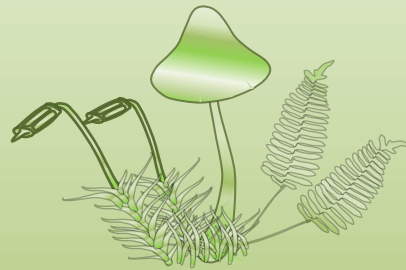


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A new record for Turkish mycota from Akdağmadeni (Yozgat) province: *Russula decolorans* (Fr.) Fr. Epicr.

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Türkiye mikotası için Akdağmadeni (Yozgat)'den Yeni Bir Kayıt: *Russula decolorans* (Fr.) Fr. Epicr.

Abstract: In this study, macrofungi samples identified as *Russula decolorans* were collected in Akdağmadeni (Yozgat) province and recorded for the first time for the Turkish mycota. A short description of new record, illustrations, locality, collection date and habitat are provided.

Key words: Akdağmadeni, biodiversity, new record, *Russula decolorans*, Turkey

Özet: Bu çalışmada *Russula decolorans* olarak teşhis edilen makrofungus örnekleri Akdağmadeni (Yozgat) yöresinden toplandı ve Türkiye mikotası için ilk kez kaydedildi. Yeni kaydın kısa bir açıklaması, fotoğrafları, konumu, koleksiyon numarası ve habitatı verildi.

Anahtar Kelimeler: Akdağmadeni, biyoçeşitlilik, *Russula decolorans*, Türkiye, yeni kayıt

1. Introduction

The *Russula* Pers. (*Russulaceae*, *Russulales*, *Basidiomycota*) which was erected by Persoon (1796), is an important genus with high diversity in the *Russulaceae* family. The members of the genus are ectomycorrhizal symbionts and have great ecological and economical importance. They are an important food source for insects and larger animals. Many species are also collected for nutritional purposes by people. Species of *Russula* are identified easily looking at some macroscopic and microscopic features such as: amyloid warty spores, mostly sphaerocysts (spherical cells in a heteromerous trama), absence of latex, the hyphae without clamp connections, colorful brittle pileus (Miller and Buyck, 2002, Liang et al. 2015).

As it is the case worldwide, studies on determining the fungal diversity in Turkey have increased. According to Sesli and Denchev (2014), 2158 macrofungi species were recorded from Turkey. Of these, 215 are Ascomycota and 1943 are Basidiomycota. Some biodiversity studies were also carried out in recent years (Kaya and Uzun, 2015; Akata and Doğan, 2015; Kaya, 2015; Kaya et al., 2015; Türkekul and Işık, 2016; Doğan and Kurt, 2016; Sesli et al., 2016a, Sesli et al., 2016b, Kaya et al., 2016; Sesli and Topcu Sesli, 2017; Sesli and Vizzini, 2017; Demirel et al., 2017; Akata et al., 2017; Kaya et al., 2017; Aktaş et al., 2017).

Akdağmadeni is a district of Yozgat province where the *Pinus sylvestris* L. populations are concentrated. *Pinus nigra* J.F. Arnold and members of the genera *Quercus*, *Rosa*, *Crataegus*, *Populus*, *Pyrus*, *Corylus*, *Salix* and *Juniperus* are some other components of forest vegetation.

2. Materials and Method

Macrofungi samples were collected from Davulbaz village-Akdağmadeni (Yozgat) district in spring 2014. Specimen were photographed and morphological and

ecological characteristics were recorded in their natural habitats. Then the samples were brought to the laboratory and spore print was obtained. Some chemical reagents (KOH 5%, melzer's reagent, cotton blue, safranin etc.) were used for the examination of microscopic structures. Characteristic features related to lamellae, structure of pileipellis, basidium, basidiospores, and cheilocystidia were obtained. The taxon was identified with the aid of Phillips (1981), Moser (1983), Kränzlin (2005), and Jordan (1998). All materials were stored in the fungarium in Department of Biology, Gaziosmanpaşa University, Tokat.

3. Results

The systematic of the new species is in accordance with Kirk et al. (2008) and Index fungorum (<http://www.indexfungorum.org>: accessed 04 October 2017). Short description locality, collection date, habitat, photograph of basidiomata, microphotographs of basidiospores, cheilocystidia, elements of pileipellis, basidia of the newly recorded species are provided below.

Fungi

Basidiomycota

Russulaceae

Russula decolorans (Fr.) Fr. Epicr. syst. mycol. (Upsaliae): 361(1838)

Syn: *Agaricus decolorans* Fr., Syst. Mycol. (Lundae) 1: 56 (1821) = *Myxacium decolorans* (Fr.) P. Kumm., Führ. Pilzk. (Zerbst): 91 (1871) = *Russula rubriceps* (Kauffman) Singer, Mycologia 35(2): 151 (1943) = *Russula decolorans* var. *albida* A. Blytt & Rostr., in Blytt, Skr. Vidensk Selsk. Christiania, Kl. I, Math.-Natur.(no. 6): 107 (1905) = *Russula decolorans* var. *cichoriata* Melzer & Z. Schaef., Holubinky (Praha): 21 (1944) = *Russula decolorans* var. *cinnamomea* Melzer, Holubinky (Praha): 21 (1944) = *Russula decolorans* var. *tenera* Melzer, Holubinky (Praha): 20 (1944).

Pileus 50-110 mm across, subspherical at first then flattened-convex with incurved margin and flattening, finally with a depression, surface even to slightly venose-tuberculate, dull to silky, ocher-orange to orange red, margin obtuse and margin slightly striate in old age. **Flesh** white, thick, quickly turning gray-black when cut, sometimes also turning orange-red in places, odorless, taste mild. **Lamellae** whitish at first, soon cream-colored with a green-yellow tone, lightly graying when bruising, narrow, close. **Stem** 45-90(100) × 10-25 mm cylindrical, firm, often with clubshaped base, solid when young, soon stuffed to hollow, surface longitudinally venosa, white when young, graying and then blackening with age and bruised or handled, ring absent. **Spores** hyaline, ovate to elliptical, 8.5-11.9 × 7-8.8 µm, ornamented with warts of varying height, spines of various heights up to 1.5 µm, spore print deep cream to pale ochre. **Basidia** clavate, 30-48 × 10-14 µm, with 4 sterigma. **Cheilocystidia** fusiform, 65-100 × 11-12 µm. **Pileipellis** cylindrical, generally flexuous and branched hairs with one or two septa, 2-4 µm across, Edible (Figure 1).

Habitat, solitary or in scattered groups in coniferous forests under coniferous trees, on moist to wet, nutrient-

and nitrogen-poor, chalk-free soils (Kränzlin, 2005; Jordan, 1998).

Akdağmadeni (Yozgat)-Davulbaz village, among needle litters in *Pinus sylvestris* forest, 39° 36' 246" K, 035° 52' 790" D, 17.06.2014, 1581 m, ISIK 713.

4. Discussions

Russula is represented by more than 2000 species worldwide (Kirk et al., 2008) and with 100 taxa in Turkey (Sesli and Denchev, 2014). *Russula decolorans* grow on acidic, moist soils in montane coniferous forests or on high moors. This species may be confused with *R. paludosa* Britzelm. because of the similarity of pileal colors. *R. decolorans* can be distinguished from *R. paludosa* by having larger and more strongly ornamented spores. Though the flesh of *R. decolorans* quickly turns gray-black when cut (especially in the stipe), *R. paludosa* does not turn gray, spotting yellowish appearance on the bruised places (Kränzlin, 2005).

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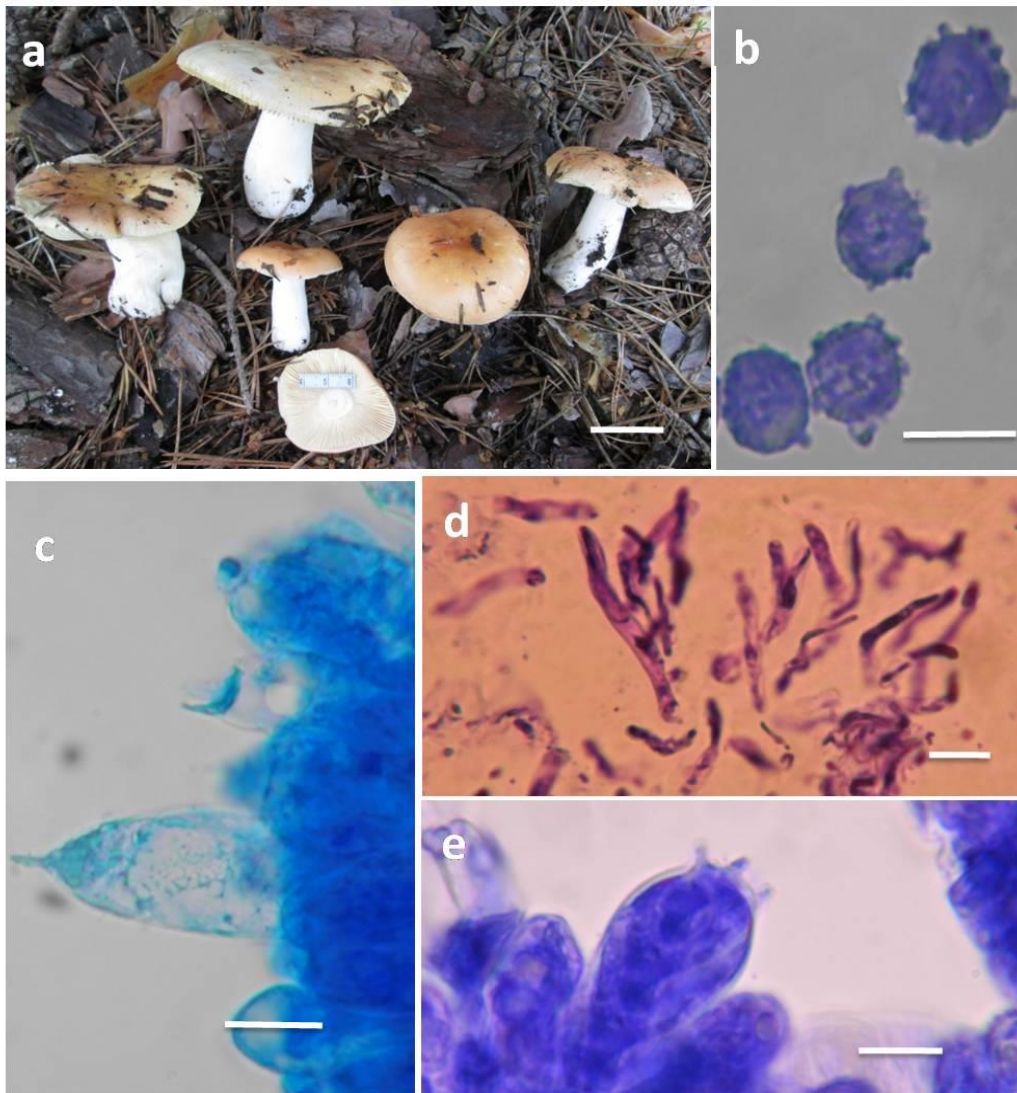


Figure 1. *Russula decolorans*: a- basidiomata, b- basidiospores (in cotton blue), c- cheilocystidia (in cotton blue+KOH), d- elements of pileipellis (in safranin), e- basidia (in cotton blue) (scale bars: a= 30 mm; b,c,d and e= 10 µm).

References

- Akata I, Doğan HH (2015). Orbiliaceae for Turkish Ascomycota: Three New Records. *Bangladesh J. Bot* 44(1): 91-95.
- Akata I, Altuntaş D, Allı H (2017). Macrofungi of Kazdağı National Park (Turkey) and its close environs. *Biological Diversity and Conservation* 10(2): 17-25.
- Aktaş S, Öztürk C, Pamukçu D (2017). Nallıhan (Ankara) İlçesi Makrofungusları. *The Journal of Fungus* 8(1): 60-67.
- Demirel K, Uzun Y, Keleş A, Akçay ME, Acar İ (2017). Macrofungi of Karagöl–Sahara National Park (Şavşat-Artvin/Turkey). *Biological Diversity and Conservation* 10(2): 32-40.
- Doğan HH, Kurt F (2016). New macrofungi records from Turkey and macrofungal diversity of Pozantı-Adana. *Turkish Journal of Botany* 40: 209-217.
- Jordan M (1998). *The Encyclopedia of Fungi of Britain and Europe*. Frances Lincoln, 384p., London.
- Kaya A, Uzun Y (2015). Six new genus records for Turkish Pezizales from Gaziantep Province. *Turkish Journal of Botany* 39: 506-511.
- Kaya A, Uzun Y, Karacan İH, Kaya ÖF, Yakar S (2015). Macromycetes determined in Islahiye (Gaziantep/Turkey) district. *Biological Diversity and Conservation* 8(3), 209-217.
- Kaya A (2015). Contributions to the Macrofungal Diversity of Atatürk Dam Lake Basin. *Turk J Bot* 39: 162-172.
- Kaya A, Uzun Y, Karacan İH, Yakar S (2016). Hyaloriaceae Lindau, A New Family Record for Turkish Mycobiota. *The Journal of Fungus* 7(1): 24-28.
- Kaya A, Uzun Y, Karacan İH, Yakar S (2017). New additions to Turkish Agaricales. *Biological Diversity and Conservation* 10(2):8-13.
- Kirk PF, Cannon PF, Minter DW, Stalpers JA (2008). *Dictionary of the fungi*, 10th edn. CAB International. Wallingford, UK.
- Kränzlin F (2005). *Fungi of Switzerland*. Volume 6. Russulaceae 2. Verlag Mykologia, 319 p., Switzerland.
- Kirk P (2011). Index Fungorum. URL: <http://www.indexfungorum.org> (accessed 04 October 2017).
- Liang JF, Li YK, Zhang X, Yuan Y, Cao Z (2015). Morphological and molecular evidence for a new species of Russula (Russulaceae) from southern China. *Phytotaxa* 202 (2): 094–102.
- Miller SL, Buyck B (2002). Molecular phylogeny of the genus Russula in Europe with a comparison of modern infrageneric classifications. *Mycological Research* 106(3): 259-276.
- Moser M (1983). *Keys to Agarics and Boleti*. Gustav Fischer Verlag, 535 p., Stuttgart.
- Phillips R (1981). *Mushrooms and Other Fungi of Great Britain & Europe*. Pan Books Ltd., 288p, London.
- Sesli E, Denchev CM (2014). Checklists of the myxomycetes, larger ascomycetes, and larger basidiomycetes in Turkey. 6 th. *Mycotaxon Checklists Online*: 1–136.
- Sesli E, Vizzini A, Baroni TJ, Antonín V, Saar I (2016a). *Rhodocybe tugrulii* (Agaricales, Entolomataceae), a new species from Turkey and Estonia based on morphological and molecular data, and a new combination in *Clitocella* (Entolomataceae). *Phytotaxa* 267 (1): 001–015.
- Sesli E, Türkekül İ, Akata I, Niskanen T (2016b). New records of Basidiomycota from Trabzon, Tokat, and İstanbul provinces in Turkey. *Turkish Journal of Botany* 40:531-545.
- Sesli E, Topcu Sesli A (2017). *Infundibulicybe alkaliviolascens* (Tricholomataceae): Türkiye Mikotası için Yeni Bir Kayıt. *Mantar Dergisi* 8(1): 6-12.
- Sesli E, Vizzini A (2017). Two new *Rhodocybe* species (sect. Rufobrunnea, Entolomataceae) from the East Black Sea coast of Turkey. *Turkish Journal of Botany* 41: 200-210.
- Türkekül İ, Işık H (2016). Contribution to the macrofungal diversity of Yozgat Province (Turkey). *Mycotaxon* 131: 483.
-
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Multiple Shoot Regeneration from Shoot Tip and Nodal Explants of *Rotala rotundifolia* (Buch-Ham. ex Roxb) Koehne

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Rotala rotundifolia (Buch-Ham. ex Roxb) Koehne'nin Sürgün Ucu ve Boğum Eksplantlarından Çoklu Sürgün Rejenerasyonu

Abstract: *Rotala rotundifolia* (Buch-Ham. ex Roxb) Koehne, an aquatic plant belonging to the family Lythraceae, is used for the treatment of some diseases due to its medical and anti-microbial properties. This study presents multiple shoot regeneration from shoot tip and nodal explants of *R. rotundifolia* cultured on Murashige and Skoog (MS) nutrient medium containing 0.05-1.25 mg/L Kinetin (KIN) and 0.25 mg/L Gibberellic acid (GA₃) combinations for eight weeks. At the end of the second week, shoot formations began to be observed on the explants. High shoot regeneration frequencies were determined for both explants in the culture medium. The maximum number of shoots per explant was obtained from shoot tip (38.66) and nodal (30.77) explants cultured on MS medium containing 0.25 mg/L KIN + 0.25 mg/L GA₃. Whereas the minimum number of shoots per explant was determined on MS medium containing 1.25 mg/L KIN + 0.25 mg/L GA₃ for both explant types. The highest shoot lengths for shoot tip (1.87 cm) and nodal (1.79 cm) explants were obtained on MS culture medium containing 0.75 mg/L KIN + 0.25 mg/L GA₃ and 0.50 mg/L KIN + 0.25 mg/L GA₃, respectively. For *in vitro* rooting of the regenerated shoots, 2 cm long cut shoots were transferred to MS medium containing 0.25 mg/L indole-3-butyric acid (IBA). The rooted shoots were then successfully acclimatized to external conditions in the aquarium environment.

Keywords: *In vitro*, Kinetin, *R. rotundifolia*, Shoot regeneration, Tissue culture

Özet: Lythraceae familyasına ait bir su bitkisi olan *Rotala rotundifolia* (Buch-Ham. ex Roxb) Koehne, tıbbi ve antimikrobiyal özelliklerinden dolayı bazı hastalıkların tedavisinde kullanılır. Bu çalışma, 0,05-1,25 mg/L Kinetin (KIN) ve 0,25 mg/L Gibberellik asit (GA₃) kombinasyonları içeren Murashige ve Skoog (MS) besin ortamında sekiz hafta boyunca kültüre alınan *R. rotundifolia*'nın sürgün ucu ve boğum eksplantlarından çoklu sürgün rejenerasyonunu sunmaktadır. İkinci haftanın sonunda explantlar üzerinde sürgün oluşumları gözlemlenmeye başlanmıştır. Kültür ortamında her iki eksplant için yüksek sürgün rejenerasyon oranları belirlenmiştir. Maksimum eksplant başına sürgün sayısı, 0,25 mg/L KIN + 0,25 mg/L GA₃ içeren MS ortamında kültüre alınan sürgün ucu (38,66) ve boğum (30,77) eksplantlarından elde edilmiştir. Buna karşın, her iki eksplant türü için minimum eksplant başına sürgün sayısı 1,25 mg/L KIN + 0,25 mg/L GA₃ içeren MS ortamında belirlenmiştir. Sürgün ucu (1,87 cm) ve boğum (1,79 cm) eksplantları için en yüksek sürgün uzunlukları sırasıyla 0,75 mg/L KIN + 0,25 mg/L GA₃ ve 0,50 mg/L KIN + 0,25 mg/L GA₃ içeren MS kültür ortamında elde edilmiştir. Rejenerasyon sürgünlerinin *in vitro* köklendirilmesi için, 2 cm uzunluğunda kesilen sürgünler 0,25 mg/L indol-3-bütirik asit (IBA) içeren MS ortamına aktarılmıştır. Köklü sürgünler daha sonra akvaryum ortamında dış koşullara başarılı bir şekilde alıştırmıştır.

Anahtar Kelimeler: *In vitro*, Kinetin, *R. rotundifolia*, Sürgün rejenerasyonu, Doku kültürü

1. Introduction

Aquatic plants, the primary producers of the aquatic environment, are a good source of nutrients for fish, invertebrates, and birds in tropical chains (Gross et al., 2001; Oyedeji and Abowei, 2012). They also provide habitats and refuges for periphyton, zooplankton, other invertebrate species, and vertebrates (Bornette and Puijalon, 2011). Moreover, many aquatic plants such as *Bacopa monnieri* (Linn.) Pennell, *Alternanthera sessilis* R. Brown ex DC., *Hydrolea zeylanica* Vahl, *Ipomoea aquatica* Forsskal, *Limnophila indica* (L.) Druce, *Ludwigia adscendens* (Linn.) Hara, *Nymphaea nouchali* N.L. Burman, *Pistia stratiotes* Linn. and *Trapa natans* Linn. have been reported for medical use in the treatment of diseases (Swapna et al., 2011).

Rotala rotundifolia (Buch-Ham. ex Roxb) Koehne (Lythraceae family) is an aquatic and amphibian plant of South and Southeast Asia, Japan, Africa, Australia, China, India and North America (Tan et al., 2009; Bhowmik et

al., 2012). *R. rotundifolia* is reputed of antipyretic, detoxication, anti-swelling and diuresis properties and useful in treatments of cirrhosis ascetic fluids, gonorrhoea, menstrual cramps and piles in the south of China (Zhang et al., 2011). The plant has also been used for the treatment of carbuncle, furuncle, rheumatism, and arthralgia (Tan et al., 2009). In a study to determine antioxidant and total phenolic content of 31 wetland plants, aqueous extracts of *R. rotundifolia* have been reported to have the highest antioxidant capacity (Ho et al., 2012).

The aim of this study is to investigate the efficient and rapid propagation from shoot tip and nodal explants of *R. rotundifolia* cultured on MS nutrient medium containing 0.05-1.25 mg/L KIN and 0.25 mg/L GA₃ combinations. This study may help to use protocol for isolation of pharmacologically useful components from the plant and may offer an alternative method for the mass production of *R. rotundifolia* in the aquarium trade industry.

2. Materials and Method

The plants of *R. rotundifolia* were obtained from the local aquarium of Konya province of Turkey. After taxonomic studies, 3-5 cm long twigs were washed under tap water for 10 minutes. Surface sterilization was performed with 20% hydrogen peroxide (H₂O₂) for 10 min followed by rinsed thrice with sterilized distilled water by continuous stirring for 5 min each. After sterilization, shoot tip and nodal explants were isolated under sterile conditions and cultured on Murashige and Skoog (1962) medium (MS) devoid of growth variants (Table 1) for 2 weeks to obtain contamination free explants. Thereafter, the explants were cultured on MS medium supplemented with 3% sucrose, different concentrations (0.05, 0.25, 0.50, 0.75, 1.00 and 1.25 mg/L) of Kinetin (KIN) and 0.25 mg/L Gibberellic Acid (GA₃) in Magenta GA⁷ vessels (Table 3).

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

Components		Concentrations (mg/L)
Macroelements	NH ₄ NO ₃	1650.00
	KNO ₃	1900.00
	CaCl ₂ .2H ₂ O	440.00
	MgSO ₄ .7H ₂ O	370.00
	KH ₂ PO ₄	170.00
Microelements	KI	0.83
	H ₃ BO ₃	6.20
	MnSO ₄ .4H ₂ O	22.30
	ZnSO ₄ .7H ₂ O	8.60
	Na ₂ MoO ₄ .2H ₂ O	0.25
	FeSO ₄ .7H ₂ O	27.85
	CoCl ₂ .6H ₂ O	0.025
	CuSO ₄ .5H ₂ O	0.025
	Na ₂ EDTA.2H ₂ O	37.25
Vitamins	Myo-Inositol	100.00
	Nicotinic Acid	0.50
	Pyrotinic Acid	0.50
	Thiamine-HCl	0.10
	Glycine	2.00

The pH of the culture media was adjusted to 5.7±1 before the autoclaving (1.2 atmospheric pressure, 120°C for 20 min). All cultures were incubated under 16 h light photoperiod. After 8 weeks of culture, the experiment was terminated and the data were recorded for shoot regeneration and analyzed.

The regenerated shoots were rooted on agar-solidified MS rooting medium containing 0.25 mg/L indole-3-butyric acid (IBA) in Magenta GA⁷ vessels. After 4 weeks of culture, the agar medium was removed carefully from the

rooted plantlets without damaging the roots by washing under running tap water. Thereafter, the plants were transferred to an aquarium containing tap water and sand for acclimatization (23°C with 16 h light photoperiod).

The experiment was replicated 6 times. Statistical analysis was performed as One Way ANOVA using SPSS 16 for Windows and post hoc tests were performed using Duncan. Data given in percentages were subjected to arcsine transformation (Snedecor and Cochran, 1967) before statistical analysis

3. Results and Discussion

For *in vitro* shoot regeneration, shoot and nodal explants of *R. rotundifolia* were cultured on MS medium containing combinations of 0.05-1.25 mg/L KIN and 0.25 mg/L GA₃. At the end of two weeks, the first shoot formations were started to be observed. In the fifth week, red colorations were observed on the ends and leaves of some regenerated shoots. After eight weeks, *in vitro* shoot regeneration from shoot tip (Figure 1a) and nodal (Figure 1b) explants of *R. rotundifolia* was successfully achieved and then variance analysis was performed for shoot regeneration frequency, the mean number of shoots per explant, shoot length, and root regeneration frequency (Table 2). Similarly, the use of shoot tip or nodal explants for multiple shoot regeneration has been reported for some aquatic plants such as *Mentha viridis* L. (Raja and Arockiasamy, 2008), *Veronica anagallis-aquatica* L. (Shahzada et al., 2011), *Ludwigia palustris* (L.) Ell. (Fontanili et al., 2015) *Ceratophyllum demersum* L. (Karatas et al., 2015; Emsen et al., 2016; Dogan et al., 2017) and *Pogostemon erectus* (Dalzell) Kuntze (Dogan et al., 2016).

As seen in Table 2, while there was no statistically significant difference in terms of root regeneration frequency, there was a statistically significant difference in shoot regeneration frequency, the mean number of shoots per explant and shoot length for shoot tip explants ($p < 0.05$). In the analysis of variance of the nodal explants, a statistically significant difference was not detected for root regeneration frequency and shoot regeneration frequency, but a statistically significant difference was found in the 99% confidence interval for mean number of shoots per explant and shoot length ($p < 0.01$). Duncan test was performed to determine the significance level of these differences (Table 3).

The shoot regeneration frequency was recorded between 83.33-100.00% for both explants (Table 3). In the shoot tip explants, 100% shoot regeneration was observed in MS medium containing 0.05-1.00 mg/L KIN + 0.25 mg/L GA₃. The highest shoot regeneration frequency (100%) in the nodal explant was obtained in the MS medium containing 0.75, 1.00 and 1.25 mg/L KIN + 0.25 mg/L GA₃. Similarly, Manik et al. (2012) reported high shoot regeneration frequency (78-92%) from shoot tip explants of *Mentha piperata* cultured on MS medium containing 0.75-2.0 mg/L KIN.

Mean number of shoots per explants of shoot tip and nodal explants was recorded 13.17-38.66 and 13.44-30.77, respectively (Table 3). The maximum number of 38.66 and 30.77 shoots per explant were obtained from shoot tip and nodal explants on MS medium supplemented with



Figure 1. *In vitro* shoot regeneration of *R. rotundifolia*. Multiple shoot regeneration from shoot tip (a) and nodal (b) explants on MS medium containing 0.25 mg/L KIN + 0.25 mg/L GA₃ after 8 weeks of culture

Table 2. Analysis of variance of shoot tip and nodal explants of *R. rotundifolia* in MS medium containing different KIN and GA₃

Source of variance	Degree of freedom	Shoot regeneration frequency (%)		Mean number of shoots per explant		Shoot length (cm)		Root regeneration frequency (%)	
		Mean square	F value	Mean Square	F value	Mean Square	F value	Mean Square	F value
Shoot tip									
Medium	5	138.89	4.00*	220.26	3.19*	0.26	4.57 *	145.83	2.10 ^{is}
Error	12	34.72	-	69.13	-	0.06	-	69.44	-
General Total	17	-	-	-	-	-	-	-	-
* Significant at $p < 0.05$ level; is: Insignificant									
Nodal									
Medium	5	138.89	1.333 ^{is}	121.34	7.44**	0.20	5.42**	55.56	0.80 ^{is}
Error	12	104.17	-	16.30	-	0.04	-	69.44	-
General Total	17	-	-	-	-	-	-	-	-
**Significant at $p < 0.01$ level; is: Insignificant									

Table 3. Effect of different combinations of KIN and GA₃ on multiple shoot regeneration from shoot tip and nodal explants of *R. rotundifolia* after eight weeks of culture

Plant growth regulators (mg/L)		Shoot regeneration frequency (%)		Mean number of shoots per explant		Shoot length (cm)		Root regeneration frequency (%)	
KIN	GA ₃	Shoot tip*	Nodal ^{is}	Shoot tip*	Nodal**	Shoot tip*	Nodal**	Shoot tip*	Nodal ^{is}
0.05	0.25	100.00 ^a	83.33	18.83 ^b	16.55 ^{bc}	1.16 ^b	1.28 ^{abc}	100.00 ^a	100.00
0.25	0.25	100.00 ^a	91.67	38.66 ^a	30.77 ^a	1.31 ^b	1.39 ^{abc}	100.00 ^a	100.00
0.50	0.25	100.00 ^a	91.67	24.33 ^{ab}	25.92 ^{ab}	1.46 ^{ab}	1.79 ^a	100.00 ^a	100.00
0.75	0.25	100.00 ^a	100.00	23.69 ^{ab}	21.25 ^{abc}	1.87 ^a	1.67 ^{ab}	100.00 ^a	100.00
1.00	0.25	100.00 ^a	100.00	20.61 ^b	18.64 ^{bc}	1.49 ^{ab}	1.24 ^{bc}	91.67 ^{ab}	91.67
1.25	0.25	83.33 ^b	100.00	13.17 ^b	13.44 ^c	1.03 ^b	1.13 ^c	83.33 ^b	91.67

*Values followed by different small letters in the same column differ significantly at $p < 0.01$

**Values followed by different small letters in the same column differ significantly at $p < 0.05$
is: Insignificant

0.25 mg/L KIN + 0.25 mg/L GA₃, respectively. On the other hand, minimum number of shoots per explants for both explant types was determined on MS medium with 1.25 mg/L KIN + 0.25 mg/L GA₃. The results revealed that the increase in the KIN + GA₃ combination in the MS medium had a negative effect on the number of shoots per explant. These results are in line with Bhattacharyya and Bhattacharya (2001) who cultured the shoot tip explants of *Phyllanthus amarus* Schum. &Thom. on MS medium containing 0.05-5.0 mg/L KIN and reported a decrease in the number of shoots per explant with an increase in KIN ratio. Banerjee and Shrivastava, (2008) obtained the minimum number of 8 ± 1.86 shoots per explant of *B. monnieri* cultured on MS medium with 2.0 mg/L KIN.

Shoot lengths ranged from 1.03 to 1.87 cm for the shoot tip explant and from 1.13 to 1.79 cm for the nodal explant (Table 3). The highest shoot length of shoot tip (1.87 cm) was obtained on MS medium containing 0.75 mg/L KIN + 0.25 mg/L GA₃, whereas the highest shoot length of nodal explant (1.79 cm) was obtained on MS medium supplemented with 0.50 mg/L KIN + 0.25 mg/L GA₃. In both explant types, the shortest shoots were determined in MS medium containing 1.25 mg/L KIN + 0.25 mg/L GA₃. Kaviani et al. (2013) reported that the longest shoots (1.20 cm) were obtained from the shoot tip explants of

Matthiola incana on MS medium supplemented with 2 mg/L KIN.

Root occurrences with KIN effect were recorded on *in vitro* propagation medium. For both types of explants, 100% root formation was obtained on MS medium containing 0.05-0.75 mg/L KIN + 0.25 mg/L GA₃. It has been determined that high KIN doses have an adverse effect on root formation.

In spite of root formation on the propagation medium, *in vitro* rooting studies of regenerated shoots were carried out in MS medium containing 0.25 mg/L IBA for four weeks. *In vitro* rooted plantlets were successfully acclimatized to external conditions in the aquarium environment. Similarly, successful acclimatization of *in vitro* regenerated aquatic plants had been reported for *Cryptocoryne wendtii* and *Cryptocoryne beckettii* (Stanly et al., 2011), *A. sessilis* (Gnanaraj et al., 2011), and *C. demersum* (Dogan et al., 2015).

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References

- Banerjee M, Shrivastava S (2008). An Improved Protocol for *In vitro* Multiplication of *Bacopa monnieri* (L.). World J Microbiol Biotechnol 24: 1355-1359.
- Bhattacharyya R, Bhattacharya S (2001). High Frequency *In vitro* Propagation of *Phyllanthus amarus* Schum. &Thom. by Shoot Tip Culture. Indian J Exp Biol 39: 1184-1187.
- Bhowmik S, Saha M, Datta BK (2012). Extended Distribution of *Rotala rotundifolia* (Buch.-Ham. ex Roxb.) Koehne (Lythraceae) from India. NeBio 3: 48-50.
- Bornette G, Puijalón S (2011). Response of Aquatic Plants to Abiotic Factors: a Review. Aquatic Sci 73: 1-14.
- Dogan M, Emsen B, Aasim M, Yildirim E (2017). *Ceratophyllum demersum* L. Extract As a Botanical Insecticide for Controlling the Maize Weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Egypt J Biol Pest Control 27: 11-15.
- Dogan M, Karatas M, Aasim M (2016). *In vitro* Shoot Regeneration From Shoot Tip And Nodal Segment Explants of *Pogostemon erectus* (Dalzell) Kuntze, A Multipurpose Ornamental Aquatic Plant. Fresen Environ Bull 25: 4777-4782.
- Dogan M, Karatas M, Aasim M (2015). An Efficient *In vitro* Plantlet Regeneration of *Ceratophyllum demersum* L., an Important Medicinal Aquatic Plant. Fresen Environ Bull 24: 3499-3504.
- Emsen B, Dogan M, Aasim M, Yildirim E (2016). Insecticidal Activity of *In vitro* Propagated Aquatic Plant *Ceratophyllum demersum* L. against Granary Weevil *Sitophilus granarius* L. (Coleoptera: Curculionidae). Egypt J Biol Pest Control 26: 619-624.
- Fontanili L, Lucchesini M, Mensuali-Sodi A (2015). *In vitro* Propagation and Shoot Encapsulation as Tools For Ex Situ Conservation of the Aquatic Plant *Ludwigia palustris* (L.) Ell. Plant Biosyst 149: 855-864.
- Gnanaraj WE, Marimuthu J, Subramanian KM, Nallyan S (2011). Micropropagation of *Alternanthera sessilis* (L.) Using Shoot Tip and Nodal Segments. Iran J Biotechnol 9: 206-212.
- Gross EM, Johnson RL, Hairston NG Jr (2001). Experimental Evidence for Changes in Submersed Macrophyte Species Composition Caused by the Herbivore *Acentria ephemerella* (Lepidoptera). Oecologia 127: 105-114.
- Ho YL, Huang SS, Deng JS, Lin YH, Chang YS, Huang GJ (2012). *In vitro* Antioxidant Properties and Total Phenolic Contents of Wetland Medicinal Plants in Taiwan. Bot Stud 53: 55-66.
- Karatas M, Dogan M, Emsen B, Aasim M (2015). Determination of *In vitro* Free Radical Scavenging Activities of Various Extracts From *In Vitro* Propagated *Ceratophyllum demersum* L.. Fresen Environ Bull 24: 2946-2952.
- Kaviani B, Hesar AA, Tarang A, Zanjani SB, Hashemabadi D, Ansari MH (2013). Effect of Kinetin (Kn) and Naphthalene Acetic Acid (NAA) on the Micropropagation of *Matthiola incana* Using Shoot Tips, and Callus Induction and Root Formation on the Leaf Explants. Afr J Agric Res 8: 4134-4139.
- Manik SR, Yattoo GM, Ahmad Z, Nathar VN (2012). Direct Organogenesis of *Mentha piperata* L. from Shoot Tip, Nodal and Sucker Explants. J Agr Tech 8: 663-669.

- Murashige T, Skoog F (1962). A Revised Medium for Rapid Growth and Bioassays with Tobacco Tissue Cultures. *Physiol Plant* 15: 473-497.
- Oyedjeji AA, Abowei JFN (2012). The Classification, Distribution, Control and Economic Importance of Aquatic Plants. *Int J Fish and Aqua Sci* 1:118-128.
- Raja HD, Arockiasamy DI (2008). *In vitro* Propagation of *Mentha viridis* L. from Nodal and Shoot Tip Explants. *Plant Tissue Cult Biotechnol* 18: 1-6.
- Shahzad A, Parveen S, Fatema M (2011). Development of a Regeneration System Via Nodal Segment Culture in *Veronica anagallis-aquatica* L. - An Amphibious Medicinal Plant. *J Plant Interact* 6: 61-68.
- Snedecor GW, Cochran WG (1997). *Statistical Methods*. The Iowa State University Press, Iowa. USA.
- Stanly C, Bhatt A, Keng CL (2011). An Efficient *In vitro* Plantlet Regeneration of *Cryptocoryne wendtii* and *Cryptocoryne beckettii* through Shoot Tip Culture. *Acta Physiol Plant* 33: 619-624.
- Swapna MM, Prakashkumar R, Anoop KP, Manju CN, Rajith NP (2011) A Review on the Medicinal and Edible Aspects of Aquatic and Wetland Plants of India. *J Med Plants Res* 5: 7163-7176.
- Tan QG, Cai XH, Feng T, Luo XD (2009). Megastigmane-type Compounds from *Rotala rotundifolia*. *Chin J Nat Med* 7: 187-189.
- Zhang LJ, Yeh SF, Yu YT, Kuo LMY, Kuo Dr YH (2011). Antioxidative Flavonol Glucuronides and Anti-HBsAg Flavonol from *Rotala rotundifolia*. *J Tradit Complement Med* 1: 57-63.

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A New *Mycena* Record for the Mycobiota of Turkey

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Türkiye Mikobiyotası İçin Yeni Bir *Mycena* Kaydı

Abstract: The mycenoid species, *Mycena pterigena* (Fr.) P. Kumm. (Mycenaceae) is given as new record from Turkey. A brief description of the species and photographs related to its macro and micromorphologies are provided.

Key words: New record, *Mycena*, Trabzon, Turkey

Özet: Mycenoid bir tür olan *Mycena pterigena* (Fr.) P. Kumm. Türkiye'den yeni kayıt olarak verilmiştir. Türün kısa betimlemesi ve makro ve mikromorfolojilerine ilişkin fotoğrafları verilmiştir.

Anahtar Kelimeler: Yeni Kayıt, *Mycena*, Trabzon, Türkiye

1. Introduction

Mycena (Pers.) Roussel is a large genus of the family *Mycenaceae* Underw., with about five hundred known species worldwide (Kirk et al., 2008). The members of the genus are generally small mushrooms rarely exceeding a few centimeters in diameter and are characterised by very fragile and thin, membranous, conical to campanulate pileus; adnate to arcuate or decurrent lamellae; 1-4-spored basidia; intricate cystidia; white spore print; and smooth, hyaline spores. Almost all the members of the genus are saprotrophic and play a vital role in litter decomposition especially in forests and woodlands. Species of the genus have cosmopolitan distribution, and are usually determined on the debris of conifers, other woody plants and rarely on or among the debris of grasses, mosses, ferns (Pegler, 1986; Singer, 1986).

On 8 October 2016 some small pinkish mycena samples were collected from Uzungöl Nature Park within the Çaykara district of Trabzon province and identified as *Mycena pterigena* (Fr.) P. Kumm. Tracing the current checklists (Sesli and Denchev, 2014; Solak et al., 2015) and the latest macrofungal studies (Akata et al., 2016; Denğiz and Demirel, 2016; Kaya et al., 2016; Öztürk et al., 2016; Sesli et al., 2016; Taşkın et al., 2016; Uzun and Acar, 2016; Kaya et al., 2017; Uzun and Kaya, 2017; Uzun et al., 2017a; 2017b, 2017c), it was founded that the taxon has not been recorded from Turkey before.

The work aims to contribute to the mycobiota of Turkey.

2. Materials and Method

Macrofungi specimens were collected from Uzungöl Nature Park within the Çaykara district of Trabzon province during field trips in 2016 Morphological and ecological characteristics of the samples were recorded and they were photographed in their natural habitats. Then the specimens were transferred to the fungarium. Necessary microscopic investigations were carried out within the fungarium. Photographs related to micromorphology were also obtained during these investigations. The samples were identified with the help of Redhead (1984) and Miller (2004).

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

3. Results

Basidiomycota R.T. Moore

Agaricales Underw.

Mycenaceae Roze

Mycena pterigena (Fr.) P. Kumm.

Synonym: *Agaricus pterigenus* Fr., *Agaricus pterigenus* Fr. var. *pterigenus*, *Agaricus rubeolarius* With., *Mycena pterigena* (Fr.) P. Kumm. var. *pterigena*.

Macroscopic features: Pileus 2-4 mm across, cylindrical when young then conical to campanulate, slightly sulcate, translucent-striate, glabrescent to somewhat pruinose, pale salmon to pale pink, somewhat pale brownish at the centre. Flesh membranous, odor mild. Lamellae broadly adnate, decurrent, whitish to pale rose. Stem 10-35 × 0.2-0.3 mm, cylindrical, threadlike, concolorous with the cap or paler when young, then becomes transparent and grayish brown when mature, thicker at the base or somewhat bulbous (Fig. 1).



Figure 1. Basidiocarps of *Mycena pterigena*.

Microscopic features: Basidia 20-25 × 8-10 µm, broadly clavate, 4-spored (Fig. 2a). Cheilocystidia abundant, 19-24 × 11-15 µm, clavate to almost spherical, covered apically with fairly numerous, simple, rarely furcate, cylindrical projections up to 11 µm long (Fig. 2b). Pileipellar hypha also covered with warts or short cylindrical excrescences

(Fig. 2c). Spores 7-10 × 3-5 µm, narrowly to broadly elliptic, hyaline, smooth (Fig. 2d).

Specimen examined: Trabzon, Çaykara, Uzungöl Nature Park, roadside on dead *Pteridium* sp. stem, 40°36'N-40°19'E, 1470 m, 08.10.2016, Yuzun 5275.

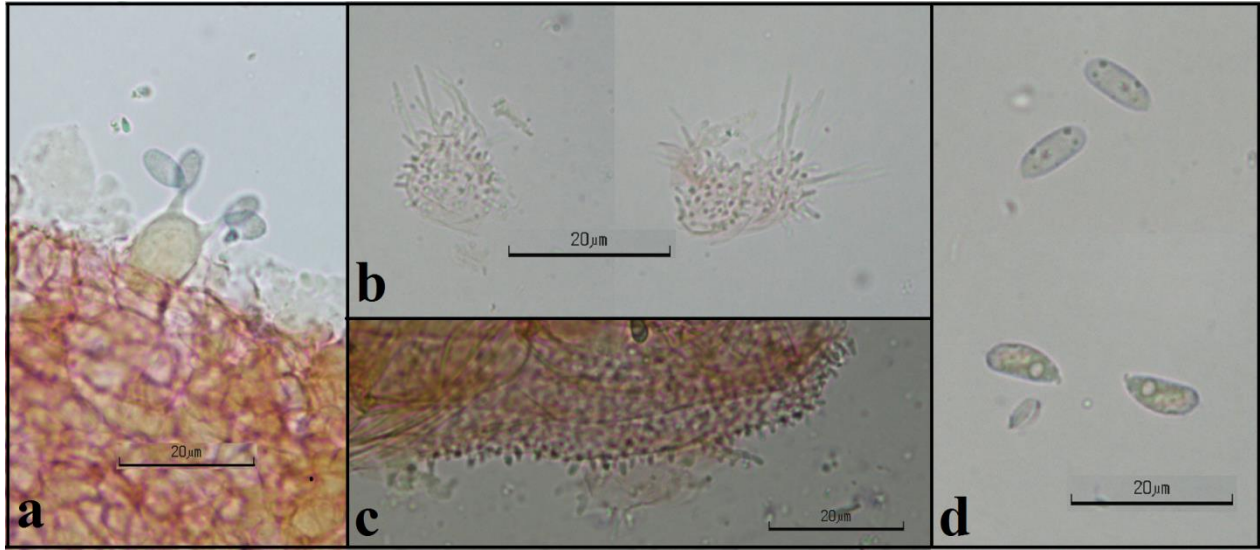


Figure 2. A four spored basidium (a), cheilocystidia (b), pileipellar hypha (c) and basidiospores (d) of *Mycena pterigena*.

4. Discussions

Current checklists on Turkish macromycota (Sesli and Denchev, 2014; Solak et al., 2015) indicate that 62 *Mycena* species have so far been recorded from Turkey. With this addition of *Mycena*, current species number of the genus *Mycena* increased to 63.

Like *Mycena pterigena*, *M. lohwegii* also determined from fern associated habitats, but it has a greyish pileus when young, has a larger fruit body, shorter projections on its cheilocystidia, larger spores and colonizes rhizomes (Miller, 2004).

References

- Akata I, Uzun Y, Kaya A (2016). Macrofungual diversity of Zigana Mountain (Gümüşhane/Turkey). *Biological Diversity and Conservation* 9(2): 57-69.
- Cannon PF, Kirk PM (2007). *Fungal Families of the World*. Wallingford, UK: CAB International.
- Denğiz Y, Demirel K (2016). Şirvan (Siirt) Yöresinde Yetişen Makrofunguslar Üzerinde Taksonomik Bir Araştırma. *Yüzüncü Yıl University Journal of the Institute of Natural & Applied Sciences* 21(2): 112-123.
- Kaya A, Uzun Y, Karacan İH, Yakar S (2016). Contributions to Turkish Pyronemataceae from Gaziantep province. *Turkish Journal of Botany* 40(3): 298-307.
- Kaya A, Uzun Y, Karacan İH, Yakar S (2017). New additions to Turkish Helotiales and Orbiliales. *Kastamonu Üniv. Journal of Forestry Faculty* 17(2): (in print)
- Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008). *Dictionary of the Fungi*, 10th ed. Wallingford, UK: CAB International.
- Miller A (2004). *Trial Key to the Mycenoid Species in the Pacific Northwest*. Pacific Northwest Key Council, Victoria, USA.
- Öztürk Ö, Doğan HH, Şanda MA (2016). Some new additions to Turkish mycobiota from Sakarya region. *Biological Diversity and Conservation* 9(1): 97-100.
- Pegler DN (1986). *Agaric flora of Sri Lanka*. Kew Bulletin Additional Series 12: 1-519.
- Redhead SA (1984). Two fern-associated mushrooms, *Mycena lohwegii* and *M. pterigena*, in Canada. *Le Naturaliste Canadien* 111: 439-442.
- Sesli E, Denchev CM (2014). Checklists of the myxomycetes, larger ascomycetes, and larger basidiomycetes in Turkey. 6th edn. *Mycotaxon Checklists Online*. (<http://www.mycotaxon.com/resources/checklists/sesli-v106-checklist.pdf>): 1-136.
- Sesli E, Türkeul İ, Akata I, Niskanen T (2016). New records of Basidiomycota from Trabzon, Tokat, and İstanbul provinces in Turkey. *Turkish Journal of Botany* 40(5): 531-545.
- Singer R (1986). *The Agaricales in modern taxonomy*. 4th ed. Königstein, Koeltz Scientific Books.

- Solak MH, Işılođlu M, Kalmıř E, Allı H (2015). Macrofungi of Turkey Checklist Vol II. Üiversiteliler ofset, İzmir.
- Tařkın H, Dođan HH, Büyükalaca S, Colwez P, Moreau PA, O'Donnell K (2016). Four new morel (*Morchella*) species in the elata subclade (*M. sect. Distantes*) from Turkey, *Mycotaxon*, 131(2): 467-482.
- Uzun Y, Acar İ, Akçay ME, Kaya A (2017a). Contributions to the macrofungi of Bingöl, Turkey. *Turkish Journal of Botany* 41(5): 516-534.
- Uzun Y, Kaya A (2017). A Hypogeous *Lactarius* sp., New to Turkish Mycobiota. *The Journal of Fungus* 8(2): (in print).
- Uzun Y, Kaya A, Karacan İH, Yakar S. (2017b). New additions to Turkish Hyaloscyphaceae. *The Journal of Fungus* 8(1): 13-19.
- Uzun Y, Acar İ (2016). *Peziza granularis* Donadini Türkiye Mikobiyotası için Yeni Bir Kayıt. Yüzüncü Yıl University Journal of the Institute of Natural & Applied Sciences 21(1): 39-42.
- Uzun Y, Kaya A, Karacan İH, Yakar S (2017c). New additions to Turkish Agaricales. *Biological Diversity and Conservation* 10(2): 8-13.

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Biochemical characterization of four different genotypes of Flax (*Linum usitatissimum* L.) seeds

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Dört farklı genotipe sahip keten (*Linum usitatissimum* L.) tohumlarının biyokimyasal karakterizasyonu

Abstract: Flaxseed (*Linum usitatissimum* L.) is an oilseed used in industrial and natural health products. It accumulates many biologically active compounds having strong phytochemicals and antioxidant properties. This study aims to provide an entire portfolio of bioactive compounds present in four different genotypes of flaxseed (Avangard, Bony Doon, Linda and Linton). Biochemical diversity among these four different types of seed with respect to oxidant and antioxidant parameters (phenolics, flavonoids, β -karoten, lycopene, DPPH scavenging activity, MDA, proline, SOD, CAT, APx and GR) were investigated. According to results, Linda types are found to have unusual phytochemicals and antioxidant profile since its oxidative status tends to the production of oxidant molecules which was reflected in differences in radical scavenging, MDA and antioxidant enzyme systems. Other flaxseeds demonstrate noticeable activities with their antioxidant potential together with their high phytochemicals. Therefore, they may be utilized as a promising source of therapeutics since they might provide an appropriate source of antioxidants.

Key words: Flaxseed, Phytochemicals, Bioactive compounds, Antioxidant enzymes

Özet: Keten (*Linum usitatissimum* L.) tohumları, endüstri ve doğal sağlık ürünleri alanında kullanılan yağlı tohumlardır. Biyolojik olarak aktif pek çok fitokimyasal ve antioksidan özelliklere sahip maddeyi yapılarında bulundurabilirler. Bu çalışma, dört farklı genotipteki keten tohumlarının (Avangard, Bony Doon, Linda ve Linton) biyoaktif içeriklerinin araştırılmasını ve özetlenmesini hedeflemiştir. Buna nedenle oksidan ve antioksidan parametrelerinin (fenolikler, flavonoidler, β -karoten, likopen, DPPH radikal yakalama aktivitesi, MDA, prolin, SOD, CAT, APx ve GR) dört farklı keten tohumundaki biyokimyasal çeşitliliği araştırılmıştır. Sonuçlara göre Linda türü keten tohumlarının diğer çeşitlere oranla beklenmedik bir şekilde farklı fitokimyasal ve antioksidan profiline sahip olduğu, bu tohumların oksidatif durumlarının oksidan molekülleri üretmeye eğilimli olduğu DPPH radikal yakalama, MDA ve antioksidan enzim aktivite sonuçlarına göre belirlenmiştir. Ayrıca diğer keten tohumlarının fark edilebilir şekilde yüksek oranda antioksidan ve fitokimyasal potansiyele sahip oldukları gösterilmiştir. Bu nedenle terapötik olarak önemli özellikleri ve antioksidan içerik açısından zengin olmaları nedeniyle keten tohumlarından daha fazla faydalanılmalıdır.

Anahtar Kelimeler: Keten tohumu, Fitokimyasallar, Biyoaktif bileşikler, Antioksidan enzimler

1. Introduction

Potential plant-derived agents that would influence human health have been in the focus of recent researches. Since ancient times, the biological activities of plants producing a wide range of phytochemicals have been known. One of the possible source of such bioactive compounds is flax (*Linum usitatissimum*), which is widely distributed in Mediterranean and temperate climate zone. The seeds of this plant have been demonstrated to have considerable functions in pharmaceutical and food industry (Czemplik et al., 2012). Even though main well-known beneficial properties are generally linked with its oil composition, ample of bioactive phytochemicals are also present in their seeds (Goyal et al., 2014).

Antioxidants are the compounds functioning in the neutralization of free radicals and therefore a healthy organism should have enough antioxidant ingredients to protect its functionality. Due to their antioxidant properties, flax seeds have been linked to the prevention and treatment of hypercholesterolemia, diarrhea, cardiovascular disorders, diabetes and certain types of cancers especially breast and colon cancers (Prasad, 2000, 2009; Wang et al., 2005; Amawi et al., 2017). However, considering the huge genetic variations in different

genotypes of this plant in Turkey, there were a few reports about the phytochemical comparison of different genotypes of flaxseed cultivated in different areas of Mediterranean region.

This study focused on the analysis of bioactive features of four different genotypes of flaxseed which are Avangard, Bony Doon, Linda and Linton type. In this concern, we elucidated the biochemical diversity with respect to enzymatic and non-enzymatic antioxidant systems. Accordingly, amount of phenolics, flavonoids, β -karoten, lycopene, DPPH scavenging activity, MDA and proline contents were evaluated with biochemical assays together with activities of main antioxidant enzymes which are superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APx) and glutathione reductase (GR)..

2. Materials and Method

Four different genotypes of naturally growing flaxseeds; Avangard, Bony Doon, Linda and Linton were kindly provided by Dr. Sibel Day from Ankara University. Before biochemical characterization, seeds were powdered with liquid nitrogen, mortar and paste. Then, the prepared samples were used for the homogenization. They were homogenized in homogenization solution (50 mM KH_2PO_4 , 5mM EDTA, %1.15 KCl, %2 PVP, pH 7.4) with

Ultraturrax™ homogenizator and then centrifuged at 9,000 g for 10 min at +4°C. Afterwards, supernatants were collected, their total protein contents were measured (Lowry et al., 1951) and then stored at -85°C until used.

Total phenolic contents

Total amount of phenolic compounds in homogenates of four different flaxseeds were determined spectrophotometrically according to method previously described (Taga et al., 1984) with slight modifications. Gallic acid with various concentrations (0.01–1.0 mM) was used as standard phenolic compound. Briefly, 20 µl of standards or homogenates of seeds (10 mg/ml) were mixed with same amount of Folin and Ciocalteu's phenol reagent (2N) and kept at dark for 3 min. Then, 20 µl of 35% sodium carbonate (w/v) and 140 µl dH₂O were added and incubated for 10 min. Next, absorbance values were measured at 725 nm and the results were calculated as mean ± standard error of mean (SEM) from gallic acid calibration curve and expressed as mg of gallic acid equivalents per mg of protein containing homogenates.

Total flavonoid contents

Total flavonoid contents of flaxseeds were determined using protocol (Pal et al., 2010) with slight modifications. Simply, 50 µl of homogenates (10 mg/ml) were mixed with 215 µl of ethanol (80% v/v), 5 µl of aluminium nitrate (10% w/v) and 5 µl potassium acetate (1 M) in microtiter plates and incubated for 40 min at room temperature. Absorbance values were recorded at 415 nm. Total flavonoid contents were calculated according to following formula; Total flavonoid contents (µg/mg protein) = (A₄₁₅ + 0.01089) / 0.002108

β-Carotene and lycopene contents

In order to determine β-carotene and lycopene contents, homogenates of four different flaxseeds were re-extracted with equal amount of acetone:hexane (4:6) mixture and filtered through Whatman No.4 filter paper. After re-extraction process, absorbance of the filtrates was measured at 453, 505 and 663 nm. β-carotene and lycopene contents were determined according to following equations (Pal et al., 2010).

$$\beta\text{-carotene content (mg/100 mg)} = 0.216A_{663} - 0.304A_{505} + 0.452A_{453}$$

$$\text{Lycopene content (mg/100 mg)} = -0.0458A_{663} + 0.372A_{505} - 0.0806A_{453}$$

Radical (DPPH) scavenging activity

Free radical scavenging activities of seeds were determined by monitoring 2,2-diphenyl-1-picrylhydrazyl (DPPH) reduction. Gallic acid (0.01–0.10 mM) was used as standard antioxidant molecule. In brief, 20 µl of various concentrations of standards or homogenates containing different amount of protein (0.1, 0.2, 0.25, 0.33, 0.5, 1.0, 2.0, 5.0 mg/ml) were mixed with 180 µl of DPPH (0.06 mM in methanol) and incubated for 1 h at dark in microtiter plates. Blank measurements were also performed without standards or homogenates. Reduction of DPPH radical was determined after reading absorbance at 517 nm, as percent discoloration of DPPH. Protein concentration providing 50% inhibition (IC₅₀) was calculated from the graph of RSA vs. protein amount (Turkoglu et al., 2007).

Malonedialdehyde (MDA) contents

MDA contents of flaxseeds were evaluated after homogenization of 0.1 gr of fresh seeds in 5% of trichloroacetic acid (TCA) solution and centrifugation at 12,000 g for 15 min. Equal amounts of 0.5% thiobarbituric acid (TBA) which was prepared in 20% TCA was added to homogenates and incubated at 100°C for 25 min. After incubation, samples were chilled on ice and centrifuged at 10,000 g for 5 min. Then, absorbance of supernatants were determined at 532 and 600 nm. MDA contents were calculated by using extinction coefficient of MDA (155 mM⁻¹.cm⁻¹) (Savicka and Skute, 2010).

Proline contents

Proline contents of homogenates were determined after homogenization of 0.2 gr of seeds in 2 ml of 3% sulfosalicylic acid and centrifugation at 14,000 g for 5 min. Hundred microliter of supernatant were added onto a solution containing 0.2 ml of ninhydrin solution (0.31 g ninhydrin, 7.5 ml acetic acid, 5 ml 6M fosforic acid), 0.2 ml of phosphoric acid and 0.1 ml of 3% sulfosalicylic acid. This mixture was then incubated at 96°C for 1 hour and then 1 ml of toluene was added. The absorbance of upper phase was determined at 520 nm. Standard calibration curve obtained with proline standards (0.01 mM – 1.5 mM) was used to calculate proline contents of flaxseeds (Ábrahám et al., 2010).

Catalase (CAT) activity

The decomposition of hydrogen peroxide (H₂O₂) was followed directly by the decrease in absorbance at 240 nm and the difference in absorbance per unit time was the measure of CAT activity (Aebi, 1984). According to modified method, 240 µl of phosphate buffer (50 mM, pH: 7.0), 10 µl of homogenates (0.5 mg/ml) and 50 µl of H₂O₂ (50 mM) were mixed in UV transparent 96 well plate and the change in absorbance was monitored at 240 nm. One unit of CAT activity was defined as the amount of substrate consumed in 1 min by 1mg total protein containing homogenate.

Total superoxide dismutase (SOD) activity

Total SOD activities were measured by following the inhibition of pyrogallol autoxidation spectrophotometrically (Marklund and Marklund, 1974). According to method, 250 µl of Tris buffer (50 mM Tris, 10 mM EDTA, pH: 8.2) were mixed with 30 µl pyrogallol solution (20 mM) and different amount of homogenates in 96 well plates and the rate of adduct formation were followed at 440 nm. One unit of SOD activity was calculated as the amount of protein causing 50% inhibition of pyrogallol autoxidation.

Glutathione reductase (GR) activity

GR activities of seed homogenates were determined with a method we have modified (Foster and Hess, 1980) for the microplate measurements. In this method, 225 µl phosphate buffer (150 mM K₂HPO₄, 25 mM EDTA, pH: 7.8), 25 µl of homogenate (5 mg/ml), 25 µl of NADPH (2.5 mM) and 25 µl of oxidized glutathione (6 mM) were mixed in UV transparent 96 well plate and the decrease in absorbance at 340 nm were followed for 2 minutes. GR activity were calculated as the amount of NADPH (ε₃₄₀ =

6.22 mM⁻¹.cm⁻¹) oxidized in one minute with one mg protein containing seed homogenates.

Ascorbate peroxidase (APx) activity

APx activities of seed homogenates were determined according to a method (Sairam and Saxena, 2000) we have modified. Accordingly, 50 µl of homogenates (1 mg/ml), 100 µl of phosphate buffer (100 mM K₂HPO₄, pH: 6.6) and 10 µl of ascorbate solution (1.5 mM) were mixed in UV transparent microplate wells and enzymatic reaction was initiated with the addition of 50 µl of H₂O₂ (5 mM). The change in the absorbance was followed at 290 nm and the APx activity was calculated as the amount of ascorbate oxidized in one minute with one mg protein containing seed homogenates.

Statistical analyses

All the assays were carried out at least in triplicate measurements. The results are expressed as mean values and standard error of mean (SEM). Antioxidant activities of flaxseeds were analyzed using one way variance (ANOVA) test followed by appropriate post-hoc test (Tukey Test) and values with $p < 0.05$ were considered as statistically significant. IC₅₀ values were calculated with Linear Regression Analysis and associated 95% confidence limits for each treatment were determined. For all those statistical calculations GraphPad Prism 6.0 software were utilized.

3. Results and Discussion

Antioxidants are vital molecules which protect the living organism from the deleterious effects of oxidation. The inhibition or retention of oxidation prevents the possible structural protein or DNA damages caused by reactive oxygen species which are produced by several reactions in living systems (Maity et al., 2014). As being vital in particular conditions for the maintenance of the life, antioxidants have been studied by so many researchers. Presence of natural antioxidant molecules in nutraceuticals; especially plant origin are of considerable interest since they have imperative constituents with free radical scavenging and contribute to antioxidative reactions directly. Among them; phenolic compounds, flavonoids, β-carotene and lycopene contents correlates well with the antioxidant potential. In the present study, biological contents of four different genotypes of flaxseeds were investigated to elucidate their potential for nutritional and therapeutic use and the results are summarized in Figure 1.

According to results, Linda type of flaxseed has the lowest protein content as compared to the other seeds (Figure 1A). Moreover, it has the lowest radical scavenging activity (IC₅₀: 0.0096 mg/ml) (Figure 1B). Highest DPPH radical scavenging were obtained from seeds in the order of Bony Doon (IC₅₀: 0.0047 mg/ml), Avangard (IC₅₀: 0.0057 mg/ml) and Linton (IC₅₀: 0.0059 mg/ml). In parallel with the radical scavenging activity, the main indicator of the free radical induced oxidative stress; MDA levels were also determined to be highest in Linda type flaxseeds (3.096±0.034 µM/mg) indicating the low antioxidant potential which leads to oxidatively modified products to be accumulated (Figure 1C). MDA contents of Linton, Avangard and Bony Doon types were significantly lower than that of Linda types. MDA levels

has the order of Avangard> Bony Doon> Linton>Linda in different genotypes of flaxseeds. Figure 1D demonstrates that Linton type of seeds were the richest considering the proline contents (0.321±0.019 mM/mg) and Linda (0.221±0.022 mM/mg), Avangard (0.193±0.019 mM/mg) and Bony Doon (0.118±0.012 mM/mg) follows this genotype. On the contrary to these oxidative parameters, Linda type of seeds has the highest amount of phenolics and flavonoid content. It has 21.99±0.74 mM GAE/mg of total phenolics but the others had approximately half of this value (13.90; 11.61 and 12.80 mM GAE/mg) (Figure 1E). In parallel with the phenolic contents, highest flavonoid (9.51±0.34 mg/mg) was also present in Linda types (Figure 1F). It is interesting to note that; amount of total phenolics and flavonoid was inversely proportional to β-carotene and lycopene contents. β-carotene (11.65 mg/100 mg) and lycopene (6.87 mg/100 mg) contents of Linda types of flaxseeds were as half of the other types (Figure 1G and 1H). β-caroten contents of Linton (20.16±2.56 mg/100mg), Avangard (20.22±1.01 mg/100mg) and Bony Doon (21.04±0.39 mg/100mg) were significantly higher ($p < 0.05$) than Linda types. Similarly lycopene contents were two times higher. We may confer from the data that the differences in the scavenging ability order could be due to reduction in β-caroten and lycopene contents in the flaxseeds which may have roles in radical scavenging activity.

Antioxidant enzyme activities in different genotypes of flaxseeds

The major antioxidant enzymes are superoxide dismutase (SOD) isozymes; SOD-1 and SOD-2 which neutralizes superoxide radicals in cytoplasm and mitochondria, respectively. Other protective mechanisms against oxidative stress could be classified as CAT which catalyzes the decomposition of hydrogen peroxide to water, a function that is shared with APx in plants. Additionally, GR helps oxidative glutathione to be reduced back to the glutathione for the antioxidant activities at the expense of cellular NADPH. The levels of these key antioxidant enzymes reflects the physiological oxidative conditions of flaxseeds which are modulated with the changes in cellular redox status.

The activities of main detoxification enzyme for superoxide radical (SOD) was found to be highest in Linda types of seeds (4.685±0.468 U/mg) which are directly correlated well with the high MDA content and low DPPH scavenging activity (Figure 2A). Additionally, CAT activity has the lowest in Linda (Figure 2B). This status reflects the presence of oxidative conditions in Linda seeds. The lowest SOD activity was observed in Linton (1.935±0.194 U/mg) type which was statistically different ($p < 0.05$) than the others. Avangard and Bony Doon had approximately the same SOD activities (2.238±0.224 and 2.543±0.254 U/mg, respectively). Any difference were observed in APx activity (Figure 2C), while GR activities were highly diverse in different types of flaxseeds. Accordingly, highest GR activities were observed in Avangard (3.371±0.282 mU/mg) and Bony Doon (2.438±0.398 mU/mg) so as to Linton (1.607±0.608 mU/mg) and Linda (1.241±0.289 mU/mg) followed Avangard.

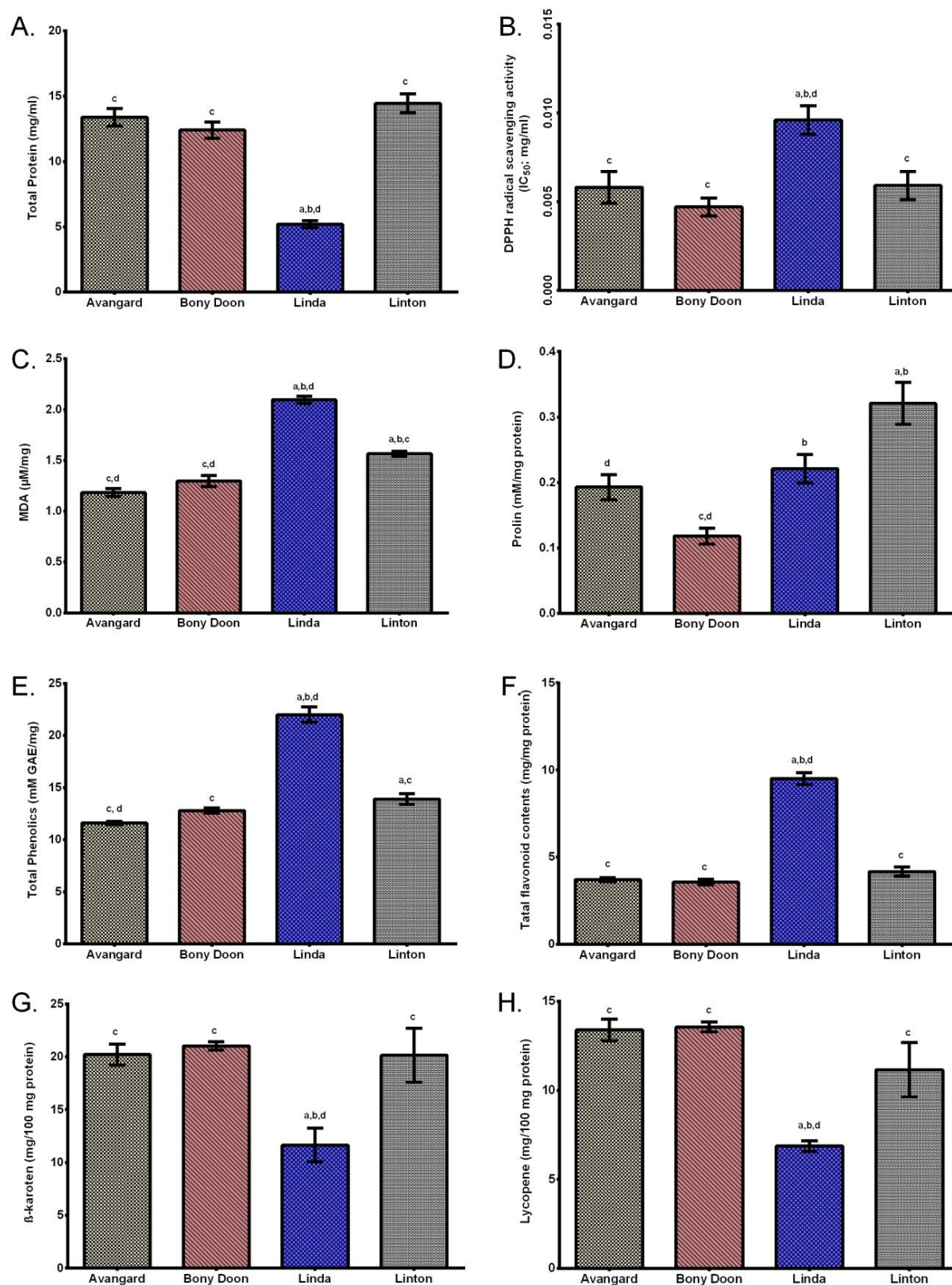


Figure 1: Antioxidant and oxidant potential of four different genotypes of flaxseeds. (A): Total protein content; (B): DPPH radical scavenging activity; (C): MDA content; (D): Prolin content; (E): Total phenolic contents; (F): Total flavonoid contents; (G) β-carotene contents; (H): lycopene contents of four different genotypes of flaxseeds. ^arepresents the significant differences as compared to Avangard (P<0.05); ^brepresents the significant differences as compared to Bony Doon (P<0.05); ^crepresents the significant differences as compared to Linda (P<0.05); ^drepresents the significant differences as compared to Linton (P<0.05).

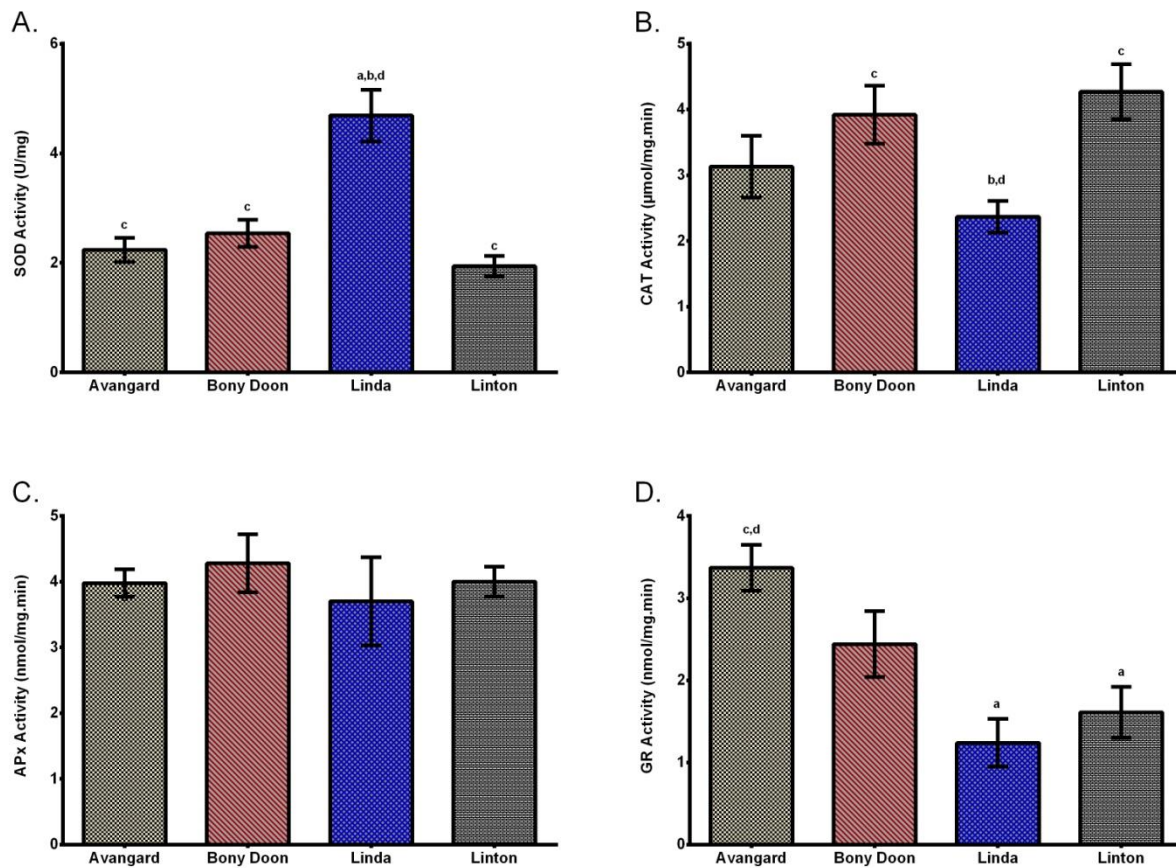


Figure 2: Antioxidant enzyme activities in four different genotypes of flaxseeds. (A): SOD activity; (B): CAT activity; (C): APx activity; (D): GR activity in different genotypes of flaxseeds. ^arepresents the significant differences as compared to Avangard ($P < 0.05$); ^brepresents the significant differences as compared to Bony Doon ($P < 0.05$); ^crepresents the significant differences as compared to Linda ($P < 0.05$); ^drepresents the significant differences as compared to Linton ($P < 0.05$).

The discrepancies in the enzymatic activities may be attributed to relative increase or decrease in enzyme activities as a compensatory mechanism to oxidative stress or direct inhibitory effects of free radicals or decrease in the total amount of antioxidant molecules in the system.

4. Conclusion

Flaxseed (*Linum usitatissimum* L.) has been the focus of interest of nutritionists due to potential health benefits. However, biochemical characterizations of all genotypes and their antioxidant efficacies have not been thoroughly documented. Further research in the context of *in vitro* and *in vivo* antioxidant potential of flaxseed is essential, as limited research attention has been received by them so far. In this article, an attempt was made to characterize antioxidant potential of four different genotypes of flaxseed; Avangard, Bony Doon, Linda, Linton. Linda types are found to have somewhat different phyto-

chemicals and antioxidant profile since its oxidative status tends to the production of oxidant molecules which was reflected in differences in radical scavenging, MDA and antioxidant enzyme systems. Other flaxseeds demonstrate noticeable activities with their antioxidant potential together with their high phytochemicals. Therefore, they may be utilized as a promising source of therapeutics since they might provide an appropriate source of antioxidants.

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Declaration of interest

The authors have no conflicts of interest to disclose.

References

- Ábrahám E, Hourton-Cabassa C, Erdei L, Szabados L (2010). Methods for Determination of Proline in Plants. In: Methods Mol. Biol. pp 317–331.
- Aebi H (1984). In Methods in Enzymology. In: Catalase Vitro. Academy Press, NY, pp 121–126.
- Amawi H, Ashby C, Samuel T, Peraman R, Tiwari AK (2017). Polyphenolic nutrients in cancer chemoprevention and metastasis: Role of the epithelial-to-mesenchymal (EMT) pathway. *Nutrients* 9(8): 911.

- Czemplik M, Kulma A, Bazela K, Szopa J (2012). The biomedical potential of genetically modified flax seeds overexpressing the glucosyltransferase gene. *BMC Complement Altern Med* 12:251.
- Foster JG, Hess JL (1980). Responses of superoxide dismutase and glutathione reductase activities in cotton leaf tissue exposed to an atmosphere enriched in oxygen. *Plant Physiol* 66: 482–487.
- Goyal A, Sharma V, Upadhyay N, Gill S, Sihag M (2014). Flax and flaxseed oil: an ancient medicine & modern functional food. *J Food Sci Technol* 51: 1633–1653.
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951). Protein measurement with the Folin phenol reagent. *J Biol Chem* 193: 265–275.
- Maity P, Samanta S, Nandi AK, Sen IK, Paloi S, Acharya K, Islam SS (2014). Structure elucidation and antioxidant properties of a soluble β -d-glucan from mushroom *Entoloma lividoalbum*. *Int J Biol Macromol* 63: 140–149.
- Marklund S, Marklund G (1974). Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem* 47: 469–474.
- Pal J, Ganguly S, Tahsin KS, Acharya K (2010). In vitro free radical scavenging activity of wild edible mushroom, *Pleurotus squarrosulus* (Mont.) Singer. *Indian J Exp Biol* 48: 1210–1218.
- Prasad K (2000). Flaxseed: a source of hypocholesterolemic and antiatherogenic agents. *Drug News Perspect* 13: 99–104.
- Prasad K (2009). Flaxseed and cardiovascular health. *J Cardiovasc Pharmacol* 54: 369–377.
- Sairam RK, Saxena DC (2000). Oxidative stress and antioxidants in wheat genotypes: possible mechanism of water stress tolerance. *J Agron Crop Sci* 184: 55–61.
- Savicka M, Skute N (2010). Effects of high temperature on malondialdehyde content, superoxide production and growth changes in wheat seedlings (*Triticum aestivum* L.). *Ekologija* 56: 26–33.
- Taga MS, Miller EE, Pratt DE (1984). Chia seeds as a source of natural lipid antioxidants. *J Am Oil Chem Soc* 61: 928–931.
- Turkoglu A, Duru ME, Mercan N, Kivrak I, Gezer K (2007). Antioxidant and antimicrobial activities of *Laetiporus sulphureus* (Bull.) Murrill. *Food Chem* 101: 267–273.
- Wang L, Chen J, Thompson LU (2005). The inhibitory effect of flaxseed on the growth and metastasis of estrogen receptor negative human breast cancer xenografts attributed to both its lignan and oil components. *Int J Cancer* 116: 793–798.

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Octospora Hedw., A New Genus Record for Turkish Pyronemataceae

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Octospora Hedw., Türkiye Pyronemataceae'leri İçin Yeni Bir Cins Kaydı

Abstract: The genus *Octospora* Hedw. is given as new record for the macromycota of Turkey, based on the collection and identification of the taxon *Octospora itzerottii* Benkert. Brief description of macroscopic and microscopic characters and photographs related to macro and micro morphology of the taxon are provided.

Key words: Biodiversity, *Octospora*, new record, *Pezizales*, Turkey

Özet: *Octospora* Hedw. cinsi, *Octospora itzerottii* Benkert. taksonunun toplanması ve teşhis edilmesi neticesinde, Türkiye makromikotasi için yeni kayıt olarak verilmiştir. Taksona ait makroskopik ve mikroskopik karakterlerin kısa betimlemesi ve türün makro ve mikromorfolojisine ilişkin fotoğrafları verilmiştir.

Anahtar Kelimeler: Biyoçeşitlilik, *Octospora*, yeni kayıt, *Pezizales*, Turkey

1. Introduction

Octospora Hedw is a genus of operculate ascomycetes within the order *Pezizales* and the family *Pyronemataceae* Corda. The genus has a cosmopolitan distribution and comprises 84 species (Kirk et al., 2008). The members of the genus are characterized by moss associated apothecia and ellipsoid to globose or rounded, sometimes ornamented, guttulate spores. The hyphal structure of the margin of apothecia is another distinguishing character of the members of the genus (Yao and Spooner, 1996).

Itzerott reports (1977) that the members of *Octospora* are relatively common in western Europe. Eighty one octosporoid fungi were reported to exist in Europe (Benkert, 2007). But current checklists (Sesli and Denchev, 2014; Solak et al., 2015) and the studies published after the preparation of the checklists (Akata et al., 2016; Denğiz and Demirel, 2016; Sesli et al., 2016; Taşkın et al., 2016; Uzun and Acar, 2016; Uzun et al., 2017) indicate that any member of the genus *Octospora* have so far been reported from Turkey.

The work aims to make a contribution to the mycobiota of Turkey by adding a new ascomycete taxa.

2. Materials and Method

Octospora samples were collected from Nurdağı district of Gaziantep Province in 2015. Ecological and morphological characteristics of the samples were recorded and they were photographed in their natural habitat. Then the samples were brought to the fungarium, dried in air conditioned room and prepared as fungarium materials in polyethylene bags. Micromorphological investigations were carried out under a Nikon eclipse Ci trinocular light microscope and a DS-Fi2 digital camera and a Nikon DS-L3 displaying apparatus were used for microstructural photographing. The samples were identified mainly with the help of Benkert (1998, 2007, 2009). They are kept at Karamanoğlu Mehmetbey University, Science Faculty, Department of Biology.

3. Results

The systematics of the taxon is given in accordance with Kirk et al. (2008), and the Index Fungorum (www.indexfungorum.org; accessed 31 July 2017). A brief description, habitat, locality, collection date, and accession number of the taxon are provided.

Ascomycota Whittaker

Pezizales J. Schröt.

Pyronemataceae Corda

Octospora Hedw.

Octospora itzerottii Benkert, Öst. Z. Pilzk. 7: 53 (1998)

Macroscopic and microscopic features: Apothecia 1-2.5 mm in diameter, surface concave at first, later plane to convex or pulvinate, hymenium orange, margin paler and not membranaceous (Fig. 1). Asci 170-260 × 15-20 µm, cylindrical, tapering below, mostly 4-spored but also 3-, 5- and 6-spored, spores uniseriate. Paraphyses cylindrical, enlarged towards the apex, 5-8 µm wide at the tips (Fig. 2a). Spores 21-26 × 11-13 µm, ellipsoid, smooth, with one or two large and several small oil droplets (Fig. 2b).

Ecology: *Octospora itzerottii* grows on mosses *Pterygoneurum ovatum* and *P. subsessile* (Eckstein, 2017).

Specimen examined: Gaziantep, Nurdağı, Kömürler village, mixed forest, on moss (*Pterygoneurum ovatum* (Hedw.) Dixon), 37°09'N-36°48'E, 535 m, 03.04.2015, K.11588.

4. Discussions

Generally the members of the genera *Inermisia*, *Lamprospora*, *Neottiella* and *Octospora* within the family *Pyronemataceae* have tiny, disc-shaped apothecia coloured in shades of orange to red. Due to their small size morphological similarities, they can easily be overlooked and it is very hard to separate their species from each other (Itzerott, 1977).

Octospora itzerottii is a 4-spore taxon and has similarities in terms of morphology, ecology and even micromorphology with *Octospora crosslandii* (Dennis & Itzerott) Benkert. But the latter species have 8-spore asci while *O. itzerottii* generally have 4-spore asci. Medium spore size of *O. crosslandii* (17-21 × 10-12) is also smaller than that of *O. itzerottii*.

The current checklists (Sesli and Denchev, 2014; Solak et al., 2015) compiled 34 pyrenomatous macrofungi existing in Turkey. After the latest versions of the checklists 28

members of Pyronemataceae have also been added to these lists and the total taxa number reached to 62 (Keleş et al., 2014; Türkoğlu and Castellano, 2014; Demirel et al., 2015; Karacan et al., 2015; Kaya and Uzun, 2015; Kaya et al., 2016; Kaya, 2016). As a result of this study, the number of pyrenomatoid species known from Turkey has increased from 62 to 63.

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Figure 1. Ascocarps of *Octospora itzerottii*.

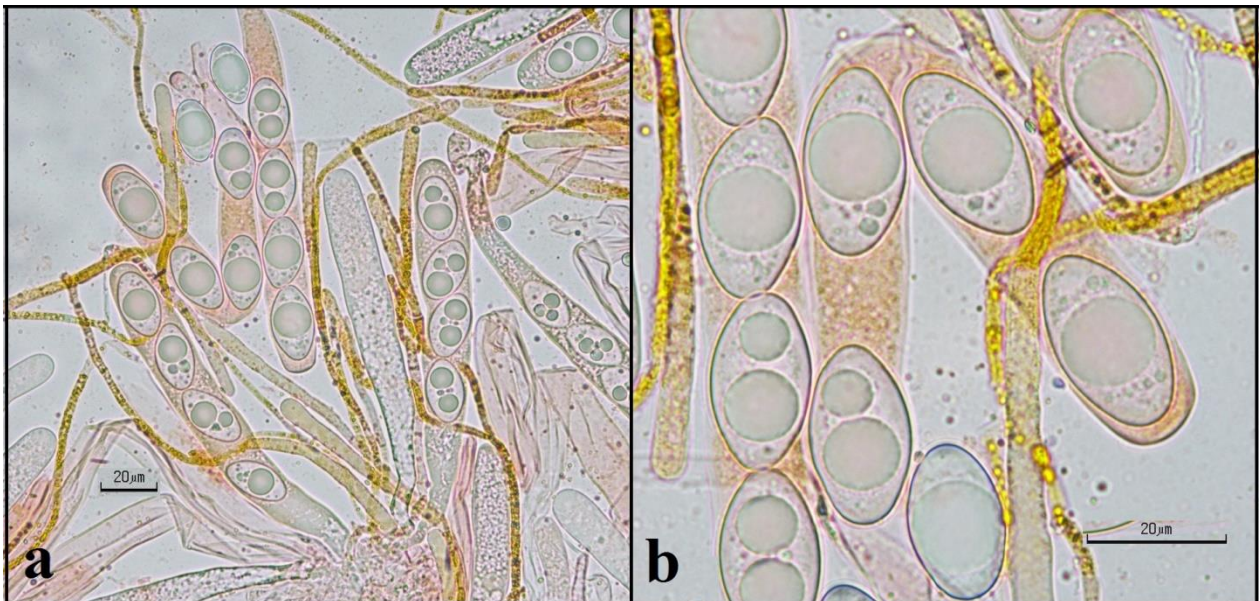


Figure 2. Asci, paraphyses (a) and ascospores (b) of *Octospora itzerottii*.

References

- Akata I, Uzun Y, Kaya A (2016). Macrofungal diversity of Zigana Mountain (Gümüşhane/Turkey). *Biological Diversity and Conservation* 9(2): 57-69.
- Benkert D (1998). Beiträge zur Kenntnis bryophiler Pezizales-Arten. 8. Viersporige Taxa der Gattung *Octospora*. *Österreichische Zeitschrift für Pilzkunde* 7: 39-63.
- Benkert D (2007). Zur Kenntnis des Vorkommens bryophiler Pezizales (Ascomycota) in Südost-Europa. *Mycologia Montenegrina* 10: 7-21.

- Benkert D (2009). Zwei neue Arten bryophiler Pezizales (Ascomycota) aus der Bundesrepublik Deutschland und Auflistung der aus Deutschland bisher nachgewiesenen Arten mit Kurzdiagnostik. Zeitschrift für Mykologie 75: 51-68.
- Demirel K, Uzun Y, Akçay ME, Keleş A, Acar İ, Efe V (2015). Van Yöresi Makromantarlarına Katkıları. Mantar Dergisi 6(2): 13-23.
- Denğiz Y, Demirel K (2016). Şirvan (Siirt) Yöresinde Yetişen Makrofunguslar Üzerinde Taksonomik Bir Araştırma. Yüzüncü Yıl University Journal of the Institute of Natural & Applied Sciences 21(2): 112-123.
- Eckstein J (2017, October). *Octospora itzerottii*: Retrieved from the <http://octospora.de> Web site: <http://octospora.de/Oitzerottii.htm>
- Index Fungorum (2017). <http://www.indexfungorum.org/Names/Names.asp>. Accessed 31 July 2017.
- Itzerott H (1977). The Genus *Octospora* and an Attempt to Solve Its Taxonomic Problems. Kew Bulletin 31(3): 497-500.
- Karacan İH, Uzun Y, Kaya A, Yakar S (2015). *Pulvinula* Boud., a new genus and three pulvinuloid macrofungi taxa new for Turkey. Biological Diversity and Conservation 8(2): 161-164.
- Kaya A (2016). Contributions to the macrofungal diversity of Atatürk Dam Lake basin. Turkish Journal of Botany 39(1): 162-172.
- Kaya A, Uzun Y (2015). Six new genus records for Turkish Pezizales from Gaziantep Province. Turkish Journal of Botany 39(3): 506-511.
- Kaya A, Uzun Y, Karacan İH, Yakar S (2016). Contributions to Turkish Pyronemataceae from Gaziantep Province. Turkish Journal of Botany 40(3): 298-307.
- Keleş A, Demirel K, Uzun Y, Kaya A (2014). Macrofungi of Ayder (Rize/Turkey) high plateau. Biological Diversity and Conservation 7(3): 177-183.
- Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008). Dictionary of the Fungi. 10th ed. Wallingford, UK: CAB International.
- Sesli E, Denchev CM (2014). Checklists of the myxomycetes, larger ascomycetes, and larger basidiomycetes in Turkey. 6th edn. Mycotaxon Checklists Online. (<http://www.mycotaxon.com/resources/checklists/sesli-v106-checklist.pdf>): 1-136.
- Sesli E, Türkekul İ, Akata I, Niskanen T (2016). New records of Basidiomycota from Trabzon, Tokat, and İstanbul provinces in Turkey. Turkish Journal of Botany 40(5): 531-545.
- Solak MH, Işıoğlu M, Kalmış E, Allı H (2015). Macrofungi of Turkey, Checklist, Vol. II. İzmir, Turkey: Üniversiteler Ofset (in Turkish).
- Taşkın H, Doğan HH, Büyükalaca S, Colwez P, Moreau PA, O'Donnell K (2016). Four new morel (*Morchella*) species in the elata subclade (M. sect. *Distantes*) from Turkey, Mycotaxon, Volume 131 (2), 467-482.
- Türkoğlu A, Castellano MA (2014). New records of some Ascomycete truffle fungi from Turkey. Turkish Journal of Botany 38(2): 406-416.
- Uzun Y, Acar İ, Akçay ME, Kaya A (2017). Contributions to the macrofungi of Bingöl, Turkey. Turkish Journal of Botany 41(5): 516-534.
- Uzun Y, Acar İ (2016). *Peziza granularis* Donadini Türkiye Mikobiyotası için Yeni Bir Kayıt. Yüzüncü Yıl Üniversitesi Fen Bilimleri Enstitüsü Dergisi 21(1): 39-42.
- Yao YJ, Spooner BM (1996). Notes on British species of *Octospora*. Mycological Research 100: 175-178.

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