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The checklist of Turkish *Pezizales* species

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Türkiye *Pezizales* türleri kontrol listesi

Abstract: This study presents a checklist of the species belonging to order *Pezizales*, determined in Türkiye. The list includes 264 species belonging to 81 genera and 15 families. In the list, the current name of the species, distributions in Türkiye (at province level), and the literature related to obtained data are provided.

Key words: Macrofungi, *Ascomycota, Pezizomycetes, Pezizales*

Özet: Bu çalışma Türkiye'de tespit edilmiş *Pezizales* takımına mensup türlerin bir kontrol listesini sunmaktadır. Liste 81 cins ve 15 familya içinde yer alan 264 tür içermektedir. Listede, türlerin güncel isimleri, Türkiye'deki yayılışları (il bazında) ve veri kaynağı olarak kullanılan literatür verilmiştir.

Anahtar Kelimeler: Makromantarlar, *Ascomyota, Pezizomycetes, Pezizales*

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1. Introduction

Pezizales J. Schröt. is a large order of the subclass *Pezizomycetidae.* It contains the operculate discomycetes which are the most readily recognized cup fungi. General characteristics of the members of *Pezizales* can be summarised as follows: Ascocarp mostly an epigeous cup-shaped or disc-like, fleshy, sometimes stalked apothecium ranging between less than one milimeter to several centimeters. Some form hypogeous ascomata and rely on animals for their spore dispersal while the epigeous fruiting bodies have an active spore dispersal mechanism. In general, asci cylindrical with an operculum. Most of the members are terrestrial and saprotrophic on soil, burnt ground, decaying wood, compost or dung, some may form ectomycorrhiza with trees (Maia et al., 1996; Webster and Weber, 2007).

Kirk et al (2008) gives the existing family, genera and species numbers as 16, 199 and 1683 respectively. Until the end of 2022, 264 of them have also been presented from Türkiye. Here we present the current names, distribution (at province level) and the relative literature.

The study aims to establish a list of *Pezizales* members in our country and to make a contribution to the mycobiota of Türkiye.

2. Materials and Method

The studies on Turkish macromycetes including the members of *Pezizales* were traced and a list of the taxa belonging to the order *Pezizales* were prepared together with their distribution localities. The list was also cross-checked with the checklists (Solak et al., 2007, 2015; Sesli and Denchev, 2008, 2014; Sesli et al., 2020; Akata et al., 2022; Solak and Türkoğlu, 2022) presented so far, and latest contributions. During preparation of the list, only the taxa presented in a peer reviewed article or a full text conference paper were considered. The taxa which were not reported by a paper or full text proceeding but anonymised by the previously presented checklists, were also included in the list. But those presented in other conference papers, graduate theses or project reports were not considered. Those taxa which were presented only at generic level (i.e. *Peziza* sp.) but not reported at species level were not included in the list.

3. Results

A list of 264 species of *Pezizales* which were reported in Türkiye were compiled. The species were listed in alphabetical order. Colour photographs of some species were also presented (Fig. 1).

Ascobolaceae Boud. ex Sacc.

Ascobolus behnitziensis Kirschst.: Karaman (Uzun and Kaya, 2016).

Ascobolus carbonarius P. Karst.: Gaziantep (Uzun et al., 2018).

Ascobolus crenulatus P. Karst.: Gaziantep (Uzun et al., 2018).

Ascobolus foliicola Berk. & Broome: Gaziantep (Uzun et al., 2018).

Ascobolus furfuraceus Pers.: Diyarbakır (Demirel et al., 2016); Gaziantep (Kaya et al., 2014); Trabzon (Akata et al., 2012).

Ascobolus immersus Pers.: Gaziantep (Uzun et al., 2018).

Saccobolus glaber (Pers.) Lambotte: Gaziantep (Uzun et al., 2018).

Thecotheus holmskjoldii (E.C. Hansen) Chenant.: Gaziantep (Uzun et al., 2018).

Thecotheus lundqvistii Aas.: Karaman (Çetinkaya et al., 2020).

Thecotheus pelletieri (P. Crouan & H. Crouan) Boud.: Gaziantep (Kaya and Uzun, 2015).

Ascodesmidaceae J. Schröt.

Lasiobolus cuniculi Velen.: Gaziantep (Uzun et al., 2018).

Lasiobolus papillatus (Pers.) Sacc.: Aydın (Güngör et al., 2014).

Caloscyphaceae Harmaja

Caloscypha fulgens (Pers.) Boud.: Adana (Doğan and Kurt, 2016); Adıyaman (Kaya, 2009); Antalya (Öztürk et al., 2003); Artvin (Demirel et al., 2017); Balıkesir (Altuntaş et al., 2017); Bolu (Yağız et al., 2006a; Servi et al., 2010); Gaziantep (Kaya, 2009; Uzun et al., 2015); Kahramanmaraş (Kaya, 2009; Kaya et al., 2009); Karabük (Yağız et al., 2005); Karaman (Doğan and Öztürk, 2006); Kastamonu (Akata et al., 2010); Kayseri (Kaşık et al., 2003; Türkoğlu and Gezer, 2006); Kocaeli (Doğan et al., 2021); Konya (Alkan et al., 2010; Kaşık et al., 2010); Mersin (Doğan et al., 2010, 2012); Nevşehir (Doğan and Türkoğlu, 2006); Sinop (Afyon et al., 2004); Trabzon (Akata et al., 2014; Akata and Uzun, 2017).

Discinaceae Benedix

Discina ancilis (Pers.) Sacc.: Adana (Doğan ve Kurt, 2016); Artvin (Demirel et al., 2017); Balıkesir (Solak et al., 2002); Bolu (Servi et al., 2010); Erzurum (Sadullahoğlu et al., 2021); Isparta (Güngör et al., 2015); İzmir (Solak et al., 1999); Kahramanmaraş (Kaya, 2009); Konya (Alkan et al., 2010); Kütahya (Allı et al., 2017); Yozgat (Türkekul and Işık, 2016).

Discina brunnea (Underw.) Raitv.: Hakkâri (Acar et al., 2018).

Discina melaleuca Bres.: Antalya (Solak et al., 2014b); Denizli (Gezer et al., 2011); Uşak (Türkoğlu ve Yağız, 2012).

Gyromitra ambigua (P. Karst.) Harmaja: Erzurum (Gücin et al., 2000).

Gyromitra esculenta (Pers.) Fr.: Aksaray (Doğan and Türkoğlu, 2006); Antalya (Gezer, 2000); Aydın (Allı and Işıloğlu, 2007; Allı et al., 2007); Balıkesir (Solak et al., 2002; Altuntaş et al., 2017); Bartın (Özkazanç and Yeşilbaş Keleş, 2019); Bolu (Afyon and Konuk, 2001b ; Yağız et al., 2006a); Denizli (Gezer et al., 2007, 2011a,b; Türkoğlu et al., 2007; Türkoğlu, 2008); Eskişehir (Köstekci et al., 2005); Isparta (Güngör et al., 2015); İzmir (Solak et al., 1999); Karaman (Öztürk et al., 2001); Kastamonu (Afyon and Konuk, 2001b); Konya (Alkan et al., 2010); Kütahya (Allı et al., 2017); Muğla (Işıloğlu, 2001; Güngör et al., 2013, 2016); Nevşehir (Doğan and Türkoğlu, 2006); Osmaniye (Solak et al., 2012); Uşak (Türkoğlu et al., 2008); Yozgat (Türkekul and Işık, 2016).

Gyromitra gigas (Krombh.) Cooke: Artvin (Demirel et al., 2017); Bingöl (Uzun et al., 2009); Diyarbakır (Acar et al., 2015); Kastamonu (Afyon et al., 2005; Yağız et al., 2006b; Akata et al., 2010); Kütahya (Allı et al., 2017).

Gyromitra infula (Schaeff.) Quél.: Artvin (Demirel et al., 2004, 2017); Gümüşhane (Sesli, 2007; Akata et al., 2016); Eskişehir (Köstekci et al., 2005); Kastamonu (Akata et al., 2010); Trabzon (Sesli, 1998).

Gyromitra longipes Harmaja: Muğla (Güngör et al., 2013).

Gyromitra tasmanica Berk. & Cooke: Muğla (Güngör et al., 2013).

Helvellaceae Fr.

Balsamia vulgaris Vittad.: Muğla (Allı ve Doğan, 2019).

Barssia gunerii H.H. Doğan, Bozok & Taşkın: Osmaniye (Doğan et al., 2018).

Barssia hellenica Kaounas, Agnello, P. Alvarado & Slavova: Gaziantep (Uzun et al., 2018).

Dissingia leucomelaena (Pers.) K. Hansen & X.H. Wang: Adıyaman (Kaya et al., 2004; Kaya, 2005, 2009, 2010); Ankara (Akata et al., 2019); Antalya (Öztürk et al., 2003; Gezer, 2000; Solak et al., 2014b); Aydın (Allı et al., 2007); Balıkesir (Solak et al., 2002; Altuntaş et al., 2017); Bolu (Yağız et al., 2006a; Afyon and Konuk, 2001b); Burdur (Solak et al., 2013); Çorum (Alkan et al., 2016); Denizli (Köse et al., 2006; Türkoğlu et al., 2007; Türkoğlu, 2008; Gezer et al., 2011a,b; Kaşık et al., 2013); Erzurum (Öztürk et al., 2000; Sadullahoğlu et al., 2021); Gaziantep (Kaya, 2009; Kaya et al., 2012; Kaya et al., 2014; Uzun et al., 2015); Hatay (Baba et al., 2013; Güngör et al., 2016); Isparta (Güngör et al., 2015); İzmir (Solak et al., 1999); Kahramanmaraş (Kaya, 2006, 2009, Kaya et al., 2009); Karaman (Öztürk et al., 2001); Kırıkkale (Güler et al., 2013); Kilis (Solak et al., 2014a); Konya (Aktaş et al., 2003; Alkan et al., 2010; Kaşık et al., 2010, 2017; Çevik et al., 2021); Kütahya (Allı et al., 2017); Malatya (Işıloğlu and Öder, 1995a; Işıloğlu, 1997); Manisa (Gücin and Öner, 1982); Mersin (Doğan et al., 2007, 2012; Güngör et al., 2015); Muğla (Işıloğlu, 2001; Güngör et al., 2016; Tırpan et al., 2018); Niğde (Kaşık et al., 2001; Berber et al., 2022); Osmaniye (Solak et al., 2012); Sakarya (Doğan et al., 2021); Şanlıurfa (Kaya, 2015); Tokat (Yıldız et al., 2019); Trabzon (Akata et al., 2014); Uşak (Türkoğlu and Yağız, 2012); Van (Demirel et al., 2015); Yozgat (Kırış et al., 2012; Türkekul and Işık, 2016).

Helvella acetabulum (L.) Quél.: Adıyaman (Kaya, 2005, 2010); Ankara (Güler and Mutlu, 2003; Akata et al., 2019); Antalya (Öztürk et al., 2003); Artvin (Demirel and Işıloğlu, 1993); Aydın (Allı et al., 2007); Batman (Demir et al., 2007); Bingöl (Uzun et al., 2017); Bitlis (Kaya, 2001); Bolu (Yağız et al., 2006a); Burdur (Solak et al., 2013); Çankırı (Öztürk et al., 2010); Denizli (Köse et al., 2006; Gezer et al., 2007a,b; 2008, 2011a,b; Türkoğlu, 2008; Kaşık et al., 2013); Diyarbakır (Acar et al., 2015; Demirel et al., 2016); Erzincan (Keleş and Demirel, 2010; Allı, 2011); Erzurum (Öztürk et al., 2000; Keleş et al., 2016; Sadullahoğlu et al., 2021);

Eskişehir (Köstekci et al., 2005); Gaziantep (Uzun et al., 2015); İzmir (Solak et al., 1999); Kahramanmaraş (Kaya, 2006, 2009; Kaya et al., 2009); Karaman (Kaşık et al., 2000; Doğan and Öztürk, 2006); Kastamonu (Yağız et al., 2006b); Kayseri (Kaşık et al., 2002, 2003; Türkoğlu and Gezer, 2006); Kilis (Solak et al., 2014a); Konya (Kaşık et al., 2010; Çevik et al., 2021); Kütahya (Allı et al., 2017); Malatya (Gücin, 1987; Işıloğlu and Öder, 1995a; Işıloğlu, 1997); Mersin (Doğan et al., 2012; Güngör et al., 2015, 2016); Muğla (Işıloğlu, 2001; Tırpan et al., 2018); Niğde (Berber et al., 2022); Osmaniye (Günay and Demirel, 2006); Sakarya (Doğan et al., 2021); Samsun (Pekşen and Karaca, 2003); Şanlıurfa (Kaya, 2015); Tokat (Yıldız et al., 2019); Trabzon (Sesli, 2007; Akata et al., 2014); Uşak (Türkoğlu et al., 2008); Van (Demirel et al., 2015; Şelem et al., 2019); Yalova (Allı et al., 2017); Yozgat (Kırış et al., 2012; Türkekul and Işık, 2016).

Helvella atra J. König: Kırıkkale (Güler et al., 2013); Konya (Çevik et al., 2021); Kütahya (Allı et al., 2017); Mersin (Doğan et al., 2010); Rize (Sesli, 2007); Samsun (Pekşen ve Karaca, 2003); Trabzon (Akata et al., 2014; Akata ve Uzun, 2017); Tokat (Türkekul, 2008), Yalova (Doğan et al., 2021).

Helvella compressa (Snyder) N.S. Weber: Gaziantep (Uzun et al., 2015).

Helvella corium (O. Weberb.) Massee: Hakkâri (Kesici and Uzun, 2021).

Helvella costifera Nannf.: Adıyaman (Kaya et al., 2004; Kaya, 2005, 2010); Ankara (Akata et al., 2019), Erzincan (Allı, 2011); Gaziantep (Kaya et al., 2014; Uzun et al., 2015); Kahramanmaraş (Kaya, 2009); Kütahya (Allı et al., 2017); Şanlıurfa (Kaya, 2015).

Helvella crispa (Scop.) Fr.: Adana (Işıloğlu and Öder, 1995b); Antalya (Solak et al., 2014b); Bartın (Özkazanç and Yeşilbaş Keleş, 2019); Gümüşhane (Akata et al., 2016); İstanbul (Akata, 2017); İzmir (Yılmaz Ersel and Solak, 2004); Kahramanmaraş (Kaya, 2009); Kocaeli (Akata et al., 2018); Manisa (Gücin and Öner, 1982); Mersin (Işıloğlu and Watling, 1992); Muğla (Işıloğlu, 2001; Güngör et al., 2016); Sakarya (Doğan et al., 2021); Samsun (Pekşen and Karaca, 2003); Sinop (Afyon et al., 2004); Trabzon (Akata et al., 2014; Akata and Uzun, 2017); Zonguldak (Afyon and Konuk, 2002).

Helvella cupuliformis Dissing & Nannf.: Yalova (Allı et al., 2017).

Helvella elastica Bull.: Adana (Güngör et al., 2015); Artvin (Demirel et al., 2017); Balıkesir (Altuntaş et al., 2017); Bursa, Kocaeli, Sakarya, Yalova (Doğan et al., 2021); Denizli (Türkoğlu et al., 2007); Gümüşhane (Akata et al., 2016); İstanbul (Akata, 2017); Kocaeli (Akata et al., 2018); Kütahya (Allı et al., 2017); Trabzon (Akata et al., 2014; Akata ve Uzun, 2017).

Helvella ephippium Lév.: Karaman (Kaşık et al., 2000); Kütahya (Allı et al., 2017).

Helvella fibrosa (Wallr.) Korf: Kahramanmaraş (Kaya, 2009); İzmir-Manisa road (Solak, 1998); Konya (Çevik et al., 2021); Niğde (Berber et al., 2022); Sakarya (Doğan et al., 2021).

Helvella fusca Gillet: Aydın (Allı et al., 2006; Allı ve Işıloğlu, 2007; Allı et al., 2007); Niğde (Berber et al., 2022).

Helvella helvellula (Durieu & Mont.) Dissing: Aydın (Güngör et al., 2015).

Helvella lactea Boud.: Tokat (Türkekul, 2003); Trabzon (Akata et al., 2014); Trabzon (Akata ve Uzun, 2017).

Helvella lacunosa Afzel.: Adana (Doğan ve Kurt, 2016); Adıyaman (Kaya et al., 2004; Kaya, 2005, 2009, 2010); Ağrı (Demirel et al., 2002); Aksaray (Doğan ve Türkoğlu, 2006); Ankara (Akata et al., 2009); Antalya (Solak et al., 2014b); Artvin (Demirel ve Işıloğlu, 1993); Aydın (Allı et al., 2006, 2007); Batman (Demir et al., 2007); Bingöl (Uzun et al., 2017); Bitlis (Kaya, 2001); Bolu (Yağız et al., 2006a); Bursa, Kocaeli, Sakarya (Doğan et al., 2021); Çankırı (Öztürk et al., 2010); Denizli (Köse et al., 2006; Gezer et al., 2007a,b, 2008, 2011; Türkoğlu et al., 2007; Türkoğlu, 2008); Diyarbakır (Demirel et al., 2016); Erzincan (Keleş ve Demirel, 2010; Allı, 2011); Erzurum (Öztürk et al., 2000; Demirel et al., 2003; Keleş et al., 2016; Sadullahoğlu et al., 2021); Eskişehir (Köstekci et al., 2005); Gaziantep (Kaya et al., 2014); Hatay (Güngör et al., 2016); Iğdır (Uzun, 2010); İstanbul (Akata, 2017); Kahramanmaraş (Kaya, 2006, 2009; Kaya et al., 2009); Karabük (Yağız et al., 2005); Kars (Demirel ve Uzun, 1996); Kastamonu (Yağız et al., 2006b; Özkazanç ve Yılmaz Oğuz, 2017); Kayseri (Kaşık et al., 2002); Kilis (Solak et al., 2014a); Konya (Alkan et al., 2010; Çevik et al., 2021); Kütahya (Allı et al., 2017); Malatya (Gücin, 1987; Işıloğlu ve Öder, 1995a; Işıloğlu, 1997); Manisa (Gücin ve Öner, 1982); Mersin (Işıloğlu ve Öder, 1995b); Muğla (Işıloğlu, 2001; Güngör et al., 2016); Nevşehir (Doğan ve Türkoğlu, 2006); Osmaniye (Günay ve Demirel, 2006); Tokat (Türkekul ve Zülfükaroğlu, 2010); Trabzon (Akata et al., 2014); Tunceli (Demirel ve Nacar, 2000); Uşak (Türkoğlu et al., 2008); Van (Demirel et al., 2015; Şelem et al., 2019); Yozgat (Türkekul ve Işık, 2016).

Helvella latispora Boud.: Erzincan (Keleş ve Demirel, 2010); Kütahya (Allı et al., 2017).

Helvella leucopus Pers.: Adana (Işıloğlu ve Watling, 1992); Adıyaman (Kaya et al., 2004; Kaya, 2005; Kaya, 2009a,b); Afyonkarahisar (Oskay ve Kalyoncu, 2006); Balıkesir (Solak et al., 2002; Yılmaz ve Işıloğlu, 2002); Bingöl (Uzun et al., 2009); Bitlis (Kaya, 2001); Bursa (Solak ve Gücin, 1992); Denizli (Köse et al., 2006; Türkoğlu et al., 2007; Gezer et al., 2008); Diyarbakır (Acar et al., 2015; Demirel et al., 2016); Erzincan (Keleş ve Demirel, 2010; Allı, 2011); Erzurum (Öztürk et al., 2000); Gaziantep (Kaya et al., 2012); Iğdır (Uzun, 2010); Isparta (Güngör et al., 2015); İzmir (Solak et al., 1999); Kahramanmaraş (Kaya, 2006, 2009; Kaya et al., 2009); Karabük (Yağız et al., 2005); Kayseri (Atila ve Kaya, 2013); Malatya (Gücin, 1987); Malatya (Işıloğlu, 1997; Işıloğlu ve Öder, 1995a); Muğla (Işıloğlu, 2001); Niğde (Berber et al., 2022); Samsun (Pekşen ve Karaca,

2003); Şanlıurfa (Kaya, 2015); Trabzon (Sesli, 2007); Van (Demirel et al., 2015; Şelem et al., 2019); Yozgat (Kırış et al., 2012).

Helvella macropus (Pers.) P. Karst.: Trabzon (Akata ve Kaya, 2012b).

Helvella monachella (Scop.) Fr.: Gaziantep (Kaya et al., 2019).

Helvella palustris Peck: Kütahya (Allı et al., 2017).

Helvella pezizoides Afzel.: Trabzon (Akata ve Kaya, 2012b).

Helvella philonotis Dissing: Aydın (Allı et al., 2006; Allı and Işıloğlu, 2007; Allı et al., 2007).

Helvella phlebophora Pat. & Doass.: Trabzon (Uzun, 2019).

Helvella solitaria P. Karst.: Antalya (Öztürk et al., 2003); Aydın (Allı et al, 2006; Allı et al., 2007); Balıkesir (Altuntaş et al., 2017); Bingöl (Uzun et al., 2017).

Helvella spadicea Schaeff.: Aksaray (Doğan ve Türkoğlu, 2006; Türkoğlu et al., 2007); Ankara (Öztürk et al., 2017); Denizli (Köse et al., 2006); Karaman (Öztürk et al., 2001; Doğan ve Öztürk, 2006); Kayseri (Kaşık et al., 2002); Konya (Kaşık ve Öztürk, 2000; Aktaş et al., 2003; Çevik et al., 2021); Mersin (Doğan et al., 2007); Nevşehir (Doğan ve Türkoğlu, 2006).

Paxina queletii (Bres.) Stangl: Adıyaman (Kaya, 2005; 2009, 2010); Ağrı (Demirel et al., 2002); Aydın (Allı et al., 2007); Balıkesir (Altuntaş et al., 2017); Bingöl (Uzun et al., 2017); Bolu (Yağız et al., 2006a); Denizli (Türkoğlu, 2008); Diyarbakır (Acar et al., 2015); Erzincan (Keleş ve Demirel, 2010; Allı, 2011); Erzurum (Keleş et al., 2016); Gaziantep (Uzun et al., 2015); Kahramanmaraş (Kaya, 2009); Karaman (Doğan ve Öztürk, 2006); Kastamonu (Yağız et al., 2006b); Kütahya (Allı et al., 2017); Malatya (Gücin, 1987); Mersin (Doğan et al., 2007); Trabzon (Akata et al., 2014); Uşak (Türkoğlu ve Yağız, 2012); Van (Demirel ve Koçak, 2016; Şelem et al., 2019); Yozgat (Kırış et al., 2012; Türkekul ve Işık, 2016).

Incertae Sedis

Coprotus disculus Kimbr., Luck-Allen & Cain: Hakkâri (Acar and Quijada, 2022).

Coprotus ochraceus (P. Crouan & H. Crouan) J. Moravec: Siirt (Akçay et al., 2022).

Psilopezia nummularia Berk.:Burdur (Çolak and Kaygusuz, 2017).

Morchellaceae Rchb.

Disciotis venosa (Pers.) Arnould: Balıkesir (Solak et al., 2002); İzmir (Yılmaz Ersel and Solak, 2004).

Morchella americana Clowez & Matherly: Van (Doğan et al., 2016).

Morchella anatolica Işıloğlu, Spooner, Allı & Solak: Muğla (Işıloğlu et al., 2010).

Morchella angusticeps Peck: Antalya (Solak et al., 2014b); Aydın (Allı et al., 2006, 2007); Bingöl (Uzun et al., 2017); Çanakkale (Solak et al., 2003); Denizli (Gezer et al., 2008; Türkoğlu, 2008); Erzincan (Allı, 2011); Erzurum (Sadullahoğlu et al., 2021); Hatay (Baba et al., 2013); Muğla (Güngör et al., 2016); Osmaniye (Solak et al., 2012); Uşak (Türkoğlu et al., 2008); Yozgat (Türkekul and Işık, 2016).

Morchella atrotomentosa (Moser) Bride: Aydın (Allı et al., 2006).

Morchella conifericola Taşkın, Büyükalaca & H.H. Doğan: Kahramanmaraş, Kastamonu, Kayseri (Taşkın et al., 2016).

Morchella costata Pers.: Antalya (Solak et al., 2014b); Ardahan (Uzun, 2010); Balıkesir (Solak et al., 2002); Çanakkale (Solak et al., 2003); İzmir (Solak et al., 1999); Kars (Demirel and Uzun, 1996); Kastamonu (Yağız et al., 2006b); Muğla (Güngör et al., 2016); Yozgat (Türkekul and Işık, 2016).

Morchella crassipes (Vent.) Pers.: Aydın (Allı et al., 2006, 2007); Bingöl (Uzun et al., 2009); Denizli (Türkoğlu, 2008); Erzincan (Allı, 2011); Hatay (Güngör et al., 2016); Isparta (Afyon, 1994); Muğla (Güngör et al., 2016); Osmaniye (Solak et al., 2012).

Morchella deliciosa Fr.: Adıyaman (Kaya et al., 2004; Kaya, 2005, 2009); Antalya (Solak et al., 2014b); Ardahan (Uzun, 2010); Artvin (Demirel et al., 2017); Balıkesir (Solak et al., 2002); Bingöl (Uzun et al., 2017); Bursa (Solak and Gücin, 1992); Çorum (Alkan et al., 2016); Denizli (Kaşık et al., 2013); Diyarbakır (Acar et al., 2015); Gaziantep (Kaya, 2009; Kaya et al., 2014; Uzun et al., 2015); Hatay (Baba et al., 2014); İzmir (Solak et al., 1999); Kahramanmaraş (Kaya, 2009; Kaya et al., 2009); Karaman (Öztürk et al., 2001); Kastamonu, Sinop (Afyon and Konuk, 2001a); Kayseri (Atila and Kaya, 2013); Konya (Alkan et al., 2010; Kaşık et al., 2010); Kütahya (Allı et al., 2017); Mersin (Doğan et al., 2007, 2010, 2012); Muğla (Güngör et al., 2016); Osmaniye (Solak et al., 2012); Sinop (Afyon et al., 2004); Şanlıurfa (Kaya, 2015); Uşak (Türkoğlu and Yağız, 2012); Van (Demirel et al., 2015).

Morchella dunalii Boud.: Antalya, Aydın, Çanakkale, Denizli, Kars, Mersin, Muğla, Samsun, Uşak, Yozgat (Doğan et al., 2016).

Morchella elata Fr.: Adana (Doğan and Kurt, 2016); Adıyaman (Kaya et al., 2004; Kaya, 2005, 2009); Antalya (Solak et al., 2014b); Aydın (Allı et al., 2006, 2007); Batman (Demir et al., 2007); Bayburt (Keleş et al., 2016); Bolu (Yağız et al., 2006a); Çorum (Kaşık et al., 2011); Denizli (Köse et al., 2006; Gezer et al., 2007a,b, 2008, 2011; Türkoğlu et al., 2007; Türkoğlu, 2008; Kaşık et al., 2013); Diyarbakır (Acar et al., 2015; Demirel et al., 2016); Erzincan (Allı, 2011); Gaziantep (Kaya, 2009; Uzun et al., 2015); Hatay (Baba et al., 2013); Isparta (Güngör et al., 2015); İzmir (Solak et al., 1999); Kahramanmaraş (Kaya, 2006, 2009; Kaya et al., 2009); Kastamonu (Akata et al., 2010); Kastamonu, Sinop, Bolu (Afyon and Konuk, 2001a); Kayseri (Kaşık

et al., 2003; Türkoğlu and Gezer, 2006); Konya (Aktaş et al., 2003; Alkan et al., 2010; Çevik et al., 2021); Kütahya (Allı et al., 2017); Malatya (Işıloğlu, 1997); Mersin (Doğan et al., 2007; Güngör et al., 2016); Muğla (Işıloğlu, 2001; Yılmaz Ersel and Solak, 2005); Osmaniye (Günay and Demirel, 2006; Solak et al., 2012); Samsun (Pekşen and Karaca, 2003); Sinop (Afyon et al., 2004); Tokat (Yıldız et al., 2019); Uşak (Türkoğlu et al., 2008); Van (Demirel et al., 2015; Şelem et al., 2019); Yozgat (Kırış et al., 2012; Türkekul and Işık, 2016).

Morchella esculenta (L.) Pers.: Adana (Işıloğlu and Öder, 1995b; Doğan and Kurt, 2016); Adıyaman (Kaya et al., 2004; Kaya, 2005, 2009a,b); Afyonkarahisar (Oskay and Kalyoncu, 2006); Ağrı (Demirel et al., 2002); Aksaray (Doğan and Türkoğlu, 2006; Türkoğlu et al., 2007); Ankara (Akata et al., 2009); Antalya (Gezer, 2000; Öztürk et al., 2003; Solak et al., 2014b); Ardahan (Uzun, 2010); Artvin (Demirel, 1994, 1997; Demirel et al., 2010); Aydın (Allı et al., 2006, 2007); Balıkesir (Solak et al., 2002; Altuntaş et al., 2017); Batman (Demir et al., 2007); Bayburt (Keleş et al., 2016); Bingöl (Uzun et al., 2017); Bolu (Yağız et al., 2006a); Burdur (Solak et al., 2013); Bursa (Solak and Gücin, 1992); Çanakkale (Solak et al., 2003); Çankırı (Öztürk et al., 2010); Çorum (Alkan et al., 2016); Denizli (Köse et al., 2006; Gezer et al., 2007a,b, 2008, 2011; Türkoğlu et al., 2007; Türkoğlu, 2008; Kaşık et al., 2013); Diyarbakır (Acar et al., 2015); Edirne (Watling and Gregory, 1977; Stojchev et al., 1998); Erzincan (Keleş and Demirel, 2010; Allı, 2011); Erzurum (Demirel et al., 2003; Sadullahoğlu et al., 2021); Gaziantep (Kaya, 2009; Kaya et al., 2012; Uzun et al., 2015); Iğdır (Uzun, 2010); Isparta (Afyon, 1994; Güngör et al., 2015); İzmir (Solak et al., 1999); Kahramanmaraş (Kaya, 2006, 2009; Kaya et al., 2009); Karabük (Yağız et al., 2005); Karabük, Bolu (Afyon and Konuk, 2001a); Karaman (Kaşık et al., 2000; Öztürk et al., 2001; Doğan and Öztürk, 2006); Kars (Demirel and Uzun, 1996); Kastamonu (Yağız et al., 2006b); Kastamonu, Bolu (Afyon and Konuk, 2001a); Kayseri (Kaşık et al., 2002, 2003; Türkoğlu and Gezer, 2006); Konya (Aktaş et al., 2003; Alkan et al., 2010; Kaşık et al., 2010; Çevik et al., 2021); Kütahya (Allı et al., 2017); Malatya (Gücin, 1987; Işıloğlu and Öder, 1995a); Manisa (Gücin and Öner, 1982); Mersin (Işıloğlu and Watling, 1992; Doğan et al., 2007, 2010, 2012); Muğla (Işıloğlu, 2001; Solak et al., 2005; Güngör et al., 2016); Niğde (Kaşık et al., 2001a,b; Berber et al., 2022); Osmaniye (Günay and Demirel, 2006; Solak et al., 2012); Sakarya (Doğan et al., 2021); Samsun (Pekşen and Karaca, 2003); Sinop (Afyon et al., 2004); Şanlıurfa (Kaya, 2015); Tokat (Türkekul, 2003, 2008; Türkekul and Zülfükaroğlu, 2010; Yıldız et al., 2019); Trabzon (Baydar and Sesli, 1994; Sesli, 2007; Akata et al., 2014; Akata and Uzun, 2017); Tunceli (Demirel and Nacar, 2000); Uşak (Türkoğlu et al., 2008); Van (Demirel et al., 2015; Şelem et al., 2019); Yalova (Allı et al., 2017); Yozgat (Kırış et al., 2012; Türkekul and Işık, 2016).

Morchella esculentoides M. Kuo, Dewsbury, Moncalvo & S.L. Stephenson: Van (Şelem et al., 2019).

Morchella eximia Boud.: Antalya (Solak et al., 2014b); Muğla (Güngör et al., 2016); Osmaniye (Solak et al., 2012).

Morchella fekeensis H.H. Doğan, Taşkın & Büyükalaca: Adana, Kahramanmaraş, Sivas, Yozgat (Taşkın et al., 2016).

Morchella fluvialis Clowez, P. Alvarado, M. Becerra, Bilbao & P.-A. Moreau: Adana (Doğan et al., 2016).

Morchella galilaea Masaphy & Clowez: Adana (Taşkın et al., 2015).

Morchella hortensis Boud.: Antalya (Solak et al., 2014b); Muğla (Yılmaz Ersel and Solak, 2005; Güngör et al., 2016); Osmaniye (Solak et al., 2012).

Morchella importuna M. Kuo, O'Donnell & T.J. Volk: Adana, Antalya, Mersin, Kastamonu, Samsun, Yozgat (Doğan et al., 2016).

Morchella inamoena Boud.: Muğla (Güngör et al., 2016).

Morchella intermedia Boud.: Antalya (Solak et al., 2014b); Bolu (Yağız et al., 2006a); Çanakkale (Solak et al., 2003); Manisa (Gücin and Öner, 1982).

Morchella magnispora Büyükalaca, H.H. Doğan & Taşkın: Adana, Aydın, Kahramanmaraş, Mersin, Muğla, Uşak (Taşkın et al., 2016).

Morchella mediterraneensis Taşkın, Büyükalaca & H.H. Doğan: Adana, Denizli, Kahramanmaraş, Kastamonu, Konya, Kayseri, Mersin, (Taşkın et al., 2016).

Morchella populiphila M. Kuo, M.C. Carter & J.D. Moore: Erzurum (Sadullahoğlu et al., 2021); Van (Acar and Uzun, 2017).

Morchella prava Dewsbury, Moncalvo, J.D. Moore & M. Kuo: Van (Keleş et al., 2018; Şelem et al., 2019).

Morchella pseudoviridis Jacquet.: Muğla (Yılmaz Ersel and Solak, 2005; Güngör et al., 2016); Osmaniye (Solak et al., 2012).

Morchella rielana Boud.: Antalya (Solak et al., 2014b); Denizli (Kaşık et al., 2013).

Morchella semilibera DC.: Adıyaman (Kaya, 2005, 2009a,b); Aksaray (Türkoğlu et al., 2007); Ankara (Güler and Mutlu, 2003); Bayburt (Uzun et al., 2004; Keleş et al., 2016); Bingöl (Uzun et al., 2009); Çorum (Alkan et al., 2016); Diyarbakır (Acar et al., 2015; Demirel et al., 2016); Erzincan (Keleş and Demirel, 2010; Allı, 2011); Gaziantep (Uzun et al., 2015); Kahramanmaraş (Kaya, 2009; Kaya et al., 2009); Karaman (Doğan ve Öztürk, 2006); Kayseri (Kaşık et al., 2002, 2003; Türkoğlu and Gezer, 2006); Konya (Kaşık et al., 2010); Kütahya (Allı et al., 2017); Nevşehir (Doğan and Türkoğlu, 2006); Samsun (Pekşen and Karaca, 2003); Tokat (Türkekul, 2003); Trabzon (Sesli, 2007); Uşak (Türkoğlu and Yağız, 2012); Van (Demirel et al., 2015; Şelem et al., 2019); Yozgat (Kırış et al., 2012).

Morchella steppicola Zerova: Kırşehir (Alkan et al., 2019).

Morchella tridentina Bres.: Muğla (Yılmaz Ersel and Solak, 2005; Güngör et al., 2016); Adana, Antalya, Aydın, Çanakkale, Kahramanmaraş, Kastamonu, Konya, Mersin, Muğla, Samsun, Uşak (Doğan et al., 2016); Denizli (Kaygusuz et al., 2019).

Morchella vaporaria Brond.: Konya (Çelik et al., 2020).

Verpa bohemica (Krombh.) J. Schröt.: Adıyaman (Kaya, 2010); Artvin (Demirel et al., 2017); Bingöl (Uzun et al., 2017); Diyarbakır (Acar et al., 2015); Erzincan (Keleş and Demirel, 2010; Allı, 2011); Erzurum (Sadullahoğlu et al., 2021); Konya (Çevik et al., 2021); Mersin (Doğan et al., 2007); Tokat (Yıldız et al., 2019); Van (Demirel et al., 2015).

Verpa conica (O.F. Müll.) Sw.: Adana (Işıloğlu ve Watling, 1992; Işıloğlu ve Öder, 1995b); Adıyaman (Kaya, 2005, 2009a,b); Aydın (Allı et al., 2007); Balıkesir (Solak et al., 2002); Bayburt (Uzun et al., 2004); Bingöl (Uzun et al., 2017); Bolu (Yağız et al., 2006a); Diyarbakır (Acar et al., 2015; Demirel et al., 2016); Erzincan (Allı, 2011); Giresun (Sesli, 2007); Kahramanmaraş (Kaya, 2006, 2009); Muğla (Solak ve Yılmaz Ersel, 2005); Niğde (Berber et al., 2022); Samsun (Sesli, 1999; Pekşen ve Karaca, 2003); Van (Demirel et al., 2015).

Otideaceae Eckblad

Otidea alutacea (Pers.) Massee: Artvin (Demirel et al., 2004); Balıkesir (Altuntaş et al., 2017); Bursa, Sakarya (Doğan et al., 2021); Gümüşhane (Uzun et al., 2006); Konya (Çevik et al., 2021); Rize (Keleş et al., 2014); Trabzon (Akata et al., 2014; Akata and Uzun, 2017); Uşak (Türkoğlu and Yağız, 2012).

Otidea bufonia (Pers.) Boud.: Denizli (Allı et al., 2008).

Otidea cantharella (Fr.) Quél.: Artvin (Demirel et al., 2017); Balıkesir (Şen et al., 2014); Erzincan (Keleş and Demirel, 2010); Karabük (Yağız et al., 2005); Rize, Trabzon (Sesli, 2007); Trabzon (Sesli, 1998); Uşak (Türkoğlu et al., 2008); Van (Demirel et al., 2015).

Otidea cochleata (L.) Fuckel: Balıkesir (Altuntaş et al., 2017); Manisa (Gücin and Öner, 1982a); Trabzon (Akata et al., 2014).

Otidea leporina (Batsch) Fuckel: Manisa (Gücin and Öner, 1982a,b).

Otidea nannfeldtii Harmaja: Balıkesir (Altuntaş et al., 2021).

Otidea onotica (Pers.) Fuckel: Ankara (Akata et al., 2009); Gümüşhane (Akata et al., 2016); Kocaeli (Akata et al., 2018) Trabzon (Akata et al., 2014; Akata and Uzun, 2017).

Pezizaceae Dumort.

Adelphella babingtonii (Berk. & Broome) Pfister, Matočec & I. Kušan: Bolu (Sesli et al., 2020).

Galactinia granulosa (Gillet) Le Gal: Adıyaman (Kaya, 2009a,b); Antalya (Öztürk et al., 2003); Aydın (Allı et al., 2007); Konya (Alkan et al., 2010; Kaşık et al., 2010); Mersin (Doğan et al., 2002; Doğan et al., 2010).

Geoscypha ampelina (Gillet) Van Vooren & Dougoud: Denizli (Türkoğlu, 2008).

Geoscypha violacea (Pers.) Lambotte: Adıyaman (Kaya, 2009a,b); Antalya (Solak et al., 2014b); Eskişehir (Köstekci et al., 2005); Şanlıurfa (Kaya, 2015).

Hydnobolites cerebriformis Tul. & C. Tul.: Rize, Trabzon (Uzun and Kaya, 2018).

Iodophanus carneus (Pers.) Korf: Bingöl (Uzun et al., 2017).

Legaliana badia (Pers.) Van Vooren: Ankara (Güler and Mutlu, 2003); Bingöl (Uzun et al., 2009); Bursa, Yalova (Doğan et al., 2021); Gümüşhane (Akata et al., 2016); Manisa (Gücin ve Öner, 1982); Trabzon (Akata ve Uzun, 2017); Yozgat (Türkekul ve Işık, 2016).

Marcelleina atroviolacea Brumm.: Gaziantep (Uzun et al., 2018).

Marcelleina persoonii (P. Crouan & H. Crouan) Brumm.: Balıkesir (Solak et al., 2002).

Marcelleina rickii (Rehm) Graddon: Gaziantep (Uzun et al., 2018).

Pachyella celtica (Boud.) Häffner: Antalya (Kaşık et al., 2002); Denizli (Türkoğlu, 2008); Kocaeli, Sakarya (Doğan et al., 2021).

Pachyella clypeata (Schwein.) Le Gal: Trabzon (Uzun and Kaya, 2019).

Pachyphlodes citrina (Berk. & Broome) Doweld: Rize, Trabzon (Uzun and Kaya, 2018).

Pachyphlodes conglomerata (Berk. & Broome) Doweld: Giresun, Trabzon (Uzun and Kaya, 2018).

Paragalactinia michelii (Boud.) Van Vooren: Mersin (Doğan et al., 2002).

Paragalactinia succosa (Berk.) Van Vooren: Artvin (Demirel et al., 2017); Gümüşhane (Akata et al., 2016); Kocaeli (Doğan et al., 2021); Konya (Çevik et al., 2021); Trabzon (Sesli, 1998b; Akata et al., 2014); Yozgat (Türkekul and Işık, 2016).

Paragalactinia succosella (Le Gal & Romagn.) Van Vooren: Aydın (Çolak and Kaygusuz, 2017b).

Peziza ammophila Durieu & Lév.: Mersin (Akata and Yaprak, 2013).

Peziza arenaria Osbeck: Adıyaman (Kaya, 2010).

Peziza arvernensis Roze & Boud.: Denizli (Türkoğlu, 2008; Gezer et al., 2011); Kahramanmaraş (Kaya, 2009); Karaman (Kaşık et al., 2002); Kütahya (Allı et al., 2017); Sakarya (Doğan et al., 2021); Trabzon (Akata et al., 2014); Yozgat (Kırış et al., 2012; Türkekul ve Işık, 2016).

Peziza cerea Sowerby: Trabzon (Baydar ve Sesli, 1994; Akata ve Uzun, 2017).

Peziza depressa Pers.: Artvin (Demirel et al., 2017); Bitlis (Kaya, 2001); Kastamonu (Akata et al., 2010); Kütahya (Allı et al., 2017); Muğla (Güngör ve Allı, 2016); Osmaniye (Solak et al., 2012); Sakarya (Doğan et al., 2021); Tokat (Sesli and Türkekul, 2000); Trabzon (Sesli, 2007; Akata et al., 2014).

Peziza domiciliana Cooke: Aydın (Allı et al., 2007); Karaman (Kaşık et al., 2002); Kocaeli (Akata et al., 2018); Kütahya (Allı et al., 2017); Trabzon (Akata et al., 2014); Van (Demirel et al., 2015).

Peziza echinospora P. Karst.: Antalya (Güngör et al., 2014).

Peziza fimeti (Fuckel) E.C. Hansen: Kütahya (Allı et al., 2017); Niğde (Berber et al., 2022); Van (Uzun et al., 2014; Demirel et al., 2015); Yozgat (Türkekul and Işık, 2016).

Peziza granularis Donadini: Hakkâri (Acar and Uzun, 2016).

Peziza lobulata (Velen.) Svrček: Muğla (Güngör et al., 2015).

Peziza micropus Pers.: Denizli (Türkoğlu, 2008); Muğla (Güngör and Allı, 2016); Sakarya (Doğan et al., 2021).

Peziza moravecii (Svrček) Donadini: Aksaray (Doğan and Türkoğlu, 2006).

Peziza pseudoviolacea Donadini: Adıyaman (Kaya, 2015); Gaziantep (Uzun et al., 2015).

Peziza punctispora (Pfister) Donadini: Muğla (Çolak et al., 2015).

Peziza repanda Wahlenb.: Adana (Işıloğlu and Watling, 1992); Ağrı (Demirel et al., 2002); Bingöl (Uzun et al., 2009).

Peziza saniosa Schrad.: Trabzon (Akata and Kaya, 2012a; Akata and Uzun, 2017).

Peziza varia (Hedw.) Alb. & Schwein.: Diyarbakır (Acar et al., 2015); Karaman (Öztürk et al., 2001).

Peziza vesiculosa Bull.: Antalya (Gezer, 2000); Antalya, Muğla (Işıloğlu and Öder, 1995b); Aydın (Allı et al., 2007); Balıkesir (Solak et al., 2002); Denizli (Gezer et al., 2007); Denizli (Gezer et al., 2007); Denizli (Gezer et al., 2008); Denizli (Türkoğlu et al., 2007); Isparta (Afyon, 1994); Karabük (Yağız et al., 2005); Kastamonu (Özkazanç and Yılmaz Oğuz, 2017); Malatya (Işıloğlu, 1997); Van (Demirel and Koçak, 2016).

Phylloscypha boltonii (Quél.) Van Vooren & Hairaud: Mersin (Kaplan et al., 2020).

Phylloscypha phyllogena (Cooke) Van Vooren: Diyarbakır (Acar et al., 2015); Mersin (Işıloğlu and Watling, 1992); Yozgat (Türkekul and Işık, 2016).

Plicaria carbonaria Fuckel: Trabzon (Kaya and Uzun, 2018).

Sarcopeziza sicula (Inzenga) Agnello, Loizides & P. Alvarado: Kütahya (Altuntaş et al., 2021).

Sarcosphaera coronaria (Jacq.) J. Schröt.: Adana (Işıloğlu and Öder, 1995b); Adıyaman (Kaya et al., 2004; Kaya, 2010); Ankara (Akata et al., 2009; Öztürk et al., 2017); Antalya (Gezer, 2000; Öztürk et al., 2003; Solak et al., 2014b); Aydın (Allı et al., 2007); Balıkesir (Altuntaş et al., 2017); Bolu (Yağız et al., 2006a); Denizli (Köse et al., 2006; Gezer et al., 2007a,b, 2011; Türkoğlu et al., 2007; Türkoğlu, 2008); Gaziantep (Kaya et al., 2014; Uzun et al., 2015); Hatay (Güngör et al., 2016); İstanbul (Akata, 2017); Kastamonu (Yağız et al., 2006b; Akata et al., 2010); Konya (Öztürk et al., 2000; Alkan et al., 2010; Çevik et al., 2021); Kütahya (Allı et al., 2017); Mersin (Doğan et al., 2010, 2012; Güngör et al., 2015); Muğla (Güngör et al., 2016); Niğde (Öztürk et al., 1997; Berber et al., 2022); Osmaniye (Solak et al., 2012); Sakarya (Doğan et al., 2021); Şanlıurfa (Kaya, 2015); Tokat (Yıldız et al., 2019); Uşak (Türkoğlu et al., 2008); Yozgat (Türkekul and Işık, 2016).

Terfezia arenaria (Moris) Trappe: Aydın (Türkoğlu et al., 2015); Malatya (Işıloğlu and Öder, 1995a).

Terfezia boudieri Chatin: Batman (Demir et al., 2007); Gaziantep (Kaya et al., 2012); Karaman (Doğan and Öztürk, 2006); Niğde (Kaşık et al., 2001); Şanlıurfa (Kaya, 2015); Uşak (Türkoğlu and Yağız, 2012).

Terfezia cistophila Ant. Rodr., Bordallo, Kaounas & A. Morte: Trabzon (Uzun and Kaya, 2019).

Terfezia claveryi Chatin: Adana (Doğan and Kurt, 2016); Denizli, Şanlıurfa, Konya, Aksaray, Diyarbakır, Karaman, Yozgat (Türkoğlu et al., 2015); Konya (Çevik et al., 2021); Niğde (Berber et al., 2022).

Terfezia leptoderma (Tul. & C. Tul.) Tul. & C. Tul.: Uşak (Castellano and Türkoğlu, 2012); Denizli, Uşak (Türkoğlu and Castellano, 2014).

Terfezia olbiensis (Tul. & C. Tul.) Sacc.: Gaziantep (Uzun et al., 2015); Konya, Nevşehir, Uşak (Türkoğlu and Castellano, 2014).

Tirmania pinoyi (Maire) Malençon: İzmir (Yılmaz Ersel and Solak, 2004).

Pseudombrophilaceae Ekanayaka, K.D. Hyde, Q. Zhao & E.B.G. Jones

Pseudombrophila merdaria (Fr.) Brumm.: Gaziantep (Kaya and Uzun, 2015).

Pseudombrophila ripensis (E.C. Hansen) Brumm.: Adıyaman (Kaya, 2015).

Pyronemataceae Corda

Acervus epispartius (Berk. & Broome) Pfister: Trabzon (Akata and Kaya, 2013).

Aleuria aurantia (Pers.) Fuckel: Bartın (Afyon et al., 2000); Eskişehir (Köstekci et al., 2005); Gümüşhane (Akata et al., 2016); İstanbul (Akata, 2017); Kastamonu (Akata et al., 2010; Özkazanç and Yılmaz Oğuz, 2017); Kocaeli (Akata et al., 2018); Kocaeli, Sakarya (Doğan et

al., 2021); Rize (Sesli, 2007); Trabzon (Akata et al., 2014; Akata and Uzun, 2017).

Aleuria exigua Rifai: Gaziantep (Kaya et al., 2016).

Aleuria splendens (Quél.) Gillet: Kocaeli, Sakarya (Doğan et al., 2021).

Anthracobia macrocystis (Cooke) Boud.: Trabzon (Kaya and Uzun, 2018).

Anthracobia melaloma (Alb. & Schwein.) Arnould: Antalya (Allı et al., 2011; Solak et al., 2014b); Niğde (Berber et al., 2022); Trabzon (Akata et al., 2014).

Cheilymenia catenipila J. Moravec: Gaziantep (Kaya et al., 2016).

Cheilymenia fimicola (Bagl.) Dennis: Trabzon (Akata et al., 2012).

Cheilymenia granulata (Bull.) J. Moravec: Trabzon (Akata and Uzun, 2017); İstanbul (Akata, 2017).

Cheilymenia megaspora (Gamundí) J. Moravec: Hakkâri (Kesici and Uzun, 2021).

Cheilymenia pulcherrima (P. Crouan & H. Crouan) Boud.: Gaziantep (Uzun et al., 2018).

Cheilymenia theleboloides (Alb. & Schwein.) Boud.: Adıyaman (Kaya, 2015).

Cheilymenia vitellina (Pers.) Dennis: Gaziantep (Kaya et al., 2016).

Ciliaria confusa (Cooke) Boud.: Elazığ (Sesli et al., 2020).

Genea hispidula Berk. ex Tul. & C. Tul.: Trabzon (Uzun and Kaya, 2019).

Genea klotzschii Berk. & Broome: Samsun (Türkoğlu and Castellano, 2014).

Genea lobulata (Mor.-Arr., J. Gómez & Calonge) P. Alvarado & Mor.-Arr.: Niğde (Berber et al., 2019; Berber et al., 2022).

Genea sphaerica Tul. & C. Tul.: İzmir (Türkoğlu et al., 2015).

Genea verrucosa Vittad.: Muğla (Türkoğlu and Castellano, 2014).

Geopora arenicola (Lév.) Kers: Adana (Doğan and Kurt, 2016); Adıyaman (Kaya, 2019a,b); Ankara (Akata et al., 2019); Aydın (Allı et al., 2007); Bingöl (Uzun et al., 2009); Diyarbakır (Acar et al., 2015); Gaziantep (Kaya et al., 2012, 2014; Uzun et al., 2015); Iğdır (Uzun, 2010); Kahramanmaraş (Kaya, 2009); Karaman (Öztürk et al., 2001; Doğan and Öztürk, 2006); Malatya (Işıloğlu, 1997); Şanlıurfa (Kaya, 2015); Van (Demirel et al., 2015).

Geopora arenosa (Fuckel) S. Ahmad: Adıyaman (Kaya et al., 2004); Aksaray (Türkoğlu et al., 2007); Antalya (Solak et al., 2014b); Aydın (Allı et al., 2007); Balıkesir (Şen et al., 2014); Denizli (Türkoğlu, 2008); Kahramanmaraş (Kaya, 2006); Kayseri (Kaşık et al., 2002, 2003).

Geopora clausa (Tul. & C. Tul.) Burds.: Trabzon (Uzun and Kaya, 2019).

Geopora cooperi Harkn.: Bolu, Denizli, Burdur, Muğla (Türkoğlu et al., 2015); İzmir (Solak et al., 2002).

Geopora sepulta (Fr.) Korf & Burds.: Van (Demirel et al., 2015).

Geopora sumneriana (Cooke) M. Torre: Adana (Doğan and Kurt, 2016); Adıyaman (Kaya, 2009); Ankara (Akata et al., 2019); Balıkesir (Solak et al., 2002); Bingöl (Uzun et al., 2009); Çankırı (Öztürk et al., 2010); Denizli (Köse et al., 2006; Gezer et al., 2011a,b); Gaziantep (Kaya, 2009; Uzun et al., 2015); Kahramanmaraş (Kaya, 2006, 2009; Kaya et al., 2009); Kayseri (Kaşık et al., 2003); Kocaeli (Doğan et al., 2021); Konya (Aktaş et al., 2003; Alkan et al., 2010; Kaşık et al., 2010; Çevik et al., 2021); Kütahya (Allı et al., 2017); Mersin (Doğan et al., 2007, 2010, 2012); Nevşehir (Doğan and Türkoğlu, 2006); Osmaniye (Solak et al., 2012); Tokat (Yıldız et al., 2019);Uşak (Türkoğlu et al., 2008); Van (Demirel et al., 2015); Yozgat (Türkekul and Işık, 2016).

Geopyxis carbonaria (Alb. & Schwein.) Sacc.: Bingöl (Uzun et al., 2010); Muğla (Tırpan et al., 2018).

Geopyxis majalis (Fr.) Sacc.: Gaziantep (Kaya et al., 2016).

Geopyxis vulcanalis (Peck) Sacc.: Konya (Çevik et al., 2021); Gaziantep (Kaya et al., 2016).

Humaria aurantia (Clem.) Häffner Benkert & Krisai & Broome) Lambotte: Gaziantep (Kaya et al., 2016).

Humaria hemisphaerica (F.H. Wigg.) Fuckel: Ankara (Akata et al., 2009); Ardahan (Uzun, 2010); Artvin (Demirel et al., 2017); Bolu (Servi et al., 2010); Gümüşhane (Sesli, 2007; Akata et al., 2016); İstanbul (Akata, 2017); Karaman (Kaşık et al., 2000); Kastamonu (Akata et al., 2010; Özkazanç and Yılmaz Oğuz, 2017); Konya (Çevik et al., 2021); Rize (Keleş et al., 2014); Samsun (Pekşen and Karaca, 2003); Trabzon (Sesli, 1998; Akata et al., 2014; Akata and Uzun, 2017); Kocaeli, Sakarya (Doğan et al., 2021).

Hydnocystis piligera Tul.: Aydın (Kaygusuz et al., 2018).

Hypotarzetta insignis (Berthet & Riousset) Donadini: Gaziantep (Kaya and Uzun, 2015).

Inermisia gyalectoides (Svrček & Kubička) Dennis & Itzerott: Gaziantep (Uzun et al., 2018); Konya (Çevik et al., 2021).

Kotlabaea deformis (P. Karst.) Svrček: Gaziantep (Kaya et al., 2016).

Lamprospora campylopodis W. D. Buckley: Trabzon (Uzun and Kaya, 2019).

Lamprospora carbonicola Boud.: Gaziantep (Uzun et al., 2018).

Lamprospora dictydiola Boud.: Gaziantep (Uzun et al., 2018).

Lamprospora miniata De Not.: Gaziantep (Uzun et al., 2018); Niğde (Berber et al., 2022).

Melastiza chateri (W.G. Sm.) Boud.: Trabzon (Akata et al., 2011).

Melastiza cornubiensis (Berk. & Broome) J. Moravec: Manisa (Gücin and Öner, 1982a).

Neottiella rutilans (Fr.) Dennis: Trabzon (Akata and Kaya, 2013).

Octospora areolata (Seaver) Caillet & Moyne: Gaziantep (Uzun et al., 2018).

Octospora axillaris (Nees) M.M. Moser: Gaziantep (Uzun et al., 2018).

Octospora coccinea (P. Crouan & H. Crouan) Brumm.: Gaziantep (Uzun et al., 2018).

Octospora excipulata (Clem.) Benkert: Gaziantep (Uzun et al., 2018).

Octospora gemmicola Benkert: Gaziantep (Uzun et al., 2018).

Octospora grimmiae Dennis & Itzerott: Gümüşhane (Uzun and Kaya, 2019).

Octospora itzerottii Benkert.: Konya (Çevik et al., 2021); Gaziantep (Uzun et al., 2017).

Octospora leucoloma Hedw.: Gaziantep, Konya (Uzun et al., 2018); Konya (Çolak and Kaygusuz, 2017); Niğde (Berber et al., 2022).

Octospora lilacina (Seaver) Svrček & Kubička: Trabzon (Uzun and Kaya, 2019).

Octospora musci-muralis Graddon: Gaziantep (Uzun et al., 2018); Niğde (Berber et al., 2022).

Octospora neerlandica Benkert & Brouwer: Niğde (Berber et al., 2022).

Octospora orthotrichi (Cooke & Ellis) K.B. Khare & V.P. Tewari: Gaziantep (Uzun et al., 2018).

Octospora polytrichi (Schumach.) Caillet & Moyne: Gaziantep (Uzun et al., 2018); Niğde (Berber et al., 2022).

Octospora rustica (Velen.) J. Moravec): Gaziantep (Uzun et al., 2018).

Octospora tuberculata (Seaver) Caillet & Moyne: Gaziantep, Giresun (Uzun and Kaya, 2022).

Octospora tuberculatella (Seaver) Caillet & Moyne: Trabzon (Uzun and Kaya, 2019).

Parascutellinia violacea (Velen.) Svrček: Konya (Çevik et al., 2021); Van (Keleş, 2019).

Picoa juniperi Vittad.: Afyon, Antalya, Denizli, Elazığ, Konya, Nevşehir (Türkoğlu and Castellano, 2014); Kayseri (Türkoğlu et al., 2015); Konya (Çevik et al., 2021); Niğde (Berber et al., 2022); Uşak (Türkoğlu and Yağız, 2012).

Picoa lefebvrei (Pat.) Maire: Elazığ, Şanlıurfa (Gücin et al., 2010); Aksaray, Denizli, Konya (Türkoğlu et al., 2015); Konya (Çevik et al., 2021); Niğde (Berber et al., 2022); Şanlıurfa (Kaya, 2015).

Pulvinula alba (Velen.) Svrček: Trabzon (Uzun and Kaya, 2020).

Pulvinula archeri (Berk.) Rifai: Gaziantep (Karacan et al., 2015).

Pulvinula carbonaria (Fuckel) Boud.: Gaziantep (Karacan et al., 2015).

Pulvinula convexella (P. Karst.) Pfister: Ankara (Akata et al., 2019); Gümüşhane, Bayburt (Uzun and Kaya, 2019).

Pulvinula johannis Lantieri: Gaziantep (Kaya et al., 2016).

Pulvinula laeterubra (Rehm) Pfister: Gaziantep (Karacan et al., 2015).

Pyronema domesticum (Sowerby) Sacc.: Gaziantep (Kaya et al., 2016).

Pyronema omphalodes (Bull.: Fr.) Fuckel: Konya (Çevik et al., 2021); Gaziantep (Kaya and Uzun, 2015).

Ramsbottomia crechqueraultii (P. Crouan & H. Crouan) Benkert & T. Schumach.: Trabzon (Uzun and Kaya, 2019).

Rhodoscypha ovilla (Peck) Dissing & Sivertsen: Gümüşhane (Akata et al., 2016); Trabzon (Akata and Kaya, 2013).

Scutellinia armatospora Denison: Trabzon (Akata et al., 2011).

Scutellinia barlae (Boud.) Maire: Muğla (Allı et al., 2011).

Scutellinia crinita (Bull.) Lambotte: Konya (Çevik et al., 2021); Van (Keleş, 2019).

Scutellinia kerguelensis (Berk.) Kuntze: Trabzon (Uzun and Kaya, 2021).

Scutellinia legaliae Lohmeyer & Häffner: Burdur (Çolak and Kaygusuz, 2018).

Scutellinia scutellata (L.) Lambotte: Adıyaman (Kaya, 2010); Bingöl (Uzun et al., 2017); Gümüşhane (Akata et al., 2016); Karaman (Doğan and Öztürk, 2006); Kastamonu (Akata et al., 2010); Konya (Kaşık et al., 2010; Çevik et al., 2021); Kütahya (Allı et al., 2017); Samsun (Pekşen and Karaca, 2003); Sivas (Kırış et al., 2012); Trabzon (Akata et al., 2014; Akata and Uzun, 2017); Van (Demirel and Koçak, 2016); Yalova (Allı et al., 2017).

Scutellinia trechispora (Berk. & Broome) Lambotte: Gaziantep (Kaya et al., 2016).

Scutellinia umbrorum (Fr.) Lambotte: Gaziantep (Kaya, 2009); Muğla (Güngör and Allı, 2016; Tırpan et al., 2018); Yalova (Allı et al., 2017).

Sepultariella patavina (Cooke & Sacc.) Van Vooren, U. Lindem. & Healy: Gaziantep (Kaya and Uzun, 2015).

Sepultariella semi-immersa (P. Karst.) Van Vooren, U. Lindem. & Healy: Gaziantep (Uzun et al., 2018).

Smardaea planchonis (Dunal ex Boud.) Korf & W.Y. Zhuang: Gaziantep (Kaya et al., 2016).

Sowerbyella rhenana (Fuckel) J. Moravec: Şanlıurfa (Kaya, 2015).

Spooneromyces helveticus Breitenbach & Krazlin: Rize (Keleş et al., 2014).

Stephensia bombycina (Vittad.) Tul. & C. Tul.: Samsun (Türkoğlu and Castellano, 2014).

Tricharina gilva (Boud. ex Cooke) Eckblad: Gaziantep (Kaya and Uzun, 2015).

Tricharina ochroleuca (Bres.) Eckblad: Gaziantep (Kaya et al., 2016).

Tricharina praecox (P. Karst.) Dennis: Gaziantep (Kaya et al., 2016).

Trichophaea gregaria (Rehm) Boud.: Trabzon (Uzun and Kaya, 2019).

Trichophaea hemisphaerioides (Mouton) Graddon: Adana (Doğan and Aktaş, 2010).

Trichophaea pseudogregaria (Rick) Boud.): Van (Keleş and Şelem, 2017).

Trichophaea woolhopeia (Cooke & W. Phillips) Boud.: Konya (Çevik et al., 2021); Muğla (Güngör et al., 2015, 2016).

Trichophaeopsis bicuspis (Boud.) Korf & Erb): Gaziantep (Kaya et al., 2016).

Rhizinaceae Bonord.

Rhizina undulata Fr.: Artvin (Demirel, 1994); Bolu (Yağız et al., 2006); Mersin (Doğan et al., 2010); Sinop (Afyon et al., 2004); Uşak (Türkoğlu and Yağız, 2012).

Sarcoscyphaceae Le Gal ex Eckblad

Kompsoscypha chudei (Pat. ex Le Gal) Pfister: Gaziantep (Kaya and Uzun, 2018).

Microstoma protractum (Fr.) Kanouse: Balıkesir (Solak et al., 2002).

Pithya cupressina (Batsch) Fuckel: Gaziantep (Kaya and Uzun, 2018); Niğde (Berber et al., 2022).

Pithya vulgaris Fuckel: Karaman (Doğan and Işıloğlu, 2002); Konya (Alkan et al., 2010); Yalova (Allı et al., 2017).

Pseudopithyella minuscula (Boud. & Torrend) Seaver: Gaziantep (Kaya and Uzun, 2018).

Sarcoscypha coccinea (Gray) Boud.: Antalya (Gezer, 2000); Bursa, Yalova (Doğan et al., 2021); Çanakkale (Solak et al., 2003); Denizli (Gezer et al., 2007a,b; Türkoğlu, 2008); Gümüşhane (Akata et al., 2016); İstanbul (Selik, 1964; Akata, 2017); Manisa (Gücin and Öner, 1982); Muğla (Güngör et al., 2016); Samsun (Pekşen and Karaca, 2000, 2003); Trabzon (Akata et al., 2014; Akata and Uzun, 2017).

Sarcosomataceae Kobayasi

Plectania ericae (Donadini) Roqué: İstanbul (Uzun and Kaya, 2018).

Plectania melastoma (Sowerby) Fuckel: Trabzon (Akata et al., 2012).

Plectania rhytidia (Berk.) Nannf. & Korf: Trabzon (Akata et al., 2012; Akata and Uzun, 2017).

Pseudoplectania melaena (Fr.) Sacc.: Antalya (Allı et al., 2011; Solak et al., 2014).

Pseudoplectania sphagnophila (Pers.) Kreisel: Uşak (Türkoğlu and Yağız, 2012).

Strobiloscypha cupressina B. Perić & Pfister: Gaziantep (Kaya and Uzun, 2018).

Tarzettaceae Ekanayaka, K.D. Hyde, Q. Zhao & E.B.G. Jones

Tarzetta catinus (Holmsk.) Korf & J.K. Rogers: Aydın (Allı et al., 2007); Balıkesir (Yılmaz and Işıloğlu, 2002; Solak et al., 2002); Bolu (Yağız et al., 2006a); Denizli (Türkoğlu, 2008); Erzincan (Allı, 2011); Isparta (Güngör et al., 2015); İzmir (Yılmaz Ersel and Solak, 2004); Kahramanmaraş (Kaya, 2009); Kilis (Solak et al., 2014a); Kocaeli, Sakarya (Doğan et al., 2021); Kütahya (Allı et al., 2017); Mersin (Güngör et al., 2015); Muğla (Güngör et al., 2016; Solak and Yılmaz Ersel, 2005); Trabzon (Akata et al., 2014; Akata and Uzun, 2017).

Tarzetta cupularis (L.) Svrček: Adıyaman (Kaya, 2009); Isparta (Afyon, 1996); Kastamonu (Akata et al., 2010); Trabzon (Akata et al., 2014; Akata and Uzun, 2017).

Tuberaceae F. Berchtold & J. Presl

Choiromyces meandriformis Vittad.: Bolu, Samsun, Uşak (Türkoğlu and Castellano, 2014).

Reddellomyces parvulosporus (G.W. Beaton & Malajczuk) Trappe, Castellano & Malajczuk: Muğla (Ünal et al., 2016).

Reddellomyces westraliensis (G.W. Beaton & Malajczuk) Trappe, Castellano & Malajczuk: Muğla (Ünal et al., 2016).

Tuber aestivum (Wulfen) Spreng.: Antalya, Artvin, Bolu, Burdur, Denizli, Düzce, Hatay, İstanbul, İzmir, Kırklareli, Muğla, Ordu, Osmaniye (Türkoğlu et al., 2015); Konya (Alkan et al., 2018).

Tuber borchii Vittad.: Kahramanmaraş (Kaya, 2009); Konya (Çevik et al., 2021); Aydın, Muğla, Samsun, Tekirdağ (Elliot et al., 2016).

Tuber brumale Vittad.: Niğde (Öztürk et al., 1997); Osmaniye, Samsun (Türkoğlu and Castellano, 2014).

Tuber excavatum Vittad.: Denizli (Türkoğlu and Castellano, 2014); Trabzon (Uzun and Yakar, 2018).

Tuber ferrugineum Vittad.: Antalya, Aydın, Denizli, Muğla (Elliot et al., 2016).

Figure 1. Colour photographs of some *Pezizales* members. *Acervus epispartius* (a), *Aleuria aurantia* (b), *Caloscypha fulgens* (c), *Gena lobulata* (d), *Gyromitra esculenta* (e), *Gyromitra infula* (f), *Helvella crispa* (g), *Helvella elastica* (h), *Helvella latispora* (i), *Legaliana badia* (j), *Marcelleina persoonii* (k), *Melastiza cornubiensis* (l), *Neottiella rutilans* (m), *Pachyella clypeata* (n), *Peziza saniosa* (o), *Plectania rhytidia* (p), *Sarcoscypha coccinea* (r), *Tuber puberulum* (s)

Tuber fulgens Quél.: Kırklareli (Akata et al., 2020).

Tuber macrosporum Vittad.: Edirne, Tekirdağ (Doğan, 2021).

Tuber mesentericum Vittad.: Denizli (Castellano and Türkoğlu, 2012; Türkoğlu and Castellano, 2014).

Tuber nitidum Vittad.: Burdur (Türkoğlu et al., 2015); Denizli (Castellano and Türkoğlu, 2012); Osmaniye (Türkoğlu and Castellano, 2014).

Tuber oligospermum (Tul. & C. Tul.) Trappe: Şanlıurfa (Akata et al., 2022).

Tuber puberulum Berk. & Broome: Aydın, Denizli, Muğla, Osmaniye (Elliot et al., 2016); Artvin, Trabzon (Uzun and Yakar, 2018).

Tuber rufum Picco: Antalya, Denizli, Kastamonu, Konya, Muğla (Türkoğlu and Castellano, 2014).

4. Discussions

As a result of this study, 264 *Pezizales* species have been determined to exist in Türkiye. Fifteen of the existing 16 family of the *Pezizales* were found to have members in Türkiye. Among them the most crowded one is *Pyronemataceae* with 92 species. Together with *Pyronemataceae,* 6 of the families are represented with 10 or more than 10 speies (*Pezizaceae* 46, *Morchellaceae* 33, *Helvellaceae* 29, *Tuberaceae* 15, *Ascobolaceae* 10) in Türkiye. The other families *Discinaceae, Otideaceae, Sarcoscyphaceae, Sarcosomataceae, Ascodesmidaceae, Pseudoombrophilaceae, Tarzettaceae, Caloscyphaceae* and *Rhizinaceae* were represented in Türkiye with 9, 7, 6, 6, 2, 2, 2, 1 and 1 species respectively (Table 1). IndexFungorum (accessed on 30 December 2022) currently doesn't include the members of the genera *Coprotus* (2) and *Psilopezia* (1) in any of the above families and keeps under Incertae sedis.

The taxa are distributed in 81 genera. *Morchella* Dill. ex Pers., *Helvella* L., *Peziza* Dill. ex Fr., *Octospora* Hedw. and *Tuber* P. Micheli ex F.H. Wigg. were found to be the most crowded first five genera with 30, 24, 17, 16 and 12 species respectively. *Scutellinia* (Cooke) Lambotte is the sixth crowded one with 8 species. Two of the genera (*Cheilymenia* Boud., *Otidea* (Pers.) Bonord.) included 7 species while the other five included (*Ascobolus* Pers., *Geopora* Harkn., *Gyromitra* Fr., *Pulvinula* Boud., *Terfezia* (Tul. & C. Tul.) Tul. & C. Tul.) 6 and one included (*Genea* Vittad.) 5 species. *Lamprospora* De Not. and *Trichophaea* Boud. includes 4 species in Türkiye. Eight of them (*Aleuria* Fuckel , *Discina* (Fr.) Fr., *Geopyxis* (Pers.) Sacc., *Marcelleina* Brumm., Korf & Rifai, *Paragalactinia* Van Vooren , *Plectania* Fuckel, *Thecotheus* Boud., *Tricharina* Eckblad) were represented with 3 species while 19 (*Anthracobia* Boud., *Barssia* Gilkey, *Coprotus* Korf & Kimbr., *Geoscypha* (Cooke) Lambotte, *Humaria* Fuckel, *Lasiobolus* Sacc., *Melastiza* Boud., *Pachyella* Boud., Pachyphlodes Zobel, *Phylloscypha* Van Vooren, *Picoa* Vittad., *Pithya* Fuckel, *Pseudombrophila* Boud., *Pseudoplectania* Fuckel, *Pyronema* Carus, *Reddellomyces* Fuckel, *Sepultariella* Van Vooren, U. Lindem. & Healy, *Tarzetta* (Cooke) Lambotte, *Verpa* Sw.) are represented with two species. The rest of the 38 genera are repsented in Türkiye with only one member.

Table 1. Distribution of the Turkish Pezizales species in families.

Family name	# of taxa
Ascobolaceae	10
Ascodesmidaceae	2
Caloscyphaceae	1
Discinaceae	9
Helvellaceae	29
Incertae sedis	3
Morchellaceae	33
Otideaceae	7
Pezizaceae	46
Pseudombrophilaceae	2
Pyronemataceae	92
Rhizinaceae	1
Sarcoscyphaceae	6
Sarcosomataceae	6
Tarzettaceae	2
Tuberaceae	15

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Protective effects of resveratrol on permethrin-induced fetotoxicity in rats

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Sıçanlarda permetrin kaynaklı fetotoksisite üzerine resveratrolün koruyucu etkileri

Abstract: Synthetic pyrethroid insecticides have been widely used for years to prevent harmful effects of insects and control disease vectors. In this study, the effects of resveratrol against the potential toxicity of permethrin, an effective pyrethroid derivative, on the fetus were investigated. Accordingly, *Wistar* female rats were divided into four groups as Control, Sham, Permethrin, and Permethrin + Resveratrol. Lung, liver, kidney and small intestine of developing fetuses were evaluated histopathologically. Also, Bone Morphogenetic Protein-4 (BMP-4) in bone tissue development and Fibroblast Growth Factor-1 (FGF-1) expressions in lung were examined immunohistochemically. All structures in the Control and Sham groups were normal. Permethrin caused epithelial damage, regression in bronchial and primitive alveolar development in the lung; congestion, edema and sinusoidal dilatation around the central vein in the liver; tubular epithelial degeneration, regression in glomeruli and tubule formation in the kidney; epithelial degeneration and irregularity in the villus structure in the small intestine. Immunohistochemical results indicated that permethrin administration decreased BMP-4 levels in bone tissue and FGF-1 levels in lung. Resveratrol application was found to greatly alleviate histopathological and immunohistopathological variability in all tissues. Oral consumption of permethrin by pregnant rats caused growth retardation and tissue damage in many different tissues in offspring. Intake of resveratrol during pregnancy showed protective effects against fetotoxicity caused by permethrin.

Key words: Permethrin, Resveratrol, Fetotoxicity, FGF-1, BMP-4

Özet: Sentetik piretroid insektisitler, yıllardır böceklerin zararlı etkilerinden korunmak ve hastalık vektörlerinin kontrolü için yaygın olarak kullanılmaktadır. Bu çalışmada, etkili bir piretroid türevi olan permetrinin fetüs üzerindeki olası toksisitesine karşı polifenolik bir bileşik olan resveratrolün potansiyel etkileri araştırılmıştır. Çalışmada *Wistar* dişi sıçanlar Kontrol, Sham, Permetrin ve Permetrin+Resveratrol olmak üzere dört gruba ayrıldı. Histopatolojik olarak fetüslerin akciğer, karaciğer, böbrek ve ince bağırsak gelişimleri değerlendirildi. Ayrıca immunohistokimyasal olarak kemik doku gelişiminde Bone Morphogenetic Protein 4 (BMP-4) ve akciğerde Fibroblast Growth Factor-1 (FGF-1) ekspresyonlarına bakıldı. Kontrol ve Sham grubunda tüm yapılar normal görünümde izlendi. Permetrin; akciğerde epitelyal hasara, bronşiyal ve primitif alveolar gelişimde gerilemeye; karaciğerde santral ven çevresinde konjesyon, ödem ve sinüzoidal dilatasyona; böbrekte tübül epitelinde dejenerasyona, glomerül ve tübül oluşumunda gerilemeye; ince bağırsakta da epitelyal dejenerasyona ve villüs yapısında düzensizliğe neden olduğu saptanmıştır. İmmünohistokimyasal sonuçlarda permetrin uygulaması ile kemik dokuda BMP-4 ve akciğerde FGF-1 ekspresyonlarında azalma saptanmıştır. Resveratrol uygulaması sonrasında tüm dokularda histopatolojik ve immünohistokimyasal değişiklikler büyük oranda hafiflemiştir. Gebe sıçanların oral yolla permetrin tüketimi, yavrularda birçok farklı dokuda gelişme geriliği ve doku hasarına neden olmaktadır. Gebelik sürecinde resveratrol alımı ise permetrinin neden olduğu fetotoksisiteye karşı koruyucu etkinlik göstermektedir.

Anahtar Kelimeler: Permetrin, Resveratrol, Fetotoksisite, FGF-1, BMP-4

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1. Introduction

Synthetic pyrethroids constitute 30% of the insecticides used worldwide (Elser et al., 2022). However, these products pose a threat to public health by spreading to the environment outside of their intended targets and accumulating in drinking water and agricultural products. Depending on the ingestion of insecticide residues in foods, toxic effects can be observed in the central nervous system, cardiovascular system, urogenital system, metabolic and endocrine systems in humans and animals (Mossa et al., 2018). Permethrin (Perm), is a pyrethroid-derived insecticide that is frequently used in agriculture, veterinary, forestry, public and environmental health spraying, the protection of stored products, and livestock (Nguyen et al.,

2023). Although they are considered to be safer in terms of use and environmental impact than Perm's other insecticide types (organophosphate derivative, carbamate), there is no clear reliability about long-term exposure. Perm and its metabolites can be found indoors and outdoors, in soil and dust. Exposure of humans to perm can occur through inhalation, consumption of contaminated food, or through the skin. Perm is hydrolyzed to a significant extent in the gastrointestinal tract after it is taken into the body, and 40-60% of the amount taken orally passes into the systemic circulation. Perm, due to its lipophilic properties, accumulates in tissues with high lipid content such as liver, kidney and breast, as well as fat and nervous system, and is metabolized in the liver and excreted through the kidneys (Ding et al., 2013). Experimental studies have shown that Perm adversely affects the peripheral and central nervous system in mammals, causes neurotoxic effects by inducing oxidative stress in the neonatal rat brain, increases malondialdehyde levels by causing lipid peroxidation, has advers effects on the liver and endocrine system, and causes degenerative and necrotic damage in the kidney, increases apoptotic cell death, decreases sperm concentration in rats, and causes heart diseases (Wang et al., 2016; Romero et al., 2017; Curtis et al., 2021). These undesirable effects of Perm can cause fetal toxicity and developmental delay in offspring, especially during pregnancy. The bone morphogenetic proteins (BMPs) have been described as a potent inducer of bone and cartilage tissue formation (Tateiwa and Kaito, 2023). In this context, especially BMP-4 is considered as an important factor in the formation and repair of endochondral bone (Ye et al., 2022). Moreover, fibroblast growth factor-1 (FGF-1) belongs to the FGF family and has been shown to inhibit fibroblast collagen production and differentiation into myofibroblasts, and reverse the epithelial-mesenchymal transition by inhibiting TGF-β1 signaling pathways (Gasser et al., 2022). FGF-1 participates in many vital functions in the cell, such as the axial structuring of embryonic tissues, identification and differentiation of cell types, morphogenesis of organs and systems such as the vascular system, and regulation of cell proliferation and movement (Xiao et al., 2021). In addition, it has a role in many biological events from tissue repair to cancer cell growth and spread (Coleman et al., 2014). In addition to being an angiogenic factor, it is involved in endothelial cell migration and proliferation, and also contributes to the development of mesenchymal and neuroectodermal cells (Fernandes-Freitas and Owen, 2015).

Resveratrol (Res), is a polyphenolic compound of the phytoalexin group (Chen et al., 2023). Polyphenolic compounds show antioxidant properties by destroying free radicals and minimizing the harmful effects of oxidative stress (Borsoi et al., 2023). Res has anti-inflammatory, antioxidant, and anticarcinogenic properties (Chen et al., 2023). Discovered in grape seeds and peel, Res is especially found in dark fruits. Res, which has a role in the defense mechanism of plants against fungicides, has been found to have many different properties in experimental studies (Bohara et al., 2022). In a cell culture study, Res was found to suppress the epithelial-mesenchymal transition in the YKG1 glioblastoma cell line (Pektas et al., 2021). Its protective efficacy against spinal cord ischemia-reperfusion injury has been demonstrated in rats (Aslan et al., 2021). Its reparative efficacy against ovarian deformation induced by metabolic syndrome has been reported (Pektaş et al., 2014). Other studies have reported anti-inflammatory effects in the heart (Pektas et al., 2017), vasodilation due to eNOS induction in vascular smooth muscles (Pektas et al., 2018), and protective effects against diabetes-induced kidney damage (Koca et al., 2016). In another study, apoptotic effects of Res metabolites were detected on *Ehrlich Ascites Carcinoma*, a resistant rodent cancer type (Bozkurt et al., 2020).

To date, there are very few animal studies in the literature showing the toxic effects of Perm on the fetus by crossing the placenta. On the other hand, the benefits of Res, which can be taken with natural foods and have epigenetic protective effects as well as antioxidant effects, during pregnancy are not known. This study compiled clues to fetotoxicity and potential effects of Res in rats given Perm on days 7 and 14 of gestation.

2. Materials and Method

2.1. Animals and the experimental procedures

This study was approved by Afyon Kocatepe University Animal Ethics Committee (49533702/58). In the study, 24 adult female *Wistar* Albino rats with a weight of 220 ± 20 *g* were used. During the study period, rats were housed in temperature and humidity-controlled rooms (20 – 22°C) with a 12-hour light-dark cycle in polycarbonate cages with standard pellet food and regular water provided. After ovulation times were determined in order to achieve pregnancy in the rats, they were caged with male rats by the one-to-two method between 17:00 and 21:00 p.m. when they were in the proestrus stage. The next day, vaginal smears were taken from female rats, sperm (+) in the smear and those showing diestrus phase in the following tests were considered pregnant and included in the study. Embryo development is mostly affected by teratogenic agents during the organogenesis period. Therefore, our applications were performed between the 6th and 17th days, which is the organogenesis stage. Pregnant (+) rats were divided into 4 groups with 6 rats in each group. No application was made to the Control group of pregnant rats during the 18-day of experimental period (Group 1). In the Sham group, on the 7^{th} and 14^{th} days of pregnancy, 0.5 cc - 5% alcohol + corn oil mixture at a ratio of 1/1, additionally only 0.5 cc - 5% alcohol application on the 7^{th}, 10^{th}, 13^{th}, and 16^{th} days of pregnancy were given with gavage (Group 2). Perm (300 mg/kg) dissolved in a 1/1 ratio of corn oil + alcohol solution was applied by gavage to the Perm group on the 7^{th} and 14^{th} days of pregnancy (Group 3). In the Perm + Res group, on the 7^{th} and 14^{th} days of pregnancy, Perm (300 mg/kg) was dissolved in alcohol and corn oil solution prepared at a ratio of 1/1, in addition, on the 7^{th}, 10^{th}, 13^{th}, and 16^{th} days of pregnancy resveratrol (50 mg/kg) dissolved in %5 alcohols were given with gavage. On the 18^{th} day of pregnancy, fetuses and placentas were removed from pregnant rats via cesarean-section under CO_2 anesthesia. Afterward, the rats were sacrificed by taking blood from their hearts. Removed baby rats were fixed in a 10% neutral buffered formaldehyde solution.

2.2. Chemicals, immunostaining and the histochemical staining

In experiments, Perm (Permethrin, 45614, Sigma), Res (Resveratrol, 51852282, Molecula), BMP-4 primary antibody (PA5-19683, Thermo Scientific), FGF-1 primary

antibody (sc-7910, Santa Cruz), and Secondary antibody kit (Thermo, Cat. No: 85-9043) were used.

Sections taken on adhesive slides were stained immunohistochemically using appropriate primary antibodies to evaluate BMP-4 and FGF-1 expressions in the samples after deparaffinization and rehydration processes. Expressions of BMP-4 in the bone and FGF-1 in the lung tissues were evaluated. The prepared preparations were examined under a light microscope (Nicon-Eclipse E600, Tokyo, Japan) and their images were taken.

After the excised fetuses were cut transversely and horizontally, they were left in 10% neutral formaldehyde solution for fixation. Three fetuses from each mother rat were included in the study, and the fetal samples were embedded in paraffin blocks after passing through the tissue follow-up method stages described. Sections of 5-micron thickness were taken from paraffin blocks. Sections were stained with hematoxylin-eosin (HE) for histopathological examinations. The images of lung, liver, kidney, and small intestine (jejunum) tissues in the sections stained with HE were examined under the light microscope (Nicon Eclipse E600, Tokyo, Japan), and the histopathological changes were recorded and visualized.

Histopathological changes in the lung, liver, kidney and small intestine (jejunum) were evaluated semi-quantitatively (−, no lesions; +, mild; ++, moderate; +++, severe) according to the Gibson-Corley scoring (Gibson-Corley et al., 2013).

After the experiment, bronchoalveolar development and infiltration scoring were performed in the lungs of the fetuses. In the 18th day of rat fetuses, terminal and respiratory bronchiole development; precursors of alveolar canals and sacs were observed to have developed in the lung.

In the lung, bronchoalveolar development and infiltration scoring were performed. In the evaluation of lung maturation, there are glandular stage, canalicular stage and saccular stage, and glandular-canalicular stage and canalicular-saccular stage as intermediate stages, according to the developmental order. Pseudo-glandular stage is observed on the 18th day of pregnancy in the lung maturation of rat fetuses, and canalicular stage is observed on the 19th and 20th days of pregnancy. Since the fetuses were removed on the 18th day in our study, the pseudo-glandular stage in lung development begins to end and the transition to the canalicular stage begins as of the developmental stage. At this stage, the major ducts have branched and started to develop, terminal bronchioles have formed, and respiratory bronchioles will begin to form. In the light of this information, lung development scoring was done by modifying the scoring in previous studies. In three lung sections belonging to each group, 10 different areas were scanned and the lung development level was scored (1: 25-40%, 2: 40-60%, 3:60-80%, 4:80-100%) as a percentage (Burri, 1984).

Congestion, infiltration and parenchymal damage in the liver were evaluated. In the kidney, the developmental stage was evaluated by looking at the parameters of cortex-medulla differentiation, glomerular development and tubule development. The kidney development was scored (1: 25-40%, 2: 40-60%, 3: 60-80%, 4: 80-100%) as a percentage (Simsek et al., 2009) by examining ten different areas in 3 different fetal kidney sections and also tubular damage was graded semi-quantitatively.

In the intestine tissue samples, changes in the villus epithelium of the jejunum region were evaluated and scored semi-quantitatively.

2.3. Statistical analysis

Immunohistochemical data were represented as mean ± standard error of the mean (SEM) throughout the study. Student's t test for unpaired data or one-way ANOVA followed by the *Bonferroni* post hoc analysis were performed for statistical comparisons in which p value less than 0.05 was considered significant. *$p<0.05$ significantly different from the Sham; #$p<0.05$, significantly different from the Perm-treated rats.

3. Results

3.1. Histopathological modifications in the lung, liver, kidney, and the small ıntestine tissues

In the Control and Sham groups, bronchiole structures and alveolar canal sacs and precursors of these structures were formed; these structures were observed to be regular and normal. Vena centralis, portal area, and sinusoidal structures of liver were evaluated as normal. In kidney tissue examination, primitive glomerular capillary network, *Bowman's space*, and tubule structures were observed. Villi and crypt structures were observed regularly in small intestine sections (Fig. 1). No significant difference was found in the scoring of the Control and Sham groups (Table 1).

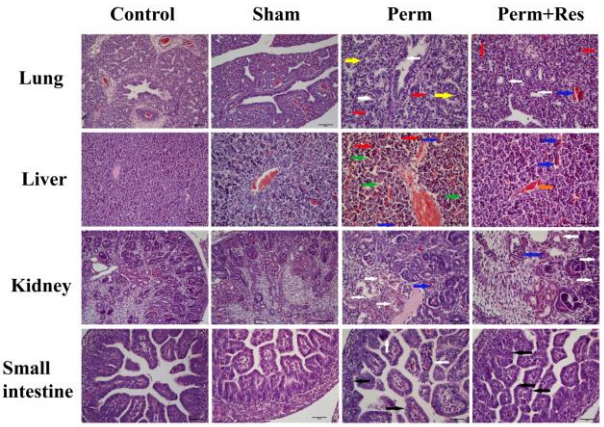

Figure 1. Representative H-E stained sections from the experimental groups of fetal tissues. Control and Sham groups have normal morphology in all tissues. In perm group histopathological changes were seen in all tissues. Histopathological changes in all tissues were greatly alleviated after resveratrol administration. Infiltrative cells (red arrow), epithelial degeneration (white arrow) and areas of delayed alveolar structure development (yellow arrow) congestion (blue arrow), dilatation in sinusoidal areas (green arrow), apoptotic changes (black arrow), vena centralis dilatation (orange arrow). It was observed that congestion and epithelial degeneration decreased in the Perm + Res group.

HE evaluations of Perm group revealed degenerative and infiltrative findings in lung parenchymal structure and deterioration in bronchiolar / alveolar epithelium. It was observed that the development of the primitive alveolar structure was slower in the samples of Perm group than in the Control and Sham groups. In liver samples, there was significant venous edema and congestion, and dilatation in

sinusoidal areas next to the infiltrative areas in the parenchyma. When kidney sections were examined, retardation was detected in the development of glomerular and tubular structures. Degenerative and vacuolar changes were observed in the tubular epithelium, and there was also congestion in the capillary area. Looking at the villi and crypt structures; irregular, degenerative and apoptotic changes were seen in some villi epithelium (Fig. 1). In the scoring of the Perm group, tissue growth retardation, congestion, edema, and degenerative changes were found to be higher compared to the Control and Sham groups (Table 1). In the examinations of the Perm + Res group, congestion in the lung and degenerative changes in the epithelial region were observed. Infiltrative cells were observed intensively in the parenchymal area. In the examination of the liver tissue, there were signs of congestion in the venous areas and dilatation of the vena centralis, but these findings were observed to be milder than the damage findings in the Perm group. A decrease in glomerular congestion was observed in kidney sections. It was observed that the degenerative appearance in the tubules was less than in the Perm group. In the small intestine sections, mild signs of damage and apoptotic cells were observed in the villi (Fig. 1). In the scoring of the Perm + Res group, it was determined that the variables seen in the Perm group were alleviated (Table 1).

Table 1. Scoring of histopathological changes of lung, liver, kidney, and small intestine.

		Control	Sham	Perm	Perm+Res
		(+) Scores			
Lung	Bronchoalveolar growth	4	4	2.67	3
	Infiltration	0	0	2	1.5
Liver	Congestion	0	0	2.17	1.83
	Parenchymal damage	0	0	2.33	1.5
Kidney	Kidney growth	4	4	3	3.33
	Tubular damage	0	0	2.16	1.33
Small intestine	Epithelial damage	0	0	1.5	1

3.2. Immunohistochemical changes of BMP-4 in the bone and FGF-1 in the lung tissues

BMP-4 expressions in the bone tissue were expressed as a percentage. Intense BMP-4 expression was observed in the area of chondrogenesis of the Control and Sham groups. Evidently, there was a significant decrease in BMP-4 expression in the sections belonging to the Perm group compared to Control. In the Res-treated group, BMP-4 levels were found to be significantly increased compared to the Perm group (Figure 2 and Figure 3a).

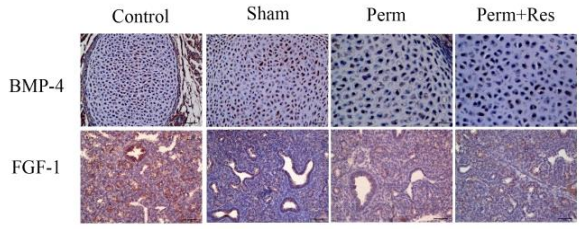

Figure 2. Representative photomicrographs of immuno-histochemical staining of fetal tissues. Immunohistochemical staining for BMP-4 in fetal bone and FGF-1 in fetal lung. In the Perm group, deterioration in the structure of the lacunae and decrease in the staining intensity with BMP-4 were evident. Staining with FGF-1 showed decrease in staining in the Perm group.

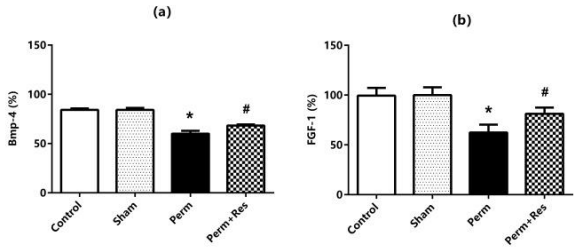

Figure 3. Relative expressions of BMP-4 (a) and FGF-1 (b). *p<0.05 significantly different from the Control; #p<0.05, significantly different from the Perm-treated rats.

FGF-1 expressions in lung sections were scored by evaluating the staining intensity in the epithelial area. According to the results, intense FGF-1 expression was observed in the Control and Sham groups. In cross-sections of Perm-treated rats, a significant decrease in FGF-1 expression was observed, especially in regions with intense degenerative changes in the bronchiole epithelial structure. In FGF-1 expressions, a partial increase was detected in the Perm + Res group compared to Per group (Fig. 2, 3b).

4. Discussions

Perm is one of the first insecticides with limited persistence in soil with its high insecticidal activity and low mammalian toxicity (Wang et al., 2016). They are widely used because they are considered to be extremely safe in terms of use and effects on the environment compared to other insecticide groups. Due to its widespread use in cities and environmental settlements, it also affects non-target organisms as well as target organisms. Although it is claimed that synthetic pyrethroid insecticides, including Perm, are much safer than other agents, it has been revealed that they can cause damage to many organs and may have teratogenic effects, especially if exposed during pregnancy. Perm frequently targets the nervous system in target organisms (López-Aceves et al., 2021). It can cause neurotoxicity, paralysis and even death in the organism by damaging voltage-dependent sodium channels in neurons. The liver is an organ that plays a vital role in the detoxification of xenobiotics. Perm is a potent inhibitor of cytochrome P4501A, which causes significant accumulation of some chemicals associated with fatal toxicity (Ding et al., 2013). In a study, it was shown that Perm destabilizes and damages the redox system of the liver in neonatal and adult rats. In addition, Perm disrupts the antioxidant balance, causes lipid peroxidation, protein oxidation and increases apoptotic cell death (Gabbianelli et al., 2013). Since exogenous chemicals and their metabolites are removed from the body via kidney tissues, they are target organs for many toxic agents. Prethyroid insecticides containing Perm are also among these agents. There are three main mechanisms responsible for Perm-induced nephrotoxicity; accumulation of xenobiotics and macromolecules induced by xenobiotics in renal tissue; accumulation in renal tissue of toxic metabolites biosynthesized in other organs; accumulation in the kidney due to bioactivation of xenobiotics into reactive metabolites. Particularly, the pars recta of the proximal tubules are more sensitive to chemicals since it is the site of enzymes that metabolize xenobiotics, especially P450 (Dekant and Vamvakas, 1996). Reactive oxygen radicals

consist primarily of arachidonic acid metabolism, the xanthine oxidase system, and activated neutrophils. Reactive free oxygen radicals (mainly hydroxyl radical and superoxide anion) cause oxidative stress in the cell by causing oxidation of membrane lipids, protein denaturation, DNA degradation, and sulfhydryl enzyme inactivation. This increases membrane permeability, leading to protein and nucleic acid degradation and ultimately to aging, cell damage, and death in cells. Under normal conditions, antioxidant enzymes render the reactive oxygen radicals formed in the organism harmless. However, agents such as Perm can inhibit antioxidant enzymes with different mechanisms, causing an increase in reactive oxygen radicals in the environment and damage to the cell. Pesticide-induced oxidative stress has been the focus of toxicological research in recent years as a possible mechanism of toxicity. Various studies have been conducted to determine whether oxidative stress in humans or animals is caused by various agents in this group and is associated with their toxic effects (Agrawal and Sharma, 2010; Gabbianelli et al., 2013; López-Aceves et al., 2021). It has been shown that oxidative stress increases in tissues after exposure to prethyroid insecticides such as Perm and that increased oxidative stress plays a fundamental role in the pathogenesis of many organ damage (Wang et al., 2016). Romero and his team showed that Cypermethrin, one of the prethyroid insecticides, causes apoptotic, necrotic and autophagic cell death by inducing oxidative stress that causes DNA damage in neuroblastoma cells (Romero et al., 2017). Although the target organs of prethyroids are different, Perm-mediated oxidative stress has been shown in humans and animals caused neurotoxicity (Nasuti et al., 2014), hepatotoxicity (Roma et al., 2012), nephrotoxicity (Guvenc et al., 2013), immunotoxicity (Jin et al., 2010), cardiotoxicity (Dhivya Vadhana et al., 2010). However, Res is a molecule that shows antioxidant potential by destroying free radicals, binding metal ions, decreasing the activity of enzymes involved in the formation of reactive oxygen species, and increasing antioxidant enzyme activities (Chen et al., 2023). In this study, we aimed to investigate the damage of Perm, which is used in many different areas and to which we are unintentionally exposed, on different organs that will be caused by exposure in the prenatal period, and the experimental model in which we planned the possible effectiveness of Res. When the lung findings were evaluated in our study, it was found that the bronchoalveolar development of the Control and Sham groups was normal and no infiltration was observed. The bronchoalveolar development of the entire Sham group was determined as stage 4. While growth stage 2 and severe infiltration were observed in the Perm group, decreased infiltration and growth were evaluated as stage 3 in the Res-treated group. In a similar study, it was shown that infiltration of the chick embryo increased with Perm injection and tissue development decreased (Curtis et al., 2021). However, Res is known to be a powerful antioxidant and tissue-protector (Aslan et al., 2021; Bohara et al., 2022). In this context, it can be said that the effects of Perm and Res are compatible with the literature. Although the protective effects of Res do not show high potency, this may be dose related. In liver sections, parenchymal damage and congestion were not observed in the Control and Sham groups, while severe congestion and parenchymal damage were observed in the Perm group. Furthermore, Res treatment was found to reduce Perm-induced congestion and parenchymal damage. In studies investigating the effects of Perm on the liver, it was shown that Perm causes hepatotoxicity by increasing lipid peroxidation (Roma et al., 2012; Gabbianelli et al., 2013). From this point of view, this may be the reason for Perm-dependent congestion and parenchymal damage in our study. However, the free radical scavenging activity of Res, whose hepatoprotective activity has been shown in many studies (Pektas et al., 2017; Aslan et al., 2021), may have suppressed Perm-dependent variables. In another similar study, the effects of Perm on the kidney were examined histopathologically, and it was shown that Perm causes necrotic and degenerative changes in the tubule epithelium and also increases apoptotic cell death (Guvenc et al., 2013). Liang et al. (2013) also found that a metabolite that causes nephrotoxicity and hepatotoxicity in rats after 60 days of Perm administration increased in urine. When kidney sections were evaluated in our study, it was found that tubular development was sufficient (Stage 4) and no damage occurred in the Control and Sham groups; tubular damage and growth retardation (Stage 2) were observed in the sections of Perm-treated rats. Similarly, our results indicate that Perm-derived metabolites may be associated with kidney damage. On the other hand, our results show that Res treatment reduces Perm-induced changes. This may be related to the free oxygen radical scavenging activity of Res and facilitating the excretion of Perm-derived metabolites. When the intestine results were evaluated, epithelial damage was observed in the sections of Perm-treated rats, while no epithelial damage was observed in the Control and Sham groups. In parallel with the findings of our study, degeneration, necrosis, and cell infiltrations of epithelial and cartilage tissues in the gills of Deltamethrin, one of the prethyroid insecticides, were detected in a study conducted in a fish of the Carassius gibelio species. In the same study, degenerative and necrotic changes with edema between the submucosa and mucosa in the intestine, enlargement of the blood vessels of the serosa and atrophy of the muscularis and submucosa, shortening and thickening of the villi were observed (Gey and Ersan, 2020). Moreover, it has been reported that Res protects intestinal barrier integrity, improves antioxidant capacity, and alleviates inflammation in the jejunum of ducks exposed to acute heat stress (Yang et al., 2021). In general, both the effects of Perm on tissues and the influences of Res on the Perm-derived variants are in line with similar studies. BMP-4 is a protein found in demineralized bone and cartilage tissue, involved in growth and differentiation. In our study, it was shown that BMP-4 levels decreased in Per group compared to the Control group and were normalized with Res supplementation. It has been reported in many studies that pesticides reduce BMP-4 levels in bone tissue (Feng et al., 2016). Supportingly, chlorpyrifos exposure has been shown to reduce BMP-4 expression, which is essential for cranial neural crest morphogenesis and chondrogenesis in *Xenopus laevis* embryos (Tussellino et al., 2016). In the same study, it was reported that chlorpyrifos inhibited development by affecting the bone formation signaling pathway. A similar activity may be valid for Perm in our study as well. However, Res is known to regulate BMP-4 levels (Min et al., 2020). Therefore, we can say that the influences of Perm and Res on BMP-4 levels in the bone tissues are also compatible with previous studies. FGF-1 protein; it is a

myth of the FGF family, which is responsible for many biological procedures such as embryo development, cell growth, morphogenesis, organogenesis, and tissue repair (Coleman et al., 2014). A decrease in FGF-1 expressions in proportion to the damage to the lung tissues with Perm was observed. In rats with Res added to the treatment, FGF-1 expressions increased compared to Perm groups according to our study. In a similar study, it was reported that Paraquat which is known as a pesticide, increases connective tissue growth factor expression and impairs lung fibroblast proliferation and viscoelasticity (Zhang et al., 2014). It has also been reported that Res has a synergistic protective effect against cardiotoxicity and hepatotoxicity in experimental studies (Lu et al., 2022; Xu et al., 2022). Thus, it is seen that the immuno-histochemical results of FGF-1 in lung sections are very similar to the literature. Histological observations in our study revealed that Perm caused histopathological changes in lung, liver, kidney and small intestine structures in rat fetuses. It has been determined that Res partially reduces or prevents these developmental and toxicological changes caused by Perm. Thanks to the antioxidant properties of Res, it can be said that it plays a role in alleviating the toxicity on the organs by reducing the oxidative damage caused by Perm. In this study, besides examining the possible side effects of Perm, an insecticide that is widely used in daily life, and revealing its fetotoxic effects, it is aimed to reveal the therapeutic and/or protective effects of foods that are widely recommended in terms of epigenetic effects and taken from natural sources on the side effects of this agent. In this study, the effects of Perm and Res on the organogenesis period were examined in many organs - there is no previous study on this subject in the literature. We think that it can contribute to some extent as a guide both in the prevention of developmental abnormalities due to the use of Perm and in the protection from these possible toxic effects. However, due to the scarcity of studies on Perm-related organ damage and the antioxidant activity of Res on this damage, there is a need for experimental studies planned in larger series on this subject.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

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Pseudoporpoloma pes-caprae (*Tricholomataceae*): A new record for the mycota of Türkiye

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Pseudoporpoloma pes-caprae (*Tricholomataceae*): Türkiye mikotası için yeni bir kayıt

Abstract: Basidiocarps of *Pseudoporpoloma pes-caprae* (Fr.) Vizzini & Consiglio were collected from Trabzon. Main morphological characters were noted, photographs were taken and the flora of the collection site was determined at the field. Basidiocarps were sectioned under binocular research microscope, treated with ammonia solution and subsequently examined under Zeiss Axio Imager A2 Research microscope. The collection has conical to bell-shaped, convex, generally umbonate, 50-85 mm, fibrillose, greyish or ocher-brown pileus; whitish, cream or pale grey, emarginate adnate to nearly free lamellae; cylindrical, tapering based, solid, longitudinally fibrillose and whitish stipe; subglobose to ellipsoid, smooth, hyaline and about 6-8 × 4-6 µm sized basidiospores.

Key words: Agaric, microscopy, taxonomy, Türkiye

Özet: *Pseudoporpoloma pes-caprae* (Fr.) Vizzini & Consiglio'nun bazidiyokarpları Trabzon'dan toplandı. Arazide ana morfolojik karakterler not edildi, fotoğraflar çekildi ve toplama sahasının florası belirlendi. Bazidiyokarplardan binoküler araştırma mikroskobu altında kesitler alındı, amonyak çözeltisi ile muamele edildi ve daha sonra Zeiss Axio Imager A2 araştırma mikroskobu altında incelendi. Toplanan örnek konik ve/veya çan şeklinde, dışbükey, genellikle tepe çıkıntılı, 50-85 mm, lifli, grimsi veya koyu sarı-kahverengi şapka; beyazımsı, krem veya soluk gri, saptan hafif ayrık veya hemen hemen serbest lamellere; silindirik, tabana doğru sivrilen, dolu, uzunlamasına lifli ve beyazımsı sapa; elipsoyit, pürüzsüz, şeffaf ve yaklaşık 6-8 × 4-6 µm büyüklüğünde bazidiyosporlara sahiptir.

Anahtar Kelimeler: Agarik, mikroskopi, taksonomi, Türkiye

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1. Introduction

The genus *Pseudoporpoloma* Vizzini & Consiglio was erected by Vizzini et al. (2016) to host *Agaricus pes-caprae*, a relatively small, grassland species. This monotypic genus is morphologically distinguished with a tricholomoid habit, a conical, radially fibrillose pileus; parallel to subparallel and cylindrical celled pileipellis; ellipsoid to oblong, smooth, thin walled and amyloid basidiospores. *Pseudoporpoloma* seems to be close to *Pseudotricholoma* (Singer) Sánchez-García & Matheny or *Tricholoma* (Fr.) Staude.

Before Vizzini et al. (2016), *Pseudoporpoloma pes-caprae* was known with different names such as *Agaricus pes-caprae* Fr., *Tricholoma pes-caprae* (Fr.) Quél., *Gyrophila pes-caprae* (Fr.) Quél., *G. aggregata* f. *pes-caprae* (Fr.) Quél., *Agaricus pes-caprae* var. *multiformis* (Schaeff.) Cooke, *Tricholoma pes-caprae* var. *multiforme* (Schaeff.) Massee, *Porpoloma pes-caprae* (Fr.) Singer, *P. pes-caprae* var. *multiforme* (Schaeff.) Bon or *Agaricus multiformis* Schaeff. A taxon with any of the above names was not collected from Turkey before the present study (Vizzini et al., 2016; Sesli et al., 2020).

The collecting site is a meadow surrounded by some woodland and bushes. Main trees and shrubs of the area are *Alnus glutinosa* (L.) Gaertn., *Carpinus betulus* L., *C. orientalis* Mill., *Corylus avellana* Thunb., *Picea orientalis* (L.) Peterm., *Quercus hartwissiana* Steven, *Rosa canina* L., *Rubus fruticosus* Pollich and *Smilax excelsa* L.

Pseudoporpoloma pes-caprae is regarded as endangered in red list of countries like Austria, Germany, Poland, Sweden and Switzerland (Vizzini et al., 2016). The fact that this species has not been recorded until today, unfortunately, supports that it may be in danger of extinction in Turkey as well. It is necessary to focus on studies that reveal the abundance levels of fungi in Turkey as well as in the world.

2. Materials and Method

Basidiocarps were collected from Trabzon, Maçka, Mataracı neighborhood on 05.11.2022 (Fig. 1). Main morphological properties, such as the shape, color, texture and size of the pileus and stipe were noted, and some photographs were taken in the field. Some fruiting bodies were collected, dried, cataloged and placed in the fungarium cabinet. During the microscopic studies, thin sections were taken from the pileus surface and lamellae of the exsiccatum. After treatment with ammonia solution, they were examined under a research microscope and photographed. In order to obtain basidiospores, a piece of lamella was cut and squeezed. The width and length of 30-40 of the displayed basidiospores were measured, their arithmetic average was taken, and the lower and upper

standard deviation limits were determined. Identifiation of the species was made according to Breitenbach and Kränzlin (1991), Knudsen and Vesterholt (2008) and Vizzini et al. (2016). The exsiccatum is kept at a personal fungarium in Trabzon University, Fatih Faculty of Education, Biology department, Trabzon, Turkey.

3. Results

Tricholomataceae

***Pseudoporpoloma pes-caprae* (Fr.) Vizzini & Consiglio, in Vizzini, Consiglio, Ercole & Setti, Phytotaxa 243(3): 274 (2016)** (Fig.1)

Pileus broadly conical to bell shaped, convex, umbonate, extremely fragile, 40–85 mm; margin uplifted, lobed, often splitting with age; surface dry, radially fibrillose, greyish-brown to yellowish-ochre, darker at the centre and paler towards the margin. Lamellae ventricose, white, smoke color, greyish or cream, crowded to subdistant, emarginate or almost free, intervenose. Stipe cylindric, tapering towards the base, longitudinally fibrillose, solid, whitish, smoke color, 30-85 × 5-15 mm, slightly yellowing towards the base, with a whitish ring-zone in young basidiomes. Context whitish and mild. Smell and taste farinaceous. Spore print whitish.

Basidia slenderly clavate, 25-40 × 6.5-8.5 µm, usually tetraspored and sterigmata up to 5 µm long. Hymenophoral trama, hyaline, made up of regular to subregular hyphae up to 15 µm wide. Basidiospores subglobose to ellipsoid, smooth, hyaline, 6-8(–8.5) × 4-5(–6.5) µm, thin-walled, amyloid, usually with drops; hilar appendix long and prominent. Marginal cells rare, often basidia-like, thin-walled, versiform, flexuous, mostly bent and 20-31 × 5-8 µm. Pileipellis is a cutis of parallel to subparallel, cylindrical, variously interwoven, somewhat gelatinized, 2-6 µm wide hyphae. Subpellis consists of vesicular to largely elliptic hyphae. Clamp-connections present at all tissues.

Specimens examined: Türkiye, Trabzon, Maçka, Mataracı neighborhood, 40°50'59.28"N / 39°37'39.37"E, meadow, in groups, 05.11.2022, E. Sesli 4602.

4. Discussions

Turkish *Pseudoporpoloma pes-caprae* collection is characterized with conical to bell-shaped, convex, umbonate, fibrillose, greyish or ocher-brown pileus; whitish, cream or pale grey, emarginate adnate to nearly free lamellae; cylindrical, solid, longitudinally fibrillose and whitish stipe; subglobose to ellipsoid, smooth, hyaline and about 6-8 × 4-6 µm sized basidiospores.

Before the present study the genus *Porpoloma* Singer or *Pseudoporpoloma* have not been reported from Turkey and *Pseudoporpoloma pes-caprae* is the first record of this genus in Turkey. *Pseudoporpoloma* is a monoytpic genus and the old name *Porpoloma* has included about 25 records to date in the world (Index Fungorum, 2023). The new record is close to some European *Porpoloma* species such

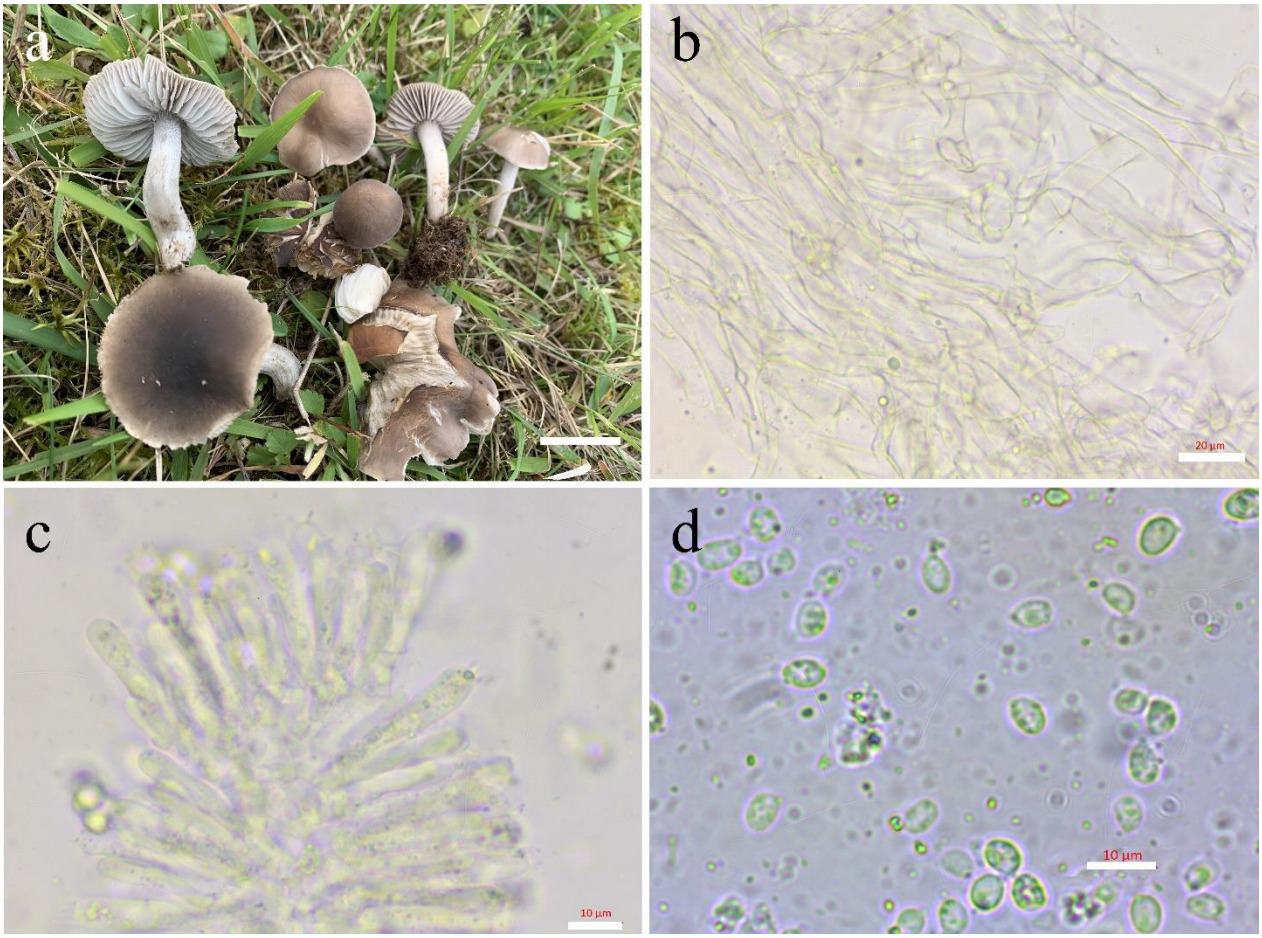

Figure 1. *Pseudoporpoloma pes-caprae*: a- Basidiocarps, b- Pileipellis, c- Basidia, d-Basidiospores (Scale bars: a: 40 mm, b: 20 µm, c and d: 10 µm)

as *P. spinulosum* (Kühner & Romagn.) Singer and *P. metapodium* (Fr.) Singer. *Porpoloma spinulosum* differs from *Pseudoporpoloma pes-caprae* with strongly yellowing stipe, aromatic smell, larger (40-120 mm), viscid and dark greyish brown pileus; crowded, cream colored or pale yellowish lamellae; smaller (4-6 × 3-4 µm) basidiospores and longer (25-50 µm) marginal cells. Another close but different species, *P. metapodium* has slightly reddening, pale to dark greyish brown, larger (40-100 mm) pileus; brownish grey lamellae, pale brownish grey stipe and narrow (3-4 µm) basidiospores (Breitenbach and Kränzlin, 1991; Knudsen and Vesterholt, 2008; Vizzini et al., 2016).

Conflict of Interest

There is no conflict of interest with any institution or person.

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Status of onion production in Türkiye and in the world, effects of abiotic and biotic stress factors

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Türkiye' de ve dünyada soğan üretiminin durumu, abiyotik ve biyotik stres faktörlerinin etkileri

Abstract: Onion plant is an indispensable additive for meals in the world and in our country. It is an important strategic agricultural product containing phytochemicals effective in the treatment of various diseases, as a medicinal and aromatic plant, as well as for consumption as food for humans. In the light of the statistical information examined, it is seen that there are changes in the supply of the onion plant to the market from year to year. The reasons for this seem to be annual land planning, input prices and human factors effective during production, as well as the damage rates of abiotic and biotic factors. In this study, the status of onion cultivation in Türkiye and in the world and the effects of abiotic and biotic factors encountered in cultivation are explained. In order to prevent fluctuations in supply to and prices in the market, it should be at the forefront of annual product planning, and producers should focus on raising awareness and training activities for growing healthy onions with high tolerance to diseases and pests.

Key words: Onion, production, disease, pests

Özet: Soğan bitkisi dünyada ve yurdumuzda yemeklerin vazgeçilmez katkı maddesidir. İnsanlar için besin olarak tüketiminin yanında tıbbi ve aromatik bitki olarak da çeşitli hastalıkların tedavisinde etkili fitokimyasalları içeren önemli stratejik bir tarım ürünüdür. İncelenen istatistiksel bilgiler ışında soğan bitkisinin yıldan yıla piyasaya arzında değişimler olduğu görülmektedir. Bunun nedenleri olarak yıllık arazi planlaması, girdi maliyetleri ve yetiştiricilik sırasında etkili olan beşeri faktörlerin yanında abiyotik ve biyotik etmenlerin zarar oranlarının etkili olduğu görülmektedir. Çalışmada yurdumuzda ve dünyada soğan yetiştiriciliğinin durumu ve yetiştiricilikte karşılaşılan abiyotik ve biyotik etmenlerin etkileri açıklanmıştır. Piyasaya arzda ve fiyatlarda dalgalanmaların yaşanmasına mahal vermemek için yıllık ürün planlamasında ön sırada yer alması ve hastalık ve zararlılara karşı toleransı yüksek sağlıklı soğan yetiştiriciliği için üreticilerin bilinçlendirilmesi ve eğitim faaliyetlerine ağırlık verilmesi gerekmektedir.

Anahtar Kelimeler: Soğan, üretim, hastalık, zararlılar

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1. Introduction

Onion is a plant species in the *Alliaceae* family (Brewster, 1994; Vural et al., 2000). Taxonomic name is *Allium cepa* L. (GBIF, 2023). Although the onion is a two-year plant species, the varieties that produce the bulb from the seed have been developed through breeding programs. Wild species of onion produce a smallbulb from seed in the first year, main bulb is produced in the second year and it has the potential to produce seeds by forming flowers. The onion consists of the root and the bulb the 2-5 layers of skin that surrounds and protects this bulb, remaining under the ground and the leaves that remain above the ground and have a green color (Sekara et al., 2017).

In addition to its consumption as a dry onion, the onion plant is also used as a green onion in salads and as a spice by being dried and powdered. It takes its importance in human nutrition from its taste and flavor characteristics. It is valuable due to the phytochemical content (Kumar et al., 2022). The onion plant has a worldwide importance (Brewster, 1994).

It is possible to classify the onion plant in several different ways. It is possible to make the first of these classifications as those that show maturation depending on the day length of the climate demands. This classification is as early varieties, mid-early and late varieties (Beşirli et al., 2021). Another classification is as summer and winter onions. Summer ones; has thick flesh, juicy, light color, long shape, coarse and loose structure. Due to its non-bitter taste, it is suitable for table use. It can not be stored for long time. Winter onions, on the other hand, have a tight structure. The color is dark and has a bitter taste. Unlike summer onion varieties, they could be stored for long time (Beşirli et al., 2021).

A suitable temperature and day length are important factors for growing the onion plant. In addition to these, although it can be grown in arid environment, abundant rainfall in spring is the most important factor affecting onion yield. Onion plants generally require a hot and dry environment during the day and a cool environment at night. During the growing season, there is a temperature demand between 12-30 °C. For seed germination, the soil temperature at the

time of sowing should be 10-13 °C. Increasing temperatures from the head-tie period to the harvest time are important for the development of the onion plant. In order to determine the harvest time, it is sufficient for the leaves to dry and fall and the color of the bark surrounding the bulbs to be darkened (Sekara et al., 2017).

Another important factor in onion cultivation is soil structure. Onions are grown in loose, water-holding capacity is medium, deep and soft enough to allow root and bulb development, easily workable, fertile and humus, loamy and light clay soils. The pH requirement for onions, which is sensitive to high acidity is between 6-6.5 (Beşirli et al., 2021).

Onions that are harvested and sold in a year are the varieties that produced directly from the seed, but mostly the production is made with shallots. However, by sowing directly with seeds, shallots are obtained in the first year and onions obtained by planting onions from these pinches in the second year are sown in the third year and harvested as seeds. Obtaining seeds again from an onion seed is at the end of three years.

Sowing frequency should be 25-30 cm between rows and 8-12 cm on the row in onions produced from seeds. The amount of seeds to be used per decare can vary between 0.5-1 kg. Before sowing, the soil should be plowed at a depth of 30 cm and sowing should be done while soil is in a moist. If the necessary conditions are met, seed germination will take place in 2-3 weeks (Beşirli et al., 2021).

The second option in onion production is from shallots with a diameter of 1-2 cm. For the planting of shallots, the distance between rows should be 25-30 cm and the distance on the rows should be 5-7 cm. 25-30 kg shallots are calculated per decare (Beşirli et al., 2021).

In the cultivation of onions, maintenance and fertilization are important issues that affect yield and quality, as in other vegetables. In order to remove weeds for maintenance, hoeing can be done two or three times, or the soil can be loosened by plowing between rows with the help of a tractor. Thus, both the weed removal process is carried out and the growth of onion bulbs in the soil is facilitated.

Although the fertilizer requirement may vary according to the soil structure and the amount of elemental matter, 8-10 kg/da of nitrogen, 8-10 kg/da of phosphorus and 12-15 kg/da of potassium are recommended for average yield (Anonymous, 2023b).

Onion can be grown without the need for irrigation in places where the precipitation amount is above 400 mm, but once a week irrigation is recommended until the head tying period in arid regions. Although the water demand depends on the climatic features, the soil structure is also one of the factors affecting irrigation.

Considering the necessary conditions for onion cultivation, it is seen that onion cultivation is carried out in many countries around the world.

2. Materials and Method

In the study, the status of onion cultivation in Türkiye and in the world, the description of abiotic and biotic factors that cause problems in onion cultivation, their symptoms on plants and control practices have been revealed by literature search.

3. Results

3.1. Onion production in the world

Onion cultivation is carried out in many areas in the world and it is made more than many green vegetables due to its consumption throughout the world. According to the United Nations Food and Agriculture Organization, onions are the third most produced product after tomatoes and potatoes. When the data in some years are compared, it is seen that there are years when more onion than potatoes are grown and the product is obtained (FAO, 2023).

Again, according to FAO data, 49,961,471.15 tons of onions were produced in an area of 2,915.706 hectares in 2000. This data increased to 79,142,982.25 tons from 4,214,126 ha by 2010. Considering that this increase is due to the increasing world population, it can be considered normal. However, these numerical data reached 104,563,843.11 t in 5,530,475 ha in 2020 and finally 106,592,088.85 t in 5,778,769 ha in 2021 (FAO, 2023). When these data are evaluated, it is seen that the cultivation area doubled in the ten-year period after 2000 and a product that has almost doubled again. However, in the ten-year period after 2010, it is seen that the increase in the amount of product obtained with the increase in the cultivation area is not sufficient (TÜİK, 2023). The reason for this could be effect of human and environmental factors.

The onion production by regions around the world, it is seen that the Asian region has a share of 65.1%. The Asian region is followed by Africa with 11.7%, America with 11.6% and finally Europe with 11.3% (FAO, 2023).

Considering the production averages for the years 2000-2021, China took the first place in onion production with 20.925.874.48 tons. India follows this ranking with 15,334,316.82 tons. America ranks third with 3,352,171.32 tons, and Türkiye ranks fourth with 2,013,635.91 tons onion production. This is followed by Egypt, Iran, Pakistan, Sudan, Russia and Brazil (FAO, 2023).

3.2. Onion production in Türkiye

Totally, 99,836 hectares were planted and 2,200,000 tons of onions were obtained in 2000. It is seen that these data have decreased by 2010. As a matter of fact, 1,900,000 tons of onion production was realized from the cultivation area, which decreased to 62,688 ha. Looking at the data in 2020 and 2021, it is seen that 2,280,000 t and 2,500,000 t of products were obtained in 68,491 ha and 69,895 ha, respectively (TÜİK, 2023).

When the data on the decrease in the cultivation areas and the amount of product obtained are evaluated, it is seen that there are fluctuating values in the production amounts despite the production in less areas.

Onion is produced in almost every province in Türkiye. Production in many provinces is offered to the domestic market. However, onions produced in large areas are sent to the provinces with less production in the Country or to foreign markets.

According to TÜİK data, at the years of 2010- 2021, provinces with the highest onion production are Ankara,

Amasya, Adana, Çorum, Hatay, Eskişehir, Konya, Tokat and Bursa (TÜİK, 2023).

Onion planting was done on 155,309 ha and 165,767 ha in Ankara in 2020 and 2021, respectively. From these areas, 567,788 t onions were harvested in 2020 and 835,269 t onions in 2021. In Amasya, it is seen that the onion planting, which was done on 89,290 ha in 2010, decreased to 81,534 ha in 2020 and to 70,387 ha in 2021. Due to the decrease in cultivation areas, it is observed that the amount of onions harvested also decreases. Onion production amount, which was approximately 312 thousand t in two thousand and ten years, decreased to 286,078 t in 2021 (TÜİK, 2023).

Considering the TÜİK data, it is seen that there is a decrease in the cultivation area and harvested product amount in onion production in Adana, Bursa and Tokat in 2020 and 2021 compared to previous years. When the TÜİK data is examined, it is seen that there is an increase in the onion cultivation area and production amount in Konya and Çorum (TÜİK, 2023).

The onion cultivation area in Konya, which was approximately 7,000 ha in 2010, increased to over 27,000 ha in 2020. This increase in cultivation areas was reflected in the amount of production and over 110 thousand tons of onions were harvested in 2020 and 2021 (TÜİK, 2023).

In Çorum, the cultivation area, which was approximately 31,000 ha in 2010, increased to 105,739 ha in 2021. The amount of production increased in direct proportion to the cultivation area and the amount of product, which was 73,673 t in 2010, reached approximately 300,000 t in 2021 (TÜİK, 2023).

Among the provinces where there is an increase in onion production, also in Eskişehir. There are fluctuations in onion production in Adana and Hatay provinces. When compared the TÜİK data of the years; 2020 and 2021, it is seen that there is a decrease. Despite the decrease in cultivation area and production amount, approximately 200,000 tons of onions were produced on 61 thousand hectares in Hatay in 2020. By 2021, these data have decreased to 167,653 tons per 50 thousand ha (TÜİK, 2023).

Vegetables grown for root and bulb are 3.874.902 tons and 3.860.298 tons, respectively in Türkiye in 2020 and 2021. It is seen that the largest production share of the vegetables grown for their roots and bulbs is the onion. The most produced vegetable is tomato with 13.000.000 tons in 2020 and 2021. Watermelon comes in the second place with the production amount of the two years, approximately 3.500.000 tons. Onion production comes in third place (TÜİK, 2023).

Onion production is an important food in the world as well as in Türkiye. This importance is also reflected in the amount of production, and it is among the top five vegetables produced the most. The fact that it is in the third place in our country brings this importance into consideration.

3.3. Challenges encountered in onion production

The amount of onion production increases parallel to increase in population. However, in some years, it is seen that its production has decreased. In some years, although the cultivation area is large, the production amount is below the expected amount. The factors affecting the yield can be caused by climatic reasons as well as biotic stresses.

Although the onion yield in Türkiye is above the world average of 32 tons/ha and 20 tons/ha, there are decreases in production over the years and problems occur in meeting the demand. As with other vegetables and fruits, there are reasons that affect onion production and yield. It is possible to classify these causes as abiotic and biotic.

3.3.1. Abiotic factors

Abiotic reasons can be listed as climate and soil characteristics, soil temperature and moisture at the time of germination, sowing method and time, harvest time, storage conditions of shallots and onions after harvest to be marketed.

Decreases in spring and summer rains due to global warming, which ranks first among the changes in climatic conditions, have led to decreases in irrigation waters (Çaltı and Somuncu, 2019). For this reason, people have turned to the production of products that have low water demand and will be affected at a minimum level by the low rainfall. Again, since the spring rains are less at the seed sowing times, they will delay the seed germination, affect the emergence and development stages and reduce the onion yield. The opposite is also possible. Too much rainfall at the time of sowing may cause the seeds or shallots to rot.

Sowing time and harvest time are among the factors affecting yield and product quality. As a matter of fact, the early sowing time may affect the first shoots that are subject to spring frosts. Late planting time may cause the onion head size to be insufficient. The early harvest time may affect the winter hardiness, as they are not fully mature, they may be crushed during loading. These situations will reduce the quality of the onion and therefore its market value. If post-harvest storage conditions are not met, shallots to be used as seeds may rot and may cause a slight decrease in the next planting amount. If the necessary conditions are not taken into account in the storage and transfer of onions to be put on the market, the onions may sprout and rot may occur. This will again reduce the market value (Beşirli et al., 2021).

It is possible to add the method of sowing to abiotic constraints. That is, shallots thrown deep during planting may cause late emergence, while onions planted close to the surface may dry out by not benefiting from soil moisture. These reasons are the main factors affecting onion yield and market quality in general.

3.3.2.Biotic factors

In addition to the abiotic factors that affect onion production, there are also biotic factors that have a great impact. Pests are the leading biotic factors. There are many diseases and pests that cause yield loss in onion production. The biotic factors that cause economic losses in onion production in our country; diseases and pests such as white rot (*Sclerotium cepivorum*), septoria (*Septoria apiicola*, *Septoria licopersici*), gray mold (*Botrytis cinerea*), onion downy mildew (*Peronospora destructor*), onion smut (*Urocystis cepula*), onion psyllid (*Bactericera tremblay*), onion fly (*Delia antiqua*), thrips (*Thrips tabaci* and *Frankliniella occidentalis*), leek moth (*Aceolepiopsis assectella*), wireworm (*Agriotes* spp.), leaf gallery flies

(*Liriomyza trifolii, L. bryoniae, L.huidobrensis, Phytomyza horticola*) and stem and bulb nematode (*Dityenchus dipsaci*) (EPPO, 2023). Biotic factors is presented in the form of the biological structure of the pest, damage symptoms and control methods. Weeds are also significant biotic constraints in onion production.

3.3.2.1. White rot diseases (*Sclerotium cepivorum*)

Sclerotium cepivorum, which is found in the fungal kingdom, is in the *Sclerotiniaceae* family. It causes economic losses in plants such as onions (*Allium cepa* L.), garlic (*Allium sativum* L.), leeks (*Allium ampeloprassum* L. (syn. *Allium porrum*) in the *Alliaceae* family (Anonymous, 2017).

Sclerotia, the winter spores of the fungus, become free in rotten plant tissue. They can remain dormant in the soil for many years. On the plant, they can remain dormant in a free state for several weeks. Volatile and water-soluble substances in host plants stimulate the germination of these sclerotia. These substances are especially found in *Allium* species. For the germination of sclerotia, the ambient temperature must be 9-21°C (Schwartz and Mohan, 2016; Anonymous, 2017). The wide temperature range is an indicator of the development of the pest and the extent of the damage. At the required temperatures, mycelium formation begins at several points and infects the roots of the host plant. Special cells that develop to infect the host plant in parasitic fungi are called apppressorium. With the formation of this cell, the hyphae that develop and spread on the surface of the roots and within the tissues can grow to reach the roots of neighboring plants. Sclerotia that migrate to the soil after all plant tissue has rotted remain dormant until new host plants are planted (Anonymous, 2017).

Its symptoms in the plant appear when the roots of the plant begin to develop. If the fungus infects the plant early, the plant rots and dies before it can develop. If the plant is infected during the late developmental stage, it is seen that the bulb is covered with a white cover around it. It is possible to see 0.35-0.50 mm sized sclerotia on the white fungal cover. One sclerot can infect about 30 adjacent plants. Contamination from underground can continue exponentially and drying occurs on the rows. If the onions taken from the diseased field are not kept at appropriate temperatures, the development of sclerots continues in the warehouses and rots are seen in the stored products (Anonymous, 2017).

Monitoring the disease throughout the growing season is important in preventing its spread and increasing yield. For this reason, as the temperatures begin to be suitable for the development of sclerotia, soil samples and onion samples should be collected with appropriate instructions, investigations should be made, and necessary precautions should be taken with the detection of diseased plants. Disease spread can be reduced by removing infected plants from the soil if necessary.

The disease is transmitted to the plant from its roots and from the sclerotids in the stem (CABI, 2015). Irrigation water, tools and equipment and production material are effective in the spread of the disease factor (EPPO, 2015a,b). Clean equipment, clean tools and equipment should be used to reduce the spread. Tools and equipment should be thoroughly cleaned after processing on contaminated soil.

Since the fungus distributed worldwide, its economic impact is also great. Although the disease is seen in a few plants at first, with the rapid spread of sclerotia, losses in plants are great in the following years in areas where Allium species are cultivated. Sclerotia, which is the causative agent of the disease, causes greater damage because it multiplies and spreads more and faster under 24°C. For this reason, it should be stored at appropriate temperatures and conditions.

In the following years, planting of *Allium* species should be avoided in contaminated areas, and rotation should be made for at least 5 years. Care should be taken to ensure that *Allium* species such as onions are clean. There is no registred formulation in Türkiye that can be used to combat this pest. For this reason, cultural and physical measures should be used.

3.3.2.2. Septoria leaf spot disease (*Septoria apiicola, Septoria licopersici*)

Septoria leaf spot disease is a important fungal disease that includes two species of the genus *Septoria*, belonging to the *Mycosphaerellaceae* family, and causes significant damage to cultivated plants.

Symptoms of the disease are in the form of brown spots on the leaves and petioles. The typical sign of the disease is that the spots increase in size up to 3 mm in diameter and their centers are light brown. When the severity of the disease is high, there is a significant decrease in yield. The severity of the disease is closely affected by the precipitation regimes (Anonymous, 2010).

This fungus causes disease on tomatoes, lettuce, parsley, zucchini, celery and onions. The use of clean seeds is at the forefront of the cultural methods recommended in the fight against this disease. Apart from this, cleaning of equipment and vehicles, destruction of contaminated plants and seeds, and alternation in contaminated soil are other recommended methods of management.

3.3.2.3. Gray mold disease (*Botrytis cinerea*)

Gray mold (*Botryotinia fuckeliana / Botrytis cinerea*) is a common fungus. It can survive in the bulbs of plants such as onions or as sclerotia in the soil. The resistance of sclerotia to cold and drought causes these diseases to persist in the soil for a long time.

It takes the nutrients it needs for the germination and development of spores from the cell sap of the plants under suitable humidity and temperature conditions on the plant tissues. It develops necrotrophically. It completes its development in a few days and goes into sporulation. The most suitable environmental conditions for disease development are 20-25 °C temperature and 95-98% humidity. In unsuitable environmental conditions, spores can maintain their vitality for a few days (Anonymous, 2023a).

In order to combat this disease factor, proper cultivation and maintenance of the plant is of great importance. In order to prevent the infection of onion bulbs in storage conditions, ventilation and controlled temperature and humidity conditions should be provided (Schwartz and Mohan, 2016).

3.3.2.4. Downy mildew disease (*Peronospora destructor*)

It is a type of water mold belonging to the *Peronosporaceae* family. It is especially harmful to *Allium* genus plants. The most damaging plant is the onion. It is known that the seeds are not contaminated, but it can preserve its existence as an oospore in the soil for many years (Anonymous, 2021). The spores of the disease agent are 7-18 µm in size, non-divided. Spores are violet in color (Kaynaş, 2010).

The disease is seen in onion plants by causing chlorotic lesions on their leaves. The color changes in the green leaves are in the form of pitting in the areas where the lesions are, especially in the bottom and middle areas. The lesions are covered with a layer of fungus and their middle part becomes white. The spots coalesce and the leaf dries up. Onion heads wrinkle and shoot. When the disease occurs in the early developmental stages of the plant, it is devastating (Schwartz and Mohan, 2016).

In order to combat the disease, the diseased onions seen in the field should be removed from the soil and destroyed. Diseased plant parts that remain after the onions are harvested should also be destroyed. In addition, it is not possible to store it in the warehouse for a long time. Oospores spread easily through the air. For this reason, diseased plants should not be contacted with clean areas and clean plants. Resistant varieties should be planted in disease-infected lands. In onion cultivation, especially open to the wind and well-drained lands should be preferred. Since the irrigation method is also effective in the spread of the disease factor, especially the sprinkler irrigation method should be avoided in contaminated lands (Beşirli et al., 2021).

Most of the fungicides are registered for onion is for downy mildew disease includes active gradients of %80 Thiram, %50 Captan, %60 Mancozeb + %5 Mandipropamid, %80 Mancozeb, %65 Dodine, %80 Fosetyl-Al, %80 sulfur, 160 g/l Cyazofamid, 190 g/l cupper sulfate + 35 g/l Cymoxanil, 200 g/l Azoxystrobin + 125 g/l Difenoconazole, 300 g/l Ametoctradin + 225 g/l Dimethomorph, 300 g/L Phosphorous Acid (mono ve di-potasyum tuzları) + 75 g/L Ametoctradin, 375 g/l Fluazinam + 150 g/l Azoxystrobin, 500 g/l Fluazinam and 625 g/ Propamocarb-HCI + 62,5 g/l Fluopicolide (Anonymous, 2023c).

3.3.2.5. Onion smut disease (*Urocystis cepula*)

Urocystis cepula, which belongs to the *Urocystidaceae* family, known as smut fungi of the *Basidiomycota* phylum, is the causative agent of onion smut disease. The spores of the fungus are brown, have thick walls and are elliptical in size of 12-15 µm (Kaynaş, 2010).

The agent causes more losses in shallot cultivation. The bulb develops in straight lines on the leaves and bark. The spores of the fungus fill in the lines over time. The emergence of the disease in the early stages of the development of the plant causes curls and developmental delays in the plant (Schwartz and Mohan, 2016).

At the beginning of the cultural measures recommended in the fight against the disease is the application of crop rotation for 8-10 years. Other important measures are the elemination of contaminated plant residues after harvest. The use of clean production material in production is also very beneficial in terms of preventing contamination to the field. Formaldehyde 40% and Thiram 80% are the drugs recommended for chemical control. Administering the disease at recommended doses as soon as it first appears will stop the spread (Anonymous, 2010; Beşirli et al., 2021).

3.3.2.6. Onion psyllid (*Bactericera tremblay*)

Onion psyllid, a member of the *Hemiptera* order, is 2-3 mm long and black in color. Larvae are 1-3 mm in length and yellow in color. The distinguishing features of the insect are the yellow spots on the thorax, the transparent appearance of its wings and prominent veins. The development of the insect continues throughout the summer, it has been determined that they complete their development in 18-25 days. They lay their eggs between the leaves and on the body of the plant and complete their development in this region (Anonymous, 2010).

Adults and larvae cause damage by feeding on onion leaves. Due to the growth hormone-like substances they secrete during their feeding, abnormal development and spiral curling occur in the plant. Therefore, it reduces the market quality of onions offered to the market as green. (Anonymous, 2010).

Cultural methods play an important role in the fight against this pest. Suggested cultural methods can be listed as cleaning the weeds in the field, doing the onion planting and planting operations as early as possible, good fertilization, irrigation and hoeing in order for the plant to develop well and to be affected by the pest at a minimum level. %80 Thiram is registered active gradient for the disaese (Anonymous, 2023b).

3.3.2.7. Onion fly (*Delia antiqua*)

Belonging to the *Anthomyiidae* family, this species is similar to small blackflies, but most are dull gray. It is a cosmopolitan pest. The onion fly has an ash gray body and resembles a house fly. The male has a longitudinal stripe on the abdomen that the female does not. The legs are black, the wings are transparent, and the compound eyes are brown. The eggs are white and elongated and are laid in groups on the shoots, leaves and bulbs of the host plants and in the nearby soil. The larvae are white and cylindrical and hatch in 3 to 8 days. Each batch of larvae tends to stay together and collectively form large burrows in the bulbs. Sometimes more than 50 maggots from eggs laid by several females can feed on one onion. The larvae moult three times, feed for about 20 days and grow to a length of about 1.0 cm. The pupa is brown, ringed, oval and 7 mm (0.28 in) long. Pupation occurs in the soil with the pupal phase from the spring belt lasting two or three weeks. Late generation pupae spend the winter in the soil (Anonymous, 2009a,b).

Adults begin to appear after mid-March and early April, depending on climatic conditions. Adult insects lay their eggs in the folds of the plant and into the soil. Larvae enter the plant and cause damage and secondary bacterial infections. As a result, regression and decay in plant growth occur (Schwartz and Mohan, 2016).

As a cultural control, it is recommended to plant quite late than the first generation time of the pest. The cultural struggle that can be applied on contaminated lands is to make deep plowing and to prefer chemical fertilizers instead of farm manure (Anonymous, 2010). What is recommended as a chemical control method is soil spraying before sowing, seed spraying during planting, and green

parts spraying against adult individuals during plant development stages. It is sufficient to see 2-3 adult individuals per 100 plants in order to be able to spray green parts (Anonymous, 2010).

3.3.2.8. Thrips (*Thrips tabaci* and *Frankliniella occidentalis*)

Thrips tabaci is a worldwide pest of the order *Thysanoptera*. Adult insects are 0.8-0.9 mm long and yellowish in color. It has fringe wings, which is the typical feature of this set. It has been determined that the development of the insect on the plant is completed in about 2 weeks and they give 4-6 generations in a plant development season (Anonymous, 2022b). The adults of *Frankliniella occidentalis* are between 1-1.4 mm (Anonymous, 2020b).

The form of damage caused by these pests on the plant; Adults and nymphs feed on plant sap on leaves, stems and fruits of plants. The leaves on which it feeds turn whitish or silver after a while. In arid areas, the damage is even greater. They carry virus diseases and infect healthy plants (Anonymous, 2010).

Among the cultural measures recommended in the fight against these insects, the most important one is the destruction of plant residues infested with the larvae of the pest. Soil tillage and weeds should be struggled. Among the recommended chemical control methods, the recommended drugs are Pirimicarb 50% and Deltamethrin 25 g/l (Anonymous, 2010).

3.3.2.9. Leek moth (*Acrolepiopsis assectella*)

Acrolepiopsis assectella is a species of moth in the family *Acrolepiidae* from the class *Insecta*. Its variegated brown and white wings are around 15-16 mm long. The body length of the adult is usually 1 cm. The head of the larva is brown and the body is yellowish white and is around 1 cm (Anonymous, 2010).

The damage starts with the emergence of the plants in April and continues with 3 offspring throughout a year until October. Female moths usually lay their eggs near the soil and on the underside of leaves. Eggs hatch in 4-6 days in spring and 8-11 days in autumn. As soon as the larvae hatch, they begin to gnaw the leaf. In leeks they prefer to eat the youngest leaves, but in onions they prefer to feed on the hollow parts of the leaves and form a hole by going up to the middle part of the plant. These pests usually stay away from the flower parts of plant because the flowers contain saponin which inhibits the growth of larvae. They go through five larval stages. Larval periods consist of 11-23 day processes. If the pupation process will be in an empty field, it can spend the winter in this way (Anonymous, 2017).

There is no chemical method or medicine recommended for the fight against leek moth. However, it can be recommended to plant together with insect-repellent plants, which is one of the cultural methods. Among the insect traps, pheromone traps can play a role in reducing the population level of this pest.

3.3.2.10. Wireworm (*Agriotes* spp.)

It is one of the harmful insect species from the members of the *Elateridae* family of the Insecta class. The main damage to plants are made bythe larvae of these insects. The female beetle produces up to 150 eggs and lays them in the soil at a depth of 10-15 cm, singly or in clusters of 30-40. It takes a long period of 1-6 years for the larva to become adult (Anonymous, 2020a).

In the spring, the larvae move to the upper layers of the soil and feed by cutting the plant roots that develop in this region. They also cause injuries by getting inside the onion bulbs. Secondary bacterial and fungal diseases develop from these parts and cause the bulbs to rot (Anonymous, 2010).

For the control of the pest, tilling the soil with a plow after the harvest will cause the larvae feeding in the upper soil layer to stay on the surface and die, which is a very effective cultural control practice. The criterion for chemical control is the presence of 6-15 larvae at a depth of 25 cm in the soil. Chemical control is applied with formulations such as Chlorpyrifos-ethyl 25% in the form of empty field spraying before sowing and planting (Anonymous, 2020a).

3.3.2.11. Onion leaf miner (*Liriomyza trifolii, L. bryoniae, L. huidobrensis, Phytomyza horticola*)

They are species in *Insecta* class, *Diptera* order, *Agromyzidae* family. The length of the adults is between 1.3-2.3 mm. Adults lay their eggs on onion leaves. Larvae that emerge from the eggs enter under the epidermis layer of the leaves and feed by opening galleries. The pupal stage of flies that develop holomethobol lasts 7-14 days at 20-30°C. It has been determined that onion leaf miner give 10 offspring and lay 400 eggs during plant development (Anonymous, 2022a).

During the feeding of females and larvae on leaves, symptoms such as deformities, yellowing and drying are observed (Schwartz and Mohan, 2016). There are damages in the form of growth retardation in plants and, most importantly, the decrease in market quality in green onion plants (Anonymous, 2010).

In the cultivation of green onions in the greenhouse, controlling the entrance and exit of the greenhouse in order to prevent the infection of insects and the control of weeds that will host the insects cause a significant decrease in the pest population. Mulching is also reported to be effective in killing pupae in the soil (Anonymous, 2022a). Chemical-impregnated sticky traps are also recommended for greenhouse cultivation (Anonymous, 2010).

3.3.2.12. Stem and bulb nematode (*Ditylenchus dipsaci*)

It is a member of the *Anguinidae* family in the class *Tylenchoidea*, the majority of which are plant parasitic nematodes in the phylum *Nematoda*. It is in thread form and its body length is 1-1.5 mm and the body is transversely striated. Stem and bulb nematode is a soil-borne, motile endoparasitic nematode. It develops in the intercellular spaces in the cortex of the plant by infecting the part of the onion plant where the root and the bulb meet. It has been determined that it completes its development at 15 °Cin 20 days (Brzeski, 1991). A female produces 200-500 eggs. Nematodes form collective associations called nematode wool on infected onion bulbs at harvest time. Contamination can occur from soil as well as from contaminated production material (Yavuzaslanoglu et al., 2015). It has been determined that the stem and bulb nematode is common in onion growing areas in Türkiye (Yavuzaslanoglu et al., 2019).

The nematodes that enter the onion bulbs continue to develop and reproduce within the plant. Onion bulbs have a lower weight and soft structure than expected (Ecevit and Akyazı, 2010). Stem and bulb nematode causes shapeless curls in the leaves of onions and splits in the onion bulb. The development of infected seedlings regresses and rots (Yavuzaslanoglu et al., 2015).

Since the stem and bulb nematode is a nematode subject to quarantine, quarantine practices are carried out to prevent its spread. In addition, since the host spectrum is very wide, control methods should be applied to keep the population level below the economic damage threshold in order to maintain economic production in areas where it is contaminated. It has been noted that 3–4 years of rotation with non-host plants significantly reduces the nematode population (Hooper, 1984; Roberts and Grathead, 1986). Suggested cultural methods other than the rotation; Planting clean seeds in clean soil, using resistant varieties, cleaning the equipment used in contaminated land and preventing its transfer to clean soils, cleaning processes should not be in the direction of stream or irrigation water since there is a possibility of transportation with irrigation water. It was determined that onion cultivars had partial resistance to stem and bulb nematode. Varieties with low nematode growth from commercial onion varieties are recommended for cultivation in contaminated areas (Yavuzaslanoglu, 2019). Chemical drugs recommended in the fight against nematodes are applied in the form of empty field applications and in combination with solarization in greenhouse production (Anonymous, 2010).

3.3.2.13. Weeds

In order to increase the yield and quality obtained in onion cultivation, weed control is a group that can be included in biotic stresses as well as the problems mentioned above. It has been reported that there is 70% yield loss due to weeds, which is one of the important factors affecting onion yield (Kaya and Üremiş, 2020). A study conducted to determine weeds in onion fields in Hatay, one of our important onion production provinces in Türkiye, shows that weeds can affect production as much as other pests (Kaya and Üremiş, 2019).

By controlling weeds at the right time and in the right way, the damage of weeds to onion development and growth can be reduced, at least until the head is tied. These control methods include hoeing, interrow spreading and herbicide use (Roberts and Grathead, 1986).

4. Discussions

As a result, onion cultivation in the world and in Türkiye is greatly affected by natural and human factors. There are fluctuations in production and supply to the market from year to year. Planning production and abiotic and biotic factors that are effective during production have a significant effect on production amounts. The development of abiotic and biotic factors on the onion plant affects each other. Changes in precipitation regimes with the effect of global warming affect the biology of diseases and pests and increase the damage rates. In addition, drought stress increases crop losses by decreasing plant tolerance against other diseases and pests.

In order to maintain the regular supply of onion, which is an indispensable additive of meals and as a medicinal aromatic plant, to the market and to prevent price fluctuations, it should be at the forefront of annual product planning and training activities should be emphasized to raise awareness of producers for growing healthy onions with high tolerance to diseases and pests.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

First author prepared the manuscript, second author edited the manuscript.

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Composition of some trace elements in wheat plant and soil

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Buğday bitkisi ve toprağındaki bazı eser elementlerin bileşimi

Abstract: Zinc, copper, nickel, and manganese are essential nutrients for plants. However, excessive accumulation in the plant can lead to significant risks and problems in terms of human health after consumption. Also, the accumulation of chromium, cadmium and lead elements in plants can have a toxic effect on human health. This study aimed to determine the concentrations of copper (Cu), chromium (Cr), cadmium (Cd), nickel (Ni), manganese (Mn), zinc (Zn), and lead (Pb) trace elements in wheat plants and soil. Mean trace element levels in soil samples taken from the city center Mn 556.9 mg kg^{-1}, Ni 62.45 mg kg^{-1}, Cr 24.98 mg kg^{-1}, Zn 40.75 mg kg^{-1}, Cu 17.25 mg kg^{-1}, Pb 7.65 mg kg^{-1}, Cd as 1.63 mg kg^{-1} and the average trace element levels in soil samples taken from villages Mn 418.7 mg kg^{-1}, Zn 48.53 mg kg^{-1}, Ni 32.34 mg kg^{-1}, Cu 15.93 mg kg^{-1}, Cr 13.7 mg kg^{-1}, Cd 1.033 mg kg^{-1} was determined. Cd, Cr, and Pb concentrations were not detected in wheat samples. Average Cu (4.462 mg kg^{-1}), Mn (30.03 mg kg^{-1}), and Zn (20.39 mg kg^{-1}) concentrations in wheat samples were determined at lower levels compared to soil samples. In the process of transporting trace elements from the soil to the plant, even if the plants are grown under the same conditions, the trace element levels accumulated in the plant may differ.

Key words: Mineral content, wheat, soil, ICP-OES

Özet: Çinko, bakır, nikel ve mangan bitkiler için temel besin maddelerindendir. Ancak, bitkide aşırı birikmeleri tüketim sonrasında insan sağlığı açısından önemli risklere ve sorunlara yol açabilmektedir. Ayrıca, bitkilerde krom, kadmiyum ve kurşun elementlerinin birikmesi insan sağlığı üzerinde toksik etkiye sahip olabilir. Bu çalışmada, buğday bitkilerinde ve toprakta Cu, Cr, Cd, Ni, Mn, Zn, ve Pb eser elementlerinin konsantrasyonlarının belirlenmesi amaçlanmıştır. Şehir merkezinden alınan toprak örneklerinde ortalama eser element seviyeleri Mn 556.9 mg kg^{-1}, Ni 62.45 mg kg^{-1}, Cr 24.98 mg kg^{-1}, Zn 40.75 mg kg^{-1}, Cu 17.25 mg kg^{-1}, Pb 7.65 mg kg^{-1}, Cd 1.63 mg kg^{-1} olarak ve köylerden alınan toprak örneklerinde ortalama eser element seviyeleri Mn 418.7 mg kg^{-1}, Zn 48.53 mg kg^{-1}, Ni 32.34 mg kg^{-1}, Cu 15.93 mg kg^{-1}, Cr 13.7 mg kg^{-1}, Cd 1.033 mg kg^{-1} olarak belirlenmiştir. Buğday örneklerinde Cd, Cr ve Pb konsantrasyonları tespit edilmemiştir. Buğday örneklerindeki ortalama Cu (4.462 mg kg^{-1}), Mn (30.03 mg kg^{-1}) ve Zn (20.39 mg kg^{-1}) konsantrasyonları ise toprak örneklerine göre düşük seviyelerde belirlenmiştir. Topraktan bitkiye eser elementlerin taşınması sürecinde, bitkiler aynı koşullarda yetiştirilse bile bitkide biriken eser element seviyelerinde farklılık gösterebilmektedir.

Anahtar Kelimeler: Mineral içerik, buğday, toprak, ICP-OES

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1. Introduction

One of the most important economic sectors in the world, especially in developing nations, is agriculture, and agricultural practices have a direct impact on food safety (Tudi et al., 2021). Considering various soil pollutants, trace elements and heavy metals pose a serious danger to human and animal health due to their toxicity and persistence. While some trace elements like Cu, Zn, and Ni are necessary for the human body and plant growth, the accumulation of toxic elements like Cd, arsenic (As), Pb, and Cr is harmful to biological systems (Rana, 2008; Järup, 2003).

The bioaccumulation and persistence of trace elements in agricultural soil systems are becoming a serious global health and environmental problem (Wang et al., 2016). Trace and toxic elements accumulate in agricultural soils due to natural sources, urbanization, industrial activities, or atmospheric transport, and the products grown in these soils are affected by these trace elements. The accumulation of these trace elements has often been studied (Rezapour et al., 2019; Zhang et al., 2019).

Through the food chain, the movement and bioaccumulation of trace elements in soils and crops not only have a detrimental impact on the environment and the safety of food but also negatively affect the reproductive, nervous, and immune systems of people and other animals (Khan et al., 2019). For instance, continued consumption of little amounts of Cd can cause the metal to build up in the body, weakening bones or affecting kidney function (Wiggenhauser et al., 2016). Relevant research also revealed that endocrine disruption in both people and animals can be caused by a combination of trace elements, even at low concentrations (Ma et al., 2016).

Among the cereals, wheat is very popular in many countries in terms of being an important food source. Wheat accounts for approximately 30% of the world's grain production (Giraldo et al., 2019). Wheat is a member of the family *Triticeae* and belongs to the genus *Triticum* (Hussain et al., 2022). Like many other plants, wheat is vulnerable to trace elements. The wheat plant is an important source of various trace elements such as Zn, Cu, Mn, and Ni that are important to humans. The wheat plant can also contain various toxic elements like Cd, Pb, and Cr.

According to the findings, mercury and arsenic uptake was maximum for wheat grown in a petri dish. Due to cadmium, nickel, mercury, and copper adsorption on soil phases, the wheat grown in a pot had high levels of manganese and iron while the wheat produced in a petri dish had considerable quantities of nickel, copper, and cadmium. A decrease in the intake of trace elements was observed after the addition of liquid fertilizers in wheat, except for manganese (Stanisić Stojić et al., 2016).

Trace elements stress plants, which results in a range of reactions from germination to growth to metabolic reactions and, in the case of wheat, yield reductions. For instance, it has been demonstrated that Pb is incredibly detrimental to wheat, causing stunted growth and raised membrane permeability, which ultimately results in the production of ROS (Gang et al., 2013). The accumulation of ROS in plant tissues causes electrolyte leakage and threatens the integrity of cell membranes (Emamverdian et al., 2015). Higher lead concentrations during the *Eruca sativa* planting stage, the researchers found, decreased germination. The quantity of soluble protein rose along with the concentration of Pb (Faheed, 2005). As Pb concentration rose, so did the amount of soluble protein. The germination of seeds was hindered when silver (Ag) (10 mM) was applied, and it was completely prevented when the Ag concentrations were increased. High Zn concentrations promote seed germination, whereas Pb treatment prevents seed sprouting (Ashraf et al., 2008).

The bioavailability of trace elements in the soil is a process that needs to be monitored as they are not degraded by microorganisms, chemicals, or other means. Trace element levels in the soil can affect the growing conditions, photosynthesis, yield, and nutritional value of wheat (Rizwan et al., 2018). This process changes the quality of wheat. Wheat is one of the most important staple foods all over the world. Trace elements and their toxicity in crops, particularly wheat, have become a regular concern. This study aimed to determine the concentrations of Cd, Cu, Cr, Mn, Ni, Pb, and Zn trace elements in wheat plants and soil.

2. Materials and Method

2.1. Materials

The wheat and soil samples were collected from Karaman city center (No:1) and 4 different villages (Lale (No:2), Kızılyaka (No:3), Sudurağı (No:4), Yollarbası (No:5)). It is important to collect the samples to be analyzed. Soil samples were taken from the fields at a depth of 10-15 cm using a Cr-Ni spatula. Wheat samples were selected from ears that were ripe and ready to be harvested before harvest time. Soil and wheat samples were collected at the same place and at the same time. The samples were kept in transparent bags. Three separate samples were taken from each selected region.

Pre-drying was done by laying the collected wheat and soil samples in a dust-free environment in the open air. It was then ground into powder by grinding in a porcelain mortar. The powdered samples were sieved through 100 and 150 mesh sieves, respectively. The sieved samples were dried in an oven at 80-90 ^{o}C for 2 hours.

2.2. Wheat and soil sampling

1 g of wheat sample was put into the beaker (50 ml) and 20 ml of HNO_3 (w/w, 65%) was added. After waiting for 10 hours, 7 ml of $HClO_4$ (w/w, 70%) was added. It was heated slowly for 6-7 hours in a fume hood. The heating was cut off and cooled near the end of the acid. 7 ml of H_2O_2 (w/w, 30%) was added and heating continued until a clear liquid was obtained. The volume was made up to 10 ml with ultrapure water (Milli-Q Direct 8, Water Purification System, Merck) by filtration through the blue band filter paper and made ready for analysis (Karapınar et al., 2017; Kaya et al., 2017; Karapınar and Kılıçel, 2020).

1 g of soil sample was placed in the beaker (50 ml) and 16 ml of HNO_3/HCl (w/w, 1/3) was added. It was kept for 7 hours. After it was heated to dryness in a fume hood, 15 ml of 2 M HNO_3 was added and after waiting for 4 hours, it was filtered through a blue band filter paper and made ready for analysis by making it up to 20 ml with ultrapure water (Türkdoğan et al., 2003; Bermudez et al., 2012).

2.3. Analytical technique trace elements detection

Inductively Coupled Plasma Optic Emission Spectroscopy (ICP-OES) was used to analyze the samples at room temperature.

Merck provided the ultra-high purity reagents (certified >99.99%). To create working solutions, 1000 mg l^{-1} standard solutions (CRM, Merck) were diluted in 0.5% HNO_3. Working standards were used for calibration curves with a six-point range (including zero).

Accelerating Inductively Coupled Plasma Optic Emission Spectroscopy with Axially Observed Plasma (Agilent 720 Series, Santa Clara, ABD) was employed for this work (Table 1).

Table 1. Analytical conditions for trace elements analysis using ICP-OES

Apparatus	**Agilent Technologies 720**
RF forward power	1.0 kW
Plasma gas (Ar) flow	15 l min^{-1}
Auxiliary gas (Ar) flow	1.5 l min^{-1}
Nebulizer gas (Ar) flow	0.75 l min^{-1}
Replicates	3
Pump rate	2.0 ml min^{-1} (peristaltic pump)
Plasma position	Axial
Rinse time	15 sec
Total sample usage	2 ml
Torch	Standard one piece quartz axial
Spray chamber type	Glass cyclonic (single-pass)
Nebulizer type	Sea Spray

A specially created CCD detector is included with the Agilent 720, enabling true simultaneous measurements and complete wavelength coverage from 167 to 785 nm (Table 2). The two-dimensional image from the echelle lenses is ideal for the continuous angled arrays in the CCD detector. The thermally stabilized optical system has no moving parts, which provides excellent long-term stability.

The method of selecting the trace elements has been validated by repeated studies of certified reference material. For every six measurements, the specified components were examined in six blank control samples and certified standards. A computerized laboratory data management

system was utilized to perform calibration measurements on standards, control standards, and blank samples.

Table 2. The studied wavelength and LOD values of the elements

Elements	Studien wavelength (nm)	Detection limit (µg/l)
Cd	228.802	0.01
Cr	206.158	0.005
Cu	327.395	0.01
Mn	259.372	0.005
Ni	216.555	0.005
Pb	182.143	0.005
Zn	213.857	0.02

The data (mean, standard deviation) from all samples were assessed three times using IBM SPSS Statistics version 20 software and the Student *t*-test. The significance level was set as $p < 0.05$.

3. Results

The World Health Organization Expert Committee (WHO, 1996) examines trace elements, Pb and Cd potentially toxic elements, Co and Ni elements that may be necessary, Zn, Cu, and Cr basic elements in terms of their nutritional importance in humans.

Cd, Cr, Cu, Ni, Mn, Zn, and Pb trace elements concentrations in wheat and soil samples are given in Tables 3 and 4.

Trace element concentrations in soil samples taken from the city center were determined as Mn> Ni> Zn> Cr> Cu> Pb> Cd. Trace element concentrations in soil samples taken from villages were generally determined as Mn> Zn> Ni> Cu> Cr> Cd.

According to the reported reports, the average trace element levels in the surface soil of arable lands were determined as 20 mg kg^{-1} for Ni, 900 mg kg^{-1} for Mn, 70 mg kg^{-1} for Zn, 0.1 mg kg^{-1} for Cd, 55 mg kg^{-1} for Cu, 14 mg kg^{-1} for Pb, and 100 mg kg^{-1} for Cr (Kabata-Pendias and Mukherjee, 2007; Kabata-Pendias, 2011). According to previous reports, it was determined that Zn, Mn, Pb, Cu, and Cr in wheat soil were below the values given, but above the values given for Ni and Cd.

Although the concentration of Ni and Cd in soil samples was higher than in previous reports, the concentration of Cd and Ni in wheat samples could not be determined. Ni trace element was detected at low levels only in the samples taken from the city center. It is thought that the Ni concentration in the samples taken from the city center is due to urbanization and industrialization.

According to the international legislation on wheat grains and foodstuffs (FAO/WHO) and the Turkish food codex communiqué (28157/2011), the average concentrations of Mn, Cu, Zn, and Ni were below the permissible limits (Berg and Licht, 2002; Joint FAO, 2017).

In our study, the amounts of Pb and Cd in wheat were lower than in previous studies (Nan et al., 2002; Lavado et al., 2007; Chandra et al., 2009; Douay et al., 2008). Ni, Zn, and Cu concentrations in wheat samples are in agreement with previous studies (Nan et al., 2002; Lavado et al., 2007; Kirchmann et al., 2009; Singh et al., 2010).

Zn is one of the trace elements that are part of a few bioactive enzymes in plants. Many plant oxidases include copper, which can encourage redox reactions. Also, plants have a lot of copper in their chloroplasts, which helps the chlorophyll stay stable (Zhang et al., 2018). Trace element concentrations can vary between various crop tissues and between different locations of the same crop. Wheat's ability to bioabsorb and transfer elements from the soil to it is influenced by a variety of factors, including cation exchange capacity, soil pH, plant species, organic matter content, plant species, and age (Abdelhafez and Li, 2015). Based on their physiological mechanisms, many plants have an innate capacity to metabolize a variety of heavy metals (Ran et al., 2016).

In addition, values similar to Cu and Zn concentrations were observed in grain samples grown in an industrial town (Huang et al., 2008). Cr concentrations in wheat samples were lower than in India, China, and previous studies (Balaji et al., 2000; Al-Dayel and Al-Kahtani, 2002; Huang et al., 2008; Singh et al., 2010).

Table 3. Average concentrations ± SD of trace elements in soil (mg kg^{-1}) (SD: Standard Deviation)

Soil	Cd	Cu	Cr	Mn	Ni	Pb	Zn
1	1.63 ±0.08	17.25 ±1.23	24.98 ±1.78	556.9 ±23.3	62.45 ±3.54	7.65 ±0.9	40.75 ±3.12
2	1.11 ±0.05	28.45 ±2.56	12.67 ±0.13	560.7 ±22.4	27.56 ±1.45	*	65.01 ±5.45
3	0.83 ±0.02	14.70 ±1.67	12.34 ±0.56	356.9 ±18.1	31.67 ±1.34	*	42.37 ±3.55
4	0.87 ±0.07	11.52 ±0.89	17.23 ±1.01	468.5 ±20.2	35.89 ±2.02	*	57.70 ±6.67
5	1.32 ±0.13	9.061 ±1.12	12.56 ±0.78	288.7 ±17.1	34.23 ±2.78	*	29.03 ±2.03

*Not determined

Table 4. Average concentrations ± SD of trace elements in wheat (mg kg^{-1}) (SD: Standard Deviation)

Soil	Cd	Cu	Cr	Mn	Ni	Pb	Zn
1	*	1.15 ±0.33	*	15.91 ±2.33	2.44 ±0.54	*	7.85 ±1.11
2	*	2.65 ±0.26	*	23.71 ±2.41	*	*	15.01 ±1.44
3	*	4.56 ±0.88	*	35.51 ±4.12	*	*	12.33 ±1.05
4	*	3.87 ±0.65	*	46.42 ±2.54	*	*	17.71 ±1.47
5	*	10.08 ±1.34	*	28.62 ±1.54	*	*	49.03 ±2.56

*Not determined

Table 5. Average trace element concentrations in previous studies in wheat (W) and soil (S) samples (mg kg^{-1})

Soil	Cd	Cu	Cr	Mn	Ni	Pb	Zn	Reference
W	0.038	3.510	0.022	33.26	0.240	-	27.35	Kirchmann et al., 2009
W	0.097	9.023	41.72	87.20	5.956	13.76	13.49	Tudi et al., 2021
W	0.055	5.229	0.108	-	0.148	0.177	27.78	Huang et al., 2008
W	1.43	14.7	9.21	34.5	1.42	21	34.2	Setia et al., 2023
W	0.1	10.7	4.0	51.4	1.6	1.3	62.6	Özturk and Arici, 2021
W	0.05	25	0.6	100	5.0	6.0	70	Kacar and Inal, 2008
W	-	4.462	-	30.03	2.44	-	20.39	This study
S	0.732	22.65	44.48	641.8	25.95	33.74	69.30	Tudi et al., 2021
S	-	7.94	-	395	8.38	10	-	Bermudez et al., 2012
S	0.16	26	67.41	-	35.84	28.63	98.58	Huang et al., 2008
S	2.42	26.3	28.9	324	28.4	36.8	55.9	Setia et al., 2023
S	0.15	14.5	24.98	352	34.29	20.4	35.98	Ozturk and Arici, 2021
S	0.41	38.9	60	488	29	27	70	Kabata-Pendias, 2011
S	1.152	16.2	15.96	446.3	38.36	7.65	46.97	This study

Cu ($p<0.03$), Mn ($p=0.004$), and Zn ($p<0.002$) levels were significantly different between wheat and soil samples. Ni ($p<0.002$) levels in the samples taken from the city center differed significantly between wheat and soil samples.

The average trace element concentrations of previous studies from similar and different regions are given in Table 5. Cd, Cr, and Pb elements were not detected in wheat samples. It was determined that the average Cu, Mn, Ni, and Zn concentrations in wheat were at lower levels compared to previous studies. Also, It was determined that the average Cu, Cr, and Pb levels in the soil were lower than in previous studies and that Cd, Mn, and Zn were at average levels.

4. Discussions

The present study demonstrates the importance of bioaccumulation and the transfer of trace elements between wheat and soil. The Pb concentration was determined only in the soil samples taken from the city center. Trace element concentrations of Cd, Cr, and Pb were not detected in wheat samples. Cu, Mn, and Zn concentrations in wheat samples were determined at very low levels compared to soil samples. It has been determined that the process of element transport from soil to plant may differ in trace element concentrations even if the plants are grown under the same conditions.

Conflict of Interest

There is no conflict of interest with any institution or person.

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A new variety of the *Bupleurum* (*Apiaceae*) from Türkiye

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Türkiye'den yeni bir *Bupleurum* (*Apiaceae*) varyetesi

Abstract: Some interesting *Bupleurum* (*Apiaceae*) specimens with very branched stems, (1–)2-rayed inflorescences and ivory-white bracteoles were collected from Tepebaşı District (Eskişehir Province). The specimes, at first glance, resembled the *Bupleurum pendikum* (sect. *Aristata*) species, which is endemic in Türkiye, as a habit. However, as a result of detailed examination, it was determined that there were some differences. Based on these differences, the specimens were presented to the scientific world as a new variety and were named *Bupleurum pendikum* var. *eskisehiricum*. The fact that the inflorescences are (1–)2 rays (not (2–)3–4(–5)); the bracteoles are 2.6–3.7 mm broad, ovate-elliptic and with arista up to 0.5 mm long (not 1.2–2 mm broad, lanceolate and with arista 1–1.5 mm long); the petals 0.6–0.8 mm broad (not 0.4–0.5 mm broad); the filaments 0.7–0.8 mm long (not c. 0.5 mm long) and the mericarps 2.7–3.1 × 1.1–1.3 mm (not 2.2–2.4 × 0.9–1 mm) are the most obvious attributes that separate *Bupleurum pendikum* var. *eskisehiricum* from *B. pendikum* (var. *pendikum*), that is a close taxon. Here, a detailed description of the new variety, informative photographs, and some ecological preferences were given.

Key words: *Bupleurum*, Eskişehir, new variety, taxonomy, Türkiye

Özet: Tepebaşı ilçesinden (Eskişehir) çok dallanmış gövdeli, (1–)2-ışınlı çiçek durumlu ve fildişi-beyazı bırakteollü olan bazı ilginç *Bupleurum* (*Apiaceae*) örnekleri toplandı. Örnekler ilk bakışta habit olarak Türkiye'de endemik olan *Bupleurum pendikum* (sect. *Aristata*) türüne benzetildi. Fakat yapılan detaylı inceleme sonucunda bazı farklar olduğu tespit edildi. Bu farklılıklara dayanarak, örnekler bilim dünyası için yeni bir varyete olarak tanıtıldı ve *Bupleurum pendikum* var. *eskisehiricum* olarak adlandırıldı. Çiçek durumu ışınlarının (1–)2 adet olması ((2–)3–4(–5) değil); bırakteollerinin 2.6–3.7 mm eninde, yumurtamsı-eliptik ve en fazla 0.5 mm uzunluğunda kılçıklı olması (1.2–2 mm eninde, mızraksı ve 1–1.5 mm kılçıklı değil); petallerinin 0.6–0.8 mm eninde olması (0.4–0.5 mm eninde değil); filamentlerinin 0.7–0.8 mm uzunluğunda olması (c. 0.5 mm değil) ve merikarpının 2.7–3.1 × 1.1–1.3 mm olması (2.2–2.4 × 0.9–1 mm değil) *Bupleurum pendikum* var. *eskisehiricum*'u yakın takson olan *B. pendikum* (var. *pendikum*)'dan ayıran en belirgin özelliklerdir. Burada; yeni varyetenin detaylı betimlemesi, bilgilendirici fotoğrafları ve bazı ekolojik tercihleri verilmiştir.

Anahtar Kelimeler: *Bupleurum*, Eskişehir, yeni varyete, taksonomi, Türkiye

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1. Introduction

Bupleurum L. is a genus of approximately 180 species in the world (Sheh and Watson, 2005). *Bupleurum* is also the genus including the most taxa in Türkiye and has become mostly adapted to the steppe habitat represented by 48 species. Approximately 44% of these taxa are endemic (Snogerup, 1972; Duman, 2000; Snogerup and Snogerup, 2001).

Some interesting *Bupleurum* specimens were collected during the "Biorestoration Observation Studies" held in 2022 within the scope of the Trans-Anatolian Natural Gas Pipeline Project (TANAP). The fact that they are ivory-white bracteoles and very branched stems are the attributes of the specimens that first draw attention. Specimens of the plants with flowers and ripe fruit, which has adapted to the dry marly places in oak and pine clearings, were collected around the end of August. It was decided at the conclusion of the investigation conducted and by considering the most recent revision studies and the *Flora of Turkey and the East Aegean Islands* that the specimens are a new variety for the subsect. *Aristata* in sect. *Aristata* Godron (Snogerup, 1972; Snogerup and Snogerup, 2001).

Aristata is the second largest subsection of the section after subsect. *Juncea* (27 taxa) and includes a total of 11 taxa in Türkiye. Together with the new variety described here, the number of taxa of the *Aristata* subsection in Türkiye has risen to twelve (Snogerup, 1972; Duman, 2000; Snogerup and Snogerup, 2001).

2. Materials and Method

Specimens belonging to the new variety were collected in August from SE of Takmak Village in Tepebaşı District of Eskişehir Province in Türkiye. The related literature (Snogerup, 1972; Duman, 2000; Snogerup and Snogerup, 2001; Aksoy et al., 2011), the specimens in the high-resolution photographs in the B, C, E, FI, G, GB, H, JE, LD, M, P and PRC herbaria were utilized in the identification and evaluation of the specimens (Thiers, 2023). A Leica EZ4 stereo microscope and a Samsung A33 5G mobile telephone were used in the examination of the specimens and the taking of photographs, whereas a ruler with a sensitivity of 0.5 mm was used in the measurements.

2.1. Specimens examined

Bupleurum pendikum Snogerup. TÜRKİYE. [İstanbul] Constantinople. In collibus graminosis prope "Pendik", [8] Julio 1906, *G.V.Aznavour 5074* (M, M-0172797! [holotype]; LD, LD1075014; LD, LD1075078; JE, JE00006889; JE, JE00006890; C, C10008365; G,

G00367571; G, G00367575; G, G00367642; G, G00367647; B, B-10-0367149; E, E00000316; E, E00000317; GB, GB-0048804; GB, GB-0048805; FI, FI-014719; H, H1394748; PRC, PRC451949 [isotypes], virtual images); Kütahya, Keles-Tavşanlı, 36 km SE Keles, 1000 m, 07.07.1980, *M.Nydegger 15181* (E, E00091560; P, P06871068, virtual images); Kocaeli: Yuvacık dam area, Çilekli village, openings of *Pinus nigra* forest in steppe, 1089 m, 07.07.2006, *N.Aksoy 6324, A.Efe & N.Güneş* (DUOF 1125, fig. 1 in Aksoy et al., 2011).

3. Results

3.1. Taxonomic treatment

Bupleurum pendikum var. eskisehiricum Hamzaoğlu, **var. nov.** (sect. *Aristata*, subsect. *Aristata*).

Diagnosis: *Bupleurum pendikum* var. *eskisehiricum* is related to *B. pendikum*. It differs from *B. pendikum* (var. *pendikum*), mainly by rays (1–)2 pieces (not (2–)3–4(–5) pieces); bracteoles 2.6–3.7 mm broad, ovate-elliptic and with arista up to 0.5 mm long (not 1.2–2 mm broad, lanceolate and with arista 1–1.5 mm long); umbellules 9–15-floweted (not 6–8-flowered); petals 0.6–0.8 mm broad (not 0.4–0.5 mm broad); filaments 0.7–0.8 mm long (not c. 0.5 mm long); mericarps 2.7–3.1 × 1.1–1.3 mm (not 2.2–2.4 × 0.9–1 mm).

Type: TÜRKİYE. Eskişehir, Tepebaşı, SE of Takmak village, 930 m a.s.l., degraded oak and pine forest clearings, marly places, 29.08.2022, *E.Hamzaoğlu 8045* (holotype GAZI!, isotypes ANK!, HUB!).

Description: Annual, glabrous, (8–)15–40 cm, erect to ascending, densely pseudo-dichotomously branched from base to apex, with (5–)15–50(–70) umbels. Stem flexuous, terete, striate, up to 2 mm diameter at base, greenish above, purplish below. Leaves sessile, linear-subulate, acuminate; lower withered after anthesis; cauline 1.5–4.5 × 0.6–1.5 mm, with midrib and marginal vein. Peduncles 5–20(–30) mm, longer than longest rays. Rays (1–)2, very unequal, 2–11 mm long. Bracts 3(–4), 2/5 or more as long as longest ray, 4–5 mm long, elliptic-lanceolate, distinctly brownish 3-nerved with broad scarious margin, becoming ivory-white and ± semi-transparent. Bracteoles 5, exceeding the flowers and enclosing them before and after anthesis, ivory-white and semi-transparent, with entire margin, 4.5–7.1 × 2.6–3.7 mm, enlarging until ripening of fruits, ovate-elliptic, apiculate, arista up to 0.5 mm long, strongly brownish 3-veined, veinlets many, conspicuous, pinnate; after anthesis becoming semi-transparent throughout but as young herbaceous along the veins and in apical part. Umbellules 9–15-flowered, pedicels ± equal, 0.4–0.9 mm long. Petals milky whitish and semi-transparent, with brownish midrib, 0.9–1.3 × 0.6–0.8 mm, irregularly lobulate-dentate especially at apex, bend irregularly 4-lobed, inflexed lobe narrow, bifid. Stamen 1.0–1.2 mm long, anthers 0.4–0.5 mm long, filament 0.7–0.8 mm long. Stylopodium 0.5–0.8 mm broad, narrower than ripe fruit, styles 0.6–0.7 mm long, about equalling stylopodium radius. Ripe mericarps 2.7–3.1 × 1.1–1.3 mm, semi-circular in transect, dark brown to blackish, smooth, ridges filiform but in ripe fruit inconspicuous, oil ducts invisible.

Phenology: Flowering time July–August, fruiting time August–September.

Etymology: The type locality of the taxon, which is from Eskişehir, inspired the scientific name of the new variety (*Bupleurum pendikum* var. *eskisehiricum*).

3.2. Distribution and habitat

Specimens of *Bupleurum pendikum* var. *eskisehiricum* were collected from SE of Takmak Village (Tepebaşı, Eskişehir). It is estimated that the variety grows in marly places in degraded forest openings, approximately between 890 and 950 m a.s.l. There are many degraded forests and marly places around Eskişehir Province. The probability that the taxon is also grown in this places is rather high, but there is still no data about this. Consequently, at present, the taxon is an endemic of Türkiye and when the area of distribution is considered, it is an element of the Irano-Turanian phytogeographic region (Figure 1).

4. Discussions

4.1. Taxonomic notes

The first comprehensive information in Türkiye about the *Bupleurum* genus *Aristata* subsection was included in Volume 4 of the work titled *Flora of Turkey and the East Aegean Islands*, but as subsect. *Glumacea* (Boiss.) Wolff *Bupleurum sulphureum* is one of the 11 species of the subsection known from Türkiye and it is endemic. This species usually grows on calcareous steppes in Central Anatolia and its periphery (Snogerup, 1972). The work titled "*Bupleurum L. (Umbelliferae) in Europe – 1. The annuals, B. sect. Bupleurum and sect. Aristata*" prepared by Snogerup and Snogerup (2001), is a broad-scope study, which includes all the species of the *sect. Bupleurum* and

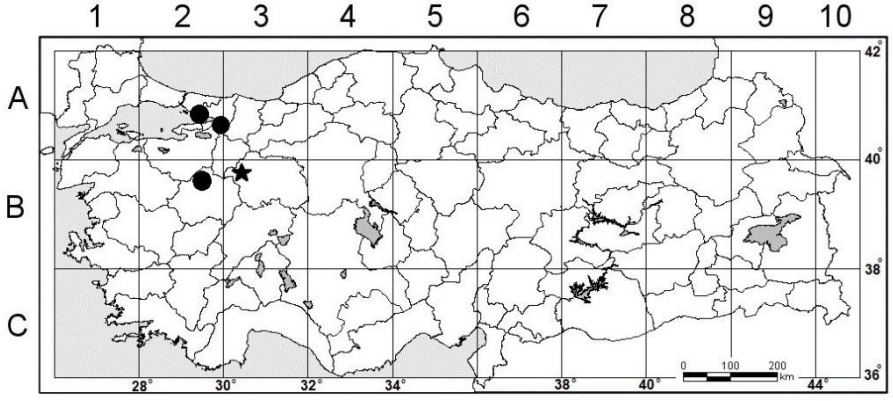

Figure 1. Distribution map of *Bupleurum pendikum* var. *eskisehiricum* (★) and *B. pendikum* (var. *pendikum*) (●).

Figure 2. *Bupleurum pendikum* var. *eskisehiricum*. A: habit (holotype), B: natural view of the umbel (with 2 rays)

Aristata sections in Europe (incl. Türkiye-in Europea, i.e. Thrace). In this study, a total of 14 species were given belonging to the subsect. *Aristata*.

The moment the *Bupleurum pendikum* var. *eskisehiricum* was seen for the first time, the most interesting aspect was that it was ivory-white bracteoles, very branched stems, and (1–)2-rayed inflorescences. When it is compared with the *Bupleurum pendikum* (var. *pendikum*) specimens, the most striking difference of the *B. pendikum* var. *eskisehiricum* variety were its few rayed inflorescences, ovate-elliptic bracteoles, very flowered umbellules, and larger mericarps (Figures 2 and 3, Table 1).

In the species belonging to the subsect. *Aristata* are bracteoles 1/3–2/3 as wide as long, enclosing the flowers before and after anthesis. Whitish bracteoles are encountered only in the *Bupleurum gracile* d'Urv., *B. aira* Snogerup, and *B. pendikum* in subsect. *Aristata* (Snogerup and Snogerup, 2001; Aksoy et al., 2011). *Bupleurum pendikum* var. *eskisehiricum* is similar to *B. pendikum* (var. *pendikum*) in terms of shape and size of bracts, shape and color of petals. However, in the *B. pendikum* var. *eskisehiricum* specimens, there are ovate-elliptic and 2.6–3.7 mm broad bracteoles, (1–)2-rayed inflorescences, 9–15-flowered umbellules, 0.6–0.8 mm broad petals, 0.7–0.8 mm long filamens, and 2.7–3.1 × 1.1–1.3 mm mericarps (Aksoy et al., 2011).

4.2. Conservation status

According to the existing data, *Bupleurum pendikum* var. *eskisehiricum* is a variety only known from the type locality. Approximately 300 individuals were counted in the type locality. The taxon grows dry marly places in oak and pine clearings in SE of Takmak Village. There are agricultural areas in the very close surroundings of the individuals belonging to the taxon. On the other hand, there

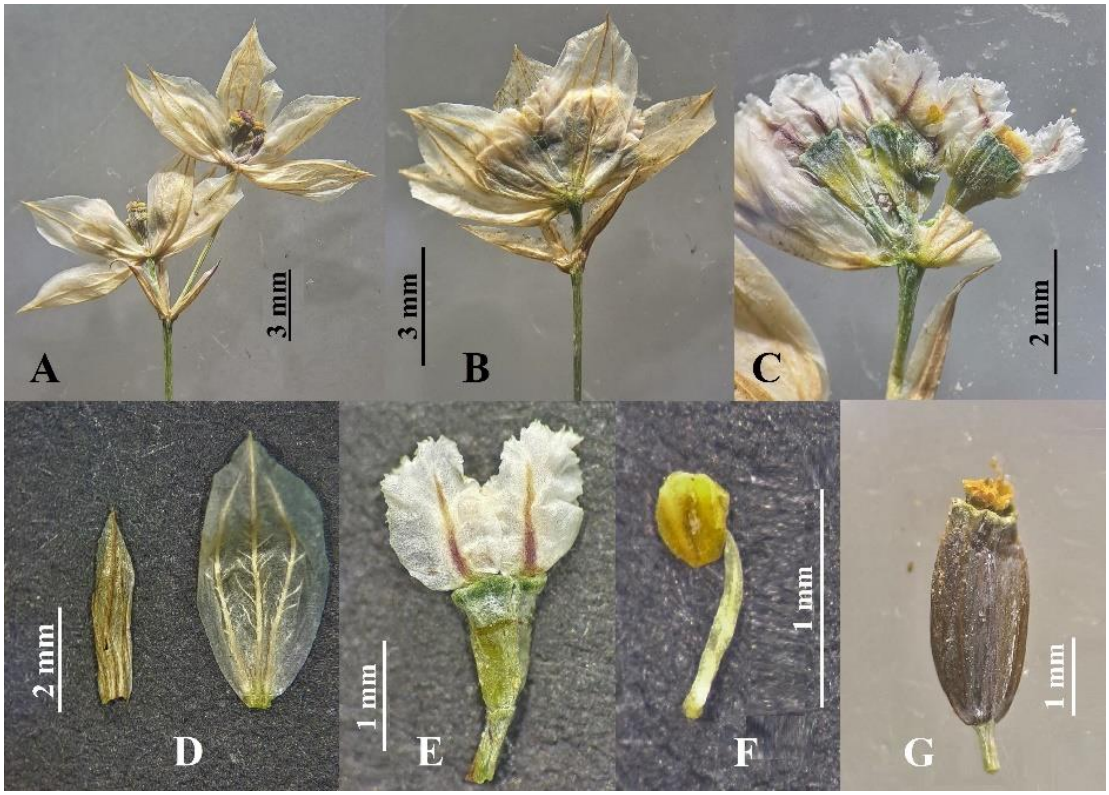

Figure 3. Inflorescence and flower parts of *Bupleurum pendikum* var. *eskisehirucum*. A: inflorescence with 2 rays, B: inflorescence with 1 ray, C: inside view of the inflorescence, D: bract (left) and bracteol (right), E: flower (ovary and petals), F: stamen (anther and filament), G: mericarp.

Table 1. Diagnostic characters of *Bupleurum pendikum* var. *eskisehiricum* and *B. pendikum* (var. *pendikum*).

Characters	***Bupleurum pendicum* var. *eskisehiricum***	***Bupleurum pendicum* (var. *pendicum*)**
Rays	(1–)2	(2–)3–4(–5)
Bracteoles	2.6–3.7 mm broad, ovate-elliptic and with arista up to 0.5 mm long	1.2–2 mm broad, lanceolate and with arista 1–1.5 mm long
Umbellules	9–15-flowered	6–8-flowered
Petals	0.6–0.8 mm broad	0.4–0.5 mm broad
Filaments	0.7–0.8 mm long	c. 0.5 mm long
Mericarps	2.7–3.1 × 1.1–1.3 mm	2.2–2.4 × 0.9–1 mm

is a very low probability of these areas becoming completely agricultural lands in the future. When the areas where the taxon could be grown are considered, it is estimated that *B. pendikum* var. *eskisehiricum* showed a distribution on an area smaller than 100 km^2. When the existing or envisaged threats are evaluated together, the taxon being known from only one address at present (area of life less than 10 km^2) and the breadth of the area of distribution calculated (less than 100 km^2), it was decided that it would be suitable to propose the Critically Endangered [CR B1ab(i)+CR B2b(ii)] classification for the extinction risk of the taxon (IUCN Standards and Petitions Committee, 2023).

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Wild mushrooms from Eastern Black Sea Region (Türkiye): Element concentrations and their health risk assessment

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Doğu Karadeniz Bölgesi (Türkiye)'den yabani mantarlar: Element konsantrasyonları ve sağlık risk değerlendirmesi

Abstract: The aim of this study is to determine the mineral contents of wild edible mushrooms. The potassium (K), magnesium (Mg), calcium (Ca), manganese (Mn), iron (Fe), zinc (Zn), copper (Cu), nickel (Ni), cadmium (Cd) and lead (Pb) contents of twenty four edible mushrooms, collected from East Black Sea Region, Türkiye, were analyzed. The studied mushrooms varied widely in their content of both essential and toxic deleterious elements. The minimum and maximum mineral contents of mushrooms were determined as mg/kg dw for K (4573-15645), Mg (173-1421), Ca (24-711), Mn (5.34-90.64), Fe (44.78-236.95), Zn (24.81-119.03), Cu (11.02-174.01), Ni (0.95-2.86), Cd (0.05-22.57) and Pb (0.01-2.07). The potassium content was found to be higher than those of the other minerals in all the mushrooms. In addition to the metal contents, the daily intakes of metal (DIM) and Health Risk Index (HRI) values of edible mushrooms were also calculated. Lead and cadmium were present but at concentrations that are not hazardous to human health except for *Russula vinosa*. The K, Mg, Zn, and Ni concentrations were determined to be high in *Russula integra*. Mushrooms have become increasingly attractive as functional foods for their potential beneficial effects on human health. Due to the toxic minerals they carry, mushrooms should be taken into consideration during their consumption as human food. The differences and similarities between mineral contents were established by Principal Component Analysis. Also, mushrooms are important in the ecosystem because they are able to biodegrade the substrate and to collect heavy metal.

Key words: Edible mushrooms, minerals, toxic element

Özet: Bu çalışmanın amacı, yenilebilir yabani mantarların mineral içeriklerini belirlemektir. Doğu Karadeniz Bölgesi'nden toplanan yirmi dört yenilebilir mantarın mineral içerikleri Potasyum (K), magnezyum (Mg), kalsiyum (Ca), mangan (Mn), demir (Fe), çinko (Zn), bakır (Cu), nikel (Ni), kadmiyum (Cd) ve kurşun (Pb) analiz edilmiştir. İncelenen mantarlar, hem temel hem de toksik elementleri büyük farklılıklar göstererek içermektedirler. Mantarların minimum ve maksimum mineral içerikleri K (4573-15645), Mg (173-1421), Ca (24-711), Mn (5.34-90.64), Fe (44.78-236.95), Zn (24.81-119.03), Cu (11.02-174.01), Ni (0.95-2.86), Cd (0.05-22.57) ve Pb (0.01-2.07) için mg/kg km olarak belirlenmiştir. Tüm mantarlarda potasyum içeriği diğer minerallerden daha yüksek bulunmuştur. Metal içeriklerinin yanı sıra yenilebilir mantarların Günlük Metal Alımları ve Sağlık Risk İndeksi değerleri de hesaplanmıştır. Kurşun ve kadmiyum *Russula vinosa* dışında insan sağlığına zararlı olmayan konsantrasyonlarda belirlenmiştir. *Russula integra* da K, Mg, Zn ve Ni konsantrasyonlarının yüksek olduğu belirlenmiştir. Mantarlar, insan sağlığı üzerindeki potansiyel yararlı etkileri nedeniyle fonksiyonel gıdalar olarak giderek daha çekici hale gelmiştir. Mantarlar, taşıdıkları toksik mineraller nedeniyle insan gıdası olarak tüketilmeleri sırasında dikkate alınmalıdır. Mineral içerikleri arasındaki farklılıklar ve benzerlikler Temel Bileşen Analizi ile de belirlenmiştir. Ayrıca mantarlar, ağır metal toplamak için substratı biyolojik olarak parçalayabildikleri için ekosistemde önemlidirler.

Anahtar Kelimeler: Yenen mantarlar, mineraller, zehirli element

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1. Introduction

Wild grown mushrooms are considered as a popular delicacy in many countries mainly in Europe, Asia and Africa. Their therapeutic application includes the prevention and treating of diseases such as hypertension, hypercholesterolemia, and cancer. It has been documented that these curational properties are mainly due to their chemical composition. Mushrooms are organisms that can grow both in uncontaminated rural ecosystems and in urban areas with high industrial pollution (Karaman et al., 2012; Rakić et al., 2014). They are important in terms of pharmaceuticals as well as their ecological values (Sirić et al., 2016). Therefore, it is thought that mushrooms have a high potential to be used in the treatment of some diseases such as cancer, obesity, hypertension, and hyperglycemia, which threaten human beings and have a high prevalence (Guggenheim et al., 2014; Guillamón et al., 2010).

Mushrooms are considered not only as spice and food ingredients, but also as a nutritional supplement in the human diet which also plays the role of functional foods. Both wild and cultivated mushrooms have many beneficial compounds for human health. For thousands of years mushrooms have constituted a valuable source of nutrients since they contain carbohydrates, protein, dietary fibre, vitamins and minerals, but are low in calories, fat and contain negligible amounts of cholesterol (Kozarski et al., 2015; Nowakowski et al., 2021). Since mushrooms are low-calorie foods, they are particularly preferred in diets. In addition, they are rich in vitamins, elements (both macro and microelements), and proteins (Gargano et al., 2017).

Some mushroom species have therapeutic value due to their primary (e.g., polysaccharides and polysaccharide–protein complexes, etc.) and secondary metabolites (e.g., alkaloids, terpenoids, phenolic compounds, etc.) and are also known as medicinal mushrooms in the literature (De Silva et al., 2013; Duru and Çayan, 2015; Gargano et al., 2017). Studies have shown that mushrooms have many biological/pharmacological activities (such as neuroprotective, cardiovascular, antioxidant, antimicrobial, antitumor, etc.). These activities are thought to be due to compounds that they contain (Gargano et al., 2017; Muszynska et al., 2018; Nowakowski et al., 2021).

In addition to the benefits mentioned above, fungi are also responsible for the cycle of elements in nature, as they also fulfill the functions of breaking down organic materials (Sesli et al., 2008). With the increasing interest of humans in wild mushroom species due to their nutritional properties, researchers have started to focus on whether the concentrations of elements accumulated in these organisms pose a risk to human health (Işıldak et al., 2004; Kalač and Svoboda, 2000; Mleczek et al., 2016a; Severoğlu et al., 2013; Sirić et al., 2016). Some mushroom species can accumulate certain metals at higher concentrations than other organisms living in the same ecosystem (Falandysz et al., 2008; Kalać, 2010; Rakić et al., 2014; Sesli et al., 2008; Severoğlu et al., 2013; Sirić et al., 2016). The biosorption of elements by mushroom species is a well-known mechanism studied by many researchers (Mleczek et al., 2016a; Sesli and Dalman, 2006; Sesli et al., 2008). When toxic metals and metalloids such as Hg, Pb, Cd, As, etc. accumulate in fruiting bodies of mushrooms at high concentrations, people who feed on these mushrooms can also accumulate them in their bodies. In this way, these metals can cause various adverse effects on human metabolism (Falandysz and Borovicka, 2013; Mleczek et al., 2016b; Rzymski et al., 2016). This makes controlling the toxic element content of wild mushroom species a priority issue (Agrawal and Dhanasekaran, 2019; Rashid et al., 2018). High metal accumulation in mushrooms is also under the scrutiny of researchers as they are indicators of metal pollution in the ecosystem as well as their negative effects on human health (Li et al., 2017).

Despite many positive aspects of mushroom consumption, there are risks associated with their ingestion, such as poisoning with pesticides or harmful elements, e.g. mercury (Hg), lead (Pb), cadmium (Cd) and arsenic (As), which might accumulate in mushrooms (Sirić et al., 2017).

Wild mushroom species can be classified as edible, inedible, poisonous, soil-grown, wood-grown, parasitic or saprophytic, etc.. These differences in the way of life of mushrooms have a major impact on their metal accumulation capacity (Kalać, 2010; Mleczek et al., 2016a). In addition, areas where mushroom species grow provide important clues in environmental researches in terms of metal accumulation (Kalac, 2001; Rakič et al., 2014; Sirič et al., 2016).

The purpose of this study was to determine K, Mg, Ca, Mn, Fe, Zn, Cu, Ni, Cd and Pb in some edible collected from Bayburt, Gümüşhane, Rize and Artvin (East Black Sea, Turkey). In addition, the daily intakes of metal (DIM) and Health Risk Index (HRI) values of edible mushroom species were also calculated, and their potential effects on human health were discussed.

2. Materials and Method

2.1. Samples

Fully matured fruiting bodies of mushroom samples were collected from Rize, Artvin, Gümüşhane and Bayburt Eastern Black Sea, Turkey. Information on the habitats and taxonomic records of mushroom species are given in Table 1. Colour slides of the macrofungal specimens were taken in their natural habitats during fields studies. After relevant notes were taken of their morphological and ecological features, they were put in private prepared boxes and brought to the laboratory. Their spore prints were taken and spore dimensions were measured using an ocular micrometer. Then, dried specimens were placed in locked polyethylene bags and kept in a deep freezer at -20 ℃ to protect against parasites.

Identification of the specimens was performed according to Breitenbach and Kranzlin (1984–2000), Bresinsky and Besl (1990), Buczacki (1989) and Dahncke and Dahncke (1989). All specimens are kept in the Herbarium of Yuzuncu Yıl University, Department of Biology (VANF). Table 1 shows the taxa of wild edible macrofungi.

2.2. Method

Atomic absorption spectrophotometer (Varian Techtron Model AAS 1000, Varian Associates, Palo Alto, CA) was used for the determination of the minerals (Ca, Mg, K, Fe, Zn, Cu, Mn, Pb, Ni and Cd) in dried fruit bodies of macrofungi. Each dried mushroom sample was weighed as 4-5 g and placed in a porcelain crucible and ashed at 550 °C for 18-20 h. then the ash was dissolved in 1ml concentrated HNO_3, evaporated to dryness, heated again at 550 °C for 4 h, treated successively with 1 ml HNO_3 and 1 ml H_2O_2 and then diluted with double deionized water up to a volume of 25 ml. Three blank samples were treated in the same way. The species, which were digested in an acid solution of HNO_3 were determined by the AAS system using different lamps, and calibrated with related minerals in different concentrations for different micronutrients (AOAC, 1990). To check for possible contamination by reagents or glassware, blanks containing 4ml of ultrapure concentrated HNO_3 and 4 ml H_2O_2 were run together with analytical samples and every batch of analytical samples was run together with the standard matrix. The values of Ca, Mg, K, Fe, Zn, Cu, Mn, Pb, Ni and Cd were calculated as mg/kg dw. Detection limit is defined as the concentration corresponding to three times the Standard deviation of ten aborbance measurements of blank solution divided by the slope of calibration curve for each element ($3xs_b/m$). Detection limits of elements as mg/kg by AAS were found to be 0.012 for K, 0.003 for Mg, 0.015 for Ca, 0.029 for Mn, 0.060 for Fe, 0.013 for Zn, 0.041 for Cu, 0.063 for Ni, 0.032 for Cd and 0.10 for Pb. The results were within limits of quantification for above minerals (calculated as 10-fold of standard deviation from ten replicates of the instrumental blank solution: $10xs_b/m$) 0, 2, 4, 8 and 16 mg/g or mg/kg, respectively. Mushrooms were selected normally harvested for consumption (pileus+stipe). For all the mushroom species, at least three samples were analysed.

2.3. Determination of DIM and HRI values

DIM and HRI analyses of mushrooms were performed following the method given in the literature (Cui et al., 2004; Liu et al., 2015). While calculating DIM values of which details were also given in the supplementary file, RfD^o values set by USEPA (2002) were taken into consideration.

Table 1. Taxa of wild edible macrofungi collected from Bayburt, Gümüşhane, Rize and Artvin regions.

	Macrofungi taxa	Collection locality, habitat/substrate
1	*Agaricus bisporus* (J.E. Lange) Imbach	Gümüşhane, Torul, Köprübaşı district, apple trees garden
2	*Chlorophyllum rhacodes* (Vittad.) Vellinga	Rize, İkizdere, Çamlık district, in conifer forest
3	*Macrolepiota procera* (Scop.) Singer	Artvin, Şavşat, Sahara National Park, in conifer forest
4	*Amanita rubescens* Pers.	Rize, Çamlıhemşin, Ayder Plateau, in conifer forest
5	*Armillaria ostoyae* (Romagn.) Herink	Gümüşhane, Kürtün, Örümçek Forest, in conifer forest
6	*Pleurotus dryinus* (Pers.) P. Kumm.	Gümüşhane, Özkürtün, Karaçukar Forest, in conifer forest
7	*Pleurotus ostreatus* (Jacq.) P. Kumm.	Bayburt, Akşar, on willow
8	*Cerioporus squamosus* (Huds.) Quél.	Bayburt, Akşar, on stumps of poplar tree
9	*Boletus edulis* Bull.	Artvin, Şavşat, Karagöl National Park, in conifer forest
10	*Neoboletus praestigiator* (R. Schulz) Svetash., Gelardi, Simonini & Vizzini	Gümüşhane, Zigana Mountein, in conifer forest
11	*Leccinum scabrum* (Bull.) Gray	Gümüşhane, Torul, Günay Village, mixed forest
12	*Suillus luteus* (L.) Roussel	Gümüşhane, Torul, Günay Village, mixed forest
13	*Lepista nuda* (Bull.) Cooke	Rize, Çamlıhemşin, Ayder Plateau, in conifer forest
14	*Lepista personata* (Fr.) Cooke	Bayburt, Aydıntepe, Centrum, garden
15	*Hydnum repandum* L.	Artvin, Şavşat, Karagöl National Park, in conifer forest
16	*Lactarius deliciosus* (L.) Gray	Gümüşhane, Torul, Günay Village, mixed forest
17	*Lactifluus piperatus* (L.) Roussel	Rize, Çamlıhemşin, Ayder Plateau, in conifer forest
18	*Lactarius salmonicolor* R. Heim & Leclair	Gümüşhane, Zigana Mauntein, in conifer forest
19	*Lactifluus volemus* (Fr.) Kuntze	Rize, Çamlıhemşin, Ayder Plateau, in conifer forest
20	*Russula delica* Fr.	Rize, Çamlıhemşin, Ayder Plateau, in conifer forest
21	*Russula integra* (L.) Fr.	Rize, İkizdere, Çamlık village, in conifer forest
22	*Russula adusta* (Pers.) Fr.	Rize, Centrum, mixed forest
23	*Russula vinosa* Lindblad	Rize, Çamlıhemşin, Ayder Plateau, in conifer forest
24	*Neoboletus erythropus* (Pers.) C. Hahn	Rize, Çamlıhemşin, Ayder Plateau, in conifer forest

3. Results and discussion

Information about the taxonomic details, substrates, and edibility of the mushroom species analyzed in this study are given in Table 1. The concentrations of K, Mg, Ca, Mn, Fe, Zn, Cu, Ni, Cd and Pb in mushrooms are presented in Table 2 (in mg/kg dry weight). Additionally, DIM and HRI values of mushrooms were calculated and documented in Table 3.

Data on potassium and nine metals most frequently determined in mushrooms from Gümüşhane, Rize, Artvin, Bayburt in Turkey are given for 24 species in Table 2. All the metal concentrations were determined on a dry weight basis. The metal contents varied across the locations and the mushroom species sampled. The variations could be as a result of differences in substrate composition which is determined by the ecosystem as well as the differences in the absorption of individual metals by the mushroom species.

Heavy metals can pass through to mushrooms from the contaminated soil and environment. Soil's composition, contaminated water, air and soil, metal based pesticides and fertilizers, industrial emissions are the main factors that affect the heavy metal concentrations in mushrooms.

The acid–base balance, the blood pressure and the regulation of osmotic pressure of the organism are controlled by potassium, which is mainly present in intracellular fluid (Yellen, 2002). Potassium content was higher than other minerals in all mushrooms in this study, varying between 4573 (*Lepista personata*) and 15645 mg/kg dw (*Russula integra* (L.) Fr.

In general, most of the mushrooms studied contained considerably high amounts of minerals. The levels of essential elements in mushroom species were higher than those of toxic elements. Gençcelep et al. (2009) reported the potassium contents of wild edible mushrooms as being between 12600 and 29100 mg/kg dw. Wang et al. (2014) found that potassium content was between 16000 and 37000 mg/kg in dry matter. Sanmee et al. (2003) reported that potassium accumulation in mushrooms could rise up to 45200 mg/kg. Liu et al. (2012) reported that the lowest potassium value (1300 mg/kg dw) was measured in *Melanoleuca gigantea* and *Melanoleuca arcuata*, the highest potassium value (4600 mg/kg dw) was found in *Boletus griseus*. The greatest concentrations of K were obtained in *C. cibarius* (41823.3 mg/kg), whereas the lowest was in *Boletus edulis* (11266.9 mg/kg) (Cvetkovic at al., 2015). Usual potassium content in mushrooms varies between 20000 and 40000 mg/kg dw (Gençcelep et al., 2009; Kalač, 2009). Literature data showed that K concentration of mushroom samples was between 19000 and 54073 mg/kg (Alaimo et al., 2018; Mleczek et al., 2020). In this study, potassium levels were lower than reported values in the literature. Showing that mushrooms are an excellent source of potassium in the human diet.

Magnesium content 173 mg/kg dw in *Cerioporus squamosus* and 1421 mg/kg dw in *Lactarius volemus*. The level of magnesium reported in this study was relatively low compared to earlier published reports (Demirbaş, 2000) which was magnesium content ranged from 330 mg/kg dw in *Tricholoma anatolicum* to 6560 mg/kg dw in *Morchella*

Table 2. Amounts of total K, Mg, Ca, Mn, Fe, Zn, Cu, Ni, Cd and Pb of wild edible mushrooms from Bayburt, Gümüşhane and Rize Region of Turkey (mg/kg dw).

No	Mushrooms	K	Mg	Ca	Mn	Fe	Zn	Cu	Ni	Cd	Pb
1	*Agaricus bisporus*	7854	880	138	14.30	140.04	46.00	23.46	1.84	0.25	1.06
2	*Chlorophyllum rhacodes*	10900	536	309	13.83	147.92	66.15	18.01	2.19	0.12	1.13
3	*Macrolepiota procera*	6810	738	197	11.21	83.47	60.22	47.56	1.55	0.34	0.67
4	*Amanita rubescens*	15527	256	262	18.46	236.95	61.07	34.47	2.54	0.14	0.96
5	*Armillaria ostoyae*	8749	255	51	17.76	218.25	33.45	35.96	1.09	6.30	1.03
6	*Pleurotus dryinus*	8098	441	338	21.42	197.76	57.66	24.35	1.91	0.84	1.19
7	*Pleurotus ostreatus*	9042	704	452	18.75	160.01	53.97	36.55	1.74	0.16	1.66
8	*Polyporus squamosus*	7348	173	24	10.08	201.25	25.03	20.99	1.52	0.18	1.04
9	*Boletus edulis*	6140	394	270	20.14	142.18	39.45	22.58	1.91	0.05	1.30
10	*Neoboletus praestigiator*	7275	231	96	9.40	71.45	42.95	23.31	0.95	0.36	1.64
11	*Leccinum scabrum*	6005	373	163	16.20	64.04	33.58	35.94	1.39	0.46	1.07
12	*Suillus luteus*	11127	393	117	12.02	225.17	57.86	33.21	1.64	0.09	1.19
13	*Lepista nuda*	7322	420	146	33.25	119.18	58.64	23.38	2.08	0.83	2.07
14	*Lepista personata*	4573	667	306	90.64	49.94	24.81	14.64	1.59	0.96	0.48
15	*Hydnum repandum*	11455	1145	47	23.31	64.43	79.05	39.34	1.66	0.52	1.61
16	*Lactarius deliciosus*	4867	661	73	15.94	77.42	28.35	11.02	1.31	1.38	0.46
17	*Lactarius piperatus*	7364	773	289	14.10	165.05	55.23	17.34	2.54	0.59	1.05
18	*Lactarius salmonicolor*	11947	781	199	38.05	119.12	65.10	40.31	2.28	0.47	0.59
19	*Lactarius volemus*	8437	1421	711	6.38	59.08	63.49	145.92	2.12	0.72	0.12
20	*Russula delica*	7887	438	133	5.34	101.88	61.56	14.89	2.03	1.24	0.97
21	*Russula integra*	15645	1249	275	11.77	44.78	119.03	72.01	2.86	0.16	0.73
22	*Russula nigricans*	11133	731	177	25.41	102.54	39.38	12.05	2.22	2.73	1.05
23	*Russula vinosa*	8673	1190	574	32.22	89.09	77.77	174.01	2.30	22.57	1.37
24	*Neoboletus erythropus*	6815	417	309	9.32	91.69	46.58	22.69	1.45	0.33	0.01
	Mean±SD	8791 ±2885	636 ±343	235.6 ±166.1	20.38 ±17.47	123.86 ±59.11	54.01 ±20.67	39.33 ±39.72	1.86 ±0.47	1.74 ±4.62	1.01 ±0.48
	Minimum	4573	173	24	5.34	44.78	24.81	11.02	0.95	0.05	0.01
	Maximum	15645	1421	711	90.64	236.95	119.03	174.01	2.86	22.57	2.07

deliciosa. In our previous study, the concentration levels of Mg in *Morchella vulgaris* 1920 mg/kg, *Helvella lacunosa* 1190 mg/kg, *Lepista nuda* 3410 mg/kg were found (Gençcelep et al., 2009). Liu et al. (2012) reported that the magnesium contents of the mushrooms ranged from 84 mg/kg dw in *Aspropaxillus giganteus* (Sowerby) Kühner & Maire to 550 mg/kg dw in *Macrocybe gigantea*.

Previously reported magnesium contents in mushrooms varied between 800 and 1800 mg/kg dw (Kalač, 2009). The lowest magnesium value, 248 mg/kg dw *Boletus tomentipes* Earle, was found by Li et al. (2011). Sanmee et al. (2003) reported that mature *Astraeus hygrometricus* (Pers.) Morgan had 1600 mg/kg of Mg concentrations. In this study, magnesium concentrations of same mushrooms species were found low. As a result, environmental factors are very important to amount of metal concentrations in mushrooms. Magnesium levels in this study are in agreement with the value reported in the literature.

In the present study, the calcium contents of the mushrooms ranged from 24 mg/kg dw in *Cerioporus squamosus* to 711 mg/kg dw in *Lactarius volemus*. In our previous study, the concentration levels of Ca in *Morchella vulgaris* 870 mg/kg, *Helvella lacunosa* 470 mg/kg, *Lepista nuda* 8800 mg/kg were found (Gençcelep et al., 2009). Previously reported calcium contents of mushrooms varied from 100 to 500 mg/kg dw (Kalač, 2009). The calcium contents in our mushroom samples are lower than the values reported in the literature. The accumulation of metals in mushrooms has been found to be affected by environmental and fungal factors. But, it seems to be higher when compared to the concentrations obtained by Sanmee et al. (2003) (100-2400 mg/kg dw). The results of nutritionally valuable minerals show that twenty four mushroom species contained high amounts of potassium, calcium, magnesium and iron. Most of them contain little lead, nickel, cadmium or copper. Minerals in the diet are required for metabolic reactions, transmission of nerve impulses, rigid bone formation and regulation of water and salt balance (Kalač et al., 2004).

Manganese was also determined in all mushrooms. The manganese content of the mushrooms studied in the present work ranged from 5.64 mg/kg dw in *Russula delica* to 90.64 mg/kg for *Lepista personata*. The Mn toxic limits for plants is 400–1000 mg/kg (Zhu et al., 2011). Some literature reported Mn levels in mushrooms of as high as 21.7–74.3 mg/kg (Mendil et al., 2004), and 14.2–69.7 mg/kg (Soylak et al., 2005) and 7.1-81.3 mg/kg, 5.54-135 mg/kg dw (Falandysz et al., 2017). Literature data showed that the Mn concentrations of the mushrooms ranged from 0.081 to 188.8 mg/kg (Ayaz et al., 2011; Wang et al., 2017). Other studies have also reported varying metal concentrations in

mushrooms (Falandysz et al., 2017; Pelkonen et al., 2006). The manganese values in this study are found almost the same in the literature.

Fe is found in the structure of hemoglobin, whose main function is to carry oxygen, and therefore is an important element. It is known that about 70% of the Fe in the human body is used for hemoglobin production. Fe is the main structural component of myoglobin, which is abundant in muscle cells, as well as hemoglobin. Fe deficiency causes anemia in organisms (Gupta, 2014). The Fe contents of the mushrooms analyzed in the present study were found to range between 44.78 (*Russula integra*) and 236.95 (*Amanita rubescens*) mg/kg dw. According to the literature data, Fe concentrations of the mushroom samples were between 0.04 and 10558 mg/kg (Niemiec et al., 2018; Rasalanavho et al., 2020). Iron values in mushroom samples (as reported) ranged from 31.3-1190 mg/kg (Sesli and Tüzen, 1999), 56.1-7162 mg/kg (Işıloğlu et al., 2001), 50.1-842.0 mg/kg (Gençcelep et al., 2009), respectively. The iron values in the present study are in lower than with reported values in the literature.

Mushrooms are known as zinc accumulators and the sporophore: substrate ratio for Zn ranges from 1 to 10 mg/kg (Işıloğlu et al., 2001). The zinc content was the lowest (24.81 mg/kg dw) in *Lepista personata*, whereas it was highest (119.03 mg/kg dw) in *Russula integra*. The reported literature zinc content ranged between 22.10 and 185 mg/kg dw (Gençcelep et al., 2009; Kalač et al., 2004; Kaya and Bağ, 2010). Sarıkürkçü et al. (2012) found the highest Zn values in *Helvella leucopus* and *Tricholoma auratum* (354 and 356 mg/kg, respectively). The lowest Zn content was found in *Lyophyllum decastes* and *Rhizopogon roseolus* (46 and 47 mg/kg, respectively). In this study, some mushroom species have higher zinc content more than 50 mg/kg, this counts total samples of %50. Therefore, metal accumulation may be more owing to soil pollution. Zn is an essential nutrient that has an important role in biological systems. Zinc is necessary for the functioning of various enzymes and plays an essential role in DNA, RNA, and protein synthesis. The major symptoms of zinc deficiency are delayed growth and slow maturation (WHO, 2004).

Copper with other metals could be transported from the anthropogenic sources of emission to remote areas with moving air masses, but they are largely deposited locally (Nygård et al., 2012). Minimum and maximum values of copper were 11.02 and 174.01 mg/kg dw in *Lactarius deliciosus* and *Russula vinosa*. Copper contents of mushroom samples in the literature have been reported to be in the range of 4.71-51.0 mg/kg (Tüzen et al., 1998) and 10.3-145 mg/kg (Sesli and Tüzen, 1999). Copper contents found in this study parallel those reported in the literature. In this study, *Leccinum versipelle* (102.40 mg/kg) and *Russula delica* (128.94 mg/kg) were collected near the downtown of Erzincan, therefore copper contents of these samples were found higher than the others. In our previous study, the concentration levels of Cu in *Pleurotus ostreatus* 47.1 mg/kg and *Lepista nuda* 26.6 mg/kg were found (Gençcelep et al., 2009). Cu is an essential element. Enzymes containing copper are important for the body to transport and use iron. In 1996, a joint FAO/International Atomic Energy Agency/ WHO official report set an upper limit for the safe range of population mean exposures for adults of 0.2 mg/kg body weight per day (WHO, 1996). According to Gast et al. (1988) Cu may be harmful to both humans and animals when its concentration exceeds the safe limits. Our results are lower than some values reported in literature: 13.4–50.6 mg/kg (Soylak et al., 2005) and 15–73 mg/kg (Sesli et al., 2008).

Nickel was determined all mushrooms. *Russula integra* var. *integra* contained high nickel content with an amount of 2.86 mg/kg dry matter. The reported Ni values for wild-growing mushrooms were 0.4-15.9, 0.4-2, 1.72-24.1 mg/kg (Işıloğlu et al., 2001; Kalač et al., 2004; Soylak et al., 2005), respectively. The Ni levels are generally in agreement with previous studies. The obtained Ni levels in almost all mushrooms are higher than (1 mg/kg dw) the allowed amount (0.05-5 mg/kg) of National Academy of Sciences (1975) for plants and foods (1975) (Table 2). Nickel has been linked to lung cancer and the tolerable upper intake level for this toxic element is reported as 1 mg/day.

Cd is mostly used in batteries, TV screens, lasers, cosmetics, paint and galvanized steel. The most common route of Cd exposure is inhalation and therefore, cigarette smoking is the primary source of contact with the element. Foods most commonly contaminated with Cd include leafy vegetables, crustaceans, offal, rice, water and dietary supplements (Abernethy et al., 2010). Cd exposure induces oxidative stress, deregulation of transport pathways and modification of DNA expression. It also inhibits the synthesis of heme component, deregulates mitochondrial potential by inducing apoptosis and binding with sulfhydryl groups. From a clinical viewpoint, Cd adversely affects kidneys, bones, hematopoiesis, cardio-vascular, immune and endocrine systems. The toxicity of Cd is enhanced by interaction with Pb and As (Bernhoft, 2013). It is a human carcinogen belonging to Group 1 according to the IARC classification (IARC, 2020). Cadmium is known as a principal toxic element, since it inhibits many life processes. Mushroom, in particular, can be very rich in cadmium. Cadmium was measured as small amount detected (0.05 mg/kg dw) in *Boletus edulis* and it was the highest in *Russula vinosa* (22.57 mg/kg dw) which is relatively high compared to reported literature data (Mendil et al., 2005; Kalač et al., 2004). Cd levels were found generally lower than 1.0 mg/kg for the other mushrooms species, in this study. Long-term exposure to high levels of Cd may lead to considerable accumulation in the liver and kidneys, particularly the renal cortex, resulting in kidney damage (WHO, 1989). Thus, cadmium seems to be the most deleterious one among heavy metals in mushrooms. It is acceptable daily or weekly intake may be easily reached by consumption of an accumulating mushroom species (Kalač et al., 2004).

The presence of Pb in the central nervous system may result in behavioural and developmental disorders. Long-term exposure to Pb can cause learning disabilities, attention deficit disorders, brain damage, muscle weakness, anaemia, and renal dysfunction. The symptoms of Pb poisoning include insomnia, depression, headache, fatigue, memory loss, hypertension and cardiovascular diseases. Lead is a cumulative toxin that can primarily affect the blood, nervous system, and kidneys. In the blood at high concentrations, lead inhibits red blood cell formation and eventually results in anemia.The main sources of Pb exposure are urban pollution, some medicines and supplements, electric cables, batteries, petrol, paint, water,

Table 3. DIM and HRI values of wild edible mushroom species

		DIM (µg/kg body weight/ serving)							HRI						
	Ebdible Mushroom	Mn	Fe	Zn	Cu	Ni	Cd	Pb	Mn	Fe	Zn	Cu	Ni	Cd	Pb
1	*Agaricus bisporus*	6.12	60.02	19.71	10.05	0.79	0.11	0.45	0.043	0.20	0.07	0.25	0.04	0.21	0.12
2	*Chlorophyllum rhacodes*	5.92	63.40	28.35	7.71	0.93	0.05	0.48	0.042	0.21	0.09	0.19	0.04	0.10	0.13
3	*Macrolepiota procera*	4.80	35.77	25.81	20.38	0.66	0.14	0.28	0.034	0.12	0.09	0.51	0.03	0.29	0.07
4	*Amanita rubescens*	7.91	101.5	26.17	14.77	1.08	0.06	0.28	0.056	0.34	0.08	0.37	0.05	0.12	0.07
5	*Armillaria ostoyae*	7.61	93.54	14.33	15.41	0.46	2.70	0.44	0.054	0.31	0.05	0.39	0.02	5.40	0.12
6	*Pleurotus dryinus*	9.18	84.76	24.71	10.43	0.82	0.36	0.51	0.065	0.28	0.08	0.26	0.04	0.72	0.14
7	*Pleurotus ostreatus*	8.03	68.58	23.13	15.66	0.75	0.07	0.71	0.057	0.23	0.07	0.39	0.03	0.14	0.20
8	*Polyporus squamosus*	4.32	86.25	10.72	9.00	0.65	0.07	0.45	0.031	0.28	0.04	0.23	0.03	0.14	0.12
9	*Boletus edulis*	8.63	60.94	16.90	9.68	0.82	0.02	0.55	0.061	0.20	0.06	0.24	0.04	0.04	0.15
10	*Neoboletus praestigiator*	4.02	30.62	18.40	10.00	0.40	0.15	0.70	0.028	0.10	0.06	0.25	0.02	0.31	0.19
11	*Leccinum scabrum*	6.94	27.44	14.39	15.40	0.06	0.20	0.45	0.049	0.10	0.05	0.39	0.03	0.40	0.12
12	*Suillus luteus*	5.15	96.51	24.79	14.25	0.70	0.04	0.51	0.036	0.32	0.08	0.36	0.04	0.07	0.14
13	*Lepista nuda*	14.25	51.08	25.13	10.02	0.89	0.35	0.88	0.101	0.17	0.08	0.25	0.05	0.71	0.24
14	*Lepista personata*	38.84	21.40	10.63	6.27	0.68	0.41	0.20	0.277	0.07	0.03	0.16	0.03	0.82	0.05
15	*Hydnum repandum*	10.00	27.18	33.88	16.86	0.71	0.22	0.70	0.071	0.09	0.11	0.42	0.03	0.44	0.19
16	*Lactarius deliciosus*	6.83	33.18	4.72	4.72	0.56	0.59	0.19	0.048	0.11	0.02	0.12	0.02	1.18	0.05
17	*Lactarius piperatus*	6.08	70.74	7.43	7.43	1.09	0.25	0.45	0.043	0.24	0.03	0.18	0.05	0.50	0.12
18	*Lactarius salmonicolor*	16.32	51.05	17.27	17.27	0.98	0.20	0.25	0.116	0.17	0.06	0.43	0.05	0.40	0.07
19	*Lactarius volemus*	2.73	25.32	27.21	62.54	0.90	0.31	0.05	0.019	0.09	0.09	1.56	0.05	0.61	0.08
20	*Russula delica*	2.28	43.66	26.38	6.38	0.87	0.53	0.41	0.016	0.15	0.09	0.16	0.04	1.06	0.11
21	*Russula integra*	5.04	19.20	51.01	30.86	1.22	0.07	0.31	0.036	0.06	0.17	0.77	0.06	0.13	0.08
22	*Russula adusta*	10.89	43.95	16.87	5.16	0.95	1.17	0.45	0.077	0.15	0.06	0.13	0.05	2.34	0.12
23	*Russula vinosa*	13.80	38.18	33.33	74.58	0.98	9.67	0.58	0.098	0.13	0.11	1.86	0.05	19.34	0.16
24	*Neoboletus erythropus*	4.00	39.30	19.96	9.72	0.62	0.14	0.01	0.028	0.13	0.07	0.24	0.03	0.28	0.01
	Rf D^0 (µg/kg body weight/day)	140[3]	300[2]	300[3]	40[3]	20[2]	0.5[2]	3.6[2]							

dust, air, soil, cereals and vegetables grown in soil rich in Pb. A significant source of exposure to lead is via the diet (Markowitz, 2000; Miracle, 2017).

Pb concentrations of mushroom samples were generally low, except *Lepista nuda* with an amount of 2.07 mg/kg dw. The Pb levels of all other samples were not higher compared to the reported Pb values for mushrooms by Tüzen et al. (1998) (2.35 mg/kg), Kalaĉ and Svoboda (2000) (0.5-20 mg/kg) and Kaya and Bağ (2010) (2.166 mg/kg). Sarıkürkçü et al. (2012) found the lowest Pb value in *Lyophyllum decastes* (0.5 mg/kg). This is followed by *Morchella esculenta* (0.9 mg/kg). In *Rhizopogon roseolus*, *Volvoriella gloiocephala* and *Cyclocybe cylindracea*, Pb contents were found equal or above 4.0 mg level (6.2, 5.9 and 4.0 mg/kg), respectively. Pb is used for a number of industrial, domestic, and rural purposes for example, in lead batteries and in leaded petrol (Çayır et al., 2010). The Food and Agriculture Organization and World Health Organization (2004) have set the provisional tolerable weekly intake of Pb at 25 µg/kg bodyweight. For a person with a bodyweight of 60 kg, these mushrooms do not pose a health risk.

The present work, therefore, aims: (1) to evaluate the metal content in several species of wild edible mushrooms harvested from Türkiye, (2) to assess the contribution of mushrooms to the daily intake of several toxic elements, (3) to determine the possibility of evaluated mushrooms as bioindicators of environmental contamination.

3.1. DIM and HRI of the mushrooms

Some metal species are accumulated at high rates by wild edible mushrooms. This can threaten human health. Some metals such as Cu and Cd are found in high concentrations in the human body to perform some metabolic functions. However, the presence of some metals such as Cd and Cr in high concentrations in the body has a toxic effect (Fu et al., 2020). Therefore, DIM and HRI values for fungi species examined in this study were calculated and the results are given in Table 4. According to the data in the Table 4, the amounts of all metals except Cu and Cd in mushrooms were not at levels that pose a threat to human health. However, the Cd concentration in *Armillaria ostoyae* (DIM: 2.70 µg/kg body weight/ serving), *Russula adusta* (DIM: 1.17 µg/kg body weight/serving), *Lactarius deliciosus* (DIM: 0.59 µg/kg body weight/serving), *Russula delica* (DIM:

0.53 µg/kg body weight/serving), and *Russula vinosa* (DIM: 9.67 µg/kg body weight/serving); the Cu concentration in *Russula vinosa* (DIM: 74.58 µg/kg body weight/serving); and *Lactifluus volemus* (DIM: 62.54 µg/kg body weight/serving) were above the reference doses. The reference doses in question can be updated by the competent authorities by following up-to-date developments. For example, while DIM determined for Cd

according to USEPA (2002) was 1.0 µg/kg body weight/serving, this value was updated as 0.36 µg/kg body weight/serving according to EFSA (2011). It was also determined that the HRI values of the Cu concentration in *Lactifluus volemus* (HRI: 1.56), *Russula vinosa* (HRI: 1.86), *Macrolepiota procera* (HRI: 0.51) and *Russula integra* (HRI: 0.77); the Cd concentration in *Armillaria ostoyae* (HRI: 5.40), *Lactarius deliciosus* (HRI: 1.18), *Russula delica (*HRI: 1.06), *Russula adusta* (HRI: 2.34), and *Russula vinosa* (HRI: 19.34) were above 1.0. Therefore, consumption of these mushroom species was considered to be risky in terms of these metals.

3.2. Principal component analysis

Principal component analysis (PCA) is a procedure which is used to reduce dimensions of multivariate data allowing to understand trends and variations in the data set by a reduced number of latent variables. In order to determine whether there exist trends and variations amongst the elements accumulated, PCA was carried out. PCA showed three components accounting for 68.977% of the variability (Fig. 1). A component loading of greater than 0.7 matrix indicates elements which are closely related to each other. The first principal component (36.285%) showed Zn (0.871), K (0.870) and Ni (0.850). The second principal component (19.754%) showed a closer relationship amongst Cu (0.910), Ca (0.694), Mg (0.497) and Cd (0.865). The third principal component (12.938%) showed Pb (0.796) and Fe (0.698) to have a common association in mushrooms.

4. Conclusions

In this study, metal contents of 24 different edible mushroom species collected from Bayburt, Gümüşhane, Rize and Artvin Region of Türkiye were analyzed, and using the data obtained, DIM and HRI values were calculated. According to JECFA (1993), Cd concentrations of *Armillaria ostoyae*, *Russula adusta*, *Lactarius deliciosus, Russula delica* and *Russula vinosa*; the Cu concentration in *Russula vinosa* and *Lactarius volemus*

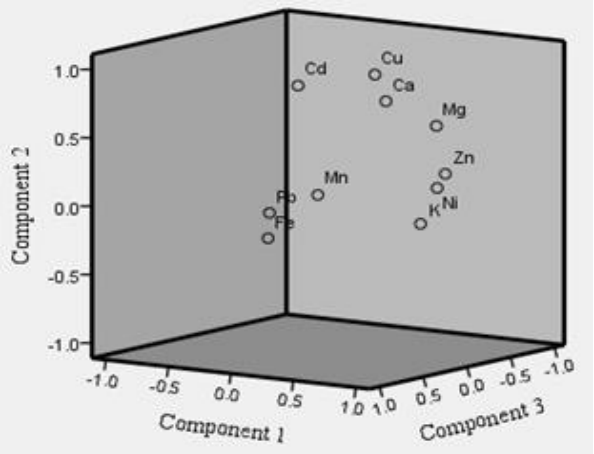

Figure 1. Principal component analysis threeplot of minerals contents of wild mushroom

were above legal limits. Based on these values, HRI values of Cu in *Lactifluus volemus*, *Russula vinosa*, *Macrolepiota procera* and *Russula integra;* the Cd concentration in *Armillaria ostoyae*, *Lactarius deliciosus*, *Russula delica*, *Russula adusta* and *Russula vinosa* were also found to be above 1.0. Therefore, it was concluded that these species collected from Rize Ayder Plateau have been polluted by Cd and Cu, and their long-term consumption may have adverse effects on the biosynthesis reactions in the human body, trigger cancer formation, and cause gastrointestinal symptoms and liver toxicity. These findings reveal that urgent measures should be taken in terms of industrial pollution in the area where samples are collected. Since the sampling area has high traffic intensity, it has been concluded that the emissions of the vehicles should be controlled in terms of legal limits and that the consumption of mushrooms in this region should not be preferred until necessary measures are taken.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

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The influence of tannins purified from Eastern Mediterranean Region plants (*Pinus brutia* Ten. and *Quercus coccifera* L.) on carbon mineralization: Antimicrobial and antimutagenic evaluation

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Doğu Akdeniz Bölgesi bitkilerinden (*Pinus brutia* Ten. ve *Quercus coccifera* L.) saflaştırılan tanenlerin karbon mineralizasyonu üzerindeki etkisi: Antimikrobiyal ve antimutajenik değerlendirme

Abstract: Tannins, which are polyphenols with a wide variety of quality-quantity that control the carbon and nitrogen cycle in forest ecosystems, are very interesting because of their protein binding abilities and forming a complex structure with other compounds. In this study, the purified tannin content of *Pinus brutia* Ten. and *Quercus coccifera* L., the two dominant plant species of the Eastern Mediterranean region, and the effect of these tannins on C dynamics in a forest soil (O and A horizon) were evaluated. In addition, antimicrobial effects of tannin extracts on *Bacillus subtilis*, *Staphylococcus aureus* and *Proteus mirabilis* bacteria by disc diffusion method and antimutagenic effects on *Allium cepa* root tip cells were evaluated. Total phenol (TP) and condense tannins (CT) concentrations of *P. brutia* and *Q. coccifera* leaves ranged from 0.78–1.33 µg/100mg DW and 4.68–1.35 µg/100mg DW, respectively. With the addition of tannin extract to the soils, C mineralization (27th day) was significantly reduced compared to the control group. Both *P. brutia* tannin extract (PTE) and *Q. coccifera* tannin extract (QTE) exhibited antibacterial activity in the range of 8±0.2–35±1.1 mm zone diameter by inhibiting their microbial growth against test microorganisms. In addition, tannin treatments caused a dose-dependent mitotic index decrease in onion root tip cells and a serious inhibition by showing toxic effects on mitotic division stages. As a result, our data showed that C mineralization in soil is affected by different tannin sources and these tannin extracts have significant antimicrobial activity against pathogens and cytotoxic activity in *A. cepa* root tip cells.

Key words: Carbon mineralization, tannin, antimicrobial activity, antimutagenic activity

Özet: Orman ekosistemlerinde karbon ve nitrojen döngüsünü kontrol eden çok çeşitli kalite-niceliğe sahip polifenoller olan tanenler, protein bağlama yetenekleri ve diğer bileşiklerle kompleks bir yapı oluşturmaları nedeniyle oldukça ilgi çekicidir. Bu çalışmada, Doğu Akdeniz bölgesinin iki baskın bitki türü olan *Pinus brutia* Ten. ve *Quercus coccifera* L.'nın saflaştırılmış tanen içerikleri ve bu tanenlerin bir orman toprağında (O ve A horizonu) C dinamiklerine etkisi değerlendirilmiştir. Ayrıca tanen ekstraktlarının disk difüzyon yöntemi ile *Bacillus subtilis, Staphylococcus aureus* ve *Proteus mirabilis* bakterileri üzerindeki antimikrobiyal etkileri ve *Allium cepa* kök ucu hücreleri üzerindeki antimutajenik etkileri değerlendirilmiştir. *P. brutia* ve *Q. coccifera* yapraklarının toplam fenol (TP) ve kondanse tanen (CT) konsantrasyonları sırasıyla 0.78–1.33 µg/100mg DW ve 4.68–1.35 µg/100mg DW arasında değişmiştir. Topraklara tanen ekstraktı ilavesi ile C mineralizasyonu (27. gün) kontrol grubuna göre önemli ölçüde azalmıştır. Hem *P. brutia* tanen ekstresi (PTE) hem de *Q. coccifera* tanen ekstresi (QTE), test edilen mikroorganizmalara karşı 8±0.2–35±1.1 mm zon çapı aralığında antibakteriyel aktivite sergilemiştir. Ayrıca tanen uygulamaları soğan kök ucu hücrelerinde doza bağımlı mitotik indeks azalmasına ve mitotik bölünme evrelerinde toksik etki göstererek ciddi bir inhibisyona neden olmuştur. Sonuç olarak, elde edilen veriler topraktaki C mineralizasyonunun farklı tanen kaynaklarından etkilendiğini ve bu tanen ekstraktlarının patojenlere karşı önemli antimikrobiyal aktiviteye ve *A. cepa* kök ucu hücrelerinde sitotoksik aktiviteye sahip olduğunu göstermiştir.

Anahtar Kelimeler: Karbon mineralizasyonu, tanen, antimikrobial aktivite, antimutajenik aktivite

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1. Introduction

Tannins (condensed-CT and hydrolyzable-HT), which are from polyphenolic plant secondary compounds, affect many ecosystem processes such as soil formation, organic matter mineralization, humus formation, carbon and nitrogen cycle, and they are common in plant flora (Norris et al., 2011; Ingold et al., 2021). Tannins, which constitute an essential part of the carbon pools of the forest ecosystem, are found in different proportions (up to 40%) in the bark, wood, fruit and leaves of the plants. This ratio varies depending on the plant species, environmental factors (precipitation, temperature, salt or water stress, etc.), presence of infection, extraction method-time (soxhlet, maceration, etc.) and solvent used (Kraus et al., 2003; Abilleira et al., 2021). In woody species, tannin concentrations in the leaf are usually between 150 and 250 mg/g DW (Kraus et al., 2004a). Especially the O and A horizons of forest soils with plant communities rich in tannins (such as pine, oak, acacia) have a large amount of

plant-derived tannin content and they meet about 90% of the world tannin production (Pizzi, 2003).

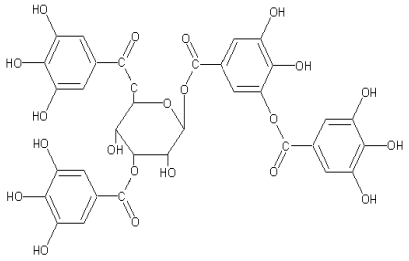

Figure 1. Molecular structure of tannic acid

Soil, which is the main source of organic and inorganic compounds among all ecosystem components, carbon and nitrogen mineralizations occurring in its structure changes over time under the influence of biotic/abiotic factors (Lal, 2002). The microbiological group, which will contribute to the nutrient mobilization required for the life cycle on Earth (Treseder and Lennon, 2015; Bardgett, 2016), has various enzymatic mechanisms for decomposition (Waldrop and Firestone, 2004; Shah et al., 2016). However, soil physical properties (texture, structure, pore size, etc.) and organic materials (plant-animal residues, secondary metabolites) contribute to the relationship between the microbial composition and nutrient cycle in the soil from different perspectives (Cai et al., 2016; Zhang and Marschner, 2017). Tannins, which are a secondary metabolite, can affect the nutrient cycle in terms of edaph by creating toxicity and inhibiting enzyme activities in microorganisms. For example, for tannins, it has been stated that NO_3, which is the source of N that plants can absorb, reduces mycotoxin production in order to reduce the loss in soil, thereby inhibiting nitrification and thus suppressing nitrogen mineralization (Zhang and Laanbroek, 2018; Peng et al., 2018; Elrys et al., 2019). Again, tannins added in the presence of cellulose in the soil showed a negative effect on carbon mineralization by reducing cumulative soil respiration (Madritch et al., 2007). Therefore, tannins help to reduce nutrient loss by slowing down the rapid transition of compounds from organic to inorganic form in the soil. Tannins, which are environmentally friendly secondary metabolites, have an important place in the field of pharmacology due to their many therapeutic properties (anticancer, antioxidant, antimicrobial, etc.) (Gomes de Melo et al., 2010; Ekambaram et al., 2016).

P. brutia (Turkish pine, Pinaceae) and *Q. coccifera* (Kermes oak, Fagaceae) are two important species distributed in the Eastern Mediterranean region of Turkey. In both species, they are used for the production of wood, tannin, seeds and resin, as well as preventing soil erosion (Petins et al., 2021; Abilleira et al., 2021; Jaramillo et al., 2022; Ghazghazi et al., 2022). Valuable secondary metabolites (monoterpenes, tannins, flavonoids, lignans and saponins) in *Pinus* and *Quercus* genera (Ito et al., 2002; Sakar et al., 2005; Sancho-Knapik et al., 2017; Kanchan et al., 2020) provide the use of these plant extracts for phytotherapy purposes (treatment of diabetes, diarrhea, hyperpigmentation and wounds) (Bulut et al., 2017). In addition, the antioxidant (Makhlouf et al., 2018; Zhang et al., 2021), anti-inflammatory (Alizade Naini et al., 2021; Kuo et al.,2021; Yang et al., 2021), antibacterial (Semwal et al., 2018; Mitić et al., 2019; Elkady et al., 2021) and antidiabetic (Zulfqar et al., 2020) effects of *Pinus* and *Quercus* extracts reflect the pharmacological properties of these plants. In recent years, the preference of compounds of natural origin in food additives due to the harm they cause to humans and nature has made medicinal-aromatic plants focus of attention in terms of gastronomy, pharmacology and medicine and has led to an increase in research in this direction.

The majority of tannins, which constitute a significant part of the compounds in the dead plant material that constituent the soil organic matter, originate from the leaves (Qualls and Bridgham, 2005). Therefore, the leaves of forest trees selected as tannin source during the research period were preferred. Antimicrobial activities and antimutagenic effects of tannins have been demonstrated in some previous studies. In this study, it was tried to determine the level of tannins purified from *P. brutia* and *Q. coccifera* grown in the Eastern Mediterranean Region in terms of these effects. Thus, determining the effect of low and high doses of tannins mixed in the soil on carbon mineralization will help to better understand the function of these polyphenols in nature, and also to support the pharmacological importance of these environmentally friendly compounds as an alternative to synthetic inputs.

2. Materials and Method

2.1. Site, plant and soil descriptions

The research area was chosen from Cukurova University Balcalı campus (18.024 da) located in the Eastern Mediterranean Region (Adana, Turkey). The local climate is a subtropical humid Mediterranean climate type with an annual average temperature of 18.7 °C, annual average precipitation 668.7 mm, and annual average humidity of 66% (Adana meteorological station, Adana Meteorology Bureau, 2021) (Fig. 2). The vegetation in the natural habitats of the campus is concentrated on the Pliocene clay deposits and conglomeratic series. The physicochemical properties of the soil (0-10 cm deep) are shown in Table 1. The samples were obtained from areas that best represent the plant communities (*Q. coccifera* and *P. brutia*) and are protected as much as possible from natural and human destruction (Fig. 3). The dominant and characteristic plant species of this region is *Q. coccifera*.

2.2. Physicochemical analysis of plant and soil samples

Soil samples were taken from the rhizosphere of the plant samples (0-10 cm deep). Soil samples were air-dried and sieved (<2mm) for physical and chemical analysis.

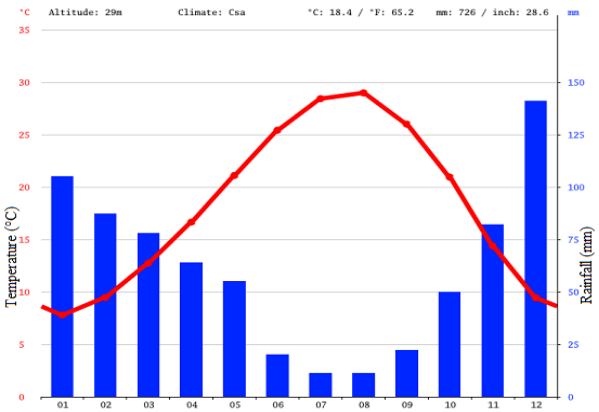

Figure 2. Climate diagram of Adana, Turkey (https://en.climate-data.org/)

Figure 3. *Pinus brutia* (Station 1) (A) and *Quercus coccifera* (Station 2) (B) populations

Leaf samples were cut from parts of the plants up to 1.5 m high to include young shoots and perennial branches, and the dried leaves were ground into powder with a blender. All samples were stored at +4 °C until analysis.

Soil texture was determined by Bouyoucos hydrometer (Bouyoucos, 1951), soil pH was measured at a soil-to-water ratio of 1:1 (w/v) with a pH meter, $CaCO_3$ content (%) by a Scheibler calcimeter, field capacity (%) was determined by vacuum pump. Total nitrogen (%N) and organic carbon (%C) contents of dried and ground plant and sieved soil samples were determined by Kjeldahl method and Anne method (Walkley and Black, 1934).

2.3. Quantitative phytochemical analysis

2.3.1. Tannin extraction

Tannin extract was performed with minor modifications to the procedure described by Makkar (2003). This tannin extract was used in all quantitative assay. For the tannin extract, the ground leaf samples (100 mg) were extracted with cold acetone (70%) in a magnetic stirrer (12 h). After, the extract was twice kept in an ultrasonic homogenizer mixer for 5 min. The extracted samples were centrifuged (3000 rpm, 15 min, 4 °C) and supernatant was stored at +4 ˚C for analysis.

2.3.2. Determination of total phenolic content

Total soluble phenols (TP) in the extract were determined by Folin-Ciocalteu method (Makkar, 2003). In this method, different concentrations of tannic acid (20–80 µg/mL) were used as standard phenolic compound. 500 µL of Folin-Ciocalteu reagent is added to 50 µl of extract in a test tube. After 3 min, 2500 µL of 2% (w/v) Na_2CO_3 were added and mixed well. The final volume was made up to 4 mL with dH_2O. The solution was incubated for 1 h under dark room conditions and measured at 725 nm (T60 UV Visible Spectrophotometer). TP was determined using the calibration curve of tannic acid standard ($y = 0{,}0819x+0{,}1112$ $R^2 = 0{,}9911$).

2.3.3. Determination of total tannins

Polyvinylpolypyrrolidone (PVPP) was used to separate tannin phenols from non-tannin phenols, condensed tannins (CT) were determined by the butanol-HCl-iron reagents (Makkar, 2003). 2 mL tannin extract and 2 mL dH_2O were added to 200 mg of PVPP (tannins precipitate with PVPP) into a test tube. The mixture was vortexed, then kept in cold water for 15 min, again vortexed and centrifuged (3000 rpm, 15 min, 4 °C). Supernatant was stored at +4 ˚C for analysis. The extracts were prepared as in the total phenol content determination and measured at 725 nm. Total tannins (TT) were calculated as the difference between TP and non-tannin phenols (NTP). TP and TT were expressed as tannic acid equivalent (TAE).

2.3.4. Determination of condensed tannins

250 µl of tannin extract, 1.5 mL of Butanol-HCl reagent (95:5 v/v) and 50 µl of Fe ($FeCl_3$ solution) were added to a test tube and vortexed. The test tube was kept in a water bath at 97-100 °C for 1 h. Then the absorbance of solution was measured spectrophotometrically at 550 nm (Bate-Smith, 1975).

2.4. Measurement of the carbon mineralization

Moist soil samples (80 g–80% field capacity) and 40 mL saturated $Ba(OH)_2$ (as an alkaline trap) were placed in 0.75 L incubation vessels for aerobic incubation. Incubation vessels were closed and incubated at 28 °C for 30 d. Empty vessels were used as blanks. The trap was titrated with $C_2H_2O_4$ every 3 d after the precipitation of the carbonates and the CO_2 resulting from microbial respiration was measured. In order to determine the effect of grain on C mineralization, after *Pinus* and *Quercus* soils were prepared as described above, the first day *P. brutia* tannin extract (PTE) (0.8 µg) and *Q. coccifera* tannin extract (QTE) (1.27 µg) were separately added to the soils and incubated (the amount of tannin was determined in accordance with their content). On the 11th and 23rd d of incubation, 2.5 times the first dose of tannin was added to both soil groups and incubation was continued. The effect of tannin on carbon mineralization was calculated separately for a total of 39 days and compared with the control. Cumulative CO_2 production throughout the incubation (39 d) was determined according to a nonlinear equation. First order kinetic model was used for organic carbon mineralization (Bernal et al., 1998).

$C_m=C_o(1-e^{-Kt})$

$C_{m:}$ The mineralised carbon (%C) at time *t* (days)

C_o: The potentially mineralisable C (%C)

K: The C mineralization rate constant (days^{-1})

2.5. Antibacterial activity of tannin extracts

Tannin extracts were tested against the Gram-positive bacteria strains *Bacillus subtilis* (ATCC® 6633), *Staphylococcus aureus* (ATCC® 29213), and the Gram-negative *Proteus mirabilis* (ATCC® 25933) bacteria strain by disc diffusion assay. Fresh bacterial cultures grown overnight were seeded onto Muller-Hinton Agar (MHA) (20 mL) plate medium using a sterile cotton swab. Sterile discs (6 mm in diameter) loaded separately with different concentrations of tannin extract (10-75 µl) were placed on MHA plates inoculated with bacteria strains from fresh culture (1.5 x 10^8 CFU/mL). Acetone (70%) was used as negative control. The inoculated plates were incubated at 37 °C for 24 h and the diameters of the inhibition zones (mm) were measured after incubation (Bauer et al., 1966).

2.6. Onion root sprouting, tannin extract treatment and preparation of mitotic phases from root apical meristem cells

Onion (*A. cepa*) root apical meristems (48 h aged and 2-3 cm length) were used to determine the cyto-genotoxic potential of tannin extracts. Mitotic abnormalities and chromosome morphology were analyzed to evaluate the cyto-genotoxic effects induced by tannin extracts on onion

root tip cells. For this purpose, onion bulbs of similar size were first rooted in distilled water (25–27°C) and after the roots reached a length of approximately 1.5-2 cm, the onion roots were treated with *Pinus* and *Quercus* leaf tannin extracts at different concentrations (1, 2, 4%) for 20 h. Then the roots were fixed in carnoy's fixative (ethyl alcohol:glacial acetic acid (3:1 v/v). The control group was kept in distilled water simultaneously with the treatment groups and their bulbs (1.5-2 cm) were fixed directly in carnoy's fixative. All groups were removed from carnoy's fixative after treatment and maintained in ethyl alcohol (80%). Preparations were prepared according to Feulgen's squash technique (Rencüzogulları et al., 2001). The prepared preparations were examined under a bright-field light microscope to observe and score cellular abnormality.

2.7. Statistical analysis

All experimental results were done in triplicate (n=3). The significant differences between groups were performed via the one-way analysis of variance (ANOVA) and Tukey HSD test. Statistical analyzes were performed with the IBM SPSS Statistics Version 24 package program. $p < 0.05$ was considered as statistically significant.

A clear and complete information should be provided about the materials, and the procedures followed.

3. Results and Discussion

3.1. Plant leaves and soil physiochemical properties and tannin contents

Physicochemical properties of plant leaves and soil samples shows Table 1. Both soil samples are of the same color (brown-red) and have a sandy loam texture. The pH of soils is slightly basic and lime-free and there is no significant difference between them ($p < 0.05$). Field capacity of *P. brutia* and *Q. coccifera* soils were 26.23% and 28.34%, respectively. Although the highest C/N ratio in both leaf and soil was determined in *P. brutia* soil at 52.37 and 15.67, respectively, the highest nitrogen content was observed in *Q. coccifera* soil at 1.34% and 0.26%, respectively. Also, although the highest TT content (40 µg/100mg) was determined in *Q. coccifera* leaves, CT content was higher in *P. brutia* leaves (4.68 µg TAE/100mg) (Table 1). Crop production inputs are the main source of carbon and nitrogen in soils (Updegraff et al., 1995; Bridgham et al., 1998). The C/N ratio is significantly affected by the diversity of tree species, plant litter, roots and microbial communities in the research areas (Landesman and Dighton, 2010). Differences in quality and quantity of aboveground and underground inputs transferred from tree species to soil have indirect effects on soil pH, C/N ratio, organic matter and tannin content (Lovieno et al., 2010). In a study, changing C/N ratio, organic matter and pH were observed in the soil with different tree species (*Mytilaria laosensis* and *Cunninghamia lanceolata*) (Wan et al., 2015). Leaf nutrient content, which is a very important component in the nutrient cycle, is affected by various edaphic factors (physicochemical structure and nutrient content of the soil, moisture, temperature, microorganisms, etc.) (Liu et al., 2017; Wu et al., 2023). In our analyzes, the effectiveness of edaphic factors on plants was once again demonstrated by the consistency of C and N ratios obtained in soil and leaves on a species basis.

Tannins, which contribute to the nutrient cycle by affecting the organic matter degradation, mineralization, carbon and nitrogen ratios in the soil (Kraus et al., 2003), constitute an important part of terrestrial biomass carbon (Hernes and Hedges, 2000). The highly variable tannin concentrations in plants are associated with a large amount of genetic and environmental variation (Northup et al., 1995; Siemens et al., 2002). Even in different parts of a plant such as leave, root, stem, fruit, there are different tannin concentrations (Kraus et al., 2004b). In this study, tannin production in plants also differed according to species. In previous studies, *P. muricata* (198 mg CE/g), *P. radiata* (93.7 mg CE/g), *P. rijida* (70.4 mg CE/g), *P. densiflora* (19.6 mg CE/g) (Ku et al., 2007), *P. pinaster* (5.15 mg CE/g) (Chupin et al., 2013), *Q. incana* (38.96-46.85 mg CE/g) (Makkar and Singh, 1991) condensed tannin and *Q. robur* (44-57 mg TAE/g) (Gonzalez-Hernandez et al., 2003) total tannin contents were determined in different pine and oak species, which is consistent with our results.

3.2. Carbon mineralization in soils of tannin extract treatment

A significant decrease in C mineralization was observed from the first day in both soils with added tannin compared to the control group and in both treatment groups, this microbial activity is very close and parallel to each other ($p<0.01$). It can be said that C mineralization in *Q. coccifera* soil was slightly higher than *P. brutia* soil among the control groups. This can be explained by the *Q. coccifera* organic matter being more suitable for soil microorganisms. Although the acceleration in the carbon mineralization of the control and treatment groups continued periodically until the 23rd day, a serious slowdown was observed in the carbon mineralization rate after the last tannin addition to the treatment soils and even after the 27th day, the mineralization activity tended to stop (Figs. 4, 5). The significant difference in carbon mineralization between control and application soils clearly

Table 1. Physicochemical properties of plant leaves and soil samples (0–10 cm deep)

Plant material	Physicochemical properties												Tannin contents of leaves (µg TAE/100mg)		
	Soil									Leave					
	Texture (%)														
	Sand	Silt	Clay	Field capacity (%)	pH	$CaCO_3$ (%)	C%	N%	C/N	C%	N%	C/N	Total tannin	TP	CT
Pinus brutia	57.93 ±0.09	27.13 ±0.13	14.93 ±0.04	26.23 ±0.77	7.80 ±0.05^{a}	1.45 ±0.75^{a}	22.56 ±0.43^{a}	0.14 ±0.00^{b}	15.67 ±0.59^{a}	52.86 ±0.31^{a}	1.01 ±0.02^{b}	52.37 ±0.74^{a}	29 ±0.72^{b}	0.78 ±0.0^{b}	4.68 ±0.12^{a}
Quercus coccifera	50.35 ±0.27	13.20 ±0.50	36.43 ±0.28	28.34 ±1.36	7.26 ±0.02^{b}	0.26 ±0.18^{b}	23.55 ±0.77^{a}	0.26 ±0.00^{a}	8.97 ±0.34^{b}	50.94 ±0.41^{b}	1.34 ±0.02^{a}	38.04 ±0.86^{b}	40 ±0.81^{a}	1.33 ±0.04^{a}	1.35 ±0.03^{b}

Note: The experiments were conducted in triplicate independently (n= 3), and the data are expressed as the means ± standard error (SE) with p<0.05

shows the antimicrobial effect of tannin on soil microorganisms (Halvorson and Gonzalez, 2008; Adamczyk et al., 2013). In addition, the decrease in C mineralization in the control groups in the last days of the experiment may be associated with the presence of more difficult to decompose or resistant organic materials in the environment. Fallen leaves to the soil are one of the main sources of primary (carbohydrate, protein, oil), secondary metabolites (polyphenols, alkaloids, etc.) and inorganic compounds. Since carbohydrates and proteins are generally susceptible to microbial degradation, they can quickly become involved in the nutrient cycle (Weiss and Simon, 1999). But, tannins, a class of polyphenols, can affect the biogeochemical cycle in ecosystems by limiting microbial activity (Kuiters, 1990; Kraus et al., 2003). Again, tannins added to the soil may be involved in ionization and oxidation reactions that generate some reactive substances such as phenolate ions and quinones (Rimmer, 2006). However, tannins can polymerize (with reversible ionic or irreversible covalent bonds) the C and N in the soil solution, turning them into more difficult to break down, stubborn substances or heavier molecules (Zibilske and Bradford, 2007). Thus, tannins can help prevent the loss of available C and N and extend their residence time in soil. This interaction appears as an important natural ecological cycle

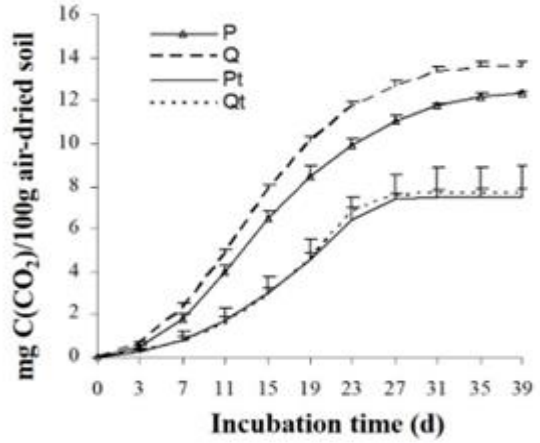

Figure 4. Cumulative C mineralized of soils during 39 days at 28ºC (mean ± standard error, mg $C(CO_2)$/100g oven-dried soil, n=3), P: *P. brutia* soil without treatment, Q: *Q. coccifera* soil without treatment, Pt: *P. brutia* soil with tannin added, Qt: *Q. coccifera* soil with tannin added

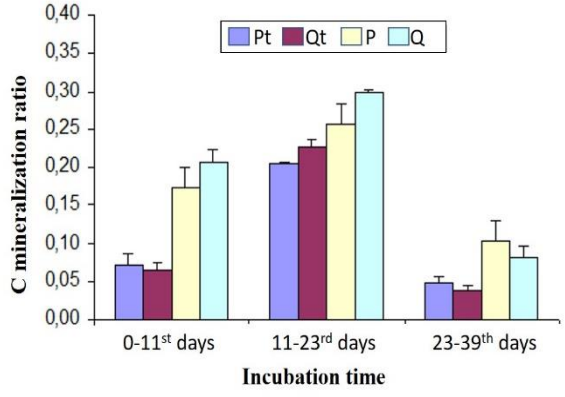

Figure 5. Soil carbon mineralization rates (mean ± standard error, n=3), P: *P. brutia* soil without treatment, Q: *Q. coccifera* soil without treatment, Pt: *P. brutia* soil with tannin added, Qt: *Q. coccifera* soil with tannin added

to preserve soil identity in the longer term by preventing rapid organic matter loss in the soil.

3.3. Antibacterial effect of tannin extracts

The antimicrobial potentials of PTE and QTE were evaluated against Gram-positive (*B. subtilis*, *S. aureus*) and Gram-negative bacteria (*P. mirabilis*) by disc diffusion method. Zones of inhibition (ZOI) exhibited by tannin extracts at different concentrations (10, 30, 45, 60 75 µl) and negative control (70% acetone) against each bacterial strain are shown in the Table 2 and Figure 6. ZOI (mm) indicates that microorganisms could not proliferate around the sample-loaded disc. The ZOIs of PTE and QTE were larger and clearer compared to the control. Antibacterial potentials of tannin extracts were dose dependent against *B. subtilis*, *S. aureus* and *P. mirabilis*. PTE and QTE applied to pathogenic microorganisms at different concentrations had the potential to inhibit their microbial growth at varying rates. The microorganism most affected by both tannin extracts was *S. aureus* and the most resistant microorganism was *B. subtilis*.

It is a frequently encountered and sometimes inevitable fact that different secondary metabolites are included in the extract along with the target molecule during the extraction stage (Jones and Kinghorn, 2012). The fact that PTE is the most effective inhibitory extract against the tested microorganisms can be explained by the species-specific composition of the *Pinus* tannin, as well as the inclusion of other phytochemicals in the extract along with tannin during the extraction. The variety and concentration of phytochemicals of different plant species cause variable inhibitory effects on each microorganism (Nouri et al., 2014; AlSheikh et al., 2020). The difference in ZOIs obtained in the study is due to both the extract concentration and the varying degrees of antibacterial properties of the species-specific phytochemicals. Overall, in the present study, PTE and QTE showed significant antimicrobial potential against the tested microorganisms. Similarly, antimicrobial properties of some *Pinus* and *Quercus* extracts have been shown against *B. subtilis*, *S. aureus* and *P. mirabilis* bacteria, supporting our results. Zone diameters of 24 mm and 20 mm were obtained by applying *Q. infectoria* ethanol extract against *B. subtilis* and *S. aureus* bacteria, respectively (Satirapathkul and Leela, 2011). In another study, *Quercus variabilis* ethanol extract exhibited 10.89mm inhibition for *S. aureus* (Zhou et al., 2019). In the literature, in terms of *Pinus* extracts, on *B. subtilis* and *S. aureus* bacteria the aqueous extract of *Pinus massoniana* formed 21.8 mm and 13.7 mm (Feng et al., 2010) zone diameters and the aqueous extract of *Pinus roxburghii* formed 22.6 mm and 21.6 mm (Kaushik et al., 2013) zone diameters, respectively. Kim et al. (2013) observed that essential oils obtained from 3 *Pinus* species (*Pinus densiflora*, *Pinus thunbergii*, *Pinus rigida*) had mild antimicrobial activity against *B. subtilis*, and the most effective essential oil for *S. aureus* belonged to *P. thunbergii*. In another study, it was stated that the essential oil of *P. roxburghaii* had moderate inhibitory activity against *S. aureus* (Hassan and Amjid, 2009). The antimicrobial properties in plants are attributed to the presence of some secondary metabolites such as tannins,

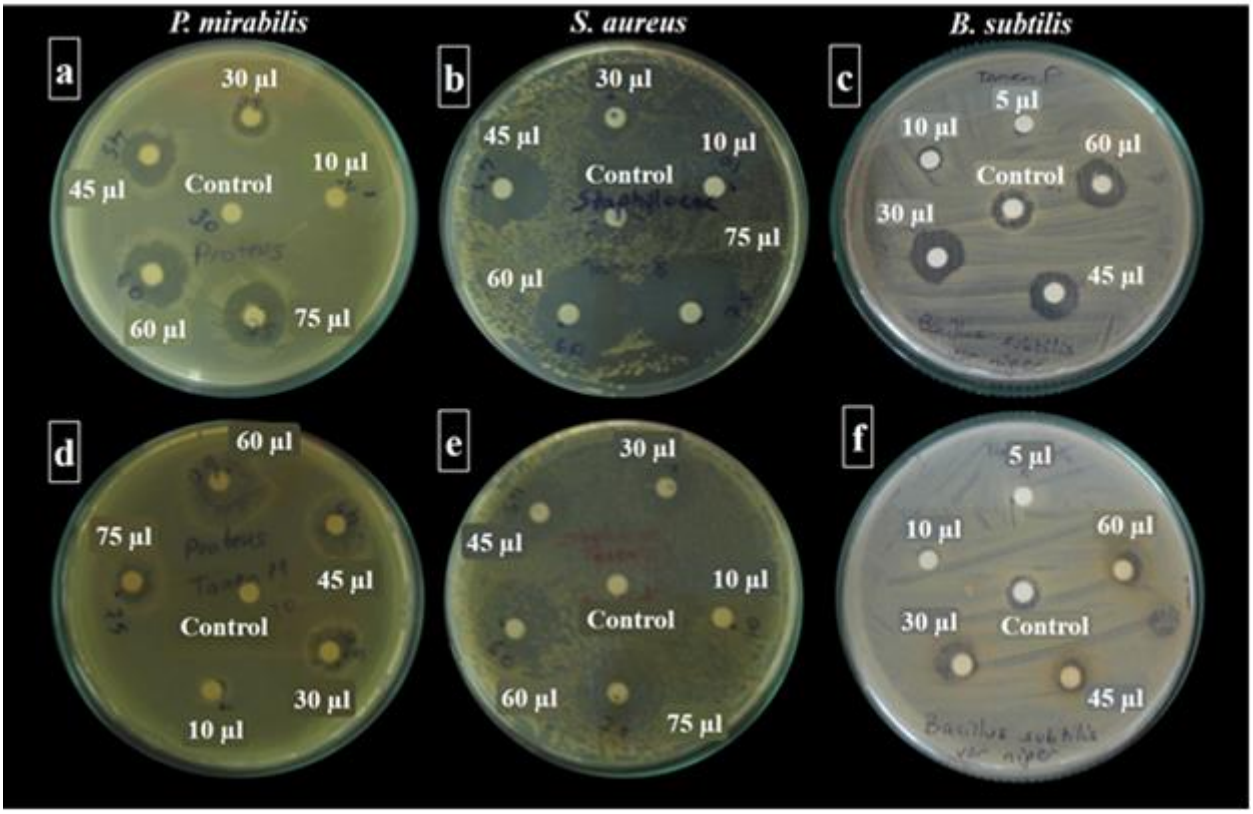

Figure 6. Antibacterial activities of (a-c) *P. brutia* tannin extract, (d-f) *C. coccifera* tannin extract, Acetone (70%) as negative control (all the experiments were performed in triplicate)

Table 2. Antibacterial activity of tannin extracts against pathogenic strains

Pathogenic bacteria	Extract	ZOI (mm)					
		Control	Tannin extract concentrations (µl)				
		Acetone (70%)	10	30	45	60	75
B. subtilis	*P. brutia*	10±0.2	NA	17±0.3	18±0.4	18±0.3	-
	Q. coccifera	7±0.4	NA	12±0.2	8±0.1	9±0.4	-
S. aureus	*P. brutia*	NA	16±0.8	17±0.5	27±0.3	30±0.4	35±1.1
	Q. coccifera	NA	8±0.2	20±0.2	24±0.5	30±0.7	31±0.9
P. mirabilis	*P. brutia*	NA	NA	14±0.4	18±0.7	21±0.6	23±0.5
	Q. coccifera	NA	NA	14±0.2	16±0.3	20±0.3	21±1.4

Note: Data are means of triplicate (n=3) ± standard error, $p<0.05$. NA (no activity)

quinones, alkaloids, phenols, flavonoids, terpenoids, glucosinolates. Tannins bind to the cell wall of bacteria and inhibit protease, thereby preventing the growth of bacteria. Thus, the ability of tannins to disrupt the metabolism and protein synthesis of microorganisms imparts important antibacterial properties to these compounds (Wafa et al., 2016; Chandra et al., 2017).

3.4. Effect of tannin extracts on mitotic index, mitotic phase frequency and abnormality of *A. cepa* root meristem cells

The mitotic index (MI), which is considered an indicator of cell cycle progression, is an important test to detect biotic or abiotic factors that pose a risk to DNA during cell divisions (Ray et al., 2013). PTE and QTE (1, 2, 4% (w/v)) were evaluated effects on mitotic phase frequency and mitotic abnormalities in onion root tip cells. MI caused by tannin extract treatments at all stages of mitosis is given in Table 3 in comparison to the control group. The obtained data show that MI in root apical meristem cells varies according to the growth period. MI (48 h) in untreated meristem cells (control) was 5.05±0.21. All doses of PTE and QTE significantly reduced MI in onion root tip cells compared to control. Dose-related decrease in MI (2.26±0.09% - 0.13±0.04%) is quite evident, especially in PTE (1-4%) treated samples. Also, both PTE and QTE significantly decreased the % rates of mitosis stages in onion root tip cells ($p<0.001$). In general, tannin extracts of both plants inhibited metaphase, anaphase and telophase at the highest dose due to toxicity. Although MI below 50% is considered a non-lethal effect (Kundu and Ray, 2017), it has been observed that tannin extract has an arresting effect on the cell cycle progression mechanism in meristematic cells and this explains the anti-proliferative activity of tannin extract in overdose. 1% solutions of PTE and QTE increased the percentage of abnormal cells by 4.35±1.32 and 6.25±1.17, respectively, compared to the control, while at the 2% concentration, this ratio regressed to 1.23±0.64 and 3.56±1.09, respectively. In addition, QTE treatment caused more abnormal cells (%) in onion root meristem cells. At the highest concentration (4%) of both tannin extracts, no abnormal cells could be detected; This may be

Table 3. The MI, the ratio of mitosis stages and abnormal cells in the root tip cells of *A. cepa* treated with tannin extracts

Treatments	Concentration (%)	Prophase (%)	Metaphase (%)	Anaphase (%)	Telophase (%)	MI (%)	abnormal cells (%)
Control		15.75±1.10	10.00±0.81	6.00±1.08	18.75±0.85	5.05±0.21	ND
PTE	1	11.00±0.44**	2.83±0.48**	1.83±0.36**	7.00±0.62***	2.26±0.09***	4.35±1.34*
	2	7.91±0.28***	1.33±0.44***	0.75±0.37**	6.75±0.39***	1.66±0.11***	1.23±0.64*
	4	1.33±0.44***	ND	ND	ND	0.13±0.04***	ND
QTE	1	11.16±0.40**	5.16±0.50*	3.50±0.55*	9.25±0.68**	2.90±0.16**	6.25±1.17*
	2	9.08±0.35***	2.66±0.51**	2.66±0.68**	6.41±0.58***	2.08±0.14***	3.56±1.09*
	4	1.83±0.66***	ND	ND	ND	0.17±0.06***	ND

*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$. ND: Not determined

associated with the inhibition caused by toxicity in the cells and the proportionally low number of cells.

In this study, it is clear that tannin extracts have a cytotoxic effect by inhibiting mitosis in onion root tip cells. Unlike other polyphenols, tannins stand out with their ability to bind to proteins, pigments, basic and large molecule compounds, and exhibit antioxidant, anticancer and antimicrobial activities (Gomes de Melo et al., 2010). It is stated that tannins provide proliferation in healthy cells and rapid closure of the wound (Antunes-Ricardo et al., 2015), as well as have a toxic effect especially against cancer cells. For example, Cuphiin D1 (CD1), the hydrolyzable tannin isolated from *Cuphea hyssopifolia* showed antiproliferative activity against HL-60 cancer cell line (Wang et al., 2000), while ellitannin isolated from *Cistus ladanifer* showed antiproliferative activity against M220, MCF-7/HER2 and JIMT-1 cancer cell lines (Barrajon-Catalan et al., 2010). The fact that bioactive molecules of plant origin, such as tannin, act in animal cells, similar to plant cells (inhibit or encourage) (Ray et al., 2013; Barman and Ray, 2022), gives an idea about the working mechanisms of these important molecules.

4. Conclusion

Tannins extracted from 2 different plant sources (*P. brutia and Q. coccifera*) caused significant differences on C mineralization in soils. This result may be due to the different resistance of the substance components used in the study against microbial decomposition. The presence of these substances resistant to decomposition in the soil prevents the rapid decomposition of organic materials of vegetable or animal origin added to the soil and provides a support in terms of soil fertility. In addition, the present study reveals the significant antimicrobial activity of PTE and QTE against gram positive and gram negative pathogens. In this respect, such extracts are among the natural agents against antibiotic-resistant microorganisms. Again, although tannin has known therapeutic potential, cyto-genotoxic effects (increased mitotic abnormality and mitotic index) induced by tannin extract on onion root tip cells were demonstrated with this study. For this reason, the indiscriminate use of medicinal and aromatic plants containing tannins should be avoided and carefully recommended for drug treatment by paying attention to the dosage. In order to determine and understand the biotic and abiotic reaction interactions between tannin and tannin-like phenolics and soil organic matter composition (humic acid, fulvic acid, glomalin, etc.), its place and effect in the nutrient cycle it should also be evaluated in terms of different plant species and thus more work is needed in this scope. *In vitro* and *in vivo* comparative studies with other pharmacological models of the Eastern Mediterranean region, which has an extremely rich biodiversity, and the purification and identification of possible molecule(s) responsible for pharmacological activity will further increase the importance of the flora of this region.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Contributions

The authors contributed equally.

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Pollen performance of pomegranate under high-temperature stress

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Yüksek sıcaklık stresi altında narın polen performansı

Abstract: The high-temperature stress (30 °C, 35 °C, 40 °C) response of pollen performance in *Punica granatum* was analyzed. Pollen germination rate and tube length were significantly inhibited after 35 °C and 40 °C treatment. According to cumulative stress response index values, 40 °C had the most destructive impact. High-temperature stress caused various abnormalities at tubes, especially at apex and the most common abnormalities were marked change of elongation direction and swelling. Although dense callose accumulation and increase in apex-localized reactive oxygen species was noticed at the apex after 35 °C and 40 °C temperature treatment, the most harmful temperature was stated as 40 °C.

Key words: High-temperature, pollen germination, pollen tube, *Punica granatum*

Özet: Bu çalışmada *Punica granatum*'da polen performansının yüksek sıcaklık stresine (30 °C, 35 °C, 40 °C) olan tepkisi analiz edildi. Polen çimlenme oranı ve tüp uzunluğu 35 °C ve 40 °C uygulamasından sonra istatistiksel olarak anlamlı bir şekilde azaldı. Kümülatif stres tepki indeksi değerlerine göre, 40 °C polen performansı üzerinde en yıkıcı etkiye sahipti. Yüksek sıcaklık stresi polen tüplerinin özellikle uçlarında çeşitli anormalliklere neden oldu. En yaygın görülen anormallikler uzama yönünün değişmesi ve tüp uçlarının şişmesiydi. 35 °C ve 40 °C uygulamalarından sonra tüp uçlarında yoğun kalloz birikimi ve reaktif oksijen türlerinde artış görülmesine rağmen, en yıkıcı yüksek sıcaklık stresi 40 °C olarak belirlendi.

Anahtar Kelimeler: polen çimlenmesi, polen tüpü, *Punica granatum*, yüksek sıcaklık

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1. Introduction

Punica granatum L. (pomegranate), a member of the *Punicaceae* family, is one of the oldest plants in the world which has commercial and cultural importance. Since it is a prominent crude ingredient for the pharmaceutical, cosmetic, beverage, and food industries, the economic value of pomegranate fruits is very high (Khemakhem et al., 2021). Pomegranate fruits are frequently preferred in people's daily diets due to its vitamin, mineral, phenols, flavonoids, and antioxidant content (Boussaa et al., 2020). Also, demand for pomegranate fruit has been increasing rapidly, especially in recent years, after the protective impacts of pomegranate against Alzheimer's disease and cancer were revealed in all details (Teniente et al., 2023). Parallel with increasing demand, the definition of the agents influencing the yield of pomegranate and the studies to increase the yield has accelerated, and these topics have started to attract more attention from researchers (Moga et al., 2021). Reproductive biology is the most important features that needs to be studied while carrying out studies about these topics in such an important medical and economic plant.

Pomegranate has perfect flowers and functional staminate flowers on the same tree. Staminate flowers are small and don't form fruits due to the they have rudimentary ovary. However, they are important and necessary for successful pollination due to they produce a large number of fertile pollen grains. Perfect flowers are large and, have functional ovary which forms fruit (Wetzstein et al., 2011). Although they also produce pollen grains, pollen viability of perfect flowers is largely lower than pollen viability of staminate flowers (Engin and Hepaksoy, 2023). Besides, pomegranate shows protogynous dichogamy that pistil gains reproductive activity much before the anther dehisces (Morbey and Ydenberg, 2001; Engin and Hepaksoy; 2003). When the pistil of the hermaphrodite flower gains reproductive activity, anthers of the hermaphrodite flower have not yet begun to pollen shedding (Çetinbaş-Genç and Ünal, 2017). So, the pistil of the hermaphrodite flower is mostly pollinated and fertilized by pollen grains of the male flowers. In this way, fertilization and therefore fruit formation mostly depend on pollen performance (PP) of male flowers in pomegranate (Engin and Hepaksoy, 2003). For this reason, it is very important to know the impacts of various factors that adversely affect PP while carrying out studies to increase yield in pomegranate because it could enable the producer to take precautions against these factors.

PP can be monitored by various parameters. The first parameter to be used to examine PP is pollen germination (PG), because PG is the first step pollen tube (PT) formation, which will ensure the move of generative nucleus to the embryo sac (ES) in the latter stages (Cascallares et al., 2020). Since the PT should be sufficiently lengthy to arrive the ES, it is also very important to evaluate the PT length when examining PP. Also, the cumulative stress response index (CSRI) computed using PG rate and PT length can also give an idea about PP (Dai et al., 1994). PT enter the ES through the micropylar gap of the ovule, and the diameter of this gap is generally proportional to the PT diameter. A morphological

abnormality in the PT may prevent the PT entering the ES via the micropyle opening. Therefore, it is very important for the PTs to maintain their unique dome-tipped cylinder shape during the PT elongation process for successful fertilization, and abnormalities seen in the PTs are considered a negative condition in PP evaluations (Srinivasan et al., 1999). Moreover, alterations in the unique construction of the PT wall mainly consisting of callose and cellulose (also pectin) are also used to evaluate PP. Cellulose is found throughout the PT including the apex, while callose is found along the PT except for the apex (Hao et al., 2013). The absence of callose at the apical region is necessary for the PT elongation (Wang et al., 2003). That's why the accumulation of callose at the PT apex is considered a low PP. Also, tip-localized ROS is essential to ensure the proper PT elongation and regulate the stress responses (Scholz et al., 2020). Due to the tip-localized ROS is over accumulate under abiotic stress conditions, changes in tip-localized ROS accumulation can be utilized as a mark to measure the damage in PP under stress conditions (You and Chan, 2015).

High-temperature stress (HTS) is a restraining agent for both the vegetative and generative growth of plants. Especially the HTS exposure during the sexual reproduction process causes significant losses in product productivity (Ferguson et al., 2021). Although it is known that gametophytes are susceptible to temperature changes, pollen grains, which are male gametophytes, are more sensitive to temperature changes as they have an active interaction with the environment. Sudden temperature changes or extreme day-night temperature differences caused by global warming reduce the PP of the species and cause a decrease in yield (Zhu et al., 2021). That's why PP is mostly used as an indicator for the determination of impacts generated by temperature change (Mesihovic et al., 2016). In many studies, the impacts of HTS on the evaluation factors mentioned above have been examined separately. For example, HTS has been shown to reduce PG and PT elongation in many species such as groundnut (Kakani et al., 2002), olive (Koubouris et al., 2009), tobacco (Parrotta et al., 2016) and almond (Sorkheh et al., 2018). Also, it has been determined that HTS causes changes in tube morphology in various species such as *Arabidopsis thaliana* (Boavida and McCormick, 2007) and tea plants (Wang et al., 2016). Also, it has been noticed that HTS changes the ROS accumulation and cell wall properties in PTs of tomatoes (Muhlemann et al., 2018). However, the number of studies in which a general evaluation is made by using all parameters is very few. Many researchers have conducted various studies examining the quality of pomegranate pollen (Gadze et al., 2011; Engin and Gökbayrak, 2016) and have carried out various studies to increase PP with various plant growth regulators (Gökbayrak and Engin, 2018; Korkmaz and Güneri, 2019). However, there are no studies indicating the impacts of HTS on pomegranate pollen grains.

The main objective of this study to investigate the PP of pomegranate under HTS, using the PP evaluation factors such as germination rate, PT length, PT morphology, callose accumulation, tip-localized ROS accumulation. The results can provide useful information to increase PP, fertilization success, and fruit set for plants grown in heat-prone regions, especially pomegranate.

2. Materials and Method

Staminate flowers were selected in 2019 spring from three different healthy trees of *Punica granatum* L. located in Akçakoca/Düzce (Türkiye). No chemical was applied to the trees since 2014 summer. Collected pollen grains were germinated at 30 °C, 35 °C and 40 °C for 3 h in PG media with 12% sucrose (Brewbaker and Kwack, 1963; Korkmaz and Güneri, 2019). Germinated pollen grains at 25 °C were used as control. PG rates, PT lengtHTS and PT abnormality rates were calculated by counting 150 pollen grains for each group. Observations were made by a light microscope. CSRI was computed to appreciate the HTS response of PTs using PG rates and PT lengtHTS of control and treatment groups with Dai's equation (Dai et al., 1994). PTs were labelled with 0.1% aniline blue for callose accumulation and with 5-(and 6-)chloromethyl-2′,7′-dichlorodihydrofluorescein diacetate (H_2DCFDA) for tip-localized ROS, and investigated respectively at 455-nm wavelengtHTS and 500-nm with an Olympus BX-51 fluorescence microscope (Chen et al., 2007). Fluorescence intensities (FIs) of aniline blue and H_2DCFDA were calculated in a 100-μm^2 zone of the 60 tube tip by the "Rectangle Selection" option of ImageJ. Statistical examinations were executed with the SPSS 16.0 software. The significance of the difference between groups of data was defined by the one-way analysis of variance (ANOVA) with a threshold P value of 0.05. Different characters in graps specify the statistically significant differences and error bars show the standard deviations.

3. Results

To observe the preliminary impact of HTS on PP, PG rate and PT length were calculated. Based on the results, no statistically significant alterations were monitored in PG rate and PT length at 30 °C treatment when compared with the control. Nevertheless, the PG was significantly decreased by 15.62% at 35 °C and 32.44% at 40 °C when compared control. Besides, PT length was significantly decreased by 12.16% at 35 °C and 22.7% at 40 °C in comparison with the control (Figs. 1a,b). The CSRI score of HTS treated groups was reckoned using the PG and PT length. According to the CSRI score, all groups showed negative data, indicating all high-temperature treatments had a minus impact on PP. CSRI score was -2.96 for 30 °C, -27.28 for 35 °C and -55.22 for 40 °C. As the CSRI scores show, the minimum detrimental impact was determined at 30 °C while the maximal detrimental impact was identified at 40 °C. To distinguish between the impact of HTS treatments on tube morphology, PT abnormalities were examined and it was determined that HTS caused various abnormalities at PTs, especially at apex. The most common abnormalities were sharp change of elongation direction and swelling (Fig. 1c). PT abnormality rate was significantly increased by nearly 2-fold at 30 °C and 35 °C while it was significantly increased by nearly 3-fold at 40 °C (Fig. 1d).

To examine the impact of HTS on the callose distribution of PT wall, PTs were stained by aniline blue. According to aniline blue staining, callose was localized throughout the PT except apex at control and 30 °C. However, intensive callose accumulation was noticed at the apex after 35 °C and 40 °C treatment (Fig. 2a). FI of callose was significantly increased by 59.31% at 35 °C and 196.58% at 40 °C when compared control (Fig. 2b). To investigate the

impact of HTS on the tip-localized ROS accumulation, PTs were labelled by H_2DCFDA. According to results, although PTs of all groups demonstrated an obviously tip-localized ROS, the ROS was more intense especially after 35 °C and 40 °C treatment, when compared with the control (Fig. 2c). FI of ROS was significantly increased by 24.77% at 35 °C and 43.79% at 40 °C when compared control (Fig. 2d).

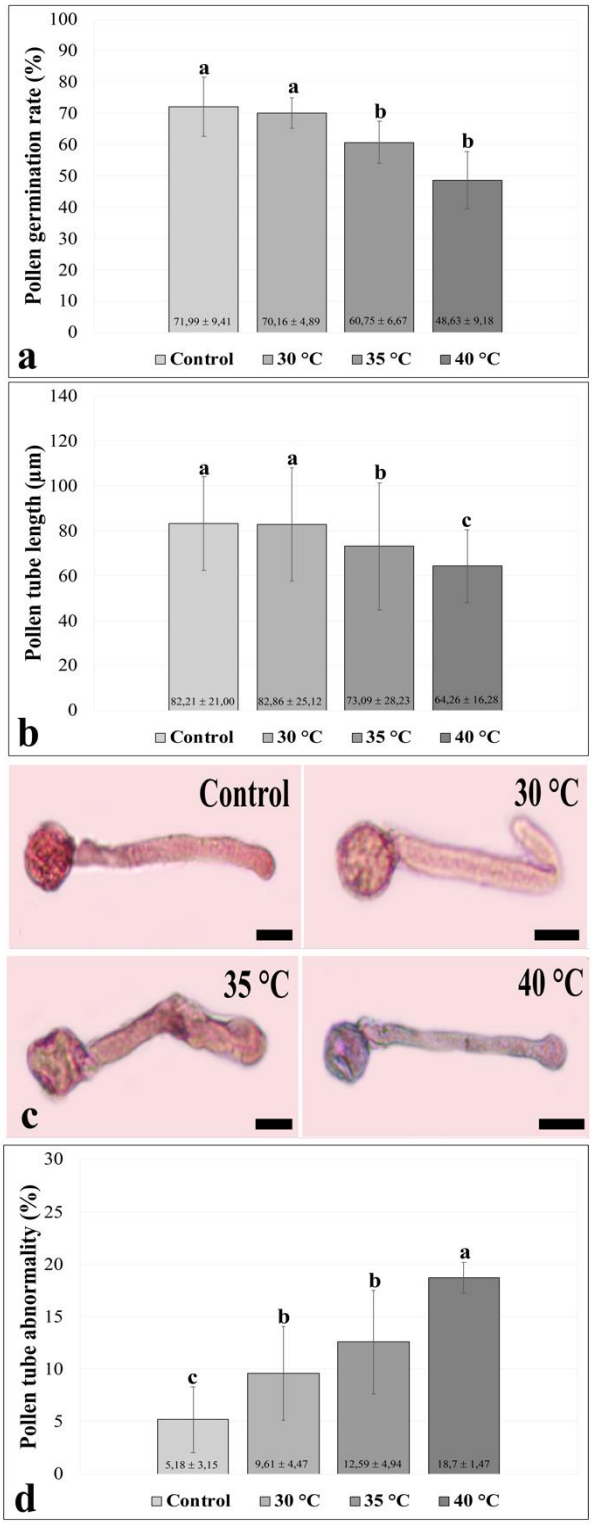

Figure 1. The impact of HTS on PG, PT length and PT morphology in *P. granatum*. **a.** PG rate, **b.** PT length, **c.** Exemplary images of PT abnormalities, **d.** PT abnormality rate. Bar: 10 µm.

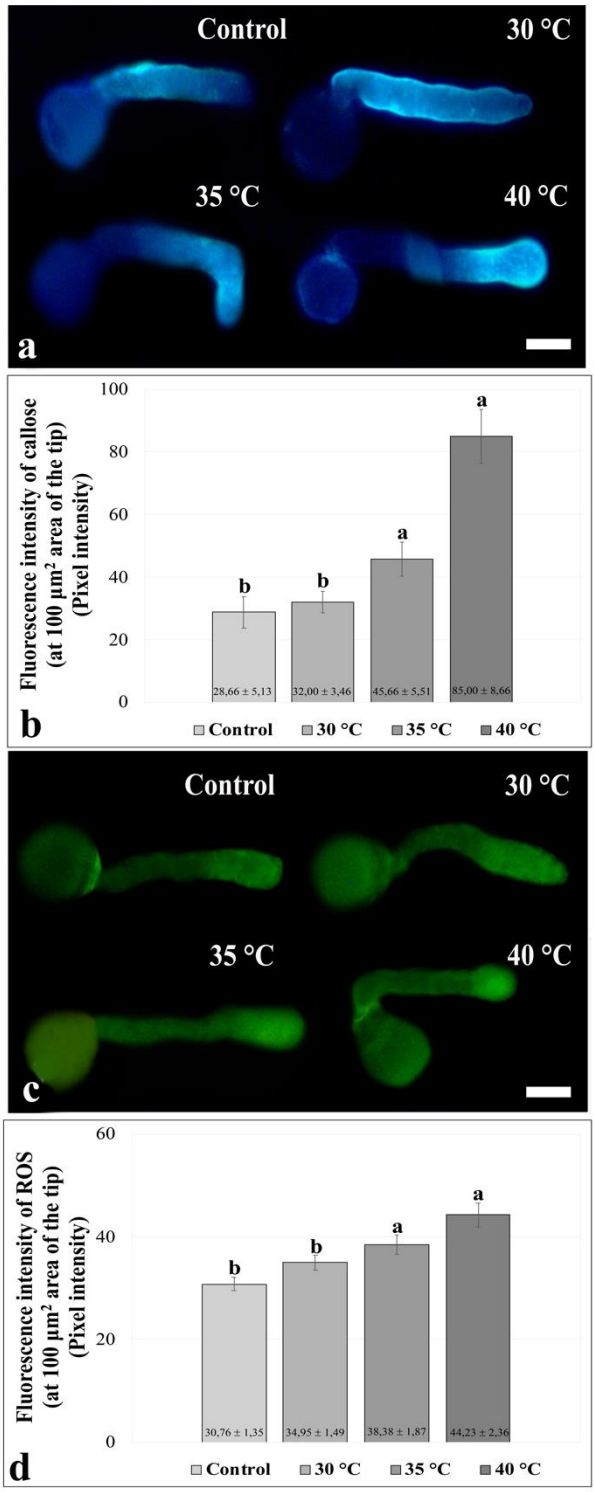

Figure 2. Impact of HTS on callose distribution, tip-localized ROS accumulation and ROS detoxification system in *P. granatum.* **a.** Exemplary images of callose accumulation, **b.** FI of callose accumulations, **c.** Exemplary images of tip-localized ROS accumulation, **d.** FI of tip-localized ROS accumulations. Bar: 10 µm.

4. Discussions

This section should underly the meaning of your research, or highlight the importance of your study and how it may be able to contribute to and/or help fill existing gaps in the field.

It has been known that low or high temperature mostly have opposite impacts on PP (Krawczyk et al., 2022). For instance; PG and PT length were decreased by high-temperature treatment at 30 °C in hazelnut (Çetinbaş-Genç et al., 2019) and, decreased by high-temperature treatment above 35 °C in cotton and tobacco (Song et al., 2015; Parrotta et al., 2016). In pomegranate, PG rate and PT length were remarkably inhibited after 35 °C and 40 °C treatment while 30 °C did not make any significant change. To reveal the most devastating temperature on PP, CSRI values of treatment groups were calculated. CSRI values showed that although all treatment temperatures had an unfavorable effect on PP, 40 °C had the most devastating effect with high negative value. Negative CSRI datas also have been stated in PTs of almond that exposed to 30 °C and 40 °C (Sorkheh et al., 2018) and in PTs of hazelnut that subjected to 30 °C (Çetinbaş-Genç et al., 2019).

Angiosperm PTs are generally linear structures with a dome-shaped tip. It has been known that various temperature stress can alter this PT morphology (Srinivasan et al., 1999). Distribution of PT morphology in angiosperm PTs, especially at apex, usually means that the growth is inhibited. (Wang et al., 2016) have been stated the swelling of PT in cold inhibited PTs of tea. Moreover, (Çetinbaş-Genç et al., 2019) have been noticed that low and high temperature obviously disrupts the morphology of PT tips in hazelnut. Parallelly with these literatures, it was detected that HTS caused various abnormalities at PTs after all treatment groups while the most increase in PT abnormality was detected after 40 °C.

Temperature stress can also alter the PT wall properties, especially accumulation of callose (Parrotta et al., 2019). In angiosperm PTs, callose is abundantly localized compound along the PT except the apex (Qin et al., 2012). Moreover, callose accumulation at the apex is considered a decrease in PP because callose accumulation at apex prevents the transfer of sperm nuclei, reducing fertilization success (Wang et al., 2003). Based on our findings, callose at PT tips increased after 30 °C and 40 °C treatment. Unconventional callose accumulation is produced by modifications in actin structure and organization (Parrotta et al., 2022). Corruption of actin cytoskeleton obstructs the generation of functional PT by blocking PG and PT growth (Cai et al., 2011). And also, it has been known that various stress conditions, especially the temperature stress cause callose deposition on the PT tip (Kapoor and Geitmann, 2023). So, it can be hypothesized that 30 °C and 40 °C temperature disrupts the actin skeletal structure, both preventing PT elongation and causing callose accumulation at the PT tips.

Tip-localized ROS is requise to ensure PT elongation (Swanson and Gilroy, 2010). Due to the tip-localized ROS is over accumulate under abiotic stress cases, alterations in tip-localized ROS accumulation can be utilized as a mark to measure the harmfull effects in PP under stress conditions (Aloisi et al., 2022). Wang et al. (2016) and Parrotta et al. (2019) noticed that cold stress improved the ROS signal in the PT apex of *Camellia sinensis* (L.) Kuntze and *Nicotiana tabacum* L., respectively. Parallelly with those results, we observed that the apex-localized ROS accumulation enhanced after 30 °C and 40 °C treatment. It has been known that regular accumulation of ROS at the PT apex directly or indirectly regulates the actin filament organization (Zonia, 2010). So, it can be hypothesized that 30 °C and 40 °C temperature increase the tip-localized ROS disrupting actin skeletal structure, both preventing PT elongation and causing callose accumulation at the PT tips.

5. Conclusion

High-temperature stress is negatively affected the PP of pomegranate. PG rate and PT length are decreased and, PT abnormality, callose accumulation at apex and tip-localized ROS accumulation are increased after 35 °C and 40 °C high-temperature treatment. These findings show that HTS may prevent the pollination and fertilization processes in pomegranate.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

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Effects of thymoquinone on valproic acid-induced oxidative stress in perinatal rat brain

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Timokinonun perinatal sıçan beyninde valproik asit indüklü oksidatif stres üzerine etkileri

Abstract: Thymoquinone (TQ), bioactive molecule of black cumin, has antioxidant and neuroprotective effects. TQ's hypoglecemic effect while applied prenatally is reported. This study is aimed to find the TQ dose with maximum antioxidant and minimum side effects in valproic acid (VPA) induced oxidative stress. Pregnant Wistar rats were injected i.p. with 400 mg/kg/ml of VPA on embryonic day 12.5 (E12.5). Repeated dose groups were injected i.p. from E11.5- E14.5; RC- repeated control: did not receive TQ, R1: 0.5 mg/kg/ml of TQ, R2: 2 mg/kg/ml of TQ, R3: 4 mg/kg/ml of TQ, R4: 8 mg/kg/ml of TQ. Single dose groups were injected i.p. on E12.5; SC- single control: did not receive TQ, S1: 8 mg/kg/ml of TQ, S2: 15 mg/kg/ml of TQ. Pups were sacrificed on postnatal day 7. Glutathione (GSH), malondialdehyde (MDA) and superoxide dismutase (SOD) levels were measured via ELISA method. Prenatal VPA exposure decreased GSH and SOD levels in RC and SC compared to naïve group. R3 group showed improved GSH and SOD levels compared to RC. No significant difference in MDA levels was found between groups. Antioxidant effects of TQ on VPA induced oxidative stress has been showed in R3 group. This dose can be used to investigate TQ's effects on other parameters that are affected by prenatal VPA exposure.

Key words: Oxidative stress, thymoquinone, valproic acid, perinatal brain, rat

Özet: Timokinon (TQ), çörek otunun aktif maddesi, antioksidan ve nöroprotektif etkileri olan bir maddedir. Prenatal dönemde uygulandığında hipoglisemi etkisi bildirilmiştir. Bu çalışmanın amacı TK'nun perinatal sıçan beyninde Valproik Asit (VPA) indüklenmiş oksidatif streste kullanımı için maksimum antioksidan ve minimum yan etkiye sahip dozunun bulunmasıdır. Gebe Wistar sıçanlara 12,5. embriyonik günde (E12.5), i.p. 400mg/kg VPA enjeksiyonu uygulanmıştır. Tekrarlayan TK doz gruplarına (R) E11,5-E14,5 arası, Tek doz TK gruplarına (S) ise E12,5'de i.p. TK uygulanmıştır. RC: tekrarlayan kontrol, TQ yok; R1: 0.5 mg/kg/ml TK; R2: 2 mg/kg/ml TQ, R3: 4 mg/kg/ml TQ, R4: 8 mg/kg/ml TQ; SC- tek doz kontrol, TQ yok; S1: 8 mg/kg/ml TQ, S2: 15 mg/kg/ml TQ. Yavrular postnatal 7. günde sakrifiye edilerek total beyin dokularında ELISA yöntemiyle glutatyon (GSH), malondealdehit (MDA) ve süperoksit dismütaz (SOD) seviyeleri ölçülmüştür. Prenatal VPA uygulaması RC ve SC gruplarında naive gruba göre GSH ve SOD miktarlarını azaltmıştır. R3 grubunda kontrol grubuna göre artmış GSH ve SOD seviyeleri ölçülmüştür. Hiçbir grupta MDA seviyelerinde farklılık görülmemiştir. TK'nun VPA indüklü oksidatif stres üzerinde yan etki olmaksızın antioksidan etkisi R3 grubunda gösterilmiştir. Bu doz prenatal VPA asit maruziyetinin olumsuz olarak değiştirdiği davranış ve beyin morfolojisi gibi diğer parametrelerin incelenmesinde kullanılmak üzere önerilmektedir.

Anahtar Kelimeler: Oksidatif stres, timokinon, valproik asit, perinatal beyin, sıçan

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1. Introduction

Valproic acid (VPA) is an effective drug that is prescribed for epilepsy, migraine and bipolar disorder. VPA is accepted as an environmental risk factor in Autism Spectrum Disorders (ASD) aetiology and is used in experimental models for this cause (Schneider and Przewlocki, 2005). Rats with in utero exposure of VPA on embryonic day 12.5 (E12.5) are reported to show autism-like behaviours and oxidative stress (Schneider and Przewlocki, 2005). These effects are related to VPA's histone deacetylate inhibitor activity (HDACi) (Dufour-Rainfray et al., 2011) and destructive effects on embryonic folate mechanism (Wegner and Nau, 1992).

ASD are complex neurodevelopmental disorders with a symptomatic spectrum that is correlated with biochemical changes, such as redox imbalance and oxidative stress related mitochondrial disruptions (Kaur et al., 2014). Experimental models are widely used to understand aetiology (Schneider and Przewlocki, 2005) and to improve symptoms (Kang and Kim, 2015).

Oxidative stress is a result of imbalance in cellular pro-oxidant and anti-oxidant mechanisms which cause an increase in reactive oxygen and nitrogen species (ROS, RON). Even though oxidative stress' effects in ASD pathogenesis is unclear, it is known that developing brain is vulnerable to oxidative damage (McQuillen and Ferriero, 2004). Compared to other organs, the brain consumes 20% of the total oxygen and is highly dependent on oxidative metabolism, it has high unsaturated lipid content with a relatively low antioxidant, activity all of which results in vulnerability to oxidative stress (Dringen et al., 2000). Blood brain barrier is not fully develop after 6 months post-birth, enabling easy transmission of ROS and RON to the brain (Adinolfi, 1985). Besides metabolic disturbances reported in people with ASD (James et al., 2006), decreased methionine and glutathione (GSH) levels and DNA hypo-

methylation (Hishida and Nau, 1998), disrupted superoxide dismutase (SOD) and increased malondialdehyde (MDA) due to lipid peroxidation (Gao et al., 2016) are reported.

Thymoquinone (TQ) is the bioactive molecule of black cumin (Ahmad et al., 2015). It is traditionally used as a miracle herb for headache, fever, rheumatism and coughing in India, the Middle East and North Africa (Burits and Bucar, 2000). TQ is reported to have gastro-protective (Kanter et al., 2006), anti-diabetic (El-Ameen et al., 2015), anti- inflammatory (Chehl et al., 2009), anti-histaminic (El-Ameen et al. 2015), anti-oxidant (Solati et al., 2014) and anti-cancer (Attoub et al., 2013) properties in preclinical studies. It is also reported to modulate oxidative stress through an increase in GSH and SOD and decrease in MDA in experimental disease models (Ince et al., 2013; Fanoudi et al., 2019).

Prenatal VPA exposure induces disrupted antioxidant defence. Several antioxidant substances are being used during the induction period to prevent or decrease the effect of VPA (Gao et al., 2016; Bambini-Junior et al., 2014). Effects of TQ supplementation before and after prenatal VPA exposure on excitatory/inhibitory balance, oxidative stress, morphological and behavioural anomalies should be studied in offsprings with prenatal VPA exposure. However, consecutive administration of TQ is reported to have negative outcomes due to its hypoglycaemic properties (Hawsawi et al., 2001) which also have teratogenic effects in pregnancy (AbuKhader et al., 2013). With known antioxidant and neuroprotective effects of TQ, a study to explore the effective dose for VPA induced models is necessary. This study is designed to find the dose of TQ administration with most effective antioxidant activity and least hypoglycaemic effect. With suggested route of administration and dose of TQ, a follow up study will be conducted to research further behavioural, morphological and biochemical parameters affected by in utero exposure to VPA.

2. Materials and Method

2.1. Animals

Wistar albino rats (bred in Bursa Uludag University Experimental Animal Research Centre) were mated (2:1) and their offspring were tested in this study. Conception was confirmed by the presence of vaginal plug and spermatozoa in vaginal smear samples (E0.5). All animals were housed in standard laboratory conditions with a 12 h / 12 h light-dark cycle, controlled temperature (20-24 °C) and humidity (40 - 60%), and access to food and water ad libitum. The study was approved by Bursa Uludag University Animal Research Ethics Committee (date: 2020— 03/ 09).

2.2. Protocol for symptomatic modelling and supplementation

According to literature and previous works of the lab, single dose of 400 mg/kg/ ml i.p. injections of VPA (Depakin, lyophilize solution, Sanofi, Aventis, Paris, France) on E12.5 is enough to constitute ASD- like symptomatic model in rats (Kim et al., 2011). TQ (Bldpharm, Shanghai, China resolved in ethyl alcohol (EtOH) and diluted to respected doses with saline) was administered according to experimental group of the animal (Hawsawi et al., 2001). All pregnant rats were handled from E0.5 to E15.5, weighed regularly. After injections, food was removed from the cages and blood glucose levels were measured by a drop of blood from the tail veins after 2 hours to detect any possible hypogliceamia (AccuCheck Glucometer, Roche, Basel, Switzerland) and dams were observed for possible side effects.

2.3. Experimental groups

Group 1 (N; Naïve, n= 8): Animals that received no injections during pregnancy.

Group 2 (RC; Control for Repeated Dose, n= 8): VPA (400 mg/kg) on E12.5 + Solvent (EtOH+ Saline, kg/ml) between E11.5- E14.5.

Group 3 (SC; Control for Single Dose, n= 7): VPA (400 mg/kg) on E12.5 + Solvent (EtOH+ Saline, kg/ml) on E12.5.

Group 4 (R1; Repeated Dose, n= 10): VPA (400 mg/kg) on E12.5 + TQ (0.5 mg/kg) between E11.5- E14.5.

Group 5 (R2; Repeated Dose, n= 6): VPA (400 mg/kg) on E12.5 + TQ (2 mg/kg) between E11.5- E14.5.

Group 6 (R3; Repeated Dose, n= 9): VPA (400 mg/kg) on E12.5 + TQ (4 mg/kg) between E11.5- E14.5.

Group 7 (R4; Repeated Dose, n= 0): VPA (400 mg/kg) on E12.5 + TQ (8 mg/kg) between E11.5- E14.5.

Group 8 (S1; Single Dose, n= 7): VPA (400 mg/kg) on E12.5 + TQ (8 mg/kg) on E12.5.

Group 9 (S2; Single Dose, n= 0): VPA (400 mg/kg) on E12.5 + TQ (15 mg/kg) on E12.5.

2.4. Postnatal evaluations

Day of birth was accepted as postnatal day 0 (P0). Offspring were allowed to be raised by their mothers. On P7, pups were sacrificed and total brain tissues were collected for further analysis.

2.5. Oxidative stress markers

GSH and MDA content and SOD activity of total brain tissues were evaluated by ELISA method according to manufacturer's protocols (Bioassay Technology Laboratory Rat ELISA Kit, Cat. No: E1101Ra; E0156Ra; E0168Ra).

2.6. Statistical analysis

Normality of distribution was assessed using Shapiro Wilk test. Comparisons between groups were analysed using one-way ANOVA (α= 0.05 in all cases) followed by post-hoc Tukey test. All statistical analyses were performed on Sigma Plot V.2 and significance was accepted as $p< 0.05$. Plots display group averages with standard errors.

3. Results

Pregnant rats were observed after injections for any side effects and blood glucose levels were measured after 2 hours (Hawsawi et al., 2001). Glucose level lower than 2 mM is accepted as moderate hypoglycaemic, and none of the rats showed a decrease to this level (Won et al., 2012). Mothers from groups R4 and S2 showed abnormal levels of blood glucose (more than 11 mM) and death in upcoming hours/days after injections. Therefore, these groups were removed from the study.

Oxidative stress markers were measured from total brain tissues of pups on P7. Comparisons of repetitive and single dose groups were made for GSH and MDA contents and for SOD activity.

For GSH, there were no significant difference between N and RC groups. When N is compared to R1, R2 and R3, there was a significant decrease in R1 and R2 in terms of GSH content with no significant difference in R3 group ($p< 0.001$; $p< 0.001$). In comparison of RC and dose groups R1, R2 and R3, there was a significant decrease in GSH content for R1 and R2 and increase for R3 ($p= 0.031$; $p< 0.001$; $p= 0.020$) (Fig 1A). Dose groups are also compared in between and there found to be a significantly lower level of GSH content was found in R2 compared to R1 and higher level in R3 compared to both R1 and R2 ($p< 0.001$; $p< 0.001$; $p< 0.001$). For single dose comparisons, SC and S1 groups displayed significant decrease in levels of GSH content compared to N group, whereas there was no difference between SC and S1 ($p= 0.033$; $p= 0.002$) (Fig 1B).

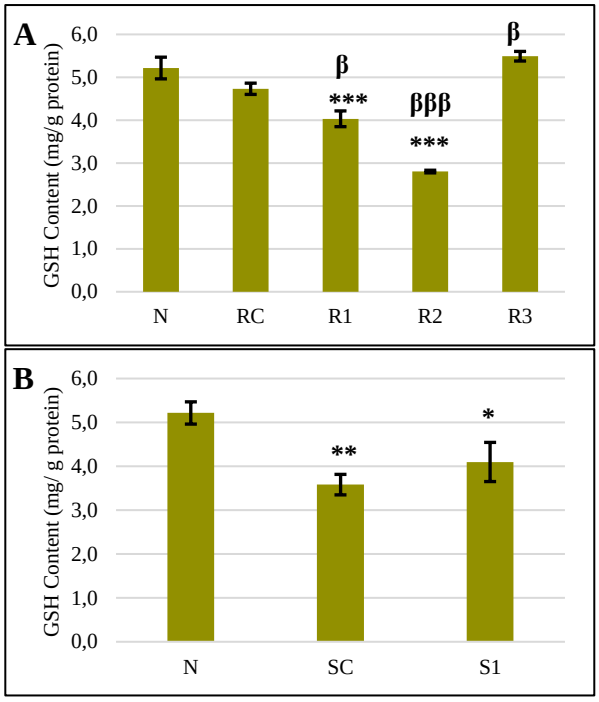

Figure 1. GSH content of total brain tissue (A) for repeated doses of TQ, (B) for single dose of TQ. Data are means ± SE of 6-9 offsprings in each group. (N: Naive group, RC; control group for repeated dose, SC; control group for single dose, R1; repeated doses of 0.5 mg/kg TQ, R2: repeated doses of 2 mg/kg TQ, R3: repeated doses of 4 mg/kg TQ, S1: single dose of 8 mg/kg TQ).

* $p< 0.05$ compared to naive group;

** $p< 0.01$ compared to naive group;

*** $p< 0.001$ compared to naive group;

β $p< 0.05$ compared to control group;

β β β $p<0.001$ compared to control group.

For MDA, there were no significant difference between N and RC groups. When N is compared to R1, R2 and R3, there was no significant difference between either group. In comparison of RC and dose groups, there was not any differences to be shown (Fig 2A). Dose groups are also compared in between and there found to be a significantly higher level of MDA content in R3 compared to R1 ($p= 0.003$) but no differences were found between R1 and R2; R2 and R3. For single dose comparisons, there was no significant difference between N, SC and S1 groups in levels of MDA content (Fig 2B).

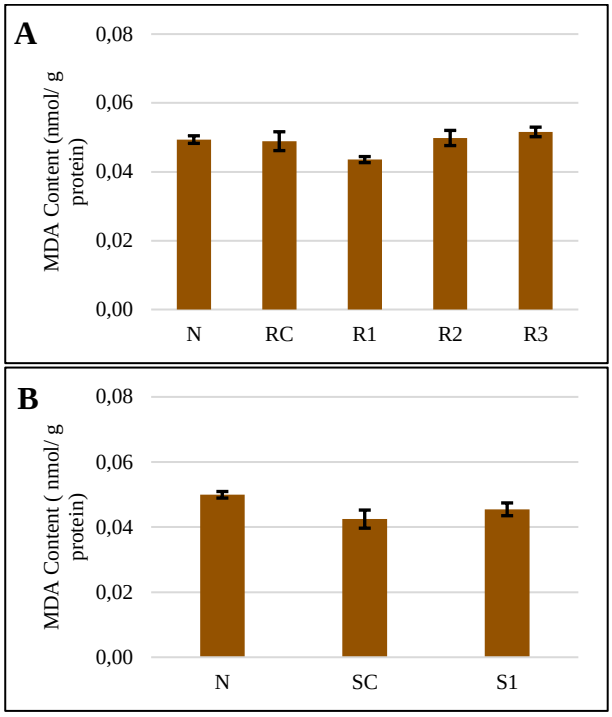

Figure 2. MDA content of total brain tissue (A) for repeated doses of TQ, (B) for single dose of TQ. Data are means ± SE of 6-9 offsprings in each group. (N: Naive group, RC; control group for repeated dose, SC; control group for single dose, R1; repeated doses of 0.5 mg/kg TQ, R2: repeated doses of 2 mg/kg TQ, R3: repeated doses of 4 mg/kg TQ, R5: single dose of 8 mg/kg TQ).

For SOD, RC showed a significant decrease compared to N ($p< 0.001$). When N is compared to R1, R2 and R3, there was a significant decrease in R1 and R3 and no difference in R2 ($p< 0.001$; $p< 0.001$). In comparison of RC and dose groups, R1, R2 and R3 groups displayed increased SOD activity compared to RC group ($p< 0.001$; $p< 0.001$; $p< 0.001$) (Fig 3A). Dose groups are also compared in between and there found to be significantly higher levels of SOD activity in R2 and R3 compared to R1 ($p< 0.001$; $p< 0.001$), and lower level in R3 compared to R2 ($p= 0.002$). For single dose comparisons, SC and S1 groups displayed significant decrease in levels of SOD activity compared to N group ($p< 0.001$; $p< 0.001$), whereas S1 group had significantly higher level compared to SC ($p< 0.001$) (Fig 3B).

4. Discussions

The developing brain is highly vulnerable to oxidative stress with partially developed blood brain barrier, higher iron concentrations and lower antioxidant defence (Panfoli et al., 2018). VPA disrupts pro-oxidant/ anti-oxidant balance due to being a HDACi (Dufour-Rainfray et al., 2011) and has a destructive effect on embryonic folate metabolism (Wegner and Nau, 1992).

In utero exposure to VPA is used to model ASD like symptoms (Schneider and Przewlocki, 2005) with its effects on disrupted excitatory/inhibitory balance (El-Ansary and Al-Ayadhi, 2014), neuronal migration (Schmitz and Rezaie, 2008) and oxidative stress (Gao et al., 2016) which are also included in ASD pathogenesis. Supplementary substances with antioxidant effects such as

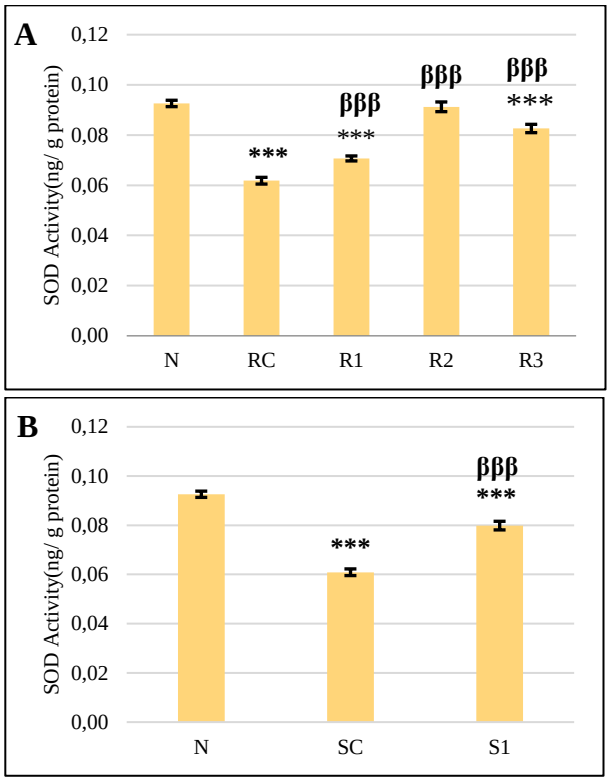

Figure 3. SOD activity of total brain tissue (A) for repeated doses of TQ, (B) for single dose of TQ. Data are means ± SE of 6-9 offsprings in each group. (N: Naive group, RC; control group for repeated dose, SC; control group for single dose, R1; repeated doses of 0.5 mg/kg TQ, R2: repeated doses of 2 mg/kg TQ, R3: repeated doses of 4 mg/kg TQ, 5: single dose of 8 mg/kg TQ).
*** p< 0.001 compared to naive group;
β β β p<0.001 compared to control group.

fingolimod (Gao et al., 2016), resveratrol (Bambini-Junior et al., 2014), curcumin (Mirza and Sharma, 2019) and S-adenosyl methionine (Al-Askar et al., 2017) are reported to regulate disrupted oxidative status and improve behavioural symptoms in VPA induced models of ASD. TQ is used in this study as it has antioxidant (Solati et al., 2014) and neuroprotective effects reported in previous studies (Ornoy et al., 2020).

Methionine is the precursor of S-adenosylmethionine, a molecule used in DNA methylation, and its concentration can be negatively affected by decreased folate content (Banerjee and Matthews, 1990). Methionine is also the precursor of major intracellular antioxidant- GSH (Finkelstein, 1998). VPA allows epigenetic changes and oxidative imbalance by hyper-acetylation and disrupted folate metabolism. Consistent with these findings, in utero exposure to VPA is reported to cause decreased methionine (Alonso-Aperte et al., 1999) and GSH content with DNA hypo-methylation (Hishida and Nau, 1998). Our study showed a decreased GSH content in VPA groups compared to naïve groups, and TQ supplementation managed to level up this decrease in R3 group. Hegazy et al. (2015) also reported decreased GSH levels in VPA model. VPA acts as an environmental factor that predisposes the organism to oxidative stress by disrupting redox/methylation balance, resulting in a decline to capacity of synchronisation in neural systems (Deth et al., 2008). Cabungcal et al. (2007), suggested that impaired GSH metabolism during development results in cognitive decline in juvenile and adult rats relative to schizophrenia.

VPA has been reported to cause an imbalance in oxidative status. Chaudhary and Parvez (2012) reported a decreased SOD activity in brain tissues of VPA exposed groups whereas Bambini-Junior et al. (2011) reported no SOD level differences in liver tissue of adult rats with in utero VPA exposure. Gao et al. (2016) used fingolimod to attenuate effects of VPA and reported increased SOD activity in supplemented groups. In our study, we showed a decrease in SOD activity in VPA-only group and increase in TQ groups for all doses compared to VPA groups, consistent with literature.

Lipid peroxidation can be measured with TBARS levels or MDA content. Chaudhary and Parvez (2012) reported increased TBARS levels and Gao et al. (2016) reported increased MDA content as a result of increased lipid peroxidation after prenatal VPA exposure. Fingolimod (Gao et al., 2016) is suggested to decrease levels of increased MDA due to VPA induction. Ornoy et al. (2020), however, reported no significant differences in levels of MDA content, as well as Bambini-Junior et al. (2011) for TBARS levels. In our study, there was no significant difference in MDA contents for either group.

TQ stimulates glucose uptake and use in tissues and cells leading to decrease in blood glucose level (Fararh et al., 2010). In our study, mothers that received 8 mg/kg for 4 days and 15 mg/kg for a day were removed from the study due to their losses in following days. During the injection days, chorionic somatomammotropin (CS) is being secreted at an optimum level (Tonkowicz et al., 1983). With CS levels high in blood, an increase in insulin levels and lipolysis would occur. All in all, hypoglycaemia caused by TQ and hyperlipidaemia caused by CS could result in acute pancreatitis (Gianfrate and Ferraris, 1998). Pancreatitis can also be caused by VPA. Acute pancreatitis is reported as a side effect of VPA in humans and it is also supported by experimental studies (Eisses et al., 2015). Role of TQ on HDACi, how and in what concentration it can contribute to VPA induced models requires further research.

In this study, several doses were used to reach the effective dose for maximum antioxidant capacity with minimum hypoglycaemic effect of TQ. Doses were determined in respect to literature. Hawsawi et al. (2001) used both *Nigella sativa* seeds and TQ in several doses to study effects on blood glucose in non- pregnant rats. They used *i.p*. injections of 0.5, 1, 2, 4, 6 and 8 mg/kg of TQ for repeated days of 1, 4, 7, 10 and 14 and reported that doses from 0.5 to 6 mg/kg were tolerable and showed no side effects. Animals injected with dose of 8 mg/kg, however, died after a week with signs of peritonitis. Al-Enazi (2007) administered TQ orally in a dose 10 mg/kg to pregnant rats from E1.5 to E19.5 and reported balanced oxidative status. AbuKhader et al. (2013) tested acute effects of TQ on pregnant rats with doses of 15, 35 and 50 mg/kg on either E11.5 (embryonic development- organogenesis) or E14.5 (early fetal development). They reported total fetal resorption in rats that received 35 or 50 mg/kg of TQ on E11.5. 15 mg/kg of TQ, however, showed no adverse effects on both E11.5 and E14.5 (AbuKhader et al., 2013). Reported LD_{50} for TQ is 57.5 mg/kg in non-pregnant rats (Kim et al., 2011). In the light of these studies, we used 0.5, 2, 4 and 8 mg/kg of i.p. TQ to be given between E11.5-E14.5, and 8 and 15 mg/kg of i.p. TQ to be given only on E12.5 to assess glycaemic state and VPA induced oxidative stress.

In conclusion antioxidant effects of TQ after VPA exposure have been studied. TQ is reported to induce hypoglycaemia which is a known teratogenic factor. Therefore, it was necessary to search for a dose with minimum hypoglycaemic and maximum antioxidant effect. Our results show that 4 mg/kg/ml of TQ given for 4 days between E11.5- E14.5 display regular blood sugar levels with highest antioxidant activity compared to the control and other dose groups. We propose that this dose can be used for further research to investigate TQ's effects on ASD-like behaviour and other parameters that are induced by in utero exposure to VPA.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

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Determination of relationship between lichen diversity value and photosynthetic pigment content in Bursa province (Türkiye)

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Bursa ilinde (Türkiye) liken çeşitlilik değeri ve fotosentetik pigment içeriği arasındaki ilişkinin belirlenmesi

Abstract: In this study, 48 epiphytic lichen species on the trunk of oak trees from seven localities were reported in Bursa province. The correlation between Lichen Diversity Value (LDV) and photosynthetic pigment content at a location were evaluated for each locality. A negative correlation was found between LDV and photosynthetic pigment contents. The LDV was higher in rural areas, and decreased in areas affected by anthropogenic and agricultural activities. LDV values were increased from 21% to 47% from degraded areas exposed to environmental pollution and stress to undisturbed areas, whereas total photosynthetic pigment contents decreased from 39% to 19%, and Phaeophytinization ratio (PR) values from 36% to 30%.

Key words: Air pollution, epiphytic lichen, lichen diversity, *Parmelina tiliacea,* photosynthetic pigment

Özet: Bu çalışmada, Bursa ilinde yedi lokaliteden meşe ağaçlarının gövdelerinde bulunan 48 epifitik liken türü rapor edilmiştir. Aynı lokasyondaki Liken Çeşitlilik Değeri (LDV) ile fotosentetik pigment içeriği arasındaki korelasyon herbir lokasyon için değerlendirilmiştir. LDV değeri ve fotosentetik pigment içerikleri arasında negatif bir korelasyon bulunmuştur. LDV değerinin kırsal alanlarda yüksek olduğu, antropojenik ve tarımsal faaliyetlerden etkilenen alanlarda ise azaldığı tespit edilmiştir. LDV değerleri, çevre kirliliğine ve strese maruz kalan bozulmuş alanlardan bozulmamış alanlara doğru %21'den %47'ye yükselirken, toplam fotosentetik pigment içeriği %39'dan %19'a ve Feofitinizasyon oranı (PR) değerleri %36'dan %30'a düşmüştür.

Anahtar Kelimeler: Hava kirliliği, epifitik liken, liken çeşitliliği, *Parmelina tiliacea*, fotosentetik pigment

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1. Introduction

Lichens do not have a protective cuticle layer like high plants; therefore, they are continuously exposed to pollutants in the air. Therefore, for a long time, lichens have been used as biological indicators to monitor air quality in both urban and rural environments. For over 140 years, lichens have been known to be extremely sensitive to air pollution due to the adverse effects of pollutants on the primer metabolism of both the algal and the fungal partners in the lichen thalli (Brodo et al., 2001). The epiphytic lichen diversity and community structures have been known to vary based on air pollution and environmental changes (Giordani, 2007; Cristofolini et al., 2008).

The decrease of lichen diversity in response to environmental conditions is widely used for a long time as an indicator of air pollution (Poličnik et al., 2008; Munzi et al., 2009,2014; Ozimec et al., 2016).

For assessing the effects of environmental stress in a short time is very important to monitor of the changes in physiological parameters in response to air pollution of lichens (Pisani et al., 2009; Sujetoviene and Galinyte, 2016). In several studies has been demonstrated a correlation between air pollution and photosynthetic pigment content of lichens (Riddel et al., 2012; Seed et al., 2013).

This study was aimed to determine the relationship between changes in photosynthetic pigment contents and lichen diversity values in localities.

2. Materials and Method

2.1. Study area

This study was conducted at seven localities in Bursa province. Bursa province is located between 39°30'-40°37'N and 28°06'-29°58'E in the southeast part of the Marmara region of Türkiye. It is usually dominated by a Mediterranean climate and is a transitional region between the Mediterranean and Black Sea climates (Öztürk, 2010). The mean annual temperature (1987-2012) in Bursa province (alt. 155 m) is 14.6 °C, the mean annual rainfall is 691 mm, at Mudanya district (alt. 13 m) in Bursa province is 16.7 °C and 614 mm, at Karacabey (alt. 15 m) is 14.7 °C and 585 mm, and at Orhaneli (alt. 484 m) 12.5°C, and 655 mm, respectively (TSMS, 2013).

2.2. Collection of lichen samples

Lichen samples were collected from total 21 oak trees at seven localities in Bursa province in the year of 2014-2015 (Figure 1, Table 1). Lichen samples on the trunk of three oak trees in each localities were collected using the methods specified by Asta and his colleagues (Nimis et al., 2002). The lichen diversity value (LDV) is the sum of the frequencies calculated for each aspect on a tree. The LDV of the locality was the arithmetic mean of the sums of frequencies for each sampling tree in a locality. For the analysis of pigment content and chlorophyll integrity, thalli of *Parmelina tiliacea* (Hoffm.) Hale was collected from each sampled localities. *Parmelina.tiliacea* was not found in

Table 1. Main characteristics of sampling sites

Locality	District - Site	Alt. (m)	Coordinates	Descriptions
1	Karacabey - Bayramdere	40	40°23′35″N 28°22′31″E	Trees in the picnic area
2	Karacabey - Kıranlar	206	40°16′09″N 28°19′00″E	Trees in the village cemetery
3	Karacabey - Örencik	340	40°18′10″N 28°17′21″E	Trees at the village cemetery in the Karadağ Mountain
4	Karacabey - Taşpınar	110	40°15′02″N 28°38′12″E	Trees in the slopes near to an asphalt roadside
5	Mudanya - Esence	140	40°20′22″N 28°40′42″E	Trees in the agricultural area
6	Osmangazi - Dürdane	328	40°20′02″N 29°05′30″E	Trees at the roadside cemetery on the Bursa - İstanbul highway
7	Orhaneli - Karaoğlan	744	39°50′43″N 28°59′15″E	Trees in the rural area at 70 km south-west of the city of Bursa

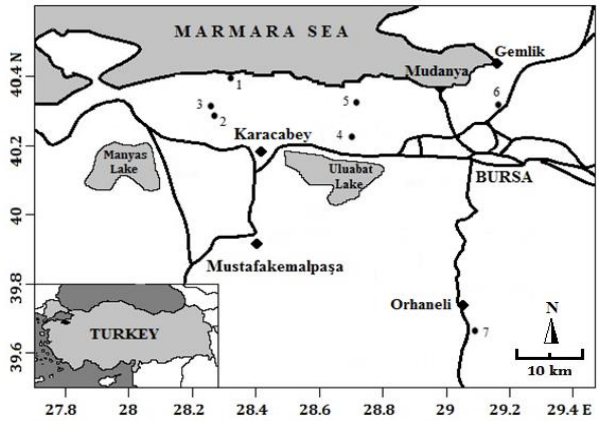

Figure 1. Map of study area and sampling sites (1-7)

the trees sampled for LDV value in the 4th and 5th localities. For photosynthetic pigment analysis, samples were taken from other trees in these localities.

2.3. Photosynthetic Pigment Analyses

20 mg of each lichen samples were used for the analysis of pigment content and chlorophyll integrity. Samples were first rinsed five times for 1 min each in $CaCO_3$-buffered acetone to remove lichen substances. After evaporation of acetone, the samples were extracted in the dark for 40 min. at 65°C in 5 ml of dimethyl sulfoxide (DMSO) and then allowed to cool down to room temperature (Barnes et al., 1992). The extracts were filtered with Whatman no 3 filter paper and were diluted with addition to 5 mL of DMSO. Extraction was repeated three times for each sample. The absorbances at 665, 649, 480, 435 and 415 nm were determined spectrophotometrically (Beckman Coulter DU 730). Concentrations of chlorophyll a, chlorophyll b and total carotenoids were calculated using the equations of Wellburn (Wellburn, 1994). The ratio of the absorbances at 435 and 415 nm, known as Phaeophytinization ratio (PR) (Ronen and Galun, 1984), was used to assess chlorophyll degradation to phaeophytin.

2.4. Statistical Analyses

The statistical analyzes were performed using SPSS version 22 software package. The level of significance was taken as $p \leq 0.05$ all tests. In terms of the content of photosynthetic pigments to test whether differences between localities was used One-Way Analysis of Variance (ANOVA). The determination of grouping localities was obtained CANOCO ordination graph of groups according to the results of PCA analysis using the 5.4 software package (Ter Braak and Smilauer, 2002). A linear regression analysis was used to determined the degree of correlation expressed by multiple correlation coefficient (R^2) and probability (P) between the physiological parameters and lichen diversity values (LDV).

3. Results

A total of 48 epiphytic lichen species was recorded (Table 2). The most common species with the frequency of presence were *Lecidella elaeochroma* (Ach.) M. Choisy (40.95%), *Lecanora chlarotera* Nyl. (28.33%), *Parmelia sulcata* Taylor (25.48%), *Physcia adscendens* (Fr.) H.Olivier (23.81%), *Physconia grisea* (Lam.) Poelt (22.62%), *Xanthoria parietina* (L.) Th.Fr. (20.48%) and *P. tiliacea* (16.90%).

Parmelina tiliacea (Hoffm.) Hale is one of the most common lichens in the Mediterranean basin. It was used as an air pollution bioindicator in many regions (Núñez-Zapata et al., 2011). Photosynthetic pigment contents and chlorophyll degradation in thalli of *P. tiliacea* were summarized in Table 3. Photosynthetic pigments (chlorophyll a, chlorophyll b, total chlorophyll and total carotenoid) and PR values have statistically significant differences between the localities. Depending on environmental stress, the content of photosynthetic pigments, especially chlorophyll a is decreased while the conversion of chlorophyll a to pheophytin is increased. The high OD435/415 ratio (PR) indicates that the degradation of chlorophyll a is low.

The highest LDV (153.3) and the lowest PR values (0.95) were determined in locality 3. This area was less affected by environmental pollutants, and located in the mountainous area. The second highest LDV (128.7) was observed in locality 7. This locality are different in terms of elevation (744 m) than the other localities. On the contrary, the lowest LDV (59.7) and the second highest PR values (1.16) were calculated at locality 2. This locality was located near to the village which highly affected by various environmental pollutants. The LDV was higher in rural areas (localities 3, 5 and 7) and decreased in localities with traffic, settlements, and agricultural areas (1, 2, 4 and 6).

Seven localities evaluated in PCA analysis were divided into four groups according to their LDV value and photosynthetic pigment content (Figure 2).

The lowest LDV (59.3) and the highest PR values (1.21) were calculated at site I (Loc. 2, 4) where located near to the village which highly affected by various environmental pollutants. Site II (Loc. 1, 6, 7) is located in the area where the high of anthropogenic activities and traffic density. In this region, LDV and PR values were 78 and 1.08, respectively. Site III (Loc. 5) is located in a region where increased of agricultural activities. It has the second highest LDV and the second lowest PR values, but the species

Table 2. The frequency of epiphytic lichens found at the localities

	Localities									Localities							
Species	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**%**	**Species**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**%**
Alyxoria varia	9	-	-	6	-	-	-	3.57	*Parmelia sulcata*	-	-	25	-	33	23	26	25.48
Amandinea punctata	-	-	25	1	23	16	-	15.48	*Parmelina carporrhizans*	-	-	3	-	-	-	2	1.19
Athallia holocarpa	-	-	3	-	1	-	8	2.86	*P. pastillifera*	-	-	-	-	-	-	8	1.90
Bacidia rosella	-	1	-	23	-	-	-	5.71	*P. tiliacea*	3	3	21	-	-	43	1	16.90
Bactrospora corticola	5	-	-	-	2	-	-	1.67	*Pertusaria albescens*	-	-	3	-	-	3	-	1.43
Buellia disciformis	-	-	22	-	-	-	2	5.71	*P. leioplaca*	-	3	-	-	-	4	-	1.67
B. griseovirens	4	-	3	-	-	-	1	1.90	*P. pertusa*	-	-	7	-	-	-	-	1.67
Candelariella vitellina	-	-	-	26	-	1	1	6.67	*Phaeophyscia orbicularis*	-	6	5	-	-	-	-	2.62
Catillaria nigroclavata	-	-	1	5	-	-	-	1.43	*Phlyctis argena*	6	10	-	-	-	2	-	4.29
Evernia prunastri	-	-	-	-	27	-	13	9.52	*Physcia adscendens*	11	8	16	17	41	-	7	23.81
Gyalecta truncigena	-	4	-	1	-	-	-	1.19	*P. aipolia*	-	8	-	-	1	-	5	3.33
Hyperphyscia adglutinata	-	-	-	51	8	-	13	17.14	*P. stellaris*	-	6	15	1	-	-	-	5.24
Hypogymnia farinacea	-	-	-	-	-	-	20	4.76	*Physconia distorta*	-	-	3	-	-	-	2	1.19
Lecanora carpinea	1	3	15	-	2	4	30	13.10	*P. enteroxantha*	-	-	11	-	-	11	18	9.52
L. chlarotera	19	-	37	-	7	15	41	28.33	*P. grisea*	-	48	6	41	-	-	-	22.62
L. hagenii	-	-	6	-	-	-	-	1.43	*Pleurosticta acetabulum*	-	-	3	-	-	-	49	12.38
L. rugosella	-	-	14	-	-	-	-	3.33	*Pseudevernia furfuracea*	-	-	1	-	-	-	20	5.00
Lecidella elaeochroma	24	-	28	6	34	32	48	40.95	*Ramalina fastigiata*	-	-	-	-	-	1	7	1.90
Lepraria incana	11	-	7	-	24	16	-	13.81	*R. fraxinea*	-	-	12	-	7	-	14	7.86
L. lobificans	24	-	-	-	-	-	-	5.71	*Rinodina exigua*	3	-	4	-	9	-	-	3.81
Melanelia subaurifera	-	-	-	-	-	5	-	1.19	*R. sophodes*	-	-	15	-	-	-	5	4.76
Melanelixia glabratula	-	-	5	-	-	-	2	1.67	*Scoliciosporum umbrinum*	2	-	3	-	49	-	-	12.86
Melanohalea elegantula	2	-	-	-	44	-	5	12.14	*Strigula affinis*	-	-	-	8	-	-	-	1.90
Opegrapha herbarum	31	-	-	3	3	-	-	8.81	*Xanthoria parietina*	-	41	2	27	1	-	15	20.48

Table 3. Mean±SD of photosynthetic pigment contents (mg g^{-1}) in thalli of *P. tiliacea*, and lichen diversity values (LDV) in the localities.

	Localities						
	1	**2**	**3**	**4**	**5**	**6**	**7**
LDV	64.7±4.0	59.7±8.3	153.3±4.0	76.0±11.3	106.7±19.0	75.3±14.2	128.7±31.7
Chlorophyll a	1.87±0.05	2.29±0.18	1.04±0.07	3.05±0.24	2.35±0.10	1.74±0.17	1.59±0.04
Chlorophyll b	0.59±0.06	0.65±0.05	0.35±0.05	0.84±0.09	0.72±0.03	0.52±0.08	0.49±0.01
Total Chlorophyll	2.46±0.04	2.94±0.23	1.39±0.10	3.89±0.32	3.06±0.13	2.26±0.25	2.08±0.05
Total Carotenoid	0.51±0.02	0.60±0.04	0.36±0.06	0.75±0.06	0.56±0.02	0.52±0.06	0.47±0.02
PR	1.01±0.11	1.16±0.01	0.95±0.08	1.25±0.13	1.06±0.01	1.13±0.05	1.10±0.01

PR: Phaeophytinization ratio

diversity is the lowest. The most number of species, the highest LDV (153.3) and the lowest PR values (0.95) were determined in Site IV (Loc. 3). This area was less affected by environmental pollutants, and located in the mountainous area (Table 4).

Consequently, there is a positive correlation (R^2: 0.895) between lichen diversity value and sites. On the other hand, a negative correlation (R^2: -0.642) was found between lichen diversity value and photosynthetic pigment contents (Table 5). LDV value was increased, while the PR value was decreased in sites.

4. Discussions

In this study, seven localities evaluated in PCA analysis were divided into four groups according to their LDV value and photosynthetic pigment content. The four groups are site I (Loc. 2, 4), site II (Loc. 1, 6, 7), site III (Loc. 5) and site IV (Loc. 3) (Figure 2).

According to alteration of naturality, 6 subclasses was determined in the province of Bursa (Güvenç, 2017). According to these subclasses, localities 3, 5, and 7 are found in high naturality zone. These localities are relatively remote areas from the settlement. Localities 4, 6 are found in medium naturality zone. These two localities are located along the roadside and are therefore under the influence of vehicle traffic. Localities 1 and 2 are found in low naturality zone. These two localities are close to the settlement areas and are under human influence. LDV and Shannon diversity values were increased from low to high naturality, whereas total carotenoid and PR values were decreased.

According to the our results, a negative correlation was found between LDV and photosynthetic pigment contents. Locality 7 has the highest LDV and the lowest total chlorophyll contents. Von Arb et al. (1990), were observed a very good correlation between the air pollutants (NO, NO_2, and O_3) and some physiological parameters of *P.*

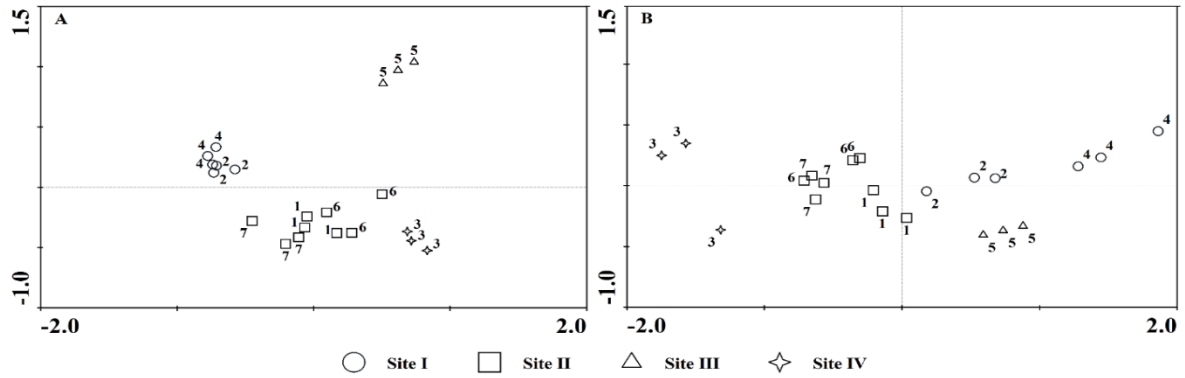

Figure 2. Ordination diagram of PCA analysis of localities (**A**: according to the lichen diversity values of localities, **B**: according to the photosynthetic pigment contents of localities)

Table 4. Mean±SD of photosynthetic pigment contents and lichen diversity values (LDV) in sites

	Site I	Site II	Site III	Site IV	F	Sig.
Localities	2, 4	1, 6, 7	5	3		
Number of sampling trees	6	9	3	3		
Number of species	24	31	20	33		
LDV	59.33±20.40	78.00±14.21	111.00±13.23	153.33±4.04	28.09	0.000
Chlorophyll a	2.67±0.46	1.73±0.15	2.35±0.10	1.04±0.07	28.91	0.000
Chlorophyll b	0.74±0.12	0.53±0.07	0.72±0.03	0.35±0.05	19.34	0.000
Total Chlorophyll (a+b)	3.42±0.58	2.26±0.21	3.06±0.13	1.39±0.10	27.58	0.000
Total Carotenoids	0.67±0.09	0.50±0.04	0.56±0.02	0.36±0.06	19.14	0.000
Total Chlorophylls/Carotenoids	5.05±0.16	4.52±0.27	5.49±0.03	3.86±0.47	24.71	0.000
Chlorophyll a/b	3.59±0.16	3.28±0.25	3.27±0.02	2.99±0.39	4.76	0.014
PR	1.21±0.05	1.08±0.08	1.06±0.01	0.95±0.08	10.34	0.000

PR: Phaeophytinization ratio

Table 5. Correlation between the photosynthetic pigments contents and lichen diversity value in the sites

	Pearson Correlation Sig. (1-tailed)							
	LDV	**Chlorophyll a**	**Chlorophyll b**	**Total Chlorophylls**	**Total carotenoids**	**Total Chlorophylls /Carotenoids**	**Chlorophyll a/b**	**PR**
Sites	0.895*	-0.691*	-0.614**	-0.679*	-0.752*	-0.428***	-0.633**	-0.771*
LDV		-0.524**	-0.465***	-0.514**	-0.565**	-0.380***	-0.550**	-0.642**

*: p<0.001 **:p<0.01 ***:p<0.05

sulcata in the northern part of Switzerland and its bordering area. Their results show that chlorophyll content increases with pollution.

Another similar result was obtained from *Usnea* sp. In parallel to the increase of air pollutants emitted by road traffic was increased the content of chlorophyl a+b in thalli of *Usnea* sp. and degradation of chlorophyll a (Carreras and Pignata, 2001). Their results demonstrate that chlorophylls would be effected by air pollutants emitted by traffic and as a compensatory mechanism the lichen would increase their synthesis. Our results are similar to those of Ra et al. (2005). They were indicated that the concentrations of total chlorophyll, chlorophyll a/b, carotenoids, and phaeophytinization ratio were generally higher in samples from the polluted areas than the clean area.

In present study, the highest total chlorophyll content (3.89), the highest PR value (1.25) and rather low LDV value (76.0) were determined at locality 4. This locality is located near the roadside in region which has a medium level alteration of naturality. We think that this locality is affected by air pollutants such as NOx, CO_2, CO and SO_2 emitted by vehicles on the road. Air pollutants emitted from the motor vehicles was as important as pollutants resulting from residential and industrial facilities (Mitreski et al., 2016). The concentrations of pollutants are higher in near the source and is decreasing with the distance from the source.

The common epiphytic species as *P. tiliacea* is very appropriate to determinate the correlation between photosynthetic pigment contents and lichen diversity value.

Conflict of Interest

Authors have declared no conflict of interest.

Authors' Contributions

The authors contributed equally.

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Flora of Davda Mountain (Karaman / Türkiye)

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Davda Dağı Florası (Karaman / Türkiye)

Abstract: The research area in this study is Davda Mountain and its surroundings, located between Karaman and the volcanic Karadağ Mountain. Throughout the visits during the vegetation periods between 2013 and 2019 years, 332 taxa were identified belonging to 44 families and 202 genera. Fifty (15.4%) taxa are endemic among the identified plants. The order of the phytogeographic region elements are Iran-Turanian 70 (21.1%), Mediterranean 34 (10.2%) and Euro-Siberian 4 (1.1%). Also, the order of most common families are; *Asteraceae, Lamiaceae, Fabaceae, Poaceae, Brassicaceae, Boraginaceae, Apiaceae* and *Caryophyllaceae*. The genera with the most taxa are *Astragalus, Alyssum, Salvia, Centaurea, Galium, Valerianella, Verbascum* and *Euphorbia*. Similarities and differences were tried to be revealed with the studies in the nearby regions.

Key words: Endemic, flora, phytogeographic region, volcanic

Özet: Bu çalışmadaki araştırma alanı, Karaman ile volkanik Karadağ dağı arasında bulanan Davda Dağı ve çevresini oluşturmaktadır. 2013 - 2019 yılları arasında vejetasyon dönemlerinde belirli zamanlarda ziyaret edilerek 44 familya ve 202 cinste ait 332 takson tespit edilmiştir. Tanımlanan bitkilerden 50 (%15.4) taksonu endemiktir. Fitocoğrafik bölge elementleri, İran- Turan 70 (%21.1), Akdeniz 34 (%10.3) ve Avrupa-Sibirya 4 (%1.1) sıralamasıyla yer alır. En fazla rastlanılan familyaların sırası ise; *Asteraceae, Lamiaceae, Fabaceae, Poaceae, Brassicaceae, Boraginaceae, Apiaceae* ve *Caryophyllaceae* sırasını izler. En çok takson içeren cinsler ise *Astragalus, Alyssum, Salvia, Centaurea, Galium, Valerianella, Verbascum* ve *Euphorbia* şeklinde yer alır. Yakın bölgede yer alan çalışmalarla benzerlik ve farklılıkları ortaya çıkarılmaya çalışılmıştır.

Anahtar Kelimeler: Endemik, flora, fitocoğrafik bölge, volkanik

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1. Introduction

More than 374,000 plant species have been described worldwide, and 308.312 of these are known as vascular plants (Christenhusz and Byng, 2016). Also about 12.000 taxa were identified in Türkiye, and about 1/3 of them are endemic (Güner et al., 2012; Özhatay et al., 2022). Discovering, identifying and introducing plants species by registering literature is the basic step to benefit them as their natural resources. Registering the regional flora, as well as the country, is therefore of utmost importance in terms of determinig the local values. Hence, the project of Illustrated Flora of Turkey was started and the 3rd volume was published (Güner and Ekim, 2014; Güner et al., 2018; Güner et al., 2022).

Similar to Anatolia, there is a significant biodiversity in the Karaman Province. The geomorphologic structure and related climatic factors of both Anatolia and Karaman Province enable the habitat of a rich plant diversity (Davis, 1965- 1985; Dönmez and Yerli, 2018; Noroozi et al., 2019; Güner et al., 2000; Maassoumi and Ashouri, 2022). Adequate research has been made to considering the plant diversity around the Karaman Province and Davda Mountain (Ünal, 1987; Ketenoğlu and Serin, 1988; Ünal and Ocakverdi, 1991; Akman et al., 1996; Serin, 1996; Bağcı et al., 1996; Koçak and Özhatay, 2000; Sağlam and Ünal, 2007; Ünal and Sağlam, 2008a,b; Geven et al., 2010; Özhatay and Koçak, 2010-2011; Yücel et al., 2011; Koçak and Özhatay, 2013; Geven et al. 2015; Akdağ and Doğu, 2016; Bağcı et al., 2016; Çeçen et al., 2018; Ertuğrul and Tugay, 2018; Hamzaoğlu et al., 2022). In addition, new taxa have also been published from these locations in recent years (Aytaç et al., 2020; Celep et al., 2020; Çeçen et al., 2015, 2016; Dinç and Bağcı, 2018; Doğru – Koca et al., 2016; Eker and Tekşen, 2017; Ulukuş and Tugay, 2018; Şirin et al., 2019, 2020; Dinç and Doğu, 2020; Çeçen and Özcan, 2021; Eker and Sağıroğlu, 2021). According to Ertuğrul and Tugay (2018), 2145 taxa are distributed in the Karaman province, 543 are endemic and 2 of them rare taxa.

Literature research, records in the flora of Turkey and the geographical and biological richness of the area (Peşmen, 1972, Çeçen et al., 2018, 2019) were influential in the decision to study the Davda Mountain. The study area is located between volcanic Karadağ and Karaman city center, about 19 km north of Karaman, (Şenel, 1997). Local people know this area as Tilki Tepe or Tilki Kaya (Çeçen et al., 2019).

While investigating the 2nd locality of the *Ferula parva* Feryn et Bornm., which was given as a suspecious taxon in the Flora of Turkey, it was revealed that some taxa (*Lepidium latifolium* L., *Dianthus cyri* Fisch & Mey., *Cicer arietinum* L. (cultivated plant), *Lythrum tribracteatum* Salzm. ex Spreng., *Glaucium corniculatum* (L.) Curtis, *Eryngium bithynicum* Boiss., *Galium verum* L., *Tripleurospermum decipiens* (Fisch. & C. A. Mey.) Bornm., *Malvella sherardiana* (L.) Jaub. & Spach, *Alhagi maurorum* Medik. var. *turcorum* (Boiss.) Meikle, *Centaurea kotschyi* (Boiss. & Heldr.) Hayek,

Centaurea patula DC., *Centaurea squarrosa* Roth, *Centaurea balsamita* Lam., *Centaurea solstitialis* L., *Carthamus dentatus* (Forsk.) Vahl., *Anchusa azurea* Mill. var. *macrocarpa* (Boiss.&Hohen.) D.F. Chamb., *Salvia sclarea* L., *Salvia virgata* Jacq., *Phlomis pungens* Willd. var. *pungens*, *Atriplex tatarica* L., *Polygonum arenarium* Waldst. & Kit., *Polygonum aviculare* L., *Euphorbia chamaesyce* L., *Euphorbia aleppica* L., *Euphorbia petiolata* Banks & Sol. *Allium leucanthum* K.Koch, *Crypsis alopecuroides* (Piller & Mitterp.) Schrad., *Aegilops caudata* L., *Plantago lanceolata* L., *Taraxacum syriacum* Boiss., *Ferula parva* Freyn & Bornm., *Morina persica* L., *Inula anatolica* Boiss. *Onopordum anatolicum* Boiss. *Crepis macropus* Boiss. & Heldr., *Salvia cyanescens* Boiss. & Balansa, *Phlomis nissolii* L., *Teucrium polium* L., *Statice echinus* L., *Kraschenninnikovia ceratoides* (L.) Gueldenst. *Carex stenophylla* Wahlenb, *Bassia prostrata* (L.) Beck, *Noaea mucronata* (Forssk.) Asch. & Schweinf. subsp. *Mucronata)* were recorded from Davda Mountain and its environs during the 1911 botanical trip of József Andrasovszky (Andrasovszky, 1912, 1914, 1917; Çeçen et al. 2019).

The lack of sufficient plant records from Davda Mountain except for *Astragalus unalii* Çeçen, Aytaç & Mısırdalı and Andrasovszky's records in studies on plant diversity around Karadağ, revealed the necessity of listing the plants of the region (Ünal, 1987; Ünal and Ocakverdi, 1991; Çeçen et al. 2019). The absence of a floristic study specific to region, was also an important factor in choosing Davda Mountain as research area.

The study aims to determine the flora of Davda Mountain, which has not been studied before.

1.1. Geographical features of the research area

Davda mountain, is geographically located in the Central Anatolian Region, in the Karaman Basin to the southwest of the Greater Konya Basin, approximately 19 km northeast of Karaman city centre. The region is bordered by Çiğdemli Village to the south, Göztepe Village to the east, Yuvatepe Village to the northwest, Kilbasan Village to the northeast, Karadağ to the north and Karalgazi Village to the west. The research area is mainly consists of a hill in the plain area with an altitude between 1019 and 1385 m, and situated between 37°14′-37°18′ north latitudes and 33°05′-33°12′ east longitudes (Figure 1, red line zone). Phytogeographically, it is located in the south of the Irano-Turanian Floristic Region, in the C4 square according to Davis (Davis, 1965-1985). According to Güner et al. (2012), the Central Anatolia Region falls under the Konya Section. A part of the research area has been protected with barbed wire for protection. The conservation area (Figure 1, green line zone) was registered as Davda General Hunting, and water fountains for wild animals (TOB, 2022-2023; Büyükşar et al., 1992).

1.2. Geology, soil and climate characteristics of the research area

The volcanism in Karadağ region, located in the north of Karaman province, is divided into Pliocene and Pliocene-Quartern. Pliocene aged Mercik andesite is a neoautochthonous cover rock. The oldest volcanics (3.2 million years) in the Karadağ region are the Mercik andesites in the south. There is a volcanic eruption center at

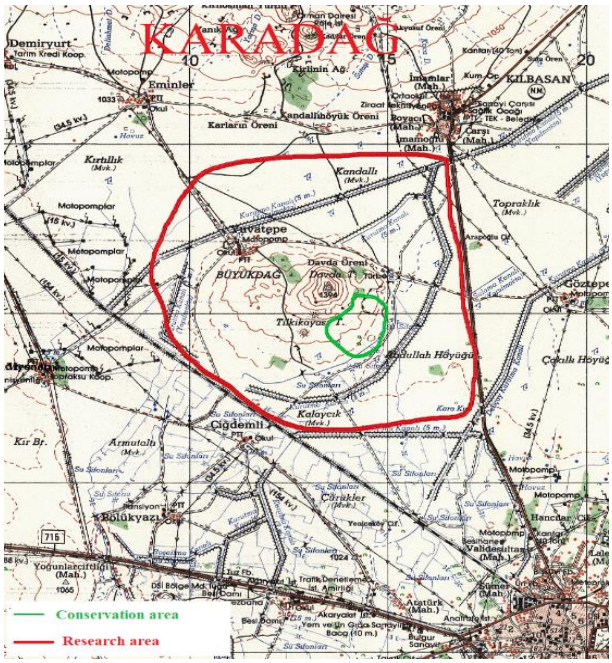

Figure 1. Simplified topographic map of the research area.

Büyükdağ Tilkikayası hill. The old andesites around the hill were seperated by a younger side chimney. Mercik andesites mostly consist of lava flows and pyroclastics (Gül et al., 1984; Ulu and Balcı, 2009). The large soil group of the research area consists of brown, colluvial, volcanic and salty sodic (barren) soils (Büyükşar et al. 1992).

According to the nearest observation stations (Karaman), the research area is located in the bioclimatic layer of "Arid, sub-tropical Mediterranean climate" (Akman, 1999). Decreased precipitation amounts according to the seasons are listed as Karaman KISY. Within the framework of this information, Karaman is included in the 1st type of the Eastern Mediterranean precipitation regime. The total annual precipitation is 329.9 mm. Ombro-thermic (rainfall-temperature) diagram of the research area (Figure 2) was drawn by calculating the average temperatures and monthly average precipitation amounts (Çeçen et al., 2018).

2. Materials and Method

Information related to geology and the soil structure of the research area were obtained from Büyükşar et al. (1992), Ulu and Balcı (2009) and Bilgiç (2009). Topographic map (Figure 1), which was prepared by Şenel (1997) was obtained from the State Hydraulic Works (DSI) Konya Regional Directorate. Meteorological data about the climate of the research area were obtained from the Ministry of Forestry and Water Affairs, Konya Meteorology 8th Regional Directorate (Çeçen et al., 2018). The climate diagram of the Karaman station near the research area were drawn according to the Gaussen method, the annual drought index of the study area was calculated according to De Mortenne and Gottman method, and the climate type and bioclimatic layer were calculated according to the Emberger method (Akman, 1999).

The research material include vascular plant species collected from and the near environ of Davda Mountain, during field trips between 2013-2019. Field records were prepared according to herbarium techniques,

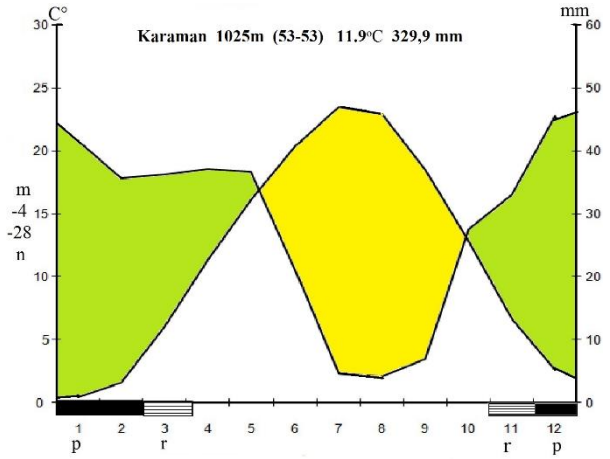

Figure 2. Climate diagram of Karaman province

Abbreviations Used; a: Meteorology Station, b: Meteorology Station Altitude (m.), c: Temperature and precipitation observation year, d: Average annual temperature (C°), e: Average annual precipitation (mm.), m: Lowest of the coldest month mean temperature (C°), n: Absolute minimum temperature (C°), p: Absolute frost months, r: Possible frost months, KISY: Winter, Spring, Autumn, Summer, cm: Centimeters, ºC:Degrees Celsius, m: Meters mm: Millimeters

and plant samples were numbered and pressed in accordance with the relevant literature (Saya and Mısırdalı, 1982; Seçmen et al., 2008).

Flora of Turkey (Davis, 1965-1985; Davis et al., 1988; Güner et al., 2000, 2012, 2014, 2022) was the main source for the identification of the specimens. In cases where it was insufficient, Flora of Europe, Iraq, Iran, Russia, Palestine (Tutin et al. 1964-1981; Towsend and Guest, 1966-1985; Rechinger, 1965-1977; Zohary, 1966-1986; Komarov et al., 1934-1964) were also used. The samples are kept at Karamanaoğlu Mehmetbey University Biodiversity Application and Research Center Herbarium (KMUB).

The list of diagnosed plants is given in alphabetical order and according to the APG III (Angiosperm Phylogeny Group [Angiospermi Phylogeny Group]) system of the Turkish Plants List (Vineary Plants) considering APG IV (Reveal & Chase, 2011; Güner et al., 2012). In the list, current names of the taxa were given without any synonyms, and the current names and status changes of the taxa are in accordance with Plants of the World Online (POWO, 2023) and International Plant Name Index (IPNI, 2023) web pages, and Güner et al. (2012).

Taxa are presented together with the author name, locality number, collection date, voucher number, endemism status, IUCN red data category, phytogeographical region, and life form. Endemism status of the taxa are given in accordance with the "The Red Book of Plants of Turkey" considering the recent changes made (Ekim et al., 2000; Güner et al., 2012; Çeçen et al., 2016; Sağıroğlu et al., 2006).

2.1. Plant collection stations

1- C4-Karaman-Davda Mountain: Büyükdağ Hill, volcanic rocky slopes, 1100-1230 m,

2- C4-Karaman-Davda Mountain: Davda Ruins Hill, volcanic rocky slopes, 1100-1385m,

3- C4-Karaman-Davda Mountain: Davda Tomb, volcanic rocky slopes, 1045-1230 m

4- C4-Karaman-Davda Mountain: Tilki Kaya Hill, volcanic rocky slopes rocky slopes, 1100-1280 m

5- C4-Karaman-Davda Mountain: Conservation Area, volcanic rocky slopes rocky slopes, 1035-1170 m,

6- C4-Karaman- Around Yuvatepe Village, steppe, 1019-1120 m,

7- C4-Karaman- From Davda Mountain to Çiğdemli Village, steppe, 1100-1230 m,

8- C4-Karaman-Davda Mountain: Abdullah Mound, volcanic rocky slopes rocky slopes, 1100-1230 m

9- C4-Karaman-Davda Mountain: Kandallı locality, steppe, 1100-1230 m

10- C4-Karaman-Davda Mountain: Kalaycık locality, steppe, 1100-1230 m

11- C4-Karaman- From Davda Mountain to Eminler Village, steppe, 1010-1055 m

12- C4-Karaman- From Abdullah Höyük to Kılbasan village, steppe, 1030-1100 m

2.2. List of the nearest researches used for comparison

1. Flora of Davda Mountain (Karaman) Florası (Çeçen, 2023).

2. Plants of Karadağ (Karaman) Bitkileri (Ünal and Ocakverdi, 1991).

3. Flora of Çakırdağı (Karaman) Florası (Çeçen et al., 2018).

4. Contributions to the flora of Vegetation of Hacıbaba (Karaman- Kazımkarabekir) Mountain (Serin, 1996).

5. Flora and Vegetation of Konya-Karapınar Region (Bağcı, 1996).

6. Flora of The Region Between Ayrancı Dam, Karakükürtlü Mountain, Alahan and Karaman 1,2 (Ünal and Sağlam, 2008).

7. Flora of Büyükeğri Mountain (Mut, İçel) and Its Surroundings (Şirin and Ertuğrul, 2015).

3. Results

Aspleniaceae

1. ***Asplenium ceterach*** L., 2, 30.06.2017, ÖÇ 2315. Geophyte.

Ephedraceae

2. ***Ephedra foeminea*** Forssk., 1, 10.06.2017, ÖÇ 2220, Kamephyte.

Amaranthaceae

3. ***Atriplex tatarica*** L. var. ***tatarica*** 6, 12.06.2019, ÖÇ 2366, Kamephyte.
4. ***Bassia prostrata*** (L.) Beck., 12, 10.06.2017, ÖÇ 2377, Kamephyte.
5. ***Chenopodium album*** L. subsp. ***album*** var. ***album***, 6, 16.08.2019, ÖÇ 5595, Therophyte.
6. ***Cyathobasis fruticulosa*** (Bunge) Aellen, 2, 16.09.2019, ÖÇ 4678, Endemic (VU), Irano–Turanian, Kamephyte,
7. ***Kraschenninnikovia ceratoides*** (L.) Gueldenst., 3, 19.05.2017, ÖÇ 2376, Kamephyte.

8. ***Noaea mucronata*** (Forssk.) Asch. & Schweinf. subsp. ***mucronata***, 2, 16.08.2019, ÖÇ 5589, Kamephyte.

9. ***Salsola tragus*** L. subsp. ***tragus***, 12, 16.08.2019, ÖÇ 5554, Kamephyte.

10. ***Sueda altissima*** Pall. 12, 30.07.2018, ÖÇ 4664, Kamephyte.

Amaryllidaceae

11. ***Allium flavum*** L. subsp. ***tauricum*** (Basser ex Reichb.) Stearn. var. ***tauricum***., 2, 5, 30.05.2019, ÖÇ 5139, Mediterranean, Geophyte.

12. ***Allium lycaonicum*** Siehe ex Hayek., 6, 19.05.2017, ÖÇ 2176, Geophyte.

13. ***Allium proponticum*** Stearn & Özhatay subsp. ***proponticum,*** 2, 30.06.2017, ÖÇ 2269, Endemic (LC), Geophyte.

14. ***Allium scabriflorum*** Boiss., 5, 10.06.2017, ÖÇ 2298, Endemic (LC), Irano–Turanian, Geophyte.

Anacardiaceae

15. ***Pistacia palaestina*** Boiss., 10, 13.05.2017, ÖÇ 2312. 2, 16.08.2019, ÖÇ 5567, Mediterranean, Fanerophyte.

Apiaceae

16. ***Bupleurum lycaonicum*** Snogerup, 2, 10.06.2017, ÖÇ 2223, Endemic (NT), Mediterranean, Therophyte.

17. ***Bupleurum sulphureum*** Boiss. & Balansa, 3, 30.06.2017, ÖÇ 2291, Endemic (LC), Irano–Turanian, Therophyte.

18. ***Bupleurum turcicum*** Snogerup, 2, 10.06.2017, ÖÇ 2250, Endemic (NT), Mediterranean, Therophyte.

19. ***Coriandrum sativum*** L. 12, 10.06. 2017, ÖÇ 2093, Therophyte.

20. ***Daucus carota*** L., 12, 16.08.2019, ÖÇ 2321, Hemicryptophyte.

21. ***Echinophora tournefortii*** Jaub. & Spach, 2, 16.08.2019, ÖÇ 5598**,** Irano-Turanian, Hemicryptophyte.

22. ***Eryngium bithynicum*** Boiss. 6, 24.06.2015, ÖÇ 2079, Endemic (LC), Irano-Turanian, Hemicryptophyte.

23. ***Eryngium campestre*** L. subp. ***campestre*** var. ***virens*** Link, 1, 30.06.2017, ÖÇ 4665, Hemicryptophyte.

24. ***Falcaria vulgaris*** Bernh., 12, 03.05.2017. ÖÇ1961, Hemicryptophyte.

25. ***Ferula parva*** Boiss. & Heldr., 2,30.06.2017, ÖÇ 2282, Endemic (VU), Irano–Turanian, Geophyte.

26. ***Hohenackeria exscapa*** (Steven) Grande, 2, 09.04.2017, ÖÇ 2031, Irano-Turanian, Therophyte.

27. ***Johrenia dichotoma*** DC., 4, 30.06.2017, ÖÇ 2303, Irano–Turanian, Hemicryptophyte.

28. ***Malabaila secacul*** Banks & Sol. subsp. ***secacul***, 5, 03.05.2013, ÖÇ 1953; 2, 30.06.2017, ÖÇ 2316., Hemicryptophyte.

29. ***Opopanax hispidus*** (Friv.) Griseb., 1, 03.05.2013, ÖÇ 1955, Hemicryptophyte.

30. ***Scandix stellata*** Banks & Sol., 4, 30.04.2017, ÖÇ 2086, 4. 13.05.2017, ÖÇ 2154, Therophyte.

31. ***Scandix iberica*** M.Bieb., 5, 03.05.2013, ÖÇ 1954, Therophyte.

32. ***Torilis leptophylla*** (L.) Rchb., 2, 08.05.2015, ÖÇ 2225, Hemicryptophyte.

33. ***Torilis ucranica*** Spreng., 2, 08.05.2015, ÖÇ 2178, 1, 24.06.2015, ÖÇ 2260, Hemicryptophyte.

34. ***Zosima absinthifolia*** (Vent.) Link., 10, 13.05.2017, ÖÇ 2080, Hemicryptophyte.

Apocynaceae

35. ***Cynanhum acutum*** L. subsp. ***acutum***, 7, 30.07.2018, ÖÇ 4674, Hemicryptophyte.

36. ***Vinca herbacea*** Waldst. & Kit., 7, 30.04.2017, ÖÇ 2084, Geophyte.

37. ***Vincetoxicum canescens*** (Willd.) Decne. subsp. ***canescens***, 3, 10.06.2017, ÖÇ 2131, Hemicryptophyte.

Asparagaceae

38. ***Leopoldia comosa*** (L.) Parl., 3, 19.05.2017, ÖÇ 2142, Mediterranean, Geophyte.

39. ***Muscari neglectum*** Guss., 4. 24.06.2015, ÖÇ 2076, Geophyte.

40. ***Muscari tenuiflorum*** Tausch., 10, 13.05.2017, ÖÇ 2110, Geophyte.

41. ***Ornithogalum fimbriatum*** Willd.,1, 19.05.2017, ÖÇ 2108, Mediterranean, Geophyte.

42. ***Ornithogalum narbonense*** L. 1, 19.05.2017, ÖÇ 2119, Mediterranean, Geophyte.

43. ***Ornithogalum neurostegium*** Boiss. & C.I. Blanche ex Boiss., 10, 13.05.2017, ÖÇ 2053, Geophyte.

Asteraceae

44. ***Achillea aleppica*** DC. subsp. ***zederbaueri*** (Hayek) Hub.-Mor., 2, 30.06.2017, ÖÇ 2333, Endemic (LC), Irano-Turanian, Hemicryptophyte.

45. ***Achillea lycaonica*** Boiss. &Heldr., 2, 30.06.2017, ÖÇ 2251, Endemic (LC), Irano-Turanian**,** Hemicryptophyte.

46. ***Achillea santolinoides*** Lag. subsp. ***wilhelmsii*** (K.Koch) Grcute, 6, 24.06.2015, ÖÇ 2231; 2, 30.06.2017, ÖÇ 2332, Irano-Turanian**,** Hemicryptophyte.

47. ***Anthemis cretica*** L. subsp. ***anatolica*** (Boiss.) Grierson, 2, 24.06.2015, ÖÇ 2190, Hemicryptophyte.

48. ***Anthemis fimbriata*** Boiss., 4, 13.05.2017, ÖÇ 2101, Endemic (VU), Mediterranean, Therophyte.

49. ***Artemisia absinthum*** L. 12, 30.06.2017, ÖÇ 2322, Hemicryptophyte.

50. ***Artemisia santonicum*** L. subsp. ***santonicum***, 3, 30.06.2017, ÖÇ 2382, Kamephyte.

51. ***Carduus nutans*** L. subsp. ***nutans*** sensu lato, 4, 30.06.2017, ÖÇ 2379, Hemicryptophyte.

52. ***Carlina oligocephala*** Boiss. &Kotschy subsp. ***oligocephala***, 2, 30.06.2017, ÖÇ 2296, Therophyte.

53. ***Carthamus dentatus*** (Forsk.) Vahl., 11, 16.08.2019, ÖÇ 5553, Hemicryptophyte.

54. ***Centaurea balsamita*** Lam., 8, 25.06.2019, ÖÇ 4449, Irano-Turanian, Hemicryptophyte.

55. ***Centaurea kotschyi*** (Boiss. & Heldr.) Hayek var. ***persica*** (Boiss.) Wagenitz, 1, 30.06.2017, ÖÇ 2317; 12, 30.07.2018, ÖÇ 4668, Irano-Turanian, Hemicryptophyte.

56. ***Centaurea patula*** L. 1, 16.09.2019, ÖÇ 5588, Therophyte.

57. ***Centaurea solstitialis*** L. subsp. ***solstitialis***, 8, 25.06.2019, ÖÇ 4453, Therophyte.

58. ***Centaurea virgata*** Lam., 2, 30.06.2017, ÖÇ 2238, Irano-Turanian, Hemicryptophyte.

59. ***Chardinia orientalis*** (L.) Kuntze, 2, 24.06.2015, ÖÇ 2285, Irano-Turanian, Hemicryptophyte.

60. ***Cichorium intybus*** L., 6, 30.06.2017, ÖÇ 2334, Hemicryptophyte.

61. ***Cnicus benedictus*** L., 4, 03.05.2013, ÖÇ 1945, Therophyte.

62. ***Condrilla juncea*** L., 11, 30.06.2017, ÖÇ 2369, Hemicryptophyte.

63. ***Cota austriaca*** **(**Jacq.) Sch. Bip., 5, 13.05.2017, ÖÇ 2127, Hemicryptophyte.

64. ***Cousinia iconica*** Hub.-Mor., 2, 30.06.2017, ÖÇ 2236, Endemic (NT), Irano-Turanian, Hemicryptophyte.

65. ***Crepis macropus*** Boiss. & Heldr., 2, 16.08.2019, ÖÇ 5573, Endemic (LC), Irano–Turanian, Hemicryptophyte.

66. ***Crepis sancta*** (L.) Bornm. subsp. ***obovata*** (Boiss.&Noö)Babc., 2, 30.06.2017, ÖÇ 2265, Therophyte.

67. ***Cyanus depressus*** (M. Bieb.) Sojak, 12, 10.06.2017, ÖÇ 2378, Therophyte.

68. ***Cyanus pichleri*** **(**Boiss.) Holub. subsp. ***extrarosularis*** (Hayek & Siehe) Wagenitz & Greuter, 10, 12.05.2017, ÖÇ 2055, Endemic (LC), Hemicryptophyte**.**

69. ***Echinops spinosissimus*** Turra subsp. ***spinosissimus***, 3, 30.06.2017, ÖÇ 2287, Mediterranean, Hemicryptophyte.

70. ***Filago pyramidata*** L., 4, 10.06.2017, ÖÇ 2252, Therophyte.

71. ***Inula anatolica*** Boiss., 12, 30.07.2018, ÖÇ 4676, Hemicryptophyte.

72. ***Koelpina linearis*** Pall. 3, 19.05.2017, ÖÇ 2062, Irano-Turanian, Hemicryptophyte.

73. ***Lactuca orientalis*** (Boiss.) Boiss. 2, 30.06.2017, ÖÇ 2295, Irano-Turanian. Hemicryptophyte.

74. ***Lactuca serriola*** L., 3, 30.07.2018, ÖÇ 2301. 2, 16.08.2019, ÖÇ 5572**,** Hemicryptophyte.

75. ***Lactuca viminea*** (L). J.Presl & C.Presl, 2, 30.06.2017, ÖÇ 2263, Hemicryptophyte.

76. ***Leontodon asperrimus*** (Willd.) Endl., 3, 10.06.2017, ÖÇ 2195, Irano-Turanian, Hemicryptophyte.

77. ***Onopordum bracteatum*** Boiss. & Heldr. subsp. ***bracteatum***, 2, 30.06.2017, ÖÇ 2352, Mediterranean, Hemicryptophyte.

78. ***Picnomon acarna*** (L.) Cass., 12, 30.07.2018, ÖÇ 4672, Therophyte.

79. ***Picris strigosa*** M. Bieb. subsp. ***strigosa***, 1, 03.05.2013, ÖÇ 1949, Irano-Turanian. Hemicryptophyte.

80. ***Rhaponticum repens*** (L.) Hidalgo, 2, 16.08.2019, ÖÇ 5586, Irano-Turanian, Hemicryptophyte.

81. ***Scolymus hispanicus*** L. subsp. ***hispanicus***, 26, 16.09.2018, ÖÇ 2284, Mediterranean, Hemicryptophyte.

82. ***Scorzonera cana*** (C.A.Mey.) Griseb. var. ***jacquiniana*** (W. Koch) D.Chamb., 4, 13.05.2017, ÖÇ 2136, Hemicryptophyte.

83. ***Scorzonera mollis*** M. Biebsubsp. ***szowitzii*** (DC.) Chamb. 2, 30.04.2017, ÖÇ 2030, Irano-Turanian, Geophyte.

84. ***Scorzonera pseudolanata*** Grossh., 10, 24.06.2015, ÖÇ 2211, Irano-Turanian, Geophyte.

85. ***Senecio vernalis*** Waldst. & Kit., 4, 03.05.2013, ÖÇ 2003, Therophyte.

86. ***Taraxacum leucochlorum*** Soest. 2, 13.05.2017, ÖÇ 2038**,** Endemic (CR)**,** Irano-Turanian, Geophyte.

87. ***Taraxacum oliganthum*** Schott. &Kotschy ex Hand. Mazz. 2, 19.05.2017, ÖÇ 2017. Irano-Turanian, Hemicryptophyte.

88. ***Taraxacum syriacum*** Boiss**.,** 1, 30.06.2017, ÖÇ 2308, Hemicryptophyte

89. ***Tragopogon buphthalmoides*** (DC.) Boiss. var. ***latifolius*** Boiss., 6, 30.06.2017, ÖÇ 3248, Hemicryptophyte.

90. ***Tragopogon latifolius*** Boiss. var. ***angustifolius*** Boiss., 5, 13.05.2017, ÖÇ 2104, Hemicryptophyte.

91. ***Tragopogon porrifolius*** L. subsp. ***longirostris*** (Sch.Bip.) Greuter, 4, 13.05.2017, ÖÇ 2102, Hemicryptophyte.

92. ***Tripleurospermum decipiens*** (Fisch. & C.A.Mey.) Bornm., 2, 03.05.2013, ÖÇ 2156, Hemicryptophyte.

93. ***Xanthium spinosum*** L., 12, 30.06.2017, ÖÇ 2280, Hemicryptophyte.

94. ***Xeranthemum annuum*** L., 8. 25.06.2019, ÖÇ 4458, Therophyte.

Boraginaceae

95. ***Alkanna pseudotinctoria*** Hub. - Mor. 1, 06.2015, ÖÇ 2240, Endemic (LC)**,** Irano-Turanian, Hemicryptophyte.

96. ***Alkanna orientalis*** (L.) Boiss. var. ***orientalis***, 1, 03.05.2013, ÖÇ 2025, Hemicryptophyte.

97. ***Anchusa leptophylla*** Roem. & Schult. subsp. ***leptophylla***, 2, 16.08.2019, ÖÇ 5584, Hemicryptophyte.

98. ***Anchusa hybrida*** Ten., 5, 10.06.2017, ÖÇ 2215, Mediterranean, Hemicryptophyte.

99. ***Asperugo procumbens*** L., 4, 03.05.2015, ÖÇ 2005, Euro-Siberian, Therophyte.

100. ***Buglossoides arvensis*** (L.) I. M. Johnst. subsp. ***sibthorpiana*** (Griseb.) R. Fren., 5, 30.04.2017, ÖÇ 2041, Therophyte.

101. ***Echium italicum*** L., 6, 30.06.2017, ÖÇ 2323. Mediterranean, Hemicryptophyte.

102. ***Heliotropium europaeum*** L., 12, 30.06.2017, ÖÇ 2327, Irano-Turanian, Therophyte.

103. ***Moltkia coerulea*** (Willd.) Lehm., 10, 13.05.2017, ÖÇ 2061, Irano–Turanian, Hemicryptophyte.

104. ***Myosotis lithospermifolia*** Hornem. 10, 13.05.2017, ÖÇ 2089,Therophyte.

105. ***Myosotis stricta*** Link ex Roemer & Schultes, 6, 10.06.2017, ÖÇ 2188, Mediterranean, Therophyte.

106. ***Nonea melanocarpa*** Boiss., 7, 30.04.2017, ÖÇ 2068. 10, 13.05.2017, ÖÇ 2135, Irano-Turanian, Therophyte.

107. ***Onosma stenoloba*** Hausskn. ex Riedl, 4. 13.05.2017, ÖÇ 2087. 2, 16.08.2019, ÖÇ 5594. Endemic (LC), Irano- Turanian, Hemicryptophyte.

108. ***Rochelia disperma*** (L. fil.) C. Koch var. ***microcalycina*** (Bornm.) Edmondson, 6, 03.05.2015, ÖÇ 2148, Endemic (LC), Irano–Turanian, Therophyte.

Brassicaceae

109. ***Aethionema arabicum*** (L.) Andrz. ex DC., 1, 03.05.2013, ÖÇ 1948; 5, 19.05.2017, ÖÇ 2120, Therophyte.

110. ***Alyssum contemptum*** Schott & Kotschy, 4, 13.05.2017, ÖÇ 2039, Irano-Turanian,Therophyte.

111. ***Alyssum dasycarpum*** Stephex Willd.,7, 30.04.2015, ÖÇ 2088,Therophyte.

112. ***Alyssum desertorum*** Stapf., 7, 30.05.2015, ÖÇ 2146, Therophyte.

113. ***Alyssum hirsutum*** M.Bieb. subsp. ***hirsutum***, 2, 10.06.2017, ÖÇ 2133, Therophyte.

114. ***Alyssum linifolium*** Stephan ex Willd. var. ***linifolium***, 3, 30.04.2017, ÖÇ 2106, Therophyte.

115. ***Alyssum mouradicum*** Boiss. & Balansa, 1, 08.05.2015, ÖÇ 2091, Kamephyte.

116. ***Alyssum murale*** Waldst. & Kit. subsp. ***murale*** var. ***murale***, 4, 13.05.2017, ÖÇ 2020, Hemicryptophyte.

117. ***Aubrieta canescens*** (Boiss.) Bornm. subsp. ***canescens,*** 4, 30.04.2017, ÖÇ 2010, Endemic (LC), Hemicryptophyte.

118. ***Boreava orientalis*** Jaub. & Spach., 2, 03.05.2015, ÖÇ 2015, Therophyte.

119. ***Brassica elongata*** Ehrh & Beitr., 10, 13.05.2017, ÖÇ 2009, Hemicryptophyte.

120. ***Clypeola jonthlaspi*** L., 7, 30.04.2017, ÖÇ 2017; 4, 13.05.2017, ÖÇ 2105, Therophyte.

121. ***Conringia clavata*** Boiss., 7, 30.04.2017, ÖÇ 2006, Therophyte.

122. ***Crambe tatarica*** Sebeök var. ***tatarica,*** 10, 13.05.2017, ÖÇ 2111, Hemicryptophyte.

123. ***Descurainia sophia*** (L.) Webb ex Prantl.subsp. ***sophia***, 7, 30.04.2017, ÖÇ 2004, Therophyte.

124. ***Draba verna*** L., 14, 09.04.2015, ÖÇ 2008, Therophyte.

125. ***Erysimum crassipes*** Fisch. & Mey., 6, 30.06.2017, ÖÇ 2247; 2, 16.08.2019, ÖÇ 5578, Hemicryptophyte.

126. ***Isatis glauca*** Aucher ex Boiss. subsp. ***iconia*** (Boiss. & Heldr.) P. H. Davis, 10, 13.05.2017, ÖÇ 2213, Endemic (LC), Irano-Turanian. Hemicryptophyte.

127. ***Isatis tinctoria*** L. subsp. ***tomentella*** (Boiss.) P.H. Davis., 7, 10.06.2017, ÖÇ 3257, Hemicryptophyte.

128. ***Lepidium draba*** L. 2, 30.06.2017, ÖÇ 2331, Therophyte.

129. ***Lepidium latifolium*** L., 2, 30.06.2017, ÖÇ 4659, Hemicryptophyte.

130. ***Mathiola longipetala*** (Vent.) DC. subsp. ***bicornis*** (Sibth. & Smith) P. W. Ball., 4, 24.06.2015, ÖÇ 2227, Therophyte.

131. ***Microthlaspi perfoliatum*** (L.) F.K. Mey, 5, 19.05.2015, ÖÇ 2002, Therophyte.

132. ***Neslia paniculata*** (L.) Desv. subsp. ***thracica*** (Velen) Bornm., 1, 03.05.2013, ÖÇ 1957, Therophyte.

133. ***Rapistrum rugosum*** (L.) All., 4, 03.05.2015, ÖÇ 2129. Therophyte.

134. ***Sisymbrium altissimum*** L., 12, 30.06.2017, ÖÇ 2325, Therophyte.

135. ***Strigosella africana*** (L.) Botsch., 1, 08.05.2015, ÖÇ 2234, Therophyte.

Campanulaceae

136. ***Asyneuma virgatum*** (Labill.) Bornm. subsp. ***virgatum***, 1, 24.06.2015, ÖÇ 2226; 2, 10, 30.06.2017, ÖÇ 2264, Hemicryptophyte.

Cannabaceae

137. ***Celtis tournefortii*** Lam., 4. 16.08.2019, ÖÇ 5569, Fanerophyte.

Caprifoliaceae

138. ***Cephalaria syriaca*** (L.) Schrad.,12, 30.06.2017, ÖÇ 2281, Hemicryptophyte.

139. ***Dipsacus laciniatus*** L. 7, 30.06.2017, ÖÇ 2224, Hemicryptophyte.

140. ***Morina persica*** L.var. ***persica***, 4, 30.05.2019, ÖÇ 5148, Irano-Turanian, Hemicryptophyte.

141. ***Scabiosa argentea*** L., 1, 4, 30.06.2017, ÖÇ 2157; 2, 16.08.2019, ÖÇ 5599**,** Hemicryptophyte.

142. ***Scabiosa rotata*** M.Bieb, 7, 30.06.2017, ÖÇ 2328, Irano-Turanian, Hemicryptophyte.

143. ***Valerianella carinata*** Lois., 1,13.05.2017, ÖÇ 2198, Therophyte.

144. ***Valerianella coronata*** **(**L.) DC., 4. 13.05.2017, ÖÇ 2206, Therophyte.

145. ***Valerianella lasiocarpa*** L., 2, 14.05.2015, ÖÇ 2092, Irano-Turanian, Therophyte.

146. ***Valerianella pumila*** **(**L.) DC., 4. 19.05.2017, ÖÇ 2144, Therophyte.

147. ***Valerianella vesicaria*** (L.) Moench, 4. 13.05.2017, ÖÇ 2107, Therophyte.

Caryophyllaceae

148. ***Bolanthus minuartioides*** (Jaub. &Spach) Hub.-Mor., 8, 14.05.2015, ÖÇ 2166. Endemic (LC), Mediterranean, Hemicryptophyte.

149. ***Bufonia calyculata*** Boiss. & Balansa, 2, 30.06.2017, ÖÇ 2314; 2, 16.08.2019, ÖÇ 5580, Endemic (LC), Hemicryptophyte.

150. ***Dianthus cf. stramineus*** Boiss. & Heldr. 2, 30.06.2017, ÖÇ 2283; 2, 16.08.2019, ÖÇ 5583, Endemic (DD), Hemicryptophyte.

151. ***Dianthus pallens*** Sm. var. ***oxylepis*** Boiss., 3, 30.06.2017, ÖÇ 2273, Hemicryptophyte.

152. ***Gypsophila laricina*** Schreb., 2, 16.08.2019, ÖÇ 5579, Endemic (LC), Irano-Turanian, Kamephyte.

153. ***Gypsophila perfoliata*** L. var. ***perfoliata,*** 8. 25.06.2019, ÖÇ 3397, Kamephyte.

154. ***Gypsophila viscosa*** Murr., 6, 03.05.2013, ÖÇ 1959, Irano-Turanian, Therophyte.

155. ***Herniaria incana*** Lam., 1, 10.06.2017, ÖÇ 2096, Hemicryptophyte.

156. ***Holosteum umbellatum*** L. var. ***glutinosum*** (M. Bieb.) Gay, 7, 30.04.2017, ÖÇ 2042, Therophyte.

157. ***Minuartia hamata*** (Hausskn.) Mattf. 1, 03.05.2013, ÖÇ 1951, Kamephyte.

158. ***Minuartia isaurica*** McNeill. 4, 08.05.2015, ÖÇ 2210, Endemic (VU), Mediterranean.

159. ***Minuartia meyeri*** (Boiss.) Bornm., 2, 19.05.2017, ÖÇ 2137, Irano-Turanian, Therophyte.

160. ***Minuartia multinervis*** (Boiss) Bornm., 3, 30.06.2017, ÖÇ 2256, Therophyte.

161. ***Paronychia chionaea*** Boiss. subsp. ***chionea*** var ***chionea***, 4, 19.05.2017, ÖÇ 2045; 2, 10.06.2017, ÖÇ 2134, Hemicryptophyte.

162. ***Silene longipetala*** Vent., 5, 13.05.2017, ÖÇ 2151; 5, 30.05.2019, ÖÇ 5140, Hemicryptophyte.

163. ***Silene spergulifolia*** (Desf.) Bieb., 4. 13.05.2017, ÖÇ 3253; 3, 30.06.2017, ÖÇ 2248, Irano-Turanian, Hemicryptophyte.

164. ***Silene subconica*** Friv., 1, 03.05.2013, ÖÇ 1956. 3, 19.05.2017, ÖÇ 3148, Therophyte.

165. ***Telephium imperati*** L. subsp. ***orientale*** (Boiss.) Nyman, 2, 10.06.2017, ÖÇ 2095, Hemicryptophyte.

Cistaceae

166. ***Fumana aciphylla*** Boiss.,3, 19.05.2017, ÖÇ 2141, Irano-Turanian, Kamephyte.

167. ***Fumana thymifolia*** (L.) Spach, 3, 19.05.2017, ÖÇ 2124. 10, 4, 10.06.2017, ÖÇ 2197, Kamephyte.

168. ***Helianthemum microcarpum*** Coss. ex Boiss., 1, 08.05.2015, ÖÇ 2098, Therophyte.

169. ***Helianthemum salicifolium*** (L.) Mill., 2,10.06.2017, ÖÇ 2196, Therophyte.

Convolvulaceae

170. ***Convolvulus arvensis*** L., 6, 29.05.2015, ÖÇ 1114; 10, 13.05.2017, ÖÇ 5571**,** Hemicryptophyte.

171. ***Convolvulus compactus*** Boiss. 3, 30.06.2017, ÖÇ 2324, Mediterranean and Irano-Turanian, Hemicryptophyte.

172. ***Convolvulus scammonia*** L., 6, 10.06.2017, ÖÇ 2143, Mediterranean, Hemicryptophyte.

173. ***Cuscuta campestris*** Yuncker, 5, 30.06.2017, ÖÇ 2174 (Host: ***Sideritis libanotica*** subsp. ***linearis*** or ***Ebenus hirsuta),*** Vascular Parasite.

Cyperaceae

174. ***Carex stenophylla*** Wahlenb. subsp. ***stenophylloides*** (V. I. Krecz.) Egorova, 11, 16.08.2019, ÖÇ 5601, Irano-Turanian, Geophyte.

Euphorbiaceae

175. ***Euphorbia aleppica*** L., 11, 10.06.2017, ÖÇ 2185, Therophyte.

176. ***Euphorbia chamaesyce*** L., 11, 16.08.2019, ÖÇ 5896, Hemicryptophyte.

177. ***Euphorbia kotschyana*** Fenzl., 2, 16.08.2019, ÖÇ 5591; 2, 10.06.2017, ÖÇ 2186, Mediterranean, Hemicryptophyte.

178. ***Euphorbia petiolata*** Banks & Sol., 11, 30.06.2017, ÖÇ 2254, Therophyte.

Fabaceae

179. ***Alhagi maurorum*** Medik. subsp. ***maurorum***, 7, 24.06.2015, ÖÇ 2239, Irano-Turanian, Kamephyte.

180. ***Astragalus angustifolius*** Lam. subsp. ***angustifolius***, 4, 30.05.2015, ÖÇ 2191, Kamephyte.

181. ***Astragalus commixtus*** Bunge., 5, 10.06.2017, ÖÇ 2158, Irano-Turanian, Therophyte.

182. ***Astragalus gaeobotrys*** Boiss. & Bal., 5, 09.04.2017, ÖÇ 2076, Endemic (EN), Mediterranean, Hemicryptophyte.

183. ***Astragalus hamosus*** L., 12, 13.05.2017, ÖÇ 2149, Hemicryptophyte.

184. ***Astragalus hirsutus*** Vahl. 3, 30.04.2017, ÖÇ 2073, Endemic (LC), Kamephyte.

185. ***Astragalus mesoginatus*** Boiss., 4, 24.06.2015, ÖÇ 2217; 2, 30.06.2017, ÖÇ 2243, Endemic (LC), Irano-Turanian, Hemicryptophyte.

186. ***Astragalus microcephalus*** Willd. subsp. ***microcephalus***, 5, 30.06.2017, ÖÇ 2259, Irano-Turanian, Kamephyte.

187. ***Astragalus suberosus*** Banks & Sol. 10, 13.05.2017, ÖÇ 2078, Irano-Turanian, Kamephyte.

188. ***Astragalus tmoleus*** Boiss. var. ***bounacanthus*** (Boiss.) Chamb., 2, 16.08.2019, ÖÇ 5571; 4, 13.05.2017, ÖÇ Endemic (LC), Kamephyte.

189. ***Astragalus triradiatus*** Bunge., 8, 13.05.2017, ÖÇ 2161, Therophyte.

190. ***Astragalus unalii*** Çeçen, Aytaç & Mısırdalı, 3, 19.05.2017, ÖÇ 2116, Endemic (CR), Hemicryptophyte.

191. ***Ebenus hirsuta*** Jaub. & Spach., 2, 16.08.2019, ÖÇ 5587**,** 30.06.2017, ÖÇ 2343, Endemic (LC). Irano-Turanian, Hemicryptophyte.

192. ***Hedysarum pannosum*** (Boiss.) Boiss., 2, 10.06.2017, ÖÇ 2193, Irano-Turanian, Hemicryptophyte.

193. ***Lathyrus nivalis*** Hand. &Mazz., 10, 13.05.2017, ÖÇ 2147, Endemic, Irano-Turanian, Therophyte.

194. ***Lens culinaris*** Medik subsp. ***orientalis***(Boiss.) Ponert., 1, 08.05.2015, ÖÇ 2209, Therophyte.

195. ***Medicago fischeriana*** (Ser.) Trautv., 1, 24.06.2015, ÖÇ 2229, Therophyte.

196. ***Medicago radiata*** L., 1, 03.05.2013, ÖÇ 1944, Irano-Turanian, Therophyte.

197. ***Medicago rigidula*** (L.) All. var. ***rigidula***, 20, 30.05.2013, ÖÇ 2065, Therophyte.

198. ***Medicago x varia*** Martyn, 4, 24.06.2015, ÖÇ 2230, Therophyte.

199. ***Onobrychis oxyodonta*** Boiss. var.***armena*** (Boiss. &Huet) Aktoklu, 2, 30.06.2017, ÖÇ 2258, Hemicryptophyte.

200. ***Ononis pusilla*** L., 2, 30.06.2017, ÖÇ 2359, Mediterranean, Hemicryptophyte.

201. ***Trifolium pratense*** L. var**.** ***pratense,*** 8. 25.06.2019, ÖÇ 4451, Therophyte.

202. ***Trigonella coerulescens*** (M. Bieb.) Halácsy subsp. ***coerulescens,*** 7, 30.04.2017, ÖÇ 2074, Therophyte.

203. ***Trigonella filipes*** Boiss., 4. 24.06.2015, ÖÇ 2219, Therophyte.

204. ***Trigonella velutina*** Boiss.,14, 13.05.2015, ÖÇ 2125, Therophyte.

205. ***Vicia cracca*** L. subsp. ***cracca***, 4. 24.06.2015, ÖÇ 2203, Euro-Siberian, Hemicryptophyte.

206. ***Vicia caesarea*** Boiss. &Balansa, 4, 13.05.2017, ÖÇ 2109, Endemic (LC), Irano-Turanian. Hemicryptophyte.

Fagaceae

207. ***Quercus trojana*** Webb subsp. ***trojana***, 2, 30.06.2017, ÖÇ 2305, Mediterranean, Fanerophyte.

Geraniaceae

208. ***Erodium gruinum*** (L.) L'Hérit., 3, 19.05.2017, ÖÇ 2123, Mediterranean, Therophyte.

209. ***Erodium cicutarium*** (L.) L'Hérit. subsp. ***cicutarium***, 4, 30.04.2017, ÖÇ 2066, Therophyte.

210. ***Geranium molle*** L. 4, 30.04.2017, ÖÇ 2016, Therophyte.

211. ***Geranium tuberosum*** L., 5, 09.04.2017, ÖÇ 2048, Irano-Turanian, Geophyte.

Lamiaceae

212. ***Ajuga chamaepitys***(L.) Schreb. subsp. ***chia*** (Schreb.) Arcang., 3, 03.05.2013, ÖÇ 1947, Hemicryptophyte.

213. ***Ajuga chamaepitys*** (L.) Schreber. subsp. ***mesoginatus***, 10, 13.05.2017, ÖÇ 2057, Mediterranean, Hemicryptophyte.

214. ***Ballota larendana*** Boiss. & Heldr., 1, 2, 24.06.2015, ÖÇ 2216, Endemic (LC), Irano-Turanian, Hemicryptophyte.

215. ***Clinopodium graveolens*** (M.Bieb) Kuntz. subsp. ***rotundifolium*** (Pers.) Govaerts, 1, 3, 30.04.2015, ÖÇ 2090, Therophyte.

216. ***Lamium amplexicaule*** L. var. ***amplexicaule***, 4, 30.04.2017, ÖÇ 2029, Therophyte.

217. ***Marrubium globosum*** Montbret & Aucher ex Bent. subsp. ***globosum***, 3, 30.06.2015, ÖÇ 2072, Endemic (LC), Irano-Turanian, Hemicryptophyte.

218. ***Micromeria myrtifolia*** Boiss. & Hohen., 1, 24.06.2015, ÖÇ 2233. 2,10.06.2017, ÖÇ 2182, Kamephyte.

219. ***Nepeta congesta*** Fisch. & C.A.Mey. var. ***congesta***, 1, 03.05.2013, ÖÇ 1960; 12, 30.07.2018, ÖÇ 4661, Endemic (LC), Irano-Turanian, Hemicryptophyte.

220. ***Nepeta italica*** L., 2, 10.06.2017, ÖÇ 2173, Hemicryptophyte.

221. ***Phlomis armeniaca*** Willd., 2, 24.06.2015, ÖÇ 2207, Irano-Turanian, Hemicryptophyte.

222. ***Phlomis pungens*** Willd. var. ***pungens***, 2, 16.08.2019, ÖÇ 5557**,** Hemicryptophyte.

223. ***Phlomis nissolii*** L., 6, 30.06.2017, ÖÇ 2170. Endemic (LC), Irano-Turanian, Hemicryptophyte.

224. ***Salvia absconditiflora*** (Montbret & Aucher ex Benth.) Greuter & Burdet., 3, 19.05.2017, ÖÇ 3252, Endemic (LC), Irano-Turanian, Hemicryptophyte.

225. ***Salvia candidissima*** Vahl subsp. ***candidissima***, 2, 30.06.2017, ÖÇ 2242, Irano-Turanian, Hemicryptophyte.

226. ***Salvia ceratophylla*** L., 4, 19.05.2017, ÖÇ 3254, Irano-Turanian, Hemicryptophyte.

227. ***Salvia cyanescens*** Boiss. & Balansa, 2, 30.05.2018, ÖÇ 2342, Endemic (LC), Irano-Turanian. Hemicryptophyte.

228. ***Salvia sclarea*** L., 11, 24.06.2015, ÖÇ 2266, Hemicryptophyte.

229. ***Salvia virgata*** Jacq., 8, 25.06.2019, ÖÇ 4454. Irano-Turanian, Hemicryptophyte.

230. ***Scutellaria orientalis*** L. subsp**.** ***pinnatifida*** Edm., 7, 30.04.2017, ÖÇ 2071, Hemicryptophyte.

231. ***Sideritis bilgerana*** P. H. Davis, 4, 30.06.2017, ÖÇ 2307, Endemic (VU), Mediterranean, Hemicryptophyte.

232. ***Sideritis lanata*** L., 4, 24.06.2015, ÖÇ 2212, Mediterranean, Therophyte.

233. ***Sideritis libanotica*** Labill. subsp. ***linearis*** (Benth.) Bornm., 2, 16.08.2019, ÖÇ 5582, Mediterranean, Hemicryptophyte.

234. ***Stachys burgsdorffioides*** (Benth.) Boiss. subsp. ***burgsdorffioides***, 6, 30.04.2017, ÖÇ 2054, Irano-Turanian, Therophyte.

235. ***Stachys cretica*** L. subsp**.** ***vacillans*** Rech.f., 5, 30.05.2019, ÖÇ 5142, Mediterranean, Hemicryptophyte.

236. ***Teucrium polium*** L. subsp. ***polium***, 2, 30.06.2017, ÖÇ 2232, Kamephyte.

237. ***Thymus leucostomus*** Hausskn. & Velen., 2, 24.05.2013, ÖÇ 2257, Endemic (NT), Irano-Turanian, Kamephyte.

238. ***Thymus sipyleus*** Boiss., 2, 03.05.2015, ÖÇ 2189 ; 1, 24.06.2015, ÖÇ 2255, Kamephyte.

239. ***Ziziphora taurica*** M. Bieb subsp. ***taurica***, 3, 30.06.2017, ÖÇ 2253, Therophyte.

240. ***Ziziphora tenuior*** L.,1, 10.06.2017, ÖÇ 2192. Irano-Turanian, Therophyte.

Linaceae

241. ***Linum austriacum*** L. subsp. ***austriacum***, 7, 30.06.2017, ÖÇ 2261. Hemicryptophyte.

Malvaceae

242. ***Alcea biennis*** Winterl., 6, 30.06.2017, ÖÇ 2309**,** Hemicryptophyte.

243. ***Malva neglecta*** Wallr.,12, 30.06.2017, ÖÇ 2249, Hemicryptophyte.

244. ***Malvella sherardiana*** (L.) Jaub. & Spach, 11, 30.07.2018, ÖÇ 4662, Hemicryptophyte.

Nitrariaceae

245. ***Peganum harmala*** L.,12, 30.06.2017, ÖÇ 2326, Kamephyte.

Oleaceae

246. ***Jasminum fruticans*** L.,1, 30.05.2017, ÖÇ 2310, Mediterranean, Fanerophyte.

Orobanchaceae

247. ***Orobanche anatolica*** Boiss. & Reuter, 6, 19.05.2017, ÖÇ 2139, Vascular parasite.

248. ***Orobanche pubescens*** d 'Urv., 4, 10.06.2017, ÖÇ 2180, Vascular parasite.

Papaveraceae

249. ***Fumaria asepala*** Boiss., 2, 19.05.2017, ÖÇ 2056, Irano-Turanian, Therophyte.

250. ***Fumaria vaillantii*** Lois., 3, 19.05.2017, ÖÇ 2064. Therophyte.

251. ***Glaucium corniculatum*** (L.) Rudolph subsp. ***corniculatum***, 5, 30.06.2017, ÖÇ 2262, Hemicryptophyte.

252. ***Hypecoum pendulum*** L., 2, 10.06.2017. ÖÇ 2121, Therophyte.

253. ***Hypecoum procumbens*** L. subsp. ***procumbens***, 6, 30.05.2019, ÖÇ 5144, Mediterranean, Therophyte.

254. ***Papaver dubium*** L. subsp. ***dubium***, 7, 19.04.2017, ÖÇ 2027, Therophyte.

255. ***Papaver macrostomum*** Boiss. & Huet ex Boiss., 4, 19.05.2017, ÖÇ 2063. Irano-Turanian,Therophyte.

256. ***Papaver rhoeas*** L., 6, 10.06.2017, ÖÇ 2162, Therophyte.

257. ***Roemeria hybrida*** (L.) DC. subsp. ***hybrida***, 10, 13.05.2017, ÖÇ 2013**,** Therophyte.

Plantaginaceae

258. ***Linaria corifolia*** Desf., 12, 13.05.2017, ÖÇ 2160, Endemic (LC), Irano-Turanian, Hemicryptophyte.

259. ***Linaria simplex*** (Willd.) DC, 4, 10.06.2017, ÖÇ 2183. Mediterranean? Therophyte.

260. ***Plantago lanceolata*** L., 7, 10.06.2017, ÖÇ 2177, Hemicryptophyte.

261. ***Veronica biloba*** Schreber, 11, 19.05.2015, ÖÇ 2007, Irano-Turanian,Therophyte.

262. ***Veronica bozakmanii*** M.A. Fisch, 6, 19.05.2015, ÖÇ 2052, Irano-Turanian-Therophyte.

263. ***Veronica cuneifolia*** D. Don. subsp. ***cuneifolia***, 4, 30.04.2017, ÖÇ 2244, Endemic, Therophyte.

Plumbaginaceae

264. ***Acantholimon venustum*** Boiss. var. ***venustum***, 4, 30.06.2017, ÖÇ 2272 ; 2, 16.08.2019, ÖÇ 5585, Kamephyte.

265. ***Plumbago europaea*** L., 12, 30.07.2018, ÖÇ 4671, Euro-Siberian, Hemicryptophyte.

Poaceae

266. ***Aegilops cylindrica*** Host, 2, 10.06.2017, ÖÇ 2194, Irano-Turanian, Therophyte.

267. ***Aegilops triuncialis*** L. subsp. ***triuncialis***, 4, 30.05.2019, ÖÇ 5131, Therophyte.

268. ***Alopecurus arundinaceus*** Poir., 1, 03.05.2013, ÖÇ 1942, Euro-Siberian, Geophyte.

269. ***Arrhenatherum palaestinum*** Boiss., 10, 03.05.2013, ÖÇ 2184, Irano-Turanian, Geophyte.

270. ***Avena barbata*** Pottex Link subsp. ***barbata***, 2, 30.05.2019, ÖÇ 5135, Mediterranean, Therophyte.

271. ***Avena sativa*** L., 2, 10.06.2017, ÖÇ 2168, Hemicryptophyte.

272. ***Bromus cappadocicus*** Boiss. & Balansa. subsp. ***cappadocicus***, 5, 19.05.2017, ÖÇ 2187, Therophyte.

273. ***Bromus japonicus*** Thunb. subsp. ***japonicus***, 6, 10.06.2017, ÖÇ 2152, Therophyte.

274. ***Bromus squarrosus*** L., 4, 30.06.2017, ÖÇ 2329, Therophyte.

275. ***Bromus sterilis*** L., 7, 24.06.2015, ÖÇ 2221, Therophyte.

276. ***Crypsis alopecuroides*** (Piller & Mitterp.) Schrad., 7, 16.08.2019, ÖÇ 5556, Hemicryptophyte.

277. ***Cynodon dactylon*** (L.) Pers. var. ***villosus*** Regel, 1, 16.08.2019, ÖÇ 5555, Hemicryptophyte.

278. ***Echinaria capitata*** (L.) Desf., 1, 03.05.2013, ÖÇ 1946, Therophyte.

279. ***Eremopyrum confusum*** Melderis subsp. ***Sublanigunosum*** (Drop) Cabi & Doğan, 6, 03.05.2013, ÖÇ 1943, Therophyte.

280. ***Festuca valesiaca*** Schleich. ex Gaudin, 5. 30.05.2019, ÖÇ 5132, Hemicryptophyte.

281. ***Gaudiniopsis macra*** (M.Bieb.) Eig. subsp. ***macra***, 3, 08.05.2013, ÖÇ 2094, Irano-Turanian, Therophyte.

282. ***Hordeum bulbosum*** L., 3, 10.06.2017, ÖÇ 2085, Geophyte.

283. ***Koeleria eriostachya*** Pančić, 5, 10.06.2017, ÖÇ 2268, Hemicryptophyte.

284. ***Melica ciliata*** L. subsp. ***ciliata***, 5. 30.05.2019, ÖÇ 5134, Hemicryptophyte.

285. ***Melica persica*** Kunth. subsp. ***inaequiglumis*** (Boiss.) Bor, 3, 30.05.2015, ÖÇ 2118, Hemicryptophyte.

286. ***Oryzopsis coerulescens*** (Desf.) Hack.,14, 03.05.2017, ÖÇ 2153, Hemicryptophyte.

287. ***Pennisetum orientale*** Rich.,10, 19.05.2017, ÖÇ 2100, Irano-Turanian, Geophyte.

288. ***Phragmites australis*** (Cav.) Trin ex Steudel. 7, 30.07.2018, ÖÇ 2202, Euro-Siberian, Hidrofit.

289. ***Poa bulbosa*** L.,7, 30.06.2017, ÖÇ 2117, Geophyte.

290. ***Stipa holosericea*** Trin., 2, 10.06.2017, ÖÇ 2172. Irano-Turanian, Hemicryptophyte.

291. ***Stipa lessingiana*** Trin. & Rupr.,1, 19.05.2017, ÖÇ 2228, Hemicryptophyte.

292. ***Taeniatherum caput-medusae*** (L.) Nevskisubsp. ***crinitum*** (Schreb.)Melderis, 7, 10.06.2017, ÖÇ 2138, Irano-Turanian, Therophyte.

293. ***Vulpia ciliata*** Dumart.subsp. ***ciliata***, 2, 02.05.2015, ÖÇ 2140, Therophyte.

Polygonaceae

294. ***Atraphaxis billardieri*** Jaub. & Spach. subsp. ***billardieri***, 4, 30. 06.2017, ÖÇ 2306***;*** 5, 30.05.2019, ÖÇ 5136, Irano-Turanian. Kamephyte.

295. ***Polygonum arenarium*** Waldst. & Kit., 6, 10.06.2019, ÖÇ 5574, Therophyte.

296. ***Polygonum aviculare*** L., 6, 30.07.2018, ÖÇ 4675, Therophyte.

Primulaceae

297. ***Androsace maxima*** L., 4. 13.05.2017 ÖÇ 2021, 3, 30.06.2017, ÖÇ 2318, Therophyte.

Ranunculaceae

298. ***Adonis flammea*** Jacq., 7, 30.04.2017, ÖÇ 2070, Therophyte.

299. ***Ceratocephala testiculatus*** (Crantz.) Roth., 10, 30.04.2017, ÖÇ 2023, Therophyte.

300. ***Cosolida orientalis*** (Gay) Schröd., 12, 10.06.2017, ÖÇ 2246, Therophyte.

301. ***Consolida raveyi*** (Boiss.) Schröd.,4, 6, 30.06.2017, ÖÇ 2279, Endemic (LC). Irano-Turanian, Therophyte.

302. ***Consolida regalis*** S. F. Gray subsp. ***paniculata*** (Host) Soo, 2, 30.06.2017, ÖÇ 2304, 12, 30.07.2018, ÖÇ 4660; 1, 30.06.2017, ÖÇ 2241, Therophyte.

303. ***Delphinium venulosum*** Boiss., 2, 16.08.2017, ÖÇ 2288, Endemic (LC), Irano-Turanian, Therophyte.

304. ***Ranunculus cuneatus*** Boiss., 3, 30.05.2017, ÖÇ 2059, Hemicryptophyte.

Resedaceae

305. ***Reseda lutea*** L. var. ***lutea***, 1, 03.05.2013, ÖÇ 1958, Hemicryptophyte.

Rhamnaceae

306. ***Rhamnus hirtellus*** Boiss., 3, 10.06.2017, ÖÇ 2130, Endemic (LC), Irano-Turanian, Fanerophyte.

Rosaceae

307. ***Amygdalus orientalis*** Miller., 4. 13.05.2017, ÖÇ 2075, Irano-Turanian, Fanerophyte.

308. ***Crataegus azarolus*** L.var. ***azarolus***, 1,2, 30.06.2017, ÖÇ 2270; 2, 12, 30.07.2018, ÖÇ. 4666, Fanerophyte.

309. ***Potentilla recta*** L., 2, 10.06.2017, ÖÇ 2114, Hemicryptophyte.

310. ***Rosa pulverulenta*** M. Bieb, 2, 30.05.2018, ÖÇ 2300, Fanerophyte.

Rubiaceae

311. ***Asperula lilaciflora*** Boiss. subsp. ***phrygia*** (Bornm.) Schönb.-Tem., 3, 10.06.2017, ÖÇ 2171; 3, 30.06.2017, ÖÇ 2267, Endemic (LC), Hemicryptophyte.

312. ***Asperula stricta*** Boiss. subsp. ***stricta***, 2, 10.06.2017, ÖÇ 2199; 5, 30.05.2019, ÖÇ 5143, Mediterranean, Hemicryptophyte.

313. ***Cruciata taurica*** (Pall. Ex Willd.) Ehrend. 10, 13.05.2017, ÖÇ 3233, Irano-Turanian, Therophyte.

314. ***Crucianella disticha*** Boiss., 2,10.06.2017, ÖÇ 2181, Endemic (LC), Irano-Turanian, Therophyte.

315. ***Galium aparine*** L., 3, 4, 13.05.2017, ÖÇ 2115, Therophyte.

316. ***Galium incanum*** Sm. subsp. ***elatius*** (Boiss.) Ehrend., 3, 10.06.2017, ÖÇ 2201, Irano-Turanian, Kamephyte.

317. ***Galium setaceum*** Lam., 5, 30.06.2017, ÖÇ 2387, Therophyte.

318. ***Galium verticillatum*** Danthoine ex Lam., 1, 10.06.2017, ÖÇ 2169, Mediterranean, Therophyte.

319. ***Galium verum*** L. subsp. ***glabrescens*** Ehrend., 2, 30.06.2017, ÖÇ 2293, Irano-Turanian, Hemicryptophyte.

Rutaceae

320. ***Haplophyllum vulcanicum*** Boiss. & Heldr., 4,5, 13.05.2017, ÖÇ 2126, Endemic (VU), Irano-Turanian, Kamephyte.

321. ***Ruta thesoides*** Fisch ex DC., 12, 10.06.2017, ÖÇ 2214; 1, 30.06.2017, ÖÇ 2290, Hemicryptophyte.

Santalaceae

322. ***Viscum album*** L. subsp. ***album***, 2, 30.07.2018, ÖÇ 4677, Vascular parasite.

Scrophulariaceae

323. ***Scrophularia scopolii*** Hoppeex Pers. var. ***scopolii***, 4, 30.04.2017, ÖÇ 3357. Hemicryptophyte.

324. ***Scrophularia xanthoglossa*** Boiss. var. ***decipiens*** (Boiss. & Kotschy) Boiss., 3, 19.05.2017, ÖÇ 2060. Irano-Turanian. Hemicryptophyte.

325. ***Verbascum campestre*** Boiss. & Heldr., 5, 19.05.2017, ÖÇ 2083 ; 12,10.06.2017, ÖÇ 2163. Endemic (NT), Irano-Turanian, Hemicryptophyte.

326. ***Verbascum cheiranthifolium*** Boiss. var. ***cheiranthifolium***, 6, 30.06.2017, ÖÇ 2335, Hemicryptophyte.

327. ***Verbascum glomeratum*** Boiss., 8, 10.06.2017, ÖÇ 2165, Irano-Turanian, Hemicryptophyte.

328. ***Verbascum lasianthum*** Boiss. ex Benth., 2, 16.08.2019, ÖÇ 5570; 12, 30.07.2018, ÖÇ 4663, Hemicryptophyte.

329. ***Verbascum vulcanicum*** Boiss. & Heldr. var. ***vulcanicum,*** 12. 30.05.2019, ÖÇ 5137, Endemic (LC), Irano-Turanian, Hemicryptophyte.

Urticaceae

330. ***Parietaria judaica*** L., 1-3, 30.06.2017, ÖÇ 2289, Kamephyte.

331. ***Urtica dioica*** L., 7, 13.05.2017, ÖÇ 2122, Hemicryptophyte.

Violaceae

332. ***Viola occulta*** Lehm., 4, 30.04.2017, ÖÇ 2044; 10, 13.05.2017, ÖÇ 2132, Therophyte.

4. Discussions

Three hundred and thirty two taxa belonging to 44 families and 212 genera were identified in the study area. Two of of belong to Pteridophyta and 320 belong to Magnoliophyta (one taxon from Pinophytina subdivision and 321 taxa from Magnoliphytina subdivision). Out of the 332 identified taxa, 51 are endemic and the endemism rate is 15.4%.

Among the determined taxa, 70 (21.1%) belong to the Iranian-Turanian, 34 (10.3%) Mediterranean, and 4 (1.1%) Euro-Siberian phytogeographic regions. The remaining 224 taxa (67.5%) are either unknown or widely distributed. According to these data, the study area mainly consists of Irano-Turanian element plants, but since it is close to the border of the Mediterranean phytogeographic region, a large increase is observed in Mediterranean element plants (Table 1).

Table 1. Comparison of the phytogeographic elements percentages with the neighbouring studies

	1	**2**	**3**	**4**	**5**	**6**	**7**
Research area	Davda Mount	Karadağ	Çakırdağı	Hacıbaba	Karapınar	Ayrancı	Büyükeğri
Number of total taxa	332	521	516	1027	227	250	330
Iranian-Turanian (%)	21.1	25.5	29,8	21,9	21,5	16,5	14.3
Mediterranean (%)	10,3	11,6	12,1	20,5	5,2	12,1	27.3
Euro-Siberian (%)	1,1	3,1	2,5	3,1	2,2	1,6	2.7
Unknown or widely distributed (%)	67,5	59,8	55,6	48,3	48,4	69,8	54.7

The findings of research area and the researches carried out in neighbouring regions show similar ratios in terms of Iran-Turanian phytogeographic region elements. Since the Hacıbaba and Ayrancı study areas are near to the Mediterranean phytogeographic region, and cover a wider surface area, the Mediterranean phytogeographic region elements seems to be closer with the research area. The Euro-Siberian phytogeographic region elements show less distribution in research area because of the distance from the compared areas (Table 2).

The most common families in the Flora of Turkey are *Asteraceae, Fabaceae, Brassicaceae, Lamiaceae, Caryophyllaceae, Poaceae, Apiaceae* and *Scrophulariaceae* respectively. Similar distribution is also observed in research area and the neighbouring regions, though some small changes in the order of the families. The order of *Scrophulariaceae* has been replaced by *Boraginaceae* due to the transfer of some genera to other families. The taxa number of the most common 8 families in study area is 216, constituting 65.1% of the total flora (Table 2). Due to its cosmopolitan structure and easy spreading with pappus, as well as its adaptation to different habitats, *Asteraceae* is the most crowded family in the region. The steppe characteristics of the research area and the neighbouring ares seems to favor *Brassicaceae* and *Poaceae* in terms of taxa number (Ekim, 2014; Kılıç, 2022).

Table 2. Families with the highest number of taxa in the research area and nearby study areas

	1	**2**	**3**	**4**	**5**	**6**	**7**
Research area	Davda Mount	Karadağ	Çakırdağı	Hacıbaba	Karapınar	Ayrancı	Büyükeğri
Asteraceae (%)	50	15.1	12,2	14,9	12,7	12,3	13,4
Lamiaceae (%)	28	8.4	6,7	6,9	7	6,1	7,5
Fabaceae (%)	27	8.1	10,6	9,5	9,9	5,7	8,4
Poaceae (%)	27	8.1	6,9	7,2	8,3	13,6	6,7
Brassicaceae (%)	26	7.8	7,4	9	8,4	8,8	6,4
Boraginaceae (%)	23	6.9	5,5	4,2	3,9	6,1	5,5
Apiaceae (%)	18	5.4	3,2	3,5	4,3	3	4,3
Caryophyllaceae (%)	17	5.1	4,8	6	5,5	4,8	6

Table 3. The genera with the highest number of taxa in the area of study and areas nearby

	1	**2**	**3**	**4**	**5**	**6**	**7**
Research area	Davda Mount	Karadağ	Çakırdağı	Hacıbaba	Karapınar	Ayrancı	Büyükeğri
Astragalus	11	10	17	22	3	20	5
Alyssum	7	6	11	13	3	12	4
Salvia	6	4	6	11	2	12	4
Centaurea	5	8	11	16	6	15	7
Galium	5	6	9	13	2	15	1
Valerianella	5	3	8	6	0	2	1
Verbascum	5	4	4	7	2	10	2
Allium	4	5	9	5	2	7	1

Astragalus L., *Verbascum* L., *Allium* L., *Centaurea* L., and *Silene* L. are the most crowded genera in Flora of Turkey. Similar distributions were also observed in research area and the neighbouring regions with some small exceptions. *Astragalus* is the richest genus in the research area and the studies in closer regions except Karapınar and Büyükeğri. Taxa richness order of *Astragalus* and *Centaurea* order in the research area is the same with the flora of Turkey. *Verbascum* and Allium which has the 2nd and 3rd crowded genera in the Flora of Turkey, had the 7th and 8th places in the current study. Though *Alyssum* L., *Galium* L., *Salvia* L., and *Valerianella* Mill. don't take place among the most crowded 10 genera in Flora of Turkey, they do in current study. The underlying reason could be the domination of steppe vegetation in the area. The fact that other genera contain different numbers of taxa can be attributed to habitat differences and the lack of a detailed investigation due to the wider surface areas of the other regions (Ekim, 2014).

Fifty one (15.4%) of the 332 determined taxa are endemic. Though the research area does not have a prominent altitude, the isolated volcanic character of the region could be underlying reason of increased endemism rate. The endemism rate is also similar to the nearby study areas (Table 4). The threatened endemic plants categories of the the study area are listed as CR (2), EN (1), VU (6), NT (6), LC (35) and DD (1). Rare taxa do not show distribution in the research area.

The distribution of the determined taxa according to Raunkiaer's life forms are as follows: 146 Hemicryptophytes, 118 Therophytes, 31 Kamephytes, 23 Geophytes, 9 Phanerophytes, 4 Vascular parasites, and 1 Hydrophyte (Table 5).

In addition to the records of Andrasovszky and *Astragalus unalii* Çeçen, Aytaç & Mısırdalı (Çeçen et al. 2016) as new species, 332 existing in the area were also listed. The floristic composition of the region has also become known. *Ferula parva* Feryn et Bornm., a suspicious record of 4th volume of the Flora of Turkey (Çeçen et al. 2019), was also determined in the region.

Table 4. Endemic taxa in the research area and close study areas

	1	2	3	4	5	6	7
Research area	Davda Mount	Karadağ	Çakırdağı	Hacıbaba	Karapınar	Ayrancı	Büyükeğri
Number of total taxa	332	521	516	1027	227	834	330
Number of endemic taxa	51	70	88	190	29	168	56
Endemism rate (%)	15.4	13.5	17.3	18.5	12.2	20	16.9

Table 5. Life forms of plants in the research area and nearby study areas

	1	2	3	4	5	6	7
Research area	Davda Mount	Karadağ	Çakırdağı	Hacıbaba	Karapınar	Ayrancı	Büyükeğri
Hemicryptophytes	146	43.9	-	38,8	41,8	-	40,5
Therophytes	118	35.6	-	39	31,7	-	37
Geophytes	31	6.9	-	9,5	11	-	5,3
Kamephytes	24	9.4	-	6,6	8,6	-	13,2
Phanerophytes	9	2.7	-	5,1	4,8	-	1,8
Vascular parasites (%)	4	1.2	-	1	0,4	-	0
Hydrophyte	1	0.3	-	0	1,7	-	2,2

Dianthus cyri Fisch et Mey. and *Lythrum tribracteatum* Salzm. ex Spreng. taxa could not be collected in the region. The herbarium samples on which Andrasovszky bases these records are in the Budapest herbarium. Although we have not contacted the curators, samples have not been reached so far. *Dianthus cyri* Fisch et Mey. one of the *Dianthus* taxa in our area may have been misdiagnosed as it is described as an annual taxon. Both of Andrasovszky's samples in Budapest and those collected from other regions should be validated and added to the flora of Turkiye in future.

Grazing pressure outside the protected area has decreased due to the decreasing sheep and goat farming in the region. Afforestation and protection of a part of the study area are considered beneficial. But the construction of houses and hobby gardens seem to be increasing in the region, especially depending on Covid 19 disease. Prevention of such attempts which disrupt the vegetation of the natural region, will contribute to the continuity of the endemic plants of the region.

Considering the revival and increase in the natural plant diversity of the protected area in our study area, it will be better to take whole area under protection in order not to damage the vegetation outside the protected area. As a result, flora lists of such narrow areas and efforts should be made to protect every taxon that constitutes plant richness in our region, as in the whole country.

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