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SEDIMENT-FRIENDLY FORMULAS: A REVIEW ON THE SEDIMENT QUALITY GUIDELINES

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ABSTRACT. Aquatic ecosystems play an important role in maintaining ecological balance such as climate regulation, irrigation, flood control, aquaculture, and especially water supply. Sediments are located at the linkup of the solid-liquid interface, therefore they form an important part of the water body. The purpose of this review article is to reveal the importance of sediment pollution with heavy metals and understand how usage of accordingly sediment quality guidelines and indices.

1. INTRODUCTION

Heavy metal contamination in sediments is a matter of environmental concern, because of their non-degradable, toxic and persistent features [1]. The amount of heavy metals is impacted by two main sources, anthropogenic and natural, which present metals into the aquatic ecosystem by different sources, such as sewage runoff, industrial waste, and agriculture discharges [2]. Thus, these sources cause the entrance of heavy metals into the aquatic system and they are distributed between the liquid phase and sediment during the transportation [3]. A large portion of heavy metal get accumulated in the sediment but only a small quantity of it stays dissolved in water [4]. There is not sufficient information to determine which sources are most influential for metals and locations specifically. Some heavy metal(loid)s such as cadmium, lead, arsenic, and mercury have toxic effects on organisms [5].

Keyword and phrases. Sediment, sediment quality guideline, aquatic, environment

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Heavy metals entering the aquatic environment precipitate to sediment and affect the health of the aquatic ecosystem. Sediments are main reservoir of heavy metals. They are vitally significant components of the aquatic systems because they have a long duration of stay. Also, sediments play an important role in preserve the trophic status of the system. The importance of the sediments not only relevant to the transport of heavy metals but also, they are a secondary heavy metal source [6]. When water parameters change that balance will collapse and heavy metals in the sediment will be released into the water [7,8]. The release processes are carried out by ion exchange, desorption, and dissolution [9] (Figure 1). Therefore, it is possible to have an idea about the pollution level of the wetland by looking at the metal concentrations in the surface sediments.

Sediment pollution is dangerous because of the food web and finally becoming detrimental to organisms. Heavy metals can pass from soil to seed, making plants toxic. When organisms feed on this plant, larger animals feed on these organisms, by the time heavy metals get more effective in the process of biomagnification. It is a significant point to analyze the metal pollution in the sediment during the investigation of the contaminated aquatic environment. Measuring the abundance and structure of organisms in the area may demonstrate the sediment ecosystem health but these measurements are expensive and requires a lot of time. The ecological risk caused by heavy metals is about to be evaluated different “sediment quality standards” have been developed.

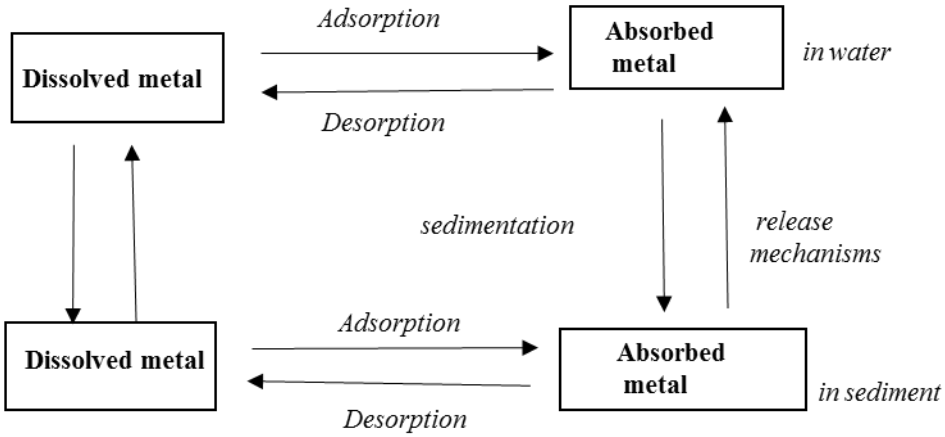

FIGURE 1. Interaction of heavy metals in water and sediment [10].

2. Sediment Quality Guideline (SQG)

Sediment quality guideline has been developed in order to understand the effects of heavy metal pollution accumulating on sediment on the organisms since the reference values or "background" used before 1980 were insufficient to understand the effect of organism [10]. In the last 20 years, many studies have been conducted that demonstrate the importance of determining and maintaining sediment pollution in order to maintain aquatic ecosystem quality using this method [11-16]. Emprical-SQG is based on both land and laboratory data, which shows the response of benthic organisms exposed to metal concentration in sediment. It focused primarily on two approaches to assessing pollution in sediment, these are the relationship between the response of the toxicity and contamination of the sediment [17-19].

Sediment quality criteria established by the National Oceanic and Atmospheric Administration (NOAA) are used to understand about contaminated sediment in the aquatic environment, specially TEL and PEL and the ERL and ERM [20]. TEL is the level of sediment contamination where toxic response begins in benthic organisms. PEL is the level of sediment contamination in which a large percentage of benthic populations show a toxic response. In summary, sediment contamination below the TEL value is acceptable, while the concentration above the PEL value is unacceptable. Further work and evaluation are required for the value between TEL and PEL. The 10th (ERL: Effects range-low) and the 50th (ERM: Effects range-median) percentile of the effects outputs were determined for each metal. The value below the ERL means that effects would rarely occur, above the ERM means that effects would frequently occur [18]. There have been references to such effects levels by different sources for heavy metals and a table has been created (Table 1).

Table 1. Some SQG values

	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn	Reference
TEL*	5.90	0.60	37.30	35.70	0.17	18.00	35.00	123.00	[19,20]
PEL*	17.00	3.53	90.00	197.00	0.486	36.00	91.30	315.00	[19,20]
ERL**	33.00	5.00	80.00	70.00	0.15	30.00	35.00	120.00	[18,20]
ERM**	85.00	9.00	145.00	390.00	1.30	50.00	110.00	270.00	[18,20]

Some of the Methods Used in the Assessment of the Sediment Pollution

Different indices have been developed to determine metal concentrations and anthropogenic effect levels in the sediment such as enrichment factor (EF), metal enrichment index (MEI), geo-accumulation index (Igeo), contamination factor (CF), pollution load index (PLI), and (Table 2). Reference values are generally source data of Turekian and Wedepohl [17].

Enrichment Factor (EF): It is used to determine whether the metals contained in the sediment are naturally (from rocks) or anthropogenic sources and also calculate the rate of pollutants in sediments [21]. Thus, it is possible to understand the status and degree of environmental pollution with this index.
In the literature, it is the most used index in the evaluation of metal pollution in sediment [11-16]. Classification values are given in the Table 2. It is formulated as follow [22]:

$$EF = \frac{C_n/C_{ref}}{B_n/B_{ref}} \quad (1)$$

Cn= It means that concentration of heavy metals in sample, Cref= It means that concentration of the heavy metals in the reference value, Bn= It means that reference element amount in the samples, Bref= It means that the value of the reference element in the reference environment, Fe is accepted as the reference element [23].

Metal Enrichment Index (MEI): This index is applied in an industrial area when investigating anthropogenic relationship with heavy metal accumulation [24, 25]. Classification values are given in the Table 2. This index has been reported to be more suitable for use in metal deposits in surface sediment and is also called surface sediment index and it is formulated as follow [24]:

$$MEI = \frac{C_A - C_B}{C_A} \quad (2)$$

Where C_A and C_B stands for total concentration of individual heavy metals, and stands for background level, respectively.

Geo-accumulation Index (Igeo): It is a frequently used index to evaluate the degree of anthropogenic and geogenic accumulated pollution loads and also this index has been reported to be successful in detecting heavy metal accumulation especially in the sediment due to industrial activity [13, 26]. In addition, it was used by most researchers to detect the severity of the pollution and quality of sediment [13, 14, 27, 28]. Classification values are given in the Table 2. It is formulated as follow [29]:

$$I_{geo} = log_2 \frac{C_n}{1.5 \times B_n} \quad (3)$$

Cn= Concentration of heavy metals in sample, Bn= amount of the reference element in the reference environment, 1.5= natural oscillation coefficient

Contamination Factor (CF): This method evaluates the enrichment of metals based on the background concentrations of each metal in sediments. It is found by dividing each metal concentration in sediment by the background value. Classification values are given in the Table 2. It is formulated as follow [30,31]:

$$CF = C_s / C_{ref} \quad (4)$$

Where Cs: concentrations of the element in the sediment sample, Cref are and the background value of the element.

Pollution Load Index (PLI): This index shows the magnitude of heavy metal pollution in sediment and also Igeo also use for this reason. Classification values are given in the Table 2. It is formulated as follow [32]:

$$PLI = (C_{f1} \times C_{f2} \times C_{f3} \ldots . \times C_{fn})^{1/n} \quad (5)$$

n = number of metals and CF = contamination factor.

TABLE 2. Some sediment quality classification.

Indices	Sediment quality classification for multiple indices to assess heavy metals	Reference
EF	EF<1 = no enrichment	[22]
	1<EF< 3 = minor enrichment	
	3<EF< 5 = moderate enrichment	
	5<EF<10 = moderately severe enrichment	
	10>EF>25 = severe enrichment	
	25>EF>50 = very severe enrichment	
	EF>50 extremely severe enrichment	
MEI	1= no enrichment	[24]
	2=low enrichment	
	3=moderate enrichment	
	4=strong enrichment	
	5=extremely enrichment	
Igeo	Igeo≤0 = practically uncontaminated	[29]
	0<Igeo<1 = uncontaminated to moderately contaminated	
	1<Igeo<2 = moderately contaminated	
	2<Igeo<3 = moderately to strongly contaminated	
	3<Igeo<4 = strongly contaminated	
	4<Igeo<5 = strongly to extremely contaminated	
	Igeo≥5 = extremely contaminated	
CF	CF<1 = low contamination	[30]
	1≤CF<3 = moderate contamination	
	3≤CF<6 = considerable contamination	
	CF≥6 = high contamination	
PLI	PLI <1 no pollution	[32]
	PLI is >1 deterioration	

Many indexes have been used in the literature to evaluate the current state of heavy metal in sediment. Some studies using these indices are summarized in Table 3.

TABLE 3. Summary of some studies using indices

Indices	Researched metals	Situation	Region	Reference
Igeo	As, **Cd**, Cr, Cu, Hg, Ni, Pb, Zn	moderately to strongly contaminated	Lake Taihu, China	[27]
EF, Igeo, CF	Cu, Cr, Ni, Zn, Pb, Mn	low ecological risk	Weihe River, China	[33]
CF,EF	Al, **As**, Cr, Cu, **Fe**, **Mn**, Ni, Pb, **Zn**	natural and anthropogenic sources	Lake Naivasha, Kenya	[11]
CF, PLI	Cr, Mn, Co, Ni, Cu, Zn, **Pb**	considerable contamination	Lishui River, China	[34]
EF, Igeo, PLI, CF	Pb, **Cd**, Cu, Cr, Ni, Hg, Zn, Mn, Fe	strongly/extremely polluted	Mashavera River, Georgia	[35]
EF, Igeo, CF	Pb, Cu, **Cd**, Ni, Cr, Zn	moderate-considerable pollution	Baltic Sea, Lithuanian zone	[36]
EF, Igeo, PLI, CF	Cu, Cr, **Pb**, Zn	very severe enrichment	Kuala Perlis, Malaysia	[37]
EF, Igeo	As, Cd, Pb, Cr, Co, Ni, Zn, Cu, Fe, Al	highly polluted	Gökçekaya Dam Lake,Turkey	[38]
EF, MEI, Igeo	Fe, Cr, Zn, Cu, Co, Ni, Mn , **Pb**, Cd	moderate contamination	Niger Delta Region, Nigeria	[39]
CF, PLI	Pb, **Cd**, As, **Hg**	moderate contamination	Danube Delta, Romania	[40]
EF, Igeo	Pb, Cu, Cr, **Ni**, **As,** Mn, Al, Fe, Zn	high contamination	Lake Beyşehir, Turkey	[41]
EF, Igeo	**Cd**, Cr, Cu, Ni, Pb, Zn	moderately severe enrichment	Laizhou Bay, China	[42]
PLI, Igeo	Fe, Cu,Pb, Mn, Ni, Cr	unpolluted by heavy metals	Benin River, Nigeria	[43]
PLI, Igeo	Mn, Ni, **Pb**, Cu, **Cd**	slightly polluted	Tigris River, Baghdad, Iraq	[44]
EF, Igeo, CF, PLI	As, Cd, **Co**, Cr, **Cu**, Mn, Ni, **Zn**, **Pb**	moderately polluted	Tigris River, Turkey	[45]

3. CONCLUSION

Numerous indices have been developed to understand the current health status of a wetland sediment. Thanks to these indices, it is possible to understand whether the presence of a metal in the sediment is natural or anthropogenic and also its level. But do these indices only have advantages? There are many advantages such as predicts toxic response, field tests and large database of lab, and easy to use. However, they also have some disadvantages such as difficult to separate effects from a mixture of contaminants, the area between thresholds, poor documentation for formulations, test errors and most data for metals. In this study, some indices frequently used in the literature are included. Studies in the literature have also shown that despite their disadvantages, they have very useful uses in terms of saving and protecting a wetland.

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ISOLATION OF ENTOMOPATHOGENIC FUNGI FROM TURKEY SOIL AND TESTING OF DIFFERENT DOSES ON *GALLERIA MELLONELLA* (LEPIDOPTERA: PYRALIDAE)

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ABSTRACT. Isolation of biological control agents which would be used with pests control could be done with different methods. The most sensitive of these methods is the insect bait method. Besides, it is known that the effects of different entomopathogenic fungi isolates, which would be used as biological control agents varies in different hosts. In this study, since the target pest organism is the larval period of *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae), this pest is used for insect bait method and as a result of isolation from 180 soil samples, 48 *Beauveria bassiana* Bals.-Criv. Vuill. (Hypocreales: Cordycipitaceae) and 1 *Beauveria brongniartii* (Sacc.) Petch (Hypocreales: Cordycipitaceae) was detected but *Metarhizium spp.* wasn't detected. Furthermore, it is known that the precise determination of the entomopathogenic fungi isolates, plays a crucial role in the success of a biological control program. In this study, as biological control agent, four concentrations ($1x10^4$, $1x10^5$, $1x10^6$ and $1x10^7$ conidia/ml) of 10 different isolates of *Beauveria bassiana* Bals.-Criv. Vuill. (Hypocreales: Cordycipitaceae), which were obtained from soils of our country and a standard strain from Denmark were applied to *Galleria mellonella* Linnaeus (Lepidoptera: Pyralidae) larvae. When Lethal times (LT_{50}) compared, it was found that the highest concentration of each isolates was also the most effective one.

1. INTRODUCTION

Due to the growing world population, natural ecosystems are being rapidly altered to acquire more agricultural land for human use. As an outcome the damage on natural ecosystems increase. The commercial and permanent farming system used

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for the production of sufficient nutrients should be both highly efficient and sustainable additionally should have minimal environmental pollution. On the other hand, natural life needs to be preserved. Achieving both goals is one of the most important problems of the 21st century. One of these solutions is biological control [1]. Chemical pesticides are used in the control of many harmful insects [2] and have proven to be effective in many cases [3]. They are particularly effective in controlling weed and plant diseases. However, they are ineffective in controlling certain insect such as silkworms [4,5]. As insects develop resistance to the chemicals used [6]. Moreover, because of the degree of pollution caused by chemicals, methods of fighting insects that are less harmful to both the environment and humans are having been developed [1,7,8,9,10]. At this stage, the idea of biological control was born, and attempts were made to prevent pests by using various living organisms [11,12,13]. Various living groups, from the smallest microorganisms to vertebrates, are being used in biological control including bacteria, fungi, nematodes, birds and insects [14]. Microbial biological control agents offer effective, environmentally harmless and cheaper long-term economic methods to deal with harmful insects [13,15]. Numerous entomopathogenic fungi are in use for the control of pests [16]. *Beauveria bassiana* Bals.-Criv. Vuill. (Hypocreales: Cordycipitaceae) and *Metarhizium anisopliae* (Metschn.) Sorokin (Hypocreales: Clavicipitaceae) are the most studied entomopathogenic fungi species, both having very wide host range [17,18] and have wide geographical distribution [15]. There are numerous successful biological control experiments carried with *B. bassiana* [19,20]. This species is known to be able to infect more than 200 species of insects [21,22]. It was reported to be the most common and most effective fungus against ground beetles that can survive the winter [21,22,23].

It was stated that the effects of isolates obtained from different regions in different hosts might be different [24,25]. In addition, accurate determination of the concentration to which entomopathogenic fungus would be administered was an important factor affecting its success as a control agent. [26,27] In many studies, it was stated that the entomopathogenic fungus isolated from the area to be applied would be more successful in that area than the entomopathogenic fungus isolated from another region. [24,25,27,28]. Therefore, this study was designed to investigate the effects of different strains and concentrations of *B. bassiana* and *M. anisopliae* using *Galleria mellonella* larvae, which were preferred as experimental animals in many studies [29]. However, since there was not any *M. anisopliae* species in our isolation studies, our studies were conducted on *B. bassiana* strains. In this study, four different concentrates for each of 10 strains of *B. bassiana* isolated from various regions of Turkey with the standard strain of *B. bassiana*, whose pathogenity was proven on insects, were tested on the larvae of *G. mellonella*.

2. Materials and Methods

2.1. Galleria mellonella

The reproduction and development of *G. mellonella* larvae used in this study was carried out in glass jars and in a dark artificial medium environment in the laboratory with a 27 °C temperature, which is suitable environment for larvae [30]. *Galleria* larvae emerging from their eggs after 4 weeks (fourth stage of larvae) was used for the insect bait method [31]. During the insect bait method, *Galleria* larvae were kept in 500 ml of water which was heated up to 56 °C to prevent larvae from pupation and their inactivation was fulfilled [31,32,33].

2.2. Collection of soil samples, insect-bait and soil dilution method

During May and August of 2007-2008, a total of 180 soil samples were collected from soils around Turkey and the points where soil samples were taken were randomly selected [34]. Soil samples of approximately 1 kg were collected from cultivated areas, meadows and forest floors. Soil samples were preferably taken from the surface of the soil from 5 - 20 cm depth [21,35,36,37,38,39,40]. These samples were taken into plastic bags to prevent drying of the soil after the labeling process, where values such as the name of the regions, vegetation type and soil temperature were recorded [21,36,41].

The insect-bait method was originally developed for isolation of entomopathogenic nematodes found in soil, but this method also provides isolation of entomopathogenic fungi [42]. The insects to be used in this method should be easily produced and that are sensitive to fungi should be preferred. The most common insect species used for this purpose is the big wax moth *G. mellonella* [31,32,33]. In the isolation of entomopathogenic fungi, baiting of soil samples with *Galleria* larvae is a very common tool [21,36,37,39,43,44,45].

Soil samples obtained from the different field were taken into 250 ml volume plastic containers without waiting too long. In dry soil samples, distilled water was added, considering that for entomopathogenic fungi infection is better in moist conditions. Inactivated fourth stage *Galleria* larvae were placed in five of the soil samples that are placed in plastic containers [46]. After the mouth of the plastic containers was closed, the plastic containers were inverted so that *Galleria* larvae remained under

the soil [40,47,48]. The samples were kept at room temperature at 20-25 °C [46,49]. The soil samples, which were inverted, were checked for a total of 15 days each 3 days apart to see if the larvae were infected [50].

The larvae thought to be infected were removed from the soil to provide surface sterilization for the cadaver with 1% sodium hypochlorite in 2-3 minutes, then washed with distilled water [31,43,51,52,53,54]. Then the larvae were transferred to sterile petri dishes with damp filter paper in them [55,56]. With daily checks, the larvae were investigated whether the cause of death was entomopathogenic fungus [56,57]. At the same time, distilled water was added to the filter paper in the petri dishes at regular intervals due to the high humidity requirement of entomopathogenic fungi (Figure 1) [56,58,59,60].

FIGURE 1. *Galleria* larvae infected with *Beauveria bassiana* and *Beauveria bassiana* spores that grow on the larvae.

When entomopathogenic fungi started to produce conidia on cadavers and, the samples that taken here were added to the selective media [59]. Fungi thought to be entomopathogen were placed on the media containing SDA + dodine + chlorotetracycline (prepared by adding 0,46 g/l dodine and 5 mg/l chlortetracycline to SDA media) and procreation of entomopathogenic fungi was provided after 7 days of incubation at 27 °C [61,62, 63,].

Soil dilution method, another isolation method of entomopathogenic fungi from soil, was also carried. Soil samples collected from various regions of Turkey were placed

in sterile tubes containing 10 ml distilled water. Each test tube was mixed in centrifuge for 20 seconds. Then 1 ml was taken from the mixture and added to the test tubes with 9 ml distilled water and thus 10 times dilution was achieved [38,60,74]. Then, a mixture of 0.5 ml from each test tube was added to Veen's Medium, the selective medium of entomopathogenic fugus, and spread with the help of a sterile L glass rod. Media were left to fungal development for 14 days at 26°C ± 2°C. Afterwards, samples that may be *Beauveria spp.* and *Metarhizium spp.* which reproduce in media were planted in SDAY media to obtain pure culture [64].

2.3. Selection of experimental strains

The fungus samples isolated by both methods were selected according to the colony characteristics in SDAY media in the first stage. Considering the colonial characteristics of the species that may be *B. bassiana* and *Metarhizium anisopliae* (Metschn.) Sorokin (Hypocreales: Clavicipitaceae), the specimens that did not provide these characteristics were eliminated. At the begining the color of *B. bassiana* colonies were white and then go to yellowish pinkish [65,66,67], while the color of *M. anisopliae* colonies were yellow to green or dark plant green to pink [66,68].

In order to distinguish the entomopathogens from the selected samples, the fungi were planted in PDA media [69]. Cultures were incubated at 27 °C for 14 days so that they can produce conidia [15]. Then five *Galleria* larvae were left in these cultural environments and the larvae were expected to wander in the petri dish for a certain period of time, allowing them to take conidia on them [70]. In the second stage, these larvae were taken from the culture environment and placed in petri dishes with sterile filter papers [60]. Drying papers were moistened with sterile distilled water to provide moisture for fungal growth [66,71]. The conditions of the larvae were observed every day and checked whether the cause of death was the fungus. The larvae infected with entomopathogenic fungi were recorded, while others were eliminated.

2.4. Obtaining entomopathogenic fungus species

Spores taken from larvae infected with entomopathogenic fungus were placed in SDA media with 1 ml dodine (prepared by dissolving 1 gr dodine in 9 ml distilled water solution) and 500 µl chloramphenicol (prepared by dissolving 1 gr chloramphenicol in 10 ml of a 96% solution of ethanol) added and incubated at 26°C

± 2°C. After 7-14 days of incubation, entomopathogenic fungus cultures that were freed from soil bacteria and saprophytic fungi were obtained [72,73].

2.5. Preparing slide and microscopic examination

Entomopathogenic fungus strains were placed in SDAY media and preparations of entomopathogenic fungus strains were made and examined under a microscope [65,74]. In this microscopic examination, the elimination of non-entomopathogenic fungi by taking into account several key features of entomopathogenic fungi. During the preparation of the slide, very small amounts of fungal samples were taken from the culture medium where the entomopathogenic fungus had been produced. Then the previously prepared lactophenol solution was dripped on microscope slide in a small drop and the sample was left on it. Entomopathogenic fungus samples taken from the medium with the inoculating loop, the medium particles on the inoculating loop were dissolved without getting too close to the bunsen flame. Thus, entomopathogenic fungi spread on microscope slide without damaging its structure and a cover glass was placed on it and examined under a microscope.

On prepared slide, the genus *Beauveria* tried to be observed the most characteristic features of the indicated conidiogenous cells densely clustered or in whorls or solitary, colorless, short, with base globose or flask-like and extending apically and repeatedly branching a short distance below each of several apically-formed conidia (Figure 2) and as for that the genus *Metarhizium* tried to be observed on prepared slide, such as conidiogenous cells with rounded to conical apices, arranged in dense hymenium; conidia aseptate, cylindrical or ovoid, forming chains usually aggregated into prismatic or cylindrical columns or a solid mass of parallel chains, pale to bright green to yellow-green, olivaceous, sepia or white in mass [65,75,76].

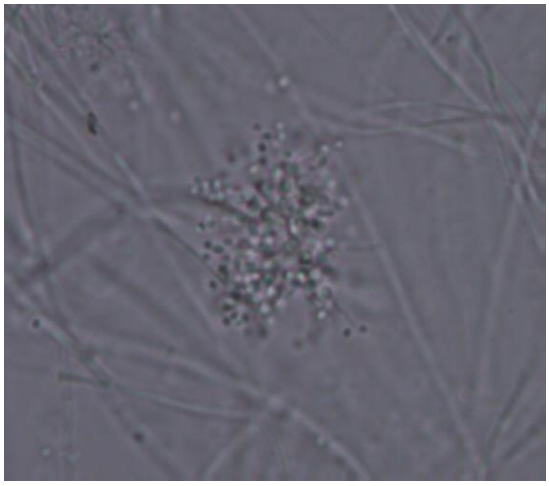

FIGURE 2. It was tried to observe that *B. bassiana* conidiaphore, sporogenic cell and conidias.

The absolute species identification of entomopathogenic fungus samples, which were isolated from soil by using insect bait method and soil dilution method and thus removed from saprophytic fungi and bacteria using selective media.

2.6. Used experimental strains

Until this stage of our studies, 48 strains of *B. bassiana* and 1 strain *Beauveria brongniartii* (Sacc.) Petch (Hypocreales: Cordycipitaceae) were identified in consequence of isolation and species identification from soil samples collected from various regions of Turkey. 48 *B. bassiana* strains that obtained from isolation were tested on *Galleria* larvae and 10 *B. bassiana* strains with the best pathogenity were selected. The type species is KVL 03-129 *B. bassiana* 2002 standard strain from Copenhagen University, Denmark. The locations where these 10 examples were taken and the vegetation types of these places are listed in Table 1.

TABLE 1. *Beauveria bassiana* strains, vegetation type of the place and area where it was isolated.

	Code Of The Strain	Where soil sample was taken	Type of vegetation where soil sample was taken
1. Strain	DenK1	Denizli - Kocabey	Plantation
2. Strain	SB1(b)	Ankara - Beytepe	Meadow Field
3. Strain	Ank 12	Ankara - Çalış Köyü	Plantation
4. Strain	Lül 5	Tekirdağ - Lüleburgaz	Tree Planted Area
5. Strain	MerK	Mersin - Kargıpınar	Plantation
6. Strain	BarK2	Bartın - Kumluca	Woodland
7. Strain	Kon-2	Konya - Cihanbeyli	Plantation
8. Strain	Ordu	Ordu - Merkez	Hazelnut Tree Gardens
9. Strain	AyAk	Aydın - Akbük	Olive Tree Gardens
10. Strain	Erz	Erzincan - Tercan, Aşkale	Meadow Field

2.7. Preparation of spore solutions

The spore solution of each strain was prepared from cultures that had been produced for 14 days in SDAY media that prepared in 50 ml tubes before the experimental setup was prepared. 10 ml distilled water containing 1% Tween 80 was added to these media. Then the conidia suspension of each strain was transferred into 50 ml volume tubes and mixed for 3 minutes at 1500 rpm to separate the spores and micelles from each other [68]. Conidial concentration in suspension was detected under phase contrast microscope using Neubauer chamber [66,73].

0.1 ml of spore solution was placed on Neubauer chamber which has four large squares, each 1 mm^2 in size. The number of spores in 1 mm^3 was determined by going into them by 4 in order to get an average of the number of spores in these four square areas. This number was then multiplied by 10^3, resulting in the number of sports in 1 ml. The number of spores was found by multiplying this number by the dilution factor [63].

In our study, 4 different concentrations were prepared for each fungus strain and standard strain and were tested on larvae in the experiments to test the 10 fungal strains whose pathogenity was to be tested on *G. mellonella* larvae. These concentrations were $1x10^4$, $1x10^5$, $1x10^6$ and $1x10^7$ conidia/ml [77].

2.8. Testing of fungal strains on *Galleria mellonella* larvae

4 different spore solutions ($1x10^4$, $1x10^5$, $1x10^6$ and $1x10^7$ conidia/ml) prepared for each selected strain of entomopathogenic fungus were added as 1 ml to the sterile petri dish containing sterile filter paper. 10 inactivated fourth stage instar *Galleria* larvae were placed in each petri dish. The dose of each entomopathogenic fungus strain was applied to 10 *Galleria* larvae and the dosage applications were repeated three times. Thus, 30 larvae were used for each dose. All these procedures were carried out in sterile conditions to prevent contamination.

In this experiment, each strain was put into plastic bags separately each as a group, thus preventing contamination and moisture loss and were removed to the environment where 27 °C and dark conditions were achieved. The established experiments were checked every day and the dead larvae found to be infected were soaked in 1% sodium hypochlorite for 2 minutes to ensure surface sterilization and then washed with distilled water and transferred to petri dishes containing sterile filter paper under sterile conditions. The moisture was provided by adding 1 ml of distilled water. These petri dishes were placed in separate plastic bags and checked daily for the observation of entomopathogenic fungi on cadavers in the same environment. Nevertheless, sterile distilled water has been added to these petri dishes to ensure the high humidity environment required for fungi. In addition, control groups were formed in the same way as the experiments, but the control group was not given spores and sterile distilled water was added in 1% Tween 80 [60].

2.9. Statistical methods

The pathogenity of these 10 strains, whether the dose applied and the effect of the strain were investigated by Anova based on the pathogenity of the type species. Groups (strains) were compared among themselves with LSD testing.
The EPA probit analysis program was used based on the killing potential of *Galleria* larvae of all fungal strains used in the experiment on the fifth day of the experiment, and thus LD_{50} (the dose required to kill 50% of the subjects) values were calculated. In addition, with the Kaplan-Meier test, LT_{50} values, in other words the time it took for 50% of *Galleria* larvae to die, and standard error (SE) were calculated [78].

3. RESULTS

Whether the dose and strain effects of these 10 strains were investigated with Anova according to the pathogenicity effects of the standard strain and the results indicated that these two factors were statistically significant. (F_{strain} = 2,06, F_{dose} = 9,30, P<0,05). The strains were compared among themselves with the LSD test (Table 2).

TABLE 2. Comparison of different doses of *Beauveria bassiana* strains which tested on *Galleria mellonella* with each other and with the control group.

Dose (con/ml)	Dose (con/ml)	Mean differences	Standard Eror	Sig.	95% Limits	Confidince
					Sub Limit	Upper Limit
$1x10^7$	$1x10^6$	,791	,774	,308	-,733	2,315
	$1x10^5$	1,871	,732	,011	,429	3,312
	$1x10^4$	2,310	,715	,001	,901	3,719
	C	4,609	,987	,000	2,666	6,552
$1x10^6$	$1x10^7$	-,791	,774	,308	-2,315	,733
	$1x10^5$	1,080	,702	,125	-,303	2,463
	$1x10^4$	1,519	,685	,027	,170	2,868
	C	3,818	,965	,000	1,918	5,718
$1x10^5$	$1x10^7$	1,871	,732	,011	-3,312	-,429
	$1x10^6$	1,080	,702	,125	-2,463	,303
	$1x10^4$	,439	,637	,491	-,815	1,694
	C	2,738	,932	,004	,903	4,572
$1x10^4$	$1x10^7$	2,310	,715	,001	-3,719	-,901
	$1x10^6$	-1,519	,685	,027	-2,868	-,170
	$1x10^5$	-,439	,637	,491	-1,694	,815
	C	2,298	,919	,013	,490	4,107
C	$1x10^7$	4,609	,987	,000	-6,552	-2,666
	$1x10^6$	3,818	,965	,000	-5,718	-1,918
	$1x10^5$	2,738	,932	,004	-4,572	-,903
	$1x10^4$	2,298	,919	,013	-4,107	-,490

A high rate of successful mortality was obtained by comparing all fungal strains and their doses with the control group on *Galleria* larvae. The results of the statistical analysis also supported this result and it was found that the average death time of the control group according to LSD test was different from the average death time of all tried doses (P<0,05) (Table 2).

It was found that there was no difference between the results of concentration applications of fungus strains $1x10^7$ and $1x10^6$ con/ml ($P>0.05$) (Table 2) also there was no difference in concentration applications of fungi strains $1x10^6$ to $1x10^5$ and $1x10^5$ to $1x10^4$ con/ml ($P>0.05$) (Table 2). It was found that the concentration application of $1x10^7$ con/ml was different from $1x10^5$ and $1x10^4$ con/ml, and the concentration application of $1x10^6$ con/ml was different from $1x10^4$ con/ml ($P<0.05$) (Table 2). According to these results, it was found that the effective dose in terms of efficacy (the dose that killed in the shortest time) was the highest of the applied doses, and the effectiveness decreased with respect to the high dose as the applied dose decreased. This result was supported by both graphs and statistical results (Figure 3) (Table 2). In this study, Erz strain was found to be most effective compared to other strains according to average death times and graphs (Table 3) (Figure 2).

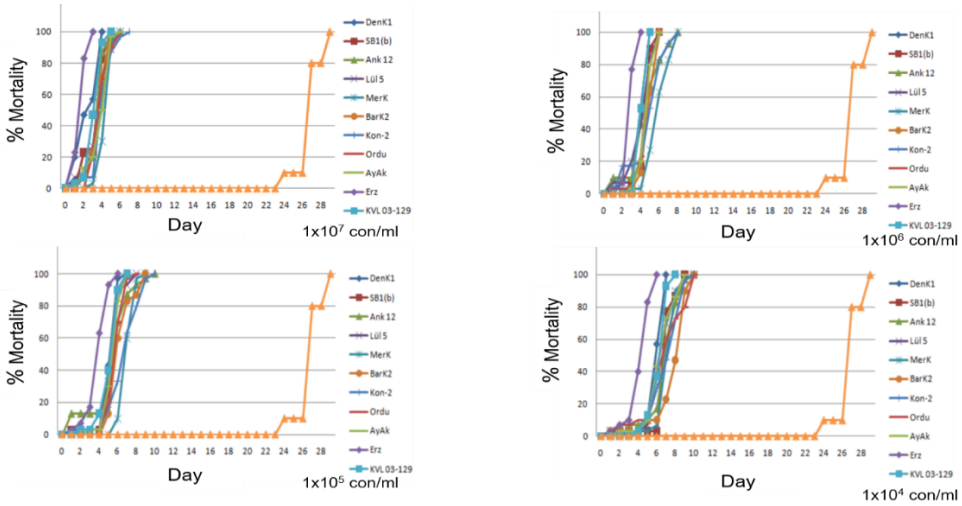

FIGURE 3. It was observed that different doses of 10 *Beauveria bassiana* strains and KVL 03-129 which was a type species, to *Galleria mellonella* on time-dependent mortality rate graphic**.**

TABLE 3. Average death times of used different doses of *Beauveria bassiana* strains and KVL03-129 which was a type species, applied to *Galleria mellonella* larvae.

Fungal Strain	Applied Dose (con/ml)	Average death times (day)	Standard Deviation
DenK1	$1x10^7$	2,80	1,22
	$1x10^6$	4,30	0,92
	$1x10^5$	5,52	0,85
	$1x10^4$	6,33	0,66
SB1(b)	$1x10^7$	3,70	1,02
	$1x10^6$	4,87	0,68
	$1x10^5$	5,71	1,04
	$1x10^4$	7,20	1,21
Ank 12	$1x10^7$	3,90	0,80
	$1x10^6$	4,83	1,78
	$1x10^5$	5,77	2,20
	$1x10^4$	7,10	1,85
Lül 5	$1x10^7$	3,97	1,10
	$1x10^6$	4,40	1,30
	$1x10^5$	5,70	1,06
	$1x10^4$	6,80	1,58
MerK	$1x10^7$	4,73	0,64
	$1x10^6$	6,13	1,41
	$1x10^5$	7,33	0,71
	$1x10^4$	7,44	1,45
BarK2	$1x10^7$	3,97	0,96
	$1x10^6$	5,20	0,76
	$1x10^5$	6,57	1,20
	$1x10^4$	8,07	1,32
Kon-2	$1x10^7$	4,40	1,13
	$1x10^6$	5,17	1,70
	$1x10^5$	6,97	1,77
	$1x10^4$	7,37	1,56
Ordu	$1x10^7$	4,17	0,91
	$1x10^6$	4,87	0,97
	$1x10^5$	6,03	1,07
	$1x10^4$	7,17	2,13
AyAk	$1x10^7$	4,20	1,10
	$1x10^6$	4,97	0,72
	$1x10^5$	5,80	0,85
	$1x10^4$	6,77	1,46
Erz	$1x10^7$	1,93	0,64
	$1x10^6$	3,17	0,53
	$1x10^5$	4,17	1,05
	$1x10^4$	4,57	1,14
KVL 03-129	$1x10^7$	3,55	0,85
	$1x10^6$	4,47	0,51
	$1x10^5$	5,50	1,04
	$1x10^4$	6,53	0,89
Control	0	27,1	1,32

TABLE 4. LT_{50} values of used different doses of *Beauveria bassiana* strains and KVL03-129 which was a type species, applied to *Galleria mellonella* larvae

Fungal Strain	Applied Dose (con/ml)	LT_{50} (day)*	Standard Eror	% 95 Confidince Limits
DenK1	$1x10^7$	3	0,15	2,69-3,30
	$1x10^6$	4	0,28	3,43-4,56
	$1x10^5$	6	0,06	5,88-6,12
	$1x10^4$	6	0,19	5,62-6,37
SB1(b)	$1x10^7$	4	0,22	3,55-4,44
	$1x10^6$	5	0,07	4,85-5,14
	$1x10^5$	6	0,20	5,83-6,16
	$1x10^4$	7	0,26	6,77-7,22
Ank 12	$1x10^7$	4	0,09	3,81-4,18
	$1x10^6$	5	0,35	4,29-5,70
	$1x10^5$	6	0,20	5,60-6,39
	$1x10^4$	7	0,26	6,48-7,51
Lül 5	$1x10^7$	4	0,21	3,58-4,41
	$1x10^6$	5	0,16	4,67-5,32
	$1x10^5$	6	0,10	5,80-6,19
	$1x10^4$	7	0,31	6,39-7,61
MerK	$1x10^7$	5	0,07	4,85-5,14
	$1x10^6$	6	0,23	5,53-6,46
	$1x10^5$	7	0,17	6,65-7,34
	$1x10^4$	8	0,13	7,74-8,25
BarK2	$1x10^7$	4	0,17	3,65-4,34
	$1x10^6$	5	0,17	4,65-5,34
	$1x10^5$	6	0,18	5,63-6,37
	$1x10^4$	9	0,12	8,75-9,24
Kon-2	$1x10^7$	4	0,35	3,30-4,70
	$1x10^6$	6	0,22	5,56-6,43
	$1x10^5$	7	0,28	6,43-7,56
	$1x10^4$	8	0,29	7,41-8,58
Fungal Strain	Applied Dose (con/ml)	LT_{50} (day)*	Standard Eror	% 95 Confidince Limits
Ordu	$1x10^7$	4	0,17	3,65-4,34
	$1x10^6$	5	0,12	4,74-5,25
	$1x10^5$	6	0,16	5,67-6,32
	$1x10^4$	7	0,36	6,27-7,72
AyAk	$1x10^7$	5	0,08	4,83-5,16
	$1x10^6$	5	0,12	4,76-5,23
	$1x10^5$	6	0,12	5,75-6,24
	$1x10^4$	7	0,26	6,48-7,51
Erz	$1x10^7$	2	0,11	1,77-2,22
	$1x10^6$	3	0,11	2,78-3,21
	$1x10^5$	4	0,18	3,63-4,36
	$1x10^4$	5	0,15	4,69-5,30
KVL 03-129	$1x10^7$	4	0,09	3,80-4,19
	$1x10^6$	4	0,19	3,62-4,37
	$1x10^5$	6	0,10	5,78-6,21
	$1x10^4$	7	0,08	6,84-7,15
Control	0	27	0,31	26,39 – 27,61

* The time it takes for 50% of the larvae to die

Moreover, LT_{50} values that obtained in the Kaplan-Meier test (Table 4) and LD_{50} values that obtained through the EPA probit analysis program (Table 5) support this result. Statistical analysis also showed that Erz was significantly different from Ank12, MerK and Kon - 2 strains ($P<0.05$), but not different from other strains ($P>0.05$).

TABLE 5. LD_{50} values of used *Beauveria bassiana* strains and KVL03-129 type species, were calculated according to their potential to kill *Galleria mellonella* larvae on the 5th day

Fungal Strain	LD_{50} (Killing Dose)	95% Confidince Limits
DenK1	$8,6x10^4$	$5,2x10^4$ - $1,4x10^5$
SB1(b)	$1,9x10^5$	$1,1x10^5$ - $3,1x10^5$
Ank 12	$2,8x10^5$	$1,4x10^5$ - $5,4x10^5$
Lül 5	$1,8x10^5$	$1x10^5$ - $3,3x10^5$
MerK	$1,7x10^6$	$9,2x10^5$ - $3,7x10^6$
BarK2	$3,4x10^5$	$1,5x10^5$ - $6,3x10^5$
Kon-2	$7x10^5$	$3,4x10^5$ - $1,5x10^6$
Ordu	$3,1x10^5$	$1,6x10^5$ - $6,1x10^5$
AyAk	$2x10^5$	$1,1x10^5$ - $3,8x10^5$
Erz	$6,5x10^2$	0,00 - $4,5x10^3$
KVL 03-129	$8,5x10^4$	$5x10^4$ - $1,4x10^5$

Within ten days, all strains caused high levels of white muscardine disease in every dose. LT_{50} values of Erz and DenK1 strains were found to be lower (effective in a shorter time) than standard strains and LT_{50} values of Merk and AyAk strains were found to be higher at a dose of $1x10^7$ con/ml. At a dose of $1x10^6$ con/ml, LT_{50} value of the Erz strain was also found to be lower than the standard strain. At this dose, the LT50 value of the DenK1 strain and the standard strain were the same, while other strains had a longer LT_{50} value than the standard strain. At this dose, the DenK1 strain had the same LT_{50} value as the standard strain, while other strains had a higher LT_{50} value than the standard strain. Kon-2 strain was one of the strains with the highest LT_{50} value at this dose. LT_{50} value of the Erz strain was low compared to the standard strain at dose of $1x10^5$ con/ml, while the Merk and Kon-2 strains were high. At dose of $1x10^4$ con/ml, LT_{50} value of Erz and Denk1 strains were low compared to the standard strains, while those of Merk and BarK2 and Kon-2 strains were high. It was found that the effectiveness of all fungal strains were not statistically different between them compared to the standard strains (Table 4) ($P>0.05$).

3. Discussion

Sensitive insects such as *G. mellonella* and *Tenebrio molitor* Linnaeus (Coleoptera: Tenebrioidae) were used in isolation of entomopathogenic fungus strains from soil [29,31,32,45]. In our study, *G. mellonella* was preferred as the target organism due to the fact that *G. mellonella* was well known the parasite of bee's wax so it had more devastating economical effect for the beekeeping industry [46,79,80,81,82].
Insect bait method was first developed for detection of entomopathogenic nematodes using *Galleria* larvae in the soil by Akhurst *et al.* and then, Zimmermann (1986) and Mietkiewski *et al*. stated that this method was also successful in the isolation of entomopathogenic fungi [42,47,83,84]. Keller *et al.* (2003) stated in a study that this method was more effective [37]. Meyling (2007) stated that the insect bait method was more advantageous than the soil dilution method. He stated that entomopathogenic fungi became difficult to find due to the use of fewer soil samples in soil dilution method; the number of spores decreased further by dilution; and that the reproduction in the medium of other opportunistic saprophytic fungi put this method at a disadvantage. Using more soil in insect bait method made this method more selective for isolation of entomopathogens [33]. Kessler *et al.* (2004) also emphasized in their study that insect bait method was a more sensitive method than the soil dilution method [38]. Dhoj *et al.* (2008) attempted to isolate entomopathogenic fungi using the insect bait method and soil dilution method from 46 soil samples they collected from Nepal between 2002 and 2005. As a result, they isolated 78 entomopathogenic fungi samples by insect bait method and 5 entomopathogenic fungi samples by soil dilution method [50]. Our findings were similar to those of these researchers. On the other hand, Imoulan *et al.* (2019) and Gürlek *et al.* (2018) isolated entomopatojenic fungus by soil dilution method [85,86]. In our study, the number of fungi isolated by insect bait method was found to be greater than the number of fungi isolated by soil dilution method. Those findings and our result showed us that insect bait method could be more precise method.

The soil is the classical place of isolation for entomopathogenic fungi [45,86] and many entomopathogenic fungi can be found in both agricultural soil and natural habitats [21,37,44,87,88]. In this study, 48 *B. bassiana* and 1 strain *B. brongniartii* were isolated and *Metarhizium spp.* was not isolated. This result showed us that there was not *Metarhizium spp.* in the soils that we studied. In addition, Sahin (2006) stated

that he isolated 19 *B. bassiana* strains from soil samples collected from Kahramanmaraş province, but failed to isolate *Metarhizium spp.* [89]. Our findings were similar to those of these researcher. But, Sevim *et al.* (2010) stated that they isolated 62 entomopathogenic fungus strains from 301 soil samples they collected from their research sites by *Galleria* bait method and that of these 62 entomopathogenic fungi, the species of *Metarhizium* genus was the most abundant [90]. Moreover, Keppanan *et al*. (2018) stated that entomopathogenic fungi of the genus *Metharizium*, genus *Beauveria* and genus *Isaria* were isolated from agricultural areas of Thekkady region of India by the *Galleria* bait method [55].

In this study, 28 of the fungi were isolated from farmland samples while 21 were isolated from uncultivated soil samples. In other studies, entomopathogenic fungi were found in different habitats in different countries at different rates. Sevim *et al.* (2010) stated that they isolated entomopathonic fungus both from agricultural and nonagricultural soils [90]. Meyling and Eilenberg (2006) found that *B. bassiana* was widespread in soils in agricultural areas of Denmark, while *M. anisopliae* was rare [87]. This conclusion was consistent with our findings. However, Sun *et al*. (2008) showed that insect pathogen fungi were more likely to be found in orchard soils than in farmland soils, and Vanninen (1995) noted that in Finland, insect pathogen fungi were more likely to be found in forest soils than in agricultural soils [91,92]. Imoulan *et al.* (2019) found that *Beauveria* species were recovered more frequently from forested soils than from farmland [86]. As a result of all these studies, it was understood that entomopathogenic fungi may have different proportions in farmland and uncultivated soils.

The reason *B. bassiana* was more isolated in insect bait method than *B. brongniartii* may be that *B. bassiana* infected and killed larvae more quickly. Keller *et al.* (2003) noted that they isolated *M. anisopliae* strain more than *B. brongniartii* strains by insect bait method. They stated that the cause of this condition was *M. anisopliae* could kill *Galleria* larvae faster than *B. brongniartii* [37]. Also, Keppanan *et al*. (2018) found that *M. anisopliae* infected *Galleria* larvae used in the insect bait method faster than other entomopathogenic fungus species [31]. This findings may explain why *B. bassiana* was more isolated by insect bait method than *B. brongniartii* in our study. Chase *et al.* (1986) found that 0.55 g/l dodine in the medium only allowed optimal isolation of *B. bassiana*, while reducing the amount of dodine to 0.46 g/l allowed isolation of *B. bassiana* as well as other entomopathogenic fungal strains [62]. In this study, *B. brongniartii* was isolated alongside *B. bassiana* by adding 0.46 g/l dodine to the SDA medium and this result was in harmony with the findings of Chase *et al.*

In this study, isolated *B. bassiana* strains were tested on *Galleria* larvae before the experiment was initiated, and infective 10 fungal strains were selected from among these strains. In the experiment, 4 different doses of these strains and the type species were used, and a control group was formed to determine whether Tween 80 solution had an effect on the survival of *Galleria* larvae and to show that uninfected larvae were healthy compared to those exposed to the doses. The dose range used in such studies usually varies depending on the host and the manner of administration, but quantities between $1x10^4$ - $1x10^8$ con/ml were used for every 10 insects. Furthermore, in such experiments, the purpose of testing different doses on the target organism was to observe small changes in virulence [17,23,31,44,93,94].

The standard strain used in this study, KVL 03-129 (ARSEF 8032), was a strain from the ARS entomopathogenic fungus collection and was isolated from agricultural region soil using *Galleria* bait method by Nicolai V. Meyling on September 18, 2002. In this study, entomopathogenic fungus strains isolated from Turkey soil were isolated by the same method, resulting in a healthy result in pathogenicity experiments. Because in biological control studies, strains isolated from homologous hosts were more successful in using entomopathogenic fungi as a biological control agent [23,85,95,96]. In addition, while it was common to search for the same origin agent as the target organism in biological control studies, successful controls were also obtained with natural enemies that did not come from the natural area of the target pest [23,31,85]. Klingen *et al.* (2002) observed in a study that the *M. anisopliae* strain (ARSEF 5520) was highly pathogenic to *G. mellonella*, but was not pathogenic to *Delia floralis* (Fallen) (Diptera: Anthomyiidae) larvae, although it was an isolated strain using *D. floralis* in bait method [44].

All tried doses of these 10 strains and standard strains were found to be highly successful on *Galleria* larvae compared to the control group. Klingen *et al.* (2002) experimented with a dose of $1x10^7$ con/ml of 3 different strains of *B. bassiana* on *Mamestra brassicae* (Linnaeus) (Lepidoptera: Noctuidae) and indicated that these strains were highly successful compared to the control group [44]. Trudel *et al.* (2007) tested the doses of $1x10^4$, $1x10^5$, $1x10^6$, $1x10^7$, $1x10^8$, $1x10^9$ and $1x10^{10}$ con/ml of 6 different *B. bassiana* strains on *Pissodes strobi* Champion & G.C. (Coleoptera: Curculionidae) and obtained a successful mortality result according to the control group [16]. Özdemir *et al.* (2020) tested the dose of $1x10^6$ and $1x10^8$ con/ml of *B. bassiana* and *M. anisopliae* on *Callosobruchus maculatus F.* (Coleoptera: Chrysomelidae: Bruchinae) at two different tempreture (22 – 26 ^{0}C) [97]. They obtained a successful mortality result too. Our findings were similar to those of these researchers.

In this study, the highest mortality in the shortest period of time of all fungal strains tried on *G. mellonella* was achieved by the application of the highest dose ($1x10^7$ con/ml) of each entomopathogenic fungus strains. Kim and Kim (2008) experimented with 5 different doses of the *Lecanicillium attenuatum* Zare & W.Gams (Hypocreales: Cordycipitaceae) strain, was a entomopathogenic fungus, on cotton aphids and found that the most effective dose was 10^8 con/ml [98]. Santoro *et al.* (2008) experimented with various strains of *B. bassiana* on *Alphitobius diaperinus* Panzer (Coleoptera: Tenebrionidae) and they also stated that the most effective dose was $1x10^8$ con/ml [99]. Yücel *et al.* (2018) experimented 4 different doses ($1x10^5$, $1x10^6$, $1x10^7$, $1x10^8$ con/ml) of *B. bassiana* strain, which was isolated from mycosed larvae and adults of *H. postica* in Adana and Iğdır, on *Hypera postica* (Gyllenhall) (Coleoptera: Curculionidae) and found that its efective dose was $1x10^8$ con/ml [100]. These results were consistent with our findings. It was understood from these findings that the high concentration of the entomopathogenic fungus to be used as a biological control agent could give a better result. However, in biological control studies conducted by other researchers, it was stated that the high dose of different entomopathogenic species applied on harmful insects was low in virulence [101,102]. The virulence of the entomopathogenic fungus was stated by researchers that it not only depends on the strain of the fungus applied, but also on the dose, formulation and frequency of the dose applied [101].

As a result of the observations made during the experiments, LT_{50} values and growing time of spores on *Galleria* larvae were decreased due to the increase in concentration. Vu *et al.* (2008) stated that as conidial concentration increased, LT_{50} value decreased [103]. Luz *et al.* (1999) noted that entomopathogenic fungal spores applied to insects grow later and occur in smaller amounts on cadavers due to a decrease in concentration [77]. In experiments conducted by different researchers on the effectiveness of different doses of enomopathogen fungus strains on *G. mellonella* larvae, they emphasized that the higher concentration applied to larvae caused the lower LT_{50} value [104,105]. These findings were in line with our conclusions. It was understood from these findings that the virulence of different fungus species and different strains of the same fungus species could vary against the same insect pest.

So far as LD_{50} and LT_{50} results, it was found that the most effective strain was Erz strain and the weakest strain was MerK strains (Table 3-4). According to the results of the highest concentration application of all fungal strains, Erz and DenK1 strain were observed to kill 50% of *Galleria* larvae at the end of the second and third day (Table 4). As noted in many biological control application studies, this was

considered a successful outcome [106,107,108]. It was also observed that DenK1 strain was more effective at the highest and lowest doses than the standard strain (Table 4). The weakest strain at the lowest dose was the BarK2 strain (Table 4). However, statistical studies were shown that the isolated strains and the standard strains did not show a significant difference in their pathogenic effects against *G. mellonella* larvae or their potential as a biological control agent. This result was consistent with the results obtained by other researchers. In their study, they found that there was no significant difference between the mortality outcomes of different strains of *B. bassiana* tried on *G. mellonella* larvae [109]. But in another study, different isolates of each of the 4 species of entomopathogenic fungi, including *B. bassiana*, resulted in very different mortalities in the host organism being tried [57]. Ultimately, it was observed that these 10 *B. bassiana* strains attempted and the standard strain achieved successful mortality on *G. mellonella* larvae. According to statistical tests, the highest dose tried on larvae was found to provide the most successful control in each strain. This conclusion was paralleled with the results of studies conducted by other researchers [60,106,108,]. The effects of fungal strains were found not to be statistically significant, but overall the most successful strain was found to be Erz strain and the most unsuccessful strain was MerK strain.

However, these studies were carried out under laboratory conditions at a constant temperature, high humidity and dark conditions. These factors had positive or negative effects on the pathogenesis of entomopathogenic fungi, depending on the situation. Because pathogenicity depended not only on biochemistry, physiology, and molecular biology of disease development of the entomopathogenic fungus, but also on the environment. Relative humidity, UV rays, temperature and nutrient availability in these factors affected the performance of a successful mycoinsecticid [21,110]. Many researchers noted that UV rays render the conidias of entomopathogenic fungi inactive [110,111]. In addition, Wojda *et al*. (2009) stated that *G. mellonella* larvae were colony-dwelling insects, and when these larvae came together, the ambient temperature rised to 40 °C, thus providing protection against pathogens. This temperature had a negative effect on the development of fungal infection [112].

In later studies, it was aimed to look at how environmental conditions and insect behavior have an effect on the pathenities of *B. bassiana* strains that were tried on *Galleria* larvae. It was also thought that studying the effects of these strains on different pest insects could create useful data for subsequent biological control studies.

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ALTHENIA ORIENTALIS (POTAMOGETONACEAE) IN TURKEY: HABITAT CONDITIONS, MORPHOLOGY AND ANATOMY

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ABSTRACT. *Althenia* F.Petit is a submerged aquatic plant genus, which is represented by two species in the world. *Althenia* species are found mainly in brackish water lagoons and in salt lakes. The genus has only one species in Turkey, *Althenia orientalis* (Tzvelev) García-Mur. & Talavera which was collected from Turkey in 1959 and published in 1975. Since then, it could not be collected again and was assessed as extinct in Turkey. We performed field studies to collect the species in May 2017 and June 2017. We managed to find the species in its original locality. We measured chemical and physical properties of the water bodies it is growing. Additionally, we provide the stem anatomy of the species based on Turkish material for the first time. The single locality of the species in Turkey is a hypersaline lagoon lake (Lake Tuzla, Adana). Only dense *Ruppia maritima* L. population accompanies the species. *Althenia orientalis* grows in slightly alkaline and warm waters. The population size is small, covering approximately 500 m^2 area. The plants future presence is threatened by severe human pressure (i.e. pollution due to construction waste deposits and dense agricultural activities).

The genus *Althenia* F.Petit was originally included in Zannichelliaceae Chevall. family [1-2]. However, all genera in the family were transferred to Potamogetonaceae Bercht. & J.Presl [3-4]. Recent molecular phylogenetic studies also support Zannichelliaceae as a synonym of Potamogetonaceae [5]. On the other hand, Talavera and Garcia-Murillo [6] continue to treat it under the family Zannichelliaceae. The genus *Althenia* was first described from France with the type species as *A. filiformis* F.Petit [1]. For a long time, the genus was known mainly from the Mediterranean basin ranging from Morocco, Algeria, Portugal, Spain, Baleares,

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Greece, and Sicily to Turkey. Later, new records were found from southern Russia and southern Iran [7-8]. Therefore, *Althenia* is considered to be a Eurasian-African genus. According to Garcia-Murillo and Talavera [9] *Althenia* s.str. consists of two species: *A. filiformis* and *A. orientalis* (Tzvel.) Garcia-Mur. & Talavera. They also described another subspecies: *A. orientalis* subsp. *betpakdalensis* (Tzvelev) García-Mur. & Talavera. However, Talavera and Garcia-Murillo [6] suggested that two subspecies were ecotypes and can be found in same ponds. Some authors proposed a broader concept for the genus *Althenia.* Ascherson and Graebner [2] treated *Althenia* and closely related genus *Lepilaena* J.Drum. ex Harv. as two sections of the genus *Althenia*. Recent studies by Macfarlane et al. [5] and Ito et al. [10] also suggested inclusion of all the previously defined *Lepilaena* species within *Althenia* sensu lato (s.l.) based on their molecular and morphological research.

It was given the species status to *A. orientalis* in 1986 [9]. Both *A. orientalis* and *A. filiformis* are monoecious plants with male flowers having two sporangiate anthers. Female flowers have long styles and peltate stigmas [11]. The leaf sheaths of *A. filiformis* are with conspicuous nerves and the leaf blade with one lateral nerve on each side of the midvein. On the other hand, the leaf sheaths of *A. orientalis* are without nerves and also leaf blade does not have conspicuous lateral nerves [5].

The genus *Althenia* is a typical brackish aquatic plant growing in waters close to sea and rarely found in continental salt lakes. It can occasionally be found in freshwaters, too. *Althenia* species are under collected due to several difficulties encountered during collection. One reason is that the color of the plant is very similar to substrate color. The other important reason for collection difficulty is the species sporadic occurrence which makes it sometimes unable to collect from the same locality every year [12]. Additionally, most of the time *Althenia* species coexists with *Ruppia* species, which are usually dominant in the vegetation. Therefore, it becomes difficult to distinguish *Althenia* species among dense *Ruppia* vegetation. The presence of such dense *Ruppia* populations also effects the growth of *Althenia* species negatively due to increased competition for nutrients and light. Kipriyanova and Romanov [8] also indicated that *A. orientalis* has a narrow ecological niche and is a poor competitive plant. Therefore, they suggested that it should be included in the Red Data Book of Novosibirsk Region as a rare species.

Herbarium specimens belonging to *A. orientalis* was first collected from Turkey by E. Hennipman in 1959. However, the occurrence of the species in Turkey was only published later by Hartog [13] as *A. filiformis*. In The Flora of Turkey [7], it was listed as *A. filiformis*. However, Güner et al. [14] in the Checklist of the Flora of Turkey, treated it as *A. orientalis*. Nevertheless, the species has been never collected

again after its initial record from Turkey. Later, as a result of comprehensive surveys in the Çukurova Delta region, Çakan et al. [15] assessed *A. orientalis* as an extinct species in Turkey. Therefore, it was very important to rediscover the species in Turkey and to determine the state of its population.

Consequently, in this study our aims are 1) to confirm the presence of *A. orientalis* in Turkey, (2) to evaluate the status of its population in Turkey, (3) to find the physical and chemical features of its habitat, (4) to prepare a new description of the species based on Turkish material and to study the stem anatomy of the species.

2. Materials and Methods

2.1. Herbarium specimen collection and limnological measurements

Field studies were performed in saline lake, Lake Tuzla in May 2017 and June 2017 (Figure 1). Herbarium specimens were collected and stored in AIBU Herbarium. Ten environmental variables were measured with a YSI-Professional Plus. Measured environmental variables are: Dissolved oxygen concentration (mg l^{-1}), percent oxygen saturation (%DO), water temperature (Tw, °C), electrical conductivity (EC, µS cm^{-1}), specific conductivity (SPC, µS cm^{-1}), pH, atmospheric pressure (mmHg), total dissolved solid (g l^{-1}), salinity (ppt) and ammonium (mg l^{-1}). Geographical data (elevation and coordinates) were recorded by using Magellan eXplorist 610.

2.2. Anatomical studies

Samples were taken to represent all parts of the plants and preserved in 70% alcohol solution for diagnosis and subsequent anatomical studies. The internode areas of the stem of the specimens were cut about 0.05 mm thick with the aid of razor blades. Samples were put into safranin or toluidine blue dye and transferred to distilled water. Stem fragments were examined under the light microscope at 4x, 10x and 40x magnifications. The stele types, shape of endodermal cells, pseudohypodermis, presence of subepidermal bundles and interlacunar bundles in the cortex were determined and photographed.

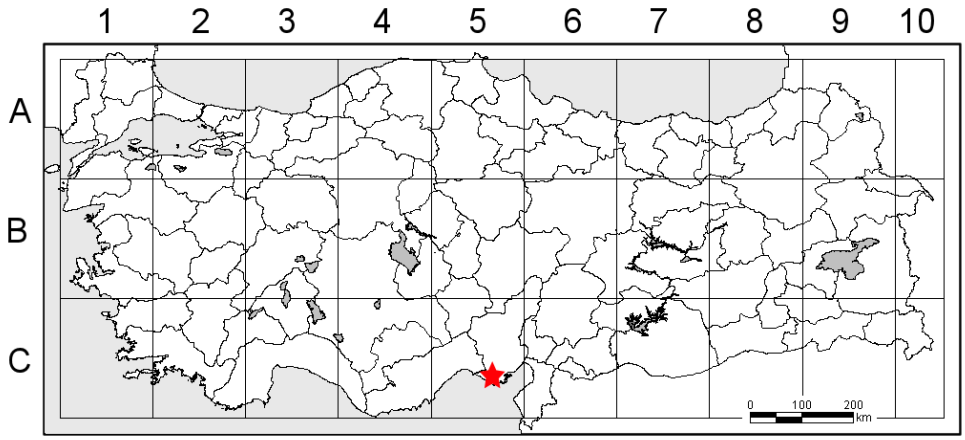

FIGURE 1. Collection site of *A. orientalis*, Lake Tuzla, Adana.

3. RESULTS

3.1. Taxonomy

Althenia orientalis (Tzvelev) García-Mur. & Talavera in Lagascalia 14: 108 (1986). Type: "Altaj occidentalis, pag. Aul, lacus amare salsus Gorczini, 26 VI 1921, V. Veresczagin" — (LE).
Sin: *Althenia filiformis* subsp. *orientalis* Tzvelev in Bot. Zhurn. (Moscow & Leningrad) 60: 390 (1975).
A. filiformis subsp. *betpakdalensis* Tzvelev in Bot. Zhurn. (Moscow & Leningrad) 60: 390 (1975).
A. orientalis subsp. *betpakdalensis* (Tzvelev) García-Mur. & Talavera in Lagascalia 14: 112 (1986).

Description:

Annual or perennial, rhizomatous aquatic plants. Stem slightly branched; mostly pale green, up to 15 cm. Leaves sessile, all submerged, alternate, entire, pale green, linear to broadly lanceolate, 1 veined, midrib without lacunae, 17-31 x 0.1-0.2 mm. Stipules adnate to leaf base, persistent, conspicuous, with auricles, c. 2 mm. Flowers unisexual, axillary; female flowers with 3 perianth segments. Fruit stipitate, c. 1.4-1.6 mm, ellipsoid, beak equal to fruit or longer.

3.2. Anatomy

Stem anatomy photographs of the species are given in Figure 2. Stele is circular type, endodermis is U-type, interlacunar bundles and subepidermal bundles are absent, and pseudohypodermis is 1-layered.

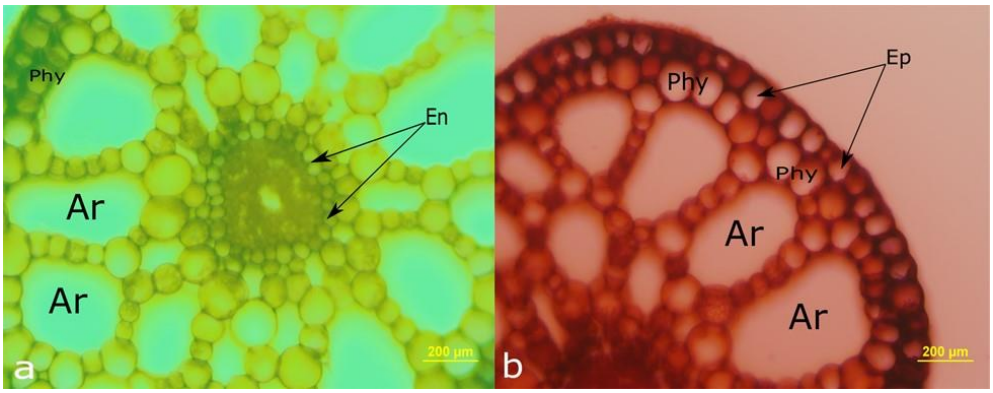

FIGURE 2. Stem anatomy of *A. orientalis* a) General view (4×), b) Epidermis and cortex (10×). Ar: aerenchyma, En: endodermis, Ep: epidermis, Phy: pseudohypodermis.

3.3. Collection Site

Althenia orientalis is a rare plant that has been collected in lagoon lake from Lake Tuzla in Adana (36° 40’ 50’’**N,** 35° 4’ 47’’**E)** in June 2017 in shallow waters (approximately 10-30 cm deep). Living fresh plant population size was small, covering a small area at the edge of the lake (Figure 3a). Additionally, plant remains in seed were observed in parts of the lake where the water dried up completely (Figure 3c, 3d). Total area of the population is approximately 500 m^2. Dense *Ruppia maritima* population coexists with *A. orientalis* (Figure 3b). There is too much agricultural activity around the lake. Freshwater coming from the fields surrounding the Lake via canals changes the salinity of the lake (Figure 4b).

FIGURE 3. a) Habitat of *A. orientalis.* b) *A. orientalis* with *Ruppia maritima* L. in lake Tuzla (Adana). Arrows shows *A. orientalis.* c) *A. orientalis* in dried up aquatic habitat. d) Inflorescence of *A. orientalis.*

FIGURE 4. a) Fresh material of *A. orientalis* (rhizomatous) b) Freshwater canals coming from agricultural fields.

3.4. Water chemistry measurements

Althenia orientalis was collected in slightly alkaline (pH = 8.05) and warm waters (water temperature = 34.3 °C). Salinity level and ammonium ion concentration were extremely high (Table 1).

TABLE 1. Water chemistry measurements for *A. orientalis* (see materials and methods for abbreviations).

Alt. (m)	**Tw** (°C)	**%DO**	**DO** (mg l^{-1})	**EC** (µS cm^{-1})	**TDS** (g l^{-1})	**Salinity** (ppt)	**pH**	**Ammonium** (mg l^{-1})
11	34.3	112	6.53	63041	34.7755	35.04	8.05	33.3

4. DISCUSSION

The submerged aquatic plant *A. orientalis* was first collected from Turkey in 1959 and published in 1975 [13] as *A. filiformis*. Since then, the species could not be collected again and was thought to be regionally extinct in Turkey [15]. In 2017, during our field works, we manage to find the species in its original habitat. The species grows in a single aquatic habitat in Turkey, which is a lagoon at the Eastern Mediterranean coast (Lake Tuzla, Adana). When we analyze the stem anatomy of *A. orientalis*, we see that it is similar to *Ruppia maritima*, which occur in the same

habitat [16]. Taxonomically closest genus to *Althenia* is *Zannichellia* L. in Turkey. *Zannichelia palustris* L. is the only representative of this genus in Turkey. We observed that in both species (*A. orientalis* and *Z. palustris*) interlacunar and subepidermal bundles are absent. However, some *Potamogeton* L. and *Stuckenia* Börner species have interlacunar or subepidermal bundles. We have observed only circular stele type in *A. orientalis* but four different types of stele can be seen in other *Potamogeton* species growing in Turkey [6, 16-17]. The habitat of *A. orientalis* had the most extreme values for the recorded water chemistry parameters compared to other Potamogetonaceae species in Turkey [18]. These values were for water temperature (34.3 °C), salinity (35.04 ppt), electrical conductivity (EC, 63041 μS cm^{-1}) and ammonium ion concentrations (33.30 mg l^{-1}). High evaporation rates during certain periods of the year cause these values when no freshwater is received via irrigation canals. The species grows in slightly alkaline waters (pH = 8.05). We recorded only *Ruppia maritima* as accompanying species in the lake abundantly. Two species co-occur most of the time together in the other Mediterranean countries and in Russia [12-8]. Kipriyanova and Romanov [8] found the species in a hyperhaline lake in Russia where its habitat is subjected to complete drying out during certain years. They also stated that the species is an ancient Mediterranean relict with a possible Paleogene origin.

According to Conde-Álvarez et al. [19], *A. orientalis* grows in shallow and temporary hypersaline water bodies where *Ruppia* sp. and *Chara* sp. accompany it. Although *A. orientalis* is very rare in the Iberian Peninsula, its habitat is unprotected because *A. orientalis* and *Ruppia maritima* grow in brackish habitats [20]. Casha and Mifsud [21] indicated that *A. orientalis* can tolerate broad salinity variations and can survive under hypersaline and drought conditions during the summer months. They reported that it produces drought resistant seeds and observed it as being annual in Malta Island. In the Turkish population, we collected both rhizomatous perennials and annual plants in seed under complete dried up conditions (Figure 3 and Figure 4).

Althenia orientalis was assessed as Data Deficient (DD) by IUCN [22]. There is no clear information about IUCN category of *A. orientalis* mainly due to confusion with *A. filiformis*. Some authors accept monotypic treatment of the genus *Althenia* [12]. Therefore, it is difficult to define the true range of each species and their red list categories. There are possible risks for *A. filiformis*. These possible risks are similar for *A. orientalis*, too. *Althenia orientalis* has a distribution in Eurasia, extending from North Africa, all the Mediterranean basin to Siberia. However, it is a rare species throughout its distribution area [8, 22].

As stated above, *A. orientalis* is known only from a single site in Turkey (Figure 1). The size of the population is small, covering roughly 500 m^2 area at the one edge of Tuzla lake. The habitat of the species is surrounded by agricultural fields. In addition to excess nutrient loading from these agricultural fields, excess freshwater from the irrigation canals alters the salinity of the lake. The area is also close to a region of rapid urbanization and tourism activities which can also effect the species population. Additionally, construction wastes create a pollution around the lake. We can also expect climate warming to change the population of *A. orientalis* [23]. Possible effects of climate change will be increasing temperatures, decrease in snow and ice cover, rising sea levels, earlier arrivals of spring, droughts and upward migration of vegetation zones [24]. Some of these factors will have a direct impact on the population of *A. orientalis*. Additionally, in Turkey the Mediterranean region will be among the most affected regions where especially temporary and shallow ponds and lakes will be subjected to change. In addition to increased temperatures, there will be a decrease in annual precipitation in the Mediterranean region of Turkey [24]. Due to all these reasons, the habitat of the species is under several threats. Precautions should be taken to protect the species and its habitat.

As a conclusion, our study confirms the presence of *A. orientalis* in Turkey and provides valuable information about its population size, habitat properties and morphological and anatomical characters of the samples from Turkey. Data provided here can be used for future monitoring of this rare species.

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THE FISH FAUNA OF A KARST SPRING; ÖZLEN STREAM (ANTALYA, TURKEY)

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ABSTRACT. Most of the ichthyological research in Turkish freshwaters are focused on large rivers. However, recent evidence indicated that local endemics and/or threatened fish species may be trapped in small springs such as karst-fed streams. In small karst-fed streams the downstream end of the underground flow reaches the surface and drain to the sea in a very short distance. Thus, fish fauna in such water bodies should be investigated for the presence of any threatened fish species. In this study, the fish fauna of a karst-fed small stream, Özlen Stream, where no ichthyological information exist, was investigated on a seasonal basis. A total of 8 fish species (*Anguilla anguilla*, *Carassius gibelio*, *Squalius fellowesi*, *Oncorhynchus mykiss*, *Gambusia holbrooki*, *Mugil cephalus*, *Liza saliens* and *Salaria fluviatilis*) were identified. *C. gibelio*, *O. mykiss* and *G. holbrooki* are exotic and invasive fish species for Turkish freshwaters. Furthermore, *C. gibelio* and *G. holbrooki*, are considered a serious threat to natural fish populations in Turkey. Presence of such invasive fish species even in a small river indicates that they are expanding their distribution area in Turkey.

1. INTRODUCTION

Turkey has a very unique freshwater fish fauna including species originated both from Europe and Asia [1]. According to the latest checklist, the Turkish freshwater fish fauna is composed of 377 species [2].

There has been a growing interest on fisheries and the freshwater fish diversity in Turkey during the last decades [1-6] which significantly contributed to the available knowledge on their distribution, taxonomy and ecology. However, there are still

Keyword and phrases. Karst spring, invasive, Eşen river, mosquito fish

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some gaps particularly on the fish fauna of karst-fed rivers or karst-springs where the downstream end of the underground flow reaches the surface and drain to the sea in a very short distance. Such karst-springs are mainly located in the Southern Anatolia, primarily in Mediterranean Region [3]. Their flow regime depends on the precipitation rates or snow-melt. Thus, some of them can be exposed to drought intermittently [7]. Recent research has shown that local endemics and/or threatened fish species may be trapped in such small springs [6]. Thus, fish fauna in such water bodies should be investigated for the presence of any threatened fish to prepare species conservation plans.

The rivers and streams located in Mediterranean regions are unique habitats in an ecological point of view. The streams in the Mediterranean region (also called med-streams) comprising the highest number of threatened freshwater species in Europe [8] are considered as global hotspots for biodiversity and endemism [9]. Unfortunately, they are very sensitive to environmental alterations resulting from human impacts. For example, in Antalya (Turkey), the drainage of municipal sewage directly to the aquifers feeding the groundwater flow is considered to be a serious concern for drinking water supply. It also creates a major threat to biodiversity in both groundwater-fed freshwaters and the Antalya Bay which may play a role as the final sink for surface runoff and groundwater flows [10]. Investigation and monitoring of the flora and fauna in such streams might brought new insight to the biodiversity and distribution of aquatic organisms. Therefore, the present study aims to represent the freshwater fish fauna of a karst-spring, Özlen Stream (Antalya, Turkey), where no ichthyological information exists on the stream.

2. Materials and Methods

2.1. The study area

The study area, Özlen Stream, is located on the southern west coasts of Antalya (Fig. 1). Özlen Stream is approximately 4 km long and originates from a spring near Karadere village (Kaş, Antalya) and drains into the Mediterranean Sea from the Patara coasts. There is a fish farm located nearby the headwaters of Özlen Stream.

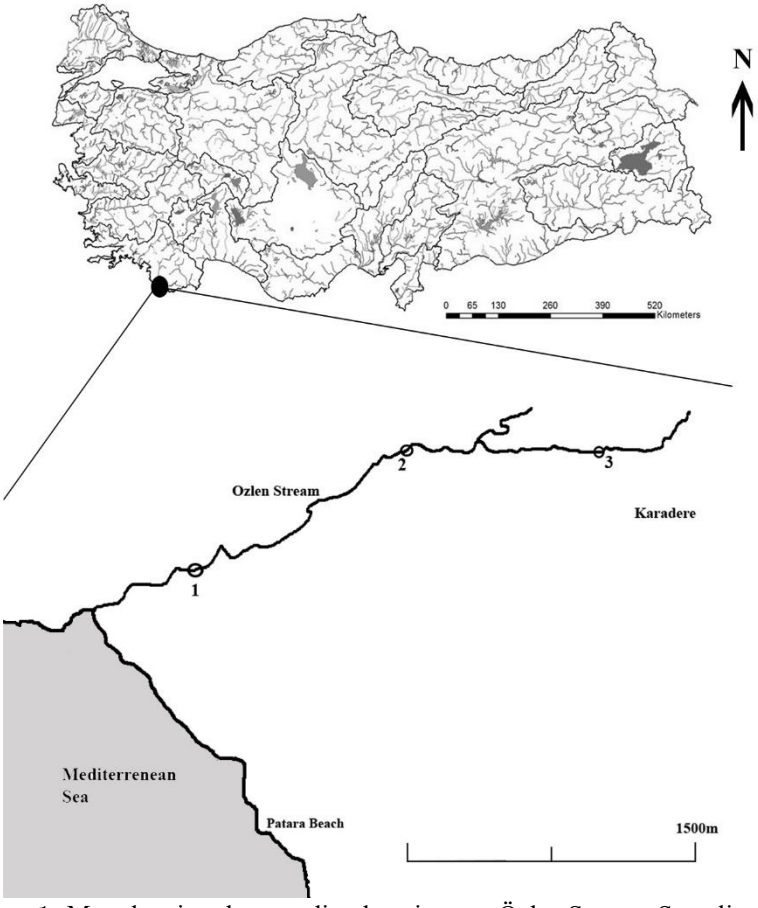

FIGURE 1. Map showing the sampling locations on Özlen Stream. Sampling stations 1: 36° 19.901'N 29° 13.940'E; 2: 36° 20.394'N 29° 14.674'E; 3: 36° 20.423'N 29° 15.394'E

2.2. Fish Sampling and Processing

Fish sampling was carried out by electrofishing with a backpack electrofishing gear (Samus 725-MP) on a seasonal basis from September 2018 to August 2019. Sampling was performed by wading in an upstream direction which included all representative habitats. Fish were caught from 3 different sampling stations on the stream including headwaters and river mouth (Figure 1). Fish specimens were euthanized using an overdose of MS-222 and fixed in formaldehyde solution (10%) and brought to the laboratory for processing. Samples were transferred to 70% ethanol for further processing. The standard length (SL) were measured (nearest to 0.1 mm) with a digital compass. Meristic characteristics including branched and

unbranched rays in dorsal (D), ventral (V), anal (A) and pectoral (P) fins, lateral line scales, the number of gill rakers, pyloric caeca and pharyngeal teeth were also noted with a stereoscopic binocular microscope (Leica EZ4) where needed. Fish were identified according to Geldiay and Balık [11], Banarescu and Bogutskaya [12], Smith and Darwall [13] using metric and meristic characteristics mentioned above.

3. Results

A total of 89 fish specimens which belong to 8 species and 7 families (Anguillidae (1), Cyprinidae (1), Leuciscidae (1), Salmonidae (1) Poeciliidae (1), Mugilidae (2) and Blenniidae (1)) were identified from the Özlen Stream. Morphometric measurements and counts for each fish species are given below. The classification of fishes in the present study is adopted from Eschemeyer's Catalog of Fishes [14].

Family: Anguillidae
Anguilla anguilla (Linnaeus, 1758) (European eel)
Material examined: Özlen Stream (Stations 1, 2, 3) (22.09.2018, 3 specimens; 02.03.2019, 3 specimens, 18.05.2019, 4 specimens; 10.08.2019, 9 specimens; SL: 12.2-27.8 cm).
Diagnostic characteristics: D 243-265, A 178-196, P 17-18, C 9-11.

Family: Cyprinidae
Carassius gibelio (Bloch, 1782) (Prussian carp)
Material examined: Özlen Stream (Station 1) (18.05.2019, 4 specimens; SL: 12.2-15.5 cm).
Diagnostic characteristics: D III-IV 15-17, A III 5, V: II 6, P: I 13, peritoneum black.

Family: Leuciscidae
Squalius fellowesii (Günther, 1868) (Aegean chub)
Material examined: Özlen Stream (Station 3) (10.08.2019, 7 specimens; SL: 8.4-14.2 cm)
Diagnostic characteristics: D III 71/2-91/2, A III 61/2- 91/2, lateral line scales 40-42, gill rakers 7-10, pharyngeal teeth 2.5-5.2.

Family: Salmonidae
Oncorhynchus mykiss (Walbaum, 1792) (Rainbow trout)
Material examined: Özlen Stream (Station 2) (18.05.2019, 1 specimen, SL: 22.4 cm)
Diagnostic characteristics: D: III-IV 10, A: III-IV 9, P: I 12, V: II 9, C: 19, lateral line scales: 139.

Family: Poeciliidae
Gambusia holbrooki Girard, 1859 (Mosquitofish)
Material examined: Özlen Stream (Stations 1, 2) (22.09.2018, 9 specimens; 02.03.2019, 11 specimens, 18.05.2019, 14 specimens; 10.08.2019, 15 specimens; SL: 2.3-5.2 cm)
Diagnostic characteristics: D I-II 6-7, A I-II 7-8, lateral line scales 29-31

Family: Mugilidae
Mugil cephalus Linnaeus, 1758 (Flathead grey mullet)
Material examined: Özlen Stream (Station 1) (22.09.2018, 2 specimens; 10.08.2019, 3 specimens; SL: 15.6-25.6 cm).
Diagnostic characteristics: D1 IV, D2 I 9, A III 8, P 17, V 15, Sq, 42-43, pyloric caeca 2

Liza saliens (Risso, 1810) (Leaping mullet)
Material examined: Özlen Stream (Stations 1) (22.09.2018, 1 specimen; SL: 23.2 cm).
Diagnostic characteristics: D1 IV, D2 I 9, A III 8, P 16, V I 5, Sq 44, pyloric caeca 8.

Family: Blennidae
Salaria fluviatilis (Asso, 1801) (Freshwater blenny)
Material examined: Özlen Stream (Station 2) (22.09.2018, 2 specimens; 18.05.2019, 1 specimen; 10.08.2019, 3 specimens; SL: 2.1-7.8 cm).
Diagnostic characteristics: D XII-XIII, A 18-19, P 13-14, V 3-4.

4. Discussion

Özlen Stream is located very close to Eşen River (150 km long) which has a very large drainage area (1200 km2). Although, there is a partial data on the fish fauna of Eşen River [4], this is the first study reporting the ichthyofauna of a karst-fed small stream, Özlen Stream (~ 4 km long). We observed a direct connection between Özlen Stream and Eşen River via irrigation channels during the field studies. Thus, it is possible to observe similar species in both rivers. A total of 10 fish species have been reported from the downstream regions of Eşen River [4]. *Carassius carassius* reported by Onaran *et al.* [4] seems to be a misidentification. *A. anguilla, S. fluviatilis, Leuciscus cephalus, M. cephalus* and *L. saliens* were also reported by Onaran *et al.* [4]. *L. cephalus* specimens are treated as a different species described as *Squalius fellowesii* according to the latest study by Özuluğ and Freyhof [5]. *Liza ramada* and *Oedalechilus labeo* which reported by Onaran *et al.* [4] were not caught

in the current study. Furthermore, this is the first study reporting the occurrence of *O. mykiss* and *G. holbrooki* in the sampling are. In an another study, 24 fish species have been reported from several streams discharging to Antalya Bay (Mediterranean Sea) [15]. The use of one type of fishing gear to catch fish may have had an effect on the lower abundance of fish in the sampling localities. Combining different fishing methods may decrease the possibility of missing any fish species inhabiting the stream. On the other hand, several physico-chemical and hydromorphological parameters may affect the fish assemblages [6]. Geological characteristics and topographic structures also have a crucial impact on the distribution of fish. However, no such information is available for Özlen Stream.

A total of 8 fish species were identified from the stream. *A. anguilla* was observed throughout the stream course and its abundance was higher in the headwaters of the Özlen stream. This might indicate that the stream continuum is very important for European eel in Özlen Stream. European eel is categorized as Critically endangered (CR) by the International Union of Conservation for Nature (IUCN) [16]. The only report on the fish fauna of Eşen River indicates that European eel also inhabits Eşen river. However, no European eel was caught in the downstream regions of Eşen River during this study (data not given here). Thus, Özlen Stream seems to play a major role in the life cycle of this fish species in the sampling area.

Three non-native and invasive fish species (*O. mykiss*, *C. gibelio* and *G. holbrooki*) were caught during the field studies. There was only 1 specimen of *O. mykiss* which probably escaped from the trout farm located on the headwaters of the stream. There is a debate whether this species naturalized or not in Turkish freshwaters [1, 17]. However, current findings support that rainbow trout cannot sustain its population reproductively in Özlen Stream. *C. gibelio* and *G. holbrooki*, are considered a serious threat to natural fish populations in Turkey. Such invasive fish species, particularly *G. holbrooki*, seems to be expanding its distribution area in Turkey [17-18]. Their intentional or unintentional release into the irrigation channels which are directly connected to the Özlen Stream might be responsible for their occurrence in Özlen Stream. These irrigation channels also create a connection between Özlen Stream and Eşen River. Thus, it is also possible that Eşen River might be the main source of these invasive fish. The presence of such fish species may impact fish populations particularly, fish with complex life cycles such as *A. anguilla*. There were no *G. holbrooki* in the downstream regions of Eşen River (data not given here). Past experience supports that mosquito fish inhabits slow moving and shallow waters. The high flow rate might have limited their occurrence in Eşen River. However, a more comprehensive study including the sampling points in the tributaries of Eşen River is needed to validate their absence or presence in Eşen River.

The lower rates of annual precipitation create a major stress on the med-streams located in Mediterranean region of Turkey [19]. Intermittent drought is very common in such small med-streams [20]. Although, there was a decrease in the flow rates of Özlen Stream during summer months, current findings indicate that Özlen Stream is a perennial running water. Water withdrawal for irrigational purposes and the intensive use of pesticides in the greenhouses located in the sampling area creates a major threat to aquatic life both in Özlen Stream and Eşen River and eventually the recipient Mediterranean Sea. Furthermore, the presence of invasive species in Özlen Stream is another threat to native biota inhabiting the stream. Recent research has shown that local endemics and/or threatened fish species may be trapped in small springs [6]. Thus, such small karst-fed streams may play a crucial role in the fate of local endemic fish species and/or threatened fish species that inhabits the stream. Thus, fish fauna in such water bodies should be investigated before human impacts reach to an irreversible point.

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EFFECTS OF HARVESTING LEAVES FROM DIFFERENT HEIGHTS OF SUMMER SNOWFLAKE (*Leucojum aestivum* L.) ON BULB DEVELOPMENT AND GALANTHAMINE CONTENT

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ABSTRACT. This research was carried out to optimize the optimum leaf length that could be used to obtain maximum galanthamine without damaging the growth of 9-10 g weight summer snowflake (*Leucojum aestivum* L.) during October 2017 and July 2019 for two years. The leaves were harvested at ground level, 5, 10, 15 cm above soil level and control (not harvested). Using a total of 600 bulbs, 30 bulbs were planted in each replication. Equally proportionate sand-soil substrate was used for planting. The length of leaf at harvest (cm), the number of leaves, the percentage of galanthamine in the leaves ranged 15.225-20.775 cm, 2.20-3.60 number, and 0.067-0.094% for the first year and it ranged 19.8-23.4 cm, 4.50-5.50 number, and 0.063-0.096% for the second year in the same order. In the second year, the amount of galanthamine in bulbs ranged from 0.326-0.376%. Harvesting the leaves from soil level negatively affected the bulb quantity and the least bulb was obtained. In control application without leaf harvest, one bulb weight and galanthamine amount reached the highest value. It has been determined that different leaf harvest heights have effects on bulb development and the amount of galanthamine in leaves and bulbs.

1. INTRODUCTION

Turkey has an important place in gene centers of world and has a rich population of plants. The number of taxa exceeds 12000 and the number of endemic species exceed 4000 plants. Geophytes constitute a great richness including approximately 850 species [1, 2]. Some of the geophytes come to the forefront with their medicinal properties. Summer snowflake (*Leucojum aestivum* L.) is a perennial plant in the narcissus (Amaryllidaceae) family that stands out with features like beautiful flowers

Keyword and phrases. Alkaloid, bulb, galanthamine, hplc, leaf.

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and grows in wet and humid areas [3, 4]. Summer snowflake bulbs are used in landscaping in parks, gardens and buildings (around pools and moist areas, along with other ornamental plants [5-7].

Most of the alkaloids of the plants in the Amaryllidaceae family are noted for their antitumor and antiviral properties as well as anticholinesterase activity [8] One of these alkaloids Galanthamine is obtained from plants of the Amaryllidaceae family, like *Galanthus* L.*, Leucojum* L.*, Narcissus* L. and *Lycoris* Herb. since the 1960s, galanthamine has been commercially obtained from natural bulbs of summer snowflake and has been used as a valuable industrial resource [9-11]. It is the most important alkaloid used in the symptomatic treatment of Alzheimer's disease [12]. Its most popularly used alkaloid, lycorine, has an anti-tumor effects [13]. Galanthamine has also been used for many years in the treatment of polio (Poliomyelitis), nerve and muscle disorders [14]. The flowers are also collected and dried for evaluation in brewing and preparation of chamomile tea.

Galanthamine was first isolated from *Galanthus woronowii* Losinsk in 1952. But later the pharmacological properties of the summer snowflake bulb attracted the attention of the pharmaceutical industry. Due to their small size and variability in galanthamine content, *Galanthus* spp. Losinsk species were soon replaced by *L. aestivum* [15]. In Europe, galanthamine is currently obtained from *L. aestivum* and culture forms of the genus *Narcissus* L. [16]. Galanthamine alkaloids, as well as chemicals having different pharmacological effects, such as lectins and chelidonic acid, can also be obtained from the bulbs and leaves of summer snowflake. The plant bulbs are also used to produce ethanol [17, 18]. The amount of alkaloid and especially galanthamine varies greatly depending on plant genotype and geographical locations [19, 20].

The summmer snowflake is found in in the Black Sea natural expansion area of the lakes and in areas close to the Marmara region located within the forest (*Fraxinus angustifolia* Vahl.) ecosystems [21] in the provinces of Beyşehir country of Konya and Erzurum [22].

It is known that the side effects of active substances obtained from plants are less compared to those obtained by chemical synthesis. Therefore, the use of active substances obtained from both summer snowflake and other medicinal plants in the pharmaceutical industry has gained importance in recent years.

Like all other bulbous plants, obtaining the bulbs from their natural habitats, with improper use of forest areas, urbanization, agricultural practices, excessive and

irregular grazing, etc. in the natural ecosystem are among many reasons that are affecting their growth and development negatively and is resulting in danger of their extinction. The pressure on these plants has increased due to their medicinal properties in recent years. The export of the summer snowflake bulbs growing under wild conditions is prohibited after last permission to remove 3 million summer snowflake bulbs from the wild in the previous years. Only the bulbs obtained after cultivation are allowed to export. This issue is considered by the Ministry of Agriculture and Forestry as it is a wild growing bulbous plant [23]. Turkey's has been engaged in collecting and exporting of bulbs collected from wild to various countries in the world, especially in European countries for a long time. Both bulbs and leaves of the Turkish summer snowflake are in high demand from abroad especially Bulgaria in recent years.

Klosi et al. [4] found the average galanthamine content of summer snowflake bulbs as 0.13% in the first week of April (flowering phase) and 0.14% in late June at the end of flowering period. It is also stated that galanthamine was present in the leaves of the plant as an important source [4, 24]. Georgievaa et al. [20] mentioned that galanthamine or lichorine was found as the main alkaloids in the clusters of shoots obtained under *in vitro* conditions and that the galanthamine content ranged 28 - 2104 μg g^{-1} in bulbs and 454 μg g^{-1} dry weight in shoots.

Petruczynik et al. [25], in their studies examining various extraction procedures using maceration followed by extraction in the ultrasonic bath, found 0.0949 mg mL^{-1} galanthamine and 0.1926 mg mL^{-1} lorin that is significantly higher concentration of alkaloids investigated. Berkov et al. [10] reported 3 fold higher galanthamine concentration (average 74 μg g^{-1} dry weight) from the cultures obtained under light conditions compared to the galanthamine concentration (average 39 μg g^{-1} dry weight) obtained from the clusters under dark conditions. Furthermore, they obtained 5 times higher galanthamine than the concentration obtained from the shoots obtained under *in vitro* conditions.

Many studies on the ecological requirements, anatomy, physiology, galanthamine content, medicinal properties and production methods of summer snowflake have been mentioned in previous studies [13, 18, 19, 21, 26, 27]. However, it has not been discussed how the harvesting of leaves from different heights affects the development of the plant and the amount of galanthamine.

Therefore, the aim of this study was to determine the effect of different leaf heights harvest of summer snowflake (*L. aestivum*) on bulb growth and the potential amount of galanthamine in leaves and bulbs.

2. Materials and Methods

2.1 Experimental Plan and Applications

This work was carried out between October 2017 and July 2019 (two years) at Uşak University Faculty of Agriculture and Natural Sciences under greenhouse conditions. The summer snowflake bulbs had an average weight of 9-10 g and a circumference of 7-8 cm at the start of the planting and were planted in randomized complete blocks design with 4 replications. The experiment determined the most appropriate level of 4 leaf harvesting heights (the leaves cut at the ground level, 5, 10 and 15 cm above soil) to obtain maximum galathamine. The leaves were not harvested under control treatment in order.

A total of 600 bulbs were used in the study and each replication included 30 bulbs. Sand and soil were mixed in 1:1 percentage to fill the crates for growth and development of summer snowflakes. After planting, the bulbs were watered, irrigated and the other maintenance operations were carried out to the extent needed. Leaf lengths were measured. The leaves harvest operations were performed at the time of the yellowing and drying of the upper parts of the leaves. The leaves left on the soil were waited until they were completely dried by watering when needed.

The bulbs were not removed from the crates during first year and they spent dormant period in the soil, that were watered periodically at drying. Leaf lengths and numbers were measured in the first and second years. They were harvested during two years. At the end of the second year, the bulbs were removed from the soil. The leaves of different length obtained during two years were dried and the galanthamine values were determined for the two years. The number of leaves per plant, fresh leaf weight, leaves height, the amount of galanthamine in the leaves were measured during both years. The number of harvested bulbs, the weight of bulbs and the amount of galanthamine in the bulbs were determined in the second year. The amount of galanthamine in harvested bulbs and harvested leaves was analyzed using HPLC.

2.2 HPLC Device Galanthamine Analysis Method

2.2.1 Sample Preparation

300 mg of leaves were weighed, dried and ground and added to 30 ml of 0.1 M HCL solution. After extraction in the ultrasonic bath for 15 minutes, it was filtered with filter paper and the extract was taken to the refrigerator until analysis.

2.2.2 Analysis Method

Analyzes were performed with Agilent brand 1260 HPLC device. Galanthamine hydrobromide (C17H21NO3 HBr) was used as standard. Galanthamine hydrobromide equivalent to 100 mg of Galanthamine was weighed and dissolved in deionized water to make up to 100 ml of deionized water. Working standard solutions of five different content percentages (20, 40, 60, 80, 100 ppm) were prepared using a standard stock solution at a content percentages of 100 ppm. Injected into HPLC. Content percentages graph was generated against peak areas [28] (Fig. 1). Chromatographic System: Detector: UV 288 nm; Column: 4.6-mm′ 15.0-cm; 5-mm packing L1 Mobile Phase A: 4.0 g L^{-1} Monobasic Potassium Phosphate solution was prepared. The pH was adjusted to 6.5 with 5 N Sodium Hydroxide. (90%) Mobile Phase B: Acetonitrile (10%); Flow Rate: 1.2 mL min^{-1}; Injection Volume: 20 µL.

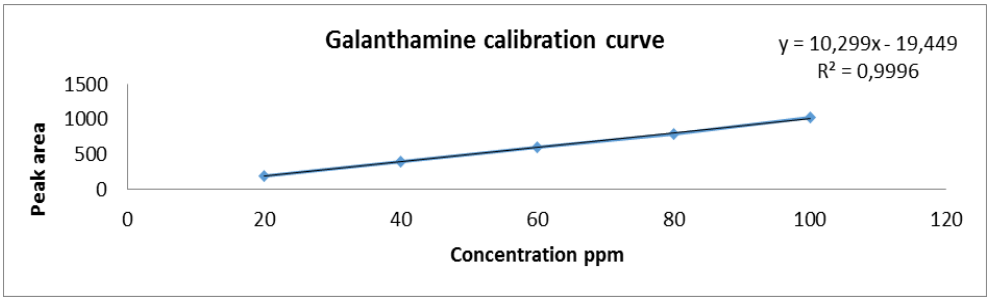

FIGURE 1. Galanthamine calibration curve

2.3 Statistical Analysis

Statistical analysis of the mean values of the measured characters was made with SPSS package program and the differences between the averages were determined by Duncan test [29].

3. Results and Discussion

Table 1. Average values and duncan groups of the measured parameters

Treatments	Number of bulbs	Bulb weight (g)**	One bulb weight (g)*	Galanthamin percentage in bulbs (%)**	Number of leaves	
					First year **	Second year
Harvesting at 15 cm height	66.500	418.000 bc	6.368 b	0.342 b	2.975 b	4.875
Harvesting at 10 cm height	68.000	439.775 ab	6.495 b	0.339 b	3.175 ab	4.950
Harvesting at 5 cm height	60.250	363.900 cd	6.083 b	0.326 b	2.200 c	5.200
Harvesting at 0 cm surface level	55.500	349.125 d	6.343 b	0.328 b	3.175 ab	4.500
Control	61.750	498.600 a	8.085 a	0.376 a	3.600 a	5.500
General Average	62.400	413.880	6.675	0.342	3.090	5.025

** The differences between the mean values were statistically significant at the 0.01 level.
* The differences between the mean values were statistically significant at the 0.05 level.

Table 1. Continued…

Treatments	Fresh leaf weight (g) **		Galanthamine percentage in leaves (%)		Leaf length (cm)	
	First year **	Second year **	First year **	Second year **	First year **	Second year *
Harvesting at 15 cm height	20.598 c	22.873 c	0.067 c	0.063 d	18.840 c	21.375 bc
Harvesting at 10 cm height	36.240 b	41.995 b	0.080 b	0.069 cb	20.675 a	21.950 ab
Harvesting at 5 cm height	37.210 b	44.400 b	0.082 b	0.076 b	15.225 d	21.800 ab
Harvesting at 0 cm surface level	52.070 a	75.738 a	0.094 a	0.096 a	19.750 b	19.800 c
Control	47.845 a	69.375 a	0.079 b	0.066 cd	20.775 a	23.400 a
General Average	38.793	50.876	0.080	0.074	19.508	21.945

** The differences between the mean values were statistically significant at the 0.01 level.
* The differences between the mean values were statistically significant at the 0.05 level.

100% output was observed in all of the planted bulbs. Every two years; number of leaves, fresh leaf weight, galanthamine percentage in leaves, leaf length, and second year; number of bulbs, bulb weight, one bulb weight, galanthamin percentage in

bulbs obtained from the experiment. Variance analysis results, mean values and duncan tests are given below (Table 1).

The maximum (68.0) and minimum (55.5) number of bulbs was obtained once the leaves were harvested at height of 10 cm and at the ground level respectively. These treatments showed numerical variability in average number and that did not show any statistically significant difference (Table 1).

The differences between average bulb weight and single bulb weight values were found significantly different ($p<0.01$). The maximum weight was obtained in control treatments during both years (498.600 g and 8.085 g). The minimum bulb weight (349.125 g) was obtained when the leaves were harvested at the ground level (Table 1).

It was found that the differences between the mean values of bulb galanthamine percentage were significantly different ($p<0.01$) and the highest percentage was obtained from control treatment (0.376%). It was seen that the differences between the average values obtained from other treatments were not significantly different (Table 1).

The differences between the average number of leaves were significantly different ($p<0.01$) in first year, and not significant in second year. The maximum values in the first and second year were noted as 3.600 and 5.500 respectively in control treatments (Table 1).

The differences between the average values of fresh leaf weight were significantly different ($p<0.01$) for two years. The maximum value was obtained when the leaves were harvested at the ground level in each years (52.070 g in the first year and 75.738 g in the second year). However, the differences between control averages were not significantly different (Table 1).

The differences between the average values of galanthamine obtained from the leaves were found significantly different ($p<0.01$) during both years. In both years; the maximum galanthamine percentage was obtained when the leaves were harvested at the ground level (0.094% during the first year, 0.096% in the second year), and the least galanthamine percentage was obtained when the leaves were harvested at 15 cm above soil level (0.67% during the first year and 0.063% in the second year) (Table 1).

The differences between the mean values of leaf height were significantly different ($p<0.01$) during both years. The maximum leaf height of (20,775 cm and 23,400 cm) was obtained from the control treatments in the first and second years respectively.

Comparing results of all treatments; it is seen that the bulb formation is less in the control treatment; whereas the control treatment had larger bulbs, and highest bulb weight was obtained. It was noted that when the leaves were harvest at the ground level the plants lacked photosynthesis that affected both the number of bulbs and bulb weight negatively. The bulb weight increased proportionally with each increase in leaf length at the time of harvest from soil level to 5 cm and 10 cm. The bulb weight and number of bulbs increased in direct proportion to the increase in the length of leaves remaining above the ground level.

It is seen that the percentage of galanthamine tend to increase (excluding control treatment) with increase in length of leaves. It was noted that the amount of galanthamine in the leaf was not high in the control treatment. This situation can be explained; when the leaves are not harvested, with the drying of the leaves, galanthamine moves from leaf to bulb and accumulates in the bulb [30]. The amount of galanthamine in the bulb was higher, when the leaves were not harvested (control treatment). At the same time the maximum galanthamine amount after control treatment was obtained at 15 cm harvesting lengths of leaves, but there were no differences among the other treatments mean values.

According to Ayan et al. [27] ; GA_3 and NAA treatments were carried out together in shade and light environment on summer snowflake that showed improvement of leaf length to 38.07 cm and 47.10 cm, the number of leaves and number of bulblets that ranged 5.28- 6.20 and between 0.63-1.33 in the same order. They found that the length of the plants obtained from GA_3 treatment was longer compared to the plants obtained after NAA treatment. The leaf heights in this experiment was shorter compared to the leaf heights noted by other researchers in their experiments.

Berkov et al. [19] found that galanthamine content in summer snowflake populations collected from different regions of Bulgaria was 0.003-0.08% in the northern regions and 0.42% in the southern regions and increased up to 0.65% in some single plant samples. According to geographic distributions, the presence of a large number of chemotypes was mentioned and they indicated that the amount of galathamine varies greatly among chemotypes. The findings of the researchers are in parallel with the findings in this research.

Yildirim et al. [24] have determined that hormone application has an effect on summer snowflake growth and yield criteria. Most galanthamine was determined in bulbs, followed by leaves and roots. They also stated that the leaves can be used to obtain galanthamine. Galanthamine general mean values were determined as 0.062 in roots, 0.089 in leaves and 0.245 in bulbs. While the rate of galanthamine in the leaf was similar, the amount of galantamine in the bulbs were found to be higher in our research.

Many researchers have reported that amount of galanthamine in the leaves of summer snowflake is very important source for obtaining and production of raw material in the pharmaceutical industry with 0.30% percentage in bulbs and 0.34% in leaves [26, 31-33].

Kreh [30], in a study performed on a daffodil species and their fresh bulb weight measurements noted that the amount of galanthamine content (in November) decreased slightly immediately after planting. Furthermore they foıund that the amount of galanthamine increased by 400% three months post planting, and the amount of galanthamine reached to the peak from mid-April to the beginning of June. Thereafter, it decreased with the aging of leaves. In the same study, it was noted that the amount of galanthamine increased dramatically in the above-ground parts before flowering, it fell slowly until the end of flowering and then remained stable for a certain time and reached the least level when the leaves were completely dry.

In another study conducted in daffodils, it was noted that galanthamine content varied according to plant type, harvest period and weather conditions. Galanthamine was found to be the maximum between 0.2% and 0.5% in leaves of eight daffodils. They found the highest percentage of galanthamine when the growth rate was at the top of the bud formation stage [26, 34, 35].

Galanthamine content in dry leaves of summer snowflake varies from low to 0.5% (usually 0.1-0.3%) according to population and geographical regions [15], and in another study, galanthamine content ranged 0.9-2.6 mg g^{-1} [36]. The galanthamine values we obtained from the leaves were lower than those obtained from these studies. It can be said that these differences are caused by ecological factors, a complex physiological cycle of the plant and different chemotypes [24].

4. Conclusion

According to the results; it has been determined that the moving of the leaves of the summer snowflake has an effect on the bulb weight and galanthamine content both in leaves and bulbs. The control treatment stands out in terms of galanthamine yield in bulbs (since bulb weight and galanthamine percentage were higher). Excluding control treatment, harvesting of leaves at 10 and 15 cm could be recommended for bulbs to gain weight. The leaves harvesting at the ground level may be recommended when obtain galanthamine from leaves (every two years fresh leaf yield was highest when the leaves were harvested at the ground level). However, when the leaves were harvested at the ground level it had negative impact on both bulb induction and weight. In this case, the development bulbs was negatively influenced when the leaves were harvested at the ground level. It is vital importance that, in order to avoid such problems instead of harvesting the leaves at the ground level, it would be appropriate to harvested them at a height 10 or 15 cm. Inappropriate height of leaves at the time of harvest could have negative effects on bulb development. Because the leaves of the plant have important role in photosynthesis and consequently on the development of the bulbs. Therefore they must be cut at appropriate length/height to allow photosynthesis for a long time.

It is presumed that this study will significantly influence in designing of future studies. And also; studies should be made for determining and detection of different summer snowflake chemotypes with high galanthamine content to achieve high value products. This research will make an important contribution to the studies of summer snowflake.

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SYNOPSIS OF THE GENUS *COTA* (ANTHEMIDEAE, ASTERACEAE) IN TURKEY

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ABSTRACT. The morphological features of *Cota* were studied based on our specimens collected between 2005 and 2019 in Turkey and herbarium specimens from various herbaria. As a result of the obtained systematic and floristic findings of the last years, *Cota* now 22 taxa (17 species, 2 subspecies, 3 variety), of which 9 are endemic to Turkey which suggests an endemism rate of about 41%. In this study, *C. coelopoda* var. *longiloba* and *C. coelopoda* var. *bourgaei* are considered a synonym of *C. coelopoda*, and one new lectotyes are designed. Moreover, main synonyms, nomenclatural types, ecological data, diagnostic keys and distribution of taxa are also given for each accepted taxon and morphological characters have been discussed.

1. INTRODUCTION

Asteraceae is represented by the greatest number of taxa in *Angiospermae* from all over the world. It has nearly 1600-1700 genera and ca. 24000 species distributed around the globe except for Antarctica [1]. According to the recent generic conspectus of *Asteraceae*, the tribe *Anthemideae* Cass. consist of 111 genera and ca. 1800 species [2].

Cota J. Gay was earlier classified as a section in the genus *Anthemis* L. in the Flora of Turkey [3]. After the generic and infrageneric concepts of *Anthemis* were changed, *Anthemis* sect. *Cota* was accepted as a genus, *Cota* [4, 5, 6].

Keyword and phrases. Asteraceae, Cota, Synopsis, Turkey

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Cota species are mainly distributed in Europe (except north of Europe), North Africa, Caucasica, and Central Asia [7, 8]. Turkey is one of the centers of diversity for the genus *Cota*, with especially widespread in the Meditarranean and Irano-Turanian phytogeographic regions of Turkey [9]. *Cota* is represented by 17 species and 20 taxa, of which 9 are endemic in Turkey [10].

During the revisionary study, *Cota hamzaoglui* Özbek & Vural has been published as a new species for science [11]. The genus *Cota* has been studied from a anatomical, morphological and palynological by authors [9, 12, 13].

The aim of this study is to present the current taxonomic arrangement and distribution of taxa of *Cota* in Turkey, providing synonymy, nomenclatural types, ecological data and diagnostic keys for species identification.

2. Materials and methods

The studied specimens were collected in Turkey between 2005 and 2019. Specimens for morphological studies were dried according to standard herbarium techniques and stored in the Gazi University Herbarium (GAZI). Relevant literature (Grierson & Yavin 1975, Fernandes 1976, Feinbrun-Dothan 1978, Pignatti 1982, Iranshahr 1986, Shishkin 1995, Güner *et al.* 2000) were examined [3, 14-19]. In addition, a large number of herbarium specimens were checked from the ANK, AEF, BOLU, DUOF, EGE, ERC, GAZI, HUB, ISTE, KNYA, and VANF herbaria. The acronmys are according to Thiers 2020+ [20]. Images of the type specimens of some *Cota* species studied were obtained from the Kew (K), Edinburgh (E), Berlin (B), Linnean Herbarium (LINN), Vienna-Herbarium (W), University Vienna Herbarium (WU), and Geneva (G) virtual herbaria. Authors of plant names are written according to International Plant Names Index (IPNI) [21].

3. Results

In the Flora of Turkey, *Anacyclus* L. is represented by four species. These species are *A. clavatus* (Desf.) Pers., *A. latealatus* Hub.-Mor*., A. nigellifolius* Boiss and *A. anatolicus* Behçet & Almanar [22, 23]. As a results of recent molecular phylogenetic study, three *Anacyclus* L. species were transferred from *Anacyclus* to *Cota* (except *A. clavatus*). This study was based on molecular data and using DNA barcoding markers [24]. Therefore, we did not include these three species in the identification key.

Identification key for *Cota* species in Turkey:

1. Perennials……...……..…….2
– Annuals ……...……...11
2. Leaves simple, serrate or shallowly pinnately lobed; phyllaries 2–3-seriate ……2. ***C. euxina***
– Leaves 2–3 pinnatisect; phyllaries 4– seriate……..…3
3. Capitula discoid……...…….....4
– Capitula radiate…….....8
4. Paleae distinctly longer than disc flowers (8–11 mm)…….....…5
– Paleae as long as disc flowers or slightly longer (4–7 mm)…….…….6
5. Stem and leaves lanate or subglabrous; leaves 2–3 pinnatisect……....7
– Stem and leaves yellowish pilose or sericeous hairs; leaves 2– pinnatisect …….. 9. **C. *fulvida***
6. Leaves 2– pinnatisect, oblong–elliptic……....3. ***C. virescens***
– Leaves 2–3 pinnatisect, obovate–oblanceolate or elliptic…....1. ***C. tinctoria***
7. Stem 12–30 cm; primary segments 3–4 paired, secondary segments 1–3 paired…….…….…….… 8. ***C. hamzaoglui***
– Stem 40–60 cm; primary segments 4–8 paired, secondary segments 4–8 paired…….... 7. ***C. oxylepis***
8. Ray flowers yellow or cream……..….1. ***C. tinctoria***
– Ray flowers white…….…….....9
9. Capitula campanulate; basal leaves petiolate……...6. ***C. antitaurica***
– Capitula hemispherical; basal leaves not petiolate……..……...10
10. Paleae narrowly lanceolate; phyllaries dark brown or black, margined ciliate……...……...….5. ***C. melanoloma***
– Paleae linear–oblong; phyllaries pale brown, only ciliate at apex …….…….. 4. ***C. triumfettii***
11. Paleae distinctly longer than disc flowers (8–10 mm)…..… 13. ***C. dipsacea***
– Paleae as long as disc flowers or slightly longer (3–7) mm…….…..……12
12. Ray and disc flowers pink……..……...17. ***C. pestalozzae***
– Ray flowers white or cream; disc flowers yellow……...……...….13
13. Decumbent or prostrate, leaves fleshy……..……16. ***C. halophila***
– Erect or ascending; leaves not fleshy……..…….....14
14. Stems radiating from base; leaves oblanceolate; 1–2- pinnatisect ……..…..15. ***C. wiedemanniana***
– Stems not radiating from base; leaves ovate, oblong or elliptic, 2-3- pinnatisect…….15
15. Paleae truncate……...……14. ***C. palestina***
– Paleae acuminate……...…..……...16
16. Subglabrous; leaves ovate–oblong or elliptic; achenes distinctly 8–10 ribbed

on each side…….10. ***C. altissima***

– Pubescent; leaves oblong or oblong–elliptic; achenes with 1–3 ribbed on each side.....17

17. Peduncles becoming thickened, acuminate apex of the paleae is long; (2–3 mm); leaves 2–3- pinnatisect ……...…….. 11. ***C. coelopoda***

– Peduncles not becoming thickened or slightly thickened; acuminate apex of the paleae is short (1–1.5 mm); leaves 2-pinnatisect……… 12. ***C. austriaca***

1. ***Cota tinctoria*** (L.) J.Gay, Fl. Sicul. Syn. 2: 867 (1855).

1. Capitula discoid……..……...……....…...1c. var. ***discoidea***
– Capitula radiate……...……2
2. Ray flowers yellow……..…....1a. var. ***tinctoria***
– Ray flowers white or cream…….....1b. var. ***pallida***

1a. var. ***tinctoria***

≡ *Anthemis tinctoria* L., Sp. Pl: 896 (1753). ═ *Anthemis tinctoria* L. var. *orientalis* Eig et var. *parviflora* Eig, Palestine J. Bot., Jerusalem ser. 1: 211 (1938). ═ *Anthemis debilis* Fed., Fl. URSS 26: 867 (1961).

Lectotype: Described from Europe (foto Hb. Cliff. 414/2!).

Specimens examined: A1 Tekirdağ: Gelibolu to Şarköy, 22 km from Şarköy, clearings of *Pinus brutia* forest, 40 m, 05 June 2007, *M.U.Özbek* 2338 (GAZI!); Kırklareli: Demirköy road, between Yenice to Mahyadağ, 26 June 1968, *A Baytop* (ISTE 13983!); Edirne: Keşan to Malkara, 20 km from Malkara, clearing of *Pinus brutia* and *Quercus* forest, 195 m, 22 July 2006, *M.U.Özbek* 2142 (GAZI!); Çanakkale: 20 km from Eceabat to Gelibolu, roadside, 5 m, 22 July 2007, *M.U.Özbek* 2141 (GAZI!); Bozcaada, Latif shrubbery, ca. 20 m, 15 June 1976, *Ö.Seçmen* 1697 & *E.Leblebici* (EGE!); Gökçeada, between Kaleköy to Zeytinli, N. of state farm, ca. 10 m., 18 June 1976, *Ö.Seçmen* 2075 & *E.Leblebici* (EGE!); Balıkesir: Marmara Island, around Saraylar village, 30 m, 13 July 1978, *E.Tuzlacı* (ISTE 40366!). **A2** İstanbul: around Fatih forest, geophysics research area, 14 June 1972, *H.Argöksel* & *E.Tuzlacı* (ISTE 22329!); Bursa: Bursa to Keles, 40 km from Keles, clearings of forest, roadside, 415 m, 28 June 2006, *M.U.Özbek* 2109 (GAZI!). **A3** Ankara: Beypazarı to Nallıhan, İnözü valley, 900–1200 m, *Quercus pubescens* alliance, 26 June 1973, *Y.Akman* 8364 (ANK!). **A4** Ankara: Kızılcahamam, Soğuksu National Parks, around Kuzcapınar, ca. 1300 m, 03 August 1989, roadside, *Ö.Eyüboğlu* 1989 (GAZI!); Kırıkkale: Delice, Büyükavşar village, around Büyükhemit, ca. 1150 m, 16 June 1990, clearings of *Quercus* forest, *C. Birden* 1157 (GAZI!); Çankırı: Atkaracalar, Dumanlı Mt., Bozkuş upland, steppe, 1250–1450 m, 10 July 1992, *A.Duran* 1617 (GAZI!); Karabük: Safranbolu, steppe, ca. 350 m, 21 June 1985,

M.Demirörs 1264 (ANK!). **A5** Samsun: Ladik, above Aslantaş road, mixed forest, 900 m, 22 August 1978, *Ş.Yıldırımlı* 1212 (HUB!); Kastamonu: Tosya, Seki village, around Seki stream, ca. 1000–1200 m, 10 June 1975, *M.Kılınç* 2095 (ANK!); Amasya: Akdağ, between Suluova to Ladik, above Eğribük village, near Elmatepe, under *Quercus* forest, 1500 m, 24 June 1977, *K.Alpınar* (ISTE 37877!). **A6** Ordu: Fatsa, Yapraklı village, 10 m, 15 August 1978, *B.Yıldız* 2226 (HUB!); Tokat: Artova, Arabacı Musa village, *Cupressus–Salix* forestration area, ca. 1200–1300 m, 11 June 1981, *R. İlarslan* 1418 (ANK!). **A7** Giresun: Şebinkarahisar to Tamdere, above Asarcık village, roadside, 1790 m, 27 July 2007, *M.U.Özbek* 2657 (GAZI!). **A8** Gümüşhane: pass between Köse and Gümüşhane, 2000 m., N. slope in scrub (igneous), 02 August 1957, *P.H.Davis* 31958 & *Hedge* (EGE!); Bayburt: Bayburt to Aşkale, Kop Mountain, high mountain steppe, 2332 m, 28 July 2007, *M.U.Özbek* 2669 (GAZI!); Erzurum: 27 km from Erzurum to Tortum, 1950 m., Ghalk hills (steppe), 29 July 1966, *P.H.Davis* 47527 (EGE!); Rize: İkizdere, around Tozköy, 1450 m, meadows, 06 August 1986, *A.Güner* 7077 (GAZI!); Artvin: above Artova, ca. 800 m, 26 June 1957, *P.H.Davis* 30038 & *Hedge*, (ANK!). **A9** Artvin: Kaçkar stream, along the streambed, 1850–2400 m, 31 July 1982, *N.Demirkuş* 1679 (HUB!); Kars: Sarıkamış, Karakurt, environs Agop, 1550–1600 m, 14 July 1979, *O.Güneş* 1363 (HUB!). **B1** İzmir: Bozdağ to Ödemiş, above Allahdiyen village, rocky slopes, 750 m, 23 June 2007, *M.U.Özbek* 2322 (GAZI!); Çanakkale: 18 km from Ayvacık, clearings of *Pinus brutia* forest, 400 m, 04 June 2007, *M.U.Özbek* 2330 (GAZI!); Manisa: 2 km from Akhisar to Gördes, roadside, 185 m, 27 May 2005, *M.U.Özbek* 1856 (GAZI!); Balıkesir: Edremit, Kaz Mts, rocky slopes, 1500 m, 26 July 1968. *Pamukçuoğlu* & *Quezel* s.n. (HUB!); 24 km E. of Balıkesir, 300 m, 7 August 1978, *A.Öztürk* 78-186a (VANF!). **B2** Bursa: Uludağ, clearings Karabelen forest, 13 August 1960, *K.Karamanoğlu* s.n. (AEF!); Kütahya: Tavşanlı to Domaniç, around Tunçbilek, meadows, 805 m, 02 June 2007, *M.U.Özbek* 2303 (GAZI!); Afyonkarahisar: Dazkırı, E. of Darıcılar village, steppe, c. 1050 m, 18 June 1984, *Z.Aytaç* 1257 (GAZI!). **B3** Bilecik: Bozüyük, Ahı Dağı, *Pinus nigra forest*, c. 770 m, 04 July 1978. *E.Yurdakulol* et al. (ANK!); Eskişehir: Sivrihisar to Mihallıçık, steppe, 1160 m, 09 June 2005, *M.U.Özbek* 1867 (GAZI!); Ankara: Polatlı to Sivrihisar, around Acıkır, steppe, 705 m, 22 June 2005, *M.U.Özbek* 1885 (GAZI!); Afyonkarahisar: Sincanlı, Kumalar Mt., around Taşoluk, Mandal hill, steppe, 1300–1400 m, 19 July 1997, *E.Akçiçek* 1836 (GAZI!); Konya: 24 km from Akşehir to Gelendost, steppe, 1050 m, 06 July 2005, *M.U.Özbek* 1902 (GAZI!); Isparta: Şarkikaraağaç, Kızıldağ Natural Park, *Cedrus libani* forest, 1250 m, 25 June 1994, *B.Mutlu* 813 (HUB!). **B4** Ankara: Hüseyingazi, steppe, 1400–1500 m, 12 August 2008, *M.U.Özbek* 2768 (GAZI!). **B5** Nevşehir: Göreme, 5 km E. of Göreme, 1110 m, 18 May 1989, volcanic tuff, *M.Vural* 4678 et al. (GAZI!); Aksaray: Hasan Dağı, N.W. of Hasan Dağı, above *Quercus* forest, ca. 1750 m, 12 July 1973, *A.Düzenli* 351 (ANK!); Kayseri: Bakırdağ, between Saraycık to Sarıkaya village, under *Abies*

cilicica forest, 1545 m, 04 August 2007, *M.U.Özbek* 2689 (GAZI!); Yozgat: Natural Park, under *Pinus nigra,* ca. 1500 m, 26 June 1979, *Birand* 53 & *Sayın* (ANK!). **B6** Adana: Tufanbeyli, around Akpınar village, steppe, 1443 m, 04 August 2007, *M.U.Özbek* 2692 (GAZI!); Kayseri: Akkışla, Ganişeyh village, around castle, roadside, 1850 m, 18 July 2007, *M.U.Özbek* 2642 (GAZI!); Kahramanmaraş: Andırın, Geben to Andırın, steppe, 1329 m, 05 August 2007, *M.U.Özbek* 2691 (GAZI!); **B7** Erzincan: Kemaliye, around Sandık village, stony slopes, 1170 m, 02 July 2007, *M.U.Özbek* 2467 (GAZI!); Elazığ: Maden, ca. 1300 m, 02 June 1957, *P.H.Davis* & *Hedge* s.n. (ANK!); Tunceli: Pertek, ca. 1100 m, 06 June 1957, *P.H.Davis* & *Hedge* 29152 (ANK!). **B8** Erzincan: Tercan, around Tercan dam, slopes of Çatalkaya hill, steppe, 1440 m, 18 July 2009, *Ö.Güneş* 1017 (DUOF!). **B9** Bitlis: Tatvan, Sorgun, above Van Lake, *Quercus infectoria* shrubs, volcanic base, 1650–1750 m, 09 July 1972, *H.Peşmen* 2961 (HUB!); foot of Nemrut Dağ, 6000 ft., 3 July 1954, *P.H.Davis* 23586 & *C.Folunia* (EGE!); Erzurum: Karaköse to Erzurum 54 km, alpine meadows, 2300 m., 20 July 1956, *H.Birand* 529 & *K.Karamanoğlu* (ANK!); Muş: Malazgirt, Karıncalı village, steppe, 1598 m, 14 July 2007, *L.Behçet* 3296 et al. (VANF!). **C1** Muğla: Datça, Bozdağ (Kocadağ), above Mesudiye village, 700 m, 03 July 1983, *E.Tuzlacı* (ISTE 51522!). **C2** Muğla: Köyceğiz, Ekincik village, around Kürkçüler, 500 m, *Pinus brutia* forest, 19 May 1991, *A.Güner* 9087 et al. (GAZI!). Denizli: Honaz Dağı Natural Park (entrance Cankurtaran), under *Pinus brutia* forest, 1135 m, 10 June 2008, *M.U.Özbek* 2740 (GAZI!). **C3** Antalya: Akseki, Çukurköy, around Istarlas, clearings of macchie, 850–1050 m, 03 June 1996, *A.Duran* 3872 (GAZI!). **C3** Burdur: Gölhisar to Altınyayla 12. km, inner valley, rocky slopes, 665 m, 30 June 2005, *M.U.Özbek* 1898 (GAZI!); Isparta: Sütçüler, between Ayvalı to Darıbükü, serpantine, 850–1100 m, 06 June 1975, *H.Peşmen* 2160 *& A.Güner* (HUB!); S. of Beyşehir Lake, around Üstünler, under *Quercus*, ca. 1150 m, 20 June 1986, *M.Küçüködük* 459 (KNYA!). **C4** Konya: Bozkır, Sorkun upland, 16 June 1968, *R.Çetik* 1198 et al. (ANK!); Karaman: Pınarbaşı to Kızılyaka, 3 km N.W. of Pınarbaşı, clearings of *Quercus*, 1205 m, 28 June 2005, *M.U.Özbek* 1893 (GAZI!); İçel: 2 km from Sertavul to Mut, steppe, 1430 m, 07 July 2005, *M.U.Özbek* 1905 (GAZI!); Çamlıyayla to Tarsus, around Ulaş village, roadside, 300–400 m, 08 June 2008, *M.U.Özbek* 2722 (GAZI!); Adana: Pozantı to Ulukışla, Gülek Boğazı, stony slopes, 1170 m, 02 July 2006, *M.U.Özbek* 2124 (GAZI!). **C6** Kahramanmaraş: Ahır Dağı, around Yalnızardıç, 1400–1500 m, 27 June 1992, steppe, *H.Duman* 4888 & *Z.Aytaç* (GAZI!); Gaziantep: İslahiye, 150 m E. of Koçcağız village, macchie, 990 m, 19 May 2006, *Ş.Çakır* 633 (VANF!). **C8** Mardin: N.E. of 5 km from Mardin, c. 900 m, 26 May 1957, *P.H.Davis* 28608 & *Hedge* (ANK!). **C10** Hakkari: Yüksekova to Şemdinli, Sopatan pass, *Quercus* forest, 1600 m, *T.Ekim* 7973 (GAZI!).

Flowering time: May–September.

Habitat: Steppe, fields, clearings of forest, roadsides, meadows, rocky and stony slopes, s.l.–1830 m.
Distribution: Europe, Caucasia, W. of Syrian, Iran.

1b. var. ***pallida*** (DC.) Özbek & Vural, Türk. Bitkileri List. 147 (2012).
Type: [Turkey A2 İstanbul] Constantinople, 1822. Olivier (G–DC., photo!).
Specimens examined: **A2** İstanbul: Aydos hill, 02 July 1974, *E.Tuzlacı & Y.Doğantan* (ISTE 20019!); Bursa to Keles, 40 km from Keles, clearings of forest, 415 m, 28 June 2006, *M.U.Özbek* 2108 (GAZI!); Kocaeli: Yuvacık dam, Aksığın–Servetiye road, roadsides, 169 m, 07 July 2006, *N.Aksoy* 6199 (DUOF!). **A3** Ankara: Çayırhan to Beypazarı, gypsum slopes, 560 m, 14 June 2005, *M.U.Özbek* 1883 (GAZI!); Beypazarı, Dedenindoruk hill, steppe, 600 m, 08 May 2008, *E.Ergin* 1653 (DUOF!). **A4** Ankara: Ayaşbeli, Akkaya hill, 1250–1400 m, 27 June 1986, steppe, *M.Vural* 4191 (GAZI!); Çankırı: Tosya, Gavurdağı (Üç Oluklar picnic area), clearings of forest, 1070 m, 06 June 2007, *M.U.Özbek* 2358 (GAZI!); Kastamonu: Ilgaz Mountain, 1830 m, 05 September 1994, clearings of forest, *N.Adıgüzel* et al. 1776 (GAZI!); Bartın: Ulus, between above Çerçi village and Arıt road, riverside, 560 m, 14 May 2017, *B.Tunçkol* 4256 (DUOF!). **A5** Kastamonu: Alçıcılar village, c. 900 m, *Pinus nigra* forest, 20 July 1993, *A.A.Dönmez* 3630 et al. (GAZI!). **A7** Giresun: Tirebolu, S.W. of İnköy village, 10–50 m, 03 July 1997, rocky slopes, *H.Duman* 6451 (GAZI!); Trabzon: Çaykara, Ataköy, roadside, 670 m, 27 July 2006, *M.U.Özbek* 2157 (GAZI!). **A8** Rize: Ardeşen, between Fırtına Köprüsü to Bakoz, sandy areas, 100 m, 28 June 1980, *A.Güner* 2860 (HUB!); Erzurum: between Erzurum to İspir, E. of Rizekent village, Hanboğazı stream, *Populus tremula* forest, 2250 m, 14 August 1976, *A.Tatlı* 5309 (HUB!); Artvin: Sarıbudak uplands, *Pinus–Picea* mixed forest, 1850–2000 m, 20 July 1982, *N.Demirkuş* 1607 (HUB!). **A9** Erzurum: Olur, Kekikli village uplands, Şirinönü, 1750–2300 m, 01 August 1984, *N.Demirkuş* 2312a (HUB!); Kars: Sarıkamış to Karakurt 6. km, *Pinus sylvestris* forest, 1950–2000 m, 20 August 1979, *O.Güneş* 1493 (HUB!). **B2** Bursa: Uludağ, above hotels, alpinic steppe, 2050 m, 24 July 2008, *M.U.Özbek* 2761 (GAZI!); Kütahya: Simav, Akdağ, under *Pinus nigra*, 1840 m, 22 June 2012, *M.Altıntaş* 1130 (DUOF!). **B3** Eskişehir: Eskişehir to Kütahya, 35 km from, *Quercus* forest, 960 m, 02 June 2007, *M.U.Özbek* 2300 (GAZI!); Afyonkarahisar: Bayat, Otlugedik, N. slopes, c. 1450 m, 27 June 1975, *M.Vural* 297 (GAZI!, KNYA!); Konya: Sultandağları, above Dereçine village, 1220 m, 10 July 1975, *G.Gökmeci* (ISTE 32878!) **B4** Ankara: Gölbaşı to Haymana, Research and Application Station of Field Crops, steppe, 1037 m, 06 June 2005, *M.U.Özbek* 1864 (GAZI!); Kırıkkale: Keskin, Böbrek Dağı, Müsellim village, N. of Öteyüz, under *Quercus*, 1100 m, 29 June 1991, *Ü.Güler* 1390 (GAZI!). **C3** Antalya: Elmalı to Fethiye, 75 km from Fethiye, rocky slopes, 1170 m, 10 June 2008, *M.U.Özbek* 2732 (GAZI!). **C5** Konya: Ereğli, Aydos Dağı, Sayıntaş, 1700 m, marmoreal rocky slopes, 27 June 1976, *S.Erik* 1681 (HUB!).

Flowering time: June–July.
Habitat: Steppe, fields, 200–2300 m.
Distribution: Greece.

1c. var. ***discoidea*** (All.) Özbek & Vural, Türk. Bitkileri List. 147 (2012).
≡ *Chamaemelum discoideum* All., Fl. Pedem. 1: 188 (1785). ═ *Anthemis discoidea* (All.) Willd., Sp. Pl. 3: 188 (1803).
Type: [N. Italy] Pedemonti circa Cuneum, Allioni.
Specimens examined: A2 İstanbul: Beykoz, Kirazlı, 100 m, macchie, 23 April 2001, *H.Altınözü* 3499 (HUB!). **A3** Sakarya: Geyve, around Bağlarbaşı village, 10 June 1972, *T.Baytop* (ISTE 22364!); Bolu: Kale and Tekneci stream, 1000 m, roadside, 12 July 1990, *İ.Kılınç* 1235 (GAZI!); Düzce: Gölyaka, Kızık pasture, around Soğuksu, 1462 m, 5 May 2011, *N.Aksoy* 5622 (DUOF!); Yığılca, Hasanlar dam, around Orhan Gazi mosque, roadsides, 191 m, 18 May 2008, *N.Güneş* 2245 (DUOF!). **A5** Amasya: Direkli village, around Bayeyn, ca. 700 m, steppe, 21 June 1987, *S.Peker* 1347 (GAZI!). **A9** Kars: Posof, Ilgar Mt. (Çiçek) to Al village, 1580–2730 m, 16 June 1986, *N.Demirkuş* 3579 (HUB!). **C2** Denizli: Acıpayam to Olukbaşı village (Abas village), Geyran uplands, around Güvercin oluğu, conservation areas, 1400–1670 m, 04 July 1997, *Z.Aytaç* 7629 (GAZI!). **C3** Isparta: Sütçüler, Kuzca village, Totabeli, *Pinus nigra* forest, rocky slopes, river edge, 1400–1500 m, 05 June 1975, *H.Peşmen* 2086 & *A.Güner* (HUB!); Antalya: Kemer, Tahtalıdağ, above Çukuryayla, alpinic steppe, 1500–2000 m, 28 July 1979, *H.Peşmen* 4466 & *A.Güner* (GAZI!). **C4** Konya: Ermenek, Kazancı town, Yeşilköy, through Göksu river, 700–800 m, 11 April 1984, *H.Sümbül* 2794 (HUB!). **C5** Adana: Pozantı, against Horoz village, rocky and stony slopes, 1290 m, 22 June 2007, *M.U.Özbek* 2432 (GAZI!); İçel: Çamlıyayla (Namrun) to Tarsus, 43 km from Tarsus, rocky slopes, 950 m, 08 June 2008, *M.U.Özbek* 2721 (GAZI!). **C6** Antakya: Antakya to Yayladağ, around Şenköy, 850–900 m, macchie, 24 June 1989, *A.Güner* 7227 (HUB!).
Flowering time: May–August.
Habitat: Roadsides, hillsides, open area of forest, s.l.–1300 m.
Distribution: South Europe, West Syrian.
Note: This species differs from the other perennial *Cota* species by its capitula radiat or discoid, paleae oblong or linear–oblong, ray flowers are yellow, white or cream and leaf segments narrow. It is the most widely distributed species between other *Cota* species growing in Turkey. During our field study, an abnormal specimen (province of İzmir, West Anatolia) was detected with ray flowers similar to disc flowers.

2. *Cota euxina* (Boiss.) Özbek & Vural, Türk. Bitkileri List. 147 (2012).

≡ *Anthemis euxina* Boiss., Fl. Orient., 3: 282 (1875). ≡ *Cota euxina* (Boiss.) Holub, Folia Geobot. Phytotax. 9: 270 (1974). ≡ *Anthemis tinctoria* L. var. *euxina* (Boiss.) Grierson, Notes Roy. Bot. Gard. Edinburgh 33 (3): 428 (1975). ≡ *Cota tinctoria* L. subsp. *euxina* (Boiss.) Oberpr. & Greuter, Willdenowia 33 (1): 41 (2003).
Type: [W. Caucasus]: ad littus marinum Euxini inter Poti et St Nicolaum Abchasiae, *Ruprecht* (E!).
Specimens examined: A2 İstanbul: Karaburun, E. of Karaburun, sandy places, 0–5 m, 26 August 2007, *M.U.Özbek* 2695 (GAZI!); Ormanlıköy, seaside, 04 October 1967, *A.Baytop* & *G.Atila* (ISTE 12134!); E. of Karaburun, 09 October 1967, *A.Baytop* (ISTE 12217!).
Flowering time: July–October.
Habitat: Sandy places, s.l.–5 m.
Distribution: Georgia.
Note: In the Flora of Turkey, this taxon was evaluated as *Anthemis tinctoria* L. var. *euxina* (Boiss.) Grierson (Grierson & Yavin, 1975). However, it can be easily distinguished from *C. tinctoria* by several noticeable morphological characters. *C. euxina* differs from *C. tinctoria* in having leaves simple, serrate or shallowly pinnately lobed (not pinnatisect), phyllaries 2–3-seriate (not 4-seriate), paleae 4–4.5 mm (not 6–7 mm) and achenes obovate and 1–1.5 mm (neither oblong nor 1.75–2.5 mm). It was re-evaluated at the species level because of these morphological differences [10].

3. *Cota virescens* (Bornm.) Özbek & Vural, Türk. Bitkileri List. 148 (2012).
≡ *Anthemis tinctoria* L. var. *virescens* Bornm., in Notizbl. Bot. Gart. Berlin-Dahlem. 7: 19 (1917). ═ *Anthemis meinkeana* Rech. f., in Ann. Naturhist Mus. Wien 57: 90 (1949) Ic: Ark. Bot. 5: t.31 (1960). ≡ *Cota tinctoria* L. var. *virescens* (Bornm.) Oberp. & Greuter, Willdenowia 33: 41 (2003).
Type: [Turkey C5 Adana] Haruniye, published erroneously as Alexandrette (İskenderun), *Meincke* 148.
Specimens examined: C6 Osmaniye: Hasanbeyli, Gökçedağ road, around TV station, clearings of *Quercus* forest, 1280 m, 06 July 2008, *M.U.Özbek* 2753 (GAZI!).
Flowering time: June–July.
Habitat: Clearings of *Quercus* forest, 1220–1520 m.
Distribution: Endemic. East Mediterranean element.
Note: As a result of morphological studies on this taxon, it has been observed some differences, such as branching of the stem, the width of involucre, the shape of leaves and the length of disc flowers. In *C. virescens*, stem unbranched or sometimes 2-branches, whereas much branched in *C. tinctoria*. At the same time in *C. virescens* involucre 1.7–2 cm, while in *C. tinctoria* 1–1.5 cm. Also, in *C. virescens*, leaves 2-pinnatisect, the length of disc flowers 4.5–5 mm, while in *C. tinctoria* leaves 2–3-

pinnatisect, the length of disc flowers 3–4 mm. Because of these permanent and distinctive features, this taxon has been evaluated at the species level [10].

4. *Cota triumfetti* (All.) J. Gay, Fl. Sicul. Syn. 2: 867 (1855).
≡*Anthemis tinctoria* var. *triumfettii* L., Sp. Pl. 896 (1753) as triumfelti. ═ *Anthemis rigescens* Willd., Hort. Berol. 1 (6): t. 62 (1806). ═ *Anthemis dumetorum* Sosn., Věstn. Tiflissk. Bot. Sada n.s., 3-4: 160 (1927).
Type: Described from Europe (Hb. Linn. (Stockholm) 355 / 13, photo!).
Specimens examined: A1 Kırklareli: Demirköy, between Hamdibey to Yeşilce village, under mixed forest, 362 m, 23 July 2006, *M.U.Özbek* 2149 (GAZI!). **A4** Zonguldak: Gipfelgebiet des steineger Bergrasen, 21 August 1973, 1970 m, *F.Holtz* (Nr.01.277) & *P.Hanel* (EGE!); Kastamonu: zwischen Kastamonu und Ilgaz, 3 km nördlich des Ilgazdaği geçidi Lichtung im Tannenwald (*Abies nordmanniana*), 19 August 1973, 1650 m, *F.Holtz* (Nr.01.246) & *P.Hanel* (EGE!). **A6** Tokat: Niksar to Akkuş, 12 km from Akkuş, clearings of forest, 990 m, 25 July 2006, *M.U.Özbek* 2153 (GAZI!). **A7** Trabzon: Sümela to Maçka, 10 km from Maçka, under mixed forest, 800 m, 26 July 2006, *M.U.Özbek* 2154 (GAZI!); Giresun: Tamdere to Giresun, Aksu village, around Karagöl, subalpine meadows, 2000 m, 27 July 2007, *M.U.Özbek* 2661 (GAZI!). **A8** Rize: 25 km from İkizdere to Başköy (Cimil), high mountain steppe, 1860 m, 27 July 2006, *M.U.Özbek* 2163 (GAZI!); 2 km from Çamlıhemşin to Ayder, roadside, 485 m, 28 July 2006, *M.U.Özbek* 2168 (GAZI!); 2 km from Çamlıhemşin, Ayder to Kavrun uplands, clearings of forest, roadside, 1440 m, 28 July 2006, *M.U.Özbek* 2170 (GAZI!); İkizdere, around Başköy (Cimil), high mountain meadows, 2100 m, 29 July 2007, *M.U.Özbek* 2688 (GAZI!); Çaykara to Rize, Uzungöl, roadside, 1150 m, 28 July 1994, *N.Gören* (ISTE 68083!); Erzurum: Tuzla– Tortum, around Ban village, 09 August 1981, *N.Demirkuş* 1211 (HUB!); between Erzurum to İspir, W. of Rizekent village, Hanboğazı stream, *Populus tremula* forest, 2250 m, 14 August 1976, *A.Tatlı* 5309 (HUB!; KNYA!); Bayburt: between Bayburt to Aşkale, Kop pass, c. 2350 m, 16 August 1984, *Ö. Seçmen* et al. (EGE!). **A9** Artvin: Ardanuç to Ardahan, 5 km from Bülbülan upland, high mountain steppe, 2300 m, 29 July 2006, *M.U.Özbek* 2176 (GAZI!); Kars: Arpaçay, Gülyüzü to Zinzal, 2020 m, 21 July 2000, mountain meadows, *M.Vural* 8254 et al. (GAZI!); Hanak, Çat village, valley of Kımılık stream, 2050–2100 m, meadows, 11 August 1983, *A.Güner* 5321 & *B.Yılmaz* (HUB!); Sarıkamış to Karakurt 10 km, around Halibey bridge, *Pinus sylvestris* forest, 2000 m, 13 July 1979, *O.Güneş* 1329 (HUB!); Göle, 30–40 km from Kars, Balçeşme meadows, 15 August 1981, *N.Demirkuş* 1293 (HUB!); **B9** Van: Muradiye, Derviş upland, Hacı Cave, *Populus* alliance, 08 July 2001, 2400–2500 m, *O.Karabacak* s.n. (EGE!).
Flowering time: May–August.

Habitat: Roadside, rocky slopes, under mixed forest, clearings of forest, sub–alpin meadows, high mountain steppe, 400–2134 m.
Distribution: Spain, France, Switzerland, Italy, Romania, Caucasia.
Note: As stated in the Flora of Turkey, *C. triumfettii* is similar to *C. tinctoria* but usually stem about 60 cm and branched fastigiately above. Also, leaves longer (3–4 cm) with flat and not infolded segments [3].

5. *Cota melanoloma* (Trautv.) Holub, Folia Geobot. Phytotax. 9 (3): 270 (1974).

1. Stem and leaves greyish–green; primary segments 3–4 paired, upper leaves obovate–oblong……...……5a. subsp. ***melanoloma***
– Stem and leaves green; primary segments 5–6 paired, upper leaves ovate…….. ……5b. subsp. ***trapezuntica***

5a. subsp. ***melanoloma***
≡ *Anthemis melanoloma* Trautv., Bull. Soc. Imp. Naturalistes Moscou 41 (1): 461 (1868). ═ *Anthemis extrarosularis* Freyn & Sint., Oesterr. Bot. Z. 44: 146 (1894).
Type: [Turkey B8 Erzurum] in montibus Ssaganlug, Lagowski (photo E!).
Specimens examined: A4 Kastamonu: Ilgaz Dağı, Küçük Hacet, alpine, 2500 m, 02 August 2005, *M.U.Özbek* 1918 (GAZI!). **A8** Rize: İkizdere, Around Başköy (Cimil), 2200 m, meadows, 24 July 1984, *A.Güner* 6066 (GAZI!); Çamlıhemşin, Yukarı Amlaket upland, 2300 m, meadows, 10 August 1984, *A.Güner* 6611 & *M.Vural* (GAZI!); İkizdere, Gülyayla to Cihantepe, 2400–2500 m, meadows, 25 July 1985, *A.Güner* 6588 & *M.Vural* (GAZI!); Erzurum: İspir to Erzurum, 1 km N. of Gölyurt pass, 2220 m, 18 July 1984, *Max Nydegger* (ISTE 19282!); Bayburt: Bayburt to Aşkale, Kop Dağı, 2332 m, 28 July 2007, high mountain meadows, *M.U.Özbek* 2666 (GAZI!). **A9** Erzurum: Horasan, 7 km N. of Sıçankale village, around Çarıykı, 01 July 1974, *A.Yürül* s.n. (EGE!); Ardahan: Bülbülan to Kutul uplands, 15 July 1981, *N.Demirkuş* 1156 (HUB!); Posof, around Doğrular village, 1800–2200 m, 30 July 1985, *N.Demirkuş* 3140 (HUB!); Posof, Ilgar meadows (Çiçek Dağı), 2000–2400 m, 04 August 1982, *N.Demirkuş* 1752 (HUB!); Ardahan: Ardanuç to Ardahan 5.km, rocky slopes, 1050 m, 29 July 2006, *M.U.Özbek* 2173 (GAZI!); 15–20. km from Ardanuç to Ardahan, under *Quercus*, 1360 m, 29 July 2006, *M.U.Özbek* 2175 (GAZI!). **B7** Sivas: Divriği, Mursal village, 1800–2200 m, serpantine steppe, 17 August 1996, *A.A.Dönmez* 4929 (HUB!); Tunceli: Pülümür, Sülbüs Dağı, clearings of *Quercus* forest, 1600–2000 m, 17 June 1980, *Ş.Yıldırımlı* 3375 (HUB!); Pülümür to Mutu, 1840 m, *Hub.– Mor.* 11193 (ANK!).
Flowering time: July–August.
Habitat: Volcanic and limy slopes, high mountain meadows, clearings of mixed forest, open area of *Quercus* forest, 1050–2600 m.
Distribution: Russia.

5b. subsp. ***trapezuntica*** (Grierson) Oberpr. & Greuter, Willdenowia 33 (1): 40 (2003).
≡ *Anthemis melanoloma* Trautv. subsp. *trapezuntica* Grierson, Notes Roy. Bot. Gard. Edinburgh 33 (3): 411 (1975).
Type: [Turkey A8 Trabzon] Bayburt to Of, 3 km N. of pass top, 2400 m, Stainton & Henderson 6179 (holo E!).
Specimens examined: A7 Trabzon: Sümela to Maçka, 10 km from Maçka, under mixed forest, 800 m, 27 July 2006, *M.U.Özbek* 2154 (GAZI!); Soğanlı pass to Çaykara 3. km, high mountain meadows, 2275 m, 28 July 2007, *M.U.Özbek* 2679 (GAZI!). **A8** Rize: İkizdere, Başköy (Cimil), around Çermaniman peak, high mountain meadows, 2610 m, 29 July 2007, *M.U.Özbek* 2687 (GAZI!); Çamlıhemşin, between Yukarı Kavrun to Pornag to Arçevit, alpinic steppe, 2200–3000 m, 10 August 1980, *A.Güner* 2944 (HUB!). **A9** Artvin: Ardanuç, Kurdevar Dağı, 2800 m, 29 July 1982, *N.Demirkuş* 1634 (HUB!); Ardahan: Bülbülan to Kutul uplands, road through, 13 July 1981, *N.Demirkuş* 1107b.(HUB!).
Flowering time: July–August.
Habitat: Volcanic and limy slopes, high mountain meadows, clearings of mixed forest, 800–3000 m.
Distribution: Endemic. Euro–Sibirian element.
Note: This species differs from the other perennial *Cota* species by its phyllaries all dark-margined and paleae narrowly lanceolate. Also, stems are generally unbranched and 1-capitulate.

6. ***Cota antitaurica*** (Grierson) Holub, Folia Geobot. Phytotax. 11 (1): 83 (1976).
≡ *Anthemis antitaurica* Grierson, Notes Roy. Bot. Gard. Edinburgh 33: 211 (1974).
Type: [Turkey B6 Adana] d. Saimbeyli, Bozoğlan Dağı above Obruk Yayla, 2000–2100 m, rocky slopes, rare, 07 July 1952, *P.H.Davis* 19746 (holo. K!).
Specimens examined: B6 Adana: Saimbeyli – Tufanbeyli, Bozoğlan Mt., above Obruk highland, Karlıktepe, rocky slopes, 1650–1900 m, 20 June 2008, *B. Bani* 6120 (GAZI!); ibid., 1800–1850 m, 05 July 2008, *M.U.Özbek* 2752 (GAZI!).
Flowering time: June–July.
Habitat: Rocky slopes, 1600–1900 m.
Distribution: Endemic. Irano–Turanian element.
Note: *Cota antitaurica* is only known from the type locality. After 1952, it was collected for the second time by Dr. Barış Bani (*B.Bani* 6120). This species differs from the other perennial *Cota* species by its campanulate involucre, densely white-lanate indumentum, petiolate basal leaves and narrowly dark-margined phyllaries.

7. ***Cota oxylepis*** Boiss., Diagn. Pl. Orient. ser. 2, 5: 109 (1856).

≡ *Anthemis oxylepis* Boiss., Fl. Orient. 3: 282 (1875).
Lectotype: [Turkey. C5 Niğde] supra Bulgarmaden, *Balansa* (K!); **paralectotype**: [Turkey. İçel] Gullek Boghas, 1160 m, (1853), Kotschy s.n. (K!).
Specimens examined: C5 Niğde: Ulukışla, Maden village, around Bulgar maden, stony slopes and screes, 2100 m, 25 July 2007, *M.U.Özbek* 2655 (GAZI!); ibid., 26 July 2007, *M.Vural* 10066 et al. (DUOF!).
Flowering time: July.
Habitat: Stony slopes and screes, 1160–2134 m.
Distribution: Endemic. Irano–Turanian element.
Note: This local endemic species differs from the other perennial *Cota* species by its paleae longer than disc flowers and discoid capitula. *C. oxylepis* is closely related to *C. hamzaoglui*, but it differs from *C. hamzaoglui* by its subglabrous to sparsely lanate (not densely lanate) indumentum of the lower surface of the leaf lamina, primary segments of the lamina in 4–8 pairs (not 3 or 4 pairs), secondary segments in 4–8 pairs (not 1–3 pairs), and achenes 3–3.5 mm long (not 2–2.5 mm long).

8. *Cota hamzaoglui* Özbek & Vural, Turkish J. Bot. 35 (4): 331–336 (2011).
Holotype: [Turkey A2 Bursa] Uludağ, above hotels, between cable cars and old volfram mine, 2050–2100 m., among *Juniperus* and *Vaccinium* subalpine shrubs, 31.07.2009, *M.U.Özbek* 2812 & *M.Vural* (holo. GAZI!; iso. BOZOK Hb!., BULU!, HUB!, ANK!).
Paratype: ibid., 2100 m, 29 July 2006, *Hamzaoğlu* 4403 et al. (BOZOK Hb!., BULU!, HUB!, ANK!).
Specimens examined: A3 Ankara: Nallıhan, Karacasu village, Sarıçal Dağı, under *Pinus nigra* forest, 1460 m, 05 July 2010, *G.Turgut* 1390 (GAZI!); ibid., 07 August 2010, *M.U.Özbek* 2827 (GAZI!); ibid., *Juniperus communis* subsp. *nana*, clearings of *Pinus nigra* forest, 1650 m, 07 August 2010, *M.U.Özbek* 2829 (GAZI!). **B2** Kütahya: Gümüşdağı, around Radar station, clearings of *Pinus nigra*, 1822 m, 09 July 2019, *M.U.Özbek* 3156 (GAZI!); Gediz, Murat Dağı, from the ski center to the summit, serpantine, 2050 m, 02 August 2015, *Koç* 2128 & *Hamzaoğlu* (GAZI!). **B3** Bilecik: Bozüyük, between Çiçekliyayla and Karagöl, clearings of *Pinus nigra,* 1779 m, 07 July 2019, *M.U.Özbek* 3155 (GAZI!); Eskişehir: Gölcük Plateu, Türkmen Mountain, 1650 m, July 2011, *A.Ocak* et al. s.n. (GAZI!).
Flowering time: July–August.
Habitat: *Juniperus* and *Vaccinium* subalpine shrubs, clearings of *Pinus nigra*, 2050–2100 m.
Distribution: Endemic, Euro–Sibirian element.
Note: *Cota hamzaoglui* differs from *C. fulvida* by its sparsely lanate (not sericeous) indumentum, leaf lamina 1–2.5 cm long, 0.5–1 cm wide (not 1.5–5 cm long, 0.75–1.5 cm wide), primary segments of the lamina in 3 or 4 pairs (not 4–6 pairs), and secondary segments in 1–3 pairs (not 4–8 pairs) [11].

9. *Cota fulvida* (Grierson) Holub, Folia Geobot. Phytotax. 11 (1): 83 (1976).
≡ *Anthemis fulvida* Grierson, Notes Roy. Bot. Gard. Edinburgh 33 (2): 213 (1974).
Type: [Turkey B3 Afyon] Sultandagh in jugis alpinis supra Engeli (Engilli), 1850 m, 28 June 1899, *Bornmüller* 4656 (holo. E!, iso. K! W!).
Specimens examined: C3 Isparta: Yenişarbabemli, above Melikler pastures, clearings of *Quercus vulcanica* and *Pinus nigra* forest, dry stream bed, 1680–1800 m, 27 August 2012, *M.U.Özbek* 2852 & *M.Arslan* (GAZI!).
Flowering time: June–July.
Habitat: Under *Pinus nigra* forest and *Juniperus communis* subsp. *nana*, clearings of *Quercus vulcanica* and *Pinus nigra* forest, 1680–1850 m.
Distribution: Endemic. Irano–Turanian element.
Note: *Cota fulvida* was collected for the first time by Bornmueller in 1899. Field studies were carried out between 2006 and 2010, but it could not be collected. In 2012, it was collected from another locality (Isparta) except its type locality. In *C. fulvida* the paleae is longer than the disc flowers and also it has discoid capitula, such as *C. oxylepis* and *C. hamzaoglui*. However it differs from the these species by its sericeous indumentum, primary segments of the lamina in 4-6 pairs, and secondary segments in 4-8 pairs.

10. *Cota altissima* (L.) J. Gay, Fl. Sicul. Syn. 2: 867 (1855).
≡ *Anthemis altissima* L., Sp. Pl. 2: 893 (1753) emend. Sprengel. Syst. Veg. 3: 594 (1826). ═ *Anthemis cota* L., Sp. Pl. 2: 893 (1753) emend. Vis. Fl. Dalm. 2: 78 (1847).
Type: Described from Italy (Hb. Linn. 1016/3, photo!).
Specimens examined: A1 Çanakkale: 5 km from Gelibolu, fields, 20 m, 05 June 2007, *M.U.Özbek* 2334 (GAZI!); Truva, roadside, 50 m, 04 June 2007, *M.U.Özbek* 2332 (GAZI!); Gökçeada, s.l, 18 June 1976, *Ö.Seçmen* 2057 & *E. Leblebici* (EGE!); Tekirdağ: Eriklice village, *Olea europae* garden, 10 m, 05 June 2007, *M.U.Özbek* 2339 (GAZI!); Balıkesir: 5 km from Gömeç to Burhaniye, roadside, 70 m, 04 June 2007, *M.U.Özbek* 2329 (GAZI!). **A2** İstanbul: Kartal to Yakacık, 17 May 1950, *A.Berk* s.n. (HUB!); Bakırköy, 16 June 1950, *A.Berk* s.n. (HUB!). **A3** Bolu: Mudurnu, Adapazarı to Mudurnu old way, 475 m, 24 May 2009, *S.S.Kanoğlu* 1579 & *A.Kaya* (DUOF!). **A5** Amasya: Yukarı Baraklı village, around Boyarlık, ca. 850 m, steppe, 01 October 1987, *S.Peker* 1742 (GAZI!). **A8** Artvin: Ardanuç, ca. 500 m, 27 June 1957, *P.H.Davis* 3017 & *Hedge* (ANK!). **B1** İzmir: Kemalpaşa to Ulucak, fire area, 25 May 1970, *S.Oflas* 14 (EGE!); Gümüldür, 26 May 1964, *Regel* s.n. (EGE!); Kemalpaşa, around Ovacık village, ca. 690 m, 17 June 1977, *Ö.Seçmen* 1146 (EGE!). **B7** Elazığ: around Fırat University, 1100 m, 29 June 1974. *N.Çelik* s.n. (HUB!); Erzincan: İliç, Bağıştaş, edge of Karasu, 870 m, 17 May 1980, *Ş.Yıldırımlı* 2774 (HUB!). **C2** Denizli: between Çivril to Işıklı, fallow fields, ca.

800–830 m, 06 June 1983, *Y.Gemici* 1936 et al. (EGE!); 15 km S. of Denizli, meadows, ca. 540 m, 05 June 1974, *C.Ödemiş* s.n. (EGE!). **C3** Antalya: Kemer, Ovacık, ca. 1100–1200 m, *P.H.Davis* 15191 (ANK!); Aksu, ca. 20 m, 15 April 1966, *G.Oğuz* s.n. (EGE!). **C4** Konya: Ermenek, Kazancı town, wetland, 1150 m, 18 June 1983, *H.Sümbül* 1962 (HUB!); Ermenek, Danan village, around Ayaplı, through Göksu river, 600 m, 15 September 1983, *H.Sümbül* 2482 (HUB!; BOLU!). **C5** Adana: 2 km from Adana to Karataş, roadside, 15 m, 06 May 2007, *M.U.Özbek* 2257 (GAZI!); Düziçi (Haruniye), Yukarı Hacılar district, Ağhüsneler street, landside, 630 m, 01 June 2006, *M.U.Özbek* 2004 (GAZI!); İçel: Çamlıyayla (Namrun), around Namrun castle, roadside, 20 June 2007, *M.U.Özbek* 2398 (GAZI!). **C6** Osmaniye: Hasanbeyli, around radar station, clearings of *Quercus* forest, 1300 m, 21 June 2007, *M.U.Özbek* 2403 (GAZI!); Antakya: İskenderun, Akarca village, Amanos Mts., landside, 385 m, 02 June 2006, *M.U.Özbek* 2006 (GAZI!); Arsuz, around Kale village, clearings of forest, roadside, 0–5 m, 07 June 2008, *M.U.Özbek* 2713 (GAZI!); Hassa, Akbez town, Yeniyapan village, landside, 685 m, 02 June 2006, *M.U.Özbek* 2010 (GAZI!); Kahramanmaraş: Engizek Dağı, around Aksu district, 1000–1100 m, 12 June 1987, fields, *H.Duman* 1388 (GAZI!); Gaziantep: İslahiye, 2 km E. of Edilli village, riverside, 630 m, 20 May 2006, *Ş.Çakır* 726 (VANF!). **C7** Şanlıurfa: Siverek to Hilvan 30 km., around Küllaplı village, steppe, 620 m, 11 June 1980, *A.Güner* 2283 & *M.Koyuncu* (HUB!); Siverek, 16 June 1962, *Regel* s.n. (EGE!). **C8** Diyarbakır: 5 km S. of Diyarbakır, c. 650 m, 31 May 1957, *P.H.Davis* 28755 & *Hedge* (ANK!); Diyarbakır to Silvan, fields, 24 June 1954, *P.H.Davis* 22102 (ANK!).

Flowering time: April–June.

Habitat: Roadside, fields, steppe, clearings of *Quercus* forest, ruderal areas, s.l.–1300 m.

Distribution: South Europe, Russia, Central Asia, Iran, Palaestina.

Note: This species differs from the other annual *Cota* species by its paleae acuminate, inner phyllaries short ciliate and leaves ovate–oblong or elliptic. During our field study, an abnormal specimen (province of Şanlıurfa, South-eastern Anatolia) was detected with ray flowers similar to disc flowers.

11. *Cota coelopoda* **(**Boiss.) Boiss, Diagn. Pl. Orient. ser. 2 (3): 21 (1856).

≡ *Anthemis coelopoda* Boiss., Diagn. Pl. Orient. ser. 1 (11): 12 (1849). ═ *Cota bourgaei* Boiss. Fl. Orient. 3: 284 (1875). ═ *Anthemis cota* L. var *brevicuspidata* Eig, Palestine J. Bot., Jerusalem ser. 1: 204 (1938). ═ *Anthemis cota* L. var. *longicuspidata* Eig, loc. cit. (1938). ═ *Cota coelopoda* **(**Boiss.) Boiss. var. *bourgaei* (Boiss.) Özbek & Vural, Türk. Bitkileri List. 146 (2012). ═ *Cota coelopoda* **(**Boiss.) Boiss. var. *longiloba* (Grierson) Özbek & Vural, Türk. Bitkileri List. 147 (2012).

Lectotype: [Turkey B1 İzmir] in planitiebus circa Smyrnam (İzmir), *Boissier* s.n. (G!); **paralectotype**: (C2 Aydın) circa Gheyra (Karacasu), *Boissier* s.n. (G! K!).

Specimens examined: A4 Kastamonu: Ilgaz Dağı, Çatören village, clearings of forest, 1230 m, 06 June 2007, *M.U.Özbek* 2351 (GAZI!). **B1** Manisa: 6 km from Kula to Salihli, Alaşehir–Denizli crossroad, *Pinus* plantation area, 98 m, 02 May 2007, *M.U.Özbek* 2216 (GAZI!); 5 km from Sarıgöl to Buldan, roadside, 640 m, 03 June 2007, *M.U.Özbek* 2311 (GAZI!); İzmir: Kemalpaşa, entrance of Sarılar village, under *Olea europae*, 185 m, 28 May 2006, *M.U.Özbek* 1986 (GAZI!); Kemalpaşa, Ovacık köyü, around Forest Management of Bayındır, meadows, 785 m, 29 June 2006, *M.U.Özbek* 2111 (GAZI!); between Tire to İncirliova, 2 km from Kazımpaşa fountain, landside, 930 m, 28 May 2006, *M.U.Özbek* 1982 (GAZI!); Kemalpaşa, around Ovacık village, roadside, 750 m, 28 May 2006, *M.U.Özbek* 1985 (GAZI!); Bozdağ, around Ovacık village, roadside, 1170 m, 27 May 2006, *M.U.Özbek* 1976 (GAZI!); Gölcük, Gölcük to Ödemiş, former road, slopes, 820 m, 27 May 2006, *M.U.Özbek* 1977 (GAZI!); Salihli to İzmir road, parting of the way Ödemiş to Bozdağ, roadside, 120 m, 27 May 2006, *M.U.Özbek* 1971 (GAZI!). **B3** Isparta: Şarkikaraağaç, entrance of Kızıldağ Natural Park, 1300 m, 01 June 1996, *B.Mutlu* 1399 (HUB!). **B4** Ankara: Kazan, roadside, 09 June 2005, *M.U.Özbek* 1881 (GAZI!). Kırıkkale: Keskin, Böbrek Dağı, Köprüköy, cemetery, steppe, 550 m, 22 June 1991, *Ü.Güler* 1736 (GAZI!). **B5** Aksaray: between Taşpınar to Hasan Dağı, roadside, 1075 m, 28 June 2005, *M.U.Özbek* 1890 (GAZI!); Hasan Dağı, slopes of N.W, Türkmenlik, sparcely *Quercus* forest, ca. 1450 m, 15 June 1973, *A.Düzenli* 353 (ANK!); Niğde: 11 June 1952, *P.H.Davis* 18851 & *R.Çetik* (ANK!); Nevşehir: Ürgüp, ca. 1200–1300 m, *P.H.Davis* 19127 & *R.Çetik* (ANK!). **B7** Erzincan: Kemaliye, 13 km from Salihli to Bağıştaş, rocky and stony slopes, 1270 m, 04 July 2007, *M.U.Özbek* 2525 (GAZI!); İliç, Bağıştaş, edge of Karasu, 870 m, 17 May 1980, *Ş.Yıldırımlı* 2773 (HUB!); İliç, above Çöpler village, Munzur Mts., Çal Mt., 1300–2000 m, 30 May 1979, *Ş.Yıldırımlı* 1823 (HUB!); Sivas: Divriği, Dumluca Mt., 30 May 1968, *T.Baytop* (ISTE 12887!); Tunceli: Ovacık, Karagöl Valley, stony slopes, 1300 m, 07 July 1980, *Ş.Yıldırımlı* 3494 (HUB!); Tunceli–Pülümür 16 km, ca. 1000 m, 07 June 1957, *P.H.Davis* & *Hedge* 29218 (ANK!). **B9** Muş: Malazgirt, around of Karıncalı village, steppe, 1509 m, 29 May 2006, *L.Behçet* 655 et al. (VANF!). **C2** Denizli: Honaz Dağı, between Honaz to Menteş, fields, 16 May 1973 *E.Tuzlacı* (ISTE 24695!). **C3** Burdur: Dinar to Burdur 20. km, clearings of *Quercus* and *Pinus nigra* forest, 1115 m, 11 June 2008, *M.U.Özbek* 2747 (GAZI!); Tefenni to Burdur 18.km, steppe, 1115 m, 10 June 2008, *M.U.Özbek* 2737 (GAZI!); Isparta: Karabağlar, near Eğridir, 01 June 1955 *A.Baytop* & *T.Baytop* s.n. (HUB!); 2–3 km. S.W. of Gönen, steppe, ca. 500 m, *H.Peşmen* 3954 & *B.Yıldız* (HUB!); Karabağlar, near Eğridir, 01 June 1955, *Hub.–Mor.* (ISTE 4322!). Antalya: Korkuteli to Elmalı, entrance of Çukurelma village, roadside, 1130 m, 10 June 2008, *M.U.Özbek* 2731 (GAZI!). **C4** Konya: Divanlar village, fields, ca. 1150 m, 26 June 1983, *H.Dural* 1464 (KNYA!). **C5** Konya: Ereğli, Aydos Dağı, Delimahmutlu, karasırt, 1600 m, 26

June 1976, *S.Erik* 1527 (HUB!); Ereğli, Aydos Dağı, İvriz, rocky slopes, 1500 m, 15 May 1977, *S.Erik* 1828 (HUB!); İçel: Çamlıyayla (Namrun), around Namrun castle, roadside, 20 June 2007, *M.U.Özbek* 2397 (GAZI!); Gözne, Darısekisi village, 20 June 2007, *M.U.Özbek* 2391 (GAZI!); Niğde: 5 km from Ulukışla to Pozantı, *Cedrus* afforestation area, 1250 m, 28 June 2005, *M.U.Özbek* 1891 (GAZI!); near Ulukışla, 18 June 1971, *T.Gözler* (ISTE 20489!). **C6** Kahramanmaraş: Süleymanlı, between Ilıca to Beşer, 800 m, 14 June 1981, *B.Yıldız* 2703 (HUB!); Osmaniye: Düziçi (Haruniye), Mezdağı road, around Soğucak upland, roadside, 1150 m, 06 July 2008, *M.U.Özbek* 2756 (GAZI!); Düziçi (Haruniye), Hacılar district, landside, 21 June 2007, *M.U.Özbek* 2406 (GAZI!); Düziçi (Haruniye), Mezdağı road, around Soğucak highland, roadside, 1150 m, 06 July 2008, *M.U.Özbek* 2756 (GAZI!); Gaziantep: İslahiye, 500 m. NW of Koçcağız village, 1080 m, 29 June 2006, *Ş.Çakır* 905 (VANF!). **C7** Şanlıurfa: Siverek to Hilvan 30 km, around Küllaplı village, steppe, 620 m, 11 June 1980, *A.Güner* 2283 & *M.Koyuncu* (HUB!).
Flowering time: May–July.
Habitat: Steppe, fields, roadside, 70–2000 m.
Distribution: Former Yugoslavia, Bulgaria, Greece, North Syrian, North Iran.
Note: According to Flora of Turkey and A Checklist of the Flora of Turkey this species has been evaluated as three variety [3,10]. In *Cota coelopoda* var. *coelopoda*, leaves 2(–3)-pinnatisect, secondary segments rather broad, 1–2 mm broad and involucre 0.5–1 cm broad. In *C. coelopoda* var. *longiloba* and *C. coelopoda* var. *bourgaei* leaves are (2–)3-pinnatisect, secondary segments narrow and 0.5–1 mm broad. *C. coelopoda* var. *longiloba* differs from *C. coelopoda* var. *bourgaei* by its involucre 1.5–2 cm broad (not 1–1.5 cm broad) and secondary segments of leaves slender, 6–8 mm (not 2–5 mm). Because of these small morphological differences, we think that this species cannot be considered as separate taxa.

12. *Cota austriaca* (Jacq.) Sch. Bip., Oesterr. Bot. Wochenbl. 4: 155 (1854).
≡ *Anthemis austriaca* Jacq., Fl. Austriac. 5: 22 (1778). ═ *Anthemis zangelana* Sosn. ex Grossh. loc. cit. (1949).
Type: Described from Austria.
Specimens examined: A1 Tekirdağ: Şarköy, 5 km from Gölcük, open area of *Quercus* forest, 300 m, 05 June 2007, *M.U.Özbek* 2343 (GAZI!); between Tekirdağ to Hayrabolu 19 km, fields, *A.Baytop* & *N.Özhatay* (ISTE 19819!); around Çorlu, 20 May 1969, *G.Ertem* (ISTE 15174!); Edirne: Keşan to Malkara, 20 km from Malkara, roadside, 75 m, 22 July 2006, *M.U.Özbek* 2143 (GAZI!); Sarayiçi, 14 July 1981, *G.Çakırer* & *H.Çakırer* (ISTE 47022!); between Keşan to İpsala, 19 May 1970, *A.Baytop* & *F.Öktem* (ISTE 17955!); Kırklareli: between Çakıllı to Vize, 11 June 1968, *A.Baytop* (ISTE 13201!); Kırklareli, roadside, 08 June 1977, *E.Palaoğlu* (ISTE 38059!); Çanakkale: Truva, roadside, 50 m, 04 June 2007, *M.U.Özbek* 2333 (GAZI!). **A2** Bursa: Bursa to Keles, 2 km from Soğukpınar, clearings of *Quercus*

forest, 840 m, 21 July 2006, *M.U.Özbek* 2139 (GAZI!). **A3** Ankara: Ayaş to Beypazarı, roadside, 560 m, 16 May 2005, *M.U.Özbek* 1823 (GAZI!); Beypazarı, Kumkaya, 28 April 1986, *N.Özsoy* 1008 (GAZI!); Bolu: Hamzabey road, 08 June 2000, *N.Sümer* 1168 (BOLU!); Yeniçağ, lakeside, 07 June 2001, *N.Sümer* 1702 (BOLU!). **A4** Ankara: Kızılcahamam, around Kargasekmez, steppe, 11 May 2007, *M.U.Özbek* 2280 (GAZI!); Ayaşbeli, Akkayatepe, 1250–1400 m, 27 June 1986, steppe, *M.Vural* 4194 (GAZI!); Çubuk II Dam, 18 May 1986, ca. 1100–1200 m, *F.Demircioğlu* 1091 (GAZI!); Ayaşbeli, Akkayatepe, 1200 m, 27 June 1986, fallow fields, *M.Vural* 4206 (GAZI!); Çubuk, Karagöl, steppe, 1530 m, 04 August 1974, *S.Erik* 678 (HUB!); Ayaş Mts., Ayaşbeli, *Astragalus microcephalus* and *Astragalus lycius* alliance, ca. 1150 m, 19 May 1975, *Y.Akman* 3072 (ANK!); Kırıkkale: Delice, between İmirli to Cingeyli village, fields, 900 m, 03 June 1990, *A.A.Dönmez* 2334 & *A.Güner* (HUB!); Delice, Samsun road, 700 m, 29 April 1989, *A.A.Dönmez* 1243 (HUB!). **A8** Rize: Rize, coastal road, 10 m, roadside, 25 May 1985, *A.Güner* 6428 (HUB!); Dinek Mt., between Balışeyh–Kılevli, 900 m, 27 May 2001, *S.A.Demir* 1045 (BOZOK!). **B1** Balıkesir: 12 km from Akhisar to Balıkesir, meadows, 185 m, 27 May 2005, *M.U.Özbek* 1851 (GAZI!); İzmir: Kemalpaşa, around Ovacık village, roadside, 750 m, 29 June 2006, *M.U.Özbek* 2112 (GAZI!); Kemalpaşa, entrance of Sarılar village, under *Olea europae*, 185 m, 29 June 2006, *M.U.Özbek* 2114 (GAZI!); Kemalpaşa, from Yukarıkızılca to Mahmut Dağ, bushes, 250–300 m, 3 May 1991, *A.Aksoy* 13 (ERC!); Kemalpaşa, from Cumali to Mahmut Dağ, fire area, 650–700 m, 4 July 1991, *A.Aksoy* 564 (ERC!). **B2** Balıkesir: Bandırma, between Tatlısu to Şahinburgaz, *Cistus* alliance, 21 May 1976, *Y.Akman* 9232 (ANK!); Yeşilhisar village, Savaştepe, 05 July 1980, *G.Çakırer* & *H.Çakırer* (ISTE 45274!); Afyonkarahisar: Afyonkarahisar to Uşak road, meadows, 1030 m, 02 May 2007, *M.U.Özbek* 2210 (GAZI!); Uşak: Banaz, edge of railway, meadows, 925 m, 02 May 2007, *M.U.Özbek* 2212 (GAZI!); roadside, 660 m, 02 May 2007, *M.U.Özbek* 2213 (GAZI!); Kütahya: Tavşanlı to Domaniç, entrance of Tunçbilek, meadows, 805 m, 02 June 2007, *M.U.Özbek* 2301 (GAZI!); Simav to Selendi, 5 km from Çıkrıkçı, open area of *Cistus,* limestone soils, 1050 m, 03 June 2007, *M.U.Özbek* 2309 (GAZI!). **B3** Eskişehir: Ankara to Eskişehir, 15 km from Eskişehir, around organized industry, afforestation area, roadside, 900 m, 02 June 2007, *M.U.Özbek* 2298 (GAZI!); Kütahya road, 16 June 1968, *A.Pamukçuoğlu* s.n. (HUB!); Karakütük fire tower, ca. 1700 m, 30 June 1970, *T.Ekim* 54 (ANK!); Konya: Akşehir, Vahapgazi (Kesikbaş), 1040 m, 27 May 1974, *G.Dökmeci* & *Y.Doğantan* (ISTE 28607!); Sultandağları, above Engili village, around Harlak, 1350 m, 07 July 1975, *G.Dökmeci* (ISTE 32707!); Doğanhisar, around Kartaltepe, ca. 1650 m, 10 June 1979, *H.Ocakverdi* 464 (KNYA!). **B4** Ankara: Ankara to Şereflikoçhisar, 20 km from Şereflikoçhisar, edge of Tuz Lake, 910 m, 31 May 2007, *M.U.Özbek* 2295 (GAZI!); Beytepe, Maslak valley, steppe, 950 m, 26 May 1975, *S.Erik* 1165 (HUB!);

Kırıkkale: Keskin, Böbrek Dağı, Çilkili village, around S. of Kızılözü, 600 m, 21 June 1992, *Ü.Güler* 1711 (GAZI!); Dinek Dağı, between Balışeyh to Kılevli, roadsides, 900 m, 27 May 2001, *S.A.Demir* 1045 (ERC!); Konya: Akşehir to Konya, 30 km from, around Bahçecik village, steppe, 19 June 2007, *M.U.Özbek* 2378 (GAZI!). **B5** Kayseri: Yahyalı, around Eskidut, ca. 2500 m, 05 July 1982, *N.Demirkuş* 1978 (HUB!). **B7** Erzincan: İliç, around Yakuplu village, 1200 m, 31 May 1979*, Ş.Yıldırımlı* 1869 (HUB!). **C1** Aydın: Eski Çine road, Çine valley, around Kayırlı dere, rocky slopes, 200 m, 23 April 2006, *M.U.Özbek* 1932 (GAZI!). **C2** Muğla: 38 km from Muğla to Kale, Çakmak village, roadside, 835 m, 30 May 2006, *M.U.Özbek* 1997 (GAZI!). **C3** Burdur: 10 km from Karamanlı to Burdur, steppe, 1100 m, 04 May 2007, *M.U.Özbek* 2239 (GAZI!); Karamanlı to Burdur 10. km, steppe, 1100 m, 04 May 2007, *M.U.Özbek* 2283 (GAZI!); Karamanlı to Tefenni, roadside, 1130 m, 29 June 2005, *M.U.Özbek* 1897 (GAZI!); Isparta: 20 km from Akşehir to Gelendost, steppe, 1535 m, 06 July 2005, *M.U.Özbek* 1901 (GAZI!); Antalya: Akçay to Elmalı 6. km, roadside, 1060 m, 31 May 2006, *M.U.Özbek* 1999 (GAZI!). **C4** Konya: Seydişehir, around mine, under *Juniperus excelsa*, ca. 1550 m, 16 June 1981, *H.Ocakverdi* 1981 (ANK!); Obruk to Kızıltepe, 1250 m, 15 June 1982, *H.Dural* 978 (KNYA!); Sarayköy to Takkalı Hill, E. of slopes, ca. 1400 m, 30 May 1979, *H.Dural* 92 (KNYA!); S. of Kadınhanı, *Hordeum vulgare* fields, ca. 1100 m, 27 May 1982, *R.Çetik* 1979 et al. (KNYA!); Karaman: Pınarbaşı to Kızılyaka, 3 km N.E of Pınarbaşı, clearings of *Quercus* forest, 1205 m, 28 June 2005, *M.U.Özbek* 1894 (GAZI!). **C5** Niğde: between Çiftehan to Alihoca village, rocky slopes, 1300 m, 22 June 2007, *M.U.Özbek* 2445 (GAZI!); Kamışlı, Asar upland, landside, 1290 m, 22 June 2007, *M.U.Özbek* 2446 (GAZI!); Melendiz Dağları, Okçu village, N.W of slopes, ca. 1700 m, 04 July 1986, *B.Eyce* 639 (KNYA!); Adana: Pozantı to Ulukışla, 35 km. from Ulukışla, roadside, 1060 m, 04 June 2006, *M.U.Özbek* 2019 (GAZI!). **C7** Şanlıurfa: 20 km from Şanlıurfa to Akçakale, fields, 380 m, 08 May 2007, *M.U.Özbek* 2277 (GAZI!); Ceylanpınar, 5 km from Beyazkule to Viranşehir, 04 May 1995, 550 m, roadside, *N.Adıgüzel* 1996 (GAZI!). **C8** Mardin: Kızıltepe, fields, ca. 600 m, 26 May 1967, *P.H.Davis* 28656 & *Hedge* (ANK!).

Flowering time: April– June.

Habitat: Steppe, fields, roadsides, clearings of forest, meadows, s.l.–2500 m.

Distribution: Central and East Europe, Balkans, Crimea, Russia, Iran.

Note: *Cota austriaca* and *C. coelopoda* are closely related species, but it differs usually a more slender plant with leaves that are only 2-pinnatisect (not becoming 3-pinnatisect as in *C. coelopoda*), and on peduncles that become only slightly swollen at maturity (not peducles thickened). Moreover, *C. austriaca* acuminate apex of the paleae is always shorter than *C. coelopoda.*

13. *Cota dipsacea* (Bornm.) Oberpr. & Greuter, Willdenowia 33 (1): 40 (2003).

Type: [Turkey C1 İzmir] in montis Mesogis (Aydın Da.) pontibus superioribus, *Bornmueller* 1906: 9643 (photo B!).
≡ *Anthemis dipsacea* Bornm, Mitth. Thüring. Bot. Vereins n.s. 23: 23 (1908). Ic: Bornm., op. cit. 24: 71, f 5 (1909).
Specimens examined: B1 İzmir: Bozdağ, Gündalan upland, steppe, 1300–1500 m, 03 June 2007, *M.U.Özbek* 2317 (GAZI!); ibid., serpentine, 1515 m, 29 June 2006, *M.U.Özbek* 2115 (GAZI!); Armutlu to Bayramlı, *Pinus brutia* forest, 410 m, 04 June 2007, *M.U.Özbek* 2323 (GAZI!); Bozdağ – Ödemiş, supra Allahdiyen village, rocky slopes, 1000 m, 27 May 2006, *M.U.Özbek* 1972 (GAZI!); Bozdağ – Ödemiş, 10 km from Bozdağ, calcareous places, 1000 m, 27 May 2006, *M.U.Özbek* 1974 (GAZI!); Tire to İncirliova 20 km, hillsides, 1020 m, 27 May 2006, *M.U.Özbek* 1980 (GAZI!).
Flowering time: April–June.
Habitat: Mountain steppe, among dwarf scrub, serpentine, clearings of *Pinus brutia* forest, 410–1515 m.
Distribution: Endemic, Mediterranean element.
Note: This endemic species differs from the other annual *Cota* species by its paleae distinctly longer than disc flowers.

14. *Cota palaestina* Reut. ex Unger & Kotschy, Ins. Cypern: 240 (1865).
≡ *Anthemis palestina* (Reut ex Kotschy) Reut ex Boiss., Fl. Orient. 3: 283 (1875). ═ *Anthemis melanolepis* Boiss., Fl. Orient. Suppl. 297 (1888).
Lektotype: Lebanon prope Berthrum (Beirut), *Gaiillardot* 2416 (in Grierson in Notes R.B.G. Edinb. 33: 216 (1974).
Specimens examined:
C4 İçel: Anamur, 14 June 1976, *Y. Akman* & *Quezel* 6183 (ANK!). **C6** Antakya: St Peter (Pierre) Church, dried–up stream bed, 180 m, 02 June 2006, *M.U.Özbek* 2009 (GAZI!); ibid., 115 m, 07 May 2007, *M.U.Özbek* 2276 (GAZI!); ibid., 115 m, 09 April 2008, *M.U.Özbek* 2703 (GAZI!); ibid., 08 June 2008, *M.U.Özbek* 2715 (GAZI!); Antakya, St. Peter's Church, 150–300 m., edge of fields, 27 April 1957, *P.H.Davis* & *Hedge* 27239 (ANK!); Gaziantep: İslahiye, 3 km W. of Koçcağız village, 680 m, 5 May 2007, *Ş.Çakır* 1216 (VANF!).
Flowering time: April–May.
Habitat: Hillsides, macchie, fields, dried–up stream bed, s.l.–900 m.
Distribution: Syrian, Lebanon, Palaestina, Cyprus. East Mediterranean element.
Note: Because of truncate paleae this species differs from the other annual *Cota* species.

15. *Cota wiedemanniana* (Fisch. & C.A.Mey.) Holub, Folia Geobot. Phytotax. 9 (3): 270 (1974).

≡ *Anthemis wiedemanniana* Fisch. & C.A.Mey., Index Seminum.[St. Petersburgh (Petropolitanus)] 2: 27 (1836).
═ *Anthemis ormenioides* Boiss., Diagn. Pl. Orient. ser. 1(4): 5 (1844). ═ *Cota ormenioides* Boiss., Diagn. Pl. Orient. ser. 2 (3): 21 (1856).
Type: [Turkey] in Natolia, Wiedemann (LE?).
Specimens examined: A3 Ankara: Beypazarı, Sarıyer Dam, meadows, 470 m, 16 May 2005, *M.U.Özbek* 1824 (GAZI!). **A4** Ankara: Kızılcahamam, around Kargasekmez, steppe, 11 May 2007, *M.U.Özbek* 2281 (GAZI!); Kızılcahamam Soğuksu Natural Park, around Vel hill, 1350–1400 m, 30 May 1990, clearings of forest, *M.U.Özbek* 1478 (GAZI!); between Kazan to Örencik village, 830–900 m, dried–up river bed, 16 June 1989, *A.Güner* 7163 (HUB!); Ayaş Mts., Çamıllı village, stepe, 26 May 1975, *Y.Akman* 6563 (ANK!); Mürtet plain, 15 May 1960*, R.Çetik* & *P.H.Davis* 20293 (ANK!); Etlik, 14 May 1928, *Müller* 69 (ANK); Cebeci, 20 June 1932, *W.Kotte* 573 (ANK!). Kırıkkale: Keskin, Sarkın village, Kırklaryediler Dağı, 1400 m, steppe, 19 June 1993, *A.A.Dönmez* 3272 (HUB!); 5 km S. of Sulakyurt, steppe, 1000 m, 25 May 1990, *A.A.Dönmez* 2026 (HUB!) Dinek Dağı, between Balışeyh–Kılevli, roadsides, 900 m, 27 May 2001, *S.A.Demir* 1046 (BOZOK!). **A5** Çorum: Osmancık, around Tepeköy, ca. 800 m, 15 June 1975, *M.Kılınç* 3000 (ANK!). **B1** İzmir: Bozdağ, around Ovacık village, roadside, 1170 m, 27 May 2006, *M.U.Özbek* 1975 (GAZI!); Gölcük to Ödemiş, former road, slopes, 820 m, 27 May 2006, *M.U.Özbek* 1978 (GAZI!); Kemalpaşa, Ovacık village, around Management of Bayındır Forest, meadows, 785 m, 28 May 2006, *M.U.Özbek* 1984 (GAZI!); Manisa: Manisa Dağı, 750 m, 27 May 1980, *A.Baytop* (ISTE 44523!). **B2** Manisa: Alaşehir, after 5 km from Kozluca village, clearings of forest, 1080 m, 03 June 2007, *M.U.Özbek* 2313 (GAZI!); Kütahya: Simav to Selendi, 5 km from Çıkrıkçı, clearings of *Cistus*, limestone soils, 1050 m, 03 June 2007, *M.U.Özbek* 2308 (GAZI!). **B3** Eskişehir: Türkmen Mt., Türkmenbaba tomb, ca. 1800 m, 24 July 1976, *T.Ekim* 2199 (ANK!); Isparta: Şarkikaraağaç, Kızıldağ Natural Park, between forest house to entrance of park, mixed *Cedrus libani* and *Quercus coccifera* forest, 1100–1250 m, 27 May 1994, *B.Mutlu* 526 (HUB!); Afyonkarahisar: Kumalar Mt., between Taşoluk to Şuhut, Sarıyer top, rocky slopes, 1400–1500 m, 24 June 2000, *E.Akçiçek* 2751 (GAZI!); Emirdağ, around Kemerkaya to Özburun, steppe, 1340 m, 30 July 1993, *Z.Aytaç* 6347 et al. (GAZI!); Konya: Akşehir, Sultan Dağları, around Cankurtaran, 1500 m, meadows, 19 June 2007, *M.U.Özbek* 2369 (GAZI!). **B4** Konya: Akşehir to Konya, 30 km from Konya, around Bahçecik village, steppe, 1200 m, 19 June 2007, *M.U.Özbek* 2376 (GAZI!); Ankara: Polatlı, around Acıkır, 840–850 m, 06 July 1990, steppe, *Z.Aytaç* 3083 & *H.Duman* (GAZI!); Bala, Beynam, 19 May 1969, *Ö.İnceoğlu* (HUB!); Beytepe, steppe, 04 July 1976, *S.Erik* 1469 (HUB!); Polatlı, around Yenikent, 20 km from Ankara, ca, 900 m, 08 June 1984, *Y.Akman* 12936 (ANK!); Kırıkkale: Keskin, Böbrek Mt., N.E. of Müsellim village, steppe, 1400 m, 02 June 1991, *Ü.Güler* 1143 (GAZI!); Dinek Dağ, between Balışeyh to Kılevli

village, 900 m, 27 May 2001, *S.A.Demir* 1046 (ERC!). **B5** Kayseri: Develi, 10 June 1971, *A.Baytop* (ISTE 20295!); Aksaray: N. of Hasandağ, clearings of *Quercus* forest, ca. 1400 m, 13 June 1973, *A.Düzenli* 352 (ANK!). **B6** Kahramanaraş: Göksun, Ericek village, Yonca river, 1500 m, wet meadows, 19 June 1981, *B.Yıldız* 2994 (HUB!); Malatya: Gölbaşı, 04 June 1968, *A.Pamukçuoğlu* s.n. (HUB!). **B7** Erzincan: İliç, edge of Karasu, fields, 900 m, 28 April 1980, *Ş.Yıldırımlı* 2715 (HUB!); Kemah, Maksutuşağı village, edge of Karasu, 1100–1300 m, 29 May 1979, *Ş.Yıldırımlı* 1727 (HUB!); Tunceli: Pülümür, Kuzdere hill, 1500–1700 m, 14 June 1980, *Ş.Yıldırımlı* 3088 (HUB!). **B9** Bitlis: Tatvan, Sorgun, above Van Lake, *Quercus infectoria* shrubs, 1650–1750 m, 09 July 1972, *H.Peşmen* 2964 (HUB!); Van: Zeve Campus of Yüzüncü Yıl University, ruderal steppe, 1670 m, 7 May 2014, *A.Öztürk* s.n. (VANF!); Ağrı: Patnos, Top Dağı, field edge, 1600 m, 23 June 2007, *H.Emcik* 1068 (VANF!). **C3** Burdur: Tefenni to Burdur 18. km, steppe, fields, 1115 m, 10 June 2008, *M.U.Özbek* 2737 (GAZI!). **C4** Konya: Şereflikoçhisar, around Tuz Lake, roadside, steppe, 18 June 1986, *S.Erik* 3838 & *İ.Verter* (HUB!); Konya to Sarayönü 13 km, edge of fallow fields, 30 May 1956, *H.Birand* s.n. (ANK!); 14 km from Konya–Beyşehir, around stony mine, ca. 1400 m, 08 June 1979, *H.Dural* 99 (KNYA!); Obruk to Kızıltepe, 1200 m, 15 June 1983, *H.Dural* 979 (KNYA!); Karaman, above Kızılyaka district, *Quercus ithaburensis* alliance, ca. 1375 m, 20 June 1984, *M.Serin* 1910 (KNYA!); Ulumuksine village, *Pinus nigra* plantation area, ca. 1500 m, 08 June 1989, *A.Tatlı* 890 et al. (KNYA!); W. of Sille to Büyükgevele hill, roadside, ca. 1350 m, 16 June 1979*, H.Dural* 97 (KNYA!). **C5** Konya: Ereğli, Aydos Dağı, around Dedeman mine, *Juniperus* forest, 1600 m, 01 June 1978, *S Erik* 2952 (HUB!); Ereğli, Aydos Dağı, Burna, *Pinus nigra* forest, 1600 m, 18 May 1977, *S.Erik* 1989 (HUB!). **C6** Kahramanmaraş: Göksun, 1 km S. of Mürsel village, steppe, 1450 m, 20 June 2006, *Z.Aytaç* 8987 et al. (DUOF!). **C8** Mardin: 19 km from Mardin, *Quercus brantii* forest, rocky slopes, ca. 930 m, 23 May 1956, *H.Birand* 86 (ANK!); Mardin to Diyarbakır 24 km, ca. 1000 m, 27 May 1957, *P.H.Davis* 20838 & *Hedge* (ANK!).

Flowering time: May–June.

Habitat: Limestone slopes, steppe, meadows, roadside, clearings of forest, 400–1800 m.

Distribution: Russia, Iran.

Note: This species can be easily distinguished from the other species within the genus. Leaves is 1–2-pinnatisect and stems are usually much branched and radiating from base.

16. *Cota halophila* (Boiss. & Balansa) Oberp. & Greuter, Willdenowia 33 (1): 40 (2003).

≡ *Anthemis halophila* Boiss. & Balansa., Fl. Orient. 3: 285 (1875). = *Anthemis alexandrettae* Eig, Palestine. J. Bot. Jerusalem Ser. 1: 211 (1938).
Type: [Turkey C5 İçel] in arenis maritimis Ciliciae ad Mersina, *Balansa* 1095 (iso. E!).
Specimens examined: C6 Antakya: İskenderun, Arsuz, Kale village, sands and sandy soil near seashore, 0–5 m, 07 May 2007, *M.U.Özbek* 2271 (GAZI!); ibid., 0–5 m, 09 April 2008, *M.U.Özbek* 2700 (GAZI!).
Flowering time: April.
Habitat: Sands and sandy soil near seashore, s.l.–5 m.
Distribution: Endemic. East Mediterranean element.
Note: Because of decumbent or prostrate and with fleshy leaves this endemic species differs from the other *Cota* species.

17. *Cota pestalozzae* Boiss., Diagn. Pl. Orient. ser. 2 (3): 21 (1856).
≡ *Anthemis pestalozzae* Boiss., Diagn. ser. 1(11): 12 (1849).
Type: [Turkey C2/3 Antalya] prope Elmalu, 1846, *Pestalozza* (photo G!).
Specimens examined: C3 Antalya: Manavgat to Akseki, 8 km from Akseki, calcareous slopes, 920 m, 20 May 2007, *M.U.Özbek* 2288 (GAZI!); Akçay, Gömbe, around Çukurbağ village, rocky slopes, 1470 m, 31 May 2006, *M.U.Özbek* 1998 (GAZI!); Manavgat to Akseki, 8 km from Akseki, calcareous slopes, 950 m, 31 May 2007, *M.U.Özbek* 2000 (GAZI!); Akseki, Otluk Dağı, Otlukbeli hill, among shrubs, 07 May 1995, 1300–1400 m, *A.Duran* 2227 (GAZI!); Akseki, Çimi village, meadows, 1500 m, May 1984, *A.Güner* 5611 & *M.Koyuncu* (GAZI!); Akseki, Geyran upland, N. of Kocaoluk, stony slopes, 1400 m, 02 June 1996, *A.Duran* 3835 (GAZI!). **C4** Konya: Akseki to Seydişehir, 30 km from Seydişehir, Alacabel pass, rocky slopes, 1825 m, 20 May 2007, *M.U.Özbek* 2290 (GAZI!); ibid, 31 May 2007, *M.U.Özbek* 2002 (GAZI!); ibid., *A.cilicica* forest, 1650–1750 m, 19 June 1997, *Z.Aytaç* 7565 (GAZI!); Konya to Karaman, Kızılyaka district, Ortagüney hill, *Juniperus excelsa* forest, ca. 1600 m, 25 May 1984, *M.Serin* 1911 (KNYA!); Karaman: Ermenek, between Ermenek to Anamur, limestone cliffs, 1370 m, 12 May 2010, *E.Ergin* 2257 et al. (DUOF!); İçel: Anamur, around Güngören village, *Pinus brutia* forest, 1200 m, 20 May 1995, *A.A.Dönmez* 4517 (HUB!).
Flowering time: May–June.
Habitat: Limestone screes, 800–1850 m.
Distribution: Endemic, Mediterranean element.
Note: This species can be easily distinguished from the other species within the genus. Ray and disc flowers are violet-pink.

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PHYSIOLOGICAL AND BIOCHEMICAL RESPONSES OF COTTON TO TIMES AND TYPE OF STRESS MODULATOR IN SALINE CONDITIONS

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ABSTRACT. Among the different agronomic techniques used to reduce the negative effects of salinity, external applications of stress modulators are considered as an efficient approach for salinity stress alleviation. An experiment was conducted as a factorial arrangement based on a complete randomized block design in 3 replications to evaluate the foliar application effects of different stress modulators on the physiological and biochemical responses of cotton cultivated in a saline condition. The involved factors included foliar application time (flowering and flowering+bolling stages) and 4 stress moderator types (control, Salicylic acid, Glycine Betaine, and Sodium Nitroprusside Application times had no significant impacts on the plant physiological attributes. Foliar application of salicylic acid further increased the activities of enzymatic or non-enzymatic antioxidants in cotton as compared to the other osmotic modulators. Salicylic acid spraying enhanced the contents of Chlorophyll a (76.4%), Chlorophyll b (47.7%), carotenoids (73.3%), proline (90.8%), catalase (82.6%), superoxide dismutase (74.5%), and guaiacol peroxidase (98.1%) in comparison to the control treatment. Overall, The modulatory effectiveness of the enzymes in reducing salinity stress by augmenting their antioxidant activities could be classified as salicylic acid > glycine Betaine > sodium nitroprusside.

1. INTRODUCTION

Environmental stress accounts for 71% of crop yields. It is estimated that about 20% of crop yield loss is caused by salinity stress. About 7 million lands under agricultural crops in Iran are influenced by salt stress impacts since this country has got the highest percentage of saline lands in the world after India and Pakistan [1]. Salinity stress is one of the major environmental stresses affecting plant growth by decreasing

Keyword and phrases. Cotton, Foliar application, salinity stress, stress modulator

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water uptakes, disturbing nutrient uptakes, reducing ion toxicities, and producing oxygen free radicals [2, 3]. One of the biochemical changes that occur under such environmental stresses as salinity is the production of Reactive Oxygen Species (ROSs) through electron-chloroplast transfer chain closure and NADP+/NADPH ratio reduction[4]. Oxygen-free radicals prevent photosynthesis by damaging cell membranes and eventually killing the plant. Plants use both antioxidant and non-antioxidant enzymatic systems to encounter the oxidative stress induced by the accumulation of oxygen free radicals [5]. Synthesis and accumulations of glutathione peroxidase, superoxide dismutase, catalase, peroxidase, and glutathione are their enzymatic mechanisms of alleviating oxidative stress and tocopherol, ascorbic acid, carotenoid, and glutathione synthesis and accumulations are their non-enzymatic reduction mechanisms [2]. It has been reported that plant antioxidant activities and the negative effects of stresses can be increased and decreased by using some modulators, respectively[6-8]. The effects of SNP [9], SA, GB, and putrescine [10] on salinity stress tolerance in cotton has been already documented.

SNP is an NO-releasing compound and one of the stress-reducing agents. Today, NO is classified in the group of plant growth regulators. SNP moderating role in environmental stresses, such as salinity, drought, heavy metals, and mineral deficiency, has been reported in many studies [10]. It is believed to have the dual toxic or protective roles, depending on its concentration, as well as plant type, tissue, and age, and the stress type applied to the plant [9]. It reduces leaf water depletion, ion leakage, and transpiration by stimulating stomach closure and thereby increases stress tolerance [11]. The external application of SNP has been evidenced to decrease stress tolerance by reducing hydrogen peroxide and malondialdehyde, enhancing antioxidant enzymatic activities, regulating proteins at post-translational levels, and increasing cell division. Its external application in cotton augmented the activities of oxygen-reducing enzymes and decreased H2O2 accumulation under salinity stress. Growth and photosynthesis include net photosynthesis rate and transcription [12]. It has been reported that foliar application of 0.05 M SNP enhances yield and yield components, increases the contents of pigments, total soluble sugars, proline, total free amino acids, phenols, and soluble proteins, besides antioxidant activities and activities of antioxidant enzymes [10].

GB is a quaternary ammonium and the most common organic solution that accumulates in plant cells in response to stress. It acts as a cytoplasmic osmolyte and protects plant enzymes and membranes against decay. GB accumulation in response to stress has been proven in many crops, including sugar beet, spinach, barley, and wheat [6]. Nevertheless, natural GB production is not enough to protect plants under severe stresses. In such circumstances, the external application of GB may be a

useful solution to overcome environmental stresses in plants. Various studies have reported the relationship between stress tolerance and external GB use [8]. The use of GB can naturally improve stress tolerance in those plants that are unable to synthesize it [13]. GB not only acts as a protective protein for maintaining normal intracellular osmotic pressure, but also stabilizes the quaternary structures and activities of enzymes and protein complexes, thus keeping membrane integrity under salt, drought, and cold stress conditions [6]. It can also effectively protect Photosynthesis System II (PSII) under salinity stress [14]. The modulatory effects of external GB consumption on reducing the impacts of drought stress [15], salinity [16] and cold stress [7] has been evidenced in cotton.

Salicylic acid (SA) or ortho-hydroxy benzoic acid belongs to a group of phenolic compounds that have been identified as important signal molecules for modulating plant responses to environmental stresses [17]. Increased photosynthetic activity, decreased transpiration, water retention in tissues, enhanced cytosolic content [4], augmented antioxidant enzyme activities [5], and free proline accumulation are the physiological effects of external SA application. In cotton, it has been reported that salinity significantly decreases its growth and yield, but the foliar application of 200 ppm of SA reduces salinity stress effects by enhancing the activities of antioxidant enzymes [18]. In addition to alleviating the impacts of salinity stress by SA application, Hameed and Ali (2016) reported that 1 mM of foliar application in cotton increased heat stress tolerance by enhancing the activities of antioxidant and non-antioxidant enzymes [19]. The positive benefits of SA intakes in lowering copper-induced oxidative stress [20], plant pests [21], and drought stress [18] have been also evidenced.

Although under stress conditions, plant cells reduce the negative effects of oxygen free radicals by accumulating antioxidant and non-antioxidant enzymes, it is well known that the external applications of these stress modulators can increase the productions of enzymatic and non-enzymatic antioxidants and enhance salt tolerance in plants. In environmental stress conditions, such as salinity stress, the response of crops to the type of stress modulator may be different. Although, in many studies, the effect of only one modulator has been reported by researchers, but determination of the most suitable stress modulator was not intended by researchers. Therefore, in this research, the effects of some modulator times and types on the physiological and biochemical traits of cotton under saline conditions were investigated.

2. Materials and Methods

The experiments were conducted as a factorial arrangement in a randomized complete block design with three replications. Factors were application time (flowering and flowering+bolling stages) and stress types of moderators (control, SA (2 mM), GB (100 mM), and SNP (100 µM). In control, No stress modifiers were used, and water was sprayed. The experiments were carried out at a private farm located at 57◦ 44' East latitude and 36◦ 13' North longitude with a height of 990 m above sea level during 2017-2018. According to the soil analysis results (Table 1), the studied soil was of a loamy-clay type with a pH of 7.2, EC of 10.5 ds/m, and total N, P, and K contents of 0.02%, 110and 4 mg/kg, respectively. The data on the rainfalls and mean temperatures during the cotton growing stages are presented in Table 2.

Table 1. Physicochemical properties of soil on experimental site

Manganese ($mg\ kg^{-1}$)	Sodium	Zinc	Copper	Iron	Phosphorus PPm	Potash	Nitrogen (%)	Sand %	Clay	Silt	EC (dS m^{-1})	$pH_{(1:5)}$
7	40.5	0.55	0.46	2.42	4	110	0.02	63	13	24	10.5	7.2

Table 2. Average temperature and total rainfall in growing season in two years.

Month	Average temperature (◦C)		Total rainfall (mm)	
	2017	2018	2017	2018
January	5.9	5.76	15	1.6
February	6.5	9.99	49.9	20
March	12.5	17.1	21.4	2
April	18.9	17.9	29.4	32.2
May	26.9	23.3	14.2	17.8
June	30.86	29.52	0	0.7
July	31.02	2.8	2.8	0.0
August	29.86	30.4	0.0	0.0
September	25.7	26.3	0.0	6.5
October	19.1	17.5	0.0	35.1
November	13.7	12.5	2.0	0.6
December	7.21	6.59	0	1.2

The experimental filed went under fallow and wheat cultivations during the first and second years. Seed-bed preparation included moldboard plowing in the autumn and harrowing and leveling in the spring. According the soil analysis results (Table 1), 100 kg P_2O_5 ha^{-1} as triple super phosphate and 100 kg K_2O ha^{-1} as potassium sulfate, together with one-third of 150 kg N as urea, were uniformly broadcasted before

sowing. Another N fertilizer was top-dressed at the first hand-weeding (about 40 days after emergence) and early flowering stages. Cotton seeds were obtained from Agricultural Jihad in Sabzevar and sown on April, 15th, 2017 and April, 20th, 2018. Each plot contained four 5-m-long rows spaced 50 cm from each other, along which the plants were planted at the equal distances of 20 cm (10 plants.m^{-2}). After completing the planting stage and at the 5-6 leaf stages, the plants were thinned in the rows at a distance of 20 cm from each other to obtain an optimum density. Irrigations were done every 10 days during the growing season according to the local custom. Weeding (weed removal) was done manually. Triton 100 X was utilized as a surfactant to better absorb foliar applications of the modulators.

Plant sampling

All the assays were performed on the 4th extended leaf, 30 days after spraying from 5 randomly plants.

The amounts of photosynthetic pigments were determined by using Arnon method [22]. The contents of Chlorophyll and carotenoids were measured via spectrophotometry at 645, 663, and 470 nm and expressed in mg/g fresh weight (mg g^{-1}F.W).

$$\text{Chlorophyll a} = (19.3 \times A_{663} - 0.86 \times A_{645})\ V/100W$$
$$\text{Chlorophyll b} = (19.3 \times A_{645} - 3.6 \times A_{663})\ V/100W$$
$$\text{Carotenoides} = 100(A_{470}) - 3.27(\text{chl. a}) - 104(\text{chl. b}) / 227$$

Proline concentration

The method applied by Bates et al. [23] was employed to measure leaf proline content. To this aim, 0.2 g of the leaf tissue was weighed and thoroughly gelled in 3 ml of 3% sulfosalicylic acid in Chinese mortar. Upon centrifuging the homogenates at 18,000 rpm for 15 min, 2 ml of the filtered extract was transferred to the gut tubes and 2 ml of ninhydrin reagent and 2 ml of glacial acetic acid were added to all the tubes. Then, they were placed in 100°C water for 1 hour. After cooling the tubes, 4 ml of toluene was added to each tube. The tubes were vortexed for 15-20 s to allow formations of two separate phases. The top phase was read at 520 nm by the spectrophotometer (Uv/Vis model T70+ , German). Proline concentration in mg/g of fresh leaf tissue was determined using the standard curve. The unit was expressed as mg/g fresh weight.

Enzyme Extraction

For enzyme extraction, 0.5 g (fresh weight) of the frozen leaf tissue was grounded with mortar in liquid N2 to obtain a fine powder, which was then homogenized in 5 mL of an extraction buffer containing 50 mM cold buffered monophosphate (pH 7.8), 0.1 mM EDTA, 0.3% TritonX-100, and 4% polyvinylpolypyrroidone. The homogeneous material was centrifuged for 15 min at the gravity of 15,000 g at 4°C. The upper transparent phase was applied to measure enzyme activity [24].

Catalase activity measurement

CAT (E.C. 1.11.1.6) activity was determined using the method of Aebi [25]. For a total volume of 3 mL, 30 µl of the extract was applied to 50 mM buffered monophosphate (pH 7.0) and 2% H_2O_2. Enzyme activity was measured at 240 nm for 2 min using a spectrophotometer (Cary 100) and expressed as changes in the absorbance units (ΔA) min^{-1} mg $protein^{-1}$

$$(Unit)/_{min} = 2(1 - \frac{\Delta Asample}{\Delta Acontrol})$$

Superoxide dismutase activity measurement

Superoxide dismutase (SOD; E.C. 1.15.1.1) activity in the leaf extracts was measured using the inhibition measurement of nitroblue tetrazolium (NBT) photochemical reduction [26]. The reaction mixture had a final volume of 3 ml and contained 2.25 ml of 50 mM potassium phosphate buffer (pH=7.8), 200 µL of EDTA (0.66 mM), 300 µL of methionine (10 mM), 150 µL of NBT (66 µM), 50 µL of riboflavin (3.35 µM), and 50 µL of the enzyme extract. The reaction was carried out in a transparent glass cuvette at 25°C under the same white light intensity for all the samples. A special cuvette for each sample was placed in the dark as a blank (zero reaction time control). After shining the light for 15 min, the light absorption at 560 nm was read and the absorption difference was set to zero for 15 min. NBT inhibitory ability in each sample was determined by the enzymatic extract of each sample as compared to the light absorption difference of 0 and 15 minutes for the control reaction (without enzyme) according to the following formula: One unit of superoxide dismutase activity equivalent to 50% inhibition of nitrobutetrazolium-to-formamazone photobleaching. Finally, the enzyme activity was reported based on superoxide dismutase unit/min/g fresh weight.

Ascorbate peroxidase activity measurement

Ascorbate peroxidase (APX; E.C. 1.11.1.1) activity was determined as described by Nakano and Asada [27]. The 3-mL reaction mixture contained 312.5 mM sodium phosphate buffer (pH=7), 0.5 mM sodium ascorbate, 0.1 mM ethylene diamine tetraacetic acid, 2.2 1 mM hydrogen peroxide, and 50 µL of the enzyme extract. The reaction was started by adding hydrogen peroxide to the reaction mixture and then, changes in the light absorption relative to the control reaction without hydrogen peroxide were recorded. The conversion rate of ascorbate to dehydroascorbate was measured for 60 s at 290 nm and finally, the enzyme activity was determined by using ascorbate extinction coefficient of 18 mM^{-1} cm^{-2} expressed as the change in µM ascorbate consumed per min/g fresh weight.

Glutathione peroxidase (GPX) measurement

Guaiacol peroxidase (EC 1.11.1.7) activity was measured according to the method of Fielding and Hall [28]. In this approach, 3 mL of the reaction mixture containing 100 mM potassium phosphate buffer (pH=7), 20 mM guaiacol, 10 mM H_2O_2, and 50 mM enzyme extract was allowed to increase absorption via guaiacol oxidation at 470 nm, which was measured for 3 min. Enzyme activity was expressed as the change in µM ascorbate consumed per min/g fresh weight by using the extinction coefficient of 18 mM^{-1} cm^{-2}.

Hydrogen peroxide (H_2O_2) extraction and measurement

Hydrogen peroxide (H_2O_2) was calculated with minor modifications using Sagisaka's test [29]. First, 0.5 mg of fresh leaf was homogenized with 5 mL of 0.1% TCA (w/v) in an ice bath and centrifuged at 18000×g for 15 min. The supernatant (0.5 mL) was added to 0.5 mL of 10 mM K-phosphate buffer (pH=7) and 1 mL of 1 M KI. The absorptions in the samples were determined with a spectrophotometer at 390 nm. The H_2O_2 concentration was expressed as nmol H_2O_2 mg $protein^{-1}$

Sodium and potassium measurements

Hamada and El-Enany [30] method was used to measure leaf sodium and potassium content. In summary. 0.5 g dry matter of leaves washed and then 10 ml of concentrated nitric acid was added and kept at room temperature for 48 hours. In order to remove all vapors, the specimens were placed on a heated oven thermostat for 2 hours. After leaving acidic vapors and viewing a colorless solution, 100 ml of

distilled water was added to each sample. Using Whatman filter paper, the samples were get smooth and sodium and potassium values were measured by Flame Photometer (Jenway, Models PFP7, UK).

Membrane stability index measurement

Membrane stability index was measured based on the electrical conductivity of ions leaking from the leaf cells into deionized water. For this purpose, 0.1 g of the fresh leaves were immersed in the test tubes containing 20 ml of deionized water. Then, one sample was kept at 40°C and another sample at 100°C for 30 min. The electrical conductivities of the samples were measured and recorded by using a conductivity meter after they reached room temperature. The membrane stability index was calculated based on the following formula:

$$\text{Membrane stability index} = \left[1 - \left(\frac{C_1}{C_2}\right)\right] * 100$$

where C_1 and C_2 represent the electrical conductivities of the samples at 40 and 100°C, respectively.

Statistical analysis

The experiment was arranged as a factorial arrangement based on completely randomized block design with three replicates. In a combined analysis of data, the interaction of year, stress modulator type, and application times was non-significant; therefore, the data were combined for both years and presented with the interaction of application times and stress modulator type. Data were subjected to analysis of variance (ANOVA) by using the software of SAS (SAS 9.1, USA). The means were compared using the least significant difference test ($P \leq 0.05$).

3. Results and discussions

Chlorophyll pigments

Spraying time affected the amount of chlorophyll pigments and spraying at the flowering compared to the flowering+bolling stage had more additive effects on chlorophyll pigments namely chlorophyll a, chlorophyll b, and total chlorophyll, while the contents of carotenoids at the flowering+bolling stage were higher than those of foliar applications at the flowering stage. Among the stress modulators,

foliar application with GB had more positive effects on chlorophyll pigment content, which was not significantly different from the case of foliar application with SA (Table 3).

TABLE 3. Means of chlorophyll pigments content as affected by time and type of stress modulator application

	chlorophyll *a* (mg. g^{-1}FW)	chlorophyll *b* (mg. g^{-1}FW)	Total chlorophyll (mg. g^{-1}FW)	Cartrenoid (mg. g^{-1}FW)
		Application times		
flowering	1.56±0.22 a	1.45±0.14a	3.01±0.47 a	0.76±0.08 a
flowering+bolling stage	1.35±0.28 b	1.20±0.19 b	2.55±0.33b	0.84±0.13 a
		Stress modulator type		
Control	0.74±0.13 c	0.86±0.12 c	1.59±0.22 c	0.53±0.12 d
GB	1.81±0.38 a	1.68±0.26 a	3.49±0.63 a	1.10±0.20 a
SNP	1.59 ±0.38b	1.24±0.26 b	2.83±0.61 b	0.70±0.12 c
SA	1.70±0.40 ab	1.52±0.25 ab	3.23±0.63 a	0.86±0.11 b

Values are mean ± SE (n = 6). Letter denotes significant differences between treatments at $p \leq 0.05$, Similar letter within the same columns not significant difference based on FLSD.

Both in the foliar applications at the flowering and flowering+bolling stages, spraying with GB produced more chlorophyll a contents compared to those produced by the other stress modulators although the difference between GB and SA in spraying at the flowering+bolling stage was less than that of spraying at the flowering stage. Foliar application with SNP at the flowering+bolling stage also decreased chlorophyll a content compared to that at the flowering stage. Similar changes in chlorophyll b content were observed, except that foliar application with SNP at the flowering+bolling stage increased chlorophyll b content compared to its application at the flowering stage (Table 4).

TABLE 4. Interaction effect of time and type of modulator application on photosynthetic pigment

Time of spraying	Modulator type	chlorophyll a (mg. g^{-1}FW)	chlorophyll b (mg. g^{1} FW)	Total chlorophyll (mg. g^{-1}FW)	Caratenoid (mg. g^{-1} FW)
Flowering	Control	0.73±0.20 c	0.92±0.17 b	1.65±0.36 d	0.55±0.17 c
	GB	1.76±0.47 a	1.61±0.22 a	3.37±0.62 ab	0.58±0.09 c
	SNP	1.35±0.49 b	0.82±0.27 b	2.17±0.67 c	0.83±0.22 b
	SA	1.57±0.54 ab	1.45±0.34 a	3.02±0.82 b	0.81±0.14 b
Flowering+bolling	Control	0.73±0.19 c	0.79± b	1.53±0.30 d	0.51±0.19 c
	GB	1.84±0.67 a	1.75±0.50 a	3.60±1.17 a	0.86±0.10 b
	SNP	1.83±0.62 a	1.66±0.41 a	3.49±0.99 ab	1.34±0.39 a
	SA	1.84±0.65 a	1.59±0.40 a	3.43±1.05 ab	0.93±0.18 b

Values are mean ± SE (n = 6). Letter denotes significant differences between treatments at $p \leq 0.05$, Similar letter within the same columns not significant difference based on FLSD.

Twice foliar applications at the flowering+bolling stage produced more carotenoid contents resulted from all the stress modulators compared to once foliar application at the flowering stage. This increase of carotenoid content was higher for SNP application than for the other modulators, while foliar application with SA had the lowest enhancement (Table 4). Studies have shown that photosynthetic pigment is one of the important determinants of photosynthetic efficiency and plant growth [31]. Chloroplast and photosynthetic system degradations, chlorophyll photooxidation, reaction with ROSs, degradation of chlorophyll precursors, inhibition of new chlorophyll biosynthesis, activation of chlorophyll-degrading enzymes, such as chlorophyllase, and hormonal abnormalities are the reasons for lowered chlorophyll contents in saline conditions [32]. Consistent with these results, the reductions of chlorophyll a, b, and total chlorophyll contents, as well as carotenoids of plants exposed to salinity stress, were reported by El-Beltagi et al. [18]. It was also observed that salinity significantly reduced chlorophyll a and b contents, whereas SA treatment decreased the trend of chlorophyll content reduction [20]. SA increasing role of chlorophyll content has been attributed to stimulations of the enzymes related to chlorophyll biosynthesis or inhibition of photosynthetic system disturbance, thereby alleviating chlorophyll degradation. Foliar application of SA in saline conditions augmented the contents of chlorophyll a, b, total chlorophyll, and carotenoids, which is in agreement with the results of this research. The enhancing effects of SA on photosynthetic capacity could be ascribed to its stimulating effects on Rubisco activity and pigment content [17]. The external application of GB in maize under salinity stress had an additive effect on chlorophyll pigments as one of the effective factors in increasing photosynthetic capacity under salinity conditions [3]. Liu et al., [12] evidenced that chlorophyll a content in cotton decreased by 25.27% in salinity conditions, However, 0.1 mM SA foliar application increased chlorophyll a content by 2.10% compared to no salinity stress and 36.46% compared to salinity stress conditions. Foliar spraying with SNP had greater positive effects on the contents of chlorophyll a, So that it increased by 27.68% and 70.64% compared to those of non-stress and stress conditions, respectively.

Antioxidant enzymes

Foliar application time had no significant effects on CA enzyme activity (Table 5). Cultivation in a saline condition significantly decreased CA enzyme activity and the external applications of the stress modulators significantly enhanced CA enzyme activities compared to the control treatment, showing that foliar application with SA had more positive additive effects than those of the other modulators. There was no statistically significant difference between the external applications of SA and SNP

(Table 5). Although foliar applications with GB and SNP at the flowering+bolling stage augmented CA enzyme activity compared to its application at the flowering stage, the external SA application caused a 21% decrease in its activity (Table 6). Foliar application time had a significant effect on SOD activity and foliar application at the flowering+bolling stage enhanced SOD enzyme activity up to 26.89% as compared to its application at the flowering stage (Table 5). Foliar application with SA among the other modulators in this study had more additive impacts on SOD enzyme activity though showing no statistically significant difference with the external application of GB (Table 5). Foliar application at the flowering+bolling stage for all the modulators had higher SOD enzyme activities compared to foliar treatments at the flowering stage. The enhancement rates of SOD enzyme activity with twice spraying at the flowering+bolling stage were 39.39, 36.08, and 18.56% higher for GB, SA, and SNP compared to its once application at the flowering stage, respectively (Table 6).

TABLE 5. Means of antioxidant enzymes as affected by time and type of stress modulator application

	CAT (units/mg proteins)	SOD (units/mg proteins)	APX (units/mg proteins)	GPX (units/mg proteins)
		Application times		
flowering	66.8±4.65 a	35.8±2.66 b	54.5 ±0.64a	0.51±0.04 b
flowering+bolling stage	65.7 ±4.01a	45.8±2.16 a	54.1±0.78a	0.58±0.31 a
		Stress modulator type		
Control	53.24±2.2 c	23.10±1.02 c	50.54±2.15 c	0.27±0.02 c
GB	67.94±2.41 b	46.80±2.06 a	55.62±0.28 b	0.65±0.04 a
SNP	69.72±3.02 ab	44.15±3.31 b	54.71±0.42 b	0.66±0.05 a
SA	74.24±5.98 a	48.75±3.35 a	56.12±1.41 a	0.61±0.02 b

Values are mean ± SE (n = 6). Letter denotes significant differences between treatments at p≤0.05, Similar letter within the same columns not significant difference based on FLSD.

TABLE 6. Interaction effect of time and type of modulator application on antioxidant enzymes

Time of spraying	Modulator type	CAT (units/mg proteins)	SOD (units/mg proteins)	APX (units/mg proteins)	GPX (units/mg proteins)
Flowering	Control	56.1±1.61 b	22.7±3.91d	51.8±1.59 b	0.25±0.02 c
	GB	60.3±5.93 ab	39.1±1.51 c	54.9±0.63 a	0.78±0.06 a
	SNP	68.0±6.31 a	40.4±3.44 b	55.1±0.56 a	0.61±0.04 ab
	SA	82.9±7.36 a	41.3±3.82 b	56.0±1.40 a	0.67±0.08 a
Flowering+bolling	Control	50.4±4.38 b	23.5±3.60 d	49.2±1.88 b	0.28±0.03 c
	GB	75.6±8.12 a	54.5±2.02 a	56.3±0.54 a	0.51±0.02 b
	SNP	71.4±7.12 a	47.9±1.52 ab	54.3±0.45 a	0.71±0.03 a
	SA	65.5±1.51 a	56.2±2.45 a	56.1±1.14 a	0.54±0.05 b

Values are mean ± SE (n = 6). Letter denotes significant differences between treatments at p≤0.05, Similar letter within the same columns not significant difference based on FLSD.
Superoxide dismutase (SOD), catalase (CA) , Ascorbate peroxidase(APX), guaiacol peroxidase (GA).

Foliar application time had no significant effects on APX activity, but the external applications of the stress modulators significantly increased APX activity, while the additive effects of external SA application was greater than those of the other modulators (Table 5). APX activity responses to the modulator times and types were different. Although there was no significant difference between foliar applications with SA at the flowering+bolling and flowering stages, foliar applications with SNP and GB at the flowering+bolling stage decreased and increased APX activities compared to its application at the flowering stage, respectively (Table 6).

Gaiacol peroxidase activity was affected by foliar application time and its application at the flowering+bolling stage created more gaiacol peroxidase activity compared to its application at the flowering stage (Table 5). Although the external applications of the stress modulators enhanced guaiacol peroxidase activity, no significant differences were observed between the modulators in this respect (Table 5). Foliar applications with SA and GB at the flowering+bolling stage increased guaiacol peroxidase activities and foliar application with SNP decreased its activity compared to foliar application at the flowering stage (Table 6).

CAT, POX, and SOD are the antioxidant enzymes that protect cells from oxidative stress caused by oxygen free radicals. As can be seen in the table, applications of the external stress modulators have increased the enzymatic activities, while catalase and superoxidase have provided the most and least reactions to their applications, respectively. The enhanced peroxidase activities under salinity stress in canola [6] and cotton [9] leaves reduced the damaging effects of salinity stress. SA induced the activities of these antioxidant enzymes in D. superbus under saline conditions [20]. The increase in growth caused by SA consumption has been linked to an increase in antioxidant activities, which protect plants from oxidative damage [19]. Studies have shown that SA plays an important role in modulating the activities of antioxidant enzymes against salinity stress, while making plants more capable of withstanding salinity damage [4, 18-20]. No difference in SOD enzyme activity was observed in the cotton mutants capable of synthesizing GB and in those mutants lacking this ability [2]. However, under salinity stress, SOD activities were increased by both types of mutants and were significantly higher in transgenic mutants after 14 days of salinity stress. Nawaz and Ashraf [3] reported that foliar application of GB enhanced SOD activity in maize, especially during the vegetative time, under salinity stress conditions. This increase in SOD activity could protect the photosynthetic system from the oxidative damage caused by salt stress. In wheat, it was reported that foliar application of GB led to a significant increase in SOD activity in saline conditions. Its spraying at a concentration of 50 mM enhanced SOD activity by 24%, but it was not statistically significantly different from the case of spraying with 75 mM [33]. In

soybean, it was shown that salinity stress significantly augmented the activity of CAT enzyme and pre-treatment with GB significantly enhanced its activity in salinity stress conditions, while increasing GB concentrations led to little change in its activity in non-saline conditions[34]. In mungbean, it was reported that salinity stress significantly enhanced GPX activity. Under this condition, the external use of Pro or GB augmented the activity of GPX enzyme, the highest activity of which was observed 48 hours after GB consumption [35]. Enhanced salinity tolerance has been also reported with increasing SOD activities with external GB applications in sompe crops [6, 8, 14-16].

Membrane stability index, proline content and content of sodium and potassium

Membrane stability index was not affected by foliar application time and the interaction between the stress modulator times and types. The external application of GB enhanced membrane stability index, which was not significantly different from that induced by SA foliar application. The external application of SNP augmented membrane stability index by 3.61% compared to the control treatment, which was statistically similar to that yielded by SA foliar application (Table 7).

TABLE 7. Means of membrane stability index, proline content and content of sodium and potassium as affected by time and type of stress modulator application

	EL (%)	Proline (mg. g^{-1}FW))	Na (mg. g^{-1}dW)	K (mg. g^{-1}FW)
Application times				
flowering	45.57±1.01 a	60.17±4.9 b	54.49±2.53 a	42.50±2.27 a
flowering+bolling stage	46.14±2.39 a	63.33±6.6 a	54.00±2.61 a	45.16±3.39 a
Stress modulator type				
Control	47.3±3.51 a	37.17±6.06 d	56.17±1.66 a	34.17±2.81 c
GB	43.9±0.88 b	67.83±3.44 b	55.66±1.09 a	44.48±1.86 b
SNP	46.5±0.96 a	59.00±6.41 c	54.62±2.13 a	43.50±2.22 b
SA	45.58±1.06 ab	83.00±4.72 a	50.54±1.7 b	53.17±2.10 a

Values are mean ± SE (n = 6). Letter denotes significant differences between treatments at $p \leq 0.05$, Similar letter within the same columns not significant difference based on FLSD.

The least electrolytic leakage indicating membrane stability index was obtained with GB foliar application. GB has been reported to not only act as a protective protein in maintaining intracellular osmotic pressure, but also stabilize quaternary structures and the activities of complex enzymes and proteins, which help keeping membrane integrity under stress conditions caused by salt, cold, and frost [6, 7]. It has been

reported that in cotton mutants capable of GB synthesis under saline stress, electrolytic leakage is lower than that induced in the mutants lacking GB synthesis capability [2].

Proline accumulation was influenced by foliar application time. Foliar application effects at the flowering+bolling stage were higher than those at the flowering stage. Under control conditions, the accumulated proline content was 123% lower than that caused by SA spraying, which had the highest proline content. Foliar applications of all the stress modulators produced more proline contents (82.48, 58.73, and 123% for GB, SNP, and SA, respectively) compared to those of the control (Table 7). The additive effects on proline contents were similar at those of the different foliage times, except for SNP, with which foliar application at the flowering+bolling stage caused a greater increase in proline content compared to that produced by its application at the flowering stage (Table 8).

TABLE 8. Interaction effect of time and type of modulator application on proline and potassium content

Time of spraying	Modulator type	Proline (mg. g^{-1}FW))	K (mg. g^{-1}FW)
Flowering	Control	38.3±1.6 d	36.6±3.41 c
	GB	67.0±1.07 b	48.6±1.22 b
	SNP	54.0±2.53 c	42.0±2.07 b
	SA	81.3±2.97 a	42.6±1.58 b
Flowering+bolling	Control	36.0±1.38 d	31.6±2.07 c
	GB	68. 7±2.57 b	40.3±1.83 b
	SNP	64.0±2.22 b	45.0±2.07 b
	SA	84.7±2.43 a	63. 7±1.95 a

Values are mean ± SE (n = 6). Letter denotes significant differences between treatments at $p \leq 0.05$, Similar letter within the same columns not significant difference based on FLSD.

In saline conditions, proline accumulation is inhibited as a defense mechanism in plants. Proline is a non-toxic preservative for osmotic regulation under salinity and other environmental stresses [6, 13]. On the other hand, proline accumulated in plants enhances antioxidant capacity and neutralizes hydroxyl free radicals [6]. In this investigation, the external applications of the stress modulators increased proline contents in cotton. According to our findings, SA foliar application in cotton was reported to augment proline content in a saline condition. This increase in proline content enabled the plant to be more resistant to stress through osmotic adjustment. In addition, proline acts as an energy source that can help improve salinity tolerance [18].

Sodium and potassium contents were not affected by spraying time and the stress moderator effects on them were not significant although spraying with the stress modulators slightly decreased and increased their contents compared to the control treatment, respectively. The decreasing effect on sodium uptake by foliar application with SA was not statistically significant with that triggered by its application with GB (Table 7). While foliar application with SA at the flowering+bolling stage decreased Na^+ content, its application with GB increaseditsuptake compared to that obtained by foliar application at the flowering stage. As with sodium content, potassium content was enhanced with the external applications of SNP and SA at the flowering+bolling stage and foliar application with GB reduced potassium content compared to when it was applied at the flowering stage (Table 8).

4. Conclusion

Overall, the results of this experiment revealed that in saline conditions, external applications of the stress modulators had additive effects on the enzymatic and non-enzymatic antioxidants. Among the modulators used in this experiment, the external application of SA had more improvement effects on the studied physiological and biochemical traits. Although the external applications of GB were not statistically different from those of SA in most of the studied traits, the external application of SNP increased the traits compared to the control treatment; however, this increase was less than those caused by SA and GB applications. The external applications of the stress modulators did not have great effects on the uptakes of ions, such as sodium and potassium, although their uses decreased and increased sodium and potassium uptakes, respectively. The results of our investigation demonstrated that applications of the stress modulators could reduce the effects of salinity stress on cotton growth and development under saline conditions by enhancing the activities of antioxidant enzymes. Furthermore, the external applications of SA and GB at the flowering+bolling stage and SNP application at the flowering stage could augment the activities of antioxidant enzymes and result in salt stress toleration in cotton.

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POLLEN MORPHOLOGY OF SELECTED ALLERGENIC SPECIES AT BEŞEVLER 10. YEAR CAMPUS, ANKARA UNIVERSITY, TURKEY

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ABSTRACT. Although airborne pollen is invisible to the eye, it has been known as a major source to respiratory allergic reactions. For this reason, relationship between the morphological characteristics of airborne pollen and their potential tendency as an allergen are still obscure. In the present study, we selected 29 allergenic species at Beşevler 10. year campus and investigated their detail pollen characteristics using light and scanning electron microscopies. 11 of the allergic plants on campus are also important for beekeeping.

1. INTRODUCTION

Recently, allergic diseases have developed pandemic health problem. That's why allergic diseases are considered to be one of the most important contemporary public health problems affecting up to 15–35% of humans worldwide. In our country, it has been reported that 25-30% of the population is affected by one or more allergic diseases [1]. Generally, abundant allergenic pollen plants are located in suitable green space of urban areas, producing allergenic pollen. University campuses have very important green space and plant diversity. A large number of allergic pollen spreads from campus plants and they disperse in the atmosphere of the city. Allergies during college years impact the quality of life by interfering with the daily activities, poor attendance to college, sleep disturbances, and inability to perform academical as well as extracurricular activities. In Basra University, majority of medical students were discovered symptoms of different allergies to 55.6% of them were allergic to dust, 26% to pollen, 18.1% to food and 10.5% to drugs [2]. An epidemiological study was performed in the students of Hacettepe University from various parts of Turkey to find the prevalence of asthma and allergic diseases and search for geographical

Keyword and phrases. Allergy, plant, pollen, beekeeping, Beş evler 10. year campus, Ankara, Turkey

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differences in Turkey by Kalyoncu et al. [3]. A questionnaire related with symptoms of asthma and allergic diseases was distributed to 4600 students and filled by 4331 students (1884 males, 43.5%-2447 females, 56.5%). They found that the current prevalences of the seasonal and perennial pollen hypersensitivities were 5.2%. Again, Kalyoncu et al. found that the first-year university students to determine prevalence of asthma, and allergic diseases and prevalence % of asthma, current wheeze and seasonal rhinitis were 2.1, 6.9 and 12.7 in boys, and 2.5, 7.2 and 14.5 in girls [4]. No such study has been conducted for the Beşevler 10. year campus.

The pollen morphology studies are used in systematics, melissopalynology, jeopalynology and aeropalynology [5–7]. Pollen characters such as number and position of the apertures and details of sculpturing of the exine are of taxonomic values among of allergenic pollen grains. To provide scientific basis for campus green space construction, the pollen morphology of allergenic plants were reviewed in this study. In this study, pollen grains of 29 widely herbs, shrubs, beside planted campus trees are examined and measured and photographed by light (LM) and scanning electron (SEM) microscopes.

2. Materials and Methods

The materials used for the present study were collected from the Beşevler 10. Year campus during February-December 2019. The study area is situated at an altitude of 860 m above sea level. The taxonomic status of studied species, their life form and span, and collector names were given in Table 1. Pollen slides were prepared using the technique of Wodehouse [8]. Pollen grains were placed in glycerin jelly, stained with safranin, and studied under a light microscope. Photographs were taken with a Leica DM 1000 digital photomicrograph system (Germany). Measurements were based on 20 or more pollen grains for each species.

For SEM studies, dry pollen grains were mounted on stubs and coated with gold. Morphological observations were made with a ZEISS EVO 40 Scanning Electron Microscope. The terminology used is mainly that of Faegri and Iversen [9], and Punt et al. [10,11].

TABLE 1. The taxonomic status of studied species, their life form and span, and collector name

Family	Species	Life form	Life span	Collector	Blooming time	Origin
Sapindaceae	*Acer pseudoplatanus*	Tree Deciduous	Perennial	Aydan Acar Şahin	March-May	Native
Betulaceae	*Betula pendula*	Tree Deciduous	Perennial	Aydan Acar Şahin	April-May	Native
Betulaceae	*Carpinus betulus*	Tree Deciduous	Perennial	Aydan Acar Şahin	July-August	Native
Cupressaceae	*Chamaecyparis lawsoniana*	Tree Evergreen	Perennial	Aydan Acar Şahin	March-April	Exotic
Cupressaceae	*Cupressus arizonica*	Tree Evergreen	Perennial	Aydan Acar Şahin	March-April	Exotic
Oleaceae	*Fraxinus excelsior*	Tree Deciduous	Perennial	Aydan Acar Şahin	March-April	Native
Juglandaceae	*Juglans regia*	Tree Deciduous	Perennial	Nur Münevver Pınar	May	Native
Moraceae	*Morus alba*	Tree Deciduous	Perennial	Nur Münevver Pınar	May	Native
Platanaceae	*Platanus orientalis*	Tree Deciduous	Perennial	Aydan Acar Şahin	March-May	Native
Salicaceae	*Populus alba*	Tree Deciduous	Perennial	Nur Münevver Pınar	March-April	Native
Fagaceae	*Quercus robur*	Tree Deciduous	Perennial	Nur Münevver Pınar	April	Native
Salicaceae	*Salix babylonica*	Tree Deciduous	Perennial	Nur Münevver Pınar	April	Native
Ulmaceae	*Ulmus campestris*	Tree Diciduous	Perennial	Aydan Acar Şahin	February-March	Native
Betulaceae	*Corylus avellana*	Shrub Deciduous	Perennial	Aydan Acar Şahin	February-March	Native

TABLE 1. The taxonomic status of studied species, their life form and span, and collector name (continued)

Family	Species	Life form	Life span	Collector	Blooming time	Origin
Anacardiaceae	*Cotinus coggygria*	Shrub Deciduous	Perennial	Nur Münevver Pınar	May-June	Native
Cupressaceae	*Juniperus oxycedrus*	Shrub Evergreen or Tree Evergreen	Perennial	Aydan Acar Şahin	late October to early November	Native
Oleaceae	*Ligustrum japonicum*	Shrub Evergreen	Perennial	Nur Münevver Pınar	late May to early June	Exotic
Asteraceae	*Artemisia vulgaris*	Herb	Perennial	Aydan Acar Şahin	July-September	Native
Amaranthaceae	*Atriplex laevis*	Herb	Annual	Aydan Acar Şahin	May-July	Native
Asteraceae	*Cirsium arvense*	Herb	Perennial	Aydan Acar Şahin	May-October	Native
Poaceae	*Cynodon dactylon*	Herb	Perennial	Nur Münevver Pınar	April-November	Native
Amaranthaceae	*Chenopodium album*	Herb	Annual	Nur Münevver Pınar	May-August	Native
Apiaceae	*Daucus carota*	Herb	Biennial	Nur Münevver Pınar	June	Native
Poaceae	*Dactylis glomerata*	Herb	Perennial	Nur Münevver Pınar	May-July	Native
Poaceae	*Phleum exaratum*	Herb	Perennial	Nur Münevver Pınar	June-August	Native
Poaceae	*Poa pratensis*	Herb	Perennial	Nur Münevver Pınar	May-August	Native
Asteraceae	*Senecio vulgaris*	Herb	Annual	Aydan Acar Şahin	March-August	Native

3. Results and Discussion

A total of 29 allergenic plants were collected from Beşevler 10. year campus of Ankara University-namely-trees; *Acer pseudoplatanus, Betula pendula, Carpinus betulus, Chamaecyparis lawsoniana, Cupressus arizonica, Fraxinus excelsior, Juglans regia, Morus alba, Platanus orientalis, Populus alba, Quercus robur, Salix babylonica, Ulmus campestris,-* shrubs*; Corylus colurna, Cotinus coggygria, Juniperus oxycedrus, Ligustrum japonica,- herbs; Artemisia vulgaris, Atriplex laevis, Cirsium arvense, Cynodon dactylon, Chenopodium album, Daucus carota, Dactylis glomerata, Phleum exaratum, Poa pratensis, Senecio vulgaris, Urtica dioca* and *Xanthium spinosum.* The allergenic plants collected belonged to 12 families identified included Amaranthaceae, Anacardiaceae, Apiaceae, Asteraceae, Betulaceae, Cupressaceae, Fagaceae, Juglandaceae, Moraceae, Oleaceae, Platanaceae, Poaceae, Salicaceae, Sapindaceae and Ulmaceae. Among them 11% are exotic taxa and 89% are native (Table 1 and Figure 1). The use of some exotic species as ornamentals in towns and cities release large amounts of pollen with a demonstrated allergenic capacity [12,13]. It has been established that 51% of exotic plants used in Ankara landscape cause allergic reactions [13]. Among the studied most allergenic plants in campus, the percentage of the herbaceous was 21.42%, the woody was 42.85% and the shrubby was 35.73%.

When spring allergy season first starts, causing you to sniffle and sneeze, tree pollen is to blame. Trees start producing pollen as early as February in the campus. The trees keep producing pollen through June and produce exhibiting the highest pollen production levels between anemophilous species [13]. General blooming times of all allergenic plants are February-December. A significant portion of allergenic pollen is produced by larger perrennial plants, shrubs and trees. Pollen production per inflorescens in perrennial grasses was up to 3.5 times higher than annual [14]. The percentage of the perrennial plant was 11%, and the annual was 89% in the campus (Figures 1-2).

The main palynological features of the allergenic specimens that were examined are summarised in the Table 2 and are shown in Figures 3-9. The size, symmetry, shape, aperture, ornamentation of the pollen were determined and the results were compiled.

Table 2. The main palynological features of the allergenic specimens of Beştepe 10. Year campus

Species	Polar axes (P) (µm)	Equatorial axes (E) (µm)	Pollen shape	Exine thickness (µm)	Intine thickness (µm)	Ornamentation	Aperture type	Aperture percullarities	Polarity	Pollen unit	Pollen coating	Tectum type	Ubish Bodies	Pollination type Abiotic-wind / Biotic-insect)s/ Both
Acer pseudoplatanus	23-32 µm	24-41	Oblate Or Spheroidal	1.25 - 1.75	0.25-1	Striate-Reticulate -Perforate	Tricolpate	Aperture Membran Smooth	Isopolar	Monad	Pollenkitt	Eutectate	+	Both
Betula pendula	16-32	23-34	Suboblate Or Spheroidal	1-2	0.75-1	Granulate	Triporate, Rarely Diporate Or Tetraporate	Vestibulum, Oncus, Operculum	Isopolar	Monad	--	Pertectate	+	Wind
Carpinus betulus	32-40	38-45	Suboblate	1-1,25	0.5	Granulate	Tetra Or Pentporate	Oncus, Operculum	Isopolar	Monad	-----	Pertec	+	Wind
Chamaecyparis lawsoniana	25-32	25-32	Spheroidal	0.5	0,1-0,3	Granulate	Inaperturate Pseudoporate	---	Isopolar	Monad	------	Eutectate	+	Wind
Cupressus arizonica	25-30	25-30	Spheroidal	0.6-1	0,3-0,5	Granulate	Inaperturate-Pseudoporate	---	Isopolar	Monad	-----	Eutectate	+	Wind
Fraxinus excelsior	17-25	18-28	Spheroidal, Suboblate	1-1.5	0,75-1	Reticulate	Tricolpate	Aperture Membrane Granulate	Isopolar	Monad	Pollenkitt	Semitectate	-----	Wind
Juglans regia	35-46	42-47	Spheroidal, Suboblate	1-1.5	0,25-0,5	Scabrate	Pantoporate	Operculum, Annulus	Isopolar	Monad	----	Eutectate	----	Wind
Morus alba	17-21	18-23	Spheroidal	0.9	1	Granulate	Triporate, Rarely Tetraporate	Operculum	Isopolar	Monad	---	Pertectate	---	Wind
Platanus orientalis	17-25	17-25	Spheroidal	2,5-3	0.75-1	Reticulate	Tricolpate Rarely Tetracolpate	Aperture Membrane Granulate	Isopolar	Monad	----	Semitectate	+	Wind
Populus alba	22-36	22-36	Spheroidal	1-1.3	0,5-1	Granulate	Inaperturate	----	Isopolar	Monad	Pollenkitt		+	Wind
Quercus robur	21-25,7	23.7 - 28.7	Spheroidal Rarely Suboblate or Oblate	1,2-1,5	0.5-1	Verrucate	Tricolpate	---	Isopolar	Monad	----	Semitectate	+	Wind
Salix babylonica	14-15	18-25	Spheroidal	0,9-1,5	0.25-6	Reticulate	Tricolpate	Aperture Membrane Granulate	Isopolar	Monad	Pollenkitt	Semitectate	----	Insects
Ulmus campestris	25-35	28-35	Spheriodal	0.9-3	1-1,17	Rugulate	Tetraporate Or Hekzaporate	---	Heteropolar	Monad	---	Semitectate	---	Wind

Species	Polar axes (P) (µm)	Equatorial axes (E) (µm)	Pollen shape	Exine thickness (µm)	Intine thickness (µm)	Ornamentation	Aperture type	Aperture percullarities	Polarity	Pollen unit	Pollen coating	Tectum type	Ubish Bodies	Pollination type Abiotic-wind / Biotic-insect)s/ Both
Corylus avellana	20-25	25-30	Suboblate	1,2-1,5	0.5	Granulate	Triporate Rarely Tetraporate	Annulus, Operculum, Oncus	Isopolar	Monad	----	Semitectate	+	Wind
Cotinus coggygria	25-30	20-32	Subprolate	1-1,5	0.5-1	Striate-Reticulate	Tricolporate	----	Isopolar	Monad	----	Semitectate	----	Insects
Juniperus oxycedrus	20-36	20-36	Spheroidal	0.6-1.5	0,3-0,8	Granulate	Inaperturate, -Pseudoporate	----	Heteropolar	Monad	-----	Eutectate	+	Wind
Ligustrum japonicum	25-30	30-34	Spheroidal	1.9	1	Reticulate	Tricolporate	Aperture Membrane Psilate	Isopolar	Monad	-----	Semitectate	----	Insects
Artemisia vulgaris	10-25	10-25	Spheroidal	2-3	0,75-1	Scabrate	Tricolporate	Aperture Membrane Granulate	Isopolar	Monad	-----	Semitectate	-----	Wind
Atriplex laevis	13,5-25	13,5-25	Spheroidal	1-1.6	0,5-0,8	Scabrate	Pantoporate	Operculum	Isopolar	Monad	Pollenkitt	Semitectate	+	Both (Mostly Wind)
Cirsium arvense	25-32	30-35	Suboblate	3-5	0,75-1	Echinate-Perforate	Tricolporate Rarely Tetracolporate	----	Isopolar	Monad	Pollenkitt	Semitectate	-----	Insects
Cynodon dactylon	25-30	25-30	Spheroidal	1-1.25	0,5-0,75	Granulate	Monoporate	Annulus, Operculum	Heteropolar	Monad	-----		+	Wind
Chenopodium album	20-35	20-35	Spheroidal	1-1.25	0,3-0,	Scabrate	Pantoporate	Operculum	Isopolar	Monad	Pollenkitt	Semitectate	+	Both (Mostly Wind)
Daucus carota	20-27	12-17	Perprolate	3,6	1.1	Rugulate-Perforate	Tricolporate	Costae	Isopolar	Monad	---	Semitectate	+	Wind
Dactylis glomerata	26-38	26-38	Spheroidal	0.75-1	0,98-1,25	Granulate	Monoporate	Operculum, Annulus	Heteropolar	Monad	----	Semitectate	+	Wind
Phleum exaratum	25-30	25-30	Spheroidal	1-1.25	0,5-0,75	Granulate	Monoporate	Operculum, Annulus	Heteropolar	Monad	----	Semitectate	+	Wind
Poa pratensis	20-25	20-25	Spheroidal	0,7-1	0,7-1	Granulate	Monoporate	Operculum, Annulus	Heteropolar	Monad	----	Semitectate	+	Wind
Senecio vulgaris	20-23	17-22	Spheroidal or Suboblate	3-4	1	Echinate	Tricolporate	Operculum	Isopolar	Monad	Pollenkitt	Semitectate	----	Insects
Urtica dioca	10-15	12-16	Suboblate	0.7-1.0	0.1-0.4	Granulate	Triporate	Operculum, Annulus, Oncus	Isopolar	Monad	----	Eutectate	+	Wind
Xanthium spinosum	20-25	22-27	Spheroidal	1-1.5	0.75-1	Scabrate	Tricolporate	Operculum	Isopolar	Monad	Pollenkitt	Semitectate	----	Insects

*The information about the presence of ubisch bodies in pollen was adopted by PalDat (Palynological Database) [15]

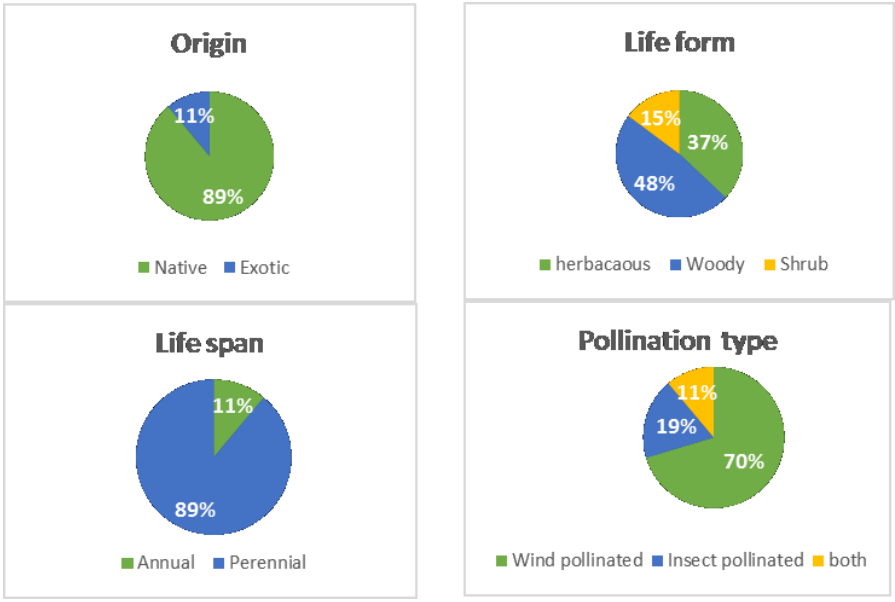

FIGURE 1. The percentages value of studied species, their origin, life form, life span and pollination type.

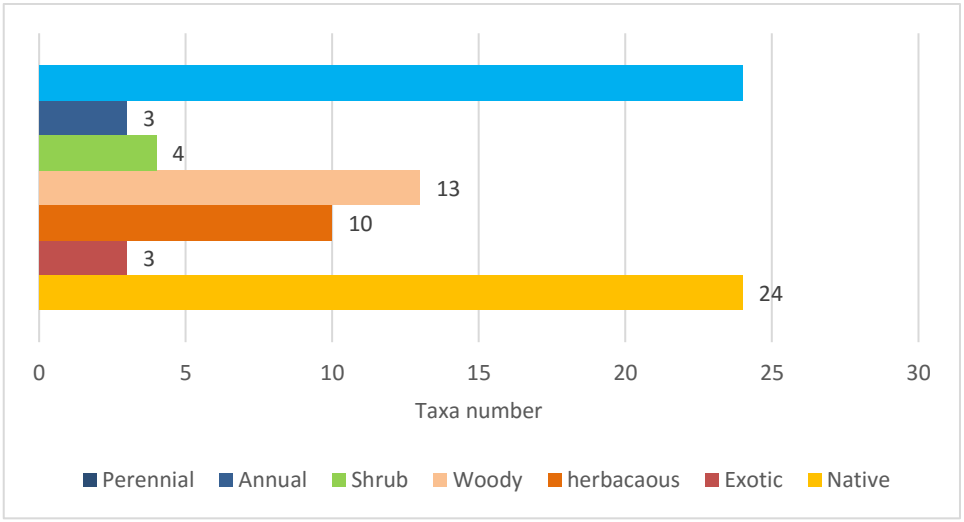

FIGURE 2. The taxa number based on the main characters of the studied taxa

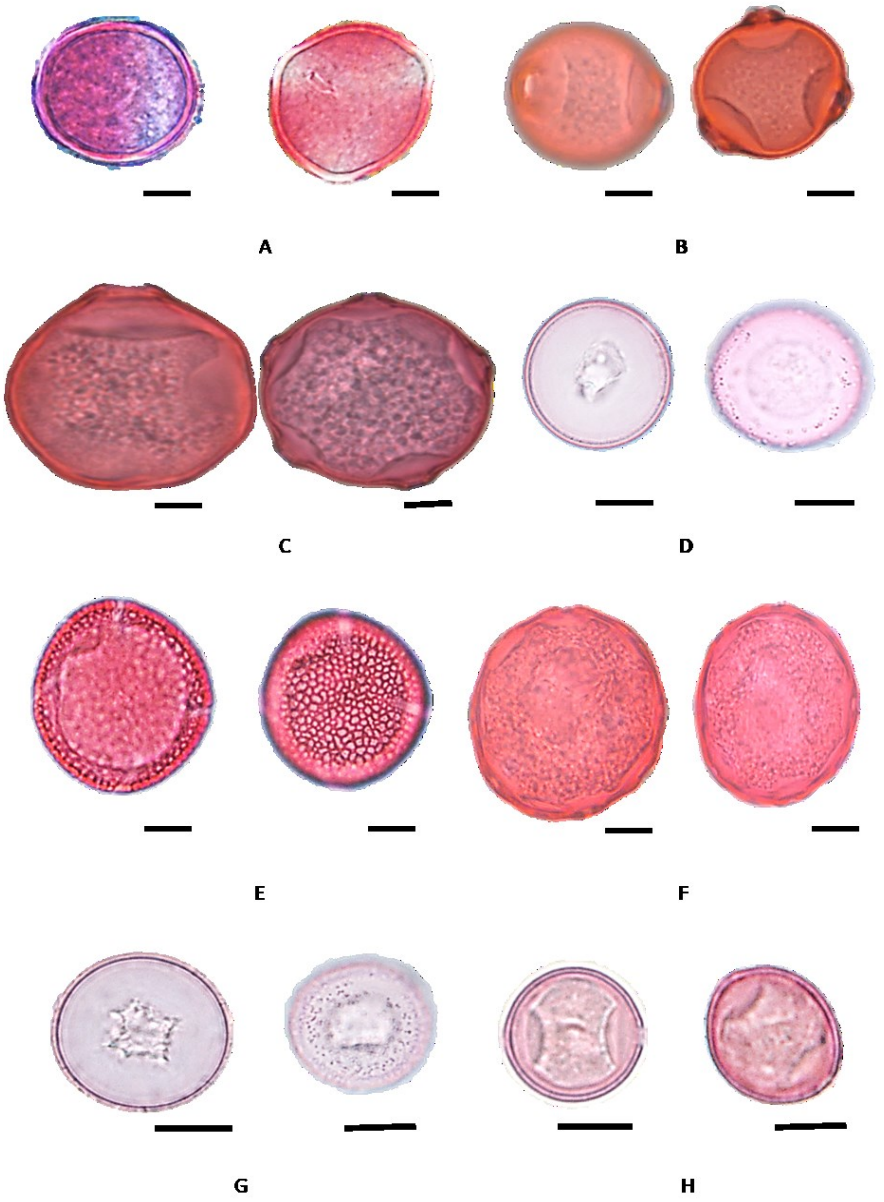

FIGURE 3. Light microscopy photos (LM) of pollen of allergic trees. A. *Acer pseudoplatanus*, B. *Betula pendula*, C. *Carpinus betulus*, D. *Cupressus arizonica*, E. *Fraxinus excelsior*, F. *Juglans regia*, G. *Chamaecyparis lawsoniana*, H. *Morus alba* (Bar=10˘m).

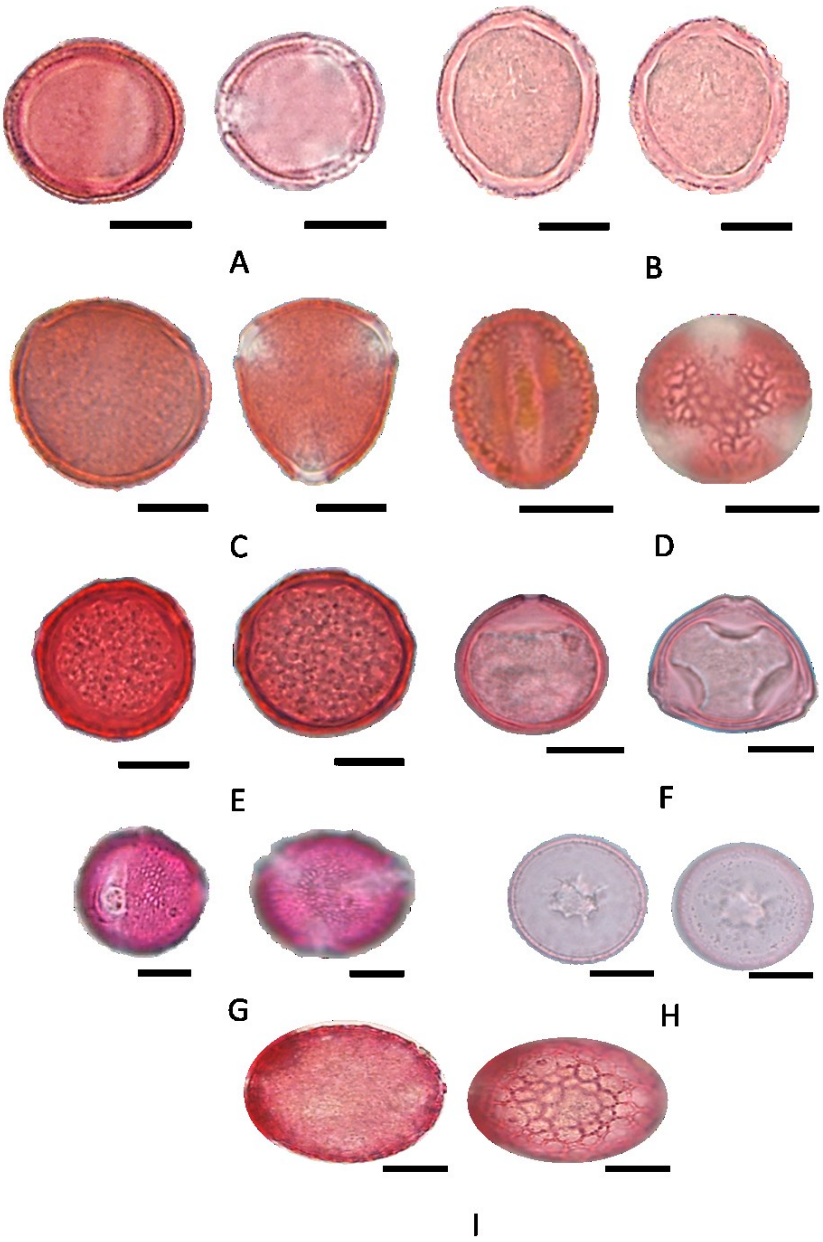
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FIGURE 4. Light microscopy photos (LM) of pollen of allergic trees. A. *Platanus orientalis*, B. *Populus alba*, C. *Quercus robur*, D. *Salix babylonica*, E. *Ulmus campestris,* F. *Corylus colurna,* G. *Cotinus coggygria,* H. *Juniperus oxycedrus,* I. *Ligustrum japonica* (Bar=10µm).

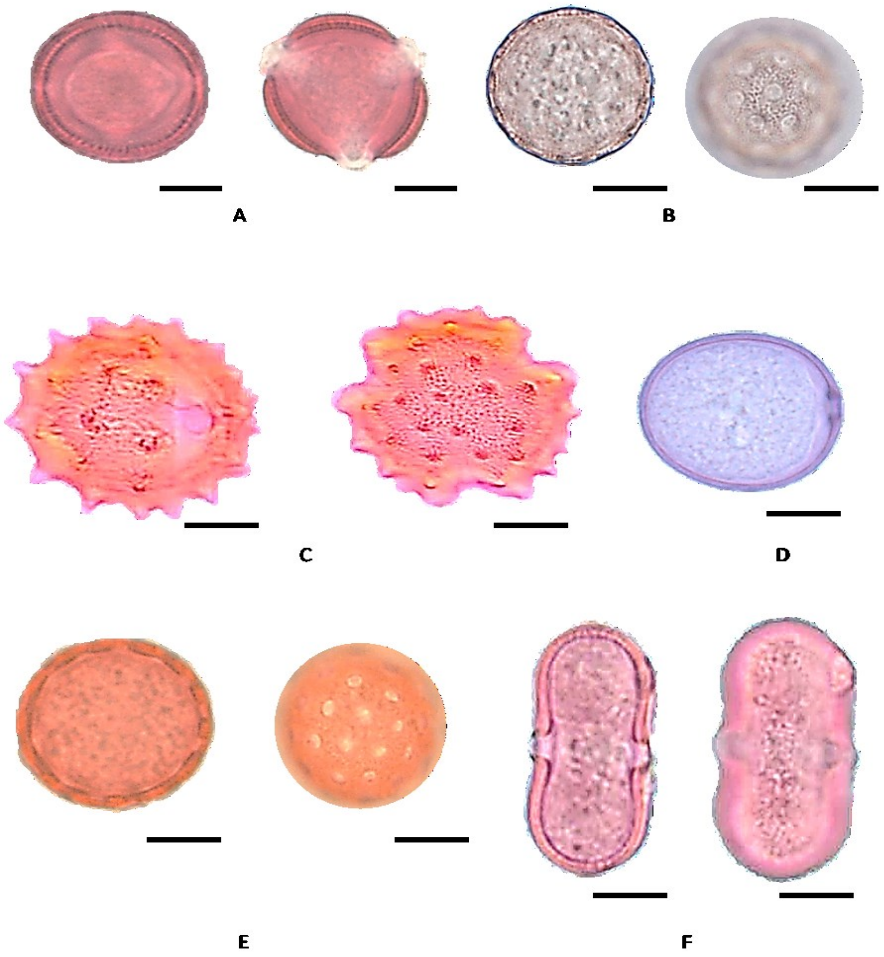

FIGURE 5. Light microscopy photos (LM) of pollen of allergic trees. A. *Artemisia vulgaris,* B. *Atriplex laevis*, C. *Cirsium arvense*, D. *Cynodon dactylon,* E. *Chenopodium album* F. *Daucus carota* (Bar=10µm).

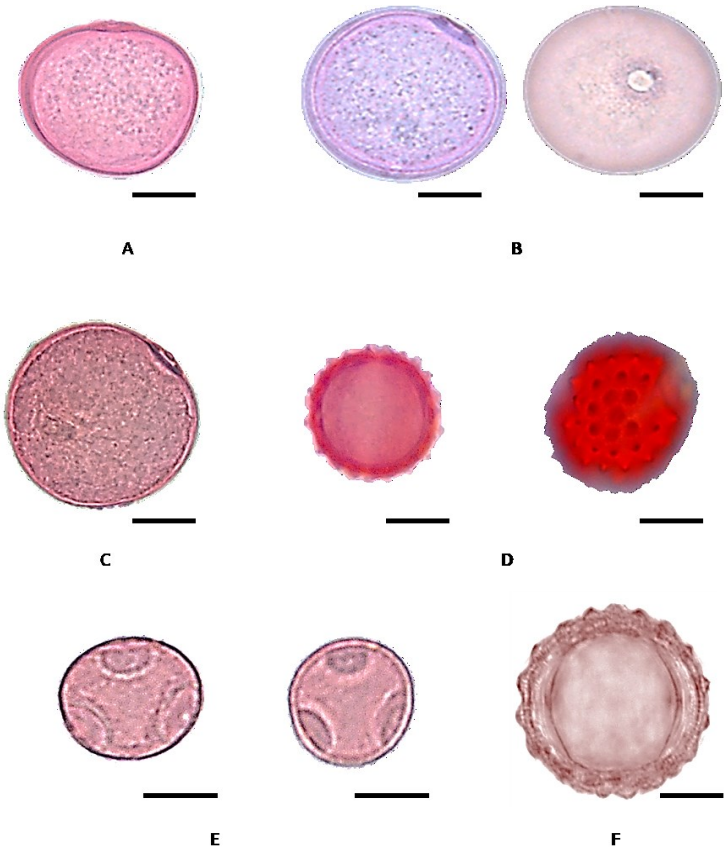

FIGURE 6. Light microscopy photos (LM) of pollen of allergic trees. A. *Dactylis glomerata,* B. *Phleum exaratum*, C. *Poa pratensis*, D. *Senecio vulgaris,* E. *Urtica dioca,* F. *Xanthium spinosum* (Bar=10µm).

The pollen grains of the allergenic plants are usually radially symmetrical and isopolar, rarely heteropolar (*Cynodon dactylon*, *Dactylis glomerata*, *Juniperus oxycedrus*, *Phleum exaratum*, *Poa pratensis* and *Ulmus campestris*). Allergenic pollen shapes of campus is oblate, spheroidal (22), suboblate (8), oblate (2), subprolate (1) and perprolate (1). Subprolate and perprolate shaped pollen grains were seen in insect pollinated. The polar axis ranging from 13.5 to 46 µm and the equatorial axis from 12 to 47 µm in this study. The dimensions are smaller in *Artemisia vulgaris* and larger in *Carpinus betulus*. Size of pollen grains vary in size (12-300 um), but are light enough to allow the wind to carry them for miles. So, in

general, wind-pollinated plants pollen grains are relatively small in size [16]. The pollen with large size, are less likely to irrittate lower airways [17].

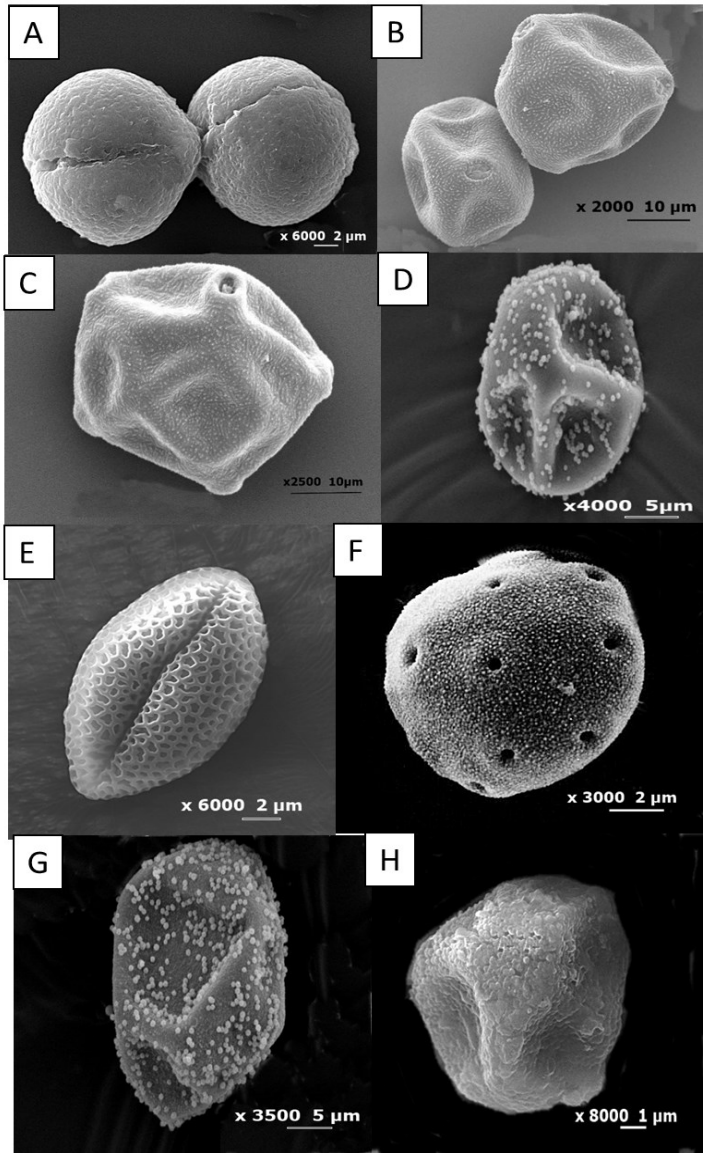

FIGURE 7. Scanning electron microscopy photos (SEM) of pollen of highly allergic trees. A. *Acer pseudoplatanus*, B. *Betula pendula*, C. *Carpinus betulus*, D. *Cupressus arizonica*,

E. *Fraxinus excelsior*, F. *Juglans regia*, G. *Chamaecyparis lawsoniana*, H. *Morus alba*. (Bar=10µm).

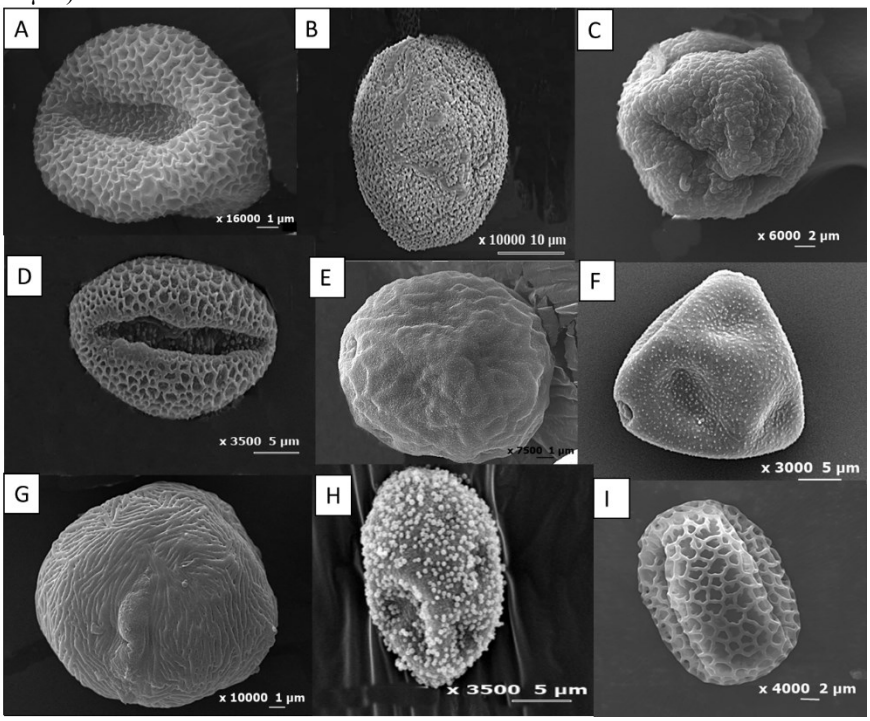

FIGURE 8. Scanning electron microscopy photos (SEM) of pollen of highly allergic trees. A. *Platanus orientalis*, B. *Populus alba*, C. *Quercus robur*, D. *Salix babylonica*, E. *Ulmus campestris,* F. *Corylus colurna,* G. *Cotinus coggygria*, H. *Juniperus oxycedrus,* I. *Ligustrum japonica* (Bar=10µm).

We could define several pollen types according to their number of aperture and surface ornamentations. The distribution of the apertures on the surface of the pollen grain is an important diagnostic feature. Six aperture types were recorded in the study (Table 2, Figure 3). This includes; inaperturate (*Chamaecyparis lawsoniana, Cupressus arizonica, Juniperus oxycedrus* and *Populus alba*), monoporate (*Cynodon dactylon, Dactylis glomerata, Phleum exaratum and Poa pratensis*), triporate or tetraporate or pentaporate (*Betula pendula*, *Carpinus betulus, Morus alba, Ulmus campestris* and *Corylus avellana*), pantoporate (*Juglans regia, Atriplex laevis* and *Chenopodium album*), tricolpate or tetracolpate (*Acer pseudoplatanus, Fraxinus excelsior, Platanus orientalis, Quercus robur* and *Salix babylonica*), tricolporate (*Artemisia vulgaris, Cirsium arvense, Cotinus coggygria, Daucus carota, Ligustrum japonicum* and *Senecio vulgaris*). All grass pollen grains are allergenic and

monoporate, having a single circular or oval pore. Worldwide, at least 40% of allergic patients are sensitized to grass pollen allergens [18]. Gymnospermae allergenic species (juniper and cypress) and poplar (Angiospermae) that can be carried long distances by the wind have inaperturate pollen.

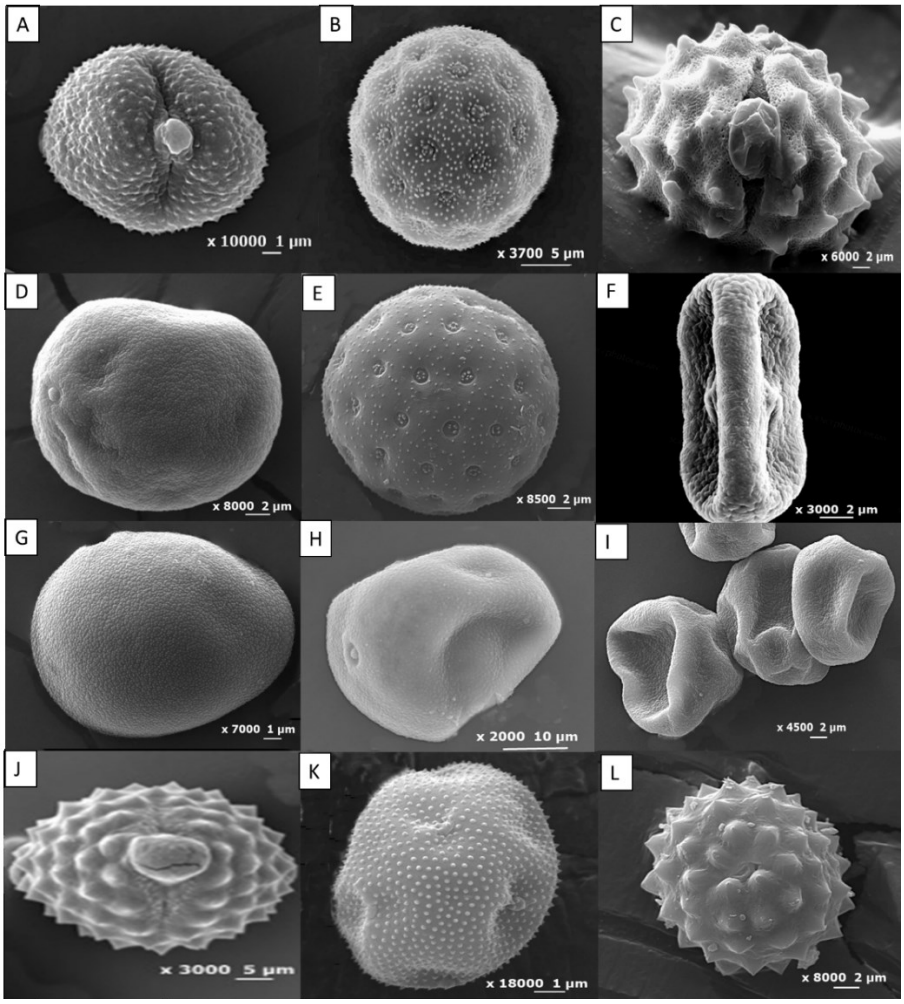

FIGURE 9. Scanning electron microscopy photos (SEM) of pollen of highly allergic weeds. A. *Artemisia vulgaris* B. *Atriplex laevis*, C. *Cirsium arvense*, D. *Cynodon dactylon,* E. *Chenopodium album* F. *Daucus carota,* G. *Dactylis glomerata,* H. *Phleum exaratum*, I. *Poa pratensis*, J. *Senecio vulgaris,* K. *Urtica dioca,* L. *Xanthium spinosum* (Bar=10µm).

Surface pattern recorded for this study as evident in Table 2 and Figure 3, include echinate *Senecio vulgaris*), echinate-perforate (*Cirsium arvense*), granulate (*Betula pendula, Carpinus betulus*, *Chamaecyparis lawsoniana, Corylus avellana, Cupressus arizonica, Cynodon dactylon, Dactylis glomerata, Juniperus oxycedrus, Morus alba, Phleum exaratum, Poa pratensis, Populus alba, Urtica dioca),* reticulate (*Fraxinus excelsior, Ligustrum japonicum, Platanus orientalis* and *Salix babylonica*), rugulate (*Ulmus campestris*), regulate-perforate (*Daucus carota*), scabrate (*Artemisia vulgaris, Atriplex laevis, Chenopodium album*, *Juglans regia, Xanthium spinosum*), striate-reticulate-perforate (*Acer pseudoplatanus*) striate-reticulate (*Cotinus coggygria*) and verrucate (*Quercus robur*). We observed that granulate is the most common ornamentation between allergic plants. Generally, granulate ornamentation was observed in pollen of wind-pollinated plants. In general, wind-pollinated plants should produce large quantities of pollen to increase pollination success [16]. In Kermanshah, 56 allergenic pollens were directly collected from nature and examined by a light and scanning electron microscope by Masoumi [15]. In our study, pollen grains were classified as reticulate, striate-reticulate, granulate, psilate, echinate, echinate-perforate, scabrate, rugulate, rugulate-perforate, verrucate and striate-reticulate-perforate in terms of ornamentation (Figure 10). Also, Moon et al. said that the echinate surface ornamentation could act as a stimulus for allergy by accumulated more allergen in the pollen ectexine [15].

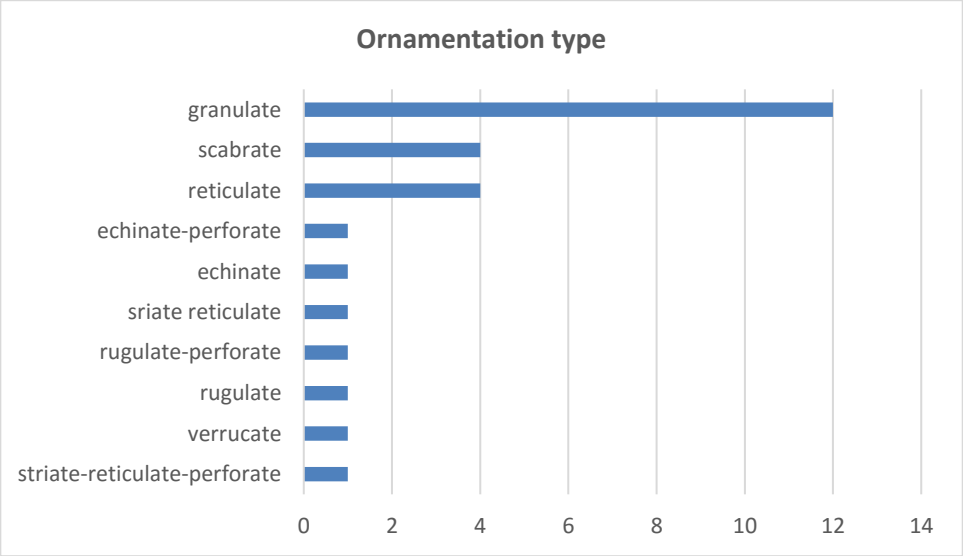

FIGURE 10. Ornamentation types of studied taxa.

Pollenkitt is a viscous material that coats grains of pollen and plays important roles in pollen dispersion and plant reproduction. Oily layer pollenkitt play a role in allergy and asthma. Because lipids can be ligands to allergenic proteins [19,20]. Pollen grains of *Acer pseudoplatanus, Atriplex laevis, Chenopodium album, Cirsium arvense, Fraxinus excelsior, Populus alba, Salix babylonica* and *Senecio vulgaris* have pollenkitt.

Most angiosperms with a secretory tapetum produce not only tryphine and pollenkitt, but also Ubisch bodies (orbicules) at the final stages of pollen maturation. It has been investigated whether orbicules also contain allergens [21]. Orbicules were detected in pollen belonging to the Betulaceae, Chenopodiaceae, Fagaceae, Poaceae, Polygonaceae and Urticaceae families. However, some clinically important *Ambrosia coronopifolia* Torr. and A. Gray, *Artemisia vulgaris* L. (Asteraceae) and *Olea europaea* L. (Oleaceae) species do not have orbicules [22,23]. It is stated that the orbicules can act as vectors in the allergen distribution in the atmosphere [23]. In our studied taxa, the orbicules are found in *Acer pseudoplatanus, Betula pendula*, *Carpinus betulus, Chamaecyparis lawsoniana, Corylus avellana, Cupressus arizonica, Juniperus oxycedrus, Platanus orientalis, Populus alba*), *Quercus robur, Ligustrum japonicum, Atriplex laevis, Cynodon dactylon, Dactylis glomerata, Phleum exaratum, Poa pratensis, Chenopodium album* and *Daucus carota* pollen. Due to their small size, orbicules can easily penetrate deeper into the lower respiratory airways than the pollen grains if dispersed into the atmosphere and inhaled by individuals [24].

Flowers provide nectar and pollen as rewards [25,26]. Despite the importance of floral diversity for proper bee nutrition, urban development has drastically altered resource availability and diversity for these important pollinators. Lau et al. (2019) have said that understanding the floral resources foraged by bees in urbanized areas is key to identifying and promoting plants that enhance colony health in those environments. They also reported that “predominant” and “secondary” pollen types belonged to the families Arecaceae, Sapindaceae, Anacardiaceae, Apiaceae, Asteraceae, Brassicaceae, Fabaceae, Fagaceae, Lythraceae, Myrtaceae, Rhamnaceae, Rosaceae, Rutaceae, Saliaceae, and Ulmaceae. Among these families, their pollen are found in highly allergic plants [27]. In Table 1, you can see the families of allergic plants in Beşevler 10. Year Campus. Allergenic *Quercus robur, Daucus carota, Acer pseudolatanus, Populus alba, Artemisia vulgaris, Juglans regia, Ligustrum japonicum, Cirsium arvense, Chenopodium album,* and *Senecio vulgaris* might respectively represent dominant, secondary, minor pollen yields for bee species in Beşevler 10. Year Campus. The pollen and nectar yields of allergenic *Salix* species are dominant and secretion honey is produced by collecting the

secretions of insects living on trees in September by honey bees [28]. It's relatively common for the pollen and other plant allergens to contaminate honey which can cause allergies. Symptoms from a honey allergy may resemble common pollen allergy symptoms [29,30]. Dutau and Rance (2009) have said that allergy to pollen grains, particularly of the Asteraceae family (mugwort, chamomilla, dandelion) are a risk factor for allergy to honey and royal jelly [31].

4. Conclusion

In this study, we have selected 29 allergenic species in Beşevler 10. year campus and investigated the detail pollen morphological properties of them using light and scanning electron microscopies. The relationship between the morphological characteristics of airborne pollen and their potential tendency as allergens has been tried to be revealed. In addition, 11 of the allergic plants on campus were observed to be important for beekeeping.

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ANTIOXIDANT AND PHYSIOLOGICAL ANALYSIS OF *TRITICALE* UNDER COLD ACCLIMATION CONDITIONS *IN VITRO* AND *EX VITRO*

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ABSTRACT. *Triticale* is an artificial species that originated about 130 years ago from between wheat and rye, and the first commercially viable cultivars were released in the 1960s. The crop exhibits high yield, promising long term potential, elevated grain quality, and better resistance to pathogens, desirable amino acid content, and high adaptation ability to adverse climate conditions. Sudden decreases in climate can pose significant losses in many crops including *Triticale*. Understanding plant response to cold acclimation could help developing crops resilient to cold. In this study, we aim to compare the antioxidants and physiological content of *Triticale* under cold acclimation *in vitro* and *ex vitro*. In our study, five triticale cultivars, *Ümran Hanım*, *Alper Bey*, *Mikham 2002*, *Tatlıcak*, and *Melez 2001* were used as the plant material. *Triticale* seeds were planted in 15 cm sand pods. They were maintained in 20/18^{0}C (day / night) greenhouse with a 12 h day length for 10 days to initiation germination. After 2 weeks the plants were transferred at 4±1^{0}C for cold acclimation for 30 days. Callus was transferred to a hormone-free MS medium for 1 month. All cultures were kept under fluorescent light with 15000 lux and 16 h/8 h light/dark cycle at 25±1^{0}C. The culture media was subsequently refreshed and kept under fluorescent light with 1500 lux and 16 h/8 h light/dark cycle at 4±1^{0}C under cold acclimation. Our results revealed that the cold acclimation changed the activities of APX (Ascorbate Peroxidase), SOD (Superoxide Dismutase), and CAT (Catalase) under both *ex vitro* and *in vitro* conditions. The highest correlation between enzyme activities and cold resistance was observed in the sugar content of in vitro stress callus. Our results indicated as closely related to proline, sugar content and antioxidant enzyme activities at cold acclimation in the evaluation of cold tolerance of *Triticale* cultivars.

Keyword and phrases. Antioxidant activity, callus, cold acclimation, proline, *Triticale*.

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1. INTRODUCTION

Low temperature is a notable abiotic stress that bring about important agricultural casualties around the world [1, 2]. The rapidly changing temperature is noted to adversely affecting plant growth and productivity. Plants are complex organisms, exhibiting a wide variation in their ability to maintain growth during chilling and lower temperatures [3]. Many plant species developed cold resistance to low non-freezing temperatures, a physiological process known as cold acclimation [4, 5]. Cold acclimation is a dynamic process in which plants acquire tolerance to sub-zero temperatures when exposed to low temperatures [6]. Plants respond to acclimation through several of biochemical, molecular genetics, ecological, and physiological alterations involving changes in carbohydrate, proline, protein content, and enzymatic activities [7].

Soluble carbohydrates and free proline may inhibit water loss during the acclimation [8]. Cold acclimation increases the level of proline via changes in enzyme activities in the proline metabolism pathway that in turn enhances cold-resistance [9]. Proline is also strongly associated with the plant cold stress since free proline increases during the acquisition of the cold resistance in plant species. The antioxidant enzymes in the plants are known to play a major role in the regulation against stress. Plant species produce various types of antioxidants, such as APX (Ascorbate Peroxidase), CAT (Catalase), and SOD (Superoxide Dismutase) to reduce the stress triggered by the elevated oxidative level [10, 11]. *Triticale* is used both for food and feed and is superior to other cereals in terms of nutritional quality [12]. Therefore, *Triticale* has gradually transformed into an important crop worldwide. *Triticale* can also contribute to environmental quality via better conservation of the soil. Resistance to diseases, the ability to grow in low pH, and persistence to drought are among other superiorities [13, 14]. The main breeding goals of *Triticale* breeding programs are increasing grain yield, nutritional quality, and plant height. Although traditional breeding methods have been routinely employed, incorporating desirable genes into the released cultivars to induce stress resistance via genetic engineering can be an alternative approach to enhance the *Triticale* breeding [15]. The selection and development of stress-resistant genotypes require an efficient screening method. Cell and tissue culture can be efficient methods for increasing the plant productivity and quality of plant materials. These methods can be also be used for understanding molecular and cellular basis of abiotic and biotic stress factors in plants and ultimately eliminating the crop yield losses due to abiotic and biotic stress factors. *In vitro*, culture techniques are among the available methods for improving cultivars resistant to a number of biotic and abiotic stress factors in the context of sustainable agriculture [16]. In recent decades, the *in vitro* plant tissue culture selection pressure method has been one of the most commonly used techniques for the selection of

genotypes resistant to environmental stress. *In vitro* culture has been used to obtain cold-resistant plants as there is a relation between cellular machinery and *ex vitro* plant with desirable traits. On the other hand, it is often uneasy to analyze the response of plants to various abiotic stresses in the field or in greenhouse conditions, due to the complex and unstable nature of these stresses. *In vitro* tissue culture is an extremely powerful tool to have a deeper understanding of physiology and biochemistry in molecular plant breeding under adverse environmental conditions [17].

The purpose of this study was to evaluate the cold acclimation stress in a number of *Triticale* plants using the proline content and antioxidant capacity of plants derived from *in vitro* culture and greenhouse (*ex vitro*).

2. Materials and Methods

Plant Material

In our study, the five most widely planted *Triticale* cultivars, *Ümran Hanım*, *Alper Bey*, *Mikham 2002*, *Tatlıcak*, and *Melez 2001* were used as the plant material. *Triticale* seeds were planted in 15 cm pods in the sand. They were maintained in $20/18^0$C (day/night) greenhouse with a 12 h day length for 10 days for the initiation of the germination. Two weeks later the plants were transferred at 4 ± 1^0C for cold acclimation and maintained there for 30 days.

Callus Induction

The mature seeds were sterilized with 1% NaOCl for 5 minutes, washed several times with sterile distilled water, and rinsed with several changes of sterile distilled water overnight at 4^0C. The mature embryos were cultivated in Petri dishes containing full MS medium. The plant material was kept in MS for 30 days at 25±1 and in 16 hours light/8 hour dark photoperiod at 1500 lux illumination intensity. Mature embryos were removed aseptically using forceps and placed on MS medium [18] with 2 mg L^{-1} glycine, 4 mg L^{-1} 2,4-D (2,4-dichlorophenoxyacetic acid), 100 mg L^{-1} myo-inositol, 0,5 mg L^{-1} nicotinic acid, 0,5 mg L^{-1} pyridoxine HCl, 0,1 mg L^{-1} of thiamine HCl vitamins, 1,95 g L^{-1} of MES, 50 mg L^{-1} of ascorbic acid, 20 g L^{-1} of sucrose, solidified with 7 g L^{-1} of agar and the pH adjusted to 5.8 prior to autoclaving. In order to sterilize the vitamins and hormones, 0.22 μm of porous cellulose nitrate filters were used. The mature embryos were incubated in total darkness at 25 ± 1^0C temperature for one month.

Cold Acclimation

Callus was transferred to a hormone-free MS medium for one month. All cultures were kept under fluorescent light with 15000 lux and 16 h/8 h light/dark cycle at 25 ± 1^0C. The culture media were subsequently refreshed and kept under fluorescent light with 15000 lux and 16 h/8 h light/dark cycle at 4 ± 1^0C under cold acclimation. The total culture duration was one month.

Proline Estimation

Proline content was detected with the method of Bates et al. [19]. Briefly, 100 mg of plant material was homogenized in 5 mL of 3% aqueous sulfosalicylic acid and centrifuged at 4^0C for 15 min at 4800 rpm. 2 mL of extract was mixed with 2 mL of acid-ninhydrin and 2 mL of glacial acetic acid in test tubes. Samples were kept for 1 h at 100^0C. The reaction was completed in an ice bath. 4 mL of toluene was used for reaction mixture extraction. The absorbance of color reaction product was measured at 520 nm using toluene for a blank. The proline concentration was determined from a calibration curve.

Soluble Sugars Determination

For soluble sugars determination, a total of 50 mg of tissue per embryogenic callus was grounded in a mortar, homogenized in 1 ml of ethanol (80%) and centrifuged at 5000 rpm for 10 min at 4^0C. Supernatants were transferred into other tubes and the pellets were homogenized again in 0,5 mL ethanol (80%),and centrifuged as above. The second supernatant was added to the first one. Total soluble sugars were measured by a modified method of Watanabe et al. [20]. Briefly, a total of 1 mL of extract was reacted with 3 mL freshly prepared anthrone reagent (50 mg anthrone, 50 mL of H_2SO_4 95%) at 100^0C for 10 min. After cooling on ice, the total sugar content was determined at 620 nm by a spectrophotometer using glucose as standard.

Enzyme Extraction and Assay

Samples for the assay of SOD (Superoxide Dismutase), APX (Ascorbate Peroxidase), and CAT (Catalase) contents were collected from the newly proliferated leaves at the end of 3 months. Fresh leaves tissue (500 mg) was homogenized in 5 mL 10 mM potassium phosphate buffer (pH:7.0) containing 4% (w/v) PVP (polyvinylpyrrolidon). The homogenate was centrifuged at 12000 rpm for 30 minutes at 4^0C, and the resulting supernatant was used as an enzyme extract. SOD (Superoxide Dismutase) activity was assayed by monitoring the inhibition of

photochemical reduction of NBT (nitroblue tetrazolium chloride) at 560 nm as described by Agarwal and Pandey [21] in a reaction mixture containing 13 mM methionine, 75 mM NBT (nitroblue tetrazolium chloride), 0.1 mM EDTA (ethylenediamine tetraacetic acid), 50mM phosphate buffer (pH:7.8), 2 μM riboflavin, and 0,02 cm^3 of enzyme extract. CAT (Catalase) activity was measured by monitoring the decrease in absorbance at 240 nm in 50 mM phosphate buffer (pH:7.5) containing 20 mM H_2O_2. One unit of CAT (Catalase) activity was defined as the amount of enzyme that was used 1 μmol H2O2 [22]. APX (Ascorbate Peroxidase) activity was measured according to Nakano and Asada [23]. The reaction mixture contained 50 mM potassium phosphate buffer (pH:7.0), 0.5 mM ascorbic acid, 0.1 Mm H_2O_2,and 0.1 mL of enzyme extract in a total volume of 1 mL. The concentration of oxidized ascorbate was calculated by 150 decreasing in absorbance at 290 nm.

Statistical Analysis

Each experiment was repeated three times. Analysis of variance was conducted using a one-way ANOVA test using SPSS 13.0 and means were compared following Duncan's multiple testing procedures test. The alpha cut-off value of 0.05 was considered throughout the statistical tests.

3. Results

Proline Assay

Proline level is increased in the leaves and callus of all the *Triticale* cultivars in both *in vitro* and *ex vitro* conditions after acclimatization. In fact, a dramatic increase in proline was observed in the acclimation period in all cultivars. Proline values indicated a narrow range of variation among cultivars for 7 days under *in vitro* conditions, ranging from 0,923 to 1,167 nmol g^{-1} FW (Fresh Weight), except the *Tatlıcak* cultivar (Figure 1). The highest proline value for 7 days, was found in *Tatlıcak* (1,370 nmol g^{-1} FW), which was followed by *Melez* 2001, *Mikham 2002*, *Alper Bey* and *Ümran Hanım*. All cultivars indicated a slight increase in proline concentration on the 14^{th} day compared to *ex vitro* (Figure 1). The lowest amount of proline was found in *Alper Bey*, but the highest amount of proline was noted in *Alper Bey* for 14-day assay. The highest amount of proline for 21-day assay was found in *Alper Bey* and *Tatlıcak*, and the lowest in *Mikham 2002* and *Melez 2001 in vitro* and *ex vitro* (Figure 1).

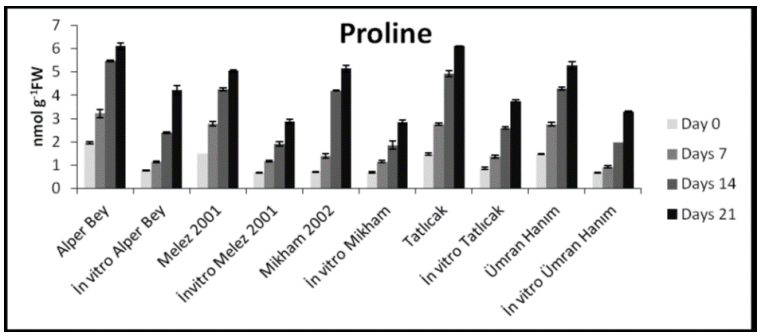

FIGURE 1. Changes of proline activity in five *Triticale* cultivars treated cold acclimation.

Sugar Content

The sugar content in 5 the cultivars of *Triticale* (*Ümran Hanım*, *Alper Bey*, *Mikham 2002*, *Melez 2001*, and *Tatlıcak*) were studied under low-temperature conditions. Figure 2 shows a gradual increase in sugar content in the leaves and callus of 5 the cultivars with increasing cold treatment. The callus and leaves of *Tatlıcak* showed 0.75 (leaves) to 1.11 (callus) nmol g^{-1} FW increase on the 7th day at *in vitro* conditions. Similarly, Tatlıcak showed 0.91 (leaves) to 1.29 (calli) nmol g-1 FW increase at the end of the 14th day under low-temperature conditions. Moreover, *Tatlıcak* slightly increased at the end of the 21st day by exhibiting 1.31 (leaves) to 1.39 (callus) nmol g^{-1} FW under the low-temperature conditions.

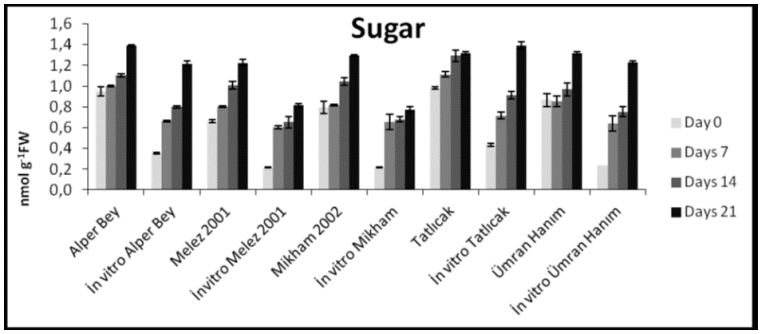

FIGURE 2. Changes of sugar content in five *Triticale* cultivars treated cold acclimation.

Antioxidant Enzyme Activity

Triticale leaves and callus were exposed to the cold stress, significantly affecting antioxidant enzyme activities after the 7th days of cold acclimation

(Fig. 3, 4-5). SOD (Superoxide Dismutase) activity was significantly higher in all tested cultivar leaves compared to cold-stressed callus. Among all the tested cultivars, *Tatlıcak* and *Alper Bey* indicated a higher SOD (Superoxide Dismutase) activity, whereas *Melez 2001*, *Mikham 2002*,and *Ümran Hanım* had a similar trend under *in-vitro* and *ex-vitro* conditions (Fig. 3).

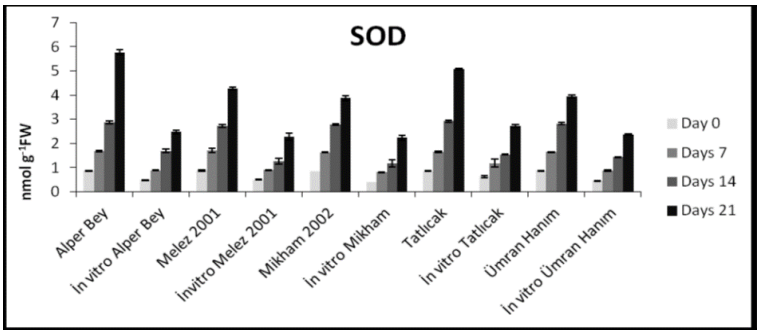

FIGURE 3. Changes of SOD (Superoxide Dismutase) in five *Triticale* cultivars treated cold acclimation.

Moreover, we observed that *Melez 2001* had higher activity at the end of the 7th day. As shown in Fig. 4, APX (Ascorbate Peroxidase) activity of five *Triticale* cultivars was obvious on the 6 activity days.

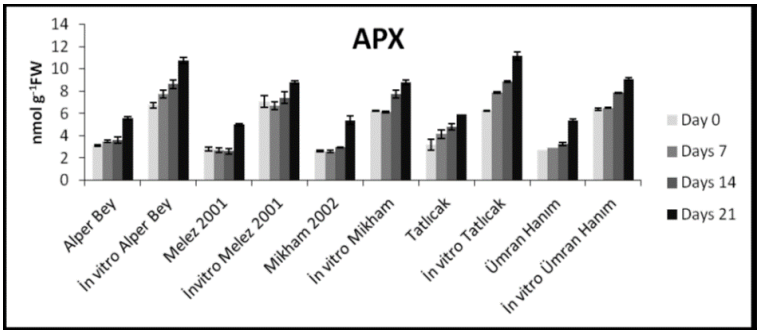

FIGURE 4. Changes of APX (Ascorbate Peroxidase) in five *Triticale* cultivars treated cold acclimation.

There was a detectable APX (Ascorbate Peroxidase) level difference between *ex vitro* plant and stressed callus. The APX (Ascorbate Peroxidase) activity in all tested cultivars was significantly increased by cold stress. Increase *ex vitro* tested plants

were higher than in the other cultivars. CAT (Catalase) activity also increased considerably under low-temperature conditions. The activity of CAT (Catalase) reduced progressively and significantly at the end of the 14th and the 21st day acclimation. Nevertheless, the activity increased significantly in callus stressed at the end of the 7th day (Fig. 5). The highest CAT (Catalase) activity was observed in the cold-acclimated leaves of the *Tatlıcak*, whereas the lowest level of activity at CAT (Catalase) was in *Mikham 2002 in vitro* callus (Fig. 5).

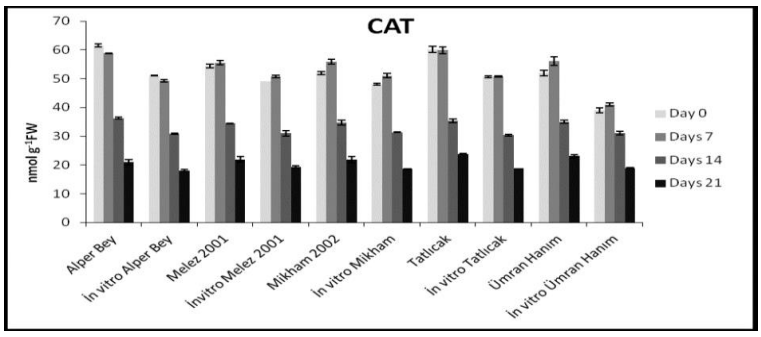

FIGURE 5. Changes of CAT (Catalase) in five *Triticale* cultivars treated cold acclimation

4. DISCUSSION

Earlier studies focusing on cold stress have indicated that the extent of cold stress adaptation in the whole plant is also reflected in callus tissue [11]. Extensive research has been conducted on understanding the physiological mechanisms underlining the cold stress in plants [24]. However, there have been a few studies on cellular level studies from cereal plants regarding cold acclimation at the tissue culture [25, 26]. The exposure of plants to cold stress induces many changes at the physiological and molecular levels [27, 28]. Pinpointing the cold resistance mechanisms under field conditions may not be straight forward due to several of other environmental factors. Cell and tissue culture have been useful for studying cold resistance mechanisms as they allow detection of relatively rapid responses, short generation time, and the use of controlled environmental conditions. Therefore, studies focusing on cold stress are preferably applied to in vitro cultures with the environmental control room set to low temperatures [29] Tissue culture is an important strategy that allows a controlled and uniform environment for studying physiological and molecular mechanisms in plants at the cellular differentiation under cold stress conditions [30]. In this study, physiological responses of *Triticale* under cold stress were studied. The results of the present study indicated that *Triticale* callus tissues respond quite differently to cold stress in comparison to the whole plant during acclimation. In this study,

through an analysis of the changes in biochemical properties in cultivated *Triticale* callus and whole plants, we found regular changes in proline, sugar content, and antioxidant enzyme activities at cold acclimation in the evaluation of cold tolerance of *Triticale* cultivars. These results suggested that the proline, sugar and antioxidant enzyme activities were important influential factors. The proline content in triticale increased in cold applications (7, 14 and 21days) (Figure 1). It has been reported that the proline levels in pea increases under cold acclimation [31]. In previous studies, it was determined that the proline content in wheat leaves increased significantly under cold stress. Proline levels have proven to be the main factors of cold resistance. The proline content in the *Triticale* plant and callus increased in cold applications. However the all *Triticale* cultivars exhibited different proline content of resistance to cold (Figure 1). The findings in our study are in coherence with the previous reports. Vera-Hernandez et al. [32] notified that cold stress increased the proline content in the amaranth. Esim and Atici [33] reported that cold stress remarkably improved proline content in wheat. The degree of cold resistance appeared to be directly related to the proline content of the callus and seedling confirm that tolerance is induced by cold levels. The results suggested that the cold-induced *proline content* might be an adaptive property for the survival and stability of the growth rate of *Triticale* seedlings under cold stress conditions. According to the carbonhydrate analysis, our study also has demonstrated that *in vitro* and *ex-vitro* were strongly improved with cold resistance. *Ex vitro* analysis showed similar changing trends that were gradually increased in planted *Triticale* cultivars, but callus was significantly increased and then recovered after being exposed to cold (Fig. 2). The results indicated that after 21st day of cold treatments at 4^{0}C, sugar content in *in vitro* of the *Tatlıcak, Ümran Hanım* and *Alper Bey* cultivars callus underwent drastic changes to acclimatize to the cold stress condition. Therefore, those cultivars have showed highly resistance in response to cold stress. This result indicated that the increases in sugar content were potentially an important factor in the cold stress of plant species. Carbohydrates have shown osmoprotectant properties as they prevent dehydration of cytoplasm and help to interact with lipid bilayer [34]. The interaction with lipid bilayer is key to providing resistance to cold, and this formation is closely related to sugar content in the cell membrane. The results shown in Fig 2 indicate that the *in vitro* acclimatization 21 days had the highest sugar content when compared to the *ex-vitro* treatments. This reveals the richness in tissue culture and the content of sugar, and thus it potentially improves cold stress response. This is consistent with a previous study in which Upadhyaya et al. [11] reported remarkably high sugar content in callus derived from rice plants exposed to cold stress comparing to acclimatized seedlings. The simultaneous activity of multiple antioxidant enzymes plays an important role in the conservation of the plant cell against stress factors. All cultivars tested in the present paper showed changes in antioxidant enzyme activities

during cold acclimation. This noticeable increase in SOD (Superoxide Dismutase) and APX (Ascorbate Peroxidase) was related to cold tolerance rather than with resistance against low temperature. This was in turn connected mainly with the increased ability to spread vital function during cold acclimatization. Changes in SOD (superoxide dismutase), APX (Ascorbate Peroxidase) and CAT (Catalase) levels of cold-induced activities during acclimation were more variable in *ex vitro* tested plants than callus. SOD (Superoxide Dismutase) is generally known as the first step of defense against oxidative stress [35]. We observed that SOD (Superoxide Dismutase) responses of *Triticale* seedlings exposed from cold stress were three times higher than from *Triticale* callus exposed from cold stress. Our results have confirmed that the plants under stressful conditions undergo oxidative stress (Fig. 3). These findings have been consistent with studies, suggesting SOD (Superoxide Dismutase) activity increases in *Passiflora alata*, and wheat and pea plants under cold stress [31, 36, 37]. APX (Ascorbate Peroxidase) activity demonstrated the same trends as SOD (Superoxide Dismutase) activity. Samples from leaves of *ex vitro* plants showed roughly half of the APX (Ascorbate Peroxidase) observed in *in vitro* callus. Callus also indicated lower APX activity when compared to the leaves of triticale. APX (Ascorbate Peroxidase) level was gradually increased in *Triticale* cultivars grown *in vitro* and *ex-vitro* at days 7, 14 and 21, due to the activity of APX (Ascorbate Peroxidase) required to protect against the cold stress. The observed differences in APX (Ascorbate Peroxidase) activity among cultivars included in this research might be the results of their genetic properties in terms of cold-resistance. Similar results were noted in *Brassica napus* under cold stress [38]. Joudmand [39] reported that under low-temperature conditions, the application of silicon can effectively mitigate the negative effects of cold stress on barley plants. The results obtained in their study demonstrated that the activity of antioxidative enzymes and concentrations of soluble carbohydrates and proteins in the leaf apoplasm were increased upon cold acclimation and particularly on Si treatment. However, further and more detailed studies are needed, particularly on the involved mechanisms. CAT (Catalase) enzyme requires no supply of reducing equivalents [40] that provide an important advantage especially in the stress conditions where photosynthesis rate reduces and the plant energy reservation. Thus, there is a strong correlation between CAT (Catalase) activity and stress tolerance in plants. Our results confirmed that cultivar *Tatlıcak* as a cold resistant cultivar displayed a higher level of this corporation under cold treatment. Our results are inconsistent with the results reported in wheat where the spring type displayed lower CAT activity than the winter type under cold acclimation [41] and soybean plants under cold treatment [42]. High activity of CAT (Catalase) displayed cold resistance in some of the wheat cultivars [43]. Based on our observations, antioxidant enzyme mechanisms after cold-acclimation seems to be linked to the re-establishment of the cold resistance.

In conclusion, the findings reported in the present study clearly show that cold exposure can improve cold resistance in triticale, which might be attributed partly to the elevation of the sugar and proline levels as well as to the activities of antioxidant enzymes. The obtained results clarify that the antioxidant enzyme level was higher in the *ex vitro* plants, a fact that might result from the environmental factors that are not present for the *in vitro* growing conditions.

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INVESTIGATION OF FUNGI SPORES CONCENTRATION IN NIGDE ATMOSPHERE (TURKEY)

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ABSTRACT. Atmospheric fungi spores investigated in Niğde province with the help of Burkard volumetric trap between January 2014 -December 2014. As total of 70 561 spores belonging to 39 Fungi taxa were counted. The taxa which were observed are the following *Cladosporium*, *Alternaria*, *Melanomma*, *Leptosphaeria*, *Ustilago*, *Pleospora*, *Exosporium*, *Penicillium/Aspergillus*, Elipsoidal basidyospores, *Epicoccum*, *Drechslera*, *Stemphylium*, Multi-septate ascospores, 1-septate ascospores, Myxomycetes, *Pithomyces*, *Torula*, *Didymella*, *Puccinia*, *Chaetomium*, *Curvularia*, Non-septate ascospores, *Oidium*, *Nigrospora*, *Periconia*, Diatrypaceae, *Venturia*, *Arthrinium*, *Xylaria*, *Agrocybe*, *Fusarium*, *Botrytis*, *Peronospora*, *Coprinus*, *Sporormiella*, *Tetracoccosporium*, *Exosporiella*, *Melanospora* and *Boletus*. The concentration of spores which was determined in the atmosphere was 69,46% *Cladosporium*, 7,21% *Alternaria*, 2,98% *Melanomma*, 2,69% *Leptosphaeria*, 2,28% *Ustilago*, 2,15% *Pleospora*, 1,98% *Exosporium*, and the remaining 11.09% belong to 32 taxa which named as an others.
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1. INTRODUCTION

Fungal spores constitute one of the most important groups of airborne allergens. Recently, allergic diseases have developed pandemic health problem. Fungi can grow in a wide temperature range from -5 to 50 C° and higher [1]. Allergic diseases are considered to be one of the most important public health problems affecting up to 15–35% of individuals worldwide. Determination of the airborne spore types and

Keyword and phrases. Allergy, Fungi spores, Niğde, Turkey

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their concentration in highly populated cities are important in terms of helping patients suffering from diseases such as allergies. For this reason; the studies about the airborne fungal spore of different areas have been carried out by many researchers [2-13]. In our country, it has been reported that 25-30% of the population is affected by one or more allergic diseases [14]. Determination of fungal spore concentration in the atmosphere is important in terms of planning the treatment of sensitive individuals. About 100 fungal spores can cause an immunological response in susceptible people when inhaled with air. *Alternaria* and *Cladosporium* are recognized as the most important fungal species responsible for respiratory diseases such as asthma, eczema, rhinitis and chronic sinusitis. Besides these most popular types of fungi, limited studies can be found on *Didymella* and *Ganoderma*, although their allergenic properties have been clinically proven. Therefore, it is very important to monitor the concentration of allergenic fungal spores in the air, especially in densely populated areas. The spores produced by *Alternaria* and *Cladosporium* are considered the most important and together they can make up 93% of the total fungal spores found in the air. *Alternaria* alternative spores are one of the most frequently identified allergic fungal spores in atmosphere *A. alternata* has been studied most extensively of all *Alternaria* species and it has been found that Alt a 1 was defined to be the most important isolated major allergen. *Alternaria* spores can cause respiratory diseases such as asthma or chronic sinusitis [15-20].

Airborne fungal spore concentrations change continuously according to the meteorological factors. Recently many studies focused on diurnal and seasonal distribution of fungi spores in the atmosphere of urban and rural area [21, 22]. Çeter and Pınar [6] determined fungi spores whole year in Ankara atmosphere, when highest concentration observed in July and the lowest concentration in January. They were observed strong positive correlation between spores concentration and monthly mean temperature, strong negative correlations between monthly mean relative humidity, precipitation spore concentrations, while wind speed has a slightly positive impact [6]. *Alternaria* and the *Cladosporium* are the primary fungal spores that trigger respiratory allergic reactions in Europe and the most abundant fungi in indoor and outdoor environments. The most common and abundant airborne fungal spores reported for Mexico City are *Cladosporium, Aspergillus, Alternaria, Ulocladium, Geotrichum, Penicillium* and *Physarum*. However, the different spore taxa that are present in the air, their concentrations, and their propagation directly depend on the geographic region, the meteorological parameters and climatic conditions, the land use and vegetation type of each locality, and the air pollution level [23].

The aim of this study is to determine the fungal spores found in the atmosphere of Niğde province on daily, weekly, monthly and yearly basis.

2. Materials and Methods

2.1 Study area

The altitude of the province of Niğde from sea level is 1,300 meters. Western parts have wavy flatness and other parts have a mountainous structure. It is neighbors with Aksaray in the northwest, Nevşehir in the north, Kayseri in the northeast, Konya in the west and southwest, İçel in the south, Adana in the southeast and east. It is located between the 37 ° 25 'north and 38 ° 58' north parallels, and the meridians 33 ° 10 'west and 35 ° 25' west in the west (Figure 1).

The continental climate is dominant in the province of Niğde, with hot and dry summers, cold and snowy winters. There is very little forest existence. It constitutes 1.7% of the provincial lands, and increases to 3 percent with the heathland. 50% of the provincial lands are cultivated-planted areas, from wheat fields, apple orchards and vineyards; 37% consists of meadows and pastures. The rest is made up of land that is not suitable for cultivation. Average annual rainfall is 349 mm with at least one of the wettest regions of Turkey. Most of this precipitation occurs during the winter months.

Figure 1. Location map of the studied area.

2.2. Aerobiological and meteorological data

Aerobiological data were collected during one year period (between January 2014 until December 2014) by placing the Burkard device at a height of about 12 meters from the ground on the entrance of the Niğde Ömer Halisdemir University Faculty of Arts and Sciences Building. Preparation and spores count procedures followed the

recommendations of the Spanish Aerobiological Network (REA: Red Española de Aerobiología)[24]. Four transverse traverses were counted on each slide, at a magnification of ×10 and x40. Counts were made daily bases and total daily counts were converted to number of spores per cubic meter of air [25-27]. The Spores were identified and counted at genus level. Meteorological data of Niğde province for the year of 2014, the monthly Average temperature, monthly total rainfall amount, monthly average relative humidity and monthly average wind speed were obtained from the General Directorate of Meteorology. The meteorological data obtained were presented in tables and graphs.

3. Results

3.1 Meteorological data of Niğde province

Meteorological data (Table 1) and graphics of the meteorology station in Niğde are given below (Figure 2).

Table 1. Meteorological data of Niğde province in 2014

YEAR / MONTH	1	2	3	4	5	6	7	8	9	10	11	12
Monthly Average Temperature (°C)	3	4.4	8	13.6	16.8	20.1	24.9	25.1	18.5	12.2	5.7	5.7
Monthly Total Precipitation (mm)	32.7	15	58	43.2	88	65	8.7	48.1	59.1	41.7	27.3	44.3
Monthly Average Relative Humidity (%)	66.5	53.6	54.3	42.3	45.6	42.8	33.5	37.2	53.7	63.2	65.1	71.9
Monthly Average Wind Speed (m / sec)	2.4	2.5	3	3	2.5	2.6	2.6	2.7	2.2	2.3	2.2	2.7

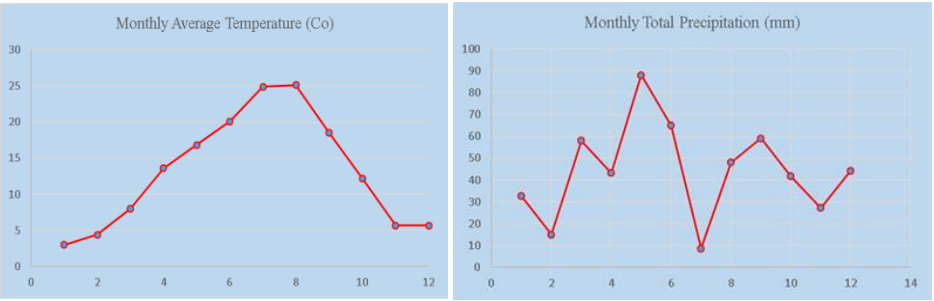

FIGURE 2. Monthly changes of meteorological parameters in Niğde province (2014)

3.2. Atmospheric fungal spore data

70561 spores belonging to 39 fungi taxa were counted in a one-year study between January 2014 and December 2014 with the Burkard volumetric trap in the atmosphere of Niğde. The determined taxa were *Cladosporium, Alternaria, Melanomma, Leptosphaeria, Ustilago, Pleospora, Exosporium, Penicillium/Aspergillus,* Ellipsoidal basidiospores, *Epicoccum, Drechslera, Stemphylium,* Multi-septal ascospores, 1-septal ascospores, Myxomycetes, *Pithomyces, Torula, Didymella, Puccinia, Chaetomium, Curvularia,* Non-septate ascospore, *Oidium, Nigrospora, Periconia, Diatrypaceae, Venturia, Arthrinium, Xylaria, Agrocybe, Fusarium, Botrytis, Peronospora, Coprinus, Sporormiella, Tetracoccosporium, Exosporiella, Melanospora,* and *Boletus*. There are 69.46% *Cladosporium*, 7.21% *Alternaria*, 2.98% *Melanoma*, 2.69% *Leptosphaeria*, 2.28% *Ustilago*, 2.15% *Pleospora*, 1.98% *Exosporium* and the rest of the total spore concentration detected in the atmosphere 32 taxa, 11,09% of which are expressed as other taxa. According these data, spore calendar of Niğde Province was prepared (Figure.3).

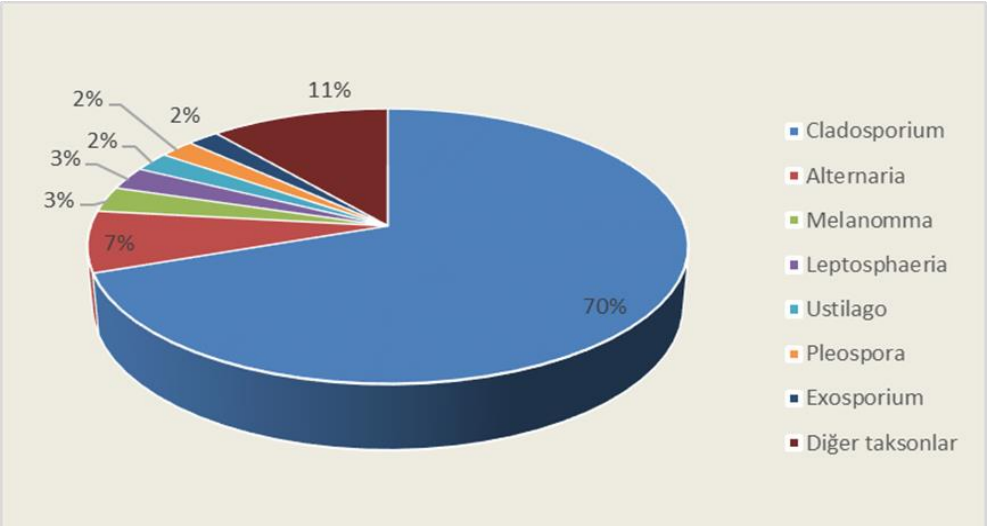

FIGURE 3. Percentage distribution graph of fungal spores detected in Niğde atmosphere.

In January, 647 spores belonging to 25 fungi taxa were counted in Niğde atmosphere. There are mostly *Cladosporium, Alternaria, Melanomma, Ustilago, Leptosphaeria*, Myxomycetes, and *Exosporium* spores were found in the atmosphere.

695 spores belonging to 24 fungi taxa were detected in the atmosphere of Niğde province in February. The spores which were detected were *Cladosporium, Ustilago, Melanomma, Leptosphaeria, Alternaria, Pleospora*, and *Exosporium* were most common.

In the atmosphere of Niğde, 3130 spores belonging to 28 fungi taxa were counted in March. In the analysis of air preparations. spores belonging to *Cladosporium, Leptosphaeria, Melanomma, Pleospora, Alternaria* and Multi-septal ascospores were mostly observed.

In April, 4280 spores belonging to 32 fungi taxa were detected in the atmosphere of Niğde province. *Cladosporium, Alternaria, Leptosphaeria, Pleospora, Exosporium, Melanomma*, Ellipsoidal basidyospore, Multi-septal ascospore, *Ustilago, Penicillium/Aspergillus* and Myxomycetes spores were most common.

7143 spores belonging to 35 fungi taxa were detected in the atmosphere of Niğde province in May. The spores of *Cladosporium, Alternaria, Ustilago, Leptosphaeria, Melanomma, Pleospora, Exosporium*, Ellipsoidal basidiospores, Multi-septal ascospore and *Pithomyces* are most common.

TABLE 2. The monthly spore concentrations for fungal taxa which are detected in Niğde atmosphere in 2014

Taxa	1	2	3	4	5	6	7	8	9	10	11	12	Total
Alternaria	38	18	94	314	340	1412	772	977	782	160	158	22	5087
Cladosporium	430	478	1947	2423	5073	8023	5591	8869	11835	2572	1365	406	49012
Botrytis						13	15	20	22	7	3		80
Pithomyces	9	12	43	36	50	54	19	45	24	14	14	10	330
Epicoccum	3	3	24	26	24	98	63	183	119	27	26	11	607
Periconia	8	4	5	21	19	30	19	20	14	7			147
Torula				23	20	60	46	28	115	11	15	12	330
Stemphylium	7	2	17	44	43	23	26	155	118	16	18	17	486
Drechslera		2	18	40	45	30	37	184	133	10	19	11	529
Exosporium	16	13	45	174	142	234	156	216	292	79	19	14	1400
Tetracoccosporium			7	11				7		6			31
Xylaria				9	13	29	13	15	7	20	12		118
Sporormiella		1	8	7	9	7	8	7	9	7	0		63
Leptosphaeria	21	22	206	234	250	430	165	238	215	56	32	28	1897
Curvularia	1	3	14	13	14	13	28	40	42	16	12	10	206
Nigrospora			15	13	14	10	18	44	18	9	9	9	159
Chaetomium	1	7	8	27	14	14	20	18	71	11	15	9	215
Fusarium	1		11		11	10	10	12	38	5			98
Melanomma	25	24	190	147	211	652	259	180	318	66	16	16	2104
Venturia	3	2	10		12	33	25	17	14	9		7	132
Melanospora	1	1			4								6
Didymella	7	17	42	25	21	21	11	16	99	8	10	10	287
Pleospora	15	19	182	220	193	126	239	50	416	33	14	14	1521
Oidium	9	9	21	22	8	8	18	9	56	8	12	7	187
1-septal ascospore	4	3	26	19	40	72	53	24	98	22	9	11	381
Coprinus	2		14	11	11				10	7	8	7	70
Agrocybe	1	3	7		22	26	16	8	9	10	1		103
Boletus											1	4	5
Puccinia				6	11	15	72	66	46	6	14	7	243
Ustilago	21	40	27	70	257	435	334	248	115	41	20		1608
Peronospora	1			10	10	14	7	15	8		1	6	72
Multi-septal ascospore	1	8	68	77	61	88	20	45	17	25		10	420
Elipsoidal basidyospore			45	88	86	182	46	35	86	41	44	16	669
Myxomycetes	18	3	27	61	14	127	27	39	36	16			368
Penicillium/Aspergillus				64	46	410	169	117	169	27			1002
Exosporiella						18			9				27
Diatrypaceae				18	9	14	33	9	30	14		10	137
Arthrinium	4	1	9	11	9	36	21	14	15	8			128
Non-septate ascospore				16	37	36	13	17	49	24			192
TOTAL	647	695	3130	4280	7143	12803	8369	11987	15454	3398	1950	705	70561

In June, 12803 spores which belonging to 35 fungi taxa were detected in the atmosphere of Niğde. They are *Cladosporium, Alternaria, Melanomma, Ustilago, Leptosphaeria, Penicillium/Aspergillus, Exosporium*, Ellipsoidal basidiospore, Myxomycetes, *Pleospora*, *Epicoccum*, Multi-septal ascospore, 1-septal ascospore, *Torula* and *Pithomyces* spores were most common.

8369 spores belonging to 34 fungi taxa were detected in the atmosphere of Niğde province in July. *Cladosporium, Alternaria, Ustilago, Melanomma, Pleospora,*

Penicillium/Aspergillus, Leptosphaeria, Exosporium, Puccinia, Epicoccum and 1-septal ascospores were the most common.

11987 spores belonging to 35 fungi taxa were detected in the atmosphere of Niğde province in August. The spores of *Cladosporium, Alternaria, Ustilago, Leptosphaeria, Exosporium, Drechslera, Epicoccum, Melanomma, Stemphylium, Penicillium/Aspergillus, Puccinia* and *Pleospora* were most common.

In September, 15454 spores belonging to 36 fungi taxa were detected in the atmosphere of Niğde province. *Cladosporium, Alternaria, Pleospora, Melanomma, Exosporium, Leptosphaeria, Penicillium/Aspergillus, Drechslera, Epicoccum, Stemphylium, Torula, Ustilago, Didymella*, 1-septal ascospore, Ellipsoidal basidyospore, *Chaetomium* and *Oidium* spores were found most frequently.

In October, 3398 spores which weree belonging to 35 fungi taxa were detected in the atmosphere of Niğde province. They are *Cladosporium, Alternaria, Exosporium, Melanomma, Leptosphaeria, Ustilago,* Ellipsoidal basidyospore, *Pleospora, Epicoccum, Penicillium/Aspergillus,* Multi-septal ascospore, Non-septal ascospore, 1-septal ascospore and *Xylaria* spores were most common.

1950 spores belonging to 26 fungi taxa were detected in the atmosphere of Niğde in November. The spores are spores of *Cladosporium, Alternaria,* Ellipsoidal basidyospore, *Leptosphaeria, Epicoccum, Ustilago, Drechslera, Exosporium, Stemphylium* and *Melanomma* were most common.

In December, 705 spores belonging to 25 fungi taxa were detected in the atmosphere of Niğde province. They are *Cladosporium, Leptosphaeria, Alternaria, Stemphylium, Melanomma,* Ellipsoidal basidyospore, *Exosporium, Pleospora, Torula, Epicoccum, Drechslera,* 1-septal asccospore, *Pithomyces, Curvularia, Didymella,* Multi-septal ascospores and *Diatrypaceae* spores were encountered (Table 2).

4. Discussion

Atmospheric fungal spores were investigated in the atmosphere of Niğde in a 12-month period between January 2014 and December 2014. Fungal spores were found during the working period in all months. That fungi taxa can tolerate wide living conditions, besides their ability to grow on a very wide substrate and habitat, their spores can be transported to long distances by wind can be listed among the main reasons for this result.

Additionally, in a significant part of the study high concentrations of fungal spores were found, the reason which plays an important role for this situation is that Fungi

species have large habitats and produce a large number of spores and release them to the atmosphere.

70561 spores belonging to 39 fungi taxa were detected in the analyzes performed over 12 months in the atmosphere of Niğde. Of these spores 69.46% *Cladosporium*, 7.21% *Alternaria*, 2.98% *Melanomma*, 2.69% *Leptosphaeria*, 2.28% *Ustilago*, 2.15% *Pleospora*, 1.98% *Exosporium* and the remaining 11.09 % belonging to 32 taxa, which are expressed as other taxa.

The taxa with spores detected in Niğde atmosphere are respectively *Cladosporium*, *Alternaria*, *Melanomma*, *Leptosphaeria*, *Ustilago*, *Pleospora*, *Exosporium*, *Penicillium/Aspergillus*, Elipsoidal basidyospor, *Epicoccum*, *Drechslera*, *Stemphylium*, Multiple septal ascospores, 1-septal ascospores, Myxomycetes, *Pithomyces*, *Torula*, *Didymella*, *Puccinia*, *Chaetomium*, *Curvularia*, Non-septal ascospore, *Oidium*, *Nigrospora*, *Periconia*, *Diatrypaceae*, *Venturia*, *Arthrinium*, *Xylaria*, *Agrocybe*, *Fusarium*, *Botrytis*, *Peronospora*, *Coprinus*, *Sporormiella*, *Tetracoccosporium*, *Exosporiella*, *Melanospora* and *Boletus*.

The concentrations of the taxa whose spores are found in the atmosphere are determined as daily, weekly, monthly and yearly and the obtained results are presented by tables and graphics.

Çeter [28] found 869 598 spores belonging to 35 taxa in his study conducted in the atmosphere of Kastamonu for 2 years between 2006-2007 and Çeter [29] determined 429264 spores belonging to 35 taxa in a study conducted in Ankara atmosphere for a period of 1 year [29]. In the study conducted in Adana atmosphere for 1-year, 197009 spores belonging to 34 taxa were detected [30], and 639282 spores belonging to 35 mushroom taxa were detected in 1-year period in Samsun atmosphere [31]. All study results show that spores of fungi are in high concentrations in the atmosphere. This situation arises as a result of the wide distribution of fungi and their release of high amounts of spores into the atmosphere. In our study, lower concentrations were found compared to the studies mentioned above. One of the reasons for this is thought to be due to the drier climatic conditions of the province of Niğde and the arid vegetation.

During the study, the lowest spore concentration was detected in December-February. The lowest spore concentration of the whole study period was seen in January 2014 with 647 spores. During this quarter *Cladosporium*, *Alternaria*, *Melanomma*, *Ustilago* and *Leptosphaeria* ascospores were found dominantly. Similarly, there are studies showing that low temperature and high relative humidity negatively affect spore concentration [29, 32, 33].

Kramer stated that fungal spores were low in the Kansas atmosphere, increased as of April, the highest spore concentrations were detected in June, July and August, and a decrease in spore concentration was observed as of September [34].

Çeter stated that the increase in temperature cause the increase on the fungal spore concentration. It has been stated that the average temperature and minimum temperature values exceeding 10-15 C° significantly increase the fungal spore concentration [28]. In our study, the average temperature being over 15 C° since April caused an increase in spore concentration.

Royes *et al.* [35] emphasized that temperature, amount of precipitation, relative humidity and Cooperman *et al*. [36] emphasized that the increase in temperature are important factors affecting the increase in spore concentration.

Studies have determined that the increase in temperature increases the spore concentration in the atmosphere, but when the temperature increases after a certain degree comes together with low rainfall and humidity, it has been found that the spore concentration is negatively affected [32, 37, 38, 39]. Although the temperature tolerances are different, many fungal spores show their best development in the range of 20-28 C°. When triggered by factors such as high temperature, low rainfall and low humidity, it has a negative effect on spore concentration.

The spore concentration has increased in the atmosphere of Niğde in May and June. In addition to the high average temperature values in July and August, the concentration of spore was found to be relatively low due to drought due to low rainfall. In September, due to the more favorable conditions, the highest spore concentration of the working period was determined. Çeter detected decreases of 25% and 50% in spore concentration in the months of July and August with the effect of heat and drought in the atmosphere of Kastamonu [28].

Çeter et al. [40] found 540908 spores belonging to 46 fungi taxa in a two-year period in their study in the atmosphere of Çankırı, 64% *Cladosporium*, 6% Ellipsoidal Basidyospores, 5% *Alternaria*, 5% *Leptosphaeria*, 4% *Pleospora* of these spores were determined as dominant taxa. The highest spore concentrations were detected in June-July. Similarly, the highest sports concentration was observed in the Gümüşhane atmosphere in June by Akdoğan et al [41] and determined the highest spore concentration in the Sinop atmosphere in July by Çeter et al. [42].

Li and Kendrick stated that rain, wind speed, humidity, temperature and vegetation are important factors affecting the concentration of spore in the air [37]. As emphasized in the studies conducted in the atmosphere of Ankara [29], Adana [30], Samsun [31], and Kastamonu [28], in this study it has been observed that, meteorological factors such as temperature, precipitation, relative humidity and wind speed are important. Low temperature and high relative humidity have been cited as

important factors reducing the concentration of fungal spores [32, 33]. The low number of fungal spores in the Niğde atmosphere in the December-February period is the result of this effect.

Like all living things, fungi thrive within certain temperature values. Studies have shown that some species of fungi are mesophilic and show optimal growth between 20-28 °C, some species are psychrotolerant and show optimal growth below 20 C° [43]. Many studies indicate that the fungal spore concentration increases with temperature [32, 37, 38, 39]. However, after a point, in temperature it has a negative effect on spore concentration. When triggered by low precipitation and low humidity values, it has a negative effect on fungal growth and atmospheric spore concentration decreases. As a result of a similar effect in July and August, the spore concentration was found to be low.

It is not sufficient to explain the fungal spore concentration only with general meteorological parameters. Since fungi are parasites, pathogens and saprophytes adaptable, many factors such as the presence and availability of the substrate where they develop, the vegetation period of the host on which they develop as a pathogen or parasite, the microclimate formed in the habitat affect the development and spore production capacity of fungi.

As a result of this study in which atmospheric fungal spores of Niğde city center were examined, the amount of spores identified in the atmosphere in a day was determined as m^3, and the data obtained by calculating weekly, monthly and annual amounts were presented in tables and graphs.

The variation of atmospheric spore concentration with meteorological factors has been studied and discussed.

It has been determined that the sensitivity to fungal spores varies geographically between 3 and 80% [44]. Determination of atmospheric spore concentration and preparation of the spore calendar will be a guide in the treatment of patients with fungal spore sensitivity, it will be possible to prevent labor loss and prevent unnecessary treatment expenses with more effective sensitization and treatment.

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ZOOPLANKTON OF SAKARYABAŞI-WEST POND, CENTRAL ANATOLIA

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ABSTRACT. The study was conducted between March 2017 and February 2018 to determine the effects various applications and environmental impacts on zooplankton composition in Sakaryabaşı-West Pond. Zooplankton samples were monthly collected with plankton net vertically and horizontally from two stations as triplicated. At the time of sampling, water temperature, dissolved oxygen, pH, electrical conductivity, water depth and light transparency were measured at the stations. Rotifera was the highest proportion in the pond during the study, followed by Copepoda and Cladocera, respectively. At this research, 11 families from Rotifera, 22 species from Cladocera, 1 family from Cladocera, 1 family from Copepoda. Proportionally most common species were *Lepadella patella* (6.38%), *Mytilina ventralis* (5.32%), *Lecane lunaris* (4.79%) from Rotifera; *Alona rectangula* (8.51%) from Cladocera. Cyclopoidae copepods was formed as adults (17.55%), nauplii (25.53%). During the study period the average zooplankton abundance ranged from 3 ± 0.5 x10^3 to 36 ± 2 x10^3 / m^3. The highest abundance value was determined in October and the lowest abundance value was determined in January. As a result, regular monitoring of zooplankton in Sakaryabaşı-West Pond as an indicator of the ecological health of the water bodies, will be important in terms of being a warning for the negative impacts of the pond.

1. INTRODUCTION

Most of the lakes and ponds are very important ecologically and they have risk of eutrophication or drying. The first step in the ecological improvement of such lakes is to reveal their condition by examining the structure of their biological communities. In aquatic systems, the zooplankton is functionally important by consuming phytoplankton and bacteria and as a prey for higher levels of fish in the

Keyword and phrases. Sakaryabaşı-West Pond, zooplankton, water quality parameters.

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food chain. Therefore, it is an important indicator of ecosystem health [1]. Water quality, hydrology, nutrient content and presence of submerged plants, being exposed to predation by planktivorous fish in water bodies caused differences in shape and movements of zooplankton in their communities [2] [3] .

Sakaryabaşı-West Pond is fed by one of the karst springs that originates Sakarya River and that was converted to a pond with the construction of a set in the 1970s. This pond also supplies water to the Fish Culture and Research Station of Ankara University through a concrete channel. Because of its spring origin, the water temperature of the pond is stable and decreases to a minimum of 13°C in the winter [4]. The lake exceeded the eutrophication limit due to agricultural activities and intensive fertilizer use at the around of the West Pond and the lake was hypertrophic in terms of total phosphorus concentration [5].

The zooplankton composition of the pond was determined by Demir and Kırkağaç in 2005. In 2006, grass carp was stocked in order to control the aquatic plants in the pond and than the effects of grass carp on zooplankton composition was exhibited [6]. However, uncontrolled fishing eliminated grass carp from the pond and the existence of European catfish, tilapia and carp from unknown sources was determined over the years.

In this study, it is aimed to reveal to what extent some lake management activities and environmental conditions in the West Pond effected the zooplankton composition and to determine its present situation.

2. Materials and Methods

Sakaryabaşı-West Pond is located at 39º21º15º-39º21º37º and 31º02º 22º -31º 02º 59º in Central Anatolia (Figure 1). The volume and surface area of the pond is 26000m^3 and 0.92 ha, respectively. It supplies water to Ankara University Sakaryabasi Fish Culture and Research Station through a concrete channel with a flow of 430 l s^{-1}. The retention time of the pond was calculated as 0.6 day^{-1}. The altitute of the pond from sea level is about 870 m. Total hardness value of the pond is between 49-55 FS^0 and calcium hardness is between 36.7-76.9 mg / l. The pond water is in hard water class. Also, the pond is eutrophic in terms of orthophosphate and total phosphorus concentrations [7,8,4,6,9]. The fisheries station produces 40 tons of rainbow trout per year together with some other fish species such as cyprinids, European catfish, tilapia, and sturgeon. In recent years, it has been reported that cyprinids, European catfish and tilapia are found in the Pond.

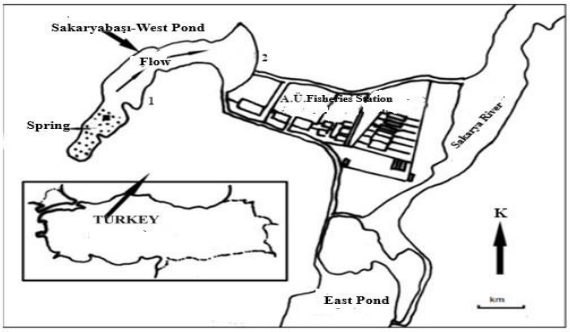

FIGURE 1. Sakaryabaşı-West Pond and sampling stations

The research was carried out for one year between March 2017 and February 2018. Zooplankton samples were collected from two stations: one near the spring, the other is near the set (Figure 1). Samples were collected vertically and horizontally by a plankton net with 55µm mesh size, triplicately for qualitative and quantitative analysis. The water temperature (°C), dissolved oxygen (mg/l), pH, conductivity (µS/cm) (CONSORT C5020T MODEL), depth (cm) and Secchi depth (cm) were measured *in situ*.

Zooplankton samples were taken into 250 ml containers, fixed with 4% formaldehyde and transferred to the laboratory [10,11]. The usual taxonomic literature was used for identifying the zooplankton samples under invert and binocular microscopes [12,13,14,15,16,17,18,19]. Zooplankton samples were counted from five subsamples each containing 1 ml under inverted microscopes [12]. Abundances were calculated according to [10,20,21]. The zooplankton abundances values were given as individual per m^3.

Statistical analysis were carried out by using SPSS 17 Statistic Program. Variance analysis (ANOVA), Duncan multiple range test and t-test were computed to evaluate the data [22].

3. RESULTS

Zooplankton community composed of 11 families and 22 species from Rotifera, 1 family and 2 species from Cladocera and 1 family and 1 genus from Copepoda were

identified (Table 1). In Sakaryabaşı-West Pond, the presence of zooplankton species in stations by months is given in Table 2. Cyclops sp. from cyclopoid copepod was mostly found in nauplii stage as well as copepodit and adult stage. Lecane lunaris, Lepadella patella ve Mytilina ventralis were mostly found species in the stations by the months while the other rotifer species were encountered only in one month during the study.

TABLE 1. Zooplankton list of Sakaryabaşı-West Pond

Division	Class/Subclass	Order/Family	Species
Rotifera	Monogononta	Gastropodidae	*Ascomorpha ecaudis* (Perty,1850)
		Collothecidae	*Collotheca ornata* (Ehrenberg, 1830)
		Lepadellidae	*Colurella obtusa* (Gosse, 1886)
		Euchlanidae	*Euchlanis dilatata* (Ehrenberg, 1832)
		Branchionidae	*Keratella cochlearis* (Gosse, 1851) *Keratella valga* (Ehrenberg, 1834) *Notholca acuminata* (Ehrenberg, 1832)
		Lecanidae	*Lecane bulla* (Gosse, 1851) *Lecane flexilis* (Gosse, 1886) *Lecane furcata* (Murray, 1913) *Lecane hornemani* (Ehrenberg, 1834) *Lecane luna* (Müller, 1776) *Lecane lunaris* (Ehrenberg, 1832) *Lecane obtusa* (Murray, 1913)
		Lepadellidae	*Lepadella patella* (Müller, 1773)
		Mytilinidae	*Mytilina bisulcata* (Lucks, 1912) *Mytilina mucronata* (Müller, 1773) *Mytilina ventralis* (Ehrenberg, 1830)
		Testudinellidae	*Pompholyx sulcata* (Hudson, 1885) *Testudinella elliptica* (Ehrenberg, 1834)
		Trichotriidae	*Trichotria pocillum* (Müller, 1776)
		Trichocercidae	*Trichocerca cylindrica* (Imhof, 1891)
Arthropoda	Branchiopoda	Cladocera/ Chydoridae	*Alona rectangula* (G.O. Sars, 1862) *Chydorus sphaericus* (O.F. Müller, 1776)
	Maxillopoda	Copepoda/ Cyclopidae	*Cyclops* sp.

TABLE 2. The presence of zooplankton species by months in Sakaryabaşı-West Pond

	Months											
	March	Apr	May	June	July	August	Sept	Oct	Nov	Dec	Janu	Febr
Rotifera												
Ascomorpha ecaudis	-	-	-	-	-	-	1*	-	-	-	-	-
Collotheca ornata	1.2*	-	1	1	-	-	-	2	-	-	-	-
Colurella obtusa	-	-	-	-	-	-	-	2	-	-	-	-
Euchlanis dilatata	-	-	-	-	-	2	1.2	2	-	-	-	-
Keratella cochlearis	-	-	-	1	-	-	-	-	-	-	-	-
Keratella valga	-	-	-	-	-	-	-	2	1.2	1	2	-
Lecane bulla	-	-	-	1	-	-	-	1	-	-	-	-
Lecane flexilis	-	-	-	1	2	-	-	1	-	-	-	-
Lecane furcata	-	-	-	1.2	-	-	-	-	-	-	-	-
Lecane hornemani	-	-	-	1	-	-	-	-	-	-	-	-
Lecane luna	-	-	-	-	-	-	-	1	-	-	-	-
Lecane lunaris	-	-	1.2	1.2	2	1	-	2	1	-	-	1
Lecane obtusa	-	-	-	2	-	-	-	-	-	-	-	-
Lepadella patella	-	-	-	1.2	1	1	1	1.2	-	2	-	-
Mytilina bisulcata	-	-	-	-	-	1.2	1.2	-	-	-	-	-
Mytilina mucronata	-	-	-	-	-	1.2	1	-	-	-	-	-
Mytilina ventralis	-	-	-	2	2	1.2	1	2	-	-	-	-
Notholca acuminata	-	-	-	-	-	-	-	-	-	-	1	-
Pompholyx sulcata	-	-	-	-	-	-	-	2	-	-	-	-
Testudinella elliptica	-	-	-	2	-	-	-	-	-	-	-	-
Trichotria pocillum	-	2	-	-	-	-	-	2	2	2	-	-
Trihocerca cylindrica	-	-	-	-	-	-	-	2	-	-	-	2
Cladocera												
Alona rectangula	1	2	1	1	2	1.2	-	1.2	-	-	-	-
Chydorus sphaericus	2	2	-	-	-	-	-	2	-	-	-	-
Copepoda												
Cyclops sp.	2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	-	-	1.2	-
Nauplii	2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	-	1	2	1.2

*1 : 1.station, 2: 2.station

Zooplankton abundance values of Sakaryabaşı-West Pond according to months and stations are given in Table 3. The variations in the average zooplankton abundance according to months and stations was evaluated by variance analysis and the differences were found statistically significant ($p<0.05$). However, the average zooplankton abundance values between the two stations in the months were evaluated with the t-test and the differences were found to be significant, except for May, June, July and November ($p <0.05$). During the study, the average zooplankton abundance changed between 3±0.5x103 and 36±2x103 individual /m3 .

TABLE 3. Zooplankton abundance according to months and stations in Sakaryabaşı-West Pond (Mean ± standard deviation) ($x10^3$) (individual / m^3)

Months	Stations	
	1	2
March	$5 \pm 1^{de*A**}$	14 ± 2^{dB}
April	9 ± 2^{cdA}	31 ± 0.5^{bB}
May	19 ± 4^{b}	17 ± 1^{c}
June	31 ± 2^{a}	31 ± 2^{b}
July	9 ± 2^{cd}	10 ± 0.6^{e}
August	$30 \pm 2^{a\,A}$	$14 \pm 1^{d\,B}$
September	12 ± 0^{cA}	17 ± 0.5^{cB}
October	12 ± 1^{cA}	36 ± 2^{aB}
November	5 ± 0.6^{e}	5 ± 1^{fg}
December	9 ± 2^{cdA}	$5 \pm 0.6^{f\,B}$
January	12 ± 0.6^{cA}	3 ± 0.5^{gB}
February	10 ± 0.4^{cA}	5 ± 1^{fgB}

*Means with the different small letters in the same line are significant statically ($p<0.05$).
** Means with the different capital letters in the same column are significant statically ($p<0.05$).

In the zooplankton community, the highest proportion in the pond belonged to group of Rotifera, followed by Copepoda and Cladocera, respectively (Figure 2). Considering the proportional distribution, the dominant species are *Lepadella patella* (6.38%), *Mytilina ventralis* (5.32%), *Lecane lunaris* (4.79%) from Rotifera; *Alona rectangula* (8.51%) is from Cladocera. The ratio of adults of *Cyclops* sp from Copepoda was 17.55%, and the nauplii stage was about 25.52%.

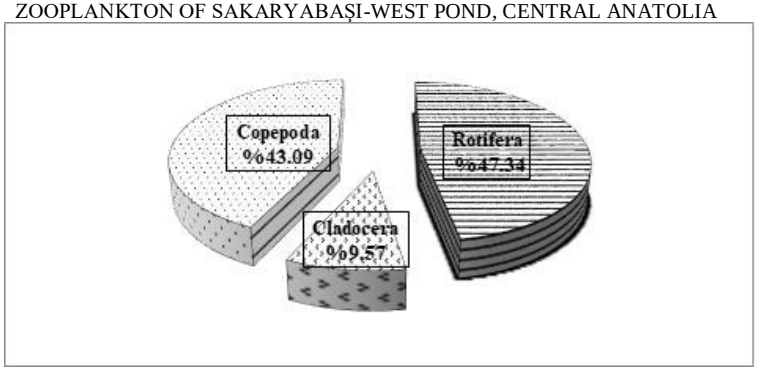

FIGURE 2. The proportional distribution of the zooplankton groups of Sakaryabaşı-West Pond (%).

The proportional distribution of the zooplankton groups at the stations according to the months in Sakaryabaşı-West Pond is given in Table 4. During the study, Rotifera was present in all stations and proportionally the highest value was in June (12%), October (10.6%) and August (7.4%), respectively. Cladocera was not encountered at the stations in September, November, December, January and February. On the other hand, Copepoda was found at the stations except November.

TABLE 4. The proportional distribution of the zooplankton groups according to the stations and months in Sakaryabaşı-West Pond (%)

Stations	Groups	Months											
		March	April	May	June	July	August	September	October	November	December	January	February
1	Rotifera	0.5	-	1.6	7	0.5	5.3	2.1	2.1	1	0.5	0.5	0.5
	Cladocera	0.5	-	1	0.5	-	1	-	0.5	-	-	-	-
	Copepoda	-	2	2.1	2.1	1.6	3.2	1.6	1	-	2.1	2.1	2.8
2	Rotifera	0.5	0.5	0.5	5	1.6	2.1	3.7	8.5	1	1	0.5	0.5
	Cladocera	0.5	3.2	-	-	0.5	0.5	-	1	-	-	-	-
	Copepoda	3.2	5.3	3.7	3.7	1	1.6	1.6	1.6	-	-	0.5	1

During the study, The water parameters were determined in terms of average temperature, dissolved oxygen, conductivity, pH and they were ranged from 14.80±0.65ºC to 28.56±0.55ºC (Figure 3), from 4.18±20 mg/l to 7.41±0.85mg/l (Figure 3), from 0.71±0.01 µS/cm to 0.85±0.03 µS/cm (Fig.4) and from 6.44±0.02 to 8.15±0.15, respectively. The average depth of Sakaryabaşı-West Pond was changed between 193±5.7cm and 198±2.8 cm in the 1. station, 213±5 cm and 223±5.7 cm in the 2. station, average transparency was about 156±5.7 cm and 218±2.8 cm (Figure 3).

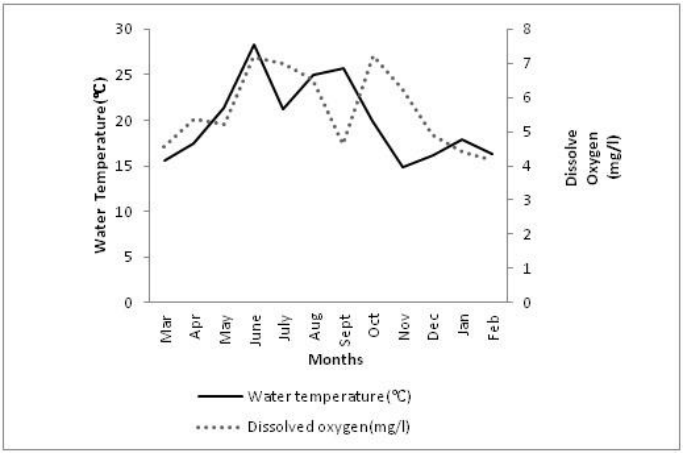

FIGURE 3. Average water temperature (ºC) and dissolved oxygen (mg/l) values in Sakaryabaşı-West Pond during the study

The differences in the average water temperature values, dissolved oxygen concentrations, conductivity and pH values according to the months were found statistically significant ($p < 0.05$), while the differences in the same parameters between the two stations were not statistically significant according to the t-test ($p > 0.05$). However, the differences in the depth values of stations by the months were found statistically insignificant ($p > 0.05$), but the differences between two stations in the months were found significant ($p < 0.05$). Average transparency values according to the months and the stations were both found to be significant statistically ($p < 0.05$) (Figure 4).

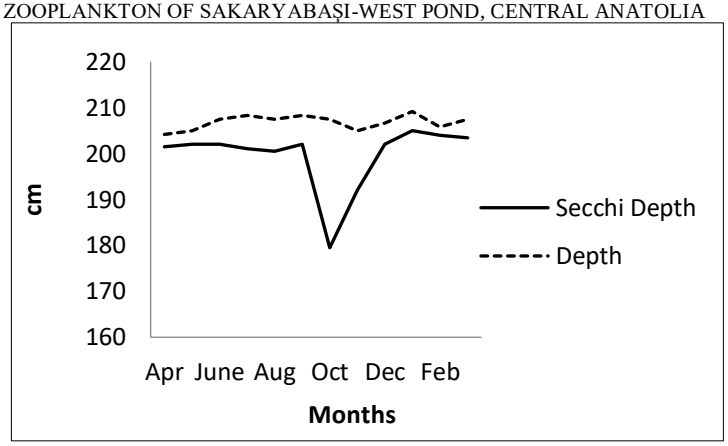

FIGURE 4. Average water depth (cm) and transparency (cm) values in Sakaryabaşı-West Pond during the study

4. CONCLUSION AND DISCUSSION

The zooplankton composition of Sakaryabaşı-West Pond was determined initially by [4]. The researchers reported totaly 25 zooplankton species, including 22 species from Rotifer, 2 species from Cladocera and one from Cyclopoid Copepoda. One year later, grass carp was stocked to the same pond for macrophyte control and the effects of grass carp on the other biological communities and water quality were investigated. In that study, as an indirect effect of aquatic plant elimination of grass carp, zooplankton species richness were decreased and the community consisted of totally 12 species; 10 Rotifera, 1 Cladocera and 1 Copepoda [6]. Over the years, the grass carp has disappeared from the pond with anthropogenic effects. Thus, zooplankton composition was found to be similar in this study to the findings of the [4]. In all three studies, the dominant group in zooplankton is Rotifera.

In zooplankton community, the most frequently found species from Rotifera was *Mytilina ventralis* (5.03%), *Lepadella lunaris* (4.52%) and *Collotheca ornata* (4.02%), respectively. Those three dominant rotifers species can be find in various trophic levels and are generally cosmopolitan species, seen in shallow waters with intense submerged plants [14]. *Ascomorpha ecaudis, Colurella obtusa, Keratella cochlearis, Lecane hornemani, Lecane luna, Lecane obtusa, Notholca acuminata, Pompholyx sulcata, Testudinella elliptica* were found once during the research. [4] reported that *Lecane luna* and *Lepadella patella* were found every month, *Asplanchna* sp., *Brachionus* sp., *Cephalodella gibba, Colletheca pelagica, Colurella obtusa, Euchlanis dilatata, Hexarthra* sp., *Keratella cochlearis, Lophocharis*

salpina, Monostyla lunaris, Mytilina mucronata, Notholca acuminata, Polyarthra dolichoptera, Proales daphnicola, Squatinella mutica, Trichotria pocillum, Trichocerca cylindrica, Trichocerca relicta, Testudinella sp. were found a few times during their study in Sakaryabaşı-West Pond. Kırkağaç and Demir [6], stocked grass carp and also placed a cage without fish in the Pond and reported that there was no differences in zooplankton composition and species richness (*Ascomorpha* sp., *Cephalodella gibba, Colurella obtusa, Lecane luna, Lepadella patella, Monostyla bulla, Mytilina mucronata, Testudinella* sp, *Trichocerca* sp.) neither inside the cage nor outside the cage in Sakaryabaşı-West Pond.

It is seen that the species richness of the Rotifera showed a change over the years. *Ascomorpha ecaudis, Collotheca ornata, Keratella valga, Lecane bulla, Lecane flexilis, Lecane furcata, Lecane hornemani, Lecane obtusa, Mytilina bisulcata, Mytilina ventralis* ve *Pompholyx sulcata* were reported first time only in this research. However, the findings about Cladocera species richness, is same with the results of [4]. *Alona rectangula* was not reported by [6], but in this study it was determined again. *Cyclops* sp. from Copepoda found mostly in nauplii stage, this is is similar to the results of the other studies.

Due to anthropogenic effects of Sakaryabaşı-West Pond, it is thought that the variations in zooplankton composition and species richness were effected indirectly from fishing activities. However, it was considering that the factors such as winds and water birds could cause the variation in Rotifera species [23]. Annual estimation of zooplankton composition in lakes and reservoirs is not always predictable. The time period in which each zooplankton species is most intense throughout the year can be variable [24]. Planktivorous organisms and the increases in the amount of nutrients can change the balance among the species which is another important factor in differentiation of the zooplankton composition. Besides this, elimination of invertebrates and planktivorous fishes by fishing causes significant variations in zooplankton community [25].

Zooplankton abundance was changed between 3 ± 0.5 x10^3 and 36 ± 2 x10^3 individual/m^3 during the study. The highest abundance value was in October, whereas the lowest one in January Demir ve Kırkağaç [4] reported the average zooplankton abundance about changing between 1 and 43 individual l^{-1}, reached to its highest abundance value in July, the lowest one in November. [6] determined the plant biomass increased 7 times in the cage without grass carp compared to the outside of the cage, and the average zooplankton abundance was generally high in the Pond; changed between 2 and 16 individual / l inside the cage and 3 and 52

individual / l outside the cage. The highest values were reached in July in the cage, in September outside the cage, and the lowest values in April and March, respectively.

In this study, the average zooplankton abundance values remained within the values ranges given in the other two studies. However, the variations of zooplankton abundance values in months were not found to be similiar. The structure and seasonal distribution of zooplankton communities in the pond are thought to vary depending on various factors such as the meteorological and hydrological parameters, the coastal areas exposed to human effects and the presence of macrophytes.

In Sakaryabaşı-West Pond, average water temperature values changed between 14.80±0.65°C and 28.56±0.55 °C. The highest value was measured in June, the lowest one in November. It was reported that pond mixing constantly by feding source, so there is no thermal stratification and average water temperature changes between 13° C (November) and 24° C (August) [4,6]. [26] made seasonal measurements in Sakaryabaşı-West Pond and reported the lowest water temperature was about 17.48°C in January and the highest one in July as about 20.10°C. It is thought that the lowest water temperature value and the season that measured were not changed by the years in the pond due to feed by geothermal spring, But upper limits of water temperature and the season to measure can vary because of the water volume of the pond, its stagnant water character, the seasonal variability of the water surface exposed to sunlights and the shallow lake feature. In this study, zooplankton abundance was increased with the water temperature rises. It was reported that water temperature is limiting factor of zooplankton distribution and encouraged the phytoplankton and zooplankton production in springs [27].

In Sakaryabaşı-West Pond, averaged dissolved oxygen concentrations changed between 4.18±0.20 mg/l and 7.41±0.85 mg/l during the study. Dissolved oxygen values determined in the study weren't similiar with the previous studies in the pond [4,6,25]. While the water temperature and dissolved oxygen values are expected to show an inversely change in water bodies, the water temperature and dissolved oxygen values increased together in April, May and June in this study. It is thought that the macrophytes and the sampling time in the pond caused increasing in both parameters. It is reported that dissolved oxygen concentration is the one of the limiting factor of zooplankton abundance [28].

During the study, the average pH values changed between 6.44±0.02 and 8.15±0.15. It is seen that the lowest and highest pH values in the Pond were measured in this study when compared with the previous studies in the pond [4,6,25]. In zooplankton distribution, pH is important. *Keratella cochlearis tecta* is inhabitant of alkaline

waters [29]. It was reported that acidity waters caused decreases in biomass of cyclopoids [30]. In this study, *Keratella* and *Trichocerca* from Rotifera represented with various species and cyclopoids from copepoda mostly in nauplii stage in high abundance were determined.

In Sakaryabaşı-West Pond, conductivity was measured between 0.71±0.01 µS/cm and 0.85±0.03 µS/cm during the study. It is reported that there is a positive correlation between zooplankton biomass and conductivity [31].

During the study, the depth and the transparency of the stations were measured in the pond. The values were both found to be lower than the values reported by [5] and [4]. Those differences in water depth is due to the measurement locations. Sakaryabaşı-West Pond and around of the pond are sometimes subject to recreational regulations. In this study, Çifteler Municipality removed the emergent *(Pragmites* sp.) and submerged macrophytes by the mechanical method from the Pond in September and this regulation caused the increase of turbidity in the Pond.

According to the results of some physical and chemical properties, Sakaryabaşı-West Pond was generally classified as "middle water" between II and III water classes in Continental Surface Water Quality Management [32].

Zooplankton distribution of wetlands depends on the seasons which are associated with daylight, presence and quantity of the food, water clarity, fish composition and size. The zooplankton composition of Sakaryabaşı-West Pond that was determined in 2005, was changed by the manipulation of the pond for controlling macrophytes by herbivorous fish. Any application or irrigation in the lakes and ponds, sediment deposits, agriculture and domestic wastes that may occur in lakes and ponds will affect the trophic level of the waters, therefore, the composition and abundance of zooplankton. Zooplankton of Sakaryabaşı-West Pond should be monitored regulary as an indicator of the ecological health. This is important for the protection and sustainability of the Sakaryabaşı-West Pond.

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