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Research article

Monitoring of heavy metals and essential trace elements in aquatic plant *Ranunculus sphaerospermus* Boiss. & Blanche (Ranunculaceae), sediments, and water of volcanic Haydarlar Lake, Türkiye

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Abstract

Aquatic ecosystems contain communities of organisms that are dependent on each other and on their environment. Monitoring of trace element and heavy metal concentrations is important to understand the possible environmental risks in natural aquatic environments. In the present study, concentrations of some heavy metals and trace elements in aquatic plant *Ranunculus sphaerospermus*, sediments, and water samples of volcanic Haydarlar Lake were analyzed by using ICP-OES. The concentrations were found in the following ranges: 82.11 - 97.38, 9174.50 - 9942.29, and 0.63 - 0.89 for Al; 10.29 - 17.43, 30.60 - 55.60, and 0.81 - 0.98 for B; 1038.44 - 1682.30, 4017.26 - 4503.54, and 1276.61 - 1541.41 for Ca; 120.69 - 178.41, 6894.50 - 8103.47, and 0.51 - 0.69 for Fe; 2503.51 - 2983.38, 1118.50 - 1693.38, and 69.43 - 93.82 for K; 563.38 - 783.22, 885.32 - 1122.47, and 108.55 - 143.36 for Mg in the plant (mg kg⁻¹), sediment (mg kg⁻¹), and water (mg L⁻¹) samples, respectively. The concentrations of Ca, Fe, and K elements in sediments and the content of Ca and K in lake water samples were found as higher than the acceptable limit, while concentrations of all elements in *R. sphaerospermus* were determined to be within acceptable limits. Transfer factors (TF) of the heavy metal and essential elements from sediment to the plant samples were evaluated. The trends of TF for all samples studied were in the following order; K>Mg>B>Ca>Fe>Al. Consequently, the approach used in this study could contribute to pollution monitoring in the future.

Keywords: Accumulation; aquatic plants; lava flow area; mineral nutrients; transfer factors; water daisy

1. Introduction

Ranunculus sphaerospermus Boiss. & Blanche (Ranunculaceae) is submerse type, herbaceous, annual orbiennial aquatic plant. The leaves in the water are filamentous, the roots are in the sediment, and the white flowers are on the surface of the water. Its laminate leaves are absent. Submerged and spreading capillary leaves, numerous and rigid segments, usually do not collapse when removed from the water. Peduncle of fruit is 5-6 cm. Petals of flower are broadly obovate shaped, contiguous throughout anthesis, 9-25 mm; nectar pit elongated, more or less pyriform. Receptacle hairy, somewhat elongated in fruit. Carpels are shorter than 1 mm, somewhat rounded,

glabrous, or slightly hairy around the base of the style. It spreads in shallow and still or fresh waters with low water flow rates, in puddles, swamps, and lake edges. It is native to the Balkans, South and West Asia, Egypt, Himalayas in alt. 0-1730 m. Its flowering time is January-September. *R. sphaerospermus* (water daisy) is known as "su çiçeği", "su düğünçiçeği" and "su papatyası" and is widely distributed in Türkiye (Cook, 1965; Guner, 2012a; Tas and Topaldemir, 2021).

Like other plants, water daisy needs macroelements (N, Ca, P, K, Na, Cl, Mg, etc.) and trace elements (Fe, Zn, Cu, Mn, Al, B., etc.) for their structure, growth, and metabolic activities in maintaining of proper life. Their uptake from the environment is crucial because these elements cannot be synthesized

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metabolically by the organism itself (Zeiner and Cindrić, 2017; Hocaoglu-Ozyigit and Genc, 2020). For instance, Boron (B) is one of these elements, an essential nutrient for vascular plants, cyanobacteria, algae, fungi and animals and has a largely structural role in development and other metabolic functions in organisms (Herrera-Rodríguez et al., living 2010). Concentration of B ranges between 0.1–0.5 mg/L⁻¹ in freshwaters and 10-300 mg kg⁻¹ in soils depending on amount of rainfall, amount of organic matter, soil type and pH (Howe, 1998). In plants, B plays important roles in seed and fruit formation, pollen health, fertilization, protein synthesis, formation and transport of carbohydrates, Calcium (Ca) transportation, and formation of hormones especially auxins in plant metabolism (Jones and Jacobsen, 2012). Deficiency of B causes vegetative and reproductive growth, necrosis, the significant losses in crop quality and yield, reduces the plant fertility, and inhibits the cell expansion in plants (Herrera-Rodríguez et al., 2010; Vatansever et al., 2017). Excess amounts of B leads to significant changes in the structure, physiology, and activity of numerous enzymes and consequently, has negative effects such as altered metabolism, osmotic imbalance, reduced root cell division, increased membrane leakiness in metabolism during the life cycle of plants (Camacho-Cristóbal et al., 2008; Herrera-Rodríguez et al., 2010; Vatansever et al., 2017). Ca serves as an essential element and is taken by the root tips of plants as Ca⁺² ions. Its concentration in the plants varies between 1000 to 20000 ppm (Tewari et al., 2021). Ca plays roles in the development of the end points of plant tissues, the formation of roots and flowers, the structural and physiological stability of plant tissue, as well as cell division, cell wall formation, cell expansion and activation of enzymes (Aydin, 2017). Ca-deficient plants show various symptoms such as blossom-end rot of fruits, necrosis in young leaves, cell breakdown, and loss of membrane integrity (Olle and Bender, 2009). The term heavy metal is generally used for metals such as arsenic, iron, cadmium, nickel, copper, mercury, chromium, manganese, and lead with a density higher than 4-5 g cm^{-3} (Caparrós et al., 2022). However, aluminum (Al) with a density of 2.70 g cm⁻³ was presented in the heavy metal category due to its contribution to pollution and toxicity according to the results of many research studies (Ghori et al., 2019; Ozyigit et al., 2022; Riyazuddin et al., 2022). In the meantime, although it is not considered as an essential nutrient, low concentrations can sometimes affect plant growth or induce some other necessary metabolic activities. Common effects of Al toxicity include decrease in shoot biomass, total leaf number and size, photosynthetic activity, chlorosis and/or necrosis of leaves, and decrease (Ozyigit et al., 2013; 2019; Dogan et al., 2014). However, slightly higher Al intake is not only very toxic for plants but also is harmful to gill-breathing aquatic animals such as fish, amphibians and some arthropods, causing osmoregulatory failure by destructing the hemolymph ions and plasma (Jaishankar et al., 2014). On the other hand, iron (Fe) is a crucial essential micronutrient for plant growth and development. Its deficiency leads the interveinal chlorosis in plant leaves and reduces crop productivity (Vatansever et al., 2017). In addition, magnesium (Mg) is the most important element for chlorophyll structure, photosynthesis, fat formation, protein synthesis, uptake and transport of phosphorus in the plant body. Mg deficiency can cause reduction in photosynthesis and plant growth (Aydin, 2017). Potassium (K) plays roles in metabolic processes such as photosynthesis, protein synthesis, and carbohydrate translocation. K deficiency can cause

regression in plant resistance mechanism, development, and photosynthesis (Jones and Jacobsen, 2012; Aydin, 2017).

During the last few decades, many studies have focused on trace element analyses and heavy metal accumulation in environment since monitoring of pollution and its effect on plant and animal species in natural habitats is vital (Sungur et al., 2013; Ozturk et al., 2017; Ghori et al., 2019; Ozturk et al., 2020; Haq et al., 2021; Ozyigit, 2021; Ozyigit et al., 2022). However, studies on mineral nutrient and heavy metal accumulations in organisms living especially in aquatic ecosystems are very limited (Kaptan and Tekin-Ozan, 2014; Yilmaz et al., 2015; Yilmaz et al., 2021a,b).

The aim of the present work was to determine some heavy metals (Al and Fe) and essential trace element (B, Ca, Mg, and K) concentrations and correlation between levels of their accumulation in sediments, water and plant tissue of *R. sphaerospermus* from the Haydarlar Lake. Also, in this work, the transfer coefficient of the studied elements from sediment to the plant samples was calculated by dividing the concentration. Since transfer factor (TF) is an indicator of heavy metal mobility in soils and quantifies the existing variations in the bioavailability of elements to plants, the knowledge of the optimum TF of heavy metals and trace elements from soil to plant is of crucial importance.

2. Materials and methods

2.1. Sampling and study area

The present study is based on the content of some trace elements and heavy metals of widely distributed aquatic plant *Ranunculus sphaerospermus*, sediments, and lake water. The plant, sediment and lake water samples used in this study were collected from six different localities of Haydarlar Lake (Hatay province) in the East Mediterranean region of Türkiye during vegetation period in March-April 2021 (Fig. 1).



Fig. 1. Ranunculus sphaerospermus, (a) Habitus (b-d) General view, (e) flower.

Hatay province, has diverse geographical, topographical, and ecological features that include mountains, plains, rivers, and lakes with a rich biodiversity. A number of ethnobotanical,

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ecological, palynological, and genetic diversity studies have been carried out for Hatay province and its vicinity (Altay and Karahan, 2012; Karahan et al., 2012; Ilhan et al., 2017; Altay et al., 2018; Alsaadi et al., 2020).

Volcanic Haydarlar Lake is located in the northern part of Hatay Province (36°44' N-36°32' E) near Syria border line and its altitude is 313 m. a.sl. The surface area of the lake changes from 50 to 75 ha seasonally with a maximum depth of 15 m. in winter seasons (Fig 2). The main habitat type around the lake consists of the lava flow area, which is assumed to have formed throughout the 5 periods between 1.57 million and 26 thousand years ago and is called "Leçelik" in Turkish (Belguzar, 2017). The study area has a climate type close to the oceanic climate, semi-humid-semi-arid, medium temperature which is (Mesothermal) with a mean annual rainfall of about 684.8 mm and an annual temperature of 17.7°C. Annual precipitation is at a maximum level in winter, while it decreases in spring. The mean minimum and maximum temperatures are measured as 6.3 and 28.9°C, respectively in January and July (Aytac and Semenderoglu, 2014; Altay et al., 2016; Topuz et al., 2016). It is used for irrigation and farming by local people.



Fig. 2. The study area (up) and localities (down) of the studied aquatic plants *R. sphaerospermus* samples.

For the present study, the plant samples were dried and identified according to related literatures (Cook, 1965; Guner, 2012b). The sampling bottles were immersed about 10 cm below the water surface. Approximately 0.5 L of water for each sampling site were collected. Samples were added 10% HNO_3 and transferred to the laboratory by placing in an ice bath. Sediment samples were also collected using a grab sampler and transported to the laboratory.

2.2. Sample preparation

Plant samples of *R. sphaerospermus* were weighted and then dried in an oven at 80°C during 2 days. Samples were ground and passed through 1.5-mm sieve after the drying process. The grinding device was cleansed with 96% ethanol and distilled water after each process. The samples were scaled to 0.2 g and put in Teflon vessels, and 10 ml of 65% HNO₃ for plant samples and 6 ml of 65% HNO₃, 3 ml of 37% HCl and 2 ml of 48% HF for sediment samples were added into each vessel. Water samples are transferred to a new Falcon tube containing 1% HNO₃ for appropriate dilution. Following this, the plant and sediment samples were digested at 165°C using Mars Microwave and left to cool following the microwaving process. The samples were then transferred into conical 50 ml centrifuge tubes also carefully being washed using distilled water and then filling the tubes up to 50 ml (Karahan et al., 2020).

2.3. Determination of Al, B, Ca, Fe, Mg, and K

Ultrapure chemicals of analytical grade were used for this study. Water (Human-Zener Power I) was used as a solvent in dilution procedures in the all experimental steps of the study. For linearity, elemental values of plant, sediment and water samples were measured in triplicate. The EPA 3051A analytical method for ICP-OES was applied using MARS microwave to dissolve the all samples. The B, Al, Fe, Ca, K, and Mg concentrations of the samples were established using ICP-OES (PerkinElmer Optima 7000 DV).

2.4. Transfer factors (TF) of the elements from sediment to the plant samples

The transfer coefficient was calculated by dividing the concentration of heavy metal and mineral nutrient elements in plant samples by the total heavy metal concentration in the sediments (Chizoruo et al., 2017).

$$TF = \frac{Cplant}{Csediment}$$

Where, Cplant = element concentration in plant tissue, mg kg^{-1} fresh weight and Csediment = element concentration in sediment, mg kg^{-1} dry weight (mg kg^{-1} Dw).

3. Results

In this study, trace element (boron, magnesium, phosphorus and potassium) and heavy metals (aluminum and iron) concentrations were determined for *R. sphaerospermus*, as well as for its habitat, from different six sampling localities of Haydarlar Lake sediments and water, and results are presented in Table 1 and Fig. 2.

According to our results, the content of Al was found as

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Table 1

Trace element and heavy metal concentrations in R. sphaerospermus, sediments and lake water samples and legal concentration limits.

			samples (mg kg ⁻¹) (mean			
LOC. NO	Al	В	Ca	Fe	K	Mg
21	86.47	13.56	1275.88	141.22	2741.68	641.11
	± 1.83	± 0.20	± 19.99	±1.23	± 30.43	±16.45
22	82.11	10.29	1038.44	120.69	2503.51	563.38
	± 2.06	±0.23	± 14.17	± 2.92	± 34.83	± 14.49
3	91.23	15.79	1389.51	155.02	2833.59	683.41
	±1.23	± 0.16	± 11.49	± 2.87	± 22.01	±19.38
24	83.20	11.23	1163.48	132.51	2673.21	581.20
	±1.59	± 0.14	± 18.26	± 2.38	± 68.57	± 10.95
? 5	88.22	14.01	1493.21	146.63	2796.00	703.23
	± 1.47	± 0.26	± 26.10	±1.31	±43.27	±17.25
6	97.38	17.43	1682.30	178.41	2983.38	783.22
	± 1.68	± 0.27	± 13.34	±2.26	± 74.56	± 13.00
Iean	88.10	13.72	1340.47	145.74	2755.23	659.26
lean	±1.64	±0.21	±17.23	±2.16	± 45.61	±15.25
egal limits ^a	15-100	3–90	1000-20000	50-200	3000-10000	100-15000
		Sedimer	nt samples (mg kg ⁻¹) (me	an ± SD)		
OC. NO	Al	В	Ca	Fe	K	Mg
1	9464.03	38.81	4277.08	7552.66	1397.71	974.06
	±214.76	± 0.55	± 88.84	±131.39	±32.66	±15.29
2	9174.50	30.60	4017.26	6894.50	1204.50	904.51
	± 196.87	± 0.75	± 98.77	± 175.58	±27.68	± 18.11
3	9748.41	44.17	4387.88	7748.21	1247.55	955.32
•	±142.12	±0.51	±76.89	±113.44	±15.90	±15.79
4	9355.69	33.07	4184.31	7409.56	1118.50	885.32
•	±229.64	±0.43	±84.32	±127.30	±25.29	±19.58
5	9641.29	45.84	4421.69	7794.31	1408.41	1043.69
	±189.72	±0.56	±51.69	±138.29	±18.55	±22.30
6	9942.29	55.60	4503.54	8103.47	1693.38	1122.47
	±225.99	± 0.69	±70.47	±108.22	±40.52	±21.01
	9554.37	41.35	4298.63	7583.78	1345.01	980.89
Iean	±199.85	±0.58	±78.50	±132.37	±26.77	±18.68
egal limits ^b	10000-40000	2-100	1440–2867	5-10	200-250	1000-40000
legal mints	10000-40000		samples (mg L ⁻¹) (mean		200-230	1000-40000
OC. NO	Al	B	Ca	Fe	K	Mg
V1	0.68	0.84	1377.63	0.60	77.88	122.43
	±0.02	±0.02	±18.36	±0.01	±1.71	±1.08
V2	0.63	0.89	1298.50	0.51	69.43	108.55
. 2	±0.01	±0.02	±23.59	±0.01	±1.13	±2.27
V3	0.72	0.81	1421.29	0.57	71.43	131.23
15	±0.02	±0.01	±20.10	±0.01	±1.34	±1.07
V4	0.69	0.82	1276.61	0.61	81.36	115.58
-	±0.02	±0.02	±17.88	±0.01	±0.90	±1.65
75						
V5	0.77 ±0.01	0.91 ± 0.02	1486.56	0.62 ± 0.02	85.60 ± 1.21	127.78
			±29.93			±2.66
V6	0.89	0.98	1541.41	0.69	93.82	143.36
	±0.02	±0.02	±14.14	±0.02	±1.56	±2.32
lean	0.73	0.87	1400.33	0.60	79.92	124.82
	±0.02	±0.02	±20.67	±0.01	±1.31	±1.84
Legal limits ^c	0.3–1	<1	75-800	0.3–5	20–50	50-150

^aKarahan et al. (2020), ^bKabata-Pendias and Mukherjee (2007), ^cUnited States Environmental Protection Agency (USEPA, 2002) and Water Pollution Control Regulations of Türkiye (WPCRT, 2004).

82.11 – 97.38 (mean 88.10), 9174.50 – 9942.29 (mean 9554.37), and 0.63 – 0.89 (mean 0.73); the content of **B** was found as between 10.29 – 17.43 (mean 13.72), 30.60 – 55.60 (mean 41.35), and 0.81 – 0.98 (mean 0.87); the content of **Ca** ranged between 1038.44 – 1682.30 (mean 1340.47), 4017.26 – 4503.54 (4298.63), and 1276.61 – 1541.41 (mean 1400.33); the content of **Fe** ranged between 120.69 – 178.41 (mean 145.74), 6894.50 – 8103.47 (mean 7583.78), and 0.51 – 0.69 (mean 0.60); the content of **K** ranged between 2503.51 – 2983.38 (mean 2755.23), 1118.50 – 1693.38 (mean 1345.01), and 69.43 – 93.82 (mean 79.92); the content of **Mg** ranged between 563.38 – 783.22 (mean 659.26), 885.32 – 1122.47 (mean 980.89), and 108.55 - 143.36 (mean 124.82) in the plant (mg kg⁻¹), sediment (mg kg⁻¹), and water (mg L⁻¹) samples, respectively (Table 1).

Considering the total accumulation values for all elements (ppm) indicated that Al mean value is the highest as 9554.37 in the sediment and the lowest 0.73 in the water samples; B mean value are highest as 41.35 in the sediment and lowest as 0.87 in the water samples; Ca mean value are the highest as 4298.63 in the sediment and the lowest as 1340.47 in plant samples; Fe mean value are the highest as 7583.35 in the sediment and the lowest as 2755.23 in the plant samples and the lowest as 659.26 ppm in

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the sediment and the lowest as 124.82 in water samples (Table 1).

When compared to the limit values recommended by the related literatures, concentrations of all elements in *R. sphaerospermus* was determined to be within acceptable limits. The concentrations of Ca, Fe, and K elements in sediments were found as above the acceptable limit, while contents of Al, B and Mg in normal limits for sediments. Moreover, the content of Ca and K in lake water samples were also found as above the acceptable limit, while contents remained in normal limits for freshwaters. (USEPA, 2002; WPCRT, 2004; Kabata-Pendias and Mukherjee 2007; Karahan et al. 2020).

The results obtained from our experiment show that TF mean value ranges were: 0.009 for Al, 0.334 for B, 0.310 for Ca, 0.019 for Fe, 2.075 for K, and 0.671 for Mg, respectively and the trend of TF for heavy metal and essential elements in plant samples studied were in order of: K>Mg>B>Ca>Fe>Al. The transfer or mobility of trace elements and heavy metals from soil or sediments to plant body could be influenced by the physicochemical characteristics of sediments and plant samples and is altered by innumerable environmental and anthropogenic factors (Aktaruzzaman et al., 2013). The highest and lowest mean TF values were found as 2.075 for K and 0.009 for Al, respectively.

Table 2

Transfer factor (TF) of heavy metal and trace elements from sediments to the plant body.

Transfer factors (<i>TF</i>)						
Samples	Al	В	Ca	Fe	K	Mg
1	0.009	0.350	0.298	0.019	1.962	0.658
2	0.009	0.336	0.258	0.018	2.078	0.623
3	0.009	0.358	0.317	0.020	2.271	0.715
4	0.009	0.340	0.278	0.018	2.390	0.656
5	0.009	0.306	0.338	0.019	1.985	0.674
6	0.010	0.313	0.374	0.022	1.762	0.698
Mean	0.009	0.334	0.310	0.019	2.075	0.671

4. Discussion

There are some previously performed studies with the aquatic plant species used in this study. In a similar study, some ecological properties of three different aquatic plants and water samples of Gölmarmara lake were analyzed and concentrations of P were found between 20 to 40 ppm and K between 18 to 80 ppm in *R. sphaerospermus* and concentrations of Mg were found between 32.11 to 47.54 ppm, K between 10.97 to 11.00 ppm,

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and Ca between 19.23 to 31.87 ppm in lake water. All concentrations were within acceptable limits for both for freshwater and plant samples (Yildiz and Ozdemir, 2005). In another study performed in the Gevaş district of Van (Türkiye), the levels of Al and Fe of the same species (R. trichophyllus) were found above the acceptable limits, whereas the levels of K, Ca and Mg were found to be within the normal ranges (Budag and Firat, 2015). When compared with our results, the concentrations of Al, B, Ca, Fe, K and Mg were found to be within the acceptable levels for plants. In another study, soil geography of Hassa district was investigated and it was reported that soil properties of Haydarlar Village had been found as; pH 7.76, K 203, Ca 320, and Mg 28.8 mg kg⁻¹. Similarly, the contents of Ca, Fe, and K in the sediment samples were determined as high level according to our findings (Atasoy, 2017). This accumulation is related to the volcanic lands surrounding the lake which occupy a very large area.

K and Mg elements had the highest TF value and TF value of Mg was higher than Ca (Table 2). This is an expected result, because Mg^{+2} ions are highly mobile in the phloem unlike Ca (Tang and Luan, 2017). Moreover, Dogan et al. (2014) reported that long term exposure of Al can effect uptake and transport of Ca, Mg and P nutrients. In our results, value of TF for Ca was lower than K and Mg. Hence, the lower value of TF for Ca than K and Mg in our plant samples may be attributable to high level of Al in sediments.

5. Conclusion and recommendations

Haydarlar Lake has been used for land irrigation and farming by local people especially in summer periods. It is also an important location for migratory birds in the wetland ecosystem. Since the contents of Al and Fe in the water samples are within the acceptable limits, it can be regarded as safe for agricultural products and animal health. In this study, the level of pollution and essential nutrients in this aquatic ecosystem were determined for the first time. In addition, due to its interesting geography and the freshwater flowers that cover the lake surface like a blanket, it has a high ecotourism potential.

Conflict of interest: The author declares that he has no conflict of interests.

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Research article

The Effects of LEDs with different CCT values on growth characteristics of *Triticum aestivum* L. (wheat) and *Hordeum vulgare* L. (barley)

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Abstract

Light Emitting Diode (LED)s are used extensively in almost everywhere in our daily life and they have different color temperatures such as Correlated Color Temperature (CCT) which is represented by °K (Kelvin). In this study, wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) were cultivated in laboratory environment under LED with different CCT values (2000, 3000, and 6000°K). After cultivation, plant height and weight values, the quantity of chlorophyll and carotene, the amount of leakage of electrolyte and element absorption capacity from the soil, POX (Peroxidase) and SOD (Superoxide Dismutase) enzyme activities of cultivated plants were determined. Results were evaluated on Statistical Package for the Social Sciences Program (SPSS 22) and significant differences were obtained on plants which were grown at different color temperatures of light. It was concluded that measurements from plants which were grown under LED light with cold color temperatures (6000°K) were more consistent than those of plants grown under other lights with different temperatures. It has been deduced that cold color temperatures are closer to the optimum light values required for a plant to grow faster.

Keywords: Barley; Correlated Color Temperature (CCT); lighting, Light Emitting Diode (LED); plant lighting; wheat

1. Introduction

LED stands for Light Emitting Diode. LED consists of two elements of a treated substance, which is referred as p-type and n-type semiconductors. LEDs are indeed semiconducting diodes that give out light via photons from p-n junction parts (Dupuis and Krames, 2008). The temperature of a light source which has the same coordinates with the color of luminescence of a black object at a certain temperature is described as "Color Temperature". However, the term "Correlated Color Temperature (CCT)" is used for light sources such as fluorescent lamps whose color values do not exist on black objects. CCT values of this kind of light sources are accepted as the temperature of the luminescence of black object which gives out the closest color to them under the same observation and radiance conditions (Wyszecki and Stiles, 1982). LEDs are produced with CCT values between 1000°K and 6500°K that enables LEDs to be used in various application fields. Luminous

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efficacy of LEDs decreases at higher CCT values. CCT values of white power LEDs vary between 3200-9000°K (Gurbuz, 2012). As given in Figure 1, the temperature between 3200-3300°K is termed warm white, 3500-4500°K is termed natural white, 5500-6500°K is termed pure white or day light and 6500-9000°K is termed cold white.

The light is an irreplaceable source of energy. Plants need light for the formation of chlorophyll, photosynthesis, the transformation of inorganic substances to organic substances, the formation of shoot, leaf, flower and fruits (Eris, 2007). The source of light which is needed for the growth of plants is either sun or artificial lights (Kim et al., 2005; Ohashi-Kaneko et al., 2007). The light from the sun consists of rays with different wave lengths. About 390 nm and 760 nm of wavelength of the light in the spectrum are termed visible light. Red-orange light in visible light spectrum is the light with the longest wavelength (600-700 nm). The energy of photons of visible wavelength from the electromagnetic rays from the sun is used for the photosynthesis by plants (Eris, 2007; Yang et al., 2012). Light is not just a source of energy for photosynthesis, it also controls different growth processes (Zhu et al., 2008). Several factors related to light such as light density, wavelength have effects on plant growth parameters (differences of node lengths, length of the plant, branch pattern, size of leaves and biomass) (Lee et al., 2014). The use of LED light has recently become widespread for additional lighting before sunrise or after sunset due to its superior advantages (Eris, 2007).

Leaves, which are one of the most important organs of plants, are necessary for photosynthesis. Photosynthesis abilities of leaves i.e. their efficiency of using light is directly proportionate to the chlorophyll concentration which is the pigment of green color (Kirbay and Ozer, 2015). Another parameter that light affects is the substance called carotene. Carotene is the pigment that gives the colors ranging from light yellow to red in plants. Some carotenoids functions as the presubstance of vitamin A and therefore, they are important for the synthesis of the necessary vitamin A. Beta-carotene has certain beneficial properties such as protecting photons against harmful light during photosynthesis, acting as antioxidants, protecting against cancer, increasing immune response, inhibiting tumor growth (Kahyaoglu and Kivanc, 2007).

Caglayan and Ertekin (2011) analyzed the technological superiorities and differences of LED light in comparison with traditional growth lamps, discussed their effects on plants and concluded that LEDs are preferred in agriculture due to their distinguished properties. Bourget (2008) and Morrow (2008) stated that solid-state light sources are ideal for the designs of plant lighting and they investigated the effects of wave lengths on plant photoreceptors in order to provide optimal production, to effect plant morphology and metabolism. Lee et al. (2014) investigated the effect of different LED lights that used commonly on buckwheat and Tartary wheat, while Koc et al. (2009) analyzed technological properties of the sources of LED type lights and mentioned their properties in terms of plant and animal production. Furthermore, Ozkok et al. (2016) concluded in their studies that, a safe lighting support was obtained through LED lightings, sprout growth was healthier, the growth of plants could be under control and increase in productivity and quality could be achieved. They also stated that a homogeneous radiance could be created and a significant level of energy saving was also possible.

In the study by Wu et al. (2007) where pea seeds were analyzed, sprouts were incubated under different LED lights and

in dark for 4 days. There was no difference in stem diameter in sprouts grown under LED lights and in dark; however, they have observed that stem length, leaf extent and sprout weight were affected by the quality of the light to a large extent. Caglayan and Ertekin (2015) compared LEDs with traditional sources of light in their study about plant growth rooms, and they concluded that LEDs are perfect sources of light that can provide benefits for plant growth environments such as plant tissue, growth rooms and cabins. (Chen et al., 2017) investigated the effects of alternative red and blue lights provided by LEDs on growth and nutritional properties of lettuce. In this research, red and blue LED lights were applied at different time periods and in different combinations in order to reveal the effects on red and blue lights on plant. Alternating irradiation of red and blue LEDs was observed to significantly increase the growth rate and biomass of lettuce when applied at varying time intervals. It was also observed that it increases the ascorbic acid content and reduces the nitrate content, leading to higher nutritional value.

There are limited numbers of studies related to the effects of LED lights in plant growth. Distinct from other studies in literature, current study aims to determine the potential effects of white LED lighting systems with 2000-6500°K CCT value. Within this context, white LED lighting systems with 2000°K, 3000°K and 6000°K color temperature values were designed and their potential effects on barley and wheat grown under this light were studied.

2. Materials and methods

LED Luminaires with 2000°K, 3000°K and 6000°K CCT values were designed to illuminate the environments where the wheat and barley were planted. CCT, CRI and LUX values of these LED elements were determined by spectrometer (Gossen M600A) during designing stage. The light parameters of the LEDs are given in Table 1. LED Luminaires with different CCT values were installed separately on working plane where *T. aestivum* L. and *H. vulgare* L. are planted after all measurements were carried out and the design stage was completed.

Table 1	
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Light	parameters
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CCT (Correlated Color Temperature)	2000 [°] K	3000°K	6000°K
CRI (Color-Rending Index)	26.34	71.7	71.4
LUX (Light Intensity Level)	97.73	1426.4	1209.2
Light of Wavelength	595 nm	596 nm	442 nm

The soil used in the study was obtained from a productive, uncontaminated field where agricultural activities have been performed. $\frac{1}{2}$ of the used soil has soil, $\frac{1}{4}$ has fertilizer and $\frac{1}{4}$ has pearlite. 750g of soil was placed into plastic pot with a capacity of 1 kg.

At first, 750g of soil was placed into the bottom of the pot, then 7g of wheat and 5g of barley were weighted and planted which then were covered with 100 g of soil. As seen in Fig. 1, three specimens were prepared and watered in accordance with the field capacity. Wheat and barley seeds in pots were exposed to almost 13 hours of light daily under LED luminaires with 2000°K, 3000°K and 6000°K CCT values.

The wheat was harvested after 15 days and the barley was harvested after 11 days. First, the wet weight and height of the samples harvested at different color temperatures were determined and consequently, electrolyte leakage, chlorophyll levels, POX, SOD enzyme activities and mineral element



Fig. 1. Wheat (a) and barley (b) plants grown under different color temperature

concentrations were determined.

2.1. Determination of parameter values

0.1 g of rinsed fresh plant sample (leaf) was placed in each of 6 test tubes with 4 ml of pure water and kept at 4° C for 24 hours. Then the amount of ion passing into the pure water in the tubes was measured with a conductometer. The response level of the plant was determined by setting a parallelism between the electrolyte leakage and the damage on the cell based on the measured value (Osma et al., 2014).

Total chlorophyll and carotenoid content in fresh leaves was estimated by using The Lichtenthaler and Buschmann (2001) method. Fresh leaf tissue with an amount of 0.5 g was grounded in a mortar and pestle which contained 5 ml acetone (80%). The solution had 663 and 646 nm (chlorophyll) and 440 nm (carotenoids) optical densities. Photosynthetic pigments were expressed as mg/g-1 FW (Osma et al., 2018).

The determination of antioxidant enzyme activities was performed on six replicate samples in the following manner. Plant leaves (0.5 g) were blended with 10 mM potassium phosphate buffer (pH = 7.0) which contains 4% (w/v) polyvinylpyrrolidone. The homogenized pulp was centrifuged at 12.000 x g for 30 minutes at 4°C. Then the extract was isolated to determine the type of enzyme. After adding the plant extract to 50 mM phosphate buffer (pH = 7.0) which contains 1 mM guaiacol and 0.5 mM H₂O₂, POX was determined by monitoring the increase in absorbance at 470 nm. One unit of POX activity was defined as the amount of enzyme that caused an increase in absorbance of 0.01 per min (Osma et al., 2014).

The reduction in absorbance of nitroblue tetrazolium (NBT) dye was monitored and in this way the activity of superoxide dismutase (SOD) was determined (Dhindsa et al., 1981). The reaction consisted of a mixture of 75 μ M NBT, 13 mM methionine, 0.1 mM EDTA, 2 μ M riboflavin, 50 mM sodium carbonate, 50 mM phosphate buffer (pH = 7.8) and 0.1 mL of plant extract. The reaction was launched by irradiating

tubes which contained the mixture using two 30-W fluorescent lamps for 15 minutes and ended by switching off the light. The absorbance of the mixture at 560 nm was measured instantly. The maximum color producing reaction mixture without enzymes was used as control. A mixture of non-irradiated complete reaction was used as a blank. The amount of enzyme, which reduces the absorbance by % compared to the tubes without enzyme, was determined as one unit of activity (Osma et al., 2014).

0.5 g of plant and sediment samples were arranged via precision scale and then put into Teflon cells. 5 ml of 65% HNO₃, 3 ml of 37% HCl and 2 ml of 48% HF (Merck) was added to the milled soil samples, as well as 8 ml of nitric acid (65%) to the milled plant samples (Osma et al., 2018). Samples were put into a microwave oven (Berghof-MWS2). Microwave was heated to 175°C gradually and held constant for 20 minutes. Samples and chemicals were filtered by using Whattman filters into 50 ml sterile tubes and they were diluted to 50 ml with ultrapure water to perform ICP-OES analysis. Before performing spectroscopic analysis (Merck), standards were prepared using 1,000 ppm multi element solution. ICP-OES measurements were performed after the calibration by using the standards (Osma et al., 2018).

Various analyses were performed by using the data obtained from our study. In the statistical comparison of obtained data $p \le 0.05$ value was determined as significant and average values, standard deviation, ANOVA test multiple comparisons of the samples in confidence interval of 95% were performed.

3. Results and discussion

It was observed that the different color temperatures of the light have a significant effect on the plant growth. In this study, the amounts of chlorophyll and carotene, electrolyte leakage, mineral element capacity, catalase and peroxidase enzyme activities were also determined. Significant differences were











Fig. 4. Carotenoid and electrolyte leakage in wheat and barley leaves of growth under LEDs with different CCT.



Fig. 5. POX and SOD activity in wheat and barley leaves of growth under LEDs with different CCT.

observed in the parameters that have an effect on wheat and barley in accordance with the color temperature of the light (Table 2). When the average post-harvest height measurement of the plants was examined, it was seen that it varied between 14.6-16.2 cm in wheat and 13.1-13.4 cm in barley. In addition, it was observed that the optimum value of average length was obtained at 6000°K in both plants. Additionally, the weight of the plants changed between 14-15.3 g in wheat and 11.3-11.4 g in barley after harvesting and 6000°K was again optimum light value for the maximum weight gain (Fig. 2).

Table 2

Statistical evaluation of data (*p<0.05; **p<0.01; ***p<0.001 significant).

	Wheat	Barley
Chl a	***	***
Chl b	**	Nd
Chl a+b	***	***
Carotenoid	***	*
Electrolyte leakage	*	**
CAT activity	Nd	Nd
POX activity	***	***

The amount of chlorophyll a varied between $8.15\pm0.63 - 15\pm0.2 \text{ mg ml}^{-1}$ in wheat and $9.3\pm0.37 - 13\pm0.3 \text{ mg ml}^{-1}$ in barley after harvesting. The amount of chlorophyll b varied between $4.2\pm0.3 - 7.2\pm2.4 \text{ mg ml}^{-1}$ in wheat and $4.5\pm0.6 - 5.4\pm0.2 \text{ mg ml}^{-1}$ in barley. The amount of chlorophyll a+b ranged between $12.4\pm0.7 - 22.2\pm0.8 \text{ mg ml}^{-1}$ in wheat and $13.8\pm0.4 - 19.1\pm0.5 \text{ mg ml}^{-1}$ in barley as seen in (Fig. 3).

The amount of carotenoid in wheat was $7.6\pm0.8 - 23.9\pm1.4$ mg ml⁻¹ and it was $10.3\pm1.6-16.3\pm1.8$ mg ml⁻¹ in barley. The amount of chlorophyll and carotenoid was more at 6000°K (Fig. 4). The amount of electrolyte leakage in wheat was between $74\pm12.5 - 222\pm14.2 \ \mu$ S cm⁻¹ and it changed between $49\pm2.4 - 62\pm3.9 \ \mu$ S cm⁻¹ in barley. The loss of electrolyte in wheat and barley grown under the light with 2000°K color temperature was more (Fig. 4).

SOD enzyme activity in wheat was between 148-184 EU/g, and its POX enzyme activity was between 54320-66711 EU/g. On the other hand, it was determined that SOD enzyme activity in barley was between 96-122 EU/g and its Peroxidase enzyme activity was between 31733-47315 EU/g. It was observed that both of the enzyme activities were pronounced in samples grown at 2000°K when the obtained data was analyzed statistically. It

was also determined that there are significant differences in both wheat and barley with respect to type of light (Fig. 5).

The effect of minerals is undisputable in the growth and developments of all the living creatures that obtain necessary elements form soil. It was observed that 7 nutrition elements i.e. Mg, K, P, Ca, Zn, Cu, Fe have statistically significant differences in terms of element absorption capacity in wheat and barley grown on the different color temperatures of the light. When the data was analyzed, mineral element concentration in the samples grown at 2000°K appears to be lower than others. Especially, the concentration of Mg element in chlorophyll structure changed depending on the color temperature (Table 3).

Koksal et al. (2013) analyzed the growth parameters of plant length (cm) and it was determined that additional lighting with red-orange LED light created statistical difference in terms of plant length, number of leaves, number of flowers and biomass weight, while in this study, it was determined that LED light with higher color temperature increased plant height and weight compared to LED light with lower color temperature. Lee et al. (2014) found in their study that the levels of phenolic compounds tended to increase when LED was used in growing wheat plants. In our study, it was observed that the amount of chlorophyll and carotene in the plant increased as the color temperature of the used white LED luminaires was increased. Bourget (2008) and Morrow (2008) reported in their work that LED lighting systems have many advantages such as spectral composition, small size, durability, long operating life, wavelength specificity, and the ability to control photon output linearly with electrical input current. They stated that they are ideal for use in plant lighting designs and allow wavelengths to match plant photoreceptors to provide more optimal production and influence plant morphology and metabolism. Our study results showed that as the color temperature of LED luminaires, which has superior properties compared to other luminaires, increases, and the amount of magnesium element that the plant takes from the soil increases. Likewise, as the color temperature of the LED luminaires increased, the electrolyte leakage amount in the cells decreased. The research by Jiang (2021) was mainly conducted with pak choi (Brassica rapa L.) to detect the difference between physiological response and gene expression under treatments of varying LED spectra. An attempt has been made to determine the ideal LED lighting "Recipes" (which includes luminous intensity and LED spectrum) for particular species and varieties. In our study, different color temperatures

Table 3

Element concentration in wheat and barley leaves of growth under LEDs with Different CCT. (μ g/g dw) (*p<0.05; **p<0.01; ***p<0.001 significant).

Element	ССТ	Barley	Significant	Wheat	Significant
	2000°K	2593.2 ± 131.2		2264 ± 87.7	
Mg	3000°K	2751.9 ± 225.3	Nd	2513.3 ± 16.7	*
	6000°K	2740.1 ± 152.2		2488.5 ± 66.4	
	2000°K	5709.9 ± 106.7		10022.9 ± 71.6	
Р	3000°K	6259.3 ± 479.7	Nd	10715.2 ± 177.8	***
	6000°K	6343.3 ± 797.8		10115.1 ± 49.9	
	2000°K	13.1 ± 0.3		14.3 ± 0.1	
Cu	3000°K	15.4 ± 0.5	*	14.4 ± 0.2	***
	6000°K	15 ± 0.7		13.5 ± 0.2	
	2000°K	45.8 ± 0.7		44.2 ± 1.2	
Zn	3000°K	49.7 ± 2.2	Nd	41.1 ± 0.8	*
	6000°K	48.1 ± 1.9		41.2 ± 0.6	
	2000°K	35389.2 ± 587.1		61256.7 ± 1171	
Κ	3000°K	41765.7 ± 2350.8	Nd	61785.2 ± 372.4	Nd
	6000°K	44705.5 ± 4448		61581.9 ± 306.1	
	2000°K	112.1 ± 1.8		91.4 ± 4.9	
Fe	3000°K	106.6 ± 4.8	*	121.8 ± 1.5	***
	6000°K	206.5 ± 40		117.5 ± 2.2	
	2000°K	1566.8 ± 12.7		1527 ± 78.1	
Ca	3000°K	1450.1 ± 109.1	Nd	2034.6 ± 92.5	***
	6000°K	1449.4 ± 45.3		1584 ± 46.3	

of LED luminaires were evaluated and the ideal color temperature for plant growth was tried to be determined.

4. Conclusion

In this study, unlike other studies in literature, the potential effects of white LED lighting systems with 2000-6000°K CCT values on wheat and barley were determined. Following results can be drawn:

- White LED luminaires which are the most commonly used LEDs in lighting applications were chosen and Color temperature as well as wave length has significant effects on plants.
- Physiological and biochemical effects of light on plants grown in laboratory environment created a significant effect on the enzymes and plant morphology.
- The LED light has various effects on plants such that when

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color temperature increased, the length and weight values of plants, amounts of chlorophyll, carotene and mineral absorption capacity also increased. However, the amount of electrolyte leakage and antioxidant enzyme activity decreased inversely.

• As a result, it was concluded that CCT values can be directly proportional with the plant productivity.

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Research article

Determination of extracellular hydrolytic enzyme capabilities of some *Anoxybacillus* isolated from hot spring environments

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Abstract

The development of microbial enzymes was a crucial event in the industrial sectors as a result of the tremendous growth of biotechnology in recent years. Popularity of waste management and bioremediation processes have both made extensive use of microorganisms' whole cells and their enzymes. The pharmaceutical, textile, food, cosmetics, leather, paper, energy, biomaterials, fine chemicals, cellulose, and detergent sectors are some of the uses area of microbial enzymes. Depending on different uses, researchers can search for novel bacterial strains that might exhibit previously unrecognized enzymatic activity. Also for searching for plasmids that could be used as cloning vectors to tackle medication resistance in thermophilic microorganisms. The *Anoxybacillus flavithermus* bacteria, which were isolated from a hot spring in the Turkish city of Afyon, was employed in this investigation. The ability of the identified bacteria to produce extracellular hydrolytic enzymes was tested. For this, the activities of catalase, urease, and lipase as well as the hydrolysis of starch, casein, xylan, and asparagine were researched. Additionally, tests for antibiotic resistance were studied on the isolated bacteria using four different antibiotics (erythromycin, chloramphenicol, rifamycin, and ampicillin). All identified strains fermented starch as carbon and energy sources, and after 24 hours of incubation, amylase activity was detected at 50°C and pH 7.0. All strains were determined to be catalase-positive, and with a few exceptions, the majority of *A. flavithermus* strains were also found to be urease and caseinase positive. Industrial products that can be obtained from bacteria found in extreme environments will be effective in the development of future technology.

Keywords: Anoxybacillus flavithermus; bacillaceae; casienase; extremozyme; gram-positive

1. Introduction

First appearance of the Bacillaceae on the Earth before 2 billion years ago, until today, the Bacillaceae family are evolved in a dramatic diversification of capabilities and take a majority of niches on our planet (David and Alm, 2011). The Bacillaceae was determined by Fisher in 1895 belong to phylum Formicates. Bacillaceae are belong to Gram-positive family, shaped by rod-or coccus, heterotrophic, may produce endospores which are oval, round or cylindrical, some members of this family are

motile by peritrichous flagella. Bacillaceae are aerobic, facultative or strict anaerobes (Vos et al., 2009). *Anoxybacillus* genus is belonging to Bacillaceae family (known as *Bacillus flavothermus*) this strain was first discovered in a hot spring of New Zealand (Heinen et al., 1982). They were identified at the year of 2000 as a relatively new genus. *Anoxybacillus* means small rod living with-out oxygen (Bevilacqua et al., 2016).

Anoxybacillus genus is one of the best thermophilic bacteria among the other bacilli, in extreme habitats, to produce valuable enzymes biotechnologically, because most of these

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bacteria have amylolytic and glucosidic activities and the ability to degradation of carbohydrate (Cihan, 2013). The *Anoxybacillus* can be aerobes, facultative anaerobes, or facultative aerobes. The application of *Anoxybacillus* increased compared to other members of Bacillaceae due to their thermo stable enzyme as sources for many biotechnological processes, such as metabolic studies, bioremediation applications, genomic analysis, and biosorption (Ozdemir et al., 2011a).

Recent investigations confirm that the main spore-formers in various types of dairy powders are *B. licheniformis*, *Anoxybacillus*, and *Geobacillus* (Eijlander et al., 2019). *Anoxybacillus* can produce a variety of enzymes that degraded carbohydrates. (Derekova et al., 2008). Solid-state fermentation was employed to produce -amylase from rice husks using *A. flavithermus* that was isolated from a hot spring in Türkiye (Ozdemir et al., 2011b). Amylase enzyme can be used in industrial processes like process of brewing, baking, textiles, paper industry, bioethanol production and detergent (Lama et al., 2009). The whole cells of *Anoxybacillus* are potentially useful as alternative resources for bioremediation and to immune stimulate fish against pathogens and renewable energy generation (Cihan et al., 2013).

Xylanolytic activity was found in several species of *A. flavithermus* (Kambourova et al., 2007), *A. pushchinoensis* (Kacagan et al., 2008), *Anoxybacillus* sp. (Wang et al., 2010), and from many strains isolated from hot spring in Türkiye. Also; for separation of heavy metals from aqueous solutions, some species of *Anoxybacillus* are useful and act as a model to developed the biosorption system (Duran et al., 2009). The new studies indicate that lipases enzyme that produced from *A. flavithermus* may be useful for various processes such as medical, food, cosmetic, detergent and leather and textile industries (Hasan et al., 2006).

Extremozymes (heat stable enzymes) produced by thermophilic bacteria, which normally found in extreme condition (pH, temperature, water activity, radiation), its great in industrial interest used in the field of textile, detergent, cosmetic, food and molecular biology. The species that obtained from the various extreme habitats can produce commercially valuable extracellular enzymes (Bischoff et al., 2006).

In current study, the different strains of *Bacillus cereus*, and *A. flavithermus*, isolated from hot spring from Afyon city of Türkiye were analysed for their hydrolytic enzyme capabilities.

2. Materials and methods

Anoxybacillus bacteria, which were isolated, morphological and biochemical tests and 16S rRNA analysis by Ozdemir et al. (2011c) within the scope of the study identified bacteria and isolated locations were given at Table 1.

2.1. Biochemical tests

2.2.1. Gram staining

Before the imaging, isolated bacteria were air dried and fixed on the glass slide by heating and stained by using Gram stain. Samples were covered with few drops of Gentian violet (which is a mixture of methyl violet and crystal violet) for a minute and washed with the tap water. Samples were treated with Gram's Iodine and allowed to effect for a minute, rinsed and dried. Then samples decolorized in absolute ethyl alcohol for about 30 seconds, rinsed and dried in air and heat fixed. The stained samples were imaged by using Olympus CX 31 system microscope with Olympus SC 30 Digital color camera. Images were photographed by light microscopy at highest magnification lens (X100) under oil immersion.

Table 1

The name of studied bacteria and their location.

Samula	Accession Number	Name Hot
Sample	- Identified Bacteria	spring/phase
Seq1	KJ434779 Anoxybacillus	Ömer/Soil
Seq2	KJ434780 A. flavithermus	Ömer/Soil
Seq3	KJ434781 A. flavithermus	Ömer/Soil
Seq4	KJ094998 A. flavithermus	Gecek/Soil
Seq5	KJ434782 Bacillus firmus	Ömer/Soil
Seq6	KJ434783 Anoxybacillus sp.	Ömer/Water
Seq7	KJ434784 A. flavithermus	Ömer/Soil
Seq8	KJ434785 A. flavithermus	Ömer/Soil
Seq9	KJ434786 A. flavithermus	Gecek /Water
Seq10	KJ094999 A. mongoliensis	Ömer/Soil
Seq11	KJ434787 A. flavithermus	Ömer/Water
Seq12	KJ434788 A. flavithermus	Gecek/Soil
Seq13	KJ095000 A. flavithermus	Gecek/Soil
Seq14	KJ434790 A. flavithermus	Gecek/Soil & Water
Seq15	KJ434791 B. cereus	Ömer/Soil
Seq16	KJ434792 A. flavithermus	Gecek/Soil & Water
Seq17	KJ434793 A. kestanboliensis	Ömer/Soil
Seq18	KJ095001 A. flavithermus	Gecek/Soil & Water
Seq19	KJ434794 Bacillus sp.	Ömer/Soil

2.2.2. Tween 80 hydrolysis test

Nutrient agar isolates which contained 3% Tween 80 were planted as an intense line with help of loop. Petri dishes were left for 1-day incubation at 50°C. Following incubation, at the rate of 0,001% Rhodamine B was added so as to enclose the surface of the petri dishes. Zones which occurred around the colonies were considered as positive for lipase activity (Karnetova et al., 1984).

2.2.3. Xylan hydrolysis test

Nutrient agar isolates which contained 1% Xylan were planted in an intense line with the help of loop. Petri dishes were left for 1-day incubation at 50°C. After incubation zones which occurred around the colonies were considered as positive for xylen activity (Karnetova et al., 1984).

2.2.4. Skim milk powder hydrolysis test

Isolates were planted in Nutrient agar which contained 1% Skim milk powder in the form of an intense line with help of loop. Petri dishes were left for 1 day's incubation at 50°C. After incubation, a clear zone around the colonies was accepted as positive for protease activity (Yin et al., 2010).

2.2.5. Starch hydrolysis test

Nutrient agar isolates (1% soluble starch) were planted by loop in the form of an intense line. Petri dishes were incubated for a day at 50°C. After the incubation, Lugol solution was added on the medium. Zones were observed around the colony, clear zones consider as positive starch digestion and purple zones accepted as negative starch digestion was assessed in areas that the starch hydrolyzed (Aygan et al., 2008).

2.2.6. Urease test

Nutrient agar plates containing 0.3% uric acid was sowed from alkalophilic isolates in a 20 mm diameter area. Petri dishes were left at 50°C for 24-48 h incubation. The transparent zone formed around the planted area following incubation was evaluated as positive for urease production (Honarbakhsh et al., 2014).

2.2.7. Asparaginase test

Nutrient agar plates containing 1% L-asparaginase milk dust were inoculated on halophilic isolates in 20 mm diameter petri dishes. Petri dishes were left at 50°C for 24-48 h incubation. Following incubation, 0.001% phenol was added and the pH was adjusted to 6.5. Color change from yellow to red around the colony was considered positive for L-asparaginase enzyme production (Gulati et al., 1997; Ogun, 2020).

2.2.8. Catalase test

Bacteria strains were isolated for 18-to-24 hours and samples were taken from the culture. Samples placed onto the microscope slide and drop of 3% H₂O₂ was added on it. The readability is improved by observing for bubble development on a dark background.

2.2.9. Antibiotic resistance test

The agar diffusion method with filter paper disk was used for antibiotic resistance test. Individual disk was containing a content concentration of a different antibiotic. The specific effectiveness of different antibiotics provides the basis for a resistance spectrum of the organism. In this study the Oxoid mark of antibiotics were used, Chloramphenicol (30 mg), Ampicillin (10 mg), Rifamcyin (30 mg) and Erythrosine (10 mg) were used for anti-biogram.

3. Results

The first test was performed as gram test. The test is study on staining of bacteria with gram dye. In this study all strains of bacteria were shown positive results with gram stain test. Also, the cells are often arranged in pairs or chain, straight, violet color and rod-shaped (Fig. 1A). The bacteria were checked for lipase test. In this study, Tween 80 was used for medium; all the bacteria that we used show negative results (Fig. 1B)

The xylan hydrolysis test is the composed of xylanolytic enzyme system of hydrolytic enzymes, the function of these enzymes is conversion of xylan to constituent sugar. According to our results there is no zones appear around the colonies of our bacteria that we used in this study, so the result is negative for this test (Fig. 1C).

Casein is a large protein molecule made of amino acids. It is the main protein found in milk and accounts for its white colour. Caseinases, which hydrolyse protein in stages to amino acids, were secreted outside of bacteria cells. If a clear zone, a zone of casein hydrolysis, evaluated it as positive. In this study 4 strains show negative result including Seq11, Seq12, Seq16, Seq18 and *Staphylococcus* and other isolates shows positive (Fig. 1D).

These forms are bonded by 1,4-glycosidic amylase and oligo-1,6-glucosidase. Enzymes are able to hydrolyse starch by

breaking the glycosidic linkages between the sugar subunits. For detection of hydrolyse; Iodine reagent reacts with starch and produces a blue or dark colour; therefore, a clear zone surrounding the growth was revealed as any microbial starch hydrolysis. In this study, all strains show positive result (Fig. 2A). Ammonia, CO₂, and water are the by-products of urea's nitrogen and carbon bond assault by the urease enzyme. In this study, 15 strains show positive result including Seq2, Seq4, Seq5, Seq6, Seq7, Seq8, Seq9, Seq10, Seq11, Seq12, Seq13, Seq15, Seq17, Seq18, and Seq21 and other isolates shows negative (Fig. 2B).



Fig. 1. Samples from (A) Gram staining, (B) Tween 80 hydrolysis test, (C) Xylan hydrolysis test, (D) Skim milk powder hydrolysis test.



Fig. 2. A samples from (A) Starch hydrolysis test, (B) Urease test, (C) Catalase test.

Catalase test was applied to all bacteria strains that we isolated it in this study and all shows bubbles of O_2 , so the result is catalase test positive (Fig. 2D).



Fig. 3. The samples of antibiotic test exposed to Anoxybacillus strain.

When the growth of bacteria has been inhibited, a clear zone was appeared around the paper disk, the diameter of this zone related with the sensitivity of the bacteria to the antibiotic and minimum inhibitory concentration (MIC) is reached at this point. The zone of inhibition appears surrounding the 4 types of antibiotic disk that were used in this study which are Chloramphenicol, Ampicillin, Rifamcyin and Erythromycin (Fig. 3).

4. Discussion

Thermophilic organisms; It is widely preferred in scientific researches because it is used in the production of enzymes, antibiotics, various biochemical substances and insecticides. Bacillus genus is one of the most studied thermophilic bacterial groups. These organisms are usually gram-positive, aerobic, rod-shaped bacteria that form endospores. Formation of endospores makes this breed resistant to unfavourable conditions. Among the reasons why Bacillus genus is preferred in the production of industrial enzymes: rapid and easy reproduction, using a wide variety of carbon and nitrogen sources and different substrates, and creating high yield products can be counted. In addition, organisms included in this group can be isolated from soil, fresh water and seas, plant rhizospheres, foods, intestinal systems of some living things, some insect larvae and even from areas with extreme pH and temperature (Tarakcioglu, 2016).

In this study, the starch hydrolysis test for 21 strains isolated from hot spring in Türkiye showed positive result for all strains and this result agrees with the concept of a study conducted previously in Afyonkarahisar, Türkiye by a team of researchers, who found that amylase enzyme positive of the *A*. *flavithermus* (Ozdemir et al., 2015). Also a similar result was obtained from a study in Ramadi (Dhafer, 2007).

This study showed that all 19 strains which isolated from hot spring in Türkiye positive result for catalase test and this result are in agreement with most published data (Heinen et al., 1982; Claus and Berkeley, 1986; Rainey et al., 1994; Pikuta et al., 2000; Belduz et al., 2003; Sharp et al., 2021). It's also agrees with the concept previously reported by several researchers, who found that catalase enzyme positive of the *A. flavithermus* (Ozdemir et al., 2015; Dhafer, 2007; Dai et al., 2011).

In current result, negative result were obtained for sample Seq1, Seq3, Seq19 of *A. flavithermus* and this result supported the published data, which described the urease enzyme negative (Pikuta et al., 2000; Belduz et al., 2003). In contrast to these, positive results were obtained for urease enzyme in *A. flavithermus* numbered as; Seq2, Seq4, Seq7, Seq8, Seq9, Seq11, Seq12, Seq13, Seq15, Seq17. And positive result to *A. mongoliensis* sample Seq10 and *A. kestanboliensis* sample Seq18. The result of this study agree with the concept previously reported by Filippidou et al., (2015) who found urease enzyme positive for *A. geothermalis* Strain GSsed3.

The positive result of casein indicated in A. flavithermus of this study agree with the concept previously reported by several researcher who found casein positive (Pikuta et al., 2000; Belduz et al., 2003; Yavuz et al., 2004). Also, negative results were obtained for sample Seq11, Seq12, and Seq19 of A. flavithermus for casein hydrolysis. The current results coincide with the results obtained by Shahinyan et al., (2017) who showed that casein negative for A. flavithermus DSM 2641. A possible explanation for this finding is that may be the mutation in the gene may have occurred, or the sample Seq11, Seq12, Seq19 sub strain of A. flavithermus. The consensus sequences among bacterial species are needed for identification of bacteria. However, it does not reflect the physiological variations among bacterial strains. Since microbial adaptive physiology to environment is highly variable among the same species. Therefore, biochemical tests represent a reliable approach that reflects the actual bacterial physiology. Furthermore, this technology allows researchers to search for novel bacterial strains that may have previously unreported enzymatic activity.

A. flavithermus isolated represent a promising candidate for many kind of biotechnology approach. The extracellular xylanase crude extract produced by this bacterium with the best inducer substrate was further characterized for their optimal temperature, pH, and stability (Goh et al., 2013). The ability of xylanases to produce xylose from commercial xylan, has an economic importance for the conversion of plant biomass into fuels and chemicals.

In this study, 19 strains isolated on Nutrient agar at 50°C from samples from hot springs from Afyon city, were tested against different antibiotic to screen for candidate isolates that may serve as a candidate one for further characterization and evaluating the possibility of possessing a novel plasmid that may be used as cloning vector. Antibiotic resistance capacity is due to transfer of antibiotic resistant genes from the others and its get conveyed by plasmids and this dissemination is rapidly occur and even among bacteria that are distantly related, horizontal gene transfer occurs frequently (Amábile-Cuevas and Chicurel, 1992). This process is important because it caused to increasing of drug resistance when one bacterial cell acquires resistance, resistance genes can quickly transfer to numerous species (Raghunath, 2008).

5. Conclusion

In the present study, all of the identified bacteria belonging to the family Bacillaceae, *Bacillus* and *Anoxybacillus* genus, they are thermophilic bacteria can live and survive at high temperature and pH and under extreme environmental condition. *A. flavithermus* is a relatively new species and the knowledge of the physiology, metabolism, and metabolomics of the *A*. *flavithermus* is incomplete, quite limited, and seldom compared with other members of Bacillaceae that well-studies. The majority of the available information indicates that this strain produces intriguing enzymes that are thermostable and tolerant of alkaline pH. The importance of bacteria used in industry has increased. Because they can use as the source of thermostable enzymes. Thermal stability enables these enzymes to be active in the presence of chemical denaturants and to resist harsh process conditions, the stability natural enzymes are thus mutually beneficial for the industry and biotechnology in different areas.

In this study, hydrolytic enzymes of some *Anoxybacillus* strains from hot spring samples from Afyon, Türkiye were screened. In order to identify these strains, some biochemical tests and molecular identification based on 16S rRNA gene were performed. As a result, it was identified as a strain of *A. flavithermus*. Isolates were first Gram stained and examined under the light microscopy. Catalase tests were performed. The isolates were then subjected to some physiological tests on nutrient agar plates for 1-2 days: growth at 50°C and pH 7. Isolates were also screened for the extracellular enzyme hydrolysis at 50°C such as starch, xylan, casein and asparagine

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Research article

Determination of heat shock proteins in certain Bacillus species

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Abstract

In this study, the effects of four different temperatures on the sporulation and development of 6 *Bacillus* species, 2 native and 4 reference were investigated. The SDS PAGE analysis emerged that two different proteins, 40 and 39 kilodalton (kDa), were produced by *Bacillus sphaericus* ATCC 2362 after 24 and 48, respectively hours at 48°C, by local isolate 4 after 24 and 48 hours at 42°C and by local isolate 31 after 24 and 48 hours at 48°C. Additionally *Bacillus firmus* (ATCC 14573) produced a 40 kDa protein after 48 hours at 52°C and *Bacillus thuringiensis* var. *israelensis* produced a 42 kDa protein after 48 hours at 42°C. At temperatures of 48 and 52°C, after 12, 24 and 48 hours incubation, vegetative and heat resistant spore counts were determined to reduce by 10^4 - 10^6 fold according to bacterial counts. As a result, the data revealed that at 48 and 52°C spore vitality fell by a significant degree. Additionally, SDS PAGE analysis results showed that high temperature resistance was provided by different heat shock proteins a 40 kDa protein produced by *B. firmus* ATCC (14573), 40-39 kDa proteins produced by *B. sphaericus* (ATCC 2362), and local isolates 4 and 31 and 42 kDa protein produced by *B. thuringiensis* var. *israelensis*.

Keywords: Bacillus spp.; electrophoretic; SDS-PAGE; sporulation; heat shock protein

1. Introduction

Bacteria develop different types of adaptation for varying environmental conditions. The response of microorganisms to stress occurs through a number of regulatory mechanisms (Segal and Ron, 1998). A range of stress studies using ethanol, heat, hydrogen peroxide, salt, low-high pH, and heavy metals were completed for species in the Bacillus genus. It was found that the bacteria produce specific proteins depending on the stress factors to protect themselves under induced stress conditions (Browne and Dowds, 2001; Melly et al., 2002; Periago et al., 2002; Gomes and Simao, 2014). Heat stress is a significant stress factor with negative effects on bacterial development. According to Segal and Ron (1998), first studies on heat shock in bacteria were performed on Escherichia coli K-12. This species has σ^{32} factor with the ability to recognize the promoter region of the specific heat shock operon. Together with the transcriptional activator of this factor, half-life of the transcript in cytoplasm is very short. In addition to temperature, production of many other proteins are induced due to changes in other conditions. However, within these proteins one group is only induced by temperature which is called heat shock proteins. Different research groups completed many studies on the effects of different stress conditions on species in the Bacillus genus (Burke Jr et al., 1983; Hecker et al., 1989; Elcin et al., 1995; Antelmann et al., 1997; Browne and Dowds, 2001; Berber et al., 2003; Berber et al., 2004; Beladjal et al., 2018; Hantke et al., 2019; Vahdani et al., 2019; Xie et al., 2019). In these studies bacteria from Bacillus genus that includes gene groups producing different special proteins against stress factors were generally chosen (Hantke et al., 2019; Schafer et al., 2019). Additionally, as members of this genus can live in high alkali environments and includes alkalophilic and facultative alkalophilic species with industrial importance they are enriched in stress mechanisms. Facultative alkalophilic Bacillus species can grow at pH 10 in addition at neutral pH values. However, obligate alkalophilic Bacillus cannot grow at pH below 8.5 (Horikosi and Akiba, 1982). Alkalophilic Bacillus species are

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known to be industrially important due to the enzymes and metabolites they produce (Krulwich and Guaffanti, 1989).

The aim of this study was SDS PAGE identification of heat shock protein of 6 *Bacillus* bacteria species, four of which were reference and 2 were native, all grown at high temperatures.

2. Materials and methods

In this study, two local *Bacillus* isolates previously defined as facultative alkalophilic (unpublished data), in project no. TBAG-2103(101T146) *B. thuringiensis* var. *israelensis and B. sphaericus* ATCC 2362 obtained from Prof. Dr. Cumhur Cokmus (Ankara University, Faculty of Science, Biology Dept.), and *B. firmus* (ATCC 14573) and *B. alcalophilus* (ATCC 27647) references species obtained from Dr. Arthur A. Guffanti and Dr. Terry A. Krulwich (Department of Biochemistry, Mount Sinai School of Medicine) were used.

2.1. Preparation of bacterial spore suspensions and creation of stress conditions

B. thuringiensis var. israelensis and B. sphaericus (ATCC 2362) strains were prepared from synchronized cultures on Nutrient broth, after which were linearly seeded on NYSM agar and left for five days until they fully sporulated. The B. firmus (ATCC 14573), B. alcalophilus (ATCC 27647) reference species and facultative alkalophilic local Bacillus isolates 4 and 31 were prepared from synchronized cultures in alkali broth medium (starch 10g, peptone 5g, yeast extract 5g, NaCl 5g, KH₂PO₄ 1g, MgSO₄.SO₄ 0.2g, 1000 ml distilled water, pH 9.5-10) Then, these cultures were linearly seeded on alkali agar media and left for five days for full sporulation. Samples were taken at different time point during sporulation and visualized under a phase-contrast microscope. Then well-sporulated, cultures were gathered on the petri surface with sterile distilled water and stock spore suspension with a concentration of 4.4 x 10^{10} (9.7x10⁸-1.33x10¹¹) spores/ml was prepared. The prepared spore stock solution was stored in a refrigerator at $+4^{\circ}$ C.

From the spore stock solutions prepared for each bacteria. 100 μ l samples were taken and seeded on 50 ml broth (alkali or nutrient) in 100 ml Erlenmeyer flasks. Then, the seeded media were shaken at 150 rpm and left to grow for 48 hours in incubators at four different temperatures. Samples were taken from the bacteria cultures at 12th, 24th and 48th hours and transferred to sterile Eppendorf tubes. The samples were stored in a deep freeze at -70°C until bacterial counts, protein extraction and electrophoresis procedure.

2.2. Total spore counts

To identify total bacteria counts after 12, 24 and 48 hours at four different temperatures, 100 μ l of bacterial samples stored at -70°C were transferred to sterile Eppendorfs containing 900 μ l sterile physiologic serum and diluted in series to 10⁻¹, 10⁻², 10⁻³, 10⁻⁴, 10⁻⁵, 10⁻⁶, 10⁻⁷, 10⁻⁸, 10⁻⁹, and 10⁻¹⁰ rates. Before each dilution, 100 μ l bacteria suspension was added to sterile petri dishes containing sufficient NYSM agar or alkali agar and bacterial suspensions were homogeneously mixed with the medium. Later sterile petri dishes were left for 24 hours in 30°C incubators for spore colony development. At the end of this time petri dishes containing from 30-300 colonies were taken and counted and the total bacteria count per ml was determined. Counts were made as triplicates on 4 parallels.

2.3. Electrophoresis and sample preparation

Electrophoresis procedure was completed according to Laemmli (1970). Briefly, bacterial samples stored in a deep freezer were transferred to 10 ml sterile glass tubes, centrifuged at 5000 rpm for 5 minutes at +4°C and the supernatant was removed. After washing the pellet three times in sterile serum physiologic, it was transferred into a porcelain crucible containing 5 ml sterile serum physiologic. Later it was sonicated for 5 minutes at 48 W. The total cell protein extract was lyophilized for 12 hours and concentrated. Then the resulting protein extracts were treated with 40-50 μ l SDS sample buffer (0.06 M Tris, 2.5% glycerol, 0.5% SDS, 1.25% β -mercaptoethanol and bromophenol blue) and boiled in a hot water bath for 5 minutes at 16,000 rpm.

2.3.1. Preparation of gels and electrophoresis

Acrylamide bisacrylamide stock solution contents are given Table 1. The prepared solution was filtered with Whatman No: 1 paper. It was stored at +4°C.

Table 1

Acrylamide bisacrylamide stock solution.

Component	Amount
Acrylamide	28.8 g
Bisacrylamide	0.12 g
Distilled water	100 ml

Separation gel tampon (1.5 M Tris-HCl, pH 8.6); The required amount of Trisma Base and SDS was weighed and taken into a clean flask, dissolved in some distilled water, adjusted to pH 8.6 with 6 N HCl and completed to the final volume. The solution was filtered through Whatman No.1 filter paper and sterilized in an autoclave for 15 minutes and stored at $+4^{\circ}C$.

Stacking gel buffer (0.5 M Tris-HCl, pH 6.8) The required amount of Trisma Base and SDS was weighed and taken into a clean flask, dissolved in some distilled water, adjusted to pH 6.8 with 6 N HCl and completed to the final volume. The solution was filtered through Whatman No.1 filter paper and sterilized in an autoclave for 15 minutes and stored at $+4^{\circ}$ C.

Running buffer contents are given Table 2. The prepared solution was filtered with Whatman no:1 paper. It was stored at $+4^{\circ}C$.

Table 2
Running buffer.

Component	Amount		
Trisma Base	1.21 g		
Glycine	5.76 g		
SDS	1.00 g		
Distilled water	1000 ml		

Sample Buffer contents are given Table 3. Sample in resolving gel were stained with Coomassie Brillant Blue stain. Staining buffer content are given Table 4. The prepared solution was filtered with Whatman No: 1 paper. It was stored at room temperature %12 resolving gel prepared and poured into the lower. %5 stacking gel prepared and poured upp. After the electrophoresis process was finished, the molecular weights of the proteins were calculated using the software program Lab.

Image version 2.6 (Halle, Germany).

Table 3

Sample buffer.

Component	Amount
0.5 M Tris-HCl pH 6.8	5.12 ml
Glycerol	8.00 ml
Distilled water	3.00 ml
SDS	2.00 ml
2-β-mercaptoethanol	4.00 ml
Bromophenol Blue	0.025 g

Table 4

Staining buffer

-	
Component	Amount
Coomassie Brillant Blue	1.50 g
Isopropyl Alcohol	250.0 ml
Glacial Acetic Acid	100.0 ml
Distilled water	650.0 ml

2.4. Statistical analysis

To determine differences between the temperature groups in terms of spore counts, the variance analysis technique was used. The difference between temperature groups was tested at the p=0.05 significance level. The Duncan Multiple Comparison test was used for two-way comparisons of the temperature groups. All analyses were performed with the SAS packet program.

3. Results

3.1. Electrophoretic results

The protein profiles belonging to *B. firmus* (ATCC 14573) species at 12, 24 and 48 hour time points at four different temperatures $(35, 42, 48, 52^{\circ}C)$ are given in Fig. 1.



Fig. 1. Protein profiles from *B. firmus* (ATCC 14573) species developed at different temperatures, obtained with SDS PAGE.

As observed in Fig. 1, very significant differences observed between the 35, 42 and 48°C protein profiles at 12, 24 and 48 hours. The molecular weight of protein no. 1 is 177 kDa and it disappears at 48 and 52°C. At temperatures 35, 42 and 48°C, the protein profiles appear very weak after 12 hours. At 52°C, the 40 kDa molecular weight protein, labeled as, number 2, was produced after 48 hours.

The protein profiles of *B. alcalophilus* (ATCC 27647) species over 12, 24 and 48 hour durations at four different temperatures (35, 42, 48, 52°C) are given in Fig. 2. Protein profiles at 35, 42, and 48°C appear similar. However, the 116 kDa molecular weight protein band labeled number 1 was synthesized at 35 and 42°C, but the amount reduced at 48°C. The molecular weight band shown by number 2 is 79 kDa, and it was synthesized after 48 hours at 42°C. At 52°C, all bands synthesized at other temperatures were lost.

The protein profiles obtained after 12, 24 and 48 hour at temperatures of 35, 42, 48, and 52°C from B. sphaericus ATCC 2362 species are given in Fig. 3. At 35 and 42°C, B. sphaericus ATCC 2362 protein profiles at 12, 24 and 48 hours are very similar. When 48 and 52°C are examined, the protein amounts reduced, and at 52°C it appears there was no band. The 179 kDa and 149 kDa molecular weight proteins together shown by number 1 were protected at 35 and 42°C but were lost at 48 and 52°C. The number 2 band at molecular weight 109 kDa appeared to be only synthesized at 35°C after 12 and 24 hours. The molecular weight of the band at number 3 was calculated as 90 kDa and this band was synthesized at 42°C temperature after 12, 24 and 48 hours, while it disappeared at 48 and 52°C. The bacteria grown at 48°C only synthesized the double band with molecular weight 40 and 39 kDa shown by number 4 after 24 and 48 hours incubation.



Fig. 2. Protein profiles from *B. alcalophilus* (ATCC 27647) species developed at different temperatures, obtained with SDS PAGE.

The protein profiles obtained after 12, 24 and 48 hours at temperatures of 35, 42, 48, and 52°C from *B. thurinrgiensis* var. *israelensis* are given in Fig. 4. At 35°C, there was no band formation after 12 hours, however after 24 and 48 hours, intense band profiles were observed. At 35°C, after 48 hours the density of protein labeled as number 3 with molecular weight 98 kDa was observed to increase. At 42°C, there was no band observed after 12 hours; however after 24 and 48 hours the band profile was densely observed. The band with molecular weight 178 kDa shown by number 1 and the 152 kDa band shown with number 2 were identified to intensely increased after 24 and 48 hours at 42°C. At 48°C, the bacteria did not grow well after 12 and 24 hours, while after 48 hours apart from the 42 kDa molecular weight band labeled number 4, no band formed. At 52°C, after







Fig. 4. Protein profiles from *B. thurinrgiensis* var. *israelensis* developed at different temperatures, obtained with SDS PAGE.

The protein profiles obtained after 12, 24 and 48 hour at temperatures of 35, 42, 48, and 52°C from local isolate 4 are given in Fig. 5. When protein synthesis at 35 and 42°C temperatures from local isolate 4 are examined, the band profiles appear very similar. The protein shown with number 1 with molecular weight 154 kDa, appeared after 12 and 24 hours at 35°C and 42°C. The amount of this protein synthesized at 42°C was identified to be higher than the amount synthesized at 35°C. The protein with molecular weight 97 kDa shown with number 2 was observed at 35°C and 42°C and this band also was observed after 48 hours. The amount of this protein synthesized at 35°C was higher than the amount synthesized at 42°C. At 48°C and 52°C, the bacteria did show good development after 12 hours. However, the twin band shown by number three with molecular weight 40 and 39 kDa was observed to develop at 42°C after 24 and 48 hours, at 48°C after 24 and 48 hours and at 52°C after 48 hours.

The protein profiles obtained after 12, 24 and 48 hour at temperatures of 35°C, 42°C, 48°C, and 52°C from local isolate 31 are given in Fig. 6. At the first two temperatures of 35°C and 42°C the band profiles appear similar. The band shown at

number 1 with molecular weight 154 kDa and the band shown at number 2 with molecular weight 100 kDa were observed to be synthesized after 24 and 48 hours at 35°C and 42°C. These bands were lost at 48°C and 52°C. Band number 3 with molecular weight 97 kDa was produced at 35°C, 42°C and 48°C after 24 and 48 hours, but was lost at temperature 52°C. Band number 4 with molecular weight 52 kDa was produced after 24 and 48 hours at temperatures 35°C and 42°C; however it appeared to increase at 42°C. At 48°C, the band was not observed after 12 hours, but an increase was observed after 24 and 48 hours. The twin band shown by number 5 with molecular weight 40 and 39 kDa appeared to increase in amount at 48°C after 24 and 48 hours. Finally, at 52°C, no band formation was observed after 12, 24 and 48 hours.



Fig. 5. Protein profiles from local isolate 4 developed at different temperatures, obtained with SDS PAGE.



Fig. 6. SDS PAGE photograph showing development of isolate 31 at 35°C, 42°C, 48°C, and 52°C.

3.2. Bacterial development and sporulation results

The six different *Bacillus* species used in our study were found to have varying vegetative or spore state in a temperature (35°C, 42°C, 48°C, 52°C) and time-dependent manner. The timedependent variation in counting results of these six bacteria species against temperature are shown in Table 5.

When the correlation between the temperatures and counting results for the 6 different bacteria species used in the study are examined, temperature was found to be very effective

Table 5

Count results for 6 bacteria used in the study (s: spore count, v: vegetative count, h: hour)

	35°C					42°C							
		12	. h	24. h		48. h		12. h		24. h		48. h	
	*	V	S	V	S	V	S	\mathbf{V}	S	\mathbf{V}	S	\mathbf{V}	S
B.firmus	5.9×10^{10}	7.3x10 ⁴	$1.4x10^4$	9.3x10 ⁶	1.9×10^{6}	3.9x10 ⁶	3.97x10	⁷ 4.9x1	0 ⁴ 7.5x10	⁴ 3.1x10 ⁵	3.10x10 ⁵	3.75x10 ⁵	4.8×10^4
B. alcalophilus	5.48x10 ¹⁰	3.6x10 ⁷	3.69x10 ⁷	1.63×10^{6}	1.4×10^{7}	3.15x10 ¹⁰	3x10 ⁹	1.70x1	0 ⁴ 1.45x10	⁵ 4.98x10 ⁵	$3x10^{4}$	1.41×10^{6}	1.9×10^{6}
B. sphaericus	8.7×10^{3}	$8.33 x 10^{6}$	1.83x10 ⁸	4.11×10^{9}	3.36x109	1.3x10 ⁹	1.43x10	⁹ 1.36x1	0 ⁶ 4.9x10	⁵ 4.5x10 ⁹	3.99x10 ⁹	1.64×10^{6}	5.91x10 ⁶
B. thuringiensis	3.3x10 ⁹	2.11×10^{6}	3.11×10^{6}	1.25×10^{7}	1.77×10^{6}	8x10 ⁶	3x10 ⁹	8.9x1	$0^5 4x10^4$	4.77x10 ⁵	1.6x10 ⁵	1.1×10^{5}	8.9x10 ⁵
4 Local isolate	1.33x10 ¹¹	4.03×10^{7}	1.02×10^{7}	1.8×10^{6}	3.14x10 ⁶	1.3x10 ⁹	3.6x10 ⁶	3.3x1	0 ⁷ 3.4x10	⁵ 1.1x10 ⁷	3.5×10^{7}	9x10 ⁷	3.45×10^{6}
31 Local isolate	5.38x10 ⁹	1.36×10^{5}	1.96x10 ⁵	1.12×10^{8}	1×10^{6}	1.35x10 ⁹	9x10 ⁶	4.4x1	0^4 8x10 ⁴	4.5x10 ⁶	5.7x10 ⁶	6.6×10^7	6.4×10^{7}
			4	8°C				52°C					
	12. h 24. h		24. h	48. h			12. h		24. h		48. h		
	V	S	\mathbf{V}	S	V	5	5	V	S	V	S	V	S
B.firmus	$4x10^{2}$	1.9x10 ³	7.9x10 ⁴	1.6x10	⁵ 1.64x	10^4 7.1x	10 ³ 8	sx10 ²	3x10 ²	2x10 ⁴	.81x10 ⁴	1.37×10^{2}	3.1×10^{2}
B. alcalophilus	4.5×10^{3}	6.5x10 ³	6.8x10 ³	4.3x10	³ 5.3x	10 ⁵ 3.1x	10 ⁵ 1.2	27×10^4	1.31x10 ⁴	9.9x10 ³	8x10 ²	8.7×10^{3}	1.08×10^4
B. sphaericus	3.68×10^4	1.57x10 ⁴	⁴ 1.7x10 ⁵	3.13x1	0 ⁶ 3.43x	10 ⁵ 4.6x	4.10 ⁵ 4.	6x10 ³	4.5×10^{3}	3.8×10^{3}	8.5×10^{3}	1.79×10^4	3.4×10^4
B. thuringiensis	1.48x10	3x10 ³	1.55x10	⁴ 1.12x1	0 ⁴ 3.1x	10 ³ 5.91	x10 3.3	33x10 ³	1.5x10 ²	5.54x10 ³	1.8×10^{2}	3.1x10 ³	5.91×10^{2}
4 Local isolate	1.13x10 ⁶	5x10 ³	3.1x10 ⁶	1.35x1	0 ⁶ 1.13x	105 3.35	x10 ⁵ 3.	5x10 ³	5x10 ³	3.5x10 ³	8x10 ²	1.54×10^4	2.97×10^4
31 Local isolate	1.6x10 ³	3.9x10 ³	1.5x10 ³	1.72x1	0 ⁴ 3.73x	10 ⁵ 3.34	x10 ⁵ 6	5x10 ²	3.3x10 ³	1.3x10 ³	7x10 ²	1.16x10 ⁴	1.01×10^4

on heat resistant spore counts and this is shown in Table 6.

Table 6

Effect of temperature on heat resistant spore counts for six bacteria used in the study.

I	B. firmus (ATCC 14573)	p<0.0026
	B. alcalophilus (ATCC 27647)	p <0.0012
	B. sphaericus (ATCC 2362)	p<0.0001
	B. thuringiensis var. israelensis	p<0.0001
	Local isolate 4	p<0.0001
	Local isolate 31	p<0.0012

4. Discussion

In our research, the development of 6 different Bacillus species was shown to reduce significantly when temperatures increased from 35°C to 52°C. Richter and Hecker (1986) in a study of rel A and rel A⁺ species of Bacillus subtilis, determined that heat shock increased the synthesis of various proteins. At 52°C cellular proteins were inhibited, while heat specific (heat shock) proteins were preserved. When temperatures rose from 37°C to 52°C synthesis of protein with 66 kDa weight was identified. In our study in B. firmus (ATCC 14573) 40 kDa weight protein and in local isolates 4 and 31-40 kDa and 39 kDa weight protein synthesis was determined. Additionally, B. cereus was found to synthesize six different proteins at 43°C; 76 and 66 kDa within 0-10 minutes, 57 and 39 kDa in 10 to 20 minutes and 98 and 43 kDa within 30-40 minutes (Browne and Dowds, 2001). When heat shock is applied to live cells, the synthesis rates of heat shock proteins are observed to increase (Gomes and Simao, 2014). Another study using heat shock stimulation of *B. subtilis* identified 97 and 66 kDa molecular weight proteins in membrane and 40 and 23 kDa molecular weight proteins in the cytosol (Qoronfleh and Streips, 1987). In our study at 35°C and 42°C temperatures, in local isolates 4 and 31, 97 kDa weight protein was synthesized and these results are in accordance with Qoronfleh and Streips (1987). We found that B. thuringiensis var. israelensis at 35°C and 42°C synthesized 98 kDa protein. At 52°C B. firmus (ATCC 14573) reference bacteria synthesized a 40 kDa protein. After 48°C temperature, B. thuringiensis synthesized a 42 kDa weight protein. Additionally, local isolates 4 and 31 and B. sphaericus (ATCC 2362) bacteria were observed to synthesize 40 kDa weight protein. A different study by Todd et al. (1985) applied heat shock to *B. subtilis* and found four heat shock proteins with molecular weights 84, 69, 32 and 22 kDa were produced. This study found that the 177 kDa molecular weight protein synthesized at 35°C and 42°C by *B. firmus* (ATCC 14573) and shown as band number 1 disappeared at higher temperatures (Fig. 1).

However the 40 kDa molecular weight at 52°C is a heat shock protein. When B. alcalophilus (ATCC 27647) species was exposed to 35°C, 42°C and 48°C, the amount of 166 kDa molecular weight protein shown by number 1 decreased at 48°C and disappeared at 52°C. After 42°C temperature application. band number 2 was intensely synthesized and it reduced at 48°C and was not observed at all at 52°C (Fig. 2). At 35°C and 42°C, B. sphaericus (ATCC 2362) species developed a doublet band with 179 and 149 kDa molecular weight shown with number 1, which then disappeared at 48 and 52°C. Stated differently, these proteins are susceptible to high temperature with the same situation present for bands shown with numbers 2 and 3. The doublet protein band shown by number 4 with molecular weight 40 and 39 kDa was not synthesized after 52°C temperature application (Fig. 3). For B. thuringiensis var. israelensis a similar situation exists as for B. sphaericus (ATCC 2362). The bands shown by 1, 2 and 3 were intensely synthesized after 35 and 42°C applications. The band shown by number 4 with molecular weight 42 kDa was synthesized at 48°C but was lost at 52°C (Fig. 4).

When local isolates 4 and 31 are examined, they synthesized a protein with molecular weight 154 kDa in band number 1 at 35°C and 42°C. The 97 kDa molecular weight protein shown with band number 2 produced by local isolate 4 and the 100 kDa molecular weight bands in local isolate 31 were not synthesized at 48 and 52°C. The twin bands, shown by band 5 in local isolate 31 and by band 3 in local isolate 4 with 40 and 39 kDa molecular weight, were intensely synthesized at 48°C and 52°C. This band is thought to be a heat shock protein that can be synthesized at high temperature.

Heat shock studies of *Bacillus* species generally comprise of two stages preliminary shock and lethal shock. In our study we obtained results by applying four different temperatures over a 48 hour period. We identified two local isolates used in our study as being heat resistant. If the genes coding for the special proteins synthesized can be identified, we believe they may be used in industry and for other applications.

Detailed studies have been carried out on genes and the control mechanism of these genes which code for heat shock proteins created specifically after heat shock of bacteria (Connors et al., 1986; Ron et al., 1999; Melly and Setlow, 2001; Versteeg et al., 2003). Many researchers have used high resolution gel electrophoresis (2D) to describe heat stress proteins in heat stress studies (Richter and Hecker, 1986; Connors et al., 1986; Qoronfleh and Streips, 1987; Antelmann et al., 1997; Browne and Dowds, 2001; Periago et al., 2002; Rosen and Ron, 2002; Han et al., 2008). In our study when high resolution gel electrophoresis is used the number of proteins may increase. A study by Elcin et al. (1995) encapsulated B. sphaericus (ATCC 2362) species in CMC (carboxymethylcellulose). The results of the study showed that at 50°C the unencapsulated spores reduced 10²-10³ fold after 60 days, while the number of encapsulated spores did not change. In our study we noted that at 50°C the number of heat resistant spores of *B. sphaericus* (ATCC 2362) species reduced 10⁴ times. The data obtained in both studies show that B. sphaericus (ATCC 2362) is not resistant to heat.

When the counts at 35°C for *B. firmus* (ATCC 14573), *B. alcalophilus* (ATCC 27647), *B. thuringiensis* var. *israelensis.* and *B sphaericus* (ATCC 2362) are compared with the counts at 52°C, a severe reduction is observed. For local isolates 4 and 31, the results again identified a severe reduction from 35°C to 52°C. However, compared with the results from other species at 52°C, the number of heat resistant spores appears to be higher. A variety of stress studies have been performed for bacteria in the *Bacillus* genus, used in a variety of areas. Studies of the alkalophilic *Bacillus* within the genus have generally focused on characterization of the microorganisms and the enzymes they

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produce, but studies related to stress were not found. It is possible that alkalophilic Bacillus may include gene groups that can produce different proteins depending on stress factors compared to other Bacillus species. Enzymes and proteins from alkalophilic bacteria are used in a variety of industrial fields such as detergent production and the paint industry. The gene groups of these bacteria, which can produce proteins and enzymes resistant to heat, can be determined and cloning studies can be made into different bacteria. After identifying heat shock proteins in our study, description of proteins with different techniques like 2D electrophoresis will be performed and later micro sequencing analysis will identify genes coding for these proteins. Additionally, the identification of gene groups coding for heat shock proteins determined in this study and the σ factor regulating transcription of these genes will contribute to studies in this area.

Recent research has determined that 4 out of 20 isolates from *B. sphaericus* were moderately toxic to larvae of the mosquito pathogen (Suryadi et al., 2016). We believe the use of heat shock proteins from two reference species that are mosquito pathogens used in our study will be a new area of use in biological intervention.

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Research article

Determination of terrestrial EUNIS habitat types of Mount Ganos (Işıklar), Tekirdağ, Türkiye

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Abstract

In this study, it is aimed to determine the terrestrial European Union Nature Information System (EUNIS) habitat types of Mount Ganos (Işıklar) and its surroundings. Field studies were carried out from April to October 2021. Reference areas were determined for Maximum Likelihood (ML) classification during the field studies. To increase the accuracy and obtain the highest possible level of EUNIS habitat types, we used both reference areas observed in the field studies and processed land cover and habitat maps. These are; Landsat Satellite Images classified with ML, Corine Land Cover, and European ecosystem maps. Regarding both biodiversity and social activities, Mount Ganos is among the most significant natural areas in the Tekirdağ district. The northern slopes of the mountain have a rainier and more humid climate than the southern slopes which Mediterranean climate is dominant. The presence of various climate types and the remarkable altitude variations also contribute to the habitat diversity of the Mount Ganos. Many natural areas have been degraded due to anthropogenic effects such as mineral extraction, agricultural, tourism, and urbanization activities in the Mount Ganos region until today. In this study, a total of 9 ecosystems and 29 habitat types were determined for Mount Ganos according to the EUNIS classification. 21 of them were identified at level 3 and 8 of them ranged between 2 and 6 levels. The intensive unmixed crops (I1.1) are the most-covered EUNIS habitat type with 16173.16 hectares. This is followed by low and medium altitude hay meadows (E2.2, 9350.63 ha), Meso- and eutrophic Ouercus, Carpinus, Fraxinus, Acer, Tilia, Ulmus, and related woodland (G1.A, 7548.73 ha) and Pseudomaquis (F5.3, 5926.65 ha). With this study, a portrait of the habitat destruction created by humans has also been drawn. The results of this study can be used by decision-makers to conserve the remaining natural habitats on Mount Ganos.

Keywords: Biodiversity; classification; ecosystem; GIS; land cover

1. Introduction

Habitat is briefly defined as terrestrial, freshwater, or marine systems where an organism continues its vital activities (Mitchell, 2005; Diehl, 2013; Hall et al., 2013). In addition to terrestrial and marine habitats, there is a proposal to consider airspace as a habitat (Diehl, 2013). Classification of habitats (including natural, semi-natural, and man-made habitats) can be accepted as a practical tool for nature and species conservation. It is also important in generating inventories of natural places; conservation or monitoring studies, and target setting in ecological restorations (Davies et al., 2004; Moss, 2008; Janssen et al., 2016; Chytrý et al., 2020).

European Union Nature Information System (EUNIS) Habitat classification based on hierarchy was designed by the European Topic Center for Biodiversity for the European Environment Agency (EEA) (Davies and Moss, 1998; Davies et al., 2004; Moss, 2008). This classification system includes much

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more detailed habitat definitions relative to Corine Land Cover and Palaearctic Habitat classification since it is based on syntaxonomic units (Moss and Davies, 2002).

Although there are some efforts to determine habitat types in Türkiye by using the EUNIS habitat classification, these efforts covered only limited areas, and they are not adequate relative to Europe EUNIS-based studies (Cakmak and Aytac, 2021). Similar to flora studies, conducting inventories of habitat types is important for conservation, monitoring, and assessment studies. Mount Ganos is one of the hotspot areas in the Thrace region because of its rich species and habitat diversity. So with this study, we also aimed to determine the terrestrial EUNIS habitat types of Mount Ganos and its surroundings, and also to contribute to the classification of habitats in Türkiye.

2. Materials and methods

2.1. Study area

Mount Ganos (Işıklar) is located in the south-eastern part of Tekirdağ province in Türkiye (40°48'06.4"N, 27°18'59.4"E). The study area, which includes Mount Ganos, is within the borders of 3 different districts: Süleymanpaşa in the north, Malkara in the northwest, and Şarköy in the south and southwest. The total terrestrial area of the study area is 52827.23 ha.

2.2. Field studies

Field studies were carried out from April to October in 2021. Reference areas were determined for Maximum Likelihood (ML) classification in the field studies. Plant samples representing reference areas were collected during field studies. The coordinates of the reference areas where the plants were collected were determined by the Magellan 510 GPS device. Identification of plants was carried out in Tekirdağ Namık Kemal University, Plant Morphology and Anatomy Laboratory. Flora of Turkey and the East Aegean Islands (Davis, 1965-1985; Davis et al., 1988; Guner et al., 2000) and Resimli Türkiye Florası (Illustrated Flora of Turkey) (Guner et al., 2018) were used in identification studies.

2.3. Determining EUNIS habitat types

ML classification was performed on Landsat Satellite Images (LSI) (covering April and October 2021) in the ArcMap (version 10.5) tool to determine the main ecosystem types of EUNIS (E, G, H, I, J, etc.) based on reference areas. During the process, we obtained 15 meters resolution satellite images with the help of panchromatic and multispectral bands of the LSI and Pan-Sharpening process. This process and also radiometric and geometric corrections of the LSI were performed with the GRASS GIS software. To increase the accuracy and obtain the highest possible level of EUNIS habitat types we used not only reference areas observed in the field studies, but also ML classification performed LSI, Corine Land Cover (Copernicus Land Monitoring Service, 2022), and European ecosystem map (EEM) provided by European Environment Agency (European Environment Agency, 2022) (100 m resolution).

3. Results and discussion

were determined according to the EUNIS classification (Fig. 1). 21 of them were identified at level 3 and 8 of them ranged between 2 and 6 levels (Table 1). The intensive unmixed crops (I1.1) are the most-covered EUNIS habitat type with 16173.16 hectares (ha). This is followed by low and medium altitude hay meadows (E2.2, 9350.63 ha), Meso- and eutrophic *Quercus*, *Carpinus*, *Fraxinus*, *Acer*, *Tilia*, *Ulmus*, and related woodland (G1.A, 7548.73 ha) and Pseudomaquis (F5.3, 5926.65 ha).

In the European ecosystem map (EEM) containing the EUNIS data presented by the European Environment Agency (EEA), habitat types are given only up to level 2 at the European scale (including Türkiye) (European Environment Agency, 2022). There are 18 different level 2 habitat types in and around Mount Ganos in EEM. In addition to these habitat types, we observed 4 new habitat types at level 2 with this study: C2 (as C2.3), D4 (as D4.1), J4 (as J4.5), and J5 (as J5.3). F5 habitat is shown in a very narrow area in the EEM. Contrary, it covers a large part of the southern slopes of Mount Ganos. The area determined as scree (H2) in the EEM was defined by us as permanent non-tidal, smooth-flowing watercourses (C2.3). In this habitat type, it was observed that the river bed was intact and stone sets were built on its edges. It is thought that the Geographical Information Systems (GIS) methods which EEM is based on, evaluate this area as H2 due to the stone set. A part of the southern slopes of Mount Ganos was specified in the EEM as miscellaneous inland habitats with very sparse or no vegetation (H5). However, in the field studies carried out by us in the relevant areas, it was determined that algae, lichens, or bryophytes were not dominant and vascular plants were not very sparse. On the contrary, habitat types in these areas were observed as E1.1, E1.2, and F5.3 where vascular plants were dominant. In addition to these habitats, the H3.2D habitat type which is associated with chasmophytic species such as Sedum album L., Melilotus albus Desr., M. spicatus (Sm.) Breistr., Senecio vulgaris L., Thymus atticus Celak., T. sibthorpii Benth., and Thymbra spicata L. was determined. A similar situation applies to the C1 and G2 habitat types given by the EEM. Areas specified as G2 in the EEM were defined as FB and G1.D in field observations. C1 was defined as J5.3.

Permanent non-tidal, smooth-flowing watercourses (C2.3) habitat type was observed in regions with intensive agricultural activities. This includes slow-flowing rivers, streams, brooks, rivulets, and rills which are associated with benthic and mesoeutrophic macrophyte communities (Davies and Moss, 1998; Davies et al., 2004). Considering the nutrition provisioning by fertilization of agricultural fields, the observation of mesoeutrophic (Groenlandia densa (L.) Fourr., Nymphaea alba L., Potamogeton natans L., P. nodosus Poir., P. perfoliatus L., and Stuckenia pectinata (L.) Börner) species in this area confirms this habitat type. Slow-flowing rivers have vegetation rich in submergent species such as G. densa, P. nodosus, and N. alba. Because the flow rate is slow, such habitats have a suitable environment for the development of submergent and freefloating species. However, changing the flow rate in rivers depending on the season or climate can damage this habitat type, which contains emergent-submergent species important for nitrogen removal from water (Albert et al., 2005; Tanaka, 2006; Fayvush and Aleksanyan, 2022). We observed that calcareous seasonal water bodies are formed in some areas with the effect of meso-eutrophic rivers. In these areas, species related to the D4.1 habitat were found such as Phragmites australis L., Glyceria maxima (Hartm.) Holmb., and Juncus subnodulosus Schrank.

In this study, a total of 9 ecosystems and 29 habitat types

Table 1

EUNIS habitat types of Mount Ganos and its surroundings.

EUNIS Habitat Type	EUNIS Habitat Name	Percentage (%)	Area (ha)	
	B-Coastal habitats			
B2.2	Unvegetated mobile shingle beaches above the drift line	0.006	3.31	
	C-Inland surface waters			
C2.3	Permanent non-tidal, smooth-flowing watercourses	0.064	34.03	
	D-Mires, bogs and fens			
D4.1	Rich fens, including eutrophic tall-herb fens and calcareous flushes and soaks	0.009	4.59	
	E-Grasslands and lands dominated by forbs, mosses or lichens			
E1.1	Inland sand and rock with open vegetation	0.092	48.45	
E2.2	Low and medium altitude hay meadows	17.700	9350.63	
	F-Heathland, scrub and tundra			
F5.3	Pseudomaquis	11.219	5926.65	
F5.4	Spartium junceum fields	0.020	10.36	
FB	Shrub plantations	1.636	864.43	
	G-Woodland, forest and other wooded land			
G1.11	Riverine Salix woodland	0.280	148.07	
G1.7	Thermophilous deciduous woodland	4.312	2277.88	
G1.712	Sub-Mediterranean Quercus petraea-Q. robur woods	0.674	355,86	
G1.76	Balkano-Anatolian thermophilous Quercus forests	3.840	2028.55	
G1.A	Meso- and eutrophic Quercus, Carpinus, Fraxinus, Acer, Tilia, Ulmus, and related woodland	14.289	7548.73	
G1.A1C3	Moesian oak-hornbeam forests	1.620	855,74	
G1.D	Fruit and nut tree orchards	5.104	2696.14	
G2.1	Mediterranean evergreen Quercus woodland	0.004	2.10	
G3.7	Lowland to montane Mediterranean Pinus woodland (excluding Pinus nigra)	1.977	1044.59	
G3.75	Pinus brutia forests	0.541	285.78	
G3.F12	Native pine plantations	0.964	509.36	
G4.B	Mixed Mediterranean Pinus - thermophilous Quercus woodland	2.468	1303.61	
G5.2	Small broadleaved deciduous anthropogenic woodlands	0.749	395.87	
G5.5	Small mixed broadleaved and coniferous anthropogenic woodlands	0.258	136.52	
	H-Inland unvegetated or sparsely vegetated habitats			
H3.2D	Basic and ultra-basic inland cliffs	0.607	320.59	
U	arly or recently cultivated agricultural, horticultural and domestic habitats			
I1.1	Intensive unmixed crops	30.615	16173.16	
	J-Constructed, industrial and other artificial habitats			
J1.2	Residential buildings of villages and urban peripheries	0.675	356.53	
J2.3	Rural industrial and commercial sites still in active use	0.008	3.99	
J3.2	Active opencast mineral extraction sites, including quarries	0.089	46.88	
J4.5	Hard-surfaced areas of ports	0.005	2.71	
J5.3	Highly artificial non-saline standing waters	0.174	92.12	
		Total Area	52827.23	

E1.1 habitat type is related to thermophilic vegetation. It was observed in the southern slopes and calcareous areas of Mount Ganos. Species such as Poa bulbosa L., Bromus tectorum L., and Cynodon dactylon (L.) Pers. associated with E1.1 were found. Sedum album and Thymus species, which can also be seen in the H3.2D habitat type were also observed in E1.1. E1.1 is rich in biodiversity and can be considered on priority status in terms of protection (Cakmak and Aytac, 2020). At low and medium altitude hay meadows (E2.2), species related to this habitat type, such as Poa pratensis L., Alopecurus pratensis L., Trifolium dubium Sibth., Arrhenatherum elatius (L.) P.Beauv. ex J.Presl & C.Presl, Dactylis glomerata L., Daucus carota L., Galium album Mill., Medicago sativa L., Orchis coriophora L., and O. laxiflora Lam., were observed. Grass-dominated hay meadows are characterized by their highly diverse vegetation (Rodríguez-Rojo et al., 2017). However, it has decreased significantly since the mid-20th century (Sullivan et al., 2018) due to the damage caused by agricultural activities (especially the disposal of the area for agricultural land) and grazing pressure in Mount Ganos and its surroundings.

F5.3 habitat type associated with sclerophyllous evergreen and deciduous shrubs was determined in the southern part of Mount Ganos. In areas where F5.3 habitat type was determined, Quercus coccifera L. was dominant, but also Juniperus oxycedrus L., Carpinus orientalis Mill., Pistacia terebinthus L., Paliurus spina-christi P. Mill., and Quercus pubescens Willd. were identified. It is thought that a part of the F5.3 in this study area was formed by the degradation of the thermophilic deciduous woodlands (G1.7). Such that, transitional areas can be seen between G1.7 and F5.3 habitats, where Quercus petraea (Matt.) Liebl., Q. robur L., Q. frainetto Ten., and Q. cerris L. are found together. In addition to the transition areas, pseudomaquis were also observed in deciduous forests, especially in the northwest of Uçmakdere. On the northern slopes of Mount Ganos, where the effect of the Mediterranean climate decreased, it was observed that Carpinus orientalis Mill. was more dominant in pseudomaguis instead of *Quercus* coccifera L.. Čarni et al. (2018) also stated that maquis formations dominated by deciduous species were observed in the regions where the effect of the Mediterranean climate decreased. Along with F5.3, Spartium junceum L. dominated F5.4 habitat type was determined near roads on the slopes facing the sea.

Grape fields, which play an important role in the livelihood



Fig. 1. EUNIS habitat map of Mount Ganos and its surroundings.

of the people of this region, are dense in the south of the study area. It is observed that these areas, which are considered especially wine gardens, have recently been turned into orchards (G1.D) (mostly cherry).

Shrubby Salix alba L. and Populus alba L. dominated G1.11 constitute 0.280% of the study area. Urtica dioica L., which is used by the local people for ethnobotanical purposes (food, health, etc.), was frequently observed in this habitat. G1.11 habitat in the study area is generally used by people for recreational activities. It was determined that this habitat type sustained damage when precipitation is below the season normal or when the stream dries out periodically, especially due to human activities.

G1.7 habitat type (including G1.712 and G1.76) covers 8.83% of the study area. It is dominated by *Quercus* species such as *Q. petraea* (Matt.) Liebl., *Q. robur* L., *Q. frainetto* Ten., and *Q. cerris* Blanco. Also, sometimes accompanied by *Carpinus* orientalis Mill. and Castanea sativa Mill.. In addition, thermophile calcicolous plants such as Limodorum abortivum (L.) Sw. associated with G1.712 and G1.76, and Veronica officinalis L. were also observed. G1.712 is generally found in the southern parts of Mount Ganos. However, G1.76 was found only in the north of Mount Ganos. In this study area, the use of *Quercus* species as wood charcoal by the people and mining activities can be shown as threat factors for this habitat type.

G1.A habitat type consisting of *Acer L., Carpinus L., Fraxinus* Tourn. ex L., and *Tilia L.* composition, especially dominated by *Quercus* species, was observed on the northfacing slopes of Mount Ganos. In terms of tree vegetation, G1.A is the largest (14.289%) habitat type and covers an area of 7548.73 hectares. There are many hiking routes in the areas where the G1.A habitat type was determined. In addition, the

local people in the vicinity harvest the *Tilia tomentosa* Moench throughout the flowering season for extra income. It was observed that sometimes insensible tourism and harvesting activities damage this habitat type. To sustain this habitat type, which is entirely composed of natural trees, it is recommended that authorized institutions follow human activities here and raise awareness of the local people on this issue.

Although the I1.1 habitat type covers 30.615% of the study area, forest vegetation (excluding FB and G1.D habitat types) constitutes 43.196% of the study area. According to the report of the General Directorate of Forestry of Türkiye (2020), the total forest assets of Tekirdağ province are 101174 hectares. The forest assets in the study area constitute almost one-fourth (22819.31 ha) of Tekirdağ. Considering that the total area of Tekirdağ is 634 thousand (study area 52827.23) ha, Mount Ganos is important for the forest assets of Tekirdağ. In addition to forest assets, the study area includes hay meadows (E2.2) rich in plant diversity, especially in the Melen region of Mount Ganos.

Coniferous trees constitute 3.483% (excluding mixed forests with broadleaved trees) of the study area. We observed habitat types that there are G3.75 (only *Pinus brutia* Ten.), G3.7 (*P. brutia* is dominant, but *Ilex aquifolium* L. is present), and G3.F12 (reforestation with *P. brutia*). It was observed that reforestation works were carried out with *Pinus brutia* species, which is naturally distributed in this region as well.

We thought that reforestation works are mostly carried out in areas where G1.7 and F5.3 habitats have been destroyed since short *Quercus coccifera* L. and *Q. petraea* (Matt.) Liebl. were observed in these areas. Most of the areas with G3 habitat type are picnic areas managed by private and municipal enterprises. The most important factors threatening this habitat type are the high potential of human-induced fires and the conversion of these areas into arable lands.

Most of the habitats determined in this study were evaluated in the LC category in the European Red List of Habitats (Terrestrial and freshwater habitats) (Janssen et al., 2016). Only the Low and medium altitude hay meadows habitat type (E2.2) has been evaluated in the VU, and the lower levels of some habitat types (e.g., E1.1a-j) are in the VU-CR category. The abovementioned study is useful in determining or evaluating the threatened categories of habitats in Europe. However, habitat types in Türkiye were not included in this study. For this reason, there is no data on how accurate it is to consider the threatened categories of habitats according to this study at the scale of Türkiye.

Mount Ganos is among the important natural areas of the Tekirdağ district in terms of both biodiversity and social activities. While the Mediterranean climate is seen on the southern slopes of the mountain, the northern slopes have a rainier and more humid climate compared to the southern parts. The presence of different climate types and the remarkable differences in altitude (for example, the sudden rise to 300-400 meters above sea level) also increase the diversity of habitats in the region. Besides, it is one of the leading tourism activity areas of Tekirdağ. At the same time, mining activities are carried out in some regions of Mount Ganos. Therefore, human pressure on the biodiversity in and around Mount Ganos is high. Determining the EUNIS habitat types may contribute to possible conservation or monitoring studies in this region.

Since the habitat types were determined on a European scale in 1998, studies to identify EUNIS habitat types and threat

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factors on related habitat types have been common for the last 10 years in Türkiye (Arslan et al., 2012; Mergen and Karacaoglu, 2015; Geven et al., 2016; Ciftci and Hasbenli, 2018; Tezel et al., 2020; Cakmak and Aytac, 2020, 2021).

Türkiye is quite different from Europe in terms of ecology, climate, topography, and biodiversity. Therefore, new habitat types have been defined in Türkiye due to the differences in the existing syntaxonomic units which define EUNIS habitat types (Cakmak and Aytac, 2021). However, probably, these syntaxonomic units may also be associated with existing habitat types. Apart from these differences, EUNIS is almost entirely compatible with Türkiye. Habitats are an important part of ecosystems, and the existence of some species depends on the continuity of their habitats. Especially due to climate change, the risk of species extinction specific to certain habitats is high (Pompe et al., 2010). For this reason, it is important to increase studies on the determination of habitat types and plant communities specific to these habitat types at the scale of Türkiye.

Conservation of specialized habitats may become even more important in comparison to the conservation of a single threatened species. Thus, it can enable the conservation of all species in related habitats.

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Review article

A review on antimicrobial activities of some culinary herbs and spices against *Staphylococcus aureus*

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Abstract

Food safety is of great importance all over the world as it concerns consumer health. All employees in the food chain must comply with the hygiene rules. One of the important issues that threaten food safety is contamination with microorganisms. Numerous people are affected by contaminated and/or poorly preserved food and outbreaks have occurred. The World Health Organization (WHO) draws attention to human health and economic losses in this respect. From ancient times, herbs and spices are utilized in Türkiye and various parts of world to enhance the flavor of food and their sensory properties. It is also possible to prevent the development of *Staphylococcus aureus*, which causes food poisoning, thanks to the antibacterial properties of culinary herbs or spices. Thus, using natural antimicrobial substances from spices and herbs may be an alternative for inhibition/elimination of growth of *S. aureus* extending the shelf life without synthetic preservatives. This review aims to explain foodborne diseases and their global burden, staphylococcal food poisoning, natural antimicrobials, some edible herbs in Türkiye: their culinary uses and antibacterial efficacy against *S. aureus*.

Keywords: Antimicrobial; culinary herbs and spices; food poisoning; Staphylococcus aureus

1. Introduction

The importance of foodborne diseases in terms of public health around the world has been emphasized in the literature. It is also known that the economic and social burden that arises especially for underdeveloped countries is remarkable when the morbidity and mortality rates are taken into account (Odeyemi, 2016; WHO, 2020, Pires et al., 2021). Different types of bacteria, viruses, parasites, prions, and fungi may cause foodborne diseases. Amongst them, bacteria have a major role in being the causative agent in foodborne diseases (Ishaq et al., 2021). As a result of burden assessments for foodborne diseases, the global importance of *Staphylococcus aureus* has been indicated (Fetsch and Johler, 2018). It is obvious that food safety should be handled carefully in the food chain from farm to table for human health (Sousa, 2008; Odeyemi, 2016). In this context, the standards ensure a strict controlling system with the traceability principle in every stage of food production. Unfortunately, developing countries face widespread food safety problems compared to industrialized countries. The reason for this can be explained simply by the use of traditional methods in marketing products (Sousa, 2008).

Staphylococcal intoxications are associated with enterotoxins, one of the virulence factors of this bacterium, which affect the digestive system of humans (CDC, 2018). The prevalence of food intoxication caused by *S. aureus* is reported to be at high rates worldwide (Fetsch and Johler, 2018). The relationship between *S. aureus* enterotoxins and food poisoning have been noted in individuals who ate a sponge cake infected with *S. aureus* and showed signs of food poisoning (Dack et al.,

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1930). Since then, there have been some food poisoning outbreaks caused by different foods depending on culturally eating habits that differ between countries (Hennekinne et al., 2012). 87% of vomiting cases by *S. aureus* outbreaks were reported in the U.S. between 1998 and 2008 (Bennet et al., 2013). In another study, *S. aureus* was detected in 102 of 971 food samples in Italy (Vitale et al., 2015).

Antimicrobials are of great importance in controlling foodborne diseases caused by bacteria. In this respect, various food preservatives are currently used. However, due to the fact that natural components are preferred by consumers as preservatives in foods in the sector and the need to find some alternative antimicrobials, the interest in herbs and spices, which have been used for centuries, has intensified (Bondi et al., 2017). Various spices and herbs have been used as flavoring, fragrant, and coloring agents in foods for centuries. Furthermore, in the respect of ethnobotanical importance, local people utilize spices and herbs in folk medicine. In various studies, medicinal properties such as antimicrobial, antioxidant, antidiabetic, and anti-inflammatory activities, etc. of these spices and herbs have been reported. They are frequently used for microbial control in food products, especially due to its antimicrobial properties on microorganisms (Chakraborty et al., 2020; Karakol and Kapi, 2021). In recent years, the use of natural preservatives has been increasing day by day due to the natural antimicrobial properties of herbs and spices compared to many synthetic antimicrobial substances used in the food industry. The plant itself, extracts, and essential oils obtained from plants show antimicrobial effects in food products and can extend the shelf life of foods (Gyawali and Ibrahim, 2014). Researchers are interested in herbs and spices to preserve foods and extend product shelf-life in the last decades. Various studies show that herbs and spices have the potential to be used in some food processing stages such as maintenance and ensuring shelf life, prevention of microbial degradation and oxidation reactions (Babuskin et al., 2014; Márquez-Rodríguez et al., 2020; Pérez-Santaescolástica et al., 2022). With the emergence of some new technologies, natural antimicrobial compounds from herbs or spices may be utilized alternatively in the food industry.

2. Foodborne diseases and their global burden

Since food is a remarkable vehicle for pathogens of public health, it is the main cause of foodborne illness through microbial contamination. The World Health Organization (WHO) stated that foodborne diseases are one of the global healthcare challenges that cause many complications and deaths all over the world and the incidence of these diseases is increasing globally. Food safety, especially for food spoilage and food poisoning, has a pivotal concern around the world. The morbidity and mortality rates of foodborne diseases are notable in developing countries as well as in less developed countries (Odeyemi, 2016; WHO, 2020). Annually, more than 2 million deaths from foodborne diseases were reported in developed countries. Unfortunately, mostly affected patient groups from foodborne diseases are infants, children, elderly people, and immunocompromised patients. According to the WHO, 125.000 children die from foodborne illnesses each year, and unfortunately, children under the age of 5 are at potential risk (WHO, 2015). According to Centers for Disease Control and Prevention (CDC) estimation, 47.8 million people suffer from foodborne diseases, and amongst them, 128.000 hospitalization and 3.000 death are reported each year in the United States

(CDC, 2018). On the other hand, foodborne diseases affect the socioeconomic development of countries leading to increased treatment costs, loss of work efficiency, tourism, and trade. Treatment costs were reported to be about \$15.6 billion each year by the U.S. Department of Agriculture (USDA). Moreover, it was reported by WHO that these costs rise up to \$110 billion higher each year when productivity and medical needs are deducted in low- and middle-income countries (WHO, 2020).

Due to the changing living conditions (increased eating out, consuming ready-made food, etc.) depending on the increasing industrialization and urbanization, great sensitivity is expected from each employee/individual in the food chain is in order to protect human health from farm to table within the scope of food safety. Despite the growth of the industry, sufficient practices of food handlers or the hygiene control of street food cannot be adequately controlled. Microorganism activity becomes inevitable due to reasons such as breaking the cold chain, not paying attention to the hygienic conditions of the personnel, the sanitation principles, the necessary storage, cooking, and heating temperatures in food and beverage enterprises. The incidence of foodborne infections and food poisoning considerably increased due to the consumption of food contaminated with pathogens or their toxins (Sousa, 2008; Odeyemi, 2016). Food safety problems are more common in developing countries than in industrialized countries, due to the use of traditional methods in the marketing of products. However, there are certain standards for every stage of the food process, handling and distribution in industrialized countries and applications are carried out with strict supervision and traceability principle. On the other hand, street foods are considerably popular in developing countries. In terms of food safety, this may affect the increase of biological, chemical, and physical hazards related to food quality (Sousa, 2008).

Foodborne diseases are mainly observed as infections or intoxications caused by ingestion of food that contain microbiological or chemical agents which occurs via contamination of food during food production, food transfer, and food chain. The source of foodborne pathogens is indicated as water, contact with infected farm animals, pets and humans. WHO reported more than 200 diseases caused by insecure foods with harmful bacteria, viruses, parasites or chemical substances (WHO, 2020). Mostly gastrointestinal problems and also neurological, gynecological, and immunological symptoms are seen in foodborne diseases. Foodborne pathogens may cause severe diarrhea which is a major problem worldwide. Thirty one foodborne pathogens are known to cause foodborne illness. According to CDC, thirty one known pathogens affects 9.4 million (6.6-2.7 million) people. Top five pathogens contributing to domestically acquired foodborne illnesses are declared as Norovirus, Salmonella nontyphoidal, Clostridium perfringens, Campylobacter spp., and S. aureus (CDC, 2018).

3. Staphylococcal food poisoning

Staphylococcus belongs to the family of Micrococcaceae according to Bergey's Manual of Determinative Bacteriology. The genus includes more than 30 species with a wide range of distribution in nature. Approximately 241.000 cases are reported annually due to S. aureus which is a Gram-positive, facultative anaerobe, and salt-tolerant (up to 20% NaCl) bacterium with an ability to resist drying and heat (Bhunia, 2018). S. aureus is commonly found in the skin and mucosal membranes of humans which the persistent and intermittent colonization ratios have
been reported to be 20-30% and 60%, respectively. *S. aureus* can also grow in animals such as dairy cattle, sheep, goats, etc. Unfortunately, the development of mastitis in these animals causes contamination in milk. Furthermore, *S. aureus* can contaminate foods via air, dust, and surfaces (Argudín et al., 2010).

The potential danger of this bacterium is associated with its virulence factors such as adhesion proteins, enterotoxins, superantigens, toxic shock syndrome toxins, exfoliative toxins (ETs), pore-forming hemolysins, ADP-ribosylating toxins, and proteases. Food poisoning is related to a superfamily of secreted virulence factors called staphylococcal enterotoxins (SEs), SE-like toxins (SEls) and toxins. *S. aureus* toxins are structurally similar, resistant to heat and proteolytic enzymes, having a property of superantigenicity causing disruptions in adaptive immunity, and having emetic activity (Bhunia, 2018).

Staphylococcal intoxications occur after intake of enterotoxins produced by enterotoxigenic staphylococci and act on the digestion system (Fig. 1). S. aureus generally localizes on human nasopharynx mucous membranes and skins of animals. Especially employee hygiene in food and beverage enterprises plays important role in food contamination with S. aureus. Any career that includes enterotoxin-producing staphylococci, transfer of bacteria to food, optimal growth conditions for S. aureus, and food with a sufficient amount of toxins are indispensable for these diseases. Therefore, hand washing has great importance because the bacteria can multiply on food contaminated with S. aureus and produce toxins. The contamination of food with S. aureus toxin does not show itself by spoilage or bad odor. The symptoms of food poisoning associated with S. aureus are generally characterized by severe vomiting, cramping, and sometimes diarrhea. Symptoms are generally observed within 30 minutes to 8 hours after ingestion of S. aureus toxin (CDC, 2018). Staphylococcal food poisoning may be related to meat and meat products such as sausage, poultry, and egg products, dairy products, raw milk, salads, ready-to-eat foods like bakery products such as cream-filled pastries, cakes, and sandwich paddings (Argudín et al., 2010).



Fig. 1. *S. aureus* and their enterotoxins causes intoxications with several symptoms. The figure is adapted from Smith, 2015; Fetsch and Johler, 2018.

4. Natural antimicrobials from plants

Plants, including herbs and spices, have various phytochemicals which are, bioactive chemicals derived from plants that have potential effects against different diseases. These compounds are produced by a secondary metabolism of the plants. It has been reported that plants produce phenolics, phenolic acids, sulfur-containing compounds, quinones, flavonoids, alkaloids, phenolic diterpenes, and vitamins, especially flavonoids and polyphenols, and tannins by their secondary metabolism. Plants produce these metabolites to protect themselves against biotic or abiotic stress conditions. The secondary metabolites with antimicrobial activity are reported from fruits, vegetables, seeds, herbs and spices, animal tissues, bacteria, and fungi to ensure food security (Argudín et al., 2010).

5. Herbs and spices: the role in the food industry

During food processes, different chemical additives with various biological activities such as protection against microbial activities, coloring, giving pungency, and preventing oxidation reactions are added into foods and beverages. However, there is controversy about the advantages and disadvantages of these artificial chemicals in terms of safety and efficacy. Nowadays, consumer trends have turned to all-natural products, or at least to foods and beverages containing extracts or chemicals from ethnobotanically valuable herbs or spices. Depending on the demand of these customers, manufacturers give more importance to the use of natural food products compared to previous years in the food industry. For this purpose, many studies focusing on various natural resources approved as GRAS (Generally Recognized As Safe) for use in foods have been performed and also are still being conducted (Bondi et al., 2017). The major issue in this perspective for the food manufacturers is to provide expected shelf life and quality. Spices, herbs, and their chemicals are utilized especially for their properties of antibacterial, antifungal, and antiviral activities.

6. Some edible herbs in Türkiye and their antibacterial efficacy against *S. aureus*

Medicinally important plants have been used for the treatment of diseases among the people for centuries in Türkiye as well as all over the world. Edible herbs are grown according to the local climate and geographical features, and people transfer this knowledge from generation to generation in their own traditions and customs (Ceylan and Akar Sahingoz, 2019). It is estimated that about five hundred plants are used in Turkish folk medicine. Some of them are locally utilized as tea, poultice or tinctures for their therapeutic efficacies. On the other hand, their culinary usage is very common between local people.

Spices and herbs have been investigated in the literature for their antibacterial effects against *S. aureus*. Some of these spices and herbs are given below.

6.1. Falcaria vulgaris Bernh.

F. vulgaris (with the local name of Kazayağı, Pekaz) belongs to the Apiaceae family. It is an annual, biannual, or perennial highly branched, dull plant. The stem is flat, rounded and striated. It can be grown up to 25-100 cm. This plant is generally consumed as salad (Iğdır, Van), pickle (Erzurum, Iğdır, Van), or as a spice in cheese (Van), either prepared as a meal after boiled in milk (Iğdır) cooked as a vegetable (Erzurum, Van), or consumed fresh (Iğdır) in Türkiye. In general, young stems of plants are utilized for culinary purposes (Dogan et al., 2014). This plant has ethnomedicinal properties and it has been reported to be used traditionally for skin ulcers, stomach and liver ailments, kidney and bladder stones since ancient times. Furthermore, there are several studies in the literature showing antibacterial and antioxidant activities of *F. vulgaris* against

several bacteria including *S. aureus*. Based on the results of detailed studies, it has been suggested that the potential for such biological activities is probably due to the high carvacrol content (about 30%) (Jaberian et al., 2013; Kohsari et al., 2019). Recently, the potential efficacy of silver nanoparticles synthesized by *F. vulgaris* aqueous extract was determined against multidrug-resistant *S. aureus* (Kohsari et al., 2019). Moreover, Hekmati et al. (2020) reported the antibacterial potential of essential oils from the flower, leaf, and stem parts of *F. vulgaris* against several bacteria including *S. aureus*. Shahbazi (2017) also reported the antibacterial efficacy of methanol extracts for *F. vulgaris* leaves with small inhibition zones.

6.2. Urtica dioica L.

U. dioica L. (with the local name of Isirgan Otu/nettle) belongs to the Urticaceae family. The members of Urtica are perennial plants with oppositely arranged serrated and hairy and stinging leaves and small greenish-white flowers (Kregiel et al., 2018). U. dioica L. is consumed both raw and cooked. Young branches and especially the upper parts are used in soups, salads, pastries, meatballs, mixed herb roasts, bulgur, and rice pilafs (Karaca et al., 2015). In Erzurum, this plant is used in several ways such as ingredients in soups, or boiled, or fried with ingredients such as eggs and onions (Cetinkaya and Yildiz, 2018). U. dioica L. has also ethnobotanical value and has been utilized in Turkish folk medicine for years especially in gastric and rheumatic pains, liver insufficiencies, and also in colds. Furthermore, its medicinal efficacy in rheumatism, eczema, urinary tract infections, arthritis, anemia, hay fever, diuretic, astringents, skin complaints, gout, sciatica, neuralgia, hemorrhoids, and hair problems has been emphasized in the literature (Gulcin et al., 2004). The potential antibacterial effect of U. dioica L. against S. aureus has been examined in some studies. Also, Gulcin et al. (2004) reported the potency of water extracts of U. dioica L. for this bacterium. In another study conducted by Zenão et al. (2017), the ethanol extracts of U. dioica L. leaves were found to be successful against methicillinsensitive and methicillin-resistant strains of S. aureus. Salehzadeh et al. (2014) investigated U. dioica L. methanol extracts against sixteen isolates of methicillin-resistant S. aureus and antibacterial efficiency was demonstrated with inhibition zones ranging between 10-21 mm except one isolate.

6.3. The genus Rumex

Rumex (with the local name of Labada, Evelik Otu, Carşaf, Efelek, Efelik, Labada, Lapaza, Mancar, Mancarotu, Pancarotu, Yapalak, Tırşık) belongs to the Polygonaceae family. Rumex genus has been recorded to have nearly 200 species distributed worldwide (Vasas et al., 2015). It is one of the oldest known herbs of Türkiye. It resembles spinach in structure. This plant is encountered in all areas of Türkiye but especially in Eastern Anatolia. In the region of Erzurum, Kars, Ardahan and Bingöl, this herb is cooked as stuff, soup, and beetroot (Cetinkaya and Yildiz, 2018). Twenty-eight species belonging to Rumex genus have healing properties in various diseases such as bacteriarelated dermatologic conditions, dysentery, and enteritis. Different parts of this herb including roots, leaves, flowers, tubers, aerial parts, stems or whole plant were investigated in many studies for their chemical compounds (Vasas et al., 2015). The fruit parts of R. pulcher L. is reported to be generally utilized

in coughs as decoctions or internal use. There are several studies examining the antibacterial efficacy of some *Rumex* species against various bacteria. Tamokou et al. (2013) reported the antibacterial activity of *R. abyssinicus* Jacq. against *S. aureus*. Furthermore, the inhibitory effect of the ether and ethanol extracts of *R. crispus* L. has been indicated on the growth of *S. aureus* (Yildirim et al., 2001). In another study, Tukappa and Londonkar (2013) demonstrated that extracts from leaves of *R. vesicarius* L. had significant efficacy against *S. aureus*. In a study testing several extracts prepared with different solvents and various parts of *R. vesicarius* L. including whole plant, leaves, stems, flowers, roots, and fruits, ether extract of roots has been found to be considerably effective to inhibit the growth of this bacterium (Mostafa et al., 2011).

6.4. Capsicum annuum L.

It is one of the most economically important genera in the Solanaceae family. It contains more than twenty five known species. Among these species, the most common and most economically used are C. annuum, C. baccatum, C. chinensis, C. frutescens and C. pubescens (Aguilar-Melendez et al., 2009). Especially C. annuum L. (red pepper) is grinded to obtain spice to give flavor and bitterness to the dishes. It is grown in the Mediterranean, Aegean, Marmara, and Southeastern Anatolia regions of Türkiye. In addition to being used as a spice since ancient times, red pepper types can be either consumed fresh or used in industry as canned, tomato paste, pickles, hot sauce, dried, powdered and chili peppers in processed meat products. With its high antioxidant activity, it reduces the free radical activity in the human body and prevents the peroxidation reaction (Eggersdorfer and Wyss, 2018). Red pepper has a high content of vitamin E and vitamin C together with phenolics, carotenoids and alkaloids. The active ingredient of red pepper is capsaicinoid type "capsaicin" and capsinoids (Kawabata et al., 2009). The antimicrobial activity of capsaicin in red pepper has been determined on various Gram-negative and Gram-positive bacteria (Bacon et al., 2017; Baenas et al., 2019). Dorantes et al. (2008) examined the effect of C. annuum on S. aureus from fresh cheese samples. They reported that S. aureus increased significantly in numbers from log2 to log6 during one-day storage. On the other hand, 5% C. annuum extract showed a significant inhibitory effect during the 7-day storage period. In a study conducted by Naseer et al. (2021), it was reported that Capsicum extract had lower MIC (Minimum Inhibitory Concentration) values against multidrug-resistant S. aureus. Mokhtar et al. (2017) isolated polyphenols and capsaicinoids from C. annuum and they reported the antimicrobial activity of phenolic extracts and capsaicinoid and capsain extracts against S. aureus. In another study, the antibacterial efficacy of methanol extracts belongs to two different C. annuum species have been observed against S. aureus (Koffi-Nevry et al., 2012).

6.5. Gundelia tournefortii L.

The local name of *G. tournefortii* L. is Kenger, Eşek dikeni, Çakırdikeni, Çengel otu, Henger, Kalağan, Kengi Otu, Kepre, Kingar, Tatlı Sakız Otu, Kanak Sakızı in Türkiye and it belongs to the Asteraceae family. It has been specified that the localization of the plant is especially in Cyprus, Egypt, Iran, Israel, Türkiye, Azerbaijan and Turkmenistan (Haghi et al., 2011; Apuhan and Beyazkaya, 2019). The stem, leave, seed, and flower parts of *G. tournefortii* are cooked in pastries, soups, and stuffing in Türkiye (Ceylan and Akar Sahingoz, 2019). In addition to being a good source of nutrients, it has been used in herbal medicine for cramps, dyspepsia, migraine, inflammation of the liver, biliary tract inflammation, cirrhosis, parotitis, vitiligo, diarrhea, bronchitis, and chronic liver diseases from ancient times (Sarac et al., 2019). There are many studies focusing on the biological activities of the extracts obtained by different solvents from G. tournefortii. The inhibitory effects of methanol extracts of G. tournefortii, and its synergistic/antagonistic effects with some antibiotics against S. aureus have been demonstrated (Darwish et al., 2002). On the other hand, in a study in which only the extracts of the plant were tested against S. aureus, the ethanol extracts of G. tournefortti was found to be effective on this bacterium (Ayoubi et al., 2016). Ceylan et al. (2019) investigated methanolic extracts of eight plants against several bacteria including S. aureus and reported MIC value for G. tournefortii as 1000 µg/mL. In another study conducted by Sarac et al. (2019), it was observed that water extracts of seeds from G. tournefortii have a moderate level of antimicrobial activity with a MIC value of 0.3125 mg/mL. Similar moderate activity for the chloroform, ethyl acetate, methanol, and aqueous extracts of G. tournefortii was observed by Dastan and Yousefzadi, 2016. Nowadays, silver nanoparticles (AgNPs) have come into prominence for having good features for feasibility and efficiency in biomedical applications. In a recent study, AgNPs prepared with fresh leaves of G. tournefortii L. have been tested for the potential inhibitory effect on bacterial growth of S. aureus and inhibition was recorded at 2 and 4 mg/mL of AgNPs with G. tournefortii (Han et al., 2020).

6.6. Chenopodium album L.

This plant is an annual herbaceous plant (with the local name of Sirken otu, Ak Kazayağı, Ak Sirken, Ak Pazı, Hoşkıran, Küllü ot, Yabani ıspanak) belongs to the Chenopodiaceae (Spinachaceae) family in Türkiye, which grows up to 150 to 200 cm (Atalay and Kamalak, 2019). Lambs quarter (English) and Bathu (Hindi) are the plant's known names around the world (Said et al., 2020). Chenopodiaceae family includes widely distributed more than 100 genera. Its vermifugal and laxative effect has been emphasized in the literature. The ethnobotanical utilization of this plant against wounds, high blood pressure, abdominal pain, intestinal ulcers, hepatic and liver disorders, piles, diarrhea, eczema, and eye inflammation was also stated. Above-ground parts of Chenopodium sp. are cooked and consumed as a vegetable like spinach. It is also boiled and roasted (Kaya et al., 2004; Yadav et al., 2007; Said et al., 2020). Also, it can be consumed as a raw vegetable in salads or wraps (Yucel et al., 2018). Cretan Turks add the leaves and fresh sprouts of Chenopodium sp. to chipohorta mix and also they use this herb in boiled salads (Kok et al., 2020). Besides the nutritional features of C. album, biological activities as an antioxidant, antibacterial, antipruritic, and antinociceptive were also investigated in several studies (Pandey and Gupta, 2014). Navak et al. (2010) demonstrated potential inhibitory efficiency of C. album with 17.3 mm zone of inhibition against S. aureus. Singh et al. (2011) indicated considerable antibacterial activity against S. aureus by the aqueous extracts of C. album L. The methanol extract of C. album exhibits antibacterial activity against S. aureus with an inhibition zone of 13.0 mm at 100% concentration (Parkash and Patel, 2014). Lone et al. (2017) recorded significant antibacterial activity against S. aureus by different extracts of *C. album* with 28 ± 0.14 mm inhibition zone at a concentration of 500 µg/mL. In one of the studies emphasizing the potential antibacterial effects of the plant, a considerable inhibition percentage (88.86%) for *S. aureus* growth was recorded via the methanol extracts of the plant by Said et al. (2020). On the other hand, Amjad and Alizad (2012) reported no antibacterial potential for methanol extract of *C. album*. More recently, whereas efficacy of methanol extract of *C. album* leaves against *S. aureus* was shown after 24 h, no activity for aqueous extracts of the plant was shown by Suleman et al. (2021). The variability in results may be due to the amount used in the experiments, regional differences, type of solvent, and bacteria used.

6.7. Crocus sativus L.

C. sativus, commonly known as saffron, is a perennial flowering plant of Iridaceae family with a bitter taste and fragrant smell. Saffron, one of the oldest spices, is commonly utilized as a spice in Anatolia and worldwide. The local name of C. sativus in Türkiye is safran. It has high economic value since it is the most expensive spice in the world. The definition of saffron covers the stigmas of the plant, spice obtained from these stigmas and the plant itself. The petal, leaves and stigmas of C. sativus have traditionally been used in folk medicine for over 3000 years as it has various bioactive chemical compounds with antispasmodic, sedative, stomachic, stimulant, emmenegogue, anti-tumoral, anti-mutatic and possible application areas (Cinar, 2019; Jadouali et al., 2019; Shadmehri et al., 2019). Saffron is preferred in various cuisines and recipes due to its coloring and flavoring properties (Soureshjan and Heidari, 2014). Its aroma is described as honey-like but with metallic notes (Mzabri et al., 2019). Saffron dyes 100 thousand times liquid its own weight in yellow. It is widely used in Iranian, Arabian, Central Asian, European, Indian, and Moroccan dishes (pilaf cuisine, various halvah, dolma, soup, etc.) (Cinar, 2019). Saffron is utilized in various traditional dishes such as Paella Valencia, bouillabaisse, Milanese risotto, saffron cake, meatballs, chelow kabab, mrouzia, biryani, gulab jamun and kulfi (Mzabri et al., 2019). Many studies demonstrating the antidepressant, antiinflammatory, anticoagulant, analgesic, antihypertensive, anticancer, and anti-tumor activities of aqueous and ethanolic extracts of C. sativus highlight the pioneering role of this plant for the pharmaceutical industry (Shadmehri et al., 2019). Okmen et al. (2016) tested methanolic, ethanolic and aqueous extracts of saffron against two S. aureus and five coagulase-negative staphylococci and recorded moderately inhibitory effect on the growth of *S. aureus* by aqueous extracts of *C. sativus* (8-10 mm). Jafari-Sales and Pashazadeh (2020) examined the potential inhibitory effects of methanol extracts of C. sativus L. on various Gram-negative and Gram-positive bacteria. Researchers observed that saffron extracts applied in varying concentrations showed reasonable inhibition on S. aureus and they indicated higher inhibition on Gram-positive bacteria than Gram-negative bacteria by the saffron extracts. Esmaeelian et al. (2021) tested saffron corm extracts, which were extracted via ultrasound and solvent utilization, on S. aureus and E. coli. No effect of extracts applied at a dose of 150 mg/mL on disk diffusion was observed in both bacteria. They reported that doses of 300 and 600 mg/mL showed inhibitory activity on S. aureus. Wali et al. (2020) extracted saffron petals by different solvents namely hexane, dichloromethane and ethanol which were then examined on various bacteria including S. aureus at the concentrations of 50015.63 µg/mL. They reported that a concentration of 500 µg/mL for ethanol extract provided the highest inhibition on *S. aureus*. On the other hand, there are also studies showing that there is no antibacterial activity against *S. aureus*. Soureshjan and Heidari, (2014) observed no antibacterial potency of saffron onion at all tested concentrations of extract *S. aureus*.

6.8. Erodium cicutarium (L.) L'Hér. ex Aiton

The genus Erodium (Geraniaceae) is widespread all over the world except Antarctica with 74 species. The greatest species diversity is found in the Mediterranean region with 62 species. In the flora of Türkiye, it is known by local names such as Dönbaba, İğnelik or locally as Çoban İğnesi, İğnelik Otu, Leylek Gagası, and it is known to have 26 species. The members of the genus Erodium can be annual, biennial, or perennial. E. cicutarium is consumed particularly as a vegetable in Ankara, Aksaray, and Afyonkarahisar. Furthermore, it is used in different cuisines all over Türkiye such as it can be added to pastries, roasted, fried with eggs or onion, and eaten raw as a snack. This herb is also of ethnobotanical importance as it is a potential analgesic or a treatment option for snake bites and hemorrhoids. The plant contains a wide variety of phenolics, volatile and amino acids, and vitamins K and C. (Sargin et al., 2015; Celikler et al., 2020). There are studies showing antimicrobial, antioxidant, anti-inflammatory, and antiviral activities of E. cicutarium (Nikitina et al., 2007; Nikolova et al., 2010). In a study by Nikitina et al. (2007), bacteriostatic effects of 7 plant species belonging to the Geraniaceae and Rosaceae families were examined on a total of 32 bacteria (Gram-negative and Gram-positive). The water extracts of the above-ground parts which are obtained from E. cicutarium showed a higher bacteriostatic effect than the ethanol extracts. Quave et al. (2008) investigated antimicrobial and antibiofilm activities of various herbal extracts against S. aureus. The ethanol extracts, which were obtained from the aerial parts of E. ciconium and E. malacoides did not inhibit biofilm formation. Whereas E. ciconium extract had no effect against Methicillin-resistant S. aureus (MRSA), expected activity was observed for E. malacoides extract. The essential oils of E. cicutarium and E. ciconium, collected from Serbia, were studied against several Gram-positive and Gram-negative bacteria including S. aureus. E. cicutarium had moderate antimicrobial activity on tested bacteria with the highest activity on S. aureus (Stojanović-Radić et al., 2010).

6.9. Nigella sativa L.

N. sativa is an aromatic (bitter and pungent) annual plant with black-colored seeds and a member of the Ranunculaceae family. It is commonly known as black seed, black cumin, Ajaji, Kalika, Kalaunji, and Habbatul-Barakah. In Türkiye, the local name of *N. sativa* is çörek otu. The seeds of the black cumin plant are traditionally used as a spice, food additive, functional foods and nutraceuticals (Paarakh, 2010). In the culinary use of *N. sativa* with its unique smell, the seeds are used in the food industry to garnish or flavor bakery products, cheese (Armenian string cheese Majdouleh or Majdouli), confectionery, and liqueurs. Also, it can be added to recipes including fruit, vegetables, salads, poultry, yogurt, pickles, sauces, and salads. Its consumption with honey and syrup is quite common (Fawzy Ramadan, 2015; Hassanien et al., 2015). Besides the use of *N. sativa* seeds as a spice, they also have applications for food preservation.

N. sativa has traditionally been used as a folk medicine for many years. It is seen that its seeds and oil have healing properties in many health problems such as cough, jaundice, fever, skin diseases, anorexia, diarrhea, flatulence, etc. It has been reported that the seed oil is used as a local anesthetic. Several biological activities for N. sativa are indicated in the literature including carminative, anti-tumor, antidiabetic, gastroprotective, pulmonar, nephroprotective, hepatoprotective, immunomodulatory, antioxidant, antibacterial, diuretic, antifungal, antihelminthic, antiimplantation etc. (Paarakh, 2010). There are numerous studies evaluating the antibacterial activities of black cumin. The effects of different solvents or extraction methods on the antibacterial activity of black cumin were also studied. Khalid et al. (2011) investigated three extraction method namely cold water, hot water and methanol for N. sativa seeds and amongst them cold water extracts were successful against S. aureus. In another study using n-hexane for the extraction of black cumin, potentially inhibitory effect against S. aureus was reported by researchers (Abraham et al., 2019). The existence of these potential activities has led researchers to find their active ingredients. Halawani (2009) examined two compounds from black cumin for antibacterial efficacy on S. aureus. It has been determined that thymoquinone and thymohydroquinone can kill bacteria at doses of 3 and 6 μ g/mL, and 400 and 800 μ g/mL, respectively. In another study, a significant bactericidal activity of thymoquinone against S. aureus was detected (Chaieb et al., 2011). In recent years, studies on the antimicrobial properties of essential oils and their components, which have been revealed by detailed studies, have attracted attention. In a study by Salman et al. (2008), it was reported that S. aureus had sensitivity to essential oil of N. sativa seeds. Similarly, Emeka et al. (2015)studied inhibitory/suppressive/lethal potency of N. sativa oil on the growth of S. aureus. Most isolates were found to be susceptible to different concentrations of oil samples. Mouwakeh et al. (2019) reported antimicrobial activities of N. sativa essential oil and its compounds (thymoquinone, carvacrol, and p-cymene) against methicillin-susceptible and resistant S. aureus strains. Georgescu and Raita (2019) compared the antibacterial efficacy for 0.1 and 0.2 w/w of N. sativa seed oil samples to control groups against Staphylococcus spp. from kneaded, sheep's milk Romanian cheese and observed that 0.2 w/w of N. sativa seed considerable inhibition in number oil provided of Staphylococcus spp.

Some of the biological activities and chemical composition of the plants mentioned above and the bacterial species, in which their antimicrobial activities are observed, are given in Suppl. Table 1.

7. Some other herbs and spices used to preserve foods and extend product shelf-life

In terms of food hygiene, recently, researchers have focused on the preservative effects of extracts and/or fractions from many herbs and spices against various food pathogens. In one such study, Márquez-Rodríguez et al. (2020) analyzed the brute phenolic extract of *Hibiscus calyces* as well as organic and aqueous phase and also fractions. They found that the F1 fraction, which is the richest fraction in phenolic acids, was successful in terms of MIC and MBC values for tested bacteria including *S. aureus*. Then, in situ studies revealed that beef steak slices sprayed with the hibiscus phenolic extract (HE) at the concentration of 500 mg/L and stored at 4 ± 1 °C for 15 days had decreased bacterial number. Also, no bad smell was observed during the 15 days of the storage. Weerakkody et al. (2011) evaluated the synergistic effect of extract combinations of Alpinia galangal and Rosmarinus officinalis against the growth of S. aureus. Frozen cooked, peeled, and deveined shrimps were inoculated by three strains of S. aureus at 8°C for 16 days. The mixture of A. galangal and R. officinalis extracts at the concentrations of 5 mg/mL and 10 mg/mL, respectively had no significant inhibition on S. aureus. However, they reported that the mix of A. galangal and R. officinalis extracts prolonged the shelf life of shrimp up to 8 days when stored at the tested temperature. Study results showed that S. aureus was completely inhibited in treated yoghurts with herb essential oil and the stability was provided for 29 days. Sayyari et al. (2021) investigated probable antibacterial efficacy by Foeniculum vulgare essential oil at the concentrations of 1%, 1.5%, and 2%, which were nanocoated with basil seed gum or Lepidium perfoliatum and also their mix, against S. aureus PTCC1431 on days 7, 14, 21 and 28. They obtained successful results from the test group of the mixed-coating samples with 2% of the fennel essential oil. Kanatt et al. (2010) demonstrated the potency of pomegranate peel extract against S. aureus in chilled chicken meat products with prolonged shelf life by 2-3 weeks. Abdolshahi et al. (2018) tested essential oils of Thymus vulgaris L., Mentha piperita L., and Ziziphora tenuior alone or mix against S. aureus in industrial doogh. During refrigerated storage, inhibition in bacterial growth was recorded on days 1 and 7. Furthermore, it has been reported that the components of essential oil which were obtained from Alpinia pahangensis, Origanum vulgare, Origanum dictamnus, Mentha piperita, Lavandula hybrida, Zataria multiflora, and Hofmeisteria schaffneri have been found to be successful against S. aureus. Some herbs and spices utilized in antibacterial food packaging are given in Suppl. Table 2.

8. Conclusion

Foodborne diseases are one of the global health problems that cause many complications and deaths all over the world. Food poisoning caused by staphylococci is one of the most common food-borne diseases via contamination by food or food processing equipment. Various synthetic antimicrobials are utilized in the food industry. However, in past decades, a tendency to use natural food products has started in line with the

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demands of customers. Herbs and spices for culinary purposes have been added to different recipes to give flavor, aroma, pungency and color to food from time immemorial. On the other hand, these herbs and spices are known to have unique compounds with properties of medicinal and numerous biological activities.

Extracts or their compounds, especially essential oils, are used in medicine, food, cosmetics, etc. Since natural extracts from herbs and spices have positive effects on shelf life, microbial degradation, and oxidation reactions, the feasibility of utilizing these natural materials such as extracts/their essential oils/other compounds in food processing for various purposes has come into prominence. More recently, it has been emphasized in the literature that there are synergistic or antagonistic effects between these compounds. Most studies focused on these interactions to increase expected impacts. Herbs and spices with medicinal importance have been used in the treatment of diseases among the people in Türkiye as well as all over the world for centuries. Studies to date focusing on the antibacterial properties of extracts from herbs and spices and their compounds have shown promising activity against S. aureus. More detailed experiments will provide comprehensive information on the applicability of these natural biological resources in the food industry. These natural herbs or spices can serve as alternative antibacterial agents against S. aureus and food preservation can be achieved with these ecological materials. In this context, herbs and spices that have been shown to be effective against S. aureus, which also causes food poisoning, can be used as an alternative to food preservation chemicals or may be integrated into food packaging material. Technologies to ensure the safety and quality of food, such as active packaging, which prolongs the shelf life of food products, and intelligent packaging, which reduces food waste by sensing changes in the food product, have become prominent in recent years. Therefore, some edible culinary herbs and spices that are traditionally used as folk medicine and have also been found to have antibacterial activity against S. aureus, can be used in antimicrobial active packages.

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Supplementary

Suppl. Table 1. Chemical composition, biological activities and antimicrobial properties of some herbs and spices.

Plant Name	Chemical Composition	Biological Activity	Antibacterial Activity	References	
	α-Pinene, Octanal, Limonene, Carvacrol,	Antioxidant,	Bacillus cereus, Serratia marcescens, Escherichia coli,		
Falcaria vulgaris Bernh.	Germacrene-D, Spathulenol, Germacrene-B, Salvial-4(14)-en-1-one, Isospathulenol, Phytol	Antimicrobial, Antidiabetic	Staphylococcus aureus, Enterobacter aerogenes, Klebsiella pneumoniae, Pseudomonas aeruginosa, B. megaterium	Jaberian et al., 2013 Choobkar et al., 2017	
Urtica dioica L.	Isorhamnetin rutinoside, Isoquercetin, Rutin, Ferulic acid, Caffeoylmalic acid, Caffeic acid, Quinic acid	Antioxidant, Anti-inflammatory, Antirheumatic, Antimicrobial, Antiulcer, Antiproliferative	Candida albicans, S. aureus, K. pneumonia, Listeria monocytogenes, Proteus vulgaris	García et al., 2021 Mahmoudi et al., 2021	
Rumex sp.	Rutin hydrate, Butyl gallate, Cardamonin, Phenoxodiol, Shikonin, Gallic acid, 3'-hydroxy-b- naphthoflavone, Anthraquinones, Tocopherols	Anti-inflammatory, Antioxidants, Antianalgesic, Antimicrobial	S. aureus, B. subtilis, E. coli, K. pneumoniae, Salmonella typhi, Streptococcus pneumoniae, L. monocytogenes	Hafaz et al., 2021 Saoudi et al., 2021	
Capsicum annuum L.	β-Tocotrienol, α-Tocotrienol, Capsidiol, Oxylipin, Capsianoside I methyl, Methyl cinnamate, Dihydrocapsaicin, Capsianoside F, Nordihydrocapsiate Capsaicin, Capsianoside C, Chlorogenic acid, Naringenin-7-O-glucoside, p-Coumaric acid, Naringenin, Liliolide	Antioxidant, Antiviral, Antimicrobial, Anticancer, Anti-inflammatory, Antiobesity, Analgesic, Antifungal	Salmonella sp. E. coli, P. aeruginosa, S. aureus, B. subtilis, Streptococcus mutans, Helicobacter pylori	Chilczuk et al., 2021 Hernández-Pérez et al., 202 Molina et al., 2021	
Gundelia tournefortii L.	Lupeol, Lupeol-trifluoroacetate, Palmitic acid, β-amyrin, Ursolic acid, 4-hydroxybenzoic acid, 3,4-dihydroxybenzoic acid, Caffeic acid, Myo-inositol, Quinic acid	Antibacterial, Anticancer, Diabetes, Antiparasitic, Anti-inflammatory	E. coli, P. aeruginosa, S. aureus	Bati et al., 2021 Asadi-Samani et al., 2013 Kadan et al., 2021	
Chenopodium album L.	Quercetin, Rosmarinic Acid, Benzoic acid, Ferulic acid, Rutin, Syringaldehyde, p-Coumaric Acid, Vanillin, Epicatechin, Syringic acid,	Antioxidant, Antibacterial, Antifungal, Anticancer	S. aureus, B. subtilis, B. polymexia, S. faecalis, P. aerugenosa, Shigella dysenteriae, S. typhimurium, P. vulgaris	Saini and Saini, 2020 Karacelik and Sahin, 2021	

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Plant Name	Chemical Composition	Biological Activity	Antibacterial Activity	References
Chenopodium album L.	Caffeic acid, Chlorogenic Acid, Catechin, Gallic acid, Protocatechuic acid, Myristic acid, cis-10-pentadecanoic acid, α-linolenic acid	Antioxidant, Antibacterial, Antifungal, Anticancer	S. aureus, B. subtilis, B. polymexia, S. faecalis, P. aerugenosa, Shigella dysenteriae, S. typhimurium, P. vulgaris	Saini and Saini, 2020 Karacelik and Sahin, 2021
Crocus sativus L.	Crocin, Picrocrocin, Safranal, Coumaric acid, Quercitrin, Gallic acid, Ellagic acid, Ellagic acid, Catechin, Epicatechin	Antidepressant, Antioxidant, Anticarcinogenic, Anti-inflammatory Antibacterial	S. aureus, L. monocytogenes, S. enterica subsp. bungori, E. coli	Caser et al., 2020 Mzabri et al., 2019 Zara et al., 2021
<i>Erodium cicutarium</i> (L.) L'Hér. ex Aiton	Gallic acid, Protocatechuic acid, 3-O- galloyl shikimic acid, 3- O-(6"-O-galloyl)-β-D- galactopyranoside, Corilagin, Didehydrogeraniin (dehydrogeraniin), Geraniin, Hyperin, Isoquercitrin, Methyl gallate 3-O-β-D- glucopyranoside, Rutin	Anti-inflammatory, Antimicrobial, Antiviral	E. coli, S. aureus, Azotobacter sp., Pseudomonas sp., B. polymyxa, B. subtilis, K. pneumoniae	Bussmann et al., 2010 Munekata et al., 2019
Nigella sativa L.	Thymoquinone, Thymohydroquinone, Dithymoquinone (nigellone), <i>p</i> -cymene, carvacrol, 4-terpineol, <i>t</i> -anethole, α-pinene, Thymol, Carvone, Limonene, Citronellol, Linoleic acid, Oleic acid, Eicosadienoic acid	Antidiabetic, Anti-inflammatory, Antioxidant, Antimicrobial, Anticancer	P. aeruginosa, K. pneumoniae, Acinetobacter baumannii, S. aureus, Yersinia enterocolitica	Srinivasan, 2018

Suppl. Table 2. Some herbs and spices utilized in antibacterial food packaging.

Plant	Applications Microorganisms		References	
		S. aureus,		
		L. monocytogenes,		
Garlic (Allium sp.)	Edible film	B. cereus,	Pranoto et al., 2005	
Oregano (Origanum sp.)		E.coli O157:H7,	Seydim and Sarikus, 2006	
		S. enteritidis,		
		L. plantarum		
	Edible coating	S. aureus,		
		B. subtilis,		
Satureja hortensis L.		S. enteritidis,	Krasniewska et al., 2014.	
		E. coli,		
		Penicillium expansum		
		E. coli,		
Hibiseus sabdariffa I		S.enterica,		
Hibiscus sabdariffa L.	Sprayed	S. aureus,	Márquez-Rodríguez et al., 2020	
		L. monocytogenes,		
		B. cereus		
Thymus vulgaris L.,	Film Coating	E. coli,		
Origanum majorana L.,		E. con, S. aureus	De Souza et al., 2020	
Ocimum basilicum L.		S. aureus		
	Edible Film	E. coli,	Alp and Aksu, 2010 Mohammadian et al., 2021	
Urtica dioica L.		S. aureus		
		Enterobacteriaceae	Mohammadian et al., 2021	
Portulaca oleracea L.	Edible Films	E. coli,	Qoeroti et al., 2021	
Fortulaca oleracea L.	Edible Fillis	S. aureus	Queroti et al., 2021	
	s sativus L. Edible Films	E. coli,		
		S. sonnei,	Hashemi and Jafarpour, 2020	
Crocus sativus L.		S. typhi,		
		S. aureus,		
		B. cereus		
	ativa L. Active pad or sachet	E. coli,		
Nigella sativa L.		S. aureus,	Hosseini et al., 2021	
		Aspergillus flavus		
	F '1	E. coli,	Same at al. 2021	
Cranberry (Vaccinium sp.)	Films	S. aureus	Severo et al., 2021	
Amaranthus sp.	Films	S. aureus	Kanatt, 2020	
Olive (Olea sp.)	Edible Films	S. aureus,	García et al., 2020	
Onve (Oteu sp.)	Edible Thins	E. coli	Garcia et al., 2020	
Sonneratia caseolaris L.	Edible Films	S. aureus,	Nguyen et al., 2020	
Sonnerana cascolaris L.	Edible Fillis	P. aeruginosa	Nguyen et al., 2020	



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Derleme / Review article

Kırgızistan çeltik üretimine genel bir bakış

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Öz

Çeltik, Antarktika hariç tüm kıtalarda yetiştirilen dünyanın en değerli gıda ürünleri arasında yer almaktadır. Küresel ölçekte, ekim alanı açısından değerlendirildiğinde buğdaydan sonra ikinci sırada yer almakla birlikte verimi yaklaşık olarak toplam buğday verimine eşittir. Kırgızistan, çeltik yetiştiriciliği bakımından önde gelen ülkeler arasında yer almamakta, bu bölgede sadece yerel ölçekte üretim yapılmaktadır. Kırgızistan'ın güney bölgesi çeltik tarımına elverişli olduğu için son 10 yılda nispeten çeltik ekim alanları ve tüketimi artmıştır. Çeltiğe ilginin artmasına; nüfus artışı, ekonomik olarak getirinin olması, diğer ülkelere ihracat etme durumu gibi birçok faktör neden olmaktadır. Kırgızistan'da çeltik yetiştirilen başlıca bölgeler olan Oş, Calal-Abad ve Batken güney bölgelerinde yer almaktadır. Özgen ve Ak-Turpak çeltik çeşitleri tadı bakımından diğer çeşitlere göre daha üstündür. Araştırmalara göre insanlar için yararlı olan birçok elementleri içermektedir ve diğer çeltiklere kıyasla yüksek protein içeriğine de sahiptir. Bu çalışmada Kırgızistan'daki çeltik tarımının genel özellikleriyle birlikte, Özgen ve Ak-Turpak çeltiklerinin önemi ortaya konulmuştur.

Anahtar kelimeler: Ak-Turpak; çeltik, çeltik üretimi, Oryza sp.; Özgen

An overview of rice production in Kyrgyzstan

Abstract

Rice is among the most valuable food products globally, grown on all continents except Antarctica. It ranks second after wheat in terms of planted area globally but is approximately equal to wheat for grain harvest. Although Kyrgyzstan is not among the leading countries in terms of paddy cultivation and only local production is carried out in this region. Since the southern region of Kyrgyzstan is suitable for paddy farming, paddy cultivation areas and consumption have increased relatively in the last 10 years. The increase in the interest in paddy is caused by many factors such as population growth, economic return, and export to other countries. The main paddy fields in Kyrgyzstan are located in Osh, Jalal-Abad, and Batken regions. Uzgen and Ak-Turpak paddy are much superior to other varieties in terms of taste. According to studies, they contain many elements that are beneficial for humans, and the protein content is much richer than other paddy types according to World standards. In this study, the importance of Uzgen and Ak-Turpak paddy has been revealed along with the general characteristics of paddy farming in Kyrgyzstan.

Keywords: Ak-Turpak; Oryza sp.; paddy; rice production; Uzgen

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1. Giriş / Introduction

Hindistan ve Çin'de ilk defa kültüre alınmış olan çeltiğin, binlerce yıldır tarımı yapılmaktadır (Vavilov, 1926; Ding, 1956; Poehlman ve Sleper, 1995; Lu ve ark., 2022). En eski kültüre alınan bitkilerden biri olan çeltik, buğdaygiller ailesinin bir üyesidir (Panda ve ark., 2020). Çeltik bitkisi, dünyanın üç büyük gıda mahsulünden biridir ve dünya nüfusunun yaklaşık yarısının temel besinini oluşturur (Andrew-Peter-Leon ve ark., 2021). Çeltik diğer buğdaygiller ailesinin üyelerinden farklı olarak su içinde de yetişebilen ve toprak altı kısımları ile suda erimiş yararlı oksijenden faydalanılabilen yegâne tahıl bitkisidir (Rijal ve Devkota, 2020). Ayrıca birbirinden farklı özelliklerdeki toprak tiplerinde de yetiştirilebilmektedir. Bu bitkinin tarımı, kuru toprak yapısı ile birlikte suya sahip, su seviyesi altındaki arazilerde, deniz seviyesinde ve denizden 2500 metreye kadar ulaşan farklı çevre şartlarında yapılabilmektedir. Bundan dolayı çeltik bitkisinin yetiştiği bölgeler oldukça geniş farklılıklar gösterebilmektedir (Sürek, 2003; Seck ve ark., 2012).

İnsan günlük kalori ihtiyacının karşılanmasında önemli bir yeri olan çeltik, oldukça değerli bir tahıl bitkisidir (Bhar ve ark., 2021). Buğdaydan sonra en fazla üretilen tahıllardan olan çeltik, insan beslenmesinde çok önemli bir yere sahiptir (Akay, 2010; Fukagawa ve Ziska, 2019). Küresel ölçekte 160 milyon hektardan fazla alanda gerçekleşen çeltik üretiminin yaklaşık % 90,6'lık kısmı dünya nüfusunun % 60'ının yaşadığı Asya kıtasında yapılmaktadır (Bandumula, 2018). Çeltik bitkisinin doğal habitatları olan Çin, Hindistan ve Endonezya küresel üretimin ilk üç sırasında yer almaktadır. Antarktika kıtası hariç tüm kıtalarda ekimi yapılan çeltik bitkisinin 100'den fazla ülkede tarımı yapılmaktadır. Tarımsal üretimde ekili tarlaların % 11'i çeltikten oluşup yıllık ortalama 700 milyon tondan fazla ürün elde edilmektedir (Alam ve ark., 2009; Nadir ve ark., 2017).

1.1. Çeltiğin Anavatanı / Homeland of paddy

Bazı kaynaklarda bu bitki Uzakdoğu Asya'da bulunan nehir ve çay kenarları gibi sulak alanlarda doğal olarak vetismekte iken tarihsel sürec iceresinde geleneksel ıslah ile günümüze kadar gelmiştir (Gaikwad ve ark., 2021; Singh ve ark., 2022). Bununla beraber bazı bilim insanları çeltik (Oryza L.)bitkisinin tarihini, kıtaların tarihi kadar eskiye götürmektedir. Aynı zamanda dört büyük kıtada 20 farklı yabani çeltik cinsi bulunmaktadır (Sadia, 2012; Gross ve Zhao, 2014). Kıtalar birbirinden ayrılmadan önce dünya yüzeyinde var olduğu ve kıtaların ayrılmasıyla dünyanın farklı kıtalarına yayıldığını kabul edenler de bulunmaktadır. Afrika, Asya, Avustralya ve Amerika kıtalarında bulunan bazı çeltik genomların birbirinin aynı olması bu görüşü desteklemektedir (Chang, 2000). Güney Asya ve Güneydoğu Asya'da yabani çeltiklerin ilk kültür formlarının farklılaşması ve çeşitlenmesi, yaklaşık 10.000 ile 15.000 yıl önce Neotermal çağda iklim değişiklikleriyle hızlanmıştır. Zamanla Hindistan ve Çin topraklarında kültüre alınarak yeryüzüne yayılmıştır (Vaughan ve ark., 2008; Sadia, 2012; Spengler ve ark., 2021).

Çeltik, dünyada yetiştirilen binlerce çeşidi ile genetik çeşitlilik açısından oldukça zengindir (Swamy ve Kumar, 2013; Akay ve ark., 2018; Filiz ve ark., 2018). Çeltik, buğdaygiller (Poaceae Barnhart) familyasındaki Oryzoideae Kunth ex Beilschm altfamilyasının bir oymağı olan Oryzeae Dumort. altında sınıflandırılmaktadır. Genom içeriklerine göre *Oryza* cinsi içerisindeki türler 4 ana tür kompleksi altında sınıflandırılabilir: O. granulata, O. officialis, O. sativa ve O. ridelyi kompleksleri. Dünya'da genellikle kısa, orta veya uzun tane boyutu kategorisine giren farklı çeltik türleri vardır. Çeltik, kültüre alınmış çeltik türleri ve yabani türler olmak üzere iki temel başlıkta incelenebilir. Kültüre alınmış çeltik türleri O. sativa ve O. glaberrima, yabani çeltik türleri ise O. rufipogon, O. nivara, O. barthii, O. longistaminata, O. meridionalis ve O. glumaepatula'dır. Bahsi geçen çeltikler diploid kromozom taşırlar ve AA genomuna sahiptirler (2n=24) (Garris ve ark., 2005; Mauleon ve ark., 2014; Arvas ve ark., 2022). Bununla birlikte su rejimine göre çeltikler; kır çeltiği (upland), sulanarak yapılan üretim (Irrigated Lowland) ve derin su şartlarında yapılan üretim (deepwater) çeltiği olarak sınıflandırılabilir (Sürek, 2003; Kaya ve ark., 2017). O. sativa ssp. indica çeltiği tropikal bölgelere daha fazla uyum sağlarken, O. sativa ssp. japonica çeltiği ılıman bölgelere uyum sağlamıştır. Hindistan, Çin ve Malezya gibi Uzakdoğu ülkelerinde ekilen indica cesitleri, celtik ticaretinde % 75 pay ile ilk sırada yer almaktadır (Akay, 2010). Çeltikte bu kadar çok çeşidin bulunmasının sebebi, her ülkenin bölgenin hatta vörenin kendine göre değişen damak tadı ve kalite anlayışına sahip olmasıdır (Kaya ve ark., 2017; Akay, 2022).

2. Küresel ölçekte çeltik üretimi ve tüketimi / Paddy production and consumption on a global scale

İnsanlar için çeltikten elde edilen pirincin birçok sektörde önemi vardır. Çeltik özellikle Asya'da yaşayan insanların çok önemli bir gıda kaynağı olduğu gibi bazen geleneksel ilaç, bazen katkı maddesi ve bazen de kozmetik için kullanılan bir araç olmuştur. Önceleri sadece Asya kıtasında kültüre alınmış olmasına rağmen günümüzde ise dünyanın hemen her ülkesinde çeltik hakkında geniş güncel bir kaynak birikimi oluşmuştur (Reshmi ve Nandini, 2018; Carcea, 2021).

Celtik, son çeyrek asırda tahıllar arasında tartışmasız üstün bir besin kaynağıdır. Genellikle protein kalitesi bakımından yulafın altında olup buğday ve mısırın üzerinde yer almaktadır (Kiple ve Ornelas, 2000). FAO istatistiklerine göre 2019 yılında 755.473.800,00 ton celtik üretilmistir. Kıtalara göre 1994-2019 yılları arası ortalama celtik üretim payı incelendiğinde, Asya'nın % 90,6'sını, ikinci sırada Amerika'nın (% 5,2), üçüncü sırada Afrika'nın (% 3,5), dördüncü sırada Avrupa'nın (% 0,6) ve beşinci sırada Okyanusya'nın (% 0,1) yer aldığı görülmektedir (FAO, 2022). Dünya çeltik üretimi 2020 ve 2021 yılları arasında 504,17 milyon tonun üzerinde gerçekleşmiştir (USDA, 2022). Asya kıtasında Çin, yaklaşık 200 milyon ton ile ana üreticidir. Çin'i, yaklaşık 150 milyon ton ile Hindistan ve yaklaşık 50 milyon ton ile Endonezya izlemektedir. Bununla birlikte kişi başına en fazla pirinç tüketimine sahip ülke, yılda 269 kg ile Bangladeş (2017'de 154 ülke karşılaştırmasına göre) olmuştur ve onu ikinci olarak Laos ve üçüncü olarak Kamboçya izlemektedir. Çin ise yılda kişi başına 125 kg ile 15. sırada yer almaktadır (Carcea, 2021).

Yeşil devrimden sonra gelişen teknolojiler, kimyasal ilaçların kullanılması ve yüksek verimli çeşitlerin kullanımının etkisiyle verim (ton/hektar) hızla artmıştır (Arvas ve Kaya, 2019). Son yıllarda çeltik üzerinde birçok biyoteknolojik yöntemlerle (Kaya ve Karakutuk, 2018; Taalat ve ark., 2021; Muhammed Azharudheen ve ark., 2022; Gain ve ark., 2022) ve gen transformasyon metotlarıyla genetiği değiştirilmiş birçok çeltik bitkileri elde edilmiştir (Wu ve ark., 2017; Kaya ve ark., 2020; Vega Rodríguez ve ark., 2022).

3. Çeltiğin genel besin değeri / General nutritional value of paddy

Pirinç, karbonhidratlar, proteinler, lipidler, mineraller ve vitaminlerden oluşmaktadır (Akay, 2020). Pirincin besin değeri varyete çeşitleri (Chaudhari ve ark., 2018), toprak özellikleri (Rahman ve ark., 2008), çeltik yetiştirme koşulları (Jabeen ve ark., 2020), genetik yapı ve iklim koşulları (Rathna Priya ve ark., 2019) gibi birçok faktöre göre değişir. Pirinç tanelerinin ana bileşenleri karbonhidratlar ve proteinlerdir. Ayrıca pirinç; yağlar, lifler, vitaminler ve mineraller içerir (Şekil 1) (Rahman ve ark., 2008).



Şekil 1 / **Figure 1.** Çeltik tahıl tanesinin genel şekli (Kabuk, kepek, tohum ve endosperm) / The general shape of the rice grain (paddy husk, bran, germ, and endosperm) (Sabirkulova, 2022).

Diğer tahıllar gibi çeltik de enerji veren bir besin olarak kabul edilir. Tablo 1'de belirtildiği gibi hem beyaz hem de esmer pirincin kimyasal bileşimine bakılırsa, karbonhidratların ve özellikle nişastanın tahıl kuru maddesinin yaklaşık % 80'ini oluşturduğunu ve proteinlerin de % 7 civarında olduğu görülmektedir. Çeltik bitkisinin glutamik ve aspartik asit bakımından amino asit içeriği yüksektir ancak lizin sınırlı oranda bulunmaktadır (Acquistucci ve ark., 2009). Esmer pirinç, beyaz pirinçte bulunan lipit miktarının dört katından fazlasına ve özellikle çoklu doymamış yağ asitlerine sahip olsa da hem beyaz hem de esmer pirinç, az yağlı gıda olarak kabul edilir. Bununla beraber, esmer pirinç lif, mineral ve vitaminler bakımında zengindir (Bodie ve ark., 2019; Prom-U-Thai ve Rerkasem, 2020; Vici ve ark., 2021).

Karbonhidratlar: Enerji kaynağı olarak sindirilebilir karbonhidratların çoğu çeltik tanesinin endosperm tabakasında bulunur. Öğütülmüş pirinç, nişasta ve serbest şekerler ile nişasta yapısında olmayan polisakkaritler dâhil olmak üzere birkaç karbonhidrattan oluşur. Gövde çoğunlukla selüloz ve hemiselüloz gibi nişasta olmayan polisakkaritlerden oluşur ve az miktarda nişasta içerebilir. Kepek ve tohum esas olarak selüloz ve hemiselüloz gibi nişasta olmayan polisakkaritlerden ve kısmen serbest şekerlerin yanı sıra az miktarda nişastadan oluşur (Hashimoto ve ark., 1987; Cheng ve ark., 2010; Acquistucci ve ark., 2009; Zhukova ve ark., 2013).

Protein: Çeltik tanelerinin nişastadan sonra ana bileşeni proteinlerdir. Tahıllar arasında çeltik proteinleri, en iyi aminogramlara ve yüksek sindirilebilirliğe sahiptir. Proteinler albümin, globülin, glüten ve prolaminlerle temsil edilir (Puncha-Arnon ve Uttapap, 2013; Aiyswaraya ve ark., 2017). Protein içeriği yetiştirilen çeşide bağlı olarak değişebilmekle beraber, erken veya geç olgunlaşma, toprak verimliliği ve su stresi gibi yetiştirme koşullarından da etkilenebilir. Esmer pirinçteki protein içeriği, birçok numunenin analizine dayalı olarak kuru madde bazında % 5-17 arasında değişmektedir (Fatchiyah ve ark., 2020). Genellikle endospermin dış tabakasındaki protein miktarı, iç tabakadakinden daha fazladır (Cao ve ark., 2010). Bu nedenle öğütme ve mekanik işlemeye daha az duyarlı olan esmer çeltik, daha fazla protein içermektedir. Çeltik glüteni, tahılın ana rezerv proteinidir. Hem pirincin hem de çeltiğin toplam proteininin % 70-80'inden fazlasını oluşturur. Çeltik glüteninin amino asit bileşiminin belirlenmesi, yüksek düzeyde dikarbonat amino asitleri göstermektedir. Amino asitlerin yaklaşık % 60'ı amid formundadır (Абилдаева, 2017). Tohumlar ve kepek, mineraller ve vitaminler bakımından yüksektir. Çeltik, demir ve çinkonun yanı sıra iyi bir tiamin, riboflavin ve niasin kaynağıdır (Kennedy ve Burlingame, 2003).

Tablo 1 / Table 1

Beyaz ve esmer pirincin besin bileşenlerinin karşılaştırılması (%) / Comparison of nutritional components of white and brown rice (%) (Khalua ve ark., 2019).

Besin	Beyaz pirinç	Esmer pirinç	
Suyu (g)	12,0	12,0	
Enerji (Kcal)	334	341	
Proteinler (g)	6,7	7,5	
Lipitler (g)	0,4	1,9	
Mevcut karbonhidratlar (g)	80,4	77,4	
Nișasta (g)	72,9	69,2	
Çözünür karbonhidratlar (g)	0,2	1,2	
Toplam Diyet lifi (g)	1,0	1,9	
Çözünür diyet lifi (g)	0,08	0,12	
Çözünmeyen diyet lifi (g)	0,89	1,8	
Mineraller			
Sodyum (mg)	5	9	
Potasyum (mg)	92	214	
Kalsiyum (mg)	24	32	
Magnezyum (mg)	20	0	
Fosfor (mg)	94	221	
Demir (mg)	0,8	1,6	
Bakır (mg)	0,18	-	
Çinko (mg)	1,3	-	
Selenyum (µg)	10	-	
Vitaminler			
Tiamin (mg)	0,11	0,48	
Riboflavin (mg)	0,03	0,05	
Niasin (mg)	1,3	4,7	
Vitamin C (mg)	0,0	0,0	
Vitamin E (mg)	0,02	0,7	

Lipidler: Çeltikte bulunan lipidler esas olarak germ, alöron tabakası ve alt-alöron tabakasında bulunur. Çeltik lipidlerinin çoğu nötrdür. Gliserolün başlıca oleik, linoleik ve palmitik asit olmak üzere üç yağ asidi ile esterleştirildiği trigliseritlerdir. Çeltik tanesinde trigliseritlerin yanı sıra serbest yağ asitleri, sterol ve digliseritler de bulunur. Çeltik tanesi ayrıca asilsterolglikozit ve sterolglikozit gibi lipid konjugatlarını, glikolipidleri ve fosfatidilkolin serebrozit gibi ve fosfatidiletanolamin gibi fosfolipitleri içerir. Yağ asitleri arasında palmitik ve linoleik asitler büyük bir oran oluştururken, oleik asit daha az miktardadır (Walter ve Marchesa, 2011; Ye ve ark., 2016).

Mineraller: Mineral elementler çeltik bitkisi sağlığı için gerekli besinlerdendir ve vücut aktivitesinin etkin işleyişinde hayati rol oynarlar. Mineral içeriği, gübreleme ve toprak koşulları dâhil olmak üzere yetiştirme koşullarından büyük ölçüde etkilenir. Çeltikte bulunan inorganik elementler arasında silisyum çeltikte baskındır. Fosfor minerali ise öncelikle bitkisel fosfor olarak, özellikle kepekte bulunur (Wang ve ark., 2011; Liu ve ark., 2017).

Vitaminler: Çeltik, nişasta, protein, vitaminler ve çeşitli mineraller dâhil olmak üzere insan vücudunun ihtiyaç duyduğu çeşitli besinler açısından zengindir. Çeltik tanesi, tiamin (B1), riboflavin (B2), niasin (B3), piridoksin (B6), siyanokobalamin (B12) ve yağda çözünen E vitamini, tokoferoller gibi suda çözünen vitaminler içerir. A, D ve K vitamini gibi yağda çözünen diğer vitaminleri önemli miktarda içermez. Vitaminler esas olarak endosperm ve kepek tabakalarında bulunur; böylece öğütülmüş çeltik daha az vitamin içerir (OECD, 2019; Mishra ve ark., 2020; Ding ve ark., 2022).

Bunların yanı sıra kepekli tahıllardan zengin bir diyet, rafine tahılların tüketildiği ve pirincin de istisna olmadığı bir diyetten daha sağlıklı bir seçenek olarak kabul edilir. Son epidemiyolojik araştırmalar, tam tahıl tüketiminin metabolik bozukluklar, özellikle tip 2 diyabet, kardiyovasküler hastalıklar ve bazı kanser türlerinin riskini azaltabileceğini göstermiştir. Zengin esmer pirinç, beyaz pirince göre daha yüksek lif içeriği nedeniyle iştah kontrolüne ve kilo kaybına yardımcı olabilir. Bu aynı zamanda LDL kolesterolün azalmasına da yardımcı olur. Esmer pirincin sağlığa yararlı bilesenlerinin iceriğini arastıran bu ve diğer çalışmalar, bilim camiasını rafine tahıllar yerine tam tahıl tüketimini teşvik etmeye sevk etmiştir. Ayrıca, bugüne kadar, bazı popülasyonlarda anemi, bodur büyüme ve kseroftalmi gibi eksiklikleri iyileştirmek için yararlı olan demir, çinko ve beta-karoten gibi besinlerle biyolojik olarak güçlendirilmiş yeni çeltik çeşitlerinin üretildiği de dikkate alınmalıdır. Çeltik, tam tahıllı versiyonunda esmer pirinç olarak tüketilirse faydaların en yüksek olacağı belirtilmektedir (Carcea, 2021).

4. Kırgısiztan'da çeltik üretimi / Paddy production in Kyrgyzstan

Coğrafi yakınlıktan ve coğrafi şartlardan dolavı Kırgızistan'daki çeltik tarımının tarihi çok eski yıllara dayanmaktadır. Ekili çeltiğin kökeni birçok araştırmacı tarafından incelenmiştir. Çoğu bilim adamı ata türlerinin çoğunun artık var olmadığına ve modern çeşitlerin şu anda bilinen türlerden geliştirildiğine inanmaktadır (Dabrowski ve ark., 2021). Kırgızistan'daki çeltiğin öneminin tüm dünyada olduğu gibi Kırgısiztan'da da artmasına karşılık ekim alanı ve üretimin, gerek küresel ısınma ve beşeri koşullardan dolayı azalıp çoğaldığı, istikrarın tam sağlanamadığı görülmektedir. Kırgızistan'da özellikle Oş ve Calal-Abad bölgeleri çeltik tarımı için, gerek iklim gerekse topografik açıdan en ideal koşulları ihtiva etmekte ve çeltik üretiminin yarısından fazlası bu bölgeden sağlanmaktadır. Kırgızistan'da çeltik tarımının yüzlerce yıllık bir geçmişinin olduğu bilinmekle beraber, tarımın ilk olarak nerede ve ne zaman başladığına dair kesin bir kanıt bulunmamaktadır. Fakat İpek Yolu üzerinden eski cağlarda girdiği görüsü hâkimdir. Nitekim ilk celtik fabrikası da 1916 yılında yine Oş şehrinde kurulmuştur. Çeltiğin, Kırgız Cumhuriyeti'nin ilanından önce Oş, Batken ve Calal-Abad bölgelerindeki varlıklı ailelerce tarımının yapıldığı ve tüketildiği ifade edilmektedir (Smailov ve ark., 2020).

Son yıllarda Kırgızistan'da çeltik üretiminin önemi giderek artmaktadır. Kırgız Cumhuriyeti Ulusal İstatistik Komitesinin verilerine göre 2020 yılında Kırgızistan'da toplam olarak 570.888 hektar alanı tarımsal üretim için kullanılmıştır. Yaklaşık olarak 11.927 hektar çeltik ekimi için kullanılmaktadır. Bununla beraber Kırgızistan'da ekim alanlarının tüm tarımsal üretim alanları içerisindeki payı sadece % 2,08'dir. Buradan elde edilmiş olan ürün, global çeltik üretiminin çok az bir kısmına karşılık gelmektedir (FAO, 2022). Çeltik üreten ülkeler arasında Kırgızistan son sıralarda yer almaktadır (OECD, 2019). Aynı zamanda Kırgızistan'da üretilen çeltiklerin verimi dünya ortalamasının altındadır. Kırgızistan'da çeltik yetiştiriciliği ana alanları Oş (Özgen, Kara-Kulja, Karasu ve Apavan), Calal-Abad (Suzak, Bazarkorgon, Nooken ve Aksu) ve Batken (Kadamjai-Ak-Turpak ve Leylek) olmak üzeri üç bölgeden oluşmaktadır (Şekil 2). Kırgız Cumhuriyeti Ulusal İstatistik Komitesinin verilerine göre en çok çeltik üretilen bölge % 48 ile Calal-Abad iken ekim alanı ve çeltik verimliliği en yüksek yer Özgen ilçesidir (Oş bölgesi) (Smailov ve ark., 2020).



Şekil 2 / **Figure 2.** Kırgızistan'da çeltik tarımı yapılan bölgeler / Regions of paddy cultivation in Kyrgyzstan (Britannica, 2022).

5. Kırgızistan'da çeltik tarımını etkileyen coğrafi faktörler / Geographical factors affecting paddy cultivation in Kyrgyzstan

Kırgızistan'dan çeltik tarımının dağılışını etkileyen en önemli coğrafi faktörlerden biri iklimdir. Çeltik, tropikal ve subtropikal habitatların doğal bitkisidir. Bu perspektifle iklimatik etkiler içerisinde çeltik tarımını en çok etkileyen, sıcaklık ve kullanılabilir suya ulaşımdır. Kırgızistan'da Calal-Abad, Oş ve Batken bölgelerinin iklimleri çeltik tarımına en uygun yerlerdir (Devkota, 2011).

Kırgısiztan'da çeltik ekiminin dağılışını etkileyen en önemli faktörlerden biri de topografyadır. Genel olarak çeltik tarımı için düz, düze yakın veya çok iyi düzenlenmiş toprak parçası tercih edilmektedir ki bu durum, çeltik tarımı için gereken sulamanın kolay ve rahatça yapılabildiği ova ve vadi tabanlarını göstermektedir. Nitekim Malezya (Kedah eyaleti), Endonezya (Sumatra Eyaleti), Tayland (Patani eyaleti) gibi uzakdoğu ülkelerinde düz ovalarda ve engebeli alanlarda teraslamalarla ve tavaların bu teraslarda kurulması sonucunda rahatlıkla çeltik yetiştiriciliği yapılabilmektedir (Firdaus ve ark., 2020). Celtik tavaları hazırlanırken az eğimli olmalarına dikkat edilmesi ve tavalardaki suvun bitkinin gelisimi icin suda çözünmüş O₂ (oksijen) bakımından önemli olduğundan devamlılığının sağlanması gereklidir. Bu bağlamda, Kırgızistan'da en geniş çeltik ekiliş alanlarına sahip Calal-Abad, Oş ve Batken bölgelerinde tavaların ideal özellikleri taşıdıkları

gözlemlenmektedir (Monfreda ver ark., 2008).

Kırgısiztan'da çeltik tarımının dağılışını etkileyen coğrafi faktörler arasına toprak özellikleri de girmektedir. Çeltik, toprak isteği bakımından çok seçici olmayıp bünyesi kumlu-tınlıdan ağır killi olanlara kadar çeşitli topraklarda yetiştirilebilse de, yüksek düzeyde verim almak için toprağın bitki besin maddelerince zengin yumuşak ve su geçirmeyen killi bir yapıda olması gerekmektedir (Taseva, 2021). Celtiklerin su icerisinde köklerinin ideal gelişimi (çeşitlere göre farklılık gösterebilir) için 20-25 cm derinlik ve 4,5-7,5 arasındaki pH değerine sahip topraklarda gerçekleşmektedir. Çeltik tarımı için, tuzlu toprakların yıkanması ve remediasyonunun da ayrı bir önemi vardır. Bunlarla beraber çeltik tarımı için alüvyon yapılı topraklar en uygun toprak çeşidi olmasına karşın, toprak yapısındaki kum ve kireç oranı arttıkça uygunluğun giderek azaldığı bilinmektedir. Aynı zamanda çeltik bitkisi, her ne kadar tuzlu toprakları sevmese de, verimsiz, kireççe zengin ve çorak topraklara orta derecede dayanma özelliğine sahip çeşitleri de bulunmaktadır (Taşlıgil ve Şahin, 2011; Nádvorníková ve ark., 2018).

6. Kırgızistan'daki bazı önemli çeltik çeşitleri / Some important paddy varieties in Kyrgyzstan

6.1. Özgen çeltiği / Ozgen paddy

İsmini Kırgızistan'da bulunan Özgen şehrinden alan Özgen çeltiği çeltik türü, diğer çeltik çeşitlerinden sadece tadı ile değil, aynı zamanda faydalı özelliklerinin bolluğu ile de farklılık göstermektedir. Özgen çeltiğinde % 90 nişasta, % 13 protein (normal pirinçte yaklaşık % 6), B2 vitamini, riboflavin,% 0,5 yağ, bol miktarda lif içeriği ve insan vücudu için yararlı diğer mineraller bulunmaktadır. Özgen çeltiğinin tüketimi eski zamanlara dayansa da besinsel özelliklerinin bilimsel çalışmalar ile desteklenmesinden sonra bazı hekimler tarafından özellikle diyet yemekleri menüsünde de tüketilmesinin tavsiye edildiği belirtilmektedir (Taşeva, 2021).

Özgen bölgesine başlıca dört nehir akmaktadır. Bu nehirlerden biri olan Karadarya nehri Doğu Türkistan sınırına yakın, yüksek dağlardan akarak Özgen iline gelmektedir. Bu nehir yatağının civarında çok fazla faydalı mineraller bulunduğu bilinmektedir. Dolayısıyla bu nehrin suyuyla beslenen ve büyüyen Özgen çeltiği mineral bakımından daha da zengin olmaktadır. Bununla birlikte çeltik çeşitlerinin verimi yalnızca tarlaların sulanacağı nehre ve ekildiği toprağa değil, aynı zamanda bu alanda belirli bir tür çeltiğin ne kadar süreyle yetiştiğine bağlı olarak değişmektedir. Bu bölgelerin dönüşümlü olarak tarımsal faaliyetlerde kullanılmasının birçok faydasının olacağı tavsiye edilmektedir. Ara verilmeden sürekli çeltik ekilmesi halinde veriminin azalacağı, toprak kalitesinin düşeceği belirtilmektedir (Mir, 2022).

Kırgızistan, Rusya ve Kazakistan'da yaygın olarak bilinen

"Uzgen pirinci", Özgen çeltiğinden elde edilmektedir. Özgen bölgesinde eski zamanlardan günümüze kadar geleneksel yöntemler ile tarımı yapılan Özgen çeltiğinin son 20 yılda verimi ve kalitesi nispeten gelişim göstermiştir (Tablo 2). Bu gelişim sebepleri arasında bazı gübreler ve tarımda kullanılan kimyasalların olduğu belirtilmiştir. Günümüzde halen çeltik yetiştiricileri tarafından geleneksel yöntemler ile yetiştirilmeye devam edilmektedir (Time.kg, 2021). Özgen celtiğinin en eski kökeni "Ak-uruk" (Arpa-şalı) ve "Kara-kıltırık" çeltik çeşitleri olduğu kabul edilmektedir. "Ak-uruk" çeşidinin ekili alanda hektar başına verimi 4.500-5.000 kilo civarında ve erken büyüme dönemi 90-100 gündür. "Kara-kıltırık" çeşidinde ise erken büyüme dönemi 110-120 gün ve ekili alanda hektar başına verimi 3000-3500 kilo civarında ve tane verimi % 50-55 oranındadır. Genel olarak yüksek kalitesi, tat ve lezzetinden dolayı yerel üreticiler tarafından küçük alanlarda ekimi yapılan bu çeltik çeşidinin düşük verimden dolayı son yıllarda ekime son yıllarda ekimi yok denecek kadar azalmıştır. Bununla beraber bu cesidin ekiminin vapılmamaya başlanmaşından dolayı bu türün yok olma sınırına yaklaştığı belirtilmektedir (Smailov ve ark., 2014).

Hauptvogel ve ark. (2012) tarafından yapılan çalışmada Özgen pirinci üzerinde protein, kül, yağ, karbonhidrat, enerji değeri, nişasta ve diyet lifi içeriğini belirlemek için kimyasal içerik analizleri yapılmıştır. Ak-uruk çeltiğinde protein içeriğinin ortalama % 8,04 olduğu bazı numunelerinde % 7,59 ile % 9,45 arasında değiştiği belirtilmiştir. Bu değerlerin, geleneksel veri tabanında beyan edilen protein içeriğinden % 2,33 daha yüksek olduğu görülmektedir. Pirinç örneklerinin kül içeriği ortalama % 1,68 iken, "Ak-uruk" çeşidinde bu oranın % 1,74 ile % 2,59 arasında değişmiştir. Pirinç nispeten az miktarda yağ içerir (500 mg/100g), ancak test edilen Özgen pirinç örneklerinde yağ içeriği % 0,97-2,74 arasında değişmiştir. "Akuruk" çeşidindeki karbonhidrat miktarının 72,11 g ile 76,80 g arasında ve nişasta içeriğinin de ortalama % 70,29 değerinde olduğu belirtilmiştir. Son olarak da enerji değeri 1480,25 kJ/100 g ile 1519,79 kJ/100 g olarak tespit edilmistir.

6.2. Ak-Turpak çeltiği / Ak-Turpak paddy

Orta Asya Cumhuriyetlerinde eski çağlardan beri yetiştirilen ana kültivarın ismi "Arpa Shalı"dır veya diğer adıyla "Devzira"dır. Bu ana kültivardan doğal seleksiyon yöntemiyle "Ak-uruk", Kara kıltırık, Tuya-tiş, Kazım (Özgen bölgesinde) ve Kadamjai bölgesinde (Ak-Turpak) "Caydarı devzire" veya "Ak devzire" ve "Kılcıksız devzire" gibi çeşitler meydana gelmiştir. Bu kadar farklı çeltik çeşitlerinin meydana gelmesinde toprak yapısı, iklim, su rejimi gibi çeşitli faktörlerin etkili olduğu belirtilmektedir.

Bu çeşitlerin ayırt edici özelliği, büyüme mevsiminin erken olgunluğunun 90-110 gün olması ve güncel verilere göre hektarda 5-6 ton civarı düşük verim elde edilen çeşitler olması-

Tablo 2 / Table 2

Kırgızistandaki çeltik tarımının Oş bölgesindeki ve ilçeler arasındaki 2019 ve 2020 yıllara göre ekim alanları, üretim ve verimlilik istatistiği / Cultivation area, production and productivity statistics of paddy rice cultivation in Kyrgyzstan in Osh region and between districts by 2019 and 2020 (NSCKR, 2020).

Yıl		Oş Bölgesi	Aravan İlçesi	Karakulja İlçesi	Kara-su İlçesi	Özgen İlçesi
	Üretim alanı (ha)	3377	153	85	109	3030
2019	Üretim (ton)	11212,9	1128,8	212,6	253,9	9617,6
	Verimlilik	3170	120	90	104	3170
	Üretim alanı (ha)	3479	120	90	204	3165
2020	Üretim (ton)	11618,5	1142,5	226,4	242,3	10007,3
	Verimlilik	3150	4000	2520	2330	3160

dır. Ancak kalite göstergeleri açısından yurtdışından getirilen, yüksek verimli olmasına karşın geç olgunlaşan (yetiştirme mevsimi 140-160 gün) çeşitlerden çok daha üstündürler. Batken bölgesinde geçmişten günümüze yetiştirilen "Devzira" çeltik çeşidi atasal çeşit olması bakımından çok önemlidir. Ak-Turpak ilçesine yakın köylerin çoğunda bu çeltik çeşidi avantajlı yerler arasında olduğundan Suu boyunda bulunan köylerde de "Devzira" çeşidinin yetiştirilmesi için en uygun bölgeler arasında almaktadır. Ak-Turpak devzirası, Özgen çeltiğinden daha beyaz olduğundan "Ak Devzira" ismiyle tanınmaktadır. Yerel üreticiler, Ak-Turpak devzirasının kalitesinin değişmesini önlemek için periyodik olarak tohumlarını kaliteli tohumlar ile değiştirirler, yani en iyi tohumları elde etmek için, olgunlaşma döneminde manuel olarak elleri ile en kaliteli çeltik tanelerini koparır ve böylece temiz tohumları gelecek sene için muhafaza ederler (Smailov ve ark., 2020).

Kerima Makhmudova'a göre 9. yüzyıldan itibaren Akdevziradan yapılmış pilavın düğünlerde ve diğer önemli törenlerde saygın ve özel ikram olarak sunulması bir gelenek haline gelmiştir. Şu anda Ak-Turpak bölgesi genelinde 2.500 hektarda Ak-Turpak çeltik yetiştirilmekte ve yaklaşık 7.000 ton pirinç üretilmektedir. Kırgızistan'da yetiştirilen çeltiğin bir kısmı, Tacikistan Cumhuriyeti'nde işlenmekte ve işlendikten sonra bir kısmı "Üretim Yeri Tacikistan" damgasıyla dış ülkelere ihraç edilmektedir. Bununla birlikte halihazırda Ak-Turpak bölge idaresinde 20'nin üzerinde modern taşlama makinesi faaliyet göstermektedir (Nádvorníková ve ark., 2018; Smailov ve ark., 2020; Otunchieva ve ark., 2021).

Ak-Turpak pirincinin taneleri iri, renkleri açık gri pigmentlidir. Bitkinin yetiştirilme ortam sıcaklığı ve nemi ekosisteme uygundur. Normal bir hasat alabilmek için sıcaklığın 10 santigrat derecenin üzerinde olması gerekir. Büyüme mevsimi boyunca, ortalama sıcaklık en az 15 derece olmalıdır. Çimlenme için 13 ve 16 dereceye ek olarak tam sağlıklı bitki eldesi için 15-19 dereceden düşük olmaması gerekmektedir. Sap kalınlığı 3-6 mm, yükseklik 50 cm'den 1 m'ye kadar cıkabilmektedir. Pirinç taneleri yuvarlak ve ovaldir. 1000 tane ağırlığı 26 gramdır. Celtiğin büyüme mevsimi 90 gündür. Celtik ılık ve nemli toprağa ekilir. Çeltik tohumları 10-12 derecelik bir sıcaklıkta hızla filizlenir. Ortalama 15 gün içinde, suyun yüzeyinde üç ile dört çeltik yaprağı belirir ve yapraklar kalınlaşır (Smailov ve ark., 2020). Son yıllarda Batken bölgesinde üretilen çeltik (Ak-Turpak), temelde % 100'ü beyaz olan ve diyet yemeklerinde kullanıldığından popülerlik kazanmıştır. Batken bölgesinde, çeltik ekim alanlarının genişlemesi çok sınırlıdır. Bu nedenle en acil sorunlardan biri olarak verim ve kaliteyi artırmaya, toprak verimliliğini ve ekolojiyi korumaya yönelik önlemlerin arttırılmasıdır. Tüm bunlar, Kırgızistan'ın sürdürülebilir kalkınma planları ve FAO / BM Dünya Gıda Programi'nin (WFP UNP) ortak misyonuyla tamamen örtüşmektedir (FAO, 2010).

Batken bölgesi için yukarıdaki faktörler dikkate alındığında, çeltik ekimi ve daha fazla verimi ekonomi için büyük önem taşımaktadır. Bu nedenle, toprak verimliliğini korurken çeltik yetiştiriciliği ve verimini artırmaya yönelik sorunları çözmek önemlidir (Smailov ve ark., 2020).

7. Sonuç ve öneriler / Conclusion and recommendations

Son yıllarda Kırgızistan'da çeltik ekim alanında artışlar görülmekte ve aynı zamanda çeltik verimlerinin de nispeten arttığı gözlenmektedir. Ancak pirinç tüketimi iç üretim miktarından çok fazladır. Çeltik üretim alanlarının sınırlı olması ve üretimin özellikle sadece "Sulanarak yapılan üretim (Irrigated Lowland)" metodu ile yapılmasından dolayı; ayrıca sulak yerler ile belirli alanlarda ekim yapma zorunluluğu gibi sebeplerden ötürü üretimi sınırlandırılmış ve ithalatı kaçınılmaz hale gelmiştir.

Nüfus artışı ile birlikte mutfak kültüründe de pirinç tüketiminin artmasıyla ülke çapında tüketim daha da artmıştır. Kırgız halkının yerli pirincleri tercih etmesinden dolayı ithal pirinç tüketiminde nispi düşüşler görülmüştür. Son yıllarda yurt dışından gelen pirinçlerin fiyat performans avantajlarının bulunması bu çeşitlerin yerli tüketiciler tarafından tercih edilmesine neden olmaktadır. Bu nedenlerden dolayı yerel Kırgız çeltik çeşitlerinin üretiminde verimliliğin arttırılması ve fiyatının ithal çeltiklerle rekabet edebilecek seviyeye çekilmesi gerekmektedir. Bunlara ilaveten geleneksel ıslah yöntemlerine alternatif metotların kullanılması gerekmektedir. Yerel çeltik çeşitlerinin ve Kırgız yemek kültürün değişmez ürünlerinden olan Özgen ve Ak-Turpak çeltikleri üzerinde alternatif metotların denenerek verim ve kalitesinin artırılması önerilmektedir. Özellikle moleküler markörler yardımıyla kuraklığa toleranslı Özgen çeltikleri elde edilebilir. Aynı zamanda bitki doku kültürü metotları ile in vitro şartlarda çok daha kısa zamanlarda verimi yüksek Özgen çeltikleri elde edilebilir.

Biyoteknolojik metotların yardımıyla, rekombinant gen teknolojileri kullanılarak farklı organizmalardan gerekli genler izole edilerek çeltiklere transfer edilebilir ve böylelikle elit çeltikler elde edilebilir. Bunların yanı sıra Kırgızistan coğrafi şartları baz alınarak kuraklığa toleranslı olan kır çeltiğine benzer özelliklerde yeni nesil çeltikler elde edilebilir.

Ayrıca, coğrafi bakımdan nispeten yüksek kesimlerde ve normalden çok daha kısa vejetasyon süresine sahip çeltik çeşitlerini modern yöntemler ile yetiştirerek ve yağışa/kar erimesine bağlı olarak gelişen "kır çeltikçiliği" yapılabilir. Unutulmamalıdır ki küresel ısınma, kullanılabilir su kaynaklarının azlığı gibi sebeplerden dolayı kır çeltiğinin önemi giderek artmaktadır. Günümüzde küresel çeltik üretiminin % 12'si kır çeltiğinden oluşmaktadır. Kırgızistan'da hâlihazırda herhangi bir kır çeltiği üretimi mevcut değildir. Kırgızistan'da çeltik verimi dünya ortalamasının altında olduğu için bahsi geçen teknikler kullanılarak verim ve kalite artışları sağlanarak dünya ortalamasının üzerine çıkabilirse, pirinçte ithalat sorununun büyük ölçüde azalacağı düşünülmektedir.

Yerel çeltikler gibi Kırgız coğrafyasının vazgeçilmezlerinden olan çeltiklerin maliyetlerinin de azaltılması hedeflenmelidir. Özgen çeltik çeşitleri ile ata formları geniş bir varyasyona sahip olacağından ıslah programlarının yapılması başarıyı artırabilir. Özgen çeltiğinde verimin arttırılması Kırgızistan'ın çeltik ihtiyacında yurtdışı bağımlılığını önemli derece azaltabilecektir.

Bu nedenle, tam tahıl üretimi ve tüketiminin teşviki için, tarım bilimi, biyokimya, biyoteknoloji, işleme, depolama, pazarlama ve tüketici bilinçlerini içeren disiplinler arası bir program oluşturulması önemlidir. Son olarak, hazırlanacak çeltik ıslah programlarında, gelecekte çeltiğin tam tahıllı pirinç olarak tercih edilen tüketimini hesaba katmalı bu konuyla ilgili olarak kalite özelliklerini dikkate alarak modern biyoteknolojik yöntemler kullanmaya odaklanılmalıdır.

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