International Journal of

JAEFS

Agriculture, Environment and Food Sciences

Volume 7 • Issue 3 • September 2023

e-ISSN: 2618-5946

edit PUBLISHING

www.jaefs.com

International Journal of
JAEFS
Agriculture, Environment and Food Sciences

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Int J Agric Environ Food Sci 7 (3) September 2023

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E-mail: editor@jaefs.com
E-mail: jaefseditor@gmail.com
Publisher: Gültekin Özdemir
Publishing Service: Edit Publishing
Address: Dicle Teknokent Yiğit Çavuş Mah. Silvan Yolu Üzeri Kat:2 No:26, Diyarbakır, Türkiye
WhatsApp: +90 850 309 59 27
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Journal Name	International Journal of Agriculture, Environment and Food Sciences
Web Page	https://dergipark.org.tr/jaefs - https://jaefs.com/
Abbreviation	Int. J. Agric. Environ. Food. Sci.
Subjects	Agriculture, Environment and Food Sciences
E-ISSN	2618-5946
DOI	10.31015
Publisher	Gültekin Özdemir
Publising Service	Edit Publishing
Language	English
Frequency	Quarterly (March, June, September, December)
Price Policy	Article Processing Charges (APCs) are paid by authors or their institution. This fee will be requested regardless of the acceptance/rejection condition of the article. https://dergipark.org.tr/en/pub/jaefs/price-policy
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RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(3): 468-475
DOI: https://doi.org/10.31015/jaefs.2023.3.1

Magnetopriming enhance germination and seedling growth parameters of onion and lettuce seeds

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Abstract
The main objective of this study was to improve seed quality by using magnetic field (MF) as a priming method to increase germination percentages (GP) and seedling emergence percentages (SEP) in onion and lettuce seeds. MF treatments on pre-hydrated seeds, significantly increased GP (up to 80% for onion, 87% for lettuce) and SEP (up to 76% for onion, 86% for lettuce) in both species. Magnetic treatments in other saying magnetopriming helped to increase germination and seedling emergence speed in treated seeds as well. The shortening of mean germination time allowed the treatments to establish uniform and well-developed seedlings. Our findings indicate that magnetopriming could be used as a pre-germination treatment before sowing.
Keywords: Magnetic field, Pre-germination treatment, Seed quality

Citation: Sari, M.E., Demir, I., Yildirim, K.C., Memis, N. (2023). Magnetopriming enhance germination and seedling growth parameters of onion and lettuce seeds. International Journal of Agriculture, Environment and Food Sciences, 7 (3), 468-475

Received: April 11, 2023
Revised: June 21, 2023
Accepted: June 23, 2023
Published Online: July 21, 2023
Revised: September 11, 2023

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Available online at
https://jaefs.com/
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INTRODUCTION

Pre-sowing seed treatments are called "pre-germination" or "seed priming" help to eliminate the problems that arise during the period from seed sowing to seedling emergence. Seed priming treatments improved germination related parameters like water uptake, speed of germination, fresh/dry weight and length of seedlings and vigor indices (Shine et al., 2011). Priming is an approach that involves hydration of seeds adequately to allow metabolic events before germination, despite preventing radicle emergence to occur (Heydecker and Coolbear, 1977; Paparella et al., 2015). Seed priming is an easy, low-risk and low-cost method, which is successfully applied either to poor germinating seed lots or to seeds, which are sown under different stress conditions (Sung et al., 1998; Jisha et al., 2013).

The most common priming techniques include hydro-priming, where controlled water intake is provided with only water, osmopriming using osmotic solutions (PEG, KNO3, KH2PO4 etc.) and matrixpriming using solid media such as vermiculite. Today, although hydro-priming and pre-germination treatments using different chemicals are widely used commercially, the use of physical priming treatments (magnetic field, ultraviolet rays, ultrasound, ionizing radiation etc.) as a priming method, especially in vegetable seeds, is not common. Physical methods that protect plant health and improve storage properties can be a good alternative to increase plant production (Aladjadjyan, 2012; Arujo et al., 2016). Physical priming methods came into prominence as environment-friendly technologies, not requiring washing of seeds after treatment and not producing waste material compared to osmotic priming methods.

Magneticbiology is a new multidisciplinary science with biophysics at its

center, covering various branches of science such as engineering, physics, chemistry and biology. This field studies the biological effects of electromagnetic (oscillating) or static low-intensity magnetic fields (MF) on tissues (without tissue heating) (Kataria, 2017a). MF is both a very old and a very recent area of plant research. MF is an unavoidable physical factor affecting living organisms. There is a MF of 50 microteslas (μT) naturally possessed by the earth around us (Belyavskaya, 2004). Some studies were conducted evaluating the response of plants to MF at different intensities, including near-zero (0-40 nT), low (up to 40 mT), and very high values (up to 30 T) (Teixeira da Silva and Dobranszki, 2015). Results show that biochemical, molecular, cellular and various effects on the whole plant occur in many plant species (Sen and Alikamanoğlu, 2016; Podlesna et al., 2019; Mohammadi and Roshandel, 2020; Jovičić-Petrović et al., 2021; Vashisth et al., 2021).

The biological effect of MF depends on the plant characteristics, such as species, variety, age of the material, number of chromosomes and structure of the target organ or tissue, the frequency of the current, the magnet poles, the MF intensity, the duration of the application and the correct dose of application. From previous studies, the most determining element for the MF effect for germination and seedling growth characteristics was the determination of a specific exposure period to the species (Aladjadjiyan and Ylieva, 2003; De Micco et al., 2014).

Studies investigating the effects of MF on plants have increased considerably. Magnetopriming has become a popular method among physical seed treatments before planting due to its economic and environment-friendly properties and proven benefits. The results of this non-destructive seed priming method increases seed germination and vigor, shoot development, fresh weight, plant height, normal seedling rate and ultimately increasing yield without harming the environment have been published for many plant species (Aladjadjiyan, 2002; Vasilevski, 2003; De Souza et al., 2006; Carbonell et al., 2008; Shine et al., 2011; Bhardwaj et al., 2012; Bilalis et al., 2013; Efthimiadou et al., 2014; Baghel et al., 2016; Kataria et al., 2017b; Razmjoo and Alinian, 2017; Ivankov et al., 2021). According to the previous studies, magnetopriming treatment not only enhances the germination percentages of seeds with low viability, but also gives good outcomes even under abiotic stress conditions (Rochalska and Orzeszko-Rywka, 2005; Thomas et al., 2013; Hozayn et al., 2018). For instance, Kataria et al. (2017c) found that the negative effect of salinity on germination and seedling vigour of maize and soybean can be alleviated by magnetopriming with static magnetic field of 200 mT for 1 h. MF treatment enhanced water uptake and improve activity of hydrolytic enzymes (α amylase and protease) in treated seeds as compared to untreated seeds under both non-saline and saline conditions. Ultimately, magnetopriming increased the percentages of germination and seedling vigour.

Onion and lettuce are widely consumed vegetables worldwide. These species are also important for Turkey in terms of seed trade, planting and production amounts and economic value. In 2021, approximately 2,625,000 tons of onion and 540,000 tons of lettuce were produced in 77,000 and 21,000 hectares, respectively, in Turkey (TUIK, 2022). However, seeds of these species are sensitive and have relatively short storage life. In order to reduce seed consumption and production cost physcial pre-sowing treatments can be used to improve germination and emergening traits. So, quantifying to possible effects of magnetic treatment on the germination and seedling emergence of onion and lettuce could have important implications for the seed sector and gene banks.

The aim of this study was to improve seed quality utilizing magnetopriming. In the present study, we tried to increase the germination and seedling emergence percentages and speed of lettuce and onion seeds, which have emergence and germination problems in plant production. Seeds of chosen species are sensitive to low-high temperature or salinity stress during germination and emergence period, and can lose their viability rapidly during the storage period. Although in recent years the studies on vegetable seed priming have increased in Turkey, not enough research has been performed on physical seed priming treatments.

MATERIALS AND METHODS

Seed material

Lettuce (*Lactuca sativa* L.) seeds were obtained from Ataturk Central Horticultural Research Institute-Yalova and onion (*Allium cepa* L.) seeds were provided by a private company (Beta Ziraat). Initial germination percentage (included abnormal seedlings) and moisture content of the seed lots were determined according to the ISTA (2016) rules (Table 1). Afterwards seeds were hermetically sealed and stored at 4°C until used.

Table 1. Germination percentages (%) and moisture contents (%) of seed lots.

Species	Variety	Germination rate (%)	Moisture content (%)
Onion	Panko	75	8.0
Lettuce	Grise Maraichere	88	5.0

Hydro-priming of seeds

Before hydro-priming treatment seeds were first moistened. Moistening was carried out by placing seeds in petri dishes containing two Whatman filter papers and spray moistening with water until seeds reached 30% moisture. Hydro-priming of lettuce and onion seeds was done by humidifying seeds in hermetic packages at 15^{0}C,

for 8 and 16 hours in dark conditions. Magnetopriming was rapidly done after the hydro-priming treatment.

Magnetic field generation and treatment

The applied static magnetic field was created by hollow rectangle magnets with a maximum and permanent strength of 800 militesla (mT) (Figure 1). Before the treatments, the magnetic field strength between the poles was measured and checked with a gaussmeter model Lakeshore 460-3.

(a) (b)

Figure 1. (a) Gaussmeter and static magnetic field generator (b) Seed sample between the poles.

Seeds of two different species were preliminarily hydro-primed at two different periods of time (8 and 16 hours), before magnetic exposure. Subsequently seeds were exposed to the static magnetic field of stationary 800 mT for four different time periods (1, 2, 5, and 10 mins). 350 healthy seeds were put in the petri dishes, 150 seeds for germination and seedling tests and 50 as substitute. Before magnetic field exposure, the seeds were placed at equal distances from each other in petri dishes without overlapping (Yinan et al., 2005; Aladjadjiyan, 2010; Feizi et al., 2012). The seeds were kept at room temperature throughout the experiment (22±1°C). When the magnetic field treatment was completed, seeds were dried at 25°C for 24 hours, control seeds were kept under similar conditions but in the absence of static magnetic field (local geomagnetic field only).

Germination test

Germination of the seeds was determined by using "between papers" method (ISTA, 2016). One hundred fifty seeds in three replications of 50 seeds each were placed between two layers of moist germination papers using with distilled water in controls and all other treatments. The germination papers with seeds were rolled and placed in a plastic bag to avoid surface evaporation of moisture. Plastic bags were placed in the germination incubator in an upright position. Germination papers were checked and moistened when necessary.

For lettuce seeds germination test performed at 20°C for 7 days and onion seeds 20°C for 12 days. For each treatment, the number of germinated seeds was scored two times per day and considered germinated when the radicle was approximately 2 mm long or more. On the final day of each practice, seedlings with normal and abnormal growth were determined and expressed as a percentages (%).

Mean germination time (MGT) was calculated based on the equation of Ellis and Roberts (1980).

$MGT = (\Sigma n \times D) / \Sigma n$

n: The number of seeds, which were newly germinated on that day

D: Number of days counted from the beginning of germination

Seedling emergence test

In the seedling emergence experiment, 50 seeds with 3 replicates from each seed lot were sown at a depth of approximately 2-2.5 cm in the seedling pots in which perlite:peat was used at a ratio of 1:2.

Seedling growth pots were placed in the growth chamber, with temperature adjusted to 22±2°C. The seedlings were counted daily. The tests were carried out with 16 hours of lighting and 8 hours of darkness. The relative humidity was at 60-65% and the light intensity was measured as 6500 lux. Seedling emergence tests were executed for 20 days. The occurence of cotyledon leaves on the surface was the criterion for emergence. At the end of the experiment, the seedlings were classified as: normal, or abnormal (no roots or shoots formed, glassy, spiral rootlets, necrosis detected in the cotyledon leaves or not developed at all, inseparable from the seed coat). Seedling emergence time (SET) was calculated using the same method used to determine MGT.

Statistical analysis

The data obtained at the end of the study was subjected to analysis of variance, and the differences between the means was compared using the LSD test at the 5% level. Obtained percentage values were subjected to $\sqrt{n}$ transformation. All statistical calculations were made using the JMP 8.0 package program.

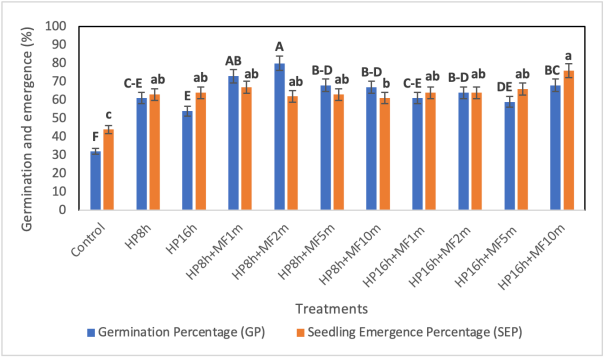
RESULTS AND DISCUSSION

Seed quality parameters

Variance analysis results of magnetic treatments of germination percentages (GP) ($P<0.01$), seedling emergence percentages (SEP) ($P<0.05$), MGT ($P<0.01$) and SET ($P<0.01$) showed statistically significant differences in onion seeds (Fig. 2). All treatments including sole hydro-priming treatments, significantly improved seed quality parameters (GP and SEP) compared to those of the control seeds. The highest GP result was observed in the hydro-priming HP8h+MF2m treatment (80%), significantly higher compared with the lowest GP obtained in the untreated control seeds (32%) (Fig. 2). In other treatments, results ranging from 54% (HP16h) to 73% (HP8h+MF1m) were achieved. Similarly, SEP was

positively affected by magnetic field treatments, the best result was obtained in HP16h+MF10m treatment (76%) compared to other treatments and control seeds (Fig. 2). As with the germination rate, the control group presented the lowest result (44%) in the SEP. In other treatments, close SEP values, 61% to 67% have been obtained.

Higher germination occured in first 8 hours HP and after magnetic treatments than HP treatments (8-16h) alone and 16 hours HP treatment before magnetic exposure. Seeds obtained higher germination percentages when exposure time reduced in HP 8h treatments.



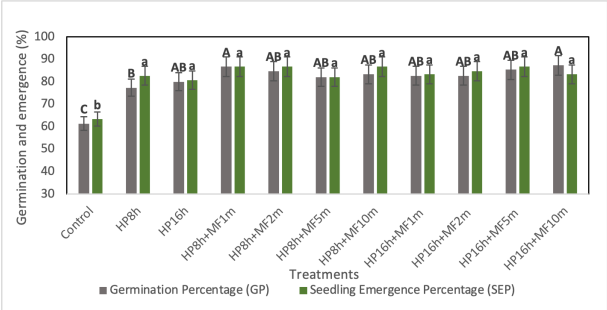
*The difference between the values shown in the similar columns with the same letter was not significant ($p < 0.05$).

Figure 2. Germination and seedling percentages of onion seeds. Bars in the same column represent SEM value.

A statistically significant difference was achieved between the effects of hydro-priming and magnetopriming on GP ($p < 0.01$), SEP ($p < 0.01$), MGT ($p < 0.01$) and SET ($p < 0.01$) in lettuce seeds (Fig. 3).

According to the results of the GP, the lowest result was 61% in the control group and the highest rate was 87% in the HP16h+MF10m treatment. In all treatments, germination values varying between 77% and 85% were significantly higher than the control group (Fig. 3). The lowest SEP (63%) was obtained by the control group, while the highest seedling emergence rate was 86% in 4 different treatments (HP8h+MF1m, HP8h+MF2m, HP8h+MF10m, HP16h+MF5m). In other treatments were included in the same group with the treatments that gave the highest statistical value (Fig. 3).

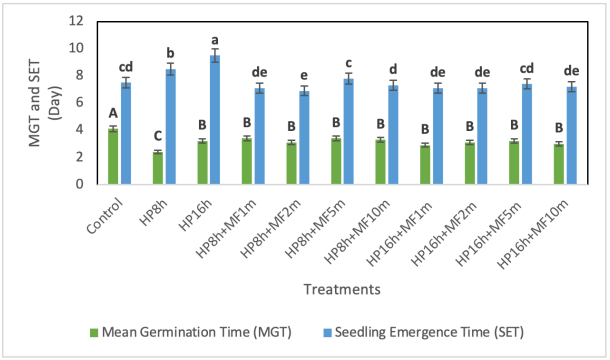
Observations were also made regarding the effect of magnetopriming on germination and seedling emergence speed. The MGT of all exposed seeds was quite lower than control seeds in other saying exposed seed showed better performance and nearly same results were observed for SET measurements (Fig. 4). As seen in Figure 4 which presents MGT of treated onion seeds; HP8h was the fastest treatment regarding the germination speed (2.4 day), and the slowest germination was in the control seeds (4.1 day). All other treatments attained similar results and were not statistically significanlty different.



*The difference between the values shown in the same columns with the same letter was not significant ($p < 0.05$).

Figure 3. Germination and seedling percentages of lettuce seeds. Bars in the same column represent SEM value.

According to the SET results, HP16h showed the slowest emergence (9.5 day), while the fastest emergence was 6.9 day in HP8h+MF2m treatment, which also provided the highest GP (Fig. 4). Overall, shorter seed exposures (1-2 minutes) resulted in higher germination and faster seedling emergence time.



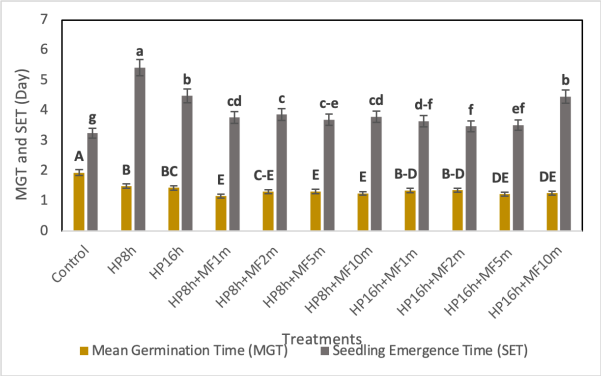
*The difference between the values shown in the same columns with the same letter was not significant ($p < 0.05$).
**The lettering in the mean germination time and seedling emergence time values indicates the "a" value which is the slowest.

Figure 4. Mean germination time (day) and seedling emergence time (day) of onion seeds.

The MGT of all exposed lettuce and onion seeds were quite lower than control (Fig. 5). While the slowest germination was 1.9 day in the control group, the fastest germinated seeds were observed in HP8h+MF1m treatment at 1.1 day. In other treatments, they presented values close to each other (1.2-1.4 days). Within the context of SET, the slowest (5.4 day) SET was in the treatment HP8h, the fastest seedling emergence was achieved in the control group with 3.2 day, contrary to all other parameters (Fig. 5).

Effects of hydro-priming on seed germination and seedling growth is well known for many years. Magnetopriming is successfully used for seed treatment, promoting higher germination ratio, well established seedling stage and ultimately increased crop yield (Shine et al., 2017; Ivankov et al., 2021; Ziaf et al., 2022). Although magnetopriming is known as a physical priming method

applied to dry seeds, there are also few studies where it is applied to pre-soaked or hydrated seeds with successful results (Florez et al., 2007; Feizi et al., 2012; Kubisz et al., 2012; Mousavizadeh et al., 2013). According to Feizi et al. (2012), exposure of hydro-primed and dry tomato seeds to magnetic field significantly improved mean germination time, root, shoot and seedling length and vigor index compared to untreated seeds. Kubisz et al. (2012) found that 20 mT magnetic field treatment after 12 hours soaked onion seeds (cultivar Eureka) led to increased energy of germination (40 to 62%) and germination capacity (by about 6%). These results are in line with our findings, enhancement in the germination and seedling parameters were observed after hydro-priming, and also increased further by hydro-priming followed with magnetic field treatment. We presumed that wet seeds may respond better to magnetopriming since physiological activity was initiated. However, hydro-priming prior to magnetopriming did not provide any extra advantage to the treatment. Particularly, given wet seeds required drying after magnetic treatment causing additional operations and expenses.



*The difference between the values shown in the columns with the same letter was not significant ($p < 0.05$).
**The lettering in the mean germination time and seedling emergence time values indicates the "a" value which is the slowest.

Figure 5. Mean germination time (day) and seedling emergence (day) time of lettuce seeds.

Germination and seedling growth parameters in our results were in agreement with the observations reported for magnetopriming of onion and lettuce seeds (Soltani and Kashi, 2004; De Souza et al., 2008; Kubisz et al., 2012; Mousavizadeh et al., 2013; Hozayn et al., 2015; Zalama and Fathalla, 2020). In the present study, magnetopriming not only helped with seed germination but also promoted proper vigour and desired seedling growth. Alexander and Doijode (1995) demonstrated that, germination and seedling emergence rate, root and seedling lengths, fresh and dry weights were increased in low viability rice and onion seeds after magnetic field treatment. In onion seeds an increase in germination (36.6%) and emergence (127.3%) was recorded compared to low viability control seeds. This is valuable in getting faster and well-developed seedlings in field conditions. Capability of lettuce seed water uptake increased when treated with a stationary magnetic field ultimately increasing the germination rate. Latef et al. (2020) reported that, growth parameters were increased by static magnetic field (SMF) treatment. They stated that this positive impact of SMF on lettuce was due to the development of osmoregulation substances, secondary metabolites, stimulation of the reactive oxygen species scavenging system via the improvement of enzymatic and non-enzymatic antioxidants and hence, the mitigation of lipid peroxidation, thereby improving the quality of lettuce leaves.

The positive effects of magnetopriming were also shown on accelerating seed germination and seedling growth (Martinez et al., 2009; Feizi et al., 2020; Ghanbarpouri et al., 2021; Alvarez et al., 2021). Our study displayed the potential of magnetic seed stimulation to decrease germination and seedling emergence time. Shorter seed exposures (1-2 minutes) resulted in higher germination and faster seedling emergence time in onion seeds. Coherent results were obtained in another study in which germination and seedling growth parameters and mean germination time were also measured. The study conducted with 2 different onion seed lots, MGT were decreased 30 minutes/60 mT treatment in high quality seeds and 60 minutes/30 mT treatment density in low quality seeds compared to the control group (Hozayn et al., 2015). Similar results were obtained in our study in lettuce seeds, while the slowest germination was 1.9 days in the untreated seeds, the fastest germinated seeds were monitored in HP8h+MF1m treatment at 1.1 days. Contrary to all other parameters, the fastest seedling emergence was achieved in the untreated lettuce seeds with 3.2 days (Fig. 5). Garcia et al. (2001) point out that, exposed lettuce seeds germinated earlier than the control seeds which were treated 1-10 mT stationary magnetic field, which could be due to increasing the water uptake into the cells. In the same way, Shine et al. (2011) also showed that, seeds treated with magnetic field of 250 and 300 mT for 30 min showed a significant increase in water uptake (54% and 90% respectively) after 40 minute of imbibition. This can be due to the small invisible perforations on the seed coat which allows water uptake faster so increase germination rate. However, despite the many studies conducted to understand the effect of magnetic field treatments on living organisms, the exact mechanism, especially in plants, remains unclear. According to the literature, several theories have been proposed including, influencing the permeability of cell membrane and ion transport, causing changes in osmotic pressure and capacity of cellular tissue and then accelerating seed water absorption process (Labes, 1996; Garcia et al., 2001; Soltani et al., 2006; Kataria et al., 2015), improving seed coat membrane integrity and reducing the cellular leakage and electrical conductivity (Vashisth and Nagarajan, 2010), increasing free Ca^{2+} concentration which possibly signals the cells to enter into an early

mitotic cycle, enhancing enzymes activity in seed (Shine et al., 2011).

This study has shown that, sole hydro-priming and magnetopriming combinations with hydro-priming led to a considerable enhancement in germination and seedling emergence rate of both onion and lettuce seeds (Fig. 2 and Fig. 3). Also ensured increased speed in the germination and seedling emergence days, except for lettuce seedling emergence time (Fig. 4 and Fig. 5). Magnetopriming presumably stimulates the seeds and triggers the germination and seedling emergence processes. However, the effect of the treatment still needs to be confirmed with a larger number of seed lots and in the field environment.

CONCLUSION

Increasing the seed quality with pre-sowing treatments is considered an important step for plant production. Pre-sowing treatment of seeds with magnetic field has been proven to have beneficial effects on many crops. Our results suggest that magnetopriming of pre-hydrated onion and lettuce seeds could improve seed quality parameters compared to untreated seeds. Magnetopriming can also be used on dry seeds that have not been moistened, this provides more economical and faster results by bypassing the moistening and drying stages applied in conventional priming methods.

Determining the effectiveness of MF treatments in different species that have not been studied before will produce important results for seed science and technology. Since the exposure time and dose may vary according to the species and even varieties, the determination of the appropriate dose and time is crucial for magnetopriming.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

All authors declare that they have no conflicts of interest

Author contribution

Mustafa Emre Sarı (M.E.S), Ibrahim Demir (I.D) and Nurcan Memiş carried out experimental part of the study. M.E.S., I.D. and Kutay Coşkun Yıldırım (K.C.Y) reviewed the manuscript, K.C.Y done statistical analysis, M.E.S and I.D. designed the experiments and M.E.S conceived the principal idea and wrote the paper.

Ethical approval

Ethics committee approval is not required.

Funding

This study did not obtain any external funding.

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgement

Prof. Dr. Yalçın Elerman and his physics labratory team for technical support in using magnetic field device. This publication has been prepared by using the results of Mustafa Emre Sarı's PhD thesis.

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RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(3): 476-487
DOI: https://doi.org/10.31015/jaefs.2023.3.2

Plate waste in food service: Nudging intervention

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Citation: Ergul, Y., Arslan, S., Atan, R.M., Sahin, K. (2023). Plate waste in food service: Nudging intervention. International Journal of Agriculture, Environment and Food Sciences, 7 (3), 476-487

Received: March 15, 2023
Revised: June 20, 2023
Accepted: June 22, 2023
Published Online: July 21, 2023
Revised: September 11, 2023

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Available online at
https://jaefs.com/
https://dergipark.org.tr/jaefs

Abstract
Food waste is a threat to global sustainability. The study aim is to determine the effect of nudge interventions to reduce food waste at lunch. In this experimental study, which was planned in this cross-sectional type, survey data on food waste attitudes were collected from the participants. Volunteers who benefited from the cafeteria service were assigned to the survey (n=157), excluding those who did not use the cafeteria regularly, had a history of food allergy, and declared a diagnosis of chewing-swallowing disorder or celiac disease (n=3). The sociodemographic data and subjective waste amounts of the participants based on the survey, the waste was measured rationally in the pre-nudging and nudging period. The primary outcome is that nudge interventions applied to reduce food waste at lunch can contribute to the total amount of food waste and in which foods to reduce waste. Despite the nudging intervention, the total amount of waste increased, only vegetarian food and bread waste decreased. These changes are not statistically significant. According to the subjective evaluation data, vegetarian food and bread group foods are wasted more. Subjective and rational evaluation results are inconsistent. Short-term nudging intervention is not effective and different strategies are needed to reduce the amount of food waste.
Keywords: Food waste, Plate waste, Nudging, Food Service

INTRODUCTION

Food waste; It covers all losses caused by agricultural production of food components, losses in processing, transportation, and storage processes of edible foods, and inability to consume foods offered for consumption in some way (purchase more than necessary, improper storage conditions, expired products, etc.) (Heller & Keoleian, 2015). Food losses and waste, along with hunger and malnutrition, rank first among global nutrition problems. Food losses and waste damage the economies of countries as well as all other components in the food chain. In addition, food waste directly threatens environmental, social, and economic sustainability. For this reason, food waste has recently become one of the important issues that come to the fore all over the world (Godfray et al. 2010). The Food and Agriculture Organization (FAO) reported that approximately 30% of the food produced for human consumption in the world is lost or thrown away every year (Parfitt et al. 2010). Approximately 1.3 billion tons, in other words, 190 kg of food per person is thrown away every year (Wu et al. 2019). Under the United Nations Sustainable Development Goal 12.3, it calls for a 50% reduction in global food waste per capita at retail and consumer levels by 2030 (Hanson et al. 2015). Reducing food waste is one of the key points identified in the future nutrition strategy of 9 billion people. (Richardson et al. 2021; Ravandi et al. 2019)

It remains unclear how much food is wasted in mass nutrition systems and what factors are affected by wasting behavior (Wu et al. 2019). Studies in the field of waste emphasize the need for more studies that reflect both subjective and objective data on this issue (Hanson et al. 2015; Richardson et al. 2021; Ravandi et al. 2019; Thongplew et al. 2021; Lorenz et al. 2017; Aires et al. 2021; Leverenz et al. 2021; Ellison et al. 2019). Studies on mass feeding focus on school canteens and often describe the wasteful behavior of adolescents and young adults (Wu et al. 2019; Richardson et al. 2021; Lorenz et al. 2017; Ellison et al. 2019; Whitehair et al. 2013). There is a need for constructive and consistent methods to reduce waste (Whitehair et al. 2013).

Nudging is one of the strategies to reduce food waste. 'Nudging' are adjustments in the electoral environment that can change behavior without eliminating individuals' choices or without economic incentives (Thaler et al. 2009). Lin et al. evaluated the concept of nudges in two categories (Lin et al. 2017). Although Type 1 nudges aim to change the behavior of the consumer at the point of decision-making, it is not intended for the decision-maker to realize it (For example, placing healthy drinks on the upper shelves and unhealthy drinks on the lower shelves on refrigerators). Type 2 nudges, on the other hand, aim to encourage long-term reassessment of how the individual made a certain decision (For example, to encourage walking or to train to abstain from alcohol) (Lin et al. 2017). In many studies, it is seen that nudges interventions are applied to reduce food waste (Ellison et al. 2019; Whitehair et al. 2013; Pinto et al. 2018; Ahmed et al. 2018). In addition, although educational campaigns are popular due to their ease of implementation and low cost, the effectiveness of these interventions remains unclear. However, a systematic review stated that most studies do not evaluate the level of food waste (Metcalfe et al. 2020).

In this research, the primary outcome aims to determine the effect of nudging interventions applied to reduce food waste at lunch on the level of waste. In this context, we conducted a field experiment in the cafeteria of a university that receives catering services. Our study aims to reduce food waste by increasing awareness of food waste among university staff through informative and educational posters, brochures, and flyers placed in the cafeteria where food is served. Secondly, by using individual surveys before nudging, it is aimed to subjectively and rationally determine consumer food wastes for food waste and to define the factors affecting the amount of waste.

DATA AND METHODS

Study type and sample

This research was carried out as an experimental field study, which was planned in this cross-sectional type, in a state university staff cafeteria located in the Marmara Region of Turkey and receiving catering services. This study consists of 3 stages. All processes involved in this study are shown in Suppl 1. In the first stage, there is a survey application regarding the sociodemographic characteristics and subjective waste disposal status of the participants. In the second stage, the pre-nudge period, plate wastes were collected without any intervention, in the third stage, nudging was made and the collection of waste continued. The wastes collected in the second and third stages are defined as the rational waste amount. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and approval was obtained from the Bandirma Onyedi Eylül University Health Sciences Non-Interventional Research Ethics Committee (Date: 07.02.2022 and Approval number 2022-10).

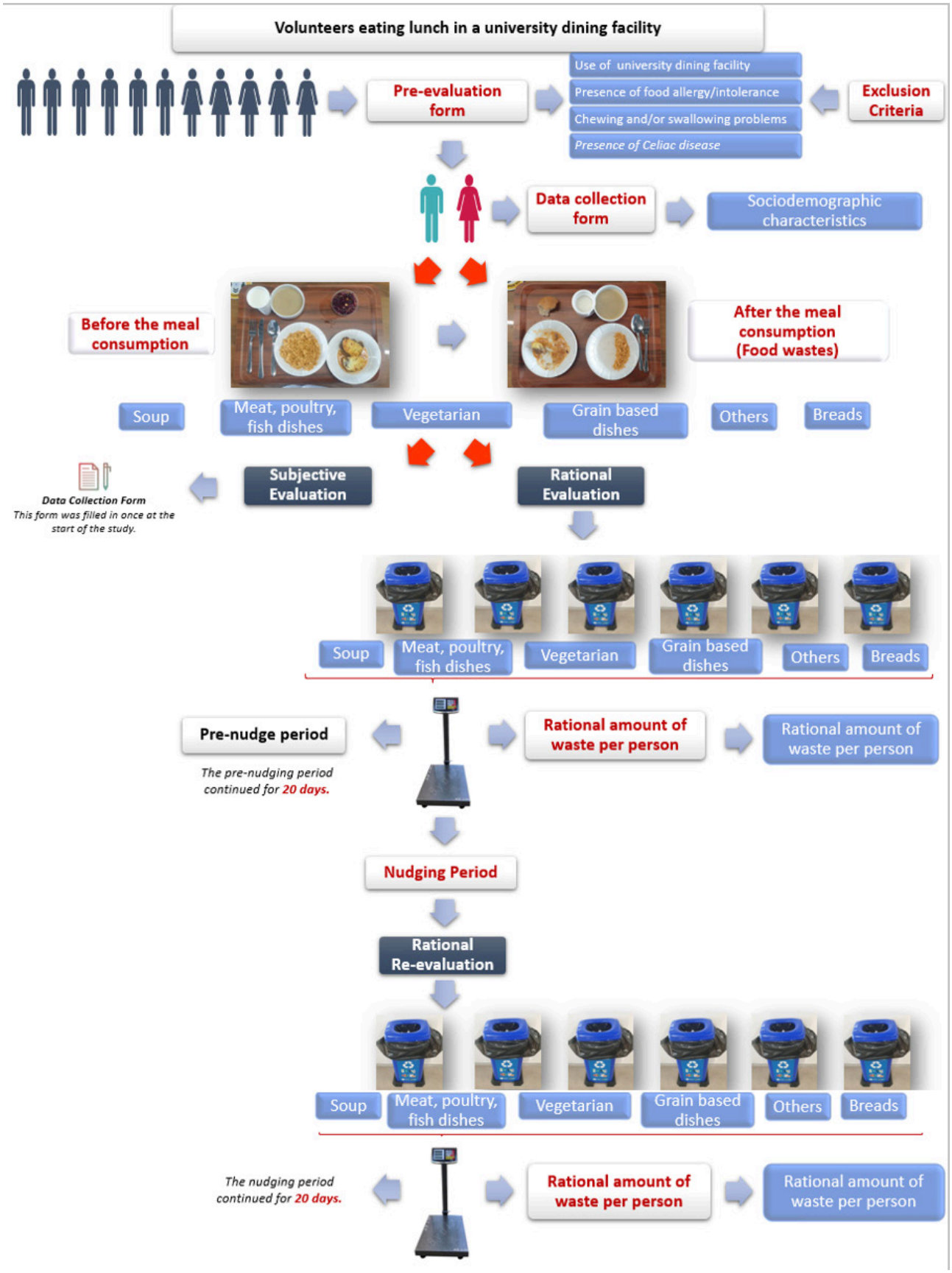
A questionnaire was applied to the participants to determine the subjective waste amounts, and some cases were excluded. Volunteering was essential to participate in the research (n=157). Those who did not use the cafeteria regularly had a history of food allergy and declared that they had a chewing-swallowing disorder or celiac diagnosis by the physician were excluded from the survey part of the study (n=3). In the evaluation of rational waste, it is aimed to reach all individuals who consume their lunch. No evaluation was made for days with Public Holidays. Since it was not known which plate belonged to which participant during the waste collection period, no exclusion was made.

Intervention procedures

Design

Firstly, a data collection form was distributed to the participants in 20-25-minute face-to-face interviews. This form consisted of 25 questions and collected information about preliminary evaluation, general characteristics, subjective evaluation, and factors affecting the amount of waste. The data form was filled in for once on the first step of the study. In the data collection form, questions were asked about the pre-evaluation questions regarding the inclusion criteria, general characteristics, subjective evaluation of waste disposal, and influencing factors. The data collection form was prepared by making use of similar studies in the literature (Lorenz et al. 2017; Aires et al. 2021).

Secondly, the process steps are listed for the rational evaluation, the wastes from the lunch were meticulously collected during the study period, in accordance with the Covid-19 precautions, with each type of food in a separate container. The wastes were collected from the dinner plates of the individuals participating in the study by the researcher. The waste collection time is a process that takes about 2 minutes at the end of the food consumption of individuals. The amount and types of waste were recorded on a daily form by the reserachers. While preparing the form, similar studies in the literature

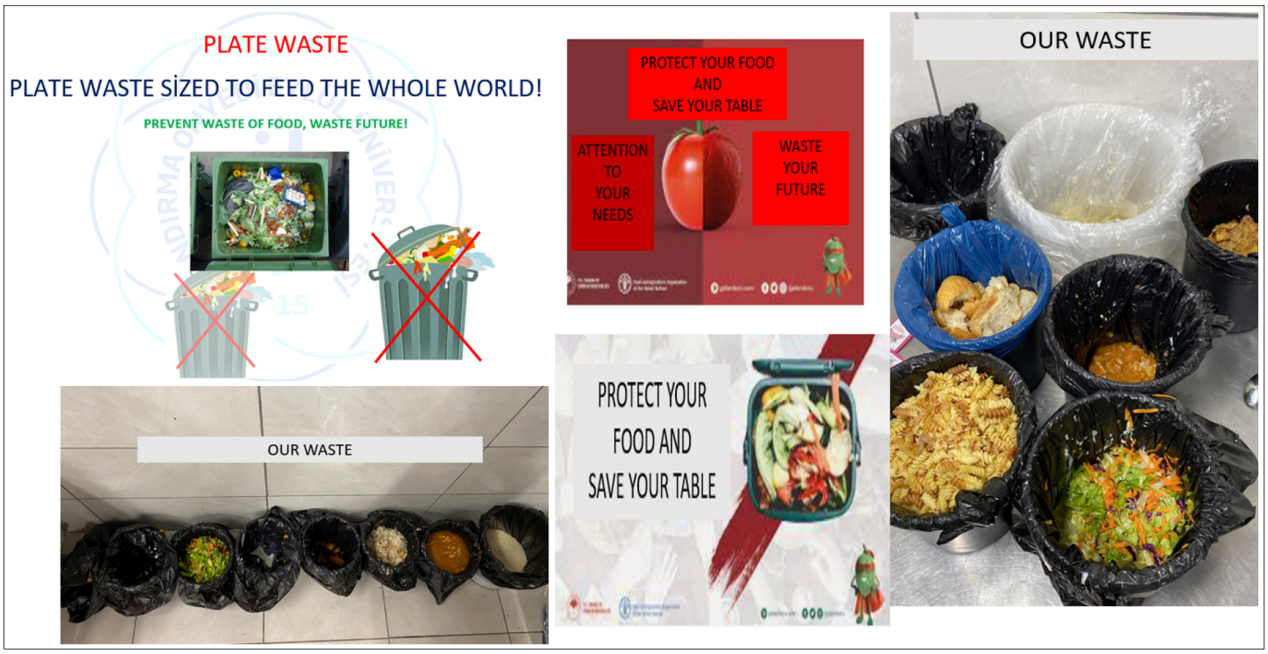

Volunteers eating lunch in a university dining facility
Pre-evaluation form
Use of university dining facility
Presence of food allergy/intolerance
Chewing and/or swallowing problems
Presence of Celiac disease
Exclusion Criteria
Data collection form
Sociodemographic characteristics
Before the meal consumption
After the meal consumption (Food wastes)
Soup
Meat, poultry, fish dishes
Vegetarian
Grain based dishes
Others
Breads
Subjective Evaluation
Rational Evaluation
Data Collection Form
This form was filled in once at the start of the study.
Soup
Meat, poultry, fish dishes
Vegetarian
Grain based dishes
Others
Breads
Pre-nudge period
The pre-nudging period continued for 20 days.
Rational amount of waste per person
Rational amount of waste per person
Nudging Period
Rational Re-evaluation
Soup
Meat, poultry, fish dishes
Vegetarian
Grain based dishes
Others
Breads
The nudging period continued for 20 days.
Rational amount of waste per person
Rational amount of waste per person

Suppl 1

were used (Lorenz et al. 2017; Elhatusaru 2018).

The waste collection period was planned to be five days a week for 8 consecutive weeks from the beginning of the study. The first 20 days period is the pre-nudge period, the second 20 days is the nudging period. For lunch, participants receive a choice of soup, cereal-based meal, vegetarian or meat-poultry-fish, and two other food types. Other foods are in the form of pairs such as yogurt and salad or salad and compote or pickles and ayran or dessert and fruit. In our research, these pairs are specified as other 1 and other 2. The bowl containing the leftovers of soup, main course (meat-poultry-fish), vegetarian meal, grain-based meals (rice/pasta/pastry), bread, and other foods (such as yoghurt, ayran, desserts, salad) was weighed with a calibrated scale in a tared manner. The calibration of the scales used was checked before the research. The container containing the wastes was weighed with a calibrated electronic scale with a precision of 0.1 g. Only complex dishes, such as dishes that cannot be mixed and the amount of waste that cannot be determined separately, are not included in the counting and total waste amounts. While determining the rational waste amounts, the samples in which the type and amount of waste could not be distinguished were not included in the total number of meals.

The Daily Record Form of Waste Amount and Types, which includes the rational evaluation of waste, was filled in by the researchers. The number of meals



Supp 2

has also been revised in cases where it is likely to be encountered (such as accidentally spilling the food). The average amount of waste per person was calculated by proportioning the collected waste and the number of people who consumed lunch that day. Afterward, nudging interventions were made to all participants on reducing waste and preventing waste, with stimuli such as posters, brochures, flyers, e-mails, and messages. The aim was to nudge in individuals with the size of waste by revealing the factors leading to food waste and capturing the attention of the participants with rhetorical questions and cultural statements and idioms about food waste. After the nudging, individuals will not be evaluated subjectively (no data collection form will be applied), and rational evaluation has been made by collecting waste only.

Nudging

In recent years, research on waste reduction by nudging intervention has become popular. Consumer behavior is the main subject of nudging research, which is preferred as an alternative, low-cost, and simple technique to reduce the amount of food waste at home or outside the home (Von Kamake & Fischer, 2018; Vidal et al. 2022; Qi et al. 2022). Some sample stimuli (brochure, mini handout, poster, email content, message content, etc.) prepared regarding the awareness intervention are presented in Suppl-2. While preparing the sample stimuli, the visuals on the publicly accessible www.SofranaSahipCik.com website prepared by the Ministry of Agriculture and Forestry for the prevention and reduction of food losses and waste were taken as a basis. Awareness-raising intervention and rational evaluation of waste continued for 20 days.

Variables

Independent variables: Individual characteristics such as age, gender, marital status, income level, household type, body mass index, type of food, and days of the week were determined.

The definitions of some variables are given as follows.

Type of food: The meals served at lunch will be evaluated as soups, main courses by evaluating the general menus of the establishment, vegetarian food, rice-pastas, and other (dessert with milk/sorbet, salad, fruit, yoghurt, ayran). Such a grouping is also present in similar study models related to the field (Lorenz et al. 2017; Aires et al. 2021).

Subjective waste rate: By using the subjective waste evaluation questionnaire, it will be questioned how much waste individuals leave at lunch from the food groups given as examples in the photographs. According to the plate size, it is scaled as whole, half, one-quarter, and three-quarter. While creating the related questions and scale, the methodology of a similar study in the literature was used (McCray et al. 2018).

Rational amount of waste per person: Each type of food will be collected in a container and the total amount will be weighed. The value obtained by proportioning the result of weighing to the number of plates used in that dish is the rational amount per person.

Rational amount of waste per person (g) = Total amount of waste (g) (according to the type of food) / Number of plates used

For example, if the total amount of waste for soup is 9 kg, the number of plates used is 110 bowls; 9.000g/110 bowl=81.8g soup is the rational waste amount per person.

Percentage of the rational waste amount per capita (%): It is found by dividing the rational per capita waste amount by the meal portion size.

Percentage rational waste amount per capita = (Rational waste amount per capita/Meal portion size) x100

Data analysis

Statistical evaluation of the data was made with the IBM SPSS (Statistical Package for Social Sciences-Chicago, IL, USA) 23.0 statistical package program. The normal distribution of data for numerical variables was evaluated with the Shapiro-Wilk test. Paired Samples t-test or Wilcoxon test according to the distribution of the data in case of repetition of two variables to compare the results obtained in the nudging period. In all statistical analyzes, the level of significance was taken as $p<0.05$.

RESULTS

Data on the general characteristics of the research population are presented in Table 1. The mean age and body mass index of the research group were found to be 35.27±8.88 and 24.63±4.53, respectively. It is seen that the research group varies in terms of social variables such as education, income, occupation, and household type. Individuals with different characteristics were included in the study in terms of the presence of disease and the status of applying a special diet. The statements of the participants regarding the subjective waste assessments are shown in Table 2. When the subjective evaluations of each food group/plate were questioned separately, it was found that about 80% of the participants did not leave any waste for the soup group. The number of people who leave all of them as waste for soups is quite low. When evaluated for meat-poultry-fish dishes, the rate of those who leave more than half of their plate as waste is around 15%. There is a similar trend for Vegetarian and Grain-based meals. The rate of leaving more than half of the portion size of other foods served on plates and glasses as waste was determined as 12%. The bread group was determined as the food left as waste the most. One out of every three participants left some of their bread as waste, and the rate of those who left all of their bread as waste was found to be relatively high compared to other food groups.

Table 1. Sociodemographic characteristics of the participants

Variables	Range	n	Valid percentage (%)
Gender	Male	102	65.0
	Female	55	35.0
Marital status	Married	82	52.2
	Single	74	47.1
	Not specified	1	0.6
Age	18-29	44	28.0
	30--40	60	38.2
	41 and above	44	28.0
	Not specified	9	5.7
Using the cafeteria	Everyday	56	35.7
	1-2 times a week	34	21.7
	3-4 times a week	57	36.3
	Every fifteen days	5	3.2
	1 time per month	5	3.2
Education	Primary education	5	3.2
	High school	10	6.4
	Associate-bachelor	58	36.9
	Graduate	83	52.9
	Not specified	1	0.6
Income status	Not declared	13	8.3
	Minimum wage and below	10	6.4
	Above the minimum wage and twice	45	28.7
	More than twice the minimum wage	89	56.7
Employment	Not declared	48	30.6
	Administrative	78	49.7
	Academician	17	10.8
	Employee	10	6.4
Household Type	Alone	58	36.9
	Living with family-friend	99	63.1
A special diet situation	Yes	10	6.4
	No	147	93.6
Presence of disease	No	126	80.3
	Yes	31	19.7
Constantly variable	**Mean±SD**	**Min-Max**	
Age (n=148)	35,27±8,88	18-62	
BKI (n=155)	24,63±4,53	15.47-42.93	

The proportional values of the answers given by the participants regarding the reasons for leaving waste are summarized in Figure 1. For all reasons, participants had the opportunity to give more than one answer. There are different reasons for taste. Tasteless, hot, smell, and salty were among the most common causes. In terms of structure, sticky and tough have been the most stated reasons. In terms of cooking methods, cooking with the boiling method and raw food are stated as the reason for leaving waste. Large portion sizes, incompatible menus, not being hungry enough, and not being hot enough are among the frequently mentioned reasons. Apart from these, being on a diet and concerns about hygiene were also stated as reasons for leaving waste.

Total waste amounts according to food groups related to pre-nudge and nudging periods are presented in Table 3. The changes in the amount of waste in both periods were not statistically significant ($p>0.05$). Considering the average values, it was seen that the average decreased only in bread and vegetarian meals, but this change was not statistically significant. It is seen that the average of the total waste amount value is higher than the pre-nudge in the nudging period, but this change is not statistically different.

The rates of subjective waste assessments according to sociodemographic characteristics are shown in Figure 2. Considering the gender variable, it was determined

Table 2. Waste rates based on the visual statement

Meals	Waste rates based on visual statement n (%)				
Soups 	 **No Waste**[a]	 **¼ waste**	 ½ waste	 ¾ waste	 **All waste**
	125 (79.6%)	15(9.6%)	10 (6.4%)	7(4.5%)	1(0.6%)
Meat-poultry-fish dishes 	 **No Waste**	 **¼ waste**	 ½ waste	 ¾ waste	**All waste**
	91(57.9%)	43(27.4%)	12(7.6%)	8(5.1%)	3(1.9%)
Vegeterian[b] 	 **No Waste**	 **¼ waste**	 ½ waste	 ¾ waste	**All waste**
	99(63%)	34(21.7%)	9(5.7%)	6(3.8%)	9(5.7%)
Grain based dishes 	 **No Waste**	 **¼ waste**	 ½ waste	 ¾ waste	**All waste**
	102(64.9%)	31(19.7%)	18(11.5%)	5(3.2%)	1(0.6%)
Others 	  **No Waste**	  **¼ waste**	  ½ waste	  ¾ waste	 **All waste**
	117(73.3%)	24(15.3%)	7(4.5%)	6(3.8%)	5(3.2%)
Breads 	**No Waste**	 **¼ waste**	 ½ waste	 ¾ waste	 **All waste**
	102(65%)	14(8.9%)	10(6.4%)	5(3.2%)	26(16.6%)

[a] In all food groups, 1-3 people responded as "I never choose that food in order not to generate waste". These participants were evaluated as No Waste.

[b] In the catering service, vegetarian meals are offered not only to individuals who are vegetarian, but also to all individuals as an alternative to meat meal. All individuals have the opportunity to receive every meal under equal conditions.

Table 3. Rational amount of waste per person (g) before and during intervention

Variables	Mean ± SE		p-value
	Pre-nudge	Nudging	
Soup	13.61±5.95	13.85±5.48	0.897
Meat	25.82±11.91	37.79±18.78	0.021
Veg	39.62±29.42	36.40±21.47	0.695
Grain	14.24±4.45	15.83±6.20	0.359
Other 1	8.07±4.41	9.00±7.91	0.647
Other 2	9.79±7.47	12.20±7.48	0.316
Bread	4.73±1.58	4.59±2.45	0.833
Total	115.88±8.69	129.66±8.19	0.256

that female participants left more waste than male participants in all food groups. Subjective waste rates according to being married or single are close to each other (±1-7%). According to age groups, the participants who did not want to indicate their age left to waste in the soup, meat products, and bread groups compared to other individuals. When the age groups are evaluated according to each other, it can be said that there is no standard tendency for all food groups. Considering their income, it is seen that the participants who do not want to indicate their income leave waste above the average in the soup, meat, vegetable, cereal, other 2, and bread groups. In addition, low-income individuals also reported that they left more waste than the average in the soup, meat-poultry-fish, vegetable, and grain-based foods group. Considering the body mass index, it was determined that underweight individuals left waste above the average in all food groups except the other 2.

Charts containing the evaluation of rational waste amounts based on day-to-day and food groups before and during the intervention are presented in Figure 3. Considering the soup waste, it is observed that the changes vary on a day-to-day basis during the intervention, and there is no linear change. In terms of control and intervention days, it was seen that the highest amount of waste was on the 10th, 16th, and 19th days of the intervention. When looking at meat-poultry-fish dishes, a similar linear trend was not observed, and there were changes on a day-to-day basis. When the control days and the intervention days are compared, it is seen that there is no linear trend on the days with the lowest waste, and the lowest wastes are on the control days. It was observed that the control and intervention days in vegetarian wastes progressed in parallel with each other, but the highest amount of waste was observed on the 17th day of the intervention. The lowest waste values in the other 1 and other 2 meal groups were similar on control days. In the bread group, there was a linear decrease in the first 3 days of the intervention, but the waste values increased and decreased in the following days. Although the days with less waste for the bread group were in the intervention period, these decreases are not linear.

DISCUSSION

In this study, which was conducted to determine subjectively and rationally the food waste generated at lunch in a food service establishment and to examine the effects of nudging interventions on food waste, it was determined that significant levels of food waste were formed and nudging intervention did not have a positive effect on food waste.

Nutrient loss and waste create nutritional, economic, social, and environmental impacts in both developed and developing countries (Aires et al. 2021). Reducing nutrient loss and waste; It is thought that it will make positive contributions to reducing production costs, increasing the efficiency of mass nutrition systems, improving food safety, and improving environmental sustainability (FAO, 2019). Prevention of food waste the Sustainable Development Goals is a matter of international concern to reduce it by half at the global level by 2030 (Sustainable Development Goals). In a study in which strategies to reduce food waste were developed, it was shown that by changing the shape and size of the plate and reducing the portion size, the waste was reduced statistically significantly. However, it was also emphasized that such a strategy is costlier than an educational intervention (Richardson et al. 2021). In this study, it is aimed to reduce the amount of waste by making a nudging intervention with posters, brochures, and informative notes at the least cost.

Plate waste in food services occurs due to different stages of food production. The first of these is the residues arising from the storage and preparation stages. The second is food that is not prepared and served, resulting from inadequate planning of the quantity to be produced. Another is considered as food (plate leftovers) that is served but not consumed (Aires et al. 2021). One of the largest sources of food waste in Europe is the food service industry, which includes the hospitality and healthcare sectors (Beratta et al. 2013, Service 2013). The accommodation sector includes staff and non-profit and non-profit catering establishments such as school canteens and cafeterias (Pirani & Arafat 2016). In this study, which was carried out in the university cafeteria, it was determined that food

waste before and after the nudging intervention had a high daily average of 115.88±8.69 g and 129.66±8.19 g, respectively. Factors such as menu planning, preferences of consumers, inadequate personnel training, excessive portion amount, and use of poor-quality products are effective in the formation of food waste (Ferreira et al. 2013, Tekiner ve ark. 2021). In this study, many topics that can contribute to the literature on the subjective reasons for leaving waste came to the fore. According to our data, taste, structure, and cooking methods were found to be the main topics that often affect waste. The participants had different comments about the taste, bitter taste and salty were the most common answers. Odor is also one of the most important reasons for leaving waste. Being sticky and tough often affects the amount of waste. The fact that the food remains raw and that the method of boiling is used while cooking is also among the reasons stated by the participants. Large portion sizes, incompatible menus, not being hungry enough, and not being hot enough are among the frequently mentioned reasons. Apart from these, being on a diet and concerns about hygiene were also stated as reasons for leaving waste.

In this study, which aimed to determine subjectively and rationally the food waste formed at lunch in the food service and to determine the effect of nudging interventions applied to reduce food waste on the level of waste, according to subjective evaluations, the least amount of waste was left in the soup group, the most in the bread group, and the waste level of the intervention

Figure 1. The causes of waste

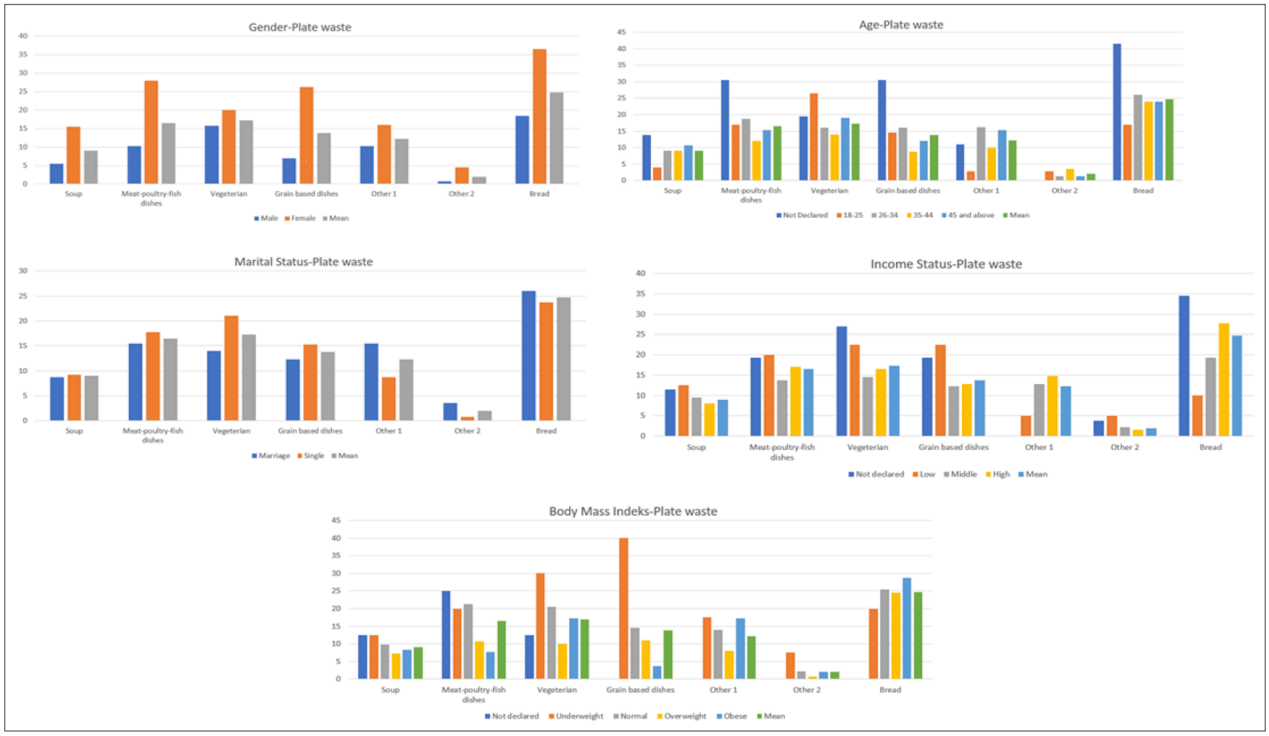


Figure 2. Variation of subjective waste rates (%) according to sociodemographic characteristics

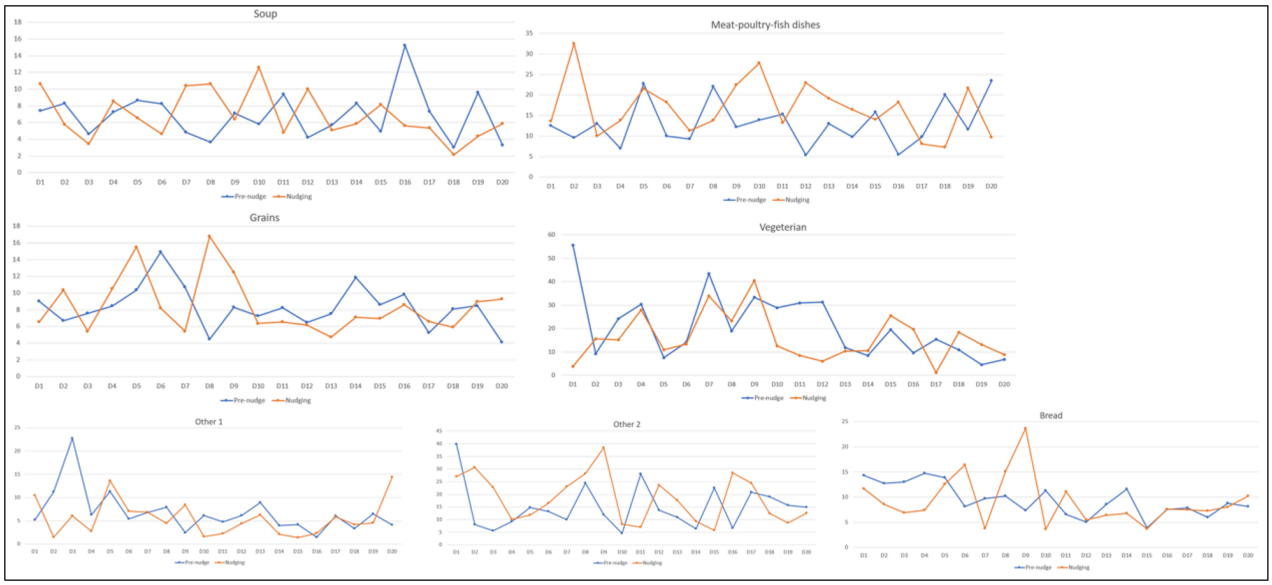


Figure 3. Day-to-day change of percentage (%) rational waste amount per capita according to meal types pre-nudge and nudging

was at the expected level. It was determined that there was no linear change. When evaluated with sociodemographic data, it was observed that subjective waste amounts may cause proportional changes according to being female, income status, and being weak. Gender is known to influence food choices and waste. It has been suggested that women waste more than men on average, which may be related to their tendency to organize food more than men. Similarly, women's subjective waste disposal rates were found to be higher than the average in this study (Richardson et al. 2021, Beardsworth et al. 2002, Painter et al. 2016). According to the subjective evaluations, although the food group the group leaves most frequently, it is seen that the least amount and ratio of bread waste is left in the rational evaluation. Regardless of the study, it was observed that the participants took their bread waste with them when leaving the cafeteria or took them with them to feed the animals. While planning the methodology of the research be done, the situation of the participants taking some foods with them should also be taken into consideration.

In a review on food waste, it was reported that information campaigns can be effective in reducing waste by up to 28%. It has been reported that applications such as changing plate sizes, cooking lessons, refrigerator cameras, advertising, and information sharing are costly and studies are still needed to make evidence-based decisions (Reynolds et al. 2019). Recent studies have focused on the concept of nudging to improve food waste behaviors (Whitehair et al. 2013, Pinto et al. 2018, Vidal et al. 2022). Whitehair et al. (2013) reported that simple to-the-point prompt-type messages in a university cafeteria resulted in a 15% reduction in food waste, while the addition of more personalized feedback-based messages did not result in a further reduction in the amount of food waste. According to Pinto et al. (Pinto et al. 2018) in a university canteen, it was found that after the education campaign, plate waste per capita decreased from 76.50 g to 64.67 g, and the waste consumption index decreased by about 15%. In another study (Vidal et al. 2022), it was reported that the total daily food residue decreased by 19.29 g after nudging strategies were applied in school canteens. On the other hand, nudging strategies are not always effective in reducing food waste. Shaw et al. (Shaw et al. 2018) found that there was no significant change in the amount of avoidable food waste in both low-income and high-income households after they forwarded brochures on the environmental impacts and economic impacts of avoidable food waste. A systematic review study revealed that nudge interventions caused undesirable increases in food waste in secondary school students. Also, most of the studies reviewed did not measure the amount of food waste (Metcalfe et al. 2020). In our study, measuring the amount of food waste after nudge intervention is an important finding. Interestingly, after nudging, a significant increase was detected in the residue of meat dishes, while no significant change occurred in other food wastes. When the literature is examined, it is unclear which intervention is effective on food waste behavior. Accordingly, it is not known whether restricting consumer choice or having choices is more effective in reducing food waste. Although it is concluded in this study that nudging is not effective in reducing food waste, more studies are needed to shed light on the subject.

While making rational waste evaluations in our research, Richardson et al. (Richardson et al. 2021) similar to his study, we removed the inedible parts of the food (such as bones, bones, shells, and fruit seeds) in waste measurements such as. We also presented the percentage values of the edible waste of the leaf-only edible foods, and the gross and net calculation of the portion values were presented in the same way. This is the strength of our study. In our study, due to the non-standard number of groups and the low number of answers given to the options in subjective evaluations, a statistical comparison test was not performed between subjective wastes and demographic variables, and the rates were compared. In the prospective studies to be planned on the subject, inferences can be made about the extent to which demographic variables can affect the subjective waste amounts by having a higher sample size, ensuring the homogeneity of the groups, and randomization. In this study, the effects of a low-cost, short-term nudge intervention were revealed. In other studies, to be planned on the subject, the effects of nudging interventions can be investigated in populations with a larger and stratified sample duration longer than 4 weeks.

CONCLUSION

In this study, some data were presented regarding the amount of waste that adults left subjectively at lunch, the reasons for leaving waste, and the amount of rational waste they left before and during the nudging intervention. It also presents the evidence in the context of its sample during the nudging period and that the four-week intervention alone will not be sufficient in the strategy of reducing food waste. Future studies should focus on adding different strategies in addition to nudging.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

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

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript.

All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval was obtained from the Bandirma Onyedi Eylül University Health Sciences Non Interventional Research Ethics Committee (Date: 07.02.2022 and Approval number 2022-10)

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

We would like to thank all voluenteers that take a part in this study.

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RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(3): 488-499
DOI: https://doi.org/10.31015/jaefs.2023.3.3

Pioneering anther culture-based embryogenesis in *Solanum aethiopicum* L.

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Citation: Shimira, F., Kara, E., Taskin, H. (2023). Pioneering anther culture-based embryogenesis in Solanum aethiopicum L. International Journal of Agriculture, Environment and Food Sciences, 7 (3), 488-499

Received: May 30, 2023
Revised: June 20, 2023
Accepted: June 23, 2023
Published Online: August 21, 2023
Revised: September 11, 2023

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Available online at
https://jaefs.com/
https://dergipark.org.tr/jaefs

Abstract
This study aimed to establish a compelling approach for inducing embryogenesis through *in vitro* anther culture in Scarlet eggplant (*Solanum aethiopicum* L.), the most consumed and popular eggplant among indigenous vegetables on the African continent. While *in vitro* androgenesis has been favorably employed in brinjal (*Solanum melongena* L.) breeding, there has been no attempt to induce embryogenesis in a large germplasm of its relative, *Solanum aethiopicum*. In two distinct experiments, the largest germplasm collection of *Solanum aethiopicum* gr. Gilo was assessed for embryogenesis induction using C medium supplemented with different concentrations of hormones. In the first experiment, callus induction was successful with an overall rate of 36.6 calli/100 anthers, but embryo formation was unsuccessful. Statistical analysis revealed a dependency of the rate of callus induction on accessions. In the second experiment, only four selected accessions of *Solanum aethiopicum* gr. Gilo were used and compared to two Turkish eggplant genotypes of *Solanum melongena* in two distinct treatments. The results showed that in the first treatment (I), only the accession GKE12 had a satisfactory outcome with a rate of embryo formation of 0.82/100 anthers and 0.41/100 anthers corresponding to the rate of developed embryos. In the second treatment (II), only controls, which were Adana and Kemer cultivars of *Solanum melongena* formed embryos with a rate of 7.26/100 anthers and 1.15/100 anthers, respectively. The obtained embryo/seedling of *Solanum aethiopicum* gr. Gilo was found to be diploid. Overall, this study demonstrated that with the right combinations of hormones, it is possible to induce embryogenesis and produce a diploid of *Solanum aethiopicum*, the world's second most popular cultivated eggplant after brinjal. These findings could potentially contribute to the breeding of eggplants for enhanced genetic variation and resistance.
Keywords: African eggplant, Embryo formation, *In vitro* androgenesis, Microspore-derived embryos, Plant breeding

INTRODUCTION

In vitro anther culture is a useful technique for generating doubled haploid plants in economically variable crops such as eggplant. Indeed, haploid plants are very useful in breeding programs. They help with the detection of recessive mutations and the attainment of F1 hybrid vigor (Alpsoy and Şeniz, 2007). Anther culture is used in addition to conventional breeding methods to produce pure (homozygous) lines. They are produced through androgenesis by generating doubled haploid (DH) plants. Consequently, hybrid plants are produced by crossing two pure (homozygous) lines with desirable characteristics (Seguí-

Simarro, 2016).

The *in vitro* anther culture technique has been consistently used to induce the establishment of plants from microspores via direct and indirect embryogenesis. Since the 1980's, anther culture has been used in eggplant to produce double-haploid plants from microspore-derived embryos (Rotino, 2016). The generation of androgenic doubled haploid (DH) lines from haploid microspores/pollen represents an attractive option to conventional breeding techniques for the production of pure lines or 100% homozygous lines for hybrid seed production in high-value crops. It is well documented that DH technology is one of the most efficient and cost-effective methods for accelerating the development of pure lines from anther culture (Salas et al., 2012; Calabuig-Serna et al., 2020). DH technology is still a long way from becoming an omnipresent technique for producing pure lines on a regular basis. In some cases, DHs are obtained, confirming the recalcitrant character of eggplant species/cultivars. This technology is considered to be species-dependent. It is also affected by the microspore's developmental stage and other factors, such as the physical and chemical settings of the *in vitro* culture system (Salas et al., 2012).

This technique has been optimized and extensively used for more than four decades, for commercial and experimental purposes, by which accelerated generation double-haploid parental lines of F1 hybrids are achieved. Furthermore, microspore-derived plants facilitate genetic analysis due to their complete homozygosity characteristics (Rotino, 2016). A handful of studies have been conducted to improve eggplant (*Solanum melongena* L.) through anther culture (Khatun et al., 2006). Other researchers like Kumar et al. (2003), Alpsoy and Şeniz (2007), Salas et al. (2011), and Başay and Ellialtıoğlu (2013) have all successfully applied anther culture to *Solanum melongena*. Salas et al. (2011) evaluated androgenesis induction through anther culture by comparing both common eggplant accessions to other related species, including one cultivated scarlet eggplant (*Solanum aethiopicum*) accession that produced 21.5 calli/100 anthers while no embryo was observed.

Sources indicate that determining the optimal development stage of microspore/pollen for successful androgenesis in different eggplant genotypes, particularly *Solanum melongena*, is more challenging. Thus, in most cases, visual references or morphological criteria differ between cultivars, or even between buds from the same plant donor. However, it has been reported that the proper growth phase from a morphological standpoint should have sepals and petals equal or petals 1-2 mm higher than sepals (Vural et al., 2019). Consequently, Salas et al. (2012) disclosed that younger anthers with mostly young and mid-microspores are preferable for anther culture. Mir et al. (2021) claim that at this younger stage, microspores are near the beginning of pollen mitosis, allowing embryogenesis to be induced more appropriately. However, this condition is not always met because all microspores in an anther are not always at the same stage. Different stages, on the other hand, exist side by side within the same anther. Younger anthers are also preferred because their walls are less thick, allowing media components and growth factors to diffuse and attain microspores inside the locules during *in vitro* anther culture.

In summary, considerable media formulations and inductive treatments have been employed to generate double haploids through anther culture in varied eggplant F1 hybrids and cultivars in alternative experimental scenarios (Başay and Ellialtıoğlu, 2013). The practical usefulness of anther culture in comparison to other androgenesis induction techniques, such as isolated microspore culture, has made it one of the most widely used techniques in eggplant, primarily in cultivated eggplant (*Solanum melongena* L.), with the target of acquiring double haploid parents for conventional breeding (Kashyap et al., 2003; Vural and Ari, 2020). To the best of our knowledge, there has been little research into androgenesis induction in *Solanum melongena* relatives. This is the primary reason why scarlet eggplant (*Solanum aethiopicum* L.), another cultivated eggplant that has long been neglected in scientific research (Shimira et al., 2021), was chosen for determining anther culture's performance in this study. As a result, embryogenesis was attempted on the largest germplasm collection made up of several accessions of *Solanum aethiopicum* gr. Gilo in this study.

The objectives of this research were to uncover the androgenic capacity of African eggplant using a germplasm collection of *Solanum aethiopicum* landraces originated from Rwanda, as well as the accessions/landraces' effects on the ability to induce haploid embryos and convert them to embryo-derived plantlets.

MATERIALS AND METHODS

Plant materials and growing seasons

Plant materials used for the in vitro anther culture study were from the collection of *Solanum aethiopicum* gr. Gilo originated from Rwanda (Shimira et al., 2021) and were maintained in a greenhouse located at the horticultural research application area of Cukurova University (37°01'46.1"N 35°22'02.7"E). For the purpose of this experiment on embryogenesis induction, there were two growing seasons. The first growing season in 2020 lasted from June to December. The second growing season in 2021 lasted from May to November. Well-developed eggplant plantlets were moved from plant growing trays to an experimental field bed for the study. The plants were planted in a randomized block configuration with eight rows and three replications of each accession spaced apart by 1.4 meters and 0.75 meters, respectively. Water-soluble nutrients were applied to the plants, and

drip irrigation was employed to keep the soil moist. Weeds were removed once a week, and other agronomic practices, such as pruning and chemical application, were carried out to manage pests and diseases.

For the first growing season, the whole eggplant germplasm collection of *Solanum aethiopicum* gr. Gilo made up of 60 different accessions was grown. Unfortunately, due to the quality of the seeds only 52 accessions were able to grow and provide enough flower buds for *in vitro* anther culture. Meanwhile, in the second growing season, fewer accessions of *Solanum aethiopicum* gr. Gilo (GKE 12, GKE20, MZE24 and MZE53) were grown with two local cultivars of *Solanum melongena* (Adana and Kemer).

Samples collection

An *in vitro* anther culture experiment was carried out at the Prof. Dr. Saadet BÜYÜKALACA - Tissue Culture Laboratory (Horticulture Department, Çukurova University) in Adana, Türkiye, from October 2020 to January 2021 (first experiment), then from September 2021 to March 2022 (second experiment) to evaluate androgenesis capacity and embryo formation of scarlet eggplant (*Solanum aethiopicum* gr. Gilo) accessions chosen for their genetic diversity.

Flower buds were collected from donor plants in the greenhouse according to the three stages established by cytological examination (See below). In the most cases, the collected flower buds were in stages 1 and 2. A few samples were collected every morning and immediately transported into appropriate containers, as shown in Figure 1.

Figure 1. Eggplants' flower buds

(A) Flower buds in the greenhouse just before collection,

(B) Flower buds in the containers after collection

Control of suitable flower bud sizes and the development stage of microspores/pollen

The optimal development stage of microspores/pollen and also the ideal size of flower buds were evaluated using microscope observations. According to Vural et al. (2019), the best stage for microspores is when they are uninucleate before the beginning of initial pollen mitosis or binucleate at the start of cytokinesis. These observations permitted the connection between floral bud sizes and microspore developmental stages to be identified (Figure 2).

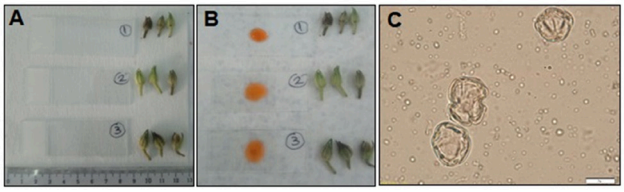

Figure 2. Flower bud size selection

(A) Flower bud types based on size, (B) Microspore staining with acetocarmine on labeled slides,

(C) Microspores observation under a microscope (Magnification 100X ~ 10 µm)

A few samples of freshly collected flower buds of *Solanum aethiopicum* gr. Gilo were used. Firstly, individual anthers were dissected out from flower buds and then crushed/squashed on petri dishes using a scalpel to expose micropores. Afterward, those microspores were stained with acetocarmine. The obtained mix was put on microscope slides and covered with glass slides for further cytological observation. From the cytological examinations, three suitable development stages for anther culture were established.

Media preparation

The protocol for media preparation used in this study was first proposed by Dumas de Vaulx et al. (1982) for anther culture in eggplants. Three distinct nutrient media (C, R, and V3) containing different plant growth regulators at various concentrations were used (Table 1).

The anther culture initiation is performed on C medium for the first 13 days. Then, R medium is used for sub-culturing untill embryos are obtained. Lastly, V3 medium is used for embryo growth and development. Details on the different concentrations of plant growth regulators are given in Table 2.

Experimental design for in vitro embryogenesis

• Experiment 1: "Effects of S. aethiopicum accessions and culture media on in vitro androgenesis induction"

In this experiment, the entire germplasm collection of *Solanum aethiopicum* gr. Gilo made up of 52 different accessions originated from Rwanda was essayed on C medium supplemented with 2.4-D (5 mg/l) and kinetin (5 mg/l). The experiment was conducted using 26 replications for each accession. Here, one accession that did not reach same number of replications was excluded from the study.

• Experiment 2: "Effects of selected S. aethiopicum accessions, 2 Turkish eggplant (Solanum melongena) varieties and 2 hormanal treatments on in vitro androgenesis induction"

In this experiment, the 4 accession lines (GKE12, GKE20, MZE24, and MZE53) of *Solanum aethiopicum* gr. Gilo and 2 local eggplant (*Solanum melongena*) cultivars (Adana

Table 1. Details on C, R and V3 nutrient media (mg/L)

	C medium	R medium	V3 medium		C medium	R medium	V3 medium
Macro nutrients				**Vitamin and amino acids**			
KNO_3	2150	2150	1900	Myo-inositol	100.00	100.00	100.00
NH_4NO_3	1238	1238	1650	Pyrodoxin HCl	5.500	5.500	5.500
$MgSO_4$-$7H_2O$	412	412	370	Nicotinic acid	0.700	0.700	0.700
$CaCl_2$-$2H_2O$	313	313	440	Thyamine HCl	0.600	0.600	0.600
KH_2PO_4	142	142	170	Calcium panthotenate	0.500	0.500	0.500
$Ca(NO_3)_2$-$4H_2O$	50	50	-	Vitamin B_{12}	0.030	-	-
NaH_2PO_4-H_2O	38	38	-	Biotin	0.005	0.005	0.005
$(NH_4)_2SO_4$	34	34	-	Glycin	0.100	0.100	0.200
KCl	7	7	-				
Micro nutrients				**Chelated Irons**			
$MnSO_4$-H_2O	22.130	20.130	0.076	Na_2-EDTA	18.65	18.65	37.30
$ZnSO_4$-$7H_2O$	3.625	3.225	1.000	$FeSO_4$-$7H_2O$	13.90	13.90	27.80
H_3BO_3	3.150	1.550	1.000				
KI	0.695	0.330	0.010				
Na_2MoO_4-$2H_2O$	0.188	0.138	-				
$CuSO_4$-$5H_2O$	0.016	0.011	0.030				
$CoCl_2$-$6H_2O$	0.016	0.011	-				
$AlCl_3$-$6H_2O$	-	-	0.050				
$NiCl_2$-$6H_2O$	-	-	0.050				

Table 2. Plant growth regulators and their concentrations

Growing Medium	Plant growth regulators		Sucrose	Agar
	2.4-D	Kinetin		
C medium	5*	5*	100**	8**
	1*	1*	100**	8**
R medium	0.01	-	30**	8**
V3 medium	-	-	30**	8**

*: mg/L, **: g/L

and Kemer) were tested on two distinct treatments and evaluated for *in vitro* endrogenesis induction on C medium. The experiment was conducted utilizing 31 replications for each accession/genotype. For treatment 1, C medium was supplemented with 2.4-D (5 mg/l) and kinetin (5 mg/l) while, for treatment 2, a 1 mg/l of 2.4-D and 1 mg/l of kinetin were supplemented in C medium.

Initiation of Anther Culture

For the disinfection step, a 20% sodium hypochlorite (NaOCl) solution was used for 15 minutes, succeeding four rinses with sterile distilled water (Figure 3).

By working under a sterile bench (laminar flow hood), the aseptic condition was maintained throughout the procedure of Başay and Ellialtıoğlu (2013). After dissecting the flower buds and carefully removing their filaments, anthers were carefully placed on the appropriate media to circumvent anthers dropping under the media surface. Petri dishes are sealed before being placed into the incubator (Shimira et al., 2019). Figure 4 illustrates the whole process of *in vitro* anther culture.

Excised anthers were cultured in the induction medium (C), complemented by proper plant growth regulators (Table 2), and installed at 35 °C in an incubator under darkness for 8 days, according to the protocol developed by Dumas de Vaulx et al. (1982). Figure 5 shows newly cultivated peti dishes in the incubator. Afterwards, petri dishes were kept in the growth chamber at 25 °C for 16 hours under fluorescent light (50 mol/m-2.s-1). Anthers were transferred to the differentiation medium on the 13th day (R). Sub-culturing can begin once embryos appear in V3 medium (Salas et al., 2012). In this study, R medium was renewed every 30 days while waiting for embryos development.

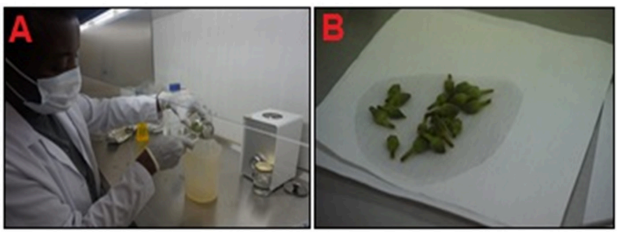

Figure 3. Sterilization process

(A) Flower buds sterilization under the bench,

(B) Sterilized flower buds on sterile tissue paper

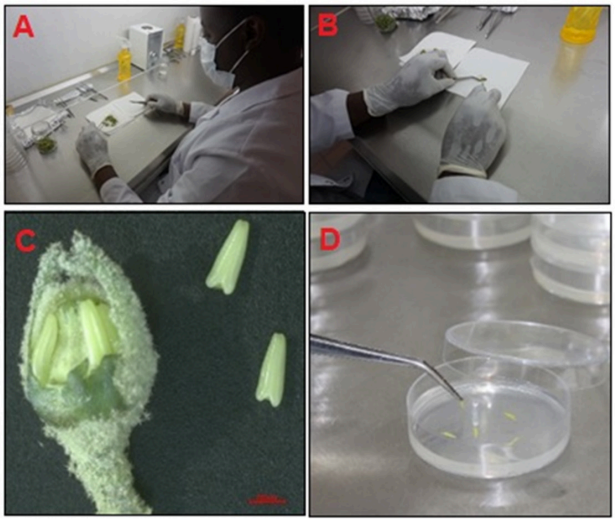

Figure 4. Anther culture process

(A) (B) (C) Flower bud and anthers, (D) Anther placement on media

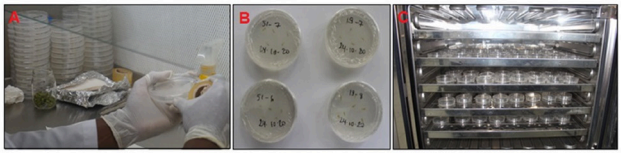

Figure 5. Major steps after anther culture

(A) The sealing of petri dish, (B) Sealed petri dishes ready to be incubated,

(C) Incubator containing newly cultured anthers

Embryo growth and development

The monitoring and observation of changes and callus development as well as embryo development were carried out by using a stereo microscope, Olympus SZ61 (Olympus Corporation, Shinjuku, Tokyo, Japan). This microscope, with high quality optics, allowed the thorough following of anthers' transformation to callus (Figure 6) and eventually the observation of a few embryos (Figure 7).

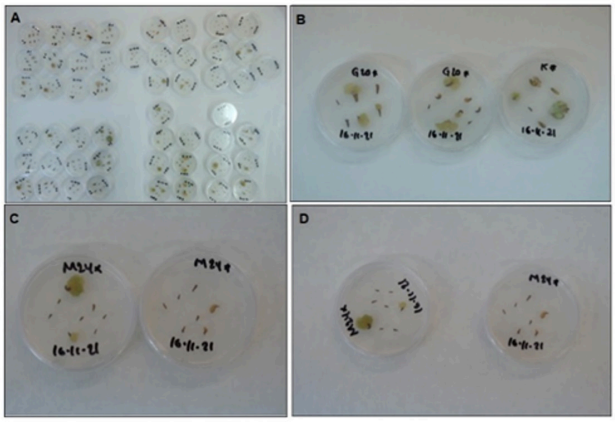

Figure 6. Petri dishes in the growing room (A), (B), (C) and (D) Petri dishes with some developed anthers

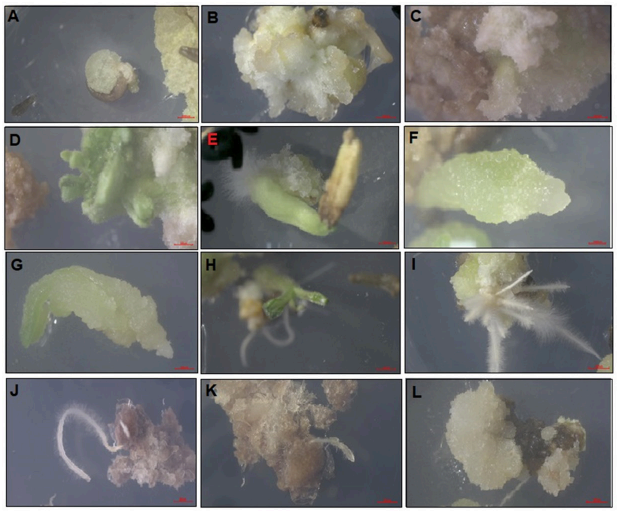

Figure 7. Embryo-like and embryo formations (A) to (L) Picture are shown at 300 µm

The protocol used by Vural and Ari (2020) for embryo conversion and acclimatization was slightly modified to meet the needs of our experiment. In brief, V3 medium was used after embryos were obtained to ensure their optimal growth and development. Firstly, embryos were transferred from petri dishes (R medium) to small glass flasks containing V3 medium (7 cm in height) (Figure 8A, Figure 8B, and Figure 8C). To obtain overall healthy embryos, grown embryos with slightly longer shoots and roots (or mature enough) were separated from their small siblings and transferred to new individual jars of superior width and height (7 cm, 8.5 cm, and 13.5 cm) containing V3 medium (Figure 8D).

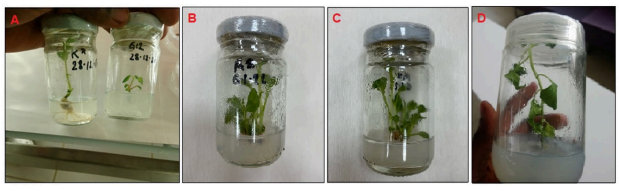

Figure 8. Eggplant seedlings into small jars

After forming strong shoots and roots, *in vitro* embryo-derived plantlets were transferred to pots with a mixture of peat moss and perlite (3:1 v/v) for acclimatization (Figure 9). They were later moved to a greenhouse and subjected to a gradually decreasing humidity and progressively rising illumination schedule.

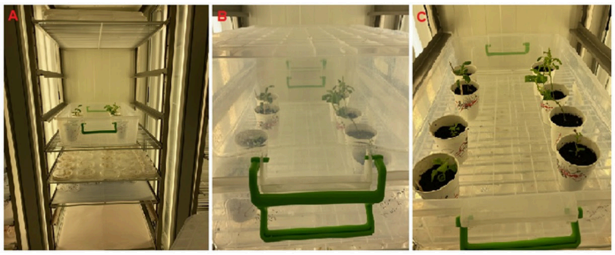

Figure 9. Embryo derived seedlings during acclimatization

Ploidy level analysis

Flow cytometry was employed to investigate the ploidy level. Fresh and young leaf samples from ex vivo embryo-derived plantlets (eggplant regenerants) were collected from the acclimation greenhouse and transported directly to the lab for ploidy determination. At this point, the research method used by Shimira et al. (2019) was employed. In that regard, leaf samples (0.5 cm^2 of leaf tissue per plantlet in individual petri dishes) were chopped with a harsh razor blade after adding 400 µl of extraction buffer (Figure 10). Afterwards, all these samples were incubated for 30 to 60 seconds. Following that, samples underwent filtration using a specialized filter (Partec 50 m Cell Trics®), and 1.6 ml of staining buffer was added to the sample tube for a brief incubation of 30 to 60 seconds before taking readings on a flow cytometer (Figure 11).

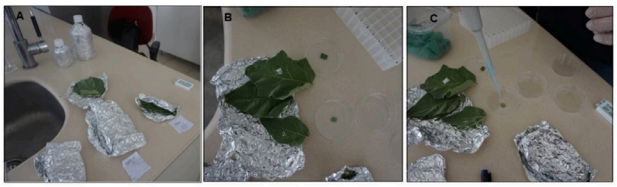

Figure 10. Collected leaf samples for flow cytometry analysis

Figure 11. Flow cytometry analysis

Data collection and analysis

The anthers were examined for callus initiation after four weeks of incubation. Furthermore, *in vitro* cultures were scored for the frequency of callus induction. The frequency was calculated as the ratio between the numbers of anthers responding to callus induction or regeneration to that of the total number of anthers inoculated as described by Kumar et al. (2003). For statistical analysis, descriptive statistics were conducted, and for advanced statistics, JMP (version 15.2.1, SAS Inc., Cary, NC, USA) statistics package software was used. An ANOVA test ($p \leq 0.05$) was conducted to evaluate general significant variations, and then a Fisher's least significant difference (LSD) test for multiple comparisons was used to sort different eggplant accessions into clusters, with significant differences at a p value < 0.05.

RESULTS AND DISCUSSION

Fist experiment

The numbers of cultured anthers and formed calli are described in Table 3. Unfortunately, since no embryos were obtained, only the frequency of callus formation was calculated in percentage per accession.

Results show that callus formation ranged between 55.90% and 20.23%, with the highest percentage for accession MZE34 and the lowest percentage for accession GKE8. The average callus formation percentage is 36.36 calli/100 anthers for the whole germplasm of *Solanum aethiopicum* gr. Gilo. For instance, the highest mean value of cultured anther was observed in GKE13 accession with values of 8.35±1.24 while the highest mean value of formed calli was noticed in MZE32 accession with 1.59±1.89.

The statistical analysis (one-way ANOVA) shows that accessions significantly influenced callus induction (Table 4). The statistical significance is shown by the small value of the P-value (compared to α=0.05). This can be taken as evidence that the means are different. Similarly, the mean comparisons through the least significant difference (LSD) test confirmed that the means were statistically different (Table 5).

Second experiment

For the second experiment, the number of cultured anthers as well as the number of formed calli and embryos are described in Table 6. Both the formation frequency of calli and embryos as well as the development of embryos were thoroughly calculated.

In the first treatment, results show that the GKE12 accession (*S. aethiopicum* gr. Gilo) was the only accession to have a good response regarding embryo formation and development, with 0.82 formed embryos per 100 anthers and 0.41 developed embryos per 100 anthers. Although, it was not the only accession to have formed embryos. Adana and Kemer eggplant (*Solanum melongena*) cultivars used here as control genotypes formed embryos with 0.61 formed embryos per 100 anthers and 1.19 formed embryos per 100 anthers, respectively. Briefly, the only developed embryo/

Table 3. Frequency of calli formation for the first experiment

N°	Accession ID	Petri Numbers	Number of Cultured Anthers	Frequency of Calli Formation	
				Number	%
1.	GKE1	26	186	72	38.71
2.	GKE2	26	190	46	24.21
3.	GKE5	26	177	88	49.72
4.	GKE7	26	201	71	35.32
5.	GKE8	26	173	35	20.23
6.	GKE9	26	175	54	30.86
7.	GKE11	26	202	74	36.63
8.	GKE12	26	207	68	32.85
9.	GKE13	26	217	58	26.73
10.	GKE14	26	157	85	54.14
11.	GKE15	26	196	72	36.73
12.	GKE16	26	182	44	24.18
13.	GKE17	26	180	57	31.67
14.	GKE18	26	182	59	32.42
15.	GKE19	26	180	78	43.33
16.	GKE20	26	200	77	38.50
17.	MZE22	26	204	96	47.06
18.	MZE23	26	183	85	46.45
19	MZE24	26	199	97	48.74
20.	MZE26	26	183	55	30.05
21.	MZE27	26	170	64	37.65
22.	MZE28	26	197	70	35.53
23.	MZE29	26	201	90	44.78
24.	MZE30	26	202	64	31.68
25.	MZE32	26	199	71	35.68
26.	MZE33	26	195	78	40.00
27.	MZE34	26	195	109	55.90
28.	MZE35	26	191	46	24.08
29.	MZE36	26	207	62	29.95
30.	MZE37	26	200	86	43.00
31.	MZE38	26	194	59	30.41
32.	MZE39	26	182	64	35.16
33.	MZE40	26	190	65	34.21
34.	MZE41	26	189	102	53.97
35.	MZE42	26	187	61	32.62
36.	MZE43	26	203	70	34.48
37.	MZE44	26	167	59	35.33
38.	MZE46	26	181	89	49.17
39.	MZE47	26	183	40	21.86
40.	MZE48	26	207	67	32.37
41.	MZE49	26	190	68	35.79
42.	MZE50	26	207	96	46.38
43.	MZE51	26	173	53	30.64
44.	MZE52	26	180	70	38.89
45.	MZE53	26	174	64	36.78
46.	MZE54	26	205	70	34.15
47.	MZE55	26	211	49	23.22
48.	MZE56	26	177	51	28.81
49.	MZE58	26	192	73	38.02
50.	MZE59	26	199	71	35.68
51.	MZE60	26	189	75	39.68

Table 4. Statistical anlysis on callus induction (First experiment)

Analysis of Variance					
Source	**DF**	**Sum of Squares**	**Mean Square**	**F Ratio**	**Prob > F**
Accessions	50	518.1568	10.3631	3.6274	<.0001
Error	1301	3716.846	2.8569		
C. Total	1351	4235.003			
Effect Tests					
Source	**Nparm**	**DF**	**Sum of Squares**	**F Ratio**	**Prob > F**
Accessions	50	50	518.1568	3.6274	<.0001

seedling was attained from GKE12.

Regarding callus induction in the first treatment, there was a great variation within the 4 different accessions of *S. aethiopicum* gr. Gilo and the two control *Solanum melongena* cultivars (Adana and Kemer) as shown in Figure 12. For instance, the highest mean values of cultured anther and formed calli were observed in the MZE24 accession, with values of 2.97±1.91 and 3.23±1.58, respectively.

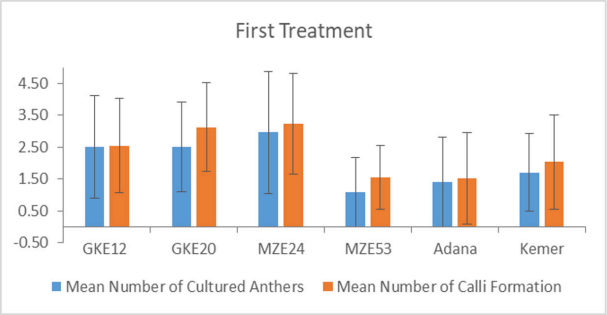

Figure 12. Callus Induction Details with Mean and SDs (Experiment II – First treatment)

In the second treatment, results show that none of the eggplant accessions (*S. aethiopicum* gr. Gilo) were able to form embryos. Embryo formation and development were observed in the two control *Solanum melongena* cultivars; Adana and Kemer, with 7.26 formed embryos per 100 anthers and 1.15 formed embryos per 100 anthers, respectively. For the frequency of developed embryos/seedlings, these values slightly decrease for Adana and Kemer, with 6.70 developed embryos per 100 anthers and 0.57 developed embryos per 100 anthers, respectively.

Regarding callus induction in the second treatment, there was a great variation within the 4 different accessions of *S. aethiopicum* gr. Gilo and the two control *Solanum melongena* cultivars (Adana and Kemer) as shown in Figure 13. For instance, the highest mean value of cultured anther was observed in the GKE20 accession with values of 2.35±1.26, while the ultimate mean value of formed calli was observed in the MZE24 accession with 2.97±1.43.

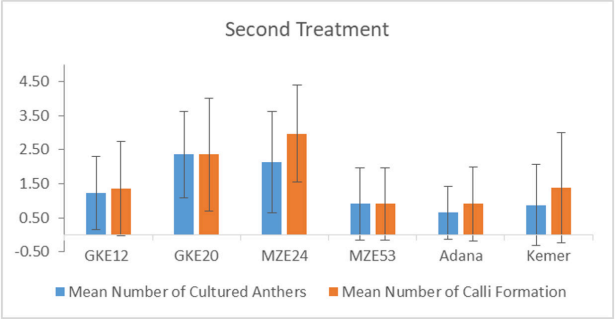

Figure 13. Callus Induction Details with Mean and SDs (Experiment II – Second treatment)

The statistical analysis (Two-way ANOVA) shows that accessions/genotypes and treatments (I and II) significantly influenced callus induction (Table 7). The statistical significance is shown by the small value of the P-value (compared to α=0.05). This can be taken as evidence that the means are different. Similarly, the mean comparisons through the least significant difference (LSD) test confirmed that means were statistically different (Table 8).

ANOVA results indicate that there was also a significant difference between treatments. That means that the factor "treatment" has an influence on the result obtained with regard to callus induction. At the accession/genotype level, we also found a significant difference. At the interaction level (Treatments*Accession/Genotype), we observed no significant difference.

Flow cytometric assessment of embryo-derived plantlets

Ploidy levels were measured in all obtained plantlets of *Solanum aethiopicum* gr. Gilo from the second experiment on *in vitro* androgenesis through anther culture. As shown in Table 9, 100% of all obtained plantlets were found to be diploid.

DISCUSSION

In the first experiment, the overall callus formation rate was 36.36 calli/100 anthers and there was no embryo formation. This value was greater than the value obtained by Salas et al. (2011), when they assessed androgenic capacity via anther in various genotypes of common eggplant and related species, including *Solanum aethiopicum*. They obtained 21.5 calli/100 anthers for the

Table 5. Details on LSD test (First experiment)

N°	Accessions	LSD results on the number of formed calli
1.	GKE01	2.77±1.53 EFGHIJK
2.	GKE02	1.77±1.21 MNOP
3.	GKE05	3.38±1.83 ABCDEF
4.	GKE07	2.73±1.64 EFGHIJK
5.	GKE08	1.35±1.41 P
6.	GKE09	2.08±1.57 JKLMNOP
7.	GKE11	2.85±1.93 CDEFGHIJK
8.	GKE12	2.62±1.68 EFGHIJKLM
9.	GKE13	2.23±1.66 IJKLMNOP
10.	GKE14	3.27±1.46 BCDEFGH
11.	GKE15	2.77±1.63 EFGHIJK
12.	GKE16	1.69±1.44 NOP
13.	GKE17	2.19±1.50 IJKLMNOP
14.	GKE18	2.27±1.80 IJKLMNO
15.	GKE19	3.00±1.92 CDEFGHI
16.	GKE20	2.96±1.95 CDEFGHIJ
17.	MZE22	3.69±1.78 ABCD
18.	MZE23	3.27±2.07 BCDEFGH
19	MZE24	3.73±1.59 ABC
20.	MZE26	2.12±1.53 IJKLMNOP
21.	MZE27	2.46±1.50 GHIJKLMN
22.	MZE28	2.69±1.93 EFGHIJKL
23.	MZE29	3.46±1.86 ABCDE
24.	MZE30	2.46±2.10 GHIJKLMN
25.	MZE32	2.73±1.93 EFGHIJK
26.	MZE33	3.00±1.72 CDEFGHI
27.	MZE34	4.19±1.60 A
28.	MZE35	1.77±1.63 MNOP
29.	MZE36	2.38±1.17 HIJKLMNO
30.	MZE37	3.31±1.76 ABCDEFG
31.	MZE38	2.27±1.80 IJKLMNO
32.	MZE39	2.46±1.45 GHIJKLMN
33.	MZE40	2.50±1.96 FGHIJKLMN
34.	MZE41	3.92±1.60 AB
35.	MZE42	2.35±1.72 IJKLMNO
36.	MZE43	2.69±1.67 EFGHIJKL
37.	MZE44	2.27±1.71 IJKLMNO
38.	MZE46	3.42±1.60 ABCDE
39.	MZE47	1.54±1.42 OP
40.	MZE48	2.58±1.65 EFGHIJKLMN
41.	MZE49	2.62±1.30 EFGHIJKLM
42.	MZE50	3.69±1.74 ABCD
43.	MZE51	2.04±1.15 KLMNOP
44.	MZE52	2.69±2.15 EFGHIJKL
45.	MZE53	2.46±1.27 GHIJKLMN
46.	MZE54	2.69±2.09 EFGHIJKL
47.	MZE55	1.92±1.71 LMNOP
48.	MZE56	1.96±1.46 LMNOPQ
49.	MZE58	2.81±1.88 DEFGHIJK
50.	MZE59	2.73±1.61 EFGHIJK
51.	MZE60	2.88±2.01 CDEFGHIJK

Table 6. Frequency of calli and embryos formation for the second experiment

Treatments	Access. IDs	Petri Numbers	Number of Cultured Anthers	Frequency of Calli Formation		Frequency of Embryo Formation		Frequency of Embryo Development	
				Number	%	Number	%	Number	%
I. (5 mg/l 2.4-D and 5 mg/l kinetin)	GKE12	31	244	78	31.97	2	0.82	1	0.41
	GKE20	31	243	78	32.10	0	0.00	0	0.00
	MZE24	31	234	92	39.32	0	0.00	0	0.00
	MZE53	31	200	34	17.00	0	0.00	0	0.00
	Adana	31	163	44	26.99	1	0.61	0	0.00
	Kemer	31	168	53	31.55	2	1.19	0	0.00
II. (1 mg/l 2.4-D and 1 mg/l Kinetin)	GKE12	31	213	38	17.84	0	0.00	0	0.00
	GKE20	31	231	73	31.60	0	0.00	0	0.00
	MZE24	31	234	66	28.21	0	0.00	0	0.00
	MZE53	31	204	28	13.73	0	0.00	0	0.00
	Adana	31	179	20	11.17	13	7.26	12	6.70
	Kemer	31	174	27	15.52	2	1.15	1	0.57

I: First treatment [C medium complemented with 2.4-D (5 mg/l) and kinetin (5 mg/l)].

II: Second treatment [C medium complemented with 2.4-D (1 mg/l) and Kinetin (1 mg/l)].

Table 7. Statistical analysis on callus induction (Second experiment)

Analysis of Variance					
Source	DF	Sum of Squares	Mean Square	F Ratio	Prob > F
Accessions/Genotypes	11	206.2876	18.7534	10.3803	<.0001
Error	360	650.3871	1.8066		
C. Total	371	856.6747			
Effect Tests					
Source	Nparm	DF	Sum of Squares	F Ratio	Prob > F
Treatments	1	1	43.35753	23.9991	<.0001
Acc/Gen	5	5	148.4005	16.4284	<.0001
Treatments*Acc/Gen	5	5	14.52957	1.6085	0.157

Table 8. Details on LSD test (Second experiment)

	Level	Least Sq Mean		Level	Least Sq Mean
Accessions / Genotypes	GKE12	1.87 ^{B}	**Genotypes *Treatments**	I, GKE12	2.52 $^{A, B}$
	GKE20	2.44 ^{A}		I, GKE20	2.52 ^{A,B}
	MZE24	2.55 ^{A}		I, MZE24	2.97 ^{A}
	MZE53	1.00 ^{C}		I, MZE53	1.10 $^{D, E, F}$
	Adana	1.03 ^{C}		I, Adana	1.42 $^{D, E}$
	Kemer	1.29 ^{C}		I, Kemer	1.71 $^{C, D}$
Treatments	I	2.04 ^{A}		II, GKE12	1.23 $^{D, E, F}$
	II	1.35 ^{B}		II, GKE20	2.35 $^{A, B, C}$
				II, MZE24	2.13 $^{B, C}$
				II, MZE53	0.90 $^{E, F}$
				II, Adana	0.65 ^{F}
				II, Kemer	0.87 $^{E, F}$

Levels not connected by same letter are significantly different

Table 9. Ploidy levels in all *S. aethiopicum* gr. Gilo plantlets

Treatments	Accession IDs	No of embryo-derived plantlets	Haploid plantlets	Diploid plantlets
I (5 mg/l 2.4-D and 5 mg/l kinetin)	GKE12	1	-	1
	GKE20	-	-	-
	MZE24	-	-	-
	MZE53	-	-	-
	Adana	-	-	-
	Kemer	-	-	-
II (1 mg/l 2.4-D and 1 mg/l Kinetin)	GKE12	-	-	-
	GKE20	-	-	-
	MZE24	-	-	-
	MZE53	-	-	-
	Adana	12	-	12
	Kemer	1	-	1

I: First treatment [C medium with 2.4-D (5 mg/l) and kinetin (5 mg/l)].
II: Second treatment [C medium with 2.4-D (1 mg/l) and Kinetin (1 mg/l)].

sole genotype of *Solanum aethiopicum* utilized in their study, and it also failed to generate embryos. The results from ANOVA demonstrated the existence of a significant statistical difference among the entire germplasm collection of *Solanum aethiopicum* gr. Gilo with regards to callus induction.

For the second experiment (the first treatment), only one accession, GKE12 (*S. aethiopicum* gr. Gilo) formed embryos at a rate of 0.41 embryos per 100 anthers that gave plantlets. Controls made from Solanum melongena cultivars; Adana and Kemer demonstrated embryo production in both the first and second treatments, which is consistent with previous researchers' findings in anther culture studies of common eggplants. These researchers found embryo formation rates of 3,67 embryos/100 anthers in 'Kemer' (Alpsoy 2007), 14.2 embryos/100 anthers (Başay et al., 2011), 0.7–60.9 embryos /100 anthers (Salas, 2011) as well as 2.49–4.49/ embryos/100 anthers (Başay and Ellialtıoğlu, 2013).

Successful strategies for increasing the number of embryos and embryo-derived plantlets in *Solanum melongena* can be replicated in *Solanum aethiopicum*. For instance, Emrani Dehkehan et al. (2017) demonstrated that an individual supplement of 1 mg/l zeatin riboside and 10 mg/l mannitol in C medium (containing NAA and Kinetin) can positively affect anther culture outcomes. The highest embryo-derived plantlets obtained in this case were 25% and 66.6% for zeatin riboside and mannitol, respectively. Vural and Ari (2020) also demonstrated that a combination of activated charcoal, maltose, and silver nitrate in the original induction medium by Dumas de Vaulx et al. (1982) (DDV medium) had a triple synergistic effect on the high embryo yield of eggplant (*Solanum melongena* L.). And it can produce 3.9 times more embryos than the original DDV medium.

Obtaining doubled haploid plants in this type of eggplant, also known as the closest relative to brinjal, is a significant step forward for DH technology. This technology is reported to have the potential to speed up the process of generating new parental pure lines in several different species, including eggplant, which is considered a species that is moderately recalcitrant with regards to this technology. Eggplant breeding programs are primarily concerned with the release of hybrid varieties with better attributes. For instance, eggplant F1 hybrids, which are produced by crossing two parental homozygous plants, customarily outperform parental lines in terms of various agronomic traits (Mir et al., 2021).

CONCLUSION

This study demonstrated that embryogenesis induction through androgenesis can be successfully performed in one of the brinjal relatives, such as *Solanum aethiopicum* at a lower rate. *Solanum aethiopicum* accessions had significantly lower androgenic potential than *Solanum melongena* varieties. These findings highlight potential limitations for the widespread adoption of anther cultures in brinjal (*Solanum melongena* L.) relatives. Only the GKE12 accession responded and produced one embryo. The species was discovered to be the limiting factor in androgenic responses. The knowledge on androgenic responses gained in this study about local landraces of *Solanum aethiopicum* from Rwanda will provide a foundation for further research to develop doubled haploids to support ongoing efforts in the common eggplant (brinjal) breeding program as well as other breeding efforts in other cultivated eggplants.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

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

Author contribution

The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and

that they have not been published before.

Ethical approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgement

Our sincere appreciation goes to Asst. Prof. Dr. Gökhan Baktemur, Dr. Şenay Karabıyık and Mr. Serhat Yıldız for guidance and technical assistance. We also extend our gratitude to Mahmut Yarar, Miyase Büyükküpeli, Fildaus Nyirahabimana, Ceren Mutludağ, Muhammed Halil Yutaz and Senem Uğur for their profound assistance during field and laboratory work. Authors are also grateful to Cukurova University—Scientific Research Projects Coordinating Office for financial support (Project No: FDK-2020-13106).

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RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(3): 500-507
DOI: https://doi.org/10.31015/jaefs.2023.3.4

Postharvest mycobial contaminants of white button mushroom (*Agaricus bisporus*) and their management using plant essential oils

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Citation: Adhikari, H.S., Jha, S.K. (2023). Postharvest mycobial contaminants of white button mushroom (Agaricus bisporus) and their management using plant essential oils. International Journal of Agriculture, Environment and Food Sciences, 7 (3), 500-507

Received: March 10, 2023
Revised: August 05, 2023
Accepted: August 09, 2023
Published Online: September 11, 2023

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Available online at
https://jaefs.com/
https://dergipark.org.tr/jaefs

Abstract
Being highly perishable, mushrooms' quality and shelf life is affected by various factors during postharvest conditions, among which fungal contamination is the main cause. The goal of this study is to identify and manage fungal contaminants present in mushrooms during postharvest conditions. A total of 23 fungi were isolated as contaminants from the samples of *Agaricus bisporus* collected from three major vegetable markets in Kathmandu city, Nepal. *Aspergillus niger*, *Aspergillus flavus*, and *Rhizopus stolonifer* were found to be the most frequent fungal contaminants. These were treated with various concentrations of essential oils (EOs) of *Cinnamomum tamala*, *Mentha spicata*, *Zanthoxylum armatum*, and *Eucalyptus citriodora* using poisoned food technique. Significant ($p<0.05$) inhibition of mycelial growth and spore germination was found in all testedfungi by all EOs. A strong inhibitory action of *M. spicata* oil was recorded against *A. flavus* and *R. stolonifer* while, *A. niger* was best controlled by *C. tamala* oil at the concentration of 20 µl/ml. These results suggest that EOs of three tested plants could be a good alternative to control fungal contaminants and extend the shelf life of *Agaricus bisporus* in postharvest conditions.
Keywords: *Aspergillus niger*, Botanicals, White button mushroom, Fungal contaminants, Fungitoxic effect

INTRODUCTION

White button mushrooms i.e., *Agaricus bisporus* (J.E. Lange) Imbach belong to the class Basidiomycetes and the family Agaricaceae is commonly recognized as 'Gobre chyau' in Nepal. It is widely cultivated all over the world and plays a key economic role in the worldwide mushroom market, accounting for 15% of all mushroom production (Royse et al, 2017). It is commonly cultivated in the peri-urban area of the Kathmandu valley and the hilly region of Nepal (Shrestha, 2014). In Nepal, it is the second most cultivated mushroom that contributes 10% of total mushroom production (Raut, 2019). Additionally, this mushroom possesses a variety of therapeutic potentials including antioxidant, antibacterial, and immunomodulating activities (Ramos et al., 2019; Usman et al., 2021).

Postharvest loss refers to the quantifiable deterioration of food quantity and quality after harvest; this condition is greater for perishable crops in less developed countries (Hodges, 2011). Quantitative loss is more of an issue in these countries than qualitative loss (Humble and Reneby, 2014). The FAO estimates that 40 to 50 percent of the world's horticulture crop yield is lost in postharvest conditions (FAO, 2018). The mushrooms are highly perishable in nature and have a poor shelf life (3-5 days), so their postharvest loss is 30-35% globally (Thakur et al., 2022). Many postharvest problems such as color changes, tissue damages, cap opening,

weight loss, turgidity, bacterial contaminations, etc. occur rapidly during storage degrading its quality and quantity (Wang et al., 2017). Additionally, during the mushroom's growing and postharvest stages, different mold fungi can contaminate the crop, which harms the yield and shelf life of the mushrooms (Sharma et al., 2009; Biswas, 2014). Different researchers have conducted studies on multiple aspects of fungal contamination and diseases of *Agaricus bisporus* (Dandge, 2012; Adhikari and Jha, 2020; Wang et al., 2020) and they and they reported *Mycogone perniciosa*, *Verticillium fungicola*, *Cladobotryum dendrites*, *Trichoderma* spp., *Chaetomium* spp., *Aspergillus* spp., *Penicillium* spp., *Monilia* sp., *Geotrichum* sp., *Fusarium* spp., *Rhizopus* sp., *Mucor* sp., *Alternaria* sp., *Curvularia* sp., *Penicillium* sp., etc. were the major contaminants of *Agaricus bisporus*. These fungal contaminants produce several mycotoxins, which pose a risk to human health because they can lead to serious and incurable health problems including cancer in developing countries (Omotayo et al., 2019). Various kinds of synthetic fungicides are commonly used to reduce such microbial contamination in the field and postharvest conditions of mushrooms in Nepal (Raut, 2013; Adhikari and Jha, 2020). However, the extensive use of synthetic fungicides has, however, resulted in the emergence of infections that are resistant to them, and concerns have been expressed regarding the long-term impact on the environment and public health (Ons et al., 2020).

Plant essential oils (EOs) are secondary metabolites made up of volatile aromatic compounds which have a significant role in the defense of the plants (Hyldgaard et al., 2012). Such EOs often possess antibacterial, antifungal, antiviral, and insecticidal properties (Falleh et al., 2020). These properties make EOs a probable substitutes for synthetic fungicides (Bassolé and Juliani, 2012). Such that essential oil can be very helpful in extending the shelf life of food and minimizing losses in postharvest conditions of agricultural products (Farzaneh et al., 2015; Prakash et al., 2015). Plant essential oils are non-toxic and biologically decomposable, they are less hazardous to public health and the environment as well. The present study aimed to isolate and identify fungal contaminants associated with *Agaricus bisporus* in postharvest conditions. Furthermore, the study also aims to evaluate the antifungal activity of EOs of four selected plant species (*Cinnamomum tamala*, *Mentha spicata*, *Zanthoxylum armatum*, and *Eucalyptus citriodora*) against some most frequent fungal contaminants.

MATERIALS AND METHODS

Isolation and Identification of Mycobial Contaminants

Fruitbodies of *Agaricus bisporus* samples were collected from three major vegetable market of Kathmandu, Nepal. The base, stipe, and pileus of the sample were cut into 3 mm pieces and plated onto potato dextrose agar (PDA) plates. A week later, each fungus was pure cultured from the numerous colonies of fungi. Fungal isolates were identified based on micromorphological and cultural characteristics by following standard literature (Barnett and Hunter, 1972; Watanabe, 2010). The three fungi (*Aspergillus niger*, *Aspergillus flavus*, and *Rhizopus stolonifer*) among the most abundant were selected as test fungi to evaluate the extent to which EOs were inhibitory to fungi.

Extraction of Plant Essential Oils

Fresh leaves of four plant species i.e., *Cinnamomum tamala*, *Mentha spicata*, *Eucalyptus citriodora*, and *Zanthoxylum armatum*, were harvested from the garden of Central Department of Botany (Tribhuvan University) at Kirtipur and private farmland at Champadevi, Kathmandu. Fresh leaves were then dried in shade and kept in the dark until extraction of EOs. The shade-dried leaves were then hydrodistilled for 6 to 8 hours using Clevenger's equipment (Sovová and Aleksovski, 2006). The extracted EOs were then dried using anhydrous sodium sulfate (Na_2SO_4), and then kept in storage at below 10°C temperatures until use.

Antifungal Effect of Plant Essential Oils

The poisoned food technique was used to evaluate the antifungal activity of EOs on the in vitro growth and development of fungal contaminants (Grover and Moore, 1962). Five different concentrations of EOs viz 1.25, 2.5, 5, 10, and 20 µl/ml were prepared with 50% acetone. First, 1 ml of each concentration of essential oil was added to sterilized petriplates, and then 9 ml of melted PDA was added. The test fungus of 4 mm diameter, which was growing aggressively, was then inoculated into each petriplates. Instead of essential oil, distilled water and 50% acetone were utilized in control setups. On the seventh day, observations were made. Five replications were maintained and fungi toxicity of Essential oils was assessed by measuring the percentage of mycelial growth that was inhibited, which was computed as;

Inhibition of Mycelial Growth (%) = [(Mc–Mt)/ Mc] × 100

[Where; Mc= mean colony diameter in control sets and Mt= mean colony diameter in treatment sets].

The effect of EOs on the spore germination of test fungus was examined using the hanging drop technique. After a 24-hour incubation period, the data from five replicates were collected. The formula below was used to determine the percentage of spore germination (Király et al., 1974).

Spore germination (%) = (Gg/Gt) × 100

[Where; Gg = number of spores germinated per microscopic field and Gt = number of spores per microscopic field.]

Data Analysis

Microsoft office Excel 2019 was used for data entry and preparation of necessary graphs. The occurrence

frequency of fungal contaminants found in *Agaricus bisporus* was compared by using frequency rank curve. Analysis of variance (ANOVA) and a post-hoc Tukey's HSD (honestly significant difference) test with a p-value of 0.05 were used to evaluate data means using the statistical programme for social science (SPSS) version 23 software.

RESULTS AND DISCUSSION

Postharvest Mycobial Contaminants

A total of twenty-three fungi were isolated as contaminant, where two of the fungi remained unidentified. *Aspergillus niger*, *Aspergillus flavus*, and Rhizopus stolonifer were found as the most frequent mycobial contaminants (Figure 1).

In *Agaricus bisporus*, seven species of the genus *Aspergillus* (*A. niger, A. flavus, A. brevipes, A. fumigatus, A. clavatus, A. versicolor, Aspergillus* sp), 3 species of genus *Chaetomium* (*C. globosum, C. spirale, Chaetomium* sp.), 2 species of *Trichoderma* (*T. harzianum, T. viride*) *Mucor hiemalis, Penicillium notatum, Rhizopus stolonifer, Alternaria alternata, Geotrichum* sp., *Gliocladium* sp., *Fusarium oxysporum, Nigrospora sphaerica, Cladosporium* sp., and two unidentified species were isolated. Among these fungal contaminants, the frequency rank curve (Fig 1) reveals that the most frequent fungal species were *Aspergillus niger* (50.00%) followed by *Aspergillus flavus, Rhizopus stolonifer, Aspergillus brevipes,* and *Aspergillus fumigatus* with the frequency value 40.00 %, 36.67%, 33.33%, and 26.67% respectively. This result thus shows that *Agaricus bisporus* is vulnerable to fungal contaminations, in particular, that of *Aspergillus*. These results confirm the work in previous literatures (Dandge, 2012; Kertesz and Thai, 2018; Adhikari and Jha, 2020; Wang et al., 2020; Mishra, 2022) wherein reported that these fungal species are the major contaminants of *Agaricus bisporus*.

Antifungal Effect of Plant Essential Oils

The results of the antifungal bioassay of essential oil revealed that all four essential oils significantly ($p<0.05$) inhibited the mycelial growth and spore germination of all test fungi. Since the mycelial growth of *A. niger* was 0.70±0.08 cm at a concentration of 20 µl/ml, *C. tamala* oil had the best effects on *A. niger* compared to *A. flavus* and *R. stolonifer*. *C. tamala* oil has the best effects over *A. niger* than *A. flavus* and *R. stolonifer* as the mycelial growth of *A. niger* was 0.70±0.08cm at 20 µl/ml concentration (Table 1). Meanwhile, *M. spicata* oil had shown better antifungal effects over *A. flavus* (0.81±0.06cm) and *R. stolonifer* (0.85±0.05cm) than *A. niger* (0.93±0.01cm) at a concentration of 20µl/ml. But, at the lowest concentration i.e, 1.25µl/ml, *E. citriodora* showed the best effect on the *A. niger* (3.40±0.10cm) and *A. flavus* (4.24±0.32cm), while *M. spicata* oil showed the better effect on *R. stolonifer* (4.81±0.01cm).

In most of the concentrations of *C. tamala* oil, a better effect was found in *A. niger* than *R. stolonifer*. *E. citriodora* oil had shown greater inhihitory effects over *A. niger* than *R. stolonifer*. Similarly, *Z. armatum* oil had shown better inhihitory effects over *A. flavus* (0.99±0.09cm) than *A. niger* (1.13±0.20cm) and *R. stolonifer* (1.13±0.15cm) at 20µl/ml concentration. whileamong all EOs, *Z. armatum*

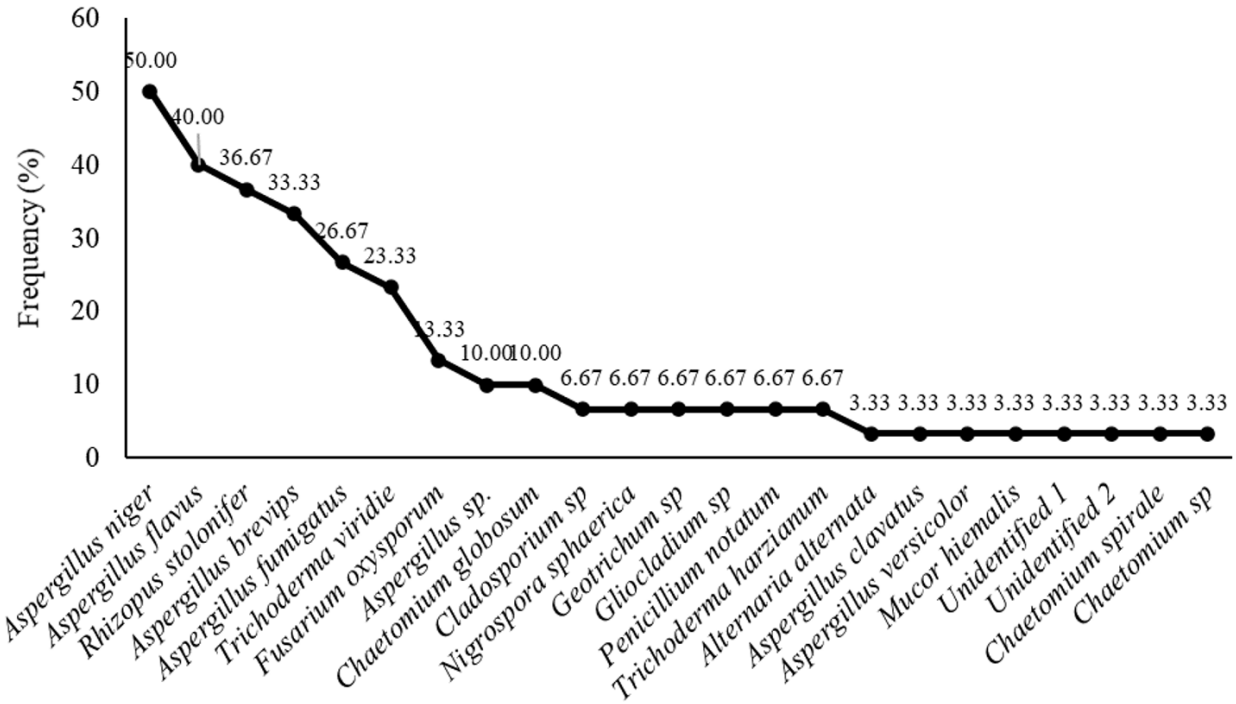

Figure 1. Frequency rank curve and rank of most commonly found fungal species in Agaricus bisporus.

Table 1. Antifungal effect of different EOs against radial mycelial growth (Mean±SD) of mycobial contaminants

Fungi	Concentrations	*E. citriodora*	*M. spicata*	*C. tamala*	*Z. armatum*
Aspergillus niger	20 µl/ml	0.95±0.05^{A}	0.93±0.01^{A}	0.70±0.08^{A}	1.13±0.20^{A}
	10 µl/ml	1.42±0.13^{B}	1.27±0.10^{B}	1.54±0.15^{B}	1.66±0.31^{B}
	5 µl/ml	2.24±0.08^{C}	1.79±0.12^{C}	2.74±0.09^{C}	2.01±0.09^{B}
	2.5 µl/ml	2.68±0.11^{C}	2.39±0.16^{D}	3.35±0.06^{C}	3.19±0.23^{C}
	1.25 µl/ml	3.40±0.10^{D}	4.57±0.08^{E}	4.16±0.25^{D}	5.21±0.03^{D}
	Negative Control	7.35±0.17^{E}	5.76±0.06^{F}	4.80±0.10^{E}	5.80±0.37^{E}
	Control	7.54±0.07^{E}	6.39±0.05^{G}	7.32±0.16^{E}	6.52±0.12^{E}
Aspergillus flavus	20 µl/ml	0.94±0.08^{a}	0.81±0.06^{a}	1.66±0.07^{a}	0.99±0.09^{a}
	10 µl/ml	1.64±0.14^{b}	1.18±0.08^{b}	2.34±0.13^{b}	1.28±0.12^{ab}
	5 µl/ml	3.03±0.11^{c}	2.01±0.08^{c}	2.52±0.10^{b}	1.62±0.10^{b}
	2.5 µl/ml	3.44±0.07^{d}	3.88±0.18^{d}	3.37±0.22^{c}	2.31±0.22^{c}
	1.25 µl/ml	4.24±0.32^{e}	4.63±0.12^{e}	4.46±0.17^{d}	4.46±0.27^{d}
	Negative Control	5.09±0.09^{f}	6.06±0.18^{f}	5.13±0.12^{e}	5.97±0.29^{e}
	Control	6.23±0.23^{g}	6.76±0.17^{g}	6.87±0.11^{f}	6.48±0.50^{e}
Rhizopus stolonifer	20 µl/ml	0.99±0.04$^{\alpha}$	0.85±0.05$^{\alpha}$	1.07±0.02$^{\alpha}$	1.13±0.15$^{\alpha}$
	10 µl/ml	2.52±0.04$^{\beta}$	2.47±0.04$^{\beta}$	2.03±0.05$^{\beta}$	1.85±0.08$^{\beta}$
	5 µl/ml	4.50±0.06$^{\gamma}$	3.13±0.12$^{\gamma}$	5.15±0.02$^{\gamma}$	2.36±0.16$^{\beta}$
	2.5 µl/ml	4.25±0.14$^{\delta}$	3.46±0.05$^{\delta}$	5.15±0.35$^{\delta}$	3.66±0.02$^{\gamma}$
	1.25 µl/ml	6.59±0.08$^{\eta}$	4.81±0.01$^{\eta}$	6.12±0.11$^{\eta}$	5.23±0.16$^{\delta}$
	Negative Control	7.82±0.05$^{\theta}$	6.51±0.06$^{\theta}$	7.21±0.03$^{\theta}$	6.89±0.03$^{\eta}$
	Control	8.05±0.12$^{\theta}$	7.24±0.10$^{\mu}$	7.56±0.09$^{\mu}$	7.44±0.15$^{\theta}$

(Note: The values of each EO in each test fungi sharing the same letters within a group in column are not significantly different in Tukey multiple range tests, $p < 0.05$.)

oil had shown less inhihitory effect on the mycelial growth of both fungal contaminants. Overall, these findings are more or less supported by findings reported by many researchers (Prakash et al., 2012; Zaidi and Dahiya, 2015; Barbosa et al., 2016; Kedia et al., 2016; Adhikari and Jha, 2017; Xiang et al., 2020; Piras et al., 2021).

At a concentration of 20 µl/ml of oil, the inhibitory effect of EOs on the spore germination of a few distinct fungal contaminants was found to be similar to the effect on mycelial growth, but at higher concentrations, the result was different. Of the four oils tested, *M. spicata* had the least antifungal impact, whereas *C. tamala* had the strongest inhibitory effect against *A. niger* (Fig. 2A). *C. tamala* oil demonstrated the greatest inhibition (79.30%) at a 20 µl/ml oil concentration, followed by *Z. armatum* (76.94%), *E. citriodora* (76.60%), and *M. spicata* (76.56%) against the *A. flavus* (Figure 2B). However, *M. spicata* oil demonstrated the maximum suppression (84.48%) of the spore germination of *Rhizopus stolonifer* Figure 2C). followed by *Z. armatum* (78.80%), *E. citriodora* (71.02%), and *C. tamala* (69.80%) at a 20 l/ml oil concentration. Eugenol, cinnamaldehyde, cinnamyl alcohol, cinnamyl acetate, cinnamic acid, and many other phytochemical constituents found in it might be the substances that give it its antifungal effects (Haddi et al., 2017).

Mentha oil had shown the best inhibitory effect (49.25%) at the lowest concentration (1.25µl/ml) too. Similarly, spore germination of *A. flavus* was also best controlled by *M. spicata* oil (77%) at the concentration of 20µl/ml whereas the effect of other EOs is 71.87-67.64%. These results are more or less supported by results from previous literatures (Thompson, 1986; Caccioni and Guizzardi, 1994; Bluma et al., 2008; Guerra et al., 2015; Adhikari and Jha, 2017; Teia et al., 2018; Hu et al., 2019). The different chemical compositions of the essential oils may be the cause of the variation in antifungal activity at the same concentration (Nazzaro et al., 2017). Thus, essential oils are among the significant plant-based substance that have a strong inhibitory effect on the mycelial growth and spore germination of fungal contaminants (Kalemba and Kunicka, 2003; Prakash et al., 2012; Ghalem, 2016; Hu et al., 2019). So, to preserve the quality and extend the shelf life of mushrooms in postharvest conditions, EOs can be used as a good alternative (Ju et al., 2019) to synthetic fungicides (Nuvan, Bavistin DF and Dicholrophus 76 EC, Mancozeb and Carbendazim etc.) used at vegetable markets in Kathmandu. The Food and Drug Administration (FDA) has categorized EOs as "Generally Recognized as Safe" (GRAS) and has determined that they are safe for human health due to their natural origin and widespread market acceptance (Edris, 2007). For instance, cinnamaldehyde and eugenol from C. tamala essential oil were approved by the Food and Drug Administration (FDA) to be used as GRAS compounds in human food (Gomes et al., 2011).

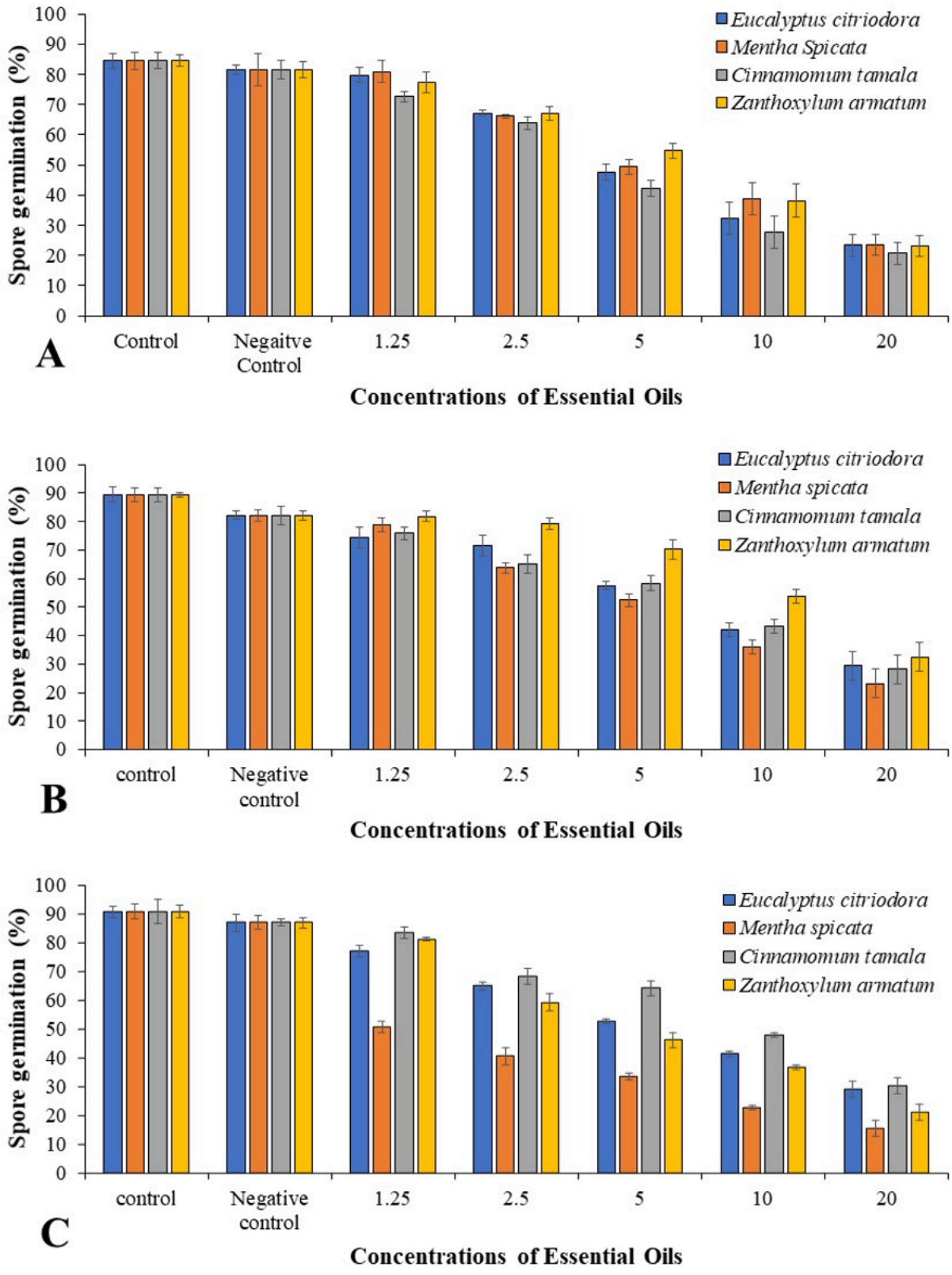

Figure 2. Antifungal effect of different EOs against spore germination of A. niger (A), A. flavus (B), and R. stolonifer (C). (Error bars represent standard errors of the means).

CONCLUSION

Mycobial contaminants are responsible for the postharvest loss of *Agaricus bisporus*, among which *A. niger, A. flavus,* and *R. stolonifer* are the most frequent. Regarding the control of these contaminants, the mycelial growth of *A. niger* and *A. flavus* were best inhibited by the EOs of *C. tamala* whereas *R. stolonifer* was best controlled by the essential oil of *E. citriodora*. The germination of all test fungal spores was also inhibited by the essential oil of *C. tamala* in comparison to that of other EOs. Therefore, the current study offers plant essential oils as a substitute input to prevent the postharvest deterioration of mushrooms. Such botanical pesticides are preferable because it minimizes the use of chemical load, minimize the cost ratio, avoid health hazards, and are eco-friendly as well.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

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

Author contribution

HSA designed the research experiment, performed the entire research and wrote the manuscript. SKJ supervised the entire research work.

Ethical approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

Data will be made available on request to corresponding author.

Consent for publication

All authors consented to the publication of this manuscript.

Acknowledgements

Authors are thankful to Prof. Dr. Mohan Siwakoti (HoD, CDB TU) for the laboratory facility. Authors are indebted to Assoc. Prof. Dr. C. P. Pokhrel, Assoc. Prof. Dr. L. B. Thapa, Assoc. Prof. Dr. Laxmi Joshi Shrestha and Mr. Sujan Balami for their valuable guidance during this study. Authors are also grateful to Mr. Bhawani Bhandari and the mushroom reseller of Kathmandu city for their help during the laboratory work and fieldwork respectively.

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RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(3): 508-516
DOI: https://doi.org/10.31015/jaefs.2023.3.5

Determination of fruit characteristics of some fig genotypes (*Ficus carica* L.) obtained by selection breeding in the eastern Mediterranean region

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Abstract
Fig (*Ficus carica* L.) is a fruit species whose cultural history is old, what is making it related to the *Ficus* genus of the *Moraceae* (Mulberry) family. The study was conducted in Kahramanmaras and Osmaniye, that are located in the Eastern Mediterranean Region, in 2016-2022. Fig cultivation is extensive in that area and at the same time in the natural fig plantation regions. Pomological and phenological analyzes were carried out on 20 fig genotypes determined in the research. The fruit weight of the fig genotypes varied between 80.79 - 40.37 g, and the average peel thickness was between 3.79-2.28 mm. The highest soluble solids value is in the genotypes 46-OS-1 at 25%, whereas the lowest soluble solids value is in the genotypes 46-TR-9 and 80-DZ-2 at 17%, and the highest acidity value is 46-OS-3 at 0.37%. The Figs genotypes were divided into two main groups in terms of fruit shape: flattened spherical and round. All genotypes were either short or moderately necked apart from one genotype. The majority of genotypes were found in yellow tones, some in purple tones, and the color of the fruit flesh was mostly in amber tones in terms of the color of the fruit skin. As a result of the study, it was revealed that Kahramanmaras and Osmaniye provinces in the Eastern Mediterranean Region have fig natural distribution areas and new varieties can be developed by selection breeding in these localities.
Keywords: *Ficus carica* L., Fig, Mediterranean region, Selection

Citation: Ugur, R., Gundesli, M.A., Ozatar, H.O., Ozen, M. (2023). Determination of fruit characteristics of some fig genotypes (*Ficus carica* L.) obtained by selection breeding in the eastern Mediterranean region. International Journal of Agriculture, Environment and Food Sciences, 7 (3), 508-516

Received: April 9, 2023
Revised: August 05, 2023
Accepted: August 7, 2023
Published Online: September 11, 2023

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Available online at
https://jaefs.com/
https://dergipark.org.tr/jaefs

INTRODUCTION

The figs belong to the species *Ficus carica* of Moraceae family of the Urticales order, and, what is more, there are many wildlife and cultivated subspecies (Çaliskan and Polat, 2012a). *F.carica* L., also known as the Anatolian Fig, is the species that is most commonly used in fruit cultivation, and abundant in natural plantations (Hepaksoy et al. 2004). The native land of figs is considered to be Anatolia (Kuden et al. 2005).

Fig, known as a subtropical-climate plant, is a major source of food for wild animals (Watson and Dallwitz, 2004) and can be found over a wide geographical area serving as the migratory routes for wild animals from the Mediterranean basin to Iran and the North Caucasus (Özbek, 1978). For this reason, it is believed that it has spread worldwide from this particular area. Although the fig is a subtropical climate plant, it requires cooling (Şahin and Ürel, 1992).

As was mentioned before, the native land of figs is Anatolia, where this fruit has cultivated in the early periods of human history, and played a particularly significant role among other fruit species. In this respect, Turkey is a major producer of table as well as dried figs (Çalışkan, 2012b). Turkey accounts for 320,000 tons of the world's fig production which is representing only 1,318,588

tonnes of production alone. 85.500 tons (58%) of dried fig production, which was 148.000 tons in 2021, were produced by Turkey, 25.000 tons (17%) by Iran, 10.000 tons (7%) by Spain (FAO, 2023).

Most of Turkey's fig production is composed of dried varieties which are produced primarily in the Aegean Region. Table figs production is mainly in the Marmara region located in the Eastern Mediterranean and Central Black Sea regions (Çalışkan and Polat, 2008).

In the regions where fig production is placed, it is naturally abundant on land and roadsides, apart from cultivated fig areas. Moreover, figs are favored by the local people who even sometimes growing it in their area. The figs also usually receiving local names, for instance, the name of the region or the owner of the area where it was grown. It is possible to develop new fig varieties from these wild genotypes with different fruit flavor characteristics (Çalışkan and Polat, 2012b).

The Eastern Transitional Region Agricultural Research Institute conducted the study in 2016-2022 years. The trees that were growing on the sides of the fields and roads and in front of the houses would have original characteristics according to the selection criteria that were mentioned in the study.

MATERIAL AND METHOD

Material

The survey and selection studies were carried out in Kahramanmaraş and Osmaniye provinces, and the coordinates data of the selected fig genotypes was recorded (Table 2). 9 fig genotypes were selected from Kahramanmaraş Onikisubat district, 2 fig genotypes from Pazarcık district and 2 fig genotypes from Türkoğlu district. In Osmaniye, 7 genotypes of figs, 2 Bahçe, 3 Düziçi, and 2 Hasanbeyli were included in the research (Table 2). In total, 20 genotypes from both regions were chosen and analyzed in the selection study (Table 2).

Method

Survey and selection studies were implemented in Kahramanmaras and Osmaniye provinces in 2016-2022. Fruit weight, ostiole width, fruit skin thickness, fruit acidity, and fruit color were examined during the research. It was determined that 9 genotypes from Kahramanmaras and 4 genotypes from Osmaniye showed promising characteristics after applying weighted grading to selected fig types in the study (Table 1).

The fruit shape index (fruit size/fruit length) and ostiole width were measured at 0.001 mm in precision digital balance. Measurements were made on 10 fruit samples from each genotype with a precision digital caliper. The selected fig genotypes were divided into three groups of oblate, spherical (global), and long (pyriform) fruits. Fruit shape indices were obtained by dividing fruit size by fruit length. According to these measurements, fruits with an index value of 1.1 were considered flat fruit, fruits with an index value between 0.9-1.1 were considered spherical, and fruits smaller than 0.9 were considered as long-oval fruits (Aksoy et al. 1992; Upov, 2003). The number of soluble solids (% SS) was measured with a hand refractometer.

Table 1. Local information and coordinates of selected fig genotypes.

No	Genotype	General Information About Genotype			
		Province	District	Location	Coordinate
1	46-OS-1	Kahramanmaras	Onikisubat	Karadere	36°37'11"N – 36°39'28" E
2	46-PZ-2	Kahramanmaras	Pazarcik	Kizkapanlı	37°22'28"N – 37°17'38" E
3	46-OS-3	Kahramanmaras	Onikisubat	Sir	37°28'51"N – 36°38'48" E
4	46-OS-4	Kahramanmaras	Onikisubat	Yenicekale	36°35'58"N – 36°28'21" E
5	46-OS-5	Kahramanmaras	Onikisubat	Dongele	37°33'45"N – 36°38'30" E
6	46-PZ-6	Kahramanmaras	Pazarcik	Yumaklicerit	37°34'27"N – 37°32'17" E
7	46-TR-7	Kahramanmaras	Turkoglu	Beyoglu	37°17'31"N – 36°46'47" E
8	46-OS-8	Kahramanmaras	Onikisubat	Derekoy	37°35'27"N – 37°01'34" E
9	46-TR-9	Kahramanmaras	Turkoglu	Beyoglu	37°17'14"N – 36°45'21" E
10	46-OS-10	Kahramanmaras	Onikisubat	Sucati	37°46'05"N – 36°38'23" E
11	46-OS-11	Kahramanmaras	Onikisubat	Suleymanlı	37°52'56"N – 36°49'39" E
12	46-OS-12	Kahramanmaras	Onikisubat	Suleymanlı	37°52'46"N – 36°49'44" E
13	46-OS-13	Kahramanmaras	Onikisubat	Suleymanlı	36°37'31"N – 36°49'46" E
14	80-BH-1	Osmaniye	Bahce	Yesilyurt	37°12'23"N – 36°10'23" E
15	80-DZ-2	Osmaniye	Duzici	Karsıyaka	37°14'14"N – 36°27'27" E
16	80-DZ-3	Osmaniye	Duzici	Yenice	37°16'17"N – 36°28'28" E
17	80-DZ-4	Osmaniye	Duzici	Tasoğlu	37°14'38"N – 36°27'02" E
18	80-BH-5	Osmaniye	Bahce	Bekdemir	37°14'26"N – 36°34'48" E
19	80-HS-6	Osmaniye	Hasanbeyli	Kaypak	37°09'56"N – 36°27'15" E
20	80-HS-7	Osmaniye	Hasanbeyli	Kaypak	37°09'51"N – 36°27'44" E

Table 2. The pointing system applied according to the weighted grading method in selected fig genotypes (Aksoy et al., 2003; Caliskan and polat, 2008; Upov, 2003)

Chracter	Weighting Factor	Classification			Point
			2021	2022	
Fruit Size (g)	30	Biggest	71.37-82,31	70.07-79.29	15
		Big	60.39-71.36	60.82-70.06	25
		Medium	49.41-60.38	51,56-60.81	20
		Small	38.43-49.40	42.31-51,55	10
Thickness of peel (mm)	10	Thin	2.00-2.45	2.01-2.88	8
		Medium	2.46-2.91	2.89-3.76	10
		Thick	2.92-3.37	3.77-4.62	6
Ostiole Width (mm)	20	More open	2.45-4.51	3.25-4.68	10
		Open	4.52-6.58	4.69-6.12	15
		Close	6.59-8.65	6.13-7.54	20
Acidity (%)	15	Low	0.27-0.31	0.26-0.29	10
		Medium	0.32-0.36	0.30-0.33	15
		High	0.37-0.40	0.34-0.37	5
Total Soluble Solids (%)	25	Low	16.00-18.66	17.00-20.00	5
		Medium	18.67-21.33	20.01-23.01	10
		High	21.34-24.00	23.02-26.00	15
Total	**100**				

RESULTS

The first fruit-bearing was distributed between the 4th week of March and the 3rd week of April. It was determined that the earliest first fruit-bearing was observed in the 80-DZ-2 genotype, and the latest first fruit-bearing was observed in 7 genotypes, all from Kahramanmaras region. Similarly, in the second fruit bearing, genotypes from the Kahramanmaras region were found to give bearing later (Table 3).

Average fruit weight for both years ranged from 80.79-40.37 g. The average fruit weight was determined to be 50.04 g. The table indicates that the highest average fruit weight is in genotype 46-TR-7, followed by the genotypes 70.79 g with 46-OS-8 and 68.32 g with 46-OS-3. The lowest fruit weight was found in genotype 46-PZ-2 with 40.37 g. In the weighted grading tests using fruit weight, We can seen that five genotypes obtained 25 points, only one of the genotypes belonging to Osmaniye the province obtained 25 points and two of them obtained 20 points. There were no significant differences in the standard deviations of the in fruit weight for years and annual averages for years.

Fruit sizes ranged from 54.93 to 35.77 mm and the highest fruit size were found in the genotypes 46-TR-8 (54.93 mm), 46-TR-9 (54.16 mm), and 46-TR-7 (52.86 mm). Average fruit size was observed to be 42.73 g and the lowest for genotype 80-BH-1. In the study where fruit length varied from70.92-39.19 mm, the average fruit length was found to be 45.14 mm. The highest fruit length were observed in genotypes 46-TR-7 and 46-OS-8, with values of 70.92 and 51.73 mm, respectively. When the fruit indices are examined in general, it is seen that the index values of the fig genotypes are close to 1 and show a global structure with these data. However, in some genotypes, the value may be said to be slightly greater than 1 and these may be assessed as flatter. It can be mentioned that the fruit structure of all genotypes was slightly-flat and round (Table 4).

The fruit acid values obtained according to the titration method and the resulting weighted rating scores are shown in the table below. Table-4. As of 2021, it was understood that the average fruit acidity in terms of citric acid varied between 0.40-0.27 in selected fig genotypes, and the average acidity was 0.32. As of 2022, it was seen that the average fruit acidity in terms of citric acid vary between 0.37-0.26 in selected fig genotypes and the average acidity is 0.32. According to the table, no change was found in the average fruit acidity values of 2021 and 2022 (Table 5).

Fruit ostiole width were separately for 2021 and 2022 and the averages of two years were seen in the selected fig genotypes, While the average fruit ostiole width in 2021 is 5.18 mm, it was seen that the fruit ostiole width in 2022 is 4.87 mm. In the mean of both years, as seen in the table, the ostiole width varied between 8.10-3.01 mm. The mean ostiole width of both years was 5.02 mm. The highest ostiole width was found to be 8.10 mm, 7.41 mm, and 7.35 mm in fig genotypes coded 46-PZ-2, 46-TR-7, and 46-OS-8, respectively. The lowest ostiole width values were in 46-OS-11 (3.01 mm) and 46-OS-12 (3.13 mm) fig genotypes (Table 5).

The fruit soluble solids values of the selected fig genotypes for the year 2021-2022 (Table 11). Fruit soluble solids varied between 17-25% and the highest fruit soluble solids were found in the genotypes 46-OS-1 (25%), 46-OS-3 (24%), and 46-OS-4 (23%). It was

Table 3. Average bearing dates of selected fig genotypes for the years 2021-2022

No	Genotype	Bearing		Maturity period
		Firts Bearing	Second Bearing	
1	46-OS-1	3rd week of April	3rd week of June	20-30 August
2	46-PZ-2	2nd week of April	2nd week of June	15-25 August
3	46-OS-3	3rd week of April	3rd week of June	20-30 August
4	46-OS-4	3rd week of April	3rd week of June	20-30 August
5	46-OS-5	2nd week of April	3rd week of June	20-30 August
6	46-PZ-6	2nd week of April	2nd week of June	15-25 August
7	46-TR-7	1st week of April	2nd week of June	15-25 August
8	46-OS-8	2nd week of April	2nd week of June	15-25 August
9	46-TR-9	2nd week of April	1st week of June	10-20 August
10	46-OS-10	3rd week of April	3rd week of June	20-30 August
11	46-OS-11	3rd week of April	3rd week of June	20-30 August
12	46-OS-12	3rd week of April	3rd week of June	20-30 August
13	46-OS-13	3rd week of April	2nd week of June	15-25 August
14	80-BH-1	2nd week of April	2nd week of June	15-25 August
15	80-DZ-2	4th week of April	1st week of June	10-20 August
16	80-DZ-3	1st week of April	1st week of June	10-20 August
17	80-DZ-4	1st week of April	1st week of June	10-20 August
18	80-BH-5	2nd week of April	2nd week of June	15-25 August
19	80-HS-6	2nd week of April	2nd week of June	15-25 August
20	80-HS-7	2nd week of April	3rd week of June	20-30 August

Table 4. Fruit size and shape of selected fig genotypes (2021-2022)

No	Genotype	Fruit Weight (g)	Fruit Size (mm)	Fruit Lenght (mm)	Fruit Index (with/ lenght)	Fruit Shape
1	46-OS-1	45,73	37,25	45,81	0,81	long-oval
2	46-PZ-2	40,37	37,29	48,68	0,77	long-oval
3	46-OS-3	68,32	50,55	47,08	1,07	spherical
4	46-OS-4	61,59	42,84	46,11	0,93	spherical
5	46-OS-5	52,75	51,21	43,78	1,17	long
6	46-PZ-6	60,11	36,21	39,55	0,92	spherical
7	46-TR-7	80,79	52,86	70,92	0,75	long-oval
8	46-OS-8	70,79	54,93	51,73	1,06	spherical
9	46-TR-9	65,91	54,16	41,18	1,32	long
10	46-OS-10	56,76	41,74	45,23	0,92	spherical
11	46-OS-11	59,07	40,70	43,60	0,93	spherical
12	46-OS-12	55,70	41,35	44,03	0,94	spherical
13	46-OS-13	65,79	39,26	41,63	0,94	spherical
14	80-BH-1	59,84	35,77	40,31	0,89	spherical
15	80-DZ-2	78,55	42,93	45,76	0,94	spherical
16	80-DZ-3	45,74	40,27	40,70	0,99	spherical
17	80-DZ-4	67,57	40,85	43,79	0,93	spherical
18	80-BH-5	47,56	37,84	41,10	0,92	spherical
19	80-HS-6	45,01	36,05	39,19	0,92	spherical
20	80-HS-7	53,37	40,53	42,68	0,95	spherical
Highest		**80.79**	**54,93**	**70,92**	**1,32**	-
Lowest		**40.37**	**35,77**	**39,19**	**0,75**	-
Mean		**59,04**	**42,73**	**45,14**	**0,95**	-
SD		±10,90	±6,20	±6,68	±0,13	-

Table 5. Fruit quality values of selected fig genotypes

No	Genotype	Acidity (%)	Ostiole Width (mm)	Total Soluble Solids (%)	Peel Thickness (mm)	Peel Color	Flesh Color
1	46-OS-1	0,36	5,06	25,00	2,53	Yellow	Dark amber
2	46-PZ-2	0,33	8,10	22,50	2,63	Yellow-green	Red
3	46-OS-3	0,37	4,19	24,00	2,95	Yeşil	Amber
4	46-OS-4	0,37	4,36	23,50	2,70	Yellow-green	Amber
5	46-OS-5	0,34	5,03	20,50	2,86	Yellow	Amber
6	46-PZ-6	0,30	3,33	18,00	2,30	Yellow-green	Kırmızı
7	46-TR-7	0,28	7,41	18,00	3,79	Yellow-green	Kırmızı
8	46-OS-8	0,35	7,35	21,00	3,22	Yellow	Light amber
9	46-TR-9	0,31	6,31	17,00	2,85	Yellow	Amber
10	46-OS-10	0,34	4,52	22,50	2,64	Purple	Amber
11	46-OS-11	0,32	3,01	19,50	2,56	Yellow-green	Amber
12	46-OS-12	0,32	3,13	20,50	2,59	Light yellow	Light amber
13	46-OS-13	0,35	6,22	22,00	2,45	Purple	Light amber
14	80-BH-1	0,33	4,34	21,50	2,31	Yellow	Light amber
15	80-DZ-2	0,28	5,98	17,00	2,69	Yellow-green	Dark amber
16	80-DZ-3	0,28	3,38	18,50	2,45	Yellow	Dark amber
17	80-DZ-4	0,33	4,19	21,50	2,57	Lihgt purple	Red
18	80-BH-5	0,31	4,67	18,00	2,40	Yellow	Dark yellow
19	80-HS-6	0,28	4,83	17,50	2,28	Yellow	Red
20	80-HS-7	0,27	5,10	17,50	2,52	Yellow	Red
Highest		**0,37**	**8,10**	**25,00**	**3,79**	-	-
Lowest		**0,27**	**3,01**	**17,00**	**2,28**	-	-
Mean		**0,32**	**5,02**	**20,28**	**2,66**	-	-
SD		±0,03	±1,42	±2,44	±0,34	-	-

Table 6. Average scores (% citric acid) obtained as a result of weighted grading evaluations for all pomological properties of 2021 and 2022.

No	Genotype	Criteria and Genotypes Scores					
		Fruit Weight	Peel Thickness	Ostiole Width	Acidity	Soluble Solids	Total Score
1	46-OS-1	300	80	300	75	375	1.130
2	46-PZ-2	300	80	200	225	375	1.180
3	46-OS-3	750	100	400	75	375	1.700
4	46-OS-4	750	80	400	75	375	1.680
5	46-OS-5	600	100	300	75	250	1.325
6	46-PZ-6	600	80	400	150	125	1.355
7	46-TR-7	450	60	200	150	125	985
8	46-OS-8	450	100	200	75	250	1.075
9	46-TR-9	750	100	300	225	125	1.500
10	46-OS-10	600	80	300	75	375	1.430
11	46-OS-11	600	80	400	225	125	1.430
12	46-OS-12	600	80	400	225	250	1.555
13	46-OS-13	750	80	300	75	250	1.455
14	80-BH-1	600	80	400	225	250	1.555
15	80-DZ-2	450	80	300	150	125	1.105
16	80-DZ-3	300	80	400	150	125	1.055
17	80-DZ-4	750	80	400	225	250	1.705
18	80-BH-5	300	80	300	225	125	1.030
19	80-HS-6	300	80	300	150	125	955
20	80-HS-7	600	80	300	150	125	1.255
Highest		**750**	**100**	**400**	**225**	**375**	**1.705**
Lowest		**300**	**60**	**200**	**75**	**125**	**955**
Mean		**540**	**83**	**325**	**150**	**225**	**1.323**

Table 7. Quality situations of selected fig genotypes according to weighted grading scores

No	Genotype	Quality Situation
1	46-OS-1	Poor
2	46-PZ-2	Medium
3	46-OS-3	High Quality
4	46-OS-4	High Quality
5	46-OS-5	Medium
6	46-PZ-6	Medium
7	46-TR-7	Poor
8	46-OS-8	Poor
9	46-TR-9	Quality
10	46-OS-10	Quality
11	46-OS-11	Quality
12	46-OS-12	High Quality
13	46-OS-13	Quality
14	80-BH-1	High Quality
15	80-DZ-2	Poor
16	80-DZ-3	Poor
17	80-DZ-4	High Quality
18	80-BH-5	Poor
19	80-HS-6	Poor
20	80-HS-7	Medium
High Quality		**5 genotype**
Quality		**4 genotype**
Medium		**4 genotype**
Poor		**7 genotype**

observed that the soluble solids were 20.28% and the lowest fruit soluble solids were in the genotype 80-DZ 2. In the weighted grading tests, it was determined that 5 genotypes obtained 15 points, and the lowest number of genotypes was 9 in Kahramanmaraş and Osmaniye. However, when we look at the table in general, it was seen that the majority of the fruit soluble solids ratios in the selected fig genotypes are above 20%. When the standard deviations in the fruit soluble solids values were taken into account, it is observed that a small amount of standard deviation difference occurs every year in the fig genotypes. It was concluded that the shell thickness of the selected genotypes were not significantly difference over the years (Table 5).

Peel thickness varied between 3.79-2.28 mm, and the highest peel thickness were found in 46-TR-7 (3.79 mm), 46-OS-3 (2.95 mm), and 46-TR-9 (2.85 mm) genotypes. It was observed that the average peel thickness was 2.66 mm and the lowest peel thickness value was in the genotype 80-HS-6. In the weighted grading tests performed according to the peel thickness, it was agreed that 4 genotypes obtained 10 points, and all other genotypes belonging to Kahramanmaraş and Osmaniye provinces obtained 8 points except 46-TR-7. It was observed that a small amount of standard deviation difference occurs each year in the standard deviations of the crust thickness of the years and annual averages. This situation led to the conclusion that the peel thickness values for the selected genotypes did not differ dramatically from year to years (Table 5).

By the study method, the scores obtained by the fig genotypes selected according to these characteristics after the weighted grading of the pomological characteristics and the total weighted grading score were shown in Table 2. When the scores of each fig genotype in the features that are weighted according to the ratios in the table of relative values in Table 2. The 80-DZ-4 (1.705), 46-OS-3 (1.700), and 46-OS-4 (1.680) genotypes received the highest weighted rating scores. The lowest scores were 955 and 985 points in the 80-HS-6 and 46-TR-7 fig genotypes, respectively (Table 6).

It was concluded that 9 genotypes were promising, 4 genotypes were of medium quality, and 7 genotypes were not of the expected quality in the study (Table 7).

DISCUSSION

August and continued until the first month of September. In many studies conducted in previous years, it has been stated that the beginning of maturation in figs differs according to ecologies and genotypes. Sen et al. (1993) stated that it ranged from 20-31 July to 1-15 August in Antalya conditions, while Ilgin (1995) reported that it varied between 20-31 July and 15-31 August in Kahramanmaraş conditions. Aksoy et al., (2003), determined that it varies between 1-15 August and 15-31 August in Erbeyli (Aydın) ecology, while Caliskan (2003) observed that it changes between 1-15 August and 15-

31 August in Dörtyol conditions. Simsek (2008) stated that this change occurred between 20-30 July and 15-31 August in Diyarbakır conditions, and Caliskan (2010) stated that this change occurred between 1-15 August and 15-31 August in Hatay central location conditions. As part of the project, the periods of intense ripening of the fig variety were determined as 15-30 July and 1-30 August, and it was seen that they are among the dates reported by other researchers. In a study conducted by Ilgin (1995), it was reported that the harvest time in fig genotypes in Kahramanmaraş was short in 36 genotypes, long in 14 genotypes and very long in two genotypes. Caliskan (2003) reported that in Dörtyol conditions it was short in 2 genotypes, medium in 23 genotypes, and long in 5 genotypes. Simsek (2008) determined that 28 genotypes were medium in Diyarbakir region, long in 11 genotypes and very long in three genotypes. Caliskan and Polat (2012) reported that the harvest period in fig genotypes in Hatay ecology was short in 3 genotypes, very long in 3 genotypes and medium in others. Simsek (2019) stated that the fig genotypes they selected in Tarsus ecology were short in 1 genotype, medium in 4 genotypes and long in 19 genotypes.We find that the harvest times for the fig genotypes in this study are similar to those identified by other researchers.

It was determined that the fruit weight values of the selected fig genotypes ranged from 80.79 to 40.37 gr based on the data obtained in the study. The average fruit weight values for the years 2021-2022 are 59.04 gr. was found to be. Aksoy et al., (1992), in their study, determined that the highest value in terms of average fruit weight was 708 Darpak with 76.00 g, and the lowest value was 31.50 g with 1119 Fethiye Kaya-2 variety.

Küden et al. (1998), in their study to determine some fig varieties that can be recommended to the Çukurova Region, determined the average fruit weight as 117.89 g in the Bursa Siyah variety and 36.69 g in the Bird fig variety. Caliskan (2003) indicated that the fruit weights for the selected fig genotypes ranged from 19.369 to 61.76 g (2001) and 20.45-56.90 g (2002), in his study conducted in Dörtyol. Gozlekci et al., (2004) determined the fruit weight of 7, 85-88, 18 g in 169 fig genotypes they selected from the Western Mediterranean Region. Alper (2006), in his research in Şanlıurfa, found that the fruit weight of the fig genotypes was 20.34-72.60 g, while Simsek (2008) determined that the fruit weight of the fig genotypes selected in Diyarbakır was 31.29-76,859 g (2006).) and 23.66-75.77 g (2007) were found. Caliskan (2010) found the fruit weight of the fig genotypes between 14.92-115.22 g (2008) and 9.66-93.06 g (2009) and Çalışkan and Polat (2012a) found the fruit weight between 12.29-98.38 g in figs in Hatay ecology. Şimşek (2019) stated that fruit weight values in fig genotypes selected in Tarsus ecology vary between 22.37 g (garbage figs) and 90.16 g (Black Figs). Aljane et al. (2007) determined the fruit weight of 10 local fig cultivars in Southern Tunisia between 24.5-106.7 g. Messaoudi and Haddadi (2008) found fruit weight between 27.0-87.5 g in 14 local fig genotypes in Morocco. Gaaliche et al. (2012) stated that the fruit weight of 17 local fig genotypes they selected from Northern Tunisia was 34.54-96.45 g. In our study, the 2-year average fruit weight values for selected fig genotypes between 40.37-80.79 g and an average of 59.04 g were found to be relatively high compared to previous studies.

It is undesirable in female figs because the ostiole width is wide, allowing many diseases and harmful factors, especially fruit internal rot, to enter the fruit (Can 1993; Çalışkan and Polat, 2012). It was determined that the ostiole width of the selected fig genotypes ranged between 3.01 mm and 8.10 mm, and the mean ostiole width was 5.02 mm. Gozlekci (2011) determined that the genotypes selected from the Western Mediterranean Region between 0.02-19.80 mm. Alper (2006) reported that the genotypes they selected from Şanlıurfa varied between 0.12-7.25 mm. Şimşek (2008) found the ostiole width between 1.30-7.62 mm in the genotypes he selected from Diyarbakır. Çalışkan and Polat (2012a) reported that fig genotypes in Hatay varied between 0.60-21.01 mm.

Although the fruit size of figs varies according to their genetic characteristics, they can be affected by appropriate climatic and care conditions. In our study, it was determined that the average fruit size values for the years 2021-2022 varied between 54.93 mm and 34.77 mm, and the average fruit size value was 42.73 mm. Bostan and Islam (1999), in their study, found that the average fruit size varies between 45.20 cm and 55.10 cm. The fruit length values obtained from our study ranged from 70-92 mm to 39.19 mm. Koyuncu (1998), in his research, stated that the average fruit size varies between 22.00 mm and 39.80 mm. Fruit size is a genotype and variety and can be affected by suitable climatic and care conditions (Polat and calikan 2008).

CONCLUSION

Anatolia is the homeland of figs and has very rich plant diversity. Twenty fig genotypes were determined and recorded at the end of the study. As a result of the examination and analysis, it was obtained that 13 of 20 fig genotypes were promising in terms of fruit characteristics and it was concluded that it would be appropriate to include them in the selection II stage. It has been thought that it is too early to put the results obtained from 13 promising local fig genotypes, which are the most important output of the project, into practice as of this stage of the project. After this step, a second selection breeding should be carried out and these 13 genotypes selected fig genotypes should be compared with standard varieties of figs. It will be possible to transfer the results to achieve and develop new varieties of alternative figs after this study is completed.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Declaration of interests

The authors have no conflict of interest to declare.

Author contribution

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

Ethics Committee Approval

Ethics committee approval is not required. This article does not contain any studies with human participants or animals performed by any of the authors.

Funding

The authors declared that this study has received no financial support.

Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Anknowledgement

This study was published by the project team with the evaluation of the data obtained from the TAGEM project numbered TAGEM/BBAD/16/A08/H07/1-3 between the years 2016-2022

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RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(3): 517-532
DOI: https://doi.org/10.31015/jaefs.2023.3.6

Exploring the relationship between leaf water potential, defoliation, and grape berry physical properties of Merlot (*Vitis vinifera* L.) grapevine

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Citation: Candar, S., Korkutal, I., Bahar, E., Aktas, F.B. (2023). Exploring the relationship between leaf water potential, defoliation, and grape berry physical properties of Merlot (*Vitis vinifera* L.) grapevine. International Journal of Agriculture, Environment and Food Sciences, 7 (3), 517-532

Received: April 10, 2023
Revised: July 26, 2023
Accepted: August 1, 2023
Published Online: September 13, 2023

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Available online at
https://jaefs.com/
https://dergipark.org.tr/jaefs

Abstract
The aim of this study was to examine the impact of leaf water potential and defoliation treatments on the physical properties of grape berries. The research was conducted over two consecutive years (2019-2020) using 'Merlot'/41B graft combination grapevines grown in the Chateau Kalpak vineyards located in Tekirdağ, Şarköy. The experiment involved four distinct water stress levels (S0, S1, S2, and S3), which were determined based on leaf water potential measurements. These stress levels were subjected to different irrigation levels. Additionally, defoliation treatments were applied, including Control (C), Full Window (FW), Right Window (RW), and Left Window (LW). The results showed that the effects of water stress and defoliation treatments on berry physical properties were statistically insignificant. However, in the second year of the study, the FW treatment was observed to have led to changes in the desired direction for grapevines. This was likely due to the cumulative decrease in water reserves caused by reduced precipitation over multiple years, making the effects of FW treatment more prominent. Moreover, the study found that both current and past vegetation period conditions influence vine production year, leaf water potentials (Ψleaf), and stress levels. Finally, the data revealed that berry weight and % dry weight increased with higher stress levels.
Keywords: Abiotic stress, Grapevines, Leaf removal, Drought, Berry quality, Merlot

INTRODUCTION

Climate is one of the most important factors that affect the life cycle of vines. Temperature, wind, frost, and precipitation are among the most influential climate factors that affect vine growth and development. Additionally, the water status of the vines in the vineyard varies according to topography, cultivation practices, and soil characteristics (Jasse et al., 2021). Global climate change has led to decreased water resources, which has a significant impact on the grapevine life cycle. Adequate water availability is crucial for sustainable viticulture (Medrano et al., 2015). The amount of precipitation that falls as rain is undoubtedly important for grapevine yield and quality. However, the water-holding capacity of soil also exerts a strong influence on these factors (Blaschek et al., 2019). The water-holding capacity of soil is influenced by several factors, including soil texture, topography, and the amount of precipitation. In turn, grapevine water status is affected by both the water-holding capacity of soil and the size of the canopy (Van Leeuwen et al., 2006). The water status of grapevines is known to vary depending on whether water deficiency in the soil occurs before or after veraison (Gambetta et al., 2020). According to Korkutal et al. (2019), grapevines are more sensitive to water restriction before veraison compared to after veraison. During the early stages of berry development, water deficiency can have a significant impact

on cell division and expansion, ultimately affecting both the size and structure of the berry, as noted by Bondada and Shutthanandan (2012). Furthermore, Flexas and Medrano (2002) have reported that excessive water stress can lead to a reduction in the size of grape berries. Properly managed water deficit can have a positive impact on various aspects of grapevine growth and development. This includes promoting slower leaf growth and higher water use efficiency, leading to improved cluster characteristics, berry composition, and ultimately, wine quality (De Orduna, 2010; Bahar et al., 2011; Savoi et al., 2016; Korkutal et al., 2019; Blancquaert et al., 2019; Vilanova et al., 2019). The physiological and metabolic responses to water stress also promote the formation of secondary metabolites in the berries, which are responsible for imparting desirable organoleptic properties. This is primarily attributed to the smaller berry size and higher skin-to-pulp ratio, resulting in a relatively higher skin content of tannins, anthocyanins, total phenolics, and other compounds.

Vineyard management practices, such as irrigation, training systems, leaf removal, and cluster thinning, can have a significant impact on grapevine growth and development (Alem et al., 2019). Additionally, both environmental conditions and viticulture practices can affect berry weight and composition at various stages of development (Dai et al., 2011). The attainment of optimal berry maturity and wine quality, particularly in cool climates, relies on striking a balance between leaf area and yield, as highlighted by King et al. (2015). Numerous researchers have endeavored to elucidate the impact of cultivation practices on grapevine, employing different varieties and treatments to explore this topic (Smart et al., 1990; Deloire et al., 2005; Poni et al., 2009; Korkutal and Bahar, 2013; Bahar et al., 2017; Candar et al., 2019; Korkutal et al., 2019; 2020; 2021b; Candar et al., 2020a; 2020b; Alço et al., 2023).

On the other hand, Dai et al. (2011) stated that the weight and composition of berries undergo changes depending on the genetics of the vine, environmental factors, and cultivation methods.

Leaves are vital organs that carry out crucial physiological functions in grapevines. These include establishing photosynthesis, transpiration, and carbon balance, as well as regulating the microclimate within the canopy (Kliewer and Dokoozlian, 2005). Additionally, they help maintain the plant and soil water budget balance and accumulate sugar and nitrogen in the berry (Nicotra et al., 2011; Rossouw et al., 2017; Wang et al., 2019). The amount of carbon that leaves absorb during photosynthesis is directly related to the total biomass produced by grapevines. The physiological activity of leaves is influenced by several factors, such as size, age, climatic conditions, general characteristics of the terroir, and genetic differences (Peppe et al., 2011; Tozer et al., 2015). This activity, in turn, affects the total leaf area on grapevines, yield, and biochemical processes during the ripening period. However, leaf shape and size may not always be effective in achieving desired outcomes (Chitwood et al., 2016; Candar et al., 2021).

Defoliation practices can significantly impact the production-consumption balance of the vine (Bowen, 2009). Various impacts arise from these conditions, encompassing reduced transport of photosynthesis products to the cluster, restricted root growth, and diminished water efficiency (Hunter et al., 1995; Medrano et al., 2007; Poni et al., 2008; Palliotti et al., 2013; Vaillant-Gaveau et al., 2014). Removing leaves during berry ripening can eliminate a source of carbon and nitrogen, resulting in a reduction in sugar and nitrogen accumulation (Rossouw et al., 2018) and potentially impacting the quality of the berries (Bubola et al., 2022). Moreover, reducing the total leaf area of the vine with defoliation treatments may weaken grapevine growth in the following years and cause a decrease in yield (Bahar et al., 2018). In some cases, the impact of leaf removal treatments on clusters and yield was not always statistically significant. However, treatments where the main shoot leaves were left on the plant showed slightly higher values compared to other treatments (Korkutal et al., 2017).

Understanding how grapevine varieties respond and their limits of adaptability is crucial in maintaining a balanced product load and canopy architecture that aligns with the targeted yield and quality, and in implementing effective vineyard management (Candar, 2022). When it comes to leaf area management, the seasonal effects of each vegetation period play a significant role in determining the outcome. Therefore, planning for canopy management practices should be done annually, based on long and medium-term meteorological evaluations, and these practices should be adjusted according to the phenological period and short-term meteorological evaluations (Candar et al., 2022).

Grape berries are complex and versatile biochemical units that undergo successive processes of change during their development and maturation, which influence their size, composition, color, texture, taste, and aroma (Kunter et al., 2013). The histochemical structure of grapes is composed of sugars, organic acids, phenolic substances, minerals, and flavoring substances. The process of berry ripening is a physiological period that has a significant impact on the composition of the berries and, subsequently, on the quality of the wine, depending on the characteristics of the grape variety. Throughout the ripening process, grapes undergo numerous physical and biochemical changes, including alterations in weight, volume, hardness, sugar content, acidity, color, and aroma. According to Chen et al. (2018), berry size is one of the factors that affects grape quality.

Schalkwyk (2004) states that several factors influence

berry weight and size, including genetic origin, berry set, number of berries per cluster, berry position within the cluster, number of seeds per berry, number of clusters per vine (bud load), climate, water conditions, fertilization, soil type, rootstock, variety, and degree of maturity. The author also notes that the weight of clusters and berries can vary from season to season and from region to region within the same variety.

Various factors such as variety, irrigation, and canopy management can affect berry size and the proportional distribution of skin, berry flesh, and seed within the berry. These differences can also alter the ratio of berry flesh/skin ratios and the amount of solutes that pass from the skin to the wine (Roby and Matthew, 2004; Matthews and Nuzzo, 2007; Barbagallo et al., 2011).

This research focused on the 'Merlot' grape cultivar and aimed to investigate the effects of four different levels of pre-dawn leaf water potential (LWP, Ψpd) and four defoliation treatments on the physical properties of grape berries.

MATERIALS AND METHODS

Location and plant material

The study was conducted at the Chateau Kalpak vineyard in the Şarköy district of Tekirdag, in coordinates 40° 39' 12.00" N and 27° 03' 20.00" E, during the 2019 and 2020 vegetation periods for two consecutive years. The grapevines used in the study were of the 'Merlot'/41B combination and were planted with a 2.1 m and 1.0 m in-row spacing, and a 70 cm stem height. The grapevines were trained using the double arm cordon training method in the Espalye system.

'Merlot' is a wine grape variety that originates from France and has been cultivated in Turkey since the early 1990s. The population of this variety in Tekirdağ shows significant morphological variation (Aktaş, 2021). 'Merlot' grape cultivar is a moderately to strongly vigorous variety that tends to produce a lot of offshoots and suckers. Its semi-erect to horizontal bearing requires sufficient trellising, and it is better to prune it short for better fertility. In certain climatic conditions, there is a risk of coulure. The cultivar is well-suited to clay-limestone terroirs. However, it is rather sensitive to winter and spring frosts (due to early budburst) and may not be well-adapted to intense drought conditions. The berries of 'Merlot' are medium in size, while the bunches are small to medium and winged. 'Merlot' grapes produce round, powerful, and richly-colored wines with relatively low acidity. These full-bodied and structured wines, with rather supple tannins, can be aged in wood barrels. The aromas of 'Merlot' wines are complex and elegant (Plantgrape, 2023a).

According to Plantgrape (2023b), the 41B rootstock is known for its ability to adapt to limestone soils and its resistance to chlorosis. It can withstand up to 60% of "total" limestone, 40% of "active" limestone, and an ICP of 60. Additionally, it has a good capacity to absorb magnesium from the soil. However, the 41B is susceptible to temporary water excess during the spring, and its resistance to drought is moderate. It may not be well-suited for overly compact soils. Grafts with 41 B MGt exhibit moderate to high vigor, and they usually have good compatibility, though some issues have been reported with 'Merlot' and 'Pinot' cultivars, which are still frequently grafted onto this rootstock. The initial growth of the plant can be slow, and the 41B promotes the compactness of grape clusters, while delaying the vegetative cycle of grafts. Compared to other rootstocks, the fruits produced by 41B grafted varieties are slightly less rich in sugar and slightly more acidic. The 41B is sensitive to both water stress and humidity excess in the soil and may be susceptible to the decline of the grapevine trunks (Plantgrape, 2023b).

Methods

To ensure homogeneity among the grapevines measured during the 2019-2020 vegetation period, plants with extreme differences in the number of clusters and shoots were excluded from the experiment. Additionally, no empty plants were included among the trial grapevines. The number of clusters and shoots were equalized again the following year when the shoots were approximately 30 cm long. The study involved 144 homogeneous vines subjected to four stress levels [S0 (Control=no irrigation), S1 (-0.3/-0.5 MPa), S2 (-0.5/-0.7 MPa), and S3(<-0.7 MPa)] and four defoliation treatments: Control (C), Full Window (FW), Right Window (RW), and Left Window (LW).

Water Availability (Stress levels)

Irrigation was carried out as needed based on the predawn leaf water potential (LWP, Ψpd) measured at five to seven-day intervals. The irrigation was adjusted according to the predetermined stress levels, and the Ψpd was checked the next day to ensure that it was within the desired range.

The S0 treatment, which served as the control, did not receive any irrigation and was left to random precipitation. For S1, the stress level was set to -0.4 to -0.6 MPa, and the Ψpd was maintained within this range through irrigation. Similarly, for S2, the stress level was set to -0.5 to -0.7 MPa, and the Ψpd was maintained within this range through irrigation. For S3, the stress level was set to ≤ -0.7 MPa, and the Ψpd was kept below this value through irrigation.

Defoliation Treatments

The defoliation treatments (DT) were carried out about two weeks after the onset of veraison. These treatments were performed by removing shoots and leaves from the eighth node and creating a window by eliminating all the leaves between the seventh and thirteenth nodes. The experiment included four different defoliation

treatments: Control (C), Full Window (FW), Right Window (RW), and Left Window (LW). For the FW treatment, shoots and leaves were removed from the eighth node. For the RW treatment, all the leaves between the seventh and thirteenth nodes on the west side of the row were removed. For the LW treatment, all the leaves between the seventh and thirteenth nodes on the east side of the row were removed. The C treatment served as the control and no defoliation was performed. During the defoliation process, special attention was paid to ensure that the grapes were between 15 and 17 °Brix according to Alço (2019).

Analysis and Measurements

Phenological development stages were recorded during the experimental years, following the guidelines of Lorenz et al. (1995). Climate data were obtained from the Turkish State Meteorological Service (MGM).

For measurements, a random sample of 18 clusters was taken from three vines in each replication. From these clusters, berries were randomly selected from all parts to determine the berry characteristics, as described by Carbonneau et al. (1991). In order to determine the characteristics of the berries, 48 representative berries from each replication of each treatment were randomly selected and their width and length were measured using a digital caliper (Mitutoyo, Japan). The values obtained were given in centimeters, following the guidelines of the International Organization of Vine and Wine (OIV, 2021). The volume of 100 representative berries was determined in cm^3 per berry using the overflow method in a measuring cylinder, as described by Bahar et al. (2011). The weight of the berries was determined using an analytical balance scale with a sensitivity of 0.001 g to obtain the fresh weight of the berries. To determine the dry weight, 48 representative berries were dried in an oven (Elektro-mag, Turkey) at 65-70°C for 72 hours and then weighed again using the analytical balance scale. The dry weight of the berries was given in g per berry, as recommended by OIV (2021). The fresh weight and dry weight of berries were used to calculate the weight values per 100 berries, using proportions. The percentage of dry weight was determined using the formula (dry weight of berries x 100) / fresh weight of berries, as described by Bahar et al. (2011). The density of the berries was calculated by dividing the berry mass by the berry volume. The berry skin area was calculated using the formula $4\pi r^2$, and the values obtained were expressed in cm^2 per berry, according to Barbagallo et al. (2011). The ratio of berry skin area to berry flesh volume was determined by dividing the berry skin area by the berry flesh volume, and the resulting value was expressed as a proportion (Palma et al., 2007).

Trail Design and Statistical Analysis

The experiment utilized a Divided Plots Trial Design, in which the main plot comprised of water stress levels, and each sub-plot was made up of defoliation practices. A total of 144 vines were examined, with four different water stress levels and four defoliation treatments. Each treatment was replicated three times, with three plants in each replication. The data collected from the berries underwent ANOVA to test for statistically significant differences between the treatments. As no statistically significant differences were detected, no multiple comparison test was conducted. Apart from ANOVA, bivariate relationships between the data were also analyzed. Additionally, a principal component analysis (PCA) was conducted to examine the physical components of the grape clusters and berries. The data analysis was carried out using the R statistical environment (R Core Team, 2016).

RESULTS AND DISCUSSION

Climate and phenology

In 2019, the total precipitation recorded was 378.4 mm, while in 2020 it was 290.00 mm. The long-term average precipitation between 1939 and 2019 was 589.5 mm. Based on this data, it can be observed that the precipitation in 2019 was 211.10 mm less than the long-term average, and in 2020, it was 299.5 mm less than the long-term average. The average temperature for 2019 was 15.60°C, while the average for 2020 was 15.30°C.

During the experimental years, phenological developmental stages were recorded and analyzed. The results showed that budburst (EL 05) occurred on 11 April in 2019 and 15 April in 2020. Full bloom (EL 23) was observed on 2 June in 2019 and 8 June in 2020, while berry set (EL 27) was recorded on 9 June in 2019 and 14 June in 2020. The verasion stage (EL 35) was observed on 20 July in 2019 and 24 July in 2020. Finally, the harvest (EL 38) was conducted on 15 September in 2019 and 16 September in 2020. The data indicate that the phenological developmental stages occurred 4-6 days later in 2020 compared to 2019. The harvest was carried out in 2019 when experimental parcels reached an average °Brix of 24.39, and average of 24.80 °Brix in 2020.

Berry Width

The results of the ANOVA revealed that the main effects of different leaf water potential and defoliation treatments were not statistically significant in both years. However, in 2019 in terms of the defoliation main effect (DME), it was observed that the C treatment had the highest berry width with a value of 12.89 mm, while the LW treatment had the lowest value of 12.72 mm. When the berry width is arranged from the largest to the smallest based on LWP Main Effect (LWPME), the following measurements were observed: S1 with 13.13 mm, S0 with 12.93 mm, S2 with 12.61 mm, and S3 with 12.53 mm. The ranking of berry width, from the largest to the smallest, based on the 2019 LWPME x DME interactions reveals that the S1

x C interaction has the highest value of 13.36 mm, while the S2 x FW interaction was the lowest with a 12.33 mm value (Figure 1A).

In 2020, during the examination of the main effects of LWP and defoliation treatments, it was discovered that both main effects did not have a significant impact, but there were measurable effects on FW and LW, with values ranging from 12.81 mm to 13.21 mm. When considering the main effect of LWP, the treatment of S3 had a higher numerical value of 13.17 mm, while the treatment of S1 had a lower numerical value of 12.79 mm (Figure1B). The effect of the interaction between LWPME and DME on berry width, was observed a higher value of 13.55 mm for S3 x LW. Conversely, the S1 x C interaction had a low value of 12.50 mm. No significant variations were observed in berry width means between the two years.

The available data is consistent with Kotseridis et al. (2012) findings that the treatment of leaf removal did not result in a change in berry size in the 'Cabernet Sauvignon' grape variety. In addition, Alco et al. (2023) reported that defoliation did not result in significant variations in berry width in the 'Gamay' grape cultivar. On the other hand, Korkutal et al. (2021) reported that the treatment of defoliation and tip removal at different phenological periods resulted in an increase in berry size in the cv. 'Michele Palieri' berries. According to Candar (2018), the effect of defoliation treatments on lateral shoots in the 'Merlot' grape cultivar varies from year to year. It has been reported that reducing defoliation during extraordinarily rainy years results in an increase in berry width. However, during years with average precipitation, more defoliation tends to increase berry width more than lateral shoots, based on several years of observations.

Therefore, the conflicting results are actually consistent with the general literature. The grapevine's response to defoliation is imprecise and depends on various factors, including the cumulative effects of the year, cultivar genetics, cultural practices, and timing of the treatment. The same argument can be applied to the LWP means as well. Although increasing water stress in 2019 resulted in smaller berry width, in 2020, the smallest berry width was observed in the C treatment. It is thought that the effect of precipitation, which was 299.50 mm less than the long-term average in 2020, outweighed the impact of cultural practices.

Berry Length

Statistical analysis showed that the changes in LWPs, defoliation treatment, and the effects of their interactions on berry size years were insignificant in both experimental years.

The ANOVA revealed that the RW treatment resulted in the smallest berry size value of 12.65 mm, whereas the FW treatment resulted in the largest berry size value of 12.98 mm. When LWPME was considered, the S1 treatment showed the highest berry length value of 13.08 mm, whereas the S2 treatment had the lowest berry length value of 12.90 mm in year of 2019. In 2020, FW treatments resulted in a small berry size of 12.71 mm in terms of DME, whereas the RW treatment produced the largest berry size value of 13.06 mm (Figure 2).

The statistical analysis of LWPME revealed that the S1 treatment had the smallest berry length value of 12.81 mm, while the S3 treatments resulted in the highest berry length value of 13.03 mm. Regarding the berry size interactions in 2020, the S3 x LW combination had the highest berry length value of 13.32 mm, while the S0 x TP combination had the lowest berry length value of 12.45 mm. Observed variations in berry lenght means between two years were not significant.

Studies by Kotseridis et al. (2012) on cv. 'Cabernet-Sauvignon' and Kılıç (2019) on cv. 'Red Globe' with

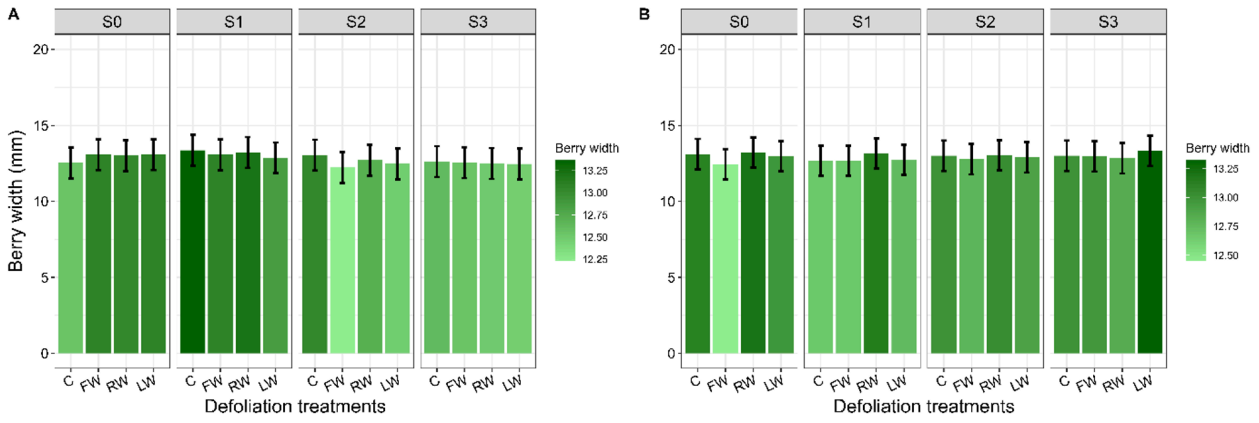

Figure 1. Effects of stress levels and defoliation treatments on berry width. Results expressed as mean of repetitions ± standard error. A; 2019, B; 2020. The results of variance the analysis did not show a statistically significant difference between the means. S0; control of stress treatments, S1; Ψpd between -0.3/-0.5 MPa, S2; Ψpd between -0.5/-0.7 MPa, and S3; Ψpd <-0.7 MPa. C; control of defoliation treatments, FW; full window, RW; right window and LW; left window.

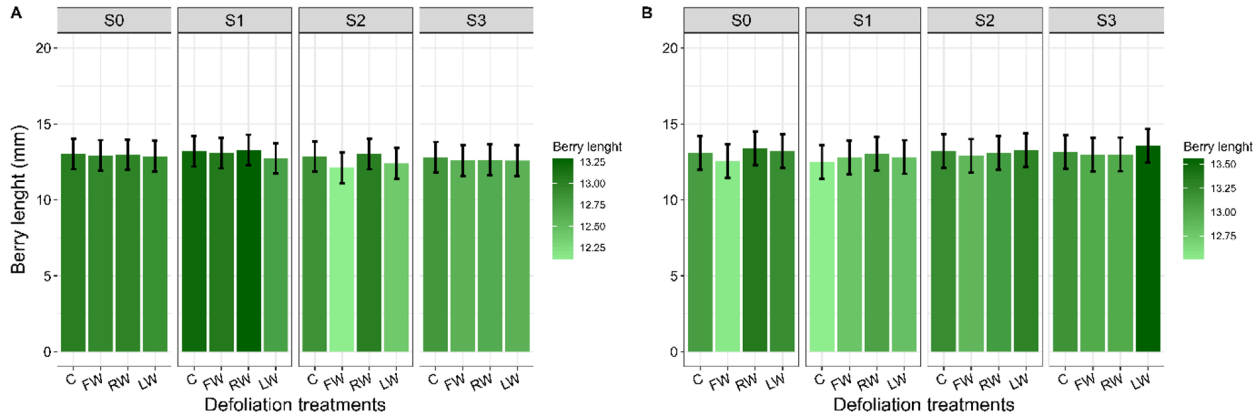

Figure 2. Effects of stress levels and defoliation treatments on berry length. Results expressed as mean of repetitions ± standard error. A; 2019, B; 2020. The results of variance the analysis did not show a statistically significant difference between the means. S0; control of stress treatments, S1; Ψpd between -0.3/-0.5 MPa, S2; Ψpd between -0.5/-0.7 MPa, and S3; Ψpd <-0.7 MPa. C; control of defoliation treatments, FW; full window, RW; right window and LW; left window.

controlled defoliation revealed no changes in berry size. In contrast, Sabır et al. (2010) reported an increase in berry size with tip removal in 'King's Ruby' grape cultivar, but found no effect on the '2B-56' grape cultivar. Candar (2018) was unable to establish the effect of mild water stress on berry size reduction that occurred with increasing main shoot length in cv. 'Merlot' grape berries, while Öner (2014) reported that mild stress influenced the development of berry width and length. Alco et al. (2023) reported that, in addition to leaf reduction interventions at different times and forms after veraison, changes in the direction of decreasing berry width and length are influenced by the amount of precipitation during the vegetation period in cv. 'Gamay'. Based on the available data, the reduction in berry width and height values was observed in relation to leaf water potential values compared to the control, although it was not statistically significant.

Berry Fresh Weight

In 2019, it was found that DME had a significant effect on the fresh weight of berries at LSD 5% level.

The RW treatment has been determined to be in the first importance group with a value of 1.37 g, while the LW and FW treatments are in the last importance group with values of 1.24 g and 1.20 g, respectively. No statistically significant effect on berry fresh weight was found with LWPME. The highest value of 1.32 g was obtained with the S0 treatment, while the lowest value of 1.22 g was obtained with the S3 treatment (Figure 3A).

The interaction between S0 and RW was found to result in a numerically high berry fresh weight of 1.46 g, while the interaction between S3 and FW was found to result

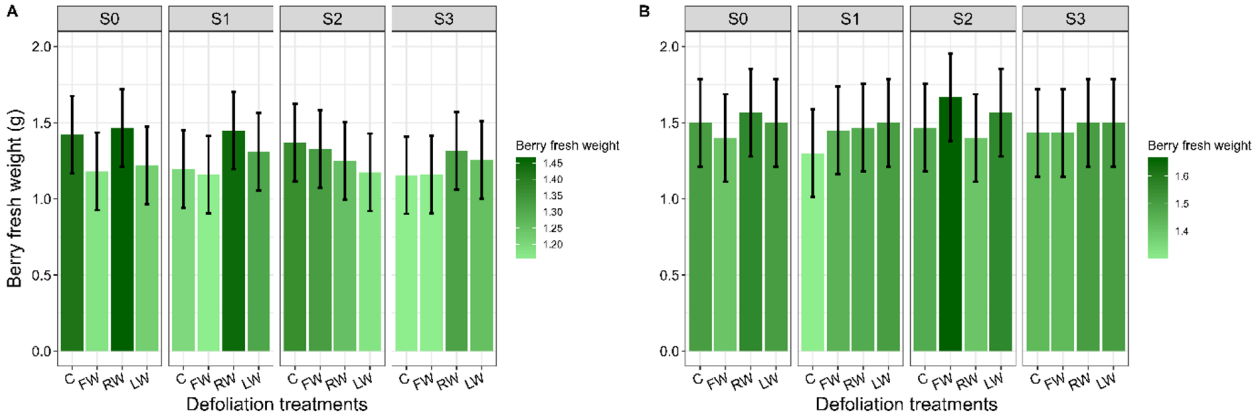

Figure 3. Effects of stress levels and defoliation treatments on berry fresh weight. Results expressed as mean of repetitions ± standard error. A; 2019, B; 2020. The results of variance the analysis did not show a statistically significant difference between the means. S0; control of stress treatments, S1; Ψpd between -0.3/-0.5 MPa, S2; Ψpd between -0.5/-0.7 MPa, and S3; Ψpd <-0.7 MPa. C; control of defoliation treatments, FW; full window, RW; right window and LW; left window.

in a numerically low fresh fruit weight of 11.15 g. In 2020, it was determined that defoliation and LWP had no statistically significant effect on berry fresh weight. Among the DME treatments, LW had the highest fresh weight with 1.51 g, while C had the lowest fresh weight with 1.42 g. The LWP means ranged from 1.42 g in S1 treatments to 1.52 g in S2 treatments, and no trend of variation proportional to stress levels was detected. Regarding the effect of treatment interactions, the highest value of 1.66 g was obtained with the S2 x FW interaction, while the lowest value of 1.30 g was obtained with the S1 x C interaction.

Although there was a statistically significant difference in fresh berry weight means between the two experimental years, this difference was not significant in terms of treatment main effects across the two years. The fresh berry means were 1.27 g in 2019 and 1.47 g in 2020 (Figure 3B).

Dimovska et al. (2000) studied the effects of defoliation treatments on the 'Beogradska Besemena' grape cultivar, while Bubola et al. (2019) investigated the same on the 'Istrian Malvasia' grape cultivar. Both studies reported a significant increase in berry weight due to defoliation. However, findings of Candar (2018) and Alco et al. (2023) suggest that defoliation did not have a significant effect on berry fresh weight in cv. 'Merlot' and cv. 'Gamay' grapes. This study predicts that berry wet weight would be lower in 2020 due to below-average rainfall compared to previous years. However, the study also reports that fresh berry weight in 2020 was higher. It is hypothesized that the higher berry set in 2019 had a more significant impact than the annual precipitation in determining the fresh berry weight in 2020. Conversely, in 2020, the weaker berry set is likely responsible for the lower berry wet weight.

Berry Dry Weight

In both experimental years, the treatment of DME and LWPME did not result in any statistically significant effects on berry dry weight. In 2019, the highest berry dry weight of 0.36 g was recorded from the C treatments, while the lowest value of 0.33 g was observed from the FW treatments. Regarding the main effect of LWP, the smallest berry dry weight of 0.34 g was numerically measured in the S0 treatments, whereas all other treatments resulted in a weight of 0.35 g. Upon ranking the LWPME x DME interactions in terms of their effect on berry dry weight from largest to smallest, the S3 x LW interaction was found to have the most substantial impact, with a value of 0.40 g. On the other hand, the S0 x FW interaction had the least effect, with a value of 0.30 g, and was ranked last (Figure 4A).

In 2020, the highest berry dry weight in terms of DME was observed in the LW treatments, with a value of 0.40 g. Conversely, the lowest dry weights were recorded in the C and FW treatments, both with a value of 0.37 g. Regarding LWPME, it was found that the lowest berry dry weight of 0.37 g was associated with the S1 treatment, while the highest berry dry weight of 0.39 g was linked to the S2. There was a statistically significant difference in berry dry weight means between 2019 and 2020, with a mean of 0.35 g in 2019 and 0.38 g in 2020 (Figure 4B).

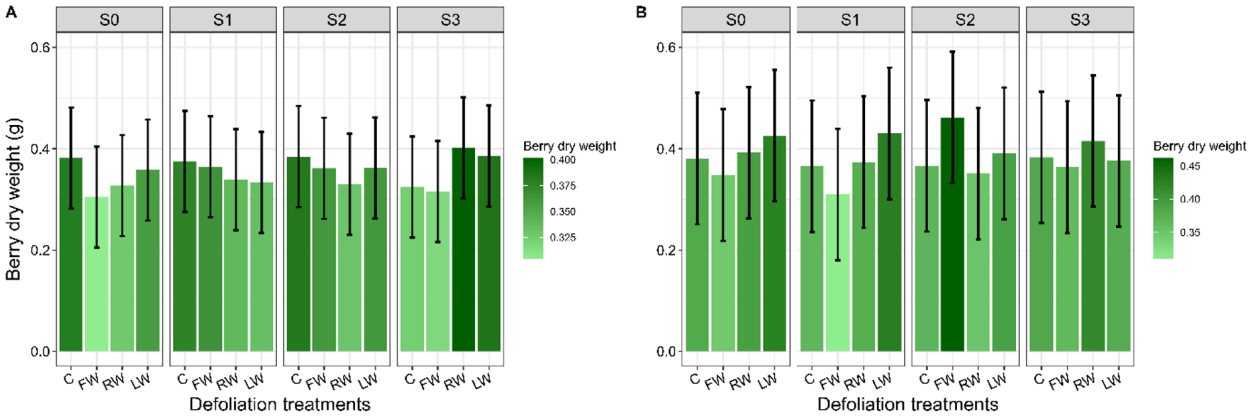

Figure 4. Effects of stress levels and defoliation treatments on berry dry weight. Results expressed as mean of repetitions ± standard error. A; 2019, B; 2020. The results of variance the analysis did not show a statistically significant difference between the means. S0; control of stress treatments, S1; Ψpd between -0.3/-0.5 MPa, S2; Ψpd between -0.5/-0.7 MPa, and S3; Ψpd <-0.7 MPa. C; control of defoliation treatments, FW; full window, RW; right window and LW; left window.

Similar to the observations made regarding fresh weights, it can be concluded that the evaluations for berry dry weights can also be repeated. The differences in the two-year averages are likely attributed to variations in berry set. While the increased stress level tended to increase the dry weight of the berry, this effect was negligible and statistically insignificant in the main effects of LWP over the two-year period. On the other hand, the defoliation treatments did not yield any

significant or linear effects on berry dry weight. Candar (2018) reported that defoliation had no significant effect on berry dry weight, while Korkutal et al. (2017) and Alço et al. (2023) observed that the treatment time was more effective than the main effect of defoliation on berry dry weight. In contrast, Korkutal et al. (2021a) found that the defoliation and tipping treatments resulted in a statistically significant increase in berry dry weight.

Percentage of Berry Dry Weight

The statistical analysis showed that there was no significant difference in berry dry weight% due to the interactions between LWPME and DME in both experimental years. When it comes to DME, the highest dry weight percentage of 29.40% was observed in the LW treatment, whereas the lowest dry weight percentage of 25.73% was found in the RW treatment. Regarding the main effect of LWP on % dry weight, the values were 26.19% for S0, 28.15% for S1, 28.17% for S2, and 29.09% for S3, respectively. The highest dry weight percentage of 31.53% was observed in the S1 x FW interaction, while the lowest dry weight percentage of 22.50% was found in the S0 x RW interaction (Figure 5A).

effect percentage was 27.90% in 2019 and 25.91% in 2020 (Figure 5B). The year averages formed different statistical groups, indicating that there was a significant difference between the two years.

Similarly, according to Candar (2018), the main effect of the year on % berry dry weight was more significant than that of the defoliation treatments. However, Korkutal et al. (2017) and Alço et al. (2023) reported positive results on % berry dry weight due to the defoliation treatments.

Berry Density

Although the main effects of different stress levels and defoliation treatments on berry density in 2019 were not statistically significant, the lowest density of berries, in terms of DME, was recorded in the FW treatment at 0.95 g L^{-1}, while the highest density was observed in the C treatment at 1.01 g L^{-1}. Regarding berry density and its main effect on LWP in 2019, the S3 treatment had the highest value at 0.94 g L^{-1}, while the S1 treatment had the lowest value at 1.02 g L^{-1}.

In 2019, the highest density value in the LWPME x YUAET interaction was recorded at 1.10 g L^{-1} in the S0 x RW interaction, while the lowest density value was recorded

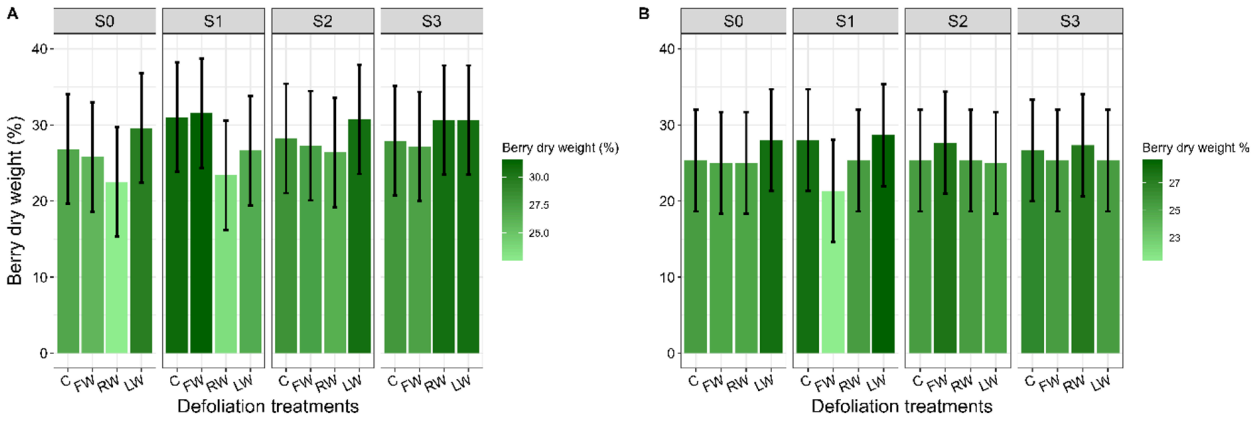

Figure 5. Effects of stress levels and defoliation treatments on berry dry weight %. Results expressed as mean of repetitions ± standard error. A; 2019, B; 2020. The results of variance the analysis did not show a statistically significant difference between the means. S0; control of stress treatments, S1; Ψpd between -0.3/-0.5 MPa, S2; Ψpd between -0.5/-0.7 MPa, and S3; Ψpd <-0.7 MPa. C; control of defoliation treatments, FW; full window, RW; right window and LW; left window.

In 2020, LW had the highest dry weight percentage of 26.75%, whereas FW had the lowest dry weight percentage of 24.83% in terms of DME. As for the main effect of LWP, the highest dry weight percentage of 26.16% was observed in the S3 treatment, while the lowest dry weight percentage of 24.83% was found in the FW treatments. In terms of interaction effects, the highest percentage of 2020 was observed in S1 x LW at 26.00%, while the lowest percentage of 21.33% was found in S1 x FW. Although the variations in the main effects of LWP and defoliation were not found to be statistically significant in the two-year average, the main

at 0.87 g L^{-1} in the S3 x TP interaction. However, in 2020, it was found that the changes in LWP and defoliation treatments had an insignificant effect on berry density compared to the LSD 5% significance level (Figure 6A).

The FW treatment was found to produce the highest berry density response at 1.13 g L^{-1} and the LW treatment produced the lowest response at 1.08 g L^{-1}. Regarding LWPME, the S3 treatment had the highest density at 1.15 g L^{-1}, while the S1 treatment had the lowest density at 1.06 g L^{-1}. In 2020, the S2 x FW combination produced the highest density value at 1.16 g L^{-1}, while the S1 x C interaction resulted in the lowest density at 0.98 g L^{-1}.

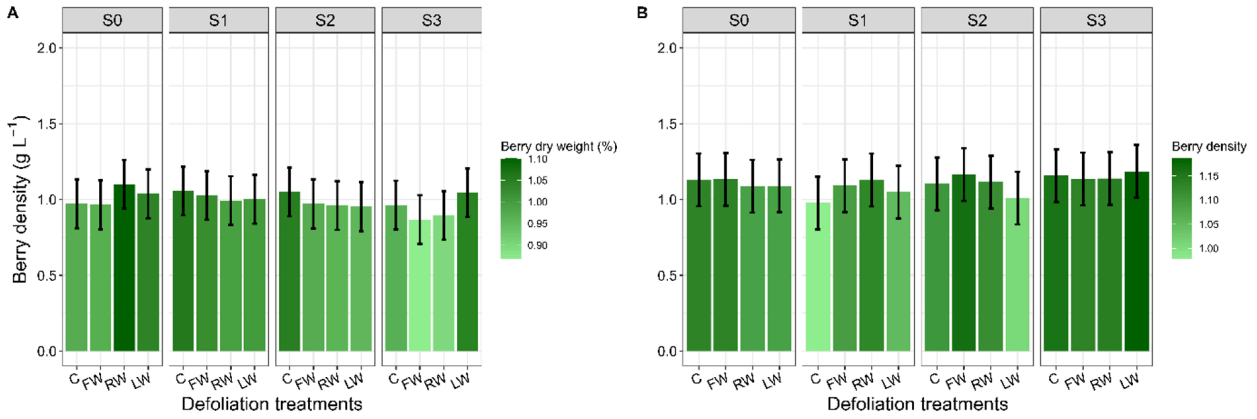

Figure 6. Effects of stress levels and defoliation treatments on berry density. Results expressed as mean of repetitions ± standard error. A; 2019, B; 2020. The results of variance the analysis did not show a statistically significant difference between the means. S0; control of stress treatments, S1; Ψpd between -0.3/-0.5 MPa, S2; Ψpd between -0.5/-0.7 MPa, and S3; Ψpd <-0.7 MPa. C; control of defoliation treatments, FW; full window, RW; right window and LW; left window.

The change in berry density between 2019 and 2020 was found to be statistically significant, with the mean density increasing from 0.99 g L^{-1} in 2019 to 1.10 g L^{-1} in 2020 (Figure 6B).

According to Candar (2018), a significant and linear relationship could not be established between defoliation and berry density in cv. 'Merlot'. However, in a recent study by Alço et al. (2023), it was highlighted that the timing of the treatment may be more crucial than the defoliation treatment itself in cv. 'Gamay'. In line with Alço et al. (2023) findings, Korkutal et al. (2021a) also supports the notion that the timing of defoliation treatments is crucial. In their research on the 'Michele Palieri' grape cultivar, defoliation performed during the berry set period resulted in reduced berry density, but the main effects of defoliation were not statistically significant. However, in a study by Bahar and Öner (2015) on the 'Cabernet-Sauvignon' grape cultivar, leaf removal treatments were found to increase berry density. It can be concluded that different cultivars may respond differently to defoliation treatments, primarily due to the cultivar genotype, treatment methods and timing, and terroir characteristics.

Berry Volume

The interactions among LWP, defoliation treatments and their mean values were not found to be statistically different for berry volume for the two experimental years, and mean of years were similar. In 2019, the treatment with the highest DME berry volume value was RW, with 1.39 cm^3, whereas the defoliation treatment with the lowest numerical value was LW, with 1.24 cm^3. The berry volume in terms of LWPME ranged from high to low as follows: S0, 1.31 cm^3; S2 and S3, 1.30 cm^3; and S1, 1.26 cm^3. The lowest value in the LWPME and DME interactions was observed in S1 x C and S1 x FW interactions, which

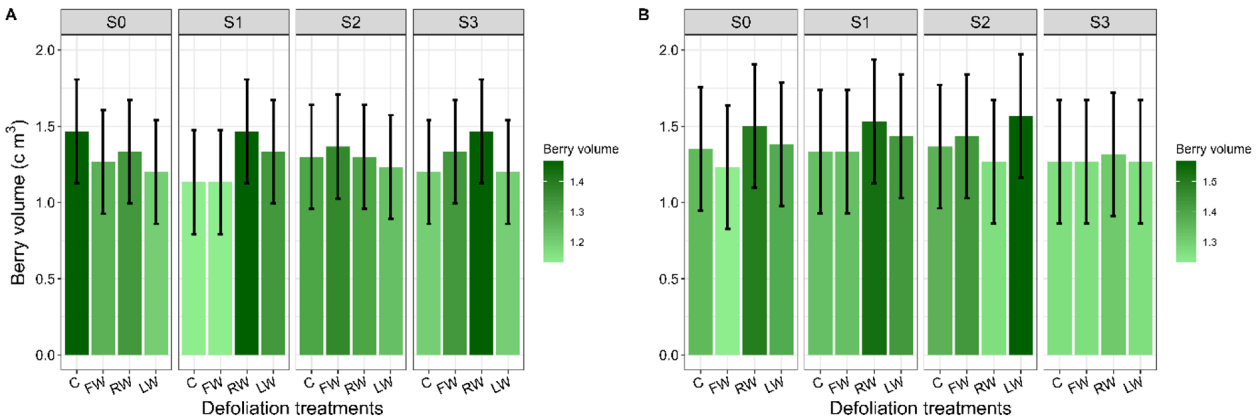

Figure 7. Effects of stress levels and defoliation treatments on berry volume. Results expressed as mean of repetitions ± standard error. A; 2019, B; 2020. The results of variance the analysis did not show a statistically significant difference between the means. S0; control of stress treatments, S1; Ψpd between -0.3/-0.5 MPa, S2; Ψpd between -0.5/-0.7 MPa, and S3; Ψpd <-0.7 MPa. C; control of defoliation treatments, FW; full window, RW; right window and LW; left window.

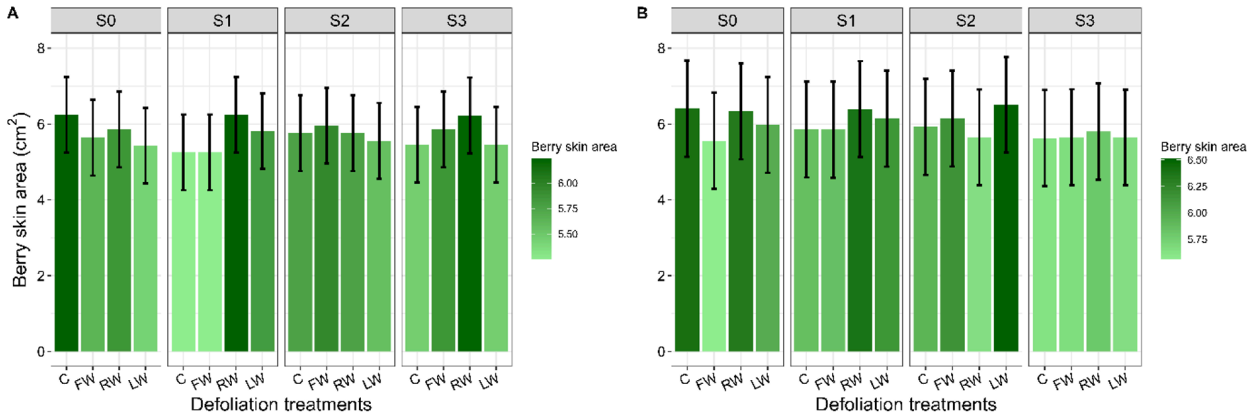

Figure 8. Effects of stress levels and defoliation treatments on berry skin area. Results expressed as mean of repetitions ± standard error. A; 2019, B; 2020. The results of variance the analysis did not show a statistically significant difference between the means. S0; control of stress treatments, S1; Ψpd between -0.3/-0.5 MPa, S2; Ψpd between -0.5/-0.7 MPa, and S3; Ψpd <-0.7 MPa. C; control of defoliation treatments, FW; full window, RW; right window and LW; left window.

reached a value of 1.13 cm^3 (Figure 7A).

In 2020, the FW treatment had the smallest DME berry volume of 1.31 cm^3, while the LW treatment had the highest with 1.41 cm^3. Among the LWPME treatments, the highest numerical value of 1.40 cm^3 was observed in S1 and S2, whereas the lowest value of 1.27 cm^3 was observed in S3. Examining the effect of interactions in 2020, the S2 x LW interaction had the highest berry volume value of 1.56 cm^3, while the S0 x FW interaction had the lowest value of 1.23 cm^3 (Figure 7B).

Korkutal et al. (2021a) found that defoliation performed during El 27 and EL31 periods increased berry volume, while treatments applied during the EL35 period decreased it. Alço et al. (2023) reported a significant increase in berry volume from veraison to maturity. However, defoliation during the 15-17° Brix period resulted in a relative reduction in berry volume, regardless of the treatment form. Candar (2018) also reported that defoliation treatments applied during the same period did not cause a significant change in berry volume similarly to Rogiers et al. (2004) which, highlighted the effect of adherence regimen in different years on volume. In 2020, despite the lower total precipitation, an increase in berry volume was detected, although it was not statistically significant. This could be due to weaker berry set in 2020, resulting in higher berry volumes.

Berry Skin Area

The interactions between LWP, defoliation treatments main effects and their interaction values did not show any significant statistical differences for berry skin area in the two experimental years, and mean of years were alike. In 2019, the FW treatment had the lowest DME berry skin area value of 5.80 cm^2, while the LW treatment had the highest value of 6.06 cm^2. Concerning LWPME, the smallest berry skin area was recorded as 5.68 cm^2 in the S3 treatment, while the highest berry skin area value of 6.06 cm^2 was observed in the S0 and S1 treatments (Figure 8A).

In 2019, when sorting LWPME x DME interactions based on berry skin area data from largest to smallest, the S0 x C and S1 x RW interactions were the lowest with 6.23 cm^2, while the S1 x C and S1 x FW interactions had the smallest numerical value with 5.25 cm^2. In 2020, the treatment with the smallest LWPME berry skin area value was FW with a value of 5.80 cm^2, and the treatment with the highest value was LW with a value of 6.06 cm^2 (Figure 8B).

In terms of LWPME, the treatment with the lowest numerical value for berry skin area was S3, with a value of 5.68cm^2. The highest numerical value for berry skin area was observed in treatments S0 and S1, with a value of 6.06 cm^2. When considering the interactions of the main effects, the combination of S2 and LW resulted in the highest berry skin area value, with 6.50 cm^2, while the combination of S0 and FW had the lowest value, with 5.56 cm^2.

Schalkwyk (2004), reported that the skin area/grape juice volume ratio is a crucial factor for wine quality. Large berries tend to produce more water and have a high grape juice ratio, while small berries offer higher color and flavor for red varieties. According to Candar (2018), defoliation treatments in various forms did not result in any statistically significant effects on the berry skin area. However, Alco et al. (2023) found that severe topping as a defoliation treatment caused a significant increase in the berry skin area from veraison to maturity but decreased it during the 15-17° Brix period across all treatment forms. In this study, the FW and S3 treatments resulted in the numerically lowest berry skin area, according to the mean of the experimental years. This finding is consistent with Alco et al.'s observation that more severe defoliation can reduce the berry skin area.

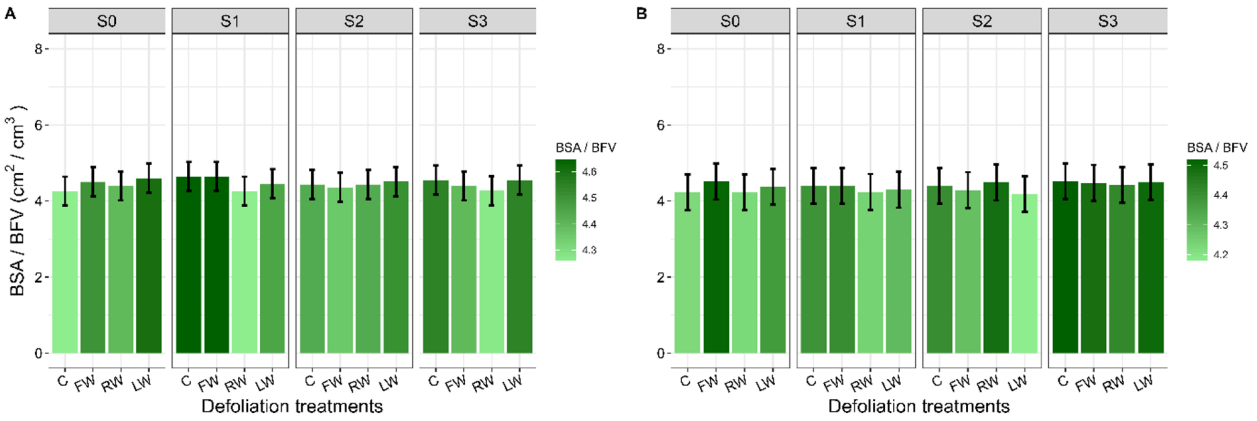

Figure 9. Effects of stress levels and defoliation treatments on berry skin area to berry flesh volume ratio. Results expressed as mean of repetitions ± standard error. A; 2019, B; 2020. The results of variance the analysis did not show a statistically significant difference between the means. S0; control of stress treatments, S1; Ψpd between -0.3/-0.5 MPa, S2; Ψpd between -0.5/-0.7 MPa, and S3; Ψpd <-0.7 MPa. C; control of defoliation treatments, FW; full window, RW; right window and LW; left window.

Ratio of Berry Skin Area to Berry Flesh Volume

There were no statistically significant differences observed in the interactions between LWP, defoliation treatments, and their main effects for berry skin area/berry flesh volume in the two experimental years. The two year means were similar, indicating that there was no significant effect of LWP or defoliation treatments on the berry skin area/berry flesh volume ratio.

When examining the berry skin area/berry flesh volume values in 2019 in terms of DME, the RW treatments had the lowest value of 4.33 cm^2/cm^3, while the LW treatments had the highest value of 4.52 cm^2/cm^3. In terms of LWPME, it was found that S1 had the highest value of 4.50 cm^2/cm^3, whereas S2 had the lowest value of 4.43 cm^2/cm^3 for berry skin area/berry flesh volume (Figure 9A).

When considering the interactions, the highest value for berry skin area/berry flesh volume was 4.64 cm^2/cm^3, observed in S1 x C and S1 x FW treatments, while the lowest value was 4 cm^2/cm^3 in S0 x C and S1 x RW treatments. The total value was calculated to be 25 cm^2/cm^3.

When ranking the values of berry skin area/berry flesh volume for 2020 from largest to smallest in terms of DME, the FW treatment had the highest value of 4.41 cm^2/cm^3, while the LW treatment had the lowest value of 4.33 cm^2/cm^3. Examining the LWP berry skin area/berry flesh volume, the S3 treatments had the highest value of 4.47 cm^2/cm^3. In terms of interactions, the S2 x LW combination had the lowest berry skin area/berry flesh volume ratio, with a value of 4.17 cm^2/cm^3 (Figure 9B).

Various factors such as variety, irrigation, canopy management can affect berry size, as reported by Sofo et al. (2012), Matthews and Kriedemann (2006), Matthews and Nuzzo (2007). Bahar et al. (2011) also stated that small grape berries have a higher berry skin area/berry flesh volume ratio than large berries, which leads to the transfer of more phenolic substances from the skin to the unit volume. Candar (2018) reported that although different responses are observed depending on the changes in physiological activity due to factors such as precipitation, humidity, and light intensity received during the vegetation period and in the total year, decreasing the total leaf area tends to decrease the berry skin area/berry flesh volume. According to Alço et al. (2023), severe topping defoliation caused a significant decrease in berry skin area/berry flesh volume towards the harvest date. However, higher berry skin area/berry flesh volume values were calculated with defoliation performed during the 15-17° Brix period. In this study, the FW and S1 treatments had the highest berry skin area/berry flesh volume ratio compared to the two-year mean.

Correlations of Berry Variables

Although no significant relationships were found between LWP levels, defoliation treatments, and berry characteristics at the $p \leq 0.05$ level according to the ANOVA results, we examined these relationships using the Pearson correlation test (Table 1).

The correlation coefficient between berry width and berry lenght is 0.852, indicating a strong positive correlation between these two variables. In general, the chart shows that there are positive correlations between berry weight and several other variables, such as berry length, berry volume, and berry skin area. However, the correlation between berry width and berry density is only weakly positive, and there is a negative correlation between berry width and bsa/bvol.

Similarly, there is a strong positive correlation between berry lenght and berry width, as well as moderate

positive correlations between berry lenght and berry fresh weight, berry dry weight, berry volume, and berry skin area. There is also a weak positive correlation between berry lenght and berry density.

Other relationships in the chart include a strong positive correlation between berry fresh weight and berry dry weight, as well as moderate positive correlations between berry fresh weight and berry volume, berry dry weight %, and berry skin area. There is also a weak positive correlation between berry fresh weight and berry density.

Finally, the chart shows a strong positive correlation between berry skin area and berry volume, as well as moderate positive correlations between berry skin area and berry fresh weight, berry dry weight , and berry skin area to berry flesh volume ratio. There is also a weak negative correlation between berry skin area and berry dry weight %.

Principal Component Analysis (PCA)

To evaluate the interaction between stress levels, defoliation treatment, and the studied berry variables, we employed Principal Component Analysis (PCA). The dataset comprising eight treatments and nine berry variables was analyzed using the covariance matrix. However, two different biplots were created to investigate the effects of stress levels and defoliation treatments on berry variables separately.

According to the cumulative proportion of variance for the LWP biplot, PC1 explains 55.15% of the total variance, while PC1 and PC2 together explained 86.41% of the total variance. PC1, PC2, and PC3 combined explain 100% of the total variance. Therefore, PC1 and PC2 are the most important components in explaining the variability in the LWP data. Similarly, for the defoliation treatments, PC1 explains 68.93% of the total variance, PC1 and PC2 combined explain 92.14% of the total variance, and

Table 1. Correlations of selected berry variables

	bw	bl	bfw	bdw	bdwper	bvol	bden	bsa	bsa/bvol
bw	1.000								
bl	0.852***	1.000							
bfw	0.191	0.326**	1.000						
bdw	0.106	0.160	0.537***	1.000					
bdwper	-0.036	-0.109	-0.309**	0.630***	1.000				
bvol	0.171	0.316**	0.696***	0.356***	-0.235*	1.000			
bden	0.055	0.026	0.303**	0.192	-0.064	-0.415***	1.000		
bsa	0.199	0.327**	0.687***	0.315**	-0.272**	0.967***	-0.383***	1.000	
bsa/bfv	-0.180	-0.304**	-0.694***	-0.326**	0.266**	-0.969***	0.403***	-0.994***	1.000

Coefficient statistical significance indicated by * symbol (absent > 0.05, * indicates < 0.05, **indicates < 0.01, *** indicates < 0.001). bw; berry widht, bl; berry length, bfw; berry fresh weight, bdw; berry dry weight, bdwper; berry dry weight %, bvol; berry volume, bden; berry density, bsa; berry skin area, bsa/bvol; berry skin area to berry flesh volume ratio.

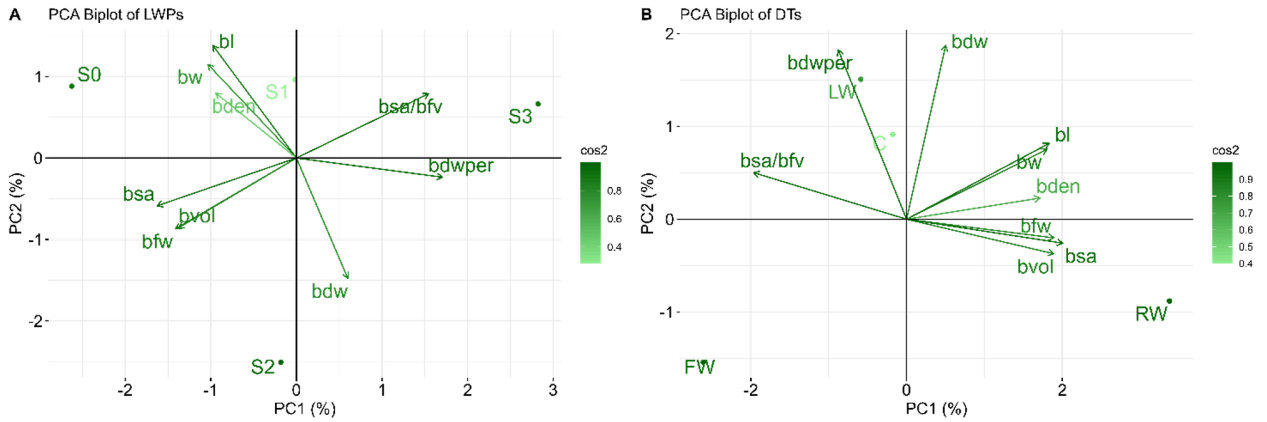

Figure 10. Principal component analysis (PCA) with the mean values of variables. A; PCA biplot of LWPs', B; PCA biplot of DTs'. All variables are displayed. The size and color of the arrows indicates the contribution strength of the variable. The color of lables reflects the magnitude of the contribution to the component. Bw; berry widht, bl; berry length, bfw; berry fresh weight, bdw; berry dry weight, bdwper; berry dry weight %, bvol; berry volume, bden; berry density, bsa; berry skin area, bsa/bvol; berry skin area to berry flesh volume ratio.

PC1, PC2, and PC3 combined explain 100% of the total variance, as indicated by the cumulative proportion of variance.

Both PCA correlation plots showed that there was a fair separation of the samples based on the treatments and variables.

Upon examination of the LWPs biplot, it is evident that variable S0 exhibits a robust negative correlation with Dim.1. This suggests that it carries a substantial weight in the first principal component and significantly contributes to the overall variability in the data. Similarly, variable S1 also negatively correlates with Dim.1, but it bears a comparatively smaller weight than S0. Conversely, variable S2 negatively correlates with Dim.2, implying that it carries a considerable weight in the second principal component and contributes significantly to the variability in that component. Lastly, variable S3 positively correlates with both Dim.1 and Dim.2, signifying that it holds a moderate weight in both principal components and contributes to the variability in both components.

Among the berry variables examined for LWP levels, berry fresh weight exhibits the highest loading (-0.809) on the first principal component, followed closely by berry volume (-0.788) and berry skin area (-0.933). These findings indicate that these three variables are highly associated with the first principal component and contribute significantly to the variability explained by this component. In contrast, the percentage of berry dry weight has the highest loading (0.979) on the second principal component, followed by the berry skin area to berry flesh volume ratio (0.886). These results suggest that these two variables are strongly associated with the second principal component and contribute the most to the variability explained by this component.

Since variables with high loadings on a specific principal component contribute the most to the variability explained by that component, it appears that the size and shape of the berry are strongly associated with the first principal component. This is evident from the high loadings of berry fresh weight, berry volume, and berry skin area (Figure 10A).

In the DTs biplot, it is observed that berry width exhibits the highest loading (0.888) on the first principal component, followed by berry length (0.902), berry volume (0.931), and berry skin area (0.987). These findings indicate that these four variables are highly associated with the first principal component and contribute the most to the variability explained by this component. On the other hand, the percentage of berry dry weight has the highest loading (-0.432) on the second principal component, followed by bsa/bfv (-0.966). These results suggest that these two variables are strongly associated with the second principal component and contribute the most to the variability explained by this component.

The first principal component appears to be related to berry weight, berry length, berry volume, and berry skin area via the high loading of these criteria. Conversely, the second principal component appears to be related to bsa/bfv and the percentage of berry dry weight, as indicated by the high loading of bsa/bfv and the negative loading of the percentage of berry dry weight (Figure 10B).

CONCLUSION

In the study, it was found that the effects of water stress and defoliation treatments on berry physical properties were statistically insignificant. However, in the second year of the study, it was observed that the treatment of FW led to changes in the desired direction for grapevines. It is believed that the cumulative decrease in water reserves, resulting from reduced precipitation during the vegetation period over multiple years, caused the effects of FW treatment to become more prominent in this criterion. Additionally, the study found that vine production year, leaf water potentials (Ψleaf), and stress levels are influenced by both current vegetation period conditions as well as those from previous years. When examining the data for berry weight and % dry weight in both years, it was noted that these criteria increased with higher stress levels. Thus, it should not be assumed that cultivation practices will yield the same results for each grape cultivar, terroir, or year. Vineyard management strategies aimed at improving berry properties should be tailored to the production target, variety, and year.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Declaration of interests

The authors have no conflict of interest to declare.

Author contribution

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

Ethics Committee Approval

Ethics committee approval is not required. This article does not contain any studies with human participants or animals performed by any of the authors.

Funding

The authors declared that this study has received no financial support.

Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

This study is a part of the master's thesis of the 4th author. The authors would like to express their gratitude to Mr. Bülent KALPAKLIOĞLU, the owner of Kalpak Tarım Ürünleri Ltd., for

providing permission to conduct this research in his vineyard for two years.

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RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(3): 533-539
DOI: https://doi.org/10.31015/jaefs.2023.3.7

The effect of two isolates of *Beauveria bassiana* (Bals.) Vull. on the larvae of confused flour beetle [*Tribolium confusum* du Val., 1863 (Coleoptera: Tenebrionidae)]

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Citation: Bayindir Erol, A., Erdogan, O., Karaca, I. (2023). The effect of two isolates of *Beauveria bassiana* (Bals.) Vull. on the larvae of confused flour beetle [*Tribolium confusum* du Val., 1863 (Coleoptera: Tenebrionidae)]. International Journal of Agriculture, Environment and Food Sciences, 7 (3), 533-539

Received: June 2, 2023
Revised: July 26, 2023
Accepted: August 1, 2023
Published Online: September 18, 2023

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Available online at
https://jaefs.com/
https://dergipark.org.tr/jaefs

Abstract

The confused flour beetle cause economic losses in stored products, especially in products obtained from wheat. Although using insecticides against storage pests is an effective method, their frequent and continuous use can lead to resistance and toxicity issues in non-target organisms. In this study, the effect of local *Beauveria bassiana* isolates (BMAUM LD.2016 and BMAUM M6-4) on the 3rd instar larvae of confused flour beetle (*Tribolium confusum*) was determined under laboratory conditions. As a result of the experiment, both isolates of *B. bassiana* were found to be more effective on the larvae in the spraying method compared to the dipping method. In the spraying method, mortality rates on the 9th day were 72% in BMAUM LD.2016 isolate, 34% in the dipping method, respectively. Mortality rates of BMAUM M6-4 isolate were recorded as 96% in the spraying method and 8% in the dipping method. In the spraying method, the mortality date (LT_{50}) was determined as 6.09 days for BMAUM LD.2016 isolate and 3.90 days for BMAUM M6-4 isolate. The LT_{50} value could not be calculated in the dipping method, since the mortality rates were below 50% for both isolates. BMAUM M6-4 isolate caused higher mortality in larvae than BMAUM LD.2016 isolate. As a result, *B. bassiana* isolates have shown a high level of effectiveness against *T. confusum*, demonstrating that they can be used as isolates for insect control.

Keywords: *Beauveria bassiana,* Biological control, Entomopathogenic fungi, Mortality rate, *Tribolium confusum*

INTRODUCTION

Grain production ranks first among cultivated crops in Türkiye and in the world. Wheat and barley are the leading grains produced in Türkiye The annual production of wheat, which is cultivated on an area of approximately 6 million 800 thousand hectares, is 18 million tons while that of barley, which is cultivated on an area of approximately 3 million 200 thousand hectares, is 6 million tons annually (TSI, 2021). After harvest, grains need to be stored and protected with minimal losses for a long time until consumption. For this, it is important to identify the organisms that adversely affect the quality and quantity of the products (Bağcı et al., 2014). Stored-grain insects cause damage to by feeding on grain prodcuts directly and indirectly, resulting in a decrease in the seed quality weight. nutritional values, and consequently commercial value (Boxall, 2001). Every year, approximately 10% to 40% of stored grains around the world are damaged both qualitatively and quantitatively by insects, especially in tropical and subtropical regions of developing or underdeveloped countries (Tripathi et al., 2009).

Stored foods are destroyed by mites and various harmful beetles and moths insects

(Rajendran and Sriranjini, 2008). The beetles (Coleoptera) include many harmful species such as *Sitophilus granarius* (L.) (Curculionidae), *Tribolium castaneum* (Herbst) (Tenebrionidae), *Oryzaephilus surinamensis* (L.) (Silvanidae), and *Rhyzopertha dominica* (Bostrichidae), *Tribolium confusum* du Val. (Tenebrionidae) (Hill, 1990; Hodges et al., 1996; Lord, 2007). Confused flour beetles, particularly in products derived from wheat (such as flour, etc.), pasta, dried fruits, biscuits, and nuts, causes damage and leads to losses (Karunakaran et al., 2004).

Farmers use different insecticides to control stored-product insects, such as malathion (Arthur and Zettler 1991, 1992), deltamethrin (Arthur, 1997), cyfluthrin (Arthur, 1994; 1999), bioresmethrin (Ardley, 1976) and chlorpyrifos-methyl (LaHue, 1997)]. Although these chemicals are effective their frequent and continuous use can lead to serious problems such as resistance and toxicity on non-target organisms (Isman, 2006; Daglish, 2008; Watts and Williamson, 2015). Hence, there has been a trend towards using safer alternative control methods (Upadhyay and Ahmad, 2011). In this context, pathogens have been used as biological control agents to control stored product insects. Such pathogens include entomopathogenic fungi, protozoa, viruses, and nematodes (Moore et al., 2000). Field and laboratory studies have shown that entomopathogenic fungi (EPF) are quite successful in controlling many pests of stored grains (Batta, 2004; Vassilakos et al., 2006; Sabbour et al., 2012).

Currently, approximately 700 species of entomopathogenic fungi belonging to 90 genera have been identified (Roberts and Humber, 1981). Among these, EPFs such as *Beauveria bassiana* (Balsamo) Vuillemin, *Metarhizium anisopliae* (Metschn.) Sorokin 1883, and *Isaria fumosorosea* Wize (=*Paecilomyces fumosoroseus* (Wize) A.H.S. Br. & G. Sm.), have been used in various studies for the control of stored-product insects (Bello et al., 2001; Padin et al., 2002; Khashaveh et al., 2011; Shafighi et al., 2014; Kubilay Er et al., 2016). *B. bassiana* and *M. anisopliae* are reported as the most extensively studied fungi species in the control of stored-product insects (Rumbos and Athanassiou, 2017). Particularly, it has been determined that *B. bassiana* isolates are effective against stored grain insects, including *Sitophilus oryzae*, *Rhyzopertha dominica*, and *Tribolium castaneum* (Padin et al., 1996; Bello et al., 2001).

In this study, the effects of *B. bassiana* BMAUM LD.2016 and BMAUM M6-4 isolates obtained from Isparta province (Western Turkey) on the 3rd instar larvae of of the confused flour beetle, *T. confusum*, were investigated under laboratory conditions.

MATERIALS AND METHODS

Rearing of *Tribolium confusum*

The adult individuals of *T. confusum* were obtained from infested seeds of wheat varieties in Pamukkale University, Faculty of Applied Sciences, Department of Organic Agricultural Management, Genetic Stock Unit. The larvae were cultured in a laboratory conditions in plastic pots (20 x 20 cm in size) containing a mixture of bran and flour. These containers were kept in plant growth chambers with a temperature of 25°C, relative humidity of 60±5%, photoperiod 16:8 (light:dark) lighting conditions.

Preparation of *Beauveria bassiana* isolates and spore suspensions

The study utilized the *B. bassiana* BMAUM M6-4 isolate, which was isolated using the Galleria trap method from soil samples collected from agricultural fields in the city center of Isparta (Zimmermann, 1986), and the *B. bassiana* BMAUM LD.2016 isolate, which was isolated from adult *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) collected on the campus of Süleyman Demirel University and found to be highly pathogenic (Baydar et al., 2016).

B. bassiana isolates used in the experiment were cultured in potato dextrose agar (PDA-39 g/l, Difco) medium. For this purpose, PDA was prepared using distilled water (dH_2O) and poured into glass Erlenmeyer flasks (500 ml) and sterilized in an autoclave at 121°C for 15 minutes. PDA medium cooled at room temperature was poured into plastic Petri dishes (90 mm) at a volume of 20 ml. Spore discs (0.5 cm) of BMAUM M6-4 and BMAUM LD.2016 isolates were transferred to the center of Petri dishes containing PDA medium in a sterile cabinet. The Petri dishes, sealed with Parafilm, were incubated for 14 days in a dark, cooled incubator at a temperature of 25°C and a relative humidity of 75%. After 14 days of incubation, the spores developed on PDA plates of BMAUM M6-4 and BMAUM LD.2016 cultures were gently scraped and collected. These spores were then added to 50 ml of sterile distilled water containing 0.05% Tween 80 to prepare spore suspensions. In order to calculate the spore density from the prepared suspension, a 10^{-2} dilution was made and counted with the help of Thoma Slide under the light microscope, and spore suspensions were prepared at a density of 1 x 10^8 conidia/ml for each *B. bassiana* isolate (Fancelli et al., 2013).

Application of *B. bassiana* isolates to *T. confusum* larvae

Each *B. bassiana* isolate was applied to five 3rd instar larvae of *T. confusum* using spraying and dipping methods. Each experiment was carried out with 10 replications according to the randomized plots design.

Spraying method

In the spraying method, spore suspensions of *B. bassiana* isolates were sprayed on the 3rd instar larvae in a Petri dish (90 mm) from a distance of 20 cm with a hand sprayer. As a control, sterile distilled water containing 0.05% Tween 80 was sprayed onto the 3rd instar larvae. In order to provide high humidity, the bottoms of Petri

dishes were covered with blotting paper, and 1 ml of sterile distilled water was impregnated on these papers. Experiments were carried out with 10 replicates and 5 individuals in each replication. Numbers of live larvae were recorded at 1, 3, 5, 7 and 9 days after spraying. The spraying experiment was carried out in a plant growth chambers with 25±1°C temperature, 60±5% humidity, photoperiod 16:8 (light:dark) lighting conditions.

Dipping method

In the dipping method, the larvae of the 3rd instar larvae were placed in a cheesecloth and kept in spore suspensions of *B. bassiana* isolates for 5 seconds, and then transferred to Petri dishes containing moistened blotting paper. In control applications, third instar larvae were dipped in sterile distilled water containing 0.05% Tween 80. Experiments were carried out with 10 replicates and 5 individuals in each replication. Numbers of live larvae were recorded at 1, 3, 5, 7 and 9 days after dipping. The dipping experiment was carried out in a plant growth cabinet with 25±1°C temperature, 60±5% humidity, photoperiod 16:8 (light: dark) lighting conditions.

Data analysis

Data for this study was subjected to analysis of variance (ANOVA), and the differences between means were compared using the Tukey's multiple comparison test at a significance level of $P \leq 0.05$ (Tukey, 1949). The data analysis was performed using the IBM® SPSS® Statistics software (Version 20.0, August 2011, SPSS Inc., Chicago, IL, USA) statistical package program. Abbott formula is used to determine the percentage of mortality rates (Abbott, 1925). In addition, the estimated time (LT_{50}) to kill 50% of the insects was determined by the Probit analysis program (Throne et al., 1995).

RESULTS AND DISCUSSION

Spraying and dipping methods of *B. bassiana* BMAUM LD.2016 showed that this isolate was effective on the 3rd instar larvae of *T. confusum* and percentage mortality rates in the larvae (Fig. 1). Mortality rates increased depending on the application days in both spraying and dipping methods. The highest mortality rates in 3rd instar larvae were recorded as 72% for the spraying method and 34% for the dipping method (Fig. 1).

Percentage of mortality rates resulting from the application of the second *B. bassiana* isolate, BMAUM M6-4, on 3rd instar larvae of *T. confusum* using the spraying and dipping methods are given in Figure 2. Percentage mortality rates increased with time after the treatment. In contrast, in the dipping method, the mortality rate was recorded as 6% on the third day and 8% on the ninth day of counting. In the spraying method, the highest mortality rate of 96% was observed in the 3rd stage larvae, while in the dipping method, the highest mortality rate was recorded as 8% (Fig. 2).

The results obtained in this study are in agreement with results of previous studies. The mortality rate was determined as 40% on the 7th day after the application of *M. anisopliae* isolate at $8x10^{10}$ spore/ml concentration to *T. confusum* larvae (Michalaki et al., 2006). When applied to adults of *S. oryzae* and *T. castaneum*, *B. bassiana* isolate resulted in mortality rates of 63.3% and 26.7% respectively, while *M. anisopliae* isolate resulted in mortality rates of 50.0% and 20.2% respectively (Batta, 2008). In another study, *B. bassiana* (BbWeevil™) was applied at a concentration of 1,000 mg/kg to adults of *S. granarius*, *Oryzaephilus surinamensis*, and *T. castaneum*, resulting in mortality rates of 88.33%, 78.31%, and 64.99% respectively (Khashaveh et al., 2011). The highest mortality rate was recorded as 57.35% 21 days after the application of *B. bassiana* (RacerTM) to the 3rd instar larvae of *T. confusum* at 0.9×10^{8} conidia/kg concentrations (Rehman et al., 2018). Çetinpolat et al. (2019) reported that the application of *B. bassiana* isolate to *T. confusum* larvae resulted in 97.4% mortality at a dose of 500 ppm and 100% mortality at a dose of 1000 ppm. In a study conducted with four isolates of *B. bassiana* (GN22-1, HP15, HP5-2, HP3-1) on *T. castaneum* adults, it was reported that after 13 days of application at a concentration of 1×10^{8} conidia/ml, the mortality rates were recorded as follows: GN22-1 - 72.85%, HP3-1 - 48.88%, HP15 - 47.37%, and HP5-2 - 30.43% (Uçar et al., 2020). *B. bassiana* (Bals.-Criv.) Vuill. (Hypocreales: Cordycipitaceae) WG-50 and WG-51 isolates, when applied at a dose of 1×10^{8} conidia/kg to *T. castaneum* adults, recorded mortality rates of 88.1% and 83.4% respectively after 21 days (Wakil et al., 2021). *B. bassiana*, when applied at a dose of 1×10^{7} conidia/ml to *T. confusum*, showed effects of 87.5%, 97.5%, and 100% at 24, 48, and 72 hours respectively (Youssra Sekrane et al., 2022).

When the mortality rates of the 3rd instar larvae of *T. confusum* were examined depending on time, the LT_{50} value, which indicates the time required for half of the *T. confusum* larvae to die in the spraying method, was calculated as 6.09 and 3.90 days in BMAUM LD.2016 and BMAUM M6-4 isolates, respectively (Fig. 3 and Fig. 4). In the dipping method, the mortality rate for both *B. bassiana* isolates remained below 50%, so the LT_{50} value could not be calculated.

Unlike the LT_{50} values we obtained in our study, Uçar et al. (2020) reported that LT_{50} values as 28.813 days for HP5-2, 17.186 days for HP3-1, 10.327 days for GN22-1, and 18.615 days for HP15 when different isolates of *B. bassiana* were applied to *T. castaneum* adults.

CONCLUSION

Results show that BMAUM M6-4 isolate of *B. bassiana*, isolated from agricultural fields, was more effective than BMAUM LD.2016 isolate on *T. confusum* larvae. The percentage of mortality rates obtained with the spraying method used in the experiment were found to be higher than the dipping method. In future studies, the interaction of these isolates with pesticides used against stored-

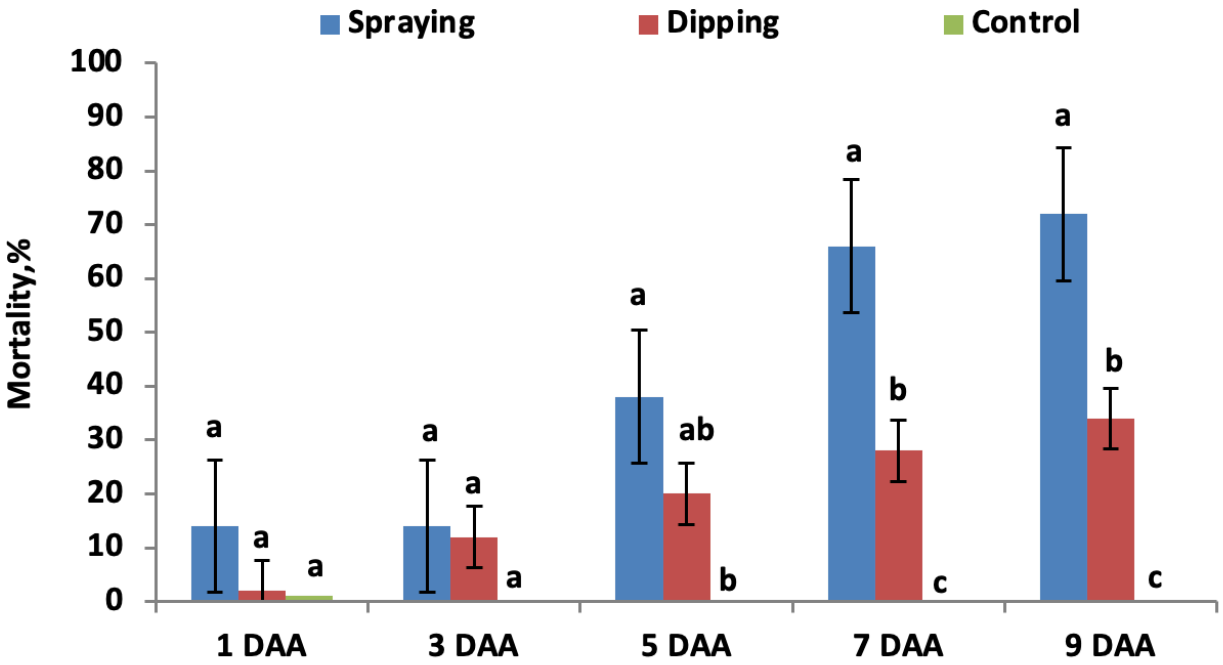

Figure 1. Percentage mortality rates of *B. bassiana* BMAUM LD.2016 isolate applied on *T. confusum* larvae with spraying and dipping methods. (The differences between the means (±standard error) of the columns indicated with different letters for each day are statistically significant (Tukey's HSD test P<0.05)). DAA: Days after application

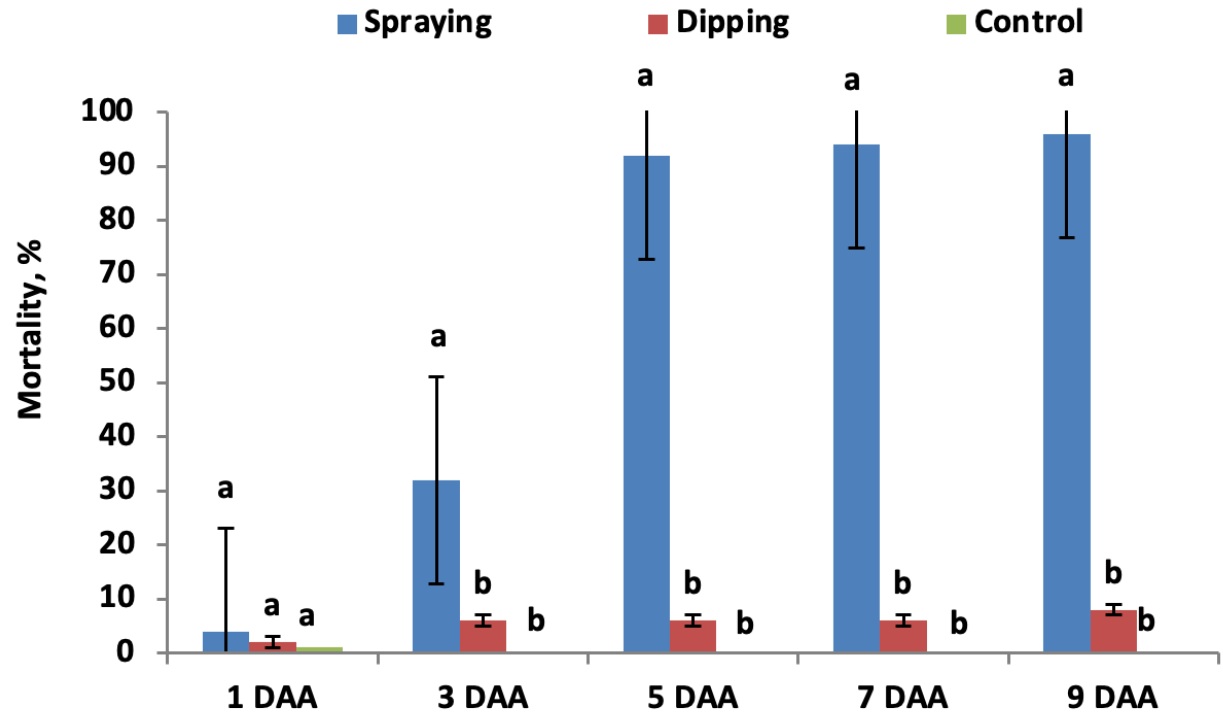

Figure 2. Percentage mortality rates of *B. bassiana* BMAUM M6-4 isolate applied on *T. confusum* larvae with spraying and dipping methods. (The differences between the means (±standard error) of the columns indicated with different letters for each day are statistically significant (Tukey's HSD test P<0.05)). DAA: Days after application

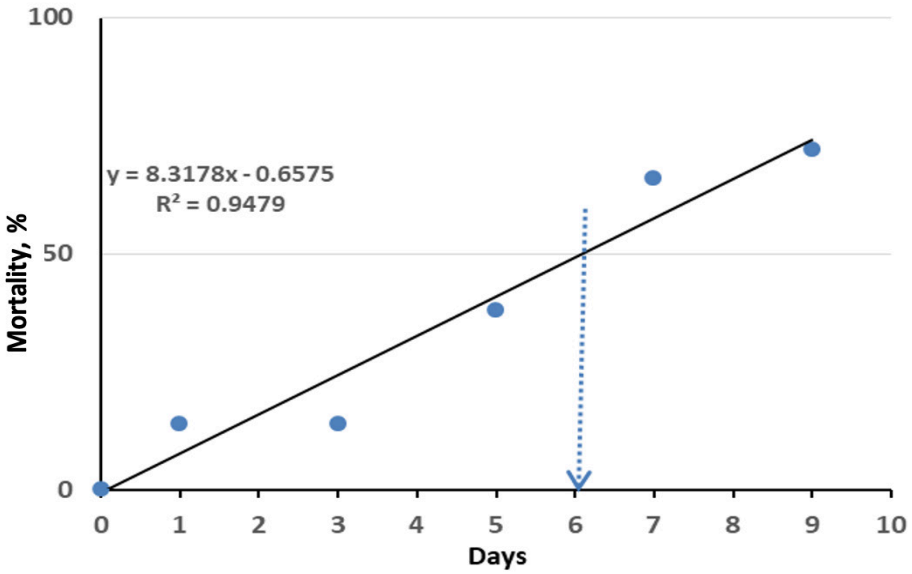

Figure 3. Mean LT_{50} values of *B. bassiana* BMAUM LD.2016 isolate in spraying method

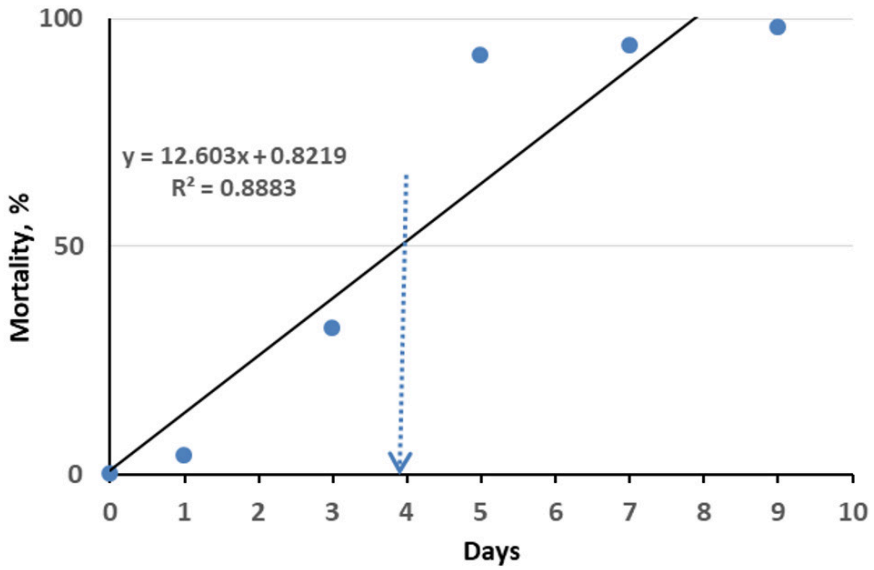

Figure 4. The mean LT_{50} values of *B. bassiana* BMAUM M6-4 isolate in spraying method

product insects should be investigated. Furthermore, the development of the use of entomopathogenic fungal isolates, including for other stored-product insects, would be beneficial for integrated pest management.

Abbreviations

ANOVA: Analysis of variance, *B. bassiana*: *Beauveria bassiana*, DAA: Days after application, EPF: Entomopathogenic fungi, PDA: Potato dextrose agar.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Declaration of interests

The authors have no conflict of interest to declare.

Author contribution

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

Ethics Committee Approval

Ethics committee approval is not required. This article does not contain any studies with human participants or animals performed by any of the authors.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(3): 540-549
DOI: https://doi.org/10.31015/jaefs.2023.3.8

Optimizing phosphine fumigation efficiency in hazelnut industry: Determining optimal exposure time for stored product pest control

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Citation: Guncan, A., Altunc, Y.E., Karayar, S., Bahat, T.N., Sezer Yuksel, E. (2023). Optimizing phosphine fumigation efficiency in hazelnut industry: determining optimal exposure time for stored product pest control. International Journal of Agriculture, Environment and Food Sciences, 7 (3), 540-549

Received: July 17, 2023
Revised: August 04, 2023
Accepted: August 09, 2023
Published Online: September 18, 2023

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Available online at
https://jaefs.com/
https://dergipark.org.tr/jaefs

Abstract

Hazelnut, as with many other stored products, are susceptible to infestation by a variety of stored insect pests. Phosphine fumigation is a widely used method to control pests in stored products, including hazelnut kernels. This study aimed to determine the optimal exposure time for phosphine fumigation for management of stored product pests in hazelnuts. Four treatments with different exposure times (3, 4, 5, and 6 days) were conducted using various development stages of *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), *Tribolium confusum* Jaqcquelin du Val, (Coleoptera: Tenebrionidae), *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae), *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae), and *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) together with the control group. The trials were conducted in a commercial chamber of a hazelnut processing facility. The insects were placed in plastic containers within jute sacks filled with hazelnuts, fumigations were done under gas-proof sheet and the survival rate was assessed after treatments. The results showed that a 3-day exposure period was sufficient to fully eradicate the pupal and adult stages of *O. surinamensis*. For *T. castaneum*, 100% mortality was achieved in both larvae and adults from 3 days of exposure, but the pupal stage required at least 5 days. For *T. confusum*, all larvae and adults died in all exposure periods, but the pupal stage required at least 4 days. In the case of the moth species, a 100% mortality rate was achieved in the larval and pupal stages of both *E. kuehniella* and *P. interpunctella* at all exposure periods. The mortality rate of *E. kuehniella* eggs was 99% after 3 and 4 days of exposure, and a fumigation period of 5 days was required to control the entire population. However, only 67% of *P. interpunctella* eggs were controlled after 3 days of exposure. The time and stage factors were found to be significant in the egg stage of *P. interpunctella*. The results suggest that a 5-day exposure period is the most effective for controlling tested stored product pests in hazelnuts.

Keywords: Hazelnut, Phosphine, Exposure period, Stored product pests

INTRODUCTION

Phosphine is a common utilized fumigant for the control of stored product pests, which can cause significant economic losses by contaminating and damaging stored grains, dry fruits, nuts, and other harvested commodities. The use of phosphine is in demand for stored product pest control, due to its low cost, versatility, high efficiency, free from toxic residue, easy accessible and use. However, the effectiveness of phosphine in controlling stored product pests dependent upon a number of factors, including the concentration of phosphine, the temperature and humidity of the storage environment, and the durations of exposure (Daglish et al., 2002).

Exposure time is a crucial factor that can influence the effectiveness of phosphine in pest control. Long-term application periods are generally more effective for controlling stored product pests (Hole et al., 1976), as enables the phosphine to reach all areas of the storage facility and ensure that pests are exposed to lethal concentrations of the fumigant. Previous studies have also demonstrated that longer exposure durations are more effective than the application of high concentrations over shorter periods (Bell, 1976; Ahmedani et al., 2007; Fukazawa & Takahashi, 2017; Agrafioti et al., 2020). This is because high concentrations may induce narcosis in pests, which can reduce the toxic effect of phosphine by decreasing metabolism (Bell, 1979; Winks, 1985; Chaudhry, 2000). In addition, one of the important reason for development of phosphine resistance is short exposure time and the cost of the treatment increased with longer exposure times. Therefore, it is important to balance the need for prolonged exposure with the need to minimize the risk of phosphine resistance and the cost of treatment (Athanassiou et al., 2016; Chadda, 2016). In the hazelnut processing industry, where time is a limited factor, the goal is to achieve a pest-free product in the shortest possible time due to their intensive production plans.

Studies have examined the effect of various exposure times on the mortality of stored product pests treated with phosphine. For instance, Ahmedani et al. (2007) found that increasing the exposure time of phosphine from 1 to 5 days resulted in a significant increase in the mortality of *Trogoderma granarium* Everts (Coleoptera: Dermestidae) larvae, with 100% mortality achieved at the 5-day exposure duration. Another study by Bell et al. (1984) found that complete mortality of diapausing *T. granarium* larvae was achieved in a 4-day exposure period with phosphine, with the exposure time required for 100% mortality increasing to 7 days in proportion to the age of the diapausing larvae. Collins et al. (2005) determined that different exposure times were necessary to reach 99.9% mortality ($LT_{99.9}$) of *Rhyzopertha dominica* (Coleoptera: Bostrichidae) adults at varying phosphine concentrations. Minimum and maximum $LT_{99.9}$ were determined to be between 5 and 8.8 days, depending on the concentrations of phosphine in susceptible strains, while $LT_{99.9}$ was between 3.7 and 14 days in strong resistant strains.

Effective fumigation requires adherence to established practices and procedures to ensure that pests are fully eliminated. Even a mortality rate of 90% after fumigation is considered a failure and a mortality rate of over 99.9% is generally required (Hole et al., 1976). Poor fumigation methods may only kill visible pests, but leave behind stages that can survive and quickly reproduce, leading to a new infestation (Chadda, 2016). Additionally, the efficacy of fumigation depends on the developmental stage of the species. Immobile stages exibiting reduced metabolism may experience an increased likelihood of survival during phosphine fumigation (Hole et al., 1976; Rajendran et al., 2001; Chadda, 2016). As a result, the optimal and successful fumigation procedure should provide complete mortality in all stages of the species within the planned exposure period.

Hazelnuts, like many other stored commodities, are prone to pest infestations during the postharvest period (Hagstrum et al., 2013). Several insect species, including *Cadra cautella* (Walker), *Ephestia kuehniella* Zeller, *Plodia interpunctella* Hübner (Lepidoptera: Pyralidae), *Tribolium castaneum* (Herbst), *Tribolium confusum* Jacqueline du Val (Coleoptera: Tenebrionidae), *Oryzaephilus surinamensis* (L.), and *Oryzaephilus mercator* (Fauvel) (Coleoptera: Silvanidae), commonly infest stored hazelnuts as well as other dried fruits and nuts (Alkan, 1959; Yasan & Kiper, 1972; Ozman-Sullivan et al., 2009; Hagstrum et al., 2013; Moraglio et al., 2018). The control of these stored product pests in the hazelnut industry is crucial for preserving the quality and value of the nuts. One of the primary objectives of the hazelnut industry is to identify the most efficient and cost-effective method that achieves the desired level of mortality. Hence, there is a necessity to optimize fumigation operations in order to strike the perfect balance between effectiveness and duration. This study aimed to establish the most time-efficient duration for phosphine fumigation for the hazelnut industry. To accomplish this, trials were conducted involving five prevalent stored hazelnut pests and various developmental stages of these pests.

MATERIALS AND METHODS

Insect rearing and handling of stages

A total of five species including *Oryzaephilus surinamensis*, *Tribolium confusum*, *Tribolium castaneum*, *Ephestia kuehniella*, and *Plodia interpunctella* were used in the experiments. The coleopteran species were reared in 1-liter glass jars with different food sources. A diet consisting of five parts oatmeal, five parts cracked wheat, and one part dry yeast was provided to *O. surinamensis*, while *T. confusum* and *T. castaneum* were given a diet of ten parts wheat flour and one part dry yeast. The jars were covered with fine muslin cloth to allow for aeration. Plastic containers (1.2 liter) used for mass rearing of the lepidopteran species. *E. kuehniella* was provided with a diet of ten parts wheat bran, half a part wheat flour, and a quarter part corn flour, while *P. interpunctella* was reared on mixture of wheat bran (90 g), dry yeast (10 g), honey and water mixture (18 g), glycerol (46 g), sunflower oil (3 g), and water (15 ml). Once adults emerged, they were daily removed to copulation cages. All jars and containers were kept in a growth chamber at a temperature of 26 °C, 65%±5 relative humidity, and a 16:8 photoperiod (light:dark). Mass production of the species has been continuously ongoing for more than three years in the Laboratory of Entomology, Department of Plant

Protection, at Ordu University in Türkiye.

In the experiments, the pupae (0-24 hours old) and adults (0-15 days old) of *O. surinamensis* were collected for use. To obtain young pupae, mature larvae were sieved and separated from the stock culture, and the pupae were collected the following day. In the case of *T. confusum* and *T. castaneum*, mature larvae (5-6th stage), pupae (0-24 hours old), and adults (0-15 days old) were collected by sieving, and the age of the stages was equalized. In the lepidopteran species, eggs (0-24 hours old), mature larvae (4-5th stage), and pupae (0-24 hours old) were collected for the trials. All stages of individuals were handled with a fine brush.

Chemical and fumigation chamber

Fumigation trials were conducted under gas-tight polyethylene sheeting in chamber of a commercial hazelnut processing facility in Ordu (Sagra Grup Gıda Üretim ve Tic. A.Ş., Ordu, Türkiye). Aluminum phosphide (Detia Gas EX-B, 57% DP, bag) was used to generate phosphine. An average of circa 20 tons of raw (shelled) hazelnuts, placed inside jute sacks, were transferred to the chamber on pallets. One bag of aluminum phosphide (34 g) per 11 m^3 was applied in all treatments as a registered dose. A total of 230-260 jute sacks of hazelnuts were fumigated under sheeting in a heated chamber, with a calculated volume of 85-88 m^3 (eight bags, 272 g aluminum phosphide used). In all trials, temperature and humidity were recorded as 20-25 °C, 60%±15 relative humidity, using a Hobo data logger (Onset, USA).

Fumigation trials

The studies consisted of four treatments in which fumigations were conducted at four different exposure times including 3, 4, 5 and 6 days. We used five replications for different stages of insects. Then, a total of 50 pupae and 100 adults of *O. surinamensis*; 50 larvae, 50 pupae and 100 adults of *T. confusum* and *T. castaneum*; 150 eggs, 100 larvae, and 50 pupae of *E. kuehniella* and *P. interpunctella* were exposed in each time unit. The insects were placed with 30 cc cylindrical plastic containers inside the jute sacks. Three holes (1 cm diameter) were opened on the lid of the containers and covered with muslin cloth. Additionally, pinholes were opened on the sides of the containers to maximize the penetration of gas. For the feeding stages of the insects, a food supply (identical to the rearing diet) was included in the containers. To ensure the same exposure conditions in each treatment, the containers of replications were placed in same pattern. The gas-proof sheet was closed and surrounded by sandbags to prevent air escape. After reaching the targeted exposure time, the chamber and sheeting were opened and ventilated for 24 hours. The plastic containers were then transferred to the laboratory and kept in test cabinets with the same conditions as previously mentioned. The hole procedure was implemented for the control group, which was not exposed to phosphine in same conditions. After 24 h, the survival of the insects was evaluated by disturbing them with a brush. Adults and larvae that exhibited no observable movement were recorded as dead. Eggs and pupae that failed to hatch after 15 days were also classified as dead.

Statistical analysis

Statistical analysis was performed by generalized linear model (GLM) with a binomial distribution (i.e., dead or alive) with a logit link function. The Wald Chi-squared test was used to assess statistical significance of the fixed factors i.e. time (length of exposure), pest species and their stages. If significant, post-hoc pairwise comparisons based on estimated marginal means were performed using the least significant difference (LSD) test ($P<0.05$). All analysis was performed using the R software (R Core Team, 2022) with the following packages: brglm2 (Kosmidis et al., 2020) for bias reduction in GLM, car (Fox & Weisberg, 2011) for analysis of GLM, and emmeans (Lenth, 2022) for pairwise comparisons.

RESULTS

The mortality rate of control groups in all species was below 5% (Table 1), therefore, there was no need to correct the mortality rate. Mortality increased with the length of exposure and varied among the insect species and development stages. [species (Wald $X^2=13.76$, d.f.=4, $P=0.0081$); time (Wald $X^2=34.80$, d.f.=3, $P<0.0001$); stage (Wald $X^2=8.60$, d.f.=3, $P=0.0351$)].

Both the pupal and adult stages of *O. surinamensis* were fully eradicated in fumigations even after just 3 days of exposure (Figure 1).

In *T. castaneum*, there were no significant differences found for either time (Wald $X^2=2.37$, d.f.=3, $P=0.4984$) or stage (Wald $X^2=4.21$, d.f.=2, $P=0.1220$). The mortality rate of both larvae and adults reached 100% starting from 3 days of phosphine exposure, while mortality rates were 96% in 3-day exposure and 98% in 4-day fumigation in the pupal stage (Figure 2). Total mortality (100%) were observed after 5-day exposure (Figure 2). There was no significant difference between mortality rates in pupal stages of *T. castaneum* within the exposure times (Wald $X^2=1.99$, d.f.=3, $P=0.5743$).

Similarly, no significant differences were found in either exposure times (Wald $X^2=3.15$, d.f.=3, $P=0.3686$) or developmental stages (Wald $X^2=3.14$, d.f.=2, $P=0.2083$) for *T. confusum*. Complete mortality was observed in all larvae and adults of *T. confusum* across all exposure periods (Figure 3). However, 3-day exposure duration was found to be insufficient for complete control of the pupal stage. Achieving 100% mortality required a 4-day exposure time (Figure 3). No significant difference was observed in the mortality rates of *T. confusum* pupal stages across the different exposure times (Wald $X^2=2.71$, d.f.=3, $P=0.4384$).

Table 1. The mean percentage mortality of the control group of the treatments.

Species	Duration (d)	Mortality (%)			
		Egg	Larvae	Pupae	Adult
Tribolium confusum	3	-	0	0	0
	4	-	0	0	1
	5	-	0	0	0
	6	-	0	2	0
Tribolium castaneum	3	-	2	0	0
	4	-	4	2	0
	5	-	0	4	0
	6	-	2	0	0
Oryzaephilus surinamensis	3	-	-	2	2
	4	-	-	4	2
	5	-	-	2	4
	6	-	-	0	2
Ephestia kuehniella	3	4	0	4	-
	4	1.33	0	2	-
	5	2	0	2	-
	6	1.33	0	2	-
Plodia interpunctella	3	2	0	0	-
	4	0.44	0	4	-
	5	2.67	0	0	-
	6	1.33	0	2	-

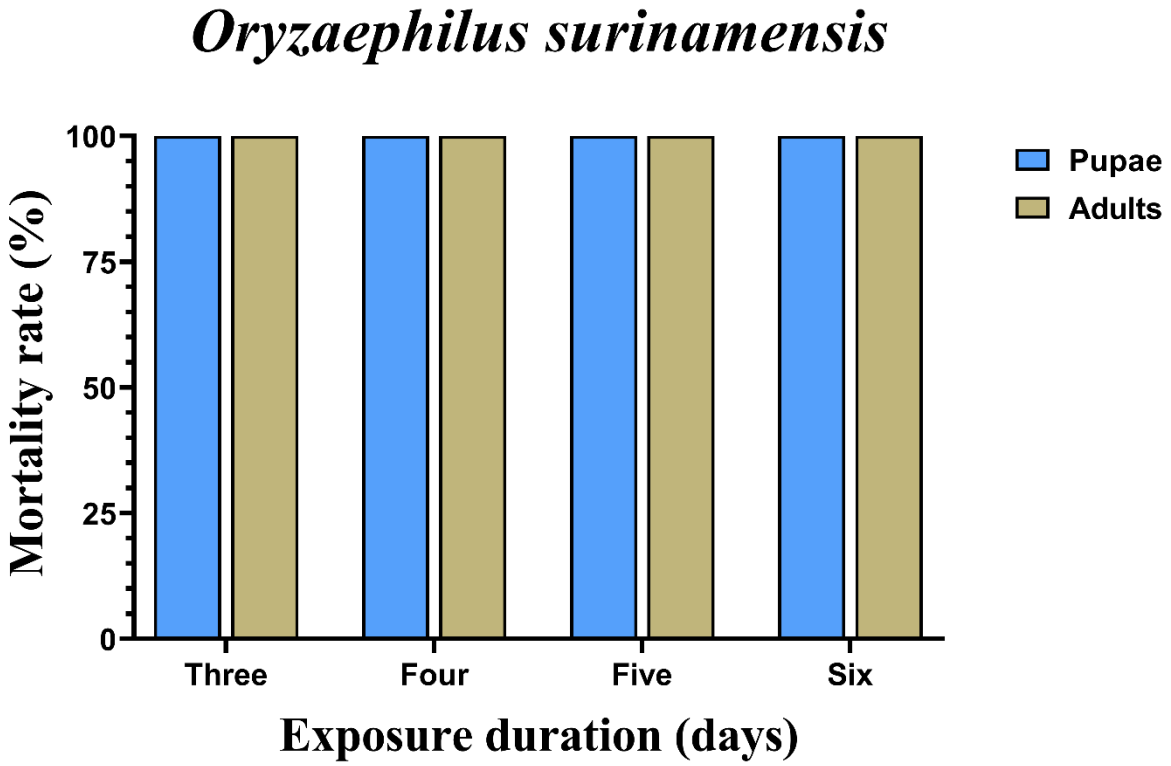

Figure 1. Mortality rate (%) (mean ± S.E.) of the pupae and adult stages of the *Oryzaephilus surinamensis* within the different phosphine exposure times

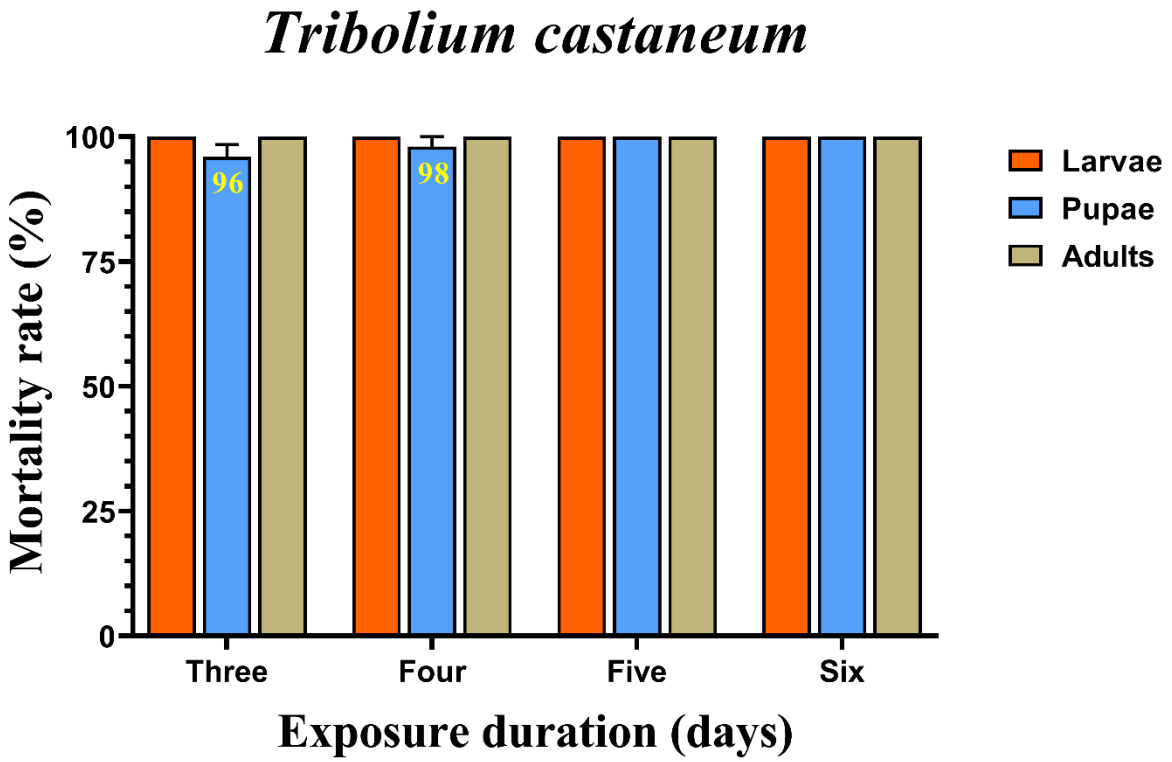

Figure 2. Mortality rate (%) (mean ± S.E.) of the larvae, pupae and adult stages of the *Tribolium castaneum* within the different phosphine exposure times

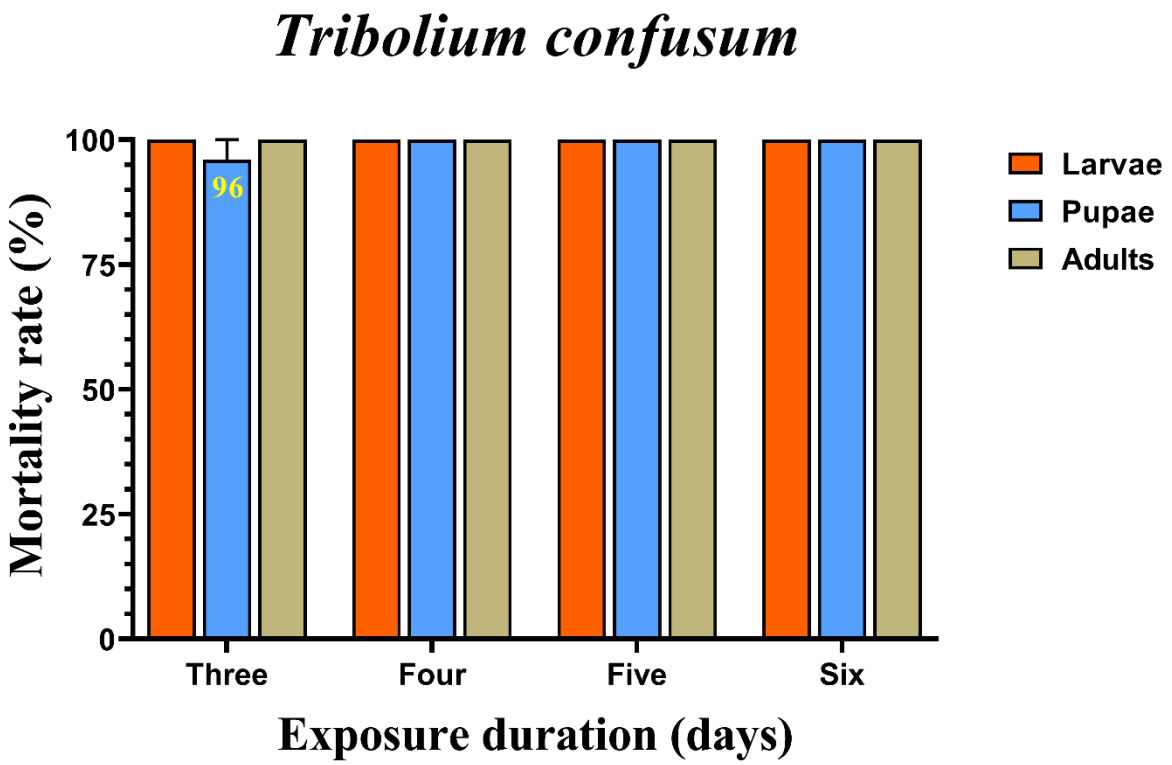

Figure 3. Mortality rate (%) (mean ± S.E.) of the larvae, pupae and adult stages of the *Tribolium confusum* within the different phosphine exposure times

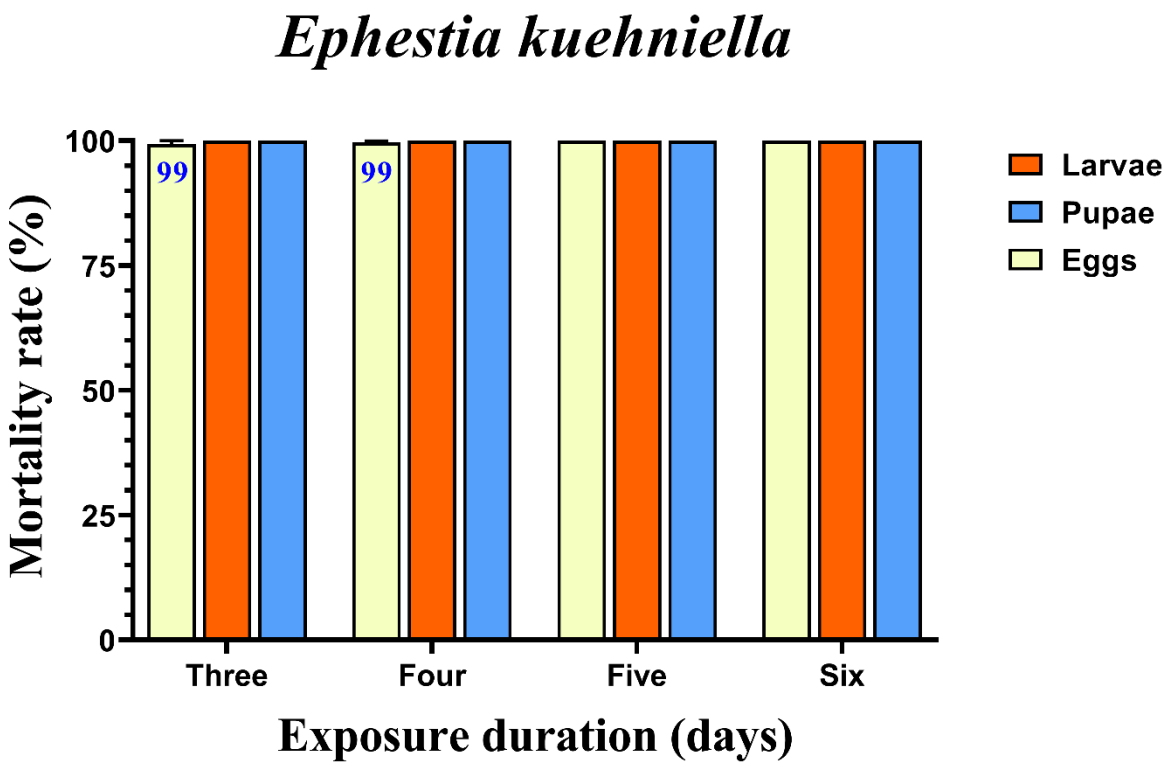

Figure 4. Mortality rate (%) (mean ± S.E.) of the egg, larvae and pupae stages of the *Ephestia kuehniella* within the different phosphine exposure times

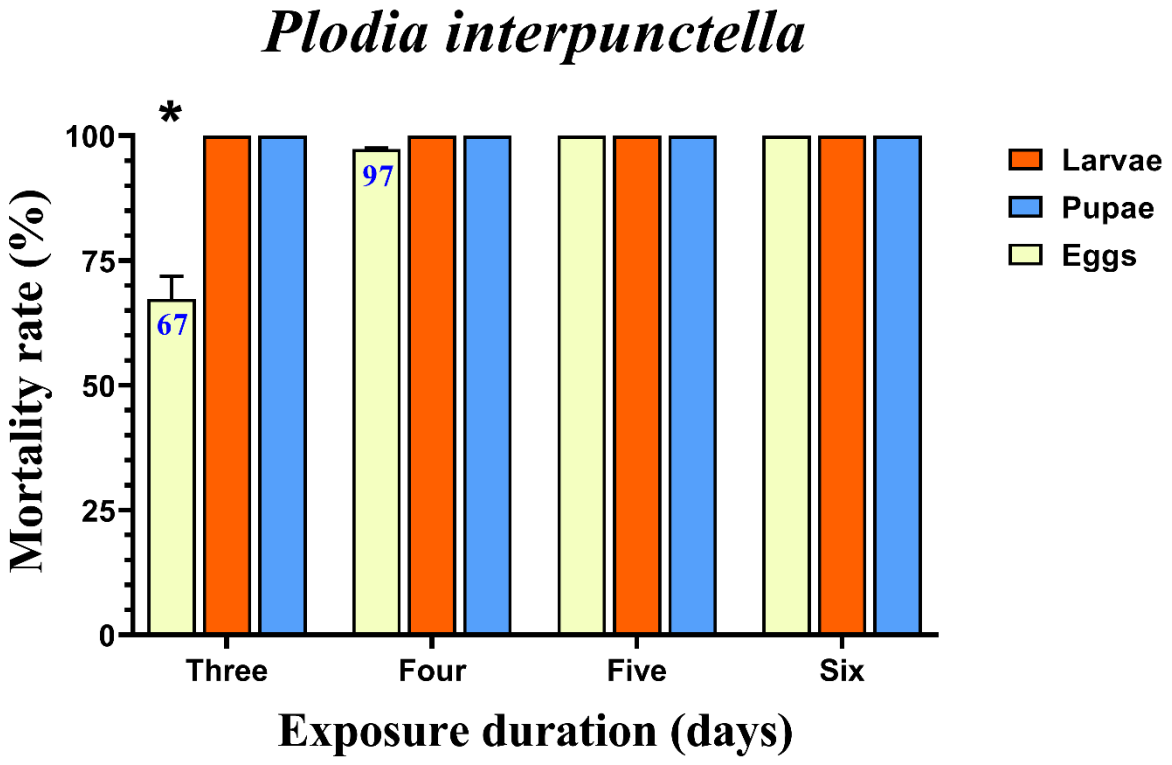

Figure 5. Mortality rate (%) (mean ± S.E.) of the egg, larvae and pupae stages of the *Plodia interpunctella* within the different phosphine exposure times. (GLM, binomial distribution, Wald χ^2 test followed by LSD post hoc multiple comparison test, * $P<0.05$)

Fumigation trials resulted in a 100% mortality rate in the larval and pupal stages of *E. kuehniella* all exposure periods (Figure 4). There were no significant differences identified in either time (Wald X^2=1.19, d.f.=3, P=0.7543) or stages (Wald X^2=0.89, d.f.=2, P=0.6415) for *E. kuehniella*. The mortality rate of eggs of *E. kuehniella* was 99% after 3 and 4 days of exposure, and no significant difference were found in mortality of eggs among the exposure periods (Wald X^2=1.03, d.f.=3, P=0.7949). A fumigation period of 5 days was required to control the entire population (Figure 4).

Significant differences were found between exposure times (Wald X^2=80.93, d.f.=3, P<0.0001), and developmental stages of *P. interpunctella* (Wald X^2=9.65, d.f.=2, P=0.0080). Throughout all exposure periods, complete mortality was observed in all larvae and pupae of *P. interpunctella* (Figure 5). However, the mortality rate of *P. interpunctella* eggs was only 67% after 3 days of exposure. Subsequently, the mortality rate increased to 97% after 4 days of exposure, and reached 100% after 5 days of fumigation, demonstrating a statistically significant difference between exposure periods (Wald X^2=80.74, d.f.=3, P<0.0001).

DISCUSSION

Phosphine fumigation was effective in controlling the entire population of *O. surinamensis* at all exposure periods. Both the pupal and adult stages were affected equally. In line with our findings, Hole et al. (1976) also reported that at 25 °C, *O. surinamensis* was the most susceptible species to phosphine fumigation in all exposure periods (2, 4, and 7 days) when compared to other twelve stored product insect species. They found that at the 7-day exposure period, much lower concentrations were needed to kill 100% of the insect species compared to shorter exposure periods. At this point, the needed phosphine concentration for absolute control of *O. surinamensis* was 0.013 mg/l, while it was 0.32 for the weevil species. In the ambient temperatures similar with our study (20-25 °C), Ferizli et al. (2007) found that 5 days of exposure duration is required for complete control of the egg, larvae, pupae, and adult stages of *O. surinamensis* and *C. cautella*. In another study, Athanassiou et al. (2016) also found that in trials of phosphine fumigation at low pressure and 48 hours of exposure, 100% mortality was only observed in *O. surinamensis* adults among the seven species of stored product pests. The species with the next highest mortality was *T. confusum* larvae, with 99.8% mortality in the 48 hours of exposure to low-pressure fumigation. They found that the maximum mortality was 75.6% in the pupal stage of *T. confusum* at all exposure periods. Although the fumigation was performed under normal air pressure in our study, we were unable to achieve 100% control at 3 days of exposure and required at least 4 days of exposure. We found 100% mortality in other stages such as larvae and adults in 3 days of exposure period and above. This finding supports the work of Aulicky et al. (2015) in which they also reported the absolute effectiveness of 3 days of exposure on adults and larvae. Our results for *T. castaneum* were similar to those for *T. confusum* for adults and larvae. Additionally, the pupal stage could not be 100% controlled at 3 days of exposure period in *T. castaneum*, and a longer (5 days) exposure duration was needed to kill all the pupae. Winks, (1984) also indicated that the eggs and pupal stages of *T. castaneum* are more tolerant and absolute control requires longer exposure times.

The biological stage of an insect is recognized as a crucial factor influencing the effectiveness of phosphine fumigation. Previous studies have indicated that low metabolism stages, such as early eggs and pupae, tend to enter a state of inactivity or "switch off" during exposure to phosphine for certain periods (Bell, 1976; Winks, 1984; Chaudhry, 1997). However, the development of tolerant stages continues, and they soon advance to the next susceptible stage (Bell, 1979; Nakakita & Winks, 1981; Winks & Waterford, 1986; Chadda, 2016). Therefore, longer exposure periods are required to target these insect stages. Bell (1976) reported that eggs of *E. kuehniella* and *P. interpunctella* were tolerant to phosphine fumigation. The study revealed that to achieve complete control of *E. kuehniella* eggs, 3 and 4 days of exposure were necessary at 25 °C and 20 °C, respectively. Similarly, at these same temperature conditions, 3 and 5 days of exposure were required for *P. interpunctella* eggs. This finding aligns with our own research, where we also observed that a 5-day exposure to phosphine at 20-25 °C resulted in 100% control of both *E. kuehniella* and *P. interpunctella* eggs. Temperature plays a crucial role in reducing exposure times. For instance, Phillips et al. (1999) reported that *P. interpunctella* eggs achieved 100% mortality after only 2 days of exposure at 32 °C, whereas it took 6 days of exposure at 5 °C to achieve the same mortality rate. However, exposing higher temperatures can influence the quality of the hazelnuts (Guiné et al., 2015).

The age of the eggs of moths during exposure to phosphine is important, as older eggs are more susceptible due to metabolism during embryonal development (Bell, 1976)., young eggs (1 day old) of *E. kuehniella* and *P. interpunctella* survived until they were older (4 days old). However, when experiments were conducted with older (2-4 days) eggs, 100% mortality was achieved even at a 24-hour exposure period (Bell, 1976). Additionally, Mbata et al. (2004) also found that in the low pressure applications the required exposure time decreases as the egg age of *P. interpunctella* increases. Based on these findings, we suppose that, as we used 0-24 hour old eggs of *P. interpunctella* in our trials, they were much more tolerant to phosphine until they reached nearly hatching age, which is around 4-7 days (Mbata & Osuji, 1983).

The optimum exposure time also depends on the

susceptibility of the pest strain to phosphine. In the control of resistant strains, exposure duration is more critical than the concentration of phosphine (Chaudhry, 1997; Daglish, 2004). Based on earlier studies, the required exposure period increases when resistant populations are present (Rajendran et al., 2001; Collins et al., 2005; Fukazawa & Takahashi, 2017). Even under high concentrations (3000 ppm) of phosphine, the percentage of immobilized adults of resistant *R. dominica*, *O. surinamensis*, and *T. castaneum* strains increases with exposure duration, while susceptible populations are 100% affected after the shortest exposure time (Lampiri et al., 2021). In our study, we did not determine the phosphine-resistant conditions of the population used in the trials. However, there was no indication of resistance based on the mortality data of the species. But, we determined the endurance of the immobile stages. Only the eggs and pupae stages of the species were not fully controlled at the shortest exposure periods, and longer times were required.

CONCLUSION

In conclusion, the study found that phosphine fumigation is an effective method for controlling stored hazelnut pests, with a mortality rate of 100% in all species tested. *O. surinamensis* was fully eradicated in fumigations even after just 3 days of exposure, with no significant differences found for either time or stage factors. Similarly, *T. castaneum* and *T. confusum* also had a high mortality rate, but longer exposure periods (min. 5 days) were required to completely control the pupal stage. In the case of moth species, the study found that fumigation resulted in a 100% mortality rate in the larval and pupal stages of both *E. kuehniella* and *P. interpunctella* at all exposure periods. However, in the egg stage of *P. interpunctella*, the time and stage factors were found to be significant and 5 days of exposure period was required to control the entire population.

Hazelnuts are commonly fumigated prior to transfer to warehouses or before processing. The fumigation implementations must be fully successful due to the phosphine doesn't have an insecticidal residual effect. Our results indicate that the most effective phosphine fumigation duration for jute sack-stored hazelnuts is 5 days of exposure. We believe that, this optimization of hazelnut fumigation duration is crucial for hazelnut processing facilities and will enhance their ability of management with stored product pests. However, phosphine resistance should be investigated before the management to ensure the effectiveness.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

ESY is presently employed as Food Engineer at Sagra Grup Gıda Üretim ve Tic. A.Ş. The remaining authors declare no apparent conflicting financial interests or personal associations with the aforementioned company that could potentially influence the findings presented within this manuscript.

Author contribution

AG: conceptualization, data curation, formal analysis, resources, writing - review & editing, YEA: conceptualization, investigation, data curation, formal analysis, writing draft - review & editing, ŞK: investigation, TNB: investigation, ESY: conceptualization, resources

Ethical approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

This study was performed within the facilities of the Sagra Group (OYAK). The authors would like to thank the Sagra Grup Gıda Üretim ve Tic. A.Ş. for granting us access to their facilities for conducting our experimental trials.

This study is also presented as an oral presentation at the X. International Congress on Hazelnut held on 5-9 September 2022 at Oregon State University in Corvallis, Oregon, USA and published as an abstract.

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RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(3): 550-562
DOI: https://doi.org/10.31015/jaefs.2023.3.9

Comparison of physical and quality characteristics of silage maize and silage sorghum under deficit irrigation conditions

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Citation: Keten Gokkus, M., Degirmenci, H. (2023). Comparison of physical and quality characteristics of silage maize and silage sorghum under deficit irrigation conditions. International Journal of Agriculture, Environment and Food Sciences, 7 (3), 550-562

Received: July 21, 2023
Revised: August 12, 2023
Accepted: August 15, 2023
Published Online: September 17, 2023

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Available online at
https://jaefs.com/
https://dergipark.org.tr/jaefs

Abstract

Silage sorghum has the feature of being an alternative to silage maize in many ways. Considering this feature, the nutritional contents and physical properties of silage maize and silage sorghum were examined. The aim of this study was to compare the physiological and quality characteristics of silage maize and silage sorghum under different irrigation treatments (M100-S100, M80-S80, M60-S60, M40-S40, and M20-S20). This study examined the physiological characteristics (chlorophyll content, plant height, stem diameter, and number of leaves) and quality characteristics (acid detergent fiber (ADF), neutral detergent fiber (NDF), and protein content (HP)) of second-crop silage maize and silage sorghum. Chlorophyll contents were measured before and after irrigation. These measurements showed that irrigation had no effect on the chlorophyll content in both plants in the middle of the growth period, and chlorophyll contents decreased towards the harvest. There was no significant difference between silage maize and silage sorghum plant height values. In the mean values for both years in which the plants were examined, stem diameter values and numbers of leaves were higher in sorghum compared to maize ($p<0.05$). There was no significant difference between maize and sorghum in terms of their protein contents (8.47% and 8.25%, respectively), acid detergent fiber (ADF), or neutral detergent fiber (NDF) values. In this case, it was seen that sorghum can be an alternative to maize in terms of nutritional quality. The protein contents of both plants decreased from the 100% irrigated treatment to the 20% irrigated treatment ($p<0.01$). This study will provide valuable information to feed producers and researchers in terms of comparing the physiological and quality characteristics of silage maize and silage sorghum under deficit irrigation conditions.

Keywords: Water, Protein content, Chlorophyll content, Acid detergent fiber

INTRODUCTION

Considering the scarcity of water resources, water must be used economically (Fereres and Soriano, 2007). The management of water resources is one of the most important problems to be solved in the 21st century (Kuscu, 2010). In particular, using modern technologies in irrigation water management is the most important parameter to be considered for the achievement of maximal plant production (Panda et al., 2003). Deficit irrigation is one of the most useful methods applied in this context. The purpose of deficit irrigation is to increase plant production while using less water. For this reason, the development of deficit irrigation programs is important (Igbadun et al., 2008). In general, water can be saved by expanding deficit irrigation programs and determining the deficit irrigation program suitable for each plant (Oktem, 2008; Kuscu, 2010).

It is crucially important to increase food production sufficiently to feed the growing population of the world and manage limited water resources for agricultural use worldwide (Asrey et al., 2018). According to Chai et al. (2014), although deficit irrigation is one of the solutions for water saving in agriculture, it may be insufficient alone for food production. In addition to deficit irrigation, alternative plants that have similar characteristics to each other need to be grown. Animal feed, which is an important input in the livestock sector, is provided from plants such as silage maize, silage sorghum, sudangrass, alfalfa, vetch, and sainfoin in Turkey. Maize is a widely used plant in silage making, followed by sorghum-sudangrass hybrids and other sorghum species (Geren and Kavut, 2009). Silage maize is grown in irrigation conditions because it has high levels of seasonal crop water consumption (Mustek and Dusek, 1980). This situation causes a problem for fodder supply. Sorghum species have great potential in terms of proving an alternative to maize both in arid and irrigated agricultural areas (Arslan, 2016).

Chlorophyll content can be used in the evaluation of plant water stress and cold tolerance, as well as the detection of ozone damage (Rose and Haase, 2002; Perks et al., 2004; Demirel et al., 2010). Carol et al. (2017) determined the crop water stress index of maize by implementing complete and deficit irrigation methods in Utah conditions. While 700 mm of water was sufficient for irrigation, they used 480 mm water in their deficit irrigation program. They found the chlorophyll content of the well-irrigated treatment as 36.2, while that of the deficit irrigated treatment was 34. In the study conducted by Yamamoto et al. (2002), chlorophyll contents varied between 15 and 60 during the 54-day plant growing season after plantation. Jangpromma et al. (2010) cultivated sorghum varieties in arid conditions (no irrigation) in Thailand and evaluated their chlorophyll contents. Accordingly, the chlorophyll content values of the varieties differed between 21.58 and 39.55. Cetin (2017) emphasized that there are multiple factors that affect the chlorophyll content of a plant; therefore, it is necessary to increase the number of studies about chlorophyll contents.

The diameter of the stem of a plant has the highest impact on its yield. Grain and silage plant height, stem diameter, and number of leaves are the features to be considered when choosing maize varieties (Torun, 1999). El-Samnoudi et al. (2019) applied different irrigation treatments to the sorghum plant grown in Egyptian conditions. They named the treatment where all of the water that evaporated from the evaporation vessel was met I100, the treatment where 85% of it was met I85, and the treatment where 70% of it was met I70. They reported that plant height and stem diameter values decreased from the I100 treatment to I70 treatment groups. They reported that the plant height values varied between 148.8 cm and 132.65 cm, and the stem diameter values varied between 2.01 cm and 1.7 cm. Keskin et al. (2018) evaluated the quality characteristics of sorghum cultivars under irrigated conditions. They reported that plant height values were between 197.1 cm and 299.4 cm, and numbers of leaves varied between 9.5 and 12.5. Uzun et al. (2017) investigated the responses of silage maize and sorghum in wet and dry conditions (in natural precipitation conditions) in Turkey. They used 2 Maize (Rx-893, Karadeniz Yıldızı) and 7 sorghum (Jumbo, Grazer, Hayday, El Rey, Gozde, Rox E., Suma) varieties. The authors reported that the varieties grown under irrigated conditions had higher feeding quality and yield compared to those grown under rainfed conditions. While the heights of the maize varieties grown in irrigated conditions were in the range of 191.2-197.3 cm, those of sorghum varieties ranged between 330.7 cm and 189 cm.

In animal nutrition, especially in ruminant rations, acid detergent fiber (ADF) has started to be used as an energy indicator. ADF, which is included in the structural carbohydrates of plants, consists of cellulose and lignin (Tekce and Gul; 2014). Neutral detergent fiber (NDF) is effective on carbohydrates that make up a large part of ruminant rations, the milk fat of ruminants, milk components, the acetic acid/propionic acid ratio in the rumen, dry matter consumption, microflora, and microfauna in the rumen (Ferreira and Mertens, 2007; Hansey et al., 2007). An increase in the amount of irrigation water applied to silage maize (0-480 mm) increased the dry matter yield from 9.3 to 23.8 t/ha and NDF values from 524 to 555 g/kg, but crude protein decreased from 78 to 52 g/kg, and water-soluble carbohydrates decreased from 88 to 31 g/kg (Islam et al., 2012). Sorghum protein content was reported as 10.14% in irrigated conditions and 14.86% in dry conditions in Kansas. Dry conditions increased the protein content of sorghum (Liu et al., 2013). Sorghum is the world's fifth most important cereal crop. It is a drought-tolerant plant. It has a higher protein content compared to maize, but its content of digestible protein is lower (Dowling et al., 2002).

Although there are previous studies on silage maize and sorghum, no studies were found to compare chlorophyll contents based on irrigation schedules. The number of articles where certain physiological and quality parameters were compared under an irrigation program is low. The aim of this study is to measure and compare the physiological and quality characteristics of silage maize and sorghum under a deficit irrigation program. Because the chlorophyll contents of these two plants were not compared before, this study will fill a gap in the relevant literature. The comparison of physiological and quality characteristics based on irrigation schedules will also help other studies conducted on this subject. The results of this study will be beneficial for many plant

producers and researchers.

MATERIALS AND METHODS

Site, Soil, Climate, and Agricultural Operations

This study was carried out on the soils of Kahramanmaraş Eastern Mediterranean Transition Zone Agricultural Research Institute and Kahramanmaraş Sütçü İmam University Laboratories. It was conducted in the second crop growing seasons in 2018 and 2019. In the growing periods of the plants (June-September), the long-term average lowest temperature was measured in September at 18.3°C, and the highest temperature was in August at 36.0°C. In the years when this study was conducted, the lowest temperature was measured at 9.0°C in September 2019, and the highest temperature was measured at 43.4°C in June 2019. The average temperature values during the growing period of the plants varied between 24.9°C and 29.3°C in the first year and between 26.0°C and 29.3°C in the second year.

The physical properties of the soils are shown in Table 1. Soil pH values were slightly alkaline and would not cause a problem in terms of agricultural production. It was found that the electrical conductivity values of the soil were not at a level that would cause a salinity-related issue. The amount of organic matter was found to be low for both years. The lime-related parameters showed the soil to be "highly calcareous" in both years of the study. The concentration of phosphate was found to be "low" in both years. While the potassium values of the examined soil in 2018 were found to be "sufficient", they were found to be "excessive" in 2019 (Table 2).

In the study, while the silage maize (Zea mays L.) plant was selected as the Colonia variety, which is a variety that can be used as a second crop and is adapted to the region, the Es Foehn cultivar was used as silage sorghum (Sorghum bicolor). Silage sorghum and silage maize plants were planted in the third week of July for both years. The horizontal and vertical distances between the rows were 70 cm and 15 cm, respectively. The study was planned with a randomized complete block factorial design with three replications. After the soil was plowed, 8 kg of fertilizer with nitrogen and phosphor contents was applied to the soil before planting. As the plants started to develop, nitrogen-containing fertilizer was applied to the soil at a rate of 10 kg per decare. The nitrogen-containing fertilizer was applied via fertigation. The length and width of each plot were 8 m and 3.5 m respectively. The total working area was 1590 m^2, and the distance between the plots was 2 meters while the distance between the blocks was 3 meters. To determine the irrigation time and amount, soil moisture was measured by the gravimetric method. The moisture content of the soil samples taken from a depth of 90 cm was measured according to the dry weight percentage calculation method. Irrigation was started when the usable water holding capacity of the soil was consumed by 50% (Dagdelen and Gurbuz, 2008). Considering these values, it was seen that the irrigation interval changed between 5 and 7 days. Soil moisture values were taken from all irrigation treatments, but irrigation was made only based on the soil moisture statuses of the M100-S100 treatments. Irrigation was started around the morning hours. The M100-S100 treatment involved meeting the entire water requirement of the plant (control treatment), while the treatment named M80-S80 corresponded to a 20% reduction in the water applied to the plant compared to the control treatment, M60-S60 corresponded to a 40% reduction, M40-S40 corresponded to a 60% reduction, and M20-S20 corresponded to an 80% reduction. The irrigation program was started on 8 July in 2018 and on 23 July in 2019. Irrigation was completed 10 days before harvesting. The irrigation program was applied using a drip irrigation system.

Table 1. Physical properties of the soil in the study area

Years	Depth	Field Capacity		Wilting point		Bulk Density	Soil Texture
		Pw (%)	mm	Pw (%)	mm	g cm^{-3}	
2018	0-30	29.35	110.06	19.80	74.25	1.25	CL
	30-60	28.25	106.79	19.60	74.08	1.26	CL
	60-90	19.65	71.33	13.95	50.63	1.21	SCL
2019	0-30	21.45	105.53	12.19	59.97	1.64	SiL
	30-60	23.35	107.88	14.28	65.97	1.54	SiL
	60-90	23.54	114.40	13.03	63.32	1.62	SL

Table 2. Chemical properties of the soil in the study area

Years	Soil depth (cm)	pH	EC (dS/m)	Organic matter (%)	P_2O_5 (kg da^{-1})	K_2O (kg da^{-1})	Lime (%)
2018	0-30	7.92	0.018	1.51	5.22	59.18	21.22
2019	0-30	7.80	0.023	1.03	3.66	62.00	19.85

Chlorophyll Content, Physiological, and Quality Measurements

Chlorophyll content

During plant development, measurements were taken before and after each irrigation step with a SPAD-502 chlorophyll meter (Minolta Co, Tokyo, Japan) device from 5 leaves of all treatment groups. The SPAD 502 chlorophyll meter is a small, handy device that measures light transmittance at red (650 nm, chlorophyll absorption) and near-infrared (960 nm) wavelengths, thus making it possible to take measurements without harming the plant (Minolta 1989, Ling et al., 2011). While measuring chlorophyll content, the device was held in such a way that it would not cast a shadow on the leaf, and readings were taken from 5 plants in each plot. The measurements were made from the area between the leaf edges and the leaf veins of the plants between 11:00 and 14:00. These measurements were made 1 day before and 1 day after irrigation.

Physiological measurements

Plant height (cm), stem diameter (mm), and numbers of leaves were measured by using an average of 10 plants from each plot at irrigation times throughout the growing season. Plant height was measured with a steel measuring tape from the ground level to the uppermost point of the plant. Stem diameter was measured by using an electronic caliper. The number of leaves was determined by counting the leaves on the stem.

Quality parameters

To measure ADF and NDF after the harvest, the dried samples were ground in a mill with a sieve allowing particles to pass at a diameter of 1 mm. Samples weighing approximately 0.5 g were placed in filter bags, and each bag was closed with glue and then weighed afterward. The empty weight (blind) of the filter bags was also measured. A mixture to be used for ADF analyses and a mixture to be used for NDF analyses were prepared. The prepared samples were placed in an ANKOM 200 Fiber Analyzer. The prepared ADF mixture (NDF with similar preparation steps) was poured on the samples placed in the device, and the device was operated. The samples were boiled at 100°C for approximately 90 minutes. When the 90-minute boiling period was over, the samples were mixed with hot water twice for 10 minutes. After the samples were removed from the device, they were kept in acetone. Afterward, the samples were kept in a fume hood to allow the acetone to evaporate. The samples, with acetone evaporated, were dried in an oven at 80°C until they reached a constant weight. After they were removed from the oven, they were put into a desiccator to bring them to room temperature. The samples at the room temperature were weighed and measured according to Equation 1 (Van Soest, 1963).

$$\%ADF, \%NDF = \frac{[W3-(W1xC1)x100]}{W2} \quad \text{Eq. 1}$$

In the equation,

W1: Tare of bags

W2: Sample weight

W3: Weight of "sample + bag" after drying

C1: Blind weight (weight/tare of the empty bag after drying)

To measure protein contents, after the harvest, the dried samples were ground in a mill with a sieve allowing particles to pass at a diameter of 1 mm. 0.2 g of the ground samples was taken and put into Kjeldahl tubes. The empty weight (blind) of the filter bags was also measured. 25 ml of sulfuric acid and a catalyst (potassium sulfate) tablet were added to the samples. Afterward, the tubes were kept at a high temperature for 5 hours in the wet burning unit. 25 ml of boric acid was prepared. After wet burning, the tubes and boric acid were placed in the Gerhart brand distillation unit. After the desired color change (green-blue) was observed in the flask containing boric acid, it was titrated with hydrochloric acid. The amount of hydrochloric acid that was consumed was recorded when the color turned pink. The amount of crude protein (AOAC, 1990) was determined using the Kjeldahl method according to Equation 2.

$$\%Nitrogen = \frac{(V1-V0)xNx0.014}{m} x100 \quad \text{Eq. 2}$$

In equality,

V1 = Amount of HCl solution spent in titration, ml

V0 = Amount of HCl solution spent in titration for the blank sample, ml

N = Normality of the HCl solution used in titration, 0.1 N

0.014 = Mil-equivalent weight of nitrogen; m: Amount of food sample taken, g or ml

Protein= %Nitrogen x 6.25

Statistical Analysis

The data that were collected in the study were analyzed according to the factorial experimental design in random blocks. An analysis of variance (ANOVA) was conducted to determine the levels of differences between the groups of data. The investigated plant height, stem diameter, number of leaves, ADF, NDF, and protein content were analyzed using a standard ANOVA test using the general linear model (SAS Institute, 1996). The significance of the differences was tested by the "F" test (Gomez and Gomez 1984). When differences were found in the ANOVA,

Duncan's Test (grouping) was applied to determine the source of the significant differences.

RESULTS AND DISCUSSION

Chlorophyll Content

Seven irrigation steps were applied to the plants in 2018, and 8 were applied in 2019. Figure 1 shows the chlorophyll contents of the silage maize and sorghum before and after irrigation in 2018 and 2019. In 2018, the chlorophyll content of the silage maize increased in all treatments from the vegetative period to the milk stage, and then, it decreased after the milk stage. While the highest chlorophyll content was 56.93 in M100 in the 4th irrigation, the lowest was 39.73 in M20 in the 7th irrigation. Other measurements varied between these values. No significant difference was found between before and after irrigation in terms of chlorophyll content. It was shown that irrigation did not change the chlorophyll content in the medium term. Thus, it was also understood that irrigation did not change the chlorophyll content in the short term. Chlorophyll content tended to be high in the non-water-stressed treatments while it decreased in the water-stressed treatments. The chlorophyll content of maize increased in the M100 and M80 treatments until harvesting, while it decreased from the milk stage to harvesting in the M60, M40, and M20 treatments and reached the lowest value at harvest in 2019. While the highest chlorophyll content value was 47.53 for M60 in the 1st irrigation, the lowest was 26.70 for M20 in the 8th irrigation. In the evaluation of both years together, it was seen that the progression of measurement times did not affect the chlorophyll contents significantly in a short time (1 to 2 days). The chlorophyll content of maize increased in all treatments from the vegetative period to the harvest in 2018.

The highest chlorophyll content of the silage sorghum was 57.57 for S100 in the 7th irrigation, and the lowest was 37.23 for S100 in the 1st irrigation in 2018. The S100 treatment had a higher chlorophyll content than the other treatments. Other measurements varied between these values. It was determined that irrigation did not significantly change the chlorophyll contents of the sorghum plants in the short term, as in the case of maize. The chlorophyll contents showed a high trend in the treatments that were not under water stress and decreased in the treatments which were subjected to water stress. The content of chlorophyll in sorghum increased until the 4th irrigation, and then, it decreased from this point to the harvest and reached lowest point at harvest in 2019. While the highest chlorophyll content was 40.23 for S100 in the 4th irrigation, the lowest was 29.93 for S20 in the 8th irrigation. The S100 treatment had a higher chlorophyll content than the other treatments. The values of the other treatments varied between these values in 2019. Considering the results in both years, no significant differences were observed between the chlorophyll contents of silage maize and silage sorghum. Both plants showed similar trends as a response to water stress.

Many researchers have reported that chlorophyll contents decrease along with increasing stress (Demirtas and Kırnak, 2009; Pouyafard et al., 2016). Moreover, Yolcu (2014) reported that as irrigated treatments have more soil moisture, the nitrogen in the soil is transmitted to the leave, and it has an increasing effect on the chlorophyll content there. Kabay and Şensoy (2016) observed that when plants were negatively affected by any adverse environmental condition, there was a decrease in the chlorophyll contents, yield, and quality of these plants. Several researchers have found different results regarding the chlorophyll contents of silage maize and sorghum. The chlorophyll content of maize has been found in the range of 36.44 to 70.78 by other researchers (Hokmalipour and Darbandi 2011; Tunali 2012; Kappes et al., 2013; Kappes et al., 2014; Yolcu 2014; Carol et al., 2017; Galindo et al., 2019). The chlorophyll content of sorghum has been reported in the range of 40 to 52.54 (Kassahun et al., 2010; Vinodhana and Ganesamurthy 2010; Kaplan and Kara 2014; Mahama 2014; Abunyewa et al., 2016; Kumari et al., 2016; Sory et al. 2017; Kiran et al. 2018).

Physiological Characteristics

The average plant height was taken in the harvest period. The results of the ANOVA on the physiological characteristics of the silage maize and sorghum plants are shown in Table 3. There was no significant difference between the maize and sorghum plants in 2018, in 2019, and in terms of the average of the two years (Table 4). No significant difference was found in the plant heights of maize and sorghum between the values of the two years. According to the average of two years, the highest plant height was obtained from the 100% irrigated treatments. The lowest plant height was obtained from the 40% and 20% irrigated treatments. Deficit irrigation reduced plant height values in both plants. Ashraf et al. (2016) found plant height values of 157 to 203.7 cm in maize, while Galindo et al. (2019) found values of 215 cm to 255 cm. El-Samnoudi et al. (2019) found plant height values to be 132.65 cm to 148.8 cm in sorghum, while Kaplan et al. (2019) found plant height values of 203 cm to 255.35 cm.

The results of the ANOVA on the stem diameters of the silage maize and sorghum plants are shown in Table 5, and the results of the Duncan's test groups formed according to the ANOVA results are given in Table 6. While the mean stem diameter of maize was 22.34 mm, that of sorghum was 20.48 mm in 2018. No significant difference was found between the stem diameters of maize and sorghum in 2019. The mean stem diameter of maize was 21.62 mm, and that of sorghum was 19.87 mm as the average of two years (Table 4). It is known that having a large stem diameter is an important factor for the achievement of high yield. Cruz et al. (2008) reported

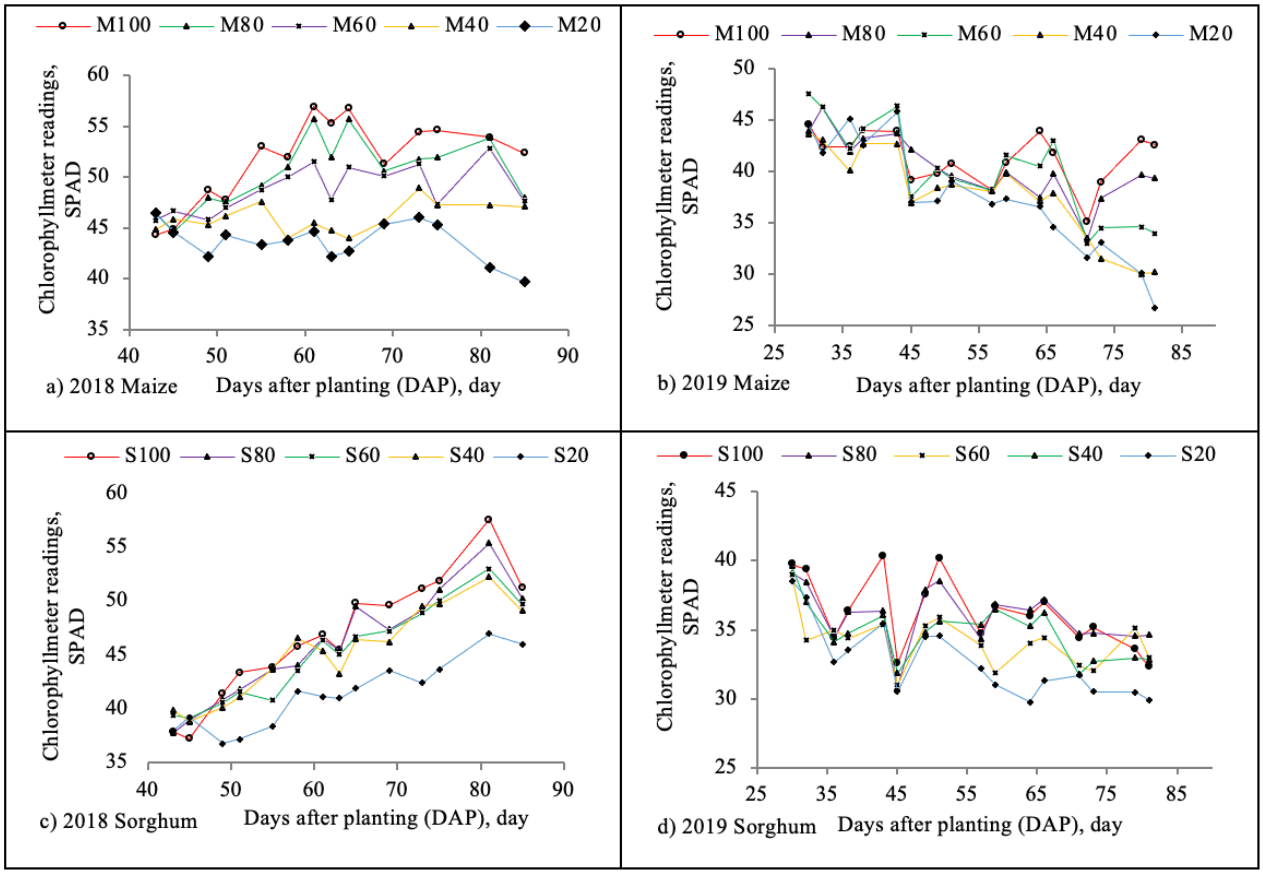

Figure 1. Plots of chlorophyll content values in silage maize and silage sorghum

Table 3. ANOVA results of height values of silage maize and sorghum at irrigation levels

Source of variation	Degrees of freedom	Mean square	F
Year	1	844.35	4.86*
Species	1	394.95	2.27
Irrigation levels	4	3024.58	17.42**
Year*Species	1	293.04	1.69
Year*Species	4	26.04	0.15
Species*Irrigation levels	4	587.82	3.39*
Year*Species*Irrigation levels	4	57.31	0.33
Error	36	173.64	

Table 4. Plant heights of silage maize and silage sorghum

Irrigation levels	2018			2019			Average of 2 years		
	Maize	Sorghum	Avg	Maize	Sorghum	Avg	Maize	Sorghum	Avg
100%	218.37	240.05	229.21a	222.67	246.33	234.49a	220.52	243.19	231.85a**
80%	212.94	228.58	220.76a	217.89	231.44	224.66a	215.41	230.01	222.71ab
60%	209.68	219.33	214.50a	222.44	222.22	222.33ab	216.06	220.77	218.41b
40%	194.16	199.37	196.76b	214.67	201.22	207.94bc	204.41	200.29	202.35c
20%	190.13	185.71	187.91b	207.22	187.22	197.22c	198.67	186.46	192.57c
Avg	205.05a	214.60a	209.83b	216.97a	217.68a*	217.33a	211.01a	216.14a	

that the larger the stem diameter, the greater the plant's capacity to store photo-assimilates that contribute to grain filling. In this case, it was understood that more yield could be obtained from the maize plant compared to the sorghum plant. While the highest stem diameters were 22.74 mm and 22.24 mm in the 100% and 80%

irrigated treatments, respectively, the lowest stem diameter was 19.61 mm in the 20% irrigated treatments in 2018. No significant difference was found among the irrigation treatments in 2019. According to these results, it was understood that in the 100% irrigated treatments, the stem diameters values were the greatest. Deficit irrigation caused a decrease in the stem diameters of the plants. The stem diameters of sorghum were previously found to be in the range of 16.07-20.1 mm by some researchers (Snider 2012; El-Samnoudi et al., 2019).

The results of the ANOVA on the number of leaves of the silage maize and sorghum plants are shown in Table 7, and the results of the Duncan's test groups formed according to the ANOVA results are given in Table 8. The maize plants had greater numbers of leaves compared to the sorghum plants. There was no significant difference in the numbers leaves between the maize and sorghum plants in 2018. The number of maize leaves was found to be 12.33/plant, while the number of sorghum leaves was found to be 11.71/plant in 2019. Orak and İptaş (1999) and Sade et al. (2002) stated that the number, weight, and ratio of leaves are important parameters for silage plants. Vartanlı and Emeklier (2007) reported that photo-assimilation increased along with an increase in the number of leaves. Yield has a positive association with photo-assimilation. According to these results, it was understood that the 100% irrigated treatments had the highest numbers of leaves, and deficit irrigation caused a decrease in the number of leaves.

Quality Features

The results of the ANOVA on the ADF values of the silage maize and sorghum plants are shown in Table 9, and the results of the Duncan's test groups formed according to the ANOVA results are given in Table 10. The ADF value of maize was 24.49%, and the ADF value of sorghum was 27.13% in 2018. ADF is expected to be low for the easier digestion of feed (Van Soest 1994; Yavuz 2005). Therefore, it was thought that maize feed could be digested more easily than sorghum feed. Maize and sorghum were found in the same group in terms of their ADF values in 2019 and based on the average of two years. While the ADF of maize was found as 25.72%, that of sorghum was 25.17% in 2019. In the average values of both years, the ADF of maize was 25.17%, while it was 26.15% in sorghum.

The was no significant difference among the irrigation treatments in 2018, in 2019, and considering the two-year average values. ADF values varied between 24.52% and 26.85% in 2018, while they varied between 24.40 and 26.27% in 2019. As the average of both years, these values varied between 24.46% and 26.35%. When the values were examined in total, the lowest ADF values were found in the 100% irrigation treatment. It was understood that the 100% irrigated treatments were more easily digestible. Seif et al. (2016) reported that ADF values increased in maize under low irrigation conditions and found maize ADF values of 22.1% to 29.5%. Teixeira (2014) reported sorghum ADF values of 21.98%-23.64%. The results of this study coincided with the results reported by the aforementioned researchers.

The results of the ANOVA on the NDF values of the silage maize and sorghum plants are shown in Table 11, and the results of the Duncan's test groups formed according to the ANOVA results are given in Table 12. In 2018, the NDF values of maize and sorghum were 50.03% and 53.68%, respectively. In 2018, these values for maize and sorghum were respectively 52.46% and 49.77%. A low NDF value is desired in animal nutrition since the structures that make up NDF cannot be digested by intestinal enzymes (Saki et al., 2010). In other words, feeds with high NDF values slow down the digestion in animals and cause a feeling of satiety. Therefore, NDF reduces the amount of feed consumed by the animal (Van Soest, 1994; Yavuz 2005). Maize and sorghum were in the same group in terms of their average NDF values of the two years. Nocek and Russell (1988) reported that NDF must be between 32.3% and 68.3% for silage maize to be suitable for animal feeding. In this study, NDF values were found higher in the deficit irrigation treatments. This situation showed that deficit irrigation reduces the digestibility of feed. Likewise, Seif et al. (2016) reported that NDF increased in maize under water stress conditions.

The results of the ANOVA on the protein content values of the silage maize and sorghum plants are shown in

Table 5. ANOVA results of stem diameters of silage maize and sorghum at irrigation levels

Source of variation	Degrees of freedom	Mean square	F
Year	1	26.77	2.55
Species	1	45.72	4.36*
Irrigation levels	4	25.82	2.46*
Year*Species	1	0.18	0.02*
Year*Species	4	0.69	0.07
Species*Irrigation levels	4	0.25	0.02
Year*Species*Irrigation levels	4	2.28	0.22
Error	36	10.47	

*, **: Significant at p<0.05 and p<0.01 levels, respectively

Table 6. Stem diameter of silage maize and silage sorghum

Irrigation levels	2018			2019			Average of 2 years		
	Maize	Sorghum	Avg	Maize	Sorghum	Avg	Maize	Sorghum	Avg
100%	22.98	22.51	22.74^{a}	23.18	21.02	22.10^{a}	23.08	21.77	22.42^{a}
80%	22.97	21.52	22.24^{a}	22.47	20.00	21.23^{a}	22.72	20.76	21.73^{a}
60%	22.53	21.11	21.82^{ab}	21.06	19.34	20.19^{a}	21.79	20.22	21.00^{ab}
40%	22.13	19.18	20.65^{bc}	19.39	18.41	18.89^{a}	20.76	18.79	19.77^{ab}
20%	21.11	18.12	19.61^{c}	18.40	17.55	17.97^{a}	19.76	17.83	18.79^{b}
Avg	22.34^{a}	20.48^{b}	21.41^{a}	20.89^{a}	19.26^{a}	20.08^{a}	21.62^{a}	19.87^{b}	

Table 7. ANOVA results of numbers of leaves of silage maize and sorghum at irrigation levels

Source of variation	Degrees of freedom	Mean square	F
Year	1	1.35	2.40
Species	1	2.54	4.52*
Irrigation levels	4	9.71	17.27**
Year*Species	1	0.66	1.18
Year*Species	4	1.36	2.42
Species*Irrigation levels	4	0.32	0.58
Year*Species*Irrigation levels	4	0.33	0.59
Error	36	0.56	

*, **: Significant at p<0.05 and p<0.01 levels, respectively

Table 8. Numbers of leaves in silage maize and silage sorghum

Irrigation levels	2018			2019			Average of 2 years		
	Maize	Sorghum	Avg	Maize	Sorghum	Avg	Maize	Sorghum	Avg
100%	13.78	13.44	13.61^{a}	13.44	12.22	12.83^{a}	13.61	12.83	13.22^{a}
80%	13.22	13.22	13.22^{a}	12.78	11.89	12.33^{ab}	13.00	12.56	12.77^{ab}
60%	12.33	12.89	12.61^{a}	12.11	11.78	11.94^{bc}	12.22	12.33	12.27^{b}
40%	11.78	11.22	11.50^{b}	12.00	11.44	11.72^{bc}	11.89	11.33	11.61^{c}
20%	11.00	10.33	10.66^{b}	11.33	11.22	11.27^{c}	11.17	10.78	10.97^{d}
Avg	12.42^{a}	12.22^{a}	12.32^{a}	12.33^{a}	11.71^{b}	12.02^{a}	12.37^{a}	11.96^{b}	

Table 9. ANOVA results of ADF values of silage maize and sorghum at irrigation levels

Source of variation	Degrees of freedom	Mean square	F
Year	1	1.98	0.29
Species	1	16.44	2.40
Irrigation levels	4	6.87	1.00
Year*Species	1	38.35	5.60*
Year*Species	4	1.49	0.22
Species*Irrigation levels	4	6.60	0.96
Year*Species*Irrigation levels	4	1.98	0.31
Error	36	6.85	

*, **: Significant at p<0.05 and p<0.01 levels, respectively

Table 10. ADF levels of silage maize and silage sorghum

Irrigation levels	2018			2019			Average of 2 years		
	Maize	Sorghum	Avg	Maize	Sorghum	Avg	Maize	Sorghum	Avg
100%	22.87	26.18	24.52^{a}	23.62	25.18	24.40^{a}	23.25	25.68	24.46^{a}
80%	23.44	28.12	25.77^{a}	26.13	26.42	26.27^{a}	24.78	27.27	26.02^{a}
60%	24.98	27.07	26.02^{a}	27.48	24.56	26.01^{a}	26.23	25.81	26.02^{a}
40%	24.32	27.47	25.89^{a}	25.01	24.43	24.72^{a}	24.67	25.95	25.30^{a}
20%	26.86	26.86	26.85^{a}	26.41	25.29	25.85^{a}	26.63	26.08	26.35^{a}
Avg	24.49^{b}	27.13^{a}	25.81^{a}	25.72^{a}	25.17^{a}	25.45^{a}	25.11^{a}	26.15^{a}	

Table 11. ANOVA results of NDF values of silage maize and sorghum at irrigation levels

Source of variation	Degrees of freedom	Mean square	F
Year	1	8.32	0.56
Species	1	3.51	0.23
Irrigation levels	4	17.85	1.19
Year*Species	1	150.63	10.05**
Year*Species	4	10.06	0.67
Species*Irrigation levels	4	9.41	0.63
Year*Species*Irrigation levels	4	2.23	0.15
Error	36	14.98	

*, **: Significant at p<0.05 and p<0.01 levels, respectively

Table 12. NDF levels of silage maize and silage sorghum

Irrigation levels	2018			2019			Average of 2 years		
	Maize	Sorghum	Avg	Maize	Sorghum	Avg	Maize	Sorghum	Avg
100%	46.81	51.85	49.32^{a}	50.50	49.73	50.11^{a}	48.65	50.79	49.72^{a}
80%	49.08	54.38	51.73^{a}	52.59	51.51	52.05^{a}	50.84	52.94	51.89^{a}
60%	49.74	53.84	51.78^{a}	54.39	49.49	51.94^{a}	52.06	51.66	51.86^{a}
40%	50.24	52.97	51.60^{a}	51.10	49.61	50.35^{a}	50.67	51.29	50.98^{a}
20%	54.31	55.41	54.85^{a}	53.72	48.54	51.13^{a}	54.01	51.97	52.99^{a}
Avg	50.03^{b}	53.68^{a}	51.86^{a}	52.46^{a}	49.77^{b}	51.11^{a}	51.24^{a}	51.73^{a}	

Table 13. ANOVA results of protein contents of silage maize and sorghum at irrigation levels

Source of variation	Degrees of freedom	Mean square	F
Year	1	1.43	2.38
Species	1	0.71	1.18
Irrigation levels	4	4.76	7.90**
Year*Species	1	2.87	4.77*
Year*Species	4	0.20	0.34
Species*Irrigation levels	4	0.63	1.05
Year*Species*Irrigation levels	4	0.76	1.26
Error	36	0.60	

*, **: Significant at p<0.05 and p<0.01 levels, respectively

Table 14. Protein contents of silage maize and silage sorghum

Irrigation levels	2018			2019			Average of 2 years		
	Maize	Sorghum	Avg	Maize	Sorghum	Avg	Maize	Sorghum	Avg
100%	8.19	9.78	8.98^{a}	9.95	9.34	9.46^{a}	9.07	9.56	9.31^{a}
80%	8.35	8.81	8.57^{ab}	8.97	8.10	8.53^{b}	8.66	8.45	8.55^{b}
60%	8.10	8.11	8.10^{ab}	8.82	8.25	8.53^{b}	8.46	8.18	8.32^{bc}
40%	8.10	7.53	7.81^{bc}	8.02	8.00	8.00^{b}	8.06	7.76	7.91^{bc}
20%	7.75	7.36	7.55^{c}	8.46	7.25	7.85^{b}	8.11	7.30	7.70^{c}
Avg	8.09^{a}	8.31^{a}	8.51^{a}	8.84^{a}	8.18^{b}	8.20^{a}	8.47^{a}	8.25^{a}	

Table 13, and the results of the Duncan's test groups formed according to the ANOVA results are given in Table 14. While the protein content of maize was 8.09%, that of sorghum was 8.31% in 2018. The highest protein content was 8.84% for maize, while it was 8.18% for sorghum in 2019. Maize and sorghum were in the same group in terms of their protein contents considering the average of the two years. Regarding the average values of the two years, while the protein content of maize was 8.47%, that of sorghum was 8.25%. Mison (1990) reported that the minimum protein content should be 7% for the maintenance of the microbes in the rumen of animals. According to these results, it was understood that the protein contents of the plants examined in this study were at a suitable level. While the highest protein content was 9.31% in the 100% irrigated treatment, the

lowest protein content was 7.70% in the 20% irrigated treatment based on the average values of the two years. According to these results, it was determined that water stress caused a decrease in protein contents. Simsek et al. (2011) concluded that increasing the amount of irrigation increased the protein content of the plant. Mahama and Doka (2019) found high protein contents in their full irrigation treatment. Many researchers have found different results regarding the protein contents of maize and sorghum. Kizilsimsek et al. (2016) reported protein contents of maize as 6.37% to 8.48%. Singh (2018) showed the protein content of sorghum to vary from 3.68% to 6.71%. Hajibabaei and Azizi (2012) emphasized that protein content is affected by many interactions like environmental, agricultural, and genetic factors. Additionally, these factors reduce the protein content of maize in cases of drought and soil moisture deficiency.

CONCLUSION

The chlorophyll contents of the plants did not show a significant decrease or increase from before to after irrigation since the measurement intervals of chlorophyll content were short. This showed that irrigation did not change the chlorophyll content of plants in a short time. It was observed that the highest values were in the 100% irrigation treatments for both plants during the plant growing period. The chlorophyll contents decreased in the period from vegetative development to the harvest, especially in the treatments that were irrigated at a rate of 60% or lower. No significant difference was found in plant height values between maize and sorghum. The stem diameter of the maize plant was higher than that of the sorghum plant. Since stem diameter is a feature related to yield, it may be more economical to grow maize at an irrigation rate of 60%. As the water stress increased, the number of leaves tended to decrease. In this study, it was observed that physiological parameters (plant height, stem diameter, and number of leaves) regressed relatively at water stress levels over 60%.

Many interactions such as variety, environment, and agricultural factors affect silage feed quality (ADF, NDF, protein content). Low ADF, low NDF, and high protein content are decisive criteria for feed quality. According to the average of two years, the irrigation treatments did not change the ADF values of maize and sorghum, and ADF values were not significantly different between maize and sorghum. A similar situation was observed for NDF. There was no significant difference in protein content between maize and sorghum. An increase of 20% or more in the deficit of irrigation caused a decrease in protein content. When the silage maize and sorghum were evaluated in terms of feed quality, there was no significant difference between them. In this case, it was seen that sorghum can be an alternative to maize in terms of nutritional quality. Similarly, there was not much difference between the physiological characteristics of the plants. Consequently, silage sorghum can be grown as an alternative to maize for silage feed. If irrigation resources are limited, sorghum is more resistant to adverse environmental conditions than maize. Therefore, sorghum can be grown and used as fodder in arid and semiarid regions. If sorghum cultivation is made prevalent in these regions, one of the greatest problems in animal husbandry can be resolved.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Declaration of interests

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

Mualla KETEN GOKKUS: Conceptualization; data curation; investigation; methodology; formal analysis; visualization; writing –original draft; writing review and editing.

Hasan DEGIRMENCI: Data curation; Supervision; writing – original draft; writing review and editing.

Ethics Committee Approval

Ethics committee approval is not required. This article does not contain any studies with human participants or animals performed by any of the authors.

Funding

This study was supported by Kahramanmaras Sütçü İmam University Scientific Research Projects Unit. in the scope of the project numbered 2017/6-33 M.

Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

Authors are thankful to Kahramanmaras Sütçü İmam University Scientific Research Projects Unit for their financial supports. This study was produced from Mualla Keten Gokkus's Doctoral Thesis.

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RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(3): 563-572
DOI: https://doi.org/10.31015/jaefs.2023.3.10

Hazard and risk assessment in a dairy products factory in Iğdır province using the Fine Kinney Risk Method: recommendations for mitigation

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Abstract

Failure to implement adequate preventive measures in workplaces leads to the occurrence of occupational diseases and accidents at work. Effectively managing and accurately defining these risks is paramount. Risk assessment begins by assigning scores to identify existing workplace hazards, assess the likelihood of potential risks, determine the level of urgency, and determine the necessary precautions. It is a continuous cycle of implementation, monitoring and review. The aim of this study is the identification of existing or potential hazards and risks in a dairy products factory in Iğdır province, Turkey. The Fine Kinney risk assessment methodology was applied, and the results provided recommendations for mitigating identified risks based on relevant regulations. Initially, brainstorming sessions were held with relevant employees and managers to gain a general overview of the health and safety culture in the work environment. In addition, observational analysis and weekly checklists were used for assessment purposes. When examining the current status and operational procedures of the company in detail, various hazards containing risks that require immediate precautions have been identified. Electric shock (RS: 1440), injuries related to electricity (RS: 720), explosion (RS: 540), and risks associated with poisoning or drowning (RS: 720) have been determined to be present. It has been concluded that most of the environmental risks in the facility arise from factors such as defective or outdated equipment, lack of ergonomic workstations, inadequate training, and insufficient supervision.

Keywords: Food industry, Risk assessment, Fine Kinney, Occupational health and safety

Citation: Ozbakir, O. (2023). Hazard and risk assessment in a dairy products factory in Iğdır province using the Fine Kinney Risk Method: recommendations for mitigation. International Journal of Agriculture, Environment and Food Sciences, 7 (3), 563-572

Received: July 24, 2023
Revised: August 15, 2023
Accepted: August 19, 2023
Published Online: September 20, 2023

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Available online at
https://jaefs.com/
https://dergipark.org.tr/jaefs

INTRODUCTION

The food industry is one of the largest industries in the world in terms of commercial volume. In this sector, food is purchased in raw form, processed and packaged before being placed on the market for human consumption. This process involves many critical steps and serves the basic need for nourishment. Factors such as population growth, industrialization and changing living conditions have rapidly increased the demand for and consumption of processed foods. The food industry covers a wide range of subsectors, each having distinct characteristics and features (Taş & Olum, 2020).

According to the statistics published annually by the Social Security Institute, the "food production" sector ranks among the top ten in terms of the number of workplaces, the number of employees and the number of occupational accidents (Can & Kargı, 2019). These data indicate that the sector faces significant occupational safety challenges and has a high incidence of work-related accidents. Food manufacturing jobs are often manual and require manual

dexterity, attention, and care (Tuğçe & Bayhun, 2021). Additionally, the chemicals and machinery used in this sector can pose risks (Pawlak, et al., 2014). Protecting workers from these hazards through proper equipment and compliance with occupational safety regulations is critical to preventing work-related injuries and illnesses.

The number of accidents at work in our country has shown that accidents in the food industry account for a significant proportion of the total number of accidents at work (Ozdemir & Serin, 2022). A significant number of accidents in this sector require hospital treatment, particularly due to incidents such as slips, trips and falls resulting in fractures or falls from heights. Other accidents are related to material handling and transportation, accidents involving the use of hand tools, and accidents caused by falling objects. These accidents often require more than 3 days of rest. (Parlak et al., 2020).

Whilst there are some shortcomings in the recording of occupational disease statistics in our country, a review of global statistics shows that approximately 5% of the food manufacturing workforce is exposed to work-related illnesses each year (Newman & Newman, 2015). A significant proportion of these conditions manifest as musculoskeletal disorders due to repetitive loading and unloading. In addition, continuous packing and similar tasks can cause discomfort in the upper limbs (Ariyanto, 2021). Furthermore, work-related stress and mental fatigue are recognised in the international literature as occupational diseases (Khamisa et al., 2015).

The food manufacturing industry also has common occupational diseases such as occupational asthma and allergic rhinitis related to exposure to flour and other organic dusts. (Talini et al., 2002). Skin disorders are also associated with exposure to food chemicals and other chemicals used in cleaning processes. Hearing loss due to working in noisy environments is another common occupational disease in the sector (Stevenson, 1989).

Respiratory system diseases caused by working with various substances such as enzymes, animals, grains, and flours are taken seriously by insurance companies in United Kingdom, which provide coverage for workers against accidents and occupational illnesses. Workers handling such substances and materials often suffer from high rates of respiratory diseases such as chronic cough and asthma (McDonald et al., 2005).

Chemicals in liquid, gas, or vapor form are used in the food industry for cooling, sterilizing, separating, and disinfecting purposes. However, these substances can also cause certain diseases. For instance, carbon monoxide (CO) is present in cooking rooms with smoke and fumes, grain silos, and fish storage areas, and its detection can be extremely difficult. CO poisoning can have fatal consequences (Moreau & Neis, 2009). Amonyak ciltte yanıklara, tahrişe ve su tutulmasına neden olabilir. Prolonged exposure and inhalation may cause bronchitis and pneumonia. Therefore, being aware of these chemicals and taking the necessary precautions is very important for those working in the food sector. (Gürler, 2020).

The polyvinyl chloride film used in packaging can cause thermal degradation when heated. It also emits an unpleasant odour and vapours that can irritate the eyes, nose and mouth. Narrowing of the airways can result from this condition (Akçay et al., 2020). Among workers in this sector, skin diseases are also a common problem. The most common skin conditions are contact dermatitis and eczema. To maintain cleanliness and sterility, continuous hand washing and disinfection with soap and ammonia-based solutions is essential. However, this can lead to contact dermatitis on the skin as a result of reduced hand moisture. Skin problems can also result from exposure to chemicals and additives. Skin problems such as eczema and allergic reactions can be caused by peptides and proteins produced during fermentation. Contact dermatitis has been reported to be caused by enzymes such as trypsin, chymotrypsin and protease. The use of personal protective equipment is the most effective way to protect against these diseases (Dickel et al., 2002). The correct use of gloves can prevent skin contact with external factors and can contribute to the prevention of disease. However, gloves made from latex material can cause skin allergies or hinder the skin's ability to breathe.

Infectious diseases are also common in sectors that work with animals. Workers in the meatpacking and dairy industries who have direct contact with infected animals are at higher risk for parasitic diseases and infections (Ogun & Akkoyunlu, 2019). Therefore, it is essential for workers in these sectors to adhere to personal hygiene rules and use appropriate protective equipment.

The advancement of technology plays a significant role in ensuring the healthy and safe nutrition of society. In the agricultural economy, dairy and dairy products account for a substantial portion, approximately 45%, of the animal protein requirement. However, in our country, only 27% of milk production takes place in modern facilities (Orhan, 2016). With technological advancements changing production processes and enabling faster production, addressing the hazards and risks that arise in production has become inevitable.

The dairy products manufacturing sector involves a series of process stages from the intake of milk to the final product's shipment, and these stages bring along a range of potential hazards and risks.

The aim of this study is to identify and assess the risks encountered by employees in the dairy products factory and contribute to the improvement activities that need to be implemented. Given the high importance of the industry, hygiene conditions should be at advanced levels, and it is crucial for the employees to adhere to

occupational health and safety guidelines and follow procedures. Within the framework of occupational health and safety, risk assessment studies conducted in workplaces not only ensure the safety of the workplace but also contribute to quality, reliability, and international reputation.

MATERIALS AND METHODS

This study was conducted in a cheese factory located in Iğdır Organized Industrial Zone. The factory operates for 45 hours per week, and the workers work from 07:30 to 17:00 on Mondays to Fridays, and from 8:00 to 12:00 on Saturdays. There are a total of 26 workers in the factory. The research was conducted between January 2022 and February 2022. Data for the study were collected through observational analyses and photographic reports.

The data related to occupational risks were collected from various areas of the factory, including milk collection, production, maintenance, storage, and offices. After preliminary observations indicated higher occupational risks for workers in these areas, specific locations were selected for the study (Figure 1.). The data collected from the observations were categorized based on departmental areas to group the types of hazards or exposure risks according to the work area. The potential study subjects included all 26 workers present in the various work areas. While it was not possible to examine all workers actively working at the same time, random visits conducted at different hours of the workday maximized the observation of workers' natural behaviors.

Data regarding occupational hazards, regulations, training, and risks faced by the workers were collected through observations, informal interviews, and a review of occupational health literature (Figure 1.). The

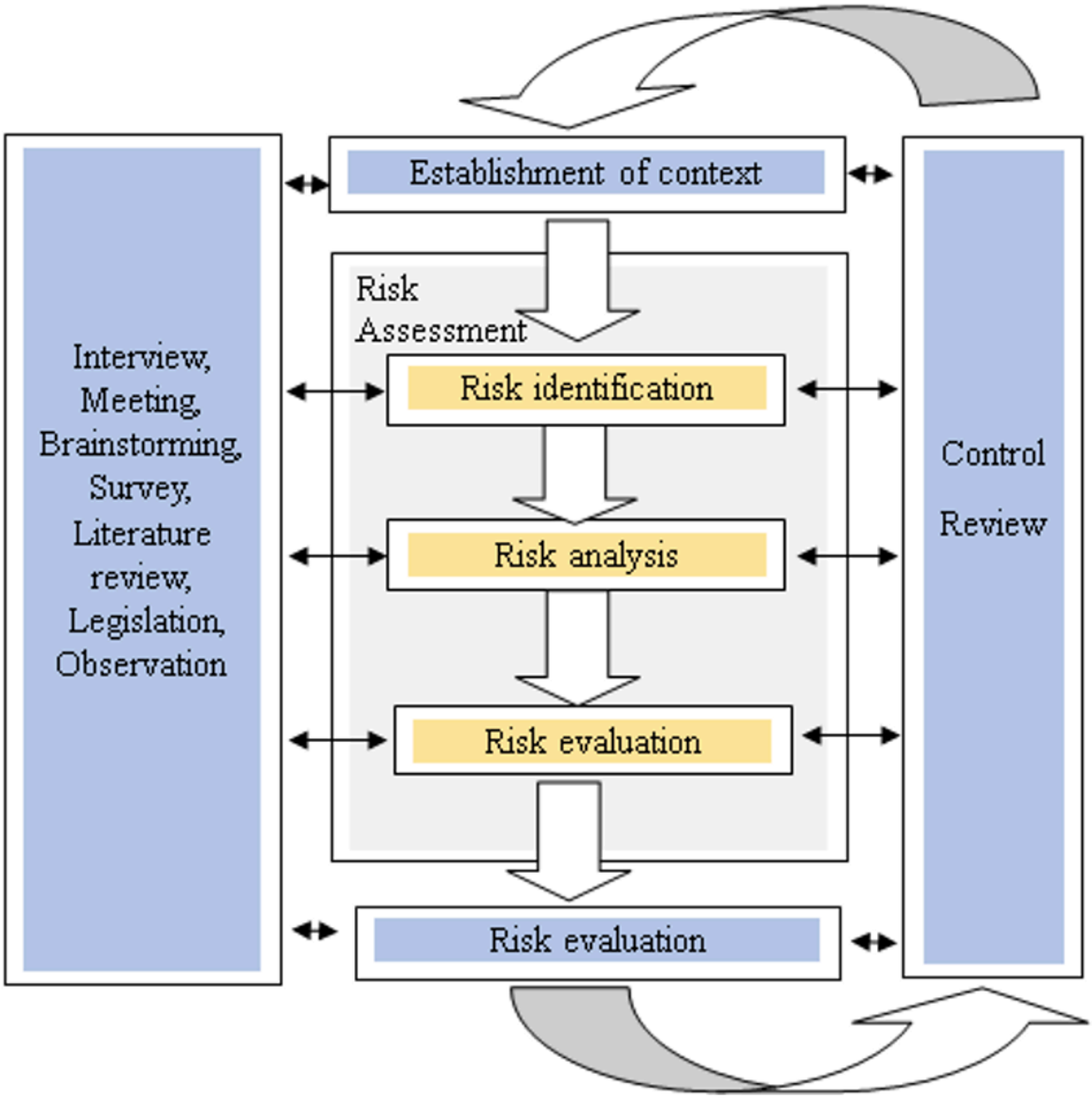

Figure 1. Risk assessment process

Table 1. Fine Kinney likelihood, frequency and severity rating

LIKELIHOOD	**Likelihood**	**Likelihood (%)**	**Likelihood of occurrence**
	0,2	2/100	Practically Impossible
	0,5	5/100	Very Unlikely
	1	10/100	Quite Unlikely
	3	30/100	Rare but Possible
	6	60/100	Highly Likely
	10	100/100	Very High Probability
FREQUENCY	**Frequency**	**Quantitative Frequency**	**Qualitative Rating**
	0,5	Very Rare	Once a year or less
	1	Quite Rare	Once or a few times a year
	2	Rare	Once or a few times a month
	3	Occasional	Once or a few times a week
	6	Frequent	Once or more per day
	10	Continuous	Continuously, more than once per hour
SEVERITY	**Severity**	**Severity**	**Rating**
	1	Should be Considered	Insignificant or trivial
	3	Significant	Low job loss, minor damage, first aid
	7	Serious	Significant damage, external treatment, workday lost
	15	Very Serious	Disability, loss of limb, environmental impact
	40	Bad	Fatality, total disability, severe environmental impact
	100	Catastrophe	Multiple fatalities, major environmental disaster

observations allowed us to make our own determinations about the types of risks encountered by the workforce, while the interviews provided insight into the workers' history and perspectives on occupational health in the cheese factory (Figure 1.).

The factory/production area was visited four times, each visit lasting between 1 to 3 hours. Each visit focused on a different department or workstation, with particular attention to all tasks performed in that area. Short follow-ups were conducted when further details were needed. During the first two visits to the production area, the workers provided guidance, and procedures in all areas were explained. Subsequently, the workers were asked questions about certain activities or aspects that may have seemed unclear. Smaller areas such as the warehouse, maintenance, and offices were each visited twice for a duration of 30 minutes to 1 hour. The milk intake area was visited three times for approximately 1 hour each time, including a specific visit to observe the cleaning of the milk tank. Short follow-ups were conducted again when additional data was required.

In this research, an electronic table was used to define the hazards, their causes, and consequences textually. Subsequently, the probability and severity (impact) of the risk occurrence were determined. Risk assessment was conducted using the Fine Kinney method to assess the risk levels.

This method is used to analyze the costs and risks of projects and provide information to decision makers. It essentially aims to identify the potential risks of the project and assess the impacts of these risks by considering various factors (Oturakci et al., 2015). These factors may vary depending on the project's characteristics and the organization's priorities. For each factor, a weight is determined, and the probability and impact of the risk are rated (Table 1.). Then, using the weight and rating factors, a risk score is calculated (1) (Table 2.). This risk score is used to determine the project's risk level. It assists decision-makers in determining the risk level and priorities of projects and contributes to the development of strategies for risk management.

The purpose of choosing the Fine Kinney method in the study is to provide both quantitative and qualitative assessment opportunities for risk evaluation. This approach quantitatively assesses the frequency of risk occurrence, the likelihood of recurrence, and its frequency over a continuous time frame, while qualitatively describes the frequency of risk occurrence using conceptual expressions such as rarely, occasionally, frequently, or continuously (Gul et al., 2018). This makes the study more comprehensive. Based on the obtained risk value, decision and action steps assist in determining priorities according to the severity of the risk. Depending on the risk level, it anticipates appropriate measures for managing acceptable risks, keeping moderate risks under control, and urgently addressing very high risks (Ak, 2020).

RS(risk score)=l(likelihood)xf(frekans)xs(severity) (1)

Table 2. Fine Kinney risk score, decision and action rating

Risk Score	Decision	Action
less than 20	Acceptable Risk	Emergency action may not be necessary
20-70	Certain Risk	Should be included in the action plan
70-200	Significant Risk	Should be closely monitored and addressed in the annual action plan
200-400	High Risk	Should be addressed in the short-term action plan
400+	Very High Risk	Work should be stopped and immediate action should be taken.

RESULTS AND DISCUSSION

In the dairy product manufacturing sector, farmers transport milk to the factory either using their own means or with the help of similar tanks provided by the factory owners. With the cooling feature of these tanks, the quality of the milk is preserved, and it is generally transported on vehicles. After the raw milk is accepted at the factory, samples are taken to determine both its quality and fat content. For the production of white cheese, the incoming milk undergoes processes such as filtering, cleaning, and separation, and then it is pasteurized to prepare it for cheese making under suitable conditions. The milk is placed in the cheese vat, and the rennet is added. The milk with added rennet is gently stirred and left to ferment for a certain period. When the curd matures after fermentation, it is cut. Then, the whey is removed, and the curd is subjected to pressing and straining. After the whey is removed, the curds are cut and placed in molds, where they are allowed to rest for a certain period. After the salting process is carried out, the cheeses are packaged and kept under appropriate conditions for maturation. In the production of Kashar cheese, pasteurized milk is placed in the fermentation tank. Then, the coagulated cheese is broken into small pieces using mixers, and the whey is removed. The cut cheeses are placed in molds and allowed to rest in the curing room for 24 hours. Afterward, the packaging process is carried out, and the cheese is introduced to the market. For the production of butter, raw cream is used. After the processes applied to raw milk, the separated cream is pasteurized and cooled. Then, the necessary steps for butter production are carried out.

As a first impression, the level differences on the floor, humps, and pits, open drainage channels, materials left in the middle, and exposed cables create high risk (*l: 3, f: 6, s: 15*) at the workplace. These risks can be eliminated by preparing Occupational Health and Safety (OHS) guidelines, providing training, and ensuring the continuous implementation of instructions (Zimolong & Elke, 2006).. Additionally, the observation indicates that the continuous wetness of the floor (*l: 4, f: 3, s: 15*) may cause employees to experience slip-related injuries. To address this issue, both the drainage channels in the production area and outside the building should be cleaned and covered with grates.

It has become a routine practice for personnel other than authorized electricians to intervene in electrical malfunctions that occur during work in the facility (*l: 6, f: 6, s: 40*). To prevent this, it is essential to provide the employees with necessary training and instructions, specifying that only authorized personnel are allowed to intervene. The fact that the panels of the existing machinery and machine control panels in the facility are left open exposes them to unauthorized intervention (*l: 3, f: 6, s: 40*), indicating a lack of proper instructions and trained personnel.

Cleaning and preparation tasks for boilers are critical operations conducted in enclosed spaces, and having inexperienced personnel perform these tasks (*l: 6, f: 6, s: 15*) could lead to severe consequences for both the employees' health and safety and the equipment's integrity. In such hazardous tasks, involving unskilled individuals may lead to potential risks that could result in disasters and significant material and human losses (Khatri et al., 2021).

The exposure of electrical cables to open areas in the facility, leading to deformation due to contact with water, moisture, and external elements, can pose risks (*l: 6, f: 3, s: 40*). However, appropriate measures can be taken to protect such cables from impacts and direct water contact. These measures can be implemented by passing the cables through suitable conduits, trays, and closed channels (Tosun, 2022).

The absence of residual current devices, inadequate grounding, and lack of lightning protection systems in electrical installations can lead to significant safety risks (*l: 6, f: 1, s: 40*). It is mandatory to have grounding and lightning protection systems regularly measured and inspected by authorized institutions and organizations at least once a year. Residual current devices are crucial devices that detect leakage currents in electrical panels, helping to prevent hazards such as electric shocks and fires (Pekeroğlu, 2017).

The lack of designated emergency exit points and exit routes (*l: 3, f: 2, s: 40*) and the absence of teams to respond to emergencies (*l: 6, f: 2, s: 15*) can result in ineffective management of emergency situations in the facility and pose serious security threats. Therefore, after preparing emergency response plans, it is of great importance to identify the locations of exit routes and doors according to the needs.

Employing unauthorized, untrained, and unaware personnel for tasks that require working at heights can increase the risk of falling from heights (*l: 3, f: 3, s: 40*)(Table3.). Taking effective safety measures and implementing appropriate permit procedures in situations involving work at heights is of utmost importance (Kamardeen, 2011).

In tasks involving measurements in laboratories or facilities, especially in microbiological experiments and analysis studies, frequent contact of personnel with sensitive materials such as milk and dairy products using bare hands can pose hygiene and health risks (*l: 6, f: 6, s: 7*). Appropriate hygiene measures and hand hygiene should be ensured, personnel should be conscious, trained, and cautious, and suitable personal protective equipment should be used during analyses.

The absence, malfunction, or incorrect installation of any safety equipment required for steam boilers (*l: 6, f: 3, s: 15*) can jeopardize the safe and healthy operation of the boilers (Table3.). İmportant measures must be taken to ensure the safety of the boilers. These measures include conducting annual inspections and tests of the boilers by qualified technical personnel, obtaining user manuals from the manufacturer or installer of the boilers, and ensuring that daily operation, maintenance, and inspection instructions are fully implemented by competent personnel (Landi et al., 2022).

The lack of regular maintenance of compressors (*l: 3, f: 2, s: 7*), continuous operation above the maximum operating pressure (*l: 3, f: 3, s: 15*), and the absence or malfunction of safety equipment on the compressors (*l: 3, f: 3, s: 7*) can jeopardize the safe and efficient operation of the compressors (Table3.). Periodic checks of the compressors should be conducted regularly, at least once a year. It is essential that the compressors display information such as the name of the manufacturer, the year of manufacture, the maximum operating pressure, and the type and quantity of compressed gas in a readable manner. This information provides details about the technical specifications of the compressors and is crucial for safe usage. It is critical to never operate the compressors above their maximum operating pressure, as such situations can subject the equipment to excessive stress and potentially lead to explosions (Aydoğan & Rüştü, 2022).

The inadequate thermal comfort conditions in the facility (*l: 3, f: 6, s: 7*), the presence of noisy machines and equipment (*l: 3, f: 3, s: 7*), and the insufficient lighting in various areas of the facility (*l: 3, f: 2, s: 7*) can pose significant occupational health and safety issues. Therefore, solutions and arrangements need to be implemented to address such problems. The lack of suitable thermal comfort conditions can cause discomfort and reduced productivity for the workers in the working environment. To mitigate this issue, proper heating and cooling arrangements should be made throughout the facility, ensuring that heat is evenly distributed. Specifically, for personnel working in noisy areas such as boiler rooms and generators, appropriate ear protectors should be provided and encouraged to be used (Rînjea et al., 2022). Proper illumination of workspaces enhances visual comfort for workers and prevents occupational accidents. Open areas, external pathways, passages, and similar places should have a minimum lighting level of 20 lux, while rough material handling, transfer, storage, and similar tasks areas should have a minimum lighting level of 50 lux. By ensuring appropriate lighting in the work areas, the working conditions of the employees can be improved, and the risk of work-related accidents can be reduced (Onur, 2012).

Tasks performed continuously while standing in the production process (*l: 3, f: 2, s: 7*) and the use of unhealthy desks, chairs, and furniture in jobs that require sitting (*l: 3, f: 1, s: 7*), as well as manual lifting and carrying tasks in offices, canteens, boiler rooms, production areas, and all other buildings and facilities (*l: 3, f: 2, s: 7*), and work involving screens (*l: 1, f: 3, s: 7*), can lead to health issues in the muscular and skeletal system of the employees (Table3.). Therefore, providing ergonomic training to all personnel working within the facility is essential. It is crucial to inform the employees, especially about the potential muscular and skeletal system diseases and other issues that may arise due to manual lifting and carrying tasks, work involving screens, and desk jobs (Engür & Chaush, 2019).

During the analysis and experiments, situations such as direct skin contact with acids, bases, and other chemicals or inhalation of these chemicals (*l: 1, f: 2, s: 15*) can pose serious risks. Additionally, unauthorized, untrained, and unaware personnel entering the laboratory and interfering with work (*l: 3, f: 2, s: 15*) and misuse of hand tools (*l: 1, f: 2, s: 7*) are significant issues concerning laboratory safety. It is of vital importance for laboratory workers to use appropriate personal protective equipment when working with chemicals. Having Material Safety Data Sheets for all chemicals in the laboratory and ensuring the availability of suitable personal protective equipment based on this information ensures the safety of the employees. Identifying personnel with access to the laboratory and prohibiting entry of unauthorized individuals is a critical step for laboratory safety. Allowing only trained and conscious personnel to enter the laboratory prevents potential accidents and hazards (Yılmaz & Bilici, 2020). Furthermore, it is essential to use hand tools only for designated purposes and prepare relevant instructions. Misuse of hand tools can lead to accidents and equipment damage. Therefore, providing training to employees on the proper use of hand tools and promoting safe usage is necessary.

During the interviews, several negative situations have been identified among the employees, including stress, job disillusionment, and bullying (*l: 3, f: 2, s: 3*), as well as

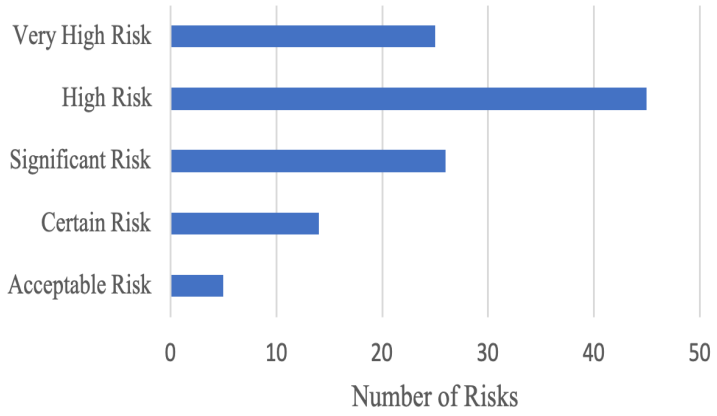

Figure 2. Distribution of risk score by number

Table 3. Risk assessment decision chart

Decision	Identified Risks	*l*	*f*	*s*	*RS*
Very High Risk	Due to the lack of authorized electrician, electric shock incidents may occur.	6	6	40	1440
	The use of portable electrical tools and equipment for transportation.	3	6	40	720
	Uncared and open electrical panels.	6	3	40	720
	Open panels of machines.	6	3	40	720
	Wet floor due to cheese whey and presence of movable electrical cables.	6	3	40	720
	Unauthorized, untrained personnel performing any work in confined areas.	6	6	15	540
	High ambient noise resulting in hearing loss.	6	6	15	540
	Slippery conditions due to the continuous wet floor.	6	6	15	540
High Risk	Lack of ventilation in confined space work.	3	3	40	360
	Maintenance of the generator by unauthorized and untrained individuals.	3	3	40	360
	Lack of grounding and residual current devices for electrical machines.	3	2	40	240
	Lack of safety equipment for steam boilers.	6	3	15	270
	Direct contact of personnel with dairy products using bare hands.	6	6	7	252
	Non-usage of the PPE during electrical maintenance and repairs.	6	3	15	270
	Undefined emergency exit routes.	3	2	40	240
	Non-usage of personal protective equipment (PPE) for chemical handling.	3	2	40	240
	Insufficient equipment usage while working at heights.	3	3	40	360
	Insufficient workplace health and safety training.	6	3	15	270
Signif. Risk	Continuous operation of the compressor at high pressure.	3	3	15	135
	Inadequate thermal comfort conditions.	3	6	7	126
	Unauthorized personnel entering the laboratory.	3	2	15	90
	Maintenance and repair works carried out by unauthorized personnel.	3	3	15	135
	Inadequate workplace hygiene conditions.	3	2	15	90
	Non-ergonomic equipment usage.	3	3	15	135
	Insufficient flow of information and communication.	3	6	7	126
	Long working hours.	3	2	15	90
	Absence of a trained emergency response team.	6	2	15	180
Certain Risk	Unauthorized access to the chemical storage area.	3	1	7	21
	Inadequate number of fire extinguishers.	1	3	15	45
	Manual lifting and carrying of heavy objects.	3	2	7	42
	Lack of clear delineation of responsibilities.	3	2	7	42
Accep. Risk	Inadequate warehouse stacking arrangement.	1	2	7	14
	Monotonous work routine.	0,5	1	7	3,5
	Misuse of hand tools for purposes other than their intended use.	3	2	3	18

Figure 3. Photographic report on working environment hazards. The different risks identified are illustrated in the photographic report. Physical and ergonomic risk. Physical and ergonomic risk (1,2,3), ergonomic risk (2,4,5), accident risk (1,8,9), physical risk (1,2,5,7) and (9), physical and accident risk (1,2,3,4,6), accident risk (8), hygiene risk (6).

incidents of violence and threats (*l: 3, f: 2, s: 3*). Moreover, unprofessional behavior (*l: 3, f: 2, s: 3*), lack of respect for employees' ideas, and insufficient information flow (*l: 3, f: 2, s: 7*) have been highlighted, alongside the issue of unclear definition of employees' authority and responsibilities (*l: 3, f: 2, s: 7*). It is essential to prevent any acts of violence and threats during work and strictly prohibit such behaviors within the hierarchical structure of the company. Providing employees with information about the company's business objectives and goals, as well as listening to their thoughts and opinions on work-related matters, are crucial steps that will enhance employee motivation and commitment (Serap, 2007). Clear definition of employees' authority and responsibilities, along with transparent task distribution, will increase work efficiency and prevent conflicts. Similarly, ensuring adequate and regular information flow will enable employees to be guided with accurate

information and carry out their tasks more effectively (Kocabaş et al., 2018).

During the observations and interviews conducted at the workplace, a total of 115 risks have been identified (Figure 3). A detailed analysis of these risks should be carried out, and appropriate measures should be taken (Figure 2). According to the reports, some of the identified risks have been categorized as 5 urgent risks requiring immediate improvement and intervention. Additionally, 14 risks have been evaluated within short-term plans, and the necessary measures have been planned accordingly. Furthermore, 26 risks have been considered significant and are expected to be carefully monitored and addressed within the annual plan, aligning them with long-term objectives. It is crucial to manage and keep these significant risks under control in line with the business's long-term goals (Figure 3).

On the other hand, 45 risks have been clearly classified as risks that need to be evaluated in the company's investment and future plans. Appropriate risk management strategies should be developed, taking into account the impact of these risks on the company's strategic decisions (Figure 2). Lastly, 25 risks fall within acceptable risk limits for the business and do not require inclusion in a specific plan. However, regular monitoring and evaluation of these risks are still essential to ensure that they can be kept under control with necessary measures if needed (Figure 3). All the data obtained forms the basis for determining the business's risk management strategies and implementing occupational safety measures. This way, the aim is to effectively manage the risks at the workplace and ensure the safety of the employees. The successful management of risks by the company holds great significance for sustainability and success.

CONCLUSION

The Fine Kinney method, which is used as a fundamental tool in occupational health and safety management in workplaces where industrial food production is carried out, is highly effective in evaluating and rating environmental risks. In the food sector, where various occupational hazards can cause occupational diseases, it facilitates the development of measures and actions to control risks, improve the working environment, and ensure the health and safety of employees.

Industrial food production involves risks at different levels. In the study, while serious risks were present at RS:1440 levels, 25 risks requiring urgent action were identified with a risk score above 540. In addition, 45 risks were evaluated as high risk and included in the annual plan for preventive measures. The study and investigations suggest a great responsibility for the employer in establishing occupational health and safety procedures in the workplace and taking action for precautions. Continuous training and increased inspections, especially in education, are among the first tasks to be carried out in this regard.

The Hazard Analysis and Critical Control Points (HACCP) method, widely used in the food production sector, can be used to carry out this study. However, since it requires teamwork, effective implementation of the risk assessment procedure is necessary.

These methodologies and practices help identify potential risks in the workplace and implement effective measures to protect the health and safety of employees. They also play a crucial role in enhancing the sustainability and productivity of the workplace. Ensuring the health and safety of employees not only improves their well-being but also positively impacts job performance.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Declaration of interests

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethics Committee Approval

Ethics committee approval is not required. This article does not contain any studies with human participants or animals performed by any of the authors.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(3): 573-580
DOI: https://doi.org/10.31015/jaefs.2023.3.11

Anatomical observations on formation and development of adventitious root primordium in canes of *Vitis* sp.

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Abstract

Understanding the anatomical aspects of adventitious root primordium formation can provide valuable insights into the improvement of propagation techniques, rootstock selection, and overall vineyard management practices in *Vitis* spp. This work was carried out to investigate anatomical root primordium formation in the rooted cuttings of Cabernet Sauvignon (CS, *Vitis vinifera* L.) and the rootstock Kober 5BB (*Vitis berlandieri* x *V. riparia*) with their relationship to stem anatomy. One-node cuttings were grown under temperature-controlled conditions for 8 weeks. After removal of the roots and calli, the stem parts were fixed in a fixative solution. A revised method of safranin staining was applied to the 90 µm thick cross-sections made with a hand microtome. It was observed that root primordia were derived from the two different regions of the cane tissues: from the groups of cells close to the outside of the conductive tissue system and from the cell groups in the deeper site, close to the pith. Cultivars showed significant differences in terms of the regions where they had their root primordial initials. Number of potential root primordia was statistically higher in CS. Both CS and 5BB had root angles in the range of 83° to 86°. It was concluded that grapevine cuttings had only induced root primordia and the capacity to produce them was dependent on the genotype. Formation and development of root primordia and the anatomical differentiation of the cell groups were similar in Cabernet Sauvignon and 5BB.

Keywords: Cutting, Grapevine, Staining, Stem tissue

Citation: Gokbayarak, Z., Engin, H. (2023). Anatomical observations on formation and development of adventitious root primordium in canes of Vitis sp. International Journal of Agriculture, Environment and Food Sciences, 7 (3), 573-580

Received: May 31, 2023
Revised: August 21, 2023
Accepted: August 23, 2023
Published Online: September 20, 2023

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Available online at
https://jaefs.com/
https://dergipark.org.tr/jaefs

INTRODUCTION

Root formation is essential for vegetative propagation and plant growth. Root formation process varies by the genotype, and woody species are generally more difficult to root than herbaceous species (Hackett, 1988). Formation of root is still not fully understood, and it is not clear why cuttings of different cultivars have different rooting potentials. Information about anatomical events relating to root primordium formation is beneficial to improve our understanding rooting process in plants. The term of adventitious root (AR), by broad definition, refers to roots that arise from aerial plants parts, or underground stems (Hayward, 1983). In another sense, it refers to roots that do not arise from root pericycle tissue. The organization of adventitious roots structure and its development sequence is similar in all features to that of true roots. AR root primordia can be formed by stem cambium at specific sites where branch and leaf traces, parenchyma, or primary or secondary rays intersect with cambium tissue (Beakbane, 1961).

In grapevine growing, cuttings have been used to study root formation, but anatomical observations on initiation and development of root primordium generally are lacking. Van der Lek (1925) reported that adventitious roots of

Vitis vinifera after wounding were originated from the medullary rays. Subsequent anatomical studies on grapevine cuttings or canes were performed by Fujii (1955) and Han (1983) in which root primordia were reported to arise from outer part of vascular strands or from the cambium and the phloem ray, respectively. Furthering such studies would clarify developmental events leading to root formation.

Literature is abundant with the studies carried out on the rooting capacity of grapevine cuttings. However, studies on the events leading to formation of root primordia inside a grapevine cutting are not sufficient. The duration and location of these events can vary between taxa and even between conventional and micro propagated material (Lovell and White, 1986). Anatomic examination of root differentiation through staining techniques by taking thin sections from grapevine canes would reveal the regions where the root primordia are formed in the stem and contribute to a detailed understanding of the action mechanism of different applications on grapevine rooting.

Current methods of micro technique for microscopy that involve epoxy embedding and thin sectioning allow observation of subtle differences between cells less evident in cuttings. Today, new micro technical methods have been developed and taking thin sections (via microtomy) and staining techniques can enable observing events in cells especially in hard and woody species.

Understanding the anatomical aspects of adventitious root primordium formation can provide valuable insights into the physiological and molecular mechanisms underlying root development in *Vitis* spp. Furthermore, this knowledge can contribute to the improvement of propagation techniques, rootstock selection, and overall vineyard management practices. This research was carried out to examine origin sites of root primordia of *Vitis* fruit and rootstock cultivars and post-differentiation changes in the root primordia. Some quantification as to the frequency of occurrence of primordia at different sites was also done on the diameter and number of the root primordia.

MATERIALS AND METHODS

Stock Plants and Cuttings

Both Cabernet Sauvignon (CS, *Vitis vinifera*) and rootstock 5BB *(V. berlandieri* x *V. riparia*) are known for their ease in rooting. 2-node cuttings of 1 year-old CS and 5BB were planted in a rooting medium containing peat: perlite (2:1) after the basal node was removed and cut in the middle. The cuttings were grown in 1.9 L pots where their 2-3 cm basal parts were buried. Every pot had 10 cuttings for each treatment. The plants were maintained in a growth chamber at 24°C with a photoperiod of 16 h light (~ 900 µmol m^{-2} s^{-1} irradiance) and 8 h darkness. The mean diameter of the cuttings was ~1 cm. Following the period of 8 weeks, rooted stem parts were rid of any roots and callus as well as top growth. They were fixed in a fixation solution composed of glacial acetic acid, formaldehyde, and ethyl alcohol (FAA, 5: 10: 50) and stored in 100 ml dark brown bottles with low light transmission.

Microtechniques

For the purpose of visualization, cane parts were processed following protocols of Hacke (2015). Samples were removed from the FAA medium to section with a microtome, Before examination for root primordium development, the cross-sections were cleaned under a stereo microscope (Olympus SZ61, Olympus Corp., Japan) with the help of arrowhead needles and scalpel. During this process, the callus tissue and dead bark around the root exit site was removed without damaging the exit points in a solution containing ethanol and pure water (1:1) so that the tissues would not soften. There was no callus-root formation. Before taking the cross-sections, the surface of the samples was dried and tightly wrapped with an adhesive resin tape close to the top to prevent the tissues from disintegrating. For each stem part, 5-6 mm internode fragments were sectioned.

Taking cross-sections as thin as possible is of great importance for successful microscopic examinations. For this purpose, a classical sliding semi-automatic microtome (Reichert Jung, Germany) was used. The thickness of the cross-sections taken was 90-100 µm on average, and very sharp blades were used and changed frequently. After adjusting the microtome blade to a section thickness about 100 µm, a drop of 98% ethanol was placed on the surface of the sample, and the excess on the sample was removed with the help of a wet watercolor brush. This process prevented the blade contact point from getting wet and bending the cross section. The cross-sections were later put into petri dishes containing 98% ethanol before proceeding to the staining procedure. The cross-sections taken were examined under the microscope (Olympus SZ61) and checked for traces and cracks that might result from the cutting edge of the microtome blade, and such samples were not included in the staining test.

Staining of the cross-sections were carried out by the modified methods of different researchers (Bond et al., 2008; Hacke, 2015). Sections were washed three times with distilled water for 10 minutes followed by immersion in 1g/L safranin solution for 20 minutes. Later they were rinsed three times in distilled water to remove excess stain. Then they were placed in 0.75% bromophenol blue solution (including 10% glycerol and 10% acetic acid) for 25 minutes and again rinsed three times in distilled water.

Microscopy

Observations of stem tissues and root primordia were carried out under stereo zoom microscope (Olympus SZX7, Olympus Corp., Japan) equipped with a digital

camera (Olympus LC20, Olympus Corp., Japan) and, when permitted, under the light microscope (Olympus CX41, Olympus Corp., Japan). The LCmicro software (Tokyo, Japan) was used for measurements and counting. Cross-sectional diameter of root primordium was calculated on each cross-sections considering at last 5 primordia, then from a total of 75 primordia for each cultivar. Sites for the root primordia were also determined. Root exit angle was determined by measuring the angle with respect to the horizontal line against the slope of the root exiting the epidermis.

Statistical Analysis

Values represent the means of three replicates per cultivar. Data for diameter of root primordium and for the other parameters were collected from n=75 and 45, respectively. They were evaluated by ANOVA (analysis of variance) and comparisons between the mean values were made according to the Tukey's t-test at $p< 0.05$.

RESULTS AND DISCUSSION

Formation and development of root primordia and the anatomical differentiation of the cell groups were similar in Cabernet Sauvignon and 5BB. It has been observed that many cells in the canes of CS and 5BB had the ability to go back to meristematic status and form roots.

Statistical analysis showed that genotype played a major role in the characteristics belonging to AR primordia formation in the stem tissues (Table 1). It was observed that both cultivars had quite a high number of dense cell groups that might be considered as potential primordia. In terms of the site for these primordia, CS had considerably more root primordia, most of which originated near the vascular elements. On the other hand, 5 BB produced almost twice the number of root initials in its deeper region compared to its vascular system. Root diameters in CS were comparably thinner than those in 5BB. Root angle was similar in both genotypes. Root angle was quantified as the average 83° in 5BB and 86° in CS.

Cell groups divided to form many new cell groups and these groups turned into root primordium (Fig. 1). In each cell group, division continued (Fig. 1a) and took the appearance of root primordia initials (Fig. 1b). Once initiated, it was observed that root primordium (Fig. 1 b, c) enlarged until it reached the outside of the tissue system and emerged towards the outer bark surface of the cane (Fig. 1d).

It was determined that the root primordia were generally stemmed from two different regions of the tissues (Table 1, Figure 2). Although the roots originated from the cells in files outside and between the conductive tissues, connections of cell groups extending to the pith were also observed in some of the samples. Roots which arose within the phloem or vascular cambium in both 5BB (Figure 2a) and CS (Figure 2b) indicated a direct AR formation that did not involve callus formation. The roots grew through the cortex and penetrated the epidermis (Figure 2c, d). Figure 2e shows a more highly magnified view of the xylem and phloem elements in a cleared root of CS and figure 2f shows view of the xylem elements of the two cleared root of 5BB.

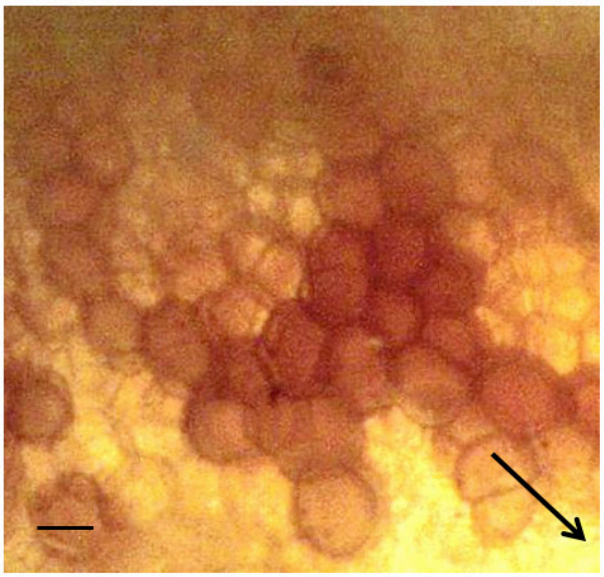
Another group of cells in the deeper regions extending to the pith were also observed to be competent to form AR initials (Figure 3). These cell groups were divided to form other cell files, and these cell files turned into root primordia. Some root primordia could also be observed between the pith and xylem or rays (Figure 3b). New root primordia continued to develop and connected to the nearest conductive tissue system (Figure 3c, d). Figure 3e shows a more highly magnified view of the new root with cambial continuity initiated from pith and xylem elements in 5BB cross section. The new root was grown outward through the cortex and penetrated the epidermis forming an angle of 83-86° with the cane (Figure 3f).

The anatomical observations in this research have confirmed that *Vitis* species do not have pre-formed root primordia in their canes, as also stated by Smart et al. (2003). They have what is called an induced primordium, which is formed upon a stimulatory effect as wounding by cutting or application of growth regulators (Tailor et al. 2022). Initiation of the primordium is usually

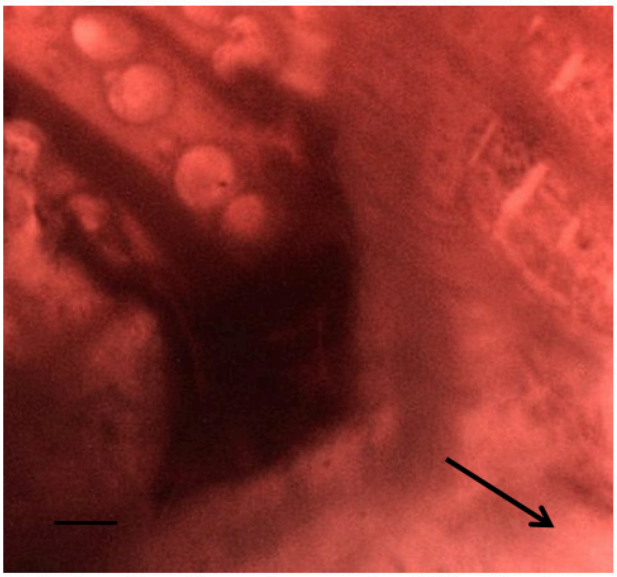
Table 1. Measurements of root primordia number, root diameter, root angle and number of root primordia in 5BB and CS

	Cultivars	
	5BB	**Cabernet Sauvignon**
Total number of potential root primordia (n)	11,83 b*	25,37 a
Number of root primordia close to the outside of the conductive system (n)	4,300 b	16,73 a
Number of root primordia from deep and pith region (n)	7,53 b	8,63 a
Root diameter (µm)	353,70 a	153,30 b
Root angle (°)	83,47 a	86,41 a

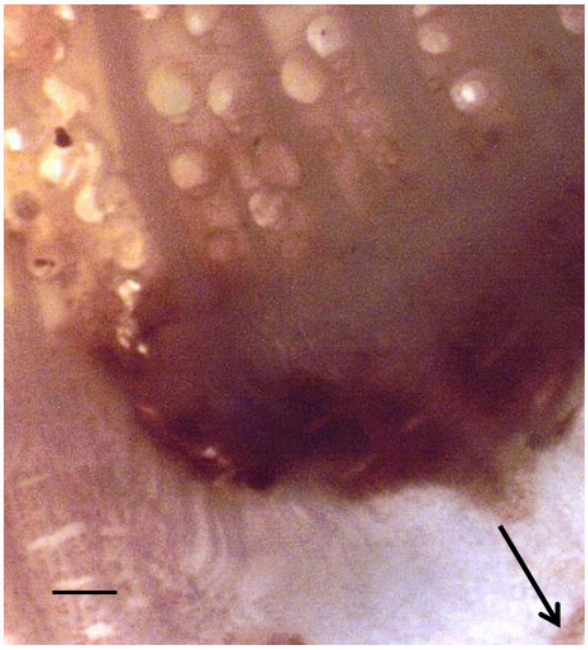
*Means in each column followed by the same letters are not significantly different at p <0.05 according to Tukey's t-test.



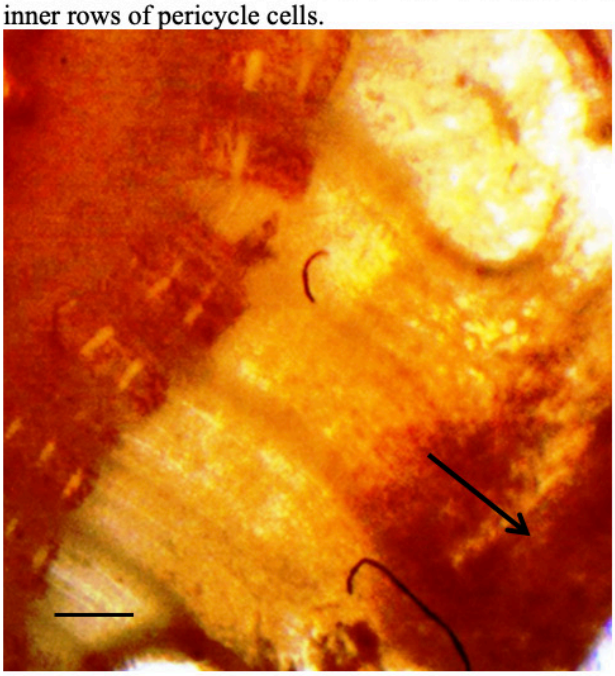
a) A site for primordium initiated. Pericycle cells going through radial expansion and one tangential division.



b) Each cell continues to expand radially, forming an arc. Outer cells continue radial enlargement and tangential division. Protoplasmic content increases in some of the endodermal cells. Radial cell lines were formed by tangential divisions of both the outer and inner rows of pericycle cells.



c) Cells of radial growth by the root primordium. The root primordia is almost traversing the cortex.



d) A root primordium just completing penetration through the cortex and epidermis.

Figure 1. Root primordium formation and development in 5 BB. The arrow shows the direction of penetration of a root primordium. Bar=100 µm.

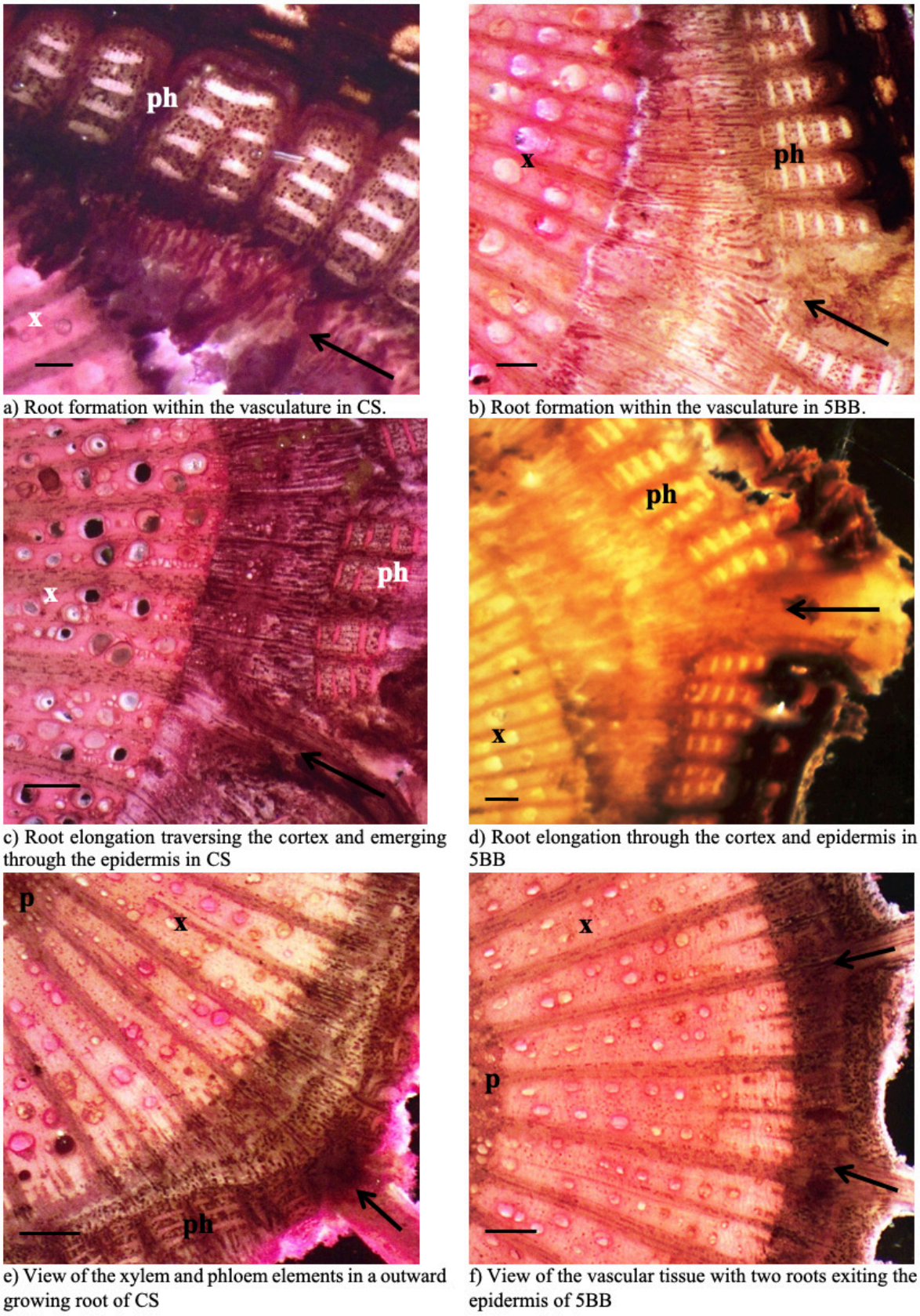
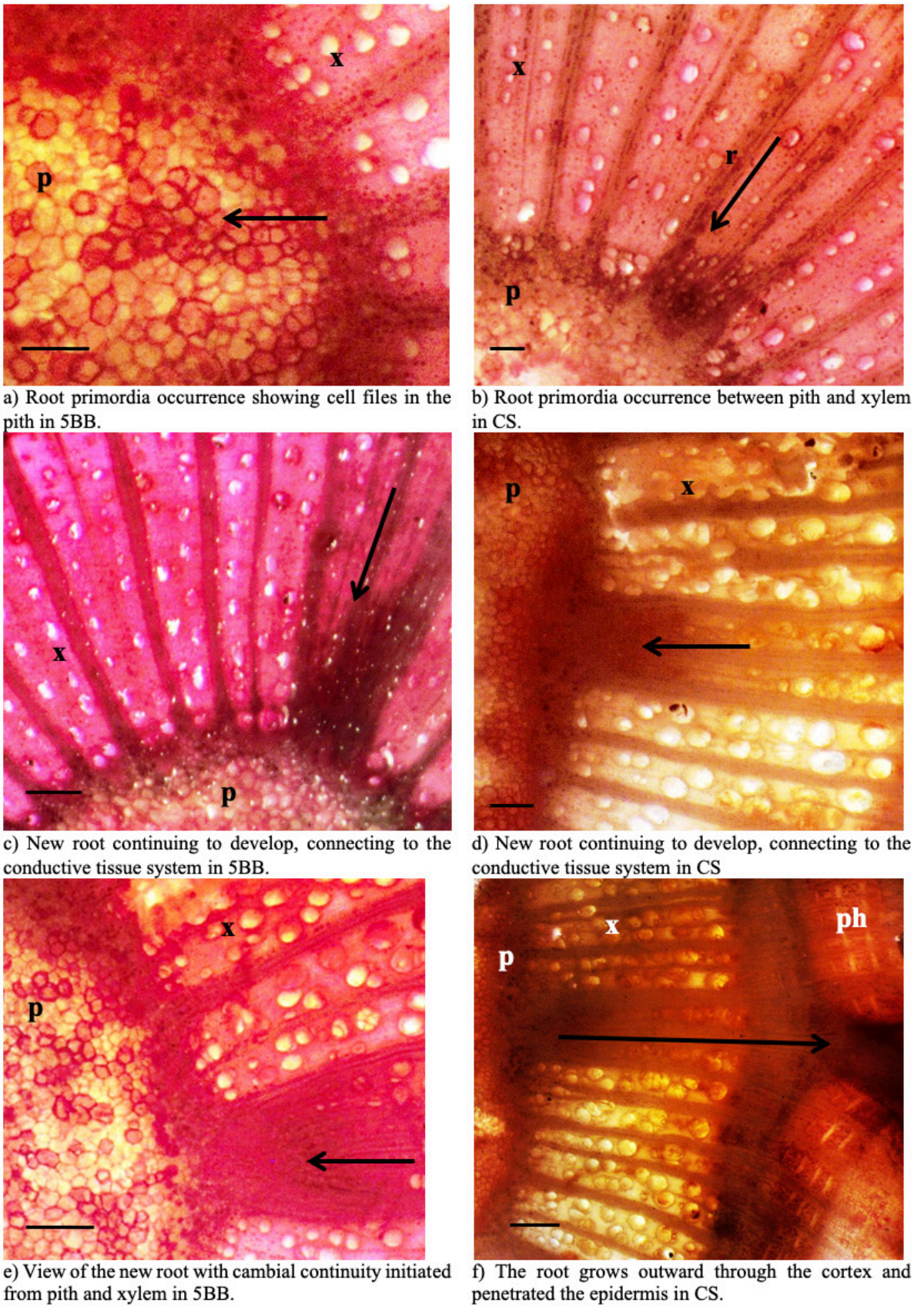


Figure 2. Root formation site and roots elongating through the cortex and emerging out of the epidermis in 5BB and CS cross sections. Arrows indicate root primordia in the vascular tissue. ph = phloem, x = xylem, p = pith, Bar=100 µm.



a) Root primordia occurrence showing cell files in the pith in 5BB.

b) Root primordia occurrence between pith and xylem in CS.

c) New root continuing to develop, connecting to the conductive tissue system in 5BB.

d) New root continuing to develop, connecting to the conductive tissue system in CS

e) View of the new root with cambial continuity initiated from pith and xylem in 5BB.

f) The root grows outward through the cortex and penetrated the epidermis in CS.

Figure 3. Root formation site and the new root primordium with cambial continuity initiated from deep and the pith, and roots passing through the cortex and epidermis in 5BB and CS cross sections. Arrows indicate new root development in cane tissues. ph = phloem, x = xylem, p = pith, r = ray, Bar=100 µm.

accounted for the dense cell groups with large nuclei and small vacuoles (Cameron and Thomson, 1969) and in this study *vinifera* had considerably had more compared to the *Vitis* hybrid. However, it does not mean that all of these cell lines would be determined to be a developing root (Lovell and White 1986). This is the case here too in which the number of the committed initial primordia was less than root primordia.

Once the root primordia initiated, it grows and enlarges until it reaches the outer bark surface. Root primordia could be formed by stem cambia at specific sites where branch and leaf traces, parenchyma, or primary or secondary rays that intersect with cambium tissue (Roy et al., 1987). Our results show that rooting is not restricted to the cambium zone and may develop at pith and cortex or any point along the vascular tissues and through xylem if there is retention of meristematic capacity in certain cells. In both cultivars, initiation of cell division for root formation took place in parenchyma cells of the phloem, cortex, cambial region, xylem, and pith. In studies of propagated other species, cells leading to root formation could have been in cells within or just external to the vascular cambium (Vieitez and Vieitez, 1983; Samartin et al., 1986; Hicks, 1987; Ranjit et al., 1988; Isfendiyaroğlu and Ozeker, 2008). Formation of root primordium occurred in the same way in both cultivars and no significant difference was determined. *Vitis vinifera* cuttings have been accepted as easy to root, compared to other *Vitis* hybrids and higher number of potential root primordia and them being located near epidermis in conjunction with vasculature would certainly support this notion. In addition to this, reactions of root formation are probably related with the presence or content of endogenous auxin in such cuttings (Koll et al., 2012).

Root diameter is considered one of the characteristics used for determination of root system architecture (Dumont et al. 2016). The rootstock 5BB is known for abundant propagule production due to its profuse rooting. Considerable difference in root diameter between 5 BB and Cabernet Sauvignon show genetical variations. The enlargement of the root diameter is associated with the inhibition of cortical cell division (Koll et al., 2012). The reason why the 5BB had thicker roots is perhaps due to higher levels of endogenous auxin concentrations and subsequent changes in cell number and size of the cortical area reflected in the root diameter.

Root angle is another factor for root system architecture, determining whether a plant develops deep or shallow roots, as it determines the direction of root elongation (Kitomi et al., 2015; Uga et al., 2015). Previous work with root angle states that roots in the two growth angle categories 0° to 45° and 45° to 90° from the horizontal line (Ramalingam et al., 2017). Many studies have examined the relationship between root angle and development in other crop species (Kato et al., 2006; Ali et al., 2015; Dathe et al., 2016). However, until now, root angle analysis studies on grapevines have not been done sufficiently. In the current study, both Cabernet Sauvignon and 5BB had root angles in the range of 83° to 86° and the difference was important, although in their study Schmitz et al. (2021) reported genotype differences in adventitious root angle between three grape cultivars. Another observation in this study was that the roots forming from deep sites and pith were at an angle close to 90° and that those from outside of the conductive system were at an angle of 70°-80°, but this needs confirmation with further studies.

CONCLUSION

Rooting ability in woody trees highly depends on their ability to turn a group of cells to dedifferentiate into meristematic form and then, develop into root primordia initials. Following sequential events that lead to adventitious root formation in the stems have been lacking in grapevines since taking and staining thin slices have proved to be not as easy and successful as that in herbaceous plants. However, successful efforts have widened our view on formation and development of root primordia and increase our understanding of the effects of wounding after cutting, growth regulators, and stress factors. This study indicated that *Vitis* species have induced primordia and the site they derive is genotype dependent. Further anatomical studies in which time-course changes from planting a cutting to uprooting are observed would allow, especially in hard-to-root species, to sequence adventitious root initiation and development inside stems.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Declaration of interests

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

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

Ethics Committee Approval

Ethics committee approval is not required. This article does not contain any studies with human participants or animals performed by any of the authors.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(3): 581-587
DOI: https://doi.org/10.31015/jaefs.2023.3.12

Chemical composition of meat from different species of animals

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Citation: Yaranoglu, B., Zengin, M., Gokce, M., Varol Avcilar, O., Berhun Postaci, B., Erdogan, C., Odabas, E. (2023). Chemical composition of meat from different species of animals. International Journal of Agriculture, Environment and Food Sciences, 7 (3), 581-587

Received: July 26, 2023
Revised: August 28, 2023
Accepted: September 01, 2023
Published Online: September 21, 2023

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Available online at
https://jaefs.com/
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Abstract
The study aimed to comparatively examine meats in terms of chemical composition originating from beef, lamb, chicken, and rabbit consumed in Türkiye and to reveal their superior aspects compared to each other and also to determine the place of rabbit meat, which is not commonly consumed among other meats. As material of the study 48 meat samples were used, 12 from each of the beef, lamb, chicken, and rabbit species provided that each of them belonged to a different animal. Moisture, ash, crude protein, and crude fat levels of the meat samples were compared between meat sources. The highest value in terms of fat and ash ratio were determined in lamb meat ($P<0.001$). Chicken meat had the highest protein ratio. Rabbit and lamb meats were followed, respectively ($P<0.001$). In terms of moisture beef meat had the highest values and there was no significant differences between other source of meat. In conclusion, as rabbit meat had higher protein ratio and lower fat ratio compared with other meat sources, it would be beneficial to expand the production and consumption. Chicken meat was advised to include in diets of patients suffering from obesity or cardio-vasculer diseases because of the highest protein and lower fat content. Lamb meat should be an indispensable part of diets due to its rich ash content. As the highest moisture content beef it could be preferred for making different kinds of meat by-products and consumed by the majority of people.
Keywords: Ash, Crude fat, Crude protein, Meat, Moisture

INTRODUCTION

Population growth and the rapid decrease in natural resources reveal the importance of healthy and adequate nutrition. For this reason, plant and animal production is very important. The fact that animal products are more valuable in nutrition and increases the importance of the livestock sector. Consumers' access to these products at lower costs and the amount of animal protein consumption in diets are important parameters that give information about the development level of countries (Frunză et al., 2023).

According to the OECD-FAO data of 2021, while meat consumption per capita was 35.2 kg in the world, this rate was 69.5 kg in developed countries and 27.6 kg in developing countries. In terms of animal species, per capita consumption of cattle, lamb, pork, and poultry in 2021 was reported as 6.3 kg, 1.8 kg, 11.8 kg, and 15.1 kg respectively in the world. It was reported that 11.1 kg of beef, 4.2 kg of lamb, and 21.9 kg of chicken meat were consumed in Türkiye.

In recent years, the quality of the yields obtained from animals has gained importance as well as the quantity. In parallel with the increase in their education

level and socio-economic status, consumers also consider nutrient content and quality characteristics in product selection. Meat quality has become a more demanded issue due to the awareness of consumers about eating quality and nutritional value. The sensory qualities of meat also have great importance in terms of consumer preference. At the purchasing stage, consumers generally evaluate the color and oiliness of raw meat as quality criteria. The flavor of the meat consumed is related to softness, juiciness, aroma, and taste. A quality meat should be soft, high in moisture, containing more muscle fibers than connective tissue, pink in color, and have a suitable aroma (Kumar et al., 2023; Nutautaitė et al., 2023).

Moisture, crude protein, crude fat, and ash ratio constitute the chemical composition of meat. It is varied according to the species of animal, genotype, sex, age, body condition score, nutritional status, and muscle structure of the animal (Ketoon et al., 2014). Since chicken meat is rich in protein with low fat content, it is an indispensable food source for patients with cardiovascular system and also for people undergoing obesity treatment. In addition, in economic terms, its consumption is in the first ranks in Türkiye, since its price is more reasonable compared to beef and lamb meat. Rabbit meat is an important functional food with high nutritive value due to its lower fat content, higher unsaturated fatty acids, and containing essential amino acids and some vitamin groups. In studies based on red meat consumption levels, the most consumed red meat is beef and lamb, sheep, goat, and kid meat follow it (Akçay and Vatansever, 2010; Kaygısız et al., 2022).

In terms of today's economic conditions, meat of beef and lamb are among the foods that people from all walks cannot easily consume. Chicken meat is preferred more than beef and lamb meat because the price of the chicken meat is relatively cheaper. While rabbit meat production is made professionally as an alternative meat source in China, Egypt, and many European countries; it is a meat product that is not widely consumed in Türkiye (Poławska et al., 2013; Saygin and Demirbaş, 2017; Saygın and Demirbaş, 2018).

The study aimed to comparatively examine meats originated from beef, lamb, chicken, and rabbit consumed in Türkiye in terms of nutrient content, and to reveal their superior aspects compared to each other, and to determine the place of rabbit meat, which is not commonly consumed among other meats.

MATERIALS AND METHODS

This study was carried out with the permission of Balıkesir University Animal Experiments Local Ethics Committee dated 18/05/2023 and numbered 2023/4-3.

Material

In the study, a total of 48 meat samples, 12 from each of the beef, lamb, chicken, and rabbit species, were used, provided that each of them belonged to a different animal. Beef samples were obtained from 2-3 years old Holstein steers, lamb samples were from 3-6 months Kıvırcık × Merino crossbred lambs, chicken samples were obtained from Ross 308 broiler hybrids slaughtered at 42 days of age, and rabbit meat samples were from New Zealand rabbits slaughtered at 3 months of age. Within the scope of the study, the chemical composition of the meat was investigated using the methods reported in AOAC (2000).

Method

For determination of moisture, the meat samples were dried at 110°C for 24 hours as the drying method (AOAC, 950.46) Weights were recorded and moisture content was calculated. The Kjeldahl method (AOAC, 928.08) was used for crude protein determination. Analysis was carried out by following digestion, distillation and titration steps. Ash content was determined by weighing the sample after it was incinerated at 550°C for 4 hours (AOAC, 920.153). Crude fat content was determined by the Soxhlet extraction (AOAC, 991.36) method as extracting the samples with ether by melting the fat in it.

Statistical Analysis

The research findings were analyzed with the SPSS 25.0 package program. Significant differences between the groups were determined by one way analysis of variance. Tukey test was used for multiple comparisons between groups with significant differences. The significance level was determined as 0.05.

RESULTS AND DISCUSSION

The chemical composition ratios of meat samples obtained from different species of animals in the study were given in Table 1.

Beef had the highest moisture content ($P<0.001$) shown in Table 1. Lamb, rabbit, and chicken meat had lower values and no significant difference was found between them (Figure 1).

Water has the highest proportional value in the composition of meat (65-80%). It is an important thermoregulator and solvent as well as taking important roles in cell and organ metabolism also transport of metabolites and wastes (Ketoon et al., 2014). The water holding capacity of the meat is an important factor affecting the appearance, color, tenderness, taste, and aroma of the meat (Apple and Yancey, 2013). The amount of water in the meat is an important factor affecting the profitability in terms of weight loss during the waiting processes of the meat (resting, packaging, freezing, transportation). At the same time, meat that does not lose its water depending on the cooking methods and is able to keep its content is evaluated quality meat category (Belichovska et al., 2017; Lima et al., 2022).

Table 1. Chemical composition values of meat samples obtained from different species of animals

Meat Sample	Moisture (%)	Ash (%)	Crude Fat (%)	Crude Protein (%)
Beef (n = 12)	75.52 ± 0.64 a	1.28 ± 0.09 a	0.81 ± 0.10 a	20.67 ± 0.50 a
Lamb (n = 12)	71.15 ± 0.75 b	2.25 ± 0.05 b	2.99 ± 0.57 b	22.16 ± 0.28 ab
Rabbit (n = 12)	72.34 ± 0.93 b	1.36 ± 0.03 ac	0.68 ± 0.06 a	23.65 ± 0.45 b
Chicken (n = 12)	71.79 ± 0.35 b	1.60 ± 0.06 c	0.69 ± 0.11 a	27.60 ± 0.49 c
P	***	***	***	***

a, b, c: Values within a column with different superscript differ significantly at P<0.05, ***: P<0.001

In the study moisture values determined from beef were similar to the values found in other studies (Ngom et al., 2022; Salim et al., 2023; Santana et al., 2023). However, the moisture value in Korean Hanwoo cattle was reported to be lower (69.21%) than the determined value (Bostami et al., 2023). The reason for this could be interpreted as the proportionally higher fat content in the meat composition of Korean Hanwoo cattle. The moisture content in lamb meat was similar (Junkuszew et al., 2020; Khal-Azzawi and Albashr, 2022; Lima et al., 2022), lower (Mioč et al., 2007; Meng et al., 2023; Radzik-Rant et al., 2023) or higher than (Romero-Bernal et al., 2017; Liang et al., 2023) other reported researches. The moisture content reported for chicken meat was similar (Silva Frasao et al., 2021; Xu et al., 2021) to the rates found in the study. Since most of the chicken meat offered for commercial consumption in the market is hybrid, it is expected that the data obtained would be uniform. The moisture content found for rabbit meat in the study was similar to the moisture rates reported in other studies (Bhatt et al., 2023; Kumar et al., 2023).

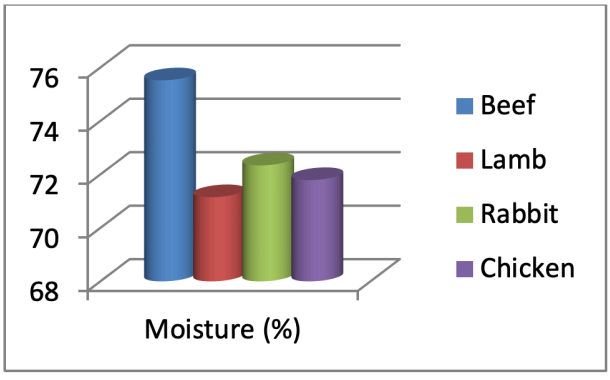

Figure 1. Moisture content of meat samples obtained from different species of animals

While the highest value in terms of ash ratio was determined for lamb (Figure 2); beef ash ratio was found to be lower than chicken meat (P<0.001). There was no significant difference between beef and rabbit meat also chicken and rabbit meat in terms of ash content as shown in Table 1.

Ash is the mineral substance of the meat and very important for human health. It is generally stored in teeth and bones (calcium, phosphate, magnesium). Apart from this, there are also mineral substances stored in body fluids (iron, sodium, potassium), enzymes (zinc), and nucleotides (phosphorus) (Ketoon et al., 2014). Because of mineral deficiencies caused significant discomfort in the body, it is very important to take them with food sources (Williams, 2007; Soriano-Santos, 2010; Pereira and Vicente, 2013; Romero-Bernal et al., 2017).

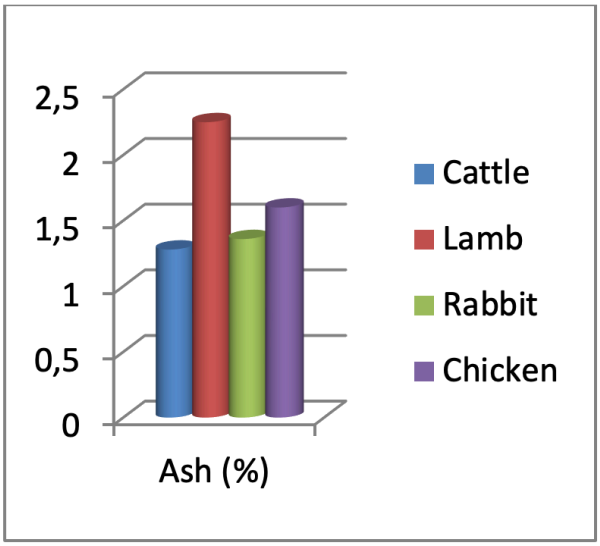

Figure 2. Ash content of meat samples obtained from different species of animals

The ash ratio in the lamb meat composition is higher than the values reported in the previous studies (Lima et al., 2022; Mercan et al., 2022; Liang et al., 2023). For the beef meat composition values although Santana et al. (2023) founded higher ash values; some of researchers found similar values (Oliveira et al., 2021; Bostami et al., 2023). Rabbit ash ratios found in the study were similar (Cardinali et al., 2015; Galeano-Díaz et al., 2023) higher (Neagu et al., 2023; Nutautaitė et al., 2023) than the other studies. Chicken ash values were found lower (Hashim et al., 2013; Cullere and Dalle Zotte, 2018) or higher (Sugiharto et al., 2022; Fathi et al., 2023) than previous reports.

While chicken meat had the highest value in terms of crude protein; it was determined that rabbit, lamb, and beef meat contain protein at decreasing rates, respectively (P<0.001) (Figure 3). There were no significant differences between lamb and rabbit meat in

terms of crude protein ratio. Beef and lamb meat had the lowest protein ratio as shown in Table 1.

Since proteins are the basic building blocks of the organism, they are key elements that should be included in diets. Proteins are essential nutrients that enable the development of muscles and organs in youngs and direct the body functions in adults. An adult person needs about 70-80 g of protein per day. About half of this value should be from animal origin. Protein content in meat is under the influence of many factors such as species, genotype, age, gender, and ration composition. Animals with the same genotype may have different nutrient contents. (Pereira and Vicente, 2013; Marangoni et al., 2015). In human nutrition, proteins from animal origin have an important place in terms of essential amino acids and fatty acids include. It is generally desirable to have a higher protein content in meat products (Akçapınar and Özbeyaz, 2021).

The protein values found for cattle in the study were similar to some of the values found in the researches (Hamed Hammad Mohammed et al., 2020; Pouzo et al., 2023; Santana et al., 2023), higher than some (Salim et al., 2023) and lower than some (Oliveira et al., 2021; Ngom et al., 2022). While the values reported for sheep were in agreement with (Costa et al., 2009; Romero-Bernal et al., 2017; Radzik-rant et al., 2023) or higher than literature reports and findings (Mioč et al., 2007; Lima et al., 2022; Latoch et al., 2023; Lunesu et al., 2023; Uushona et al., 2023). The rates found for chicken meat were higher than the reported values (Cullere and Dalle Zotte, 2018; Xu et al., 2021; Sugiharto et al., 2022; Weng et al., 2022; Fathi et al., 2023). The protein values found for rabbit meat were similar to the results found in the other studies (Belichovska et al., 2017; Cullere and Dalle Zotte, 2018; Frunză et al., 2023; Galeano-Díaz et al., 2023).

While the highest value in terms of fat ratio was determined in lamb meat ($P<0.001$) (Figure 4); there was no significant difference between chicken, beef, and rabbit meats (Table 1).

Fats are related tenderness, flavor, and aroma of meat products. In terms of human health, unsaturated fatty acids desired to be higher in the composition of fatty acid profile of meat and saturated fatty acids should be lower. It is important for meat products to include lower fat content in the diets of individuals who have problems with the cardio-vascular system or obesity. The fat ratio in the meat composition varies depending on various factors (Williams, 2007; Pereira and Vicente, 2013; Marangoni et al., 2015). Since the muscle development is higher in male animals, the protein ratio is proportionally higher, fat content is lower (Ngom et al., 2022; Santana et al., 2023). Carcass score is also one of the parameters to be taken into account as it affects fatness (Rubayet Bostami et al., 2018).

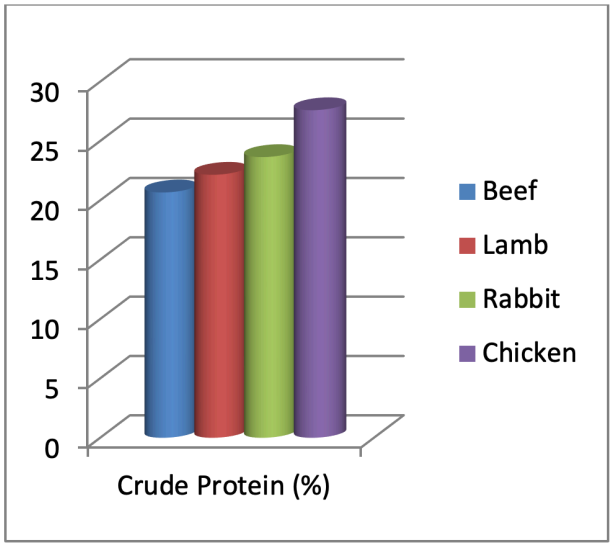

Figure 3. Crude protein content of meat samples obtained from different species of animals

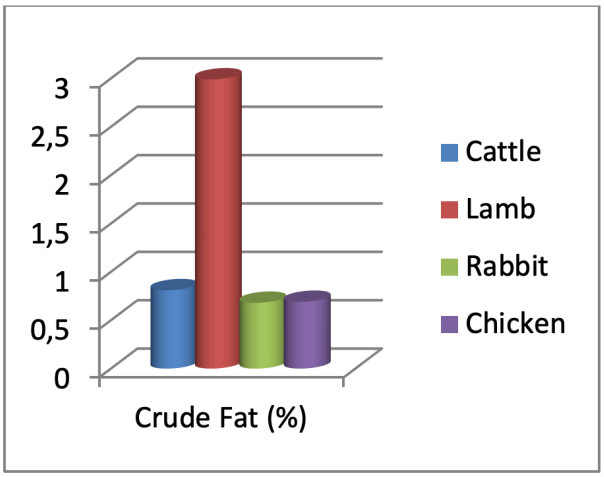

Figure 4. Crude fat content of meat samples obtained from different species of animals

The fat values presented in the literature reports were similar (Romero-Bernal et al., 2017), lower (Mioč et al., 2007), or higher (Junkuszew et al., 2020; Lima et al., 2022; Latoch et al., 2023) for lambs; higher for beef (Rubayet Bostami et al., 2018; Hamed Hammad Mohammed et al., 2020; Ngom et al., 2022; Santana et al., 2023); higher (Cullere et al., 2018; Xu et al., 2021) and similar (Weng et al., 2022; Fathi et al., 2023) for chicken; similar (Cardinali et al., 2015; Dalle Zotte et al., 2018; Kumar et al., 2023) and higher (Haque et al., 2016; Frunză et al., 2023; Neagu et al., 2023; Nutautaitė et al., 2023) for rabbit meat.

CONCLUSION

As a result of the study, it was determined that different species of meat animals have varied rates of moisture, ash, crude fat, and crude protein levels. Chicken meat had the highest protein ratio. Rabbit and lamb meats were followed it. Beef meat had the highest values of

moisture value. The highest value in terms of fat and ash ratio were determined in lamb meat. Chicken meat was advised to include in diets of patients suffer from obesity or cardio-vasculer diseases because of higher protein and lower fat content. Lamb meat should be an indispensable part of diets due to its rich mineral content. As the highest moisture content beef meat could be preferred for making different kinds of meat by-products and consumed by the majority of people. As rabbit meat had higher protein and lower fat ratio compared with other meat sources, it would be beneficial to expand the production and consumption of rabbit meat.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

Experimental design was planned by BY, MZ, MG, ÖVA; meat samples were collected by BY, chemical composition analysis were performed by MZ, MG, BBP, ÇE, EO; statistical analysis were done by BY and ÖVA; results were evaluated by BY, MZ and ÖVA; manuscript was written by BY and all authors contributed to the final version of the manuscript.

Ethics committee approval

This study was carried out with the permission of Balıkesir University Animal Experiments Local Ethics Committee dated 18/05/2023 and numbered 2023/4-3.

Funding

This study was supported by the TUBITAK (TUBITAK-2209-1919B012002536).

Data availability

Data could be shared if requested from corresponding author (B. Yaranoğlu).

Consent for publication

Not applicable

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RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(3): 588-595
DOI: https://doi.org/10.31015/jaefs.2023.3.13

The utilization of vaporized ethyl pyruvate for decontamination of lettuce from *E. coli* O157:H7

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Citation: Ucak Ozkaya, G. (2023). The utilization of vaporized ethyl pyruvate for decontamination of lettuce from *E. coli* O157:H7. International Journal of Agriculture, Environment and Food Sciences, 7 (3), 588-595

Abstract

The objective of this study was to utilize vaporized ethyl pyruvate (EP) as a means to enhance the safety of lettuce for human consumption. For this purpose, the antimicrobial activity of EP was evaluated on lettuce dipping-inoculated with *Escherichia coli* O157:H7 ATCC 25150. Inoculated samples for antimicrobial analysis and non-inoculated samples for organoleptic analysis (color and sensorial analysis) were treated with 0, 42, 105, and 420 ppm EP and then stored at 4 °C for 7 days and 10 °C for 5 days. Following a storage period of 7 days at a temperature of 4 °C, it was observed that the EP concentrations of 42, 105, and 420 ppm resulted in reductions of 0.8, 1.5, and 3.4 log, respectively, in the population of *E. coli* O157:H7 on lettuce. After a period of 5 days at a temperature of 10 °C, the presence of *E. coli* O157:H7 was observed to decrease by 1.3, 2.1, and 2.2 log. This reduction in bacterial count was attributed to the application of 42, 105, and 420 ppm of EP, respectively. In conclusion, based on the evaluation of organoleptic and color properties, it is suggested that the treatment involving a concentration of 42 ppm EP at 10 °C for 3 days can be a viable non-thermal method for effectively inhibiting bacterial growth.

Keywords: Vaporized ethyl pyruvate, *E. coli* O157:H7, Decontamination, Lettuce, Food safety

Received: July 26, 2023
Revised: August 22, 2023
Accepted: August 24, 2023
Published Online: September 21, 2023

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Available online at
https://jaefs.com/
https://dergipark.org.tr/jaefs

INTRODUCTION

Lettuce (*Lactuca sativa* L.), a member of the cabbage family, is a significant fresh vegetable, and its leaves are widely seen in salads and sandwiches (Mou, 2008, 2009). Lettuce is characterized by its low caloric, fat, and sodium content, while also being a source of dietary fiber, iron, folate, and vitamin C. In addition, lettuce is a notable provider of various bioactive compounds. Lettuce has been found to contain bioactive chemicals that exhibit anti-inflammatory, cholesterol-lowering, and anti-diabetic properties both *in vitro* and *in vivo*. (Kim et al., 2016). Therefore, lettuce may substantially contribute to increasing the nutritional content of diets (Kenny & O'Beirne, 2009). Unfortunately, vegetables are susceptible to infection by pathogenic bacteria, including *Listeria monocytogenes*, *Escherichia coli* O157:H7, and *Staphylococcus aureus*, throughout different stages of production, encompassing harvest, storage, and transportation. (Abadias et al., 2008). It is advisable for customers to engage in the practice of washing fresh vegetables and fruits as a precautionary measure against potential microbial contamination resulting from the presence of pathogens. Although the act of washing fresh vegetables under running tap water effectively eliminates dirt and other particles, it does not completely eliminate surface bacteria. Tap water has little or no impact on microorganisms found in fresh vegetables at 10^3-10^9 CFU/g (Koseki et al., 2001).

The market for minimally processed vegetables (MPV) has shown significant growth in recent years due to the heightened consumer awareness. The fresh-cut produce industry frequently uses chlorine solutions as a prevalent technique for mitigating the microbial load on vegetables (Tirpanalan et al., 2011). Nevertheless, the significance of discovering novel decontamination technologies is amplified by public health apprehensions regarding the potential development of trihalomethanes, a known carcinogen, as well as legislative restrictions on chlorine usage (Millan-Sango et al., 2016). Researchers have investigated three distinct categories of alternatives to chlorine disinfection. The three categories of sanitization methods include chemical sanitizers such as chlorine dioxide, hydrogen peroxide, and ozone; natural antimicrobials such as organic acids, plant extracts, and protective cultures; and physical decontamination methods such as irradiation, ultraviolet (UV) treatment, and electrolyzed water (Meireles et al., 2016).

Therein, we utilized ethyl pyruvate (EP), a straightforward derivative of pyruvic acid, as a means to eliminate *E. coli* O157:H7 contamination on lettuce. According to the evaluation conducted by the United States Food and Drug Administration (FDA), EP has been categorized as being safe for consumption in food products. A research group first reported that vaporized EP could be used to decontaminate green onion and baby spinach (Durak et al., 2012) followed by parsley decontamination by Tornuk and Durak (2015), control of postharvest quality and fungal damage of strawberry and cherry fruits by Bozkurt et al. (2016), reduction of *Salmonella* Enteritidis in raw chicken meat by Çetin et al. (2019), inactivation of *Listeria monocytogenes* on sausage surface by Cetin et al. (2019) and recently EP treatment against *Escherichia coli* O157:H7 and *Salmonella* Typhimurium on cherry tomatoes by Ucak Ozkaya et al. (2021). This study aimed to examine the efficacy of vaporized EP decontamination in reducing the presence of *E. coli* O157:H7 on lettuce samples.

MATERIALS AND METHODS

Materials

Lettuce was obtained from a local grocery shop in Istanbul and kept at 4 °C prior to use. The strain *E. coli* O157:H7 ATCC, which was acquired from Acibadem University, was employed in order to assess the antibacterial efficacy of vaporized ethyl pyruvate on lettuce. The stock cultures, maintained at -80 °C with 15% glycerol, were streaked onto Nutrient agar (Merck, Darmstadt, Germany) and incubated at 37 °C overnight. Following this, a single colony was cultured in Nutrient broth (Merck, Darmstadt, Germany) and subsequently incubated at a temperature of 37 °C for a period of 24 h. The initial bacterial inoculum concentration for the dipping inoculation technique to introduce contamination to lettuce was approximately 10.4 log CFU/mL for *E. coli* O157:H7.

Preparation and inoculation of lettuce

Prior to conducting tests for EP treatment, the initial verification of the absence of *E. coli* O157:H7 in the samples was performed. Initial washing with tap water for 10 min was carried out to remove unwanted residues and reduce native bacteria. Following that, the samples were subjected to a deionized water rinse and then dried within a biosafety cabinet at ambient temperature for a duration of 30 min, while being exposed to ultraviolet (UV) treatment. The lettuce samples that were contaminated with *E. coli* O157:H7 were subjected to a drying process at ambient temperature for a period of 2 h subsequent to the inoculation procedure.

Application of vaporized ethyl pyruvate

Lettuce samples that were purposely contaminated with *E. coli* O157:H7 were subjected to treatment using vaporized ethyl pyruvate (EP, 98% purity; Sigma-Aldrich, St. Louis, United States). Specifically, the experiment involved the placement of three lettuce leaves that had been inoculated with microorganisms. Each lettuce leaf was individually placed in a food container with a closed lid. The food container had dimensions of 18.00 cm × 25.50 cm × 9.00 cm and was manufactured by Bora Plastic in Istanbul, Turkey. To maintain humidity, a sponge soaked in 20 mL of deionized water was included in the container. Additionally, Kim Wipes tissues from Kimberly-Clark in Rosewell, GA were used. These tissues were treated with different amounts of EP, specifically 105, 260, and 1.050 µL, which corresponded to concentrations of 42, 105, and 420 ppm, respectively. Samples containing microorganisms and the Kim Wipes tissues added EP were put into the container. Following the closure of the container, EP-treated samples with microorganisms and control samples were subjected to storage conditions of 4 °C for a duration of 7 days, and stored at 10 °C for a period of 5 days.

Microbiological analysis

The spread plate technique was utilized to conduct microbiological analyses at various time intervals during storage. Specifically, analyses were conducted on 0, 1, 3, 5, and 7 days of storage at 4 °C, and on 0, 1, 3, and 5 days of storage at 10 °C. The samples with bacteria, both control and EP-treated, were enumerated for *E. coli* O157:H7 using Sorbitol MacConkey agar (Merck, Darmstadt, Germany). The homogenization of samples was conducted using a Stomacher device (MiniMix 100, Interscience, St. Nom, France) for a duration of 2 min in sterile 0.1% peptone water (1:2, w/v). Subsequently, 1 mL of the resultant mixture was subjected to serial dilution, with each dilution being added to test tubes containing 9 mL of sterile peptone water. The plates were prepared by applying suitable dilutions, followed by incubation at a temperature of 37 °C for a duration of 24 h. After the incubation period, the colonies were counted and expressed as logarithm of colony forming units per gram

(log CFU/g).

Determination of inhibition level

The assessment of growth inhibition levels induced by various concentrations of ethyl pyruvate on *E. coli* O157:H7 was conducted utilizing Equation 1, as reported by Sagdic (2003):

$$GIL(\%) = \frac{(P_C - P_T)}{P_C} \times 100 \qquad (1)$$

The variables and represent the microbial populations of control and EP-treated samples, respectively, at a specific time.

Color analysis

The color of non-inoculated, EP-treated samples and control samples was measured using a colorimeter (Konica Minolta CR-400, Osaka, Japan) at 4 °C on days 0, 1, 3, 5, and 7, and at 10 °C on days 0, 1, 3, and 5. The luminosity value (L^*), chromaticity on the green to red axis (a^*), and chromaticity on the blue to yellow axis (b^*) were measured in triplicate, and the average values were reported.

Sensory assessment

The sensory evaluation was carried out on control (no EP treatment) and EP-treated (42, 105, and 420 ppm) lettuce samples without microorganisms. The samples marked with three-digit numbers were tested on days 0, 1, 3, 5, and 7 at 4 °C and days 0, 1, 3, and 5 at 10 °C. The 20 panelists conducted a simultaneous evaluation of the samples, scoring them based on color, odor, texture, and overall quality.

Statistical analysis

Statistical analysis was conducted using JMP statistical software (version 9.0, 2010, SAS Institute, Cary, NC) after performing all experiments three times on dependent samples. The study employed a two-way analysis of variance (ANOVA) and Tukey's multiple comparison test to examine the disparities in bacterial populations, color, and sensory attributes between lettuces treated with EP and a control group. The data in the tables were presented in the form of mean values accompanied by their corresponding standard deviations. Statistical significance was determined at a significance level of $p<0.05$.

RESULTS AND DISCUSSION

EP-treatment based inactivation of *E. coli* O157:H7 on lettuce

The evaporative nature of the EP facilitates its swift transfer to the stored materials. This particular capability confers a benefit in the elimination of pathogenic bacteria. In the present study, three different EP concentrations, two different storage temperatures, and seven days of storage time were used. Lettuce leaves contaminated with *E. coli* O157:H7 were treated with ethyl pyruvate in a vaporized form. The treated leaves were then stored at 4 °C for a duration of 7 days and at 10 °C for a duration of 5 days. Table 1 displays the outcomes of microbial count pertaining to *E. coli* O157:H7 when subjected to EP treatment. The initial population level of *E. coli* O157:H7 that adhered to the lettuce samples was estimated to be approximately 10.4 log CFU/g. The application of EP at concentrations of 42 and 105 ppm did not yield a statistically significant impact on the decontamination of the samples ($p>0.05$). Nevertheless, the inhibition level of the 420 ppm EP treatment on lettuce leaves after one day of storage at 4 °C was found to be 1.2 log CFU/g ($p<0.05$). On days 3 and 5, the reduction amount of 420 ppm EP was 2 and 3.3 log CFU/g, respectively. On the 7th day of storage, all EP concentrations exhibited a substantial reduction in comparison to the control samples ($p<0.05$). However, the most effective EP concentration was determined to be 420 ppm during the storage period at 4 °C.

On the first day at 10 °C, the concentration of EP at 420 ppm led to a reduction of 0.8 log in comparison to the control samples. The reduction levels of 42, 105, and 420 ppm EP were 0.9, 1.0, and 1.7 log on the 3rd day of storage, respectively ($p<0.05$). Following a storage period of 5 days, the samples exhibited a notable decrease in the presence of *E. coli* O157:H7. Specifically, the log reductions for *E. coli* O157:H7 were 1.3, 2.1, and 2.2 when treated with EP concentrations of 42, 105, and 420 ppm, respectively. These findings suggest that the application of EP at varying concentrations effectively deactivated the bacteria in the samples compared to the control group. However, no statistically meaningful difference was identified between them on the 5th day at 10 °C ($p>0.05$). The day when all concentrations are most effective in the inactivation of *E. coli* O157:H7 was found to be the 5th day.

The inactivation of *E. coli* O157:H7 on lettuce was effectively achieved through the application of EP concentrations at 4 °C and 10 °C. Nevertheless, the antimicrobial effect of EP was greater at 4 °C. Several research studies have reported that 10 °C exhibits greater efficacy in terms of bacterial inactivation (Durak et al., 2012; Ucak Ozkaya et al., 2021). While the dipping method was used for inoculation in the present study, the study conducted by Ucak Ozkaya et al. (2021) involved the attachment of bacteria onto the surface of tomato samples through the utilization of the spot inoculation technique. This means that lowering the bacterial density in a specific region could potentially be a more feasible task. Nonetheless, another study indicated that the inoculation method did not significantly affect the effectiveness of UV application on the reduction of bacteria (Guo et al., 2019). In addition, the initial

number of microorganisms attached to the lettuce samples was high. A study on this subject indicates that the effectiveness of the antimicrobial agent employed may be influenced by the concentration of inoculation (Tornuk & Durak, 2015).

Various decontamination methods have been employed to prolong the shelf life of products and eliminate pathogenic bacteria present in fresh fruits and vegetables. Of these, essential oils (Rossi et al., 2019), allyl isothiocyanate (Guo et al., 2018), and ozone (Aday & Caner, 2014) were the most commonly used vaporized antimicrobials. Numerous research endeavors have been undertaken to examine the effects of EP on the eradication of pathogenic bacteria present in unprocessed vegetables and fruit (Bozkurt et al., 2016; Durak et al., 2012; Tornuk & Durak, 2015). Durak et al. (2012) conducted an investigation to evaluate the antimicrobial efficacy of EP in the process of decontaminating green onions and spinach contaminated with *E. coli* O157:H7. The EP treatment demonstrated efficacy in achieving a reduction of greater than 4.7 log in the population of *E. coli* O157:H7 on green onions following the completion of the storage period. These findings were determined to be consistent with our own results. The study conducted by Tornuk and Durak (2015) yielded similar findings, as they utilized EP treatment on fresh parsley to deactivate *S. aureus* and *E. coli* O157:H7.

GILs of *E. coli* O157:H7 at 4 °C and 10 °C

The GILs of *E. coli* O157:H7 inoculated on lettuce stored at 4 °C and 10 °C are shown in Figure 1. The figure presented clearly illustrates the relationship between inhibition levels and concentration dependence. The concentration of 420 ppm EP at 4 °C exhibited a greater inhibition rate compared to that observed at 10 °C at the end of the storage. On the fifth day, it was observed that the inhibition rate was higher for concentrations of 42 ppm and 105 ppm at 10 °C as compared to 4 °C. However, a concentration of 420 ppm exhibited a lower effect at 10 °C. The GILs obtained by the 42 ppm and 105 ppm EP concentrations at 10 °C had nearly the same rate.

Lettuce is a highly perishable vegetable, and as such, it is advisable to consume it in its fresh state. However, it can also be preserved for a few days by storing it at a temperature range of 4-5 °C (Hoza et al., 2020). The reason behind utilizing 10 °C in our research was to increase the impact of ethyl pyruvate on the duration of storage under elevated thermal conditions. Nevertheless, the data collected from the experiment revealed that the antimicrobial effectiveness of EP was relatively diminished at 10 °C. These findings align with the conclusions drawn by Ijabadeniyi et al. (2020). Besides storage temperature, the duration of storage and the concentration of antimicrobial agents are also influential factors that impact the efficacy of decontamination. The findings of this study provide clear evidence that higher concentrations of EP were effective in eliminating microbial activity. Furthermore, the extent of the logarithmic decrease in microbial activity was positively associated with the length of time the samples were stored.

Color and sensorial evaluation of lettuce

The color values (*L**, *a**, and *b**) of lettuce without *E. coli* O157:H7 after EP treatment are summarized in Table 2. A reduction in *L** values was observed on day 3 at 4 °C when comparing the control sample to those treated with 105 ppm and 420 ppm ($p<0.05$). The *L** value of lettuce samples was maintained by the 42 ppm and 105 ppm EP applications on the seventh day. A reduction in the *L** value was observed in both the control and 420 ppm EP-treated samples upon completion of the storage duration. The treatment of 420 ppm was found to exhibit the minimum *L** value. Regarding the storage at 10 °C, it was observed that the application of 105 ppm and 420 ppm EP resulted in the preservation of the *L** value on day 1, in contrast to the control group. However, a decline in the *L** value was noted in lettuce samples treated with 42 ppm EP. As a result of the presence of 420 ppm EP, a decrease in *L** value was seen at the end of storage. The EP with concentrations of 105 ppm and 420 ppm, in general, maintained the *L** value of lettuce during the storage period.

Applications of 105 ppm and 420 ppm EP increased *a** value during storage at 4 °C. The *a** value was better retained in the 42 ppm EP lettuce samples and the control sample. The observed increase in the *a**

Table 1. Inactivation of *E. coli* O157:H7 on lettuce by vaporized EP at 4 °C for 7 days and 10 °C for 5 days.

EP concentration (ppm)	*E. coli* O157:H7 count (log CFU/g)								
	4 °C					10 °C			
	0 day	1 day	3 days	5 days	7 days	0 day	1 day	3 days	5 days
0 (control)	10.4 ± 0.0^{aA}	10.1 ± 0.2^{aA}	10.5 ± 0.1^{aA}	10.4 ± 0.5^{aA}	10.3 ± 0.3^{aA}	10.4 ± 0.0^{bA}	10.4 ± 0.1^{bA}	10.8 ± 0.1^{aA}	10.7 ± 0.8^{aA}
42	10.4 ± 0.0^{aA}	10.2 ± 0.1^{abA}	9.7 ± 0.1^{bcB}	9.6 ± 0.8^{bcA}	9.5 ± 0.2^{cAB}	10.4 ± 0.0^{aA}	10.1 ± 0.2^{abAB}	9.9 ± 0.2^{bB}	9.4 ± 0.2^{cB}
105	10.4 ± 0.0^{aA}	9.9 ± 0.2^{abA}	9.4 ± 0.2^{abcB}	9.0 ± 0.6^{bcA}	8.8 ± 1.2^{cB}	10.4 ± 0.0^{aA}	10.1 ± 0.7^{aAB}	9.8 ± 0.5^{aB}	8.6 ± 0.1^{bB}
420	10.4 ± 0.0^{aA}	8.9 ± 0.7^{bB}	8.5 ± 0.4^{bC}	7.1 ± 1.6^{cB}	6.9 ± 0.4^{cC}	10.4 ± 0.0^{aA}	9.6 ± 0.1^{abB}	9.1 ± 0.1^{bcC}	8.5 ± 1.3^{cB}

$^{A-C}$: The same superscript uppercase letters show no significant ($p > 0.05$) differences between ethyl pyruvate concentrations within the same storage times. $^{a-c}$: The same superscript lowercase letters show no significant ($p > 0.05$) differences between storage times within the same ethyl pyruvate concentration.

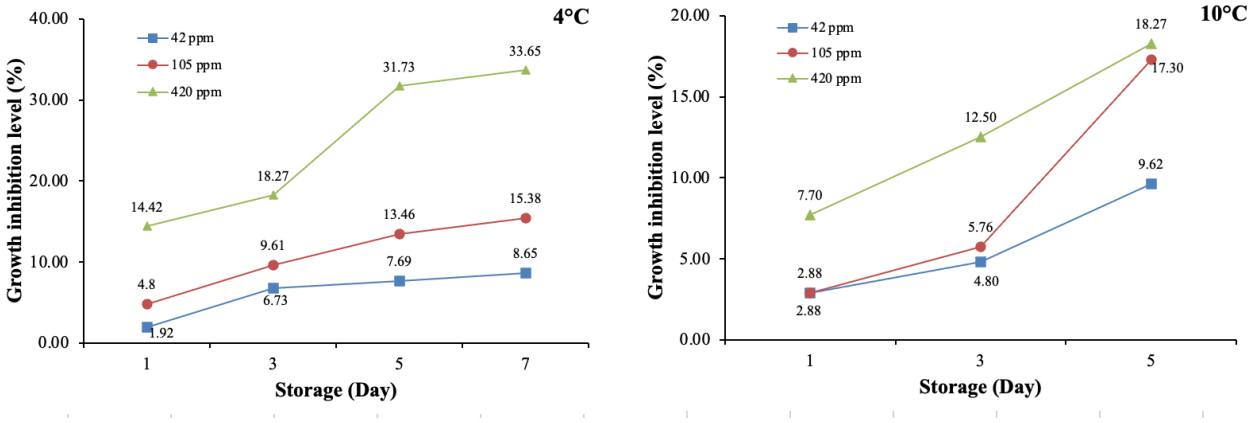

Figure 1. GILs of vaporized EP at different concentrations against *E. coli* O157:H7 on lettuce at 4 °C and 10 °C.

value corresponded with the rise in EP concentration, indicating an inability to sustain the green color. The 42 ppm EP application caused a decrease in *a** value during storage at 10 °C. Nevertheless, the observed rise was considered statistically insignificant on the 1st and 3rd days of storage ($p>0.05$). Upon completion of the storage period, it was observed that the utilization of 105 and 420 ppm EP increased the *a** value of samples ($p<0.05$).

The storage period exhibited an increase in the *b** value of samples due to the presence of 42 ppm EP at 4 °C. The samples exhibited comparable *b** values on the 3rd and 5th days of storage in the 105 ppm EP application. The 420 ppm EP showed the highest *b** value on the 7th day of storage. Upon completion of the storage period, it was noted that the utilization of 105 ppm of EP led to a reduction in the *b** value in comparison to the control sample. Regarding the storage conditions at 10 °C, no discernible variations were observed in the *b** values when comparing the control sample with the lettuce samples treated with EP, upon completion of the storage period ($p>0.05$).

The sensory scores of the samples are presented in Table 3. The data indicated a negative correlation between EP concentration and color scores relative to the control group at 4 °C. Upon evaluation of the concentrations concerning time, a decrease in the color scores was observed. After conducting an assessment of each concentration with respect to its corresponding time frame, no statistically significant change in the scores was detected ($p>0.05$). However, the application of 420 ppm EP on the 1st day and 105 ppm and 420 ppm EP applications on the 3rd day yielded a reduction in

Table 2. Color values for control and EP-treated lettuce stored at 4 °C and 10 °C.

	4 °C				10 °C			
Days	0 ppm (control)	42 ppm	105 ppm	420 ppm	0 ppm (control)	42 ppm	105 ppm	420 ppm
*L**								
0	57.11±5.1^{aB}				57.11±5.1^{aB}			
1	65.85±3.4^{aA}	61.33±3.2^{bAB}	65.89±1.3^{aA}	60.57±3.2^{bA}	58.34±2.3^{aAB}	53.95±1.0^{bB}	56.97±2.4^{aA}	58.29±2.3^{aA}
3	64.33±7.1^{aA}	60.21±5.9^{abAB}	55.94±4.4^{bcB}	51.55±5.4^{cC}	61.33±4.3^{aA}	57.33±1.6^{abA}	59.68±8.6^{aA}	53.37±7.4^{bA}
7	58.93±1.8^{bB}	64.14±7.1^{aA}	59.07±5.1^{aB}	53.35±4.0^{cBC}				
*a**								
0	-14.39±1.6^{aA}				-14.39±1.6^{aA}			
1	-16.12±0.6^{cB}	-12.45±2.7^{aA}	-15.53±0.4^{bcC}	-14.30±0.1^{bB}	-17.24±2.1^{aB}	-16.34±0.6^{aB}	-16.82±0.1^{aC}	-16.32±0.5^{aC}
3	-16.07±1.4^{cB}	-15.10±2.1^{bcB}	-12.59±3.6^{abAB}	-10.25±3.1^{aA}	-16.71±1.5^{bB}	-15.93±1.5^{bB}	-12.01±2.0^{aA}	-10.31±2.0^{aA}
7	-16.37±1.3^{bB}	-15.24±3.1^{bB}	-11.02±3.8^{aA}	-9.83±1.7^{aA}				
*b**								
0	25.28±3.5^{aB}				25.28±3.5^{aB}			
1	31.33±2.1^{aA}	23.16±4.1^{cC}	31.86±1.1^{aA}	27.50±0.7^{bAB}	32.44±3.9^{aA}	27.88±1.0^{bAB}	27.64±0.8^{bAB}	29.37±1.2^{bA}
3	29.53±4.7^{aA}	27.81±4.4^{abAB}	23.76±3.5^{bB}	24.45±4.6^{bB}	30.97±4.5^{aA}	28.60±3.5^{aA}	29.88±3.9^{aA}	26.71±5.8^{aAB}
7	29.47±3.4^{aA}	31.20±6.4^{aA}	23.72±6.2^{bB}	29.11±3.6^{aA}				

$^{A-C}$: The same superscript uppercase letters show no significant ($p > 0.05$) differences between ethyl pyruvate concentrations within the same storage times. $^{a-c}$: The same superscript lowercase letters show no significant ($p > 0.05$) differences between storage times within the same ethyl pyruvate concentration.

Table 3. Sensory scores of control and EP-treated lettuce stored at 4 °C and 10 °C.

	4 °C				10 °C			
Days	0 ppm (control)	42 ppm	105 ppm	420 ppm	0 ppm (control)	42 ppm	105 ppm	420 ppm
Color								
0	7.5±1.5^{aAB}				7.5±1.5^{aA}			
1	5.7±1.3^{bC}	7.3±0.9^{aA}	6.4±1.0^{bAB}	4.7±1.4^{cB}	6.2±1.3^{aB}	6.1±1.4^{aB}	4.9±1.2^{bB}	3.8±1.5^{cB}
3	8.2±1.2^{aA}	6.7±1.7^{aAB}	4.0±1.9^{bC}	3.2±1.9^{bC}	7.9±1.1^{aA}	7.1±1.1^{aAB}	4.9±2.1^{bB}	3.9±1.9^{bB}
7	6.7±1.5^{aBC}	5.6±2.0^{aB}	5.3±2.1^{abBC}	3.6±2.0^{bBC}				
Odor								
0	6.6±1.6^{aAB}				6.6±1.6^{aA}			
1	6.2±1.1^{aB}	6.5±1.1^{aA}	5.4±1.1^{aB}	3.6±2.1^{bB}	5.8±1.2^{aA}	5.5±1.3^{aB}	4.8±1.2^{abB}	3.7±1.8^{bB}
3	7.7±1.4^{aB}	6.7±1.2^{abA}	5.2±1.7^{bcB}	4.1±2.2^{cB}	7.0±1.4^{aA}	6.6±1.4^{aAB}	5.4±1.9^{abAB}	4.0±2.4^{bB}
7	6.2±1.6^{aA}	6.1±1.6^{aA}	4.4±2.0^{abB}	3.6±2.7^{bB}				
Appearance								
0	7.1±1.5^{aB}				7.1±1.5^{aA}			
1	5.4±1.3^{bcC}	7.0±1.0^{aA}	6.1±1.3^{abA}	4.7±1.6^{cB}	6.4±1.3^{aA}	6.0±1.3^{aA}	4.7±1.2^{bB}	3.8±1.1^{bB}
3	8.3±0.9^{aA}	6.1±2.3^{bAB}	3.9±1.9^{cB}	3.2±1.7^{cC}	7.1±1.9^{aA}	6.3±2.2^{abA}	5.0±1.9^{bcB}	3.8±1.9^{cB}
7	6.1±1.3^{aBC}	5.3±2.0^{abB}	4.1±2.0^{bcB}	3.5±1.9^{cBC}				
Texture								
0	7.3±1.2^{aA}				7.3±1.2^{aA}			
1	5.3±1.4^{bcB}	7.2±1.0^{aAB}	6.1±1.3^{bB}	4.7±1.5^{cB}	6.2±1.5^{aB}	6.3±1.4^{aA}	4.8±1.3^{bB}	4.3±1.6^{bB}
3	7.9±1.5^{aA}	6.0±1.7^{bB}	4.2±1.9^{cC}	3.1±1.8^{cC}	7.2±1.1^{aAB}	6.6±1.4^{aA}	4.9±2.3^{bB}	3.1±1.6^{cB}
7	5.9±1.7^{aB}	6.2±2.0^{aAB}	5.2±2.1^{abBC}	3.6±2.2^{bBC}				
Overall quality								
0	7.6±0.9^{aA}				7.6±0.9^{aA}			
1	5.4±1.3^{bcB}	7.1±1.0^{aAB}	6.2±1.0^{abB}	4.7±1.4^{cB}	6.2±1.3^{aB}	6.2±1.3^{aB}	5.0±1.2^{bB}	3.9±1.2^{cB}
3	8.1±1.1^{aA}	6.3±1.8^{bB}	4.6±2.1^{cC}	3.7±2.1^{cB}	7.3±1.2^{aA}	6.7±1.4^{aAB}	5.0±2.0^{bB}	3.4±1.8^{bB}
7	6.1±1.3^{aB}	6.2±1.7^{aB}	5.1±2.0^{abBC}	3.6±2.2^{bB}				

$^{A-C}$: The same superscript uppercase letters show no significant ($p > 0.05$) differences between ethyl pyruvate concentrations within the same storage times. $^{a-c}$: The same superscript lowercase letters show no significant ($p > 0.05$) differences between storage times within the same ethyl pyruvate concentration.

odor scores when compared to control samples. As for appearance and texture scores, the 42 ppm EP application demonstrated results that were identical to those of the control sample. The application of concentrations of 105 ppm and 420 ppm of EP led to a decrease in both visual appearance and textural quality ratings over the course of the storage duration. Looking at the overall acceptability scores, lower scores were obtained for all EP concentrations compared to the control sample. In addition, the control sample experienced a decrease in scores as a result of storage.

The lettuce samples exhibited better preservation of their color and odor properties when subjected to the 42 ppm EP application at 10 °C. The 420 ppm EP resulted in the lowest scores for both color and odor. The control sample and the samples treated with the 42 ppm EP exhibited greater appearance and structure scores during the storage period at 10 °C. After the storage period, a decline in both visual and structural characteristics was observed in comparison to the control group, as evidenced by the corresponding scores. At the end of the storage at 10 °C, a deterioration in both appearance and texture characteristics was observed in comparison to the control group, as evidenced by the corresponding scores. In the overall assessment, the lettuce samples that were treated with 105 ppm and 420 ppm EP were rated less acceptable than the samples from the control group.

The importance of preserving or improving the sensory attributes of fresh-cut fruits and vegetables during antimicrobial treatments is paramount due to the common consumption of these products in their raw state. The objective of the current study was to evaluate and compare the sensory and color traits of lettuce samples treated with EP and those that were untreated (control). The application of EP demonstrated a protective effect on the sensory and color properties of lettuce samples. The optimal attainment of color and sensory attributes is achieved through the utilization of 42 ppm EP. Previous research has indicated that baby spinach samples treated with EP exhibited lower sensory attributes compared to control samples when stored at 4 °C and 10 °C (Durak et al., 2012), which aligns with the findings of our own study.

CONCLUSION

This study investigated the antimicrobial activity of EP treatments at various concentrations against *E. coli* O157:H7 that were inoculated onto fresh lettuce stored for a duration of 7 days at 4 °C and for 5 days at 10 °C. The efficacy of EP at varying concentrations was observed in the inactivation of *E. coli* O157:H7 on fresh lettuce. The control sample exhibited a consistent bacterial load at 4 °C, whereas an increase in bacterial load was observed at 10 °C. While it is possible to observe bacterial growth at a storage temperature of 10 °C, it is clear that the utilization of EP demonstrates effective control over bacterial development in the samples. It was also determined that different storage temperatures also changed the effectiveness of EP. Although storing lettuce samples at 10 °C did not appear to be efficient in inactivating bacteria, 42 ppm and 105 ppm EP treatments at the end of storage showed a better rate of bacterial decrease than at 4 °C. Nevertheless, the results indicated that the 420 ppm EP yielded better results at 4 °C. Despite the successful inactivation of bacteria, increasing the concentration of EP had adverse effects on the color and sensory attributes of lettuce after the third day of storage.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author contribution

The author read and approved the final manuscript. The author verifies that the Text, Figures, and Tables are original and that they have not been published before.

Ethics committee approval

Ethics committee approval is not required. This article does not contain any studies with human participants or animals performed by any of the author.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(3): 596-606
DOI: https://doi.org/10.31015/jaefs.2023.3.14

Examination of crop pattern change in the economic sustainability of agriculture

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Citation: Tok, N., Tunalioglu, R., Cobanoglu, F. (2023). Examination of crop pattern change in the economic sustainability of agriculture. International Journal of Agriculture, Environment and Food Sciences, 7 (3), 596-606

Received: July 28, 2023
Revised: August 20, 2023
Accepted: August 22, 2023
Published Online: September 23, 2023

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Available online at
https://jaefs.com/
https://dergipark.org.tr/jaefs

Abstract

Türkiye, in the 1980s, passed from an import substitution economic model to a free market economy. The agriculture sector has been the most affected by this transition. In this period, it was decided to abandon some production activities on the grounds that the deficit in the was due to country's budget was due to price supports in agriculture. Farmers whose agricultural potential has narrowed their field of activity have changed their crop patterns by embarking on new searches. In this study, the results of the product pattern changes of the local farmers after the national policy change were examined in a sample field (Akhisar), and the problems experienced after the unplanned production pattern changes and the solution proposals in the transition period from Turkey's protectionist agricultural policies to the liberal agricultural policies were examined. It is intended to be an example and a guide. In total, the opinions of 42 olive producers regarding their expectations from the state and other stakeholders in solving the problems they are experiencing after this change were analyzed with the qualitative method using the data obtained using the semi-structured question technique and the inductive approach. As a result, it was determined that the individual and courageous decisions taken by the farmers about product change forty years ago were not wrong, but in this process, the farmers experienced decreases in productivity and quality in production due to changes in climate, land structure and diseases. It has been determined that farmers need medium and long-term policies (structural and social tools) rather than short-term policies (price tools) currently applied. In addition, it has been concluded that the state should share the cost of crop pattern choices with farmers in order to ensure the sustainability of agriculture in the country.

Keywords: Agricultural policy, Crop pattern, Carmers' choice, Table olive, Qualitative method

INTRODUCTION

Agriculture, when Türkiye as in the world is evaluated, in terms of economic and social structures, is a sector of strategic importance to its geography and biodiversity (Atamer Balkan and Meral, 2017). Today, developed countries in the agricultural sector of the world have started to use knowledge (technology)-intensive production techniques by completing labor-intensive and capital-intensive production techniques. Türkiye has not yet completed the first and second production techniques. For this reason, it frequently updates its agricultural policies by renewing local programs and regional and national development plans. In this update, it acts in accordance with the constraints of international organizations (WTO, EU, IMF, etc) and agreements with obligation (Çağatay et al., 2010).

Due to the importance of agriculture in the economy and structural features in Türkiye, it has been supported by different policies since 1923 when the Republic was established. Although the main purpose is supplying security, the welfare of the producers and increasing efficiency, the applications have always been different. Because the number of populations living in the countryside for many years was considerable and the level of education was low, the political and economic sanctions of the country created differences. However, regardless of the liberal or protectionist policies, state support in the agricultural sector has always continued and has shown a change (Eğri, 2014).

The biggest change in agricultural policies in Türkiye, apart from the structural policies in the first years of the Republic founded, was experienced in the 1950s, 1980s and 2000s. The most important of these is the change in national economic policies under the name of "24 January Stabilization Program Decisions" and the most affected was the agricultural sector. Because in accordance with the liberal policies adopted in 1980 and the free market economy understanding, structural-social changes are foreseen in agriculture. The state decided to support the agricultural sector less, on the grounds of reducing intervention in the economy (Kılıç, 2020).

In accordance with this stabilization program, the number of agricultural crops supported in 1980 decreased from 24 to 18 in 1985 to 10 in 1990 (tobacco, wheat, barley, rye, maize, paddy, oat, sugar beet, poppy and chickpea). In this period, the decrease in the ratio of the amount of crop purchased to the total production amount adversely affected the producer as well as the decrease in the number of supported crops. For example, this decrease caused tobacco production to decrease from 75% to 39% and wheat production from 10% to 3% between 1980-1990 (Öztürk et al., 2008).

After the agricultural policies implemented after the 24 January 1980 decisions led to a decline in tobacco production, international tobacco companies entered the domestic market. These companies with eye-catching advertising activities led to an increase in cigarette consumption, in other words, policies relating to tobacco control in Türkiye kept the Turkish tobacco producers away from production and encouraged foreign enterpreneurship.

Tobacco production in Türkiye in the ongoing years

-In 1996 'Law numbered 4207 on the Prevention of the Harms ofTobacco Products and Control',

-In 2002 with 'the law numbered 4733 on the Organization and Duties of Tobacco and Alcohol Market Regulatory Agency', eliminating Tekel's (Institution that buys tobacco on behalf of the state) effectiveness,

-In 2004 with 'World Health Organization Framework Contract on Tobacco Control', it was taken continuously under control (Saraçoğlu and Öztürk, 2020).

Prior to changing agricultural policies in Türkiye, the largest tobacco producer province is Manisa. Even in some districts of Manisa such as Akhisar, tobacco production is carried out as monoculture (Anonymous, 2021d; Şahin and Taşlıgil, 2013). Tobacco production restricted by law in the said process negatively affected the economies of many regions, towns and small towns with a high income such as Akhisar. Hence, before "1980-24 January stability decisions" and the legislation on tobacco, while *tobacco* was the primary crop in all villages and towns of Akhisar, afterward tried to earn income, the production pattern has necessarily changed in favor of *olives*. In the 1990s in Akhisar, the tobacco cultivation area, which was 11 thousand hectares, decreased approximately 72% to three thousand hectares (Karabacak, 2017). In contrast to the contraction in valid production areas for many crops, when olive came to the top of the most important crops in agriculture in the world in recent years, olive gloves increased rapidly (Doğan, 2017).

Thus, Akhisar farmers tried to earn the income they lost in tobacco production, instead of the one-year crop plant, which has lost value with political decisions and laws since the 1990s, by substıtuting the *perennial olive plant*, which has become increasingly important in the world (Güner et al., 2010). In this change, from before 2000 to today, a farmer's trust in the knowledge and experience of many years plays a major role in olive cultivation as in tobacco (Ünsal, 2008). In addition, the farmer's crop pattern preference is also important to the aim of maintaining the current quality of life by providing an adequate level of income to her family (Günden, 2016).

The agricultural sector due to the low elasticity of supply and demand has been supported in Türkiye as in all the developed and developing world countries. Yet, there are some problems in Türkiye's agricultural policy. These problems are the insufficiency of structural and social policies that cause national planning and difficulties in production. For this reason, producers try to solve their problems locally and individually. Farmers who rely on their traditional knowledge and experience, especially in regions with high agricultural potential, change their production patterns themselves when national planning is delayed. Farmers who produce under risky conditions in rural areas endure only production costs in the short term, while they have to undertake all the costs arising from long-term agricultural policy changes. Therefore, making such important decisions in agriculture forces farmers to deal with many different problems in the long run. This research was precisely for this very reason, and the short, medium and long term problems experienced by the farmers in the field were evaluated by interviewing them personally.

In this study, it was aimed to examine the situation of the farmers with high agricultural production experience in Akhisar, which was chosen as the sample area, after

they changed their product patterns from tobacco planting (annual) to olive planting (perennial). In this process, the progress of farmers in olive cultivation, the problems they experience during and after the crop pattern change, the individual solutions they developed against these problems, detailed problems including their expectations from the state, non-governmental organizations and private sector, and the collected data were analyzed in accordance with the qualitative research method.

MATERIALS AND METHODS

Research area

The Aegean region has 47,5% of the table olive production area in Türkiye. Manisa province has 53,3% of the Aegean region. Akhisar district has 59,9% of Manisa province's production area. In other words, Akhisar district has 31,4% of the table olive production area, 14,9% of the table olive production area in Türkiye. Olive cultivation is still carried out in 63% of the current agricultural area in Akhisar. In Akhisar, 75% of the olives are evaluated as table olives and the varieties of Domat, Gemlik, Ayvalık and Uslu are commonly produced (Tiryakioğlu Ligvani and Artukoğlu, 2015). Akhisar produces 80% of production (Domat predominantly and Ayvalık) in Türkiye's green table olives, and 35% of black olive production (Gemlik, Uslu) (Kayalı et al, 2008). The research area has 30% of the olive area of the Akhisar district which is seven villages (Zeytinliova, Medar, Mecidiye, Ballıca, Dereköy, Büryaniosmaniye, Sünnetçiler) (Anonymous, 2021a; Anonymous, 2021d) (Figure 1).

Data collection

This research was carried out by Aydın Adnan Menderes University in the Akhisar district/Manisa/Aegean region with the same support (non-monetary support-transportation and technical interview support) of the Akhisar Chamber of Agriculture. The purposeful and snowball sampling technique (Patton, 2015) was used to select information-rich participants and comprehensive questions were prepared for the purpose of the research. Thus, the survey was supported by a qualitative natural query design within the existing area (Guba and Lincoln, 1994). The sample in accordance with the purpose of the research includes 42 table olive producers who have

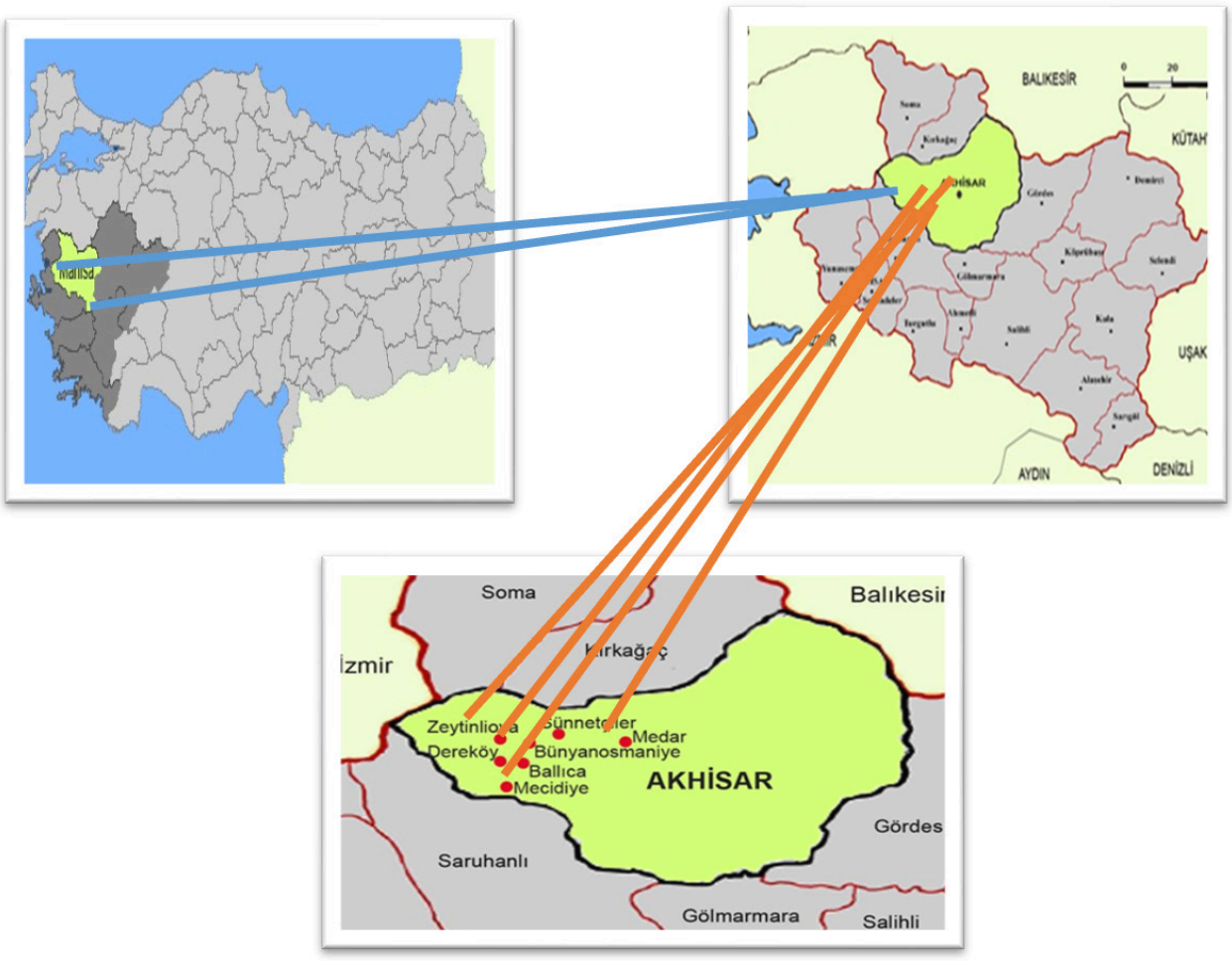

Figure 1. Research Area (Türkiye-Manisa-Akhisar and villages map)

Table 1. Farmers interviewed within the scope of the research

Interview No	Akhisar (villages)	Age (Year)	Education	Professional Experience (years) (Olive cultivation)	Non-agricultural activity
F1	Zeytinliova	62	University	45	Yes (supply)
F2	Zeytinliova	62	Middle School	50	Yes (Commerce)
F3	Zeytinliova	63	Middle School	35	-
F4	Zeytinliova	48	High school	25	-
F5	Zeytinliova	55	High school	35	-
F6	Zeytinliova	35	University	19	Yes (Commerce)
F7	Zeytinliova	32	University	8	Yes (Agricultural Engineer)
F8	Zeytinliova	50	Middle School	40	-
F9	Zeytinliova	58	Middle School	45	Yes (Formerly-Mayor)
F10	Zeytinliova	54	High School	30	---
F11	Zeytinliova	76	High School	40	---
F12	Zeytinliova	67	Primary School	1	
F13	Medar	56	University	40	Yes (Teacher)
F14	Medar	42	Primary School	5	Yes (Handicraftsman)
F15	Medar	48	Middle School	25	----
F16	Medar	64	University	30	Yes (Retired teacher)
F17	Medar	75	Middle School	70	--
F18	Dereköy	40	Primary School	30	---
F19	Dereköy	29	High School	20	Yes (Commerce)
F20	Dereköy	54	High School	30	--
F21	Dereköy	58	Primary School	30	--
F22	Dereköy	50	Primary School	40	--
F23	Dereköy	48	College	30	Yes (Commerce)
F24	Dereköy	56	Middle School	35	--
F25	Dereköy	31	Middle School	16	--
F26	Ballıca	68	Primary School	50	Yes (Commerce)
F27	Ballıca	60	Primary School	35	--
F28	Ballıca	53	Primary School	45	--
F29	Ballıca	65	Primary School	50	--
F30	Ballıca	58	Primary School	35	--
F31	Ballıca	40	High School	10	--
F32	Ballıca	52	Primary School	20	--
F33	Ballıca	54	Primary School	44	--
F34	Ballıca	50	Primary School	40	--
F35	B.Osman	65	Primary School	40	--
F36	B.Osman	66	Primary School	55	--
F37	B.Osman	62	Primary School	30	--
F38	Mecidiye	55	Primary School	40	--
F39	Mecidiye	53	Middle School	18	--
F40	Mecidiye	67	Primary School	55	--
F41	Sünnetçiler	59	Primary School	49	--
F42	Sünnetçiler	63	Primary School	25	--

their own olive orchards in seven villages (at an average distance of 15 km from each other) of the Akhisar district and actively work in the district suggested by the Akhisar Chamber of Agriculture. The sample size in the research is well above the norms in qualitative research (Saunders and Townsend, 2016). All interviews with these producers between 2020/February-July 2020 (It took a long time as the interviews had to be suspended due to the covit 19 outbreak) were held face-to-face and voice recordings were taken (Miles and Huberman, 1994). The 24,5-hour

data obtained from these interviews took an average of 30-40 minutes each. Each interviewer (farmer) has been given a code number in accordance with the research ethics (Farmer 1=F1 etc) (Table 1).

Semi-structured interviews

In this study, in-depth semi-structured interviews were chosen similar questions to all the participants were asked and a guide was created. Thus, it was possible to ask more questions in the flexible structure to clarify the subject (Patton, 2003). All interviews are digitally recorded and put down on paper. The producers who communicate with stakeholders (public, non-governmental organizations, universities etc) in determining the innovations with leadership in each village and solving the problems were interviewed in more detail. The special notes taken during and after these interviews have turned into new research questions and codes (Patton, 2015). In a sense, these notes have enriched the encodings.

Data analysis

The data collected in this study were anaylized with a qualitative method and the stage of thematic qualitative data analysis defined by Miles and Huberman (1994) was applied. Qualitative data analysis includes the analytical meticulousness of the researcher, creativityü analytical thinking ability, professional equipment and discipline. Therefore, reducing the raw information by creating a framework from the data obtained constitutes the first part of the analysis (Patton, 2015). Although thematic analysis includes a coding process used in many qualitative research methods, it requires a systematic structure. The researcher can interpret different concepts and ideas by associating them with each other using thematic analysis (Boyatzis, 1998). In this study, first of all, the producer interviews in the villages where the study was carried out and notes taken on the field were examined. Then, familiarization, coding, generating themes, reviewing/renaming themes etc. steps were followed. After that, the data obtained were categorized as first-order, second-order and illustrative data. With this approach, first-order codes are the codes that define the 'analytic concepts' that characterize the specific problems and expectations for a solution that farmers experience in cultivation and marketing in the field. Second-order codes are codes that associate with specific problems in first codes within the framework of theoretical concepts and categories. It is used for (Corbin and Strauss,2015). Illustrative data, on the other hand, are defined as auxiliary codes that are defined from specific to general (induction) in dealing with specific problems and recommendations (Gioia et al., 2013). (Figure 2). In fact, the purpose of Inductive analysis is to reveal the underlying concepts of the data and the relationships between these concepts through coding. This process is called the "theory building process" (Strauss and Corbin, 1990).

RESULTS AND DISCUSSION

The first order code (specific deduction) is grouped under three headings according to the qualitative analysis method. These are cultivation, marketing and recommendation codes. This each specific deduction (illustrative data) is also a summary of the most repetitive general deductions in farmer interviews and is detailed in almost all of the research areas (Table 2).

If the farmers in the region are defined by their socio-demographic characteristics, the average age of all men table olive producers is 55 years, their average education is 7,8 years and their professional experience is 33,7 years. 52% of these producers are primary school graduates, 19% secondary school, 14% high school and 15% university graduates (Table 1). These results coincide with the producers' information in the study on olive establishments in the Aegean region, their average age is 54,4 years, their education average is 6,13 years (Adıgüzel and Kızılaslan, 2019), and the average age in Çanakkale 53,6 years, the average education 7,5 years, and the professional experience of the producers 24,9 years (Özsayın et al., 2018).

Cultivation

The most important elements of success in the garden facility are the geographical location and structure of the land and the planting of local species and varieties adapted to the region. As with many other plants olive plant also has many varieties, but geography is the biggest limiting factor in cultivation (Barranco and Rallo, 2000). The biggest success in cultivation is the correct and timely cultural processes (irrigation, fertilization, spraying and pruning) and harvesting. In this context, the general and common problems of table olive producers in the region related to cultivation have been evaluated under four main headings; efficiency in production, input, irrigation and labor.

Production

Significant decreases are observed in the quality and quantity of olive production in the region. The reasons for this decline are the drought in the region, irrigation difficulties and tree drying. However, the Akhisar region has been known as a district with a high agricultural potential for a long time since its ground water is high (Güryıldız, 2021). As a matter of fact, one of the factors in the producer's transition from tobacco to table olive production is the presence of ground water in the region. Although producers use this water carefully with the drip irrigation technique, they have started to experience problems due to the decrease in ground water and climate change. Irrigation has reached serious proportions in some villages. On the other hand, Verticillium dahliae Kleb (wilt) disease in the soil caused the drying of the olive trees over time due to the cultivation of cotton or vegetables on this land. This disease spreads rapidly with

Table 2. Table olive farmers data structure

First order codes	Second order codes	Illustrative data
Cultivation	*Production*	Decreases in effieciency, quality and drying of trees are a problem in production. The causes are insufficient ground water, continual variation and verticillium disease. Raw size is directly related to irrigation in table olives. Groundwater reduction and climate change are growing concerns (F1) (F3).
	Input	The most important input items in olive production are considered as pesticides, fertilizer and diesel oil. These items are also supported by the state. (F2).
	Labor	Skilled labor and labor costs in pruning and cultivation, and harvesting are refferred to here as a labor (F23).
Marketing	*Storage*	A storage system must be established by the state or non-governmental organizations (F4) (F7).
	Organization	Farmers need cooperatives where they can exchange information, market and sell without intermediaries under one roof (F1) (F8) (F10).
Recommendation	*Climate*	Special measures need to be taken due to climate change and a decrease in ground water. Individual solutions should be supported in these measures (F5) (F8) (F10) (F25)
	Price	Import of all inputs used in production, high exchange rate, high costs, and insufficient support negatively affect prices (F27) (F33).
	Training	Special trainig is needed for the farmer to include sustainable production techniques and technology (F13) (40) (42).

heavy tillage and pruning residues from irrigation water. Therefore, the farmer in production sees the certified olive sapling support given by the state as a remedy for the decrease in productivity and the drying of the trees and has started new plantings. Locally adapted (local) table olive varieties for new plantings: Domat, Uslu (both geographically indicated) and the use of Cherry varieties were not a problem. Yet, the planting of Gemlik/Trilye and Ayvalık/Edremit varieties, whose adaptation is not known but whose sapling supply is easily and quickly rooted, has caused problems (Türkyarar et al., 2008).

The farmer states her problems and individual solutions in production as follows:

'Climate change is my biggest problem. There have been no seasonal rains for seven years here. Low ground water and even drip irrigation are troublesome. I have land in two different places. There is no irrigation cooperative in the village where I have the second land. Therefore, I can irrigate half of my total land because I have to do everything individually' (F22).

'Climate change, rainfall times and weather conditions changed. I have a problem with irrigating and my orchard, has dropped yields because it is no easy for me to reach underground water as before. I also have drying of trees in my orchard, but I don't know the reason, I am planting saplings instead' (F5).

'Climate change related diseases have increased in olive trees in recent years. My trees are drying up and my yield and grain quality have dropped' (F8).

'I made a variety change with my own decision to increase efficiency. I converted Domat variety to Gemlik variety with the vaccine but Gemlik variety is quickly affected by the cold in this region, then I regretted it' (F2).

'The yield in my orchard, has dropped. I planted a Gemlik variety in my garden, which is a Domat variety, inherited from my father to increase it, but I am not satisfied expect for the dusting benefit' (F3).

'I planted Gemlik and Ayvalık saplings because I think they are more durable than Domat variety in order to increase my yield in my newly established garden after the support of certified saplings from the state' (F25) (F40).

Input

As in other agricultural production activities, fertilizers, pesticides and diesel oil are the most important components of olive cultivation. In a study conducted in the Aegean region, it was found that variable costs (fertilizer, pesticide, diesel oil, pruning) accounted for almost half (47,17%) of the total costs (Adıgüzel and Kızılaslan,2019). In all villages interviewed, all of the

farmers stated that the inputs are imported and they are constantly affected by the increase in the exchange rate, so the cash support of the state for diesel oil, fertilizer and pesticide is not sufficient.

Farmers' views on input costs;

'Input prices are very very high, supports are insufficient. The state provides diesel oil and fertilizer support, but very insufficient, there are problems with plant protection. Plant husbandry costs are high' (F1).

'Agricultural inputs are expensive and state subsides are insufficient. This is reflected in our investments and we can not make our investments when the support is not enough' (F5).

*'The most important problem in olive production is high input prices. I've been tired of struggling with climate change * related diseases in recent years'* (F8).

'Input prices are high. The input is imported and its price is increasing with foreign currency. The farmer continuous with the minimum wage' (F33).

'Input prices have been increasing since 2002, the price of the crop I sell is the same. No one protects us' (F34).

Labor

Table olive production in olive cultivation requires more care for quality fruit. As in all fruit cultivation, the biggest cost item is labor costs in pruning and harvesting. Professional labor in harvesting and pruning is important as the olive tree produces fruit in two-year shoots, as which affects the fruit quality of the following year. Therefore, both skilled workers supply is difficult and the cost is high. As a matter of fact, Tiryakioğlu Ligvani and Artukoğlu (2015) found that labor wages in table olive establishments were 16%, Adıgüzel and Kızılaslan 14,18%, Artukoğlu et al. (2010) found the ratio of workforce expenses 19,31% to harvest costs 29,55% within variety costs, once again Artukoğlu et al. (2012) calculated the harvest labor as 25,26% in another study.

Farmers with regard to workforce quality and costs;

'I have a workforce problem, especially in pruning, labor wages are very high' (F6).

'I can not find the number of workforce I want when I want, especially in the harvest, I say ı need 15 people today, but 10 people come and my work is not done' (F26).

'I can not find workforce as before. Professional pruners got old or pass away. I employ more women workers in the harvest because the table oil harvest is gentle and we use ladders as the crowns of the trees are short. After the start of immigration from Syria, I prefer them because of low wages, but they are not as qualified as I would like' (F34).

'I have a lot of workforce problem. Two workers fell off the stairs this year, they have no insurance, I always paid the health expenses, unfortunately there is no support from the state' (F33).

Marketing

As in other fields of activities, the quality of the crop is important in marketing-oriented agricultural production. Yet, the most important thing is to produce in accordance with the target group that this production appeals to. The principle of modern marketing that started at the end of the 20th century, 'Produce and sell with the quality you can sell' instead of 'I sell whatever I produce' is the basic principle of the strategic remarketing methods (Aslan Çetin, 2018). Since olive fruit is not consumed raw, it is processed into table and oil. Therefore, it must be processed before marketing. The three biggest problems of farmers in raw grain supply in marketing are the price problem due to the limitation of individual storage facilities and lack of organization.

Storage

In Türkiye, in "2005 dated and 5300 numbered on Agricultural Crops Licenced Storage" law, with the possibility of producers' safe, insured and healthy warehouse, instead of selling their cops during harvest season when prices are low, it is aimed to provide the opportunity to market the crop when the prices are high. Farmers in this region complain about the lack of licensed warehouses. After harvesting, they find a solution by placing the raw grain n low-capacity tanks and pools with their own means to wait for the price to rise (Anonymous, 2005).

'I have no raw sales. I don't sell my crop right away and keep 95% of it in a polyester tank in less salt water, so I make my own storage' (F1).

'The most important thing to do is to be licensed warehousing and sponsored by the state. There are big companies in the market and I don't want to be crushed. Large supplies must be made in warehousing, the state or the exchange of commerce should make the storage' (F6).

Organization

There is only one agricultural development marketing cooperative established in the region according to the law 'Cooperatives dated 1969 and numbered 1163' (Anonymous, 1995). Therefore, the producer does not have any power in the grain sales price. However, in agricultural marketing, the greatest power is the producer organization (Türkyarar et al., 2008).

In Türkiye, farmers have traditional problems regarding coming together, establishing a cooperative and continuing the cooperative established.

'We established a cooperative twice exactly, it just didn't work out. We establishe a third cooperative, but I don't think it will be successful, either' (F6).

'Since there is no cooperative in marketing, we sell at the price of the trader. Since our crop is for export, it has been

affected by the exchange rates. In marketing, companies set a specific price for raw grain. The producer sells at this price necessarily. We must have a cooperative. The producer must be organized; otherwise, we will dependent on the trader' (F12).

'The marketing problem is too much, but if there is a cooperative, it can be solved. Yet, unfortunately, there is not much cooperative. The state should support this issue. There should be a cooperative system like Spain' (F15).

Recommendation

Years ago farmers relied on geographical limitations, land structure and fund of knowledge in their preferences for table olive production instead of tobacco. Because they took a serious economic risk with the transition decision from one-year plant to perennial plant production in agriculture. Yet, farmers in the region are interested and curious producers who follow television programmes and social media that care about training that is open to innovations. For this reason, they share thoughts, experiences and trainings on changing variety, use of inputs, irrigation, climate change, prices and training in their conversations with each other in village cafes.

Climate

In Türkiye, in recent years as well as in the world, in dated disasters such as matingly increasing drought, frost, hail, tornado, flood, hurricane, flooding etc. are the most important problems (Bayraç and Doğan, 2016). In Türkiye, as in Aegean Region, some areas in the national drought map are the most troubled regions. It is estimated that vegetative production will be affected most by uneven rainfall or drought in the coming years. Although the olive is a Mediterranean plant that is resistant to heat and relatively drought, it takes place in this region. As a matter of fact, in a study conducted in the Aegean Region (Çolakoğlu and Tunalıoğlu, 2010), it was stated that the climate limiting data that affect the yield in olive cultivation the most are average maximum temperature, humidity, wind and total precipitation, respectively. On the other hand, Tunalıoğlu and Durdu (2012), as a result of possible climate change and drought, olive cultivation was intensive and foreseen that problems would be in seven cities in the west of Türkiye (Muğla, Aydın, İzmir, Manisa, Balıkesir, Çanakkale and Bursa). It was calculated that the drought in question would reduce the yield of olive plants by 61% in the Manisa region (Tunalıoğlu and Durdu, 2012).

As a result of these expectations, TARSİM (Agricultural Insurance Pool) was established in order to protect the farmer against climate change within the scope of "Agricultural Insurance Law dated 2005 and numbered 5363". This organization guarantees farmers' crops by insuring them with 50% grant support from the Ministry of Agriculture and Forestry. Thus, a sustainable risk management strategy is implemented through TARSİM against the risks of climate change in agriculture (Anonymous, 2021c).

In the production of table olives, the farmers in the region mostly complain about hoarfrost, frost, hail and drought and they take out insurance from TARSİM.

'Climate changed, rainfall times and weather conditions changed. I have problems with irrigation. My trees are drying out'(F5).

'Climate change, frost and hail damage in winter, extremely cold during flowering and sometimes drought, I've been having bad days as a farmer for years' (F32).

'There is TARSİM and it is effective against hail and frost. Getting insurance from TARSİM provides (50%) state grant and bank loan interest rate cut. But even though I don't have state support and interest rate cut, I will get TARSİM done. Because my olive variety is Gemlik and I am in danger of frost when it is harvested late' (F21).

'I am getting TARSİM done. It is important in terms of protecting me against natural disasters and risks. I also benefit from the state support and interest rate cuts. I do it even with no interest rate cut because I feel safe' (F23).

'My olive trees in my orchard are productive for six years, I have been making TARSİM because of frost and hail damage against natural disasters and risks, so I also benefit from the state's discounted credit' (F29).

'Climate change is the biggest problem. There has been no seasonal rainfall in this village for 7 years. I have problems with irrigation from time to time. Fortunately, TARSİM has existed for 7 years and it protects against increasing natural disasters and risks. Even though the state didn't have a grant support (50% grant), I would have it done but if there was no interest rate cut, I would not have it done' (F30).

'The ground land are damaged in the first hoarfrost. Its risk is high. Even though there is no interest rate cut and state support, I can get TARSİM done because my land is on the ground' (F31).

Price

The most important marketing problem in the region is the low price and instability due to the inability to stock raw grain. Similar problems are similar in different regions. In a study conducted in the Aegean Region on this issue, it is suggested that the collection problem n forward sales of table raw grain olives where the state will play an important role in eliminating the fluctuations in product prices, can be solved by obtaining a bank guarantee (Adıgüzel and Kızılaslan, 2019). In another study conducted in the Southeast Anatolia Region, it was stated that the instability in the product price is the most important problem affecting marketing activities negatively. It is also stated that the low price has a negative effect of 46,2% on marketing (Özgürsoy, 2006).

The opinions of the local producers on this issue are as

follows.

'Traders in the region are very effective in terms of price. Large table olive processing enterprises agreed among themselves and shared the market. There was competition between them in the beginning. Now they get along with each other and make us buy from elsewhere' (F3).

'It must be cooperative and licensed warehousing' (F4).

'We have problems in marketing. Some years no traders come. When the trader does not come, the crop is waiting and I am looking for remedies' (F5).

'Another important problem I have in the region is related to marketing (sales). There are 4 big companies in the region. These companies determine the price and the farmer becomes victim' (F6).

'The price is low and there is a problem of trust between the buyer and seller' (F12).

'Product prices are very unstable. I sell my crop at the price almost 10 years ago, we are in a pathetic situation as raw grain' (F33).

'The price of the product I have been selling since 2002 is the same. They should audit the prices' (F34).

Training

Local olive farmers are extremely sensitive to training. In the first years when the farmers started producing table olives, they participated in the training given by the Manisa Agriculture and Forestry Provincial Directorate, Akhisar District Directorate of Agriculture and Foresty, Akhisar Chamber of Agriculture and the Olive Research Institute in Bornova/İzmir. These trainings have been in the nature of courses, seminars and conferences, especially on the use of correct ınput, pruning and harvesting. However, in countries such as Egypt (Anonymous,2021b) which has recently begun to have a say in the production of table olives, farm visits, group meetings and practical demonstration practices are preferred in training (Mansour et al., 2019).

In recent years, farmers have preferred training that they can reach via television or social media. Their expectations from training are mostly aimed at solving the problems they experience due to climate change.

'I certainly follow and apply new varieties and cultivation practices. My social environment is vey wide. I get information from my acquaintances' (F1).

'I formerly received many trainings on cultivation and table olive processing, but now I have touble in adapting to technology. Trainings should be more technological, I need them' (F2).

'I attended courses on olive cultivation and technical issues at regular intervals. I last attended training in 2008, but I want training on drought' (F10).

'I never miss plant protection and pruning traning' (F16).

'I get training from (local) state of agriculture, but I mainly get help from relatives and family elders' (F19).

'If m head is stuck, I will go to university (Aegean University), I will go to Chamber of Agriculture and District Agriculture. I also don't need to attend training' (F23).

'I learn from books, I watch radio and television and youtube, and I get my own training' (F26).

'I decide everthing myself, because I often watch agricultural programs on television early in the morning and learn a lot from farmer programs on the internet' (F30).

CONCLUSION

Agriculture is a risky sector. Productivity and efficiency in production are very important for minimizing the mentioned risks and for economic sustainability. Therefore, countries have to adapt to different uses of tools (price, social, structural) in national and international agricultural policies. Adaptation to these changes is provided by the state and institutionally in developed countries and by farmers and locally in developing countries. When it comes to crop pattern changes in local adaptation, just as in this research, the individual decisions of the farmers are valid. As a matter of fact, farmers in Akhisar made a pattern change about forty years (1980-2020) ago, relying on their knowledge and the adaptation of the preferred crop in the region to adapt to the change in national policy. However, they lost their income in preferring *perennial* olive fruit from the *one-year* tobacco plant. For this reason, they migrated to nearby cities and towns in order to compensate for the economic losses they experienced until they were harvested from olives. As a result, farmers have tried to adapt to a new production activity on the one hand, and to their new social life with migration on the other hand. Today, Akhisar farmers have completed the transition from labour-intensive production to capital-intensive production in olive cultivation, despite all the risks and devoted efforts they took forty years ago, farmers are being tested again with their crop pattern preferences. In fact, the basis of the problems, as mentioned above, is that the state prefers structural and social tools in agricultural policies more than the price tool. However, there are mostly middle and long-term problems in perennial plants such as olives. The effective policy tool to be used in solving these problems should be structural and social tools, not prices.

The problems faced by farmers in their production can be grouped into two groups general and specific problems. General problems; frequent devaluation due to the functioning of the national economy, the persistently preferred floating exchange rate and input is also external dependency. Actually, input support (plant, pesticide, fertilizer) is provided by the Ministry of Agriculture and Forestry in order to alleviate these problems in the general economy in Türkiye. However,

these supports are within the scope of price tools and lose their importance before reaching the farmer. Therefore, the table olive producer has to endure constantly increasing costs. Specific (local) problems are related to product quality and yield. The most important of these are the disasters experienced due to climate change (drought, hail, frost, etc.), reduced groundwater, drying of trees caused by Verticilium dahliae, credit problems (very high private bank loan rates, low interest and low loan rates for state banks) in some investments such as irrigation, infrastructure or new garden facilities, for raw/semi-finished olives lack of institutional stocking establishment, frequent variation in order to increase yield and quality.

Farmers state that due to the mentioned issues above, a new crop pattern in the region will not be healthy in the short or medium term from now on. As long as climate change does not force them, they will continue table olive production. However, in order to make sustainable production, they need structural policies and the costs of this process to be undertaken by the state. In fact, these demands are highly viable. Because the executing agency which is responsible for agricultural policy is the Ministry of Agriculture and Forestry in Türkiye. In this sense, Türkiye is a lucky country because the ministry is one of the most well-organized ministries in the country. There are currently geographically present 81 provinces and organized directorates in 911 districts. In addition, there are Agricultural faculties of Bornova Olive Research Institute, Aegean University and Aydın Adnan Menderes Universities working on R&D in olives in the region. It is sufficient for the ministry to establish a functional structure that takes into account regional and local problems and to lead it, with only this existing infrastructure and organization presence. It is possible to provide *training and consultancy* services through the existing R&D institutions and universities within its structure, to provide *surveillance services* with well-organized district organizations, and to receive the support of the Chamber of Agriculture, the Commercial Exchange and the Chamber of Commerce in the region for the *sustainability and success* of these services.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

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

Author contribution

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

Ethics committee approval

Ethics committee approval is not required. This article does not contain any studies with human participants or animals performed by any of the authors.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgments

Many thanks to**,** The Head of the Akhisar Chamber of Agriculture and the technical staff for their support in selecting the villages, farmers to be interviewed in this research and in transportation also all farmers.

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RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(3): 607-620
DOI: https://doi.org/10.31015/jaefs.2023.3.15

Determination of the tolerance of physiological, morphological, and yield parameters of landrace durum wheat (*Triticum durum* Desf.) to high-temperature stress

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Citation: Okan, M., Alp, A., Haliloglu, K. (2023). Determination of the tolerance of physiological, morphological, and yield parameters of landrace durum wheat (*Triticum durum* Desf.) to high-temperature stress. International Journal of Agriculture, Environment and Food Sciences, 7 (3), 607-620

Received: July 31, 2023
Revised: September 03, 2023
Accepted: September 05, 2023
Published Online: September 25, 2023

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Available online at
https://jaefs.com/
https://dergipark.org.tr/jaefs

Abstract

In the South-eastern Anatolia Region, where the climate is favorable to cultivation for durum wheat, there have been notable reductions in both yield and quality due to biotic and abiotic stress factors in the region. Primary one of these stresses is high-temperature stress. High-temperature stress, specifically during the late flowering stage and early grain filling stage, results in substantial reductions in both crop yield and quality. In this study, several practical, easily and rapidly quantifiable physiological, morphological, and yield-related parameters that may be used in durum wheat improvement programs in the region for high-temperature stress tolerance were investigated. Ninety landrace durum genotypes and 4 standard cultivars were used. The study was carried out at GAP (South-eastern Anatolia Project) International Agricultural Research and Training Centre in an air conditioning room according to an augmented design under optimum conditions and stressful conditions where high-temperature stress was created. Compared to optimum conditions, there were reductions in flag leaf greening time by 20%, days to maturity by 7%, spike length by 10%, peduncle length by 18%, grain filling time by 23%, number of spikelets on the spike by 12%, number of grains on the spike by 39%, and thousand-kernel weight by 33%, while grain filling rate increased under high-temperature stress conditions. The genotypes 82, 83, 87, 88, 99, and 103 and the standard varieties Artuklu and Sümerli prevailed in both optimum and stress conditions. The fact that leaf erectness, flag leaf greening time, grain filling time, and leaf chlorophyll content among morphological and physiological characteristics had a significant correlation with yield components under both conditions indicates that these characteristics can be used as selection criteria for tolerance to stressful conditions in the region.

Keywords: Landrace, Durum wheat, High-temperature stress, Tolerance, Selection

INTRODUCTION

Wheat provides about 20% of the calories required for human nutrition due to its nutrients and is the staple food of about 40 countries, accounting for 35% of the world's population (Dereli, 2016). The South-eastern Anatolia Region contains a diverse range of landrace wheat genotypes that may be used in improvement studies and enables the creation of a gene pool with a narrowed genetic base (Özberk et al., 2010). These landrace materials managed to survive in the region from the past to the present under the pressure of natural selection and reached the present day despite different biotic and abiotic stresses due to their extensive adaptation abilities (Özberk et al., 2005). The landrace genotypes,

especially Bağacak, Sorgül, Beyaziye, Menceki, Iskenderi, and Havrani, have been still preferred by many farmers in the region, and they give a satisfactory yields from these genotypes. There are many stress factors that have negative effects on yield and yield components in wheat cultivation. High-temperature stress among these factors is primary one among the environmental factors that adversely affect the growth, development, and yield of wheat (Kılıç 2020). It has been reported that under high temperature stress conditions, grain filled duration, grain size, grain numbers per spike and maturatiy time are adversely affecting wheat yield. (Lal et al., 2021). Ullah et al., (2022) stated that temperatures above 30 °C during the flowering period of wheat shorten the grain filled duration, which causes pollen loss, reduces the starch and protein accumulation in the grain, and yield loss occurs. In fact, Asseng et al., (2011) reported that when the average temperature rose by 2 °C during the growth and development stages of wheat, there were losses in wheat yield of up to 50%. However, Gibson and Paulsen (1999), Yang et al., (2002) and Pradhan et al., (2012) found that wheat exposed to high-temperature stress at 35/20 °C (day/night) for 10 and 20 days after flowering, 35/20 °C (day/night) for 10 days after flowering, and 36/30 °C (day/night) for 21 days after flowering had losses of 18%–29%, 50%, and 39% in grain weight, respectively. It is very important to determine the morphological, physiological, and yield tolerance levels of wheat to high temperatures and to use genotypes with high tolerance levels in improvement in the cross-breeding studies to be carried out in the region. This study aimed to identify the reactions of some morphological, physiological, and yield components of 90 landrace and 4 standard durum wheat genotypes collected from the South-eastern Anatolia Region to high-temperature stress.

MATERIALS AND METHODS

This study was carried out in the air conditioning room of the Directorate of the GAP International Agricultural Research and Training Centre. 90 genotypes from the landrace durum wheat and 4 standard durum wheat cultivars (Diyarbakır-81, Fırat-93, Sümerli, and Artuklu) collected from different provinces of the South-eastern Anatolia Region and stored in the gene bank of the Directorate of GAP International Agricultural Research and Training Centre were used. The study was carried out in an augmented experimental design for one year. The materials used in the study to determine the genotypes tolerant to high temperatures were subjected to two treatments: optimum ambient conditions and stress conditions. Under optimum conditions, the genotypes were grown in a control environment with optimum environmental conditions (22/14 ± 1 °C Day/night) from the sowing period to the ripening period. Under stress conditions, the genotypes were grown in the control environment where optimum temperature conditions were provided from the sowing period until the end of the flowering period (Zadoks 70), and 10 days after the full flowering period and at the beginning of the grain filling period (39/26 ± 1 °C Day/night), they were divided into 4 groups for 3 days and exposed to temperature stress. The pots with dimensions of 20 x 20 cm and a volume of 5 liters were used during the sowing process of the study. The filling material was turf. Eight wheat grains were sowed in each pot from seeds previously blended from a single spike. After the stem elongation period, plants were thinned to leave five plants in each pot. Genotypes were treated with 6.5 g/pot of fertilizer 15-15-15 (N, P, and K) at a mixture of 1.3 g/kg/pot (5 x 1.3) before being sowed in pots. The plants were fertilized with 2 g/L water-soluble urea (46%), once at stem elongation and once at flowering, to provide adequate nutrition. The genotypes underwent an air conditioning setting with a 16-hour light and 8-hour darkness cycle. The moisture content of the pots was measured with a soil moisture meter at 3-day intervals to prevent drought stress in the genotypes, and when the moisture amount fell below 50%, the pots were irrigated. The characteristics studied are described below.

Figure 1. Sowing of genotypes in the air conditioning room

Chlorophyll content of flag leaf

Flag leaves of five plants were randomly selected from each pot and measured with the manual device SPAD-502 at the grain filling period before and immediately after the stress, and the data were averaged.

Days to heading

The number of days elapsed between plant emergence and the date when ½ of the spikes emerged from the flag leaf sheath in 70% of the plants in each pot was calculated.

Greening time of flag leaf

The number of days elapsed between the emergence of the flag leaf and the period when the leaf turned yellow at the rate of approximately 95% was determined in days.

Days to maturity

The number of days elapsed between the emergence date of the genotypes and the date when the genotypes in each pot turned yellow at the rate of 95% was calculated.

Plant height

The height of the stem with five random spikelets from each pot was determined by measuring from the soil level to the tip of the top spikelet.

Flag leaf area

The flag leaf area was determined in cm^2 by measuring the width and length of the flag leaf of five plants from each pot after the flag leaf on the main stem of the plants had fully developed and multiplied by a factor of 0.72.

Spike length

After the plants reached their physiological maturity, the spike length of the labelled plants was determined by measuring the distance from the lower node of the spike to the tip of the uppermost spikelet (excluding the awns).

Leaf erectness

The angle of 0-90° between the leaf blade and the stem was used as a scale in order for the plant to absorb the rays better and minimize negative effect of high temperature and it was measured visually during the heading period.

Peduncle length

The length between the upper node of the plant and the first spikelet node of the spike was calculated by taking the average of five plants selected from each pot at the yellow maturity period.

Grain filling duration

The number of days from the flowering date to physiological maturity was calculated.

Grain filling rate

This was calculated as the average single grain weight of the plant divided by the grain filling time.

Number of spikelets on the spike

This was determined by counting the number of spikelets on each spike in five spike samples collected from each pot.

Number of grains per spike

This was determined by counting the grains from five spike samples collected from each pot.

Thousand-kernel weight

This was calculated by counting 400 grains from each pot and multiplying the resulting weight by 2.5.

Data Assessment

Statistical analysis of the data was carried out using JMP 13.2 (Copyright © 2007 SAS Institute Inc.) software. A MSD test was used to compare the difference between averages. A pairwise correlation was analyzed by using the scatterplot matrix in the Jump-Pro13 software to determine the correlation between the characteristics.

RESULTS AND DISCUSSION

The study aimed to identify the reactions of some morphological, physiological, and yield components of 90 landrace and 4 standard durum wheat genotypes collected from the South-eastern Anatolia Region to high-temperature stress. The results of variance analyses showed statistically significant ($P \leq 0.01$) differences between the genotypes in terms of the studied characteristics (Table 1 and Table 2)

Under optimum conditions, the highest (55.3) flag leaf chlorophyll content was recorded in genotype no. 68, and the lowest (31.9) flag leaf chlorophyll content was recorded in genotype no. 47. Under stress conditions, genotype no. 13 had the highest leaf chlorophyll content (39.2), and genotype no. 36 had the lowest leaf chlorophyll content (8.4). However, 19 landrace genotypes exceeded the highest standard cultivar (Artuklu) under both optimum and high-temperature stress conditions. Compared to optimum conditions, leaf chlorophyll content declined significantly under stress conditions (Table 1). This suggests that such decline was mainly due to the acceleration of metabolism and consequently desiccation of the leaves in the genotypes exposed to 39 °C high-temperature stress for 3 days. During the stress period, flag leaf chlorophyll content decreased linearly in all cultivars and high stress contitions flag leaf chlorophyll content was 80%–91% lower than the plants under control conditions (Mirosavljević et. al., (2021). Indeed, Nawaz et al., (2013) reported that high-temperature stress corrupted SPAD content in plants under controlled conditions. Similarly, Efeoğlu and Terzioglu (2009) reported that chlorophyll deposition was restricted in wheat cultivars exposed to 45 °C for eight hours under high-temperature stress conditions, and 37 and 45 °C temperatures significantly affected chlorophyll fluorescence and photosynthesis in the main leaves of wheat cultivars.

The days to heading showed very close values under optimum and stressful conditions. Under optimum conditions, genotype no. 88 headed the earliest (60.1 days), while genotype no. 46 headed the latest (104.8 days). Under stress conditions, genotypes 88 and 46 headed the earliest (61.3 days) and latest (104.6 days), respectively. Under optimum conditions, the number of landrace genotypes that exceeded the highest standard cultivar (Artuklu) was 84, while the number of landrace genotypes that exceeded the highest standard cultivar (Sümerli) under high-temperature stress conditions was

81 (Table 1). This study showed that the genotypes were late in heading compared to the standard cultivars. The early heading is an important criterion for wheat in the South-eastern Anatolia region, where wheat is frequently exposed to high-temperature stress. Indeed, Bilgin and Korkut (2005) reported that the grain filling duration was higher in early-headed genotypes, and accordingly, the amount of nutrients that entered the grain was higher. However, Karaman (2017) reported that late-headed genotypes were exposed to higher temperatures during the grain-filling period, and the yield of these genotypes was adversely affected.

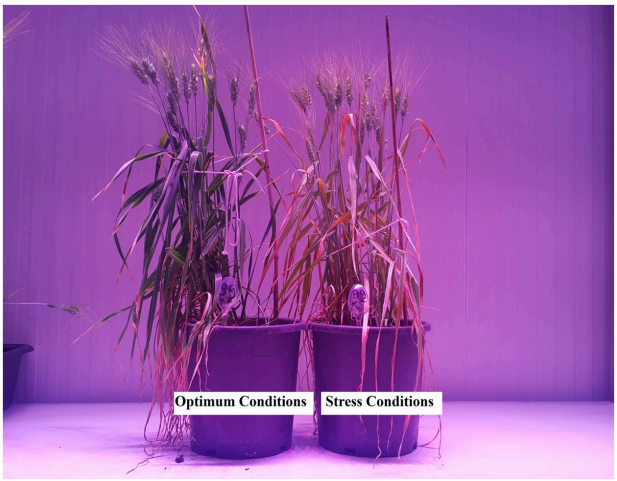

Figure 2. Negative effect of high-temperature stress on the morphological and physiological characteristics of wheat

The longest (70.9 days) flag leaf greening time was recorded in genotype no. 87, and the shortest (46.7 days) flag leaf greening time was recorded in genotype no. 61 under optimum conditions. Under stress conditions, the longest flag leaf greening time (58.9 days) was recorded in genotype no. 54 and the shortest flag leaf greening time (37.7 days) in genotype no. 27. Under optimum conditions, the number of landrace genotypes exceeding the highest standard cultivar (Artuklu) was 1, while under high-temperature stress conditions, the number of landrace genotypes exceeding the highest standard cultivar (Artuklu) was 6. Compared to optimum conditions, the greening time for flag leaf under high-temperature stress conditions was 20% less in both landrace genotypes and standard cultivars (Table 1). This suggests that it resulted from the yellowing and wilting of the flag leaves, the death of the leaves, and their inability to photosynthesize after exposure to high-temperature stress for 3 days during the study. The findings of the present study are compatible with those of the study by Al-Khatib and Paulsen (1984) who reported that high temperatures above 30 °C inhibited the photosynthesis process, caused vegetative and generative growth to stop, accelerated senescence, and disrupted photosynthetic activities. Mirosavljević et al. (2020), higher crop greenness could be related to the additional increase of photosynthetic activity during the grain filling period, supporting a higher grain yield of crops.

The largest (39.59 cm^2) flag leaf area was recorded in genotype no. 45 and the smallest (12.67 cm^2) was recorded in genotype no. 49 under optimum conditions. Under stress conditions, the largest flag leaf area (23.0 cm^2) was recorded from genotype no. 24 and the smallest (2.37 cm^2) from genotype no. 49. Under optimum conditions, the number of landrace genotypes exceeding the highest standard cultivar (Fırat-93) was 29, while under high-temperature stress conditions, the number of landrace genotypes exceeding the highest standard cultivar (Fırat-93) was 13. Compared to optimum conditions, there was a reduction of approximately 100% under high-temperature stress conditions (Table 1). Bhuta (2006) reported a positive correlation between flag leaf area and yield. Öztürk (2011) also reported that wheat with a larger flag leaf area prevailed in terms of both biological and grain yield. Similarly, High temperatures at grain filling had a more negative effect on the photosynthetic-related parameters, resulting in higher grain weight and grain yield reduction (Mirosavljević et. al., (2021).

The highest flag leaf erectness was recorded in Artuklu, Sümerli, and Fırat-93 (81°) cultivars, while the lowest flag leaf erectness (53°) was recorded in genotype no. 11. There was no landrace genotype that exceeded the highest standard cultivars under both optimum and high-temperature stress conditions. During the period until the grain filling period, the same values were observed under optimum and stress conditions (Table 1).

The highest plant height (97.8 cm) was obtained from genotype no. 42 and the lowest plant height (45.3 cm) was recorded from genotype no. 47 under optimum conditions. Under stress conditions, the highest plant height (95.3 cm) was recorded from genotype no. 42 and the lowest plant height (42.8 cm) from genotype no. 87. Under optimum conditions, the number of landrace genotypes exceeding the highest standard cultivar (Artuklu) was 87, while under high-temperature stress conditions, the number of landrace genotypes exceeding the highest standard cultivar (Artuklu) was 79. The plant height of landrace genotypes was longer than the standard cultivars (Table 1). This suggests that the main reason why plant height showed no significant difference between optimum and stressful conditions was the generative period of wheat exposed to high-temperature stress, the completion of plant height development in wheat, and no effect of stress during the same period. Indeed, Tatar (2011) reported that the plant height of wheat was not affected by stress after the flowering period.

Genotype no. 22 showed the longest (27.7 cm) peduncle length and genotype no. 52 showed the shortest (5.8 cm) peduncle length under optimum conditions. Under stress conditions, genotype no. 19 had the longest peduncle

length (23.6 cm); whereas, genotype no. 12 had the shortest peduncle length (3.9 cm). However, the number of landrace genotypes exceeding the highest standard cultivar (Diyarbakır-81) under optimum conditions was 73, while the number of landrace genotypes exceeding the highest standard cultivar (Artuklu) under high-temperature stress conditions was 53 (Table 1). The landrace wheat genotypes prevailed over the standard cultivars with respect to the length of the peduncle, and when analyzed over the averages, the peduncle length decreased by 18% under high-temperature stress conditions compared to optimum conditions. Likewise, in the study by Çekiç (2007) reported that the upper internode length of wheat decreased by 31.6% under stress conditions compared to optimum conditions.

Genotype no. 34 showed the longest spike length (10.37 cm) and genotype no. 56 showed the shortest (4.07 cm) spike length under optimum conditions. Under stress conditions, the longest spike length (9.60 cm) was recorded in genotype no. 41 and the shortest spike length (3.30 cm) in genotype no. 56. Under optimum conditions, the number of landrace genotypes exceeding the highest standard cultivar (Artuklu) was 67, while under high-temperature stress conditions, the number of landrace genotypes exceeding the highest standard cultivar (Artuklu) was 44. It appears that high-temperature stress negatively affected the spike length, and when analyzed over the general averages, it was 10% shorter under high-temperature stress conditions compared to optimum conditions (Table 2). Findings of the present study are similar to the study by Al-Otayk (2010), who reported that temperature stress had a negative impact on the spike length, and the study by Roy et al., (2013), who reported that spike length was shorter under temperature stress conditions compared to optimum conditions.

Under optimum conditions, the longest (48.5 days) grain filling duration was recorded in genotype no. 97 and the shortest (23 days) grain filling duration was recorded in genotype no. 46. Under stress conditions, the longest grain filling duration (43.1 days) was obtained in genotype no. 97 and the shortest grain filling duration (15.4 days) was obtained in genotype no. 53. Under optimum conditions, the number of landrace genotypes exceeding the highest standard cultivar (Artuklu) was 1, while under high-temperature stress conditions, the number of landrace genotypes exceeding the highest standard cultivar (Artuklu) was 3 (Table 2). During grain filling, heat stress decreased wheat grain yields due to reduced individual grain weights (Kaur et al., 2021). Heat stress (35 °C) post - anthesis shortened the grain filling duration and limited resource allcation to grain (Bergkamp et. Al., 2018). In the study, grain filling duration of the genotypes exposed to high-temperature stress at 39–26 °C night/day for 3 days decreased by 23% compared to optimum conditions. Likewise, many studies have reported that grain filling duration is negatively affected under high-temperature conditions (Stone and Nicolas (1995); Castro et al., (2007); Nawaz et al., (2013); Modhej et al., (2015)).

The longest grain filling rate (1.39 mg/day) was recorded in genotype no. 46 and the shortest grain filling rate (0.56 mg/day) was recorded in genotype no. 36 under optimum conditions. Under stress conditions, the longest grain filling rate (1.59 mg/day) was recorded in genotype no. 21, and the shortest grain filling rate (0.45 days) was recorded in genotype no. 37. However, the number of landrace genotypes that exceeded the highest standard cultivar (Fırat-93) under optimum conditions was 30, while the number of landrace genotypes that exceeded the highest standard cultivar (Fırat-93) under high-temperature stress conditions was 26 (Table 2). In the study, genotypes exposed to high-temperature stress at 39–26 °C night/day for 3 days increased the grain filling rate compared to optimum conditions. The main reason here is predicted to be the avoidance of stress by the genotypes exposed to such stress in order to minimize the stress effect. However, it was found that the genotypes with a higher grain filling rate achieved better values for thousand-kernel weight. Similarly, the study conducted by Chinnusamy and Chopra (2003) to investigate the starch development in the grain during the grain-filling period under high-temperature stress conditions emphasized that grain-filling rate was an important characteristic under high-temperature conditions.

Under optimum conditions, the longest (142.4 days) days to maturity was obtained in genotype no. 42 and the shortest (113.1 days) days to maturity was obtained in genotype no. 47. Under stress conditions, the longest (128.9 days) days to maturity was recorded in genotype no. 46 and the shortest (104.4 days) was obtained in genotype no. 33 and 36. Under optimum conditions, the number of landrace genotypes exceeding the highest standard cultivar (Artuklu) was 74, while under high-temperature stress conditions, the number of landrace genotypes exceeding the highest standard cultivar (Artuklu) was 74. It was observed that landrace durum wheat genotypes had longer days to maturity periods than the standard cultivars (Table 2). However, a long days to maturity was not a desirable trait in the South-eastern Anatolia region where wheat is frequently exposed to high-temperature stress. This is because it is considered that genotypes with a longer days to maturity period would be exposed to more stress due to the high temperatures that occur in the region, especially during the grain-filling period, and this suggests that it would cause the grains of these genotypes to be weak and wrinkled. In the study, days to maturity of the genotypes that were exposed to high-temperature stress at 39–26 °C night/day for 3 days decreased by 7% compared to optimum conditions. Similarly, Alam et al., (2013)

reported significant reductions in heading, flowering, and days to maturity under high-temperature stress conditions.

Under optimum conditions, the highest number of spikelets on the spike (18.6 pieces) was recorded in genotype no. 38, and the lowest (6.1 pieces) was recorded in genotype no. 61. Under stress conditions, the highest number of spikelets on the spike (17.1 pieces) was recorded in genotype no. 44 and the lowest (5.2 pieces) in genotype no. 61. Under optimum conditions, the number of landrace genotypes exceeding the highest standard cultivar (Sümerli) was 62; whereas, under high-temperature stress conditions, the number of landrace genotypes exceeding the highest standard cultivar (Sümerli) was 38. Landrace wheat genotypes had higher values in terms of the number of spikelets on the spike compared to standard cultivars (Table 2). The results of the study showed that the number of spikelets on the spike decreased by 12% in the genotypes which were exposed to high-temperature stress at 39–26 °C night/day for three days compared to optimum conditions. Pimentel et al., (2015) and Youldash (2017) reported that high temperatures reduced the number of spikelets on the spike by 21%.

Under optimum conditions, the highest number of grains on the spike (25.8 pieces) was observed in genotype no. 103 and the lowest (12.3 pieces) was observed in genotypes no. 6 and 47. Under stress conditions, the highest number of grains on the spike (19.1 pieces) was recorded in the Artuklu cultivar and the lowest (7.8 pieces) in genotypes no. 6 and 47. Under optimum conditions, the number of landrace genotypes exceeding the highest standard cultivar (Artuklu) was 1, but there was no landrace genotype exceeding the highest standard cultivar (Artuklu) under high-temperature stress conditions (Table 2). The results of the study showed that the genotypes that were exposed to high-temperature stress at 39- 26 °C night/day for three days decreased the number of grains on the spike by 41% compared to optimum conditions. Similarly, Pimentel et al., (2015) reported that the number of grains on the spike reduced by 39% due to the effect of high temperature, and the number of grains on the spike was the yield component that was affected mostly by high temperature. However, numerous researchers reported that high-temperature stress conditions negatively affected the number of grains on the spike (Al-Otayk, 2010; Youldash (2017) Tomás et al. (2020).

Under optimum conditions, the highest thousand-kernel weight (38.0 g) was obtained in the Fırat-93 cultivar and the lowest weight (19.1 g) was obtained in genotype no. 37. Under stress conditions, the highest thousand-kernel weight (33.9 g) was recorded in Fırat-93, and the lowest weight (14.8 g) was recorded in genotype no. 71 (Table 3). There was no landrace genotype that exceeded the highest standard cultivar (Fırat-93) under both optimum and high-temperature stress conditions (Table 2). The study showed that in the genotypes which were exposed to high-temperature stress at 39- 26 °C night/day for three days, high-temperature stress decreased the thousand-kernel weight by 33% compared to optimum conditions. This decrease is considered to be associated with the shorter generative period of the genotypes that were exposed to high temperatures during the grain-filling period in order to minimize such stress, which resulted in weak and spindly grains. Pimentel et al., (2015) reported that high temperatures reduced the thousand-kernel weight by 27%. Similarly, many researchers reported that high-temperature conditions caused substantial losses in thousand-kernel weight (Kaur and Behl, 2009; Castro et al., 2007; Modhej et al., 2015; Jamil et al., 2019)).

Assessment of the correlation between the characteristics studied under optimum conditions by correlation analysis

The results of the correlation analyses between the characteristics studied showed that there was a significant and positive correlation between the number of grains on the spike, which affected the grain yield under optimum conditions, and flag leaf erectness, flag leaf greening time, grain filling time, SPAD, and the number of spikelets on the spike, but a significant and negative correlation between the number of grains on the spike and peduncle length, plant height, and days to heading. While a significant and positive correlation was found between thousand-kernel weight another trait that affected grain yield and flag leaf erectness, grain filling rate, and SPAD, there was a significant and negative correlation between thousand-kernel weight and plant height. A negative correlation was found between plant height and characteristics such as grain filling time, the number of grains on the spike, and thousand-kernel weight, all of which have a direct effect on yield. A significant and positive correlation was found between flag leaf erectness and grain filling time, the number of grains on the spike, and thousand-kernel weight, but there was a significant and negative correlation between flag leaf erectness and days to maturity, plant height, and days to heading. Leaf erectness had a positive effect on yield and yield components (Table 3).

Assessment of the correlation between the characteristics studied under stress conditions by correlation analysis

The results of the correlation analyses between the characteristics studied showed that there was a significant and positive correlation between the number of grains on the spike, which affected the grain yield under high-temperature conditions, and flag leaf erectness, flag leaf greening time, grain filling time, SPAD, the number of spikelets on the spike and the thousand-kernel weight, but a significant and negative correlation between the number of grains on the spike and peduncle length,

plant height, and days to heading. While a significant and positive correlation was found between thousand-kernel weight another trait that affected grain yield under high-temperature stress conditions and flag leaf erectness, flag leaf area, grain filling rate, and the number of spikelets on the spike, there was a significant and negative correlation between thousand-kernel weight and peduncle length, plant height, days to heading, and number of spikelets on the spike (Table 4).

Table 1. Results of analysis on the averages of the studied characteristics

Genotypes	SPAD		D.T.H. (day)		G.T.F.L (day)		F.LA. (cm²)		F.L.E. (0-90)		P.H. (cm)		P.L (cm)	
	O.C	S.C	O.C	S.C	O.C	S.C	O.C	S.C	O.C	S.C	O.C	S.C	O.C	S.C
1	48,2	37,4	85,3	85,1	65,4	56,4	22,7	14,82	63	63	69	77,8	16	13,9
2	49,2	30,1	80,3	80,1	60,4	51,4	21,7	19,32	58	58	79	82,8	21	15,9
3	45,1	23,3	85,3	84,1	66,4	43,4	29,82	19,58	63	63	79	92,8	25,7	22,9
4	46,6	18	66,3	65,1	60,4	49,4	16,94	11,8	68	68	54	57,8	14,3	14,9
6	41	11,6	85,3	86,1	59,4	48,4	23,55	9,71	58	58	74	77,8	11,3	5,3
7	45,3	24,6	64,3	65,1	62,4	50,4	18,14	13,67	73	73	54	52,8	11	10,6
8	38,7	34	91,3	84,1	57,4	48,4	32,17	9,6	58	58	69	77,8	15,3	12,6
9	48,6	18,9	87,3	92,1	60,4	55,4	21,98	5,13	63	63	79	72,8	10	9,9
11	41,6	13,8	73,3	82,1	49,4	48,4	18,31	18,59	53	53	79	77,8	21	16,6
12	43,9	11,1	89,3	92,1	61,4	49,4	32,06	14,82	58	58	59	52,8	9,7	3,9
13	45,9	39,2	83,3	80,1	60,4	41,4	23,08	11,12	58	58	74	62,8	14,7	11,9
14	49,6	19,3	85,3	88,1	49,4	38,4	19,82	13,33	63	63	69	77,8	22,3	18,9
16	41,9	16,6	77,3	78,1	54,4	45,4	29,43	17,8	58	58	69	72,8	15,7	11,9
17	40,7	15,3	89,3	86,1	59,4	43,4	21,21	16,24	58	58	69	72,8	12	10,6
18	46,2	12,8	84,3	85,1	53,4	46,4	15,31	6,2	63	63	69	67,8	18,7	13,9
19	43,6	15,2	84,3	86,1	56,4	43,4	28,22	13,4	68	68	79	92,8	26,3	23,6
21	46	21,8	94,3	95,1	56,4	45,4	29,37	15,1	68	68	74	82,8	17	17,6
22	44,9	18,4	79,3	80,1	52,4	43,4	24,3	15,69	68	68	79	77,8	27,7	22,3
23	41,4	12,4	76,3	73,6	51,15	43,65	21,78	12,57	65,5	65,5	77,8	70,3	23	22
24	46,7	25,3	81,3	79,6	56,15	44,65	30,44	23	70,5	70,5	77,8	80,3	23	16,7
26	54,2	17,6	81,3	82,6	61,15	48,65	24,48	11,88	70,5	70,5	67,8	60,3	21,7	12
27	52,3	28,5	83,3	82,6	58,15	37,65	23,85	13,15	65,5	65,5	77,8	65,3	21,3	14
28	47,9	15,7	90,3	89,6	59,15	46,65	22,25	11,21	70,5	70,5	47,8	60,3	11,3	10
29	49,2	11,4	82,3	80,6	64,15	45,65	22,13	11,93	65,5	65,5	77,8	75,3	25,3	16
31	37,6	10,5	83,3	79,6	66,15	40,65	19,95	11,33	60,5	60,5	52,8	65,3	20,3	17,4
32	45,2	18,7	81,3	82,6	51,15	48,65	24,63	12,69	70,5	70,5	62,8	75,3	21,3	13,4
33	45,7	9,6	69,3	66,6	59,15	42,65	20,79	10,73	70,5	70,5	72,8	55,3	25,7	17,4
34	44,7	23,8	84,3	79,6	68,15	46,65	27,72	13,73	70,5	70,5	82,8	80,3	22,7	16
36	38,6	8,4	70,3	69,6	61,15	39,65	19,44	10,53	70,5	70,5	52,8	55,3	14,3	7,7
37	47,3	17,2	90,3	89,6	60,15	47,65	27,51	5,29	60,5	60,5	82,8	70,3	18	15,7
38	52,5	20,2	98,3	87,6	57,15	52,65	35,38	22,74	70,5	70,5	87,8	75,3	16,3	10,7
39	51	22,7	84,3	86,6	59,15	54,65	20,23	18,74	75,5	75,5	92,8	80,3	20,7	13,7
41	49,6	18,3	92,3	91,6	68,15	48,65	19,04	18,03	65,5	65,5	77,8	75,3	16	7,7
42	50,8	24,8	100,3	93,6	65,15	53,65	15,36	9,35	65,5	65,5	97,8	95,3	24,3	18,4
43	49	19,6	90,3	87,6	63,15	49,65	28,51	9,4	65,5	65,5	87,8	85,3	25,7	17
44	52,7	18	91,3	89,6	65,15	57,65	15,9	10,57	70,5	70,5	62,8	60,3	17	11,4
45	49,5	22,7	89,8	91,6	63,65	54,9	39,59	19,72	70,5	70,5	75,3	74	11,5	12,9

46	46,6	12	104,8	104,6	61,65	56,9	21,95	6,25	60,5	60,5	75,3	74	18,2	14,9
47	31,9	11,6	67,8	69,6	49,65	42,9	25,36	7,4	65,5	65,5	45,3	44	12,8	9,9
48	46	16,6	85,8	90,6	48,65	46,9	22,98	20,45	60,5	60,5	70,3	79	18,2	14,9
49	48,5	18	99,8	91,6	67,65	55,9	12,67	2,37	75,5	75,5	65,3	64	14,2	13,2
51	44,3	16,3	90,8	91,6	59,65	52,9	31,03	10,6	60,5	60,5	65,3	74	15,2	11,2
52	39,1	17,5	89,8	90,6	62,65	42,9	38,24	15,01	60,5	60,5	50,3	64	5,8	4,5
53	45,3	15,5	102,8	104,6	67,65	56,9	20,67	6,2	65,5	65,5	75,3	79	16,2	14,5
54	50,9	24,1	98,8	98,6	68,65	58,9	16,67	3,94	70,5	70,5	65,3	74	14,8	13,2
56	46,5	12,5	85,8	85,6	49,65	51,9	32,99	11,65	65,5	65,5	70,3	74	17,2	11,5
57	49,5	16,2	91,8	95,6	58,65	50,9	22,72	9,53	60,5	60,5	80,3	79	15,2	13,2
58	48,4	19	79,8	82,6	54,65	48,9	23,87	10,85	60,5	60,5	75,3	74	26,5	19,9
59	48,5	19,1	89,8	89,6	65,65	52,9	24,98	8,68	65,5	65,5	55,3	64	13,5	8,9
61	46,2	11,3	85,8	90,6	46,65	42,9	27,21	5,05	65,5	65,5	55,3	49	14,8	10,2
62	40,8	16,1	87,8	89,6	57,65	45,9	25,63	13,07	55,5	55,5	65,3	69	8,2	8,2
63	46,2	24,2	81,8	81,6	67,65	58,9	28,01	9,53	65,5	65,5	55,3	69	13,2	12,9
64	47,9	18,3	81,8	81,6	65,65	55,9	27,52	13,41	70,5	70,5	70,3	69	18,5	16,5
66	44,7	12,4	87,8	87,6	57,65	47,9	32,32	14,82	60,5	60,5	65,3	74	10,2	5,2
67	52,3	8,7	74,1	66,3	62,9	47,65	26,33	10,57	70,5	70,5	66,5	62,8	16,2	13,1
68	55,3	13,8	76,1	74,3	58,9	51,65	28,71	8,18	65,5	65,5	71,5	67,8	15,5	16,4
69	53,8	16,7	74,1	72,3	51,9	43,65	22,37	17,23	70,5	70,5	76,5	77,8	20,2	20,8
71	41,3	18,6	84,1	82,3	55,9	46,65	29,18	15,5	60,5	60,5	86,5	72,8	24,8	15,1
72	52,4	14,2	78,1	77,3	56,9	50,65	22,95	13,82	55,5	55,5	71,5	57,8	22,2	17,1
73	50,3	18,8	84,1	82,3	52,9	47,65	28,73	9,26	60,5	60,5	66,5	67,8	17,2	16,1
74	46,5	14,8	88,1	82,3	67,9	46,65	31,01	14,21	60,5	60,5	71,5	67,8	13,8	9,1
76	47,2	16,4	79,1	77,3	52,9	47,65	19,06	8,18	65,5	65,5	71,5	72,8	15,8	16,1
77	44,4	14,7	83,1	80,3	55,9	48,65	17,69	9,08	60,5	60,5	61,5	57,8	17,2	11,8
78	45,3	18,2	84,1	84,3	58,9	49,65	20,5	13,66	70,5	70,5	76,5	72,8	18,5	20,1
79	48,8	12,9	79,1	84,3	50,9	50,65	24,47	13,66	70,5	70,5	76,5	67,8	21,8	17,4
81	43,1	18,5	84,1	88,3	61,9	50,65	33,82	17,82	60,5	60,5	76,5	67,8	9,8	11,4
82	54,2	29,8	68,1	68,3	66,9	52,65	23,46	19,34	80,5	80,5	56,5	57,8	11,8	9,4
83	47,1	13	62,1	61,3	56,9	48,65	24,15	12,18	80,5	80,5	56,5	47,8	7,5	8,8
84	50,1	20,7	84,1	89,3	59,9	53,65	21,54	13,82	60,5	60,5	71,5	72,8	17,2	13,4
86	43,1	8,5	78,1	71,3	56,9	41,65	28,71	8,39	60,5	60,5	76,5	72,8	14,5	16,4
87	46,2	28,8	64,1	63,3	70,9	54,65	26,66	21,06	80,5	80,5	51,5	42,8	8,2	11,1
88	46,8	12,5	60,1	61,3	61,9	48,65	26,34	9,5	80,5	80,5	51,5	47,8	9,2	9,1
89	45,7	15,6	83,6	86,6	61,9	46,4	34,34	18,42	65,5	65,5	81,5	75,3	19,3	10,8
91	45,1	19	83,6	85,6	62,9	50,4	29,73	16,17	60,5	60,5	76,5	70,3	8,7	10,1
92	49,2	21,6	80,6	83,6	55,9	46,4	15,52	12,14	60,5	60,5	56,5	70,3	14,3	15,1
93	46,2	19,2	77,6	81,6	55,9	49,4	25,58	14,29	60,5	60,5	76,5	70,3	19	22,4
94	50,5	20,7	80,6	86,6	58,9	50,4	29,79	9,27	60,5	60,5	76,5	75,3	15,7	15,4
96	45	17,5	77,6	81,6	60,9	47,4	18,97	11,43	70,5	70,5	76,5	70,3	20	21,4
97	40,5	19,5	67,6	70,6	64,9	46,4	21,13	17,12	80,5	80,5	46,5	55,3	10,7	6,1
98	45,2	25,8	77,6	80,6	50,9	44,4	21,77	14,25	70,5	70,5	66,5	80,3	22	13,4
99	43,2	19,8	60,6	61,6	63,9	52,4	21,44	19,23	80,5	80,5	51,5	50,3	8	5,8
101	46,7	17	78,6	80,6	48,9	38,4	25,88	12,05	70,5	70,5	71,5	80,3	20,3	17,1
102	41,9	16,3	79,6	82,6	62,9	48,4	21,89	12,18	70,5	70,5	66,5	60,3	19,3	15,1

103	45,5	15,8	63,6	65,6	66,9	53,4	26,37	19,69	80,5	80,5	51,5	65,3	15	16,1
104	45,4	20,8	82,6	86,6	64,9	45,4	19,17	7,88	75,5	75,5	61,5	65,3	12,7	13,1
106	46,1	15,3	89,6	86,6	60,9	47,4	30,21	12,8	70,5	70,5	61,5	75,3	13	10,1
107	41,6	22,7	86,6	88,6	66,9	48,4	20,86	9,7	70,5	70,5	61,5	75,3	13	10,1
108	48,4	17,9	75,6	82,6	54,9	50,4	24,89	13,34	70,5	70,5	71,5	75,3	21,3	16,1
109	49,1	17,8	75,6	79,6	55,9	47,4	25,33	19,18	70,5	70,5	66,5	70,3	17,7	19,4
110	47,5	21,2	74,6	79,6	50,9	47,4	20,8	14,41	70,5	70,5	76,5	75,3	26	22,4
Artuklu	49,5	21,8	62,4	65	68,8	56	25,03	17,14	81	81	50	57	10,8	13
D. Bakır-81	44,7	18,7	64,2	64,6	63,6	49,4	24,55	15,35	79	79	50	55	11,8	12,3
Fırat-93	48,2	19,7	65,2	66,6	62,8	48,6	26,9	18,48	81	81	46	50	9	9,1
Sümerli	46,4	19,3	65,4	64	64,4	49,6	21,12	13,13	81	81	50	49	9,1	9,7
Ov. Av.	46,4	18,4	81,5	81,7	59,6	48,6	24,6	13,03	66,9	66,9	68,4	69,1	16,6	13,7
Land. Av.	46,4	18,3	82,2	82,4	59,37	48,5	24,57	12,89	66,4	66,4	69,2	69,7	16,9	13,8
Std. Cul Av.	47,2	19,8	64,3	65,1	64,9	50,9	24,4	16,1	80,5	80,5	49	52,7	10,2	11
Max. volue	55,3	39,2	104,8	104,6	70,9	58,9	39,59	23	81	81	97,8	95,3	27,7	23,6
Min volue	31,9	8,4	60,1	61,3	46,65	37,65	12,67	2,37	53	53	45,3	42,8	5,8	3,9
NLPHSC	19	19	84	81	1	6	29	13	0	0	87	79	74	53
Varyans	15,92	33,09	95,24	91,72	32,44	21,78	27,31	19,1	49	49	130,09	116,34	27,33	19,39
Std. Dev.	3,99	5,75	9,76	9,58	5,7	4,67	5,23	4,37	7	7	11,41	10,79	5,23	4,4
CV	3,87	11,75	3,63	3,63	1,83	4,77	8,4	10,27	3,24	3,24	5,87	6,78	11,99	14,03
$LSD_{(0.05)}$	5.55**	6.74**	8.82**	8.86**	3.41**	7.19**	6.35**	4.26**	6.89**	6.89**	11.85**	13.92**	5.78**	5.75**

**; İndicates Significant differences P≤0.01. Abbreviations; OC: Optimum conditions, SC: Stressful conditions, NLPHSC: Number of landrace genotypes, exceeding the highest standard cultivar, CV: Coefficient of variation, LSD: Least significant difference, SPAD: Flag leaf chlorophyll content, HT:Days to heading, GTFL: greening time of flag leaf, FLA: Flag leaf area, FLE: Flag leaf erectness, PH: Plant height, PL: Peduncle length. Ov. Av: overall average, Land. Av: landrace average, Std. Cul Av: Standard cultivar average, Std. Dev: standard Deviation

Table 2. The highest and lowest averages of the analyzed characteristics

Genotypes	S.L.(cm)		G.F.T. (day)		G.F.R. (mm/day)		D.T.M.(day)		N.S.S.		N.G.S.		T.G.W	
	O.C	S.C	O.C	S.C	O.C	S.C	O.C	S.C	O.C	S.C	O.C	S.C	O.C	S.C
1	6,3	6,2	40,2	30,1	0,72	0,99	132,4	121,4	12,1	10,7	15,4	11	28,8	29,2
2	6,1	5,6	39,2	33,1	0,69	0,67	123,4	116,4	12,9	12,5	18,7	15	26,9	21
3	6,7	6,6	37,2	30,1	0,66	0,71	128,4	121,4	12,9	12,3	14,3	11	24,6	20,7
4	5,5	5,2	40,2	34,1	0,86	0,98	113,4	107,4	10,7	10,1	17,9	15	30,9	28,5
6	5,9	5,6	38,2	29,1	0,88	0,94	128,4	118,4	13,3	10,5	12,3	7,8	29,7	23,6
7	5,7	5,2	40,2	36,1	0,67	0,68	116,4	111,4	13,1	11,5	21,1	13	26,6	23,2
8	6,5	5,8	37,2	32,1	0,7	0,82	132,4	123,4	13,3	12,1	17,3	11	25,8	19,5
9	6,1	5,4	45,2	27,1	0,67	0,84	138,4	127,4	14,3	11,9	21	12	29,6	22,5
11	5,7	5,6	35,2	23,1	0,83	1,13	113,4	110,4	13,5	13,1	19	10	27,6	21,6
12	8,3	8	31,2	23,1	1,11	0,98	127,4	118,4	14,9	14,1	18,2	12	35,6	23
13	6,9	6,8	33,2	24,1	0,89	0,7	123,4	109,4	12,1	11,1	12,9	8,3	29,9	20,7
14	4,5	3,8	35,2	28,1	0,71	0,82	126,4	119,4	13,5	10,1	16	12	30,1	22,5
16	5,5	4,4	36,2	31,1	0,79	0,87	120,4	113,4	11,5	9,3	16,1	8,8	28,9	26,2
17	5,5	5,2	35,2	25,1	0,8	0,81	129,4	116,4	15,3	12,7	17,1	13	28,4	20,3
18	5,1	5	31,2	27,1	1,21	1,02	123,4	118,4	11,5	10,9	13,9	9,9	33,9	27,6
19	5,7	5,2	32,2	26,1	0,94	0,73	122,4	116,4	14,9	14,3	17,7	16	31	20,7
21	6,1	5,8	36,2	19,1	0,84	1,59	136,4	118,4	15,3	12,1	18,7	12	30,7	26,5

22	7,9	7,8	36,2	32,1	0,81	0,85	122,4	118,4	14,5	13,9	17,1	13	29,5	27,3
23	6,4	6,2	32,5	27,1	0,83	0,61	117,4	110,4	12,4	11,3	17,8	13	28,1	18,1
24	4,8	4,6	35,5	28,1	0,73	0,7	122,4	115,4	15,8	14,7	19,4	17	25,7	21,3
26	6,8	5,8	36,5	28,1	0,89	0,73	125,4	117,4	12,6	11,9	19,1	9,5	32,4	22,1
27	6,6	5,8	41,5	21,1	0,75	0,94	130,4	110,4	13,4	13,3	21,1	11	30,9	21,5
28	8	6,6	36,5	24,1	0,88	0,73	132,4	120,4	14,8	14,1	21,6	17	32,1	19,2
29	6,4	5,6	41,5	25,1	0,91	0,77	130,4	113,4	12,2	11,3	17,8	12	31,9	20,8
31	6,6	6,2	42,5	17,1	0,7	1,33	133,4	106,4	13,4	12,1	17,3	10	29,5	24,7
32	6	5,6	34,5	33,1	1	0,7	123,4	122,4	14	11,1	17,1	9,6	34,5	24,7
33	7	6,2	37,5	26,1	0,56	0,64	114,4	104,4	13,2	12,3	20,8	12	25,9	18,2
34	10,4	8,6	39,5	27,1	0,79	0,58	128,4	113,4	15,6	15,1	19,7	12	31,2	17,2
36	7,2	6,6	39,5	24,1	0,54	0,6	119,4	104,4	15,2	13,9	18,7	15	21,2	15,8
37	4,4	4	29,5	28,1	0,65	0,45	123,4	122,4	12,6	11,9	17,8	11	19,1	15,9
38	9,8	8,6	26,5	23,1	1,08	0,99	131,4	118,4	18,6	15,1	20,6	13	28,8	24,7
39	9,6	9,2	39,5	31,1	0,83	0,9	130,4	123,4	16,8	16,1	19,9	16	31,7	25,7
41	10,2	9,6	41,5	28,1	0,75	0,89	139,4	126,4	15,6	14,9	19,2	13	31,1	26,7
42	7,2	6,4	33,5	25,1	0,85	0,69	142,4	125,4	15,2	13,7	21,7	14	28,6	18,7
43	7,6	6,8	34,5	24,1	0,88	0,78	130,4	117,4	16,6	16,5	19,8	15	30,4	20,4
44	7	6,6	33,5	25,1	0,99	0,8	130,4	123,4	17,4	17,1	21,2	13	29,3	21,6
45	8,5	7,5	35	28,4	0,94	0,89	129,1	123,9	18,3	15,6	19	15	33,2	24,6
46	6,9	4,7	23	19,4	1,39	1,26	134,1	128,9	15,1	11,6	18,7	16	31,6	23,6
47	5,3	4,7	37	34,4	0,66	0,69	113,1	108,9	11,5	7,6	12,3	7,8	25,2	23
48	7,5	6,5	35	17,4	0,75	1,14	125,1	112,9	13,1	12,4	19	15	26,8	19,1
49	6,3	5,9	27	26,4	1,15	0,82	133,1	121,9	15,5	15	18	15	30,9	21,1
51	6,5	5,5	36	27,4	0,74	0,66	130,1	119,9	16,1	15,2	14,9	13	27,2	17,4
52	8,7	8,3	35	24,4	0,93	1,23	132,1	120,9	15,5	12,4	18,6	9,6	32,9	29,2
53	6,1	5,5	31	15,4	1,07	1,02	139,1	123,9	14,9	13,8	20,8	14	33,5	15
54	7,5	7,1	33	18,4	0,76	0,87	140,1	123,9	18,3	16,4	19,9	12	25,6	15,4
56	4,1	3,3	42	31,4	0,6	0,79	132,1	119,9	13,1	10,8	16,2	12	29,4	24,1
57	6,3	5,7	38	21,4	0,81	0,9	137,1	121,9	15,7	15	18,3	11	31,4	18,5
58	6,5	6,1	37	28,4	0,72	0,89	123,1	116,9	14,3	11,8	17,5	11	27,3	24,5
59	8,1	6,3	39	26,4	0,71	0,88	133,1	118,9	17,3	16,2	19,3	16	28,7	22,5
61	5,1	4,5	32	23,4	0,99	1,1	124,1	118,9	6,1	5,2	17,4	11	32,1	25
62	6,1	5,3	37	26,4	0,78	0,85	129,1	118,9	12,3	11	16,6	13	29,5	21,8
63	5,9	5,3	44	34,4	0,58	0,58	132,1	121,9	13,1	12	16	11	26,6	19,1
64	6,7	5,9	35	32,4	0,8	0,77	124,1	119,9	14,3	11,4	14,8	14	28,6	24,3
66	6,3	5,9	33	29,4	0,93	0,8	125,1	119,9	15,7	13,4	14,4	10	31,1	17,7
67	6,2	5,6	38	36,4	0,91	0,76	124,4	116,9	12	11,8	21,5	12	34	24,4
68	6,6	5,6	39	35,4	0,79	0,79	125,4	120,9	12,8	10,6	20,8	13	30,3	23,8
69	6,4	5,4	38	31,4	0,75	0,66	117,4	108,9	13,8	12,2	17,3	14	27,8	20,5
71	6	4,8	34	24,4	0,74	0,63	122,4	111,9	14,8	12,6	17,3	9,8	27,3	14,8
72	6,4	5,4	38	26,4	0,73	0,73	122,4	111,9	13	11,2	14,4	13	27,1	18,8
73	6,6	5,4	43	25,4	0,68	0,86	129,4	110,9	12,6	11,2	17,8	9,4	31,6	21
74	6,6	6,4	43	29,4	0,69	0,73	135,4	116,9	13	13,2	17,8	9,6	29,4	21
76	6,6	6	40	33,4	0,66	0,59	124,4	118,9	12,2	12,4	19,3	13	30,8	19,5
77	5,8	5,6	39	33,4	0,6	0,62	124,4	118,9	12	12	18,3	11	23	20,7

78	6,2	5,8	39	24,4	0,68	0,96	127,4	113,9	14,6	13,2	17,1	13	25,9	22,3
79	7,6	6,6	39	30,4	0,8	0,8	123,4	119,9	13,8	9,6	17,8	10	30,5	23,7
81	7,8	5,6	37	31,4	0,84	0,72	123,4	120,9	16,2	12,4	16,5	11	30,4	22,3
82	6,6	6	43	38,4	0,8	0,79	117,4	113,9	15	14	21,1	19	33,7	30,2
83	5,6	5,4	44	37,4	0,72	0,78	114,4	107,9	13,6	12,6	23,9	18	31,3	29,2
84	6,4	5,6	37	31,4	1,03	0,97	125,4	122,9	11	11,4	14,3	12	37,3	29,8
86	7,2	6,2	36	34,4	0,7	0,6	118,4	116,9	15	12,6	16,8	12	24,7	20,7
87	5,8	5,2	46	42,4	0,7	0,59	117,4	112,9	12,6	12,4	22,5	17	31,6	25,5
88	6,4	5,6	45	38,4	0,67	0,67	113,4	109,9	14,6	13,8	23,9	18	29,9	25,9
89	6,5	5,6	39,5	31,1	0,68	0,67	127,9	123,4	14	12,3	17,1	12	26,8	21
91	7,3	6	37,5	28,1	0,9	0,71	124,9	117,4	14,6	11,5	14,7	10	28,4	20
92	5,3	4,6	35,5	33,1	0,97	0,6	122,9	124,4	12	8,9	15,7	12	33,9	19,8
93	7,1	6,6	42,5	39,1	0,83	0,54	124,9	126,4	14,8	11,5	14,5	13	31	21,5
94	6,3	5	38,5	33,1	0,83	0,66	122,9	124,4	13,2	10,9	15,8	13	31,4	22
96	5,3	4,8	41,5	29,1	0,73	0,84	123,9	118,4	12,6	8,9	14,9	13	30,1	24,5
97	5,1	5	48,5	43,1	0,61	0,54	121,9	119,4	14,2	12,9	21,6	17	29,4	24
98	6,3	4,2	32,5	25,1	1	1,05	116,9	114,4	12,6	9,3	15,4	11	32	26,1
99	5,5	5	45,5	43,1	0,78	0,74	114,9	114,4	14,8	13,5	23,1	19	33,1	32,5
101	5,3	4,8	33,5	26,1	0,99	1,04	116,9	114,4	13,8	11,7	17	12	32,7	27
102	5,9	5,6	41,5	31,1	0,76	0,76	127,9	121,4	11	9,3	16,1	13	31,3	23,7
103	6,3	6,4	47,5	39,1	0,67	0,64	117,9	113,4	15,4	14,9	25,8	18	31,6	25,4
104	6,3	6	42,5	25,1	0,69	0,97	130,9	116,4	13,4	12,9	20,3	16	28,9	24,2
106	10,1	9,2	34,5	25,1	1,11	1,14	128,9	117,4	12,4	11,9	15,5	12	37,6	28,4
107	6,5	5,6	35,5	27,1	0,81	0,76	128,9	119,4	15,8	12,5	16,2	13	31,4	20,5
108	6,3	6	41,5	30,1	0,76	0,78	122,9	119,4	12,4	11,3	17,4	14	31,1	23,5
109	5,9	4,8	37,5	27,1	0,89	0,9	118,9	117,4	14,4	12,9	20,3	10	33,1	24,3
110	6,1	6	40,5	24,1	0,92	0,91	121,9	113,4	12,4	11,5	19,3	11	34,9	21,6
Artuklu	5,9	5,7	48,2	39,4	0,69	0,8	118,6	112,4	12,6	11,7	25,7	19	32,9	30,8
D. Bakır-81	5,8	5,4	41	36,8	0,76	0,79	113,2	109,8	12,7	11,8	23,2	18	30,6	28,3
Fırat-93	5,4	5,2	45	38,2	0,85	0,89	117,4	112	12,4	11,3	21,5	17	38	33,9
Sümerli	5,2	5	45,6	39	0,71	0,76	117,2	111,4	12,9	12,4	23,2	18	31,5	29
Ov. Av.	6,52	5,86	37,86	29,14	0,81	0,82	125,4	117,2	13,85	12,36	18,29	13	30,01	22,91
Land. Av..	6,5	5,9	37,5	28,7	0,81	0,82	125,8	117,5	13,9	12,4	18	13	29,9	22,6
Std. Cul Av	5,8	5,3	44,9	38,3	0,75	0,81	116,6	111,4	12,65	11,8	23,4	18	33,25	30,5
Max. volue	10,4	9,6	48,5	43,1	1,69	1,59	142,4	128,9	18,6	17,1	25,8	19	38	33,9
Min. volue	4,1	3,3	23	15,4	0,54	0,45	113,1	104,4	6,1	5,2	12,3	7,8	19,1	14,8
NLPHSC	67	44	1	3	29	25	74	74	62	38	1	0	0	0
Varyans	1,45	1,27	22,51	34,71	0,02	0,03	47,14	28,53	3,48	3,88	7,99	7	10,39	15,47
Std. Dev.	1,2	1,13	4,74	5,89	0,15	0,19	6,87	5,34	1,87	1,97	2,83	2,7	3,22	3,93
CV	4,95	5,42	5,74	6,59	7,73	12,9	1,42	1,41	2,95	4,77	4,53	4,8	5,2	8,57
LSD $_{(0.05)}$	0.97**	0.96**	6.88**	6.19**	0.19**	0.32**	5.43**	5.06**	1.24**	1.80**	2.65**	2**	4.89**	6.34**

**: İndicates Significant differences P≤0.01. Abbreviations; OC: Optimum conditions, SC: Stressful conditions, NLPHSC: Number of landrace genotypes, exceeding the highest standard cultivar, CV: Coefficient of variation, LSD: Least significant difference, SL: Spike length, GFT: Grain filling time, GFR: Grain filling rate, DTM: Days to maturity, NSS: Number of spikelets on the spike, NGS: Number of grains per spike, TGW: Thousand-kernel weight, Ov. Av: overall average, Land. Av: landrace average, Std. Cul Av: Standard cultivar average, Std. Dev: standard Deviation

Table 3. The results of the correlation analysis of the characteristics studied under optimum conditions

Characteristics	SPAD	G.F.T	G.F.R	D.T.H	G.T.F.L	D.T.M	P.H	P.L	F.L.A	F.L.E	S.L	N.S.S	N.G.S
G.F.T	0,009												
G.F.R	0,17	-0,665**											
D.T.H	0,103	-0,667**	0,434**										
G.T.F.L	0,097	0,275**	-0,084	0,061									
D.T.M	0,17	-0,235*	0,172	0,859**	0,318**								
P.H	0,273**	-0,391**	0,138	0,500**	-0,196	0,356**							
P.L.	0,189	-0,270**	0,024	0,175	-0,352**	0,046	0,633**						
F.L.A	-0,1	-0,042	-0,015	0,096	-0,032	0,034	0,094	-0,163					
F.L.E	0,195	0,429**	-0,105	-0,526**	0,320**	-0,339**	-0,427**	-0,205*	-0,148				
S.L	0,16	-0,167	0,221*	0,352**	0,267**	0,363**	0,256*	0,021	0,224*	-0,028			
N.S.S	0,114	-0,194	0,077	0,366**	0,330**	0,355**	0,244*	-0,049	0,176	0,103	0,495**		
N.G.S	0,284**	0,386**	-0,163	-0,341**	0,339**	-0,126	-0,271**	-0,220*	-0,112	0,657**	0,061	0,224*	
T.G.W	0,264*	0,082	0,603**	-0,06	0,052	0,009	-0,203*	-0,187	0,035	0,280**	0,146	-0,108	0,151

İndicates Significant differences ***:** p<0.05, ****:** P≤0.01. Abbreviations; SPAD: Flag leaf chlorophyll content, GFT: Grain filling time, GFR: Grain filling rate, DTH: Days to heading, GTFL: Greening Time of flag leaf, DTM: Days to maturity, PH: Plant height, PL: Peduncle length, FLA: Flag leaf area, FLE: Flag leaf erectness, SL: Spike length, NSS: Number of spikelets on the spike, NGS: Number of grains on the spike, TGW: Thousand-kernel weight.

Table 4. The results of the correlation analysis of the characteristics studied under high-temperature stress conditions

Characteristics	SPAD	G.F.T	G.F.R	D.T.H	G.T.F.L	D.T.M	P.H	P.L	F.L.A.	F.L.E	S.L	N.S.S	N.G.S
G.F.T	0,109												
G.F.R	-0,053	-0,555**											
D.T.H	0,039	-0,731**	0,396**										
G.T.F.L	0,213*	0,17	-0,045	0,191									
D.T.M	0,131	-0,088	0,031	0,703**	0,466**								
P.H	0,189	-0,442**	0,164	0,588**	-0,025	0,428**							
P.L	0,058	-0,159	-0,04	0,036	-0,156	-0,037	0,499**						
F.L.A	0,257*	0,263*	0,006	-0,276**	-0,063	-0,199	0,075	0,008					
F.L.E	0,09	0,475**	-0,132	-0,541**	0,188	-0,250*	-0,423**	-0,098	0,185				
S.L	0,081	-0,204*	0,152	0,195	0,112	0,139	0,153	-0,106	0,194	0,044			
N.S.S	0,111	-0,163	-0,074	0,176	0,313**	0,121	0,197	-0,1	0,157	0,198	0,519**		
N.G.S	0,069	0,389**	-0,133	-0,366**	0,289**	-0,125	-0,268**	-0,072	0,231*	0,700**	0,032	0,392**	
T.G.W	0,104	0,462**	0,384**	-0,390**	0,077	-0,114	-0,336**	-0,215*	0,316**	0,467**	0,019	-0,214*	0,321**

İndicates Significant differences ***:** p<0.05, ****:** P≤0.01. Abbreviations; SPAD: Flag leaf chlorophyll content, GFT: Grain filling time, GFR: Grain filling rate, HT: Days to Heading, GTFL: Greening time of flag leaf, DTM: Days to maturity, PH: Plant height, USL: Peduncle length, FLA: Flag leaf area, FLE: Flag leaf erectness, SL: Spike length, NSS: Number of spikelets on the spike, NGS: Number of grains on the spike, TGW: Thousand-kernel weight.

CONCLUSION

The study revealed that there are landrace genotypes that may have the potential to tolerate high-temperature stress one of the main causes of yield losses in the South-eastern Anatolia Region. Under high-temperature stress conditions, the genotypes with early heading time, longer flag leaf greening time, more erect flag leaf, longer grain filling time, and higher grain filling rate prevailed in terms of the characteristics that affected the yield, such as the thousand-kernel weight and number of grains on the spike. Therefore, the preference of genotypes with these parameters in the improvement programs to minimize the effect of high-temperature stress in the South-eastern Anatolia Region is considered to increase improvement success. Besides, it is recommended that practical, easy, and fast-measurable characteristics such as days to heading, flag leaf erectness, flag leaf greening time, grain filling time, and grain filling rate be utilized as selection criteria in identifying genotypes tolerant to high-temperature stress in the region. Furthermore, it is thought that landrace durum genotypes 82, 83, 87, 88, 99, and 103, as well as Artuklu and Sümerli, standard durum wheat, distinguished by their morphological and

physiological parameters that positively affect grain yield and yield components that are directly associated with grain yield, can be included as parents in crossbreeding programs in the development of cultivars tolerant to high-temperature stress.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

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

Ethics committee approval

Ethics committee approval is not required. This article does not contain any studies with human participants or animals performed by any of the authors.

Funding

This study was supported by Dicle University Scientific Research Projects Coordination Unit with project number ZİRAAT.21.005 and by the General Directorate of Agricultural Research and Policies with project number TAGEM/TBAD/A/22/A7/P1/5088.

Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

This study was produced from the Ph.D. thesis titled "The determination of DNA markers related to Heat Stress and relating morphological and physiological characters being selection criteria in some durum wheat landraces" conducted by Mustafa OKAN in the Department of Field Crops, Institute of Science and Technology, Dicle University. We thank the Scientific Research Coordination Unit and the General Directorate of Agricultural Research and Policies for their support.

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RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(3): 621-632
DOI: https://doi.org/10.31015/jaefs.2023.3.16

Environmental effects of tourism activities in Niksar Çamiçi Plateau in the context of sustainable tourism: a qualitative research

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Citation: Gunduz, C., Atak, O. (2023). Environmental effects of tourism activities in Niksar Çamiçi Plateau in the context of sustainable tourism: a qualitative research. International Journal of Agriculture, Environment and Food Sciences, 7 (3), 621-632

Received: August 10, 2023
Revised: August 30, 2023
Accepted: September 01, 2023
Published Online: September 25, 2023

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Available online at
https://jaefs.com/
https://dergipark.org.tr/jaefs

Abstract

Tourism, as a booming industry, plays a significant role in shaping economies and cultural exchanges worldwide. However, the rapid growth of tourism has brought about both positive economic impacts and negative environmental consequences. This research delves into the environmental effects of tourism activities through the lens of sustainable tourism. Focusing on Niksar Çamiçi Plateau, a transition zone between Central Anatolia and the Black Sea Region, situated at an altitude of 1350 meters, the study aims to identify the specific environmental impacts resulting from tourism activities in this unique location. The research adopts participant observation and semi-structured interviews as data collection methods, enabling a comprehensive understanding of tourists' and locals' behaviors and practices concerning the environment. The findings highlight the pressing issues of unplanned development, urban sprawl, migration, and environmental pollution in regions experiencing concentrated tourism activities. In response to these challenges, the study proposes a set of sustainable tourism solutions to mitigate environmental degradation. Recommendations include promoting responsible tourism practices, reducing the carbon footprint, promoting environmentally friendly accommodation and transportation, and implementing effective waste management strategies. Additionally, raising awareness among tourists and local communities about the importance of preserving natural resources and cultural heritage emerges as a crucial approach. This study contributes to the growing body of knowledge on sustainable tourism by providing valuable insights into the complex relationship between tourism and the environment, particularly in Niksar Çamiçi Plateau. By advocating for sustainable practices, the research aims to strike a balance between economic growth and environmental conservation, ensuring a harmonious coexistence between tourism and the ecosystem.

Keywords: Sustainable Tourism, Environmental Impacts, Niksar Çamiçi Plateau, Responsible Tourism, Environmental Conservation

INTRODUCTION

Tourism is defined as a significant economic sector on a global scale (Gössling, et al., 2023; Scott et al., 2012; Hardy, et al., 2002). The advancement of transportation and communication technologies has contributed to the growth of the tourism industry, leading to a noticeable increase in the number of people traveling for tourism purposes worldwide (UNWTO, 2020). The relationship between tourism and the environment is a dynamic interplay where the impacts of tourism activities on natural and cultural surroundings, as well as efforts to minimise negative effects and promote responsible practices, shape the overall

ecological and social sustainability of destinations (Hall, 2021). Recognising the significant role of tourism in the global economy and understanding its evolving relationship with the environment underscores the need for a concerted effort towards sustainable practices that preserve both the allure of destinations and the integrity of our planet for future generations.

However, the rapid expansion of the tourism sector has also increased its environmental impacts (Zhong et al., 2011). Mass tourism and unplanned growth can give rise to environmental issues (Razali et al., 2018). Especially in areas where tourism activities are concentrated, negative effects such as unplanned urbanization, migration, environmental pollution, and the assimilation of cultural values are more commonly experienced (Türker, 2020).

This study focuses on the environmental effects of tourism activities and takes Niksar Çamiçi Plateau as an example. Situated on the transition route from the Central Anatolia Region to the Black Sea Region, Niksar Çamiçi Plateau is a tourist destination known for its natural riches and pine forests (TİKTM, 2021). The study aims to identify the environmental impacts of tourism activities in the plateau settlement and evaluate them from the perspective of sustainable tourism.

Tourism is a complex socio-economic phenomenon that interacts with cultural and natural environments (Briassoulis & Straaten, 2000). Tourism mobility can affect the natural environment, socio-cultural structure, and economy of the visited regions (Mejjad et al., 2022; Rezaei et al., 2020; Karakaya et al., 2013). The environmental impacts of tourism can manifest in various dimensions in areas with concentrated tourism activities. These impacts can lead to concerns about environmental sustainability, such as environmental pollution, ecosystem disturbances, depletion of natural resources, and a decrease in plant and animal species (Sharpley & Telfer, 2014).

Sustainable tourism is an approach that aims to manage tourism activities in a balanced manner, considering economic, socio-cultural, and environmental aspects (Yfantidou & Matarazzo 2017; Gündüz, 2016; Liu, 2003). Sustainable tourism focuses

on meeting the needs of local communities and preserving the natural environment to increase positive impacts of tourism and minimize negative ones (Koçoğlu et al., 2020; Swarbrooke & Horner, 2007). Touristic activities cause an increase in carbon footprints worldwide. Sustainable management of touristic activities and reduction of carbon footprints are critical in terms of minimising environmental impacts on a global scale and protecting the natural resources of future generations (Çelik, 2022). Adopting an effective waste management strategy in the tourism industry is an important step towards protecting the environment without harming natural beauty, maintaining the quality of life of local communities, and ensuring the long-term sustainability of tourist destinations (Obersteiner Eet al., 2021). Informational signage plays a critical role in raising environmental awareness in tourist areas, as it provides visitors with an opportunity to understand local ecosystems, natural resources, and sensitive areas and guides them in minimising environmental impacts (Cole, 2011).

The primary objective of this study is to investigate the environmental impacts of tourism activities on the Niksar Çamiçi Plateau from a sustainability perspective. Understanding the effects of tourism on the plateau's environment is critical for implementing measures for environmental conservation and formulating policies for sustainable tourism (Wang et al., 2017). To accomplish this aim, a comprehensive analysis of the environmental impacts of tourism in the plateau area will be conducted using participant observation and semi-structured interviews.

Previous research on sustainable tourism has predominantly emphasised its benefits, such as economic growth, employment generation, and cultural exchange (Agrawal et al., 2022; Atak, 2016; Gündüz, 2022; Loureiro & Nascimento, 2021; Aktaş & Çiçek, 2019; Gkoumas, 2019; Atasoy et al., 2018; Ağca, 2016; Demircan, 2016; Carter et al., 2015; Hardy et al., 2002). However, there is a growing body of literature underscoring the necessity of striking a balance between tourism development and environmental conservation. Studies have highlighted cases where unregulated tourism has resulted in deforestation, pollution, habitat destruction, and disruption of local ecosystems (Mejjad et al., 2022; Wang et al., 2021; Barletta et al., 2010; Davenport & Davenport, 2006). To address these concerns, researchers and policymakers have advocated for the adoption of sustainable practises aimed at minimising negative impacts while maximising the benefits of tourism (Özgit & Öztüren, 2021; Vernon et al., 2005).

The environmental impacts of tourism, together with factors such as heavy visitor flow, infrastructure needs, and waste generation, can lead to degradation of natural areas, increased water and energy consumption, and loss of biodiversity. The management of these impacts and the adoption of sustainable tourism practices are vital to ensuring that natural and cultural resources are protected for future generations (Mikayilovet al, 2019). This study is crucial for comprehending the environmental impacts of tourism activities in regions endowed with natural and cultural wealth, such as the Niksar Çamiçi Plateau. By revealing how the rapid expansion of the tourism sector affects environmental resources and societal structures, it will offer policy recommendations for managing tourism in harmony with principles of sustainability. Furthermore, this research will promote the development of sustainable tourism practises by encouraging the engagement of environmentally

conscious tourists and local communities. The findings from the Niksar Çamiçi Plateau will serve as a model for addressing environmental impacts in similar tourism destinations.

In conclusion, this study represents a significant stride towards understanding and managing the environmental ramifications of tourism activities on the Niksar Çamiçi Plateau. Assessing the environmental impacts of tourism based on scientific evidence is vital to ensuring the preservation of natural resources for future generations (Shasha et al., 2020). Additionally, the research will contribute to advocating for eco-friendly practises within the tourism sector by providing policy recommendations for the eco-conscious management of tourism activities.

MATERIALS AND METHODS

The Study Area

The research was conducted in Niksar Çamiçi Plateau, located in the transition area from the Central Anatolian Region to the Black Sea Region, at an approximate altitude of 1350 meters (TİKTM, 2021). The plateau is situated approximately 16 kilometers away from Niksar district center and can be reached by car within approximately 25 minutes. The plateau experiences the highest population density during the summer months when tourism activities are at their peak (Gündüz & Topaloğlu, 2019).

Sampling Techniques and Sample Size

This study employed a qualitative research design. Data were collected using participant observation and semi-structured interviews. In qualitative research, the researcher is a key instrument for data collection (Kılıç et al., 2020). The researcher gains the opportunity to gather information on-site by obtaining permission from and joining the group being observed (Uzuner, 1999).

A purposive sampling method, specifically criterion sampling, was employed to determine the study group. Criterion sampling involves selecting individuals, events, objects, or situations with specific characteristics related to the problem being studied (Büyüköztürk et al., 2009; Yıldırım & Şimşek, 2004). The study group of 14 individuals, comprising local residents and business owners, who were directly involved with tourism activities in the plateau. Within the scope of the research, 3 visits were made to the plateau region in July, August and September 2021. During these visits, a group of fourteen residents and tradesmen in the highland talked about the environmental impacts of tourism by Gökçe et al. (2015) were asked questions prepared by making use of the study.

Methods of Data Analysis

The data analysis process involved thematic analysis, which is a common approach in qualitative research. Thematic analysis allows the researcher to identify, analyze, and report patterns or themes within the data (Braun & Clarke, 2006). The transcribed interviews and observation notes were carefully reviewed, and key themes related to the environmental impact of tourism were identified.

The identified themes were then organized into a table to provide a comprehensive overview of the environmental effects. The table included the specific environmental impacts (e.g., noise pollution, air pollution, water pollution, etc.) as rows, and three columns labeled "exists", "partially", and "does not exist" to indicate the presence and intensity of each impact. Additionally, the table presented the months in which these effects were most pronounced to identify seasonal patterns.

The analysis also involved identifying prominent issues through direct observation. The researcher observed areas such as the campsite, health center, gendarmerie post, and restaurant zone, and documented the environmental pollution problems using photographs and notes.

Ethical Considerations

The study adhered to ethical principles by obtaining informed consent from the participants and ensuring their anonymity. Ethical approval for this study was received from the Tokat Gaziosmanpaşa University Social and Humanity Sciences Research Ethics Committee, numbered 12/03 and dated 09.19.2022. The study was carried out in accordance with the ethical rules and standards determined by the research institution.

Limitations

It is important to acknowledge certain limitations of this research. The sample size was relatively small due to the low population and limited tradesmen in the highlands, and it represented a specific group of people in a specific geographic area. Therefore, the findings may not be fully generalizable to other locations or communities. In addition, the research focused only on the environmental impacts of tourism in the Niksar Çamiçi Plateau and did not address other aspects of sustainability or its economic impacts. In conclusion, this study used qualitative research methods to investigate the environmental impact of tourism on the Niksar Çamiçi Plateau. The thematic analysis was used to identify key environmental issues related to tourism activities. The findings offer valuable insights into the challenges faced by the plateau and offer recommendations for sustainable tourism development. However, it would be beneficial to work with larger and more diverse sample groups in order to comprehensively cover the broad aspects of sustainability in the tourism sector.

RESULTS AND DISCUSSION

The fundamental objective of this study is to comprehensively examine the ecological repercussions

stemming from tourism activities within the confines of the Niksar Çamiçi Plateau, within the overarching framework of sustainable tourism practises. Utilising a quantitative methodology, the investigation delves into the variations in population density on the plateau across different months. This population density computation is underpinned by data gathered through comprehensive interviews with local residents and merchants. Each participant was requested to assess the relative population density of the plateau on a scale of one hundred points. The resultant Figure 1 is a composite representation of the average scores derived from these assessments.

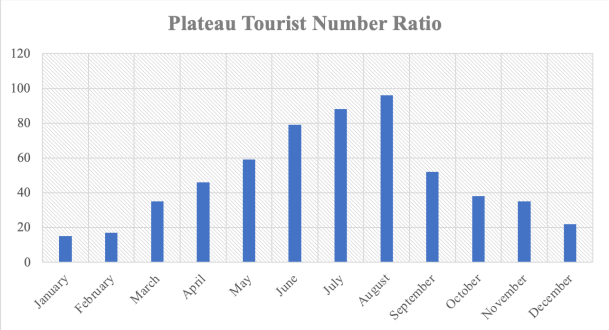

Figure 1. Plateau Tourist Number Density by Month

Analysing the population density trends depicted in Figure 1 serves as a pivotal conduit for deciphering the cyclic fluctuations in environmental impacts. The initial months of January and February exhibit a palpable reduction in plateau inhabitants, signifying a discernible decrease in both local residents and tourists, a pattern attributed to the prevailing winter conditions. Conversely, the onset of March signals a noticeable resurgence in population density, which correlates with the advent of spring. April and May are characterised by a more pronounced escalation in population density, distinctly underscoring the heightened allure of the plateau to tourists as the natural environment undergoes rejuvenation.

The zenith of tourist density materialises during the months of June and July. As the summer vacation season unfolds, the plateau transforms into a preferred destination for vacationers and explorers. This juncture imposes elevated pressures on the plateau's ecological system, thereby accentuating the necessity for structured environmental oversight. August similarly witnesses a substantial population density, reaffirming the plateau's enduring appeal as summer approaches its zenith.

The month of September witnesses a gradual decrease in the tourist influx, coinciding with the conclusion of the summer holiday period. During this phase, the number of visitors, including both local residents and merchants, displays a discernible decline. The subsequent months of October and November experience a further attenuation in population density as the winter season approaches, accompanied by a concomitant decrease in interest in the plateau. December marks the nadir of occupancy, mirroring the seasonal retreat during the wintertime.

These findings furnish invaluable insights into the seasonal dynamics of tourism activities at the Niksar Çamiçi Plateau and their associated environmental consequences. Adherence to the principles of sustainable tourism is of paramount importance in safeguarding the plateau's ecosystem integrity and counteracting the encroachment of environmental degradation. The implications of this study hold relevance for the domain of tourism management and strategic planning, offering an elemental blueprint for the forthcoming tourism progression of the Niksar Çamiçi Plateau.

The results gleaned from the interview, which included participants from the Niksar Çamiçi region, provide noteworthy insights into the prevalence and intricate interplay of diverse environmental pollutants. The ensuing analysis corroborates the observations delineated in Table 1:

Noise Pollution and Air Pollution: The data indicate that 43% of the participants reported experiencing noise pollution, and 50% identified air pollution as a concern in the region. Both noise and air pollution show their highest presence during June and July, with June being one of the busy months for both pollutants. This similarity in peak periods suggests a possible correlation between noise and air pollution, which can be attributed to the increased vehicular activities and tourist influx during the plateau season and weekends. For example, Participant-4 mentioned experiencing noise annoyance due to vehicle sounds near the Ünye highway on some days, which likely contributes to air pollution through vehicle emissions. Moreover, Participant-13 highlighted that noise levels increase during the plateau season, indicating a possible relationship between increased tourist activities and elevated air pollution during this period.

Air Pollution and Water Pollution: The data show that 50% of the participants reported air pollution as a concern, while 29% identified water pollution. When the Air Quality News Bulletins prepared by the Ministry of Environment, Urbanisation, and Climate Change (2022) are examined, the particulate matter pollutant (PM10) values between Çamiçi Plateau and Ünye also confirm this pollution. For example, while the value measured by Kardemir Karabük Station, which is an iron and steel industrial zone, is 39, the value measured by Ünye and Tokat stations has reached 48. This shows that the air pollution rate is higher than the industrial situation in the region.

Interestingly, both air and water pollution demonstrate similar busy months, with the plateau season being a critical period for both pollutants. Participants-6 and -14

Table 1. Regional Pollution Impact Analysis and Participant Feedback

Effects	Present (Number of Participants)	Partially Present (Number of Participants)	Absent (Number of Participants)	Busy Months	Example Responses
Noise Pollution	n=6 (43%)	n=5 (36%)	n=3 (21%)	June-July	Participant-4: "We experience noise annoyance due to vehicle sounds near the Ünye highway on some days." Participant-2: "The noise increases near the gendarmerie station at times and can affect the quality of sleep." Participant-13: "Noise levels increase during the plateau season and can be disturbing on weekends."
Air Pollution	n=7 (50%)	n=4 (29%)	n=3 (21%)	Plateau season (Weekends)	Participant-6: "Air pollution from the nearby highway affects the plateau air." Participant-14: "Air pollution, possibly caused by tourists coming to the area during weekends (barbecues), increases." Participant-3:"During the plateau season, weekend traffic increases, leading to a decline in air quality."
Water Pollution	n=4 (29%)	n=3 (21%)	n=7 (50%)	May-June	Participant-9: "We see some signs of pollution in the water resources during the months of May and June (when the plateau becomes touristically crowded). Participant-1: "In some areas, water sources get polluted due to environmental contamination." Participant-5: "With the start of the plateau season, the water quality of some streams decreases."
Soil Pollution	n=10 (71%)	n=4 (29%)	n=0 (0%)	May-June-July-August	Participant-7: "In May and June, soil pollution becomes more apparent, and we can see litter around." Participant-8: "We don't experience any issues with soil pollution; our lands are clean and productive." Participant-4:"Agricultural pesticides and chemicals can affect our soil; we need to be careful."

mentioned that air pollution from the nearby highway and recreational activities during weekends and the plateau season contribute to air pollution in the region. Given that the route from the Central Anatolian region to the Middle Black Sea region traverses through this plateau, the occurrence of elevated air pollution is posited, primarily during the summer season, attributable to vehicular congestion and halts. This observation suggests a potential connection between increased tourism-related activities and higher air pollution levels, which may subsequently impact water quality. For instance, pollutants emitted into the air can deposit into

water bodies through wet and dry deposition, leading to contamination. Or it is possible for the garbage left from the vehicles to pollute the water resources by the wind. Participant-5's response also supports this finding, stating that the water quality of some streams decreases with the start of the plateau season.

Water Pollution and Soil Pollution: The data indicate that 29% of the participants reported water pollution, and a concerning 71% identified soil pollution in the region. In Image 1, there is a visual of the soil pollution detected by the researcher in Çamiçi Plateau. This pollution can also affect water resources from time to time, as stated by the participants.

Both water and soil pollution exhibit their highest presence during May and June. Intensification of agricultural activities can also be among the causes of water pollution. However, in this study, only questions about pollution originating from tourism were asked to the participants. Participants-9 and -1 reported observing signs of water pollution during these months, while Participant-7 mentioned that soil pollution becomes more apparent in May and June, with visible litter observed in the area. This close alignment in peak periods suggests a possible link between water and soil pollution, particularly during the busy months. Agricultural runoff and improper waste disposal may contribute to both water and soil contamination during this period. For example, Participant-4 highlighted the potential impact of agricultural pesticides and chemicals on soil pollution, supporting the idea of a relationship between agricultural activities and soil contamination, which can, in turn, affect water quality.

Image 1. Environmental Pollution Observed by the Researcher in Niksar Çamiçi Plateau

Air Pollution and Soil Pollution: The data show that 50% of the participants reported air pollution, while 71% identified soil pollution. Although no direct correlation is evident in the data between air and soil pollution, it is essential to consider the potential indirect effects of air pollution on soil quality. Airborne pollutants, such as particulate matter and gaseous pollutants, can deposit onto the soil surface, leading to soil contamination over time. While the data do not explicitly reveal a relationship, it is plausible that the presence of air pollution may have implications for soil health. Further research is needed to explore this potential connection more comprehensively.

The findings from the interview support the interrelationships between different environmental pollutions in the Niksar Çamiçi region. The numerical data in the table align with the observed patterns, indicating potential correlations between noise pollution and air pollution, air pollution and water pollution, and water pollution and soil pollution. Soil and water pollutions mentioned in this study have been evaluated from a touristic point of view, not from an agricultural point of view. Pollutions seen in Image 1 originate from tourism and have a negative impact on soil and water. These findings underscore the importance of taking an integrated and holistic approach to environmental management to effectively address the interconnected environmental challenges in the region. Implementing targeted pollution control measures and seasonal management strategies can contribute to a sustainable and healthy environment for the residents of Ünye.

The data presented in Table 2 provide insight into the effects of various factors on flora, fauna, construction, infrastructure and informative signage in the Niksar Çamiçi Plateau region. Figure 2 shows an example of human damage to trees in the plateau region. Findings on the subject are discussed below:

Image 2. An example of the damage done by the picnickers to the trees in the Çamiçi Plateau

Alterations in Flora: A significant proportion, approximately 64.3%, of the surveyed participants conveyed the occurrence of perturbations in the indigenous flora, notably accentuated during the bustling months of July and August. Participant-3 made specific note of observing pronounced disruptions in the botanical realm during the summer months, resulting in a decline in certain plant species. Image 2 below shows the damage done to the trees by the tourists who come to the plateau for a picnic. Similarly, Participant-2 documented compromised flora within camping vicinities, characterised by indications of desiccation and diminished botanical diversity. In contrast, Participant-4

Table 2. Regional Deterioration Impact Analysis and Participant Feedback

Impact Type	Present (Participant Count)/ Percentage Present	Partially Present (Participant Count)/ Percentage Partially Present	Absent (Participant Count)/ Percentage Absent	Busy Months	Example Responses
Disturbance in Flora	n=9 (64.3%)	n=3 (21.4%)	n=2 (14.3%)	July-August	Participant-3: "During the summer months, we observe serious disturbances in the flora, and some plant species have decreased." Participant-2: "In some camping areas, the flora has been damaged, and we noticed withered plants and reduced plant diversity." Participant-4: "We generally didn't detect any disturbances in the flora, and we observed the preservation of natural beauty."
Disturbance in Fauna	n=5 (35.7%)	n=3 (21.4%)	n=6 (42.9%)	Plateau season	Participant-9: "During the plateau season, we haven't noticed a significant disturbance in wildlife, and animals are generally present." Participant-6: "We observe partial disturbances in animal presence, and some animals' behaviors have changed." Participant-8: "We haven't observed any negative impact on animal presence during the plateau season, and the richness of natural life continues."
Excessive and Unplanned Construction	n=13 (92.9%)	n=1 (7.1%)	n=0 (0.0%)	All months except winter	Participant-10: "In some areas, we believe construction is unplanned, which is visually disturbing." Participant-11: "We observed only one example of construction at the plateau, and we didn't detect excessive construction." Participant-9: "We think there is excessive construction at the plateau, and it disrupts the natural balance."
Insufficiencies in Infrastructure	n=11 (84.6%)	n=2 (15.4%)	n=0 (0.0%)	Plateau season	Participant-14: "We occasionally come across infrastructure insufficiencies, but mostly there are adequate infrastructure services." Participant-1: "During the plateau season, we didn't detect any infrastructure insufficiencies, and infrastructure services are generally in good condition." Participant-3: "During the plateau season, we face infrastructure insufficiencies, and there are interruptions in water and electricity supply."
Insufficiencies in Informative Signboards	n=5 (35.7%)	n=7 (50.0%)	n=2 (14.3%)	June-July-August	Participant-7: "During June, July, and August, we feel the lack of informative signboards for visitors." Participant-8: "Most of the time, informative signboards are insufficient, and they lack essential information for tourists." Participant-11: "We have enough informative signboards, and visitors usually have no trouble finding their way."

documented an absence of discernible floral disturbances and attested to the persistence of the area's innate natural allure. The observed disturbances within the floral ecosystem during the zenith of tourist activity can be attributed to escalated anthropogenic engagements, such as camping and recreational excursions, which can culminate in trampling, littering, and the infringement of natural habitats. To alleviate these impacts and uphold the region's biodiversity, the implementation of sustainable tourism protocols and the institution of initiatives for habitat preservation stand as imperatives.

Disturbances in Fauna: Roughly 35.7% of the respondents conveyed the existence of disturbances within the fauna domain during the plateau season. Participant-9 remarked upon the paucity of discernible faunal perturbations during this interval, while Participant-6 alluded to partial perturbations in animal presence coupled with shifts in behavioral patterns. Conversely, Participant-8 noted an absence of deleterious effects on animal presence and underscored the profusion of natural vitality during the plateau season. The identified faunal disturbances during this period may be correlated with heightened human presence, resultant clamour, and the concomitant interactions between humans and wildlife. To safeguard indigenous wildlife, the imperative lies in the adaptation of conscientious tourism practises, the preservation of unobstructed pathways for wildlife mobility, and the abstention from any encroachments upon their habitats.

Unbridled and Unplanned Urbanisation: A substantial majority, accounting for 92.9% of the participants, underscored the pervasiveness of uncontrolled and haphazard urban development across the region, a phenomenon recurring throughout the year excepting the winter months. Participant-10 and Participant-9 both chronicled the encroachment of rampant construction activities, with Participant-10 lamenting the visual blight consequent to unregulated construction undertakings. Yet, Participant-11 cited a solitary illustration of construction, implying the absence of universally conspicuous, unbridled development. The ubiquity of unchecked and unplanned construction endeavours can culminate in the degradation of habitats, aesthetic pollution, and a transformed physical milieu. The countering of this predicament necessitates the enforcement of stringent zoning mandates, the institution of environmental impact assessments, and the advocacy of sustainable developmental paradigms.

Insufficiencies in Infrastructure: Around 84.6% of the participants reported insufficiencies in infrastructure during the plateau season. Participant-14 and Participant-1 mentioned that infrastructure services were generally adequate during this period. However, Participant-3 highlighted infrastructure insufficiencies, including interruptions in water and electricity supply. The presence of insufficiencies in infrastructure can impact the quality of tourism experiences and local residents' well-being. To enhance the region's infrastructure and ensure a positive visitor experience, investment in essential services such as water, electricity, waste management, and transportation is crucial.

Insufficiencies in Informative Signboards: Approximately 35.7% of the participants reported the presence of insufficiencies in informative signboards, with 50% partially present during the busy months of June, July, and August. Participant-7 and Participant-8 mentioned the lack of informative signboards during these months, while Participant-11 stated that they have enough informative signboards. Insufficient informative signboards can lead to navigation difficulties for visitors and negatively impact their overall experience. To enhance visitor satisfaction and ensure safety, adequate and well-placed informative signboards with essential information should be provided in key locations. In addition, it is important for highway authorities to put up warning signs in order to increase environmental awareness and prevent littering on the roadsides.

The findings from the survey highlight the diverse impacts of various factors on the flora, fauna, construction, infrastructure, and informative signboards in the Ünye region. These impacts are closely related to tourism activities and human interactions with the environment. Sustainable tourism practices, environmental protection measures, and improved infrastructure services are vital to ensure the long-term preservation of the region's natural beauty, biodiversity, and overall ecological integrity. Collaborative efforts between local authorities, stakeholders, and the community are essential to address these impacts and promote responsible and sustainable tourism practices in the Niksar Çamiçi Plateau region.

CONCLUSION

Consequently, this qualitative research delved into the environmental ramifications of tourism activities within the Niksar Çamiçi Plateau, employing the lens of sustainable tourism. The investigation illuminated the intricate interplay between tourism development and its ecological repercussions. The findings underscored the multifaceted challenges stemming from haphazard expansion, urban sprawl, population migration, and environmental pollution in regions concentrated in tourism activities. This issue aligns with the findings of Özdemir & Tabak (2019) and Deniz (2019), whose studies also yielded analogous results. These challenges, particularly the interconnected predicaments of noise, air, water, and soil pollution, manifest prominently during peak tourism months and the plateau season. Gökçe et al. (2015) similarly contended that such issues are more pronounced during periods of high plateau visitation. The tourism sector is a sector that can harm the environment and increase its carbon footprint due to its structure (Lenzen et al., 2018). No study has been

found on reducing the carbon footprint.

The research underscores the imperative of embracing sustainable tourism practises to mitigate environmental degradation within the Niksar Çamiçi Plateau and analogous tourist destinations. An array of recommendations has been offered to overcome the identified difficulties. Chief among them is the advocacy for responsible tourism practises that inculcate conscientious behaviour among tourists, fostering the safeguarding of natural environs and cultural heritage. This sentiment is echoed by Oğuz & Yılmaz (2019), who, in their research, affirmed that environmental awareness significantly influences responsible tourism practises. Furthermore, endorsing ecologically friendly lodging and transportation alternatives such as eco-lodges and electric vehicles stands as an effective measure for curtailing the carbon footprint generated by tourism activities.

Interview participants underscored that the sites within the plateau compromise water resources and soil integrity. Consequently, the implementation of effective waste management strategies that prioritise recycling and proper waste disposal is pivotal in curtailing pollution and conserving the plateau's pristine allure. Oliver et al. (2021) also contend that attitudinal variables exert influence over recycling behaviours, substantiating earlier findings that vacationers display a diminished inclination to recycle. Thus, initiatives aimed at enhancing tourist awareness upon visiting the plateau become of paramount importance. Equally crucial is the role of awareness campaigns targeted at both tourists and local communities, accentuating the significance of preserving natural assets and cultural heritage. These endeavours can engender a sense of environmental stewardship and sustainable tourism values, involving all pertinent stakeholders.

Likewise, enhancing infrastructure and augmenting informative signage emerge as indispensable for elevating the overall tourist experience and mitigating the adverse consequences of inadequate infrastructure. Environmental information signs are important tools that help the community and visitors understand environmental impacts and increase environmental awareness. These signs are used in natural areas, historical sites, parks, and other public places. It provides information to visitors on issues such as the value of the environment, sensitive ecosystems, local flora and fauna, and sustainable tourism practices, and contributes to making informed decisions and exhibiting environmentally friendly behaviors (Rezaei et al., 2023; De Sausmarez, 2007). Participants emphasised that these information signs are insufficient during the summer months. It is recommended that these deficiencies be corrected by local governments.

A subset of research participants emphasised that water shortages during the summer months adversely impact both tourists and plateau inhabitants. Strategic investments in water supply, electricity, and other essential amenities will undoubtedly contribute to a more sustainable tourism milieu. This sentiment aligns with the findings of Adeola & Evans (2020) in their exploration of Africa, affirming the constructive role of robust infrastructure in advancing tourism.

It is important to consider the suggestions to ensure the development of sustainable tourism in Niksar Çamiçi Plateau by ensuring the balance between tourism and the environment. These recommendations aim to strike a harmonious balance between economic growth and environmental protection. Tourism and environmental factors engage in a complex interaction where the dynamics of traveller behaviour, local ecosystems, and resource use intricately shape the sustainability of destinations (Kallmuenzer et al., 2019). The research provides a broader perspective on the principles of sustainable tourism by considering the complex interaction of tourism and environmental factors from an insightful perspective. The unique ecosystem and cultural heritage of Niksar Çamiçi Plateau are preserved for future generations, offering an exemplary model for local governments, policymakers, and other stakeholders. While Corazza (2020) emphasised that the sustainability training to be given to managers is of critical importance, Yüksek et al. (2019) provide an important perspective on how tourism stakeholders' awareness of sustainable tourism contributes to overall sustainability efforts. In this context, the aim of the study is to encourage a more in-depth cooperation between tourism development and the sensitive ecosystem, so that a balanced approach is adopted, considering both economic progress and environmental protection.

Understanding the limitations of the study and the encountered problems is crucial, as it necessitates careful consideration when making generalisations; for instance, the effects of focusing on a specific time frame or sample size should be evaluated. In this context, some recommendations for future researchers can be made: Exploring similar environmental and sustainable tourism issues in different regions can contribute to understanding both overall trends and regional variations. A more detailed examination of tourist behaviours can deepen our comprehension of influencing factors and offer insights for designing effective awareness campaigns. Sustainability heavily relies on the active involvement and conscientious actions of local communities, as they play a pivotal role in preserving the ecological balance and cultural heritage of their regions (Bergquist, 2007). The role of local community engagement holds critical importance in understanding factors influencing the success of sustainable tourism. Investigating the impacts of significant environmental challenges, especially climate change, on sustainable tourism can be vital for shaping future approaches. Analysing the strategies

of local governments and policymakers can aid in understanding effective planning and implementation, thereby contributing more effectively to the realisation of sustainable tourism.

COMPLIANCE WITH ETHICAL STANDARDS

This research adhered to ethical principles and guidelines while conducting the study. Informed consent was obtained from all participants, and their voluntary participation in interviews and observations was ensured. The study also maintained the anonymity and confidentiality of the participants' information. Ethical approval was obtained from the relevant institutional review board for the study.

Peer-review

Externally peer-reviewed.

Conflict of interest

The author declare no conflict of interest regarding this research. The study was conducted in an impartial manner and there were no external influences or financial interests that could affect the research results.

Author Contribution

Authors designed the study, collected data through participant observation and semi-structured interviews, conducted data analysis and wrote the first draft of the article.

Ethics committee approval

Ethical approval for this study was received from the Tokat Gaziosmanpaşa University Social and Humanity Sciences Research Ethics Committee, numbered 12/03 and dated 09.19.2022. The study was carried out in accordance with the ethical rules and standards determined by the research institution.

Financing

This research did not receive any specific grants from any funding agency in the public, commercial, or not-for-profit sectors. The work was fully funded by the author.

Data availability

The data and materials used in this study are provided for academic and research purposes upon request from the relevant author, subject to ethical considerations and the consent of the participants.

Release

All participants gave written consent for publication of the study's findings. The use of direct citations and descriptive information in this article was approved by the participants.

Thanks

The authors would like to express his gratitude to all the participants who contributed to this research by sharing their views and experiences. Their valuable contributions were instrumental in enriching the findings and conclusions of the study. The authors would also like to thank the residents and shopkeepers of Niksar Çamiçi Plateau for the support and guidance they received throughout the research process.

Acknowledgements

This article has been produced by making use of the oral presentation at the III. International Sustainable Tourism Congress held in 2021

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RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(3): 633-638
DOI: https://doi.org/10.31015/jaefs.2023.3.17

A study on bitter gourd (*Momordica charantia* L.) callogenesis optimization based on hormone balance and explant types

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Citation: Ozsan Kilic, T., Onus, A.N. (2023). A study on bitter gourd (*Momordica charantia* L.) callogenesis optimization based on hormone balance and explant types. International Journal of Agriculture, Environment and Food Sciences, 7 (3), 633-638

Received: August 15, 2023
Revised: September 12, 2023
Accepted: September 14, 2023
Published Online: September 27, 2023

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Available online at
https://jaefs.com/
https://dergipark.org.tr/jaefs

Abstract

Bitter gourd known as *Momordica charantia* L. is an important summer vegetable, belongs to the *Cucurbitaceae* family, grown in tropical areas in the world. Due to its fruits being rich in vitamins, minerals and good dietary fibers, the bitter gourd has many health-protective and pharmacological properties. The goal of the current study was to comparatively assess the indirect regeneration capability using *in vivo* leaf, internodal, and petiole explants of bitter gourd. To serve the purpose Murashige and Skoog (MS) media augmented with various concentrations of auxin and cytokinin for callus formation potent. The study was conducted by evaluating the selection of various auxin and cytokinin concentrations and combinations in MS basic medium. The rate of explant development decreased as cytokinin concentration increased. Although the lowest cytokinin concentration utilized in this study (1.0 mg L^{-1} BAP), it had a beneficial impact on explant development. When compared to the auxin NAA, the auxins IAA and 2,4-D were more beneficial on calli weights. It has been well proven that calli cannot be produced without the presence and balance of plant growth regulators. Experimental results demonstrated that callogenesis of bitter gourd from different explants might be successfully and effectively utilized in cell suspension cultures, genetic transformation, and callogenesis may also be adaptable to other species that are closely related.

Keywords: Bitter Melon, Calli Formation, Internode, Leaf, Petiole

INTRODUCTION

The bitter melon, commonly known as the bitter gourd, belongs to the *Cucurbitaceae* family and a highly nutritious food, due to its high level of bioactive compounds with therapeutic uses. The bitter gourd plant is particularly rich in antioxidants, peptides, alkaloids, glycosides, vitamins A, B, and C, flavonoids, beta-carotene, and lutein. It has a low carbohydrate and fat content as well as few calories (Alina et al., 2016). *Momordica charantia* L. is used in folk healthcare to treat lung and digestive disorders as well as hepatitis, cancer, diabetes, HIV/AIDS, contraceptives, and viral infections (Alexandra and Dorica, 2010; Jin et al., 2019).

It is known that studies to reveal valuable bioactive components that are desired to be obtained from medicinally valuable plants and to increase existing components can support traditional agriculture on industrial scale, thanks to biotechnological approaches. The callus culture technique is an efficient method used for the production of secondary metabolites, which is a specific issue. Of course, as in every tissue culture technique, success in the callus culture

technique depends on some certain specific parameters. Explant source, type of explant, a basic nutrient medium used, type and concentration of carbon source, type, concentration, and combinations of various plant growth regulators such as auxin and cytokinin are the main factors affecting the potential success of callus cultures (Tariq et al., 2014). Although the callus culture technique is an efficient way to produce valuable bioactive components, there is a need to develop an effective callus formation system crop-specific in order to reduce the application cost of the techniques while increasing the yield and quality of the products.

Studies have been carried out on micropropagation, organogenesis and somatic embryogenesis of *M. charantia* L. by using various explants such as cotyledon, leaf, nodal, shoot tip, stem, root fragments by some researchers until today (Huda and Sikdar, 2006; Munsur et al., 2009; Paul et al., 2009; Thiruvengadam et al., 2010; Tang et al., 2011). However, callus and suspension culture studies have not been extensively conducted. In terms of genetic transformation studies and studies in the field of genetic engineering, callus and cell suspension cultures are needed effectively, but there is a need to increase the number of studies to be conducted in this field, since there is no sufficient and detailed research in the literature on *M. charantia* L. callus cultures (Thiruvengadam et al., 2006; Sultana and Rahman, 2012; Alina et al., 2016).

The current study aimed to organize and efficient callus culture protocol for bitter gourd by using three different explant types, namely leaf, internode, and petiole, and various plant growth regulator concentrations and combinations by evaluating the development of explants, and weights of formed calli.

MATERIALS AND METHODS

Plant cultivation and preparation of explants

For the callogenesis process, bitter gourds that were firm, ripe, uniform in color and appearance, and free of any flaws were harvested from a private garden in Antalya (36°53'10.1" N 30°45'23.4" E), Turkey. Bitter gourds, which were transferred to Akdeniz University, Faculty of Agriculture, Department of Horticulture, were washed, and cleaned, and then seeds were separated. The bitter gourd seeds at the same size, appearance, and maturity, were used (Figure 1). The three seeds were sown in plastic pots (13 x 35 cm size) filled with a 2:1 peat: perlite mortar mixture ratio.

After 30 days from seed sowing, while the bitter gourd plants were at the four-five leafy stage, explants of leaf, internodes, and petioles were removed and taken to the laboratory (see Figure 2). To serve the surface sterilization process, *in vivo* leaf, internode, and petiole explants were immersed in a 15% sodium hypochlorite solution (5% active substance) for ten minutes, and then rinsed three times with sterile distilled water.

Figure 1. Bitter gourd seeds

Figure 2. Surface sterilization (a), culturing the bitter gourd explants (b, c, d)

Media preparation, culture conditions, and setting up callus cultures

To see the calli development in bitter gourd, 19 different media (which also include a control medium) combinations were used in the present study (Table 1). The basic media was Murashige and Skoog medium (MS, 1962), supplemented with different concentrations and mixtures of BAP, NAA, 2,4-D, and IAA. All of the explants used in the study were cultured at a specific size (0.5 to 1.0 cm), and maintained under growth chamber settings at temperature of 24±2 °C, a photoperiod of 16 hours of light and 8 hours of darkness, and a light intensity of 3000 $E.m^{-2}.s^{-1}$. Developed explants and calli were subjected to 3 subcultures with 30 days intervals.

Evaluated parameters

Developments of leaf, internode, and petiole explants (%), and formed callus fresh weights (g) were evaluated

Table 1. Combinations of Media Utilized for Callogenesis Process

Media No	Media Combinations						
	MS (g L^{-1})	BAP (mg L^{-1})	NAA (mg L^{-1})	2,4-D (mg L^{-1})	IAA (mg L^{-1})	Sucrose (g L^{-1})	Agar (g L^{-1})
1 (cont.)	4.4	-	-	-	-	30	6
2	4.4	1.0	0.5	-	-	30	6
3	4.4	1.0	1.0	-	-	30	6
4	4.4	1.0	-	-	0.5	30	6
5	4.4	1.0	-	-	1.0	30	6
6	4.4	1.0	-	0.5	-	30	6
7	4.4	1.0	-	1.0	-	30	6
8	4.4	2.5	0.5	-	-	30	6
9	4.4	2.5	1.0	-	-	30	6
10	4.4	2.5	-	-	0.5	30	6
11	4.4	2.5	-	-	1.0	30	6
12	4.4	2.5	-	0.5	-	30	6
13	4.4	2.5	-	1.0	-	30	6
14	4.4	5.0	0.5	-	-	30	6
15	4.4	5.0	1.0	-	-	30	6
16	4.4	5.0	-	-	0.5	30	6
17	4.4	5.0	-	-	1.0	30	6
18	4.4	5.0	-	0.5	-	30	6
19	4.4	5.0	-	1.0	-	30	6

after each subculture. Three subcultures were carried out to promote the weight of the calli obtained. To determine the developments of leaf, internode, and petiole explants (%) the following equation was separately used for each.

Leaf, internode, and petiole explants development (%) = (number of developed leaf, internode, and petiole explants/ number of total cultured leaf, internode, and petiole explants) × 100

Statistical analysis

The experiment of callogenesis process was carried out as 3 replicates and 3 subcultures were conducted for each explant type. Four petri dishes with 10 explants of each leaf, internode, and petiole explant parts were utilized in each repetition. At the end of 3 subcultures for each explant type, the mean values were evaluated, and obtained result data were subjected to Duncan test at $p < 0.05$ level by using SPSS (Version 17; Chicago, IL, USA) statistics software.

RESULTS AND DISCUSSION

Evaluations of explant types

In terms of both explant development and produced calli weights, the results of experiments definitely indicated that there was a statistically important distinction among three different types of explants. Regarding explants types, in comparison, the responses of leaf explants were better than internode and petiole explants (Table 2).

Evaluations of media combinations

Regarding the percentages of explants development, there were statistically important distinctions among various media combinations (Table 3). The medium number 6 which was augmented with 1.0 mg L^{-1} BAP + 0.5 mg L^{-1} 2,4-D, was the best among all 19 media combinations regarding percentages of explants development, while the control medium and medium number 18 (5.0 mg L^{-1} BAP + 0.5 mg L^{-1} 2,4-D) had no positive effects on explant developments.

Regarding the calli weights, statistically important variations were found among media combinations (Table 3). MS basic medium supplemented with 2.5 mg L^{-1} BAP + 0.5 mg L^{-1} IAA (medium No. 10) and 1.0 mg L^{-1} BAP + 0.5 mg L^{-1} 2,4-D (medium No. 6) were the best when the effects of different media combinations on explant development. On the other hand, there were no favorable impacts of control medium or medium number 7 (1.0 mg L^{-1} BAP + 1.0 mg L^{-1} 2,4-D) on calli weights.

It is widely known that several factors influence the synthesis and accumulation of secondary metabolites in explants cultivated *in vitro*, as well as the callogenesis process. Genotype, different types of explants, composition, and combination of media, carbohydrate supply, plant growth regulators types, concentrations and combinations, and culture conditions are among the parameters that have been found to be crucial (Siatka, 2019).

Table 2. Explant Development (%) and Calli Weights Based on Explant Types

Explants	Percentages of explants development	Mean values of formed calli weights
Leaves	$65.56^{c} \pm 21.31$	$0.21^{c} \pm 0.13$
Internodes	$19.97^{b} \pm 15.87$	$0.12^{b} \pm 0.10$
Petioles	$5.64^{a} \pm 7.01$	$0.06^{a} \pm 0.11$

*According to the Duncan multiple comparison results, the difference between the means with the same letter is insignificant.

Table 3. Explant Development (%) and Calli Weights Based on Media Combinations

Media	Percentages of explants development	Mean values of formed calli weights
1	$0.00^{a} \pm 0.00$	$0.00^{a} \pm 0.00$
2	$32.41^{bc*} \pm 18.33$	$0.18^{cde*} \pm 0.07$
3	$29.45^{abc*} \pm 20.87$	$0.14^{b-e*} \pm 0.14$
4	$35.00^{bc*} \pm 30.00$	$0.16^{b-e*} \pm 0.13$
5	$40.47^{bc*} \pm 18.04$	$0.12^{b-e*} \pm 0.12$
6	$50.28^{c} \pm 34.53$	$0.21^{e} \pm 0.10$
7	$21.21^{abc*} \pm 2.95$	$0.05^{ab*} \pm 0.11$
8	$38.33^{bc*} \pm 29.04$	$0.20^{de*} \pm 0.12$
9	$26.30^{abc*} \pm 47.17$	$0.12^{a-e*} \pm 0.16$
10	$37.69^{bc*} \pm 36.11$	$0.22^{e} \pm 0.13$
11	$25.38^{abc*} \pm 24.79$	$0.14^{b-e*} \pm 0.14$
12	$25.00^{abc*} \pm 43.30$	$0.09^{a-d*} \pm 0.14$
13	$42.41^{bc*} \pm 33.05$	$0.13^{b-e*} \pm 0.13$
14	$38.33^{bc*} \pm 42.49$	$0.14^{b-e*} \pm 0.14$
15	$29.17^{abc*} \pm 41.23$	$0.14^{b-e*} \pm 0.14$
16	$24.17^{abc*} \pm 47.63$	$0.09^{a-e*} \pm 0.14$
17	$26.95^{abc*} \pm 45.48$	$0.12^{b-e*} \pm 0.15$
18	$18.05^{ab*} \pm 31.85$	$0.07^{a-d*} \pm 0.11$
19	$24.26^{abc*} \pm 41.50$	$0.07^{abc*} \pm 0.11$

*According to the Duncan multiple comparison results, the difference between the means with the same letter is insignificant.

Thiruvengadam et al. (2006) and Sultana and Rahman (2012) reported a positive trend about calli inducing using leaf explants of bitter gourd on MS basic medium fortified with 1.0 mg L^{-1} 2,4-D. Paul et al. (2009) obtained the best bitter gourd calli formation on MS media fortified with 0.5 mg L^{-1} NAA and 5.0 mg L^{-1} BAP, in other words, relatively high cytokinin concentration was necessity for calli formation in their study. On the other hand, in the present study, the best explants that developed calli and showed best explant development were obtained in MS media suplemented with low BAP (1.0 mg L^{-1}) and low 2,4-D (0.5 mg L^{-1}) concentrations. Also, the same media combination above stated along with a relatively high BAP (2.5 mg L^{-1}) and low IAA (0.5 mg L^{-1}) combinations were found to be effective on calli weights. Callus induction for *Momordica cymbalaria* was reported by Jeyaprakasam et al. (2021). Researchers cultured explants (leaf, root tubers, internode, fruit) of *Momordica cymbalaria* for callus induction in MS medium supplemented with different growth regulators at various concentrations. It was demonstrated that callus was induced from leaf and internode explants when MS medium was supplemented with 1 mg L^{-1} and 5 mg L^{-1} of 2,4-D, respectively. Chung et al. (2016) reported that the highest callus frequency occurred when culturing *Momordica dioica* leaf explants with 1 mg L^{-1} NAA and 0.5 mg L^{-1} TDZ. It was also demonstrated that TDZ combined with IAA or 2,4-D, both of which induced less callogenesis than the NAA plus TDZ combination.

As reported by numerous researches on different plant types, the callogenesis process could not be conducted in a medium without plant growth regulators of auxin and cytokinin (Elaleem et al., 2009; Ozsan and Onus, 2020). However, media combination should be supplemented with appropriate concentration and combination of auxin and cytokinin to trigger calli formation as well as explant types (Lestari et al., 2019). Results of current study and results of previous studies indicated the importance of precense of auxins and cytokinins as well as the balance between auxin and cytokinin concentration for calli formation (Baharan et al., 2015; Efferth, 2019).

CONCLUSION

The increase in cytokinin amount led to a decrease in the explant development rates, while the lowest cytokinin amount (1.0 mg L^{-1} BAP) which was used in the current study had a positive effect on explant development. The

auxins IAA and 2,4-D at 0.5 mg L^{-1} concentration were favorable to calli weights compared with the NAA as auxin source. The findings of the present study also clearly revealed that there were differences among explant types regarding callus induction and weights and leaf explants came into prominence. It is believed that more diverse media combinations should be optimized based on the explant types used for callus formation in future research. It is assumed that the findings of the present study may serve as a guide for scientists working on bitter gourd breeding and biopharmaceuticals employing callus and cell suspension cultures.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

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

Author contribution

The contribution of the authors to the present study is equal. Both authors read and approved the final manuscript. Both authors verify that the text, figures, and tables are original and that they have not been published before.

Ethics committee approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

Thanks to Research Assistant İsmail BOYAR from Akdeniz University for his contribution to statistical analysis.

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RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(3): 639-649
DOI: https://doi.org/10.31015/jaefs.2023.3.18

Unveiling the phytochemical variability of fatty acids in world marigold (*Calendula officinalis* L.) germplasm affected by genotype

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Citation: Barut, M., Tansı, L.S., Karaman, S. (2023). Unveiling the phytochemical variability of fatty acids in world marigold (*Calendula officinalis* L.) germplasm affected by genotype. International Journal of Agriculture, Environment and Food Sciences, 7 (3), 639-649

Received: August 14, 2023
Revised: September 11, 2023
Accepted: September 12, 2023
Published Online: September 28, 2023

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Available online at
https://jaefs.com/
https://dergipark.org.tr/jaefs

Abstract

Marigold is an annual herbaceous medicinal and aromatic plant, native to the Mediterranean region. Although marigold flowers have attracted considerable attention, the noteworthy characteristics of marigold seeds have often been overlooked. The industrial sector holds keen interest in marigold due to the presence of calendic acid in its seeds. Moreover, calendic acid exhibits promising anti-cancer properties, adding to the growing interest in the medicinal potential of this plant. In this study, a total of 31 marigold genotype seeds from fifteen different countries were used as experimental material. The observed seed oil content exhibited a range of values spanning from 6.00% to 20.33%, with a mean value of 11.59%. GC/MS analysis was conducted to evaluate the chemical variability associated with genotypic changes. Notably, the main fatty acids observed in the oil of these genotypes were α-calendic acid (ranging from 6.91% to 51.42%), linoleic acid (ranging from 30.50% to 48.25%), oleic acid (ranging from 8.26% to 22.50%), and palmitic acid (ranging from 3.86% to 9.28%). Particularly noteworthy is the emergence of genotypes PI 420376, PI 545694, PI 545701, PI 578109, PI 597588, PI 597591, and PI 597594, boasting calendic acid content exceeding 50%. Furthermore, the values of calendic acid exhibit significant variation across countries. The range extends from the United Kingdom, displaying one of the lower values, to Ontario, Canada, which represents countries with notably higher values. Consequently, there exists a necessity to enhance the proportion of calendic acid within marigold through strategic plant breeding techniques. This can be achieved through the selection and development of marigold cultivars with higher calendic acid contents.

Keywords: Calendic acid, Genotype, Fatty acid profiles, Plant genetic diversity, Polyunsaturated fatty acids

INTRODUCTION

Marigold (*Calendula officinalis* L.), a member of the Compositae family, is an oilseed plant indigenous to the Mediterranean region (Krol et al., 2016). It is a versatile plant, valued for its beauty, therapeutic properties, and culinary uses. While its flowers have attracted considerable attention, the often overlooked, marigold seeds possess a number of impressive properties. The oil is obtained from the seeds of the plant is valued for its potential health benefits. It contains polyunsaturated fatty acids like linoleic acid and calendic acid and it finds extensive usage in skincare items due to its hydrating and revitalizing characteristics, making it highly sought after (Ahmad and Ahsan, 2020). It has the potential to support skin health, facilitate the recovery process for wounds and minor burns, minimize the visibility of scars, and enhance the overall complexion (Orchard and van Vuuren, 2019).

Within this highly adaptable plant lies an exceptional fatty acid recognized as calendic acid, which has garnered considerable attention (Olennikov et al., 2022). Calendic acid, also referred to as trans,trans,cis-8,10,12-octadecatrienoic acid, is a unique polyunsaturated fatty acid (Avato and Tava, 2022). It is characterized by its distinctive molecular structure, containing three double bonds along its carbon chain. This structural feature sets calendic acid apart from other fatty acids and contributes to its notable properties and potential health benefits. Calendic acid and linoleic acid are both fatty acids that belong to the omega-6 family. They share a structural similarity but differ in terms of the number and position of double bonds along their carbon chain (Crombie and Holloway, 1985). Calendic acid and linoleic acid both have 18 carbon atoms in their chain. Linoleic acid is a polyunsaturated fatty acid with two double bonds, located at positions 9 and 12 along the carbon chain. Linoleic acid serves as the precursor for the synthesis of various other omega-6 fatty acids, including calendic acid (Cahoon et al., 2001). Calendic acid stands out among various fatty acids essential for important bodily functions due to its notable cytotoxic effects (Verma et al., 2018). This cytotoxic action primarily operates through lipid peroxidation and the reduction of lcf1 gene expression, which plays a role in encoding long-chain fatty acyl-CoA synthetase (Suzuki et al., 2001; Dulf et al., 2013; Garaiova et al., 2023). Moreover, it might inhibit tumour growth, induce apoptosis (programmed cell death) in cancer cells, and modulate inflammatory responses (Garaiova et al., 2023). Both α-calendic acid and β-calendic acid have exhibited anti-cancer properties in laboratory-based in vitro studies (Yuan et al., 2014; Dubey et al., 2019).

Germplasm collections serve as vital repositories of plant genetic diversity (Çelik et al., 2023; Nadeem et al., 2020; Nadeem et al., 2021; Yılmaz et al., 2021). Various institutions, botanical gardens, and research organizations have initiated efforts to collect, conserve, and document marigold germplasm from different regions. These collections ensure the preservation of valuable genetic resources, allowing breeders and researchers to access diverse genetic material for future breeding programs and scientific studies (Barut et al., 2020). To understand the genetic diversity present within marigold germplasm, breeders and researchers conduct comprehensive phenotypic and genetic characterization studies. Through these studies, breeders attain valuable knowledge regarding the diversity of traits and identify promising genotypes displaying sought-after attributes (Kurt et al., 2020; Güneş and Tonçer, 2023).

The industrial sector has shown a keen interest in marigold due to the discovery that its seeds contain approximately 60% calendic acid (Cromack and Smith, 1998). Marigold seed holds significant importance due to its rich composition of calendic acid and the potential for increasing its content through plant breeding. Moreover, it is important to investigate genetic diversity, apply breeding techniques, and understand the basic mechanisms that regulate fatty acid biosynthesis (Dulf et al., 2013). This knowledge can be applied to other plants and contribute to broader research and development efforts in plant breeding, lipid metabolism, and functional crop improvement. The objective of this work is to elucidate the role of genetic factors, specifically the genotype of marigold plants, in the modulation of differences observed in the fatty acid profiles.

MATERIALS AND METHODS

Seed samples

The field study was conducted at the experimental area of the Department of Field Crops at Çukurova University, located in Adana (37°00'55.20" N, 35°21'25.80" E), Türkiye, during the 2021 cultivation period. In this study, a total of 31 marigold (*Calendula officinalis* L.) genotype seeds were used as an experiment material (Table 1). These genotypes were obtained from the United States Department of Agriculture (USDA). The area generally experiences a Mediterranean climate, characterized by warm and arid summers, along with temperate and rainy winters. The soil composition at the location consisted of clay-loam texture, containing a minimal amount of organic material (1.11%). The soil was tilled using a field cultivator. In situations where rainfall was lacking, irrigation was carried out on a weekly basis using sprinklers after the sowing. Weed management was executed using a hoe. Throughout the experiment, no pesticides were applied. For plant fertilization, N and P_2O_5 were administered to the plots at a dosage of 25 kg/ha in the form of diammonium phosphate (DAP) (18–46–0). However, potassium was not applied during the research due to the soil's ample potassium content.

Oil extraction and preparation of fatty acid methyl esters (FAME)

Marigold seeds were isolated and milled. A 5 g sample was mixed with 117 ml of n-hexane, and subsequently, seed oil extraction was performed using an ultrasonic bath set at 55 °C for a duration of 45 minutes. Following this, the n-hexane was removed from the extract through evaporation at 70 °C using a rotary evaporator. The remaining oil was then quantified at the Department of Field Crops, Faculty of Agriculture, Çukurova University. To analyze the composition of oil fatty acids, the oil underwent methylation to produce fatty acid methyl esters (Stefanoudaki et al., 1999). Prior to analysis, the fatty acids were transformed into methyl esters by agitating a mixture of 0.5 ml of oil and 5 mL of hexane for 5 minutes. Subsequently, 0.5 mL of 2 N methanolic potassium hydroxide was added to the solution and shaken for an additional 5 minutes, followed by centrifugation for 5 minutes.

Table 1. List of investigated marigold germplasm

No	Genotype	Name	Origin	Improvement status
1	Ames 24244	NU 40517	England, United Kingdom	Uncertain
2	PI 279690	Orange Shaggy	England, United Kingdom	Cultivated
3	PI 293762	A 23846	Former, Soviet Union	Cultivated
4	PI 420253	34	Portugal	Cultivated
5	PI 420375	35	Spain	Cultivated
6	PI 420376	44	Spain	Cultivated
7	PI 506435	-	Ukraine	Cultivated
8	PI 535879	'Promyk'	Poland	Cultivar
9	PI 545694	86-3A	India	Cultivated
10	PI 545699	NU 40598	England, United Kingdom	Cultivated
11	PI 545701	NU 52322	Illinois, United States	Cultivated
12	PI 560148	31	Finland	Cultivated
13	PI 578105	Ames 19025	Kazakhstan	Cultivated
14	PI 578106	Ames 19026	Alma-Ata, Kazakhstan	Cultivated
15	PI 578107	880608	Germany	Cultivated
16	PI 578109	883077	Algeria	Uncertain
17	PI 597588	NU 40010	Maryland, United States	Cultivated
18	PI 597589	'Ball's Orange'	England, United Kingdom	Cultivar
19	PI 597591	'Radio'	England, United Kingdom	Cultivar
20	PI 597592	'Pacific Beauty Lemon'	Maryland, United States	Cultivated
21	PI 597593	NU 45275	Former Serbia and Montenegro	Cultivated
22	PI 597594	NU 48884	Ontario, Canada	Cultivated
23	PI 600911	'Orange Gitana'	Netherlands	Cultivar
24	PI 603111	'Orange Baby'	New York, United States	Cultivar
25	PI 607418	CAL 44/89	Algeria	Uncertain
26	PI 613018	'Orange Sunshine'	England, United Kingdom	Cultivar
27	PI 613019	'Pacific Beauty Cream'	England, United Kingdom	Cultivar
28	PI 613020	W-F Formula Blend	California, United States	Cultivated
29	PI 618688	NU 40508	England, United Kingdom	Cultivar
30	PI 662007	Orange King Improved	England, United Kingdom	Cultivar
31	PI 675148	Beauty Mixed	Illinois, United States	Cultivar

Gas chromatography ± mass spectrometry (GC/MS) analysis

GC-MS analyses were carried out in the Department of Biology at Kahramanmaras Sutcu Imam University. After the oil was extracted, 1 µl of the esterified sample was injected to the GC-MS device. Qualification of the oil was assessed using an Agilent 5975C Mass Spectrometer coupled with an Agilent GC-6890II series. The GC was equipped with an HP-88 capillary column (100 m x 250 µm m x 0.20 µm film thickness) and He was used as carrier gas with a flow rate of 0.8 mL/min. The GC oven temperature was programmed as follows: 170 °C (1 min), 230 °C at 15 °C/min and then kept at 230 °C at 20 min. The injector temperature was 250 °C. The mass spectrometer was operating in EI mode at 70 eV. The split ratio was 20:1, and the mass range analyzed 35-400m/z with a scan speed of 1000 amu/s. To identify the compounds, the Wiley7n.1, Famdbwax.L, and Famedb23.L libraries were utilized.

Statistical analysis

Statistical software JMP® (version 14.0, SAS Institute Inc., Cary, NC, 1989-2019) was used to conduct principal components on correlations and constellation plot analysis. In order to construct the heat map, Flourish studio was used. The Metan package within the R Studio software was utilized to calculate correlations among the fatty acids employing the Pearson coefficient.

RESULTS AND DISCUSSION

The biovariability of fatty acids

The seed oil content and fatty acid composition of the seeds of 31 different marigold genotypes were analyzed and the results are presented in Table 2. The seed oil content ranged from 6.00% to 20.33% with a mean of 11.59%. Variations in marigold seed oil content have also been observed in the works of other researchers; 13.60% to 21.70% (Dulf et al., 2013), 14.88% to 19.76% (Krol et

al., 2016), 15.81% to 20.10% (Król and Paszko, 2017), 13.30% to 15.40% (Zarrinabadi et al., 2019). Król and Paszko (2017) reported that marigold plants cultivated in Mediterranean climates possess notably lower amounts of oil compared to those cultivated in temperate climates. However, despite the prevailing Mediterranean climate in our region, the ratios of seed oil align closely with the values reported in the literature. The chemical composition of marigold oil varied according to genotypes. The representative GC-MS chromatogram of the fatty acids is provided in Figure 1. The heatmap for the fatty acids of marigold samples is presented in Figure 2. Ten compounds were found, representing 90.47% to 100% of the total seed oils. The main fatty acids in the seed oil of these genotypes were α-calendic acid, linoleic acid, oleic acid, and palmitic acid, respectively.

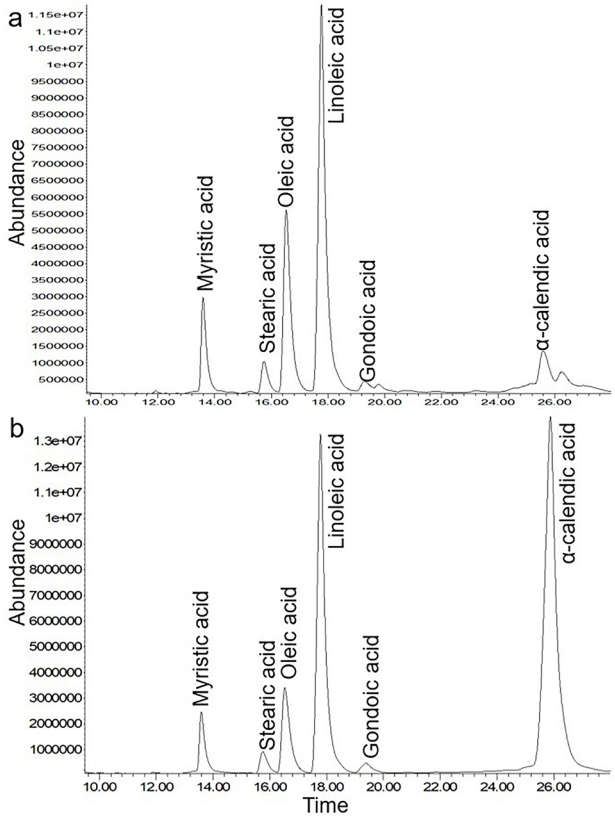

Figure 1. GC/MS chromatogram of the different genotypes a: Ames 24244, b: PI 597594.

The α-calendic acid was the highest polyunsaturated fatty acid (PUFA) in the genotypes. The α-calendic acid ranged from 6.91% to 51.42% with a mean of 40.12%. Our results showed that the highest α-calendic acid was found in PI 597594, while the lowest percentage was found in Ames 24244. When contrasting the α-calendic acid levels in marigold with previous studies, diverse results were observed; 59.00% (Feder et al., 2009), 51.40% to 57.60% (Dulf et al., 2013), 38.79% to 53.43% (Krol et al., 2016), 40.10% to 51.44% (Król and Paszko, 2017), 42.92% to 50.98% (Rahimi et al., 2020), 45.95% to 46.27% (Salama and Sabry, 2023). There is a broad range of calendic acid values based on the countries. The range extends from UK (6.91%), which represents one of the lowest values, to Ontario, Canada (51.42%), which is among the countries with the highest values. There seems to be diversity in the calendic acid content even within genotypes from the same geographical region. For example, Ames 24244 and PI 545699, both originating from England, United Kingdom, have distinct calendic acid values of 6.91% and 47.97% respectively. This suggests that even within close geographical regions, plants might possess varying calendic acid levels due to growing conditions, soil structure, or other factors. Additionally, there is variability in calendic acid values across countries from different continents. For instance, plants from geographically distant areas like India (51.27%) and Canada (51.42%) showcase similar elevated calendic acid values. This highlights the possibility of achieving comparable outcomes in diverse geographical locations, attributed to the intricate interplay of plant genetic structures, cultivation methodologies, and environmental factors. Through a process known as elongation and desaturation, linoleic acid can be converted into calendic acid by introducing an additional double bond at position 8 (Cao et al., 2013). Different genotypes may have distinct genetic traits that influence the production and accumulation of calendic acid in their seeds. Moreover, environmental conditions, such as temperature, sunlight, soil composition, and moisture levels, can influence the biosynthesis and accumulation of calendic acid in seeds. Furthermore, the content of calendic acid can vary during different stages of plant development. It is possible that certain marigold genotypes have higher levels of calendic acid at specific growth stages, which may contribute to the observed differences in content among genotypes. In addition, variations in the activity or expression of enzymes involved in biosynthetic pathways can affect the production and accumulation of calendic acid. Differences in the regulation of these pathways among marigold genotypes can contribute to variations in calendic acid content. Understanding these factors and their interplay is crucial for researchers and breeders aiming to develop marigold genotypes with desired calendic acid profiles.

The linoleic acid was found in high quantities for all genotypes. The results indicate that the contents of linoleic acid were detected between 30.50% to 48.25% with a mean of 35.98%. Various outcomes concerning the linoleic acid content in marigold have also been documented by researchers; 28% (Feder et al., 2009), 28.50% to 31.90% (Dulf et al., 2013), 30.70% to 36.63% (Krol et al., 2016), 31.99% to 36.52% (Król and Paszko, 2017), 21.20% to 28.01% (Rahimi et al., 2020), 26.56% to 26.81% (Salama and Sabry, 2023). According to Özgül-Yücel's (2005) findings, the fixed oil derived from marigold seeds in Turkey is distinguished by its elevated levels of

linoleic acid and relatively low amounts of calendic acid. The predominant factor influencing the ratio of calendic acid is believed to be primarily associated with the timing of harvest (Barut et al., 2022).

The oleic acid was the highest monounsaturated fatty acid (MUFA) in the genotypes. The oleic acid ranged from 8.26% to 22.50% with a mean of 12.91%. Diverse results based on the oleic acid content of marigold were also reported by the researchers; %4 (Feder et al., 2009), 3.64% to 5.78% (Krol et al., 2016), 2.78% to 6.08% (Rahimi et al., 2020), 5.87% to 6.72% (Salama and Sabry, 2023). On the other hand, palmitic acid was the highest saturated fatty acid (SFA) in the genotypes. The palmitic acid ranged from 3.86% to 9.28% with a mean of 5.20%. Upon comparing the palmitic acid levels in marigold with previous investigations, varying outcomes were

Table 2. Fatty acid profile of marigold genotypes

Fatty acids		**C 14:0**	**C 16:0**	**C 16:1**	**C 17:0**	**C 18:0**	**C 18:1**	**C 18:2**	**C 20:1**	**C 22:0**	**C 18:3n6**	**Total**	**MUFAs**	**PUFAs**	**SFAs**
RT (min)		**11.879**	**13.679**	**14.479**	**14.679**	**15.679**	**16.479**	**17.879**	**19.279**	**21.879**	**25.679**				
Genotype	**Seed oil cont. (%)**	**Relative peak area (%)**													
Ames 24244	6.00	0.16	8.55	nd	nd	3.53	21.82	47.63	1.67	0.20	6.91	90.47	23.49	54.54	12.44
PI 279690	8.00	0.16	5.15	nd	nd	2.23	13.38	35.64	1.21	0.03	40.03	97.83	14.59	75.67	7.57
PI 293762	11.67	0.06	3.97	0.11	nd	2.31	12.10	33.34	1.51	0.01	45.55	98.95	13.71	78.89	6.35
PI 420253	15.00	0.10	4.73	nd	nd	2.09	11.00	33.45	1.19	0.04	47.32	99.91	12.18	80.77	6.96
PI 420375	6.00	0.13	8.96	nd	nd	4.07	17.87	44.66	1.77	0.21	17.26	94.93	19.64	61.92	13.38
PI 420376	16.00	0.11	4.18	0.06	nd	1.96	8.68	31.71	1.32	0.01	51.31	99.33	10.05	83.01	6.27
PI 506435	9.33	0.11	5.55	0.27	nd	2.44	15.57	34.90	1.23	0.11	39.62	99.81	17.08	74.52	8.21
PI 535879	16.00	0.08	4.10	nd	nd	2.24	14.34	33.59	1.30	0.06	43.99	99.71	15.65	77.58	6.49
PI 545694	14.00	0.07	4.26	0.17	nd	2.07	8.89	31.21	1.42	0.49	51.27	99.85	10.47	82.48	6.90
PI 545699	12.67	0.14	4.26	nd	nd	2.12	11.29	32.65	1.31	0.04	47.97	99.78	12.60	80.62	6.56
PI 545701	11.33	0.11	4.34	0.18	nd	1.94	11.47	30.50	1.12	nd	50.04	99.71	12.78	80.54	6.39
PI 560148	12.00	-	3.99	nd	nd	1.90	9.79	34.65	3.46	nd	45.98	99.77	13.25	80.63	5.89
PI 578105	10.00	0.09	5.44	nd	nd	2.27	14.56	35.48	1.24	0.14	31.92	91.14	15.80	67.40	7.94
PI 578106	6.00	0.10	5.76	nd	nd	2.50	10.97	34.19	1.59	0.22	43.09	98.42	12.56	77.28	8.58
PI 578107	8.67	0.15	8.48	0.14	0.22	3.79	22.50	48.25	1.86	0.21	11.64	97.23	24.50	59.88	12.85
PI 578109	9.33	0.07	4.42	nd	nd	2.20	9.40	31.79	1.30	0.11	50.66	99.95	10.70	82.45	6.80
PI 597588	20.33	0.08	4.13	0.01	nd	2.03	8.77	32.50	1.27	0.04	51.12	99.95	10.05	83.62	6.28
PI 597589	10.00	0.09	5.24	nd	nd	1.65	10.28	33.46	1.49	0.05	47.46	99.72	11.77	80.92	7.03
PI 597591	12.33	0.08	3.86	nd	nd	1.91	8.26	33.24	1.41	0.02	50.20	98.97	9.67	83.44	5.85
PI 597592	11.00	0.05	4.21	nd	nd	2.08	15.92	33.39	1.65	0.09	42.09	99.47	17.56	75.48	6.42
PI 597593	8.67	0.11	6.70	nd	nd	2.99	16.84	44.56	1.58	0.05	24.84	97.68	18.42	69.40	9.86
PI 597594	17.33	0.07	4.19	nd	nd	1.99	8.43	32.06	1.34	0.01	51.42	99.50	9.77	83.48	6.26
PI 600911	10.33	0.09	5.14	nd	nd	2.71	18.96	40.97	1.31	0.03	29.38	98.58	20.27	70.35	7.97
PI 603111	13.67	0.07	4.09	nd	nd	1.88	11.32	34.90	1.48	nd	46.01	99.76	12.79	80.92	6.05
PI 607418	12.00	0.07	4.03	nd	nd	1.89	11.88	32.94	1.49	nd	45.97	98.27	13.37	78.92	5.99
PI 613018	8.67	0.12	6.42	nd	nd	3.20	14.70	43.81	1.34	0.14	23.70	93.42	16.04	67.51	9.87
PI 613019	18.00	0.06	4.21	nd	nd	2.00	14.30	34.51	1.34	0.02	43.56	100.00	15.64	78.07	6.29
PI 613020	13.00	0.07	4.28	nd	nd	1.63	10.24	32.54	1.43	0.02	48.47	98.69	11.67	81.01	6.01
PI 618688	12.00	0.07	4.75	nd	nd	2.14	12.22	33.91	1.45	0.12	44.79	99.45	13.66	78.70	7.09
PI 662007	7.33	0.18	9.28	nd	0.20	3.31	14.23	46.23	2.07	0.21	21.21	96.92	16.30	67.44	13.18
PI 675148	12.67	0.07	4.37	nd	nd	2.00	10.38	32.61	1.21	0.08	48.91	99.64	11.59	81.52	6.52
Min	6.00	0.05	3.86	0.01	0.20	1.63	8.26	30.50	1.12	0.01	6.91	90.47	9.67	54.54	5.85
Mean	11.59	0.10	5.20	0.13	0.21	2.36	12.91	35.98	1.49	0.10	40.12	98.28	14.44	76.09	7.75
Max	20.33	0.18	9.28	0.27	0.22	4.07	22.50	48.25	3.46	0.49	51.42	100.00	24.50	83.62	13.38

(C14:0 (Myristic acid), C 16:0 (Palmitic acid), C 16:1 (Palmitoleic acid), C 17:0 (Heptadecanoic acid), C 18:0 (Stearic acid), C 18:1 (Oleic acid), C 18:2 (Linoleic acid), C 20:1 (Gondoic acid), C 22:0 (Behenic acid), C 18:3n6 (α-calendic acid)), nd: not detected.

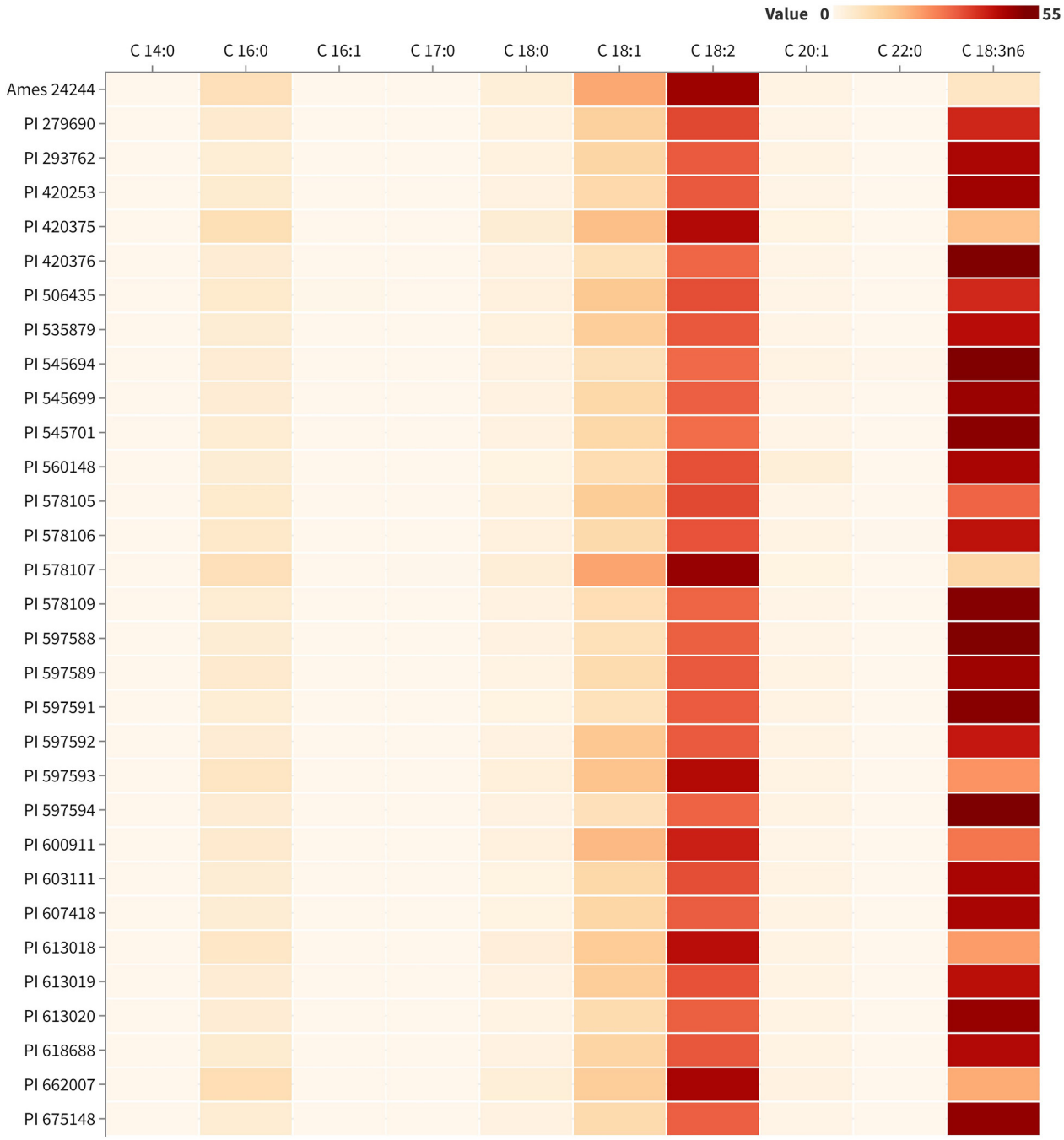

Figure 2. Heatmap of fatty acid profiles for different marigold genotypes (Value=%).

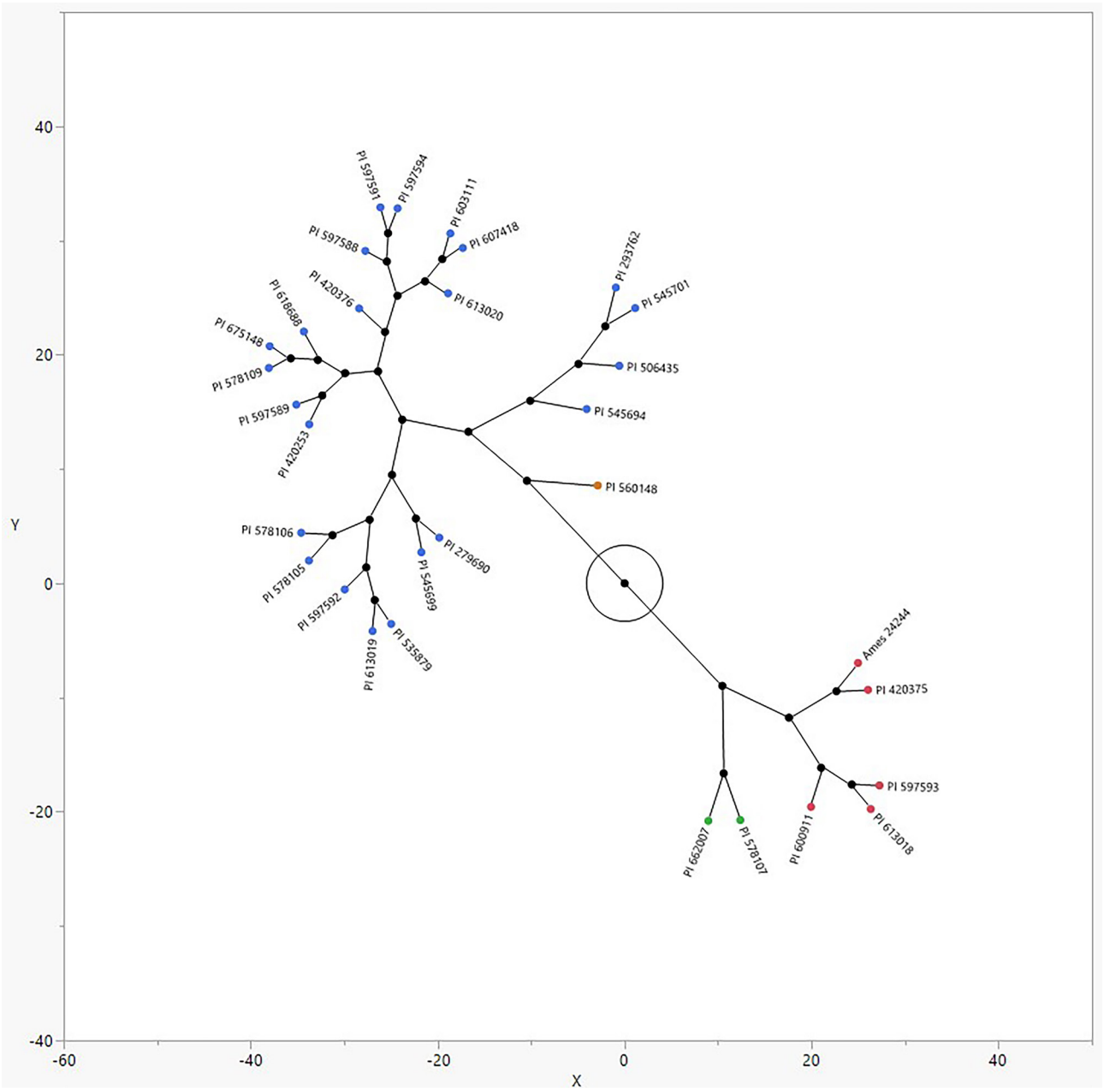

Figure 3. Constellation plot analysis of marigold genotypes.

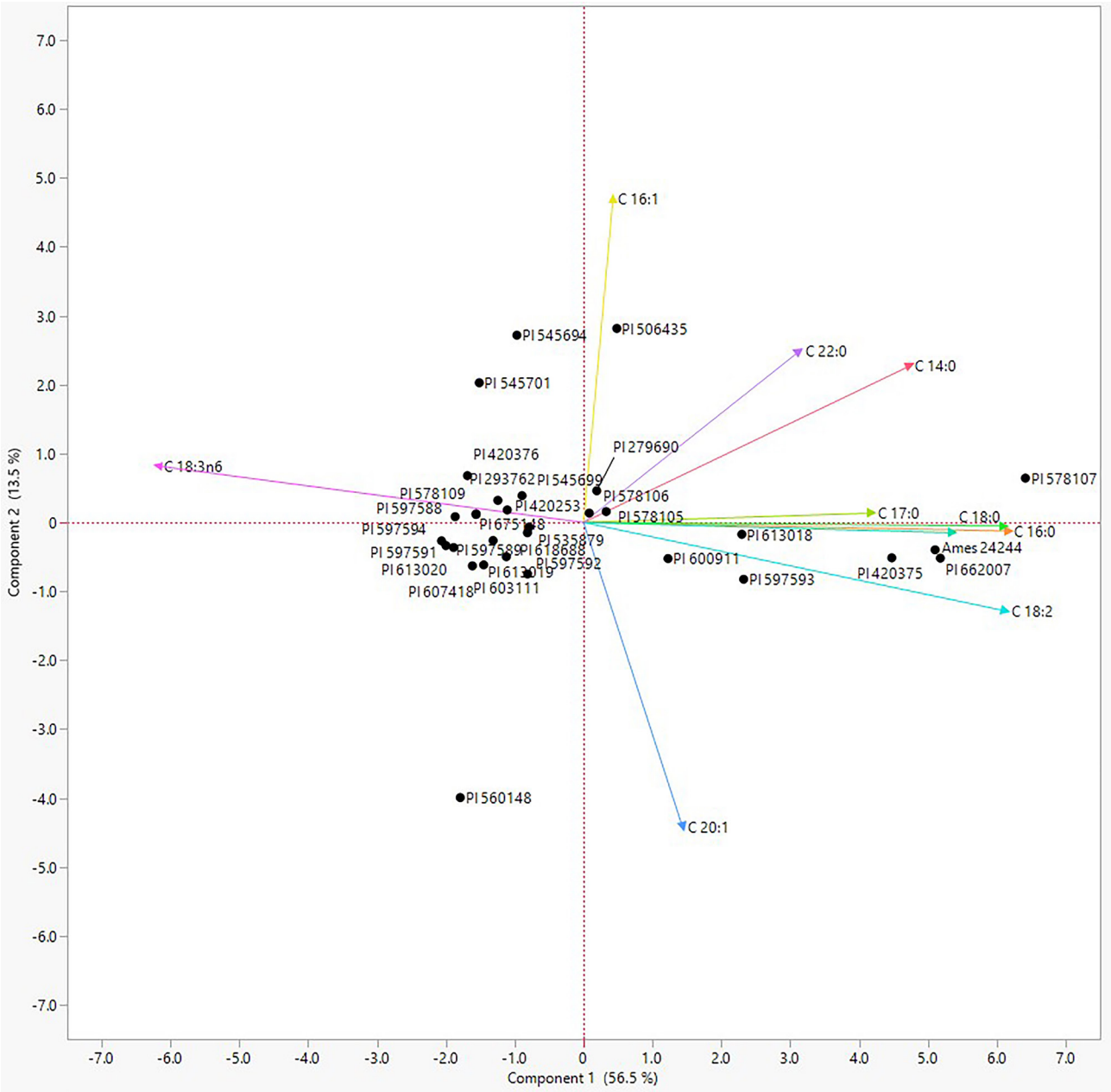

Figure 4. Principal component analysis performed on correlations among genotypes based on fatty acids. (C14:0 (Myristic acid), C 16:0 (Palmitic acid), C 16:1 (Palmitoleic acid), C 17:0 (Heptadecanoic acid), C 18:0 (Stearic acid), C 18:1 (Oleic acid), C 18:2 (Linoleic acid), C 20:1 (Gondoic acid), C 22:0 (Behenic acid), C 18:3n6 (α-calendic acid)).

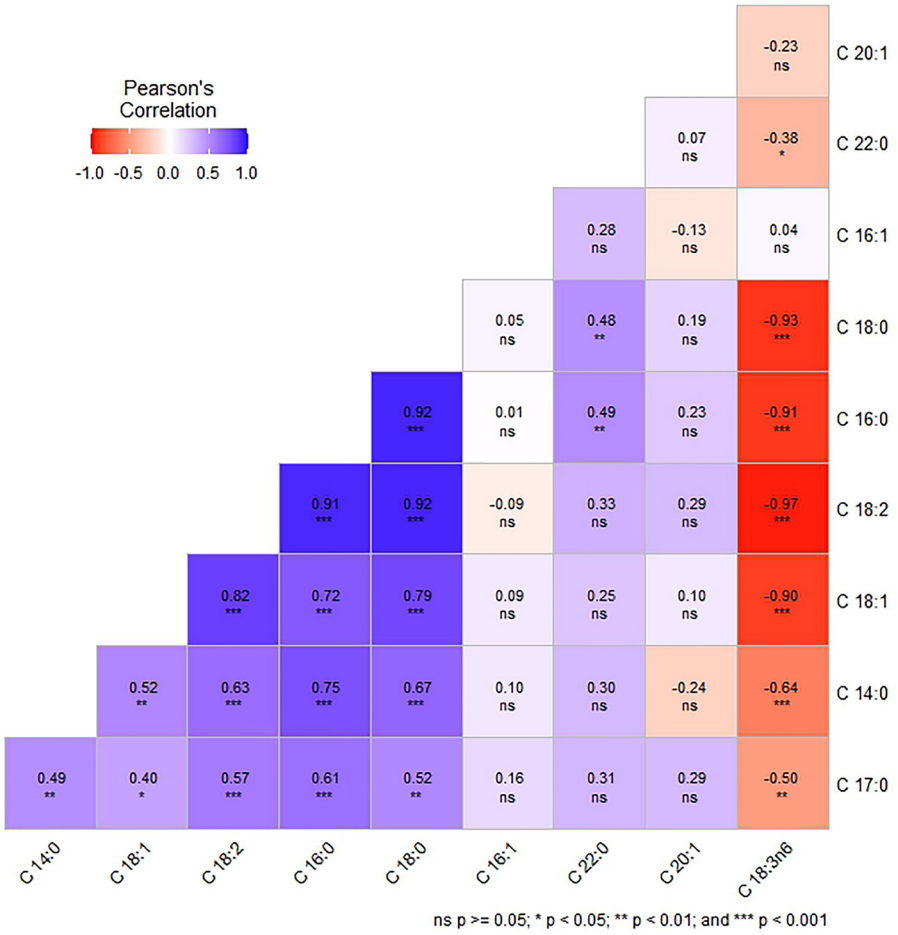

Figure 5. Correlation analysis among the fatty acids (C14:0 (Myristic acid), C 16:0 (Palmitic acid), C 16:1 (Palmitoleic acid), C 17:0 (Heptadecanoic acid), C 18:0 (Stearic acid), C 18:1 (Oleic acid), C 18:2 (Linoleic acid), C 20:1 (Gondoic acid), C 22:0 (Behenic acid), C 18:3n6 (α-calendic acid)).

observed; 3.43% to 5.39% (Krol et al., 2016), 3.77% to 5.65% (Król and Paszko, 2017), 13.12% to 18.62% (Rahimi et al., 2020), 5.23% to 5.32% (Salama and Sabry, 2023). In addition, it was determined that the oil composition of marigold contains a small amount of myristic acid, palmitoleic acid, heptadecanoic acid, stearic acid, gondoic acid, and behenic acid. SFAs level of studied marigold genotypes ranged from 5.85% to 13.38% with a mean of 7.75%. MUFAs level of studied marigold genotypes ranged from 9.67% to 24.50% with a mean of 14.44%. PUFAs level of studied marigold genotypes ranged from 54.54% to 83.62% with a mean of 76.09%. Dulf et al. (2013) reported SFAs level 6.39 to 7.34%, MUFAs level 5.09 to 6.99%, and PUFAs level 60.4 to 66.4%.

Constellation plot, principal component biplot, and correlation analysis of the genotypes based on the fatty acids

To explore the genetic diversity of marigold genotypes, a constellation plot analysis was performed (Figure 3). The utilization of a constellation plot provided optimal outcomes in discriminating the genetic diversity among marigold genotypes. This plot was divided into two main groups: A and B. These main groups were also divided into two subgroups: A1, A2, B1, and B2. Most of the marigold genotypes (23 genotypes-blue color) were located in Group A1. Subgroup A2 had one genotype (orange color). Five genotypes were located in Subgroup B1 (red color), and two genotypes were found in subgroup B2 (green color). The analysis of genotype and fatty acid composition revealed associations as depicted in Figure 4. PC1, the primary principal component, accounted for 56.5% of the standardized dataset's variability, while PC2, the secondary principal component, explained 13.5% of the variation. Together, these two components encompassed a total of 70% of the overall variation. Correlation analysis was performed to observe the relationship between fatty acids (Figure 5). The correlation graph indicates that calendic acid was negatively correlated with myristic acid, palmitic acid, heptadecanoic acid, stearic acid, oleic acid, linoleic acid, and behenic acid. The graph depicting correlations reveals that there is a negative correlation between calendic acid and several other fatty acids including myristic acid, palmitic acid, heptadecanoic acid, stearic

acid, oleic acid, linoleic acid, and behenic acid. This suggests that as the level of calendic acid increases, the levels of these other fatty acids tend to decrease.

CONCLUSION

Through the analysis of fatty acids in marigold seeds using GC/MS, this study revealed significant chemical variability among different genotypes from various countries. The main fatty acids identified in the seed oil included α-calendic acid, linoleic acid, oleic acid, and palmitic acid. There exists a broad spectrum of calendic acid contents among genotypes collected from 15 countries. The range spans from the UK, registering one of the lowest contents, to Ontario, Canada, which stands as one of the nations exhibiting the highest contents. Furthermore, there is variability in calendic acid contents across countries located on different continents. As an instance, plants from geographically distant regions such as India and Canada display comparably high calendic acid contents. This underscores how analogous outcomes can arise across diverse geographical locations, owing to the intricate interplay of plant genetic structures, cultivation methodologies, and environmental factors. To meet the growing demand for calendic acid, it is crucial to enhance its production through plant breeding methods. This can be achieved by selectively breeding marigold cultivars with higher levels of calendic acid. This research lays the foundation for further exploration and advancement in the field of marigold cultivation and calendic acid production. The findings will contribute to a deeper understanding of the phytochemical diversity within marigold and will have implications for future breeding programs aimed at developing marigold cultivars with specific fatty acid profiles to meet various market demands and enhance its health-promoting properties.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

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

Author contribution

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

Ethics committee approval

Ethics committee approval is not required. This article does not contain any studies with human participants or animals performed by any of the authors.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(3): 650-659
DOI: https://doi.org/10.31015/jaefs.2023.3.19

Impact of gamma radiation on the agronomic properties of naked barley genotypes

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Citation: Ergun, N., Akdogan, G., Unver Ikincikarakaya, S. (2023). Impact of gamma radiation on the agronomic properties of naked barley genotypes. International Journal of Agriculture, Environment and Food Sciences, 7 (3), 650-659

Received: August 01, 2023
Revised: August 25, 2023
Accepted: August 27, 2023
Published Online: September 27, 2023

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Available online at
https://jaefs.com/
https://dergipark.org.tr/jaefs

Abstract
The usage of naked barley in the food industry is increasing day by day due to its health benefits. As a result, research on breeding naked barley have gained popularity. In these breeding studies, a wide variation in desired traits is needed to achieve higher success in selection. One of the best methods for obtaining genotypic variation, which is crucial for breeding studies on naked barley, is mutation. To obtain genotypic variation in certain agronomic parameters in naked barley genotypes, the impact of different gamma radiation doses on M_1 and M_2 plants of two naked barley genotypes was evaluated in this research. The seeds were treated with gamma irradiation using Cobalt 60 gamma source at six different doses, along with non-irradiated control samples. While the values at low doses were found to be comparable to the control in the majority of the traits, 250-300 Gy caused significant decreases in the majority of the traits in the M_1 generation of both genotypes. Plant height, number of spikelets per spike, and number of grains per spike at the M_2 generation were all negatively impacted by 250–300 Gy, although spike length, grain weight per spike, and thousand grain weight were positively impacted by the same doses. The mutant population generated by gamma irradiation of seeds of different naked barley genotypes was found to have suitable variation for the selection of desired traits. In addition, this material can be used to select individuals with outstanding agronomic characteristics.
Keywords: Naked barley, Mutation, Gamma irradiation, M_1 and M_2 plants, Variation

INTRODUCTION

Barley (*Hordeum vulgare* L.) is an important cultivated crop that ranks second after wheat in terms of cultivation area and production amount among cool-season cereals. Currently, 146 million tonnes of barley grain are produced on 48 million hectares throughout the world (FAOSTAT, 2022). Globally, 60% of barley is used in animal feed, 40% in the malt industry, 5% as seed, and 3% for human consumption (Ullrich, 2011). Barley is an important source of feed and malt as well as an important food crop in some countries of the world. It is also the most abundant source of functional ingredient-rich cereals and the most available species for functional food crops. Barley is extremely rich in nutrients and functional components (Zeng et al., 2020). Depending on the separation of grains and husks after harvest, barley is classified into two main categories as hulled and naked. The recessive nud gene determines the naked caryopsis trait in barley (Duan et al., 2015).

Naked barley is grown mainly for food as it contains greater nutrients, including beta-glucan and total dietary fiber, and is easier to process than hulled types

(Meints et al., 2021). Despite its advantages as a food, the number of naked barley varieties released worldwide is still limited compared to its hulled counterpart. The neglect of naked barley in breeding programs and the limited genetic resources required for breeding studies are the two most prominent causes of this limitation (Dickin et al., 2012; Meints et al., 2021). A wide genetic diversity of genotypes provides for an efficient and successful breeding program for the development of new varieties. Crossing and mutation are the most common methods for creating genetic variation.

Mutations, which are sudden changes in the genome of plants, are commonly used in barley breeding and genetic research. The primary advantage of using mutations in breeding studies is the potential to improve one or two traits without affecting the rest of a well-adapted cultivar's genotypic structure (Dyulgerova & Dyulgerov, 2020). Mutant plants can be released as a new variety or used as a parent in crossing studies (Ahloowalia et al., 2004). In addition, mutation methods allow rapid generation of desired traits when genetic diversity or traits are unavailable in a germplasm collection (Maluszynski et al., 2009). Mutation breeding is one of the most effective ways for increasing genetic diversity for commercially important agricultural traits (Chaudhary et al., 2019).

Physical and chemical mutagens are utilized in mutant breeding studies to change the genetic structure of plants and increase variation. Gamma (γ) rays are the most popular physical mutagens used as mutation sources by the breeders because they are easy to use and can penetrate deep into a biological substance, are less harmful to the environment and humans, and are easily accessible (Suprasanna et al., 2015; Ulukapi & Nasircilar, 2015). In addition, gamma rays cause point mutations and small deletions that have less destructive effects on the organism than other physical mutagens (Suprasanna et al., 2015). As a gamma ray source, Cobalt 60 (^{60}Co), the radioactive isotope of Cobalt, is widely used in mutation breeding due to its short half-life and high energy (Shu et al., 2012).

This study aimed to evaluate the effect of six different gamma radiation doses on M_1 and M_2 plants of naked barley cv. Yalin and naked barley line YAA7050-14. In addition, genotypic variation in agronomic parameters affecting the yield of naked barley was obtained using gamma radiation. The differences between the impacts of the gamma ray doses compared to the non-irradiated control were determined by analysis of variance. The measurement of the variation created was based on the standard deviations and coefficients of variation of the traits.

MATERIALS AND METHODS

Materials

Elite seeds of the naked barley cv. Yalin and the naked barley line YAA7050-14 developed by the Central Research Institute for Field Crops (CRIFC) were used as plant material. As the physical mutagen source, gamma-rays obtained from the 381 Gray/hour Cobalt 60 (^{60}Co) source in the Ankara Nuclear Research and Training Centre (ANAEM) were used.

Methods

For each dose and control group, 500 seeds of naked barley cv. Yalin and line YAA7050-14, both healthy and having nearly 12% moisture, were prepared separately. The prepared seeds were irradiated with gamma rays at doses of 0 (Control), 100, 150, 200, 250, and 300 Gray (Gy). Seeds in the control group and irradiated at different doses were sown in a randomized complete block design with three replications separately in the experimental field of the CRIFC to grow M_1 plants.

The field trials were conducted in the Yenimahalle location in the first year (2017-2018 growing season) of the study for observations to be taken from M_1 plants, and seeding was done manually one day after irradiation. The plots consisted of four rows one meter in length, spaced by 30 cm. Twenty five healty seeds were sown in each row. The second-year (2018-2019 growing season) trials with M_2 plants were also done manually at the Ikizce location. Sowing was done in 1 m long rows with 30 cm row spacing in this trial. In each replication, the number of rows seeded was equal to the number of main spikes gathered in the M_1 generation.

Measurements and observations on M_1 and M_2 plants were made according to Senay & Ciftci 2005, Spencer-Lopes et al., 2018. Measurements related to height and length are given in centimeters (cm) and measurements related to weight are given in grams (g). During the field study, the data obtained from M_1 and M_2 plants were subjected to an analysis of variance according to the randomized complete blocks design, separately for the cv. Yalin and the line YAA7050-14. F test was used to determine the statistical significance level of the differences, and Duncan's multiple comparison method was used to group the means (Montgomery, 2013).

RESULTS AND DISCUSSION

Based on the results of variance analysis (ANOVA) performed on collected data from M_1 plants at different gamma-ray doses, significant differences in all attributes except spike length were detected between doses at 0.01 and 0.05 in both genotypes (Table 1).

While the control plant height in cv. Yalin was 114.3 cm, after 250–300 Gy gamma irradiation, plant height (PH) reduced by 15% to 97.0 cm (Table 2). The PH values in M_1 plants of line YAA7050-14 was decreased significantly at high gamma doses compared to the PH values in the control (Table 3). While the average PH was 108 cm in the control, the average PH was 96.2 and 94.0 cm at 250 and 300 Gy doses, respectively. When the results of similar

Table 1. ANOVA results for the traits of M_1 plants of cv. Yalin and line YAA7050-14.

	Yalin (M_1)				YAA7050-14 (M_1)			
	Replication	Doses	Error		Replication	Doses	Error	
DF	2	5	10		2	5	10	
	Mean square			CV	Mean square			CV
PH	50.18	177.85*	18.78	4.1	13.99	95.30**	11.98	3.4
NS	0.03	1.07*	0.029	3.0	0.56	3.65**	0.28	9.3
SL	0.19	0.38^{ns}	0.34	5.2	0.21	0.21^{ns}	0.72	2.7
NSS	2.17	6.47**	0.86	3.1	2.05	5.26**	0.43	2.2
NGS	1.00	31.16**	0.81	3.3	1.76	11.93**	0.38	2.4
FT	3.85	121.20**	4.12	2.8	0.77	27.03**	2.05	2.1
GW	0.01	0.11**	0.003	5.0	0.01	0.08**	0.004	7.4
SR	14.89	314.63**	14.47	5.4	1.82	188.00**	12.85	4.9

* Statistically significant at 0.05 level; ** Statistically significant at 0.01 level; ns Not statistically significant; PH Plant height; NS Number of fertile spikes; SL Spike length; NSS Number of spikelets per spike; NGS Number of grain per spike; FT Fertility of spike; GW Grain weight per spike; SR Survival rate; DF Degrees of freedom; CV Coefficient of variation (%)

studies were evaluated, it was reported that there was a decrease in plant height in barley (Ashmawy et al., 2016) at increasing mutagen doses. Possible reasons for this decrease in plant height include damage at DNA and chromosome levels (Stoilov et al., 2013), decreased photosynthetic activity and increased oxidative stress (Choi et al., 2021), negative effects on the synthesis and balance of plant hormones such as gibberellins, brassinosteroids, and strigolactones (Barboza et al., 2013; Marzec & Alqudah, 2018; Sarkar et al., 2004), damage to apical meristems (Sayed Hussien Elsayed et al., 2014), gas exchange in leaves, mineral uptake and utilization by roots (Singh et al., 2013). Increasing yield under irrigated conditions by shortening plant height, increasing stem robustness, and resistance to lodging through mutation breeding were the main objectives of many studies in barley and other cereals (Ahuja et al., 2014). As a result of these studies, dwarf and semi-dwarf mutant genotypes took their place in modern agriculture (Gruszka et al., 2011). The finding of shorter genotypes compared to the control in our study shows that the desired variation in plant height has occurred in the naked barley genotypes used as material.

In both genotypes, the decrease observed in plant height was not seen in spike length (SL). When all gamma doses were considered, although there was a slight decrease in SL only at 250 and 300 Gy doses, control and all other doses were in the same statistical group (Table 2 and Table 3). In similar studies conducted with different mutagen doses, it was found that there was no or very little difference in SL depending on the dose increase (El-Degwy & Hathout, 2014), while it is also possible to see results that SL decreased at high doses (Ashmawy et al., 2016). In our study, our results are in parallel with the findings of these researchers in terms of the presence of plants with longer spike and shorter spike plants together and similar to the control. SL in barley varies with the length and number of internodes on the spike axis. The variation observed at high doses is thought to be caused by differences in the length and number of internodes on the rachis. Mutant barley genotypes with lax spikelet arrangement (*lax*-a) with long internodes on the rachis or dense spikelet arrangement (*dsp*) with short internodes, in other words "*compact spikes*", are examples of sources of variation in spike length (Terzi et al., 2017). Disruption of cell division and growth in apical meristems after mutagen treatments and negative effects on the synthesis and release of plant hormones such as auxin and enzymes active in spike formation (Terzi et al., 2017; Wang et al., 2018) could be among the possible reasons for differences in spike length.

In M_1 plants of the cv. Yalin, higher values were obtained in the number of fertile spikes (NS) per plant counted at higher doses compared to the control (Table 2). While the average NS in the control was 5.3, the NS at the 300 Gy dose was 6.4. NS in M_1 plants of line YAA7050-14 was higher at high doses than in the cv Yalin. A decrease in surviving rate occurred after the 200 Gy dose in M1 plants (Table 3). One possible explanation for this occurrence is that the surviving plants are more tillering when the number of plants per unit area decreases at high doses. Barley with a high tiller number (*hnt1*) or no tillering (*uniculm2*) has been developed by mutation breeding (Okagaki et al., 2018; Ye et al., 2019). In our study, the coexistence of plants with less or more spike numbers per plant than the control shows sufficient variation in this material for selection for this trait.

The number of spikelets (NSS) counted in the main spike was around 31 in the control plants of cv. Yalin, however, the NSS decreased to 28 at the 300 Gy dose. The number of grains (NGS) counted in the same spikes showed a similar trend. The number of grains in the spike at doses of 250 and 300 Gy, however, was found to be 20% lower than the control, indicating that the adverse effects of high doses were more severe in this trait. The adverse effect might result from the decrease in the fertility rate

(FT) of the spike. While the FT was around 95% at 0-150 Gy doses, this value decreased to 86.6% at 250 Gy dose and to 77.9% at 300 Gy dose. Due to the decrease in the NGS at high gamma-ray doses, the grain weights per spike (GW) also decreased at a similar rate (Table 2). NSS in the main spikes of YAA7050-14 and the NGS obtained from the same spikes were also negatively affected by high gamma-ray doses (Table 3). While there were approximately 32 spikelets in one spike in the control, the NSS decreased to 28 at the dose of 300 Gy. This decrease in the NGS at high gamma-ray doses was higher than the decrease in the NSS. The increase in the number of sterile spikelets in the spike at doses of 250-300 Gy caused the decrease in FT. While the FT of the control was approximately 91%, the FT decreased to 83% at high doses of gamma rays. Possible reasons for the decrease in the NSS according to increasing doses may include the negative effects on the synthesis and release of enzymes and auxin group plant hormones that are effective in the beginning of the formation of the spike draft in barley (Wang et al., 2019) and the disruption of cell division and growth in the apical meristems (*phytomere* 2) (Terzi et al., 2017) where the spike is formed after mutagen applications. In some similar studies conducted in barley, it was found that increasing mutagen doses decreased the number of grains in the spike (Ashmawy et al., 2016; Dyulgerova & Dyulgerov, 2020). The findings of our study are in parallel with the findings of these researchers. It can be clearly seen that the main reasons for the decrease in the NGS are the reduction in the total number of spikelets per spike and the lower fertility at high gamma doses in both genotypes. The most common cause of the fertility reduction after mutagenic applications is chromosomal abnormalities and the resulting non-functional gametes. In addition, defects in different stages of meiosis and reduced pollen fertility are among the main causes of reduced fertility (Lavinscky et al., 2017; Pagliarini, 2000). The results of similar studies (Choi et al., 2021; Gowthami et al., 2017; Nazarenko & Lykholat, 2020) on mutagen application in barley and other cereals reported a decrease in FT with increasing mutagen dose. The results of these researchers are similar to the results of our study and support our findings.

Average grain weight (GW) per spike in control cv. Yalin was 1.3 g, while GW in M_1 plants at 250 and 300 Gy doses was 0.9 g (Table 2). Whereas in the YAA7050-14 line, GW measured in the control was 1.0 g, it decreased to 0.7 and 0.6 g after irradiation at 250 and 300 Gy doses, respectively (Table 3) The decrease in the NGS caused a decrease in the GW. Previous studies conducted in barley (Akgün et al., 2019; Karakoca & Akgün, 2020), reported that the GW obtained after high-dose mutagen application was reduced. These researchers confirm the results of our study. The most important reasons for the decrease in GW at 250 and 300 Gy gamma ray doses in both naked barleys are the increase in the NGS in the spike at these doses. As a result, it is possible to accept the decrease in GW at 250-300 Gy doses as an indirect result of the decrease in FT and NGS in the spike at these doses.

The percentage of M_1 plants of the cv. Yalin that survived from germination to harvest (SR) decreased by roughly 30% as gamma-ray doses increased. While the SR was about 96% in the control and low gamma-ray doses, the SR was found 66% at 300 Gy (Table 2). The change in SR in the YAA7050-14 line was similar to the cv Yalin. While SR value was 97% in the control, it decreased to 72% at 300 Gy dose (Table 3). Results from previous studies (Ahumada-Flores et al., 2020; Nazarenko & Lykholat, 2020) indicate that high doses of mutagenic applications reduce SR in barley and wheat. The results of our study in terms of SR are consistent with the results of these studies. High mutagen doses increase the mutation frequency, however, they may also reduce the SR and, in some cases, it is not possible to reach the number of plants sufficient for M_2 generation (Rybinski et al., 2003). In this respect, SR (viability) is one of the important parameters used in determining the effective dose (ED_{50}) in mutagen applications(Ahumada-Flores et al., 2020). Plant death after mutagen treatments can occur at any time between germination and maturation and these plants are usually sterile (Nielen et al., 2018). Biochemical changes in photosynthetic pigments (*chlorophyll*-a, *chlorophyll*-b and *xanthophyll*) and the effect of free radicals (Marcu et al., 2013), adverse effects on the synthesis and balance of plant hormones (Bitarishvili et

Table 2. Means of the traits studied in M_1 plants of cv. Yalin at different gamma-ray irradiation doses

Doses	PH	NS	SL	NSS	NGS	FT	GW	SR
Control	114.3 a*	5.3 b	11.2 a	31.3 a	29.9 a	95.2 a	1.3 a	95.7 a
100	110.6 a	5.2 b	11.8 a	30.7 ab	29.3 a	95.5 a	1.3 a	96.0 a
150	110.9 a	5.1 b	11.2 a	30.8 a	29.3 a	95.2 a	1.3 a	95.0 a
200	106.8 ab	6.4 a	11.0 a	30.2 ab	26.9 b	89.4 b	1.0 b	92.0 a
250	97.5 bc	5.4 b	11.5 a	27.7 c	24.0 b	86.6 b	0.9 b	76.3 b
300	95.5 c	6.4 a	10.8 a	28.3 bc	22.4 c	77.9 c	0.9 b	66.0 b
Mean	105.9	5.6	11.2	29.8	26.9	90.0	1.1	86.8

* Means indicated with the same letter belong to the same statistical group; PH Plant height; NS Number of fertile spikes; SL Spike length; NSS Number of spikelets per spike; NGS Number of grain per spike; FT Fertility of spike; GW Grain weight per spike; SR Survival rate

Table 3. Means of the traits studied in M_1 plants of line YAA7050-14 at different gamma ray irradiation doses

Doses	PH	NS	SL	NSS	NGS	FT	GW	SR
Control	108.0 a*	5.0 b	10.4 a	31.5 a	28.7 a	90.9 a	1.0 a	97.0 a
100	102.5 a	4.4 b	10.5 a	29.7 b	27.2 ab	91.4 a	1.0 a	96.0 a
150	106.8 a	5.4 b	10.2 a	30.4 ab	26.7 b	87.5 ab	1.0 a	92.7 a
200	103.4 a	6.8 a	10.0 a	29.5 b	26.5 b	89.6 a	0.9 ab	91.7 a
250	96.2 b	5.2 b	9.9 a	28.8 bc	24.3 c	84.1 b	0.7 bc	89.3 a
300	94.0 b	7.3 a	9.9 a	27.7 c	23.2 c	83.5 b	0.6 c	71.7 b
Mean	101.8	5.7	10.2	29.6	26.1	87.8	0.9	89.7

* Means indicated with the same letter belong to the same statistical group; PH Plant height; NS Number of fertile spikes; SL Spike length; NSS Number of spikelets per spike; NGS Number of grain per spike; FT Fertility of spike; GW Grain weight per spike; SR Survival rate

Table 4. ANOVA results for the traits of M_2 plants of cv. Yalin and line YAA7050-14.

	Yalin (M_2)				YAA7050-14 (M_2)			
	Replication	Doses	Error		Replication	Doses	Error	
DF	2	5	10		2	5	10	
	Mean square			CV	Mean square			CV
PH	3.05	35.83^{ns}	11.88	4.0	6.10	23.20^{ns}	10.42	4.0
SL	0.004	0.20^{ns}	0.08	2.6	0.09	0.50^{ns}	0.11	3.4
NSS	0.01	0.50^{ns}	0.17	1.4	0.95	2.00^{ns}	0.92	3.2
NGS	0.78	1.98**	0.22	1.7	1.97	0.52^{ns}	1.35	4.0
GW	0.001	0.003*	0.001	2.1	0.002	0.005^{ns}	0.005	5.3
TKW	0.16	1.50^{ns}	0.67	1.7	1.23	2.27^{ns}	0.80	1.9

* Statistically significant at 0.05 level; ** Statistically significant at 0.01 level; ns Not statistically significant; PH Plant height; NS Number of fertile spikes; SL Spike length; NSS Number of spikelets per spike; NGS Number of grain per spike; FT Fertility of spike; GW Grain weight per spike; SR Survival rate; DF Degrees of freedom; CV Coefficient of variation (%)

al., 2018) are among the most important reasons that decrease the SR at high doses. In addition, chromosomal and biological damage (Khah & Verma, 2015), disruptions in the division of somatic cells and inhibition of the activity of RNA polymerase and the effects of genes responsible for cell division (Ahumada-Flores et al., 2020) are the other most important reasons for this decrease. Furthermore, environmental stress factors experienced in field trials may also increase mortality rates after mutagen application (Nielen et al., 2018).

Table 4 shows the results of the analysis of variance on the data obtained from M_2 plants in the second year of the study (Table 4). The differences between the doses in PH, SL, NSS and TGW were not statistically significant in M_2 generation. In terms of NGS and GW, the differences between the doses were statistically significant in cv. Yalin and insignificant in line YAA7050-14. Although the differences between different gamma ray doses in most of the traits examined in M_2 generation were not statistically significant, it was determined that 250 and 300 Gy gamma ray doses decreased the PH, NSS, and NGS compared to the control and these effects were realized at different levels according to genotypes. On the other hand, SL, GW, and TGW values obtained from high doses such as 250 and 300 Gy were higher than the control and other doses. In addition, the variation obtained in most of the traits examined in M_2 plants was higher in 250 and 300 Gy doses compared to the control and other doses.

Descriptive statistics of the examined traits related to M_2 plants of cv. Yalin are given in Table 5. The control plants of cv. Yalin had an average plant height (PH) of 85.1 cm. The PH of the main stem in control ranged from 72.0 cm to 94.0 cm. In M_2 plants the highest variation in PH was observed at 200 and 250 Gy doses (Table 5). The lowest and highest PH values of 200 Gy were 65.0 cm and 102.0 cm, respectively. At 250 Gy dose, PH values varied between 59.0-94.0 cm. The highest coefficients of variation (CV) in plant height were 9.0% and 8.9% at 200 and 250 Gy doses, respectively. The coefficient of variation of the control for this trait was 6.6%. Coefficients of variation for spike length (SL) also increased after gamma irradiation. The CV of the control was 6.3%, while the CV of the 300 Gy dose was 8.3%. At 300 Gy dose, the variation around the average in SL increased, and the lowest and highest spike lengths varied between 9.7 cm and 13.5 cm, respectively. Control and M_2 plants obtained by gamma irradiation at different gamma doses had very similar mean and CV values for the NSS. However, the number of grains per spike (NGS) changed after gamma irradiation compared to the control. The

Table 5. Descriptive statistics of the traits examined in M_2 plants of cv. Yalin

Doses		PH	SL	NSS	NGS	GW	TGW
	min-max	72.0-94.0	9.6-12.7	25.0-34.0	21.0-33.0	0.98-1.62	40.0-53.1
Control	X-SD	85.1±5.58	11.0±0.70	30.1±2.30	29.3±2.51 a*	1.38±0.14 ab	46.9±2.98
	CV	6.6	6.3	7.6	8.6	10.4	6.3
	min-max	80.0-104.0	8.9-13.0	25.0-35.0	18.0-35.0	0.77-1.73	35.8-52.4
100 Gy	X-SD	91.3±6.08	10.9±0.79	30.5±2.23	28.5±3.54 a	1.33±0.22 bc	46.5±3.47
	CV	6.7	7.2	7.3	12.4	16.5	7.5
	min-max	71.0-103.0	9.1-12.5	25.0-34.0	24.0-34.0	0.93-1.63	35.6-52.7
150 Gy	X-SD	89.7±7.01	10.9±0.63	30.4±2.22	29.5±2.41 a	1.39±0.13 a	47.2±3.10
	CV	7.8	5.8	7.3	8.2	9.3	6.6
	min-max	65.0-102.0	7.8-12.5	21.0-34.0	18.0-33.0	0.85-1.61	37.8-53.4
200 Gy	X-SD	83.5±7.48	11.0±0.79	29.8±2.29	28.7±2.68 a	1.35±0.16 ac	47±3.16
	CV	9.0	7.2	7.7	9.4	11.7	6.7
	min-max	59.0-94.0	9.8-13	25.0-33.0	19.0-33.0	0.88-1.68	39.7-52.6
250 Gy	X-SD	83.0±7.38	11.4±0.82	29.8±2.08	27.2±3.90 b	1.3±0.22 c	47.8±2.85
	CV	8.9	7.1	7.0	14.3	17.2	6.0
	min-max	67.0-99.0	9.7-13.5	27.0-34.0	20.0-34.0	0.94-1.65	40.9-52.8
300 Gy	X-SD	84.6±7.08	11.4±0.91	30.8±1.84	28.4±3.60 ab	1.38±0.19 ab	48.5±2.38
	CV	8.3	7.9	6.0	12.6	13.4	4.9

* Means indicated with the same letter belong to the same statistical group; PH Plant height; SL Spike length; NSS Number of spikelets per spike; NGS Number of grains per spike; GW Grain weight per spike; TGW Thousand grain weight; X-SD Mean-Standard deviation; CV Coefficient of variation (%)

Tablo 6. Descriptive statistics of the traits examined in M_2 plants of line YAA7050-14

Doses		PH	SL	NSS	NGS	GW	TGW
	min-max	71.0-89.0	8.5-10.7	20.0-36.0	19.0-35.0	0.85-1.64	36.8-51.5
Control	X-SD	80.5±4.24	9.6±0.46	30.0±2.75	29.3±3.02	1.36±0.16	46.5±3.10
	CV	5.3	4.8	9.2	10.3	11.9	6.7
	min-max	65.0-88.0	8.3-11.5	22.0-38.0	19.0-38.0	0.81-1.71	30.3-52.3
100 Gy	X-SD	80.5±4.74	9.8±0.63	30.5±2.73	29.9±3.95	1.33±0.22	45.6±4.79
	CV	5.9	6.4	9.0	13.2	16.5	10.5
	min-max	71.0-95.0	8.0-10.7	25.0-35.0	23.0-34.0	0.99-1.64	34.2-51.4
150 Gy	X-SD	81.1±5.26	9.3±0.67	29.7±2.22	29.1±2.36	1.33±0.15	45.6±3.74
	CV	6.5	7.2	7.5	8.1	11.1	8.2
	min-max	51.0-90.0	7.7-11.5	21.0-36.0	19.0-35.0	0.82-1.73	37.9-51.7
200 Gy	X-SD	77.3±7.19	9.6±0.76	29.8±3.17	29.1±3.45	1.37±0.20	47±3.14
	CV	9.3	8.0	10.6	11.9	14.5	6.7
	min-max	49.0-91.0	8.5-11.2	21.0-36.0	15.0-36.0	0.75-1.73	37.5-50.9
250 Gy	X-SD	74.7±8.66	9.8±0.60	30.5±3.33	28.7±4.62	1.32±0.23	46.1±2.81
	CV	11.6	6.2	10.9	16.1	17.3	6.1
	min-max	62.0-89.0	8.0-12.7	26.0-38.0	20.0-34.0	0.9-1.83	32-51.7
300 Gy	X-SD	78.2±6.45	10.5±0.85	31.9±2.56	29.0±3.16	1.43±0.21	47.9±3.11
	CV	8.2	8.1	8.0	10.9	15.0	6.5

PH Plant height; SL Spike length; NSS Number of spikelets per spike; NGS Number of grains per spike; GW Grain weight per spike; TGW Thousand grain weight; X-SD Mean-Standard deviation; CV Coefficient of variation (%).

coefficients of variation increased relative to the control at 250 and 300 Gy gamma-ray doses, reaching 14.3% and 12.6%, respectively. At 300 Gy gamma-ray dose, 34 grains per spike were observed, indicating a positive variation in this trait. A similar situation was observed in grain weight per spike (GW), which is closely correlated with NGS. The lowest and highest GW in control were 0.98 g and 1.62 g, respectively. The highest CV for this trait among M_2 plants was found at 250 Gy with 17.2%. For this dose, the lowest and highest GW values were 0.88 g and 1.68 g, respectively. The following highest CV was found at 100 Gy. At the 100 Gy dose, a 1.73 g spike grain

weight value was reached. The coefficients of variation in TGW were found to be similar in control and M_2 plants obtained after gamma irradiation at different doses. The highest CV for this trait was found at 100 Gy with 7.5%. The highest mean value in terms of TGW was determined at 300 Gy gamma-ray dose with 48.5 g.

Table 6 shows the descriptive statistics of the characteristics examined in M_2 plants of the naked barley line YAA7050-14. The main stem PH of the control ranged from 71.0 cm to 89.0 cm. In M_2 plants obtained after gamma irradiation, the greatest variation in PH was observed at 250 Gy dose with decreased 9.0% (Table 6). At 250 Gy, 49.0 cm and 91.0 cm were the lowest and highest PH values, respectively. The CV of the control group for this characteristic was 5.3 %. Gamma irradiation also increased the coefficient of variation for spike length. The CV of the control was 4.8%, while the CV of the 300 Gy dose was 8.1%. At 300 Gy SL varied between 8.0 cm and 12.7 cm. The coefficient of variation of NGS increased compared to the control at 250 and 100 Gy, reaching 16.1% and 13.2% respectively. At 100 Gy gamma dose, 38 grains per spike were observed, indicating a positive variation in this characteristic. A similar trend was observed for the grain weight per spike. The lowest and highest GW measured in the control were 0.85 g and 1.64 g respectively. The highest CV for this characteristic in M_2 plants was found at 250 Gy with 17.3%. At this dose, the lowest and highest GW values were 0.75 g and 1.73 g, respectively. In M_2 plants, the maximum GW value was found at 300 Gy dose with 1.83 g. The coefficients of variation for TGW were found to be similar in control and M_2 plants obtained after gamma irradiation at different doses. The highest CV for this characteristic was found at 100 Gy with 10.5% (Table 6).

At high gamma ray doses, plants close to the control were observed in the M_2 generation as well as plants with much shorter height than the control at these doses. The presence of genotypes shorter than the control indicates that the desired variation in terms of plant height has occurred in the naked barley genotypes used as material and that short and lodging resistant genotypes can be selected from this variation. When the results of similar studies on mutagen application in barley were evaluated (Ashmawy et al., 2016), similar results were reported that PH decreased with increasing doses.

A closer evaluation of the spike length data in this study shows that plants with short and long spikes were found together compared to the control, i.e. the variation in spike length at high doses was higher than at the control and other doses (Tables 5 and 6). In similar studies investigating the effects of mutagen applications on spike length, it was found that there was no or very little difference in spike length depending on the dose increase (El-Degwy & Hathout, 2014), while it is possible to see the results that spike length decreased at high doses (Ashmawy et al., 2016). The presence of genotypes with longer spike length compared to the control in the material obtained from the study shows that there is a very promising variation for increasing the yield potential.

In M_2 generation of both naked barley genotypes, 250 and 300 Gy doses increased the number of sterile spikelets in the spike and caused a decrease in the NGS. Although the NGS decreased at high gamma ray doses in M_2 generation, plants with grain numbers close to the control and higher grain number were also observed (Tables 5 and 6). In other words, it can be said that there was enough variation in both genotypes as a result of gamma ray application to make a selection in the desired direction for this trait. Increasing the NGS is among the main objectives of mutation breeding in terms of its positive relationship with yield (Ahuja et al., 2014).

The results show that high doses of gamma radiation, such as 250-300 Gy, induce a variation with higher TGW in these genotypes, and it is possible to select plants with higher values than the control from this material. In many studies, it has been reported that TGW decreases with increasing mutagen doses (Dyulgerova & Dyulgerov, 2020; El-Degwy & Hathout, 2014; Singh & Datta, 2010), while in some studies, similar to our results, it has been reported that there is no difference with mutagen applications or an increase in TGW can be achieved (Cheng et al., 2015). The presence of plants with higher TGW compared to the control, which is both one of the most important yield factors (Ataei, 2006), as well as one of the most important quality parameters in barley (Jilal et al., 2013), indicates that there is sufficient variation in this material to make target-oriented selection.

The negative effects of high gamma ray doses and the decrease in M_2 generation compared to the control were not as effective and significant as in M_1 generation. The reason for this could be the somatic effects of gamma irradiation and the non-heritable morphological differences in the plants (Raina et al., 2022) and the DNA repairs after mutagen application (Viana et al., 2019).

CONCLUSION

The results of this study showed that within the mutant population created by gamma irradiation of the seeds of naked barley cv. Yalin and naked barley line YAA7050-14, a variation suitable for selection in the desired direction was formed with respect to plant height, spike length, number of grains per spike, grain weight per spike, and thousand grain weight characteristics. In addition, it is possible to select individuals with superior characteristics to the controls from this material. Gamma irradiation at 250 Gy caused the highest variation in both barley genotypes in this study, followed by 300 Gy. These doses could be recommended for the creation of genetic variation in naked barley. Consequently, it can be said that gamma irradiation is an effective way to create variation for use in naked barley breeding studies.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

All authors declare that they have no conflicts of interest

Author contribution

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

Ethics committee approval

Ethics committee approval is not required. This article does not contain any studies with human participants or animals performed by any of the authors.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

Acknowledgements

The authors are grateful to Dr. Ali Senay from the Ankara Nuclear Research and Training Center, for his contribution to gamma irradiation. We would also like to thank the CRIFC for the provision of the experimental fields and other research facilities. This study is based on Namuk ERGÜN's PhD thesis completed at Ankara University, Graduate Scholl of Natural and Applied Sciences under the supervision of Prof. Dr. Saime ÜNVER İKİNCİKARAKAYA.

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RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(3): 660-668
DOI: https://doi.org/10.31015/jaefs.2023.3.20

Determination of free, esterified, bound bioactive compound contents of *Euphorbia cyparissias* organs and their biological activities

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Citation: Karaaslan, A. (2023). Determination of free, esterified, bound bioactive compound contents of Euphorbia cyparissias organs and their biological activities. International Journal of Agriculture, Environment and Food Sciences, 7 (3), 660-668

Received: August 18, 2023
Revised: September 15, 2023
Accepted: September 17, 2023
Published Online: September 29, 2023

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Available online at
https://jaefs.com/
https://dergipark.org.tr/jaefs

Abstract

Euphorbia plants have long been used as herbs in numerous traditional medicines in Anatolia. They were employed for the treatment of microbial infections, skin wounds and gastrointestinal diseases. *Euphorbia* species are rich sources of phenolic acids, flavonoids and many other natural compounds with antioxidant effects. In the context of this study the phenolic content, antioxidant activity and antidiabetic effect of *Euphorbia cyparissias* (*E. cyparissias*) leaf, flower and stalk extracts were evaluated. Three separate phenolic fractions namely free, esterified and bound extracts were prepared from leaf, stalk, and flower organs. Enzymatic treatment was utilized to remove bound phenolics from the cellular structures. A total of nine different extracts obtained from *E. cyparissias* organs. The highest phenolic fraction was bound phenolics in all three assayed extracts. The highest total phenolic compound (TPC) was found as bound phenolic fraction form in leaf extracts (21.088±0.32 mg GAE/g). Similarly the leaf samples displayed the highest total flavonoid contents (TFC) as bound form (1.798±0.02 mg CE/g). Four different methods were employed to determine the antioxidant potencies of the extracts. In parallel with the TPC and TFC results the bound fraction of leaf extract displayed the highest antioxidant capacities when evaluated with DPPH, ABTS and CUPRAC assays. According to FRAP analysis, free phenolic compounds of the leaves had the highest antioxidant potential. Free, esterified and bound phenolic compound fractions were all displayed inhibitory activity against α-amylase and α-glycosidase enzymes which is associated with their antidiabetic effects. Especially esterified phenolic compounds displayed significant inhibitory activity against α-amylase while bound fractions found in stalks and flowers exhibited stronger α-glycosidase activities.

Keywords: *Euphorbia cyparissias*, Phenolic compounds, Flavonoids, Antioxidant, Antidiabetic

INTRODUCTION

It has long been known that plants can be utilized in medicinal purposes because of their curing and healing abilities originating from their rich phytochemical contents. There are many studies in the literature showing that plant organs confer antimicrobial (Passari et al., 2015), antioxidant (Huang et al., 2011), anticancer (Rajasekar et al., 2012), and anti-diabetic activities (Gad et al., 2006) under *in vitro* and *in vivo* conditions. Currently plants are being used by the nutraceutical and pharmaceutical industries for the production of phyto-tablets, food-supplements and drugs. Recently, plant based diets were suggested by the researchers because of lower risk of cardiac diseases associated with regular daily

consumption of fruits, vegetables and the other plant tissues (Salehin et al., 2023). Therefore, the antioxidant, antimicrobial and biological activities of plant species should be evaluated to provide alternative phyto-chemical sources to the industry.

Anatolia harbors a myriad of different endemic plant species. One of those plants is *Euphorbia* genus. *Euphorbia* is a member of *Euphorbiaceae* family which is generally known as spurge, falls into a diverse genus of perennial flowering plants, and comprises more than 2000 species (Özbilgin et al., 2012). *Euphorbia* exhibits biological activities associated with medicinal plant attributes and has been widely used by the local people because of its curing, healing, protecting and antimicrobial properties. *Euphorbia* is originated from Asia and Arabian Peninsula and distributed up until to Anatolia. Especially in the Southeastern Region of Anatolia local people use this plant while they are producing grape molasses because of its food protecting ability. It was known that use of *Euphorbia* in the production of grape molasses confers better stability and longer shelf life to molasses.

Previously, total phenolic compounds (TPC), total flavonoid compounds (TFC), and antioxidant activities of three different *Euphorbia* species were evaluated and the results showed their high phyto-chemical contents and antimicrobial activity against *Staphylococcus aureus* (Budhathoki et al., 2016). The reported data also indicated that *Euphorbia* extracts contain a remarkable amount of phenolic acids, flavonoids, tannins and glycosidic compounds which indicate the high potential of *Euphorbia* extracts for the food and drug applications. In another study, it was determined that *Euphorbia tirucalli* contains a significant amount of bioactive compounds including alkaloids and phenolic derivatives while extracted with ethyl acetate (Le et al., 2021). Ethyl acetate fraction of the extract displayed antimicrobial activity against *Xanthomonas axonopodis*. In a similar fashion, methanolic extract of *Euphorbia* plant conferred significant antiproliferative activity against MiaPaCa-2 cell line of pancreatic cancer (Munro et al., 2015).

There are similar reports in the literature regarding the bioactive compound content of *Euphorbia* and its functional properties. However these studies were focused on the free phenolic content of the *Euphorbia* plant. Recently it was shown that besides the free fraction of phenolic compounds; esterified and bound fraction of the phenolic compounds also display functional properties and those two fractions should be measured to determine the exact amount of TPC (Acosta-Estrada et al., 2014).

Phenolic compounds draw significant attention because of their free radical scavenging activity and health promoting effects. It was shown that phenolic compounds are related to protecting human health, inhibition of tumor formation, and DNA protecting ability (Bernatoniene et al., 2023). Flavonoids and tannins also display strong biological activities such as inhibition of cancer cell proliferation, antioxidant activity and anti-diabetic activity (Maphetu et al., 2022). However to get a complete profile of the phenolic compounds and assess their health promoting potential three fractions of the phenolic compounds should be isolated and their biological activities determined separately.

In general, phenolic compounds fall into three separate classes according to their chemistry and existing forms in plant organs such as free phenolics, esterified phenolic compounds and insoluble ones which covalently bonded to the cellular structural components (Wu et al., 2021). In general, aqueous, methanolic, ethanolic and most of the solvent extracts of plant organs contain free phenolic compounds rather than the bound ones. Bound phenolic compounds are covalently linked to the cellulose micro-fibrils or pectin matrix existing in the cell wall of plant cells. Thermal treatments, alkalization or enzymatic treatments could be employed to remove such compounds from their existing compartments after the removal of free phenolic compound fraction and esterified phenolics (Acosta-Estrada et al., 2014; Shahidi et al., 2016). High blood glucose levels, type-2 diabetes, and insulin resistance are the common diseases among people which are associated with uncontrolled depolymerization of carbohydrates. α-amylase and α-glucosidase are the two common enzymes that control the digestion of carbohydrates in the human body and thus increase the blood glucose level. Therefore it is necessary to explore drugs or natural compounds to inhibit the activity of these enzymes. Plant extracts display significant enzyme inhibitory activities that can be used in the remedy of diabetes (Başyiğit et al., 2020). *E. cyparissias* plant should also be evaluated to assess its antidiabetic activity.

In the context of this study *E. cyparissias* plants grown in the Sanliurfa region were collected and leaf, stem and stalk organs were used as the bioactive compound sources. Three different phenolic compound fractions such as free, esterified and bound have been isolated and their biological activities were determined. TPC, TFC, hydrolysable tannin content, antioxidant and antidiabetic activities of each phenolic compound fraction were determined.

MATERIALS AND METHODS

Materials

Euphorbia plant employed in this study was obtained from a local market in Şanlıurfa. The plants were transported to the laboratory under suitable conditions and stored in a refrigerator until the analyses. The organs underwent a thorough washing process, being rinsed three times with distilled water, succeeded by manual

cleaning. The drying trials were conducted openly, without exposure to sunlight, and at room temperature. Post-drying, the organs were fragmented using a waring commercial lab blender (Conair, Stamford, CT, USA). For the analytical processes, the enzymes Vegazym HC, Fructazym MA-LG, and EnerZyme P7 were procured from Erbslöh (Geisenheim, Germany). The necessary chemicals and standards were sourced from Sigma or Merck (Darmstadt, Germany), unless exceptions are explicitly mentioned. Throughout the procedures, solvents of standard analytical quality were employed.

Extraction conditions

Free phenolics

Free phenolic extraction was conducted according to the method described by Ambigaipalan et al (2016) with slight changes. 1 g finely grounded plant material was dipped into 10 mL ethanol–water mixture (42–58; v/v). This mixture was subjected to extraction in a shaker at room temperature for a duration of 30 min. Following this, the mixture was centrifuged at 4000 rpm for 5 min, leading to the collection of the resulting supernatant. The solid residue that remained was then subjected to an oven-drying process at 40 °C for 48 h, and this material was reserved for subsequent bound phenolic extraction. This entire procedure was repeated three times, after which the resulting mixture was acidified to a pH of 1.5–2 using 6 M HCl. The final solution underwent three extraction cycles with an equivalent volume of diethyl ether–ethyl acetate (1:1, v/v) using a separator funnel. The organic fractions derived from this process were filtered through sodium sulfate and then evaporated at 40 °C until complete dryness was achieved. Obtained phenolic compounds were dissolved in 10 mL methanol and incubated in a deep freezer at –20 °C.

Esterified phenolics

Esterified phenolics were isolated according to the method reported by Ambigaipalan et al (2016) with minor modifications. The water phase remaining after free phenolic extraction was combined with an equivalent amount of 4 M NaOH. This mixture was then exposed to nitrogen in a shaker at room temperature for 4 h to liberate the esterified phenolic compounds. Subsequently, the mixture was acidified to a pH of 1.5–2 using 6 M HCl. After centrifugation at 4000 rpm for 5 min, the esterified phenolic compounds were subjected to three successive extractions using a 1:1 mixture of diethyl ether and ethyl acetate. This process was conducted as previously described. The organic phase was evaporated, and phenolic compounds were dissolved in 10 mL methanol and incubated in a deep freezer at –20 °C.

Bound phenolics

The approach utilized in this study followed the procedure outlined by Cuevas Montilla et al (2011) albeit with certain modifications. The residual solids obtained after the extraction of free and esterified phenolic compounds were subjected to oven-drying at 40 °C for a duration of 48 h. These dried solids were then combined with 10 mL water at 50 °C, and the pH of the mixture was adjusted to 4.5. For the process of cellulosic and pectolytic hydrolysis, a mixture of 2 µL vegazym and 1 µL fructazym was introduced to the mixture. The hydrolysis took place in a shaker at 55 °C for a period of 1 h. In the case of proteolytic hydrolysis, 2 µL EnerZyme P7 was added to the mixture under conditions of pH 6.5 and 55 °C, again for a duration of 1 h. It's worth noting that the specified conditions for each enzyme were determined based on their respective specifications. Subsequent to centrifugation at 4000 rpm for 5 min, the resulting supernatant was collected and preserved at a temperature of –20 °C in a freezer for subsequent analyses. Meanwhile, the solid residue derived from the centrifugation step underwent oven-drying at 90 °C for 1 h in order to deactivate the enzymes. Subsequently, this residue was allowed to dry for 48 h at 40 °C. The conducted experimental protocols for the extraction steps were summarized by Figure 1.

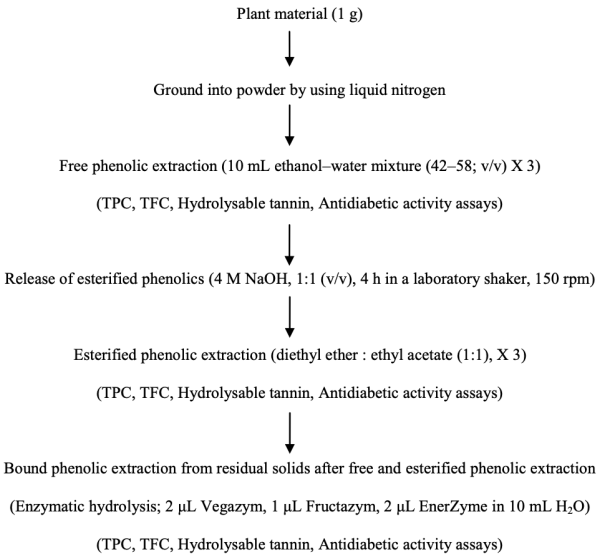

Figure 1. Free, esterified, and bound phenolic fractions' extraction from *E. cyparissias* organs.

Total phenolic content

Two mL Folin–Ciocalteu's phenol reagent after tenfold dilution and 0.4 mL of either diluted extract or gallic acid solution (5–100 mg/L) were mixed in a flask. To this mixture, 1.6 mL of a 7.5% (w/v) sodium carbonate solution was added. Following the addition, the solution was kept at room temperature during 1 h time period. Subsequently, the absorbance of the samples was determined at 765 nm by utilizing a UV–Vis spectrophotometer (Model UV-1280, Shimadzu Corp) (Singleton et al., 1965).

Total flavonoid content

The method described for determining the TFC was employed as described in a previous study (Zhishen et al., 1999). In this procedure, a 10 mL volumetric flask was utilized. To the flask, 1 mL of diluted extract or catechin in the range of 50 to 250 mg/L, was added. Subsequently, 4 mL distilled water and 0.3 mL sodium nitrite (5%, w/v) were introduced. The flask was then left at room temperature for a duration of 5 min. Following the incubation period, 0.3 mL aluminum chloride (10%, w/v) was incorporated into the flask. After an additional 6 min, a mixture consisting of 2 mL of 1 M sodium hydroxide and 2.4 mL of distilled water was added to the solution. The final solution's absorbance was spectrophotometrically determined at 510 nm.

Total hydrolysable tannin content

The determination of the overall hydrolysable tannin content (HTC) was carried out using a spectroscopic approach (Willis, 1998). A combination of 1 mL of diluted extract or tannic acid (ranging from 125 to 2500 mg/L) with 5 mL of potassium iodate solution (2.5%, w/v) was prepared. This mixture was kept at room temperature for 6 min. The solution's absorbance was spectrophotometrically determined at 550 nm. The obtained results were expressed as tannic acid equivalent (TAE).

Antioxidant activity

In the DDPH (2,2-Diphenyl-1-picrylhydrazyl) assay, a series of extracts dilutions, as well as trolox (ranging from 20 to 1000 µmol/L), were combined with a solution of DPPH (25 mg/L). This mixture was then allowed to incubate at room temperature for a duration of 30 min. Subsequently, the absorbance of the incubated solution was gauged at a wavelength of 515 nm using a UV–Vis spectrophotometer. A trolox curve was employed to measure the antioxidant capacity (Çam et al., 2009).

For the ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) assay, a solution of 0.96 mg ABTS was prepared in a 25 mL volumetric flask by adding 10 mL distilled water and 5 mL potassium persulfate (2.45 mM). The mixture was then brought to a final volume of 25 mL with distilled water. This solution was incubated in darkness at room temperature for 16 h. To adjust the absorbance of the radical solution, 0.2 M sodium phosphate buffer (pH 7.4) was employed to attain a target absorbance of 0.700 ± 0.02 at 734 nm. For subsequent steps, a stock solution (2000 µL) was prepared, and the diluted extract or trolox (ranging from 0.1 to 2 mM) was mixed with it. This mixture was then incubated at room temperature for a period of 6 min. Following incubation, the solution's absorbance was spectrophotometrically determined at 734 nm. A trolox curve was employed to measure the antioxidant capacity (Çam et al., 2009).

For the FRAP (ferric reducing antioxidant power) assay, a volumetric flask was used to combine the following components: 25 mL 30 mM acetate solution, 2.5 mL 10 mM solution of 2,4,6-Tris(2-pyridyl)-s-triazine, and 2.5 mL 20 mM solution of iron (II) chloride. Subsequently, this mixture was blended with 150 µL serially diluted extracts or trolox (ranging from 40 to 300 µmol/L). The resulting solution was then incubated at room temperature for a duration of 30 min. Following incubation, the absorbance of the solution was measured at 593 nm using a UV–Vis spectrophotometer (Benzie et al., 1996).

0.4 mL serial dilution of extracts put into flasks was used for the CUPRAC (cupric reducing antioxidant capacity) assay. To this flask, 1 mL 0.01 M copper (II) chloride, 1 mL 7.5×10^{-3} ethanolic neocuproine solution, and 1 mL 1 M ammonium acetate solution at pH 7 were added. Subsequently, distilled water was used to bring the total volume to 4.1 mL. This mixture was then incubated at room temperature for a duration of 30 min. The absorbance of the solution after incubation was measured at 450 nm using a UV–Vis spectrophotometer. To determine the antioxidant activity of the samples, the regression equation derived from the trolox curve was applied (Apak et al., 2007).

Antidiabetic activity

For the α-glucosidase measurement, a mixture of 50 µL diluted extract, 1250 µL potassium phosphate buffer with a pH of 6.8, and 50 µL α-glycosidase enzyme solution was incubated at 37 °C. Following a 5-min incubation period, the enzymatic reaction was initiated by introducing 125 µL 10 mM 4-nitrophenyl α-D-glucopyranoside to the prepared blend. After allowing the reaction to proceed for 20 min, 2000 µL 0.1 M sodium carbonate was introduced to halt the reaction process. The resulting solution's absorbance was then measured at a wavelength of 400 nm (McDougall et al., 2005).

For the α-amylase assay, a mixture of 1 mL diluted extract, 1 mL starch solution (1% w/v), and 1 mL 20 mM sodium phosphate buffer (pH 6.9) was incubated at a temperature of 37 °C. After an initial 5-min incubation period, the enzymatic reaction was initiated by introducing 1 mL the α-amylase solution. Following a 30-min incubation, the reaction was halted by adding 1 mL color reagent. This color reagent was composed of a solution containing 5.31 M sodium potassium tartrate, which was prepared using 2 M NaOH and 96 mM 3,5-dinitrosalicylic acid. The reaction mixture was subsequently boiled for 5 min, and the absorbance of the resulting solution was measured at a wavelength of 540 nm. For the corresponding enzymatic control and blank samples, the preparation involved excluding the extract and the enzyme, respectively (McDougall et al., 2005).

Statistical analysis

Extractions and analyses were carried out in a minimum of two separate instances. Data underwent examination

utilizing the one-way ANOVA within the SPSS 22.0 statistical software package for Windows (SPSS, Inc., Chicago, USA). Distinctions between means were assessed through Tukey's HSD test at a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSIONS

Free, esterified and bound phytochemical content of Euphorbia cyparissias organs

Three different parts of *Euphorbia* plants namely stalk, flower and leaf were used as plant material to extract free, esterified, and bound phenolic fractions. Water/ethanol mixtures were utilized to extract the free phenolic compounds and enzymatic extraction techniques were employed for the further extraction of esterified and bound phenolics remaining in the plant tissues after the removal of free phenolic compounds. Similar extraction procedure was also employed for the isolation of flavonoids and hydrolysable tannins from the *E. cyparissias* tissues as well with specific extraction buffers. In general, *E. cyparissias* leaves contained the highest amount of free, esterified and bound phenolic compounds compared to euphorbia stalks and flowers. While leaf tissues contain 14.22±0.07 mg GAE/g, *E. cyparissias* flowers contain 8.654±0.04 mg GAE/g, the stalks only contain 6.013±0.8 mg GAE/g free phenolic compounds (Table 1). According to the obtained results, *E. cyparissias* leaf is a rich source of free phenolic compounds compared to flower and stalk organs of the plant. The phenolic content *of E. tirucalli* L. was determined as 0.305 mg/g gallic acid equivalent (GAE) by de Araujo et al (de Araújo et al., 2014)). The free phenolic content of *E. cyparissias* was significantly higher than the free phenolic content of *E.tirucalli L.* Yener et al. examined the chemical and biological profile of the *Euphorbia* species grown in different regions of Anatolia and determined a statistically significant difference in TPC (ranging from 63.03 to 372.27 µg pyrocatechol equivalents/mg extract) of the examined samples (Yener et al., 2018). The difference between phenolic contents could be attributed to the difference in extraction methods, species type, soil structure and the climatic conditions in which the plant grows (Yener et al., 2018). The leaf, flower and stalk organs were also assessed in terms of their esterified phenolic compound content. It should be noted that, the esterified phenolic concentrations of the leaf (1.731±0.2 mg GAE/g) and stalk (1.756±0.35 mg GAE/g) are higher than that of flower organs (1.182±0.08 mg GAE/g) (Table 1). An enzymatic treatment was applied for the removal of bound phenolic compounds from the E. *cyparissias* organs and to determine the bound phenolic content of the samples. In a similar fashion the leaf of the E. *cyparissias* contain the highest amount of bound phenolic compounds (21.088±0.32 mg GAE/g) which was followed by flower (16.09±0.2 mg GAE/g) and stalk (8.159±0.94 mg GAE/g). In general the assayed leaf samples had the highest amount of free, esterified and bound phenolic compounds in comparison to the other two organs; the stalk was the second phenolic-rich organ which was followed by flower. The free, esterified and bound flavonoid content of the *E. cyparissias* organs were also investigated (Table 1). The highest amount of free flavonoids was detected in the leaf samples (1.471±0.06 mg CE/g) and followed by flower (1.399±0.37 mg CE/g) and stalk organs (0.614±0.09 mg CE/g). The highest amount of bound flavonoids were detected in leaf samples. According to the obtained results the leaf and flower contain higher amounts of flavonids than that of found in stalks. In general the flavonoid content of the *E. cyparissias* was higher compared to the other nine *Euphorbia* species analyzed in a previous study. The difference between the results could be species-specific or stemmed from the variation in employed phytochemical extraction techniques (Yener et al., 2018). There are at least four flavonoids in the *Euphorbia* species such as naringenin, aromadendrin, apigenin and luteolin (Soliman et al., 2021) which are known for their positive impact on human health. The obtained results indicated that *E. cyparissias* organs could be employed for the preparation of flavonoid-rich plant extracts to be used in the food and health industry. Hydrolysable tannin is an important group of plant natural compounds and include gallic acid, ellagitannins, and gallotannins which exert positive impact on human health due to their anti-ulcerative, anti-microbial, and anti-tumor activities (Orabi et al., 2015). Interestingly the flowers of *E. cyparissias* contain the highest amount of free and esterified hydrolysable tannin (Table 1). The free, and esterified hydrolysable tannin fractions were detected at the highest amount in flower compared to leaf and stalk organs. It should be noted that bound hydrolysable tannins were found at the highest amount in leaves (Table 1).

Antioxidant activities of free, esterified and bound phytochemical fractions extracted from Euphorbia cyparissias organs

There are different subgroups of phenolic compounds existing in plant tissues and thus different antioxidant capacity assays should be employed to assess the actual radical scavenging activity and reducing power (Arnao et al., 1999). In the context of this study four different antioxidant activity assays were employed to determine their potential antioxidant activities, namely DPPH, $ABTS^{+}$, FRAP and CUPRAC. In terms of the effect of phenolic fraction type on antioxidant activity; the bound fraction had the highest radical scavenging potency followed by free fraction and finally esterified fraction according to DPPH assay (Table 2). Similar results were also obtained in the $ABTS^{+}$ antioxidant capacity determination assay as well. Bound fractions were displayed better at radical scavenging potency according to $ABTS^{+}$ assay which is an electron-transfer based system and CUPRAC reducing power assay. However in FRAP assay the free fractions

Table 1. The free, esterified and bound phenolic fractions of phenolics, flavonoids and hydrolysable tannin content of *E. cyparissias* organs. Different letters in the same column indicate statistically significant difference (p<0.05).

Sample Name	Phenolic Fraction	Phenolic (mg GAE/g)	Flavonoid (mg CE/g)	Hydrolysable tannin (mg TAE/g)
***E. cyparissias* Stalk**	*Free*	6.013±0.8^{c}	0.614±0.09^{c}	15.591±0.7^{d}
	Esterified	1.756±0.35^{b}	0.094±0.00^{a}	2.062±0.03^{a}
	Bound	8.159±0.94^{d}	1.017±0.02^{d}	ND
***E. cyparissias* Leaf**	*Free*	14.22±0.07^{e}	1.471±0.06^{g}	18.614±1.03^{e}
	Esterified	1.731±0.2^{b}	0.263±0.01^{b}	5.408±0.08^{b}
	Bound	21.088±0.32^{g}	1.798±0.02^{h}	15.797±0.9^{d}
***E. cyparissias* Flower**	*Free*	8.654±0.04^{d}	1.399±0.37^{f}	32.642±1.13^{g}
	Esterified	1.182±0.08^{a}	0.569±0.03^{c}	25.570±1.8^{f}
	Bound	16.09±0.2^{f}	1.082±0.21^{e}	9.839±0.74^{c}

Table 2. The antioxidant activities of free, esterified and bound phenolic fractions extracted from *E. cyparissias* stalk, leaf and flower organs. Different letters in the same column indicate statistically significant difference (p<0.05).

Sample Name	Phenolic Fraction	DPPH (µmol Trolox/g)	ABTS (µmol Trolox/g)	FRAP (µmol Trolox/g)	CUPRAC (µmol Trolox/g)
***E. cyparissias* Stalk**	*Free*	182.012±2.02^{d}	135.052±1.03^{d}	212.085±1.8^{e}	163.598±1.79^{d}
	Esterified	71.364±1.23^{b}	19.645±0.4^{a}	77.872±0.8^{a}	63.915±0.9^{a}
	Bound	191.114±1.09^{e}	155.702±1.21^{e}	193.081±0.913^{d}	170.018±1.86^{e}
***E. cyparissias* Leaf**	*Free*	174.032±1.5^{c}	266.255±1.72^{g}	302.514±1.17	307.336±1.7^{g}
	Esterified	69.378±0.3^{b}	40.78±0.8^{b}	89.33±0.76^{c}	104.67±0.5^{c}
	Bound	269.853±1.7^{h}	421.130±2.05	263.439±1.52^{f}	372.682±1.43
***E. cyparissias* Flower**	*Free*	227.335±1.63^{f}	239.495±1.8^{f}	298.689±1.86^{h}	254.63±1.33^{f}
	Esterified	60.060±0.58^{a}	45.734±0.7^{c}	83.953±0.81^{b}	84.44±0.135^{b}
	Bound	233.773±2.02^{g}	356.601±1.6^{h}	286.853±2.18^{g}	321.534±1.147^{h}

exhibited stronger reducing power compared to other two fractions (Table 2). In general, it was obvious that leaf samples had stronger antioxidant activity compared to stalk and flower samples. The antioxidant activity of plant extracts in general is correlated with their phyto-chemical contents (Mohammed Fazil Ahmed et al., 2012); the higher phytochemical content refers to higher radical scavenging strength. The high phenolic content of the leaf samples could be attributed to their high antioxidant power. The antioxidant activities of *Euphorbia* species were reported in previous studies. Remarkable antioxidant potencies were also detected in the *Euphorbia* species collected from different Anatolian regions (Yener et al., 2018). Four different plant organs (leaves,stems, flowers and roots) obtained from *E. hirta* were assayed to determine the antioxidant activities of the samples. Besides, cyanoferrate method was employed to assess the reducing power of the tissues. Similar to the results obtained in this study they also concluded that the extract of leaves displayed a maximum DPPH scavenging and reducing power activities (Özbilgin et al., 2012).

Antidiabetic activities of free, esterified and bound phytochemical fractions extracted from Euphorbia cyparissias organs

Diabetes mellitus is one of the most common chronic disease types affecting the life quality of human beings. The main cause of this disease could be related to inadequate insulin activity or disturbed secretion of insulin hormone into the bloodstream (Petersmann et al., 2019). The pharmaceutical industry developed many drugs to cure the patients with diabetes mellitus. One of the most common drugs to be used against type 2 diabetes mellitus (T2DM) is metformin (MET). MET displays elongated anti-hyperglycemic effects, does not interfere with cardiac health, a decreased possibility of hypoglycemia risk with affordable price (Lee et al., 2021). However, some of the patients developed intolerance against the use of MET to control the negative effects of T2DM (McCreight et al., 2016). Therefore it is necessary to investigate alternative medicines or natural treatment methods to overcome such side-effects of current drugs. There are many studies in the literature describing the antidiabetic activity of plant extracts (Temiz, 2021; Keskin et al., 2022). There are investigations depicting that pomegranate (Alsataf et al., 2021) and *Quercus infectoria* (Başyiğit et al., 2020) display antidiabetic activity. Flower extracts of *E. hirta* exhibited antidiabetic effect when orally administered to alloxan diabetic mice and also showed in vitro antioxidant activity (Başyiğit et al., 2020). In a similar fashion, Subramanian et al. (Subramanian

Table 3. The enzyme inhibitory activities of free, esterified and bound phenolic fractions extracted from *E. cyparissias* stalk, leaf and flower organs. Different letters in the same column indicate statistically significant difference ($p<0.05$).

Sample Name	Phenolic Fraction	α-amylase IC_{50} (mg/mL extract)	α-glycosidase IC_{50} (mg/mL extract)
***E. cyparissias* Stalk**	Free	14.505±0.5^{d}	12.02±0.08^{e}
	Esterified	2.938±0.02^{a}	11.32±0.22^{e}
	Bound	30.971±0.9^{g}	0.18±0.01^{a}
***E. cyparissias* Leaf**	Free	24.357±0.12^{f}	0.49±0.05^{a}
	Esterified	4.403±0.05^{b}	25.24±0.8^{f}
	Bound	4.595±0.128^{b}	31.80±0.94^{g}
***E. cyparissias* Flower**	Free	15.712±0.82^{e}	5.24±0.2^{c}
	Esterified	2.939±0.07^{a}	6.91±0.3^{d}
	Bound	12.60±0.21^{c}	3.35±0.41^{b}

et al., 2011) reported that 30 day of oral administration of extracts obtained from *E. hirta* leaves displayed antidiabetic activity in an animal-model experiment. The antidiabetic activity of the extracts obtained from *E. cyparissias* plant was assessed in the context of this study. Enzyme inhibitory activities of stalk, leaf and flower extracts are given in Table 3. The antidiabetic effect of the *Euphorbia* plant was calculated by measuring the α-amylase and α-glycosidase enzyme activities in the stalk, leaf and flower parts of the plant. According to the α-amylase enzyme activity measurement, the highest antidiabetic effect was determined in the esterified phenolic fractions in the flower and stalk. The α-glycosidase enzyme activity measurement depicted that the highest antidiabetic effect was present in the form of the bound phenolic fraction in the plant stalk. The lower IC_{50} means the stronger antidiabetic activity. Esterified phenolic extracts displayed the lowest IC_{50} values in stalk and flower organs as can be seen in Table 3.

CONCLUSIONS

There are more than 8000 individual phenolic compounds in plant species which establish one of the biggest classes of secondary metabolites. Phenolic compounds are involved in the defense mechanism of the plants against microorganisms and detrimental effects of abiotic stresses. In recent years phenolic compounds draw significant attention due to their antioxidant, antidiabetic and antiproliferative activities. Many studies were conducted to show their promoting effects on human health. However most of these studies focused on the free phenolic compounds rather than esterified and bound forms. *Euphorbia* is a genus comprising more than 2000 species and contains a significant amount of phenolic compounds. Until today, only free phenolic compounds extracted from *Euphorbia* species and their biological activities were evaluated. In the context of this study three different organs of *E. cyparissias* (stalk, leaf, flower) were used as phytochemical sources. Free, esterified, and bound forms of phenolics were isolated from these organs to evaluate their biological activities. The results showed that all three organs contain a significant amount of esterified and bound phytochemicals beyond their free forms. Esterified and bound natural compounds displayed strong antioxidant and antidiabetic activity that indicated their importance for the production of functional extracts. Especially esterified leaf extracts displayed strong biological activities and thus can be used for the production of functional extracts for food and pharmaceutical industries. Overall, the obtained results in this study showed the importance of *E. cyparissias* extracts to be used in pharmaceutical and food industries. The flowers, stalks and leaves can be used to produce phyto-tablets and food supplements after extensive *in vitro* and *in vivo* studies.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The author declare no conflict of interest.

Author contribution

The author collected the data, analyzed the results and wrote the paper.

Ethics committee approval

Ethics committee approval is not required.

Funding

No financial support received for this study.

Data availability

Data are available upon reasonable request.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(3): 669-689
DOI: https://doi.org/10.31015/jaefs.2023.3.21

Effects of postharvest edible coating applications on storage life and quality of some apple cultivars

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Abstract

In this study, the effects of postharvest calcium chloride dihydrate ($CaCl_2.2H_2O$), rosehip essential oil (REO) (cold pressed), and $CaCl_2.2H_2O$+REO combination edible coating applications on some fruit quality parameters and storage life of 'Starkrimson Delicious' ('S. Delicious'), 'Golden Delicious' ('G. Delicious') and 'Granny Smith' ('G. Smith') apple cultivars were investigated. For this purpose, the fruit was divided into four groups after harvest: 1st group: Fruit was dipped in distilled water (control), 2nd group: Fruit was dipped in 1.5% $CaCl_2.2H_2O$, 3rd group: Fruit was dipped in 2% REO, 4th group: Fruit was dipped in 1.5% $CaCl_2.2H_2O$+REO (%2) to form an edible coating on fruit. Before and during storage at periodical intervals weight loss, fruit color, fruit flesh color, fruit flesh firmness, soluble solids content, titratable acidity, pH, respiration rate, ethylene production, sensory evaluations (overall quality, taste and aroma, odor, decay) superficial scald, and superficial scald severity analyzes were performed. In the 'S. Delicious', the lowest weight loss was in $CaCl_2.2H_2O$+REO during and at the end of storage, REO had the lowest in the 'G. Delicious'. 'G. Smith' had lower weight loss in $CaCl_2.2H_2O$ and the control group than the others. The REO preserved the fruit flesh firmness better than others with the least loss. REO was the most effective treatment in suppressing ethylene production in all cultivars, followed by $CaCl_2.2H_2O$+REO. In 'S. Delicious' and 'G. Smith', fruit color and vividness of fruit color were best preserved by REO. As a result, postharvest edible coating applications of $CaCl_2.2H_2O$, REO, and $CaCl_2.2H_2O$+REO in 'S. Delicious', 'G. Delicious' and 'G. Smith' had positive contributions in maintaining fruit quality attributes during storage.

Keywords: Apple, Calcium chloride dihydrate, Cold storage, Ethylene production, Rosehip essential oil

Citation: Dilmacunal, T., Yigit, E. (2023). Effects of postharvest edible coating applications on storage life and quality of some apple cultivars. International Journal of Agriculture, Environment and Food Sciences, 7 (3), 669-689

Received: August 13, 2023
Revised: September 13, 2023
Accepted: September 14, 2023
Published Online: September 29, 2023

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Available online at
https://jaefs.com/
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INTRODUCTION

Apple is one of the world's most traded fruit, and consumer demand is changing rapidly (Bayav and Armağan, 200; Bayav, 2023) Apple is an important fruit type that has been cultivated in Türkiye for many years, ranks first among other temperate climate fruit in terms of cultivated area and production (Küden et al., 1997; Bayav and Karlı, 2020; 2021), and contributes to employment in marketing, packaging, processing and storage stages (Burak and Ergun, 2001) World apple production reached 93,144,358 tons. China ranks first in the world with a production of 45,983,400 tons, followed by the Türkiye (4,493,264 tons) and the USA (4,467,206 tons) (FAO, 2023). Türkiye increased its apple production by 55.53% in the last ten years (2012-2021), from 2,888,895 to 4,493,264 tons. Apple production ranks first when pome and stone fruit production in Türkiye is evaluated (Güner, 2019; Bayav et al., 2023). Isparta accounts for 20.4% of Türkiye's total apple production, with investments in processing, storage and

R&D infrastructure as well as production (TEPGE, 2019).

Apples are rich in bioactive compounds such as antioxidants, organic acids, phenolic substances and vitamins (Kuşçu and Bulantekin, 2016; Ozturk et al., 2022). Many cultivars are used in apple cultivation in the world, and 'Starking Delicious' and 'Golden Delicious' cultivars are among the widely cultivated cultivars in Türkiye (Mordoğan and Ergun, 2002). Among the apple cultivars cultivated in Türkiye, 'S. Delicious', 'G. Delicious', and 'G. Smith' apple cultivars occupy an important place in 4,817,500 tons (TurkStat, 2023). It is reported that 25-40% of fresh produce in Türkiye is lost after harvest for various reasons (Sayılı et al., 2006). Approximately 40% of the apples produced in Türkiye are consumed fresh, and late harvested apple cultivars such as 'Granny Smith', 'Braeburn', 'Pink Lady', 'Golden Delicious' group, 'Red Delicious' group and 'Fuji' group are among the apple cultivars that can be stored for a long time. It is reported that long-term storage is only possible in cold storages where important environmental factors affecting the storage period such as temperature, relative humidity, atmospheric composition and ethylene removal in the environment can be well controlled. However, in our country, due to the insufficient capacity of cold storage where the ambient factors can be fully controlled and high storage costs, a significant portion of the cultivars suitable for long-term storage are stored in uncontrolled conditions, which are called ordinary cold storages and do not have the possibility of mechanical cooling (Üstün, 2018).

The high fruit losses that occur during postharvest storage and the introduction of restrictive measures to control methods with plant growth regulators applications in order to reduce these losses have recently led researchers to natural applications. The fact that the toxic effect of the chemical substances used does not completely disappear in short-term stored products causes natural applications to be preferred as protection measures used to delay postharvest losses (Öz and Süfer, 2012). However, using safe practices to preserve fruit quality is an important activity to help prevent climate change (Kahramanoğlu, 2019). Recently, there has been a growing interest in using of natural compounds to maintain fruit quality and extend shelf life (Dursun, 2019). Therefore, alternative strategies are being developed to reduce losses due to postharvest spoilage that are perceived as safe by the consumer and pose no risk to human health and the environment (Wilson et al., 1997). In recent years, consumers have also become increasingly interested in consuming natural products (Pinheiro et al., 2012) and tend to buy fruits and vegetables free from diseases and defects and not treated with pesticides. However, importing countries also enforce strict import regulations on maximum residue limits on consumed portions of fruits and vegetables (Njombolwana et al., 2013). In recent years, essential oil treatments and calcium salts have been developed as alternatives to chemical methods to maintain the quality of fruits and vegetables, among which calcium chloride is the most widely used and successful. Essential oils, which have natural ingredients and are obtained from various plants, have recently been preferred against the disadvantages of chemicals after harvest, especially due to their antibacterial and antimicrobial properties. It has been reported that essential oil application is considered a safe practice for postharvest quality and decay control in fresh produce (Sivakumar and Bautista-Banos, 2014). Studies using postharvest calcium applications to extend the postharvest shelf life of fruits are also among the alternative practices (Poovaiah et al., 2003; Ranjbar et al., 2018; Gemeda, 2021). Depending on the salt type and calcium concentration, postharvest calcium immersion can significantly increase calcium content without causing fruit damage (Conway and Sam's, 2001; Hussain et al., 2012).

According to our research, no study investigated the effects of postharvest edible coating applications of rosehip essential oil (cold pressed) or calcium chloride dihydrate ($CaCl_2.2H_2O$) in combination with rosehip essential oil on the storage life and fruit quality of horticultural products. This study was conducted to investigate the effects of postharvest edible coating applications of $CaCl_2.2H_2O$, rosehip essential oil, and a combination of $CaCl_2.2H_2O$+rosehip essential oil treatments on the storage life and fruit quality of 'Golden Delicious', 'Starkrimson Delicious' and 'Granny Smith' apple cultivars throughout the the cold storage period of 6 months.

MATERIALS AND METHODS

The study was carried out at Isparta University of Applied Sciences, Faculty of Agriculture, Department of Horticulture Laboratories, Eğirdir Fruit Research Institute Postharvest Physiology Laboratory and Yuvalı Village Cold Storage in 2021. The study used apple fruit from three different cultivars: 'Starkrimson Delicious' ('S. Delicious'), 'Golden Delicious' ('G. Delicious'), and 'Granny Smith' ('G. Smith'). These cultivars were grafted onto a 15-year-old MM 106 rootstock and grown under conventional farming conditions at an altitude of 1276 meters in the Asaraltı locality of Yuvalı village, located in the Eğirdir district of Isparta province. The latitude of the orchard is 37.71, longitude 30.94, Asaraltı locality, island 188, plot 26 (Anonymous, 2023). In 2021, the average temperature was 12.3 °C and the average rainfall was 568.4 mm (Anonymous, 2022).

Healthy fruit was selected from the harvested fruit before the application by removing those damaged by any disease or pest, mechanically damaged and those with broken stems. Fruit was washed with tap water to remove dust and dirt. The washed fruit was kept in a cool, shady place for an hour to drain and dry (Shehata et al., 2020). The fruit of each apple cultivar were

divided into four groups; Group 1: Control group and the fruit was immersed in distilled water for 5 minutes; Group 2: The fruit was treated with $CaCl_2.2H_2O$ (Tekkim Extra pure, Food quality) at a dose of 1.5% (Saftner and Conway, 1998; Chardonnet et al., 2003; Trentham et al., 2008; Hussain et al., 2012; Gago et al., 2016) for 5 minutes; Group 3: Rosehip essential oil (REO) coating was prepared by dissolving REO (Botalife, cold-pressed 100% pure Manolya Natural Aromatic Products Food Industry and Trade Co.Ltd.) in 0.5% Tween 80 and immersing it for 5 minutes in 2% final solution concentration obtained by adding distilled water (Paladines et al., 2014; Martínez-Romero et al., 2017; Martínez-Romero et al., 2019); Group 4: $CaCl_2.2H_2O$+REO combined application, the fruit was immersed in the $CaCl_2.2H_2O$ solution prepared as above at a dose of 1.5% for 5 minutes, (Saftner and Conway, 1998; Chardonnet et al., 2003; Trentham et al., 2008; Hussain et al., 2012; Gago et al., 2016), after the dipping treatment the excess solution was drained and then fruit was immersed in the REO solution prepared at a dose of 2% as described above for 5 minutes, (Paladines et al., 2014; Martínez-Romero et al., 2017; Martínez-Romero et al., 2019).

In the experiment, the treatments were performed in each apple cultivar in three replicates with 15 fruit in each replicate. The fruit was not subjected to pre-cooling. After the treatments, the excess solution of the fruit was drained in a cool and shady place (Shehata et al., 2020) and placed in plastic perforated 20 kg capacity crates separately for each cultivar and treatment. Apples were placed in the normal atmosphere (NA) commercial cold storage at 0 °C temperature and 90-95% relative humidity.

Weight loss

The fruit was labelled, weighed, and placed in storage for weight loss analyzis. The analyses described below were performed as beginning analyses prior to cold storage and periodically throughout the storage period on fruit samples removed from storage conditions. Weight loss (WL) was calculated by weighing the same fruit samples labelled and prepared before storage according to the equation presented below:

WL: (A1-A2/A1)x100 (A1: Initial weight, A2: Period weight) and given as %.

Fruit flesh firmness

Fruit flesh firmness (FFF) measured by removing the fruit peel with the help of Fruit Peeler-Italy in equal thickness and measured with the 11.1 mm probe of a digital penetrometer (Labor Teknik) and given in Newton (N).

Soluble solids content

Soluble solids content (SSC) was measured with a digital refractometer (Atago Pocket PAL-1 Japan) in blended and filtered juice and given in Brix° (Cemeroğlu, 1992).

Titratable acidity and pH

Titratable acidity (TA) was measured using a Hanna instruments HI221 model digital pH meter with a probe immersed in 10 ml of juice and titrated with 0.1 N NaOH using a Titrette model digital burette and titrated according to the formula A = S x N x F x E x 100/C (A: the amount of acid (mg malic acid/100mL), S: the amount of sodium hydroxide used (mL), N: normality of sodium hydroxide used, F: factor of sodium hydroxide used, C: the amount of sample taken (mL), E: equivalent value of the respective acid (malic acid)) (Karaçalı, 2002). pH was measured using a Hanna instruments HI221 model digital pH meter with a probe immersed in 10 mL of fruit juice.

Fruit color and fruit flesh color

For fruit color analyses, the fruit was labelled, color measured (Minolta CR-300 Model Japan) and placed in storage. Fruit color analyzes were performed on the same apple samples during storage. The analyses were performed as beginning analyses prior to storage and throughout storage on fruit samples removed from storage conditions periodically. Fruit flesh color analyzes were performed on the fruit samples at harvest date and during the cold storage removed from cold storage at monthly intervals.

Fruit color (FC) and fruit flesh color (FFC) were read according to CIE L^*, a^*, b^* values and evaluated as L^*, a^*, b^*, hue angle (h°=arctanb/a) and chroma ($C^*=(a^2+b^2)^{1/2}$).

Respiration rate and ethylene production

Respiration rate and ethylene production were measured simultaneously in a single gas sample from each jar. Measurements were made in S/SL inlet split mode with a gas sampling valve using a fused silica capillary column (GS-GASPRO, 30 m x 0.32 mm I.D.) in 1 a mL gas sample. Agilent brand GC-7890A model gas chromatography with a thermal conductivity detector (TCD) for respiration rate measurement, a flame ionization detector (FID) for ethylene production, and loaded into a computer to which it is connected. It was made using the Chemstation REV. B. 04.03 (16) package program. The carrier gas flow is 1.7 mL/min in constant flow mode. The temperatures of the furnace, TCD and FID detectors are 40 (isothermal), 250°C and 250°C, respectively. Gas flows for high purity hydrogen (H_2) and dry air used as carrier gas in FID are 30 and 300 mL/min, respectively. High purity helium (He) (makeup) and reference flow rates used as carrier gas in TCD are 7.0 and 20 mL/min, respectively. 0.5 kg of fruit was placed in 2 L jars and kept at 20^{0}C for 24 hours. Then, 10 mL of air was taken from the jars and reading was done in gas chromatography. The readings were evaluated according to Anonymous (2020) for respiration rate and according to Dixon and Hewett (2001) for ethylene production. Ethylene production is given in µL.C_2H_4/kg.h, and respiration rate in mL.CO_2/kg.h.

Sensory evaluations

In sensory evaluations, overall quality was evaluated on a scale of 1-9 (1≤4: unmarketable, 5: marketable, 6-8: good, 9: very good) (Dilmaçünal, 2009), the odor was evaluated on a scale of 0-5 (0: none, 1: very little, 2: little, 3: medium, 4: much, 5: very much) (Peña et al., 2013), taste and aroma was evaluated on a scale of 1-5 (1:very bad, 2: bad, 3: medium, 4: good, 5: very good) (Dilmaçünal, 2009), decay as % of fruit with rot in each analysis period (Yılmaz, 2019; Şener et al., 2022).

Superficial scald and superficial scald severity

Superficial scald (SS) as % of fruit with SS in each analysis period, and superficial scald severity (SSS) according to Mditshwa et al. (2018) (0: no superficial scald, 1: 1-25% very slight, 2: 26-50% slight, 3: 51-75% moderate, 4: 76-100% severe).

Statistical evaluation

In the study, the data obtained in terms of soluble solids content, pH, titratable acidity, fruit flesh firmness, and respiration rate were analyzed by using the variance analysis technique in factorial. In the experiment, there are three levels of variety factor, four levels of treatment factor and seven levels of month factor. The number of observations in subgroups was three. Tukey test, one of the multiple comparison methods, was used to determine the differences between factor level means.

It was determined that the data obtained in terms of ethylene production amount characteristic did not meet the prerequisites of parametric tests such as normality and homogeneity of variances as a result of Anderson Darling (Normality) and Levene (Homogeneity) Test respectively. Therefore, the Kruskal-Wallis test was used to determine whether the differences between the level medians of the factors were statistically significant. Dunn-Bonferroni test, one of the nonparametric multiple comparison methods, was used to determine the differences between the medians. Repeated measures analysis of variance technique in factorial order was applied to the data obtained in terms of fruit and flesh color (L^*, a^*, b^*) and weight loss characteristics. In the study, there were three levels of variety factor, four levels of treatment factor and six levels of time factor. Initial values of the time factor were included in the analysis as covariates. Repeated measurements were carried out at the levels of the time factor. Tukey test, one of the multiple comparison methods, was used to determine the differences between factor level means. Since the data obtained in terms of overall quality, taste- aroma, odor and decay characteristics did not meet the prerequisites for parametric tests, the differences between the level medians of any of the factors were compared separately in each combination of the remaining two. Statistical significance was determined by the Kruskal-Wallis test.

RESULTS AND DISCUSSION

Weight loss (WL)

In general, fruit weight loss is normally attributed to fruit senescence or water loss and is also used as a quality index for the postharvest life of fruit (Sati and Qubbaj, 2021). Through transpiration, WL is one of the major causes of quality deterioration in fresh horticultural crops after harvest. It causes not only direct quantitative losses, but also losses in appearance, textural quality (loss of softness, crispness and juiciness) and nutritional quality. If the WL is more than 10%, the fruit surface becomes prone to quality defects such as wilting and shriveling and the product becomes unmarketable (Hussain et al., 2012). In general, the WL was higher in the control group compared to the treated fruit during storage. According to the storage period, the highest (4.13%) WL was obtained in the 'S. Delicious' in the 6th month. The WL increased in all treatments with the extension of the storage period. In 'S. Delicious' the lowest WL (2.59%) obtained from $CaCl_2.2H_2O$+REO at the end of the storage (Table 1).

Chardonnet et al. (2003) reported that $CaCl_2$ infiltration provides an increase in both total and cell wall-bound Ca in apple tissue during storage and reaches its maximum with $CaCl_2$ application in fruit stored for 4 or 6 months, thus protecting the cell wall. In 'G. Delicious' the lowest WL (0.80%) was in REO at the end of the storage. In 'G. Smith' $CaCl_2.2H_2O$ resulted in lower fruit weight loss than other applications during storage (Table 1), and was recorded as the most successful treatment in limiting weight loss in this cultivar. It is concluded that the reason for the obtained results could be related to the application of REO, which creates a modified atmosphere around the apple fruit as well as $CaCl_2.2H_2O$ protecting the cell integrity of the fruit. Although it varies depending on the cultivar, weight losses of more than 5% may cause noticeable wrinkling in apples during marketing. The 'G. Smith' can be counted among the cultivars in which the least wrinkling was observed due to its peel structure. Previous studies have also reported that essential oil and Ca applications limit weight loss in fruit during storage. This effect is known to be successful in fruit by directly preserving both the physiological metabolism associated with ripening and the tissue firmness of the fruit (Shirzadeh et al., 2011; Paladines et al., 2014; Gago et al., 2016; Martínez-Romero et al., 2017; El-Dengawy et al., 2018; AL-Saikhan, 2018; Mahmoud et al., 2019; Martínez-Romero et al., 2019; Gemeda, 2021; Mazumder et al., 2021; Sati and Qubbaj, 2021; Singh et al., 2022). Saftner and Conway (1998) investigated the effects of postharvest $CaCl_2$ applications on the firmness-water relations, cell wall calcium levels, and postharvest life of the apple. It was reported that $CaCl_2$ had positive effects on minimizing salt-related damage to the fruit of the 'G. Delicious' apple cultivar and preserving fruit-water relationships and postharvest life.

Table 1. Effect of postharvest $CaCl_2.2H_2O$, REO, and $CaCl_2.2H_2O$+REO applications on the weight loss (%) of fruit during storage in 'S. Delicious', 'G. Delicious' and 'G. Smith' apple cultivars

Cultivar	Application	Storage period (months)					
		1	2	3	4	5	6
'S. Delicious'	$CaCl_2.2H_2O$**	c0.68Cc*	a1.54BCb	a1.60BCb	a2.20Bd	a3.40Ab	a3.80Ab
	REO***	b1.39Ca	b1.20Cc	a1.40Cc	a2.63Bb	a3.20ABc	a4.03Aa
	$CaCl_2.2H_2O$+REO	b1.40Ba	b1.43Bb	b1.40Bc	b2.40ABc	b2.40ABd	b2.59Ac
	Control	a1.13Cb	a2.55Ba	a2.44Ba	a3.60Aa	a3.79Aa	a4.13Aa
'G. Delicious'	$CaCl_2.2H_2O$	a1.23Ac	c0.90Aab	c0.97Aab	b0.77Ac	c0.97Ab	c1.04Ab
	REO	a2.06Aa	c1.00Ba	b0.93Bb	c1.13ABb	c0.93Bb	c0.80Bc
	$CaCl_2.2H_2O$+REO	a1.60Ab	c0.80Ab	c1.08Aa	c0.76Ac	c1.30Aa	c1.33Aa
	Control	b0.67Bd	c0.87Bab	c0.74Bc	b2.81Aa	c0.87Bb	c1.31Ba
'G. Smith'	$CaCl_2.2H_2O$	b1.03Cc	b1.23BCd	b1.20BCc	a2.20ABb	b2.20ABc	b2.40Ab
	REO	a2.13ABa	a1.60Bb	a1.41Bb	b2.20ABb	b2.80Aa	b3.00Aa
	$CaCl_2.2H_2O$+REO	a1.56Bb	a2.00ABa	a1.60Ba	a2.60ABa	a2.80Aa	a3.01Aa
	Control	b0.60Cd	b1.40ABc	b1.60ABa	c2.00Ac	b2.40Ab	b2.40Ab

*Uppercase letters on the right side of the numbers indicate the difference between each cultivar x treatment combination for storage period; right lowercase letters indicate the difference between each cultivar x storage period combination for treatments; left lowercase letters indicate the difference between each treatment x storage period combination for cultivars. **$CaCl_2.2H_2O$: Calcium chloride dihydrate, *** REO: Rosehip essential oil

Fruit flesh firmness (FFF)

Fruit texture is one of the important quality characteristics of horticultural crops (Sati and Qubaj, 2021). Fruit freshness is generally reported to be directly proportional to the firmness value (Amin, 2016) and firmness is a critical factor affecting customers' decision to purchase apple fruit (Amin, 2016; Singh et al., 2022). In 'S. Delicious' and 'G. Delicious' cultivars, the highest FFF (31.14 N and 26.69 N) was obtained from REO. In the 'G. Smith', the $CaCl_2.2H_2O$ had a slightly higher FFF than the others. In general, REO maintained FFF better than the other treatments, followed by $CaCl_2.2H_2O$. It is concluded that the reason for the obtained results could be related to the effect of REO acting like a modified atmosphere around the apple fruit as well as the protection attribute of the $CaCl_2.2H_2O$ on the cell integrity of the fruit (Table 2). Chardonnet et al. (2003) reported that $CaCl_2$ infiltration provides an increase in both total and cell wall-bound Ca in apple tissue during storage and reaches its maximum with $CaCl_2$ application in fruit stored for 4 or 6 months, thus protecting the cell wall. Similar to our findings, Shirzadeh et al. (2011), Salem and Moussa (2014), Gago et al. (2016), and Gemeda (2021) reported positive effects of postharvest $CaCl_2$ applications on apple fruit.

Positive effects were reported in some previous research such as Serrano et al. (2005) in postharvest treatments of eugenol, thymol, menthol and eucalyptol, Shirzadeh and Kazemi (2012) in essential oil treatments of thyme and lavender, Paladines et al. (2014) in REO combined with *Aloe vera* gel on peach, nectarine, plum and sweet cherry, Öztürk et al. (2018) in *Aloe vera* treatments in 'Piraziz' apple cultivar, Amin (2016) in essential oil treatments on apple cv. of 'Anna', Martínez-Romero et al. (2017) in REO combined with *Aloe vera* or *Aloe arborescens* gels in plum fruit, Mahmoud et al. (2019) in essential oil applications in apple cv. of 'Anna'. Similarly, Martínez-Romero et al. (2019) reported higher firmness values in REO coated plum fruit during storage at room conditions. Singh et al. (2022) reported that applying REO combined with *Aloe vera* gel in the form of edible coating in pomegranate arils had higher firmness values than those without treatment.

The study findings displayed a correlation between fruit flesh firmness and weight loss. In particular, apples with firmer textures experienced lower weight loss rates. Wei et al. (2010) reported that enzymes affecting cell wall structure in apples play an important role in fruit softening, and found β-Gal and α-L-Af more effective than polygalacturonase and pectin methyl esterase on the storage period and quality of apples, especially when ripening and softening begins. Likewise, Shirzadeh et al. (2011) found successful postharvest Ca applications to prevent fruit softening and reduce weight loss. In this study, the FFF decreased in all treatments with increasing storage period (Table 2). Similarly, Shirzadeh et al. (2011), Hussain et al. (2012), Paladines et al. (2014), Salem and Moussa (2014), Amin (2016), Gago et al. (2016), Martínez-Romero et al. (2017), AL-Saikhan (2018), Mahmoud et al. (2019), Martínez-Romero et al. (2019), Gemeda (2021), Mazumder et al. (2021), Sati and Qubbaj (2021) and Singh et al. (2022) reported a decreasing in FFF during storage in different fruit cultivars treated with $CaCl_2$ and essential oils.

Titratable acidity (TA) and pH

TA is one of the most important parameters in evaluating fruit quality during storage, and low TA indicates accelerated senescence (Sati and Qubbaj, 2021). In the 'S. Delicious' the treatment with the lowest TA loss

Table 2. Effect of postharvest $CaCl_2.2H_2O$, REO, and $CaCl_2.2H_2O$+REO applications on the fruit flesh firmness (N) of fruit during storage in 'S. Delicious', 'G. Delicious' and 'G. Smith' apple cultivars

Cultivar	Storage period (months)							
	Application	0	1	2	3	4	5	6
'S. Delicious'	$CaCl_2.2H_2O$**	a84.52Aa*	B71.17Bb	A75.62ABa	B66.72BCa	b57.83Ca	c26.69Da	Ab26.69Dab
	REO***	A75.62Aa	B71.17Aa	B66.72Aab	Ab71.17Aab	b66.72Aa	B26.69Ba	A31.14Ba
	$CaCl_2.2H_2O$+REO	A80.07Aa	B75.62ABab	B66.72Cb	b66.72BCab	b66.72Ca	a31.14Da	b22.24Db
	Control	A80.07Aa	A75.62Ab	B62.28Bb	b57.83Bb	b57.83Ba	a35.59Ca	A26.69Cab
'G. Delicious'	$CaCl_2.2H_2O$	B57.83BCb	B75.62Aa	B62.28Ba	c53.38CDb	c44.48Dc	A48.93CDa	B17.79Ea
	REO	A80.07Aa	C62.28Cbc	B66.72BCa	b62.28BCa	ab75.62ABa	B26.69Db	B26.69Da
	$CaCl_2.2H_2O$+REO	B71.17Aa	C62.28Ac	C48.93Bb	c40.03Bc	c44.48Cc	B17.79Cb	B17.79Ca
	Control	A75.62Aa	A71.17ABab	B62.28Ca	b57.83Cab	b62.28BCb	B22.24Db	A22.24Da
'G. Smith'	$CaCl_2.2H_2O$	A84.52Ba	A97.86Aa	A80.07Ba	a84.52Ba	a80.07Ba	B40.03Ca	A31.14Ca
	REO	A80.07Ba	A97.86Aa	A75.62Ba	a80.07Ba	a80.07Ba	A35.59Ca	A31.14Ca
	$CaCl_2.2H_2O$+REO	A84.52ABa	A84.52Ab	A75.62Ba	a84.52ABa	a88.96Aa	a35.59Ca	a31.14Ca
	Control	A75.62Ba	A75.62Bc	A80.07ABa	a81.76ABa	a19.8384.52Aa	A35.59Ca	a31.14Ca

*Uppercase letters on the right side of the numbers indicate the difference between each cultivar x treatment combination for storage period; right lowercase letters indicate the difference between each cultivar x storage period combination for treatments; left lowercase letters indicate the difference between each treatment x storage period combination for cultivars. **$CaCl_2.2H_2O$: Calcium chloride dihydrate, *** REO: Rosehip essential oil

(9.4%) during storage was $CaCl_2.2H_2O$, followed by REO (12.1%) at the end of the storage. In the 'G. Delicious' the treatment with the lowest TA loss was REO (21.3%) at the end of the storage. In the 'G. Smith' TA increased in all treatments during storage and the highest value was in REO followed by $CaCl_2.2H_2O$+REO (Table 3). Consumers prefer apples that meet certain quality criteria, including maintaining fruit titratable acidity. Treatments have proven effective in achieving this, likely through a decrease in fruit respiration rate. This decrease leads to less organic acid consumption, thereby preserving fruit acidity during storage better than untreated samples. According to Reyes-Medina et al. (2017), applying $CaCl_2$ can delay ripening and prevent fruit from spoiling. According to Fidler (1973), storage of most apple cultivars at relatively high CO_2 concentrations combined with low oxygen delays TA loss (Argenta et al., 2000). It is thought that REO acted like an modified atmosphere surrounding the fruit and maintained the TA thanks to slowing down the metabolic process. Similar findings reported in previous researches of Rabiei et al. (2011), Shirzadeh et al. (2011), Hussain et al. (2012), Paladines et al. (2014), Salem and Moussa (2014), Amin (2016), Gago et al. (2016), Martínez-Romero et al. (2017), AL- Saikhan (2018), Martínez-Romero et al. (2019), Hussain et al. (2019), Gemeda (2021), Mazumder et al. (2021), Sati and Qubbaj (2021) and Singh et al. (2022).

The pH contents of 'S. Delicious' and 'G. Delicious' increased during storage compared to the initial values. At the end of storage, the highest (4.07) pH was recorded in REO, and the treatment with the highest pH increase (8.82%) at the end of storage compared to the initial values was REO, followed by $CaCl_2.2H_2O$+REO (7.30%). At the end of storage, the highest pH content (3.97) was recorded in REO, and the treatment with the highest pH increase (7.87%) at the end of storage compared to the initial values was control. In 'G. Smith' cultivar, pH contents increased in $CaCl_2.2H_2O$ during storage compared to the initial values, while there was a slight decrease in the other treatments, and the highest (3.36) pH content at the end of storage was recorded in the control. REO was more effective in 'S. Delicious' and 'G. Delicious', in maintaining the pH content of the fruit, while in 'G. Smith', $CaCl_2.2H_2O$ had a higher pH than the initial values of other treatments. However, the effects of treatments on the pH of cultivars were not statistically significant during storage (Table 3). Shirzadeh et al. (2011) and Tamalea et al. (2021) reported that postharvest $CaCl_2$ application did not have any effect on the pH of fruit. Singh et al. (2022) reported that pomegranate arils treated with REO had higher pH content than those treated with *Aloe vera* gel.

Soluble solids content (SSC)

The soluble solids content (SSC) of fruit is a good index for determining fruit quality and ripeness, and SSC increases with ripeness (Sati and Qubbaj, 2021). The increase in SSC is attributed to the enzymatic conversion of higher polysaccharides such as starches and pectins to simple sugars during ripening (Gemeda, 2021). The SSC increased in the 'S. Delicious' cultivar compared to the initial values, with the highest increase (51.44%) in the control group. In 'G. Delicious', an increase was recorded compared to the initial values except for $CaCl_2.2H_2O$+REO, and the highest (12.19 Brix°) SSC was recorded in the control at the end of the storage. In the 'G. Smith', SSC increased during storage compared to the initial value (Table 4).

Similar to the findings obtained in this study in 'S. Delicious' and 'G. Delicious', Hussain et al. (2012) reported that postharvest $CaCl_2$ treatment had a lower SSC than

Table 3. Effect of postharvest $CaCl_2.2H_2O$, REO, and $CaCl_2.2H_2O$+REO applications on the titratable acidity (mg malic acid/100 mL) and pH of fruit during storage in 'S. Delicious', 'G. Delicious' and 'G. Smith' apple cultivars

Cultivar	TA - Storage period (months)							
	Application	0	1	2	3	4	5	6
'S. Delicious'	$CaCl_2.2H_2O$**	c0.317Ba*	c0.440Aef	c0.301Ba	c0.321Ba	c0.324Bb	c0.272Ba	c0.291Ba
	REO***	c0.325ABa	c0.379Aa	c0.262BCa	b0.293BCa	c0.322ABab	c0.248Ca	c0.290BCa
	$CaCl_2.2H_2O$+REO	c0.327BCa	c0.403Aa	c0.295BCa	c0.301BCa	b0.352ABa	c0.266Ca	b0.273Ca
	Control	c0.353Aa	c0.356Aab	c0.266Ba	b0.294ABa	c0.347Aa	c0.233Ba	b0.270Ba
'G. Delicious'	$CaCl_2.2H_2O$	b0.686Aa	b0.633Aa	b0.418BCa	b0.415BCa	b0.470Ba	b0.356Cb	b0.388Cb
	REO	b0.612Ab	b0.519Bb	b0.426Ca	b0.325Db	b0.474BCa	b0.414Ca	b0.478BCa
	$CaCl_2.2H_2O$+REO	b0.613Ab	b0.506Bb	b0.407Ca	b0.391CDa	b0.341Dc	b0.374CDab	b0.314Dc
	Control	Bb0.576Ab	b0.643Aa	b0.336Cb	b0.325Cb	b0.411Bb	b0.404Bab	b0.321Cc
'G. Smith	$CaCl_2.2H_2O$	a0.104Ac	a0.852Ca	a0.909BCb	a0.935Ba	a0.986Ba	a0.709Db	a0.623Ec
	REO	a0.125A	a0.884Ca	a0.951Bb	a0.845CDb	a0.790DEb	a0.744EFab	a0.709Fa
	$CaCl_2.2H_2O$+REO	a0.143Aa	a0.884Ca	a0.103Ba	a0.908Ca	a0.906Ca	a0.801Da	a0.692Eab
	Control	a0.121A	a0.789Cb	a0.894Bb	a0.931Ba	a0.751CDb	a0.704DEb	a0.645Ebc
Cultivar	**pH - Storage period (months)**							
	Application	**0**	**1**	**2**	**3**	**4**	**5**	**6**
'S. Delicious'	$CaCl_2.2H_2O$**	B3.75Ba*	a4.01ABa	a3.90ABa	a4.02ABa	b4.07ABa	a4.15Aa	a3.94ABa
	REO***	a3.74Ba	a4.10Aa	a3.94ABa	a4.03ABa	a4.20Aa	a4.10Aa	a4.07ABa
	$CaCl_2.2H_2O$+REO	a3.70Ba	a4.14Aa	a4.11Aa	a4.02ABa	a4.11Aa	a4.05Aa	a3.97ABa
	Control	a3.77Ba	a4.20Aa	a4.09ABa	A3.94ABa	a4.05ABa	a4.25Aa	a3.94ABa
'G. Deliicous'	$CaCl_2.2H_2O$	a3.77Ba	a4.20Aa	a4.09ABa	a3.94ABa	a4.05ABa	a4.25Aa	a3.94ABa
	REO	a3.70Ba	a4.14Aa	a4.11Aa	a4.02ABa	a4.11Aa	a4.05Aa	a3.97ABa
	$CaCl_2.2H_2O$+REO	b3.75Ba	a4.01ABa	a3.90ABa	a4.02ABa	b4.07ABa	a4.15Aa	a3.94ABa
	Control	ab3.56BCb	ab3.71ABa	b3.81ABa	a4.03ABa	a3.90ABa	b3.28Cb	a3.84ABa
'G. Smith'	$CaCl_2.2H_2O$	b3.27Ba	b3.26Ba	b3.35Ba	a3.36Aa	b3.25Ba	c3.24Ba	b3.34Aa
	REO	c3.37Aa	b3.28Aa	c3.42Aa	c3.41Aa	b3.27Aa	c3.29Aa	c3.14Ba
	$CaCl_2.2H_2O$+REO	C3.34ABa	c3.25Ba	b3.38ABa	b3.66Aa	b3.16Ba	b3.31Ba	c3.27Ba
	Control	b3.45Aa	c3.29Aa	b3.42Aa	b3.43Aa	b3.18Aa	b3.14Aa	b3.36Aa

*Uppercase letters on the right side of the numbers indicate the difference between each cultivar x treatment combination for storage period, right lowercase letters indicate the difference between each cultivar x storage period combination for treatments, left lowercase letters indicate the difference between each treatment x storage period combination for cultivars. **$CaCl_2.2H_2O$: Calcium chloride dihydrate, ***REO: Rosehip essential oil

the control in the 'Red Delicious' cultivar. Similarly, Sati and Qubbaj (2021) reported that $CaCl_2$, gum arabic+$CaCl_2$ and cactus mucilage+$CaCl_2$ treatments effectively maintained the SSC of tomato fruit during storage. Shirzadeh et al. (2011) reported that postharvest $CaCl_2$ treatment preserved SSC in the 'Jonagold' cultivar. Gago et al. (2016) found effective the $CaCl_2$ in preserving SSC in 'G. Delicious'. Salem and Moussa (2014) reported an insignificant effect of postharvest $CaCl_2$ treatment on SSC in the apple cv. of 'Anna'. Gemeda (2021) reported a higher SSC in postharvest $CaCl_2$ treatments compared to the control similar to the findings obtained in this study in 'G. Smith'. Hussain et al. (2019) reported a higher SSC in postharvest $CaCl_2$ treated pear fruit than in the control. Similar to the 'S. Delicious' cultivar in this study, Amin (2016) reported a higher SSC in the essential oil treated 'Anna' cultivar compared to the control.

Similar to the 'G. Delicious' in this study, Martínez-Romero et al. (2019) reported higher values of ripeness index in plums in the postharvest REO treatment compared to the control. Paladines et al. (2014) reported a higher ripeness index in the control group of peach, nectarine, plum and sweet cherry compared to postharvest treatment of *Aloe vera* gel+REO. Singh et al. (2022) found successful the REO+*Aloe vera* gel treatment in the preservation of SSC better than control in the pomegranate arils. With extending storage period, an increase was recorded in the SSC of the fruit in all treatments. Similarly, Shirzadeh et al. (2011), Hussain et al. (2012), Paladines et al. (2014), Amin (2016), Gago et al. (2016), Martínez-Romero et al. (2017), AL-Saikhan (2018), Martínez-Romero et al. (2019), Hussain et al. (2019), Gemeda (2021), Sati and Qubbaj (2021) and Singh et al. (2022) reported an increase in SSC during storage.

Respiration rate (RR)

Respiration metabolism is known to be a normal process in the postharvest life of fruit, mainly consuming

Table 4. The effect of postharvest $CaCl_2.2H_2O$, REO, and $CaCl_2.2H_2O$+REO applications on the soluble solids content (Brix°) of fruit during storage in 'S. Delicious', 'G. Delicious' and 'G. Smith' apple cultivars

Cultivar	Storage period (months)							
	Application	0	1	2	3	4	5	6
'S. Delicious'	$CaCl_2.2H_2O$**	b8.63Db*	a12.20Aa	a11.93Aba	a12.00ABa	b12.10Aab	b11.03BCc	b10.76Cb
	REO***	a10.30CDa	b10.03Dc	a11.20BCa	a11.10BCb	a12.53Aa	a12.26Aab	a12.00ABa
	$CaCl_2.2H_2O$+REO	b10.36Ea	a10.70DEbc	a11.93ABCa	a11.43CDab	a12.50ABa	a12.86Aa	a11.73BCa
	Control	c7.66Bc	b10.83Ab	b11.83Aa	b11.43Aab	b11.70Ab	a11.70Abc	a11.60Aa
'G. Delicious'	$CaCl_2.2H_2O$	a9.56Db	b11.40BCb	b10.30Db	b10.53CDb	a13.06Aa	a11.80Ba	a11.63Ba
	REO	ab10.00Bb	a10.76ABb	b8.39Cc	a10.60ABb	b10.96ABb	ab11.60Aa	ab11.53Aa
	$CaCl_2.2H_2O$+REO	a11.66Aa	a10.96ABb	b10.53Bb	a11.26ABb	b10.50Bb	c10.43Bb	b10.80ABb
	Control	a11.13Ea	a13.40ABa	a13.90Aa	a13.20ABC	a12.83BCa	a11.80DEa	a12.19CDa
'G. Smith'	$CaCl_2.2H_2O$	c7.33Cb	b11.43ABa	a11.60Aa	a11.33ABa	c10.76ABa	b10.46Bb	a11.60Aa
	REO	b9.53Ba	a10.83Aab	a11.03Aab	a11.06Aa	b11.43Aa	b11.40Aa	b11.16Aa
	$CaCl_2.2H_2O$+REO	c9.63Ba	a10.43ABbc	b10.60ABb	a11.23Aa	b10.73Aab	b11.30Aa	ab11.06Aa
	Control	b9.39Ca	c10.00BCc	b11.23Aab	b11.23Aa	c10.50ABb	b10.36ABC	b10.09BCb

*Uppercase letters on the right side of the numbers indicate the difference between each cultivar x treatment combination for storage period, right lowercase letters indicate the difference between each cultivar x storage period combination for treatments, left lowercase letters indicate the difference between each treatment x storage period combination for cultivars. **$CaCl_2.2H_2O$: Calcium chloride dihydrate, *** REO: Rosehip essential oil

carbohydrates, and organic acids, leading to weight loss and quality deterioration. Respiration metabolism is also accompanied by ethylene production and causes fruit senescence. This means that a lower RR and ethylene production may play an important role in better preservation of fruit quality (Fan et al., 2022). At the end of storage, the lowest RR (43.02 μLCO_2/kg.h) was in REO and the highest RR (50.56 μLCO_2/kg.h) was recorded in the control group in 'S. Delicious'. The lowest RR, at the end of storage, in 'G. Delicious' was in $CaCl_2.2H_2O$+REO (35.27 μLCO_2/kg.h), followed by REO with 39.16 μLCO_2 / kg.h. In 'G. Smith', the lowest RR at the end of storage was in the control with 58.407 μLCO_2/kg.h, followed by REO with 58.501 μLCO_2/kg.h. In general, it can be said that REO and $CaCl_2.2H_2O$+REO were more successful treatments in suppressing the RR of the fruit. Hussain et al. (2012), Gemeda (2021) and Mazumder et al. (2021) reported a positive effect of postharvest $CaCl_2$ applications on the suppression of RR during storage. According to Paladines et al. (2014), Martínez-Romero et al. (2017) and Singh et al. (2022), REO+*Aloe vera* gel had lower RR than the control. Similarly, Martínez- Romero et al. (2019) reported that REO exhibited lower RR than the control. Similar to Mazumder et al. (2021), it was observed in this study that the RRs of fruit increased in all treatments with an extended storage period (Table 5).

Ethylene production (EP)

Ethylene is considered as the main ripening stimulant synthesized during the ripening stage of fruit under growing or storage conditions (Mazumder et al., 2021). EP increased during storage in the 'S. Delicious' cultivar. At the end of storage, the lowest EP was recorded in REO with 11.473 μLC_2H_4/kg.h and the highest EP was in the control group with 44.331 μLC_2H_4/kg.h. In the 'S. Delicious', the EP of the fruit treated with REO was significantly lower than the other treatments, therefore, it can be said that REO was quite successful in suppressing EP for this apple cultivar compared to other treatments. 'G. Delicious' showed an increase in EP at the end of storage. The lowest EP was obtained in REO with 3.156 μLC_2H_4/kg.h, followed by $CaCl_2.2H_2O$+REO with 24.282 μLC_2H_4/kg.h at the end of storage. As in the 'S. Delicious', it was noted that the EP of REO-treated fruit was significantly lower than the other treatments in the 'G. Delicious'. Therefore, it can be said that for the 'G. Delicious', REO successfully suppressed the EP of the fruit compared to the other treatments. In 'G. Smith', EP generally increased during storage. At the end of storage, the lowest EP was recorded in REO with 0.442 μLC_2H_4/kg.h, followed by $CaCl_2.2H_2O$ with 2.716 μLC_2H_4/kg.h. At the end of storage, the highest EP was recorded in the control group with 4.300 μLC_2H_4/kg.h. As in the case of 'S. Delicious' and 'G. Delicious', it was noted that the EP of the REO-treated fruit was significantly lower than the other treatments in the 'G. Smith'. Therefore, it can be said that for 'G. Smith' as well, REO was quite successful in suppressing the EP of the fruit compared to the other treatments. REO was the most effective treatment in suppressing EP in all cultivars, followed by $CaCl_2.2H_2O$+REO at the end of storage (Table 6).

Shirzadeh et al. (2011) and Mazumder et al. (2021) found successful postharvest application of $CaCl_2$ in effectively reducing EP during storage. Rabiei et al. (2011) reported that postharvest application of thyme and lavender essential oils had positive effects on the suppression of EP during storage. Shirzadeh and Kazemi (2012) reported that postharvest application of thyme essential oil had positive effects on the suppression of EP during storage, while postharvest application of Ca was found

Table 5. Effect of postharvest $CaCl_2.2H_2O$, REO, and $CaCl_2.2H_2O$+REO applications on respiration rate (μLCO_2/kg.h) of fruit during storage in 'S. Delicious', 'G. Delicious' and 'G. Smith' apple cultivars

Cultivar	Storage period (months)			
	Application	1	3	6
'S. Delicious'	$CaCl_2.2H_2O$**	a50.48Ab*	a33.37Ba	b47.82Aa
	REO***	a49.76Ab	a24.76Ba	b43.02Aa
	$CaCl_2.2H_2O$+REO	a43.90Ab	a30.34Ba	b48.57Aa
	Control	a67.32Aa	b30.87Ca	ab50.56Ba
'G. Delicious'	$CaCl_2.2H_2O$	b6.01Cc	a26.24Bb	a62.50Aa
	REO	a50.74Aa	a29.77Bb	b39.16Ab
	$CaCl_2.2H_2O$+REO	a37.24Ab	a27.12Bb	b35.27Bb
	Control	c8.52Bc	a40.96Aa	b42.08Ab
'G. Smith'	$CaCl_2.2H_2O$	a45.07Bab	a27.67Ca	a61.77Ab
	REO	a52.89Aab	a22.53Ba	a58.50Ab
	$CaCl_2.2H_2O$+REO	a43.32Bb	a23.51Ca	a77.89Aa
	Control	b55.34Aa	b26.48Ba	a58.41Ab

*Capital letters on the right side of the numbers denote the difference between each cultivar x treatment combination in terms of storage time, right lower case letters denote the difference between each cultivar x storage time combination in terms of treatment, left lower case letters denote the difference between each cultivar x storage time combination in terms of the cultivar. **$CaCl_2.2H_2O$: Calcium chloride dihydrate, ***REO: Rosehip essential oil

Table 6. Effect of postharvest $CaCl_2.2H_2O$, REO, and $CaCl_2.2H_2O$+REO applications on ethylene production (μLC_2H_4/kg.h) of fruit during storage in 'S. Delicious', 'G. Delicious' and 'G. Smith' apple cultivars

Cultivar	Storage period (months)			
	Application	1	3	6
'S. Delicious'	$CaCl_2.2H_2O$**	5.537	16.499	27.777
	REO***	5.285	3.557	11.473
	$CaCl_2.2H_2O$+REO	0.014	15.117	32.345
	Control	12.923	16.076	44.331
'G. Delicious'	$CaCl_2.2H_2O$	25.405	19.280	33.601
	REO	0.013	4.534	3.156
	$CaCl_2.2H_2O$+REO	15.303	22.378	24.282
	Control	25.118	31.177	28.059
'G. Smith'	$CaCl_2.2H_2O$	0.017	4.948	2.716
	REO	0.012	1.167	0.442
	$CaCl_2.2H_2O$+REO	0.015	3.201	3.437
	Control	0.007	4.966	4.300

**$CaCl_2.2H_2O$: Calcium chloride dihydrate, ***REO: Rosehip essential oil

more effective than lavender essential oil. Paladines et al. (2014) indicated that REO combined with *Aloe vera* gel effectively reduced the EP of peach, nectarine, and plum fruit during storage. Singh et al. (2022) stated that the EP of pomegranate arils treated with an edible coating of *Aloe vera* gel combined with REO was lower than those without treatment. Martínez-Romero et al. (2017) reported that the application of REO combined with *Aloe vera* or *Aloe arborescens* gels was effective in delaying the EP of plum fruit during storage, and the EP of treated fruit was lower than the control group. Martínez-Romero et al. (2019) stated that the EP of plum fruit coated with REO was significantly lower during storage compared to the control group. In this study, an increase was found in the EP in all apple cultivars and postharvest treatments during storage. Similar to our findings, Mazumder et al. (2021) and Singh et al. (2022) reported an increase in the EP of fruit with an extended storage period.

According to the Kruskal-Wallis test results, when the effects of $CaCl_2.2H_2O$, REO and $CaCl_2.2H_2O$+REO combined treatments on the EP of fruit during storage in 'S. Delicious', 'G. Delicious' and 'G. Smith' apple cultivars were evaluated according to the cultivar x month and application x month combination, the difference between the rank means of the months was statistically significant ($P<0.05$) The difference between the first and sixth month EP was not statistically significant, while the third month EP was lower than these two months and statistically significant ($P<0.05$) (Table 7).

Fruit color (FC)

Postharvest fruit quality is influenced by various quality

Table 7. Effect of postharvest $CaCl_2.2H_2O$, REO, and $CaCl_2.2H_2O$+REO applications on ethylene production (μLC_2H_{24}/kg.h) of fruit during storage according to cultivar x month and application x month combination in 'S. Delicious', 'G. Delicious' and 'G. Smith' apple cultivars according to the Kruskal-Wallis test

Cultivar	By cultivar x month combination			
	Storage period (months)			
	Application	1	3	6
'S. Delicious'	$CaCl_2.2H_2O$**	2C*	5B	8A
	REO***	5.66A	2B	7.33A
	$CaCl_2.2H_2O$+REO	2C	5B	8A
	Control	2.66C	4.3B	8A
'G. Delicious'	$CaCl_2.2H_2O$	5B	2C	8A
	REO	2C	5B	8A
	$CaCl_2.2H_2O$+REO	5B	8A	2C
	Control	2.33	7.67	5.00
'G. Smith'	$CaCl_2.2H_2O$	2C	8A	5B
	REO	2B	7.33A	5.66A
	$CaCl_2.2H_2O$+REO	2C	5B	8A
	Control	2.00	7.00	6.00
Cultivar	**By application x month combination**			
	Storage period (months)			
	Application	1	3	6
'S. Delicious'	$CaCl_2.2H_2O$**	4.6B*	2B	2C
	REO***	10A	6A	9A
	$CaCl_2.2H_2O$+REO	2.3B	8A	5B
	Control	9A	10A	10A
'G. Delicious'	$CaCl_2.2H_2O$	10.33A	5.66B	11A
	REO	2C	2C	5C
	$CaCl_2.2H_2O$+REO	5B	7.33B	2D
	Control	8.6A	11A	8B
'G. Smith'	$CaCl_2.2H_2O$	10.16A	9.3A	5B
	REO	5B	2C	2C
	$CaCl_2.2H_2O$+REO	8.83A	5B	9A
	Control	2C	9.66A	10A

*According to the Kruskal-Wallis test, capital letters indicate differences between cultivars in each treatment x month combination. **$CaCl_2.2H_2O$: Calcium chloride dihydrate, ***REO: Rosehip essential oil

attributes such as weight loss, firmness, SSC and TA as well as color and their changes during storage (Paladines et al., 2014). In the 'S. Delicious' cultivar, fruit brightness (L^*) increased in all treatments except $CaCl_2.2H_2O$+REO. The highest increase of 19.5% compared to the beginning was realized in $CaCl_2.2H_2O$, followed by REO with 18.2% and control with 8%. In $CaCl_2.2H_2O$+REO, fruit brightness decreased by 11.5%. REO was the treatment that best preserved FC and vividness of FC with the highest Hue angle (h°) (212.33) and Chroma (C^*) values (31.17). In 'G. Delicious', brightness increased in all treatments except for $CaCl_2.2H_2O$+REO compared to the beginning. The highest increase was recorded in REO with 5.1%, while there was a 1% decrease in $CaCl_2.2H_2O$+REO (Table 8). h° and C^* were highest in the control group (107.36 and 47.99, respectively), followed by $CaCl_2.2H_2O$+REO (106.91 and 47.73, respectively). According to these data, control and $CaCl_2.2H_2O$+REO were the treatments in which FC and vividness of FC of the 'G. Delicious' were best preserved (data not presented in the table). In this cultivar, staining on the peel towards the end of storage, especially in the $CaCl_2.2H_2O$, is thought to be caused by the treatment.

Gago et al. (2016) and Tamalea et al. (2021) reported that postharvest $CaCl_2$ positively affected FC. In the 'G. Smith' cultivar, a slight decrease in brightness was recorded in all treatments except REO compared to the beginning. In REO, fruit brightness increased by

Table 8. Effect of postharvest $CaCl_2.2H_2O$, REO, and $CaCl_2.2H_2O$+REO applications on fruit color (CIE L*a*b*) during storage in 'S. Delicious', 'G. Delicious' and 'G. Smith' apple cultivars

Cultivar	Application	Storage period (months) 0	1	2	3	4	5	6
				L*				
'S. Delicious'	$CaCl_2.2H_2O$**	32.57a*	40.56a	40.56a	35.19a	40.21a	39.04a	38.92a
	REO***	32.94a	36.47a	35.15a	33.85a	35.08a	32.54a	38.92a
	$CaCl_2.2H_2O$+REO	36.45a	33.30a	33.64a	36.66a	36.42a	35.13a	32.26a
	Control	37.76a	35.09a	35.59a	36.03a	40.34a	35.84a	40.78a
'G. Delicious'	$CaCl_2.2H_2O$	76.37a	76.29a	76.00a	71.23a	78.36a	78.78a	77.90a
	REO	71.32a	70.93a	71.73a	71.12a	72.37a	71.22a	74.97a
	$CaCl_2.2H_2O$+REO	76.99a	76.13a	76.28a	76.56a	77.20a	77.39a	76.23a
	Control	77.04a	75.78a	76.50a	76.03a	76.59a	75.88a	78.76a
'G. Smith'	$CaCl_2.2H_2O$	68.37a	65.23a	61.21a	68.35a	65.20a	67.45a	67.04a
	REO	64.65a	64.79a	67.49a	66.03a	65.06a	65.79a	68.61a
	$CaCl_2.2H_2O$+REO	69.04a	67.02a	65.54a	66.19a	69.62a	66.02a	65.59a
	Control	68.48a	68.04a	66.04a	68.39a	68.10a	68.00a	65.89a
				a*				
'S. Delicious'	$CaCl_2.2H_2O$**	27.28a	30.64a	30.64a	26.90a	29.38a	27.47a	26.90a
	REO***	27.33a	28.82a	29.04a	26.96a	28.07a	29.44a	26.34a
	$CaCl_2.2H_2O$+REO	30.62a	27.30a	25.41a	28.88a	28.31a	28.88a	25.39a
	Control	25.39a	29.09a	28.04a	25.07a	26.01a	29.80a	25.43a
'G. Delicious'	$CaCl_2.2H_2O$	-13.47a	-14.40a	-13.36a	-13.66a	-13.72a	-12.15a	-6.75a
	REO	-16.07a	-16.19a	-14.06a	-13.89a	-15.42a	-12.78a	-13.03a
	$CaCl_2.2H_2O$+REO	-16.98a	-16.32a	-16.19a	-16.65a	-16.46a	-15.41a	-13.88a
	Control	-15.18a	-15.13a	-13.90a	-15.44a	-13.55a	-14.00a	-14.32a
'G. Smith'	$CaCl_2.2H_2O$	-21.44a	-21.55a	-20.86a	-20.69a	-20.35a	-18.53a	-15.40a
	REO	-21.69a	-20.80a	-20.78a	-20.77a	-19.10a	-18.14a	-18.61a
	$CaCl_2.2H_2O$+REO	-21.58a	-20.24a	-21.02a	-20.21a	-18.96a	-19.71a	-16.76a
	Control	-21.94a	-21.06a	-20.97a	-19.55a	-17.32a	-17.40a	-14.57a
				b*				
'S. Delicious'	$CaCl_2.2H_2O$**	12.14a	16.45a	16.45a	14.16a	18.10a	16.45a	15.36a
	REO***	12.64a	15.13a	14.33a	12.92a	14.40a	14.40a	16.67a
	$CaCl_2.2H_2O$+REO	16.07a	12.87a	13.08a	22.39a	16.45a	14.16a	10.81a
	Control	15.38a	14.51a	14.91a	12.95a	17.18a	12.69a	15.02a
'G. Delicious'	$CaCl_2.2H_2O$	42.47a	42.90a	42.77a	41.76a	45.07a	49.10a	45.41a
	REO	41.51a	40.62a	41.85a	41.32a	42.41a	45.08a	43.97a
	$CaCl_2.2H_2O$+REO	45.62a	43.71a	44.38a	44.66a	45.39a	46.22a	45.67a
	Control	42.16a	41.64a	42.29a	43.49a	45.02a	47.35a	45.80a
'G. Smith'	$CaCl_2.2H_2O$	40.97a	41.35a	41.92a	42.17a	40.96a	41.28a	42.48a
	REO	39.53a	39.03a	41.18a	41.70a	39.85a	40.01a	41.28a
	$CaCl_2.2H_2O$+REO	42.74a	43.06a	40.77a	41.76a	40.22a	40.84a	41.71a
	Control	42.47a	42.21a	40.69a	42.71a	41.90a	41.29a	39.58a

*Lowercase letters indicate the difference between treatments in each cultivar x storage time combination. **$CaCl_2.2H_2O$: Calcium chloride dihydrate, ***REO: Rosehip essential oil

6.1% at the end of storage compared to the beginning. There was a 1.9% decrease in brightness at the end of storage in $CaCl_2.2H_2O$, a 3.8% decrease in control, and a 5% decrease in $CaCl_2.2H_2O$+REO. h° (114.27) and C* (45.28) were the highest in REO (data not presented in the table). According to these data, as in the 'S. Delicious' cv., REO was the treatment that best preserved the FC and vividness of FC of the 'G. Smith'. Paladines et al. (2014) reported that REO combined with *Aloe vera* gel delayed the color change of peach, nectarine, plum and sweet cherry fruit and a higher h° value was obtained compared to other treatments. Martínez-Romero et al. (2017) reported that the highest h° in plum fruit was obtained in fruit treated with REO after harvest. Similarly, Martínez-Romero et al. (2019) obtained higher h° values in plums treated with REO. Singh et al. (2022) reported that the color of pomegranate arils with REO+*Aloe vera* gel edible coating treatment was better preserved than those without treatment.

Table 9. Effect of postharvest $CaCl_2.2H_2O$, REO, and $CaCl_2.2H_2O$+REO applications on fruit flesh color (CIE L*a*b*) during storage in 'S. Delicious', 'G. Delicious' and 'G. Smith' apple cultivars

Cultivar	Application	Storage period (months)						
		0	1	2	3	4	5	6
				L*				
'S. Delicious'	$CaCl_2.2H_2O$**	83.86a*	84.03a	82.70a	82.38a	81.80a	80.93a	81.31a
	REO***	83.20a	83.40a	83.65a	83.91a	80.95a	80.91a	80.98a
	$CaCl_2.2H_2O$+REO	83.81a	82.42a	83.62a	72.04a	79.11a	82.65a	80.98a
	Control	84.06a	81.85a	82.59a	82.23a	83.25a	81.41a	80.99a
'G. Delicious'	$CaCl_2.2H_2O$	81.92a	83.95a	83.97a	83.01a	83.44a	78.53a	81.49a
	REO	78.51a	82.93a	82.91a	83.19a	82.97a	84.04a	82.98a
	$CaCl_2.2H_2O$+REO	82.48a	83.94a	83.94a	83.68a	84.22a	84.91a	83.71a
	Control	82.48a	84.87a	84.87a	83.93a	85.19a	85.17a	82.99a
'G. Smith'	$CaCl_2.2H_2O$	81.67a	82.56a	82.10a	82.79a	81.43a	81.84a	80.22a
	REO	82.32a	82.83a	81.19a	82.05a	81.03a	82.20a	82.30a
	$CaCl_2.2H_2O$+REO	82.11a	83.13a	80.98a	82.77a	81.65a	80.98a	79.74a
	Control	82.30a	82.66a	82.19a	82.88a	82.25a	81.89a	80.61a
				a*				
'S. Delicious'	$CaCl_2.2H_2O$**	-9.64Aa	-9.34Aa	-9.74Aa	-8.43Aa	-8.17Aa	-8.43Aa	-8.62Aa
	REO***	-8.54Aa	-9.78Aa	-9.09Aa	-8.51Aa	-9.37Aa	-8.09Aa	-8.20Aa
	$CaCl_2.2H_2O$+REO	-9.05Aa	-9.31Aa	-8.91Aa	-8.66Aa	-7.29Aa	-8.50Aa	-8.69Aa
	Control	-7.77Aa	-8.86Aa	-8.08Aa	-8.98Aa	-7.99Aa	-8.57Aa	-8.75Aa
'G. Delicious'	$CaCl_2.2H_2O$	-7.05ABa	-9.48Aa	-9.48Aa	-8.22ABa	-8.19ABa	-7.78ABa	-6.75Ba
	REO	-7.38Aa	-8.06Aa	-8.06Aa	-8.29Aa	-8.18Aa	-9.59Aa	-8.27Aa
	$CaCl_2.2H_2O$+REO	-7.99Aa	-8.98Aa	-8.98Aa	-9.57Aa	-7.46Aa	-7.78Aa	-7.80Aa
	Control	-8.86Aa	-7.56Aa	-7.56Aa	-7.79Aa	-8.53Aa	-7.99Aa	-7.04Aa
'G. Smith'	$CaCl_2.2H_2O$	-13.35Aa	-11.72ABa	-9.11Ca	-9.57BCab	-10.00BCa	-7.73Ca	-9.23BCa
	REO	-12.28Aab	-10.17ABCab	-10.52ABCa	-11.19ABa	-9.26BCa	-9.13BCa	-8.42Ca
	$CaCl_2.2H_2O$+REO	-10.98Ab	-8.31Bb	-9.63ABa	-9.91ABab	-9.87ABa	-8.22Ba	-8.55ABa
	Control	-11.53Aab	-10.14ABab	-9.76ABa	-8.42Bb	-8.06Ba	-8.06Ba	-8.06Ba
				b*				
'S. Delicious'	$CaCl_2.2H_2O$**	26.06a	23.82a	26.75a	24.26a	23.68a	25.68a	27.60a
	REO***	24.71a	24.27a	23.35a	22.99a	23.96a	22.45a	26.28a
	$CaCl_2.2H_2O$+REO	24.26a	25.86a	23.00a	28.75a	24.72a	26.05a	29.48a
	Control	21.83a	27.83a	25.19a	27.03a	23.23a	23.15a	27.03a
'G. Delicious'	$CaCl_2.2H_2O$	22.46a	28.78a	28.78a	26.80a	26.95a	26.49a	26.47a
	REO	24.00a	28.92a	28.92a	26.47a	27.31a	26.55a	29.98a
	$CaCl_2.2H_2O$+REO	21.05a	26.68a	26.68a	26.52a	27.93a	25.24a	27.17a
	Control	26.18a	31.25a	31.25a	27.47a	31.82a	29.82a	26.54a
'G. Smith'	$CaCl_2.2H_2O$	25.29a	22.88a	18.87a	21.25a	20.51a	15.65a	22.67a
	REO	22.55a	19.25a	21.22a	22.03a	19.53a	19.58a	19.20a
	$CaCl_2.2H_2O$+REO	22.19a	15.37a	19.70a	20.11a	21.19a	17.97a	18.86a
	Control	21.32a	19.23a	18.68a	16.70a	17.44a	18.72a	20.91a

*Right uppercase letters denote the difference between each cultivar x treatment combination and storage time, right lowercase letters denote the difference between each cultivar x storage time combination. **$CaCl_2.2H_2O$: Calcium chloride dihydrate, ***REO: Rosehip essential oil

Table 10. Effect of postharvest $CaCl_2.2H_2O$, REO, and $CaCl_2.2H_2O$+REO applications on the overall quality (points) of fruit during storage in 'S. Delicious', 'G. Delicious' and 'G. Smith' apple cultivars

Cultivar	Storage period (months)								
	Application	0	1	2	3	4	5	6	Mean
'S. Delicious'	$CaCl_2.2H_2O$**	9	9	9	9	9	8	8	8.71
	REO***	9	8.66	8.33	8.33	8	8	7.66	8.28
	$CaCl_2.2H_2O$+REO	9	9	9	9	8.33	8.33	8	8.66
	Control	9	9	9	9	8.33	8	7	8.47
'G. Delicious'	$CaCl_2.2H_2O$	9	9	9	9	9	7.66	7	8.52
	REO	9	8	7	5.66	5	4.33	4.33	6.18
	$CaCl_2.2H_2O$+REO	9	9	9	9	8.33	8.33	7.66	8.61
	Control	9	9	9	8.66	9	7.66	7.33	8.52
'G. Smith'	$CaCl_2.2H_2O$	9	9	9	9	8.66	7	7.33	8.42
	REO	9	9	9	9	9	8.66	8.66	8.90
	$CaCl_2.2H_2O$+REO	9	9	9	9	9	8.33	8	8.76
	Control	9	9	9	9	9	6.66	6.66	8.33

$CaCl_2.2H_2O$: Calcium chloride dihydrate,*REO: Rosehip essential oil

Table 11. Effect of postharvest $CaCl_2.2H_2O$, REO, and $CaCl_2.2H_2O$+REO applications on the overall quality (points) of fruit during storage in 'S. Delicious', 'G. Delicious' and 'G. Smith' apple cultivars according to cultivar x month and application x month combination according to Kruskal-Wallis test result

Cultivar	By cultivar x month combination							
	Storage period (months)							
	Application	0	1	2	3	4	5	6
'S. Deliicous'	$CaCl_2.2H_2O$**	14A*	14A	14A	14A	14A	3.5B	3.5B
	REO***	16.5A	13.16A	9.83AB	9.83AB	16.5A	6.5B	4.66B
	$CaCl_2.2H_2O$+REO	14.5A	14.5A	14.5A	14.5A	7.5B	7.5B	4B
	Control	15A	15A	15A	15A	9B	6B	2C
'G. Delicious'	$CaCl_2.2H_2O$	14A	14A	14A	14A	14A	4.5B	2.5C
	REO	20A	16.5AB	14.16B	10.5C	7.5CD	4.16D	4.16D
	$CaCl_2.2H_2O$+REO	14.5A	14.5A	14.5A	14.5A	7.83B	7.83B	3.33B
	Control	14.5A	14.5A	14.5A	11.5A	14.5A	4.33B	3.16B
'G. Smith'	$CaCl_2.2H_2O$	14.5A	14.5A	14.5A	14.5A	11.83	3B	4.16B
	REO	12.00	12.00	12.00	12.00	12.00	8.50	8.50
	$CaCl_2.2H_2O$+REO	13.5A	13.5A	13.5A	13.5A	13.5A	6.5B	3B
	Control	14A	14A	14A	14A	14A	3.5B	3.5B
Cultivar	**By application x month combination**							
	Storage period (months)							
	Application	0	1	2	3	4	5	6
'S. Deliicous'	$CaCl_2.2H_2O$**	5.00	5.00	5.00	5.00	5.50	7.00	7.50
	REO***	5.00	5.50	5.166AB*	5.5A	6.5A	5.5A	5.33A
	$CaCl_2.2H_2O$+REO	5.00	5.00	5.00	5.00	4.00	5.00	5.5A
	Control	5.00	5.00	5.00	5.50	3.00	7.00	5.00
'G. Delicious'	$CaCl_2.2H_2O$	5.00	5.00	5.00	5.00	5.50	5.50	3.00
	REO	5.00	2.50	2.33B	2B	2B	2B	2B
	$CaCl_2.2H_2O$+REO	5.00	5.00	5.00	5.00	4.00	5.00	4B
	Control	5.00	5.00	5.00	4.00	6.00	5.67	6.33
'G. Smith'	$CaCl_2.2H_2O$	5.00	5.00	5.00	5.00	4.00	2.50	4.50
	REO	5.00	7.00	7.5A	7.5A	6.5A	7.5A	7.6A
	$CaCl_2.2H_2O$+REO	5.00	5.00	5.00	5.00	7.00	5.00	5.5A
	Control	5.00	5.00	5.00	5.50	6.00	2.33	3.67

*According to the Kruskal-Wallis test, capital letters indicate differences between cultivars in each cultivar x month and treatment x month combination. **$CaCl_2.2H_2O$: Calcium chloride dihydrate,*** REO: Rosehip essential oil

Fruit flesh color (FFC)

In the 'S. Delicious' cultivar, a slight decrease in brightness was recorded in all treatments at the end of storage compared to the beginning (Table 9). When the beginning (109.07) and end of storage (107.36) values of FFC values of h° were compared, the least change (1.56) was observed in the REO. $CaCl_2.2H_2O$+REO had the highest (30.73) C*. In the 'G. Delicious', brightness increased in all treatments except for $CaCl_2.2H_2O$ at the end of storage compared to the beginning. The highest h° (106.03) was in $CaCl_2.2H_2O$+REO. The highest C* (31.10) was in REO (data not presented in the table). Gago et al. (2016) reported that postharvest $CaCl_2$+1-MCP treated fruit of 'G. Delicious' were brighter, had higher h° and C* than the control. In the 'G. Smith', all treatments had very similar values at the end of storage and there was a slight decrease in brightness in all treatments except for $CaCl_2.2H_2O$ compared to the beginning. In the 'G. Smith' cv., $CaCl_2.2H_2O$+REO had the highest h° (114.40), while $CaCl_2.2H_2O$ had the highest C* (24.48). The higher h° was obtained by Paladines et al. (2014) in *Aloe vera* gel+REO treatment, Martínez-Romero et al. (2017) in REO, and Martínez-Romero et al. (2019) in REO treatments. Singh et al. (2022) reported that REO+*Aloe vera* gel preserved the color better than the untreated ones. Tamalea et al. (2021) also stated positive effects of postharvest $CaCl_2$ application.

Sensory evaluations

Overall quality

In the 'S. Delicious' cultivar, $CaCl_2.2H_2O$ and $CaCl_2.2H_2O$+REO were of good quality (8 points) at the end of storage, followed by REO (7.66 points) and control (7 points). In the 'G. Delicious', $CaCl_2.2H_2O$+REO had the highest score (7.66 points) at the end of storage, followed by control and $CaCl_2.2H_2O$, and REO had the lowest score (4.33 points). In the 'G. Delicious', staining was observed on the fruit peel starting from the 2nd month in REO. In previous studies, there is no such finding related to this in any fruit species and cultivar in which postharvest essential oil applications were made. It is thought that the staining may be related to a reaction of the peel structure of the 'G. Delicious' apple cultivar to the treatments. At the end of storage REO had the best score in the 'G. Smith' cultivar (8.66 points), followed by $CaCl_2.2H_2O$+REO (8 points) and $CaCl_2.2H_2O$ (7.33 points). The lowest score (6.66 points) was recorded in the control (Table 10). Ullah et al. (2007) reported that $CaCl_2$ significantly preserves the sensory quality of fruit by slowing down metabolic changes.

According to Kruskal-Wallis test results, when the effects of $CaCl_2.2H_2O$, REO and $CaCl_2.2H_2O$+REO combined treatments on the overall quality of fruit during storage in 'S. Delicious', 'G. Delicious' and 'G. Smith' apple cultivars were evaluated according to the cultivar x month and application x month combination, the difference between the rank means of the months was statistically significant ($P<0.05$) (Table 11).

Taste and aroma

At the end of storage in the 'S. Delicious' cultivar, the taste and aroma of the fruit was evaluated as good with 4 points in $CaCl_2.2H_2O$, $CaCl_2.2H_2O$+REO and control group and 3.66 points in REO. The reason for the lower score of the fruit in REO may be a greasy feeling caused by the application. At the end of storage in the 'G. Delicious' cultivar, it was observed that $CaCl_2.2H_2O$+REO and REO were better (4 points) than other treatments for preserving the taste and aroma of fruit. While the fruit treated with $CaCl_2.2H_2O$ were evaluated as medium (3 points) by the panellists, they stated a decrease in fruit taste and perceived a slight bitterness. The control group fruit had 2.66 points in the evaluation made by the panellists. In the 'G. Smith' cultivar, the taste and aroma of the fruit treated with REO and $CaCl_2.2H_2O$+REO were

Table 12. Effect of postharvest $CaCl_2.2H_2O$, REO, and $CaCl_2.2H_2O$+REO applications on the taste and aroma (points) of fruit during storage in 'S. Delicious', 'G. Delicious' and 'G. Smith' apple cultivars

Cultivar	Storage period (months)							
	Application	0	1	2	3	4	5	6
'S. Delicous'	$CaCl_2.2H_2O$**	5.00	5.00	5.00	4.66	4.66	4.33	4.00
	REO***	5.00	5.00	4.66	4.66	4.66	4.33	4.00
	$CaCl_2.2H_2O$+REO	5.00	5.00	5.00	5.00	4.66	4.33	4.00
	Control	5.00	5.00	5.00	4.33	4.33	4.00	3.66
'G. Delicous'	$CaCl_2.2H_2O$	5.00	5.00	5.00	5.00	4.33	4.00	3.00
	REO	5.00	4.33	4.66	4.00	4.00	4.00	4.00
	$CaCl_2.2H_2O$+REO	5.00	5.00	5.00	4.66	4.00	4.33	4.00
	Control	5.00	5.00	5.00	5.00	4.33	4.00	2.66
'G. Smith'	$CaCl_2.2H_2O$	5.00	5.00	5.00	5.00	4.33	4.00	4.33
	REO	5.00	5.00	5.00	5.00	5.00	4.00	5.00
	$CaCl_2.2H_2O$+REO	5.00	5.00	5.00	5.00	5.00	4.00	5.00
	Control	5.00	5.00	4.66	5.00	4.33	4.33	3.66

**$CaCl_2.2H_2O$: Calcium chloride dihydrate, ***REO: Rosehip essential oil

evaluated as very good, and these treatments had the highest score (5 points) at the end of storage. At the end of storage, $CaCl_2.2H_2O$ (4.33 points) also positively affected on the preservation of the taste and aroma of the fruit, while the fruit in the control group had a lower score (3.66 points) compared to the other treatments. According to the taste and aroma evaluations of the fruit, the fruit was of edible quality at the end of storage in all cultivars and all treatments. REO and $CaCl_2.2H_2O$+REO were slightly more prominent than the other treatments in terms of taste and aroma in all cultivars (Table 12). The findings obtained in this study are consistent with the literature (Paladines et al., 2014; Gemeda et al., 2021; Tamalea et al., 2021; Singh et al., 2022).

According to Kruskal-Wallis test results, when the effects of $CaCl_2.2H_2O$, REO and $CaCl_2.2H_2O$+REO combined treatments on the taste and aroma of fruit during storage in 'S. Delicious', 'G. Delicious' and 'G. Smith' apple cultivars were evaluated according to the cultivar x month and application x month combination, the difference between the rank means of the months was statistically significant ($P<0.05$) (Table 13).

Table 13. Effects of postharvest $CaCl_2.2H_2O$, REO, and $CaCl_2.2H_2O$+REO applications on the taste and aroma (points) of fruit during storage in 'S. Delicious', 'G. Delicious' and 'G. Smith' apple cultivars according to cultivar x month and application x month combination according to Kruskal-Wallis test results

Cultivar	By cultivar x month combination							
		Storage period (months)						
	Application	0	1	2	3	4	5	6
'S. Delicious'	$CaCl_2.2H_2O$**	14.50	14.50	14.50	11.00	11.00	7.50	4.00
	REO***	15.00	15.00	11.67	11.67	11.67	8.33	3.67
	$CaCl_2.2H_2O$+REO	14.00	14.00	14.00	14.00	10.50	7.00	3.50
	Control	16A*	16A	16A	9B	9B	5.5B	5.5B
'G. Delicious'	$CaCl_2.2H_2O$	15A	15A	15A	15A	8.33B	5B	3.66B
	REO	18.5A	12.5AB	15.5A	9.5B	7B	7B	7B
	$CaCl_2.2H_2O$+REO	15.5A	15.5A	15.5A	12AB	5C	8.5BC	5C
	Control	15A	15A	15A	15A	8.33B	5B	3.66B
'G. Smith'	$CaCl_2.2H_2O$	14.5A	14.5A	14.5A	14.5A	7.5A	4B	7.5A
	REO	12.5A	12.5A	12.5A	12.5A	12.5A	2B	12.5A
	$CaCl_2.2H_2O$+REO	12.5A	12.5A	12.5A	12.5A	12.5A	2B	12.5A
	Control	15.00	15.00	11.67	15.00	8.33	8.33	3.67
Cultivar	By application x month combination							
		Storage period (months)						
	Application	0	1	2	3	4	5	6
'S. Delicious'	$CaCl_2.2H_2O$**	5.00	5.00	5.00	4.00	6.00	6.00	5.00
	REO***	5.00	6.00	4.50	5.50	5.67	6.33	3.5B
	$CaCl_2.2H_2O$+REO	5.00	5.00	5.00	5.50	5.50	5.50	3.50
	Control	5.00	5.00	5.50	3.00	5.00	4.50	6.00
'G. Delicious'	$CaCl_2.2H_2O$	5.00	5.00	5.00	5.50	4.50	4.50	3.67
	REO	5.00	3.00	4.50	2.50	2.33	3.67	3.5B
	$CaCl_2.2H_2O$+REO	5.00	5.00	5.00	4.00	2.50	5.50	3.50
	Control	5.00	5.00	5.50	6.00	5.00	4.50	4.50
'G. Smith'	$CaCl_2.2H_2O$	5.00	5.00	5.00	5.50	4.50	4.50	6.33
	REO	5.00	6.00	6.00	7.00	7.00	5.00	8A
	$CaCl_2.2H_2O$+REO	5.00	5.00	5.00	5.50	7.00	4.00	8.00
	Control	5.00	5.00	4.00	6.00	5.00	6.00	4.50

*According to the Kruskal-Wallis test, capital letters indicate differences between cultivars in each cultivar x month and application x month combination.

$CaCl_2.2H_2O$: Calcium chloride dihydrate,* REO: Rosehip essential oil

Table 14. Effect of postharvest $CaCl_2.2H_2O$, REO, and $CaCl_2.2H_2O$+REO applications on the odor (points) of fruit during storage in 'S. Delicious', 'G. Delicious' and 'G. Smith' apple cultivars

Cultivar	Application	Storage period (months)						
		0	1	2	3	4	5	6
'S. Delicous'	$CaCl_2.2H_2O$**	0	0	0	0	0	0	0
	REO***	0.66	0.66	0	0.66	0	0	0.33
	$CaCl_2.2H_2O$+REO	0	0	0	0	0	0	0
	Control	0	0	0	0	0	0	0.33
'G. Delicous'	$CaCl_2.2H_2O$	0	0	0	0	0	0	0
	REO	0	0.66	0	0.33	0.66	0.33	0.33
	$CaCl_2.2H_2O$+REO	0	0	0	0	0.33	0	0.33
	Control	0	0	0	0	0.33	0	0
'G. Smith'	$CaCl_2.2H_2O$	0	0	0	0	0	0.33	0.33
	REO	0	0.33	0	0	0	0	0.33
	$CaCl_2.2H_2O$+REO	0	0	0	0	0	0.33	0.33
	Control	0	0	0.33	0	0	0	0

$CaCl_2.2H_2O$: Calcium chloride dihydrate, * REO: Rosehip essential oil

Table 15. Effect of postharvest $CaCl_2.2H_2O$, REO, and $CaCl_2.2H_2O$+REO applications on the percentage of decay (%) during storage in 'S. Delicious', 'G. Delicious' and 'G. Smith' apple cultivars

Cultivar	Application	Storage period (months)						
		0	1	2	3	4	5	6
'S. Delicous'	$CaCl_2.2H_2O$**	0ns	0	0	0	0	0	0
	REO***	0	0	0	0	0	3	0
	$CaCl_2.2H_2O$+REO	0	0	0	0	0	0	0
	Control	0	0	0	0	0	3	3
'G. Delicous'	$CaCl_2.2H_2O$	0	0	0	0	0	10	0
	REO	0	0	0	0	8	28	0.33
	$CaCl_2.2H_2O$+REO	0	0	0	0	0	0	0.33
	Control	0	0	0	0	2	26	0
'G. Smith'	$CaCl_2.2H_2O$	0	0	0	0	0	7	10
	REO	0	0	0	0	0	0	0
	$CaCl_2.2H_2O$+REO	0	0	0	0	1	1	0
	Control	0	0	0	0	0	2	5

nsNot significant, **$CaCl_2.2H_2O$: Calcium chloride dihydrate, *** REO: Rosehip essential oil

Odor

In this study, 'S. Delicious', 'G. Delicious' and 'G. Smith' apple cultivars were also evaluated for any bad odor other than fruity odor during storage. According to the evaluations made by the panellists, although a slightly different odor was perceived during storage in the REO in general and at the end of storage in the $CaCl_2.2H_2O$+REO in the 'G. Delicious', in the $CaCl_2.2H_2O$ and $CaCl_2.2H_2O$+REO in the 'G. Smith', it was not at a level that would affect the fruit taste, as can be seen in Table 14, where taste and aroma values are given. The findings obtained in this study are consistent with the findings of Paladines et al. (2014), Gemeda et al. (2021), Tamalea et al. (2021) and Singh et al. (2022).

According to the Kruskal-Wallis test results, when the effects of $CaCl_2.2H_2O$, REO and $CaCl_2.2H_2O$+REO treatments on fruit odor during storage in 'S. Delicious', 'G. Delicious' and 'G. Smith' apple cultivars were evaluated according to the cultivar x month and application x month combination, the difference between the rank mean of months was not statistically significant ($P<0.05$).

Decay

Table 15 shows that no rot was detected in any cultivar

and treatment until the 4th month of storage. While no decay was detected in the 'S. Delicious' cultivar in the 4th month of storage, 8% decay was detected in the REO and 2% in the control group in the 'G. Delicious' cultivar; 1% decay was detected in the $CaCl_2.2H_2O$+REO in 'G. Smith' cultivar. At the end of the storage period, no decay was detected in 'S. Delicious' cultivar in treatments other than control, in 'G. Delicious' cultivar in $CaCl_2.2H_2O$ and the 'G. Smith' cultivar in REO and $CaCl_2.2H_2O$+REO, while the highest decay was in the control group of the 'G. Delicious' cultivar with 40%. As can be seen from the data obtained, the effect of treatments on the decay rate of apple cultivars was variable. It was observed that all treatments significantly reduced the fruit decay rate in 'S. Delicious' cultivar; $CaCl_2.2H_2O$ and $CaCl_2.2H_2O$+REO in 'G. Delicious' cultivar, and REO and $CaCl_2.2H_2O$+REO in 'G. Smith' cultivar compared to the control group.

Salem and Moussa (2014) reported that $CaCl_2$ treatment protected the apple cv. of the 'Anna' against various rotting agents during storage. Sohail et al. (2015) reported that postharvest application of $CaCl_2$ reduced the decay rate in peach fruit. Eric et al. (2015) reported that postharvest application of $CaCl_2$ reduced the decay rate in tomato fruit. Gago et al. (2016) reported the decay rate in the 'G. Delicious' cultivar was significantly reduced in $CaCl_2$ application compared to the control group. El-Dengawy et al. (2018) reported that postharvest $CaCl_2$ immersion of guava fruit stored under room conditions reduced the percentage of fruit decay. Similar to our findings, Rabiei et al. (2011) reported that thyme and lavender essential oils significantly reduced the decay rates of apple cv. of the 'Jonagold'. Mahmoud et al. (2019) reported that essential oil treatment had positive effects against decay agents in the 'Anna' apple cultivar. Gemeda et al. (2021) reported that calcium immersion treatments increase the likelihood that fruit is less susceptible to rot during storage, and the researcher related the higher decay rate in untreated fruit to the result of lower tissue strength and cellular disorganization.

According to the Kruskal-Wallis test results, when the effects of $CaCl_2.2H_2O$, REO and $CaCl_2.2H_2O$+REO on the percentage of fruit decay during storage in 'S. Delicious', 'G. Delicious' and 'G. Smith' apple cultivars were evaluated according to the cultivar x month and application x month combination, the difference between the rank mean of months was not statistically significant ($P<0.05$).

Superficial scald (SS) and superficial scald severity (SSS)

SS is one of the most common postharvest physiological disorders of some apple cultivars, although its development's aetiology and biochemistry are not yet fully understood. SS involves the synthesis of (E,E)-alpha-farnesene, a sesquiterpene, and the primary products of its oxidation, namely the accumulation of conjugated trienols in the fruit epidermis and hypodermis. This causes the rupture of cell membranes, leading to polyphenoloxidase-mediated browning of the fruit peel (Gago et al., 2016). Typical symptoms of SS are brown or black spots on the fruit peel during storage (Zanella and Rossi, 2015). In this study, except for the 'G. Smith' apple cultivar, no symptoms of SS were observed in other cultivars. In the 'G. Smith' apple cultivar, no SS was observed in any treatment until the 6th month of storage. At the end of the storage period, SS was detected in the control group and $CaCl_2.2H_2O$ treated fruit. In the $CaCl_2.2H_2O$-treated fruit, SS was recorded as 3%, while 7% SS was detected in the control group. In the fruit treated with $CaCl_2.2H_2O$, SSS was evaluated as 0.25%, while in the control group SSS was higher (1%) (Table 16).

Shatat and Fadhil (2010) reported that postharvest $CaCl_2$ application effectively reduced SS during storage in the 'G. Smith' apple cultivar. Gago et al. (2016) reported that

Table 16. Effect of postharvest $CaCl_2.2H_2O$, REO, and $CaCl_2.2H_2O$+REO applications on superficial scald (%) and superficial scald severity (%) of fruit during storage in 'G. Smith' apple cultivar

Cultivar	Application	Superficial scald Storage period (months)						
		0	1	2	3	4	5	6
'G. Smith'	$CaCl_2.2H_2O$**	0ns	0	0	0	0	0	3
	REO***	0	0	0	0	0	0	0
	$CaCl_2.2H_2O$+REO	0	0	0	0	0	0	0
	Control	0	0	0	0	0	0	7
		Superficial scald severity Storage period (months)						
	$CaCl_2.2H_2O$**	0ns	0	0	0	0	0	0.25
	REO***	0	0	0	0	0	0	0
	$CaCl_2.2H_2O$+REO	0	0	0	0	0	0	0
	Control	0	0	0	0	0	0	1

nsNot significant, **$CaCl_2.2H_2O$: Calcium chloride dihydrate,*** REO: Rosehip essential oil

$CaCl_2$ effectively reduced SS during storage in apple fruit compared to the control group. According to the findings obtained, REO and $CaCl_2.2H_2O$+REO were successful in preventing the occurrence of SS in fruit. Based on the fact that an oxidation mechanism is effective in the emergence of SS (Gago et al., 2016) and that the emergence of SS is also prevented in environments where oxygen is reduced (Poirier et al., 2020). It is thought that the emergence of SS in fruit treated with REO and $CaCl_2.2H_2O$+REO was prevented in this study because REO forms a barrier and modified atmosphere in the fruit peel. Indeed, respiration rate (Table 5) and ethylene production (Table 6) support this finding.

CONCLUSION

As a result, when all the data were evaluated together, it was revealed that the postharvest edible coating applications in the form of $CaCl_2.2H_2O$, REO and $CaCl_2.2H_2O$+REO combination had positive contributions to the storage life of the fruit and on the parameters constituting the fruit quality during storage in 'S. Delicious', 'G. Delicious' and 'G. Smith' apple cultivars. However, it was observed that the effects on some quality characteristics differed according to the cultivars. In the 'S. Delicious', REO was slightly more prominent than the other treatments, but the other treatments were also effective in preserving fruit quality compared to the control group. In the 'G. Delicious', REO was prominent in preserving fruit quality characteristics. However, REO can be recommended for short-term storage since the formation of spots on the fruit peel starting from the 2nd month will negatively affect the market value. For this cultivar, other treatments were effective in preserving fruit quality compared to the control group. In the 'G. Smith', $CaCl_2.2H_2O$ and REO applications had a more positive contribution to the quality characteristics of the fruit than $CaCl_2.2H_2O$+REO. However, REO and $CaCl_2.2H_2O$+REO were more successful in preventing the emergence of SS, which is an important problem for the 'G. Smith' apple cultivar. The study results recommend the use of $CaCl_2.2H_2O$ for the 'S. Delicious' apple cultivar, $CaCl_2.2H_2O$+REO for the 'G. Delicious', and REO for the 'G. Smith' cultivar.

It is thought that the results obtained will contribute to the prevention of losses that may occur in the fruit of 'G. Delicious', 'S. Delicious' and 'G. Smith' apple cultivars during storage and encourages using of alternative natural practices that can replace chemical substances that negatively affect on human health and the environment in apple storage.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

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

Author contribution

This manuscript was produced from the master dissertation entitled 'Effect of post-harvest rosehip essential oil and calcium chloride application on storage time and fruit quality in some apple cultivars' prepared by Emine YİĞİT at the Institute of Graduate Education, Isparta University of Applied Sciences under the supervision of Assist Prof. Dr. Tuba DİLMAÇÜNAL. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original.

Ethics committee approval

Ethics committee approval is not required. This article does not contain any studies with human participants or animals performed by any of the authors.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(3): 690-695
DOI: https://doi.org/10.31015/jaefs.2023.3.22

Effect of phosphorus fertilization on phenolic compounds and antioxidant activity in *Galanthus elwesii* Hook.

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Citation: Bati Ay, E., Kara, S.M., Acikgoz, M.A. (2023). Effect of phosphorus fertilization on phenolic compounds and antioxidant activity in Galanthus elwesii Hook. International Journal of Agriculture, Environment and Food Sciences, 7 (3), 690-695

Received: August 24, 2023
Revised: September 11, 2023
Accepted: September 13, 2023
Published Online: September 29, 2023

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Available online at
https://jaefs.com/
https://dergipark.org.tr/jaefs

Abstract

Snowdrop is a genus of high medicinal value with alkaloids such as galantamine, and lycorine of the *Amaryllidaceae* family. The present study was conducted to have an effect on the effects of phosphorus (P) treatment on antioxidant activity and phenolic compounds in *Galanthus elwesii* Hook. The plants were exposed to different concentrations of P (0, 3, 6, and 12 kg da^{-1}). The study was carried out in the 2018-2019 growing season. *G. elwesii* were harvested based on different growing stages (flowering and fruit ripening). In this study, the bulb and roots of the plant were used. Total flavonoid content (TFC), total phenolic content (TPC), phenolic compounds, and antioxidant activity were determined in harvested bulb and roots. The highest TPC was detected as 358.36 mg GAE/g in the flowering period of the plant, and the lowest TPC determined as 80.13 mg GAE/g in the fruit ripening period in the treatment P 6 kg da^{-1}. The highest TFC was detected as 108.07 mg QE/g with the flowering period of the plant, and the lowest TFC was determined as 52.33 mg QE/g in the fruit ripening period in the treatment P 6 kg da^{-1}. The main phenolic component of *G. elwesii* was determined to be gallic acid (GA). In antioxidant activity, while the highest 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity (72.30%) was in the flowering period in the treatment P 6 kg da^{-1}, the highest ferrous ions chelating activity (66.77%) was detected in the fruit ripening period in the treatment P 6 kg da^{-1}. As a result, it was determined that TPC, TFC and DPPH activity in *G. elwesii* of flowering period >fruit ripening period.

Keywords: *Galanthus elwesii,* Phosphorus, Alkaloids, Blbous plants, Growth and development periods

INTRODUCTION

Snowdrop is among the most significant species of the *Amaryllidaceae* family (Semerdjieva et al., 2019). The species in the *Amaryllidaceae* family contain up to 150 alkaloids called *Amaryllidaceae* alkaloids such as nivalin, galantamine, tazettin, and lychorenin, which have high biological activities, and according to their structures, they also have anticancer (Ay et al., 2023), anti-inflammatory (Kang et al., 2012), anti-diabetic (Ghane et al., 2018), and anti-bacterial, anti-malarial, acetylcholinesterase and butyrylcholinesterase inhibition (Pesaresi et al., 2022; Ay et al., 2023) effects. The active agents of snowdrop species, minimize the damage they cause to the body by blocking free radicals and have antioxidant effects that prevent chain reactions that lead to premature aging as well as many diseases. Natural antioxidants that are common in plants are phenolic compounds, nitrogen compounds, polyphenols, carotenoids, ascorbic acid, and selenium (Fernandez-Lopez et al., 2020). The antioxidant activity of phenolic

compounds was investigated by many researchers and the structural characteristics of flavonoids, which are among the compounds providing antioxidant activity, were identified (Deveci et al., 2018). Flavonoids are phenolic compounds among the most common groups of secondary metabolites in various food and medicinal plants.

Factors that cause stress in the medicinal plant may be of living origin such as disease-causing agents and pests, or may also be of inanimate origin such as salinity, drought, low and high temperatures, radiation, and deficiencies or excesses of nutrients (Taiz and Zeiger, 2010). The deficiency or excess of a nutrient element may have positive or negative effects on the availability and toxicity of another nutrient element to the plant, which may create a stress effect in terms of plant development. The effects of increasing doses of macronutrients on plant growth in some medicinal and aromatic plants and especially on secondary metabolites have recently become increasingly important. P is an essential macronutrient that plays a role in many physiological processes such as cell division nucleic acid synthesis, , membrane stability, respiration, enzymatic activities, photosynthesis and development in plants (Orme˜no and Fernandez, 2012; Abbas et al., 2018; Kalayu, 2019).

Although the medicinal value of the *Amaryllidaceae* family is already known, the number of studies conducted on the antioxidant capacity, and total phenolic and flavonoid contents of this plant is quite a few. No studies were detected in the literature regarding the effects of fertilizer applications on total phenolic and flavonoid contents and antioxidant capacity, especially in snowdrop.

In light of these, the purpose of this research investigate the effects of P on phenolic compounds and antioxidant activity in *G. elwesii*.

MATERIALS AND METHODS

Material and design

The study was conducted in the 2018-2019 growing seasons in a land previously used as agricultural land in the Suluova district of Amasya. In the study, *G. elwesii* species with a bulb diameter larger than 4 cm, obtained from commercial companies, was used. The study was created in the "Randomized Complete Block Design" trial design with 3 replications. Three different concentrations of P (0, 3, 6, and 12 kg da^{-1} P_2O_5 - 0.43% w/v) were applied. After the drying process was completed, the root and bulbs were macerated.

Phytochemical analysis

Preparation of plant samples to determine antioxidant activity

After the drying process was completed, the plants were macerated. Then the methanol was evaporated by rotary and the extracts were obtained.

Determination total phenolic content (TPC)

TPC of the *G. elwesii* plant extracts was found according to the method suggested by Slinkard and Singleton (1977), using. The results are expressed as mg gallic acid/g (mg GAE/g) in the dried samples.

Determination total flavonoids content (TFC)

TFC was determined with the quercetin standard solution by using the methods suggested by Park et al. (2008) and total flavonoids were expressed as mg equivalents of quercetin (mg QE/g) per g of the dried fraction.

Determination phenolic compounds

The amounts of gallic acid, caffeic acid, campherol, formonenitin, p-coumaric, cinnamic, and ferulic acid were determined with high-performance liquid chromatography (HPLC).

Antioxidant activity

DPPH Free Radical Scavenging Activity

In the present study, DPPH free radical scavenging method was used to determine the antioxidant activity (Brand-Williams et al., 1995).

Ferrous ions chelating assay

The chelating activity (Fe^{2+}) of the extracts in iron ions was determined according to the method proposed by Decker and Welch (1990).

Statistical Analysis

Evaluation of the obtained data was done by using JUMP statistical package program. The significance control of the differences between the means was performed using the Duncan test.

RESULTS AND DISCUSSION

Total phenolic content

TPC founded from *G. elwesii* samples treated with P fertilizer are shown in Figure 1. The highest TPC during the flowering period was found to be 358.36 mg GAE/g with 6 kg/da P treatment. The lowest TPC during fruit ripening period was found to be 80.13 mg GAE/g with 6 kg/da P treatment. When the related figure is examined, it is seen that the total phenolic content increased by 117.12% in the 6 kg/da P treatment during the flowering period.

Ay et al., (2018) conducted a study to determine the total phenolic content of *G. elwesii* . The highest phenolic content in *G. elwesii* extracts was found to be 42.63 mg GAE/g in the fruit ripening period, the lowest phenolic content was determined as 18.15 mg GAE/g in the fruit ripening period. In the study of Korcan et al. (2018) in which they determined the phenolic compounds, total flavonoid substance, antioxidant capacity, and

antimicrobial activity in bulbs of a narcissus species *Narcissus papyraceus*, the amount of total phenolic substance in bulbs and bulbs skins was determined as gallic acid equivalent of 98 mg GAE/g and 584 mg GAE/g, respectively. Erenler et al., (2019) conducted a study to determine the antioxidant activity and total phenolic content of *Galanthus krasnovii*. The total phenolic contents of dichloromethane- ,hexane-, and ethyl acetate extracts were 60.95 mg GAE/g, 71.90 GAE/g, and 58.90 mg GAE/g, respectively. Albayrak and Elmacı (2017) found that the effect of increasing nitrogen and sulfur doses on the total phenolic substance and antioxidant activity in bulbs was insignificant, and the total phenolic substance content was 63.16 mg 100 g^{-1} and 76.92 mg 100 g^{-1}.

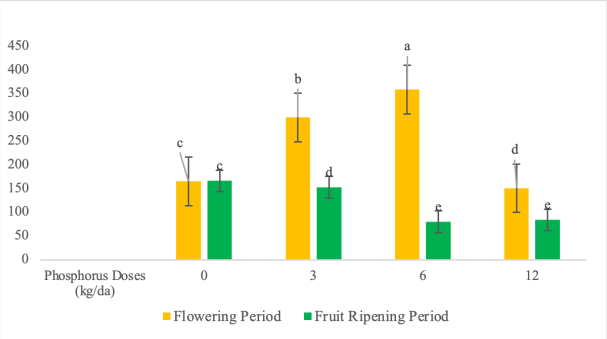

Figure 1. Total phenolic substance content (mg GAE/g) values of *G. elwesii* plants treatment with different doses of phosphorus (P) fertilizer according to different development periods.

Total flavonoid content

TFC obtained from *G. elwesii* samples treated with P fertilizer are shown in Figure 2. The highest TFC during the flowering period was found to be 108.07 mg QE/g with 6 kg/da P treatment. The lowest TPC during fruit ripening period was found to be 52.33 mg mg QE/g with 6 kg/da P treatment. As seen in Figure 2, the total flavonoid content of *G. elwesii* plant decreased by 51.57 % in the fruit ripening period compared to the flowering period.

Ay et al. (2018) reported that the highest flavonoid content in *G.elwesii* extracts was determined in the fruit ripening period, and the lowest flavonoid content was found in the the fruit ripening period. Korcan et al. (2018) determined the total flavonoid content in *Narcissus papyraceus* bulbs and bulb skins as 8.75 mg QE/g sample and 5.04 mg QE/g sample as quercetin equivalents, respectively. In a study that was conducted by Deniz (2016), when the flavonoid amounts of *Crocus* L. taxa extracts were compared, although the highest amount was found in *C. cancellatus* subsp. as 60.71 mg QE/g in the ethanolic areial extract of the mazziaricus taxon, the lowest value was in *C. pallasii subsp*. Pallasii taxon as 5.65 mg QE/g in the ethanolic underground extract. This study results support previous study results.

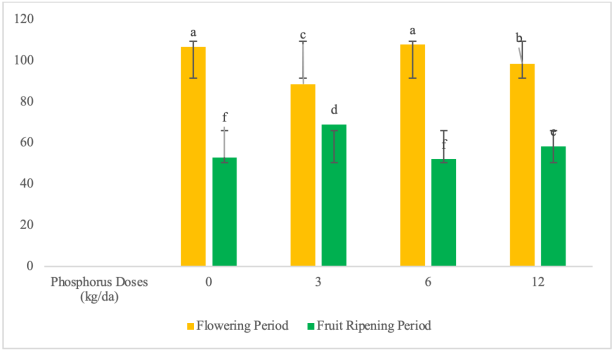

Figure 2. Total flavonoid content (mg QE/g) values of *G. elwesii* plants treatment with different doses of phosphorus (P) fertilizer according to different development periods.

Determination phenolic compounds

The phenolic substances identified were caffeic acid, gallic acid, p-coumaric acid, ferulic acid, canferol, cinnamic acid, syringer, vanillic acid and formonentin. Only gallic acid and caffeic acid were detected in the bulb and root that were harvested during flowering and fruit ripening periods, but p-coumaric acid, ferulic acid, cinnamic acid, syringer, canferol, formonentin, and vanillic acid could not be detected. When the amount of gallic acid was evaluated, it was found that the amount of gallic acid in the fruit ripening period ranged between 167.39-313.03 µg/ml. Although the amount of gallic acid in the underground organs during the flowering period varied between 153.84-1039.78 µg/ml. During the flowering period, the amount of caffeic acid was found to be 0.04-0.09 µg/ml in the bulb and root. Caffeic acid could not be detected in the bulb and root organs during the flowering period. Studies conducted on the determining phenolic compounds with this species were very limited. In the phenolic component determination study of *Galanthus elwesii*, Ay et al., (2018) found gallic acid, caffeic acid, myricetin, kaemferol, formononetin, and quercetin. Prakash et al., (2007) determined phenolic compounds such as gallic acid, ferulic acid, protocatechic acid, quercetin and campherol in their study conducted for HPLC and LC-MS/MS in four types of bulbs (red, purple, white, and green). At the end of their study, they found that the amount of ferulic acid varied between 13.5 and 116, the amount of gallic acid between 9.3 and 354, the amount of protocatechic acid between 3.1 and 138, the amount of quercetin between 14.5 and 5110, and the amount of campherol between 3.2 and 481 µg/g. In the present study, it was found that the main phenolic component of *G. elwesii*, which was applied at different doses of P, was gallic acid.

Antioxidant activity

DPPH free radical scavenging activity obtained from *G. elwesii* samples treated with P fertilizer are shown in Figure 3. The highest DPPH free radical scavenging

activity during the flowering period was found to be 72.30% with 6 kg/da P treatment. The lowest DPPH free radical scavenging activity during fruit ripening period was found to be 49.85% with 3 kg/da P treatment. As seen in Figure 3, DPPH free radical scavenging activity of *G.elwesii* plant decreased by 53.05% in the fruit ripening period compared to the flowering period.

Ferrous ions chelating assay obtained from *G. elwesii* samples treated with P fertilizer are shown in Figure 4. The highest ferrous ions chelating assay during the fruit ripening period was found to be 66.77% with 6 kg/da P treatment. The lowest ferrous ions chelating assay during the flowering period was found to be 50.55% with 12 kg/da P treatment. As seen in Figure 4, DPPH free radical scavenging activity of *G.elwesii* plant decreased by 24.29% in the the flowering period compared to fruit ripening period.

Antioxidant studies on *Galanthus* species are very limited. As a result of our study, the DPPH ranking was found as fruit ripening period <flowering period according to the harvest time in the bulbs and roots. Aydin et al., (2015) reported that ethanol extracts obtained from bulbs and leaves of *Sternbergia lutea* species had higher antioxidant activity than bulbs extracts (86.60%) and leaf extracts (68.10%). Deniz (2016) investigated two plant taxa of the genus *Crocus* L., which are in the *Iridaceae* family, including very important geophytes in terms of chemical contents, and spreads in Denizli. The highest total antioxidant activity was detected in *C. pallasii subsp*. pallasii taxon, the underground extract (90.25%), the highest free radical scavenging activity was detected in the underground extract of the *C. cancellatus* subsp. mazziaricus taxon prepared with acetone (90.3%). Turan (2016) conducted a study to determine the antioxidant activities, phenolic substances, and flavonoids, the phenolic components with spectroscopic methods in the *Cyclamen alpinum Dammann* ex. *Sprenger* and *Cyclamen parviflorum Pobed*. In antioxidant activity trials, the highest activity was detected in the areial parts part of *C. parviflorum* (91.39%), but the lowest activity was detected in the underground part of *C. alpinum* (13.11%). Korcan et al., (2018) performed a study to determine the phenolic compound, total flavonoid substance, and total antioxidant capacity and antimicrobial activity in *Narcissus* Species *Narcissus papyraceus* bulbs grown in Izmir. The remaining DPPH rank of ethanol extracts of the plants at 30 µg/ml plant concentration is bulb peels (88.96%) > bulbs (55.5%). In the plant extracts that were prepared with water, the remaining DPPH rank of 30 µg/ml concentration was bulb peels (66.1%) > bulbs (25.3%). In a study conducted by Ay et al. (2018) to determine antioxidant activity of *G. elwesii* in flowering period and fruit ripening period, and reported that the highest DPPH free radical scavenging activity was in the fruit ripening period of *Galanthus elwesii* extracts. It was reported in the same study that antioxidant activity showed significant differences according to growth periods and plant organs.

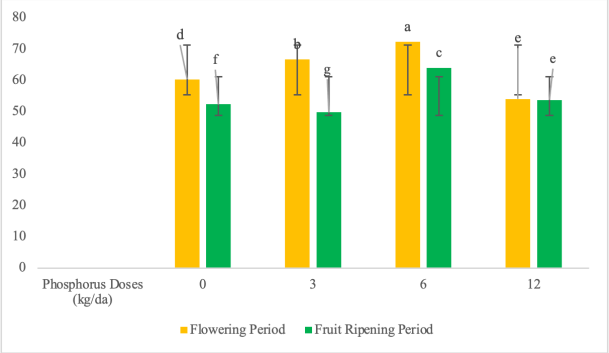

Figure 3. DPPH free radical scavenging activity (%inhibisyon) values of *G. elwesii* plants treatment with different doses of phosphorus (P) fertilizer according to different development periods.

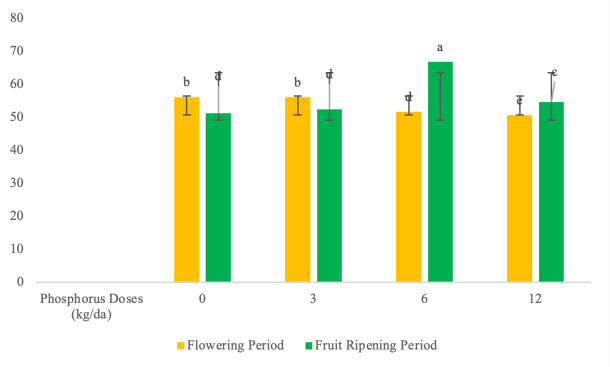

Figure 4. Ferrous ions chelating assay (%inhibisyon) values of *G. elwesii* plants treatment with different doses of phosphorus (P) fertilizer according to different development periods.

CONCLUSION

In the present study, the highest amount of total phenolic was determined as 358.36 mg gallic acid/g in *G. elwesii* species during the flowering period of the plant, and the lowest total phenolic content was determined as 80.13 mg gallic acid/g in the fruit ripening period. The highest amount of total flavonoid content was determined in the flowering period of the plant. Although it was found as 108.07 mg QE/g, the lowest total flavonoid content was determined as 52.33 mg QE/g in the fruit ripening period with 6 kg/da P treatment. gallic acid was the major phenolic component of *G. elwesii*. The highest DPPH free radical scavenging activity in the *G. elwesii* was determined to be 72.30% with 6 kg/da P treatment during the flowering period of the plant, while the lowest DPPH free radical scavenging activity was detected as 49.85% with 3 kg/da P treatment in the fruit ripening period of the plant. The highest ferrous ions chelating assay during the fruit ripening period was found to be

66.77% with 6 kg/da P treatment. The lowest ferrous ions chelating assay during the flowering period was found to be 50.55% with 12 kg/da P treatment. When the *G. elwesii* is evaluated according to different harvest times, it is seen that the highest total flavonoid , total phenolic content and DPPH free radical scavenging activity are in the flowering period. Only the amount of ferrous ions chelating assay was determined to be the highest during the fruit ripening period, which shows that the amount of secondary metabolite will vary according to the organ in the plant, harvest time, and many factors. In the present study, it is possible to express the total flavonoid , total phenolic content and DPPH free radical scavenging activity fruit ripening period <flowering period.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The authors declare that they have no competing, actual, potential or perceived conflict of interest.

Author contribution

Ebru Batı Ay: Investigation, Project administration, Writing, Funding acquisition; Şevket Metin Kara: Supervisor, Editing; Muhammed Akif Açıkgöz: Editing.

All the authors verify that the text, figures, and tables are original and that they have not been published before.

Ethics committee approval

Ethics committee approval is not required. This article does not contain any studies with human participants or animals performed by any of the authors.

Funding

We would like to thank the Scientific Research Projects Unit (BAP) of Ordu University for providing support to this research, as a part of the TF-1645 BAP Project.

Data availability

Not applicable.

Consent to participate

Not applicable.

Consent for publication

Not applicable.

Acknowledgments

This study is a part of Dr. Ebru BATI AY's doctorate thesis.

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RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(3): 696-702
DOI: https://doi.org/10.31015/jaefs.2023.3.23

Determining the energy balances of black carrot cultivation in Türkiye

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Abstract

Black carrot (known as *Daucus carota* ssp. sativus var. atrorubens Alef.) production of Turkey is increasing day by day. New production fields are added to existing ones and production are ever increasing. Black carrot is processed as concentrated and used as a natural colorant for the color of purple and tones in food and beverage industries. Black carrot is also consumed as a fermented beverage. However, the studies carried out on black carrot culture in Turkey are highly limited. This study was carried out to determine energy balances in black carrot production since it is a significant source of income. Results revealed that direct and indirect total energy input in black carrot cultivation was 58 014.8 MJ/ha, total energy output was 119 560 MJ/ha, Output/input ratio (OI) was 2.06 and net energy ratio (NER) was 1.06.

Keywords: Black carrot, Net energy ratio, Energy balance, Energy output, Energy input

Citation: Yalcin Dokumaci, K. (2023). Determining the energy balances of black carrot cultivation in Türkiye. International Journal of Agriculture, Environment and Food Sciences, 7 (3), 696-702

Received: January 25, 2023
Revised: August 10, 2023
Accepted: August 22, 2023
Published Online: September 29, 2023

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Available online at
https://jaefs.com/
https://dergipark.org.tr/jaefs

INTRODUCTION

Carrot belongs to the Daucus carota L. species of Apiacea family. Among the carrot species, black carrot (Daucus carota ssp. sativus var. atrorubens Alef.), is consumed as a fresh vegetable and concentration. Black carrot is a plant originating from Turkey, Middle and Far East and it is known that it has been cultivated for at least 3000 years. Black carrot contains various beneficial substances such as sugar, vitamin A and carotene. A previous study revealed the ascorbic acid content of black carrot as 26.40 mg/100 ml (Kirca, 2004). In recent years, black carrot has drawn attention with its rich anthocyanin content (1 750 mg/kg) and specific quality criteria (Kirca et al. 2006). Black carrot has high antioxidant activity and is a potential anthocyanin pigment source. Anthocyanins are the most popular natural food colorings providing the carmine color to foods and commonly preferred as an alternative of synthetic food dyes.

Anthocyanins were proved to have threpuatic effects on vascular diseases especially on artery thickening, cancers and diabetes, nerve degenerations and some eye diseases (Kong et al. 2003; Wrolstad, 2004). The fruits colored with black carrot juice provide several benefits with their high anthocyanin contents to chronicle diseases. There are also several anthocyanin-containing pharmaceutical products on market.

The anthocyanin pigment of black carrot is formulized together with ferulic, p coumaric, sinapic acid and p-hydroxybenzoic acid. The such formulation makes it more resistant against hydration, food pH and light (Khrandare et al. 2011).

Black carrot provides a wonderful strawberry red color with acidic pH values.

The extract is used in fruit juice coloring, softening, preservation, jellifying and pastry sector. Since it is a natural additive, it is not indicated with an e-number on the food and beverage labels. It also contains non-anthocyanin phenolics compounds and these compounds help to get pure and fresh fruit juices (Downham and Collns, 2000).

Black carrot is highly consumed as a fermented beverage in Turkey and India. Fermented carrot juice has an appetizing characteristic (Canbas and Deryaoglu, 1993). It also contains lactic acid. Lactic acid provides the sour taste of the juice, it is a peptic refresher, regulates pH of digestive system and allows the body to benefit more from some minerals (Misoglu, 2004).

There are many researches related energy balances for different vegetables and fruits, previously. Those are rose oil (Gokdogan and Demir, 2013), pumpkin seed (Haciseferogullari and Acaroglu, 2012), apple (Strapatsa et al. 2006; Rafiee et al. 2010; Yilmaz et al. 2010), potato (Hamedani et al. 2010; Mohammadi et al. 2008), cucumber (Mohammadi and Omit, 2010), barley (Mobtaker et al. 2010), cherry (Kizilaslan, 2009), grape (Kocturk and Engindeniz, 2009), apricot (Gezer et al. 2003), camelina (Şeflek et al. 2018) and sugar beet (Erdal et al. 2007; Haciseferogullari et al. 2003). However, researches carried out on black carrot culture are highly limited (Celik et al. 2010).

In addition to these, subsoil crops are examined, Allali et al. (2017) conducted in Morocco, the energy consumption value in onion production was found to be 107 483 MJ/ha, while this value was found as 74 270 MJ/ha in potato production. Özgöz et. al, (2017) determined that Chisel + Disk harrow spring tillage system in potatoe production, net energy value 102 217,25 MJ/kg, energy use efficiency 2.29, energy productivity 0,64 kg/MJ and specific energy is 1.57 MJ/kg. In another research on root edible vegetables, Çelik et al. (2010), compared organic and traditional cultivation of black carrot in Turkey conditions. In that study, the energy requirements obtained in the production of black carrots were stated that as 37 758.82 MJ/ha in organic farming and 75 335.72 MJ/ha in traditional.

Eregli town of Konya has the first place in black carrot production in Turkey. It was recorded that black carrot trading firms contracted for about 150-160 thousand tons production in the year 2018. Such contracted amount corresponds to about 4000 ha of production site.

Beside the yield increases in black carrot culture, production costs should also be reduced for profitable production. Agricultural machinery activities constitute the greatest cost item in black carrot culture and energy balances should be evaluated to reduce the machinery and energy costs in black carrot culture. Therefore, it was studied the energy balance of black carrot cultivation that is sustained traditionally. In line with all these, the relations between the energy output and input parameters of the black carrot were calculated in this study and compared in energy units.

MATERIALS AND METHODS

This research was carried out in Kuzukuyusu village of Eregli in 2018. The villagers intensely deal with black carrot culture. Trials were performed in 3 replications on 3m x 50 m plots according to randomized block design. Soil texture of the experimental field was loam. Soils were alkaline (pH=8.44) and nonsaline, and had sufficient potassium, deficient phosphorus, and highly lime content.

In the region, black carrot is sown in the same fields every year. Traditional production processes are summarized in Figure 1.

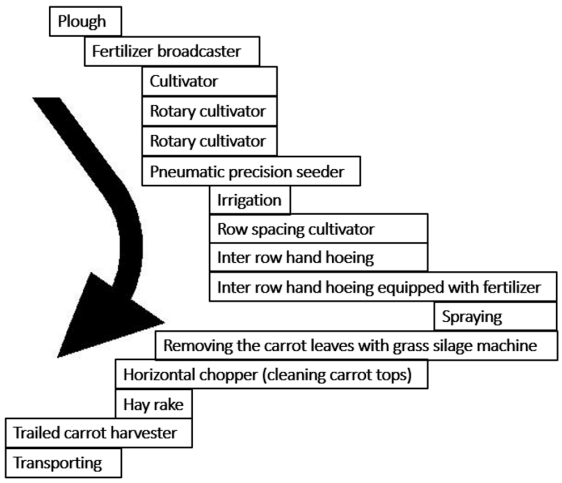

Figure 1. Black carrot production processes

The experimental field was plowed on 10 March 2018 with a moldboard plough. Diammonium phosphate fertilizer was applied to the field at 400 kg/ha using a disc fertilizer broadcaster. The field was tilled by a cultivator, and the seedbed was prepared with a rotary tiller. The ridge cultivation sites were formed with a ridger roller. Sowing was performed on 25 March 2018 using a high precision vacuum sowing machine at 2 cm in-row spacing. Black carrot seeds were sowed to the narrow row sowing surface with three rows in a ridge with 7.5 cm row spacing. Sprinkler irrigation was performed four times and for 2 hours in the first month to ensure of seed emergence. A cross-sectional view of a ridge is presented in Figure 2. Un-coated black carrot seeds were used in the experiments, and the sowing norm was 3,500 g/ha.

Interrow weeding was performed 4 times with a four-foot cultivator arranged to 75 cm row spacings. Manual weeding was made for the weeds growing in the rows also 4 times. A fertilizing rotary hoe was used to provide 200 kg/ha urea and 15 kg/ha ammonium nitrate separately. Herbicide was applied two times with a sprayer. Sprinkler irrigation was performed 13 times and each of them took

6 hours. A submerged 45 kW pump and a total of 8,630 m^3/ha irrigation water were used in irrigations.

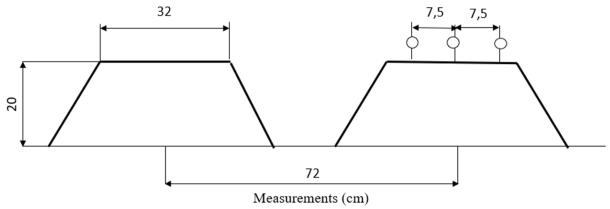

Figure 2. Cross-section of a ridge sowing

Greens were cut before the harvest with a weed silage machine. Heads were cleaned with a horizantal weed chopper, and greens were collected with a finger disc hay rake. Carrots were pulled with a carrot harvester, and harvested carrots were transported to a washing facility in a trailer. An average yield of 61,000 kg/ha was obtained in experiments.

During the experiments, tractor fuel consumption was evaluated by using a fuel gauge (a Rudolf Schmitt-brand, ±0.5%). Effective work performances were calculated by taking into account forward speeds and work widths.

The parameter provided in Table 1 were taken into consideration to define the energy balance of the black carrot. The values of energy equivalents of black carrots are indicated in Table 2.

The energy balances of all inputs in all cultivation processes which are hoeing, tillage, sowing, irrigation, herbicide application, fertilizing, harvest, and transport were measured. As the Table 3, input energy and output energy values were either measured by field conditions or quoted from the former literature.

Machine manufacturing energies of each implement used in black carrot culture were calculated as Equation 1 (Yavuzcan, 1994).

$$M_p = (M_u + F) \times 0.82 + Y_d \text{ (MJ)} \quad (1)$$

M_p : Machinery manufacturing energy
M_u : Material production energy
F : Factory energy
Y_d : Replacement energy

Table 1. Definition of energy parameters

Parameter	Definition	Unit
Direct energy inputs (E_d)	Diesel input	MJ/ha per year
Indirect energy input (E_i)	Machinery + seed + fertilizers + herbicides, etc.	MJ/ha per year
Total Energy input (E_T)	$E_T = E_d + E_i$	MJ/ha per year
Energy Output (EO)	Biomass energy	MJ/ha per year
Energy use efficiency	Energy Output (MJ per ha) / Energy Input (MJ per ha)	-
Energy productivity	Yield (kg per ha) / Energy input (MJ per ha)	kg/MJ per year
Specifit energy	Energy Input (MJ per ha) / Yield (kg per ha)	MJ/ha per year
Net Energy	Energy Input (MJ per ha) - Energy input (MJ per ha)	MJ/ha per year

Table 2. Energy equivalents

Input	Energy equivalents	Reference
Human labor	1.87 MJ/kg	Fluck (1992)
Water*	2.95 MJ/m^3	Calisir (2007)
Seed	2.5 MJ/kg	Kaltschmitt and Reinhardt (1997)
Transporting	9.22 MJ/t.km	Kaltschmitt and Reinhardt (1997)
Herbicide	290 MJ/kg	Canavate and Hernanz (1999)
N	78.1 MJ/kg	Canavate and Hernanz (1999)
P_2O_5	17.40 MJ/kg	Canavate and Hernanz (1999)
Material production coefficient of tractor	49.453	Acaroglu (1998)
Material production coefficient of steel	35.216	Acaroglu (1998)
Fuel and oil	40.035	Kaltschmitt and Reinhardt (1997)
The output energy equivalent of black carrot	1.96 MJ/kg	Celik et al. (2010)

Table 3. Machinery used in black carrot cultivation and manufacturing energies of this machinery

Agricultural Practices	Work performance (h/ha)	Characteristics	Machine manufacturing energy (MJ/kg)
Tractor	-	61 kW, 0.155 kg kW/h, 3340 kg, 6000 h/life	71.36
Plough	1.42	350 kg, 2300 h/life, 20 l/ha, work width 1.4 m	49.35
Cultivator	0.91	400 kg, 2300 h/life, 8 l/ha, work width 2.75 m	48.96
Disc fertilizer broadcaster	0.14	100 kg, 1000 h/life, 2.5 l/ha, work width 10 m	104.93
Rotary cultivators	1.25	700 kg, 2300 h/life, 21 l/ha, work width 2.35 m	48.96
Sprayer x 2	0.29	140 kg, 750 h/life, 1.5 l/ha, work width 10 m	102.26
The vacuum-type pneumatic precision seeder	2.5	525 kg, 1200 h/life, 6 l/ha, work width 2.8 m	63.34
Disc ridge	1.43	1200 kg, 2300 h/life, 10 l/ha, work width 2.1 m	49.15
Inter row hoeing x 4	1	250 kg, 2300 h/life, 7 l/ha, work width 2.8 m	56.76
Fertilizing inter row hoeing x 2	1.33	350 kg, 1200 h/life, 4 l/ha, work width 2.8 m	57.24
Forage harvester	2.85	500 kg, 1000 h/life, 25 l/ha, work width 1.3 m	105.09
Horizontal weed chopper	2	200 kg, 2000 h/life, 15 l/ha, work width 1.3 m	101.09
Finger disc hay rake	0.55	150 kg, 1000 h/life, 7.5 l/ha, work width 2.1 m	105.09
Pulled carrot harvester	10	2500 kg, 2500 h/life, 80 l/ha, work width 0.7 m	74.88

Energy inputs per unit area were calculated using manufacturing energies, the mass of the machine, economic life and work performance of the machines (Yavuzcan, 1994). The equation 2 was used in calculations:

$$M_{pe} = \frac{G.M_p}{T.W} \quad (2)$$

M_{pe} : Machine energy (MJ/ha)
G : Machine mass (kg)
T : Machine economic life (h)
W : Work performance (ha/h)

RESULTS AND DISCUSSION

The energy inputs in the mechanization of black carrot production were shown in Table 4. The ratio of tillage practices was 22.38% in total, sowing was 3.48%, maintenance operations were 17.92% in total, and harvesting operations were followed by 56.22%. The harvesting process is carried out in four stages (grass silage, scooping with a horizontal shredder, grass silage and digging up roots). High energy inputs in the harvesting process were followed by the harvester 36.78%, grass silage 10.71%, horizontal shredder 5.93% and grass rake 2.79%. Fuel-oil energy constituted 55.43% of the mechanization processes. In the region, modified potato harvesters and intensive labor are used in black carrot harvest. However, because of increasing labor costs and problems in finding available labor, pull-type or self-propelled storage-type black carrot harvesters

Table 4. Energy inputs values in the mechanization of black carrot culture

Agricultural practices	Tractor Manufacturing Energy (MJ/ha)	Tool-Machine Manufacturing Energy (MJ/ha)	Fuel + oil Energy (MJ/ha)	Labor Energy (MJ/ha)	Total (MJ/ha)
Tractor (MJ/h)	39.72	-	-	-	-
Plow	56.40	10.74	800.70	2.65	870.49
Single disc fertilizer spreader	5. 56	2.12	100.09	0.26	108.03
The second tillage in spring (Cultivator)	48.96	8.51	320.28	1.70	379.45
Rotary cultivators	48.96	21.29	840.75	2.34	913.34
Disc ridger	56.80	32.05	400.35	2.67	491.87
The vacuum-type pneumatic precision seeder	99.30	69.28	240.21	4.68	413.47
Inter row hoeing x 4	158.88	24.68	1,120.96	7.48	1,312.00
On-row hoeing x 4	-	-	-	314.16	314.16
Fertilizing inter row hoeing	52.83	20.86	160.14	2.49	236.32
Sprayer x 2	23.04	10.90	120.10	1.08	155.12
Forage harvester	113.20	151.31	1,000.87	5.33	1,270.71
Horizontal weed chopper	79.44	20.21	600,53	3.74	703.92
Finger disc hay rake	21.85	8.41	300,26	1.03	331.55
Pulled carrot harvester	397.20	745.20	3,202.,0	18.70	4,363.90
Total	**1,162.42**	**1,125.56**	**9,208.04**	**368.31**	**11,864.33**

Table 5. Direct and indirect energy inputs in black carrot culture

Direct Energy Inputs	MJ/ha	(%)
Tractor Energy	1,162.42	2.00
Fuel-oil Energy	9,208.04	15.87
Labor Energy	368.31	0.63
Tool-machine Energy	1,125.56	1.94
Indirect Energy Inputs		
Fertilizer Energy	1,987,5.95	34.26
Herbicide Energy	272.51	0.47
Seed Energy	8.75	0.02
Irrigation Energy	25,458.85	43.88
Transportation Energy	534.76	0.92
Total Input	**58,014.8**	**100**

Table 6. Consumption of energy and energy ratios in black carrot culture

Total input (MJ/ha)	58,014.8
Total output (MJ/ha)	119,560
Net Energy (MJ/ha)	61545,2
Energy use efficiency	2,06
Energy productivity (kg/MJ)	1,05
Specific energy (MJ/kg)	0,95

have started to be imported. These harvesters should be modified and made suitable for regional conditions. Also, to reduce this energy input, combined carrot harvesters that complete the harvest in one pass can be used. For this, it is necessary to invest in mechanization in enterprises. Among the other mechanization implementations, tillage-group machines constitute 2,655 MJ/ha energy input (22%). Such a case was because of the small-granulation seed bed requirement of black carrot seeds. The third group was composed of maintenance-care processes.

According to the Table 5, direct and indirect energy inputs of black carrot cultivation were given. Considering the general energy inputs, irrigation energy comprised the most energy input with 44%, and it was followed by fertilization energy with 34% and fuel-oil energy with 16%. Regional black carrot cultivated soils contain low organic matter and high lime. Therefore, fertilization and irrigation energy inputs of the present study were relatively high. Fuel-oil energy input may be reduced especially by using vertical rotary cultivator and combined tillage combinations. In this study, the total energy input was found to be 58 014, 8 MJ/ha, and this value which was determined by Celik et al. (2010) was found to be lower than the input value (75,335 MJ/ha) obtained in traditionally produced black carrots in the same region.

It was shown in Table 6, some basis indicators of the energy performance of black carrot production using the energy accounting approach commonly used in the energy literature. The net energy balance was determined as 61 545.2 MJ/ha in black carrot. When this value is evaluated in terms of energy use, it reveals that black carrot agriculture has passed the sustainability test. Among the energy inputs, the high level of irrigation and fertilization energy inputs draws attention.

Energy use efficiency, energy productivity and specific energy values were determined as 2.06, 1.05 kg/MJ and 0.95 MJ/kg, respectively. Celik et al. (2010) reported the energy use efficiency and energy porductivity in ten conventional black carrot agriculture as 1.30 and 0.66 kg/MJ. The energy use efficiency and energy productivity values obtained from the research were found to be high. This is due to the higher yield value of black carrot and the low total energy input.

It has been reported that the energy use efficiency, energy productivity and specific energy values are between 1.70 and 1.77, 0.47 and 0.49 kg/MJ, and 2.03 and 2.12 MJ/kg in potato production in different soil cultivation systems in Central Anatolian conditions (Özgöz et al., 2015). When compared with potato, the value of energy use efficiency and energy productivity was found to be higher, and the amount of energy (specific energy) required to obtain one kg of product was found to be lower. It was reported that the specific energy for vegetable production in Turkey is 1.14 MJ/kg for tomato, 0.98 MJ/kg for melon and 0.97 MJ/kg for watermelon (Çanakçı et. al., 2005).

CONCLUSION

To increase the energy output rate, it is necessary to increase the yield of black carrots. For this, the solution of the seed problem is necessary. The local population in the region produces black carrot seeds and is used in the planting process. Calibration and coating of these seeds are not performed. For this reason, yield values remain low due to field exit. On the other hand, research should be carried out especially about ridge-sowing, smooth sowing, on-row sowing distances, ridge dimensions and closing wheels of the seeders. In this way, field yield levels may ebe improved. Despite all these unfavorableness, energy use efficiency value being 2,06 indicates that black carrot agriculture is sustainable in Turkey. Research should be conducted on the use of seeds with a shorter vegetation period. Thus, a reduction in irrigation energy input can be achieved. At the same time, with the modification studies on the combined self-propelled potato harvesters, the mechanization energy input will be able to reduce by harvesting the black carrot at once.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

The author announced that for this research article, I have no actual, potential or perceived conflict of interest.

Author contribution

The author read and approved the final manuscript. The author confirms that they have not been published before and that the Text, Figures, and Tables are original.

Ethics committee approval

Ethics committee approval is not required. This article does not contain any studies with human participants or animals performed by any of the authors.

Funding

The author declared that this study has received no financial support.

Data availability

All data obtained or generated during the investigation appear in the published article.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(3): 703-717
DOI: https://doi.org/10.31015/jaefs.2023.3.24

Export competitiveness of Türkiye's agricultural machinery and equipment sector

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Abstract
The aim of the study is to determine the competitiveness of Turkey's agricultural machinery and equipment sector. Balassa's Revealed Comparative Advantage (RCA) approach was used in the period 2002-2021 by using International Trade Center database. Balassa's Revealed Comparative Advantage RCA2 Index for 39 agricultural machinery and equipment product groups (their sub-product groups of 69, 82, 84, 87) related to Harmonized System 6-digit product classification, Vollrath's Relative Export Advantage-RXA, Net Export Index-NEI, and Export-Import Rate Index-EIRI indices were calculated. According to the results, while Turkey has a competitive advantage in 13 of the agricultural machinery and equipment product group exports, it has a competitive disadvantage in the other 26 product groups.
Keywords: Agricultural Machinery and Equipment, Revealed Comparative Advantages, Vollrath Index, Net Export Index, Export-Import Rate Index

Citation: Aktas Cimen, Z., Ertekin, C. (2023). Export competitiveness of Türkiye's agricultural machinery and equipment sector. International Journal of Agriculture, Environment and Food Sciences, 7 (3), 703-717

Received: August 01, 2023
Revised: August 21, 2023
Accepted: August 22, 2023
Published Online: September 29, 2023

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Available online at
https://jaefs.com/
https://dergipark.org.tr/jaefs

INTRODUCTION

The advantages of free trade and international specialization are demonstrated by Ricardo's theory of comparative advantage. Analyzing a country's foreign trade structure is required to ascertain its comparative advantage on a product or sectoral basis (Kilicarslan, 2019). Developing countries like Turkey have changed the dynamics of the global economy (Aktas Cimen & Kutlu, 2023; Reddy, 2018). According to the International Monetary Fund (2020), despite the expectation of a slowdown in global trade in 2023; expectations for emerging economies are stronger than for developed economies. A good, strong, and innovative one in the global economy has a competitive advantage (Saricoban & Yalcin, 2020).

Competitiveness in the global environment is defined as the ability of an industry (firm, country) to trade in the global market in a sustainable manner (Fronberg & Hartmann, 1997; Esterhuizen & Van Rooyen, 2001). This study focuses on the analysis of Turkey's agricultural machinery and equipment sector. It evaluates Turkey's competitiveness in global markets in the relevant sector in terms of the position of exported products and reveals the competitiveness of the sector. It is of great importance in terms of planning targets and forming government policies in order to increase the competitiveness of the sector (Startiene & Remeikiene, 2014). It is also useful for seeing the results of policy changes and assessing its contribution to economic well-being. In order to analyze the sectoral changes that may occur due to various factors, it would be more accurate to evaluate the past years with a holistic approach (Akyuz et al., 2020).

The first index to measure competitiveness using post-trade data was introduced by Liesner (1958) and Balassa (1965) developed this index. Later, Vollrath (1991)

said that Balassa did not include enough imports and made a double counting error, and he developed Balassa's index and revealed the Vollrath Index.

In this study the competitiveness of Turkey's exports of agricultural machinery and equipment will be determined using the Revealed Comparative Advantage (RCA) approach. In order to achieve this, RCA coefficients were generated for each product group using the International Trade Center (ITC) Trade Map, annual time series, export and import data (x1000 USD), and 6-digit product classification. In the analysis, 39 different product groups in the 6-digit classification within the 69, 82, 84, 87 product codes were interpreted separately.

Overview of Agricultural Machinery and Equipment Exports

Turkey and the world's exports of agricultural machinery and equipment between 2002-2021 and their share in total exports were shown in Table 1. Table shows that Turkey's exports of agricultural machinery and equipment have been in an increasing trend in the 20-year period examined, excluding the 2009 financial crisis. Turkey's agricultural machinery and equipment exports, which emerged in December 2019 and had a global impact in a short time, decreased by 8.69% in 2020, but decreased by 2.66% in 2021. Agricultural machinery and equipment, which had a share of 0.29% in Turkey's exports in 2002, increased to 0.48% in 2009, 0.75% in 2020, 0.73% in 2021. The share of world agricultural machinery and equipment exports in the World exports shows a partially stable outlook. Agricultural machinery and equipment exports, which had a share of 0.40% of the world's exports in 2002, share declined to 0.37% in 2006. However, although the share of agricultural machinery and equipment exports in world exports increased to 0.44% in 2008, it declined to 0.41% in 2009 due to the financial crisis.

World exports of agricultural implements and equipment, whose share in world exports fluctuated slightly between 2010 and 2021, accounted for 0.41% of exports in 2021. Although it increased by 7.69% in 2020 after the Covid-19 pandemic, it decreased by 2.38% in 2021. So, the epidemic seems to have affected Turkey's agricultural machinery and equipment exports more than the World's agricultural machinery and equipment exports. The increase in the share of agricultural machinery and equipment exports in total exports both in Turkey and in the world in 2020 may be due to the decrease in total exports due to the prohibitions and restrictions introduced to prevent the Covid-19 epidemic. However, while the share of agricultural machinery and equipment exports in the world's total exports in the 2002-2021 period examined, the share of agricultural machinery and equipment exports in Turkey's total exports is increasing.

Table 1. General Outlook of Exports of Agricultural Machinery and Equipment in Turkey and the World (2002-2021)

Year	Total Export of Turkey (1000 US $)	Total Agricultural Machinery and Equipment Exports of Turkey (1000 US $)	Rate (%)	Total Export of World (1000 US $)	Total Agricultural Machinery and Equipment Exports of World (1000 US $)	Rate (%)
2002	35761981	103085	0.29	6432105964	25693391	0.40
2003	47252836	228288	0.48	7498530918	29587322	0.39
2004	63120949	262052	0.42	9110737596	36383071	0.40
2005	73476408	283701	0.39	10360495753	40514027	0.39
2006	85534676	317204	0.37	11979108568	44807336	0.37
2007	107271750	414278	0.39	13809800618	54540516	0.39
2008	132027196	564629	0.43	16007659828	70206795	0.44
2009	102142613	485673	0.48	12384813282	50780145	0.41
2010	113883219	532938	0.47	15098981170	54769620	0.36
2011	134906869	626603	0.46	18141372916	70406035	0.39
2012	152461737	806325	0.53	18399990900	72907729	0.40
2013	161480915	853309	0.53	18858726557	74700191	0.40
2014	166504862	1022732	0.61	18862399126	73441172	0.39
2015	143844066	945804	0.66	16416895796	63892286	0.39
2016	142606247	860669	0.60	15923096945	62107793	0.39
2017	156992940	951580	0.61	17561440015	70440948	0.40
2018	167923862	1127056	0.67	19327897410	75898338	0.39
2019	180870841	1243226	0.69	18750885146	73187422	0.39
2020	169657940	1264995	0.75	17488466269	73160595	0.42
2021	225264314	1646014	0.73	22112533133	90904116	0.41

Source: Prepared by using Trade Map data (Trade Map, 2023).

Literature Review

In the literature research, only one scientific study was found regarding the competitiveness of Turkey's agricultural machinery and equipment sector. Berk & Erdem (2019) compared the agricultural machinery and tractor exports of Turkey with some selected countries with RCA, RXA, and Relative Trade Advantage (RTA) and Revead Competitiveness (RC) indices between 2008 to 2017. According to the results, the Turkish agricultural machinery and tractor sector is highly sensitive to local currency and inflation rates. RCA index was a minimum 0.68 in 2008 and a maximum of 1.15 in 2015.

While Kosekahyaoglu & Ozdamar (2005) comparatively analyzed Turkey and Czech Republic, Hungary, Poland, and Estonia, which are members of the European Union, in terms of sectoral competitiveness and foreign trade structures, the different forms of RCA, NEI, lnRCA2,3 and Donges Comparative Export Performance (CEP) indices. Altay & Gurpinar (2008) used RCA, RXA, NEI, The Relative Import Advantage (RMA) , RTA, RC, Export Smiliraty Index (ESI) to determine the international competitiveness of the Turkish furniture industry. Sarica (2016) examined the competitiveness of Turkey's foreign trade in agricultural products with the help of RCA, RC, RTA and Revelaed Export Advantage Index (lnRXA). Erkan (2013), RCA2, RXA2, and EIRI to examine the competitiveness of Turkey's textile and apparel industry exports between 1993 and 2009. Erkan & Batbayli (2017) revealed the global markets in terms of exports on a sectoral basis for the Black Sea Economic Cooperation Organization (BSEC-12) countries and Turkey with the "export similarity index"; Balassa's RCA and Vollrath's RXA Indexes to measure the competitiveness of 12 BSEC members in the period of 2000-2014. Saricoban & Kosekahyaoglu (2017b) used RCA and RXA2 indices to measure Turkey's export competitiveness in agricultural product groups. Cestepe & Tuncel (2018) used RTA, RC and Vollrath index to determine the international competitiveness of the Turkish iron and steel industry for the period of 2007-2016. Magezi & Okan (2019) investigated the competitiveness of Turkey and EU countries in forest products trade for the period of 2006-2016 by using the RCA, RXA, RMA, RTA, Cross Relative Export Advantage, Cross Relative Import Advantage CRMA, and Logarithmic Cross Relative Competitiveness indexes. Akyuz et al. (2020) used the RCA, RXA, RMA, RTA, lnRXA, and RC indexes to determine the competitive position of the Turkish forest products industry for the period of 2001-2017. Saricoban & Yalcin (2020) used RCA2, RXA, and NEI indices to determine the export competitiveness of Turkey's carpet industry by RCA index. Ozbas & Yildirim (2022) used the RCA, RXA, and RTA indexes to determine the top ten products in which Turkey has the highest competitive power for the period of 2001-2019. Ortikov et al. (2019) determined Uzbek foreign trade in agriculture with different groups of countries. Agrarian trade competitiveness and territorial and commodity structure changes were analyzed between 2000 and 2018 by using "product mapping approach" method, Herfindahl-Hirschman Index, Lafay Index LI, and NEI. According to the results, Uzbek agricultural exports are competitive with regard to Asian and CIS countries, and limited when compared with other territories. Erdem (2020) searched the competitiveness of the world dried product sector such as apples, prunes, apricots, figs, and grapes. In this study, the data was subjected to the RCA, RXA, RMA, RTA and RC indexes for 2007 to 2017 data of China, USA, Chile, Germany, Iran, the Netherlands, South Africa, France, Uzbekistan, Argentina, Spain, Turkey, and India. Results showed that the world dried product sector is very responsive to economic crises and to local currency rate. The RCA index was found to be 4.66 in 2007 for Turkey and it decreased to 4.45 by 2009 during the World economic crisis. The other breaking point was 2013 when Turkey experienced both economic and political crises. Saptana et al. (2021) determined the competitiveness of shallot in Indonesia. The results showed that shallot farming in Indonesia has both competitive and comparative advantages. While the highest competitive and comparative advantages were found in the dry season in the upland of Malang district with the coefficient values of PCR (Private Cost Ratio) of 0.268-0.508 and DRCR (Domestic Resource Cost Ratio) of 0.208–0.323, the lowest competitive advantage was found in the lowland of East Lombok district in the dry season with a coefficient value of PCR 0.728–0.844. So, it is more profitable for Indonesia to increase domestic shallot production than to import. Improving shallot competitiveness can be carried out by implementing advanced technology, agricultural infrastructure, capacity building of farmers' resources, and government incentive policies to increase productivity and competitiveness sustainability. Torayeh (2013) analyzed the export competitiveness of Egypt's agricultural exports in the European Union between 1998 and 2010 by RCA index and CEP index. Results showed that although Egypt's exports of fruit & vegetables to the EU are growing, it is limited to the competition from other MEDC which has grown dramatically in the last years. The results revealed that while Egypt is losing its comparative advantages in Saudi market, Russian and Ukrainian markets are found to be more optimistic. Egypt experienced a progressive trend in gaining a comparative advantage in exporting agricultural products in comparison to the main rivals. Zhang and Sun (2022), examined the static distribution of agricultural trade comparative advantage in countries along the Belt and Road (B&R) and China by utilizing the Balassa RCA index, Revealed Symmetric Comparative Advantage index and the ordinary least squares correlation analysis. The results showed that the initial comparative advantage of most agricultural products along the B&R and China deteriorated, simultaneously, but the initial comparative disadvantage of most and

some agricultural products along the B&R and China improved, respectively. Pakravan and Kalashami (2011) searched Iran, U.S, and Turkey's pistachio export by RCA using agricultural and total economy export, then forecasted by using Auto-Regressive Integrated Moving Average approached for 2008-2013. The results showed that, Turkey and Iran had comparative advantage in pistachio export in 1982-2007, but US did not. Also, forecasting RCA index, based on both commodity baskets, show the improvement of US Pistachio export situation, unlike the values of RCA index forecasting for Iran and Turkey is falling. Long (2021) analyzed the international competitiveness of six China's representative agricultural products by TC and RCA index between 1994 and 2013. The results indicated that, China should vigorously promote the production and foreign trade in traditional agricultural products. At the same time, comprehensive measures should be taken to enhance the international competitiveness of disadvantaged agricultural products.

METHOD

In the study, the RCA method was used. RCA coefficients compare the domestic specialization of a country in a sector with the specialization of the world or any country (Erkan, 2013).

In the analysis of the study, Balassa's Comparative Advantage (RCA2) method, which is frequently used in the literature to measure competitiveness with post-trade data, was later developed by Vollrath in 1991, the RXA, NEI and EIRI were used.

Balassa's RCA_2

The first index to measure competitiveness using export data is the Liesner Index (L-RCA), which was introduced by Liesner in 1958. Balassa (1965) developed the RCA1-Revealed Comparative Advantage Index by making the L-RCA index more functional (Saricoban & Kosekahyaoglu, 2017a). Balassa changed the RCA Index in 1977, 1979 and 1986 (Jagdambe, 2019). Balassa's RCA2 Index compares a country's share in total exports of a product or industry with its share in the world (or group of countries) total exports of the product or industry under consideration (Esterhuizen & Van Rooyen, 2001; Mykhnenko, 2005). In other words, it is used to calculate the relative advantage or disadvantage of a country in a product or sector (Startiene and Remeikiene, 2014). Balassa's RCA_2 Index is formulated as follows (Balassa, 1965);

$$RCA_2 = \frac{X^j_{kt}/X^j_t}{X^w_{kt}/X^w_t} \qquad (1)$$

Where;

X^j_{kt} Export of 'k' good (or sector) in 't' period of country 'j',

X^j_t Total exports of country 'j' in period 't',

X^w_{kt} Total world exports of good (or sector) 'k' in period 't',

X^w_t World total export values in the 't' period. The results of this index are interpreted as follows (Saricoban et al., 2017; Sarıçoban & Kösekahyaoğlu, 2017_b);

- If RCA2>1, country 'j' export share of 'k' good (or sector) is greater than the world's share of 'k' (sector) export. This indicates that country j has competitive power in the export of 'k' good (sector).

- If RCA2<1, the export share of 'k' good (or sector) of country 'j' is smaller than the export share of 'k' good (sector) of the world. This indicates that country j has a competitive disadvantage in the export of 'k' good (sector).

- If RCA2=1, the export share of 'k' good (sector) of country 'j' is equal to the world's share of export of 'k' good (sector). This indicates that there is a balance in the export competitiveness of goods 'k' (sector).

In summary, if RCA2>1, then that country has a comparative advantage in the product (or industry) and is relatively more specialized in terms of exports (Bojnec & Ferto, 2006).

Hinloopen and Van Marrewijk (2001) made a fourfold classification as follows in order to make the RCA Index results easier to interpret. This classification is detailed;

Class 1, 0<RCA≤1, No advantage, no competitiveness,

Class 2, 1<RCA≤ 2, Weak competitiveness,

Class 3, 2<RCA≤ 4, Medium advantage,

Class 4, 4<RCA, Strong competitiveness. Class 1 relates to sectors that do not have a comparative advantage, while class 2-3-4 relates to roughly all sectors with comparative advantage.

Vollrath's RXA

Vollrath's RXA Index is based on the RCA Index developed by Balassa (1965). Unlike Balassa's RCA Index, the RXA Index prevents double couting and increases the reliability of the results (Saricoban & Yalcin, 2020). The RXA Index is defined as the relationship between the rate of exports of some products of a country in the world market and the rate of exports of all other products of this country in the world market (Hambalkova, 2006). The index results provide the opportunity to compare the domestic specialization of a country in a certain sector (or product group) with the world specialization. Vollrath's RXA Index is calculated using the following equation (Fronberg & Hartmann, 1997; Saricoban & Kosekahyaoglu, 2017a; Saricoban & Yalçın, 2020):

$$RXA = \frac{X^{j}_{kt}/X^{j}_{-kt}}{X^{-j}_{kt}/X^{-j}_{-kt}} \quad (2)$$

Where;

X^{j}_{kt} Export of 'k' good (or sector) in 't' period of country 'j',

X^{j}_{-kt} Total exports of country 'j' excluding commodity 'k' in period 't',

X^{-j}_{kt} Total world exports of 'k' good (or sector) excluding '' in the 't' period,

X^{-j}_{-kt} World total exports excluding X^{-j}_{kt} and X^{-j}_{-kt} in the 't' period.

Index results are interpreted as follows (Hambalkova, 2006; Saricoban & Yalcin, 2020);

- RXA>1, the country has a comparative advantage (competitive advantage) in the evaluated product category,

- If RXA<1, it indicates that the country has a comparative disadvantage (competitive disadvantage).

RXA Index results can be divided into 4 groups and interpreted in more detail (Hinloopen and Van Marrewijk, 2001);

Class 1: 0<RXA≤1, No advantage, no competitiveness (specialization),

Class 2: 1<RXA≤2, There is poor competitiveness (weak specialization),

Class 3: 2<RXA≤4, Moderate advantage/competitiveness (medium specialization),

Class 4: 4<RXA means strong competitiveness (strong convergence).

NEI

According to Gnidchenko and Salnikov (2015), an appropriate comparative advantage index should reflect net trade. The RCA Index is calculated only with export values and does not take into account import data. NEI, also an RCA method, is interpreted as a country's relative ability to profit from trade in a particular product (Gnidchenko & Salnikov, 2021). The index describes an assessment of a country's trade with the rest of the world. NEI, which is calculated by dividing the export and import difference of a particular product group by the sum of exports and imports, takes values between "-1" and "+1" (Balassa & Noland, 1989). NEI is formulated as follows (Saricoban & Kosekahyaoglu, 2017a; Saricoban & Yalcin, 2020.);

$$NEI^{j}_{kt} = -\frac{X^{j}_{kt} - M^{j}_{kt}}{X^{j}_{kt} + M^{j}_{kt}} \quad (3)$$

Where;

Xjkt Export of 'k' good (or sector) in 't' period of country 'j'

Mjk Import of 'k' good (or sector) in 't' period of country 'j'.

NEI^{j}_{kt} =-1; Negative values indicate full imports in that product group (or sector). Import is more important and the country has a competitive disadvantage in that product group (or sector),

NEI^{j}_{kt} =1; Positive values indicate full exports in that product group (or sector). Export is more significant and demonstrates the nation's advantage in that product group (or sector),

NEI^{j}_{kt} =0; It expresses a balanced situation in trade and the existence of maximum intra-industry trade.

NEI value of '-1' or '+1' indicates that there is no intra-industry trade (Bozduman & Erkan, 2019).

EIRI

The fact that only export-related data is used in the measurement of competitiveness is criticized in some studies. Bowen (1983) states that it would be a more accurate approach to measure competitiveness with a method based on net exports (export-import), which includes not only exports but also imports. The index provides information about the level of specialization in the goods exported by a country and is formulated as follows (Mikic, 2005; Erkan, 2013; Saricoban & Kosekahyaoglu, 2017a);

$$RCA_{4} = \frac{X^{j}_{kt}/X^{j}_{t}}{M^{j}_{kt}/M^{j}_{t}} \quad (4)$$

Where,

X^{j}_{kt} Country 'j' exports of good (or sector) 'k' in period 't',

X^{j}_{t} Total exports of country 'j' in period 't',

M^{j}_{k} Country 'j' imports of good (or sector) 'k' in period 't',

M^{j}_{t} Total imports of country 'j' in period 't'.

If;

-RCA4>1, if country 'j' specializes in commodity 'k' (or sector) and has a comparative advantage (competitive advantage) in the export of this commodity;

-RCA4<1 indicates that country 'j' is disadvantaged in commodity (or sector) 'k', that is, its export performance is low (Mikic, 2005).

RESULTS AND DISCUSSION

In the analyzes, the RCA coefficients in Turkey's agricultural machinery and equipment sector exports were calculated based on the "Trimmed Mean (TM)" values. TM, eliminating the highest and lowest values in a series and taking the arithmetic mean of the remaining values (Statistics, 2023). At the next stage, the distribution characteristic of the average RCA values of the sectors (volatility around the period average) was determined by means of the Coefficient of Variance (CV) (Kucukkiremitci, 2006). That is, the higher the CV, the higher the deviation from the mean. A product with a comparative advantage has a low CV value, indicating that its competition is stable. Erkan & Batbaylı (2017) stated that if the CV value is below 15 in the RCA and RXA indexes used by the BSEC member countries to determine their comparative advantage in global markets, it indicates that the competition of the product groups is stable.

A total of 49 product groups were selected for analysis, and 39 product groups formed as a result of combining some product groups are presented in Table 2. Abbreviations used in tables;

-Product Code: PC

-2002-2011 Average: A (arithmetic average of 10 years RCA coefficients)

-2012-2021 Average: B (arithmetic average of 10 years RCA coefficients)

-The Superiority Rating expresses the status of superiorities relative to the appropriate average RCA values.

Balassa's RCA Results

RCA coefficient values for Turkey's agricultural tools and equipment product group are presented in Table 3.

According to the Table, the TM values show that 13 out of 39 product groups have a competitive advantage and 26 have a competitive disadvantage. Turkey has a strong competitive advantage in 4 out of 13 product groups, a moderate advantage in 3 and a weak advantage in 6. The product group coded '843780' has the highest competitive advantage, the product group coded '843410' has the highest moderate advantage and the product group coded '843610' has the highest weak advantage.

Among the 26 product groups in which Turkey is disadvantaged, the arithmetic average of RCA values decreased in the second period compared to the first period of only 6 product groups did change. However, the increase in the arithmetic mean of RCA values in the second period compared to the first period of 20 product groups, which are disadvantaged in competition, is remarkable and the changes that should be especially emphasized. The arithmetic mean of the RCA values of the product groups '870110', '820190', '843319', '820160' and '843311' decreased in the second period. The most disadvantageous is the product group with the code '843311'.

Vollrath's RXA Results

The results according to TM values are presented in Table 4.

According to the table, Turkey has a competitive advantage in 13 product groups and a competitive disadvantage in 26 product groups in 39 agricultural tools and equipment exports. Among the product groups in which Turkey has an advantage, 4 have a strong advantage, 3 have a moderate advantage and 6 have a weak advantage. The increases in the RCA values of the products coded '843780' and '843790', which are in the first two ranks where Turkey has a strong advantage, in the second period indicate an increase in their competitiveness. However, the decline in the RCA value of the product group coded '843352', which ranks third, in the second period indicates a loss of competitiveness. The increase in the competitiveness of the other 3 product groups in the second period, except for the product group coded 843352 in the strong superiority group, is promising for Turkey and the sector. This result indicates that the policies implemented were successful. The CV value of the first 2 products with strong competitive advantage varies between '26.79' and '28.49' and is partially lower than the other product groups. This finding indicates that exports of the first 2 product groups with strong competitive advantage are more stable, albeit partially.

According to TM values, 2 of the 3 product groups coded '843410', '843210', '843629', which are ranked as moderately superior, reached strong superiority in the second period, except for the product group coded '843410'. These changes should be particularly emphasized. The product group coded "843210' has the lowest CV value of '32.11' and seems to be more stable in competition.

26 product groups are disadvantaged according to TM values. However, 20 disadvantaged product groups have reached an advantageous position in the second period compared to the first period. In addition, the fact that the product groups coded '843240', '841939', '843290', '871620' and '842490' had no competitiveness in the first period, but increased to weak superiority in the second period indicates increases in competitiveness. However, the decline in the RCA value of the product group coded '870110', '820190', '820150', '843319', '820160' and ' 843311' in the second period indicates a decrease in competitiveness. Products in the weak superiority classification are on the advantage/disadvantage border

Table 2. List of Product Codes and Labels of Agricultural Machinery and Equipment Products

	Product code	Product label
1	690990	Ceramic troughs, tubs and similar receptacles of a kind used in agriculture; ceramic pots, jars and similar articles of a kind used for the conveyance or packing of goods (excluding general-purpose storage vessels for laboratories, containers for shops and household articles)
2	820150	Secateurs and similar one-handed pruners and shears, incl. poultry shears, with working parts ...
3	820160	Hedge shears, two-handed pruning shears and similar two-handed shears, with working parts of base metal
4	820190	Scythes, sickles, hay knives, timber wedges and other hand tools of a kind used in agriculture, horticulture or forestry, with working parts of base metal (excluding spades, shovels, mattocks, picks, hoes, rakes, axes, billhooks and similar hewing tools, poultry shears, secateurs and similar one-handed pruners and shears, hedge shears, two-handed pruning shears and similar two-handed shears)
5	820840	Knives and cutting blades, of base metal, for agricultural, horticultural or forestry machines (excluding those for wood-working)
6	842490*	Parts of fire extinguishers, spray guns and similar appliances, steam or sand blasting machines
7	843210	Ploughs for use in agriculture, horticulture or forestry
8	843221	Disc harrows for use in agriculture, horticulture or forestry
9	843229	Harrows, scarifiers, cultivators, weeders and hoes for use in agriculture, horticulture or ...
10	843230**	Seeders, planters and transplanters for use in agriculture, horticulture and forestry
11	843240***	Manure spreaders and fertiliser distributors for use in agriculture, horticulture and forestry
12	843280	Agricultural, horticultural or forestry machinery for soil preparation or cultivation; lawn ...
13	843290	Parts of agricultural, horticultural or forestry machinery for soil preparation or cultivation ...
14	843311	Mowers for lawns, parks or sports grounds, powered, with the cutting device rotating in a horizontal ...
15	843319	Mowers for lawns, parks or sports grounds, powered, with the cutting device rotating in a vertical ...
16	843320	Mowers, incl. cutter bars for tractor mounting (excluding mowers for lawns, parks or sports ...
17	843330	Haymaking machinery (excluding mowers)
18	843340	Straw or fodder balers, incl. pick-up balers
19	843351	Combine harvester-threshers
20	843352	Threshing machinery (excluding combine harvester-threshers)
21	843353	Root or tuber harvesting machines
22	843359	Harvesting machinery for agricultural produce (excluding mowers, haymaking machinery, straw ...
23	843360	Machines for cleaning, sorting or grading eggs, fruit or other agricultural produce (excluding machines for cleaning, sorting or grading seed, grain or dried leguminous vegetables of heading 8437)
24	843390	Parts of harvesting machinery, threshing machinery, mowers and machines for cleaning, sorting ...
25	843490	Parts of milking machines and dairy machinery, n.e.s.
26	843410	Milking machines
27	843610	Machinery for preparing animal feedingstuffs in agricultural holdings and similar undertakings (excluding machinery for the feedingstuff industry, forage harvesters and autoclaves for cooking fodder)
28	843621	Poultry incubators and brooders
29	843629	Poultry-keeping machinery (excluding machines for sorting or grading eggs, poultry pickers of heading 8438 and incubators and brooders)
30	843680	Agricultural, horticultural, forestry or bee-keeping machinery, n.e.s.
31	843691	Parts of poultry-keeping machinery or poultry incubators and brooders, n.e.s.

32	843699	Parts of agricultural, horticultural, forestry or bee-keeping machinery, n.e.s.
33	841939	Dryers (excl. lyophilisation apparatus, freeze drying units, spray dryers, dryers for agricultural products, for wood, paper pulp, paper or paperboard, for yarns, fabrics and other textile products, dryers for bottles or other containers, hairdryers, hand dryers and domestic appliances)
34	843710	Machines for cleaning, sorting or grading seed, grain or dried leguminous vegetables
35	843780	Machinery used in the milling industry or for the working of cereals or dried leguminous vegetables (excluding farm-type machinery, heat treatment equipment, centrifugal dryers, air filters and machines for cleaning, sorting or grading seed, grain or dried leguminous vegetables)
36	843790	Parts of machinery used in the milling industry or for the working of cereals or dried leguminous vegetables or machines for cleaning, sorting or grading seed, grain or dried leguminous vegetables, n.e.s.
37	870110	Pedestrian-controlled agricultural tractors and similar tractors for industry (excluding tractor units for articulated lorries)
38	870190****	Tractors (excluding those of heading 8709, pedestrian-controlled tractors, road tractors for semi-trailers and track-laying tractors)
39	871620	Self-loading or self-unloading trailers and semi-trailers for agricultural purposes

Source: Prepared by using Trade Map data (Trade Map, 2023).
*As of 2017, product code 842490 also includes data for 842482 Agricultural or horticultural mechanical appliances, whether or not hand-operated, for projecting. For analysis, this product code was collected by us since 2017 and analyzed with the product code 842490 as before 2017.
**As of 2017, product code 843230 also includes product codes 843231 No-till direct seeders, planters and transplanters and 843239 Seeders, planters and transplanters (excl. no-till machines).
***Since 2017, product code 843240 also includes product codes 843241 Manure spreaders (excl. sprayers) and 843242 Fertiliser distributors (excl. sprayers and manure spreaders).
****From 2017, product code 870190, 870191 Tractors, of an engine power <= 18 kW (excl. those of heading 8709, pedestrian controlled tractors, road tractors for semi-trailers and track-laying tractors), 870192 Tractors, of an engine power > 18 kW but <= 37 kW (excl. those of heading 8709, pedestrian-controlled tractors, road tractors for semi-trailers and track-laying tractors), 870193 Tractors, of an engine power > 37 kW but <= 75 kW (excl. those of heading 8709, pedestrian-controlled tractors, road tractors for semi-trailers and track-laying tractors), 870194 Tractors, of an engine power > 75 kW but <= 130 kW (excl. those of heading 8709, pedestrian controlled tractors, road tractors for semi-trailers and track-laying tractors) and 870195 Tractors, of an engine power > 130 kW (excl. those of heading 8709, pedestrian-controlled tractors, road tractors for semi-trailers and track-laying tractors).
Note: The product codes were determined by the researchers with reference to the codes of the Republic of Turkey Ministry of Commerce, GTIP NO: 842441-842449-842482-8432-8433-8436-8478-870110-870191-870192-870193-870194-870195-871620 (Ticaret Bakanlığı (TB), 2023) was selected.

in terms of RCA coefficients. Therefore, they are priority product groups that should be taken into consideration in order not to lose competitive advantage.

NEI Results

The results of the analysis of Turkey's competitiveness and especially the level of specialization in Turkey's foreign trade in agricultural machinery and equipment with NEI are given in Table 5.

According to the table, Turkey has specialized in exports of 23 product groups and has a competitive advantage. However, Turkey has not been able to specialize in the exports of 16 product groups and has no competitive advantage. The 3 product groups with the highest level of specialization in Turkey's agricultural machinery and equipment exports are coded '843210', '843221', '843780' respectively. The competitiveness of the product groups coded '843352' and '843330', which are in the competitive advantage group, declined in the second period. In the product groups coded '843691', '843710', '843390', '843680', '843490', imports were more important in the first period and had a competitive disadvantage, while exports were more important in the second period and they reached a competitive position. This result indicates that the competitiveness of the sector has increased. Another positive development is that Turkey's imports decreased in 12 of the 16 product groups in which Turkey is a full importer. However, there is an increase in the imports of product groups coded '820160', '843359', '843353', '870110'. It should be emphasized that most of the product groups of the agricultural machinery and equipment sector increased their level of specialization in the second period and had a competitive advantage.

EIRI Results

The results of the analysis with the EIRI, which measures Turkey's intra-industry trade and is used only to determine Turkey's own trade performance, are presented in Table 6.

According to the table, Turkey has a competitive advantage in the exports of 24 product groups in the agricultural machinery and equipment sector. In other words, its trade performance is high. However, it has a disadvantage in the exports of 15 product groups and its export performance is low. The 3 product groups with the highest trade performance in Turkey's agricultural

Table 3. Balassa Index and Turkey's Agricultural Machinery and Equipment Product Group RCA Coefficients and Superiority Degrees

	PC	A	B	TM
Strong Superiority	843780	14.97	21.59	18.34
	843790	5.02	5.23	5.04
	843352	6.59	3.50	4.90
	843710	4.04	4.77	4.29
Moderate Superiority	843410	4.01	3.84	3.84
	843210	2.59	3.32	2.92
	843629	1.55	3.42	2.48
Weak Superiority	843610	1.11	2.95	1.97
	870190	1.52	2.21	1.87
	843230	1.06	2.34	1.71
	843221	1.29	2.03	1.65
	843229	1.35	1.31	1.31
	843621	0.57	1.66	1.06
Disadvantages	843240	0.74	1.21	0.96
	841939	0.63	1.24	0.93
	843290	0.37	1.22	0.79
	871620	0.47	1.21	0.78
	870110	0.99	0.52	0.72
	843699	0.61	0.78	0.69
	843320	0.58	0.70	0.64
	820190	0.65	0.52	0.58
	843490	0.35	0.75	0.56
	843691	0.51	0.63	0.56
	843340	0.18	0.90	0.52
	842490	0.18	1.04	0.50
	843359	0.49	0.51	0.50
	843390	0.36	0.58	0.48
	843360	0.28	0.51	0.38
	843280	0.29	0.43	0.34
	820840	0.22	0.42	0.32
	843353	0.17	0.48	0.32
	843330	0.28	0.36	0.32
	690990	0.10	0.24	0.16
	820150	0.16	0.12	0.14
	843680	0.08	0.20	0.14
	843319	0.07	0.05	0.06
	820160	0.05	0.04	0.04
	843351	0.04	0.06	0.04
	843311	0.03	0.02	0.02

machinery and equipment product group are coded '843210', '871620' and '843352' respectively. However, despite the increase in the export performance of the product group coded '871620' in the second period, the decrease in the export performance of the product group coded '843210' should be emphasized. The noteworthy development is that, in general, the specialization levels of product groups increased in the second period and it is observed that they have a competitive advantage in exports. While specialization increased in 21 out of 24 product groups with specialization in the second period, it declined in 3 product groups. In 11 of the 15 product groups where there is no specialization, there is an improvement in specialization in the second period. No results were obtained for the product groups coded '843221', '843353' and '870110' due to the lack of import

Table 4. Vollrath's Index and Turkey's Agricultural Machinery and Equipment Group RCA Coefficients and Superiority Degrees

	PC	A	B	TM	CV
Strong Superiority	843780	16.84	26.77	21.84	28.49
	843790	5.20	5.45	5.23	26.79
	843352	6.90	3.59	5.08	50.29
	843710	4.15	4.97	4.42	33.94
Moderate Superiority	843410	4.12	3.95	3.94	37.29
	843210	2.63	3.39	2.98	32.11
	843629	1.56	3.51	2.52	47.09
Weak Superiority	843610	1.12	3.02	1.99	61.89
	870190	1.53	2.24	1.89	27.54
	843230	1.06	2.37	1.72	49.32
	843221	1.29	2.06	1.66	40.47
	843229	1.35	1.32	1.32	27.06
	843621	0.57	1.67	1.06	73.11
Disadvantages	843240	0.73	1.21	0.96	37.48
	841939	0.63	1.24	0.93	43.71
	843290	0.37	1.23	0.79	62.57
	871620	0.47	1.22	0.78	74.12
	870110	0.99	0.52	0.72	67.70
	843699	0.60	0.78	0.68	44.47
	843320	0.57	0.70	0.63	35.27
	820190	0.65	0.52	0.57	22.71
	843691	0.51	0.62	0.56	39.34
	843490	0.35	0.75	0.55	45.61
	843340	0.18	0.90	0.52	75.12
	842490	0.18	1.05	0.50	128.98
	843359	0.49	0.51	0.50	32.36
	843360	0.28	0.51	0.38	49.77
	843280	0.29	0.42	0.34	48.84
	820840	0.22	0.42	0.32	46.30
	843330	0.28	0.36	0.32	46.60
	843353	0.17	0.48	0.32	63.13
	690990	0.10	0.24	0.16	68.67
	843680	0.08	0.20	0.14	63.23
	820150	0.16	0.12	0.13	44.60
	843319	0.07	0.05	0.06	69.40
	820160	0.05	0.04	0.04	71.44
	843351	0.04	0.06	0.04	104.37
	843311	0.03	0.02	0.02	38.41
	843390	0.36	0.57	0.47	40.12

Table 5. NEI and Turkey's Agricultural Machinery and Equipment Group RCA Coefficients and Superiority Degrees

	PC	A	B	TM
There is Specialization	843210	0.97	0.94	0.96
	843221	0.89	0.98	0.95
	843780	0.86	0.93	0.90
	871620	0.76	0.89	0.84
	843352	0.81	0.78	0.80
	843790	0.71	0.83	0.78
	843240	0.54	0.80	0.68
	843230	0.27	0.69	0.48
	843290	0.22	0.73	0.48
	843330	0.52	0.38	0.47
	843410	0.38	0.49	0.45
	843280	0.25	0.53	0.42
	843610	0.17	0.54	0.36
	843699	0.28	0.39	0.35
	843629	0.21	0.47	0.34
	843229	0.10	0.27	0.19
	870190	0.16	0.21	0.18
	843320	0.00	0.19	0.09
	843691	-0.04	0.16	0.07
	843710	-0.05	0.16	0.05
	843390	-0.04	0.10	0.04
	843680	-0.28	0.31	0.02
	843490	-0.27	0.30	0.01
No Specialization	843351	-0.93	-0.89	-0.93
	843311	-0.86	-0.85	-0.86
	820150	-0.86	-0.79	-0.83
	820160	-0.77	-0.82	-0.80
	843360	-0.86	-0.67	-0.78
	690990	-0.71	-0.68	-0.70
	843319	-0.69	-0.49	-0.60
	843359	-0.43	-0.59	-0.52
	841939	-0.65	-0.29	-0.48
	842490	-0.69	-0.19	-0.47
	843340	-0.68	-0.11	-0.42
	820840	-0.58	-0.20	-0.41
	843621	-0.70	0.01	-0.37
	843353	-0.15	-0.52	-0.37
	820190	-0.12	0.10	-0.01
	870110	0.27	-0.29	-0.01

Table 6. EIRI and Turkey's Agricultural Machinery and Equipment RCA Coefficients and Superiority Degrees

	PC	A	B	TM
Competitive Advantage	843210	132.91	76.27	98.55
	871620	45.45	132.84	73.85
	843352	28.89	64.22	39.77
	843780	28.63	64.97	38.27
	843790	10.59	16.13	13.39
	843240	5.61	17.02	10.64
	843230	2.95	14.76	7.16
	843290	2.60	9.69	5.90
	843410	5.23	6.87	5.78
	843330	9.52	3.68	5.62
	843280	3.08	6.41	4.42
	843610	2.59	7.23	4.14
	843629	2.58	5.28	3.64
	843229	3.05	4.55	3.48
	843699	3.22	3.27	3.20
	870190	3.30	2.61	2.64
	843680	1.01	3.19	2.01
	843320	1.64	2.57	1.99
	843691	1.83	2.25	1.96
	843490	0.96	2.97	1.82
	843710	1.52	2.28	1.82
	843390	1.78	1.76	1.75
	820190	1.24	1.80	1.48
	843621	0.37	2.72	1.05
	820840	0.44	0.98	0.68
	843340	0.32	1.28	0.68
	842490	0.29	1.27	0.64
Competitive Disadvantage	841939	0.36	0.79	0.56
	843359	0.80	0.38	0.53
	843319	0.32	0.50	0.39
	690990	0.27	0.27	0.26
	843360	0.12	0.28	0.19
	820160	0.21	0.15	0.17
	820150	0.12	0.16	0.14
	843311	0.11	0.11	0.11
	843351	0.06	0.09	0.05
	843221	-	636.58	-
	843353	-	0.46	-
	870110	-	1.20	-

or export values.

CONCLUSION

In this study, the competitiveness of Turkey's exports of agricultural implements and equipment for the period 2002-2021 is empirically analyzed using the Explained Comparative Advantage approach. Balassa's RCA2 Index, Vollrath's RXA, NEI and EIRI were used in the analysis.

Balassa's RCA index coefficients for Turkey's exports of agricultural implements and equipment are consistent with the results of Vollrath's RXA Index. According to the results of Turkey's global export competitiveness analyzed with the RXA Index, Turkey has a competitive advantage in exports of 13 out of 39 products. Turkey has a strong competitive advantage in 4, a moderate competitive advantage in 3 and a weak competitive advantage in 6 of the 13 products. Except for the product group coded '843352' in the strong advantage group, the competitiveness of the other 3 product groups increased in the second period. In addition, the arithmetic average of the RCA values of 9 product groups, 3 of which

have medium and 6 of which have weak competitive advantage, and 20 product groups with competitive disadvantage, are higher in the second period compared to the first period. These findings indicate an increase in the competitiveness of the sector. It is also evidence of the success of the policies implemented.

According to the NEI results on Turkey's own trade performance and specialization level, Turkey has specialized in exports of 23 out of 39 product groups, while it has not specialized in 16. A very important development for the agricultural machinery and equipment sector is the decrease in imports in 12 of the 16 product groups in which it is an importer. It is noteworthy that most of the product groups of the agricultural machinery and equipment sector increased their level of specialization in the second period and had a competitive advantage. According to the results of the EIRI, which measures the level of specialization, Turkey specialized in the exports of 23 product groups, while it did not specialize in the exports of 13 product groups. However, the high rate of decline in specialization levels in the second period is noteworthy. The 3 product groups with the highest level of specialization in Turkey's exports of agricultural tools and equipment are coded '843210', '843352' and '843780', respectively.

The findings of the study show that Turkey's export competitiveness in the agricultural machinery and equipment product group has increased over time despite increasing global competition. In order to ensure continuity in increasing competitiveness, it may be a better approach to focus on factors that will provide competitive advantage such as R&D and marketing instead of focusing on production and cost control. Policies to accelerate technological progress and the creation of attractive conditions for foreign investments that can create technology transfer can be effective in increasing competitiveness. In addition, it may be useful to make the necessary planning for the training of the labor force that will create technological progress. Considering the dependence on imports in the production of exported products in the Turkish economy, a stable exchange rate policy will be effective in increasing exports.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

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

Author contribution

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

Ethics committee approval

Ethics committee approval is not required. This article does not contain any studies with human participants or animals performed by any of the authors.

Funding

No financial support was received for this study.

Data availability

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

International Journal of Agriculture, Environment and Food Sciences 2023; 7(3): 718-724
DOI: https://doi.org/10.31015/jaefs.2023.3.25

Morphological, biochemical and health promoting properties in seed propagated quince fruits found in coruh valley in Türkiye

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Abstract

Pome fruits including apples and pears widely recognized species and shows rich morphological and biochemical properties. However, the studies on the other pome fruits including quince are scarce in literature. Quince is one of the most diverse specie in the pome fruits and, in particular, its fruits are rich in bioactive compounds. Türkiye, China and Uzbekistan are leading country for world quince production. Due to self-pollination characteristics, it is possible to obtain high quality quince genotypes from seeds. In this study, some important fruit properties of ten seed propagated quince genotypes naturally found in Aras valley, located in the eastern Anatolia region of Türkiye were investigated. The genotypes differed each other for most of the morphological, biochemical and human health promoting properties. Fruit weight were in range of 205-389 g among genotypes. Seven genotypes were found pear-shaped (pyriformis) and the rest of the genotypes were apple-shaped (maliformis). Fruit firmness ranged from 5.12 kg/cm^2 to 8.30 kg/cm^2, respectively. Fruit skin Chroma and Hue values were found between 47.34-65.67 and 71.98-89.17, respectively. SSC (Soluble Solid Content), Vitamin C and total phenolic content of the genotypes ranged from 9.7-13.4%, 4.2-11.2 mg per 100 g FW (fresh weight), 290–432 mg gallic acid equivalent per 100 g FW, respectively. This work constitutes an important step in the conservation of quince genetic resources in the eastern Anatolia.

Keywords: Genetic resources, Characterization, Diversity, Quince

Citation: Ilhan, G. (2023). Morphological, biochemical and health promoting properties in seed propagated quince fruits found in coruh valley in Türkiye. International Journal of Agriculture, Environment and Food Sciences, 7 (3), 718-724

Received: August 18, 2023
Revised: September 27, 2023
Accepted: September 28, 2023
Published Online: September 29, 2023

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Available online at
https://jaefs.com/
https://dergipark.org.tr/jaefs

INTRODUCTION

More recently there is an increasing interest to exotic fruits, including quinces that have distinctive taste and aroma. The characteristic flavor of exotic fruits is one of their most attractive attributes to consumers. They are found in general as semi wild in nature and are an important employment and income sources for rural peoples. In addition, these fruits are often inexpensive and rich in vitamins and human health promoting substances and can be used in a wide range of food products. Nowadays, food industries are looking at how to use these exotic fruits to obtain new products (Lee et al., 2020; Garcia-Vallejo et al., 2023).

The origin of the quince (*Cydonia vulgaris* Pers.), which is among the temperate climate fruit species, is North West Iran, North Caucasus, Caspian Sea and North Anatolia. Quince cultivation in the world has been known since ancient times. It is reported that quince passed from Anatolia to Greece and Rome in the years before Christ and was cultivated in Greece in 650 years (Abdollahi, 2019).

According to FAO data, quince is grown in about 50 countries in the world. Türkiye ranks first with 199.311 tons of production and followed by China with

a production of 112.000 tons. Uzbekistan placed in the third with a production of 96.200 tons (FAO, 2022).

In Türkiye, due to the increase in quince usage areas and consumption in recent years, significant increases are evident in the quince production area and production amount. When the quince production amounts and production areas of Türkiye for the last five years are examined, it is understood that this increase is more especially in 2017 and 2018. In these years, the production area has increased 1.5 times and the production amount has doubled compared to previous years (TUIK, 2022). Quince is a species whose table consumption as a fruit has been limited. The fruit features play an important role for restricting table consumption. In accordance with this situation, studies on quince are few in literature.

Considering the fruit shape, quinces shows apple-shaped (maliformis) and pear-shaped (pyriformis). In the world, most of the quinces grown are in pear shaped (Ercisli et al., 2015; Abdollahi, 2019). There are some structural differences between pear-shaped quince and apple-shaped quince. In pear-shaped quinces, the fruit flesh is soft and there are fewer stone cells. On the other hand, the most distinctive feature of apple-shaped quinces is that their fruits are dry, their flesh is hard and are more aromatic than those in the form of pear-shaped (Velickovic et al., 2001; Rodríguez-Guisado et al., 2009; Pinar et al., 2016).

Compared to apples and pears, the number of cultivars in quince is very limited throughout world. The main reason for this is that the quince is self-fertile. For example, in Türkiye, the number of quince cultivars around 30, while this number is around 400 for apples and 350 for pears. Another reason is that quince fruits not easily consumed as fresh and in general processed into jams, jellies, marmalades, fruit juices and preserves (Patel et al., 2011; Najman et al., 2023). However, more recently its commercial importance and popularity among consumers has continued to expand to markets in Türkiye (Yildiz et al., 2019; Gunes and Poyrazoglu, 2022; Sonmez and Sahin, 2023).

There are around 30 quince cultivars in Turkey, but there are numerous seed propagated quince genotypes in every region. Especially in the central, northern and eastern Anatolian regions, quince populations consist of promising genotypes that have completely grown from seeds. Traditionally, farmers have retained genotypes at field or orchards that emerge from seed which have good fruit characteristics. The seed propagated quince genotypes from the Aras valley shows great variability from an agro-morphological point of view. Many of them, despite being practically unknown in the scientific literature, presented very interesting productive characters.

Opposite to cultivars, seed propagated quinces more resistant to adverse soil and climatic conditions. Most seed propagated quinces may have high content of phytochemicals that vital for human health compared with cultivated one (Pinar et al., 2016). They have rich gene combinations that could be important for breeding new commercial quince cultivars with improved aroma and resistance to biotic and abiotic stressors.

More recently there were an increasing interest in nutraceuticals and functional foods which had better organoleptic properties and high human health promoting content. Exotic and less known fruits including quince are rich sources of nutraceuticals and studies concentrated on their quality and bioactivity (Muzykiewicz et al., 2018; Sonmez and Sahin, 2023). Exotic fruits exhibit rich morphological and biochemical diversity and all those traits can be influenced by environmental conditions and genotypes (Najman et al., 2023). Thus, genotype selection is important task for appropriate cultivar development and this is more important for species which had less number of cultivars. Therefore, it is crucial to make detailed comparative studies on quince genotypes related to important plant traits and phytochemical content.

In the literature, there was limited information about the comparison of morphological, biochemical and human health promoting substances in quinces, in particular seed propagated ones. Thus, in this study, we aimed to determine and compare some important morphological, biochemical and human health promoting features of ten seed propagated quince genotypes, naturally growing in Aras valley in Türkiye.

MATERIALS AND METHODS

Plant material

The fruits of 10 genotypes and cv. Ekmek were sampled at full commercially maturation stage in Aras valley (Kagizman district) located northeastern Anatolia region of Turkey during October in 2021 and 2022. A total of 40 health fruits per genotype and cultivar were randomly harvested from different parts of trees and quickly transferred to the laboratory in cold chain for morphological measurements, biochemical and human health content analysis.

Morphological measurements

The harvest time was determined when the pubescence of fruit skin can be easily removed by hand in genotypes had pubescence on fruit skin. However, for the other genotypes, which had no pubescence, it was determined when green skin color turns to yellow. Fruit weight (g) was measured with a digital scale sensitive to 0.01 g (Scaltec SPB31). Fruit firmness was determined with non-destructive Acoustic Firmness Sensor (Aweta B.V., The Netherlands) expressed as kg/cm^2. Fruit shape index (SI) was calculated with the following equation (Ercisli et al., 2009)

$$SI:\frac{W+T}{2L}$$ where W: Width, T: Thickness and L: Length

Fruit dimensions (length, width) were determined by digital caliper. The pubescence of fruit skin was determined by observation and expressed as low, medium and high. Shape index were determined by using fruit width and length.

The color of the fresh harvested quince fruits was assessed on both sides of the fruits by using a handheld tri-stimulus colorimeter (Minolta Chroma Meter CR-400) and a CIE standard illuminant C to determine the CIE color space co-ordinates, *L*, a*, b**, C, and hue° (*h°*) values. C was the color intensity of the skin, and the *h°* distinguishes one color from another and is described using common color names such as green, blue, red, yellow, etc. Hue value refers also to the lightness or darkness of a color. It defines a color in terms of how close it is to white or black. The colorimeter was calibrated against a standard and the results are expressed as the mean of three replications (Mc Lellan et al., 1995).

Biochemical Composition

Sample Preparation and Extraction

To conduct biochemical analyses, the harvested quince fruits from genotypes and cv. Ekmek was immediately frozen and stored at −80 °C. For doing analysis, the frozen quince fruits were taken and thawed to 24–25 °C. A blender was used to homogenise the fruit samples (100 g lots of fruits per genotype) and a single extraction procedure (3 g aliquots put inside tubes and extracted for 1 h with 20 mL buffer including acetone, water (deionized), and acetic acid (70:29.5:0.5 *v/v*) was used for total phenolic content analysis.

Soluble Solid Content (SSC)

SSC of fruits were determined by a hand refractometer (Kyoto 250 RH, Japan). A single drop of juice from each sample was mounted on the prism of dry refractometer and data was recorded in percent.

Titratable Acidity (TA)

Titratable acidity of quince samples obtained from 10 genotypes and cv. Ekmek was done by titration method. For analysis, 5 g fruit pulp was homogenized and followed by mixing with 20 ml of distilled water and then filtered to obtain pure extract. The pure extract (5 ml) was titrated with sodium hydroxide solution and phenolphthalein. This titration continues until end point reaches light pink color. Results expressed as %.

Vitamin C

Assessment of vitamin C in each sample was determined by using RQflex (Merck, Germany). Vitamin C was expressed as mg of vitamin C per 100 g fresh weight.

Total Phenolic Contents

Folin–Ciocalteu method according to Singleton and Rossi (1965) was used for determination of total phenolic content (TPC) of the samples. The TPC results was expressed as mg of gallic acid equivalents (GAE) per 100 g of fresh sample.

Statistical Analysis

The data of both years were pooled because there were no differences between years. SPSS software and procedures used for analysis and Least Significant Difference (LSD) method at $p < 0.05$ was used to analyze of variance tables.

RESULTS AND DISCUSSION

Morphological traits

Table 1 presents average fruit weight, shape index, color indices (chroma and hue), fruit firmness, pubescence, stone cell situation and shape of 10 seed propagated quince genotypes and cv. Ekmek. There were statistically significant differences among genotypes and cv. Ekmek in terms of fruit weight, shape index, color indices (chroma and hue) and fruit firmness at $p<0.01$ level (Table 1).

We found a wide variation in fruit weight. The genotype AV7 gave the highest fruit weight as 396 g and followed by AV9 as 386 g, AV4 as 378 g, respectively. The lowest fruit weight was obtained from AV1 as 233 g. The cultivar Ekmek gave fruit weight as 298 g. Six genotypes gave the higher fruit weight than cv. Ekmek and rest of the 4 genotypes gave fruit weight close to cv. Ekmek or lower values than cv. Ekmek (Table 1). Quince is an interesting self-pollinated fruit species that shows great variation among cultivars in terms of fruit weight. Ercisli et al. (2009) reported fruit weight between 255-530 g among local quince cultivars. It could be interesting to use AV7, AV9 and AV4 with higher fruit weight in future quince breeding programs. Tok (2020) reported fruit weight between 96-362 g among 60 local quince genotypes sampled in western Anatolia. Bak et al. (2015) investigated fruit weight on a number of quince genotypes and found between 188 and 345 g. Yilmaz (2007) also reported a great variation among local quince genotypes (between 240-597 g) in Türkiye. Gungor (1989) also found a wide variation among local quince genotypes in Türkiye between 164-595 g. Ercisli et al. (2015) found fruit weight 175-329 g among 4 quince cultivars in Türkiye.

With respect to shape index, most of the genotypes had shape index over 1.0 indicating the majority of genotypes had pyriformis fruit shape. The AV6 genotype with 1.20 shape index value showed more elongated fruits than the others. Previously Ercisli et al. (2009) reported shape index between 0.88-1.21 and indicating most of the quince genotypes had pyriformis fruit shape which shows similarities with our results. They also reported shape index as 0.88 for cv. Ekmek which shows

great similarities with our result on cv. Ekmek. Bak et al. (2015), Ercisli et al. (2015) and Tok (2020) also reported shape index over 1.0 in fruits of among a large number of quince genotypes indicating similarities with our present results.

Quince genotypes showed great variations on fruit skin color indices (Chroma and Hue values). The Chroma value of genotypes and cv. Ekmek were between 47.34-65.67 and hue value ranged from 71.98 to 89.17 (Table 1). Tok (2020) reported Chroma and Hue value between 46-61 and 73-89 among a large number of quince genotypes sampled from Bolu region in Turkey. Ercisli et al. (2015) also reported chroma and hue values between 56.83-65.22 and 92.70-96.47 among 4 quince cultivars. Our results on Chroma and Hue are good agreement with Ercisli et al. (2015) and Tok (2020). The results are also indicating that color saturation of some genotypes are better than cv. Ekmek indicating their fruits attractiveness.

Fruit firmness ranged from 5.12 kg/cm^2 to 8.30 kg/cm^2, the genotypes with the softest and the hardest being cv. Ekmek and AV7 genotype, respectively. It is also important to point out the relevant differences recorded for this parameter between the ten genotypes and cv. Ekmek. Tok (2020) reported fruit firmness between 4.80-6.46 kg/cm^2 among a large number of quince genotypes sampled from Bolu region in Turkey. Bolat and Ikinci (2015) found fruit firmness as 7.73 kg/cm^2 in Esme quince cultivar in Turkey. Quince cultivars and genotypes show great variation in terms of fruit weight as well as fruit firmness (Ercisli et al., 2009; Tok, 2020)

With respect to pubescence on fruit skin, 5 genotypes had high, 3 genotypes had low and 2 genotypes had medium pubescence. The cv. Ekmek had medium pubescence on its fruit skin (Table 1). The 4 genotypes had low, 3 genotypes had medium and rest of 3 genotypes had high stone cell in its fruits. Thus AV1, AV4, AV5 and AV9 genotypes also recorded low values of stone cell. This tendency to produce only a stone cell free well-formed fruit is a highly desirable cultivar trait in quince and even in pears. Previous studies described variability in pubescence and stone cell formation of Quince cultivars and ecotypes (Ercisli et al., 2009; Guney et al., 2019). These promising ecotypes can be considered for future research on the quince cultivar development.

The obtained diversity on most of the searched morphological parameters on quince genotypes may have important because quince is self-pollinated species and thus obtained greater gene diversity could increase crop genetic diversity.

Biochemical Traits

ANOVA test shows significant differences between the quince samples including ten genotypes and cv. Ekmek (p<0.01) in vitamin C, SSC, titratable acidity, and total phenolic content (Table 2)

AV4, AV9 genotypes and cv. Ekmek had the lowest vitamin C content (4.2, 5.0 and 5.8 mg/100 g, respectively). In contrast, vitamin C in fruits of AV1, AV2 and AV6 genotypes were much more abundant (11.2, 10.9 and 10.4 mg/100 g, respectively). The other genotypes AV3, AV7, AV8, AV10 and AV5 had vitamin C content as 9.4, 8.9, 8.2, 7.2 and 6.2 mg/100 g, respectively. Previous studies reported that the concentration of vitamin C in quince cultivars are relatively low change between 3-24 mg/100 g (Ercisli et al., 2009; Wojdylo et al., 2013; Rasheed et al., 2018). The studies also indicated that vitamin C in quince fruits can vary according to cultivars and genotypes, the fruits ripening degree and growing conditions (Rasheed et al. 2018).

The genotypes affected significantly the amounts of SSC. The highest SSC content was obtained from AV10 genotype (13.4%), and followed by AV7 (13.1%) and AV1(11.2%), respectively (Table 2). The AV9 genotype had the lowest SSC content (9.7%). Tok (2020) found SSC content on a large number of Quince genotypes between 9.46-13.68% indicating similarities with our present results on SSC. Ercisli et al. (2009) reported SSC content between 11.80-16.00% in a number of local quince cultivars in Northeastern Anatolia. Bak et al. (2015) found SSC in quince genotypes between 8.18-11.80% also indicating similarities with present findings. SSC along with acidity is important quality and harvest criteria for most of the fruit species and in particular used for harvest time determination.

Titratable acidity of quince samples were between 0.90-1.25% (Table 2). Previous studies are also indicated that quince genotypes shows differences on titratable acidity. Ercisli et al. (2009) found titratable acidity between 0.54-1.51% on quince samples. Tok (2020) reported titratable acidity between 0.90-1.19% on a large number of quince genotypes. Bolat ve Ikinci (2014) found titratable acidity as 0.63 on Esme quince cultivar. Calhan and Koyuncu (2018) found SSC and titratable acidity as 13.40% and 0.88% in quince fruit. Our SSC and titratable acidity results were in agreement with previous reports and titratable acidity is also affected by environmental conditions, genetic background of used plant materials, harvest time etc. (Ercisli, 2009; Bolat and Ikinci, 2014).

Total phenolic content varied from 290 mg GAE to 432 mg GAE per 100 g in ten samples. Quince fruits is accepted one of the richest source of phenolic content (Rop et al., 2011). Phenolic content of quince fruits was the highest on skin (peel) and followed by flesh (Karakaya and Balta, 2021). Previously total phenolic content was reported as 282 mg Esme quince cultivar grown in central Anatolia (Karadeniz et al., 2005). In Spain it was determined as 40-100 mg GAE in flesh and 200-430 in peel on 9 quince cultivars. (Legua ve ark., 2013). Several factors including genetic background, harvest period, cultural treatments may have affact phenolic content (Legua et rk., 2013; Wojdylo ve ark., 2013; Blanda ve ark., 2020).

Table 1. Morphological traits of 10 quince genotypes

Genotypes	Fruit weight (g)	Shape index	Chroma	Hue	Fruit firmness (kg/cm²)	Pubescence	Stone cell	Shape
AV1	233 ± 11.3	1.12± 0.08	47.34± 2.1	73.44± 3.1	6.45± 0.81	Low	Low	Pyriformis
AV2	304 ± 14.2	1.07± 0.11	55.03± 2.7	85.23± 4.0	6.90± 0.77	High	Medium	Pyriformis
AV3	286 ± 10.0	0.91± 0.07	49.67± 2.0	75.56± 3.6	8.11± 0.60	Medium	High	Maliformis
AV4	378 ± 12.2	1.10± 0.14	60.08± 3.2	71.98± 3.2	6.10± 0.73	Medium	Low	Pyriformis
AV5	290 ± 13.1	1.16± 0.11	54.37± 2.7	83.08± 4.1	5.85± 0.66	Low	Low	Pyriformis
AV6	341 ± 14.4	1.20± 0.13	65.67± 4.0	77.20± 4.3	7.11± 0.59	High	Medium	Pyriformis
AV7	396 ± 15.7	0.96± 0.05	63.11± 4.2	80.65± 3.8	8.30± 0.84	High	High	Maliformis
AV8	255 ± 12.0	0.88± 0.10	61.44± 3.9	87.48± 3.6	7.74± 0.45	High	High	Maliformis
AV9	386 ± 20.1	1.15± 0.07	58.98± 3.5	89.17± 3.4	5.78± 0.38	Low	Low	Pyriformis
AV10	314 ± 141	1.06± 0.08	52.20± 2.6	75.30± 4.2	7.30± 0.65	High	Medium	Pyriformis
Ekmek	298 ± 13.1	0.90± 0.05	62.11± 2.9	82.80± 4.4	5.12± 0.47	Medium	Low	Maliformis
Significance	**	**	**	**	**			
LSD5%	12.5	0.10	4.2	6.8	1.43			

***:p<0.01*

Table 2. Biochemical traits of 10 quince genotypes

Genotypes	Vitamin C (mg/100 g)	SSC (%)	Titratable acidity (%)	Total phenolic content (mg GAE/100 g)
AV1	11.2 ± 0.4	12.7± 0.4	1.02± 0.05	345±17
AV2	10.9± 0.4	11.0± 0.3	0.95± 0.06	325±11
AV3	9.4± 0.3	12.7± 0.4	1.16± 0.09	407±19
AV4	4.2± 0.2	10.5± 0.4	1.25± 0.08	290±12
AV5	6.2± 0.2	12.0± 0.3	1.10± 0.05	390±15
AV6	10.4± 0.5	11.5± 0.3	0.90± 0.10	382±18
AV7	8.9± 0.3	13.1± 0.4	1.14± 0.07	298±9
AV8	8.2± 0.4	10.6± 0.4	1.10± 0.06	432±20
AV9	5.0± 0.2	9.7± 0.2	0.98± 0.10	307±15
AV10	7.2± 0.3	13.4± 0.4	1.06± 0.10	333±16
Ekmek	5.8± 0.2	10.7± 0.2	0.90± 0.05	358±18

CONCLUSION

As a conclusion of the study, there were enough variability among local quince genotypes in Aras valley and some genotypes was found promising for future breeding activities to use them in cross breeding studies.

COMPLIANCE WITH ETHICAL STANDARDS

Peer-review

Externally peer-reviewed.

Conflict of interest

Author declare that they have no conflicts of interest

Ethics committee approval

Ethics committee approval is not required. This article does not contain any studies with human participants or animals performed by any of the authors.

Funding

This study did not obtain any external funding.

Data availability

Not applicable.

Consent for publication

Not applicable.

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