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RESEARCH ARTICLE

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Some physical properties of bitter gourd (*Momordica charantia* L.) seeds and kernels

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Abstract

A noteworthy member of the *Cucurbitaceae* family, the bitter gourd (*Momordica charantia* L.) is a slender, vine-like annual summer vegetable. Despite the health promoting features, it is among the neglected vegetables in terms of both production values and scientific studies. The current study was aimed to assess some physico-mechanical properties of bitter gourd seeds and kernels viz., sizes, geometric shapes, angle of repose, densities, 1000-seed and kernel weight and, coefficient of friction on several surfaces (aluminum, chrome, iron, plastic, rubber, cardboard, glass and MDF wooden panel) in order to determine to important parameters to design seed sowing machines the materials to be used in storage and their design, the screening/separation/classification processes and the processing of bitter gourd into a commercial product. It has been observed that the seeds of bitter gourd have similar geometrical properties with the seeds of melon, watermelon, squash and cucumber from the *Cucurbitaceae* family. While closer values were observed in terms of length and width, it was determined that the seeds of bitter gourd were thicker. The lengths, widths, and thicknesses of these seeds/kernels with a moisture content of 16% (w.b.) according to the wet base were found to be 14.176/11.517, 7.562/5.922, and 4.076/2.815 mm, respectively. The angle of repose, thousand grain weight and true density of seeds/kernels were found 28.467/26.982°, 202.931/118.359 g and 0.919/1.659 g/cm^3 respectively. Obtained results most likely can serve bitter gourd to be grown in large amounts in different part of the world where the climate is suitable for growing.

Keywords: Bitter melon, Cucurbitaceae, Physical properties, Vegetable seed

INTRODUCTION

The bitter gourd (*Momordica charantia* L.) is a thin vine-like annual summer vegetable and is one of the worthy members of the *Cucurbitaceae* family (Grover and Yadav, 2004; Yan et al., 2019). It is well-known for different kinds of names in the world, such as bitter melon, balsam pear or karela, kugua, and kudret nari. It is commonly grown in tropical parts of the world and is popular due to its medicinal value. Although the demand for this vegetable has increased in recent years, only production data for the years 2021 (66 tons), and 2022 (84 tons) are given by the Turkish Statistical Institute (TUIK) (TUIK, 2023). These values show the potency of bitter gourd, which has many benefits, but yet to get enough attention as plant. There is no doubt that importance given to these neglected crops will increase in next coming years.

The fruits of the bitter gourds have a rough texture with a pointy apex and are 8-15 centimeters in length and 4-10 centimeters in breadth. The fruit, which starts off green in color and grows to yellow and golden yellow, has approximately 25 dark

red, bean-like seed within. Seeds or seedlings are used to cultivate bitter gourds, but cultivating with seedlings is advisable because the germination rate is not 100% (Baryeh, 2002; Ünal et al., 2013).

In agricultural processes sowing, caring for, harvesting, and handling activities for the crop are key factors, and research on developing tools and equipment and conducting modeling studies associated with these processes is equally important. It has been observed that the basic physical properties of seeds and kernels of various vegetables have a very important role in the design, use and control of processing equipment (Amin et al., 2004). Understanding the characteristics of biological materials such as seeds are vital for engineers as well as food technologists, processors, breeders, and experts from other domains who could find novel applications for it (Mohsenin, 1986; Pradhan et al., 2013).

The physical characteristics of the seeds or kernels provide the basis for the design of various kinds of cleaning, classifying, separating, and oil extraction equipment. When developing machines for separating, sorting, grinding, and flow ability characteristics the size and form of the seeds are crucial. On the other hand, moisture content is required to predict the drying period of seeds.. For the designing of aeration, drying, preservation, and transport-related machinery, an understanding of gravimetric qualities such as bulk and true density, 1000-seed mass, and porosity are crucial considerations because these have an effect on the resistance of mass to airflow (Amin et al., 2004; Dash et al., 2008; Ekinci et al., 2010; Pradhan et al., 2013). When designing a pneumatic conveyor, the terminal velocity is crucial (Vilche et al., 2003; Dursun and Dursun, 2005; Pradhan et al., 2013). The angles of repose, friction coefficient as well as other frictional qualities are essential when creating transportation equipment, grain containers, and some other storage facilities (Pradhan et al., 2013). Typically, threshing is done with handmade threshing equipment on a firm floor. Thus, its physical characteristics must be well understood in order to maximize the bitter gourd's threshing efficiency, pneumatic transportation, and preservation (Unal et al., 2013; Yilmaz and Altuntas, 2020).

The bitter gourd has so many significant therapeutical and health-promoting properties for good wellness. Despite having several beneficial qualities, the bitter gourd is hardly seldom planted and unfortunately one of the neglected vegetables in the world. For widespread cultivation of the bitter gourd all over the world, it is necessary to steer with some agricultural processes. Similar to other crops, the physical characteristics of bitter gourd seed are crucial for the design of machinery for handling, selecting and collecting, processing, as well as preserving kernels.

In recent years, some research has been conducted on various crops such as chickpea seed (Konak et al., 2002), faba bean (Haciseferogullari et al., 2003), watermelon seed (Koocheki et al., 2007), and bottle gourd seed (Pradhan et al. 2013) for the purpose of studying the physical characteristics of seeds in order to similar aims (Aghkhani et al., 2012; Ünal et al., 2013; Pradhan et al., 2013). However, there were limited studies carried out concerning the physical properties of bitter gourd seed.

Thus, in the present study, the physico-mechanical properties of bitter gourd seeds and kernels were characterized in order to determine their potential. The current study documents a number of physical characteristics of bitter gourd seeds and kernels, including their principal dimensions, geometric shapes mean diameter, sphericity, 1000 seed and kernel weight, porosity, angle of repose, as well as coefficient of friction when placed on a variety of surfaces, such as aluminum, 304 stainless steel (chrome), ST-37 black sheet (iron), plastic, rubber, cardboard, glass and MDF wooden panel. The current study aims to find the results to be used in the cleaning, separation, transportation, storage, drying and sowing of bitter gourd seeds and kernels.

MATERIALS AND METHODS

Plant material

Bitter gourd fruits were obtained at approximately similar size, appearance and ripeness from center of Antalya province (36°53'10.1" N 30°45'23.4"E) in Türkiye. Fruits were washed of under running tap water to remove dust, dirt, stones, and chaff matter. The seeds were manually separated from cleaned fruits and experiments were carried out by removing damaged, broken, and hollow seeds in order to perform uniform operations with a standard measurement system (Figure 1).

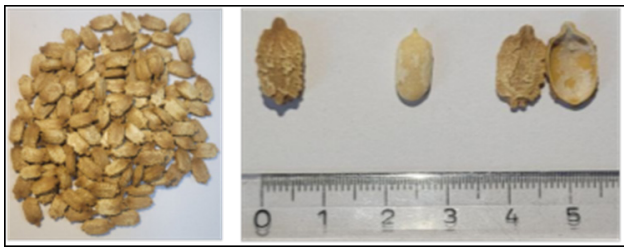

Figure 1. Bitter gourd seeds, kernel and shells

Method

Digital calipers were used with 0.01 precision (Mitutoyo, 150x0.01) for determining the dimensional properties of bitter gourd seeds (length (L_s), width (W_s) and thickness (T_s)) and kernels (length (L_k), width (W_k) and thickness (T_k)). In addition, the shells of the seeds were opened and their shell thickness T_{ss} (mm) was measured. In the abbreviations of the measured parameters, the subscript "s" for seeds and "k" for kernels are used. Experiments were carried out with 100 randomly selected seeds. By using these measurements, the geometric mean

diameter (D_{gs}/D_{gk}) (mm), sphericity (S_{ps}/S_{pk}) (%), aspect ratio (R_{as}/R_{ak}) and seed surface areas (S_s/S_k) (mm^2) of the seeds/kernels, calculated using Equation 1, 2, 3 and 4 (Mohsenin, 1986; Omobuwajo et al., 1999; Sacilik et al., 2003; Oyelade et al., 2005; Kabas et al., 2006; Saracoglu et al., 2010; Altuntas and Naneli, 2017; Kobuk et al., 2019).

$$D_g = LWT^{\frac{1}{3}} \quad (1)$$

$$S_p = \frac{D_g}{H} \quad (2)$$

$$R_a = \frac{W}{H} \quad (3)$$

$$S = \pi D_g^2 \quad (4)$$

D_g : Geometric mean diameter (mm),
L : Length (mm),
W : Width (mm),
T : Thickness (mm),
S_p : Sphericity (%),
R_a : Aspect ratio,
S : Surface area (mm^2).

A pipe with a diameter of 63 mm and a length of 90 mm was used for the angle of repose measurement. This pipe was placed in the middle of a 50x50 cm cardboard plate and filled with bitter gourd seeds and kernels. Afterwards, this piece of pipe was slowly lifted, allowing the spilled seeds and kernels to form a conical structure on the cardboard plate (Cekim and Ozarslan, 2020). The height of the cone formed as a result of the process and the diameter of the circular area formed at the base were measured with 30 repetitions. In order to facilitate diameter measurements, circles were drawn with 0.5 cm radius differences starting from the center of the cardboard plate with the help of a compass, and diameter readings were made using these circles. After the measurements, the angle of repose was calculated with Equation 5 (Kaleemullah and Gunasekar, 2002; Ozguven and Vursavus, 2005; Yilar and Altuntas, 2017).

$$\gamma = tan^{-1}\left(\frac{2h}{d}\right) \quad (5)$$

γ: Angle of repose (°),
h: Cone height (mm),
d: Cone diameter (mm).

A plate mechanism was used to determine the static coefficient of friction. This plate measures 30x45x1.8 cm and has a movable system that is fixed on one side and can be lifted from the other side. Due to a mechanical protractor located on the fixed side, the angle value of the raised plate with the horizontal can be read. In order to determine the static coefficient of friction on different surfaces, experiments were carried out by fixing different materials such as aluminum, 304 stainless steel (chrome), ST-37 black sheet (iron), plastic, rubber, cardboard, glass and MDF wooden panel on the plate. A tube with a diameter of 63 mm and a length of 90 mm, open on both sides, was placed on the different friction surfaces and filled with bitter gourd seeds and kernels. Then, the screw plate system was lifted up on one side, when the pipe started to slide, the system was stopped and the angle value with the horizontal was read, the tangent of this value was taken and the measurements were carried out with 10 repetitions (Suthar and Das, 1996; Ozarslan, 2002; Ertekin et al., 2006; Celik et al., 2007; Yilar and Altuntas, 2017). After the measurements, the static coefficient of friction was calculated with Equation 6.

$$\mu_s = \tan\alpha \quad (6)$$

μ_s: Static coefficient of friction,
α: Angle of gradient (°).

The results of the static coefficient of friction determination experiments on different friction surfaces were compared with the SPSS (Version17; Chicago, IL, USA) statistical data analysis program and groupings were formed.

Single (W_s/W_k) and 1000 grain (W_{ts}/W_{tk}) weights of bitter gourd seeds/kernels were determined by measuring 100 repetitions with a balance which is the sensitivity of 0.001 g (AND, GF-600). In order to determine the 1000 grain weight of the seeds, 100 seeds were randomly selected and their masses were weighed and the measurements were carried out by multiplying the found value by 10.

A cylindrical scale measuring 250 ml was used to determine the bulk density (P_b) of the seeds and kernels. Bitter gourd seeds and kernels, whose mass were determined, were poured into this container from a height of 150 mm and their volume was determined. Bulk density was found by dividing the mass of the bitter gourd seeds by the volume. For the true density (P_t), the liquid displacement method was used and toluene, which has a lower density than water, was preferred as the liquid (Cekim and Ozarslan, 2020). True density was calculated using Equation 7 and all measurements were made in 10 repetitions (Alayunt, 2000).

$$P_t = \frac{mxd_t}{m_t} \quad (7)$$

P_t: True density (g/cm^3),
m: Measured weight of grain (g),
d_t: Toluen density (g/cm^3),
m_t: Weight of toluene displaced with grains (g).

After determining the true density of the seeds and kernels, the porosity was calculated using Equations 8 (Suthar and Das, 1996; Ozarslan, 2002; Akbolat et al., 2008a; Cekim and Ozarslan, 2020; Ertugrul et al., 2022).

$$\varepsilon = \left(1 - \frac{P_b}{P_t}\right) x100 \quad (8)$$

ε: Porosity (%)

Color measurements of bitter gourd seeds and kernels were measured with a PCE brand CSM3 color measuring device. The L* value represents the degree of lightness, darkness, brightness, or black/white from 0-100. Pure white indicates 100L*, while pure black indicates 0L*. The a* value represents the redness-greenness of the color and the b* value represents the yellowness-blueness ratio. Positive a* and b* values indicate red and yellow, and negative a* and b* values indicate green and blue, respectively. In addition, chrome (C*) and hue angle (°) values were also measured (Akbolat et al., 2008b; Akman, 2022). Measurements were made with 100 repetitions.

In order to determine the moisture content of the seeds and kernels of bitter gourd according to the wet basis, 12 samples were kept at 105°C for 24 hours and the masses of the samples were measured before/after and calculated with the help of Equation 9 (Yagcioglu, 1999).

$$\%N_y = \frac{W_s}{W_s + W_k} x100 \quad (9)$$

N_y: Moisture content wet basis (%) (w.b.),
W_s: Water weight in the grain (g),
W_k: Dry matter weight (g)

RESULTS AND DISCUSSION

Geometric Properties

It can be said that the lengths of seeds and kernels are about twice their width. The average values of the length, width, thickness, geometric mean diameter, sphericity, aspect ratio and surface area of the seeds/kernels were 14.176/11.517 mm, 7.562/5.922 mm, 4.076/2.815 mm, 7.579/5.759 mm, 53.5/50.1 (%), 1.863/2.111, and 180.765/104.457 mm^2, respectively. In addition, the average value of the shell thickness of the seeds was found to be 0.85 mm (Table 1).

In a study on the physical properties of karingda seeds, the length, width and thickness values were found to be 10.60, 6.18 and 2.37 mm, respectively. Approximately 55% of these seeds are between 9.5-11.5 mm in length (Suthar and Das, 1996). In a study to determine the physical properties of pumpkin seeds at 6.46% dry basis (d.b.) moisture content, the length, width, thickness, geometric mean diameter and sphericity values were found respectively 18.16±1.40 mm, 9.80±0.24 mm, 2.67±0.38 mm, 7.72±0.75 mm and % 43.00±0.42 (Paksoy and Aydın, 2004).

Length of 3 different watermelon seeds in Sarakhsy, Kolaleh and Red varieties, 15.597, 13.455 and 18.972 mm; widths, 9.190, 8.401, 10.720 mm; thicknesses, 3.107, 2.912, 2.988 mm; geometric mean diameters, 7.620, 6.893, 8.456 mm; sphericity, 0.490, 0.513, 0.446%, surface area, 182.963, 149.684, 225.031 mm^2 (Seyed and Elnaz, 2006). Results from their study clearly show that watermelon seeds are similar in shape (length and width) to the seeds of bitter gourd, but thinner. In particular, the sphericity values of bitter gourd and watermelon seeds were highly similar.

In a study of the physical properties of watermelon seeds with 6% dry base moisture content, the average length was found to be 10.8, width 6.8, thickness 2.3, geometric mean diameter 5.5 mm. Sphericity and thousand grain weight were determined as 51.5% and 94.10 g, respectively (Paksoy et.al., 2010). Compared to the seeds of bitter gourd, it was observed that the length, thickness, geometric mean diameter and weight of one thousand seeds of watermelon seeds were less, but their widths and sphericities were close to each other.

In a study on melon seeds with different moisture content, the lowest (2.8%) and highest (25%) moisture content (d.b.) values were found to be length, width, thickness, geometric mean diameter, sphericity, surface area and aspect ratio, respectively; 13.91/14.37 mm, 8.49/8.88 mm, 1.71/2.16 mm, 5.81/.6.44 mm, 0.42/0.45%, 106.87/132.09 mm^2 and 61.15/61.94%. At the lowest (1.1%) and highest (23%) moisture content (d.b.) values in the kernel of the melon seed, the same parameters were respectively; 13.24/14.08 mm, 7.71/8.21 mm, 1.47/1.92 mm, 5.30/6.01 mm, 0.40/0.43%, 88.53/.114.40 mm^2 and 58.35/58.56% (Obi and Offorha, 2015). According to the potency bitter gourd study, while the length and width values were similar, it was seen that these seeds were thicker than melon seeds. This situation caused the geometric mean diameter, sphericity and seed surface area values of bitter gourd seeds and seeds to be larger.

The fact that the results obtained from present study clearly indicated that the physical properties of the bitter gourd vegetable are similar to the seeds of other vegetables in the *Cucurbitaceae* family, such as pumpkin, melon, and watermelon, and assumed that results of present study will guide the design of sowing machines, seed screening systems and systems used for the transportation/storage of these seeds and similar machines for bitter gourd.

Gravimetric Properties

The averages of angle of repose, single grain weight, one thousand grain weight, bulk density, true density and porosity values of seeds/kernels were 28.467/26.982°, 0.192/0.116 g, 202.931/118.359 g, 0.456/0.540 g/cm^3, 0.919/1.659 g/cm^3, 5.526/2.642 cm^3 and 49.788/67.107%, respectively (Table 2). As a result of the examinations, it was observed that a large part of the seed weight was formed by the kernel.

Table 1. Geometric Properties of Bitter Gourd Seeds and Kernels.

Bitter Gourd	Geometric Properties	Maximum	Minimum	Average ± Standard Deviation
Seed	Length (L_s) (mm)	15.400	12.670	14.176 ± 0.679
	Width (W_s) (mm)	9.060	6.290	7.562 ± 0.503
	Thickness (T_s) (mm)	5.770	3.290	4.076 ± 0.340
	Geometric mean diameter (D_{gs}) (mm)	8.585	6.417	7.579 ± 0.348
	Sphericity (S_{ps}) (%)	62.4	47.3	53.5 ± 2.700
	Aspect ratio (R_{as})	2.145	1.282	1.863 ± 0.141
	Surface area (S_s) (mm^2)	231.402	129.287	180.765 ± 16.543
	Shell thickness (mm)	1.270	0.650	0.850 ± 0.096
Kernel	Length (L_k) (mm)	12.870	9.870	11.517 ± 0.661
	Width (W_k) (mm)	8.840	4.250	5.922 ± 0.595
	Thickness (T_k) (mm)	3.330	2.310	2.815 ± 0.195
	Geometric mean diameter (D_{gk}) (mm)	6.692	4.740	5.759 ± 0.291
	Sphericity (S_{pk}) (%)	60.0	44.1	50.1 ± 2.600
	Aspect ratio (R_{ak})	2.908	1.518	2.111 ± 0.236
	Surface area (S_k) (mm^2)	140.675	70.573	104.457 ± 10.374

Table 2. Gravimetric Properties of Bitter Gourd Seeds and Kernels.

Bitter Gourd	Geometric Properties	Maximum	Minimum	Average ± Standard Deviation
Seed	Angle of repose ((°)	35.700	21.301	28.467 ± 3.670
	Single grain weight (W_s) (g)	0.251	0.116	0.192 ± 0.025
	One thousand grain weight (W_{ts}) (g)	206.570	201.390	202.931 ± 1.624
	Bulk density (P_{bs}) (g/cm^3)	0.468	0.442	0.456 ± 0.010
	True density (P_{ts}) (g/cm^3)	1.052	0.777	0.919 ± 0.110
	Porosity () (%)	57.361	40.158	49.788 ± 6.175
Kernel	Angle of repose ((°)	34.026	19.871	26.982 ± 2.950
	Single grain weight (W_k) (g)	0.152	0.066	0.116 ± 0.016
	One thousand grain weight (W_{tk}) (g)	121.345	112.873	118.359 ± 1.783
	Bulk density (P_{bk}) (g/cm^3)	0.580	0.511	0.540 ± 0.019
	True density (P_{tk}) (g/cm^3)	1.933	1.338	1.659 ± 0.303
	Porosity () (%)	73.123	59.668	67.104 ± 6.265

In one of the previous study conducted by Western Mediterranean Agricultural Research Institute (BATEM) clearly showed that some physical and fatty acid properties of bitter gourd seeds were determined by the and 1000 seed weight of bitter gourd seeds was found to be 183.20 ± 6.56 g (Golukcu et al., 2014). In current study, it was seen that the 1000 seed weight of the seeds was close, but 20 grams heavier and on average 202.931 g. In a study on pumpkin seeds, which have similar geometric properties with the seeds of bitter gourd, the weight of thousand seeds was found in the range of 144-295 g (Durgut, 2008). It has been observed that the thousand seed weights of the seeds of the bitter gourd showed similar characteristics.

Single grain and kernel weights of karingda seeds were found to be 0.099 and 0.062 g, respectively (Suthar and Das, 1996). Grain bulk densities of pumpkin seeds at different moisture levels were found to be between 450 and 625 kg/m^3 (Paksoy and Aydin, 2004), in another study, it was observed that the grain density of terebinth fruits increased depending on the increase in moisture content (Aydin and Ozcan, 2002).

In a study of three different watermelon seeds (Sarakhsy, Kolaleh and Red), the true density, bulk density and porosity were found as 861.754/866.669/863.036 kg/m^3, 416.333/527.265/451.616 kg/m^3 and 51.681/39.143/47.604%, respectively (Seyed and Elnaz,

2006). It was observed that the porosity values of bitter gourd seeds and the prosthesis values of watermelon seeds were very close to each other, and this situation is thought to be since the two seeds are similar in shape.

In a study on melon seeds with different moisture content, the lowest (2.8%) and highest (25%) moisture content (d.b.) values were found to have bulk density, true density, 1000 seed weight and porosity values, respectively; 408.04/500.50 kg/m^3, 820.00/1189.00 kg/m^3, 43.60/168.43 g and 45.27/57.52%. At the lowest (1.1%) and highest (23%) moisture content (d.b.) values in the kernel of the melon seed, the same parameters were respectively; 474.80/539.00 kg/m^3, 1039.40/1229.50 kg/m^3, 87.33/105.33 g and 54.33/56.12% were found (Obi and Offorha, 2015). It was observed that 1000 seed weights of bitter gourd seeds and kernels were approximately 1.5 times higher than melon seeds and 1.08 times more than kernels.

In a study on the determination of gravimetric properties of pumpkin seeds, the surface area, sphericity, bulk density, true density and porosity values of these seeds were found to be 533.38 mm^2, 0.47, 0.94 kg/m^3, 1.15 kg/m^3 and 17.55%, respectively (Aremu et al., 2016). The surface area of squash seeds is 2.95 times larger than that of bitter gourd seeds. The porosity value of bitter gourd seeds was 2.83 times greater than that of pumpkin seeds.

The gravimetric properties of bitter gourd seeds and the similarities with other vegetables in the *Cucurbitaceae* family show that the height of the piles to be formed in the storage of seeds gives important values about the circular area they will cover and the equipment to be used in their transportation.

Color Properties

The results for some color values such as L*, a*, b*, C* and h° of bitter gourd seeds and kernels are given in Table 3. Since the L* value is seen as + and is closer to the value of 100, it can be said that these seeds have a shiny surface. The a* value is on the red side, with it being closer to 0. In b* value, it can be said that these seeds are yellow/orange as they are closer to yellow. While the brightness values of the seeds and kernels are close to each other, it can be said that the color of the kernels is closer to yellow, although it is seen that there is a decrease in a*, b* and C* values.

Table 3. Color Parameters of Bitter Gourd Seeds and Kernels.

Bitter Gourd	Color Parameters	Maximum	Minimum	Average ± Standard Deviation
Seed	L*	71.180	49.710	58.843 ± 4.946
	a*	17.010	6.870	11.873 ± 2.203
	b*	29.410	21.730	26.594 ± 1.717
	C*	33.660	23.380	29.172 ± 2.053
	h°	73.810	57.440	66.048 ± 3.801
Kernel	L*	77.370	67.010	72.903 ± 2.271
	a*	7.860	5.460	6.946 ± 0.591
	b*	25.180	15.630	20.587 ± 2.295
	C*	26.380	16.560	21.730 ± 2.352
	h°	72.660	69.700	71.327 ± 0.766

Aydos (2022), examined the color properties of the bitter gourd fruits by drying in microwave, tray dryer and vacuum dryer. L*, a* and b* values were 57.85, 7.03 and 38.53 in microwave, 64.71, 6.30 and 42.24 in tray dryer and 61.36, 12.27 and 37.60 in vacuum dryer. The color of the seed and the bitter gourd fruits themselves are seen to be close to each other in a visual examination.

Frictional Properties

The static coefficient of friction occurring on different friction surfaces of bitter gourd seeds and kernels are given in Table 4. The static friction coefficients of these seeds/kernels on different surfaces such as aluminum, 304 stainless steel (chrome), ST-37 black sheet (iron), plastic, rubber, cardboard, glass and MDF wooden panel surfaces are average, 0.569/0.339, 0.467/0.324, 0.504/0.337, 0.342/0.321, 0.590/0.284, 0.522/0.314, 0.341/0.227, and 0.411/0.289, respectively. In the statistical analysis, when going from low static coefficient of friction to high, plastic and glass are in the first group, MDF wood panel is in the second, 304 stainless steel is in the third, ST-37 black sheet and cardboard is in the fourth, aluminum and rubber are in the fifth group. When the static coefficient of friction for the kernels is evaluated, it is seen that the lowest friction is seen in glass, the highest is seen in aluminum, 304 stainless steel, ST-37 black steel, plastic, and cardboard.

The static coefficient of friction calculations of pumpkin seeds on plywood and galvanized sheet surfaces varied between 0.18 and 0.64 (Paksoy and Aydin, 2004). In a study on watermelon seeds of Sarakhsy, Kolaleh and Red varieties, plywood, galvanized sheet, glass, rubber and fiberglass materials were used as friction surfaces to determine the static coefficient of friction. The static coefficient of friction of these three seeds was found

Table 4. Static Coefficient of Friction of Bitter Gourd Seeds According to Different Materials.

	Materials	Static Coefficient of Friction ±Standard Deviation
Seed	Aluminum	0.569^{e}±0.067
	304 Stainless Steel (Chrome)	0.467^{c}±0.025
	ST-37 Black Sheet (Iron)	0.504^{d}±0.018
	Plastic	0.342^{a}±0.032
	Rubber	0.590^{e}±0.074
	Cardboard	0.522^{d}±0.026
	Glass	0.341^{a}±0.023
	MDF Wooden Panel	0.411^{b}±0.022
Kernel	Aluminum	0.339^{d}±0.034
	304 Stainless Steel (Chrome)	0.324^{cd*}±0.051
	ST-37 Black Sheet (Iron)	0.337^{d}±0.039
	Plastic	0.321^{cd}±0.043
	Rubber	0.284^{b}±0.044
	Cardboard	0.314^{bcd*}±0.040
	Glass	0.227^{a}±0.014
	MDF Wooden Panel	0.289^{bc*}±0.016

* According to the Duncan multiple comparison results, the difference between the means with the same letter is insignificant.

in plywood, 0.56, 0.48, 0.61, galvanized sheet, 0.38, 0.40, 0.43, glass, 0.26, 0.31, 0.26, rubber, 0.66, 0.56, 0.68, fiberglass, 0.30, 0.36, 0.34, respectively (Seyed and Elnaz, 2006). In plywood, glass and rubber materials, bitter gourd seeds showed similar static friction coefficient values with watermelon seeds.

In a study on melon seeds, it was observed that the increase in moisture content of melon seeds and kernels increased the mean values of the geometric, gravimetric and frictional properties studied. Plywood showed the highest coefficient in all of the static coefficient of friction experiments performed at different moisture contents. According to the angle of inclination obtained on different structural materials results on pumpkin seeds, galvanized steel, mild steel, stainless steel, plywood and glass were used. Inclination angles (friction coefficients) on these surfaces were found to be 29.24° (0.559), 27.04° (0.510), 29.04° (0.555), 30.00° (0.577) and 25.52° (0.477), respectively (Aremu et al., 2016).

The frictional properties of bitter gourd seeds and the similarities with other vegetables in the *Cucurbitaceae* family include important details for designers in the selection of materials used in seed storages and in sowing machines.

Moisture Content

The moisture content of bitter gourd seeds was 16.212 ± 2.376% (w.b.), and 11.50 ± 0.316% (w.b.) of kernels. In a study on karingda seeds, it was stated that the moisture content of seeds has an important effect on their physical properties (Suthar and Das, 1996). As the moisture content of edible pumpkin seeds increased, the bulk density, true density, % porosity, surface area and final velocity values increased (Paksoy and Aydın, 2004). This shows that the specified values have increased due to the water absorption ability of the seeds. The average moisture contents of watermelon seeds in Sarakhsy, Kolaleh and red cultivars were found to be 4.55, 5.02 and 4.75% (w.b.), respectively (Seyed and Elnaz, 2006). In another study on watermelon seeds, bulk density, true density, porosity, surface area and sphericity decreased with increasing moisture content of the seed (Paksoy et. Al., 2010). A study was conducted on the physicomechanical properties of melon seeds at different moisture contents. This study was carried out with moisture content values of 2.8%, 7, 12, 17 and 25 in seeds, and 1.1%, 6, 11, 16 and 23 in kernels (Obi and Oforha, 2015).

CONCLUSION

In this study, some physical properties of bitter gourd seeds and kernels such as linear dimensions, geometric mean diameter, shell thickness, sphericity, surface area, true and bulk densities, porosity, static coefficient of friction, color and moisture content were determined. These properties are necessary for cleaning, separating, transporting, storing and drying the seeds and kernels of bitter gourd. The developments of a sowing machine for this seed and the features that must be known in the design of any machine equipment for this seed have been obtained in this study. It has been observed that the physical properties of pumpkin, cucumber, watermelon and melon seeds and the seeds of bitter gourd are similar. Since these vegetables are from the same family, it is normal for them to exhibit similar properties in terms of seed physical properties. On average, the length of the bitter gourd seeds/kernels was 14.176/11.517 mm, their width was 7.562/5.922 mm, and their thickness was 4.076/2.815 mm. The angle of repose, which is an

extremely important criterion in the storage of bitter gourd seeds, was found to be 28.467° on average. The static coefficient of friction tests performed on glass and plastic surfaces were lower than the other surfaces. The highest friction occurred in aluminum and rubber materials. It is an undeniable fact that the commercial demand of the bitter gourd vegetable, which is added to its beneficial properties day by day, will increase. Since the demand for this vegetable assumed to be getting increased in next coming years, the physical properties of seeds and kernels revealed in the present study have a high potential to be used in the stages of cultivation, transportation, storage and product processing. But it must be also emphasis that further work should be conducted on this important crop to get complete profile of the physical properties of bitter gourd seeds.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

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

Author contribution

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

Ethical approval

Ethics committee approval is not required.

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Data availability

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Not applicable.

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RESEARCH ARTICLE

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The effect of boiling and baking process on total phenolic compounds and antioxidant capacity of Osmanoğlu and Sarıaşlama chestnuts grow in Bursa

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Abstract

Chestnut is a fruit with high carbohydrate content, low-oil ratio and protein content, including minerals, vitamins, amino acids and phenolic compounds which can have antioxidant properties. It has been determined that the amount and composition of phenolic components could change in according to environmental and growing conditions, and the variety of chestnuts having impact on protein content. This study was conducted to investigate the possible effects of boiling and baking on some physical and chemical properties, antioxidant activity and total phenolic content of Osmanoğlu and Sarıaşlama chestnut varieties grown in Bursa. According to the results of the present study, some physical and biochemical properties of raw chestnut were determined as humidity 48.78–56.57 %, ash 1.11–1.27 g 100 g^{-1}, water activity 0.705–0.844, pH 7.03–7.29, color L* 60.52±2,64; a* 0.13±0,18; b* 12.39±1.57. The total phenolic compounds content was 129.17±6.23 mg GAE (gallic acid equailent) kg^{-1} in raw chestnuts, 180.97±18.25 mg GAE kg^{-1} in boiled chestnuts, and 149.86±7.95 mg GAE kg^{-1} in baked chestnuts. Also, the antioxidant capacity found like 0.42±0.01 µmol AAE (ascorbic acid equivalent) g^{-1} for raw chestnuts, 0.31±0.03 µmol AAE g^{-1} for boiled chestnuts, and 0.40±0.01 µmol AAE g^{-1} of baked chestnuts. It was determined that while the application of the boiling process caused a significant decrease in the antioxidant activity of chestnuts, the application of the baking process did not cause any meaningful change on the antioxidant capacity of the chestnuts. It was determined that there was a significant increase in the total phenolic compounds content with the boiling and baking process compared to raw chestnuts.

Keywords: Chesnut, Total phenolic compounds content, Antioxidant capacity

INTRODUCTION

Chestnut was a general name given to trees of the genus *Castanea* and has the edible seeds of these trees. Although it can be grown in all over the world except in Antarctica, most production in the world is made in China and Bolivia (Atasoy and Altıngöz, 2011). Turkiye ranks third in this ranking, with 62,904 tons in 2017 (Faostat, 2018). The main chestnut varieties grown in Turkiye were Osmanoğlu, Sarıaşlama, Hacı İbiş, Hacıömer and Mahmutmolla.

Chestnut has been an important source of carbohydrates and proteins in human nutrition since ancient times (Jaynes, 1979; Payne et al., 1983) and these proportions change according to the type, variety and ecological conditions in which chestnuts grows (Payne et al., 1983). Chestnuts are rich in carbohydrates and low in oil (1.5-2%) and protein (2.5-3%), unlike other hard-shelled fruits. Chestnut, which is a nutritious and high-calorie food, is rich in vitamins B1, B2

and C. Regarding its mineral content, chestnut is also a source of K, Mg, Fe, Mn and Cu (Borges et al., 2008). They are an excellent source of dietary fiber; It provides 8.1 g (approximately 21% Reference Daily Intake) fiber per 100 g. Dietary Fiber helps to lower blood cholesterol levels by limiting excessive cholesterol absorption in the intestines (URL– 4, 2017). Compared to other hard-shelled fruits, it contains the lower oil and higher carbohydrate (Turkomp, 2022). Although the sugar content is equal to the 10% of carbohydrate composition, the remaining part is starch and dietary fiber, which causes it to be a fruit with a low glycemic index.

Chestnut, which is rich in carbohydrates, is a healthy food due to its cholesterol-free, low glycemic index, little calories, rich in protein and vitamin C (Ribeiro et al., 2019). The oil and high fiber content of chestnuts make them a healthy food and a well energy source. Recently, chestnut consumption has been increasing daily with the health benefits of chestnuts, changes in dietary habits and gluten-free nutrition approaches (Wani et. al., 2017). The protein content of chestnut contains essential amino acids tryptophan, lysine, and methionine (Wani et. al., 2017). The amino acid profile of chestnut is dominated by L-aspartic acid, followed by L-glutamic acid, Leucine, L-alanine and Arginine (Borges et al., 2008). Furthermore, chestnut contains important components that have a positive effect on health; these are antioxidant compounds such as L-ascorbic acid, vitamin E, carotenoids, and polyphenols (especially gallic and ellagic acids) (Barreira et al., 2009; de Vasconcelos et al., 2010, De Vasconcelos et al., 2007; De Vasconcelos et al., 2009; Gonçalves et al., 2010; Neriet al., 2010; Barros et al., 2011).

Chestnut fruits (Ribeiro et al., 2007) and leaves (Calliste et al., 2005) have been shown to contain phenolic compounds (Barreira et al., 2008). Phenolic compounds are secondary metabolites found in fruits, vegetables, and grains. They influenced the color, taste and aroma of fruits and vegetables (Wollgast and Anklam, 2000; Havsteen, 2002;). The antioxidant activities of phenolic compounds are related to a number of different mechanisms, such as free radical scavenging, hydrogen donation, $^1O_2$ quenching, metal ion chelation, and acting as a substrate for $O_2^{\cdot -}$ and $OH^{\cdot}$ radicals. A direct relationship was found between the antioxidant capacity of plants and their total phenolic content (Robards et al., 1999; Barreira et al., 2008). Natural antioxidants, phenolic acids and their derivatives present in the diet or prepared synthetically have been shown to have chemopreventive (preventing the harmful effects of chemicals) properties (Fang, Yang and Wu, 2002; Barreira et al., 2008).

Like almonds, hazelnuts, walnuts and pistachios, chestnuts are in the group of hard-shelled fruits. Tree-grown fruits such as hazelnuts, chestnuts, walnuts and pecans have high antioxidant content (Blomhoff et al., 2006; Barros et al., 2011). In recent years, chestnut has also attracted attention in the health, pharmaceutical and material sectors, apart from food products. The use of chestnuts to develop gluten-free products, for high cholesterol, diabetes and celiac patients is becoming widespread. Studies on the antioxidant functions of chestnuts and its effects on diet-induced obesity have been increasing. The variety of chestnuts in the market has led to increase in the use and consumption of chestnut. However, consumers prefer fresh chestnuts because they think it is healthier. In addition, chestnut flours are used in the formulations of products made for celiac patients. (Li et al., 2022; Liu et al., 2020; Niazi et al., 2018). Fresh chestnuts are generally consumed after boiling, baking, or roasting processes. The aim of this study to investigate the effects of heat treatments applied by boiling and baking chestnuts on antioxidant activities in chestnuts. Thus, the effect on the nutritive properties of chestnuts during industrial processing can be optimized.

MATERIALS AND METHODS

The type of chestnut used in this study is *Castanea sativa* Mill. It was obtained from Kurşunlu village of Karacabey district of Bursa province and from Cumalıkazık, Yiğitali and Kirazlı villages of Bursa center in October 2015. Chestnut varities used in this study were Osmanoğlu and Sarıaşlama. After the inner and outer shells of the raw chestnuts were cleaned, they were cut into small pieces using a shredder. The prepared samples were used for physical analysis and antioxidant analyses. For boiled chestnuts, 200 g chestnuts were boiled in 1000 ml water for 30 min. For the chestnuts baked in the oven were drawn as (+) using a knife and then baked in the oven at 180˚C for 30 min. Then it was separated from its inner and outer shells and then was made homogeneous by crushing.

Physicochemical Analysis

The water activity of raw chestnut samples was measured from the prepared homogenate at 25˚C with a water activity analyzer (Novasina, LabMaster). For determination the total dry matter, 4 g of the raw chestnut samples were weighted from the homogenate then the weighted sample were taken into petri dishes. The weights were taken by keeping them in a vacuum oven at 70ºC (200 mmHg) for 24 hours and calculated as % dry matter. For pH determination, suspension was prepared by taking 5 g of homogeneous sample and diluting it with 25 ml of distilled water. The resulting suspension was mixed at 20 min intervals and measured with a pH meter (Thermo Scientific, Orion3Star, Singapore) at 20ºC. For ash determination, 4 g of the homogenate was taken (prepaid) and weighed in a porcelain crucible. Then, 95% ethanol was poured into the crucibles and burned until charred. The charred sample was burned in the muffle furnace at 550ºC until there were no black spots.

Color measurement; Chestnut fruit flesh color was

determined by measuring the peeled chestnuts with a colorimeter (Konica Minolta, CR-400, Japan) in the Hunter (L*, a*, b*) color measurement system at room temperature. Flesh color of chestnut fruit was measured in terms of L*, a*, and b*. L* stands for brightness/darkness, a* stands for redness (+)/greenness (-), and b* stands for yellowness (+)/blueness (-).

Determination of total phenolic content (TPC)

The total phenolic content in extracts of raw, boiled and baked chestnuts was determined according to the Singleton et al. (1999) method with some modifications. The Folin-Ciocalteu method is based on the absorbance measurement according to the color intensity formed by the reagent that gives the method its name (Huang et al., 2005). A calibration curve was drawn by using gallic acid solutions. 1 mL of chestnut fruit extract were taken and placed in balloon bottles. 45 mL of distilled water, 1 mL of 2N Folin-Ciocalteu reagent and 3 mL of 3% Na_2CO_3 solution were added after 3 min. After 2 hours, the absorbance of the mixture was read in the spectrometer at 720 nm. The results of the samples were calculated as mg GAE kg^{-1} wet weight.

Determination of total antioxidant activity

The antioxidant activity of raw, boiled and baked chestnuts was determined according to the DPPH (2,2-Diphenyl-1-picrylhydrazyl) antioxidant activity method. 0.1 ml of diluted extract was taken, and 3.9 ml of DPPH solution ($6x10^{-5}$ M) was added. After the mixture was kept at dark for 30 min, absorbance values of the samples were read at 515 nm and antioxidant activity values in the samples were calculated according to the calibration graph drawn with ascorbic acid. The results were given as µmol AAE g^{-1} wet weight (Coklar and Akbulut, 2016).

Statistical analysis

Statistical analyzes were performed using the SPSS 20.0 package program. The significance of differences between the physicochemical properties of chestnut fruit cultivars were determined by using an independent two-sample t-test (0.01 ve 0.05 confidence interval). The effects of boiling and baking processes on chestnuts on the total antioxidant activity and total phenolic content were determined using a one-way MANOVA Duncan multiple comparison method at a 95% confidence interval.

RESULTS AND DISCUSSION

In terms of nutritional physiology, preserving vitamins and minerals in foods depends on processing and storage conditions. During processing and storage, the physical and chemical state of water affects the quality of food. Similarly, chestnut varieties should also be considered when the quality properties of them were evaluated. Water activity is known as the ratio of the vapor pressure of water in food to the vapor pressure of pure water at the same temperature. Water activity has an important role in the deterioration of foods rather than the amount of water chestnuts contain (Erdal 2013). In our study, the water activity value was found to be 0.705–0.844. In terms of water activity (aw) values, the average value of water activity of the Sarıaşlama variety was higher than the Osmanoğlu variety ($p<0.01$). Erdal (2013), the quality of chestnut fruit before and after harvest, found water activity between 0.952 and 0.963 in peeled chestnuts. Erdal (2013) determined that storage conditions (temperature, humidity and time) have an affect on water activity values. Erdal (2013) worked with chestnuts obtained from the Nazilli region of Aydın province but the samples we used were obtained from Bursa province. Thus these differences may be due to the difference in the growing environment of chestnuts.

Total dry matter content may vary depending on the type and variety of chestnut and the conditions during storage. In our study, the total dry matter content was determined as 48.82±1.19% in the chestnut fruit of the Sarıaşlama variety and 46.96±1.17% in the Osmanoğlu variety. Moisture content was determined as 51.1% in the Sarıaşlama variety and 53.04% in Osmanoğlu. There was no statistical difference between Sarıaşlama and Osmanoğlu cultivars in terms of total dry matter ($p>0.01$). The moisture content was found by Yıldız et al., (2009), Otles and Selek, (2012), Fatih et al., (2013), Mert and Ertürk, (2017) 54.84%, 26.14–44.99%, 52.6-56.9%, 46.52-59.47% as, respectively. Neri et al., (2010) determined between 42.27-52.89% of the moisture content of chestnut fruits in Italy. In addition, the total dry matter amount values found in our study are consistent with the results determined in previous studies.

The term pH is used to describe the degree of acidity or, in other words, the strength of acidity. The factor affecting pH is the active hydrogen ion concentration. The pH value was 7.18 of Sarıaşlama cultivar and 7.26 in Osmanoğlu cultivar and the difference was determined to be statistically significant ($p<0.01$). In our study, it was observed that the pH of raw chestnuts varied between 7.03 and 7.29.

Ash refers to the sum of the mineral substances in the food, and the amount of mineral substance varies from food to food. In our study, the amount of ash was found to be 1.11±0.14% in the chestnuts of the Sarıaşlama variety and 1.27±0.15% in the chestnuts of the Osmanoğlu variety. There was no statistical difference between Sarıaşlama and Osmanoğlu cultivars in terms of ash ratio ($p>0.01$). Ertürk et al., (2006) found the amount of ash 1.02-3.22 g 100 g^{-1} in their study. In the study conducted in the Erfelek region of Sinop province, the ash amount of chestnut was determined as 1.40-4.92 g 100 g^{-1} (Üstün et al., 1999). Yıldız et al., (2009) found the amount of ash 1.078 g 100 g^{-1}. In the study conducted with chestnuts grown in the Marmara region, they stated

that the amount of ash varied between 2.09 g 100 g^{-1} and 4.39 g 100 g^{-1} and the average was 3.00 g 100 g^{-1} (Mert and Ertürk, 2017). The amount of ash we determined in our study is similar to other studies.

Table 1. Some physicochemical properties of Sarıaşılama and Osmanoğlu raw chestnut cultivars

	Sarıaşlama	Osmanoğlu
Water activity	0.784±0.02**	0.728±0.01
Total dry matter (%)	48.82±1.19	46.96±1.17
pH	7.18±0.05	7.26±0.04**
ash (%)	1.11 ±0.14	1.27±0.15
L*	61.02±3.37	60.01±2.50
a*	-0.05±0.02	0.29±0.02
b*	12.94±1.90	11.84±1.20

** The difference is statistically significant ($p<0.01$).

Color is a visual property that occurs depending on the spectral distribution of light. The main reasons why color gains importance in foods are taking a role in the pleasing of food in terms of consumer preferences; The change in the color of the food gives an idea about the ripening and the deterioration with the change in taste and texture. In our study, the average color values of raw chestnuts were L* 60.52±2.64; a* 0.13±0.18; b* 12.39±1.57. Erdal, (2013) found L^*96.07, a^*-3.89, b^*7.44. Similarly, Algül et.al., (2016) determined L^*89.93, a^*-2.18, b^*5.20 in their research. In our study, between the sarıaşlama and osmanoğlu cultivars in terms of color parameters (L, a*, b*) were not found statistical difference ($p>0.05$). Color values were L* 61.02±3.37, a*-0.05±0.02, b* 12.94±1.90 in Sarıaşlama cultivar, L* 60.01±2.50, a* 0.29±0.02, b* 11.84±1.20 in osmanoğlu cultivar.

Abe et al., (2010) expressed the antioxidant activity of chestnut as 6.2 µmol Trolox equivalent g^{-1} d.m. using the DPPH method. Otles and Selek, (2012), determined the total antioxidant capacity of raw chestnuts according to the FRAP method as 9.08-14.15 mM $FeSO_4$ g^{-1} d.m. were found to vary within the range. The fact that the obtained data is lower than the values found in the literature due to the chestnut cultivars used in the present study and the differences in growing conditions.

In the study examining the total vitamin C content of chestnuts (ascorbic acid + dehydroascorbic acid) and antioxidant activity in raw and cooked chestnuts, the antioxidant content of roasted and boiled chestnuts decreased by 51% and 88%, respectively (Barros et al., 2011). Li et al., (2016) investigated the effects of different cooking methods on the content of important nutrients and volatiles in Chinese chestnuts. They reported that the compounds such as reducing sugar, sucrose, organic acids and total flavonoids of boiled, roasted and fried chestnuts were significantly lower than raw chestnut varieties after cooking ($p<0.05$). The total polyphenol content (2.24 mg/g) in raw chestnut remained unchanged after roasting (2.26 mg/g) but it decreased after boiling (2.03 mg/g) and frying (2.08 mg/g). Total flavonoid content (2.62 mg/g) also decreased after boiling (2.12 mg/g), roasting (2.25 mg/g) and frying (2.13 mg/g) processes (Li et al., 2016). Barros et al., (2011) and Li et al., (2016) determined that the antioxidant value of raw chestnuts was higher and heat treatment caused a decrease in the antioxidant value of chestnuts but Gonçalves et al., (2010) determined that the antioxidant value of roasted and boiled chestnuts is higher than that of raw chestnuts. Gonçalves et al., (2010) investigated

Table 2. Changes in the amount of TPC and DPPH in the boiling and baking process of chestnut varieties.

	Sarıaşlama		Osmanoğlu	
	DPPH µmol AAE g^{-1}	TPC mg GAE kg^{-1}	DPPH µmol AAE g^{-1}	TPC mg GAE kg^{-1}
Raw	0.41±0.01 c	121.06±7.45 a	0.42±0.01 b	137.28±5.00 a
Baked	0.23 ±0.04 a	175.11±13.39 b	0.30±0.02 a	186.83±23.10 b
Boiled	0.38±0.01 b	144.11±10.46 ab	0.42±0.01 b	155.61±5.43 ab

Different letters in each column indicate statistical difference ($p<0.05$)

The antioxidant activity values of chestnut samples are shown in Table 2. While it was determined as 0.41±0.01 µmol AAE g^{-1} of raw chestnut in Sarıaşlama variety, antioxidant activity values decreased with baking and boiling processes ($p<0.05$). When the antioxidant activity was examined after the cooking processes with raw chestnut in the Osmanoglu type, there was no significant change in the antioxidant activity with the boiling process ($p>0.05$), but a decrease occurred in the baking process ($p<0.05$). Neri et al., (2010) determined the antioxidant activity of chestnut by ABTS method as 4.77-8.15 µmol Trolox equivalent g^{-1} d.m. found to be in the range.

the metabolic composition of chestnut (*Castanea sativa* Mill.) during cooking. As a result of this research, they concluded that roasted chestnuts have higher gallic acid and total phenolic content, and boiled chestnuts have higher gallic and ellagic acids compared to raw chestnuts. Contrary to what is known, studies conducted in recent years have also shown that new antioxidant compounds can be formed with heat treatment and increase the number of phenolic compounds. It is also observed that antioxidant and phenolic components increase with the inactivation of enzymes that inhibit antioxidant activity and the liberation of some phenolic compounds by heat treatment (Pinelo et al., 2005).

Total phenolic content of boiled chestnut in Sarıaşlama cultivar was similar to that of baked chestnut, but higher than raw chestnut. Total phenolic content of boiled chestnut in Osmanoğlu cultivar was similar to that of baked chestnut, but higher than raw chestnut ($p<0.05$). The total amount of phenolic substance was found to be 121.06±7.45 mmol gallic acid equivalent/g sample in raw chestnut (Table-2). Total phenolic content of raw and baked chestnuts was similar and higher than boiled chestnuts in both cultivars ($p<0.05$). When the researches were examined, it was seen that different results were recorded depending on many factors such as fruit variety, the season in which the fruit is harvested, the fraction of the fruit, and the climatic conditions in which it is grown (Coklar and Akbulut, 2016).

Neri et al., (2010) determined the total phenolic content of raw chestnuts as 1120,6 mg GAE/kg dm. In another study conducted on chestnuts collected from 12 different locations in Turkey, the amount of TPC was 500–3200 mg GAE kg^{-1} dm. (Otles and Selek, 2012). Suárez et al., (2012) determined in their study that the TPC amount of chestnuts obtained from 20 different locations in Spain varied from 196-431 mg GAE kg^{-1} dm. and the average was 284 mg GAE kg^{-1} d.m. In another study, Chang et al. (2020) determined the phenolic content of five types of chestnut produced in China between 243,5-586 mg GAE kg^{-1} dm. in different cultivars. As seen in previous studies, the amount of TPC in chestnut varies according to the place where it is grown and the type of chestnut.

CONCLUSION

As a result of the research, according to the studies on chestnut fruits, physicochemical analyzes are generally compatible. It is thought that the differences in some parameters were caused by the type and variety of chestnut and the ecological conditions in which it is grown. In antioxidant analysis, the total amount of phenolic substances in raw chestnuts was higher than others. The difference may be due to genetic and environmental factors. While the boiling process caused a significant decrease in antioxidant capacity according to the DPPH method, the baking process did not cause any change. It was observed that there was a significant increase in the total phenolic content with the boiling and baking process.

As a result, this study, some physicochemical and antioxidant properties of two different chestnut species grown in the Bursa region were determined and the effects of boiling and baking on chestnuts' antioxidant properties and phenolic substance content of chestnut were investigated. Chestnut, which is not widely consumed raw, is generally used in confectionery, bakery, and pastry products. Chestnuts used commercially in confectionery and pastry products are first boiled. The boiling process is important in this respect. In addition, chestnuts are also consumed by baking. In our study, in which we examined the antioxidant substance content and total phenolic substance changes when chestnuts were treated with these two most consumed cooking techniques, it can be concluded that the best method is the baking method.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

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

Author contribution

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

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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REVIEW ARTICLE

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Enabling circularity for food safety: the rooftop farming model

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Abstract

Population growth, environmental change, urbanization, consumption patterns, waste, the global political climate, conflicts, and infectious diseases all exert a strain on global food security. Access to sufficient and nutritious food is becoming increasingly problematic, particularly for individuals living in less developed and developing countries. More individuals are becoming aware of the significance of developing a "Green Economy" approach to ensure global food security. A multifaceted strategy is required to ensure global food security. This study emphasizes the need for regional self-sufficiency for the transition to a green economy, and as a model proposal, it is suggested that it would be beneficial to encourage and develop farming operations on the roofs of residential, commercial, and public housing in urban regions. Rooftop farming, as one of the urban agriculture practices, can be utilized as a strategic instrument to achieve a variety of key aims, including enhancing the local economy, reducing inequality, internalizing responsible production and consumption by society, improving the quality of urban life, and more. Furthermore, because of the novel production processes seen in urban agriculture, circularity in production and consumption, a key component of the transition to a green economy, can be realized. "Self-sufficiency" is one of the concepts underlined in this study. The study also includes real-life cases highlighting the potential benefits of rooftop farming.

Keywords: Food security, Self-sufficiency, Circular economy, Urban Farming, Rooftop farming

INTRODUCTION

If the current situation continues, the world population is expected to reach 10 billion by 2050 (UN, 2017), and the urban population will also increase to 66% (WHO, 2016). Many reasons, such as the ever-increasing global food demand, the physical distance between production and consumption locations, intensive agriculture, and livestock farming shaped by gene technologies, synthetic fertilizers, and pesticides, will lead to further increasing pressure on the ecosystem (Boneta et al., 2019).

It can be said that agricultural land is declining, especially due to the increasing urbanization rate and increasing population, and traditional food production, which is largely based on agriculture and livestock farming, is no longer sustainable in terms of environmental costs in its current state. These developments also pose a food security problem. The situation has been exacerbated by COVID-19, which

has hampered the international food trade (Xi et al., 2022). In addition to population growth, the increasing demand for food due to protein-intensive and high-calorie diets is considered an important problem (Godfray et al., 2010). Due to these stated circumstances, the need to rethink food systems emerges. It is observed that the number of academic debates and research focusing on the green economy is increasing. It can be said that efforts to create an academic conceptual framework in this direction will not be meaningful on their own unless they are supported by practice. For this reason, innovative practices supported by innovative perspectives are needed.

In the literature, it is seen that recommendations for ensuring food security are concentrated on more efficient production or more limited consumption (Beltran-Peña et al., 2020). Examples; reducing meat consumption and preferring a diet based on plant-based consumption, lowering food waste, increasing yield in production in existing arable areas, ensuring efficiency in resource use in production, and using environment-friendly irrigation (Davis et al., 2014; Kummu et al., 2012; Garnett et al., 2013; Pretty, 2018; Springmann et al., 2018; Rosa et al., 2018).

The rate of urbanization has made it necessary for food production areas to be increasingly located near the main consumption centers (Gupta & Mehta, 2017). The approach of "food self-sufficiency", which refers to the ability to provide the necessary food within the city or country borders, has a long history (Ligutti & Rawe, 1940). It is aimed regionalizing food systems by increasing local food production and thus shortening supply chains (Zasada et al., 2019). The phenomenon of self-sufficiency in food has made urban agriculture practices more popular. Through urban agriculture, food crops are grown within the city boundaries, and urban dwellers have access to healthy, fresh produce (Hume et al., 2021). While urban households historically supplemented their foods from their gardens, in times of economic upswing (e.g. Industrial Revolution, post-World War II) has been experienced a shift from producing food at home to sourcing food products produced in different geographies from distribution channels (Burgin, 2018). Rooftop farming may be considered a model to meet food safety in a circular way (improve air quality, heat flow, and reduce corridors for local flora and fauna) (Skanavis et al., 2017). Gupta & Mehta (2017) state that rooftop farms can help achieve six important sustainable development goals of the United Nations. These are:

Food Security

According to the Food and Agriculture Organisation of the United Nations (FAO), which estimates that the global population will rise by one-third between 2009 and 2050, and already 9.3% of the world's population (approximately 689 million people) has problems accessing food (Manríquez-Altamirano et al., 2020). Climatic changes and the increase in the number and impact of natural events with destructive effects (droughts, floods, storms, etc.), plant and animal diseases, epidemics, and increases in the costs of production factors (water, electricity, fertilizers, pesticides, oil, etc.), the use of land for animal feed or bio-fuels instead of food, relatively small fluctuations in global supply or demand can lead to large fluctuations in international prices, speculative or manipulative price or supply interventions on foods, increasing urbanization rate, changing lifestyle and food consumption patterns, and increasing spoilage and wastage make access to food increasingly difficult (Wong et al, 2020; Misselhorn et al., 2012). It is also estimated that every one (^{0}C) degree increase in global warming will lead to a 10 per cent decrease in the suitable agricultural land (Despommier, 2013). These conditions have made 'food security' an issue that is being discussed, debated, and researched more and more every day. Financial, energy-based and food crises, as a result of globalization, have made the fragility and interrelatedness of lifestyles more salient (Kinda, 2021). Especially after the 2007-2008 food crisis, food security has become more questionable in some countries and food self-sufficiency has become an important goal of agricultural policies. Possible global food shortages are considered a national security issue (Clapp, 2017).

The fluctuations in world food markets, which started with the COVID-19 pandemic, led to a different level of concern with Russia's attack on Ukraine. Global epidemics, decreases and fluctuations in agricultural output depending on climatic change, the use of food as a political trump card in international relations and many similar factors have made the issue of food security more topical than ever (Clapp, 2017).

Food Self-sufficiency

Food self-sufficiency means the ability of a country to provide the food it needs through its domestic production (FAO, 1999). Food self-sufficiency was set by the first six members of the European Union as an objective of their agricultural policy in the 1950s and 1960s (Zobbe,

2001). However, towards the end of the 20th century, as globalization increased its influence, the approach of food self-sufficiency began to be disregarded.

Food security and food self-sufficiency are not the same concepts. Food security focuses on the accessibility of food regardless of its source. In this respect, it does not matter where the food is produced; it is sufficient for consumers to have access to food. However, the concept of food self-sufficiency focuses on reducing imports and supplying food requirements based on the geography of the country. There is no avoidance of food imports in food security (Wegren & Elvestad, 2018). Clapp (2017), defines food self-sufficiency on a national basis as a country's ability to produce at least as much food as it consumes. She has created the information in Table.1 by taking into account the categorization developed by FAO. In the table, the point at which food production equals food consumption is indicated by 100 and is expressed as the point where self-sufficiency begins.

Table 1. Food Self-sufficiency Situation of some Countries

	Food Self-sufficiency Ratio		
	<85%	=85-115%	>115%
Consumption equal to or above a balanced diet	Produce less food than they consume, and meet their food requirements. The hunger rate is < 5% e.g.: Japan, South Korea, Greece, Italy, Mexico, Kuwait	Produce food close to what they consume, and can meet their food requirements. The hunger rate is <5 % e.g.: South Africa, Brazil, Germany, Turkey, Austria Sweden,	Produce more food than they consume, and can easily meet their food requirements. The hunger rate is <5 % e.g.: Canada, Australia, Argentina, USA, Hungary, Kazakhstan, Russia,
Consumption below the balanced diet rate	Produce less food than they consume, and have a high hunger rate is >25% e.g.: Liberia, Zimbabwe, Namibia, Bolivia, Haiti, Mongolia, Yemen, Mozambique	Produce food close to what they consume, and have moderate (5-25%) and high (>25%) levels of hunger e.g.: India, Tanzania, China, Guinea, Cambodia, Malawi, Chad, Zambia	Produce more food than they consume, and have low to moderate levels of hunger (5-14.9%) e.g.: Guyana, Vietnam, Thailand, Paraguay

Source: Clapp (2017)

According to the table, countries such as Australia, Canada, Argentina, Russia, and the USA stand out in terms of self-sufficiency, and Liberia, Bolivia, Zimbabwe, Namibia, Yemen, Mongolia, Haiti, and Mozambique are the countries that are the farthest away from self-sufficiency. Even if a country has the necessary natural, financial and economic resources, vision, management skills, infrastructure and distribution systems, effective agricultural programs and policies to become fully self-sufficient, dynamics such as comparative advantage, political and economic relations between countries may prevent this. A country cannot be expected to isolate itself from global markets based on food self-sufficiency.

In practice, very few countries are able to achieve food self-sufficiency (Wegren & Elvestad, 2018). The traditional meaning of food security favors interaction between markets. Increased trade volume is thought to be in favor of food security (Dithmer & Abdulai, 2017). In a sense, food self-sufficiency is contrary to the spirit of globalization. Because it will lead to a decrease in the integration between countries and foreign trade volume. Since a country with a food surplus cannot export the surplus, the excess supply will lower domestic prices, which may lead to production cuts. However, instability in commodity prices due to economic, political, or climatic factors enables countries to put food self-sufficiency on the agenda as a protective measure. They develop various strategies to increase self-sufficiency to be minimally affected by the anomalies brought about by unpredictability. These can take the form of increasing domestic food production, expanding the modes of production, improving the distribution of domestically produced food, improving food production technologies, reducing food loss, waste, and hoarding, and even aligning domestic consumption levels with supply (Gráda, 2009).

Innovative Approaches for Food Safety

The world's population is increasing day by day. In 1950, the population was approximately 2.5 billion and reached approximately 6.08 billion in 2000. As of 2021, approximately 7.9 billion people live in the world, and with the same growth rate, it is estimated to be 9.7 billion in 2050, and 10.4 billion in 2080. Similarly, the rate of urbanization is also increasing. The global urbanization

rate of 50% in 2009 is expected to increase to 68% in 2050 (UN, 2018). The increasing population and urbanization rate create significant pressure on nature (Deelstra and Girardet, 1999). The current economy and development model have come under increasing scrutiny as the effects of climate change have become more apparent, and efforts to adopt an environmentally friendly growth model have increased (UNEP, 2009). The abandonment of the linear economy model and the transition to an environmentally friendly circular economy have been discussed more and more in recent years. The green economy approach can be considered an important political tool to ensure food security. Through various innovative tools and strategies to be developed within this approach, global monitoring, strategy development, and management capacity can be established.

Urban Agriculture

When the history of humanity is taken into account, it is seen that there have been some breaking points where paradigmatic changes in production and consumption patterns have occurred. With the Industrial Revolution, a new phase was entered in production technologies, and logistics facilities were carried to a higher level than ever before. Similarly, after the two world wars, war technologies evolved into production technologies. These revolutionary developments in production and distribution have undoubtedly increased consumption opportunities and facilitated consumer access to goods and services. This has resulted in radical transformations in consumption patterns. Until the Industrial Revolution, consumption was limited to local production possibilities. Goods and services produced in geographically close neighborhoods were purchased, and agricultural and animal production was processed to be consumed within the year with storage techniques such as drying, salting, canning, etc. The family's main source of nutrition was the small gardens and fields near the house. Surplus food was distributed or exchanged among neighbors, friends, and/or relatives. In short, the physical distance between the places of production and consumption was quite short. With the transition to modern times, especially in industrialized countries, the role of local production in meeting the family's food needs has declined significantly. Grapes started to come from Chile, computers from Taiwan, and bottled drinking water traveled thousands of kilometers (Unmüßig et al., 2012). Increasing urbanization has been a major accelerator. However, situations that pose a threat to food access (such as economic and financial crises and wars) have once again highlighted the importance of micro-scale agricultural activities. For example, during World War I (1917-1918), 'amateur gardeners' played an important role in overcoming food shortages (Cole, 1993). Moreover, during extraordinary periods such as the Great Depression and the Second World War, home gardens were able to produce more than 40 per cent of fresh food in the USA (Burgin, 2018). More than %50 human being currently live in urban areas. This rate is estimated to reach 70 per cent in 2050 (%66 for least developed regions and %86 for the most developed regions) (Parfitt et al., 2010). The fact that cities are responsible for using %75 of the global resources (TFPC 1999), however, they only account for 2 per cent of the global surface area, makes it necessary to position cities in a critical position for food security (Kumar et al., 2019). Globalization and increasing urbanization have caused the food supply chain in the world to spread over long distances. The place of production is separated from where the products are consumed, leading to long transport distances and associated environmental impacts (Grewal & Grewal, 2012). The continuous development of infrastructure in cities also leads to increased demand for energy, water, food, and other resources. The intensity and impacts of many environmental problems such as decreasing availability of natural ecosystems, poor air quality, polluted water, the urban heat island effect, and the loss of natural habitats have become serious (Goldstein et al., 2016). It is estimated that food production in the world will decrease by more than 50% and the population will reach 9 billion by 2050 due to climate change (Kumar et al., 2019). All these changes, trends, and forecasts indicate that there will be more pressure on food security (Corbould 2013; Buehler & Junge, 2016; Shrestha et al., 2020) and a need for a sustainable food supply in cities (Lawrence et al., 2022). A food security problem is of great concern globally due to the intense environmental impacts of urban populations, limited agricultural land resources, and the fact that traditional agricultural production can technically be increased to a certain extent, consumption patterns are no longer sustainable, there is widespread waste, and traditional and industrial agriculture are becoming increasingly difficult. Worsening global warming and climate change will make traditional agricultural activities more difficult, risky, and uncertain. Therefore, innovative strategies are needed to increase the production of food crops without any detrimental impact on the environment (Grard et al., 2015). Discussions on urban metabolism are becoming increasingly important (Meerow et al., 2016), and resource flows, food security, and regional self-sufficiency are receiving increasing attention (Zasada et al., 2019).

Urban Agriculture Concept

Urban agriculture has a long history. Examples of urban agriculture, which developed as a measure against the food security problem of cities that emerged with the establishment of cities, date back to Ancient Egyptian societies and examples can be found throughout history until after the world wars (Calvet-Mir and March, 2019). The fight against climate change, the search for alternatives to traditional commercial agriculture that have a larger carbon footprint, the adoption of

sustainable agricultural methods, and the aim of reducing and overcoming environmental depression, economic bottlenecks, and social problems as well as meeting the needs and demands of people are important motivators for urban agriculture (Wong et al., 2020; Yusoff et al., 2017). There are the following definitions of urban agriculture in the literature:

> "Raising food crops and livestock in an urban environment to feed the local population" (Pfeiffer et al., 2014:1),
>
> "An urban design solution to the environmental impacts of urban food needs" (Goldstein et al., 2016:984),
>
> "Food or animal cultivation or processes carried out in urban areas or around urban centers to generate income" (Yusoff et al., 2017:272).

Urban agriculture, which is considered a complement to rural agriculture, includes different scales from commercial agricultural facilities to household-level production (van Veenhuizen, 2014) and is widely practiced by society in areas of rapid urbanization, cities, and towns (Yusoff et al., 2017). It is estimated that urban agriculture provides about %15-20 of the world's food supply (Gerster-Bentaya, 2013; Nadal et al., 2017). Urban agriculture activities can be carried out in small-intensive urban farms where traditional agriculture is practiced, in public spaces such as schools, in the gardens and/or roofs of residences and businesses, on balconies and windowsills (Burgin, 2018; Schupp & Sharp, 2012). The term Zero-Field Farming (ZFarming) is used for urban farming activities that do not use agricultural land or open space (Buehler & Junge, 2016). However, there is currently no taxonomy to categorize urban agriculture practices (Goldstein et al., 2016).

Benefits of Urban Agriculture

Urban agriculture has become an area of increasing interest. This is based on increasing urban food demand, global and local environmental impacts, and growing concerns about food security (Sanyé-Mengual et al., 2018). Urban agriculture, which can be realized in different urban settings and in different forms, contributes positively to the economic, social, and environmental sustainability of cities (Ackerman, 2011). There are many studies in the literature on the potential benefits of urban agriculture. For example, Goldstein et al. (2016) grouped these benefits into three categories: supply chain efficiency, urban symbiosis; on-site environmental benefits. The proximity to urban processes makes urban agriculture a viable practice to increase food security by reducing the environmental impact of long transportation distances in the conventional food cycle (Buehler & Junge, 2016). Because of its proximity to other urban processes, it can contribute to closing the urban metabolism cycle and achieving sustainability goals by reusing urban waste products as agricultural inputs (Hume et al., 2021). Crop planning that takes into account the region's consumption preferences, demand volumes, and fluctuations, and high-yielding food crops can enable environmentally and cost-effective, efficient production and consumption, and can be useful to avoid waste (Sanyé-Mengual et al., 2016). For example, in conventional food production, the average distance of arrival is estimated to be 2800 km on average (Peters et al., 2009). In addition to the environmental destruction caused by this transport, it also causes waste due to the loss of freshness and spoilage of food products. According to Peters et al. (2009), the distance traveled by food can be reduced to 49 km by reorganizing the food system.

In the literature, the benefits of urban agriculture are generally expressed under the headings of environment, economy, and social (e.g. Hui, 2011; Noseir, 2014), but some studies (e.g. Yusoff et al., 2017) also include a fourth category called health and nutrition. In this study, as a result of the literature review, Table 2, which includes the benefit categories of 'environment, economy, social structure, health and nutrition, education, and motivation', was created and shown below.

In addition to the benefits mentioned above, there are also some difficulties in the implementation of urban agriculture. High investment costs, the need for structurally sound multi-story buildings, the need for technical knowledge for soilless agriculture applications (such as aquaponics, hydroponics, and aeroponics), and the need for artificial light during periods of lack of sunlight can be given as examples of these difficulties. There is also a misperception that urban agriculture is "unnatural" (Specht and Sanyé-Mengual, 2017), especially for systems that move away from traditional agriculture (e.g. soilless agriculture). This may slow down the acceptance and popularization of such systems.

Types of Urban Agriculture

Although there is no clear taxonomy in the literature, different types of urban agriculture can be mentioned, ranging from family and community gardens to commercial ventures (Opitz et al., 2016). For example, Yusoff et al. (2017) identified three types of urban agriculture: community farming, vertical farming, and rooftop farming. Each approach has similar practices and objectives but also has some distinctive characteristics. Urban farming types are differentiated according to the purpose(s) of the establishment (food security, contribution to the family budget, local development, education, R&D, social motivation, aesthetics, etc.), scale, and level of technology utilization. These activities can be carried out both indoors and outdoors. There are urban agriculture types known as vertical agriculture, greenhouse agriculture, container agriculture, roof agriculture, reserved area agriculture, and warehouse agriculture. The applications can be soil-based, as in

Table 2. Benefits of Urban Agriculture in the Literature

Benefit category	Description of benefit	Author(s)
Environmental	Reducing water use, eliminating or minimizing the need for pesticides and fertilizers thanks to soilless/closed field applications	Ercilla-Montserrat et. al. (2018)
	Reducing the burden on agricultural land and new landscape opportunities	Thomaier et. al. (2015)
	Development of environmental management skills	Lydecker & Drechsel (2010), Lanarc-Golder (2013)
	Increasing urban biodiversity	Howe & Wheeler (1999), McClintock (2010), Arosemena and Hammond (2012), Guitart et. al. (2012), Smith et. al. (2013), Sanyé-Mengual et. al. (2013)
	Saving up to 95% in water use thanks to aeroponic and hydroponic methods	Kalantari et. al. (2018), Perez (2014)
	Can help remove potentially hazardous wastewater	Möller Voss (2013)
	Reducing urban heat island effects	Chen & Wong (2005), Grewal & Grewal (2012), Haberman et. al. (2014), Johnson et. al. (2015), Hussain et. al. (2020)
	Can help manage municipal waste for food production	Shrestha et. al. (2020))
	Prevents overuse of natural resources and high waste production	Deelstra & Girardet (1999).
	Wastewater (recycling of rainwater and domestic wastewater) and organic solid waste can be turned into resources for the cultivation of crops	Hussain et. al. (2020)
	Potential to integrate the requirements of agricultural production into the flow of resources in the city (supporting the circular economy)	Xi et. al. (2022).
	Contributes to reducing the environmental impact of consumption by providing food from remote farms without the need for transport	Puri & Caplow (2009)
Economic	Utilization of idle areas for agricultural purposes and bringing them into the economy	van Veenhuizen (2006)
	Ability to reduce food waste throughout the supply chain	Despommier (2013)
	Growing fresh produce all year	Despommier (2013)
	It also can attract new investment opportunities	Toledano (2019)
	It can produce a much larger amount of food per square meter compared to traditional forms of agriculture	Kalantari et. al. (2018)
	Can grow a variety of crops at any time throughout the year	Platt (2007), Sivamani et. al. (2014)
	Shortening urban agri-food supply chains	Van der Schans & Wiskerke (2012)
	High-quality niche products can be sold at high prices	Hinrichs (2000)
	Development of local and environmentally friendly economies	Howe & Wheeler (1999), McClintock (2010), Arosemena and Hammond (2012), Guitart et. al. (2012), Smith et. al. (2013), Sanyé-Mengual et. al. (2013, 2015), Altieri et. al. (1999), Bon et. al. (2010), Kortright & Wakefield, (2011), Nadal (2015), Manríquez-Altamirano et. al. (2020)
	Reduced food costs by saving on transport, storage, and product loss due to local production of food	Puri & Caplow (2009), Manríquez-Altamirano et. al. (2020), Goldstein et. al. (2016)
	Local job creation	Manríquez-Altamirano et. al. (2020)
	From an economic point of view, urban agriculture also has a positive impact on job creation	Surls et. al., 2015

Table 2. Benefits of Urban Agriculture in the Literature (continued)

Benefit category	Description of benefit	Author(s)
Social	Urban revitalization through increasing green areas for recreation purposes	Wackernagel & Rees (1996), Yusoff et. al. (2017)
	Establishing economic confidence in society	Giedych (2015), Lyson et. al. (1995), Yusoff et. al. (2017)
	Helps reduce the problem of food shortages and limited space for agriculture	Giedych (2013), Smith (2005), Yusoff et. al. (2017)
	Improving food safety	Kalantari et. al. (2018), Carney 2011, Goldstein et. al. (2016), Badami & Ramankutty (2015), Maxwell (2003)
	It can build a strong and vibrant partnership and enhance social cohesion	Yusoff et. al. (2017)
	Increased social interactions, contribution to social and cultural integration, social development	Boneta et. al. (2019), Feenstra (1997), Sharp et. al. (2002), Lachowycz & Jones (2011), Yusoff et. al. (2017)
	Can support the low-income group of society	Shrestha et. al. (2020)
	Increased social well-being, sense of community cohesion, and political participation	Armstrong (2000), Hale et. al. (2011), Morgan (2015)
	Social welfare and social resilience	Yusoff et. al. (2017), Morgan (2015), Mok et. al. (2014), Tornaghi (2014)
Health and Nutrition	Healthier nutrition	Kumar (2015), Yusoff et. al. (2017)
	Increasing green spaces can support public health	Shrestha et. al. (2020)
	Increased organic fruit and vegetable consumption and exercise	Mansfield & Mendes (2013), Taylor & Lovell (2012), Wood et. al. (2020)
Education	Bringing urban people back to nature, opening minds to global issues, and educating children about the natural life cycle of the landscape environment	Cabannes (2006), Yusoff et. al. (2017)
	Green training opportunities	Yusoff et. al. (2017)
Motivation	Promote individual food production	Block et. al. (2012), Vogl et. al. 2004, Ercilla-Montserrat et. al. (2019)
	Awareness of climate change prevention	Lwasa et. al. 2014
	Promote sociocultural relations	Calvet-Mir et. al. (2016), Zasada 2011
	Promoting the participation of young people and voluntary workers and skills development for job training programs	Pfeiffer et. al. (2014), Wood et. al. (2020)
	Ideas for beautifying the environment	Okvat & Zautra (2011)
	Leisure activities and exercise	Lachowycz & Jones (2011)
	Development of aesthetic values	Yusoff et. al. (2017)
	Urban self-efficacy, well-being, self-satisfaction, lifestyle, and urban sustainability	Hamilton et. al. (2014), Specht et. al. (2016), Mok et. al. (2014)

traditional agriculture, or in the form of hydroponics, aquaponics, or aeroponics, which are becoming increasingly widespread and use much less water. High-rise commercial and residential buildings and buildings belonging to government institutions can be used for these purposes (Xi et al., 2021). In Almeria (Spain), the largest vegetable producer in Southern Europe, soilless food production is carried out in greenhouses, and most of the vegetables sold in the market are produced using soilless techniques (Specht and Sanyé-Mengual, 2017). Urban agriculture practices vary in all corners of urban environments and ecosystems. Many studies have reported that urban agriculture is practiced on floors, balconies, roofs, and walls of buildings (Hui, 2011).

Roof Farming as Urban Agriculture Practice and Examples

Rooftop farming has become one of the most popular urban agriculture practices in the last 20 years. Rooftop farming practices are sprouting up around cities (Mok et al., 2013). Rooftop farming is the establishment of an ecosystem on the roof of any building, regardless of its intended use (residential, commercial or public), using different methods. In addition to plant products, livestock production (such as poultry, and bee breeding) can also be carried out. Rooftops can be used open and/or closed

and can be established and operated with or without soil. Even the most primitive planting of perennial plants or herbs in pots or large planting beds can become an important food source when economies of scale are achieved. In developed countries, especially shopping malls and large roofs of production facilities in organized industrial zones can be used to provide economies of scale. Real-life examples of rooftop farms that can offer many concrete outputs such as reducing the potential of cities to create heat islands, reducing energy costs by providing insulation of buildings, ensuring circularity by using rainwater and waste food for production purposes, a rich landscape visuality, developing the local economy, reducing the environmental pressure caused by supply from long distances thanks to local consumption, social integration are given below.

"FOOD from the SKY"

Thornton Budgens supermarket in North London has created a rooftop permaculture garden of organic fruit, vegetables, and herbs with the "Food From The Sky" project. The aim of the project is summarized as increasing the food security of the community and proving that food products can be grown in cities and sold efficiently locally without waste. Starting in 2010 with 10 tons of compost and 300 recycling bins, the project collects enough produce to be sold every Friday with the support of more than 20 volunteers.

"Trent Rooftop Garden"

Trent University in Canada has an (educational purpose) rooftop garden. The garden provides food for the university restaurant. The Rooftop Garden was first established in 1996 as a laboratory on the roof of Trent's Environmental Science Complex. The project leader, Professor Tom Hutchinson, was fascinated by the food-producing capacity of urban areas and established this agroecosystem. In addition to being a research space, it contributes to the education of students. Despite its small size, about 500 kg of crops are harvested annually in this area.

"Rooftop Garden for Hotels"

A 4,000-square meter rooftop garden has been installed on the 14th-floor roof of the Fairmont Royal York hotel in Toronto, Canada. The freshly harvested herbs are used in approximately 6000 meals a day served at the hotel during the summer months. In addition to herbs, tomatoes, beans, and various fruits are also grown.

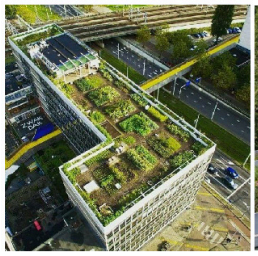
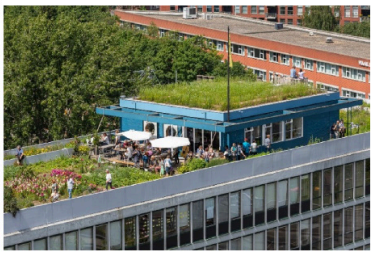
"Rooftop Garden for Hotels"

"Eagle Street Rooftop Farm"

Rooftop Farm is an internationally renowned, commercially operated 6,000-square meters rooftop farm producing organic vegetables on top of a warehouse in Brooklyn, New York. Offering seasonal produce, the farm's customers are restaurants in the area. The farm offers apprenticeship programs throughout the season and volunteer opportunities.


"Brooklyn Grange"

Brooklyn Grange was founded in 2010 and builds green spaces and hosts educational programs and events. It promotes sustainable urban living by expanding access to locally grown produce. The 5.6-acre rooftop farm is spread over three rooftops and produces around 50 tons of organic food per year. Their produce is sold at farmers' markets and through retailers. In addition, more than 30% of their harvest is sent free of charge to community members with limited access to wealth. They also run workshops that have helped educate thousands of students.

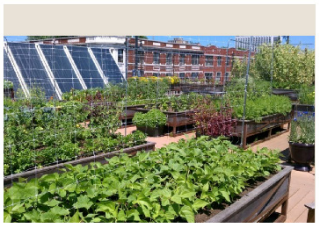
"Dakakker"

DakAkker is a 1000 m2 rooftop farm located on top of an apartment building in Rotterdam. The largest open-air rooftop farm in Holland (and one of the largest in Europe). DakAkker was established as an example of utilizing old buildings to provide alternative food sources within the city (Milanovic et al., 2018). It produces fruit and vegetables for sale to local restaurants, grows edible flowers, and has six beehives.


"Rooftop Farms for Restaurants"

Rosemary's restaurant in New York uses the products grown in the garden on the roof of the establishment in its kitchen.

There are many examples of restaurants with this concept in developed metropolises. Uncommon Ground, a restaurant in Chicago, likewise offers local food produced on its roof to its customers. So much so that it has received the title of the country's first certified organic rooftop farm. All of the perennial vegetables and herbs that are grown on the roof are included in the menu.

"Lufa Farms"

Lufa Farms in Montreal, Canada, produces 40 crops throughout the year, using cost-effective hydroponic methods. It provides food for 0.2% of Montreal's population. It is estimated that only 14 such greenhouses on the roofs of shopping centers would be sufficient to feed Montreal (Maughan, 2015). With a vision to create a better food system that includes local agriculture of all shapes and sizes on rooftop farms, the business delivers thousands of food baskets of rooftop-grown vegetables directly to thousands of customers every day.


"Gotham Greens"

This business was established in New York in 2009 and works in urban agriculture. Lettuces, herbs, and sauces grown throughout the year are distributed fresh locally. One of its facilities (established in Chicago in 2015) is the world's largest rooftop greenhouse (about seven thousand square meters). According to the enterprise, which defines itself as farmers living in apartments, they see green fields where others see gray. This business strives to help put better food on tables through environmental, educational, and community initiatives by partnering with local schools, community leaders, and non-profit organizations.


CONCLUSION

Global food security is under increasing pressure from population growth, climate and other environmental changes, urbanization, consumption patterns, wasta, the global political climate and conflicts, and infectious diseases. Increasing challenges to equitable food access hinder access to adequate and nutritious food, especially for people in less developed and developing countries. The need to develop a "Green Economy" approach to global food security is increasingly recognized. A multifaceted approach is needed to ensure global food security (Misselhorn et al., 2012). This is because the food security problem is multi-scale and cross-sectoral in nature. Addressing this problem requires various actors to work to ensure continuous improvements in human development and reduce pressure on the environment. In this context, especially in recent years, innovative business models have been sought to ensure food security through the green economy phenomenon. In addition, a green economy should be compatible with natural cycles and obtain and process food from regional ecosystems (Unmüßig et al., 2012).

In this study, the importance of regional self-sufficiency for the transition to a green economy is tried to be emphasized and as a model suggestion, it is tried to be expressed that it would be useful to encourage and expand farming activities on the roofs of residential, commercial, and public housing in urban areas. When we look at the past, we see that urban agriculture has been used especially in periods of high social stress. World wars are good examples of this (Mok et al., 2014). The COVID-19 pandemic has also highlighted the current food insecurity problems and once again emphasized the importance of local food production (Lal, 2020). Urban agriculture has become more important than ever before with the addition of factors such as bottlenecks in traditional agriculture due to global warming, the environmental burden caused by global food mobility, and the use of food as a political tool in international relations (Hume et al., 2021).

Urban agriculture has the potential to ensure food security (Nogeire-Mcrae et al. 2018). It can overcome many challenges facing traditional agriculture, such as climate change, loss of arable land, rapid population growth and urbanization, depletion of water resources, and soil pollution due to chemical pesticides and fertilizers (Kozai et al., 2016). In addition, thanks to the innovative production approach witnessed in urban agriculture, circularity in production and consumption, which is an important element of the transition to a green economy, can also be achieved. One of the concepts particularly emphasized in this study is "self-sufficiency". This self-sufficiency is an important approach to food security. Increasing localization in food production can provide economic independence and regional development (Calvet-Mir et al., 2012). In addition to ensuring food security, the proposed rooftop farming model can also help reduce the footprint of food production (use of rainwater, reuse of food waste in production processes, up to 95% less water use, no need for fertilizers and pesticides used in traditional agriculture, etc.), community education and participation, and strengthen ties between communities (Yusoff et al., 2017). Real examples of these potential contributions of rooftop farming were also included in the study. The introduction to the study also includes Gupta & Mehta's (2017) view that rooftop farms can help achieve six key sustainable development goals (SDGs). These are zero hunger, health and quality of life, industry, innovation, and infrastructure, sustainable cities and communities, climate action, and life on land. However, following a literature review and case studies, it is recognized that rooftop farming is also an opportunity to help achieve the other four SDGs. These are:

In summary, the systematic spread of rooftop farming as one of the urban agriculture practices in cities can be used as a strategic tool for the realization of many critical situations such as strengthening the local economy, reducing inequalities, internalizing responsible production and consumption by society, increasing the quality of urban life, and ensuring food security. Especially in the first quarter of the 21st century, when research and discussions are intensifying for the transition to a green economy, rooftop farming can make a difference as a multidimensional practice based on circularity instead of a linear economy.

The role of central and local governments in the process (policy development, planning, promotion, providing financial incentives, organizing trainings, etc.) is also critical for success. It is believed that rooftop farming will provide significant improvements in terms of sustainability with a systematic approach in which geographical information systems are actively used, location-dependent food types are identified and local distribution is planned with knowledge-based modern technologies.

In this context, new academic discussions and research are a must. With the increase in the number of empirical

studies centered on rooftop agriculture in the literature, it is clear that this strategic approach will find more application areas. Academic research should first focus on calculating the potential of rooftop agriculture for different geographical locations and then analyze techniques to increase the productivity of rooftop agriculture and improve the distribution channels of urban agriculture.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The author declare that for this research article, he has no actual, potential, or perceived conflict of interest.

Author contribution

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

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RESEARCH ARTICLE

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Chemo-enzymatic synthesis of chiral precursor molecules with chiral ring hydroxyenone and acetoxyenone structures

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Abstract

A biocatalytic transformation has the potential to perform organic reactions that are quite challenging to achieve with synthetic organic chemistry. They also catalyze these reactions with a chemo and enantio selective manner. The discovery and development of new chemoenzymatic methods for the synthesis of these chiral structures is essential to the production of a wide range of bioactive compounds. In this study, two important pharmaceutical precursors were synthesized chemoenzymatically and subjected to biocatalytic conversions with different dehydrogenases. One of these compound is an α-acetoxy enone structure 4-methoxy-2-oxacyclohex-3-enyl acetate and the other is an α-hydroxy ketone 6-hydroxy-3-methoxycyclohex-2-enone. To obtain these pharmaceutical precursors, 3-methoxy-cyclohex-2-enone was prepared using 1,3-diketone as a starting material. After obtaining this material, α-acetoxy enone was synthesized by chemical acetylation and α-hydroxy ketone prepared by enzymatic deacetylation. The structure of these products was elucidated by NMR analysis. In addition, biocatalytic reduction reactions involving the enzymes galactitol dehydrogenase (GatDH), shikimate dehydrogenase (SDH) and diaphorase were carried out with these products.

Keywords: Acetoxy enone, Biotransformation, Dehydrogenase, Lipase, α-hydroxyketon

INTRODUCTION

Drug discovery and development are among the most important translational science activities that contribute to human health and well-being. However, the development of a new drug is a complex, expensive and lengthy process (Çelik *et. al.* 2021). The sequence of biologically active molecules alters their physiological properties, which highlights the importance of preparing stereoisomers of the precursor molecule (Zhuozhuo, *et. al.* 2022). Since enzymes and biological catalysts have asymmetric structures, they can perform selective and targeted biological functions in nature. Moreover, special attention is paid to the currently available methods for their asymmetric synthesis (Ari, 2022). They are used in the synthesis of biologically active molecules, such as pharmaceutical molecules and agricultural control agents, where optical purity (% ee) is the most important criterion for such structures (Csuk, *et. al.* 1990). The human body is also enantioselective and consequently enantiopure drugs are essential for the treatment of disease. Therefore approximately 50 % of drugs produced in pharmaceutical industry are chiral (Mane, 2016).

One of the most demanded chiral structures in pharmaceutical industry is chiral alcohols. They are also found in the structure of many bioactive compounds.

α-hydoxy ketones are the versatile chemical structure that contain a functional group at the α carbon attached to a chiral carbon atom. Asymmetric catalysis has emerged as a general and powerful approach to prepare chiral compounds. Developing new chiral ligands and catalysts that can effectively induce asymmetry in reactions is crucial in modern chemical synthesis (Huang, 2022). Since the carbonyl group can be easily converted into other functional structures such as diols, halo or amino derivatives, these structures are important bioactive building blocks (Kataoka, *et. al.* 2003). Reduction products of alpha hydroxy ketones are diol structures (Faber, 1996). Diols are likewise as important structures as α hydroxy ketones considering the chiral information they carry and the functionality of the alcohol groups. Vicinal diols are used in the synthesis of aldehydes and ketones (Noyori, *et. al.* 2006). They are also known to be used in the synthesis of 1,2-diketones and the formation of α-ketoalcohols (Demir, *et. al.* 2007). Biocatalytic methods can be used effectively in the synthesis of the chiral structures such as enzymatic oxidation and reduction, deacetylation and kinetic decomposition reactions (Heiba, *et. al.* 1974).

In this study, the reactions of two chiral α-hydroxy ketone formed by biocatalytic deacetylation of α-acetoxy enone and α-acetoxyenone structures to be used as substrate in biocatalytic reduction with dehydrogenase enzymes. The first structure, 4-methoxy-2-oxacyclohex-3-enyl acetate, is a 2-ring polyoxo ketone, and this type of substances are important structural units of many bioactive molecules as shown in Figure 1 (Noyori, 2006). Among efficient asymmetric synthesis methods, metal-acting radical reactions using $Mn(OAc)_3$ are one of the most widely used approach (Faber, 1996). Reactions using $Mn(OAc)_3$ have been used in the development of many chemo- and stereoselective synthesis methods (Demir, *et. al.* 2007). The use of *in situ* preparations of $Mn(OAc)_3$ from KMnO4/$Mn(OAc)_2$ is also available in the literature (Heiba, *et. al.* 1974).

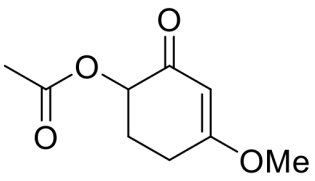

Figure 1. Structure of 4-methoxy-2-oxacyclohex-3-enyl acetate.

6-Hydroxy-3-methoxycycyclohex-2-enone structure can be obtained by biocatalytic deacetylation of the acetoxy enone molecule with lipase or esterase enzymes. Among enzymatic methods, lipase enzymes are particularly used to obtain optically pure compounds by kinetic dissociation reactions. In this study, the experimental design was to obtain optically pure product by subjecting the racemic starting material to kinetic dissociation. By taking the advantage of enantioselective deacetylation property, the enzyme lipase is planned to employ for this kinetic resolution reaction. Due to the nature of kinetic resolution reactions, the product formed with 50% yield at the best resolution level and the untransformed starting material can be obtained with high optical purity as well.

The other group of biocatalysts used in this study are dehydrogenase enzymes. Galactitol dehydrogenase (EC 1.1.1.16) and shikimate dehydrogenase (EC 1.1.1.24) and diaphorase are the enzymes that we selected to carry out for the biocatalytic reduction reactions . As a result of these catalytic reductions, it was our aim to obtain a cyclic chiral alcohol or diol structures. Galactitol dehydrogenase (galactitol:NAD^+oxidoreductase; GatDH) was first isolated from the galactitol-utilizing mutant bacterium *Rhodobacter sphaeroides* (Carius *et.al*. 2010). This homotetrameric enzyme reduces galactitol and catalyses its conversion to L-tagatose (Demir, *et. al.* 2004). This enzyme has also been used in biotransformation studies for the reduction reactions of molecules such as hydroxyacetone and hydroxyketone. The other enzyme used in this study for bioreduction conversion shikimate dehydrogenase (SDH) was isolated from *Corynebacterium glutamicum* (Schoepe, *et.al.,* 2006). *Corynebacterium glutamicum* is a microorganism often used in industry for bioconversion of aromatic structures. It also appears to provide an attractive route for sustainable indole production from tryptophan in *Corynebacterium glutamicum* in the bioconversion production process (Mindt M. *et. al*. 2021). As for diaphorase a commercially available enzyme that is frequently reported in biotransformation studies, especially in the reduction of double bond structures (Fronza *et.al* 1996)

Here we aim to identify new enzymes that can be used in the synthesis of chiral molecules with pharmaceutical value. To this end, it is essential to screen enzyme candidates applicable to chemoenzymatic methods. The starting materials we have chosen here to create chiral centres by reduction reactions are 4-Methoxy-2-oxacyclohex-3-enylacetate and the esterase reaction product 6-Hydroxy-3-methoxycycyclohex-2-enone. While the enzymes we selected have the catalytic properties to give the candidate reactions, the range of non-specific substrates is unknown. An important factor in the selection of these enzymes is that they have been isolated, characterized and produced in quantities that can be used in chemoenzymatic studies. As a result of the optimization studies performed with these enzymes, 4-Methoxy-2-oxacyclohex-3-enylacetate, 6-Hydroxy-3-methoxycycyclohex-2-enone did not form any reduction product as a result of the analysis of the products isolated from the enzymatic reaction medium. The deacetylation product obtained by metal-activated radical reactions using $Mn(OAc)_3$ and lipase enzyme reactions was previously developed but was successfully applied again

with some modifications. This was followed by screening of the products with some of the new and previously unapplied dehydrogenase enzymes in the literature that may enable the synthesis of more advanced chiral structures.

MATERIALS AND METHODS

Synthesis of Substrates Used in Enzymatic Reduction Reactions

Two substrates were prepared for the bio-reduction reactions catalysed by galactitol dehydrogenase, shikimate dehydrogenase and diaphorase. First one is acetoxyenone (4-methoxy-2-oxocyclohex-3-enlylacetate) and deacetylation product of this structure alphahydroxyketone (6-hydroxy-3-methoxycycyclohex-2-enone). First, 3-methoxycyclohex-2-enone was synthesized from cyclohexane-1,3-dione by methylation method. This substance was then acetylated with manganese-III acetate. The resulting 4-methoxy-2-oxocyclohex-3-enlyacetate was converted to 6-hydroxy-3-methoxycycyclohex-2-enone by lipase and esterase enzymes. This material was used as starting material for enzymatic reduction reactions.

Synthesis of 3-Methoxycyclohexan-2-enone

4.500 g of 1,3-cyclohexanedione was taken into a 100 ml flask and 1 mL of CH_3COOH was added and stirred for a while in the heating mantle to dissolve. Then 50 ml of methanol was added to this medium and the back cooling process was started. The formation of the product was monitored by TLC (hexane: ethyl acetate 1:1). After the reaction was completed (approximately 12 hours), the product was extracted twice with ethyl acetate at a volume ratio of 1:1. The pH was adjusted to 7-8 with saturated $NaHCO_3$ solution. A silica-filled column was used for purification of the product by column chromatography. The column was conditioned at (2:1) Hexane:EtOAc and the product was fractionated at (1:1) Hexane: EtOAc. The structure of the product was determined by ^{1}H-NMR analysis.

Synthesis of 4-Methoxy-2-Oxocyclohex-3-Enyl Acetate

Synthesis of 4-Methoxy-2-Oxocyclohex-3-Enyl Acetate was carried out following the method described in Demir et al. (2004) with some modifications. As organic solvent benzene was used for this reaction. For this first anhydrous benzene was prepared. Na was added to the benzene in round bottom flask and the reaction was carried out at 70 °C in argon gas environment. Evaporated water is condensed and collected in a separate flask and this process was repeated several times.

For the reaction, potassium permanganate ($KMnO_4$) was dissolved in 10 mL of acetic acid (CH_3COOH) and 90 mL of anhydrous benzene was added. Reaction was performed in a round bottom flask and placed in a heating mantle. After reflux started the mixture was filtered and the residual $KMnO_4$ was removed from the medium. The medium turned to a purple colour due to the formation of manganese(III)acetate. Then the flask was placed into the heating mantle again and total amount of 1260 mg of enone was added slowly at a molar ratio of 1:4 Enone: $KMnO_4$. Since the separation of undissolved $KMnO_4$ from the medium by filtration was laborious, $Mn(OAc)_3.2H_2O$ was also used instead of $KMnO_4$. For this, $Mn(OAc)_3.2H_2O$ was kept in an oven for 12-24 hours to obtain anhydrous $Mn(OAc)_3$. For the acetylation step 3 g manganese (III) acetate ($Mn(OAc)_3$) in 10 ml acetic acid (CH_3COOH) in a 250 ml flask is used and 90 ml benzene was added to start the reaction. Reaction performed under reflux with Dean-Stark trap and 45 minute later 1000 mg enone was added slowly and the reaction was continued for 2-3 days. TLC controls were performed with 1:1 Hexane: Ethyl acetate system. For extraction, benzene was evaporated, and the product was dissolved in ether and extracted with ethyl acetate. The extraction mixture was neutralized with saturated aqueous $NaHCO_3$ solution. The column was conditioned in 1:1 EtOAc-Hexane and the product collected for separation. Structure analysis of the product was performed by^1 H-NMR.

Enzymatic Hydrolysis of 4-Methoxy-2-Oxocyclohex-3-Enyl Acetate

The starting material 4-methoxy-2-oxacyclohex-3-enylacetate (20 µl) which was prepared in previous steps was dissolved in 200 µl DMSO and added to 10 ml 50 mM phosphate buffer (pH: 7) containing the enzyme Porcine Liver esterase (30 mg) or Lipase (40 mg). The reaction was incubated at 30°C with 100 rpm rotation for 50 hours for racemic product and 22 hours for optically enriched product formation. The reaction progress was monitored by TLC in 1:1 EtOAc:Hexane mobile phase. Product analysis was carried out by HPLC (Chiralpak AD, 90:10 Hexane:Isopropanol, 0.8 ml/min) for determination of the enantiomeric excess (ee) and by H^1 – NMR for structural analysis.

Producing Galactitol Dehydrogenase and Shikimate Dehydrogenase from Recombinant E. coli Cells

Recombinant cells (*E. coli BL21* were grown in sterile LB (Luria Broth) agar containing 100 mg/ml ampicillin and then transferred to 10 mL LB liquid media in 50 mL falcon tube. Ampicillin was added at a concentration of 100 µg/mL. After an overnight incubation (150 rpm 37 °C) cells were transferred to 250 mL volume of the same medium and incubated at the same conditions for approximately 3-4 hours until OD value reaches 0.6-0.7 at 600 nm. At the end of this period, IPTG (Isopropyl-D-thiogalactopyranoside) was added to the culture medium with a final concentration of 1 mM for transcription initiation. IPTG was sterilized before the procedure by passing through a 0.2 µm filter (Millipore®).

After 5-8 hours incubation, cells were separated from the medium by centrifugation (5000 rpm 4 °C, 10 min) and then supernatant was discarded. Cells were resuspended in phosphate buffer (50 mM, pH:7.5) containing 10 mM NaCl, 10 mM imidazole, 10 mL 2 mg lysozyme for extraction. Crude extract was prepared by sonication (2 min, 2 sec intervals) and centrifugation (20000 rpm, 10 min at 4° C). For long-term storage, it was lyophilized for 1-2 days or stored in 20% glycerol at -20° C.

When we stored lyophilized cells instead crude extract, cells were suspended in 50 mM phosphate buffer (pH: 6.5), lysozyme was added and incubated in an ice bath for 5 min. The cells were lysed with the help of a sonicator for a total of 2 min, 2 sec intervals and then the mixture was centrifuged at 9000 rpm for 10 min. The crude extract was used directly in enzymatic reactions or kept in 20% glycerol at -20 °C for long term storage.

Bradford Protein Detection Method

Standard Preparation: Protein was determined according to the Bradford method. 500 µl Bovine Serum Albumin (Sigma) from 1mg/ml stock solution was diluted to 0.5 mg/ml with 500 µl of water. 500 µl of the 0.5% solution was taken and diluted to 0.25 mg/ml with 500 µl of water. A 0.125 solution was also obtained by the same procedure. A blind sample, i.e. one without protein, was also prepared. 50 µl of each of these solutions was added to spectrophotometer cuvettes containing 1.5 ml of Bradford reagent.

Sample Measurement: The samples to be measured for protein concentration were diluted 1:100 and added to 1.5 ml of Bradford reagent and their optical absorbance at 595 nm was measured and protein values were determined.

Biocatalytic Reactions of 4-Methoxy-2-Oxocyclohex-3-Enylacetate with Dehydrogenase Enzymes

Recombinant enzymes (GatDH and ShDH) prepared as described above were added to 50 mM phosphate buffer. For reactions with GatDH, 1 mM $MgCl_2$ was added to the reaction medium. To this mixture 6 mg of NADH in 300 µL DMSO was added. The reaction mixture was left at 35 °C with a rotation speed 80 rpm for 2 days. Reactions with diaphorase enzyme were carried out with 4 mg of lyophilized enzyme (Sigma, 6-8 U/mg). After 24 h, same amount of enzyme was added to the reaction mixture. Product formations were monitored by TLC (1:1 EtOAc: Hexane). The reaction medium was extracted twice with ethyl acetate, once with concentrated NaCl solution and dried over magnesium sulfate (MgSO4). Product was separated on a silica column (Hexane: Ethyl acetate 1:1). Control experiments were carried out with enzyme-free and substrate-free reaction mixtures.

Biocatalytic Reactions of 6-Hydroxy-3-Methoxycyclohex-2-Enon with Dehydrogenase Enzymes

6-Hydroxy-3-methoxycyclohex-2-enon (10 mg) was dissolved in 300 µL DMSO and added to 50 mM pH 6-8 phosphate buffer containing 6 mg NADH, the enzyme used for this conversion were GatDH (28 U) and SDH (100 mg total protein of crude enzme). It was left at 35°C with a rotation speed 80 rpm for 2 days. Reactions with diaphorase enzyme were carried out with 4 mg of lyophilized enzyme (Sigma, 6-8 U/mg). Reactions with enzyme-free and substrate-free mixtures were also performed as control experiments. Product formation was monitored by TLC (EtOAc: hexane 1:1). The reaction medium was extracted twice with ethyl acetate, once with concentrated NaCl solution, dried with magnesium sulphate and cleaned on a silica column (1:1 Ethyl acetate: Hexane). The resulting product was analysed by ^{1}H-NMR.

RESULTS AND DISCUSSION

Synthesis of 4-Methoxy-2-Oxacyclohex-3-Enyl Acetate

We aimed to synthesize a cyclic acetoxy enone structure (4-methoxy-2-oxocyclohex-3-enlylacetate) to use in enzymatic reduction reactions. The first step to obtain this substance was to synthesize 3-methoxy-cyclohex-2-enone from cyclohexane-1,3-dione to prepare the starting material for the acetylation reaction.

The synthesis of enone structure from cyclohexane-1,3-dione was carried out in acidic medium (1:50 mixture of acetic acid and methanol). The product was monitored by TLC and the RF value of the product was determined as 0.802 (EtOAc:Hexane 2:1) and product formation was observed within the first 6 hours. Starting material was completely consumed after 16 hours. According to the proton MNR analysis, the methoxy group in the molecule appeared at 3.63 ppm, the olephinic proton at 5.29 ppm and the other -CH_2 peaks were 2.36-2.33, 2.29-2.25, 2.29-2.25. (^{1} H NMR ($CDCl_3$, 400 MHz):δ 5.29 (s, 2H), 3.63 (s, 3H), 2.36-2.33 (m, 2H), 2.29-2.25 (m, 2H), 1.95-1.88 (m,2H)). Based on this, the results showed that the substance obtained is the structure of the expected enone (Figure 4.2).

4-methoxy-2-oxacyclohex-3-enyl acetate was synthesized as a starting material for two different enzymatic reactions (enzymatic reduction and deacetylation reactions). α, β α' acetoxylation products of unsaturated ketones are important chiral ligands. Many methods have been reported related to synthesis of these compounds. The method had been used in this work is found to be advantageous because there is no arylated by-product is formed. The reaction mechanism as it is shown in the figure 2 proceeds radically.

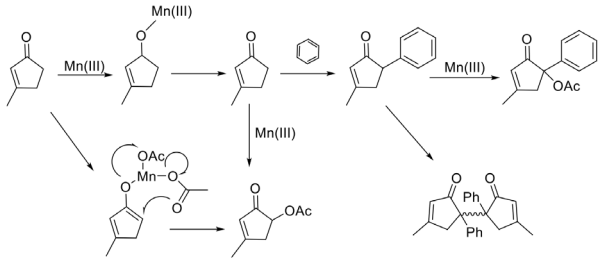

Figure 2. Mechanism of formation of α' acetoxylation products of α, β, unsaturated ketones

3-Methoxy-cyclohex-2-enone acetylated at the 3 enyl position by adding 3 equivalents of manganese acetate in acetic acid: benzene medium (Figure 3). The oxidation product was obtained with 40-60% conversion rate in 48-54 hours. Since the reaction is not proceeded enantioselective the product obtained as racemic mixture (Demir, 1991).

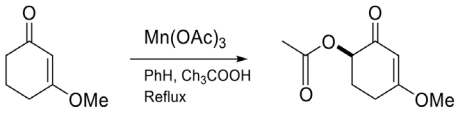

Figure 3. Synthesis of 4-methoxy-2-oxacyclohex-3-enyl acetate

After product formation observed by TLC (Rf: 0.320; 2:1 EtOAc: Hexane) at the end of 20 hrs, anhydrous manganese acetate was added to the reaction medium. To our observation using anhydrous manganese acetate was an important factor affecting the efficiency of the reaction. The reaction was terminated after 54 hours when there is no change was observed. After isolation and purification of the product, it was analysed by NMR (Figure 4). Structure of the product was easily determined by the proton NMR spectrum where the methyl peak of the acetyl group was observed at 2.18 ppm and the alpha proton was observed as dd at about 5.30 ppm. (^{1}H NMR ($CDCl_3$, 400 MHz): δ 5.41 (d, *J=1*.5 Hz, 1H), 5.30 (dd, , *J=5*.3 and 12.6 Hz, 1H), 3.71 (s, 3H), 2.67 (dddd, *J=1*.6, 5.2, 12.0 and 17.4Hz, 1H), 2.52 (ddd, *J=2*.9, 5.3 and 17.8 Hz, 1H), 2.27-2.20 (m,1H), 3.71 (s, 3H), 2.15-2.06 (m, 1H)).

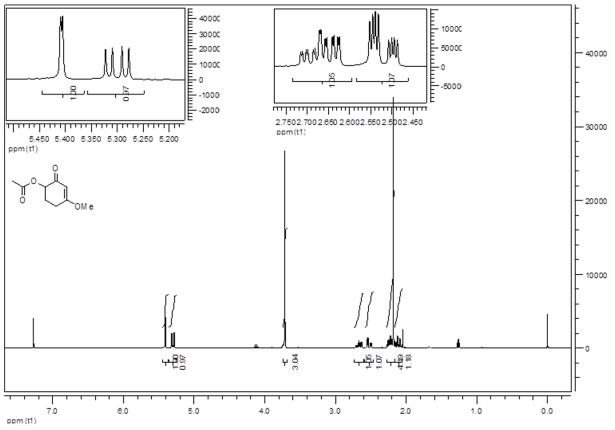

Figure 4. NMR spectrum of 4-methoxy-2-oxacyclohex-3-enyl acetate

Asymmetric Hydrolysis of 4-Methoxy-2-Oxacyclohex-3-Enyl Acetate Catalysed by the Enzymes Lyase and Esterase: Synthesis of 6-Hydroxy-3-Methoxycyclohex-2-Enone

The alpha hydroxy enone structure was formed by deacetylation of the acetoxy enone structure. Enzymes Porcine Liver Esterase (EC. 3.1.1.1.1) (PLE) and Amanolipase (EC. 3.1.1.3.3) were chosen because these enzymes carry out acetyl group transfers (e.g. in amino acid synthesis) in the cell and these reactions are enantioselective reactions (Figure 5). Due to these properties, they are used in biotransformation studies in acetyl group transfers, especially in deacetylation reactions (Tanyeli, 2002). In this way, enantiospecific synthesis of some valuable alcohols of pharmaceutical importance is carried out (Zelinski, 1994).

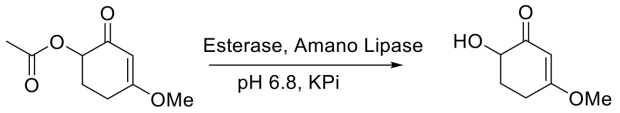

Figure 5. Synthesis of 6-hydroxy-3-methoxycyclohex-2-enone.

The GC-MS analysis of the product we synthesized shows that the molecular weight of product is 142.1 and this can be seen in the mass analysis, in addition, the mass of the molecule formed because of a proton break (m/z=141.1) can also be observed. In the mass analysis of alcohol-containing compounds, the observation of the M-1 peak accompanied by the molecular ion (M^+) peak and the presence of water exit (M^{-18}) peaks are quite distinctive. The M^{-18} peak of the compound was observed at 124.1. The alpha hydroxyketone, which is expected to be formed because of deacetylation by esterase reaction, was analysed by proton NMR as well (Figure 6). The methyl group of the acetyl group of this substance is not observed in the spectrum and the alpha proton peaked at a lower ppm (4.06 ppm) as expected. While the peak should be split into three, it was split into two because the substance is chiral. Apart from these, the proton of the hydroxy group is seen as a doublet at 3.83 ppm, which proves that the substance is the desired product hydroxy enone structure. H NMR ($CDCl_3$, 400 MHz): δ 5.42 (d, *J=1*.6 Hz, 1H), 4.06 (ddd, *J=1*.2, 5.5 and 13.1 Hz, 1H), 3.83 (d, *J=1*.2 Hz, 1H), 3.73 (s, 3H), 2.63 (dddd, *J=1*.7, 5.0, 12.6 and 17.7 Hz, 1H), 2.49 (ddd, *J=2*.2, 5.2 and 17.9 Hz, 1H), 2.37 (dtd, *J=2*.3, 5.2 and 12.7 Hz, 1H), 1.84 (dq, *J=5*.3 and 12.7 Hz, 1H).

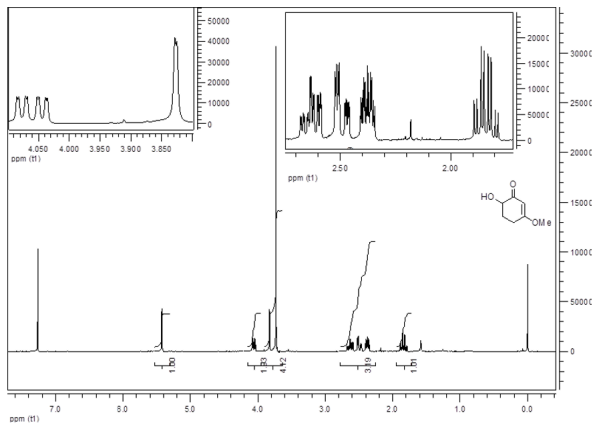

Figure 6. NMR spectrum of 6-hydroxy-3-methoxycyclohex-2-enon

Production of Galactitol Dehydrogenase and Shikimate Dehydrogenase Enzymes and Determination of Enzyme Activity

During an enzyme production, it is necessary to know the time when the enzyme is expressed at the highest level and therefore the time point for enzyme extraction. In this study, *E. coli* strains BL21 and DH5α carrying genes encoding galactitol dehydrogenase and shikimate dehydrogenase enzymes (D GatDH pET and CglSDH-L correspondently) were used for enzyme production. After induction with IPTG, production was terminated after 5-8 hours, and cells were collected by centrifugation. After obtaining the crude extract, enzyme-catalysed reactions were carried out with this crude extract. The enzyme activity in the crude extract was measured via the reduction of GatDH 1,2-hexanedione and the specific activity in the crude extract was calculated as 0.8-1 U/mg. For the enzyme shikimate dehydrogenase, the activity could not be calculated since the natural substrate of the enzyme could not be obtained. However, experiments were carried out with a crude extract mixture with a determined total protein amount (100 mg) to able to corelate the amount of the biocatalyst.

Biotransformation of 4-Methoxy-2-Oxacyclohex-3-Enyl Acetate 2 And 6-Hydroxy-3- Methoxycyclohex-2-Enone with 3 Dehydrogenase Enzymes

Galactitol dehydrogenase, shikimate dehydrogenase and diaphorase are the enzymes selected for biocatalytic conversion of the acetoxy enone 4-methoxy-2-oxacyclohex-3-enyl acetate and the cyclic alpha hydroxy enone 6-hydroxy-3-methoxycyclohex-2-enone. Bioconversion reactions were carried out under optimum reaction conditions for each enzyme according to the reduction reaction in which the co-enzyme (NADH) is oxidized. For dehydrogenase enzymes, the reduction reaction takes place mostly at relatively acidic pH levels compared to oxidation reactions. For this reason, enzymatic reduction reactions were tested at different pH levels in the range of pH 6-8. The reactions were carried out completely in buffer medium, i.e. water. In our study, DMSO (1%) was added to the reaction medium due to the poor solubility of the substrates we synthesized for the reduction reactions in water.

First, acetoxyenone structure was used for reduction transformations (Figure 7). All three enzymes mentioned above were used to test the biocatalytic reduction of this structure. TLC checks at regular intervals from the first 30 minutes of the reactions and a product was observed in all enzymatic transformations during the reactions. However subsequent NMR analysis showed that this compound was a α-hydroxy ketone structure formed by the hydrolysis of the acetyl group. It is thought that this hydrolysis product is not formed because of an enzymatic conversion under the specified conditions (pH: 6-8, 25-30 °C). However, these hydrolysis products, which occurred at different rates and conversion yields for each enzyme, were analyzed by HPLC to determine the enantiomeric excess. The results showed that the products had ee values in the range of 15-22% in experiments with GatDH and SDH. Experiments were also carried out with a control group in buffer medium only and in buffer medium containing NADH. In these control experiments, the deacetylation product was also formed, it was observed that it occurred much slower and in lower yields than the enzymatic reactions. This result was difficult to interpret in terms of biocatalytic conversions. It is well known phenomena that the enzymes are promiscuous for their natural substrate and sometimes also for their natural reaction type. But the evidences here are not clear enough to conclude that this reaction occurred due to this type of a promiscuous mode of action of the correspondent enzymes. Therefore, we need to have more evidence of this kind of deacetylation reactions in aqueous media at different pH to be certain about if this this conversion observed as a result of an enzymatic reaction.

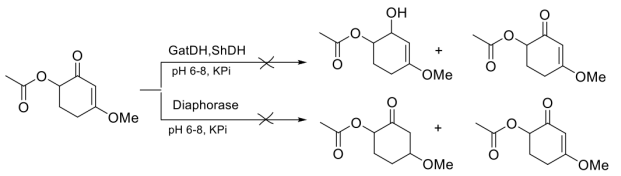

Figure 7. Expected reactions of 4-methoxy-2-oxacyclohex-3-enyl acetate with GatDH, SDH and diaphorase enzymes.

Another starting material 6-hydroxy-3-methoxycyclohex-2-enone was synthesized for bio-reduction reactions. Bioconversion conditions that have been used in previous bio-reduction reactions were again relatively alkaline and aqueous at 25-30 °C. However, no product formation was observed under any of the conditions (Figure 8).

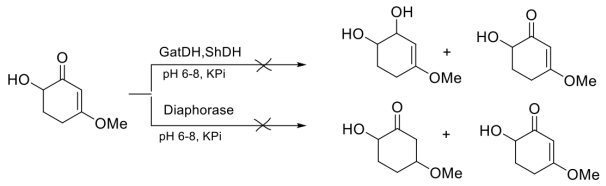

Figure 8. Expected reactions of 6-Hydroxy-3-methoxycyclohex-2-enone with GatDH, SDH and diaphorase enzymes.

For our biodegradation experiments, the enzyme diaphorase was also used since to examine the bio-reduction of double bond of the enone structure. However, according to the NMR and GC- MS analyses no such product was observed as a result of the enzymatic bioconversions with this enzyme for both of the substrates 4-Methoxy-2-oxacyclohex-3-enyl acetate and 6-hydroxy-3-methoxycyclohex-2-enone.

CONCLUSION

This study covers the optimisation of chemoenzymtic synthesis of pharmaceutically important chiral cyclic enol structures. The first step was the synthesis of starting material 3-methoxy-cyclohex-2-enone starting from the cyclic diketone. This was followed by the synthesis of 4-methoxy-2-oxacyclohex-3-enyl acetate by alpha acetylation reaction and the enzymatic synthesis. The formation of the products of these reactions was confirmed by structure determination using proton NMR analysis. The synthesized product was used for biocatalytic digestion of dehydrogenase reactions as well as acetylation with lipase and esterase enzymes to obtain a second substrate for dehydrogenase conversions. The formation of this substrate was also confirmed by both proton NMR and GC-MS analysis and the enantiomeric excess of this chiral structure was calculated by HPLC. It was found necessary to use the racemic starting materials for the enzymatic reactions which was planned to be carried out with GatDH, SDH and diaphorase enzymes. Depending on success of these trials then we could proceed with the optically pure substrates. For this reason, the reaction was first performed until all the product was completed which results in racemic mixture of deacetylation product. For the subsequent experiments, reactions terminated at the end of the 20-24 hour therefore we could obtain 50% pure chiral deacetylation product due to kinetic resolution of the starting material. Following the synthesis of starting materials (4-Methoxy-2-oxacyclohex-3-enyl acetate and 6-hydroxy-3-methoxycyclohex-2-enone) GatDH, SDH enzymes were produced using recombinant *E. coli* cells carrying plasmids containing the genes encoding the enzymes for use in biocatalytic reduction reactions and prepared as crude extracts for biotransformation reactions.

Biocatalytic reduction reactions of substrate in acetoxy enone structure in aqueous medium with DMSO as co-solvent did not produce the expected reduction products. NMR analysis of the product showed that the reduction product of the carbonyl group was not formed but the acetyl group was cleaved. It would be stated as this phenomenon was occurred due to the activity of the other enzymes available in the medium for the bio-reduction reaction performed with crude enzyme SDH. But the same observation was obtained with the pure dehydrogenase GatDH which was used for this reaction. The reason for this may be that the acetyl group is not formed because of an enzymatic reaction but because of spontaneous deacetylation in aqueous medium. HPLC analysis of the substance showed that product has formed with a low enantiomeric excess (ee: 16%) and this also suggests that both of the hypotheses may suggest a explanation.

The fact that the expected product was not formed should not be attributed to the deacetylation of the substrate, but to the fact that the enzyme did not convert this substance into a product under the specified conditions. Because the alpha hydroxy ketone formed as a result of deacetylation was also not converted into any reduction product Moreover deacetylation reaction took place quite slow (up to 16 hours), therefore it is not expected to be competitive with the reduction reaction.

As it is stated before each substrate was also reacted with another enzyme diaphorase for a possible reduction reaction in double bond structure. Deacetylation product that has been formed with GatDH and SDH was not observed with this enzyme under the specified conditions and within the specified time. In biotransformation studies, dehydrogenase reactions are carried out in buffer solution under acidic or basic pH conditions, depending on the direction of the reaction. Therefore, this type of biotransformation method was used in our study. The reactions took place at temperatures where the enzymes showed optimum activity and the results obtained at different pH values did not differ and the expected reduction product was not obtained. Under these conditions, it can be concluded that the dehydrogenase enzymes we used do not prefer the substances 4-methoxy-2-oxacyclohex-3-enyl acetate and 6-Hydroxy-3-methoxycyclohex-2-enone as substrates for bio reduction reactions. These pharmaceutically important compounds can be tested with a different dehydrogenases for optimisation of enantioselective reduction reactions.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

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

Author contribution

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

that they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Investigation of generative high temperature tolerances of some cotton (*Gossypium hirsutum* L.) varieties

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Abstract

The potential of cotton genotypes to form buds, flowers and bolls is not sufficient to achieve cotton seed yield targets. Despite global warming buds, flowers and bolls that mature in cotton plants must be successfully transformed into products. However, this is related to the generative tolerance of the genotype to high temperature. In study aims to scan the negative effects of high temperature stress on the generative development on cotton varieties registered in Turkey in the last 10 years. The experiment was established in the GAP International Agricultural Research and Training Center trial field in 2020, with 4 blocks according to the Augmented design. Six standards (Tamcot Spnhix, SJU86, AGC208, ST468, ST474, Carmen) and 88 cotton varieties registered in Turkey National Variety List were used as trial material. In this study, high temperature pollen vitality stress index (HTPVSI) and high temperature shedding stress index (HTSSI) properties were investigated. According to the results of the experiments we conducted, it was determined that the HTPVSI values ranged between 0.17-1.26, the HTPVSI averages of the standards were 1.17, and the HTPVSI averages of the genotypes were 0.99. It has been determined that HTSSI values vary between 0.30-1.71. It was determined that the mean HTSSI values of the standards were 0.89 and the genotypes were 1.00. It was determined that there was a wide variation among the genotypes screened for generatively high temperature stress. Using HTSSI and HTPVSI features is recommended as a selection criterion since it is an important trait for screening genotypes in terms of tolerance or sensitivity to generative high temperature stress in cotton plants. In our study, the results were not similar to each other in terms of HTPVSI and HTSSI traits, due to the low share of flower shedding after applying HTSP (High Temperature Shock Practice: 96 hours of uninterrupted exposure to high temperature during generative periods) in the shedding rate. When the examined HTSSI and HTPVSI traits were examined together, no cotton genotypes were found to be generatively tolerant. In terms of sensitivity of genotypes to high temperature, 18 cotton genotypes were found in the medium tolerant group and 25 cotton genotypes were found in the sensitive group.

Keywords: Cotton, Generatively High Temperature, Pollen Vitality, Shedding

INTRODUCTION

Cotton plays an important economic role in the global economy due to its widespread use in the textile industry and providing job opportunities in the countries where it is grown (Khan, 2013; Yaşar, 2023). Cotton (*Gossypium hirsutum* L.), an important species of the mallow family and cultivated in nearly a hundred countries with temperate and tropical climates, is one of the indispensable raw materials of the industrial industry. In the 2021 and 2022 cotton production

seasons, the world's four largest cotton-producing countries are India (5.9 million tons), China (5.7 million tons), the USA (4 million tons), and Brazil 2.7 million tons, respectively. In Turkey, seed cotton production increased by 26.9% in 2021 and amounted to 2.25 million tons (Anonymous, 2022). Considering the average data of the last 10 years in Turkey, the cotton cultivation area is 462 thousand hectares, the amount of fiber produced is 835 thousand tons, and the fiber yield is 19.3 kg ha^{-1}. (TUIK, 2022). In Turkey, cotton is grown intensively, especially in the Southeastern Anatolia Region, Aegean Region, Adana, and Antalya regions with the determining effect of climate factors. Approximately 59.31% of the cotton produced in our country is produced in the Southeastern Anatolia Region (Aytaç et al., 2020). However, due to the fact that the climate conditions of the Southeastern Anatolia Region are dry and hot in summer, high temperature has a negative and significant effect on the vegetative and generative periods of cotton. Cotton is frequently exposed to many biotic and abiotic stresses during its growth stages (Li et al., 2019; Yaşar, 2022). According to the International Intergovernmental Panel on Climate Change report, air temperatures are expected to increase by 0.2 °C every 10 years, with global warming being the key factor of high temperature stress. And from 2020 to 2080, the world temperature is predicted to increase by 0.5–5.44 °C (IPCC, 2007; IPCC 2018). Temperature trends display that the global average temperature may increase by 1–4 °C by the end of the 21st century (Driedonks et al., 2016). Although the temperature requirement of the cotton plant varies according to the growth stage, in conditions where it does not fall below 15°C, leaf, bud, flower, and boll development takes place and it tends to grow continuously, and temperatures of 25–32°C are sufficient for optimum growth (Reddy et al., 1997; Burke and Wanjura, 2010; Yaşar et al., 2019). If the temperature rises above 36°C, a significant decrease in fruit set is observed (Luo, 2011; Nasim et al. 2016; Singh et al., 2007). The optimum temperature values for the first development stages of cotton (main stem elongation, leaf area development, and biomass production) are 30/22°C day/night. Heat stress can be defined as the emergence of morphological, physiological, and biochemical changes in the plant that exceed the thermal capacity of the plant above the desired optimum temperature in its life cycle. Accordingly, since registered commercial cultivars with little resistance to high temperature stress have a narrow genetic base with limited genetic gain, these cultivars may increase their susceptibility in a stressful environment (McCarty et al., 2008; Wang et al., 2017; Ma et al., 2018). While the cotton plant produces four times more fruit branches at 30/22°C than at 20/12 °C, it produces fewer monopodial branches (Reddy et al., 1992). Going on of the daily maximum temperature affects the germination of cotton plants in the vegetative period, root and tiller growth, sympodial and monopodial branches, internode distance, photosynthesis, respiration, and ATP formation. In the generative period, it affects biomass, boll number per plant, boll size and weight, cellulose accumulation and fiber yield, fiber quality, fiber length, strength and micronaire value (Wahid et al., 2007; Bibi et al., 2008; Pettigrew, 2008; ITC, 2011; Loka and Oosterhuis, 2016). The high daily maximum temperature negatively affects pollination and fertilization in cotton plants (Kakani et al., 2005); It causes early maturation by bud-flower shedding (Reddy et al., 1995). It has been emphasized that temperature values between 33 °C and 40 °C have increasingly serious effects on pollen vitality and germination (Barrow, 1983). With the effect of the predicted global warming, temperature increases in the generative period depending on the severity and duration of the temperature can cause significant yield losses by causing a decrease in fertilization and pollen vitality, a decrease in the number of bolls, boll weights and hundred seed weights. It is of great importance to determine the negative effects of high temperatures in terms of species and varieties. Many techniques are used to identify high temperature tolerant varieties. In terms of high temperature stress, pollen vitality test and boll shedding are two important features in the generative period. The main aim of this study is to determine the response and tolerance status of some domestic and foreign origin cotton (*Gossypium hirsutum* L.) genotypes in the GAP International Agricultural Research and Training Center inventory, especially originating and registered cotton varieties in Turkey, to high temperatures in the vegetative period. At the same time, determining the parents with special characteristics and including them in the breeding program (Demiray et al., 2019) is to facilitate the researchers in breeding studies and to minimize the environmental effects in selection.

MATERIALS AND METHODS

Material

In this study, 94 cotton (*Gossypium hirsutum* L.) genotypes registered in Turkey National Variety List of domestic and foreign origin, cotton varieties originating from especially Turkey and registered were used as plant material (Table 1).

Experimental Design

The trial field was established in the trial area of the GAP International Agricultural Research and Training Center, in the cotton growing season of 2020, with 4 blocks according to the Augmented Design. ST474, Tamcot Sphinx, SJU86, ST468, AGC208, and Carmen genotypes were included as standard in the experiment. In the experiment, each of the parcels consisting of two rows is 4.0 m long and 1.4 m wide. In the experimental area established under field conditions, the cotton plant was subjected to high temperature shock practice (HTSP) by being placed in a low tunnel for an uninterrupted 96 hours during the peak flowering period. With the

Table 1. Some information about cotton genotypes

Origin of Material	Names of Cotton Varieties
ABD (USA)	Tamcot Sphinx, SJU86, AGC208
BASF Turkish Chem. Inds. and Trade Ltd. Comp.	Fiona, Carla, ST498, ST468, Carmen
Bayer Turkish Chem. Inds. Ltd. Comp.	Claudia, Gloria, Candia, Flora
Birlik Seed. Inds. and Trade. Ltd. Comp.	Bir781, Bir949, Cosmos, Bir138
Caso Seed. Inds. and Trade Ltd. Comp.	Caso 9048
EMTZARI	Furkan
EMARI	TYA193, Ceykot340, TYA366, ADN701, MAY355, MAY455, MAY505, TMK122, TMN18, MAY344, Nihal, ADN413, ADN710, ADN712, ADN123, ADN811, Gelincik, Sarıgelin, Çukurova1518, Bossa159, Teksa415, Yıldırım63, Ayzek595, Gapkot732, Ceykot 92
GAP ARI	ZN 243
GAP IARTC	Kartanesi
Golden West Seed Trade Ltd. Comp.	Optasia, Esperia, Bomba, GW2345, Babylon, Famosa, Fantom, Penta (Golda), Primera
Livagro Agr. Seed. Ltd. Comp.	Zara
May-Agro Seed Inds. and Trade Incorp. Comp.	Gaia, ST474, MAY404
Monsanto Nutr. and Agr. Trade. Ltd. Comp.	DP332, ST478, DP396, DP499, SG125
Özaltın Agr. Bus. Inds. and Trade Incorp. Comp.	Lodos, Özaltın404, Özaltın112
Özbuğday Agr. Bus.and Seed Incorp. Comp.	Lider (Mig119), Diva (Teks)
CRI	SC2009, SC2079, Efe, Ergüven, Harem1, Harem2, ES1, ES2, Sezener76, Özbek105, İpek607, Gürelbey, Aydın110, Şahin2000
Progen Seed Incorp. Comp.	Kaira, Lima, Astoria, Edessa, BA440, Carisma, PG2018, BA525, Flash
Tiriyo Seed. Ltd. Comp.	Zena1010, Zena1040, Zena1018

EMTZARI (East Mediterranean Transitional Zone Agricultural Research Institute), EMARI (East Mediterranean Agricultural Research Institute), GAPARI (GAP Agricultural Research Institute), GAP IARTC (GAP International Agricultural Research and Training Center), CRI (Cotton Research Institute)

help of the thermometer placed in the low tunnel, during the hot hours of the day (13:00-16:00), when the temperature is above 50°C, the low tunnel was opened from the sides to reduce the temperature. Observations were taken before high temperature shock application were recorded as Control. Observations taken at the end of the high temperature shock application period were recorded as Stress. Control and Stress observations were taken separately from 3 of the same plants, which were previously coded and selected in each plot, and the average of the observations was taken.

Pollen Vitality

Flowers blooming on the same day from 3 plants selected randomly from each plot and coded were used as material. In order to determine the pollen vitality levels of pollen belonging to the cotton genotypes in the experiment, 2,3,5, Triphenyl Tetrazolium Chloride (TTC) dye solution was prepared as specified by Norton (1966). Two coverslips were prepared for each genotype and counting was performed with light microscopy in 3 regions on each coverslip. During the count, the pollen stained red was considered as live, the pollen stained pink as semi-live and the pollen not stained at all as non-living. The living, semi-living and non-living pollen counts of the genotypes were determined.

Shedding Ratio (Buds-Flowers-Bolls)

High temperature shock practice (HTSP) before (control) and after (stress) periods were taken separately. Two-row parcels were created for each genotype. Three plants were randomly marked in these parcels. Bud/flower/boll numbers of the marked plants were taken separately as control and stress. Calculated using Formula 1 after counting.

$$\text{Shedding Ratio (\%)} = \left[\frac{\text{HTSP Before (B - F - B) Numbers} - \text{After(B - F - B)Numbers}}{\text{HTSP After (B - F - B) Numbers}}\right] x1 \quad (1)$$

HTSP: High temperature shock practice

B-F-B: Bud/Flower/Boll

Pollen vitality test and shedding rate data obtained after control and stress were analyzed according to Augmented Design. It was calculated over the corrected values obtained after the analysis (Roger, 1985). High temperature stress indices for both properties examined were calculated according to Formula 2 according to the method of Fischer and Maurer (1978) and evaluated by modifying it according to Ekinci et al., (2012).

$$\text{HTSSI and HTPVSI: } \frac{\frac{GN - GS}{GN}}{\frac{AN - AS}{AN}} \qquad (2)$$

HTSSI: High temperature shedding stress index, HTPVSI: High temperature pollen vitality stress index

GS: Value of genotype under stress conditions, GN: Value of genotype under normal conditions

AS: Average of all genotypes under stress conditions, AN: Average of all genotypes under normal conditions

Regarding the evaluation of genotypes after calculating HTSSI & HTPVSI values; If HTSSI & HTPVSI ≤ 0.5 it was evaluated as "Tolerant", If 0.5< HTSSI & HTPVSI ≤1 as "Medium Tolerant" and If HTSSI & HTPVSI >1 as "Sensitive" (Khanna-Chopra and Viswanathan, 1999).

RESULTS AND DISCUSSION

Pollen Vitality

When controlled conditions were examined, pollen vitality percentages varied between 73.01 and 98.86%; While the average pollen vitality of the standards was 91.57%, the average vitality percentage of the genotypes was 89.88%. After high temperature shock practice (HTSP), pollen vitality rates of the experiment varied between 1.10-78.55%; While the average pollen vitality of the standards was 7.61%, the average of the genotypes was 19.43%. The histogram of the high temperature pollen vitality stress index (HTPVSI) feature is given in Figure 1a. High temperature pollen vitality stress index (HTPVSI) values varied between 0.17-1.26, the HTPVSI mean of the standards was 1.17, and the HTPVSI of the genotypes was 0.99. As a result of the evaluation made in terms of HTPVSI feature, it was determined that there are 54 Sensitive, 36 Medium Tolerant and 4 (ADN701, Optasia, Lima and Diva (Tex)) Tolerant cotton genotypes (Table 2, Figure 1). After high temperature shock practice (HTSP), it was determined that all cotton genotypes experienced stress in pollen vitality and as a result, their vitality values decreased. The findings we obtained, indicate that high temperature reduces pollen vitality. The findings of Song et al. (2015) and Alas (2022) show parallelism. Our findings show that flowers exposed to high temperature stress weaken and kill pollen vitality, or that semi-alive pollen weakens germination functions or causes loss of fertilization ability and stigma functions; It is similar to the findings of Barrow, (1983); Sato et al. (2002); Foolad, (2005); Firon et al. (2006); Maheswari et al. (2012); Ekinci et al. (2012); Dhatt and Kaur (2017) and Aladizgeh (2021).

Shedding (Bud-Flower-Boll)

When the bud, flower and boll (B-F-B) numbers were examined under controlled conditions, it was found that they varied between 11.84 and 20.51 per/plant; it was determined that the average number of B-F-B of the standards was 15.37 per/plant, and the genotypes were 15.99 per/plant. B-F-B numbers after high temperature shock practice (HTSP) varied between 7.72 - 17.17 per/plant; the average number of B-F-B of the standards 11.80 per/plant; genotypes were determined as 11.86 per/plant. In this context, it was determined that the shedding rates ranged from 8.50% to 79.34%, standards shedding rates were 30.41%, and the genotypes were 36.32%. The histogram of the high temperature shedding stress index (HTSSI) feature is given in Figure 1b. In the evaluation made within the scope of high temperature shedding index values, HTSSI values varied between 0.30 - 1.71; HTSSI values of the standards were found to be 0.89 and genotypes to be 1.00. In this context, it has been determined that there is a wide variation among genotypes. As a result of the evaluation made in terms of HTSSI feature, it was determined that 40 cotton genotypes were Sensitive, 51 cotton genotypes were Medium Tolerant and 3 cotton genotypes (Nihal, Lodos, Bir781) were Tolerant (Table 2, Figure 1). Our study states that there are yield losses as a result of small or dry boll formation as well as boll shedding due to the effect of high temperature in the cotton plant; by Yfoulis and Fasoulas (1978); Wullschleger and Oosterhuis (1990); Rawson (1992); Reddy et al. (1999); Zhao et al. (2005); Hatfield et al. (2008, 2011); Oosterhuis (2009) and Karademir et al. (2012) are similar to their research.

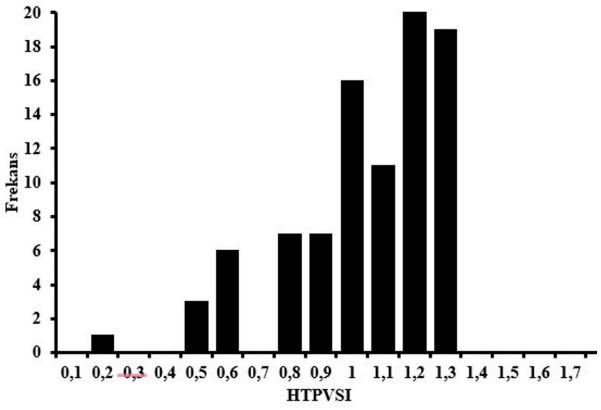

Figure 1a. Histogram for the HTPVSI feature

The variation of genotypes HTPVSI and HTSSI is given in Figure 2.

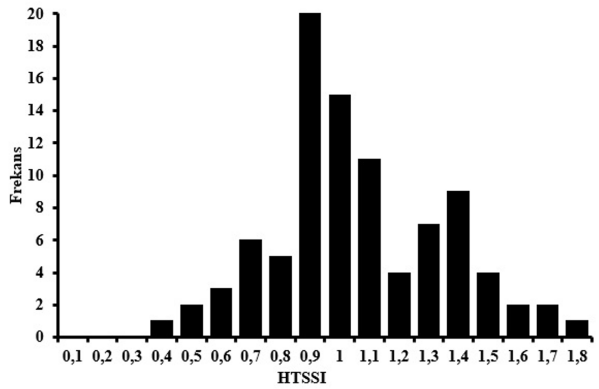

Figure 1b. Histogram for the HTSSI feature

In Figure 2, it is understood that as the genotypes get closer to the origin, there is more generatively tolerance in terms of both traits. In terms of HTPVSI and HTSSI characteristics examined, the Tolerance Zone (HTPVSI ≤0.5 and HTSSI≤ 0.5) was marked as TZ. Sensitivity Zone (HTPVSI >1.00 or HTSSI>1.0) was marked SZ. The Medium Tolerance Zone (0.5< HTPVSI ≤ 1.0 or 0.5<HTSSI≤1) was marked as MTZ. However, the Medium Tolerance Zone consists of three parts: MTZ, Gray I and Gray II. Although the Gray I region is tolerant in terms of the HTSSI feature, it is seen to be in the Medium Tolerant group in terms of the HTPVSI feature. Similarly, although the Gray II region is tolerant in terms of the HTPVSI feature, it is noticed that it is in the Medium Tolerant group in terms of the HTSSI feature. Therefore, Gray I and Gray II zones

Table 2. Genotype Numbers of Sensitive, Medium Tolerant and Tolerant groups according to HTSSI and HTPVSI

Groups	In terms of HTSSI	In terms of HTPVSI	In Terms of Both Traits
Sensitive	**40**	**54**	**25** Astoria, MAY455, Efe, Ergüven, Harem1, Sezener76, Babylon, Carisma, PG2018, Furkan, Kartanesi, Claudia, Gloria, ST478, Çukurova1518, BA525, Gürelbey, Aydın110, Bossa159, Ayzek595, Gapkot732, Caso9048, Flora, Flash, SJU86
Medium Tolerant	**51**	**36**	**18** (Ceykot340, TYA366, Bomba, MAY355, TMK122, Özaltın404, Özaltın112, ADN413, BA440, Sarıgelin, Famosa, Fantom, Penta (Golda), Candia, Şahin2000, Teksa415, Yıldırım63, Gaia)
Tolerant	**3** (Nihal, Lodos, Bir781)	**4** (Optasia, ADN701, Lima, Diva (Teks))	**0**

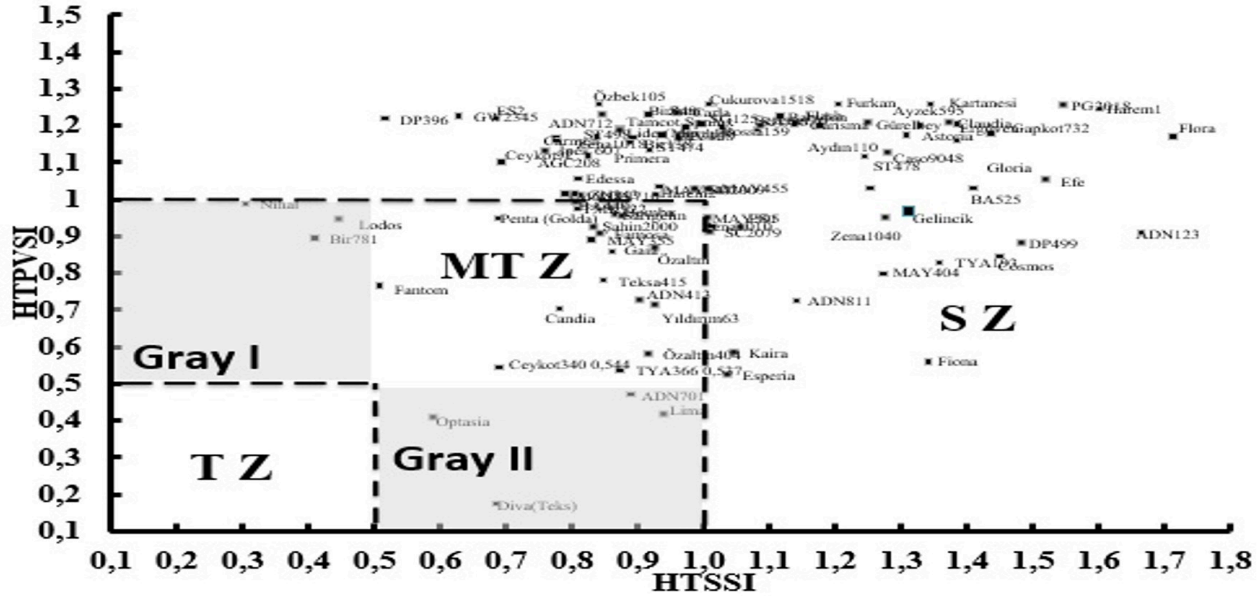

Figure 2. HTPVSI and HTSSI Change Graph of Genotypes

were included in the Medium Tolerant Zone (Figure 2). In terms of both traits, 25 cotton genotypes (Astoria, MAY455, Efe, Ergüven, Harem1, Sezener76, Babylon, Carisma, PG2018, Furkan, Kartanesi, Claudia, Gloria, ST478, Çukurova1518, BA525, Gürelbey, Aydın110, Bossa159, Ayzek595, Gapkot732, Caso9048, Flora, Flash and SJU86) were located in the Sensitive region (SZ) while 18 cotton genotypes (Ceykot340, TYA366, Bomba, MAY355, TMK122, Özaltın404, Özaltın112, ADN413, BA440, Sarıgelin, Famosa, Fantom, Penta (Golda), Candia, Şahin2000, Teksa415, Yıldırım63 and Gaia) were located in the Medium Tolerant region (MTZ). In terms of both traits, the cotton genotype in the Tolerant group could not be determined (Table 2). Gray I and Gray II regions can be considered as more advantageous regions than MTZ regions. Although flower shedding, which is included in the shedding ratio, is directly related to pollen vitality, boll and bud shedding is not related to pollen vitality. The results do not show similarity with each other in terms of HTPVSI and HTSSSI properties because the share of flower shedding in the shedding ratio (B-F-B) is low. Shedding of buds, which are sensitive to the effect of high temperature stress in cotton cultivation, may result in the shedding of bolls under more severe stress conditions. As a result, serious yield losses will be inevitable.

CONCLUSION

It was concluded that boll and bud shedding occurred much more than flower shedding since HTSP (96 hours of uninterrupted exposure to high temperatures during generative periods) in our study created very severe heat stress for genotypes. Therefore, severe and prolonged high temperatures have become inevitable to cause serious yield losses. It is recommended for the screening of genotypes in terms of tolerance or susceptibility to generatively high temperature stress in cotton plants by using HTPVSI and HTSSI features. In addition, it is suggested that it would be beneficial to use HTPVSI and HTSSI traits in selection in breeding programs. Prolonged and severe high temperatures will inevitably cause yield losses. HTPVSI and HTSSI characteristics were examined together, and no genotype was included in the generatively tolerant group. In terms of sensitivity of genotypes to high temperature, 18 cotton genotypes were found in the medium tolerant group and 25 cotton genotypes were found in the sensitive group.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

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

Author contribution

This article was produced from the Y.G.D. PhD thesis, and the supervisor of the thesis is R.E. and the co-supervisor is A.B. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Effects of different industrial cannabis (*Cannabis sativa* (Linnaeus 1753) (Cannabaceae)) genotype extracts on *Diuraphis noxia* Kurdjumov, 1913 *Myzus persicae* Sulzer, 1776 and *Aphis fabae* Scopoli, 1763 (Hemiptera: Aphididae)

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Abstract

In this study, the effect of methanol extracts of three different genotypes (Narlısaray, Kavacık, Maltepe) of *Cannabis sativa* L. on *Diuraphis noxia* Kurdjumov, *Myzus persicae* (Sulzer) and *Aphis fabae* (Scopoli) (Hemiptera: Aphididae) were investigated. In the first stage of the study, 10% concentrations of each cannabis extract were applied on the 2nd and 3rd nymphal stages of aphid species by spraying method. After the end of 24 - 48 and 72 hours of the applications, the alive and dead individuals were recorded and mortality rates were determined. In the second stage, the genotype with the highest effect was used in dose-death trials and LD_{50} and LD_{90} values at different doses (2.5%, 5%, 7.5% and 10%) were specified. In the census after 72 hours, Narlısaray genotype showed the highest mortality rate with 54.04% on *D. noxia*. While the effect of Kavacık genotype on *M. persicae* was found as 23.13%, the highest toxicity record of the same genotype was determined on *A. fabae* (as 91.76%). According to the dose measurement studies of Kavacık genotype on *A. fabae*, LD_{50} and LD_{90} values were calculated to be 0.33 and 0.110 (mg/individual), respectively. At the results of study, it has been observed that extracts of different genotypes of the industrial cannabis plant are found effective on aphid species and it is thought that they can be used in controlling of these pests.

Keywords: Aphids, Bio-pesticide, Biological control, *Cannabis sativa*, Plant extract

INTRODUCTION

Recently, many different plant-based products (botanical pesticides) are cultivated intensively in agricultural areas to control pest insects which affect negatively yield and yield parameters. Aphids (Hemiptera: Aphididae) are one of the most important harmful insects that cause damage to various cultivated plants (such as cereals, fruit trees, vegetables, etc.) and restrict crop yields (Baumann et al., 1995). Approximately, 5000 species belonging to 493 genera and 24 subfamilies of aphids are well-known in the world (Favret, 2019; Baki et al., 2020). So far, nearly 570 aphid species have been identified in Turkey (Görür, 2020). Aphids cause significant damage to plants by being a vector for plant virus diseases as well as causing direct sucking damage to the plant. Aphids cause fumagine by promoting the development of saprophytic fungi due to the honeyed substance they secrete. (Von Dohlen et al., 2006; Stevens and Lacomme, 2017; Helvacıoğlu and Akşit, 2020; Satar, 2020).

As with many plant pests, the most commonly used method of controlling aphids is also chemical control. Therefore, with the intensive usage of synthetic insecticides, aphids have developed stronger resistance to many insecticides over time (Elbert et al., 2008). As a result of excessive use of insecticides, the

environment and human health are adversely affected, the natural balance is disturbed, and residue problems occur on the plant parts (Grdıša and Gršić, 2013; Gill and Garg, 2014, Rother, 2018). Due to these and similar effects, alternative natural plant-derived compounds have been sought, which have shorter degradation times, are effective only on the target organisms, and have little negative impact on the environment (Arnason et al., 1989; Feng and Isman, 1995; Wewetzer, 1995; Hedin et al., 1997; Momen et al., 1997; Liao et al., 2017; Kunbhar et al., 2018). Plant-based extracts and essential oils attract attention as a good alternative to chemicals due to the range of bioactive chemicals they contain against plant pests (Isman, 2000; Kim et al., 2003b; Govindarajan et al., 2016; Khan et al., 2017; Sammour et al., 2018). Many of the volatile compounds found in plants are rapidly decomposed in nature and do not accumulate in the environment like other chemicals, so they could be preferred in biological control (Arnason et al., 1989; Hedin et al., 1997; Regnault-Roger et al., 2012). These compounds pose a low risk to non-target organisms, i.e., predators and parasitoids, and they are mostly non-toxic to mammals (Scott et al., 2003). Today, many researchers reveal the insecticidal activities of essential oils and their chemical components. (Regnault-Roger et al., 1993; Regnault-Roger and Hamraoui, 1995; Golob et al., 1999; Weaver and Subramanyam, 2000; Kéita et al., 2001; Lee et al., 2001; Papachristos and Stamopoulos, 2002; Kim et al., 2003a; Isman and Miresmailli, 2011; Miresmailli and Isman, 2014; Regnault-Roger et al., 2012; Pavela and Benelli, 2016; Chaubey, 2019; Feng et al., 2020; Gaur and Kumar, 2020; Sayed et al., 2021). Herbal extracts or oils obtained from plants have different advantages over pests when compared to chemical insecticides. Secondary metabolites derived from some plant species act on physiological or behavioral adaptations in the target organism and they contain many components with mechanisms that slow down the evolution of insects in these parts. When their side effects (toxicities) are evaluated for mammalians, very few compounds were found to be toxic to mammalians (Isman, 2006). Cannabis ((*Cannabis sativa*) *Linnaeus* 1753) *(Cannabaceae)*) is one of the plants that attract the attention of researchers being as a potential botanical insecticide due to the terpenoids (limonene, linalool and pinene) and phenolic compounds in it (McPartland and Sheikh, 2018).

In this study, the toxicity of the extract obtained from different genotypes of *C. sativa* against *Diuraphis noxia* (Kurdjumov, 1913), *Myzus persicae* (Sulzer, 1776) and *Aphis fabae* (Scopoli, 1763) (Hemiptera: Aphididae) was evluated, aiming their usage in integrated control of aphids on arable crop plants.

MATERIALS AND METHODS

Producing of the plant extracts

The leaves of Narlısaray genotype were obtained from Yozgat Bozok University Boğazlıyan Vocational School Agricultural Experimental Area, and the local Kavacık and Maltepe genotypes were from the campus trial areas in 2020. The leaves of the cannabis plant were dried in room conditions without direct sunlight and were ground with the help of a grinder and stored in dark conditions. A hundred g of ground plant samples were weighed and 500 ml of methanol was added to it, and it was kept in an Erlenmeyer flask for 24 hours. The plant suspensions were filtered through filter paper to separate the plant parts. Herbal extracts were obtained by evaporating the solvent in the obtained suspension with an evaporator (Buchi R-3). The obtained extracts were stored in the refrigerator at + 4°C (Alkan and Gökçe, 2012).

Plants rearing

In order to feed different aphid species, different plant species were grown for each aphid. Broad bean (*Vicia faba* (Fabaceae)), pepper (*Capsicum* sp. (Solanaceae)) and wheat (*Triticum* sp. (Poaceae)) plants used in the experiments were grown in the plastic containers (200 ml) containing soil: peat in a 1:1 ratio. Production was carried out in the climate room of Yozgat Bozok University, Faculty of Agriculture, at 25±1 °C and 60±5% relative humidity and under 16:8 (light: dark) lighting conditions.

Aphids rearing

Individuals of the last stage of nymph and/or adult D. noxia, M. persicae, and A. fabae were transferred to broad beans, pepper, and wheat plants when they reached a sufficient height (15 cm) and leaf number (over 6 pieces) to be used in the experiments, and they were reared in separate environments. The initial population of aphid cultures transmitted to the clean plants was obtained from the mass production at Yozgat Bozok University, Faculty of Agriculture, Department of Plant Protection. The reared aphids were placed into the cages (50x50x50 cm) covered with tulle in order to prevent the mixing of individuals. To ensure the continuity of mass production, aged and decaying plants were replaced with clean ones at weekly intervals. Rearing of aphid species was performed in a climate room with 25±1 °C, 60±5% relative humidity, and 16:8 (light: dark) lighting conditions.

Toxicity tests

Single Dose Death Trials

In the experiments, 2nd and 3rd instar aphid nymphs were used. The aphids that were selected to carry out contact toxicity studies were transferred to petri dishes with a 90 mm in diameter. 10% (w/v) concentrations of the extracts were used in single-dose death trials, and 10% (w/v) concentrations were sprayed onto each petri dish with a 20 ml small handheld sprayer (Erdoğan and Yıldırım, 2013). For the control group, 50% acetone/water was sprayed with the same method. The treated individuals were transferred to petri dishes

containing clean plant leaves with the help of a sable brush. At the end of the application, petri dishes were incubated at 26±1 °C, 65±5% relative humidity, and 16 hours of illumination. Experiments were set up with 10 replications, and 10 individuals were used in each replication. Mortality rates of the aphid individuals were recorded after 24, 48 and 72 hours.

Dose - Death Trials

Contact effect dose-death trials were conducted with plant extracts whose effect was determined as a result of single dose death trials. For this purpose, LD_{50} and LD_{90} values were determined by applying the genotype extracts with promising results on pests at different doses (2.5%, 5%, 7.5%, and 10%). Experiments were set up with 10 replications, and 10 individuals were used in each replication. Mortality rates in individuals were recorded after 24 and 48 hours.

Statistical analysis

The single-dose contact data were calculated as a percentage and then normalized using arcsin transformation. The data then were subjected to variance analysis (ANOVA) (P≤0.05) and the Tukey test ((P≤0.05) for differentiating treatments using the SPSS® 20 statistical software program. Probit analysis was used to calculate LD_{50}, and LD_{90} values and 95% confidence intervals for dose-response contact bioassay data using SPSS® 20 statistical software.

RESULTS AND DISCUSSION

Single Dose Death Results

In the study, the insecticidal activity of methanol extract obtained from 3 different genotypes of cannabis plant on *D. noxia*, *M. persicae* and *A. fabae* was tested. The Narlsaray genotype was found to be more effective against D. noxia than the other two cannabis genotypes at all times. While Kavacık (21.03%) genotype showed the lowest efficiency on *D. noxia*, Narlısaray genotype caused 62.32% mortality after 72 hours (F=26.23; df= 3.36; P<0.05). (Table 1).

Table 1. Toxicity of cannabis extracts on *Diuraphis noxia*

	Mortality rates (%)±SEM		
Genotypes	**24th hour**	**48th hour**	**72th hour**
Control	3.03±0.35 b	5.62±0.63 b	5.62±0.63 c
Maltepe	8.73±0.29 b	17.38±0.10 b	25.32±0.15 b
Narlısaray	35.58±0.17 a	49.89±0.29 a	62.32±0.16 a
Kavacık	2.90±0.50 b	13.07±0.59 b	21.03±0.56 b

*Means in a column followed by a different lowercase letter represents results are significantly different according to Tukey test (p<0.05)

In studies conducted with *M. persicae*, the Kavacık genotype was found to be more effective than the other two genotypes at all times. After 72 hours, the Kavacık genotype caused 27.27% mortality on *M. persicae* nymphs (F=4.02; df= 3.36; P<0.05) (Table 2).

The Kavack genotype had the highest toxicity on Aphis fabae and M. persicae in the different genotype extracts of cannabis. At the end of 72 hours, Kavacık genotype caused 96.55% mortality on *A. fabae* nymphs. On the other hand Maltepe genotype showed the lowest efficiency with 71.39% mortality (F=64.79; df=3.36; P<0.05) (Table 3).

Dose Mortality Results

As a result of the single-dose screening test, Kavacık genotype showed the highest toxicity amongst all *C. sativa* genotypes against *A. fabae*. According to the dose measurement results of the Kavacık genotype, the LD_{50} value was calculated as 0.40 mg/individual after 24 hours, while the LD_{90} value was found to be 0.159 mg/individual. At the 48th hour of the study, the LD_{50} value was calculated as 0.33 mg/individual, while the LD_{90} value was determined as 0.110 mg/individual (Table 4).

Table 4. Results of dose-death trial of *Cannabis sativa*-Kavacık genotype on *Aphis fabae*

DISCUSSION

As a result of screening the insecticidal activity of different cannabis genotypes on the tested insects, the Narlısaray genotype caused the highest mortality rate on *D. noxia* nymphs (64.32%), while the Kavacık genotype had the highest mortality rate on *M. persicae* (27.27%) and *A. fabae* (96.55%). The study showed that the numbers of dead individuals were recorded after 24 and 72 hours, and the mortality percentage increased as the exposure time to the extract increased in all aphid species, depending on the time. Ahmed et al. (2020) found the efficacy of *Artemisia argyi* (L.) extract against *Brevicoryne brassicae* L. and while the LC_{50} value was found to be 38.6 mg/mL at the 24th hour, it was 3.91 mg/mL at the end of 72th hour in the study. Yadav and Patel (2018) concluded that the efficacy of *Cassia angustifolia* on *M. persicae* was 46.67% at the end of the 24th hour, while the mortality rate increased to 93.33% at the end of the 72nd hour.

The current study's findings suggest that the contents of all cannabis genotype extracts have different toxic effects on the aphid species tested. Peña-Cerda et al. (2017) evaluated the total phenolic and flavonoid

Table 2. Toxicity of cannabis extracts on *Myzus persicae*

	% Mortality rates ±SH		
Genotypes	**24th hour**	**48th hour**	**72th hour**
Control	3.03±0.35 b	7.94±0.20 b	7.94±0.20 b
Maltepe	1.22±0.33 b	6.67±0.55 b	10.17±0.79 b
Narlısaray	4.08±0.48 b	12.02±0.40 ab	19.38±0.11 ab
Kavacık	23.56±0.30 a	26.01±0.21 a	27.27±0.17 a

*Means in a column followed by a different lowercase letter represents results are significantly different (ANOVA $p < 0.05$, Tukey test).

Table 3. Toxicity of cannabis extracts on *Aphis fabae*

	% Mortality rates ±SH		
Genotypes	**24th hour**	**48th hour**	**72th hour**
Control	5.44±0.44 c	5.44±0.44 c	5.44±0.44 c
Maltepe	50.15±0.19 b	63.29±0.14 b	71.39±0.11 b
Narlısaray	65.76±0.60 b	75.48±0.43 b	77.67±0.46 b
Kavacık	84.33±0.35 a	93.13±0.40 a	96.55±0.44 a

*Means in a column followed by a dizfferent lowercase letter represents results are significantly different (ANOVA $p < 0.05$, Tukey test).

Table 4. Results of dose-death trial of *Cannabis sativa*-Kavacık genotype on *Aphis fabae*

	LD_{50} (mg/individual) (confidence interval)	**LD_{90}(mg/individual) (confidence interval)**
24th hour	0.40 (0.33-0.46)	0.159 (0.122-0.246)
48th hour	0.33 (0.27-0.38)	0.110 (0.91-0.246)

contents of the 10 different genotypes of *Ugni molinae* and stated that the different genotypes had different rates of phenolic and flavonoid compounds. According to Pavel et al. (2014), the obtained essential oil contents and their amounts from different *Mentha* genotypes were different. Moreover, they tested the effectiveness of essential oils obtained from different genotypes of *Mentha* on *Culex quinquefasciatus* (Say, 1823) (Diptera: Culicidae) larvae, and while some genotypes caused high mortality in *C. quinquefasciatus* larvae, others caused lower mortality rates. They stated that this difference was due to the different chemical contents and amounts of them in each genotype.

The sensitivity of some insect species to different plant substances could be variable. Similarly, the different mortality rates observed at aphid species following application of plant extracts derived from different genotypes of cannabis could be attributed to the different compounds and their rates in the plants. Alghamdi (2018) tested the efficacy of *Moringa oleifera* and *Eruca sativa* on *Macrosiphum rosae* and *A. fabae*. While *Moringa oleifera* caused 63.5% mortality, *M. rosae* caused 72.5% mortality in *A. fabae*. *Eruca sativa* caused 97.5% mortality in *M. rosae* and 92.4% mortality in *A. fabae*. Salari et al. (2010) tested the efficacy of *Otostegia persica* extract on three different aphid species and a warehouse insect pest, and after 72 hours, percentages of mortality rates were found to be 57.9% on *M. persicae*, 70.8% on *A. fabae*, 89.5% on *A. gossypii,* and 34.4% on *Trilobium castaneum,* respectively.

There are also many different studies associated with the effectiveness of extracts from different plants against these pest species. For instance, Czerniewicz et al. (2018) tested the toxic effects of plant essential oils obtained from *Santolina chamaecyparissus*, *Achillea millefolium*, *Tanacetum vulgare*, *Tagetes patula,* and *Artemisia absinthium* on *M. persicae* and found LC_{50} values of 0.34%, 0.34%, 0.47%, 0.61% and 0.69%, respectively, after 24 h. The ethanol extract of *Nerium oleander* with a 10% concentration showed 70% mortality on *M. persicae* nymphs (Nia et al., 2018). Mohmed (2019) indicated that *Carissa macrocarpa* extract, caused the toxic effect at 46.6% on *A. fabae*.

CONCLUSION

When the literature was examined, no study was found on the insecticidal activity of cannabis extract on *D. noxia*, *M. persicae,* and *A. fabae*; thus, this research represents the first study on this plant with these pests. It has been aimed at obtaining information about the possibilities of using some industrial cannabis genotypes, whose importance has increased day by day in recent years, especially in the biological control of agricultural pests. It is thought that the chemical differences between these genotypes should be revealed, and their effectiveness against pests should be determined in future studies.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

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

Author contribution

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

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

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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The sensitivity of radish cultivars to high temperatures during germination and seedling growth stages

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Abstract

A laboratory experiment was conducted at constant temperatures of 20, 23, 26, 29, 32, 35, 38, and 41^{0}C to identify the response of six radish cultivars with different root sizes and colors to high-temperature stress during germination, emergence, and early seedling growth stage. Also, the optimal temperature was determined by calculating the relationship between seedling length and temperature via a polynomial regression model. The results showed that no seed germination was detected at 41^{0}C, while emergence and seedling growth were not observed at 38 ^{0}C and above. Similar germination percentages, mean germination time, and germination index were obtained between 20 ^{0}C and 35 ^{0}C. The root length reduced at 32 ^{0}C and it was more sensitive to high temperatures than shoots because the root/shoot length ratio showed a decreasing trend by increasing temperatures and longer root length was recorded at lower temperatures than 32 ^{0}C. There were genotypic variations among radish cultivars for temperatures and 'Kırmızı İnci', 'Beyaz İnci', and 'Siyah İnci' produced a better performance than the others under high temperatures. The regression analysis predicted the optimal temperatures as 21.5 ^{0}C and 22.6 ^{0}C for root and shoot length, respectively. This study indicates that high temperatures inhibited seedling growth rather than seed germination performance of radishes.

Keywords: *Raphanus sativus* L., Germination, Seedling growth, High temperature

INTRODUCTION

Radish (*Raphanus sativus* L.) is an important and common vegetable consumed directly or indirectly in salads by humans for its roots with high vitamin and mineral contents throughout the world (Banihani, 2017). There are several types of radish cultivars in terms of root shapes, colors, and vegetation periods (Gunay, 2005). It is successfully grown in cool climates and high temperatures negatively affect its growth and tuber yields (Jia et al., 2020). Its cultivation concentrates in the Mediterranean region during the autumn seasons in Türkiye. Nowadays, fast-maturing varieties with small-sized roots are grown in vials under greenhouse conditions, even in pots for kitchen gardening (Khan et al., 2022). Under these climatic conditions, it is frequently subjected to high temperatures during germination, emergence, and seedling growth stages, resulting in irregular plant density.

Seed germination is mainly controlled by several environmental factors such as temperature, water, and oxygen availability along with seed viability and vigor. Among these factors, the temperature has a key role in regulating water

uptake by seeds, which affect adversely or favorably germination and seedling establishment (Bradford, 2002). Since extraordinary temperatures prevent the radicle protrusion from the seeds during germination in some plant species, the temperature requirements for germination are vital for a successful stand establishment and high-yielding production. In radish, Abdel (2015) announced that radish cultivars germinated better at 12 ^{0}C than 20 ^{0}C. Recently, Bakhshandeh and Gholamhossieni (2019) found that the base, optimum, and ceiling temperatures for radishes were 9.64, 21.3, and 33.0 ^{0}C, while Khan et al. (2022) reported these temperatures as 15, 20, and 40 ^{0}C, respectively. Studies on the existence of thermo-dormancy and germination responses of the radish cultivars with different sizes, colors, and vegetation periods to high temperatures are limited. Therefore, the study aimed to determine the germination, emergence, root, and shoot development of six radish cultivars with different root shapes and colors under eight constant temperatures starting from 20 ^{0}C and to calculate the optimum temperature using the polynomial regression model.

MATERIALS AND METHODS

A laboratory experiment was arranged to determine the optimum and high temperature for germination of radish cultivars with different root sizes at the seed sciences laboratory of Eskişehir Osmangazi University, Türkiye. The seeds of six radish cultivars namely 'Beyaz İnci', 'Siyah İnci', 'Kırmızı İnci', 'Alçin' (medium), 'Toros Beyazı' (big), and 'Cherry Belle' (small) produced by Sim Arzuman Seed Company in Türkiye were used, and their thousand seed weights were 8.20, 8.38, 8.67, 14.43, 8.83, and 7.40 g, respectively.

The germination test was arranged with 4×50 replication/seed at constant temperatures of 20, 23, 26, 29, 32, 35, 38, and 41^{0}C under continuous dark. The seeds were permitted to germinate between three layers of filter papers wetted with 21 mL distilled water. The rolled filter papers were placed in sealed plastic bags to prevent evaporation. They were put in incubators with respective temperatures. The criterion for germination was a radicle protrusion of 2 mm. At the end of the experimental period (7th day), the final germination percentage (GP, Eq. (1)), mean germination time (MGT, Eq. (2)), and germination index (GI, Eq. (3)) were calculated as follows.

$GP = n/N \times 100$ (Eq. 1)

n represents germinated seeds and N total seeds.

$MGT = \Sigma(Dn)/\Sigma n$, (ISTA, 2018) (Eq. 2)

n is germinated seeds on day D, and D is the day number from the start of the experiment.

GI = Number of germinated seeds/days of first count + . . . + Number of germinated seeds/days of final count (Salehzade et al., 2009). (Eq. 3)

The emergence test was also performed with 200 seeds from each cultivar and temperature in a seedling tray filled with a mixture of peat: perlite: vermiculite (3:1:1). They were exposed to constant temperatures in growth chambers with 70% relative humidity, and the 2 mm of hypocotyl length above the medium surface were considered as emergence criterion.

The optimal temperature was estimated by a polynomial regression equation between temperature and the lengths of the root and shoot. In this model, the independent variable (temperature) was assumed to X-axis and the dependent variable (length) on the Y-axis. The optimum temperature was calculated as the peak value (-b/2a) from the regression equation ($y= ax^2+bx+c$) to the minimum temperature of 20 ^{0}C (Fallahi et al., 2017; Wang et al., 2020).

RESULTS

Analysis of variance showed significant differences in germination parameters among radish cultivars, temperatures, and cultivar × temperature interaction (Table 1). 'Kırmızı inci' gave the highest germination percentage and germination index, while the lowest MGT was obtained from it. At 26 ^{0}C, the maximum germination percentage and index were recorded, but the shortest time for germination was obtained. Germination parameters were depressed at 38 ^{0}C. Moreover, the emergence percentage of radish cultivars reduced at 35 ^{0}C, and no emergence was observed at 38 ^{0}C.

All radish cultivars germinated at all temperatures except for 41^{0}C, but higher temperatures than 29 ^{0}C reduced germination percentage (Figure 1a). A clear difference among radish cultivars for germination percentage was observed at 38 ^{0}C. The differences among the temperatures may result from genotypic factors because we determined significant differences among radish cultivars concerning germination percentage and Kırmızı İnci had the highest germination at 38 ^{0}C, followed by Beyaz İnci and Toros Beyazı.

MGT varied relatively with radish cultivars by increasing temperatures (Figure 1b). Temperatures between 20 ^{0}C and 35 ^{0}C gave similar MGT, while Kırmızı İnci possessed shorter MGT than the other cultivars. A similar trend was observed for GI and the highest GI was calculated in Kırmızı İnci at all levels of temperature (Figure 1c). Although the emergence percentage of radish cultivars responded differently to high temperatures and Kırmızı İnci was slightly affected, fluctuated, and finally dropped at 38 ^{0}C when the temperature increased (Figure 1d).

The seedling growth of radish cultivars was significantly depressed by increasing temperatures. As expected, Toros Beyazı, which is the cultivar with the highest seed weight and root size, produced longer shoots and roots than the other cultivars and it had heavier seedling fresh weight. However, Cherry Belle and Alçin

Table 1. Germination parameters and emergence percentage of radish cultivars under increasing temperatures

Factor	Germination (%)	Mean germination time (day)	Germination index	Emergence (%)
Cultivar (A)				
Beyaz İnci	94.9^{b}	1.22^{d}	42.9^{b}	94.3^{ab}†
Kırmızı İnci	96.6^{a}	1.16^{e}	44.9^{a}	96.3^{a}
Siyah İnci	93.1^{c}	1.56^{a}	36.7^{e}	93.2^{bc}
Toros Beyazı	94.1^{bc}	1.32^{b}	41.1^{cd}	97.0^{a}
Alçin	90.4^{d}	1.27^{c}	41.7^{c}	90.9^{c}
Cherry Belle	91.0^{d}	1.35^{b}	40.8^{d}	93.3^{b}
Temperature (B)				
20^{0}C	97.3^{a}	1.46^{b}	37.4^{d}	96.0^{ab}
23^{0}C	97.2^{a}	1.09^{d}	46.6^{b}	93.8^{b}
26^{0}C	98.2^{a}	1.04^{ef}	47. 9^{a}	95.0^{ab}
29^{0}C	97.6^{a}	1.04^{f}	47.8^{a}	96.6^{a}
32^{0}C	95.0^{b}	1.09^{de}	46.1^{b}	96.0^{ab}
35^{0}C	96.8^{ab}	1.14^{c}	44.9^{c}	87.2^{c}
38^{0}C	70.2^{c}	2.33^{a}	18.8^{e}	-
Analysis of Variance				
A	**	**	**	**
B	**	**	**	**
A×B	**	**	**	**

**: significant at 1%. †: Letter(s) connected with the means in each column show the significance levels at 5%.

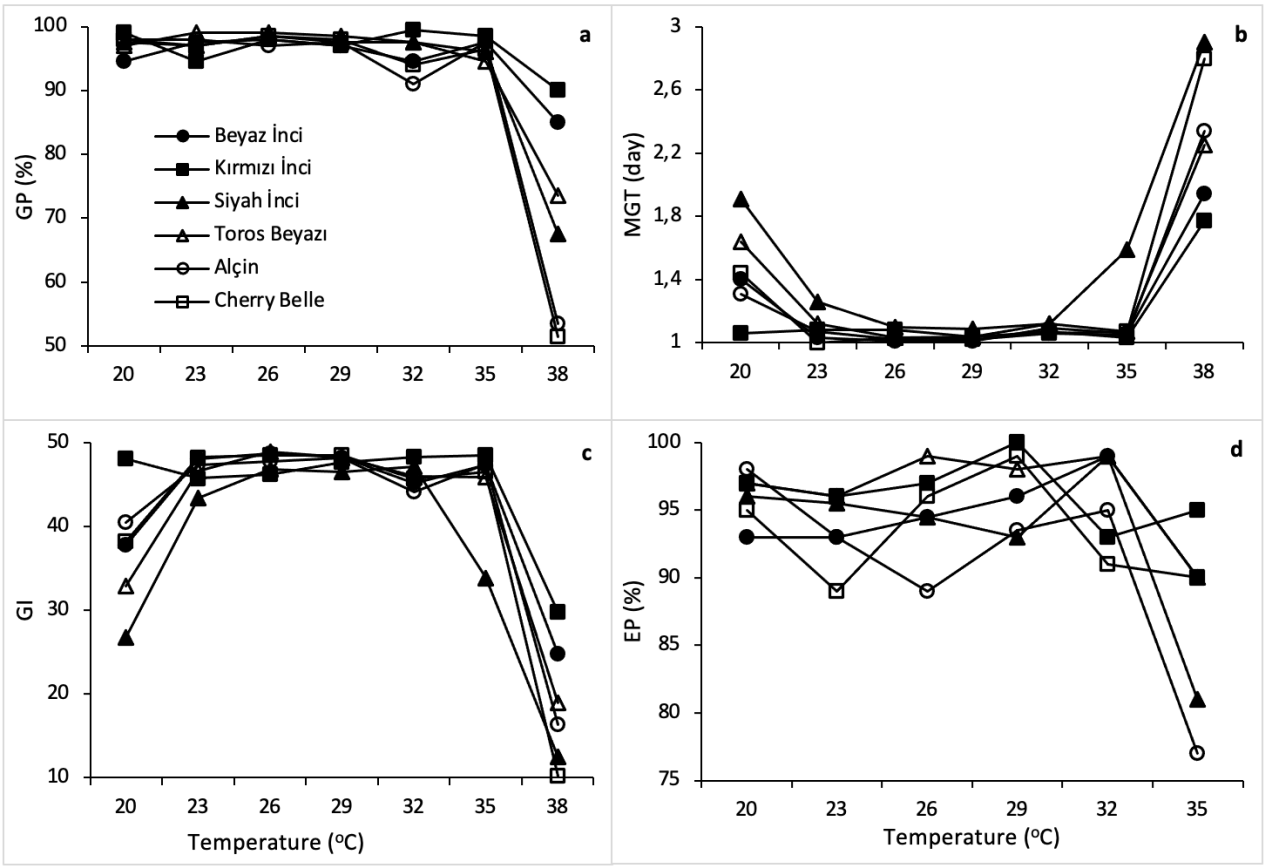

Figure 1. The interaction effects of radish cultivar × temperature on germination percentage (GP, a), mean germination time (MGT, b), germination index (GI, c), and emergence percentage (EP, d).

Table 2. Seedling growth parameters of radish cultivars under increasing temperatures

Factor	Shoot length (cm)	Root length (cm)	Seedling fresh weight (mg plant^{-1})	Seedling dry weight (mg plant^{-1})	Root/Shoot ratio
Cultivar (A)					
Beyaz İnci	6.07^{b}	8.53^{a}	118^{b}	1.44^{b}	1.44^{b}†
Kırmızı İnci	5.80^{b}	7.60^{bc}	103^{c}	1.28^{c}	1.28^{c}
Siyah İnci	5.32^{c}	7.34^{c}	88^{d}	1.43^{b}	1.43^{b}
Toros Beyazı	8.28^{a}	8.83^{a}	194^{a}	1.00^{d}	1.01^{d}
Alçin	5.10^{c}	8.01^{b}	103^{c}	1.52^{ab}	1.52^{ab}
Cherry Belle	4.13^{d}	6.60^{d}	86^{d}	1.57^{a}	1.57^{a}
Temperature (B)					
20^{0}C	5.68^{d}	10.80^{a}	113^{c}	2.05^{a}	2.05^{a}
23^{0}C	7.49^{a}	10.18^{b}	135^{a}	1.40^{bc}	1.39^{bc}
26^{0}C	6.92^{b}	8.83^{c}	137^{a}	1.36^{c}	1.36^{c}
29^{0}C	6.21^{c}	8.98^{c}	127^{b}	1.50^{b}	1.49^{b}
32^{0}C	4.73^{e}	5.96^{d}	98^{d}	1.32^{c}	1.32^{c}
35^{0}C	3.66^{f}	2.18^{e}	82^{e}	0.62^{d}	0.63^{d}
38^{0}C	-	-	-	-	-
Analysis of Variance					
A	**	**	**	**	**
B	**	**	**	**	**
A×B	**	**	**	**	**

**: significant at 1%. †: Letter(s) connected with the means in each column show the significance levels at 5%.

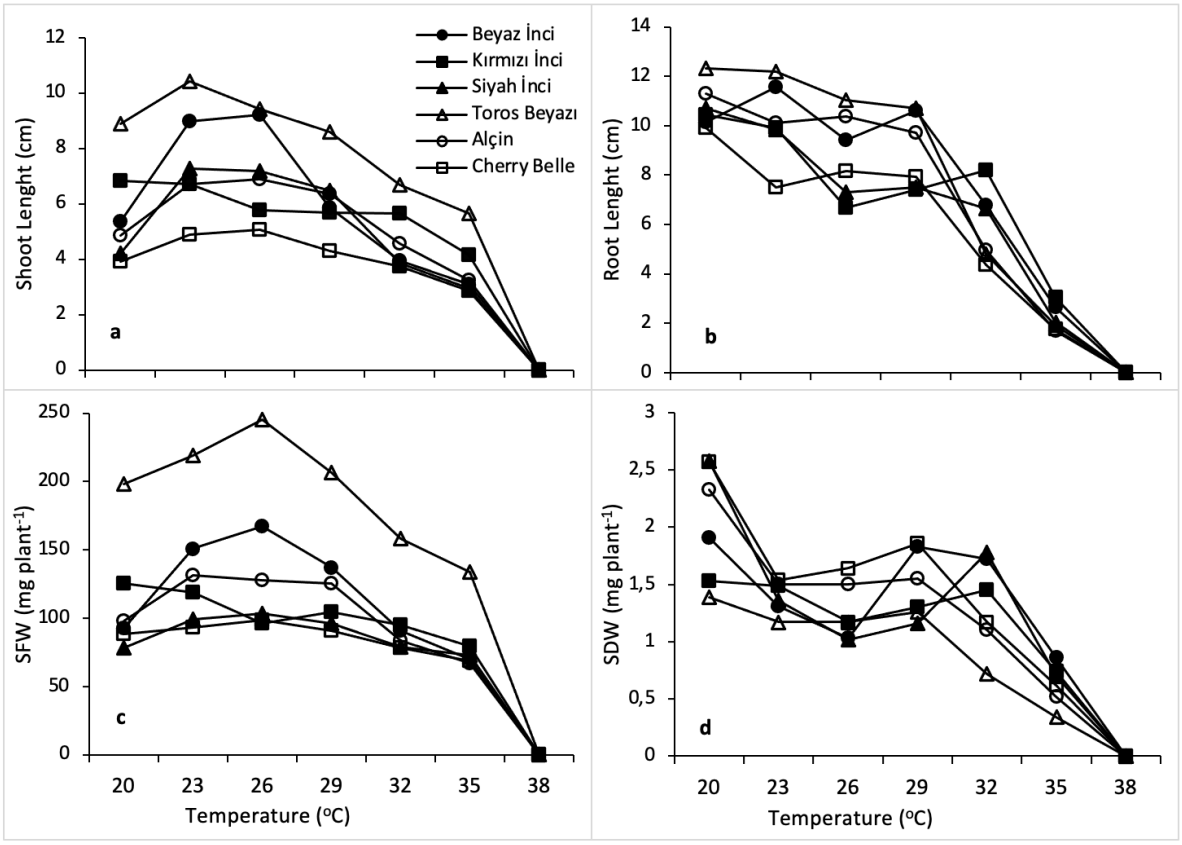

Figure 2. The interaction effects of radish cultivar × temperature on shoot length (a), root length (b), seedling fresh weight (c), and seedling dry weight (d).

cultivars accumulated more dry weight compared to the others; reflecting that the cultivars were influenced by high temperatures. Shoot length reached the highest level at 23 ^{0}C and a dramatic reduction occurred at 32 ^{0}C although root length was diminished with each increase in temperature. Similar trends were determined for seedling fresh and dry weights that were reduced at 32 ^{0}C. It was confirmed by the reduction in root/shoot length ratio that was lessened by increasing temperature. The ratio showed that low temperatures prominently inducted the root growth and each increase in temperature led to inhibition in root length.

The shoot length of radish cultivars was significantly changed by increasing temperatures and Toros Beyazı exhibited the highest shoot length at all levels of temperatures. A temperature of 38 ^{0}C did not allow the seedling growth of radish cultivars. None of the radish cultivars produced the shoots at 38 ^{0}C. Temperatures of 23 ^{0}C and 26 ^{0}C resulted in an increase in the shoot length of the cultivars except for Kırmızı İnci (Figure 2a). On the other hand, the root length of radish cultivars except for Beyaz İnci was reduced by each increase in temperature, Toros Beyazı with high-sized roots and seed weight had higher root length until 29 ^{0}C than the others (Figure 2b). No longer root length than germination criterion occurred at 38 ^{0}C. Seedling fresh weight peaked at 26 ^{0}C for Toros Beyazı, Beyaz İnci, Siyah İnci, Alçin, and Cherry Belle (Figure 2c) and it was considerably decreased at higher temperatures than 26 ^{0}C. The lower seedling dry weight was observed at temperatures above 20 ^{0}C and the horizontal course was observed between 23 ^{0}C and 29 ^{0}C (Figure 2d). There was a significant drop in seedling dry weight at 23 ^{0}C. The root/shoot length ratio showed that increased temperature led to a reduction in root growth; indicating the roots of radish were more sensitive to high temperatures rather than shoot growth (Figure 3).

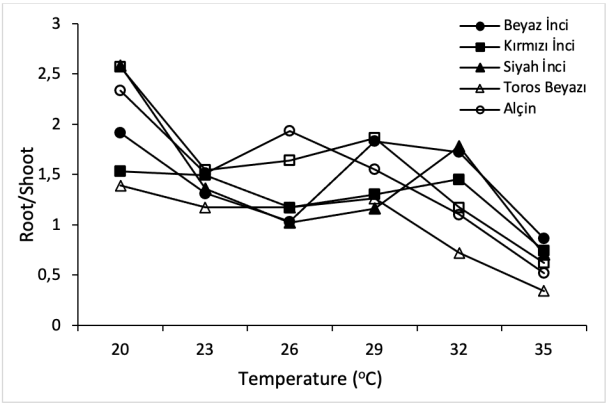

Figure 3. The root/shoot length ratio of radish cultivars as affected by temperatures

The relationship between temperature and root and shoot length was determined by a polynomial regression equation and significant relationships were calculated ($P < 0.01$) (Figure 4). Shoot length increased with the temperature of 22.6 ^{0}C computed by the equation of $y=-0.3223x^2+1.7117x+4.681$, $R^2=0.907^{**}$. The shoot length gradually reduced at higher temperatures. However, the relationship between root length and the temperature was significant and root length was shortened at higher temperatures than 21.5 ^{0}C which was calculated by the equation of $y = -0.4018x^2+1.2219x+9.642$, $R^2= 0.964^{**}$.

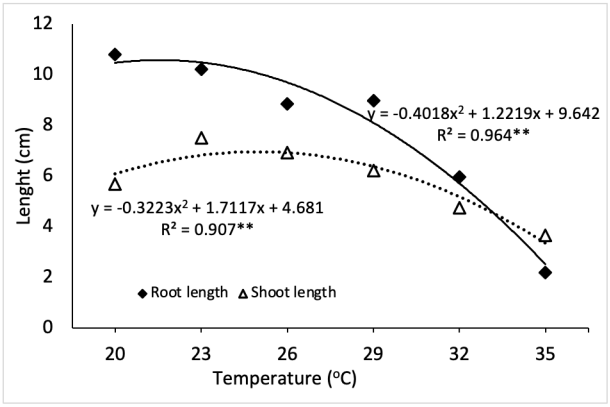

Figure 4. The relationship between the length of shoot and root and increasing temperatures for radish cultivars.

DISCUSSION

Temperature is a critical factor for seed germination of several plants especially those grown all seasons. Radishes are exposed to various temperatures from germination to harvest because it is easily adapted to different environmental conditions using varieties with short maturating, root sizes, and colors in both open fields and greenhouse. In this study, the responses of different radish cultivars to high temperatures were investigated during germination and early seedling growth stages. The temperature of 41 ^{0}C did not allow the seeds of all radish cultivars to germinate, but they showed differences in germination percentage at 38 ^{0}C. Kırmızı İnci and Beyaz İnci germinated better, while Alçin and Cherry Belle had the minimum germination percentage at this temperature. Similar results were reported by Cavusoglu and Kabar (2007) who found a 22.0% germination percentage of radish at 38 ^{0}C. Also, it was not determined as thermo-dormancy temperature for radish, which is consistence with the findings of Steiner et al. (2009) who reported no significant changes in germination with temperatures between 10 ^{0}C and 35 ^{0}C, but Bakhshandeh and Gholamhossieni (2019) and Shah et al. (2022) demonstrated the inhibitory effects of temperature at 33 ^{0}C and 40 ^{0}C, respectively. Also, Dell'Aquila (2005) determined a significant reduction in germination percentage at 35 ^{0}C in radishes and Elson et al. (1992) found 63% germination at 35 ^{0}C and zero at 40 ^{0}C in broccoli. The variation in temperatures may

result from genotypic factors because there were distinct results for the germination of radish cultivars at 38 ^{0}C in this study. The result revealed that the most suitable temperature for separating germination performance was 38 ^{0}C which was higher than that reported by Steiner et al. (2009), 35 ^{0}C. The higher MGT or lower GI were recorded at 20 ^{0}C and 38 ^{0}C, with differences in radish cultivars. These findings are in agreement with the results of Khan et al. (2022) and Dell'Aquila (2005), who determined retardation in mean germination time and reduction in germination index at 40 ^{0}C. Although the emergence percentage was changed by radish cultivars and temperatures, it was inhibited at 35 ^{0}C without any significant decrease in Kırmızı İnci when the temperature increased.

Seedling growth of radish cultivars was also restricted by increasing temperature; however, shoot and root parts were differently influenced. An increase in temperature from 20 ^{0}C to 23 ^{0}C enhanced shoot length and seedling fresh weight, and then, they began to drop at 26 ^{0}C. Contrarily, root length, and seedling dry weight declined with increasing temperature. This tendency was confirmed by the polynomial regression models for shoot and root length (Figure 4). Rowse and Finch-Savage (2003) calculated a similar curve for GR_{50} in carrots. In addition, decreased root/shoot length ratio reflects that root growth was reduced more severely than shoot growth, and a lower temperature for root length (21.5 ^{0}C) than shoot length (22.6 ^{0}C) was calculated. This result supports the findings reported by Steiner et al. (2009) who demonstrated that the root system development was negatively influenced by high temperatures.

CONCLUSION

Extreme temperatures are harmful abiotic stress factors affecting germination of the crop plants (Bakhshandeh et al. 2020). In this study, radish cultivars responded differently to increasing temperatures for germination performance and seedling growth. Among the radish cultivars, Kırmızı İnci showed the best germination and seedling growth performance under high temperatures. The results revealed that there was no thermo-dormancy in the radish. Seedling growth was more sensitive to high temperatures than germination. Furthermore, root length was much more negatively influenced by high temperatures than shoot length, which limited emergence performance. For these reasons, the optimum temperature for radish was calculated using root and shoot lengths that were at 21.5 ^{0}C and 22.6 ^{0}C. It was concluded that emergence failure in radish under high temperatures resulted from the inhibition of shoot and root growth rather than germination inability and insensitive cultivars such as Kırmızı İnci should be preferred for high temperatures.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

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

Author contribution

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

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Determining the temporal and spatial variation of the land cover according to CORINE(1990-2018) in the basin of Kesis Stream (Southern Türkiye)

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Abstract

Human has interfered with nature in many different ways and tried to benefit from it since the dawn of time. Especially during and after the Industrial Revolution, human pressure on nature exploded. Due to this increasing pressure, global warming and climate change have occurred, resulting in events such as landslides, floods, and droughts. In this study, CORINE land cover data were used to determine the temporal variation of land use/cover (1990-2018) in the basin of Kesis Stream. CORINE land use/cover data, which was created for periods of ten and six years (1990-2000-2006-2012-2018), was processed through geographic information systems (GIS) and presented with various figures, graphics, and tables. Accordingly, the discontinuous urban fabrics in the basin covered an area of 1.09 km^2 in the 1990-2000 period, while they covered an area of 1.35 km^2 with a partial increase in 2018. The basin is mainly covered with forest and agricultural fields. While the forest lands (1990-code; 311, 312, 313, 324) covered an area of 410.29 km^2 in 1990, they gradually increased to 446.39 km^2 in 2018. While the agricultural lands covered an area of 368.04 km^2 in 1990 (code-1990; 211, 212, 242, 242), they decreased to an area of 326.85 km^2 in 2018 a significant decrease. According to these results, it can be asserted that the morphological structure of the basin, with steep and deep valleys has restricted adverse human activities and reduced forest destruction in the last 28 years with the implementation of nature protection laws.

Keywords: Determination, Temporal-Spatial Variation, CORINE Land use/cover, Basin of Kesis Stream

INTRODUCTION

Human has used nature for their purposes since the dawn of time and has continued to benefit from it by changing its conditions. Human pressure on nature increased the use of natural resources especially during the Industrial Revolution and exploded today (Karaosmanoglu et al.,2022). Human interventions in the natural cause a variety of environmental problems such as air, water, and soil pollution, wrong land use, climate change, drought, and flood. Determining the effects of human interventions in nature and their temporal and spatial changes has been one of the important research topics, especially in recent years. The rapid population growth, industrialization, and technological advancements experienced since the Industrial Revolution have increased ground cover destruction and improper land use. This has brought about many natural and human environmental problems (Pektezel, 2016). Ekinci and Pektezel. (2012) emphasized that improper land use is one of the most important current problems encountered by humanity. Kahan et al.(2015) It has been stated that the formation of the upper soil layer accelerates and changes in the land cover by converting the forest destruction surfaces in the high slope landslide areas

into agricultural land and horticulture activities.The basic principle of proper land use is based on the use of the land as agriculture, grassland and forest by taking into account the topography (altitude, slope, slope exposure, landforms), parent material and soil characteristics (Atalay and Gündüzoğlu, 2015). Accordingly, human beings need to use the natural environment where they live properly, rationally, and sustainably.

All kinds of human activities outside of the sustainability approach not only impair the quality of water, soil and air, but also make access to food difficult. Therefore, land use planning and an understanding of ecosystem service accessibility are critical criteria for the survival of communities (Ding et al., 2015; Parveen et al., 2018). If the sensitive balance between natural and human environmental components is maintained, it becomes possible to continue a sustainable life and development (Pektezel, 2016). Land use land cover (LULC) maps play a significant and primary role in planning, management, and monitoring programs at local, regional, and national levels. It is necessary to monitor the ongoing process of LULC patterns for a while (Hamad, 2020). Information on LULC patterns play an important role in the development plan of any area. In addition, the information on the change in LULC is important for investigating the type and magnitude of land conversion and the associated land and environmental degradation taking place in a given area (Tiwari et al., 2021). Samie et al. (2017) they determined that dramatic changes in land use are associated with factors such as climatic, socio-economic, geophysical and proximity. Therefore, the importance of understanding land use mechanisms and developing models for future changes is emphasized.

Determining the current spatial distribution of LULC classes and examining the changes occurred during the constitutes an important basis for studies carried out in many economic and socio-cultural fields (Kaya et al., 2020). Determining the current status of the land cover/land use classes distributed over the earth as well as identifying their spatial distributions and examining the temporal changes constitute an important basis for studies carried out in economic, ecological, social, military and many other fields(Sertel et al., 2018). Researchers such as; Di Gregorio and Jansen.(2000) Karnieli and Rozenstein.(2011) stated that land cover and land use are used together. However, while emphasizing the importance of clearly defining these terms, they stated that land cover is the physical and biological surface cover on the ground such as discontinuous urban fabrics, forests, agricultural lands, semi-natural areas, and water resources.

On the other hand, the land use refers to human activities in areas described as settlement, industry, trade, agriculture, forestry and recreation (Kaya et al., 2020). More effective and sustainable land management can be achieved by detecting and monitoring land use/cover properties. One of the most widely applied methods in land use/cover is the CORINE system (Sarı and Özşahin, 2016). Several studies have been conducted to determine the current status and temporal change of land use/cover in Türkiye (Özdemir and Bahadır, 2008; Gülersoy, 2013; Gülersoy, 2014; Kaya and Toroglu, 2015: Bayrak et al., 2021; Timur et al., 2021). The use of Geographic Information System (GIS) and Remote Sensing (RS) techniques has increased rapidly in land cover/use change studies and has contributed to studies. Remote sensing is of great importance in the determination and numerical inquiry of land cover/use changes (Üzülmez, 2021).

As can be understood from the studies mentioned above, processes such as the eco-systemic changes of the natural environment, the effects of human intervention on the environment, the current state of the environment and its temporal changes can be determined. In the clear and understandable determination of these processes by the researchers; Geographical information systems(GİS), remote sensing(RS) techniques as well as programs and models other created for purposes are used. Thus, human can obtain significant knowledges thanks to advanced programs, models and techniques. In the light of this knowledges, human can easily learn the eco-systemic changes, temporal changes and the effects of human intervention in the natural environment where he lives in. Thanks to this knowledges, human can gain the opportunity to live in a sustainable natural environment compatible with the environment. In here, the basin of Kesis Stream was chosen as the research area. Basin of Kesis Stream; It is located in the southern of Türkiye with its rugged topographic structure consisting of steep and deep valleys (Figure 1). Spatial and temporal change of land use/cover on such a basin is the main subject of the research. For this reason, the current and temporal environmental change of the area will be examined by analyzing the coordination of information on the environment(CORINE) land use/cover data of the basin.

MATERIALS AND METHODS

Materials

By using RS techniques by the European environment agency, land cover classification is made with the help of satellite images called CORINE. CORINE has created forty-four(44) land cover classifications for this purpose. Positive and negative interventions by human beings to the environment and nature's own eco-system changes can be obtained by using CORINE data. Detection of land cover changes, especially in development; It is of great importance in making economic, ecological and social decisions. Therefore, the analysis and evaluation of the CORINE data of the research area was deemed appropriate. CORINE data should be used by geographers, urban planners, environmental engineers, and all branches related to human and nature.

Alos-Palsar (12.5 x 12.5) meter resolution physical map was used as location map to determine the general topographic view of the study area (Figure 1). CORINE land use/cover data for the years 1990-2000-2006-2012-2018(https://land.copernicus.eu/pan-european/corine-land-cover, 2022), with the help of programs and modules such as Arc.Map, Excell, Coral-Drawn, various shapes, graphics and tables produced in accordance with the purpose create research materials.

Area of Study

The study area is located in the Mediterranean region in southern Türkiye, between 37° 19′ 00″- 37° 51′ 00″ north latitudes and 36° 12′30″- 36° 36′50″east longitudes(Figure 1) (Karaosmanoglu et al., 2022). This area covers 826.49km^2 and has a very rugged structure and an altitude increasing from 163 m to 2300 m from south to north. It has steep and deep V-shaped valleys carved by rivers. When it is examined from the climate characteristics, it is seen that its temperature decreases from 19 °C to 12.4 °C from south to north and a decrease is detected in annual average temperature values. The precipitation values of the basin, on the other hand, increase from 743.2 mm to 1473 mm from south to north. In the basin of the Kesis stream, soil formation (pedogenesis) realize according to these climatic characteristics. Under the effects of the Mediterranean climate conditions that are effective in the basin; brown forest soils, red Mediterranean soils, and alluvial and colluvial soils formed in areas where hydrographic processes were effective. The vegetation of the study area, on the other hand, formed under the effects of the basin's landforms, climatic characteristics, soil types forming accordingly, and these three factors. Accordingly, plant species such as maquis, Pinus brutia, Pinus nigra, Cedrus libani from south to north are widely distributed in the basin (Karaosmanoglu et al., 2022).

Methods

The methods used in the study included the model flow of CORINE land use/cover created in the basin by the purpose, acquisition of CORINE land use/cover data with the help of RS techniques, processing of the CORINE data in integrated GİS based on Arc.Map, as well as the use of Coral-Drawn and Excel programs. The model flow of the aforementioned CORINE land use/cover is presented below (Figure 2).

RESULTS AND DISCUSSIONS

A previous study conducted in the basin of Kesis Stream (Karaosmanoglu et al., 2022) reported that climate, landforms, soil, and vegetation were effective on land use/cover. According to this study, it is possible to assert

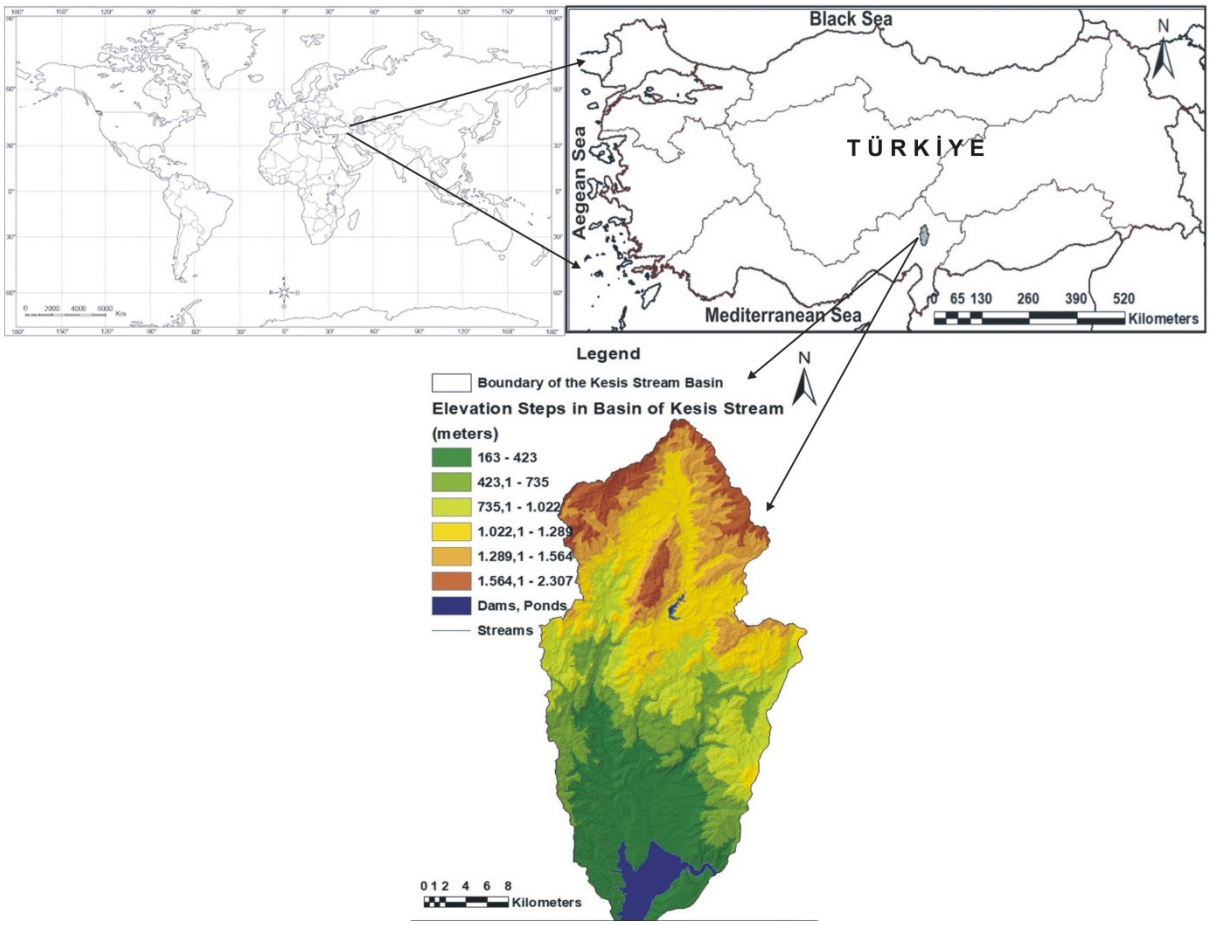

Figure 1. Location map of the Study Area(Karaosmanoglu, et al., 2022)
(Source: https://asf.alaska.edu/data-sets/sar-data-sets/alos-palsar)

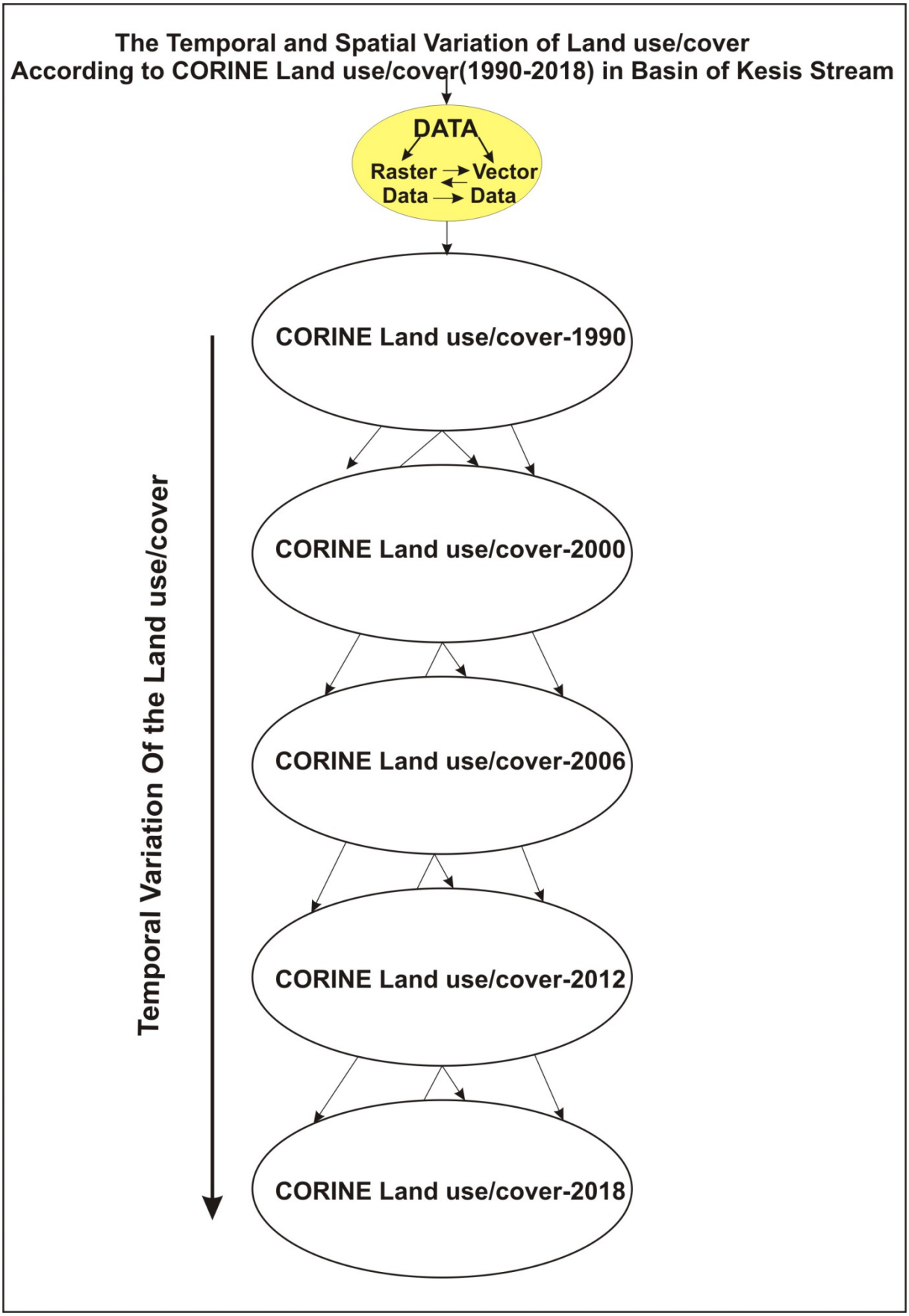

Figure 2. Model Flow of the CORINE Land use/cover in the basin of Kesis Stream (1990-2018)

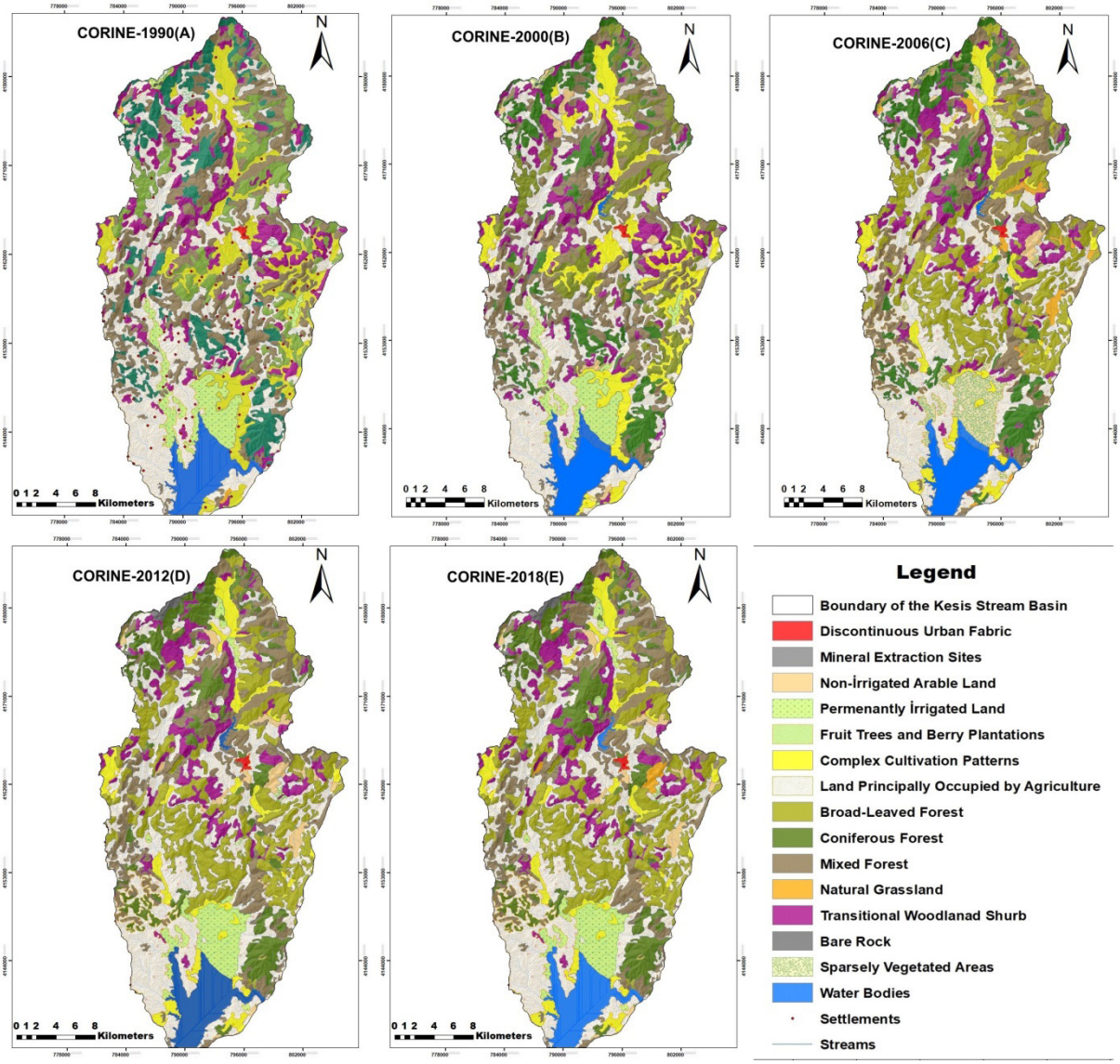

Figure 3. Area Distribution of the CORINE Land use/cover in the basin of Kesis Stream (CORINE 1990/A, CORINE 2000/B, CORINE 2006/C, CORINE 2012/D, CORINE 2018/E).

that climate and landforms have a fundamental effect, especially on the formation of land use/cover in the basin. Thus, it can be asserted that land class elements such as soil and vegetation in the basin are also shaped under the control of climate and landforms. In this context, the temporal and spatial changes of the CORINE land use/cover classes of the basin will be discussed below.

The areas occupying a large area in the basin according to the CORINE(1990-2018) 28-year temporal data of the study area included areas used for agricultural purposes, forest lands with intense vegetation, natural grassland, water bodies, bare rock, sparsely vegetated areas and discontinuous urban fabric and the lands covering a limited area. According to CORINE land use/cover, major changes were observed in the presence of land use/cover in the basin of Kesis Stream in the period of 1990-2018(Figure 3).

Area Distribution of the CORINE Land use/cover-1990 in the basin of Kesis Stream

According to the data of CORINE-1990 in the Basin of Kesis Stream, it was determined that agricultural lands and forest lands covered with vegetation covered a large area (Table 1, Figure 3). In the basin, the lands covering a smaller area were natural grasslands, bare rocks, water bodies, sparsely vegetated areas and discontinuous urban fabrics (Table 1, Figure 3/A). In the basin, the discontinuous urban fabric had an area of 1.09 km^2 (0.13%) the non-irrigated arable land had an area of 1.69 km^2 (0.20%), the permanently irrigated land had an area of 39.17 km^2 (4.74%), the complex cultivation patterns had an area of 101.98 km^2 (12.35%), lands principally occupied by agriculture had an area of 225.20 km^2 (27.25%), broad-leaved forest lands had an area of 75.81 km^2 (9.18%), coniferous forest lands had an area of 87.40

Table 1. Area and Ratio Distribution of the CORINE Land use/cover-1990 in the basin of Kesis Stream.

Code-1990	Land use/cover Classes	Area(km$^{2)}$	Ratio (%)
112	Discontinuous urban fabric	1.09	0.13
211	Non-Irrigated Arable land	1.69	0.20
212	Permanently irrigated land	39.17	4.74
242	Complex Cultivation Patterns	101.98	12.35
243	Lands principally occupied by agriculture	225.20	27.25
311	Broad-leaved Forests	75.81	9.18
312	Coniferous Forest	87.40	10.57
313	Mixed Forest	155.35	18.80
321	Natural Grassland	7.47	0.90
324	Transitional Woodland Shrub	91.73	11.09
332	Bare Rock	0.27	0.03
333	Sparsely Vegetated areas	1.74	0.21
512	Water Bodies	37.59	4.55
	Total Area of the Land use/cover	**826.49**	**100**

km^2 (10.57%), mixed forest lands had an area of 155.35 km^2(18.80%), natural grassland had an area of 7.47 km^2 (0.90%), transitional woodland shrub areas had an area of 91.73 km^2 (11.09%), bare rocks had an area of 0.27 km^2 (0.03%), lands with sparse vegetation had an area of 1.74 km^2 (0.21%), and water bodies had an area of 37.59 km^2 (4.55%) (Table 1, Figure 3/A).

Considering these data in the study area, it was determined that while the total area of agricultural lands was 368.04 km^2 (44.53%), the total area of forest lands was 410.29 km^2 (49.64%). While discontinuous urban fabric areas, bare rocks and natural grasslands had a very limited area and percentage in the basin, water bodies were 37.59 km^2 (4.55%) (Table 1, Figure 3/A). According to the CORINE-1990 land use/cover classification of the basin, it was determined that forest lands occupied the most, followed by agricultural lands. The sum of forest lands and agricultural lands was 94.17% with an area of 778.33 km^2. Here, the other land use/cover classes of the basin had very limited area and proportional values such as only 5.84% with an area of 48.16 km^2.

Area Distribution of the CORINE Land use/cover-2000 in the basin of Kesis Stream

According to CORINE-2000 data, agricultural lands and forest lands covered with vegetation showed a wide distribution in the study area, similar to CORINE-1990 data (Table 2, Figure 3/B). In the basin, water bodies, grasslands, bare rocks, sparsely vegetated areas and discontinuous urban fabrics covered limited areas (Table 2, Figure 3/B).

According to table 2 data in the study area, the total area of non-irrigated arable lands, permanently irrigated lands, complex cultivation patterns and lands principally occupied by agriculture was 363.9 km^2 (44.03%), while the total area of broad-leaved forests, coniferous forests, mixed forests and transitional woodland shrubs was 418.25 km^2 (50.60%). While forest lands corresponded

Table 2. Area and Ratio Distribution of the CORINE Land use/cover-2000 in the basin of Kesis Stream

Code-2000	Land use/cover Classes	Area(km$^{2)}$	Ratio(%)
112	Discontinuous urban fabric	1.09	0.13
211	Non-Irrigated Arable land	1.19	0.14
212	Permanently irrigated land	36.50	4.42
242	Complex Cultivation Patterns	101.72	12.31
243	Land principally occupied by agriculture	224.49	27.17
311	Broad-leaved Forest	80.55	9.75
312	Coniferous Forest	84.83	10.26
313	Mixed Forest	157.24	19.02
321	Natural Grassland	7.47	0.90
324	Transitional Woodland Shrub	88.16	10.67
332	Bare Rock	0.27	0.03
333	Sparsely Vegetated areas	1.74	0.21
512	Water Bodies	41.24	4.99
	Total Area of the Land use/cover	**826,49**	**100**

to half of the basin, agricultural lands covered a large area in second place (44.03%). In the basin, total area of forest lands and agricultural lands was 782.15 km^2 (94.64%) and very large area. The study area covered a very limited area such as 6.26% with an area of 51.81 km^2 in discontinuous urban fabrics, sparsely vegetated areas, natural grasslands, bare rocks, and water bodies. When CORINE-1990 and CORINE-2000 data were compared, it was determined that there was a decrease of 4.14 km^2 in the total area of agricultural lands and an increase of 7.96 km^2 in the total of forest lands (Table 1, 2). The discontinuous urban fabrics, sparsely vegetated areas, natural grasslands, bare rocks and water bodies covered an area of 48.16 km^2 according to CORINE-1990 data, while there was a decrease of approximately 3.82 km^2 with an area of 44.34 km^2 in CORINE-2000 data (Table 1, 2). Thus, it is found that while a limited decrease was observed in agricultural lands in the temporal period from 1990 to 2000, there was a partial increase in forest lands.

Area Distribution of the CORINE Land use/cover-2006 in the basin of Kesis Stream

According to CORINE-2006 data, agricultural lands and forest lands covered with vegetation covered a large area in the basin, similar to previous years (Table 3, Figure 3/C). In the basin, water bodies, natural grasslands, bare rocks, sparsely vegetated areas and discontinuous urban fabrics showed a limited distribution (Table 3, Figure 3/C). When CORINE-2000 and CORINE-2006 data were compared, it was determined that total area of non-irrigated arable lands, permanently irrigated lands, complex cultivation patterns and lands principally occupied by agriculture were 363.9 km^2 according to CORINE-2000 data and 332.07 km^2 according to CORINE-2006 data (Tables 2 and 3); accordingly, there was a decrease of 31.83 km^2. In this case, the area of broad-leaved forests, coniferous forests, mixed forests and transitional woodland shrubs increased from 418.25 km^2 to 444.2 km^2, resulting an increase of 25.95 km^2 (Table 3, Figure 3/C).

In the study area, total area of agricultural and forest lands was 782.15 km^2 (94.64%) based on CORINE-2000 data and 776.27 km^2(93.93%) based on the data of CORINE-2006 showing a limited decrease. In the basin, discontinuous urban fabrics, sparsely vegetated areas, natural grasslands, bare rocks and water bodies covered an areas of 44.34 km^2 based on CORINE-2000 data and 50.02 km^2 based on CORINE-2006 data, resulting in a slight increase of 5.68 km^2.

Area Distribution of the CORINE Land use/cover-2012 in the basin of Kesis Stream

The CORINE-2012 data indicated that agricultural lands and forest lands covered with vegetation covered a large area of 770.97 km^2 in total (93.28%) in the study area similar to previous years (Table 4, Figure 3/D). In the basin, water bodies, natural grasslands, bare rocks, sparsely vegetated areas and discontinuous urban fabrics had a limited area covering 55.52 km^2 (6.71%) (Table 4, Figure 3/D).

When CORINE-2006 and CORINE-2012 data were compared, it was determined that while there was an increase of 7.13 km^2 from 332.07 km^2 to 339.2 km^2 in agricultural lands, there was a decrease of 12.43 km^2 in forest lands from 444.2 km^2 to 431.77 km^2. While water bodies, natural grasslands, bare rocks, sparsely vegetated areas and discontinuous urban fabrics had an area of 50.02 km^2 according to CORINE-2006 data, they had an area of 55.52 km^2 according to CORINE-2012 data and there was an increase of 5.5 km^2 between both data. When these data were analyzed, it was determined that while agricultural lands showed a partial increase in the previous six (6)-year period, forest lands showed a partial decrease for this period. In addition, a very limited increase was observed in the total area of water bodies,

Table 3. Area and Ratio Distribution of the CORINE Land use/cover-2006 in the basin of Kesis Stream

Code-2006	Land use/cover Classes	Area(km^2)	Ratio(%)
112	Discontinuous urban fabric	1.20	0.14
211	Non-Irrigated Arable land	12.71	1.54
212	Permanently irrigated land	37.70	4.56
222	Fruit Trees and Berry Plantations	0.35	0.03
242	Complex Cultivation Patterns	47.57	5.75
243	Land principally occupied by agriculture	233.74	28.28
311	Broad-leaved Forest	155.69	18.84
312	Coniferous Forest	81.42	9.85
313	Mixed Forest	117.83	14.27
321	Natural Grassland	5.05	0.62
324	Transitional Woodland Shrub	89.26	10.80
332	Bare Rock	1.14	0.14
333	Sparsely Vegetated areas	4.43	0.54
512	Water Bodies	38.40	4.64
	Total Area of the Land use/cover	**826.49**	**100**

natural grasslands, bare rocks, sparsely vegetated areas and discontinuous urban fabrics compared to the previous period.

vegetated areas and water bodies had a very limited area of 52.9 km^2 (6.4%) in total (Table 5, Figure 3/E).

Table 4. Area and Ratio Distribution of the CORINE Land use/cover-2012 in the basin of Kesis Stream.

Code-2012	Land use/cover Classes	Area(km^2)	Ratio(%)
112	Discontinuous urban fabric	1.35	0.16
211	Non-Irrigated Arable land	12.87	1.56
212	Permanently irrigated land	37.48	4.54
242	Complex Cultivation Patterns	56.20	6.80
243	Land principally occupied by agriculture	232.65	28.15
311	Broad-leaved Forest	146.20	17.69
312	Coniferous Forest	87.11	10.54
313	Mixed Forest	119.02	14.40
321	Natural Grassland	5.05	0.61
324	Transitional Woodland Shrub	79.44	9.61
332	Bare Rock	3.66	0.44
333	Sparsely Vegetated areas	4.94	0.60
512	Water Bodies	40.52	4.90
	Total Area of the Land use/cover	**826.49**	**100**

Area Distribution of the CORINE Land use/cover-2018 in the basin of Kesis Stream

According to the CORINE-2018 land use/cover data in the basin of Kesis Stream, broad-leaved forests, coniferous forests, mixed forests, and transitional woodland shrubs had an area of 446.39 km^2 (54.01%,), thus, total area of forest lands took place on the top; whereas, the total area of non-irrigated arable land, permanently irrigated areas, orchard areas, and lands principally occupied by agriculture was 327.2 km^2 (39.59%), leading agricultural lands to be ranked as the second (Table 5, Figure 3/E). In the basin, discontinuous urban fabrics, mineral extraction areas, natural grasslands, bare rocks, sparsely

When the CORINE-2012 and CORINE-2018 data of the study area were compared, it was determined that while an increase of 14.62 km^2 was observed in forest lands compared to the previous one, there was a decrease of 12 km^2 in agricultural lands (Table 4, 5). The total area of land use/cover classes such as discontinuous urban fabrics, natural grasslands, bare rocks, sparsely vegetated areas and water bodies covered an area of 55.52 km^2 according to CORINE-2012; whereas, according to CORINE-2018, the total area of land use/cover areas including discontinuous urban fabrics, mineral extraction sites, natural grassland, bare rocks, sparsely vegetated areas, and water bodies was 52.9 km^2, showing a very limited decrease of 2.62 km^2. Considering

Table 5. Area and Ratio Distribution of the CORINE Land use/cover-2018 in the basin of Kesis Stream

Code-2018	Land use/cover Classes	Area(km^2)	Ratio(%)
112	Discontinuous urban fabric	1.35	0.16
131	Mineral Extraction Sites	0.38	0.04
211	Non-Irrigated Arable land	12.87	1.56
212	Permanently irrigated land	37.48	4.54
222	Fruit Trees and Berry Plantations	0.35	0.04
242	Complex Cultivation Patterns	46.09	5.58
243	Land principally occupied by agriculture	230.41	27.88
311	Broad-leaved Forest	166.72	20.18
312	Coniferous Forest	92.62	11.20
313	Mixed Forest	116.05	14.04
321	Natural Grassland	5.05	0.61
324	Transitional Woodland Shrub	71	8.59
332	Bare Rock	3.66	0.44
333	Sparsely Vegetated areas	1.94	0.24
512	Water Bodies	40.52	4.90
	Total Area of the Land use/cover	**826.49**	

Table 6. Area Distribution of the CORINE Land use/cover (1990-2018) in the basin of Kesis Stream

CORINE Land use/cover Classes	CORINE-1990(km²)	CORINE-2000(km²)	CORINE-2006(km²)	CORINE-2012(km²)	CORINE-2018(km²)
Discontinuous Urban Fabric	1.09	1.09	1.2	1.35	1.35
Mineral Extraction Sites	0	0	0	0	0.38
Non-Irrigated Land	1.69	1.19	12.71	12.87	12.87
Permanently Irrigated Land	39.17	36.5	37.7	37.48	37.48
Fruit Trees and Berry Plantations	0	0	0.35	0	0
Complex Cultivation Patterns	101.98	101.72	47.57	56.2	46.09
Land Principally Occupied by Agriculture	225.2	224.49	233.74	232.65	230.41
Broad-Leaved Forest	75.81	80.55	155.69	146.2	166.72
Coniferous Forest	87.4	84.83	81.42	87.11	92.62
Mixed Forest	155.35	157.24	117.83	119.02	116.05
Natural Grassland	7.47	7.47	5.05	5.05	5.05
Transitional Woodland Shrub	91.73	88.16	89.26	79.44	71
Bare Rock	0.27	0.27	1.14	3.66	3.66
Sparsely Vegetated Areas	1.74	1.74	4.43	4.94	1.94
Water Bodies	37.59	41.24	38.4	40.52	40.52
Total of the Study Area	**826.49Km²**	**826.49Km²**	**826.49 Km²**	**826.49 Km²**	**826.49 Km²**

all these explanations, CORINE-2018 data on the Basin of Kesis Stream indicated that an area of 773.59 km² of forest lands and agricultural lands corresponded to the majority of the basin (93.60%). Other land use classes in the field, on the other hand, remained very limited with an area of 52.9 km² (6.4%) (Table 5, Figure 3/E).

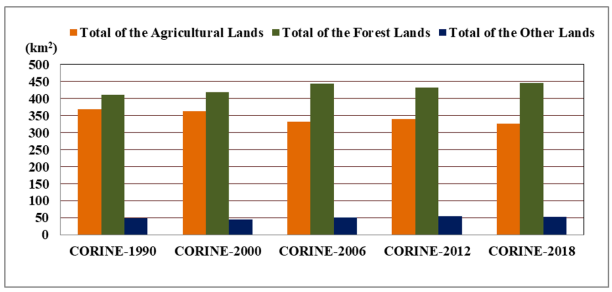

Figure 4. The total areas of the Agriculture Lands, the Forest lands and the other Lands according to CORINE-1990-2018 data in the basin of Kesis Stream.

When the CORINE (1990-2018) 28-year land use/cover of the study area was analyzed together, it was determined that while residential areas showed a partial increase, non-irrigated arable areas showed a significant increase about seven and a half (7.5) times. Permanently irrigated agricultural lands, on the other hand, generally showed a stable course with minor ups and downs. Again, in the same period, complex cultivation patterns in the basin showed a serious decrease since 2000, and by 2018, it decreased by more than half with an area of 46.09 km². In the same period, although there was no significant change, a partial increase was observed in priority areas in agriculture. While there was a partial increase in broad-leaved forest lands between 1990 and 2000, there was an increase of more than two times in 2006. Although there was a partial decrease in 2012, a significant increase was realized with an area of 166.72 km² again in 2018. There was little fluctuation in a total area of the coniferous forest until 2012; however, a partial increase was observed in 2018.

While a stable course was observed in total area of mixed forests between 1990 and 2000, there was a serious decrease in 2006, a partial increase in 2012, a partial decrease in 2018, and a significant decrease in the last 18 years in general. Transitional woodland shrubs showed a gradual decrease over time (Table 6). There was a partial decrease in the natural grasslands in the basin; whereas, a partial increase was observed in the sparsely vegetated areas. Spatial changes in natural grasslands and sparsely vegetated areas can be interpreted as a sign of partial drought depending on climate change. In addition, in the context of climate changes, erosion and transport activities increase on steep slopes of the basin due to irregularities such as sudden rapid and flooding precipitation. As a result, it can be asserted that the area of bare rocks in the basin has increased. While the surface area of water in the basin increased by 3.65 km² from 1990 to 2000, there was a partial decrease in 2006. In the period between 2012 and 2018, it is observed that there is a partial increase again and it covers an area of 40.52 km²(Table 6). The reason for the increase in the surface areas of the water in the basin, the surface area of the water has increased thanks to the ponds built on the area since 2000. It can be said that factors such as lack of precipitation due to climatic changes and drought are effective in the narrowing of water surfaces. when the land use/cover was classified as agricultural lands, forest lands and other areas in the study area, significant changes took place in the 28-year time period (Figure 4). Here, while a gradual decrease occurred in the total

area of agricultural lands, the total area of forest lands increased gradually (Figure 4). While the grassland areas, which are expressed as other areas, showed a partial decrease, the vegetation, sparse areas, bare rocks and water bodies showed partial increases.

CONCLUSION

As a result, the CORINE data of the Kesis Stream basin successfully reflected the land classification and changes of the area in the 1990-2018 period. During this time period, while the forest lands of the area gradually expanded in area, the agricultural lands, on the contrary, gradually narrowed. In the same process, It has been determined that no negative interventions such as serious deforestation and wrong land use have been made by human beings to the environment. In fact, the rugged topographic structure of the basin, consisting of steep and deep valleys, has limited environmental pollution and adverse human intervention. Again in this process, although the basin has a limited area, While dry agricultural lands and bare surface lands have expanded, natural grasslands have narrowed. While the water bodies have expanded with the pond structures, the bodies of the water have narrowed in some periods. The spatial variation of these surfaces proves that global climate change has partial effects on the field. According to these data, According to these data, the plans, projects and investments to be made in the basin today and in the future should be sustainable by preserving the current balance without polluting the field.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

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

Author contribution

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

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RESEARCH ARTICLE

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The biological activity features and mineral element analyses of some *Inula* L. species exhibit natural spread in Mugla (Turkiye)

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Abstract

Medicinal and aromatic plants (MAPs) are rich in nutrients and alternative therapies. Some MAPs become industrial crops that are grown around the world for their nutritional and medicinal properties. The aim of this study was to assess the relationship between mineral nutrient content and antioxidant properties of *Inula viscosa* (*I. viscosa*) and *Inula graveolens* (*I. graveolens*) species found in the Köyceiz region of Muğla province. In this study, the antioxidant activity values of the extracts obtained were found to be the highest in methanol and acetone extracts of *Inula viscosa*. In contrast, the lowest in hexane extracts of *Inula graveolens* species. It was determined that the methanolic extract of *I.viscosa* had the highest 137.1 (µg PE /mg) a and the hexane extract of *I. graveolens* L. had the lowest 22.40 (µg PE /mg) total phenolic content. On the other hand, the mineral content of the species (macro (%): N, P, K, Ca, Mg, and micro (ppm): Fe, Mn, Zn, Cu, B) were also taken into consideration. As a result, it was observed by the analysis that there was a significant interaction between the antioxidant activity values of the species and their mineral nutrition. The antioxidant activities of plants are influenced by a variety of factors. The plant's activity is influenced by a number of variables, including the time of harvest (flowering, seed formation, etc.), extraction technique, solvent polarity, fresh or dry plant material, mineral nutrient content, and method. It is thought to broaden perceptions of these plants beyond their nutritional value by putting the antioxidant effects of the plant on a scientific basis. In this study, *Inula graveolens* L. and *Inula viscosa* L. demonstrated the potential of plant extracts as a readily available source of natural antioxidants, potential food additives, pharmaceuticals, and pharmaceutics.

Keywords: Antioxidant activity, DPPH, *Inula* L., Mineral nutrition, Phenolic content

INTRODUCTION

Plants have been used for treatment for thousands of years and the amount of these plants has been increasing continuously since ancient times. Herbal medicines form a vital part of the culture and traditions of rural communities in developing countries. It is known that in the ages when medicine was not as developed as it is today, people used plants that grow naturally in nature (Berber, et al., 2013). Today, most of the world's population uses plants as pharmaceutical raw materials. Especially in developing countries, 80% of the population meets their health needs from traditional medicinal plants in the first place. Approximately 25% of prescription drugs in developed countries are chemicals of herbal origin (Farnsworth, 1990). In Turkey, as in all countries, plants are considered as food, tea, resin, dye, spice, and phytotherapeutic resources among

the people. The depletion of natural riches and countries' economic problems have made using natural products widespread, no matter how far technology, science, and medicine have advanced. When the World Health Organization (WHO) data are examined, the amount of medicinal plants used for therapeutic purposes is around 20,000. This number is around 500 in Turkey (Çınar, 2012; Arıkan, 2019). Plant-derived secondary metabolites, which have the potential to treat various diseases, are divided into many classes such as flavonoids, phenolic acids, phenolic glycosides, unsaturated lactones, phenylpropanoids, lignins, terpenoids, and steroids. These compounds have many applications in the food, cosmetic and pharmaceutical industries (Banerjee and Bonde, 2011).

Today, although medicinal plants require longer treatment, they are met with great interest since they are more natural than synthetic drugs and do not have many side effects (Silinsin, 2016). In this study, which was carried out considering the aforementioned reasons, *Inula graveolanes* L. and *Inula viscosa* L., the natural species of Muğla province and its districts, were preferred. There are nearly 100 species belonging to the genus *Inula* L. from the *Asteraceae* family, and it is known to spread mainly in Asia, Africa, Europe, and the Mediterranean Region. There are also annual species of members of the genus *Inula* L., known as perennial. There are 27 species in our country, and 7 of them are endemic. Some members of *Inula* L. species are used effectively in traditional medicine worldwide. Numerous biological activities, including anticancer, antibacterial, hepatoprotective, cytotoxic, and anti-inflammatory properties, are associated with the genus *Inula* (*Asteraceae*) (Zhao et al., 2006).

The demand for more efficient ways to deliver life's basics drove the industrial revolution. Biotechnology approaches are currently being employed in agriculture to create new and more efficient food, medicinal, and energy products by manipulating organisms. Ecological factors have been considered in plant cultivation since ancient times. Environmental factors affect medicinal plants more compared to that cultivated plants. This is because the quality of medicinal plants is at least as important as the yield, and even those below the quality limit are not grown regardless of the yield. Moreover, for the safe use of these plants, the sustainability of the study needs to determine the content and amounts of mineral substances and the soil values of the environment where they are grown. In summary, significant interactions were observed between secondary metabolites and mineral nutrition.

MATERIALS AND METHODS

Plant materials and extraction techniques

Through the collection phase of the plants used in the study, the Flora of Turkey (Davis, 1984; 2000) is used for the locality of the plants and the resources related to the previous flora studies within the province of Muğla were scanned so that plats were collected without damaging the ecological balance elements and natural structure. A field study program was prepared by considering factors such as flowering time, spread areas, altitude, and habitat of these species. In light of this information, *Inula viscosa* L. and *Inula graveolens* L. species, which are members of the genus *Inula* L., were collected in October 2020 from the Ağla Highland, located within the borders of Köyceğiz district of Muğla province. Diagnosis of plants was carried out by Dr. Kenan AKBAŞ and Dr. Olcay CEYLAN (Muğla Sıtkı Koçman University, Department of Biology); hence, their diagnosis was prepared following the herbarium and added to the collection.

Extracts of the collected species prepared in different solvents (hexane, acetone, methanol) were appropriately prepared for analysis in order to determine the antioxidant activity studies. In order to determine macro and micro nutrients, plant, and soil samples were taken from each locality under appropriate conditions. After the preliminary stages of mineral analysis were carried out in our laboratories, support was received from Muğla Sıtkı Koçman University Research Laboratories Center for the instrument reading phase. The names of the plant species that are the subject of the study, the localities where they were collected, the date of collection, and the herbarium codes are shown in Table 1 below.

The plants collected and brought from their locations were dried in the shade and at room temperature with little air circulation. The dried plant samples were ground with a blender, and 10 g of each sample was weighed and extracted twice in 175 mL solvent (acetone, hexane, and methanol) for 24 and 48 hours. The mixture formed as a result of the extraction was filtered through filter paper, the solvents were evaporated at 50°C in a rotary evaporator, and the remaining extract was lyophilized and extracted. The remaining extracts were stored at -20 °C until analysis.

Table 1. Collection localities of plant materials

Species Name	Locality	Altitude	Collection Date	Herbarium Code
Inula viscosa L. Aiton	Köyceğiz Ağla Highland 36°59'47"N / 28°42'38"E	178 m	October 2020	O.2116
Inula graveolens L. Desf.	Köyceğiz Ağla Highland 36°59'52"N / 28°42'36"E	217 m	October 2020	O.2115

Antioxidant Activity

The β-carotene-linoleic acid test system developed by Marco (1968) with minor modifications was used to assess lipid peroxidation inhibition activity (Tel-Cayan and Duru, 2019). As previously described, the DPPH activity was measured spectrophotometrically (Blois, 1958). With slight adjustments (Tel et al., 2012), $ABTS^{+}$ activity was calculated, as Re et al. (1999) mentioned. (Tel et al., 2012). Apak et al. (2004)'s method was used to determine the cupric-reducing antioxidant capacity (CUPRAC). To compare the $ABTS^{+}$, DPPH, β-carotene-linoleic acid, and CUPRAC assays, BHA and tocopherol were used as antioxidant standards. The metal chelating activity of plant extracts for Fe^{2+} was measured using spectrophotometry (Decker and Welch, 1990). The reference compound for activity comparison was EDTA. The antioxidant activity results were reported as the 50% inhibitory concentration.

Total phenolic and total flavonoid content analysis

The total amount of phenolic substance of the plant extract, 1mL extract solution, 45mL distilled water, and 1ml Folin-Ciocalteu reagent were placed in test tubes. After 3 minutes, 3 ml of 2% Na_2CO_3 solution was added. The mixture, left to incubate for 2 hours at room temperature, was stirred at regular intervals. The absorbance values of the samples were read at 760 nm (Slinkard and Singleton, 1977).

The total amount of flavonoid substance, 4 mL of distilled water, 1 mL of standard quercetin solutions and plant extract, and 0.3 mL of 5% sodium nitrate were placed in test tubes and incubated for 5 minutes at room temperature conditions. After the incubation, 0.3 mL of 10% aluminum chloride was added and kept in room temperature conditions without light for 5 more minutes. At the end of the incubation period, 2 mL of 4% sodium hydroxide and 2.4 mL of distilled water were added, and the absorbance values were read at 415 nm (Onar, 2015).

Analysis of mineral elements

Soil properties and elemental analysis methods

Analyzes applied to soil samples; using the texture hydrometer test, lime; based on calcimetric, organic matter (Walkley and Black, 1934) method, available Zn, Fe, Mn, and Cu; DTPA (diethylene-triamine-penta-acetic-acid) method (Lindsay and Norvell, 1978), available K, Ca and Mg content (Thomas, 1982), available Na content (Knudsen et al., 1982) were found in the extracts obtained with 1 N neutral ammonium acetate solution in atomic absorption spectrophotometer. In addition, the content of Water-Soluble Phosphorus was read calorimetrically in the spectrophotometer using the Bingham (1982) method.

Plant nutrient content analysis

According to Kacar (1992), nutrient element analysis of plant extracts was determined. P, K, Ca, Mg, and Na in the macro element class; Fe, Cu, Mn, Zn, and B contents, which are in the microelement group, were measured in the ICP-AES device at the specific wavelength of each element. The Kjeldahl method was used to determine total nitrogen in plant leaf samples. The data obtained are shown in % and ppm according to the dry matter principle.

Data analysis

Each bioassay measurement and absorbance was performed in triplicate. The results were recorded as the means ± standard error (SE) of the mean for three parallel measurements. MINITAB 16 was used for statistical analysis, and the ANOVA (variance analysis) procedure was used to determine significant differences between means, with $p<0.05$ considered significant.

RESULTS AND DISCUSSION

In this study, *Inula graveolens* (L.) DESF. and *Inula viscosa* (L.) AITON, which are plants belonging to the genus *Inula* L. of the *Asteraceae* family, were used in various solvents extraction with the β-carotene-linoleic acid system, DPPH method, ABTS method, CUPRAC method, and Metal chelation; hence, total antioxidant activity levels determined by its capacity and total phenolic and flavonoid substance contents were determined. In addition, the nutritional status and elemental contents of these plants with high medicinal value and the soil properties of the environment where they grow are also discussed in comparison with the analyzes conducted.

Total antioxidant activity of plants

The total antioxidant activity results determined by the methanol, acetone, and hexane extracts of *Inula graveolens* L. and *Inula viscosa* L. plants and the β-carotene-linoleic acid system, DPPH method, ABTS method, CUPRAC method, and metal chelating capacity are presented in Table 2.

According to the antioxidant activity results of the extracts of *Inula graveolens* L. and *Inula viscosa* L. plants, which are the subject of the study, determined by the β-carotene-linoleic acid system, *Inula viscosa* methanol extract showed the strongest antioxidant activity compared to the other tested extracts, with an IC50 value of 10.33 (µg/mL). The polarities of the solvents can be shown as the reason why the extracts obtained from the same plants with different solvents show very different antioxidant activities from each other. These various antioxidant activities of the extracts can be attributed to their effective hydrogen-donating abilities and free radical scavenging (Hayouni et al., 2007). Bayraktar (2019) determined the antioxidant activities of n-hexane and methanol extracts of *Inula graveolens* and *Inula viscosa* species and standard antioxidants with the β-carotene-linoleic acid system and reported that antioxidant activities increased with the increase in the

Table 2. Total antioxidant activity[a]

Antioxidant Activity								
	Period	Plant Species	Solvent	β-Carotene-Linoleic acid	DPPH·	ABTS·+	CUPRAC	Metal Chelating
				IC_{50} (µg/mL)	IC_{50} (µg/mL)	IC_{50} (µg/mL)	$A_{0.50}$ (µg/mL)	IC_{50} (µg/mL)
Extracts	24 hours	*Inula graveolens*	Acetone	23.61±0.82	30.71±0.41	25.43±0.65	23.66±0.47	280.3±1.35
	24 hours	*Inula graveolens*	Hexane	205.6±1.45	401.2±0.62	183.7±1.40	331.3±0.31	487.5±1.08
	24 hours	*Inula graveolens*	Methanol	19.51±0.31	25.65±0.75	20.55±0.53	17.61±0.18	205.6±1.85
	24 hours	*Inula viscosa*	Acetone	**13.54±0.48**	**28.88±0.35**	**12.34±0.28**	**16.47±0.63**	**184.2±1.96**
	24 hours	*Inula viscosa*	Hexane	195.3±1.89	355.3±1.73	168.3±1.05	291.8±1.21	365.1±1.90
	24 hours	*Inula viscosa*	Methanol	**11.51±0.25**	**19.81±0.36**	**10.25±0.14**	**12.14±0.27**	**154.1±1.44**
	48 hours	*Inula graveolens*	Acetone	21.47±0.18	28.51±0.50	21.38±0.38	23.80±0.51	282.5±1.65
	48 hours	*Inula graveolens*	Hexane	207.8±1.75	390.8±1.95	175.3±0.90	297.6±1.46	459.2±1.36
	48 hours	*Inula graveolens*	Methanol	18.75±0.56	21.33±0.23	17.85±0.42	15.83±0.17	194.3±0.97
	48 hours	*Inula viscosa*	Acetone	**13.41±0.20**	**26.51±0.61**	**12.05±0.21**	**12.85±0.36**	**177.5±1.25**
	48 hours	*Inula viscosa*	Hexane	196.7±1.29	362.4±0.90	155.8±1.70	258.9±0.86	361.0±1.86
	48 hours	*Inula viscosa*	Methanol	**10.33±0.80**	**17.63±0.34**	**9.58±0.22**	**11.93±0.19**	**139.2±1.37**
Std	BHA		α-Tocopherol	2.20±0.05	38.00±0.40	35.50±0.21	63.50±0.80	NT[b]
	EDTA			1.41±0.02	19.85±0.35	12.80±0.15	25.30±0.50	NT[b]
				NT[b]	NT[b]	NT[b]	NT[b]	5.80±0.50

a Values represent the means ± SEM of three parallel sample measurements ($p < 0.05$).
b NT: not tested.

concentrations of both plant extracts. In addition, *Crocus mathewii* and *Crocus biflorus* Mill. subsp. *isauricus* plants' different solvents in the study on the total antioxidant activities determined by the β-carotene-linoleic acid method displayed the highest antioxidant activity (83.1±1.75%) in *Crocus biflorus* Mill. subsp. *isauricus*' ethanolic extract of the underground part, the lowest antioxidant activity (27.05±0.93%) was observed in the extract of the above-ground parts of the *Crocus mathewii* species obtained with the same solvent (Yıldıztekin, 2015).

In the analysis using the DPPH (1,1-diphenyl-2-picryl hydrazyl) free radical scavenging activity determination method, the highest free radical scavenging activity value (24 h) of *Inula viscosa* was determined in the methanolic extract (19.81±0.36%). In contrast, the lowest free radical scavenging activity was determined in the lowest concentration of *Inula graveolens* hexane solvent (401.2±0.62). While the highest free radical scavenging activity was detected in the methanolic extract (17.63±0.34) of *Inula viscosa* species (48 h), the lowest free radical scavenging activity was measured in the lowest concentration of *Inula graveolens* hexane solvent (390.8±1.95%). For both periods (24 and 48 h), similar results were found in the same solutions (Table 2). The low IC50 value indicates that 50% of the free radical is removed in the DPPH free radical scavenging activity and that the antioxidant capacity is strong. The free radical scavenging activity of the extracts depends on the ability of the antioxidant compounds in the extract to give their hydrogen and the structural conformation of the compound (Fukumoto and Mazza, 2000). The DPPH radical, dark purple, gives the highest absorption at a wavelength of 517 nm. With the addition of an antioxidant to the DPPH solution, absorbance decreases, and the radical changes from purple to yellow with the effect of antioxidants (Huang et al., 2005). According to the method above, the highest scavenging value was determined in *Saponaria kotschyi* water (89.93±1.30) and the lowest in *Saponaria pumilio* water (5.17±2.39%) extract (Saraç, 2019).

ABTS activity, on the other hand, according to the BHA

standard, *Inula viscosa* methanol extract showed the strongest antioxidant activity compared to the other tested extracts, with IC50 values of 9.58 and 10.25 µg/mL, respectively, when both periods were taken into account. Following this, it was determined that *Inula graveolens* acetone extracts showed high activity with IC50 values of 12.05 and 12.34 µg/mL, respectively. In addition, acetone and methanolic extracts of *I.graveolens* and *I.viscosa* plants were determined to show high activities when the results of 24-hour and 48-hour data analysis were examined; however, stronger results were obtained through the activities of BHA and α-Tocopherol where the methanol extract used as standard antioxidants (Table 2). ABTS activity according to BHA standard, *Inula viscosa* methanol extract was determined to have the strongest antioxidant activity compared to other extracts tested when both periods were considered (Table 3). Erbil et al. (2018) found that the methanol extract of the fruit and leaf parts of the *Arum maculatum* plant was 54.8 ± 0.32% in the fruit and 41.9 ± 1.79% in the leaf. In the study by *Thymus cariensis* where Essential oil, hexane, acetone, and methanol extracts and the cation removal activity of the standards were determined using $ABTS^{+}$ cation, acetone, and methanol extracts reported high activities, he underlined that especially the methanol extract achieved higher results at the concentrations of 100 µg/ml, 200 µg/ml, and 400 µg/ml than the activities of BHA and α-Tocopherol, which they used as standard antioxidants, at the same concentrations (Küçükaydın, 2014). The determination of the highest values in the methanolic extract in both literature data showed parallelism with our study.

According to the CUPRAC analysis results, when the 24-hour and 48-hour data were examined, it was determined that the acetone and methanolic extracts of *I. graveolens* and *I.viscosa* plants showed high activity. However, especially BHA and α-Tocopherol activities where the methanol extract used as standard antioxidants displayed stronger results (Table 2.) The chelating capacity of Fe^{2+} ions of the extracts obtained from the species was calculated as the EDTA standard (mg EDTA/g) and the results are given in Table 2. As a result of the examination, while the highest activity (24 and 48 h) was observed in *I. viscosa* methanol extract, it was determined that the acetone extracts of the same plant species also had high activity. On the other hand, the lowest activity values were determined in *I. graveolens* hexane extract. The low absorbance values indicate that the metal ions are chelated before the ferrozine bonds; the metal chelating activity is high. Considering the 24-hour and 48-hour data according to the CUPRAC method, acetone and methanolic extracts of *I. graveolens* and *I. viscosa* plants were determined to show high activities (Table 2). Antioxidant activities of standards and methanolic extracts calculated using different solvents (essential oil, hexane, methanol) in *Inula graveolens* and *Inula viscosa* species at 100 µg/mL concentration (1.85% and 2.17%, respectively) displayed higher activity compared to BHA at 100 µg/mL concentration (1.70%). (Bayraktar, 2019). According to Table 2, the highest metal chelating activity (24 and 48 h) was observed in *I.viscosa* methanol and acetone extract. The low absorbance values indicate that the metal ions are chelated before the ferrozine bonds; that is, the metal chelating activity is high. The extracts' metal chelating activities differed depending on the solvent used. These findings are consistent with a previous report published in the literature (Kaska et al., 2019; Uysal et al., 2016).

Total phenolic and flavonoid contents

Phenolic compounds are highly soluble in polar solvents (Zhou and Yu, 2004), and ethanol is one of the best solvents for polyphenolic compounds that is also safe for human consumption (Shi et al., 2005; Naidoo et al., 2016). The total amount of phenolic substance was compared between species depending on the methanol solvent, the highest was determined in *Inula viscosa* species (48 h) (137.1), the lowest in *Inula graveolens* species (24 h) (118.7 (µg PE /mg)[a]. When the total amount of phenolic substance was evaluated depending on the acetone solvent, the highest was determined in *Inula viscosa* (48 h) (98.57), the lowest in *Inula graveolens* species (24 h) (90.58 (µg PE /mg)[a]. When the total amount of phenolic substance was evaluated depending on the hexane solvent, the highest was determined in *Inula viscosa* (48 h) (31.05), the lowest in *Inula graveolens* species (24 h) (22.40 (µg PE /mg)[a] (Table 3). In this context, it was concluded that the total amount of phenolic substances obtained in the species is related to both the type of solvents and the residence time. It is thought that there is a linear relationship between phenolic substances and antioxidant activity. Salim et al. (2017), extracts were prepared from the whole plant, leaf, stem, and flower parts of the plant (*Inula viscosa* L.) collected from Palestine using ethanol and methanol solvent, and the total phenolic content was examined. The results showed that methanolic extracts of all plant parts had higher total phenolic content and antioxidant activity than ethanolic extracts. However, the data presented in reference (Rhimi et al., 2017) showed that Tunisian-collected *Inula viscosa* methanol extracts had a higher value of total phenolic content (123.07 1.69 mg GAE/g extract). In addition, an EtOAc *Inula viscosa* sample from Morocco's Sefrou region was found to have a high total phenolic content value (274.4 6.94 mg GAE/g DW) (Chahmi et al., 2015).

When the total amount of flavonoid substance was analyzed depending on the methanol solvent, the highest in *Inula viscosa* (48 h) (95.78 (µg QEs /mg)[a], the lowest in *Inula graveolens* (24 h) (88.54 (µg QEs /mg))[a] has been determined. When evaluated depending on the acetone solvent, the highest was determined in *Inula viscosa* species (48 h) (80.12), the lowest in *Inula graveolens* species (24 h) (65.51 (µg QE /g)[a]. When looking at the hexane solvent, the highest was determined in

Table 3. Total phenolic content of plant extracts

Extracts	Period	Plant Extract	Solvent	Total phenolic amount	Total flavonoid amount
				µg PEs/mg extract[a]	µg QEs/mg extract[b]
	24 hours	*Inula graveolens*	Acetone	90.58±0.07	65.51±0.11
	24 hours	*Inula graveolens*	Hexane	22.40±0.05	13.25±0.05
	24 hours	*Inula graveolens*	Methanol	118.7±0.15	88.54±0.07
	24 hours	*Inula viscosa*	Acetone	98.35±0.10	79.65±0.15
	24 hours	*Inula viscosa*	Hexane	30.45±0.09	16.50±0.01
	24 hours	*Inula viscosa*	Methanol	133.8±0.12	93.50±0.08
	48 hours	***Inula graveolens***	**Acetone**	91.33±0.10	68.57±0.07
	48 hours	***Inula graveolens***	**Hexane**	22.85±0.06	13.41±0.04
	48 hours	***Inula graveolens***	**Methanol**	126.7±0.13	90.21±0.20
	48 hours	***Inula viscosa***	**Acetone**	98.57±0.12	80.12±0.18
	48 hours	***Inula viscosa***	**Hexane**	31.05±0.09	16.55±0.03
	48 hours	***Inula viscosa***	**Methanol**	137.1±0.04	95.78±0.05

aPEs: pyrocatechol equivalent; bQEs: quercetin equivalent

Inula viscosa species (48 h) (16.55), the lowest in *Inula graveolens* species (24 h) (13.25 (µg QE /g)[a] (Table 3). It has been determined in literature studies that flavonoids can prevent damage caused by free radicals in various ways (Panche et al., 2016). Gökbulut (2011) conducted pharmacognostic studies on some *Inula* species belonging to the *Asteraceae* family and naturally spread in our country.

According to the results of the study, it was determined that the species were rich in terpenic compounds and flavonoid amounts. Flower, leaf, and root parts of *Inula* species were examined separately, DPPH and ABTS in vitro tests, and antioxidant activity tests were performed from aqueous, methanol, and ethyl acetate extracts. He shared that activity levels, especially flavonoids, are higher in *Inula viscosa* than in other species. All this information supports our work.

Mineral nutrition contents

Determination of soil characteristics of species

Soil factor comes to mind when considering parameters such as productivity and quality in crop production. In some conditions, the excess or deficiency of one or more of the mineral elements negatively affects the intake of other elements. This situation affects product yield and quality negatively. Recently, the issue of determining the nutrient content of plants that are rich in medicinal and aromatic aspects, especially in our country, has started to attract attention. In light of this information, determining the nutritional status of the areas where the researched species spread and the content of macro and micronutrients in terms of their safe consumption when necessary were determined.

Considering the soil characteristics of the localities where the species grow; While the soil of *Inula graveolens* was low in salt and lime, clay textured, and rich in organic matter, the soil of *Inula viscosa* was found to be salt-free, clay loamy, slightly alkaline, and the organic matter content was moderate. The N content of the soil sample where *Inula graveolens* and *Inula viscosa* species were spread was determined as 0.15%. When the macro element contents of the localities of the species that are the subject of the study are examined, it was determined respectively, the P content (1-2 ppm); K (6.59-71.42ppm); Ca (656.12-7356.9 ppm); Mg (15.76-166.81 ppm) and Na (4-25.93 ppm). On the other hand, when the soil microelement contents were examined; it was determined respectively, Fe (2.76-34.63 ppm), Mn (39.58-12.28 ppm), Zn (0.22-0.15 ppm), Cu (1.1-0.37 ppm). The N content of the two soil samples is also 0.15%. (Table 4). The desired N level in soils has been reported as 0.11-0.15% (Chapman, 1973). The soil N level obtained in our study is in the reference range and is considered to be at an appropriate level.

The plant-available phosphorus (P) concentration of soil samples of all the species in question was determined to be between 1-2 ppm (Table 4). Olsen et al. (1954) determined as a result of their study that the P limit values should be between 7.1 and 25.0 ppm. In this case, it was determined that the % phosphorus content of both soil samples was at deficient levels. The available Potassium (K) concentration of the soil samples made in the research area has been determined to be between 6.59 – 71.42 ppm. The desired limit values in Cottenie (1980) soils should be between 201 - 250 ppm. However, all of the soil samples were found to be at deficient levels in terms of K (Table 4). The amount of available Calcium (Ca) in the soil

Table 4. Results of the species' soil analysis

Parameters		Soil sample from *Inula viscosa* locality	Soil sample from *Inula graveolens* locality
Structure (%)	**Loamy**	-	39
	Clay Loamy	53	-
pH		7.76	7.07
EC (dS/m)		0.83	0.41
Lime (%)		15.7	0.45
Organic Matter (%)		2.95	3.05
Macro Element (%)	**Nitrogen (N)**	0.15	0.15
Macro Elements (ppm)	**Phosphorus (P)**	2	1
	Potassium (K)	71.42	6.59
	Calcium (Ca)	7356.9	656.12
	Magnesium (Mg)	166.81	15.76
	Sodium (Na)	25.93	4
Micro Elements (ppm)	**Iron (Fe)**	34.63	2.76
	Manganese (Mn)	12.28	39.58
	Zinc (Zn)	0.15	0.22
	Copper (Cu)	0.37	1.1

sample of *Inula viscosa* L. type collected in the research area is very high; Magnesium (Mg) content was found to be sufficient. The Ca and Mg levels of *Inula graveolens* L. species determined in the soil were determined at very low values (Cottenie., 1980). However, the reference range of recommended microelements in soils is Cu (>0.2 ppm), Mn (15-20 ppm), Fe (6-10 ppm), and Zn (0.8-2.5 ppm) (Lindsay and Norwell, 1978). When the soil microelement contents were evaluated in general terms by looking at the reference intervals, it was determined that *Inula graveolens* L. species was at higher levels than *I.viscosa* species (Table 4). Although microelements are mineral elements that have various biochemical functions in living organisms and are vital for human health, they can have harmful effects when taken in high concentrations (Gürel, 2014). In the study conducted in Fethiye-Babadag, *Crocus mathewii,* and *Crocus biflorus* subsp. *isauricus* endemic species' soil nutrient content was investigated, macro elements were determined as respectively; N content (0.18-0.2%); P (5-2.33 ppm); K (403-213 ppm); Ca (3497-2972 ppm); Mg (124-138 ppm) and Na (25.83-21.43 ppm) and micronutrient contents were measured as respectively Fe (37.05-47.48 ppm), Mn (111-122 ppm), Zn (2.45-4.07 ppm), Cu (3.11-3.85 ppm) and B (1.12-0.9 ppm) (Yıldıztekin, 2015). The study in question showed a similar quality to our research, although there were differences in some parameters.

Determination of plant nutrient content of species

In order to determine the nutritional status of *Asteraceae* family members *Inula viscosa* L. and *Inula graveolens* L. species in their environment, which are naturally spread within the borders of Muğla province, their mineral content was compared with the analyzes made and examined (Table 5). In this context, the nitrogen content was determined as 0.28% in *Inula graveolens* species and 0.21% in *Inula viscosa* species. In addition, other macro element contents measured in leaves of *Inula graveolens* and *Inula viscosa* species and respectively were found as; P: 0.09% - 0.22%; K: 1.39%-1.2%; Ca: 0.35%-1.81% and Mg: 0.14%-0.43%. When the nitrogen content of *I.graveolens* and *I.viscosa* species was examined, it was determined that the values were 0.21-0.28%. In general, it is recommended that the N ratio in the structure of plants should be in the range of 1.5-5% (Kacar and Katkat, 2007). According to the reference range, the plant N content was determined at very low values. In addition, when the other macro element contents were measured in the leaves of *Inula graveolens* and *Inula viscosa* species, it was determined that the P and K contents were at low levels compared to the limit values reported by Kacar and Katkat (2007). When the leaf Ca and Mg contents were examined, it was found to be sufficient in *Inula viscosa* species and low in *Inula graveolens* species (Table 4). From some literature studies on *Asteraceae* family members: *Achillea millefolium* L. K: 13869.1±15.95mg/100gr Mg: 339.62±3.40mg/100gr Fe: 73.317±0.84 mg/100gr Ca: 794.82±9.528 mg/100gr; *Cichorium intybus* L. K: 124.7±2.9 mg/100gr Mg: 485.15±2.96 mg/100gr Fe: 29.256±0.34 mg/100gr Ca: 1130.66±13.56 mg/100 gr data were obtained (Ashirova et al., 2021).

The microelement changes of the species are shown in Table 5. When the results were evaluated, leaf Fe content was found to be 1.09 ppm in *Inula graveolens* species and 910.06 ppm in *Inula viscosa*. In addition,

Table 5. Macro and microelement contents of leaves of *Inula viscosa* and *Inula graveolens* species

Mineral Nutrients		*Inula viscosa* L.	*Inula graveolens* L.
Macro Elements (%)	Nitrogen (N)	0.21	0.28
	Phosphorus (P)	0.22	0.09
	Potassium (K)	1.2	1.39
	Calcium (Ca)	1.81	0.35
	Magnesium (Mg)	0.43	0.14
Micro Elements (ppm)	Iron (Fe)	910.06	1.09
	Manganese (Mn)	125.72	2.92
	Zinc (Zn)	56.16	1.51
	Copper (Cu)	11.03	1.46
	Boron (B)	53.87	33.21

when the amounts of other microelements in the leaf are examined for *Inula graveolens* and *Inula viscosa* species, the following results are found respectively; Mn: 2.92-125.72 ppm; Zn: 1.51-56.16 ppm; Cu: 1.46-11.03 ppm and B: 33.21- 53.87 ppm. When the leaf Fe content of *Inula graveolens* species, which is the subject of the study, was examined (1.09 ppm), it was found to be relatively low compared to the reference values. In comparison, relatively high values were found in *Inula viscosa* (910.06 ppm) species (Table 5).

When the leaf Fe content (1.09 ppm) of the *I. graveolens* species, which is the subject of the study, was examined, it was found to be relatively low compared to the reference values (Kacar and Katkat, 2007), while relatively high values were determined for *I. viscosa* (910.06 ppm). We may explain the situation in question with the difference in the Fe content of the soil where the species spread. That is, while the soil Fe content of *I. graveolens* species was determined as 2.76 ppm, the soil Fe content of *I. viscosa* species was found to be 34.63 ppm which is approximately 13 times of 2.76.

As a result of studies on some *Asteraceae* family members, *Achillea millefolium* L. Zn: 2.487±0.029 mg/100 gr; Cu: 0.161±0.019 mg/100 g; Cichorium intybus L. Cu: 0.140±0.016 mg/100 g; Zn: 2.996±0.034 mg/100 g; *Chamomilla recutita* (L.) Rausch. Zn: 2.440±0.028 mg/100 g; Cu: 0.343±0.039 mg/100 g (Ashirova et al., 2021). The literature review about some other parameters that are the subject of our study is as follows; Baydar and Erdal (2004) examined the leaf Fe content of İzmir thyme and reported that they measured values in the range of 47.25-97.50 ppm, Meraler (2010) looked at the leaf Mn content of mahlep plant and determined the lowest as 8 ppm and the highest as 36 ppm. As a result, when the leaf macro and micronutrient data of *I. graveolens* and *I. viscosa* species, which constitute the material of our study, were examined, it was found that there were values that were contrary or in accordance with the literature information in general.

CONCLUSION

The current study shows that the antioxidant substance amounts and mineral substance contents of the extracts prepared in methanol, hexane, and acetone solvents of *Inula graveolens* L. and *Inula viscosa* L. species belonging to the genus *Inula* L. from the *Asteraceae* family, which naturally spread in Muğla province, were analyzed. Many factors affect the antioxidant activity and phenolic content in plants; the period the plant was collected, the solvent used, the waiting duration, the method used, etc., affect the antioxidant capacity of the plant. In light of the data obtained in this study, it is suggested that the extracts obtained with different solvents can be used in easily accessible natural antioxidant sources, food supplements, food additives, pharmacology, and pharmacy, but additional studies on the subject are recommended.

Moreover, the macro and microelement contents of the species were examined, and it was determined that *Inula viscosa* L. had higher values than *Inula graveolens* L. in terms of both micro and macronutrients. This situation is thought to be due to topographical features and environmental factors. However, since it is known that the number of microelements and heavy metals must be at a limited value, and it is known that they can have toxic effects on plants and humans when exceeded, thus, the studies need to be detailed. Finally, it is our sincere hope that the findings of this study will serve as a foundation for future research to capitalize on the natural antioxidant substances found in the extracts of these two *Asteraceae* plants.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

Authors do not declare any conflict of interest.

Author contribution

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

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RESEARCH ARTICLE

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Some *lactobacillus*, *leuconostoc* and *acetobacter* strains in traditional turkish yoghurt, cheese, kefir samples as a probiotic candidate

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Abstract

Lactic acid bacteria which are important for production of fermented milk products contain may strains called *Lactobacillus*, *Streptococcus*, *Lactococcus* and *Leuconostoccus*. As a result, lactic acid bacteria are called 'milk-souring (fermenting)' organisms. In addition to the fermentation abilities of *Lactobacillus* spp., it is important for aroma, texture and acid formation and comprises the most important group of lactic acid bacteria. Their critical importance comes from their metabolic capacity and probiotic features. In this research, yogurt, cheese and kefir samples were collected from cities in Turkey and used to isolate. Isolates were identified phenotypically and genotypically characterized. The probiotic features antibacterial activity against *Staphylococcus aureus* ATCC6538, *Listeria monocytogenes* DSM12464, *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC51299, and *Salmonella* Enteritidis ATCC 130762; bile and acid salt tolerance, susceptibility to chloramphenicol, erythromycin, penicillin G, gentamicin, vancomycin, streptomycin, kanamycin, and tetracycline of isolates were determined. Isolates, were identified as *Lactobacillus paracasei* subspecies (subsp.) *paracasei*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Acetobacter ghanensis*, *Acetobacter fabarum*, *Acetobacter* subsp., *Leuconostoc pseudomesenteroides*, and *Leuconostoc mesenteroides* subsp. *mesenteroides*. Some isolates were tolerant of acid and bile salt, some strains were resistant to antibiotics, and some could inhibit pathogens. In this study, isolates were determined to have probiotic features. As a result of the study, it was determined that some isolates showed probiotic properties and had strong antibacterial activity. Isolates can be use as natural alternative in infections.

Keywords: *Lactobacillus*, *Leuconostoc*, *Acetobacter,* Probiotic properties

INTRODUCTION

Probiotic bacteria are defined as live microorganisms. When taken into the body in certain amounts, they are microorganisms with health benefits beyond basic nutrition (Coeuret et al., 2004). Probiotic, should meet some requirements, adherence to human enteric epithelial cells, like resistance to the bile and gastric acids, bile salt hydrolase activity, ability to reduce pathogen adhesion to the gastrointestinal tract, and antimicrobial activity against pathogenic bacteria (Kolacek, 2017). The use of probiotics is more generally accepted, milder, safer of foods than commonly used natural and chemical preservatives (Vieco-Saiz et al., 2019).

Dairy products have been consumed for centuries and yogurt, cheese, kefir are most popular of worldwide (Buttriss, 1997). All dairy products are unique because of fermentation type, environmental conditions and they have similar or different microflora with each other. The determination of microflora of fermented dairy

products is important for determining characteristics features and further improving of fermented dairy products. There are studies investigating the microbial content of fermented dairy products (Simova et. al., 2002, Manolopoulou et al. 2003, Zamfir et. al., 2006, Waldherr et. al., 2010, Aldrete-Tapia et. al. 2014, Garofalo et al., 2015, Sarikkha et al., 2015, Xue et. al. 2018).

The main aim of this research was to use genotypic and phenotypic methods to identify *Lactobacillus*, *Leuconostoc*, and *Acetobacter* strains. Then, the evaluation of tolerance to acidity and bile salts, antimicrobial activity, hydrophobicity, and antibiotic susceptibility were investigated.

MATERIAL AND METHOD

The study used naturally fermented milk products. These included 19 yogurts, 3 cheeses, and 1 kefir sample (Tekirdag, Antalya, Hatay, Van, Edirne cities in Turkey). The samples were stored in sterile sample containers, brought to the laboratory and stored in a refrigerator until study.

Phenotypic Characterization

Samples were seeded on MRS agar. With the aim of phenotypic characterization of those with different dimensions, shapes or colors, gram staining, catalase test, ability to create gas from glucose and ammonium from arginine, and developmental ability with different temperatures and different salt concentrations were investigated.

Genotypic Characterization of Isolates

DNA isolation comprised the stages of lysis of bacteria, removal of proteins, precipitation of DNA and purification. The Genomic DNA Purification KIT (Fermentas, FINLAND) was used to complete isolation.

For identification of bacteria with the 16S rDNA method, general bacterial primers were used benefiting from the homology of the 16S rDNA region proliferated with polymerase chain reaction (PCR). In studies, forward primer 5' AGAGTTTGATCCCTGGCTCAG- 3' and reverse primer 5'- CCGTCAATTCCTTTGAGTTT – 3' were used (Beasley and Saris 2004). In the study, 500 µl PCR tubes were completed to total volume 50 µl with 17.5 µl sterile water produced for molecular studies, 2.5 µl buffer (not containing MgCl2), 0.5 µl (deoxynucleotidetriphosphate) dNTPmix (mixture prepared with dATP, dCTP, dGTP, dTTP, each concentration 200 µM), 0.5 µl primers, 0.5 µl Taq DNA polymerase enzyme and 2 µl $MgCl_2$ and finally 1 µl DNA addition. After the tubes were inserted in the PCR chamber, PCR reaction parameters were programed as 94 °C 5-min initial denaturation, 94 °C 45 s denaturation (opening of double chains), 53 °C 1 min annealing (adhering of primers), and 72 °C 1 min extension (chain extension). This procedure was repeated 30 times. Tubes removed from the PCR were stored at -40 °C. Later PCR products were purified and then DNA array analysis. Sequence was directed ABI 3130 genetic analyzer in the BLASTN program, then NCBI web site (http://www.ncbi.nlm.nih.gov) was used.

Determination of acid tolerance of isolates

After *Lactobacillus* spp. and *Leuconostoc* spp. isolates were incubated overnight at 37 °C and *Acetobacter* spp. isolates incubated at 32°C for 72h in MRS fluid medium, seeding was performed on 10 mL fresh MRS fluid media with pH set to 3 with HCl (3M) and initial counts were identified with cultural methods. The prepared bacterial cultures were incubated for 3 hours (180 min) at 37 °C. 1 mL was taken from each of the pH 3 cultures and serial dilutions with 9 mL sterile physiologic saline up to 10^{-6} were prepared. Seeding was performed on MRS media with these dilutions. Analyses were performed in triplicate. The colonies developing on the MRS media were counted and the viability rates were identified compared to initial counts (Charteris et al. 1998).

Detection of antibiotic resistance of isolates

Eight different antibiotic disks (erythromycin, streptomycin, vancomycin, penicillin G, kanamycin, gentamycin, chloramphenicol, and tetracycline,) were used to investigate the resistance of the isolates to antibiotics.

Sterile MRS agar medium was cooled to 45-50 °C and active cultures of isolates on MRS fluid media were mixed at rates of 100 µL. Antibiotic disks were placed on the petri dishes, after incubation the diameter of the inhibition zones forming around the disks was measured. Analyses were completed with three replications (Sadrani et al. 2014).

Determination of bile salt resistance of isolates

Tolerance of isolates to bile salt was identified according to the method of Kotsou et al. (2008). Active cultures (2236 g) were centrifuged for 5 min and pellets were diluted with MRS broth. 0.3% bile salt or MRS broth for the control group, were added and 50 µL of the inoculum mentioned above was added and left for incubation at 37 °C for 24 hours. Analyses were performed with three repeats and bile salt resistance of isolates seeded on MRS agar at 0 and 24 hours were determined.

Determination of hydrophobicity of isolates

The hydrophobicity ability of isolate was determined according to the method reported by Perez et al. (1998). From fresh bacteria cultures, 2 mL was taken, vortexed with 0.4 mL xylene for 120 s and then absorbance measured at 600 nm with a spectrophotometer (Shimadzu 1208). The analyses had three repetitions. The cell surface hydrophobicity was calculated with the aid of the following formula.

Hydropobicity (%) = [(Ao-A)/Ao]x100

The A_0 and A values are the absorbance values before

and after extraction with xylene.

Determination of antibacterial activities of isolates

Fresh cultures were prepared from isolates in MRS fluid media, cultures were centrifuged and after obtaining cell-free solution, the supernatant was passed through a cellulose acetate filter with 0.2 µm pore size. For antibacterial activity, 18-hour cultures of the chosen test bacteria [*Enterococcus faecalis* ATCC51299, *Listeria monocytogenes* DSM12464, *Salmonella* Enteritidis ATCC 13076, *Staphylococcus aureus* ATCC6538, and *Escherichia coli* ATCC 25922] were poured onto petri dishes containing nutrient agar and wells with 6 mm diameter were opened. Supernatant from the isolate to be tested was pipetted into each well and the diameters of the inhibition zones forming around the wells were measured and recorded after incubation. Analyses were performed in triplicate (Arıcı et al. 2004).

Statistical analysis

Acid tolerance and bile tolerance of isolates calculated with two-way ANOVA using the Graph Prism 7.0 program. The % hydrophobicity values of the isolates were calculated with one-way ANOVA using the Graphprism 7.0 program. Differences were considered significant at p value <0.01.

RESULTS AND DISCUSSION

Identification of Isolates

A total of 105 isolates were obtained from 23 samples. With the aim of genotypic identification of gram (+) and catalase (-) samples among isolates assessed in terms of morphology after phenotypic identification analyses.

While the study continued, permission was granted and samples were stored in the laboratory. With the aim of isolation, general bacterial primers were used for identification of bacteria with the 16S rDNA method using homology proliferated in the 16S rDNA region with polymerase chain reaction (PCR).

After determining the basal sequence, this sequence was compared with the database using a program on the internet (http://www.ncbi.nlm.nih.gov./BLAST/). Screening results determined which microorganism the researched array sequence may belong to and the percentage similarity. Among the isolates, 95-99% similarity was identified with reference strains for 1 isolates with *Leuconostoc pseudomesenteroides* (TDP 71), 2 isolates with *Leuconostoc mesenteroides* subsp. *mesenteroides* (TDP 22, TDP 50), 2 isolates with *Acetobacter fabarum* (TDP 54, TDP 90), 1 isolate with *Acetobacter* spp. (TDP 69), 3 isolates with *Acetobacter ghanensis* (TDP 21, TDP 38, TDP 40), 4 isolates with *Lactobacillus paracasei* subsp. *paracasei* (TDP 1, TDP 2, TDP 3, TDP 28), and 19 isolates with *Lactobacillus delbrueckii* subsp. *bulgaricus* TDP (37, 41, 56, 57, 58, 59, 63, 66, 70, 72, 88, 89, 92, 93, 95, 97, 98, 100, 103).

Antibacterial activities of isolates

The antagonism ability of the bacterial isolates was ordered according to the size of the zones of inhibition against *Salmonella* Enteritidis ATCC 13076, *S.aureus* ATCC6538, *L.monocytogenes* DSM 12464, *E.faecalis* ATCC 51299, *E.coli* ATCC 25922 (Table 1). When the antibacterial properties of isolates are investigated, most inhibition activity was identified against *Salmonella* Enteritidis ATCC 13076 and *Enterococcus faecalis* ATCC 51299. All isolates were effective against *Enterococcus faecalis* ATCC 51299 (8.7-15.9 mm). The largest inhibition zone against *Salmonella* Enteritidis ATCC 13076 was 10.3 mm with isolate TDP 63. Only 9 isolates created zones against *Staphylococcus aureus* ATCC 6538. TDP 50 and TDP 90 isolates had the largest zones against *Staphylococcus aureus* ATCC 6538 (8.5 mm). The antibacterial effect of *Lactobacillus paracasei* subsp. *paracasei* was stronger compared to *Lactobacillus delbrueckii* subsp. *bulgaricus*.

Acetobacter isolates were more effective against *Escherichia coli* ATCC 25922 (14.5-15.9 mm) than the others. *Leuconostoc mesenteroides* subsp. *mesenteroides* exhibited inhibitory ability against all test bacteria, albeit with small zones.

Studies of *Lactobacillus* spp. isolated from milk products showed they were effective against, *Escherichia coli*, *Salmonella typhimurium*, and *S.aureus* (Patra et al., 2011, Tambekar and Bhutada, 2010; Abosereh et al. 2016). Akpinar and Yerlikaya, (2021) reported that; many of the *Lactobacillus paracasei* strains from kefir and raw milk showed higher antagonistic effects than *Leu. mesenteroides* strains. Pisano et al., (2022) reported that *L. plantarum* from cheese reduced by 3–4 log10 CFU/g *L.monocytogenes* ATCC 7644. Some researchers reported that *Leuconostoc mesenteroides* strains had antimicrobial activity against *S. aureus* and *E. coli* (inhibition zones ranging from 7.42 to 16.00 mm) using the agar well diffusion method. (Rani and Agrawal, 2008; Ryu and Chang, 2013). Haghshenas et al. (2015) found that strains of *Acetobacter syzygii 38Lac, A. indonesiensis 10HN L.*, and *A. cibinongensis 34L* were able to exhibit antimicrobial activity to important human pathogens.

The data obtained in the study were higher compared to data stated by Abosereh et al. (2016), but similar to data from studies by Etöz (2006), Rani and Agrawal, 2008, Patra et al. (2011), Tambekar and Bhutada (2010), and Ryu and Chang, 2013.

Acid tolerance of isolates

Among the isolates, *Lactobacillus delbrueckii* subsp. *bulgaricus*, and *Lactobacillus paracasei* subsp. *paracase*i were identified to be more susceptible to low pH (Figure 1). The acid tolerance of isolates was assessed with two-way ANOVA and results differed according to time and bacteria (p<0.0001).

Table 1. Antibacterial activity of isolates determined by agar spot assay (mm)

Isolate Number	S. Enteritidis ATCC 13076	S.aureus ATCC6538	L.monocytogenes DSM12464	E. faecalis ATCC51299	E. coli ATCC 25922
TDP1	9.1±0.1	8.2±0.3	9.7±0.7	10.7±0.8	6.5±0.5
TDP 2	9.3±0.6	7.9±0.2	9.8±0.9	10.7±0.7	6.4±0.2
TDP 3	9.0±0.7	7.3±0.5	9.4±0.4	10.5±0.5	6.4±0.1
TDP 21	-	-	-	15.9±1	9.5±0.7
TDP 22	8.5±0.4	8.2±0.2	8.9±0.3	10.8±0.4	6.4±0.6
TDP 28	9.9±0.5	8.4±0.4	10.1±1	10.5±1.2	6.7±0.3
TDP 37	8.9±0.2	-	-	9.4±0.5	-
TDP 38	8.1±0.5	-	9.5±0.2	15.3±1.3	9.1±0.8
TDP 40	-	-	9.7±0.7	14.5±0.9	9.3±0.6
TDP 41	8.7±0.3	-	-	9.4±0.5	-
TDP 50	8.5±0.3	8.5±0.2	8.5±0.4	9.6±0.9	7.1±0.3
TDP 54	8.3±0.2	-	9.3±0.8	15.6±1.1	9.1±0.9
TDP 56	8.9±0.3	-	-	9.7±1	-
TDP 57	8.5±0.1	-	-	8.8±0.6	-
TDP 58	8.1±0.7	-	-	9.6±0.8	-
TDP 59	8.9±0.4	8.2±0.3	8.4±0.6	9.5±0.6	-
TDP 63	10.3±0.9	-	-	9.1±0.5	-
TDP 66	8.2±0.8	-	-	9.7±0.7	-
TDP 69	-	-	9.5±0.4	15.3±0.6	9.1±0.4
TDP 70	8.3±0.6	-	-	9.8±1.2	-
TDP 71	-	-	-	14.3±0.4	8.8±0.2
TDP 72	8.5±0.3	-	-	8.9±0.9	-
TDP 88	8.9±0.6	-	-	9.0±0.5	-
TDP 89	9.2±0.9	8.1±0.2	8.1±0.3	9.4±0.2	-
TDP 90	8.2±0.3	-	8.9±0.4	15.0±0.8	9.4±0.3
TDP 92	8.0±0.8	-	-	9.2±0.7	-
TDP 93	8.9±0.5	-	-	9.6±0.5	-
TDP 95	8.9±0.2	8.5±0.3	-	9.4±0.2	-
TDP 97	9.0±1	-	8.1±0.3	8.7±0.6	-
TDP 98	8.9±0.4	-	-	9.4±0.9	-
TDP 100	9.3±0.4	-	-	10.8±0.7	-
TDP 103	8.1±0.5	-	-	9.9±0.2	-

When the properties of *Lactobacillus* spp. are investigated in studies, isolates were identified to preserve their viability at pH 3 (Prasad et al., 1999; Maragkoudakis et al. 2006; Minelli et al. 2004). *Lactobacillus paracasei* were inhibited at pH 2, while they were reported to be resistant at pH 3 (Schillinger et al. 2005; Abosereh et al. 2016). Some studies have reported that *Leuconostoc mesenteroides* spp. from natural yogurt and whey have demonstrated the ability to survive at low pH (Perea et al., 2007; Rani and Agrawal, 2008) Haghshenas et al. (2015), found that *Acetobacter* strains had high survival rate (> 44–78%) in traditional dairy products, after conditions (pH 2.5 for 3 hour).

Data analyzed by two-way ANOVA using the Graph Prism 7.0 program. Statistical differences between bacteria groups are depicted on the tops of bars as follows: *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$, and ****$p < 0.0001$.

These results confirmed by many other studies Maragkoudakis et al. (2006), Prasad et al. (1999), and Haghshenas et al. (2015) while they are different to the data obtained by Schillinger et al. (2005) due to that study taking pH 2 as reference.

Antibiotic Resistance of Isolates

Isolates were resistant to Vancomycin except *Acetobacter* strains. All isolates were susceptible to tetracycline and chloramphenicol. Majority of the lactobacilli, *Leuconostoc* and *Acetobacter* strains were resistant to erythromycin

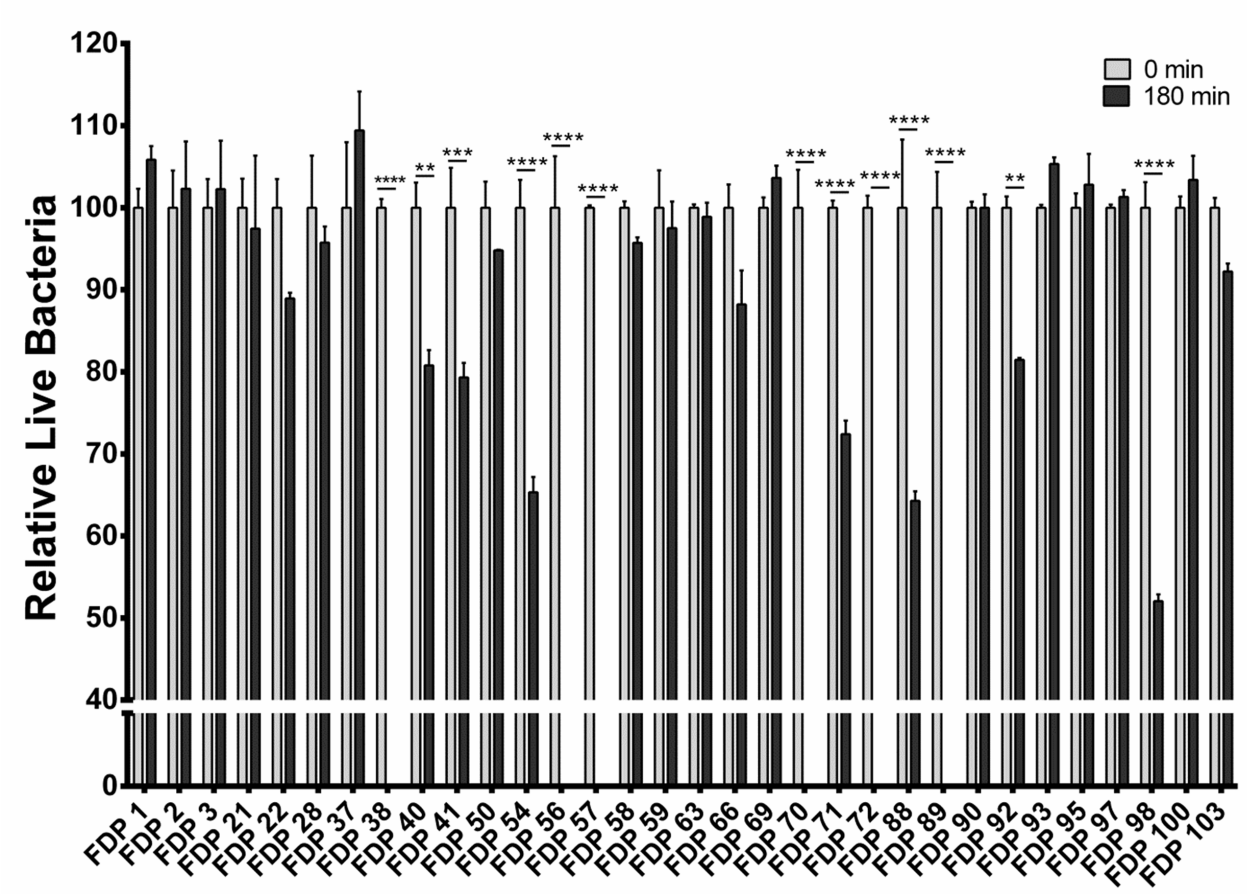

Figure 1. Acid tolerance of isolates

and penicilin G.

Previous studies confirm the generally susceptible of the *lactobacillus* spp. species studied here to erythromycin, chloramphenicol and tetracycline (Charteris et al. 1998, Katla et al. 2001, Temmerman, 2003, Erginkaya et al. 2018).

A variety of studies as this study stated that *Leuconostoc* spp. are resistant to vancomycin (Tynkkynen et al., 1998; Salminen et al.,1998; De Paula et al., 2015). While some researchers also found that *Leu. mesenteroides* strains were resistant to tetracycline and streptomycin (Akpinar and Yerlikaya, 2021).

Ahmad et al. (2004) found that *Acetobacter diazotrophicus* isolated from sugarcane was mostly resistance to test antibiotics. Haghshenas et al. (2015), determined that all examined *Acetobacter* strains show high resistance to erythromycin, vancomycin and sulfamethoxazole.

Hydrophobicity capability of isolates

In vitro studies showed that the adhesion properties of probiotic bacteria displayed non-competitive exclusion features by affecting their adhesion properties against pathogens (Ouwend et al.,1999; Gopal et al., 2001).

The hydrophobicity with xylene of isolates was identified to be between 58.75% and 11.5%. The hydrophobicity of *Lactobacillus delbrueckii* subsp. *bulgaricus* was identified to be lower compared to *Lactobacillus paracasei* subsp. *paracasei*. The hydrophobicity values were statistically different between the bacterial strains ($p<0.001$).

Lactobacillus paracasei subsp. *paracasei* and a variety of *Lactobacillus* spp. were determined to adhere to HT29 and Caco-2 cells (Minelli et al., 2004; Schillinger et al., 2005). *L. mesenteroides* spp. strains had lower hydrophobicity capability than some other strains reported in the literature (Aswathy et al., 2008, Raghavendra and Halami 2009, De Paula et al.,2015).

Bile salt resistance of isolates

All isolates were resistant against 0.3% bile salt, and were not inhibited after 24-hour incubation in a 0.3% bile salt medium (Figure 2). The bile salt tolerance values of isolates were assessed with two-way ANOVA and the results differed according to time and bacteria ($p<0.0001$).

Previous studies confirm the generally resistance of the strains isolated from milk products studied here towards to Bile salt (Abosereh et al., 2016; Prasad et al. 1999, Minelli et al. 2004;

Maragkoudakis et al. 2006)

The results in our study are parallel to data reached in

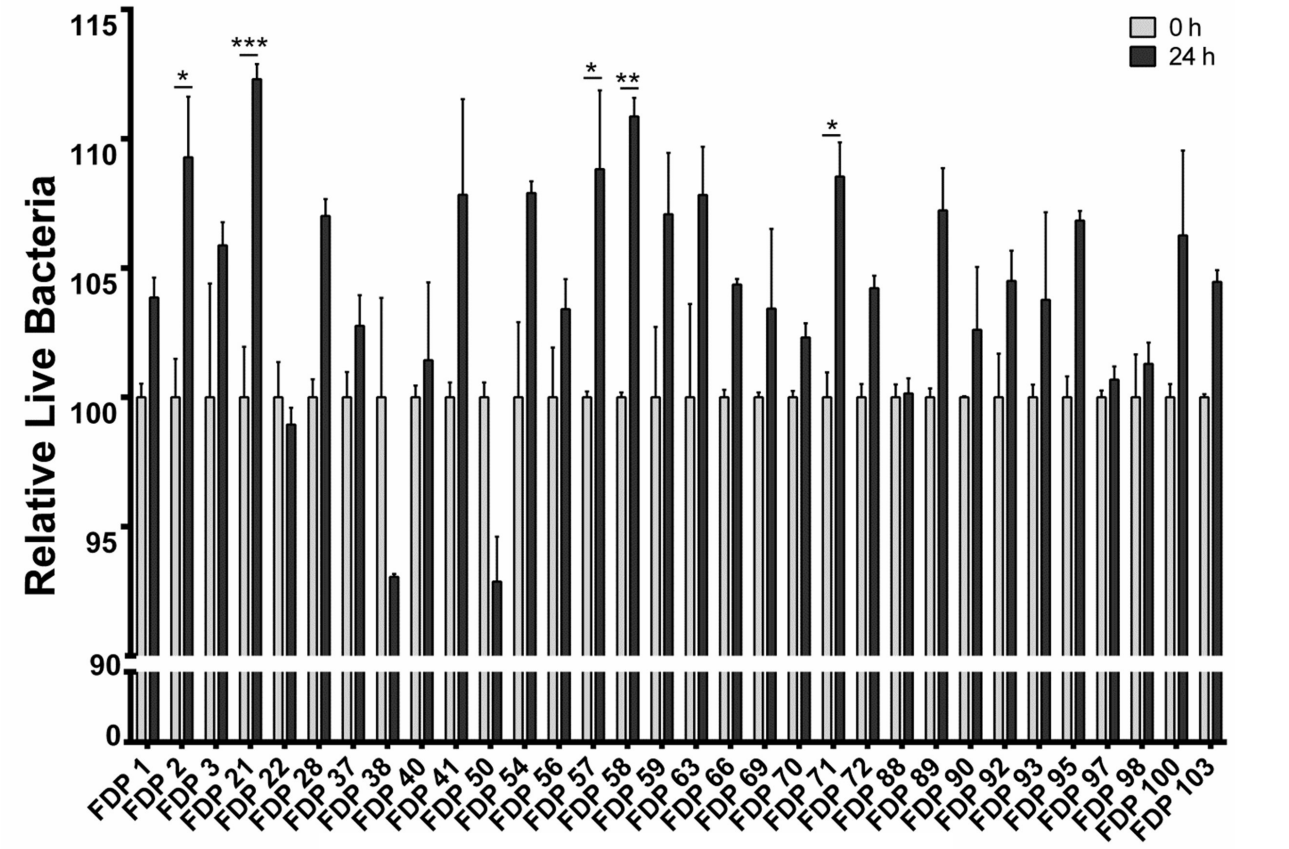

Figure 2. Bile Salt Resistance of isolates

studies by Abosereh et al. (2016), Prasad et al. (1999), Minelli et al. (2004), and Maragkoudakis et al. (2006), though there are differences according to strains.

Data analyzed by two-way ANOVA using the Graph Prism 7.0 program. Statistical differences between bacteria groups are depicted on the tops of bars as follows: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

Haghshenas et al. (2015), reported that *Acetobacter* isolates showed good tolerance to bile (1% bile; ranged from 62% to 95%).

Some researchers reported that bile salt affected the growth rate of *Leuconostoc* spp. strains and limited its viability (Allameh et al., 2012; Todorov et al., 2012; Akpinar and Yerlikaya, 2021). In contrast Some researchers have reperted that strains of *Leuconostoc* spp. can survive under different concentrations of bile salts (Chang et al., 2010; Seo et al., 2012; Nakamura et al., 2012).

CONCLUSION

Fermented milk products that do not use commercial cultures for fermentation but developed their own microbial culture through the years have their own characteristic features. Determination of the microbiota in naturally fermented products not using commercial cultures is important in terms of protecting these products and sustaining them for future generations.

Primary probiotic assessments, including high bile salt and low pH tolerance tests, hydrophobicity test, antibiotic susceptibility, and antagonistic activity test against pathogens confirmed the probiotic properties of TDP 1, TDP 93, TDP 21 isolates, which was identified *Lactobacillus paracasei* subsp. *paracasei* (from cheese), *Lactobacillus delbrueckii* subsp. *bulgaricus* (from yoghurt), and *Acetobacter ghanensis* (from kefir) respectively can be introduced as novel candidate probiotics.

There is an increasing number of studies showing that probiotics can be an important tool in the treatment and prevention of gastrointestinal tract infections and chronic inflammatory disorders. Probiotic candidates with strong antibacterial activity we obtained in our research supports these studies.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

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

Author contribution

Conceptualization; N.T.D., methodology; N.T.D and M.A., formal analysis; N.T.D, M.A., and S.Y., validition; N.T.D., investigation; N.T.D and M.A., supervision; N.T.D., writing-original draft; N.T.D, M.A., and S.Y., review and editing; N.T.D, M.A., and S.Y., All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that

they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable

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RESEARCH ARTICLE

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Total secondary metabolites and heavy metal profile of some medicinal plants frequently consumed as winter tea

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Abstract

In this study, sage (*Salvia officinalis* L.), linden (*Tilia platyphyllos* Scop.), and daisy (*Matricaria chamomilla* L.) herbs, which have rich phytochemical content and are frequently consumed in winter months for medicinal purposes, were obtained from three different herbalists located in Bingol and investigated in terms of their heavy metal contents, total flavonoid, total anthocyanin and total polyphenol contents. Some of the heavy metal (Cr, Fe, Co, Ni, Cu, Zn, As, Hg, Pb) contents of the collected herb samples were determined by ICP-MS. Total flavonoid, total anthocyanin and total polyphenol contents were determined by analyzing spectrophotometric methods. While the total phenolic content was found the most in sage (S1) ethylacetate and methanol extracts (760.1±2.0, 410.33±1.5 mg GAE/g), it was found the least in linden (L1, L3) hexane extracts (6.66±.1.3, 8.35±0.8 mg GAE/g). It was determined that total flavonoid content and total anthocyanin contents were higher in sage (S1) ethylacetate (294.31±2.1 mg CE/g and 291.31±2.1 mg MvGE/g), sage (S2) methanol (375.0±1.9 mg CE/g and 139.01*±2.0 mg MvGE/g) extracts, and less in linden (L1) and daisy (D1) hexane extracts respectively. It was determined that Cr and Cu metals were among the values suitable for consumption in terms of health in all samples except the chamomile (D1, D3) samples, and Zn metals were among the values suitable for consumption in all samples except the linden (L2) sample. Furthermore, the toxic metal Cd (0.05±0.00- 0.09±0.00 µg/g) in daisy (D1, D3) samples, as well as the other toxic metal Pb (4.50±0.01-6.43±0.01 µg/g) in sage (S1,S2,S3) and linden (L1,L2,L3) samples, were found to be among the values suitable for consumption in terms of health. As a result, when the total anthocyanin, polyphenol and flavonoid contents were compared in all groups it was found that sage had the highest value in ethyl acetate extract. Additionally, Cu and Ni values of sage, and Fe and Pb values in both sage and linden were within safe limits in terms of health. They are of utmost importance in terms of supporting the daily mineral intake. However, As, Pb, and Hg were found to be toxic in daisy extract. This result can be shown as a result that the daisy will be a bioaccumulator. Therefore, consumption of daisy tea should be limited.

Keywords: Daisy, Heavy metal, Herbal Tea, Linden, Sage, Total anthocyanin, Total flavonoid, Total polyphenol

INTRODUCTION

Phytotherapy, which means treatment with herbs, is the use of chemical substances in herbs as medicines without subjecting them to an isolation process (Dündar 2001). Many herbs around us stand out as genuinely healthy physiological figures with their chemical programs and commonly used stakeholders of life with effects such as reducing stress, relieving anxiety, soothing the stomach,

activating intestines or providing antiflatulent effects (Dündar and Aslan 2000). Benefiting from herbs for preserving body homeostasis or treating diseases is as old as the history of humanity. Thanks to these properties, herbs and phytochemicals are regarded as super ammunitions used in the body's defense and used against common risks, such as cancer, cardiovascular problems, hormonal disorders and diabetes, especially for their antioxidant effects (Evcimen and Aslan 2015). However, scientific reports that suggested that not every antioxidant substance could be used safely in under every circumstance motivated researchers to look for safer antioxidant herbs and herbal products (Dündar and Aslan 2000). Findings within this scope also update and change antioxidant herbal tea habits.

Herbal teas, which emerge as reliable fluid sources in winter periods when fluid intake and water cycle reduces, are also investigated by medicine, veterinary, biology and pharmacy disciplines. Thanks to being cheap, preferable and having positive effects, herbal teas have found a place in Turkish Pharmacopeia and the process of determining standards, licensing and determining indication fields have been going on. Many studies were conducted on using the teas made from herbs, such as classic black tea, green tea, white tea, jasmine, linden, sage, turmeric, ginger, melissa (lemon balm), parsley, daisy, rosemary, fennel, fennel-aniseed, stinging nettle, dill, thyme and rosehip as effective beverages in terms of physiological homeostasis and vitality and phytotherapeutic substances (Aslan 2018).

In color, smell and taste formation of medicinal herbs, phytochemicals are determinative as biologically active substances (Aslan, 2018). Considering the fact that there are more than ten thousand phytochemicals in the structures of herbs, they are impossible to investigate one by one (Balch and Balch 1997); (Craig 1997). Some of those that stand out in winter teas include catechins, isoflavones, indoles, carotenoids, anthocyanidins, ellagic acids and polyphenols, phenolic compounds, flavonoids, coumarins, catechins, anthocyanidins, carotenoids, lycopenes, phytates, indoles, sulfites, isothiocyanates, lignins, lactones, sponins, glucosinolates, phytosterols, phytoestrogens, saponins, terpenes, ellagic acids, quercetins.

In our study, each of the herbs investigated has medicinal effects. The scientific name for sage, which belongs to the lamiaceae family is Salvia officinalis. Sage (adaçayı in Turkish) is known to facilitate digestion and eliminate stomach gas due to its bitter taste. In addition, due to its soothing and disinfectant effects, it has been reported that it reduces sweating and saliva secretion, cleans the pores, is used as a mouthwash in rheumatic pain, tonsils, teeth, mouth and inflammation (Özer et al. 2001); (Aydıner 2006). The scientific name for linden, which belongs to the tiliaceae family is Tilia platyphyllos. Linden (ıhlamur in Turkish) is known to be used as a diaphoretic, chest emollient, sleep inducer and tranquilizer in addition to its use in strengthening cardiac muscle and nerves, activating and cleaning kidneys, treating epilepsy and migraines, and its properties such as antifebrile, fatigue eliminator and biligenic effects (Aydıner 2006); (Baytop 1999); (Şeker 2011). The scientific name for daisy, which belongs to the asteraceae family is Matricaria chamomilla. Daisy (papatya in Turkish) is known to be effective in increasing urine, whetting appetite, soothing nerves, reducing fever, eliminating diarrhea and gas, treating throat and tonsil pain, healing wounds, having biligenic effects and in the treatment of hemorrhoids, inflamed wounds, rheumatism, insomnia, flu, anemia, dizziness, eczema, waist and back pain (Aydıner 2006); (Koç 2002).

Heavy metals that are taken at high doses via the food chain also affect human health negatively (Öktüren and Sönmez 2006); (Okcu et al. 2009). For example, excess lead builds up in the bones and causes damages in the kidney, brain and nervous system functions while cadmium accumulation in the body causes serious problems that may lead to lung and prostate cancer (Kahvecioğlu et al. 2006). Herbs that are grown in regions polluted with heavy metals [such as roadsides and mineral deposits] may have high heavy metal contents (Pip 1991). Furthermore, the use of fertilizers with cadmium content and pesticides with organic mercury or lead increases the rate of herbs containing heavy metals (Arab et al. 1999).

Medicinal and aromatic herbs cover quite a large field in terms of herbs, their active ingredients and areas of consumption. These active ingredients have nutritional elements such as various vitamins, carotenes and calcium, while most of them do not have a nutritional element characteristic. These include flavonoids, polyphenols, monoterpenes, chlorophyll, dietary fiber, aliphatic sulfites, aromatic isothiocyanates, phytic acid (Stavric 1994); (Hollman et al. 1996). Flavonoids, which are the main sources of yellow, blue and red pigments in herbs, are polyphenolic compounds that are in a 2-phenyl-benzo-benzo-α-piron structure and found naturally in fruits, vegetables and beverages such as tea and wine (Herton et al. 1993); (Hollman et al. 1996). With the molecules that bind to this structure with benzene and heterocyclic rings, they get different names. These compounds in the flavonoid structure can be divided into groups, such as anthocyanins, flavonols, flavones, catechins, flavanones and isoflavonoids. Today, more than 4000 flavonoids that are naturally present in food are identified (Abacı et al. 2014)

The aim of this study is to investigate the total flavonoid, total anthocyanin and total polyphenol contents of the species sage (Salvia officinalis), linden (Tilia platyphyllos) and daisy (Matricaria chamomilla) that are sold as teas in herbalists and determine whether their heavy metal (Cr, Fe, Co, Ni, Cu, Zn, As, Cd, Hg, Pb) content are within the limits presented at World Health Organization (WHO) /

Food and Agriculture Organization (FAO) literature.

MATERIALS AND METHODS

Herbal Materials

Between the 10th and 11th months of 2019, three different plant species utilized for therapeutic purposes, such as antipyretic, sedative, muscle relaxant, and tranquilizer, were collected from three herbalists in Bingol. According to the Flora of Turkey and the Eastern Aegean Islands (Davis 1965-1985), plant species were recognized taxonomically by a faculty member of the Department of Biology, Faculty of Science, Bingöl University (Davis et al. 1988). Scientific names, family names, English names, Turkish names and used parts of the herbs that constituted the material of the study were presented in Table 1.

Table 1. Scientific, family, English and Turkish names of medicinal plant used different parts different parts

Scienctific name	***Family name***	***English name***	***Turkish name***	***Plant part used***
Salvia officinalis L.	*Lamiaceae*	Sage (S)	Adaçayı	Leafy and flowering branches
Tilia platyphyllos Scop.	*Tiliaceae*	Linden (L)	Ihlamur	Flower and buds
Matricaria chamomilla L.	*Asteraceae*	Daisy (D)	Papatya	F lowers

Preparing Samples for Heavy Metal Analysis

In the study, different parts of sage, linden and daisy herbs were used as materials. In the analyses, approximately 0.5 g was weighed from the ground samples and were placed in teflon containers of the microwave unit of CEM-Mars 6 240/50 (Corp. Matthews NC, USA) and 10 ml HNO_3 were added on top. The temperature conditions of the microwave unit were raised to 200 oC at the 25th minute. Then, this temperature was kept for 15 min and the temperature was lowered for a period of approximately 15 min. Therefore, it was enabled that the samples were turned into completely soluble states. Following the burning process, the 0.5 mL samples were cooled down to room temperature and they were completed by diluting with 1% (HNO_3-ultra pure water) on their volumes up to 15 mL.

Analysis Method

Heavy metal analyses of the medicinal herb samples were conducted with ICP-MS in three times of repetition. Heavy metal analyses of As, Cd, Cr, Cu, Fe, Co, Pb, Zn, Ni and Hg in these herbal samples were conducted according to ICP-MS method. The fundamental aspect of this method is that samples, which are turned into solutions by burning them via the microwave soluble method, are measured for concentrations of heavy metal contents by ICP-MS for certain standards. In table 2, the operating conditions of Perkin Elmer NexION 2000 model ICP-MS device used for the analysis were presented.

Analysis Methods for Bioactive Compounds

250 mg samples were obtained from dried herbs and they were solved in 5 mL hexane, methanol and ethyl acetate.

Determination of Total Polyphenolic Content

This process was conducted with Folin-Ciocalteu reactive method (Singleton et al. 1999). This method measures the required amount of the tested sample to inhibit

Table 2. ICP-MS (NexION) operating conditions

Parameter/Component	**Description / Value**
Nebulizer	MEINHARD® plus Glass Type C
Nebulizer şow	Optimized for < 2% oxides
Nebulizer gas şow rate	0,93 L/min
Spray Chamber	Glass cyclonic (bafşed), 2 °C
Injector	2.0 mm i.d.
Deşector voltage	–12 V
Analog stage voltage	–1750 V
RF Power	1600 W
Rinse time	45 second
Dwell time	50 ms
Aerosol Dilution	Set to 2.5x
Sample Delivery Rate	350 µL/min
Discriminator threshold	26
Alternating current (AC) rod offset	–4
Cones	Ni
Replicates	3

the oxidation of the reactive (Vinson et al. 2005). Total phenolics were determined as mg gallic acid equivalents (GAE)/g of dried samples.

Determination of Total Flavonol Content

Total flavonols were determined wth the vanillin method according to Butler et al. (1982). Readings in spectrophotometer were conducted at 500 nm wavelength and total flavonols were determined as mg catechin equivalents (CE)/g of dried samples (Butler et al. 1982).

Determination of Total Anthocyanin Content

In plants types, anthocyanin analyses were conducted with the pH difference method according to Wrostad (Wrostad 1976). Readings in spectrophotometer were conducted at 520 and 700 nm wavelengths and at two different pH levels as 1.5 - 4.0. The values were determined as mg malvidin-3-glucoside equivalenst (MvGE) /g of dried samples.

Statistical Analysis

The data were calculated as the mean ± standard deviation of three replicates. IBM SPSS Statistics 28.0 software was used for statistical analysis. The Friedman test was used to analyze dependent variables. The Kruskal-Wallis test was used to analyze independent variables. The Mann-Whitney U test was used to determine the difference between groups with significant differences in the Kruskal-Wallis test.

RESULTS AND DISCUSSION

In this study, heavy metal contents of herbal samples were determined in mg/kg via the ICP-MS method. The limit of detection (LOD), limit of quantification (LOQ) and recovery (R, %) values of the calibration were presented in Table 3 while mean and standard deviation values of heavy metal contents of sage, linden and daisy herbs investigated in the study were presented in Table 4 and Table 5. There were statistically significant differences in the Cr, Fe, Co, Cu, Zn, As and Hg groups ($p<0.05$), but not in the Ni, Cd and Pb groups ($p> 0.05$). (Tables 4 and 5)

Abbreviations for plants in tables are as follows:

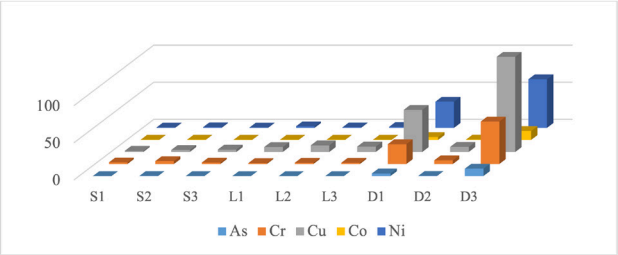
S1, S2, S3: Sage (*Salvia officinalis* L.) samples obtained respectively from the first, second and third herbalists

L1, L2, L3: Linden (*Tilia platyphyllos* Scop.)samples obtained respectively from the first, second and third herbalists

D1, D2, D3: Daisy (*Matricaria chamomilla* L.) samples obtained respectively from the first, second and third herbalists

When the heavy metal contents of the plants were examined, sage and linden were found to have heavy metal content below the toxic limits. However, it is shown in figure 1 that the daisy content is above the toxic limits (Figure 1).

Figure 1. Concentration values of the heavy metal contents of sage (S), linden (L) and daisy (D) herb samples



The solubility of phenolic compounds depends on the polarity of the solvent used, polymerization degree of phenolics and interaction of phenolics with other herbal compounds and formation of insoluble complexes. Therefore, there is no universal procedure to remove all of the phenolics or a branch of specific phenolic substances. Methanol, ethanol, acetone, water and combinations of these are frequently used for the extraction of phenolics. Free flavonoid aglycones are efficiently extracted by non-polar solvents such as methylene chloride, ethyl ether or ethyl acetate. Additionally, more polar glycosidic conjugate is solved in more polar solvents such as methanol and ethanol, thus, these organic solvents are generally used in Soxhlet extraction.

According to the statistical results, in the evaluation of

Table 3. Limit of detection (LOD), limit of quantification (LOQ) and recovery (R, %) values for the heavy metals in the study

Element	LOD (mg/L)	LOQ (mg/L)	R,%
Cr	0.017	0.056	97.380
Fe	0.223	0.746	98.743
Co	0.00032	0.00103	96.521
Ni	0.008	0.025	98.968
Cu	0.022	0.073	96.784
Zn	0.043	0.142	97.417
As	0.001	0.004	95.826
Cd	0.000165	0.00055	97.608
Hg	0.0016	0.0054	95.950
Pb	0.010	0.033	94.882

Table 4. Heavy metal contents (Cr, Fe, Co, Ni, Cu) of sage, linden and daisy herbs in the study

Herbal Material		Cr (µg/g)	Fe (µg/g)	Co (µg/g)	Ni (µg/g)	Cu (µg/g)
Herbalist 1	S1	2.18±0.01^{a}	723.50±1.22^{ab}	0.26±0.001^{bc}	0.55±0.00	1.51±0.00^{ab}
	L1	1.23±0.00^{a}	649.37±0.69^{ab}	0.26±0.00^{bc}	2.06±0.01	6.62±0.01^{ab}
	D1	26.69±0.03^{a}	8783.62±11.48^{ab}	3.9±0.04^{bc}	35.26±0.04	56.67±0.07^{ab}
Herbalist 2	S2	3.97±0.00^{a}	1410.40±0.07^{ab}	0.34±0.00^{bc}	0.86±0.00	2.70±0.00^{ab}
	L2	1.66±0.00^{a}	684.57±0.63^{ab}	0.27±0.00^{bc}	0.74±0.00	8.88±0.01^{ab}
	D2	5. 00±0.01^{a}	1338.08±1.16^{ab}	0.46±0.00^{bc}	2.10±0.00	6.84±0.01^{ab}
Herbalist 3	S3	1.97±0.00^{a}	790.49±0.45^{ab}	0.23±0.00^{bc}	0.52±0.00	3.17±0.00^{ab}
	L3	1.69±0.00^{a}	1008.96±1.57^{ab}	0.27±0.01^{bc}	1.21±0.00	7.24±0.01^{ab}
	D3	57.21±0.04^{a}	22187.48±12.00^{ab}	12.02±0.06^{bc}	65.45±0.03	128.08±0.04^{ab}
p value		0.044	0.019	0.033	p>0.05 (0.068)	0.028

Values were given as mean ± standard deviation of triplicate (n=3) determinations. Statistical analyzes were performed using the Kruskal-Wallis and post hoc Mann-Witney U test.

Table 5. Heavy metal contents (Zn, As, Cd, Hg, Pb) of sage, linden and daisy herbs in the study

Herbal Material		Zn (µg/g)	As (µg/g)	Cd (µg/g)	Hg (µg/g)	Pb (µg/g)
Herbalist 1	S1	27.87±0.04^{ac}	0.11±0.00^{bc}	Not detected	Not detected	6.43±0.01
	L1	69.09±0.10^{ac}	0.05±0.00^{bc}	Not detected	Not detected	5.92±0.00
	D1	90.36±0.14^{ac}	3.50±0.01^{bc}	0.09±0.00	Not detected	6.24±0.00
Herbalist 2	S2	92.76±0.05^{ac}	0.25±0.00^{bc}	Not detected	Not detected	6.07±0.00
	L2	25.57±0.02^{ac}	0.04±0.00^{bc}	Not detected	Not detected	4.70±0.01
	D2	32.45±0.03^{ac}	0.33±0.00^{bc}	0.09±0.00	Not detected	4.35±0.01
Herbalist 3	S3	56.83±0.03^{ac}	0.11±0.00^{bc}	Not detected	Not detected	4.50±0.01
	L3	96.38±0.17^{ac}	0.07±0.00^{bc}	Not detected	Not detected	4.53±0.00
	D3	1278.98±0.80^{ac}	9.96±0.01^{bc}	0.05±0.00	6.35±0.00^{ab}	11.71±0.00
p value		0.037	0.031	p>0.05 (0.061)	0.003	p>0.05 (0.057)

Values were given as mean ± standard deviation of triplicate (n=3) determinations. Statistical analyzes were performed using the Kruskal-Wallis and post hoc Mann-Witney U test.

the extracts of the herbs obtained from three different herbalists, and by using three different solvents, it was determined that polyphenol content values obtained from ethyl acetate extracts of all of the herbs were significantly higher, except for sage 2 and linden 2 (Table 6).

In the evaluation of the total anthocyanin content of the herbs, in terms of sage extracts, it was determined that the anthocyanin content of ethyl acetate extract of sage obtained from the 1st herbalist and anthocyanin contents of methanol extracts of sage obtained from the 2nd and 3rd herbalists were higher. According to linden results, it was determined that ethyl acetate extracts of linden obtained from the 1st and 3rd herbalists and methanol extracts of linden obtained from the 2nd herbalist were higher in anthocyanin content. For daisy, it was determined that D1 ethyl acetate extract, D2 and D3 hexane extracts were higher in anthocyanin content (Table 7).

In the comparison of flavonoid contents of herbs obtained from different herbalists, it was determined that ethyl acetate extracts of sage, linden and daisy

Table 6. Total polyphenolic substance content of extracts

		Extracts (mg GAE/g)			
	Plants	Hexane	Methanol	Ethyl acetate	p
Herbalist 1	S1	300.33±1.5	410.33±1.5	760.1*±2.0	p<0.001
	L1	6.66±.1.3	31.74±1.2	58.24*±1.0	p<0.001
	D1	18.62±1.2	25.33±1.5	38.08*±2.0	p<0.001
Herbalist 2	S2	21.68±1.4	60.42*±1.7	36.38±0.99	p<0.001
	L2	26.34±1.5	145.29*±2.0	68.11±1.3	p<0.001
	D2	9.69±0.9	16.59±1.2	25.67*±1.5	p<0.001
Herbalist 3	S3	51.67±1.5	22.0±2.0	198.74*±2.0	p<0.001
	L3	8.35±0.8	13.31±0.9	28.41*±1.2	p<0.001
	D3	11.71±1.5	21.29±2.0	34.58*±1.2	p<0.001

*Shows the significant difference between the extracts of the same herb compared to other two extracts. Values were given as mean ± standard deviation of triplicate (n=3) determinations. Statistical analyzes were performed using the Friedman test.

Table 7. Total anthocyanin content of extracts

		Extracts (mg MvGE/g)			
	Plants	**Hexane**	**Methanol**	**Ethyl acetate**	**p**
Herbalist 1	S1	12.01±1.5	97.67±1.2	291.31*±2.1	p<0.001
	L1	7.65±0.9	38.37±1.1	66.1*±1.5	p<0.001
	D1	7.07±0.97	28.41±1.4	29.67±1.8	p<0.001
Herbalist 2	S2	56.29±1.0	139.01*±2.0	84.0±1.9	p<0.001
	L2	17.09±1.3	96.29*±1.8	52.64±1.5	p<0.001
	D2	14.74±1.1	11.71±0.9	13.82±1.5	p>0.1
Herbalist 3	S3	12.61±0.98	35.34±1.4*	27.69±1.2	p<0.001
	L3	10.13±1.0	15.67±1.5	76.04*±1.9	p<0.001
	D3	27.32±1.5*	15.13±1.5	23.52±1.7	p<0.05

*Shows the significant difference between the extracts of the same herb compared to other two extracts. Values were given as mean ± standard deviation of triplicate (n=3) determinations. Statistical analyzes were performed using the Friedman test.

Table 8. Total flavonoid content of extracts

		Extracts (mg CE/g)			
	Plants	**Hexane**	**Methanol**	**Ethyl acetate**	**p**
Herbalist 1	S1	108.67±1.5	124.31±1.2	294.31*±2.1	p<0.001
	L1	16.33±0.9	55.6±1.1	111.67*±1.5	p<0.001
	D1	12.03±0.97	48.67±1.4	55.0±1.8	p<0.001
Herbalist 2	S2	118.0±1.0	375.0*±1.9	192.0±2.	p<0.001
	L2	55.37±1.3	104.3*±1.5	97.52±1.8	p<0.01
	D2	28.71±1.5	20.35±1.1	23.36±0.9	p<0.05
Herbalist 3	S3	36.61±0.98	107.0±1.4	121.34*±1.2	p<0.001
	L3	18.64±1.5	41.78±1.9	156.31*	p<0.001
	D3	52.29*±1.7	29.59±1.5	43.0±1.5	p<0.05

*Shows the significant difference between the extracts of the same herb compared to other two extracts. Values were given as mean ± standard deviation of triplicate (n=3) determinations. Statistical analyzes were performed using the Friedman test.

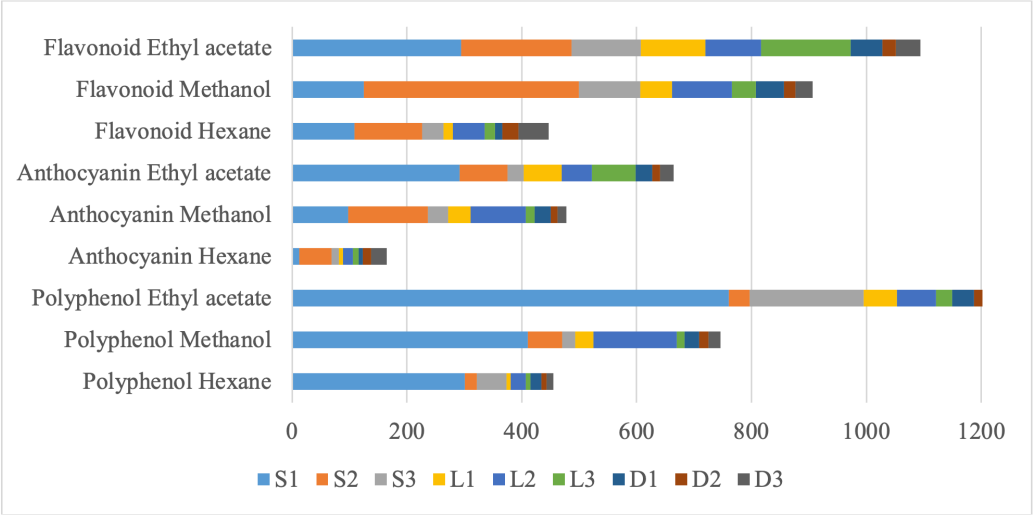


Figure 2. Bioactive components of different extracts of herbal tea samples

obtained from the 1st herbalist, methanol extracts of sage and linden and hexane extracts of daisy obtained from the 2nd herbalist, and ethyl acetate extracts of sage and linden and hexane extracts of daisy obtained from the 3rd herbalist were higher in flavonoid contents compared to other extracts (Table 8).

As seen in figure 2, considering the content of bioactive components, the best plant in terms of content is sage taken from the first herbalist. Solvents that form the best solvent medium for determining the polyphenolic, flavonoid and anthocyanin contents of different extracts of the same plants were determined as ethyl acetate, methanol and finally hexane, respectively (Figure 2).

In previous studies, it was reported that herbal substances were quite rich in micro and macro elements and teas obtained from these herbs were high in mineral contents (Sembratowicz and Rusinek-Prystupa 2014); (Stanojkovic-sebic et al. 2015); (Çolak et al. 2014). Therefore, including herbal teas in diets is of utmost importance in terms of supporting daily mineral intake. The minerals found in products result from the water and herbs used in its production. Leblebici et al. (2012) reported that Cr content of sage herb (7.30 ppm) was quite high (Leblebici et al. 2012). In our study, it was determined that daisy samples included more Cr content (Table 4). For medicinal herbs, the limit of containing Cr element is 2.0 ppm (20.000 µg/g) (WHO, 2007). For edible plants, this limit for Cu element was set at 3.0 ppm (FAO/WHO 1984). In our study, linden (1.23±0.00-1.69±0.00 µg/g) values were observed to be below Cr limit while sage (1.51±0.00, 2.70±0.00 µg/g) values were below the Cu limit. Additionally, in a study conducted in the Kisii region of Southwestern Kenya with medicinal herbs, Cr levels were determined as 2.035-.0567 ppm (Jabeen et al. 2010) while they were determined as 1.2-.29.49 ppm in the Haripur basin of Pakistan (Maobe et al. 2012).

According to Polat and Ogut (218), the Fe, As, Cd, Cr, Cu, Hg, Ni, Pb and Al metals' concentrations in six different medicinal plants *(Camellia sinensis, Tilia platyphyllos, Hypericum perforatum, Matricaria chamomilla, Salvia officinalis, Thymus vulgaris*) sold in herbal stores in Aydın, Denizli, Burdur and Isparta were determined via ICP-OES in samples sold unpackaged and as packaged tea for infusions. According to this study, more heavy metal residues were detected in the herbal tea samples sold unpackaged compared to the infused teas. At the end of the study, while the maximum amount of heavy metal in the samples sold unpackaged was Fe (302 mg/kg), Cd and Cr heavy metal residues were not identified in the samples made into infusions from packaged tea. The largest heavy metal residues were found in *Thymus vulgaris* samples sold unpackaged, and the smallest heavy metal residues were detected in the *Tilia platyphyllos* samples (Polat and Ogut 2018). Although Fe contents in types of plants are determined to be higher compared to other elements (415.65±0.13- 8783.62±11.48 µg/g), the limit of FAO/WHO for the value required in edible plants ranges below 20 ppm. Additionally, in this study, it was observed that most types of plants investigated in terms of Zn contents were quite high compared to the accepted limit values (27.4 ppm) (27.87±0.04-1278.98±0.80µg/g) (FAO/WHO 1984). While no reported toxic effect of Fe element was observed, excessive Fe, especially in children, was noted to form toxic effects as well as fatal effects of 60 mg/kg Fe intake (Kulhari et al. 2013). Small amounts of Co are a necessary element for the human body and especially, deficiency of Co may result in skin problems. Of the herbs investigated in the study, the highest amount of Co was determined in the types of daisies (3.9±0.04, 12.02±0.06 µg/g). No information for the determined limit was observed for Co element. Rajan et al. (2014) studied 4 different medicinal plants and they determined Co values as 0.284±0.099, 2.025±0.679, (0.059±0.001 ve 0.715±0.039 ppm (Rajan et al. 2014), which are quite below the values of our herbal samples. Co values we determined for sage and linden were observed to be similar to those found in the study conducted by Başgel and Erdemoğlu (2006).

High amounts of Cd can result in cancer, diarrhea, stomach problems and effects that can influence the central nervous system, and lead to death. World Health Organization's (WHO) acceptable limit is 0.3 ppm. This value for the Pb upper limit was set at 10 ppm. Accumulated lead in the body leads to acute and chronic poisoning and this can have negative effects on the kidneys, which may result in death (Heyes 1997). In our study, the amount of Hg, whose acceptable limit is 0.1 ppm, was not detected in all of the herbs while the amount of Cd could not be detected in all of the herbs, except for daisy (0.05±0.00-0.09±0.00 µg/g) (FAO/WHO 1984). Pb values for sage (4.50±0.01- 6.43±0.01 µg/g) and linden (4.53±0.00-5.92±0.00 µg/g) in our study were below the acceptable limit. Values for linden varied between 4.35 and 11.71 ppm. In a study conducted by Martín-Domingo et al. (2017) on herbal teas in Spain, it was reported that Cd content was 0.08 ppm and Pb content was 1.00 ppm (Martín-Domingo et al. 2017). These values are quite below the Cd and Pb contents of herbal samples in our study.

Natural antioxidant sources generally consist of herbal phenolics (Atoui et al. 2005). Antioxidant activities of phenolics are related to hydroxyl groups in their molecules (Ziakova and Brandsteterova 2003). The largest part of herbal phenolics comprises of flavonoids. This group includes more than 8000 known compounds (Pietta and Gardana 2003). Phenolic substances included in food compounds are of importance in terms of nutrition and health owing to their antioxidant properties. In a study conducted by İncedayı (2017), it was reported that linden plant and its beverage had total phenolic substance amounts of 7415.56±28.50 mg GAE/100g and 220.96±7.68 mg GAE/100mL, respectively (İncedayı 2017). Akış (2010) reported that the total phenolic substance content in the same plant was 5112 mg GAE/100g. Sinir et al. (2016) determined this value in carbonated erica beverage as 174.06±24.53 mg GAE/100mL and the results were deemed close to each other. The fact that the results were determined to be higher than the data obtained in this study (varies between 6.66 and 145.29 mg GAE/g) were linked to the fact that the linden concentrations that was extracted were higher (5% and 10%).

In a study that investigated the mean flavonoid contents in linden and mint samples depending on boiling, brewing and extraction processes, it was observed that boiling process for mint and linden samples and

unprocessed linden samples were significant compared to the extraction process. It was also determined that the brewing process for processed mint, processed linden and unprocessed linden samples were more significant compared to the extraction process (Aksu 2010).

In studies conducted on types of sage (*Salvia spp.*), Miliauskas et al. (2004) determined that the flavonoid content of *Salvia officinalis* L. was 3.5 mg CE/g. In our study, the mean value was 6.70 CE/g, which is higher than that study. Miliauskas et al. (2004) investigated 12 different types of plants and determined the total phenolic content of *Salvia officinalis* L. as 22.6 mg GAE/g. Dinçer et al. (2013) determined the total phenol content of *Salvia tomentosa* type between 49.27 and 66.15 GAE/g, which indicated that their results were higher than those obtained in our study. In medicinal sage type (*Salvia officinalis* L.), Arıduru and Arabacı (2013) determined that the total phenol content, according to different solvents, was between 43.55 and 11.58 mg GAE/g.

As a result, antioxidant substances reduce the harmful effects of free radicals, which are known to play roles in the aging process and illnesses, by inhibiting reactions of free radicals, preventing the damages of oxidations via connecting oxygen and metals and preventing low-density lipoprotein (LDL) and lipoprotein oxidation. Antioxidant effects of herbal products, especially flavonoids, result from phenolic compounds such as cinnamic acid derivations and coumarins. Various studies demonstrated that phenolic compounds had antiallergic, anti-inflammatory, antidiabetic, antimicrobial, antipathogenic, antiviral and antithrombotic properties, and they had preventive effects in terms of diseases such as cardiovascular disease, cancer, osteoporosis, diabetes mellitus and neurodegenerative diseases. In the early 200s, more than 8000 phenolic compounds were identified and this number increases every day. Recently, several herbal phenolics were regarded as antioxidants and they are produced commercially. In this respect, it is deemed important to know the presence of these antioxidants, which provides preventive effects in diets, food and the levels they should be taken. Most of the phenolic compounds, whose efficiency on several subjects were scientifically proven, lack studies that investigate foreseen preventive and therapeutic properties of them, and multidisciplinary studies should be conducted on this subject.

Due to social accustomedness, linden, sage and daisy teas are frequently consumed during the winter months. There are many advantages to consuming them daily in terms of both antioxidant content and macro and micro element content. Especially sage is among the winter tea form that we should consciously use. In the comparison of total anthocyanin, polyphenol and flavonoid contents of the three herbs, it was determined that the highest content belonged to sage. This also has an important in terms of meeting daily mineral intake. Another winter tea that should be emphasized is linden tea. Every other herbal tea can be used as long as they meet the quality standards. However, because herbal teas, such as daisy, linden and sage teas, can show their effects after several uses, their use should be limited. Additionally, by determining the microelement concentrations of herbal samples, which are excessively consumed by purchasing from markets, the question of whether the mineral element levels of frequently used herbs are safe in terms of health.

CONCLUSIONS

As a result, It was determined that sage ethyl acetate extract had the highest value in terms of total anthocyanin, polyphenol, and flavonoid contents when compared to other plant samples. In addition, it was determined that the Cu and Ni values in sage and the Fe and Pb values in both sage and linden were within safe limits for health.

In conclusion, considering the winter periods, when fluid intake decreases and fluid excretion increases, and detoxification process based on sweating becomes less active, herbal teas, which passes into the extracellular fluid environment and excreted via sweating and urine, should be consumed more as long as they meet the minimum quality standards. They also have importance in terms of the development process of children and youth, and geriatric period health and vitality as well as in terms of homeostasis.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

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

Author contribution

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

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable

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RESEARCH ARTICLE

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Determination of effective surface sterilization protocol in in vitro tissue culture for Giant Snowdrop (*Galanthus elwesii* Hook) bulbs

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Abstract

Giant Snowdrop (*Galanthus elwesii* Hook) is a species of snowdrop belonging to the Amaryllidaceae family. In this study, the deformation rates of the bulbs was calculated as % (percent). For sterilization G. elwesii bulbs were sterilized at different concentrations (1, 3, 5, 7, 9, 11 and 13 %) of sodium hypochlorite (NaOCl) for 5, 10, 15, 20, 25 minutes. The bulbs were rinsed with sterile distilled water 3 times for 5 minutes and then transferred to the MS medium. Contamination rates in MS nutrient medium were determined as percent (%) after 7 days. Deformation was not observed in the bulbs sterilized with 1 and 3% sodium hypochlorite solution however 100% contamination was detected. The most effective surface sterilization was obtained by soaking in 9-11% sodium hypochlorite solution for 5 and 10 minutes. As the sodium hypochlorite concentration and the application time increased, the surface sterilization of the bulbs increased, but the deformation rate of the scaly leaves of the bulbs increased due to this increase.

Keywords: Giant Snowdrop, Bulb, Sterilization, Contamination, Deformation

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INTRODUCTION

In terms of seed plant diversity and plant genetic resources, our country is one of the richest countries in the world and by means of biodiversity it is compared with the Europe. Biodiversity is the natural resources of the countries and it refers to the diversity of life at all organizational levels, from the genetic, population and species levels to communities and ecosystems, and a dynamic feature of the ecosystem. Protection, development and sustainable use of this resource should be among the main objectives (Leveque and Mounolou, 2003). Considering that the origin of the basic foodstuffs necessary for our life is formed by wild species in nature the importance of preserving biological equality will be better understood (Arpa, 2012).

Bulbous plants which are called as geophytes have underground organs such as bulbs, tubes, and rhizomes They contribute to biodiversity since most of the geophytes have a very long time to form new shallots in their natural environment and the bulb formation rate is very low (Baktır et al., 1997). Giant Snowdrop reproduces in the natural environment with the formation of seeds and new shallots. For the formation of a new bulb from the seed, a long period of 4-5 years is required. Snowdrop reproduces in the natural environment with the formation of seeds and new shallots. The removal of bulbs from nature and long life cycle of the plantlead to the decline of the species belonging to the genus *Galanthus*. Therefore, rapid propagation methods should be designed for generation of these plants (Tıpırdamaz et al., 1999).

An effective sterilization method should provide both effective sterilization on the surface of the plant material and against possible infection sources on the plant material. In *in-vitro* studies usually same type of surface sterilization methods are used however these methods might not be effective against field-borne in-seed and bulbus pathogen contamination.

On the other hand, Girmen and Zimmer (1988) found that efficiency of sterilization was different between explants and seeds in *Galanthus*, *Leucojum* and *Tulipa* plants.

In a sterilization study using NaOCl, they reported that they obtained *Aloe pretoriensis* (*Liliaceae*) seeds by soaking them in 5% NaOCl and 1% $HgCl_2$ for 30 minutes, then in Nalauryl sulfate for 10 minutes and rinsing them with distilled water four times (Groenewald et al., 1975).

According to Gochhayat et al. (2017), hybrid *Lilium* Cv. Explants of Tresor plants were subjected to surface sterilization with $HgCl_2$ at different times (Control, 3, 4, 5, 6, 7, 8, 9 minutes) and at least 8 minutes of fungal and bacterial contamination was observed 15, 30, 45 days after inoculation. In their study, they applied different doses of BAP (0.5, 1 mg/l) and 2,4-D (0.5, 1, 1.5, 2, 2.5 mg/l) hormones and the highest callus production was obtained from the application which they applied 1 mg/l BAP and 1.5 mg 2,4-D hormones together.

Farooq et al. (2022), applied different concentration of carbendazim, mercuric chloride and ethyl alcohol sterilants to bulb shells and young leaf segments of "Indian Summerset" and "Nashville" Lilium LA hybrids at different times. The maximum percentage of asepsis was found in the application of Carbendazim 0.02 % for 30 minutes, mercuric chloride 0.1% for 5 minutes, and ethyl alcohol 70% for 10 seconds in both varieties.

Kone et al. (2011), conducted a study on the effect of substrate type and bulb size on *in vivo* production of seedlings in three plantain cultivars. They determined that the application of furadan+mancozeb in all cultivars in their study was successful in reducing contamination than other applications (Javel 0.25%, water 50°C, Javel 0.25%+water 50°C). In that study, the average number of buds in the bulb obtained from the soil substrate application was higher than the other applications (sawdust, sand). In addition, the largest bulbs were obtained from the least amount of buds in all cultivars.

This study was carried out to determine an effective sterilization method against microbial pathogens found in the bulbs of *Galanthus elwesii* plant. For this purpose, *Galanthus elwesii* bulbs were kept in 1, 3, 5, 7, 9, 11, 13 and 15% sodium hypochlorite (NaOCl) solution for 5, 10, 15, 20 and 25 minutes.

MATERIALS AND METHODS

In this study, the bulbs of *Galanthus elwesii* plants were used as material. Bulbs were washed under tap water for 10 minutes, and the soil, mud and other foreign matters were removed. In order to achieve an effective surface sterilization; 1, 3, 5, 7, 9, 11, 13 and 15% sodium hypochlorite (NaOCl) solutions (Y1, Y3, Y5, Y7, Y9, Y11, Y13, Y15) were applied to the bulbs for 5, 10, 15, 20 and 25 minutes (S5, S10, S15, S20, S25). The rate of deformation that occurred in bulbs after NaOCl application was determined as percent (%) and is shown in (Table 1). The sterilized bulbs were rinsed by passing them through distilled water 3 times for 5 minutes. After rinsing, bulbs were cultured in MS (Murashige and Skoog, 1962) nutrient medium. The contamination rate that occurred 7 days after the bulbs were cultured was determined in percent % and shown in (Table 1). In the study, nutrient mediums, tools and equipment were sterilized by autoclave under 1.05 atmosphere pressure at 120°C for 21 minutes. The pH of the nutrient medium was adjusted to 6.1 using 1 N KOH and 1 N HCl. Bulbs taken into the culture medium were cultured at 25±0.5°C in a climate cabinet with 18 hours of light (350 µmol m^{-2} s^{-1}) and 6 hours of darkness. For each repetitions, 4 bulbs were used, the trials were planned as 3 repetitions and the average of the percent (%) values formed as a result of these repeated trials was calculated. Relationships between the investigated features were determined by correlation analysis (Table 1). Biplot analysis was used in the interpretation of the data and the data were evaluated on the graph (Figure 1-2).

RESULTS AND DISCUSSION

We found a significant negative correlation between the contamination and deformation rates in terms of solution concentration and treatment duration (Table 1).

Table 1. Correlation coefficients between contamination and deformation rates

	Contamination rate	Deformation rate
Contamination rate	1,000	-0,8278***
Deformation rate	-0,8278***	1,000

The resulting inverse ratio was also seen in the graph of the correlation analysis. It can be concluded that as the solution concentration and duration of the treatment were increased the contamination rate was decreased while the deformation rate was increased.

In this study, keeping the bulbs in 9-11% hydrochloric acid for 5-10 seconds both sterilized and did not degenerate. The samples in the subjects with high deformation rate were deformed which meant that their structures were deteriorated due to the effects of high hypochlorite concentration or treatment time. It was found that the samples with high contamination rate were infected by bacteria or fungi due to low hypochlorite concentration or treatment time. When the biplot graph is examined, it can be said that the subjects closer to the origin than other subjects such as Y11S15, Y11S10, Y7S25, Y7S20, Y11S5, Y9S10, Y9S5 comply with the described definition

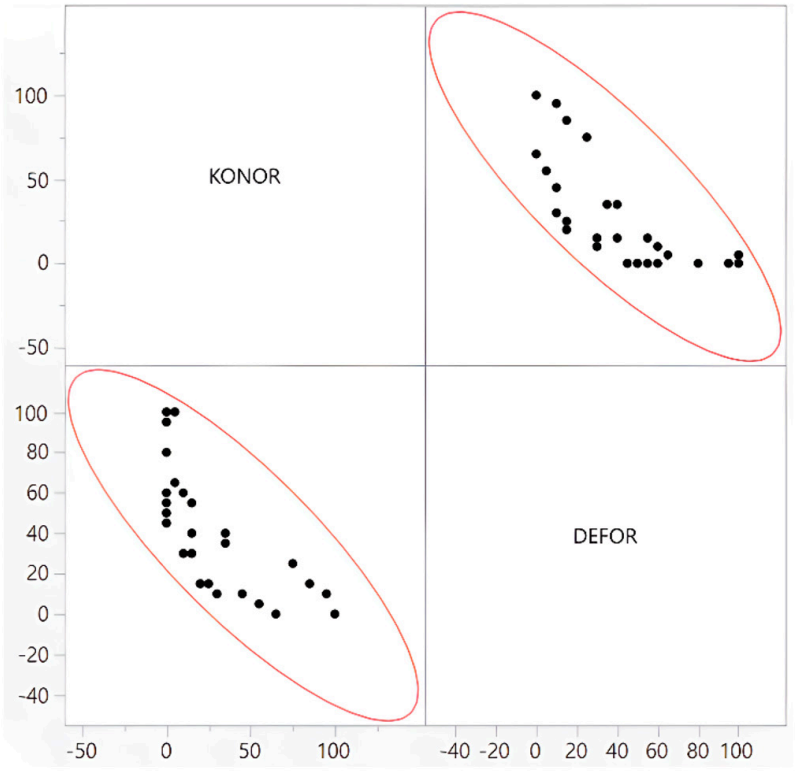

Figure 1. Correlation Analysis (Konor: Contamination rate, Defor: Deformation rate).

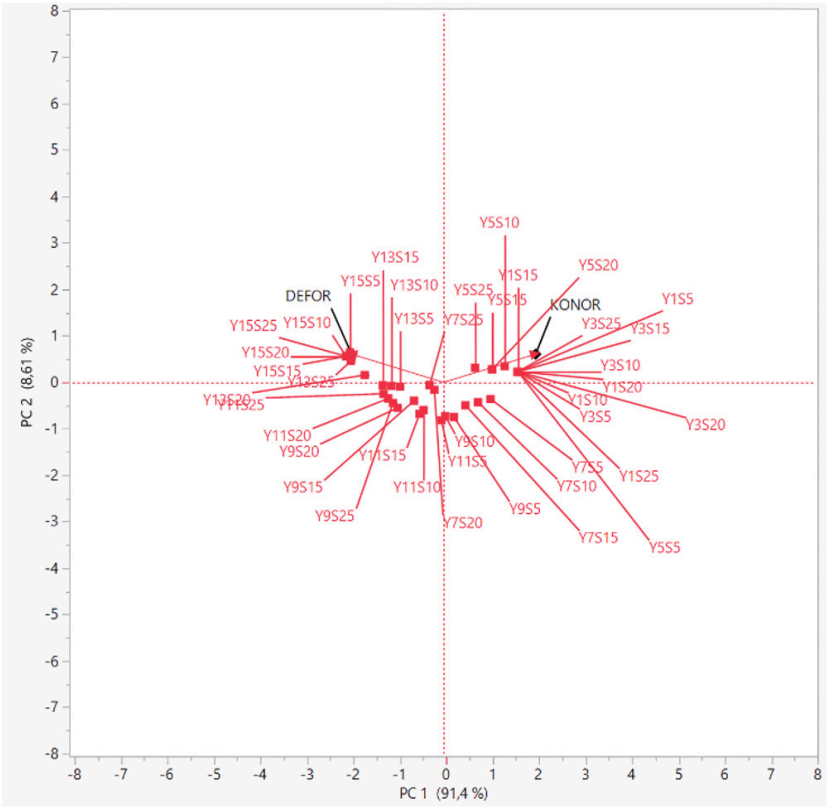

Figure 2. Graph of Biplot Analysis (Konor: Contamination rate, Defor: Deformation rate).

and undergo less contamination and deformation than other subjects, that is, they are more usable.

According to the results of the study, the average contamination rate was determined as 100% and the average deformation rate was 0% in all bulbs dipped in 1% NaOCl for 5, 10, 15, 20 and 25 minutes. The average contamination rate was determined as 100% and the average deformation rate was 0% in all bulbs dipped in 3% NaOCl for 5, 10, 15, 20 and 25 minutes.

The most effective surface sterilization was obtained by 9-11% sodium hypochlorite solution for 10 and 5 minutes. The deformation rates of scaly leaves of the bulbs was increased by high hypochlorite concentrations and treatment time.

In another study, the surface sterilization of the bulb explants of *G. nivalis* and *G. elwesii* was followed by sterilization of the whole bulb with 2 and 3% NaOCl solution for 20 minutes. It provided almost complete infection control in all of its batches, making the use of PPM or fungicides in the medium unnecessary. Therefore, they recommend the use of NaOCl in the surface sterilization of plant materials of the genus *Galanthus* (Staikidou et al., 2008).

CONCLUSION

Before starting the studies using plant tissue culture technique, the surface sterilization of the plant material to be used as material should be done effectively. If the surface sterilization of the plant material cannot be performed effectively, plant material, time and chemicals will be lost due to possible contamination problems. Therefore, it is very important to develop an effective surface sterilization protocol for the plant material used. Deterioration in tissue culture due to sterilization and hydrochloric acid (NaOCl) used can be a problem for researchers. With this study, keeping the bulbs in 9-11% hydrochloric acid for 5-10 seconds both sterilized and did not degenerate. Information has been obtained that can directly benefit those who will do tissue culture with Giant Snowdrop bulbs. Apart from these, many scientists around the world have tried sterilization in different organs and tissues of various plants by using a wide variety of substances and methods, and naturally they have reached very different results. The study provided convenience to those who do plant tissue culture studies. According to the results obtained, considering the applied concentration and time, it can be suggested that NaOCl can be used successfully in the sterilization of the bulbs of the Giant Snowdrop plant.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

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

Author contribution

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

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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An example of lettuce (*Lactuca Sativa*) seedling selection using deep learning method for robotic seedling selection system

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Abstract
Lettuce is a type of vegetable that is widely cultivated and consumed in our country and in the world. The seedling period, which is the beginning of production, is the most sensitive time for the plant. Starting production with healthy seedlings is an important parameter for quality and efficient production. In this study, a sample program for automatic seedling selection was developed for a robotic system to be used in seedling production. With the developed program, it was aimed to select seedlings with the same degree of maturity in multi-well pots. In this study, Yolo5n was used for the training model. A learning system was established on two types of lettuce (curly salad), and red curly lettuce leaf (lolo-rosso) seedlings. As a result of the training, F1 score was found as 83%; Precision was 100%; Recall was 95%; Precision Recall was 86.7%. The learning rate was 0.0005 for all given images. In view of these data, positive results were obtained for the mentioned method in seedling selection.
Keywords: Deep learning, Lettuce, Robotics

INTRODUCTION

Lettuce (*Lactuca sativa*) is a cool climate vegetable from the Asteraceae (Compositae) family, widely produced worldwide, whose leaves is eaten and can be grown year-round in open and under cover. Since it can be produced all year round, it can be easily alternated with different vegetables. It can also be grown on small home balconies and in large areas. The lettuce group is a vegetable of high economic value that is consumed as a salad ingredient in the world. However, in some countries such as China, the roots and leaves are also eaten cooked (Anonymous,2023a). Lettuce is one of the vegetables that are not very selective in terms of soil requirements. It can be grown in all kinds of soils from light soils to clayey heavy soils. The most important climatic factors in the germination and emergence of lettuce are light and temperature (Vural et al. 2000, Doğru and Çilingir, 2019).

Because of the advantages such as lettuce cultivation can be done by direct seed sowing method, seedling cultivation is more preferred. There are some quality characteristics that producers look for in ready seedlings. These are; narrow internodes of the seedlings, dark green leaf color, completed root wrapping in peat, thick and strong stem, and high dry matter content of the root and stem (Akdemir, 2018). Production with ready seedlings has reached 100% in greenhouse vegetable production and 70% in open field vegetable cultivation in our country (Yelboğa, 2014).

Seedling production is done in pans, crates and viols according to the growing season. In seedling cultivation, which is carried out by growers within their own

means, cultivation with ready seedlings produced in seedling enterprises has been preferred in recent years due to reasons such as failure to provide optimum conditions, failure to carry out maintenance processes in accordance with the technique and high cost. In modern seedling production facilities, seeds are sown untouched with advanced technologies. It has been reported that coated seeds, which are mostly used in lettuce seedling production, provide great convenience in seedlings, reduce seed loss, have a germination rate close to 100% and uniform emergence. Lettuce seeds germinate within 1-3 days at optimum temperature values in germination rooms. Immediately after germination, the vials are transferred to the greenhouse. Since the optimum ecological factors required for lettuce seedling production are easily provided in the greenhouse and maintenance processes such as irrigation, feeding, disease and pest control and growth control are carried out regularly in accordance with the technique, quality seedlings that reach the planting stage in a short time (25-35 days) are obtained (Anonymous, 2023b). In seedling cultivation under producer conditions, seedlings reach planting size in 40-50 days depending on the production period, while ready seedlings reach planting size between 25-35 days. In lettuce cultivation, planting time varies according to regions and varieties, but planting is done on tubes or flats. Considering the variety characteristics in planting, the row spacing is adjusted as 30-40 cm and the row spacing is adjusted as 20-30 cm. Mostly, depending on the variety, harvesting is done 60 - 90 days after planting. It is more convenient to harvest lettuce in the morning. The average yield per hectare is 20-40 tons. [Vural et al. 2000, Sevgican 2002)]

According to Turkish Statistical Institute data, total lettuce production in our country in 2021 was determined as 540,569 tons. Lettuce, which is so widely and intensively cultivated, is especially important to benefit from deep learning applications in order to prevent developmental differences that may arise from seedlings by obtaining homogeneous seedlings, to offer healthy seedlings to production at the right time and to minimize seed and seedling losses.

On the basis of previous studies, there are many studies with deep learning for lettuce. Especially for lettuce, studies such as disease detection and product harvest size determination were carried out. (Lu et al., 2019) monitored the growth of lettuce in greenhouses using real-time image and deep learning. They processed the images taken with the imaging system installed in the greenhouse. They developed a mask region-based convolutional neural network (Mask R-CNN) model for simultaneous segmentation. They determined lettuce growth rates according to the values of leaf areas against time. The experimental results showed that the Mask R-CNN model achieved an accuracy of up to 97.63% in predicting the leaf area. The aim of the research conducted by Hassim & Chuah (2020) was to design lettuce variety recognition with at least 90% accuracy using Convolutional Neural Network (CNN) in MATLAB. The CNN was used to classify the seven most commonly found lettuce varieties. The CNN model was trained with 7000 leaves and tested with 1800 leaves to classify the 7 lettuce varieties. Yudha Pratama et al. (2020), used Deep Learning to recognize and detect disease in hydroponic vegetables using Inception V2 algorithm and Faster R-CNN in their research. They divided the training and validation dataset rate into 3 categories. As a result of the study, they determined that the testing and validation rate was affected by deep learning model performances. In another study, Alon (2020) developed a system focusing on lettuce health recognition. With this system, it was determined whether lettuce was healthy or diseased. The system was a machine using deep learning. The machine was connected to a microcontroller raspberry pi 4b. Lettuce health recognition was done with an overall test accuracy of 97.59%. Zhang et al. (2020) suggested a method to monitor the growth of greenhouse lettuce using digital images and a convolutional neural network (CNN). Taking lettuce images as input, they trained a CNN model to learn the relationship between the images and relevant growth-related characteristics, such as leaf fresh weight (LFW), leaf dry weight (LDW), and leaf area (LA). As a result of the experiments, they showed that a CNN with digital images was a robust tool for greenhouse lettuce growth monitoring. Rizkiana et al. (2021) developed a plant growth prediction model using the Artificial Neural Network (ANN) method. The ANN model was tested using different numbers of nodes, from 1 to 7 nodes, daily average temperature, average daily humidity, EC and light intensity input in the hidden layer. Lettuce growth rate prediction was found to be accurate. In another study, Ahsan et al. (2022) used four lettuce varieties grown hydroponically. They took RGB images of lettuce leaves. The results showed that the visual geometry group 16 (VGG16) and VGG19 architectures of the developed DL identified the nutrient levels of the lettuces with 87.5% to 100% accuracy for the four lettuce varieties, respectively.

MATERIAL AND METHODS

Preparation of the Data Set

While preparing the dataset of lettuce vegetable, targeted for object detection and analysis within the project, harvest photos taken in the greenhouses were used. Since lettuce is a vegetable that diversifies as green leaf lettuce and Mediterranean lettuce (lolo rosso), it is possible to see two types of lettuce in the photographs. Many lettuce images taken in the vineyard during harvest and cultivation were collected. Among the images obtained, the images that we could not evaluate within the scope of our project were eliminated. We identified 15 images that would be reliable for our object identification study. In addition, there are 6 * 8 =

48 seedlings in the seedling tabs used in each image. The dataset used in training are shown in Figure 1 and 2.

Data Set Used in Training

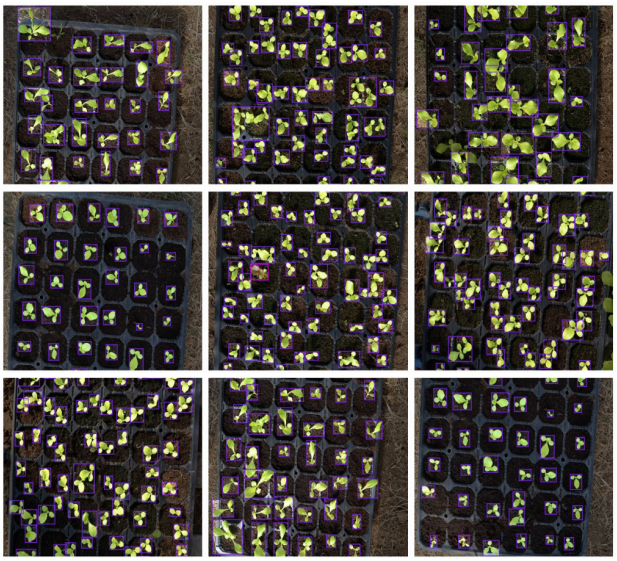


Figure 1. Images from the training sets used during the training of the models (Lettuce)

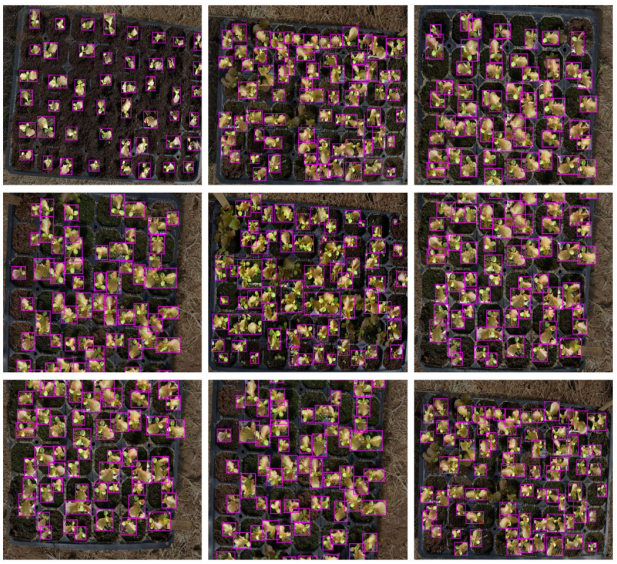


Figure 2. Images from the training sets used during the training of the models (Lolo-Rosso)

Labeling

In order for an object detection model to be able to train on a dataset, the objects targeted to be detected must be labeled/ signed in the dataset to be trained. For this reason, the parts containing the lettuce image in each of the 15 images should be marked with the bounding box area and assigned to the "lettuce seedling" or "lolo-rosso seedling" class, which is the object class it belongs to. There are many programs, websites and utilities available in the open source communities for image labeling. One of these tools is Roboflow, a popular program that is frequently used in object detection projects.

Roboflow is a website that provides all the tools needed to transform raw images into a specially trained computer vision model and distribute it for use in applications, as well as to perform field selections, markings and class labeling on images. This marking and labeling is easily done through the graphical user interface of the website. Figure 3 and Figure 4 show the Label screen.

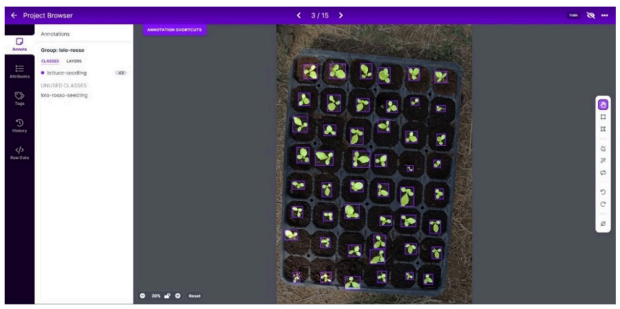


Figure 3. Label Screen (lettuce)

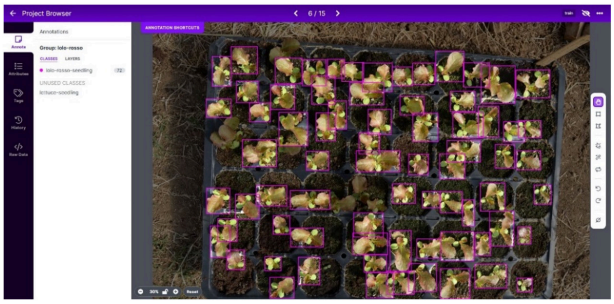


Figure 4. Label Screen (Lolo-Rosso)

Training Model Selection

In the project we carried out, the YOLOv5 family, developed as an open source of the YOLO model family developed by the CNN method, was preferred. The YOLOv5 model of the YOLO model family, which has a significant advantage over models using a two-stage network similar to RCNN, was preferred because it provided advantages in terms of accuracy values and speed ratio to versions developed before it. As explained in detail in the upper sections, the YOLOv5 model also contains models within itself. The YOLOv5n (nano) model was preferred for deep learning training.

Initiation of Training

In order to start the training of the model that will perform lettuce detection, the location of the YOLOv5 model on the computer was visited and a Python runner editor was opened there. The train.py program, which is in the main directory and provides the YOLOv5 training, was checked to be run. The execution of this Python program can be customized with various parameters.

Within the project, the parameters and regulations in the code written below were preferred.

python train.py --img 640 --batch 30 --epochs 400 --data dataset.yaml --weights yolov5n.pt

--img: The pixel size at which the images to be trained will be reduced by the YOLOv5 model. Its default value is 640x640, and it was chosen here in this way.

--batch: The number of data point packets to be used by the display card at a time while training the model.

--epochs: The number of times all training data is shown to the trained network and the weights are updated while training the model.

--data: The path to the .yaml file containing the general path and class information of the file containing the dataset

--weights: The location of the weight file containing the training coefficients to be used in training the model

As a result of running this line of code correctly, the training process of the model has started. The program first checks the YOLOv5 files and checks for any update status. Then, the training process is carried out during the determined number of cycles (epoch).

Performance Matrix

The Performance matrix requires measuring deep learning performance that consists of four core values below:

1) True Positive (TP): There is mature seeding, and the algorithm detects it as mature seeding.

2) False Positive (FP): There is no mature seeding, and the algorithm detects it as no mature seeding.

3) False Negative (FP): There is mature seeding, but the algorithm does not detect it as mature seeding.

4) True Negative (TN): No mature seeding and nothing is detected.

5) Accuracy: Accuracy is a measure that explains that a model or algorithm has been properly trained and can show the results of the training. In this study, accuracy explains how to classify mature seeding. The accuracy is calculated using the following formula:

$$\text{Accuracy} = \frac{(TP+TN)}{(TP+TN+FP+FN)} \quad (1)$$

6) Precision: Shows the ratio of positive predicted cases that are positive. In the context of this study, precision measures a small portion of the object predicted as mature seeding and immature seeding. Accuracy is calculated using the following formula:

$$\text{Precision} = \frac{TP}{(TP+FP)} \quad (2)$$

7) Recall: This is the ratio between actual positive cases that are predicted to be positive. In the context of this study, recall measures a small portion of seeding that is predicted to be mature. The recall is calculated using the following formula:

$$\text{Recall} = \frac{TP}{(TP+FN)} \quad (3)$$

8) F1 Score: Also known as an F-score or F-measure counterweight. F1 scores are a measure of model accuracy that combines precision and recall. In the context of this study, a good F1 score indicates that there are fewer false positives and false negatives. This shows that this model correctly identifies mature seeding from the existing dataset. The model or algorithm is considered perfect if the F1 score is 1. F1 scores are calculated using the following formula:

$$F1 = \left(\frac{2xPrecisionxRecall}{Precision+Recall}\right) \quad (4)$$

9) Training time: Training time is a metric used in this research to measure the time needed to conduct training on the modeling algorithm chosen for the dataset.

10) Detection Speed: Speed is a metric used in this study to measure the time required by the algorithm to process and detect seeding objects.

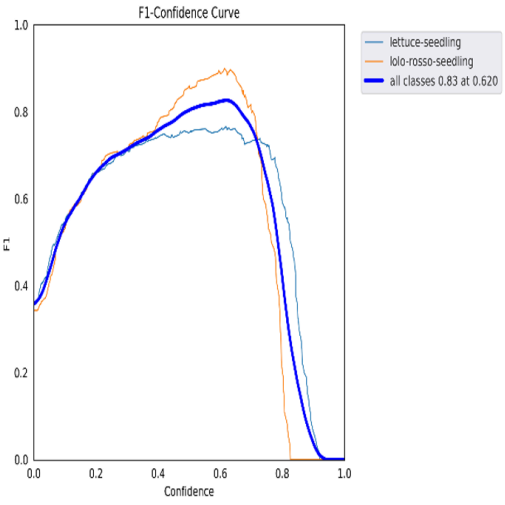
RESULT FINDINGS

Analyzing the results of YOLOv5 algorithms according to error matrix metrics

F1 Score

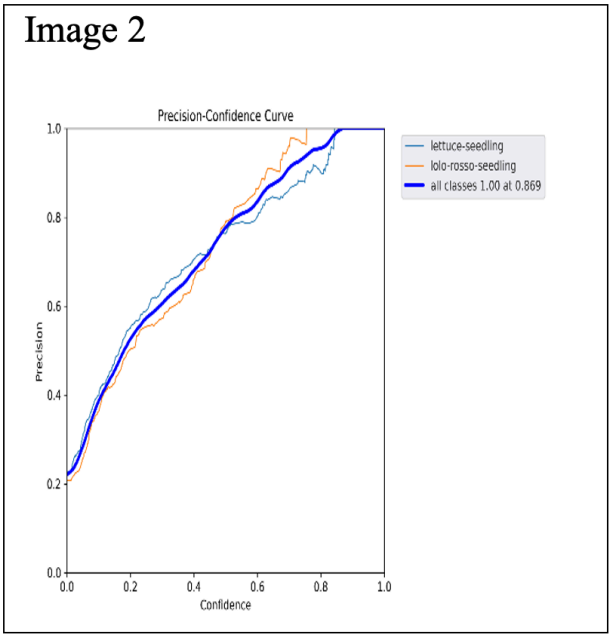
Image 1: Size: 640x640, Batch: 30, Epoch: 400, Algorithm: YOLOv5n

Image 1



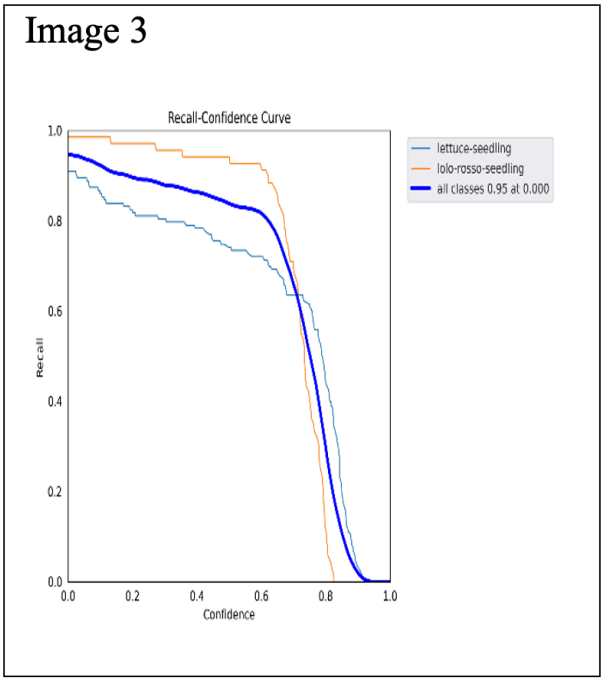
Precision

Image 2: Size: 640x640, Batch: 30, Epoch: 400, Algorithm: YOLOv5n



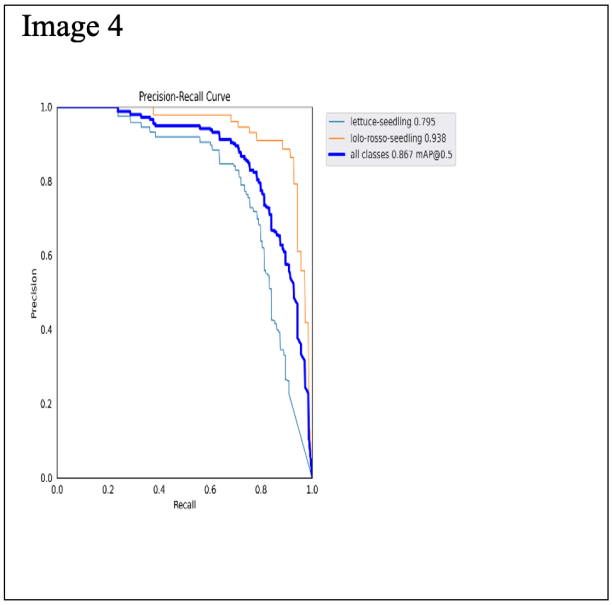
Recall

Image 3: Size: 640x640, Batch: 30, Epoch: 400, Algorithm: YOLOv5n



Precision Recall

Image 4: Size: 640x640, Batch: 30, Epoch: 400, Algorithm: YOLOv5n



Loss Function

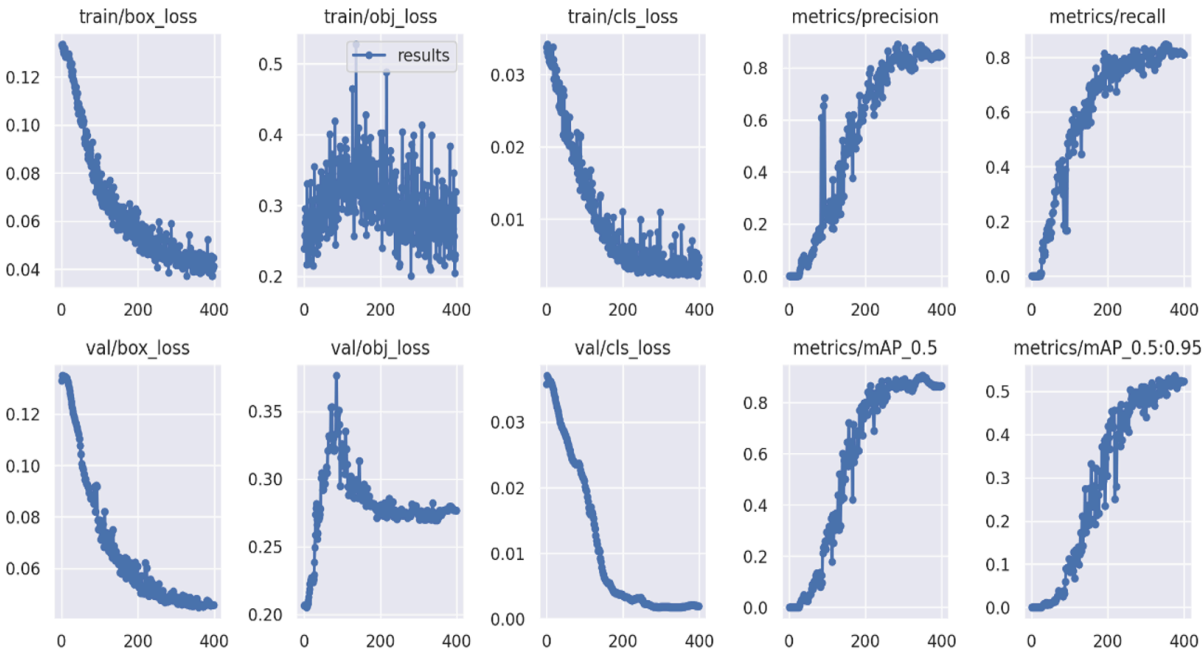
Image 5: Size: 640x640, Batch: 30, Epoch: 400, Algorithm: YOLOv5n

TRAINING RESULT

Figure 5. "Validation Batch" prediction markings resulting from the training of the models (Lettuce-Seeding)

Figure 6. "Validation Batch" prediction markings resulting from the training of the models (Lolo-Rosso Seeding)

Image 5



Configuration Files and Parameters

These algorithms use the following configuration files which are available under the yolov5 framework and contain the hyper parameters used to train the models:

Descriptions of the parameter columns in the table: "Model" : Full name of the corresponding YOLOv5 algorithm model

"size" : The size of the model's input image, in pixels

"mAP^{val}" : The mean average precision value of the

YOLOv5 model uses the configuration file "hyp.scratch-low.yaml" and the hyperparameters in it.	lr0: 0.01 lrf: 0.01 momentum: 0.937 weight_decay: 0.0005 warmup_epochs: 3.0 warmup_momentum: 0.8 warmup_bias_lr: 0.1 box: 0.05 cls: 0.5 cls_pw: 1.0 obj: 1.0 obj_pw: 1.0 iou_t: 0.20 anchor_t: 4.0 fl_gamma: 0.0 hsv_h: 0.015 hsv_s: 0.7 hsv_v: 0.4 degrees: 0.0 translate: 0.1 scale: 0.5 shear: 0.0 perspective: 0.0 flipud: 0.0 fliplr: 0.5 mosaic: 1.0 mixup: 0.0 copy_paste: 0.0
YOLOv5 model uses the configuration file "hyp.scratch-high.yaml" and the hyperparameters in it.	lr0: 0.01 lrf: 0.1 momentum: 0.937 weight_decay: 0.0005 warmup_epochs: 3.0 warmup_momentum: 0.8 warmup_bias_lr: 0.1 box: 0.05 cls: 0.3 cls_pw: 1.0 obj: 0.7 obj_pw: 1.0 iou_t: 0.20 anchor_t: 4.0 fl_gamma: 0.0 hsv_h: 0.015 hsv_s: 0.7 hsv_v: 0.4 degrees: 0.0 translate: 0.1 scale: 0.9 shear: 0.0 perspective: 0.0 flipud: 0.0 fliplr: 0.5 mosaic: 1.0 mixup: 0.1 copy_paste: 0.1

model. This value is a metric showing the performance of the model to recognize an object and is expressed as a percentage. $mAP^{val}50\text{-}95$ gives an average performance value for all targets between 50% and 95%. $mAP^{val}50$ shows the performance of the model by considering only the best match.

"Speed" : Parameter columns that specify the processing speed of the model in ms. This value indicates how long the model can process for an input image. The CPU b1 column shows the processing speed of the model using a CPU processor, while the V100 b1 and V100 b32 columns show the single and 32-core processing speed of the model using the NVIDIA V100 graphics processor.

"Params" : Shows the total number of weight parameters of the model in M (Million).

"FLOPs" : FLOPs, short for "FLoating point OPerations", indicates the number of processes the model will perform for an input image (640x640 by default) in B (Billion).

CONCLUSION

In our study,object detection accuracies in the sample training and validation images performed with the YOLOv5 Nano model and the prepared dataset were examined. When the metric data and accuracy prediction rates indicating the object detection success of the models was examined, it was confirmed that the training result of the model was successful. In a similar study (Du et al., 2020), they found that the F1 score value was 97.65% in the system of determining the maturity of lettuce with deep learning. They found that the product certainty detection value was 99.82%. In another study (Yudha Pratama et al., 2020), they found Accuracy 70%; Precision 97%; Recall 68% and F1 Score 80% in the use of deep learning medtod to recognize and detect disease in hydroponic vegetables using Inception V2 algorithm and Faster R-CNN. They measured the learning speed as 0.0002 for all data. In this study, as a result of the training, F1 score was 83%; Precision 100%; Recall 95%; Precision Recall 86.7%. The learning rate for all given pictures was found to be 0.0005. When the results of previous studies are compared with the results of our research, it is seen that similar results are obtained. However, it should be considered that these results may change when working on datasets of different sizes and diversity, or when changes are made on the hyper parameters and general operating parameters related to the training algorithms, or when a success rating based on speed performance rather than object detection success is made.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

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

Author contribution

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

Ethical approval

Ethics committee approval is not required.

Funding

No financial support was received for this study.

Data availability

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

Consent for publication

All authors consented to the publication of this manuscript.

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RESEARCH ARTICLE

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Biological effects of *Beauveria bassiana* and *Akanthomyces attenuatus* isolates on *Aphis gossypii* Glover (Hemiptera: Aphididae)

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Abstract

The biological effects of entomopathogenic species, *Beauveria bassiana* (Bals.-Criv.) Vuill and *Akanthomyces attenuatus* Zare & Gams on cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae) nymphs at $1x10^8$ conidia concentration (ml^{-1}) were investigated at the laboratory conditions. The experiments were conducted at 25°C, 65% relative humidity, and 16:8 hours of lighting in a climatic cabinets. Alive nymphs were recorded on the 1st, 3rd, 5th, 7th, and 9th days of incubation. *B. bassiana* caused higher mortality of the nymphs than *A. attenuatus* did. A statistical difference was determined between the isolates on the 7th and 9th days of the experiment. The highest mortality rates were determined in the isolate of *B. bassiana* with 72% and the isolate of *A. attenuatus* with 54% on the 9th day. The LT_{50} value for the isolates of *B.bassiana* and *A. attenuatus* was 6.02 days and 8.33 days, respectively.

Keywords: *Aphis gossypii*, *Beauveria bassiana*, *Akanthomyces attenuatus*, Entomopathogenic fungus, Biological control

INTRODUCTION

Aphis gossypii Glover (Hemiptera: Aphididae) is widely distributed in tropical, subtropical and temperate regions. It is a polyphagous species that disrupts plant growth by feeding on phloem sap and is act as a virus vector (Martin et al., 2003). Among its hosts, there are more than 50 plant families, including Asteraceae, Cucurbitaceae, Rosaceae, and Solanaceae (Guldemond et al., 1994; Basu and Patro, 2007). *Aphis gossypii* is the vector of potato virus, citrus tristeza virus, cucumber mosaic virus, and turnip mosaic virus (Kennedy et al., 1962; Blackman and Eastop 2000; Mnari-Hattab et al., 2008; Behi et al., 2019). Pesticides with active ingredients, such as, bifenthrin, deltamethrin, imidacloprid and malathion are used to control this pest. With the long-term use of these chemicals, changes occur in the genetic structure of the pest and chemical resistance occurs. (Herron et al., 2000; Herron et al., 2001; Wang et al., 2002). It is reported that this pest was resistant to 50 active substances worldwide (Hollingsworth et al., 1994; Nauen and Elbert, 2003; Seyedebrahimi et al., 2016). As a result of the intensive application of these insecticides, the natural ecosystem (including the air, non-target organisms and humans) is affected and therefore, there is a tendency towards alternative control methods (Revathi et al., 2014). In recent decades, entomopathogenic fungi have been widely used as biocontrol agents for aganist harmful insects as they are pathogenic to insects (Akbari et al., 2013). These fungi are important in pest management with their metabolite production and biosecurity levels (Wang et al., 2019). Of these, *Beauveria bassiana*, *Metarhizium anisopliae*, *Isaria fumosorosea*, and several *Lecanicillium* species have attracted to great interest with their use in controlling aphids, and

a few of them have been used commercially (de Faria and Wraight, 2007; Lacey et al., 2015; Kumar et al., 2019). Well known entomopathogenic fungi such as *Beauveria* has been found to be effective on pests of Lepidoptera (Soetopo, 2004), Coleoptera (Lord, 2001; Wraight and Ramos, 2002) and Homoptera (Wraight et al., 1998). However, *Akanthomyces attenuatus* Zare & Gams is a well-known pathogen of whitefly, aphid and thrips, and some isolates of this species have been developed as commercial biopesticides (Wang et al., 2007; Ainsworth et al., 2008; Gottel et al., 2008; Lu et al. al., 2015). In this study, the effectiveness of *B. bassiana* and *A. attenuatus* isolates isolated from *Ips sexdentatus* (Boerner, 1776) (Coleoptera: Curculionidae) adults, on the second instar nymphs of *A. gossypii* was investigated.

MATERIALS AND METHODS

Rearing of plants and aphids

For the cotton plant (*Gossypium hirsutum* L.) to be used in the experiments, cotton seeds of variety Ergüven was planted in plastic pots with an equal ratio of soil:peat. The growth and development of these sown seeds was ensured through regular irrigations. When the cotton plants reached the height (10-15 cm) to be used in the experiments, adult individuals of *A. gossypii* were transferred to these plants and aphids were reared. For the continuity of this mass production, clean cotton plants were added to the medium regularly at weekly intervals. The production of both clean-healthy cotton plants and aphids was carried out in the climatic cabinets with 25°C temperature, 60±5% relative humidity, 16:8 (light:dark) hours conditions.

The *Beauveria bassiana* (Bals.-Criv.) Vuill. strain used in the study

The *B. bassiana* strain used in this study were isolated on Malt Extract Agar (MEA) media from surface of the bark beetle, *I. sexdentatus* distributed in *Pinus nigra* Arn. stands in the Western Mediterranean Region of Turkey (Karaceylan, 2023). After performing the morphological characterization studies of the isolate, the sequence information of the ITS gene regions of the rDNA was used for molecular characterizations and ITS1-ITS4 primer pairs were used for the amplification of the ITS region. The isolate was consistent with *B. bassiana* isolates (100% sequence similarity to *B. bassiana* KP862996.1 and OK094889.1; NCBI GenBank). Based on abundant conidia production, this isolate was selected for laboratory and field testing. The *B. bassiana* solution used in the study was prepared at a concentration of $1x10^8$ conidia/ml.

The *Akanthomyces attenuatus* Zare & Gams strain used in the study

The *A. attenuatus* strain used in this study were isolated on MEA media from surface of the *I. sexdentatus* bark beetle distributed over *P. nigra* stands in the Western Mediterranean Region of Turkey (Karaceylan, 2023). After performing the morphological characterization studies of the isolate, the sequence information of the ITS gene regions of the rDNA was used for molecular characterizations and ITS1-ITS4 primer pairs were used for the amplification of the ITS region. The isolate was consistent with *A. attenuatus* isolates (>98.90% sequence similarity to *A. attenuatus* MN908945.1 and MH231313.1; NCBI GenBank). Based on abundant conidia production, this isolate was selected for laboratory and field testing. The *A. attenuatus* solution used in the study was prepared at a concentration of $1x10^8$ conidia/ml.

Application of entomopathogenic fungi against *Aphis gossypii*

Petri dishes with a diameter of 6 cm were used in the experiments. A thinly cut sponge was placed at the bottom of these Petri dishes to retain water. A layer of blotting paper was placed on this sponge, and a leaf of a cotton plant was placed on it. In each Petri dish prepared in this way, five 2nd nymph stage cotton aphid transfers were carried out. The isolates prepared at a conidia density of $1x10^8$ (ml^{-1}) on aphids, were sprayed 3 times (2±0.5 mgl/1cm^2) with a hand sprayer from a distance of 10-15 cm. Pure water was used for control application. The experiments were designed with 10 replications, allocating five nymphs in each Petri dish. The experiments were carried out in climatic cabinets with 25°C temperature, 60±5% relative humidity, 16:8 hours lighting conditions. Alive individuals were counted and recorded on the 1st, 3rd, 5th, 7th and 9th days after the applications.

Analysis of the data

Mortality rates (%) were calculated by applying the Abbott formula (Abbott, 1925). Probit analysis program was used to determine the LT_{50} (50% time to death) value. By applying one-way analysis of variance to the obtained data, the difference between the mean numbers of alive nymphs was compared by use Tukey multiple comparison test at $P<0.05$ importance level. Statistical analyzes SPSS® 20.0 package program was used to analyze data obtained.

RESULTS AND DISCUSSION

The percent mortality rates (%) that occurred as a result of the application of entomopathogenic fungus isolates used in this study on *A. gossypii* nymphs are given in Figure 1. As a result of the applications, the percent mortality rate in the *B. bassiana* isolate was recorded at a higher rate than the *A. attenuatus* isolate on all counted days. Statistical differences were recorded between *B. bassiana* and *A. attenuatus* isolates only on the 7th and 9th days of the counts. While the percent mortality rates in *B. bassiana* isolate were 13, 25, 38, 62 and 72%, respectively, the percent mortality rates in *A. attenuatus* isolates were

recorded as 6, 17, 32, 41 and 54%, respectively.

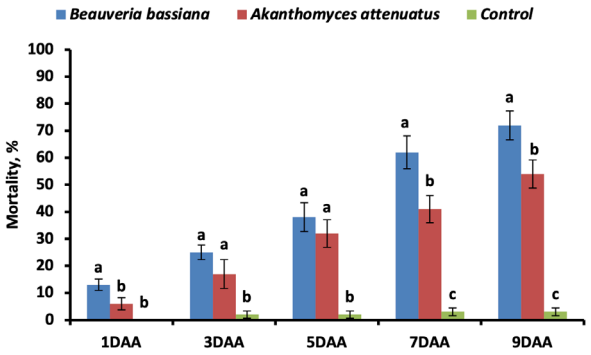

Figure 1. Percent mortality rates resulting from the application of entomopathogenic fungus isolates to *Aphis gossypii* nymphs. (The differences between the means (±standard error) of the columns indicated with different letters for each day are statistically significant (Tukey's HSD test P<0.05)). DAA: Days after application.

As a result of probit analysis, the death time% (LT_{50}) was recorded as 6.02 days in *B. bassiana* isolate and 8.33 days in *A. attenuatus* isolate (Figure 2.3).

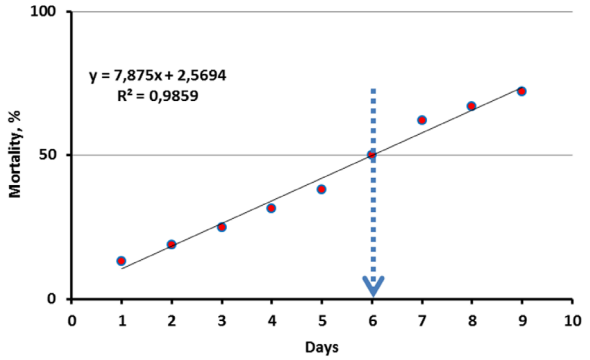

Figure 2. Time dependent mortality rates as a result of application of *Beauveria bassiana* to *Aphis gossypii* nymphs

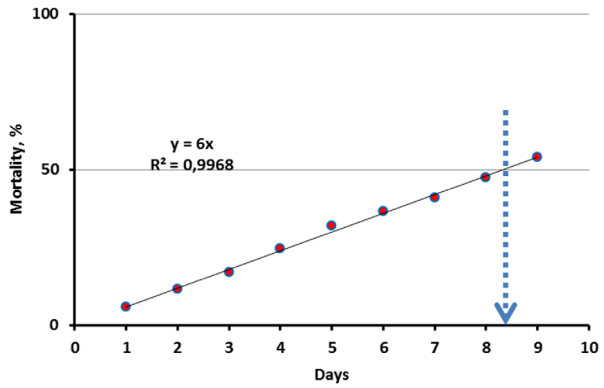

Figure 3. Time dependent mortality rates as a result of application of *Akanthomyces attenuatus* to *Aphis gossypii* nymphs

It was concluded that *B. bassiana* isolate was more effective than *A. attenuatus* isolate on *A. gossypii* nymphs. When we look at the studies, as a result of the application of *B. bassiana* Bb-5a isolate to *A. gossypii* individuals under field conditions, a 75.1% reduction in the pest population was recorded (Ramanujam et al., 2018). As a result of the application of four *Beauveria* isolates and two *Metarhizium* isolates to *A. gossypii* at different temperatures, 73.33-93.33% mortality rates and LT_{50} value 3.83-4.98 days at 25 °C; mortality rates of 82.22-100% at 30 °C and LT_{50} values were recorded as 3.23-4.02 (Tesfaye and Seyoum, 2010). *B. bassiana* (Bals.-Criv.) Vuill IRAN 429C, IRAN 108 and LRC 137 three isolates were administered to adult individuals of *A. gossypii* at a concentration of 10^8 conidia ml^{-1}, resulting in LT_{50} values of 2.90, 3.84, and 4.64 days, respectively (Mousavi et al., 2020). Application of *B. bassiana* isolated from *Hypera postica* Gyllenhal (Coleoptera: Curculionidae)'at 10^6 spores/ml concentration to *A. gossypii* adult individuals resulted in LT_{50} value of 5.66 days, and application of *B. bassiana* isolated from *Sphingonotus* sp. (Orthoptera: Acrididae) at the same concentration was recorded as 3.32 days. As a result of the application of *B. bassiana* isolated from *Sphingonotus* sp. to *A. gosyypii* at 10^4, 10^5 and 10^6 concentrations, mortality rates of 33.5-96.7% were recorded (Anonymous, 2023). As a result of the application of *B. bassiana* and *Lecanicillium lecanii* isolates to *A. gossypii* individuals, the reproduction time and reproduction rate of the pest were affected (Gurulingappa et al., 2011). *Akanthomyces attenuatus* Zare & Gams has been reported to be a pathogen of whitefly, aphids, thrips and mites. Some isolates of this species have been developed as commercial biopesticides (Wang et al., 2007; Ainsworth et al., 2008; Gottel et al., 2008; Lu et al., 2015). Considering the studies, it was noted that the net reproductive power of *A. gossypii* decreased as a result of the application of *Lecanicillium attenuatum* Zare & W. Gams CS625 (Kim, 2007). In another study, a mortality rate of 87.7% was recorded as a result of the application of *Akanthomyces muscarium* (DIKA11/1) isolate to sunn pest at a concentration of 1×10^7 conidia/ml (Gül et al., 2022). As a result of the application of *Lecanicillium attenuatum* to *A. gossypii* first-stage nymphs at 1×10^4 and 1×10^8 conidia/ml concentrations, it was noted that the life span of the pest was shortened (10.8 and 8.4 days). Total fecundity was recorded at 1×10^4 (41±7.3 nymph), 1×10^6 (26±0.8 nymph) and 1×10^8 conidia/ml concentrations (22±5.7 nymph) (Kim, 2007). As a result of the application of *Lecanicillium muscarium* (Zare & Gams) at 1×10^7 and 1×10^6 spore/ml concentrations to *A. gossypii*, a 100% mortality rate was determined (Razmjou et al, 2016).

CONCLUSIONS

As a result of this study, it was found that *B. bassiana* isolated from *I. sexdentatus* adults was more effective on *A. gossypii* nymphs than *A. attenuatus* isolate. Considering

the results obtained in this study and previous studies, it was noted that entomopathogenic fungi are a good alternative to chemical pesticides in aphid control. More studies are needed to determine the insecticidal activities of the entomopathogenic fungi used in this study as biological control agents in greenhouse and field conditions.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

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

Author contribution

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

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Technical efficiency of agroforestry production technology among smallholder farmers in Kaduna State, Nigeria

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Abstract

This study evaluated technical efficiency of agroforestry production technology among smallholder farmers in Kaduna State, Nigeria. Multi-stage sampling technique was adopted. A total sample size of 120 smallholder agroforestry farmers was used. Primary data were collected with the aid of structured and well-designed questionnaire. Analytical tools used were: descriptive statistics, farm budgeting techniques, financial analysis, stochastic production frontier model, and principal component model. About 85% of agroforestry farmers were male, while 15% were female. Also, 87.50% of agroforestry farmers were less than 50 years of age. The mean age was 45 years. Furthermore, 85% of agroforestry farmers had formal education and were literate. The household sizes were large with mean value of 6 members per household. The agroforestry systems practiced include: alley farming, shelterbelts, wind breaks, home gardens, api-silviculture, aqua-forestry, retaining tree on farm land, taungya systems, farmed parkland, and silvo-pasture. The result of the farm budgetary technique show that agroforestry farming was profitable among the smallholder farmers in the study area. The result of the maximum likelihood estimates shows that the significant factors influencing output of agroforestry production technology were: seed input ($P<0.05$), farm size input ($P<0.01$), fertilizer input ($P<0.10$), labour input ($P<0.10$), and chemical input ($P<0.01$). The significant factors influencing technical inefficiency of agroforestry production technology were: gender ($P<0.10$), marital status ($P<0.01$), education level ($P<0.01$), experience in agroforestry production ($P<0.01$) and size of households ($P<0.10$). The average technical efficiency score obtained by the smallholder agroforestry farmers was 40.18%. The constraints facing agroforestry farmers include: lack of training and capacity building, inadequate extension officers, lack of improved seeds, lack of credit facilities, lack of fertilizers, and lack of agroforestry tree seedlings. The study recommends that agroforestry tree seedlings should be made available to farmers, credit facilities should be provided for easy access to agroforestry production technologies, extension officers should be employed, and improved seeds, fertilizers should be provided for increased productivity.

Keywords: Technical Efficiency, Agroforestry Production Technology, Kaduna State, Nigeria

INTRODUCTION

Agroforestry can be defined as the practice of deliberately integrating woody vegetation (trees or shrubs) with crop and/ or animal systems to benefit from the resulting ecological and economic interactions. The Food and Agriculture Organization, FAO (2015) defined agroforestry as a collective name for land

use systems and technologies where woody perennials (trees, shrubs, palms bamboos, etc) are deliberately used on the same land management units as agricultural crops and/or animals in some form of spatial arrangement or temporal sequence. Agroforestry is a combination of forestry and agriculture, it is an intensive land management system that optimize the benefit from the biological interactions created when trees and or shrubs are combined deliberately with crop and/ or animals. The system is intentional, intensive, interactive, and integrated. Agroforestry, the integration of trees in agricultural activities has the capacity to increase soil fertility, reduce evaporation, increase nutrient recycling, reduce land degradation from erosion, and improvement of water quality. These benefits will have an impact and environmental benefits at the farm level, local and regional levels. Agroforestry helps to maintain the well-being of societies at all levels (Alemu, 2016). Agroforestry is a proven model of integrated sustainable land use system which can enhance agricultural productivity and production in a low input and in an ecological and economically feasible way in the effort of enhancing food security sustainably (Mbow, 2015). The growing of trees on the border of the crop land is a good source of income for smallholder farmers on one hand and on the other hand plays an important role in increasing soil fertility, enhances biodiversity and cleans water that ultimately reduces global warming by carbon sequestrations (Ingwe *et al*., 2009). Agroforestry systems are both stable and sustainable, it has greater diversity than do monoculture practices and can distribute production over a long period, thus provide income that is more regular with increased cash flow stability. Integrating of trees into agricultural systems may result in more efficient use of sunlight, moisture and plant nutrients than is generally possible by mono-cropping of either agricultural or forestry crops (Amunum *et al*., 2009). Agroforestry system contribute to the rural economy poverty alleviation, employment, and environmental protection at a local regional and national level (Alavalapati *et al*., 2004). Agroforestry production technology also have economic dimension since it helps maximize agricultural production by reducing soil erosion, water, and organic matter losses. The practice can increase microbial activities which can help nutrient recycling, thus increase the fertility of soil under agricultural production (Jose, 2009). Nitrogen fixing trees can also increase agricultural production and thereby reduced cost for agricultural inputs. Agroforestry practices are essential resources to combat climate because of their role in sequestering carbon and other greenhouse gases (AAC, 2014). Agroforestry production technologies are also known to increase the biodiversity resource potentials, since they provide shelter and food, they are known to support the existence of wildlife. The presence of woody perennials in agroforestry systems may affects several bio-chemical and bio-physical processes that determine the health of the soil substrate. The impacts of trees on soil include: surface litter cover and under story vegetation, amelioration of erosion, maintenance or increase of organic matter and diversity, nitrogen fixation, continuous degeneration of roots and decomposition of litter, enhancement of physical properties such as porosity, soil structure, and moisture retention due to the extensive root system and the canopy cover, and enhanced efficiency of nutrient use because the tree – root system can intercept, absorb, and recycle nutrients in the soil that would otherwise be lost through leaching. The choice of tree species is the most important factors to be considered in agroforestry systems. The choice of tree species be made after careful consideration of their benefit for rural populace and adaptability for growth. The farmers' preference of forest trees would definitely be due to their potentials and adaptability to the land area. Technical efficiency is the extent to which smallholder farmers use their resources to produce the maximum possible output.

Objectives of the Study

The broad objective evaluated technical efficiency of agroforestry production technology among smallholder farmers in Kaduna State, Nigeria. Specifically, the objectives are to:

(i) determine the socio-economic profiles of smallholder agroforestry farmers,
(ii) determine the types of crops grown, animal reared and trees under agroforestry production technologies among smallholder farmers,
(iii) analyze the profitability of agroforestry production technology,
(iv) evaluate factors influencing technical efficiency of agroforestry production technology among smallholder farmers,
(v) evaluate socio-economic factors influencing technical inefficiency of agroforestry production technology among smallholder farmers,
(vi) determine the technical efficiency scores of smallholder agroforestry farmers, and
(vii) determine the constraints faced by smallholder agroforestry farmers in the study area.

Methodology

This research study was conducted in Kaduna State, Nigeria. Kaduna State occupies between Longitudes 06° 15| and 08° 50| East and Latitudes 09° 02| and 09° 02|North of the equator. The State has land area totaling 4.5 million hectares. The state vegetation is divided into two (2), the Southern guinea savanna and Northern guinea savanna. There are two (2) seasons in Kaduna State. The seasons are: wet and dry seasons, the dry season is between October to March, and the wet season starts from April to October, in between the wet and dry seasons is the brief harmattan period which span from

November to February. The mean or average rainfall is about 1,482mm, the temperature of Kaduna State ranges from 35°C to 36°C, which can be as low as 10°C to 23°C during the harmattan period. The population of Kaduna as at 2021 was 8.9 million people. They are involved in agricultural activities. The people are involved in agroforestry production technology. Crops grown include: okra, pepper, maize, ginger, sorghum, rice, yam, cassava, millet, and tomatoes. Animal reared include: cattle, goats, sheep, rabbit, and poultry.

Research Design

A descriptive cross-sectional research design was employed in this study with the aim of describing the socio-economic profiles of characteristics of smallholder agroforestry farmers, determine the various types of crops grown, animal reared and trees under agroforestry production technologies among smallholder farmers, and to evaluate socio-economic factors influencing technical inefficiency of agroforestry production technology among smallholder farmers in the study area.

Sampling Techniques and Sample Size

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

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

Where,

n = Desired Sample Size

N = Finite Size of the Population

e =Maximum Acceptable Margin of Error as Determined by the Researcher

Methods of Data Collection

The data for this study was collected through the use of well-designed structured questionnaire. The data collected were cross sectional data from primary source, the data collected from smallholder agroforestry farmers were socio-economic profiles of the farmers, prices of production inputs, quantity of inputs used and constraints faced by farmers in the course of agroforestry production technology in the study area. Data were analyze using the following descriptive and inferential tools:

Descriptive Statistics

Data collected from field survey on smallholder agroforestry farmers were summarized through the use of mean, frequency distributions, and percentages. Descriptive statistics was used to summarize the socio-economic profiles of smallholder agroforestry farmers as stated in specific objective one (i), and determine the types of crop grown, animal reared, and trees under agroforestry production technology among smallholder farmers as stated in specific objective two (ii).

Farm Budgetary Technique

Gross margin and net farm income analysis of agroforestry production technology was estimated using the following models:

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

$$GM = \sum_{i=1}^{n} P_i Q_i - \sum_{j=1}^{m} P_j X_j \qquad (3)$$

$$NFI = TR - TC \qquad (4)$$

$$NFI = \sum_{i=1}^{n} P_i Q_i - \left[\sum_{j=1}^{m} P_j X_j + \sum_{k=1}^{k} GK\right] \qquad (5)$$

Where

P_i = Price of Agroforestry Produce ($\frac{₦}{Kg}$),

Q_i = Quantity of Agroforestry Produce (Kg),

P_j = Price of Variable Inputs ($\frac{₦}{Unit}$),

X_j = Quantity of Variable Inputs (Units),

TR = Total Revenue obtained from Sales from Agroforestry Production Technology (₦),

TVC = Total Variable Cost (₦),

GK = Cost of all Fixed Inputs (Naira)

NFI = Net Farm Income (Naira)

The farm budgetary technique was used to analyze the profitability of agroforestry production technology as stated in specific objective three (iii).

Financial Analysis

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

$$Gross\ Margin\ Ratio = \frac{Gross\ Margin}{Total\ Tevenue} \quad (6)$$

According to Olukosi and Erhabor (2015), Ben-Chendo *et al*. (2015) operating ratio (OR) is defined as:

$$Operating\ Ratio = \frac{TVC}{GI} \quad (7)$$

Where,

TVC = Total Variable Cost (Naira),

GI = Gross Income (Naira),

The financial analysis was used to analyze the profitability of agroforestry production technology as stated in specific objective three (iii).

Stochastic Production Frontier Model

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

$$Y_i = f(X_i, \beta_i)e^{v_i - u_i} \quad (8)$$

$$l_nY = \beta_0 + \beta_1 l_nX_1 + \beta_2 l_nX_2 + \beta_3 l_nX_3 + \beta_4 l_nX_4 + \beta_5 l_nX_5 + V_i - U_i \quad (9)$$

where,

Y_i = Output of Agroforestry Practices (kg)

X_i = Vectors of Factor Inputs

β_i = Vectors of Parameters

V_i = Random Variations in Agroforestry Technology

U_i= Error Term due to Technical Inefficiency

X_1 = Seed Input in kg

X_2 = Farm Size (ha)

X_3 = Fertilizer-Input in kg

X_4 = Labour-Input in mandays

X_5 = Chemical-Input in litre

$$U_i = \alpha_0 + \alpha_1 Z_1 + \alpha_2 Z_2 + \alpha_3 Z_3 + \alpha_4 Z_4 + \alpha_5 Z_5 + \alpha_6 Z_6 \quad (10)$$

where,

Z_1 = Gender (Dummy; 1, male; 0, otherwise)

Z_2 = Age of Smallholder Agroforestry Farmers in years

Z_3 = Marital Status (Dummy; 1, married; 0, otherwise)

Z_4 = Educational Level Attained

(Likert; 0, non-formal; 1, primary; 2, secondary; 3, tertiary)

Z_5 = Experience in Agroforestry Production Technology (years)

Z_6 = Size of Household (number)

α_0 = Constant Term

$\alpha_1 - \alpha_6$ = Parameters to be Estimated

U_i= Error Term due to Technical Inefficiency

Cost Saving Formula

The cost saving formula for average technical efficient (ATE) smallholder agroforestry farmers and least technical efficient (LTE) smallholder agroforestry farmers is stated as:

$$\text{Cost Savings} = \left[\left[1 - \frac{\text{ATES or LTES}}{\text{MaxTES}}\right] \times 100\right] \quad (11)$$

Where,

ATES = Average Technical Efficiency Score (Units)

LTES = Least Technical Efficiency Score (Units)

MaxTES = Maximum Technical Efficiency Score (Units)

This was used specifically to achieve objective four (iv), which is to evaluate factors influencing technical efficiency of agroforestry production technology, objective five (v), which is to evaluate socio-economic factors influencing technical inefficiency of agroforestry production technology by smallholder farmers in the study area, and objective six (vi) which is to determine the technical efficiency scores of smallholder agroforestry farmers in the study area.

Principal Component Analysis

The constraints facing smallholder farmers and militating against practice of agroforestry production technology were subjected to principal component analysis. This was used to achieve specific objective seven (vii).

RESULTS AND DISCUSSION

Socio-Economic Profiles of Smallholder Agroforestry Farmers

The socio-economic characteristics of smallholder agroforestry farmers was presented in Table 1. The socio-economic profiles under considerations were: sex, marital status, age, level of education, household size, farming experience, extension contact, membership of cooperatives, and farm size. The sex distributions of agroforestry farmers show that 85% were male, while 15% were female. The distributions of marital status categorized agroforestry farmers into single (39.16%), divorced (17.50%), and married (43.33%). About 87.50% of agroforestry farmers were less than 50 years of age. The mean age was 45 years. This means that agroforestry farmers were active, young, and energetic in their youthful age. This is in line with Luqman *et al*. (2018) who reported that majority of people in the research area were young, active age respondents and are more likely to adopt new technologies and they also have larger capacity to cultivate larger fields. About 85% of agroforestry farmers had formal education and were literate this include: tertiary (14,17%), secondary (35.83%), and primary (35%). Also, 15% of agroforestry farmers had no formal education. Farmers who have some level of education respond readily to improved technology thus increasing their productivity. According to Amaza and Tashikalma (2003), the literacy level of farmers is important as it determines the rate of adoption of improved technology for increased productivity. Also, Adekunle (2009) observed that the level of education of farmers will directly affects their ability to adapt to change and accept new ideas. Farmers who acquire some level of education are more likely to perceive new technologies than the ones who have no any form of education. Furthermore, 75% of agroforestry farmers had between 1 – 10 members as household size. The mean household size was 6 members per household. This signifies that more quality labour would be available for carrying out agroforestry production technologies. This is in line with findings of Villano and Fleming (2004). Averagely, farmers had 8 years' experience in agroforestry production technology. Agroforestry farmers with more years of farming experiences tend to be more efficient in production. Also, 73.33% of agroforestry farmers had extension contact, while 26.67% of agroforestry farmers do not have extension contact. In addition, 71.66% of agroforestry farmers were members of cooperative organizations, while 28.34% of agroforestry farmers do not belong to any members of cooperative organization. About 55.83% of agroforestry farmers had less than one hectare of farm land. The mean size of farm land was 1.25 hectares, this means that they are smallholder farmers.

Types of Crops Grown, Animal Reared and Trees under Agroforestry Production System among Smallholder Farmers

Table 2 presented the types of trees identified under the agroforestry systems, about eighteen (18) agroforestry trees were identified with various economic benefits. This include: *Parkia biglobosa, Musa species* and *Eucalyptus camadulensis* ranked first with the highest frequency (49) having 7.19% each respectively, followed by *Carica papaya* with 7.04%. This is in line with findings of Jamala *et al*. (2013). Table 3 shows the type of crops grown either as sole or in mixtures in agroforestry system. Maize ranked first with the highest frequency (58) having 11.08%, followed by rice with frequency of (56) having 10.70%, yam ranked third having frequency of (51) with 09.75%. The various types of animal reared under agroforestry system include: cattle (19.06%), sheep (15.71%), goats (16.05%), poultry (17.39%), fish (16.05%) and rabbits (15.71%) (Table 4). The various agroforestry systems practiced in the area include: alley farming, shelter belts, wind breaks, retaining tree on farm land, taungwa system, home garden, aqua-forestry, api-silviculture, silvo-pasture, farmed parkland etc.

Profitability Analysis of Agroforestry Production Technology per Cycle

Table 5 presents the results of the profitability of agroforestry production technology per cycle in the study area. The results show that the cost of seed inputs incurred by the agroforestry farmers is ₦25,500.00 carrying 10.49% of the total cost of production, the cost of fertilizer incurred was ₦51,000 and it carries 22.78% proportion of the total cost, while about 13% was incurred as the cost of purchasing chemical inputs. The total cost of labour incurred by the agroforestry farmers was ₦90,301.06 which carries 40.33% of the total cost, this carries the highest share of the total cost involved in the agroforestry production among the smallholder farmers in the study area. The total variable cost incurred by the agroforestry farmers was ₦193, 902.07 which is 86.6% of the total cost. The total fixed cost which comprises of depreciation on farm tools and rent on land was ₦30,221.62 which carries 13.49% of the total cost of production. The total revenue realized was ₦875,645.47, while the total cost of production incurred was ₦223, 888.13. The gross margin obtained was ₦651,757.34 which indicated that agroforestry farming was profitable among the smallholder farmers in the study area. The gross margin ratio obtained was 0.744, this implies that for every one (1) Naira invested in agroforestry production per hectare, 744 kobo covered interest, profits, taxes, depreciation, and expenses, while the net income was ₦624,771.78 with operation ratio of 0.221 implying that agroforestry is a profitable venture. Lower operating ratio is much preferable, the operating ratio of 0.221 signifies that the smallholder agroforestry farmers were cost effective in their handlings. This implied that

Table 1. Socio-Economic Profiles of Smallholder Agroforestry Farmers

Variables	Frequency	Percentage	Mean
Sex			
Male	102	85.00	
Female	18	15.00	
Marital Status			
Single	47	39.16	
Divorced	21	17.50	
Married	52	43.33	
Age (Years)			
31 – 40	18	15.00	
41 – 50	87	72.50	**45.0**
51 – 60	15	12.50	
Level of Education			
Non-Formal	18	15.00	
Tertiary	17	14.17	
Secondary	43	35.83	
Primary	42	35.00	
Household Size (Units)			
1 – 5	37	30.83	
6 – 10	78	65.00	**6.0**
11 – 15	05	04.17	
Farming Experience (Years)			
1 – 5	49	40.84	
6 – 10	41	34.16	**8.10**
11 – 15	21	17.50	
16 – 20	09	07.50	
Extension Contact			
Yes	88	73.33	
No	32	26.67	
Memberships of Cooperative			
Yes	86	71.66	
No	34	28.34	
Farm Size (Hectares)			
Less than 1.0	67	55.83	
i.1 - 2.0	28	23.33	**1.25**
2.1 – 3.0	15	12.50	
3.1 – 4.0	10	08.34	
Total	**120.00**	**100.00**	

Source: Field Survey (2022)

22.1% of returns from agroforestry production produce was used to cover cost of output sold and other operating expenses. This is in line with Alabi *et al.* (2023)

Table 5: Profitability Analysis of Agroforestry Production Technology per Cycle

Factors Influencing Technical Efficiency of Agroforestry Production Technology

Table 6 presents the results of the stochastic production frontier estimated through maximum likelihood method of estimation. The first stage of the stochastic frontier analysis show all the variables included in the model were statistically significant, the significant variables were: seed input, farm size, fertilizer input, labour input, and chemical input The coefficient of seed input influence the total output of the agroforestry production positively and it was significant at ($P<0.05$) probability level. The magnitude of the coefficient of seed input (0.3089) implies that percentage change in the seed input as a result of more usage will results in 30.9% increase in the total output of agroforestry production. This is in line with Idumah *et al.* (2015) who reported that seed contribute to the increase in total output which could lead to increase in the income of agroforestry farmers. Farm size influence the total output of agroforestry positively and it was statistically significant at ($P<0.01$). The elasticity of farm size 0.6701 implies that percentage change in the farm size will lead to 67.01 % increase in the total output of agroforestry production in the study area. This conforms with the findings of Amaza and Olayemi (2000) who reported that increase in farm output in

Table 2. Types of Trees under Agroforestry System in the Study Area

Trees under Agroforestry System	*Frequency	Percentage
Parkia biglobosa	49	07.19
Tamarindus indica	37	05.43
Mangifera indica	34	04.99
Azadiracta indica	46	06.75
Moringa oleifera	31	04.55
Adansonia digitata	37	05.43
Vitellaria paradoxa	43	06.31
Acacia Senegal	38	05.58
Jatropha curcas	46	06.75
Eucalyptus camadulensis	49	07.19
Tectonia grandis	29	04.25
Gwelina arborea	21	03.08
Cocus nucifera	42	06.16
Carica papaya	48	07.04
Musa species	49	07.19
Phoenix dactylifera	34	04.99
Terminalia ivorensis	27	03.96
Khaya ivorensis	21	03.08
Total	***681**	**100.00**

Source: Field Survey (2022) *Multiple Choices

Table 3. Types of Crops Grown as Sole or Mixtures in Agroforestry System

Types of Crops Grown in Agroforestry	*Frequency	Percentage
Okra	28	05.35
Pepper	34	06.50
Maize	58	11.08
Millet	39	07.45
Sorghum	48	09.17
Rice	56	10.70
Yam	51	09.75
Cassava	48	09.17
Onion	39	07.45
Tomatoes	38	07.26
Ginger	41	07.83
Vegetables	43	08.22
Total	***523**	**100.00**

Source: Field Survey (2022) *Multiple Choices

Table 4. Types of Livestock Reared in Agroforestry System

Livestock Reared in Agroforestry	*Frequency	Percentage
Cattle	57	19.06
Sheep	47	15.71
Goats	48	16.05
Poultry	52	17.39
Fish	48	16.05
Rabbits	47	15.71
Total	***299**	**100.00**

Source: Field Survey (2022) *Multiple Choices

the developing world is usually a function of farm size. Fertilizer input had a positive influence on the total output of agroforestry production, it was statistically significant at ($P<0.10$). The coefficient of the fertilizer input was 0.09120, this signifies that percentage change in the quantity of fertilizer applied as a result of more usage will result in 9.1% increase in the output of agroforestry production in the study area. Labour input and chemical input influence agroforestry production positively and were significant at ($P<0.10$) probability level respectively. The coefficient of labour and chemical input were 0.2321 and 2302 respectively, which implies that a percentage change in these inputs will results in the increase in the total output of agroforestry by 23.2% respectively in

Table 5. Profitability Analysis of Agroforestry Production Technology per Cycle

Items	Amount (Naira)	% of Total Cost
Total Revenue	**875,645.47**	
Gross Income	**875,645.47**	
Variable Cost		
Seed Input	23,500.00	10.49
Fertilizer Input	51,000.00	22.78
Insecticides	15,650.56	06.99
Herbicides	13,450.45	06.01
Labour Cost:		
(i) Land Clearing and Preparation		
(ii) Planting	11,650.56	
(iii) Weeding	23,800.00	
(iv) Fertilizer Application	7,500.00	
(v) Chemical Application	15,000.00	
(vi) Harvesting	24,600.00	
(vii) Transportation	5,400.50	
(viii) Loading and Offloading	2,350.00	
Total Labour Cost	90,301.06	40.33
Total Variable Cost	193,902.07	**86.61**
Fixed Cost		
Estimated Depreciation Value on Tools (Hoes, Machetes)	3,235.56	1.45
Rent on Land	26,750.50	11.95
Total Fixed Cost	30,221.62	**13.49**
Total Cost	223,888.13	**100.00**
Gross Margin	651,757.34	
Gross Margin Ratio (GMR)	0.744	
Net Farm Income (NFI)	624,771.28	
Operating Ratio (OR)	0.221	

Source: Field Survey (2022) USD = 760 Naira

the study area. This is in line with the findings of Yusuf and Abdulrahman (2018) who reported that labour is an important variable in agricultural production and also reported that agrochemical was positive and statistically different from zero. This implies that an increase in agrochemical to a certain level will decrease technical inefficiency because it reduces drudgery and controls weeds. The return to scale of the agroforestry farmers is 1.3236 which indicates increasing return to scale this implies that as the use of input increases, it will result in more than proportionate increase in the total output of the agroforestry farmers.

Socio-Economic Factors Influencing Technical Inefficiency of Agroforestry Production Technology

The technical inefficiency component revealed that the socio-economic factors influencing technical inefficiency were: gender, age, education level, experience in agroforestry, and size of household (Table 6). The negative sign of the coefficients implies decrease in technical inefficiency but increases technical efficiency, while the positive sign signifies increase in technical inefficiency and decrease in technical inefficiency in agroforestry production in the study area. Gender of smallholder agroforestry farmers influence technical inefficiency in agroforestry production negatively, the gender was measured as dummy such as 1, male, 0 otherwise, the coefficient of gender is -0.1714 and was statistically significant at ($P<0.10$), this implies that a unit change in gender being a male will result in the decrease in technical inefficiency or increase in technical efficiency in agroforestry production by 17.1% among the smallholder farmers, if a farmer is male will lead to increase in technical efficiency because male farmers are more energetic to carry out some task than their female counterpart would not be able to do. Marital status of smallholder farmer was significant at ($P<0.01$), the magnitude of the coefficient of marital status is -0.0365 which implies that a unit change in the marital status will result in 3.7% increase in the technical efficiency or decrease in technical inefficiency among smallholder agroforestry farmers in the study area. Education level of smallholder agroforestry farmer influence technical inefficiency negatively and it was statistically significant at ($P<0.01$). The coefficient of education level 0.2321 implying that a unit change in the education level of agroforestry farmers will result in 23.2% decrease in technical inefficiency among smallholder agroforestry farmers. Farmers with higher education level stand a chance of exploring more information of agroforestry

Table 6. Maximum Likelihood Results of the Stochastic Frontier Production Model

Variables	Parameters	Coefficient	Standard Error	t-Value
Constant	β_0	2.1396***	0.2565	8.34
Seed Input	β_1	0.3089**	0.0933	3.31
Farm Size	β_2	0.6701***	0.1175	5.70
Fertilizer Input	β_3	0.0912*	0.0453	2.02
Labour Input	β_4	0.2321*	0.099	2.34
Chemical Input	β_5	0.2302***	0.0142	16.21
Return to Scale (RTS)		**1.3236**		
Inefficiency Component				
Constant	α_0	2.6436***	0.4509	5.86
Gender	α_1	-0.1714*	0.0760	-2.25
Age	α_2	0.0021	0.0029	0.74
Marital Status	α_3	-0.0365***	0.0090	-4.05
Educational Level	α_4	-0.0231***	0.0062	-3.71
Experience in Agroforestry	α_5	-0.0358***	0.0087	-4.14
Size of Households	α_6	-0.0111*	0.0230	-1.79
Diagnostic Statistics				
Total Variance	σ^2	1.9011***		
Variance Ratio	γ	0.7221		
Log-Likelihood		-307.12		
Likelihood Ratio Test		318.31		

Source: Data Analysis (2022)

*Significant at ., **Significant at .***Significant at .

Table 7. Summary Statistics of Technical Efficiency Scores

Efficiency Score	Frequency	Percentage
0.00 – 0.20	10	08.30
0.21 – 0.40	23	19.20
0.41 – 0.60	50	41.70
0.61 – 0.80	22	18.30
0.81 – 1.00	15	12.50
Mean	40.18	
Standard Deviation	23.50	
Minimum	0.110	
Maximum	0.810	

Source: Field Survey (2022)

Table 8. Principal Component Model of Constraints Encountered by Smallholder Agroforestry Farmers

Constraints	Eigen-Value	Difference	Proportion	Cumulative
Lack of Training and Capacity Building	3.1272	0.2260	0.1702	0.1702
Inadequate Extension Officers	2.9452	0.2087	0.1664	0.3366
Lack of Improved Seeds	2.8325	0.1994	0.1606	0.4972
Lack of Credit Facilities	2.7630	0.1987	0.1534	0.6506
Lack of Fertilizers	2.6601	0.1885	0.1392	0.7898
Lack of Agroforestry Tree Seedlings	2.5020	0.1806	0.1024	0.8922
Bartlett Test of Sphericity				
Chi Square	721.22***			
KMO	0.7901			
Rho	1.00000			

Source: Field Survey (2022)

and have more ability of adopting innovation and new technologies. This is in conformity with the findings of Ogundari and Ojo (2007) who reported coefficients of educational level to be negative, meaning that this factors increases technical efficiency and decreases technical inefficiency. Experience in agroforestry influence technical inefficiency of agroforestry production negatively and was statistically significant at

(P<0.01) probability level, the coefficient of experience in agroforestry was -0.0358 implying that a unit change in years of experience in agroforestry will lead to 3.6% decrease in the technical inefficiency in agroforestry production, experience could help farmers to acquire more knowledge about agroforestry which would lead to increase in the technical efficiency in agroforestry production. This is in conformity with the Nwahia *et al*. (2020) who reported that farmers with more experience tends to be technically efficient than those that has less farming experience. Size of households influence technical efficiency in agroforestry production negatively and it was statistically significant at (P<0.10). The coefficient of size of household is -0.011, this signifies that a unit change in the number of size households will results in the decrease in technical inefficiency by 11.1% among agroforestry farmers in the study area. This in line with the finding of Nwahia *et al*. (2020) who reported that farmers with larger family size enhances labour availability as majority of the members were involved in agroforestry activities.

Technical Efficiency Scores of Smallholder Agroforestry Farmers in the Study Area

Table 7 shows the summary statistics of technical efficiency scores of smallholder agroforestry farmers. Majority (60.9%) of agroforestry farmers were between 21 to 60 % efficiency levels, this implies that most farmers were average technically efficient. The mean technical efficiency was 40.18 % leaving a gap of 59.82 % for improvement. This is in line with Yusuf and Abdulrahman (2018) who reported 61% average technical efficiency among farmers in Kogi State, Nigeria. In addition, the least technical efficiency score was 11.0 %, while the best performing smallholder agroforestry farms had the maximum technical efficiency of 81.0%. If the average smallholder agroforestry farmers were to achieve the level of technical efficiency like most of its efficient counterparts, then the average smallholder agroforestry farmers could make 50.39 % cost savings calculated as . The calculated value for the most technically inefficient smallholder agroforestry farmers reveal a cost savings of 86.42 % calculated as . This is contrary with the findings of Ogundari and Ojo (2007) who found an average technical efficiency of 81% among agroforestry farmers in South-Western Nigeria.

Constraints Encountered by the Smallholder Agroforestry Farmers in the Study Area

Table 8 presented the results of the Principal component analysis to identify the constraints encountered by agroforestry farmers in the study area, the Principal component analysis (PCA) is one of the important statistical tools which is likely related with the principles of factor analysis procedure which has the ability to transform the variables that interrelated in survey data that comprises of so many variables into nearest minimum or few number of variables that are uncorrelated. The output result of the number of principal components retained using the Kaiser Meyer criterion were six (6) based on the Eigen values that are greater than 1. The components that were retained explained about (0.8922) 89.22% of the variation in the component included in the model. The Kaiser-Meyer-Olkin measures of sampling adequacy (KMO) for cowpea farmers were 0.7901 and the Bartlett test of Sphericity of 721.22 and was statistically significant at 1 % probability level this justified the subjection of the data set for principal component analysis. Lack of training and capacity building had an Eigen value of 3.272 and it was ranked 1st in the order of importance based on perception of the agroforestry farmers while inadequate extension officers, lack of improved seeds had an Eigen values of 2.9452 and 2.8325 respectively was ranked 2nd, 3rd respectively, while lack of credit facilities and lack of fertilizers with Eigen values of 2.7630 and 2.6601 respectively were ranked 4th and 5th respectively also in the order of its occurrence measured based on the perceptions of the agroforestry farmers. This result is in line with the findings of Alabi, *et al.* (2020) who use similar approach to identify the constraints encountered by farmers in crop production. The results are also consistent with Cooker *et al.* (2018)

Conclusion and Recommendations

Based on the findings emanating from this research work concluded that agroforestry farmers were young, active, resourceful, and energetic. The agroforestry systems practiced in the area include: alley farming, retaining tree on farm land, shelter belt, wind break, home gardens, api-silviculture, aqua-culture, silvo-pastures, farmed parkland, and taungya system etc. Agroforestry systems was profitable and worthwhile. The significant factors influencing output of agroforestry production were: seed input (P<0.01), farm size input (P<0.01), fertilizer input (P<0.05), labour input (P<0.05), and chemical input (P<0.01). The significant factors influencing technical inefficiency of agroforestry production technology were: gender (P<0.05), marital status (P<0.01), education level (P<0.01), experience in agroforestry production (P<0.01) and size of households (P<0.10). The average technical efficiency score obtained by the smallholder agroforestry farmers was 40.18%. The constraints facing agroforestry farmers include: lack of training and capacity buildings inadequate extension officers, lack of improved seeds, lack of credit facilities, lack of fertilizers, and lack of agroforestry tree seedlings. Based on the findings the following recommendations were made:

(i) Agroforestry tree seedlings should be made available free to smallholder farmers
(ii) Credit facilities should be provided for smallholder farmers to access new production technologies
(iii) Improved seeds and fertilizers should be made available for smallholder farmers for increased

productivity.

(iv) Extension officers should be employed to disseminate research findings and new agroforestry production technology to smallholder farmers

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

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

Author contribution

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

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Ethics committee approval is not required.

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RESEARCH ARTICLE

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The shortest way to diffuse agricultural innovations: A network study in the paddy sector in Türkiye

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Abstract
The study revealed the diffusion processes of agricultural innovations and the influential advice sources in the diffusion process by using the network approach. The study sought to answer the questions of how agricultural innovations diffuse in the paddy sector, how agricultural innovations can be delivered to paddy farmers in the most concise form, and what are the typical characteristics of influential advice sources. Data were collected using questionnaires from paddy farmers, input suppliers, rice mills, and other relevant people and organizations in 2017. Social network analysis was used to analyze farmer advice networks, and Ordinal Logistic Regression was used to identify the common characteristics of influential actors in the network. The research results indicated that the farmers were a bridge between innovation creators and other farmers. The local pesticide dealers were the intermediary position among the paddy farmers with the innovation creators. Collective action, membership in agricultural organizations, social status, and project experience were influential factors in being an advice source. The study found that agricultural innovations in the paddy sector can diffuse most quickly from innovation creators to local pesticide dealers and farmers.
Keywords: Diffusion of innovations, Advice networks, Paddy, Ordinal logistic regression, Türkiye

INTRODUCTION

In developing countries where agriculture is the dominant sector, the diffusion of new technologies plays an essential role in income growth due to productivity in agriculture. The World Bank (2012) defines agricultural innovation, which is the product of a complex process, as a product of the relationships, resources, and capacity that emerge from the combination of actors in a wide range of fields related to agriculture.

The increasing complexity of new technologies causes the perspectives on innovations to change. Today's agricultural innovations require a wide range of participation from different actors, including joint development in institutions and technologies (Bandiera and Rasul, 2006). Van de Ven (2017) points out that innovation is a process involving many stakeholders and argues that innovation's primary function is to create shared knowledge. The common point of the definitions in the literature is that agricultural innovation is a complex process with multiple actors, focusing on the relationship between actors and continually evolving (Joffre et al., 2018; Klerkx and Begemann, 2020). Groups such as farmers, research institutes, universities, input providers, intermediary organizations, pressure groups, capital owners, and regulators occur in these systems.

Generally, the previous studies analyzing the diffusion of agricultural innovations used reductionist approaches and models. These approaches and models consider the actors in the same category as a single actor and they are limited in showing which actors in the system are more effective. Instead, the network approach is more advantageous than reductionist approaches in revealing the relationship patterns between actors with numerical and visual analysis. Demiryurek (2010) argued that the diffusion of the agricultural innovation process could be better analyzed using social network analysis (SNA). With SNA, it is possible to reveal the role of actors in the innovation network, which characteristics of the actors are helpful in these roles, and when they fulfill their roles. On the other hand, along with the decreased budgets allocated to agricultural services worldwide, the concept of leader farmers, opinion leaders, and advice sources became essential with the transition to participatory and decentralized extension (Cook et al., 2021; Lin et al., 2021). This change increased the importance of advice networks in the diffusion processes of agricultural innovation, and social network analysis emerged as an essential tool to reveal the advice sources in the networks.

Network studies in agriculture were often used to explain the diffusion of innovations, and the adoption of innovations by farmers was interpreted as a function of the farmer's position in the social network (Isaac et al., 2007; Carruthers and Vanclay, 2012). The fact that a farmer with many people in his/her social network was aware of innovations meant that other farmers in that farmer's network might also be aware of innovations and new practices (Valente, 2005). The reason for this study is to reveal the diffusion process of agricultural innovations in farmer social networks.

The literature has numerous studies on the diffusion of agricultural innovations and identifying the key players in this process (Tran et al., 2019; Parry et al., 2020). Studies on the diffusion of innovations in Türkiye have generally considered technology transfer and focused on farmers' adoption of these technologies (Hasdemir and Taluğ, 2012). In addition, some SNA studies have been conducted on the diffusion of agricultural innovations in Türkiye (Demiryürek, 2010; Aydoğan et al., 2016; Demiryürek et al., 2017; Aydoğan and Demiryürek, 2018; Aydoğan et al., 2018). However, the common feature of these social network studies (Skaalsveen et al., 2020; Lin et al., 2021) was that they examined the diffusion of agricultural innovations in a single layer, specifically focusing on the farmer level. Considering that the sources of advice change rapidly over the years, the fact that farmers' awareness of agricultural innovations also changes frequently over time makes it necessary to conduct similar studies on the same issue. Identifying the actors and their typical characteristics that are effective in the diffusion processes of agricultural innovations can contribute to the problem's solution. However, farmer advisory networks are not only composed of farmers but are a complex environment. Therefore, it would be appropriate to analyze farmers' advisory networks in layers.

Using the network approach, the study identified the diffusion processes of agricultural innovations and the sources of advice that are effective in the process. Unlike previous studies, the study analyzed the diffusion process in three layers: farmer, local, and national. In the second stage of the study, the question of the characteristics of the advice sources who are effective in the diffusion processes of agricultural innovations have brought them to this position sought answered.

MATERIALS AND METHODS

Research area

An average of 980 thousand tons of paddy is produced annually in Türkiye. The provinces with the highest paddy production are respectively Edirne (40.2%), Samsun (15.0%), Balıkesir (14.0%), Çanakkale (10.2%), and Çorum (5.9%) in Türkiye. Paddy production in Samsun is mostly carried out in Bafra, Terme, Alaçam, Çarşamba, and Yakakent districts. Bafra district paddy production areas constitute 67.6% of the total paddy production areas of Samsun Province and approximately 14.9% of Türkiye's total paddy production (Turkstat, 2022).

Bafra district is essential in terms of both agricultural production and commercials. In the Bafra district, there are 13 paddy mills, the Chamber of Agriculture, Grain Producers and Paddy Producers Union, 35 pesticide dealers and seed dealers, and 36 machine-equipment dealers. Also, Ondokuz Mayıs University Faculty of Agriculture, Black Sea Agricultural Research Institute, Samsun Metropolitan Municipality Rural Services Department, and the Rural Development Agency implement projects and research in the region. Eventually, the Bafra district was selected as a research area since it can represent the entire Samsun province and other actors related to the paddy sector (Figure 1).

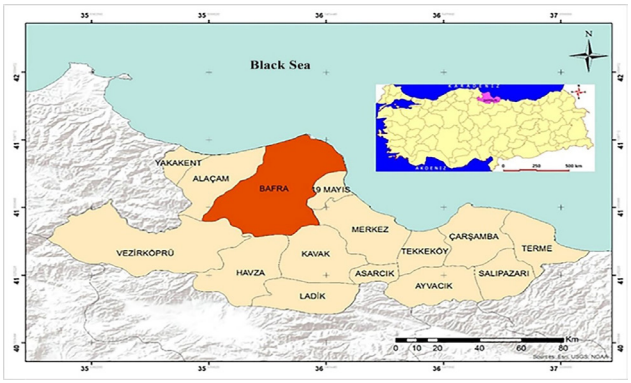

Figure 1. The research area: the map of Bafra district of Samsun province

Materials

The study's primary material is the data obtained through questionnaires and interviews with paddy farmers, farmer organizations, rice mills, pesticide dealers, seed dealers, researchers, scholars, and agricultural consultants in the Bafra district of Samsun province. Also, the data were enriched and commented on with group discussions with the stakeholders and observations. Previous studies, institutions and organizations' databases, and statistical reports were also used. The data were collected from the production period of 2017 years.

The simple random sampling method was used to determine the paddy farmers to survey (Yamane, 1967). A questionnaire was conducted with 70 farmers determined within a 10% margin of error and a 90 % confidence interval through sampling from 1798 paddy farmers that constituted the main population. In-depth interviews were conducted with three farmer organizations, eight rice mills, seven pesticide dealers, three seed dealers, two researchers, three scholars, and three agricultural consultants who agreed to participate in the study. The snowball sample technique was also used to define the advice sources and important actors from interviews with farmers and other stakeholders.

Methods

The average, frequency, and ratio statistics were performed for the paddy farmers' socioeconomic characteristics using R Statistical Software. In the research, the use of new certified paddy seeds, advanced new tools, and machinery, the seedling method in paddy farming, applying new approaches in marketing, adoption of sustainable agricultural, soil analysis, risk management practices, and participating in on-site training activities were recognized as agricultural innovations. We analyzed the diffusion process of agricultural innovations in the paddy sector and the visualization of relations by Social Network Analysis concepts. The NodeXL package program was used for SNA analysis. The analyses were conducted in a three-layer network model. The first layer (farmer layer) identified the leader farmers from whom farmers obtain advice on agricultural innovations and reveal how diffusion processes of innovation occur among farmers. For this purpose, three different relational questions were asked to the farmers: i) Which farmers do you get advice from? ii) Who are the people or institutions that you get advice about paddy in the Bafra district? iii) Who are the people or institutions where you get advice about paddy in Türkiye? A relational network database was created with the answers received. The network was named the Farmer-to-Farmer Innovation Advice (FFIA) network. This network included paddy farmers who were interviewed only within the scope of the research and other farmers with whom these farmers were in contact concerning innovations (Lin et al., 2021).

In the second layer, the Local Innovation Advice Network (LIAN) was created to reveal the diffusion process of innovations and identify influential actors in the Bafra district locally. The LIAN included the input providers (fertilizers, pesticides, seeds, so forth), processors and marketing agents (factories, intermediaries, paddy mills), and the organizations that technically support the paddy sector (universities, research institutes, public extension services), and farmers. The representatives of the institutions were asked the question of who is influential in the diffusion processes of agricultural innovations related to paddy in the research region (local layer) and throughout Türkiye (national level).

The third layer determined the influential actors in the diffusion processes of paddy-related innovations throughout Türkiye. The data used in creating the third layer were obtained from the actors interviewed in the first and second layers, and the created network was named Paddy Innovation Advice Network (PIAN). The direction of the relations in the networks created in all three layers showed the consulted or advised actors or leader farmers. The circle size showed the importance of the actors, and the distances between actors were not taken into account in all three layers.

Eigenvector centrality is an SNA method that measures the influence of an actor in a social network. An actor with high eigenvector centrality is an important actor in the network (Scott, 2011; de Nooy et al., 2018). Eigenvector centrality scores were used to determine the leadership roles of the FFIA actors. According to the eigenvector values, the actors in the network were divided into four groups by Hierarchical Clustering Analysis. The factors influencing the leadership role of actors were analyzed using the Ordinal Logistic Regression Model (OLR), one of the nonparametric analysis methods. OLR analysis was carried out to determine the factors that affect being a source of advice.

The OLR was used because the dependent variable (Y) had more than two categories. The general representation of the OLR model is as follows (Eq.1).

$$Ln\,(Y_j) = \alpha_j + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \ldots + \beta_n X_n \qquad \text{(Eq.1)}$$

On the left side of equation (1), *Ln(Yj)* is the dependent variable; on the right side of the equation, αj is the coefficient of the equation, *βi* is the coefficient of the predictor variables, and *Xi* is the predictor variable. The fourth category was accepted as the reference category in interpreting the model's coefficients.

RESULTS

The socio-economic and farming characteristics of paddy farmers

The average age and agricultural experience of the paddy farmers were 52.6 and 30.7 years, respectively. Considering that those between the ages of 18 and 40 are considered young farmers, it could be said that the farmers' age was relatively high. The farmers' average

Table 1. The socio-economic characteristics of paddy farmers

The socio-economic characteristics	Count	Average	Std. deviation
Age (year)	70	52.6	10.3
Agricultural experience (year)	70	30.7	12.9
Year of formal education	70	6.6	2.5
Household size	70	4.7	2.8
The number of women working on the farm	38	1.5	0.8
The number of men working on the farm	70	1.7	1.4
The number of agricultural organizations	70	3.4	1.1

Table 2. Farm characteristics of paddy farmers

Farm characteristics	Count	Mean	Std. deviation
Land size (ha)	70	11.4	13.9
Animal presence (LSU)	53	19.7	40.7
Paddy production area (ha)	70	8.1	10.1
GAP paddy production area (ha)	30	14.7	12.5
Labor requirements per unit area (person/ha)	70	6.0	5.0

formal schooling or education was 6.6 years (Table 1).

The household size contributes positively to farms, enabling farmers to obtain the labor force and meet farm work from the family. The household size of the paddy farmers consisted of an average of 4.7 people. An average of 1.7 of the male population in the family work on the farm, and women participate in agricultural activities on 54.3% of the farms. The average household size of paddy farmers (4.7 people) was more than the average household size (3.4 people) in Türkiye (Turkstat, 2020). Additionally, each paddy farmer was a member of at least one agricultural producer organization and an average of three agricultural producer organizations.

The study calculated that the farmers' average land size was 11.4 hectares, more than the land size (6.0 ha) per farmer in Türkiye (Table 2). The farmers' average paddy land amount was calculated as 8.1 hectares, and the average land size of farmers who practiced good agricultural practices (GAP) was 14.7 hectares. Six workers per hectare were needed for rice cultivation. Farmers' animal assets were converted into a livestock unit (LSU) to ensure homogeneity in comparison. The majority of the farmers (75.7%) had animals and an average of 19.7 LSU animals per farm.

Half of the farmers had another income source other than agricultural activity. As 71.4% of these farmers earned a wage income (active wage employee, retired, and so forth), 28.6% had commercial activities. The majority of

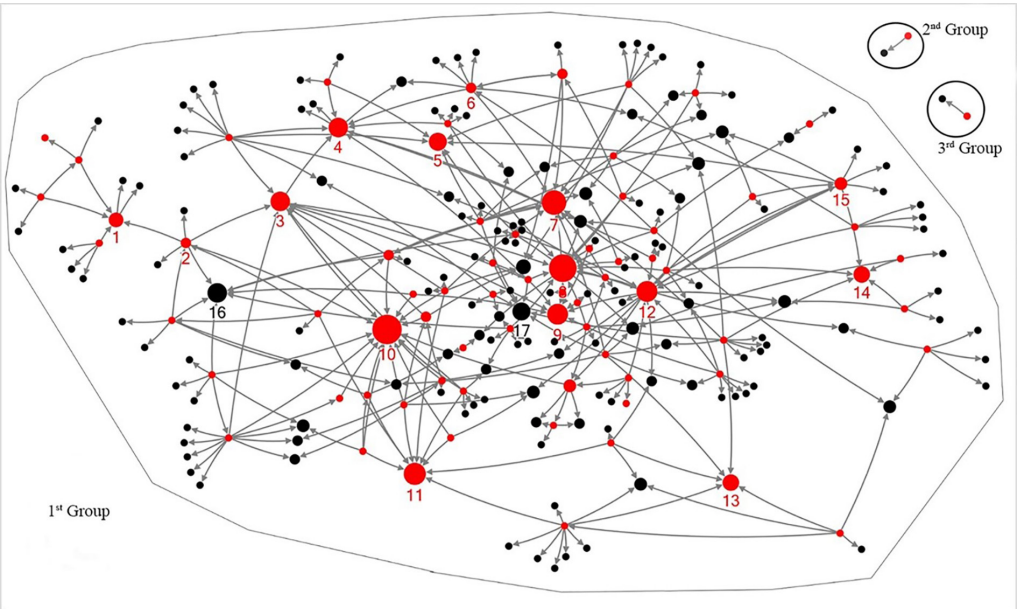

Figure 2. The model of farmer-to-farmer innovation advice network

paddy farmers (97.1%) had social security. 34.3% of the farmers carried out commercial livestock activities in the study. While more than half of the farmers (55.7%) had a soil analysis done, those applying risk management practices such as insurance and product diversification (42.9%) were lower. It can be said that participation in locally organized on-site training activities was high (61.4%) in the research area.

The Diffusion Process of Agricultural Innovations in Networks

Farmer-to-Farmer Innovation Advice (FFIA) Network

In Figure 2, the red-colored circles represented the interviewed farmers, and the black circles represented the farmers' advice sources. The FFIA network had 228 paddy farmers. The number of relations (edges) between these farmers in the network was 338. Also, the network has three separate groups (components) in the network, independent of 224 farmers with the highest number of farmers among these groups and two farmers in the group with the fewest farmers. In other words, most of the farmers in the network were in contact with each other. The distance between farmers in the network was determined as nine steps at most. The average distance in the network was 4.4 steps, and this meant that a farmer could reach any advice source after 4.4 steps (farmer). The density of the network was calculated as 0.0591. This means that only 5.9% of the relationships had the potential to be established in the network setup. The average in-degree score was calculated as 3. In other words, any farmer in the network consulted with an average of 3 different farmers about innovations.

In the FFIA network, some farmers were advice sources for other farmers. These farmers are shown in the network (Figure 2) as bigger than the others and were named with numbers and called leader farmers. The main characteristics of these leader farmers (Table 3) were that they engaged in other agricultural or non-agricultural activities and paddy cultivation in extensive lands (average 2.1 ha). Farmers 2 and farmer 7 worked as managers in agricultural farmer organizations and professional associations in the region, while farmer 1

Table 3. Some characteristics of the leader farmers

Actor Nu.	Properties
1	Retired senior manager
2	Beet Cooperative President, large-land farmer
3	Large-land farmer, a farmer who first tried paddy seedlings
4	Large-land farmer, a young entrepreneur
5	Large-land farmer, cattle breeder
6	Large-land farmer, fuel dealer
7	Large-land farmer, former president of the Bafra Chamber of Agriculture
8	Large-land farmer, Tractor dealer
9	Large-land farmers, agricultural products marketing company owner
10	Large-land farmers, agricultural products, and fertilizer dealer
11	Retired from the municipality, owner of the laser leveling system
12	Combine harvester and livestock owner
13	Organic farmer, former MP, rancher
14	Buffalo and cattle ranch owner
15	Rancher

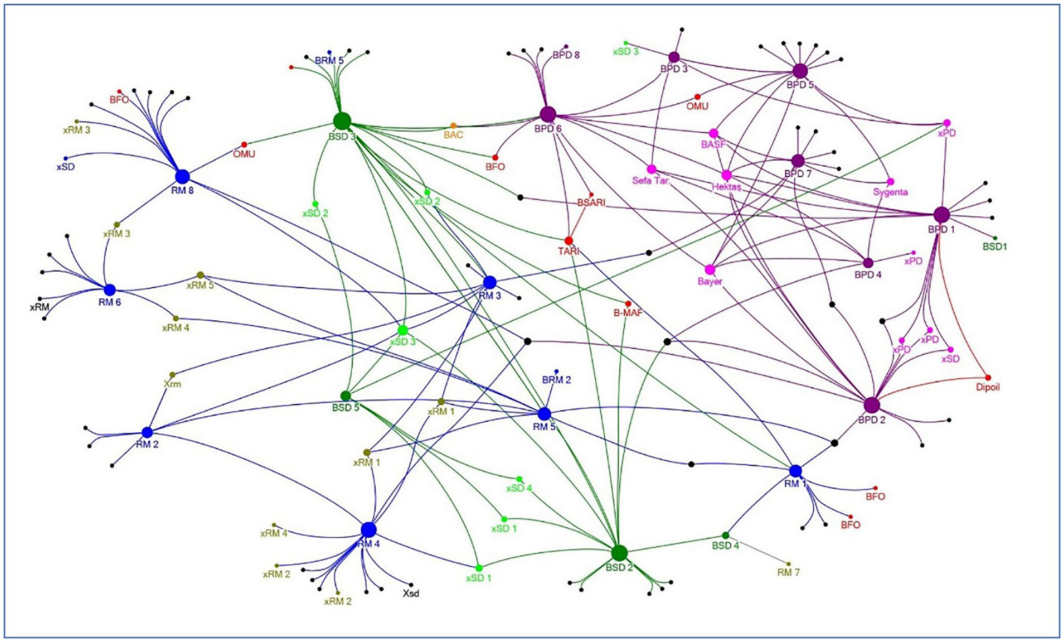

Figure 3. The actors in the local innovation advice network

had senior management experience in the private sector.

Similarly, farmers 5, 12, 13, 14, and 15 were engaged in professional cattle breeding besides paddy farming. Remarkably, the farmers in this group (5, 12, 13, 14, and 15) consisted of extended families and all family members worked in everyday agricultural activities. Farmers 3, 4, 8, and 10 were the first farmers to make seedlings, a new technique in paddy cultivation, on a large scale in Bafra. Of the other leader farmers, farmer 6 was petrol dealer, and farmers 9 and 10 owned companies selling agricultural inputs and products and paddy cultivation. The farmers 16 and 17, were the elected village headmen.

Local Innovation Advice Networks (LIAN)

In the LIAN (Figure 3), each sector and geographical area were represented differently. Blue circles symbolized rice mills (RM), green circles symbolized seed dealers (SD), purple circles symbolized pesticide dealers (PD), black circles symbolized farmers and red circles symbolized the experts working in public institutions in the Bafra district. Also, the lighter tone of each color indicated the actors outside the Bafra district.

The LIAN network has 125 actors, and the number of relations between these actors was 181. All actors in the network had a relationship, even if indirectly. The distance between actors in the network was determined as eight steps at most, an average of 3.9 steps, and it means that an actor who wanted to reach an innovation advice source anywhere in the network could reach this after four actors. The network density was calculated as 0.024. The average network degree was 2.9. It can be said that the actors in the network consulted with an average of 3 different actors regarding innovations. The LIAN was analyzed for the gatekeeper (broker) roles, and the results indicated that no gatekeeper could control the diffusion of innovations through the network. However, it could be said that the seed dealers (SD) and pesticide dealers (BPD) were closest to the gatekeeper role.

Paddy Innovation Advice Network (PIAN)

In the PIAN (Figure 4), each actor was represented with a different color and abbreviation, and the meanings of these signs representing the actors are given in Table 4. The PIAN network has 353 actors, and the number of relationships between these actors was 843. All actors in the network had a relationship, even if indirectly. The distance between actors in the network was determined as six steps at most, and the average distance was 3.6 steps. It could be said that an actor who wanted to reach an advisor anywhere in the network could reach this advisor after 3.6 actors. The network density was calculated as 0.016, it meant that only 1.6% of the potential relationships were established in the network. The average degree of the actors was calculated as 2.3.

In Figure 4, it was identified that the gatekeeper structure that could fully control the diffusion of innovation between the actors was absent. However, in the PIAN, the most influential actors in the diffusion of innovations were public institutions (BMAF), farmer organizations (BPO), pesticide dealers (PD), seed dealers (BSD), rice mills (BRM), and leader paddy farmers.

The factors influencing the leadership role of actors

The results of the goodness of fit test (Hosmer and Lemeshow test) indicated that the variables included

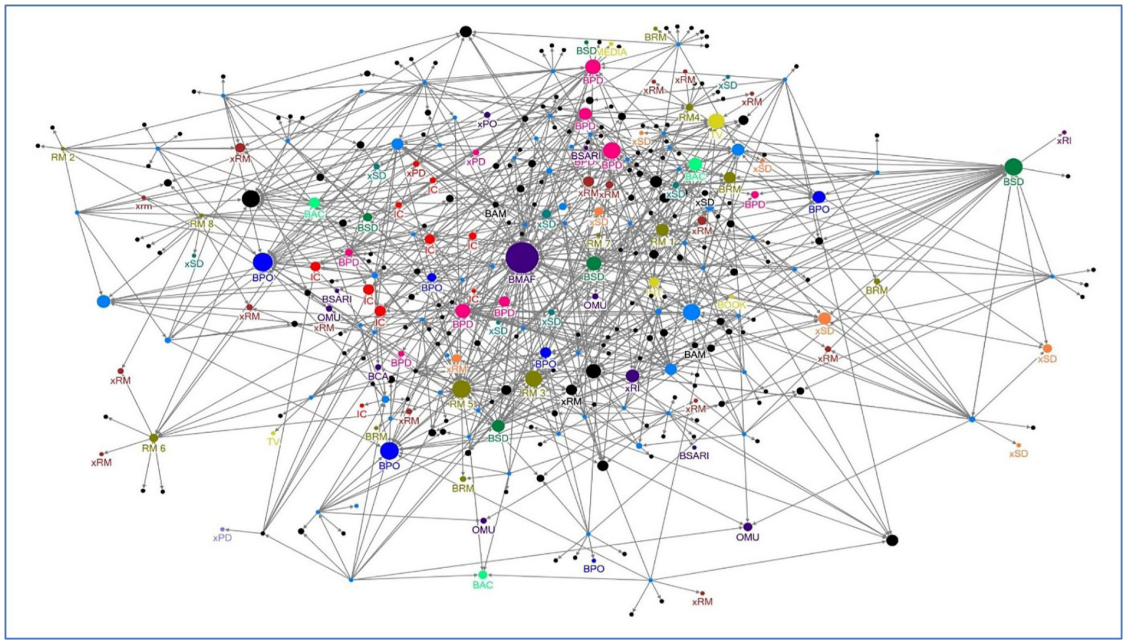

Figure 4. Paddy innovation advice network

Table 4. The meanings of the signs and abbreviations in the paddy innovation network

Signs and abbreviations	Meanings
BMAF, OMU, TARI, and BSARI	Public institutions
BPO	Farmer organizations
	Interviewed paddy farmers
BRM	Rice mills in Bafra district
TSD	Seed dealers in the Thrace region (West of Türkiye)
BPD	Pesticide dealers in Bafra district
BSD	Seed dealers in Bafra district
TV, Media, Lit.	Visual and printed media
IC	National and international companies
xRM	Rice mills in provinces outside the region
xPD	Pesticide dealers in provinces outside the region
xSD	Seed dealers in provinces outside the region
	Other actors who were not interviewed but were in the system

Table 5. Results of the ordinal logistic regression model

Predictor variables	Coeff. (β_j)	SE.	Wald	Sig.	Exp(β_j)
Age (year)	0.002	0.033	0.071	0.943	
Year of formal education	-0.836	0.446	-1.875	0.061*	0.4
Household size	0.119	0.119	1.003	0.316	
Agricultural experience (year)	-0.020	0.023	-0.881	0.378	
The number of membership in agricultural organizations	0.404	0.179	2.255	0.024**	1.5
The number of demonstrations and training attended	0.970	1.271	0.764	0.445	
Cooperation score (collective action)	2.607	1.073	2.429	0.015**	13.6
Social status (being selected head)	2.446	0.852	2.871	0.004**	11.5
The number of projects involved	1.360	0.731	1.859	0.063*	3.9

, **, * significant at 10%, 5%, and 1%, respectively*

in the model were in fit with the model ($p<0.05$). To the Nagelkerke R^2, the independent variables predicted 41.7% of the dependent variable changes and the model results are given in Table 5. Age, household size, agricultural experience, number of demonstrations, and education variables did not differ according to the groups ($p>0.05$). However, there were statistically significant differences among the groups and the predictor variables, the number of memberships in agricultural organizations ($p<0.05$), cooperation score ($p<0.05$), the social status ($p<0.05$), formal education year ($p<0.10$), and the project experience ($p<0.10$).

To the results, a high cooperation score increased the probability of farmers as a being consulted actor by 13.6 times. The increase in the social status of the farmers increased the probability of being as a consulted actor by 11.5 times. The increase in the agricultural project experience of the farmers increased the probability of being as a consulted actor by 3.9 times. As an increase in the number of agricultural producer organizations that farmers were members of, the probability of being as a consulted actor increased 1.5 times. The increase in the education period of the farmers increased the probability of being as a consulted actor 0.4 times.

DISCUSSION

Most previous studies on agricultural innovation networks have focused on relationships in the individual or institutional layer separately (Wu and Zhang, 2013; Skaalsveen et al., 2020). However, it may be more appropriate to examine all relationships in the network in layers and focus on transitions between layers. Focusing on the diffusion processes of agricultural innovations in the paddy sector, this study differs from others in examining network layers.

In the farmer-level network, the farmers have large kinship networks and have relationships with other farmers increasing the possibilities of reaching innovation sources outside their social networks. Van den Broeck and Dercon (2011) found that farmers can rely on social networks for agricultural information, and Kroma (2006) stated that farmers could rely on other farmers. Weyori et al. (2018) emphasized that leader farmers were the shortest way to disseminate information in agricultural innovation systems. In the study, it was determined that some farmers facilitated the diffusion of agricultural innovations among farmers. The research findings supported the results of previous studies, so it could be concluded that using leader farmers in

extension programs would increase the effectiveness of dissemination in the paddy sector.

In the literature, innovation agents or brokers were defined as individuals or organizations that played a catalytic role in bringing together different actors and facilitating the interactions that lead to the development of innovations (Klerkx et al., 2009). Innovation brokers are located close to farmers, regional public institutions, and private R&D institutions in the network, thereby facilitating the diffusion of innovation (Madureira et al., 2019). In the local layer in the study, the pesticide dealers (PD) were the innovation brokers. The position of pesticide dealers in the network could be interpreted as accelerating the network's information transfer. Most of the pesticide dealers in the research area were local companies and were the dealers of large national/ international companies. Thus, the arrival of a national or international innovation to the local speeding up.

Perhaps one of the most critical outputs of this study was that it revealed how the farmers, who were the advice source, came to this position. Actors central in referral networks provide significant opportunities for other actors in the network to implement new ideas and disseminate innovations (Battke et al., 2016; Guan et al., 2016; Brennecke ve Stoemmer, 2018; Tang et al., 2020). According to the research findings, farmers who cooperate have high social status, have more project experience, and participate in agricultural organizations are more likely to be a source of advice Gulati and Srivastava (2014) state that advice resources provide other actors in the network with tangible and intangible resources needed for innovation and psychological trust. Once the research findings are evaluated in this context, it can be concluded that farmers with high social status provide psychological trust to other farmers because actors with high social status are assumed to have more information about agricultural innovations due to their connections. In addition, Emerick and Dar (2021) found that field trials would be one of the most suitable options for poor farmers to reach innovations due to it is economical. Once the research results are combined with the findings of previous studies, it can be concluded that the farmers participating in field trials and making trial production with new agricultural technologies would be more likely to a more central position in the networks.

According to the research findings, cooperating farmers are more likely to be a source of advice for other farmers. The cooperation between small farmers comes to the fore with its risk-sharing and welfare-enhancing features (Colombo and Perujo- Villanueva, 2017; Wardhana et al., 2020). Vissers and Dankbaar (2013) emphasize that agricultural collaborations lead to complex network relationships and facilitate the diffusion of innovation through information exchange. The findings of previous studies and the research results are synthesized; it can be concluded that the farmers who cooperate are accepted as a source of advice by other farmers because they may access information more quickly and not take risky actions. In the diffusion process of agricultural innovations, collaborative farmers can be considered accelerators.

The study and its findings were limited to the research area and the paddy industry. A similar study in another region or sector may not yield the same results. Since social relations were examined in the research, the variables influential in being a source of advice were mainly chosen from sociodemographic characteristics. In future studies, farmers' financial indicators can be added to the model along with sociodemographic characteristics. Another limitation of the study was the determination of the common characteristics of the consulted farmers. Future studies can also focus on the typical characteristics of intermediaries and accelerators in layers outside the farmer network (regional, national, international, so forth).

CONCLUSION

The study analyzed the diffusion of agricultural innovation processes, identified influential actors in the paddy sector in a three-layered network, and indicated how agricultural innovations could be delivered to farmers fastest and in the shortest way.

The results indicated that agricultural innovations in the paddy sector first diffused within layers in social networks and that sources of advice supported diffusion processes across layers. It was concluded that the sources of advice had two roles in the social network. Their primary role is to ensure that farmers are aware of innovations by carrying the information from the innovation source from top to bottom between layers and acting as an accelerator for farmers to adopt innovations. The second role of advice sources is to provide feedback on bottom-up innovations. According to the research results, three different sources of advice emerge. The sources of advice at the outermost periphery of the social network are representatives of international companies and paddy seed breeder researchers. The representatives of international companies connect with input providers at the middle periphery of the network, whereas seed breeder researchers establish two-way connections with both local input providers and consultant farmers. In the middle periphery of the network, pesticide dealers and seed sellers act as a source of advice. Significantly, local pesticide dealers are the intermediary positions among farmers (innermost periphery) and the national network (outermost periphery). The local pesticide dealers deliver the information or technology they received from innovation creators to farmers through the leader farmers. The local pesticide dealers have remarkable two-way roles in the network. The correct delivery of the requests from farmers to the innovation creators (and vice versa) depends on the local pesticide dealers'

desire, responsibility, or communication skills. Thus, the local pesticide dealers' positions may pose a barrier to transferring information or innovations in the network. They can prevent the transfer of technology that would be newly introduced to the network for various reasons such as commercial concerns and higher commission payments by other companies.

Some farmers acknowledged the other farmers as a source of advice. The study concluded that an increase in the social and human capital of the farmers increased the probability of being a source of advice. Although it is a standard to start from leader farmers in programs to disseminate agriculture innovation, this method is open to discussion. Like leaders, farmers have more social relationships and more robust capital structures than others; their capacity to take risks is also more remarkable. They usually find the opportunity to reach the innovation aimed to be disseminated. Here, while farmers in the leader farmer's network achieve innovations quickly, the question of how farmers outside the leader farmer's network reached innovation arises. For this reason, the extension programs should include all farmers in case the farmers (isolated) might be out of the leader farmers' network and should be supported by farmer organizations and public extension services.

As a result, the study concluded that agricultural innovations could be delivered to farmers most concisely, following the ranking of innovation creators, local pesticide dealers, and leader farmers. Finally, this study positively contributes to the methodology and agricultural extension literature by proving that social network analysis could help identify influential actors and their roles in the diffusion process of agricultural innovations.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

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

Author contribution

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Ethical approval

This article does not contain any studies with human or animal subjects. Ethics committee approval is not required

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RESEARCH ARTICLE

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Pollution indices assessment of metal concentrations in Karabuk soil samples

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Abstract

Soil pollution refers to the contamination of soil by harmful substances that can have adverse effects on plant and animal life, it also negative affects the health-being of humans. The sources of soil pollution include industrial activities, agricultural practices, mining and transportation activities. The contaminants in soil can include heavy metals, pesticides, herbicides, fertilizers, petroleum products, and other chemicals. These contaminants can seep into the soil and accumulate over time, making the soil unsuitable for agriculture or other uses. Heavy metals are a significant concern in soil pollution due to their persistency and potential harm for living organisms. Therefore, it is essential to evaluate metal contamination in soil using ecological risk indices to protect human health. This assessment can help identify potential risks and enable effective management of contaminated sites. This study aimed to assess of the metal pollution levels, including Arsenic (As), Cobalt (Co), Chromium (Cr), Copper (Cu), Nickel (Ni), Lead (Pb), and Zinc (Zn), in soil samples from Karabuk using various ecological risk indices. These indices included the geo-accumulation index (Igeo), enrichment factor (EF), contamination factor (CF), contamination degree (Cd), pollution load index (PLI), and potential ecological risk (PERI). Furthermore, statistical techniques such as correlation and factor analysis were employed to determine the underlying sources responsible for these metals. Based on the results of the Cd, PLI, and PERI, it was found that the soil at T7 exhibited a very high degree of contamination, was moderately to highly polluted, and posed a moderate ecological risk, respectively. The results of the pollution indices suggest that the sources of pollution in the Karabuk soil samples are anthropogenic, meaning they are a result of human activities like industrial processes and improper waste disposal.

Keywords: Karabuk, Metal pollution Soil, Pollution indices, Risk management

INTRODUCTION

Environmental pollution such as soil, water and air pollution have become an important trouble for human life in the last years. Research-based studies and assessments are useful for understanding the state of environmental pollution and providing reference for further studies. Soil is crucial for human survival and societal development and its quality directly impacts food security, agricultural product quality, human health, and social progress. Unfortunately, as the economy and society continue to grow, human activities have caused a surge in soil pollution, particularly from heavy metals. This pollution disrupts the ecosystem balance and leading to decreased agricultural output and quality. Moreover, heavy metals enter the human body through the food chain and other pathways, accumulating over time and posing significant risks to human

health (Zhao et al., 2022; Tian et al., 2021: Zhai et al., 2018). Metal pollution is commonly contaminants in soil ambient especially As, Co, Cr, Pb, Cu, Zn and Ni.. The founding of more than normal amounts of various metals with the rapidly development of urbanization and industrialization in the soil cause negative effects for environment and human health (Sezgin et al., 2022; Zhao et al., 2022). In recent years, there has been a growing local and global concern regarding the assessment of pollution and soil remediation techniques. This is due to the detrimental effects of long-term exposure to metals, particularly heavy metals, which can result in adverse health outcomes such as lung cancer and bone fractures. (Rai et al., 2019; Chen et al., 2016). Numerous studies used different techniques have conducted for remediating soil contaminated with heavy metals. These methods include in-situ approaches like surface capping, encapsulation, electro kinetic extraction, soil flushing, chemical immobilization, phytoremediation, and bioremediation, as well as ex-situ techniques such as landfilling, soil washing, solidification, and vitrification. The primary focus of these techniques is to reduce the concentration of heavy metals in the soil, making them less accessible or bioavailable. While these methods have shown high efficacy, they are often expensive, environmentally harmful, and time-consuming. Consequently, there is a need to implement advanced technologies for pollution assessment and remediation that can effectively and safely address heavy metal-contaminated soil (Zhao et al., 2022: Nayak et al., 2020). Assessing heavy metal pollution in soil holds great significance in combating and mitigating the escalating issue of soil pollution. The commonly employed approaches for assessing heavy metal pollution locally and globally can be broadly categorized into index methods and model methods. Index methods encompass single pollution, pollution load and cumulative indexes, and more. Model methods include the enrichment factor, potential ecological risk index and others. These assessment techniques provide valuable insights into the extent of heavy metal pollution in soil and help guide remediation efforts (Zhao et al., 2022).

In this study, pollution indexes such as enrichment factor (EF), contamination factor (CF), geo-accumulation index (Igeo), contamination degree (Cd), and pollution load index (PLI) and potential ecological risk index (PERI) were calculated for evaluating metal pollution level in soil samples of Karabuk. The amounts of the metals investigated in the Karabuk soil sample were obtained using data from an open-source report published by the Turkish Atomic Energy Authority in 2015 (Turkish: Türkiye Atom Enerjisi Kurumu – TAEK). There are many elements analyses in this report. However, As, Co, Cr, Cu, Ni, Pb, and Zn concentrations were evaluated for the pollution assessment for Karabuk soil in the study. The Fe values in the report were utilized as a reference metal.

Arsenic

Arsenic (As) is one of the naturally occurring elements in abundant quantities within the earth soil. Arsenic has existed in the air, in water and on the earth surface soil. Arsenic has two forms such as organically and inorganically forms. The latter is extremely toxic. Arsenic is a chemical element without taste or smell. In addition to its natural presence in the soil, arsenic is also released from industrial activities such as the manufacture of pesticides, dyes and in the metallurgical industry (Shrivastava et al., 2015).

Uses of Arsenic

Although As is known as a toxically element, it is used in industry. Arsenic is used in the manufacture of pesticides such as rat poison and insecticides. It is also used in the wood industry. It keeps the wood for a long time without decomposition. İt thus plays a role of wood protector. It can also be used in the glazing industry.

Arsenic in Soils

Arsenic can occur in organically and inorganically forms in soils. Generally arsenic is found as ions in soils. Arsenide(As^{+3}) is most often found in reducing environments such as waterlogged soils and arsenate(As+5) in well-drained surface soils (Roberts et al., 2010). Arsenide(As^{+3}) compounds are generally more mobile than arsenate(As^{+5}) compounds in soils. Thus arsenic through arsenites can infiltrate soil surfaces to groundwater (Shumlas et al., 2016).

Arsenic Pollution and Public Health

Arsenic present on the surface of soils can contaminate groundwater through infiltration. The consumption of contaminated water has a very serious impact on public health. According to the WHO, contaminated groundwater is the greatest threat from arsenic. Inorganic arsenic is naturally present at very high concentrations in groundwater in many countries. Drinking water, crops irrigated and food prepared with contaminated water are the sources of arsenic exposure. Arsenic and its inorganic forms have been classified as carcinogenic to humans since 1980 by IARC. Epidemiological researches have shown that chronic exposure to arsenic by inhalation is the cause of primary bronchial cancers. While chronic exposure by ingestion of contaminated water is the cause of lung, skin and bladder cancers (IARC, 2012).

Cobalt

Cobalt (Co) is a chemical element discovered in 1735 by Georg Bandt. It is a naturally occurring hard gray heavy metal with atomic number 27. Cobalt is a significant element in the metabolism and grow up for animal and vegetable cells. İt is generally found in inorganic compounds in several forms. Its most abundant forms are its 2+ ions and 3+ ions (Mahey et al., 2020). It also exists in a radioactive form of cobalt. This latter form of

cobalt comes from nuclear waste and nuclear accidents. Generally, the presence of cobalt in the environment results from the burning of coal and mining of cobalt-containing ores. The production and use of cobalt-based chemicals are also sources of cobalt in the environment. Cement industries and carbide tool grinding plants are also responsible for cobalt leaching. Cobalt is also found in the fumes of thermal power plants or incinerators and in the exhaust of combustion engine vehicles (INERIS, 2006; Abraham and Hunt, 1995).

Uses of Cobalt

Cobalt is a heavy metal that is widely used in industry. Many industrial applications use cobalt. It is used in metal form or as a compound with oxides, sulfate, sulfide and chloride. It is used, among other things, in the composition of resistant alloys used in the electrical, aeronautical and automotive industries, permanent magnets, cutting tools, surgical alloys (prostheses), agricultural fertilizers and animal feed additives. Cobalt salts are used as pigments (glass, ceramics, paints, varnishes) (INERIS, 2006). Cobalt is also used in the manufacture of batteries. It also knows a pharmaceutical use as veterinary products, and feed additives for cattle. At low doses, cobalt is also an essential trace element, constituent of vitamin B12 (found in meat and dairy products) (URL-1).

Cobalt in Soils

Cobalt is a heavy metal naturally present in soils. It is also present in water and rocks. Cobalt can be found in surface water through rainwater runoff. In soils, cobalt can react with other compounds or adsorb to soil particles. It may also adsorb to sediments in water. Soils near mining and smelting operations are very rich in cobalt (URL-1).

Cobalt Pollution and Human Health

Cobalt has been classified by the IARC since 1991 and revised in 2006 as a metal that may be carcinogenic to humans if exposed by inhalation. With the conclusions of the IARC, the European Union has classified cobalt sulphate in group 1B; that is to say, as a substance that should be regarded as carcinogenic to humans through inhalation exposure (IARC, 2012).

Chromium

Chromium (Cr) is one of the most abundant elements in the earth surface soil and exists in the environment as Cr (III) or Cr (VI). Other intermediate valence state of Cr also exist in the natural environment, but they are mainly not stable. Pure chromium has a silver white color and is obtained by reaction of aluminum and chromium oxides. This reaction is done by electrolysis or from chromium iodide (Cary, 1982).

Uses of Chromium

Chromium is widely used in industry for the manufacture of rust resistant surfaces. Hard chrome plating is a very hard and slippery compound used in anti-wear coatings. It is less expensive and allows a better protection of new parts at acceptable prices. It is an electrolytic coating that is applied to metals such as steel, copper, bronze and aluminum alloys. It can be found in almost all sectors of activity such as the automotive, steel, mechanical, food, aeronautical, hydraulic, printing, glass, foundry, metallurgy, recycling of industrial waste, paper, tires, textiles, plastics, tooling and medical (Ashley et al., 2003).

Chromium in Soils

The manner in which chromium behaves in soil is significantly impacted by its speciation, which is determined by the soil's pH and redox potential. Typically, chromium is predominantly found as a chromium(III) among of their forms in mostly soil. This form is comparatively insoluble, unreactive, and poses a low risk of toxicity to living organisms (Ashley et al., 2003; Barnhart, 1997). However, certain conditions can result in the presence of chromium(VI) in the soil, such as oxidizing conditions, which can give rise to highly soluble, mobile, and toxic forms of chromium such as CrO_4^{-2} and $HCrO_4$ (James et al., 1997). Under anaerobic conditions where oxygen is limited, chromium(VI) can be reduced to chromium(III) through the presence of S^{-2} and Fe^{+2} in the soil. Such reduction can also take place in soils that have adequate organic matter and a low pH. In contrast, the oxidation of chromium(III) to chromium(VI) in soil can take place in the presence of organic matter, oxygen, manganese dioxide, moisture, and high temperatures, as seen during brush fires (EPA 1990b; Salem et al., 1989; Calder, 1988; Cary, 1982).

Chromium Pollution and Public Health

Chromium especially Chromium (IV) is a heavy metal toxic to humans. It has several forms that each have a different degree of toxicity. Among others, we have the nanoparticle, oxide and valence forms. The nanoparticle form can have adversely effect on the respiratory system following inhalation in high concentrations. In addition to the presence of Chromium in water and soils, it is also found in some organisms such as food plants. It can therefore through the food chain cause impairment of human health. Excess inhaled Chromium (IV) can cause nosebleeds or even nasal irritation. However, it is also important to mention that Chromium (III) is necessary for humans, whose deficiency can have cardiac consequences or even on diabetes (Cary, 1982).

Copper

Copper(Cu) is a reddish metal. The earth's crust contains an average of 50 mg/kg. It is corrosion resistant and has excellent thermal and electrical conductivity. It is an essential element for living organisms. Copper has 29 isotopes, two of which are natural and stable: ^{63}Cu and ^{65}Cu. The others are radioactive and artificially produced. Copper is a non-renewable resource, but it is fully

recyclable and can be re-smelted and reused. In 2008, 2.5 million tonnes of copper were recycled in Europe, which is almost 45% of total copper consumption. The main producing countries are Chile, China, Peru and the USA. Global reserves are estimated at 630 million tonnes, while global production was 18.1 million tonnes in 2013 (URL-3).

Uses of Copper

Copper is used in the electrical, electronic and telecommunications industries, for power cables, computer chips, television cables and batteries. It is used in construction for plumbing, fittings and valves. In architecture, its compounds give roofs a characteristic green color. Copper is also used in transport, machinery, marine and armaments. Finally, copper's antibacterial, fungicidal and algicidal properties make it a product used in hospitals and for aquaculture. Some of its isotopes also have medical applications (URL-3; Catherine, 2016).

Copper in Soils

Copper mining and refining activities are the main sources of copper in soils. Other sources of copper contamination in soils include landfill sites, domestic sewage, fossil fuel combustion, pulp and paper, use of organic and chemical fertilizers and forest fires. Copper alone remains immobile in soils. It gains mobility when transferred to water or the atmosphere. It is adsorbed by clays, organically matters, carbonates and iron and manganese oxides and hydroxides (Yarlagadda et al., 1995). Copper can also react with carbonates.

Copper Pollution and Public Health

Excess copper is toxic to aquatic organisms, vascular plants and farm animals. In humans, drinking water with excessive copper can cause nausea, vomiting, cramps and diarrhea. Chronic ingestion of excessive amounts of copper can cause irreversible liver and kidney damage, even death (Catherine, 2016). Excessive ingestion of copper in humans can result in severe mucosal corrosion, extensive capillary damage, liver and kidney damage and central nervous system irritation followed by depression (Singh and Kalamdhad, 2011).

Nickel

Nickel(Ni) is a chemical element found almost everywhere in the environment. İt is a bright white and hard metal. Its average concentration on the earth's crust is about 20mg kg^{-1}. İt can be present in air, and in airborne particles. Nickel originates from the incineration of household waste, the burning of coal and wood.

Uses of Nickel

Nickel is an important element in the manufacture of stainless steel, non-ferrous alloys such as coins and in the manufacture of Ni-Cd batteries. Nickel is also used as a catalyst in organic chemistry (Pichard et al., 2006).

Nickel in Soils

In nature, nickel can be found in different oxidation states (+II, +III and +IV) but is mostly found in its +II state. Its solubility in water varies greatly depending on its chemical form. Nickel compounds such as acetate, chloride, nitrate and sulfate are highly soluble, followed by carbonates and hydroxides, sulfides and disulfides, while the oxides are practically insoluble (WHO, 2021). the location of nickel in the soil is linked to its origin. Thus, geogenous nickel is adsorbed preferably on iron and manganese oxides, whereas nickel of anthropogenic origin tends to remain exchangeable and to bind to organic matter and carbonates (Baize, 1997). Like other metals, the mobility of nickel increases with the acidity of the environment.

Nickel Pollution and Public Health

Nickel compounds are mainly absorbed by humans via the respiratory route and can cause chronic bronchitis and asthma. Intoxication via skin absorption is not to be neglected. Indeed, some costume jewelry contains traces of nickel and causes numerous skin reactions, a phenomenon regularly seen in young girls. Nickel also has a chronic toxicity since the International Agency for Research on Cancer (IARC) classifies nickel compounds as carcinogenic to humans and metallic nickel as a possible human carcinogen (Pichard et al., 2006).

Lead

Lead (Pb) is an element present in several natural minerals such as sulphites, sulphates, carbonates, oxides, hydroxides and phosphates. The main mineral sources of lead are galena (PbS), cerussite ($PbCO_3$) and anglesite ($PbSO_4$). We most often encounter lead compounds associated with minerals composed of zinc, cadmium, silver and copper (Catherine, 2016; Laperche et al., 2004, Pichard, 2003).

Uses of Lead

The contamination caused by the use of lead in paint makes him infamous. Indeed, cerussite and anglesite (a lead carbonate and sulphate, respectively) providing a white color pigment were used for water pipes, gasoline additives, cable sheaths and in agricultural pesticides. In recent years these uses have greatly diminished due to pollution caused by lead. (Laperche et al., 2004; Mercier, 2000; Bonnard et al., 2006). Many scientific works spread the concerns around its use. In addition, it has been widely used in printing, metallurgy and the manufacture of accumulators, capable of providing much more energy than an ordinary battery (Bonnard et al., 2006, Laperche et al., 2004). Today, lead is used in the composition of electric batteries, automobile radiators, ammunition and alloys (Pichard, 2003). Finally, recent technological advances have favored the emergence of the use of lead. For example, lead sheet provides effective protection against radiation used in medical

imaging and radiotherapy (Catherine, 2016). The fields of use of lead and its various compounds are given in the Table 1.

Lead in Soil

Since lead is not very mobile, it attaches itself to the upper surface of soils. Lead is mainly adsorbed on clays, oxides and hydroxides, carbonates and organic matter (Basta et al., 2005). The lead-organic complexes formed are stable. An alkaline pH results in the precipitation of lead in the form of carbonates or phosphates; its complexation with organic matter makes it more soluble. According to Yarlagadda et al. (1995), lead can be found in all particle size fractions of contaminated soils.

Lead Pollution and Public Health

Humans absorb lead by three main routes; (1) inhalation of dust or fumes of lead or lead oxides, (2) ingestion of food, dust, soil or paint containing lead, (3) cutaneous absorption, for example, organic and fat-soluble lead used in the composition of creams and cosmetics (Laperche et al., 2004; Lyn, 2006; Miquel, 2001). Thus in children from 0 to 5 years old, lead poisoning mainly takes place by ingestion of dust or contaminated soil. The harmful effects of lead on the health of young children, and in particular on the development of the nervous system, are all the more recognized as they often result from an unfavorable socio-economic situation (unsanitary housing or located near industrial areas) (Catherine, 2016). Lead is physiologically and neurologically toxic to humans. Acute lead poisoning can lead to dysfunction of the kidneys, reproductive system, liver and brain. Lead can be harmful even in very low concentrations.

Lead toxicity can lead to a range of harmful effects on the body, including teratogenicity, inhibition of hemoglobin synthesis, and damage to the central and peripheral nervous systems. Other chronic symptoms such as anemia, gastrointestinal problems, and anoxia may also arise. Pregnant women may experience difficulties, and individuals may develop high blood pressure and joint/muscle pain. In summary, lead poisoning can result in an overwhelming sense of exhaustion and fatigue. It is also capable of causing damage to the gastrointestinal system and urinary tract thus causing bloody urine (Singh and Kalamdhad, 2011).

Table 1. Common uses of lead and its compounds (Catherine, 2016)

Application domain	Chemical formula
Cotton printing	$Pb(CH_3COO)_2$
Wood conservation	$PbC_{14}H_{30}O_2$
Cosmetics and disinfectant	$Pb(CH_3COO)_2$
Enamel, glaze	PbS
Semiconductor	PbS, PbSe, PbTe
Catalyst for polyurethane polymerization	$PhPb(OAc)_3$
Ceramic	$PbSi_2O_5$
Putty and matches	PbO, PbO_2 , Pb_3O_4
Makeup	PbS
Textile dyeing	$Pb(NO_3)_2$
Oxidizer in fireworks	PbO_2

Zinc

Zinc(Zn) is a blue-gray metal, moderately reactive in water, oxygen and CO_2. It has the property of releasing hydrogen in the presence of weak acids. The earth's crust contains a mean of 70 mg kg^{-1}. This makes it the 24th most abundant element. It is an essential trace element involved in cell development in particular and present in nearly 200 enzymes (Abarnou et al., 2000).

Uses of Zinc

Zinc is mainly used for the galvanization of iron and the production of alloys. It is used in the manufacture of conductive agents for electrical and electronic equipment. It is also used in construction, in the automotive industry and for railways. Its compounds are used to make plastics, pigments, lubricants, pesticides and fungicides. Zinc is used in the pharmaceutical industry as a dietary supplement for the treatment of deficiencies and dermatoses. It is involved in the human body in the maintenance of immune function, blood clotting, wound healing, thyroid function and spermatogenesis (URL-2).

Zinc in Soils

Industrial and mining activities, agricultural sludge spreading, road transport and waste incineration are the commonly resources of zinc in the environment and soils. Zinc is most often found in the oxidation state, as ZnS, and in different ionic forms in soils. Although mobile, it is most often found at the soil surface. The half-life of zinc in soil is estimated to be about 80 years (Catherine, 2016).

Zinc Pollution and Public Health

Zinc is a trace element necessary for the proper functioning of the human body. It is essential for proper development of the human body, animals and plants. A deficiency of zinc can lead to malfunctions such as dermatitis, growth retardation and poor tissue healing. Conversely, excess zinc is also toxic to living beings. In humans, the routes of exposure are ingestion (through food), inhalation (workplace fumes) and skin contact (cosmetics). Zinc chloride ($ZnCl_2$), zinc oxide (ZnO), zinc sulphide (ZnS) and zinc sulphate ($ZnSO_4$) are the most widely studied compounds in zinc toxicity. Effects of excessive zinc exposure include gastrointestinal discomfort, lung irritation (alveolar fibrosis and bronchopneumonia), anemia, liver and kidney damage, endocrine and neurological disturbances, miscarriage,

and cancers (prostate and gonads) (URL-2; Naert, 2017; Catherine, 2016).

MATERIALS AND METHODS

Karabuk province is located in the Western Black Sea region of Turkey, between the latitudes of 40°57′ and 41°34′ North and the longitudes of 32°04′ and 33°06′ East. The province has a surface area of 4,145 square of kilometer and a population of 252.058 according to 2022 estimates. The economy of Karabuk has developed based on the iron and steel industry. KARDEMIR (Karabuk Iron and Steel Works), one of Turkey's important industrial facilities, has been active in the province since 1939. In addition, the rolling mills and foundries established in the city are other institutions related to the iron and steel industry. Iron trading, transportation, and forestry are also important economic activities in the city. In recent years, the provincial economy has begun to diversify, with the establishment of textile, marble, forestry, and cement industries. The economy of the central district is mainly based on manufacturing. Additionally, Safranbolu district, with its rich historical past, is an important tourism center among the districts (URL-4). In the report, pollution indexes were evaluated for selected metals at only 37 specific locations out of the total sample points. One particular sample point, labeled as 78T008 in the report, was chosen as a reference soil sample. This specific sample point was distinct from the other 37 points evaluated and was selected due to its significantly lower metal content compared to the rest. The graphical representation of all the investigated sample points can be found in Figure 1. Collecting and analysis of Karabuk soil samples in the TAEK report, 2015 are defined as below:

Soil samples were taken from areas 20 cm in diameter and 10 cm deep from the surface. While selecting the sampling sites, care was taken to ensure that the sample place was flat, uncultivated and not accumulated by erosion. In addition, samples were taken after cleaning the stone, grass, grass and garbage from the soil surface to be sampled. Each soil sample was obtained after mixing several samples from an area of approximately 400-500 m^2 and the samples were placed in nylon bags and numbered. After, all collected samples were dried at the room temperature for 1 week in the laboratory. After drying all samples were powdered and sieved using a 200 mesh diameter stainless sieve for trace elements analysis. Trace element analyzes have been performed using the Wavelength Dispersive X-ray Fluorescence (WDXRF) method. For XRF analysis, 12 g powder soil samples have mixed with 3 g cellulose in the agate mortar for 5 minutes. Then, to pellet the samples, they were pressed for 3 minutes using a 25-ton hydraulic press with 40 mm diameter steel pellet containers. Finally, prepared pellets have been analyzed by WDXRF spectrometry to determine trace elements.

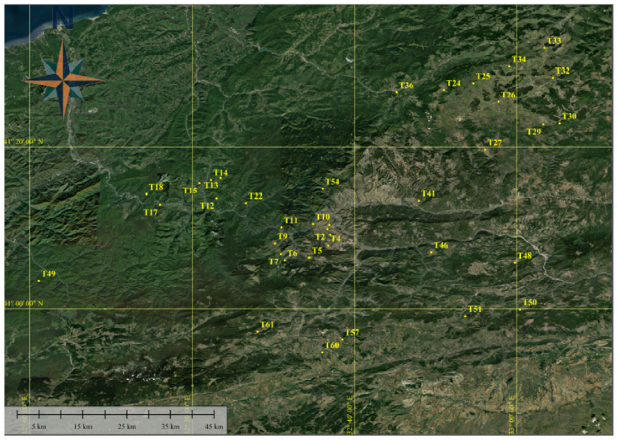

Figure 1. Soil sampling locations in Karabuk

The relationships between metal components are valuable for understanding the sources of metal resources (Lu et al., 2010). Pearson's Correlation Coefficients and Principal Component Analysis (PCA) are statistical analyses commonly utilized to explore these relationships in many previously studies. Pearson's correlation coefficient, employed in this study using IBM SPSS 21.0, measures the strength and direction of the linear correlation between pairs of pollutants present in the analyzed samples. By assessing the correlation matrix, we can identify positive correlations, indicating that metal components originate from similar sources, whether natural or anthropogenic, or are co-contaminants. Conversely, negative correlations suggest that metal pairs come from different sources in this statistical analysis (Chandrasekaran et al., 2015). PCA, another statistical technique employed in this study, is used to reduce the number of variables and derive a smaller set of latent factors or principal components (PCs) (Chen et al., 2014). These components provide insight into the relationships between observed variables. PCA facilitates the identification of metal resources, distinguishing between natural and anthropogenic origins (Saedi et al., 2012; Tokalioğlu and Kartal, 2006). In summary, Pearson's Correlation Coefficient and PCA are employed to assess the relationships between metals and their potential sources. Pearson's correlation coefficient measures the strength of linear correlation between pollutants, while PCA reduces variables and identifies latent factors or principal components, aiding in the analysis of relationships between observed variables and identification of metal resources. These statistical analyses are commonly used in environmental studies on soil samples (Sezgin et al., 2022).

Pollution indexes and Factors

In this study, pollution indexes and factors such as Igeo, EF, CF, Cd, PLI, and PERI were used to the assessment of pollution in Karabuk soil samples. Equations of all pollution indexes and factors are given in Table 2 and their classification/categorizations are shown in Table 3.

Table 2. Pollution indexes and factors equations

Pollution indexes/Factors	Equations	Explanations
Igeo (geo-accumulation index),	$Igeo = log_2\left(\frac{C_s}{1.5C_r}\right)$	Cs:The measured concentration of the elements in the soil samples, Cr:The geochemical reference value. The constant 1.5 given in the equation is used to consider the potential differences in the reference values since they are influenced by natural fluctuations and anthropogenic influence (Sezgin et al., 2022).
EF (Enrichment Factor)	$EF = \frac{\left(\frac{C_x}{C_{ref}}\right)_{sample}}{\left(\frac{C_x}{C_{ref}}\right)_{background}}$	Cx is the concentration of the investigating metal in the sample and background (reference) soil sample, Cref is the concentration of reference metals in the sample and background (reference) soil sample (Christoforidis and Stamatis, 2009).
Contamination Factor (CF)	$CF = \frac{C_s}{C_r}$	According to the pollution factor proposed by Hakanson (1980), Cs represents the metal concentration in the sediment sample, while Cr represents the metal concentration in the reference sample (Keshavarzi et al., 2015; Sezgin et al., 2019).
Contamination Degree (Cd)	$C_d = \sum_{n=1}^{n} CFs$	(Keshavarzi et al., 2015; Sezgin et al., 2019).
Pollution Load Index (PLI)	$PLI = \sqrt[n]{CF_1 x CF_2 x \ldots\ldots CF_n}$	(Hołtra and Zamorska-Wojdyła, 2020).
Ecological Risk (Er)	$Er = Tr.CF$	The contamination factor (CF) is calculated using the equation provided earlier. Tr represents the coefficients defined as "toxic-response factor" by Hakanson (1980) (Sezgin et al., 2019; Hołtra and Zamorska-Wojdyła, 2020).
Potential Ecological Risk (PERI)	$PERI = \sum_{n=1}^{n} Er$	(Hołtra and Zamorska-Wojdyła, 2020).

Igeo, which is recommended by Muller, is successfully used to pollution assessment in soil and implements a logarithmic operation to the results of sample analysis. 1.5 is uses as a constant in Igeo calculation and its allows to the eliminate of likely differentiations in the contents of the investigated elements in soil due to the likely variation in the background (Bn) and a small influences of anthropogenic activity (Sezgin et al., 2022). This index presents an opportunity of comparing with now and before concentrations (or non-industrial conditions) (Hołtra and Zamorska-Wojdyła, 2020). Table 3 presents Muller's classification for the assessment of the contamination level of Igeo (Sezgin, 2019). Enrichment factor (EF) is generally used to determine metals sources which arise from naturally or anthropogenic resources. (Sezgin et al., 2022; Wei et al., 2010; Meza-Figueroa et al., 2007). This parameter is calculated to depend on normalization of an investigated elements versus a reference element. The elements such as Al, Fe, Sc and Sr are often selected as a reference element because of they have low occurrence variabilities (Klos et al., 2011; Han et al., 2006; Turner and Simmonds, 2006). Fe was determined as a reference element among of these elements in this study. Equation of EF and classification criteria were given in Table 2 and Table 3. Contamination factor (CF), which defines a rational approach of the metal concentrations in the investigation soil samples to individual metal concentrations in reference soil

Table 3. The pollution indexes/Factors and Soil classifications/Categories

Igeo classification		
Igeo Value	**Class**	**Soil Quality**
Igeo≤ 0	0	Unpolluted
0 < Igeo ≤ 1	1	Unpolluted to moderately polluted
1 < Igeo ≤ 2	2	Moderately polluted
2 < Igeo ≤ 3	3	Moderately polluted to severely polluted
3 < Igeo ≤ 4	4	Severely polluted
4 < Igeo ≤ 5	5	Severely polluted to extremely polluted
5 < Igeo	6	Extremely polluted
Enrichment categories of EF values		
EF value	**Enrichment category**	
EF ≤ 2	Minimal enrichment	
2 < EF ≤ 5	Moderate enrichment	
5 < EF ≤ 20	Significant enrichment	
20 < EF ≤ 40	Very high enrichment	
40 < EF	Extremely high enrichment	
CF	**Cd**	**Contamination category**
CF < 1	Cd<8	Low degree of contamination
1 ≤ CF < 3	8 ≤ Cd<16	Moderate degree of contamination
3 ≤ CF < 6	16≤ Cd <32	Considerable degree of contamination
6 ≤ CF	32 ≤Cd	Very high degree of contamination
CF < 1	Cd<8	Low degree of contamination
PLI value	**Soil quality**	
0 < PLI < 1	Unpolluted	
1 < PLI < 2	Moderately polluted to unpolluted	
2 < PLI < 3	Moderately polluted	
3 < PLI < 4	Moderately to highly polluted	
4 < PLI < 5	Highly polluted	
5 < PLI	Very highly polluted	
PERI values	**Ecological risk category**	
PERI < 150	Low ecological risk	
150 ≤ PERI < 300	Moderate ecological risk	
300 ≤ PERI < 600	Considerable ecological risk	
600 < PERI	Very high ecological risk	

samples that is used as a preindustrial concentration, and contamination degree (Cd), which is defined as a total of contamination factors all investigated elements, is proposed by Hakanson (1980) to explain of the pollution of toxic substance in soil (Sezgin et al, 2022). On the other hand, one of the pollution indexes is PLI which is the geometric average of the contamination factors. This parameter determines the contribution of all metals in a sample point and it allows to assess the level of environmental pollution with a view to undertake monitoring or repair activities aimed at improving soil quality (Holtra and Zamorska-Wojdyła, 2020; Tomlinson et al. 1980;). CF, Cd and PLI equations and classification of pollution were given Table x and Table Y respectively. The CF is also used the another indexes calculation that are called ecological risk (Er) index for each elements and potential ecological risk index (PERI), which are introduced by Hakanson (1980), in the soil samples. In these equations Tr is defined as a "toxic-response factor" for a given substance and demonstrated this value for As, Co, Cr, Cu, Ni, Pb, and Zn to be 10, 5, 2, 5, 5, 5 and 1 respectively (Sezgin et al., 2019).

RESULTS AND DISCUSSION

Descriptive statistics of metal concentrations

Descriptive statics of investigated metal concentrations in this study and background (reference) soil values were given in Table 4. As seen Table 4, the means of As, Co, Cr, Cu, Ni, Pb, Zn and Fe were determined as 8.28, 15.69, 88.27, 39.89, 50.50, 17.05, 92.39 and 35329.22 mg kg^{-1}. The mean concentrations of these metals were shown severely higher than background (reference) soil concentrations (except Fe) in Table 4. According to

Table 4. Descriptive statistic of metal concentrations in Karabuk soil samples (mg kg^{-1})

	As	Co	Cr	Cu	Ni	Pb	Zn	Fe
Minimum	3,13	5,88	38,39	18,00	12,00	5,44	35,00	17889,00
Maximum	17,06	46,49	236,14	58,00	175,00	72,60	243,00	57827,15
Range	13,93	40,61	197,75	40,00	163,00	67,16	208,00	39938,15
Mean	8,28	15,69	88,27	39,89	50,50	17,05	92,39	35329,22
Std. Deviation	2,90	7,49	40,49	9,53	31,46	11,37	37,07	9014,65
Skewness	1,01	1,96	1,80	-0,28	2,34	3,54	2,06	0,30
Kurtosis	1,29	6,60	3,84	-0,09	6,68	15,70	6,86	0,02
Background Soil	6.31	5.88	38.39	18.00	12.00	7.05	35.00	22173.64

these results, it is may said that the investigated metals are come from anthropogenic sources. Skewness values of investigated metals in Karabuk soil samples were calculated positively (except Cu). If skewness value is positive that it presents the average concentration of metals are lower than their median value. On the other hand, negative skewness value is means the mean metal concentration is higher than their median value. The maximum value of skewness is Pb (3.54) among all investigated elements.

Assessment of Pollution Indexes and Factors Results

To assess the pollution levels and soil classification/ categories, as well as the potential anthropogenic effects on soil samples from Karabuk, this study was calculated Igeo, EF, CF, Cd, PLI and PERI values. The results of these pollution indexes and factors were presented in Figures 2-6. As shown in Figure 2, the Igeo results indicated that the soil quality of Karabuk is severely polluted for Ni at T7 sample point. The soil is also moderately to severely polluted for Co at the T3, for Cr at the T7, for Ni at the T2, 5, 6, 24, and 61, and for Pb and Zn at T46. Moreover, the soil quality of other sample points is determined to be unpolluted, unpolluted to moderately polluted, or moderately polluted based on the Igeo results in this study.

Figure 3 displays the results for the enrichment factor (EF) assessment for Karabuk soil samples. The EF values for Ni at T5, T6, and T7 sampling points were found to be within the range of 5 to 20, indicating a significant enrichment of Ni concentrations. In contrast, moderate levels of enrichment were observed for Co (T3, 7, 14, 15, 25, 26, 32, 49, and 51), Cr (T5 and 7), Cu (T5, 29, 48 and 54), Ni (T2-4, 18, 24-27, 29, 30, 32, 33, 36, 41, 48-51, 57, 60, and 61), Pb (T14, 18, 30, 34, 41, and 46), and Zn (T18, 26, 29, 30, 34, 41, 46, and 48) at their respective sampling points. On the other hand, minimal enrichment was also

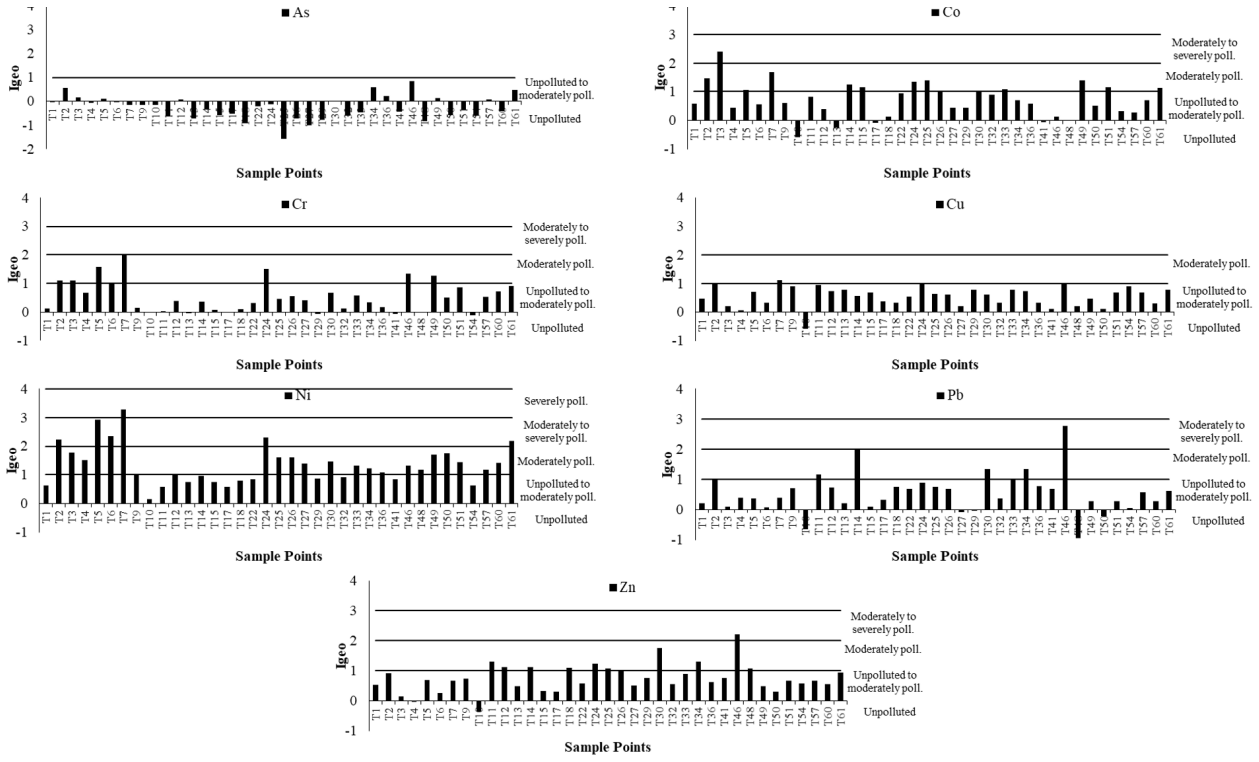

Figure 2. The Igeo values of Karabuk soil samples and soil quality

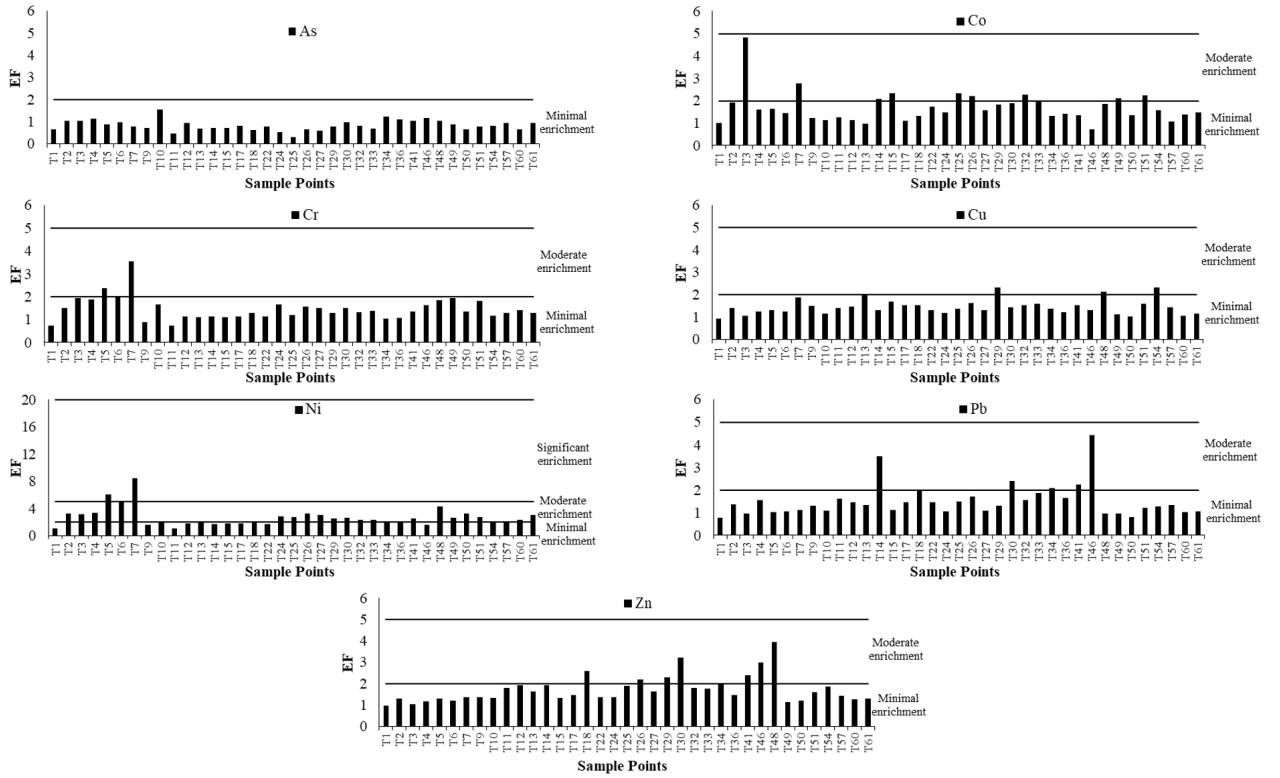

Figure 3. The EF values of Karabuk soil samples and enrichment levels

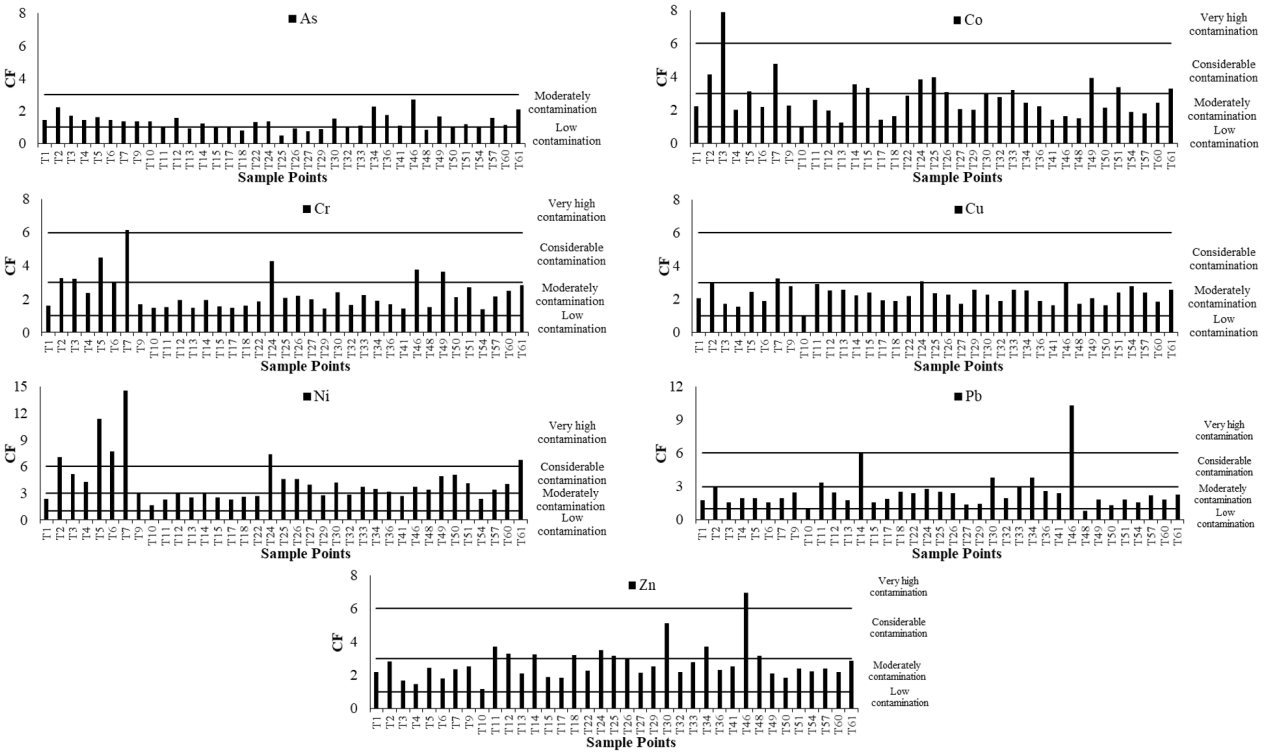

Figure 4. The CF values of Karabuk soil samples and soil contamination categories

determined at other sampling points in Karabuk soil.

Based on the results of the CF analysis presented in Figure 4, it was observed that the sample points had a high level of contamination for several metals. Specifically, the metals Co (T3), Cr (T7), Ni (T2, 5-7, 24, and 61), as well as Pb, and Zn (T46) were found to have very high contamination at the sample points that are given in the parenthesis. Furthermore, the CF analysis revealed

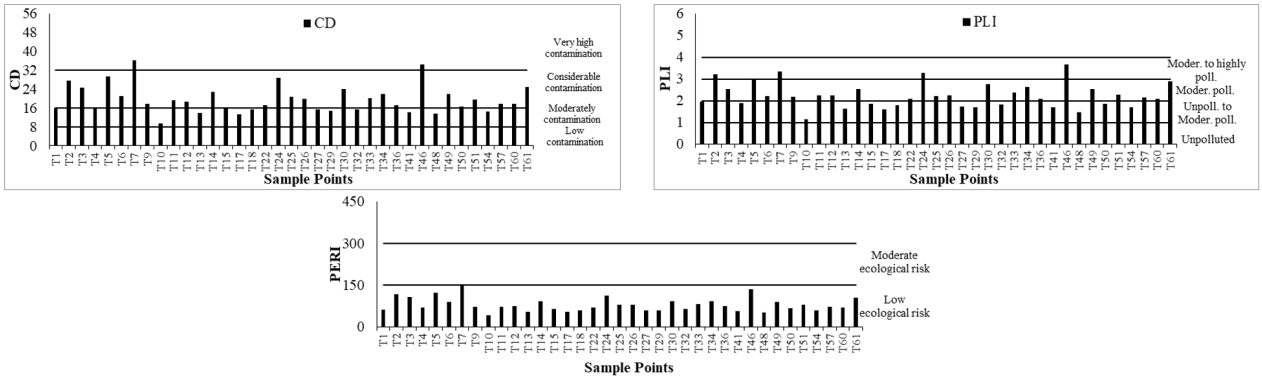

Figure 5. The CD, PLI and PERI values of Karabuk soil samples and soil categories

Table 5. Pearson's correlation matrix among the metals in Karabuk Soil Samples

	As	Co	Cr	Cu	Ni	Pb	Zn	Fe
As	1							
Co	0,190	1						
Cr	0,446**	0,564**	1					
Cu	0,300	0,293	0,464**	1				
Ni	0,275	0,500**	0,909**	0,405*	1			
Pb	0,544**	0,019	0,217	0,426**	-0,024	1		
Zn	0,412*	0,003	0,215	0,542**	0,039	0,839**	1	
Fe	0,587**	0,413**	0,549**	0,644**	0,396*	0,485**	0,464**	1

*. Correlation is significant at the 0.05 level (2-tailed).
**. Correlation is significant at the 0.01 level (2-tailed).

that moderate to considerable levels of contamination were present for the investigated metals in many sample points of the Karabuk soil samples. The very high degree and considerable degree contamination level of CF values observed in the Karabuk soil indicate a high level of contamination and suggest a higher risk of exposure to the corresponding metals. These findings suggest that the anthropogenic sources of these metals are a cause for concern.

According to the Cd values calculated by summing the CFs in Figure 5, it was found that high and considerable levels of contamination were present at all sample points except for 12 sample points (T1, 10, 13, 15,17, 18, 27, 29, 32, 41, 48, and 54). This indicates that the majority of the sample points were heavily polluted with anthropogenic sources. In addition, very high pollution was found at T7 and 46 sample points due to Cd results in Figure 5. The sample points of T2,7, 24 and 46 were found moderately polluted to highly polluted soil according to PLI results that was given in Figure 5. The other sample points were determined moderately polluted and moderately polluted to unpolluted soil for PLI results. Lastly in Figure 5, it was seen moderate ecological risk due to PERI results for T7 sample point. On the other hand, the low moderate ecological risk was determined at the other sample points for PERI results in this study.

Statistical Analysis

Multivariate statistical techniques, such as Pearson correlation and Principal Component Analysis (PCA), were used to explore the potential sources of heavy metals in sediment samples. Correlation analysis examines the relationships between quantitative variables by calculating the Pearson correlation coefficient. Table 5 displays Pearson's correlation coefficients of the elements investigated in the Karabuk soil samples. The results revealed significant positive correlations between certain pairs of elements, such as Cr-Ni and Pb-Zn. Moderate positive correlations were also observed between As-Pb, As-Fe, Co-Cr, Co-Ni, Cr-Fe, Cu-Zn, and Cu-Fe.On the other hand, the positive correlations were relatively lower between As-Co, As-Cr, As-Cu, As-Ni, As-Zn, Co-Cu, Co-Fe, Cr-Cu, Cr-Pb, Cr-Zn, Cu-Ni, Cu-Pb, Ni-Fe, Pb-Fe, and Zn-Fe.The Pearson's correlation coefficients results suggest that there is a significant positive correlation between Cr, Ni, Pb, and Zn, which is likely due to their common anthropogenic sources. However, the relatively lower positive correlations observed among the other pairs of elements suggest that their sources may be more diverse, including both natural and anthropogenic sources. Overall, the use of Pearson's correlation coefficients proved to be a useful tool in identifying the sources of the metals found in the soil samples. The results obtained help to shed light on the possible origins of the contaminants and aid in the development of strategies for mitigating their impact on the environment and human health.

PCA was conducted on the metal concentrations in the sediments to better understand the grouping of metals in Karabuk soil samples originating from the same source.

The validity of PCA was assessed using the Kaiser-Meyer-Olkin test and Bartlett's test. Principal components (PCs) with eigenvalues greater than one were deemed relevant (Vasiliu et al., 2020; Yang et al., 2014). To determine the contribution of elements to a specific group, components with factor loadings > 0.6, 0.4–0.6, and 0.3–0.4 were classified as highly, moderately, or weakly associated with elements in that group, respectively. Similar classification approaches have been employed in related studies focused on identifying sources of heavy metals in soil samples (Vasiliu et al., 2020; Maina et al., 2019; Javed et al., 2018). The results of the analysis, conducted using IBM SPSS 21.0, were presented in Table 6 and include eigenvalues and commonalities. Two eigenvalues were found to be higher than 1, with the two factors accounting for 72.043% of the total variance. In each factor column, values exceeding 0.6 were identified and highlighted in bold. The first factor, which explains 44.084% of the total variance, is heavily loaded with As, Cu, Pb, Zn, and Fe, suggesting that these metals share common sources. Co, Cr, and Ni, on the other hand, were appeared in the second factor, and indicated that they come from the same originate, with second factor explaining 23.960% of the total variance.

Table 6. The rotated component matrix for data Karabuk soil

Elements	Component		Communalities
	1	2	
As	0.650	0.301	0.513
Co	0.021	0.756	0.572
Cr	0.262	0.899	0.877
Cu	0.621	0.433	0.572
Ni	0.039	0.914	0.837
Pb	0.923	-0.086	0.859
Zn	0.906	-0.063	0.825
Fe	0.664	0.517	0.708
Eigenvalue	3.847	1.917	
% of variance	48.084	23.960	
% of cumulative	48.084	72.043	

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization. Rotation converged in 6 iterations.

CONCLUSIONS

In this study, an assessment of metal pollution (including As, Co, Cr, Cu, Ni, Pb, and Zn) in soil samples from Karabuk was presented using various pollution indices and factors such as Igeo, EF, CF, Cd, and PERI. The concentration of Fe was used as a reference metal for calculating the pollution indices. All the metal concentrations in the Karabuk soil samples were obtained from an open-source report by the Turkish Atomic Energy Authority (Türkiye Atom Enerjisi Kurumu – TAEK) in 2015. Thirty-seven sample points were used for the assessment of pollution indices and factors in this study.

The results of EF, CF, and Cd showed that there was moderate to high pollution at many sample points in Karabuk soil. Nickel (Ni) enrichment in soil was significant level at several sample points according to EF analysis. CF results showed very high contamination for Co, Cr, Ni, as well as Pb and Zn at the similar investigated sample points. Additionally, based on Cd values, high and considerable levels of contamination were present at all sample points except for 12 sample points. Moreover, the PLI results indicated moderate to highly polluted conditions at T2, T7, T24, and T46, and moderately potential ecological risk at T7 according to PERI results. The pollution near industrial facilities, such as iron and steel factories, and steel production works, particularly at T2-7, was likely due to anthropogenic activities. Pollution at other sample points may have been caused by atmospheric transportation. These findings highlight concerns regarding anthropogenic sources of metals in the Karabuk soil, especially near industrial facilities. Statistical analysis, including Pearson's correlation coefficients and principal component analysis (PCA), was employed to determine the sources of metals in Karabuk soil samples. Metals were classified into anthropogenic and natural categories. The study identified a significant positive correlation between Cr, Ni, Pb, and Zn, suggesting their anthropogenic origin. PCA revealed two factors explaining 72.043% of the total variance. Factor 1 included As, Cu, Pb, Zn, and Fe, while Factor 2 comprised Co, Cr, and Ni, commonly associated with anthropogenic sources. Overall, statistical analysis effectively determined that certain metals, particularly those linked to industrial activities, originated from anthropogenic sources. PCA provided additional insights into the contamination sources in the soil samples. According to the results obtained in this study, it was observed that there is pollution in Karabuk soil due to anthropogenic activities. However, due to limited available data on atmospheric transport, a detailed analysis could not be conducted within the scope of this study. Long-term monitoring studies are recommended to observe the seasonal variations of the pollution caused by the identified elements that pose risks to the environment and human health. Furthermore, it is advisable to develop sector-specific recommendations for reducing pollution at its source. These suggestions can guide future studies and efforts aimed at monitoring pollution and implementing measures to mitigate its effects.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

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

Author contribution

The contribution of the authors to the present study is equal.

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

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Biosynthesis of silver nanoparticles from *Arum dioscoridis* plant leaf aqueous extract: anticancer and antimicrobial properties

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Abstract

This study was carried out to synthesize silver nanoparticles (AgNPs) from *Arum dioscoridis* (AD) leaf extract and to investigate the cytotoxic and antipathogenic effects of them. The plant material had a reducing and stabilizing effect on the synthesized nanomaterial. During the plant-mediated synthesis of nanomaterials, no substances that would cause environmental pollution were used. For the structural characterization of AD-AgNPs, Ultraviolet-visible (UV-vis) Spectroscopy, Field Emission Scanning Electron Microscopy (FE-SEM), Electron Dispersive X-ray (EDX) Spectroscopy, Fourier Transform Infrared (FT-IR) Spectroscopy, Transmission Electron Microscopy (TEM), X-ray Diffractometer (XRD), Atomic Force Microscopy (AFM) and Zetasizer analyses were performed. The produced AgNPs showed maximum surface plasmon resonance at 431.67 nm and had mostly spherical morphology. The zeta potential value of the nanomaterial was -9.76 mV and the average powder crystal size was 31.48 nm. The minimum inhibitory concentration (MIC) values (mg/L) of AD-AgNPs on *Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa*, and *Candida albicans* were 0.25, 2.00, 0.125, 4.00, and 1.00, respectively. After 24 and 48 hours of application by MTT [3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolium bromid] assay, the half-maximal inhibitory concentrations (IC_{50}: µg/mL) of AD-AgNPs on human colon adenocarcinoma cell (CACO-2), human breast cancer cell (MCF-7), glioblastoma multiforme cell (T98-G), and healthy human umbilical vein endothelial cell (HUVEC) lines were determined as 2.977, 2.801, 5.694, 4.392; 2.115, 2.300, 2.612, 4.091, respectively.

Keywords: AgNPs, *Arum dioscoridis* extract, Cytotoxicity, Green chemistry, Pathogen microorganism

INTRODUCTION

Plant-based metallic nanoparticles (NPs) synthesis has risen dramatically over the last few decades (Song and Kim, 2009; Philip, 2011; Kanniah et al., 2020). Metallic NPs are more advantageous than bulk materials due to their unique size (1-100 nm), morphology, and surface distribution, as well as optoelectronic, physicochemical, and magnetic attributes (Fahmy et al., 2019; Jamkhande et al., 2019). These NPs have also been evaluated in recent years for usage in water treatment, diagnostic, cancer therapy, cell labeling, antimicrobial agent, drug delivery, and biomarker applications (Dikshit et al., 2021; Salem and Fouda, 2021; Singh et al., 2018).

The NPs are produced according to chemical, physical, and biological perspectives (Zhang et al., 2016). In a physical perspective, various methods such as microwave irradiation, ultra-sonication, and physical adsorption are used to synthesize NPs.

In a chemical perspective, decreasing agents like sodium citrate, elemental hydrogen, cetyltrimethylammonium bromide (CTAB), ascorbate, sodium hydroxide (NaOH), sodium borohydride (NaBH4), polyol process, N,N-dimethylformamide (DMF), and tollens reagent are used. Since the aforementioned approaches necessitate the use of environmentally harmful toxic chemicals or non-biodegradable agents (Gour and Jain, 2019; Heuer-Jungemann et al., 2019), scientists have adopted to produce nanoparticles by biological methods, also called "green synthesis" (Baran et al., 2022; Gur et al., 2022; Keskin et al., 2022).

Not only plants (Palithya et al., 2021), but also natural resources such as bacteria (Patil et al., 2019), fungi (Abdelkader et al., 2022), algae (Chaudhary et al., 2020), and seaweeds (Dixit et al., 2019) are frequently utilized in the biological generation of NPs. In recent years, herbal nanoparticle synthesis studies with silver metal have attracted attention (Alkhulaifi et al., 2020; Baran et al., 2021; Zubair et al., 2022). As is common knowledge, silver (Ag) plays an important role in limiting bacterial growth. Because Ag ions can interrupt DNA duplication and cell division. On the other hand, AgNPs attach to cell membrane proteins thanks to their ultra-mini dimension and trigger the manufacturing of reactive oxygen species (ROS) in the bacteria. This condition creates oxidative stress, which leads to cell death (Baran et al., 2022).

Arum dioscoridis, one of the 26 species belonging to the genus *Arum* L. of the Araceae family, is disseminated in Western Asia, North Africa, Europe, and the Mediterranean region. The distribution area of this plant, which grows wild in the natural environment, in Türkiye, is Eastern Mediterranean and Southwestern Anatolia (Yabalak, 2018). *A. dioscoridis*, which is called "Yılanyastığı" and "Gavur Pancarı" in Türkiye, is used in making "Tirşik soup" and the "pastry" in Kahramanmaraş, Kilis, Osmaniye, and Adana provincials (Çeçen et al., 2020; Sökmen et al., 2023). Since the leaves of this plant contain toxic alkaloids and can cause food poisoning when consumed without cooking, it is not considered suitable to be consumed raw (Kozuharova et al., 2020).

This work was fulfilled to synthesize AgNPs from AD leaf extract and to research the cytotoxic and antipathogenic effects of them. In the research, in which no environmentally harmful substances were used, the aqueous synthesis and stabilization of AgNPs were carried out by reducing silver nitrate ($AgNO_3$) aqueous solution with *A. dioscoridis* leaf extract. After the structural characterization of the obtained AgNPs, their cytotoxic effects in some cell lines as well as their antipathogenic activities against pathogenic yeast and bacteria were revealed.

MATERIALS AND METHODS

Material

Arum dioscoridis leaves obtained from Alatepe Village in Kilis (Türkiye) were used in the study. GPS location: 36°47'13" N, 37°11'57" D. The plant was diagnosed by Dr. Cumali Keskin, a plant taxonomist from Mardin Artuklu University (Mardin, Türkiye). $AgNO_3$ (99.8% purity), vancomycin, fluconazole, penicillin, streptomycin, colistin, fetal bovine serum (FBS), Mueller Hinton Broth (M-H Broth), and Roswell Park Memorial Institute-1640 (RPMI-1640) were obtained from Sigma-Aldrich (Germany). Dulbecco's modified eagle medium (DMEM) was acquired from Gibco (UK). [3-(4,5-dimetiltiazol-2-il)-2,5-difeniltetrazolium bromid] (MTT) was purchased from Thermo Fisher Scientific (USA). *Candida albicans* (ATCC® 10231™), *Staphylococcus aureus* (ATCC® 29213™), *Escherichia coli* (ATCC® 25922™), *Pseudomonas aeruginosa* (ATCC® 27853™), and *Bacillus subtilis* (ATCC® 11774™) utilized for antipathogenic activities of the AgNPs. CACO-2, MCF-7, T98-G lines, and HUVEC were used for the cytotoxic tests of the AgNPs.

Arum dioscoridis leaf extract preparation

The green leaves of *A. dioscoridis* were cleaned with pure water and dried at room conditionss. 5 g of the dried leaves were boiled with 0.5 L of pure water and cooled. Then, it was processed via Whatman filter papers and maintained at +4 °C for the production of AgNPs.

Manufacturing of the nanoparticles

A 30 mM $AgNO_3$ aqueous solution was adjusted from solid $AgNO_3$ for the production of AgNPs. The extract and solution at a ratio of 3:2 were left to react in a beher. The dark solution resulting from the reaction was centrifuged (6000 rpm, 5 minutes). The granular portion produced at the conclusion of the centrifugation was cleaned numerous times with pure water, and the resultant leftover (AgNPs) was maintained in a furnace at 65 °C for 24 hours to dry. The dried material was preserved for characterization processes.

Characterization of the nanoparticles

UV-vis. spectrophotometer (CARY 60, Agilent, USA) spectra of the synthesized AgNPs were ascertained in the 300-800 nm wavelength range. The size, morphology, crystallite, and surface distribution of AD-AgNPs were determined by FE-SEM (Quanta, FEG240, USA), TEM (JEM-1010, JEOL, USA), XRD (RadB-DMAX II, Rigacu, Japan), EDX spectroscopy (Quanta, FEG 240, USA), and Zetasizer (Malvern, Mastersizer 3000, UK). Debye-Scherrer equation was applied to reckon the crystal size of AD-AgNPs (Hatipoğlu, 2021a). FT-IR spectroscopy (CARY 630, Agilent, USA) was utilized to recognize the functional units in the leaf extract as well as the functional units causing the decrease at the conclusion of the reaction. AFM (Park NX10, South Korea) analysis was used to ascertain the exterior topology of the produced AgNPs.

Antipathogenic activity of the nanoparticles

The microdilution technique was utilized to assess

MIC of AgNPs against *C. albicans*, *P. aeruginosa, E. coli*, *B. subtilis,* and *S. aureus*. The wells were filled with M-H Broth medium for bacteria and RPMI-1640 for yeast. The microplates containing the medium and bacteria were treated with an AgNPs solution. Each time, 100 L was drawn from these wells and transferred to the next well. The microplates were then filled with microplate solutions arranged and regulated due to 0.5 McFarland. It was incubated for a day at 36 °C. The MIC value was calculated after incubation by ascertaining the least concentration without proliferation. Moreover, the antipathogenic activities of the AgNPs on the pathogen microorganisms were evaluated using a 30 mM $AgNO_3$ solution containing the commercial antibiotics vancomycin, colistin, and fluconazole.

MTT test to assess the cell viability

The cell viability test was performed at Dicle University Veterinary Faculty Cell Culture Laboratory. RPMI-1640 medium was used for HUVEC and CACO-2 cell lines, and DMEM was used for T98-G and MCF-7. All the cell lines were incubated in T75 culture flasks at 37 °C in 5% CO_2. 10% FBS, 100 U/mL penicillin, and 100 U/mL streptomycin were supplemented to both mediums. When the cells reached 80-90% confluence, they were taken from the flasks, and their numbers were determined by the hemocytometric method. The counted cells were inoculated into 96-well plates in 90 µL of the medium, with $7.5x10^3$ cells for HUVEC, 8 $x10^3$ cells for CACO-2, $5x10^3$ cells for MCF-7 and T98-G in each well. The cell inoculations were performed in triplicate and two plates (to perform two different time treatments, 24 and 48 hours). The AgNPs synthesized at diverse doses (500, 250, 125, 62.5, 31.25, and 15.625 µg/mL) were applied to the plates that were inoculated the next day. Ultrapure water was applied to the cells in the control group. At 24 and 48 hours after the application, an MTT test was conducted in the dark to ascertain the changes in the cell viability (Irtegun Kandemir and Ipek, 2023).

RESULTS AND DISCUSSION

UV-vis analysis

After allowing the plant extract and $AgNO_3$ solution to react, the color change was detected. The UV-vis spectra of AD-AgNPs at 30-120 minutes of the reaction are shown in Figure 1. The color shift in the suspension assisted in identifying the presence of synthesized AgNPs. In other words, UV-vis revealed the manufacturing of plant-based AgNPs by converting Ag^+ ions to Ag^0 ions (Garibo et al., 2020). As is known, free electrons in AgNPs are in charge of the surface plasmon resonance (SPR) absorption band of the nanomaterial (Alias Antonysamy et al., 2017). AD-AgNPs showed maximum absorbance at 431.67 nm. It was reported that the maximum absorbance of the AgNPs based on various plants varied between 420-480 nm (Shruthi et al., 2017; Pallela et al., 2018; Mariadoss et al., 2019; Aryan et al., 2021).

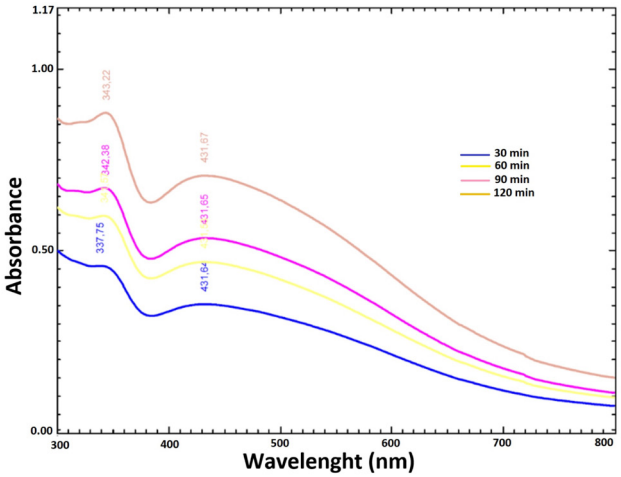

Figure 1. Time-based UV-vis absorption spectra of AD-AgNPs

EDX analysis

The EDX spectrum of AD-AgNPs verified the incidence of sharp spectral signals at nearly 3 keV for the absorption of nanocrystals in the silver area owing to surface plasmon resonance (Figure 2). This characteristic peak (3 keV) is consistent with the results of many studies with different plants (Hatipoğlu, 2022b; Ali et al., 2023; Baran et al., 2023). Other absorption peaks that emerged were due to phytochemicals in *A. dioscoridis* leaf extract. EDX analysis clearly indicated the existence of fundamental silver indicators.

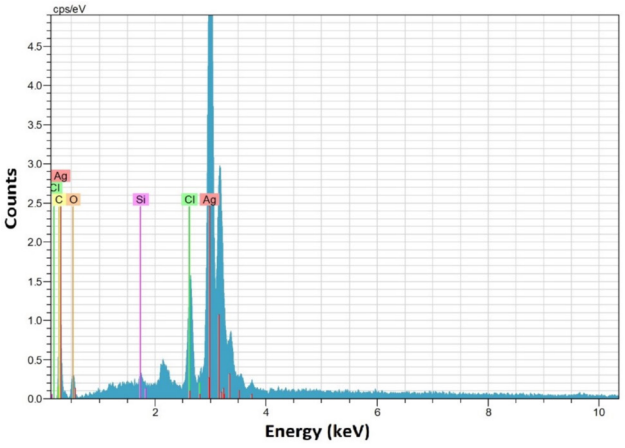

Figure 2. EDX spectrum of AD-AgNPs

FE-SEM and TEM analysis

FE-SEM (Figure 3), and TEM (Figure 4) were used to examine the shapes and sizes of biogenic AgNPs. The nanomaterial is found to be in spherical-shaped clusters that are not in direct touch with each other, especially in the TEM images. This shows that AD-AgNPs have stabilized. The results accord with those of almond (Aktepe and Baran, 2021), *Campsis radicans* and *Cascabela thevetia* (Tufail et al., 2022), *Cicer arietinum* (Baran et al., 2022), *Mimusops coriacea*, (Lopes and Courrol, 2020), *Prunus cerasifera pissardii nigra*, (Hatipoğlu, 2022a), and

Sida cordifolia (Pallela et al., 2018) plants.

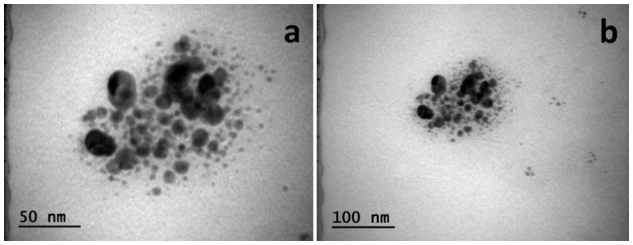

Figure 3. FE-SEM micrograph of AD-AgNPs at 1 µm scale

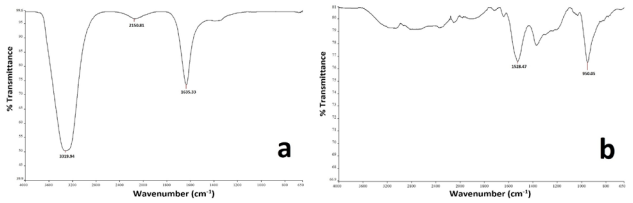

Figure 4. TEM micrographs of AD-AgNPs at 50 nm (a) and 100 nm (b) scale

FT-IR analysis

To determine possible biomolecules contained in *A. dioscoridis* leaf extract that was in charge of the decrease and stability of AgNPs, FT-IR analyses of biosynthesized AgNPs were performed. Absorption bands were observed at 1528.47 and 950.05 cm^{-1}, as seen in the FT-IR spectra (Figure 5). According to the FT-IR data, the peak at 950.05 cm^{-1} reveals the existence of metallic bonding. In addition, it can be said that the peak at 1528 cm^{-1} attached to the aromatic ring belongs to the functional units C=C, N=N, C=N. These outcomes revealed the existence of protein in the extract along with covering agents for the stability of AD-AgNPs (Debnath et al., 2019).

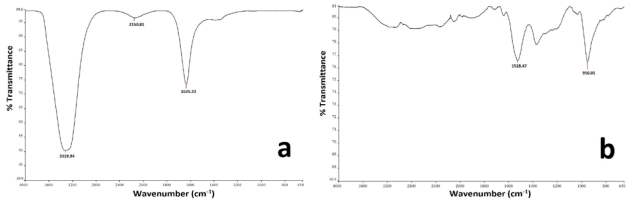

Figure 5. FT-IR spectra of AD leaf extract (a), and manufactured AD-AgNPs (b)

XRD analysis

Regarding the XRD spectrum model for the biogenic AgNPs, 111^0, 200^0, 220^0, and 311^0 diffraction peaks coinciding with 38.37, 44.28, 64.23, and 77.49 at 2θ represent the spherical crystal construction of silver (Figure 6).

Metallic silver ions are face-centered cubic, as seen by the peaks. Several investigations indicated that these diffraction peaks corresponded to the silver ion (Yusof et al., 2018; Hatipoğlu, 2021a; Khan et al., 2021; Naghmachi et al., 2022). The top angle was assessed to be 44.28 and the mean dimension of the nanomaterial was computed as 31.48 nm.

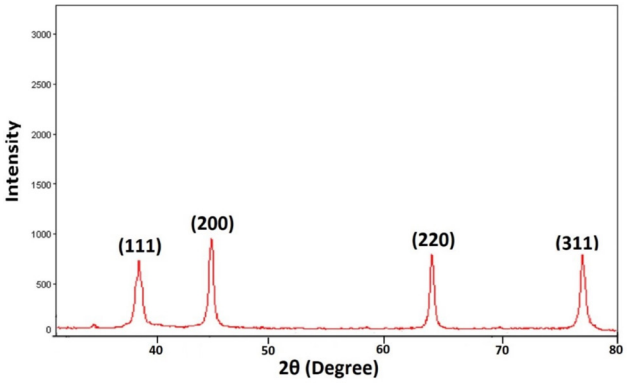

Figure 6. XRD pattern of AD-AgNPs

AFM analysis

Three-dimensional morphology and dimensions of biogenic AgNPs were obtained with the help of AFM (Figure 7). AFM analysis indicated that most of the produced AgNPs were monodisperse and spherical in form. The agglomeration and dissolution model of biogenic AgNPs in this study by AFM analysis was also well supported in the research of Nayak et al. (2020), Atalar et al. (2021), and Basavarajappa et al. (2022).

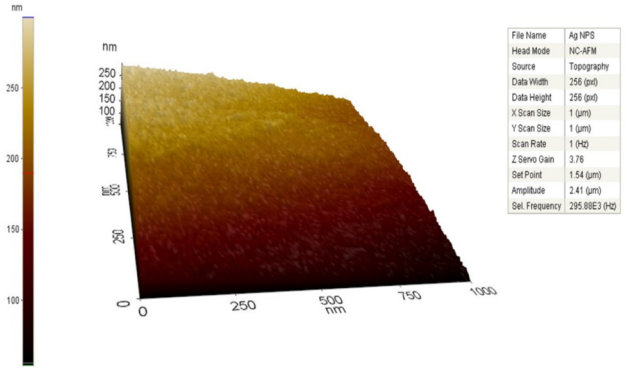

Figure 7. AFM results of AD-AgNPs

Zeta analysis

It is seen in Figure 8 that the mean particle dimension of the manufactured AgNPs ranges between 10-150 nm. The nanomaterial's zeta potential was determined to be -9.76 mV. (Figure 9). As it is known, the upper negative value of the zeta potential reveals the steadiness of AgNPs and their good distribution without clustering (Baran et al., 2022). This conclusion is consistent with the activity of AgNPs generated from other plant leaves by other investigators (Paosen et al., 2017; Aryan et al., 2021).

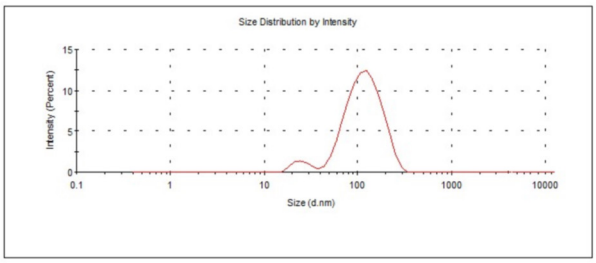

Figure 8. Particle size distribution of AD-AgNPs by density

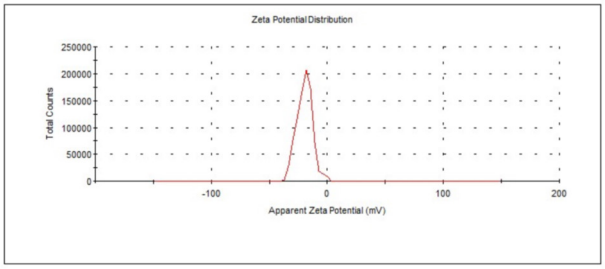

Figure 9. Zeta potential of AD-AgNPs

Antipathogenic activity

Antibiotic resistance has begun to spread around the world in recent years. For this reason, the interest in AgNPs as a substitute for antibiotics is increasing day by day. In the research, the AgNPs were found to decrease yeast and bacterial growth even at extremely low concentrations when compared to traditional antibiotics (Table 1). As compared to other species, AD-AgNPs were discovered to be far more effective towards *S. aureus* and *B. subtilis*. Because Gram-positive bacteria have a tougher polysaccharide coating than Gram-negative ones, their inhibitory effects on AgNPs are greater (Tamboli and Lee, 2013). Various research were revealed that AgNPs had inhibitory effects on pathogenic microorganisms (Hatipoğlu, 2021b; Vanlalveni et al., 2021; Basavarajappa et al., 2022; Younas et al., 2023).

Table 1. Minimum inhibitory concentration results (mg/L) of AD-AgNPs, $AgNO_3$, and standard antibiotics

Microorganisms	AgNPs	$AgNO_3$	Antibiotics
S. aureus	0.250	2.650	2.000
B. subtilis	0.125	1.320	1.000
E. coli	2.000	0.660	2.000
P. aeruginosa	4.000	0.660	2.000
C. albicans	1.000	0.660	2.000

Cytotoxic effects

The MTT assay results showing the effects of AD-AgNPs on HUVEC, CACO-2, MCF-7, and T98-G cell lines utilized in the study are presented in Figures 10, and 11. It was determined that the AD-AgNP had an antiproliferative impact on HUVEC, CACO-2, MCF-7, and T98-G cells. 24 hours after the application, IC_{50} values (µg/mL) in HUVEC, CACO-2, MCF-7, and T98-G cells were found as 2.977, 2.801, 5.694, and 4.392, respectively. As a result of the 48-hour application, these values were ascertained as 2.115, 2.300, 2.612, and 4.091, respectively.

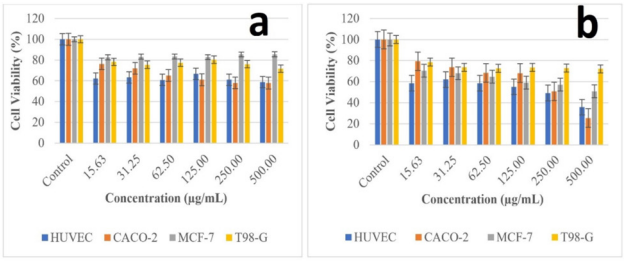

Figure 10. The cell viability percentages of AD-AgNPs at 24 (a), and 48 (b) hours

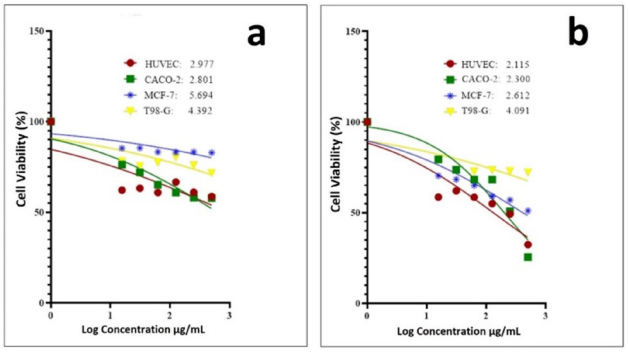

Figure 11. IC_{50} values of AD-AgNPs at 24 (a), and 48 (b) hours

Many researchers tested the silver nanomaterials they synthesized from different biomaterials on cancer cell lines. In these studies, it was reported that the antiproliferative effect levels of biogenic AgNPs (µg/mL) were 5.12-58.00 for MCF-7 (Hamouda et al., 2019; Mariadoss et al., 2019; Hamida et al., 2020), 5.37-6.20 for HCT-116 (human colon cancer cell) (Hamouda et al., 2019; Abu-Dief et al., 2020), and 5.00-90.00 for CACO-2 (Buttacavoli et al., 2018; Hamida et al., 2020; Zein et al., 2020). The IC_{50} values of the cancer cell lines used in this investigation seem to be less than the values reported by the other researchers. In addition, various researchers reported that AgNPs had high cytotoxic effects against lung adenocarcinoma cells (A549), skin cancer cells (A431), and HepG2 cell lines (Nayak et al., 2015; Wang et al., 2016; Donga and Chanda, 2021; Farshori et al., 2022; Naveed et al., 2022). Different cytotoxic effects and concentrations in the studies can be attributed to AgNPs being synthesized from different plants or having different sizes and morphological features.

CONCLUSION

This research proved the ecologically friendly, simple, and low-cost production of AgNPs from *A. dioscoridis* aqueous leaf extract. The sizes, morphological structures, and surface distributions of the manufactured AgNPs were ascertained. The impacts of the phytochemicals in the plant extract in decrease and closure (stabilization)

were proven. The biogenic AgNPs were found to have strong inhibitory impacts on *S. aureus, P. aeruginosa, C. albicans*, *E. coli*, and *B. subtilis*. In other words, it was understood that the synthesized nanomaterial had the potential to be used instead of antibiotics. The antipathogenic activity of AgNPs may be considerably enhanced through their synergistic interactions with antibiotics. AD-AgNPs were shown to decrease the growth of CACO-2, MCF-7, and T98-G cells while causing toxicity in healthy HUVEC cells.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

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

Author contribution

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

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Biotechnological potential of apple pomace for value-added products

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Abstract
Agri-food processing waste and by-products are important to be valued in an integral unit to the main process. This study focused on showing the potential valorization of apple pomace as substrate towards valuable products by a biotechnological mean. Apple pomace was fermented by *B.subtilis* at 37 °C, 150 rpm, and 72 h. Reducing sugars, total phenol content and α-amylase activity were followed throughout the fermentation. The results showed that *B.subtilis* assimilated apple pomace sugars and stimulated the release of sugars into the medium during fermentation. α-amylase activity detected in the medium also indicated the degradation of pomace by *B. subtilis*. However, the total phenol content was found to be low. The α-amylase activity at 24th h was 29.6% higher when the fermentation initiated with a former fermentation medium than that of started with the inoculum based on agar and liquid incubation media. Overall results showed –for the first time- that apple pomace can be valued towards α-amylase activity, reducing sugar and total phenol content by the activity of *B.subtilis* cells.

Keywords: Apple pomace valorization, Fermentation, *Bacillus subtilis*, α-amylase enzyme

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INTRODUCTION

Apple is among the most consumed fruits and the annual world production of apples was reported as 83.1 million metric tons in 2017. About one third of the world total production of apple is used in the production of juice, wine, jams and dried products**;** and 65% of the total amount of processed apple is juiced (Lyu et al., 2020) Apple pomace is the waste product of the juice processing plants and huge amounts of apple pomace are generated worldwide every year. Most part of the apple pomace is disposed of through storage and incineration processes whereas a fraction of it is used as livestock feed. On the other hand, its composition rich in carbohydrates with some minerals, proteins, and vitamins provides it to be used as a low-cost substrate in microbial conversions. The simple sugars in apple pomace are mainly glucose (22.7%), fructose (23.6%) and galactose (6% to 15%) (Lyu et al., 2020). Several bacteria, yeasts, and fungi have been used for the production of enzymes, ethanol, biopolymers, fatty acids, polysaccharides, and organic acids from apple pomace. β-Glucosidase by *Aspergillus foetidus*, lignocellulolytic enzymes by *Candida utilis*, pectin methylesterase by *Aspergillus niger*, pectolytic enzymes by *A. niger*, polygalacturonase by *Lentinus edodes* and pectinase by *Polyporus squamosus* are some examples for fermentation enzymes of apple pomace (Kosseva, 2011). Cellulase production (Sun et al., 2010), extraction of phenolic antioxidants (Ajila et al., 2011), production of lignocellulosic enzymes

(Gassara et al., 2012), feed additive studies such using fermented apple pulp on animal development (Ajila et al., 2015) are other examples for the fermentation studies for microbial evaluation of apple pomace.

The genus *Bacillus* is among the most important microorganisms used in industrial processes due to its high capacity of secreting enzymes such as protease, α-amylase, β-glucanase and lipolytic enzymes (Su et al., 2020). The literature reports some fermentation studies by *Bacillus* strains that use apple pomace as an agro-waste for value-added products. A fibrinolytic protease enzyme was produced by solid phase fermentation on different vegetable solid substrates including apple pulp by using a *Bacillus cereus* strain that they isolated and mutated (Venkata et al., 2014). Alkaliphilic *Bacillus subtilis* with genetic modifications was used in the fermentation of apple pomace hydrolysate for 2,3-butanediol production (Bialkowska et al., 2016). The production of polyhydroxyalkanate (PHA) was studied with different *Bacillus* species using various herbal wastes including apple pulp (Kumar et al., 2016). An amylase production by solid phase fermentation was studied with a *Bacillus thuringiensis* strain isolated in environments containing three different herbal solid substrates including apple pulp (Rana et al., 2017). Pectinase production was optimized by a co-culture of *B. subtilis* and *B. pumilus* in a submerged fermentation using apple pomace as the carbon source (Kuvvet et al., 2017).

The forementioned studies have reported the production of some value-added products from apple pomace. In the present study, different from the existing literature, we aimed to explore and suggest how apple pomace can be valued in a biotechnological process rather than focus on a specific compound. In the study, only liquid phase of fermentation medium was considered. The course of two main components in apple pomace, that is reducing sugar and total phenolic compounds, during fermentation of apple pomace by a strain of *Bacillus subtilis* was monitored. The activity of α-amylase enzyme was also measured to investigate the microbial production of a starch degrading enzyme in a fermentation process of apple pomace.

MATERIALS AND METHODS

Materials and Microorganism

Apple pomace was supplied from a commercial apple juice factory and dried in a forced-air drying oven (Zhicheng ZRD-5110) at 60 ^{0}C for 16-17 h. Then, the pomace was ground and kept in a refrigerator at +4 ^{0}C. The starch content of the pomace was found approximately 16.30 % (Sulewska et al., 2014). No method was used to hydrolyze starch content of apple pomace before fermentation. The chemicals were of among the commercial brands as Merck, Sigma-Aldrich and Applichem. *Bacillus subtilis* strain was purchased from the Public Health Agency of Turkey. Gene sequence analysis of the strain was provided by Ankara University Biotechnology Institute (Sanger et al., 1977)F. & Coulson, A. R. (1975.

Culture Media and Conditions

The culture medium used for *B. subtilis* was based on tryptic soy medium (TS Broth composition: 1.7% (w/v) peptone from casein, 0.3% (w/v) peptone from soy hydrolyzate, 0.5% (w/v) NaCl, 0.25% (w/v) glucose, 0.25% (w/v) K_2HPO_4 (pH 7)). In some experiments, tryptic soy agar (TSA) and broth (TSB) were used by introducing starch or apple pomace into the medium instead of glucose and named as induced-TSA/TSB medium. All media were sterilized at 121 °C for 20 minutes in an autoclave (ALP) before fermentation. The microorganism was incubated on induced TSA at 30 °C for 16-18 h and then inoculated into 10 ml induced TSB pre-culture medium. After 24 h incubation at 30 °C and 150 rpm in an orbital shaker (Edmund Bühler SM-30), the preculture medium was transferred into 100 ml fermentation medium that contained 2.5% (w/v) dried apple pomace, 1.7% (w/v) peptone from casein, 0.3% (w/v) peptone from soy hydrolysate and 0.5% (w/v) NaCl. Fermentation conditions were 37 °C, 150 rpm, and 72 h. The samples taken off from the medium were centrifuged at 10000 rpm (Hottich Mikro 22) and the liquid phase was analyzed for reducing sugar, total phenol content (TPC) and α-amylase enzyme activity. The cell growth could not be followed as the pomace particles were present in the medium together with the cells. Instead, the decrease in reducing sugars content and an increase in α-amylase activity -that provides also released reducing sugars- were considered as the indicator of the cell growth.

Reducing Sugar Assay

The reducing sugar analysis was carried out spectrophotometrically at 575 nm (Shimadzu 1601) according to the DNS method (Miller, 1959; Sadasivam S., 2008).

Total Phenol Content

Total phenol content of fermentation medium was measured spectrophotometrically at 760 nm (Shimadzu 1601) by using the Folin-Ciocalteu method (Keskin-Šašić et al., 2012; Škerget et al., 2005). Briefly, 1000 µL of 10% Folin reagent (v/v) was added to 200 µL of sample and mixed. After the mixture was waited for 4-5 minutes at room temperature, 800 µL of 7.5% sodium carbonate solution was added. The sample was then waited in a dark place for 30 minutes. Absorbance values of samples were read against blank at 760 nm. The total phenol concentration of samples was calculated as gallic acid equivalent (GAE).

α-Amylase Enzyme Assay

Alpha-amylase enzyme activity was measured spectrophotometrically (Shimadzu 1601A) by the Sigma

Aldrich method. One unit of enzyme activity was defined as 1 mg maltose released for 3 min under pH 6.9 and 20 °C conditions ("Enzymatic Assay of α-Amylase (EC 3.2.1.1)," n.d.). Briefly, a- 500 µL / b- 700 µL / c- 1000 µL enzyme samples mixed with 1% starch solution at 20 °C. The mixtures were incubated in a water-bath and stirred at 20 °C for 3 min. 1000 µL of colour reagent was added to each mixture and mixed. After the mixtures were waited for 15 minutes in a boiling water-bath, a- 500 µL / b- 300 µL / c- 0 µL enzyme samples were added into the mixtures. The samples were waited on ice until they reached room temperature. Then, 1000 µL distilled water was added for dilution. Absorbance values of samples were read against blank at 540 nm wavelength.

Total Antioxidant Activity

Total antioxidant activity of samples was determined in terms of free radical scavenging activity on 2,2-diphenyl-1-picrylhydrazyl (DPPH) (Savatovic et al., 2009; Yen and Chen, 1995).1 mL of sample and 3 mL methanol was mixed with 1 mL of 0.3 mM DPPH solution in methanol and after 10 min in the dark at room temperature, the absorbance at 517 nm was measured (A_{sample}). The similar procedure was repeated without sample ($A_{control}$). Percent inhibition of DPPH radicals (%inh.) was then calculated according to Eq. (1):

$$\%inh. = \frac{A_{control} - A_{sample}}{A_{control}} x100 \qquad (1)$$

In this study, all experiments and analyses were carried out at least in dublicate. The standard deviations were within ±10%.

RESULTS AND DISCUSSION

Identification of the Strain

The strain used in the study was analyzed by 16S rDNA sequences method. The 16S rDNA sequencing of the isolate showed high homology (98.76%) with *B. subtilis,* thus it is classified as a variant of *B. subtilis.* Agarose gel image and DNA sequence analysis of PCR product are given in Picture 1.

Effect of Nitrogen Source on Fermentation Products

The effect of nitrogen source on the fermentation of apple pomace by *B.subtilis* was investigated in the medium containing 2.5% (w/v) dried apple pomace, 0.5% (w/v) NaCl and ammonium sulphate or urea. The cultivation medium used in these experiments was apple pomace-induced TSA/TSB medium. The fermentation carried out in TSB involving apple pomace instead of glucose was referred to the control medium. The concentration of nitrogen was adjusted to 1.63 g/L referring TSB medium in all fermentation media. (Also, it was estimated that apple pomace had no nitrogen.) Initial pH values of fermentation media were adjusted to 5.00 ± 0.30 by K_2HPO_4.

The changes in the concentrations of reducing sugar and total phenol throughout the fermentations carried out with different nitrogen sources are shown in Figures 1 and 2, respectively. The profiles of reducing sugar concentration were similar to each other in all media where there was a decrease at the beginning of the fermentation indicating the utilization of the sugars in apple pomace as substrate by the cells (Figure 1). However, the reducing sugar concentration remained constant or slightly increased with time. This shows that the releasing rate of sugars from apple pomace by the action of enzymes was higher than the rate of consuming of them by the cells after a certain period of fermentation. The variations in total phenol concentration with time were also similar in all fermentation media (Figure 2). There were no considerable changes in phenolic content of the medium with time and nitrogen source indicating that the phenolic compounds released from apple pomace at the beginning of (or before) the fermentation were not used by the cells and remained constant throughout the fermentation. This variation also showed that the cells did not contribute the release of phenolic compounds from apple pomace. The α-amylase activity of the cells in the fermentation media was measured at 24 h and 48 h and the results are given in Table 1. The enzyme activity was higher at the 24 h of fermentation. This variation was in coherent with the periods of consuming of reducing sugar by the cells and then the increase in the concentration of reducing sugar, in order. Organic nitrogen source appeared to be more effective for α-amylase secretion by *B. subtilis* in the fermentation of apple pomace. The literature also reported that the combination of organic nitrogen sources like peptone, tryptophan or yeast extract had more stimulating effect than inorganic nitrogen sources likely ammonium salts on α-amylase production by *Bacillus subtilis* (Dash et al., 2015).

Effect of Cell Inoculum Size and Type on Fermentation Products

Fermentation of apple pomace cultured with different amounts of *B.subtilis* strains -grown on starch-induced TSA/TSB medium- was carried out to investigate the effect of inoculum size on the fermentation course. The OD values of the pre-culture media were 0.32 and 1.0. In the same experimental set, the medium in which the cells previously cultured in the starch-induced preculture medum (OD value=1) then fermentation medium containing 2.5% (w/v) dried apple pomace, 0.5% (w/v) NaCl, %0.3 peptone from soy hydrolysate, 1.7% peptone from casein and then stored at +4°C for 3 months were also tested as the preculture of the fermentation (which was called former fermentation medium thereafter).

The changes in the concentrations of reducing sugar and total phenolics as well as the pH of the medium

Table 1. α-Amylase activity the 24. and 48. hours of fermentations carried out with different nitrogen sources.

Nitrogen source	α-amylase activity U/mL	
	t=24 h	t=48 h
TSB (control)	1.920 ± 0.162	1.467 ± 0.159
Urea	1.802 ± 0.083	1.787± 0.107
Ammonium sulphate	1.526 ± 0.104	1.438 ± 0.198

Table 2. α-Amylase activity at the 24. and 48. hours of fermentations carried out with different cell inoculum sizes and types.

Samples	α-amylase activity U/mL	
	t=24 h	t=48 h
Inoculum with the culture of OD=1.0	2.311 ± 0.187	2.068 ± 0.076
Inoculum with the culture of OD=0.32	2.060 ± 0.106	1.968 ± 0.076
Inoculum with former fermentation medium	2.996 ± 0.275	2.349 ± 0.148

Table 3. α-Amylase activity at the 24. and 48. hours of fermentations carried out in the presence and absence of metal ions

Samples	α-amylase activity U/mL	
	t=24 h	t=48 h
Inoculum with the culture of OD=1.0 (without metal ions-control)	2.311 ± 0.1875	2.068 ± 0.0768
Inoculum with the culture of OD=1.0 (in the presence of metal ions)	2.483 ± 0.0128	1.153 ± 0.0514

Table 4. α-Amylase activity and total antioxidant activity of the medium for the fermentation carried out under optimal conditions (ND: not determined)

Samples	α-amylase activity U/mL		Total antioxidant activity (inh.%)			
	t=24 h	t=48 h	t=0	t=24 h	t=48 h	t=72 h
average of two runs	3.022	2.075	22.72	18.88	13.21	24.64
control	ND	ND	17.31	14.51	14.96	16.06

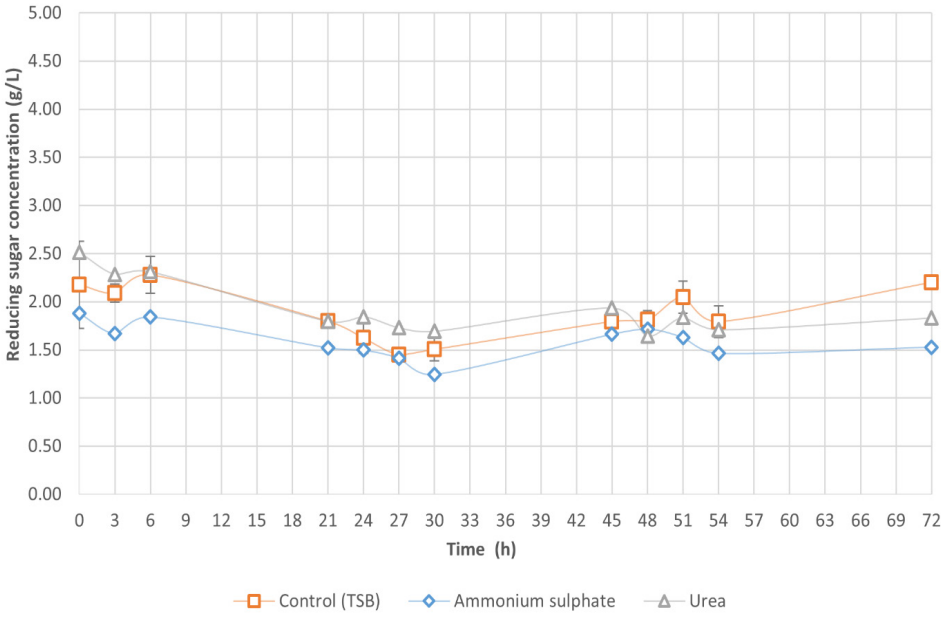


Figure 1. Effect of nitrogen source on reducing sugar concentration throughout the fermentations (T=37°C, N=150 rpm)

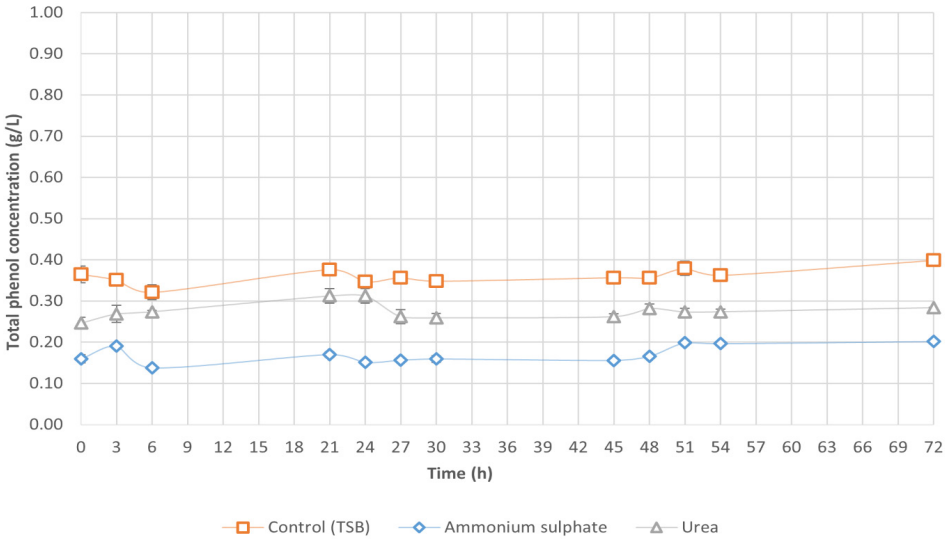


Figure 2. Effect of nitrogen source on total phenol concentration throughout the fermentations (T=37^{0}C, N=150 rpm)

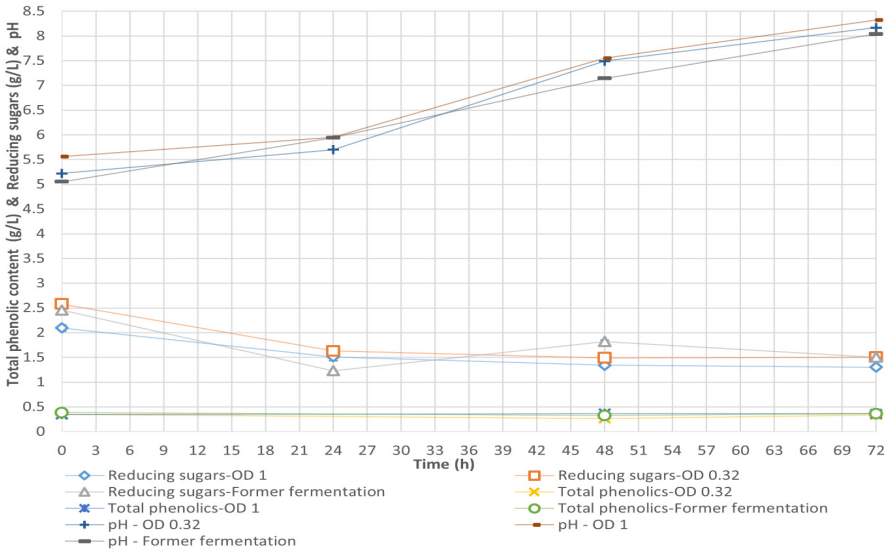


Figure 3. Effect of cell inoculum size and type on pH, concentrations of reducing sugars and total phenolics throughout the fermentations (T=37^{0}C, N=150 rpm)

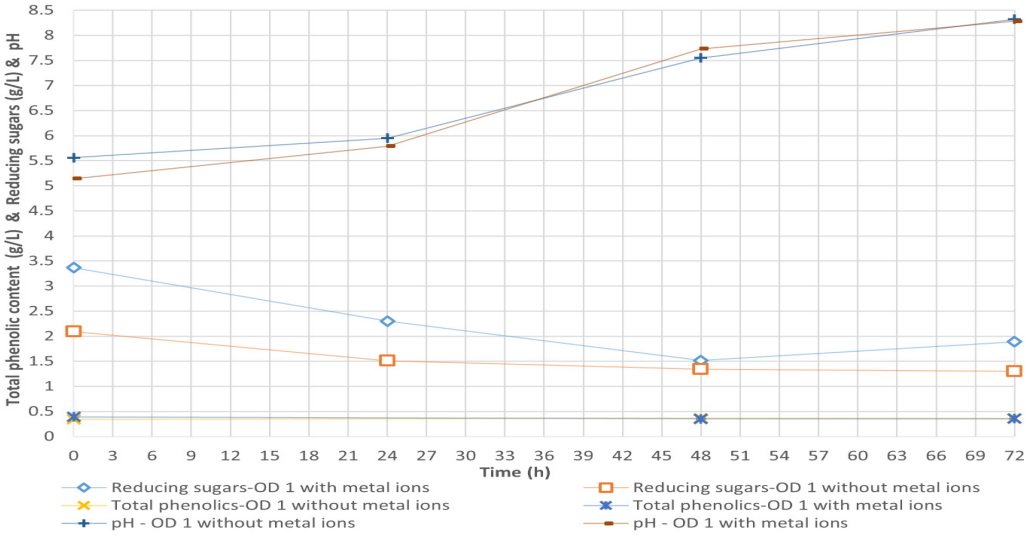


Figure 4. Effect of metal ions on pH, concentrations of reducing sugars and total phenolics throughout the fermentations (T=37^{0}C, N=150 rpm)

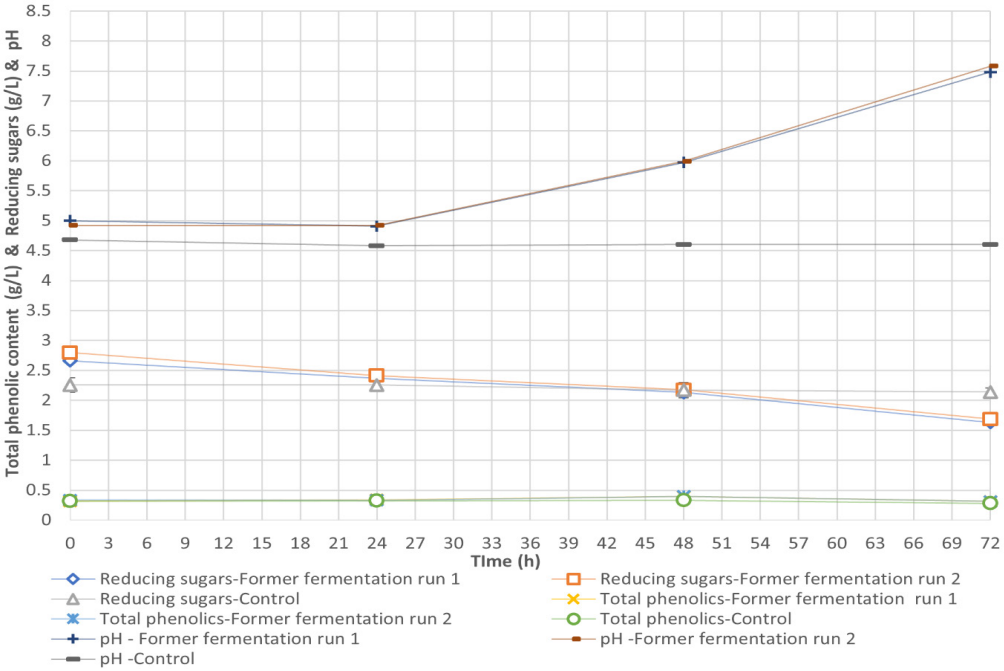
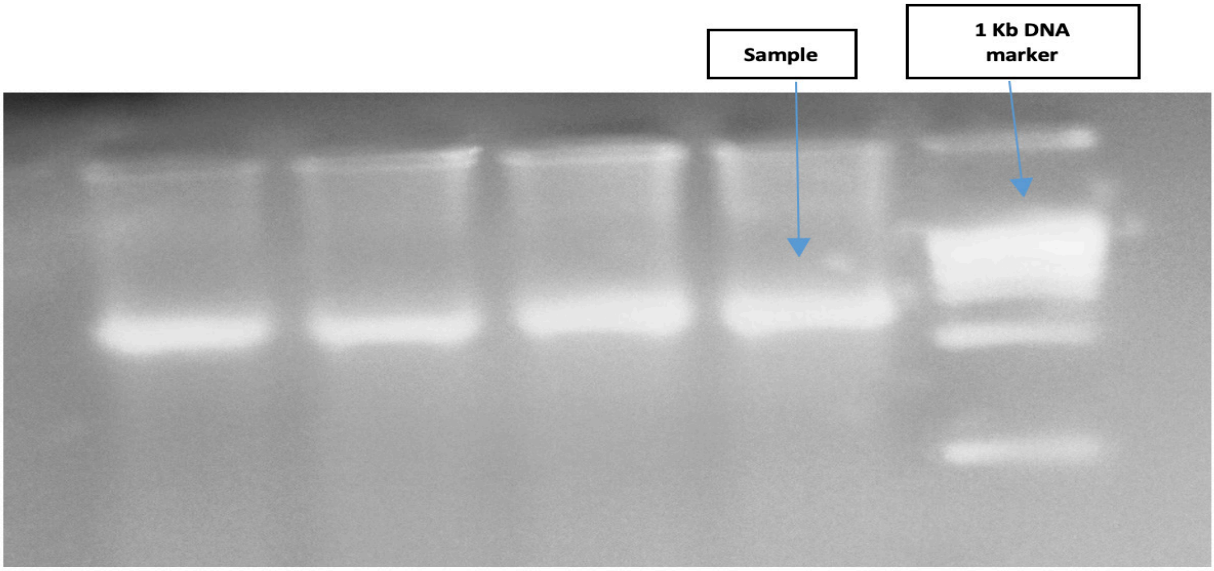


Figure 5. Comparison of fermentation course under optimal conditions with control (T=37^{0}C, N=150 rpm)



DNA sequence analysis

NNNNNGGCGCTGCTAATACATGCAAGTCGAGCGGACAGATGGGAGCTTGCTCCCTGATGTTAGCGGCGGACGGGTGAGTA
ACACGTGGGTAACCTGCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGGTTGTTTGAACCGCATG
GTTCAAACATAAAAGGTGGCTTCGGCTACCACTTACAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCAC
CAAGGCAACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGGAG
GCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCGCGTGAGTGATGAAGGTTTTCGGATCGTA
AAGCTCTGTTGTTAGGGAAGAACAAGTACCGTTCGAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCCACGGCTAAC
TACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGGGCTCGCAGGCGGTTTCT
TAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGGAACTTGAGTGCAGAAGAAGAGAG
GGAATTCNNCGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGNNTGCGACTCTCTGGTCTGTAGCTAC
CGCTGAGAAGNNAAACGTNGG

Picture 1. Agarose gel image of 16S PCR product (The sample was loaded into the gel in 4 replicates and an example of DNA sequence analysis for the product

throughout the fermentations are shown in Figure 3. The time variations of reducing sugar concentration were similar to each other in all media where there was a decrease at the very beginning of the fermentation and then remained almost constant with time. However, the rate of decrease and the following increase in reducing sugar concentration inoculated with a former fermentation medium were higher than others. The time variation of total phenol concentration during all fermentations were also similar to each other. The phenolic compounds released from apple pomace at the beginning of the fermentation were very low and did not change with time considerably. The pH of all fermentation media increased with time possibly referring the release of nitrogen metabolites to the medium.

The α-amylase activity of the cells in the fermentation media at 24 h and 48 h is given in Table 2. The enzyme activities were higher at the 24 h of the fermentations where the inoculation with a former fermentation medium was more effective for α-amylase secretion by *B. subtilis* in the fermentation of apple pomace.

Effect of Metal Ions on Fermentation Products

The effect of metal ions on the fermentation of apple pomace by *B.subtilis* strain was investigated by introducing $CaCl_2$. $2H_2O$ (0.1 g/L), $MgSO_4$. $7H_2O$ (0.1 g/L), $MnSO_4$. $4H_2O$ (0.01 g/L) and $FeSO_4$. $7H_2O$ (0.01 g/L) in the medium composed of 2.5% (w/v) dried apple pomace, 0.5% (w/v) NaCl, %0.3 peptone from soy hydrolyzate and 1.7% peptone from casein. The agar medium used in these experiments was starch-induced-TSA medium.

The changes in pH, concentrations of reducing sugar and total phenolics with fermentation time are shown in Figure 4. The fermentation course without metal ions was also shown in Figure 4 for comparison. Although there were no differences between the total phenols and pH courses with metals introduction into the medium, the rate of sugar release from the apple pomace and of consumption by the cells appeared to increase with the presence of metals.

The α-amylase activity of the cells in the fermentation media was measured at 24 h and 48 h and the results are given in Table 3. The enzyme activities of samples taken from the medium containing metal ions were slightly higher when compared to those without metal ions.

Fermentation Course Under Optimal Conditions

The abovementioned results obtained in the fermentation of apple pomace by *B. subtilis* showed the advantage of inoculation of the fermentation medium with a former fermentation medium (stored at +4 ^{0}C for 3 months). In this part of the study, the optimal apple pomace fermentation medium was used to culture *B.subtilis*. To show the repeatability of the fermentations, two runs were conducted in parallel and compared with the run without any inoculation. The fermentation medium composition was as follow: apple pomace (2.5%), peptone from casein (1.7%), peptone from soy hydrolyzate (0.3%), NaCl (0.5%).

The changes in the concentrations of reducing sugar and total phenols as well as the pH of the media throughout the fermentations are shown in Figure 5. A continuous decrease in the reducing sugar concentration with time was observed in both fermentation runs where the concentration remained constant in the control run. The decrease in sugar concentration indicated that sugars of apple pomace were utilized as substrate by the cells and that the releasing rate of sugars from pomace was lower than the rate of consumption of them. The variations in total phenol concentration with time are also similar for both fermentations where it increased up to 48 h and then decreased. The initial increase showed that the cells may contribute the release of phenolics by their enzymes. However, the decrease after a certain time indicated the loose of enzyme activities and domination of free release of phenolics from pomace over the enzymes action. The oxidation of phenolics might have also occurred. The profile of control run also showed the free release of phenolics from the apple pomace. The pH of the fermentation media increased after 24 h due to the changes in medium composition by the action of enzyme activities on the cells. The formation of alkaline compounds based ammonia might have increased the medium pH.

The α-amylase activities at the 24 h and 48 h of the fermentations are given in Table 4. Parallel fermentation runs resulted in the similar activities. The α-amylase activities were higher at the 24 h than those at 48 h. This was compatible with the change in reducing sugar concentration where it decreased at 48 h by the action of α-amylase activity to degrade starch.

Total antioxidant activity of the fermentation medium was also followed (Table 4). The control medium possessed almost the constant antioxidant activity throughout 72 h arising from the phenolic compounds present in the medium. The fermentation medium possessed more antioxidant activity compared to that of control indicating the release of phenolics by the cells at the 72 th h of fermentation. The antioxidant activity increased after 48 h where the concentration of total phenolic compounds was at its highest value.

Research Needs and Perspectives

Apple pomace has a low economical value, it is difficult to disposal; also causes pollution when it is discarded into the environment. However, valuable products can be produced in a simple fermentation medium by using this food processing chain product as shown in the present study. Phenolic compounds are useful for human and animal health, sugars are the main substrates for fermentations and enzymes produced using pomaces are cheaper than produced using synthetic medium.

To be made the fermentation medium more cheaper, urea should be preferred as the nitrogen source instead of peptone since there wasn't a significant difference in yields when peptone or urea were used. In further studies, solid biomass of fermentation medium, that is the mixture of microbial biomass and remaing pomace, can be evaluated as a feed additive or fertilizer. This study supports circular bioeconomy and sustainability by using renewable resources and eco-friendly processes for value-added products for the market.

CONCLUSIONS

The overall results of the present study showed that apple pomace can be valorized in a simple biotechnological process. It is considered that all of the outcomes have a potential for industrial usage. In the sterilized fermentation medium, the amount of sugars decreased or increased in all the experiments. Also, α-amylase activities were detected in all the experiments. These results showed that the sugars were consumed by the cells and α-amylase enzyme was started to secrete when the sugars became insufficient for the growth of the cells. *Bacillus subtilis* assimilated reducing sugars of apple pomace and secreted α-amylase enzyme, which has an important role in chemical and food industries. Phenolic compounds of apple pomace released in the first period of fermentation probably before the enzymes of *B.subtilis* started to degrade them. Therefore, by finalizing the fermentation where phenolics concentration was still high would provide the process to obtain phenolic compounds. The α-amylase activity at 24 th h was found to be 29.6% higher when the fermentation initaited with a former fermentation medium than that of started with the inoculum based on starch induced agar (Inoculum with the culture of OD=1.0 both Table 2 and Table 3) and liquid incubation media. The activity of the enzyme can be enhanced by further formulation of fermentation medium.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

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

Author contribution

This study is a part of the Master's Thesis of Sıla Sözgen. The contribution of the authors to the present study is equal. All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

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Consent for publication

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RESEARCH ARTICLE

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Comparative life history and demographic parameters of *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) on maize and oat flours

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Abstract

Ephestia kuehniella Zeller (Lepidoptera: Pyralidae), Mediterranean flour moth, is a major pest of stored food products, causing significant economic losses. Understanding the life history and population dynamics of *E. kuehniella* is crucial for developing effective pest management strategies and improving the sustainability of the food industry. In this study, we conducted an age-stage, two-sex life table analysis of *E. kuehniella* reared on maize and oat flours. The aim of the study was to compare the demographic parameters of the *E. kuehniella* populations reared on the two different types of flour including maize in laboratory conditions at 26 ± 1 °C, 60 ± 5% R.H., and a 16:8 (light: dark) photoperiod. Our findings suggest that while the flour type had a minor effect, there was no significant impact on the developmental time, survival rate, fecundity, and population growth rate of *E. kuehniella*. However, adult female longevity was significantly longer in the maize flour group compared to the oat flour group. The pre-adult survival rate was higher in the maize flour group (83%) than the oat flour group (72%). Additionally, our results indicate that fecundity of the *E. kuehniella* was slightly higher on oat flour than on maize flour, while the population growth rate was similar on both flours. Our results demonstrated that the larvae fed with maize flour consumed a significantly higher amount of flour (394 mg) than those fed with oat flour (278 mg). Furthermore, after 120 days of simulation the population growth projection of *E. kuehniella* was twice as high in oat flour compared to maize flour. These findings suggest that both maize and oat flours are susceptible to *E. kuehniella* infestation, which has important implications for the development of effective pest management strategies and the sustainability of the food industry.

Keywords: Mediterranean flour moth, Maize flour, Oat flour, Stored product pest, Life table

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INTRODUCTION

Ephestia kuehniella Zeller (Lepidoptera: Pyralidae), commonly known as Mediterranean flour moth, is an economically important insect pest of a wide range of stored food products such as grains, seeds, nuts and dried fruits (Hagstrum et al., 2013). In addition, *E. kuehniella* is one of the major pests of flourmills (Jacob and Cox, 1977) and flour is one of the most commonly infested food products with *E. kuehniella*. Larvae affect food quality and safety by contaminating stored products with feces, webbing, and cast skins, making the product unmarketable (Hill, 2003). The presence of the moth in stored food products can also result in health risks, the contamination of the product with *E. kuehniella* may be allergenic to consumers (Mäkinen-Kiljunen et al. 2001).

Ephestia kuehniella has been widely studied species due to its worldwide distribution and economic significance (Rees, 2004). In particular, the study of the life history and population dynamics of *E. kuehniella* is important for developing effective pest management strategies and minimizing economic losses in the food industry. Life tables are essential tools for understanding the ecology of pests and the demographic parameters of pests can shed light on the factors that influence its survival and reproduction, and how it adapts to different types of food sources at population level (Harcourt, 1969). Compared with traditional life table analysis, the age-stage two-sex life table can include stage differentiation and both sexes in data analysis (Chi et al. 2020). To date, some previous studies investigated the demographics of *E. kuehniella* on a variety of flours including rice, barley, maize, wheat, millet, buckwheat, sorghum, teff, and quinoa flours (Faal-Mohammad-Ali and Shishehbor, 2013; Tarlack et al. 2015; Seyedi et al., 2017; Mohammadi et al. 2020; Parra et al., 2022; Altunç et al., 2023a; Karayar, 2023).

Maize and oat flour are commonly used as ingredients in many food products, including cereal products and animal feed (Serna-Saldivar, 2012). Maize flour is particularly susceptible to infestation by *E. kuehniella*, and the pest has been found to cause significant damage to maize flour products (Hagstrum et al., 2013). However, there is limited information available on the demographic parameters and population dynamics of *E. kuehniella* in oat flour, which is also commonly used as a food ingredient. The comparison of *E. kuehniella* population dynamics in maize and oat flour can provide valuable insights into the factors that influence the population growth rate and pest management strategies.

Therefore, the aim of this study was to conduct an age-stage, two-sex life table analysis of *E. kuehniella* reared on maize and oat flours, and to compare the demographic parameters of the populations on these two different types of flour. The results of this study can provide valuable insights into the impact of these flour types on the life history and population dynamics of *E. kuehniella*, and inform the development of effective pest management strategies of *E. kuehniella* populations and for improving the sustainability of the food industry.

MATERIALS AND METHODS

Ephestia kuehniella rearing and flours

The *E. kuehniella* used in this study was obtained from a main colony that has been continuously reared for over ten years in the Laboratory of Entomology, Plant Protection Department, Ordu University. The colony was maintained using a stock colony rearing medium comprising ten parts of wheat bran, half a part of wheat flour, and a quarter of corn flour. Rearing cages, consisting of 1.2-liter plastic containers with muslin attached to the lid for aeration, were used to rear the moths. The cages were kept in a growth chamber under controlled conditions of 26 ± 1 °C, 60 ± 5% R.H., and a 16:8 (light: dark) photoperiod. Maize and oat flours procured from local markets (İngro, Konya/Türkiye) were stored in a refrigerator prior to experiments to prevent any insect infestation. The *E. kuehniella* was reared for one generation prior to experimentation to obtain the F_0 generation.

Life table experiments

After obtaining 0-24 h old adults from the F_0 generation, the moths were transferred to copulation cages and allowed for mate and oviposition during a day. The copulation cages consist of two intertwined 1.2-liter containers, with the bottom of the top container replaced with mesh to allow eggs to pass through to the bottom container. One hundred 0-24 h old eggs were obtained and individually placed in experiment cages, which were 30 cc plastic containers with 3 cm diameter holes in the lid for aeration. One gram of either maize or oat flour was added to each cage, which were then kept in the chambers under the same conditions described above for development.

After emergence, new adults (0-24 h old) were paired separately and transferred to 30 cc copulation cages. Eggs laid by each female were counted and were transferred to another 30 cc plastic cup in order to observe hatching. As new adults (0-24 h old) emerged, they were paired separately and transferred to 30 cc copulation cages. The eggs laid were counted and removed every day. In cases where both sexes did not emerge on the same day or if one of the sexes in the mating cages died, the remaining individual was mated with a one-day-old individual from the stock colony of the same flour. However, data from these individuals were not used for life table analysis. Fecundity of females were calculated form both total and hatched eggs. Observations were made at 24-hour intervals for egg hatching, longevity, and fecundity in both maize and oat flours until all individuals died. The total amount of consumed flour and pupal weights of both sexes were also measured and recorded.

Data analysis

The age-stage, two-sex life table theory (Chi et al., 2020) was utilized to evaluate the obtained data, with life table analysis performed by using the TWOSEX-MSChart computer program (Chi, 2023a). The formulae for life table parameter analysis are shown in Table 1. Means and standard errors all life table data together with the consumption and pupal weights were estimated using the 100,000 bootstrap sampling technique (Efron and Tibshirani, 1993; Huang and Chi, 2012). Comparison of data was performed using paired bootstrap tests at %5 significance level (Wei et al., 2020).

Population growth of *E. kuehniella* was projected for both maize and oat flours based on the net reproductive rate (R_0) parameter using the TIMING-MSChart computer program (Chi and Liu, 1985; Chi, 1990; Chi, 2023b). The

Table 1. Description and formulas of the life table parameters used in the study

Parameter	Description	Formula	Remarks	Reference
l_x	Age-specific survival rate	$l_x = \sum_{j=1}^{k} s_{xj}$	s_{xj} characterizes the probability that a newborn nymph will survive to age x while in stage j, and k is the number of stages	Chi and Liu (1985); Chi (1988)
m_x	Age-specific fecundity	$m_x = \frac{\sum_{j=1}^{k} s_{xj} f_{xj}}{\sum_{j=1}^{k} s_{xj}}$	f_{xj} is the age–stage specific fecundity of the individual at age x and stage j	Chi and Liu (1985); Chi (1988)
R_0	Net reproductive rate	$R_0 = \sum_{x=0}^{\infty} l_x m_x$	The total number of offspring produced by an average individual during its lifetime	Chi and Liu (1985); Chi (1988)
r	Intrinsic rate of increase	$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1$	Estimated according to the Euler–Lotka formula, with age indexed from 0 by using the iterative bisection method	Chi and Liu (1985); Chi (1988), Goodman, (1982)
λ	Finite rate of increase	$\lambda = e^r$	The increase rate per day as the stable age–stage distribution is reached	Chi and Liu (1985); Chi (1988)
T	Mean generation time	$T = \frac{\ln R_0}{r}$	T is the time required for a population to increase to R_0-times its size as the stable age distribution is reached	Chi and Liu (1985); Chi (1988)
e_{xj}	Age–stage-specific life expectancy	$e_{xj} = \sum_{i=x}^{\infty} \sum_{y=j}^{k} s'_{iy}$	s'_{iy} is the probability that an individual of age x and stage j will survive to age i and stage y	Chi (1988); Chi and Su (2006)
v_{xj}	Age–stage reproductive value	$v_{xj} = \frac{e^{r(x+1)}}{s_{xj}} \sum_{i=x}^{\infty} e^{-r(i+1)} \sum_{y=j}^{k} s'_{iy} f_{iy}$	Contribution of an individual of age x and stage j to the future population	Fisher (1958); Huang and Chi (2011); Tuan et al., (2014)

population projections were started with an initial population of 10 eggs of *E. kuehniella* and continued for a scenario of four months (120 d) storage of the flours to obtain the total population size without suppression by biotic and abiotic factors. The population growth and its variability based on the 0.025th and 0.975th percentiles of the bootstrap results of the net reproductive rate (R_0) were projected 120 days (Huang et al., 2018).

RESULTS

Developmental periods

The developmental duration of *E. kuehniella* on maize and oat flours is presented in Table 2. The average duration of the egg stage on maize flour was 4.33 ± 0.08 days, while it was 4.16 ± 0.04 days on oat flour. Similarly, the larval and pupal stages did not differ significantly between the two flour types, with mean durations of 33.85 ± 0.60 days and 15.56 ± 0.69 days, respectively, on maize flour, and 33.87 ± 0.42 days and 14.75 ± 0.56 days, respectively, on oat flour.

There were no significant differences in pre-adult (i.e. egg, larva and pupa) development durations and total longevity between the two flour types. The adult male longevity was slightly shorter on oat flour, exhibiting a mean ± SE of 14.43 ± 0.41 days, in comparison to 15.39 ± 0.94 days on maize flour; however, the difference was not statistically significant. The adult female longevity was significantly shorter on oat flour, with a mean ± SE of 8.30 ± 0.42 days, compared to 10.13 ± 0.81 days on maize flour. Male individuals were significantly live longer than female ones in maize (P = 0.00007) and oat flour (P < 0.00001) (Table 2).

The age-stage specific survival rates (s_{xj}) indicate that developmental stages of *E. kuehniella* exhibited overlapping patterns on both maize and oat flours (Figure 1). The maximum age reached by male individuals was approximately 88 and 85 days in maize and oat flours, respectively. Similarly, the maximum larvae ages were the same in both flours.

Life table parameters

The key life parameters measured for *E. kuehniella* fed on maize flour and oat flour were not significantly different, except for the oviposition day (OvD) (Table 3).

Table 2. Development duration (d) (mean ± SE) of the stages of *Ephestia kuehniella* reared on maize and oat flours

Developmental durations	*n*	Maize flour	*n*	Oat flour	*P*-value
Egg	54	4.33 ± 0.08	81	4.16 ± 0.04	0.05055
Larva	39	33.85 ± 0.60	67	33.87 ± 0.42	0.98393
Pupa	39	15.56 ± 0.69	67	14.75 ± 0.56	0.35638
Female*	16	10.13 ± 0.81	27	8.30 ± 0.42	0.04670
Male	23	15.39 ± 0.94	40	14.43 ± 0.41	0.34865
Preadult	39	53.72 ± 1.19	67	52.72 ± 0.94	0.50931
Total longevity	54	60.78 ± 1.37	81	60.28 ± 1.94	0.83551

*indicates difference is significant based on the paired bootstrap test at the 5% significance level
Standard errors (SE) were estimated with 100,000 bootstrap resamplings

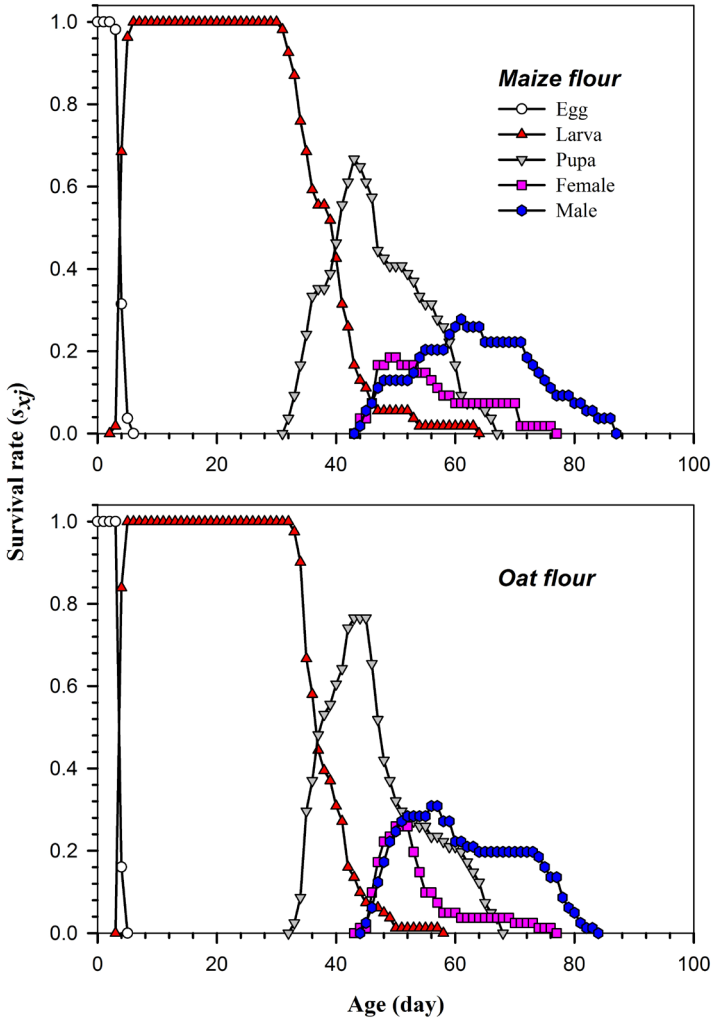

Figure 1. Age-stage survival rate (s_{xj}) of *Ephestia kuehniella* on maize and oat flours

No significant differences were observed between the two types of flour in terms of the intrinsic rate of increase (r) or finite rate of increase (λ). The mean intrinsic rate of increase was 0.0842 ± 0.0053 on maize flour and 0.0936 ± 0.0038 on oat flour. Similarly, the finite rate of increase was 1.0878 ± 0.0057 on maize flour and 1.0981 ± 0.0041 on oat flour. There were also no significant differences in the net reproductive rate (R_0), mean generation time (T), fecundity (calculated from both total eggs, F_T and hatched eggs F_H), oviposition period (OP), adult pre-oviposition period (APOP), and total pre-oviposition period (TPOP) between *E. kuehniella* reared on maize and oat flours, as shown in Table 3. However, *E. kuehniella* exhibited a significantly longer oviposition day (OvD) when fed on maize flour compared to oat flour, with a value of 7.50 ± 0.69 (Mean ± SE) on maize flour and 6.04 ± 0.28 on oat flour.

Table 3. Life table and reproduction parameters (mean ± SE) of *Ephestia kuehniella* reared on maize and oat flours

Parameters**	Maize flour	Oat flour	*P*-value
r	0.0842 ± 0.0053	0.0936 ± 0.0038	0.12865
λ	1.0878 ± 0.0057	1.0981 ± 0.0041	0.12811
R_0	99.1 ± 23.4	129.5 ± 21.1	0.33793
T	54.61 ± 1.60	51.96 ± 0.74	0.11578
F_T	334.4 ± 37.2	388.4 ± 17.5	0.18721
F_H	318.9 ± 38.3	367.8 ± 25.6	0.28567
OP	8.00 ± 4.91	6.00 ± 3.39	0.87681
OvD*	7.50 ± 0.69	6.04 ± 0.28	0.04790
APOP	1.81 ± 0.23	1.78 ± 0.35	0.93311
TPOP	53.25 ± 1.80	51.70 ± 1.19	0.47129
Pre-adult survival rate	0.83 ± 0.04	0.72 ± 0.06	0.15649

* indicates difference is significant based on the paired bootstrap test at the 5% significance level,
** r, intrinsic rate of increase (d^{-1}); λ, finite rate of increase (d^{-1}); R_0, net reproductive rate (offspring/individual); T, mean generation time (d); F_T, fecundity calculated over total eggs (eggs/female); F_H, fecundity calculated over hatched eggs; OP, oviposition period (d); OvD, oviposition days (d); APOP, adult preoviposition period (d); TPOP, total preoviposition period (d); pre-adult survival rate
Standard errors (SE) were estimated with 100,000 bootstrap resamplings.

Table 4 Weight of consumed flour (mg) and of pupae (mg) of *Ephestia kuehniella* reared on maize and oat flours

Parameter	*n*	Maize flour	*n*	Oat flour	*P*-value
Consume*	39	394.2 ± 16.5	67	278.1 ± 11.7	< 0.00001
Pupa weight	39	31.62 ± 0.46	67	30.38 ± 0.44	0.05111
Female pupa weight	17	32.74 ± 0.58	27	32.13 ± 0.69	0.50247
Male pupa weight*	22	30.75 ± 0.62	40	29.19 ± 0.48	0.01998

* indicates difference is significant based on the paired bootstrap test at the 5% significance level,
Standard errors were estimated with 100,000 bootstrap resamplings

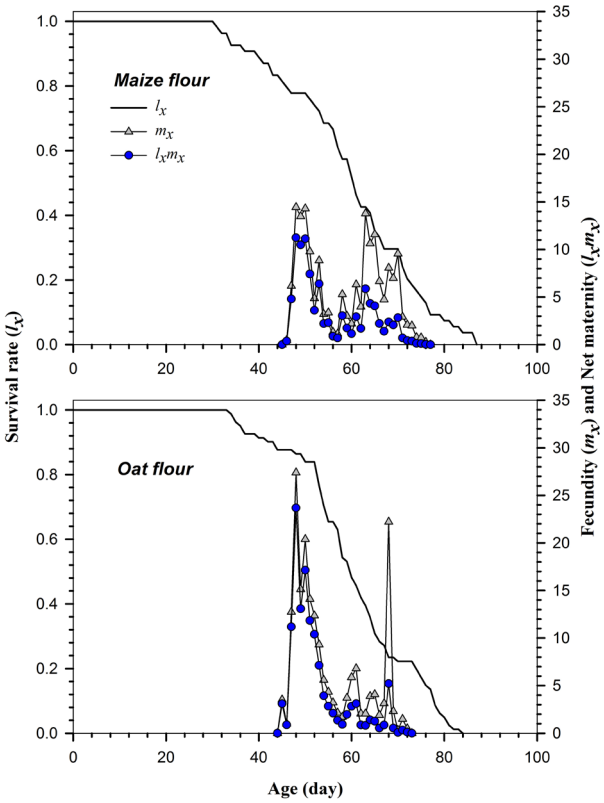

Figure 2. Age-specific survival rates (l_x), age-specific fecundity (m_x), and net maternity ($l_x m_x$) of

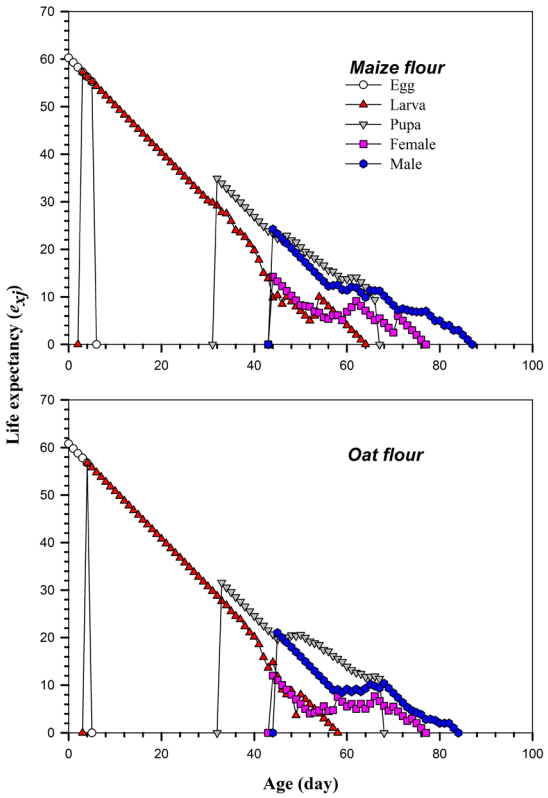

Figure 3. Age-stage-specific life expectancy (e_{xj}) of *Ephestia kuehniella* on maize and oat flours

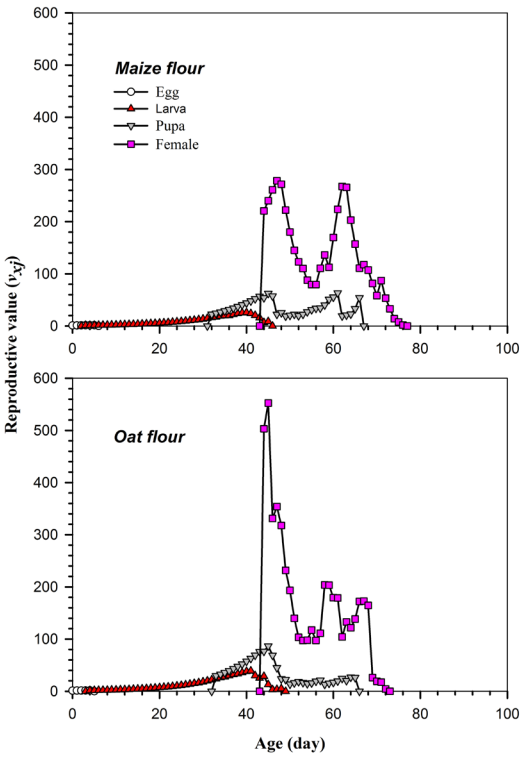

Figure 4. The age-stage specific reproductive value (v_{xj}) of *Ephestia kuehniella* on maize and oat flours

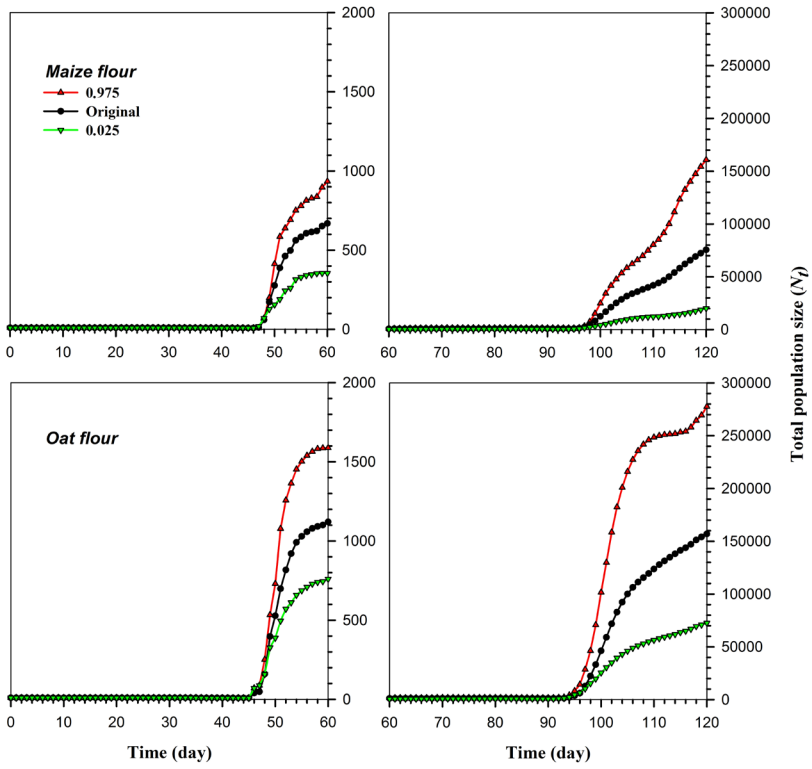

Figure 5. Total population size (N_t) after population projection of *Ephestia kuehniella* on maize and oat flours for 120 days

The age-specific survival rates (l_x) of *E. kuehniella* on both maize and oat flours showed a similar pattern of decline, beginning at 31-34 days (Figure 2). However, the maximum age-specific fecundity value (m_x) was significantly higher on oat flour (27.4 d) compared to maize flour (14.5 d). The fecundity (m_x) values exhibited fluctuations in both flours.

Ephestia kuehniella on maize and oat flours

The life expectancies (e_{xj}) for each age stage of *E. kuehniella* were also similar between maize and oat flours, with a life expectancy at birth of 60.28 and 60.78 days, respectively (Figure 3). These values represent the expected lifespan of individuals of age x and stage j after reaching age x.

The age-stage reproductive value (v_{xj}) of *E. kuehniella* on maize flour showed a peak at the later ages of adult females, while on oat flour, it gradually decreased with female age. The maximum v_{xj} value on oat flour was 552, compared to approximately half that value at 278 on maize flour (Figure 4).

Larval consumption and pupa weight

The weight of consumed flour was significantly different between the two flour types, with larvae consuming 394.2 ± 16.5 mg of maize flour and 278.1 ± 11.7 mg of oat flour. While, the weight of pupae (mixed sex) was not significantly different between the two flour types with mean weights of 31.62 ± 0.46 mg on maize flour and 30.38 ± 0.44 mg on oat flour we found difference in the male pupae of the *E. kuehniella* depend on the flour. Female and male pupa weights of *E. kuehniella* were different in both maize (P= 0.01998) and oat flours (P = 0.0005) (Table 4).

Projection of population

The total population of *E. kuehniella* over a four-month storage estimated a total population of approximately 157,000 individuals in oat flour and exceed 75,000 individuals in maize flour (Figure 5).

DISCUSSION

This study found that the life table parameters and development durations of *E. kuehniella* were mostly similar in maize and oat flours. While no previous comparison of age-stage, two-sex life table parameters of this pest reared on oat and maize flours was found in the literature. However, several studies have investigated the effect of different grain flours on demographic parameters of *E. kuehniella* (Seyedi et al. 2017; Kurtuluş et al., 2020; Mohammedi and Mehrkhou, 2020; Türkoğlu and Özpınar, 2021; Karayar, 2023). These previous studies reported variable values for life table parameters of *E. kuehniella* were diverse among the flour types. For instance, Mohammedi and Mehrkhou (2020) reported lower values for R_0 (76 offspring/female) and mean generation time T (42 d), and faster pre-adult

development (47 d) than our findings on maize flour. Pashaei et al. (2023) also reported a shorter oviposition period of 6 days, which is lower than our result. However, the age-specific fecundity (m_x) value was higher in our study (around 15 offsprings) than in other studies (around 10 offsprings). The pre-adult survival rate in this study was the same as our findings, with 83% in maize flour. Ayvaz and Karabörklü (2008) reported the longevity of female and male adults of *E. kuehniella* reared on oat and maize flours as 7.2-7.4 days and 8.3-8.4 days, respectively, which is shorter than our findings. They also reported significantly lower fecundity of 248 egg/female in oat flour and 184 egg/female in maize flour compared to our results. However, the pre-adult development duration was the same at 52 days in oat flour, while we found a longer development duration in maize flour.

In addition to the changes of the population parameters and damage caused by stored product pests depending on the commodity (Athanasiou et al. 2016; 2017), it may also be correlated with the variety of the grains (Altunç et al. 2023b).

For instance, Razmjou et al. (2022) reported significant variations in the age-stage two-sex life table parameters of *E. kuehniella* based on different maize hybrids. Similarly, Tarlack et al. (2015) found differences in the demographic parameters of *E. kuehniella* depending on the wheat flour varieties. Furthermore, Naseri and Bidar (2015) reported varying intrinsic rates of increase for *E. kuehniella* depending on barley and wheat cultivars. Although the specific varieties of the maize and oat flours used in our study are unknown, our use of standardized flours from the same brand ensured consistency in the composition of the diet. However, future studies should investigate the varietal differences in oat flour and their impact on the demographic parameters of *E. kuehniella* to better understand the effects of grain variety on the life history of this pest.

Diet may affect the qualitative and quantitative characteristics of *E. kuehniella* as well as the pupal weights (Solis et al. 2006; Moghaddassi et al. 2019; Sönmez et al. 2019; Kurtuluş et al. 2020; Türkoğlu and Özpınar, 2021). Karayar (2023), also found that pupa weight differs in *E. kuehniella* when reared on gluten free grains such as millet, buckwheat, sorghum, and tef with varying values between 25.4-30.5 mg. In barley pupal weights were found at rage of 17.1-18.6 mg and in wheat it was between 18.6-21.1 mg depending to variety of grains (Naseri and Bidar, 2015). In another study, mean pupae weight of *E. kuehniella* was 23.3 mg on maize, 17.4 mg on wheat, and 16.5 mg on barley flours (Seyedi et al. 2017). Mohammadzadeh et al. (2020) also found that the pupal weight of *E. kuehniella* is higher when reared on barley and maize compared to wheat and oat diets. We found no impact of flour type in the pupal weight of *E. keuhniella* regardless of sex. Also, our findings were higher from all those literature data with 30.4 mg in oat flour and 31.6 mg in maize flour, but this may be cause of varietal difference of the grains used in our study.

On the other hand, *E. kuehniella* is served as a host to some parasitoids and predators for mass rearing (Nielsen, 2003; Özder, 2006; Çobanoğlu et al., 2007; Yanik and Unlu, 2011), some of which are crucial for the control of stored product pests. Consequently, this pest is the subject of many biological control studies that involve rearing natural enemies, and extensive mass production of *E. kuehniella* is carried out in laboratories. Several studies have focused on optimizing the diet for time and cost-effective mass production of *Ephestia* species for finding the faster development of stages, higher fecundity, and higher pre-adult survival rates (Ayvaz and Karabörklü, 2008; Faal-Mohammad-Ali and Shishehbor, 2013; Moghaddassi et al., 2019; Nezhad et al., 2016; Pehlivan, 2021; Wang et al., 2021).

Examining our study data from a different perspective reveals that utilizing either maize or oat flour as a diet for mass rearing *E. kuehnilella* produces noteworthy outcomes. Moghadamfar et al. (2020) found that under conditions similar to ours (25°C, 50 ± 5% RH), the pre-adult development duration of *E. kuehniella* on laboratory diet consisting of wheat flour and yeast powder was around 65 days, while we observed a quicker development duration of 54 days on maize flour and 53 days on oat flour. Additionally, we observed a fecundity value of 334 and 388 eggs/female and an R_0 value of 84 and 139 offspring/female on maize and oat flours, respectively, while Moghadamfar et al. (2020) reported a fecundity value of 351 eggs/female and an R_0 value of 99 offspring/female under similar conditions. In a similar study, Seyedi et al. (2017) compared maize, wheat, and barley flours to determine the best diet for *E. kuehniella*'s biological and physiological characteristics. They found that the highest survival rate (88%) and growth index (9.8) of larvae occurred on maize flours. The authors also noted the maximum protein content and proteolytic activity in individuals fed on maize flours, two critical parameters for natural enemies' optimal development on *E. kuehniella*.

CONCLUSION

This study found that most of the life table parameters and development durations of *E. kuehniella* were similar when reared on either maize or oat flour. However, the oviposition day was significantly longer in insects fed with maize flour, and the weight of consumed flour was significantly higher in larvae fed with maize flour compared to those fed with oat flour. Furthermore, the computer simulation demonstrated that *E. kuehniella* can reach high population levels on oat flour than maize flour, highlighting the importance of effective control measures to prevent infestations and minimize crop losses. This study provides important information on the biology and population growth of *E. kuehniella* in

different types of flour, which can be useful in developing effective management strategies for this pest. In addition, the results of our study can be used as practical implications for the development of more efficient and cost-effective rearing methods for *E. kuehniella,* which is widely used effectively in the production of natural enemies, but further research is needed to determine the optimal conditions for mass rearing of *E. kuehniella* on maize and oat flours and to explore the potential for scaling up these methods to industrial-scale production.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contribution

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

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Ethics committee approval is not required.

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Not applicable.

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Not applicable.

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RESEARCH ARTICLE

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Oxalic acid: an important organic acid to increase yield and quality in lettuce

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Abstract

In the present study, the effects of different doses of oxalic acid applications on yield and quality in lettuce cultivation were evaluated. Yedikule 5107 variety was used as plant material and four different doses of oxalic acid (0-2-4-6 mM) were applied to lettuce plants from leaves. Plant weight varied between 343.02-432.57 g/plant, plant height 28.0-30.35 cm, plant diameter 26.67-28.72 cm, leaf length 21.23-22.44 cm, root collar diameter 19.11-21.49 mm and number of leaves 47.57-55.63 per plant depending on oxalic acid doses, and the highest yield was obtained from 2 mM oxalic acid application. Total chlorophyll and total phenolic contents varied between 37.47-39.31 and 67.35-103.98 mg/100g, respectively. While the highest chlorophyll value was obtained from 2 mM oxalic acid; the highest phenolic substance value was obtained from 4 mM oxalic acid application. It was determined that L^*, a^*, b^*, and C^* values varied from 47.43 to 48.76, -17.55 to -18.26, 27.89 to 28.68, and 32.95 to 34.00 depending on applications, and the highest L^* value was obtained from 2 mM oxalic acid application and the highest a^*, b^* and C^* values were observed in 2 mM and 4 mM oxalic acid applications. The antiradical values in lettuce varied between 42.36-82.64%. At the end of the study, when all these parameters were considered, it was determined that oxalic acid applications significantly and positively affect the yield and quality of lettuce.

Keywords: Oxalic acid, Lettuce, Quality, Yield

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INTRODUCTION

Lettuce (*Lactuca sativa* L.) belonging to the Compositae family is one of the cool climate vegetable species (Pink and Keane, 1993). Lettuce is among the most important and consumed types of leafy vegetables in the world (Mou, 2009). According to the data for 2021, the total lettuce production in the world is 27.011.748 tons (FAO, 2023).

Technological and scientific developments not only affect individuals' social, cultural, and economic qualities but also cause significant changes in their perspectives on life and consumption habits. Especially today, one of the areas where the understanding of being a sensitive and conscious consumer is widespread is food consumption (Altunişik et al., 2003). Instead of meeting their basic nutritional needs, people are now turning to healthy foods that can reduce their discomfort and increase the length and quality of their lives (Baslam et al., 2013).

Nowadays, the attractiveness of vegetables has increased due to their beneficial nutritional value, ease of consumption as fresh, and their entry into the group of minimally processed or fresh foods, thus leading to an increase in consumer

demand (Jiang et al., 2020). In addition, epidemiological studies have shown that there is a relationship between increased vegetable consumption and reduced risks of cancer, cardiovascular and chronic diseases (Hung et al., 2004; Pavia et al., 2006; Morris et al., 2006). The reasons why vegetables are beneficial to health are explained by the macro-micro nutrients and bioactive compounds they contain (Kris-Etherton et al., 2002; Soetan et al., 2010).

Lettuce is a type of vegetable that is rich in many vitamins, minerals, and nutritional content necessary for human health (Costa et al., 2015; Konatu et al., 2017; Lara et al., 2017). Lettuce is an important dietary vegetable because it contains a very low amount of calories and fat. It is also one of the types of vegetables rich in vitamins A, C, E, and antioxidants (Nicolle et al., 2004).

In the developing world, the population is constantly increasing and the need for food and food products is also increasing (Demirel et al., 2022). As the global population increases, the demand for food and the desire and efforts to achieve high yields in agricultural production has accelerated (Beacham et al., 2019). For this purpose, studies on different compounds that act as plant growth regulators have gained intensity in order to increase production and obtain quality products. One of the compounds that have been emphasized in recent years is oxalic acid.

Oxalic acid is naturally found in the structure of plants and is an organic acid that increases the resistance of plants to environmental stresses (Liang et al., 2009). It has been reported that it plays different roles for each organism in plants, fungi, and mammals (Shimada et al., 1997; Serna-Escolano et al., 2021). Oxalic acid, a final metabolite product of plants, has a variety of physiological effects, mostly enhancing the defense-related enzymes' activity and secondary metabolites such as phenolics to promote systemic tolerance against infections caused by bacteria, viruses, and fungi (Martinez-Esplá et al., 2014). The researchers suggested that more metabolic studies on oxalic acid should be done (García-Pastor et al., 2020).

In studies on pre-harvest oxalic acid applications in vegetables and fruits, it was determined that applications positively affected antioxidant activity, total anthocyanin, total phenolic content (Martinez-Esplá et al., 2014), fruit weight, fruit firmness, total sugar content, and color parameters (Serna-Escolano et al., 2021). It has been revealed that the post-harvest applications increase the storage life of the products and prevent quality loss (Yücel, 2005), and also increase the resistance of the fruits to post-harvest browning, ripening, and chilling damage (Yoruk, 2002; Zheng et al., 2007a, 2007b; Huang et al., 2013a, 2013b). However, when the literature is evaluated, it becomes clear that more research is needed on the effects of preharvest oxalic acid applications on vegetable production and quality characteristics. For this purpose, our study focused on the effects of oxalic acid applications on the yield and quality of lettuce.

MATERIALS AND METHODS

The study was performed under open field conditions at Isparta (Aliköy), Türkiye (37° 48′ N and 30° 38′ E, altitude 1020 m). Lettuce seedlings (cv. Yedikule) were purchased from a commercial seedling production company (Anamas Tohum Ltd. Şti., Antalya, Türkiye) and prior to planting, diammonium phosphate was applied at a rate of 200 kg/ha. Seedlings were planted on 03 September 2019 in rows 40 cm apart with an intra-row spacing of 25 cm. 17 days after planting seedlings zinc sulfate was applied at a rate of 20 kg/ha and during the growth period, ammonium sulfate was applied to 3 times (10th day and 20th day after planting) at a rate of 100 kg/ha. The soil's characteristics of the experimental area were analyzed by ISLAB Soil and Plant Analysis Laboratory and the results are presented in Table 1.

Table 1. Characteristics of the experimental area's soil

Parameters	Values	Description
EC (dS/m)	0.12	Saltless
pH	8.38	Alkali
Lime (%)	5.75	Medium
Organic Matter (%)	1.81	Low
N (ppm)	497.00	Low
P (ppm)	7.00	Low
K (ppm)	718.21	Very high
Ca (ppm)	4 900.00	High
Mg (ppm)	436.04	Medium
Fe (ppm)	1.91	Low
Cu (ppm)	1.34	Medium
Mn (ppm)	12.57	Medium
Zn (ppm)	0.31	Low

Plants were exposed to 0, 2, 4, and 6 mM oxalic acid (Merck) solutions prepared with deionized water containing 0.5% Tween 20 (Merck) as a surfactant. Foliar oxalic acid applications were made three times at 10-day intervals from seedling planting. A randomized plot design with three replications was used, and each replication consisted of 60 plants.

The temperature and relative humidity during the experimental period varied from 9.8 to 20°C and 50.1-71.6%, respectively. Table 2 shows the climatic variables for the months of September to November during the experimental period.

After plants were harvested (19.11.2019), ten plants of each replication of the experimental plot were randomly taken and the following measurements were recorded: plant weight (g per plant), plant diameter (cm), plant height (cm), root collar diameter (mm), number of leaves, dry matter (%). For determining leaf width (cm), leaf length (cm), and total soluble solid (TSS) content (brix)

Table 2. Meteorological data

	Average Temperature (°C)	Total Precipitation (mm=kg/m²)	Average Relative Humidity (%)	Average Soil Temperature (°C) 10 cm	Average Soil Temperature (°C) 20 cm
September	20	26.5	50.1	21.4	23.1
October	15.7	9.9	59.1	16.3	18.4
November	9.8	28.6	71.6	9.8	12.1

(refractometer), the 2nd and 3rd leaves of 10 lettuce plants of each replication were used.

Also, L* (Lightness), a* (Red/Green Value), b* (Blue/Yellow Value), C* (Chroma), h* (Hue), and chlorophyll SPAD values of leaves were determined by measuring in 3 different positions of the 2nd and 3rd leaves of 10 plants of each replication with a Minolta colorimeter (CR-400) and a portable chlorophyll meter (SPAD-502).

Ascorbic acid content

The content of ascorbic acid in lettuce leaves was determined with the method described by Cemeroğlu (2013). For this purpose, samples were taken from lettuce leaves and homogenized thoroughly with the help of a blender with the same amount of 2% oxalic acid. A certain amount of the mixture was weighed and made up of oxalic acid to 100 ml and then filtered. 10 ml of the filtrate was taken and titrated with 2.6 dichlorophenolindophenol solution. Results are expressed as mg of ascorbic acid/100 g of fresh weight.

Total phenolic content

Fresh leaf samples (5 g) were homogenized in 95% ethanol (20 mL) for 2 minutes. Then, the obtained homogeneous mixture was boiled for 10 minutes and centrifuged at 8000 rpm. After centrifugation, 20 ml of 80% ethanol was added to the samples filtered through filter paper, and boiled for 10 minutes. After this process, the mixture was made up to 100 ml with 80% ethanol. Then, using the Folin-Ciocalteu reagent, total phenolic content analysis was carried out according to Coseteng and Lee (1987) and the results were given as mg/100g.

Antiradical activity

The antiradical activities of lettuce leaves were determined according to the 2,2-diphenyl-1-picrylhydrazil (DPPH) method described by Dorman et al. (2003). First, the samples were mashed, filtered, and then centrifuged. Then, 450 µL of Tris-HCl buffer (50 mM, pH 7.4) was added to the samples in 50 µl. Finally, 1.00 mL of DPPH (0.10 mM, in methanol) solution was added to the mixture, and the samples were kept in the dark for 30 minutes. The absorbance reading values of the samples were obtained at a wavelength of 517 nm in the spectrophotometer, and the results were expressed as % Inhibition (DPPH).

Statistical analysis

Using the Minitab (17) Inc. Package program, data were analyzed in one-way analysis of variance (ANOVA). Significant means were compared with Tukey's multiple range test. The analysis was performed in triplicate.

RESULTS AND DISCUSSION

The effects of oxalic acid applications on plant weight, root collar diameter, plant diameter, plant height, leaf width, leaf length, and the number of leaves per plant in lettuce are given in Table 3. According to Table 3, the effects of oxalic acid applications on leaf width were found to be insignificant, whereas the effects on plant weight, plant diameter, plant height, root collar diameter, leaf length, and the number of leaves per plant were significant (P<0.05).

Wang et al. (2009), in their study on jujube, determined that oxalic acid application increased the RuBisCO activase enzyme. Moreover, García-Pastor et al. (2020) reported that with the increase of this enzyme, an increase in the rate of photosynthesis occurs, and thus vegetative growth is encouraged. They also suggested that more metabolic research on this subject should be done. Anwar et al. (2018) reported that the increase in leaf area showed an increase in water ingress to plant tissues, which played a positive role in water uptake or transport of oxalic acid to vegetative organs, thus supporting vegetative growth. These reports explain the reason for the increase in plant weight between 8.1%

Table 3. Effects of applications on plant weight, plant height, plant diameter, root collar diameter, leaf width, leaf length, and the number of leaves per plant of lettuce.

Applications	Plant weight (g/plant)	Plant height (cm)	Plant diameter (cm)	Root collar diameter (mm)	Leaf width (cm)	Leaf length (cm)	Number of leaves per plant
Control	343.02 c*	28.00 c*	27.23 b*	19.11 b*	12.07 ns	21.54 b*	47.57 c*
2 mM	432.57 a	30.35 a	28.72 a	21.49 a	12.60	22.44 a	55.63 a
4 mM	376.75 b	29.07 b	26.67 b	19.60 b	12.11	21.73 b	52.20 b
6 mM	371.10 b	28.18 c	26.70 b	19.84 b	12.02	21.23 b	54.33 a

*: Means with different letter differ significantly (P<0.05), ns = not significant.

(6 mM oxalic acid application) and 26.1% (2 mM oxalic acid application) when compared to the control group in our study. As a matter of fact, in studies conducted by different researchers, it was determined that oxalic acid applications increased the yield by 19% in rockets, 34% in pomegranate, 30% in cherries, 13% in pears, and 21% in apricots (Uludağ, 2021; García-Pastor et al., 2020; Martínez-Esplá et al., 2014; Budak and Şan, 2017).

It was determined that the oxalic acid applications increased the plant height by 0.6% (6 mM oxalic acid application), 3.8% (4 mM oxalic acid application), and 8.3% (2 mM oxalic acid application) rates compared to the control application. Supporting our results, different researchers found that oxalic acid applications increased fruit size in pears (Budak and Şan, 2017), grapes (Kök and Bal, 2019), and apricot (Kurucu, 2019) by 2.1-6.6%, 2.6%, and 4-7%, respectively. Moreover, it was also reported that it increased plant height by 41% in tomatoes (Pérez-Labrada et al., 2019) and 6.96% in the rocket (Uludağ, 2021).

Positive effects of 2 mM oxalic acid application were observed on plant diameter and root collar diameter of lettuce. It provided increases of 5.4% and 12.4% compared to the control application, respectively. Similarly, Anwar et al. (2018) stated that oxalic acid has positive effects and increases the width and length of fruits in strawberries. In a study on pears, the highest values in fruit width were obtained in the application of 1 mM oxalic acid compared to the control (Budak and Şan, 2017).

Although the effects of the applications on leaf width were found to be insignificant in our study, it was observed that 2 mM oxalic acid application increased the leaf width by 4.3% compared to the control. Also, this dose increased the leaf length by 4.1% compared to the control application. Garcia-Pastor et al. (2020) and Martínez-Esplá et al. (2014) reported that oxalic acid applications had favorable effects on fruit volume in their study on pomegranates and cherries, respectively. Despite the increase in leaf width and length, the number of leaves per plant did not decrease, on the contrary, it increased. We can see that 4 mM, 6 mM, and 2 mM oxalic acid applications increased the number of leaves per plant in lettuce by 9.7%, 14.2%, and 16.9%, respectively. This can be explained by the fact that oxalic acid increases photosynthesis, water, and nutrient uptake, and encourages vegetative growth.

Significant differences were observed in the colors and chlorophyll values (Table 4).

The positive effect of oxalic acid, which was stated in different studies (Kurucu, 2019; Budak ve Şan, 2017; Huang et al., 2013a, 2013b; Martínez-Esplá et al., 2014) on color parameters were also determined in our study. It has been found that in general 2 mM and 4 mM oxalic acid applications increased the color values compared to the control. Wang et al. (2009), in their study on jujube, determined that oxalic acid prevented the chloroplast from breaking down and the reddening of the fruit peel. This situation can be thought of as a result of the reduction of ethylene production by inhibiting the synthesis of 1-aminocyclopropane-1-carboxylic acid of oxalic acid, thus reducing the respiratory rate and lowering metabolic activity (Razzaq et al., 2015). Zheng and Tian (2005), Huang et al. (2013b), and Whangchai et al. (2006) stated in their study that oxalic acid prevented browning in postharvest lychee, banana, and longan fruits. Yoruk and Marshall (2003) found that different levels of oxalic acid prevent browning on the surface of freshly cut apples. These studies support the findings of Tang et al. (2020) who have reported that oxalic acid applications delay aging and reduce the losses in color values. As a matter of fact, our findings were found to be parallel to these reports.

Chlorophyll is the pigment, which gives plants their green color, so, while chlorophyll values are compared with the a^* values, it is seen that the results are similar to each other. In our work, the chlorophyll values increased by 1.5%, 2.4%, and 4.9% in 6 mM, 4 mM, and 2 mM oxalic acid applications respectively, compared to the control application. Wang et al. (2009) and Huang et al. (2013b) reported in their studies that oxalic acid application prevents/delays chloroplast/chlorophyll degradation.

The effects of the applications on TSS were found insignificant, but a positive effect of oxalic acid was observed with increasing doses compared to the control application. Similarly, in the studies carried out on pomegranate (García-Pastor et al., 2020), apricot (Kurucu, 2019), kiwifruit (Ali et al., 2019), and rocket (Uludağ, 2021) oxalic acid applications were found to increase the amount of TSS. Martínez-Esplá et al. (2014), have reported that it is due to the fact that oxalic acid is effective in the increase of photosynthesis. A similar circumstance was also observed in the dry matter parameter. Although the effect of the oxalic acid application on the dry matter

Table 4. Effects of applications on colors, chlorophyll, TSS, and dry matter of lettuce.

Applications	L^*	a^*	b^*	C^*	H°	Chlorophyll (SPAD)	TSS (%)	Dry matter (%)
Control	47.53 c*	-17.55 b*	27.89 b*	32.95 b*	122.18ns	37.47 b*	3.33ns	5.26ns
2 mM	48.76 a	-18.26 a	28.68 a	34.00 a	122.48	39.31 a	3.34	5.29
4 mM	48.12 b	-18.10 a	28.58 a	33.83 a	122.34	38.38 ab	3.65	5.37
6 mM	47.43 c	-17.66 b	27.98 b	33.09 b	122.26	38.04 ab	3.69	5.62

*: Means with different letter differ significantly ($P<0.05$), ns = not significant.

Table 5. Effects of applications on ascorbic acid, total phenolic and antiradical activity of lettuce.

Applications	Ascorbic acid (mg/100g)	Total Phenolic (mg/100g)	%Inhibition
Control	11.15^{ns}	72.81 b*	70.74 b*
2 mM	11.25	67.35 c	42.36 c
4 mM	11.51	103.98 a	82.64 a
6 mM	11.57	72.58 b	80.28 a

*: Means with different letter differ significantly (P<0.05), ns = not significant.

was found to be insignificant, an increase of 0.5%, 2%, and 6.8% was observed in 2 mM, 4 mM, and 6 mM oxalic acid applications, respectively. Anwar et al. (2018), in their study on strawberries, suggest that with the improvement of vegetative growth, the uptake of N, P, and K nutrients increases, and as the transportation of these nutrients to the leaves becomes easier the amount of dry matter increases. Also, Pérez-Labrada et al. (2019) found that the amount of dry matter in the leaves of the tomato plant increased and stated that this may be due to the increase in plant height.

Table 5 shows the findings related to ascorbic acid, total phenolic content, and antiradical activity of lettuce. The applications had significant effects (P<0.05) on total phenolic content and antiradical activity of lettuce.

When the table is examined, it is seen that the effects of the applications on vitamin C in lettuce are insignificant. However, a slight increase is observed depending on the doses. A similar result was found by Zhu et al. (2016), who reported that the pre-harvest application of oxalic acid increased the ascorbic acid content of kiwifruit at harvest.

In the study, it was determined that 4 mM oxalic acid application increased the total phenolic substance content by 42.8% when compared to the control application. In their studies, different researchers reported that pre-harvest oxalic acid applications increased the total phenolic content of artichoke (Martínez-Esplá et al., 2017), coriander (El-Zaeddi et al., 2017), and pomegranate (García-Pastor et al., 2020). In addition, it has been reported that the total amount of phenolic substance is preserved in studies where the oxalic acid application was applied after harvest. As a matter of fact, Koyuncu et al. (2019) stated in their storage study on pomegranate that oxalic acid application protects and increases the total phenolic content. In addition, Martínez-Esplá et al. (2017) reported that oxalic acid application increased the amount of phenolic substances by 30-50% in their study on artichokes. Also, oxalic acid applications increased the antiradical activity up to 17% (4 mM oxalic acid application) as compared to the control application. In other studies that support our results, postharvest oxalic acid application on tomato (Kant et al., 2013), peach (Zheng et al., 2007a), plum (Wu et al., 2011), mango (Zheng and Tian 2005; Zheng et al., 2007b, 2012a, 2012b), jujube (Wang et al., 2009), pomegranate (Sayyari et al., 2010), cherry (Valero et al., 2011) and banana (Huang et al., 2013a) fruits increase the antioxidant potential. Dinçay (2021), in his study on the freshly cut rocket, reported that oxalic acid application increased antioxidant activity. In addition, in different studies, it has been determined that oxalic acid applications are effective in the regulation of enzymes such as LOX, SOD, and APX in lychee and mango fruits (Zheng and Tian 2005; Zheng et al., 2007b). Wang et al. (2018) reported that oxalic acid application increased the APX enzyme in their study on melon.

CONCLUSION

In recent years, the use of plant growth regulators, which is one of the cultural practices, has become widespread, as well as the studies on different compounds that can be alternatives to plant growth regulators. In this study, the effects of oxalic acid, which can be an alternative and one of the compounds that have been emphasized recently, on the yield and quality characteristics of lettuce were investigated. As a result of the study, when all the data are evaluated together, it has been seen that oxalic acid applications in lettuce cultivation provided positive contributions to the yield and quality values. Also, it was determined that especially 2 mM and 4 mM oxalic acid doses could play an active role in increasing yield and biochemical properties, respectively.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

According to the authors, there is no potential, actual, or perceived conflict of interest with this research article.

Author contribution

The authors each contributed equally to the current work. The authors have read the final manuscript and given their approval. Every author verify that the text and tables are unique and have never been published previously.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Investigation of yield and quality parameters of some sugar beet varieties in Muş ecological conditions

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Abstract

In plant production, determining the suitable varieties suitable for the location and choosing the varieties with the best performance are among the top priorities. Biplot analysis has become widespread in recent years as an important statistical technique for plant breeders and those working in agricultural research. This study was carried out according to Randomized Complete Block Design with 18 upcountry and 2 abroad registered varieties in Muş location in 2022 year. Yield and quality components were investigated. According to the results of variance analysis, it was determined that there were statistically significant differences at the level of 1% among the varieties in terms of all the traits examined. According to the average data obtained in the study; bifurcation varied between (%) 5.4-17.8, leaf yield 13.54-24.28 t ha^{-1}, root yield 73.42-93.57 t ha^{-1}, biological yield 90.29-118.26 t ha^{-1}, sugar content (%) 16.2-19.0, plant juice purity 82.39- 88.10%, dry matter (%) 16.4-20.1, α-amino N (mg $100g^{-1}$) 0.0405-0.0498 and ash (%) varied between 2.49-3.35. According to the results of the research, in terms of root yield, G10 no and G14 no varieties came to the fore in terms of the most stable and examined traits. G12 no variety came to the fore in terms of sugar yield and G19 no variety in terms of sugar content. When the average data of all examined traits are evaluated together, G10, G11 and G14 no varieties are considered as the most stable varieties. However, varieties with high root yield, sugar content and sugar yield are the primary preferences of growers in sugar beet production. When all the data of the varieties used in the research are evaluated together; G2, G4, G8, G10, G11, G12, G14, G15 and G19 no varieties can be recommended for Muş ecological conditions. However, it was concluded that the study should be carried out in the following years for more decisive recommendations.

Keywords: Sugarbeet, Variety, Root yield, Sugar content, Biplot

INTRODUCTION

Sugar beet (*Beta vulgaris* subsp. *vulgaris*) is an important agricultural crop and a nutrient-rich commodity. It is the raw material of many industrial products and is largely used in the production of sucrose, which accounts for about 16% of global sugar production (FAO, 2018; Usmani et al., 2022). Sugar beet is also an industrial plant that has an important role in the socio-economic development of the rural population. Being a hoe plant, taking place in the agricultural rotation system, contributes to the development of sectors such as irrigation, mechanization, transportation, plant protection and fertilization. However, it is a major raw material for cosmetics, alcohol, biofuels and sugar and sugar products. It is intertwined with many different sectors such as meat, milk, medicine and transportation. It is the most important raw material source of bioethanol, which

is a source of animal feed and renewable energy (Dohm et al., 2014). The employment it creates in agriculture and industry is so high that it cannot be compared with alternative products, and it adds privilege and an effective social dimension to its activities. Sugar factories are of great importance in terms of reducing regional development disparities in our developing regions and Eastern Anatolia and contributing to employment in rural areas.

Sugar beet production in the world is carried out under climatic and agroecological conditions in many different climatic regions, from irrigated production to precipitation-based production (Hergert, 2010). Sugar beet production in the world is carried out under climatic and agroecological conditions in many different climatic regions, from irrigated production to precipitation-based production (Hergert, 2010). Although the sustainability of sugar beet production in the modern sense is quite high cost, the development in its production tends to increase with the increase in both plant breeding and agronomic developments. There are many opportunities for sugar beet production to be more sustainable and at the same time to produce more (Stevanato et al., 2019).

According to FAOTSTAT data, sugar, a strategic product, was produced in 110 countries around the world in the 2021 production period, with a total of 169 million tons of it being 35.9 million tons from beet and 133.1 million tons from cane. In the world, 253 million tons of sugar beet was produced on 4.4 million hectares of land in 53 countries in 2020, and the average yield was 57 t ha^{-1}. Approximately 80% of the sugar beet produced is produced by Russia, France, Germany, USA, Turkey, Poland, China, Egypt, Ukraine and England, respectively. Turkey ranks 5th in the world in terms of production amount and 4th in the European Continent and meets approximately 9% of the production. Although sugar beet yield in Turkey is quite stable and increasing, it has a moderate yield (55-68 t ha^{-1}) compared to other countries. Sugar beet cultivation in Turkey is carried out intensively in Konya, Eskişehir, Yozgat, Kayseri, Sivas, Aksaray, Afyonkarahisar, Yozgat, Ankara, Tokat, Karaman, Kütahya, Nevşehir and Muş provinces. The cultivation areas of these provinces constitute approximately 77.3% of Turkey's cultivation areas and 80.1% of the total production. Muş province ranks 15th in terms of production amount and 13th in terms of cultivation area among the provinces producing sugar beet in 2021 with a production amount of approximately 278 thousand tons, a cultivation area of 5.6 thousand hectares and an average yield of 56 t ha^{-1} (Yasar, 2022).

Sugar beet production follows a fluctuating course from year to year with the effect of global warming. Among the most important reasons for this are; issues such as supply-demand imbalance, drought and irrigation (Ober & Rajabi, 2010; Ghaffari et al., 2019). It is important to determine the varieties suitable for the regions in order to produce high quality and higher yield sugar beet from the unit area. For this reason, the development of new varieties through breeding studies, the testing of different varieties in different locations and the determination of stable varieties with good performance are a current issue that should be addressed in sugar beet production as in all plant groups.

This study was carried out to determine the appropriate varieties in terms of yield and quality parameters with 20 sugar beet varieties in Muş ecological conditions and to determine the correlation between the investigated traits.

MATERIALS AND METHODS

Materials

In the research, 18 up country varieties and 2 abroad registered varieties were used in the production season of 2022. Some information about the cultivars used in the study is given in Table 1.

Considering the climate data for many years, the province of Muş receives an annual average of 758.9 mm of precipitation. Most of the precipitation is in winter and in the form of snowfall. In this respect, the province of Muş can be described as a snow depot (Table 3). The climate of Muş has a suitable climate especially in terms of sugar yield. The temperature differences between day and night are around 15°C, making it a suitable location for sugar beet production. In Muş, sugar beet cultivation is carried out at the end of March and the beginning of April. Harvest begins in late September and continues until mid-November.

The soil structure of the experiment area has suitable values for the cultivation of sugar beet in terms of clay and organic matter (Table 4).

Method

The study was established in the 2022 sugar beet production season according to the Randomized Complete Block Design with 4 replications. 11 types of root and sugar types and 9 types of root type used in the research were selected (Table 1). Trial sowing was done on April 30, 2022. The seeds used in the experiment were genetic monogerms, 3.25 mm – 4.50 mm calibrated and coated seeds were used. Trial sowing, optimal sowing depth 2-4 cm, row length: 10.0 m, number of rows: 5, row spacing: 0.45 m, above row: 0.18-0.20-0.25 m, in sowing parcel area: 2.25 m x 10.00 m = 22.5 m^2. In the trial, fertilizers were given according to the soil analysis results at the recommended amounts and times for sugar beet planting. In the trial, 120 kg ha^{-1} N, 80 kg ha^{-1} P_2O_5 and 60 kg ha^{-1} K_2O fertilizers were used. Thinning was done at 18-25 cm intervals on rows during weed control and 4-6 true leaf periods. When the soil moisture fell below 50%, 5 irrigations were made with sprinkler irrigation. In the harvest, 2 rows from the sides and 1 m distance from the plot heads were taken as the edge effect and the middle

Table 1. Some information about the varieties used in the experiment

Code	Varieties	Variety owner	Registration year	Breeding country	Type	Root yield (t ha^{-1})	Sugar content (%)	Dry matter rate (%)
G1	Danicia KWS	KWS Türk Tarım Tic. A.Ş -Eskişehir	2015	Germany	Roo and, Sugar	101.54	16,14	18,7
G2	Bernanche	Dirik Dış Ticaret Memduh Zafer Dirik	2016	France	Sugar	93.36	15,12	17,71
G3	Orthega KWS	KWS Türk Tarım Ticaret A.Ş.	2019	Germany	Root	78.67	16,61	19,72
G4	Smilodon	Sesvanderhave TR Tarım Ltd. Şti.	2014	Belgium	Root and Sugar	97.40	17,13	19,72
G5	Cigogne	Dirik Dış Ticaret Memduh Zafer Dirik	2016	France	Sugar	93.52	14,85	17,47
G6	Mohican	Sesvanderhave TR Tarım Ltd. Şti.	2012	Belgium	Root	94.26	14,33	14,78
G7	Exotique	Dirik Dış Ticaret Memduh Zafer Dirik	2018	France	Root and Sugar	83.49	15,78	19,11
G8	Garrot	Dirik Dış Ticaret Memduh Zafer Dirik	2017	France	Root and Sugar	91.93	16,21	19,29
G9	Lizard	Sesvanderhave TR Tarım Ltd. Şti.	2012	Belgium	Root	9310	13,86	14,44
G10	Terranova KWS	KWS Türk Tarım Ticaret A.Ş.	2019	Germany	Root	84.33	16,63	19,70
G11	Emirata	DLF Tohumculuk Tic. Ltd. Şti.	2011	Sweden	Root and Sugar	80.57	18,11	20,15
G12	Chevalier	Dirik Dış Ticaret Memduh Zafer Dirik	2018	France	Root and Sugar	80.20	17,72	20,79
G13	Tuna	Sesvanderhave TR Tarım Ltd. Şti.	2019	Belgium	Root and Sugar	76.22	17,52	20,69
G14	Taurus	Sesvanderhave TR Tarım Ltd. Şti.	2019	Belgium	Root and Sugar	80.33	17,05	20,13
G15	Kuno	Sesvanderhave TR Tarım Ltd. Şti.	2016	Belgium	Root	103.04	13,14	15,44
G16	Premmio	DLF Tohumculuk Tic. Ltd. Şti.	2019	Denmark	Root	76.81	17,89	20,95
G17	MA4094*	DLF Tohumculuk Tic. Ltd. Şti.	--	Denmark	Root and Sugar	--	--	--
G18	Molly	DLF Tohumculuk Tic. Ltd. Şti.	2013	Sweden	Root	99.71	17,22	19,11
G19	MA4071*	DLF Tohumculuk Tic. Ltd. Şti.	--	Denmark	Root and Sugar	--	--	--
G20	Varios	Alfa Tarım Gıda İnşaat Hayvancılık Paz. San. Tic.Ltd. Şti - Balıkesir	2015	Denmark	Root and Sugar	89.39	16,62	19,55

Source: Ankara Variety Registration and Seed Certification Center-2022, *abroad registered

Table 2. Coordinates of the trial area

Location	Altitude (m)	Latitude	Longitude
Muş/TİGEM	1259	38°48'46.32"K	41°31'26.23"D

Table 3. Location climate data

Months	Average Precipitation (mm)		Average Temperature (°C)		Average Relative Humidity (%)	
	Years		Years		Years	
	1964-2022 (l.term)	2022	1964-2022 (l.term)	2022	1964-2022 (l.term)	2022
January	89.5	49.4	-7.1	-7.3	81.8	87.4
February	96.5	44.2	-5.6	-2.9	79.7	90.7
March	108.5	162.6	1.1	-0.1	70.9	89.1
April	101.9	32.0	9.2	11.3	62.1	54.9
May	69.1	91.6	14.8	13.5	58.7	64.1
June	27.1	16.0	20.1	21.1	45.2	43.4
July	7.7	0.0	25.0	25.5	33.9	23.2
August	5.4	0.0	25.0	26.6	30.9	17.3
September	15.6	17.2	20.1	21.4	35.5	25.6
October	62.8	21.4	12.8	15.1	56.0	45.4
November	86.7	42.8	4.7	6.6	68.1	73.7
December	88.1	4.2	-2.6	6.3	79.5	89.5
Average	**758.9**	**395.8**	**9.8**	**11.5**	**58.5**	**58.7**

Source: General Directorate of Meteorology -2022

Table 4. Soil characteristics of the trial field

Location	Physical Analysis					Chemical Analysis					
	Dept. (cm)	Sand (%)	Silt (%)	Clay (%)	pH	Cal. (%)	Sali-nity %	Organic matter (%)	P_2O_2 kg ha^{-1}	K_2O kg ha-1	Tekstur
Muş/TİGEM	0-30	36.64	17.45	45.91	7.4	0.26	0.40	1.28	1020.00	9080.00	Clayed

Table 5. Variance Analysis 1

Variation Sources	DF	Bifurcation	Leaf yield	Root yield	Biological Verim	Sugar Yield
Variety	19	44,6442**	43,7154**	131,494**	290,236**	4,75624**
Block	3	0,8184	0,6942	41,605	59,542	0,65777
Error	57	0,7418	2,0997	26,606	39,941	1,02008
C.Total	79	892,9764	952,3557	4139,713	7969,749	150,4863
CV (%)		8,24	7,97	6,09	6,15	6,88

***, p<0.01; *0.01<P<0.05; CV: coefficient of variation; DF: degrees of freedom*

Table 6. Variance Analysis 2

Variation Sources	DF	Sugar content	Plant juice purity	Dry matter rate	α-amino N	Ash rate
Variety	19	2,73755**	10,5708**	3,51572**	0,00002**	0,175787**
Block	3	0,38086	1,6046	0,02086	0,000006646	0,021448
Error	57	0,588	1,8627	0,28764	0,00000762	0,059408
C.Total	79	86,67208	311,8352	83,25682	0,000831	6,790555
CV (%)		4,41	1,59	2,53	6,25	8,07

***, p<0.01; *0.01<P<0.05; CV: coefficient of variation; DF: degrees of freedom*

3 rows were harvested. Harvest area: 1.35 m x 8 = 10.8 m^2 and was harvested on October 25, 2022.

Statistical Analysis

The analysis of variance of the obtained data was made with the JMP Pro 13 (© 2023 JMP Statistical Discovery LLC.) statistical package software and the traits found to be important were evaluated and grouped according to the LSD test. In addition, Biplot analyzes of the obtained data were analyzed using Genstat 14th (Copyright © 2000-2022 VSN International Ltd.), using GT (Genotype × Trait) biplot method as suggested by Yan and Thinker (2006). The graphs obtained were interpreted according to the results obtained by the researchers working on different plants.

RESULTS AND DISCUSSION

The mean squares of the results of the analysis of variance of the yield traits examined in the study are in Table 5, the mean of the squares of the variance analysis results of the quality traits are in Table 6, the averages of the yield and quality traits and the resulting groups are in Table 7 and Table 8, and the bilateral relationship between the examined traits. table is given in table 9.

According to the results of variance analysis of yield traits in the study, it was determined that there were statistically significant differences at the level of 1% between varieties in terms of bifurcation, leaf yield, root yield, biological yield and sugar yield.

According to the results of variance analysis of the quality traits in the research, it was determined that there were statistically significant differences at the level of 1% between the varieties in terms of polar ratio, plant juice purity, dry matter ratio, α-amino N content and ash ratio.

The bifurcation rate (%) of the varieties examined in the study varied between 5.4-17.8. The highest bifurcation rate was obtained from Danicia KWS (17.8%) and the lowest from MA4071 (5.4%). Çatal and Akınerdem, (2013) reported in their study that the bifurcation rates of the varieties varied between 9.3% and 24.5%. Yaşar and Kendal (2022), in their study to determine the most suitable sugar beet varieties in Muş conditions, reported that the number of bifurcation beets varied between 3.0 and 36.0 pieces per decare, which is similar to the data we obtained from our study on the number of bifurcation beets.

Leaf yield of the cultivars varied between 13.54-24.28 t ha^{-1} and the highest leaf yield was obtained from Smilodon (24.28 t ha^{-1}) variety and the least leaf yield was obtained from Tuna (13.54 t ha^{-1}). Ada and Akınerdem (2011) obtained the highest and lowest values of 32.73

Table 7. The averages of the examined traits and the resulting groups

Varieties	Bifurcation (%)	Leaf yield (t ha^{-1})	Root yield (t ha^{-1})	Biological yield (t ha^{-1})	Sugar yield (t ha^{-1})
Danicia KWS	17.8 a	17.68 de	80.81 fg	98.50 e-j	13.46 e-g
Bernanche	10.1 fg	23.60 ab	93.30 ab	116.91 a	15.67 bc
Orthega KWS	9.3 gh	14.74 g-ı	79.83 f-h	94.58 g-j	12.90 g
Smilodon	15.2 b	24.28 ab	93.97 a	118.26 a	15.43 bc
Cigogne	10.3 e-g	17.60 de	81.65 e-g	99.26 e-j	13.68 d-g
Mohican	12.3 d	21.77 bc	81.94 d-g	103.71 c-f	13.81 d-g
Exotique	15.9 c	16.87 d-f	73.42 h	90.29 j	13.38 fg
Garrot	10.6 ef	20.97 c	91.11 a-c	112.08 a-c	15.48 bc
Lizard	6.6 j-l	18.44 d	83.92 c-g	102.36 d-h	13.61 e-g
Terranova KWS	10.9 ef	16.88 d-f	86.03 b-f	102.92 d-g	14.75 c-f
Emirata	11.5 de	16.72 d-g	83.65 d-g	100.38 e-j	15.06 b-d
Chevalier	7.6 ı-k	21.28 c	92.95 ab	114.24 ab	17.14 a
Tuna	10.9 ef	13.54 ı	78.18 gh	91.72 ıj	14.37 c-f
Taurus	7.0 jk	16.55 d-g	88.88 a-e	105.44 b-e	15.34 bc
Kuno	11.3 d-f	21.75 bc	88.93 a-e	110.68 a-d	16.21 ab
Premmio	6.4 kl	16.04 e-h	80.39 f-h	96.44 f-j	14.43 c-f
MA4094	7.7 ıj	14.37 hı	80.70 f-h	95.08 f-j	14.40 c-f
Molly	13.7 c	21.37 c	89.21 a-d	110.58 a-d	14.88 b-e
MA4071	5.4 l	14.23 hı	83.55 d-g	97.79 e-j	15.78 a-c
Various	8.6 hı	14.88 f-ı	78.76 f-h	93.65 h-j	13.83 d-g
Means	**10.4**	**18.18**	**84.56**	**102.74**	**14.68**
LSD 0,05	1.22	2.05	7.30	8.95	1.43

LSD: The least significant difference

t ha^{-1}, 19.54 t ha^{-1}, Şatana (2011) 35.92 t ha^{-1}, 19.69 t ha^{-1}. Yaşar et al., (2023) reported in their study that leaf yield varied between 12.38-19.06 t ha^{-1}. The differences in their findings are thought to be due to genotpic variations between varieties and lines, and environmental and climatic factors.

Root yield of cultivars varied between 73.42-93.57 t ha^{-1}. In terms of root yields, the highest root yield was obtained from Smilodon (93.97 t ha^{-1}) variety and the lowest root yield was obtained from Exotique (73.42 t ha^{-1}) variety. Keskin (2018), in his study, reported that the beet yield varied between 77.7-111.7 t ha^{-1}, and in Canıgeniş (2012) 71.0-120.7 t ha^{-1}. Hoffman et al. (2009) reported that they obtained the highest and lowest values of 90.30 t ha-1, 69.20 t ha-1 sugar beet root yield in their study. In similar studies conducted in different ecologies, it is seen that the sugar beet root yield obtained by the researchers varies between 36.60 t ha^{-1} and 99.27 t ha^{-1} (Kurtcebe 1999; Azam Jah et al. 2003; Boyacıoğlu et al. 2014; Sefaoğlu et al. 2016). Yaşar and Kendal (2022) reported that root yield varied between 45.00-117.08 t ha^{-1} in their study. From our research findings, it can be said that the data on root yield is higher than previous studies. This can be explained by the new generation of the varieties and the suitability of Muş ecology for sugar beet farming. 80% of sugar beet yield potential is determined by climate, soil and variety factors (Pidgeon et al., 2001; Yaşar and Ekinci, 2021).

The biological yields of the varieties varied between 90.29-118.26 t ha^{-1}. The highest biological yield was obtained from Smilodon (118.26 t ha^{-1}) and Bernanche (116.91 t ha^{-1}) varieties located in the same group. The lowest biological yield was obtained from Exotique (90.29 t ha^{-1}) variety. Yaşar et al., (2023), in their study with 8 sugar beet genotypes in Muş ecological conditions, reported that the biological yields showed a change of 80.1-104.0 t ha^{-1}. These findings show parallelism with the data obtained in the study.

Sugar yields of the varieties varied between 12.90-17.14 t ha^{-1}. The highest sugar yield was obtained from Chevalier (17.14 t ha^{-1}) variety and the lowest sugar yield was obtained from Orthega KWS (12.90 t ha^{-1}) variety. Tosun et al. (2019) reported that the sugar yield varied between 14.90 and 18.67 t ha^{-1} in their research under Isparta conditions. Yaşar et al., (2023), reported in their study that it varies between 9.91-16.37 t ha^{-1}. The most important factor in sugar beet production is the high sugar yield per unit area. Since the aim in sugar beet cultivation is to produce white sugar, sugar beet root yield and sugar presence should be evaluated together.

When the averages of all cultivars are considered, the bifurcation rate is 10.4%, leaf yields are 18.18 t ha^{-1}, root yields are 84.56 t ha^{-1}, biological yields are 102.74 t ha^{-1}

Table 8. The averages of the examined traits and the resulting groups

Varieties	Sugar content (%)	Plant juice purity (%)	Dry matter rate (%)	α-amino N (mg 100g^{-1})	Ash rate (%)
Danicia KWS	16.7 h-j	87.15 ab	20,6 f-ı	0.0405 f	3.07 a-f
Bernanche	16.8 g-j	83.60 e-g	19,4 j	0.0408 f	3.16 a-d
Orthega KWS	16.2 j	83.56 e-g	21,9 bc	0.0428 c-f	3.01 a-f
Smilodon	16.4 ıj	86.13 bc	21,0 e-h	0.0470 ab	2.80 e-g
Cigogne	16.8 g-j	86.53 ab	20,7 f-ı	0.0433 b-f	3.13 a-e
Mohican	16.9 f-j	82.39 g	20,4 hı	0.0433 b-f	3.10 a-e
Exotique	18.2 a-d	85.45 b-e	22,3 b	0.0455 b-e	3.00 b-f
Garrot	17.0 e-j	88.10 a	21,7 b-e	0.0498 a	3.34 ab
Lizard	16.2 ıj	86.07 bc	20,1 ıj	0.0440 b-f	2.95 d-f
Terranova KWS	17.1 d-j	86.67 ab	21,4 c-e	0.0468 ab	2.75 fg
Emirata	18.1 a-e	86.38 a-c	21,9 b-d	0.0428 c-f	2.79 e-g
Chevalier	18.5 ab	84.52 c-f	22,4 b	0.0458 b-d	2.91 d-f
Tuna	18.4 ab	86.60 ab	20,5 g-ı	0.0440 b-f	2.99 c-f
Taurus	17.3 c-ı	85.27 b-f	20,6 f-ı	0.0440 b-f	2.93 d-f
Kuno	18.2 a-c	86.85 ab	21,9 bc	0.0463 a-c	3.09 a-f
Premmio	18.0 a-f	85.76 b-d	21,3 c-f	0.0423 d-f	3.32 a-c
MA4094	18.0 b-g	82.45 g	21,2 c-g	0.0440 b-f	3.12 a-e
Molly	16.7 h-j	83.34 fg	20,5 g-ı	0.0440 b-f	2.49 g
MA4071	19.0 a	85.77 bd	23,4 a	0.0440 b-f	3.35 a
Various	17.6 b-h	84.04 d-g	21,2 d-h	0.0418 ef	3.04 a-f
Means	**17.4**	**85.3**	**21.2**	**0.0441**	**3.0**
LSD 0,05	1.08	1.93	0.75	0.004	0.34

LSD: The least significant difference

and sugar yields are 14.68 t ha^{-1}.

The sugar content (%) of the varieties examined in the study varied between 16.2-19.0. The highest sugar content was obtained from MA4071 (19.0%) and the lowest in Lizard and OrthegaKWS (16.2%) in the same group. Ada and Akınerdem (2011) think that the differences in the findings of the highest 19.3% and the lowest 16.39% are due to genotypic variations between varieties and lines, and environmental and climatic factors. Çakmakçı and Oral (1998) reported the highest 16.91% and the lowest 14.84%, Toprak et al. (2010) obtained the highest rates of 18.68% and the lowest 15.95%. Yaşar and Kendal (2022), in their study with 8 sugar beet genotypes in Muş ecological conditions, reported that the polar ratio (%) of genotypes varied between 12.8-16.3%. The sugar content in sugar beet varies considerably depending on the variety, plant density, climatic and soil conditions, fertilization, vegetation period, harvest time, and disease and pest population.

The plant juice purity of the varieties varied between 82.39 and 88.10%, and the highest plant juice purity was obtained from Garot (88.10%) and the least from MA4094 (82.45%) and Mohican (82.39%). Doxtator and Bauserman (1952) highest 91.15% and lowest 79.34%, Alfaig et al. (2011) highest 81.18%, lowest 78.59%, Oad et al. (2001) 81.29%, 79.35% and 79.06%, Stevanato et al. (2010) 92.24%, 82.29%, Çakmakçı and Tıngır (2001) 86.33%, 85.84%.

The dry matter rate (%) of the varieties varied between 19.4-23.4. The highest dry matter rate of the varieties was obtained from MA4071 (23.4%) and the lowest dry matter rate was obtained from Bernanche (19.4%) variety. In similar studies conducted in different ecological conditions, Çelikel (1989) reported that the dry matter ratio ranged from 21.5% to 22.5, Kurtcebe (1999) reported that it ranged from 21.8% to 23.7%, and Turgut (2012) reported that it ranged from 16.4% to 17.6%. Çimrin (2001) found the highest dry matter rate 21.4% and the lowest 19.8%, Çakmakçı and Oral (1998), the highest 22.8% and the lowest 21.1%, Yarnia et al. (2008) determined it as 19.44%. As the dry matter rate increases, the sugar content increases at the same rate, since the amount of sugar in the dry matter is calculated as refined sugar content.

The α-amino N (mg 100g^{-1}) rate of the varieties varied between 0.0405 and 0.0498. The highest α-amino N rate was obtained from Garot (0.0498 mg 100g^{-1}) and the lowest α-amino N rate was obtained from Danicia KWS (0.0405 mg 100g^{-1}) and Bernanche (0.0408 mg 100g^{-1}) varieties in the same group. Can (2016), in his research conducted in Yozgat ecological conditions, reported that the α-amino N varies between 0.045 and 0.050. The highest 0.043 and the lowest 0.031 for Şatana (2011), Hoffman et al. (2009) found the ratios 0.022 and 0.017, Rashidi and Abbassi (2011) 0.025, 0.016 ratios in their research. Nitrogenous compounds, which are known as harmful nitrogen and are mostly formed by glutamine and asparagine amino acids and betaine, cannot be precipitated by liming in the sugar process because they dissolve in alkaline solutions and water, and they constitute 5% of the dry matter in molasses (Burba et al. 1996, Mahn et al. 2002). For this reason, it is desired that the nitrogenous compounds, which are expressed as harmful nitrogen, are low in the beet to be processed in the sugar process. The harmful nitrogen content of sugar beet, which is sensitive to nitrogen fertilization, is healthy and has not experienced drought stress, varies between 1.30-1.70 mmol 100g^{-1} beet (Armstrong and Milford, 1985). However, nitrogen fertilizer applications increase the harmful nitrogen content of sugar beet root and values above 2.86 mmol 100 g-1 beet affect the sugar process negatively (Akyar et al., 1980).

The ash rate (%) of the varieties varied between 2.49-3.35 (%). The highest ash rate was obtained from MA4071 (3.35%) and the lowest ash rate was obtained from Molly (2.49%). Şatana and Atakış (1999) found the highest ash rate of 2.47% and the lowest 0.90%, Alfaig et al. (2011) found the highest 0.651% and the lowest 0.560% values.

Table 9. Correlation values of the bilateral relations between the examined traits

Examined traits	Bifurcation (%)	Root yield (t ha^{-1})	Leaf yield (t ha^{-1})	Biological yield (t ha^{-1})	Sugar yield (t ha^{-1})	Sugar content (%)	α-amino N (mg 100g^{-1})	Ash rate (%)	Dry matter rate (%)
Root Yield	-0.0141								
Leaf yield	0.3403**	0.5802**							
Biological yield	0.1139	0.7164**	0.7216**						
Sugar yield	-0.1674	0.7832**	0.3699**	0.531**					
Sugar content	-0.2255	-0.2085	-0.2475	-0.2047	0.4431**				
α-amino N	0.0294	0.2148	0.1739	0.1586	0.2299*	0.0617			
Ash rate	-0.3024**	-0.0585	-0.1531	-0.1499	-0.0233	0.0386	0.0191		
Dry matter rate	-0.1082	-0.0962	-0.1594	-0.1712	0.1807	0.423**	0.2315*	0.1529	
Plant juice purity	0.1548	0.0165	-0.0331	-0.002	0.0437	0.0493	0.1079	0.0224	0.5055**

***:%1; *: %5 statistically significant at the level*

The differences in their findings are thought to be due to genotypic variations between cultivars and lines, and environmental and climatic factors.

When the averages of all varieties are considered, the sugar content is 17.4%, the plant juice purity is 85.3%, the dry matter rate is 21.2%, the α-amino N rate is 0.0441 mg $100g^{-1}$ and the ash rate is 3%.

When the correlation values of the bilateral relations between the examined traits are examined; between bifurcation rate and leaf yield, root yield and leaf, biological and sugar yields, sugar yield and sugar content, between sugar content and dry matter rate, and between dry matter rate and plant juice rate at the level of 1%. It was determined that there was a significant and positive relationship. It was determined that there was a statistically significant and negative relationship at the 1% level between bifurcation and ash rate. It was determined that there was a statistically significant and positive correlation at the 5% level between α-amino N and dry matter rate.

GGE biplot analysis

The variety*trait biplot technique uses the angles between the vectors of the traits to explain the relationship between two traits or a trait with other traits, and the location of the region where the cultivars are located depending on the traits (Figure 1). Many researchers have stated in different studies that there is a positive relationship between the vectors of two traits as the angle value (>0--<90°) gets narrower, and a negative relationship as the angle value (90°>-<180°) increases (Curcic et al., 2018; Yaşar and Kendal, 2022; Yaşar, 2023). Many researchers have reported that this technique is beneficial in the results of their research on this subject. (Gauch, 2006; Xu et al., 2014; Movahedi et al., 2020; Khan et al., 2021; Yaşar, 2023).

When Scatter Plot Figure 1 is examined; It is seen that there is a positive relationship between biological yield and root yield, leaf yield and sugar yield. At the same time, it is seen that there is a negative relationship between root yield and sugar content. This is in agreement with the data in Table 9. In terms of root yield, G10 and G14 varieties stand out in terms of the most stable and examined traits. It was determined that the most suitable variety in terms of sugar yield traits was G12, and the most suitable variety was G4 in terms of leaf yield traits. G19 variety stands out in terms of sugar content (Figure 1).

With the sector analysis, both the traits can be grouped and the most suitable varieties can be determined for each sector and trait group (Figure 2). In the research; The bifurcation rate trait is associated with G2, G6, G9 and G18 in the 1st sector, root yield, biological yield and leaf yield traits are associated with the G4 and G14 varieties in the 2nd sector, sugar yield and α-amino N rate traits are in the 3rd sector and G8, G12 and G15 varieties in sector 4 and sugar content, dry matter rate, plant juice purity and ash rate in sector 5 and associated with G16, G11 and G19 varieties. Other varieties, on the other hand, took place in sectors 6, 7, 8 and 9 and were not associated with any traits (Figure 2).

The average (vertical) and stability (horizontal) curves are created by using the average data of all the traits examined in the research with the Ranking biplot method, and information is given about the stability of the varieties according to these curves (Figure 3). Accordingly, in Figure 3, G10, G12 are the most stable varieties in terms of all traits, as they are located both above the mean curve (horizontal) and close to the stability curve (vertical). G8, G11 and G10 seem to be the suitable varieties, because they located the above or mean line of data. Other varieties are not considered suitable for Muş ecology, but it would be more accurate to repeat the experiment in the following years in order to get more stable results.

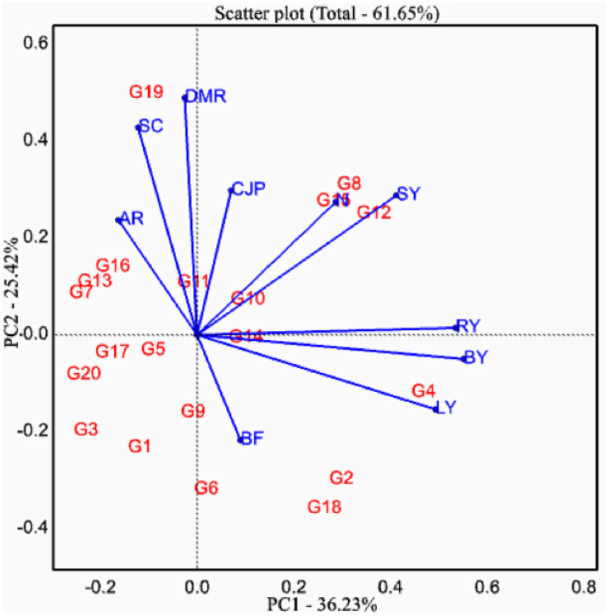

Figure 1. The relationship between varieties and traits.

According to the Comparison method, an ideal center was created according to the average of the traits and the varieties were ranked according to this center (Figure 4). Accordingly, G8, G12 and G15 varieties stand out as the most ideal varieties because they are located close to the ideal genotypes. In addition, G10, G11 and G14 varieties can be recommended as they can be considered close to the ideal genotypes. In addition, G2, G4 and G19 varieties were found to be suitable varieties because they were above the average curve.

It can be said that when recommending varieties in plant production, it is necessary to choose varieties that are located in the ideal center or close to the center and above the average curve, and varieties that are located

below the average curve should not be recommended. When these graphs are examined, they can be evaluated according to the places where the varieties are located and their distance or proximity to the traits. It has also been found that it is very convenient for us to determine the genotypes to be selected and eliminated by easily observing the genotypes above and below the mean vertical curve. These results are confirmed by the results of many researchers. (Jockovic et al., 2019; Ghaffari et al., 2021; Gholizadeh et al., 2022; Yaşar, 2023).

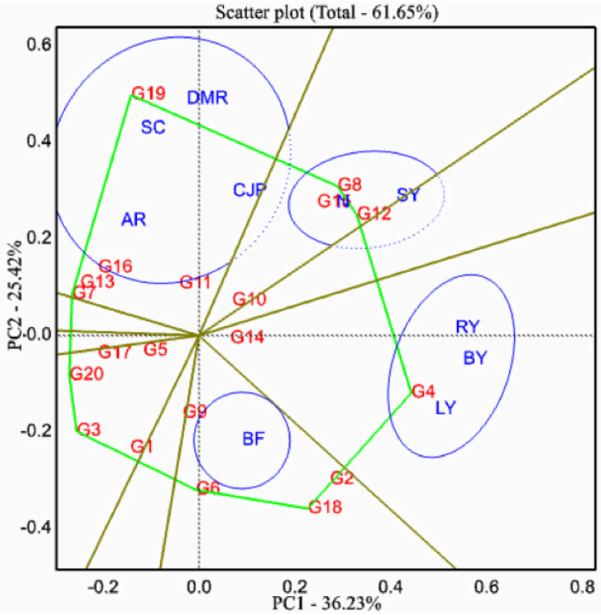

Figure 2. Grouping of varieties in terms of traits.

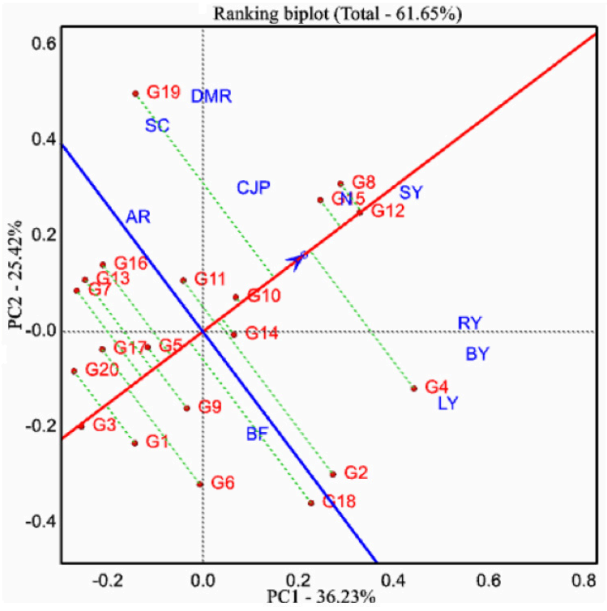

Figure 3. Ranking of varieties in terms of traits.

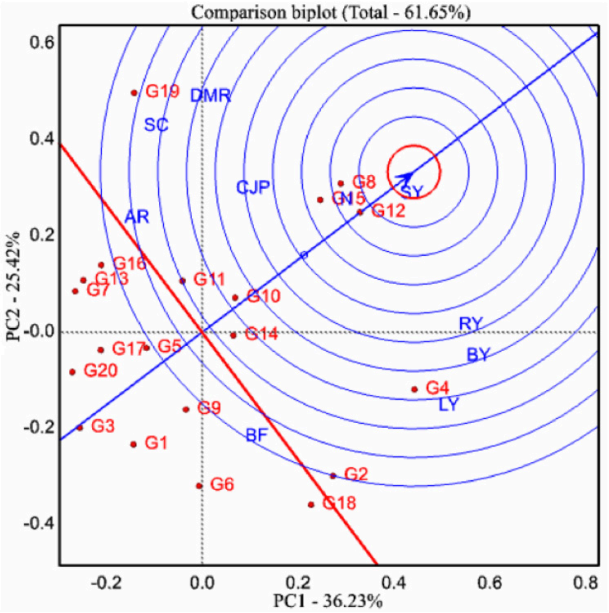

Figure 4. Ranking of vareties according to ideal center.

BF: *bifurcation;* ***RY:*** *root yield;* ***LY:*** *leaf yield;* ***BY:*** *biological yield;* ***SY:*** *sugar yield;* ***SC:*** *sugar content;* ***N:*** *α-amino N;* ***AR:*** *ash rate;* ***DMR:*** *dry matter rate:* ***CJP:*** *plant juice purity.*

CONCLUSION

In this study, In the study carried out with a total of 20 varieties, 18 registered in Türkiye and two registered abroad were tested in the 2022 sugar beet production season in Muş ecological conditions, all cultivars used in the study performed above the Muş sugar beet yield average. According to the results of the research, in terms of root yield, G10 and G14 varieties came to the fore. G12 variety came to the fore in terms of sugar yield and G19 variety in terms of sugar content. When the average data of all examined traits are evaluated together, G10, G11 and G14 varieties are considered as the most stable varieties. However, varieties with high root yield, sugar content and sugar yield are the primary preferences of growers in sugar beet production. It can be said that when recommending varieties in cultivation, it is necessary to choose varieties that are located in the ideal center or close to the center and above the average curve, and varieties that are located below the average curve should not be recommended by biplot tecnique. When all the data of the varieties used in the research are evaluated together; Chevalier, Garrot, Kuno, Smilodon, Terranova KWS, Taurus, Emirata, Bernanche and MA4071 varieties can be recommended for Muş ecological conditions. However, it was concluded that the study should be carried out in the following years for more decisive recommendations.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

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

Author contribution

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

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Phenological, morphological and physicochemical characteristics of some local olive varieties grown in Mardin (Derik)

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Abstract

This study was carried out between 2016–2017 to identify local olive varieties grown in the Derik district of Mardin province and their distinguishing characteristics. In the study the growing strength of the trees of eight local olive species, pomological characteristics of their leaves, inflorescence, fruit, and seeds, phenological development periods, total oil content, fatty acid composition, and sensory characteristics of their oils were examined. It was found that the "Mavi" variety had the highest values for fruit weight, width, and flesh ratio among the varieties examined. The variety with the highest fruit oil content (30.0%) was "Derik Halhalı", followed by the "Melkabazi" variety with oil content of 26.0%. The most abundant fatty acid in the oils of local olive varieties was cis-Oleic acid (57.60–73.51%), followed by Palmitic acid (12.90–18.57%), cis-Linoleic acid (7.97–17.76%), and Stearic acid (2.48–3.30%). It has been determined that growing "Derik Halhalı" and "Melkabazi" as oil genotype is suitable, while growing "Zoncuk", "Mavi", "Kejik", "Belluti", "Hursiki" and "Gulleki" as table genotype is suitable

Keywords: *Olea europaea* L., Morphology, Fatty acids, Sensory analysis, Mardin

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INTRODUCTION

Olive is a cultivated plant dating back to 4000 BC (Özkaya et al., 2006). The homeland of olive (*Olea europaea* L.), a member of the family Oleaceae, includes South Asia Minor and Upper Mesopotamia, including the South-eastern Anatolia region of Turkey (Sakar, 2015). Olive is accepted to have spread from its homeland to the whole world through three routes, the first of which is Tunisia and Morocco via Egypt, the second of which is the Aegean islands, Greece, Italy, and Spain along Anatolia, and the third of which are Pakistan and China via Iran (Özkaya et al., 2010). It is considered that the Sami cultivated the olive and carried out the first breeding studies (Kaplan and Arıhan, 2012). The presence of the lowest species of the olive tree in Mardin, Kahramanmaraş and Hatay in recent studies supports this judgement. However, there are also sub-varieties of olive species and wide biodiversity in this region (Sakar et al., 2017).

Since the early ages, people have not only considered the olive plant as an essential foodstuff but also made use of its oil in the treatment of internal diseases and wounds (Kaplan and Arıhan, 2012). The olive tree has symbolised peace and prosperity throughout history due to its abundant crop and easy growth in suitable climatic conditions. This has led the olive to be one of the plants frequently mentioned in sacred scriptures (Eskiyörük, 2016).

Mardin, one of the ancient cities established in Mesopotamia between the Euphrates and Tigris rivers, is a region where both table and oil olives have been

grown for many years (Acar, 2016). As of 2022, a total of 4620 tonnes of olives are produced in an area of 20123 da in Mardin province. Table olives constitute 4278 tonnes of this production. Although olive cultivation in Mardin province is concentrated in Derik, Kızıltepe and Artuklu districts, Derik district accounts for 59% of the olive production in the province with a total olive grove of 11661 da and an annual olive production of 2727 tonnes (TÜİK, 2023). Derik district is protected from cold north winds by its location on the south-facing slopes of the Mardin Threshold Mountains and is exposed to hot winds blowing from the south and southwest directions. Nevertheless, the alluvial soils with high limestone content make this region favourable area for olive cultivation (Doran et al., 2008). Today, Derik Halhalı, Zoncuk, Melkabazi, Belluti, Hursiki, Mavi, Kejik and Gulleki local olive varieties cultivated in the Derik district hold an important share in the olive production of the region. Besides, these varieties were protected by the first selection study carried out by the Olive Research Institute in 1968 and are the olive species in the National Collection (Özkaya et al., 2006).

When the structure of olive cultivation in Turkey is analysed, it is observed that although there was a significant rise in the olive grove and the number of trees between 2004 and 2022, the same is not observed in the yield per tree (TÜİK, 2023). The main reasons why Turkey has not been able to achieve the output growth that other countries with which Turkey competes in olive cultivation have achieved in recent years are low productivity and unfavourable climatic factors that increase the tendency of periodicity (Bayramer, 2015; Şahin and Aydoğdu, 2021). Also, harvesting every 2-3 years due to the harsh periodicity of local varieties in the South-eastern and Mediterranean regions causes the olive groves in those regions to be abandoned to their own fate for a long period of time. Although it varies according to the provinces, the drying problem causes greater economic losses in olive groves that are not fertilised, are not pruned, or deprived of soil cultivation and are subjected to agricultural control (Tüzün, 2003). Also, the variety confusion in olive cultivation in Turkey and the recognition of a variety with different names in different regions and even in the same region are observed in the South-eastern Anatolia Region (Dölek, 2003; Sakar, 2015). Despite having large lands that can be utilised for olive cultivation, the climatic characteristics (high summer temperatures and insufficient rainfall) of the South-eastern Anatolia Region limit the expansion of olive cultivation in the region (Akıllıoğlu et al., 2000; Sakar, 2015). The climatic disadvantage of the region can be partially eliminated, and olive cultivation can be expanded in the region by identifying the potential of local olive varieties that are naturally distributed in the region and promising varieties among them.

Given the said reasons, this study, which was carried out between 2016–2017, aimed to reveal the current situation of these varieties in the region and their potential by examining their morphological, phenological, and pomological characteristics, fatty acid composition in oils, and sensory qualities of local olive varieties grown in Derik district of Mardin province.

MATERIALS AND METHODS

Material

The first stage of this study included the identification of local olive varieties and species grown in the olive groves of the Derik district of Mardin province, among which eight local varieties with production potential in the region were selected to be utilised herein. The olive trees of the previously identified local olive varieties (Mavi, Belluti, Derik Halhalı, Zoncuk, Kejik, Hursiki, Melkabazi, and Gulleki) in the same grove and age were used as the plant material of the study between 2016–2017.

Method

The olive trees used in the study were of productive age, free from diseases and pests, and exhibited similar characteristics for development. For each variety examined, a total of eight trees were identified, and samples were taken from those trees for further examination and analysis. To identify the morphological and pomological characteristics of leaves, fruits, and flowers of olive varieties, each tree was randomly selected from different parts of the tree to represent the tree, and 50 samples were collected from each tree. Fruit samples which had been taken from each olive variety at harvest time were transported to the Postharvest Physiology Laboratory of the Faculty of Agriculture, Harran University to determine fruit and seed characteristics. Pomological and morphological characteristics of olive samples were identified following the methods and scales reported by Barranco et al., (2000), Şeker et al., (2012) and Sakar et al., (2017). Leaf samples were morphologically characterised using the methods and scales reported by Kaymak (2011) and Sakar (2015). The inflorescence of the examined olive varietieswas characterised using the method and scale of Ulaş (2001). Soxhelet extraction using n-hexane solvent was used to determine the total oil content of olives (Kadaster, 1960). The fatty acid composition of olive oils was determined by gas chromatography at the Food Technologies Laboratory of the Olive Research Institute (Önal et al., 2006). Sensory analyses (fruitiness, bitterness, and pungency) of the olive oils extracted from the studied varieties were done by the IOC method (COI/T.20/Doc.No 15/2007) at the Olive Research Institute.

RESULTS AND DISCUSSION

Morphological Characteristics

Tree characteristics

During the garden observations in July-August, the trees belonging to the olive varieties analysed hereunder were

evaluated. In general, it was determined that the growth strength of the trees belonging to the varieties examined was "strong". However, the growth strength of olive trees does not only depend on the genotypic character of the variety. The growth strength may vary depending on the genotypic character as well as the climatic characteristics of the cultivation region and the nature of the cultivation practices applied to the grove (Baktır et al., 1995; Sakar et al., 2013; Sakar, 2015; Sakar et al., 2017).

The observations revealed that the crown structure of the trees differed according to the olive varieties analysed hereunder and varied between "upright", "semi-upright", and "spreading". The varieties with "upright" crown structures were Melkabazi, Belluti, Mavi and Zoncuk; whereas, the varieties with "semi-upright" crown structures were Gulleki, Hursiki, Derik Halhalı and the variety with "spreading" crown structures was Kejik.

Turanoğlu (2015) found that branching was sparse and leaf density was low in trees of Ayvalık olive variety. Ulaş (2001) reported that Adana Topağı, Mavi, Sarı Ulak (Adana), Silifke oil, Halhalı and Kargaburnu varieties had "dense" crown density and Edremit oil, Gemlik, Yerli, Kilis oil, Nizip oil, San Ulak (İçel) and Küncülü varieties had "moderate" crown density.

Leaf characteristics

Table 1 shows the leaf characteristics of local olive varieties grown in Mardin (Derik). The leaves of the varieties examined were generally found to be of medium length; however, the "Zoncuk" variety had longer leaves than the other varieties. Also, the width of the leaves of this variety was narrower, and the leaf length/width ratio was high compared to the other varieties. It is relatively easy to distinguish the variety "Zoncuk" from the others analysed even by considering only the leaf structure (Figure 1). The leaf shape of the varieties "Derik Halhalı", "Kejik," and "Gulleki" was elliptical long, while the leaf shape of the variety "Zoncuk" was long, and the leaf shape of the other varieties was elliptical.

Inflorescence characteristics

The inflorescence of the local olive varieties were collected at the end of April (during the inflorescence period) and analysed. The results showed that the inflorescence length of the varieties varied between 16.73-29.54 mm (Table 2). The "Melkabazi" variety had the longest inflorescence length. On the other hand, the olive variety "Derik Halhalı" had a greater number of flowers per inflorescence than the other varieties (18.07 pcs/ inflorescence) among the varieties analysed in terms of the number of flowers per inflorescence. The number of flowers per inflorescence was generally low in the other varieties analysed.

The number of flowers on the inflorescence in olive trees may vary depending on the genetic characteristics of the variety, its susceptibility to periodicity, and the care procedures (fertilisation, irrigation, etc.) followed during cultivation (Sakar, 2015). However, it was also observed that there was no linear correlation between the inflorescence length and the number of flowers on the inflorescence of the olive varieties we analysed. Hence, the "Melkabazi" variety with a long straight inflorescence and the "Kejik" variety with a short compact inflorescence had similar numbers of flowers on their inflorescence (Table 2, Figure 2).

Pomological characteristics

Fruit characteristics

Table 3 presents the fruit characteristics of the local olive varieties analysed. Accordingly, the fruit weights of the analysed varieties varied between 1.33 and 7.10 g and the "Mavi" variety was identified as the olive variety with heavier fruits than the other varieties. When the varieties analysed by the fruit weight were classified based on the scale reported by Kaymak (2011), "Mavi" was classified as very heavy; "Belluti", "Melkabazi," and "Hursiki" as heavy; "Gulleki" and "Derik Halhalı" as moderate; and "Zoncuk" and "Kejik" as light. Fruit weight values are similar to the fruit weight values determined by Bolat and Güleryüz (1995) in local olive varieties grown in Çoruh Valley and by Sevgin and Caner (2020) in olive genotypes grown in Şırnak and Mardin. The fruit flesh ratio of the varieties varied between 66.91 and 91.40%, and fruit weight and fruit flesh ratio were correlated. The "Mavi" variety with the heaviest fruits had the highest flesh ratio, and the "Kejik" variety with the lightest fruits had the lowest flesh

Table 1. Leaf characteristics of local olive varieties grown in Mardin (Derik)

Varieties	Leaf Length (mm)	Leaf Width (mm)	Leaf length/width Index	Leaf Shape
Derik Halhalı	66.29 Medium	12.27 Medium	5.40	Elliptical long
Zoncuk	71.51 Long	10.86 Medium	6.58	Long
Mavi	53.68 Medium	15.11 Wide	3.55	Elliptic
Kejik	63.82 Medium	12.04 Medium	5.30	Elliptical long
Belluti	65.94 Medium	18.03 Wide	3.65	Elliptic
Hursiki	56.61 Medium	18.83 Wide	3.00	Elliptic
Melkabazi	64.05 Medium	16.23 Wide	3.94	Elliptic
Gulleki	64.43 Medium	14.59 Medium	4.41	Elliptical long

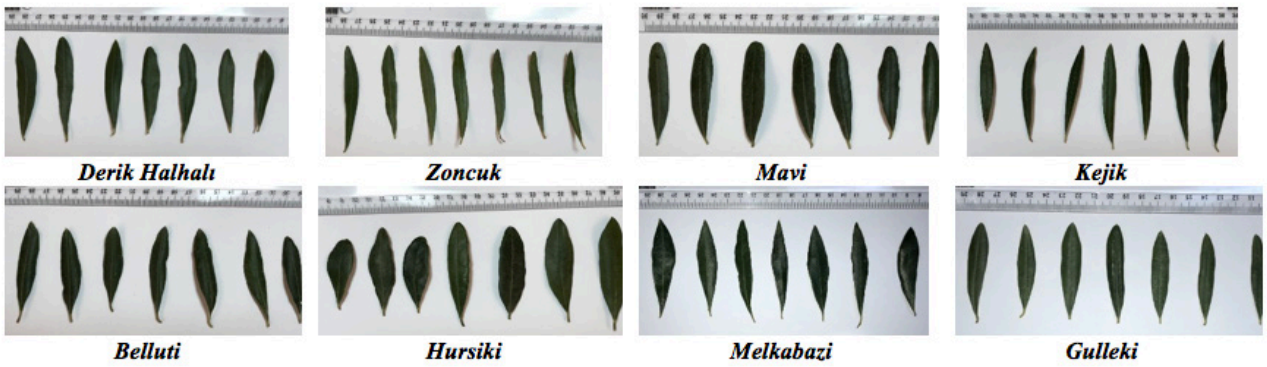

Figure 1. Leaves of local olive varieties grown in Mardin (Derik)

Table 2. Inflorescence characteristics of local olive varieties grown in Mardin (Derik)

Varieties	Inflorescence Length (mm)	Inflorescence Structure	Number of Flowers in the Inflorescence (pcs/ Inflorescence)
Derik Halhalı	26.10	Long Straight	18.07 Medium
Zoncuk	26.01	Long Straight	14.64 Low
Mavi	25.23	Short Straight	13.20 Low
Kejik	21.40	Short Compact	14.38 Low
Belluti	17.92	Short Straight	5.84 Low
Hursiki	16.73	Short Compact	11.12 Low
Melkabazi	29.54	Long Straight	15.02 Low
Gulleki	21.26	Short Straight	11.92 Low

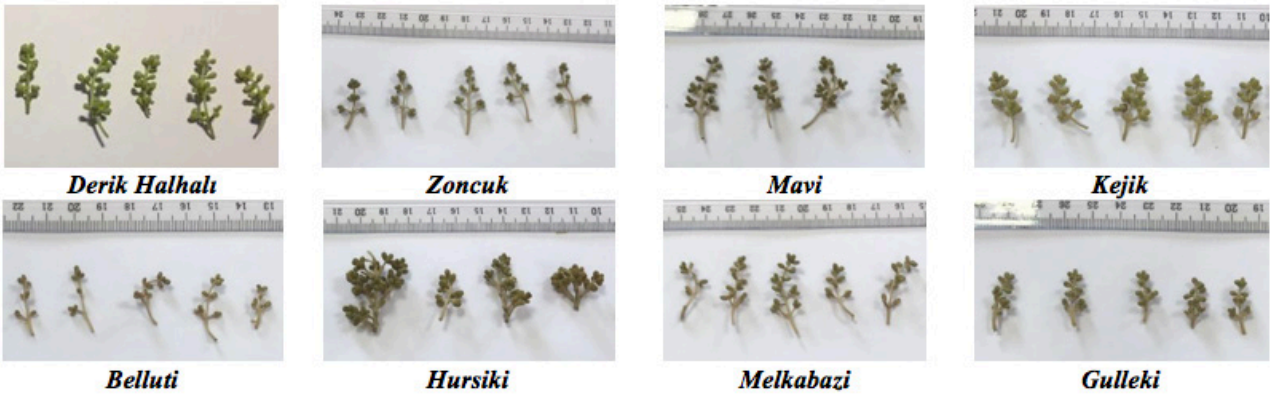

Figure 2. The inflorescence of local olive varieties grown in Mardin (Derik)

Table 3. Fruit characteristics of local olive varieties grown in Mardin (Derik)

Varieties	Fruit Length (mm)	Fruit Width (mm)	Fruit Shape Index	Fruit Weight (g)	Flesh Ratio (%)	Fruit Shape
Derik Halhalı	20.69	15.52	1.33	2.80 Medium	82.50	Elliptic
Zoncuk	16.26	12.12	1.34	1.52 Low	67.10	Elliptic
Mavi	28.07	21.30	1.31	7.10 Very high	91.40	Elliptic
Kejik	16.71	10.91	1.53	1.33 Low	66.91	Sharp
Belluti	28.20	16.06	1.75	4.52 High	76.77	Sharp
Hursiki	22.35	18.55	1.20	3.69 High	77.24	Egg
Melkabazi	29.74	15.17	1.96	4.30 High	83.72	Sharp
Gulleki	19.43	16.19	1.20	3.42 Medium	83.92	Egg

ratio. Şeker et al., (2012) reported that the fruit-flesh ratio of olive varieties grown in the Eastern Black Sea Region varied between 74.86 and 77.86%. Biricik and Başoğlu (2005) reported that the flesh ratio of olive varieties grown in the Marmara Region varied between 79.86 and 85.43%. Gündeşli and Küden (2020) reported that the flesh ratio of olive varieties grown in Çukurova (Adana) varied between 76.0 and 87.0%. Dağdelen (2008) found that the flesh ratio increased in parallel with ripening in "Ayvalık", "Domat," and "Gemlik" olive varieties grown under Edremit conditions.

Fruit length and fruit width varied between 16.26–29.74 mm and 10.91–21.30 mm, respectively, in local olive varieties. The longest fruits were found in the "Melkabazi" variety, and the widest fruits were found in the "Mavi" variety. On the other hand, the fruit shape index (fruit length/fruit width) varied between 1.20 and 1.96 in these varieties, while the highest shape index value was found in "Melkabazi" variety, the lowest shape index value was found in "Hursiki" and "Gulleki" varieties. Caner (2018) reported that olive varieties differed from each other in terms of fruit shape index. Indeed, the fruit shape index varied between 1.17 and 1.44 in the "Yamalak Sarısı" variety (Kaya and Tekintaş, 2006), 1.29 and 1.33 in the "Uslu" variety (Demir, 2018), and 1.34 and 1.53 in the "Erkence" variety (Tutar, 2010). Dölek (2003) reported that fruit widths varied between 15.10 and 18.19 mm, and Kaleci et al., (2016) reported that fruit widths varied between 20.78 and 35.07 mm in the olive varieties they analysed. Biricik and Başoğlu (2005) reported that fruit length varied between 21.32 and 29.17 mm in the olive varieties they analysed. Gündeşli and Küden (2020) stated that fruit length varied between 13.10 and 34.83 mm, and Şahin and Şeker (2022) reported that fruit length varied between 13.70 and 23.37 mm. Findings on fruit size, shape index, weight, and flesh ratio in the present study appear to be similar to those in the literature. Also, findings of the present study showed that the local olive varieties grown in Derik are similar to the nationally recognised and extensively grown olive varieties and some local varieties are well suited to the production of table olives.

The local olive varieties analysed hereunder varied in terms of fruit shape (Figure 3). The fruits of "Derik Halhali", "Zoncuk", and "Mavi" varieties were elliptical; the fruits of "Kejik", "Belluti", and "Melkabazi" varieties were sharp; and the fruits of "Hursiki" and "Gulleki" varieties were egg-shaped.

Seed characteristics

Table 4 presents the findings related to the seed characteristics of the local olive varieties analysed. The findings of the present study showed that the seed length varied between 12.35 and 23.36 mm and the seed width varied between 6.86 and 9.25 mm in the local olive varieties grown in Mardin (Derik). While the "Melkabazi"variety had the longest seeds, the "Hursiki"variety had the widest seeds. Besides, the seed shape index of the analysed varieties varied between 1.57 and 3.09. When assessing the potential of olive varieties in terms of cultivation, fruit flesh ratio is an important criterion as is fruit weight, which is directly affected by seed weight. The seed weights of the varieties analysed hereunder varied between 0.44 and 1.05 g. The "Belluti"varietyhad the heaviest seeds and the "Kejik"varietyhad the lightest seeds. Kaynaş et al., (1996) reported that seed weight varied depending on the olive variety analysed. Toplu et al., (2009) found that the seeds of the "Gemlik" olive variety were heavier in sufficiently irrigated trees compared to trees grown under water shortages. The seed weight was reported to vary between 0.38–0.72 g and 0.25–1.80 g in olive genotypes grown in Mardin and Şırnak by Sevgin and Caner (2020) and in Gaziantep province by Sakar (2015), respectively. The findings obtained here showed that the olive genotype was the direct and primary influential factor on seed characteristics. Moreover, it is consistent with the findings of previous researchers, who reported that the pomological characteristics of olive seeds varied according to the olive genotype studied.

The seed shapes of the local olive varieties in Mardin (Derik), which we analysed hereunder, differed as

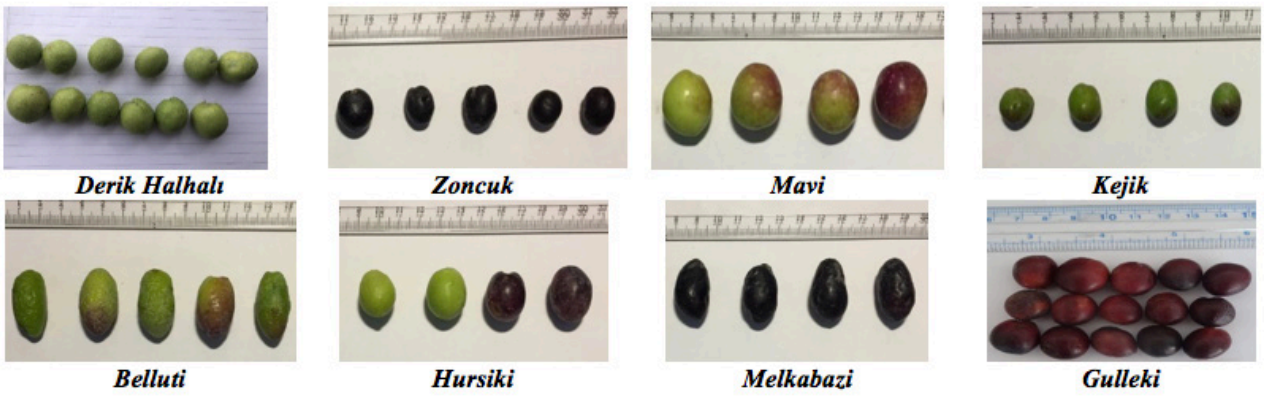

Figure 3. Grains of local olive varieties grown in Mardin (Derik)

Table 4. Seed characteristics of local olive varieties grown in Mardin (Derik)

Varieties	Seed Length (mm)	Seed Width (mm)	Seed Shape Index	Seed Weight (g)	Seed Shape
Derik Halhalı	14.08	7.69	1.83	0.49 Heavy	Elliptic
Zoncuk	12.35	6.86	1.80	0.50 Heavy	Elliptic
Mavi	14.42	8.63	1.67	0.61 Heavy	Elliptic
Kejik	14.53	7.55	1.92	0.44 Medium	Elliptic
Belluti	21.90	9.04	2.42	1.05 Very heavy	Sharp
Hursiki	17.89	9.25	1.93	0.84 Very heavy	Elliptic
Melkabazi	23.36	7.55	3.09	0.70 Heavy	Sharp
Gulleki	12.44	7.89	1.57	0.55 Heavy	Elliptic

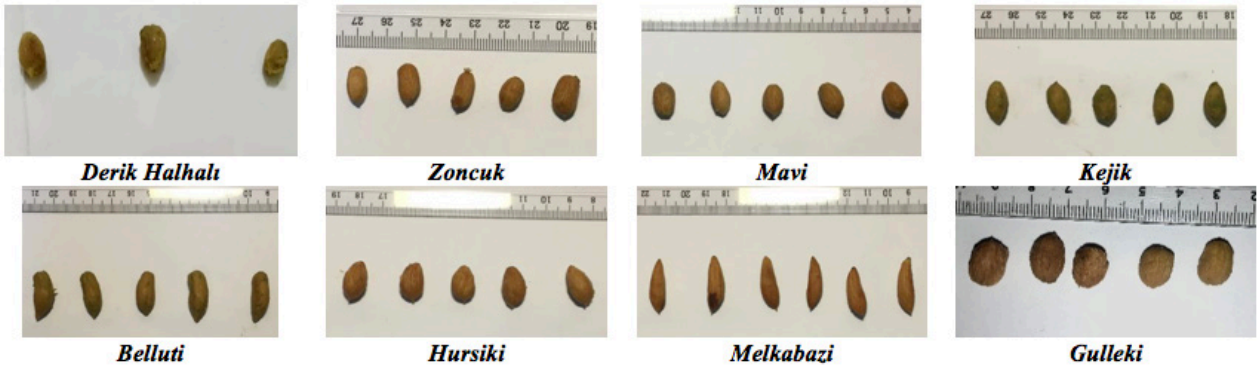

Figure 4. Seeds of local olive varieties grown in Mardin (Derik)

elliptical and sharp (Figure 4). The seeds of the "Belluti" and "Gulleki"varietieswere sharp, while the seeds of the other varietieswere elliptical.

Phenological Observations

The date when 5% of the flowers bloomed on the trees was accepted as the beginning of inflorescence in the olive varieties we analysed. Biagnami et al., (1993), who studied different varieties and ecologies, reported that the inflorescence dates of olive varieties changed when the temperature was 2°C higher, but there was no significant difference in the ripening time of the fruits. Canözer (1991) found that full inflorescence of olive varieties grown under Izmir conditions took place at the end of May. In the Derik district of Mardin province, the beginning of inflorescence of the olive varieties we analysed took place at the end of April due to the microclimate characteristics of the region. There was no difference between the varieties in terms of the beginning date of inflorescence. However, the determination of the full inflorescence date of the varieties was based on the date when 70% of the flowers bloomed on the trees. The observations revealed that full flowering took place in the middle of May for the eight local olive varieties grown in Mardin (Derik). Also, the period between the beginning of flowering and full blooming was determined to be 10 days in these varieties. The end of flowering was accepted as the date when all the flowers bloomed on the trees, and observations were made accordingly. The observations revealed that the end of inflorescence was between the end of May and the beginning of June for the varieties analysed. No distinctive differences were found between the varieties in terms of both full blooming and end-of-flowering dates.

Chemical Analyses

The total oil content of the fruits of the local olive varieties analysed herein varied between 10.2 and 30.0% (Figure 5). The variety with the highest oil content was "Derik Halhalı" and the variety with the lowest oil content was "Mavi". Previous studies conducted by different researchers reported that the total oil content of olive fruits varied between 1.0 and 40.9% depending on the variety/genotype, growing conditions, and climatic conditions (Sakar, 2009; Tutar, 2010; Sakar et al., 2013; Karanfiloğlu et al., 2017). Findings of the present study showed a distribution between the values of fruit oil content reported in the literature and, hence, were in parallel with the findings of previous researchers. The classification by the International Olive Council (IOOC) for oil olive varieties indicates that "Zoncuk", and "Melkabazi" and "Derik Halhalı", the varieties analysed here, are classified as having low or high oil content and can be considered as oil olive varieties. On the other hand, it was concluded that "Mavi", "Kejik", "Belluti", "Hursiki," and "Gulleki"varieties would not be suitable to be considered as oil varieties.

Table 5 shows the distribution of fatty acid composition in the oils of the local olive varieties analysed. The most

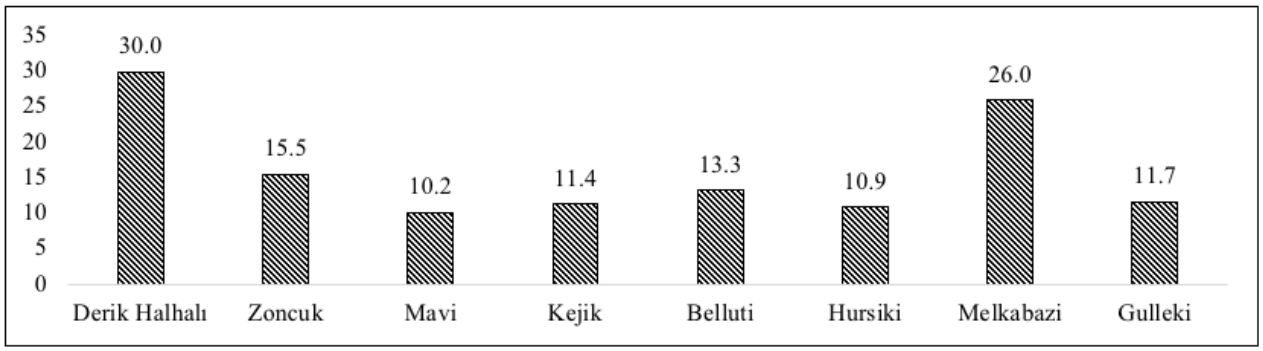

Figure 5. Total oil content (%) in the fruits of local olive varieties grown in Mardin (Derik)

Table 5. Fatty acid composition (%) of local olive varieties grown in Mardin (Derik)

	Derik Halhalı	Zoncuk	Mavi	Kejik	Belluti	Hursiki	Melkabazi	Gulleki
C16:0	14.39	12.90	18.57	18.06	16.30	16.10	16.40	15.12
C16:1	1.23	0.53	1.22	0.84	1.03	1.14	1.06	1.14
C17:0	0.17	0.20	0.10	0.07	0.13	0.10	0.13	0.04
C17:1	0.25	0.27	0.13	0.08	0.17	0.14	0.18	0.04
C18:0	3.30	3.06	2.59	3.11	2.58	2.48	2.57	2.62
C18:1	69.04	73.51	57.60	62.10	63.41	64.93	63.08	70.90
C18:2	9.58	7.97	17.76	13.76	14.07	13.33	14.32	8.01
C18:3	0.96	0.72	1.00	0.87	1.21	0.84	1.19	0.95
C20:0	0.53	0.37	0.52	0.56	0.53	0.44	0.53	0.55
C20:1	0.30	0.24	0.29	0.29	0.32	0.32	0.31	0.32
C22:0	0.15	0.18	0.15	0.14	0.16	0.11	0.17	0.17
C24:0	0.10	0.05	0.07	0.12	0.09	0.07	0.06	0.14
∑SFA	18.64	16.76	22.00	22.06	19.79	19.30	19.86	18.64
∑MUFA	70.82	74.55	59.24	63.31	64.93	66.53	64.63	72.40
∑PUFA	10.54	8.69	18.76	14.63	15.28	14.17	15.51	8.96

C14:0: Myristic acid, C16:0: Palmitic acid, C16:1: Palmitoleic acid, C17:0: Heptadecanoic acid, C17:1: *cis*-10 Heptadecanoic acid, C18:0: Stearic acid, C18:1: *cis*-Oleic acid, C18:2: *cis*-Linoleic acid, C18:3: *cis*-Linolenic acid, C20:0: Arachidic acid, C20:1: Gadoleic acid, C22: 0: Behenic acid, C24:0: Lignoceric acid

abundant fatty acid found in the olive oils of all analysed varieties was *cis*-Oleic acid, followed by Palmitic acid, *cis*-Linoleic acid and Stearic acid, respectively. This order was also reported by other researchers who analysed the oils of different olive varieties (Dağdelen, 2008; Kutlu and Şen, 2011; Sakar et al., 2017). Nevertheless, the varieties analysed in this study differed from each other with respect to palmitoleic acid and *cis*-linolenic acid content. Palmitoleic acid was higher in the oils of "Derik Halhalı", "Mavi", "Hursiki" and "Gulleki"varieties, while *cis*-Linolenic acid was higher in the oils of "Zoncuk", "Kejik", "Belluti," and "Melkabazi" varieties. The olive variety containing the highest *cis*-Oleic acid (73.51%) in its oil was identified as "Zoncuk" and the variety containing the highest *cis*-Linoleic acid (17.76%) was identified as "Mavi".

When the fatty acids in the oils of local olive varieties grown in Mardin (Derik) were classified according to their saturation levels and these classes were ranked according to their presence in the oil, the order of MUFA>SFA>PUFA was achieved. The researchers have obtained the same order in previous studies conducted in different olive varieties and in different ecologies (Stefanoudaki et al., 1999; Dıraman and Dibekoğlu, 2009; Kritioti et al., 2018; El Riachy et al., 2019). However, Toplu et al., (2009) found that SFA and PUFA increased and MUFA decreased with irrigation in the "Gemlik" olive variety, but fertilisation had no effect on the fatty acid composition. Future studies on the effects of irrigation, fertilisation, and similar cultural practices on the fatty acid composition of the olive varieties we analysed would contribute to the literature and help growers.

Sensory analyses

The sensory analyses of the olive oils revealed that the oils of the local olive varieties cultivated in Mardin (Derik) were dominated by spicy, walnut leaf, grapefruit peel, green banana, roasted terebinth seed, rocket, rosemary, green grass, green apple, green tomato, honey, pollen, forest fruits, green almond, and pistachio (fruit) odours

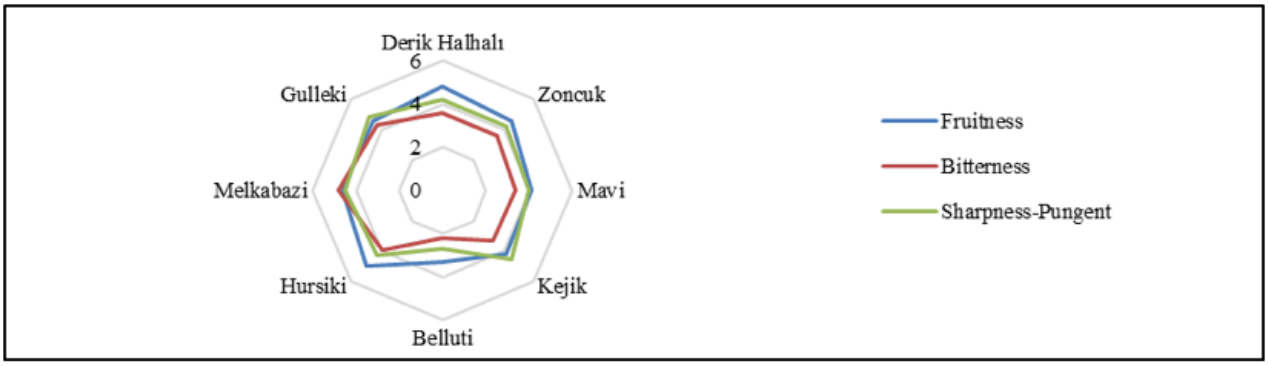

Figure 6. Tasting (sensory) analysis values of oils of local olive varieties grown in Mardin (Derik)

reflecting the characteristics of the region. The bitterness in the olive oils of the varieties analysed was felt with medium intensity and bitter almond, and green walnut inner shell bitterness was felt as particles popping on the back of the tongue. Also, the oils of these varieties were found to have a long-lasting pungent flavour. When the oils of the varieties were compared with each other, the highest fruitiness score was recorded in the oil of "Derik Halhalı" variety, the highest bitterness score was recorded in the oil of "Melkabazi," variety and the highest sharpness-pungent score was recorded in the oil of the "Gulleki" variety (Figure 6).

CONCLUSION

This study, which aimed to identify the morphological, pomological, phenological, and physicochemical characteristics of the olive varieties grown in the Derik district of Mardin province, reveal the current conditions of the olive varieties in the region, and provided a preliminary evaluation of the cultivation potential of these varieties in ecologies similar to the region where they are currently grown. Although "Derik Halhali", one of the olive varieties analysed in this study, is a nationally recognised one due to its role in the cultivation of olives for oil in South-eastern Anatolia, the "Melkabazi"variety was also identified as a variety that can be evaluated for this purpose and can be adapted to the region in general. On the other hand, "Zoncuk", "Mavi", "Kejik", "Belluti", "Hursiki," and "Gulleki"varieties were found to be suitable for production of table olive.

New studies on the performance of the local olive varieties we analysed, both in their region and in different ecological and cultivation conditions, would contribute to improving the national and international recognition of these varieties and expanding their cultivation. Furthermore, different species of these varieties and clonal propagation of the superior genotypes under protection would also contribute to the agriculture of our country as a part of the conservation and dissemination of Turkey's olive genetic resources.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

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

Author contribution

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

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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RESEARCH ARTICLE

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Experimental investigation of parametric changes in seepage time and length into the subsoil of hydraulic structures

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Abstract
Dams and hydraulic structures are built on rivers in order to protect water resources due to global warming, to collect surface waters to provide drinking water and/or irrigation water, to prevent floods and to establish hydroelectric power plants. Dams, for example, are hydraulic structures that have more or less positive or negative environmental interactions on surface water and groundwater. One of the environmental interactions of dams and similar hydraulic structures is the seepage of accumulated water in its reservoir from upstream bottom of the dam. This seepage can affect the level and location of groundwater, reduce the accumulation of water in the reservoir, and cause piping in the ground below the construction of the dam body. In order to control the seepage, the methods of increasing the seepage length by using sheet pile and clay blanket on the dam foundation are frequently used. In this study, in the physical laboratory model, the variations in the seepage lengths that occur under the hydraulic structure section in the soil with two different grain diameters of 0.85 mm and 1.5 mm, depending on the dam structure, soil and barrier structures (sheet pile and upstream clay blanket), were experimentally investigated. As a result, it was determined that the seepage occurs less in the soil with a smaller grain diameter of 0.85 mm, the smaller the soil particle diameter has a reducing effect on the seepage, and the use of sheet pile increases this effect positively. In addition, it has been determined that the clay blanket in the upstream is effective compared to the general conditions, but the use of sheet pile provides the most efficiency.
Keywords: Clay blanket, Environmental risk, Hydraulic structure, Seepage, Sheet pile

INTRODUCTION

Hydraulic structures are engineering structures designed and built to control and use natural water resources (Sedghi-Asl et al., 2012; Mohammed-Ali, 2011). These structures must have high strength in order to provide a long and high quality service lifespan. For this reason, examining and solving the problems that the structures may encounter is a very important parameter in terms of strength and service lifespan in terms of protection of water resources from global warming adverse effects and seepage related water losses, and protection from hydraulic structure collapse (especially dams) related environmental disaster risks. Hydraulic structures built on various soils are based on the life and durability of the structure by using different methods in accordance with the structure of soil. These foundations are divided into three types as rock, coarse-grained material (sand and gravel) and fine-grained material (clay and silt) foundations. Foundation soils of hydraulic structures are mostly composed of permeable sand and gravel including relatively impermeable sections. These soils, which consist

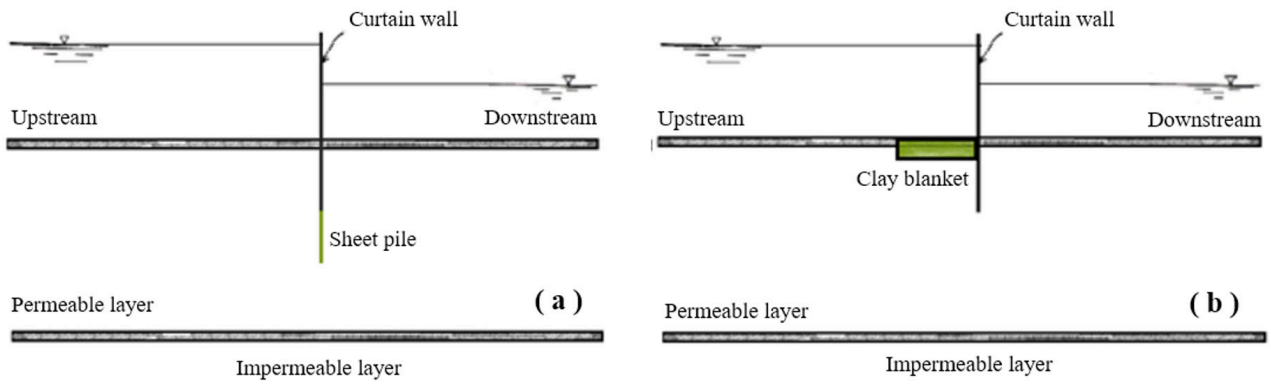

Figure 1. Schematic profile views of (a) sheet pile and (b) clay blanket examples

of material called alluvium, have a wide grain distribution range from fine sand to coarse gravel (Tosun, 2004). Having sufficient thickness of the sand and gravel layer reduces the problems in the direction of soil load transfer and structure stability to a certain level. However, the soil must be subjected to tests and analyzes due to its high permeability.

Dams, which are important hydraulic structures, together with their foundations, are structures that prevent the passage to the downstream side by creating a barrier to the water accumulating in the reservoir (Cilingir, 2007). In these structures, hydraulic load occurs as a result of the water level difference between the upstream and the downstream and seepages occur in different sections of the structure (Mohammed-Ali, 2011; Mesci, 2006). These seepages are one of the most fundamental problems affecting the stability of the structure. Seepage in the ground of structure is related to the ground medium, flow type, and limit conditions (Alghazali and Alnealy, 2015a, b, c). The seepage of water by eroding the ground causes the fine grains to be washed away and starts to be carried with larger diameter grains from the spaces formed by the movement of these grains. If this process permanently continues, the ground soil will have a porous structure. If the seepage velocity exceeds the critical value, erosion occurs on the foundation ground, which can cause dam collapse (Cilingir, 2007).

With the effect of the pressure caused by the seepage currents, the ground at the downstream boundary rises, cracks and wedge are formed in the core of the dam, and water channels are formed, creating the possibility of piping. Piping, which seriously damages the stability of the structure, is prevented by extending the seepage length by various methods (Mesci, 2006). If the amount of seepage exceeds the anticipated amount of water loss, the safety of the construction is under threat, and seepage that occurs in this context causes a swamp situation and increases the instability of the construction behavior to the maximum level (Eynur, 2004; Ullah et al., 2019). Lifting force increases in direct proportion to piping and the soil becomes more saturated with water and loses its bearing capacity (Eynur, 2004). The buoyancy force reduces the shear resistance that exists between the foundation of the structure and itself, and this situation loses the strength of the structure against overturning or sliding (Khalili Shayan and Amiri-Tokaldany, 2015). As a result of this situation, the ground of the hydraulic structure causes loss of life and property, and environmental destruction (Ullah et al., 2019). The Tigra Dam, located in India on August 4, 1917, collapsed due to seepage in the foundation and as a result of this destruction, approximately 1000 people lost their lives downstream of the dam (Ullah et al., 2019; Abay et al., 2015). The Teton Dam in the USA collapsed on June 5, 1976, for the same reason; It caused the death of 11 people and 13,000 cattle, with an estimated damage of approximately 2 billion USD (Ullah et al., 2019). It has been determined that many such collapses require a more critical and careful analysis and design of the building foundations to be built compared to other sections, and it has been stated that a problem existing in the foundation will cause the entire structure to be damaged or completely destruction (Mohammed-Ali, 2011).

Water losses due to seepage should remain within acceptable limits, if necessary, they should be removed without damaging the structure, and the related precautions should be taken into account during the design stage. The amounts and routes of possible leaks should be analyzed in detail and resolved within the framework of economical and safe conditions (Eynur, 2004). Determining the amount and route of seepage is the first step in solving the problem. Various sealing structures such as cutoff walls, source covers, downstream seepage blankets, and sheet pile trenches are used to control the resulting seepage, effectively containing water leakage at desired intervals (Figure 1.a). The low permeability layer (Figure 1.b), called the clay blanket, consists of clay material. The thickness, length, and permeability of this cover are the parameters that determine the effect of this method (Sedghi-Asl et al., 2012).

Sheet pile trenches are sealing trenches filled with cement grout or compacted clay fill, which are applied

to connect the impermeable core of a hydraulic structure foundation with the impermeable layer of the foundation. These trenches are used in shallow hydraulic structures to ensure a watertight connection between the structure's body and the foundation. If the depth of the permeable layer in the structure's foundation is high, it is necessary to utilize methods such as impermeable source covers, pressure relief wells, and downstream seepage piles (Eynur, 2004). Impermeable source covers are a common practice used to increase the seepage path and prevent leakage in soils with high permeability. As the length of the seepage path increases, there is a loss of head in the water particles, leading to a decrease in their energy.

Researcher Bligh (1910), who first declared the seepage length theory, defined the seepage length as the first seepage path in contact with the foundation, and assuming that the hydraulic slope along the seepage line is constant, the energy loss along this path varies linearly with the seepage length, thus stated that the Lifting force distribution is linear (Khalili Shayan and Amiri-Tokaldany, 2015). Assumed that the total seepage length is the sum of the horizontal and vertical distances traveled by a liquid particle from the upper bed level (Sedghi-Asl et al., 2012). Based on Bligh's theory, the hydraulic slope is assumed to be constant and equal to h/L_0 anywhere along the structure, where h represents the difference between the upstream and downstream water levels, while L_0 represents the flow seepage length. It is also suggested that the seepage factor $c=L_0/h$ should be equal to or higher than an optimum value so that the structure can resist any internal erosion. According to Bligh theory, Equation 1 given below is established:

$$L=LH+LV \quad (1)$$

Where LH, horizontal leakage length; LV is the vertical leakage length. Lane (1935), who examined more than 200 damaged hydraulic structures, formed the following Equation 2, and suggested that the horizontal and vertical seepage lengths should take different coefficients:

$$L=LH/3+LV \quad (2)$$

Lane (1935) proposed the weight-seepage theory, predicting that the vertical and horizontal flow of the seepage are different and that they lose more load in the vertical direction than in the horizontal. In this theory, in order to find the total seepage length, weight coefficients of 0.33 in the horizontal direction and 1.0 in the vertical direction are assigned and if the angle of the seepage line with the horizontal is higher than 45°, it is considered vertical, and if it is lower than 45°, it is horizontal (Sedghi-Asl et al., 2012; Khalili Shayan and Amiri-Tokaldany, 2015). Sedghi-Asl et al. (2012) created a hydraulic channel model in the laboratory by using Lane and Bligh methods to ensure seepage control. The shoreline made of trapezoidal stainless steel sheet is assumed impermeable and four upstream blankets of various lengths and four sheet piles of various depths are used. As a result of the experiments, the difference between the results of the Lane and Bligh methods was quite large and it was determined that the difference between the methods decreased with the increase in the blanket lengths and the sheet pile depth. When these two methods were compared, it was observed that the results of the Bligh method were in better agreement with the experimental data, and it was presented that this was due to the different seepage lengths applied in the two methods. Based on the results, it has been determined that the Bligh method provides an accurate and economical criterion in the safety control of structures against piping, and therefore it is presented that this method would be appropriate to use for the design of hydraulic structures installed in coastal sandy soils. It has been shown that the use of both seepage control methods together is more effective in reducing the lifting force, and it has been shown that the use of larger values of the blanket length and sheet pile depth gives more appropriate results with the experimental data and determines the optimum length values. Sedghi-Asl et al. (2010) determined that the seepage volume was effectively reduced by 60%, 70%, 75%, and 82%, respectively, by applying clay blankets of various lengths (d/D=0.2, 0.4, 0.6, 0.8) at different depths. It was determined that increasing the upstream blanket length resulted in a significant reduction in the outlet hydraulic slope.

Alghazali and Alnealy (2015a, b, c) investigated the effect of sheet pile position and inclination angle (Θ) on the uplift pressure in a physical laboratory model, based on a hydraulic structure located on a single and multi-layered soil. As a result of the study, they presented that a sheet piling inclined towards 45° upstream provides a very good efficiency by reducing the uplift pressure to 40.3%, and the seepage amount to 28.5% compared to the general situation (without sheet pile). It has also been observed that the use of a sheet pile with a slope of Θ=120° towards the downstream is beneficial in reducing the current gradient value to 5% and increasing the safety factor against piping to 3.18. On the other hand, it has been determined that the use of sheet piles both upstream and downstream significantly reduces the amount of leakage compared to other options. Alghazali and Alnealy (2015a, b, c) concluded that a sheet pile placed at 90° angle reduces the effective uplift pressure on the ground by 45% compared to the case without sheet piling, and that a sheet pile placed at 90° towards the upstream face of the system will reduce leaks compared to a sheet pile placed at 120°. They concluded that it was 42% more efficient.

Abedi Koupaei (1991) study examined the sealing structures with different methods and under various conditions, both by laboratory and numerical methods. Koupaei stated that the amount of lifting force estimated

using the theories of Bligh and Lane is less than both Khosla et al. (1936), and Finite Difference Methods (FDM). In the study of Sedghi-Asl et al. (2015), the effects of a sheet pile position on reducing seepage and flow rate under hydraulic structures were investigated by using the Finite Difference Method, and it was determined that the best position for sheet piling was upstream and downstream, respectively.

In a study by Ahmed (2011), the effects of different sheet pile configurations on buoyancy, seepage flow, and reduction of outlet slope were investigated in order to reduce leakage losses from channel beds and to design stable hydraulic structures. Consequently, it is recommended to build a clay (or very low permeability soil) core on the inner edge of the embankments. Khalili Shayan and Amiri-Tokaldany (2015) stated in their study that increasing sheet pile depth causes an increase in the leak length, a decrease in the average hydraulic slope, and thus a decrease in the amount of leakage. In this study, it was also determined that as the distance between the sheet pile and the impermeable blanket gets smaller, the increasing flow line creates a resistance and reduces the leakage, and when the sheet pile is placed at the downstream end, the leakage flow rate reaches its minimum value. In addition, in the study, it was calculated that the sheet pile placed on the upstream side affected the buoyancy force more than the upstream blanket. Khalili Shayan and Amiri-Tokaldany (2015) investigated the effects of downstream blanket and sheet pile on seepage flow, outlet slope and buoyancy reduction using a physical model created in the laboratory and two datasets obtained from GeoStudio (2007) software. Using data from these laboratory experiments, it has been observed that the best position of the sheet pile used to reduce seepage flow is at the downstream end. In addition, in the study of Khalili Shayan and Amiri-Tokaldany (2015), it has been determined that the best position of the sheet pile is the upstream side to reduce the amount of uplift pressure. The effects of sheet pile inclination on seepage flow, outlet slope, and buoyancy were evaluated using the software, and it was presented that the optimum inclination angle depends on the sheet pile position and length Khalili Shayan and Amiri-Tokaldany (2015). It was also observed in Khalili Shayan and Amiri-Tokaldany (2015) study that the effect of a sheet pile placed at the downstream end on reducing the outlet slope was greater than that of an upstream blanket. Zainal (2011) investigated the effects of the shear wall on the seepage formed in the dam foundation and concluded that the best angle to minimize the seepage flow rate, uplift pressure, and outlet slope is approximately 60°, 120° to 35° and 45° to 75°, respectively.

Ullah et al. (2019) discussed, using SEEP/W, the effect of the use of sheet pile and filter flow blankets on reducing the amount of seepage under the foundation. Ullah et al. (2019) used sheet piles, which are widely used in practice, were used under the conditions of varying depths (5m, 7.5m, and 10m) and thickness (0.5m, 1m) for leakage reduction. In the study Ullah et al. (2019), it was determined that the leakage reduction for 5 m, 7.5 m, and 10 m lengths of 0.5 m thick sheet pile decreased by 1.05%, 10.15%, and 19.75%, respectively, compared to the general situation. In sheet pile models with the same lengths and 1 m thickness, leakage improvement efficiencies were obtained as 2.29%, 12.37%, and 21.36%, respectively (Ullah, 2019). In the same study, U/S impermeable clay blankets were also used in the study to reduce the hydraulic slope and the amount of seepage in the downstream section. They modeled the 1 m thick U/S clay blanket for various lengths (50 m, 100 m, 150 m, 200 m, 250 m, and 300 m). Under the conditions where the U/S clay blanket is used (50 m, 100 m, 150 m, 200 m, 250 m, and 300 m respectively), there is a significant reduction in seepage volume compared to the dam section without control measures (55.14%, 58.65%, 59.65%, 60.44%; 60.10% and 60.14% respectively).

According to the results obtained from this study, it is possible to reduce the seepage that occurs in the foundation of hydraulic structures by taking various precautions. In addition to the seepage volume and uplift pressure examined in the studies, it is also necessary to calculate the seepage length, which should be considered in engineering calculations. In this study, unlike the literature, the seepage length parameter was investigated with two different grain diameters and application types. Seepage length, downstream transition time, and seepage path obtained by using sheet pile and upstream clay blanket related parameters were investigated with the physical model. In this way, the risk of collapse of hydraulic structures due to seepage will be eliminated and possible environmental disasters that may occur due to collapse will be prevented. In addition, since the surface waters are decreasing significantly due to global warming, the measures against the loss of water accumulated in the reservoirs by seepage into the soil in order to obtain the drinking water needed for the settlements, irrigation, and utility water needed for agriculture and livestock have been put forward in this study.

MATERIALS AND METHODS

In this study, it was aimed to examine the effect of the use of sheet pile curtains and upstream clay blankets on the amount and length of seepage in order to prevent seepage under hydraulic structures, with physical models in the laboratory in different soils (different grain diameters (d_1=0.85mm and d_2=1.5mm)). In the experiments carried out in Bursa Technical University Hydraulics Laboratory, a box-shaped apparatus made of plexiglass material with dimensions of 30x30x15cm and a thickness of 5mm was used (Figure 2). The aim of the studies is to examine the behavior of seepage lengths

under hydraulic structures in different soils (different grain diameters). It has been examined how the water injected from two different points follows under normal conditions, in sheet pile condition, and in the presence of upstream blanket. The effect of the injected trace material on the seepage length of the sheet pile and upstream blanket is discussed, taking into account the transition times from upstream to downstream. In order to keep the upstream and downstream water levels determined during the experiment, a submersible water pump designed to operate in water and to prevent water leakage in the motor body was used. Soil samples with two different grain diameters were used in the experiments and the median grain sizes were determined as a result of the sieve analysis (d_1=0.85 mm and d_2=1.5 mm). A plexiglass piece with 11 cm length, 15 cm width, and 5 mm thickness representing the hydraulic structure cross-section was used in the middle of the assembly (x= 15 cm distance). In the study, 5 cm long, 15 cm wide, and 5 mm thick sheet piles and a 6 cm long and 1.5 cm thick upstream blanket were used to reduce seepage under the structure. Blue (Point B) and red (Point A) dyes were used as trace material in the experiment and the dye tracer was injected from two different designated points (A and B) of the sand-filled assembly using a 5 ml syringe (Figure 2).

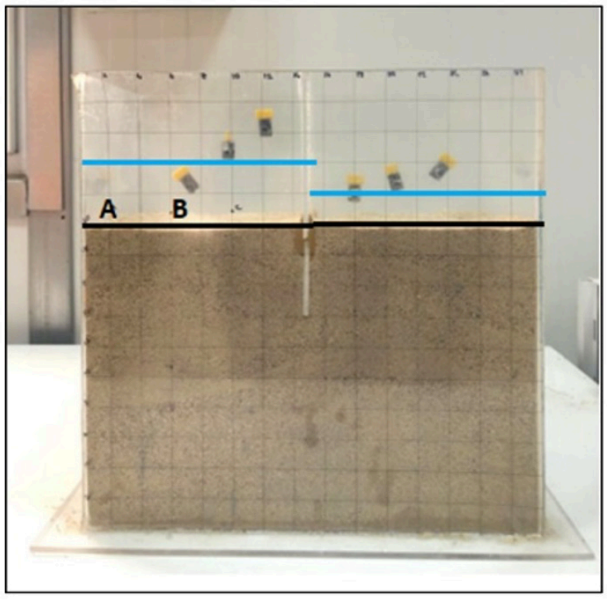

Figure 2. The experimental setup profile view

In the experiment, first of all, the ground levels determined according to the dimensions of the physical model were filled up to 20 cm in an equal way from the upstream and downstream sides. Water was added up to 24 cm on the upstream side and a submersible water pump was operated so that the water level did not exceed 22 cm at the downstream side. The reason for determining the level difference between the water levels is to create a hydraulic load, allowing seepage from the upstream side to the downstream side. After adjusting the amount of water, the trace injection points were selected. By injecting red dye from point A (2,0) where the most distant but comfortable observation can be made, and blue dye from point B, which is approximately in the middle of the upstream side, closer to the obstacle (6,0) at 30 second intervals, the progress of the trace materials is horizontal and vertical coordinate measurements were made (Figure 3). The horizontal and vertical coordinates of the seepage trajectory of the trace materials were determined depending on the time by marking the grids on the transparent front wall of the experimental setup. At the end of each test, the entire sample was emptied and washed in order to evacuate the dyes that may have remained in the ground, and the same volume of sand was placed in the apparatus to start the new test. When the tracers reached the downstream ground level, the experiments were terminated and a flow net image was obtained from the coordinates obtained on the plexiglass. In order to observe the effect of the grain diameter on the critical seepage length and the effect of the sheet pile shear and the clay blanket on the seepage length, the summary of 12 experiments is presented in Table 1.

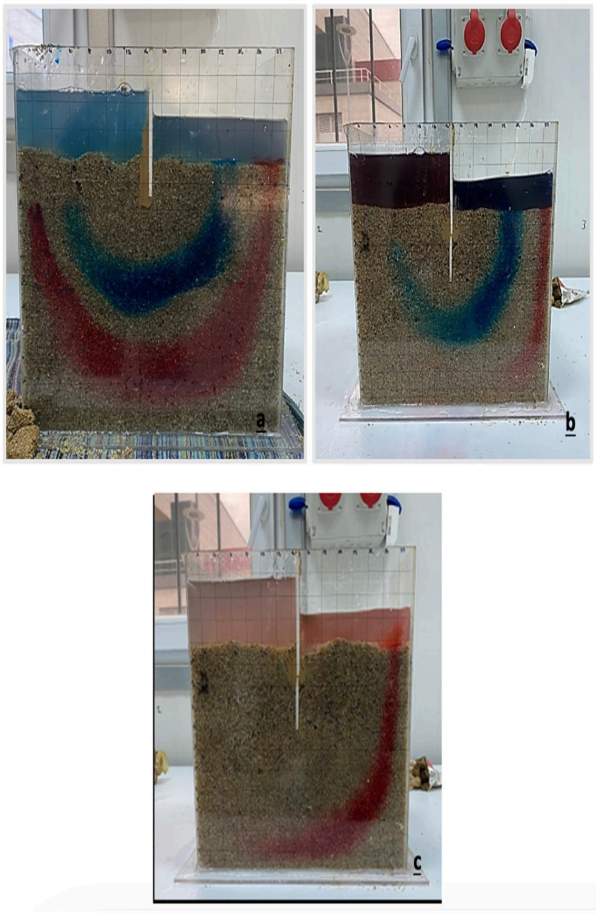

Figure 3. For identical grain diameters having experiments a) Clay blanket points A and B b) Sheet pile point B c) Sheet pile point A

Table 1. Summary of experiments

Application point	Ground 1 (d_{50}= 0.85mm)	Ground 2 (d_{50}= 1.5 mm)
Point A (2,0)	General situation Upstream blanket Sheet pile	General situation Upstream blanket Sheet pile
Point B (6,0)	General situation Upstream blanket Sheet pile	General situation Upstream blanket Sheet pile

RESULTS AND DISCUSSION

In the seepage tests performed for the same grain diameter and at the same points, it was observed that the application of clay blanket and sheet pile had the effect of increasing the seepage time and seepage length at both points ((2,0), (6,0)). In the experiment in which the sheet pile was used, it was determined that the seepage times and seepage length values increased significantly at both points when compared to the other experiments with the same grain diameter. In this study, it was calculated that the seepage length determined for the same grain diameter (d_1=0.85 mm) increased by 46.45% compared to the general situation (Table 2 (data) and Table 3 (comparison)). When the upstream clay blanket used in the experiments is compared to the general situation, it is observed that the seepage time and length increase in a high amount at the point (6,0), which is close to the dam cross-section, while the (2,0) point, which is relatively far from the dam cross-section, shows the percentage of seepage length despite the high increase in seepage time. It was observed that the increase was smaller (Table 2). The probable reason for this situation is that, as seen in Figures 4 and 5, the clay blanket placed horizontally on the upstream section is not close enough to affect the seepage trajectory of the trace material injected from the point (2,0) which is far from the dam section.

When the experiments to increase the seepage length were evaluated among themselves, it was seen that the use of sheet pile was more effective than the use of clay blanket. In the experiments carried out at (2.0) in soil with a d_1=0.85mm grain diameter, the use of sheet pile increased the seepage time by 4.5% and the seepage length by 31.4% compared to the use of upstream blanket. The percentage of seepage length increase of -1.5% in Table 3, may be due to the small

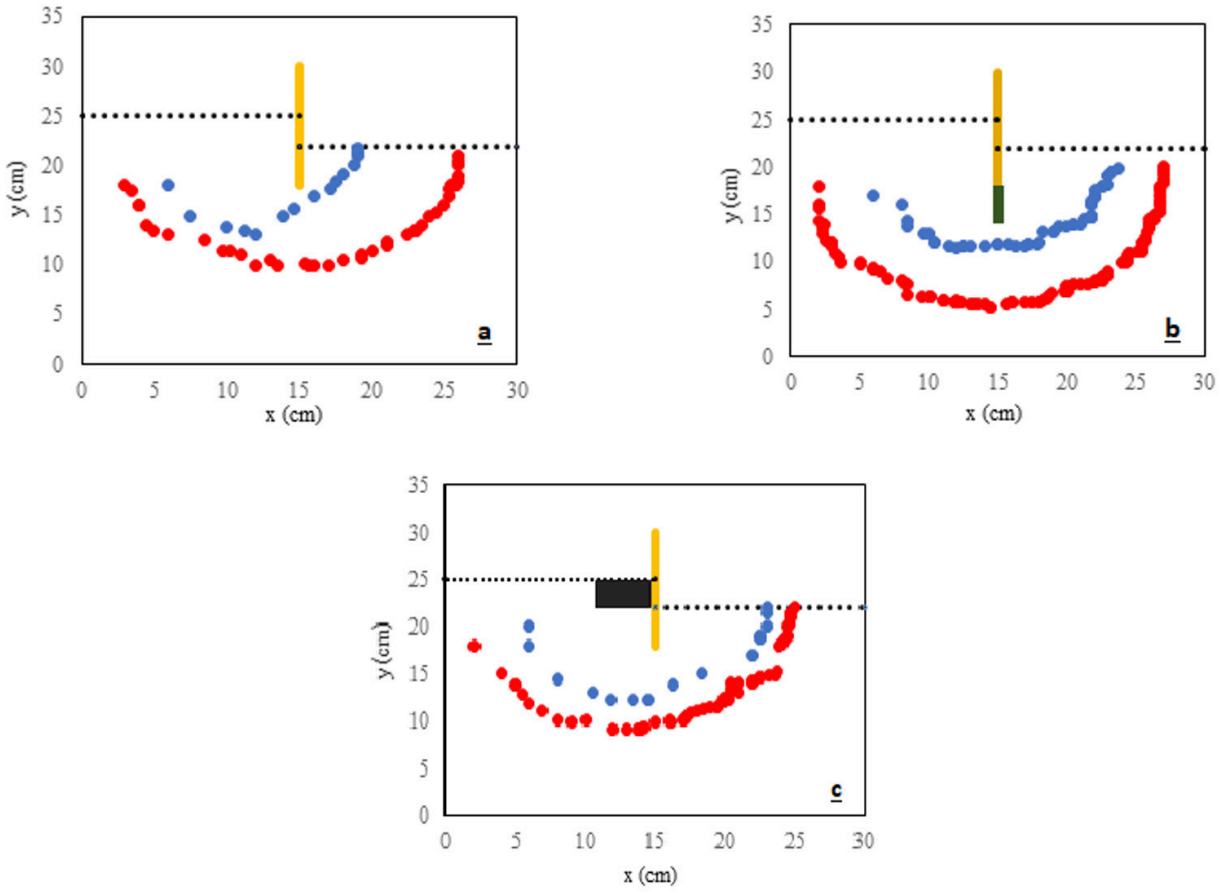

Figure 4. a) General b) Sheet pile c) Upstream clay blanket system test plots (for d_1=0.85mm)

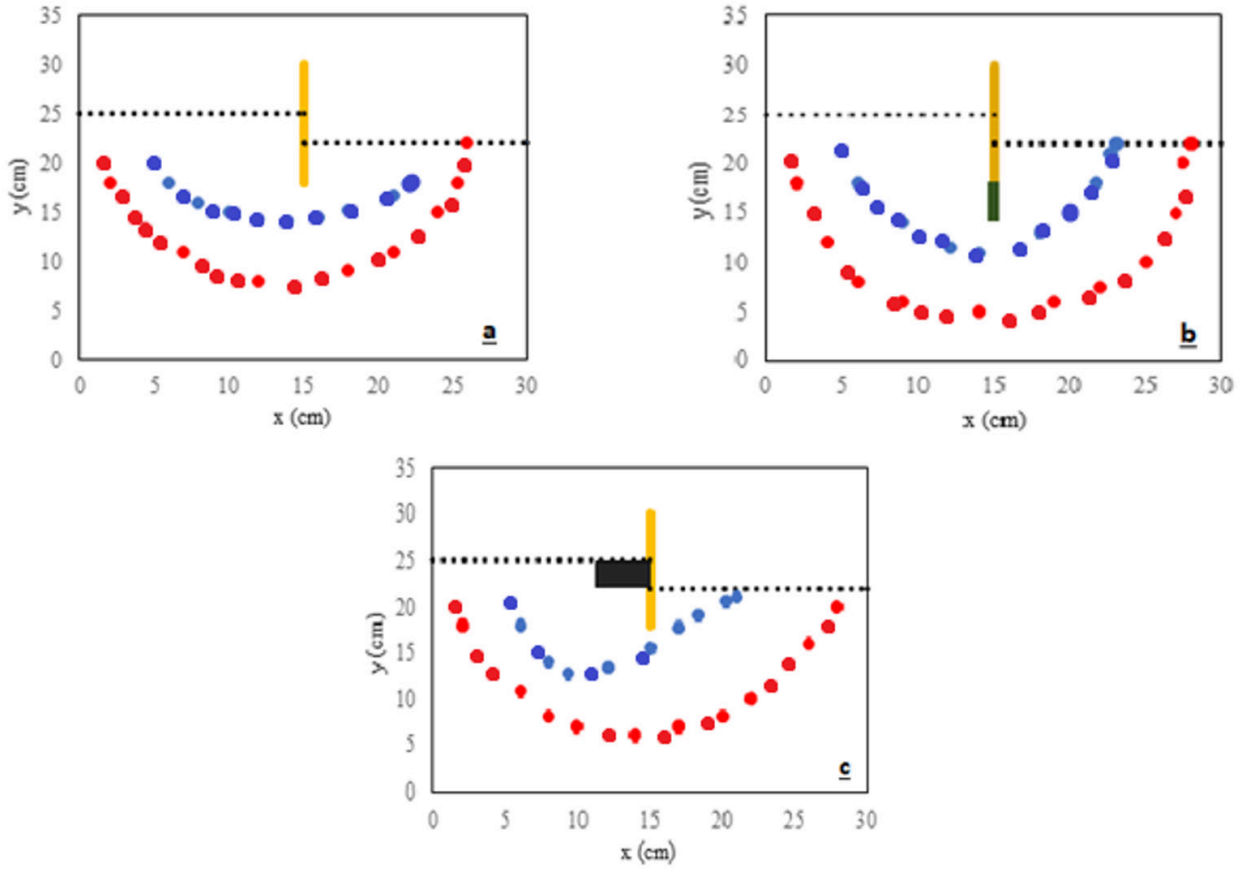

Figure 5. a) General b) Sheet pile c) Upstream clay blanket system test plots (d_2=1.5mm)

Table 2. Summary of experimental results

Grain diameter (mm)	Application point	Method	Seepage time	Seepage length (cm)
0.85	(2,0)	General situation	44min 10sec	35.31
		Upstream blanket	54min 5 sec	38.28
		Sheet pile	56min 30sec	50.30
	(6,0)	General situation	8min 12sec	19.86
		Upstream blanket	15min 57sec	27.29
		Sheet pile	25min 15sec	26.87
1.5	(2,0)	General situation	4min 3sec	36.45
		Upstream blanket	5min 3sec	38.86
		Sheet pile	5min 18sec	44.33
	(6,0)	General situation	48sec	16.76
		Upstream blanket	2min 5sec	21.08
		Sheet pile	2min 17sec	25.90

experimental deviation. In the tests carried out at (2.0) in soil with d_2=1.5mm grain diameter, the rate of seepage time was 5.0% and the seepage length was 14.1%; in the experiments performed at the point (6.0), it was observed that the seepage time increased by 9.6% and the seepage length increased by 22.9%.

When the tests performed on the same grain diameter and at different points (2,0 and 6,0) are compared, it is seen that the obstacles to increase the seepage length at the point close to the dam cross-section (6,0) are approximately 7-8 times more effective in the downstream exit time of the tracer. In the experiment with sheet pile, when compared to the general situation, the seepage time increased by 27.9% in the experiments performed at the point (2,0) on the soil with a d_1=0.85mm grain diameter, while this increase was 207.9% in the experiment performed at the point (6,0). While the seepage time increased by 30.6% in the experiments performed at (2,0) in soil with d_2=1.5mm grain diameter, the seepage time increased by 185.4% in the experiments performed at (6,0). When the experiment with a blanket is compared to the general situation, the seepage time increased by 22.5% at the point (2,0) in the soil with

Table 3. Experiment comparison table (according to the method)

Grain diameter (mm)	Main experiment	Compared experiment	Application point	Increase in seepage time to downstream (%)	Seepage length increase (%)
0.85	Sheet pile	General situation	(2,0)	27.9	42.50
	Upstream blanket	General situation	(2,0)	22.5	8.40
	Sheet pile	Upstream blanket	(2,0)	4.5	31.40
	Sheet pile	General situation	(6,0)	207.9	35.29
	Upstream blanket	General situation	(6,0)	94.5	37.40
	Sheet pile	Upstream blanket	(6,0)	58.3	-1.50
1.5	Sheet pile	General situation	(2,0)	30.6	21.60
	Upstream blanket	General situation	(2,0)	24.7	6.60
	Sheet pile	Upstream blanket	(2,0)	5.0	14.10
	Sheet pile	General situation	(6,0)	185.4	54.50
	Upstream blanket	General situation	(6,0)	160.4	25.70
	Sheet pile	Upstream blanket	(6,0)	9.6	22.90

Table 4. Experiment comparison table (according to grain diameter)

Grain diameter (mm)	Compared grain diameter (mm)	Method	Application point	Increase in seepage time to downstream (%)	Seepage length increase (%)
0.85	1.5	General situation	(2,0)	990.5	-3.10
		Upstream blanket	(2,0)	970.9	-1.50
		Sheet pile	(2,0)	966.0	13.47
		General situation	(6,0)	925.0	18.49
		Upstream blanket	(6,0)	665.6	29.45
		Sheet pile	(6,0)	1005.8	3.70

d_1=0.85mm grain diameter, while the seepage time increased by 94.5% at the point (6,0). While the seepage time increased by 24.7% in the experiments performed at (2,0) in soil with d_2=1.5mm grain diameter, the seepage time increased by 160.4% in the experiments performed at (6,0) (Table 4).

In the experiments, when the soil with d_1=0.85mm grain diameter is passed to the soil with d_2=1.5mm grain diameter, the permeability of the soil also increases, and the transition time of water from the upstream to the downstream side is greatly reduced. In the experiments performed on the same system by changing only the grain diameter, in all experiments at the point (2,0), the seepage time was 990.5%, 970.9%, 966% in the experiments at the point (6,0), it was observed that the seepage time decreased by 925%, 665.6%, and 1005.8% for the systems with general condition, upstream blanket and sheet pile, respectively (Table 4). When the seepage lengths are compared, in the experiments performed at the point (2,0), it was observed that the general situation, the system with the upstream cover and the sheet pile showed a decrease of -3.1%, -1.5% and 13.47%, respectively. Due to the relatively small dimensions of the laboratory physical scale, it is thought to cause small experimental deviations that can cause negative values. In the experiments performed at the point (6,0), the seepage time decreased by 18.49%, 29.45%, and 3.7% for the systems with the general condition, upstream blanket, and sheet pile, respectively.

CONCLUSION

Seepage is one of the main factors limiting the project lifespan of hydraulic structures and it plays a very important role in reducing seepage, protecting the collected water, and eliminating the risk of collapse of the hydraulic structure. Seepage, especially starting from under the structure, causes the risk of collapse in the structure and has the potential to cause environmental disasters, especially in dams and similar hydraulic structures. For this reason, it is necessary that the seepage length passes as far from the foundation of the structure as possible and in connection with this, the seepage length must be increased. For this reason, it is necessary that the seepage length passes as far from the foundation of the structure as possible and in connection with this, the seepage length must be increased. This subject, which was discussed in the study, was investigated on

soils with different diameters by using sheet pile and clay blanket. Both the grain diameter effect and the barriers used to increase the seepage length were examined in detail and it was determined that these barriers contributed greatly to the seepage time and length. As a result of all the experiments, the experiments with obstacles placed to increase the seepage length in soil with d_2=1.5 mm grain diameter were completed in a shorter time than the general test of soil with d_1=0.85 mm grain diameter. In this case, it was concluded that the soil grain diameter was more affected by the seepage time by obstacles such as sheet pile and clay blanket. The test with the longest seepage time is the one in which the sheet pile is used on the soil with a grain diameter of d_1=0.85 mm and the dye tracer is started from the point (2,0) which is far from the dam cross section. As a result of these experimental studies, when the examined seepage prevention methods were compared, it was determined that the most effective method for reducing seepage under the dam was to reduce the grain diameter and add sheet pile under the foundation. It is also expecting from these seepage preventing arrangements that they would be helpful for maintaining lesser environmental disaster risk related to dam etc. collapse, and/or keeping more mass of water in the reservoir with lesser leak.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of interest

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

Author contribution

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

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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