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Optimization of in vitro sterilization for pistachio (*Pistacia vera* L.) rootstocks

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Antep fıstığı (*Pistacia vera* L.) anaçlarında in vitro sterilizasyon optimizasyonu

Abstract: Pistachio (*Pistacia vera* L.) is one of the leading edible nut consumed all over the World due to its nutritional values. The plant is cultivated in most of the countries along with Türkiye which is one of the leading grower of pistachio. In Türkiye, the rootstock material is currently propagated through traditional methods and there is a need of propagating plant material using modern biotechnological techniques like plant tissue culture. The provision of contaminated free explants with minimum or no phenolic compounds in the culture medium is the prerequisite of *in vitro* regeneration protocol. The plant material used in this study was collected at different physiological stages during different months like April- June and Sep-October. The plant material was cut into 2-3 cm long nodal segments followed by cleaning with different agents like water, soap and fungicide prior to subjected to sterilizing agents. Different sterilizing agents used in this study were $HgCl_2$, Huwa-san (H_2O_2) and commercial bleach (NaOCl) for both rootstocks (UCB-1 and Buttum) with different exposure time. Sterilized explants were cultured on MS basal medium containing plant growth regulators and sub-cultured once a week for three weeks. Results revealed that $HgCl_2$ as sterilizing agent was more superior than other sterilizing agents for both rootstocks. Among rootstocks, UCB-1 was more responsive than Buttum and relatively more sterilized plants were attained. On the other hand, plant material collected during June responded better and 90.0% and 50.0% sterilized plants were attained for UCB-1 and Buttum respectively. The results revealed the significant impact of collection time, sterilizing agent type, concentration and exposure time on sterilization of *P. vera* rootstocks.

Key words: In vitro, pistachio, phenolics, rootstocks, sterilization

Özet: Antep fıstığı (*Pistachio vera* L.), besin değerleri sebebiyle dünyada en çok tüketilen kuruyemişlerden biridir. Antep fıstığı, fıstık yetiştiriciliğinde önde olan Türkiye ile birlikte birçok ülkede yetiştirilmektedir. Türkiye'de genel olarak anaç materyali geleneksel yöntemler ile çoğaltılmaktadır ve bitki materyallerinin bitki doku kültürü gibi biyoteknolojik teknikleri kullanılarak çoğaltılmasına ihtiyaç vardır. In vitro rejenerasyon protokolünün ön koşulu, kültür ortamında kontamine olmayan, minimum veya hiç fenolik bileşik içermeyen eksplantların sağlanmasıdır. Bu çalışmada kullanılan bitki materyali, Nisan-Hazıran ile Eylül-Ekim ayları arasında değişen farklı aylarda, farklı fizyolojik aşamalardayken toplanmıştır. Bitki materyali 2-3 cm uzunluğunda nod parçaları olarak kesilmiş ardından sterilizasyon ajanlarına tabi tutulmadan önce su, sabun ve mantar ilacı gibi farklı ajanlarla temizlenmiştir. Yapılan sterilizasyon çalışmada her iki anaç (UCB-1 ve Buttum) için farklı sürelerde $HgCl_2$, Huwa-san (H_2O_2) ve ticari çamaşır suyu (NaOCl) farklı sterilizasyon ajanları kullanılmıştır. Daha sonra eksplantlar, bitki büyüme düzenleyicileri içeren bazal ortamlarda kültürlendi ve üç hafta boyunca haftada bir kez kültür aktarıldı. Sonuç olarak, sterilizasyon ajanı olarak $HgCl_2$'nin her iki anaç için de diğer sterilizasyon ajanlarından daha üstün olduğunu ortaya koymuştur. Anaçlardan UCB-1, Buttum'a göre daha duyarlı ve nispeten daha steril bitkiler elde edildi. Sonuç olarak, *P. vera* anaçlarının sterilizasyonunda eksplant alım zamanı, sterilizasyon ajanının türü, konsantrasyonu ve maruz kalma süresinin önemini ortaya koymuştur.

Anahtar Kelimeler: Anaç, Antep fıstığı, fenolikler, in vitro, sterilizasyon

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1. Introduction

Application of biotechnological techniques like *in vitro* plant tissue culture is highly significant for producing plants at larger scale in short time especially for fruit trees and ornamental plants (Hesami and Daneshvar, 2016, 2018; Hesami et al., 2018 a,b). The other advantage of *in vitro* regeneration protocol is its application for genetic improvement of plants with elite characteristics and also for breeding purposes (Hesami et al., 2019). However, optimization of sterilization process is highly significant for the establishment of these protocols (Aasim et al., 2013). The contamination of explants is the major inhibiting factor for the development of successful regeneration protocol (Hesami et al., 2017). The occurance of contamination is due to the presence of different microorganism (endogenic and exogenic) in the explant (Arab et al., 2014; Da Silva et

al., 2016a). Therefore, sterilization process need more time, attention and techniques (Mihaljević et al., 2013; Jafari et al., 2016) in order to eliminate contamination with objective to attain explants without damage to proceed for in vitro regeneration process (Batti et al., 2020).

The optimization of whole sterilization process is dependent on variable factors ranging from plant age, size, location, physiological and enviromental factors and explant (Da Silva et al., 2016b). These factors later on regulate the in vitro regeneration process (Hesami et al., 2018c) and selection of sterilizing agent and exposure time are significant with focus on obtaining contamination free explants without substantial damage. The most commonly used sterilizing agents are NaOCl (sodium hypochlorite), H_2O_2 (hydrogen peroxide), $Ca(ClO)_2$ (calcium hypochlorite), $HgCl_2$ (mercury II chloride), silver based chemicals like $AgNO_3$ (silver nitrate) or NS (Nano-silver) and PPM (Mihaljević et al., 2013; Nongalleima et al., 2014). Besides that, several other commercial chemicals, antibiotics and fungicides are also employed for in vitro sterilization process (Aasim et al., 2019; Barpete et al., 2021). Another important factor in sterilization is the exposure time of explants to the specific sterilizing agents. It is already established that there is correlation between sterilizing agent, dose and exposure time on sterilization (Nongalleima et al., 2014; Hesami et al., 2018c). Therefore, it is highly important to select sterilizing agent with low phytotoxicity and high inhibition rate of contaminants (Arab et al., 2014; Jafari et al., 2016).

Türkiye is one of the leading pistachio grower (Ertürk et al., 2015) and its cultivation is mostly done in Southeastern Anatolian Region (Çağlar et al., 2017). Türkiye ranked 4th in the production of pistachio with annual production of 74.828 ton (FAO, 2018). In Türkiye, the production of pistachio rootstock is generally done with grafting. However, elevated demand of rootstocks in recent years forced the producers to use modern biotechnological techniques like plant tissue culture. Although, research carried out in Türkiye on pistachio revealed the successful development of in vitro regeneration protocols (Onay et al., 2004; Ozden-Tokatli et al., 2005; Kılınç et al., 2015), the commercial production of pistachio rootstocks is still neglegible due to certain issues during plant tissue culture. The major concern is the availability of contamination free explants alongwith inhibiton of phenolic compounds in the culture medium which hinders the sprouting of shoots from sterilized explants. Therfore, establishment of successful sterilization protocol is highly noteworthy for the development of in vitro regeneration protocol of pistachio. Keeping in view, the study was designed for the optimization of sterilization for two different rootstocks of pistachio commonly used in Türkiye. Plant material in this study was collected at different physiologial stages and subjected to different sterilizing agents and exposure time.

2. Materials and Method

The shoots with 5-10 nodes of two different pistachio rootstocks (UCB-1 and Buttum) were procured/collected from Pistachio Research Institute, Gaziantep, Türkiye. The plant material was also grafted and cultured under greenhouse conditions at Bademli Biyoteknoloji Sanayi ve Ticaret Limited Ödemiş, Izmir for the availability of continuous plant material. The plant material used in this study were collected in different months and sources (1, 2).

The leaves were removed and twigs were placed under running water to remove trashes prior to surface sterilization (Benmahioul et al., 2016). Thereafter, shoots were cut into pieces of 2-3 cm long with at least one node (nodal segment) for sterilization. Different experiments were carried out using different sterilizing agents followed by inoculation on culture mediums enriched with different plant growth regulators. In all experiments, 30 explants were used from both rootstocks for each sterilizing agent.

Experiment 1: The plant material collected during April was subjected to the following procedure of washing material with 5% liquid soap (5 ml Protex+95 ml dH2O) and washed with running under tap water followed by soaking of nodal segments with 30% fungisit (MERPAN 80 WG - Captan) for 30 min and washed under running under tap water. Thereafter, material was sterilized with 0.1 % $HgCl_2$ for 55 min followed by three times washing with sterile water. After sterilization, nodes were cultured on MS medium enriched with 2 mg/L BA + 2 mg/L AgNO3. Similar sterilization procedure was also adapted for plant material collected during May with minor modificcation of using 0.1 % $HgCl_2$ for 45 min.

Experiment 2: The plant material was procured during second week of September and during May. The plant material (nodal segments) were subjected to sterilization using three different sterilizing agents in this study. The overall procedure used for sterilization is given in Table 1. Three different sterilizing agents used were 0.1 % $HgCl_2$, 10.0% Huwa-San (H_2O_2) and 10.0% commercial bleach. After sterilization, nodal segments were cultured in glass tubes containing MS medium enriched with 2 mg/L BA + 2 mg/L $AgNO_3$. Nodal segments were subcultured once a week for 3 times and then cultured on MS medium enriched with 0.5 mg/L GA_3.

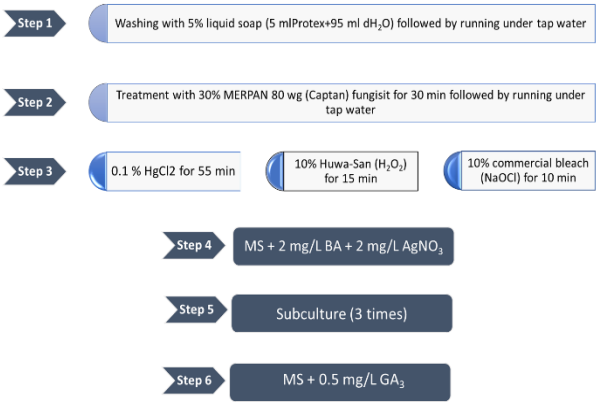

Figure 1: An overview of sterilization using different sterilizing agents

Experiment 3: The plant material (UCB-1) collected during end of October (Pistachio Research Institute) were subjected to sterilization by cleaning the nodal segments with water and subjected to 0.1% $HgCl_2$ for 45 min and rinsed with sterile dH_2O. Nodal segments were transferred to MS medium enriched with different BAP (1.0, 2.0, 3.0, 4.0, 5.0 mg/L) concentration and 25 mg/L Polyvinylpyrrolidone (PVP) for 8 weeks without any subculture.

After the completion of experiment, the success of sterilization process (%) was calculated by counting and tabulated by using formula based on non-contaminated plants and results were given in percentage.

$$\text{Sterilization (\%)} = \frac{Contamination\ free\ explants}{Total\ explants}\ x\ 100$$

The basal mediums used in this study was MS (Murashige and Skoog, 1962) medium used at 4.4 g/L, 30 g/L sucrose and medium was solidified by agar used at the rate of 6.5 g/L. The pH of the medium was adjusted to 5.8 by using 1N NaOH or 1N HCl. The incorporation of $AgNO_3$ and PVP were done prior to autoclaving the culture medium. The medium was poured into glass tubes (25 mm wide) or plastic falcon tubes (15 mm wide). After placing explants into the culture medium, tubes were placed in the growthroom equipped with White cool flourescent (experiment 1 and 3) or white LEDs (experiment 2) at 16h light photoperiod. The temperature of the growthroom was adjusted at 24± 1℃.

Analysis of variance (ANOVA) of different experiments were performed by using SPSS 20.00 for Windows. Duncan's multiple range test (DMRT) was used for Post hoc tests for comparing means difference. The data was transformed into Arcsine square root transformation (Snedecor and Cochran, 1967) before statistical analysis.

3. Results

The results revealed the clear impact of material collection timing and sterilizing agents for sterilization process for both rootstocks. The results of experiment 1 revealed the high contamination rate (100%) irrespective of exposing explants for more time. There was no shoot induction from nodal segments explants due to high contamination and possibility of dormancy phase of axillary buds at the time of collection. Besides that extensive release of phenolic compounds also inhibited the shoot induction.

Three different sterilizing agents were employed for plant material collected at different time in experiment 2. Results clearly revealed the clear impact of collection time and sterilizing agent on sterilization process. The response of rootstock was also different with each other. The samples collected during september 2020 revealed relatively low response (Figure 2) than plant samples collected during May (Figure 3) for both rootstocks.

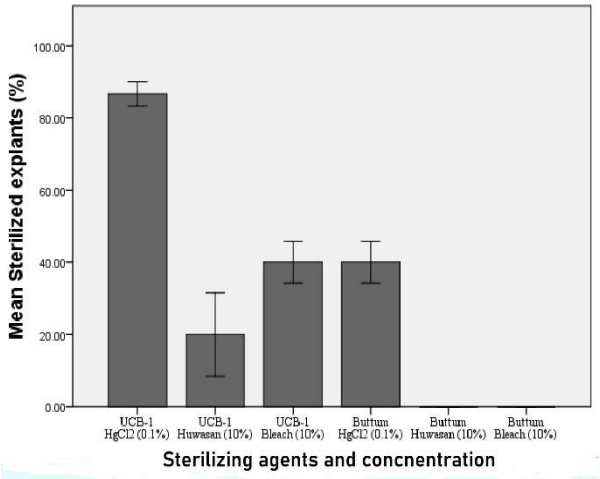

Figure 2: Optimization of sterilization of UCB-1 and Buttum rootstock material collected during month of September

The results given in Fig. 2 revealed that application of 0.1% $HgCl_2$ for 55 min was more effective than other treatments for both rootstocks and recorded 40.0% and 20.0% respectively for UCB-1 and Buttum rootstock. Whereas, application of 10% Huwa-San and 10% bleach resulted in 30% and 20% contamination free explants for UCB-1. Whereas, the response of Buttum was not good and 100% contamination was recorded for both 10% Huwa-San and 10% bleach. Comparison of both rootstocks revealed that UCB-1 was more responsive than Buttum. Whereas relatively more damage was associated with Huwa-san and bleach. On the other hand, plant samples collected during May were more responsive than september collected samples but follwed the same trend like samples collected during September. Application of 0.1% $HgCl_2$ resulted in relatively high non-contaminated nodal segments for both rootstocks and recorded as 87.0% (UCB-1) and 40.0% (Buttum). Results further revealed that other sterilizing agents also responded better and 20.0% (Huwa-San) and 40.0% (bleach) non-contaminated nodal segments was recorded for UCB-1. On the other hand, Buttum was less responsive to these two sterilizing agents and resulted in 100% contamination. Application of bleach resulted in damaging the explant which was more prominent in buttum compared to UCB-1. Regular subculture for three times found to be effictive to reduce the phenolic compounds. This decresae in phenolic compounds is possibly due to subculture and presence of $AgNO_3$ in the culture medium.

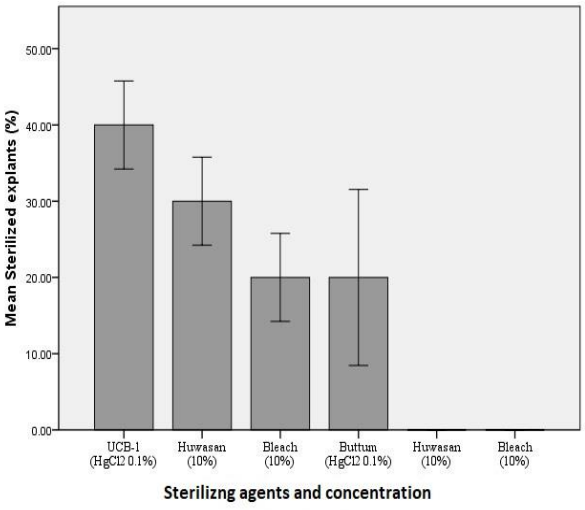

Figure 3. Optimization of sterilization of UCB-1 and Buttum rootstock material collected during month of May

After analysing the results of sterilizing agents and plant material collection time, another eperiment was desigend using only 0.1% $HgCl_2$ for plant material collected in the month of June. The results revelaed relatively low contamination than other experiments. The contaminated free nodal segments were recorded as 90.0% for UCB-1 and 50.0% for Buttum (Table 1). Results also revealed the relatively low phenolic compounds in the culture medium compared to other experiments. These results clearly revealed that collecting time of plant material and sterilizing agent is highly significant for obtaining contamination free explants for *in vitro* micropropagation of pistachio rootstocks.

Table 1. Optimization of sterilization of UCB-1 and Buttum rootstock material collected during month of June

Rootstock	Sterilizng Agent	Mean Sterilized explants (%)
UCB-1	%0.1 $HgCl_2$	90.00
Buttum	%0.1 $HgCl_2$	50.00

Plant material collected during late October was subjected to sterilization process of using 0.1 % $HgCl_2$ for 45 min followed by culture on MS medium enriched with different BAP concentrations and 25 mg/L PVP (experiment 3). Results revealed zero contamination irrespective of culturing continuously on the same medium for 8 weeks. However, explants turned into black with the passage of time due to extensive release of phenolic compounds in the culture medium. The level of phenolic compounds in the culture medium was variable with BAP concentration and minimum phenolic compounds were recorded for MS medium enriched with 3.0 mg/L BAP+25 mg/L PVP. These results suggested that phenolic compunds can be minimized by using different culture mediums and addition of subculture could lead to minimum phenolic compounds alongwith high possibility of shoot induction. The results also suggest that contamination is dependent on collection time and plant material from where samples were taken.

4. Discussion

Sterilization of plant material taken directly from field or potted plants are generally difficult to sterilize due to heavy infestation with microbes. Therefore, these materials require special treatments prior to use major sterilizing agent (Karataş and Aasim, 2014; Dogan et al., 2016). Surface sterilization of pistachio is also one of the major problem for establishing the plant tissue culture protocol (Mascarello et al., 2007). The surface sterilization protocol of such material is generally based on multisteps like cleaning of explants with water, soaps, shampoos, treating explants with fungicides or bactericide, use of antibiotics and alcohol for a certain period of time (Tilkat and Akdemir, 2013; Benmahioul, 2017; Barpete et al., 2019). Elimination of contaminants in pistachio is very important for the establishment of in vitro regeneration protocol. On the other hand, exudation of phenolic compounds from explants of plants like pistachio also cause serious problem which may lead to death of explants (Leng et al., 2009; Marin et al., 2017). Therefore, optimization of proper sterilization protocol is prerequisite for the establishment of in vitro micropropagation of pistachio. Previous studies on pistachio revealed the application of different sterilizing agents with multisteps of exposing explants to other disinfectants (Benmahioul et al., 2016). The results obtained in this study revealed the significant impact of different internal and external factors like collection time, subculture, sterilizing agent, concentration and sterilization time (Tilkat and Akdemir, 2013).

Collection of plant material is highly significant for successful in vitro sterilization of pistachio. In this study, plant materials collected at different physiological stages resulted in different response towards sterilization. The best time for the collection of plant samples was the month of June followed by May and September. Whereas, collection of plant material in other months responded variably with high in vitro contamination and exedution of phenolic compounds. The impact of season on in vitro contamination of pistachio explants has been documented by Benmahioul et al. (2016) and Benmahioul (2017). The possible reason might be the physiological age and dormancy found in the axillary bud and best time for the collection of plant material is spring or juvenile phase with more rapid cell division and growth (Benmahioul, 2017). High frequency of sterilized explants collected duuring May and June for both rootstocks confirmed the results of Benmahioul et al. (2016).

Presence of *in vitro* contamination and exudation of phenolic compounds in the culture medium are the major limiting factors for successful in vitro regeneration. In order to overcome the issue, selection of proper sterilizing agents, concentration and exposure time are important. Previous studies on pistachio revealed the use of different sterilizing agents like $HgCl_2$, H_2O_2 (Ozden-Tokatli et al., 2005) and NaOCl (Kılınç et al., 2015). In all these studies, researchers employed different concentration and treated explants for different time. The results of experiment 2 revealed the supermacy of $HgCl_2$ than other sterilizing agents for inducing high sterilization frequency. The results are in line with the findings of Benmahioul (2017), who reported more contamination on explants treated with NaOCl (84.7%) compared to $HgCl_2$ (38.7%). Whereas, Benmahioul et al. (2016) reported statisitcally insignificant impact of $HgCl_2$ (4.4%) and NaOCl (4.2%) for pistachio.

Application of Huwasan (10%) in this study resulted in 20-30% contamination free explants of UCB-1. Whereas, 100% contamination was recorded for Buttum. Contrarily, successful utilization of 10.0% H_2O_2 for surface sterilization of pistachio has been reported by Ozden-Tokatli et al. (2005). Application of 10.0% NaOCl was least effective than other sterilizng agents used in this study and resulted in 100% contamination rate. The previous study on pistachio by Tilkat et al. (2009) did not support the results and they achieved 100 % sterilized eplants of *P. vera* cv. Atli. By exposing explants to 10% (v/v) NaOCl for 30 min. These results clearly revealed the varaiable impact of sterilizing agents and success of sterilization is dependent on genotype used.

Exudation of phenolic compounds in the regeneration medium is the major threat to the in vitro regeneration of most of the plants like pistachio. In order to overcome the issue, incorporation of chemicals like PVP (Aasim et al., 2010), antioxidants like ascorbic acid (Marin et al 2017) or continuous subculture of explants are employed. The results clearly revealed the signfiicant impact of subculture on eliminating or minimizing the exudation of phenolic compounds. The positive impact of subculture on phenolic compounds has been documented for pistachio (Benmahioul, 2017). Application of PVP alongwith BAP also regulated the phenolic compounds exudation. These results suggest that proper combination of PVP+BAP can be useful for inhibiting phenolic compounds with the addition of subculture

5. Conclusion

Selection of proper sterilizing agent with concentration and sterilization time regulate the *in vitro* sterilization process for attaining healthy and contamination free explants for *in vitro* regeneration. On the other hand, this study also revealed the significance of sterilizing agent, rootstock type and plant material collection time. Application of 0.1%

$HgCl_2$, material collection during June or May alongwith continuous subculture are optimized for both UCB-1 and Buttum rootstocks. On the other hand, there is also need to perform more experiments to achieve healthy explants with no or minimum releaase of phenolic compounds.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

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Callistosporium luteo-olivaceum (*Callistosporiaceae: Basidiomycota*), an agaric new to Türkiye

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Callistosporium luteo-olivaceum (*Callistosporiaceae: Basidiomycota*), Türkiye için yeni bir agarik

Abstract: *Callistosporium luteo-olivaceum* collected from Mediterranean region of Türkiye is identified based on morphological characters and combined dataset of nuclear ribosomal ITS and LSU sequences. *Callistosporium luteo-olivaceum* is new to the country's mycobiota and it is the first report of this species from the relict endemic *Liquidambar orientalis* forest. Description of the species based on macro and micromorphological characters, colour photograph from its natural habitat and line-drawings of microscopic features are presented.

Key words: *Basidiomycota, Callistosporiaceae*, molecular phylogeny, new record, Türkiye

Özet: Türkiye'nin Akdeniz bölgesinden toplanan *Callistosporium luteo-olivaceum*, morfolojik karakterlere ve nükleer ribozomal ITS ve LSU dizilerinin birleşik veri setine dayalı olarak tanımlanmaktadır. *Callistosporium luteo-olivaceum* ülkenin mikobiyotası için yenidir ve relikt endemik *Liquidambar orientalis* ormanından bu türün ilk raporudur. Türün makro ve mikromorfolojik karakterlere dayalı betimlemesi, doğal ortamından renkli fotoğrafı ve mikroskobik özelliklerin çizimleri sunulmaktadır.

Anahtar Kelimeler: *Basidiomycota, Callistosporiaceae,* moleküler filogeni, yeni kayıt, Türkiye

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1. Introduction

In previous classification, the genus *Callistosporium* Singer belonged to the family *Tricholomataceae* R. Heim ex Pouzar within *Basidiomycota* R.T. Moore and its members have been regarded in the tricholomatoid clade (Moncalvo et al., 2002). When a relationship with the entolomatoid clade was revealed, the genus was transferred to the *Entolomataceae* Kotl. & Pouzar based on rRNA data (Matheny et al., 2006). Later, a phylogenetic study by Sánchez-García et al. (2016) showed that the genus *Callistosporium* with other genera such as *Catathelasma* Lovejoy, *Guyanagarika* Sánchez-García, T.W. Henkel & Aime, *Macrocybe* Pegler & Lodge, *Pleurocollybia* Singer, and *Pseudolaccaria* (Fr.) Vizzini, Contu & Z.W. Ge have been included within the family *Catathelasmataceae* Wasser. Recently, Raj et al. (2019) added the genus *Anupama* K.N.A. Raj, K.P.D. Latha & Manim from India to this family. Moreover, Vizzini et al. (2020) reorganized the genera *Anupama, Callistosporium, Guyanagarika, Macrocybe, Pseudolaccaria* and *Xerophorus* (Bon) Vizzini, Consiglio & M. Marchetti within the family *Callistosporiaceae* Vizzini, Consiglio, M. Marchetti & P. Alvarado.

According to Index Fungorum, the genus *Callistosporium* is represented by about twenty five species (Kirk, 2019) with worldwide distribution from arctic to tropical habitats from North and South America, Europe, and South Asia (Singer, 1970; Hongo, 1981; Redhead, 1982; Horak, 1987; Manimohan and Leelavathy, 1989; Saba and Khalid, 2014; Sánchez-García et al., 2016; Raj et al., 2019; Vizzini et al., 2020). In Türkiye, only one collection named *Callistosporium olivascens* (Boud.) Bon. was reported as belonging to the genus *Callistosporium* (Akata et al., 2020); however, this species was combined into genus *Xerophorus* (Vizzini et al., 2020). Recently, the first member, *Xerophorus donadinii* (Bon) Vizzini, Consiglio & M. Marchetti, has been presented at genus level from Türkiye (Sesli, 2021).

The genus is characterized by the basidiomata with collybioid habit often fasciculate; pileus with a mixture of yellow, brown and olivaceus colours; the subdecurrent, adnexed or emarginated lamellae; ellipsoid, smooth, inamyloid basidiospores with scarcely or weakly cyanophilic, white deposit; presence or absence of cheilocystidia, pleurocystidia and clamp-connections; a cutis of pileipellis that contains hyphae with encrusting and intracellular pigments; and lilac, violet to olive yellow staining of flesh in KOH reaction (Singer, 1986; Noordeloos, 1995; Arnolds, 2006; Vesterholt and Holec, 2008; Halama and Rutkowski, 2014; Jančovičová et al., 2016; Sánchez-García et al., 2016). *Callistosporium* live as saprotrophic on humus, litter and also other kinds of wood debris, and on the base of palm trees and *Sphagnum*, mostly in forests (Singer, 1986; Noordeloos, 1995; Vesterholt and Holec, 2008; Jančovičová et al., 2016; Sánchez-García et al., 2016).

Callistosporium luteo-olivaceum (Berk. & M.A. Curtis) Singer has been recorded from North America, Canada,

tropical south America, Europe, China, Japan, England, India, Pakistan (Bigelow and Barr, 1969; Singer, 1970; Hongo, 1981; Redhead, 1982; Horak, 1987; Manimohan and Leelavathy, 1989; Roberts, 2009; Saba and Khalid, 2014). It is a decomposer found primarily on dead conifer wood growing alone or gregariously (Kuo, 2016).

In this study, *Callistosporium luteo-olivaceum* is identified both morphologically and molecularly, and presented as the first record from Türkiye. Phylogenetic position of this species is inferred from a combined ITS and LSU rDNA dataset. The results provide taxononamically important features of the Turkish *C. luteo-olivaceum* which enables distinction of its closely related species in the genus *Callistosporium*.

2. Materials and Method

2.1. Morphological analysis

Callistosporium samples were picked up from Muğla province during 2018 season. Pictures are taken in the field. Micro-morphological observations were done using dried samples and using Leica DM500 light microscope (Leica Microsystems, Wetzlar, Germany) at magnifications up to 400× and 1000×. Samples were stained with Congo red. The following measurements were taken, which are: L^m and W^m indicating the average length and width of basidiospores, respectively; Q indicating the ratios of length/width and Q^m presenting the average quotient of the measured basidiospores. Specimens were deposited at the fungarium of Isparta University of Applied Sciences, Isparta, Türkiye.

2.2. Molecular analysis

2.2.1. DNA isolation, Polymerase chain reaction (PCR) and Sequencing

The genomic DNA was extracted from dried specimens using ZR Fungal/Bacterial DNA MiniPrep kit (Zymo research, Irvine, California) according to the manufacturer's protocol. Polymerase chain reaction was performed as described by Kaygusuz et al. (2021) using the primer pairs ITS1F/ITS4 (White et al., 1990) and LR0R/LR5 (Vilgalys and Hester, 1990) to amplify nrITS and nLSU gene regions, respectively. Same primer sets were used for sequencing reactions (Source Bioscience, Berlin, Germany). Sequence chromatograms were edited using in BioEdit 7.0.5 (Hall, 1999).

2.2.2. Phylogenetic analyses

Maximum Likelihood (ML) and Bayesian Inference (BI) methods were used for constructing phylogenetic trees. A concatenated dataset was used to perform ML analysis in RAxML v8.2.10 (Stamatakis, 2014) under the GTRGAMMA substitution model with 1.000 bootstrap replicates. The BI analysis was performed using MrBayes 3.2.2 (Ronquist et al., 2012) by Markov chain Monte Carlo (MCMC) method including six simultaneous Markov chains run for 1,000,000 generations where sampling was done for every 100^{th} generation.

3. Results

3.1. Taxonomy

Callistosporiaceae Vizzini, Consiglio, M. Marchetti & P. Alvarado

Callistosporum luteo-olivaceum (Berk. & M.A. Curtis) Singer, Lloydia 89: 117 (1946).

Macroscopic and microscopic features: Habit collybioid. Pileus (Fig. 1a) 15-30 mm diam., convex to plano-convex with centre slightly depressed, margin more or less defiexed or straight, surface smooth and slightly hygrophanous, olivaceous yellow to olivaceous grey brown. Lamellae crowded, regular, ventricose, emarginated, pale yellow or olive yellow. Stipe 35-65 × 3-10 mm, cylindrical with tapering towards at base, sometimes curved, olive brown to yellowish brown or brown, covered with white floccose at the base. Odor and taste indistinct. Spore print white.

Basidiospores (Fig. 1b) (4.5-)5.5-6.5(-7.0) × (3.2-)3.5-4.0(-4.5) µm, $L^m \times W^m$ = 5.7 × 3.9 µm, Q = 1.3-1.7, Q^m = 1.4, ellipsoid, with small hilar appendage, guttulate, smooth, thin-walled, hyaline or brownish intracellular pigment, non-dextrinoid, non-amyloid. Basidia (Fig. 1c) (20.0-)24.5-28.5(-32.0) × 5.0-6.5(-7.0) µm, clavate to narrowly clavate, 4-spored or rarely 2-spored, hyaline or violaceous brown and thin-walled. Pleurocystidia absent. Cheilocystidia (Fig. 1d) (25.0-)30.0-45.0(-47.0) × 5.5-10.0 µm, cylindrical to fusiform, hyaline and thin-walled. Hymenial trama made up of clavate, narrowly clavate to cylindrical with obtuse to subcapitate apex, (10.0–)15.0–25.0(-29.0) × 3.5-6.0 µm, hyaline and thin-walled. Pileipellis (Fig. 1e) consisting of cylindrical or clavate, 3.0-10.0 µm wide hyphae, hyaline or some with brown to light brown pigments and thin-walled. Stipitipellis a cutis of cylindrical, 3.5-10.0 µm, hyaline and thin-walled hyphae. Caulocystidia (Fig. 1f) (10.0-)15.5-26.0(-32.0) × 3.5-6.0 µm, narrowly clavate to clavate to cylindrical with obtuse to subcapitate apex, hyaline and thin-walled. Clamp connections absent in all examined tissues.

Ecology: Saprotrophic, in groups on angiosperm wood, present at elev. 5 m, on *Liquidambar orientalis* Mill., growing naturally on rich, deep and moist soils.

Collections examined: TÜRKİYE, Muğla Province, Köyceğiz district, around Döğüşbelen village, on well decayed *Liquidambar orientalis* log, alt. 5 m, 18 January 2018, O. Kaygusuz, OKA-TR1529; GenBank: OK626226 nrITS, OK625531 for nrLSU; *ibid.*, on rotten wood of *L. orientalis*, alt. 5 m, 19 January 2018, O. Kaygusuz, OKA-TR1530; GenBank: OK626227 for nrITS, OK625532 for nrLSU; *ibid.*, on wood of *L. orientalis*, alt. 6 m, 19 January 2018, O. Kaygusuz, OKA-TR1531; GenBank: OK626228 for nrITS, OK625533 for nrLSU.

3.2. Molecular analysis

The combined nrITS+nLSU dataset included sequences from 52 specimens, including 6 newly generated sequences. The ML and BI tree topologies were nearly identical. Therefore, only the ML tree is shown, along with the Maximum-likelihood bootstrap (MLB) and Bayesian posterior probabilities (BPP) values (Figure 2). The phyllogram showed that *Callistosporium luteo-olivaceum* is composed of two considerably distinct clades with sequences from different geographic areas. Sequences of *C. luteo-olivaceum* from Türkiye were clustered in the same branch as sequences produced from Austria, Italy, Japan and Pakistan, with significant statistical support (MLB = 88%, BPP = 0.92, Fig. 2).

Figure 1. ***Callistosporium luteo-olivaceum*****:** (a) basidiomes growing in its natural habitat, (b) basidiospores, (c) basidia and hymenial elements, (d) cheilocystidia, (e) pileipellis elements, and (f) caulocystidia. Scale bars: a = 10 mm, b-f = 10 µm.

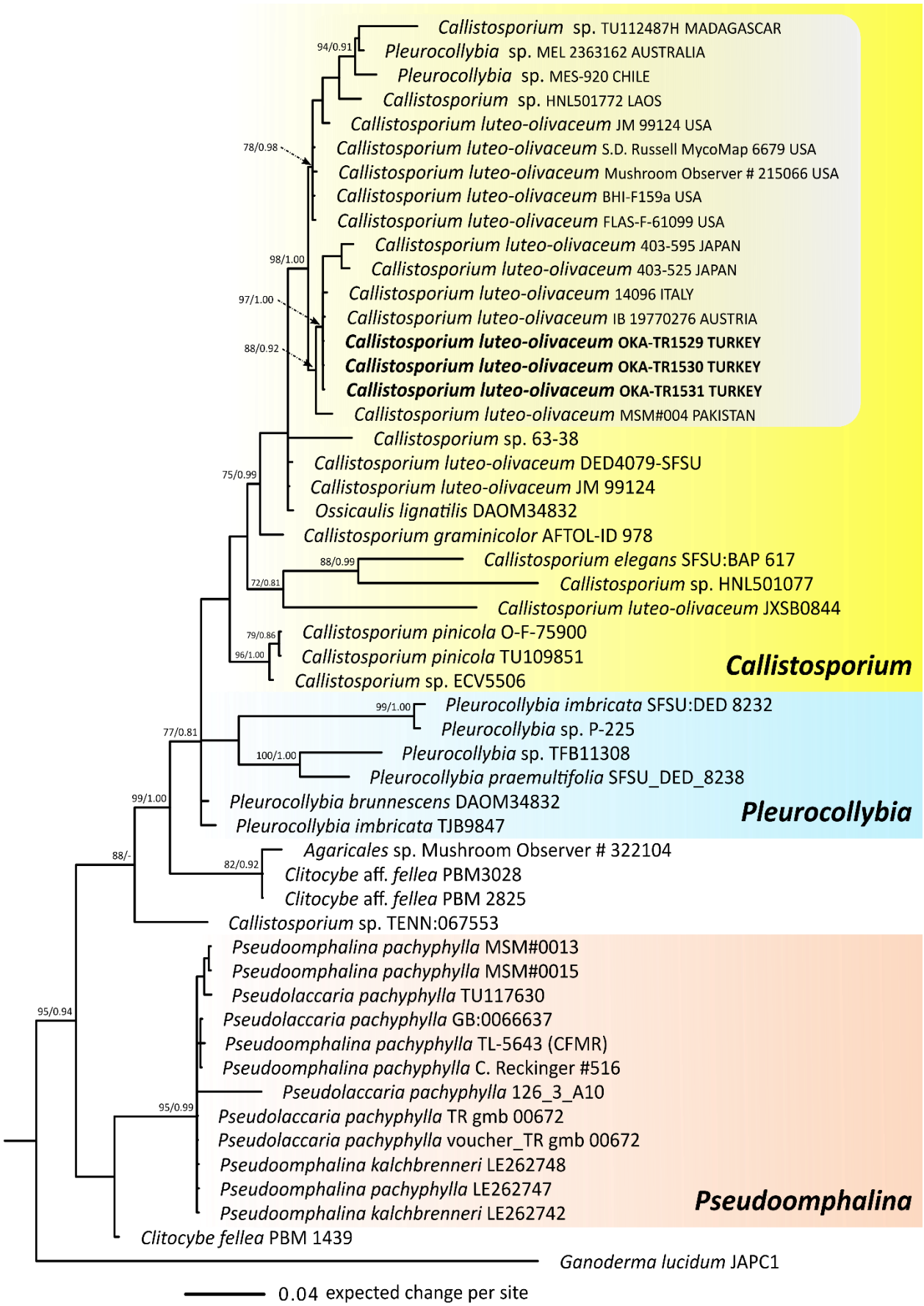

Figure 2. Maximum Likelihood (ML) tree of *Callistosporium* species based on nrITS and nrLSU sequences with the outgroup *Ganoderma lucidum*. Maximum-likelihood bootstrap (MLB) values ≥ 72% and Bayesian posterior probabilities (BPP) ≥ 0.81 are shown on the branches. Collections presented in this study are in bold.

4. Discussions

Callistosporium luteo-olivaceum is regarded as a taxonomically complicated macrofungus due to its variable characters that may arise from its worldwide distribution (Saba and Khalid, 2014). Some researchers have given different names to the species, but critical examination by Redhead (1982) have resulted in elimination of many taxa in this genus, where *C. elaeodes* Bon, *C. favrei* Singer, *C. graminicolor* Lennox, *C. luteofuscum* Singer, *C. luteofuscum* var. *major* Singer, *C. majus* Singer, and *C. xanthophyllum* (Malençon & Bertault) Bon are considered as synonyms of *C. luteo-olivaceum*.

Turkish collection of *C. luteo-olivaceum* is characterized by pileus with yellow olive tinges, frequent pale yellowish and olive yellow lamellae and a white spore print. The features of the Turkish collections of *C. luteo-olivaceum* resembles the species described by Redhead (1982).

Our phylogenetic result confirmed that *C. luteo-olivaceum* is a member of the *Callistosporium* clade within the family *Callistosporiaceae*. Genetically, *Callistosporium luteo-olivaceum* is significantly related to *C. elegans* Desjardin & B.A. Perry, *C. graminicolor* and *C. pinicola* Arnolds. However, they have distinctive morphological characteristics that differentiate each other. *Callistosporium elegans* differs from *C. luteo-olivaceum* mainly by its violet to purple-wine pileus, smaller basidiospores (4.4-5.3 × 3.0-3.7 μm) and cylindraceous, subcapitate to bilobed cheilocystidia (Vizzini et al., 2020).

Callistosporium graminicolor is separated from *C. luteo-olivaceum* by having a smaller basidiomata (12-18 mm) and larger basidiospores (6.0-8.0 × 4.0-5.5 μm) (Lennox, 1979). It also differs from *C. luteo-olivaceum* by its dark reddish to dark reddish brown color formation with KOH on lamellae and stipe (Vizzini et al., 2020). *Callistosporium pinicola* has somewhat small-sized basidiomes (5-30 mm), usually vividly yellow-brown, orange-brown, rusty-brown to red-brown colored pileus, distinctively smaller basidiospores (2.5-4.5 × 2.0-3.5 μm) and prefers decayed wood and conifers (Arnolds, 2006; Halama and Rutkowski, 2014).

Callistosporium elaeodes is morphologically close to *C. luteo-olivaceum*, but it can be distinguished by its smaller basidiomes (5-20 mm), bright olive-green pileus, thicker and green-yellow and olive-brown lamellae, and larger basidiospores (5.9-7.6 × 3.3-4.3 μm) and it is mostly associated with broadleaved trees (Vizzini et al., 2020).

With this study, the Turkish *C. luteo-olivaceum* is described, supported with its molecular and morphological features. This species is in the red-list of many European countries and we propose it could be considered as the same for conservation of the Turkish mycobiota.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

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Phytoremediation efficiencies of *Brassica napus* and *Chenopodium quinoa* in soils contaminated with Pb using chelator complexes

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Şelatör kompleksleri kullanılarak Pb ile kirlenmiş topraklarda *Brassica napus* ve *Chenopodium quinoa*'nın fitoremediasyon etkinlikleri

Abstract: Heavy metal pollution is one of the essential pollutions, and phytoremediation is one of the preferred methods to eliminate this pollution. The use of the degradable chelating agent for phytoremediation efficiency is a promising and low-cost method for removing soil contaminated with heavy metals. In this study, it was investigated whether phenanthroline and humic acid increase phytoremediation activities for *Brassica napus* L. and *Chenopodium quinoa* Wild. species and their applicability. The study was carried out under greenhouse conditions with 3 replications according to a complete random block trial design by applying 4 doses of each of the (i) control (without chelate), (ii) EDTA, (iii) nitro, (iv) pyridine, (v) 1-10 phenanthroline and (vi) humic acid treatments (0, 2.5, 5 and 10 mmol kg^{-1} The obtained results showed that the highest tolerance indices (TI) for *B. napus* was found at 2.5 mmol kg^{-1} nitro chelate. TI of *C. quinoa* was highest at 5 mmol kg^{-1} pyridine chelate. Maximum Pb accumulations were found at 5 mmol kg^{-1} 1-10 phenanthroline and 5 mmol kg^{-1} pyridine chelates in *B. napus* and *C. quinoa*, respectively. In both species, while Pb accumulations were high in roots, they were low in stems and leaves. Bioconcentration factors (BCF) were calculated highest at 2.5 mmol kg^{-1} nitro and 1-10 phenanthroline for *B. napus* and *C. quinoa*, respectively. These species were used as hyperaccumulator plants in many studies. Increasing the performance of hyperaccumulator plants to be used in cleaning the habitats exposed to heavy metal pollution will increase the efficiency of phytoremediation.

Key words: Heavy metal, pollution, accumulator plant, agricultural plants

Özet: Ağır metal kirliliği önemli kirliliklerden olup, fitoremediasyon bu kirliliği ortadan kaldırmak için tercih edilen yöntemlerdendir. Fitoremediasyon verimliliği için bozunabilir şelatlama maddesinin kullanımı, ağır metallerin topraktan uzaklaştırılması için için umut verici ve düşük maliyetli bir yöntemdir. Bu çalışmada, EDTA (etilendiamintetraasetik asit), nitro (4-nitrobenzaldehit), piridin, 1-10 fenantrolin ve hümik asidin *Brassica napus* L. ve *Chenopodium quinoa* Willd. türleri için fitoremediasyon etkinliklerini arttırıp arttırmadığı ve uygulanabilirliği araştırılmıştır. Çalışma (i) kontrol (şelat ilavesiz), (ii) EDTA, (iii) nitro, (iv) piridine, (v) 1-10 fenantrolin ve (vi) hümik asit uygulamalarının her birinden 4 doz (0, 2.5, 5 ve 10 mmol kg^{-1}) uygulayarak tam şansa bağlı blok deneme desenine göre 3 tekerrürlü olarak sera şartlarında yürütülmüştür. Elde edilen sonuçlara göre, *B. napus* için en yüksek tolerans indeksinin (TI) 2.5 mmol kg^{-1} nitro şelatta bulunmuştur. *C. quinoa*'nın TI değeri 5 mmol kg^{-1} piridin şelatta en yüksek bulunmuştur. Maksimum Pb birikimleri sırasıyla *B. napus* ve *C. quinoa*'da 5 mmol kg^{-1} 1-10 fenantrolin ve 5 mmol kg^{-1} piridin şelatlarında bulunmuştur. Her iki türde de köklerde Pb birikimleri yüksek iken, gövde ve yapraklarda düşük düzeydeydir. Biyokonsantrasyon faktörleri (BCF) en yüksek sırasıyla *B. napus* ve *C. quinoa* için 2.5 mmol kg^{-1} nitro ve 1-10 fenantrolinde hesaplanmıştır. Bu türler birçok çalışmada hiperakümülatör bitkiler olarak kullanılmıştır. Çalışma sonunda, türlerin en yüksek hiperakümülatör kapasiteleri 2.5 mmol kg^{-1} nitro ve 1-10 fenantrolin uygulamasından elde edildiği bulunmuştur. Ağır metal kirliliğine maruz habitatların temizlenmesinde kullanılacak hiperakümülatör bitkiler kadar onların performansının artırılması da fitoremediasyonun etkinliğini artıracaktır.

Anahtar Kelimeler: Ağır metal, kirlilik, akümülatör bitki, tarımsal bitkiler

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1. Introduction

Heavy metal density in the soil decreases the fertility quality of the soil. It also creates dangers for organisms due to its toxic effect (Quartacci et al., 2006). Lead (Pb), which is highly toxic, is transported to organisms by air, water and soil (Kabata-Pendias, 2004). When the Pb naturally found in soils exceeds the limit value, it causes excessive heavy metal accumulation in plants (Dürüst et al., 2004).

Phytoremediation is a method that removes heavy metal pollution from the environment using plants (Raskin et al., 1997). Phytoremediation preserves the soil's biological properties and physical structure (Khan et al., 2008; Gong et al., 2018). Plant species that can accumulate 50 to 500 times more metal than the metal concentration in the soil are used in the phytoremediation method, and these species are called hyperaccumulative plants (Clemens, 2006; Özay and Mammadov, 2013). Approximately 450 plant species (only 0.2 % of angiosperms) have been defined as hyperaccumulator (Reeves, 2006).

In phytoremediation studies, chelate addition facilitates the plant's uptake by increasing the mobility of metals in the soil and so increases the accumulator capacity of plants (Adiloğlu et al., 2015). EDTA (ethylenediaminetetraacetic acid), EGTA (ethylenglutaric acid), DTPA (diethyltriaminpentaacetic acid), SDS (sodiumdodecylsulfate), NTA (nitrilotriacetate), [S, S] - EDDS (S, S-ethylenediamindiscinucin acid), humic acid

(HA), boric acid are the most commonly used chelates (Ladislas et al., 2012). This study used five chelates which are humic acid, EDTA, 1-10 phenanthroline, nitro and pyridine.

Brassica napus and *C. quinoa* are well known as hyperaccumulator species. However, previously unused chelates (nitro, pyridine, 1-10 phenanthroline) were used in this study for studied species and Pb. We aimed to determine the phytoremediation efficiencies of species in a pot experiment.

2. Materials and Method

The soil was collected from the rural village of Suluova (Akören; 40° 52' 29.1756" N and 35° 27' 42.3144" E) without traffic and industrial in Amasya Province in Türkiye. The soil was a clay loam paddy soil with a pH (in water) of 7.76 and had high organic matter content and medium calcareous. Collected soil had aqua regia-soluble concentrations of Pb of 0.12 mg kg^{-1} (Table 1).

Table 1. Chemical properties of the soil used in the experiment.

Parameters	SoilValue
Saturation (%)	62.00
pH	7.76
EC (ds/m)	572
Lime (%)	14
Organic matter (%)	7.99
Phosphorus (kg/da)	6.73
Potassium (kg/da)	7.12
Pb (mg/kg)	0.12

The collected soil was dried in the air, crushed and passed through a coarse sieve. 3 kg portions of the soil were transferred to 20 cm diameter pots. The lead was added at the rate of 100 mg kg^{-1} as $Pb(NO_3)_2$ form to each pot. One month was waited for the soil to become homogeneous after the lead addition. We added the necessary amount of nutrients (NPK fertiliser) to the pots before planting. *B. napus* and *C. quinoa* seeds were previously surface sterilised with a 10 % sodium hypochlorite solution. 20 seeds of each species were sown in each pot.

The treatments were as follows (1) control without chelates; (2) EDTA; (3) nitro; (4) pyridine; (5) 1-10 phenanthroline and (6) humic acid at doses of 0-2.5-5.0-10 mmol kg^{-1}. Plot experiment was conducted at the greenhouse conditions with natural light, 14 hours (24.2° C) in a day and 10 hours (16.7° C) in a night and about 50 % relative humidity. There were three replicates of each treatment in a randomised block design.

Plant materials for heavy metal analysis were harvested after mature (four weeks). The harvested plants were classified as root, stem and leaf. They were rinsed with tap water and then with distilled water, dried at 65° C for 48 h, and their dry weights were measured. Plant materials were analyzed for Pb concentrations using Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) method.

Growth parameters such as root, stem and leaf length, and fresh and dry matter were used for tolerance index (TI) calculations (Wilkings, 1978).

$$\mathrm{TI} = \frac{\text{Metal applied plant growth parameters}}{\text{Control plant growth parameters}}$$

Bioconcentration Factor (BCF): The ratio between the total heavy metal concentration in the plant and soil (Mertens et al., 2005; Ladislas et al., 2012).

$$\mathrm{BCF} = \frac{\text{Heavy metal concentration in the plant biomass}}{\text{Soil heavy metal concentration}}$$

Translocation factor (TF) is the ratio of the metal concentration in the plant stem to the root's metal concentration. It was used to give an idea about the plant's heavy metal carrying ability (Padmavathiamma and Li, 2007; Badr et al., 2012; Alaribe and Agamuthu, 2015).

$$\mathrm{TF} = \frac{\text{Shoot heavy metal concentration}}{\text{Root heavy metal concentration}}$$

Data were tested statistically by analysis of variance and Tukey's honestly significant difference (HSD) tests were carried out by using SPSS 22.0 version.

3. Results

The highest tolerance indices was calculated for *B. napus* (126 %) and *C. quinoa* (165 %) at 2.5 mmol kg^{-1} nitro and 5 mmol kg^{-1} pyridine chelates, respectively. Many chelate treatments of *C. quinoa* have been found to higher tolerance indices than the *B. napus* (Figure 1).

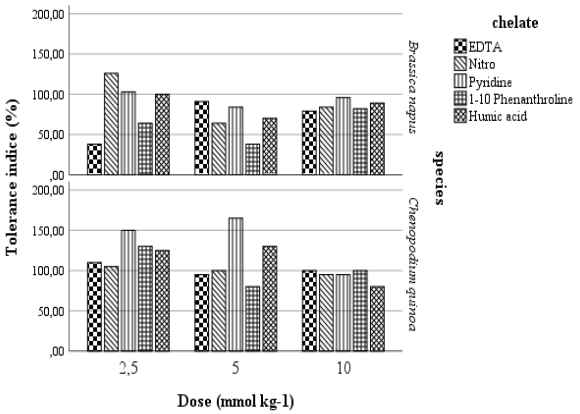

Figure 1. Tolerance Index (TI) values of *B. napus* and *C. quinoa* plant at the different chelate treatments.

We found that Pb accumulation was highest (252.13 ± 3.80) in *B. napus* at 5 mmol kg^{-1} 1-10 phenanthroline and lowest accumulation (63.80 ± 0.50) was found at 10 mmol kg^{-1} humic acid chelate. For *C. quinoa*, highest accumulation (606.00 ± 9.60) was found at 10 mmol kg^{-1} pyridine and lowest accumulation (131.46 ± 0.25) was found at 5 mmol kg^{-1} in EDTA (Table 2).

Comparing lead accumulation in plant organs of species, the accumulation capacity of the roots is higher (Figure 2).

According to the repeated measures ANOVA (RMANOVA) results, heavy metal accumulations were significantly among roots, stems, leaves and whole plants for each species considering chelate, chelate dose and chelate * chelate dose parameters (Table 3).

BCF values is higher than 1 at all chelates treatments in *B napus* and *C. quinoa*. We found the highest BCF (6.15) at 2.5 mmol kg^{-1} in nitro chelate for *B. napus* and the highest

BCF (23.31) at 2.5 mmol kg^{-1} 1-10 phenanthroline for *C. quinoa*. TF values of species were generally found to be less than 1 (Table 4).

Table 2. Pb concentration of total plant at the different chelate treatments.

Chelate	Dose (mmol kg^{-1})	Total Plant Pb (mmol kg^{-1})	
		B. napus	*C. quinoa*
Control		5.81 ± 0.03	6.01 ± 0.07
EDTA	2.5	129.93 ± 0.11	229.23 ± 2.70
	5	160.86 ± 0.95	**131.46 ± 0.25**
	10	121.83 ± 0.80	255.10 ± 0.20
Nitro	2.5	146.06 ± 0.65	215.03 ± 1.30
	5	153.06 ± 0.25	233.46 ± 1.15
	10	97.56 ± 2.15	264.80 ± 0.20
Pyridine	2.5	133.43 ± 0.05	562.70 ± 1.10
	5	132.90 ± 1.20	289.26 ± 0.35
	10	68.16 ± 0.45	**606.00 ± 9.60**
1-10 Phenanthroline	2.5	99.13 ± 0.70	516.96 ± 3.55
	5	**252.13 ± 3.80**	511.90 ± 0.70
	10	147.36 ± 0.75	225.80 ± 2.50
Humic acid	2.5	103.93 ± 0.30	472.13 ± 0.60
	5	97.26 ± 0.05	462.40 ± 16.88
	10	**63.80 ± 0.50**	290.00 ± 0.43

4. Discussions

Adding chelates to soils increases heavy metal and nutrient intake and disrupts nutrient intake balance (Chen et al., 2003; Turan and Angin, 2004; Turgut et al., 2004; Turan and Esringü, 2008). This decrease dry matter in the root, stem and leaf of the plant (Lai and Chen, 2005; Nascimento et al., 2006; Quartacci et al., 2006; Ben Rejeb et al., 2013; Zaier et al., 2014). In this study, 1-10 phenanthroline, nitro and pyridine were used for the first time. Previous studies have revealed that EDTA and humic acid increase heavy metal accumulation by plants (Ali and Chaudhury, 2016; Halim et al., 2003; Evangelou et al., 2004). EDTA has high binding capacity for Pb (Elliott et al., 1986; Quartacci et al., 2006; Safari Sinegani and Khalilikhah, 2011; Kanwal et al., 2014; Tai et al., 2018) and is used for Pb uptake at different dose treatments (Patra and Goldberg, 2003; Evangelou et al., 2004; Lai and Chen, 2005).

It has been found that EDTA application slows down the biomass increase in *Ricinus communis* L. species (Zhang et al., 2016), increases the accumulation of Pb in *Paspalum fasciculatum* and is higher in the roots than other organs (Salas-Moreno and Marrugo-Negrete, 2020). Humic acid application increase Cu accumulation and restrict Zn uptake in roots of *Chrysopogon zizanioides* (Vargas et al., 2016). Many studies have found that these chelates increase heavy metal accumulation in roots (Bhattacharya et al., 2010; Idris et al., 2016).

In the current study, chelate applications have been found to reduce growth and biomass in *B. napus* and increase growth and biomass in *C. quinoa*. For *B. napus*, Pb accumulation is highest for 5 mmol kg^{-1} 1-10 phenanthroline chelate and lowest for 10 mmol kg^{-1} humic acid chelate. The highest concentration of Pb in *C. quinoa* was found at a dose of 10 mmol kg^{-1} pyridine, and the lowest accumulation was found in 5 mmol kg^{-1} EDTA chelate. Pb accumulation in the roots of both species was higher than in the stem and leaves. These results are similar to previous studies. Generally, high Pb uptake was found in both species in nitro, pyridine and 1-10 phenanthroline applications.

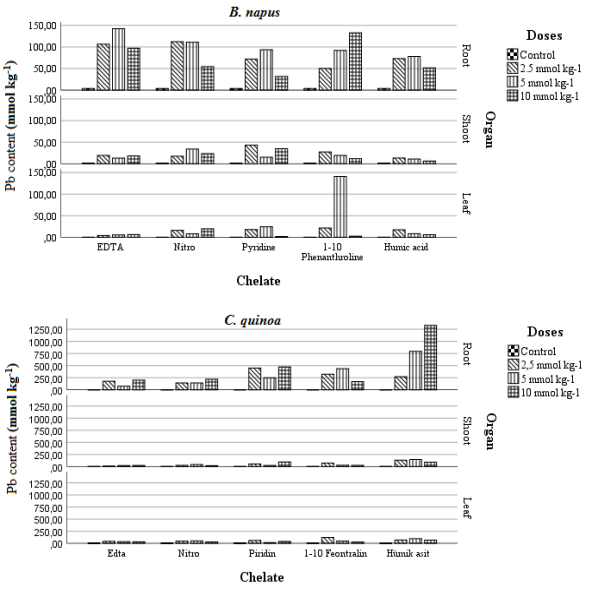

Figure 2. Pb in the plant organs at the different chelate treatments.

Table 3. RMANOVA test results between the chelate and pb concentration in root, stem, leaf and plant in *B. napus* and *C. quinoa* species. ($p<0.01$** , $p<0.05$*, ns=$p>0.05$).

		B. napus		*C. quinoa*	
Source		**F value**	**Sig.**	**F value**	**Sig.**
Chelate	Root	4229.06	0.00**	184.42	0.00**
	Stem	13938.5	0.00**	56.77	0.00**
	Leaf	26359.8	0.00**	26.9	0.00**
	Total plant	4767.32	0.00**	5089.42	0.00**
Chelate dose	Root	38764.94	0.00**	512.27	0.00**
	Stem	37898.91	0.00**	50.45	0.00**
	Leaf	35374.2	0.00**	122.57	0.00**
	Total plant	47790.31	0.00**	20488.08	0.00**
Chelate * Chelate dose	Root	3207.4	0.00**	70.15	0.00**
	Stem	5364.1	0.00**	13.9	0.00**
	Leaf	24275.97	0.00**	15.75	0.00**
	Total plant	2531.71	0.00**	2076.16	0.00**

Zayed et al. (1998) classified the plants into four groups considering BCF. BCF <0.01; non-accumulator plant, BCF = 0.01-0.1; low accumulator plant, BCF = 0.1-1.0; medium accumulator plants, BCF = 1-10; high accumulative or hyperaccumulator plant. BCF values of *B. napus* and *C. quinoa* species are greater than 1 in all applications. BCF values of *B. napus* species in nitro chelate were the highest at 2.5 mmol kg^{-1} doses, while in *C. quinoa* species, at a dose of 2.5 mmol kg^{-1}, 1-10 phenanthroline was found to be the highest in chelate. *B. napus* and *C. quinoa* show high accumulative properties.

Table 4. BCF and TF values of species at the different chelate treatments.

Chelate	Dose (mmol kg^{-1})	BCF		TF	
		B. napus	*C. quinoa*	*B. napus*	*C. quinoa*
Control		1.57	2.98	0.57	0.60
EDTA	2.5	1.61	4.80	0.22	0.29
	5	3.82	3.00	0.13	0.73
	10	2.38	6.44	0.25	0.27
Nitro	2.5	6.15	8.76	0.30	0.51
	5	5.67	8.75	0.38	0.64
	10	1.83	7.26	0.79	0.21
Pyridine	2.5	2.52	10.31	0.84	0.25
	5	1.99	6.61	0.42	0.16
	10	1.18	21.70	1.15	0.28
1-10 Phenanthroline	2.5	4.55	23.31	0.97	0.59
	5	3.16	15.98	1.73	0.17
	10	2.16	6.09	0.10	0.31
Humic acid	2.5	2.33	12.03	0.42	0.74
	5	3.75	19.71	0.24	1.46
	10	1.18	6.35	0.23	1.19

If TF> 1, chelates interact with metals to form a metal-chelate pair. It provides faster transport of metal ions to the plant's aboveground organs (Ladislas et al., 2012). TF values of *B. napus* and *C. quinoa* species were generally less than 1. TF values for *B. napus* were higher than 1 in 10 mmol kg^{-1} dose of pyridine and 5 mmol kg^{-1} 1-10 phenanthroline. TF values of *C. quinoa* were found higher than 1 in 5 and 10 mmol kg^{-1} humic acid applications. As a result, we put forward the contributions of different chelate applications in selecting plants to be used in phytoremediation studies. Also, determining the underground and aboveground parts of the selected plants' accumulator capacity gives an idea about how environments can be used.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

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Plants used in complementary medicine in the treatment of respiratory tract diseases in Türkiye

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Türkiye'de solunum yolu hastalıklarının tedavisinde tamamlayıcı tıp uygulamalarında kullanılan bitkiler

Abstract: In this study, the plants used in complementary medicine for the treatment of respiratory tract diseases in Turkey have been researched. The study was carried out in two stages: literature study and fieldwork. As a result of the study, it was determined that 73 plant species have been used as complementary medicine in the treatment of respiratory tract diseases. The genera which have the most species are *Salvia* L., *Thymus* L., *Origanum* L., *Alcea* L., *Datura* L., *Lavandula* L., *Malva* L., *Pinus* L. and *Verbascum* L. The rest of the genera are represented with one species. The most commonly used plant species are *Salvia* sp., *Thymus* sp., *Tilia* L. sp., *Mentha* L. sp., *Lavandula* sp., *Origanum* sp., *Thymbra spicata* L., *Mrytus communis* L., *Rosmarinus officinalis* L. Bioactive compounds found in many of these plants; carvacrol, alkaloids, saponins, steroids, tannins, glycosides, anthocyanins, terpenes and other secondary metabolites. According to the results of the literature and field studies it has been determined that these plants are used effectively within the scope of complementary medicine against asthma, bronchitis, chronic cough, influenza, flu, sinusitis, pharyngitis, shortness of breath and tuberculosis. Most of these plants are used in the treatment of all respiratory tract infections without distinguishing the type of infection among the public. As a result; it was determined that 73 plant species researched in this study have the potential to be used in the treatment of respiratory tract diseases. With further studies, there are possibilities to develop phytochemicals for use in the treatment of respiratory tract diseases from these plants.

Key words: complementary medicine, respiratory tract diseases, asthma and bronchitis, cough, tuberculosis

Özet: Bu çalışmada Türkiye'de solunum yolu enfeksiyonlarının tedavisinde tamamlayıcı tıp uygulamalarında kullanılan bitkiler araştırılmıştır. Araştırma literatür çalışması ve halk arasında yapılan saha çalışmaları olmak üzere iki aşamalı olarak gerçekleştirilmiştir. Yapılan çalışma sonunda 73 bitki türünün solunum yolu enfeksiyonlarının tedavisinde tamamlayıcı tıp uygulamalarında kullanıldığı belirlenmiştir. En fazla tür içeren cinsler şunlardır; *Salvia* L., *Thymus* L., *Origanum* L., *Alcea* L., *Datura* L., *Lavandula* L., *Malva* L., *Pinus* L. and *Verbascum* L. Diğer cinsler ise birer türle temsil edilmektedir. En yaygın kullanılan bitki türleri; *Salvia sp.*, *Thymus* sp., *Tilia* L. sp., *Mentha* L. sp., *Lavandula* sp., *Origanum* sp., *Thymbra spicata* L., *Mrytus communis* L., *Rosmarinus officinalis* L. Bu bitkilerin çoğunda bulunan biyoaktif bileşikler; karvakrol, alkaloitler, saponinler, steroitler, tanenler, glikozitler, antosiyaninler, terpenler ve diğer sekonder metabolitler. Yapılan literatür ve saha çalışmaları sonuçlarına göre bu bitkiler; astım, bronşit, kronik öksürük başta olmak üzere, grip, nezle, sinüzit, farenjit, nefes darlığı ve tüberküloza karşı tamamlayıcı tıp kapsamında etkili bir şekilde kullanıldığı belirlenmiştir. Ancak belirlenen bu bitkilerin büyük bir çoğunluğu halk arasında enfeksiyon tipi ayırt edilmeden tüm solunum yolu enfeksiyonlarının tedavisinde kullanılmaktadır. Sonuç olarak; bu çalışmada incelenen 73 bitki türünün solunum yolu enfeksiyonlarının tedavisinde kullanılabilme potansiyelinin olduğu belirlenmiştir. Yapılacak ayrıntılı çalışmalarla bu bitkilerden solunum yolu enfeksiyonlarının tedavisinde kullanılmaya uygun fotokimyasalların geliştirilme olanakları bulunmaktadır.

Anahtar Kelimeler: tamamlayıcı tıp, solunum yolu enfeksiyonları, astım ve bronşit, öksürük, tüberküloz

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1. Introduction

Respiratory tract infections are generally grouped under two main headings: upper (Pharyngitis, Laryngitis, Flu, Sinusitis) and lower (Asthma, Bronchitis, COPD, Tuberculosis, Pneumonia) respiratory tract infections. However, clinical findings of upper respiratory tract infections are very similar. For this reason, it has been reported that defining local infections above the larynx as upper respiratory tract infections will be more practical in terms of clinical approach (Akan, 2012).

Viruses (RSV, rhinovirus, parainfluenza, influenza A, B, adenovirus, coronaviruses) are causative agents in 80-90% of acute respiratory infections; *Streptococcus pneumoniae* (Klein 1884) Chester 1901, *Haemophilus influenzae* (Lehmann and Neumann 1896) Winslow et al. 1917, *Mycoplasma pneumonia* Somerson et al. 1963 and *Bordetella pertussis* (Bergey et al. 1923) Moreno-López 1952 are the most common agents among bacteria (Akşit, 2002; Mufson, 2000). Respiratory tract diseases are one of the most common diseases in childhood. Acute respiratory tract infections are shown as one of the most important causes of mortality and morbidity for children (Aydın et al., 2015). According to the 2018 Türkiye Statistical Institute data, respiratory tract diseases in Türkiye ranks 3rd leading cause of death (Anonymous, 2020). According to the data of the World Health Organization (WHO), respiratory

infections are responsible for 13% of the causes of death in children under the age of 5 (Aydın et al., 2015).

According to data from 2017, it has been reported that respiratory tract diseases are the third leading cause of death after cardiovascular diseases and neoplasms worldwide (Soriano et al., 2020). In addition, respiratory infections cause significant loss of workforce and lessons for students. It is reported that an adult catches cold on average 2 times a year and a child who goes to kindergarten 6-7 times (Akan et al., 2012). With this feature, it is seen that respiratory diseases have a great impact on human life as they affect the quality of life and cause loss of workforce.

The inability to fully treat some respiratory diseases, the desire for rapid recovery of the patients or the economic inadequacies, direct people to use traditional and complementary medicine methods. According to Türkiye Traditional and Complementary Medicine regulations using herbs under complementary medicine is one of the 15 accepted methods (Resmi Gazete, 2014). Scientifically unproven herbal treatment may have negative consequences such as side effects and drug interactions that may seriously affect human health. Although it is known, the tendency towards traditional and complementary medicine practices is gradually increasing. According to the World Health Organization, complementary medicine practices are used at a rate of 80% in Africa, 70% in Canada, 48% in Australia, 42% in America, 38% in Belgium, and 49% in France (Hazır and Bozkurt, 2020).

There are about 12.000 plant species and subspecies in Türkiye; it is known that about 500 of these plants are used in traditional and complementary medicine. In Türkiye, 447 varieties of medicinal and aromatic plants are traded, 139 of them exported abroad (Özgüven et al., 2005; Öztürk et al., 2017). In Türkiye, local people to collect plants from nature traditionally and continue to use them in the treatment of various diseases from past to present. Also, herbalists sell these plants, which are permitted to be sold in spice-sellers and pharmacies. Ethnobotanical and clinical studies conducted in recent years show that herbal therapies have an important place among Complementary and Alternative Medicine methods in respiratory tract infections (Yücel and Yücel, 2020).

In this study, it is aimed to determine the plants used in traditional and complementary medicine in the treatment of respiratory tract diseases in Türkiye.

2. Material and Method

This study was carried out in two stages in line with the determined objectives. Firstly all the resources which might be relevant, especially ethnobotanical studies in Türkiye, pharmacopeia, and monographs were examined (Ağca, 2011; Aksu, 2011). In the next stage, face-to-face interviews with local people were made with field studies. As a result of the studies, plants used in Türkiye in traditional and complementary medicine against respiratory tract diseases were determined, made a list, and are given as a table.

3. Results

As a result of the literature and field studies, 73 plant varieties used within the scope of traditional and complementary medicine practices were determined (Fig. 1). The names of the plants, their bioactive compounds, and their effects on respiratory tract infections are given in Table 1.

Among the plants determined in this study, the genera containing the most species are; *Salvia* L. (5.48%), *Thymus* L. (5.48%), *Alcea* L. and *Origanum* L. (4.11%); *Datura* L., *Lavandula* L., *Malva* L., *Mentha* L., *Pinus* L. and *Verbascum* L. (2.74%) (Fig. 1). Other genera are represented by only one species (1.37%). The most commonly used plant species are; *Salvia* L. sp., *Thymus* sp., *Tilia* L. sp., *Mentha* sp, *Lavandula* sp., *Origanum* sp., *Thymbra spicata* L., *Mrytus communis* L., *Rosmarinus officinalis* L.

Table 1. Plants used in complementary and alternative medicine applications in the treatment of respiratory tract diseases in Türkiye

Names of the Plants	Bioactive Compounds	Effects on Respiratory Tract Infections	References
Abies nordmanniana (Steven) Spach	Resin acids, monosaccharides, Alcohols, lignans, glycerol, gallic acid, sucrose, catechin isomers, gallocatechin isomers, etc.	Asthma and bronchitis prevention	Ermiş (1997), Yücel (2014)
Alcea officinalis L.	Tannin, alanine, arginine, lysine, asparagine, coumarin, altein, mucilage, quercetin, kaempferol, pectin etc.	Asthma and bronchitis prevention	Rotblatt (2000), Yücel (2008), Shah et al. (2011), Sağlam (2011)
Alcea pallida Waltdst. & Kit- *Alcea rosea* L.	Starch, sucrose, galactose, pectin, oil, tannin, asparagine, mucilage, etc.	Against cold and bronchitis	Yücel (2014), Şimşek et al. (2002), Altay et al. (2015), Ermiş (1997)
Allium schoenoprasum L.	Cysteine, alkanyl, anthocyanin-flavonol 3 " - acetate etc.	Against cold and flu	Yücel (2014)
Ammi visnaga L.	Resin, fixed oil, visnagin, meladinin, kellin etc.	Asthma and cough reliever	Baytop (1999), Yücel (2008), Uz (2011), Kökçü et al. (2015)
Artemisia annua L.	Resin, artemisia ketone, camphor, 1.8-cineole etc.	Against tuberculosis and bronchitis	Yücel (2014), Altay et al. (2015)
Bellis perennis L.	Polyacetylene, tannin, saponin, flavonoid etc.	Against cough and upper respiratory tract disease	Yücel (2008), Melikoğlu et al. (2015)
Borago officinalis L.	Linoleic acid, oleic acid, palmitic acid, stearic acid, eicosenoic acid, erucic acid, gallic acid, oleuropein etc.	Preventing respiratory diseases	Yücel (2014), Al-Rubaye et al. (2017)

Bryonia cretica L.	Saponin, lectin, resin, tannin, choline, etc.	Against asthma, bronchitis, and cough	Yücel (2014)
Cannabis sativa L.	Cannabinoids, oil, lecithin, choline, globulen, resin, alkaloid etc.	Prevents dyspnea and cough	Yücel (2008), Altay et al. (2015)
Castanea sativa Mill.	Tannins; potentillin, pedunculagin, castalagin and vescalagin, quercetin, flavonoids, oil, pectin, sugar, vitamin C, β-sitosterol, glycoside etc.	Preventing asthma, bronchitis, and cough	Ermiş (1997), Yücel (2008), Sargın et al. (2013), Eminağaoğlu et al. (2017)
Cichorium intybusm L.	Mucilage, fructose, levulin, coumarin glycoside, tannin, inulin, pentozones etc.	Against asthma	Öztürk and Özçelik (1991), Yücel (2008), Melikoğlu et al. (2015)
Cotinus coggygria Scop.	Tannin, flavone derivatives, gallic acid, quercetin, penta-O-galloyl-β-d-glucose, butine, taxsipholin, catechin fustine, sulfuretin etc.	Preventing cold, sinusitis, bronchitis	Yücel (2014), Melikoğlu et al. (2015)
Crataegus monogyna Jacq.	Amine, tannin, rutin, hyperoside, vitamin C, vitex, triterpene, flavone etc.	Preventing asthma	Yücel (2008), Şöhretoğlu (2011), Şengün and Yücel (2015)
Crocus pallasii Goldb.	Organic acids, sugars, crocin etc.	Preventing asthma, bronchitis, and cough	Zengin et al. (2020), Yücel (2014)
Datura inoxia Mill.	Atropine, scopolamine, saponins, flavonoids, cardiac glycosides, phenols etc.	Preventing asthma	Yücel (2014), Melikoğlu et al. (2015)
Datura stramonium L.	Atropine, saponins, tannins, alkaloids, glycosides etc.	Asthma treatment	Yücel (2014), Melikoğlu et al. (2015)
Descurainia sophia (L.) Webb ex Prantl	Bromoxynil, thifensulfuron methyl, clopyralid etc.	Against chronic cough and asthma	Yücel (2014)
Ecballium elaterium (L.) A. Rich.	Cucurbitacin, elaterin, carbohydrate, saponin, etheric oil, flavonoid, etc.	Preventing sinusitis	Yücel ve Tülükoğlu (2000), Yücel (2014)
Echinacea purpurea (L.) Moench	Elaterin, chlorogenic and caffeic acid, xyloglucan, alkaloids, amino acids and derivatives, etc.	Flu and common cold	Yale and Liu (2004), Sharma et al. (2006), Yücel (2014)
Echinophora tenuifolia L. *ssp. sibthorpiana* (Guss,) Tutin	Methyl eugenol, d-3-carene, p-cymene, α-phellandren etc.	Preventing cold, flu, and respiratory diseases	Chalchat et al. (2011), Gökbulut et al. (2013), Şengün and Öztürk, 2018 (2018)
Ephedra majör Host	Tannin, ephedrine, norephedrine, etc.	Against asthma	Asımgil (1997), Yücel (2008)
Glycyrrhiza glabra L.	Triterpenic saponins, sterol, glycosides, coumarin, linolenic acid, coumarins, tryptamine, indolo, pyrazine, pyrrolidine, salicylic acid, asparagine, betaine, chelite, glycyrrhizin, isoflavones, steroids, lecithin, protein etc.	Preventing cold, flu, dyspnea, cough; expectorant	Aksu (2011), Yücel (2014), Sargın et al. (2013)
Inula helenium L.	Helenin, isochostic acid, tomentozine, inhibitoroz, 1, 2-longidinone, iso-velleral, methyl ester, quercetin, chiapin B, inulin, etc.	Cough and upper respiratory tract diseases treatment	Yücel (2014)
Lavandula angustifolia Mill.	α and β-pinene, sabinen, o-cimene, limonene, eucalyptol, beta cis ocimene, α-terpinene, p-mentha-1,4 (8) diene, linalool, camphor, erpinen-4-ol, α-terpinol, linalyl anthralinate, geraniol acetate, B-caryophilene, B-bergamotene, B-farnesen, etc.	Preventing cough, bronchitis, asthma, respiratory diseases	Baytop (1999), Yücel (2008), Eröztürk (2002)
Lavandula x intermedia Emeric ex Loisel.	linalool, linalyl acetate, 1,8-cineole, terpinen-4-ol Lavandulyl acetate, borneol α-pinene, camphene, β-pinene, myrcene, α-phellandrene, limonene, 1,8-cineole, (Z)-β-ocimene,γ-terpinene trans-β-ocimene, terpinolene, o-cymene	Preventing cough, bronchitis, asthma, respiratory diseases	Baytop (1999), Yücel (2014), Eröztürk (2002)
Leonurus cardiaca L.	Flavonoids, terpenes, chlorogenic acid, orientein, quercetin, hyperoside rutin etc.	Against asthma	Yücel (2014)
Liquidambar orientalis Mill.	Cinnamic acid, styracin, styrol, styrone, storesinol, storegenin, terpinen-4-ol, α- terpinol, sabinene, and γ-terpinene, styrene, α-pinene, camphenene, β-pinene, cinnamyl alcohol, limonene, acetophenone, petylphenol, 3-phenyl propanol etc.	Against asthma	Asımgil (1997), Yücel (2008), Melikoğlu et al. (2015)
Malva neglecta Wallr.	Anthocyanidin compounds such as mucilage, malvidin, delphinidin and malvin, tannins, mucilage, flavones, protein etc.	Anti-pharyngitis	Polat et al. (2015), Korkmaz and Karakurt (2015), Güner and Selvi (2016)

Malva sylvestris L.	Mucilage, pectin, malvin, malvidin, glycoside, flavonol, tannin, anthocyanin; pentadecenoic, palmitic, stearic, oleic, linoleic, linolenic, arachidic, arachidonic, behenic, lignoceric and nervonic acid etc.	Cough suppressant, against asthma	Asımgil (1997), Yücel (2008), Melikoğlu et al. (2015)
Marrubium vulgare L.	2-hexenal, α-phellandrene, α-pinene, β-phellandrene, β-pinene, m-myrene, α-limonene, sabinen, caryophyllene, β-farnesene, marubin, tannin etc.	Preventing cough, bronchitis and asthma	Yücel (2012)
Matricaria recutita (L.) Rauschert	Chamazulene, 1,8-cineole, β-pinene, α-pinene, α-bisabolol, farnacene, bisabolon, terpinene, apigenin, apigetrin, apiin, luteolin, quercetin, quercitrin, quercimetrin, chrysoeryol, umbelliferon, heniarin etc.	Preventing cough, bronchitis and asthma	Şimşek et al., (2002), Yücel (2012), Melikoğlu et al. (2015)
Mentha longifolia (L.) Huds.	Mentton, 1.8 cineole, terpineol-4, menthol, pulegone, piperitone, germakrene, caryophyllene, thymol, α-humulene, α-longipinen etc.	Against cough, bronchitis, cold, dyspnea	Güneş and Özhatay (2011), Yücel (2012), Melikoğlu et al. (2015)
Mentha x piperita L.	Menttone, menthyl acetate, neomenthol, isomenthone, menthofuran, limonene, pulegon, α-pinene, β-pinene, resin, carvone, pinene, tripertene, menthyl acetate, tannin etc.	Against cough, cold, dyspnea	Ağca (2011), Yücel (2014)
Mrytus communis L.	Myrtenyl acetate, 1,8-cineol, α-pinene, linalool, limonene, linalyl acetate, geranyl acetate, α-terpineol etc.	Against asthma, bronchitis, sinusitis, cough	Yücel (2014), Melikoğlu et al. (2015)
Ocinum basilicum L.	Linalol, epi-α-cadinol, a-bergamoten, γ-cadinene, estragole, 1,8-cineol, methylchavikol, methylcinnamate, linolene, rosmarinic acid, citral, eugenol, geraniol, pinene etc.	Cough suppressant	Eröztürk (2002), Yücel (2008), Korkmaz and Karakurt (2015)
Orchis masculata (L.) L.	E-ocimen, limonene, 3-hexenyl acetate, linalool, β-Terpineol, a-pinene, sabinene, limonene, carvone, p-caryophylene, p-farnesene, myrcene, glucose, glucomannan, starch, mucilage etc.	Cough and bronchitis preventive	Yücel (2014)
Origanum majorana L.	Terpinen-4-ol, cis-sabinen hydrate, p-cymene, γ-terpinene, linalool, carvacrol, glycoside etc.	Cough and bronchitis preventive	Yücel (2012), Polat et al. (2015)
Origanum sipyleum L.	γ-terpinene, p-cymene, thymol methyl ether, carvacrol methyl ether, thymol, carvacrol etc.	Cough suppressant, respiratory tract diseases preventive	Yücel (2014)
Origanum vulgare L.	Carvacrol, terpinen-4-ol, linalol, sabinen, α-terpinen, γ-terpinen thymol, cymen, etc.	Cough and bronchitis preventive	Polat et al. (2015), Eminağaoğlu et al. (2017)
Papaver rhoeas L.	p-hydroxybenzoic acid, protocatechuic acid, ethanol, kaempferol, kerempetol, quercetin, luteolin, flavonoids, astragalin, hyperoside, glycosides, isoquercitrin etc.	Cough suppressant, bronchitis preventive	Yücel and Tülükoğlu (2000), Yücel (2014), Melikoğlu et al. (2015)
Petasites hybridus (L.) Gaertn., Mey. & Scherb.	Sesquiterpenes, alkaloids, petasin, petasol, hydroxylase, buteonate, isopetasin etc.	Cough, bronchitis and cold preventive	Yücel (2014)
Phlomis fruticosa L.	β-caryophylene, (E) -methyl isoeugenol, α-asaron, germakrene D, γ-bisabolen, α-pinene etc.	Respiratory tract diseases preventive	Yücel (2014)
Phragmites australis (Cav.) Trin. ex Steud.	Carbohydrates, glycosides, protein, asparagine, vanillic acid, hydroxy benzaldehyde, Mg, K, manganese etc.	Bronchitis preventive	Yücel (2008), Uysal et al. (2010)
Physalis peruviana L.	Carotene, polyphenols, protein, lipids, inoleic acid, oleic acid, palmitic acid, palmitoleic acid, gadoleic, erucic, lignoceric, nervonic acid, papain, Fe, Mg, Zn; Vitamin A, C, B1, B2, B6, B12 etc.	Asthma preventive	Yücel (2014)
Pinus nigra Arnold *subsp. pallasiana* (Lamb.) Holmboe	Resin, fixed oil, linoleic, oleic, anteiso, pinolenic and coniferonic acid, protein, cellulose, phenols etc.	Tuberculosis and respiratory diseases preventive	Baytop (1999), Yücel (2008), Melikoğlu et al. (2015)
Pinus sylvestris L.	Resin, fixed oil, syadonic, sciadonic acid, limonene, protein, cellulose, essential oil, etheric oil, mineral salt, vitamin B-C-K, tannin, etc.	Against cold and dyspnea	Yücel (2008), Sağıroğlu et al. (2012), Korkmaz and Karakurt (2015)
Pistacia terebinthus L.	Resin, α-pinene, β-pinene, limonene, terpinen-4-ol, α-terpineol, essential and fixed oils etc.	Dyspnea, asthma, bronchitis, cough preventive	Yücel (2014), Melikoğlu et al. (2015)
Plantago lanceolata L.	Flavonoids, hydroxycinnamic acids, carboxylic acid, coumarin, aucubin and iridoid glycoside, mucilage, saponin, alkaloid, K, Zn, tannin and silicic acid etc.	Against bronchitis, cold, sinusitis and asthma	Ertuğ (2004), Yücel (2014), Güner, and Selvi, 2016
Rosa cannina L.	Ascorbic acid, flavonoids, dimethyl sulfide, protein, tannin, Na, K, P, Mn, Mg, vitamin C, A, B, flavone, malic acid, pectin, citronellol etc.	Cough and cold preventive	Eröztürk (2002), Şimşek et al. (2002), Yücel et al. (2019)
Rosmarinus officinalis L.	Rosmarin, phenolic diterpenes, carotenoid, p-cymene, linalool, γ-terpinene, thymol, beta-pinene, α-pinene, 1,8-cineol, camphor, verbenone, borneol, etc.	Asthma and respiratory tract diseases preventive	Şimşek et al. (2002), Yücel (2008)

Salvia cadmica Boiss.	Tannin, alkaloids, phenols, tannins, glycosides, steroids, 1, 2 cyclohexanediol, salicyl alcohol, stearic acid, linolenic acid, galactose 4,6-0-nonylidene, thion, krypton, 1.8-cineol, borneol etc.	Respiratory tract diseases preventive	Şimşek et al. (2002), Koyuncu et al. (2010), Yücel (2014)
Salvia fruticosa L.	α-pinene, campene, β-pinene, 1,8-cineol, γ-terpinene, cis-tujone, trans-thujone, camphor, terpinen-4-ol, trans- (E) -caryophylene, aromadendrene, α- humulen etc.	Tuberculosis prevention	Yücel (2014), Ertuğ (2004), Koyuncu,et al. (2010)
Salvia officinalis L.	Thujon, viridiflorol, caryophyllene, apigein, polysaccharide, 1,8-cineol, linalool, borneol, salven, pinene, camphor, vitamin C and A, phytonzides; triterpenoids ursolic, oleanolic acid etc.	Respiratory tract diseases preventive	Ceylan (1996), Baytop (1999), Yücel (2008)
Salvia sclarea L.	α-thujene, α and β-pinene, camphene, myrcene, α-hexandrene, α-terpinene, p-cymene, limonene, sabinene, linalool oxide, fenchone, terpinolene, linalol, camphor, borneol, myrtenol, piperitone, β-caryophyllene , sclareol etc.	Respiratory tract diseases preventive	Ceylan (1996), Koyuncu et al. (2010), Korkmaz and Karakurt (2015)
Santolina chamaecyparissus L.	1,8-cineol and β-eudesmol, ketone, camphor, beta-phellandren etc.	Asthma, bronchitis, tuberculosis preventive	Yücel (2008, 2014)
Sedum acre L.	Edacrine, flavonol, glycoside, cedinine sedacryptine, n-methyl anabasine, thujone, borneol, 1,8-cineol, camphor, salvin, tannin, fumaric acid, borneol acid, malic acid, oxalic acid, sedamine, sedridine, cedinone, nicotine etc.	Stimulating the respiratory center	Yücel (2008, 2014)
Sinapis arvensis L.	Erythritol, Nitro-2-propanol, Furfural, Cyclopentanemethylamine, 1-Butene, 4-isothiocyanato, difluorobenzene, Eicosanoic acid, phenylmethyl ester, trigonelline, estragole, pentanenitrile, eugenol, maltose, monoacetate, tributyl acetylcitrate, docosenoic acid, phthalic acid, γ-tocopherol, tetraacetate, campesterol , γ-sitosterol	Preventing asthma and bronchitis	Yücel (2014)
Solanum nigrum L.	Gentic acid, luteolin, apigenin, kaempferol, m-coumaric acid, anthocyanidin, glycoalkaloid, tannin, saponin, linoleic acid, palmitic acid, solanine etc.	Preventing bronchitis and cough	Ermiş (1997), Yücel (2008)
Teucrium polium L.	α- β-pinene, p-cymene, germakrene D, carvacrol, α-copaene, spathulenol, α-tugen, sabinene, β-mirre, limonene, benzene, linalol, caryophyllene, α-carmine etc.	Preventing sinusitis	Yücel (2014), Altay et al. (2015)
Thymbra spicata L.	Carvacrol, gamma-terpinene, p-cymene, thymol, terpinene, borneol etc.	Preventing respiratory tract diseases	Yücel (2008), Sargın et al. (2013)
Thymus citriodorus (Pers.) Schreb.	Borneol, thymol, 3,7-dimethyl-1,6-octadien-3-ol, cyclohexene, terpene, camphor etc.	Against cough, bronchitis, cold, respiratory diseases	Koyuncu,et al. (2010), Altay et al. (2015)
Thymus leucostomus Hausskn. et Velen var. *argillaceus* Jalas	Flavonoid, linalool, resin, tannin, thymol and carvacrol	Against cough, bronchitis, cold, respiratory diseases	Polat et al. (2015), Yücel et al. (2011)
Thymus longicaulis C. Presl	Limonene, thymol, geraniol, geranyl acetate, linalool, α-terpinyl acetate etc.	Against cough, bronchitis, cold, respiratory diseases	Yücel (2008), Altay et al. (2015)
Thymus vulgaris L.	Camphor, α-β-pinene, 1.8-cineol, borneol, carvacrol, linalool, geraniol, borneol, p-cymene, γ-terpinene, thymol etc.	Against cough, bronchitis, cold, respiratory diseases	Yücel (2014), Altay et al. (2015), Melikoğlu et al. (2015)
Tilia rubra DC.	Flavonoid, glycoside, quercetin, quercitrin, apigenin, luteolin, hexadecanoic acid, 2-phenethyl benzoate, beta-ionone, geranyl acetone, farnesyl acetone and hexahydrofarnesyl acetone etc.	Respiratory diseases preventive, cough suppressant	Asımgil 1997, Yücel (2008), Polat et al. (2015)
Tussilago farfara L.	Kaempferol, glycopyranoside, arabinopyranoside, glycopyranoside, 3-O-α-rhamnopyranosyl (1-6) -β-glycopyranoside, quercetin, rhamnopyranosyl (1-6) -β-glycopyranoside, inulin, phytosterol etc.	Against respiratory diseases, cough, bronchitis and asthma	Yücel (2014), Kökçü et al. (2015)
Urtica dioica L.	Phenolic acids, isoquercitrin, kaempferol, vitamin A-B2-C-K1, histamine, folic acid, lecithin, lignan, polyphenol, sterol, polysaccharide secretin etc.	Asthma preventive	Şimşek et al. (2002), Yücel (2008), Sağıroğlu et al. (2012)
Verbascum bombyciferum Boiss.	Saponins, iridoid, phenylethanoid glycosides, flavonoids, vitamin C, minerals etc.	Bronchitis, tuberculosis prevention	Yücel (2014)
Verbascum olympicum Boiss.	Saponins, iridoid, phenylethanoid glycosides, flavonoids, minerals, mucilage etc.	Asthma ang cough prevention	Yücel (2014), Melikoğlu et al. (2015)
Vitex agnus-castus L.	Flavonoids (casticin, isovitexin, orientin), iridoids (aucubin, agnuside, eurostide), volatile oils, linoleic acid, diterpene, kineol, glycoside, resin etc.	Dyspnea preventive	Daniele et al. (2005), Yücel (2014)
Zingiber officinale Roscoe.	Resin, polyphenols, β carotene, flavonoids, tannins, Fe, Ca, P, Zn, Cu, Cr, Mg, vitamin C, lactone, mucilage, starch, oleoresin, sesquiterpene, etc.	Preventing cold and flu	Yücel (2014), Altay et al. (2015), Karahan and İlçim (2017)

The most commonly used herbs in traditional and complementary medicine against respiratory tract infections are: *Alcea officinalis*, *Lavandula x intermedia*, *Matricaria recutita*, *Mentha x piperita*, *Origanum vulgare*, *Rosmarinus officinalis*, *Salvia officinalis*, *Thymbra spicata*, *Thymus leucostomus*, *Tilia rubra*, *Zingiber officinale*. However, some plants such as *Artemisia annua* L., *Pinus nigra* subsp. *pallasiana*, *Salvia fruticosa*, *Verbascum bombyciferum* have more specific uses such as tuberculosis.

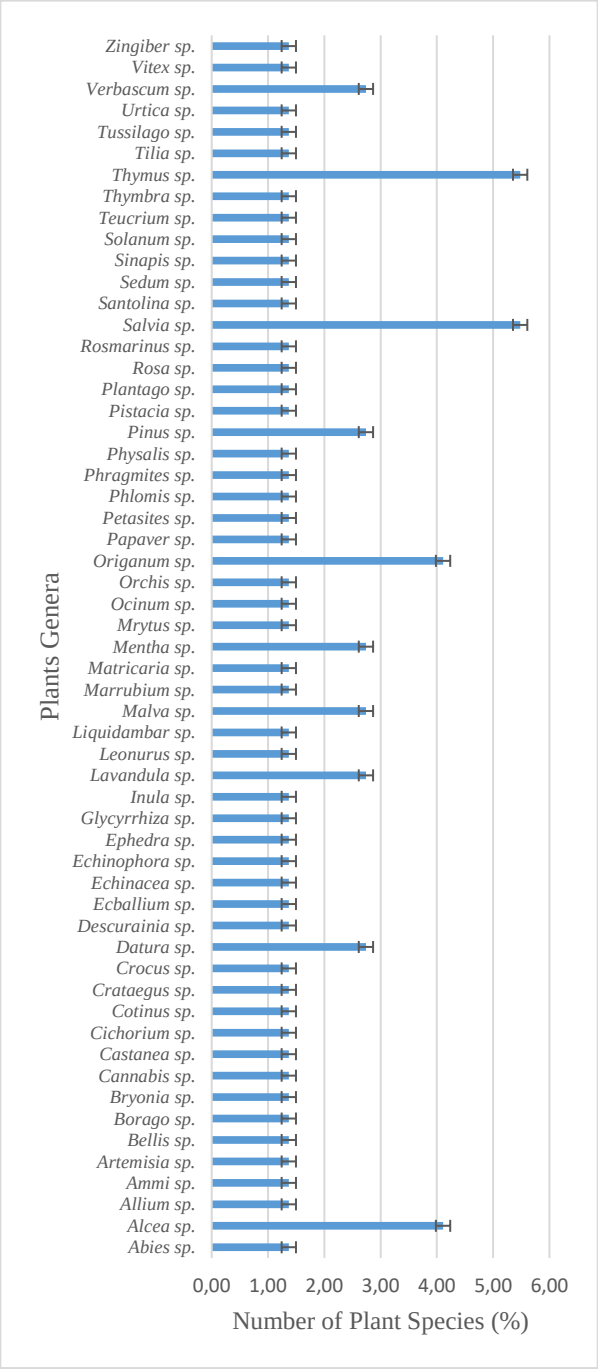

Figure 1. Distribution of plants used in respiratory diseases within the scope of complementary medicine by plant species (%)

In addition to the use of plants one by one, mixtures of various plants are also used. However, although not directly related, some plants such as *Rosa canina* and *Citrus* x *limon* are used in the treatment of respiratory diseases because they are rich in vitamin C.

When examined as a reason for use; These plants are mostly used as cough (22.32%) suppressants, followed by bronchitis (19.64%), asthma (16.96%), cold (12.50%), respiratory tract (12.50%), dyspnea (6.25%), tuberculosis (4.46%), sinusitis (2.68%), flu (1.79%) and expectorant (0.89%) (Fig. 2). Symptoms of respiratory tract diseases are close to each other and the public's lack of knowledge on this issue makes it difficult to define plants by disease type (Table 1). However, specific use is also seen against some diseases such as tuberculosis.

When these plants are examined in terms of traditional usage; *Salvia* sp., *Thymus* sp., *Tilia* sp. as in its types, it is mostly in the form of "herbal teas". In general, the use of dried plant parts is more common. Also, essential oils are used diluted, such as *Lavandula* x *intermedia*. Besides, it is generally used in the form of syrup, or paste prepared by mixing with honey or sugar.

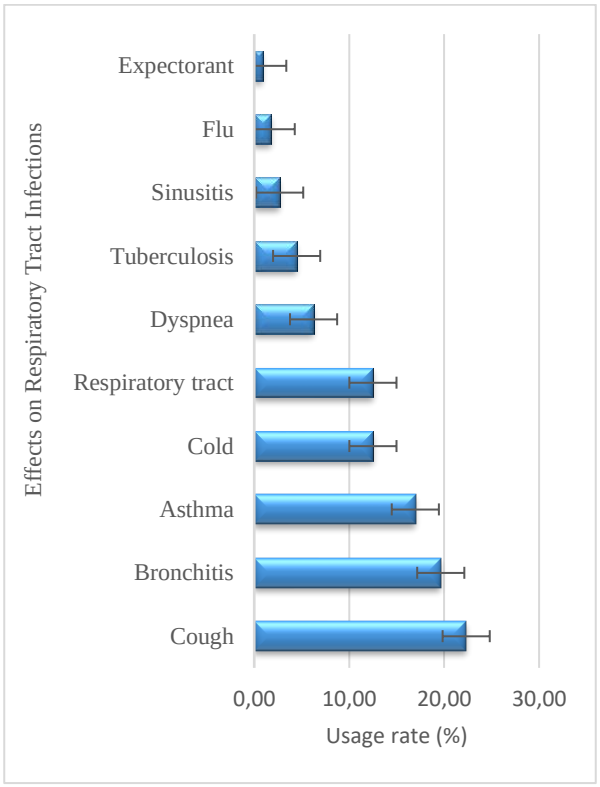

Figure 2. Distribution of plants used in complementary medicine by respiratory tract disease types (%)

The 73 plant species examined in this study contain a large number of secondary metabolites (Table 1). The most common bioactive substances in these plants; tannin (16.79%), terpinen (14.6%), flavonoid (13.87%), glycoside (11.68%) and resin (10.22%) (Fig. 3). These are followed by limonene (9.49%), linalool (8.03%), α-pinene (8.03%), β-pinene (8.03%), borneol (7.3%), camphor (7.3%), mucilage (7.3%), saponin (7.3%), thymol (7.3%), cymene (6.57%), quercetin (6.57%), vitamin c (6.57%), carvacrol (5.84%), p-cymene (5.84%), sabinen (5.84%), 1.8-cineole (5,11%), alkaloid (5.11%), protein (5,11%), coumarin (3.65%), kaempferol (3.65%) and pectin (3.65%), respectively. In addition, there are many bioactive substances such as alkaloids, eugenol, geraniol, inoleic acid, iridoid, luteolin, magnesium, vitamin a, b, asparagine, caryophylene, gallic acid, hyperoside, linoleic acid, myrcene, palmitic acid and others which are found in less

than four percent of the examined plants. The general common feature of these bioactive compounds is their high antimicrobial effect.

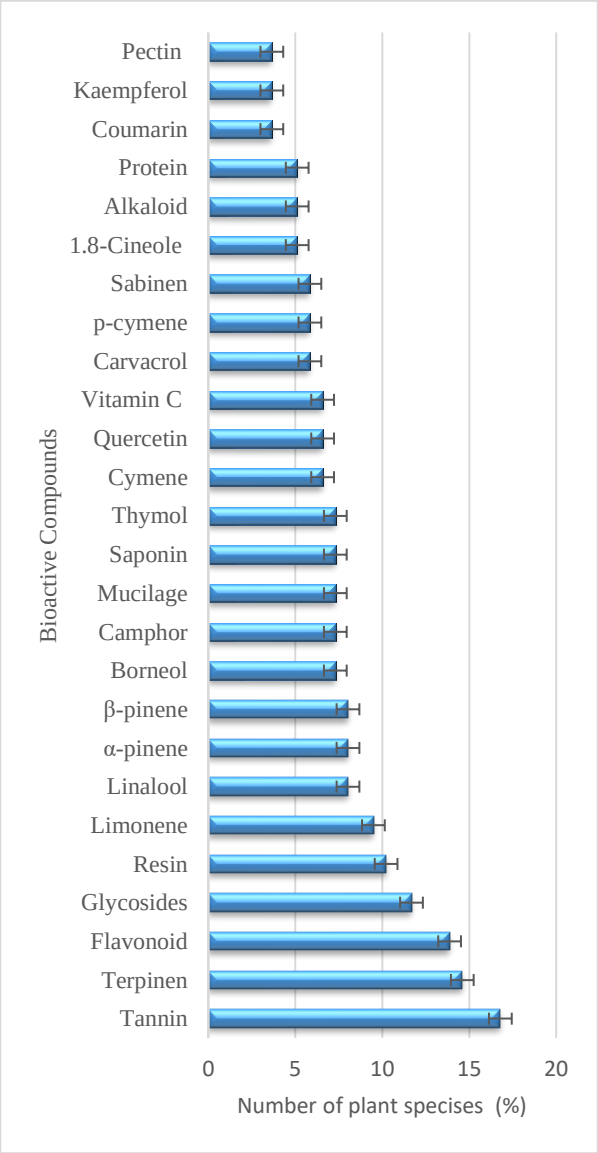

Figure 3. Number of plants containing bioactive substances (for 5 or more plant species)

4. Discussions

Traditional and complementary medicine practices are accepted as an important part of the global health system by World Health Foundation (Biçer and Balçık, 2019). World Health Foundation stated the targets about Traditional Health Strategy at 2002-2005 report, then at 2014-2023 Traditional Health Strategy Report they stated the directions and actions about it. At these reports, it is emphasized that if the traditional and complementary medicine used properly and safely, it can contribute to the modern medicine as well.

The usage of the plants at traditional and complementary medicine practices against the respiratory tract disease can have some important risks. For example, it is stated that the usage of *Echinacea purpurea* at diseases like flu and cold can cause nausea, vomit and effect the coagulation (Cupp, 2000); *Tussilago farfara* ve *Glycyrrhiza glabra* which is used at upper respiratory tract diseases can have side effects like hypertension, headache, arrhythmia and *Pinus silvestris* can cause irritation at skin and mucosa (Öztürk, 2010). Also it is known that some plants can interact with the medicines that are used at respiratory tract infections can interact. For example, *Matricaria recutita* can interact with anticoagulants and iron supplement medication., *Echinacea purpurea* can interact with anabolic steroids, amiodarone, methotrexate, ketoconazole, immunodepressants, corticosteroids and cyclosporin (Erdem and Eren, 2009). Therefore, offering the medicinal plants to consumers with decontaminating them from the risks, at certain standards and by doctors or experts will contribute to solve the problems.

As a result, 73 plants used in respiratory tract diseases were determined (This number is constantly changing with new studies being carried out every day). Some of these herbs are also featured in monographs and pharmacopeia (ESCOP, 1997; WHO, 2006, 2014, 2017; Anton et al., 2009; Demirezer, 2011). The use of herbal drugs in traditional and complementary medicine practices in respiratory tract diseases carries some important risks. Risks such as the lack of reliable scientific studies on this subject and the herb-drug interactions pose a major obstacle. The increasing use of herbal drugs against respiratory tract infections among the public since ancient times necessitates more and more detailed studies on this subject. The literature and field studies conducted during this study show that new bioactive phytochemicals can be obtained from plants to prevent respiratory diseases.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

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Molecular and morphological identification of *Cortinarius eucaeruleus* Rob. Henry (subgenus *Phlegmacium*) from Türkiye

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Türkiye'den *Cortinarius eucaeruleus* Rob. Henry (subgenus *Phlegmacium*)'un moleküler ve morfolojik belirlenmesi

Abstract: *Cortinarius eucaeruleus* is identified from central Black Sea region of Türkiye based on morphological characteristics and ribosomal DNA gene sequence analyses. This species was found on calcareous soil associated with *Quercus* in autumn season from Tokat province in Türkiye. It has distinctive morphological features such as strong and deep violaceous blue pileus, ellipsoid and densely verrucose spores. In addition to the morphological features, the ITS (internal transcribed spacer) and LSU (large subunit ribosomal) sequence analyses indicated that the studied specimen is *C. eucaeruleus* that is identified for the first time from Türkiye.

Key words: *Cortinariaceae*, molecular taxonomy, ITS, LSU, Turkiye

Özet: *Cortinarius eucaeruleus*, Türkiye'nin Orta Karadeniz bölgesinden morfolojik özellikler ve ribozomal DNA gen dizi analizlerine dayalı olarak tanımlanmıştır. Bu tür, Türkiye'de Tokat ilinden sonbahar mevsiminde *Quercus* ile ilişkili kalkerli topraklarda bulunmuştur. Güçlü ve derin morumsu mavi tüy, elipsoid ve yoğun verrukoz sporları gibi ayırt edici morfolojik özelliklere sahiptir. Morfolojik özelliklere ek olarak, ITS (internal transcribed spacer) ve LSU (large subunit) ribosomal dizi analizleri, incelenen örneğin Türkiye'den ilk kez teşhis edilen *C. eucaeruleus* olduğunu göstermiştir.

Anahtar Kelimeler: *Cortinariaceae*, moleküler taksonomi, ITS, LSU, Türkiye

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1. Introduction

Cortinarius (Pers.) Gray is the most diverse and species-rich genus of macrofungi and contains a complex taxonomic system including several subgenera, sections and other infrageneric taxa (Kirk et al., 2008). It is divided into five to nine subgenera based upon micromorphology (Moser and Horak, 1975). Among them, subgenus *Phlegmacium*, subgenus *Telamonia* and subgenus *Dermocybe* are the most diverse of the subgenera. In general, the macrofungi species in subg. *Phlegmacium* are ectomycorrhizal fungi associated with deciduous and coniferous trees and usually have a vivid colored basidiocarp (Garnica et al., 2003). They are non-hygrophanous and have a dry stipe and viscous or sticky pileus surface in moist conditions (Soop et al., 2019). This subgenus is divided into the sections and other infrageneric groups, primarily based on gill color, and then veil color, pileus color, odor and the color change that occurs with alkaline chemicals (Moser, 1983; Garnica et al., 2003). Some species in this subgenus are widely distributed, while others may show limited distribution due to host specificity, the climate and soil requirements (Garnica et al., 2003; Liimatainen et al., 2014; Soop, 2014).

More than 5000 records in *Cortinarius* genus have been published worldwide (Index fungorum, CABI Bioscience Databases). However, many species identified as *Cortinarius* have been shown to be synonyms (Moser and Horak, 1975; Moser, 1983; Breitenbach and Kränzlin, 2000; Kirk, 2011; Liimatainen et al., 2014; Brandrud et al., 2018; Soop et al., 2019). Thus, morphology-based taxonomic studies in this genus are yet to be informative and phylogenetic tools need to be undertaken for clarification within the genus. The phylogenetic relationship of the *Cortinarius* species is inferred from gene regions encoding nuclear ribosomal internal transcribed spacer (ITS), nuclear large subunit ribosomal DNA (nLSU), and the two largest subunits of RNA polymerase II (RPB1 and RPB2), and an increasing number of studies are being conducted to understand species delimitation and identification based on sequence analysis. Although some subgenera are supported as monophyletic, subg. *Phlegmacium* is shown to be polyphyletic (Høiland and Holst-Jensen, 2000; Peintner et al., 2004; Garnica et al., 2005).

Cortinarius eucaeruleus Rob. Henry is a macrofungi species grouped in the Subgenus *Phlegmacium*, section *Caerulescentes* Rob. Henry ex Moenne-Locc. & Reumaux, clade *Eucaerulei* (Soop et al., 2019). Section *Caerulescentes* develops especially in calcareous soils. The pileus can be blue, lilac-violet, silver-gray, blue-gray and wine-brown. Lamella are violaceous at first, then becoming violaceous gray. There is distinctly a margin bulb on the stem. Its flesh does not react with alkaline chemicals or gives a brownish color (Moser, 1983; Soop, 2014). The

clade *Eucaerulei* comprises species with medium-sized basidiomata that are pileocarpic with violaceous hues and found on calcareous soil with *Quercus*, *Pinus* and deciduous trees. This clade contains species such as *C. caerulescentium*, *C. perpallens*, *C. eucaeruleus* and *C. terpsichores* that are mostly distributed in Europe (Soop et al., 2019). *C. eucaeruleus* and *C. terpsichores* are the two species that are morphologically the most similar and could be synonymous (Tortelli, 2011; Ślusarczyk et al., 2015). However, sequence data separates them as two very closely related but morphologically different species (Soop et al., 2019).

In Türkiye, more than 130 *Cortinarius* species have been identified (Sesli et al., 2020). Here, we present the first report of *Cortinarius eucaeruleus* from Türkiye based on morphological, ecological and phylogenetical data. We provide sequence information for the two gene loci (nrITS and nrLSU) for better resolution of the sect. *Phlegmacium* species.

2. Material and Method

2.1. Collection and morphological analyses

The fresh basidiomata specimens belonging to the genus *Cortinarius* were collected from Yaylacık Mountain (Tokat) during field trips in autumn 2019. The specimens were photographed at their natural habitats and the macroscopic and ecological characteristics were recorded. They were wrapped in paper and placed in a box during laboratory transfer. A mature sample was selected to obtain a spore print of the sample. The samples were dried and the fungarium number was given. Microscopic studies were carried out on dry samples under a Nikon brand research microscope. Some chemicals (such as 5% KOH, NaOH, Congo red) were used to rehydrate and dye dry samples during the studies. Basidiospore measurements were done, where L_m is the average length, W_m is the average width, Q is the quotient of length/width, and Q_m is the average of all calculated Q values for all basidiospores measured. At least 30 measurements of basidiospores were made from a single basidioma in profile view and basidiospore shape was described according to Bas (1969). Authors of fungal names are cited according to the IndexFungorum (http://www.indexfungorum.org) and MycoBank (http://www.mycobank.org). The findings obtained as a result of all these studies were compared with the existing literature (Knudsen and Vesterholt, 2008; Soop, 2014; Ślusarczyk et al., 2015; Muñoz Sánchez, 2018; Tanchaud, 2020a,b) to identify the studied fungal samples. Dried mushroom samples were stored in the fungarium of Tokat Gaziosmanpaşa University, Department of Biology (GOPUF).

2.2. Molecular analyses

2.2.1. DNA extraction and PCR

Genomic DNA was isolated from about 20 mg of lamella materials of the sample using GeneMATRIX Plant & Fungi DNA purification kit (EURx, Poland) following manufacturer's protocol. Approximately 700 bp genomic sequence of the ITS1-5.8S-ITS2 region of the rDNA gene was amplified using primer pairs ITS4-ITS5 (White et al., 1990) and a 950 bp genomic sequence of the 28S LSU gene region was amplified using primer pairs LROR-LR5 (Vilgalys and Hester, 1990). Each gene amplification was performed in 30 µl volume mixture containing 3 µl 10X buffer, 3 µl dNTP mix, 3 µl degenerate primer pair (final concentration of 1 µM each), 0.3 µl Dream Taq DNA polymerase (Thermo), 10 µl gDNA and 7.7 µl sterile double distilled H_2O (ddH_2O). Sterile ddH_2O was also used for negative PCR control reactions instead of gDNA. PCR conditions for ITS amplification were set as follows: 5 min initial denaturation at 95 ºC followed by 40 cycles of denaturation at 95 ºC for 30 sec, annealing at 53 ºC for 30 sec and extension at 72 ºC for 1 min and a final extension for 10 min. The conditions for LSU amplification included the same program except that the annealing temperature was set to 48 ºC. Both PCR amplifications were verified by using 1% agarose gel electrophoresis. PCR products were sequenced from both ends using forward and reverse primers (BM Labosis Inc., Ankara).

2.2.2. Sequence analysis and phylogenetics

Sequences in both directions were checked for sequencing errors and an assembled sequence for both rDNA genomic regions was generated for further analysis. Basic Local Alignment Search Tool (BLAST) program from the National Center for Biotechnology Information (NCBI) nucleotide database was used for homology searches. Best matches from BLAST results of ITS and LSU analyses were retrieved from GenBank for phylogenetic analysis. Multiple sequence alignments were conducted using Clustal W (Larkin et al., 2007). Phylogenetic trees for each genomic region were generated using Molecular Evolutionary Genetics Analysis software (MEGA 7.0; Kumar et al., 2016). Phylogenetic analyses were inferred using the maximum likelihood (ML) and maximum parsimony (MP) methods. ML method was based on Tamura-Nei model (Tamura and Nei, 1993) with bootstrap support of 1000 replicates. Initial tree(s) for the heuristic search were automatically obtained by using Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and topology with superior log likelihood value was selected. MP trees were constructed using the Tree-Bisection-Reconnection (TBR) search method with 100 random addition replications. The bootstrap support values > 50% were marked on the branches of the tree. *Hebeloma fastibile* (Pers.) P. Kumm. was selected as an outgroup species.

3. Results

3.1. Taxonomy

Cortinarius eucaeruleus Rob. Henry, Docums Mycol. 20 (no. 77): 69 (1989) (Fig. 1)

Mycobank MB 126119

Pileus 40-100(120) mm diam., hemispherical to convex at first, later expanded, with involuted at first, then straight margin; very strong and deep violaceous blue, finely dark-lilac fibrillose. Lamellae crowded; narrowly adnate; violaceous at first, becoming violaceous gray, then finally rusty ochraceous; smooth to wavy at margin. Veil distinctly violaceous blue, sparse; cortina white with a violet tinge. Stipe 30-80 × 10-20 mm, cylindrical, central, stuffed, with a marginate bulb (up to 30 mm), pale bluish white, soon white, yellowish when old. Flesh thick; firm; white to grayish white, often violaceous to violaceous gray in stem top when young, slightly flavescent in stipe-base. Smell and

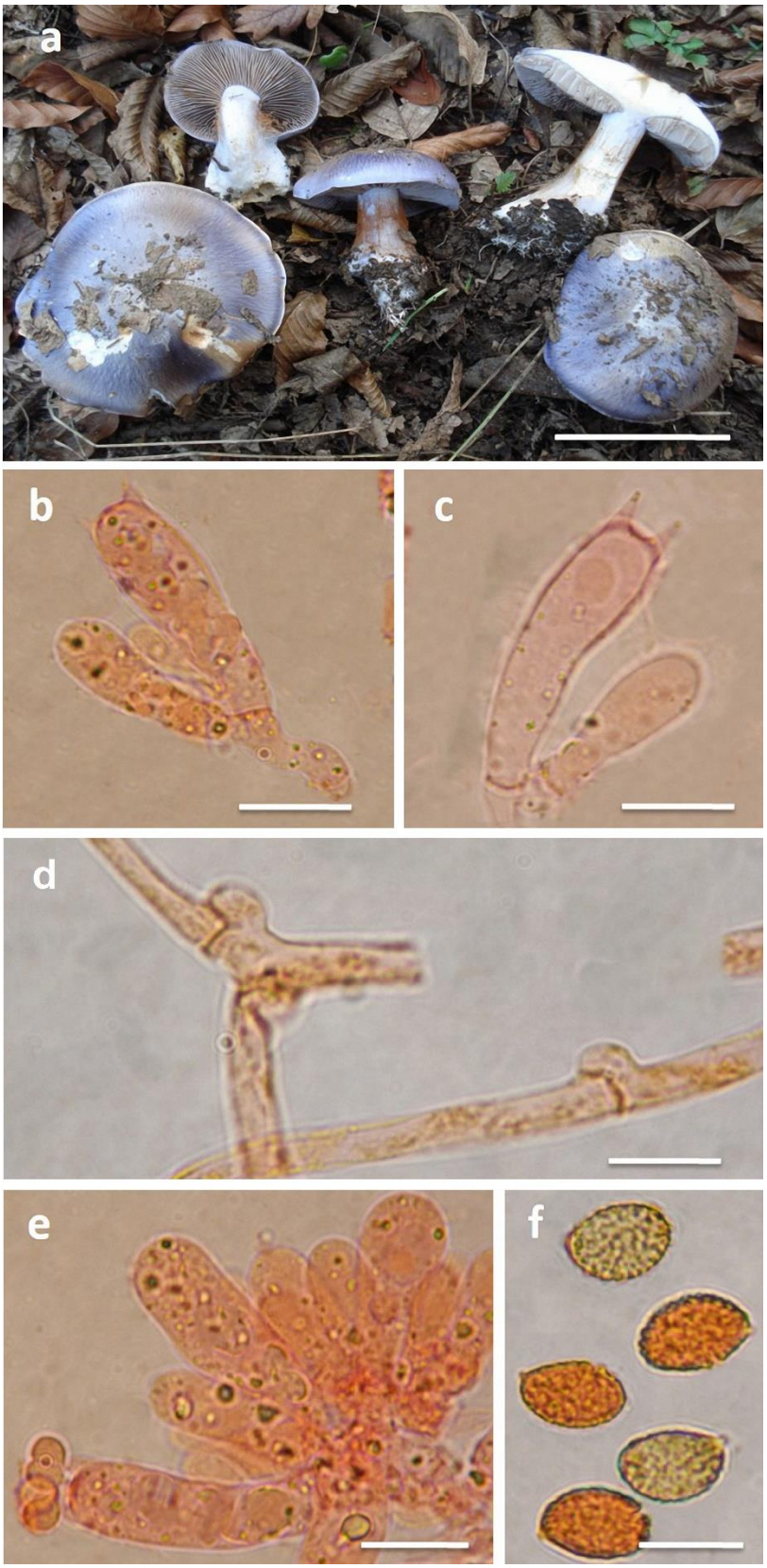

Figure 1. *Cortinarius eucaeruleus* (Collection HIS-20). a. Basidiomata in situ. b–c. basidia and basidioles. d. hyphae of pileipellis with clamp. e. basidia and basidioles. f. basidiospores. Scale bars: a = 80 mm; b–c = 10 µm, d–f = 10 µm.

taste somewhat Scleroderma-like; sweet flavor. Basidiospores 9-12.5(-13) × 5.5-7(-8) µm, $L_m \times W_m$ = 11.8 × 6.2 µm, Q = 1.4-1.6(-1.8), Q_m = 1.5, elliptic to amygdaloid, distinctly and densely verrucose. Basidia 20-25×6-8 µm, cylindrical to clavate, with 4 sterigmata and a basal clamp. Cheilocystidia not seen. Pileipellis hyphae filamentous with terminal elements 7-9 × 4-5 µm, cylindrical, septate. Clamp connections present on hyphae. Reactions of NaOH yellowish in flesh.

Ecology and distribution: On calcareous soil in broad-leaf forests, among leaf litter under of members of the genera *Carpinus* L., *Quercus* L., *Tilia* L. and *Corylus* L., rarely with members of *Fagus* L. (Soop, 2014), fruiting in temperate periods between late October and early November, present at elevation below 1050 m. Currently known from Tokat province Türkiye.

Specimen examined: TÜRKİYE. Tokat Province: Akbelen village, among leaf litters in broad-leaf forests (especially present with *Quercus*) 1044 m., 40°28'00.57"N- 36°38'47.98"E, 15 December 2019, HIS-20.

3.2. Molecular Phylogeny

We determined the ITS and LSU genomic sequences of *C. eucaeruleus* sample from Türkiye, and both sequences were deposited at GenBank under the accession numbers MT116433 and MT116800, respectively. The identified ITS region included a 712 bp length of the ITS1-5.8S-ITS2 region and the amplified LSU region included a 976 bp long 28S LSU genomic segment. BLAST results for ITS region have given significant hits to previously known species, such as *C. eucaeruleus* and *C. terpischores*. A total of twenty-six sequence for ITS and twenty-three sequence for LSU phylogeny were retrieved from NCBI databases.

Phylogenetic trees based on ITS dataset were constructed using MP and ML methods and both methods have resulted in similar topologies. Thus, we used only ML tree to infer the evolutionary relationship of the newly identified species. Based on the ITS tree, the studied sample clustered in the *C. eucaeruleus* clade with strong support (99% ML bootstrap) (Fig. 2). *Cortinarius terpischores* (JF907894) and *C. caerulescens* (MH718791) also grouped within the same clade, which indicate that they are genetically close with *C. eucaeruleus* species. Since there were no previously identified LSU sequences for *C. eucaeruleus* from different collections, the LSU-based phylogenetic tree did not provide a good phylogenetic inference as the ITS tree. Only one representative for *C. caerulescens* and *C. terpischores* were used, and they formed a clade including *C. eucaeruleus* from this study (Fig. 3).

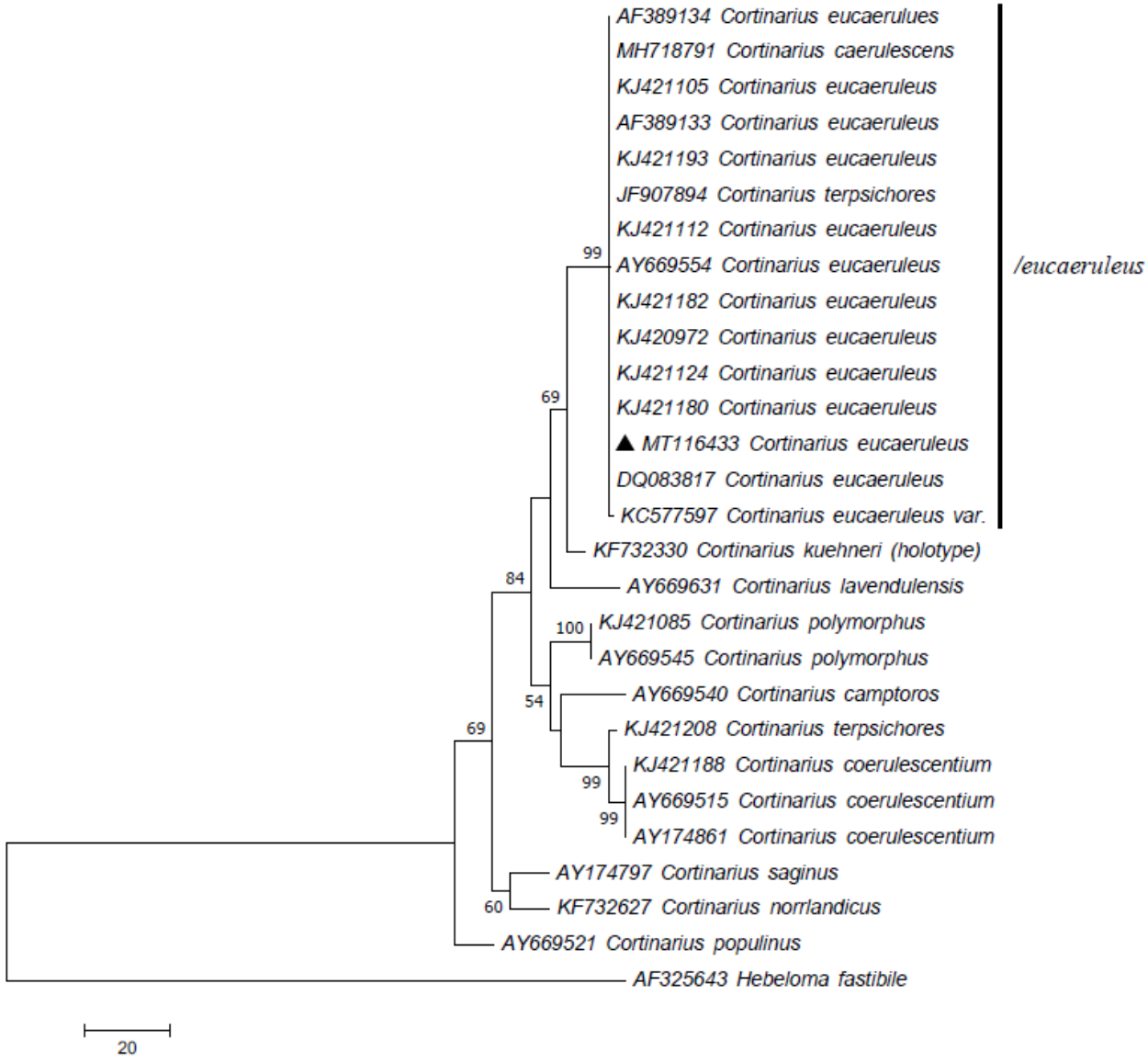

Figure 2. The ML phylogenetic tree of *Cortinarius* based on the ITS gene region. Black triangle indicates the identified species *C. eucaeruleus* in this study. *Hebeloma fastibile* was used as the outgroup species. Bootstrap test included 1000 replicates and bootstrap support values ≥ 50% were indicated on the branches. Scale bar indicates the number of substitutions per site.

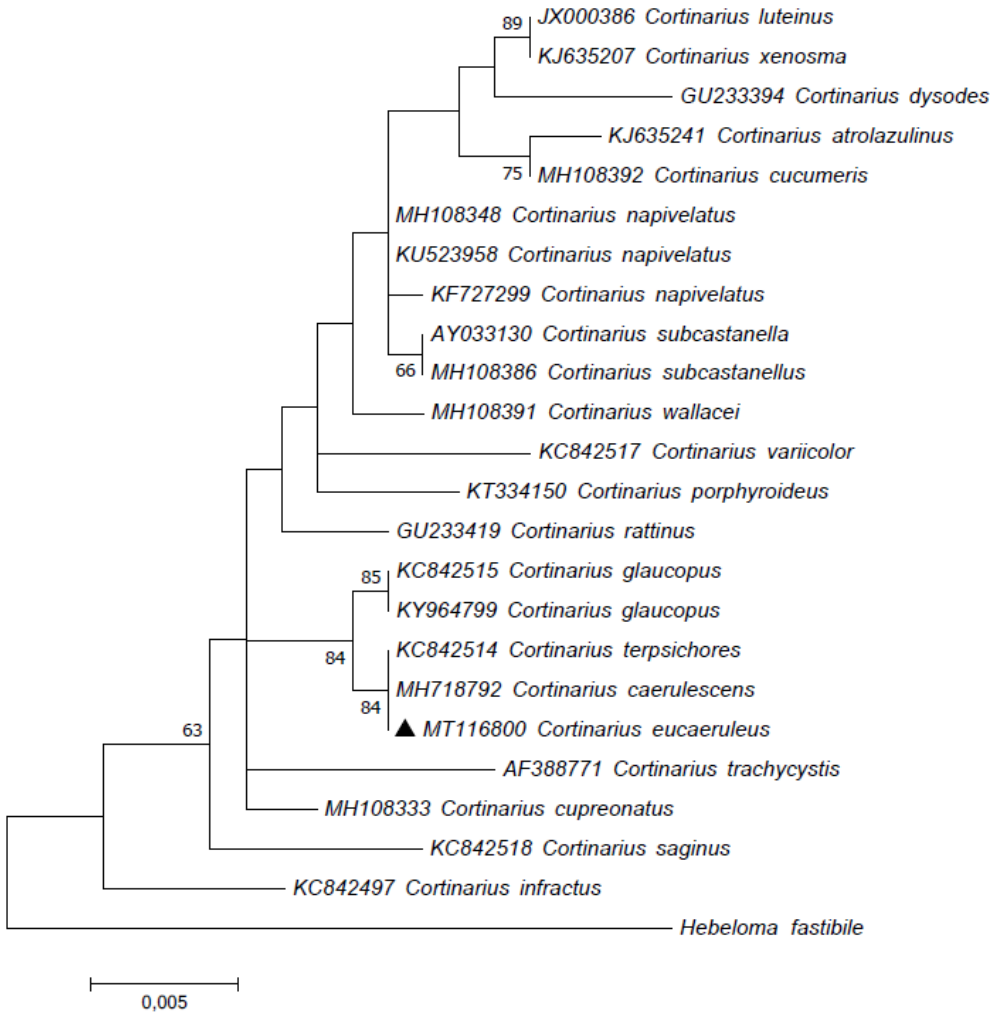

Figure 3. The ML phylogenetic tree of *Cortinarius* based on the LSU gene region. Black triangle indicates the identified species *C. eucaeruleus* in this study. *Hebeloma fastibile* was used as the outgroup species. Bootstrap test included 1000 replicates and bootstrap support values ≥ 50% were indicated on the branches. Scale bar indicates the number of substitutions per site.

4. Discussions

Cortinarius eucaeruleus has been misinterpreted as *C. terpsichores* Melot and *C. caerulescens* (Schaeff.: Fr.) Fr. In previous studies (Garnica et al., 2005; Peintner et al., 2004). Recently, some collections are re-examined by Garnica et al. (2016) at the molecular level, and they were reported as *C. eucaeruleus*. Although these species are pileocarpic fungi and have similarities in pileal color, they have distinct morphological features that distinguish them from each other. The most important differences that distinguish the three species are the spore shapes and sizes (Table 1). *Cortinarius caerulescens* has amygdaloid basidiospores while *C. eucaeruleus* and *C. terpsichores* have ellipsoid ones. *Cortinarius eucaeruleus* has bigger spore size compared to that of the other two species, e.g. 8.5-10 × 5.5-6.5 µm for *C. terpsichores* (Knudsen and Vesterholt, 2008), and 8.8-11.5 × 5-6.5 µm for *C. caerulescens* (Breitenbach and Kränzlin, 2000). Their spore ornamentations (verrucose structures) also differ from each other. The verrucose structure in *C. eucaeruleus* is strong while it is thin and densely verrucose in *C. terpsichores*, and weak to moderate verrucose in *C. caerulescens*. When their ecological preferences are compared (Table 1), *C. eucaeruleus* is generally found with *Quercus*, *Carpinus*, *Tilia* and *Corylus*, while *C. terpsichores* is associated with *Pinus*, and *C. caerulescens* with *Fagus*.

The molecular phylogenetic analysis supports a distinct clade including *C. eucaeruleus* with high bootstrap support. Based on the ITS tree, our specimen clustered in a distinct clade with other *C. eucaeruleus* species, strongly suggesting that it is *C. eucaeruleus*. The results from our phylogeny are also in congruent with the results shown in Garnica et al. (2016). *Cortinarius caerulescens* from Türkiye (Kalmer et al., 2019) and *C. terpsichores* from Italy (Osmundson et al., 2013) were also positioned in the same cluster with *C. eucaeruleus*. In Osmundson et al. (2013), the phylogeny Included *C. terpsichores* sequence from Italy but did not include any *C. eucaeruleus* or *C. caerulescens* sequences for comparison. Thus, this study could not distinguish *C. terpsichores* from *C. eucaeruleus* and *C. caerulescens* at the molecular level. Interestingly, a study by Kalmer et al. (2019) showed an ITS phylogeny which clustered *C. caerulescens* from Türkiye within the clade of *C. terpsichores*. They did 7not include *C. eucaeruleus* sequences in their molecular analysis. Thus, a relationship between *C. caerulescens* and *C. eucaeruleus* species was absent and should be addressed with further molecular studies. However, our detailed morphological description and molecular phylogeny analyses clearly indicate that *C. eucaeruleus* from Türkiye is different from *C. terpsichores* and *C. caerulescens* from other collections, which were

Table 1. Comparison of macroscopic and microscopic features of *C. eucaeruleus*, *C. terpsichores* and *C. caerulescens*.

Feature	*C. eucaeruleus*	*C. terpsichores*	*C. caerulescens*
Pileus	40-110 mm, strongly saturated violet; later fading to gray-brown from the centre; at margin darker violet.	40-90 mm, at margin with light blue colours, at centre ochraceous yellow.	50-120 mm, blue-violet, later discolouring from the centre to ochraceous buff, sometimes eventually entirely pale ochre.
Stipe	40-80 × 10-20 mm; with a marginate bulb, pale bluish white, soon white, yellowish when old.	40-80 × 8-15 mm, with a marginate bulb, pale blue, strongest towards base.	40-70 × 10-20 mm, cylindrical, base with a marginate bulb, gray-violet and longitudinally fibrillose when young, later glabrescent.
Flesh	white to grayish white with a violet in cap, violaceous to violaceous gray, slightly flavescent in stipe-base.	flesh grayish, becoming yellowish in bulb	light blue
Lamellae	violaceous when young, becoming violaceous gray	gills grayish to slightly violaceous gray	blue-violet when young, later gray-violet to ocher-brown.
Spores	9-12.5 × 5.5-7 µm; elliptic to amygdaloid, rather strongly verrucose.	8-10 x 5.5-6.5 µm; ellipsoid, finely and densely verrucose.	8.8-11.5 × 5-6.5 µm, amygdaliform, weakly to moderately verrucose.
Reactions with NaOH	yellowish in flesh	absent, sometimes yellowish	on the pileal cuticle and flesh ocher.
Ecology	with *Quercus*, *Carpinus*, *Tilia* and *Corylus*, rarely with *Fagus*, on calcareous soil.	on calcareous soil, with *Pinus* and with broad leaf forest (primarily *Fagus*)	on heavy calcareous soils under broadleaved trees, with primarily *Fagus*

recently revised by Garnica et al. (2016) through a large-scale DNA barcoding analysis. It is obvious that both micro-macro morphological and molecular analysis are needed to clarify the systematic position of many species in the subg. *Phlegmacium*. Further molecular studies including many other species of this subgenus from different geographic localities are urgently needed to have a reliable discrimination of *Cortinarius* species and understand their relationship in taxonomic studies.

5. Conclusion

Although the use of morphological and chemical characters can create consistent taxonomies for various groups, it is difficult to diagnose *Cortinarius* species due to the species richness, similar morphological characters and common ecological preferences. Due to difficulties in correct identification of *Cortinarius* species, the diagnosis and classification on these species should be supported by marker DNA sequences. Current studies now focus on both morphological ad molecular data for accurate species delimitation and nomenclature of the *Cortinarius* species. In this study, a phylogenetic relationship based on ITS dataset resulted in a reliable identification of the studied sample as *C. eucaeruleus*. While *C. eucaeruleus*, *C. terpsichores* and *C. caerulescens* share high sequence identity, our morphological results clearly indicate distinct morphological properties that separates *C. eucaeruleus* from the other two species. This study provides the first molecular and morphological identification of *C. eucaeruleus* from Türkiye. Molecular and morphological contributions from different geographic locations are necessary to understand the distribution and possible genetic and phenetic variations among the species of *Cortinarius* for an accurate taxonomic classification.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

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New chromosomal data of the genus *Dianthus* section *Dentati* (*Caryophyllaceae, Sileneae*)

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Dianthus cinsi *Dentati* seksiyonunun (*Caryophyllaceae, Sileneae*) yeni kromozomal verileri

Abstract: In section *Dentati*, the chromosome numbers were reported from only eight of 30 taxa. There is no chromosomal record relating to the other 22 taxa. In the present study, it is intended to detect the chromosome numbers of section *Dentati* taxa. Chromosome numbers of 15 taxa were obtained, 13 of which were reported for the first time and two numbers agreed with the previous reports. 14 taxa were diploid with $2x = 30$ and only one taxon was polyploid with $4x = 60$. Polyploidy might have played a role in the karyotype evolution of the genus. Although polyploidy was seen, there was no dysploidy causing to change in the basic chromosome number. According to all chromosomal data on *Dianthus*, the basic chromosome number was only one ($x = 15$). In conclusion, this study presents new data into the karyological characteristics of section *Dentati* (genus *Dianthus*) that may be useful for understanding or interpreting relationships among the sections.

Key words: Chromosome, cytotaxonomy, *Caryophyllaceae, Dianthus, Dentati*

Özet: *Dentati* seksiyonunda, toplam 30 taksonun sadece sekizinin kromozom sayıları rapor edilmiştir. Diğer 22 taksona ait kromozomal kayıt bulunmamaktadır. Bu çalışmada, *Dentati* seksiyonu taksonlarının kromozom sayılarının tespit edilmesi amaçlanmıştır. On üçü ilk kez rapor edilen, iki tanesi de önceki raporlarla uyumlu olan 15 taksonun kromozom sayısı elde edilmiştir. 14 takson $2x = 30$ ile diploid ve sadece bir takson $4x = 60$ ile poliploiddir. Poliploidi, cinsin karyotip evriminde rol oynamış olabilir. Poliploidi görülmesine rağmen, temel kromozom sayısını değiştirmeye neden olan disploidi yoktur. *Dianthus*'taki tüm kromozomal verilere göre, temel kromozom sayısı yalnızca bir tanedir ($x = 15$). Sonuç olarak, bu çalışma, *Dentati* (cins *Dianthus*) seksiyonunun karyolojik özelliklerine, seksiyonlar arasındaki ilişkileri anlamak veya yorumlamak için faydalı olabilecek yeni veriler sunmaktadır.

Anahtar Kelimeler: *Caryophyllaceae, Dianthus, Dentati*, kromozom, sitotaksonomi

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1. Introduction

Dianthus L., is a large genus of subfamily *Sileneae* DC. of family *Caryophyllaceae*, is composed of more than 300 species, and its species are widely distributed in the Asia and Mediterranean region of Europe (Şahin et al., 2016; Altay et al., 2017; Hamzaoğlu et al., 2021). However, Africa and North America have a few species of *Dianthus* (Madhani et al., 2018).

"Flora Orientalis" is the first work that contains comprehensive information about the Turkish *Dianthus* taxa (Boissier, 1867). Forty-eight of the 89 *Dianthus* species mentioned in this work are related to the flora of Türkiye. Also here, the genus *Dianthus* is grouped as *Verruculosi* Boiss., *Leiopetali* Boiss., *Fimbriati* Boiss., *Dentati* Boiss., and *Carthusiani* Boiss. under 5 groups. On the other hand, Williams (1893) divided the *Dianthus* into 3 subgenera as *Carthusianastrum* F.N.Williams, *Caryophyllastrum* F.N.Williams, *Proliferastrum* F.N.Williams, and into many sections and subsections together with those given by Boissier. The first study focusing only on Turkish taxa was done by Reeve (1967). In this revision published in "Flora of Türkiye and the East Aegean Islands", first the group key was given and the genus was divided into 5 sections as *Verruculosi*, *Leiopetali*, *Fimbriati*, *Dentati* and *Carthusiani*.

The taxa of section *Dentati* are distinguished from other taxa with short-leaf sheaths (not longer than 3 × stem diameter), dentate petal margins (not entire or fimbriate), barbellate petal surfaces (not glabrous), and not verruculose calyx (Boissier, 1867; Reeve, 1967). *Dentati* is the section containing the highest number of taxa of the genus *Dianthus* in Türkiye and includes 20 species in the Flora of Türkiye (Reeve, 1967). Some species of the section are common in Türkiye and Greece. The current literature has been taken into account in writing the taxonomic status and valid names of the section *Dentati* taxa of Turkish *Dianthus*

(Hamzaoğlu et al., 2014, 2015; Hamzaoğlu and Koç, 2018, 2019, 2020; Hamzaoğlu, 2021).

The basic and diploid chromosome numbers are the most basic characters concerning the genome of species and they are methodologically the easiest to apply. Therefore, chromosomal data for plant organisms have been reported worldwide for long years (Stace, 2000). Karyotype analysis is a basic part of cytotaxonomic studies of organisms. Therefore, it can play a significant role, particularly in taxonomically complicated species or genera (Kashmenskaya and Polyakov, 2008). In genus *Dianthus*, the basic chromosome number is $x = 15$ (Carolin, 1957) and the diploid chromosome number of many taxa is $2n = 30$ (Darlington and Wylie, 1956; Sünter, 1979; Magulaev, 1982; Zhang, 1992; Runemark, 1996; Martin et al., 2009; Şahin et al., 2016; Altay et al., 2017; Ahıskalı et al., 2020).

In section *Dentati*, the chromosome numbers were reported from only eight of 30 taxa. Six taxa are only diploid with $2n = 2x = 30$. *D. zonatus* Fenzl is polyploid, which reveals only one polyploidy level of hexaploidy ($2n = 6x = 90$). *D. barbatus* L. is diploid ($2n = 2x = 30$) and polyploid ($2n = 4x = 60$) (Darlington and Wylie, 1956; Sünter, 1979; Magulaev, 1982; Zhang, 1992; Runemark, 1996; Martin et al., 2009; Ahıskalı et al., 2020). There is no chromosomal record relating to the other 22 taxa. In the present study, it is intended to detect the chromosome numbers of other taxa. Thus, significant contributions will be made to the cytotaxonomy of section *Dentati*.

2. Material and method

2.1. Plant samples

Fifteen *Dianthus* taxa were collected from their natural habitats across Türkiye. Exsiccates were deposited at the herbarium of the Gazi University (GAZI) in Ankara. Table 1 presents the *Dentati* taxa, authors, distribution regions, collection information, and collector numbers.

2.2. Preparation and observation

The cytogenetic procedure consisted of the different processes; which were germination in petri dishes at room temperature, pretreatment by α-mono-bromonaphthalene at

Table 1. The collection information of section *Dentati* (*Dianthus*) by last taxonomic status and valid names

Taxa (alphabetically	Distribution regions and collection information
D. acrochlonis Stapf	Endemic. Türkiye. Antalya: Elmalı, Bey Mountain, Küçüksöğle village, above Serkizalan High Plateou, Kırkmar Gediği place, 2240 m a.s.l., grassy plains in doline, 28 July 2012, Hamzaoğlu **6549**, Aksoy & Koç (GAZI).
D. aculeatus Hamzaoğlu	Endemic. Türkiye. Afyonkarahisar: Between Bayat and İscehisar, Köroğlu Pass, 1500 m a.s.l., rocky igneous slopes with tuff gravels and shrub openings, 16 June 2013, Hamzaoğlu **6744**, Aksoy & Koç (GAZI).
D. armeria L.	Europe (except Eastern Europe), Caucasus, Iran and Türkiye. Zonguldak: Between Ereğli and Zonguldak, c. 10 km, 265 m a.s.l., 10 August 2012, forest clearings, Hamzaoğlu **6613**, Aksoy & Koç (GAZI).
D. aticii Hamzaoğlu	Endemic. Türkiye. Bilecik: Bilecik highway exit towards Eskişehir, 330 m a.s.l., stony slopes and steppes, 16 June 2013, Hamzaoğlu **6743** & Koç (GAZI).
D. brevicaulis Fenzl	Endemic. Türkiye. Niğde: Ulukışla, Bolkar Mountain, above Maden village, south of Meydan High Plateou, 2400 m a.s.l., 26 July 2012, calcareous rocks, Hamzaoğlu **6521**, Aksoy & Koç (GAZI).
D. erinaceus Boiss.	Endemic. Türkiye. Manisa: Spil Mount National Park, Atalanı place, around the fire tower, 1475 m a.s.l., 5 August 2012, rocks, Hamzaoğlu **6589**, Aksoy & Koç (GAZI).
D. glutinosus Boiss. & Heldr.	Eastern Aegean Islands and Türkiye. İzmir: above Yamanlar village, Karagöl road, 530 m a.s.l., 11 June 2012, forest clearings, Hamzaoğlu **6329**, Aksoy & Koç (GAZI).
D. goekayi Kaynak, Yılmaz & Daşkın	Endemic. Türkiye. Bursa: Between Soğukpınar and Karaıslah villages, 860 m a.s.l., 8 August 2012, *Quercus* sp. clearings, serpentine stony slopes, Hamzaoğlu **6596**, Aksoy & Koç (GAZI).
D. hymenolepis Boiss.	Iraq and Türkiye. Muş: Varto, Sağlıcak village, Değirmendere place, 1840 m a.s.l., 6 July 2013, grassy and stony slopes, Hamzaoğlu **6842**, Aksoy & Koç (GAZI).
D. kastembeluensis Freyn & Sint.	Endemic. Türkiye. Karabük: Between Eskipazar and Mengen, north of Çötler village, 1010 m a.s.l., *Quercus* sp. clearings, 9 September 2012, Hamzaoğlu **6679** (GAZI).
D. masmenaeus Boiss. var. *glabrescens* Boiss.	Endemic. Türkiye. Kars: Arpaçay, Dağköyü village turnout, 2010 m a.s.l., 23 July 2013, dry meadows, Hamzaoğlu **6909** & Koç (GAZI).
D. nihatii Güner	Endemic. Türkiye. Mersin: Arslanköy, Tırtar village, around Dümbelek Pass, 2185 m a.s.l., 26 July 2012, grassy plains in doline, Hamzaoğlu **6530**, Aksoy & Koç (GAZI).
D. preobrazhenskii Klokov	Armenia and Türkiye. Ağrı: Doğubayazıt, northeast of Örtülü village, Ağrı Mountain slopes, 2120 m a.s.l., grassy slopes between igneous rocks, 27 August 2012, Hamzaoğlu **6660** & Koç (GAZI).
D. roseoluteus Velen.	Bulgaria and Türkiye. Kırklareli: Between Kofçaz and Kocayazı, 685 m a.s.l., 7 July 2012, *Quercus* sp. clearings, Hamzaoğlu **6593**, Aksoy & Koç (GAZI).
D. webbianus Parl. ex Vis.	Endemic. Türkiye. Balıkesir: Edremit, Zeytinli village Kazdağı National Park, Sarıkız road, 1675 m a.s.l., 6 August 2012, rocks, Hamzaoğlu **6590**, Aksoy & Koç (GAZI).

4°C for 16 h, fixation by Carnoy's fixative at 4 °C for 24 h, storage in 70% ethanol at 4°C until use, hydrolysis by 1 N HCl at 60°C for 12 min, staining by 2% aceto-orcein for 2h, and preparation by squash method (Eroğlu et al., 2019). At least 10 metaphase microphotographs were used to detect chromosome numbers.

3. Results

3.1. Chromosomal data

Figure 1 shows the mitotic metaphase chromosomes selected from the clearest images of section *Dentati*. Chromosome numbers of 15 taxa were obtained, 13 of which were reported for the first time and two numbers agreed with the previous reports (Table 2). In genus *Dianthus*, because the chromosomes are very small and the centromere region is unclear, detailed chromosomal measurements were not made. Two different chromosome numbers ($2n$ = 30 and 60) were found and $2n$ = 30 was the most common diploid number in genus. *D. hymenolepis* was the only taxon with a different chromosome number by $2n$ = 60.

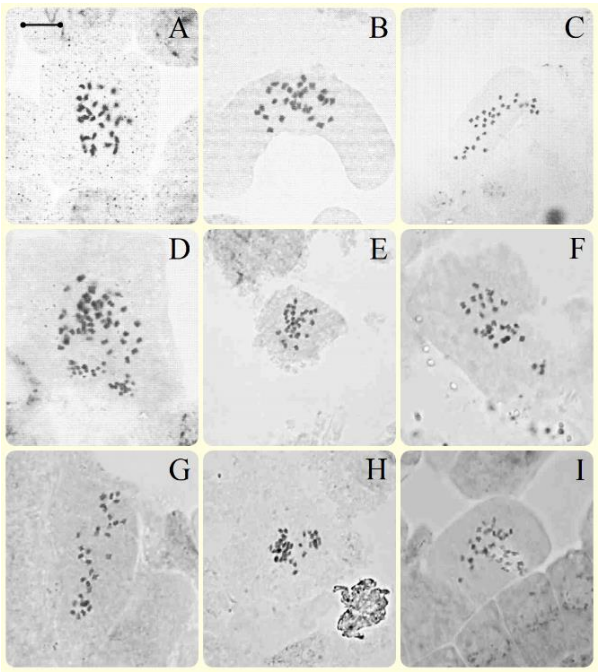

Figure 1. Mitotic metaphase chromosomes of *D. acrochlonis* (**A**), *D. aculeatus* (**B**), *D. erinaceus* (**C**), *D. hymenolepis* (**D**), *D. masmenaeus* var. *glabrescens* (**E**), *D. nihatii* (**F**), *D. preobrazhenskii* (**G**), *D. roseoluteus* (**H**), and *D. webbianus* (**I**). Scale bar: 10 µm

3.2. Basic number, ploidy levels, and polyploidy

The *Dentati* was a monobasic section by x = 15 with ploidy levels of $2x$ and $4x$. Fourteen taxa were diploid with $2n$ = $2x$ =30. *D. hymenolepis* was polyploid, which reveals only one polyploidy level of tetraploidy ($2n$ = $4x$ = 60).

4. Discussions

The chromosome numbers of 13 taxa were reported for the first time: *D. acrochlonis*, *D. aculeatus*, *D. aticii*, *D. brevicaulis*, *D. erinaceus*, *D. glutinosus*, *D. goekayi*, *D. kastembeluensis*, *D. masmenaeus* var. *glabrescens*, *D. nihatii*, *D. roseoluteus*, *D. webbianus* ($2n$ = 30), and *D. hymenolepis* ($2n$ =60). The chromosome numbers of the two species were the same as previous reports, which were *D. armeria* (Sünter, 1979) and *D. preobrazhenskii* (Borsos, 1971).

In the present study, 14 taxa were diploid with $2x$ = 30 (93.33% of the taxa) and only one taxon was polyploid with $4x$ = 60 (6.67% of the taxa). Polyploidy might have played a role in the karyotype evolution of the genus. *Dianthus* was a monobasic genus by different groups having polyploid origins, which were $3x$, $4x$, $6x$, and $8x$ (Chromosome Counts Database, CCDB, version 1.59). While all species were diploid in the section *Fimbriati* (Şahin et al., 2016), all taxa were diploid excluding *D. strictus* Sm. in section *Verruculosi* (Darlington and Wylie, 1956). However, there was no polyploidy in four varieties, which were *D. strictus* var. *strictus*, *D. strictus* var. *subenervis* (Boiss.), *D. strictus* var. *axilliflorus* (Fenzl), and *D. strictus* var. *gracilior* (Boiss.) (Altay et al., 2017).

Dianthus is a genus with geographically limited ranges and is a taxonomically difficult group characterized by a large number of endemic species (Tutin and Walters, 1993). Geography is an important factor in the evolution of *Dianthus* species in Eurasia. Although *Dianthus* does not have great interspecific ecological differentiation, it contains a large number of narrowly endemic species (especially Anatolia). This is strong evidence that the most dominant model in speciation is geographic (Valente et al., 2010). Therefore, as in other large genera, there is no clear relationship between interspecific relationships and karyotype evolution. In genus *Dianthus*, although polyploidy is seen, there is no dysploidy causing to change in the basic chromosome number. According to all chromosomal data on *Dianthus*, the basic chromosome number is one (x = 15). The basic chromosome number is x = 15 in 15 taxa in the present study, in 125 taxa in chromosome counts database (Chromosome Counts Database, CCDB, version 1.59), in four species of section *Fimbriati* (Şahin et al., 2016), and in nine taxa of section *Verruculosi* (Altay et al., 2017).

In the present study, it was reported two different chromosome numbers, the first report for chromosome numbers of 13 taxa and the same chromosome count from previous reports in two taxa. The other five species in the Flora of Türkiye could not be studied because they could not be germinated. These species were *D. elegans* d'Urv., *D. engleri* Hausskn. & Bornm., *D. raddeanus* Vierh., *D. muschianus* Kotschy & Boiss., and *D. barbatus*. However, the diploid numbers of *D. elegans* and *D. barbatus* were reported as $2n$ = 30, 60 and $2n$ = 30, respectively (Chromosome Counts Database, CCDB, version 1.59). In conclusion, this study presents new data into the karyological characteristics of section *Dentati* (genus *Dianthus*) that may be useful for understanding or interpreting relationships among the sections. In addition, polyploidy variations may probably play an important role in speciation. In this respect, the results contributed to some missing parts in *Dianthus* cytotaxonomy. However, all sections should be examined to elucidate the relationships between *Dianthus* sections and these should be supported by molecular data.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

Table 2. The chromosome counts of the investigated taxa in present and previous studies

Taxa (alphabetically)	Previous *x*: basic number, 2*n*, ploidy	Present *x*: basic number, 2*n*, ploidy	Report
D. acrochlonis		*x* = 15, 30, diploid	First report
D. aculeatus		*x* = 15, 30, diploid	First report
D. armeria	*x* = 15, 30, diploid (Sünter, 1979)	*x* = 15, 30, diploid	Equal count
D. aticii		*x* = 15, 30, diploid	First report
D. brevicaulis		*x* = 15, 30, diploid	First report
D. erinaceus		*x* = 15, 30, diploid	First report
D. glutinosus		*x* = 15, 30, diploid	First report
D. goekayi		*x* = 15, 30, diploid	First report
D. hymenolepis		*x* = 15, 60, tetraploid	First report
D.kastembeluensis		*x* = 15, 30, diploid	First report
D. masmenaeus var. *glabrescens*		*x* = 15, 30, diploid	First report
D. nihatii		*x* = 15, 30, diploid	First report
D. preobrazhenskii	*x* = 15, 30, diploid (Borsos, 1971)	*x* = 15, 30, diploid	Equal count
D. roseoluteus		*x* = 15, 30, diploid	First report
D. webbianus		*x* = 15, 30, diploid	First report

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Investigation of the allelopathic effects of lyophilized ethanol extract of *Xanthoparmelia somloensis* (Gyelnik) Hale lichen on tomato plant

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Xanthoparmelia somloensis (Gyelnik) Hale likeninin liyofilize etanol ekstraktının domates bitkisi üzerindeki allelopatik etkilerinin araştırılması

Abstract: All organisms in nature interact and compete with each other. Various groups of organisms such as algae, lichens, crops and weeds have been found as allelopathic interaction and it is known that lichens have many potent secondary metabolites. The aim of this study was to determine the allelopathic effects of lyophilized ethanolic extract of *Xanthoparmelia somloensis* (Gyelnik) Hale lichen (XS) on tomato (*Lycopersicum esculentum* L.) germination and early development stage. Obtained lyophilized ethanolic extracts of XS lichen at concentrations of 50 ppm (XS-50), 100 ppm (XS-100), 200 ppm (XS-200) and 400 ppm (XS-400) were applied to tomato seeds and seedlings. In order to determine the effects caused by the extracts, germination rate and seedling growth parameters (vigor index, length, fresh weight, relative water content and pigment contents) were analyzed. According to the obtained data, there were significant decreases in germination rate and vigor index values depending on the increase in lichen extract concentration. Similar effects were also observed in root and shoot length and pigment contents. However, results of the lowest application (XS-50) were similar to control except root length and carotenoid content. The data obtained from this study exhibited that the lichen *Xanthoparmelia somloensis* has allelopathic effects and has the potential to be used for agricultural purposes.

Key words: Allelopathy, germination, lichen, tomato, vigor index

Özet: Doğadaki tüm organizmalar birbirleriyle etkileşim ve rekabet halindedir. Algler, likenler, kültür bitkileri ve yabani otlar gibi çeşitli organizma gruplarının allelopatik etkileşime sahip oldukları ve likenlerin birçok güçlü sekonder metabolite sahip olduğu bilinmektedir. Bu çalışmanın amacı, *Xanthoparmelia somloensis* (Gyelnik) Hale liken (XS) liyofilize etanolik ekstraktının domates (*Lycopersicum esculentum* L.) çimlenmesi ve erken gelişme aşaması üzerindeki allelopatik etkilerinin belirlenmesidir. 50 ppm (XS-50), 100 ppm (XS-100), 200 ppm (XS-200) ve 400 ppm (XS-400) konsantrasyonlarında XS likeninin elde edilen liyofilize etanolik ekstreleri domates tohumlarına ve fidelerine uygulandı. Ekstraktların neden olduğu etkileri belirlemek için, çimlenme oranı ve fide büyüme parametreleri (canlılık indeksi, uzunluk, taze ağırlık, bağıl su içeriği ve pigment içeriği) analiz edildi. Elde edilen verilere göre liken ekstraktı konsantrasyonundaki artışa bağlı olarak çimlenme hızı ve vigor indeks değerlerinde önemli düşüşler tespit edildi. Benzer etkiler kök ve sürgün uzunluğu ve pigment içeriklerinde de gözlendi. Ancak en düşük uygulamanın (XS-50) sonuçları kök uzunluğu ve karotenoid içeriği dışında kontrole benzerlik gösterdi. Bu çalışmadan elde edilen veriler, *Xanthoparmelia somloensis*'in allelopatik etkilere neden olduğu ve tarımsal amaçlı kullanım potansiyeline sahip olduğunu göstermiştir.

Anahtar Kelimeler: Allelopati, çimlenme, liken, domates, vigor indeksi

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1. Introduction

Allelopathy is known as a biological phenomenon caused by biochemicals that affect the growth, existence, development and reproduction of other organisms. These biochemicals can have beneficial or harmful effects on target organisms, depending on their content and type. Allelopathic effects are often modified by additional biotic and abiotic stress factors, uncertain meteorological events, or physical, chemical, and biological soil factors, all of which can influence the residence time, persistence, concentration, and fate of allelopathic compounds in the environment (Belz and Hurle, 2005).

Lichens produce over a thousand different extracellular secondary metabolites known as lichen substances/compounds (Hauck and Huneck, 2007). Secondary metabolites produced by lichen-forming fungi have attracted attention of investigators for over 100 years. Approximately 500 compounds have been reported from lichens, of which about 350 appear to be unique. Most of these compounds are weak phenolic acids, which are produced by the fungal partner and accumulate on the outer walls of fungal hyphae. Concentrations vary considerably within and among species (Lawrey, 1995). Lichen secondary metabolites can function as allelochemicals and affect the development and growth of neighboring bryophytes, fungi, vascular plants, microorganisms, and even other lichens (Goga et al., 2018).

Lichens are compound organisms formed from a partnership of fungi living in a symbiotic relationship with a green algae or cyanobacteria. This interrelationship of lichens has been extensively studied (Green, 2008). Certain

secondary metabolites of lichens appear to have allelopathic effects on vascular plants (Lechowski et al., 2006). Although it is known that lichens produce a wide variety of secondary metabolites chemically, studies on their allelopathic properties are limited.

Lichens are known to produce acid derivatives such as terpenes, terpenoids, and steroids originating from the mevalonic acid pathway and vulpinic and pinastric acids via the shikimic acid pathway (Shukla et al., 2010). All known lichen secondary metabolites have been confirmed by usnic acid and atranorine applications to have phytotoxic effects on lichen photobionts. Both metabolites have been found to exhibit phytotoxicity by causing growth inhibition, inhibition of chlorophyll and fluorescence, decreased plant viability, and induction of oxidative stress in plant cells (Bačkor et al., 2010).

In most cases, lichens used as growth substrates positively affect plant growth and are not much affected by lichen overgrowth, but there are not many studies of negative associations, including lichen parasitism and allelopathic interactions. Extraction with acetone and water was generally used in the studies. Secondary metabolites extracted from different lichen species showed inhibitory effects on the germination of *Funaria hygrometrica* Hedwig and *Ceratodon purpureus* C. Müller spores (Frahm et al., 2000). In this manner, the aim of the study was to investigate the effects of ethanol extracts of *Xanthoparmelia somloensis* lichen on the germination and seedling growth of tomato seeds.

2. Material and method

2.1. Preparation of lichen extract

Lyophilized hydro alcoholic extract of *Xanthoparmelia somloensis* (Gyelnik) Hale lichen was prepared with 80 % of ethanol. Ground lichen was shaken in 80 % ethanol (1:20 w/v) at 300 rpm for 3 hours. After vacuum filtration, ethanol was evaporated via rotary evaporator (Buchi) and water was removed by lyophilization. Finally, fine powder lyophilized lichen extract was stored at a freezer. Lichen extract was tested at different concentrations: 0 ppm (Control), 50 ppm (XS-50), 100 ppm (XS-100), 200 ppm (XS-200) and 400 ppm (XS-400).

2.2. The effect of lyophilized lichen extract on tomato germination

Ten tomato seeds were planted in a petri dish with bi-layer filter paper moistened with hydroponic solution. Germination was followed for 6 days. Germinated seeds having at least 0.5 mm radicle were counted and noted for every day.

Germination rate (Germinated seed number/Planted seed number x 100) and Vigor Index (Seedling length (cm) x germination rate (%)) were calculated.

2.3. Growth of tomato seedlings

Tomato seeds were surface sterilized with 3 % of sodium hypochlorite for 10 minutes and washed with sterile distilled water for several times. Seeds were planted on plastic pots filled with hydroponic culture (Hoagland and Arnon, 1950). 10 days old seedlings were incubated with different lichen concentrations for 5 days to determine allelopathic effects of XS lichen at early developmental stage of tomato plants. Length, fresh and dry weight, turgid weight and relative water content were calculated for shoot and root tissues. (RWC %) = [FW-DW] / [TW-DW] x 100 (Smart and Bingham, 1974).

2.4. Chlorophyll and carotenoid contents

A piece of tomato leaf was weighted and incubated in absolute acetone for 48 hours. Samples were read at 661.6 nm, 644.8 nm and 470 nm against absolute acetone as a blank. Pigments were calculated as following formula according to Lichtenthaler (1987):

Chlorophyll a (µg/mL) = Ca = (11.24 * OD 661.6) – (2.04 * OD 644.8)

Chlorophyll b (µg/mL) = Cb = (20.13 * OD 644.8) – (4.19 * OD 661.6)

Chlorophyll a+b (µg/mL) = Ca+b = (7.05 * OD 661.6) + (18.09 * OD 644.8)

Carotenoids = Cx+c = [(1000 * OD 470) – (1.9 * Ca) – (63.14 * Cb)]/21

2.5. Statistical analysis

For statistical analysis, groups that were higher than the normality test were compared. The samples were randomly selected with at least three replications. One Way Anova and Tukey tests were used using GraphPad Prism 8 program.

3. Results

In this study, the effects of four different lichen concentrations were evaluated. It was determined that the applications inhibited the germination of the seeds depending on the increase in the concentration. While the inhibition effect caused by the extracts was not prominent at 50 ppm, the effects were started in 100-200 ppm however the main and significant effect was in the application of 400 ppm compared to control. Germination percentage declined by 76 % at 400 ppm concentration (Fig. 1).

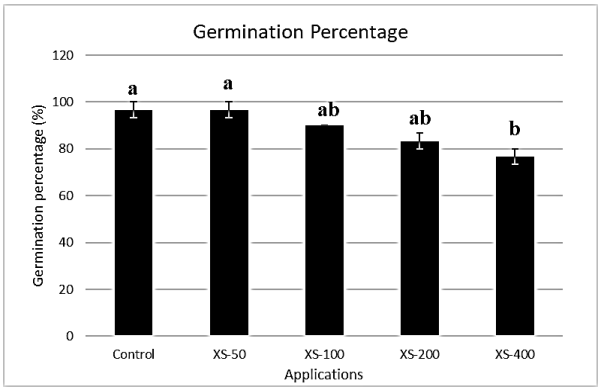

Figure 1. Germination percentage of tomato seeds exposed to *Xanthoparmelia somloensis* extracts (N=3)

Seedling vigor index was found significantly different in all extract except in 50 ppm. The 200 and 400 ppm concentrations have the higher reductions in seedling vigor index (Fig. 2).

While XS-50 caused significantly longer root length according to control, XS-400 application resulted with significantly shorter root length compared to control (Table 1). According to shoot length results, the significant decrease in shoot length was observed in XS-100, XS-200

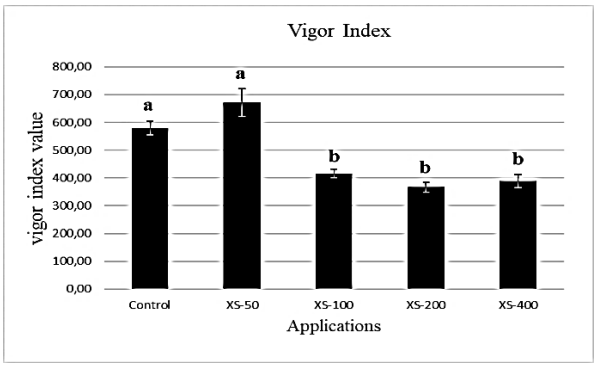

Figure 2. Vigor index parameters. Different letters on column indicates significant difference (P<0.05) (N=6)

and XS-400 applications relative to control (Table 2). Although XS-50 application resulted with shoot length decrease compared to control, it was not significant (Table 2).

Table 1. The effects of lichen *Xanthoparmelia somloensis* extracts on root parameters

	Root length (cm)	Root weight (g)	RWC (%)
Control	14.95±0.40^{b}	0.087±0.003^{ab}	119.9±2.50^{ab}
XS-50	17.66±0.48^{a}	0.101±0.007^{a}	125.4±4.05^{a}
XS-100	13.31±0.54^{bc}	0.070±0.007^{bc}	120.5±2.44^{a}
XS-200	13.33±0.36^{bc}	0.053±0.003^{c}	116.4±2.32^{ab}
XS-400	11.89±0.28^{c}	0.050±0.004^{c}	108.6±2.69^{b}

Different letters in the same column indicates significant difference (P<0.005) (N=8)

The highest root fresh weight was at XS-50 application and significantly higher than other lichen applications. Additionally, XS-200 and XS-400 caused significantly decrease in root fresh weight according to control. These two high concentrations negatively affected root fresh weight. On the other hand, while there was no significantly difference in shoot fresh weight parameter between control and XS-50, the higher concentrations of lichen extract (XS-100, XS-200 and XS-400) caused significantly decrease compared to control and XS-50. Application of the highest lichen extract concentration (XS-400) resulted with significantly decrease in relative water content (RWC) in the both of the root and shoot according to XS-50 application. However, it was not significantly different from control for RWC parameter. Growth parameters like root elongation, are even more sensitive to allelopathic effects than seed germination (Peres et al., 2009). Especially the effect on shoot was more evident with increasing concentration compared to root growth (Tables 1,2).

Table 2. The effects of lichen *Xanthoparmelia somloensis* extracts on shoot parameters

	Shoot length (cm)	Shoot weight (g)	RWC (%)
Control	8,08±0,21^{a}	0,26±0,007^{a}	98,30±1,92^{ab}
XS-50	7,69±0,13^{a}	0,24±0,0012^{a}	106,70±2,92^{a}
XS-100	6,43±0,13^{b}	0,18±0,0012^{b}	102,20±2,38^{ab}
XS-200	6,30±0,15^{b}	0,16±0,008^{b}	102,50±1,92^{ab}
XS-400	6,05±0,17^{b}	0,15±0,01^{b}	93,30±3,04^{b}

Different letters in the same column indicates significant difference (P<0.005) (N=8)

When the effects of XS ethanolic extracts on the photosynthetic pigments of tomato seedlings, lichen extract caused decrease in Chl-a content (Table 3). However, only the highest concentration (XS-400) was significantly different from control (Table 3). While there was no statistical difference between control and lichen applications for Chl-b, total chlorophyll content of XS-400 was significantly lower than control. According to Table 3, carotenoid content in tomato seedlings was obviously negatively affected by all treatment of the lichen extract (Table 3).

Table 3. The effects of lichen Xanthoparmelia somloensis extracts on pigment parameters (mg/g)

	Chl-a	Chl-b	Total Chl	Carotenoids
Control	1673,2±99,2^{a}	609,9±30,1^{a}	2283,1±128,7^{a}	484,41±34,4^{a}
XS-50	1595,7±32,4^{ab}	623,5±16,4^{a}	2219,2±47,8^{ab}	400,23±4,4^{b}
XS-100	1539,9±51,4^{ab}	608,9±21,8^{a}	2148,8±73,1^{ab}	393,07±15,1^{b}
XS-200	1445,1±58,1^{ab}	575,1±29,8^{a}	2020,2±87,3^{ab}	366,05±13,3^{b}
XS-400	1353,9±32,7^{b}	529,4±25,4^{a}	1883,3±88,1^{b}	365,14±14,6^{b}

Different letters in the same column indicates significant difference (P<0.005) (N=8)

4. Discussions

Water extracts of lichens, reduce the seed germination of several vascular plants, including *Pinus sylvestris* L. and angiosperms, trees, and shrubs and grasses (Sedia and Ehrenfeld, 2003). The different effects of lecanoric acid (*Parmotrema tinctorum* (Despr. ex Nyl.) Hale and its orsellinate derivatives on the germination and growth of *Lactuca sativa* L. and *Allium cepa* L. (strongly affected) have highlighted that the allelopathic action of certain metabolites, which can vary to a great extent between different species. As a result, in our study it is thought that the active compounds of the *Xanthoparmelia somloensis* lichen cause the germination inhibition due to the increase in concentration.

Allelochemicals were reported to influence several physiological processes during seed germination such as inhibiting amylase activity and delaying the translocation of food reserve (Kruse at al., 2000). Lowering germination rate as a result of allelochemical stress may be due to inhibition of water uptake and alteration in the activity of gibberellic acid (Tawaha and Turk, 2003).

One of the first effects of allelopathic compounds at the cell level is membrane depolarization. It causes disorders in the transport of anions and cations, and damage to membranous cell structures depends, among others on the concentration

and solubility of allelopathic substances and the pH of the environment (Einhellig, 2004).

Application of evernic acid decreased total chlorophyll content and chlorophyll a content as well as chlorophyll a to chlorophyll b ratio Bačkor et al. (2010). Also plants cultivated with usnic acid (20 or 30 μM) have demonstrated lower photosynthetic (about -40%) and respiratory (down to -80%) activities than the controls and displayed a reduction in their chlorophyll and carotenoid contents (Latkowska et al., 2006). Reduction in Chl a, Chl b and total Chl were previously reported as a result of allelochemical stress (Singh and Singh, 2009). Root exudates from sorghum were reported to inhibit the activity of hydroxyphenyl pyruvate dioxygenase which resulted in plastoquinone deficiency and therefore, disrupt the biosynthesis of carotenoids (Meazza et al., 2002). Romagni et al. (2000) reported in their study that usnic acid is a potent inhibitor of phytoene desaturase, which converts phytoene to carotenoids.

As a conclusion, ethanolic XS lichen extract has dose dependent allelopathic effects on tomato germination and on physiological and pigment parameters. Especially, the highest XS extract concentration (XS-400) caused severe negative effects in the most of the parameters (germination rate, vigor index , root length and fresh weight, shoot length and fresh weight, Chl-a, total Chl and carotenoids) in this study. On the other hand, results for the lowest XS extract concentration (XS-50) were mostly similar to control. Furthermore, XS-50 application significantly promoted root length compared to control. However, carotenoids in the XS-treated tomato seedling was significantly lower than control. In the light of these results, the ethanolic lyophilized XS extract shows allelopathic effects on tomato plant at high concentrations by negatively affecting germination rate, physiological and pigment parameters. As a further study, the different concentrations of XS extract could be tested for weed studies, because of secondary metabolite potential of lichens.

Conflict of Interest

Authors declare that there is no conflict of interest.

Authors' Contributions

ÖB, AB and MEE contributed to the study conception and design. Material preparation, data collection and analysis were performed by ÖB, AB, MEE and AA. The first draft of the manuscript was written by ÖB and AB. MEE and AB commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Karyotype analysis of *Astragalus stenosemioides* (*Fabaceae*)*

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Astragalus stenosemioides (*Fabaceae*)'in karyotip analizi

Abstract: In this research the metaphase chromosome number and karyotype of *Astragalus stenosemioides* species growing naturally in Turkey are studied. The study revealed that the chromosome number of *A. stenosemioides* is 2*n* = 16. The basic chromosome number of species is determined as *x* = 8. The karyotype formula of *A. stenosemioides* is 8m. The total length of the somatic chromosomes of *A. stenosemioides* ranges between 1.96 - 3.38 µm. The total haploid chromosome length of *A. stenosemioides* is determined as 21.36 µm. In addition, the karyotype asymmetry of the species was evaluated by different methods; Stebbins classification, TF%, AsK%, Syi and Rec, A1, A2, DI, A, and AI.

Key words: *Astragalus, Fabaceae,* karyotype, Türkiye

Özet: Bu çalışmada Türkiye'de doğal olarak yetişen *Astragalus stenosemioides* türünün metafaz kromozom sayısı ve karyotipi incelenmiştir. Çalışmada *A. stenosemioides*'in kromozom sayısının 2*n* = 16 olduğu tespit edilmiştir. Türün temel kromozom sayısı *x* = 8 olarak belirlenmiştir. *A. stenosemioides*'in karyotip formülü 8m'dir. *Astragalus stenosemioides*'in somatik kromozomlarının toplam uzunluğu 1.96 - 3.38 µm arasında değişmektedir. *A. stenosemioides*'in toplam haploid kromozom uzunluğu 21.36 µm olarak belirlenmiştir Ayrıca türün karyotip asimetrisi; Stebbins sınıflandırması, %TF, %AsK, Syi ve Rec, A1, A2, DI, A ve AI gibi farklı yöntemlerle değerlendirilmiştir.

Anahtar Kelimeler: *Astragalus, Fabaceae,* karyotip, Türkiye

Citation: Atasagun B, Aksoy A, Martin E (2022). Karyotype analysis of *Astragalus stenosemioides* (*Fabaceae*). Anatolian Journal of Botany 6(1): 44-48.

1. Introduction

Astragalus L. (Fabaceae), is one of the largest genera of vascular plants in the world, with an estimated number of 3000 species. Many species are narrow endemics (Maassoumi, 1998). It is also the largest genus in Turkey, where it is represented by nearly 484 taxa including 203 endemic in 63 sections (Hamzaoğlu, 2020; Karaman Erkul et al., 2021). In Turkey, the genus *Astragalus* is represented best in the steppe environment of high mountains. In the Irano-Turanian phytogeographic region of Turkey, which is one of the centers of diversity of the genus (Karaman Erkul et al., 2014).

Astragalus stenosemioides Bornm. Ex D.F.Chamb.& V.A. Matthews included in the *Hololeuce* Bunge section is very local endemic species growing only Erciyes Mountain in Kayseri and at a low population density (Fig. 1, Table 1).

Cytological investigations which were carried out on taxa of the genus *Astragalus* showed that chromosome numbers in these taxa were found to be 2*n* = 14, 16, 22, 24, 32, 48 and 64 (Aryavand, 1977; Dawe and Murray, 1979; Magulaev, 1980; Astanova, 1981; Spellenberg, 1981; Hung, 1984; Mu and Shue, 1985; Maassoumi, 1987; Ward and Spellenberg, 1988; Magulaev, 1989; Maassoumi, 1989; Liston, 1990; Daviña and Gómez-Sosa, 1993; Kandemir et al., 1996; Aytaç, 1997; Murray and Kelso, 1997; Gervais et al., 1999; Yan et al., 2000; Nazarova, 2004; Martin et al., 2008; Sheidai et al., 2009; Sheidai and Gharemani-Nejad, 2009; Kazem et al., 2010; Borjian et al., 2012; Ranjbar et al., 2012; Martin et al., 2019). Ekici et al. (2005) investigated that chromosome morphology of *Astragalus ovalis* Boiss. & Balansa from the section *Ammodendron* Bunge. Ekici and Çelik (2005) examined autoecological and morphological features of *A. stenosemioides*, but there is no study on the chromosome morphology of the species.

In the present study, the chromosome number and karyotype of *A. stenosemioides* has been studied. We hope that this study will contribute to future karyological studies of the genus *Astragalus*.

2. Material and Method

The karyotype was carried out on root tips. The root-tip meristems were provided from seed by germinating them on wet filter paper in petri dishes at room temperature. Firstly root tips pretreated for 16 h in α-monobromonaphthalene at 4°C, fixed in 3:1 absolute alcohol/glacial acetic acid, then the root tips were hydrolyzed with 1 N HCl for 12 min at room temperature and stained with 2% aceto-orcein for 3 h at room temperature. Stained root tips were squashed in a drop of 45% acetic acid and permanent slides were made by mounting in Depex. For karyotype analysis the photographs enlarged 10×100 were taken using a microscope with a camera attachment. The karyotypes were measured by Software Image Analyses (Bs200ProP) loaded on a

*A part of this study was presented as an abstract at International Symposium on Biodiversity and Edible Wild Species Congress, 2017.

Figure 1. The habitat of *Astragalus stenosemioides.*

personal computer. Ideograms of these taxa were arranged in decreasing length. The karyotype asymmetry of the species has been determined by different methods: Stebbins classification (Stebbins, 1971), TF% (Huziwara, 1962), AsK% (Arano, 1963), Rec and Syi index (Greilhuber and Speta, 1976), A1 and A2 (Zarco, 1986), A (Watanabe et al., 1999), DI (Lavania and Srivastava, 1999), and AI (Arano and Saito, 1980).

Table 1. Collection data of *Astragalus stenosemioides* species examined here from karyological point of view.

Species	Locality
A. stenosemioides	B5 Kayseri: Erciyes Mount, above Tekir plateau, 2316 m, 38º 32' 533" N, 035º 31' 105" E, 17.06.2016, *B. Atasagun 1078* (ERCH).

3. Results

Karyotype analysis of *Astragalus stenosemioides* was analyzed in detail. The chromosome number of *A. stenosemioides* was determined to be 2*n* = 16 in the karyomorphological study (Fig. 2,3). The smallest chromosome has a length of 1.96 µm and the largest has a length of 3.38 µm. Total haploid chromosomes length was measured as 21.36 µm. The karyotype formula of this species consists of eight median chromosome. Chromosome arm ratio is ranging from 1.06 to 1.39. Centromeric index from 3.86-7.66 and relative length from 9.18-15.81 (Fig. 2,3, Table 2). The karyotype asymmetry is as follows: Stebbins index 1A, TF% 44.85, AsK% 55.15, Syi 80.95, Rec 78.99, A1 0.19, A2 0.18, DI 8, A 0.11, AI 3.79.

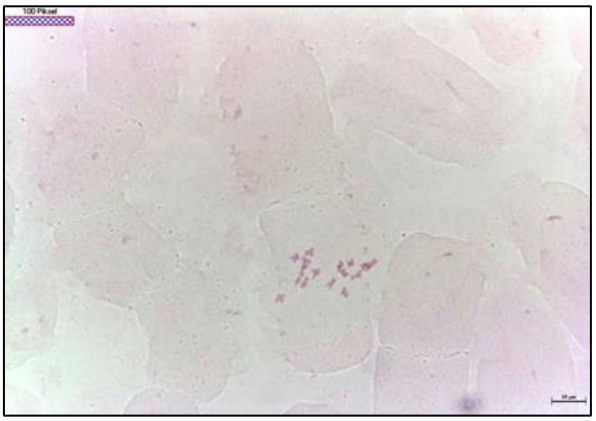

Figure 2. Metaphase chromosomes of *Astragalus stenosemioides.* Scale bar: 10 µm.

Table 2. Measurements (µm) of somatic chromosomes in *Astragalus stenosemioides* (m = median)

Chromosome	Chromosome arms (µm)		Total length (µm)	Arm ratio (L/S)	Relative length (%)	Centromeric index	Chromosome type
Pair no.	Long arm (L)	Short arm (S)					
1	1.74	1.64	3.38	1.06	15.81	7.66	m
2	1.74	1.31	3.05	1.32	14.31	6.16	m
3	1.69	1.27	2.96	1.33	13.84	5.95	m
4	1.48	1.33	2.81	1.12	13.16	6.21	m
5	1.56	1.12	2.68	1.39	12.53	5.25	m
6	1.27	1.15	2.42	1.11	11.36	5.39	m
7	1.16	0.94	2.1	1.24	9.81	4.38	m
8	1.14	0.82	1.96	1.38	9.18	3.86	m

4. Discussions

This study reports the diploid chromosome number and karyotype of *Astragalus stenosemioides.* Martin et al. (2019) reported that taxa in four different sections (*Macrophyllium* Bunge, *Hymenostegis* Bunge, *Hymenocoleus* Bunge and *Anthylloidei* DC.) of the genus *Astragalus* naturally grown in Turkey were examined in terms of cytogenetics. Somatic chromosome numbers have been reported as in section *Macrophyllum*: *Astragalus oleaefolius* DC., *A. dipodurus* Bunge, *A. cephalotes* Banks & Sol. and *A. yukselii* Karaman & Aytaç 2*n* = 16, *A. longifolius* Lam. 2*n* = 32 and *A. isauricus* Hub.-Mur. & Matthews 2*n* = 48, in section *Hymenostegis*; *Astragalus zohrabi* Bunge, *A. sosnowskyi* Grossh., *A. velenovskyi* Nabelek, *A. trifoliastrum* Hub.-Mur. & Matthews and *A. uraniolimneus* 2*n* = 16 Boiss., *A. hymenocystis* Fisch. & C.A.Mey., 2*n* = 18, *A. lagopoides* Lam., *A. hirticalyx* Boiss. & Kotschy, *A. gueruenensis* Podlech, *A. ciloensis* Podlech 2*n* = 48, in section *Hymenocoleus*; *A. vaginans* DC. 2*n* = 48 and in section *Anthylloidei*; *Astragalus szowitsii* Fisch. & Mey., *A. ermineus* Matthews, *A. zederbaueri* Stadlmann, *A. anthylloides* Lam., *A. halicacabus* Lam., *A. chardinii* Boiss.and *A. wagneri* Bunge2*n* = 16, *A. surugensis* Boiss. & Hausskn. 2*n* = 48.

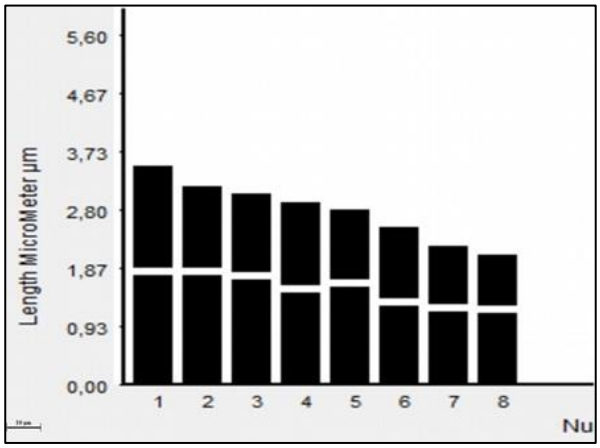

Figure 3. Ideogram of *Astragalus stenosemioides* Scale bar: 10 µm.

The chromosome number of *Astragalus stenosemioides* was determined as 2*n* = 16 in this study. In terms of chromosome number, it overlaps with *Astragalus oleaefolius*, *A. dipodurus*, *A. cephalotes*, *A. yukselii*, *A. zohrabi*, *A. sosnowskyi*, *A. velenovskyi*, *A. trifoliastrum*, *A. uraniolimneus*, *A. szowitsii*, *A. ermineus*, *A. zederbaueri*, *A. anthylloides*, *A. halicacabus*, *A. chardinii* and *A. wagneri*.

When compared in terms of karyological measurement results, the karyotype formula consists of 8m types in *Astragalus stenosemioides*. Species with the same karyotype formula; *A. oleaefolius*, *A. dipodurus*, *A. cephalotes*, *A. yukselii*, *A. sosnowskyi*, *A. velenovskyi*, *A. uraniolimneus*, *A. szowitsii*, *A. ermineus*, *A. zederbaueri*, *A. anthylloides*, *A. halicacabus*, *A. chardinii* and *A. wagneri*, while other species have different karyotype formulas.

Ekici et al. (2005) stated that the chromosome of *A. ovalis* were consisted of median (8m) chromosomes. The somatic chromosome number of *Astragalus ovalis* was determined as 2*n* = 16. Total chromosome lengths range from 1.11 µm to 1.63 µm. The karyotype formula of *A. stenosemioides* (8m) was the same as *A. ovalis*. In addition, chromosome lengths of *A.stenosemioides* were found to be longer than *A. ovalis*.

Although it has the same chromosome number and karyotype formula, it is the closest *Astragalus halicacabus* species in terms of chromosome measurement results. While the average chromosome lengths were between 1.96 µm and 3.38 µm in *Astragalus stenosemioides*; 1.89 and 3.49 µm in *A. halicacabus* species. In terms of total haploid chromosome length, it is 21.36 µm in *A. stenosemioides*, while it is 21.28 µm in *A. halicacabus* (Martin et al., 2019).

As in the *Astragalus stenosemioides* species, Atasagun et al. (2018) reported that the somatic chromosome number of *A. argaeus* was as 2*n* = 16 and it was stated that it had a different karyotype formula. However, we can state that they are different from each other in terms of chromosome measurement results. While the karyotype formula was 8m in *A. stenosemioides*, it was reported as 3m+5sm in *A. argaeus*. While the average chromosome lengths were between 1.96 µm and 3.38 µm in *A. stenosemioides*; 1.76 and 3.14 µm in *A. argaeus*, in terms of total haploid chromosome length; It is 21.36 µm in *A. stenosemioides* and 19.44 µm in *A. argaeus* (Atasagun et al., 2018).

The *Hololeuce* section includes 15 taxa in Turkey (Ekici & Ekim, 2004). In this section, two species (*Astragalus incertus* Ledeb. and *Astragalus sibthorpianus* Boiss.) were examined in terms of cytogenetics. It has been stated that *A. incertus* has 2n=4x=32 tetraploid, while *A. sibthorpianus* has 2n=16 chromosomes (Cartier, 1979; Magulaev, 1980). In this study, it was determined that *A. stenosemioides* was diploid and there was no polyploidy in the squash preparation method.

Since the genus *Astragalus* has a large number of taxa in the world, there are chromosomal studies on different taxa of the genus. However, these chromosome studies are usually on the determination of the somatic chromosome number.

The number of taxa in which detailed chromosome measurements of the genus are made is quite low. In karyotype studies, metacentric and submetacentric and very rare subtelocentric chromosome pairs are generally observed in taxa belonging to the genus. At the same time, we can talk about polyploidy, a folding of the basic chromosome numbers in different taxa of the genus. No polyploidy was found in the studied species. Rarely, it is observed in some taxa of the genus in different situations such as satellites and B chromosomes. However, no difference was observed in the taxa in our study as a result of repeated squash preparation method. If we evaluate in terms of chromosome measurement results; we can say that the chromosomes do not show a very large obvious measurement difference in the different taxa of the genus so far.

In this study, the karyotype asymmetry of *Astragalus stenosemioides* were determined for the first time. We hope that this study will contribute to the future karyological studies about the genus *Astragalus*.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

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An ignored habitat in Türkiye: Sandy steppe

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Türkiye'de yok sayılmış bir habitat: Kumlu bozkır

Abstract: The steppes habitat in Türkiye took bedrock formed of soil as the basis, and was classified by separating into groups, such as calcareous, with gypsum, volcanic, and serpentine. However, a classification was not made that took the soil texture as the basis. In this study, the stable sand dunes' soil texture observed in Karapınar, Konya, Türkiye erosion region, was evaluated for flora and syntaxonomy. Within the scope of the study, observations were conducted in the sandy habitats found to the south-southwest of the Karapınar county center, the plant species that preferred the habitat were determined, and the data obtained was compared with the steppes' habitat in the close environs and with the studies made previously. Furthermore, it emphasized the necessity of analyzing syntaxonomically the sandy steppes in Turkey, which are evaluated within the "E1.A5 - Irano-Anatolian inland dunes" habitat type according to the European Nature Information System (EUNIS).

Key words: Central Anatolia, Habitat classification, sandy flora, sandy soil, steppe, vegetation

Özet: Türkiye bozkır habitatı; toprağı oluşturan ana kayaç esas alınarak kireçli, jipsli, volkanik, serpantin vs. gibi gruplara ayrılarak sınıflandırılmış, ancak toprak bünyesini esas alan bir sınıflandırma yapılmamıştır. Bu çalışmada Karapınar (Konya, Türkiye) erozyon bölgesinde gözlenen durağan kumullar toprak bünyesi, flora ve sintaksonomi bakımından değerlendirilmiştir. Çalışma kapsamında Karapınar ilçe merkezinin güney-güneybatısında bulunan kumlu habitatlarda gözlemler yapılmış, habitatı tercih eden bitki türleri tespit edilmiş, elde edilen veriler yakın çevresindeki bozkır habitatı ve daha önce yapılmış çalışmalarla karşılaştırılmıştır. Ayrıca Avrupa Doğa Bilgi Sistemine (EUNIS) göre "E1.A5 - Irano-Anatolian inland dunes" habitat tipi içinde değerlendirilen Türkiye kumlu bozkırlarının sintaksonomik açıdan analiz edilmesinin gerekliliği vurgulanmıştır.

Anahtar Kelimeler: Bozkır, habitat sınıflandırması, İç Anadolu, kumlu flora, kumlu toprak, vejetasyon

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1. Introduction

Studies for the classification of vegetation started in the first half of the nineteenth century (Humboldt and Bonpland, 1814; Grisebach, 1838). In these studies, vegetation was considered in a general manner and classified based on physiognomy. From the beginning to the present-day, vegetation classification methodology has gradually been developed, and new classification techniques and schools have been formed based on different characteristics of vegetation (Whittaker, 1973; Mucina, 1997). The data obtained from the vegetation classifications, currently constitutes the basis of the habitat classification. For example, the European Nature Information System (EUNIS) is an original and dynamic system that classifies all the natural or artificial habitats in Europe (Evans, 2012).

If the studies are not considered that were conducted separately from each other, the regular studies related to the vegetation of Türkiye were started by Prof. Dr. Hikmet Ahmet Birand in the 1930s (Handel-Mazetti, 1909; Schwarz, 1936; Czeczott, 1939; Krause, 1940; Gür, 2010). These studies accelerated as of the 1970s with the efforts of numerous Turkish and foreign scientists. As a result of these intensive studies, a considerable number of plant communities, especially forest and steppe vegetations, were identified, mostly in central, south, west and north of Anatolia and partially in the east (Ketenoğlu et al., 2010; Özdeniz, 2017). However, unfortunately, the vegetation studies in Türkiye have slowed down, and even came to a standstill at the beginning of 21st century, though there are many areas without any vegetation data, especially in the southeast and east Anatolia.

Since the vegetation studies in Türkiye remained unfinished and were not matched with habitats, the obtained data could not be transferred to an EUNIS-like system as in Europe. Furthermore, despite conducting studies, it can also be said that detailed classification has not been made for some habitats in Turkiye and it has been handled in a very general manner. For example, the salt marsh habitat has been evaluated within the steppes' habitat for an extensive period and the salt marsh and salt steppe differentiations were made approximately 40 years later (Birand, 1961; Aydoğdu et al., 2002; 2004).

Sandy steppe is another habitat type has not been differentiated until now. In Türkiye, the steppe is classified into groups such as calcareous, gypsum, volcanic, serpentine, etc., based on the bedrock that forms the soil (Özdeniz, 2017). But a steppe classification based on soil texture has not been made. However, with its soil texture and flora, sandy steppe habitat is different from all known steppe types in Türkiye. The most significant feature that separates the sandy steppes' habitat from the other steppe varieties is the texture of the soil that develops on it. The

particles that make up the soil with a size between 2.0-0.02 mm are grouped as sand, those between 0.02-0.002 mm as silt, and those with a size less than 0.002 as clay (Aydın and Kılıç, 2010). According to accepted international standards, soils containing more than 68% sand and less than 18% clay in the first 100 cm of solum are classified as sandy (ISSS Working Group RB, 1998). Since sandy soils have larger interparticle spaces, water drainage is faster and water holding capacity is low. They are poor soils in terms of nutrients due to excessive washing in the solum. Generally they have an acidic, dry, and light character. On the other hand, they have better aeration and faster heat exchange compared to silty and clay soils. However, since they heat up rapidly, they become dry quickly in the summer (Özen and Onay, 2018; Okur et al., 2021).

Psammophytes are special plants that have adapted to living in sandy soils. Sand dunes, which are formed as a result of the accumulation of sand particles blown with the effect of wind, are not suitable for plant life, especially due to their low water holding capacities. To get rid of this negativity, perennial psammophytes have adapted to give new shoots from the underground parts of their roots and stems. In some species, the leaves are either absent or rather narrowed to minimize water loss. Like therophytes, living in only one suitable season, is also an another adaptation. Therophytes only germinate during the rainy season, give flowers and finally die, leaving seeds. Precipitation is the most important source of water in arid and semi-arid sandy areas. It is also the most fundamental factor that ensures the formation and development of a psammophyte community (Wilhelmi and Wilhite, 2002).

Depending on the sandy soil on which they live, psammophyte communities are classified as drift lines, mobile dunes, stabilized dunes and salt marshes (Mahdavi et al., 2017). Stabilized dunes are habitats where the sand does not move with the wind, but remains stable. In Europe, the sandy steppe vegetation that develop on stabilized dunes has been attributed to class *Koelerio-Corynephoretea canescentis* Klika in Klika et Novák 1941 (Mucina et al., 2016). This class includes primary plant communities that develop on sandy soils and shallow soils that cover rocky areas that are often degraded by erosion, animal or human activities. No syntaxon has been identified so far in Türkiye that belongs to this class. The syntaxa, identified in the steppe vegetation of Türkiye, were assigned to the class *Astragalo-Brometea tomentelli* Quézel 1973 (Özdeniz 2017). In this study, the stabilized dune habitat observed in the Karapınar (Konya) erosion zone was evaluated by considering the soil texture, flora and syntaxonomy, compared with the steppe habitats in close environ, and the existence of sandy steppe habitat in Türkiye was discussed.

2. Material and Method

This study aims to reveal the existence of the "sandy steppe" habitat in Türkiye. For this purpose, observations were made on 28.6.2018 in the sandy steppe habitat located at south-southwest of Karapınar (Konya, Türkiye) district center (Fig. 1). The data obtained as a result of the observations, the determined plant samples and the previous vegetation researches carried out in the region constitute the material of the study (Birand, 1970; Bağcı et al., 1996). The methodology of the study was based on the comparison the comparison of the studies carried out in the Karapınar sandy steppe habitat with the sandy-loam or clay-loam steppe habitat in the close environs in terms of soil texture, flora and syntaxonomy. As the soil texture, the international standard, which considers soils containing more than 68% sand and less than 18% clay in the first 100 cm of the solum, as "sandy soil" were regarded (ISSS Working Group RB, 1998). The identification and phytogeographical designation of the vascular plants recorded in the area were done according to Davis (1965-1985) and other resources (Davis et al., 1988; Güner et al., 2000). The determined plant species detected during field observations were regarded as flora, and syntaxons to which the Turkish steppe habitat and the European sandy steppe habitat were attributed, were regarded as syntaxonomy (Mucina et al., 2016; Özdeniz, 2017).

2.1. Brief description of the study area

Karapınar district is located Konya province in the southern part of central Anatolia (Turkiye). There are sandy areas formed by wind erosion about 5 km south-southwest of the district center (Fig. 1). The elevations in the area range between 1000 and 1060 meters. There are no lakes or streams in the area. As a result of wind erosion and the anthropogenic effects in the region, sandy steppes remained as patches among salt marshes and other steppes. The soil data related to Karapınar erosion zone were obtained from previous studies carried out in the area (Birand, 1970; Bağcı et al., 1996). The area is dominated by alluvial and regosol soils. In these soils, the sand ratio varies between 40-80% and the clay ratio varies between 7-25% (Birand, 1970; Bağcı and Dural, 1997).

The climatic data of Karpınar erosion zone was obtained from Konya meteorological station. The average annual temperature of Konya province is 11.7 °C. The highest and lowest temperatures are observed in July and January with 23.5 and -0.2 °C. The highest rainfall in Konya takes place in May and December with 43.1 and 43.2 mm respectively. The lowest rainfall is observed in August and July with 6.4 and 7.5 mm (MGM, 2022). These values indicate that Karapınar erosion zone is dominated by “semi-arid very cold Mediterranean” climate (Akman, 1999).

3. Results

Two basic plant communities were determined during the observations conducted on 28 June 2018 in the stabilized dune habitats of the Karapınar (Konya) erosion region. The first one is observed in the relatively rough terrain around Örnektepe, and is characterized by the dominance of the

Figure 1. Karapınar erosion zone and Örnektepe (adapted from “Google Earth Pro”)

Figure 2. *Alhagi maurorum* subsp. *maurorum* community around Örnektepe

Alhagi maurorum Medik. subsp. *maurorum* (Fabaceae) species (Fig. 2). The second is observed in almost flat areas in the south and southeast of Örnektepe, and is characterized by the dominance of *Stipa hohenackeriana* Trin. & Rupr. (Poaceae) species (Fig.3). A total of 75 species and infraspecies taxa were determined in these communities. Of these, 30 are Irano-Turanian and one is Mediterranean phytogeographic region element. In addition, 15 of these taxa are endemic to Türkiye (20% endemism).

Other common species in areas dominated by *Alhagi maurorum* subsp. *maurorum* are *Alkanna orientalis* (L.) Boiss. var. *orientalis*, *Anchusa leptophylla* Roem. & Schult. subsp. *incana* (Ledeb.) D.F. Chamb., *Anisantha tectorum* (L.) Nevski, *Artemisia campestris* L. subsp. *campestris*, *Astragalus matthewsiae* Podlech & Kirchhoff, *Bromus japonicus* Thunb. subsp. *japonicus*, *Centaurea pulchella* Ledeb., *Chondrilla juncea* L., *Cynanchum acutum* L. subsp. *acutum*, *Minuartia anatolica* Woronow var. *arachnoidea* McNeill, *Phleum boissieri* Bornm., *Poa bulbosa* L. and *Xeranthemum annuum* L. On the other hand, *Minuartia anatolica* Woronow var. *arachnoidea* McNeill, *Onosma roussaei* DC., *Haplophyllum vulcanicum* Boiss. & Heldr., *Centaurea pulchella* Ledeb., *Phleum boissieri* Bornm., *Cynodon dactylon* (L.) Pers., *Lomelosia argentea* (L.) Greuter & Burdet, *Artemisia campestris* L. subsp. *campestris*, *Xeranthemum annuum* L. and *Poa bulbosa* L. were found to be common in *Stipa hohenackeriana* dominated areas.

Figure 3. *Stipa hohenackeriana* community to the south and southeast of Örnektepe

3.1. The sandy steppe flora of Karapınar

Spermatophyta
Gymnospermae
Cupressaceae
Juniperus deltoides R.P.Adams

Angiospermae
Monocotyledonae
Amaryllidaceae
Allium myrianthum Boiss., Irano-Turanian element

Poaceae
Anisantha tectorum (L.) Nevski
Briza humilis M.Bieb.
Bromus japonicus Thunb. subsp. *japonicus*
B. tomentellus Boiss., Irano-Turanian element
Cynodon dactylon (L.) Pers.
Phleum exaratum Griseb.
Poa bulbosa L.
Stipa hohenackeriana Trin. & Rupr., Irano-Turanian element

Dicotyledonae
Amaranthaceae
Salsola kali L. subsp. *ruthenica* (Iljin) Soó

Apiaceae
Bupleurum sulphureum Boiss. & Balansa, Irano-Turanian element, endemic
Echinophora tenuifolia L. subsp. *sibthorpiana* (Guss.) Tutin, Irano-Turanian element
Eryngium campestre L. var. *virens* (Link) Weins
Ferulago armena (DC.) Bernardi, Irano-Turanian element, endemic

Apocyanaceae
Cynanchum acutum L. subsp. *acutum*

Asteraceae
Anthemis cretica L. subsp. *albida* (Boiss.) Grierson
Artemisia campestris L. subsp. *campestris*
Centaurea pulchella Ledeb., Irano-Turanian element
C. solstitialis L.
C. virgata Lam., Irano-Turanian element
Chondrilla juncea L.
Cirsium arvense (L.) Scop.
Cousinia birandiana Hub.-Mor., Irano-Turanian element, endemic
Crepis foetida L. subsp. *rhoeadifolia* (M.Bieb.) Čelak.
Crupina crupinastrum (Moris) Vis.
Cyanus depressus (M.Bieb.) Soják
Helichrysum plicatum DC. subsp. *plicatum*
Lactuca serriola L.
Tragopogon latifolius Boiss. var. *latifolius*, Mediterranean element
Xeranthemum annuum L.

Boraginaceae
Alkanna orientalis (L.) Boiss. var. *orientalis*, Irano-Turanian element
Anchusa leptophylla Roem. & Schult. subsp. *incana* (Ledeb.) D.F.Chamb., Irano-Turanian element, endemic
Lappula barbata (M.Bieb.) Gürke, Irano-Turanian element
Onosma roussaei DC., Irano-Turanian element

Brassicaceae
Alyssum strigosum Banks & Sol. subsp. *strigosum*
Camelina rumelica Velen.
Descurainia sophia (L.) Webb ex Prantl subsp. *sophia*

Erysimum crassipes Fisch. & C.A.Mey.
Isatis floribunda Boiss. ex Bornm., Irano-Turanian element, endemic
Meniocus linifolius DC.
Sinapis arvensis L.

Caryophyllaceae
Dianthus crinitus Sm.
Minuartia anatolica Woronow var. *arachnoidea* McNeill, Irano-Turanian element, endemic
Saponaria prostrata Willd., Irano-Turanian element, endemic
Silene conica L.
S. otites (L.) Wibel

Convolvulaceae
Convolvulus arvensis L.

Dipsacaceae
Lomelosia argentea (L.) Greuter & Burdet
L. rotata (M.Bieb.) Greuter & Burdet, Irano-Turanian element

Fabaceae
Alhagi maurorum Medik. subsp. *maurorum*, Irano-Turanian element
Astragalus lycius Boiss., endemic
A. matthewsiae Podlech & Kirchhoff, Irano-Turanian element, endemic
A. mesogitanus Boiss., Irano-Turanian element, endemic
A. microcephalus Willd., Irano-Turanian element
Hedysarum varium Willd., Irano-Turanian element
Medicago astroites (Fisch. & C.A.Mey.) Trautv., Irano-Turanian element
M. isthmocarpa (Boiss. & Balansa) E.Small, endemic
Onobrychis arenaria (Kit.) DC. subsp. *cana* (Boiss.) Hayek
O. tournefortii (Willd.) Desv., Irano-Turanian element, endemic

Lamiaceae
Ajuga chamaepitys (L.) Schreb. subsp. *chia* (Schreb.) Arcang. var. *chia*
Salvia absconditiflora Greuter & Burdet, Irano-Turanian element, endemic
S. ceratophylla L., Irano-Turanian element
Ziziphora tenuior L.

Papaveraceae
Papaver argemone L.

Plumbaginaceae
Acantholimon venustum Boiss. var. *venustum*

Ranunculaceae
Adonis flammea Jacq.
Nigella arvensis L. var. *glauca* Boiss., Irano-Turanian element

Resedaceae
Reseda lutea L. subsp. *lutea*

Rhamnaceae
Rhamnus hirtella Boiss., Irano-Turanian element, endemic

Rosaceae
Prunus orientalis Koehne, Irano-Turanian element

Rubiaceae
Galium aparine L.

Rutaceae
Haplophyllum vulcanicum Boiss. & Heldr., Irano-Turanian element, endemic

Scrophulariaceae
Verbascum cheiranthifolium Boiss. var. *asperulum* (Boiss.) Murb.

Zygophyllaceae
Tribulus terrestris L.

4. Discussions

The first study on xerophyte plant communities around Karapınar (Konya) was carried out by Birand (1970). In the study, the areas between Konya-Karapınar, south of Karapınar and between Karapınar-Ereğli were treated as two different habitats as "Die Sandwüste (sandy desert)" and "Artemisia-Steppe". A soil profile was taken from the habitat type called sandy desert and 22 relevé records were made. The soil between 0-110 cm of the soil profile is sandy, while loamy between 110-135 cm and 135-205 cm. The sand ratio of the soil in the range of 0-110 cm is given as 60.25%. Although he named it as sandy desert (die Sandwüste), the steppe habitat whose relevé records were made by Birand (1970) is not considered "sandy", according to international standards, since the proportion of sand is less than 68% (ISSS Working Group RB, 1998). When the recorded 22 relevé were examined, it can easily be seen that *Salvia cryptantha* Montbret & Aucher ex Benth. (current name *S. absconditiflora* Greuter & Burdet) and *Phlomis armeniaca* Willd. are dominant, and *Alhagi maurorum* subsp. *maurorum* and *Stipa hohenackeriana*, which were found to be among the dominant species according to our observations, are either absent or found in small numbers and with low overlap values. Furthermore, *Salvia cryptantha* and *Phlomis armeniaca* are the species belonging to the *Astragalo-Brometea tomentelli* class or its subunits (Quézel, 1973). Considering the soil texture, flora and syntaxonomy, it can easily be understand that the study conducted by Birand (1970) around Konya, Karapınar and Ereğli was carried out in clay-loamy steppe habitat, rather than sandy steppe habitat. Probably, Birand considered the areas containing dense sand in Örnektepe and its surroundings in Karapınar as "degraded steppe" and did not record the relevé, thinking that it would not reflect the truth.

The second study on xerophytic plant communities around Karapınar (Konya) was carried out by Bağcı et al. (1996). Compared to the previous two studies, it can be said that this study is more satisfactory in terms of syntaxonomy. Sixty three relevé recordings were made from xerophytic plant communities observed around Karapınar. As a result of the analysis of soil samples taken from the depths of 0-20 cm, 20-40 cm and 40-60 cm from the area, 5 xerophytic plant communities were identified growing on sandy and sandy-loam soils. Of these, the *Salsola ruthenicae-Alhagietum pseudalhagi* Bağcı, Tatlı & Kargıoğlu 1996, *Astragaletum lycio-microcephali* Bağcı, Tatlı & Kargıoğlu 1996 and the *Marrubio parviflori-Salvietum cryptanthae* Bağcı, Tatlı & Kargıoğlu 1996 prefer sandy soils with a sand ratio exceeding 68% and a clay ratio less than 18% (Bağcı et al., 1996; Bağcı and Dural, 1997). These data indicate that the sandy habitat dominated by the *Alhagi maurorum* subsp *maurorum* species in the vicinity of Karapınar was also determined by Bağcı et al. (1996).

Stipa hohenackeriana (in the study as *Stipa holosericea* Trin.) community was evaluated within the *Alhagi maurorum* subsp. *maurorum* community by Bağcı. Despite his detailed study, Bağcı et al. (1996) used the term "steppe" instead of "sandy steppe" for sandy habitats in his

study and attributed the identified communities to the *Astragalo-Brometea tomentelli* class and its subunits accordingly. However, when the table of the *Salsola ruthenicae-Alhagietum pseudalhagi* association is examined, it is clearly seen that the subunits of the *Astragalo-Brometea tomentelli* class are very weakly represented (Bağcı et al., 1996). It is also seen that Bağcı et al. (1996) evaluated the sandy steppe habitat in sandy-loam or clay-loam steppe, did not draw clear boundaries and therefore mixed the relevé record. According to the analysis, although the soil texture is "sandy", the existance of the species *Astragalus microcephalus* Willd., *A. lydius* Boiss., *A. lycius* Boiss., *Onobrychis armena* Boiss. & A. Huet, *Salvia cryptantha* and *Marrubium parviflorum* belonging to *Astragalo-Brometea tomentelli* class and its subunits, as dominant species or at higher rates in *Astragaletum lycio-microcephali* and *Marrubio parviflori-Salvietum cryptanthae* communities, also supports this confusion (Bağcı et al., 1996).

According accepted international standards and our observations, the areas around Karapınar (Konya) and its close environs with soil containing more than 68% sand and less than 18% clay, should be considered as "sandy steppe" (ISSS Working Group RB, 1998). On the other hand, a careful review of previous studies conducted in Karapınar and its close environ indicate that the authors neither not examined the sandy steppe habitat, regarding it as a "degraded habitat" nor handled as a different habitat type, evaluating it together with an onother steppe habitat, and made, if done, the syntaxonmy in accordance (Birand, 1970; Bağcı et al., 1996). In the vegetation studies conducted with the flora based Braun-Blanquet (1964) method, the relevé area was recommended as 50-100 m^2 for the steppe habitat and 1-10 m^2 for the dune and sandy steppe habitat (Whittaker, 1973). The reason why the relevé widths are determined differently for these two habitats is that the sandy steppe habitat is often found in small patches among other habitats. Sandy steppe habitat is found in small pieces in and around Karapınar. However, in previous studies, the of the relevé widths were taken as 36-100 m^2 (Bağcı et al., 1996). Due to higher relevé widths, species belonging to the sandy steppe habitat were recorded within the same sample of other steppe habitats in the environs.

The sandy steppe habitat of Karapınar had been ignored and evaluated within the other steppe type despite the studies carried out in the region. Here, we reveal that the habitat is different from other steppe habitats in the vicinity in terms of soil texture, flora and syntaxonomy. In Europe, plant communities belonging to the sandy steppe habitat on stationary dunes were attributed to *Koelerio-Corynephoretea canescentis* class, and were classified as "RLE1.1a Pannonian and Pontic sandy steppe" by taking under protection (Mucina et al.. 2016; EUNIS, 2022). On EUNIS website, the sandy steppes of Turkiye are classified in "E1A5 - Irano-Anatolian inland dunes" and their probable distribution is given. But necessary information about the general characteristics of the habitat, its floristic structure and syntaxa has not been given on the website, since necessary researches have not completely been conducted (EUNIS, 2022).

In order to overcome this deficiency and to classify the Turkish habitats properly, the syntaxonomy of the sandy steppes of Türkiye should urgently be analyzed.

Conflict of Interest

Authors declare that there is no conflict of interest.

Authors' Contributions

The authors confirm contribution to the manuscript as follows; study conception and design: Ergin Hamzaoğlu, Kuddisi Ertuğrul, analysis and interpretation of results, and draft manuscript preparation: Ergin Hamzaoğlu, data collection: Ergin Hamzaoğlu, Kuddisi Ertuğrul, Murat Koç.

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Diversity of myxomycete on Konya-Beyşehir highway route

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Konya-Beyşehir karayolu güzergahındaki miksomiset çeşitliliği

Abstract: In this study, it was aimed to determine myxomycetes growing on materials collected from forests on Konya-Beyşehir (Turkey) highway route between 2019-2020. 253 materials such as log and stump materials, forest debris and bark of living tree were collected during the fieldworks in the region. The moist chamber technique was applied to the collected materials. As a result, 80 myxomycete specimens were developed and 21 myxomycete taxa belonging to 8 families were identified. The most common species is *Perichaena depressa* Lib. and was detected on 17 different substrates. In addition, the localities of the species (station number, substrate, collection date, collection number), and photographs of the species identified from the region are also given.

Key words: Biodiversity, Konya, myxomycete

Özet: Bu çalışmada, 2019-2020 yılları arasında Konya-Beyşehir (Türkiye) karayolu güzergâhındaki ormanlardan toplanan materyaller üzerinde gelişen miksomisetlerin tespit edilmesi amaçlanmıştır. Bölgeye düzenlenen arazi çalışmalarında kesilmiş ve devrilmiş ağaç kütüğü materyalleri, orman döküntü katı ve canlı ağaç kabuğu gibi 253 adet materyal toplanmıştır. Toplanan materyallere nem odası tekniği uygulanmıştır. Arazi ve laboratuvar çalışmaları sonucunda 80 miksomiset örneği gelişmiş ve 8 familyaya ait 21 miksomiset taksonu tespit edilmiştir. En yaygın tür *Perichaena depressa* Lib. olup 17 farklı substratta tespit edilmiştir. Ayrıca türlerin lokaliteleri (istasyon numarası, substrat, toplama tarihi, toplama numarası), ve bölgeden tespit edilen türlerin fotoğrafları da verilmiştir.

Anahtar Kelimeler: Biyoçeşitlilik, Konya, miksomiset

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1. Introduction

Myxomycetes (slime moulds), also known as Mycetozoa or Myxogastria, are ameboid protists that have been considered as a special group of fungi for many years. They are called as "cıvık mantarlar" in our country (Türkiye) (Sesli et al., 2020).

Though some of them have fructifications, large enough to be seen by naked eye, most of them are quite small and they can be best observed with a hand lens or a microscope (Stephenson and Stempen, 2000). For most of its life, a myxomycete exists as a thin, free-living mass of protoplasm (Stephenson and Stempen, 2000). In their slimiest state, they creep through the forest growing in size and consuming food (Niblett, 2017). Plasmodia usually inhabit environments, such as soil, dead wood, and various plant litter and debris, which are moist and support bacteria and other decay microorganisms (Stephenson and Landolt, 1996; Clark and Haskins, 2015). After a period of feeding and growth, the plasmodium moves out of its normal habitat and into a drier, more exposed location. Here it gives rise to one or more fruiting bodies (Stephenson and Stempen, 2000).

Classification of myxomycetes was made first according to their microscopic characters (Rostafiński, 1873) and then according to the type of plasmodia and the morphogenesis of fruiting bodies (Martin et al., 1983; Lado and Eliasson, 2017). Therefore, myxomycetes have been studied in the kingdoms of plants, animals and protists by different taxonomists over time (Ing, 1999). In addition, myxomycetes are typically found in the same habitats as fungi, they were treated as taxa within the Kingdom *Fungi* (Class *Myxomycetes*) (Everhart and Keller, 2008). Baldauf and Doolittle (1997) conducted a phylogenetic analysis of highly conserved, elongation factor 1-alpha (EF-1α) gene sequences and showed that myxomycetes are not fungi. Physiology, morphology, life history, and genetic analysis support the classification of myxomycetes in the Kingdom *Protoctista* along with other eukaryotic microorganisms (Spiegel et al., 2004). With the increasing availability of genetic information, traditional myxomycete taxonomy is being increasingly challenged, and new hypotheses continue to emerge (Walker and Stephenson, 2016). Recently, Cavalier-Smith (2013), using Zoological Nomenclature, proposed a new classification of the myxomycetes based on evolutionary and phylogenetic evidence. Since this classification has not yet been consolidated (Lado and Eliasson, 2017), the classification used by researchers such as Martin and Alexopoulos (1969) and Nannenga-Bremekamp (1991) in their main works is generally used.

Myxomycetes have long attracted the attention of biologists, and always surprised them. Almost 1000 species (Lado, 2005-2022) have been detected worldwide, and 301 species have been identified in Turkey (Baba, 2021; Baba and Sevindik, 2021; Baba et al., 2021). Though Myxomycetes of the Beyşehir district have been studied (Yağız et al., 2002), any myxomycete record in our study

area have been given. A part of this study was presented as a poster presentation at EMC19. The study aims to determine the myxomycetes and species richness along the Konya-Beyşehir road.

2. Material and Method

The study area consists of a region of 80 km along Konya-Beyşehir highway route up to the Beyşehir district border within Konya province in Central Anatolian Region (Fig. 1). The coordinates of the localities are given in the table (Table 1).

Although Beyşehir district is located within the boundaries of Konya province and is takes place in the Central Anatolian Region, it mostly reflects the Mediterranean climate characteristics in terms of precipitation, and continental climate characteristics in terms of temperature conditions. As for the general climatic feature, it belongs to the main Mediterranean climate floor (Kaya and Şimşek, 2014).

Since the study area consisted of forested areas on the highway with a width of 500 m from the road, no sampling was made from the mountainous areas and interior parts of the region. *Cedrus libani* A. Rich, *Pinus nigra* J.F.Arnold, and the members of *Juniperus* L., *Pyrus* L., *Morus* L., *Ulmus* L., *Juglans* L., *Platanus* L., *Quercus* L., *Salix* L. and *Populus* L. are common around Altınapa dam lake (Yıldıztugay et al., 2006). In Urban Forests (Kent Ormanı), the dominant species is *P. nigra*. In addition, there are approximately 180 perennial woody and herbaceous plant species, including *Quercus*, *Juniperus*, *Acacia* Martius, *Populus*, *Crataegus* Tourn. ex L. (Anonymus, 2022). *Salix* and *Populus* trees are also common along the streamsides and orchards.

The study materials were collected during field studies conducted between 2019-2020. In the field, samples from rotten bark and wood, bark of living trees, leaves, needles and pine cones and dungs were collected. For each of the material, which may have spores or plasmodium on them, a collection number was given and placed in a palstic bag or small cardboard box.

Moisture chamber technique was applied to collected the materials (Gilbert and Martin, 1933), and were examined under a stereomicroscope pediodically (every day) and sporocarp developments were recorded. The developing samples were glued together with their substrates to cardboard boxes measuring 4 × 4.5 × 11.5 cm and to the central part of the suitably cut cardboard boxes.

Figure 1. Map of the study area (adopted from Google Earth)

With the aid of a stereomicroscope, features such as the type of sporocarp, colour, dimensions, stalk lengths, calcified structure of peridium (if present), type of peridium opening were determined. Microscopic features such as the spore size, colours under the microscope light, spore ornaments, ornaments of the capillitial threads, their dimensions, presence of columella, calyculus, stalk structure, peridium features, whether the capillitial system is calcified or not were determined under a light microscope. Identification of specimens wer performed using the major works such as Martin and Alexopoulos (1969), Stephenson and Stempen (2000), Neubert et al. (1993; 1995; 2000), Nannenga-Bremekamp (1991) and Poulain et al. (2011).

The names, authors and synonyms of taxa were checked from the online nomenclature information system prepared by Carlos Lado (Lado, 2005-2022). The classification sytem used by Poulain et al. (2011) was followed. In addition, some required information related to the nomenclature of myxomycetes was checked from the website www.mycobank.org, which provides regularly updated data. During identification, digital photographs of both the sporocarp structures and microscopic structures of the samples were also taken.

Table 2. The coordinates of the sample collection sites

Localities	Date	Coordinates
B1 (Altınapa Dam)	01.05.2019 11.06.2020	37°53′06″N 32°18′36″E
B2	11.06.2020	37°52′58″N 32°16′07″E
B3	11.06.2020	37°48′12″N 31°48′36″E
B4	11.06.2020	37°46′07″N 31°47′63″E
B5	11.06.2020	37°52′49″N 31°56′56″E
B6	11.06.2020	37°52′51″N 31°59′51″E
B7 (Konya Urban forest)	01.05.2019 11.06.2020 12.07.2020	37°53′47″N 32°13′53″E

The samples are kept in the Fungarium of the Selçuk University Mushroom Application and Research Centre.

3. Results

Protozoa R. Owen

Myxomycota Whittaker

Myxomycetes G. Winter

Echinosteliales G.W. Martin

Echinosteliaceae Rostaf. ex Cooke

3.1. *Echinostelium minutum* de Bary (Fig. 2a)

Location: B1, on debris barks, 01.05.2019, RB32; B7, on bark of living *Juniperus* sp., 01.05.2019, RB42; B1, near the stream, on debris bark of *Populus* sp., 11.06.2020, RB68; B3, on dried reed, 11.06.2020, RB109; B5, on log branch *Acacia* sp. Martius, 11.06.2020, RB144; B7, on debris twig, 12.07.2020, RB192.

Liceales E. Jahn

Cribrariaceae Corda

3.2. *Cribraria cancellata* (Batsch) Nann.-Bremek. (Fig. 2b)

Location: B7, on stump bark, 12.07.2020, RB192.

3.3. *Cribraria violacea* Rex (Fig. 2c)

Location: B5, on stump wood of *Populus* sp., 11.06.2020, RB140.

Liceaceae Chevall.

3.4. *Licea denudescens* H. W. Keller & T. E. Brooks (Fig. 2d)

Location: B1, on log wood, 11.06.2020, RB66; B1, on bark of living *Populus* sp., 11.06.2020, RB79; B3, on debris bark of *Salix* sp., 11.06.2020, RB110.

Physarales T. Macbr.

Badhamiaceae Locq.

3.5. *Badhamia macrocarpa* (Ces.) Rostaf. (Fig. 2e)

Location: B1, on branch of living tree, 01.05.2019, RB3; B7, on bark of living tree, 01.05.2019, RB38; B7, on debris bark of *Juniperus* sp., 01.05.2019, RB43; B1, on bark of *Salix* sp. log, 11.06.2020, RB72.

3.6. *Badhamia panicea* (Fr.) Rostaf. (Fig. 2f)

Location: B7, on branch of living tree, 01.05.2019, RB53.

Didymiaceae Rostaf. ex Cooke

3.7. *Didymium annulisporum* H. W. Keller& Schokn. (Fig. 2g)

Location: B1, on debris wood, 01.05.2019, RB12; on bark of living *Crataegus* sp. L., 01.05.2020, RB13; B1, on debris bark of *Populus* sp., 11.06.2020, RB70; B4, on debris twig bark of *Populus* sp., 11.06.2020, RB115; B5, on bark of living *Populus* sp., 11.06.2020, RB134; B7, on bark of living tree, 12.07.2020, RB207.

Physaraceae Chevall.

3.8. *Physarum cinereum* (Batsch) Pers. (Fig. 2h)

Location: B7, on bark of living tree, 11.06.2020, RB162.

3.9. *Physarum didermoides* (Pers) Rostaf. (Fig. 2i)

Location: B1, on debris bark, 01.05.2019, RB29; B7, on bark of living tree, 01.05.2019, RB38; B1, near stream, on debris bark of *Populus* sp., 11.06.2020, RB68; B2, on debris twig *Pinus nigra*, 11.06.2020, RB90; B3, on debris twig *Salix* sp., 11.06.2020, RB106; B4, on bark of living *Populus* sp., 11.06.2020, RB114; B4, on bark of living *Populus* sp., 11.06.2020, RB118; B4, on debris bark of *P. nigra*, 11.06.2020, RB126; B5, on bark of living *Populus* sp., 11.06.2020, RB134; B7, on bark of living tree, 11.06.2020, RB162; B7, on debris twig, 12.07.2020, RB173; on debris cone, 12.07.2020, RB225; on log wood, 12.07.2020, RB250.

3.10. *Physarum notabile* T. Macbr. (Fig. 2j)

Location: B1, on bark of living *P. nigra*, 11.06.2020, RB86; B4, on debris bark of *Populus* sp., 11.06.2020, RB118.

Stemonitidales T. Macbr.

Stemonitidaceae Fr.

3.11. *Comatricha nigra* (Pers. ex J. F. Gmel.) J. Schröt. (Fig. 2k)

Location: B7, on debris twig, 12.07.2020, RB184; B7, on bark of living tree, 12.07.2020, RB241.

3.12. *Macbrideola cornea* (G. Lister & Cran) Alexop. (Fig. 2l)

Location: B7, on bark of living of *Juniperus* sp., 01.05.2019, RB42; on debris bark of *Juniperus* sp., 01.05.2019, RB43.

3.13. *Paradiacheopsis fimbriata* (G. Lister & Cran) Hertel ex Nann.-Bremek. (Fig. 2m)

Location: B7, on bark of living *Juniperus* sp., 01.05.2019, RB42.

Trichiales T. Macbr.

Arcyriaceae Rostaf. ex Cokee

3.14. *Arcyria cinerea* (Bull.) Pers. (Fig. 2n)

Location: B7, on log wood, 01.05.2019, RB40; B5, on log wood of *Populus* sp., 11.06.2020, RB140; B7, on log wood, 12.07.2020, RB250.

3.15. *Arcyria pomiformis* (Leers) Rostaf. (Fig. 2o)

Location: B7, on debris wood, 12.07.2020, RB185; RB190; RB196; B7, on debris cone, 12.07.2020, RB198.

Trichiaceae Chevall.

3.16. *Arcyodes incarnata* (Alb. & Schwein.) O. F. Cook (Fig. 2p)

Location: B1, on debris bark of *Salix* sp., 11.06.2020, RB73.

3.17. *Perichaena chrysosperma* (Curr.) Lister (Fig. 2r)

Location: B7, on debris wood, 01.05.2019, RB36; on stump wood, 01.05.2019, RB40.

3.18. *Perichaena depressa* Lib. (Fig. 2s)

Location: B1, on stump wood, 01.05.2019, RB23; on debris bark, 01.05.2019, RB29; on stump wood, 01.05.2019, RB31; on stump bark of *Populus* sp., 01.05.2019, RB33; B7, on debris wood, 01.05.2019, RB36;

B1, on debris bark of *Populus* sp., 11.06.2020, RB65; B1, near the stream, on debris wood of *Populus* sp., 11.06.2020, RB67; B1, on debris twig bark of *Populus* sp., 11.06.2020, RB70; B1, on debris bark of *Salix* sp., 11.06.2020, RB73; B1, on debris bark of *Populus* sp., 11.06.2020, RB85; B3, on bark of living *Populus* sp., 11.06.2020, RB112; B4, on debris bark of *Populus* sp., 11.06.2020, RB118; B5, on bark of living *Populus* sp., 11.06.2020, RB134; B5, on log twig of *Acacia* sp., 11.06.2020, RB144; B6, on root bark of *Acacia* sp., 11.06.2020, RB149; B7, on debris twig, 12.07.2020, RB242; on bark of dead tree, 12.07.2020, RB247.

3.19. *Perichena quadrata* T. Macbr. (Fig. 2t)

Location: B1, on debris bark of *Populus* sp., 11.06.2020, RB83.

3.20. *Perichaena vermicularis* (Schwein.) Rostaf. (Fig. 2u)

Location: B7, on bark of living tree, 01.05.2019, RB38; on bark of living *Juniperus occidentalis* Hooker, 01.05.2019, RB41; on bark of living of *Juniperus* sp., 01.05.2019, RB42; B1, on log bark of *Populus* sp., 11.06.2020, RB69; B4, on bark of *Populus* sp., 11.06.2020, RB118; B6, on twig of living *J. communis* L., 11.06.2020, RB146; B6, on root bark of *Acacia* sp., 11.06.2020, RB149.

3.21. *Trichia lutescens* (Lister) Lister (Fig. 2v)

Location: B6, on twig of living *J. communis*, 11.06.2020, RB146; B7, on twig of living *J. communis*, 11.06.2020, RB160.

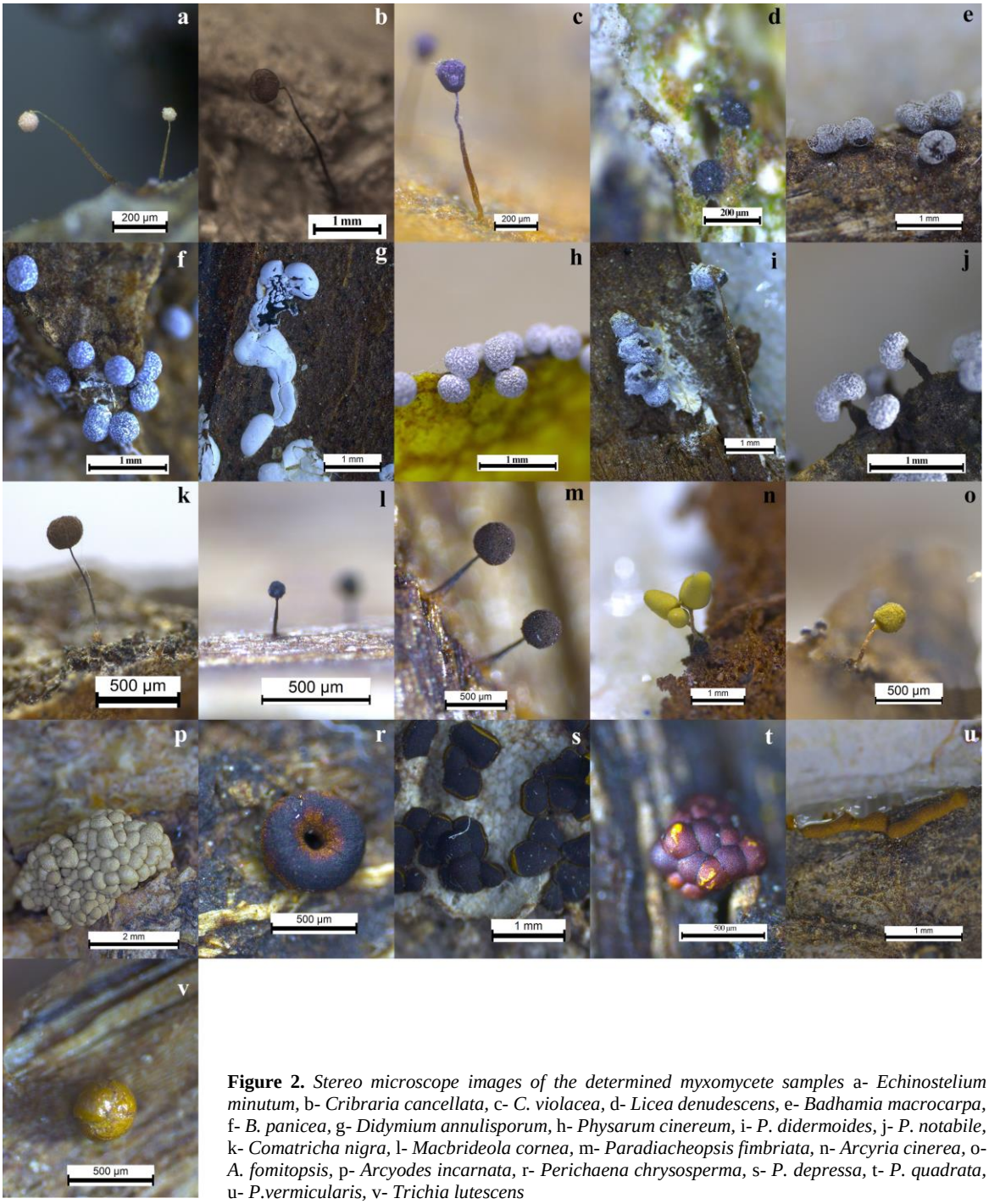

Figure 2. *Stereo microscope images of the determined myxomycete samples* a- *Echinostelium minutum*, b- *Cribraria cancellata*, c- *C. violacea*, d- *Licea denudescens*, e- *Badhamia macrocarpa*, f- *B. panicea*, g- *Didymium annulisporum*, h- *Physarum cinereum*, i- *P. didermoides*, j- *P. notabile*, k- *Comatricha nigra*, l- *Macbrideola cornea*, m- *Paradiacheopsis fimbriata*, n- *Arcyria cinerea*, o- *A. fomitopsis*, p- *Arcyodes incarnata*, r- *Perichaena chrysosperma*, s- *P. depressa*, t- *P. quadrata*, u- *P.vermicularis*, v- *Trichia lutescens*

4. Discussions

As a result of the field and laboratory studies, a total of 80 myxomycetes specimens were developed and of 21 myxomycete taxa were identified. No mature sporocarp was found in its natural environment. All specimens were developed using the moist chamber technique. Most specimens remained in the plasmodium stage, and the development of some myxomycete samples were prevented by invertebrate larvae.

The distribution of 21 determined species to the orders, is as follows: *Trichiales* 8 (38.1%), *Physarales* 6 (28.57%), *Liceales* 3 (14.29%), *Stemonitidales* 3 (14.29%) and *Echinosteliales* 1 (4.76%) (Fig. 3).

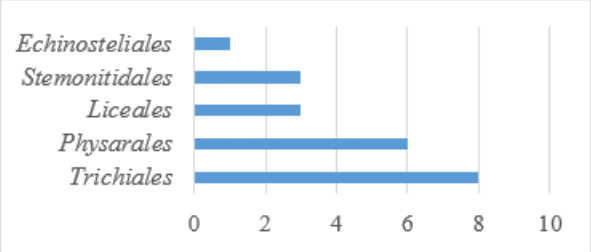

Figure 3. Order wise distribution of the determined taxa

Trichiales seems to be the most crowded order in the region. The order was represented with two families *Arcyriaceae* 2 (9.5%) and *Trichiaceae* 6 (28.57%) in the region. Within *Arcyriaceae, A. cinerea* was detected on two different substrates, and was detected on four different substrates. Within the family *Trichiaceae*, *Perichaena* is the most crowded genus with 4 species, among which *P. depressa* was the most abundant species which were detected on 17 different substrates.

Members of the order *Physarales* were distributed to two families, *Didymiaceae* and *Physaraceae*. In the region *Didymiaceae* was represented with only one (4.76%) species while *Physaraceae* was represented with 5 (23.81%) species. *Physarum didermoides* was found to be the second abundant taxon in research area and detected on 13 different substrates.

Twenty seven (33.75%) of the determined species were detected on debris bark, 20 (25%) on bark of living tree, 8 (10%) on log bark, 2 (2.5%) on root bark, 8 on log wood (10%), 7 (8.75%) on debris twigs, 2 (2.5%) on branches of living tree, 3 (3.75%) on cones, 2 (2.5%) on debris wood, and 1 (1.25%) on dried reeds (Fig. 4). As total, 71.25% of the samples were detected on bark and 12.5% on wood.

As it is the general case, 71.25% of the samples were corticolous myxomycetes detected on the bark. The bark of the branches and trunks of trees (Ing, 1994) are suitable substrates for myxomycetes (Snell and Keller, 2003; Liu et al., 2015; Policina and dela Cruz, 2020). The water holding capacity of the bark, the general shape of the tree, the surface texture of the bark, and the epiphytic cover of algae, moss, liverwort and lichens (Everhart and Keller, 2008, Stephenson and Stephen, 2000) facilitate colonization by more species (Keller and Braun, 1999).

When compared the results of this study show similarities and also some differences with those conducted in close environs and in Turkey (Ergül and Dülger, 2002; Ergül et al., 2005; Yağız and Afyon, 2005; Oran et al., 2006; Eroğlu and Kaşık, 2013a,b; Ocak and Konuk, 2018; Touray and Ergül, 2019).

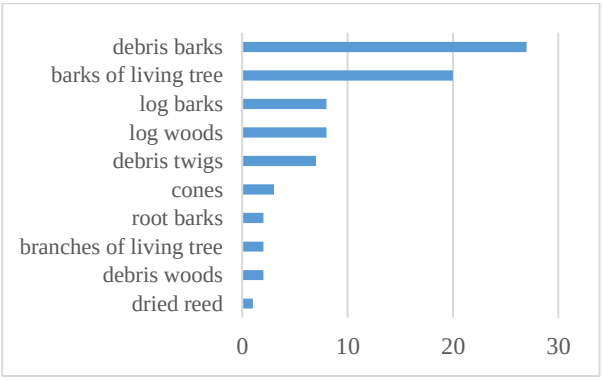

Figure 4. Substrate wise distribution of the determined taxa

Echinostelium minutum, the most common corticolous myxomycete species (Snell and Keller, 2003; Everhart and Keller, 2008), was determined for the first time on *Juniperus* and *Populus* spp. in Turkey while *Cribraria violacea* was also determined by Eroğlu and Kaşık (2013a) on *Populus* sp., and on rotten wood. Existance of this species on on *Acacia* sp. was also reported by Oran et al. (2006).

Occurrence of *Physarum didermoides* on three barks was reported by many researchers (Demirel and Kaşık, 2012; Eroğlu and Kaşık, 2013a), *P. notabile* (Ergül and Dülger, 2002; Ergül et al., 2005; Eroğlu and Kaşık, 2013b; Ocak and Konuk, 2018; Touray and Ergül, 2019).

Members of *Macbrideola* (Stephenson and Stephen, 2000) and *Paradiacheopsis* (Ing, 1994) species are common on tree bark. As it is the case in many studies *M. cornea* (Härkönen and Uotila, 1983; Härkönen, 1987; Ergül et al., 2005; Ocak and Hasenekoğlu, 2005; Yağız and Afyon, 2005; Oran et al., 2006; Yağız and Afyon, 2007; Baba, 2012; 2015; Oskay and Tüzün, 2015; Baba, 2017; Baba et al., 2018; Ocak and Konuk, 2018; Touray and Ergül, 2019; Baba et al., 2020; Baba and Sevindik, 2020) and *P. fimbriata* (Härkönen and Uotila, 1983; Härkönen, 1987; Baba and Tamer, 2008; Baba et al., 2012) were determined on the barks of *Juniperus* sp.

Perichaena depressa, which was detected on *Populus* and *Salix* spp., had the same substratec in Eroğlu and Kaşık (2013a). Additionally, we determined it on *Acacia* sp. as well.

Unlike other studies (Gücin and Öner, 1986; Demirel et al., 2006; Baba and Tamer, 2008; Baba et al., 2013; Baba and Zümre, 2015; Çağlar et al., 2016; Baba and Doğan, 2018; Zümre et al., 2019)., *Trichia lutescens* was detected on *Juniperus* sp.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

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