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Impacts of some eco-friendly methods on the storage life of tomato fruits

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ABSTRACT

The objective of current research was to explore the influence of several eco-friendly techniques, including modified atmosphere packaging (MAP), edible coating (EC), heat treatment and edible coating enriched with centaury oil (EO), on the postharvest storage of tomato fruit. Tomatoes (*Solanum lycopersicum*) cv. Newton were harvested red ripe and used in this experiment. Experimental studies were established on 5th February 2023. A total of 8 treatments were tested. They were: 1) control, 2) MAP, 3) MAP+EC, 4) EC, 5) MAP+EC+EO, 6) EC+EO, 7) hot air and 8) MAP+hot air. The experiments were continued for 30 days and the measurement points time intervals were defined as 5, 10, 15, 20, 25, and 30 days (six different measurements points), 384 fruits in total were utilized in the storage studies and 8 extra fruits were used at the start as a control to identify the fruit's initial qualities. The fruits were kept for 30 days at 6 to 8 °C and 90 to 95 % relative humidity. According to the study's findings, each treatment had a significant impact on the fruit's decay incidence, weight loss, vitamin C content fruit firmness, chilling damage and SSC. The study found that all treatments significantly improved the quality of the tomato fruits, except for SSC. The best results were obtained from the MAP+EC and MAP+EC+EO treatments. Tomato fruits can be stored at a temperature range of 6 to 8°C for up to 20 days, demonstrating the effectiveness of the treatments.

1. Introduction

One of the world's most significant fruit plants is the tomato (*Solanum lycopersicum*). Globally, roughly 180 million tons of tomato are cultivated yearly which has expanded by 165% over the past 20 years and is anticipated to keep growing in the upcoming years (Wyngaard and Kissinger 2022).

As a healthy component of a balanced diet, tomatoes are simple to incorporate and contain a variety of chemicals that are beneficial to human health (Martí et al. 2016). There has been an increase in the past ten years in consumer knowledge of the health benefits of foods and their involvement in avoiding a range of chronic illnesses and dysfunctions (Dhandevi and Jeewon 2015).

According to Cherono et al. (2018) the overall quality of fresh products, such as tomatoes, is substantially connected with the temperature of storage and storage period. Tomato flavor, firmness and color can all be significantly impacted by storage temperature. Due to their high moisture content, tomatoes have a 48-hour shelf life and are a perishable crop (Muhammad et al. 2011).

Due to their potential harm to human health, agrochemicals are becoming less accepted on a global scale (Sharma et al. 2009). As a result, studies aimed at finding alternatives to agrochemicals are extremely important.

In order to ensure product safety and increase the shelf life of fruits and vegetables wrapped in polymeric films, modified atmosphere packaging (MAP) has been utilized extensively along with refrigeration (Abadias et al. 2012).

Application of coatings that are edible either alone or in combination with essential oils, plant extracts, and active metabolites, is one of the effective postharvest handling procedures used to protect postharvest fruit quality (Chen et al. 2019). Edible films and covers can be made from a variety of materials. The most often utilized are polysaccharides (starches cellulose, derivatives, microbial gums & or vegetable etc.), proteins (gluten, zein and gelatin etc.) & lipids (lipid derivatives and waxes) (Fakhouri et al. 2007).

Essential oils have recently attracted a lot of attention as potential substitutes for chemical preservatives (Mastromatteo et al. 2011). Lemongrass essential oil shows antibacterial action towards a wide variety of microbes, especially molds, yeast and both gram positive & gram-negative bacteria (Naik et al. 2010).

According to Liu heat treatment can reduce disease symptoms, inactivate or completely kill germinating spores and delay the growth of the pathogen's germ tubes (Liu et al. 2012).

The objective of this study was to investigate the impact of edible coating (EC), centaury oil, MAP, heat treatment application and their combined effects on tomato post-harvest preservation. The findings of this study indicated that all the applied treatments had a noteworthy influence on various parameters including fruit firmness, fruit weight, decay incidence, chilling injury & vitamin C content. However, the

effects on soluble solids content (SSC) and titratable acidity (TA) were found to be negligible.

2. Materials and methods

2.1. Materials

"Newton" cultivar tomatoes (*Solanum lycopersicum*) were used in this study. Tomato fruits were collected from Yedidalga Lefke city in the Turkish Republic of Northern Cyprus on 5 February 2023. The fruits were kept throughout harvest and brought to the European University of Lefke, Research and Implementation Farm within two hours after harvest (approximately) under controlled conditions (10 C). Tomato fruits were picked for this research when they were red ripe or red matured. The other materials used in this study were modified atmosphere packaging (MAP) bags, centaury oil (EO), starch, glycerol, alcohol, heat treatment machine, weighting scale, refractometer, penetrometer, plastic shell packaging, and a cold room. All 100% of the Vesile plant centaury oil (Centaurium erythraea Rafn) used during this study was obtained from the ilksen pharmacy in the Turkish Republic of Northern Cyprus, Gemikongi. The modified atmosphere packaging (MAP) materials were ordered online from the fresh plus packaging company Türkiye with a brand of Fresh Plus map. The plastic shell for tomatoes packaging was received from Nicosia. The remaining above mentioned materials or equipment which were used were provided by the European University of Lefke research and implementation farm.

2.2. Experimental methodology

This research experiment was composed of eight distinct treatments and studies lasted up to 30 days. It was decided that there would be six separate measuring points: 5, 10, 15, 20, 25, and 30 days. For each experiment (#8) and each measurement point (#6), four replications (each with 12 fruits) were employed. Two fruits from each replication (a total of 8) for each treatment were taken out of the storage rooms at each measurement point for quality analysis. Thus, a total of 384 (8 treatments * 4 replication * 12 fruits in each replication) fruits were used in the storage trials while an additional 8 fruits served as a control at the start of the research to assess the fruit's initial quality.

A solution of diluted maize starch (2% w/v) & glycerol (0.5% v/v) was boiled at 90-95°C for 30 minutes in distilled water to create the edible coating. The same process was used to prepare an edible coating supplemented with centaury oil (0.5%) dissolved in ethyl alcohol (70%) 2:1 v/v.

A total of eight treatments were tested. These were : 1) control, 2) MAP, 3) MAP+EC, 4) EC, 5) MAP+EC+EO, 6) EC+EO, 7) hot air and 8) MAP+hot air. The fruits were divided into eight groups (number of treatments), each having 48 (12*4) fruits. The tomato fruits were dipped in the aforementioned treatments for 1 minute. After dipping, the fruits were air-dried for two hours. The application of hot air (45°C) was done by supplying a 30-minute heat treatment. A detailed explanation of the treatments is given in Table 1.

Next, the weights of each fruit were calculated and noted for subsequent analysis. After that, the fruits were organized neatly into tomato-shaped plastic containers and stored at 6°C to 8°C with 90-95% relative humidity.

2.3. Data collection

Then, at each measurement point, the new (final) weights of each fruit were recorded and used for the calculation of the (%) weight loss. A digital scale (0.01 g) was utilized for the determination of fruit weights. The percentage of weight reduction was calculated using the usual ratio approach.

The number of decayed fruits was noted in each replication of all treatments, Because of high levels of water activity, fresh tomatoes & tomato-based products are extremely sensitive to yeast and fungus development, including *Fusarium*, *Aspergillus niger* & *Penicillium* (Elahi et al. 2021).

Using a hand penetrometer, the fruit firmness (kg cm^{-2}) of each tomato fruit was determined and noted. Each fruit's firmness was measured at one location within the fruit's core and the average was utilized in the calculations.

To determine the soluble solids concentration, 1 fruit from each replication of the same treatment (4 tomatoes) were pressed and the juice extracted and put on a hand refractometer to measure the SSC of tomato fruits.

The vitamin C (VC) content was assessed by titration with iodine solution (Skinner 1997). The strong antioxidant ability of VC for preventing the reaction of iodine with starch was used to determine the VC content of the samples. Therefore, first of all the necessary iodine concentration for oxidization of a known amount of ascorbic acid was calculated as a standard solution (calibration). Then, the amount of iodine needed for the oxidization of an unknown (tomato) sample was determined. To do it, 10 ml of tomato juice, 5 ml of starch solution and 85 ml of pure water were mixed in a clean cup. Tincture of iodine was added into the solution drop by drop and stirred gently after each drop (same procedure for calibration). It was continued until there was a constant blue/black color and the number of drops required for titration was noted. The required amount of iodine for titration was then used in the formula below for estimating vitamin C mass in mg per g^{-1}:

$$\text{VC of sample (mg ml}^{-1}) = \left(\frac{\text{number of drops}}{10\ \text{(volume of sample)}}\right) \times \text{calibration}$$

The fruit of tomatoes is highly capable of suffering chilling injury (CI), a postharvest physiological problem brought on by insufficient storage temperatures. Symptoms of CI include sunken spots on the fruit (blemishes), disease susceptibility and slowing of ripening & color development. Tomato fruit was evaluated for chilling damage using a 0-4 scale. By comparing the Cl area to the total area, five grades of Cl symptoms were created.

- 0: no Cl symptom:
- 1: slight damage (<25%);
- 2: moderate damage (25% to 50%)
- 3: moderately severe damage (50% to 75%).
- 4: severe damage (>75%)

The above-described scale was used in the formula given below to determine the chilling injury (CI) incidence of each treatment.

$$CI = \{[(1 \times N1) + (2 \times N2) + (3 \times N3) + (4 \times N4)] / (4 \times N)\}.$$

N stands for the total amount of fruit that was measured, while N1, N2, N3 and N4 denote the numbers of fruit that showed various degrees of chilling harm.

Table 1. Detailed explanation of the eight treatments of current study

Number	Treatment	Definition
1	Control	A control is a group of tomatoes that did not receive the treatment being tested.
2	MAP	Modified atmosphere package ordered online from fresh plus packaging company Turkey with a brand of Fresh Plus map.
3	MAP+EC	The treatment 1 and 4 were combined.
4	EC	The edible coating contains corn starch, glycerol, and water. To make the coating, the corn starch (2% w/v) and glycerol (0.5% v/v) are added to distilled water (2.5 liter) and boiled for about 20 minutes until the mixture reaches 100 degrees Celsius. This high temperature helps to dissolve the corn starch and create a uniform, smooth texture. The resulting mixture can be used to provide a barrier on the surface of food goods, preventing moisture loss, microbial contamination & other types of degradation.
5	MAP+EC+EO	This treatment involves a combination of MAP and EC with oil to make tomato fruits last longer on the shelf. The edible coating is made from corn starch (2% w/v) & glycerol (0.5% v/v), which are dissolved in distilled water (2.5 liter) along with centaury oil (0.5%) and alcohol (1%). Centaury oil is a natural oil derived from the centaury plant, which has been used for its medicinal properties and also has potential as an antimicrobial agent.
6	EC+EO	These two were combined (corn starch 2% w/v) and glycerol (0.5% v/v) in distilled water with the addition of centaury oil (0.5%) and alcohol (1%) boiled for about 20 minutes until 100 centigrade. Both coatings can be used to protect food goods against oxidation, moisture loss, & microbiological contamination by creating a barrier on their surface.
7	Hot air	In this treatment, the tomato fruit is exposed to hot air (45°C) for a period of up to 30 minutes using a hot air machine.
8	MAP+ hot air	MAP + hot air. The treatment 2 and 7 were combined.

2.4. Statistical analysis

Microsoft Office Excel 2007 was used to compute the means & standard deviations for every treatment based on the raw data. The graphs below were created using the results. To find any statistically significant differences, the experiment's raw data were then put through an analysis of variance (an ANOVA) in SPSS 22.0. Mean separations had been examined by using the Tukey's HSD test at $P \leq 0.05$.

3. Results and Discussions

3.1. Effect of treatment on the weight loss

One of the most important determinants of the postharvest quality of fresh fruits is weight loss because it has an impact on the products' weight, appearance, texture and general acceptability, which in response influences consumers' purchasing decisions (Sabir et al. 2004). In all treatments weight loss increased as storage time passed. The control treatment resulted in the greatest weight reduction, but the modified atmosphere package with edible coating (MAP+EC) caused the least weight loss. An absolute maximum gain was seen with the control treatment (see Figure 1). Comparatively to the control, treated fruits lost relatively little weight, demonstrating that the modified atmosphere package with edible coating (MAP+EC) had an impact on reducing tomato fruit weight loss. Weight loss rose across all fruit groups for the 30 days of storage for tomato fruits, while some treatments were successful in lowering the weight loss. (Figure 1). After 25 days of storage, a few other treatments (EC+EO, EC, MAP+hot air) were likewise ineffective. The control treatment experienced the greatest weight loss during storage (11.5%) and the fruits treated with MAP combined with edible coating (MAP+EC) showed the lowest weight loss of 4.1%. The similar trend was continued till the end MAP+EC 4.1%, MAP+EC+EO 4.3%, MAP 5.3%, MAP+hot air 7.6%, EC+EO 8.2%, hot air 8.8%, EC 8.2% and control 11.5%. Additionally, successful at preserving & reducing weight loss MAP+EC+EO was used to maintain the quality of the tomato fruit. These findings make it clear that MAP+EC, EC combined with and without oil (MAP+EC+EO) & MAP are the most effective treatments for slowing weight loss.

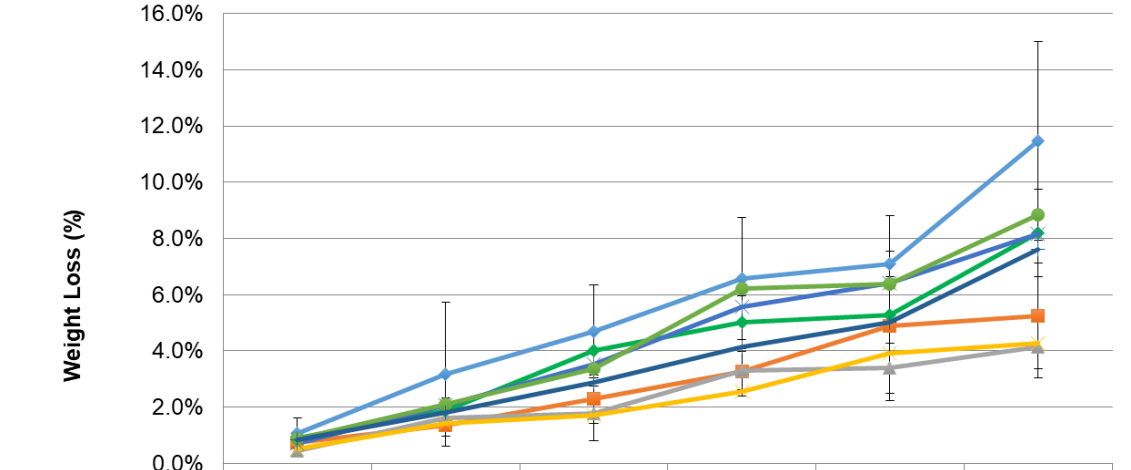
According to Kibar et al. (2018) his research results indicate that MAP and chitosan coating can significantly keep the quality of tomatoes high & reduce weight loss during storage. Olawuyi et al. (2019) also suggested that the combined treatment may significantly decrease weight loss & maintain the quality of cucumber during storage. Similar results were noted by Mangaraj and Goswami (2009).

3.2. Effects of treatments on the Decay Incidence

Tomatoes are particularly perishable by nature due to the large amount of free & bound water that is available for the growth of different microbes. Tomato fruits were safe in all groups for the first ten days of storage. After 25 days of storage, however, both edible coating and the control treatment's fruit showed high rot. The control fruits showed the greatest DI values at 75%, edible coating at 62% and EC+E0 at 50% (see Figure 2). As expected, the highest impact was noted from the hot air & MAP+hot air at 15% and was followed by the MAP+EC+E0 with 22.5%, MAP+EC with 25% and MAP with 27.5%. Similar results were previously mentioned by multiple researchers. Fallik et al. (1999) developed an innovative fast hot water treatment to enhance the quality of sweet pepper storage. Wan et al. (2020) reported similar findings on Newhall navel oranges and their findings suggested that the period of heat treatment plays a critical role in maximizing preventive measures of fruit rot and weight loss.

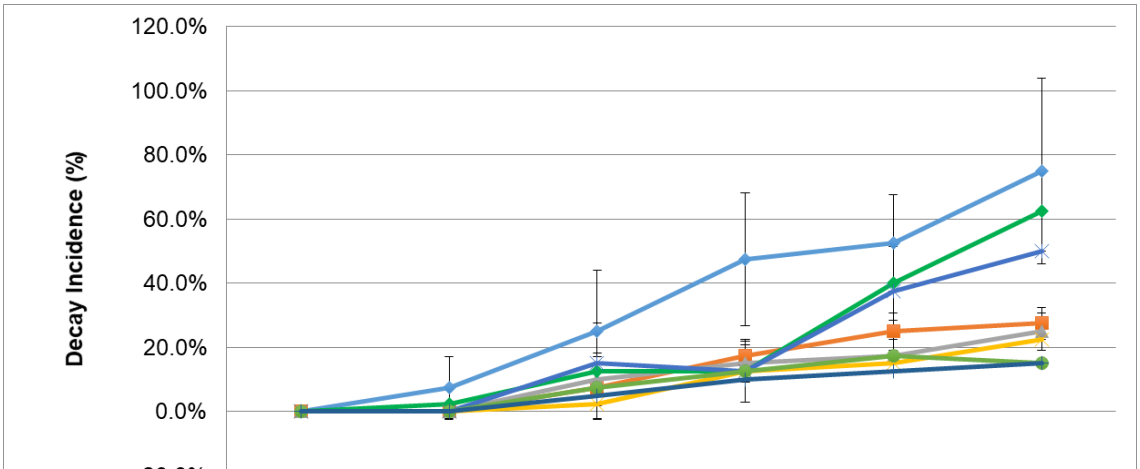
3.3. Effects of treatments on the fruit firmness

During storage, there was a trend toward lessening fruit firmness in tomato fruits. The initial fruit firmness of the control fruits, which was 0.45 kg cm^{-2} at the start of storage, decreased to 0.28 kg cm^{-2} after 30 days (Figure 3). Fruits kept in MAP+EC were reported to have a 0.26 kg cm^{-2} fruit firmness at the same time. The most effective treatment for maintaining fruit firmness appears to be the combination of EC with MAP+EC treatment. This treatment shows the most positive effect on fruit firmness in comparison to the alternative treatment with the most significant difference observed on Day 10 and beyond. The MAP & EC treatment also has a positive effect on fruit firmness. The other treatments, including, EC+EO, hot air and MAP+hot air, have



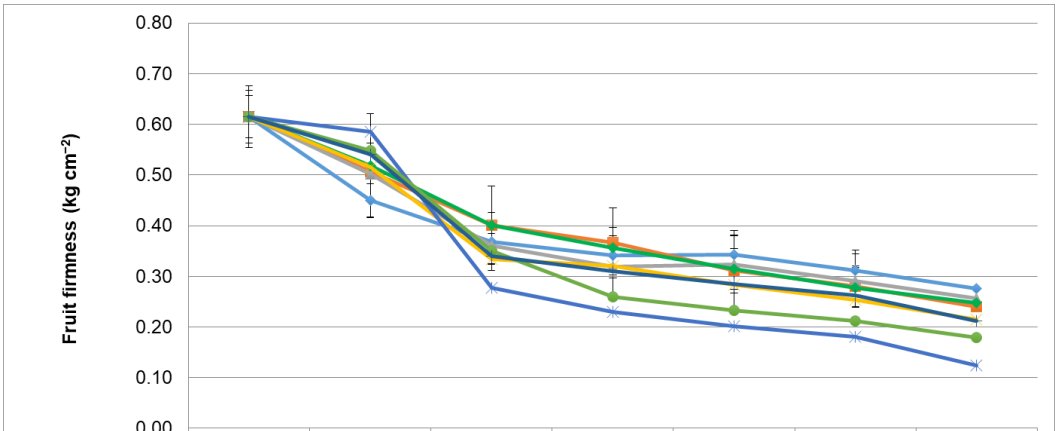
	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
Control	1.1% a	3.2% a	4.7% a	6.6% a	7.1% a	11.5%a
MAP	0.7% ab	1.4% b	2.3% cde	3.3% bc	4.9% abc	5.3% cd
MAP+EC	0.5% b	1.6% ab	1.8% de	3.3% bc	3.4% c	4.1% d
EC	0.9% ab	1.9% ab	4.0% ab	5.0% abc	5.3% abc	8.2% abc
MAP+EC+EO	0.5% b	1.4% b	1.7% e	2.6% c	3.9% bc	4.3% d
EC+EO	0.7% ab	2.1% ab	3.5% abc	5.6% ab	6.4% ab	8.2% abc
Hot Air	0.9% ab	2.1% ab	3.4% bcd	6.2% a	6.4% ab	8.8% ab
MAP+Hot Air	0.8% ab	1.8% ab	2.9% cde	4.1% abc	5.0% abc	7.6% bcd

Figure 1. Weight loss of the tomato fruits throughout the duration of 30 days of storage as influenced by the various treatments. (The means of various treatments are compared independently at each measurement point using the letters in the table below the figure. According to Tukey's HSD, different letters are employed to indicate scientific differences at the $P \leq 0.05$ level.



	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
Control	0.0% a	7.5% a	25.0% a	47.5% a	52.5% a	75.0% a
MAP	0.0% a	0.0% a	7.5% a	17.5% b	25.0% bc	27.5% bc
MAP+EC	0.0% a	0.0% a	10.0% a	15.0% b	17.5% c	25.0% bc
EC	0.0% a	2.5% a	12.5% a	12.5% b	40.0% ab	62.5% a
MAP+EC+EO	0.0% a	0.0% a	2.5% a	12.5% b	15.0% c	22.5% bc
EC+EO	0.0% a	0.0% a	15.0% a	12.5% b	37.5% ab	50.0% ab
Hot Air	0.0% a	0.0% a	7.5% a	12.5% b	17.5% c	15.0% c
MAP+Hot Air	0.0% a	0.0% a	5.0% a	10.0% b	12.5% c	15.0% c

Figure 2. Decay incident of the tomato fruits throughout the duration of 30 days of storage as influenced by the various treatments. (The means of various treatments are compared independently at each measurement point using the letters in the table below the figure. According to Tukey's HSD, different letters are employed to indicate scientific differences at the $P \leq 0.05$ level.



	Day 0	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
Control	0.62 a	0.45 c	0.37 ab	0.34 ab	0.34 a	0.31 a	0.28 a
MAP	0.62 a	0.51 abc	0.40 a	0.37 a	0.31 a	0.28 ab	0.24 ab
MAP+EC	0.62 a	0.50 bc	0.36 ab	0.32 abc	0.32 c	0.29 ab	0.26 a
EC	0.62 a	0.52 abc	0.40 a	0.36 ab	0.32 a	0.28 ab	0.25 ab
MAP+EC+EO	0.62 a	0.52 abc	0.33 ab	0.32 abc	0.28 ab	0.25 abc	0.22 ab
EC+EO	0.62 a	0.59 a	0.28 b	0.23 c	0.20 c	0.18 c	0.12 c
Hot Air	0.62 a	0.55 ab	0.35 ab	0.26 bc	0.23 bc	0.21 bc	0.18 bc
MAP+Hot Air	0.62 a	0.54 ab	0.34 ab	0.31 abc	0.29 ab	0.26 ab	0.21 ab

Figure 3. Fruit firmness of the tomato fruits throughout the duration of 30 days of storages influenced by the various treatments. (The means of various treatments are compared independently at each measurement point using the letters in the table below the figure. According to Tukey's HSD, different letters are employed to indicate scientific differences at the $P \leq 0.05$ level.

either a negative or slightly positive impact on fruit firmness compared to the control group. The ripening process is inversely correlated with the tomatoes' firmness. (Wakabayashi et al. 2000). Similar results were previously suggested by Kahramanoğlu and Usanmaz (2019) on cucumber fruit, their results suggested that cucumber fruit firmness can be preserved in MAP bags for twenty days (20d) at 4.5 ± 0.5°C & 95% RH. A similar result was noted by Wei et al. (2021) on mango fruits, their result suggested that the MAP compound treatment improved mangoes' ability to retain their commercial qualities, followed by the application of the edible coating compound treatment.

3.4. Effects of treatments on the soluble solids concentration

The results indicated that the treatments had no statistically significant impact on tomato fruit SSC, however in some treatments, SSC means were found to differ significantly over the period of storage (Figure 4). Especially MAP+EC treatment resulted in a reduced soluble solids concentration from 0 to 5 days, but after that, the concentration remained relatively stable and did not decrease as much as in the other treatments. The concentration even increased slightly between days 25 and 30. This demonstrates that the MAP+EC treatment helped to maintain the fruit quality of the tomato and minimize the breakdown of sugars. Overall, the other treatments (MAP, EC, EC+EO, hot air, and MAP+hot air) also had varying affects on the concentration of soluble solids of the tomato fruits over time, but none of them appeared to be as effective as the MAP+EC treatment in preserving the fruit's level of quality.

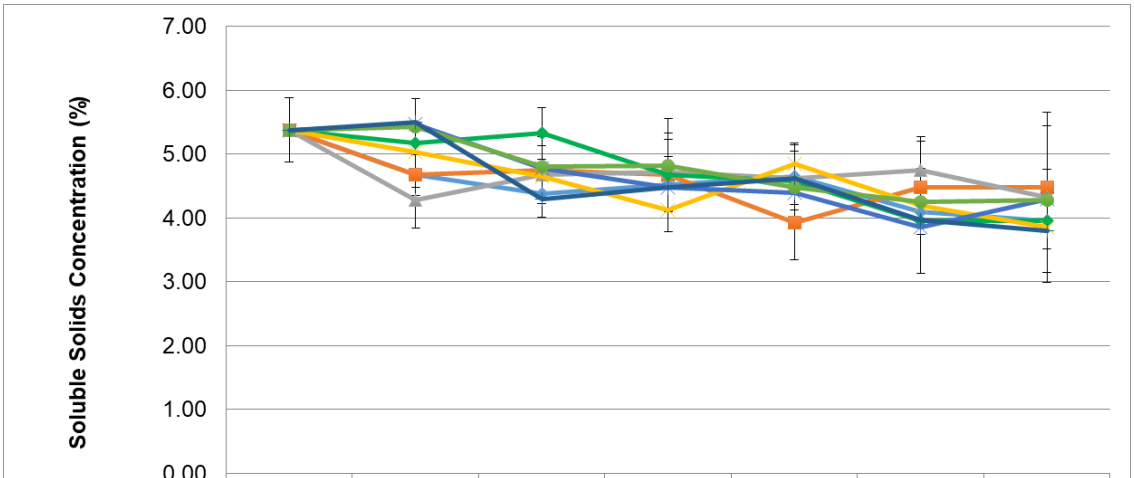
Similarly, Öztürk and Ağlar (2019) reported that the quality of fruits of cornelian cherries while in cold storage might be maintained with the help of edible coating (EC) and MAP treatments. The results of Liao et al. (2023) also showed that fresh-cut pineapples treated with EC + MAP had better performance at preserving storage quality and prolonging shelf life. Islam et al. (2022) reported that jujube fruit quality losses that developed during cold storage and shelf life may be extended by using MAP and *Aloe vera* (AV).

3.5. Effects of Treatments on the Ascorbic Acid (Vitamin C)

All treatments had the same ascorbic acid (vitamin C) content on day 5, as the control group (Figure 5). However, on day 10, the EC+EO treatment showed a content of ascorbic acid significantly lower than the control. On day 15 control, EC+EO and MAP+EC+EO treatments all showed a reduction in the amount of ascorbic acid compared to the other treatments. On day 20 EC, control, MAP, and MAP+EC treatments all showed a decrease in ascorbic acid content. On day 25, the EC treatment indicated a substantial drop in ascorbic acid content compared to the other treatments. On day 30, control and EC showed a decrease in ascorbic acid. The result showed that some of the treatments had a favorable impact on the ascorbic acid content of the tomato fruit such as MAP+hot air, MAP+EC+EO and EC+EO treatments, where the ascorbic acid content of these treatments was noted to be slightly higher than the other treatments and control. This indicates that these treatments could be successful in delaying the reduction of ascorbic acid content of tomato fruits. Similar outcomes have previously been reported by Erkan et al. (2005). He pointed out that the use of hot air delayed the elimination of VC and TSS. In further research with 'Satsuma' mandarins, Shen et al. (2013) pointed out that while hot water treatment somewhat raises VC concentration, untreated control fruits show no significant difference. The same results for MAP was noted by Kahramanoğlu and Usanmaz (2019).

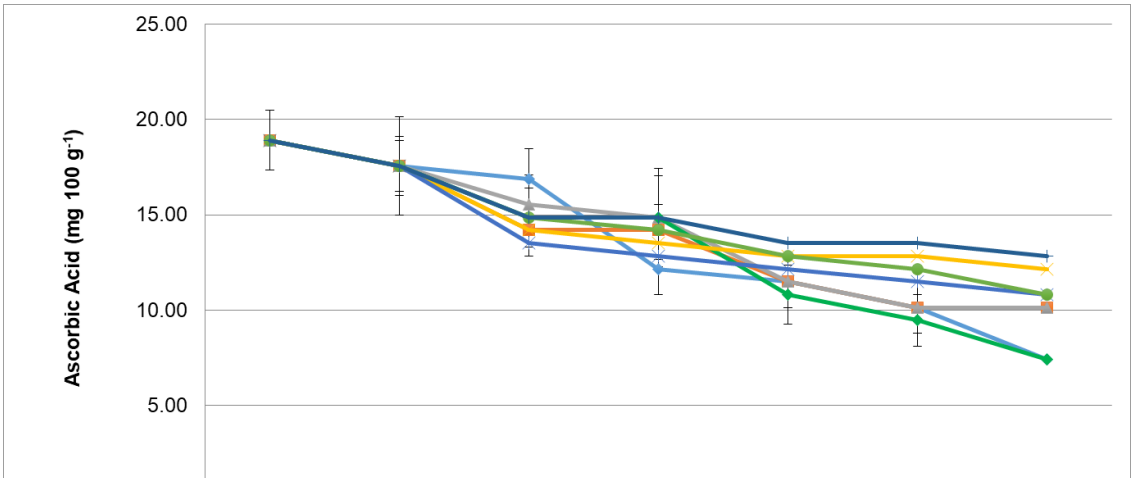
3.6. Effects of treatments on the chilling injury

All treatments were effective at minimizing chilling injury in tomatoes compared to the control treatment (see Figure 6). The treatments that consistently indicated the lowest levels of chilling



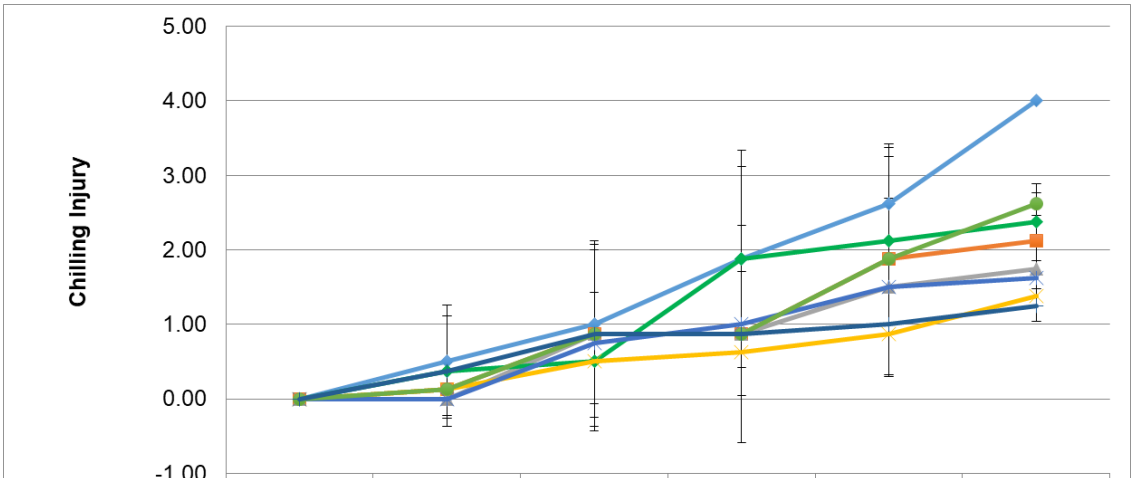
	Day 0	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
Control	5.38 a	4.68 ab	4.38 a	4.53 a	4.65 a	4.10 a	3.95a
MAP	5.38 a	4.68 ab	4.75 a	4.68 a	3.93 a	4.48 a	4.48a
MAP+EC	5.38 a	4.28 b	4.68 a	4.73 a	4.63 a	4.75 a	4.33a
EC	5.38 a	5.18 ab	5.33 a	4.68 a	4.58 a	3.95 a	3.95a
MAP+EC+EO	5.38 a	5.03 ab	4.65 a	4.13 a	4.85 a	4.20 a	3.85a
EC+EO	5.38 a	5.48 ab	4.78 a	4.48 a	4.40 a	3.85 a	4.30a
Hot Air	5.38 a	5.43 ab	4.80 a	4.83 a	4.48 a	4.25 a	4.28a
MAP+Hot Air	5.38 a	5.50 a	4.30 a	4.48 a	4.63 a	3.98 a	3.80a

Figure 4. Soluble solids of the tomato fruits throughout the duration of 30 days of storages influenced by the various treatments. (The means of various treatments are compared independently at each measurement point using the letters in the table below the figure. According to Tukey's HSD, different letters are employed to indicate scientific differences at the $P \leq 0.05$ level.



	Day 0	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
Control	18.92 a	17.57 a	16.89 a	12.16 a	11.49 a	10.14 ab	7.43 b
MAP	18.92 a	17.57 a	14.19 a	14.19 a	11.49 a	10.14 ab	10.14 ab
MAP+EC	18.92 a	17.57 a	15.54 a	14.86 a	11.49 a	10.14 ab	10.14 ab
EC	18.92 a	17.57 a	14.86 a	14.86 a	10.81 a	9.46 b	7.43 b
MAP+EC+EO	18.92 a	17.57 a	14.19 a	13.51 a	12.84 a	12.84 ab	12.16 a
EC+EO	18.92 a	17.57 a	13.51 a	12.84 a	12.16 a	11.49 ab	10.81 ab
Hot Air	18.92 a	17.57 a	14.86 a	14.19 a	12.84 a	12.16 ab	10.81 ab
MAP+Hot Air	18.92 a	17.57 a	14.86 a	14.86 a	13.51 a	13.51 a	12.84 a

Figure 5. Vitamin C of the tomato fruits throughout the duration of 30 days of storage as influenced by the various treatments. (The means of various treatments are compared independently at each measurement point using the letters in the table below the figure. According to Tukey's HSD, different letters are employed to indicate scientific differences at the $P \leq 0.05$ level.



	Day 5	Day 10	Day 15	Day 20	Day 25	Day 30
Control	0.00 a	0.50 a	1.00 a	1.88 a	2.63 a	4.00 a
MAP	0.00 a	0.13 a	0.88 a	0.88 a	1.88 a	2.13 b
MAP+EC	0.00 a	0.00 a	0.88 a	0.88 a	1.50 a	1.75 b
EC	0.00 a	0.38 a	0.50 a	1.88 a	2.13 a	2.38 b
MAP+EC+EO	0.00 a	0.13 a	0.50 a	0.63 a	0.88 a	1.38 b
EC+EO	0.00 a	0.00 a	0.75 a	1.00 a	1.50 a	1.63 b
Hot Air	0.00 a	0.13 a	0.88 a	0.88 a	1.88 a	2.63 ab
MAP+Hot Air	0.00 a	0.38 a	0.88 a	0.88 a	1.00 a	1.25 b

Figure 6. Chilling injury of the tomato fruits throughout the duration of 30 days of storage as influenced by the various treatments. (The means of various treatments are compared independently at each measurement point using the letters in the table below the figure. According to Tukey's HSD, different letters are employed to indicate scientific differences at the $P \leq 0.05$ level.

injury throughout the 30-day storage period were MAP+hot air, MAP+EC+EO, EC+EO and MAP+EC compared to control. The control showed higher levels of chilling injury compared to all the other treatments. The data suggests that MAP+hot air, MAP+EC+EO, EC+EO and MAP+EC may be effective at reducing chilling injury in tomatoes, and the addition of essential oil (EO) may further enhance this effect. Similarly, Kahramanoğlu and Usanmaz (2019) indicated that MAP enhances fruits' ability to withstand CI. Moradinezhad et al. (2013) suggested that combining hot water with MAP had a greater effect on fruit quality. Similarly, Mastromatteo et al. (2010) study on food preservation, noted that a sealed packaging system with naturally occurring antimicrobials and MAP conditions frequently provides an efficient approach to lengthen the shelf life of food (Moradinezhad et al. 2013).

4. Conclusions

Overall, the findings of this study demonstrated that the combination of MAP technology, edible coatings, essential oils and heat treatment shows promise in comparison to the control group for keeping the postharvest storage quality of tomato fruits. Further research can be conducted to optimize these treatments and explore other eco-friendly methods for enhancing post-harvest preservation and reducing losses in tomato fruits.

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Exploring *Aeolothrips* spp. Diversity: A Morpho-Molecular Examination of *Aeolothrips collaris* and *Aeolothrips intermedius* (Thysanoptera: Aeolothripidae)

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ABSTRACT

This study investigated the evolutionary relationship between *Aeolothrips collaris* Priesner, 1919 and *Aeolothrips intermedius* Bagnall, 1934 within the Thysanoptera: Aeolothripidae family. Notably, *A. collaris* DNA barcode was made for the first time and compared with other *Aeolothrips* species in GenBank. While revealing a close genetic association (82-83%) between these species, the analysis using the Neighbor-Joining method clustered *A. collaris* with *A. albicinctus* Haliday, 1836 and *A. ericae* Bagnall, 1920, while *A. albicinctus* grouped alongside *A. fasciatus* (Linnaeus 1758) and *A. duvali* Moulton, 1927 GenBank BlastN analysis supported the expected placement of *A. intermedius*. These insights highlight significant genetic connections but suggest the necessity for a broader examination involving more species and gene regions. Expanding this research could yield a comprehensive understanding of the intricate taxonomic relationships within this thrips genus, setting the groundwork for future investigations into thrips species evolution and taxonomy.

1. Introduction

The order thrips (Thysanoptera) encompasses around 6000 species worldwide, classified into two suborders: Terebrantia, consisting of 2606 species, and Tubulifera, which includes 3809 species (ThripsWiki 2024). Thrips are tiny insects, inconspicuous due to their slender bodies, typically measuring 1-3 mm in length. Their adult stage is defined by narrow, elongated wings adorned with long fringes along the edges, hence the name Thysanoptera, derived from fringed wings (Riley et al. 2018). Thrips exhibit diverse biology and inhabit various environments, engaging in activities from plant feeding and predation to consuming fungi. Their two pairs of well-developed wings are positioned outside the thrips' body. Additionally, they possess piercing-sucking mouthparts. One distinctive trait of thrips is the asymmetry in their mouthparts, where only the left mandible is present (Parker et al. 2013). Their short antennae typically consistis of VI to X segments, depending on the species (Loomans et al. 1995). Aeolothripids are identified by their broad forewings, typically rounded at the apex and often displaying shaded, colorless transverse bands. Additionally, their antennae consist of nine segments, with segments III-V elongated and parallel-sided, featuring flat, linear, or oval-shaped sensoria on antennal segments III and IV. Moreover, their ovipositor is upturned (Mirab-Balou et al. 2011). The Aeolothripidae family, belonging to the suborder Terebrantia, comprises approximately 240-250 species exhibiting a wide array of feeding behaviors. Among many species within this family, both adults and larvae act as facultative predators, preying on other small arthropods. They feed not only on floral tissue but also on thrips and mites residing within flowers (Conti 2009; Parker et al. 2013).

Thrips, through their feeding on developing flowers or vegetables, frequently become pests affecting commercial crops. Additionally, they can act as carriers of plant diseases such astospoviruses (Reitz 2009; Ullman et al. 2007). Incorrectly identifying an economically significant species can have far-reaching and serious consequences, leading to confusing data across different biological disciplines. Accurately identifying thrips species stands as the initial and crucial step in gathering genetic and other biological data necessary for effective management strategies. Morphological traits such as color, chaetotaxy, and body structure primarily underpin thrips species identification. However, their small size, secretive behavior, sexual differences, developmental stage similarities, and polymorphism (seen in color, wing development, body size, etc.) present hurdles for morphology-based identification. Given these challenges, it becomes essential to employ supplementary methods for thrips species identification and resolving taxonomic issues. Molecular tools, increasingly utilized over the past decade, have proven valuable in augmenting various biological fields, spanning from systematics to ecology (Alves-Silva and Del-Claro 2010; Tyagi et al. 2017).

Aeolothrips collaris Priesner, 1919 (Thysanoptera: Aeolothripidae) (*A. collaris*) is widely distributed, spanning from southern Europe across the Mediterranean to Madeira and the Canary Islands, further extending eastward to encompass regions in India and Bangladesh. It has also been introduced to California (Alavi and Minaei 2018; zur Strassen 2003). Additionally documented in Iran, China, India, France, Albania, Egypt, Cyprus, the Canary Islands, Mongolia, Türkiye, and Bangladesh (Mirab-Balou 2013; Tunç 1991). *Aeolothrips intermedius*

Bagnall, 1934 (Thysanoptera: Aeolothripidae) (*A. intermedius*) exhibits a widespread distribution across both Eastern and Western Europe, as well as in Iran and China (Loomans et al. 1995; Trdan et al. 2005).

1.1. Taxonomy

The two thrips suborders, Terebrantia and Tubulifera, have 9 extant families and 6 fossil families. Among these, Terebrantia comprises 8 extant and 5 fossil families, while Tubulifera includes 1 extant and 1 fossil family (ThripsWiki 2024). In the suborder Terebrantia, the family Merothripidae comprises of 5 genera with 18 species, Melanthripidae includes 6 genera with 75 species, Aeolothripidae consists of 28 genera with 250 species, Fauriellidae encompasses 4 genera with 5 species, Stenurothripidae involves 12 genera with 24 species, and Heterothripidae contains 7 genera with 24 species. Within the family Thripidae, the subfamily Panchaetothripinae contains 40 genera with 140 species, Dendrothripinae includes 15 genera with 100 species, Sericothripinae comprises of 3 genera with 145 species, and the subfamily Thripinae comprises of 240 genera with 1700 species. Uzelothripidae includes 1 genus with 1 species. In the suborder Tubulifera, within the family Phlaeothripidae, the subfamily Phlaeothripinae contains 375 genera with 2820 species, while the subfamily Idolothripinae consists of 80 genera with 715 species (Parker et al. 2013).

1.2. Host plants and feeding behavior

Aeolothrips collaris is often found dwelling among flowers on diverse plants without any noted specificity. It's believed to be a facultative predator, displaying a diverse diet that includes pollen and larvae from other thrips (Priesner 1964).

Aeolothrips intermedius primarily preys on Thysanoptera, comprising 44 species such as *Thrips tabaci* Lindeman 1889 (Thysanoptera: Thripidae), *Frankliniella* spp, and other *Thrips* spp. Additionally, it targets various other prey, including spider mites, psyllid larvae and eggs, and microlepidoptera (Loomans et al. 1995; Trdan et al. 2005). Despite being a predator, *A. intermedius* exhibits a diverse feeding behavior, from leaf cells and petal cells to pollen, expanding its diet across various areas (Parker et al. 2013).

This study aimed to make a comprehensive exploration of *Aeolothrips* diversity, merging intricate morphological assessments with precise molecular analyses. The focal points were the species *A. collaris* and *A. intermedius*. A significant milestone was achieved with the pioneering creation of the DNA barcode for *A. collaris*, enabling subsequent comparisons with related sequences in GenBank. This integrated approach not only revealed the fascinating interplay between morphological variations and genetic makeup within *Aeolothrips* but also laid the groundwork for further taxonomic advancements. The creation of *A. collaris*' DNA barcode stands as a pivotal contribution, promising enhanced insights into the genus' taxonomy and evolutionary patterns.

2. Materials and Methods

In 2021, specimens of the species were collected from Isparta and Konya provinces of Türkiye. Collection involved shaking plants on a tray, and the specimens gathered were temporarily preserved in small vials containing a mixture of 70% ethanol, stored at +4°C. These specimens were later mounted in Hoyer's medium for microscopic examination. The slides carrying the specimens are archived in the Department of Plant Protection, Faculty of Agriculture, Selçuk University, Konya, Türkiye. Subsequent to the diagnostic phase, each specimen underwent DNA isolation using the 'CTAB' protocol developed by Doyle and Doyle (1987). For the mitochondrial Cytochrome Oxidase Subunit, I (COI) gene region, the COI deg F1/R1 primers were employed, targeting a segment of approximately 350 base pairs (Timm et al. 2008). The PCR protocol mirrored that used in (Şahin Negiş et al. 2022). The taxonomic distances of the assembled specimens were manually corrected using the MEGA11 analysis program. Each species' gene region was individually aligned, and before tree analyses, overall mean distances were computed using MEGA11 to calculate the mean pairwise distances between taxa. Additionally, *Ixodes ricinus* Linnaeus (Acari: Ixodidae) was used as an outgroup for the phylogenetic tree.

3. Results

3.1. Morphological diagnosis

Aeolothrips collaris and *A. intermedius* are two distinct species of thrips found across different geographical regions and exhibiting notable differences in various morphological characteristics.

Aeolothrips collaris typically displays an antenna segment III that is predominantly yellowish white, with a brown to dark brown coloration near the apical edge. The antennal segment III is typically yellow, with brown coloring in the apical fifth or less. Segment V measures approximately 1.2 times the length of segments VI-IX combined (Fig. 1b). Moreover, the antennal segment IV is usually 4.0-4.2 times as long as its width. The pronotum exhibits a range from yellow to dark brown. Fore legs are usually lighter than the mid and hind legs (Fig. 1a). The dark transverse band on the fore wing tends to be about 1.12-1.28 times as long as it is wide (Fig. 1c). In some instances, the prothorax and abdominal segments III and IV might have a yellowish hue. Females of this species measure between 1790-2125 µm (zur Strassen 2003; Alavi and Minaei 2018). The abdomen displays marginal setae on sternites that originate either at or near the margin. Sternite VII specifically features two pairs of accessory setae arising notably in front of the margin. In males, tergites IV and V exhibit paired dorsal tubercles. Additionally, on tergite IX, the setae located at the base of the claspers are shorter than the clasper itself, accompanied by a stout curved seta positioned laterally to the clasper (Priesner 1964).

The species *A. collaris* and *A. intermedius* both belonging to the Thysanoptera: Aeolothripidae, are closely related within the *Aeolothrips* genus. Interestingly, these two species showcase both similarities and differences, which provide valuable insights into their characteristics and relationship. However, according to (zur Strassen 2003), *A. collaris* is considered synonymous with *A. intermedius*, indicating a shared identity between the two species. The comparative analysis of sampled specimens and sequence comparisons among closely related species uncovered substantial patterns in evolutionary relationships, offering insights into the identities and connections within this group.

On the other hand, *A. intermedius* has approximately three or four generations per year. Adult insects begin to appear in April and May. Females begin laying eggs after emergence, and the duration of egg laying ranges from two to four weeks. The female lays 29-73 eggs, and the incubation period for the eggs lasts for

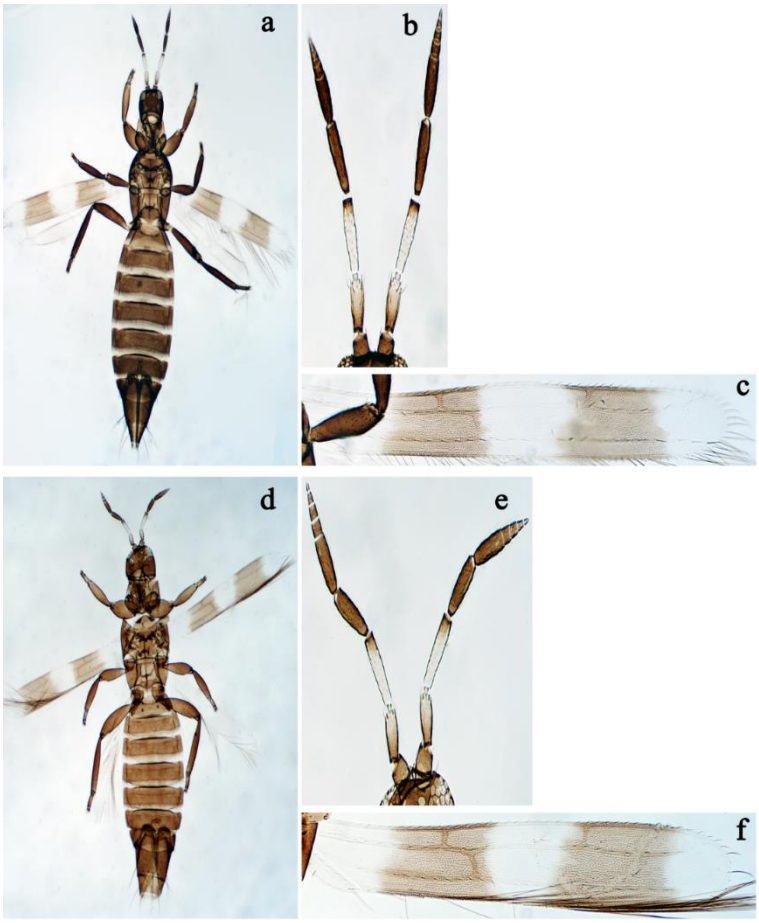

Figure 1. *Aeolothrips* female species. *A. collaris* (a-c): **(a)** body, **(b)** antenna, **(c)** right wing. *A. intermedius* (d-f): **(d)** body, **(e)** antenna, **(f)** right wing.

21 days at a temperature of 14, 6 days at a temperature of 26, and 4 days at a temperature of 38 (Loomans et al. 1995)**.**

Aeolothrips intermedius demonstrates an antenna segment III that starts as light yellow or yellowish brown but darkens notably from the middle or at least in the apical third towards the dark brown apical edge. Antennal segment IV, in this species, tends to be 3.1-3.8 times as long as its width. Antennal segment III ranges from yellow to brownish-yellow, gradually darkening to brown in the apical fourth to half. Segment V is about the same length as segments VI-IX combined (Fig. 1e). The pronotum is consistently brown. Fore legs are not lighter than the mid and hind legs (Fig. 1d). The length of the fore wing's distal dark band at the anterior margin is 1.0-1.5 times the length of the pale area between the dark bands. Its distal dark transverse band on the fore wing is typically 1.26-1.43 times as long as it is wide (Fig. 1f). The prothorax is consistently dark, and females measure between 1850-2400 µm (zur Strassen 2003; Alavi and Minaei 2018).

3.2. Molecular diagnosis

The generation of *A. collaris*' DNA barcode and its comparison with sequences in GenBank showcased a close genetic association among related species, underscoring the potential for utilizing DNA barcoding in future taxonomic studies within the genus. These findings emphasize the significance of combining multiple analytical methods to unravel the intricate relationships and evolutionary trajectories within *Aeolothrips*, setting a solid foundation for continued research in thrips taxonomy and evolution.

Utilizing the BlastN option with the *A. collaris* type, *Aeolothrips* sp. displayed identities ranging between 82-83%. Additionally, sequence matches revealed percentages of 76-77% with *Aeolothrips albicinctus* Haliday, 1836 (*A. albicinctus*), 80% with *Aeolothrips duvali* Moulton, 1927 (*A. duvali*) (which had only two sequence options), 82% with *A. intermedius*, and 79-80% with *Aeolothrips ericae* Bagnall, 1920 (*A. ericae*) and *Aeolothrips fasciatus* (Linnaeus 1758) (*A. faciatus*).

The Neighbor-Joining method (Saitou and Nei 1987), was employed to infer the evolutionary history, presenting the optimal tree. Bootstrap analysis (500 replicates) demonstrated the percentage of replicate trees wherein associated taxa clustered together, with values indicated alongside branches (Felsenstein 1985). Using the p-distance method (Nei and Kumar 2000), evolutionary distances were calculated in units of base differences per site. The analysis encompassed 42 nucleotide sequences, considering codon positions $1^{st}+2^{nd}+3^{rd}+$ noncoding. Ambiguous positions were eliminated for each sequence pair using the pairwise deletion option. The final dataset comprised 290 positions. Evolutionary analyses were performed using MEGA11 (Tamur et al. 2021). In the NJ tree, *A. collaris* clustered closely with *A. albicinctus* and *A. ericae*, while *A. intermedius*, *A. faciatus* and *A. duvali* species clustered together. As confirmed by the GeneBank BlastN percent identity (100%) result, the *A. intermedius* sample took its place in the tree together with the GeneBank sequence samples of the same species (Fig. 2).

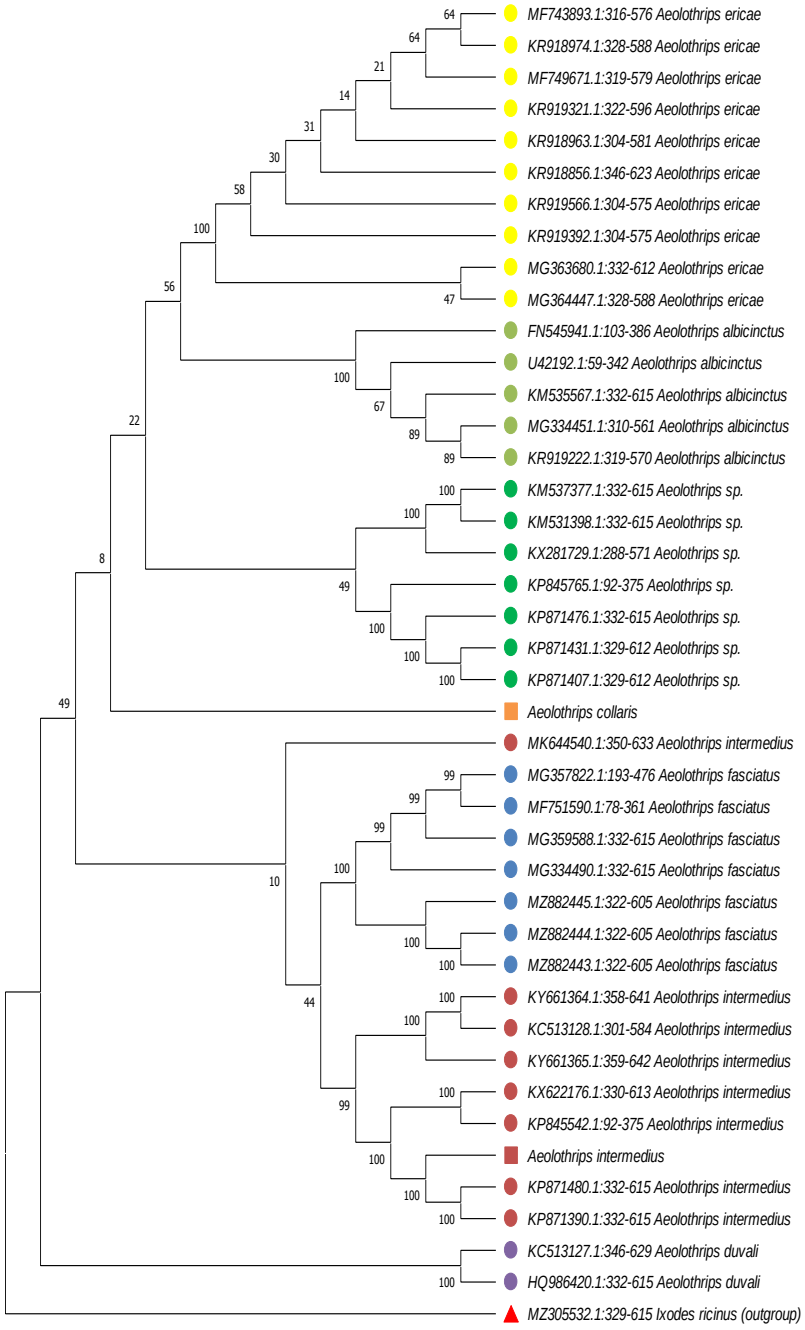

Figure 2. The NJ phylogenetic tree was constructed based on the COI gene region of *A. collaris* and *A. intermedius* species. *Ixodes ricinus* Linnaeus (Acari: Ixodidae) was utilized as an outgroup (MZ305532.1). The numbered sample sequences were obtained from GenBank. (Overall mean distance: 0.20).

In the NJ tree illustrating branching from the outgroup root species, *A. albicinctus*, *Aeolothrips ericae*, *A. intermedius*, *A. fasciatus*, *A. duvali*, and *A. collaris* species exhibit a shared ancestral positioning. Upon examining the resulting tree, it is suggested that *A. collaris*, *A. albicinctus*, *Aeolothrips sp.*, and *A. ericae* might share a closer relationship and a common ancestor. Further sampling is anticipated to enhance the comprehension of evolutionary relationships, potentially clarifying similarities or differences among species. With more extensive sampling and analysis using genetic data, it is anticipated that studies will facilitate a clearer understanding of relationships among species and their evolutionary history.

The *Aeolothrips* genus, a substantial subset within the Arthropoda class, demonstrates notable variations among its species, particularly in distinct anatomical features. Detailed examinations among species like *A. albicinctus*, *A. ericae*, *A. intermedius*, and *A. collaris* revealed morphological disparities, especially in abdominal coloration patterns, prothorax characteristics, and tonal variations in antennal segments. These pronounced discrepancies play a pivotal role in species identification and classification.

Distinctive features, such as the dark body with specific abdominal patterns in *Aeolothrips albicinctus*, or the coloration

and length differences in antennal segments in *Aeolothrips ericae*, offer crucial markers for species differentiation. Furthermore, characteristic variations in prothorax coloration and antennal segment shades between *A. intermedius* and *A. collaris* serve as prominent identifying factors. Additionally, specific sensory structures on abdominal tergite I distinguish *A. fasciatus* from other *Aeolothrips* species (zur Strassen 2003).

4. Discussion and Conclusion

The findings of this study open avenues for substantial discussion. The amalgamation of morphological and molecular analyses provided a holistic perspective on *Aeolothrips* diversity, particularly focusing on *A. collaris* and *A. intermedius*. The creation of *A. collaris*' DNA barcode, a notable first, served as a valuable tool for comparative genomic analyses, affirming close genetic affinities among certain *Aeolothrips* species. However, while shedding light on these species' genetic associations, the study also underscored the necessity for broader sampling across additional species and genetic regions to fortify taxonomic assessments. Moreover, the integration of molecular data with traditional morphological taxonomy accentuates the significance of a multidisciplinary approach in elucidating thrips diversity. This study's implications stretch beyond *Aeolothrips* alone, advocating for a more comprehensive understanding of thrips evolutionary dynamics and taxonomic frameworks. Future endeavors encompassing an expanded dataset and varied methodologies are crucial to unravel the complexities of thrips taxonomy and evolution, offering a clearer lens into the vast world of these tiny yet evolutionarily significant insects.

The distribution of the Aeolothripidae family displays an inherent asymmetry, evident both in its taxonomic structure and geographic spread. While a substantial 55% of described aeolothripid species find their primary habitat within the largely Holarctic confines, notably encapsulated within the *Aeolothrips* and *Melanthrips* genera, an equally noteworthy statistic emerges: 50% of the acknowledged genera within this family are distinctly tropics-bound (Mound and Marullo 1998) and, the feeding habits of both species are similar, and according to (House 1966), the consumption of various prey items and potentially plants might influence the behaviors of flower-dwelling predators such as *A. intermedius*, a point of particular significance. Additionally, adults of *Aeolothrips intermedius* require feeding on flowers to reach sexual maturity. In the absence of prey, a floral diet can sustain complete larval development in the predatory insect. It's emphasized that *A. intermedius* is primarily a predator of thrips (Bournier et al. 1979).

By examining and contrasting these distinct morphological features among species, a clearer understanding of their classification and identification within the *Aeolothrips* genus emerges. These findings contribute significantly to the broader understanding of evolutionary relationships and species differentiation within this taxonomic group.

While both species belong to the *Aeolothrips* genus and share habitats within the herbaceous and shrub layers, they differ in characteristics such as the coloration and proportions of antennal segments III and IV, the presence of yellow hues on specific body parts, the length-to-width ratio of the dark transverse band on the fore wing, and the size range of females. These distinctions help distinguish them from one another and aid in their taxonomical classification and ecological understanding.

This study examined the evolutionary relationship between *A. collaris* and *A. intermedius* within the *Aeolothrip*s genus. While indicating a close genetic relationship (82-83%) between these species, the analysis using the Neighbor-Joining method revealed the clustering of *A. collaris* with *A. albicinctus* and *A. ericae*, whereas *A. intermedius* grouped with *A. fasciatus* and *A. duvali*. GenBank BlastN analysis supported the anticipated position of *A. intermedius*. These findings, while highlighting significant genetic connections, underscore the necessity for a more extensive study encompassing a broader array of species and gene regions. Expanding this research could provide a comprehensive understanding of the intricate taxonomic relationships within this thrips genus and lay the foundation for future investigations into the evolution and taxonomy of thrips species.

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Determination of fungal root and stem rot agents of melons grown in Kumluca/Antalya

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ABSTRACT

Kumluca has an important place in terms of greenhouse vegetable cultivation. Melon is among one of the main vegetables grown in the district on about 3200 decare of land. Various diseases cause decrease in the yield and quality of melons grown undercover in Türkiye. Among them, Fusarium wilt and gummy stem blight diseases especially have caused significant losses in recent years. In this research, the incidence and severity of the root and stem rot disease in Kumluca were determined by surveys made in 72 melon greenhouses in this area. Plant and soil samples were taken to the laboratory and isolations were made. As a result, *Fusarium oxysporum* and *Didymella bryoniae* were the most frequently isolated pathogens from the plant samples, followed by other *Fusarium* species. Fungi with the highest isolation frequency from the soil samples were *Fusarium* spp., *Rhizoctonia solani* and *Macrophomina phaseolina*. In the pathogenicity test, *F. oxysporum*, *F. solani*, *F. verticillioides*, *M. phaseolina* and *R. solani* isolates caused severe symptoms on melon seedlings. Virulence of the *F. oxysporum* isolates on different cucurbit species was also investigated and it was determined that they caused severe wilting on melon and watermelon seedlings, while symptoms on squash and cucumber were moderate or slight. Additionally, reactions of five melon cultivars (Yusufbey, Çıtırex, Niovi, Ferdevs and Memory) commonly grown in the region against *D. bryoniae* were investigated using randomly selected four pathogen isolates. All the cultivars were susceptible to the disease.

1. Introduction

Melon (*Cucumis melo* L.) is one of the most popular fruit vegetable crops, belonging to the *Cucurbitaceae* family. It is thought to have originated from Africa, but today it is cultivated all over the world. It is known to have many health benefits, such as skin beauty, regulation of blood flow, recovery from diarrhea, etc. (Goutam et al. 2020). Türkiye is the largest melon producer in Europe and the second largest producer in the world after China, with 1724860 tonnes production on 76129 hectares of land (FAO 2020). Melon cultivation is performed in Central Anatolia, Aegean, Southeastern Anatolia, Mediterranean, Marmara, Eastern Anatolia and Black Sea regions (Ünlü et al. 2017). The Kumluca district of Antalya province is an important area in terms of vegetable production and many types of vegetables are grown. Melon cultivation is third after pepper and tomato, on approximately 3260 decare of land (Anonymous 2021). Fungal pathogens are especially important among the factors causing losses in melon production. *Didymella bryoniae*, *Fusarium* spp., *Macrophomina phaseolina*, *Monosporascus cannonballus*, *Podosphaera xanthii*, *Pythium* spp., *Rhizoctonia solani* and *Verticillium dahliae* are among the most common fungal pathogens isolated from melons (Reuveni et al. 1982; Aegerter et al. 2000; Santos et al. 2017).

In this study, surveys were performed in the melon growing areas of the Kumluca district of Antalya province and plant and soil samples were collected. Fungal agents causing root and stem rot on melon plants were isolated from the samples identified according to their cultural and morphological features. Virulence of the isolates on melon and other cucurbit seedlings were determined by pathogenicity tests. In addition, reactions of commonly grown melon cultivars against *D. bryoniae* which was the most common pathogen in the region, were determined.

2. Materials and Methods

2.1. Survey studies

During surveys made in the melon growing areas in Kumluca, in the 2019-2020 and 2020-2021 vegetation periods, 73 randomly selected melon greenhouses were investigated for root and stem rot symptoms and isolations were made from the plant and soil samples. In the greenhouses, 100 randomly selected melon plants were examined among approximately 2500 plants in each greenhouse with 1 decare of land, to determine disease incidence and severity rates (%). Disease severity on melon plants was scored by using 0-4 scale modified from Santos et al. (2017), where; 0: no visible symptoms, 1: <1 cm diameter soaked lesion on the plant stem or slight wilting, 2: >1 cm diameter soaked lesion on the plant stem or moderate wilting, 3: partially necrotic lesion on the plant stem, with severe wilting of the plant, and 4: complete necrosis with total

wilting and plant death. Disease severity rates were calculated with the Townsend and Heuberger (1943) formula. Mean rate and severity of the disease in the melon production areas were also calculated (Bora and Karaca 1970).

2.2. Isolation and identification of the pathogens

Isolations were made by transferring small plant samples including healthy and symptomatic tissues to Petri dishes with water agar (WA) or potato dextrose agar (PDA, Biolife-Italy), after surface disinfection with 1% NaOCl solution. Cultures with plant samples were incubated in a climatic chamber with 22±2ºC temperature and a photoperiod of 12 hours light-dark. Baiting technique was used for the isolations made from the soil samples. Sterile wild oat stem pieces of about 2 cm long were buried into soil samples in plastic pots and incubated for two days at room temperature, under plastic cover in order not to become dry. After incubation, oat stem pieces were surface sterilized and transferred to 1.5% WA amended with 10% lactic acid (3 ml l^{-1}). Growing hyphal tips were transferred to PDA to obtain pure cultures (Erper et al. 2008). Fungi were identified according to their cultural and morphological features (Watanabe 2002; Keinath et al. 1995).

Synthetic nutrient agar (SNA) and potato sucrose agar (PSA) were used for the identification of *Fusarium* species (Booth 1971). To verify the identification of *F. oxysporum* isolates, one isolate (KUM8-1) used in the pathogenicity test was sent to the Centre for Implementation and Research of Plant Health Clinic, Hatay Mustafa Kemal University, for molecular identification. Special primers for translation elongation factor 1-alpha (EF) gene region were used and sequences were compared with those in the GeneBank. Later the isolate was tested by qPCR, using specific primers for *F. oxysporum* f. sp. *melonis*, in the Molecular Biology Laboratory of Plant Protection Department, Faculty of Agriculture, Isparta Applied Sciences University.

2.3. Determination of the virulence of the isolates

The virulence of the selected *Fusarium* isolates on melon seedlings was determined by pot trials. In the pathogenicity trial, 16 *F. oxysporum*, 1 *F. solani*, 1 *F. semitectum*, 3 *F. equiseti*, 2 *F. verticillioides* isolates were used. Isolates were grown on SNA for seven days and spore suspensions with 10^6 conidia/ml were prepared. Melon seedlings (cv. Çıtırex) were inoculated with the isolates by the root dipping method. Disease severity was determined 7, 14 and 21 days after inoculations, by using a 0-4 scale, where 0 means healthy plant and 4 totally wilted or dead plant (Zhao et al. 2014). To determine the virulence of *M. phaseolina* and *R. solani* isolates, wheat seed inoculum of the pathogens were used. A hundred grams of wheat seeds autoclaved with 200 ml water were inoculated with 8 mm diameter agar discs taken from the 4 days old pathogen cultures. After 30 days incubation, the seeds were transferred into the soil around the roots of seedlings (Zhang et al. 2014).

2.4. Determination of the virulence of Fusarium oxysporum isolates on cucurbits

The virulence of the *F. oxysporum* isolates on melon (cv. Niovi), watermelon (cv. Crimson Sweet), squash (cv. Amelthee) and cucumber (cv. PTK 40) plants were determined by the root dipping method, using 32 isolates. Disease severity was determined 7, 14 and 21 days after inoculation by using a 0-4 scale as mentioned above (Zhao et al. 2014).

2.5. Determination of the reactions of some melon cultivars against Didymella bryoniae

Reactions of 5 commercial melon cultivars commonly grown in the region (Yusufbey, Çıtırex, Niovi, Ferdevs ve Memory), against 4 randomly selected *D. bryoniae* isolates (HV7-2, KUM6-2, SAR4-1-1, SAR2-2-3) were investigated. Melon seedlings used in the experiment were transferred to plastic pots with a sterile soil mixture. Agar pieces with pathogen mycelia taken from the growing cultures were placed on the stem of the melon seedlings near cotyledones with sterile toothpicks. Plants were kept in a moisture chamber for 72 hours and disease severity evaluations were made 7, 14 and 21 days after inoculations using a 0-4 scale (Santos et al. 2017).

2.6. Statistical analyses

All data were subjected to analyses of variance using SPSS 23® (IBM Corp., Armonk, NY, ABD) program and means were compared by Tukey's test ($P \leq 0.05$).

3. Results and Discussion

3.1. Incidence and severity of root and stem rot disease in Kumluca district

During surveys performed in the randomly selected 73 melon greenhouses in the Kumluca district, typical symptoms of the gummy stem blight and wilt diseases were observed, and it was determined that the mean incidence and severity rates of the disease were 15.76% and 11.87%, respectively. The highest incidence and severity of the disease were in Sarıcasu location, while those were lower in Beşikçi location where melon production is also less (Table1). This difference among the locations may be because of the decreased inoculum levels in some greenhouses, depending on the regular measures such as soil solarization or fungicide applications.

Table 1. Surveyed areas and numbers of melon greenhouses in Kumluca district and root and stem rot disease incidence and severity rates

Locations	Area of greenhouses surveyed (da)	Number of greenhouses surveyed	Disease incidence (%)	Disease severity (%)
Adrasan	3.5	1	20.00	13.21
Beşikçi	7.0	2	4.43	6.64
Beykonak	56.9	12	12.09	8.64
Erentepe	10	2	8.60	7.85
Güzören	7.0	2	30.00	21.00
Hacıveliler	5.0	10	21.68	16.96
Hızırkahya	23.8	4	14.78	9.35
Kavak	11.5	2	25.70	23.21
Mavikent	43.5	10	10.50	6.80
Merkez	29.1	14	9.31	6.44
Salur	15.3	6	21.24	18.84
Sarıcasu	13.1	8	48.09	35.97
Total	**225.7**	**73**	-	-
Mean		-	**15.76**	**11.87**

3.2. Fungi isolated from the plant and soil samples taken from the melon greenhouses

A total of 913 fungal isolates were obtained from the plant and soil samples. From the plant samples, *D. bryoniae* and *F. oxysporum* were the most frequently isolated pathogens. Since they were generally isolated together from the same samples, it was thought that they had combined effects on disease symptoms. Other *Fusarium* species followed these pathogens. *F. oxysporum*, *R. solani* and *M. phaseolina* were the most common fungi isolated from the soil samples. *F. equiseti*, *F. semitectum*, *F. solani* and *F. verticillioides* were the other *Fusarium* species isolated from the samples (Table 2). These *Fusarium* species were previously isolated from melon cultivation areas in Türkiye (Sağır 1988; Erzurum 2000a; Boyraz and Baştaş 2005). Fusarium wilt is among the first diseases detected on melon plants. Different species were isolated from the diseased melon plants, while *F. oxysporum* f. sp. *melonis* and *F. solani* f. sp. *cucurbitae* were mentioned as the pathogens responsible from the serious losses in melon production (Latin and Snell 1986). *F. oxysporum* f. sp. *melonis* is common in almost all melon areas of the world, including Türkiye and known as the most important pathogen causing Fusarium wilt on melons (Kurt et al. 2002; Chikh-Rouhou et al. 2021). Selected virulent *F. oxysporum* isolates, identified according to their cultural and morphological features and confirmed by molecular techniques, were tested by qPCR using Fom specific primers and determined as *F. oxysporum* f. sp. *melonis*. *D. bryoniae*, causing gummy stem blight disease, which is known as an important pathogen of cucurbits all over the world, causing economical losses especially on melon, watermelon and cucumber especially under hot and humid conditions (Gasparotto et al. 2011; Babu et al. 2015). In Türkiye, the pathogen was first reported with a prevalence of 10.79% and disease severity of 20.02% on cucumbers grown in Elazığ province (Mutlu et al. 2015). Later, it was isolated from watermelon plants in Antalya province and determined by pathogenicity experiments that the isolates can cause disease symptoms on melon, cucumber and zucchini plants, besides watermelons (Basım et al. 2016). *M. phaseolina* and *R. solani* are the other pathogens commonly isolated from melon plants. Drying symptom caused by *M. phaseolina* is known as charcoal rot and melon plants are among the host plants damaged by the pathogen (Reuveni et al. 1982; Boyraz and Karaca 1991). *R. solani* was previously reported from Italy, USA and Brasil, as an important agent causing root rot and wilting on melons (Corazza et al. 1992; Aegerter et al. 2000; Andrade et al. 2005). This pathogen was also isolated from the melon plants showing wilting symptoms in Türkiye (Sağır 1988; Tezcan and Yıldız 1991; Erzurum 2000a; Boyraz and Baştaş 2005; Duran and Özgönen-Özkaya 2016). AG 4 HG-II strain of the pathogen was recently reported to cause damping off on melon seedlings in Kyrgyzstan (Erper et al. 2022).

Table 2. Number of fungal isolates obtained from plant and soil samples taken from the melon greenhouses in Kumluca district

Fungus species	Plant samples	Soil samples
Didymella bryoniae	182	-
Fusarium equiseti	-	4
Fusarium oxysporum	314	174
Fusarium semitectum	3	1
Fusarium solani	15	1
Fusarium verticillioides	3	-
Macrophomina phaseolina	-	16
Rhizoctonia solani	-	130
Others	-	70
Total	**517**	**396**

The fungi represented by the small numbers of isolates from the soil samples were *Aspergillus* spp., *Chaetomium* spp., *Cladosporium* spp., *Clonostachys rosea*, *Mucor* spp., *Penicillium* spp., *Rhizopus stolonifer* and *Stachybotrys chartarum*.

3.3. Virulence of the fungi obtained from melon greenhouses

In the first observation made 7 days after inoculations, the virulence of *F. verticillioides* and *R. solani* isolates were rather high. Some *F. oxysporum* and *M. phaseolina* isolates also caused severe symptoms (Table 3). The virulence of most of the *Fusarium* isolates increased after 14 days, while the disease severity values caused by *D. bryoniae* isolates increased in the last observation made on the 21st day. It was determined that the virulence of the *Fusarium* isolates randomly selected for the pathogenicity test were different from each other. Most of the *F. oxysporum* isolates with *F. solani* and *F. verticillioides* isolates caused severe symptoms, while the virulence of *F. semitectum* was lower (Figure 1). There are various reports on the virulence of *Fusarium* species on melon plants. In a study made in Korea, it was found that the virulence of *F. oxysporum* isolates was high, while that of *F. equiseti* was lower (Seo and Kim 2017). *F. semitectum* was isolated from melons in Konya in low rates, but nothing was mentioned about its virulence (Boyraz and Baştaş 2005). It is known as a post-harvest rot agent of melon fruits in Brazil (Oliveira et al. 2014). In a recent study made in Türkiye, the virulence of *F. solani*, *F. oxysporum* and *F. equiseti* isolates obtained from melon areas was investigated, and *F. oxysporum* was found to be the most virulent isolate with 68.6% disease severity. This pathogen also caused decrease on plant fresh and dry weights and root lengths of melon plants. *F. equiseti* caused 46.3% severity but it did not significantly change plant growth parameters, whereas *F. solani* decreased root lengths of the plants with 53% severity (Teniz and Demirer Durak 2023). In this study, *M. phaseolina* and *R. solani* isolates also caused severe disease on melon seedlings. Our results are coherent with previous studies. It was reported that the virulence of 19 *M. phaseolina* isolates obtained from melon roots was high (Tezcan and Yıldız 1991). In a similar study on the comparison of the virulence of 26 *M. phaseolina* isolates selected among 51 isolates from different provinces in Central Anatolia, the isolates caused disease severity rates between 3.5 and 82% (Erzurum 2000b). Regarding *R. solani*, it was mentioned that the pathogen can cause severe disease especially in high inoculum rates (Andrade et al. 2005; Silva et al. 2020).

3.4. Virulence of Fusarium oxysporum isolates on different cucurbit species

Evaluations made 3 weeks after the inoculation of *F. oxysporum* isolates showed that the virulence of the isolates on melon and watermelon plants were rather high, while they caused moderate or slight wilting symptoms on squash and cucumber plants. Nine isolates did not cause any symptoms on cucumber plants (Table 4). Previous studies showed that the virulence of the isolates obtained from different plants varied. Watermelon and cucumber isolates of the pathogen caused disease only on original host plants (McMillon 1986). Similarly,

Table 3. Virulence of the fungal isolates obtained from the plant and soil samples taken from the melon greenhouses in Kumluca district

Pathogens	Isolate code	7. Day		14. Day		21. Day	
		Mean scale value	Disease severity (%)	Mean scale value	Disease severity (%)	Mean scale value	Disease severity (%)
Didymella bryoniae	HV7-2	0.0*c**	0	0.2 b	10	2.8 a	70
	KUM6-2	0.0 c	0	0.8 b	20	2.8 a	70
	SAR4-1-1	0.0 c	0	1.2 ab	35	3.4 a	85
	SAR2-2-3	0.0 c	0	0.2 b	10	0.4 b	15
Fusarium equiseti	TSA 6-1-4	2.4 ab	60	3.6 a	90	4.0 a	100
	TS8-3-2	1.4 ab	35	3.6 a	90	4.0 a	100
	TS8-3-1	1.8 ab	45	2.6 a	65	3.4 a	85
F. oxysporum	TB9-1-3	2.2 ab	55	4.0 a	100	4.0 a	100
	THV6-2-3	3.6 a	90	4.0 a	100	4.0 a	100
	TS8-2-2	2.8 a	70	3.4 a	85	4.0 a	100
	TSA4-3-2	3.6 a	85	4.0 a	100	4.0 a	100
	TS2-2-1	0.0 c	0	0.2 b	20	0.8 b	40
	TSA5-1-2	0.6 bc	25	2.6 a	70	3.2 a	80
	TG2-3-1	3.0 a	75	4.0 a	100	4.0 a	100
	TG1-4-3	3.4 a	85	4.0 a	100	4.0 a	100
	TG1-3-1	1.6 a-c	40	4.0 a	100	4.0 a	100
	TSA6-1-1	1.6 a-c	40	4.0 a	100	4.0 a	100
	SAR2-1	2.6 ab	65	3.8 a	95	4.0 a	100
	HAC2-1	3.4 a	85	4.0 a	100	4.0 a	100
	MA2-1	2.0 ab	50	3.6 a	90	4.0 a	100
	BEY6-1	1.0 a-c	25	3.0 a	75	4.0 a	100
	KUM8-1	3.4 a	85	4.0 a	100	4.0 a	100
F. semitectum	TM4-2-1	0.0 c	20	0.8 b	20	1.2 b	20
F. solani	TK11-1-3	0.0 c	0	0.0 b	0	0.8 b	80
F. verticillioides	SAR6-1	3.8 a	95	4.0 a	100	4.0 a	100
	SAR6-2	4.0 a	100	4.0 a	-	4.0 a	100
Macrophomina phaseolina	TK12-2-3	3.6 a	85	4.0 a	100	4.0 a	100
	TKa1-2-1	4.0 a	100	4.0 a	-	4.0 a	100
	TE1-2-3	3.8 a	95	4.0 a	100	4.0 a	100
	TB8-1-1	1.6 b	40	2.2 b	55	3.2 a	80
Rhizoctonia solani	TSa5-2-3	4.0 a	100	4.0 a	-	4.0 a	100
	TSa4-2-4	3.6 a	90	4.0 a	100	4.0 a	100
	TS8-1-1	3.2 a	80	4.0 a	100	4.0 a	100
	TG2-2-3	3.8 a	95	4.0 a	100	4.0 a	100
	TK12-1-2	3.6 a	90	4.0 a	100	4.0 a	100

* √+1 transformation was applied to the scale values before statistical analyses, real values were given in the table.
**There were no statistically significant differences among the means in the same column followed by the same letters, according to Tukey test ($P \leq 0.05$).

the virulence of the cucumber and melon isolates of the pathogen was high on cucumber, melon and watermelon plants but the virulence was lower on squash cultivars (Najafinia and Sharma 2009). Coherent with our results, in a study made in Korea, melon and watermelon plants were found more susceptible than cucumber (Seo and Kim 2017).

3.5. Susceptibility of melon cultivars against Didymella bryoniae

As a result of inoculations of 4 randomly selected *D. bryoniae* isolates on 5 melon cultivars, severe symptoms occurred on all tested cultivars 21 days after inoculations. The disease became more severe at an early stage on Yusufbey cultivar (Table 5). Since it is the most effective control method, research on the development of resistant genotypes have been ongoing (Virtuoso et al. 2022).

4. Conclusion

This research showed that *D. bryoniae* and *F. oxysporum* were the main pathogens of melons grown in the Kumluca district. *M. phaseolina* and *R. solani*, isolated from the soil samples, were also potential pathogens that may cause significant losses. After the ban of methyl bromide, systemic fungicides have generally been used against soil-borne plant pathogens. However, after continuous use they may lose their effectiveness because of the resistant pathogen strains. Therefore, sustainable control strategies should be developed. In this context, application of better control methods such as solarization, use of resistant varieties and biocontrol agents are gaining importance. Emphasis should be given on the determination of biological control agents effective against the pathogens causing losses in melon cultivation areas. Integrated management strategy will provide environmentally friendly control of soil borne pathogens.

Figure 1. Wilting symptoms on melon plants caused by *Fusarium oxysporum* (on the left) and *F. semitectum* isolates.

Table 4. Virulence of selected *Fusarium oxysporum* isolates on cucurbit plants three weeks after the inoculations

Origin	Isolate code	Mean scale values			
		Cucumber	Squash	Watermelon	Melon
Plant	BEY6-1	0.00*b** C	0.60 d-h B	3.80 a A	3.40 ab A
	BEY6-4	0.00 b D	0.80 b-h C	4.00 a A	2.40 b-d B
	BEY11-1	0.00 b B	0.00 h B	2.00 ab A	3.00 b-d A
	GUZ1-3	1.60 ab A	1.60 a-g A	2.20 ab A	3.60 a A
	GUZ2-1	0.40 ab B	2.60 a-d A	3.40 ab A	3.80 a A
	GUZ2-6	1.20 ab B	3.20 a-c A	4.00 a A	3.80 a A
	HAC2-1	1.80 ab A	3.00 a-c A	3.00 ab A	4.00 a A
	HAC3-1	2.20 ab A	3.40 a-c A	3.80 a A	3.80 a A
	HV8-3	0.00 b B	0.20 gh B	3.80 a A	3.20 a-c A
	HV8-5	0.00 b D	1.00 a-h C	3.80 a A	3.00 a-d B
	KUM8-1	2.00 ab A	3.40 ab A	4.00 a A	3.80 a A
	KUM9-3	1.20 ab B	1.40 a-g B	4.00 a A	4.00 a A
	KUM13-1	0.00 b B	0.20 gh B	3.40 ab A	2.40 b-d A
	MA2-1	0.00 b B	0.20 gh B	3.40 ab A	2.00 d A
	MA8-5	0.00 b C	0.40 e-h C	4.00 a A	2.20 cd B
	MA9-6	0.00 b B	0.60 f-h B	3.80 a A	3.60 a-c A
	MA10-4	0.20 ab C	1.40 a-g B	3.60 a A	3.40 ab A
	SA3-2	1.20 ab B	1.80 a-g AB	4.00 a A	3.40 ab A
	SA4-2	3.20 a A	3.00 a-c A	4.00 a A	3.80 a A
	SA6-3	1.40 ab B	2.20 a-e AB	3.80 a A	3.40 ab A
	SAR2-1	1.60 ab B	1.00a-h AB	3.00 ab AB	3.80 a A
	SAR5-3	1.80 ab A	1.40 a-g A	4.00 a A	3.80 a A
	SAR8-3	0.60 ab B	1.00 c-h B	4.00 a A	3.80 a A
Soil	TB9-1-3	2.20 ab B	3.20 a-c AB	3.60 a AB	4.00 a A
	TG1-1-1	1.00 ab B	2.60 a-d AB	1.80 ab AB	4.00 a A
	TG2-3-1	1.80 ab B	1.80 a-f AB	4.00 a A	3.40 ab AB
	TG2-3-4	0.80 ab B	3.20 a-c A	3.40 ab A	4.00 a A
	TSA4-3-5	2.40 ab A	2.60 a-d A	3.40 ab A	3.60 a A
	TSA6-1-1	0.40 ab B	2.60 a-e A	4.00 a A	3.60 a A
	TSA6-2-1	2.20 ab B	2.60 a-d AB	3.80 a A	3.60 a AB
	TSA6-3-2	3.00 a A	3.80 a A	1.40 b B	4.00 a A
	TSA6-3-3	2.20 ab A	1.60 a-f A	4.00 a A	4.00 a A

* √+1 transformation was applied to the scale values before statistical analyses, real values were given in the table.
**There were no statistically significant differences among the means in the same column shown by the same lower case letters, and in the same row shown by the same upper case letters, according to Tukey test ($P \leq 0.05$).

Table 5. Disease severity rates (%) on melon cultivars caused by four selected *Didymella bryoniae* isolates

Melon cultivars	HV7-2			KUM6-2			SAR2-2-3			SAR4-1-1		
	Disease severity (%)											
	7. day	14. day	21. day	7. day	14. day	21. day	7. day	14. day	21. day	7. day	14. day	21. day
Çıtırex	0	10	70	0	20	70	0	10	15	0	35	85
Ferdevs	5	45	100	10	60	100	10	10	25	5	70	100
Memory	5	65	100	10	100	100	5	10	60	10	80	100
Niovi	0	25	80	0	40	95	0	20	55	0	20	80
Yusufbey	55	100	100	20	90	100	15	80	80	50	85	100

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Solanum americanum: An alternative model crop in plant pathology

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ABSTRACT

The *Solanum americanum*, possesses valuable features that earn it a deserved addition to the expanding list of model plants that are utilized in the fields of plant genetics, plant breeding and biotechnology. This review attempts to comprehensively highlight the crucial role of this model plant and its genetic diversity in terms of resistance against biotic pathogens. In summary, we recommend the use of this plant in future studies focused on plant-pathogen interactions.

1. Introduction

Plant pathogens have become a major global concern, especially among crop farming communities. This concern is aggravated by significant changes in climate patterns, which have informed the importance of identifying, researching, and effectively addressing these pathogens and their economic impact. This poses a significant struggle for plant pathologists, who strive to bring to light the complete array of effects caused by these pathogens and find lasting solutions to them. In the pursuit of complete understanding and the scrutiny of viable alternatives, researchers and plant pathologists have turned to the utilization of model crops as indispensable tools in their research endeavors.

Model plants are pivotal in phytopathology as they are used in research on plant-pathogen interactions, disease resistance mechanisms, and genetic variability of plants. Plants such as *Arabidopsis thaliana* (Padole and Ingle 2017), maize (Gaut et al. 2000; Strable and Scanlon 2009), tomato (Meissner et al. 1997), wheat (Feldman et al. 2012), rice (Izawa and Shimamoto 1996) and others have been reported to be used as model crops. These crops, by virtue of valuable characteristics such as genetic tractability, and rapid growth, provide a controlled environment to investigate the complexity of plant diseases and other agronomic features. They offer insights into host responses, pathogen virulence, and underlying molecular pathways involved in resistance or susceptibility. The understanding of these interactions helps researchers gain fundamental knowledge applicable to diverse crops, aiding in the development of disease-resistant varieties.

Aside from their role in plant pathology, they also serve as valuable tools for assessing the evolutionary trajectories of plant genomes. For example, maize, as a model plant, has been used to decipher complex subjects of heterosis, epigenetics, and quantitative inheritance, thereby enhancing our comprehension of these complex phenomena (Strable and Scanlon 2009). Amongst other reported model plants, *A. thaliana* has been investigated the most and has been widely used in research studies. The preference of this plant for research studies can be attributed to its short life cycle, ability to produce a multitude of seeds, small genome size (about 143 Mbp) and the ability to generate mutants using simple agrobacterium transformation systems such as the flower dip method (Meyerowitz 2001). Despite the popularity of *A. thaliana*, another interesting crop group that are used in the understanding of plant pathogen interactions are the *Solanaceae* group. In a review paper by Arie et al. (2007), the importance of tomato (*Solanum lycopersicum* L.) in understanding the interaction between tomato and its viral, bacterial, fungal and nematode pathogens were highlighted. Also, they listed resistance and susceptibility genes discovered in tomato plants. Nevertheless, recent attention has shifted towards another member of this botanical family, *Solanum americanum* Mill., Black nightshade (Fig. 1), which is emerging as a promising candidate for plant-pathogen studies. Consequently, this paper aims to comprehensively review current research on *S. americanum*, particularly focusing on its morphology and role in plant-pathogen interaction studies.

2. *S. americanum* morphology

Solanum americanum is commonly known as Black nightshade. Despite its importance and diversity within the *Solanum* group, it has been classified as a weed and of less usage in most countries (Vandeventer et al. 1982). Often mistaken for *S. nigrum*, *S. americanum* is a diploid whilst *S. nigrum* is a polyploid. Moreover, the seeds of *S. americanum* are smaller and range from 1.2 - 1.6 mm in length, contrasting with the larger seeds of *S. nigrum* (1.8 - 2.2 mm). Phenotypically, *S. americanum* can be distinguished by its umbellate inflorescence, purplish-

blackish shiny fruits (Fig. 2), 1 - 2 mm long anthers and pollens with diameter between 19 - 27 µm (Schilling et al. 1992).

3. *S. americanum* as a model plant

When comparing *S. americanum* with established model plants such as *A. thaliana*, distinct differences and potential advantages emerge. *A. thaliana* has been a cornerstone in plant research due to its well-characterized genome and extensive genetic tools, but *S. americanum* offers a unique perspective. Numerous studies have reported and documented various genes conferring resistance to plant pathogens in this emerging model plant. For instance, Moon et al. (2021) identified *RipAZ1* in *S. americanum*, which serves as an avirulent factor against *Ralstonia solanacearum*, the causal agent of bacterial wilt in most crops. In addition, two resistance genes (*Rpi-amr1* and *Rpi-amr3*) to late blight (*Phytophthora infestans*) resistant - potato plants were reported by Lin et al. (2023). The integration of these genes into vegetable and other crop species could confer resistance against these pathogens. Other analogous investigations focusing on pathogen-microbe interactions in *S. americanum* have also been conducted, as outlined in Table 1. However, there is insufficient knowledge on other important plant pathogens. Notably, the impact of *Rhizoctonia solani* infection on *S. americanum* remains unknown. However, research on *S. nigrum* extract had proven to have high fungicidal (88%) properties against this pathogen (Pathak et al. 2020). Similar instances were observed for *Fusarium solani* and *Pseudomonas syringae* where a 10% concentration of *S. nigrum* extract were effective in inhibiting these pathogens' growth (Opande et al. 2017). These findings underscore the potential for uncovering possible resistance gene resource in *S. americanum* since these pathogens are of serious economic importance.

Beyond studies on plant pathogens, liquid and aqueous extracts from *S. americanum* have exhibited efficacy against diverse pathogens, offering potential applications in clinical studies (Afolabi et al. 2008; Cáceres et al. 1998).

Figure 1. *Solanum americanum* plant.

Figure 2. Morphology of *S. americanum* plant. A) *S. americanum* flowers (white, small, star-shaped), B) *S. americanum* flowers and leaves (leaves are ovate, arranged alternately along the stems), C) colour (initially green and may darken as it ripens), shape, and size (pea-sized) of *S. americanum* fruits.

Table 1. List of research conducted on *S. americanum*

Pathogen	Research findings	Recommendations	References
Ralstonia solanacearum	1.*RipAZ1* was discovered to be an *avirulent* gene against *Rastolnia solanacearum* 2. The 213-amino acid central region of RipAZ1 was reported to induce programmed cell death in *R. solanacearum*-infected *S. americanum* *3*. The gene can initiate early defense mechanism in the cytoplasm of the host cell.	The *S. americanum R* gene recognizing RipAZ1 offers potential for creating potato varieties resistant to both *P. infestans* and *R. solanacearum* in breeding programs	(Moon et al. 2021)
Phytophthora infestans	Nine (9) *Rpi-amr1* genes which offers broad spectrum resistance against different isolates of *P. infestans* were successfully identified and isolated from *S. americanum* Rpi-amr recognized the effectors *of Phytophthora parasitica* and *Phytophthora cactorum*	Combination of these genes and other R*pi* genes may help in the development of other crop varieties that are resistant to late blight disease	Witek et al. (2021)
Xanthomonas perforans	*X. perforans* was isolated from wild *S. americanum* on tomato fields.	*S. americanum* weed plants on tomato fields should be eliminated since they are favourable hosts for *X. perforans*.	Araújo et al. (2015)
Cariogenic *Streptoccoccus* mutants	Extracts from *S. americanum* together with *Hibiscus subdariffa*, and Garcinia kola in addition to methanol were analyzed for their role as growth inhibitors of Cariogenic bacteria. 2.5 g ml^{-1} of *S. americanum* extracts were ineffective in inhibiting growth of *Streptococcus* mutants	*S. americanum* liquid extracts should not be used in the treatment of cariogenic *Streptococcus* mutants	Afolabi et al. (2008)
Artemia salina	50 mg kg^{-1} of *S. americanum* extracts inhibited the growth of *A. salina*	*S. americanum* extracts can be used in the treatment of A. *salina*.	Cáceres et al. (1998)

4. *S. americanum* and plant viruses

Plant viruses are responsible for severe losses in the yield of economic crops. Plant viruses pose significant threats to crop production worldwide, impacting both quantity and quality of agricultural yields. Cao et al. (2020) estimated that more than 1500 viruses belonging to 26 families negatively affect plants. With the advent of new detection and sequencing methods, these numbers are expected to increase. Viruses infect crops, causing a range of detrimental effects with symptoms manifesting as leaf discoloration, distorted growth patterns, stunted growth, reduced yields, and in severe cases, plant death. Viruses are regularly transmitted through insect vectors, contaminated seeds, and agricultural tools.

Weeds and natural hosts within plant ecosystems serve as active reservoirs for these viruses. For instance, studies conducted in Poland revealed significant coinfections in weeds belonging to the *Asteraceae* family, including *Achillea millefolium*, *Sonchus oleraceus*, and *Crepis tectorum*, with pathogens such as Tomato spotted wilt virus (TSWV), Tobacco mosaic virus (TMV), Potato virus Y (PVY), Cucumber mosaic virus (CMV), and Tobacco ringspot virus (*TRSV)* (Korbecka-Glinka et al., 2021) . Additionally, in a survey conducted in Saudi Arabia by Al-Shahwan et al. (2017), *Sonchus oleraceus* was found to be infected with Alfalfa mosaic virus (AMV), Bean common mosaic virus (BCMV), Bean leaf roll virus (BLRV), Bean yellow mosaic virus (BYMV), Cucumber mosaic virus (CMV), Lucerne transient streak virus (LTSV), Pea streak virus (PeSV), Red clover vein mosaic virus (RCVMV), Tobacco streak virus (TSV), and White clover mosaic virus (WCMV).

Weeds of the Solanaceae family have been identified as significant reservoirs for numerous plant viruses in a review by Hančinský et al. (2020). The review highlighted Carolina horse nettle as a natural host for Peach rosette mosaic virus (PRMV), and petunia as a host of Potato virus B (PVB) and Tomato ringspot virus (ToRSV). Various viruses have been observed and documented on *S. americanum* across different global locations. For instance, in 2016, Potato yellow mosaic virus was recorded on both *S. americanum* and *S. pimpinellifolium* in Venezuela (Romay et al., 2016). Similarly, in the United States, Tomato chlorotic spot virus was reported on *S. americanum* (Badillo-Vargas et al. 2015), while in South America, Tomato chlorosis virus was also reported on *S. americanum* and *S. sisymbriifolium* (Arruabarrena et al. 2015). Although Tomato spotted wilt virus has not been officially reported on *S. americanum*, it has been found on other weed species such as *Amaranthus hybridus*, *S. nigrum*, *Tagetes minuta*, and *Datura stramonium* (Macharia et al. 2016). The confirmation of TWSV infection on *S. nigrum*, a close relative of *S. americanum*, prompts research questions regarding the potential for this virus to infect *S. americanum* as well.

Presently, within our research facility, there are ongoing research to investigate the susceptibility of *S. americanum* to TSWV and Tomato brown rugose fruit virus (ToBRFV). The prospect of identifying inherent resistance genes within this plant against these pathogens will serve as a roadmap in engineering resistant crop varieties. Since there are limited research studies

on the susceptibility or resistance of the plant against important plant viruses (for example Tomato leaf curl new Delhi virus-ToLCNDV, and Tomato yellow leaf curl virus- TYLCV), future research directions can be tailored to that.

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Influence of plants and spices on the formation of biogenic amines in meat

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ABSTRACT

Biogenic amines (BAs) consist of organic nitrogenous compounds produced by the amino acid's decarboxylation. They are present in various foods, such as meat products, and are associated with several health hazards. In meat, BAs are produced by the action of microorganisms that can decarboxylate amino acids. BAs can also be produced in meat naturally by enzymatic pathways. Tyramine, histamine, cadaverine, and putrescine are BAs frequently found in meat products. BAs are frequently found in fish depending on the species and time-temperature control, but can also be present in meat, particularly in canned, cured and fermented meat products. BAs are associated with various health disorders and toxicological effects including cardiovascular, respiratory and gastrointestinal system problems. Numerous factors influence the BAs generation in meat products. Factors such a handling, storage temperature, and processing procedures are essential for minimizing the risk of BAs formation to ensure food safety. Plant extracts and spices, play a multifaceted role in regulating BAs developments in diverse food items. Plant extracts containing phenolic/polyphenols, terpenoids and alkaloids have exhibited antimicrobial properties that can hinder the growth of microorganisms responsible for producing amines, consequently reducing BAs formation. Also, spices frequently contain compounds that impede the enzymatic conversion of precursor amino acids into biogenic amines.

1. Introduction

Biogenic amines (BAs) are naturally appearing nitrogen-containing compounds present in foods and beverages, particularly in fermented products. The classification of these compounds is determined by both their chemical composition and the quantity of amine groups present within their molecular structure. They can also be classified based on their origin or synthesis, which includes endogenous and exogenous sources. Endogenous sources can be further divided into dietary and non-dietary sources, while exogenous sources can be microbial or plant/animal in origin (Linares et al. 2011; Ruiz-Capillas and Herrero 2019).

BAs serve essential functions in both eukaryotic and prokaryotic cells. They have an important role and act as precursors for the synthesis of nucleic acids, alkaloids, proteins, and hormones, and function as neurotransmitters in the nervous system. Spermidine and putrescine are required for important biological functions, such as DNA and protein synthesis, and modulating RNA. In prokaryotic cells, BA production is related to defense mechanisms against acidic stress, generation of energy, and osmotic and oxidative stress responses (Premont et al. 2001; Santos 1996).

BAs are synthesized in food by bacterial decarboxylation of amino acids, particularly under certain conditions such as high temperature and humidity. Excessive consumption of BAs can lead to health issues, and the accumulation of BAs in products is an important concern for food safety and quality. The production of BAs is generally affected by various conditions and factors, such as type of meat, processing conditions, and storage temperature (Naila et al. 2010). Many microorganisms have the ability to produce BAs, and proper hygiene and sanitation practices (Wójcik et al. 2021), controlling temperature and pH during processing, and using microbial starter cultures can decrease BAs accumulation in meat (Gardini et al. 2016).

The formation of BAs is directly dependent on microorganisms with decarboxylase activity (Bardócz 1995). Various factors, such as pH, water activity, and temperature can affect the formation of BAs. The microbial load and diversity of bacteria can also impact BAs formation in food (Jairath et al. 2015). Moreover, the formation of BAs is contingent on the existence of microorganisms that produce amino acid decarboxylase and the accessibility of free amino acids, which serve as essential precursors (Ruiz-Capillas and Moral 2001).

Using specific starter cultures (Landete et al. 2007), lowering pH during fermentation (Gardini et al. 2016), reducing salt concentration, and adding food additives and preservatives are effective strategies for reducing BAs levels in fermented foods (Kongpun and Suwansakornkul 2000). Processing technologies such as high-pressure processing, irradiation, vacuum packaging, and smoking have also been shown to reduce BAs levels in food products (Naila et al. 2010).

Plant extracts have a significant impact on microbial growth and the formation of BAs. These substances can either inhibit or

promote microbial growth, depending on their composition. Plant extracts, such as thyme essential oil (EO), green tea extract, rosemary extracts, sage and tea have been successful in inhibiting the spoilage bacteria growth and reducing the accumulation of BAs in various food products (Bozkurt 2006; Cai et al. 2015; Huang et al. 2021; Lu et al. 2015; Özogul et al. 2015). Plant extracts and essential oils have also been found to decrease BAs formation, improving the safety of products (Wang et al. 2021).

Spices such as red pepper, ginger, garlic, clove, cinnamon, and others have been shown to influence the levels of BAs in food products. Red pepper's capsaicin can reduce BAs in sausages (Hirasa 1998), while cinnamon have inhibitory effects on BAs production (Shakila et al. 1996). These spices can enhance food safety by limiting the growth of BAs-producing bacteria and reducing amine levels in various dishes, including fermented sausages and anchovy products (Lu et al. 2015).

The aim of this review is to find out the effect of some food additives, plant extracts, and spice extracts on the formation of BAs in food products and especially meat products. Also, this review aims to shed light on the conditions that favor the formation of BAs, as well as the permissible limits for their presence in food products in general.

2. Classification of Biogenic Amines

BAs can be categorized according to their chemical structure (Fig. 1). Aromatic compounds, such as phenylethylamine and tyramine, aliphatic compounds, such as cadaverine, spermidine, spermine, and putrescine, and heterocyclic compounds, such as histamine and tryptamine, are among the classifications (Ruiz-Capillas and Jiménez-Colmenero 2005; Smith 1981). Furthermore, it is possible to categorize them based on the number of amine groups they contain, which includes monoamines (like tyramine and phenylethylamine), diamines (such as putrescine and cadaverine), and polyamines (including spermine and spermidine). According to certain researchers, polyamines such as spermine and spermidine should not be regarded as BAs because they are generated through a condensation process following decarboxylation (Linares et al. 2011).

BAs can also be grouped according to their origin or synthesis. BAs can be endogenous, produced by living organisms, or exogenous, produced by microbial activities in foods. Endogenous BAs are synthesized by living organisms during normal metabolism or in response to various stimuli, such as stress, infection, or injury. Exogenous BAs are produced by microbial activities in foods, such as fermentation or decay (Linares et al. 2011; Prester 2011; Santos 1996).

3. Influential Factors in the Formation of Bas

Several factors impact the formation of BAs, including composition of meat (such as protein, free amino acids, fat content, etc.), pH, and raw material source, and also, the existence of free amino acids (FAAs) is directly related to proteolysis since they provide a substrate for BAs production (Fig 2). A majority of the microorganisms present in meat during storage and treatment increase precursor concentrations through proteolytic reactions. Two key elements are required to form BAs in products: free amino acids, which serve as the basic precursors, and microorganisms that possess amino acid decarboxylases. The presence of a suitable environment that promotes the growth of these microorganisms is also essential (Ruiz-Capillas and Jiménez-Colmenero 2005).

Studies have shown that BAs can be affected by the type of meat source. A few studies have suggested that packed meat products containing only pork (e.g., cooked and cured ham) tend to have a lower level of histamine and tyramine formation than derivatives containing pork and beef (chorizo, salchichon, salami, or Bologna sausages), which tend to have higher levels of tyramine formation (Vidal et al. 1990; Wortberg and Woller 1982).

Temperature is another critical factor that can influence the production of BAs in food (Suzzi and Gardini 2003). Bacterial growth and enzyme activity are temperature-dependent, and 20°C to 37°C is the optimal temperature range for BAs formation (Karovičová and Kohajdová 2005). However, different bacterial species have different temperature requirements for BA

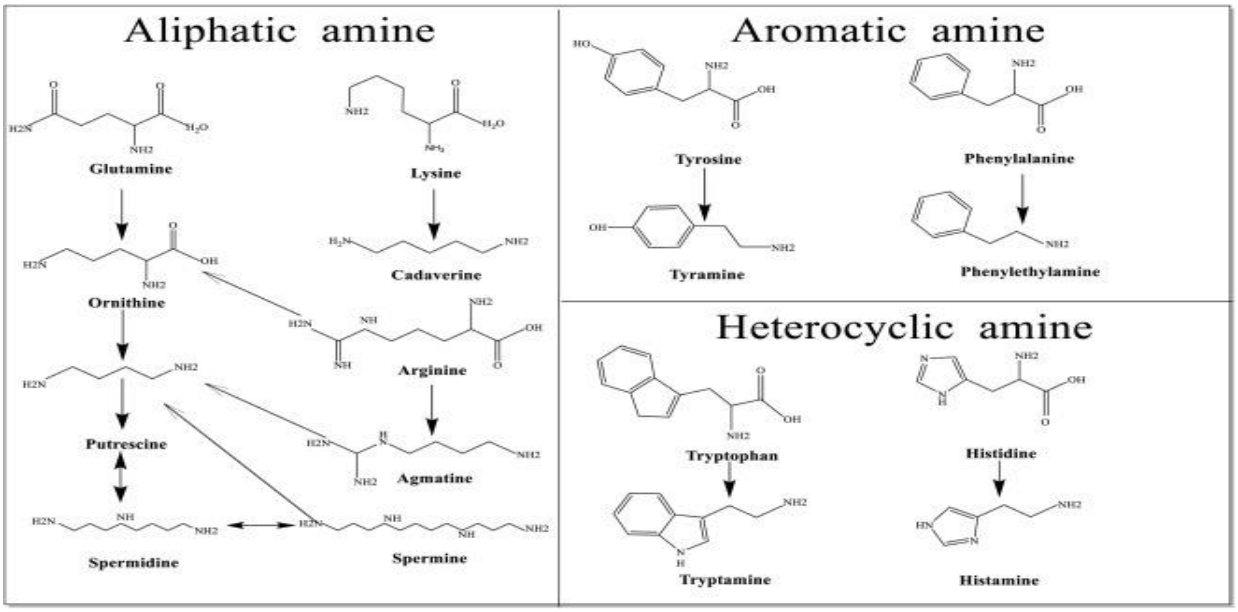

Figure 1. The precursors of BAs and their structures (Li and Lu 2020).

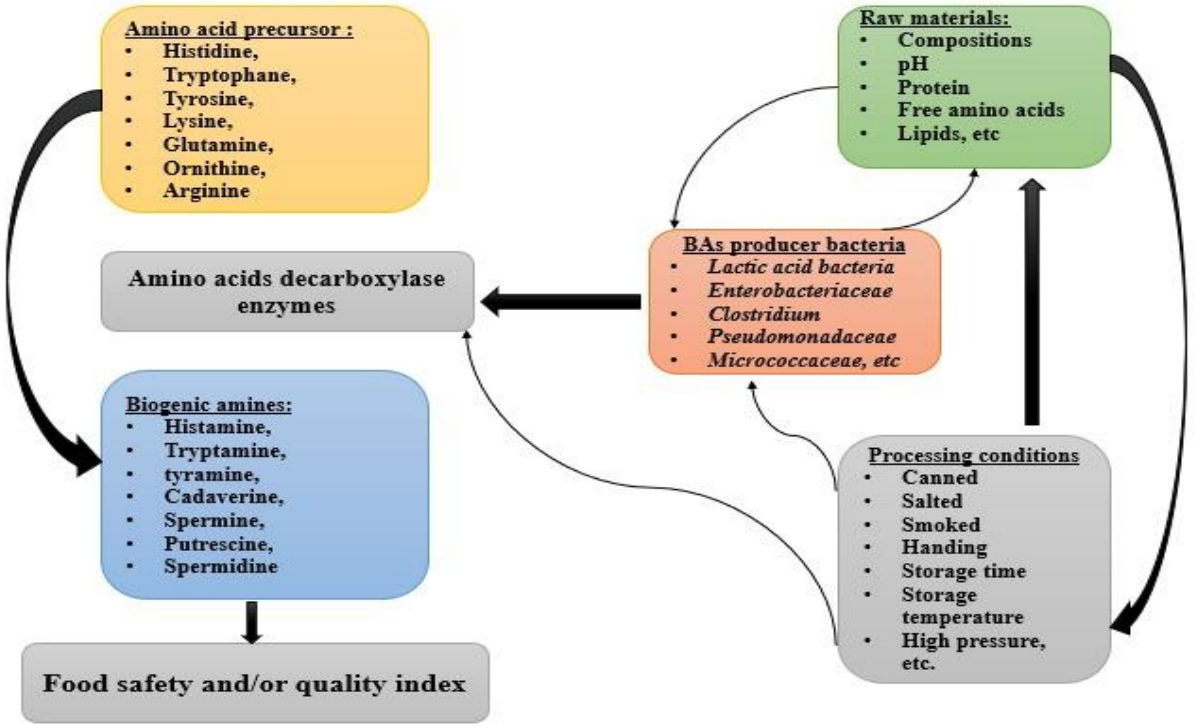

Figure 2. Effect of different factors on BAs formation (Ruiz-Capillas and Herrero 2019).

formation, and some bacteria can produce BAs at lower temperatures (Halász et al. 1994). Microbial development can also be slowed down by freezing fish between -18 and -30°C, but certain enzymatic and nonenzymatic processes and reactions may still occur (Karoui et al. 2017; Sampels 2015; Stonehouse and Evans 2015).

Due to the fact that they are amines precursors and serve as microbial development substrates, FAAs play a crucial part in the production of BAs in meat and meat products. Concentrations rise in tandem with proteolytic activities occurring during treatment and storage, primarily because of the numerous bacteria present. However, it has not been feasible to demonstrate a clear correlation between free amino acids concentrations and the production of the equivalent BAs in meat, such as fish (Eerola et al. 1996; Ruiz-Capillas and Moral 2001).

The pH balance has a substantial influence on the level of BAs due to two mechanisms that affect their production. Acidity inhibits the growth of microorganisms by affecting their growth (Jairath et al. 2015; Maijala et al. 1993). As a method of defense against acidic media, bacteria produce more decarboxylase when the pH drops because low pH encourages them to do so (Cid et al. 2008). BAs are produced as pH decreases due to increased decarboxylase activity. The decarboxylation of amino acids requires a pH above 4.5, and bacterial species such as Enterobacteriaceae and Pseudomonas can produce BAs at pH values between 5.0 to 9.0. In contrast, lactic acid bacteria require lower pH values for BA formation, between 4.5 to 6.5 (Halász et al. 1994). They underscored that inducing a swift and substantial drop in the pH of sausages can be an effective measure to inhibit the proliferation of amine-positive microorganisms, particularly the Enterobacteriaceae family, thereby safeguarding meat products from the formation of BAs (Bover-Cid et al. 2006).

4. Plant and Spices Extracts to Control Biogenic Amines

Plant and spices extracts have a significant impact on microbial growth and the formation of biomolecules. They can inhibit or promote microbial growth, depending on the specific compounds they contain. Additionally, they play a role in providing essential amino acids and stimulating their production. Understanding these effects is vital for optimizing nutrition strategies and has applications in various fields. Some reduction techniques of BAs are explained in the titles below.

4.1. Plant Extracts and Bioactive Compounds

Özogul et al. (2015) conducted a study to examine the effect of various levels of carvacrol on the BAs production by different pathogenic bacteria. The study encompassed a range of bacteria, including *Pseudomonas aeruginosa*, *Salmonella paratyphi A*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *E. faecalis*, *E. coli*, *A. hydrophila*, and *Listeria monocytogenes*. The findings revealed that all the bacteria tested had the ability to decarboxylate multiple amino acids, and the concentration of carvacrol, as well as the bacterial species, influenced the reduction in BAs formation.

Also, the use of green tea extract in Turkish dry-fermented sausage resulted in a decrease in the levels of tyramine, putrescine, and histamine as compared to the control (Bozkurt 2006). The impact of thyme essential oils on the accumulation of biogenic BAs was investigated in traditional horse meat sausages in Xinjiang. The inclusion of thyme essential oil resulted in a reduction in BAs levels (specifically, tyramine, putrescine, cadaverine, and histamine) (Křížek et al. 2018). When 5% garlic extract was added to fermented anchovies, the quantity of BAs was decreased by around 8.7% (Mah et al. 2009).

Recently, the growing demand for natural products and the shift away from synthetic additives in the food industry has led to an upsurge in the use of essential oils as natural flavor and aroma enhancers. Essential oils such as clove, which contain eugenol, possess antibacterial properties that can delay the accumulation of BAs in mackerel caused by *E. aerogenes* (Nandanie and Sakaguchi 1993). Additionally, the utilization of a blend of thymol, lemon extract, and grapefruit seed extract in conjunction with modified atmosphere packaging (MAP) successfully prevented the presence of amines in blue fish burgers (Del Nobile et al. 2009).

Furthermore, thyme essential oil decreased the overall bacterial count and particularly the Gram-negative Enterobacteriaceae family (Huang et al. 2021). Plant extracts were successful in stopping the proliferation of spoilage bacteria and also hindered the buildup of BAs (Cai et al. 2015; Lu et al. 2015). The level of BAs reduction was dependent on both the concentration of the essential oil and the type of bacteria present (Özogul et al. 2015). The primary bioactive antimicrobial agents present in thyme extracts that were effective in preventing BAs formation in sausages were carvacrol and thymol (Burt 2004). The use of rosemary extracts and sage tea was found to significantly inhibit histamine formation in sardines' muscles (Özogul et al. 2011). In a previous study, it was observed that the formation of BAs was inhibited by the antibacterial activity of plant extracts. For example, tea polyphenols were effective in reducing biosynthesis by inhibiting the growth of bacteria responsible for biosynthetic amine production (Fan et al. 2015).

4.2. Spices Extracts

Spices are aromatic plant substances that are used to flavor and color foods. They have been found to have an impact on the BAs accumulation in food. A study by Komprda et al. (2004) findings indicated that the significant red pepper content, in combination with the use of starter cultures, led to a reduced BAs content in dry fermented sausages. Red pepper, owing to its capsaicin content, is recognized for its ability to inhibit the proliferation of specific bacterial strains (Hirasa 1998). The impact of different spices, including cinnamon, clove, ginger, red pepper, on diminishing the levels of BAs in myeolchi-jeot, a traditional Korean salted and fermented anchovy preparation, was examined. Ginger extract was observed to decrease the presence of putrescine, while red pepper extract was effective in lowering cadaverine accumulation (Mah et al. 2009). Other studies have also showed that cinnamon and clove can reduce production histamine by *M. morganii* by up to 95% (Shakila et al. 1996). The inhibition of tyramine formation was observed with the addition of spice extracts such as cinnamon, clove, and anise (Shakila et al. 1996). A study showed Red drum filets (*Sciaenops ocellatus*) that were treated with 4 ml L^{-1} of spearmint, clove, and cumin oils had a low content of BAs, particularly histamine, putrescine, and cadaverine (Cai et al. 2015). A study was conducted to investigate the impact of ginger, cinnamon, and anise essential oils on the level of BAs in fermented sausage. The findings showed that the addition of 0.3% ginger, 0.3% anise, and 0.3% cinnamon essential oils resulted in reductions of total BAs by 28.58%, 34.87%, and 21.63%, respectively. These results suggest that tea polyphenol and plant essential oils, particularly anise essential oil, can be utilized to decrease the BAs formation and inhibit the growth of spoilage bacteria in fermented sausage, which can ultimately enhance its safety (Wang et al. 2021). In addition, plant extracts such as cinnamon, cloves, ginger, and fennel were found to be effective in inhibiting the growth of BAs-producing bacteria, thereby reducing levels of BAs in fermented sausages (Lu et al. 2015).

5. Conclusion

In conclusion, plant extracts have a significant impact on microbial growth and BAs accumulation in food. They can either promote or inhibit microbial growth depending on their composition and provide essential amino acids crucial for overall health and protein synthesis. Determination of microbiological compositions and amino acid contents in the samples is a critical step in understanding the formation of BAs. Reducing BAs in food is essential for food safety and quality. New extraction techniques and reducing methods to obtain high effective active ingredients from plant materials such as thyme, clove, anise, green tea and spices should be studied further in the future.

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Determination of germination characteristics and salinity and drought tolerances of Mountain Swan (*Atriplex nitens* Schkuhr)

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ABSTRACT

In this study, it was aimed to determine the appropriate seed germination temperature, salt tolerance, salinity and drought tolerance of seedlings in Mountain swan (*Atriplex nitens*). For this purpose, an experiment was established in 2021 in laboratory conditions according to the factorial experiment design in random plots and in greenhouse conditions according to the random plots experiment design with three replications. Firstly, 4 constant (10, 15, 20, 25, 30°C) and 2 variable (20/15, 25/15°C) temperatures were used in the refrigerated incubator. Total germination rates and average germination times of seeds with and without pericarp were determined. Secondly, 6 different salt concentrations (0, 100, 200, 300, 400 and 500 mM NaCl) were studied considering the optimum germination temperatures (25°C and 20/15°C). At the end of the study, germination rates (%), average germination times (days) and sensitivity indices (SI) were determined. In the last two stages, seedlings were subjected to 4 different salinity (0, 100, 200, 300, 400 and 500 mM NaCl) and 5 different drought (control, low, moderate, high and severe) tests under greenhouse conditions. At this stage, plant and root length, stem thickness, leaf area index (LAI), plant and root dry weight, root/plant ratio and tolerance percentage values were measured. As a result of the laboratory study, it was determined that seeds without pericarp had a better germination percentage than seeds with pericarp. The highest total and normal germination rates were obtained from 200 mM NaCl treatment at 20/15°C. Germination rate was determined from 0, 100 and 200 mM salt treatments at 25°C. These results showed that *Atriplex nitens* seeds could germinate in high salt concentrations and that their seedlings had high tolerance to drought and salinity.

1. Introduction

Approximately 2 billion ha of the world's agricultural areas are affected by drought and 954 million ha are affected by salinity. In Turkey, 66.9 million ha of agricultural land is affected by drought and 4.2 million ha of land is affected by salinity (SSCA 2014). Therefore, many cultivated and fodder plant species cannot be cultivated economically. As a consequence, these areas are out of production due to salinity and drought. It is necessary to search and find species and varieties that are not affected by these stress factors or that are resistant to them. One of these species is *Atriplex nitens* (Mountain swan), which belongs to the *Amaranthaceae* family. This species is an annual, fringed-rooted C3 plant that can grow in extremely saline and arid areas (Dursun and Acar 2015; Doudova et al. 2017). The plant has pericarp seeds and reproduces by seed. The plant can produce high amounts of seed and stem yields under unsoiled and rainfall conditions(Temel and Keskin 2022a; Keskin et al. 2023; Temel et al. 2024). Leaves are lanceolate and shiny, ashy green. Under wet and dry conditions without any fertiliser, the plant grows 2.0-3.0 m tall at the end of the vegetative period and 2.5-3.3 m tall at full flowering (Keskin and Temel 2022; Temel and Keskin 2022b). The flowers are sparsely racemose and monoecious, located at the very tip of the plant and at the ends of the lateral branches. The leaves of the plant are preferred as human food and the above-ground biomass produced in high quantity and medium quality is preferred as animal feed (Acar et al. 2017; Keskin and Temel 2022; Temel and Keskin 2022b; Temel et al. 2022a; Temel et al. 2022b). In crop production, low germination rates are one of the main reasons that restrict cultivation and cause great economic losses. The environmental factors affecting seed germination are temperature, water and oxygen (Emiralioğlu and Acar 2022). Temperature is the most important among these factors (Erkovan et al. 2017). Because temperature affects germination by changing water absorption, diffusion of mobile substances and enzyme activity. (Kacar et al. 2022; Başaran and Aytaş Akçin 2022).

Low temperatures reduce the swelling rate, which leads to a reduced germination rate and biological damage to the seed. High temperatures can increase the swelling rate of seeds and lead to faster radicle emergence (Kenanoğlu et al. 2019). It may also reduce the germination rate of many seeds or cause seed death (Kenanoğlu et al. 2019). Variable temperatures favourably affect germination (Kenanoğlu et al. 2019; Temel et al. 2023). Seeds of some species need variable temperatures for germination (Dimen 2016). On the other hand, even if

conditions such as temperature, water and oxygen are suitable for germination, the presence of some factors at extreme levels in the germination environment is not suitable. One of these factors is salinity, which causes many negative effects by inhibiting seed germination at high levels or by promoting the onset of dormancy at low levels (Gürsoy 2023). These effects may include reduced imbibition due to low osmotic potential, altered enzymatic activity due to toxicity, disruption of the balance of plant growth regulators (Yıldız et al. 2017) inhibition of protein metabolism (Dölarslan and Gül 2012), decreased utilisation of nutrients in seeds or inhibition of mitosis of cells (Julkowska and Testerink 2015; Khan et al. 2019).

Germination rates of seeds are generally high at optimum temperature values. At these temperatures, seed germination may not be affected much by salinity (Liu et al. 2021; Temel et al. 2023). Seedling period is another important plant development stage that should be emphasised in cultivation for the formation of healthy individuals. Because in this early developmental stage, seedlings are exposed to many stress factors such as weed competition, salinity and drought that limit crop diversity and productivity (Partheeban et al. 2017; Abbasdokhta et al. 2014; Yohannes and Abraha 2013). For these reasons, it is necessary to know the minimum, optimum and maximum germination temperatures of the species.

It is of great importance to bring saline and arid soils, which are very abundant in the world, into the economy. There is no detailed study on how Mountain swan, which can grow in such areas and has high economic value, responds to temperature, salinity and drought conditions in seed germination and seedling development. With this study, it was aimed to determine the appropriate temperatures and salinity tolerances of Mountain swan in seed germination and to prove that it can be grown in saline and arid soils by testing the degree of salinity and drought tolerance in seedling development.

2. Materials and Methods

The present research was conducted both in the laboratory (seed germination temperatures and germination period salinity tests) and controlled greenhouse conditions (seedling period salinity and drought tests) in 4 stages between 2020-2021. Pericarp and non-pericarp seeds of Mountain swan were used in the study (Figure 1).

The studies planned under laboratory conditions were established according to the factorial experiment design in random plots, and the studies under greenhouse conditions were established according to the random plots experiment design with three replicates. In order to determine the appropriate germination temperatures, seeds with and without pericarp were held in a refrigerated incubator at 4 constant (10±1°C, 15±1°C, 20±1°C, 25±1°C, 30±1°C) and 2 variables (20/15±1°C, 25/15±1°C) (12/12) temperatures. The seeds were sterilised with 2% sodium hypochlorite for 10 minutes and then washed with distilled water. After this process, the seeds (25 seeds in each treatment) were placed in petri dishes (12 cm) and covered with blotting paper. After that, 10 ml of 2% pomarsol solution was poured into the germination cups against fungal pathogens. Humidity controls were made during the experiment, and 5 ml of pure water was added when necessary. The experiment was maintained for 28 days. In daily controls, the seeds whose rootlets reached 2 mm in length were considered germinated and taken out of the germination cups (Shiade and Boelt 2020). In the second process, seeds were subjected to salinity tolerance tests at the two most appropriate temperatures (25°C and 20/15°C) and salt concentrations of 0, 100, 200, 300, 400 and 500 mM NaCl during germination. The procedures for determining the appropriate temperatures were observed, and 10 ml of pre-prepared appropriate salt solutions were poured over the seeds during moisture controls (Tan and Akçay 2019). In order to prevent salt accumulation in the germination dishes to which salt solution was added, the blotting papers were changed at 2-day intervals. At the end of the experiments, germination rates, average germination times and the sensitivity indices of the seeds were determined using the following formulae.

Germination Rate (%)= (Total number of seeds germinated at the end of 28 days / total number of seeds placed in germination cups) x 100.

Mean Germination Time/Rate (days)= $\Sigma(ix) / \Sigma i$ (Bilgili et al. 2018)

i: Number of germinated seeds on the counting day, x: Number of counting days

Sensitivity Index (SI)= Mean germination time in the salt treatment / Mean germination time in the control treatment (Altuner et al. 2019).

For seedling period salinity (0, 100, 200, 300, 400 and 500 mM NaCl) and drought tests (control, low drought, moderate drought, high drought, and severe drought), the soils (garden soil + 10% burnt farmyard manure) were filled into potsnumber 8 (diameter 47x39 cm= 35 L) at the same level. The studies were carried out in 3 repetitions. For this purpose, 18 pots were used for salinity tests and 15 pots were used for drought tests. Sowing was carried out at a sowing depth of 3-4 cm. Seedlings were watered with distilled water until the 10th day of emergence. Then, in the seedling salinity tests, salt solutions prepared previously were gradually applied with adaily increase of 50 mM

Figure 1. Seed types.

until the targeted concentrations were reached (Hariadi et al. 2011; Shavrukov 2013). When the targeted concentrations (levels) were reached, the solution was applied according to the amount of salt in the concentration determined for each treatment. These treatments were made during the period when the plants needed water (when 50% of the available water was consumed).

In the low, moderate, high and severe drought tests during the seedling period, water was added until the field capacity was reached when 50%, 75%, 90%, and 95% of the available water was consumed, respectively. For this purpose, field capacity and sustained wilting points of the soils placed in pots were determined before the experiment. Then, the moisture content of the soils was measured with a soil moisture measuring device (by burying the probe of the device at a depth of 20-30 cm in 2-3 places of the potted area). The amount of moisture decreasing from the field capacity was determined using the available water-holding capacity calibration curve determined by USDA. The pots in the control group were kept constant at field capacity throughout the experiment. These treatments continued until flowering began (Keskin and Temel 2022; Temel and Keskin 2022b), which is the most suitable harvesting period for Mountain swans. In the present study, when the plants in the control treatment began flowering, the plants in the other treatments, including the control, were harvested in this period. Plant height, root length, stem thickness, leaf area index (LAI), plant dry weight, root dry weight, root/plant ratio, salt tolerance percentage (STP) and drought tolerance percentage (DTP) were determined in each treatment before harvest (Hariadi et al. 2011; Adolf et al. 2012; Raney et al. 2014) using the formulas given below:

Root/plant ratio (%)= (root dry weight / plant dry weight) * 100

Percent salt tolerance (%)= (Plant dry weight in salt treatment/plant dry weight in control treatment) * 100

Drought tolerance percentage (%)= (Plant dry weight in drought treatment/plant dry weight in control treatment) * 100

The data obtained in seed germination temperatures and germination period salinity tests were subjected to analysis of variance in SPSS statistical package program according to the factorial arrangement in random plots experimental design, and in seedling period salinity and drought tests according to random plots experimental design, and the grouping of significant means was made according to the Duncan multiple comparison test.

3. Results and Discussion

Germination rates (%) and average germination times (days) of Mountain swan seeds obtained as a result of different temperature treatments are given in Table 1. When Table 1 is examined, it is seen that seeds without pericarp germinated in a shorter time and had higher germinations rates than seeds with pericarp in terms of seed types. This may be due to the presence of germination inhibitors in the fruit shell or the slowing down of water uptake by the fruit shell (pericarp). Previous studies have shown that the seed coat and pericarp inhibit gas diffusion. In addition, it has been revealed that it restricts water uptake or physically prevents the germination of seeds due to chemical inhibitors (Baskin and Baskin 2014; Tan and Akçay 2019). In terms of temperature, it was observed that the germination rate and speed of seeds were low at low temperatures. The best germination rate (98.0%) and speed (3.8 days) were obtained at 20/15°C and 25°C, respectively. This may be due to the slowing down of water absorption into the seed at lower temperatures. As a matter of fact, decreases in germination rate at temperatures below the optimum temperature may be due to decreases in swelling rate with decreasing temperature (Kacar etal. 2022).

Regarding seed type x temperature interaction, the fastest germination was determined in seeds without a pericarp at 30°C and the slowest germination was determined in seeds with a pericarp kept at 10°C (Figure 2). This may be since seed types do not respond to different temperatures at the same rate.

As a result of the germination test, it was determined that seeds without pericarp had a higher germination percentage and speed than seeds with pericarp. However, since it is difficult and uneconomical to remove the fruit shells (pericarps) of the plant in practice, the study was continued by using seeds with pericarps in germination-seedling period salinity and seedling period drought stages.

Total germination rates of Mountain swan seeds germinated at different temperatures and salt concentrations varied between 85.3% and 98.0%, and the highest total germination percentages were obtained from 0, 100, 200 and 300 mM NaCl concentrations. However, it was observed that normal germination rates decreased with increasing salt concentrations. The highest normal germination rates were obtained from the control (0 NaCl) treatments (Table 2). This may be due to the fact

Table 1. Germination rates (%) and germination times (days) of Mountain swan seeds as a result of different seed types and temperature treatments

Treatments	Germination rates (%)	Germination times (days)
Seed types		
Perikarplı	90.3 b	5.90 a
Perikarpsız	95.6 a	4.20 b
Temperature		
10°C	88.0 c	6.4 a
15°C	90.0 bc	6.1 a
20°C	91.3 bc	4.3 d
25°C	94.7 ab	3.8 e
30°C	95.3 ab	5.3 b
20/15°C	98.0 a	4.6 cd
25/15°C	93.3 a-c	4.9 bc
P value and significance	ST: 16.69**. T: 3.92**. ST× T: n.s.	ST: 186.79**. T: 30.310**. ST × T: 39.251**

** It is significant within the 1% probability limits. ns: non-significant. a, b, c: values shown with different letters in the same column are statistically different from each other. ST: seed type, T: temperature, ST x T: seed type x temperature interaction.

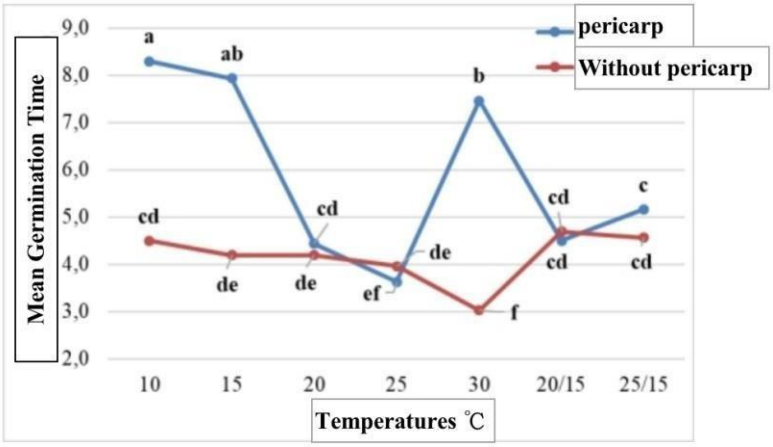

Figure 2. Effect of seed type x temperature interaction on mean germination time.

Table 2. Total and normal germination rates (%), mean germination time (day) and sensitivity index of Mountain swan seeds exposed to different temperatures and salt concentrations

Treatments	Total germination rate (%)	Normal germination rate (%)	Mean germination time (day)	Sensitivity index
Temperatures				
25°C	94.0	30.0 b	5.10 b	1.47 a
20/15°C	94.0	52.7 a	5.40 a	1.29 b
Salt concentrations				
0 mM	96.0 a	74.7 a	3.8 c	1.00 c
100 mM	98.0 a	66.0 b	3.9 c	1.01 c
200 mM	98.0 a	61.3 b	4.1 c	1.07 c
300 mM	96.0 a	34.7 c	5.5 b	1.47 b
400 mM	90.7 b	6.7 d	6.9 a	1.84 a
500 mM	85.3 c	4.7 d	7.1 a	1.89 a
P value and significance	SC: 24.3**.T: n.s. SC × T: 4.3**	SC:244.6**. T: 200.1**, SC × T: 50.1**	SC: 98.3**. T:5.4*, SC × T: 7.4**	SC: 88.8**. T:24.3*, SC × T: 9.7**

** and * are significant at the 1% and 5% probability limits, respectively. ns: non-significant. a, b, c: values shown with different letters in the same olumn are statistically different from each other. SC: salt concentration, T: temperature, SC x T: salt concentration x temperature interaction.

that increasing salt levels decreases the osmotic potential in the germination medium or damages the metabolic and physical structure of the seed. At high salt levels, osmotic potential decreases, and seed imbibition decreases (İnan et al. 2018). This results in metabolic and physical damage to the seed (Köseoğlu and Doğru 2021). It was also observed that increasing salt concentration, after a certain dose, increased the average germination time and salinity sensitivity of the seeds (Table 2).

According to these results, the average germination times and sensitivity indices obtained from 100 and 200 mM NaCl treatments were in the same statistical group as the control treatment. It is known that salinity slows down the water uptake potential of seeds in almost all plant species and thus delays germination. Because salt decreases the water potential in the environment during germination and slows down the water uptake of the seed, this, in turn, can affect the rate of biochemical events during germination and cause prolonged germination time (Tabassum et al. 2017; Öner and Kırlı 2018).

Table 2 shows that seeds at variable temperatures (20/15°C) had a higher normal germination rate and salinity tolerance level. This may be because *Atriplex nitens* may require variable temperatures for normal germination since it uses the C3 photosynthetic pathway. As a matter of fact, Kenanoğlu et al. (2019) and Temel et al. (2023) stated that seeds of some species require variable temperatures for germination. However, the average germination time of the seeds was shorter at constant temperature (25°C). This may be due to the fact that at constant temperatures, the seeds met their total temperature requirements in a shorter period and, therefore, showed rapid germination.

Accordingly, prolonged exposure to high temperatures may also increase salt damage. Indeed, Terzi et al. (2017) reported that high temperatures increased salt damage in *Salsola crassa*. The effect of temperature x salt concentration on the parameters examined was found to be statistically significant (Figure 3). Accordingly, the highest total and regular germination rates were obtained from seeds exposed to variable temperature conditions and 200 mM NaCl treatment, while the average germination time was found to be fastest in control and 100 mM NaCl treatments kept at constant temperature. It was also determined that seeds exposed to constant temperature and 100 mM NaCl treatment had higher tolerance to salinity (Figure 3).

When Table 3 was analyzed, it was observed that there were significant decreases in plant height, root length, plant stem thickness, leaf area index, plant dry weight, root dry weight and salt tolerance percentage values with increasing salt concentrations. This may be due to the decrease in salt concentrations in the growing medium, which decreases the water uptake potential of the seedlings. Accordingly, plant growth may slow down because physiological drought occurs in plants due to increased salt concentration in the growing

medium, which decreases the water uptake potential of the plant (Doğan and Çarpıcı 2016). As a result, the seedling cannot compensate for the reduced water due to transpiration. The resulting decrease in turgor pressure limits growth and development (Tan and Akçay 2019). The present study showed no linear decrease in root/plant ratio with increasing salt concentration as in other parameters. On the contrary, an increasing trend was observed. Although the root/plant ratio was generally higher in the control treatment,the highest ratios were statistically obtained from 300 and 400 mM NaCl treatments (Table 3).

Statistically significant differences were observed between the parameters examined (except root length and root/plant ratio) depending on the drought levels (Table 4). Table 4 shows that plant height, stem thickness and leaf area index decreased linearly with increasing drought. Similarly, plant dry weight and drought tolerance percentage also decreased, but these two parameters occurred after moderate drought. According to these results, the highest plant dry weight and drought tolerance percentage were obtained from control and low drought treatments. These differences may be due to the inability of plants to absorb the water they need for growth and development due to increasing drought severity. In order to realize growth (elongation in length, expansion in volume and increase in weight) in plants, a sufficient amount of water must enter the cell (Gençtan 2016). In addition, as water becomes scarce, the plant usually closes its stomata to avoid losing more water. This limits the uptake of CO2 required for fixation by photosynthesis and thus the net photosynthetic (organic matter) production (Örs and Ekinci 2015). As a result, the lack of sufficient water/humidity due to drought severity causes decreases in growth parameters. On the other hand, the highest root dry weight was determined at low and medium drought levels, which were in the same statistical group as the control group. Indeed, plants primarily react to drought stress conditions by slowing the formation of above-ground parts (Raney et al. 2014).

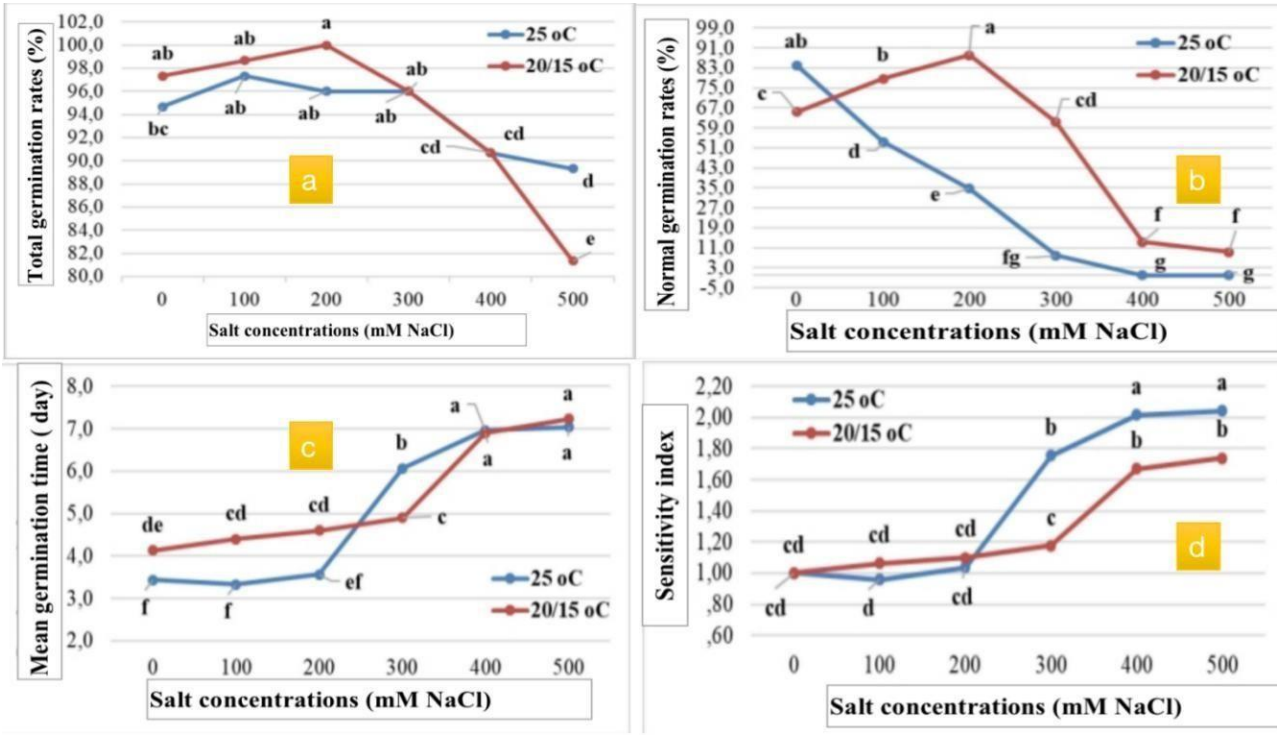

Figure 3. Effect of temperature x salt concentration interaction on total germination rate (a), normal germination rate (b), mean germination time (c) and sensitivity index (d).

Table 3. Some growth and development characteristics of Mountain swan seedlings at different salt concentrations

Doses	PH (cm)	RL (mm)	ST (mm)	LAI (cm^2)	PDW (g)	RDW (g)	RPR (%)	STP(%)
0 mM	198.7±20.1 a	1206.7±78.8 a	10.1±0.6 a	5.44±0.35 a	38.90±2.80 a	8.03±0.55 a	20.68±0.82 ab	100.00±0.00 a
100 mM	135.3±2.9 b	759.3±26.4 bc	7.8±0.3 b	1.88±0.09 b	20.10±1.29 b	3.40±0.12 b	16.98±0.52 c	51.66±3.30 b
200 mM	114.3±5.9 bc	855.3±19.9 b	6.6±0.2 c	1.37±0.07 bc	16.48±0.58 bc	3.27±0.12 b	19.82±0.04 ab	42.37±1.50 c
300 mM	104.7±2.3 c	817.3±24.0 b	6.0±0.3 cd	0.94±0.09 cd	14.84±0.48 c	3.13±0.07 bc	21.13±0.31 a	38.15±1.23 cd
400 mM	97.7±4.3 c	535.7±62.5 c	5.9±0.1 cd	0.79±0.09 d	14.14±0.18 c	2.97±0.15 bc	20.97±0.80 a	36.34±0.45 d
500 mM	88.0±5.1 c	189.3±21.9 d	5.5±0.3 d	0.53±0.12 d	12.78±0.49 c	2.40±0.21 c	18.71±0.96 bc	32.85±1.25 d
Avg.	123.1±9.5	727.3±38.9	7.0±0.4	1.82±0.41	19.54±2.22	3.87±0.47	19.72±0.42	50.23±5.61
P value and significance	19.72**	20.07**	27.85**	121.99**	56.01**	63.61**	6.00**	232.45**

**$P < 0.01$ is significant within the probability limits. a, b, c, values indicated with different letters in the same column are statistically different from each other. PH: Plant height, RL: Root length, ST: Stem thickness, LAI: Leaf area index, PDW: Plant dry weight, RDW: Root dry weight, RPR: Root/plant ratio, STP: Salinity tolerance percentage.

Table 4. Some growth and development characteristics of Mountain swan seedlings at different drought levels

DL	PH (cm)	RL (mm)	ST (mm)	LAI (cm^2)	PDW (g)	RDW (g)	RPR(%)	DTP (%)
0	218.7±10.1 a	1318.67±137.7	11.8±0.2 a	5.20±0.48 a	40.11±1.79 a	8.23±0.39 a	20.70±1.96	100.0±0.00 a
%50	198.3±13.1 ab	1350.33±101.9	11.0±0.3 ab	4.84±0.29 ab	35.37±2.14 a	7.17±0.22 a	20.44±1.57	88.16±3.19 a
%75	178.3±8.6 bc	1765.67±170.7	10.3±0.5 b	4.81±0.14 ab	29.62±1.73 b	7.30±0.72 a	24.53±1.11	74.41±7.06 b
%90	159.7±9.2 c	1281.0±161.1	8.8±0.2 c	4.03±0.33 bc	21.10±0.58 c	4.83±0.77 b	22.74±2.97	53.56±1.48 c
%95	151.0±0.6 c	1004.33±210.7	8.3±0.5 c	3.68±0.04 c	18.46±1.51 c	3.93±0.48 b	21.44±2.46	46.85±3.83 c
Ort	**181.2**±7.5	**1344.0**±89.1	**10.0**±0.4	**4.51**±0.19	**28.93**±2.28 **c**	**6.29**±0.48	**21.97**±0.90	**72.60**±5.57
P value and significance	8.87**	2.89n.s	16.33**	4.53*	31.48**	10.69**	0.63n.s.	32.89**

** and * are significant, n.s., insignificant at the 1% and 5% probability limits, respectively. a, b, c, values indicated with different letters in the same column are statistically different from each other. DL: drought level, PH: Plant height, RL: Root length, ST: Stem thickness, LAI: Leaf area index, PDW: Plant dry weight, RDW: Root dry weight, RPR: Root/plant ratio, DTP: Drought tolerance percentage.

4. Conclusion

The results of this study showed that Mountain swan seeds germinated faster and had a higher germination percentage at high temperatures (25, 30, 20/15 and 25/15℃) compared to low temperatures (10, 15 and 20℃) and seeds without pericarp have high germination rate compared to seeds with pericarp.

Although the germination percentage decreased with increasing salt concentration, it was revealed that seeds could germinate easily at high salt concentrations, and seedlings could grow at high salt and drought levels. These results suggest that *Atriplex nitens* can play an essential role in planting marginal areas abandoned due to salinity and drought, especially in bringing these areas into production as an alternative feed/food source.

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Variable rate phosphorus fertilizer recommendations for rainfed wheat

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ABSTRACT

A uniform application of phosphorus (P) fertilizers to spatially variable soils often results in under-fertilization in low P-localities and over-fertilization in high P-localities. This study aimed to evaluate the variable rate applicability of P fertilizers on a 300-ha sloping landscape under rainfed winter wheat cultivation for over 70 years. The soils were sampled (155 samples) using a random spatial sampling technique based on visual differences in soil color and topographic factors. Plant available soil P content (P_{av}) and other variables of soil samples were analyzed. The spatial variability of P_{av} was evaluated and the area was divided into three uniform zones (low, medium, high) for fertilizer P application based on the spatial variation of P_{av}. The values of P_{av} showed moderate variablity (CV= 21.3%). The fertilizer recommended by the Ministry of Agriculture and Forestry (MAF) was calculated for five identically-sized sub-regions. The results showed that P fertilizer rates calculated for all five sub-regions based on MAF were identical, suggesting that the MAF was insensitive to spatial variability of P_{av} in the study soils. Both semivariograms and surface maps of soil properties indicated a strong spatial association between P_{av} and each of plant available water content (PAWC) and aggregate stability index (ASI), suggesting that yield limitation casued by PAWC should be considered in a variable P-application program in the study area. A more comprehensive study is needed to evaluate the efficiency and cost-benefit economics of variable P application in the study soils.

1. Introduction

Application of commercial fertilizers contributes to crop growth to a considerable extent, resulting in a substantial yield increase in agricultural crops. However, uniform use of fertilizers has induced economical losses and caused pollution of the surface and groundwater, due to the spatial variability of soils, across the world (Vadas et al. 2004). The importance of the spatial variability of soil properties has long been recognized, emphasising the need for precise site-specific applications of agricultural fertilizers (Kassa et al. 2022; Sharma et al. 2022; Abera et al. 2022). Uniform fertilizer application may lead to over-fertilization in some localities and under-fertilization in others, resulting in improper fertilizer applications (Günal 2021). Site-specific fertilizer applications help equilibrate and stabilize the content of soil nutrients and yield (Sanches et al. 2021). Therefore, when high spatial variability of soil nutrient content is the case, application rates of those fertilizers should be adjusted site-specifically to optimize the nutrient supply to crops across the field (Ruffo et al. 2005).

Site-specific crop management (SSCM) considers variability in soil and crop parameters to optimize use of inputs such as fertilizers and pesticides (Sudduth et al. 1997). However, without an adequate knowledge of the spatial variability of soils, site-specific application of soil nutrients is impossible (Sawyer 1994). Conventional soil testing methods, used in determining spatial variability of soils, are costly and time-consuming. In addition, the time and cost required for intensive sampling in the SSCM can limit the implementation of a variable-rate fertilizer application.

Spatial variability management of soil chemical attributes is one of the means of precision agriculture (PA) to increase yield (Raddy et al. 2021; Beneduzzi et al. 2022). Understanding the variability in crop yield in relation to the spatial variations in soil properties can help more efficiently apply agricultural inputs on site-specific basis (Ameer et al. 2022; Ameer et al. 2023). Fertilizer application relying on soil characteristic map-based fertilizer recommendations may help reduce fertilizer input without sacrificing crop production (Yadav et al. 2023).

Several factors, such as differences in crop response to applied fertilizers and in exisiting nutrient pools in the soil, are considered in delineating nutrient management zones (MZs) on fields (Abera et al. 2022). Many researchers considered identifying existing nutrient pools in the soil to provide reliable fertilizer recommendations. Mapping of soil fertility is a practical and effective means to delineate the soils into low, medium, and high nutrient status zones (Ameer et al. 2022). These delineated internally homogeneous MZs, in terms of soil fertility and crop productivity management, can be treated separately for the precise application of fertilizers (Ameer et al. 2023). Some other techniques such as remote sensing (RS), geographical information system (GIS) (Yadav et al. 2023) and their combination (Trivedi et al. 2022) have been used successfully in

site-specific crop and fertilizer management. Modern geospatial tools such as RS, GIS, and Global Positioning System (GPS) have provided tremendously powerful means for surveying, mapping, monitoring, delineating, and charaterizing soil resources (Trivedi et al. 2022; Beneduzzi et al. 2022; Kumar et al. 2023).

In Türkiye, ferilizer recommendations under the fertilizer support program rely on the "Fertilizer Calculator" provided by the Ministry of Agriculture and Forestry of Türkiye (MAF)", which roughly considers variability in soil properties and concentrations of major nutrients in soil at the sampling time. This study aimed to 1) evaluate sensitivy of MAF-fertlizer recommendations to the varability in soil properties and 2) formulate a variable-rate P recommentation, based on spatial variation in P_{av}, for rainfed winter wheat, in a 300-ha land exhibiting differences in soil and slope properties.

2. Materials and Methods

2.1. Material

This study was carried out on a 300-ha sloping farmland, located 20 km from the center of Çankırı cith along the Çankırı-Ankara highway (Fig. 1). The study area comprises many scondary hillslopes characterized by varying aspects, steepness, and shapes, located on a sloping landscape with a general linear slope resulting from a liner increase in elevation toward the north. The area has been under rainfed winter wheat production for more than 70 years. Variation in slope properties and distribution of parent materials are key factors affecting yield variability. The prominent differences in soil color highlight substantial soil spatial variability potentially leading to variation in crop yield thoroughout the study area.

A dry sub-humid/semi-arid continental Anatolian type climate prevails in the study area (Iyigun et al. 2013). Long term mean annual precipitation ranges from 406.0 to 538.0 mm, mean annual temperature from 9.1 to 11.1°C and relative humidity from 61.0 to 66.0%. The long term means of minimum temperature range from −5.0 to −2.7°C (in January) and maximum temperatures from 26.4 to 30.9°C (in July). The lowest extreme temperature ever recorded was −25.0°C on 25 January 1950, and the highest was 42.0°C on 30 July 2000 (MGM 2024). Soils of the study area are Gypsic Haplustepts and Gypsic Ustorthents according to Soil Survey Staff (2014). The parent materials are gypsum/calcium carbonate mixed with colluvium in the majority of cases. Also, gypsum over lacustrine residuum generally appears on the flat to slightly sloping landscape positions.

2.2. Methods

2.2.1. Soil sampling and laboratory analyses

In this study, 155 soil samples were taken based on random geostatistical sampling technique from the plow depth (0-20 cm). The sampling was designed with a minimum distance between two samples set at least 5 m, ensuring an adequate number of lags at close proximity to safely model the semivariogram near the origin. A global positioning system (GPS) was used to address sample coordinates. Soil samples were transferred to a laboratory, dried at room temperature, passed through a 2-mm sieve and stored for analysis. The soil variables analyzed and the methods used in the analyses are given in Table 1.

2.2.2. Delineating uniform phosphorous application zones

The study area was conveniently divided into five equal-sized sub-regions based on visual differences in soil color and topography, and P recommendation-values were calculated with mean data for each of the sub-regions using the fertilizer recommendation calculator of the Ministry of Agriculture and Forestry of Türkiye (MAF). The recommendation for P for all five sub-regions was identical, indicating that the MAF was insensitive to spatial variability of P_{av} in the study area. For delineating variable P fertilizer management zones (MZs), the fertilizer recommendation was calculated for each of the sampling points using P_{av} and expected wheat yield. The expected wheat yield was determined based on farmer's statement, and it was taken as 4000 kg ha^{-1}. The mean and standard deviation of P_{av} and P recommendation values were calculated for the study area and then used to delineate MZs as follows:

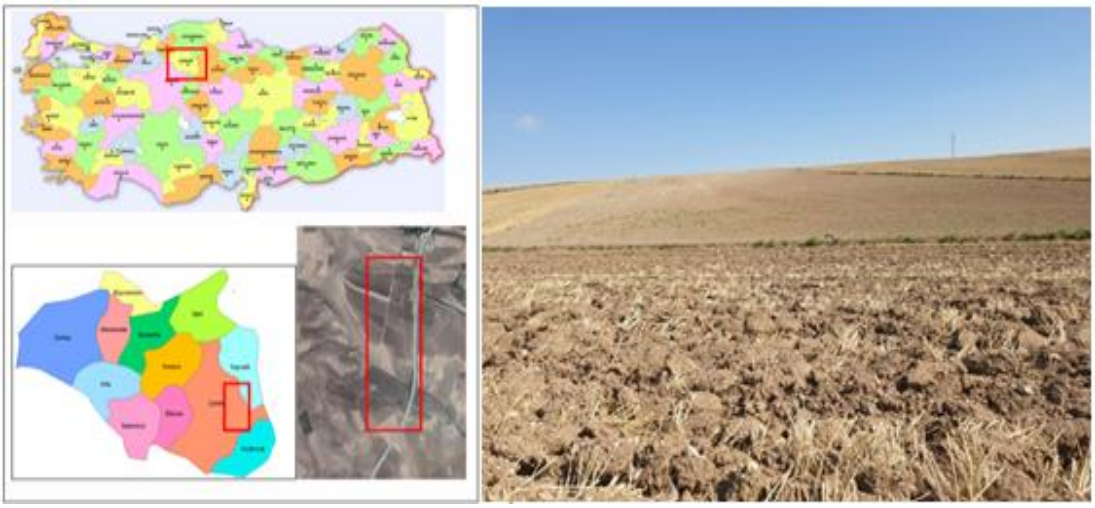

Figure 1. Location and view of the study area. The lighter colors indicate low fertility localities.

$$If\ P_{avi} < (M_{Pav} - SD_{Pav})\ then\ FP_i = M_{FP} + SD_{FP} \quad [1]$$

$$If\ P_{avi} > (M_{Pav} + SD_{Pav})\ then\ FP = M_{FP} - SD_{FP} \quad [2]$$

$$If\ (M_{Pav} - SD_{Pav}) < P_{avi} < (M_{Pav} + SD_{Pav})\ then\ FP_i = M_F \quad [3]$$

Where, SD_{Pav} is the standard deviation and M_{Pav} is the mean of P_{av}-values, and M_{FP} is the mean and SD_{FP} is standard deviation of fertilizer P recommendations, calculated using P_{av} and mean yield (4000 kg ha^{-1}) in the study area. P_{avi} is the plant available soil P content and FP_i is the fertilizer P recommended for sampling site *i*. For example, let SD_{Pav}= 3, M_{Pav}= 15, M_{FP}= 17, SD_{FP}= 4, and P_{avi}= 6, then FP_i for the site *i* can be calculated as follows: As P_{avi}= 6 < (15-3) = 12, the Eq. (1) should be used. Therefore, according to Eq. (1), FP_i= 17+4= 21 kg ha^{-1}. Thus, the 21 kg ha^{-1} application zone should include the sampling site *i*.

2.2.3. *Geostatistical analysis of spatial variability of soil variables*

A typical geostatistical analysis was conducted at three stages: an exploratory data analysis, a semivariogram analysis, and a spatial interpolation of the variable of subject (Isaaks and Srivasta 1989) The data for experimental semivariograms were modelled using commonly employed theoretical models (spherical, exponential, Gaussion models). Geostatistical analysis of soil variables of pH, EC, sand, silt, clay, OM, $CaCO_3$ and K contents and PAWC and ASI was conducted besides P_{av}. According to Webster (2001), variables with a skewness > | 1.0 | are assumed to be strongly skewed and log-transformed and those between | 0.5 | and |1.0| are assumed to be moderately skewed and square root-transformed, while those < | 0.5 | are assumed to be slightly skewed and do not need to be transformed. The data for EC were log-transformed to decrease its skewness below absolute | 0.5 |. The log-transformation resulted in a small decrease in skewness. However, after removing one data point and repeating the log-transformation this resulted in a tremendous decrease in skewness of the data for EC (Table 2). Similarly, to EC, removing four data points and then square root-transforming resulted in a substantial decrease in skewness for PAWC. As both full and reduced datasets for pH, clay content, silt content, and P_{av} were insensitive to data transformations, data were removed from those data sets until the values of skewness fell below absolute | 0.5 |. Table 2 shows descriptive statistics of full and reduced datasets and results for log- and square root-transformation for EC and PAWC, respectively.

Table 1. Soil variables and the methods used in their analysis

Soil property	Methods/device	Reference
Soil texture	By mechanical analysis	Gee and Bouder (1986)
Plant available potassium content	With a flame photometer	Kacar (1996)
Field capacity and wilting point	With pressure chambers	Cassel and Nielsen (1986)
Plant available water content	Difference between field capacity and wilting point	Cassel and Nielsen (1986)
Electrical conductivity	With an EC electrode in 1:2.5 soil-water suspension	Rhoades et al. (1999)
Soil pH	With a pH electrode in 1:2.5 soil-water suspension	Rhoades et al. (1999)
Organic matter content	By Walkley-Black method	Nelson and Sommers (1982)
$CaCO_3$ content	With a Scheibler calcimeter	McLean (1982)
Plant available P content	By Olsen method	Olsen (1954)
Aggregate stability index	By wet sieving	Kemper and Rosenau (1986)

Table 2. Descriptive statistics of soil properties in study area

Soil property	N	Min	Max	Mean	SD	Skewness	Kurtosis	CV, %
pH (1:2.5)	155	6.80	7.69	7.15	0.23	1.70	1.49	3.21
pH (1:2.5)	&128	6.90	7.20	7.06	0.055	-0.20	-0.04	0.77
EC (mS cm^{-1})	155	2.49	2630.00	472.10	521.3	3.16	9.31	110.40
#EC	&154					-0.41	9.22	
Sand (%)	155	7.97	63.56	26.24	7.97	0.24	-0.48	30.37
Clay (%)	155	16.75	69.70	53.80	7.43	-0.97	3.37	13.81
Clay (%)	&153	29.80	69.70	53.94	7.08	-0.42	0.66	13.13
Silt (%)	155	5.45	47.05	20.18	5.61	0.96	3.00	27.80
Silt (%)	&153	5.45	35.00	19.94	5.14	0.41	0.46	25.82
$CaCO_3$ (%)	155	4.65	32.76	17.12	6.22	0.38	-0.34	36.32
OM (%)	155	0.62	2.95	2.19	0.53	-1.10	0.65	18.27
OM (%)	&145	1.09	2.95	2.28	0.41	-0.77	-0.06	17.98
Na (mg kg^{-1})	155	5.90	37.69	15.78	15.78	0.70	2.57	75.91
K (mg kg^{-1})	145	13.51	65.10	38.94	12.98	-0.01	-0.92	33.33
PAWC (%)	155	2.90	52.08	20.87	11.69	0.87	-0.16	54.94
## PAWC	&151					0.38	-0.60	
ASI (%)	155	0.33	0.611	0.49	0.05	-0.21	0.20	11.16
P (mg kg^{-1})	155	3.42	20.11	15.26	3.25	-1.73	2.83	21.29
P (mg kg^{-1})	&140	12.21	20.11	16.25	1.65	-0.43	-0.41	10.15

N: Number of soil samples, Min: Minimum, Max: Maximum, SD: Standard deviation, CV (%): Coefficient of variation EC: Electrical Conductivity, OM: Organic Matter, Na: Sodium, K: Potassium, P_{av}: Plant available phosphorus, FC: Field capacity, WP: Wilting point, ASI: Aggregate stability index.
#: log-transformed, ##: Square root-transformed, &: Number of data points retained to decrease the corresponding value of skewness below | 0.50 |.

The spatial structure of soil variables including P_{av} was modeled and ordinary kriging (OK)-interpolations were conducted using geostatistical software (GS$^+$ 2022). The most suitable semivariogram model was selected based on the highest R^2 and lowest SSE-values for semivariogram fitting. In addition, cross-validation correlation coefficient (r_{CV}) was considered to judge if the theoretical semivariograms could adequately represent the experimental semivariograms. For sand content, $CaCO_3$ content, and ASI full data (155 data points); for EC log-transformed and PAWC square root-transformed reduced data; and for rest of the soil variables reduced data were used in geostatistical analysis (Tables 2 and 3).

We used variable lag-distances to increase the quality of semivariogram fits. Also, some data points were removed from some lags to increase modeling performance, especially for increasing R^2 and decreasing RSSE-values. Table 3 shows the number of data points used in semivariograms modeling. OK-interpolations were conducted using parameters (sill, range (A), and nugget variance) from the corresponding theoretical semivariograms. A minimum of 10 and a maximum of 13 neighboring data were used in OK interpolations. We applied inverse distance with varying power when the OK-interpolation performed inadequately. The data were interpolated by normal distance interpolation when r_{CV} was insignificant in both of IDW and OK-interpolations.

3. Results and Discussion

3.1. Descriptive statistics of soil properties

The variability of soil attributes plays a crucial role in defining uniform nutrient management zones. The sand and silt contents of soil textural components were highly similar in CV compared to clay content (Table 2). Sand and silt content were moderately and clay content was slightly variable according to (Mulla and McBratney 2002), who noted that a soil attribute with CV< 15% is deemed slightly, between 15 and 36% moderately and >36% highly variable. Silt content was moderately and sand and clay contents were slightly right-skewed according to Webster (2001). The values for K and Na showed highly dissimilar statistical distributions as suggested by their corresponding values of skewness, kurtosis, and CV. Values for Na were highly variable, while those for K were moderately variable. The values of $CaCO_3$ content ranged from 2.49 to 32.76 and were moderately variable and slightly right-skewed. The mean for $CaCO_3$ suggested that the majority of the study soils were highly calcareous (Table 2). The values of OM content were moderately variable and strongly left-skewed, indicating that some extremely low OM-valued localities were present in the study area. Aggregate stability index (ASI) is an important indicator of soil physical quality. Values for ASI were between 33 and 61% and showed a slightly left-skewed distribution and little variability. The values for ASI indicated that the study soils were structured weakly to moderately in strength. Soil pH has a strong influence on the soil P availability to plants (Tisdale et al. 1993). The values of soil pH ranged from 6.80 to 7.69 (Table 2). The range indicated that soil pH would be a limiting factor of P-availability, especially at some high pH-localities in the study area. Values for EC showed a highly asymmetric and flat distribution as suggested by high positive skewness and kurtosis. Also, the greatest value of CV occurred for EC. The ranges for EC and pH indicated that no salinity or alkalinity problems were the case in the study area. Values for FC and WP showed somehow dissimilar statistical distribution, while they were highly similar in variability, they were highly different in skewness and kurtosis. Plant available water content (PAWC) was highly variable (CV%= 54.7); its values ranged from 2.74 (very low) to 48.74 (very high). The value for skewness (0.79) indicates the presence of some relatively high-valued localities of PAWC across the study area.

3.2. Spatial variation of plant available soil phosphorous content as related to uniform phosphorous application zones

Values for P_{av} ranged from 6.84 to 23.53 mg kg^{-1} and exhibited a medium variability (CV= 21.29%) (Table 2). High positive skewness indicated that some high P_{av}-valued localities are present in the study area. High short-range variability (nugget variance) and short geostatistical range (A= 20 m) indicated that P-availability was affected by numerous soil factors as noted elsewhere (Trangmar et al. 1985). Table 3 shows that the geostatistical range for P_{av} is 20 m, and that the values are moderately spatially dependent according to Cambardella et al. (1994), who suggested that a variable with percent nugget effect < 25 is dependent strongly, between 25 and 75 moderately, and > 75 weakly. Figure 2b shows spatial pattern in P_{av} across the study area. The P_{av} was interpolated by OK using the parameters of semivariogram given in the Table 2, and results are shown in Table 3. The cross-validation correlation coefficient (r_{cv}) suggested that the kriging interpolation performed poorly, which may be attributed to a short-range of influence (small A) and a high short-range variability (relatively high nugget variance) for P_{av} as indicated corresponding semivariogram (Fig. 2a and Table 3). The inverse distance weighted (IDW) interpolation technique was tried using different power-values, again, the results were unsatisfying.

Table 3. Semivariogram analysis of study soils

SV	M	C_o	C	C_o/C0+C	A	RSSE	R^2	r_{CV}	n
Sand (%)	E	33.30	66.60	0.34	276.0	349.00	0.86	0.54	151
Clay (%)	E	16.80	58.00	0.29	690.0	149.00	0.94	0.62	152
Silt (%)	E	8.37	30.00	0.29	51.0	71.40	0.74	0.29	153
OM content (%)	E	0.11	0.23	0.33	192.0	6.48×10^{-3}	0.63	0.01	145
pH	E	0.001	0.003	0.47	24.0	1.84×10^{-7}	0.53	0.01	128
EC (ms cm^{-1})	G	0.25	0.50	0.50	51.9	0.07	0.49	0.60	154
$CaCO_3$ content (%)	S	6.24	33.80	18.00	25.0	430.00	0.38	0.56	150
PAWC (%)	E	0.60	1.46	0.41	21.0	0.03	0.72	0.01	150
ASI	S	6.30×10^{-3}	2.8×10^{-2}	0.23	20.0	6.00×10^{-7}	0.62	0.26	150
P (mg kg^{-1})	E	1.27	3.18	40.93	18.0	1.45	0.62	0.05	140

SV: Soil variable, M: Model type (E exponential, S: spherical, G: Gaussian), Co: Nugget variance, C: sill, A: geostatistical range, RSSE: Residual sum of squared error, R^2: Coefficient of determination for semivariogram fit, r_{CV}: Correlation coefficient between cross validated and actual values, n: number of data points (out of 155 data points (full data)) included in the geostatistical analysis, EC: Electrical conductivity, OM: Organic matter, P_{av}: Plant available phosphorus, FC: Field capacity, WP: Wilting point, PAWC: Plant available water content, ASI: Aggregate stability index.

Therefore, we used the normal distance interpolation technique to build a surface map for P_{av} (Fig. 2b). Fig. 2b shows that most of the low P_{av} sites were located in the southern and northern part of the study area. Please notice that the "north" in the GS+-produced surface maps is different from the absolute north on the Google Earth Map for the study area.

Figure 2c shows uniform P fertilizer management zones (MZs) determined based on spatial distribution of P_{av}-values shown in Fig. 2b. Three MZs were defined: High, medium and low P application zones (Fig. 2c) Medium P application sites were located mainly in northeast and southwest, while high P application sites oriented from southeast to northwest (Fig. 3c). The reverse was the case for spatial pattern for P_{av} (Fig. 2b). The M_{FP} was 24 kg P ha^{-1} for the uniform application across the study area. The uniform P application could result approximately 450 kg P to be saved. However, plants in the high P application zones (sites) would suffer P deficiency due to the application of P fertilizer in inadequate amounts, which may result in significant

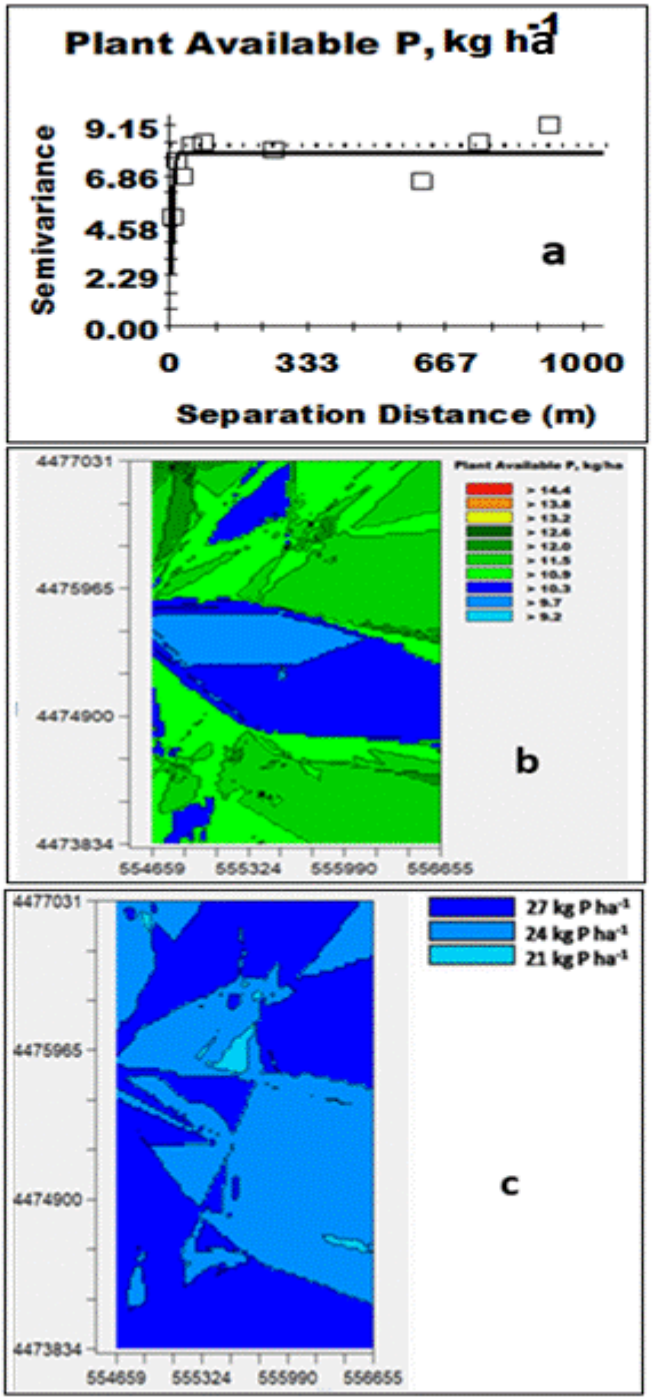

Figure 2. (a) Semivariogram and (b) spatial distribution pattern for plant available P content in study soils, (c) delineated uniform P application zones and corresponding fertilizer P requirements for each of the zone. The surface map in b was built by normal distance weighted interpolation. See text for explanation.

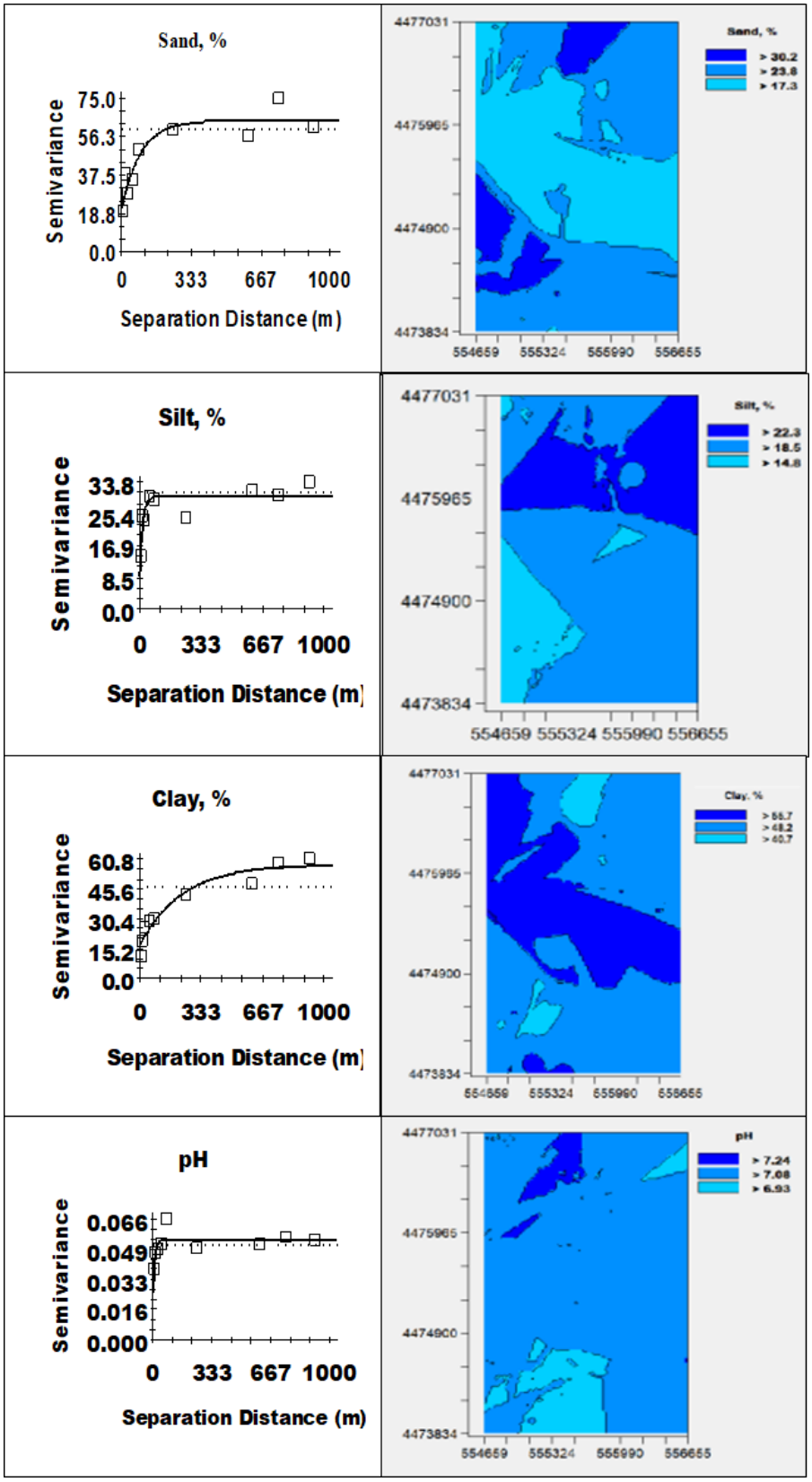

Figure 3. Semivariograms and corresponding surface maps of soil variables. The surface maps for OM content, pH, and PAWC were built by normal distance weighted interpolation.

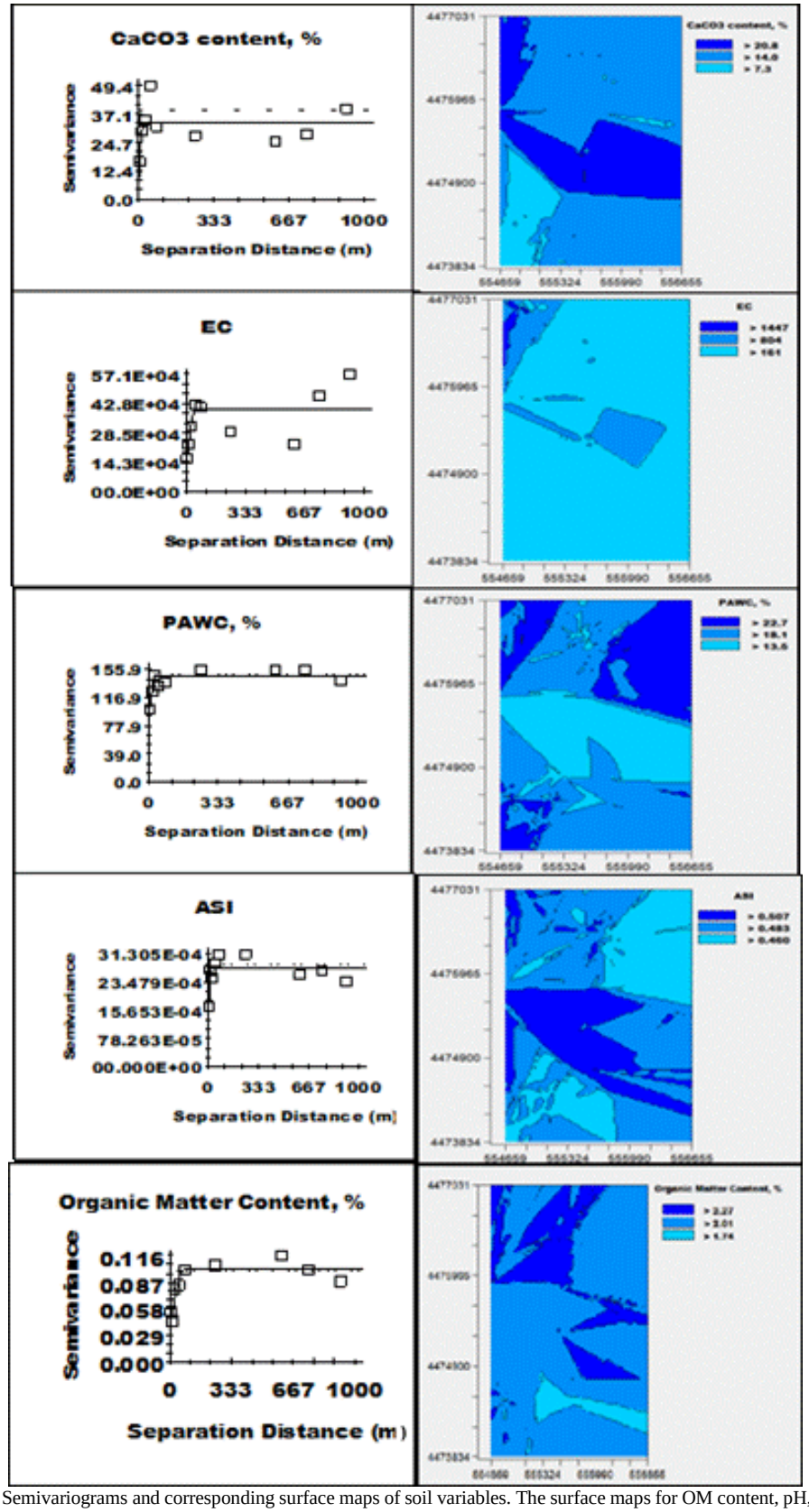

Figure 3. (continued) Semivariograms and corresponding surface maps of soil variables. The surface maps for OM content, pH, and PAWC were built by normal distance weighted interpolation.

decrease of the yield. A further study is needed to evaluate the cost: benefit economics of variable P applications in the study area. On the other hand, the size of management zone 1 is negligible (the isolated lighter blue-colored spots oriented from northwest to southeast, Fig. 2c); therefore, the study area was divided into two management zones: a medium P application zone including low P application spots and a high P application zone.

The study area is located on a sloping landscape with ridges and eroded hilltops, characterized by shallow topsoil and exposed subsoils. Overall, eroded soils, where low P_{av}–valued sites are located, had a lighter color than the other sites. Similarly, Fleming et al. (2001) reported lower yield for corn (*Zea mays* C.) on upper slope positions in Nebraska. Additionally, a strong correlation between P_{av} level and winter wheat yields in northeast Colorado was reported. In western Iowa, crop yields on footslope positions surpassed those on backslopes and side-slope positions, which was attributed to higher soil organic matter and available water content for plants in more productive localities (Bonfil et al. 2006).

The variable fertilizer application yields benefits in the majority of cases. For example, similar to our study, Bhatti et al. (1998) calculated varying rates of fertilizer for their area, divided into three homogenous MZs. They calculated the net profits as \$321 for MZ1, \$392 for MZ2, and \$416 ha^{-1} for MZ3. Their cost: benefit ratios were 4.33, 5.28, and 5.62 for MZ1, MZ2, and MZ3, respectively. They suggested that hybrid selection and hybrid-specific fertilizer management are also important in management of N fertilizers.

3.3. Spatial variability of soil properties as related to plant available soil P content in the study area

Table 3 presents the results of semivariogram analysis, and Figure 3 shows the semivariograms and spatial pattern of the soil properties within the study area. The findings in Table 3 indicate that the majority of the soil variables are moderately spatially dependent similar to P_{av}. In addition, many of the soil variables are poorly interpolated as low r-values for cross validation (r_{CV}) indicated. Silt and clay contents were highly different from the rest of the soil variables in A, while $CaCO_3$ content, FC, WP, PAWC, ASI and P_{av} were similar. In addition, the spatial structure of P_{av} was highly similar to those of ASI and PAWC in both model type and A. Except ASI and $CaCO_3$ content, all the soil attributes, including P_{av}, were moderately spatially dependent.

Figure 3 shows the spatial pattern of soil variables across the study area. Greater values of sand content and lower values of clay content are generally co-located on the medium P application zone. The spatial pattern for OM content showed no clear spatial association to the spatial pattern of P_{av} in the study area, while those for $CaCO_3$ content and wilting point showed that their greater values tended to be located in the medium P application zone. Similarly, a spatial relationship between ASI and P_{av} is evident, while soil pH and EC showed no apparent spatial relationship with P_{av}. When semivariogram and the spatial pattern for P_{av} are compared to those for rest of the soil properties, it can be concluded that the greatest spatial relationship occurred between P_{av} and ASI and PAWC. The close spatial relationship with PAWC and P_{av} should be considered in variable P application as low P_{av} and low PAWC tend to co-exist in the study area. In many cases, especially in dryland farming conditions, low PAWC is one of the most important yield limiting factors. If this is the case in the study area, the expected benefit may not be obtained from greater fertilizer applications on the low-P sites.

The results revealed that OK- interpolations for many of the soil variables performed poorly due probably to the same reasons behind unsatisfactory interpolation of P_{av}. Just like with P_{av}, we experimented with different lagging and modeling approaches to improve the success of OK-interpolation for other soil properties. Although performance for semivariogram modelling increased tremendously in many cases as indicated by increased R^2 and/or decreased RSSE, no significant improvement was the case in the corresponding r_{CV}-values. A different sampling configuration may yield a highly different modeling performance (Kravchenko 2003). Actually, there were adequate numbers of samples in close distances (between 0 and 50 m), while greater number of samples were needed in medium distances (50-200 m) to safely model semivariograms in those proximities. There are also adequate numbers of samples to calculate semivariograms in distances longer than 200 m. However, the fact that the data enabled the detection of close spatial associations between P_{av} and PAWC is very important in practice as PAWC can limit the benefit from greater P application on the low PAWC-sites.

The On Farm Management Information Systems (FMIS) seem promising to facilitate the implementation of precision agriculture for small-scale farmers in Türkiye. The On-Farm Experimentation (OFE) is an innovative process in which farmers and professional researchers collaborate to improve farm management by generating data from agronomic experiments on farmers' own fields (Tanaka et al. 2023). Lack of data availability is another key obstacle in the implementation of precision farming, worldwide (Tanaka et al. 2023; Kumar et al. 2023). Many different tools and techniques are used to gather the information needed for precision agriculture. Tools and technologies such as Global Positioning System (GPS), Geographic Information System (GIS), sensor technology, yield monitoring systems, software, and spatial interpolation of soil resources can be used for gathering the information needed for implementing variable rate application of fertilizers across the world (Kumar et al. 2023). Those same techniques and tools can be used in Türkiye to facilitate the application of variable fertilizer management across the nation.

4. Conclusions

The main objectives of this study were to identify the field scale spatial variability in soil P_{av} and some soil properties to develop uniform P management zones (MZs) for site-specific applications of P fertilizers. Characteristics of semivariograms and spatial patterns related to both P_{av} and most of the studied soil properties indicated the presence of high short-range variability of soil attributes in the study area. Results of cross-validation analyses suggested that a different soil sampling configuration with a greater sampling density is needed to safely apply geostatistics to delineate uniform P application MZs.

Two MZs were identified based on the spatial variation of P_{av}. The soil attributes such as PAWC and ASI were highly spatially related to P_{av} as their greater values co-located with greater values of P_{av}, and vice versa. The close spatial association between P_{av} and PAWC is very important in practice. A greater P application may not yield the expected benefit on low PAWC-sites as low PAWC may still limit the yield increase at those sites. The variable P application management zones (MZs), identified through observed yield differences, may not fully capture soil factors that limit yield beyond P_{av}. Therefore, the spatial

variability in soil variables should be considered along with spatial variability in yield to correctly identify the yield differences and factors responsible for the differences in developing a successful variable fertilizer application program.

An extensive literature search for this research revealed that Türkiye is just in the early stages of adopting and beginning to embrace precision agriculture, aligning with the global trend observed in numerous other nations across the world. However, the strategic support from both public and commercial sectors is still in the infancy stages. The advancement of precision agriculture (PA) in Türkiye faces obstacles and various challenges, including a lack of information, connectivity issues in rural areas, and a lack of funding. The main factor impeding the advancement of precision agriculture, and a key reason for its slow implementation, is the constraint posed by insufficient financial resources. Issues related to the adaption of the farmers, organization, and functioning of the economy to increase its profitability, employment, and staff development should be solved in order to implement precision agriculture on a national scale. The small field size and lack of financial resources are obstacles for small-scale farmers. These problems force the farmers to apply traditional methods in production.

Future studies may explore how current variable rate fertilizer and pesticide management techniques and approaches may increase food production, limit environmental effects, and cut costs. Studies should be conducted to achieve the integration of precision farming into everyday farming operations in Türkiye. The obstacles that force the farmers to apply traditional methods in production should be studied holistically, considering the technical, financial, and social aspects of the problem. The On Farm Management Information Systems (FMIS) may facilitate the implementation of precision agriculture, especially by small-scale farmers. Therefore, we propose research on the orientation of FMIS to be given priority in future studies in Türkiye.

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HSP90AB1 (SNP-4338T>C) gene polymorphism associated with thermo-tolerance in some cattle breeds in Türkiye*

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ABSTRACT

Global warming is becoming a greater threat for the agricultural sector, while molecular genetics studies still hold new opportunities, to not only detect heat-tolerant animals, but also to allow for increasing the frequencies of desired genotypes in a certain population. In this study, *HSP90AB1* gene associated with heat tolerance was investigated in four cattle breeds known as Zavot (ZAV), Sout Anatolian Yellow (SAY), South Anatolian Red (SAR), and Brown Swiss (BS) reared in Türkiye via Allele-Specific Polymerase Chain Reaction (AS-PCR). 4338T>C mutation of the *HSP90AB1* gene yielded a total of three genotypes (CC, CT, and TT) across all cattle breeds in which C allele frequency ranged from 0.34 (SAY) to 0.73 (BS), while T allele frequency varied between 0.27 (BS) and 0.66 (SAY). In BS, CC was the genotype with the highest frequency (0.50), whereas the frequency of CC was lower than CT and TT in the Anatolian breed (ZAV, SAR, and SAY). Similarly, the frequency of TT was higher in native Anatolian breeds than BS (0.05). All the populations studied were in Hardy-Weinberg Equilibrium (HWE) in terms of the *HSP90AB1* gene. This study confirmed that the *HSP90AB1* gene was polymorphic in four cattle breeds reared in Türkiye. This polymorphism has the potential to allow for improving heat tolerance to maintain animal production in the future via suitable selection studies. Therefore, this polymorphism should be conserved in Anatolian cattle breeds, while other genes related to different environmental stressors may be monitored by further studies.

1. Introduction

During the last century, numerous developments, such as an enormous increase in the production level of agricultural and industrial sectors, improvement in global transportation networks as well as a rapid increase in the human population, have negatively affected the environment which can not be easily recovered (Bayram et al. 2023). Global warming-based problems are the main concern among these negative effects. It is estimated that temperature has increased by approximately 1ºC from the beginning of the 20th century, while it is reported that this increase has reached up to nearly 2ºC per decade after 1975. The Intergovernmental Panel on Climate Change (IPCC) has documented that the increase in temperature is expected to be 2-3ºC higher at the end of the 21st century (Lu et al. 2020; Malhi et al. 2021; UNEP 2022). As highlighted by Demir et al. (2022a), climate change is considered to be a major threat to the sustainability of animal breeding systems across the globe since increased temperature not only negatively affects animal welfare but also leads to reproduction problems and a decrease in economically important yields (Demir et al. 2021a).

In this context, native animal breeds, which are believed to have developed adaptation against numerous climatic conditions, have gained more importance in maintaining animal production under environmental factors which are likely to change in the near future. Official data indicates that approximately 17 million cattle are reared in Türkiye of which 49.2% and 7.3% are represented by cosmopolitan and native breeds, respectively (TUIK 2022). Holstein Friesian, BS, Simmental, and Jersey are the most preferred among the cosmopolitan breeds, while ZAV, SAR, SAY, Anatolian Black, East Anatolian Red (EAR), and Turkish Grey Steppe (TGS) are the native Anatolian cattle breeds (Demir et al. 2021b; Demir et al. 2022b). Of these native breeds, SAR is reared in the southern part of Anatolian including Mersin, Adana, Gaziantep, and Şanlıurfa provinces for milk and beef production. SAY is also reared in the southern part of Anatolia for dual purpose, whereas it is distinct from other native cattle breeds in terms of its coat colour and climbing ability to mountainous areas. ZAV is raised by smallholder farmers in the eastern part of Anatolia including Kars and Ardahan provinces, particularly for milk production. Native Turkish cattle breeds are of lower yields in terms of milk and beef compared to cosmopolitan breeds, while they have developed excellent adaptation against local diseases and climatic conditions of Anatolia (GDARP 2009; Demir et al. 2021b).

Possessed by native breeds, adaptive traits, which have been shaped by numerous factors from domestication till the present, and their underlying reasons have always been considered interesting study areas for scientists working on molecular genetics. Indeed, many previous molecular studies have revealed numerous genes such as *KRT77*, *MYO1A*, *BoLA-DRB3* which are directly associated with environmental stressors (Duangjinda et al. 2013; Jia et al. 2019; Zhang et al. 2023) When it comes to heat stress and tolerance to higher temperatures in livestock species,

*This research was approved by the Local Ethics Committee of Animal Experiments of the Eskişehir Osmangazi University (Protocol No: HAYDEK-970/2023).

Heat Shock Proteins (HSPs) and variations in their related genomic regions have been mostly investigated (El-Zarei et al. 2019; Irivboje et al. 2020; Rawash et al. 2022; Guzmán et al. 2023). HSPs showing differences in terms of their molecular weight and biological functions are molecular chaperons activated during heat and related stress conditions. During cellular damage, HSPs are released into the blood in order to protect living cells from toxic effects and heat-related stress factors. Based on their molecular weights, HSPs are categorized into five groups such as HSP100, HSP90, HSP70, HSP60, and small HSPs whose molecular weights are estimated at 17-30 kDA (Yer 2017; Çıldır and Özmen 2019; Şenel et al. 2019). HSP90AB1 gene has been mapped to bovine chromosome 23 (with 724 amino acids) and consist of 12 exons (Prastowo et al. 2021; Hariyono and Prihandini 2022).

Several studies have confirmed that variations in HSP gene regions were directly associated with thermal tolerance in different cattle (Kumar et al. 2022), sheep (Sheraz et al. 2023), goat (Mohanarao et al. 2014), and chicken (Sheraiba et al. 2019) breeds. To illustrate, Sajjanar et al. (2015) investigated the effects of genetic variations in the *HSP90AB1* (SNP g.4338T>C) gene on heat tolerance and milk production in Sahiwal and Frieswal cattle breeds via Allele-Specific Polymerase Chain Reaction (AS-PCR). A total of three genotypes called CC, CT, and TT were declared and animals with TT genotype were reported to be more advantageous in terms of heat tolerance and respiration rate based on association analysis (Sajjanar et al. 2015). Moreover, the authors highlighted that *HSP90AB1* (SNP g.4338T>C) polymorphism could be utilized as a molecular marker to improve heat tolerance in cattle breeds. On the other hand, few studies are available in the literature attempting to investigate HSP polymorphisms in native Turkish livestock species (Öner et al. 2017; Atalay and Kök 2023; Yurdagül et al. 2023). Hence, this study aims to screen four cattle breeds (SAY, SAR, ZAV, and BS) reared in Türkiye in terms of *HSP90AB1* (SNP g.4338T>C) polymorphism which was previously reported to be associated with heat tolerance in cattle. In the case of observing the TT genotype, it is also aimed to evaluate the usefulness of this polymorphism in Marker Associated Selection (MAS) which may be taken into consideration for further management practices.

2. Material and Methods

2.1. *Ethic statement*

This research was approved by the Local Ethics Committee of Animal Experiments of the Eskişehir Osmangazi University (Protocol No: HAYDEK-970/2023).

2.2. *Sample collection and DNA extraction*

A total of 117 animals (from both sexes) belonging to ZAV (n= 30), SAY (n= 34), SAR (n= 31), and BS (n= 22) were sampled from different representative herds (at least three herds per breed) to prevent possible kinship. Blood samples of ZAV, SAR, and SAY were collected from herds located in Kars, Şanlıurfa, and Adana provinces, respectively, while blood samples of the BS breed were obtained from different herds reared in Antalya province (Figure 1). The salting-out protocol described by Miller et al. (1988) was used to total DNA extraction from blood.

2.3. *Determination of HSP90AB1 (SNP-4338T>C) gene polymorphism*

In this study, the AS-PCR protocol described by Sajjanar et al. (2015) was used to amplify a 562 base pair length of the *HSP90AB1* region to screen 4338T>C SNP in four cattle breeds. As recommended two specific primers were used to amplify the forward strand for C (CTGGAGTCACACTGAGGAAC) and G (CTGGAGTCACACTGAGGAAT) alleles, while a common primer (TGTTGGAGATCGTCACCTG) was used to amplify the reverse strand for both alleles. PCR reaction of 30 µl (4 µl 10X PCR buffer, 10 mM $MgCl_2$, 2.5 mM dNTPs, 10 pmol each primer, 50 ngP µl template DNA, 2.5 U Taq DNA polymerase, and 12.9 µl ddH_2O) was subjected to thermal cycler as follows: initial denaturation at 95ºC for 5 min, followed by 30 cycles of denaturation at 95ºC for 45 m, annealing at 63ºC for 45 s, and extension at 72ºC for 45 s, while final extension was optimized at 72ºC for 5 min. Amplified PCR fragments were separated on 1.5% agarose gel electrophoresis in which individuals with two amplifications were genotyped as CT, while others were genotyped as TT or CC based on the presence of the amplified allele.

Figure 1. Geographical representation of sampling strategy.

2.4. Statistical analysis

Popgene v.1.32 (Yeh et al. 1997) was used both to calculate allele and genotype frequencies and to test Hardy-Weinberg Equilibrium (HWE) across four cattle breeds in terms of *HSP90AB1* (SNP g.4338T>C) polymorphism. Minitab software was also utilized to compare genotype frequencies within (two dependent proportions) and between (two independent proportions) populations via z test.

3. Results and Discussion

In this study, the *HSP90AB1* gene region turned out to be polymorphic in terms of 4338T>C mutation in four cattle breeds. The allele and genotype frequencies across the cattle breeds studied are summarized in Table 1, while a representative agarose gel electrophoresis including three genotypes (CC, CT, and TT) for nine animals belonging to SAR is given in Figure 2.

C allele frequency ranged from 0.34 (SAY) to 0.73 (BS), while T allele frequency varied between 0.27 (BS) and 0.66 (SAY) across the cattle breeds studied (Table 1). The lowest (0.13) and highest (0.50) CC genotype frequencies were observed in ZAV and BS breeds, respectively. CT genotype frequency ranged from 0.35 in SAR to 0.60 in ZAV, while TT allele frequency varied between 0.05 (BS) and 0.47 (SAR) (Table 1). Chi-square approach revealed that all cattle breeds were in HWE in terms of the *HSP90AB1* (4338T>C) gene region.

Sajjanar et al. (2015) investigated the associations between the *HSP90AB1* (4338T>C) gene variations and heat tolerance parameters (heat tolerance coefficient, average respiration rate, and average rectal temperature) and milk production in a total of 200 animals belonging to Sahiwal (n= 80) and Frieswal (n= 120). *HSP90AB1* (4338T>C) gene was shown to be polymorphic in both native Indian cattle breeds (Sahiwal and Frieswal). CC, CT, and TT genotype frequencies were reported as 0.20, 0.70, and 0.10, respectively for the Sahiwal breed, while these values were 0.05, 0.78, and 0.17, respectively for the Frieswal (Sajjanar et al. 2015). Compared to animals with CT and CC genotypes, a higher heat tolerance coefficient and lower average respiration rate and rectal temperature were reported in animals with TT genotypes in both cattle breeds ($P<0.01$). The authors have suggested that the TT genotype could be utilized to improve heat tolerance in cattle breeds, while other controlling mechanisms and related genes should not be neglected (Sajjanar et al. 2015). Another study conducted by Prastowo et al. (2021) revealed that CC, CT, and TT frequencies were 0.10, 0.50, and 0.40 in twenty animals belonging to Holstein Friesian reared in Indonesia in terms of the *HSP90AB1* (4338T>C) gene polymorphism.

TT genotype frequencies in ZAV (0.27), SAY (0.47), and SAR (0.35) breeds were higher than the values reported by Sajjanar et al. (2015) for Sahiwal (0.17) and Frieswal (0.10) breeds. On the other hand, a lower TT genotype frequency was detected for the BS breed (0.05) compared to both Anatolian and Indian native cattle breeds. The differences in the distribution of genotype frequencies among different cattle breeds may be explained by their genetic origins. Cattle breeds raised in Türkiye such as ZAV, SAY, SAR, and BS as well as Holstein Friesian investigated by Prastowo et al. (2021) are descendants of *Bos taurus*. On the other hand, Sahiwal originates from *Bos indicus*, while Frieswal has been developed via crossbreeding practices between *Bos taurus* and *Bos indicus*.

Normally, indicine cattle breeds are expected to be more heat-tolerant animals compared to taurine cattle breeds (Gaughan et al. 2010). Therefore, the frequency of the TT allele, which showed superior values in terms of heat tolerance as reported by Sajjanar et al. (2015), is expected to be higher than the values reported in taurine cattle breeds. However, both the results of the current study and the values reported by Prastowo et al. (2021) showed higher TT genotype frequency in different taurine cattle breeds. On the other hand, it is noteworthy that the number of animals used in the current study and Prastowo et al. (2021) was lower compared to the study conducted by Sajjanar et al. (2015).

Table 1. Allele and genotype frequencies and chi square values for the *HSP90AB1* (SNP-4338T>C) across four cattle breeds

		Allele frequency		Genotype frequency			χ^2
Breed	**n**	**C**	**T**	**CC**	**CT**	**TT**	
ZAV	30	0.43	0.57	0.13 (4)^{Bc}	0.60 (18)^{Aa}	0.27 (8)^{Bb}	1.475^{d}
SAY	34	0.34	0.66	0.15 (5)^{Bc}	0.38 (13)^{Ab}	0.47 (16)^{Aa}	0.724^{d}
SAR	31	0.47	0.53	0.30 (9)^{Ab}	0.35 (11)^{Ab}	0.35 (11)^{Aab}	2.560^{d}
BS	22	0.73	0.27	0.50 (11)^{Aa}	0.45 (10)^{Ab}	0.05 (1)^{Bc}	0.468^{d}

Comparison of genotype rates within and between populations are given as lower- and upper-case letters, respectively ($P<0.05$). $\chi^2_{0.05;1}$: 3.84; d: Deviation from HWE is non-significant.

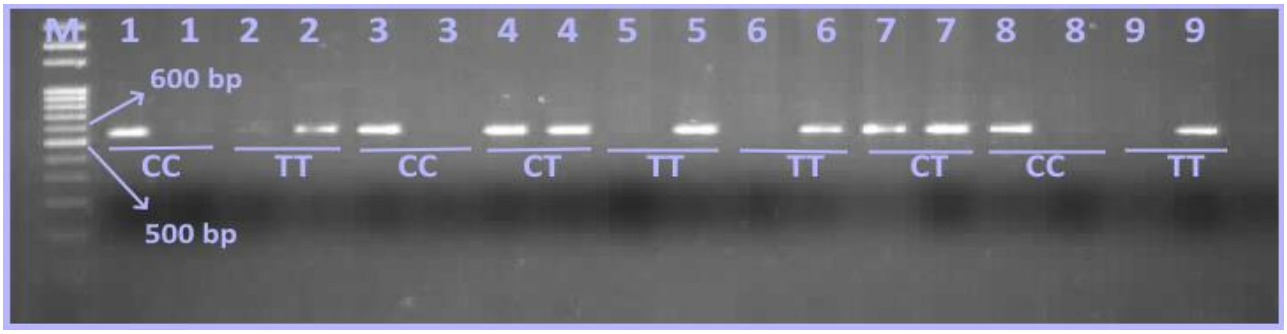

Figure 2. Image of 1.5% agarose gel electrophoresis for nine samples of SAR breed. M: Marker (100 bç- Thermo 100 bp; Cat.No: SM0241); 1, 3, and 8: animals with CC genotype; 4 and 7: animals with CT genotype; 2, 5, 6, and 9: animals with GG genotype.

Numerous studies are available in the literature indicating that native Anatolian cattle breeds are significantly tolerant to local diseases and environmental conditions compared to cosmopolitan cattle breeds (Bilgen et al. 2016; Karayel and Karslı, 2022; Çobanoğlu and Ardıçlı 2022). The results of this study are relevant to the literature because TT genotype frequency in three Anatolian cattle breeds (ZAV, SAY, and SAR) was significantly ($p<0.05$) higher than the value detected in the BS breed (0.005). Among the Anatolian cattle breeds the highest TT genotype frequencies were observed in SAY (0.47) and SAR (0.35), respectively and differences between these breeds were statistically non-significant. However, differences in TT genotype between these breeds (SAR and SAY) and ZAV were statistically significant ($P<0.05$). This finding is compatible with their geographic distributions. Indeed, SAR and SAY are mainly reared in the eastern Mediterranean and south-eastern Anatolia. These locations are of the highest temperature in Anatolia. Therefore, these breeds are thought to have developed an adaptation against higher temperatures over a long period of time.

4. Conclusion

Due to the fact that the trends in increasing temperature will continue globally in the future and threaten sustainable livestock breeding, not only thermo-tolerant animals should be detected but also their frequency should be increased via suitable management practices. In this study, the TT genotype of *HSP90AB1* (4338T>C) gene polymorphism, which was reported to be the tolerant genotype for heat stress, was detected at enough frequency in three Anatolian cattle breeds. The animals with TT genotype may be utilized in MAS programs to face increasing global warming in the future. On the other hand, other native Turkish cattle breeds need to be screened for heat-related genomic regions. Moreover, further studies may be conducted via higher-resolution molecular genotyping methods such as SNP arrays and next-generation sequencing platforms together with thermo-physiological parameters.

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