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Ecological aspects of the arid and semi-arid truffle in Turkey: evaluation of soil characteristics, morphology, distribution, and mycorrhizal relationships

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Abstract: We undertook an evaluation of the physical and chemical quality of soil characteristics, micro- and macroscopic features of morphology, distribution, and mycorrhizal relationships of *Terfezia boudieri* Chatin. The topsoil was found to be 54.73% sand, 21.97% clay, 23.30% dust, 45.27% dust-clay, 0.126% nitrogen, 1.848% organic matter, and 3.78% total lime. Investigations revealed that the soil contained 40.92 ppm P_2O_5, 613.0 ppm K_2O, 28.82 ppm Na, and 0.164 mmhos/cm salt. It had a pH of 7.12 and the soil type was determined to be sand-clay-slimy. Various characteristics were recorded including ascocarp features (hypogeous, like tubers) and colour (peridium, glebe red tones), occurrence frequency (infrequent), time of appearance (March to May), host (*Helianthemum salicifolium* (L.) Miller), traditional uses (food, aphrodisiac), eating quality (excellent), habitat (basic soil, sand-clay-slimy, and semi-arid zone), fresh weight (20-150 g) and ascospore morphology (reticulate and warty spherica, 15-18 µm, medium size). In conclusion, the habitats of *T. boudieri* and *H. salicifolium* should be protected by turning those fields into natural protected areas.

Key words: Biodiversity, *Helianthemum*, truffle, mushroom, mycorrhiza, *Terfezia*

Türkiye'de kurak ve yarı kurak alanlardaki yer mantarına ekolojik bakış: Toprak karakteristikleri, morfoloji, dağılımları ve mikorizal ilişkilerinin değerlendirilmesi

Özet: Bu çalışmada; *Terfezia boudieri* Chatin türünün mikorizal ilişkisi, dağılımı, mikro-makroskobik özelliği ile toprağın kimyasal ve fiziksel niteliği araştırıldı. Üst toprağın bazı karakteristik özellikleri; %54,73 kum, %21,97 kil, %23,30 toz, %45,27 kil-toz, %0,126 nitrojen, %1,848 organik madde, %3,78 kireç, 40,92 ppm P_2O_5, 613,0 ppm K_2O, 28,82 ppm Na, 0,164 mmhos/cm tuz, 7,12 pH ve toprak çeşidi olarak kum-kil-balçık olduğu bulunmuştur. Askokarp özelliği (hypogeous, yumru gibi), renk (peridium, kırmızı toprak), meydana gelme sıklığı (nadir, seyrek), görülme dönemi (Mart-Mayıs), konukçusu (*Helianthemum salicifolium* (L.) Miller), geleneksel kullanımı (gıda, afrodizyak), yenebilme kalitesi (mükemmel, leziz), habitat (temel toprak, kum-kil-balçık ve yarı kurak bölge), taze ağırlığı (20-150 g) ve aynı zamanda askospor morfolojisinide (ağsı, siğil şeklinde küremsi, 15-18 µm, orta boy) içeren çeşitli karakterler kaydedilmiştir. Sonuç olarak; *T. boudieri* ve *H. salicifolium* habitatları, doğal koruma alanları ilan edilerek korunması sağlanmalıdır.

Anahtar sözcükler: Biyolojik çeşitlilik, *Helianthemum*, keme, mantar, mikoriza, *Terfezia*

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Introduction

Truffles are edible hypogeous fruit bodies produced by many genera of fungi belonging to the class *Ascomycetes* G.Winter. The hypogeous ascocarps of these fungi are known as truffles. Truffle mushrooms can be classified as forest truffles, desert truffles, and semi-arid truffles. Among these, *Terfezia* (Tul. & C.Tul) Tul. & C.Tul and *Tuber* P.Micheli ex. F.H.Wigg. are classified in different taxa in *Pezizales* J.Schröt (Roth-Bejerano et al., 2004). Underground members of the *Pezizaceae* are well distributed around the globe. The genera *Tirmania* Chatin and *Terfezia* are monophyletic, and morphological species correspond to phylogenetic species. *Terfezia* clade-grouped species found in semi-arid habitats have ornamented and spherical spores. These species are adapted to exploit different types of soil in association with specific hosts. They are not mycorrhizal with trees. Although other factors might also play a role, host specialisation and edaphic tolerances may be key in the species diversity of these genera (Diez et al., 2002). *Terfezia* and *Tirmania* spp. form mycorrhizae mainly on roots of the family *Cistaceae*, including different species of the genus *Helianthemum* (L.) Miller (Dexheimer et al., 1985; Fortas & Chevalier, 1992; Gücin & Dülger, 1997), as well as other symbionts. Mycorrhizal fungi ramify through the soil, absorbing nitrogen and other minerals, which are transported back to the host plant (Ewaze & Al-Naama, 1989). These plants and their associated fungi may play a major role in the maintenance of Mediterranean shrublands and xerophytic grasslands, in terms of preventing erosion and desertification (Honrubia et al., 1992).

Pezizales are found in arid and semi-arid zones of Syria, Iraq, Iran, Lebanon, Bahrain, Jordan, Kuwait, Saudi Arabia, Tunisia, Egypt, the Sahara, Algeria, Morocco, the Kalahari Desert, Italy, France, Spain, and Turkey (Lawrynowicz et al., 1997; Al-Ruqaie, 2002; Diez et al., 2002; Moreno et al., 2002; Mandeel & Al-Laith, 2007; Trappe et al., 2008). In addition, it grows in Europe, where it is known locally as trüff, truffle, and la truffe, as well as several common Arabic names which are still in use today depending on local dialects: al-kamah, al-chamae, kamaa, kame, al-faga, and faga (Bokhary, 1987; Mandeel & Al-Laith, 2007). In Turkey, it is known locally as keme, kemi, kumi, domalan, dobalan, dolaman, dobelan, dümbelek, duvberon, tombalak, topalak, and türüf.

Terfezia boudieri Chatin is a seasonal and socio-economically important mushroom. It usually appears in arid and semi-arid zones following the rainy season from March to May in Turkey. It is rare, due to its specific, economic, and nutritional value and medical importance. It is an expensive delicacy; the cost of 1 kg of *T. boudieri* may reach as high as $15-70 in Turkey while the cost of truffles may reach as high as $60 per kg in Saudi Arabia, on a bargain basis (Bokhary, 1987), and up to 200€ per kg in France (Chang & Mshigeni, 2001). The high cost might be due to their scarcity, coupled with poor yield in certain seasons in each country. Al-Laith states that the truffle commodity is regarded as a costly delicacy (2010). The popularity of truffles is thought to be due to their nutritional value, delicious taste, and aphrodisiac feature (Langley-Danysz, 1982). Recently, publications on the macrofungi flora of Turkey have increased (Alkan et al., 2010; Demirel et al., 2010; Uzun et al., 2010; Allı, 2011).

With the exception of some *Tuberaceae* family members, truffle members of the order *Pezizales* have been neglected by science. Among truffles, *T. boudieri* is especially prized for its culinary value due to its intense aroma. Therefore, the aim of this research was to extend knowledge on the physical and chemical quality of the soil characteristics of growing fields, micro- and macroscopic features of morphology, distribution, and mycorrhizal relationships such as ecological aspects of *T. boudieri* analysed in Turkey.

Material and methods

Obtaining the mushrooms

A wild sample of fresh *Terfezia boudieri* Chatin was collected from the vicinity of Kadıköy, Baskil-Elazığ, Turkey (38°26' 541'N, 38°41' 752' E, 700 m, 13.05.2007), as shown in Figure 1. It was collected from the wild area during early spring and spring, particularly from March to May. We identified the location of the truffles from crevices that appeared in the surface of the soil above the truffles (Figure 1). The samples were cleaned without washing, cut into slices, dried at room temperature, and then stored (Figure 1).

Figure 1. The species of *Terfezia boudieri* from Kadıköy, Baskil-Elazığ, Turkey. **a** - a view of the species growing deep in the earth and the surrounding vegetation; **b, c** - ascocarps of *T. boudieri* and the state of *T. boudieri* in deep soil after removal of the surface soil; **d** - cut *T. boudieri*; **e** - mycorrhizal with *H. salicifolium*; **f** - ascospores of *T. boudieri*.

Soil sampling and methods of analysis

Before establishing the experiment, a soil sample was taken from a depth of about 10-15 cm from the surface by means of a stainless steel crab. It was air-dried in the laboratory at 25 °C, crushed with a wooden pestle, screened through a 2 mm sieve, and analysed to determine some of the physical and chemical characteristics.

Selected soil physico-chemical properties were determined with appropriate methods, as described below: distribution of soil texture such as sand, clay, and dust by the hydrometer method (Bouyoucus, 1962), $CaCO_3$ content by Scheibler calcimeter method (Allison & Modie, 1965), organic matter by modified Walkley-Black (Walkely & Black, 1934; Nelson & Sommers, 1996), total nitrogen by the Kjeldahl digestion and distillation procedure (Bremner, 1965), pH and electrical conductivity as mmhos/cm were measured in water with a 1:2.5 ratio of soil solution (Jackson, 1958; Allison & Moode, 1965), available phosphorus by the vanadomolybdate method of Murphy and Riley (1972), and Na and K were determined by flame photometer using atomic absorption spectroscopy (AAS). Furthermore, exchange acidity was extracted with 0.1 N NaOH solution (Jackson, 1958).

Results and discussion

Study area, vegetation, and mycorrhizal relationships

Terfezia boudieri Chatin is a kind of fungus that spreads on steppes, grows under soil, and appears with spring rains (Figure 1). It has mycorrhizal associations with the roots of *Cistaceae*, such as *Helianthemum salicifolium* (L.) Mill. (Figure 1). Some reports also exist on mycorrhizal associations between *Terfezia* and the roots of *Helianthemum* (Dexheimer et al., 1985; Fortas & Chevalier, 1992; Diez et al., 2002; Mandeel & Al-Laith, 2007). In our research, this mushroom was observed to

inhabit sand-clay-slimy soils and require water. In addition, this region has both great ecological and agricultural prosperity because it has sand-clay-slimy soil in impoverished steppe ecosystems within the Anatolian climate region. Some characteristics of the topsoil of the fields in which *Terfezia boudieri* grows were found to be 54.73% sand, 21.97% clay, 23.30% dust, 45.27% dust-clay, 0.126% nitrogen, 1.848% organic matter, 3.78% total lime, 40.92 ppm P_2O_5, 613.0 ppm K_2O, 28.82 ppm Na, 0.164 mmhos/cm salt, 7.12 pH, and a sand-clay-slimy soil type, as seen in Table 1. The truffles are mostly found in barren, uncultivated meadows in mountainous areas. The soils are generally rendzina, chalky, alkaline, granular, and well drained (Ammarellou & Saremi, 2008), as well as being poor in organic matter content, slightly alkaline, and saline. The limestone is covered by various densities of saline sand, capable of supporting only halophytic desert vegetation in the form of small trees and shrubs (Mandeel & Al-Laith, 2007). Sandy soils are 80%-90% sand, 4%-9% clay, 1%-8% silt, 7.9-8.5 pH, and 4%-5.4% $CaCO_3$ (Kagan-Zur & Roth-Bejerano, 2008). Analyses of Kalahari truffle-bearing sands by Taylor et al. (1995)

Table 1. Some characteristics of the topsoil in natural *Terfezia boudieri* growing fields in Baskil-Elazığ, Turkey.

(%)								(ppm)			(mmhos/cm)		
Sand	**Clay**	**Dust**	**Dust+clay**	**Nitrogen**	**Organic matter**	**Lime**		**P_2O_5**	**K_2O**	**Na**	**Salt**	**pH**	**Soil kind**
						Total	**Active**						
54.73	21.97	23.30	45.27	0.126	1.848	3.78	-	40.92	613.0	28.82	0.164	7.12	sand-clay-slimy

showed pH ranging from 5.5 to 6.5 in 8 stands and reaching 7.2 in another. $CaCO_3$ was also low, ranging from 0.3% to 3.1%. Those data were very different from those given in previous work (Taylor et al., 1995; Hashem & Al-Obaid, 1996; Mandeel & Al-Laith, 2007; Ammarellou & Saremi, 2008; Kagan-Zur & Roth-Bejerano, 2008). This difference probably arose from different growth regions, ecological habitats, environmental conditions, temperatures, rainfall, soil types, and host plants, as observed by Diez et al. (2002). Normally, abundant spring rainfall induces good ascocarp production. The formation of truffles depends on several factors such as the rainy season with its amount and timing, soil types and characteristics, water availability, and climatic conditions (Bokhary & Parvez, 1988). They are normally collected from wild areas during early spring and spring, particularly from March to May. We identified the location of the truffles from crevices that had appeared in the surface of the soil above the truffles (Figure 1). Conversely, Ammarellou et al. (2007), Ammarellou and Saremi (2008), and Kagan-Zur and Roth-Bejerano (2008) have reported on truffles that were normally harvested during mid-winter and early spring, particularly from January to March. They were different from each other.

Macroscopic and microscopic studies

Ascocarp of *Terfezia boudieri* (Figure 1) is generally hypogeous, i.e. like tuber, with basal attachment. Fresh weight ranges between 20 and 150 g per ascocarp with the colour being brown to dark brown or blackish brown, or especially glebe red tones. The size of the ascospore's diameter is about 15-18 µm (Figure 1). Various characteristics of the macro- and microscopic structure were recorded, including ascocarp features and colour (peridium, glebe red tones), occurrence frequency (infrequent), time of appearance (March to May), host (*Helianthemum salicifolium*), traditional uses (food, aphrodisiac), eating quality (excellent), habitat (basic soil, sand-clay-slimy, and semi-arid zone), fresh weight (20-150 g), and ascospore morphology (reticulate and warty spherica, 15-18 µm, medium size), as seen in Table 2. Other researchers have reported various values for the fresh weight, ascocarp features and colour, occurrence frequency, time of appearance, host plant, size of ascospores, uses, eating quality, habitat and growth region features, and ethnomycological aspects (Hashem & Al-Obaid, 1996; Diez et al., 2002; Moreno et al., 2002; Ferdman et al., 2005; Ammarellou et al., 2007; Mandeel & Al-Laith, 2007; Ammarellou

Table 2. Some characteristics of the ascocarp and ascospores of *Terfezia boudieri* from Baskil-Elazığ, Turkey.

Ascocarp features	Habitat	Host	Time of appearence	Occurence frequency	Traditional uses	Eating quality	Spore morphology
thick peridium, brown-glebe red tones, ~20-150 g	basic soil (sand-clay-slimy) and semiarid zone	*Helianthemum salicifolium*	spring (March to May)	infrequent	food, aphrodisiac feature	excellent	reticulate and warty spherical, medium

& Saremi, 2008; Kagan-Zur & Roth-Bejerano, 2008). These data were similar to the data in our work, but it was also observed that some values varied in the cited studies. This difference probably arose from different growing regions, ecological habitats, environmental and climatic conditions, soil types, and host plants, as noted by Bokhary and Parvez (1988), Diez et al. (2002), and Mandeel and Al-Laith (2007).

Conclusions

Wild fields of *Terfezia boudieri* and *Helianthemum salicifolium* need to be protected from the activities of animal pasture and agriculture (Figure 2). Furthermore, the habitats of the species should be protected by turning those fields into natural protected areas. We also recommend *T. boudieri* to consumers due to its features such as excellent texture and consistency, pleasant aroma, culinary qualities, and aphrodisiac feature.

Figure 2. Distribution of *Terfezia boudieri* in the Anatolian region of Turkey.

[1] Konya (around the Selçuklu, Karatay, and Çumra Districts); [2] Karaman (around the Karadağ, Kılbasan, and Akçaşehir villages and the Ayrancı District); [3] Ankara (around the Polatlı District); [4] Aksaray; [5] Kırşehir; [6] Eskişehir (around the Günyüzü and Çifteler Districts); [7] Kütahya (around the Altıntaş District); [8] Mardin (around the Kızıltepe and Midyat Districts); [9] Şanlıurfa (around the Ceylanpınar and Suruç Districts); [10] Elazığ (around the Baskil District); [11] Malatya; [12] Gaziantep (around the Nizip District); [13] Diyarbakır.

Regions 1, 2, 4, 9, 10, 11, and 12 represent the most dense growing places of *T. boudieri*.

Regions 3, 5, 6, 7, 8, and 13 represent the rarer growing places of *T. boudieri*.

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