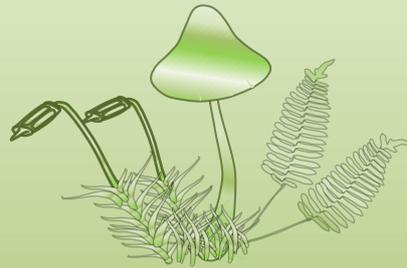


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(In memory of Prof. Dr. Kenan Demirel)

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In Vitro rapid propagation of an aquatic plant *Pogostemon erectus* (Dalzell) Kuntze

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Sucul bir bitki olan *Pogostemon erectus* (Dalzell) Kuntze'un In Vitro hızlı çoğaltımı

Abstract: The aim of this study is to investigate the multiple and rapid production of *Pogostemon erectus* (Dalzell) Kuntze by tissue culture techniques. The shoot tip and nodal explants of *P. erectus* were isolated and then cultured in Murashige and Skoog (1962) (MS) nutrient media containing thidiazuron (TDZ) and 2,4-Dichlorophenoxyacetic acid (2,4-D) in different combinations. The first shoot formation on the explants was observed on day 15. The number of shoots per explant ranged from 2.85 to 29.39 in the shoot tip explants and from 2.72 to 32.24 in the nodal explants. The maximum number of shoots per explant was obtained in the MS medium containing 0.20 mg/L TDZ + 0.10 mg/L 2,4-D for shoot tip explant (29.39) and in the MS medium containing 0.10 mg/L TDZ + 0.10 mg/L 2,4-D for nodal explant (32.24). The longest shoots in the shoot tip (1.76 cm) and nodal (1.64 cm) explants were determined in MS medium supplemented with 0.10 mg/L TDZ + 0.10 mg/L 2,4-D. Regenerated shoots were rooted in MS medium supplemented with 0.25 mg/L indol-3yl acetic acid (IAA), and then acclimatized to aquarium conditions.

Key words: In vitro, *Pogostemon erectus*, Shoot regeneration, TDZ, Tissue culture

Özet: Bu çalışmanın amacı, *Pogostemon erectus* (Dalzell) Kuntze'un doku kültürü teknikleri ile çoklu ve hızlı üretimini araştırmaktır. *P. erectus*'un sürgün ucu ve nodal eksplantları izole edilmiş ve daha sonra farklı kombinasyonlarda thidiazuron (TDZ) ve 2,4-Diklorofenoksiasetik asit (2,4-D) içeren Murashige ve Skoog (1962) (MS) besin ortamında kültüre alınmıştır. Eksplantlardaki ilk sürgün oluşumu 15. günde gözlenmiştir. Eksplant başına sürgün sayısı, sürgün ucu eksplantlarında 2.85 ila 29.39 ve nodal eksplantlarında 2.72 ila 32.24 arasında sıralanmıştır. Eksplant başına maksimum sürgün sayısı sürgün ucu eksplantı için (29.39) 0.20 mg/L TDZ + 0.10 mg/L 2,4-D içeren MS ortamında ve nodal eksplant için 0.10 mg/L TDZ + 0.10 mg/L 2,4-D içeren MS ortamında (32.24) elde edilmiştir. En uzun sürgünler, sürgün ucu (1.76 cm) ve nodal (1.64 cm) eksplantlarında 0.10 mg/L TDZ + 0.10 mg/L 2,4-D ile desteklenmiş MS ortamında belirlenmiştir. Rejenere sürgünler, 0.25 mg/L Indol-3-asetik asit (IAA) ile takviye edilmiş MS ortamında köklendirilmiş ve ardından akvaryum koşullarına alıştırmıştır.

Anahtar Kelimeler: In vitro, *Pogostemon erectus*, Sürgün rejenerasyonu, TDZ, Doku kültürü

1. Introduction

Plant tissue culture is defined as the growth of plant parts isolated from the main plant in an artificial nutrient medium under sterile conditions. This technique is mainly due to the totipotency of the plant cell. Totipotency is the ability of a single cell to generate whole genome with cell divisions (Neumann et al., 2009; Hussain et al., 2012).

The plant tissue culture medium should contain all the nutrients needed to grow a plant normally. This nutrient medium is mainly; macronutrients, micronutrients, vitamins, other organic components, plant growth regulators, carbon source and some gelling agents (Murashige and Skoog, 1962). Murashige and Skoog (MS) nutrient media are the most used nutrient medium for *in vitro* production of many plant species. Similarly, *Lachenalia viridiflora* (Kumar et al., 2016), *Rotala rotundifolia* (Buch-Ham. ex Roxb) Koehne (Dogan, 2017a), *Urginea altissima* (L.f.) Baker (Baskaran et al., 2017), *Ceratophyllum demersum* L. (Emsen and Dogan, 2018), *Brassica napus* L. (Nazi et al., 2018), *Ophiorrhiza mungos* L. var. *angustifolia* (Thw.) Hook. f. (Krishnan et al., 2018), *Hybanthus enneaspermus* (L.) F. Muell. (Shekhawat and Manokari, 2018) were propagated using the MS nutrient medium.

The pH of the nutrient medium significantly affects the activities of plant growth regulators and plant growth. For this reason, the pH of the nutrient medium is adjusted between 5.4 and 5.8. In addition, plant parts, plant growth regulators and nitrogen sources in the nutrient media are also very important for shoot regeneration. Auxins, cytokinins and gibberellins are the most commonly used plant growth regulators. The variety and concentration of the plant growth regulator may differ according to the cultivated plant species and explant varieties (Hussain et al., 2012). In addition, the correct selection of explant types (shoot tip, node, internode, etc.) has an important effect on the success of tissue culture (George et al., 2008).

The purpose of this study is to propagate rapid and multiple production of tissue culture techniques of *Pogostemon erectus* (Dalzell) Kuntze. Thus, the shoot tip and nodal explants of *P. erectus* were transferred to culture media containing different thidiazuron (TDZ) and 2,4-Dichlorophenoxyacetic acid (2,4-D) combinations. The effects of these hormones on *in vitro* production have been examined.

2. Materials and Method

Pogostemon erectus (Dalzell) Kuntze's surface sterilization has been sterilized according to the procedure previously done by Dogan (2017b). Sterilized shoot tip and nodal explants were cultured in MS (Murashige and Skoog, 1962) basal medium containing vitamins (Table 1) at 24°C for 16 hours in light and 8 hours in dark photoperiod. The shoot tip and nodal explants from 4 week old plants grown in this culture medium were transferred to MS medium supplemented with 0.10 mg/L 2,4-Dichlorophenoxyacetic acid (2,4-D) and 0.10, 0.20, 0.40 and 0.80 mg/L thidiazuron (TDZ) combinations.

Table 1. The content of Murashige and Skoog (1962) basic nutrient medium

Components		Concentrations (mg/L)
Macroelements	NH ₄ NO ₃	1650.000
	KNO ₃	1900.000
	CaCl ₂ .2H ₂ O	440.000
	MgSO ₄ .7H ₂ O	370.000
	KH ₂ PO ₄	170.000
Microelements	KI	0.830
	H ₃ BO ₃	6.200
	MnSO ₄ .4H ₂ O	22.300
	ZnSO ₄ .7H ₂ O	8.600
	Na ₂ MoO ₄ .2H ₂ O	0.250
	FeSO ₄ .7H ₂ O	27.850
	CoCl ₂ .6H ₂ O	0.025
	CuSO ₄ .5H ₂ O	0.025
	Na ₂ EDTA.2H ₂ O	37.250
Vitamins	Myo-Inositol	100.000
	Nicotinic Acid	0.500
	Pyrotinic Acid	0.500
	Thiamine-HCl	0.100
	Glycine	2.000

MS medium, vitamins, 3% sucrose and 0.65% agar were used in all culture media. Distilled water was used for the preparation of the nutrient medium. The pH of the nutrient medium was adjusted to 5.7±1 using 1N NaOH and 1N HCl, followed by sterilization at 1.2 atmospheres pressure and at 120°C for 20 minutes. In the experiments, the explants were incubated under a white LED (Light-Emitting Diode) light (1500 lux) at a temperature of 24°C and a 16 hour light photoperiod.

Regenerated shoots were transferred in culture medium containing 0.25 mg/L indole-3- acetic acid (IAA) for *in vitro* root formation, and then transferred to the aquarium environment to acclimate to external conditions. The aquarium conditions are set at 24°C temperature and 16 hours lighting. Also liquid fertilizer was added to the aquarium water.

Experiments were carried out in 100x10 mm petri dishes in 3 replicates. The data obtained from the study were analyzed using the SPSS 21 for Windows (Statistical Package for the Social Sciences) program. Duncan tests

were applied for Post Hoc tests. Percent values were subjected to arcsin transformation prior to statistical analysis (Snedecor and Cochran, 1967).

3. Results and Discussion

The correct choice of explant variety in tissue culture studies has an important effect on the success of the study. For this reason, researchers have experimented with different explant varieties such as shoot tip (Sasidharan and Jayachitra, 2017; Bourrain, 2018), node (Dobranski et al., 2017; Irshad et al., 2018), internode (Thul and Kukreja, 2010; Zhang et al., 2017), leaf (Mahindrakar et al., 2018) and root (Sharma et al., 2017).

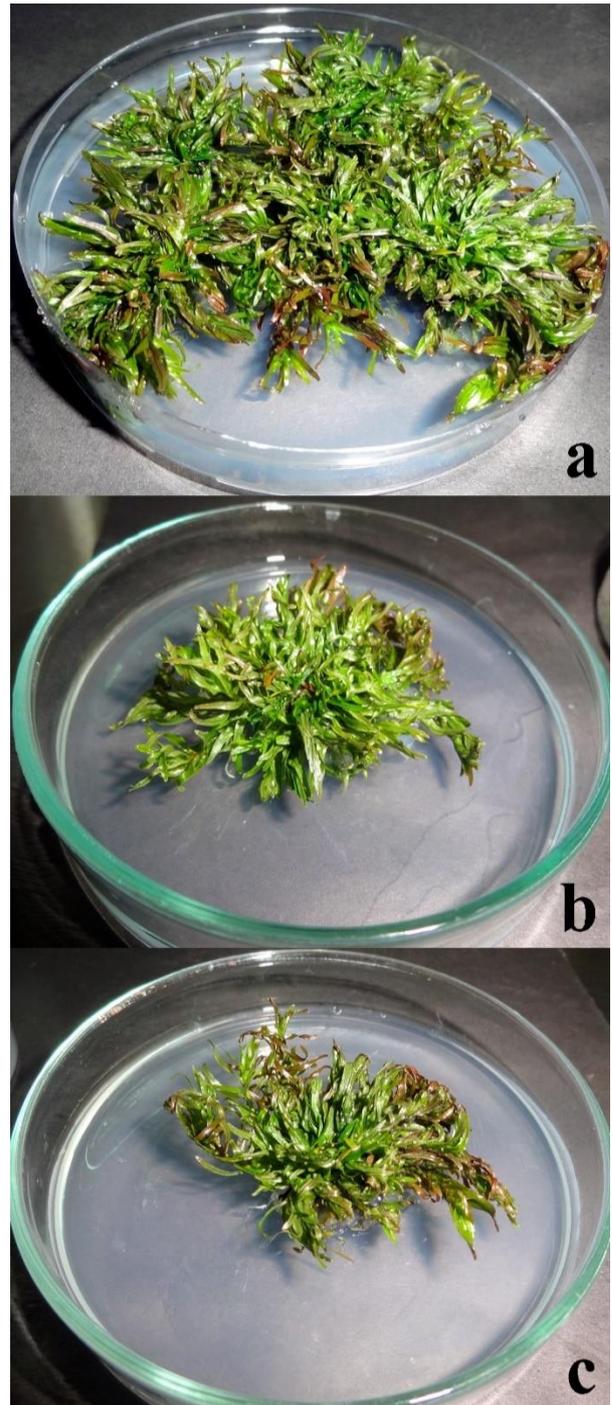


Figure 1. *In vitro* plant regeneration of *P. erectus* (a). Multiple shoot regeneration from shoot tip (a) and nodal (b) explants after 8 weeks of culture

Table 2. Analysis of variance of shoot tip and nodal explants of *P. erectus* in MS medium containing different TDZ and 2,4-D

Source of variance	Degree of freedom	Shoot regeneration frequency (%)		Mean number of shoots per explants		Mean shoot length (cm)	
		Mean square	F value	Mean square	F value	Mean square	F value
Shoot tip							
Medium	4	2518.76	33.99**	356.41	80.92**	0.08	9.28**
Error	10	74.104	-	4.405	-	0.01	-
General Total	14	-	-	-	-	-	-
** Significant at $p < 0.01$ level							
Nodal							
Medium	4	2102.01	28.37**	427.80	70.56**	0.03	18.62**
Error	10	74.10	-	6.06	-	0.002	-
General Total	14	-	-	-	-	-	-
** Significant at $p < 0.01$ level							

In the present study, shoot tip and nodal explants of *P. erectus* were cultured in MS medium containing 0.10-0.80 mg/L TDZ + 0.10 mg/L 2,4-D for rapid and multiple production under *in vitro* conditions. As a control group, these explants were also cultured in hormone-free MS nutrient medium. Similarly, the effect of TDZ hormone on *in vitro* production has been previously reported in plants such as *Aronia mitschurinii* (Mahoney et al., 2018) and *Rauvolfia tetraphylla* (L.) (Hussain et al., 2018).

After 15 days, regenerated shoots began to spread in culture media and multiple shoot formation was observed after four weeks. After eight weeks (Figure 1 a, b, c), the experiment was terminated and variance analysis was applied for percentage of shoot regeneration, number of shoots per explant and length of shoot (Table 2).

As seen in the analysis of variance, the statistically significant differences in the both explant types were found at $p < 0.01$ level for shoot regeneration percentage, number of shoots per explant and shoot length (Table 2). The Duncan test results for determining the significance level of this difference are given in Figure 2.

The percentage of shoot regeneration of the shoot tip and nodal explants ranged from 33.31% to 100.00% and 38.89% to 100.00%, respectively (Figure 2 A and B). 100% shoot regeneration frequency was reached in MS medium containing 0.10, 0.20 and 0.40 mg/L TDZ + 0.10 mg/L 2,4-D in the both explants. The lowest shoot regeneration percentages in culture media containing plant growth regulator were detected in MS medium containing 0.80 mg/L TDZ + 0.10 mg/L 2,4-D. In general, the shoot tip explants had a higher percentage of shoot regeneration than the nodal explants.

The number of shoots per explant ranged from 2.85 to 29.39 in the shoot tip explants (Figure 2 C) and from 2.72 to 32.24 in the nodal explants (Figure 2 D). The maximum number of shoots per explant was obtained in the MS medium containing 0.20 mg/L TDZ + 0.10 mg/L 2,4-D for shoot tip (29.39), in the MS medium containing 0.10 mg/L TDZ + 0.10 mg / L 2,4-D for nodal explant (32.24). In culture media containing hormones, the least number of shoots were recorded in MS medium supplemented with 0.80 mg/L TDZ + 0.10 mg/L 2,4-D in both explant types. The results showed that the increase in TDZ ratio used in

the culture medium decreased shoot numbers from explants. Generally, nodal explants give higher number of shoots than shoot tip explants.

The length of shoots from the explants varied with the effects of growth regulators and statistically significant at $p < 0.05$ level. In the medium containing growth regulators, the longest shoots for shoot tip explant was recorded as 1.76 cm in MS medium supplemented with 0.10 mg/L TDZ + 0.10 mg/L 2,4-D in the shoot explant, followed by in the MS medium supplemented with 0.20 mg/L TDZ + 0.10 mg/L 2,4-D (1.73 cm) (Figure 2 E). Short shoots were obtained in the nodal explant compared to the shoot tip explants. The highest shoot lengths for nodal explant (1.64 cm) were determined in MS medium supplemented with 0.10 mg/L TDZ + 0.10 mg/L 2,4-D, followed by in the MS medium supplemented with 0.20 mg/L TDZ + 0.10 mg/L 2,4-D (1.55 cm) (Figure 2 F).

The high rate of use of TDZ in culture media has adversely affected shoot length. Similar results were observed in *Pyrus pyrifolia* (Kadota and Niimi, 2003), *Vitex trifolia* (Ahmed and Anis, 2012) and *R. tetraphylla* (Hussain et al., 2018) plants, which were left in the TDZ medium for a long time. It has been reported that this inhibitory effect of TDZ is highly cytokine-like activity and may be due to the presence of the phenyl group in TDZ (Huetteman and Preece, 1993).

Regenerated shoots were transferred in culture medium containing 0.25 mg/L IAA for *in vitro* root formation. After four weeks, intense root formation was detected. The rooted plants are then left to aquarium conditions to acclimate to external conditions. Extensions were observed in the plant length and in the leaves after two weeks. At the end of four weeks the plants were successfully adapted to external conditions (100% survival). As the MS basal medium was carefully removed from the plants, no contamination was detected in the aquarium environment. Similarly, the acclimatization of plants propagated *in vitro* to external conditions have been reported in *Alternanthera sessilis* (L.) (Gnanaraj et al., 2011), *R. rotundifolia* (Dogan, 2017), *Pisum sativum* L. (Sharma et al., 2017), *Monochasma savatieri* Franch ex Maxim (Zhang et al., 2017) and *Enicostema axillare* (Sasidharan and Jayachitra, 2017).

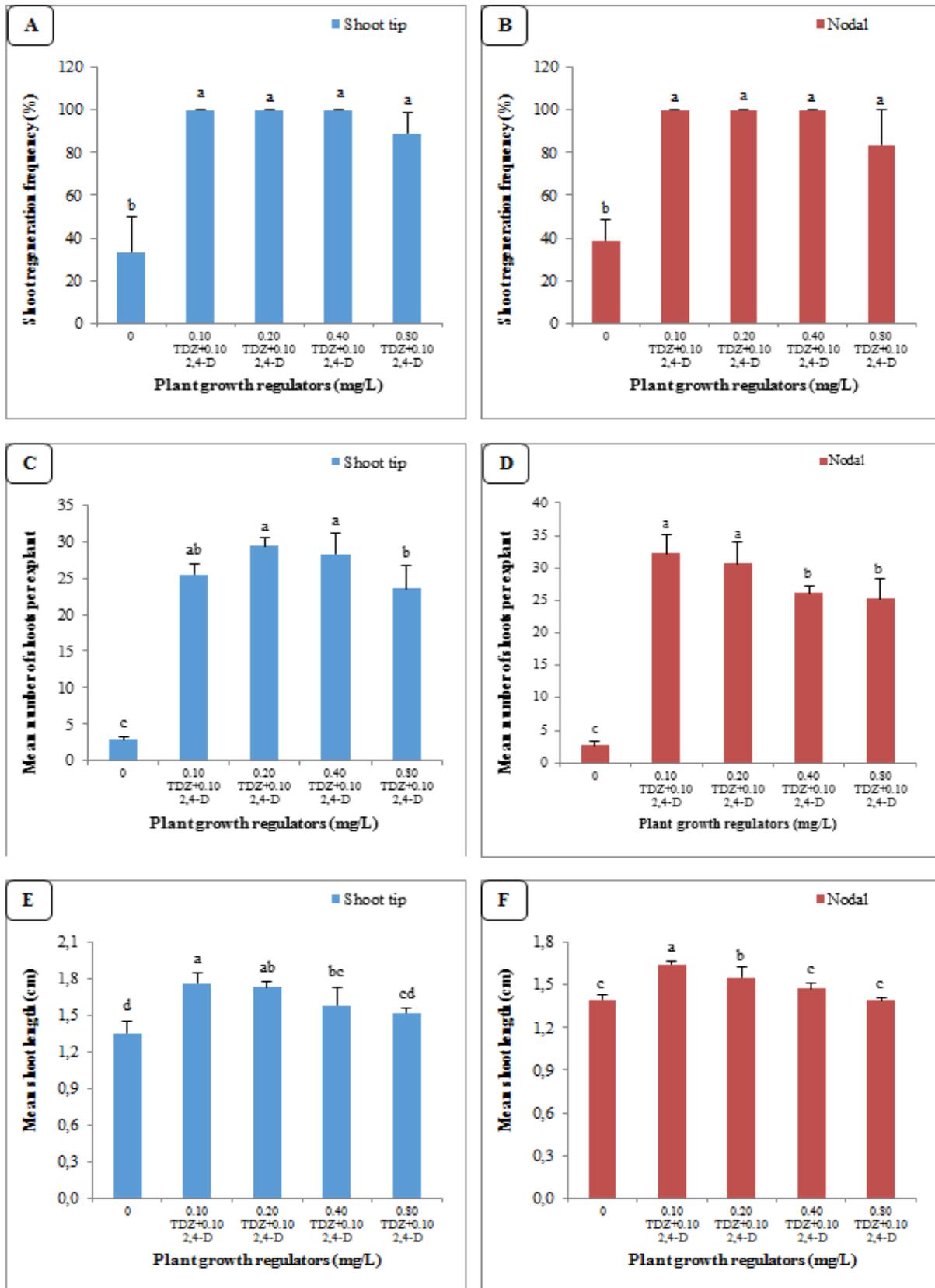


Figure 2. Effect of different combinations of TDZ and 2,4-D on *in vitro* shoot regeneration from shoot tip and nodal explants of *P. erectus* after eight weeks of culture. All values are the means of triplicates \pm SD (n = 3). Vertical bars indicate standard error of three separate experiments. Different letters indicate significantly different values (DMRT, $p < 0.05$)

Consequently, multiple shoot formation from shoot and nodule explants of *P. erectus* was successfully achieved in culture media containing different TDZ and 2,4-D combinations. The best results for the average number of

shoots were found in the lower combinations of TDZ and 2,4-D. This work presents an important protocol for the mass production of this plant. It can also help with gene transfer studies with this plant in the future.

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Edible macrofungi determined in Gürpınar (Van) district

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Gürpınar (Van) yöresinde belirlenen yenilebilir makromantarlar

Abstract: This study was carried out on wild edible macrofungi samples, collected within the boundaries of Gürpınar (Van) district between 2016 and 2017. As a result of necessary investigations related to macro and micromorphologies of the samples, 36 wild edible macrofungi species belonging to 11 families and 4 orders were determined. Eight of them belong to the phylum Ascomycota and 28 to Basidiomycota. Among the determined taxa *Agaricus bisporus*, *A. campestris*, *A. urinascens*, *Pleurotus eryngii*, *P. ostreatus* and *P. populinus* are collected and consumed by locals. *Pleurotus eryngii* is the only economically important macrofungi in the region.

Key words: Biodiversity, edible mushrooms, Eastern Anatolia, Turkey

Özet: Bu çalışma 2016 ve 2017 yılları arasında Gürpınar (Van) ilçe sınırları içinde kalan bölgeden toplanan yabani yenilebilir makromantar üzerinde gerçekleştirilmiştir. Örneklerin makro ve mikromorfolojilerine ilişkin gerekli incelemeler sonucunda, 4 ordo ve 11 familyaya ait 36 yenilebilir yabani makromantar türü belirlenmiştir. Bunlardan 8 tanesi Ascomycota bölümüne, 28 tanesi ise Basidiomycota bölümüne aittir. Belirlenen taksonlardan *Agaricus bisporus*, *A. campestris*, *A. urinascens*, *Pleurotus eryngii*, *P. ostreatus* ve *P. populinus* yerel halk tarafından toplanıp tüketilmektedir. Yörede ekonomik öneme haiz tek tür ise *Pleurotus eryngii*'dir.

Anahtar Kelimeler: Biyoçeşitlilik, yenilebilir mantarlar, Doğu Anadolu, Türkiye

1. Introduction

Turkey is a very rich country in terms of naturally growing macrofungal species, due to its unique flora and climatic conditions (Dündar et al., 2016), and currently comprises more than 2500 macrofungi (Kaya and Uzun, 2018). Fungi are an important group of organism for nature, and have a crucial role in recycling the organic matter. Beside the traditional consumption of naturally growing mushrooms as an important part of human diet, they are also used as a source of bioactive metabolites (Acharya et al., 2018). They are also emphasized by many researchers as valuable food source due to their fatty acid, vitamin, fiber, carbohydrate, protein and mineral contents (Demirbaş, 2001; Owaid et al., 2007; Türkekul, 2017). Because of their medical benefits, mushrooms have been consumed for centuries in many far eastern countries such as China and Japan (Manzi et al., 2001) as well as in many European and American countries (Boa, 2004). The findings of recent studies also indicate that many macrofungi accumulate heavy metals, radioactivity and some other toxic materials in their fruit bodies (Mat, 2000; Isıldak et al., 2004; Soylak et al., 2005; Kalać, 2010; Uzun et al., 2011; Kaya and Bağ, 2013; Korkmaz et al., 2016; Karapınar et al., 2017; Türkmen and Budur, 2018).

Gürpınar, where the research was conducted, is a district of Van province in Eastern Anatolian Region of Turkey. The district is the largest one in Turkey with a surface area of 4.063 km² (Anonymus, 2018), and surrounded by Central district of Van and Özalp to the north, Gevaş and Çatak to the west, Hakkari to the south and Başkale (Van) to the east (Fig. 1). The research area lies within the Irano-Turanian phytogeographical flora sector. Steppe vegetation is the dominant one in the region with distinct

Astragalus L. populations. *Quercus* L., *Pinus* L. and some *Juniperus* L. species form rare and mixed populations at higher elevations (Atalay, 1989). Along stream banks, *Eleagnus* Hill., *Populus* L., *Malus* Mill., *Prunus* L., *Pyrus* L. and *Salix* L. trees form small populations.

The climate of the research area is Mediterranean with an annual rainfall of 281mm and an annual average temperature of 8.1 °C (Bani and Adıgüzel, 2008).

The study aims to determine the edible macrofungal composition and local consumption of them in the region, and to make a contribution to the mycobiota of Turkey.

2. Materials and Method

The research material was collected from suitable localities (Table 1) within the boundaries of Gürpınar (Van) district between the years 2016 and 2017. During field studies, macrophotographs of the macrofungi samples were taken at their natural habitats, and the diagnostic, descriptive and ecological characteristics were recorded. Local edibility status of the collected samples were also investigated by asking the local people. Then the samples were transferred to the laboratory. Macroscopic and microscopic measurements and chemical investigations were performed in the laboratory. The specimens were identified by comparing the obtained data with the relevant literature (Moser, 1983; Breitenbach and Kränzlin, 1984-2000; Cappelli, 1984; Buczacki, 1989; Jordan, 1995; Bessette et al., 1997; Kränzlin, 2005; Phillpis, 1991, 2006; Besette et al., 2007; Garnweidner, 2010; Beug et al., 2014; Kuo and Methven, 2014). The determined macrofungi samples are kept in the fungarium of Biology Department, Science Faculty, Van Yüzüncü Yıl University.



Figure 1. Map of the research area (modified from google earth)

3. Results

The list of the determined edible taxa, are given together with their habitats, collection localities, collection dates and the personal voucher numbers. The systematics of the taxa are in accordance with Kirk et al. (2008) and Index fungorum (accessed on 10 December 2018).

Ascomycota Whittaker

Pezizomycetes O.E. Erikss. & Winka

Pezizales J. Schröt.

Helvellaceae Fr.

1. *Helvella acetabulum* (L.) Quéf.: Among needle litter under *Pinus* L. sp. trees, locality 14, 18.5.2015, Şelem 71.
2. *Helvella lacunosa* Afzel: Among leaf litter under *Populus* sp. trees, locality 14, 5.6.2016, Şelem 132.
3. *Helvella leucopus* Pers.: Among leaf litter under *Populus* sp. trees, locality 14, 18.5.2015, Şelem 116.
4. *Paxina queletii* (Bres.) Stangl: Under *Salix* sp. trees, locality 14, 5.10.2016, Şelem 131.

Morchellaceae Rchb.

5. *Mitrophora semilibera* (DC.) Lév.: Among leaf litter under *Populus* sp. trees, locality 14, 18.05.2015, Şelem 63; under *Salix* sp. trees, locality 6, 05.10.2016, Şelem 172.
6. *Morchella elata* Fr.: Among leaf litter under *Populus* sp. trees, locality 11, 03.06.2016, Şelem 230.
7. *Morchella esculenta* (L.): Pers.: Among leaf litter under *Populus* sp. trees, locality 11, 03.06.2016, Şelem 83; under *Salix* sp. trees, locality 9, 08.05.2015, Şelem 11.
8. *Morchella esculentoides* M. Kuo, Dewsbury, Moncalvo & S.L.Stephenson: Among leaf litter under *Populus* and

Salix sp. trees, locality 11, 03.06.2016, Şelem 96.

9. *Morchella prava* Dewsbury, Moncalvo, J.D. Moore & M.Kuo: Among leaf litter under *Populus* and *Salix* sp. trees, locality 11, 03.06.2016, Şelem 94.

Basidiomycota Whittaker ex Moore

Agaricales Underw.

Agaricaceae Chevall.

10. *Agaricus bisporus* (J.E. Lange) Imbach : Among grass in meadow, locality 9, 18.05.2015, Şelem 98.
11. *Agaricus campestris* L.: Among grass in meadow, locality 13, 18.05.2015, Şelem 21.
12. *Agaricus urinascens* (Jul. Schäff. & F.H.Møller) Singer: Among grass in meadow, locality 12, 5.10.2016, Şelem 316.
13. *Bovista plumbea* Pers.: Among grass in meadow, locality 10, 30.05.2017, Şelem 360.
14. *Coprinus comatus* (O.F. Müll.) Pers.: Among grass in meadow, locality 30, 18.05.2015, Şelem 51; locality 19, 03.06.2016, Şelem 248; under *Salix* sp. trees, locality 24, 03.06.2016, Şelem 225.

Hymenogastraceae Vittad.

15. *Psilocybe coronilla* (Bull.) Noordel. : Among grass in meadow, locality 9, 18.05.2015, Şelem 92; locality 36, 18.11.2015, Şelem 196.

Pleurotaceae Kühner

16. *Pleurotus eryngii* (DC.) Quéf.: On *Ferula* sp. remains, locality 33, 03.06.2016, Şelem 242; locality 18, 28.06.2017, Şelem 354; locality 1, 13.06.2017, Şelem 365; locality 2, 13.06.2017, Şelem 375; locality 7, 13.06.2017,

Table 1. Macrofungi collection localities

Locality No	Locality name	Coordinates	Altitude
1	Albenek village	N 38° 16.015'; E 43° 37.050'	2022 m
2	Alniak village	N 38° 13.126'; E 43° 42.047'	2502 m
3	Bozyiğit village	N 38° 21.965'; E 43° 34.098'	1845 m
4	Çörekli village	N 38° 21.147'; E 43° 48.146'	2001 m
5	Between Çörekli- Cevizalan villages	N 38° 21.855'; E 43° 48.062'	2025 m
6	Erkaldı village	N 38° 21.842'; E 43° 33.202'	1809 m
7	Giyimli village	N 38° 12.040'; E 43° 47.090'	2322 m
8	Günbaşı village	N 38° 18.300'; E 43° 43.051'	2168 m
9	Gürpınar central county	N 38° 19.820'; E 43° 25.010'	1745 m
10	Gürpınar central county	N 38° 19.534'; E 43° 23.867'	1751 m
11	Around Gürpınar	N 38° 17.929'; E 43° 45.243'	2010 m
12	Güzelsu village	N 38° 18.960'; E 43° 48.130'	1980 m
13	Güzelsu village	N 38° 17.925'; E 43° 48.828'	1996 m
14	Güzelsu village	N 38° 19.303'; E 43° 47.973'	1977 m
15	Güzelsu village, near castle	N 38° 19.139'; E 43° 47.991'	1986 m
16	Hacıköy village	N 38° 18.093'; E 43° 38.100'	2205 m
17	Hamurkesen village	N 38° 20.150'; E 43° 37.650'	1948 m
18	Işıkpınar village	N 38° 19.450'; E 43° 36.840'	2134 m
19	Kırgeçit village	N 38° 11.320'; E 43° 29.884'	2147 m
20	Kırgeçit village	N 38° 11.321'; E 43° 29.884'	2165 m
21	Kuşdağı place	N 38° 14.690'; E 43° 27.380'	1905 m
22	Ortaköy village	N 38° 21.873'; E 43° 38.294'	1910 m
23	Ortaköy village	N 38° 21.734'; E 43° 37.854'	1868 m
24	Örmeli village	N 38° 07.220'; E 43° 30.430'	2231 m
25	Sevindik village	N 38° 18.293'; E 43° 52.059'	2098 m
26	Sevindik village	N 38° 17.972'; E 43° 50.348'	2038 m
27	Taşdöndüren village	N 38° 15.328'; E 43° 48.910'	2031 m
28	Tepegören village	N 38° 21.506'; E 43° 53.276'	2107 m
29	Tutmaç village	N 38° 15.930'; E 43° 42.790'	2401 m
30	Üçgen village	N 38° 22.515'; E 43° 44.693'	2141 m
31	Üçgen village	N 38° 22.363'; E 43° 44.975'	2135 m
32	Yedisalkım village	N 38° 11.012'; E 43° 42.100'	2441 m
33	Yoldüştü village	N 38° 09.710'; E 43° 33.790'	2187 m
34	Yurtbaşı village	N 38° 13.933'; E 43° 47.250'	2108 m
35	Around Zerneke watchhouse	N 38° 21.562'; E 43° 39.162'	1934 m
36	Around Zerneke watchhouse	N 38° 21.676'; E 43° 38.620'	1900 m

Şelem 372; locality 29, 13.06.2017, Şelem 377; locality 32, 13.06.2017, Şelem 374.

17. *Pleurotus ostreatus* (Jacq.) P. Kumm.: On *Populus* sp. stump, locality 14, 18.05.2015, Şelem 54; locality 14, 05.06.2015, Şelem.120; locality 10, 08.11.2015, Şelem 180; locality 10, 18.11.2015, Şelem 211; locality 4, 13.11.2016, Şelem 382; locality 26, 13.11.2016, Şelem 384; on *Salix* sp. stump locality 27, 13.06.2017, Şelem 368; locality 28, 13.06.2017, Şelem 367; locality 34, 13.06.2017, Şelem 371.

18. *Pleurotus populinus* O.Hilber & O.K.Mill.: On *Populus* sp. stump, locality 15, 05.10.2016, Şelem 317.

Pluteaceae Kotl. & Pouzar

19. *Pluteus aurantiorugosus* (Trog) Sacc.: On *Populus* sp., stump, locality 6, 19.05.2016, Şelem 338.

20. *Pluteus romellii* (Britzelm.) Sacc.: On *Salix* sp. stump, locality 14, 18.05.2015, Şelem 55; on *Populus* sp. stump, locality 12, 05.10.2016, Şelem. 300.

21. *Volvopluteus gloiocephalus* (DC.) Vizzini, Contu & Justo: Among grass in meadow, locality 9, 18.05.2015, Şelem 109.

Psathyrellaceae Vilgalys, Moncalvo & Redhead

22. *Coprinellus disseminatus* (Pers.) J.E. Lange: Around *Populus* and *Salix* sp. trees, locality 15, 18.05.2015, Şelem 28; locality 22, 18.05.2015, Şelem 76; locality 23, 18.11.2015, Şelem 185.

23. *Coprinellus micaceus* (Bull.) Vilgalys, Hopple & Jacq. Johnson: Under *Salix* sp. trees, locality 9, 08.05.2015, Şelem 3; under *Populus* sp. trees, locality 12, 18.05.2015, Şelem 29; locality 30, 03.06.2016, Şelem 225;

locality 31, 03.06.2016, Şelem 227; locality 21, 03.06.2016, Şelem 252; locality 17, 28.06.2017, Şelem 351; locality 8, 28.06.2017, Şelem 353.

24. *Coprinopsis atramentaria* (Bull.) Redhead, Vilgalys & Moncalvo: Around *Populus* sp. stump, locality 14, 05.06.2015, Şelem 126; locality 23, 18.11.2015, Şelem 183; locality 5, 03.06.2016, Şelem 364; around *Salix* sp. stump, locality 15, 18.05.2015, Şelem 39; under *Robinia* sp. tree, locality 22, 21.05.2015, Şelem 88.

25. *Psathyrella candolleana* (Fr.) Maire: Around *Populus* and *Salix* sp. trees, locality 14, 05.06.2015, Şelem 123; locality 14, 05.06.2015, Şelem 173.

Strophariaceae Singer & A.H.Sm.

26. *Agrocybe dura* (Bolton) Singer: Among grass in meadow, locality 16, 28.06.2017. Şelem 361.

27. *Agrocybe pediades* (Fr.) Fayod: Among grass in meadow, locality 36, 18.05.2015, Şelem 72.

28. *Agrocybe praecox* (Pers.) Fayod: Among grass in meadow, locality 14, 05.06.2015, Şelem 58.

29. *Cyclocybe cylindracea* (DC.) Vizzini & Angelini: On *Populus* sp. stump, locality 11, 05.10.2016, Şelem 268.

30. *Pholiota aurivella* (Batsch) P. Kumm.: On *Salix* sp. stump, locality 3, 18.11.2015, Şelem 210; locality 10, 18.11.2015, Şelem 214; locality 14, 05.10.2016, Şelem 288; locality 25, 13.11.2016, Şelem 383.

Tricholomataceae Lotsy

31. *Melanoleuca brevipes* (Bull.) Pat.: Among grass in meadow, locality 14, 18.05.2015, Şelem 52; among grass under *Populus* sp. trees, locality 10], 19.05.2016, Şelem 326.

32. *Melanoleuca cognata* (Fr.) Konrad & Maubl.: Among grass under *Salix* sp. tree, locality 12, 05.10.2016, Şelem 309.

33. *Pseudoclitocybe cyathiformis* (Bull.) Singer: Among grass in meadow, locality 22, 18.11.2015, Şelem 193.

34. *Lepista personata* (Fr.) Cooke: Among grass in meadow, locality 20, 03.06.2016, Şelem 247.

Boletales E.-J.Gilbert

Suillaceae Besl & Bresinsky

35. *Suillus collinitus* (Fr.) Kuntze : Among needle litter under *Pinus* sp. trees, locality 35, 18.11.2015, Şelem 192.

Polyporales Gäum

Polyporaceae Fr. ex Corda

36. *Cerioporus squamosus* (Huds.) Quél.: On *Populus* sp. stump, locality 15, 18.05.2015, Şelem 356.

4. Discussions

As a result of this study, 36 wild edible macrofungi taxa belonging to 11 families, 4 orders and 2 classes were determined within the boundaries of Gürpınar district. Eight of the determined species belong to Ascomycota and 28 belong to Basidiomycota. Though 36 edible

macrofungi exist in the region, it is found that only six of them are collected and consumed in the region (Table 2). This benefit rate (%16.6) seems to be smaller than the average benefit rate of naturally growing wild edible mushrooms in Turkey (Table 3).

Table 2. Locally consumed taxa and their local names

Scientific name	Local name
<i>Agaricus bisporus</i>	Çayır mantarı
<i>Agaricus campestris</i>	Çayır mantarı
<i>Agaricus urinascens</i>	Çayır mantarı
<i>Pleurotus eryngii</i>	Heliz mantarı
<i>Pleurotus ostreatus</i>	Kavak mantarı
<i>Pleurotus populinus</i>	Kavak mantarı

Among the determined edible species, *Pleurotus eryngii* is the most preferable one due to its delicious taste and the belief that it is good for indigestion. It is also important for its economic potential. Especially in May and June this fungus is collected and sold in public bazaars and markets. It had a price of 50 Turkish liras per kilogram in 2018.

Collection and consumption of the members of the genus *Agaricus* L. also common in the region with the name meadow mushroom (çayır mantarı). *Coprinopsis atramentaria* was also determined in the region. Normally this mushroom is an edible one, but if it is consumed with alcohol, it becomes poisonous due to the interaction of coprine with alcohol (Michelot, 1992).

Though regarded as edible, *Helvella acetabulum*, *H. lacunosa*, *Morchella prava* and *Pholiota aurivella* should not be consumed unless cooked. Among them *H. acetabulum*, *H. lacunosa*, *Paxina queletii* and *P. aurivella* may cause gastrointestinal distress or gastric upset following ingestion (Gücin et al., 2000). Since it liquifies at maturity, *Coprinus comatus* should be consumed when young and still white. Likewise, *Pholiota aurivella* and *Cerioporus squamosus* should also be consumed when young, before the fruit bodies become hard and ligneous.

Pluteus aurantiorugosus is an attractive mushroom and could be regarded as suspicious due to its red to yellowish colour, but it is used for food in Mexico and Malaysia (Boa, 2004).

Wild edible mushrooms are among the most valuable non-wood forest products and have been collected and consumed by people for thousands of years (Boa, 2004). But, it should be avoided to eat naturally growing mushrooms if the edibility is not definitely known.

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Table 3. Local consumption percentages of wild edible mushrooms in some regions of Turkey (modified from Kaya, 2018)

Reseachs	Number of edible taxa	Local consumption	
		taxa number	%
Demirel (1996)	27	5	18,52
Yıldız and Ertekin (1997)	22	8	36,36
Solak et al. (1999)	49	26	53,06
Gezer (2000)	48	13	27,08
Demirel et al. (2002)	21	2	9,52
Demirel et al. (2003)	60	6	10
Demirel et al. (2015)	47	7	14,89
Kaya et al. (2012)	25	5	23,09
Atila and Kaya (2013)	32	7	17,71
Keleş et al. (2014)	59	6	10,17
Akata et al. (2014)	60	9	15
Uzun et al. (2015)	54	7	12,97
Kaya (2015)	40	6	12
Akata et al. (2016)	46	4	8,7
Uzun et al. (2017)	45	15	33,33
		Average	19,2

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Arsenic accumulation in some natural and exotic tree and shrub species in Samsun Province (Turkey)

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Samsun İli'ndeki bazı doğal ve egzotik ağaç ve çalı türlerinde arsenik birikimi

Abstract: The bioaccumulation of metalloids especially arsenic (As) concentrations in urban and suburban environments and bioaccumulation of As in natural and exotic tree and shrub species are not well-documented. One of the most significant sources of As are vehicular emissions and coal combustion. The bioaccumulation of As in some natural and exotic tree and shrub species in Samsun and Atakum in Central Black Sea Region of Turkey is studied. Most of the studies about As pollution were carried out in heavily polluted environments such as lead smelters. However, high As concentrations were found for some natural and exotic tree and shrub species in urban and suburban environments in this study. It has been found that *M. grandiflora* twigs had the highest As concentrations in all of the studied species. Leaf As concentrations were found to be high in *E. camaldulensis*, *P. abies*, *A. cyanophylla*, *C. vitalba*, and *L. vulgare* as compared to twigs and flowers, while twigs of *O. europaea* and *M. grandiflora* had high As concentrations in Samsun center. *E. camaldulensis* and *A. cyanophylla* had high As concentrations in their leaves in Atakum similar to Samsun city center. *M. grandiflora* twigs and *L. vulgare* leaves can be used for biomonitoring studies due to high As concentrations in their tissues.

Key words: Arsenic, Automobile emissions, Central Black Sea Region, Heavy metal

Özet: Metalloidlerin özellikle arsenik (As) biyoakümülyasyonunun kentsel ve kırsal alanlardaki doğal ve egzotik ağaç ve çalı türlerindeki konsantrasyonları pek çalışılmamıştır. Arsenik metaloidinin en önemli kaynaklarından biri taşıt emisyonları ve kömür yanmasıdır. Samsun ilindeki bazı doğal ve egzotik ağaç ve çalı türlerinde arsenik metaloidinin biyolojik birikimi incelenmiştir. Kirlilik gibi çalışmaların çoğu kurşun kirleticileri gibi yoğun kirlenmiş ortamlarda gerçekleştirilmiştir. Ancak, bu çalışmada kentsel ve kırsal ortamlarda bulunan bazı doğal ve egzotik ağaç ve çalı türlerinde yüksek arsenik konsantrasyonları bulunmuştur. İncelenen tüm türlerden, *M. grandiflora* dallarının en yüksek As konsantrasyonuna sahip olduğu bulunmuştur. *E. camaldulensis*, *P. abies*, *A. cyanophylla*, *C. vitalba* ve *L. vulgare* türlerinde dal ve çiçeklere göre yapraklarda arsenik konsantrasyonu yüksek iken, *O. europaea* ve *M. grandiflora* türlerinde ise dalların yüksek arsenik konsantrasyonlarına sahip olduğu bulunmuştur. Samsun şehir merkezi ve Atakum'da *E. camaldulensis* ve *A. cyanophylla* türlerinin yapraklarında benzer yüksek oranda arsenik konsantrasyonu bulunmuştur. *M. grandiflora* dalları ve *L. vulgare* yapraklarının yüksek arsenik konsantrasyonlarına sahip olmasından dolayı bu türler biyo-izleme çalışmalarında kullanılabilirliği belirlenmiştir.

Anahtar Kelimeler: Arsenik, Otomobil emisyonu, Orta Karadeniz Bölümü, Ağır metal

1. Introduction

Due to their immutable nature, heavy metals are a group of pollutants of much concern. Heavy metals and metalloids are known as important environmental pollutants and they are toxic even at very low concentrations. These are usually essential for biological systems, but at high concentrations, they can act in a deleterious manner by blocking essential functional groups, displacing other metal ions, or modifying the active conformation of biological molecules (Alkorta et al., 2004; Korn et al., 2007; Gill et al., 2012).

The presence of metallic and metalloid species in automotive fuels is undesirable and metallic or metalloid elements may derive from the raw product, such as nickel and vanadium in petroleum-based fuel or phosphorus in biodiesel, or they may be introduced during production and storage. In addition to this fuel burning, the wear of auto tires, fluid leakage degradation, and corrosion of metals are the other most important sources of pollution (Sadiq et al., 1989; Wei and Morrison, 1994; Monaci et al., 2000; Suzuki et al., 2009). Motor vehicles have direct and indirect impacts on the metabolism of roadside plants

and that automobile emissions caused chronic pollution in the neighboring environment in long term (Ozakı et al., 2004; Akan et al., 2013).

It has been pointed out that Arsenic (As) is an important metalloid although it ranks 20th among the elements in abundance and period 4 of the periodic table (Fishbein, 1981; Wuana and Okieimen, 2011). Koch et al. (2000) found that As is very harmful because large fraction of this metalloid in many plant tissues is not water soluble. These fractions could be bound to lipids or to cell wall components, including in soluble cellulose, calcium or magnesium pectates, or lignin. It has been known that As usually inhibits plant growth because it is affected the uptake of other nutrients and as a result of this metabolic processes such as nutrient transport are altered (Gomes et al., 2012). It is also very harmful for central cellular functions such as aerobic phosphorylation and the function of proteins (Hughes, 2002; Nagajyoti et al., 2010; Bergqvist, 2011). As is toxic for plants, animals, microorganisms, and human beings and sometimes concentration of As in the environment can be reached to toxic levels (Karimi and Souri, 2015).

As pollution in the environment from both anthropogenic and natural sources is a global problem. In many parts of the world As concentrations in the environment have exceeded the safe threshold (Gonzaga et al., 2006). It has been pointed out that automobile emissions exhibit significant increase in the accumulation of As in plants. Coal combustion is also known to be one of the main sources of As in environment. Anthropogenic activities such as mining facilities, urban wastes, sewage sludge, and dye industry are known to be the other main sources of As pollution. (Bajpai et al., 2010).

Biomonitoring has been defined as using of organisms to obtain quantitative information on environmental quality and is a remarkable contribution to traditional monitoring techniques (Gerhardt, 1999). Tree species has long been used for biomonitoring because they are very efficient at trapping atmospheric particles and play a critical role to determine the risk categories for a particular heavy metal and metalloid. They have also been used in phytoremediation and the restoration of mine areas due to their high biomass and productivity (Tomasevic et al., 2011; Favas et al., 2013; Pal et al., 2014).

Traffic intensity has long been known as a great problem for environmental pollution. Because automobile emissions one of the most striking causes of environmental pollution and alarmingly increased from year to year in Black Sea Region (Samsun Security Directorate of Traffic Bureau, 2012). Meharg and Hartley-Whitaker (2002) reported that arsenic accumulation in plant tissues was poorly understood. There were several studies about As concentrations around mine and smelter wastes, in urban environments (Tomašević et al., 2005; Nkongolo et al., 2008; Xie et al., 2009). However, aquatic species (Koch et al., 2000), lichen species (Bajpai et al., 2010), savanna trees (Gomes et al., 2012), pine species (Favas et al., 2013), and herb species (Karimi and Souri, 2015) were used. However, no study was carried out to evaluate the using of shrub and tree species for biomonitoring of As pollution mainly traffic origin in urban environments. This study is aimed to determine (i) As concentrations in leaf, twigs, and flowers of some natural and exotic tree species in Samsun city (located in Central Black Sea Region of Turkey) and suburb of Samsun (Atakum) mainly originated from traffic density (ii) which plant organ had accumulated the highest As concentration (iii) to compare As concentrations of studied plant species with As concentrations in other plant species (iv) to find which plant species may be used safely for biomonitoring of As pollution.

2. Materials and Method

Sampling

In the present study, 10 exotic and natural tree and shrub taxa (*Laurocerasus officinalis* Roemer (Rosaceae), *Eucalyptus camaldulensis* Dehnhardt (Myrtaceae), *Picea abies* (L.) Karst (Pinaceae), *Acacia cyanophylla* L. (Mimosaceae), *Clematis vitalba* L. (Ranunculaceae), *Olea europaea* L. var. *europaea* (Oleaceae), *Platanus orientalis* L. (Platanaceae), *Ligustrum vulgare* L. (Oleaceae), and *Magnolia grandiflora* L. (Magnoliaceae) were used. Two different regions (Samsun city center and Atakum (suburb of Samsun) which had different traffic densities were selected. Mean traffic density in Samsun city center is

6000 vehicles hr⁻¹, while traffic density is lower in Atakum (the suburb of the city) and mean traffic density is 2000 vehicles hr⁻¹. Leaf, needle and twig samples of studied plant species were used to determine As concentrations.

Five leaves and twigs samples per species, and per plant organ in each region were used. Plant organs were cut off with teflon coated stainless steel scissors using polyethylene gloves. All specimens were taken from the same height and at the same time. Samples were taken from the side facing the highway of the crown. It has been shown that sampling from different sides of the crown did not affect heavy metal concentrations in leaves (Bargagli, 1998).

Taxonomic nomenclature followed that of Guner et al. (2012).

Plant analysis

In the laboratory leaf and twig samples were dried to a constant weight at 60°C with a microwave oven. Dried plant samples were ground by using a hand mortar. Each time, approximately 0.5 g of ground sample was taken and digested using a mixture of HNO₃/H₂O₂ in microwave oven of 360 W for 30 mins. Each digested mixture was diluted to 100 mL volume and centrifuged before the metalloid analysis. A Varian spectra 220/880 flame atomic absorption spectrometer operating with an air/acetylene flame. The spectrophotometer was operated at 193.7 nm with a slit width of 1.0 nm. Air flow was 13.00 L /min. Lamp current was 10.0 mA B, and measurement time was 1.0 s. Pre-read delay was 5 s. The carrier gas flow was optimized to 80 mL/min prior to calibration in order to achieve the highest sensitivity (Allen et al., 1986; Allen et al., 1989; Uddin et al., 2013; Engin et al., 2015). The gps coordinates of sampling points were measured by Garmin gps map 60csx device. Sampling points were showed on map according to gps coordinates (Figure 1).

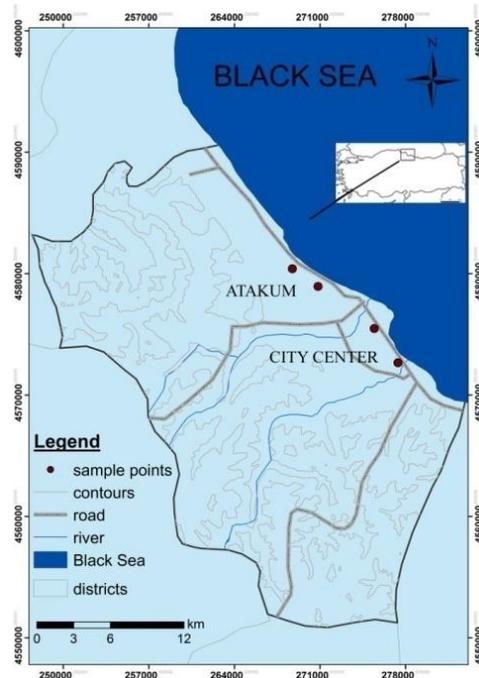


Figure 1. The map of two different regions (Samsun city center and Atakum) which had different traffic densities was selected.

Repeated multivariate analysis of variance (RMANOVA) was used to find the significant differences between studied localities and among plant organs. Tukey's honestly significant difference (HSD) tests were used to rank means by using SPSS 19.0 version. Data was tested for normality using the Kolmogorov-Smirnov test before analysis.

3. Results

It has been found that As concentrations in Samsun city center were two times higher in suburb (Atakum). There were some differences with respect to As concentrations in different plant organs. For example, As concentrations were higher in leaves than twigs in Atakum samples. However, such a pattern was not found in Samsun samples. *M. grandiflora* twigs had the highest As concentrations in all of the studied species.

The highest As concentrations were found in *M. grandiflora* and *L. vulgare*. As concentrations were reached to 3.0 and 2.0 ppm in twigs and leaves of *M. grandiflora* and *L. vulgare*, respectively in Samsun city center (Figure 2).

Leaf As concentrations were found to be high in *E. camaldulensis*, *P. abies*, *A. cyanophylla*, *C. vitalba*, and *L. vulgare* as compared to twigs and flowers, while twigs of *O. europaea* and *M. grandiflora* had high As concentrations in Samsun city center (Figure 2).

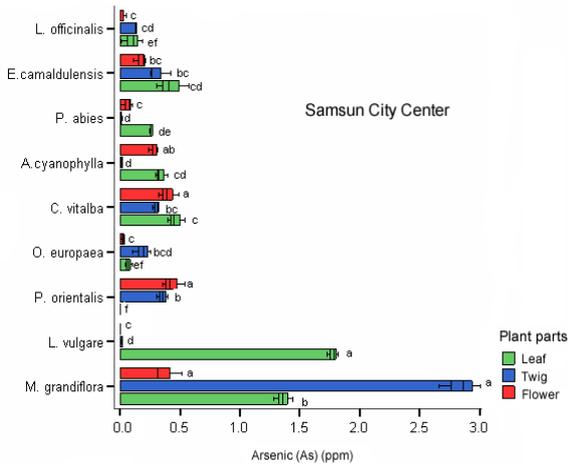


Figure 2. Mean arsenic (As) concentrations and Tukey HSD groups measured at the Samsun city center between plant species of each plant parts. (Different lower case letters indicate significant differences between plant parts. Vertical lines indicate standard error).

Eucalyptus camaldulensis and *A. cyanophylla* had high As concentrations in their leaves in Atakum similar to Samsun city center. However, twigs of *L. officinalis* and *L. vulgare* were higher As concentrations in Atakum (Figure 3).

It has been found that leaf As concentrations in leaf samples which taken from Samsun city center and Atakum were significantly different and leaf As concentrations in Samsun city center were comparatively high. However, As concentrations in twig samples in *L. vulgare* in Atakum were higher than that of twig As concentrations in Samsun city center, while twig As concentrations were found to be high in other plant species which taken from Samsun city center (Figure 4 and Figure 5).

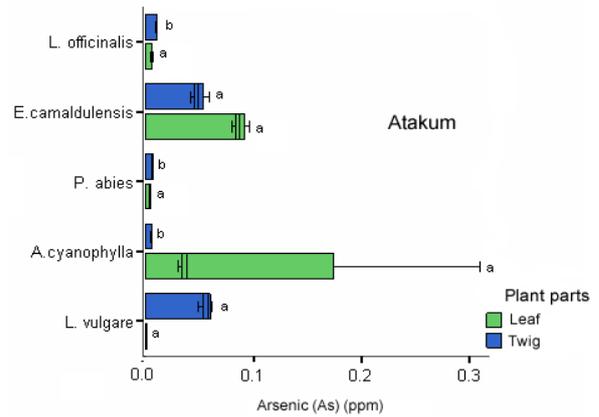


Figure 3. Mean arsenic (As) concentrations and Tukey HSD groups measured at the Atakum city centre between plant parts of each plant parts. (Different lowercase letters indicate significant differences between plant parts. Vertical lines indicate standard error).

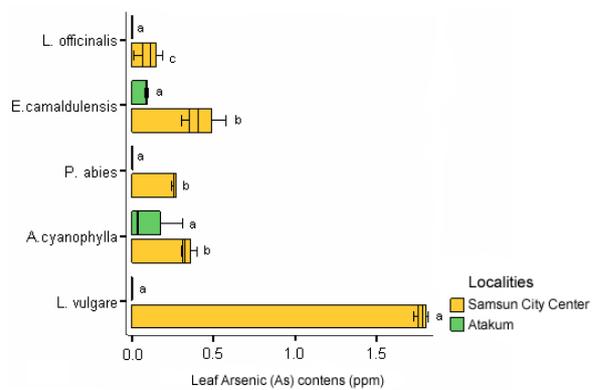


Figure 4. Mean arsenic (As) concentrations and Tukey HSD groups measured at leaves of each plant species between localities. (Different lowercase letters indicate significant differences between plant species. Vertical lines indicate standard error).

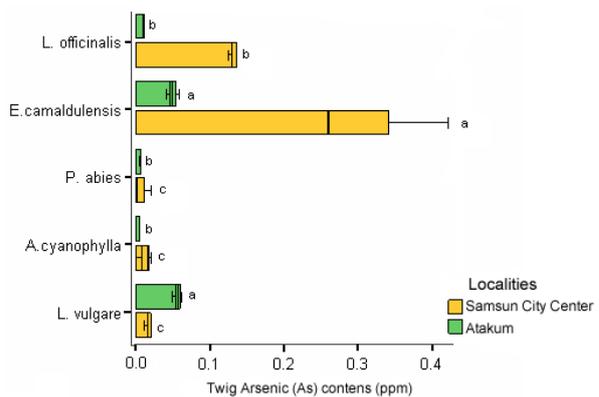


Figure 5. Mean arsenic (As) concentrations and Tukey HSD groups measured at twigs of each plant species between localities. (Different lower case letters indicate significant differences between plant species. Vertical lines indicate standard error).

4. Discussions

As concentrations may be changed regarding traffic density, tree species, and different plant parts. Plants vary in their sensitivity and accumulation of As in their tissues (Meharg and Hartley-Whitaker, 2002) For example, lower As concentrations were found in plant specimens which

taken from Atakum where traffic density was comparatively low. This is shown that As accumulation has been changed due to traffic density. High As concentrations were found in the leaves of a coniferous species (*P. abies*) in Samsun city center where traffic density was two times higher than Atakum. In coniferous species, it has been found that leaves had the highest As concentrations and this is evaluated as a plant defense mechanism against As toxicity (Favas et al., 2013). High As concentrations were also found in the leaves of *L. officinalis*, *E. camaldulensis*, *A. cyanophylla*, *C. vitalba* and *L. vulgare* as compared to twigs. Similarly, *A. cyanophylla* and *E. camaldulensis* had higher As concentrations in their leaves as compared to twigs in Atakum. These results may be indicated As accumulation in plant species is strongly species-dependent (Bergqvist, 2011).

In plant roots As compounds are sequestered in vacuoles and chelated with thiols and as a result of this the transportation of As compounds are limited (Zhao et al., 2010; Gomes et al., 2012). Zhao et al., (2010) also stated that As has low mobility and translocation from roots to aboveground parts of plants were limited except for hyper accumulator species. On the contrary to such limitations high As concentrations were found in *M. grandiflora* twigs and *L. vulgare* leaves. Because As concentrations in *M. grandiflora* twigs and *L. vulgare* leaves were above the toxicity limit. It has been reported that toxicity limit for arsenic in plants is above approximately 2 mg kg⁻¹ (Kabata-Pendias, 2010; Favas et al., 2013). Low As concentrations were found in several studies although they

were carried out in polluted environments as compared to the present study. For example, Nkongolo et al. (2008) found low arsenic concentrations in *Picea mariana* although they studied near smelter sources. Akan et al. (2013) have been reported 0.02-0.21 µg g⁻¹As concentrations in *Azadirachta indica*. Xing et al. (2016) found 0.091 mg/kg As in 25 wheat (*Triticum aestivum*) varieties near the lead smelters in China. As concentrations in some studied species were found to be rather high as compared to similar studies. 3.0 mg kg⁻¹ and 1.75 mg kg⁻¹As concentrations were found in *M. grandiflora* twigs and *L. vulgare* leaves, respectively. Dias et al. (2010) also found that As may be translocated to twigs in some plant species. In summary, As is a very important metalloid not only very toxic for plants but also influences the metabolism of the other elements such as N, P, K, Ca and Mg (Tu and Ma, 2005; Gomes et al., 2012). We suggested that As accumulator tree species may be used safely for urban-planning especially in highly-contaminated areas with As. *M. grandiflora* and *L. vulgare* can be used for biomonitoring studies due to high As concentrations in their twigs and leaves, respectively. In general, As concentrations were higher in plant specimens in Samsun city center and population density is higher in Samsun city center than Atakum and automobile emissions and coal combustion are more apparent in Samsun city center. In order to prevent As pollution heavy traffic intensity should be restricted. For example, odd- and even-numbered vehicles plates may be exit to traffic on alternating days. In addition to this, coal combustion should be prohibited and the usage of renewable resources such as natural gas should be put into practice.

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Mycena ustalis, a new record for the mycobiota of Turkey

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Mycena ustalis, Türkiye mikobiyotası için yeni bir kayıt

Abstract: The small agaric, *Mycena ustalis* Aronsen & Maas Geest. (*Mycenaceae*), is given as new record from Turkey. The macro and micromorphological photographs of the species and a brief description of the species are provided.

Key words: Biodiversity, *Mycena*, new record, Trabzon

Özet: Küçük bir agarik olan *Mycena ustalis* Aronsen & Maas Geest. (*Mycenaceae*), Türkiye'den yeni kayıt olarak verilmiştir. Türün makro ve mikromorfolojilerine ilişkin fotoğrafları ve kısa bir betimlemesi verilmiştir.

Anahtar Kelimeler: Biyoçeşitlilik, *Mycena*, yeni kayıt, Trabzon

1. Introduction

Mycena (Pers.) Roussel is a genus in the family *Mycenaceae* Underw. Members of the genus have cosmopolitan distribution, and play a vital role in litter decomposition since the majority of them are saprotrophic (Pegler, 1986; Singer, 1986). Kirk et al. (2008) reports the existence of almost five hundred *Mycena* species in the world.

The checklists, prepared by Sesli and Denchev (2014) and Solak et al. (2015) compiled 62 *Mycena* species from Turkey. In 2017 three *Mycena* species, *M. meliigena* (Berk. & Cooke) Sacc., *M. pearsoniana* Dennis ex Singer and *M. pterigena* (Fr.) P. Kumm. were added to list by Türkekül (2017), Uzun and Demirel (2017) and Uzun et al. (2017). Currently 65 species of the genus are known to exist in Turkey.

During a field trip within the boundaries of Of district of Trabzon province, some brownish *Mycena* samples were collected from the cemetery. As a result of field and laboratory studies, the samples were identified as *Mycena ustalis* Aronsen & Maas Geest. The current checklists (Sesli and Denchev, 2014; Solak et al., 2015) on Turkish mycobiota and the contributions made after the checklists (Uzun et al., 2015; Akata et al., 2016; Işık et al., 2016; Sesli et al., 2016; Taşkın et al., 2016; Türkekül and Işık, 2016; Çöl et al., 2017; Demirel et al., 2017; Kaşık et al., 2017; Uzun et al., 2017; Alkan et al., 2018; Kaya and Uzun, 2018; Kaygusuz et al., 2018), revealed that *M. ustalis* has not been reported from Turkey before.

The study aims to make a contribution to the mycobiota of Turkey.

2. Materials and Method

Basidiomata of *M. ustalis* species were collected from Of district of Trabzon province in 2017. During field studies, the ecological characteristics of the fruit bodies were recorded and macro photographs of them were taken at their natural habitats. Carrying the samples to the fungarium, they were dried in an air conditioned room, and prepared as fungarium materials in polyethylene bags. Dry materials were used for microscopic investigations. A

Leica DM500 trinocular compound microscope was used for micromorphologic measurement and investigations. By comparing the obtained macromorphological and micromorphological data with Aronsen and Maas Geesteranus (1989) and NBIC (2018), identification of the specimens were performed. The samples are kept at the fungarium of Van Yüzüncü Yıl University in Van, Turkey (VANF).

3. Results

Basidiomycota R.T. Moore

Agaricales Underw.

Mycenaceae Roze

Mycena ustalis Aronsen & Maas Geest.

Macroscopic features: Pileus 9-38 mm across, conical to campanulate when young, broadly convex to applanate at maturity, slightly umbonate, surface smooth to very finely fibrillose when young, somewhat sulcate when mature, especially towards the margin, dark blackish to bluish brown when young, brown when mature, darker at the center, paler toward the margin. Margin whitish when young, brownish when mature. Flesh thin, odor and taste not distinctive. Lamellae adnate to uncinata, white when young, dark grey to pale grey at maturity. Stem 35-70 × 3-4 mm, cylindrical to somewhat compressed, slightly curved and widened towards the base, almost concolorous with the pileus or darker, especially when mature, white puberulous when young, puberules paler to brownish at maturity (Fig. 1).

Microscopic features: Basidia 27-46 × 6.5-9.2 µm, cylindrical to slightly clavate, with 2-4 sterigmata (Fig. 2a), some clamped. Cheilocystidia 21-62 × 7.2-17 µm, clavate to fusiform (Fig. 2b). Spores 7.8-12 × 5.5-7.4 µm, ellipsoid to pip shaped, smooth (Fig. 2c).

Ecology: *Mycena ustalis* was reported to grow in grass near or on needles of *Juniperus* L. (Aronsen and Maas Geesteranus, 1989).

Specimen examined: Trabzon, Of, İrfanlı village cemetery, among grass under *Juniperus* and *Cupressus* L. sp., 40°56'N-43°15'E, 138 m, 19.11.2017, AK.2964.



Figure 1. Basidiocarps of *Mycena ustalis*

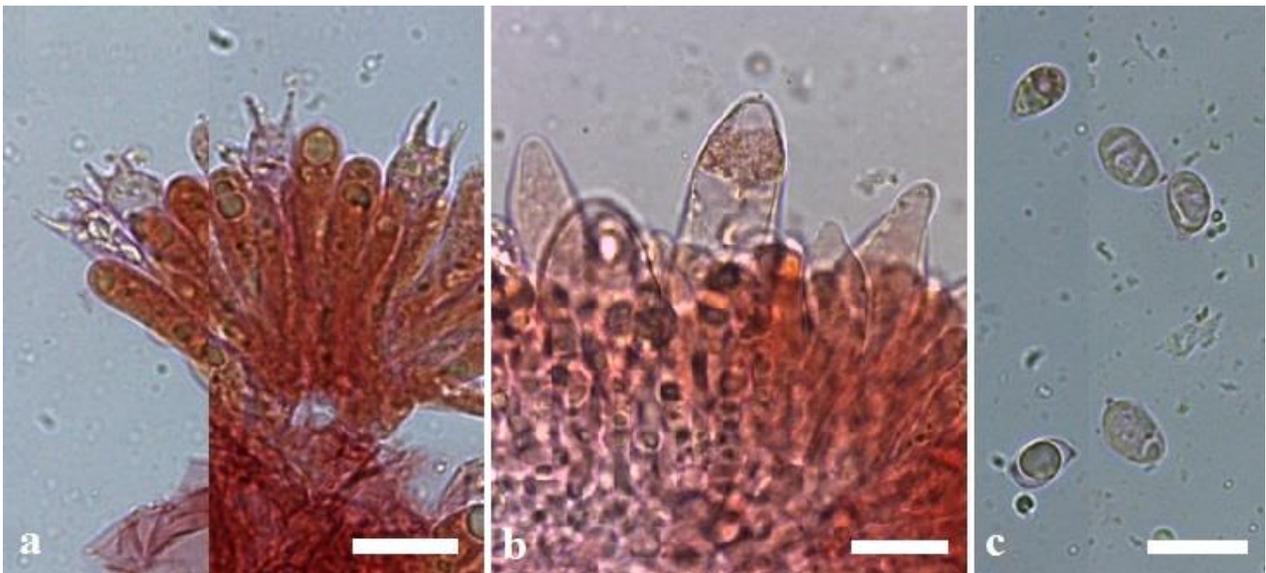


Figure 2. basidia (a), cheilocystidia (b) and basidiospores (c) of *Mycena ustalis* (Congo red) (bars 15 μ m)

4. Discussions

Mycena ustalis is a member of the section *Fragilipedes* (Fr.) Quél. (Aronsen and Maas Geesteranus, 1989). The macro and micromorphological characters of the determined sample, generally conforms those investigated by Aronsen and Maas Geesteranus (1989). Habitat of the sample is also similar, except the existance of *Cupressus* species beside the *Juniperus* species.

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Though Aronsen and Maas Geesteranus (1989) mentions about a nitrous odor of *M. ustalis*, we could not observe a distinct odor from our Turkish collection.

Morphologically, *M. leptcephala* (Pers.) Gillet is similar to *M. ustalis* as having dark blackish brown pileus and stem, but differs from the latter species by growing among needle litter under conifers.

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Cytogenetical analyses of some species of the genus *Sisymbrium* (Brassicaceae) in Turkey

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Türkiye'de *Sisymbrium* (Brassicaceae) cinsinin bazı türlerinin sitogenetik analizleri

Abstract: In this study, karyotype analyses of three species belonging to the genus *Sisymbrium* L. (Brassicaceae) and grown naturally in Turkey were conducted. These taxa are *S. officinale* (L.) Scop., *S. runcinatum* Lag. ex DC. and *S. orientale* L. The somatic chromosome number of $2n = 14$ was reported for all species. Total chromosome lengths are between 1.33 and 2.43 μm in *S. officinale*, 1.30 and 2.48 μm in *S. orientale*, 1.02 and 2.18 μm in *S. runcinatum*. The karyotypes are as follows: *S. officinale* consists of 12m + 2sm, *S. orientale* consists of 10m + 4sm and *S. runcinatum* consists of 10m + 4sm chromosome pairs. *S. runcinatum* is the most asymmetrical karyotype based on all index values.

Key words: *Sisymbrium*, chromosome, karyotype, Turkey

Özet: Bu çalışmada, Türkiye'de doğal olarak yetişen *Sisymbrium* L. (Brassicaceae) cinsine ait üç türün karyotip analizleri yapılmıştır. Bu taksonlar *S. officinale* (L.) Scop., *S. orientale* L. ve *S. runcinatum* Lag. DC. taksonlarıdır. Tüm türler için somatik kromozom sayısı $2n = 14$ olarak bildirilmiştir. Toplam kromozom uzunlukları, *S. officinale*'de 1.33 ve 2.43 μm , *S. orientale*'de 1.30 ve 2.48 μm , *S. runcinatum*'da 1.02 ve 2.18 μm arasındadır. Karyotipler aşağıdaki gibidir: *S. officinale* 12m + 2sm, *S. orientale* 10m + 4sm ve *S. runcinatum* 10m + 4sm kromozom çiftinden oluşur. *S. runcinatum*, tüm endeks değerlerine göre en asimetric karyotiptir.

Anahtar Kelimeler: *Sisymbrium*, kromozom, karyotip, Türkiye

1. Introduction

The family Brassicaceae is the richest in the United States (616 species, 148 endemic) and the second richest in Turkey (606 species, 39 subspecies, 18 varieties, and 226 endemics) in terms of species number (Al-Shehbaz et al., 2007; Al-Shehbaz, 2010; Mutlu, 2012; Mutlu and Karakuş, 2015). Forty species belonging to the family Brassicaceae in Turkey were published as new species in the last decade (Mutlu, 2012; Mutlu and Karakuş, 2015), and this number has continued to increase. The family Brassicaceae has many species of economic importance and consumed as vegetables. These species are *Brassica nigra* (L.) Koch. (black mustard), *Sinapis alba* L. (white mustard), *B. oleracea* L. var. *oleracea* (cabbage), *B. oleracea* L. var. *acephala* DC. (black cabbage), *B. oleracea* L. var. *gemmifera* DC. (Brussels cabbage), *B. oleracea* L. var. *botrytis* L. (cauliflower), *B. rapa* L. var. *rapa* (cole-seed), *Eruca sativa* Miller (rocket), *Raphanus raphanistrum* L. (wild radish), *R. sativus* L. var. *radicula* (red radish), *Lepidium sativum* L. (cress).

Brassica napus L. (rape) and *B. rapa* L. var. *oleracea* DC. (oil turnip) are species cultured to obtain oil from the seeds in family Brassicaceae. *Isatis tinctoria* L. (woad) is an important dyeing agent and *Cheiranthus cheiri* L. (gillyflower) is an important ornamental plant (Seçmen et al., 1998).

Sisymbrium is one of 98 genera within the family Brassicaceae (tribe Sisymbrieae DC.) in Turkey. The genus is represented by 11 species in Turkey. These

species are *S. altissimum* L., *S. confertum* Stev., *S. elatum* K.Koch, *S. irio* L., *S. loeselii* L., *S. officinale* (L.) Scop., *S. orientale* L., *S. polyceratum* L., *S. runcinatum* Lag. ex DC., *S. septulatum* DC. and *S. malatyanum* Mutlu & Karakuş (Hedge, 1965; Al-Shehbaz et al., 2007; Mutlu, 2012; Mutlu and Karakuş, 2015).

The different chromosome numbers have been reported in the genus *Sisymbrium* till now. In genus *Sisymbrium*, the most frequent chromosome number is $2n = 2x = 14$. The chromosome numbers of the species belonging to the genus *Sisymbrium* varies from $2n = 14$ to $2n = 42$ such as $2n = 14, 18, 20, 22, 26, 28$ and 42 (Chromosome Counts Database, <http://ccdb.tau.ac.il/home/>; Missouri Botanical Garden, <http://mobot.mobot.org/W3T/Search/ipcn.html> and Index to Chromosome Numbers in the Brassicaceae, <http://www-brassicaceae.cla.kobe-u.ac.jp/index.html>). In the present study, new chromosomal data and detailed chromosome measurements are reported in three species of genus *Sisymbrium*.)

2. Materials and Method

The species were collected from different localities in Turkey. Collection information is listed below.

S. officinale – A1 Çanakkale: Gökçeada, stony slopes, 40°14'01"N–25°54'05"E, 24 m, 05.V.2012, Asef. 349!

S. orientale – A1 Edirne: beside 80. Yıl Anadolu Lisesi, wasteland, 41°40'09"N–26°34'12"E, 49 m, 30.IV.2012, Asef. 317!

S. runcinatum – C7 Şanlıurfa: Sırrın neighborhood, wasteland, 37°09'35"N–38°48'45"E, 489 m, 20.IV.2012, Asef. 292!

The habitat and flower photos of three species are given in Figure 1. Voucher specimens have been deposited at the herbaria of Necmettin Erbakan University, Faculty of Science, Konya in 2015.

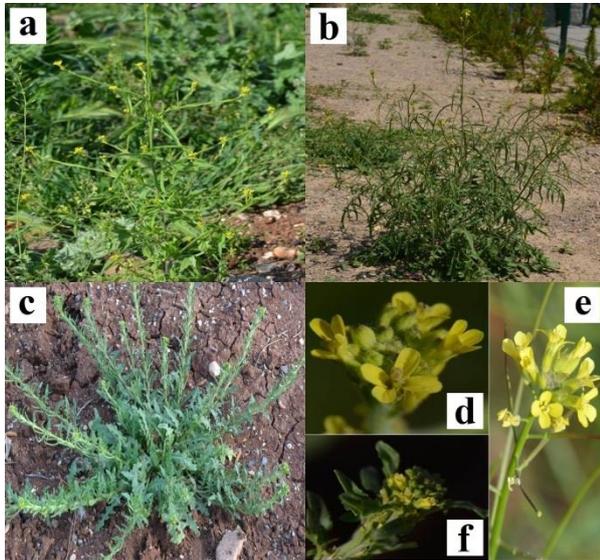


Figure 1. Habitat and flower of three species: a- *Sisymbrium officinale* (habitat), b- *Sisymbrium orientale* (habitat), c- *Sisymbrium runcinatum* (habitat), d- *Sisymbrium officinale* (flower), e- *Sisymbrium orientale* (flower) and f- *Sisymbrium runcinatum* (flower).

Karyotypes analysis were carried on somatic metaphases using Image System Analysis. Root meristems from germinating seeds collected in the wild were used. Root tips were pretreated with α -monobromonaphthalene at 4°C for 16 h and were fixed with Carnoy for 24 h at 4°C. Before staining, the materials were hydrolyzed with 1N HCl for 12 minutes at room temperature, then stained with 2% acetic orcein and mounted in 45% acetic acid. Permanent slides were made by using the standard liquid nitrogen method.

Photographs were taken through BX51 Olympus microscope. The ideograms were prepared with measurements taken on enlarged micrographs of ten well spread metaphase plates. The classification of chromosomes, the length of long and short arm, arm ratio, centromeric index and relative chromosomal length were measured by Software Image Analysis (Bs200ProP). Chromosomes were classified using the nomenclature of Levan et al. (1964). The karyotype asymmetry was determined as the interchromosomal asymmetry with parameters of Rec, A2 and CV_{CL} and the intrachromosomal asymmetry with parameters of AsK, TF, Syi, A1, A and M_{CA} (for details see Paszko, 2006; Eroğlu et al., 2013; Peruzzi and Eroğlu, 2013).

3. Results

3.1. *Sisymbrium officinale*

The metaphase chromosomes and ideogram are shown in Figure 2a. The karyotype formula is $2n = 2x = 14 = 12m + 2sm$. The sixth chromosome pairs are submedian and the others are median as observed at mitotic plates. The total

haploid length and mean haploid length of the chromosomes are 12.71 and 1.82 μm with single chromosome lengths ranging from 1.33 to 2.43 μm (Table 1). The intrachromosomal and interchromosomal asymmetry values are given in Table 2.

3.2. *Sisymbrium orientale*

The metaphase chromosomes and ideogram are shown in Figure 2b. The karyotype formula is $2n = 2x = 14 = 10m + 4sm$. The second and third chromosome pairs are submedian and the others are median as observed at mitotic plates. The total haploid length and mean haploid length of the chromosomes are 12.84 and 1.83 μm with single chromosome lengths ranging from 1.30 to 2.48 μm (Table 1). The intrachromosomal and interchromosomal asymmetry values are given in Table 2.

3.3. *Sisymbrium runcinatum*

The metaphase chromosomes and ideogram are shown in Figure 2c. The karyotype formula is $2n = 2x = 14 = 10m + 4sm$. The fourth and sixth chromosome pairs are submedian and the others are median as observed at mitotic plates. The total haploid length and mean haploid length of the chromosomes are 10.56 and 1.51 μm with single chromosome lengths ranging from 1.02 to 2.18 μm (Table 1). The intrachromosomal and interchromosomal asymmetry values are given in Table 2.

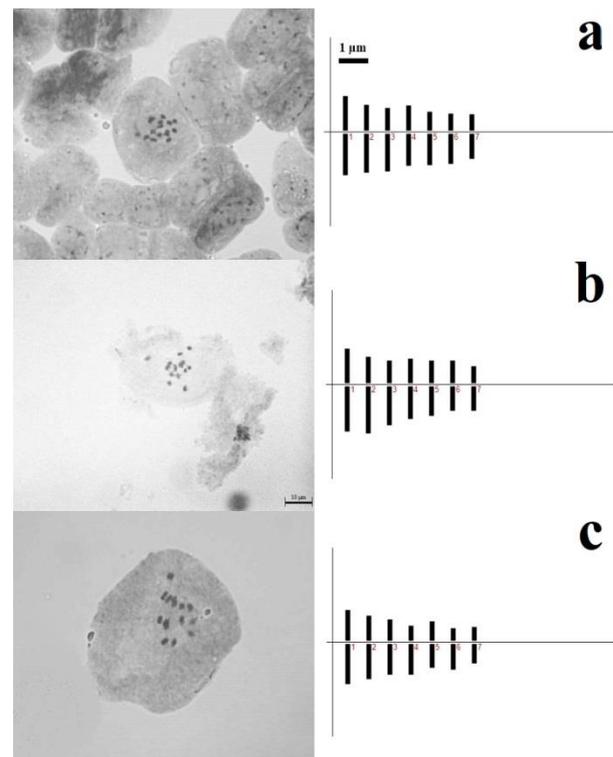


Figure 2. Somatic metaphase chromosomes and ideograms of three species: a- *Sisymbrium officinale*, b- *Sisymbrium orientale* and c- *Sisymbrium runcinatum*.

4. Discussions

Image Analysis System we used in this study made us to find the length of chromosomes and to compare different taxa more precisely. This system offers many advantages over conventional cytological and chromosomal techniques. It enables the analysis of a large number of chromosome spreads, saving the time and effort; besides,

the accuracy of the data is high and the reproducibility of the image manipulation is complete and very sensitive helping to reduce error factor to minimal.

In general, presents low level of variation in chromosome number, with $2n = 14$ in most of the species. The different chromosome numbers were reported as $2n = 14, 18, 20,$

22, 26, 28 and 42 (Chromosome Counts Database, <http://ccdb.tau.ac.il/home/>; Missouri Botanical Garden, <http://mobot.mobot.org/W3T/Search/ipcn.html> and Index to Chromosome Numbers in the Brassicaceae, <http://www-brassicaceae.cla.kobe-u.ac.jp/index.html> *Sisymbrium*).

Table 1. The karyotype data of *Sisymbrium* species. Total chromosome length (L + S), long arm length (L), short arm length (S), arm ratio (L/S), relative length (RL), centromeric index (CI), median (m), submedian (sm).

Species	Pair	L + S (μm)	L (μm)	S (μm)	L / S	RL (%)	CI (%)	Type
<i>Sisymbrium officinale</i>	1	2.43	1.33	1.10	1.21	19.12	45.27	m
	2	2.08	1.25	0.83	1.51	16.37	39.90	m
	3	1.93	1.20	0.73	1.64	15.18	37.82	m
	4	1.83	1.03	0.80	1.29	14.40	43.72	m
	5	1.61	1.01	0.60	1.68	12.67	37.27	m
	6	1.50	0.96	0.54	1.78	11.80	36.00	sm
	7	1.33	0.80	0.53	1.51	10.46	39.85	m
<i>Sisymbrium orientale</i>	1	2.48	1.40	1.08	1.30	19.31	43.55	m
	2	2.29	1.47	0.82	1.79	17.83	35.81	sm
	3	1.91	1.21	0.70	1.73	14.88	36.65	sm
	4	1.78	1.01	0.77	1.31	13.86	43.26	m
	5	1.61	0.91	0.70	1.30	12.54	43.48	m
	6	1.47	0.77	0.70	1.10	11.45	47.62	m
	7	1.30	0.77	0.53	1.45	10.12	40.77	m
<i>Sisymbrium runcinatum</i>	1	2.18	1.23	0.95	1.29	20.64	43.58	m
	2	1.84	1.07	0.77	1.39	17.42	41.85	m
	3	1.60	0.95	0.65	1.46	15.15	40.63	m
	4	1.42	0.95	0.47	2.02	13.45	33.10	sm
	5	1.33	0.73	0.60	1.22	12.59	45.11	m
	6	1.17	0.78	0.39	2.00	11.08	33.33	sm
	7	1.02	0.60	0.42	1.43	9.66	41.18	m

Table 2. The asymmetry index values of *Sisymbrium* species.

	<i>Sisymbrium officinale</i>	<i>Sisymbrium orientale</i>	<i>Sisymbrium runcinatum</i>
AsK	59.64	58.72	59.75
TF	40.36	41.28	40.25
Syi	67.68	70.29	67.35
Rec	74.72	73.96	69.20
A1	0.33	0.28	0.33
A2	0.21	0.23	0.27
A	0.20	0.17	0.20
CV_{CL}	20.59	23.36	26.54
M_{CA}	20.05	16.82	20.35

In mentioned species, the karyotype analyses and detailed chromosome measurements were obtained for the first time. The karyological data confirmed that the chromosome number of *Sisymbrium* species is $2n = 14$ with the basic number of $x = 7$. It was reported that the chromosome numbers was $2n = 14$ in *S. officinale*; $2n = 14$ and 18 in *S. orientale*; $2n = 14, 18, 28, 42, 56$ in *S. runcinatum* (Chromosome Counts Database, <http://ccdb.tau.ac.il/home/>; Missouri Botanical Garden, <http://mobot.mobot.org/W3T/Search/ipcn.html> and Index to Chromosome Numbers in the Brassicaceae, <http://www-brassicaceae.cla.kobe-u.ac.jp/index.html>;

Kamari et al., 2015). The reports include both similar and dissimilar results with our results.

All *Sisymbrium* species examined in the present study show a similar karyomorphology including only median and submedian chromosomes. According to literature survey, the cytological studies on *Sisymbrium* are generally related to the chromosomal counts. The report also confirm a similar karyomorphology including only median and submedian chromosomes for *S. orientale* and *S. runcinatum* from population of Libya (Kamari et al., 2015). Despite similar karyomorphology, the chromosome numbers are different between Turkey and Libya populations.

Karyotype asymmetry is an important parameter in karyological data (Eroğlu et al., 2013). *S. runcinatum* is the most asymmetrical karyotype based on all index values. However, the symmetrical karyotypes are different. While *S. orientale* is the most symmetrical karyotype in M_{CA}, AsK, TF, Syi, A1 and A (intrachromosomal index values); *S. officinale* is the most symmetrical karyotype in CV_{CL}, Rec and A2 (interchromosomal index values) (Table 2).

Karyological knowledge needs to be used in conjunction with other sources of data to achieve a better understanding of the cytological relationship of *Sisymbrium* species, leading to their natural classification. In this regard, karyotypes were determined in three

species of *Sisymbrium* growing naturally in Turkey, and karyological attributes of selected species were evaluated for the first time.

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Suillellus amygdalinus, a new species record for Turkey from Hakkari Province

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Suillellus amygdalinus, Hakkari'den Türkiye için yeni bir tür kaydı

Abstract: *Suillellus* genus is represented by 20 species which are generally edible. In the present study, specimens of *Suillellus* genus (*Suillellus amygdalinus*) were collected from Hakkari province, in 2014 and recorded for the first from Turkey. Short depiction and the photographs of the determined species was given and discussed briefly.

Key words: Macrofungus, *Suillellus amygdalinus*, new record, Hakkari

Özet: 20 türle temsil edilen *Suillellus* cinsi genellikle yenilebilir özelliktedir. Bu çalışmada, 2014 yılında Hakkari ilinden *Suillellus* cinsine (*Suillellus amygdalinus*) ait örnekler toplandı ve Türkiye için yeni kayıt olarak kaydedildi. Tespit edilen türün kısa betimlemesi ve fotoğrafları verilerek kısaca tartışıldı.

Anahtar Kelimeler: Makromantar, *Suillellus amygdalinus*, yeni kayıt, Hakkari

1. Introduction

Suillellus Murrill is a genus of bolete fungi in the family *Boletaceae*. The actual name of the genus *Boletus* is divided into 5 different genera as a result of the molecular studies (*Butyriboletus*, *Caloboletus*, *Neoboletus*, *Suillellus* and *Rubroboletus*) (Zhao et al., 2014). The genus *Suillellus* (*Boletaceae*) was firstly identified by Murrill in 1909. Some researchers regarded it as the synonymy of *Boletus*. Based on the results of molecular phylogenetic studies, Vizzini and his colleagues (2014) moved it to *Suillellus*.

In Turkey, many significant studies have been carried on macrofungi especially in last three to four decades. Regarding the diversity of the country, it can easily be understand that there are lots of macrofungi which are waiting to be determined. Some Turkish mycologist have periodically presented the studies which were carried out on Turkish macrofungi as checklists. Latest checklists were prepared by Sesli and Denchev (2014) and Solak et al. (2015).

Some studies were also carried out after the presentation of latest checklist. According to the checklists and the previous studies (Acar and Uzun, 2016; Akata et al., 2016; Demirel and et al., 2016; Kaya, 2016; Sesli et al., 2016; Acar and Uzun, 2017; Allı et al., 2017; Demirel et al., 2017; Uzun et al., 2017a; Uzun et al., 2017b; Acar et al., 2018; Işık and Türkekul, 2018; Kaya and Uzun, 2018; Sesli and Liimatainen, 2018; Uzun and Kaya, 2018; Uzun and Acar, 2018; Uzun et al., 2018a-b) 5 *Suillellus* species have been reported from Turkey.

This study aims to make a contribution to the mycobiota of Turkey.

2. Materials and Method

Mushroom samples were collected from Hakkari (Yüksekova-Şemdinli) province in 2014. Macroscopic and

ecological properties of the samples were recorded and they were photographed with a digital photograph machine (Canon EOS 60D camera). After the samples were transferred to the laboratory, they were dried and prepared as fungarium materials. The identification of the samples were performed with the help of the relevant literature (Thiers, 1965; Bessette and et al., 2000a; Desjardin et al., 2015). The identified samples are kept in the fungarium of Yüzüncü Yıl University, Science Faculty, Department of Biology.

3. Results

Basidiomycota R.T. Moore

Boletales E.-J. Gilbert

Boletaceae Chevall.

Suillellus amygdalinus (Thiers) Vizzini, Simonini & Gelardi

Syn: *Boletus amygdalinus* Thiers, *Boletus puniceus* Thiers.

Macroscopic and microscopic features: Pileus 40-110 mm across, widely convex when mature, margin lobed or wavy, curling downward when young, surface dry, like mountain goat skin when young, then more or less hairy and slightly paler reddish-brown colored. Flesh reddish under cuticula, the other places are yellow and turn blue immediately when cut. Hymenophore with tubes. The tubes are flat near the stipe, and 10 to 15 mm, pores red or rusty-red when young, apricot red when ripened, becomes bluish when it starts to dry or when injured. Stipe 40-100 × 15-35 mm diam., equal or thickens towards the base, solid, surface dry, reddish on a yellowish background, with bluish spots, turn blue near pileus and turns blue when cut. **Spores** 11-16(19) × 5-7.7(9) µm, thick-walled, ellipsoid, somewhat spindle-shaped, smooth, with a large drop or smaller droplets. **Basidia**; 25-38 × 8-12 µm, club-shaped and contains a large number of intercellular spaces. **Cystidia** 40-60 × 9-13 µm (Figure 1).

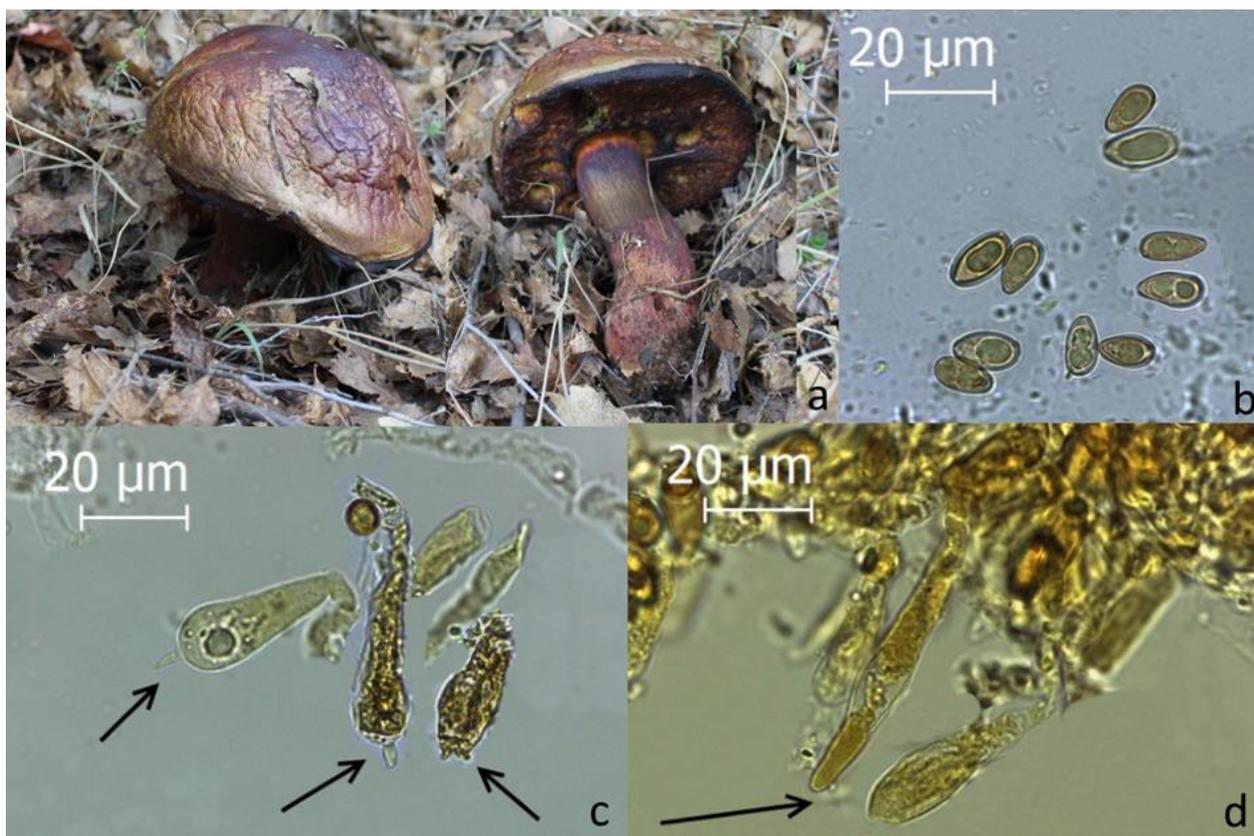


Figure 1. *Suillellus amygdalinus*: a. Basidiocarps b. Spores c. Basidia d. Cystidia (in KOH)

Specimen examined: Hakkari, Şemdinli, Öveç village, under *Quercus* sp., 37°22'322"N-44°28'495"E, 01.11.2014, Acar 761.

4. Discussions

With this study, *Suillellus amygdalinus* was added to the macrofungi of Turkey as the 6th member of the genus *Suillellus*. Five of them were compiled within the checklists prepared by Sesli and Denchev (2014) and Solak et al. (2015).

Suillellus amygdalinus is similar to *Rubroboletus satanas* (Lenz) Kuan Zhao & Zhu L. Yang, *R. eastwoodiae* (Murrill) Vasquez, Simonini, Svetash., Mikšik & Vizzini, *R. pulcherrimus* (Thiers & Halling) D. Arora, N. Siegel & J.L. Frank, *Boletus subvelutipes* Peck and *Sutorius luridiformis* (Rostk.) G. Wu & Zhu L. Yang. But all the similar taxa have larger fruit bodies. The pileus of *Rubroboletus satanas* is up to 300 mm, pale yellow or greenish yellow when young, stipe 50-300 × 40-120 mm, often very bulbous, spores 9-16 × 4.5-7 µm (Breitenbach and Kränzlin, 1991; Knudsen and Vesterholt, 2008; Šutara

et al., 2009). The pileus of *Rubroboletus eastwoodiae* is up to 220 mm, pale grey to pale olive-buff, stipe 70-140 × 60-130 mm, base abruptly bulbous, spores 11-15 × 3.5-6 µm (Ammirati et al., 1985; Breitenbach and Kränzlin, 1991; Desjardin et al., 2015). The pileus of *Rubroboletus pulcherrimus* is 90-170 mm broad, dull-brown to cream-brown, stipe 70-140 × 40-80 mm, clavate, upper large parts of stipe covered with vinaceous-red reticulations over a pallid background, spores 13-15.5 × 5-6 µm (Ammirati et al., 1985; Desjardin et al., 2015). *Boletus subvelutipes* have a pileus of 60-130 mm broad, cinnamon-brown, reddish brown or reddish orange to orange-yellow, stipe 40-100 × 9-20 mm, usually equal, sometimes slightly wide to base, spores 12-18 × 4.5-6.5 µm (Bessette et al., 2000b; Kuo, 2013). The pileus of *Sutorius luridiformis* is up to 200 mm, brownish orange when young, rust brown, olivaceous yellow or dark brown, stipe 40-120 × 10-35 mm, slightly club-shaped when young, without a network pattern, spores 10-14.5 × 4.5-7 µm (Breitenbach and Kränzlin, 1991; Muñoz, 2005; Phillips, 2006; Šutara et al., 2009).

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New locality records for two hypogeous basidiomycete species in Turkey

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İki toprakaltı bazidiyomiset türü için Türkiye’de yeni lokalite kayıtları

Abstract: New specimens of two previously recorded hypogeous basidiomycete species, *Alpova diplophloeus* (Zeller & C.W. Dodge) Trappe & A.H. Sm. and *Schenella pityophila* (Malençon & Rioussset) Estrada & Lado, were collected from Eastern Black Sea and Central Anatolian regions, and reported for the second time from Turkey. New distribution localities and brief descriptions of the species were provided together with the photographs related to their macro and micromorphologies.

Key words: *Alpova*, biodiversity, hypogeous fungi, *Schenella*, Turkey

Özet: Daha önceden kaydedilmiş olan iki toprakaltı bazidiyomiset türüne, *Alpova diplophloeus* (Zeller & C.W. Dodge) Trappe & A.H. Sm. ve *Schenella pityophila* (Malençon & Rioussset) Estrada & Lado, ait örnekler Doğu Karadeniz ve İç Anadolu bölgeleri’nden toplanarak Türkiye’den ikinci kez rapor edilmiştir. Türlerin yeni yayılış lokaliteleri ve kısa betimlemeleri, makro ve mikromorfolojilerine ait fotoğrafları ile birlikte verilmiştir.

Anahtar Kelimeler: *Alpova*, biyoçeşitlilik, toprakaltı mantarlar, *Schenella*, Türkiye

1. Introduction

Alpova C.W.Dodge and *Schenella* Malençon & Rioussset are two hypogeous basidiomycete genera within the families *Paxillaceae* and *Geastraceae*. *Alpova* was first discovered by L.E. Vehmeyer and C.H. Kauffman (Zeller, 1939) in 1922 and is characterized by globose to irregular basidiocarps; well developed and variably thickened peridium; solid gleba with gell-filled chambers separated by pallid veins; hyaline to yellow or light grayish brown, ellipsoid, obovoid, reniform or irregular basidiospores (Dodge, 1931; Trappe, 1975; Beaton et al., 1985). *Schenella* was first described by Thomas Huston Macbride (1911) and is characterized by hypogeous to erumpent, indehiscent, globose to subglobose basidiomata, two to three layered peridium, powdery or peridoliated mature gleba, distinct columella, elastic, nonseptate and smooth capillitial threads, tubular, filiform, thin walled and hyaline basidia, and ellipsoidal to globose basidiospores (Dominguez de Toledo and Castellano, 1996).

During field studies in Konya, Rize and Trabzon provinces, some hypogeous basidiomycete samples were collected and identified as *Alpova diplophloeus* (Zeller & C.W.Dodge) Trappe & A.H. Sm. and *Schenella pityophila* (Malençon & Rioussset) Estrada & Lado. Tracing the current literature it was found that both taxa have only one mention in Turkey.

The study aims to make a contribution to the mycobiota of Turkey by presenting new distributions for two hypogeous basidiomycete taxa.

2. Materials and Method

Schenella samples were collected from Ereğli district of Konya (Central Anatolian Region), and *Alpova* samples were collected from Rize and Trabzon (Eastern Black Sea Region) provinces. All the samples were photographed at their natural habitats and the ecological and descriptive characteristics were recorded during field studies. Then

the samples were transferred to the fungarium and dried in an air conditioned room before preparing as fungarium materials. Macroscopic and microscopic measurements were performed on dried samples in the fungarium. A Nikon Eclipse Ci-S trinocular light microscope were used for micromorphological investigations. Photographs related to micromorphology were taken by a DS-Fi2 digital camera aided by a Nikon DS-L3 displaying apparatus through the same trinocular microscope. The specimens were identified by comparing the obtained data with Zeller and Dodge (1918), Trappe (1975), Gross (1980), Dominguez de Toledo and Castellano (1996), Venturella et al. (2004), Trappe et al. (2007), Signore et al. (2008), Trappe et al. (2009), D’Auria et al. (2013) and Hayward et al. (2014).

The specimens are kept at Biology Department, Kamil Özdağ Science Faculty, Karamanoğlu Mehmetbey University.

3. Results

Basidiomycota R.T. Moore

Boletales E.-J. Gilbert

Paxillaceae Lotsy

Alpova diplophloeus (Zeller & C.W.Dodge) Trappe & A.H. Sm., Beih. Nova Hedwigia 51: 286 (1975)

[**Syn:** *Alpova diplophloeus* (Zeller & C.W.Dodge) Trappe & A.H.Sm. f. *diplophloeus*, *Alpova diplophloeus* f. *europaeus* Trappe, *Rhizopogon diplophloeus* Zeller & C.W.Dodge]

Basidiomata 20-40(-50) mm, globose, ovoid, irregular to reniform. Peridium up to 1 mm thick, yellowish to yellowish-brown, surface smooth, somewhat pruinose in young samples, yellowish-brown to brownish. Gleba sticky-gelatinous with gellfilled chambers of 0.5-2.5 mm broad and separated by yellow veins, pale yellow to reddish brown and finally dark brown (Fig. 1). Odor fruity. Basidia 10-15×4-5 µm, clavate, hyaline.

Basidiospores 4-5.6×2.1-2.7 µm, ellipsoid, rarely allantoid to reniform, smooth (Fig. 2).

Ecology: *Alpova diplophloeus* was reported to grow as a mycorrhizal associate among roots of *Alnus* spp. (Trappe et al., 1975, 2007). Trappe et al. (2009) claims *A. diplophloeus* as a strictly *Alnus* associate.

Specimen examined: Turkey – Rize, Ardeşen, Yukarıdurak Village, mixed forest, in soil around *Alnus* sp. roots, 41°05'N-41°06'E, 925 m, 12.08.2017, Yuzun 5782; Trabzon, Tonya, Erikbeli Village, mixed forest, in soil around *Alnus* sp. roots, 40°45'N-39°14'E, 1680 m, 22.09.2015, Yuzun 4610; Yomra, Özdil Village, *Alnus* sp. forest, 40°50'N-39°48'E, 1210 m, 25.08.2018, Yuzun 6684;



Figure 1. Basidiocarps of *Alpova diplophloeus*

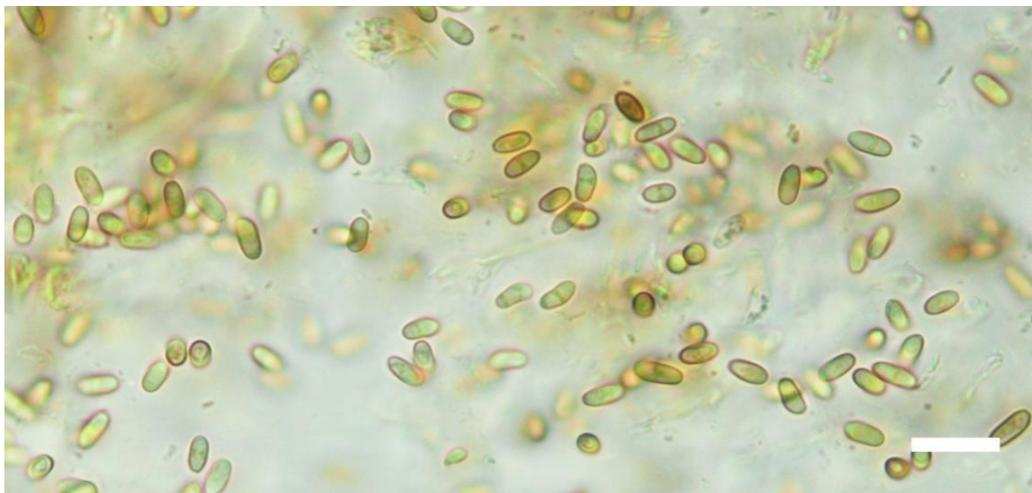


Figure 2. Basidiospores of *Alpova diplophloeus* (Melzer's solution) (bar 10 µm)



Figure 3. Basidiocarps of *Schenella pityophila*

Central district, Esenyurt Village, mixed forest, in soil under *Alnus* sp., 40°53'N-39°45'E, 745 m, 30.08.2018, Yuzun 6740; 40°54'N-39°45'E, 660 m, 02.09.2018, Yuzun 6754.

Alpova diplophloeus is the second member of the genus *Alpova* in Turkey. The first member of the genus, *Alpova corsicus* P.-A. Moreau & F. Rich., was reported by Türkoğlu et al. (2015). Though not reported in a paper,

Alpova diplophloeus was also declared as new record from Turkey by Türkoğlu A, Castellano MA, Trappe JM, Yaratanakul Gungör M in 2014 during Second Symposium on Hypogeous Fungi in Mediterranean Basin (HYPOGES2) & Fifth Congress *Tuber aestivum/uncinatum* European Scientific Group (TAUESG5) in Rabat-Morocco. That's why our collection was regarded as the second in Turkey.

Geastrales K. Hosaka & Castellano

Geastraceae Corda

Schenella pityophila (Malençon & Rioussset) Estrada & Lado, Mycologia 97(1): 147 (2005)

[Syn: *Pyrenogaster pityophilus* Malençon & Rioussset]

Macroscopic and microscopic features: Basidiomata hypogeous, 10-25 mm in diameter, spherical to ellipsoidal (Fig. 3). Peridium three layered. Exoperidium felty to cottony, easily separable, covered by numerous mycelial residues (Fig. 4a,b), that adhere part of the substrate, whitish to light ochraceous, turns red to pink when handled or exposed to the air. Mesoperidium white, fleshy, hard and compact. Endoperidium white, membranous and soft. Gleba composed of a round basal pseudocolumella, in which 150-200 conical or pyramidal and 3-1.5 mm peridioles are placed radially, white and firm when young, black and dehiscent when mature (Fig. 3). Basidia 35-40 × 2-3 µm, filiform, hyaline, enlarged towards the apex up to 4 µm. Sterigmata 2-8, finger-shaped, 3-5 × 0.5-0.6 µm. Basidiospores ellipsoidal,

radially symmetrical, 7.5-12×5-7.5 µm, smooth hyaline and thin-walled when young, 7-8 × 5-7 µm, finely verrucose and apiculated when mature (Fig. 4d,e).

Ecology: *Schenella pityophila* was reported to grow under Douglas-fir and oak trees (Trappe et al., 2007), *P. halepensis* (Rana et al., 2015), *P. ponderosa*, *P. jeffreyi* and *Arbutus menziesii* (Reha and Southworth, 2015)

Schenella pityophila was reported previously from Turkey only once from the locality in Köyceğiz district of Muğla province (Doğan, 2018).

Specimen examined: Turkey – Konya, Ereğli, Sarıca Village, pine forest, roadside, in soil, 37°27'N-34°07' E, 1100 m, 30.12.2017, FTÇ 270.

4. Discussions

New collections of two hypogeous basidiomycete species, *Alpova diplophloeus* and *Schenella pityophila*, were reported. Both taxa have previously been presented from Turkey only once.

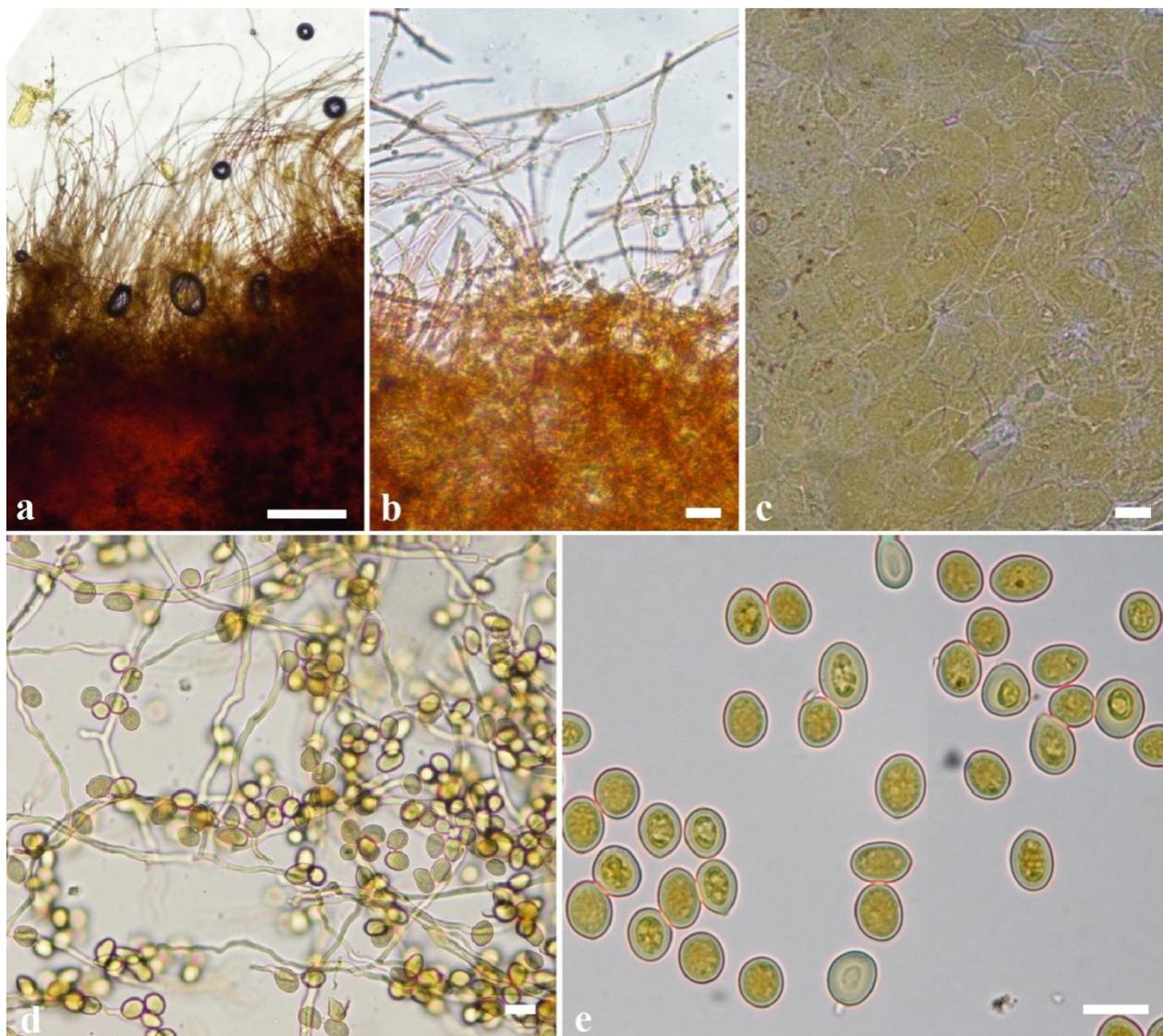


Figure 4. Mycelial layer on exoperidium (a,b), endoperidium (c) and basidiospores (d,e) of *Schenella pityophila* (a,b: Congo red, c,d,e: Melzer's solution) (bars a: 100µm, b-e: 10 µm)

Alpova diplophloeus is a member of the order *Boletales* and sixteen hypogeous members of this order belonging to four families and five genera (*Octaviania* Vittad., *Alpova* C.W.Dodge, *Melanogaster* Corda, *Rhizopogon* Fr., *Sclerogaster* R.Hesse) have so far been reported from Turkey (Demirel, 1996; Kaşık et al., 2002; Sesli and Castellano, 2009; Uzun et al., 2014; Sesli and Moreau, 2015; Türkoğlu et al., 2015; Elliot et al., 2016; Allı et al., 2017; Öztürk et al., 2017; Türkekel, 2017; Kaygusuz et al., 2018; Uzun et al., 2019).

Schenella pityophila is a member of the the family Geastraceae and 20 members of this family belonging to

four genera (*Geastrum* Pers., *Myriostoma* Desv., *Schenella* and *Sphaerobolus* Tode) have been reported from Turkey (Sesli and Denchev, 2014; Doğan, 2018).

General macro- and micromorphological characteristics of the studied samples of *A. diplophloeus* and *S. pityophila* are in agreement with those given in literature.

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