durations, respectively. The minimum SFW (3.27 mg) and SDW (0.33 mg) weights were determined for 48 h durations (Table 3). While priming durations significantly increased the FGP and seedling growth parameters in black mustard seedlings, these parameters decreased by 48 h for the priming duration. At the onset of germination, rapid water uptake slows down after seed-based metabolic activities, resulting in the emergence of radicles leading to germination (Kayacetin, 2022). Therefore, 12 and 24 h are considered optimum duration for priming (Table 3). Kayacetin (2023) in black cumin found that 24 or 36 h priming treatment durations improved germination and seedling growth. Sadeghi et al. (2011) demonstrated that soybean seed osmopriming for 12 h improved the FGP. Mirmazloum et al. (2020) in caraway showed that 24 h priming duration was recommended as the best treatment for improving the FGP when compared to unprimed seeds. Benadjaoud et al. (2022) in *Lavandula stoechas* and Bahreininejad (2023) in *Thymus daenensis* reported that germination was positively affected by priming treatments compared to the unprimed. Mehra et al. (2003) determined that seeds of brown mustard and field mustard subjected to aerated hydration for up to 24 h had the most suitable timing at 12 h, which increased the final germination, and reduced MGT. Similar findings were observed by OrzeszkoRywka and Podlaski (2003) in sugar beet; Kayacetin, 2022 in black cumin with washing and priming that MGT was shortened by seed treatments.

A significant difference was determined among the priming treatments for all the investigated characteristics (Table 4). While the maximum GP (96.67%) and MGT (2.28 d) were obtained with unprimed applications, the minimum GP (72.67%) and MGT (1.37 d) were obtained with applications at -0.8 MPa of PEG priming (Table 4). The maximum RL (2.22 cm) and SL (5.11 cm) were obtained at -0.4 MPa and -0.1 MPa of PEG priming, respectively, whereas the minimum RL (1.35 cm) and SL (3.15 cm) were obtained at 0.8 MPa of PEG priming. The maximum SFW (6.00 mg) and SDW (0.49 mg) were obtained at -0.1 MPa of PEG priming and unprimed, respectively. The minimum SFW (3.23 mg) and SDW (0.36 mg) were determined at -0.8 MPa of PEG priming (Table 4). Whereas hydro and osmopriming treatments significantly increased the FGP and seedling growth parameters in black mustard seedlings compared to the unprimed, these parameters decreased by -0.6 and -0.8 MPa PEG osmopriming treatments, respectively. MGT was decreased by seed priming treatments and priming durations compared to unprimed ones. In black cumin, Kayacetin (2023) found that a -0.2 or -0.4 MPa priming treatment improved germination and seedling growth. Faijunnahar et al. (2009) in wheat seeds determined the most successful seedling growth with -0.1 MPa osmoprimed in comparison to unprimed. These outcomes are aided by Trisnawaty et al. (2021), who detected that rice seeds primed with PEG both improved germination indices and reduced MGT. Furthermore, results revealed that priming treatments were successful techniques to improve seed germination. Hydro and osmopriming of black mustard seeds also increased germination traits and seedling growth in the study.

Conclusions

The results showed that both priming durations and priming treatments of black mustard seeds significantly affected GP, MGT, RL, SL, SFW, and SDW compared to the unprimed. This positive effect, especially on germination, shortened the MGT. In terms of the properties studied, the optimal priming durations are 12 and 24 h; and priming treatments are hyropriming and osmopriming doses (-0.1, -0.2, and -0.4 Mpa of PEG). Results revealed that the applied priming treatments can be useful in improving the ability of black mustard in terms of germination treatments. It may be concluded that priming could end up being a very effective treatment to increase fast and identical emergence to accomplish better vigor, ending up with a better stand and yield. Therefore, current findings confirm that seed priming with PEG can be employed as a novel approach for improving black mustard seed germination efficiency. This technique is a practical pretreatment for fast and uniform emergence in unsuitable climatic conditions and can be used by researchers and farmers.

Table 1. Characteristics of the mustard species used in the study

Scientific name	Common name	Other name	Origin	Seed color	Thousand seed weight (g)	Registration
Brassica nigra	black mustard	*Sinapis nigra*	Ankara/Turkey	black-brown	1.4-1.6	pure line

Table 2. Effect of priming duration × priming treatment interaction on different germination parameters of black mustard

Priming durations	Priming treatment	GP (%)	MGT (day)	RL (cm)	SL (cm)	SFW (mg)	SDW (mg)
12	Unprimed	96.67 a	2.28 a	1.44 ij	4.31 fg	4.23 hi	0.49 b
	Hydroprimed	96.67 a	1.61 b	1.51 ij	4.25 fgh	4.33 h	0.35 g
	-0.1 MPa PEG primed	97.33 a	1.56 bc	1.73 hi	4.53 f	5.78 de	0.37 efg
	-0.2 MPa PEG primed	97.33 a	1.52 cd	2.65 bc	5.71 cd	6.01 cd	0.41 cde
	-0.4 MPa PEG primed	90.67 b	1.49 de	2.71 bc	6.21 b	8.29 a	0.71 a
	-0.6 MPa PEG primed	82.00 d	1.45 ef	3.23 a	5.99 bc	4.43 gh	0.49 b
	-0.8 MPa PEG primed	76.67 e	1.38 ghi	2.30 def	3.64 j	3.92 I	0.35 fg
24	Unprimed	96.67 a	2.28 a	1.44 ijk	4.31 fg	4.25 hi	0.49 b
	Hydroprimed	97.33 a	1.55 cd	1.72 hi	4.38 fg	4.78 fg	0.41 cde
	-0.1 MPa PEG primed	97.33 a	1.45 ef	2.40 c-f	5.10 e	5.91 cd	0.45 c
	-0.2 MPa PEG primed	97.33 a	1.53 cd	2.62 bc	4.88 e	5.95 cd	0.51 b
	-0.4 MPa PEG primed	90.67 b	1.51 cde	2.82 b	6.18 b	6.24 bc	0.43 cd
	-0.6 MPa PEG primed	82.67 cd	1.41 fgh	2.18 fg	5.90 bc	5.90 cd	0.41 cde
	-0.8 MPa PEG primed	76.67 e	1.45 ef	1.14 kl	3.48 jk	4.85 f	0.40 de
36	Unprimed	96.67 a	2.28 a	1.44 ijk	4.31 fg	4.23 hi	0.49 b
	Hydroprimed	92.67 b	1.42 fg	2.58 bcd	6.80 a	5.12 f	0.39 def
	-0.1 MPa PEG primed	90.67 b	1.29 jkl	2.53 b-e	6.69 a	6.44 b	0.43 cd
	-0.2 MPa PEG primed	85.33 c	1.24 lm	2.22 efg	5.54 d	5.48 e	0.51 b
	-0.4 MPa PEG primed	85.33 c	1.35 hij	2.10 fg	4.25 fgh	4.78 fg	0.41 cde
	-0.6 MPa PEG primed	76.00 e	1.29 kl	1.96 gh	3.97 hi	4.44 gh	0.21 j
	-0.8 MPa PEG primed	72.67 f	1.41 fgh	1.22 jkl	3.13 l	3.30 j	0.39 def
48	Unprimed	96.67 a	2.28 a	1.44 ijk	4.31 fg	4.24 hi	0.49 b
	Hydroprimed	62.67 h	1.32 ijk	1.26 jkl	3.69 ij	4.34 h	0.35 fg
	-0.1 MPa PEG primed	83.33 cd	1.33 ijk	1.22 jkl	4.13 gh	5.85 d	0.41 cde
	-0.2 MPa PEG primed	81.33 d	1.27 kl	1.29 jkl	3.47 jk	4.35 h	0.25 ij
	-0.4 MPa PEG primed	72.67 f	1.21 mn	1.26 jkl	3.24 kl	2.04 k	0.25 ij
	-0.6 MPa PEG primed	68.00 g	1.17 n	1.11 l	3.24 kl	1.26 l	0.30 h
	-0.8 MPa PEG primed	64.67 h	1.24 lm	0.74 m	2.36 m	0.83 m	0.27 hi
Summary of ANOVA		**	**	**	**	**	**

** Significant at $p < 0.05$

Table 3. Effect of priming duration on different germination parameters of black mustard

Priming durations (h)	GP (%)	MGT (day)	RL (cm)	SL (cm)	SFW (mg)	SDW (mg)
12	91.05 a	1.61 a	2.23 a	4.94 a	5.30 a	0.46 a
24	91.24 a	1.60 a	2.05 b	4.89 a	5.41 a	0.44 a
36	85.62 b	1.47 b	2.01 b	4.96 a	4.83 b	0.41 b
48	75.62 c	1.40 c	1.19 c	3.49 b	3.27 c	0.33 c
Summary of ANOVA	**	**	**	**	**	**

** Significant at $p < 0.05$

Table 4. Effect of priming treatment on different germination parameters of black mustard

Priming treatment	GP (%)	MGT (day)	RL (cm)	SL (cm)	SFW (mg)	SDW (mg)
Unprimed	96.67 a	2.28 a	1.45 d	4.29 d	4.26 d	0.49 a
Hydroprimed	87.33 d	1.48 b	1.77 c	4.78 c	4.64 c	0.38 d
-0.1 MPa PEG primed	92.17 b	1.41 c	1.97 b	5.11 a	6.00 a	0.42 c
-0.2 MPa PEG primed	90.33 c	1.39 cd	2.20 a	4.90 bc	5.45 b	0.42 c
-0.4 MPa PEG primed	84.83 e	1.39 cd	2.22 a	4.97 ab	5.34 b	0.45 b
-0.6 MPa PEG primed	77.17 f	1.33 e	2.12 ab	4.77 c	4.01 e	0.35 e
-0.8 MPa PEG primed	72.67 g	1.37 d	1.35 d	3.15 e	3.23 f	0.36 de
Summary of ANOVA	**	**	**	**	**	**

** Significant at $p < 0.05$

Data Availability Statement

The data are available on request.

Acknowledgments

The author acknowledges the facilities provided by the Kalecik Vocational School, Ankara University, Ankara, Turkey.

Statement of Conflict of Interest

The author(s) declare no conflict of interest for this study.

Author's Contributions

The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

References

Asaduzzaman, M. Huqe, M.A.S. Haque, M.S. Uddin, M.N. Hossain, M.A. (2021). Seed priming improves germination and early seedling growth in wheat under control and drought conditions. Journal of Bangladesh Agricultural University, 19, 2, 184-191.

Bahreininejad, B. (2023). A predictive method for selecting the most appropriate level of water potential for *Thymus daenensis* ssp. daenensis Cleak seed priming with respect to the severity of salinity or drought stress. Journal of Applied Research on Medicinal and Aromatic Plants, 34, 100453. https://doi.org/10.1016/j.jarmap.2022.100453

Bao, J.P. Zhang, S.L. (2010). Effects of seed coat, chemicals and hormones on breaking dormancy in pear rootstock seeds (*Pyrus betulaefolia* Bge. and *Pyrus calleryana* Dcne.). Seed Science and Technology, 38, 2, 348-357.

Benadjaoud, A. Dadach, M. El-Keblawy, A. and Mehdadi, Z. (2022). Impacts of osmopriming on mitigation of the negative effects of salinity and water stress in seed germination of the aromatic plant *Lavandula stoechas* L. Journal of Applied Research on Medicinal and Aromatic Plants, 31, 100407.

Benech-Arnold, R.L. Sánchez, R.A. Forcella, F. Kruk, B.C. Ghersa, C.M. (2000). Environmental control of dormancy in weed seed banks in soil. Field Crops Research, 67, 2, 105-122.

Biswas, U. Dutta, A. (2021). Influence of pre-sowing seed priming and packaging materials on the quality of sesame (*Sesamum indicum* L.) seeds. 23, 4, 389-395. https://doiorg/10.5958/0974-4517.2021.00049.5

Bourioug, M. Ezzaza, K. Bouabid, R. Alaoui-Mhamdi, M. Bungau, S. Bourgeade, P. Aleya, L. (2020). Influence of hydro-and osmo-priming on sunflower seeds to break dormancy and improve crop performance under water stress. Environmental Science and Pollution Research, 27, 13215-13226.

Böhm, W. (1979). Methods of Studying Root Systems, Springer-Verlag, Berlin.

Darwesh, D.T.D. (2017). Plant Biodiversity and Ethnobotanical Properties of

Various Plants in Choman (Erbil-Iraq) (Doctoral dissertation. 130pp, MSc, Sutcu Imam University, Kahramanmaras, Turkey.
Dembélé, S. Zougmoré, R.B. Coulibaly, A. Lamers, J.P. Tetteh, J.P. (2021). Accelerating seed germination and juvenile growth of sorghum (*Sorghum bicolor* L. Moench) to manage climate variability through hydro-priming. Atmosphere, 12, 4, : 419.
Fallah-Toosi, A. Baki, B.B. (2013). Effect of NaCl on germination and early seedling growth of *Brassica juncea* var. Ensabi. International Journal of Agronomy and Plant Production, 4, 11, 3004-3011.
Farooq, T. Nisa, Z.U. Hameed, A. Ahmed, T. and Hameed, A. (2022). Priming with copper-chitosan nanoparticles elicit tolerance against PEG-induced hyperosmotic stress and salinity in wheat. BMC chemistry, 16, 1, 23.
Finch-Savage, W.E. Bassel, G.W. (2016) Seed vigour and crop establishment: extending performance beyond adaptation. J Exp Bot 67, 567- 591.
Gupta, S.K. (Ed.). (2016). Biology and breeding of crucifers. CRC Press.
Guragain, R.P. Baniya, H.B. Shrestha, B. Guragain, D.P. Subedi, D.P. (2023). Germination enhancement of mustard (*Brassica nigra*) seeds using dielectric barrier discharge (DBD). AIP Advances, 13(3).
Hassan, F.E. Alyafei, M.A. Kurup, S. Jaleel, A. Al Busaidi, N. and Ahmed, Z.F. (2023). Effective Priming Techniques to Enhance Ghaf (*Prosopis cineraria* L. Druce) Seed Germination for Mass Planting. Horticulturae, 9, 5, 542.
Hatami, M. Khanizadeh, P. Abtahi, F.A. Abaszadeh Dehgi, P. (2021). Influence of plant growth promoting rhizobacteria and hydro-priming on some physiological indices of lemon balm (*Melissa officinalis*). Iranian Journal of Horticultural Science, 52, 1, 11-21.
ISTA. (2003). ISTA Handbook on seedling evaluation. 3rd ed. International Seed Testing Association (ISTA), Zurich, Switzerland.
Kader, M.A. (2005). A comparison of seed germination calculation formulae and the associated interpretation of resulting data. Journal and Proceeding of the Royal Society of New South Wales, 138, 65-75.
Kartika, K. Lakitan, B. Ria, R.P. (2021). Hydro-and osmo-priming effects on upland rice exposed to drought conditions at vegetative and reproductive stages. CMUJ. Nat. Sci., 20, 3, e2021053.
Kayacetin, F. (2020). Botanical characteristics, potential uses, and cultivation possibilities of mustards in Turkey: a review. Turkish Journal of Botany, 44, 2, 101-127. https://doi.org/10.3906/bot-1909-30
Kayacetin, F. (2019). Morphological characterization and relationships among some important wild and domestic Turkish mustard genotypes (*Brassica* spp.). Turkish Journal of Botany, 43, 4, 499-515. https://doi.org/10.3906/bot-1810
Kayacetin, F. (2021). Selection of some important species in genus Brassica against drought and salt tolerance by morphological observations on germination and seedling growth parameters. Fresenius Environmental Bulletin, 30, 7 A, 9228-9236.
Kayacetin, F. (2022). Response to Direct Selection against drought stress in black cumin (*Nigella sativa* L.). Evidence-Based Complementary and Alternative Medicine, 2022, Article ID 6888187, 1-10. https://doi.org/10.1155/2022/6888187.
Kayacetin, F. Efeoglu, B. Alizadeh, B. (2018). Effect of NaCl and PEG-Induced osmotic stress on germination and seedling growth properties in wild mustard (*Sinapis arvensis* L.). ANADOLU Ege Tarımsal Araştırma Enstitüsü Dergisi, 28, 1, 62-68.
Kinay, A., Kayacetin, F. (2023). Phenology, morphology, yield and quality characteristics of mustard species (*Brassica* spp.) suitable for energy sector. Gesunde Pflanzen, 1-10. https://doi.org/10.1007/s10343-022-00817-w
Lee, I. S. Kang, C.H. Kwon, S.J. Na, Y.E. (2018). Effects of PEG priming and fungicide treatment on kenaf (*Hibiscus cannabinus* L.) seed germination. Journal of Agricultural Science and Technology, B, 278-289. https://doi.org/10.17265/2161-6264/2018.05.002
Lietzow, J. (2021). Biologically active compounds in mustard seeds: A toxicological perspective. Foods, 10, 9, 2089. https://doi.org/10.3390/foods10092089
Luo, J.M. Liu, H. Wang, J.L. Liu, Y.F. Wang, Q.Y. Huang, Y. (2022). Screening and evaluation of methods for breaking seed dormancy of wild Artemisia wellbyi. Acta Agrestia Sinica, 30, 6, 1603. https://doi.org/10.11733/j.issn.1007-0435.2022.06.035
Mayekar, V.M. Ali, A. Alim, H. Patel, N. (2021). A review: Antimicrobial activity of the medicinal spice plants to cure human disease. Plant Science Today 8, 3, 629-646. https://doi.org/10.14719/pst.2021.8.3.1152
Mehra, V. Tripathi, J. Powell, A.A. (2003). Aerated hydration treatment improves the response of *Brassica juncea* and *Brassica campestris* seeds to stress during germination. Seed Science and Technology, 31, 1, 57-70.
Michel, B.E. Kaufmann, M.R. (1973). The osmotic potential of polyethylene glycol 6000. Plant Physiol., 51, 914-916.
Mirmazloum, I. Kiss, A. Erdélyi, É. Ladányi, M. Németh, É. Z. Radácsi, P. (2020). The Effect of osmopriming on seed germination and early seedling characteristics of *Carum carvi* L. Agriculture, 10, 4, 94. https://10.3390/agriculture10040094
Neamatollahi, E. Bannayan, M. Darban, A.S. Ghanbari, A. (2009). Hydropriming and osmopriming effects on cumin (Cuminum Cyminum L.) seeds germination. International Journal of Agricultural and Biosystems Engineering, 3(9), 477-480. https://doi.org/10.12982/CMUJNS.2021.053
Nisar, NS. Mehmodd, H. Nisar, S. Jamil, Z. Ahmad, N. Ghani, N. Oladipo, A.A. Waseem, R.Q. Latif, A.A. Ahmad, S.R. Iqbal, M. Abbas, M. (2018). *Brassicaceae* family oil methyl esters blended with ultra-low sulphur diesel fuel (ULSD): Comparison of fuel properties with fuel standards. Renewable Energy 117: 393-403. https://doiorg/101016/jrenene201710087
Okello, D. Komakech, R. Gang, R. Rahmat, E. Chung, Y. Omujal, F. Kang, Y. (2022). Influence of various temperatures, seed priming treatments and durations on germination and growth of the medicinal plant Aspilia africana. Scientific Reports, 12, 1, 14180. https://doi.org/10.1038/s41598-022-18236-2
Orzeszko-Rywka, A. Podlaski, S. (2003). The effect of sugar beet seed treatments on their vigour. Plant Soil and Environment, 49, 6, 249-254.
Palle-Reisch, M. Wolny, M. Cichna-Markl, M. and Hochegger, R. (2013). Development and validation of a real-time PCR method for the simultaneous detection of black mustard (*Brassica nigra*) and brown mustard (*Brassica juncea*) in food. Food Chemistry, 138, 348-355. https://doi.org/10.1016/j.foodchem.2012.10.055
Pandey, S. Oza, K. Maitreya, B. (2022). Effect of different solutions on seed germination and growth of different species of seeds review. International Association of Biologicals and Computational Digest, 1, 2, 168-172. https://doi.org/10.1371/journal.pone.0140620
Raj, A.B. and Raj, S.K. (2019). Seed priming: An approach towards agricultural sustainability. Journal of Applied and Natural science, 11, 1, 227-234. https://doi.org/10.31018/jans.v11i1.2010
Sadeghi, H. Khazaei, F. Yari, L. Sheidaei, S. (2011). Effect of seed osmopriming on seed germination behavior and vigor of soybean (*Glycine max* L.). ARPN Journal of Agricultural and Biological Science, 6, 1, 39-43.
Shahverdi, M.A. Omidi, H. Tabatabaei, S.J. (2017). Effect of nutri-priming on germination indices and physiological characteristics of stevia seedling under salinity stress. Journal of Seed Science, 39, 353-362. https://doi.org/10.1590/2317-1545v39n4172539
Singh, S. Lal, G.M. Bara, B.M. Mishra, S.N. (2017). Effect of hydropriming and osmopriming on seed vigour and germination of Pea (*Pisum sativum* L.) seeds. J. Pharmacognosy and Phytochemistry, 6, 3, 820-824.
Thakur, M. Tiwari, S. Kataria, S. Anand, A. (2022). Recent advances in seed priming strategies for enhancing planting value of vegetable seeds. Scientia Horticulturae, 305, 111355. h
Thapa, S. Baral, B. Shrestha, M. Dahal, D.K.C. (2022). Effect ff different priming methods on germination behaviour of broadleaf mustard cv. marpha chauda paate. Tropical Agrobiodiversity (TRAB), 3, 2, 52-59. http://doi.org/10.26480/trab.02.2022.52.59
Trisnawaty, A.R. Asra, R. Panga, N.J. Sjahril R. (2021). Effect of osmo-priming with polyethylene glycol 6000 (PEG-6000) on rice seed (*Oryza sativa* L.) germination and seedling growth under drought stress," International Journal of Agriculture System, 9, 1, 40-50. https://doi.org/10.20956/ijas.v9i1.2558
Vidak, M. Lazarević, B. Nekić, M. Šatović, Z. Carović-Stanko, K. (2022). Effect of hormonal priming and osmopriming on germination of winter savory (*Satureja montana* L.) natural population under drought stress. Agronomy, 12, 6, 1288. https://doi.org/10.3390/agronomy12061288
Zhang, F. Yu, J. Johnston, C.R. Wang, Y. Zhu, K. Lu, F. Zhang, Z Zou, J. (2015). Seed priming with polyethylene glycol induces physiological changes in sorghum (*Sorghum bicolor* L. Moench) seedlings under suboptimal soil moisture environments. PLoS One, 10(10), e0140620. https://doi.org/10.1371/journal.pone.0140620 42.

IJAFLS
e-ISSN: 2602-4381
International Journal of Agriculture, Forestry and Life Sciences
Original Article | Open access | Int J Agric For Life Sci (2023) 7(2): 47-51

The Effect of Different Phosphorus Doses on Fixed Oil Component and Plant Nutritional Elements of Black Cumin (*Nigella sp.*)

Osman Gedik[1]*

[1]Kahramanmaras Sutcu Imam University, Faculty of Agriculture, Department of Field Crops, 46100 Kahramanmaras, Türkiye
***Correspondence:**ogedik@ksu.edu.tr

Citation
Gedik, O. (2023). The Effect of Different Phosphorus Doses on Fixed Oil Component and Plant Nutritional Elements of Black Cumin (*Nigella sp.*). Int J Agric For Life Sci (2023) 7(1): 47-51.

Received :3 September 2023
Accepted :21 November 2023
Published Online :22 December 2023
Year: :2023
Volume :7
Issue :2 (December)
Pages :47-51

Available online https://dergipark.org.tr/ijafls

Abstract
This study was conducted to determine the fixed oil components and macro-micro nutrients of black cumin grown at different phosphorus doses. Seed samples were obtained from two different black cumin genotypes grown for two years (in 2017-2018 and 2019-2020 winter growing seasons) by applying five different phosphorus doses (0, 3, 6, 9, 12 kg da-1 P). According to the research results, increasing phosphorus amounts did not show a significant difference in fixed oil components. The unsaturated fatty acid ratio has a much higher ratio than saturated fatty acid ratio. Linoleic acid (58.26%), an unsaturated fatty acid, and palmitic acid (11.84%), a saturated fatty acid, were found to be the highest in black cumin oil. When macro-micro nutrients are examined, it has been observed that the difference in dose and genotype x dose interaction is important. While the macro-micro elements Fe, Ca, P, Zn, and Mn increased up to a certain dose, the elements Mg, K, Na, and N increased depending on the dose increase, and the highest value was obtained from the 12 kg da-1 P application.

Key words
Çameli variety, Nigella sativa, Phosphorus dose, Fixed oil, Plant nutrients.

Introduction
Medicinal plants have been used for seasoning, food, and healing purposes for centuries (Bayram et al. 2010). Black cumin is an annual herbaceous plant in the Ranunculaceae family. While the genus *Nigella* is represented by 20-24 species in the world, it is known that 12-15 of the species naturally grow in our country's flora (Baser, 2010; Ayhan, 2012). Black cumin, which is among the medicinal and aromatic plants, is used as a raw material in the food industry, and is used as a flavor, odor, and flavoring agent in kitchens, can be easily grown in the climatic conditions of Turkey. (Ertas, 2016; Akgul, 1993; Kucukemre, 2009; Ceylan, 1997). It has a hairy and upright structure, which can usually be 35-70 cm in length. Black cumin seeds are black in color and 2.5-4 mm long (Baydar, 2013; Urusan, 2016). Traditionally, black cumin is used as a therapeutic for stomach ailments, gas expectorant, dysentery, diuretic, obesity, back pain, asthma, bronchitis, cough relief, appetite stimulant, menstrual regulator, jaundice reliever, and many other diseases (Ozguven, 2005; Baser, 2010). The main fatty acids in black cumin seeds are linoleic, oleic, and palmitic acid (Kizil et al. 2008). Black cumin seeds carbohydrates (25%), crude fiber (8.4%), contain protein (26%) and ash (4.8%). The seeds contain good amounts of carotene and minerals such as Cu, P, Zn, and Fe (Ahmad et al. 2013). Although yield values vary depending on annual rainfall (Kaya et al. 2023), one of the most important cultural processes that increase the quality and yield in plant production is the appropriate fertilization. (Anonymous, 2016). For this reason, the application time, shape, and amount of the fertilizer should be determined according to the region where the plant will be grown. Phosphorus needed in Turkish soil is in the first place after nitrogen. (Turan 2014). According to the dry matter principle, the P content of the plants generally varies between 0.05% and 1.0%. In plants; phosphorus is the least abundant macronutrient (N, K, Ca, Mg, P). Inorganic phosphorus compounds (Pi) entering the root are stored in the root or transported to the top organs of the plant. Phosphorus taken from the soil after various chemical reactions; It turns into various inorganic compounds, including enzymes, proteins, and nucleic acids. High-energy phosphate compounds play an important role in all metabolic events in plants. It is known that the amount of phosphorus in the plant is very important for seed and fruit quality. Phosphorus has a positive and significant effect on root development in plants. Phosphorus increases the resistance of plants against diseases and pests because it makes plant tissues stronger. Phosphorus accelerates generative development in plants and causes plants to come to harvest earlier (Kacar, 2015). According to Kizil et al. (2008) reported that the maximum fixed oil rate (37.4%) was obtained in 160 kg ha^{-1} P application and it was statistically similar to 120 kg ha^{-1} P dose in a study conducted in Diyarbakır ecological conditions. In this study, the effects of different phosphorus doses on fixed oil components and plant nutrients in black cumin genotypes were determined.

Materials and Methods
In this study; Seed samples used for the determination of fixed oil components and nutrients were obtained from a two-year study established in 2018 and 2020. Two different genotypes were used, with the first genotype 1 (*N. sativa*) and the second genotype 2 (Çameli variety).

Experimental Material
Seed samples obtained from two different black cumin genotypes grown by applying five different phosphorus doses in Kahramanmaras conditions for two years were used. Phosphorus doses applied in the experiment were 0, 3, 6, 9, and 12 kg da^{-1}, while the genotypes used were *N. sativa* and Çameli registered variety.

Design and Cultural Practices
The trial was repeated for two years in 2018 and 2020. The experiment was set up in a split-parcel trial design with 3 replications. Five different doses of phosphorus (0, 3, 6, 9, 12 kg da^{-1}) and two different black cumin genotypes were used. *Nigella sativa* genotypes were distributed to the main plots, and phosphorus doses to the sub-plots. In both years, the trial was established in November, the necessary agricultural applications were made and the harvest was made in June.

Table 1. Climate data of Kahramanmaraş Province trial years and long-term growing seasons (Anonymous 2020a)

		Months								
Climatic Factory	Year	November	December	January	February	March	April	May	June	Total Average
Precipitation (mm)	2017-2018	89.60	33.70	149.90	63.10	47.40	71.60	28.10	39.40	522.80
	2019-2020	39.10	198.50	88.00	72.70	173.40	61.80	18.50	0.30	652.30
	Long years	87.50	116.60	125.40	108.30	93.40	69.80	41.20	8.40	650.80
Average Temperature (°C)	2017-2018	12.20	8.90	7.40	9.70	14.20	18.40	21.70	25.40	14.74
	2019-2020	13.50	8.40	6.30	6.10	12.50	15.90	15.90	24.50	13.25
	Long years	11.50	6.80	4.90	6.40	10.60	15.50	20.30	25.30	12.60
Relative humidity (%)	2017-2018	64.20	69.00	69.50	69.40	60.80	45.30	52.60	49.10	59.98
	2019-2020	56.20	81.90	69.30	68.30	67.30	58.20	47.20	46.90	61.91
	Long years	66.68	79.85	69.99	65.62	60.00	57.59	54.95	49.67	63.04

It is seen that the average temperature values in both years in which the experiment was carried out were above the average temperature of Kahramanmaras for many years. When the relative humidity values are examined, it is seen that the average relative humidity values of both years are lower than the average relative humidity values of the long years. Considering the precipitation values, the average precipitation amount in the first year of the experiment was below the average precipitation value for many years, while the second year had a value above the average precipitation amount for long years (Table 1).

Considering the soil characteristics of the experimental area, it is clay loam, slightly alkaline, low in salinity, moderately calcareous, low in organic matter, and high in potassium (Table 2).

Table 2. Some chemical and physical properties of the study area soils (Anonymous 2020b)

Year	Texture class	Organic matter (%)	$CaCO_3$ (%)	EC (dS m-1)	pH	P_2O_5 (kg da-1)	K_2O (kg da-1)
2018	Clay (72)	1.66	3.91	0.86	7.66	6.29	53
2020	Clay-loam (69.96)	1.58	6.09	0.05	7.71	2.84	55.51

Data Collection

Fixed oil extraction

After the seed samples were ground, 15 gr samples were prepared from each sample and, the fixed oil was obtained by extraction with petroleum ether for 6 hours in the Soxhlet apparatus.

Fixed oil components (%): 0.1 g of oil was taken from the extracted oil samples, 1 ml of 2 N methanolic KOH solution was added, vortexed for 2 minutes, and waited for 15 minutes. Then, 10 ml of hexane was added to it, mixed thoroughly, and centrifuged at 7000 rpm for 10 minutes for phase separation, and 1 microliter of the upper phase was injected into the Shimadzu brand GC-FID device.

Nutrient elements (mg kg^{-1}): Necessary preparations were made using ground seed samples and measurements were made in the ICP-OES (Optima 2100 DV: Perkin Elmer Inc.) device in the ÜSKİM laboratory.

Statistical Analyses

The results obtained from the properties of plant nutrients were analyzed using the SAS 9.1 package program according to the split-parcel trial design. Differences found to be significant were subjected to the LSD multiple comparison test.

Results and Discussion

In this study, the effects of phosphorus dose-increasing applications on fixed oil components and plant nutrients in two different black cumin genotypes were investigated.

Plant nutritional elements (mg kg^{-1})

When we look at the data regarding the nutritional elements in Tables 3 and 4, it is seen that genotype, dose, and genotype x dose interaction is significant at the $p<0.01$ level in all macro-micro elements. It was found in terms of genotypes that genotype 2 had a higher value than genotype 1 in all elements except zinc (Table 4). When we look at the nutritional elements in terms of doses, the lowest values for Fe, Ca, Na, and Mn were obtained from the 9 kg da^{-1} P dose, and the

lowest value was obtained from the 0 kg da^{-1} dose for the other elements studied. It was found that Fe, Ca, and Mn reached the highest value at the 3 kg da^{-1} application; P and Zn at the 9 kg da^{-1} application; and Mg, K, Na, N at the 12 kg da^{-1} application. In terms of genotype x dose interaction, the highest values of Mg, P, and Na elements were obtained from the 12 kg da^{-1} application of genotype 1; the highest value of Fe, Ca, and Mn from the 3 kg da^{-1} application of genotype 2 and the highest value of N from 6 kg da^{-1} of genotype 2 (Fig. 1F-1N). Vatansev et al. (2013) determined Fe as 117.32 □g g^{-1}, Zn as 41.42 □g g^{-1}, and Mn as 28.56 □g g^{-1}, which were lower than the values in this study. Mamun and Absar (2018) reported Fe as 41.80 mg%, Ca as 579.33 mg%, Mg as 218.33 mg%, P as 91.5 mg%, Na as 100 mg%, and K as 510.30 mg%. Studying the nigella varieties obtained from five different sources, Takruri and Dameh (1998) obtained the highest Fe ratio (91-130 mg kg^{-1}) from the Turkey source, the highest Na ratio (419-550 mg kg^{-1}) from the Indian source, the highest K ratio (4423-5606 mg kg^{-1}) from Syria 1 source, the highest Ca ratio (1544-2005 mg kg^{-1}) from Syria 1 source, the highest Zn ratio (56-66 mg kg^{-1}) from Syria 2 source, the highest P ratio (5023-5769 mg kg^{-1}) from Syria 1 source.

Table 3. Two-year averages and LSD groupings of applied phosphorus doses in terms of properties examined in black cumin

		Fe(mg/kg)		Ca(mg/kg)		Mg(mg/kg)		P(mg/kg)	
Genotype	G1	317.84	b	5788.70	b	2550.70	b	4575.20	b
	G2	367.88	a	6533.20	a	2646.36	a	4721.10	a
Doses (kg/da)	0	322.71	d	6109.92	d	2475.50	e	4306.83	e
	3	380.03	a	6538.00	a	2581.50	c	4346.16	d
	6	377.27	b	6167.75	c	2538.58	d	4420.41	c
	9	296.66	e	5544.58	e	2629.91	b	5161.08	a
	12	337.62	c	6444.50	b	2767.16	a	5006.25	b
Year	2018	390.93	a	6933.90	a	2657.33	a	4868.80	a
	2020	294.79	b	5388.00	b	2539.73	b	4427.50	b
Genotype x Doses (GxD)	G_1 x P_0	391.55	b	5839.17	f	2302.67	f	3701.83	h
	G_1 x P_3	222.43	i	5809.33	f	2472.50	e	4679.50	e
	G_1 x P_6	395.05	b	5552.50	g	2422.50	e	4205.17	f
	G_1 x P_9	273.45	g	5522.67	g	2660.17	bc	5105.33	b
	G_1 x P_{12}	306.73	f	6219.83	e	2895.67	a	5184.17	a
	G_2 x P_0	253.88	h	6380.67	d	2648.33	bcd	4911.83	c
	G_2 x P_3	537.65	a	7266.67	a	2690.50	b	4012.83	g
	G_2 x P_6	359.51	d	6783.00	b	2654.67	bc	4635.67	e
	G_2 x P_9	319.88	e	5566.50	g	2599.67	d	5216.83	a
	G_2 x P_{12}	368.53	c	6669.17	c	2638.67	cd	4828.33	d
Mean		324.86		6160.95		2598.53		4648.15	
CV		0.71		0.74		0.80		0.45	
LSD for genotype		1.29**		24.03**		10.97**		11.09**	
LSD for doses		2.04**		37.99**		17.35**		17.54**	
LSD for G x D		9.65**		179.41**		81.94**		82.82**	

Table 4. Two-year averages and LSD groupings of applied phosphorus doses in terms of plant nutrients in black cumin

		K(mg/kg)		Zn(mg/kg)		Mn(mg/kg)		Na(mg/kg)		N(%)	
Genotype	G1	8291.43	b	65.09	a	49.11	b	78.91	b	3.44	b
	G2	8868.07	a	63.54	b	51.89	a	90.55	a	3.67	a
Doses (kg/da)	0	8127.25	e	61.47	e	49.51	d	80.93	d	3.47	d
	3	8455.50	d	64.33	b	57.90	a	84.68	c	3.51	dc
	6	8579.83	c	61.91	d	51.39	b	87.58	b	3.60	b
	9	8643.92	b	69.96	a	43.26	e	78.41	e	3.55	c
	12	9092.25	a	63.91	c	50.43	c	92.06	a	3.65	a
Year	2018	9052.70	a	58.36	b	50.12	b	103.20	a	3.58	a
	2020	8106.80	b	70.27	a	50.88	a	66.27	b	3.53	b
Genotype x Doses (GxD)	G_1 x P_0	7347.33	g	57.43	h	54.61	b	73.35	f	3.36	de
	G_1 x P_3	8379.00	e	67.37	c	49.46	f	75.17	f	3.38	de
	G_1 x P_6	8215.50	f	60.34	f	50.90	e	78.90	e	3.34	e
	G_1 x P_9	8539.17	d	71.62	a	42.64	i	68.15	g	3.50	cd
	G_1 x P_{12}	8976.17	b	68.73	b	47.96	g	99.02	a	3.65	b
	G_2 x P_0	8907.17	bc	65.52	d	44.43	h	88.52	cd	3.60	bc
	G_2 x P_3	8532.00	de	61.30	f	66.35	a	94.19	b	3.65	b
	G_2 x P_6	8944.17	b	63.50	e	51.89	d	96.27	b	3.87	a
	G_2 x P_9	8748.67	c	68.31	bc	43.90	h	88.68	c	3.62	b
	G_2 x P_{12}	9208.33	a	59.10	g	52.92	c	85.12	d	3.65	b
Mean		8579.75		64.32		50.50		84.73		3.56	
CV		0.73		0.70		0.63		1.39		1.45	
LSD for genotype		33.11**		0.23**		0.16**		0.62**		0.02**	
LSD for doses		52.35**		0.37**		0.26**		0.98**		0.04**	
LSD for G x D		247.22**		1.77**		1.26**		4.62**		0.20**	

**P≤0.01

In a study conducted by Al-Naqeep et al (2009) on three different samples in Yemen, it was reported that calcium values of 544.00 mg 100g^{-1}, 755.00 mg 100g^{-1}, 811.00 mg 100g^{-1} were obtained from Marib, Sadah, Taiz samples respectively, potassium values of 447.30 mg 100g^{-1}, 476.70 mg 100g^{-1}, 563.00 mg 100g^{-1} were obtained in the same respective order, and *Nigella* seeds were rich in potassium. They reported that the phosphorus element had values of 65.00 mg 100g^{-1}, 54.20 mg 100g^{-1}, and 77.40 mg 100g^{-1} and it was different from the data in the literature. The values of zinc 1.84 mg 100g^{-1}, 1.90 mg 100g^{-1}, 2.50 mg 100g^{-1}, sodium 44.00 mg 100g^{-1}, 73.00 mg 100g^{-1}, 80.70 mg 100g^{-1}, magnesium 219.00 mg 100g^{-1}, 260.00 mg 100g^{-1} , 234.00 mg 100g^{-1}, iron 8.60 mg 100g^{-1}, 10.70 mg 100g^{-1}, 56.60 mg 100g^{-1} were obtained from the samples. Kabir et al. (2019) found the values as potassium 1498.3 mg 100g^{-1}, phosphorus 481.5 mg 100g^{-1}, sodium 44.8 mg 100g^{-1}, calcium 366.7 mg 100g^{-1}, magnesium 355.2 mg 100g^{-1}, zinc 6.7 mg 100g^{-1}, iron 42.6 mg 100g^{-1}, manganese 3.1 mg 100g^{-1}. Uras et al. (2010) determined the P-value as 5284 mg g^{-1}, the K value as 42.18 mg g^{-1}, the Ca value as 4214 mg g^{-1}, the Mg value as 1387 mg g^{-1}, the Na value as 367.40 mg g^{-1}, the Mn value as 25.83 mg g^{-1}, the Fe value as 77.37 mg g^{-1}, and the Zn value as 78.79 mg g^{-1}. They stated that three macro-minerals, namely phosphorus, potassium, and calcium were relatively high in seeds, *N.sativa* seeds contained relatively high P, K, Ca, and Fe, these elements played an important role in the protection of human health, and the seeds would be a good alternative source of these mineral elements as nutritional supplements (Uras et al., 2010). Similarly, the three macro minerals mentioned were also found to be high in this study.

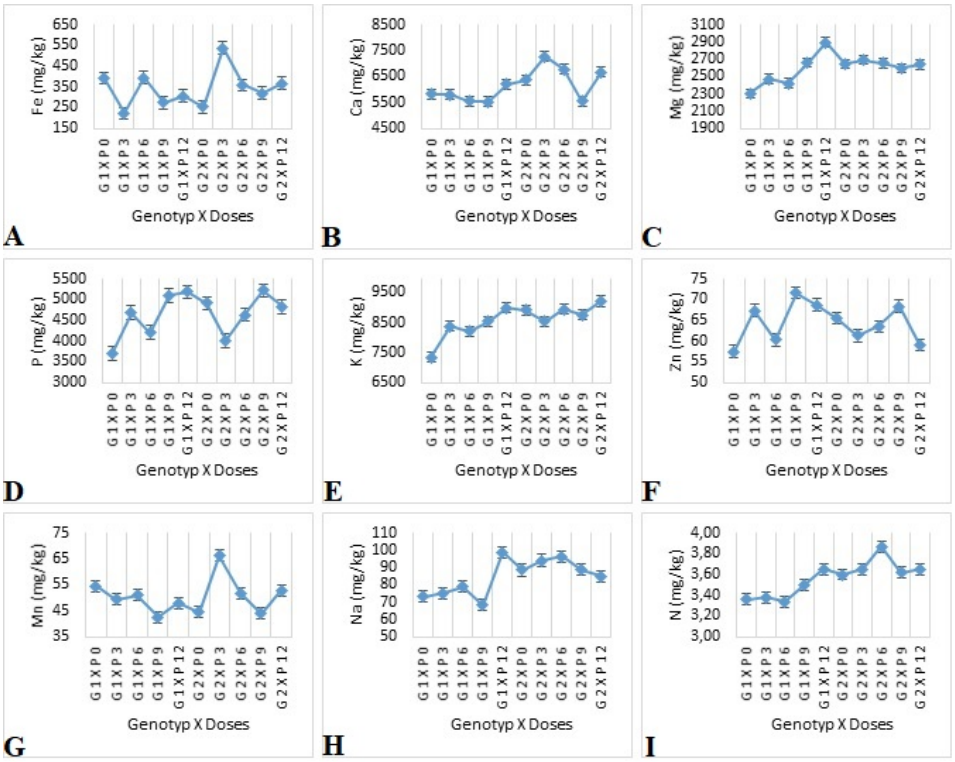

Figure 1. Two-year average values of genotype x dose interaction, which is statistically significant in the investigated characteristics

Fixed oil component (%)

When looking at the fixed fatty acids of black cumin genotypes grown in increasing phosphorus applications; In both genotypes, the rate of unsaturated fat is approximately 4.5 times higher than the rate of saturated fat. When the ratios of saturated and unsaturated fatty acids are examined in terms of doses, it is seen that the values in all doses are close to each other. A total of 19 fixed fatty acid components were determined in both genotypes. When the percentage ratios of the components are examined, it is seen that the main fatty acid is linoleic acid and while it is 57.77% in genotype 1, it is 57.95% in genotype 2. Oleic acid is in the second place, while it is 23.46% in genotype

1 and 23.50% in genotype 2. While palmitic acid is 11.74% in genotype 1, it is 11.55% in genotype 2. The highest value in saturated fatty acids was obtained in 9 kg da^{-1} P application in genotype 1, and 6 kg da^{-1} P application in genotype 2. The highest value in unsaturated fatty acids was obtained from 6 kg da^{-1} P application in genotype 1, and 9 kg da^{-1} P application in genotype 2 (Table 5,6). Since the genotypes are composed of the same species (*N. sativa*), the fatty acid components and ratios are very close to each other.

Table 5. Two-year average values of the main fixed oil components of genotype 1 (*N. sativa*) applied different phosphorus doses

			Phosphorus doses (kg da^{-1})					
No	**RT**	**FAMEs***	**0**	**3**	**6**	**9**	**12**	**Mean**
1	5.11	Caproic Acid	0.36	0.13	0.46	0.51	0.10	0.31
2	17.54	Myristic Acid	0.13	0.14	0.14	0.13	0.14	0.14
3	19.60	Myristoleic Acid	0.02	0.01	0.02	0.02	0.01	0.01
4	21.75	Palmitic Acid	11.66	11.84	11.76	11.73	11.70	11.74
5	22.98	Palmiteloic Acid	0.23	0.22	0.23	0.26	0.19	0.22
6	25.07	Heptadecanoic Acid	0.05	0.04	0.04	0.04	0.05	0.04
7	26.18	Stearic Acid	2.52	2.62	2.61	2.53	2.42	2.54
8	27.20	Oleic Acid	23.31	23.86	23.57	23.23	23.33	23.46
9	28.54	Linolelaidic Acid	0.09	0.07	0.09	0.09	0.07	0.08
10	28.99	Linoleic Acid	57.89	57.56	57.41	57.72	58.26	57.77
11	30.48	Gamma-Linolenic Acid	0.39	0.10	0.08	0.07	0.08	0.14
12	31.00	Alfa-Linolenic Acid	0.21	0.19	0.21	0.21	0.23	0.21
13	31.33	Arachidic Acid	0.29	0.29	0.29	0.29	0.29	0.29
14	32.96	Heneicosanoic Acid	2.98	2.83	2.87	3.00	3.02	2.94
15	35.44	Cis-11,14,17-Eicosatrienoic Acid	0.20	0.00	0.02	0.03	0.00	0.05
16	36.14	Arachidonic Acid	0.02	0.00	0.02	0.02	0.00	0.01
17	37.08	Tricosanoic Acid	0.03	0.03	0.02	0.02	0.03	0.03
18	41.09	Nervonic Acid	0.02	0.01	0.02	0.01	0.03	0.02
19	43.37	Cis-4,7,10,13,16,19-Docosahexaenoic Acid	0.14	0.08	0.17	0.13	0.06	0.11
Saturated fatty acids %			18.02	17.92	18.19	18.25	17.75	18.03
Unsaturated fatty acids %			82.5	82.1	81.84	81.79	82.26	82.08
Total %			100	99.98	100	100	99.98	

*FAMEs: Faty acid methyl esters

In addition, it is seen that there is no significant difference in the amount of fixed oil components with increasing phosphorus doses. Similarly, Kizil et al. (2008) reported that there was no significant change in fatty acid components in terms of P application and linoleic, palmitic, and oleic acid were the main fatty acids, and the highest linoleic acid was obtained from 0 kg da^{-1} P and 160 kg da^{-1} P application in winter planting. Uras et al. (2010) reported that from saturated fatty acids palmitic acid is 14.1% and stearic acid is 2.6%, from unsaturated fatty acids oleic acid is 20% and linoleic acid is 51.8%. According to Telci et al. (2014), palmitic acid 12.5%, linoleic acid 57.0%, oleic acid 22.8%, heneicosanoic acid 3.3%. In this study, heneicosanoic acid was obtained as 3.02% from 12 kg da^{-1} P application and the data are compatible with the studies in the literature.

Table 6. Two-year average values of the main fixed oil components of genotype 2 (Çameli variety) applied different phosphorus doses

			Phosphorus doses (kg da^{-1})					
No	**RT**	**FAMEs***	**0**	**3**	**6**	**9**	**12**	**Mean**
1	5.11	Caproic Acid	0.13	0.76	0.55	0.19	0.54	0.43
2	17.54	Myristic Acid	0.19	0.16	0.16	0.19	0.17	0.17
3	19.60	Myristoleic Acid	0.01	0.02	0.01	0.01	0.02	0.01
4	21.75	Palmitic Acid	11.60	11.54	11.56	11.52	11.54	11.55
5	22.98	Palmiteloic Acid	0.23	0.28	0.27	0.25	0.28	0.26
6	25.07	Heptadecanoic Acid	0.05	0.04	0.05	0.04	0.05	0.05
7	26.18	Stearic Acid	2.33	2.38	2.52	2.33	2.44	2.40
8	27.20	Oleic Acid	23.85	22.62	23.84	23.73	23.48	23.50
9	28.54	Linolelaidic Acid	0.10	0.11	0.09	0.07	0.10	0.09
10	28.99	Linoleic Acid	58.01	58.27	57.40	58.35	57.72	57.95
11	30.48	Gamma-Linolenic Acid	0.05	0.07	0.07	0.10	0.08	0.07
12	31.00	Alfa-Linolenic Acid	0.20	0.20	0.19	0.19	0.20	0.19
13	31.33	Arachidic Acid	0.28	0.33	0.29	0.28	0.28	0.29
14	32.96	Heneicosanoic Acid	2.87	3.01	2.85	2.77	2.91	2.88
15	35.44	Cis-11,14,17-Eicosatrienoic Acid	0.00	0.03	0.02	0.00	0.02	0.01
16	36.14	Arachidonic Acid	0.00	0.03	0.03	0.00	0.03	0.02
17	37.08	Tricosanoic Acid	0.03	0.03	0.02	0.04	0.03	0.03
18	41.09	Nervonic Acid	0.02	0.01	0.02	0.02	0.01	0.02
19	43.37	Cis-4,7,10,13,16,19-Docosahexaenoic Acid	0.07	0.14	0.13	0.08	0.15	0.11
Saturated fatty acids %			17.48	18.25	18.00	17.36	17.96	17.80
Unsaturated fatty acids %			82.54	81.78	82.07	82.80	82.09	82.23
Total %			99.99	99.99	100	100	99.99	

*FAMEs: Faty acid methyl esters

Conclusion

This study was carried out to determine the effect of increasing phosphorus doses in black cumin on fixed oil components and plant nutrients. The nutritional elements, Fe, Ca, and Mn had the highest value at the 3 kg da^{-1} P dose, P and Zn at the 9 kg da^{-1} P dose, Mg, K, Na, and N at the 12 kg da^{-1} P application. Accordingly, nutritional elements were affected at different levels by the increasing doses of phosphorus. It was observed in the applied phosphorus doses that the main fatty acids were the unsaturated fatty acids of linoleic and oleic acids, and that the main unsaturated fatty acid was palmitic acid. There was no significant difference between increasing phosphorus doses in terms of fixed fatty acid components. It was observed that the genotypes had similar ratios and characteristics in terms of fixed oil components.

Acknowledgments

This research (Project No: 2018/1-11 M) was supported by KSU Scientific Research Projects Unit.

Statement of Conflict of Interest

The author(s) declare no conflict of interest for this study.

Author's Contributions

The author confirms sole responsibility for the following: study conception and design, data collection, analysis and interpretation of results, and manuscript preparation.

References

Ahmad, A., Husain, A., Mujeeb, M., Khan, S.A., Najmi, A.K., Siddique, N.A., Damanhouri, Z.A. Anwar, F. (2013). A review on therapeutic potential of *Nigella sativa*: A miracle herb. Asian Pac J Trop Biomed. 3 (5): 337-352.

Akgül, A. (1993). Spice Science Technology, Food Technology Association Publication No:15, Damla Printing, Ankara, Pages: 72-75.

Al-Naqeep, G.N., Ismail, M.M., Al-Zubairi, A.S. Esa, N.M. (2009). Nutrients

Composition and content of three different samples of *Nigella sativa* L. cultivated in Yemen. Asian Journal of Biological Sciences. 2 (2): 43-48.
Anonymous. (2016). Principles of Fertilization Ministry of National Education, Ankara. Page: 74.
Anonymous. (2020a). Climate data for 2017-18 and 2019-2020 and long years measured at Kahramanmaraş Province Meteorology Station.
Anonymous. (2020b). Kahramanmaras Sütcü Imam University. USKIM laboratory soil analysis results.
Ayhan, B. (2012). Phytotherapeutic studies on *Nigella sativa* L. Plant. Gazi University, Department of Pharmacognosy, Master's Thesis. Page: 153.
Baser, K.H.C. (2010). Black cumin (*Nigella sativa*). Bagbahce Journal. 32(3):26-27.
Baydar, H. (2013). The Science and Technology of Medicinal and Aromatic Plants (Extended 4th Edition). Süleyman Demirel University, Publication No: 51. Page: 339.
Bayram, E., Kırıcı, S., Tansı, S., Yılmaz, G., Arabacı, O., Kızıl, S. Telci, I. (2010). Possibilities of increasing the production of medicinal and aromatic plants. VII. Agricultural Engineering Technical Congress Book. Pages:1-21.
Ceylan, A. (1997). Medicinal plants-II. Ege University Faculty of Agriculture Publications, No:481. Page: 306.
Ertaş, M.E. (2016). Determination of agronomic and quality characteristics of black cumin (*Nigella* sp.) genotypes planted in winter and summer in Tokat Kazova ecological conditions. Gaziosmanpaşa University, Department of Field Crops, Master Thesis, Page: 62.
Kabir, Y., Shirakawa, H. Komai, M. (2019). Nutritional composition of the indigenous cultivar of black cumin seeds from Bangladesh. Progress in Nutrition, 21(Supplement 1): 428-434.
Kacar, B. (2015). General Plant Physiology. 1st edition; Nobel Academic Publishing Training Consultancy Trade. Ltd. Sti. Turkey.
Kaya, A.R., Eryigit, T., Aysabar, Z. (2023). All aspects of Black Cumin (*Nigella* sp.) and Related Studies. IKSAD Publishing House. 74p, ISBN: 978-625-367-132-7.
Kizil, S., Kirici, S., Cakmak, O. Khawar, K.M. (2008). Effects of sowing periods and P application rates on yield and oil composition of black cumin (*Nigella sativa* L.). Journal of Food, Agriculture & Environment. 6 (2): 242-246.
Küçükemre, D. (2009). Effects of different row spacing and sowing norm on yield and quality of black cumin (*Nigella sativa* L.), Gaziosmanpaşa University, Department of Field Crops, Master Thesis, Page: 67.
Ozguven, M. (2005). Black Cumin *Nigella sativa* L. Research on the Place and Composition in Traditional Medicine. Journal of Nature & Health, 5(3):10-13.
Takruri, H.R.H. Dameh, M.A.F. (1998). Study of the nutritional value of black cumin seeds (*Nigella sativa* L.). J Sci Food Agric. 76: 404-410.
Telci, I. (1995). The effect of different planting density on yield, yield components and some vegetative properties of black cumin (*N. sativa* L.) in Tokat conditions. Gaziosmanpaşa University, Department of Field Crops, Master's Thesis, Page: 43.
Telci, İ., Yaglioglu, A.S., Eser, F., Aksit, H., Demirtas, I. Tekin. S. (2014). Comparison of seed oil composition of *Nigella sativa* L. and *Nigella damascena* L. during seed maturation stages. Journal of the American Oil Chemists' Society. 91: 1723-1729.
Turan, Y.S. (2014). The effect of phosphorus doses on the yield and quality of black cumin (*Nigella sativa* L.). Eskişehir Osmangazi University, Department of Field Crops, Master Thesis, Page: 76.
Uras S.S., Silahtaroğlu, S., Ilcim, A. Kökdil, G. (2010). Fatty acid, tocopherol, mineral composition, total phenolic, flavonoid, thymoquinone content and antioxidant activity of *Nigella sativa* L.. J.Fac. Pharm, Ankara, 39(3): 173-186.
Urusan, Z. (2016). Determination of agricultural and quality characteristics of some black cumin (*Nigella sativa*, *Nigella damascena*) genotypes. Atatürk University, Department of Field Crops, Master Thesis, Page: 53.
Vatansev, H., Ciftci, H., Ozkaya, A., Ozturk, B., Evliyaoglu, N. Kiyici, A. (2013). Chemical composition of *Nigella sativa* L. seeds used as a medical aromatic plant from East Anatolia Region, Turkey. Asian J. Chem., 25(10):5490-5492.

IJAFLS
e-ISSN: 2602-4381
International Journal of Agriculture, Forestry and Life Sciences
Original Article | Open access | Int J Agric For Life Sci (2023) 7(2): 52-55

Determining the Antimicrobial Activities of the Essential Oils of Some Taxa Used as Thyme

Neslihan Taşar[1]* **Ferit Can Yazdıc[2]** **Altuğ Karaman[3]** **Osman Gedik[4]**

[1]Munzur University, Tunceli Vocational School, Organic Agriculture Department, Tunceli, Türkiye
[2]Department of Biotechnology, Institue of Graduate Studies in Science, Munzur University, Tunceli, Türkiye
[3]Munzur University, Tunceli Vocational School, Department of Medical Documentation and Secretarial, Tunceli, Türkiye
[4]Kahramanmaraş Sütçü İmam University, Faculty of Agriculture, Department of Field Crops, Kahramanmaraş, Türkiye
***Correspondence:** ntasar@munzur.edu.tr

Citation
Taşar, N., Yazdıc. F.C., Kahraman, A., Gedik, O. (2023). Determining the Antimicrobial Activities of the Essential Oils of Some Taxa Used as Thyme. Int J Agric For Life Sci (2023) 7(2): 52-55.

Received	:28 September 2023
Accepted	:4 December 2023
Published Online	:22 December 2023
Year:	:2023
Volume	:7
Issue	:2 (December)
Pages	:52-55

Available online https://dergipark.org.tr/ijafls

Abstract
Many aromatic plant species that belong to the Lamiaceae family are defined as “thyme” in Turkey, and the species that contain “carvacrol” and “thymol” in the essential oil of these plants are accepted as “thyme”. Among them, Origanum, Satureja, and Thymbra genera have great importance in terms of distribution and economics. The present study was conducted to determine in vitro the antimicrobial effects of the essential oils obtained from Thymbra spicata L., Satureja cuneifolia Ten., Satureja hortensis L., Origanum onites L. and Origanum majorana L. species through hydroxylation method on the pathogenic bacteria of *Salmonella enteritidis* (ATCC 13075), *Enterococcus faecalis* (ATCC 29212), *Enterobacter aerogenes* (ATCC 13048), *Staphylococcus aureus* subsp. aureus (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Serratia marcescens* (ATCC 13880), which are resistant to many drugs. The essential oils that constituted the study material were extracted in the Neo-Clevenger device and their antimicrobial activity against gram-negative and gram-positive bacteria was investigated by using the Standard Agar Well Diffusion Test Method. It was found that the *T. spicata, S. cuneifolia, S. hortensis, O. onites,* and *O. majorana* species had antibacterial activity varying between 8.43±1.00-26.53±2.50 mm against six test strains. The fact that T. spicata species had higher antibacterial activity against all pathogens compared to standard antibiotics is important in terms of using and culturing this species and is the most striking finding of the present study.

Key words
Antimicrobial activity, *Thyme*, *Origanum*, *Satureja, Thymbra*.

Introduction
Approximately 3.000 (1/3) of the species that were registered in the flora of Turkey consist of medicinal and aromatic plants (Tan 2017). The Lamiaceae family, which includes the thyme plant, is among the most widespread plant families worldwide and contains 3.000 species of approximately 200 genera (Heywood 1978). It is the 6th largest family in our country with 45 genera and the 3rd largest family with 550 species in terms of species (Dönmez et al. 2011). Members of the mint family Lamiaceae are highly favored for the treatment of human and animal diseases due to their essential oil content (Güler et al., 2021; Babacan et al., 2022; Selvi et al., 2022). The fact that the Lamiaceae family is the source of many essential oils employed in medicine and perfumery and that it is employed for both treatment and spice shows the importance of this family. Thyme, which is among the most important plants of the Lamiaceae family, has five genera in Turkey (*Thymus, Origanum, Satureja, Thymbra,* and *Coridothymus*) (Baser et al. 1994, Davis 1982). There are 220 species of the genus Thymus on Earth and 39 species (58 taxa) in Turkey. There are 43 species of Origanum, of which 23 species (27 taxa) are found in Turkey. The genus Satureja contains 30 species, 13 of which (14 taxa) are found in Turkey. There are about 12 species of the genus Thymbra in Turkey, 2 species (4 taxa). There is only one species in the genus Coridothymus, which is also found in Turkey. In Turkey, 44.2% of Lamiaceae species, 65.2% of Origanum species, 52.6% of Thymus species, and 28% of Medicago species are endemic. This information indicates how abundant turkey is in these genera and that it is their genetic center (Davis 1988, Biskup and Saez 2002, Kintzios 2002).

Our country holds 70% of the world’s thyme market with its rich thyme reserves, and produces approximately 10.000 tons of annual production, providing the economy of the country with an income of approximately 21 million dollars.

O. onites (Izmir thyme, earth thyme, Turkish thyme) is known in Europe as "Turkish oregano" and is widely cultivated along the Aegean and Western Mediterranean coasts (Balikesir, Izmir, Ay Den, Mugla, Antalya) (up to 1400 meters above sea level). It is cultivated in the Aegean region. This variety accounts for the largest share (about 80%) of my country's thyme exports. The average plant height can reach 100 cm. Essential oil yields range from 2% to 5%. It is thought to be the chemotype that grows in this region (Baytop 1991).

O. Majorana (sweet marjoram, Alanian thyme, marjoram, sweet thyme, white thyme) is commonly found in dry grasslands, rocks and dry forests in the western regions of our country (Thrace, Marmara, Aegean and Mediterranean). The plant is 20-80 cm tall and is a perennial plant with pink or white flowers. It blooms from July to September. It grows in the Aegean region and is used for essential oils in gardens. Its essential oil is rich in terpinen-4-ol, trans-sabinete hydrate, cis-sabinete hydrate and linalool. The carvacrol content is also quite high (78-80%). European-grown samples, known as marjoram, do not contain carvacrol (Skoula et al. 2005).

T. spicata (Karakekik, Karabaşkekik, Sivrikekik) is a common species in Thrace, the Mediterranean coast, the Aegean Sea, and western and southeastern Anatolia. Used as a medicine, with a high proportion of carvacrol for its antiseptic effect, and also in spices and teas. Carvacrol content is 50-71%. The plant height is 24-43 cm, and the essential oil proportion is between 1-3.4%.

Essential oils can be obtained from flowers, roots, bark, leaves, seeds, fruits, and various parts of plants (Khorshidian et al., 2018). Essential oils are defined as “products obtained from the peels of citrus fruits by mechanical processing, steam distillation or dry distillation after separation of the aqueous phase from natural raw materials of plant origin” (ISO/DIS9235, 2013). Today, approximately 3,000 different essential oils are used commercially as condiments and fragrances (Burt 2004). The composition of essential oils varies depending on the plant species, geographical origin, climatic conditions, soil composition, phase of the vegetation cycle, and the plant parts used to extract the essential oil (Masotti et al. 2003). In general, they play an important role in defense mechanisms against bacteria and fungi and are excreted as secondary metabolites (Tajkarimi et al. 2010). The antimicrobial activity of essential oils is related to their active components (Hyldgaard et al., 2012). Their antimicrobial properties and composition (Nychas, 1995) and their mechanisms of action have been extensively studied (Lambert et al. 2001). An important property of essential oils is their hydrophobicity, which allows them to bind to the lipids of bacterial cell membranes, disrupting their structure and making them more permeable (Sikkemat et al. 2010) and this results in the escape of ions and other cellular molecules (Gustafson 1998, Cox 2000, Carson and Riley 1993, Ultee et al. 2002). Approximately 90-95% of all essential oils consist of volatile components and fatty aldehydes, alcohols and esters as well as monoterpene and sesquiterpene hydrocarbons and their oxygenated derivatives. The remaining 5-10% of the main components are hydrocarbons, fatty acids, sterols, carotenoids, waxes, coumarins and flavonoids (Luque De Castro et al. 1999). The most active antimicrobial compounds in essential oils are divided into four classes based on their chemical structures: terpenes (e.g., paracymene, limonene), terpenoids (e.g., thymol, carvacrol), phenylpropenes (e.g., eugenol, vanillin) and other compounds such as allicin or isothiocyanates (Hyldgaard et al., 2012). The chemical structure of an essential oil also affects its antimicrobial activity (Knobloch et al., 1986). Previous research has shown that using the entire essential oil has a greater effect than using the primary ingredients together

(Burt, 2004), suggesting that secondary ingredients are critical to effectiveness and may have synergistic effects (Maruyama et al., 2005). Antagonism can be observed with essential oils, namely H. The effect of multiple compounds may be weaker than when used individually (Gill et al. 2002).

In addition to the therapeutic properties of essential oils, their antimicrobial effects have been known since the early 19th century and they have been used as disinfectants. The thyme plant, which is the subject of the present study, has a wide area of use in this context with its antimicrobial, antiseptic, anthelmintic, cardiovascular, and stimulant characteristics (Cingi et al. 1991). The purpose of this study was to compare the effects of these plants, which are important for their antimicrobial properties. The antibacterial effects of the extracted essential oils on various pathogenic bacteria were studied.

Material and methods

Supply of plant material

The localities of the plant materials that were employed to obtain the essential oils that were the subject of the study are given below.

T. spicata was obtained from natural growing areas in Kahramanmaras Menzelet Dam Lake. 37°39'2.85"K, 36°48'9.96"D, 529m.

S. cuneifolia was obtained from the natural growing area around the Hamamgozu area of Kahramanmaras Goksun District. 37°52'38.10"K, 36°23'30.59"D, 1530m.

S. hortensis Malatya, Kapidere Kumlu road surroundings. 37°56'31.94"K, 37°42'5.10"D, 1055m.

O. onites was obtained from Kahramanmaras Sutçu Imam University Field Crops Department. 37°35'37.25"K, 36°48'48.96"D, 489m.

O. majorana was obtained from Kahramanmaras Sutçu Imam University Field Crops Department. 37°35'37.25"K, 36°48'48.96"D, 489m.

Essential oil extraction

The extraction process of essential oils was carried out in the laboratory of the Department of Crops, Medicinal and Aromatic Plants, Faculty of Agriculture, Kahramanmaras Sutçu Imam University. The thyme varieties in the study samples were collected in 2018. Essential oils extracted from the above-ground parts of the plant during flowering are inspected to be pure (undiluted). The dried aerial parts of the thyme species employed in the study were completely ground and Their essential oils are extracted using hydrodistillation in a Neo-Clevenger unit for three hours. To obtain essential oil, 500 ml distilled water was added to 50 grams of ground plant material and subjected to the hydro distillation process. The resulting essential oils were stored in the refrigerator at -18ºC until the working setting was established.

Microorganisms employed

The bacterial species used in the study were selected from American Type Culture Collection (ATCC) quality control strains known for their sensitivity and recommended for use by the Clinical and Laboratory Standards Institute (CLSI). In this context, Enterobacter aerogenes (ATCC 13048), Enterococcus faecalis (ATCC 29212), Salmonella Enteritidis (ATCC 13075), and Staphylococcus aureus subsp. Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 25922), and Serratia marcescens (ATCC 13880) were used in this study.

Agar well diffusion test method

The agar well diffusion test method has been used to determine the antimicrobial activity of essential oil extraction products (Russo et al., 2012) as a widely used method to evaluate the antimicrobial activity of plant or microbial extracts (Magaldi et al., 2004; Vargas et al., 2007). Muller Hinton agar (MHA) was employed for bacterial strains. Briefly, 100 µL of the pathogen sample in the logarithmic growth phase was added to 20 mL of MHA medium and poured into the Petri dish. Use a sterile cork drill (6 mm diameter) to make a hole in the MHA Petri dish and add 10 µL Necessary addition of fatty acids to each well. 10 µL of the standard antibiotic kanamycin (30 µg/mL) was also used as a positive control to determine the susceptibility of the tested microorganisms. Petri dishes were incubated overnight at 37°C, and if significant inhibition was observed, it was considered to indicate the presence of antimicrobial activity. Testing was performed in triplicate. Antimicrobial activity was assessed by measuring the diameter of the area surrounding each hole (Balouiri et al., 2016).

Statistical calculations

Statistical analysis was performed using GraphPad Prism 8 software, and all tests were performed in triplicate. Results are expressed as mean ± standard error of the mean and compared using analysis of variance (ANOVA) and least significant difference (LSD) at $p \leq 0.05$ and $p \leq 0.01$ (Steel et al., 1997).

Results

The most researched aspect of essential oils relates to their antimicrobial effects. Since these oils are complex mixtures with different components, their degree of effectiveness varies depending on the type and amount of active ingredients (Toroğlu et al., 2006). Although the information on the mechanisms of action is limited, it is suggested that this is related to the lipophilic characteristics and chemical structures of the oils (Farag et al., 1989). The essential oils that were obtained from 5 different thyme species (*T. spicata*, *S. cuneifolia*, *S. hortensis*, *O. onites*, and *O. majorana*) were tested against 6 different pathogenic microorganisms. The antibacterial activity results of the plant essential oils used in the study are shown in Table 1. The essential oils used in the study were found to exhibit broad antimicrobial activity against both gram-negative and gram-positive bacteria. *T. spicata* showed the highest antibacterial activity against all the pathogens compared to the other essential oils and standard antibiotics.

T. spicata and O. onites have strong antibacterial activity against Gram-positive bacteria, and S. cuneifolia, S. hortensis and O. onites have strong antibacterial activity against Gram-negative bacteria.

A heat map model was employed to understand the relationship between essential oils and antimicrobial activities better. In this method, the effect values of the plant species employed against bacteria are shown by the changes in blue and white. The intensity in the blue hue indicates high antibacterial activity, the white color indicates no activity, and the intermediate values indicate the corresponding gradient between the two colors (Figure 1).

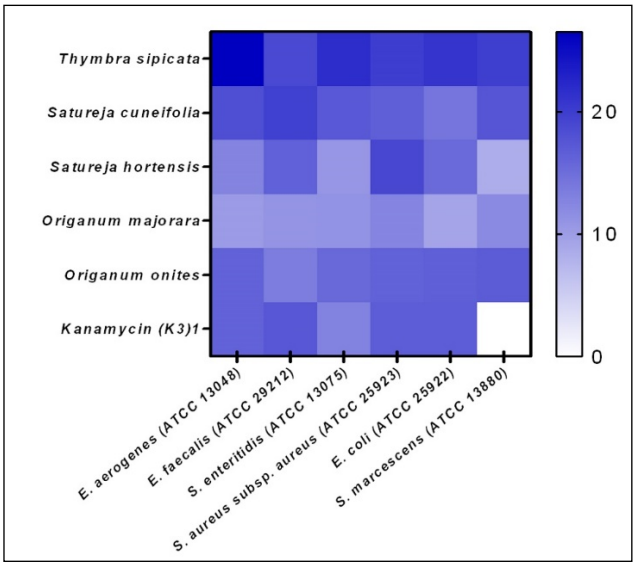

Figure 1. The comparison of antibacterial activity of essential fatty acids obtained from *T. spicata*, *S. cuneifolia*, *S. hortensis*, *O. onites*, and *O. majorana* plants and inhibition zones between pathogenic microorganisms (mm±SD).

Table 1. Data are expressed as mean ± standard deviation (SD) of three independent samples. NIZ stands for No Inhibition Zone.. 1: Kanamycin (K30), the largest zone diameter.

	Essential Fatty Acids					Standard Antibiotics
Microorganisms	***Thymbra spicata***	***Satureja cuneifolia***	***Satureja hortensis***	***Origanum majorana***	***Origanum onites***	**Kanamycin (K30)[1]**
***E. aerogenes* (ATCC 13048)**	26.53±2.50[a]**	18.30±1.42*	12.70±1.01	10.30±0.52	16.13±0.81	16.20±0.90
***E. faecalis* (ATCC 29212)**	18.93±1.90*	19.73±1.33[a]*	16.37±1.05	11.10±1.20	13.50±0.62	17.37±0.31
***S. enteritidis* (ATCC 13075)**	21.77±1.92[a]**	17.23±0.35*	10.83±1.00	11.23±0.65	15.60±0.72*	12.97±0.38
***S. aureus* subsp. *aureus* (ATCC 25923)**	20.00±1.14[a]**	16.57±0.93	19.13±0.86*	12.63±1.07	16.27±0.65	16.77±0.64
***E. coli* (ATCC 25922)**	20.97±1.63[a]**	14.30±0.40	15.43±1.40	9.40±1.28	16.47±0.83	16.80±0.17
***S. marcescens* (ATCC 13880)**	19.97±1.87[a]**	17.63±1.30**	8.43±1.00**	12.10±0.50**	16.90±0.26**	NIZ

*Significant effect; * P<0.05, ** P<0.01)*

Discussion and conclusion

Today, the increasing resistance of microorganisms to antibiotics is attracting people to investigate alternative drugs. Studies conducted to find plant-derived antimicrobial agents are becoming increasingly important. To this end, screening of active ingredients in plants represents an important step in the development of new antibiotic active ingredients. The antimicrobial agents to be obtained in these studies will show new horizons for the treatment of resistant microorganisms. A heat map model was prepared to demonstrate the relationship between essential oils and antimicrobial activities. The interaction

between the species that formed the study sample and pathogenic bacteria is explained in this method. In this study, the diameter of the area where essential oils were ground was found to vary between 26.53 ± 2.50 and 9.40 ± 1.28. pathogen strains, were higher than those reported in previous studies and this effect was statistically significant (P <0.01). These values also indicate that it has a stronger antibacterial effect than the antibiotic K30 with the largest area diameter in the control group (17.37 ± 0.31 mm). It is worth noting that even though the essential oil extracted from T. spicata had the lowest antibacterial activity (18.93±1.90), it was higher than that of the control group, showing its broad application potential.

When the positive definition of zone measurements of 7 mm and above in previous studies was considered, it was concluded that all the results obtained were high in terms of antibacterial activity (Prabuseenivasan et al., 2006; Bilenler and Gokbulut, 2019). It was found that *T. spicata*, *S. cuneifolia*, *S. hortensis*, *O. onites,* and *O. majorana* did not show antibacterial activity against gram-positive and gram-negative bacteria.

Another study examined the effects of different concentrations of thyme essential oil on various bacteria. These essential oils were found to have antimicrobial effects against Escherichia coli, Listeria monocytogenes, and Staphylococcus aureus. Studies have also found that as the concentration of essential oils decreases, so does the antibacterial effect. Studies have shown that variations in bacterial exposure can be attributed to different plant species and strains (Baydar et al., 2005).

It was also suggested that there are additives, antagonistic, and synergistic interactions between the components of essential oils (Burt 2004). In a study that examined the effects of thymol and carvacrol on *Staphylococcus aureus* and *Pseudomonas aeruginosa*, it was reported that these substances had better effects when employed together than when used alone (Lambert et al. 2001).

Akin et al. 2010) tested the essential oil obtained from *T. spicata* L. var. *spicata* through distillation method on *S. aureus* ATCC 25923, *E. faecalis* RSKK 97008, *B. cereus* ATCC 17778, E. coli ATCC 29998, *Salmonella cholerasuis* ATCC 14028, *Streptococcus mutans* NCTC 10449 and *Sarcina lutea* ATCC 9341 strains. As a result, they reported that the bacteria used were ineffective and there was no growth.

Joma (2018) tested the extracts of *T. spicata* L. var. *spicata* plant obtained with water, methanol, and hexane on *S. maltophilia* strain by disk diffusion method as 20 pl. As a result, they reported that they obtained 16 mm, 23 mm, and 10 mm zone diameters, respectively.

Also, it was reported in a previous study in which essential oils of the thyme plant were tested on 9 gram (-) and gram (+) bacteria strains that bacteriostatic effects were observed against all test microorganisms (Mahboubi et al. 2010). It was reported in some similar studies that thyme essential oil showed strong bactericidal effects on *Listeria monocytogenes* and some other bacteria (Kalemba et al. 2003).

According to the results of this study, antimicrobial activity was found in essential oils extracted from certain plants such as thyme, but this activity varied depending on the type of microorganism. The antimicrobial activity of plant essential oils varies depending on the type of microorganism tested. The results of the study show that the antibacterial effect of the essential oils used is mainly positive. Therefore, it is recognized that the use of herbal essential oils and extracts can be one of the effective solutions in this regard. The lack of mutagenicity in most plant essential oils suggests that these oils are potential sources for use as alternatives to many synthetic food additives.

Statement of Conflict of Interest

The author(s) declare no conflict of interest for this study.

Author's Contributions

NT, AK, and FCY studied antimicrobial activity. AK completed the experiments. FCY performed the statistical analysis. Plant samples were collected and identified by OG. The article was written by NT, FCY, AK, and OG. All authors edited, revised, and provided comments to the manuscript.

References

Akin, M., Demirci, B., Bagci, Y. Baser, K.H.C. (2010). Antibacterial activity and composition of the essential oils of two endemic *Salvia* sp from Turkey. *African Journal of Biotechnology*, 9, 2322-2327.

Babacan, E.Y., Polat, R., Güler, O., Moyan, A., Paksoy, M.Y. Cakilcioglu, U. (2022). An ethno veterinary study on plants used for the treatment of livestock diseases in Genc (Bingöl-Turkey). *Indian Journal of Traditional Knowledge*, 21, 81-88.

Balouiri, M., Sadiki, M. Ibnsouda, S.K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis*, 6, 71-79.

Baser, K.H.C., Ozek, T., Kürkcüoglu, M. Tümen, G. (1994). The Essential Oil of *Origanum vulgare* subsp. *hirtum* of Turkish Origin, *Journal of Essential Oil Research*, 6, 31-36.

Baydar, H. (2005). The Effects of Different Harvest Dates on Essential Oil Content Essential Oil Composition in *Origanum minutiflorum* O. Schwarz et. P.H.Davis. *Akdeniz University Faculty Of Agriculture Journal*, 18, 175-178.

Baytop, A. (1991). Wild and grown aromatic plants used in Turkey. *J. of Pharmacy*, 1, 76-78.

Bilenler, T. Gökbulut, I. (2019). Determination of Sensitivity of Hospital Pathogens Against Commercial Essential Oil. *Gumushane University Journal of Science Institute*, 9, 716-723.

Biskup, S. Saez, E. (2002). Thyme, The Genus *Thymus*. *Taylor-Francis*. London.

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods *review. Int J Food Microbiol*, 94, 223-253.

Carson, C.F., Hammer, K.A. Riley, T.V. (2006). Melaleuca alternifolia (Tea tree) oil: A review of antimicrobial and other medicinal properties. *Clin Microbiol Rev.*, 19, 50-62.

Cingi, M.I., Kirimer, N., Sarikardasoglu, I., Cingi, C. Baser, K.H.C. (1991) Pharmacological Effects of *Origanum onites* and *O. minutiflorum* Essential Oil, *IX Herbal Medicine Raw Materials Meeting, Proceedings*, Eskisehir.

Cox, S.D., Mann, C.M., Markham, J.L., Bell, H.C., Gustafson, J.E., Warmington, J.R. Wyllie, S.G. (2000). The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). *Journal of applied microbiology*, 88, 170-175.

Davis, P. H. (1982). Flora of Turkey and The East Aegean Islands. Univ. Press, Edinburgh. Vol. 7, 36-42, 297-313.

Davis, P.H. (1988). Flora of Turkey. *Uni.Press.Edinburg*. Vol. 10.

Dönmez, M., Kargıoğlu, M. Temel, M. (2011). The Morphological, Anatomical and Ecological Properties of *Stachys palustris* L. Afyon Kocatepe, *University Journal of Science*, 11, 1-9.

Farag, R.S., Daw, Z.Y. Abo-Raya, S.H. (1989). Influence of some spice essential oils on *Aspergillus parasiticus* growth and production of aflatoxins in a synthetic medium. *J. Food Sci.*, 54, 74-76.

Gill, A.O., Delaquis, P., Russo, P. Holley, R.A. (2002). Evaluation of antilisterial action of cilantro oil on vacuum packed ham. *Int. J. Food Microbiol.*, 73, 83-92.

Güler, O., Polat, R., Karaköse, M., Çakılcıoğlu, U. Akbulut, S. (2021). An ethnoveterinary study on plants used for the treatment of livestock diseases in the province of Giresun (Turkey). *South African Journal of Botany*, 142, 53-62.

Gustafson, J.E. (1998). Effects of tea tree oil on *Escherichia coli*. *Lett. Appl. Microbiol*., 26, 194–198.

Heywood, V.D. (1978). Flowering Plands of The World. *Oxford Üniv. Press, London. p*. 239.

Hyldgaard, M., Mygind, T. Meyer, R.L. (2012). Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. Frontiers in Microbiology, 3, 1-24.

ISO/DIS9235. (2013). Aromatic natural raw materials-vocabulary: *International Standard Organisation*. ISO 9235:2013. 9–10.

Joma, M.H. (2018). Investigation of the effect of *Thymbra spicata L. var. spicata* plant extracts on DNA protective activities and *Stenotrophomonas maltophilia*. Master Thesis, *Gaziantep University Institute of Science and Technology*, Gaziantep.

Kalemba, D. Kunicka, A. (2003) Antibacterial and antifungal properties of essential oils. *Current Medicinal Chemistry*, 10, 813–829.

Khorshidian, N., Yousefi, M., Khanniri, E. Mortazavian, A.M. (2018). Potential application of essential oils as antimicrobial preservatives in cheese. *Innovative Food Science & Emerging Technologies*, 45, 62-72.

Kintzios, E.S. (2002). Oregano, The Genus *Origanum* Lippia Taylor-Francis. London.

Knobloch, K., Weigand, H., Weis, N., Schwarm, H.M. Vigenschow, H. (1986). Action of terpenoids on energy metabolism. In Progress in Essential Oil Research: 16th International Symposium on Essential Oils, Brunke, E.J., Ed., *De Walter de Gruyter: Berlin, Germany*, pp. 429-445.

Lambert, R.J.W., Skandamis, P.N., Coote, P.J. Nychas, G.J.E. (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *J. Appl. Microbiol.*, 91, 453–462.

Luque De Castro, M.D., Jiménez-Carmona, M.M. Fernández-Perez, V. (1999). Towards more rational techniques for the isolation of valuable essential oils from plants. TrAC, Trends in Analytical Chemistry, 18, 708–716.

Magaldi, S., Mata-Essayag, S. Hartung de Capriles, C. (2004). Well diffusion for antifungal susceptibility testing Int. J. Infect. Dis., 8, 39-45.

Mahboubi, M. Bidgoli, F.G. (2010). In vitro synergistic efficacy of combination of amphotericin B with *Myrtus communis* essential oil against clinical isolates of Candida albicans. *Phytomedicine*, 17, 771–774.

Maruyama, N., Sekimoto, N. Ishibashi, H. (2005). Suppression of neutrophil accumulation in mice by cutaneous application of Geranium. *essential oil. J. Inflamm*., 2, 1-11.

Masotti, V., Juteau, F., Bessiere, J.M. Viano, J. (2003). Seasonal and phenological variations of the essential oil from the narrow endemic species Artemisia molinieri and its biological activities. *Journal of*

Agricultural and Food Chemistry, 51, 7115–7121.
Nychas, G.J.E. (1995). New Methods of Food Preservation (Natural Antimicrobials from Plants). Ed. Gould G.W. Publisher: *Springer US Copyright: Holder Chapman & Hall, pp* 58-89.
Prabuseenivasan, S., Jayakumar, M. Ignacimuthu, S. (2006). In vitro antibacterial activity of some plant Essential oils. *Biomedical Central*, 6, 39-45.
Russo, A., Formisano, C. Rigano, D. (2013). Chemical composition and anticancer activity of essential oils of Mediterranean sage (*Salvia officinalis* L.) grown in different environmental conditions. *Food Chem Toxicol*., 55, 42–47.
Selvi, S., Polat, R., Cakılcıoglu, U., Celep, F., Tuncay Dirmenci, T. Ertug, Z.F. (2022). An ethnobotanical review on medicinal plants of the Lamiaceae family in Turkey. *Turkish J Botany*, 46, 283-332.
Sikkemat, B.J., Bontt, J.A.M. Poolmann, B. (2010). Interactions of Cyclic Hydrocarbons with Biological Membranes. *J. Biol. Chem.*, 269, 8022–8028.
Skoula, M. Grayer, R.J. (2005). Volatile Oils of *Coridothymus capitatus, Satureja, Thymbra, Satureja spinosa* and *Thymbra calostachya* (Lamiaceae) from Crete, *Flavour Fragr. J.*, 20, 573-576.
Steel, R.G.D., Torrie, J.H. Dicky, D.A. (1997). Principles and Procedures of Statistics, A Biometrical Approach. 3rd Edition, *McGraw Hill, Inc. Book Co.*, New York, 352-358.
Tajkarimi, M.M., Ibrahim, S.A. Cliver, D.O. (2010). Antimicrobial herb and spice compounds in food. *Food Control*, 21, 1199–1218.
Tan, N., Satana, D., Sen, B., Tan, E., Altan, H.B., Demirci, B. Uzun, M. (2016). Antimycobacterial and Antifungal Activities of Selected Four *Salvia* Species. *Records of Natural Products*. 10, 593-603.
Toroglu, S. Cenet, M. (2006). The Methods Used To Determine Antimicrobial Activity Of The Plants And Their Application Area. *KSU Journal of Science and Engineering*, 9, 12-20.
Ultee, A., Bennik, M.H.J. Moezelaar, R. (2002). The Phenolic Hydroxyl Group of Carvacrol Is Essential for Action against the Food-Borne Pathogen Bacillus cereus. *Applied and Environmental Microbiology, Apr.*, 68, 1561–1568.
Valgas, C., De Souza, S.M. Smânia, E.F.A. (2007). Screening methods to determine antibacterial activity of natural products Braz. *J. Microbiol.*, 38, 369-380.

IJAFLS
e-ISSN: 2602-4381
International Journal of Agriculture, Forestry and Life Sciences
Original Article | Open access | Int J Agric For Life Sci (2023) 7(2): 56-59

Identification and Genetic Diversity of Hypovirulent Binucleate Rhizoctonia spp. Isolated from Turfgrass and Soil

Filiz Ünal[1]* **Aysun Çavuşoğlu[2]**

[1]Eskişehir Osmangazi University, Faculty of Agriculture, Department of Plant Protection, Eskişehir, Türkiye
[2]Kocaeli University, Faculty of Agriculture and Natural Sciences, Department of Plant Protection, Kocaeli, Turkey
***Correspondence:** fucar06@yahoo.com

Citation
Unal, F., Çavuşoğlu, A. (2023). Identification and Genetic Diversity of Hypovirulent Binucleate Rhizoctonia spp. Isolated from Turfgrass and Soil. Int J Agric For Life Sci (2023) 7(2): 56-59.

Received	:2 October 2023
Accepted	:19 December 2023
Published Online	:22 December 2023
Year:	:2023
Volume	:7
Issue	:2 (December)
Pages	:56-59

Available online https://dergipark.org.tr/ijafls

Abstract
The aim of this study was to obtain hypovirulent binucleate Rhizoctonia anastomosis groups from turfgrass roots and rhizosphere soils in Eskişehir, Ankara and Kocaeli. In 2022, 153 turfgrass and 78 rhizosphere soil were taken from turfgrass areas. Isolations were made from the roots and rhizosphere soils. Isolations from soils were performed by colonization of trap plant tissues. Detection of anastomosis groups of the isolates were done according to the hyphal nucleus staining, colony apparences and rDNA-ITS sequences analysis. The ITS regions of the isolates were amplified by polymerase chain reaction (PCR) using rDNA primer pair ITS 1 and ITS2. Pathogenicity tests of all isolates were conducted with pot assays in greenhouse conditions. As a result of the study, 17 BNR isolates belonging to 7 different anastomosis groups were obtained. Two AG D isolates were found pathogenic. As a result, 15 hypovirulent binucleate Rhizoctonia isolates belonging to AG A, AG B(o), AG Fa, AG Fb, AG G and AG K groups were obtained. The phylogenetic neighbor-joining tree of 17 Binucleate isolates clearly shows that the isolates are grouped into seven distinct clusters. AG Fa and AG Fb appeared on the tree as genetically close isolates. In future, studies should be conducted to investigate the effect levels of the hypovirulent strains detected in this study on the control of pathogenic fungi that cause diseases in cultivated plants.

Key words
Anastomosis group, Turfgrass, Soil, Phylogeny.

Introduction
Rhizoctonia is a huge genus with more than three hundred different host ranges. This genus includes different groups that are pathogenic and non-pathogenic to plants. The genus *Rhizoctonia* is divided into subgroups called 'anastomosis groups', taking into account hyphal union reactions. According to the number of nuclei in the hyphal compartments, they are divided into 3 groups as uninucleate (UNR), binucleate (BNR) or multinucleate *Rhizoctonia* (MNR) (Sharon et al., 2007). Binucleate *Rhizoctonia* species are divided into 19 different anastomosis groups (AG A-L, AG O - S and AG U, V, W) (Dong et al. 2017, Hyakumachi et al. 2005, Misawa and Kurose 2018, Sharon et al. others 2008, Yang et al. 2015, Zhao et al. 2019). Binuclear *Rhizoctonia* species live both freely in the rhizosphere soil and colonized in plant roots. Therefore, in the studies carried out, they can be isolated both from the soil and from the roots of the plants they host. Some of them live in the soil as saprophytes, some of them have a symbiotic relationship with plant roots. Another group, *Rhizoctonia*, has parasitic relationships with plant roots and has the ability to cause disease (Sneh et al., 1996; Herr, 1995). Nonpathogenic *Rhizoctonia* species prevent many plant pathogens from infecting their hosts through the hypovirulence mechanism (Cardoso and Echandi 1987a, b; Roberts and Sivasithamparam 1986; Gutierrez and Torres 1990; Sneh et al. 1996, Herr 1995). For this reason, Non-pathogenic *Rhizoctonia* anastomosis groups are important in terms of biological control as they are used successfully in the control against pathogenic fungi. The majority of nonpathogenic groups within the genus *Rhizoctonia* are binucleates. But, in recent years, several studies revealed that some BNR *Rhizoctonia* AGs were pathogenic on some agricultural plants (Oniki et al., 1986; Sneh et al., 1996; Yang et al., 2015; Alaei et al., 2017; Dong et al., 2017; Türkkan et al., 2018; Türkölmez et al., 2019; Basbagci and Dolar, 2020). In wheat, only BNR AG D is pathogenic. When the studies conducted in the world on turfgrass are examined, among BNR isolates; subgroups I, II and III of AG D, AG Q and AG P were not found to be pathogenic on turfgrass (Oniki et al., 1986; Sneh et al., 1996; Ünal and Cavusoglu, 2023). The genetic diversity of *Rhizoctonia* isolates has been studied using RAPD-PCR, SSR-PCR, rDNA-RFLP, rDNA-ITS sequence analysis, with universally primers, PCR, and rep-PCR (Sharon et al. 2006). Currently, the rDNA-ITS sequence analysis is the most appropriate method for the classification of *Rhizoctonia* spp. and sequence analysis of the ITS-5.8S rDNA region has been used as a suitable molecular tool the for identification of *Rhizoctonia* subgroups (Carling et al., 2002, Sharon et al., 2006, 2007, 2008).
While some BNR *Rhizoctonia* species have been reported as non-pathogen or pathogen on various hosts, there is no a comprehensive data describing their behavior and fungus-host relationships on turfgrass. The aim of this study is identification, pathogenicity and phylogenetic evaluation of binucleate *Rhizoctonia* isolates isolated from turfgrass and rhizophere soil in Eskişehir, Ankara and Kocaeli provinces.

Material and Methods
Collection of Samples and Isolation of Fungi
In the spring of 2022, turfgrass samples and rhizosphere soils were taken from large parks, recreation areas, picnic areas and feruges in Eskişehir, Ankara and Kocaeli provinces, and were put in paper bags and brought to the laboratory. Symptomatic and asymptomatic root surfaces were sterilized in 1.5% sodium hypochlorite for approximately one minute. Then, the root pieces were rinsed by soaking in sterile pure water for 30 seconds, was kept and allowed to dry on sterile filter papers, and placed on PDA to which 100 µg/ml streptomycin sulfate was added. In isolations from soil, sterile wheat stalks were used as trap plants. Soil samples taken from the surveyed grass areas were transferred to pots in the greenhouse. After the soil in the pots was watered, sterilized wheat stalks, approximately 6-7 cm long, were placed vertically in each pot and covered with a nylon bag. After waiting for 4 days, the stems were washed, dried and placed on water agar (WA) to which 10% (v/v) lactic acid was added. After 7 days, the developing similar *Rhizoctonia* hyphae were transferred to potato dextrose agar (PDA).
Pathogenicity Assays
To obtain fungal inoculums, first 500 g of wheat seeds were filled into 1- liter bottles and 120 ml of water was added. These bottles were autoclaved twice at 100°C for one hours, with a one-day break. Then, 12 pieces of agar with a diameter of 3-4 mm containing hyphae of different *Rhizoctonia* isolates were put to each of these bottles and waited in incubator at 24°C for three weeks. The inoculum obtained after incubation was dried on sterile papers and chopped in a blender. Then, this inoculum was mixed into a 5% sterilized mixture of sand, soil and fertilizer (1:2:1) (twice for 45 minutes at 121 ºC with a one-day break). Three pots (12x12 cm) were used for each application. No inoculum was added to the control pots. Then, the pots were covered with nylon cover and left to incubate for three days. Three days later, 25 *Festuca arundinaceae* variety seeds (sensitive to *Rhizoctonia* spp.) were sowed in each pot and covered with approximately soil. Then, 20 ml of pure water was dropped to each pot. The experiments were carried out in a greenhouse with 12 h daylight 60 % RH, 23±3°C. The evaluations were done one month later. Disease assessments were made using Ichielevich-Auster et al. (1985)'s 0-5 scale. Using the values of the scale, disease severity values were calculated using the Townsend and Heuberger formula.

Determination of Nucleus Numbers of Isolates
The fungi hyphae obtained from the cultures were transferred to lamella water agar medium. Then, the coverslips, sterilized by burning with alcohol, were immersed in soft PDA medium containing 0.5% agar and placed in water agar

medium. The petri dishes prepared in this way kept in the incubator on 24°C for one day. After incubation, the samples were examined under a coverslip microscope. For examination, Safranin O dye (0.5%) was dropped on a slide. Then, the coverslip taken from the water agar medium was placed on the solution on the slide (Bandoni, 1979). After the coverslips were prepared in this way, they were examined under the microscope and the number of nuclei in the hyphal septa were determined. Nucleus numbers in at least 15 septa were examined (x100 and x400). Three petri dishes were used for each sample (Ogoshi et al., 1990, Carling et al., 2002, Karaca et al., 2002).

DNA isolations, PCR studies and phylogenetic analysis of isolates

DNA isolations of fungal isolates were done using the QIAGEN (Blood and Tissue Kit) DNA isolation kit. PCR studies were carried out with ITS 1/2 primers (White et al., 1990). In the PCR analyses, the final mixture was 50 µl. For this; 25 µl GoTaq® Hot Start Green Master mix (2x), 2 µl of 5 µM each primers, 13 µl in a 2 ml ependroph tube double distilled water and 4 µl BSA were mixed. Then, the DNAs were added to 4 µl of each PCR mixture distributed in different PCR tubes. The cycle PCR protocol consists of an initial denaturation of 4 minutes at 94°C, followed by 30 cycles of 45 seconds at 94°C, 45 seconds at 55°C, and 2 minutes at 72°C, and finally at 72°C. It consisted of a 10-minute overtime. Direct sanger sequencing analyzes of PCR products were performed in a special R&D Laboratory. Sequence results were compared with isolate sequences in GenBank by BLAST analysis at NCBI. Sequences were edited and aligned (Thompson et al., 1994). The phylogenetic tree was constructed using ITS sequences of fungi using the neighbor-joining method using MEGA ver 7.0 software (Kumar et al., 2016), with sequence distance calculated by the Kimura 2-parameter model (Kimura, 1980). Bootstrap analysis was performed with 1,000 replicates to determine support for each class.

Results

As a result of the survey studies carried out in Eskişehir, Ankara and Kocaeli provinces in 2022, 153 turfgrass and 78 rhizosphere soils were taken and isolation studies of fungi were carried out from all plant and soil samples. As a result of the isolations, nuclei staining was done to display the nuclei numbers of the isolates detected at the genus level. As a result of dyeing, the nucleus number that was found in each hypha cell was two, and width of the main runner hyphae was less than 7 µm (Figure 1). As a result of mycelial staining, *Rhizoctonia* isolates with two nuclei in each hyphal compartment and a hypha width of less than 7 µm were identified (Figure 1). Considering the hypha width and the number of nuclei in the hypha compartments, colony structures and colors, 17 isolates were determined as binucleate *Rhizoctonia*. As a result of the molecular diagnostic studies conducted with these isolates, it was determined that the isolates belonged to the *Rhizoctonia* AG A, AG B(o), AG D I, AG Fa, AG Fb, AG G and AG K groups (Figure 1, Table 1).

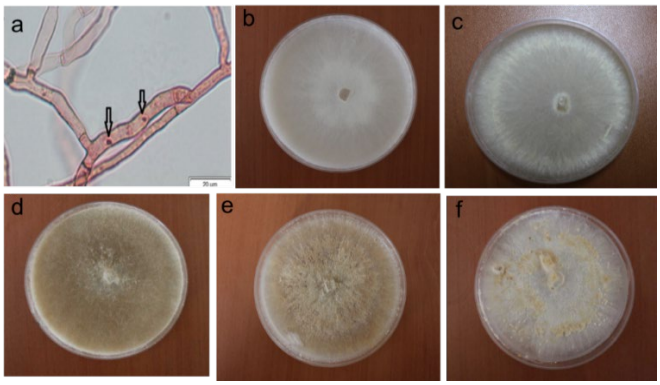

Figure 1. Binucleate *Rhizoctonia* AGs isolated from in this study: a: Binucleate hyphae, b-f: Colony appearance of some binucleate *Rhizoctonia* isolates on potato dextrose agar; (b) AG A, (c) AG D, (d) AG F, (e) AG G, (f) AG K.

When the growth of the BNR isolates obtained on PDA medium was examined, the morphological feature of the isolates matched the 'features of binucleate isolates' described in Sneh et al. (1994). While BNR AG A, D and K isolates grown on PDA were initially white and then turned beige, AG D and G isolates initially turned light yellow and turned buff within 3 weeks as they aged. Sclerotia formation was rarely observed in isolates other than AG D and G in the developing colonies. In the isolates with sclerotia development, sclerotia of 0.5-1.3 mm in diameter, close to a sphere, formed singly or in clusters, and yellow (AG G) or light to dark brown (AG D) were observed (Figure 1).

As a result of the isolations made from plant and soil samples, the most isolated group was the AG A anastomosis group. This was followed by AG B(o) and AG G with 3 isolates each. 9 isolates were isolated from soil and 8 isolates from plant roots. The most isolates were isolated from Ankara province. As a result of pathogenicity tests, two AG D I isolates were found to be pathogenic. It was determined that all isolates and groups except these two isolates were no pathogens in turfgrass. The disease severity values of the two AG D I isolates were determined as 82.50% and 88.12%.

Table 1. Anastomosis group, geographic origin, source of isolation and pathogenicity value of binucleate *Rhizoctonia* isolates used in this study

Isolate Number	Anastomosis group	Origin	Source of isolation	Pathogenicity (%)
BNR204	AG A	Ankara	Plant	Non-pathogen
BNR932	AG A	Kocaeli	Soil	Non-pathogen
BNR141	AG A	Ankara	Soil	Non-pathogen
BNR1341	AG A	Eskişehir	Plant	Non-pathogen
BNR939	AG A	Kocaeli	Plant	Non-pathogen
BNR1844	AG A	Ankara	Soil	Non-pathogen
BNR314	AG B(o)	Ankara	Plant	Non-pathogen
BNR354	AG B(o)	Eskişehir	Soil	Non-pathogen
BNR497	AG B(o)	Eskişehir	Soil	Non-pathogen
BNR98	AG DI	Ankara	Plant	82.50
BNR1484	AG DI	Kocaeli	Soil	88.12
BNR2013	AGFa	Ankara	Soil	Non-pathogen
BNR316	AGFb	Eskişehir	Plant	Non-pathogen
BNR1325	AG G	Kocaeli	Soil	Non-pathogen
BNR1552	AG G	Ankara	Plant	Non-pathogen
BNR1415	AG G	Ankara	Soil	Non-pathogen
BNR1577	AG K	Eskişehir	Plant	Non-pathogen

Rhizoctonia AG A, AG DI, DII, DIII, G and K were previously detected in wheat in Türkiye. Only AG DI, DII, DIII groups pathogens were found in wheat (Demirci 1998; Ünal and Dollar 2023). In this study, AG D was found to be a pathogen in turfgrass. All the other isolates were found to be non-pathogenic. In previous studies conducted on turfgrass, *Rhizoctonia* AG D (subgroups I, II, III), AG Q and AG P were found to be pathogen in turfgrass (Oniki et al., 1986; Sneh et al., 1996; Ünal and Çavuşoğlu, 2023). Ünal and Çavuşoğlu (2023) reported that AG P causes damping off in turfgrass. Although binucleate *Rhizoctonia* spp. has been reported to be pathogenic in some hosts, these species generally live saprophytically in soil and dead plant residues. Many studies have shown that low-virulent or non-virulent species have hypovirulent properties (Sneh et al., 1996; Tewoldemedhin et al., 2006). There are studies showing that these strains, which generally consist of binucleate species, can be used successfully in biological control studies against pathogens (Cardoso and Echandi, 1987 a,b; Roberts and Sivasithamparam 1986; Gutierrez and Torres, 1990; Sneh et al., 1996; Herr, 1995).

Phylogenetic tree was constructed by bootstrap neighbor-joining analysis of nucleotide sequences to evaluate genetic differences among isolates belonging to 17 binucleate *Rhizoctonia* anastomosis groups. The phylogenetic neighboor-joining tree belonging to BNR isolates clearly demonstrated that the isolates were grouped into 7 distinct clusters (Figure 2). It was observed that AG Fa and AG Fb subgroups formed their own small groups within the same group on the tree. This situation showed that these two species were genetically closely related species. Even though AG A isolates were in the same group, a slight intragroup genetic difference was observed between them. Isolates of belonged the other 4 groups were grouped within themselves and formed groups in different places on the tree than the groups of other species (Figure 2).

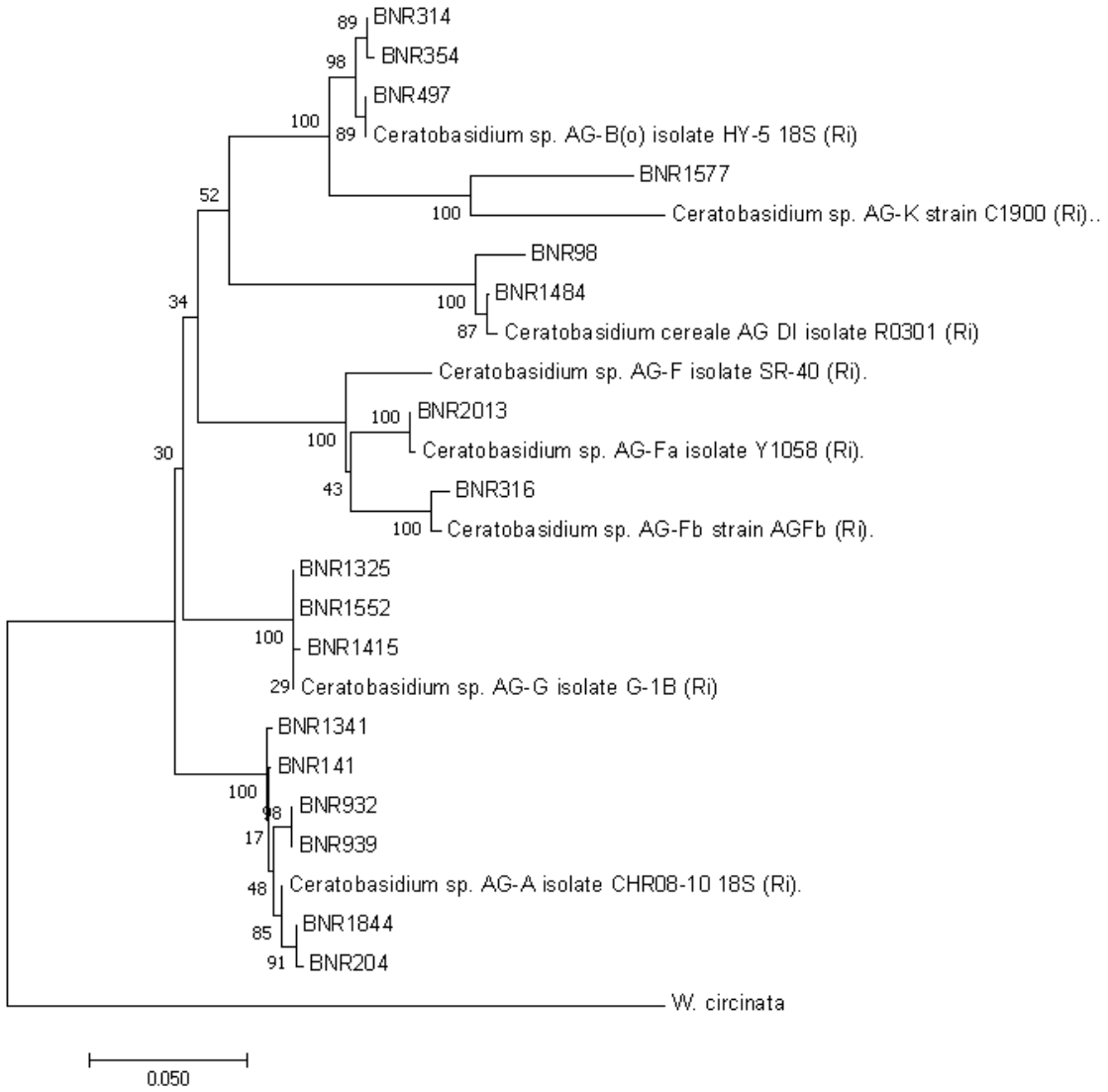

Figure 2. Neighbour-Joining phylogenetic tree of BNR isolates obtained with 1000 bootstrap replicates.

Discussions

Non-pathogenic species of *Rhizoctonia* are important as biological control agents of other pathogenic fungi. In recent studies, non-pathogenic multinucleate and binucleate *Rhizoctonia* species have been used as hypovirulent strains in the fight against pathogenic species (Sneh et al. 1996). Herr (1995) states that in the studies conducted on biological control since the 1930s, the biocontrol of diseases caused by *Rhizoctonia solani* does not have any economic commercial value, but in new approaches to this subject, new agents, especially in field crops, are identified and used, and an applicable biocontrol management system is needed. Studies conducted since 1989 have shown that isolates from different groups of binucleate *Rhizoctonia* spp. and hypovirulent *R. solani* isolates will be effective in the biocontrol of diseases caused by *R. solani* in different hosts. These are several different binucleate *Rhizoctonia* anastomosis group (AG) and hypovirulent *R.solani* AG isolates, and the effects of none of these isolates on *R. solani* are through mycoparasitism and antibiosis, but through systemic promotion of host resistance or competition for nutrients or habitat. The tested binucleate *Rhizoctonia* spp. It was also reported by the researcher that the effects of hypovirulent *R. solani* isolates on the diseases caused by *R. solani* were different, and that isolates that colonized the plant surface were effective as biocontrol agents, while non-colonized isolates were ineffective. Ichielevich-Auster et al. (1985) isolated 107 *Rhizoctonia* spp. from soil samples taken from 26 different locations in Israel. They found that 32 of the isolates were not pathogenic in 11 different hosts. Cardoso and Echandi (1987a) investigated the effect of binucleate *Rhizoctonia* against *R. solani*, which causes root rot in bean seedlings, under laboratory and greenhouse conditions, and showed that BNR isolates suppressed *R. solani* at the infection site by promoting the metabolic response in bean seedlings. The same researchers investigated the effects of 11 BNR, 1 *R. zeae*, 1 *Trichoderma hamatum* and 1 *T. harzianum* isolate as a biological control agent against *R. solani*, which causes root rot in bean seedlings. In their other study, they investigated the disease severity of BNR isolates under greenhouse conditions. They found that they reduced the in field trials, they also observed that some of the BNR isolates were more effective against root rot than *Trichoderma* isolates (Cardoso and Echandi, 1987b). Herr (1988) reported that 7 of 10 BNR isolates obtained from sugar beet cultivation areas in Ohio prevented root and crown rot caused by *R. solani* anastomosis group 2, type 2 in sugar beet. Bandy and Tavantzis (1990) recorded a 56% decrease in the infected stem tissues of potato plants inoculated with the virulent AG 3 (Rhs 27) isolate of *R. solani* and the Rhs1A1 isolate from the hypovirulent anastomosis group (AG). When potato plants were inoculated with only the Rhs1A1 isolate, a 4-fold increase in stolon dry weight and a 1.7-fold increase in stem dry weight was observed compared to control plants. Haris et al. (1994) investigated the effect of 9 BNR isolates against the collapse caused by AG 4 and AG 8 isolates of *R. solani*. All isolates inhibited the collapse caused by AG 4 in cucumber, and most of the isolates increased shoot length in plants infected with AG 4 and AG 8. Two of the binucleate isolates were also found effective against *Pythium* spp. Potvin et al. (1999) determined that binucleate nonpathogenic AG-G isolates provided effective protection against root rot caused by AG-4 in bean seedlings. Villajuan-Abgona et al. (1996) in their study in which they investigated the effect of 3 binucleate *Rhizoctonia* (BNR) against the collapse caused by AG 2-2 and AG 4 groups of *R. solani* in cucumber plant, found that BNR isolates L2 (AG Ba), W1 and W7 (AG A) were affected by the virulent isolate C4 (AG 4). They found that it provided 58-71% protection against another virulent isolate, RH 65 (AG 2-2), was between 64-75%. W7, one of the BNR isolates, was not only effective in protecting against disease, but also caused a significant increase in plant fresh weight. Muslim et al. (2003a) in their study investigating the effect of 4 hypovirulent binucleate *Rhizoctonia* isolates isolated from soil in the Gifu region of Japan on *Fusarium oxysporum* f.sp *spinaciae* (Fos)- induced wilt disease in spinach, they found that HBNR isolates reduced the severity of the disease by 56-100% and the severity of discoloration by 52-100%. In addition, they also observed that extracts obtained from roots with HBNR significantly inhibited the germination and grass tube development of Fos conidia. The same researchers determined that the hypovirulent binucleate 4 isolate of *Rhizoctonia* (G1, L2, W1 and W7) significantly reduced the disease severity values, including leaf symptoms and

color change in the stem, caused by *Fusarium oxysporum* f.sp. *lycopersici* (Fol) in tomatoes under field conditions, and Fol colonization in the roots and stems of tomato plants. The reduction in disease severity varied depending on the HBNR isolates and method of administration. Among these isolates, G1, W1 and W7 also caused an increase in fresh weight. Researchers have reported that HBNRs can be used as biocontrol agents against *Fusarium* wilt (Muslim et al. 2003 b).

Conclusions

The success of microorganisms used in biological control studies is generally more successful in the soil and climatic conditions to which they are isolated and adapted. Therefore, it is important to use local isolates rather than imported ones in these studies. It is necessary to conduct studies on the possibilities of using the domestic hypovirulent binucleate strains obtained in this study in the fight against diseases caused by pathogenic fungi. It is also necessary to focus on bioformulation studies of isolates that are found to be effective.

Statement of Conflict of Interest

The authors declares no conflict of interest

Author's Contributions

FÜ contributed to the survey, isolation, identification, pathogenicity, phylogenetic studies of BN *Rhizoctonia* isolates and writing of the manuscript, AÇ contributed to the survey, isolation and pathogenicity. All authors read and approved the final manuscript.

References

Alaei, H., Molaei, S., Mahmoodi, S.B., Saberi-Riseh, R., (2017). New anastomosis group F (AG F) of binucleate Rhizoctonia causing root and stem rot of *Pistacia vera*. Journal Crop Protection, 6(1): 1-13.

Bandoni R.J., (1979). Safranin O as a rapid stain for fungi. Mycologia, 71 (4), 873–874.

Bandy, B.R. Tavantzis, S.M., (1990). Effect of hypovirulent *Rhizoctonia solani* on *Rhizoctonia* disease, growth, and development of potato plants, American Journal of Potato Research, 67(3), 189-199.

Basbagci, G., Dolar, F.S. (2020). First report of binucleate *Rhizoctonia* AG-K causing root rot on chickpea. Archives of Phytopathology and Plant Protection, 53(13-14), 640-652.

Cardoso, J.E. Echandi, E. (1987a). Biological control of *Rhizoctonia* root rot of snap bean with binucleate Rhizoctonia-like fungi. Plant Disease, 71:167-170.

Cardoso, J.E Echandi *Rhizoctonia*, E. (1987b). Nature of protection of bean seedlings from *Rhizoctonia* root rot by a binucleate -like fungus, Phytopathology, 77, 1548-1551.

Carling, D.E., Kuninaga, S., Brainard K.A., (2002). Hyphal anastomosis reactions, rDNA-internal transcribed spacer sequences, and virulence levels among subsets of *Rhizoctonia solani* anastomosis group-2 (AG-2) and AG-BI. Phytopathology, 92 (1), 43–50.

Demirci, E. (1998). *Rhizoctonia* species and anastomosis groups isolated from barley and wheat in Erzurum, Turkey. Plant Pathology, 47 (1), 10-15.

Dong, W., Li, Y., Duan, C., Li, X., Naito, S., Conner, R.L., Yang, G., Li, C., 2017. Identification of AG-V, a new anastomosis group of binucleate *Rhizoctonia* spp. from taro and ginger in Yunnan province. European Journal of Plant Pathology, 148 (4), 839-851.

Gutierrez, P. Torres, H., (1990). Biological control of *Rhizoctonia solani* with binucleated *Rhizoctonia*. Fitopatología, 25 (2), 45-50 pp.

Haris, A.R., Schisler, D.A., Neate, S.M. Ryder, M.H. (1994). Suppression of damping-off caused by *Rhizoctonia solani*, and growth promotion, in bedding plants by binucleate *Rhizoctonia* spp., Soil Biology and Biochemistry, 26, 263-268.

Herr, L.J. (1988). Biocontrol of Rhizoctonia crown and root rot of sugar beet by binucleate Rhizoctonia spp. and Laetisaria arvalis, Annuals of Applied Biology, 113,107-118.

Herr, L.J. (1995). Biological control of Rhizoctonia solani by binucleate Rhizoctonia spp. and hypovirulent R. solani agents. Crop Protection, Volume 14 Number 3, 179-186.

Hyakumachi, M., Priyatmojo, A., Kubota, M., Fukui, H. (2005). New anastomosis groups, AG-T and AG-U, of binucleate Rhizoctonia causing root and stem rot of cut-flower and miniature roses. Phytopathology, 95 (7), 784–792.

Ichielevich-Auster, M., Sneh, B., Koltin, Y. Barash, I. (1985). Suppression of damping-off caused by *Rhizoctonia* species by a nonpathogenic isolate of *R. solani*. Phytopathology 75: 1080-1084.

Karaca, G.H., Özkoç, İ. Erper, İ. (2002). Determination of Anastomosis grouping of *Rhizoctonia solani* Kühn isolates associated with bean plants grown in Samsun, Turkey. Pakistan Journal of Biological Sciences, 5(4): 434-437,

Kimura, M.A. (1980). simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16: 111–120.

Kumar, S., G. Stecher K. Tamura. 2016. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33: 1870–1874.

Misawa, T. and Kurose, D. (2018). First report of binucleate *Rhizoctonia* AG U causing black scurf on potato tubers in Japan. New Disease Reports , 38, (1), 24-24.

Muslim, A., Horinouchi, H., Hyakumachi, M. (2003a). Suppression of *Fusarium* wilt of spinach with hypovirulent binucleate *Rhizoctonia*, J. Gen. Plant.Pathol. 69, 143-150.

Muslim, A., Horinouchi, H., Hyakumachi, M. (2003b). Biological control of *Fusarium* wilt of tomato with hypovirulent binucleate *Rhizoctonia* in greenhouse conditions, Mycoscience, 44, 77-84.

Ogoshi, A., Cook, R.J. Bassett, E.N. (1990). *Rhizoctonia* species and anastomosis groups causing root rot of wheat and barley in the Pacific Northwest. Phytopathology, 80(9),785-788.

Oniki, M., Kobayashi, K., Araki, T., Ogoshi, A. (1986). A New disease of turfgrass caused by binucleate *Rhizoctonia* AG Q. Japanese Journal of Phytopathology, 52(5), 850-853.

Potvin, C.L., Balmas, V., Charest, P.M., Hare, S.J., (1999). Development of reliable molecular markers to detect non-pathogenic binucleate *Rhizoctonia* isolates (AG-G) using PCR, Mycological Research, 103, 1165-1172.

Roberts, F.A. Sivasithamparam, K. (1986). Identity and pathogenicity of *Rhizoctonia* spp. associated with bare patch diseases of cereals at field site in Western Australia. Europan Journal of Plant Pathology, 92(5): 185-195.

Sharon, M., Kuninaga S., Hyakumachi M. Sneh B. (2006). The advancing ıdentification and classification of *Rhizoctonia* spp. using molecular and biotechnological methods compared with the classical anastomosis grouping. Mycoscience 47: 299-316.

Sharon, M., Freeman, S., Kuninaga, S., Sneh, B. (2007). Genetic diversity, anastomosis groups, and pathogenicity of *Rhizoctonia* spp. isolates from strawberry. European Journal of Plant Pathology, 117 (3), 247–265.

Sharon, M., Kuninaga, S., Hyakumachi, M., Naito, S., Sneh, B. (2008). Classification of *Rhizoctonia* spp. using rDNA-ITS sequence analysis supports the genetic basis of the classical anastomosis grouping. Mycoscience, 49 (2), 93–114.

Sneh, B., Jabaji-Hare, S., Neate, S., Dijst, G. (1996). *Rhizoctonia* species: taxonomy, molecular biology, ecology, pathology and diseases control (1st Ed.), Kluwer Academic Publishers, Dordrecht, 1-559.

Tewoldemedhin, Y.T., Lamprecht, S.C., McLeod, A. Mazzola, M. (2006). Characterization of *Rhizoctonia* spp. Recovered from crop plants used in rotational cropping systems in the Western Cape Province of South Africa. Plant Disease, 90(11):1399-1406.

Thompson, J.D., D.G Higgins T.J. Gibson. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22: 4673–4680.

Türkkan, M., Erper, I., Kılıçoğlu, M.Ç., Yazıcıoğlu, E., Özcan, M. (2018). Characterization and pathogenicity of *Rhizoctonia* spp. isolated from kiwifruit in the Middle and Eastern Black Sea region of Turkey. J Phytopathol.;166:761–774.

Türkölmez, Ş., Çiftçi, O., Derviş, S., Ulubaş Serçe, Ç. (2019). First report of binucleate *Rhizoctonia* AG-F Causing stalk and root rot of corn (*Zea mays*) in Turkey. Plant Diseases, 103(8).

Ünal F. Cavusoglu A. (2023). Characterization And Pathogenicity of *Rhizoctonia* AG P Causing Damping-off on Turfgrass. 5th Internatıonal Balkan Agriculture Congress, Edirne, Türkiye, 20- 23 September 2023, p.146.

Ünal F. Dolar F. S. (2023). Anastomosis grouping and phylogenetic analysis of *Rhizoctonia* isolates on wheat in Türkiye. Plant Protection Bulletin, 63 (2), 5-15.

Villajuan-Abgona, R., Kageyama, K. Hyakumachi, M. (1996). Biocontrol of *Rhizoctonia* damping off of cucumber by non-pathogenic binucleate Rhizoctonia. European Journal of Plant Pathology, 102(3), pp 227-235.

White, T.J., T. Bruns, S. Lee J. Taylor. (1990). Amplification and direct sequencing of fungal ribosomal rna genes for phylogenetics. In PCR Protocols; Elsevier: Amsterdam, The Netherlands, pp. 315–322.

Yang, Y. G., Zhao, C., Guo, Z. J., Wu, X. H. (2015). Characterisation of a new anastomosis group (AG-W) of binucleate *Rhizoctonia,* causal agent for potato stem canker. Plant Disease, 99, 1757–1763.

Zhao, C., Li, Y., Liu, H., Li, S., Han, C., Wu, X.A. (2019). Binucleate *Rhizoctonia* anastomosis group (AG-W) is the causal agent of sugar beet seedling damping-off disease in China. European Journal of Plant Pathology, 155 (1), 53–69.

IJAFLS
e-ISSN: 2602-4381
International Journal of Agriculture, Forestry and Life Sciences
Original Article | Open access | Int J Agric For Life Sci (2023) 7(2): 60-63

Use of Probiotics in Health Raising Newborn Calves

Serkan Özkaya[1]* Raya Myktybayeva[2] Togzhan Boranbayeva[2] Zhassulan Otebayev[2]
Cevdet Gökhan Tüzün[1] Elif Rabia Şanlı[1] Esra Bahar Çatal[1]

[1]Isparta Applied Sciences University, Faculty of Agriculture, Department of Animal Science, Isparta-Türkiye
[2]Kazakh National Agrarian University, Almaty, Kazakhstan
*Correspondence: serkanozkaya@isparta.edu.tr

Citation
Özkaya, S., Myktybayeva, R., Boranbayeva, T., Otebayev, Z., Tüzün, C.G., Şanlı, E.R., Çatal, E.B. (2023). Use of Probiotics in Health Raising Newborn Calves. Int J Agric For Life Sci (2023) 7(2): 60-563

Received :17 October 2023
Accepted :13 November 2023
Published Online :22 December 2023
Year: :2023
Volume :7
Issue :2 (December)
Pages :60-63

Available online https://dergipark.org.tr/ijafls

Abstract
The growing global population is driving up demand for animal products. This increase in demand causes the industrialized of livestock farms. In industrialized farms where intensive breeding methods are applied, calves are separated from their dams immediately after birth and a fed with bottle. Rapid growth of calves is aimed in farms where intensive breeding programs are implemented. Antibiotics have been used for many years to stimulate growth. However, the resistance of bacteria to antibiotics has had negative effects on both human and animal health. In addition, consumers' awareness and pressure on this issue have led to the ban on the use of antibiotics to promote growth and the restriction of their use in treatments. This situation has led researchers to conduct study on the use of alternative feed additives. In recent years, studies have been conducted on the use of probiotics as an alternative to antibiotics, and these studies are continuing. There are differences in information regarding the benefits of probiotics in the first four weeks of life, which is a critical period for calves. This review aims to reveal the effects of probiotics use on the growth and health of calves during the suckling period.

Key words
Calf, Probiotics, Growth, Health.

Introduction
In a dairy cattle farm, cows that comprise the herd are separated due to aging, reduced productivity, and various health reasons, including primarily udder diseases. To replace these animals, high-yield, healthy heifers must be introduced to the herd. This is why the proper breeding and nutrition of female calves, especially, is crucial for dairy cattle herds (Tüzemen and Yanar, 2004). Female calves should be raised and nurtured in a way that preserves their health and well-being for their future milk production. Furthermore, it is important to raise male calves in a healthy manner to maximize their meat production potential. However, challenges persist in raising both male and female calves during their milk-feeding period. In the United States, it has been reported that approximately 5-6% of female calves die during the milk-feeding period (Quigley, 2018). These deaths are attributed to digestive system diseases, defined as calf diarrhea, in 32% of cases, respiratory diseases in 14% of cases, and a combination of diarrhea and respiratory diseases in 7% of cases (Table 1).

Table 1: Causes of female calf mortality

Cause of death	%
Digestive system diseases	32.00
Respiratory diseases	14.10
Combination of digestive system and respiratory diseases	7.00
Due to unknown causes	25.00
Other causes*	13.30
Unreported	8.6

*Source: Urie et al. (2018), *Calves sold without specifying infection, injury, or a specific cause*

Most of these deaths correspond to the first 4 weeks of the suckling period. Deaths due to diarrhea are observed at a younger age compared to deaths caused by respiratory diseases or unknown reasons. Furthermore, it has been reported that at least one case of illness was observed in 33.8% of female calves on farms in the United States. Digestive system diseases, defined as calf diarrhea, account for 56% of these cases (Urie et al., 2018). The majority of gastrointestinal diseases in calves occur within the first 2 weeks of life and this is common worldwide. (Quigley, 2018). The development of calves during the suckling period significantly affects their future growth, productivity, and health. Diarrhea cases that occur during this period suppress calf growth and have adverse effects on their age at first calving and milk yield during the first lactation (Hadimli, 2020). Antibiotic use in preventing and treating diarrhea cases not only reduces the numbers of pathogenic bacteria in the gut flora but also non-pathogenic bacteria (Soltan, 2009). Additionally, concerns about antimicrobial resistance and the variable effectiveness of antimicrobials in preventing and treating diseases have increased the focus on researching alternative methods (Smith, 2015).

The primary effects of feed additives used to support calf growth include an increase in resistance against colonization by pathogenic bacteria and enhancement of the host's mucosal immunity, resulting in a reduction of the pathogen load and improved animal health (Choct, 2009). The intestinal microbiota plays a significant role in influencing intestinal health and disease. The large intestine, in particular, harbors diverse bacterial populations with various functions, including acting as barriers against pathogens and macromolecules in the digestive epithelium (Donohue et al., 2002). During the suckling period, the intestinal microbial flora of calves affects their health and growth. A normal intestinal flora performs functions such as the breakdown of carbohydrates and fiber, regulation of lipid intake and accumulation in the diet, production of vitamins and short-chain fatty acids, stimulation of the immune system, control of intestinal motility, and protection of the host from intestinal pathogens (Azarpajouh, 2023).

Newborn calves' digestive systems are exposed to various microorganisms from the external environment, which colonize their intestines (Azarpajouh, 2023). However, the separation of newborn calves from their mothers immediately after birth necessitates artificial feeding with whole milk and milk replacers. As a result, calves cannot rapidly acquire the microflora from their dams and other cows' saliva and feces. This situation slows down the formation of the microflora and may even disrupt the homeostatic balance in the intestine (Donohue et al., 2002; Lopes et al., 2021). Disruption of the balance of intestinal microbial flora can lead to the colonization of various enteric pathogens such as bacteria and viruses in the intestine, resulting in diarrhea (Azarpajouh, 2023). The presence of specific bacteria, such as Bifidobacterium and Lactobacillus, in calf feces has been found to decrease the occurrence of diarrhea during the suckling period, leading to increased body weight (Oikonomou et al., 2013). Moreover, it has been noted that various genera from the Bifidobacteriaceae family and Bifidobacterium are common in healthy calves (Gomez et al., 2017). Appropriate feeding and rearing strategies that can alter the intestinal microbial flora of calves in their early stages of life in a way that positively influences their health should be implemented. This is a critical stage for calves, and it is essential to ensure that they receive colostrum in their early days. Colostrum accelerates bacterial colonization in the small intestine (Malmuthuge et al., 2015a). Delaying colostrum feeding by 12 hours reduces the numbers of Bifidobacteria and Lactobacillus in the colon of calves (Fischer et al., 2018). However, the intestinal flora in newborns is more flexible and unstable in the early stages of life, whereas it becomes more stable and less affected in the later stages of life (Malmuthuge et al., 2015b). The use of microbial-based products to manipulate the intestinal flora during the suckling period can improve calf health (Malmuthuge and Guan, 2017). Probiotics, in particular, are popular feed additives for enhancing intestinal health and reducing diarrhea cases in

young calves. Probiotics are live microorganisms that, when applied in sufficient doses, can modulate the balance and activities of gastrointestinal microflora, thus improving the host's health and promoting growth (Retta, 2016).

This review aims to describe the effects of probiotic use on the healthy rearing of calves in their critical early stages of life.

Probiotics

Probiotics are a general product that can be included in the diets of animals to enhance performance and/or reduce pathogenic bacteria. While the term "probiotic" was not used until 1960 to refer to substances produced by microorganisms that promote the growth of other microorganisms, the first studies recommending the use of probiotics by bacteria had been conducted. Initially, probiotics were defined as "live microbial feed supplements, which beneficially affect the host animal by improving its microbial balance in the intestinal tract" (Fuller, 1989). In addition, probiotics were described as "mono- or mixed cultures of bacteria applied to an animal or human when native bacterial characteristics are improved, beneficially affecting the host" (Havenaar and Huisin't Veld, 1992). Moreover, probiotics were defined as "viable defined microbial preparations or products, which when administered in adequate amounts, confer a health benefit on the host" (Markowiak and Slizewska, 2017). In recent years, the technical definition of probiotics has been expanded to encompass products containing microbes or their final products (e.g., fermented dairy products, etc.). Another recommended definition for probiotics is "a preparation or product containing a sufficient number of live, defined microorganisms, which alter the microflora (by implantation or colonization) in a compartment of the host and by that exert beneficial health effects in this host" (Schrezenmeir and de Vrese, 2001). Probiotics may refer to specific bacterial or fungal species, microbial cultures, enzyme preparations, culture extracts, or combinations thereof (Yoon and Stern, 1995).

To enhance intestinal health, promote earlier consumption of concentrated feed, and increase growth in preruminant calves, milk or starter feed for young calves can be supplemented with probiotics. Bacteria-based probiotics such as Lactobacillus spp., Enterococcus spp., and Bacillus spp. are commonly used in the nutrition of young calves (Uyeno et al., 2015).

Bacteria-based probiotics are commonly used to improve intestinal health, reduce diarrhea, and promote growth in suckling calves. Some of the commonly used bacteria-based probiotics include Lactobacillus spp., Bifidobacterium spp., Bacillus spp., and Enterococcus spp. The addition of bacteria-based probiotics provides a stable, nutrient-rich environment for intestinal flora, limiting pathogen invasion, and thus enhancing host digestive efficiency and mucosal immunity (Uyeno et al., 2015; Ma et al., 2018).

The mechanism of action of probiotics involves several key processes:

Lowering pH: Probiotic bacteria produce organic acids such as lactic acid, acetic acid, and formic acid, which help reduce the pH in the gut (Dhama et al., 2008; Broadway et al., 2014). This acidic environment inhibits the growth of pathogenic bacteria.

Preventing Pathogen Attachment: Probiotics play a role in preventing pathogenic bacteria from attaching to the intestinal surface and multiplying (Bahadıroğlu, 1997; Kocaoğlu Güçlü and Kara, 2009). This interference with pathogen attachment is a crucial defense mechanism.

Stimulating the Immune System: Probiotics stimulate the host's immune system, enhancing its ability to defend against infections (Rastall et al., 2005). This immune response can be beneficial in fighting off pathogens.

Increasing Anaerobic Microorganisms: Probiotics can boost the number of anaerobic microorganisms in the gut by consuming oxygen in the rumen (Kocaoğlu Güçlü and Kara, 2009). This helps create a more favorable environment for beneficial microorganisms.

Early Rumen Development: Some research suggests that probiotics contribute to the early development of rumen flora in young ruminants. This can lead to improved feed digestibility and help prevent the formation of enteropathogens that cause diarrhea (Krehbiel et al., 2003; Wallace and Newbold, 1995).

The combined actions of probiotics help maintain a healthy gut environment, enhance the host's defense mechanisms, and promote better nutrient utilization, ultimately benefiting the overall health and development of the host animal. The mechanisms of action of probiotics are presented in Table 2. The effects of probiotics indeed can vary depending on a range of factors, including the specific animal species, the type of bacteria used as probiotics, the dosage, the duration of use, and environmental conditions. Continuous use of probiotics has been reported to enhance their effects in various studies (Kurtoğlu et al, 2004; Antunovic et al, 2005).

While there are various theories regarding the mechanisms of action of live probiotics, and they are known to contribute to health by preventing or restraining the growth of harmful bacteria in the host's gastrointestinal tract, there is no precise mechanism of action established to date. It is believed that several factors may be involved in these mechanisms (Burçak and Yalçın, 2013).

Research in the field of probiotics is ongoing, and as our understanding of these mechanisms continues to evolve, we may gain more insights into the specific ways in which probiotics exert their beneficial effects in different animal species and under varying conditions.

Table 2: Mechanism of action of probiotics

BENEFICIAL IMPACT	MECHANISM OF ACTION
Contribution to lactose digestion	▪ Probiotics help digest lactose using bacterial lactase, an enzyme necessary for lactose digestion. This can be particularly useful for individuals with lactose intolerance.
Resistance to enteric pathogens	▪ Probiotics can enhance the immune system's response to pathogens. ▪ Probiotics change the conditions in the intestinal tract, such as pH, short-chain fatty acids, and bacteriocins, making it less favourable for pathogenic bacteria to thrive. ▪ Probiotics may modify toxin binding sites, reducing their effectiveness., ▪ Probiotics can alter the composition of the gut microbiota, favoring beneficial bacteria., ▪ Probiotics form aggregates in the intestinal mucosa, preventing pathogens from binding and invading., ▪ Probiotics can regulate mucin production, further reducing pathogen attachment.
Preventive effect on bowel cancer	▪ Probiotics may bind to mutagenic substances, reducing their harmful effects. ▪ Probiotics can block the activity of carcinogens, rendering them inactive. ▪ Probiotics might inhibit enzymes produced by intestinal microorganisms that generate carcinogens. ▪ Probiotics bolster the immune system, providing defense against cancer development.
Regulation of the immune system	▪ Probiotics enhance the body's general defense mechanisms against infections and tumor formation. ▪ Probiotics increase the production of IgA antibodies, boost the phagocytic activity of white blood cells, and enhance the specific immune response to antigens.
Allergy	▪ Probiotics can help prevent the passage of antigenic (allergenic) substances into the circulatory system, potentially reducing allergic reactions.

Source: Ceyhan and Alıç, 2012.

The mechanisms of action of probiotics are multifaceted and can be summarized as follows:

pH Regulation: Probiotics lower the pH in the gastrointestinal tract by producing organic acids, especially lactic acid. This acidic environment inhibits the growth of bacteria that thrive in neutral or basic conditions.

Redox Potential: Probiotics lower the redox potential, which prevents aerobic pathogens from utilizing oxygen and inhibits their growth.

Immune System Support: Probiotics have a positive impact on the immune system by increasing lymphocyte activity, regulating antibody production, and activating phagocyte cells and antigen-specific cells.

Toxic Substances: Probiotics prevent the proliferation of microorganisms that produce toxic ammonia and amines, thereby preventing the accumulation of these harmful substances.

Digestive Function Regulation: Probiotics help improve feed utilization by regulating digestive system functions. They also contribute to digestion by synthesizing B-group vitamins.

Enzyme Production: Probiotics produce various enzymes essential for digestion, including cellulase, xylanase, lipase, protease, beta-glucanase, and amylase. These enzymes work in conjunction with the host animal's digestive system enzymes, increasing the digestibility and energy value of feeds (Karademir and Karademir, 2003).

Guillot (2003) outlined two main mechanisms for the effects of probiotics:

a) **Nutritional Effects**: These are characterized by the reduction of metabolic reactions related to the production of toxic substances, stimulation of natural enzymes, production of vitamins, and antimicrobial substances.

b) **Health Effects**: These include increased resistance to colonization, competition for adhesion to the intestinal surface, and stimulation of the immune response. In summary, probiotics play a crucial role in strengthening the intestinal barrier, increasing resistance to harmful agents, and directly triggering the immune response in the intestinal mucosa (Karademir and Karademir, 2003; Guillot, 2003; Kocaoğlu Güçlü and Kara, 2009). These multiple mechanisms collectively contribute to the overall health and well-being of the host animal.

Effects on the Growth, Performance, and Health of Calves

Studies on the effects of bacteria-based probiotics on the growth, performance, and health of calves yield inconsistent results. While some studies emphasize positive effects, others indicate no significant impact. Key findings include:

Positive Effects: Some studies, such as Ratre et al. (2019), demonstrate that

probiotic application has a positive influence on the growth of calves. These effects may promote growth. In studies where calf diarrhea cases were high (Frizzo et al., 2010; Zhang et al., 2019), probiotics have been observed to have a positive impact on growth. It appears to have the potential to support calf health and growth.

Inconsistent Results: Other studies suggest that probiotic application has no significant effect on growth. For example, Seifzadeh et al. (2016), Satık and Günal (2017), and Vazquez-Mendoza et al. (2020) have obtained different results in this regard.

This inconsistency may be attributed to various factors, including different studies addressing a range of variables such as the probiotic strains used, dosages, application durations, and possibly environmental factors. Additionally, calf age, overall health status, and farm conditions can also influence the outcomes. In conclusion, further research is needed to better understand the effects of probiotics on the growth and health of calves. It should be considered that each probiotic strain and application method may yield different results in calves.

While the effects of bacteria-based probiotics on intestinal health have been considered, some researchers have also reported their impact on rumen function. Supplementation of *Lactobacillus rhamnosus* during the first 6 weeks of the suckling period led to increased microbial diversity within the rumen, altering the dominant bacterial composition and increasing bacterial counts in rumen fluid (Zhang et al., 2019). This resulted in increased volatile fatty acids and microbial protein concentration in the rumen, as well as a decrease in rumen pH. However, it should be noted that the improvements in rumen function may not be solely attributed to the direct effect of bacteria-based probiotics but could also be a result of increased concentrate feed intake. Probiotics lower the pH of the intestinal content, produce antibacterial substances to reduce the amount of ammonia and toxic amines, support the immune system, enhance the palatability of feeds, improve the digestion of carbohydrates, and aid in the synthesis of vitamins and amino acids. Changes in the microbiota of the digestive tract also impact the health of calves, making probiotics a valuable tool for manipulating rumen fermentation to improve live weight gain and feed utilization (Szabo and Szabo, 2003; Pinloche et al., 2013).

The supplementation of lactic acid bacteria has been shown to reduce cases of diarrhea in calves during the suckling period (Signorini et al., 2012). However, the type of milk or milk replacer fed to the calves plays a significant role in the occurrence of diarrhea cases. Calves fed with whole milk tend to experience fewer cases of diarrhea compared to those fed with milk replacers (Selim and Cullor, 1997).

Various studies have reported positive effects of supplementing calves with different bacterial strains, such as Pediococcus acidilactici, Enterococcus faecium, Lactobacillus acidophilus, Lactobacillus casei, and Bifidobacterium bifidum (Renaud et al., 2019). While bacterial-based probiotics have been associated with positive effects on diarrhea cases, the exact mechanisms behind these effects remain not fully understood. Most studies have focused on calves during their pre-weaning period. Furthermore, the effects are often linked to specific bacterial species used (Newbold et al., 1995). Bacterial-based probiotics interact directly with the host by modulating the intestinal immune system, increasing mucus production by goblet cells, enhancing tight junctions, and promoting the regulation of inflammatory responses. Lactic acid-producing bacteria can create more favorable conditions for commensal microorganisms by lowering intestinal pH. Additionally, bacterial-based probiotics can produce and release antimicrobial peptides like bacteriocins in the intestinal lumen, which may help reduce the risk of pathogen infections (Aragon et al., 2010; Cazzola et al., 2010; Wang et al., 2019).

The use of probiotic supplements containing Enterococcus faecium M74 in calf milk has been observed to have positive effects on calves. These supplements improved the live weight and daily live weight gain of the calves. Additionally, they significantly improved the fecal scores of the calves and reduced the frequency of diarrhea cases. This suggests that probiotics can have a positive impact on the intestinal health and digestive system of calves (Jatkauskas and Vrotniakiene, 2010).

Probiotic supplements have been shown to increase the live weight and daily weight gain of calves, but they can also lead to an increase in total dry matter and calf starter feed consumption, resulting in a decrease in feed efficiency. Furthermore, probiotic application can affect rumen fermentation by reducing acetate levels and increasing butyrate production. It can also increase the bacterial count in feces, leading to a reduction in fecal scores. In addition, probiotics can reduce the concentration of malondialdehyde, an oxidative stress marker, and enhance the total antioxidant status, which is an indicator of the antioxidant defense mechanism. This contributes to the improvement of calf health (Wang et al., 2023). This suggests that the addition of probiotics to calf milk during the suckling period can enhance calf growth, feed efficiency, and overall health. However, the effectiveness of probiotics depends on factors such as the dosage used and the composition of bacterial strains in the probiotic supplement (Wang et al., 2023). Direct feeding of animals with microbials can lead to changes in their intestinal bacterial populations. This can enhance resistance to diseases, reduce the spread of pathogens acquired through oral routes, improve intestinal immunity, reduce disease symptoms, and ultimately improve animal health (Timmerman et al., 2005; Adams et al., 2008; Wu et al., 2021).

In some studies, partially replacing milk with probiotic yogurt has been implemented, and this application increased dry matter intake, resulting in improvements in live weight gain and feed efficiency. Furthermore, it led to increased body measurements in calves. Additionally, this approach increased the blood lymphocyte ratio, reduced the neutrophil-to-lymphocyte ratio, and modulated the immune response (Noori et al., 2016). This indicates that using probiotic yogurt as a partial substitute for milk can have positive effects on calf growth, feed utilization, and immune system modulation.

The supplementation of probiotics to calf milk increased daily live weight gain and reduced the number of E. coli in feces in some studies, such as Roodposhti and Dabiri (2012). However, it did not significantly affect dry matter intake or the immune system. While there is sufficient data available to suggest that probiotics can improve calf performance and productivity parameters, there may not be enough data to support their role in enhancing calf health and reducing disease risk (Alawneh et al., 2020). In fact, Karamzadeh-Dehaghani et al. (2021) suggested that the impact of probiotic application on calf health is insignificant, and it has no significant effects on daily live weight gain and the immune system during the milk-feeding period, nor does it affect the frequency of calf diarrhea.

Conclusion

The use of probiotics in raising calves has been the subject of many studies, and research in this area continues. Calves, especially during the critical first four weeks of their lives, are highly susceptible to digestive and respiratory diseases. Probiotics used during this period can help reduce the incidence of digestive system diseases such as calf diarrhea by adhering to the intestinal mucosa before pathogenic microorganisms and preventing the attachment of pathogenic microorganisms. Additionally, the use of probiotics during the suckling period has shown different results in terms of daily live weight gain and feed efficiency. Therefore, there is not enough evidence to support the claim that probiotics promote calf growth and their use as a replacement for antibiotics. The dosage, frequency, and duration of probiotic applications may be one reason for the varying results. Thus, more research is needed to standardize probiotic applications and provide clear conclusions.

Statement of Conflict of Interest

The author(s) declare no conflict of interest for this study.

Author's Contributions

The contribution of the authors is equal

References

Adams, M.C., Luo, J., Rayward, D., King, S., Gibson, R., Moghaddam, G.H. (2008). Selection of a novel-fed microbial to enhance weight gain in intensively reare calves. Animal Feed Science and Technology 145: 41-52.

Alawneh, J.I., Barreto, M.O., Moore, R.J., Soust, M., Al-harbi, H., James, A.S., Krishnan, D., Olchowy, T.W.J. (2020). Systematic review of an intgervention: the use of probiotics to improve health and productivity of calves. Preventive Veterinary Medicine 183: 105147.

Antunovic, Z., Speranda, M., Liker, B., Seric, V., Sencic, D., Domacinovic, M., Sperandat, T. (2005). Ibfluence of feeding the probiotic Pioneer PDFMR to growing lambs on performances and blood composition. Acta Veterinaria 55(4): 287-300.

Aragon, G., Graham, D.B., Borum, M., Doman, D.B. (2010). Probiotic therapy for irritable bowel syndrome. Gastroenterology and Hepatology 6(1): 39-44.

Azarpajouh, S. (2023). Probiotics and its impact on calf gut health. Article No. 10-01 http://www.dairyglobal.net/dairy/calves/probiotics-and-its-impact-on-calf-gut-health/ (Erişim: 23.09.2023)

Bahadıroğlu, E. (1997). Aviguard (Doğal sindirim sistemi florası). Hayvancılık Yan Sanayi ve Veteriner Hekimliği Dergisi 17(1): 5-8.

Broadway, P.A.U.L., Carroll, J., and Callaway, T. (2014). Alternative antimicrobial supplements that positively impact animal health and food safety. Agriculture, Food and Analytical Bacteriology 4(2): 109-121.

Burçak, E., Yalçın, S. (2013). Buzağı beslemede probiyotiklerin kullanımı Derleme. Lalahan Hayvancılık Araştırma Enstitüsü Dergisi 53(2): 101-114.

Cazzola, M., Tompkins, T.A., Matera, M.G. (2010). Immunomodulatory impact of a synbiotic in Th1 and Th2 models of infection. Therapeutic advances in respiratory disease 4(5): 259-270.

Ceyhan, N., Alıç, H. (2012). Bağırsak mikroflorası ve probiyotikler. Türk Bilimsel Derlemeler Dergisi 1: 107-113.

Choct, M. (2009). Managing gut health through nutrition. British Poultry 50: 9-15.

Dhama, K., Mahendran, M., Tomar, S., Chaquhan, R.S. (2008). Beneficial effects of probiotics and prebiotics in livestock and poultry: the current perspectives. Intas Polivet 9(1): 1-12.

Donohue, D.J., Donohue, A.M., Tellez, G., Hargis, B.M. (2002). New evidence that specific probiotics may reduce intestinal foodborne pathogen colonization and improve performance. In: Proceedings, Arkansas Nutrition Conference, Arkansas, USA, 56-59.

Frizzo, L.S., Soto, L.P., Zbrun, M.V., Bertozzi, E., Sequeira, G., Armesto, R.R.,

Rosmini, M.R. (2010). Lactic acid bacteria to improve growth performance in young calves fed milk replacer and spray-dried whey powder. Animal Feed Science and Technology 157(3-4): 159-167.
Fuller, R. (1989). Probiotics in man and animals. A rewiew. Journal of Applied Bacteriology 66: 365-378.
Gomez, D.E., Arroyo, L.G., Costa, M.C., Viel, L., and Weese, J.S. (2017). Characterization of the fecal bacterial microbiota of health and diarrheic dairy calves. Journal of Veterinary Internal Medicine 31(3): 928-939.
Guillot, J.F. (2003). Probiotic feed additives. Journal of Veterinary Pharmacology and Therapeutics 26:52-55.
Hadimli, H. (2020). Buzağılarda bakteriyel hastalıklar. In: Buzağı sağlığı ve yetiştiriciliği, Ed. Erdem, H., Çiftçi, E., Işık, M.K., Yorgancılar, M.Ü., Yaralı, C. (Medisan Yayınevi, Ankara, 65-70).
Havenaar, R., Huis in't Veld, J.H.J. (1992). Probiotics: A general view. In: Wood B.J.B.: The lactic acid bacteria. Vol. 1. The lactic acid bacteria in health and disease. Chapman and Hall, Bew York, NY, 209-224.
Jatkauskas, J., Vrotnialiene, V. (2010). Effect5s of probiotics dietary supplementation on diarrhoea patterns, faecal microbiota and performance of early weaned calves. Veterinarni Medicina 55: 494-503.
Karademir, G., Karademir, B. (2003). Yem katkı maddesi olarak kullanılan biyoteknolojik ürünler (Derleme). Lalahan Hayvancılık Araştırma Enstitüsü Dergisi 43(1): 61-74.
Karamzadeh-Dehaghani, A., Towhidi, A., Zhandi, M., Mojgani, N., Fouladi-Nashta, A. (2021). Combined effect of probiotics and specific immünoglobulin Y directed against Escherichia coli on growth performance, diarrhea incidence, and immüne system in calves. Animal 15: 100124.
Kocaoğlu Güçlü, B., Kara, K. (2009). Ruminant beslemede alternatif yem katkı maddelerinin kullanımı: 1. Probiyotik, prebiyotik ve enzim. Erciyes Üniversitesi Veteriner Fakültesi Dergisi 6(1): 65-75.
Krehbiel, C.R., Rust, S.R., Zhang, G., Gilliland, S.E. (2003). Bacterial direct-fed microbials in ruminant diets: Performance response and mode of action. Journal of Animal Science 81(14-Suppl-2): E120-E132.
Kurtoğlu, V., Kurtoğlu, F., Seker, E., Coşkun, B., Balevi, T., Polat, E.S. (2004). Effect of probiotic supplementation on laying hen diets on yield performance and serum and egg yolk cholesterol. Food Additives and Contaminants 21(9): 817-823.
Lopes, R.B., Bernal-Cordoba, C., Fausak, E., Silva-del-Rio, N.J.P.O. (2021). Effect of prebiotics on growth and health of dairy calves: a protocol for a systematic review and meta-analysis. PLoS One 16: eo253379.
Ma, T., Suzuki, Y., Guan, L.L. (2018). Dissect the mode of action of probiotics in affecting host-microbial interactions and immunity in food producing animals. Veterinary Immunology and Immunıpathology 205: 35-48.
Malmuthuge, N., Chen, Y., Liang, G., Goonewardene, L.A. (2015a). Heat-treated colostrum feeding promotes beneficial bacteria colonization in the small intestine of neonatal calves. Journal of Dairy Science 98(11): 8044-8053.
Malmuthuge, N., Griebel, P.J., Guan, L.L. (2015b). The gut microbiome and its potential role in the development and function of newborn calf gastrointestinal tract. Frontiers in Veterinary Science 2: 36.
Malmuthuge, N., Guan, L.L. (2017). Understanding the gut microbiome of dairy calves: Opportunities to improve early-life gut health. Journal of Dairy Science 100: 5996-6005.
Markowiak, P., Slizewska, K. (2017). Effects of probiotics, prebiotics, and synbiotics on human health. Nutrients 9(9): 1021.
Newbold, C.J., Wallece, R.J., Chen, X.B., Mclntosh, F.M. (1995). Different strains of Saccharomyces cerevisiae differ in their effects on ruminal bacterial numbers in vitro and in sheep. Journal of Animal Science 73(6): 1811-18
Noori, M., Alikhani, M., Jahanian, R. (2016). Effect of parial substitution of milk with probiotic yoğurt of different pH on performance, body conformation and blood biochemical parameters of Holstein calves. Journal of Applied Animal Research 44(1): 221-229.
Oikonomou, G., Teixeira, A.G.V., Foditsch, C., Bicalho, M.L., Machado, V.S., Bicalho, R.C. (2013). Fecal microbial diversity in pre-weaned dairy calves as described by pyrosequencing of metagenomic 16S rDNA. Associations of Faecalibacterium species with health and growth. PloS One 8(4): e63157.
Quigley, J. (2018). Preweaning morbidity and mortality in the U.S. Calf note 203. http://www.calfnotes.com (Erişim: 23.09.2023).
Pinloche, E., McEwan, N., Marden, J.P., Bayourthe, C., Auclair, E., Newbold, C.J. (2013). The effects of probiotic yeast on the bacterial diversty and population structure in the rumen of cattle. PLoS One 8:1-9.
Rastal, R.A., Gibson, G.R., Gill, H.S., Guarner, F., Klaenhammer, T.R., Pot, B., Reid, G., Rowland, I.R., Sanders, M.E. (2005). Modulation of the microbial ecology of human colon by probiotics, prebiotics and synbiotics to enhance human health:an overview of enabling science and potential applications. FEMS Microbiology Ecology 52(2): 145-152.
Ratre, P., Singh, R.R., Chaudhary, S.S., Chaturvedani, A.K., Patel, V.R., Hanumant, D. (2019). Effect of prebiotic and probiotic supplementation on growth performance and body measurement in pre-ruminant Surti buffalo calves. The Pharma Innovation Journal 8(3): 265-269.
Retta, K.S. (2016). Role of probiotics in rumen fermentation and animal performance: a review. International Journal of Livestock Production 7(5): 24-32.
Renaud, D.L., Kelton, D.F., Weese, J.S., Noble, C., Duffield, T.F. (2019). Evaluation of a multispecies probiotic as a supportive treatment for diarrhea in dairy calves: A randomized clinical trial. Journal of Dairy Science 102(5): 4498-4505.
Roodposhti, P.M., Dabiri, N. (2012). Effects of probiotics and prebiotic on average daily gain, fecal shedding of Escherichia coli, and immüne system status in newborn female calves. Asian-Australasian Journal of Animal Science 25(9): 1255-1261.
Satık, S., Günal, M. (2017). Effects of kefir as a probiotic source on the performance and health of young dairy calves. Turkish Journal of Agriculture-Food Science and Technology 5(2): 139-143.
Schrezenmeir, J., de Vrese, M. (2001). Probiotics, prebiotics, and synbiotics approaching a definition. American Journal of Clinical Nutrition 73 (Suppl1.): 354S-361S.
Seifzadeh, S., Aghjeh-Gheshlagh, F.M., Abdi-Benemar, H., Saifdavati, J., Navidshad, B. (2016). The effects of a medical plant mixture and a probiotic on performance, antioxidant activity and weaning age of newborn Holstein calves. Iranian Journal of Applied Animal Science 6(2): 285-291.
Selim, S.A., Cullor, J.S. (1997). Number of viable bacteria and presumptive antibiotic residues in milk fed to calves on commercial dairles. Journal of the American Veterinary Medical Association 211(8): 1029-1035.
Signorini, M.L., Soto, L.P., Zbrun, M.V., Sequeira, G.J., Rosmini, M.R., Frizzo, L.S. (2012). Impact of probiotic administration on the health and fecal microbiota of young calves: a meta-analysis of randomized controlled trials of lactic acid bacteria. Research in Veterinary Science 93(1): 250-258.
Soltan, M.A. (2009). Effects of essential oils supplementation on growth performance, nutrient digestiblity, health condition of Holstein male calves during pre- and post-weaning periods. Pakistan Journal of Nutrition 8(5): 642-652.
Smith, G. (2015). Antimicrobial decision making for enteric diseases of cattle. Veterinary Clinics: Food Animal Practice 31(1): 47-60.
Szabo, J., Szabo, L. (2003). Prebiotics and probiotics in animal nutrition. (Hung J) Animal Production 52: 423-440.
Timmerman, H.M., Mulder, L., Everts, H., van Espen, D.C., van der Wal, E., Klaassen, G., Rouwers, S.M.G., Hartemink, R., Rombouts, F.M., Beynen, A.C. (2005). Health and growth of veal calves fed milk replacers with or without probiotics. Journal of Dairy Science 88: 2154-2165.
Tüzemen, N., and Yanar, M. (2004). Buzağı yetiştirme teknikleri. Atatürk Üniversitesi Ziraat Fakültesi Ders Yayınları No. 232.
Urie, N.J., Lombard, E.J., Shivley, C.B., Kopral, C.A., Adams, A.E., Earleywine, T.J., Olson, J.D., Garry, F.B. (2018). Preweaned heifer management on US dairy operations: Part V. Factors associated with morbidity and mortality in preweaned dairy heifer calves. Journal of Dairy Science 101: 9229-9244.
Uyeno, Y., Shigemori, S., Shimosato, T. (2015). Effect of probiotics/prebiotics on cattle health and productivity. Microbes and Environments 30(2): 126-132.
Vazquez-Mendoza, O., Elghandour, M.M.Y., Salem, A.Z.M., Cheng, L., Sun, X., Garcia-Flor, V.L., Pilego, A.B., Vazquez-Mendoza, P., Anele, U. (2020). Effects of sodium butyrate and active Bacillus amyloliquefaciens supplemented to pasteurized waste milk on growth performance and health condition of Holstein dairy calves. Animal Biotechnology 31(3): 209-216.
Wallace, R.J., Newbold, C.J. (1995). Microbial feed additives for ruminants. Probiotics: Prospects of use in opportunistic infections. Institute for Microbiology and Biochemistry, Herborn-Dill, Germany, 101-125.
Wang, Q., Lv, L., Jiang, H., Wang, K., Yan, R., Li, Y., Ye, J., Wu, J., Wang, Q., Bian, X., Yang, L. (2019). Lactobacillus helveticus R0052 alleviates liver injury by modulating gut microbiome and metabolome in D-galactosamine-treated rats. Applied Microbiology and Biotrechnology 103: 9673-9686.
Wang, L., Sun, H., Gao, H., Xia, Y., Zan, L., Zhao, C. (2023). A meta-analysis on the effects pf probiotics on the performance of pre-weaning dairy calves. Journal of Animal Science and Biotechnology 14(3),
Wu, Y., Wang, L., Luo, R., Chen, H., Nie, C., Niu, J., Chen, C., Xu, Y., Li, X., Zhang, W., (2021). Effect of a multispecies probiotic mixture on the growth and incidence of diarrhea, immüne function, and fecal micorbiota of pre-weaning dairy calves. Frontiers in Microbiology 12: 681014.
Yoon, I.K., Stern, M.D. (1995). Influence of direct-fed microbials on ruminant microbial fermentation and performance of ruminants- A review. Asian-Australasian Journal of Animal Sciences 8(6): 533-555.
Zhang, L., Jiang, X., Liu, X., Zhao, X., Liu, S., Li, Y., Zhang, Y. (2019). Growth, health, rumen fermentation, and bacterial community of Holstein calves fed Lactobacillus rhamnosus GG during the preweaning stage. Journal of Animal Science 97(6): 2598-2608.