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Journal Cover Design Mert EKŞİ Istanbul University Faculty of Forestry Department of Landscape Techniques Bahçeköy-Istanbul, Turkey

Technical Advisory Osman Yalçın YILMAZ Surveying and Cadastre Department of Forestry Faculty of Istanbul University, 34473, Bahçeköy, Istanbul-Türkiye

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Contact H. Barış TECİMEN Istanbul University-Cerrahpasa, Faculty of Forestry, Soil Science and Ecology Dept. İstanbul, Turkey

hbarist@gmail.com Journal Web Page http://dergipark.gov.tr/ejejfs



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Epiphytic bryophyte vegetation of Beldibi and Babadağ forests (Zonguldak, Turkey)

Mevlüt ALATAŞ¹* Tülay EZER², Nevzat BATAN³

¹*Munzur University, Engineering Faculty, Department of Bioengineering, 62000, Tunceli, Turkey,
 ²Niğde Ömer Halisdemir University, Faculty of Science, Department of Biology, 51100, Niğde, TURKEY,
 ³Karadeniz Technical University, Maçka Vocational School, 61750, Trabzon, TURKEY,

Corresponding author: mevlutalatas@hotmail.com

Abstract

In this study, epiphytic bryophyte vegetation of Beldibi and Babadağ forests has been studied in northwest Turkey (Zonguldak). A total of 55 sample plots were studied. Phytosociological data, obtained from sampe plots, were classified according to the Barun Balnquet method. And also sample plots were ordinated with Detrended Correspondance Analysis (DCA) and classified with two-way indicator species analysis (TWINSPAN). The results indicated that three bryosociological unit were determined from the forests. These; *Neckeretum crispae*, *Anomodonto viticulosi-Leucodontetum sciuroidis* and, *Orthotricho straminei-Pterigynandretum filiformis*. Amanog them *Neckeretum crispae* was reported for the first time from Turkey. **Key Words:** Bryophyte, Epiphyte, Vegetation, Zonguldak, Turkey

Introduction

Bryophytes are a conspicuous and ecologically significant component of mountain forests, and grows on various substrates such as soil, rocks, living and dead trunks, branches. Nevertheless, studies of bryophyte communities from Turkey are lacking, although several studies on bryo-sociology have been carried out with successfully in Turkey as well as bryofloristic studies (Alataş et al. 2017; Alataş and Uyar 2017; Ezer 2017; Can Gözcü et al. 2018; Alataş 2018; Alataş et al. 2019). No study to classify the bryophyte vegetation of the Beldibi and Babadağ forests were made until now. The aim of this study was to reveal the epiphytic bryophyte vegetation of Beldibi and Babadağ forests using DCA and TWINSPAN multivariate analysis techniques as well as Braun-Blanquet method in nortwest Turkey. *Neckeretum crispae* which was reported for the first time from Turkey with the present paper, brings the total to 47 the number of epiphytic bryophyte syntaxa known from Turkey. Even so, further studies on this topic will be needed to determine and real composition and structure of the Turkish bryophyte vegetation.

Material and Methods

Study area

Beldibi and Babadağ forests (Zonguldak, Devrek), located in A2 grid-square according to the Henderson (1961), are located in Euxine zone of Euro-Siberian phytogeographic region (Anşin 1983). The study area, situated in the Western Black Sea Region, is located within the borders of Zonguldak province. There are Karabük district to the east of the study area, Ereğli district to the west, Zonguldak to the north, Bolu and Düzce provinces to the South (Fig. 1). Since the mountains in Zonguldak are

parallel to the coast, the climate from the coast to the inner part shows significant changes. Depending on these climate changes, different vegetation types such as Oceanic, Mediterranean and Sub-Mediterranean are seen from the north to the south of the area (Güvenç et al. 2009).

The average precipitation is 902 mm and the average annual temperature is 13.9 °C in the study area. The hottest month of the year is July and the coldest month is January (URL,1). The annual precipitation regime of the study area is KSII. This situation indicates that the study area has a oceanic climate and it is located in a transitional zone of the sub-mediterranean bioclimatic region (Akman 2011).

The Black Sea Region is particularly suitable for the development of leafy forests. While the conifer *Pinus nigra* J.F.Arnold subsp. *nigra* var. *pallasiana* Schneid is the most dominant species in the study area, the deciduous species *Fagus orientalis* Lipsky. and *Carpinus betulus* L. are the co-dominant. And also, *Castane sativa* Miller., *Quercus petraea* (Mattuschka) Liebl., *Acer platanoides* L., *Tilia rubra* DC., *Salix caprea* L., *Cornus mas* L. and, *Platanus orientalis* L. are other common species in the study area.



Figure 1. Grid system of Turkey adopted by Henderson (1961) and the study area.

Vegetation sampling

According to the Braun-Blanquet method (Braun-Blanquet, 1964), a total of 55 phytosociological relevés were taken from the lower (0-0,5 m) and middle (0,5-2m) zones of the trunks of trees which found in the different localities of the Beldibi and Babadağ forests during the different vegetation periods of 2014 (Table 1). The relevés were selected depending on the minimal area concept. For the relevés, abundance-coverage scale of Frey and Kürschner was used (Frey and Kürschner 1991; Table 2).

Phytosociological data, obtained from relevés, were evaluated in accordance with the classical method of Braun-Blanquet (1964). In addition, multivariate analysis techniques such as Two Way Indicator Species Analysis (TWINSPAN; Hill, 1979b) and Detrended Correspondance Analysis (DECORANA; Hill 1979a) were used for classification and ordination respectively. Bryophyte associations were arranged by diagnostic species (Braun-Blanquet 1964) and named according to Weber et al. (2000).

Number of relevés	Localities	Altitude (m)	Phorophyte	Date	GPS Coordinates
1-5	1	451	<i>Q.p.</i>	19.05.2014	N 41°06' 42.52" E 032°01'02.00"
6-11	2	566	T.r., F.o., C.b.	19.05.2014	N 41°07' 20.91" E 032°01'47.11"
12-18	3	539	C.b., A.p., T.r., F.o., Q.p.	20.05.2014	N 41°08'02.19" E 032°01'42.57"
19-24	4	485	C.b., A.p., F.o.	26.06.2014	N 41°07'54.02" E 032°01'16.86"
25-36	5	357	<i>P.o.</i>	27.06.2014	N 41°07'27.87" E 031°59'42.49"
37-45	6	495	C.m., S.c., Q.p., C.b., F.o.	28.06.2014	N 41°06'25.06" E 031°59'09.62"
46-51	7	583	Q.p., C.b., F.o.	12.09.2014	N 41°13'16.68" E 031°51'06.18"
52-57	8	669	Q.p., C.b.	13.09.2014	N 41°12'48.86" E 031°50'10.82"

Table 1. The list of localities.

F.o.; Fagus orientalis, C.b.; Carpinus betulus, T.r.; Tilia rubra, Q.p.: Quercus petraea, A.p.: Acer platanoides, S.c.: Salix caprea, C.m.: Cornus mas, P.o.: Platanus orientalis

Table 2. Abundance-coverage scale used for bryophytes.

scale	Abundance-coverage	scale	Abundance-coverage
+	< % 1	3	% 12,1-25,0
1	% 1,1-6,0	4	% 25,1-50,0
2	% 6,1-12,0	5	% 50,1-100

The determination of the associations in the present study was carried out via comparison with related associations in Marstaller (2006) and classified with the aid of published studies. For the identification of the epiphytic bryophyte specimens, different floras, monographies and revisions were used (Nyholm 1981; Hedenäs 1992; Smith 2004; Zander 1993; Paton 1999; Cortini Pedrotti 2001, 2006; Heyn and Herrnstadt 2004; Frey et al. 2006; Guerra and Cros 2007; Casas et al. 2009; Kürschner and Frey 2011, Plášek et al. 2015; Lara et al. 2016).

Results and Discussion

Classification of Braun-Blanquet method

As a results of phytosociological data obtained from total 55 relevés, three epiphytic bryophyte associations were determined using Braun-Blanquet (1964) method. These; *Neckeretum crispae* (Kaiser 1926) Herzog et Höffler 1944, *Anomodonto viticulosi-Leucodontetum sciuroidis* Wisn. 1930 and, *Orthotricho straminei-Pterigynandretum filiformis* Gillet 1986. The *Neckeretum crispae* was reported for the first time from Turkey.

Floristic and ecological features of these syntaxa are given below in accordance with Marstaller's (2006) sequence.

Class: Neckeretea complanatae Marst. 1986

Order: Neckeretalia complanatae Jez. and Vondr. 1962

Alliance: Neckerion compalanatae Sm. and Had. ex Kl. 1948

Associations: Anomodonto viticulosi-Leucodontetum sciuroidis Wisn. 1930

Neckeretum crispae (Kaiser 1926) Herzog et Höffler 1944

Class: Frullanio dilatatae-Leucodontetea sciuroidis Mohan 1978

Order: Orthotrichetalia Had. in Kl. and Had. 1944

Alliance: Ulotion crispae Barkm. 1958

Association: Orthotricho straminei-Pterigynandretum filiformis Gillet 1986

Description of the syntaxa

Anomodonto viticulosi-Leucodontetum sciuroidis Wisn. 1930 (Table 3)

The association was first described by Wisniewski in Poland and later recorded in Eastern Germany, Estonia, Sweden and the Netherlands respectively (Barkman 1958). Later, it was recorded by Goia and Schumacker (2004) in Romania. The association which is also the type association of the *Anomodonto-Leucodontenion sciuroidis* suballiance, wide-spreads on tree trunks in of shade, alkaline and very humid areas (Barkman 1958; Marstaller 2006).

The association occurs exclusively in the eastern and southern parts of the study area. It was determined within the total of 10 sample plots between 357-539 meters. The sample plots are situated on tree trunks that are spread on moist and shaded habitats in the eastern and southern parts of the study area. The association is similar to the European association in terms of ecological characteristics. It spreads on deciduous tree trunks in European localities such as *Carpinus betulus*, *Tilia ulmifolia*, *T. cordata*, *Fraxinus excelsior*, *Ulmus montana*, *Salix caprea*, *Acer platonoides*, *Fagus sylvatica*, *Quercus robur* and, *Alnus glutinosa* (Barkman 1958). Similarly, it occurs on *Fagus orientalis*, *Platanus orientalis*, *Cornus mas*, *Tilia rubra* and, *Quercus petraea* trunks in the study area (Fig. 2).

Number of relevés	37	38	33	34	36	17	40	41	18	31
Altitude (m)	495	495	357	357	357	539	495	495	539	357
Size of relevés (dm2)	9	4	12	12	16	12	6	9	8	16
Phorophyte	C.m.	C.m.	P.o.	P.o.	P.o.	T.r.	Q.p.	Q.p.	F.o.	P.o.
Trunk (m)	1,2	1,1	1,7	7,7	5,5	1,5	0,8	0,9	2	1,5
Exposition	к	к	к	к	к	к	KB	KB	к	К
Position of relevés	к	к	к	к	к	к	к	к	к	К
Covering (%)	88	95	90	98	92	93	90	95	91	90
Closure (%)	80	80	70	70	70	90	80	80	90	70
Base (B) / Trunk (T)	В	В	т	т	В	т	в	в	в	Т
Number of species	7	5	6	6	7	7	6	7	9	5
Characteristic species										
Anomodon viticulosus (Hedw.) Hook. & Taylor.	2	3	1	3	3	2	2	2		
Leucodon sciuroides (Hedw.) Schwägr.	3	2	3	3	3	3	4	4	2	4
Characteristic species of the Neckerion complan	atae alli	ance								
Alleniella besseri (Lobarz.) S.Olsson, Enroth & D.Quandt.	2									
Homalia trichomanoides (Hedw.) Brid.									1	
Anomodon attenuatus (Hedw.) Huebener.									1	
Characteristic species of the Neckeratalia complete	anatae o	order a	nd Ne	ckeret	ea cor	nplana	atae cl	ass		
Alleniella complanata (Hedw.) S.Olsson, Enroth & D.Quandt.	4	4	2		2	4		2	4	3
Homalothecium sericeum (Hedw.) Schimp.	2	3	4	4	4	2	3	3	1	3
Porella platyphylla (L.) Pfeiff.	2	2								
Radula complanata (L.) Dumort.	1			1		1			2	
Hypnum cupressiforme var.cupressiforme Hedw.			2	2			2	2		2
Isothecium alopecuroides (Lam. ex Dubois) Isov.									1	
Others										
Frullania tamarisci (L.) Dumort.						2	3		2	
Ptychostomum moravicum (Podp.) Ros & Mazimpaka. Pulvigera lyellii (Hook. & Taylor) Plášek. Sawicki & Ochyra				1			2			
Frullania dilatata (L.) Dumort.			1		1			1		
Hypnum resupinatum (Taylor) Schimp.					2					
Zygodon rupestris Schimp. ex Lorentz.										1

Table 3. Anomodonto viticulosi-Leucodontetum sciuroidis Wisn. 1930.



Figure 2. The tree preferences of the syntaxa.

While the general cover of the epiphytic bryophyte species within the association ranges from 88% to 98%, canopy cover is 70%-90%. A total of 17 taxa were recorded in sample plots. 4 of them are liverworts, 13 of them are mosses (3 acrocarpous, 10 pleurocarpous). An average number of taxa are 6 within the association. The high proportions of pleurocarpous species, more sensitive to drought than acrocarpous, suggests that the association is generally spread in moist habitats in the study area.

The permanency of *Anomodon viticulosus*, one of the diagnostic species of the association, is 80% in the sample plots. The permanency of another diagnostic species *Leucodon sciuroides* is 100 %. The mesophyte taxon *Anomodon viticulosus* generally grows on trunks as epiphytically and, on stone, wall and, rock as epilithically in shady semi-arid and semi-neutral environments. The mesophyte-xerophyte taxon *Leucodon sciuroides*, generally grows as epiphytically and epilithically, spreads on acidic and semi-neutral habitats in open areas. Therefore, it can be said that both diagnostic species are strongly compatible with the characteristics of the upper syntaxonomic units (class, ordo, alliance).

This association is characterized by facultative epiphytes with large proportion (41 %). The percentage of obligate epiphytes is 35% in the association. Indifferent species were represented by low percentage (24%) respectively (Fig. 3). In this situation, it can be say that the determined syntaxon in the study area is facultative epiphyte.



Figure 3. The habitat affinities spectrum of the species of syntaxa.

Syntaxonomically, *Anomodonto viticulosi-Leucodontetum sciuroidis* Wisn. 1930 was classified in the alliance *Neckerion complanatae* Sm. & Had. ex Kl. 1948, the ordo *Neckeretalia complanatae* Jez. & Vondr. 1962 and, the class *Neckereta complanatae* Marst. 1986 due to it includes of characteristic species of the upper units (Table 3).

The association, determined in the study area, show some floristic and ecologic similarity to Romanian association previously determined by Goia and Schumacker (2004). *Leucodon sciuroides, Porella platyphylla* and, *Homalothecium sericeum* are characteristic species of both associations.

Neckeretum crispae (Kaiser 1926) Herzog et Höffler 1944 (Table 4)

The association was determined with a total 13 sample plots between 357 to 539 meters of the study area. The association occurs mainly northern parts of the lower base and middle zone of trees. The trees are widespread in shade and open areas in the mesic southern and eastern slopes of the study area. *Carpinus betulus* is the most preferred tree species by the association in the study area (Fig. 2).

While the general bryophyte cover within the association ranges from 85% to 97%, canopy cover is 70%-100%. A total of 23 taxa were recorded in the sample plots belonging to the association. Among them, 7 are liverworts and, 16 are mosses (3 acrocarpous, 13 pleurocarpous). The average taxa numbers are 7 within the association.

This association highly occurs on deciduous *Carpinus betulus, Acer platanoides* and *Platanus orientalis* mixed forests as epiphytically in moist slopes of the study area. At the same time, *Exsertotheca crispa*, the main diagnostic species of the association, is hygrophytic pleurocarpous taxon. Therefore, it can be said that the association is hygrophytic. The permanency of the main diagnostic species within the sample plots is 85%.

The percentage of cortico-saxicolous members in the association is 39%. On the other hand, the percentage of epiphytic members is 26%. Indifferent species were represented by highly proportion with 35% within the association (Fig. 3). Therefore, the association determined in the study area is facultative epiphyte.

													
Number of relevés	11	20	25	12	13	14	21	22	23	24	26	45	42
Altitude (m)	566	485	357	539	539	539	485	485	485	485	357	495	495
Size of relevés (dm2)	12	9	16	12	9	12	16	16	12	9	12	16	6
Phorophyte	C.b.	A.p.	P.o.	C.b.	A.p.	C.b.	C.b.	C.b.	C.b.	C.b.	P.o.	C.b.	C.b.
Trunk (m)	1,5	1,2	2,7	1,4	1,2	1,8	1,5	1,8	1,4	1,8	4,2	2,4	1,1
Exposition	к	к	к	к	к	к	к	к	к	К	к	KB	KB
Position of relevés	к	к	к	к	к	к	к	к	к	К	KD	к	к
Covering (%)	94	90	86	85	95	91	92	91	90	97	96	88	90
Closure (%)	100	80	70	90	90	90	80	80	80	80	70	80	80
Base (B) / Trunk (T)	т	в	т	т	т	т	т	т	т	т	т	т	в
Number of species	7	7	9	8	8	5	7	8	10	7	7	8	7
Characteristic species													
<i>Exsertotheca crispa</i> (Hedw.) S.Olsson, Enroth & D.Quandt	5	4	2	4	4	5	4	4	4	3	3		
Characteristic species of the Neckerion complanatae a	alliance	e											
Anomodon viticulosus (Hedw.) Hook. & Taylor.					2								
Anomodon attenuatus (Hedw.) Huebener.												2	
Characteristic species of the Neckeratalia complanata	e orde	r and I	Necke	retea o	compla	anatae	class						
Alleniella complanata (Hedw.) S.Olsson, Enroth & D.Quandt.	1	2	3				2		2	4	4	4	3
Homalothecium sericeum (Hedw.)Schimp.	1		3		2	2	3	2	2		4	2	3
Metzgeria furcata (L.) Dumort.	1						1				1		
Radula complanata (L.) Dumort.		1	1	2	1	1	2	1			1	2	1
Isothecium alopecuroides (Lam. ex Dubois) Isov.				1	1	3		1	1	1		1	1
Hypnum cupressiforme var.cupressiforme Hedw.		2	1	2	2			2	1	2	2	2	2
Cirriphyllum crassinervium (Taylor) Loeske & M.Fleisch.			2										
Porella arboris-vitae (With.) Grolle.									2	3	1		
Lejeunea cavifolia (Ehrh.) Lindb.				1	1		2	1	1			1	
Others													
Plagiomnium affine (Blandow ex Funck) T.J.Kop.				2	2	1		3					
Leucodon sciuroides (Hedw.)Schwägr.	3	2	2										
Pterigynandrum filiforme Hedw.		2					2			2			
Frullania tamarisci (L.) Dumort.	3			1				1	1	1		2	
Isothecium myosuroides Brid.	1												
Lophocolea heterophylla (Schrad.) Dumort.				1									
<i>Bryum capillare</i> Hedw.									1				
Brachythecium mildeanum (Schimp.) Schimp.			2										
Ptychostomum moravicum (Podp.) Ros & Mazimpaka.			1										
Frullania dilatata (L.) Dumort.													1
Antitrichia curtipendula (Hedw.) Brid.													4

Table 4. Neckeretum crispae (Kaiser 1926) Herzog et Höffler 1944.

The diagnostic species of the association are *Neckera complanata, Metzgeria furcata* and *Homalothecium sericeum*. These species which are diagnostic species of *Neckeretalia complanatae* are represented by high proportion in the syntaxon. Moreover, *Exsertotheca crispa*, grows on epiphytic and epilithic habitats, is the main diagnostic species of the facultative epiphyte association. It was therefore supported that the association is classified within the alliance *Neckeria complanatae*, the ordo *Neckeretalia complanatae* and the facultative epiphyte class *Neckereta complanatae* as syntaxonomically (Table 4).

The association, recorded for the first time from Turkey with the present paper, show floristic and ecologic similarity to Italian association previously determined by Cortini Pedrotti (1988) in the

Quercus ilex forests. *Neckera complanata, Metzgeria furcata* and *Homalothecium sericeum* are characteristic species of both associations and of the upper syntaxonomic units to which they are attached.

Orthotricho straminei-Pterigynandretum filiformis Gillet 1986 (Table 5)

The association is situated in a total of 32 sample plots on the north face of middle and basal parts of trunks of deciduous tree species which are spread on all localities within an elevation range of 357-669 meters in the study area.

The association was firstly described by Ochsner in 1928 with the name of *Ulotetum crispae*. Along with the changes in the syntaxonomical nomenclature, it was re-edited by Gillet under the name of *Orthotricho straminei-Pterigynandretum filiformis* Gillet 1986 and introduced to the world of science (Marstaller 2006).

The general bryophyte cover of the association ranges from 82% to 98%. The canopy cover of the forest where *Quercus petraea* is the most dominant tree species is 70%-100%. At the same time, *Quercus petraea* is the most preferred phorophyte species by the association with 38% (Fig. 2). The total number of species in this syntaxon was twenty seven. Among them, six are liverworts and the others are mosses (10 acrocarpous, 11 pleurocarpous). An average number of 7 species was recorded in the sample plots.

The main diagnostic species of association was aero-hygrophyte-mesophyte *Orthotrichum stramineum*. Although the permanency of the species is 41%, its cover within the association is very low. The permanency of strong competitive species *Pterigynandrum filiforme*, another diagnostic species of the association, is 75% and its cover is relatively higher.

Number of relevés	1	3	6	7	46	47	48	53	54	55	57	4	8	10	49	50	52	51	2	44	19	32	39	56	43	9	16	29	35	5	27	30
Altitude (m)	451	451	566	566	583	583	583	669	669	669	669	451	566	566	583	583	669	583	451	495	485	357	495	669	495	566	539	357	357	451	357	357
Size of relevés (dm2)	12	9	8	12	6	8	4	8	q	8	12	12	6	q	6	9	6	8	q	12	12	о <i>с,</i>	8	12	6	20	6	12	8	q	20	8
Phorophyte	0.0	0 n	Tr	т. Т.г	0 n	0 n	0 n	0 n	0 n	Ch	. <u>-</u>	0 n	Tr	Fo	0 n	Ch	0 n	Fo	0 n	C h	F.O.	Po	S.c.	C h	Fo	E o	An	Po	Po	0 n	Po	Po
	Q.p.	Q.p.	1.1.	1.1.	Q.p.	Q.p.	0.p.	Q.p.	Q.p.	1 5	1.6	Q.p.	1.1.	1.0.	4.p.	4.2	Q.p.	1.0.	Q.p.	1.4	1.0.	2.6	1.6	1.0	1.0.	2.7	л.р. о т	1.0.	2.4	4.p.	2.4	1.0.
	0.0	1,1	1,2	1,2	0,9	0,0	0,7	0,0	0,9	1,5	1,0	ı K	ı K	1,0	1,1	1,2	0,9	1,7	0,9	1,4	1,9	3,0	1,0	1,0	1,5	2,1	0,7	1,0	2,4	1,2	2,1	1,4
	ĸ	ĸ	ĸ	ĸ	ĸ	ĸ	ĸ	ĸ	ĸ	ĸ	ĸ	ĸ	ĸ	ĸ	ĸ	ĸ	ĸ	ĸ	ĸ	ĸв	ĸ	ĸ	ĸ	ĸ	ĸв	ĸ	ĸ	ĸ	ĸ	ĸ	ĸ	ĸ
Position of releves	K	K	K	к	к	К	К	K	K	К	K	K	К	К	к	K	K	K	К	К	K	K	K	К	K	К	K	K	К	KD	KD	KD
Covering (%)	90	90	93	82	91	98	85	91	93	95	96	93	80	83	95	86	93	84	95	98	98	93	96	91	98	90	90	95	97	96	88	88
Closure (%)	90	90	100	100	90	90	90	90	90	90	90	90	100	100	90	90	90	90	90	80	80	70	80	90	80	100	90	70	70	90	70	70
Base (B) / Trunk (T)	Т	В	В	Т	Т	В	В	В	В	Т	Т	Т	В	Т	В	В	Т	В	Т	Т	В	Т	Т	Т	Т	В	В	Т	Т	Т	Т	т
Number of species	8	7	7	7	10	8	7	7	10	7	9	7	5	5	7	7	6	7	7	9	5	6	10	8	10	6	6	6	6	5	5	6
Characteristic species																																
Pterigynandrum filiforme Hedw.			2	2	2	1		2	3	4	4			3	2	4	3	4	2	2	2	2	1	4	2	3	2	1	1			
Orthotrichum stramineum Hornsch. ex Brid.	1	1	2	1		1		1	1	1	2	1	1		1		1															
Characteristic species of the Ulotion crispae alliance																																
Metzgeria furcata (L.) Dumort.			2	3	1				1	1	2		2			1		1		2				1	2							
Hypnum andoi A.J.E.Sm.	2						2						3		3			3	3													
<i>Lewinskya striata</i> (Hedw.) F.Lara, Garilleti & Goffinet.				1	1	1	1	1	1					1		1		1														
<i>Pulvigera lyellii</i> (Hook. & Taylor) Plášek, Sawicki & Ochyra.								3	2		1			2			2								2	2						
Frullania tamarisci (L.) Dumort.		2										1							2	3	3								2			
Orthotrichum pallens Bruch ex Brid.																				1												
Characteristic species of the Orthotrichetalia or class	rder al	nd Fru	llanio	dilatat	ae-Lei	ucodo	ntetea	sciuro	idis																							
Frullania dilatata (L.) Dumort.	2		2		2	2	1	2	2	2	2		2	3	2	2	3	2					1	2	2	3	2	2		2		2
Radula complanata (L.) Dumort.	1		1	2	1	2	1					1			1	1		2		1					2					1		1
Radula lindenbergiana Gottsche ex C. Hartm.		1																														
Leucodon sciuroides (Hedw.)Schwägr.	4	4	4	4	4	4	4	4	4	3	3	2	4	4	4	2	4		4	3	4	3	4	3		4	3	4	4	5	4	4
Homalothecium sericeum (Hedw.) Schimp.	2	2										4							2			4	2		3			3	4	2	3	3
Zygodon rupestris Schimp. ex Lorentz.																													1		2	1
<i>Lewinskya affinis</i> (Schrad. ex Brid.) F.Lara, Garilleti & Goffinet.																							1		1						1	

Table 5. Orthotricho straminei-Pterigynandretum filiformis Gillet 1986.

Others

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Alleniella complanata (Hedw.) S.Olsson, Enroth & D.Quandt.																3	4	3	3			4	2		3	
Porella platyphylla (L.) Pfeiff.			2	2	2	3	3	2	1	2	1	3			1		1	3	1	2	3					
Isothecium alopecuroides (Lam. ex Dubois) Isov.	1													1												
Hypnum cupressiforme var.cupressiforme Hedw.					2	2			2		2		3			2			1				3			2
Hypnum cupressiforme Hedw. var. lacunosum Brid.																								2		
Hypnum resupinatum (Taylor) Schimp.		3										3									2			3	3	
Amblystegium confervoides (Brid.) Schimp.																					1					
Anomodon attenuatus (Hedw.) Huebener.																						2				
<i>Ptychostomum moravicum</i> (Podp.) Ros & Mazimpaka.					1													1								
Syntrichia virescens (De Not.) Ochyra.										1	1									1						
Syntrichia latifolia (Bruch ex Hartm.) Huebener.																			1							
Orthotrichum pumilum Sw. ex anon.																				1	1					

The proportion of obligate epiphyte members within the association is the highest with 48%. Despite that the proportions of cortico-saxicolous (33%) and indifferent taxa (19%) are relatively lower within the association (Fig. 3). Therefore, it can be say that the association is epiphytic.

The association was classified into the alliance *Ulotion crispae* Barkman 1958, the ordo *Orthotrichetalia* Hadac in Klika & Hadac 1944 and, the class *Frullanio dilatatae-Leucodontetea sciuroidis* Mohan 1978, due to it included the diagnostic species of these upper sytaxonomic units (Table 5).

Additionally, the association, recorded for the first time from Turkey by Alataş and Uyar (2017), show floristic and ecologic similarity to previously determined association in the Abant Mountains, due to ecological characteristics and diagnostic species of both associations and of the upper syntaxonomic units are same.

Classification and ordination with multivariate analysis techniques

Two-Way Indicator Species Analysis (TWINSPAN) that classifies communities according to their floristic similarity, and Detrended Correspondence Analysis (DECORANA), as an ordination technique, were applied to the presence estimates of the recorded taxa in 55 sample plots. TWINSPAN classified epiphytic bryophyte vegetation into three clusters at third hierarchical level (Fig. 4). According to the indicator species clusters were as follows; *Orthotrichum stramineum* (cluster I), *Anomodon viticulosus* (cluster II) and, *Neckera crispa* (cluster III). The results obtained by TWINSPAN indicated that the classification of the communities are appropriate to that of Braun-Blanquet's table arrangements.

The Detrended Correspondance Analysis (DCA), an indirect gradient analysis technique, distributed the epiphytic communities on axis 1 and axis 2 to three groups (I, II and III) according to the ecological characteristics of the epiphytic habitats (Fig. 5). The DCA ordination agreed well with TWINSPAN clusters. According to this; axis 1 clearly represented that the groups were distributed according to the humidity gradient of epiphytic habitats. The axis 2 was not so clear. But, for axis 2 could be said that the groups were distributed according to the height gradient (from lower base to upper zones) of epiphytic habitats on the phorophytes. Results obtained by DCA ordination showed that the spatial distribution of epiphytic bryophyte communies in the study area were affected by the ecological factors of the habitats. Especially humidity the most important ecological factor in community structures and distribution.



Figure 4. Dendrogram with clusters obtained by TWINSPAN.



DECORANA Ordination Plot - devrek

Figure 5.The demonstration of plots on DCA ordination diagram.

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Determination of distribution, emergence period, population fluctuation and infestation rate of cherry fruit fly (*Rhagoletis cerasi* L.) (Diptera: Tephritidae) in cherry orchards of Mardin province (Turkey)

Mehmet Kaplan^{1*}

^{1*}Department of Plant Protection, Faculty of Agriculture, Siirt University, Turkey

Corresponding author: mehmetkaplan1971@hotmail.com

Abstract

This study was carried out to determine distribution, emergence period, population fluctuation of Cherry Fruit Fly (*Rhagoletis cerasi* L.) (Diptera: Tephritidae) in cherry orchards of Mardin province in 2013 and 2014. Studies were conducted through vegetation period in the orchards which have at least 50 cherry trees of Napolyon (Ziraat 900) varieties in each orchard. In the studies, visual yellow colored adhesive attractive traps were used in the monitoring of the pest. As a result of studies, adults of cherry fruit fly were seen first time in traps in the first week of May when the fruits were in the hay-yellow period. In addition, it was determined that the adults of the pest produced the highest density and damage in this period. It was determined that the population development of the pest continued about 21-42 days and the adult population continued until the third and last week of June, the infestation rate was between 0.1% and 0.7%.

Keywords: Cherry, Cherry Fruit Fly, Rhagoletis cerasi L., Visual traps, Mardin

Introduction

Cherry is a tasty fruit, besides it is a fruit with a very high nutritional value. In addition, in terms of both the production and marketing stages, the use of intensive workforce, creating a wide range of employment opportunities, significant currency income due to foreign sales, it has got great importance for the country's economy.

In the production and export of cherries, Turkey took the first place with 639,564 tons of cherry production in the world in 2018. Turkey exported 70 thousand tons and 162 million dollars (Anonymous, 2018a). Cherry production of Mardin province was 3.338 tons of in 2018 (Anonymous, 2018b). Although the amount of cherry production in the region seems to be relatively low, the cherry that is cultivated in the region is early and therefore the economic yield is high.

There are many pests that negatively affect quality and yield in cherry orchards directly or indirectly. Among the many factors that negatively affect yield and quality in cherry production, plant protection problems are the most important. Insect pest the cherry fly [*Rhagoletis ceras* L. (Diptera: Tephritidae)] is the most important pest of the cherry fruit both in other cherry-producing countries and in our country. The larva of the pest feeds on the fleshy parts of the fruit, causing some fruit to be dumped prematurely and cause significant economic losses too. As the quality of the harvested fruits with

worms is low, the market value also decreases. The damage of the pest is mostly seen on average, especially in June. The damage rate can be up to 80% during the epidemic years. Loss tolerance in exports is zero (Anonymous, 2011). Pesticides are widely used to control of the pest.

Recently, the increase in the use of pesticides for the controlling of harmful insect species in the orchards in the region leads to the deterioration of the natural balance between living beings. These increase the likelihood of pests in the future. The elimination of these problems is important in terms of the introduction of both environmentally sound and healthy products.

In this study, in order to make an effective struggle against the cherry fly, it was aimed to determine the distribution areas of the pest, time of emergence and population development.

Materials and Method

The main materials of the study are cherries grown in the province of Mardin (Central, Ömerli and Yeşilli) and samples of larvae, pupae and adults of *Rhagoletis cerasi* L. (Diptera: Tephritidae) which are harmful to them. materials.

The experiments were carried out cherry orchards of the towns and villages in the districts of Center, Ömerli and Yeşilli of Mardin province where pesticide was not applied and infested with pest. The sampling was carried out according to the Grigorov sampling method (1974) in the selected gardens. According to this method; all trees in the orchards with 20 trees, 21-30 trees in 21-70 trees, 31-40 trees in 71-150 trees, 41-80 trees in 151-300 trees, 15% in 301-1000 trees, and more than 1000 trees 5 were checked. In the study of determining the distribution areas and population intensities of the pest, attractive rebel traps with an ammonia salt were hung in the cherry trees in the gardens where there was no closure or closed cultivation. The traps were placed at 1.5-2 m height of the trees in the direction of the prevailing wind and they were placed at the end of the side branches of the trees (Özdem and Kılınçer 2008).

The traps were adjusted to be 2 pieces per deciare and the trap distance was 15-20 m and the attractive ammonia capsules were replaced every 3-4 weeks. Surveys during April-July months, traps were controlled by controlling the traps and spreading areas of the cherry fly for two weeks. To determine the population development of cherry fly; Mardin Central district of Sultan village, Ömerli district of the village of Anittepe and the village of Ömerli district of the village Dereyani a total of three trappings hanging in the garden were checked weekly and the number of adult flies trapped was recorded. Thus, the distribution areas of *R. cerasi* L. were determined at the time of the first exit, the peak of the population and the end of the population of the pest.

Results and Discussion

As a result of surveys carried out in Mardin province in 2016-2017, it was determined that cherry production areas were infested with Cherry fly (*Rhagoletis cerasi* L.). In the traps containing Rebel + Ammonium acetate suspended from the third week of April in the cherry orchards, the first adult emergence in the traps and the population change follow-up were determined weekly.

In the observations made at the Anttepe Village (Ömerli) in 2016, the first capture in the mass traps was observed with 3 adults on 05.05.2016, 10 adults on 12.05.2016, 8 adults on 19.05.2016, 5 adults on 26.05.2016, 2 adults on 02.06.2016 and the number of adults captured on 09.06.2016 has fallen to zero. In the following study conducted in 2017, the first adult was observed with 1 fly on 11.05.2017, 13 flies on 18.05.2017, 11 flies on 25.05.2017, 2 flies on 01.06.2017 and population dropped to zero on 08.06.2017. Taking this data into consideration, the population duration is approximately 21-28 days in Anttepe Village (Yeşilli) (Table 1.).

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		Trial location	S			Trial locations	
	Anttepe	Dereyanı	Sultan		Anttepe	Dereyanı	Sultan
Date	Vilage	Village	Village	Date	Vilage	Village	Village
		(Fly/Trap)				(Fly/Trap)	
28.04.2016	0	0	0	04.05.2017	0	0	0
05.05.2016	3	0	0	11.05.2017	1	1	4
12.05.2016	10	6	0	18.05.2017	13	9	10
19.05.2016	8	10	5	25.05.2017	11	5	5
26.05.2016	5	10	13	01.06.2017	2	2	5
02.06.2016	2	4	9	08.06.2017	0	0	6
09.06.2016	0	3	5	15.06.2017	0	0	5
16.06.2016	0	2	6	22.06.2017	0	0	3
23.06.2016	0	0	0	29.06.2017	0	0	0

Table 1. Populatio	n fluctuation of Rh	nagoletis cerasi ir	h cherry orchards	s of Mardin	province in	2016 and 2017
radie is ropanado	II IIII OI III	ingererie eereist in	· • · · · · · · · · · · · · · · · · · ·			

During the study conducted in Dereyani Village (Yeşilli) in 2016, the emergence of first grown-ups in yellow sticky visual trap has been observed with 6 flies on 12.05.2016, followed by 10 flies on 19.05.2016, 10 flies on 26.05.2016, 4 flies on 01.06.2016, 3 flies on 08.06.2016, 2 flies on 15.06 2016 and finally no flies were observed on 22.06.2016. In the following study conducted in 2017, the first adult was observed with 1 fly on 11.05.2017, 9 flies on 18.05.2017, 5 flies on 25.05.2017, 2 flies on 01.06.2017 and population dropped to zero on 08.06.2017. Taking this data into consideration, the population duration is approximately 21-35 days in Dereyani Village (Yeşilli) (Table 1).

During the study carried out in Sultan Village (Central Mardin) in 2016, the first adult capture in yellow sticky visual traps has seen observed with 5 flies on 19.05.2016, 13 flies were seen on 26.05.2016, 9 flies were seen on 02.06.2016, 5 flies were seen on 09.06.2016, 6 flies were seen on 16.06.2016 and population fell to zero on 23.06.2016, indicating a short lifespan of the population. Then in the following study conducted in 2017, the first grown-ups were observed with 4 flies on 11.05.2017, 10 flies on 18.05.2017, 5 flies on 25.05.2017, 5 flies on 01.06.2017, 6 flies on 08.06.2017, 5 flies on 15.06.2017, 3 flies on 22.06.2017 and population fell to zero on 29.06.2017. Population duration has been observed to be 28-42 days in Sultan Village (Mardin-Center) (Table 1).

At the end of the study the population growth of the pest continued for approximately 21-42 days in two years, and the end date of the population coincided with the third and last weeks of the month of June. Ecological conditions, altitude, plant and vegetation period all have an influence on the date of becoming adolescent, and the growth and end of the population. As a matter of fact, studies conducted abroad have similarities with the findings of this study; Mitić-Mužina (1960) concluded that the flight of this species under our local conditions lasted about 2 months (began in mid-May and ended in the first half of July). The life span of flies under laboratory conditions depends on the type of diet, size and abundance of flies, and can last up to 100 days (Ranner, 1988b). It is difficult to estimate the life span of adult flies in natural environment because of unpredictable environmental effects, but in most cases it lasts between four and seven weeks (Samoggia 1932, Sprengel 1932a, Wiesmann 1933b, Bohm 1949), setting the total annual adult flight phenology to 7-11 weeks (Jancke and Bohmel 1933, Bohm 1949, Stamenković et al. 1996a).

In the cherry gardens where the studies were carried out, it has been determined that the number of flies caught in traps and the duration they were seen in nature changed by each year in 2016 and 2017. Since the sampling gardens are the same in both years, this is thought to be caused by climatic conditions. In fact, Özdem and Kılınçer (2009a) emphasized that between 2007 and 2009, they tried several mass capture traps against the Cherry fly and the number of populations and population

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duration for each trial year had changed. In the cherry orchards of the province of Mardin in May, after the start of the cherry fly, the highest population was found in the straw-yellow period and the majority of the damage was found in this period. It was determined that the population decreased to zero at the end of the harvest period. In a study conducted in the cherry orchards of the province of Çanakkale, which is similar to the results of this study, it was reported that the flight of the Cherry fly continued until the end of April and mid-June (Ertop and Özpınar, 2011). However, another study conducted by Tezcan and Gülperçin (2000) between 1998-2000 reported the first sight of grown-up cherry flies in May with the pest population coming to an end towards mid-June.

In another study, Ulusoy et al. (1999) reported that *R. cerasi* began to emerge in late May in the Pozanti region and the flies were seen in the traps until mid-August; Tezcan & Gülperçin (2000) stated that *R. cerasi* is the main harmful in İzmir and Manisa provinces; reported that adults emerged at the end of April and they had determined at the end of May that their adult output had ended.

In 2016, the infestation rate was determined 0.2% in Anttepe village, 0.4% in Dereyan village and 0.6% in Sultan village by controlling 1000 fruits in each orchards during harvest time. In 2017, randomly controlled 1000 fruits were found to be infested with *R. cerasi* 0.1% in Anttepe village, 0.3% in Dereyan village and 0.7% in Sultan village. Indeed, Tezcan and Gülperçin (2000), in order to capture the *R. cerasi* (L.) In ecological cherry production gardens in İzmir and Manisa, the average size of the tree from the yellow colored visual traps made of 15x20 cm fiberglass have made checks weekly by hanging 4 (2-7) traps / tree. In 1998, the rate of infestation was found to be 0.2% in İzmir and 0.1% in Manisa. medium and late varieties have not been infested with the pest.

Conclusion

At the end of the study, it was determined that the cherry fly was distributed in all areas of Mardin Merkez, Ömerli and Yeşilli district where cherry was produced widely in Mardin province. Grown-up cherry flies have been first observed in first week of May in visual yellow-color sticky traps in gardens. Following the emergence of cherry fly, the highest population caught in the visual yellow sticky traps was observed during the straw-yellow period and most of the damage was done during this period. Approaching the harvest season, pest population decreased, hence its damage has also decreased, and the population decreased to zero in the post-harvest period. The population coincided with the third and last weeks of June. Ecological conditions, altitude, plant and vegetation periods all have an influence on the date of grown-ups emerging in nature, their spread in nature and the growth and end of the population. In every thousand fruits controlled at the cherry orchards during harvest season, respective infestation rates (worms in fruit) of 0.1% and 0.7% have been observed.

In conclusion, this study has yielded significant findings with regards to the cherry fly, its emergence in nature, population growth and disappearance in cherry production areas in Mardin province.

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A faunistic study on the geometrid moths (Lepidoptera, Geometridae) of Maden district (Elazığ Province, Turkey)*

Abdullah Çakır¹, Erdem Seven²

¹ Hilar Vocational and Technical Anatolian High School, Ergani, 21950, Diyarbakır, Turkey ² Department of Gastronomy and Culinary Arts, School of Tourism and Hotel Management, Batman University, 72060, Batman, Turkey *This study was produced from the MSc thesis of first author. Corresponding author: abdullahc21@gmail.com

Abstract

This paper is conducted on the Geometridae fauna of Maden (Elazığ) district. In total, 87 species are diagnosed in 47 genera of 6 subfamilies. Except *Charissa onustaria* (Herrich-Schäffer, [1852]), all of the determined species are new record for the research area and also 81 of the species are new record for Elazığ province. And, second location records of rare known species, *Idaea consociata* (Staudinger, 1900), *Gnophos sacraria* (Staudinger, 1895) and *Wehrliola inexpectata* Kemal & Uçak, 2018, are given from the study area.

Keywords: Lepidoptera, Geometridae, Maden, Elazığ, Turkey

Özet

Bu makale, Maden (Elazığ) ilçesi Geometridae faunası üzerine yapılmıştır. Toplamda 6 altfamilya içerisinde 47 cinse ait 87 tür tespit edilmiştir. *Charissa onustaria* (Herrich-Schäffer, [1852]) dışında, belirlenen türlerin tümü araştırma alanı için, 81'i ise Elazığ ili için yeni kayıttır. Nadir bilinen *Idaea consociata* (Staudinger, 1900), *Gnophos sacraria* (Staudinger, 1895) ve *Wehrliola inexpectata* Kemal & Uçak, 2018 türlerinin ikinci lokalite kayıtları çalışma alanından verilmiştir.

Anahtar Kelimeler: Lepidoptera, Geometridae, Maden, Elazığ, Türkiye

Introduction

Most Geometridae species are nocturnal, but they often fly preferably at dusk and others active mainly after midnight. Each species is characterized by a specific resting position: Planiform (wings are parallel to the ground), veliform (wings are vertically folded) or tectiform (wings are positioned like a roof) (Hausmann 2001). The adults of the geometrid moths are commonly small to medium in size, with broad wings. Flights of the adults are usually short distance and close to the ground. Geometridae species move in a characteristic "inching" and called to "measuring worms" because the larvae lack of prolegs (except Archiearinae). The larvae resemble twigs or leaf stems and feed on foliage. They seriously damage or destroy trees and crops, when their populations are excessive. The geometrid moths can be distinguished from other moth families by their long legs, thin abdomen and slim wings. However, in order to diagnosis of some taxa (such as *Scopula, Idaea* and *Eupithecia*), tergit/sternit and tympanal organs should be examined together with male and female genital structures (Hausmann 2001, Leraut 2009, Kemal and Seven, 2013).

Studies on the geometrid moths of Turkish fauna, and resources used for identification and distribution of the species in this survey are include: Zukowsky 1941, Doğanlar et al. 1982, S. Seven 1993,

Hausmann 2001, 2004, Karsholt and Razowski 1996, Okyar and Aktaç 1999, Schön 2002, Mironov 2003, Özdemir 2007, 2016, Leraut 2009, Hausmann and Viidalepp 2012, Rajaei et al. 2012, 2017, Kemal and Seven 2013, Seven 2014, 2015, 2018, Skou and Sihvonen 2015, Hausmann et al. 2017.

Geometridae species in the Turkish fauna are classified in Archiearinae Fletcher, 1951; Desmobathrinae Meyrick, 1886; Ennominae Duponchel, 1845; Geometrinae Stephens, 1829; Larentiinae Duponchel, 1845; Orthostixinae Meyrick, 1892 and Sterrhinae Meyrick, 1892 subfamilies (Özdemir 2007, Kemal and Seven 2013). Geometridae family is one of the species-rich group in Lepidoptera order after noctuid and pyralid moths. The number of known geometrid moths in the European continent is more than 900 (Hausmann 2001), and 638 species are reported in Turkey (Koçak and Kemal 2018). However, before the investigations, 16 geometrid species were known in Elazığ province (Koçak and Kemal 2007, 2018), and Geometridae fauna of Maden district was unknown.

Maden district is generally composed of mountainous areas and has a rich vegetation in woody and herbaceous species that contains mostly *Astragalus*, *Quercus*, *Juniperus* and *Pinus* species (Çakılcıoğlu and Civelek 2011, Tonbul et al. 2018). The area also hosts a large number of endemic and rare plant species (Khatun et al. 2012).

Material and Methods

The samples were collected from 17 localities (Figure 1) between in 2016 and 2017. A sweep net was utilized for catching of diurnal (daytime active) species and UV light traps were used for capturing of nocturnal (active at night) species. Studies were mostly carried out at night, because of majority of the members of Geometridae family were nocturnal. The samples were pinned and labelled and turned into museum materials. The Canon EOS 1100D macro lens camera was used for photographing of the samples and habitats. Also, for precise diagnosis, genitals of some species were prepared under Nikon SMZ1000 stereomicroscope by methods of Robinson (1976). At this stage the genitals were boiled with potassium hydroxide (KOH), taken to the alcohol and finally covered with Euparal between in lam-lamella. The materials are deposited in the collection of Batman University, Faculty of Science and Arts, Department of Biology.



Figure 1. Studied locations in Maden district of Elazığ province.

The studied locations (Figure 1) and coordinates are as follows: 1. K1sabekir (38°21'20.37"N 39°44'27.04"E), 2. Soğuksu (38°24'0.45"N 39°39'29.26"E), 3. Sağrılı-1 (38°21'56.99"N 39°45'13.85"E), 4. Ağadibek (38°21'21.90"N 39°40'49.27"E), 5. Gezin (38°30'45.85"N 39°32'21.89"E), 6. Altıntarla (38°24'3.23"N 39°26'58.16"E), 7. Kavak (38°23'41.95"N 39°25'52.46"E), 8. Centre (Ergani road) (38°22'42.52"N 39°41'21.59"E), 9. Karatop (38°24'16.45"N 39°43'28.17"E), 10. Yeşilova (38°32'7.90"N 39°34'37.41"E), 11. Tekevler (38°27'58.75"N 39°36'23.46"E), 12. Çitliköy (38°28'44.31"N 39°32'52.66"E), 13. Centre (Alacakaya road) (38°23'41.91"N 39°41'31.64"E), 14. Hanevleri (38°24'17.55"N 39°33'2.03"E), 15. Dutpınar (38°24'58.32"N 39°37'36.76"E), 16. Sağrılı-2 (38°22'22.46"N 39°44'11.10"E), 17. Çalkaya (38°19'57.75"N 39°34'20.68"E).



Figure 2. Habitats: a. Yeşilova, 1380 m, 22.09.2017; b. Tekevler, 1300 m, 10.11.2018

The species are evaluated in terms of their distributions as widespread, local or rare according to number of the samples and the number of localities they found. 'Widespread' is used for the species a large number of samples and found in many localities. Species observed only in a specific locality are specified as 'Local', and species with very few samples (1 or 2) are reported as 'Rare' in the conclusion part.

Results

As a result of the researches, 87 species belonging to 6 subfamilies of Geometridae family are determined in the study area. Among these, except 6 species: *Aplasta ononaria* (Geometrinae), *Aplocera plagiata, Camptogramma bilineatum* (Larentiinae), *Charissa onustaria, Nychiodes variabila* (Ennominae), *Scopula immistaria* (Sterrhinae), 81 species are new discovered from Elazığ province. Examined materials of each species are presented.

Family Geometridae Leach, 1815Subfamily Desmobathrinae Meyrick, 1886Myinodes shohami Hausmann, 1994Material. 1♂, Soğuksu, 1020 m, 36.03.2017.

Subfamily Ennominae Duponchel, 1845 Agriopis bajaria ([Denis & Schiffermüller], 1775) Material. 2♂, Kısabekir, 860 m, 10.03.2017. Aleucis orientalis (Staudinger, 1892) **Material.** 5♂ 3♀, Kısabekir, 860 m, 10.03.2017, 13.04.2017; 1♂, Centre (Ergani road), 940 m, 20.04.2017. Alsophila aceraria ([Denis & Schiffermüller], 1775) **Material.** 1∂, Centre (Ergani road), 940 m, 24.03.2017. Apochima flabellaria (Heeger, 1838) **Material.** 3∂, Centre (Ergani road), 940 m, 24.03.2017. Aspitates ochrearia (Rossi, 1794) Material. 3♂, Kısabekir, 860 m, 23.04.2016; 1♂, Soğuksu, 1020 m, 17.05.2017. Biston stratarius (Hufnagel, 1767) **Material.** 4∂, Karatop, 1045 m, 30.03.2017. Charissa dubitaria (Staudinger, 1892) **Material.** 3⁽³⁾, Tekevler, 1300 m, 05.08.2017, 12.06.2017; 1⁽³⁾, Centre (Alacakaya road), 1160 m, 29.06.2017; 5♂ 5♀, Kısabekir, 860 m, 29.06.2017. Charissa mucidaria (Hübner, [1799]) **Material.** 5♂ 5♀, Kısabekir, 860 m, 29.06.2017. Charissa onustaria (Herrich-Schäffer, [1852]) Material. 8♂ 2♀, Sağrılı-1, 920 m, 25.05.2016, 27.07.2017; 7♂ 1♀, Tekevler, 1300 m, 10.05.2017, 22.09.2017; 1♂, Dutpinar, 1150 m, 30.07.2017; 3♀, Ağadibek, 915 m, 29.09.2017. Chiasmia aestimaria (Hübner, [1809]) **Material.** 7Å 4, Ağadibek, 915 m, 22.06.2016, 24.07.2017, 28.08.2017, 29.09.2017; 1Å, Kısabekir, 860 m, 29.06.2017; 1Å, Gezin, 1295 m, 15.08.2017. Chiasmia clathrata (Linnaeus, 1758) **Material.** 2Å, Kısabekir, 860 m, 23.04.2016; 3Å, Tekevler, 1300 m, 10.05.2017; 5Å 1♀, Sağrılı-1, 920 m, 27.04.2017; 2Å, Soğuksu, 1020 m, 17.05.2017. Colotois pennaria (Linnaeus, 1761) **Material.** 1Å, Sağrılı-2, 930 m, 27.10.2017. Crocallis elinguaria (Linnaeus, 1758) Material. 13⁽²⁾, Sağrılı-1, 920 m, 11.10.2017, 19.09.2017; 1⁽³⁾, Kısabekir, 860 m, 11.10.2017; 34⁽³⁾, Gezin, 1295 m, 25.09.2017; 6Å, Tekevler, 1300 m, 22.09.2017, 06.10.2017; 1Å, Ağadibek, 915 m, 29.09.2017; 1♂ 1♀, Soğuksu, 1020 m, 04.10.2017; 4♂ 1♀, Dutpinar, 1150 m, 10.09.2017; 1♂, Sağrılı-2, 930 m, 27.10.2017. Crocallis tusciaria (Borkhausen, 1793) Material. 23∂ 2♀, Sağrılı-2, 930 m, 27.10.2017; 1∂, Ağadibek, 915 m, 22.10.2017; 2∂, Kısabekir, 860 m, 11.10.2017. Dasycorsa modesta (Staudinger, 1879) **Material.** 3Å, Kısabekir, 860 m, 10.03.2017, 04.04.2017; 1Å, Altıntarla, 1355 m, 09.09.2017; 3Å, Centre (Ergani road), 940 m, 20.04.2017. Dyscia innocentaria (Christoph, 1885) **Material.** 1∂ 3♀, Tekevler, 1380 m, 22.09.2017; 5∂, Gezin, 1295 m, 25.09.2017; 2∂, Ağadibek, 915 m, 29.09.2017. Eilicrinia cordiaria (Hübner, 1790) **Material.** 3♂ 3♀, Soğuksu, 1020 m, 01.06.2016, 17.05.2017, 05.08.2017; 6♂ 2♀, Kısabekir, 860 m, 23.04.2016, 29.06.2017; 5 2, Centre (Ergani road), 940 m, 20.04.2017; 5 11, Yeşilova 1380 m,

18.06.2017, 16.07.2017, 02.08.2017; 1 \Diamond , Tekevler, 1300 m, 10.05.2017; 5 \bigcirc , Çalkaya, 1350 m, 27.07.2017; 2 \bigcirc , Ağadibek, 915 m, 24.07.2017.

Eumera hoeferi Wehrli, 1934

Material. 2♂, Ağadibek, 915 m, 29.09.2017; 7♂, Tekevler, 1300 m, 22.09.2017; 2♂, Dutpınar, 1150 m, 30.09.2017; 1♂, Kısabekir, 860 m, 11.10.2017.

Gnopharmia irakensis Wehrli, 1938

Material. 2♂, Çalkaya, 1350 m, 27.07.2017; 5♂, Sağrılı-1, 920 m, 23.07.2017; 6♂ 3♀, Kısabekir, 860 m, 29.06.2017; 3♂ 1♀, Soğuksu, 1020 m, 07.08.2016, 05.08.2017; 1♂, Çitliköy, 1265 m, 16.07.2017; 7♂, Ağadibek, 915 m, 24.07.2017.

Gnopharmia rubraria Staudinger, 1892

Material. 14♂ 3♀, Yeşilova, 1380 m, 18.06.2017, 12.07.2017, 16.07.2017, 02.08.2017, 23.08.2017; 2♂ 1♀, Çitliköy, 1265 m, 21.06.2017; 5♂, Soğuksu, 1020 m, 17.05.2017, 05.08.2017; 1♂, Tekevler, 1300 m, 10.05.2017; 8♂ 4♀, Kısabekir, 860 m, 29.06.2017; 3♀ Sağrılı-1, 920 m, 23.07.2017.

Gnophos pseudosnelleni Rjabov, 1964

Material. 8♂, Sağrılı-1, 920 m, 25.05.2016, 15.09.2017, 19.09.2017, 11.10.2017; 2♂, Yeşilova, 1380 m, 18.06.2017; 27♂ Ağadibek, 915 m, 30.05.2017, 29.09.2017, 22.10.2017; 3♂, Centre (Alacakaya road), 1160 m, 12.06.2017; 2♂, Gezin, 1295 m, 25.09.2017; 2♂, Çitliköy, 1265 m, 21.06.2017; 4♂, Dutpınar, 1150 m, 30.09.2017; 1♂ 13♀, Soğuksu, 1020 m, 01.06.2016, 04.10.2017; 20♂, Tekevler, 1300 m, 22.09.2017, 06.10.2017; 5♂, Kısabekir, 860 m, 15.09.2017, 11.10.2017.

Gnophos sartatus Treitschke, 1827

Material. 1♂, Sağrılı-1, 920 m, 25.05.2016; 2♀, Ağadibek, 915 m, 22.10.2017; 13♀, Soğuksu, 1020 m, 01.06.2016.

Gnophos sacraria Staudinger, [1895] (Figure 3 a)

Material. 2Å, Ağadibek, 915 m, 29.09.2017

Larerannis marginaria (Fabricius, 1777)

Material. 1♂, Kısabekir, 860 m, 10.03.2017

Lycia hirtaria (Linnaeus, 1761)

Material. 1∂ 1♀, Kısabekir, 860 m, 04.04.2017.

Neognopharmia stevenaria (Boisduval, 1840)

Material. 39♂ 7♀, Soğuksu, 1020 m, 01.06.2016, 17.05.2017, 05.08.2017; 1♂, Centre (Ergani road), 940 m, 20.04.2017; 5♂, Yeşilova, 1380 m, 18.06.2017, 16.07.2017; 12♂, Kısabekir, 860 m, 29.06.2017; 2♂ 7♀, Tekevler, 1300 m, 10.05.2017, 05.08.2017; 1♀, Ağadibek, 915 m, 24.07.2017; 7♂, Sağrılı-1, 920 m, 25.05.2016, 27.04.2017, 23.07.2017; 3♂, Çitliköy, 1265 m, 16.06.2017; 2♀, Çalkaya, 1350 m, 27.07.2017.

Nychiodes variabila (Brandt, 1938)

Material. $19 \stackrel{\circ}{\circ} 2 \stackrel{\circ}{\circ}$, Gezin, 1295 m, 03.07.2017, 15.08.2017; $31 \stackrel{\circ}{\circ} 7 \stackrel{\circ}{\circ}$, Yeşilova, 1380 m, 16.07.2017, 02.08.2017, 23.08.2017; $13 \stackrel{\circ}{\circ} 1 \stackrel{\circ}{\circ}$, Sağrılı-1, 920 m, 25.05.2016, 23.07.2017, 15.09.2017, 19.09.2017; 15 $\stackrel{\circ}{\circ} 1 \stackrel{\circ}{\circ}$, Soğuksu, 07.08.2016, 05.08.20147, 12.09.2017; 17 $\stackrel{\circ}{\circ}$, Kısabekir, 860 m, 29.06.2017, 15.09.2017, 19.09.2017; 2 $\stackrel{\circ}{\circ}$, Centre (Ergani road), 940 m, 20.04.2017; 8 $\stackrel{\circ}{\circ}$, Çalkaya, 1350 m, 27.07.2017; 4 $\stackrel{\circ}{\circ}$, Tekevler, 1300 m, 05.08.2017; 15 $\stackrel{\circ}{\circ}$, Altıntarla, 1355 m, 24.08.2016, 09.09.2017; 2 $\stackrel{\circ}{\circ}$, Kavak, 1350 m, 09.09.2017; 2 $\stackrel{\circ}{\circ}$, Centre (Alacakaya road), 1160 m, 12.06.2017; 1 $\stackrel{\circ}{\circ}$, Çitliköy, 1265 m, 21.06.2017.

Peribatodes correptarius (Zeller, 1847)

Material. 2Å, Çitliköy, 1265 m, 21.06.2017.

Peribatodes rhomboidarius [Denis & Schiffermüller], 1775)

Material. 1♂, Soğuksu, 1020 m, 01.06.2017; 5♂, Yeşilova, 1380 m, 18.06.2017; 3♂ 1♀, Kısabekir, 860 m, 23.04.2016, 19.09.2017, 11.10.2017; 1♂, Kavak, 1350 m, 28.08.2016; 11♂ 1♀, Ağadibek, 915 m, 22.06.2016, 30.05.2017, 29.09.2017; 1♂, Gezin, 1295 m, 25.09.2017; 1♀, Tekevler, 1300 m, 22.09.2017.

Peribatodes umbrarius (Hübner, [1809])

Material. 1♀, Soğuksu, 1020 m, 01.06.2016; 1♂, Ağadibek, 915 m, 30.05.2017; 1♂, Tekevler, 1300 m, 22.09.2017.

Ramitia kufrana Seven, 2016 (Figure 3 b)

Material. 3[⊖], Sağrılı-1, 920 m, 27.04.2017.

Selidosema plumarium ([Denis & Schiffermüller], 1775)

Material. 6♂ 1♀, Altıntarla, 1355 m, 24.08.2016, 09.09.2017; 20♂ 1♀, Sağrılı-1, 920 m, 15.09.2017, 19.09.2017; 4♂, Kısabekir, 860 m, 19.09.2017; 1♂, Gezin, 1295 m, 15.08.2017; 1♂, Kavak, 1350, 28.08.2016, 09.09.2017; 8♂, Tekevler, 1300 m, 22.09.2017, 06.10.2017; 14♂, Ağadibek, 915 m, 29.09.2017.

Stegania dilectaria (Hübner, 1790)

Material. 3♂ 1♀, Yeşilova, 1380 m, 18.06.2017, 12.07.2017, 02.08.2017; 1♂, Kısabekir, 860 m, 29.06.2017; 1♂, Gezin, 1295 m, 15.08.2017.

Wehrliola inexpectata Kemal & Uçak, 2018

Material. 1♂, Soğuksu, 1020 m, 04.10.2017.

Wehrliola revocaria (Staudinger, 1892)

Material. 7♂, Ağadibek, 915 m, 29.09.2017; 5♂, Kısabekir, 860 m, 19.09.2017; 4♂, Sağrılı-1, 920 m, 15.09.2017, 19.09.2017.

Subfamily Geometrinae Stephens, 1829

Aplasta ononaria (Fuessly, 1783)

Material. 1♂, Kavak, 1350 m, 09.09.2017; 1♀, Yeşilova, 1380 m, 02.08.2017.

Phaiogramma etruscaria (Zeller, 1849)

Material. 25♂, Yeşilova, 1380 m, 16.07.2017, 02.08.2017, 23.08.2017; 5♂ 2♀, Çalkaya, 1350 m, 27.07.2017; 2♂ 1♀, Ağadibek, 915 m, 30.05.2017, 24.07.2017; 1♂, Kavak, 1350 m, 28.08.2016; 2♂, Gezin, 1295 m, 15.08.2017.

Proteuchloris neriaria (Herrich-Schäffer, [1852])

Material. 1♂, Yeşilova, 1380 m, 02.08.2017; 1♂, Ağadibek, 915 m, 22.06.2016.

Subfamily Larentiinae Duponchel, 1845

Aplocera plagiata (Linnaeus, 1758)

Material. 5♀, Kavak, 1350 m, 28.08.2016, 09.09.2017; 1♀, Ağadibek, 915 m, 22.10.2017; 1♂ 2♀, Gezin, 1295 m, 03.07.2016, 15.08.2017, 25.09.2017; 4♂ 1♀, Soğuksu, 1020 m, 01.06.2016, 17.05.2017; 1♂ 2♀, Yeşilova, 1380 m, 18.06.2017, 02.08.2017.

Camptogramma bilineatum (Linnaeus, 1758)

Material. 4♂, Kavak, 1350 m, 28.08.2016; 3♂ 2♀, Ağadibek, 915 m, 22.06.2016, 29.09.2017; 2♂, Altıntarla, 1355 m, 24.08.2016; 2♀, Sağrılı-1, 920 m, 23.07.2017; 4♀, Soğuksu, 1020 m, 07.08.2016; 2♂, Tekevler, 1300 m, 22.09.2017.

Cataclysme riguata (Hübner, [1813])

Material. 38°_{\circ} 19 $^{\circ}_{\circ}$, Yeşilova, 1380 m, 18.06.2017, 12.07.2017, 16.07.2017, 02.08.2017, 23.08.2017; 5 $^{\circ}_{\circ}$, Hanevleri, 1602 m, 20.07.2017; 1 $^{\circ}_{\circ}$, Altıntarla, 1355 m, 09.09.2017; 1 $^{\circ}_{\circ}$, Kavak, 1350 m, 28.08.2016.

Catarhoe permixtaria (Guenée, [1858])

Material. 2[♀], Yeşilova, 1380 m, 18.08.2017; 1♂, Sağrılı-1, 920 m, 25.05.2016.

Chesistege korbi (Bohatsch, 1909)

Material. 1♂, Sağrılı-2, 930 m, 27.10.2017; 1♀, Ağadibek, 915 m, 22.10.2017; 1♂, Kısabekir, 860 m, 11.10.2017.

Docirava mundata (Staudinger, 1892)

Material. 1♂ 1♀, Tekevler, 1300 m, 10.05.2017, 22.09.2017; 1♀, Ağadibek, 915 m, 29.09.2017.

Eulithis roessleraria (Staudinger, 1871) (Figure 3 c)

Material. 8^A, Yeşilova, 1380 m, 12.07.2017, 16.07.2017.

Euphyia frustata (Treitschke, 1828)

Material. 1Å, Tekevler, 1300 m, 22.09.2017.

Eupithecia breviculata (Donzel, 1837)

Material. 1♀, Sağrılı-1, 920 m, 25.05.2016; 1♂, Ağadibek, 915 m, 30.05.2017; 1♀, Soğuksu, 1020 m, 17.05.2017.

Eupithecia gueneata (Millière, 1862)

Material. 10♂ 6♀, Yeşilova, 1380 m, 18.06.2017, 12.07.2017, 16.07.2017, 02.08.2017, 23.08.2017; 1♂, Gezin, 1295 m, 15.08.2017.

Eupithecia impurata (Hübner, [1813])

Material. 1∂, Ağadibek, 915 m, 30.05.2017.

Eupithecia inconspicuata Bohatsch, 1893

Material. 1♂, Soğuksu, 1040 m, 17.05.2017.

Eupithecia mystica Dietze, 1910

Material. 1♂, Sağrılı-1, 920 m, 19.09.2017; 3♂ ♀, Tekevler, 1300 m, 22.09.2017; 3♂, Yeşilova, 1380 m, 12.07.2017, 18.07.2017; 1♂, Ağadibek, 915 m, 20.09.2017; 1♀, Altıntarla, 1355 m, 09.09.2017; 2♂, Gezin, 1295 m, 15.08.2017.

Eupithecia oblongata (Thunberg, 1784)

Material. 1Å, Yeşilova, 1380 m, 18.06.2017.

Eupithecia semigraphata (Bruand, [1847])

Material. 14♂ 8♀, Ağadibek, 915 m, 29.09.2017, 22.10.2017; 1♂, Sağrılı-1, 920 m, 15.09.2017; 1♂, Tekevler, 1300 m, 22.09.2017; 2♂ 3♀, Dutpınar, 1150 m, 30.09.2017; 1♂, Soğuksu, 1020 m, 04.10.2017.

Eupithecia silenicolata Mabille, 1867

Material. 1♂ 2♀, Yeşilova 1380 m, 12.07.2017, 16.07.2017; 1♂, Soğuksu, 1020 m, 17.05.2017.

Gymnoscelis rufifasciata (Haworth, [1809])

Material. 1Å, Altıntarla, 1355 m, 09.09.2017.

Larentia clavaria Haworth, [1809] (Figure 3 d)

Material. 1♂, Sağrılı-2, 930 m, 27.10.2017.

Nebula senectaria (Herrich-Schäffer, [1852])

Material. 1♂, Dutpinar, 1150 m, 30.09.2017.

Protorhoe corollaria (Herrich-Schäffer, [1848])

Material. 1Å, Kısabekir, 860 m, 23.04.2016.

Xanthorhoe fluctuata (Linnaeus, 1758)

Material. 1Å, Sağrılı-1, 920 m, 25.05.2016.

Subfamily Orthostixinae Meyrick, 1892

Orthostixis calcularia Lederer, 1853

Material. 1♂, Soğuksu, 1020 m, 17.05.2017.

Subfamily Sterrhinae Meyrick, 1892

Cleta perpusillaria (Eversmann, 1847) **Material.** 1♀, Yeşilova, 1380 m, 18.06.2017. *Cyclophora suppunctaria* (Zeller, 1847) **Material.** 1♀, Sağrılı-1, 920 m, 27.04.2017; 1♂, Kısabekir, 860 m, 29.06.2017.

Idaea consociata (Staudinger, 1900)

Material. 1♂, Ağadibek, 915 m, 30.05.2017.

Idaea camparia (Herrich-Schäffer, [1852])

Material. 1♂ 3♀, Ağadibek, 915 m, 29.09.2017, 22.10.2017; 8♂, Sağrılı-1, 920 m, 19.09.2017; 1♂, Kısabekir, 860 m, 11.10.2017; 1♀, Soğuksu, 1020 m, 17.05.2017.

Idaea degeneraria (Hübner, [1799])

Material. 1♂ 6♀, Ağadibek, 915 m, 30.05.2017, 29.09.2017;1♂ 4♀, Sağrılı-1, 920 m, 19.09.2017; 2♂, Kısabekir, 860 m, 19.09.2017; 2♂, Kavak, 1350 m, 09.09.2017; 5♂, Tekevler, 1300 m, 22.09.2017; 1♀, Dutpınar, 1150 m, 30.09.2017; 1♂, Centre (Alacakaya road), 1160 m, 12.06.2017; 1♀, Gezin, 1295 m, 25.09.2017.

Idaea determinata (Staudinger, 1876)

Material. 3♀, Çitliköy, 1265 m, 21.06.2017.

Idaea deversaria (Herrich-Schäffer, [1847])

Material. 8♂ 1♀, Kısabekir, 860 m, 29.06.2017, 19.09.2017; 4♂ 4♀, Yeşilova 1380 m, 12.07.2017, 16.07.2017, 02.08.2017; 1♂, Tekevler, 1300 m, 05.08.2017.

Idaea filicata (Hübner, [1799])

Material. 1♂ 1♀, Yeşilova 1380 m, 12.07.2017, 16.07.2017; 1♂ 1♀, Sağrılı-1, 920 m, 25.05.2016, 19.09.2017; 5♀, Tekevler, 1300 m, 22.09.2017; 2♂ 1♀, Ağadibek, 915 m, 30.05.2017, 29.09.2017, 22.10.2017; 2♂ 10♀, Kısabekir, 860 m, 29.06.2017, 15.09.2017, 19.09.2017; 1♂, Çitliköy, 1265 m, 16.07.2017.

Idaea ochrata (Scopoli, 1763)

Material. 65♀, K1sabekir, 860 m, 29.06.2017; 12♂ 24♀, Yeşilova, 1380 m, 18.06.2017, 12.07.2017, 16.07.2017; 3♀, Soğuksu, 1020 m, 01.06.2016; 2♀, Ağadibek, 915 m, 22.06.2016; 2♂, Çitkiköy, 1265 m, 21.06.2017; 1♂ 1♀, Centre (Alacakaya road), 1160 m, 12.06.2017.

Idaea ossiculata (Lederer, 1871)

Material. 3♂, Ağadibek, 915 m, 30.05.2017; 3♂ 2♀, Centre (Alacakaya road), 1160 m, 12.06.2017; 6♂, Yeşilova, 1380 m, 18.06.2017.

Idaea ostrinaria (Hübner, [1813])

Material. 2♂, Çitliköy, 1265 m, 21.06.2017; 2♂, Ağadibek, 915 m, 30.05.2017; 3♀, Soğuksu, 1020 m, 01.06.2017.

Idaea politaria (Hübner, [1799])

Material. 5[♀], Kısabekir, 860 m, 29.06.2017; 1♂, Sağrılı-1, 920 m, 23.07.2017.

Idaea rusticata ([Denis & Schiffermüller], 1775)

Material. 1♂ 5♀, Yeşilova, 1380 m, 12.07.2017, 16.07.2017; 1♂, Çitliköy, 1265 m, 16.07.2017; 1♀, Kısabekir, 860 m, 29.06.2017.

Idaea subsericeata (Haworth, [1809])

Material. 1♂1♀, Çitliköy, 1265 m, 21.06.2017.

Idaea textaria (Lederer, 1861)

Material. 3♂, Tekevler, 1300 m, 22.09.2017; 1♂, Yeşilova, 1380, 12.07.2017.

Idaea trigeminata (Haworth, [1809])

Material. 4♂, Yeşilova, 1380 m, 18.06.2017, 12.07.2017.

Rhodometra sacraria (Linnaeus, 1767)

Material. 1♂ 1♀, Kısabekir, 860 m, 19.09.2017; 1♂, Ağadibek, 915 m, 28.08.2017; 1♀, Sağrılı-2, 930 m, 27.10.2017.

Rhodostrophia auctata (Staudinger, 1879)

Material. 10♀, Yeşilova, 1380 m, 18.06.2017, 23.08.2017; 2♂, Gezin, 1295 m, 15.08.2017; 1♀, Altıntarla, 1355 m, 09.09.2017.

Rhodostrophia discopunctata Amsel, 1935

Material. 1♂, Ağadibek, 915 m, 30.05.2017; 6♂, Soğuksu, 1020 m, 01.06.2016; 25♂, Yeşilova, 1380 m, 18.06.2017; 2♂ 2♀, Çitliköy, 1265 m, 21.06.2017; 12♀, Centre (Alacakaya road), 1160 m, 12.06.2017.

Scopula decorata ([Denis & Schiffermüller], 1775)

Material. 1♂ 1♀, Yeşilova, 1380 m, 18.08.2017.

Scopula immistaria (Herrich-Schäffer, [1852])

Material. 6♂ 3♀, Kavak, 1350 m, 28.08.2016, 09.09.2017; 14♂ 5♀, Yeşilova, 1380 m, 18.06.2017, 16.07.2017, 02.08.2017, 23.08.2017.

Scopula marginepunctata (Goeze, 1781)

Material. 3° , Hanevleri, 1600m, 20.07.2017; 4° , 6° , Kısabekir, 860m 23.04.2016, 11.01.2017, 29.06.2017, 19.09.2017; 6° , Gezin, 1295m, 03.07.2016, 15.08.2017; 2° , 1 $^{\circ}$, Altıntarla, 1355m, 09.09.2017; 17° , 2° , Tekevler 1300m, 05.08.2017, 22.09.2017; 42° , 2° , Yeşilova 1380 m, 18.06.2017, 16.07.2017, 02.08.2017, 23.08.2017; 1° , 2° , Soğuksu, 1020 m, 05.08.2017; 16° , Ağadibek, 915 m, 22.06.2016, 30.05.2017, 28.08.2017, 29.09.2017, 22.10.2017; 3° , 3° , Sağırılı-1, 920 m, 15.09.2017 19.09.2017; 1° , Kavak, 1350 m, 28.08.2016; 1° , 1° , Çitliköy, 1265 m, 21.06.2017; 3° , Çalkaya, 1350 m, 27.07.2017.

Scopula minorata (Boisduval, 1833)

Material. 2♂ 1♀, Kavak, 1350 m, 09.09.2017, 1♂, Gezin, 1295 m, 15.08.2017, 1♂, Altıntarla, 1355 m, 09.09.2017.

Scopula ochraceata (Staudinger, 1901)

Material. 1♂, Yeşilova, 1380 m, 12.07.2017.

Scopula ornata (Scopoli, 1763)

Material. 1[♀], Yeşilova, 1380 m, 02.08.2017.

Scopula submutata (Treitschke, 1828)

Material. 31 \bigcirc 17 \bigcirc , Ağadibek, 915 m, 30.05.2017, 24.07.2017, 28.08.2017, 29.09.2017, 22.10.2017; 20 \bigcirc 184 \bigcirc , Yeşilova, 1380 m, 18.06.2017, 12.07.2017, 16.07.2017, 02.08.2017, 23.08.2017; 5 \bigcirc 2 \bigcirc , Soğuksu, 1020 m, 07.08.2016, 05.08.2017; 4 \bigcirc , Kısabekir, 860 m, 29.06.2017; 3 \bigcirc 14 \bigcirc , Sağrılı-1, 920 m, 15.09.2017, 19.09.2017, 11.10.2017; 1 \bigcirc 9 \bigcirc , Gezin, 1295 m, 15.08.2017; 2 \bigcirc , Sağrılı-2, 930 m, 27.10.2017; 7 \bigcirc , Çalkaya, 1350 m, 27.07.2017; 1 \bigcirc , Çitliköy, 1265 m, 16.07.2017; 2 \bigcirc , Altıntarla, 1355 m, 09.09.2017.



Figure 3. Adults of some species: a. *Gnophos sacraria*, b. *Ramitia kufrana*, c. *Eulithis roessleraria*, d. *Larentia clavaria*

Discussion and Conclusion

Geometridae fauna of Maden district was not studied in detail before. In this study, 87 Geometridae species are identified in 6 subfamilies from Maden (Elazığ) district. Except *Charissa onustaria*, all of the determined species are new record for the study area. And, 81 of them have been reported for the first time in Elazığ province.

Before the researches, 16 geometrid moths were known in Elazığ province (Koçak and Kemal 2007, 2018): Aplasta ononaria, Aplocera plagiata, Camptogramma bilineatum, Cataclysme subtilisparsata, Charissa mutilata, C. onustaria, Docirava mundulata, D. musculata, Euphyia sintenisi, Gnopharmia colchidaria, Idaea persidis, Lithostege buxtoni, Nychiodes variabila, Rhodostrophia bahara, Scopula immistaria, S. transcaspica. Among these species, Cataclysme subtilisparsata, Charissa mutilata, Docirava mundulata, D. musculata, Euphyia sintenisi, Gnopharmia colchidaria, Idaea persidis, Lithostege buxtoni, Rhodostrophia bahara and Scopula transcaspica are not found in Maden district during the surveys.

The number of the geometrid moths have reached to 97 species in Elazığ province with previously known 16 species and 81 new records. In the subfamilies, Ennominae is showed the biggest increase. Moreover, Orthostixinae and Desmobathrinae subfamilies are new reported from the fauna of Elazığ (Table 1).

Subfamilies	Before the survey	After the survey	New records
Sterrhinae	4	26	25
Larentiinae	7	21	19
Ennominae	4	35	33
Geometrinae	1	3	2
Orthostixinae	0	1	1
Desmobathrinae	0	1	1
Total	16	87	81

Table 1. Species numbers of subfamilies before and after the surveys and new record numbers in Elazığ province

The contribution of the study on Geometridae fauna is clearly seen when the number of the species in neighboring provinces (Koçak and Kemal 2018) and Maden district are compared (Table 2).

1	1		0		
Subfamilies	Maden (Elazığ)	Bingöl	Diyarbakır	Malatya	Tunceli
Sterrhinae	26	3	8	13	-
Larentiinae	21	3	11	17	4
Ennominae	35	6	18	24	4
Geometrinae	3	1	3	8	2
Orthostixinae	1	2	-	-	-
Desmobathrinae	1	-	1	1	-
Total	87	15	41	63	10

Table 2. Comparison of the species number in Maden district and neighboring provinces

Furthermore, *Idaea consociata* is known only in Turkey, Mardin province until now. *Wehrliola inexpectata* is described by Kemal and Uçak from Hakkari province (Turkey) in 2018. And, *Gnophos sacraria* is known from Israel, Egypt and Turkey (Siirt prov.) (Seven 2014, Koçak and Kemal 2018). The second locality records of these rare known species are given from the study area.

Although the vast majority of the geometrid moths are active at night, some of them also fly at daytime. The diurnal species determined in the region are: *Camptogramma bilineatum, Chiasmia clathrata, Myinodes shohami, Orthostixis calcularia, Rhodostrophia discopunctata* and *Rhodometra sacraria.*

According to the information obtained from the conducted studies in the research region, the species were evaluated in terms of their distributions in 3 categories (widespread, local and rare) by the number of samples and the number of localities they found.

Widespread species (a large number of samples and observed in many localities): Idaea ochrata, I. degeneraria, I. filicata, Scopula submutata, S. marginepunctata, Eupithecia mystica, Camptogramma bilineatum, Crocallis elinguaria, Peribatodes rhomboidarius, Gnophos pseudosnelleni, Nychiodes variabila, Gnopharmia irakensis, Selidosema plumarium, Eilicrinia cordiaria, Neognopharmia stevenaria.

Local species (collected only in a specific locality): Idaea determinata, I. subsericeata, I. trigeminata, Scopula decorata, Eulithis roessleraria, Peribatodes correptarius, Charissa dubitaria, Gnophos sacraria, Apochima flabellaria, Agriopis bajaria, Lycia hirtaria, Biston stratarius, Ramitia kufrana.

Rare species (represented by very few samples): *Idaea consociata*, *Scopula ochraceata*, *S. ornata*, *Cleta perpusillaria*, *Eupithecia inconspicuata*, *E. impurata*, *E. oblongata*, *Nebula senectaria*, *Euphyia frustata*, *Gymnoscelis rufifasciata*, *Xanthorhoe fluctuata*, *Larentia clavaria*, *Protorhoe corollaria*, *Wehrliola inexpectata*, *Alsophila aceraria*, *Larerannis marginaria*, *Colotois pennaria*.

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A new locality record of a rare fungus *Cercopemyces rickenii* (Agaricales: Tricholomataceae) in Ukraine

Maryna M. Sukhomlyn¹, Vasyl P. Heluta², Yulia M. Kulsha³, Ilgaz Akata^{4,*} and Vitaliy I. Sapsay²

 ¹Educational and Scientific Centre, Institute of Biology and Medicine, Taras Shevchenko Kyiv National University, 64 Volodymyrska Str., Kyiv, 01033, Ukraine
 ²M.G. Kholodny Institute of Botany, National Academy of Sciences of Ukraine, 2 Tereshchenkivska Str., Kiev, 01004, Ukraine
 ³ Kaniv Nature Reserve, 108 Shevchenko Str., Kaniv, 19000, Ukraine

^{4*} Ankara University, Faculty of Science, Department of Biology, Ankara, 06100, Turkey Corresponding author: fungus@hotmail.com.tr

Abstract

Cercopemyces rickenii (Bohus) Dima & L. Nagy (Agaricales: Tricholomataceae) is a rare species red-listed in Ukraine and in a number of other countries. Before now in Ukraine, the fungus was recorded in five localities, only in the steppe zone. All known localities were confined to sandy soil and plantings of *Acer tataricum* L. or *Robinia pseudoacacia* L. It is reported about sixth record of *C. rickenii*, located outside the steppe zone and the most northern in Ukraine. The fungus was found here in plantings formed by *Caprinus betulus* L. and *Acer platanoides* L. on forest soils. Using scanning electron microscopy, it was shown that the spore surface of *C. rickenii* had tuberculate ornamentation. In addition, it was shown that the basidium of the fungus could have more than four sterigmata.

Keywords: new locality, rare species, Tricholomataceae, Ukraine

Introduction

Different authors at different times included *Cercopemyces rickenii* (Bohus) Dima & L. Nagy (Agaricales: Tricholomataceae) into several genera. Therefore, this fungus was previously known as *Armillaria rickenii* Bohus, *Floccularia rickenii* (Bohus) Wasser ex Bon or *Ripartitella rickenii* (Bohus) Singer. However, recently the species was transferred to the genus *Cercopemyces* T.J. Baroni, Kropp & V.S. Evenson (Dima, 2015). Today *C. rickenii* is known from Bulgaria, Czech Republic, France, Hungary, Poland, Russia, Slovakia, Sweden, and Ukraine (Bohus, 1970; Wasser, 1971; Lange, 1995; Prydiuk 1999; Lizon, 2001; Gyosheva et al., 2006; Denchev and Assyov, 2010; Gierczyk et al., 2012; Lessoe, 2014; Rusanov, 2014; Westling, 2015). The fungus is rare in everywhere and red-listed in a number of countries. Therefore, it was proposed to be included in the European Red List (Senn-Irlet, 2011).

In Ukraine, *C. rickenii* (sub *Floccularia rickenii*) is listed in the Red Data Book as a vulnerable rare species with a disjunctive range (Didukh, 2009). Before our research, the fungus was known here only from five localities, exclusively in the steppe zone (Figure 1). However, in 2014 and 2015, this species

was found by us outside of this zone. Consequently, this article supplemented the information about distribution of *C. rickenii* in Ukraine. In addition, we expanded the morphological characteristics of the species by studying the hymenium using scanning electron microscopy.



Figure 1. Distribution of Cercopemyces rickenii in Ukraine

Material and Methods

The materials for the study were samples of *C. rickenii* collected by us on May 11, 2014 and June 25, 2015 on the territory of the Kaniv Nature Reserve (Cherkasy Region, Ukraine). The specimens were dried at room temperature. The spores and hymenium elements of the fungus were studied and photographed under a light microscope Primo Star (Carl Zeiss, Germany) using the camera Canon A 300 and the software AxioVision 4.7. For scanning electron microscopy (SEM), small pieces of dried hymenial lamellae were placed on a metal stub, then coated with gold and observed under SEM Jeol 6060LA, Japan. Species names of vascular plants are in accordance with the catalogue "Vascular plants of Ukraine. A nomenclatural checklist" (Mosyakin and Fedoronchuk, 1999).

Results and Discussion

Cercopemyces rickenii is a quite easily recognizable species in natural conditions. The fungus has rather large basidiocarps covered with conical or pyramidal scales (Figure 2A). The basidiocarps grow on the ground singly or in groups, sometimes large. *Cercopemyces rickenii* is a thermophilic species, a humus saprotroph that prefers sandy soils (Wasser, 1975, Gierczyk et al., 2012). Most European records of the fungus are associated with *Robinia pseudoacacia* L. (Wasser, 1975; Horak, 2005). However, in Sweden it was found among the bushes of *Crataegus rhipidophylla* Gand. and Rosa sp. (Bohlin and Knutsson, 2001). In Ukraine, C. rickenii was collected both under *Robinia pseudoacacia* and in monocultures of *Acer tataricum* L. (Prydiuk, 1999).

For the first time in Ukraine, *C. rickenii* was found by S.P. Wasser in 1968 on the territory of the Oleshky Forestry (Oleshky District, Kherson Region) (Wasser, 1971). Sometime later, in 1970, the fungus was collected by the same author on sand or sandy soil in other forest areas located in the steppe zone of

Ukraine, namely Berezivske Forestry (Berezivka District, Odesa Region) and Volodymyrske Forestry (Bereznehuvate District, Mykolaiv Region), as well as in the Orel Forest (Petrykivka District,



Figure 2. Morphology of the basidiocarp of *Cercopemyces rickenii*. A, B - the surface of the pileus; C - gills; D - the basidiocarp in section; E - spores (light microscope, scale bar =10 μ m).

Dnipropetrovsk Region) (Wasser, 1971, 1974, 1976). The author also reported on the localities of this species, gave a description and black-and-white photos of basidiocarps, drawings of the gill trama structure, and spore images of this fungus. Much later, after 30 years, *C. rickenii* was again found in the steppe zone, in the Dniprovsko-Orilsky Nature Reserve (Dnipro and Petrykivka districts, Dnipropetrovsk Region), on the sands in the thickets of *Acer tataricum* and *Robinia pseudoacacia* (Prydiuk, 1999, 2005). After than, until our research, the fungus was no longer found in Ukraine.

As already mentioned above, *C. rickenii* was found by us in 2014 and 2015 on the territory of the Kaniv Nature Reserve located in the forest-steppe zone of Ukraine (Figure 1). Habitats of the fungus were situated in a leveled area of a broad-leaved forest, which bordered on the edge of the forest and passed to the slope of the southern exposure. The tree tier was composed here mainly of *Caprinus betulus* L. and *Acer platanoides* L. Also in this tier there were *Quercus robur* L., *Ulmus scabra* Mill., *Betula pendula* Roth., *Acer negundo* L., *Crataegus pseudokyrtostyla* Klok., and *Fraxinus excelsior* L. The undergrowth was formed by *Caprinus betulus* L., *Acer platanoides* L., *Fraxinus excelsior* L. The grass layer formed mainly by *Stellaria holostea* L. and *Viola hirta* L.; *Asarum europaeum* L., *Galium aparine* L., *Polygonum convolvulus* L., *Alliaria petiolata* (Beib.) Cavara et Grande, *Dentaria bulbifera* L., and *Poa nemoralis* L. was also present.

The basidiocarps we found were 5–8 cm in diameter, had a yellowish-white, yellowish-creamy (with a lemon tinge), a slightly darker to the center, convex, convex-expanded, dry, matte pileus covered by concentrically arranged multi-faceted conical warts (Figure 2A and 2B). The edge of the pileus smooth, slightly bent down, with white remnants of the universal veil. Gills dense, attached, with a straight edge, white, white-cream (Figure 2C). The gill trama regular. The stipe $1.0-1.5 \times 3-6$ cm, the same color as the cap, central, solid, slightly narrowed upwards and enlarged downwards, at the top smooth, below covered by the remnants of an universal veil in a form of warts forming a rapidly disappearing ring. Flesh dense, firm, white in the pileus and stipe, unchanging on cutting (Figure 2D), sweetish. It was previously reported that young basidiocarps have a pleasant mushroom smell (Wasser, 1971) but our samples were odorless, probably due to the fact that they were quite dry. Spore-print cream. Basidiospores broadly ovoid, $4.6-5.8 \times 2.8-3.7 \mu m$, that fully corresponds to already known data (Wasser, 1971; Prydiuk, 1999; Lange 2008; Didukh, 2009; Gierczyk et al., 2012). Under a light microscope and with small magnifications in a SEM, spores appear smooth (Figure 2E and 3A). However, with a large magnification, more than \times 12 000, tuberculate ornamentation is visible on the spore surface (Figure 3B and 3C). The tubercles are arranged throughout the spore surface unevenly at a distance of $0.2-5.0 \,\mu\text{m}$. The dimensions and bulge of the tubercles are also variable. Before, it was not known that the surface of the spore of C. rickenii was ornamented. However, the same surface is typical for Cystoderma ponderosum (A.H. Smith & Singer) T.J. Baroni, Kropp, & V.S. Evenson (Franco-Molano, 1993), which was later also referred to the genus Cercopemyces (Baroni et al., 2014).

In addition, in the SEM image, it can be seen that spores have a nipple-like apiculus (Figure 3C). Basidia are clavate (Figure 3D), mostly having four sterigmata. However, for the first time, basidia with a more number of sterigmata were found for this species (Figure 3E and 3F). The sterigmata are long, up to 3.4 μ m, with spherical extensions on the apex. After maturation and separation of basidiospores these thickened apices become flattened to almost hemispherical (Figure 3F).

Thus, as a result of our research a new, sixth and the most northern locality of the rare fungus *C. rickenii* has been recorded in Ukraine. In addition, the fungus was found on forest soil in plantings mainly formed by *Caprinus betulus* and *Acer platanoides*, but not on sandy soils in plantings of *Acer tataricum* or *Robinia pseudoacacia*, as it was previously known. Using scanning electron microscopy, for the first time it was shown that the spore surface of *C. rickenii* has tuberculate ornamentation, and that the basidium of the fungus could have more than four sterigmata.



Figure 2. Morphology of basidia and basidiospores of *Cercopemyces rickenii*. SEM. A - basidiospore mass on the surface of the gill; B - basidiospore at high magnification; on the surface is clearly visible tuberculate ornamentation; C - basidiospores with apiculi; D - young basidium (in the central part of the image); E - sterigmata (in the center) with spherical extensions on the apex; F - basidium with more than four sterigmata. Scale bar for A = 5 μ m, for B, C = 1 μ m, for D = 2 μ m, for E, F = 1 μ m

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Seasonal Changes in Lipid and Fatty Acid Profiles of Sakarya Chub (*Squalius pursakensis*) from the Melen River Basin

Tuğba İnan¹, Deniz Ayas², Şerife Gülsün Kırankaya^{3*}

¹Instutute of Science, Department of Biology, Konuralp Campus, Düzce University, 81620, Düzce. Turkey. ²Faculty of Fisheries, Department of Seafood Processing Technology, Mersin University, Mersin, Turkey ³Faculty of Arts and Sciences, Department of Biology, Düzce University, Konuralp Campus, 81620, Düzce, Turkey.

Corresponding author: gkirankaya@gmail.com

Abstract

In this study, total lipids and fatty acid composition of *Squalius pursakensis* fillet samples which were obtained from Melen River Basin during winter, spring and summer seasons were evaluated. Total lipid levels were 1.80% in winter, 2.56% in spring and 5.17% in summer. The composition of fatty acids showed that total polyunsaturated fatty acids (32.05-38.90%) were highest, followed by monounsaturated (29.85-35.40%) and saturated (27.10-31.23%). The fatty acids composition of polyunsaturated fatty acids (PUFAs) in fillets of *S. pursakensis* shows a high content *n*3 fatty acids eicosapentaenoic acid (EPA C20:5*n*3) and docosahexaenoic acid (DHA C22:6*n*3) with maximum rates of 6.70% and 16.33%, respectively (p<0.05). The saturated fatty acid (SFA) content was dominated by palmitic acid with the maximum ratio of 19.03%.

Keywords: Squalius pursakensis, fatty acids, lipids, SFA, MUFA, PUFA, Melen River

Introduction

Fish is considered as an essential and available protein source for human populations all around the World (Ghaly et al. 2013). Today, fish is an important diet component in many countries and contributes to 16.6% of animal protein consumed by the world's population (FAO 1997, FAO 2012). Additionally, fish provide important nutrients for human health such as trace elements, lipid-soluble vitamins and lipids (Roos et al. 2007, Benguendouz et al. 2017).

Lipids are biomolecules that play an important role in the body. They play the role of storage of energy and lipid-soluble vitamins. Furthermore, lipids are known to serve as integral components of biological membranes and to function as key intracellular and extracellular messengers (Simopoulos 1991). Therefore, especially unsaturated fatty acids are necessary for human health (Kaya et al. 2004). However, long-chain *n*-3 PUFAs should be obtained in the diet, since they can not be synthesized in human body (Brown 2000, Alasalvar et al. 2002).

Currently, numerous scientific researches focus on fish and fish oil fatty acids due to their various benefits on health. Fish oils are considered as the best natural source of highly unsaturated fatty acids, especially acids with more than three ethylenic groups or double bonds (Gruger et al. 1964). Studies have shown that fish lipids are rich in PUFAs, especially the EPA and DHA from *n*-3 PUFAs bonds (Ugoala et al. 2009, Sharma et al. 2010). Fish have a high capacity for the transformation of C18 essential fatty acids (EFAs); C18:3 n-3 and C18:2 n-6 to C20:5 n-3, C22:6 n-3 and C20:4 n-6 and therefore, they may be an available source of such fatty acids to consumers (Henderson and Tocher 1987). These fatty acids turned out to be very important for human health. EPA and DHA can not be

synthesized in human boyd. They were only found in seafood such as fish. Thus, they need to be supplemented through dietary intake (Gunstone 1996). PUFAs, especially the EPA and DHA possess important beneficial properties such as reducing the risk of heart attack and human coronary artery disease (Leaf and Weber 1988, Kaya et al. 2004), lowering serum triacylglycerol levels, increasing membrane fluidity, and reducing thrombosis, the risk of coronary heart disease, hypertension, inflammation, and autoimmune disorders (von Schacky et al. 1985, Bønaa et al. 1990, Horrocks and Yeo 1999, Simopoulos 2002). Many studies show that consumption of fish oil rich in *n*-3 PUFA has beneficial effects on coronary heart disease (von Schacky and Harris 2007), hypertension (Bønaa et al. 1990), rheumatoid arthritis (Berbert et al. 2005), breast and colon cancer (Roynette et al. 2004), Alzheimer disease (Petot and Friedland 2004), inflammation and autoimmune disorders (Simopoulos 2002). *n*-3 PUFAs play a vital role in the development and function of the nervous system, photoreception and the reproductive system (Alasalvar et al. 2002, Sidhu 2003).

Fatty acid compositions of fish are affected by genetic properties, environmental factors and nutritional quality (Bayır et al. 2006). Although it is generally recognized that fatty acid composition may vary among fish species, there is limited information about fatty acid content of different species to be selected for diets. Additionally, it is claimed that the amount of *n*-3 PUFAs in marine fish is higher than freshwater species although studies on the fatty acid content of freshwater fish are scarce (Rahman et al. 1995).

S. pursakensis is a freshwater fish species endemic to Sakarya River, its tributaries and neighbouring basins. Although, this species is preferred as food local people, there is limited data on its nutritional value. The aim of this study was to determine the total lipid amount and fatty acid composition of *S. pursakensis* living in Melen River basin.

Material and Methods

S. pursakensis samples were obtained from four different sites in the Melen River Basin during winter, spring and summer seasons in 2018. Melen River is located on western Black Sea region in Turkey. Sampling sites selected were: Küçük Melen River (latitude: $40^{\circ}54'25''$ N; longitude: $31^{\circ}15'7''$ W), Asar Creek (latitude: $40^{\circ}47'44''$ N; longitude: $31^{\circ}14'14''$ E; latitude: $40^{\circ}49'19''$ N; longitude: $31^{\circ}11'43''$ E; latitude: $40^{\circ}50'6''$ N; longitude: $31^{\circ}8'6''$ E). Totally 30 fish specimens were caught by electrofishing. The fish samples were transported to the laboratory in ice boxes and metric measurements were carried out. The standard length ranged between 9.5 cm and 26.5 cm and the body weight from 20 g and 268 g. The fish were eviscerated, the backbones were removed and edible flesh was stored at -20° C for further analysis.

Fat and Fatty Acids Analyses

Lipid content was measured by the method of Bligh and Dyer (1959). Methyl esters were prepared by transmethylation using 2 M KOH in methanol and n-heptane according to the method described by Ichihara et al. (1996) with minor modification. Fatty acid composition was analyzed using a Gas Chromatography (GC) Clarus 500 device (Perkin–Elmer USA), one flame ionization detector (FID) and SGE (60 m x 0.32 mm ID BPX70 x 0.25 μ m, USA or Australia) column. Injector and detector temperatures were set as 260°C and 230°C respectively. During this time, the furnace temperature was kept at 140°C for 8 minutes. After that, it was increased by 4°C per minute until 220°C, and from 220°C to 230°C by increasing the temperature 1°C per minute. It was kept at 230°C for 15 minutes to complete analysis. Sample scale was 1 μ l and carrier gas was controlled at 16 ps. For split flow 40, 0 mL/minute (1:40) level was used. Fatty acids were determined using a comparison to the exit times of the FAME mix that contains 37 standard components.

Conversion factor:

Triplicate GC analyses were performed and the results were converted to mg fatty acid per 100 g total lipid using lipid conversion factors and then to mg fatty acid per 100 g edible portion of food using the

total lipid content. Details of the derivation of lipid conversion factors were published by Weihrauch et al. (1975).

Factor (Fish) = 0.956-0.143/total lipid

Fatty acid (mg/100g) = Factor x FAME (%) x lipid level (%) x 10

Atherogenicity index (AI) and thrombogenicity index (TI):

The AI and TI linked to the fatty acid composition were calculated according to Ulbricht and Southgate (1991).

AI = [(a*12:0)+(b*14:0)+(c*16:0)] / [d*(PUFA n-6+n-3)+e*(MUFA)+f*(MUFA-18:1)]

 $TI = [g^{*}(14:0+16:0+(18:0)] / [(h *MUFA)+i*(MUFA-18:1)+(m*n-6)+(n*n-3)+(n-3/n-6)]$

a, c, d, e, f=1, b=4, g=1, h, i, m=0.5 n=3

Statistical Analysis

Obtained data were statistically analyzed using by the IBM SPSS version 22 Statistical Software. Prior to the statistical analyses for evaluating lipid and fatty acid data, all data were checked for outliers (Z values were checked) and homogeneity of variance was also tested with Duncan test. One-way ANOVA (Analysis of Variance) was used to evaluate the difference in the seasons.

Results

Total lipid (%)

Lipid content of S. pursakensis is 1.80% in winter, 2.56% in spring and 5.17% in summer (p<0.05).

Fatty acids (%)

In fillets *S. pursakensis*, 29 fatty acids type were determined (Table 1). The fatty acid composition of *S. pursakensis* fillets ranged between 27.10% and 31.23% for SFAs, 29.85 - 35.40% for MUFAs and 32.05 - 38.90% for PUFAs.

The highest proportions determined were palmitic acid (C16:0, 17.41-19.03%), transoleic acid (C18:1 n9t, 11.65-18.09%), linoleic acid (C18:2 n6cis, 7.78-11.01%), eicosapentaenoic acid (EPA, C20:5 n3, 4.82-6.70%) and docosahexaenoic acid (DHA, C22:6 n3, 9.91–16.33%). It was also observed that the levels of these fatty acids changed significantly between seasons (p<0.05).

According to obtained data, major saturated fatty acid contributing to \sum SFA was palmitic acid (C16:0). Trans oleic acid (C18:1) was the most represented of the \sum MUFA. The major fatty acids identified in the PUFAs, were EPA and DHA. The highest levels of EPA and DHA were obtained during spring and summer, respectively (p<0.05).

MUFAs and PUFAs were dominant in the profile of fatty acids (Table 1). The proportions of *n*3 PUFAs (ranging between 20.01 and 24.69%) were higher than those of *n*6 PUFAs (ranging between 11.72% and 14.21%). According to obtained amount of \sum PUFA, the highest content was found in the summer (38.90%).

The ratio n3/n6 varied from 1.66 to 1.81 and showed differences between seasons. Additionally, the SFA/PUFA ratio was lowest during summer (0.80) and highest during winter (0.93). DHA/EPA ratio was 1.70 in the spring and 3.31 in the summer.

Fatty acids (mg/100 g)

The level of fatty acids in 100 g fillets of *S. pursakensis* were shown in Table 2. Amount of SFAs, MUFAs and PUFAs ranged between 455.25-1161.20 mg/100g, 460.16-1396.64 mg/100g and 492.1-182.07 mg/100g, respectively.

The highest amounts determined were palmitic acid (C16:0, 272.84-890.39 mg/100g), transoleic acid (C18:1 *n*9t, 178.87-817.40 mg/100g), linoleic acid (C18:2 *n*6c, 119.45-515.14 mg/100g),

eicosapentaenoic acid (EPA, C20:5 *n*3, 74.01-231.14 mg/100g) and docosahexaenoic acid (DHA, C22:6 *n*3, 152.16-764.06 mg/100g).

	Winter	Spring	Summer		
Fatty acids	$\overline{\mathbf{X}} \pm \mathbf{S}_{\mathbf{x}}$	$\vec{X} \pm S_x$	$\overline{\mathbf{X}} \pm \mathbf{S}_{\mathbf{x}}$		
Lauric acid (C12:0)	0.33±0.06ª	0.25 ± 0.05^{a}	0.26±0.05 ^a		
Myristic acid (C14:0)	1.40 ± 0.14^{b}	1.23 ± 0.10^{b}	0.88 ± 0.04^{a}		
Pentadecylic acid (C15:0)	0.40 ± 0.01^{b}	0.29±0.01ª	0.29 ± 0.01^{a}		
Palmitic acid (C16:0)	17.77 ± 0.20^{a}	17.41 ± 0.90^{a}	19.03 ± 1.65^{a}		
Margaric acid (C17:0)	1.25±0.07°	0.44 ± 0.02^{b}	0.29 ± 0.06^{a}		
Stearic acid (C18:0)	7.52 ± 0.46^{b}	6.18 ± 0.63^{a}	8.94±0.77°		
Arachidic acid (C20:0)	0.26 ± 0.04^{a}	0.27 ± 0.04^{a}	0.27 ± 0.04^{a}		
Behenic acid (C22:0)	0.26 ± 0.04^{a}	$0.40 {\pm} 0.05^{b}$	0.31 ± 0.02^{ab}		
Lignoceric acid (C24:0)	0.46 ± 0.02^{a}	0.63 ± 0.12^{b}	$0.96 \pm 0.06^{\circ}$		
ΣSFA	29.65	27.10	31.23		
Myristoleic acid (C14:1)	0.32 ± 0.03^{b}	0.23 ± 0.06^{a}	0.31 ± 0.01^{b}		
Pentadecenoic (C15:1)	0.12 ± 0.01^{a}	$0.14{\pm}0.02^{a}$	0.19 ± 0.01^{b}		
Palmitoleic acid (C16:1)	6.27 ± 0.16^{b}	6.08 ± 0.98^{b}	2.87±0.29 ^a		
Heptadecenoic acid (C17:1)	0.45 ± 0.06^{a}	$0.37{\pm}0.05^{a}$	$0.35{\pm}0.04^{a}$		
Trans oleic acid (C18:1 <i>n</i> 9t)	11.65 ± 1.15^{a}	$18.09 {\pm} 1.75^{b}$	17.47 ± 2.03^{b}		
Oleic acid (C18:1 <i>n</i> 9c)	$5.25 \pm 0.42^{\circ}$	4.16±0.75 ^b	2.27 ± 0.14^{a}		
Vaccenic acid (C18:1 <i>n</i> 7)	0.13 ± 0.02^{b}	$0.09{\pm}0.01^{a}$	0.10 ± 0.00^{a}		
Gadoleic acid (C20:1 <i>n</i> 9)	$0.54{\pm}0.03^{ab}$	$0.59{\pm}0.06^{b}$	0.48 ± 0.04^{a}		
Erucic acid (C22:1 <i>n</i> 9)	5.07±0.22ª	5.49±0.74 ^a	5.61 ± 0.38^{a}		
Nervonic acid (C24:1 <i>n</i> 9)	0.17 ± 0.01^{a}	0.16 ± 0.01^{a}	0.20 ± 0.00^{b}		
ΣΜυγΑ	29.97	35.40	29.85		
Linolelaidic Acid (C18:2n6t)	0.17 ± 0.01^{a}	$0.19{\pm}0.01^{a}$	0.17 ± 0.02^{a}		
Linoleic acid (C18:2 <i>n</i> 6c)	7.78 ± 0.26^{a}	9.02±0.70 ^a	11.01 ± 0.79^{b}		
α -Linolenic acid (C18:3 <i>n</i> 3)	4.69±0.31 ^b	2.05±0.14ª	2.07 ± 0.29^{a}		
Gamma linolenic acid (C18:3n6)	0.26±0.03ª	$0.35{\pm}0.04^{ab}$	0.43 ± 0.05^{b}		
Eicosatrienoic acid (C20:3n3)	0.59±0.02ª	1.03±0.03 ^b	1.35±0.17°		
Dihomo- γ -linolenic acid (C20:3 <i>n</i> 6)	$0.60{\pm}0.02^{a}$	0.74 ± 0.05^{b}	0.49 ± 0.10^{a}		
Arachidonic acid (C20:4 <i>n</i> 6)	2.98±0.02°	1.06±0.11ª	1.62±0.05 ^b		
Eicosapentaenoic acid (C20:5n3)	4.82±0.31 ^a	6.70±0.72 ^b	4.94±0.38 ^a		
Adrenic acid (C22:4n6)	0.25±0.04ª	0.36±0.07 ^a	0.49 ± 0.03^{b}		
Docosahexaenoic acid (C22:6n3)	9.91±0.15 ^b	11.38±0.64 ^b	16.33±0.64 ^c		
ΣΡυγΑ	32.05	32.88	38.90		
SFA/PUFA	0.93	0.82	0.80		
$\Sigma n7$	0.13	0.09	0.10		
$\Sigma n6$	12.04	11.72	14.21		
$\Sigma n3$	20.01	21.16	24.69		
$\Sigma n9$	22.68	28.49	26.03		
<i>n6/n</i> 3	0.60	0.55	0.58		
<i>n</i> 3/ <i>n</i> 6	1.66	1.81	1.74		
DHA/EPA	2.06	1.70	3.31		
AI	0.26	0.23	0.26		
TI	0.29	0.25	0.27		
Unidentified	8.33	4.62	0.02		

Table 1. Seasonal fatty acids profile of S. pursakensis caught from Melen River Basin (%).

 $\overline{X} \pm S_x$: means \pm SD; the values in the same line with different superscript letter (a-b-c-d-e) are significantly different (p<0.05).

Conversion Factor	0.853	0.877	0.905
Fatty acid (mg/100g)	Winter	Spring	Summer
Lauric acid (C12:0)	5.07	5.61	12.17
Myristic acid (C14:0)	21.50	27.61	41.17
Pentadecylic acid (C15:0)	6.14	6.51	13.57
Palmitic acid (C16:0)	272.84	390.88	890.39
Margaric acid (C17:0)	19.19	9.88	13.57
Stearic acid (C18:0)	115.46	138.75	418.29
Arachidic acid (C20:0)	3.99	6.06	12.63
Behenic acid (C22:0)	3.99	8.98	14.50
Lignoceric acid (C24:0)	7.06	14.14	44.92
ΣSFA	455.25	608.43	1461.20
Myristoleic acid (C14:1)	4.91	5.16	14.50
Pentadecenoic (C15:1)	1.84	3.14	8.89
Palmitoleic acid (C16:1)	96.27	136.50	134.28
Heptadecenoic acid (C17:1)	6.91	8.31	16.38
Trans oleic acid (C18:1n9t)	178.87	406.14	817.40
Oleic acid (C18:1 <i>n</i> 9c)	80.61	93.40	106.21
Vaccenic acid (C18:1 <i>n</i> 7)	2.00	2.02	4.68
Gadoleic acid (C20:1n9)	8.29	13.25	22.46
Erucic acid (C22:1 <i>n</i> 9)	77.84	123.26	262.48
Nervonic acid (C24:1 <i>n</i> 9)	2.61	3.59	9.36
ΣΜυγΑ	460.16	794.77	1396.64
Linolelaidic Acid (C18:2n6t)	2.61	4.27	7.95
Linoleic acid (C18:2 <i>n</i> 6c)	119.45	202.51	515.14
α -Linolenic acid (C18:3 <i>n</i> 3)	72.01	46.02	96.85
Gamma linolenic acid (C18:3 <i>n</i> 6)	3.99	7.86	20.12
Eicosatrienoic acid (C20:3n3)	9.06	23.12	63.16
Dihomo-γ-linolenic acid (C20:3 <i>n</i> 6)	9.21	16.61	22.93
Arachidonic acid (C20:4 <i>n</i> 6)	45.75	23.80	75.80
Eicosapentaenoic acid (C20:5n3)	74.01	150.42	231.14
Adrenic acid (C22:4n6)	3.84	8.08	22.93
Docosahexaenoic acid (C22:6n3)	152.16	255.50	764.06
ΣΡυγΑ	492.10	738.20	1820.07
$\Sigma n6$	184.86	263.13	664.86
$\Sigma n3$	307.23	475.07	1155.21
$\Sigma n9$	348.23	639.63	1217.90
$\Sigma n7$	2.00	2.02	4.68
Unidentified	127 90	103 72	0.94

Table 2. Seasonal fatty acids profile of S. pursakensis caught from Melen River Basin (mg/100g)

The amount of *n*3 PUFAs reached 1155.21 mg/100g during the summer; although amount of *n*6 PUFAs (ranging between 184.86 and 664.86 mg/100g) were lower than *n*3 PUFAs in all seasons.

Discussion

Fish are often classified on the basis of their fat content as, lean, low-fat, medium-fat and fatty fish. In the Lambertsen classification, fat content is lower than 2% by weight in lean fish, between 2-4% by weight in low-fat fish, 4-8% by weight in medium-fat fish and higher than 8% by weight in fatty fish (Lambertsen 1978). Whereas in the Polish standards fat content is lower than 2% in lean fish, between 2-7% in low-fat fish, 7-15% in medium-fat fish and higher than 15% in fatty fish (Polish Standard PN-A-86770 1999). Fish lipid content varies according to species, seasons, geographic origin as well as age and sexual maturity in the same species (Rahman et al. 1995). Lipid content of S. pursakensis varied between 1.80% and 5.17, therefore it can be considered medium fat fish especially during summer season. Lipid content of S. pursakensis is 1.80% in winter, 2.56% in spring and 5.17% in summer (p<0.05). According to Lambertsen and Polish standards, S. pursakensis can be accepted as low-fat fish in terms of annual variations in fat content In fish, lipids are stored in the liver and muscle tissues, and the stored lipids are transported from the liver and muscles to other parts of the body such as gonads when they need (Jangaard et al. 1967). After the breeding season, fish restart an intensive feeding period in order to gain their condition lost in their breeding activities. During summer and autumn seasons, metabolic rate increases and lipids are stored for using in winter and next breeding season. As the gonads begin to develop, storaged lipids in the liver, muscle and other tissues transport to the gonads, thus the lipid content is increased in gonads but decreased in the liver and muscle tissues. Additionally, temperature is a significant environmental factor affecting the feeding activities of fish which is poikilotherm animals.

In the present study, the PUFA content (32.02-38.90%) was generally higher than MUFA (29.85-35.40) and SFA (27.10-31.23). Similarly, Ateş (2013) found higher rates of PUFA in *S. pursakensis* caught from upper Sakarya River. Kaçar and Başhan (2016) also reported higher PUFA content in *Carassius gibelio*, *Carassius auratus*, *Liza abu*, *Chalcalburnus mossulensis* and *Chondrostoma regium*, whereas MUFA were higher amount in *Aspius vorax*, *Carassobarbus luteus*, *Acanthobrama marmid*, *Cyprinion macrostomum* and *Capoeta trutta* from Atatürk Dam Lake. However, Rahman et al. (1995) found that MUFA content was higher than SFA and PUFA in many freshwater fish species especially in tropical waters.

The highest content of total PUFAs was found in the summer, while Ateş (2013) found higher PUFA contents for the same species during spring and autumn (50.039-55.512%). Concerning total PUFAs, lower contents was found for *S. pursakensis* in Melen River Basin than upper Sakarya River Basin. Fish need PUFAs to provide tolerance to low water temperatures. Decreases in PUFA concentrations in lipids would therefore be expected in warmer waters (Rahman et al. 1995).

EPA and DHA were found to be higher rate in the present study. Similarly, Ateş (2013) also found higher EPA and DHA ratios for *S. pursakensis* caught from Upper Sakarya River. According to data obtained by Ateş (2013), EPA ratio was varied from 6.59% to 15.469%, and ratio of DHA was between 20.534% and 25.604%. In tropical waters, EPA and DHA ratios in freshwater fish species were found lower than 2% (Rahman et al. 1995). EPA and DHA are also dominant in marine fish species. Bayır et al. (2006) found that EPA ratio in some marine fish ranged between 6.02% (Salmon) and 18.74 (two-banded bream), and ratio of DHA varied between 10.57% (horse mackerel) and 25.85% (Anchovy). Similarly, EPA and DHA proportions in *Sardina pilchardus* in Algerian coasts were found 7.74-15.75% and 16.83-28.34%, respectively (Benguendouz et al. 2017). EPA is the most essential fatty acid of the *n*3 series for the human diet (Chen et al. 1995). On the other hand, DHA is considered more efficient then EPA as an essential fatty acid, because it improves the health value of fish (Benguendouz et al. 2017). The fatty acid composition in fish can vary depending on fishing ground and can be affected by environmental and geographical conditions (Saito et al. 1997).

The SFA/PUFA ratio estimated in this study (0.80-0.93) were higher than the one estimated by Ateş (2013) (0.46-0.65), for *S. pursakensis*. The n3/n6 fatty acid ratio is the best index for evaluating the

nutritional value of fish species (Piggott and Tucker, 1990). In the marine fish, this ratio is ranged from 1.3 (butterfish) and 15.2 (Atlantic cod), while in freshwater fish n3/n6 fatty acid ratio varied between 0.6 (carp) and 5.6 (black bass) (Hearn et al. 1987). In this study, the n3/n6 ratios ranged between 1.66 and 1.81 However, Ateş (2013) found that n3/n6 fatty acid ratio varied between 3.95 and 5.412. The n3/n6 fatty acid profile in *S. pursakensis* is convenient to obtained data for freshwater fish.

The amount of PUFAs in the muscle filets of *S. pursakensis* varied between 74.01-231.14 mg/100g for EPA and 152.16-764.06 mg/100g for DHA. Kmínková et al. (2001) found that the amount of EPA and DHA in muscle tissue of carp were 82.2-149.2 mg/100g and 24.5-56.2 mg/100g, respectively. According to these values, *S. pursakensis* has higher amount of *n*3 PUFAs. According to British Nutrition Foundation standards, it is recommended that people who have a balanced and healthy diet should consume 0.2 g of EPA + DHA per day (HMSO 1994). Obtained results in the present study suggest that EPA and DHA required for a balanced and healthy nutrition can be provided with 100 g daily consumption of *S. pursakensis* species in all seasons.

Steady growth of human populations results in higher fish consumption, in part due to their *n*3–PUFAs, which plays a decisive role in the preventing cardiovascular diseases (Leaf and Weber 1988, Bouderouna et al. 2011) and neurodegenerative syndromes such as Alzheimer's disease (Moyad 2005). Therefore, when fish are suggested as a means of improving health, both the lipid content and the PUFA profile should be considered (Rahman et al. 1995). According to the data obtained, *S. pursakensis* is a good source in terms of EPA and DHA.

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Changes in physical and hydraulic properties of a clay soil due to the irrigation of tomatoes with recycled wastewater

Azize DOĞAN DEMİR1*, Üstün ŞAHİN2

^{1*}Department of Biosystem Engineering, Faculty of Agriculture, Bingöl University, Bingöl, Turkey
²Department of Agricultural Structures and Irrigation, Faculty of Agriculture, Atatürk University, Erzurum, Turkey
Corresponding author: ademir@bingol.edu.tr

Abstract

This study was carried out investigate the effect of treated wastewater on the physical, chemical and hydraulic properties of the soil in Bingöl conditions during 2013 and 2014 years. In this study physical (bulk density, particle density, porosity, wet aggregate stability) and hydraulic properties (field capacity, wilting point, available water content, infiltration) of the soil did not change significantly under different irrigation practices. However, the porosity, available water content, and infiltration improved partly under partial root-zone drying practice with 50% water shortage. Wet aggregate stability in surface soil increased significantly with wastewater treatment. Wastewater reduced the stable infiltration rate significantly as well. Although the physical and hydraulic properties improved when compared with the pre-planting values, the wastewater treatment could not provide sustainable important effects in a short-time period.

Key words: Municipal wastewater, Soil properties, Irrigation practices

Introduction

Nowadays, wastewater treatment and reuse for agriculture in arid and semi-arid regions is an important factor in saving water resources. Physical and hydraulic properties of the soils irrigated with wastewater can be affected positively due to high organic matter content in the wastewater. The high organic matter is the main component in the improvement of soil aggregation (Nadav et al. 2013). The water retention capacity and hydraulic conductivity of the soil can be increased due to better soil aggregation.

An increase of sodicity level in the soil leads to deterioration of the porosity, bulk density, water retention, and infiltration rate. However, structural deterioration depends on the interaction between sodicity and electrolyte concentration of the soil solution (Muyen et al., 2011). Many research results reported generally negative effects on the soil properties under the wastewater irrigation conditions. Assouline et al. (2016) pointed out an increasing sodicity hazard and a decreasing hydraulic conductivity in the treated wastewater irrigation. Schacht and Marschner (2015) found lower soil hydraulic conductivity and aggregate stability values under the treated wastewater application. Assouline and Narkis (2011) reported that long-term wastewater irrigation adversely affected soil hydraulic properties. Aiello et al. (2007) indicated lower soil porosity in the wastewater application and a consequent decrease in water retention and hydraulic conductivity.

In this study, our aim is to investigate the effect of wastewater on the physical and hydraulic properties of soil in tomato plants irrigated with wastewater.

2. Material and methods

The mean elevation of the study area from sea level was 1030 m and the study area is located at latitude 38°53' 01,91" -38°53' 01,52" N and longitude 40°32' 57,82" - 40°32' 56,73" E. The region has a continental climate (Dsa; D: cold continental climate, s: dry summer, a: hot summer), with hot and dry summers and cold and snowy winters considering Köppen classification (Kottek et al., 2006).

Table 1 shows the long-term average climatic data and climatic data for the study year in Bingöl, Turkey, as obtained from the meteorology station. According to the long period (1961-2016) data obtained from the Bingöl Meteorological Station located near the experimental site, the annual mean temperature and total precipitation in the region are 12.1 °C and 943.6 mm, respectively. About 80% of precipitation falls during November-April period (761.1 mm). July (5.6 mm total precipitation and 26.8 °C mean air temperature) and August (3.2 mm total precipitation and 26.4 °C mean air temperature) are the driest and hottest months.

Year	Year Month Temperature Relative W		Wind	Daily	Evaporation	Precipition		
		(°C)	Humudity	speed	Sunshine	(mm)	(mm)	
			(%)	(m/s^{-1})	(h)			
	May	16.3	55.8	1.9	7.31	116.5	75.1	
Long	June	22.1	43.5	2.1	9.40	179.1	20.6	
term	July	26.7	35.9	2.2	9.54	231.2	5.7	
(1960-	August	26.4	35.1	2.1	9.24	221.7	3.3	
2013)	September	21.1	41.0	1.9	8.31	158.2	10.4	
	May	18.7	47.8	0.87	7.20	12	7.0	
	June	22.8	33.3	1.04	8.84	181	6.2	
2013	July	27.2	26.4	1.28	9.39	278	-	
	August	26.4	26.2	1.08	9.19	255	-	
	September	20.3	34.5	0.83	7.95	111	10.9	
	May	16.7	48.9	0.96	6.56	0.95	0.41	
	June	22.0	34.6	1.01	8.58	215	23.4	
2014	July	27.2	25.9	0.95	8.54	216.9	4.88	
	August	27.4	23.4	0.93	8.77	203.5	7.53	
	September	20.9	35.3	0.75	7.86	114	-	

Table 1. The long term average climatic data and climatic data for the study year in Bingöl, Turkey

[&]Includes the vegetation period in May and September of 2013-2014

*The precipitation and evaporation values for 2013 were measured using a rain gauge and an A-class evaporation pan installed in the study area.

A profile was dug to determine the soil properties in the study area and disturbed and undisturbed soil samples were collected from the depths of 0-30, 30-60 and 60- 90 cm of the soil profile, followed by the analyses of the physico, chemical and hydraulic properties of the soil samples (Tables 2).

The Joker-F₁ (Lycopersicon esculentum) variety was used in the study. In the trials, Joker F1 tomato variety was chosen due to the fact that it is a very strong variety that can cover the fruit, it is more resistant to diseases, it has can highly adapt to the region, it yields a hard fruit with a long shelf-life and it is suitable for temporary and long-term harvest. The seedlings were planted in the field on May 20 2013 and on May 31 2014. Prior to planting, plough cultivation was applied, large clods were crushed and the field surface was smoothed to prepare the conditions for planting. The plants were planted in 5 rows with an inter-row spacing of 100 cm and intra-row spacing of 50 cm. Weeding was carried out manually through hoeing three times until the first harvest. Nitrogenous, phosphorous and potash fertilizers were used in fertilization. Prior to planting, compound Diammonium Phosphate (DAP) (20:20:0) was applied in the dose of 50 kg per decare. Equal fertilization to each plant was carried out until reaching a cover level of 30% and irrigation conditions were met; after planting, in each irrigation, the 15:15:15 and 13-0-46 fertilizers were applied in the amount of 10 kg (50 kg in total) using a drip

irrigation system. To control the more cricket population, the Korban-4 insecticide was used after planting. The experiments were carried out using waters of two different qualities in the applications, i.e. freshwater (FW) and treated wastewater (TWW). The plants were drip-irrigated with two different water types, namely recycled wastewater (RW) collected from the Bingöl city wastewater treatment plant and freshwater (FW) obtained from an irrigation network in the region.

Domomotor		Soil layer (cm)	
Parameter	0-30	30-60	60-90	
Texture	Clay	Clay	Clay	
Sand, %	30.2	26.4	27.4	
Silt, %	28.6	29.1	27.9	
Clay, %	41.2	44.5	44.7	
Particle density	2.59	2.60	2.61	
Bulk density, Mg m ⁻³	1.30	1.31	1.36	
Porosity, %	49.8	49.6	47.9	
Wet aggregate stability, %	39.8	52.9	49.2	
Field capacity, % of weight	28.5	30.3	30.8	
Wilting point, % of weight	17.2	18.1	18.4	
AWC, mm	44.1	47.9	50.6	
pН	8.01	7.94	7.92	
EC, dS m^{-1}	0.53	0.51	0.45	
Organic matter, %	1.60	1.30	1.10	
CaCO ₃ , %	4.60	3.40	2.10	

Table 2. The physico, chemical and hydraulic properties of experimental field soil prior to the study

AWC: Available water content; EC: Electrical conductivity

Drip irrigation method was used in irrigation. The irrigation strategies comprised full irrigation (100%), deficit irrigation, which involves the use of 75% and 50% of the water used in full irrigation and partial root drying (PRD). In all applications, waters of two different qualities (clean water and wastewater) were used. In each parcel, 6 lateral pipes were installed in the middle of each plant row pair with a spacing of 100 cm; the irrigation water was applied from all lateral pipes in full and deficit irrigation applications, while the irrigation water was alternately applied in the partial root drying method [from no. 1-3-5 lateral pipes in odd-number irrigations; from no. 2-4-6 lateral pipes in even-number irrigations]. The amounts of irrigation waters were equal in wastewater and clean water applications and adjusted in accordance with the control application in which 100% full irrigation was applied. The irrigations were commenced immediately after planting and finalized on September 29, when the growing season ended.

The proportions of sand, silt, and clay fractions were detected using the Bouyoucos hydrometer method described by Gee and Bauder (1986). The cylinder method was used to determine the soil bulk density, and a pycnometer was used to determine the particle density (Blake and Hartge1986a,b). Soil porosity was calculated from the bulk density and particle density values (Danielson and Sutherland 1986). The wet-sieving method was used to measure the aggregate stability (Kemper and Rosenau 1986). The amount of water retention at field capacity (-33 kPa) and wilting point (-1.5 MPa) were determined with a pressure plate (Cassel and Nielsen 1986). Available water content was calculated as the difference between water retained at field capacity and at the wilting point.

Infiltration measurements were conducted one month after the last harvest on each plot in both experimental years by double-ring infiltrometers under constant freshwater head for 420 minutes. The measurements had also been carried out in the study field prior to the experiment. Infiltration measurement data was analyzed using regression to obtain a Kostiakov model parameter values for each treatment.

The variance analyses were carried out using the SAS software (Anonymous, 2000) and the Duncan multiple comparison test of the Minitab software was used to compare the significant averages (Kesici and Kocabaş, 2007).

3. Results and discussion

The quality of irrigation waters and irrigation quantities

The pH, EC, and TSS values in the waters given in Table 3 were suitable for irrigation considering the standards proposed by the FAO (6.5-8.4 for pH, < 0.7 dS m⁻¹ for EC, and < 50 mg l⁻¹ for TSS) and their quality were in good class (Ayers and Westcot 1985). Although SO₄²⁻(< 200 mg l⁻¹), Na⁺ (< 125 mg l⁻¹) and B (< 1 mg l⁻¹) concentrations in the waters were low according to Turkish national water pollution control regulation (WPCR) standards, recycled wastewater (RW) was the water with increasing problem due to a Cl⁻ concentration of higher than > 25 mg l⁻¹ (WPCR, 2008). Total N (> 5 mg l⁻¹) and P (> 0.65 mg l⁻¹) values in RW were too high. While the Fe (< 0.30 mg l⁻¹), Mn (< 0.10 mg l⁻¹) and Zn (< 0.20 mg l⁻¹) concentrations in RW and freshwater (FW) provided the best quality for irrigation, in RW treatment Cu (0.05-0.20 mg l⁻¹; 2nd-3rd class water), Cd (> 0.01 mg l⁻¹; 4th class water), Ni (0.02-0.05 mg l⁻¹; 1st-2nd class water), Cb (> 0.05 mg l⁻¹; 4th class water), Co (0.02-0.20 mg l⁻¹; 2nd-3rd class water), and Cr (> 0.20 mg l⁻¹; 4th class water) concentrations exhibited lower quality (WPCR 2008). Current Cu, Cd, Ni, Co, and Cr concentrations in FW also lowered irrigation water quality of this type of water. SAR value was low in both water types according to the USSL classification (Kanber and Ünlü, 2010).

D (T T •	2013		2014				
Property	Unit	RW	FW	RW	FW			
рН	-	7.76±0.34	8.15±0.22	7.73±0.24	7.57±0.08			
EC	dS m ⁻¹	0.51±0.02	0.16±0.01	0.42±0.03	0.24±0.03			
TSS	mg 1 ⁻¹	21.4±3.5	20.1±1.2	16.7±3.3	12.1±2.3			
Total N	mg 1 ⁻¹	13.8±0.6	-	12.6±0.5	-			
Total P	mg 1-1	1.94 ± 0.04	-	1.65±0.09	-			
Ca	me l ⁻¹	2.09 ± 0.29	1.10 ± 0.10	1.75±0.37	1.50 ± 0.40			
Mg	me l ⁻¹	1.32 ± 0.28	0.78±0.03	0.89±0.17	0.65 ± 0.06			
Na	me l ⁻¹	1.17 ± 0.44	0.17±0.03	1.47 ± 0.12	0.19 ± 0.01			
K	me l ⁻¹	0.35±0.11	0.05 ± 0.01	0.33±0.02	0.11±0.02			
CO ₃	me l ⁻¹	0.03±0.03	0.01 ± 0.01	-	-			
HCO ₃	me l ⁻¹	0.37±0.07	0.28±0.03	0.35 ± 0.08	0.26 ± 0.06			
SO_4	me l ⁻¹	3.21±0.34	1.35 ± 0.07	2.51±0.43	1.64 ± 0.03			
Cl	me l ⁻¹	1.24 ± 0.41	0.44 ± 0.12	1.50 ± 0.40	0.50 ± 0.26			
В	mg 1 ⁻¹	0.43±0.12	0.25±0.11	0.41±0.13	0.19 ± 0.10			
Fe	mg 1 ⁻¹	0.23±0.11	0.13±0.05	0.12±0.01	0.15 ± 0.01			
Zn	mg 1 ⁻¹	0.06 ± 0.01	0.04 ± 0.00	0.02 ± 0.00	0.01 ± 0.00			
Cu	mg 1 ⁻¹	0.09 ± 0.02	0.05 ± 0.02	-	-			
Mn	mg 1 ⁻¹	0.07 ± 0.03	0.03 ± 0.01	0.04 ± 0.003	0.01 ± 0.00			
Cd	mg 1 ⁻¹	0.06 ± 0.03	0.04 ± 0.02	0.08 ± 0.01	0.07 ± 0.01			
Ni	mg 1 ⁻¹	0.04 ± 0.01	0.04 ± 0.003	0.05 ± 0.01	0.02 ± 0.01			
Pb	mg 1 ⁻¹	0.06 ± 0.02	0.05 ± 0.01	0.04 ± 0.00	0.03 ± 0.00			
Co	mg l ⁻¹	0.19 ± 0.00	0.17±0.003	0.19 ± 0.01	0.15 ± 0.02			
Cr	mg 1 ⁻¹	0.41±0.03	0.34 ± 0.05	0.34±0.04	0.29 ± 0.04			
SAR	-	0.95 ± 0.42	0.18 ± 0.04	1.32±0.19	0.19 ± 0.01			

Table 3 I	Physical	and chemical	properti	as values	(mean+SEM)) of the wate	rs used in irrigation
Table 3. I	nysicar	and chemical	properu	es values	(mean±SEW)) of the wate	is used in migation

SEM: Standard error of the mean; RW: Recycled wastewater; FW: Freshwater; EC: Electrical conductivity; TSS: Total suspended solids; SAR: Sodium adsorption ratio

Table 4 shows the amounts of monthly irrigation water applied in the irrigation practices. Equal amounts of irrigation water were applied in the RW and FW treatments in each irrigation practice. A total of 23 irrigations were applied in both years. Seasonal irrigation quantities in full irrigation (FI) practice were

640.2 mm in 2013 and 648.1 mm in 2014. Considering the two-year average data, while Deficit Irrigation-I (DI25) and Partial Root-zone Drying Irrigation-I (PRD25) practices were irrigated with 23.3% less irrigation water when compared with the FI practice, Deficit Irrigation-II (DI50) and Partial Root-zone Drying Irrigation-II (PRD50) practices received 46.5% less water. Deep percolation below root zone from precipitation and irrigation quantities during the growing periods in both years did not occur. Reduced irrigation quantities provided less soil moisture contents in the DI and the PRD practices compared with the FI practice. However, soil moisture content did not reduce below the wilting point in either practice. Soil moisture content had the most decreased amount in the PRD50 practice between all of the practices.

Year	Practice	Month								
		May	June	July	August	September	Total			
2013	FI	10.6	37	219	207.6	166.1	640.2			
	DI25	10.6	34.2	164.3	155.7	124.6	489.3			
	DI50	10.6	31.4	109.5	103.8	83.1	338.3			
	PRD25	10.6	34.2	164.3	155.7	124.6	489.3			
	PRD50	10.6	31.4	109.5	103.8	83.1	338.3			
2014	FI	4	33.2	141.6	256	213.3	648.1			
	DI25	4	33.2	110.3	192	160	499.5			
	DI50	4	33.2	79.1	128	106.7	350.9			
	PRD25	4	33.2	110.3	192	160	499.5			
	PRD50	4	33.2	79.1	128	106.7	350.9			

Table 4. Monthly irrigation quantities applied to irrigation practices in experiment years (mm)

FI: Full irrigation, DI25 and DI50: 25 and 50% deficit irrigation, PRD25 and PRD50: 25 and 50% deficit irrigation with PRD technique

Soil physical properties

Bulk density, particle density, porosity, and wet aggregate stability values were determined as the soil physical properties in three soil layers under different irrigation water types and irrigation practice conditions and they were found statistically similar in 2013 and 2014. The figures 1-4 show combined results of experiment years for bulk density, particle density, porosity, and wet aggregate stability, respectively. As shown in these figures, soil physical properties did not change statistically with the irrigation water types and the irrigation practices in soil layers of 30-60 and 60-90 cm. In the 0-30 cm soil layer, while RW irrigation significantly increased the wet aggregate stability by 3.8% compared with the FW irrigation, particle density was decreased by 0.4% (Figs. 2 and 4). Particle density in 0-30 cm soil layer was also significantly reduced by 1.1% in the DI50 practice compared with the FI practice (Fig. 2). Although there was a significant difference between DI25 practice with the highest bulk density value and PRD50 practice with the lowest bulk density value, the values obtained in both practices were statistically similar to the value in the FI practice (Fig. 1). The DI25 practice, which had the highest bulk and particle density values in 0-30 cm soil layer, lowered porosity and also had a lower value than the FI practice (Fig. 3).



Fig. 1 The bulk density values as two-year average in three soil layers in different irrigation practices and water types. The mean values marked with different letters are significantly different (*: P < 0.05). FI: Full irrigation, DI25 and DI50: 25 and 50% deficit irrigation, PRD25 and PRD50: 25 and 50% deficit irrigation with PRD technique, RW: Recycled wastewater, FW: Freshwater



Fig. 2 The particle density values as two-year average in three soil layers in different irrigation practices and water types. The mean values marked with different letters are significantly different (*: P < 0.05). Explanations of the abbreviations are as shown in Fig 1



Fig. 3 The porosity values as two-year average in three soil layers in different irrigation practices and water types. Explanations of the abbreviations are as shown in Fig 1



Fig. 4 The wet aggregate stability values as two-year average in three soil layers in different irrigation practices and water types. The mean values marked with different letters are significantly different (**: P < 0.01). Explanations of the abbreviations are as shown in Fig 1

The bulk density values in 0-30 cm soil layer under the DI25, DI50, and PRD25 practices irrigated with RW were higher than subsoil layers. Although the bulk density values for all practices in 0-30 and 30-60 cm soil layers irrigated with both water types were near the values of pre-planting in Table 1, the values in 60-90 cm soil layer after experiments were about 5% lower. Particle density values in 60-90 cm soil layer for all practices in both water types were lower than the values in topsoil layers (0-30 and 30-60 cm) (Fig 2). The particle density values for all practices at the end of the experiment in three soil layers under RW and FW irrigation conditions were generally higher than the initial values (Table 2).

Particle densities in 0-30 and 30-60 cm soil layers resulted in about a 2% increase. In 60-90 cm soil layer, increase rates were less. The porosity values in three soil layers irrigated with both water types observed after the experiment were higher than the initial values (Table 2). The PRD50 practice generally had the highest values in all soil layers (Fig. 3). The porosity values obtained in the PRD50 practice in 0-30, 30-60 and 60-90 cm soil layers irrigated with RW were by 2.2, 3.2, and 6.9% higher than the initial values, respectively. Considering the initial wet aggregate stability values in Table 1, remarkable improvements in 0-30 and 60-90 cm soil layers were observed in the RW treatment. Wet aggregate stability in the surface soil layer (0-30 cm) was the highest in the plots fully irrigated with RW. In 30-60 and 60-90 cm soil layers, the PRD25 practice that was irrigated with wastewater provided the highest values as 5.4 and 10.0%, which are also higher values compared with the FI practice, respectively (Fig. 4). However, PRD25 treatment had the lowest value in the 0-30 cm soil layer under the wastewater irrigation conditions. The wet aggregate stability in the PRD25 practice was by 11.5% lower than the value in FI practice. This finding could be associated with the ESP value in Table 5. Compared with the other practices, ESP value in the PRD25 practice was high in 0-30 cm soil layer and low in 30-60 and 60-90 cm soil layers. The increase of ESP may importantly decrease the aggregate stability (García-Orenes et al., 2005; Schacht and Marschner, 2015).

Our results indicated that the soil salinity-sodicity was too low and non-significant changes for the OM in soil under different water types and practices were observed, and solid material content in the waters was too low as well $(12.1-21.4 \text{ mg } l^{-1})$ (Table 3). Moreover, the wetting-drying cycles due to different irrigation practices and the changes in rooting volume may affect the soil physical properties (Gould et al., 2016; Wang et al., 2015). Significant findings for bulk density, particle density, and porosity in different water types and irrigation practices could not be determined due to many reasons that are mentioned above. The particle density depends on soil mineral composition and the degree of organic matter decomposition (Rühlmann et al., 2006). Although soil OM content observed after harvesting was higher than the initial values, there was no significant difference in soil OM content under the conditions irrigated with both water resources (Table 5). Moreover, the amount of suspended solids in both water types was not considerable (Table 2). Therefore, it could be said that a very low difference in particle density between the RW and FW may be a result of less decomposition of organic matter only. Some researchers showed that irrigation with wastewater had non-significant effects on soil physical properties. Goncalves et al. (2010) and Tunc and Sahin (2015) observed that the variation of soil bulk density was insignificant in the wastewater and freshwater applied plots. Magesan (2001) expressed that treated wastewater irrigation did not change the bulk density, particle density, and porosity. Many studies have shown that wastewater irrigation improved the porosity and bulk density because of the presence of higher soil OM compared with freshwater irrigation conditions (Beigi and Banitalebi, 2013; Biswas et al., 2017; Mojiri, 2011; Mojid and Wyseure, 2013; Vogeler, 2009). However, some researchers found the opposite results for bulk density and porosity due to dispersion and sedimentation under the wastewater irrigation conditions (Abedi-Koupai et al., 2006; Aiello et al., 2007; Coppola et al., 2004). Higher wet aggregate stability under RW treatment could be explained by lower CaCO₃ content in 0-30 cm soil layer, because dissolved $CaCO_3$ in the soil under the wastewater application conditions may be induced to higher aggregation. Tayel et al. (2010) also determined a significant positive correlation between the soil aggregation and the $CaCO_3$ content in the soil. Furthermore, Ca and Mg cations in wastewater, by way of cationic bridging between clay and organic matter, could increase the formation of micro aggregates that cause aggregation (Bronick and Lal, 2005;Durán-Álvarez and Jiménez–Cisneros, 2014).

Soil	Parameter	FI		DI25		DI50		PRD25		PRD50		General	nean					
layer		RW	FW	RW	FW	RW	FW	RW	FW	RW	FW	RW	FW	FI	DI25	DI50	PRD25	PRD50
0-30	pН	7.85 ± 0.07	7.83 ± 0.07	7.82±0.06	7.77 ± 0.07	7.85 ± 0.06	7.94 ± 0.04	7.86 ± 0.05	7.91±0.03	7.87 ± 0.05	7.89 ± 0.07	7.85	7.87	7.84	7.80	7.89	7.88	7.88
	EC, dS m ⁻¹	$0.77 {\pm} 0.04$	0.70 ± 0.03	0.63 ± 0.04	$0.64{\pm}0.03$	0.56 ± 0.02	0.58 ± 0.03	0.65 ± 0.04	$0.64{\pm}0.05$	0.62 ± 0.01	0.56 ± 0.03	0.65	0.62	0.74a**	0.63bc	0.57c	0.65b	0.59bc
	OM, %	2.41 ± 0.13	2.32 ± 0.13	2.28±0.16	$2.24{\pm}0.14$	$2.20{\pm}0.16$	2.15 ± 0.21	2.33±0.14	$2.24{\pm}0.16$	2.19 ± 0.17	2.12 ± 0.10	2.28	2.21	2.36	2.26	2.18	2.29	2.16
	CaCO ₃ , %	3.73 ± 0.27	4.27 ± 0.18	3.89±0.29	4.31±0.20	4.19 ± 0.18	3.81±0.26	3.74 ± 0.18	4.24 ± 0.20	3.54 ± 0.26	3.92 ± 0.16	3.82b*	4.11a	4.00	4.10	4.00	3.99	3.73
	CEC, cmol kg ⁻¹	$32.5{\scriptstyle\pm1.2}$	$34.5{\scriptstyle\pm0.9}$	31.4±1.4	33.6 ± 0.3	31.2±1.6	33.4±1.0	35.5±1.3	33.5±1.0	31.5±1.5	32.1 ± 0.4	32.4	33.4	33.5	32.5	32.3	34.5	31.8
	ESP, %	$2.20{\pm}0.10$	$1.68{\pm}0.09$	2.03±0.15	$1.60{\pm}0.08$	1.81 ± 0.11	1.73±0.23	1.94 ± 0.17	1.86 ± 0.20	1.76 ± 0.17	1.77 ± 0.14	1.95a*	1.73b	1.94	1.82	1.77	1.90	1.77
30-60	pH	7.85 ± 0.04	$7.90{\pm}0.03$	7.78 ± 0.04	$7.82{\pm}0.04$	7.81±0.03	7.91 ± 0.04	7.92 ± 0.04	7.88 ± 0.03	7.90±0.03	$7.94{\pm}0.03$	7.85	7.89	7.87ab*	7.80b	7.86ab	7.90a	7.92a
	EC, dS m ⁻¹	$0.77{\pm}0.05$	0.63 ± 0.04	0.67 ± 0.05	$0.59{\pm}0.05$	0.70 ± 0.04	0.49 ± 0.02	$0.70{\pm}0.05$	$0.60{\pm}0.02$	0.64 ± 0.05	$0.56{\pm}0.02$	0.70a**	0.57b	0.70	0.63	0.59	0.65	0.60
	OM, %	$2.38{\pm}0.20$	$2.29{\pm}0.15$	2.23±0.15	$2.17{\pm}0.14$	2.12 ± 0.06	2.09 ± 0.26	2.21±0.15	$2.06{\pm}0.12$	2.16±0.13	$2.09{\pm}0.10$	2.22	2.14	2.34	2.20	2.10	2.14	2.12
	CaCO ₃ , %	4.11 ± 0.43	4.12 ± 0.32	3.66±0.21	4.14 ± 0.17	3.99 ± 0.18	3.98 ± 0.16	3.77±0.16	$3.98{\pm}0.21$	3.68 ± 0.23	3.82 ± 0.15	3.84	4.01	4.12	3.90	3.99	3.87	3.75
	CEC, cmol kg ⁻¹	$35.4 {\pm} 0.9$	32.6 ± 1.0	32.2±0.6	$33.4 {\pm} 0.7$	33.4±1.0	32.8±1.0	35.5±1.1	34.4±1.2	34.5±1.2	33.4 ± 1.0	34.2	33.3	34.0	32.8	33.1	34.9	34.0
	ESP, %	$2.09{\pm}0.14$	$2.01{\pm}0.18$	1.99±0.13	1.77 ± 0.11	2.06±0.12	1.65 ± 0.12	1.85 ± 0.09	1.67 ± 0.09	1.96±0.14	1.87 ± 0.10	1.99a*	1.79b	2.05	1.88	1.86	1.76	1.92
60-90	pH	7.87 ± 0.05	7.85 ± 0.06	7.92±0.04	7.92±0.03	7.89±0.06	7.91±0.02	7.86±0.04	7.91±0.03	7.91±0.03	$7.84{\pm}0.05$	7.89	7.88	7.86	7.92	7.90	7.88	7.87
	EC, dS m ⁻¹	$0.64{\pm}0.06$	0.62 ± 0.03	0.60 ± 0.02	$0.58{\pm}0.03$	$0.52{\pm}0.02$	0.57 ± 0.03	0.63 ± 0.03	$0.58{\pm}0.04$	0.56 ± 0.05	$0.53{\pm}0.03$	0.59	0.58	0.63	0.59	0.54	0.61	0.54
	OM, %	$2.39{\pm}0.20$	2.21 ± 0.12	2.01 ± 0.14	$2.0 {\pm} 0.07$	1.95 ± 0.12	1.88 ± 0.09	$1.90{\pm}0.17$	$1.87{\pm}0.16$	1.80 ± 0.15	$1.95{\pm}0.09$	2.01	1.98	2.30a**	2.00b	1.92b	1.89b	1.87b
	CaCO ₃ , %	$3.89{\pm}0.42$	4.60 ± 0.22	4.30±0.33	4.21 ± 0.35	3.71±0.13	4.21±0.23	4.45 ± 0.24	4.52 ± 0.18	3.66±0.15	$3.93{\pm}0.22$	4.00	4.30	4.24	4.26	3.96	4.48	3.80
	CEC, cmol kg ⁻¹	$34.5{\scriptstyle\pm1.2}$	33.6±1.14	35.1±1.0	$33.6 {\pm} 0.9$	33.5±1.0	32.2±0.4	35.8±0.9	34.4±0.5	37.3±1.1	32.7 ± 0.6	35.2a**	33.3b	34.0ab*	34.3ab	32.9b	35.1a	35.0a
	ESP, %	2.08 ± 0.12	$1.90{\pm}0.05$	1.92 ± 0.14	$1.88{\pm}0.10$	1.99 ± 0.20	1.88 ± 0.14	1.70 ± 0.16	1.71±0.15	$1.59{\pm}0.08$	1.93 ± 0.07	1.85	1.86	1.99	1.90	1.93	1.70	1.76

Table 5. pH, electrical conductivity (EC), organic matter (OM), CaCO₃, cation exchange capacity (CEC), and exchangeable sodium percentage (ESP) values (mean±SEM) in three different layers (0-30, 30-60, and 60-90 cm) of the soil irrigated with recycled water (RW) and freshwater (FW) under different irrigation practices in 2013-14

The values marked with the same letters for different water qualities or irrigation practices in each line of the "general mean" column of each soil layer are not significantly different (**: P<0.01 or *: P<0.05). SEM: Standard error of the mean; FI: Full irrigation, DI25 and DI50: 25 and 50% deficit irrigation, PRD25 and PRD50: 25 and 50% deficit irrigation with PRD technique

Soil hydraulic properties

Field capacity, wilting point, and available water content

The results obtained for field capacity (water retained at -33 kPa), wilting point (water retained at -1.5 MPa) and available water content were found statistically similar in both trial years. Considering the average values of two-years, generally, there were no significant changes in these parameters under different water types and practices in surface and subsoil layers. Field capacity values in two-year average data for 0-30 and 30-60 cm soil layers in the FW treatment were higher than RW treatment values (Fig. 5). Only the change in 60-90 cm soil layer was important, and the field capacity value in 60-90 cm soil layer was found 31.1% for FW and 31.5% for RW. The lowest field capacity values were determined in surface soil layer compared with sub soil layers. Field capacity values in three soil layers under different water types and practices were higher than the initial values in Table 2. Compared with the initial values, the increase rates in the RW and FW treatments were 8.4 and 8.8% in 0-30 cm soil layer, 2.6 and 3.0% in 30-60 cm soil layer, and 2.3 and 1.0% in 60-90 cm soil layer, respectively. The field capacity values were observed higher by 9.1, 4.3, and 3.6% in 0-30, 30-60 and 60-90 cm soil layers, respectively in the fully irrigated RW treatment compared to pre-planting values. Similarly, Darvishi (2014) determined that the field capacity was higher in both domestic and agronomical water irrigation conditions compared with the pre-irrigation value, and was 11% higher than the field capacity under the irrigation with domestic wastewater. Tunc and Sahin (2015) found higher field capacity values in two soil layers of 0-20 and 20-40 cm of the soils irrigated with wastewater compared with pre-irrigation. Gharaibeh et al. (2007) also found that the field capacity increased in the top and sub-layers of the soil irrigated with wastewater according to the initial values. Soil organic matter enhances useful water retention for plants due to the improvement it makes in porosity and aggregation (Huntington, 2005). As a result, observation of higher water retention at the field capacity in the soil after the experiment in our study could be explained by higher OM content compared with pre-irrigation practices (Tables 2 and 5). Reichert et al. (2009) confirmed that organic matter increased water retention at the field capacity.



Fig. 5 The field capacity values as two-year average in three soil layers in different irrigation practices and water types. The mean values marked with different letters are significantly different (*: P < 0.05). Explanations of the abbreviations are as shown in Fig 1



Fig. 6 The wilting point values as two-year average in three soil layers in different irrigation practices and water types. The mean values marked with different letters are significantly different (**: P < 0.01). Explanations of the abbreviations are as shown in Fig 1

The RW and FW treatments had a similar effect on the soil moisture retained at the wilting point in three soil layers (Fig. 6). The highest values were observed in the bottom soil layer in both water sources. After two experimental years, wilting point values in the surface soil layer were higher than the initial values in Table 1 by 5.8 and 4.7% in RW and FW treatments, respectively. Although the irrigation practices had nosignificant effect on wilting point in 0-30 cm and 60-90 cm soil layers, the DI50 and PRD50 practices in 30-60 cm soil layer provided lower values compared with the DI25, PRD25, and FI practices (Fig. 6). Moreover, these values were lower than the initial values (Table 2). Compared with pre-planting, the decreases were 2.8% for DI50 and 3.9% for PRD50 practice considering the average values for both water types. The DI and PRD practices with 50% water shortage showed lower values in the other soil layers as well. This phenomenon could be explained by the fact that soil microporosity may be reduced under lesser soil wetting (Tunc and Sahin, 2015). Generally, RW treatments in all practices caused higher wilting point values compared with FW treatment. Narrowing in soil pores because of the accumulation of both the suspended solids in the RW and the soil particles dispersed due to the higher ESP in the RW irrigation may be the reasons for this finding. Similarly, Al-Othman (2009) determined that the wilting point significantly increased in a loamy sand soil irrigated with treated domestic wastewater.

Available water contents calculated from the differences in water contents between field capacity and wilting point in three soil layers were not affected by different water types and practices (Fig. 7). The values did not change with soil depth either. Soil total porosity and pore size distribution are the important factors that affect the water retention. Our results showed that total porosity values were not affected by different water qualities and practices (Fig 3). Therefore, this confirmed the non-significant changes in available water content. Available water content in 0-30 and 30-60 cm soil layers under different water types and practices were higher than the initial values (Table 2). The RW and FW treatments increased available water content by 12.9 and 15.0% in 0-30 cm soil layer, and by 5.0 and

7.3% in 30-60 cm soil layer, respectively. When compared with the pre-planting values, the available water content under the FI, DI25, PRD25, DI50, and PRD50 practices in surface soil layer were higher by 12.5, 16.6, 13.8, 13.2, and 13.2%, respectively considering the average values for two water types. The increases in 30-60 cm soil layer were also by 7.1, 4.4, 7.3, 3.5, and 9.0%, respectively. Gharaibeh et al. (2007) determined higher available water contents than initial values in top and subsoil layers of the soil irrigated with wastewater. It could be expressed that considering the pre-planting OM values in our study, the increase of the OM content after the experiments resulted in an increase of available water content. Reichert et al. (2009) declared that the available water content had a positive correlation with the soil organic matter. Biswas et al., (2017) and Rawls et al. (2003) observed that soil organic matter increased water retention.



Fig. 7 The available water content values as two-year average in three soil layers in different irrigation practices and water types. Explanations of the abbreviations are as shown in Fig 1

Infiltration

The changes in infiltration rate expressed with equations based on the Kostiakov model were statistically similar in both experiment years. Considering the two-year average values, the infiltration rates observed in the RW and FW treatments decreased throughout the measuring time of 420 min., and while the PRD50 practices had higher values, the least values were obtained in the FI practice (Fig. 8). The fact that no difference in mean infiltration rates at the end of measurement time of 420 minutes was observed under any application indicated that neither the water qualities nor the irrigation practices had an effect on the mean infiltration rates (Fig. 9). The stable infiltration rate values in the RW treatment at the end of 420 minutes measurement were significantly lower than the values of FW treatment (Fig. 9). Decrease level was 6% compared with the FW value. Considering the pre-planting values of the first experiment year (22.3 mm h⁻¹), stable infiltration rates that were measured under the RW and FW treatments after the experiment secue for the treatment that was fully irrigated with the RW were higher than the initial value (46.9 mm h⁻¹). The highest increase rate was determined to be 13.7% in the PRD50 practice under both of the water sources (Fig. 9). Pore size distribution affects the water infiltration and percolation in the soil. Hence, relatively high infiltration rates in the PRD50 could be explained by a partial increase

in macroporosity due to the wetting-drying process in the PRD practices that may affect macroporosity (Bodner at al., 2013). Lado and Ben-Hur (2009) pointed out that both wastewater and soil properties have a combined impact on soil hydraulic properties. In our study, the soil ESP values in the RW treatment in surface soil layers were higher than the values in the FW (Table 5). Moreover, the wastewater used in the present study included TSS and Na at higher concentrations compared with FW (Table 3). The accumulation of dispersed clay and suspended solid particles in the soil pores due to the effects of these factors could decrease stable infiltration rate under wastewater irrigation. Adhikari et al. (2012) reported that water movement in soil may decrease due to clay dispersion and suspended solid accumulation in the soil pores in the saline/sodic wastewater treatments. Similarly, Gharaibeh et al. (2007) expressed that higher Na caused dispersion and so water conductivity was negatively affected, and they observed lower infiltration rates in soils irrigated with wastewater for two to five years. Mollahoseini (2013) found that saturated water movement in upper soil layers decreased significantly due to the filling of suspended particles in wastewater within the soil porosity. Assouline and Narkis (2011) determined lesser infiltration rates in the long-term wastewater-irrigated soils compared with freshwater. Bedbabis et al. (2014) observed significant decreases in infiltration rate under the wastewater treatment conditions at the end of four years. Alrajhi et al. (2017) found that the different irrigation practices (FI, DI25, DI50, PRD25, and PRD50) and water sources did not cause significant differences in the water conductivity.



Fig. 8 The infiltration rate values versus time as two-year average in different irrigation practices and water types. Explanations of the abbreviations are as shown in Fig 1



Fig. 9 The stable and mean infiltration rate values as two-year average in different irrigation practices and water types. The mean values marked with different letters are significantly different (*: P < 0.05). Explanations of the abbreviations are as shown in Fig 1

Conclusions

In this study conducted in Bingöl province conditions, it was seen that treated wastewater caused changes in physical, chemical and hydraulic properties of soils, but these changes were at levels that could be controlled and safe in the short term.

The findings showed that different water types and irrigation practices had slight effects on soil physical and hydraulic properties. Wet aggregate stability was highly improved in the plots fully irrigated with recycled wastewater.

It is concluded that soil physical and hydraulic properties with the PRD practices under the RW treatment were preserved and even slightly improved compared with the initial values.

In this study carried out for two years, where pressure on clean water resources is increasing for supply the irrigation water in today's conditions that it is concluded that treated wastewater can be used to irrigate tomato plant.

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Contribution to the flora of Murmansk Region (Russia): a new species of *Veronica* (Plantaginaceae)

Ilona V. Blinova

Polar-Alpine Botanical Garden-Institute, Kola Research Centre of Russian Academy of Sciences, Kirovsk, Murmansk Region, Russia. Corresponding author: <u>ilbli@yahoo.com</u>

Abstract

Twelve species of *Veronica* (Plantaginaceae) are recorded from Murmansk Region (Russia). We report the first localities (N $67^{\circ}35'42'' \ge 33^{\circ}24'50''$, $67^{\circ}16'58'' 32^{\circ}27'56''$) of *Veronica beccabunga* for the region. The species distribution range in Europe extends to nearly $65^{\circ}N$. The first find of *V. beccabunga* in *Lapponia Imandrae* in Russia is briefly described, and ecological data about its habitat are given. A dispersal route of this species from its northern range in Europe to the new isolated locality is suggested. The spread success could be explained by regional climatic changes along with the high degree of seed and vegetative survival of *V. beccabunga*. It is proposed to include this species in the next regional Red Data Book in the group "In need of monitoring".

Keywords: Veronica, Plantaginaceae, Murmansk Region, flora, new record

Introduction

Murmansk Region is one of the best studied in Russia botanically. The most important treatments were published in five volumes in 1953–1966 with descriptions of 1162 vascular plant species (Gorodkov 1953–1954, Pojarkova 1956–1966). Since then any additions, especially of native plants, have been made one at a time. The species total has increased mainly through alien species and various taxonomic resolutions, and it has reached 1336 species (Kostina and Filimonova 2009). The most recent, but uncompleted, work on the flora of Murmansk Region was been conducted in 2010: the list of vascular plants of Murmansk Region included 1372 species (Kostina et al. 2010, unpubl.).

Eight species of *Veronica* were included in the Flora of Murmansk Region (Russia): *V. alpina* L., *V. chamaedrys* L., *V. fruticans* Jacq., *V. longifolia* L., *V. officinalis* L, , *V. scutellata* L., *V. serpyllifolia* L., *V. tenella* All. (Kuzeneva 1966). Recently *V. tenella* has been united with *V. serpyllifolia*. Three records of alien *Veronicas* were made relatively recently: *V. gentianoides* Vahl was collected in 2010 by Gryaznova (Kozhin 2013) and in 2011 by Kostina (Borovichev et al. 2013), *V. arvensis* L. was found in 2010 by Kravchenko (Kravchenko 2011), and *V. persica* Poir. is recorded by Kostina (Kostina et al. 2010, unpubl.). One native species *V. anagallis-aquatica* L. was included in the "The list of vascular plants of Murmansk Region" (Kostina et al. 2010, unpubl.). In 2019 during preparation of this work no herbarium sheet - neither *V. gentianoides*, *V. arvensis & V. persica* nor *V. anagallis-aquatica* - was found in the public collection of KPABG. However, these herbarium specimens might be found in an unsorted

collection made by Kostina. It means that in 2019 the total number of *Veronicas* in Murmansk Region might be 12 including the newly discovered *Veronica beccabunga* L.

V. beccabunga is a herbaceous plant with succulent leaves belonging to the family Plantaginaceae. The shoot formation is monopodial long-shoot with plagiotropic shoots (Savinykh 2003). The species has a Eurasian range (Hultén and Fries 1986) (Figure 1), and it occurs widely in Russia (Borissova 1955, Elenevsky 1978). In the north it is found mainly in the middle taiga at the latitudes of Petrozavodsk and Medvezhyegorsk (Kravchenko 2007). The group of aquatic *Veronicas* is extremely polymorphic in the section *Beccabunga* (Hill) Dumort, and within *V. beccabunga* three subspecies have been distinguished: *V. beccabunga* ssp. *abscondita, V. beccabunga* ssp. *beccabunga, V. beccabunga* ssp. *muscosa* (Fischer 1985, Ellmouni et al. 2018).



Figure 1. The localities of *V. beccabunga* near the town of Apatity and in the Kandalaksha District (Murmansk Region, Russia). The new sites are indicated by shaded squares. Top right: part of the map in Hultén and Fries (1986) showing the Eurasian range of *V. beccabunga*. Murmansk Region is outlined with a black rectangle in this map.

V. beccabunga is included in the European Red List of species (Bilz and al. 2011) and in the Red Data Book of Eastern Fennoscandia and Finland (Kotiranta and al. 1998, Rassi and al. 2010). However, it was excluded from the Red Data Book of Karelia (Ivanter and Kuznetsov 2007), and is not listed in the Norwegian Red List (Kålås and al. 2010).

V. beccabunga grows in wet habitats. By spreading vegetatively, it can form carpet-like stands. In Karelia, the species occurs on swampy banks, in swamps around springs, and in ditches (Ramenskaja 1983, Kravchenko 2007).

The census of biodiversity is especially important in the regions where many plant species grow at the limits of their ranges because theses natural borders are very influenced by climatic changes. One such area is Murmansk Region (Russia) in the extreme north of Europe. The aim of the current study was to present the first discovery of *V. beccabunga* in the region and to give an overview of its isolated position in the north of its range.

Material and Methods

Study region. Murmansk Region (66–70° N) is situated in the Atlantic-Arctic climatic zone of the temperate belt, and climatically the region is very heterogeneous. Two latitudinal vegetation zones can be distinguished: tundra and taiga. The average annual temperature in Murmansk Region has been -0.32 °C in the reference period 1981–2010 (with extreme years of -3.3 °C and 1.4 °C). Since 2000 there has been a tendency to earlier onset and mainly to later end of the growing season. The actual length of the growing season reached 120 days on average, from 30 May to 27 September (Blinova and Chmielewski 2016).

Field work and lab studies. Vegetative and generative individuals of *V. beccabunga* were photographed in the field. Additional photos were made in order to show the habitat of the population. Some individuals were collected to prepare herbarium specimens. The following characteristics were studied in the lab: leaf shape, presence/absence of petiole, length of peduncle, length of pedicels, and number of flowers per inflorescence. Herbarium collections of *Veronicas* in KPABG and literature data were analysed.

Nomenclature. Species names are given in accordance with The Plant List (2010).

Results and Discussion

In July 2016 the species was found in the center of Murmansk Region (Russia) about 3.3 km north-northeast of Apatity (N 67°35′42″ E 33°24′50″). It was observed by the right bank of the River Belaya in the South Khibiny Mts which flows into Belaya Bay of Lake Imandra (Figure 1). The banks here had a rather heterogeneous vegetation. *Phalaris arundinaceae* stand prevailed on relatively high and dry sites, whereas hydrophytes were frequent in depressions (Figure 2). *V. beccabunga* was found in one such a water-logged area adjacent to the river. It was locally abundant. *Epilobium ciliatum, Myosotis scorpioides* and some grasses occurred sparsely in this habitat. Two voucher specimens were collected by Blinova on 27.07.2016 helped by Kirillova and deposited in the Herbarium of the Polar-Alpine Botanical Garden-Institute (KPABG) (Figure 3).

At the end of July of 2016 individuals of *V. beccabunga* were in late-flowering stage (Figure 4). The greater part of the inflorescences already had unripe fruits. The study material had the typical characters of *V. beccabunga* ssp. *beccabunga* (Ellmouni et al. 2018): leaves obovate or broad elliptic with clear petiole, and not longer than the subtending leaf; pedicels subpatent, up to 10 mm; number of flowers 16–20 (Figure 3 and 4). The identification was confirmed by Ellmouni and Albach (27.05.2019). Regarding the peduncle our study shows that on the apical half of shoot it is indeed less than 30 mm long, whereas on the basal half of the shoot, especially submerged in water, the peduncles are more elongated.

The two northernmost records of *V. beccabunga* in Karelia are located in the biogeographic province *Karelia pomorica occidentalis*: Sosnovets N 64°25′24″ E 34°28′24″ and Zolotets N 64°29′24″ E
$34^{\circ}39'01''$ (Kravchenko 2007). Three herbarium records at similar latitudes are known from Finland: two from municipality Hyrynsalmi (N $64^{\circ}45'23'' \ge 28^{\circ}37'14''$, N $64^{\circ}45'23'' \ge 28^{\circ}21'43''$) and one near the city of Oulu – N $65^{\circ}1'23'' \ge 25^{\circ}24'19''$ (Lampinen and Lahti 2018). One herbarium specimen was collected in Norway in the county Røyrvik of the province Nord-Trøndelag – N $64^{\circ}54'46'' \ge 13^{\circ}34'24''$ (NTNU University Museum 2019). All these records are situated near latitudes $64-65^{\circ}$, and none is located north of the Arctic Circle N $66^{\circ}33'44''$. Thus, the Apatity' record of *V. beccabunga* in *Lapponia Imandrae* (N $67^{\circ}35'42'' \ge 33^{\circ}24'50''$) in Russia is the northernmost for Europe.



Figure 2. River Belaya at the first site with *V. beccabunga* near Apatity (Murmansk Region, Russia) in larger image, and habitat of this species on its right bank in smaller image. The white line indicates the boundary of the local population. Individuals form a rather dense population in the water-logged area. Blinova, 27 July 2016.

Another interesting record was found during studies of herbarium specimens of *Veronica* in KPABG in 2019. A new specimen of *V. beccabunga* was deposited in KPABG recently. It was collected and identified by Kirillova in July 2018. This site comes from surroundings of the settlement of Nivskij in Kandalasksha district ($67^{\circ}16'58'' 32^{\circ}27'56''$). It was found in a spring flowing into the River Niva about 300 meters north of Lake Plesozero. As indicated on the sheet label the plants were growing on the sandy bottom of this spring at 20 cm depth. This was the second find of *V. beccabunga* in Murmansk Region, and it was situated about 52 km south of the first find.

From present climatic trends it has been proposed that enrichment of the northern taiga by southern species is possible (Blinova and Chmielewski 2016). In recent decades *V. beccabunga* has actively extended its range northwards along the White Sea-Baltic Canal in Karelia (Kravchenko and Kuznetsov 2009), and it is no longer red-listed in Karelia (Ivanter & Kuznetsov 2007). The second find of *V. beccabunga* in Murmansk Region from a spring by the River Niva proves the species distribution channel through the White Sea, and it explains the origin of the first find of this species in the town of Apatity from the River Belaya. The River Niva flows from Lake Imandra into the White Sea, and its chemical composition is determined greatly by the Lake (Cherepanova 2015). The River Belaya flows into Lake

Imandra. It means that a dispersal route of *V. beccabunga* from Karelia into Murmansk Region could be – White Sea-Baltic Canal in Karelia – the Kandalaksha shore of the White Sea in Murmansk Region – River Niva – Lake Imandra – River Belaya.



Figure 3. Two herbarium specimens of *V. beccabunga* ssp. *beccabunga* collected by Blinova on 27 July 2016 and deposited at KPABG in 2017.



Figure 4. Vegetative shoot and inflorescence of flowering shoot of *V. beccabunga* on the right bank of the River Belaya in the South Khibiny Mts. Blinova, 27 July 2016.

The success of such effective spread is apparently linked with a warmer and an extended growing period along with high seed vigor and various means of dispersal of this species. *V. beccabunga* shows one of the highest rates of seed viability and seed germination among other aquatic species (van Leeuwen et al. 2014). Besides, this species is commonly dispersed by plant fragments dislodged during periods of high water flow (Les and Stuckey 1985). Another revealed way of secondary dissemination of *V. beccabunga* could be by piscivorous birds (van Leeuwen et al. 2017).

Of the IUCN-criteria *V. beccabunga* could be considered as "Critically Endangered" for Murmansk Region because of its small population size and the number of occurrences. However, based on the fact that the species is a good colonizer in various parts of its distribution area and it is a new regional find, the category "In need of monitoring" is more appropriate for the next Red Data Book of Murmansk Region. The same category is proposed for another species recently found in Murmansk Region – *Eleocharis mamillata* - with a similar strategy (Blinova and Gregor 2016). Additional field research is required to study the northernmost populations of this species. Furthermore, a detailed exploration of the hydrological system of Lake Imandra is necessary, in the course of which some new populations of this species may be discovered and the actual status of *V. beccabunga* in the extreme north of Europe reassessed.

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The Antioxidant potential of ethanolic extract of edible mushroom *Lycoperdon molle* Pers. (Agaricomycetes)

Celal BAL^{1,*}, Hasan AKGÜL², Mustafa SEVİNDİK³

 ^{1,*} Oguzeli Vocational School, University of Gaziantep 27910 Gaziantep, Turkey
 ² Department of Biology, Faculty of Science, University of Akdeniz 07070 Antalya, Turkey
 ³ Department of Food Processing, Bahçe Vocational School, Osmaniye Korkut Ata University, Osmaniye, Turkey 80500
 Corresponding author: bal@gantep.edu.tr

Abstract

Mushroom play an important role in the decay of organic cover in forest ecosystems. In addition, mushrooms which are forest products are consumed as food. This study aims to determine the antioxidant activity of ethanol (EtOH) extract from edible *Lycoperdon molle* Pers. mushroom collected from Antalya (Turkey). Total antioxidant status (TAS), total oxidant status (TOS) and the oxidative stress index (OSI) were analyzed using Rel Assay Diagnostics kits. In addition, free radical scavenging activity was determined using the DPPH method. As a result of the studies, TAS, TOS and OSI values of *L. molle* were 1.855 ± 0.072 , 2.201 ± 0.085 and 0.119 ± 0.008 , respectively. The free radical scavenging activity of the fungus was at a normal level in comparison to the standards used, i.e. rosmarinic and caffeic acids. Consequently, it was determined that *L. molle* may be used as a source of natural antioxidants. As a result, it was determined that edible *L. molle*, which is one of the forest products, has antioxidant potential in addition to its nutritional properties.

Keywords: Lycoperdon molle, Antioxidant, Oxidant, DPPH, Oxidative stress.

Özet

Mantarlar orman ekosistemlerinde organic örtünün ayrıştırılmasında önemli rol oynarlar. Ayrıca orman ürünleri olan mantarlar gıda olarak tüketilmektedir Bu çalışmada Antalya (Turkey) ilinden toplanan yenilebilir *Lycoperdon molle* Pers. mantarının etanol (EtOH) ekstraktının antioksidan aktivitesinin belirlenmesi amaçlanmıştır. Mantar örneklerinin soxhlet cihazında özütleme işlemi yapılmıştır. Toplam antioksidan aktivitesi, toplam oksidan aktivitesi ve oksidatif stress indeksi Rel Assay Diagnostics kitleri kullanılarak analiz belirlenmiştir. Ayrıca serbest radikal süpürme aktivitesi DPPH metodu kullanılarak belirlenmiştir. Yapılan çalışmalar sonucunda *L. molle*'nin TAS değeri 1.855±0.072, TOS değeri 2.201±0.085 ve OSI değeri ise 0.119±0.008 olarak belirlenmiştir. Mantarın serbest radikal süpürme aktivitesinin ise kullanılan standartlar rosmarinik asit ve caffeic asite göre normal seviyelerde olduğu belirlenmiştir. Sonuç olarak *L. molle*'nin doğal antioksidan olarak kullanılabileceği belirlenmiştir. Sonuç olarak orman ürünlerinden olan yenilebilir *L. molle*'nin besin özelliklerine ek olarak antioksidan potansiyelinin olduğu belirlenmiştir.

Keywords: Lycoperdon molle, Antioxidant, Oxidant, DPPH, Oxidative stres.

Introduction

Mushrooms exhibit cosmopolitan distribution. Macrofungi belong to the order of Basidiomycetes or Ascomycetes and they can be found in soils rich in organic matter and

humus as well as various substrates such as in moist wood. Moreover, they can be found in animal dung after heavy rain or anywhere after sudden temperature changes occur and they then disappear after a short time, i.e. within several hours or at the end of the day (Girma and Tasisa 2018). The number of macrofungi species in nature is estimated to be between 53 and 110 thousand globally, however only a few are considered as nutrients and are able to be cultivated commercially (Mueller et al. 2007). Edible mushrooms have been globally consumed with increasing popularity due to their nutritional and medicinal value since Ancient Greek and Roman times (Udu-Ibiam et al. 2014). The fruiting bodies of fungi are consumed due to their variant textures and tastes. In addition, they are considerably important sources of dietary fiber, minerals, vitamins, water, proteins and carbohydrates (Kalac 2012, Yılmaz et al. 2016, Durmaz et al. 2018).

It has been found that mushrooms also have various pharmacological properties in addition to their nutritional value. Previous studies have reported that mushrooms had antioxidant, antimicrobial, anticancer, antiproliferative, DNA-protective, antiallergic, analgesic, antitumor, immunosuppressive, antiatherogenic, hypoglycemic, anti-inflammatory, hepatoprotective activities (Yang et al. 2008, Hetland et al. 2011, Patel and Goyal 2012, Ren et al. 2012, Li et al. 2013, Soares et al. 2013, Sun et al. 2014, Elsayed et al. 2014, Yıldız et al. 2015, Bal et al. 2017, Béni et al. 2018). Also, it has been found that Lycoperdon sp. mushrooms also have various pharmacological properties, in addition to their nutritional value. Previous studies have reported have antioxidant, that mushrooms antimicrobial, antiproliferative, antitumor. immunosuppressive and esterolytic activity (Colak et al. 2009, Shen et al. 2009, Sing et al. 2012, Novaković et al. 2015, Akpi et al. 2017).

Mushrooms are very valuable forest products. They are distributed in different forest ecosystems as saprotrophic, pathogenic and parasitic (Akata et al., 2018). In this context, they produce different levels of antioxidant and oxidant compounds depending on their habitats and capacities (Bal et al., 2019). In this study *L. molle* which is an edible species was used as material. In addition to the edible properties of the fungus, it was aimed to determine the presence of antioxidant potential. In addition, the oxidant level of the fungus was determined and the condition of the region where it was collected in terms of oxidant compound was determined.

In this context, this study aims to determine the antioxidant potential of the *L. molle*, an edible mushroom.

Material and Method

Mushroom Samples and Ethanolic Extract Preparation

L. molle were collected in Elmalı/Antalya, Turkey in 2018. Mushroom samples were collected from pine forest. Mushroom samples were introduced into the laboratory environment under suitable conditions. After identification of the mushroom samples, ethanol (EtOH) extraction of the mushrooms was carried out at 500C for nearly 6 hours using a Soxhlet extractor (Gerhardt EV 14). The extracts were concentrated using a rotary evaporator (Heidolph Laborota 4000 Rotary Evaporator).

TAS, TOS and OSI tests

The total antioxidant status (TAS) and total oxidant status (TOS) of the mushrooms were determined using Rel Assay kits (Rel Assay Diagnostics Kits, Turkey). Trolox was used as a calibrator in determining the TAS value and the results were expressed in mmol Trolox equiv./L (Erel 2004). Hydrogen peroxide was used as a calibrator in determining the TOS value and the results were expressed in μ mol H₂O₂ equiv./L (Erel 2005). OSI values were calculated by dividing the obtained TOS value by the obtained TAS value. OSI (arbitrary unit: AU) was calculated according to the following formula and expressed in percentage terms (Erel 2005).

 $OSI = \frac{TOS, \mu mol H_2O_2equiv./L}{TAS, mmol Trolox equiv./L X 10}$

In this current study, six mushroom samples were obtained from the mushrooms and the measurements were repeated five times.

DPPH Free Radical Scavenging Activity Assay

The free radical scavenging activity of the mushrooms was determined using 1-diphenyl-2picrylhydrazyl (DPPH). Stock solutions containing 1 mg/mL extract were prepared with DMSO. 50 μ L solution was added to 160 μ L 0.039% DPPH. The resulting solution was incubated in the dark at room temperature for 30 minutes. A reading for absorbance at 517 nm was obtained. The procedures were repeated individually for each concentration and sample (Shimada et al. 1992). In addition, caffeic and rosmarinic acids were used as reference antioxidants. Then, DPPH free radical scavenging percentages were calculated according to the formula:

Scavenging activity (%)= [(ADPPH-ASample)/(ADPPH)]x100.

Results and Discussion

Antioxidant activity

In addition to the benefits of oxygen for biological systems, it can also have side effects that are potentially harmful. Reactivity allows oxygen to take part in high-energy electron transfers and, therefore, supports a high amount of adenosine-5-triphosphate (ATP) formation via oxidative phosphorylation. Hence, it plays an important role in the development of multicellular organisms. Despite the benefits, oxygen also has the potential to harm many biological molecules such as proteins, lipids and DNA. Consequently, living organisms are always threatened by reactive oxygen species (ROS). This threat is generally balanced by the antioxidant protection system. However, this balance can be disrupted due to many environmental and inherent effects leading to oxidative stress (Burton and Jauniaux 2011; Sevindik et al., 2018).

The oxidative stress, which can manifest as a result of different environmental and inherent effects, is referred to as an imbalance between the production and elimination of reactive oxygen species (ROS) that cause multiple oxidative modifications of the basic and regulatory processes. Oxidative stress can increase due to increased levels of ROS, drug metabolism, over-expression of enzymes producing ROS or ionizing radiation as well as antioxidant enzyme deficiency (Gospodaryov and Lushchak 2012). Oxidative stress is associated with cellular

aging, acute and chronic kidney disease, neurodegenerative diseases, macular degeneration, biliary diseases, cancer and various acute and chronic pathological processes in addition to cardiovascular risk factors (obesity, diabetes, hypertension and atherosclerosis) (Burton et al. 2010; Chandrasekaran et al. 2017, Liguori et al. 2018; Sevindik, 2018).

In synthesis, given the close relationship between oxidative stress, inflammation, and aging, the oxidation-inflammatory theory of aging or oxi-inflamm-aging has been proposed: aging is a loss of homeostasis due to chronic oxidative stress that affects especially the regulatory systems, such as the nervous, endocrine, and immune systems. The consequent activation of the immune system induces an inflammatory state that creates a vicious circle in which chronic oxidative stress and inflammation feed each other and consequently, increases the age-related morbidity and mortality (De la Fuente and Miquel 2009, Liguori et al. 2018). Living organisms developed antioxidant defense systems in order to protect themselves from the negative effects of oxidative stress. These systems include some antioxidants produced in the body (endogenous) and some antioxidants derived from the diet (exogenous) (Rahman et al. 2012). It is of utmost importance to take antioxidant supplements in order to reduce oxidative stress when endogenous antioxidants are insufficient.

In this study, the antioxidant capacity of an edible mushroom, *L. molle*, was investigated. As a result, TAS, TOS and OSI values of *L. molle* were determined to be 1.855 ± 0.072 mmol/L, $2.201\pm0.085 \mu$ mol/L and 0.119 ± 0.008 , respectively. There is no previous study investigating the oxidative stress status of *L. molle*. According to previous studies, the TAS values of other medicinal mushrooms, such as *Auricularia auricula* and *Trametes versicolor* were found to be 1.010 and 0.820 mmol/L, TOS values of the same species were found to be 23.910 and 17.760 µmol/L, whereby OSI values 2.367 and 2.166, respectively (Akgül et al. 2017). Moreover, TAS, TOS and OSI values for *Fomitopsis pinicola* were reported to be 1.44, 14.21 and 0.99, respectively (Sevindik et al. 2017). In comparison to these studies, *L. molle* was found to have a higher TAS value compared to *A. auricula, T. versicolor* and *F. pinicola* a species. It is thought that the differences in TAS, TOS and OSI values stem from the antioxidant production capacity, oxidant production capacity and environmental conditions of habitat of the fungus, as well as from their substrate.

	Mushroom extract concentrations						
	0.25 mg/mL	0.5 mg/mL	1 mg/mL	2 mg/mL			
Caffeic acid	8.62±0.91	21.34±0.66	38.39±0.66	59.47±0.05			
Rosmarinic acid	6.03±0.15	7.00 ± 0.41	35.09±0.10	61.91±8.77			
L. molle	25.15±1.86	34.48±3.75	43.05±1.27	51.72±3.25			

Table1. DPPH scavenging activity of L. molle

In addition, it was found that the DPPH activity of *L. mole's* fruiting bodies extract at 0.25, 0.5, 1 and 2 mg/mL concentrations ranged between 25.15% and 51.72 % (Table 1). Caffeic and rosmarinic acids as standards displayed $59.47\pm0.05\%$ and $61.91\pm8.77\%$ activity, respectively at a 2 mg/mL extract concentration. It was observed that the crude extract of the *L. molle* fruiting bodies exhibited activity similar to the standards used. Previous studies have reported that

methanol, ethanol and acetone extracts of *L. molle* displayed high DPPH free radical scavenging activity (Barros et al. 2008, Singh et al. 2012). This current study also indicated that *L. molle's* fruiting bodies extract possesses DPPH free radical scavenging activity which has demonstrated the antioxidant potential of the studied fungus.

Wild edible and medicinal mushrooms represent important forest products world-wide (Bonet et al., 2008; Bal et al., 2019). The material used in our study, *L. molle* is quite common and edible mushroom According to the results of the study, *L. molle* has antioxidant potential. Mushrooms, which are among forest products, are very important in determining new antioxidant sources. In this study, it was determined that *L. molle* has an important place in the forest ecosystem.

Conclusion

In this study, the antioxidant status of the edible mushroom *L. molle* was determined. This fungus displayed a high TAS value and a good DPPH free radical scavenging activity. *L. molle* may be a source of antioxidant compounds. Mushrooms are not only a source of income for collectors and tourism businesses, but can also provide economic incentives for forest owners. Therefore, it can improve forest management.

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Investigation of myxomycetes in Selcen Mountain (Turkey) and its

close environs

Murat ZÜMRE^{1*}, Hayri BABA¹ and Mustafa SEVİNDİK^{2*}

¹Biology Dept. Faculty of Science and Literature, University of Hatay Mustafa Kemal, 31060 Hatay, Turkey ²Department of Food Processing, Bahçe Vocational School, Osmaniye Korkut Ata University, Osmaniye, Turkey Corresponding author: <u>sevindik27@gmail.com</u>

Abstract

In this study, myxomycetes, which have an important place in forest ecosystem, were determined. Myxomycetes act as saprophyte in the forest ecosystem. In this context, the myxomycet diversity of Selcen Mountain and its close environs was determined. This study has been made on the specimens which were obtained from 11 different station areas of Selcen Mountain and its close environs in 2011-2012. The samples were acquired from barks of trees, leaves and the materials of decayed trees. These materials were employed the Moist Chamber Culture and it was tried to develop myxomycetes sporophore. In addition myxomycetes were obtained from natural environment. As a result of field and laboratory studies 57 taxa belonging to 10 families and 21 genera were identified, and they were added to the Turkish Myxobiota. **Keywords:** Myxomycetes, Diversity, Selcen Mountain.

Key words. Myxomyceles, Diversity, Seleen

Özet

Bu çalışma 2011-2012 yılları arasında, Selcen Dağı ve yakın çevresini kapsayan 11 ayrı istasyondan toplanan numuneler üzerinde yapılmıştır. Örnekler ağaç kabukları, yaprak, çürümüş veya çürümemiş bitkisel materyallerden elde edilmiştir. Toplanan numunelere Nem Odası Tekniği uygulanmış ve miksomiset sporoforları geliştirilmeye çalışılmıştır. Ayrıca doğal ortamında gelişmiş olan miksomisetlerde toplanmıştır. Elde edilen örneklerden arazi ve laboratuvar çalışmaları sonucu 10 familyaya ait 21 cins toplam 57 takson tespit edilmiştir. Belirlenen taksonlar Türkiye Miksobiotasına ilave edilmiştir.

Anahtar Kelimeler: Myxomycetes, Çeşitlilik, Selcen Dağı.

Introduction

Myxomycetes are small, relatively homogeneous group of eukaryotic organisms. Myxomycetes are multi- nucleate, lack of cell wall and free living organisms. The plasmodium a colorless or brightly colored vegetative body of myxomycetes that consists of multinucleate protoplasm lacking a membrane. According to last classification the myxomycetes classified in kingdom protista. Some groups of myxomycetes sometimes include lime in sporangium, out of the sporangium and sometimes both in sporangium and out of the sporangium which are important for taxonomy (Farr, 1981; Ergül and Akgül, 2011).

Some species of myxomycetes most widely distributed and the others keep their living in certain habitats. The myxomycetes are shown awareness on developed substrate and are sensitve to light, moisture, temperature and pH. The myxomycetes live on bark of living trees, plant litter on the ground, aerial plant litter, standing dead wood or stumps, dead but still attached herbaceous plant parts

such as old inflorescences, downed and decayed wood or bark, decaying fruit, herbivorous animal faces and animal bone. The myxomycetes feeding with other organism (bacteria, yeast, green algea) which are living in their habitat. The material which collected from the field bring to labrotory to developed myxomycetes in moist chamber culter (Gilbert ve Martin, 1933; Ergül et al., 2005a).

Like many microorganisms, myxomycets play an important role in the forest ecosystem. Results from studies carried out across different types of terrestrial ecosystems suggest that the species associated with coarse woody debris represent one of the main components of overall myxomycete diversity (Ing, 1994; Rufino and Cavalcanti, 2007; Takahashi and Harakon, 2012). Studies may help to determine the crucial biotic and abiotic factors determining species richness and diversity of myxomycetes in major ecosystems of the world (Novozhilov et al., 2017).

The number of myxomycetes are about 1017 in the world (Lado, 2019). The number of the myxomycetes in Turkey are 286 (Ergül et al., 2005b; Baba et al., 2013; Süerdem et al., 2015; Baba, 2015; Baba and Zümre., 2015; Alkan et al., 2016; Dülger et al., 2016; Ergül et al., 2016; Baba et al., 2016; Sesli et al., 2016; Baba and Arslan, 2017; Baba and Özyiğit, 2017; Baba and Er, 2018; Baba et al., 2018; Ocak and Konuk, 2018). Aim of this study identify myxomycetes of Selcen Mountain and its close environs.

Materials and Methods Sampling Area

Yayladağı is located in Hatay city in Akdeniz region. Antakya is located at the North of the town, Mediterranean sea is in the west, Syria at the east and South, Samandağı is in the northwest and Altınözü is located at the northeast of the town. Yayladağı is located between 35 $^{\circ}$ 48 '- 36 $^{\circ}$ 04' north latitude and 35 $^{\circ}$ 55 '- 36 $^{\circ}$ 13' east longitude. Map of the Study Area is below (Figure 1).



Figure 1. Map of the research area

In this study, 11 station was stated in Selcen Mountain and and its close environs in 2011-2012. In summer, autumn, winter and spring seasons was done field studies and sporophores were collected.

Table 1. Localities, date, altitude and coordinate of study area						
No	Localities	Date	Altitude (m)	Coordinate		
1	Eski Kapı Hill	03.12.2012	501 m	35° 54' 39" N; 36° 00' 55" E		
2	Doruca Hill	03.12.2011	516 m	35° 54' 13" N; 36° 01' 00" E		
3	Karpuzlu Hill	17.12.2011	489 m	35° 54' 21" N; 36° 01' 41" E		
4	Salakçam Hill	24.12.2011	507 m	35° 54' 45" N; 36° 02' 14" E		
5	Karpuzluk Hill	05.02.2012	476 m	35° 53' 49" N; 36° 01' 41" E		
6	Dağardı Hill	11.03.2012	460 m	35° 54' 21" N; 36° 01' 41" E		
7	Ölüali Hill	18.03.2012	486 m	35° 51' 02" N; 36° 02' 29" E		
8	Habeștepe Hill	25.03.2012	540 m	35° 53' 23" N; 36° 03' 45" E		
9	Köşker Hill	28.04.2012	585 m	35° 53' 15" N; 36° 06' 36" E		
10	Ayvacık Farm	28.04.2012	589 m	35° 53' 24" N; 36° 04' 50" E		
11	Alibey Farm	28.04.2012	569 m	35° 53' 20" N; 36° 05' 28" E		

Localities, date	, altitude and	coordinate of	of study a	area was	shown in	n Table 1.
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Collecting samples and laboratory studies

Myxomycetes sporophores were collected from barks, woods, organic material debris. It was then carefully placed in cardboard herbarium boxes. In addition, the fructification of myxomycetes were as obtained from the moist chamber culture in the laboratory. The cultures were moistened with distilled water. Moisturized sporophores were examined every day under a dissecting microscope (Baba et al., 2018).

Identification of Samples

For identification of samples was used stereo microscope and high definition light microscope. With the stereo microscope general structure, shape, color, macroscopic dimensions of fructification and, lime availability or color and shape of lime were analyzed. The capillitium, whether lack of the pseudokapillitium and columella, if available shape and dimensions, strands of capillitium ornementation, branch shape of the capillitium, situation of the columella free or not, features of the pseudocapillitium, shape, color, size and ornamentation of spores were studied in detail by light microscopy.

The identification of the samples was made by using different studies (Martin and Alexopoulos 1969; Farr 1976; Thind 1977; Farr 1981; Martin et al., 1983; Neubert et al., 1993; Neubert et al., 1995; Neubert et al., 2000; Stephenson and Stempen 1994; Alexopoulos et. al., 1996; Lado and Pando 1997; Ing 1999). The fungarium samples were stored in the laboratory of Department of Biology, Faculty of Arts and Science, Mustafa Kemal University.

Results

In this study 642 myxomycetes sporophores were obtained from 11 different station of Selcen Mountain and its close environs in 2011-2012. As a result of field and laboratory studies 57 taxa belonging to 10 families and 21 genera were identified.

Systematic classification

Eukaryota Protozoa Amoebozoa Myxomycetes

Ceratiomyxales

Ceratiomyxaceae

1. Ceratiomyxa fruticulosa (O.F. Müll.) T. Macbr., Karpuzluk Hill, on Pinus sp. L. wood, Zümre. 218.

Echinosteliales

Echinosteliaceae

2. *Echinostelium minutum* de Bary, Eski Kapı Hill, on *P. brutia* wood, Zümre. 1; Dağardı Farm, on *P. brutia* wood, Zümre. 297; Habeştepe Hill, on *P. brutia* wood, Zümre. 468; Köşker Hill, on cone stamps, Zümre. 540.

Liceales

Cribrariaceae

3. *Cribraria cancellata* (Batsch) Nann.-Bremek., Eski kapı Hill, on *P. brutia* wood, Zümre. 1; Eski kapı Hill, on *P. brutia* wood, Natural, Zümre. 64; Karpuzlu Hill, on *P. brutia* wood, Zümre. 101; Karpuzlu Hill, on *P. brutia* wood, Zümre. 164; Dağardı Farm, on *P. brutia* wood, *Zümre*.322; Ölüali Hill on *P. brutia* wood, Zümre. 376; Köşker Hill, on *P. brutia* wood, Zümre. 536; Ayvacık Farm, on *P. brutia* wood, Zümre. 569.

4. *C. intricata* Schrad., Karpuzlu Hill, on *P. brutia* wood, Zümre. 104; Dağardı Farm, on *P. brutia* wood, Zümre. 305.

5. *C. macrocarpa* Schrad., Eski kapı Hill, on *P. brutia* wood, Zümre. 58; Karpuzluk Hill, on *P. brutia* wood, Zümre. 230.

6. *C. microcarpa* (Schrad.) Pers., Eski kapı Hill, on *P. brutia* wood, Zümre. 53; Salakçam Hill, on *P. brutia* wood, Zümre. 164; Karpuzluk Hill, on *P. brutia* wood, Zümre. 240; Dağardı Farm, on *P. brutia* wood, Zümre. 295; Köşker Hill, on *P. brutia* wood, Zümre. 541; Ayvacık Farm, on *P. brutia* wood, Zümre. 565.

7. *C. minutissima* Schwein, Eski kapı Hill, on *P. brutia* wood, Zümre. 11; Köşker Hill, on *P. brutia* wood, Zümre. 533; Ayvacık Farm, on *P. brutia* wood, Zümre. 566; 569.

8. C. piriformis Schrad., Habeştepe Hill, on P. brutia wood, Zümre. 462.

9. *C. vulgaris* Schrad., Karpuzlu Hill, on *P. brutia* wood, Zümre. 105; Karpuzluk Hill, on *P. brutia* wood, Zümre. 226; Ölüali Hill, on *P. brutia* wood, Zümre. 378; Habeştepe Hill, on *P. brutia* wood, Zümre. 455; Habeştepe Hill, on *P. brutia* wood, Zümre. 462.

Liceaceae

10. *Licea castanea* G.Lister, Karpuzlu Hill, on *P. brutia* wood, Zümre. 101; Karpuzluk Hill, on *P. brutia* wood, Zümre. 226; Dağardı Farm, on *P. brutia* wood, Zümre. 295; Habeştepe Hill, on *P. brutia* wood, Zümre. 463; Köşker Hill, on *Quercus* sp., Zümre. 544.

11. *L. kleistobolus* G.W. Martin, Eski kapı Hill, on *P. brutia* wood, Zümre. 42; Habeştepe Hill, on *P. brutia* wood, Zümre. 471.

12. *L. minima* Fr., Eski kapı Hill, on *P. brutia* wood, Zümre. 31; Dağardı Farm, on *P. brutia* wood, Zümre. 322; Habeştepe Hill, on *P. brutia* wood, Zümre. 456; Köşker Hill, on *P. brutia* wood, Zümre. 538; Alibey Farm, on *P. brutia* wood, Zümre. 629.

Reticulariaceae

13. Lycogala epidendrum (L.) Fr., Eski Kapı Hill, on P. brutia wood, Zümre. 1.
14. Reticularia lycoperdon Bull., Köşker Hill, on cortex of *P. brutia*, Zümre. 547.

Trichiales

Arcyriaceae

15. Arcyria affinis Rostaf., Dağardı Farm, on P. brutia wood, Zümre. 301.

16. *A. cinerea* (Bull.) Pers., Eski kapı Hill, on *P. brutia* wood, Zümre. 11; Karpuzluk Hill, on *P. brutia* wood, Zümre. 239; Dağardı Farm, on *P. brutia* wood, Zümre. 295; Ölüali Hill, on *P. brutia* wood, Zümre. 378; Habeştepe Hill, on *P. brutia* wood, Zümre. 451; Köşker Hill, on *P. brutia* wood, Zümre. 536; Alibey Farm, on *P. brutia* wood, Zümre. 601.

17. *A. denudata* (L.) Wettst., Karpuzluk Hill, on *P. brutia* wood, Zümre. 240; Habeştepe Hill, *on Quercus* sp. wood, Zümre. 464.

18. *A. ferruginea* Saut., Karpuzluk Hill, on *P. brutia* wood, Zümre. 240; Dağardı Farm, on *P. brutia* wood and bark, Zümre. 302; Habeştepe Hill, on *Quercus* sp. wood, Zümre. 469.

19. *A. incarnata* (Pers. ex J.F. Gmel.) Pers., Eski kapı Hill, on *P. brutia* wood and bark, Zümre. 4; Karpuzluk Hill, on *P. brutia* wood, Zümre. 232; Dağardı Farm, on *P. brutia* wood and bark, Zümre. 306; Köşker Hill, on *P. brutia* wood, Zümre. 561.

20. *A. pomiformis* (Leers) Rostaf., Eski kapı Hill, on *P. brutia* wood, Zümre. 31; Dağardı Farm, on *P. brutia* wood, Zümre. 296; Ölüali Hill, on *P. brutia* wood, Zümre. 380; Köşker Hill, on *P. brutia* wood, Zümre. 534; Alibey Farm, on *P. brutia* wood, Zümre. 597.

21. A. stipata (Schwein.) Lister, Ayvacık Farm, on P. brutia wood, Zümre. 564.

22. A. versicolor W. Phillips, Habeştepe Hill, on P. brutia wood, Zümre. 460.

Trichiaceae

23. Hemitrichia abietina (Wigand) G. Lister, Ölüali Hill, on P. brutia wood, Zümre. 380.

24. *Trichia botrytis* (J.F. Gmel.) Pers., Dağardı Farm, on *Quercus* sp. wood, Zümre. 303; Habeştepe Hill, on *P. brutia* wood, Zümre. 461; Köşker Hill, on *Quercus* sp. wood, Zümre. 537; Salakçam Hill, on *P. brutia* wood, Zümre. 174; Karpuzluk Hill, on *P. brutia* wood, Zümre. 225; Köşker Hill, on *Quercus* sp. wood, Zümre. 537.

25. *T. crateriformis* G.W. Martin, Karpuzluk Hill, on *P. brutia* Ten., wood, Zümre. 240; Dağardı Farm, on *P. brutia* Ten., wood, Zümre. 322; Köşker Hill, on *P. brutia* Ten. bark, Zümre. 538.

26. T. decipiens (Pers.) T. Macbr., Dağardı Farm, on P. brutia wood, Zümre. 297.

27. T. erectra Rex., Eski kapı Hill, on P. brutia bark, Zümre. 19.

28. *T. lutescens* (Lister) Lister, Eski kapı Hill, on *P. brutia* wood, Zümre. 47; Salakçam Hill, on *P. brutia* wood, Zümre. 164; Habeştepe Hill, on *P. brutia* wood, Zümre. 459.

29. T. munda (Lister) Meyl. Eski kapı Hill, on P. brutia wood, Zümre. 53.

30. *T. verrucosa* Berk., Dağardı Farm, on *P. brutia* wood, Zümre. 302; Habeştepe Hill, on *P. brutia* wood, Zümre. 450.

Physarales

Didymiaceae

31. *Diderma carneum* Nann.- Bremek., Eski kapı Hill, on *P. brutia* wood, Zümre. 62; Ölüali Hill, on *P. brutia* wood, Zümre. 367.

32. *D. radiatum* (L.) Morgan, Eski kapı Hill, on *P. brutia* wood, Zümre. 58; Ölüali Hill, on *P. brutia* wood, Zümre. 375.

33. Didymium bahiense Gottsb., Karpuzluk Hill, on P. brutia wood, Zümre. 244.

34. *D. difforme* (Pers.) Gray, Habeştepe Hill, on *P. brutia* wood, Zümre. 451; Köşker Hill, on *P. brutia* wood, Zümre. 536.

35. D. melanospermum (Pers.) T. Macbr., Ölüali Hill, on P. brutia wood, Zümre. 370.

Physaraceae

36. Badhamia nitens Berk., Salakçam Hill, on P. brutia wood, Zümre. 164.

37. *Physarum album* (Bull.) Chevall., Eski kapı Hill, on *P. brutia* wood, Zümre. 19; Karpuzluk Hill, on *P. brutia* wood, Zümre. 244.

38. P. flavicomum Berk., Eski kapı Hill, on P. brutia wood, Zümre. 10.

39. P. murinum Lister, Salakçam Hill, on P. brutia wood, Zümre. 167.

Stemonitales

Stemonitidaceae

40. *Collaria lurida* (Lister) Nann.-Bremek., Eski kapı Hill, on *P. brutia* wood, Zümre. 11; Köşker Hill, on *P. brutia* wood, Zümre. 540.

41. *Comatricha ellae* Härk., Eski kapı Hill, on *P. brutia* wood, Zümre. 3; Karpuzlu Hill on *P. brutia* wood, Zümre. 117; Salakçam Hill, on *P. brutia* wood, Zümre. 168; Karpuzluk Hill, on *P. brutia* wood, Zümre. 239; Dağardı Farm, on *P. brutia* wood, Zümre. 296; Ölüali Hill, on *P. brutia* wood, Zümre. 386; Habeştepe Hill, on *P. brutia* wood, Zümre. 452; Köşker Hill, on *P. brutia* wood, Zümre. 535, Alibey Farm, on *P. brutia* wood, Zümre. 598.

42. C. elegans (Racib) G. Lister, Eski kapı Hill, on P. brutia wood, Zümre. 31, Ölüali Hill, on P. brutia wood, Zümre. 377.

43. *C. nigra* (Pers. ex J.F.Gmel.) J.Schröt., Eski kapı Hill, on *Quercus* sp. wood, Zümre. 5; Karpuzluk Hill, on *P. brutia* wood, Zümre. 234; Dağardı Farm, on *P. brutia* wood, Zümre. 298; Ölüali Hill, on *P. brutia* wood, Zümre. 383; Habeştepe Hill, on *P. brutia* wood, Zümre. 455; Alibey Farm, on *P. brutia* wood, Zümre. 599.

44. *C. pulchella* (C. Bab.) Rostaf., Eski kapı Hill, on *P. brutia* wood, Zümre. 16; Salakçam Hill, on *Quercus* sp. wood, Zümre. 160.

45. *Enerthenema papillatum* (Pers.) Rostaf., Eski kapı Hill, on *P. brutia* wood, Zümre. 28; Dağardı Farm, on *P. brutia* wood, Zümre. 297.

46. Lamproderma arcyrioides (Sommerf.) Rostaf., Karpuzluk Hill, on P. brutia wood, Zümre. 240.

47. *L. laxum* H. Neubert, Ölüali Hill, on *P. brutia* wood, Zümre. 370; Köşker Hill, on *P. brutia* wood, Zümre. 557.

48. Paradiacheopsis rigida (Brândza) Nann. – Bremek., Eski kapı Hill, on P. brutia wood, Zümre. 23.

49. *Stemonitis axifera* (Bull.) T. Macbr., Eski kapı Hill, on *P. brutia* wood, Zümre. 14; Dağardı Farm, on *P. brutia* wood, Zümre. 298; Habeştepe Hill, on *P. brutia* wood, Zümre. 468; Köşker Hill, on *P. brutia* wood, Zümre. 539.

50. *S. flavogenita* E. Jahn, Karpuzluk Hill, on *P. brutia* wood and bark, Zümre. 226; Dağardı Farm, on *P. brutia* wood, Zümre. 318; Ölüali Hill, on *P. brutia* wood, Zümre. 373; Habeştepe Hill, on *Quercus* sp. bark, Zümre. 466; Köşker Hill, on *P. brutia* bark, Zümre. 544.

51. *S. fusca* Roth, Salakçam Hill, on *P. brutia* wood, Zümre. 180; Karpuzluk Hill, on *P. brutia* wood, Zümre. 296; Habeştepe Hill, on *P. brutia* wood, Zümre. 470; Ayvacık Farm, on *P. brutia* wood, Zümre. 570.

52. S. herbatica Peck, Alibey Farm, on P. brutia wood, Zümre. 594.

53. *Stemonitopsis amoena* (Nann.-Bremek.) Nann.-Bremek., Ölüali Hill, on *P. brutia* wood, Zümre. 382; Habeştepe Hill, on *P. brutia* wood, Zümre. 475.

54. *S. hyperopta* (Meyl.) Nann.-Bremek., Eski kapı Hill, on *P. brutia* wood, Zümre. 22; Karpuzlu Hill, on *P. brutia* wood, Zümre. 135; Karpuzluk Hill, on *P. brutia* wood, Zümre. 235; Dağardı Farm, on *P. brutia* wood, Zümre. 295; Köşker Hill, on *P. brutia* wood, Zümre. 540.

55. *S. reticulata* (H.C. Gilbert) Nann.-Bremek.& Y. Yamam., Ölüali Hill, on *P. brutia* wood, Zümre. 235; Dağardı Farm, on *P. brutia* wood, Zümre. 306; Alibey Farm, on *P. brutia* wood, Zümre. 595.

56. S. subcaespitosa (Peck) Nann.-Bremek., Köşker Hill, on P. brutia wood, Zümre. 534.

57. Symphytocarpus trechispora (Berk. ex Torrend) Nann.-Bremek., Dağardı Farm, on *P. brutia* wood, Zümre. 296.

Discussion

In this study, 57 taxa belonging to 10 families and 21 genera were identified in Selcen Mountain (Turkey) and its close environs. In our study, the distributions of families were determined as Ceratiomyxaceae 1, Echinosteliaceae 1, Cribrariaceae 7, Liceaceae 3, Reticulariaceae 2, Arcyriaceae 8, Trichiaceae 8, Didymiaceae 5, Physaraceae 4 and Stemonitidaceae 18 taxa respectively. In our study, 4 families (Cribrariaceae 7, Arcyriaceae 8, Trichiaceae 8 and Stemonitidaceae 18) constituted the majority of taxa. In this context, our study showed similarity with that of Yağız and Afyon (2005).

In literature search samples of the myxomycetes mostly have been identified on the Gymnosperms rotted wood, leaves and debris (Martin ve Alexopoulos 1969; Ergül and Akgül, 2011; Baba et al., 2018). Members of Liceales, Trichiales and Stemonitales are usually grows in conifer forests (Martin and Alexopoulos 1969; Ergül and Akgül 2011; Baba et al., 2018). The majority of the samples that were identified have been deceted on the angiosperm debris.

Corticolous myxomycetes are found on decaying leaf litter, and still others on the bark of living trees and vines. Lignicolous myxomycetes are found on rotten wood and wood cortex. Foliicolous myxomycetes are found on leaves. Finicolous myxomycetes are found on faces of the animals (Everhart and et. al., 2008). From the materials collected from the study area just from the cortex and rotten wood samples myxomycetes were obtained.

In addition, *E. minutum*, *A. cinerea*, *A. denudata* and *S. fusca* are cosmopolitan taxa (Stephenson and Stempen 1994). These species were also detected in our study area. *E. minutum*, *A. pomiformis*, *A. cinerea*, *C. ellae* and *C. nigra* have been identified in many areas of our study. In this context, it has been similar to the studies of many researchers (Ocak and Hasenekoğlu, 2003; Yağız and Afyon, 2007; Baba, 2015; Ergül et al., 2016).

Conclusion

In our study, myxomycetes were determined to be rotten in the forest ecosystem. In this context, myxomycetes were determined in Selcen Mountain (Turkey) and its close environs. As a result of field and laboratory studies 57 taxa belonging to 10 families and 21 genera were identified, and they were added to the Turkish Myxobiota. Identificated myxomycetes can contribute to determine of microhabitat located in the forest ecosystem.

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In vitro study on antioxidant, antibacterial and DNA interaction activities of extracts from *Arbutus andrachne* L.

Ceren Baskan^{1*}, Dudu Duygu Kılıc², Belgin Sırıken³, Güler Tanrikulu⁴, Melek Gül⁴, Omer Ertürk⁵

*¹Amasya University, Sabuncuoğlu Şerefeddin Health Services Vocational School, Amasya, Turkey.
 ²Amasya University, Faculty of Arts and Sciences, Department of Biology, Amasya, Turkey
 ³Ondokuz Mayis University, Faculty of Veterinary Medicine, Aquatic Animal Diseases, Samsun, Turkey.
 ⁴Amasya University, Faculty of Arts and Sciences, Department of Chemistry, Amasya, Turkey.
 ⁵Ordu University, Faculty of Arts and Sciences, Department of Biology, Ordu Turkey.

Corresponding author: ceren.yavuz@amasya.edu.tr,

Abstract

Traditionally, the fruits and leaves of *Arbutus* (*A.*) *andrachne* are well known and used for applications in antimicrobial, antioxidant and anticancer activity. This study was aimed to determine *in vitro* antibacterial, antioxidant and DNA interaction activities of the different extracts of *A. andrachne*.

A. andrachne was collected from Amasya Province during May 2018. Dried leaves powder were mixed with of suitable solvents. Then, the mixtures were extracted with the soxhlet apparatus for 4 hours. The antibacterial effects of extracts were researched on pathogens, namely *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 25953, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus cereus* ATCC 7064, *Bacillus subtilis* ATCC 6633, and *Salmonella enteritidis* ATCC 13076 using disc diffusion methods. Gentamicin and cefotaxime were used as the control for bacteria. To try to understand each plant extracts effect on the antioxidant methods, we studied the different methods as named radical scavenging activity (DPPH), metal chelating activity, ferric reducing antioxidant power assays. In the plant extracts, total phenolic and total flavonoid contents were determined by spectrophotometric methods due to investigating the effect of secondary metabolites on antioxidant activity. To explore the beneficial effect of the extracts on hydroxyl radical-mediated DNA damage, plasmid DNA pUC18 was used.

As a result, *A. andrachne* extracts had an antibacterial effect when they compared with control group antibiotics. According to disc diffusion methods, the highest antibacterial effect was identified in ethanol, chloroform and hexane extract, respectively. Ethanol extract of *A. andrachne* showed the best antioxidant activity. Moreover, extracts of *A. andrachne* had repair effects on plasmid DNA in the presence of H_2O_2 condition.

The investigated *A. andrachne* extract showed significant bioactivities. These extracts have been a promising candidate for the preparation of new natural products. However, future studies should be carried out to verify such actions in different matrices.

Keywords: Arbutus andrachne L. antioxidant, antimicrobial, DNA interaction

Introduction

In recent years, the root, trunk, leaf or fruit of various plants are utilized as a natural alternative in modern medicine. Secondary metabolites (terpenes, terpenoids, flavonoids) in the content of natural plants help to protect human health against various diseases. The antioxidant content of secondary flavonoids provides inhibition of oxidative damage associated with many diseases such as cancer, coronary heart disease, and paralysis (Özgen et al. 2009). Antioxidants are known to have a protective role against oxidative damage of reactive oxygen species on biomolecules such as DNA, lipid, and protein (Ayvaz et al. 2018; Gül et al. 2017).

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Also, the unconscious and unnecessary use of antibiotics causes a rapid increase in antibiotic-resistant microorganisms day by day. Therefore, diseases caused by resistant microorganisms pose serious public health problems. The use of medicinal herbs to reduce the harmful effects of antimicrobial agents increases with each passing day (Beattie et al. 2005). Already, the World Health Organization (WHO) has acknowledged many years ago that traditional medicine is important in health applications (WHO 2002-2005). Therefore, compounds derived from medicinal and aromatic plants with biological activity are widely investigated to treat various animal and human diseases (Kunduhoglu et al. 2011; Ünal et al. 2008).

A. andrachne L. plant belongs to the Ericaceae family and is known to have antimicrobial, antioxidant, anticancer and anti-inflammatory activity (Sıcak and Eliuz, 2019; Saklani and Kutty, 2008). The local name in Turkey is known as "Sandal tree" and "Davulga" or "Kocayemiş". The small fruit of *A. andrachne* L., which blossomed in May and localized in many different regions of Anatolia, was reported to occur in November (Baytop et al. 1999). The small fruit of *A. andrachne* L., which blossomed in May and localized in many different regions of Anatolia, was reported to occur in November (Baytop et al. 1999). The small fruit of *A. andrachne* L., which blossomed in May and localized in many different regions of Anatolia, was reported to occur in November. Its fruits are used in a wide food product such as jam, fruit jelly, marmalade and alcoholic beverages (liquor and wine). Fruits containing tannin, anthocyanin, and carotenoids are generally sweet, although unpleasant taste. The main therapeutic effects of *A. andrachne* L. are related to urinary tract infections, as well as, anticancer, antibacterial and antioxidant activities.

The main purpose of this study is to reveal the antibacterial and antioxidant effects of the *A. andrachne* L. plant obtained from the province of Amasya. For this purpose, the antibacterial effects of *A. andrachne* L. plant extracts were investigated by the disk diffusion method. Secondly, the antioxidant activities of *A. andrachne* L. specimens were determined using radical scavenging activity (DPPH), metal chelating activity, ferric reducing antioxidant power assays methods. In addition, the protective role of *A. andrachne* L. extracts on DNA damage originating from hydroxyl radicals was investigated using plasmid DNA. In the literature search, DNA interaction experiments with *A. andrachne* L. were not found. In this context, the current work differs from previous studies. These analyses were also made to determine which of the three extracts (ethanol, dichloromethane, and n-hexane) is more effective.

Materials and Methods

Sample extraction

A. andrachne L. was collected from a natural population which is located in Devret Hill (Amasya) in May 2018 (Baytop, 1999). The leaves of *A. andrachne* L. were then dried at room temperature. The plants (25 g) were extracted with the soxhlet tool (Isolab, Turkey) for 4 hours in ethanol, dichloromethane, and n-hexane (prepared by using solvents of different polarity). The extracts were filtered by Whatman No. 1 paper. The solvents were removed under a rotary vacuum until dry at 25-35 °C for 3–4 h. (Heidolph Collegiate, LV28798826, New Jersey, USA). Then the residue dissolved in Tetrahydrofuran (THF, Sigma) for antimicrobial activity (20 mg/mL). The extracts samples were stored at 4 °C in dark bottle for investigation (Bouyahya et al. 2016).

Test organisms and culture condition for antibacterial analysis

Extracts of A. andrachne L. were tested against Gram-positive and Gram-negative bacteria, including Staphylococcus aureus ATCC 25923, Staphylococcus aureus ATCC 25953, Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, Pseudomonas aeruginosa ATCC 9027, Bacillus cereus ATCC 7064, Bacillus subtilis ATCC 6633, and Salmonella enteritidis ATCC 13076. Before use, bacterial strains were subcultured overnight at 37°C for 18 to 24 h in Trypric Soy Broth (TSB, Oxoid, Hampshire, UK).

Antibacterial activity

Antibacterial activity of the *A. andrachne* L. extracts were determined by the disc diffusion method (CLSI 2010). *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 25953, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa* ATCC 9027, *Bacillus cereus* ATCC 7064, *Bacillus subtilus* ATCC 6633, and *Salmonella enteritidis* ATCC 13076 were used in bacterial strains. The

concentrations of the microorganisms were adjusted by turbidity measurements (0.5 McFarland) using serum physiologic solution. The concentration of bacterial suspensions was adjusted to 10^8 cells/mL. Then, extracts (20 mg/mL) prepared in THF were loaded 6 mm to diameter sterile blank discs (Oxoid). Inhibition zones were determined after incubation at 37 °C for 24 h. As a positive control for bacteria, gentamicin (10 µg) and ceftriaxone (30 µg) were placed in Petri dishes. All tests were performed in triplicate.

Antioxidant activity

Free radical scavenging activity

The free radical scavenging activity was determined with 1,1-diphenyl-2-picrylhydrazyl (DPPH•) using methods of Brand-Williams (Brand-Williams et al. 1995). Different concentrations of plant material (25, 50, 100, 200, 400 µg/mL) were prepared and 0.75 mL each one of these extracts was added in to the 1.5 mL of 0,1 mM DPPH• solution in methanol. This solution was also added to, butylated hydroxytoluene (BHT), and Trolox (25-400 µg/mL) which were used to as positive controls for comparing. The mixture was shaken vigorously, and the decrease in absorbance at 517 nm was measured for 30 min at room temperature. Water (0.75 mL) in place of the sample was used as control. IC50 value was calculated to use the linear regression as the concentration required for 50% reduction of the DPPH radical. The percent inhibition activity was calculated using the following equation: free radical scavenging effect $\% = [(A_0 - A_1)/A_0] \cdot 100$. (A_0 = the control absorbance and A_1 = the sample solution absorbance).

Metal chelating activity

The chelating activity of extract on ferrous ions (Fe²⁺) was measured according to the method of Decker and Welch (Decker and Welch, 1990). Aliquots of 1 mL of different concentrations (100–500 µg/mL) of extracts were mixed with 3.7 mL of deionized water. The mixture was incubated with FeCl₂ (2 mM, 0.1 mL) for 30 min. After incubation, the reaction was initiated by addition of ferrozine (5 mM and 0.2 mL) for 10 min at room temperature, and then the absorbance was measured at 562 nm. A lower absorbance indicates a higher chelating power. The chelating activity of the extract on Fe²⁺ was compared with that of EDTA at the same concentrations. Metal chelating activity was calculated using the following equation Metal chelating activity (%) = $[(A_0 - A1)/A0] \cdot 100$.

Estimation of total phenolic content

According to the methods of Slinkard and Singleton (Slinkard and Singleton, 1977), using Folin–Ciocalteu reagent was determined depending on phenolic standard gallic acid. 1 mL of the plant extract was introduced into test tube followed by 1 mL Folin–Ciocalteu's reagent. The solution was kept in the dark for 5 min and then 3 mL of sodium carbonate (2%) was added. The tubes were covered with parafilm and kept again in the dark for 1 h and were measured absorption at 765 nm with a spectrophotometer and compared to a gallic acid calibration curve. The results were expressed as mg gallic acid/g dried sample. Each assay was carried out in triplicate.

Estimation of total flavonoid content

Total flavonoid content was determined with quercetin standard solution using Park methods (Park et al. 2008). The plant extract in 0.3 mL of was introduced into test tubes followed by 3.4 mL 30% methanol, 0.15 mL of 0.5 M NaNO₂ and 0,3 M AlCl₃ reagent. After 5 min 1 mL of 1 M NaOH was added and absorption was measured at 506 nm with a spectrophotometer and compared to a quercetin calibration curve. Each assay was carried out in triplicate. The total flavonoids were described as mg of quercetin equivalents per g of the dried fraction.

DNA interaction assay

To explore the beneficial effect of the *A. andrachne* L. extracts on hydroxyl radical-mediated DNA damage plasmid pUC18 DNA (Thermo Scientific) was used. Firstly, the *A. andrachne* L. extracts were dissolved in dimethyl sulfoxide (DMSO, concentration range from 12.5 to 100 mg/mL). A reaction mixture (20 μ L final volume) containing 0.25 μ g/ μ L plasmid DNA pUC18, 1.0 mM EDTA (Ethylenediaminetetraacetic acid)-FeSO4,1 μ L of 3% H₂O₂, 0.1 g/mL *A. andrachne* L. extracts in Tris-EDTA (TE) buffer was prepared. H₂O₂ and 0.1% tetrahydrofuran treated plasmid DNAs were used as control groups. Secondly, the prepared mixture for each *A. andrachne* L. extracts were incubated at 37°C for 24 hours. 2 μ L loading dye (bromophenol blue [0.025%] and

sucrose [4%] in dH₂O) was added into the mixture (10 μ L total volume) and loaded on to the 1% agarose gel. Electrophoresis process was for 90 min at 80 V in TBE buffer running buffer (pH 8). Then, the agarose gel was stained with 0.5 μ g/L ethidium bromide after electrophoresis. The Gel was imaged under UV light (Ayvaz et al. 2018).

Results and Discussion

In the present study, the extracts from *A. andrachne* L. samples collected from Amasya were assayed for antibacterial potency against four Gram-positive and four Gram-negative bacteria of clinical importance. Antioxidant activity was evaluated by measuring with different methods. At the same time, this study exhibited also DNA damage inhibitory activities of *A. andrachne* L.

According to analyzed antibacterial activity, the extracts of the plants indicated varying degrees of activity against bacterial strains that outlined in Table 1. Ethanolic extracts from *A. andrachne* L. is the most effective on *S. aureus* (ATCC 25953), *S. aureus* (ATCC 25953) and *B. cereus*. The antimicrobial activity of dichloromethane extracts against the *P. aeruginosa* (ATCC 9027), *S. enteritidis* and *E. coli* was found to non-effective. Hexane extracts of *A. andrachne* L is the most effective on *B. cereus* and *B. subtilis*.

able	1. Zone diameters of inhibit	ion (mm) shown	ng the anti	microbial	activities	of each ex	stract from	m A. andrachi
	Microorgansims	B.s	B.c	P.a	P.a	S.a	S.a	S.e	E.c
				9027	27853	25953	25923		
	Solvent and								
_	antibiotics								
	Ethanol	14	10	10	10	19	15	11	11
	Dichloromethane	12	14	8	10	14	17	9	9
	n-Hexane	11	11	8	10	9	8	8	9
	CRO 30 µg	28	13	12	33	21	31	10	34
	CN 10 µg	19	21	23	23	20	23	26	23

Table 1. Zone diameters of inhibition (mm) showing the antimicrobial activities of each extract from A. andrachne

Microorganisms: B.s.: Bacillus subtilis, B.c.: Bacillus cereus, P.a.: Pseudomonas aeruginosa, S.a.: Staphylococcus aureus, S.e.; Salmonella enteritidis, E.c.: Eschericia coli. CRO: Ceftriaxone, CN: Gentamicin

The literature investigations revealed diverse traditional uses of *Arbutus* species based on antiseptic, antihypertensive, anti-diabetic, anti-inflammatory, anticancer and laxative properties. However, only a few of them are determined scientifically for their biological activities (Tenuta et al. 2019). The described characteristics are related to the contents of several biologically active compounds of *Arbutus* sp. (Morgado et al. 2018). One of them reported from Turkey, in the survey, the highest antibacterial activity of *A. andrachne* L. was reported against *S. aureus* (Stcak and Eliuz, 2019). In another survey carried out in Turkey, *A. andrachne* L. extracts inhibited the growth of five bacteria and the inhibition zones ranged between 8- 17 mm. The highest antibacterial activity shows was observed on *S. aureus* (17 mm) (Ergun et al. 2014). Similarly, in the present study, ethanolic extracts of *A. andrachne* L. was the most effective on *S. aureus* (19 mm) (ATCC 25953). Tenuta et al. (2019) from France reported, they reported that the most promising activity of *Arbutus* sp leaves was found mainly against Grampositive bacteria. In addition, they showed an intense antimicrobial activity against *Staphylococcus aureus*, *Bacillus subtilis* and *Pseudomonas aeruginosa*, moderate activity against *Salmonella typhimurium*, *Escherichia coli*.

Although there is a few information in the literature about *A. andrachne* L. extracts biological activity, extracts prepared from *A. unedo* L., another type of *Arbutus* genus, leaves were showed antimicrobial activity against many microorganisms *B. cereus*, *B. subtilis*, *S. aureus*, *S. epidermis*, *E. coli* and *P. aeruginosa*. The biological activity of *A. unedo* L. tree has been the subject of several studies (Delgado-Pelayo et al. 2016; Erkekoglou et al. 2017; Fonseca et al. 2015). One of them reported from Portugual, in the survey, the extracts revealed antibacterial activity against several Gram-positive bacteria, including *Bacillus cereus*, *Enterococcus faecalis* and clinical methicillin-resistant *Staphylococcus aureus* (Morgado et al. 2018; Asmae et al. 2012; Ertabaklar et al. 2009; Ferreira et al.,

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2012). In another survey carried out again in Portugual, it was determined that the antimicrobial potential of the extract were more efficient to Gram-positive bacteria (Malherio et al. 2012). Similar results were also reported for different *Arbutus* sp. in the literature. In Gram-positive bacteria, cell wall allows the essential oil and hydrophobic constituents to be in direct contact with the phospholipid bilayer of the cell membrane. Researchers reported that where they bring about their effect, causing either an increase in ion permeability and leakage of vital intracellular constituents, or impairment of the bacterial enzyme systems (Wendakoon and Sakaguchi, 1995; Malherio et al. 2012).

Natural or synthetic antioxidant compound capability which is obtained the quenching the reactive oxygen/nitrogen or radicals is determined via two different chemical processes: electron (ET) and hydrogen atom transfer (Apak et al. 2016). The antioxidant assay performed on the ethanol, dichloromethane, and n-hexane extract of *A. andrachne* L. collected in the Amasya region evidenced the presence of total antioxidant capacity via electron transfer methods. To perform DPPH, metal chelating, total flavonoid, total phenolic assay on these extracts were chosen from similar family plants works literature (Pallauf et al. 2008; Oliveira et al. 2009; Fortalazes et al. 2010). The level of DPPH activity was the highest at ethanolic extracts, so this result showed that ethanol was a good solvent for the extraction of the antioxidant moiety. The hexane solvent of which generally is used for terpenoids extraction, exhibited the lowest activity. The IC₅₀ value of ethanolic extracts was lower than dichloromethane and hexane; 146.6, 200.9, 333.3 respectively. In literature was found similar results concerning DPPH activity (Figure 1) (Figure 1) (Bilto et al. 2015).



Figure 1. DPPH radical scavenging of extracts

When the metal chelating activity was compared EDTA standard, all extracts exhibited moderated metal chelating activity. Again, ethanol was the highest activity than others (Figure 2).

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Figure 2. The extracts metal chelating studies.

Total flavonoid and total phenolics content were related to antioxidant capacity. Antioxidant result and these content values were shown similarities for each extract. The total phenolic ranged from 11,6 to 151,9 mg/g (gallic equivalent) and total flavonoid ranged from 65,2 to 71.6. *A. andrachne* L. fruit total penolic content was found 3.3 mg/g in 2010 (Table 2) (Serce et al. 2010).

Tuble 2: 1 otal phonone and navonora content of extracts						
Chemical	Total Phenolic Content	Total Flavonoid Content				
Composition	(mg GAE/g)	(mgQTE/g)				
n-Hexane	11,6061	65,20905				
Dicholoromethane	34,2140	66,13783				
Ethanol	151,8707	71,64125				

Table 2. Total phenolic and flavonoid content of extracts

Moreover, in this study, inhibitory activities of *A. andrachne* L. on hydroxyl radical-induced deoxyribonucleic acid (DNA) damage of *A. andrachne* L. extracts was investigated. According to the results of agarose gel electrophoresis, extracts were dissolved in DMSO and 0.25 μ g/ μ L plasmid DNA pUC18 was treated with 12.5, 25, 50 and 100 mg/mL extracts respectively (Figure 3).



Figure 3. Gel image of extracts of A. andrachne L.

a) Lane 1: H_2O_2 and pUC18 plasmid DNA; Lane 2: pUC18 plasmid DNA control (blank); Lane 3: DMSO control; Lane 4-7: H_2O_2 , pUC18 plasmid DNA and different concentration of ethanolic extracts (12,5-100 mg/mL)

- b) Lane 8-11: H2O2, pUC18 plasmid DNA and different concentration of dichloromethan extracts (12,5-100 mg/mL)
- c) Lane 12-15: H₂O₂, pUC18 plasmid DNA and different concentration of n-Hexanolic extracts (12,5-100 mg/mL)

Lane 2 and lane 3 was run with untreated pUC18 plasmid DNA as a control, while lanes 4-15 pointed out plasmid DNA interacted with increasing concentrations of the extracts in H_2O_2 condition. Increasing doses of *A. andrachne* L. extracts had a protective effect on hydroxyl radical-mediated plasmid DNA damage, but a low concentration of all *A. andrache* extract had no protective effect on plasmid DNA in H_2O_2 conditions. It appears that extracts of ethanol, dichloromethane, and n-hexane, exhibit relatively similar effects against plasmid DNA. The extracts did

not cause to change in Form I DNA (supercoil), but transferred from Form II DNA (open circular) (Ayvaz et al., 2018). The extracts have resulted in the cleavage of the plasmid DNA hence converting the plasmid form I to either form II and/or form III. As the concentrations of ethanol dichloromethane and n-hexane extracts increased, the mobility and band density of form I DNA increased slightly.

Conclusion

Several in vitro studies have conducted with different extracts of parts of *Arbutus* sp. Concerning the biological activity of usually *A. unedo* extracts, the majority of studies reported data based on antibacterial and antioxidant activity. We determined to total phenolic, total flavonoid and antioxidant activity of each extract as prepared from the n-hexane, dichloromethane, and ethanol. The ethanol extract was important not only suitable solvent of flavonoid and phenolic content but also antioxidant activity.

This study showed that *A. andrachne* L. species belonging to the Ericaceae family have *in vitro* significant biological activities. In conclusion, *A. andrachne* L. might be considered as a potential source which could be developed as precursors for antimicrobial and antioxidants drugs. After additional studies should be performed to analyze cytotoxic and pharmacological properties.

Conflict of interest

The authors declare no conflict of interests.

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Investigation of Çanakkale urban cemeteries within the scope of landscape planning criteria

Yavuz ALKAN

Onsekiz Mart University, Lapseki Vocational School, Department of landscape and ornamental plants, 17800 Lapseki/Çanakkale. Corresponding author: <u>valkan58@comu.edu.tr</u>

Abstract

Cemeteries are areas which have been important to humanity since history began, with a place in every period of human life and sustained importance. As a result, they are currently planned in settlement areas or in areas close to cities. The study researched the landscape infrastructure and furnishings of two city cemeteries with an important place in the current structure of Canakkale city. The landscape elements based on the planned and present structures were determined in these areas of "Canakkale Municipality City Cemetery" and "New City Cemetery". For this, literature screening and data obtained from observations represent the basis of the study. Within the scope of the obtained data, it was identified that the main criteria for cemetery planning and design were not included sufficiently in construction of the two cemeteries. The main findings in relation to landscape infrastructure and furnishings are that main and side roads do not abide by standards; there is esthetic and functional insufficiency in terms of paving, car parks, distance between graves and planting; there is insufficient multifunctional space at the entrance for collection/distribution and rest; there is insufficient seating groups and boundary elements for shade and rest; and there are no sections for maintenance, security, WC, prayer rooms, florists, or open/enclosed mourning sections in buildings at the entrance. It was identified that Canakkale Municipality City Cemetery requires 75 more fountains, while New City Cemetery requires 66 more fountains and the grave area per person is close to the recommended standard (6 m²). Additionally, the New City Cemetery was calculated to have potential for 11,266 graves. In conclusion, in line with the obtained data, attempts are made to develop recommendations to solve problems in these study areas. Keywords: Canakkale, city cemeteries, landscape planning.

Özet

Mezarlıklar insanlığın tarihiyle başlamış, her dönem insan hayatında yer almış ve önemini devam ettirerek sürdürmüş alanlardan birisidir. Bu nedenle günümüzde de yerleşim alanlarında ya da kente yakın yerlerde planlanmaktadır. Çalışmada Çanakkale kentinde mevcut yapısıyla önemli bir yer arz eden iki kent mezarlığının peyzaj altyapı ve donatıları arastırılmıştır. "Canakkale Belediyesi Sehir Mezarlığı" ve "Yeni Sehir Mezarlığı" olarak öne çıkan bu alanlarda planlanmış mevcut yapı itibariyle peyzaj ağırlıklı öğeler belirlenmiştir. Bunun için literatür taraması ile gözlem sonucunda elde edilen veriler calısmanın temelini teskil etmektedir. Elde edilen veriler kapsamında mezarlık planlama ve tasarımına yönelik başlıca kriterlere her iki mezarlık yapılanmasında çok fazla yer verilmediği tespit edilmiştir. Öyle ki; ana ve ara yollarda standartlara uyulmaması, zemin döşeme, otopark, mezarlar arası mesafe ve bitkilendirmedeki estetik ve fonksiyonel yetersizlikler, girişte yeterli ölçüde toplanma/dağılma, dinlenme gibi çok fonksiyonlu meydana yer verilmemesi, gölge ve dinlenme amaçlı oturma grupları ve sınır elemanları yetersizliği ile yine girişte yapı bileşenleri kapsamında idare, güvenlik, wc, mescit, çiçekçi, açık/kapalı taziye bölümlerinin olmaması peyzaj altyapı ve donatıları kapsamında öne çıkan bulgular olmuştur. Çanakkale Belediyesi Şehir Mezarlığı için 75; Yeni Şehir Mezarlığı için 66 çeşmeye daha ihtiyaç olduğu; kişi başına mezar alanlarının standarda yakın (6m²) olduğu da tespitler arasında yer almaktadır. Ayrıca Yeni Şehir Mezarlığı için 11266 mezar potansiyelinin olduğu da hesaplanmıştır. Sonuç olarak, elde edilen veriler doğrultusunda söz konusu çalışma alanlarına yönelik çözüm önerileri geliştirilmeye çalışılmıştır. Anahtar kelimeler: Çanakkale, kent mezarlıkları, peyzaj planlama.

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Introduction

Urbanization continuously develops with the impetus of the urban population potential increasing through internal and external migration and linked to this, involves a changing and developing dynamic city planning and design for urban furnishings. Generally during city planning, elements like population structure, groups and potential population in the future (population projections) are ignored (Özhancı and Aklıbaşında, 2017). As a result, we cannot get ahead of rapid urbanization. Linked to rapid urbanization, it is common that urban green spaces and urban lands are consumed in slapdash fashion, even encroached. However, in consolation, city cemeteries are preserved as urban green spaces and ensuring their sustainability and permanence will guarantee this important city component (Sarı and Koçak, 2005). Linked to increasing population, the dimensions of city cemeteries change and develop. Linked to rapid urbanization, cemeteries are one of the problems occurring in metropolitan cities (Afla and Reza, 2012). At the point of identifying grave locations, the effects of local and administrative management are dominantly mentioned. An unplanned and unformulated approach limits the working areas for urban planners or landscape architects and at the same time pushes the welfare of society into the background (Lehrer, 1974). At this point, planning, design and economy of use of grave locations are important dimensions. There is a need for evaluation of metropolitan cemeteries within the scope of public services in terms of variety and wealth of use and for evaluation for recreational aims (Afla and Reza, 2012; Lehrer, 1974). The principle for calculating cemetery area in urban planning changes linked to information such as population projections for the town 20-30 years later, estimated death rates, and unit grave area (Uslu, 1997). Though cemeteries were designed to be outside cities previously in history, over time they have been included in the city settlement and have taken a place as open and green spaces in human life. This situation has led to cemeteries becoming insufficient linked to the increase in population over time. It is unavoidable that during this process new locations appropriate to standards outside the city, in the style of the satellite town model, must be planned as cemeteries. Cemeteries are locations where the bodies of the dead are buried without loss of time and without harmful effects to the environment and especially humans. At the same time, they are symbolic areas representing the other dimension of life that is worth memorializing for the remaining community (Uslu, 2009). According to the urban texture "cemeteries", indexed to humans and their lives throughout our existence, with location prioritized by distance, are important urban green spaces that should be evaluated in terms of physical dimensions like soil structure, field slope, climate characteristics, dominant wind direction, ground water, aspect, elevation, architecture and planting (Uslu, 2009; Özkardaş, 2010; Capels and Senville, 2006). Additionally, factors like beliefs, traditions/customs/habits, health conditions, and burial methods shape cemeteries. The consideration of the belief that life continues after death suggests that cemeteries are eternal resting places. In line with this, attempts are made to keep the esthetic effect of cemeteries high. In fact, even food, clothing and personal items are placed on graves (Akdoğan, 1962). Additionally, the results of locating cemeteries with mistaken strategies affecting groundwater near the city comprises a risk factor in terms of the city's inhabitants. At the same time, this situation primarily affects human and environmental health but also affects welfare levels (Üçışık and Rushbrook, 2001). However, when planning these spaces rationality and necessary criteria that should be primarily considered are often ignored, making it possible they are insufficient both in terms of urban esthetics (urban identity) and benefit (functionality). This study attempts to examine the present landscaping and furnishings in two city cemeteries located in urban Çanakkale. Within the text "Çanakkale Municipality City Cemetery" is referred to as "Old Cemetery", while the "New City Cemetery" is referred to as the "New Cemetary". Within this scope, the main insufficiencies in planning and design of these areas were identified. Finally, recommendations are made about development of these areas.

Material and Methods

This study was completed in two important urban cemeteries in Çanakkale of the "Çanakkale Municipality City Cemetery" and "New City Cemetery". The "Çanakkale Municipality City Cemetery" is located in the city, while the "New City Cemetery" is located outside the city adjacent to the "15 July Democracy Bus Station". The "Çanakkale Municipality City Cemetery" is located at coordinates 40° 9' 5.79" north 26° 26' 42.36" east and has 90 da area (Figure 1).



Figure 1. Location of Çanakkale Municipality City Cemetery (adapted from Google Earth (2019))

Located outside the city, the "New City Cemetery" has coordinates 40° 9' 4.99" north 26° 27' 34.68" east and has 93.6 da area (Figure 2). The area measurements and coordinates were determined from Çanakkale Implementation Development Plan and Google Earth (2019). The study conceptualized the identification of landscape infrastructure and furnishings within the scope of these cemeteries located within the urban green space system. With this aim, initially the areas were visited for observations in an attempt to analyze the current status. For this, first planting and plant types were identified. Secondly the structural (physical) status of the cemeteries were determined. In this stage, attempts were made to access current data through contact with the Çanakkale Municipality Cemetery and Burial Operations Unit. Finally, findings obtained through analysis in terms of visuals, technical and theoretical details were evaluated within the scope of landscape planning and design criteria, cemetery planning criteria, and laws and directives, and attempts were made to recommend solutions for the areas. With this aim, comparisons were made of data from some national- and international-based studies with data from this study.

Results

The "New Cemetery" is a newly created cemetery due to the lack of burial potential in the "Old Cemetery". The area consists of 20 blocks, with grave construction on these blocks having a planned structure. According to burial density, the number of blocks can be increased to include more graves. The area criteria were identified as 93,600 m² and 90,000 m², respectively. The findings obtained for both study areas can be listed in general below. Of the cemeteries, the Municipality City Cemetery is

within the town, while the other is located adjacent to the bus station and has no limits in terms of access.



Figure 2. Location of Çanakkale New City Cemetery (adapted from Google Earth (2019))

Within the scope of insufficient landscape planning components, for the Old Cemetery it is possible to list pedestrian footpaths, more than one esthetic and functional entrance, mourning area at the entrance, sufficient/planned car park, seating groups for resting, side roads with paving in block plans, maintenance/service building providing washing (abdest) facilities, morgue, prayer room, WC, storage, security, lighting units, florist, ironmonger, stonemason, warning/information panels, and management/doctor/religious personnel services, wide/standard side roads between graves, boundary elements and fountains. For the New Cemetery, the list includes road (alles) tree planting, uniform/planned/esthetic, functional and ecological planting (Usta et al., 2018), throughout the area, square for collection/distribution/mourning at the entrance, planned car park, seating benches, paving of side roads, maintenance/service building with security, florist, ironmonger, stonemason, warning/informative panels and management/doctor/religious personnel services building with security, florist, ironmonger, stonemason, warning/informative panels and management/doctor/religious personnel services, planned car park, seating benches, paving of side roads, maintenance/service building with security, florist, ironmonger, stonemason, warning/informative panels and management/doctor/religious personnel services, more than one entrance, boundary elements and fountains (Odabaş et al., 1994).

Sufficient landscape planning components; can be stated as sound system, lighting units, sufficient metal waste containers, washing (abdest), morgue, prayer room, WC and storage facilities at the entrance, separate entry planning for vehicles and pedestrians, evergreen trees, and inclusion of small trees and bushes for the New Cemetery (Figure 3) (Odabaş et al., 1994). For the Old Cemetery, these elements can be listed as separate entry planning for vehicles and pedestrians, plant types included in planting (evergreen trees, small trees and bushes) (Figure 4) and amounts are uniform and sufficient, side roads with asphalt or parquet/keystone paving, metal waste containers, access system within the cemetery and main roads being close to standard.

Figure 3. Views from Çanakkale New City Cemetery (New Cemetery).

As seen in Table 1, the area of the Old Cemetery is $90,000 \text{ m}^2$, and is a cemetery which has fulfilled its potential for 14,500 filled graves. The area per grave was calculated as 6.2 m^2 (90000/14500) and this is at standard levels (Anonymous, 2011). Data was reached that there are currently 70 fountains (Anonymous, 2019a). However, as stated by Özkan et al. (1996), it is necessary to provide 1 fountain

per 75-100 graves, and it was identified that the cemetery should have 145 fountains (145000/100) based on the current number of graves in the cemetery.



Accordingly, it was concluded that there is a need for 75 more fountains. The New Cemetery has $67,600 \text{ m}^2$ empty area, with a total area of $93,600 \text{ m}^2$ and $26,000 \text{ m}^2$ filled grave area. Additionally, information obtained from counts by Municipality personnel on 09.07.2019 identified 4369 filled graves and 90 fountains. With the calculation 4369/100=43.69 fountains required, there were 90 fountains present. Additionally, the grave potential of the cemetery was calculated as 93600/6=15,600 graves. According to current data, with current $67,600 \text{ m}^2$ empty area, the cemetery had potential for 11,266 further grave sites (67600/6). According to the literature, this requires 15600/100 or 156 fountains. Currently there are 90 fountains showing 66 more fountains are required. Additionally, it is notable that the current status has 5.95 m^2 area per grave, so the "New Cemetery" is at standard levels.

An attempt is made to list the decorative plant groups commonly used in the Old and New Cemeteries. Decorative plants in the tree/small tree group: *Cupressus sempervirens*/Mediterranean cypress, *Cupressus arizonica*/Arizona cypress, *Cupressus macrocarpa*/Monterey cypress, *Pinus pinea*/stone pine, *Catalpa bignonioides*/southern catalpa, *Ligustrum vulgare*/wild privet, *Olea europaea*/olive, *Platanus orientalis*/oriental plane, *Morus sp.*/mulberry sp., *Melia azedarach*/chinaberry, *Pyrus communis*/European pear, *Pyrus elaeagnifolia*/oleaster-leaf pear, *Punica granatum*/decorative pomegranate, and *Ulmus minor*/field elm. Decorative plants in the shrub group: *Rosmarinus officinalis*/rosemary, *Euonymus japonica*/evergreen spindle, *Pyracantha coccinea*/scarlet firethorn, and *Rosa sp.*/rose species. Decorative plants in the creepers, climbers and ground cover group: *Lampranthus roseus*/rosy dewplant, *Narcissus pseudonarcissu*/wild daffodil, *Lilium sp.*/lily species, *Portulaca grandiflora*/moss rose, *Lavandula officinalis*/lavender, *Tradescantia zebrine*/inch plant, *Osteospermum ecklonis*/cape marguerite, *Tagetes erecta*/marigold, and *Cedrus libani glauca*/Lebanon cedar (Figure 3-4).

	Çanakkale Municipality	New City Cemetery/ New Cemetery		
	City Cemetery/Old Cemetery (m ²)	(m ²)		
Area (m ²)	90000	93600		
Empty area (m ²)	_	67600		
Full area (burials) (m ²)	90000	26000		
Number of graves (no)	14500	4369/15600		
Number of fountains	70	90		
Number of fountains required according to total number of graves (no)	145 (14500/100)	156 (15600/100)		
Number of fountains required (no)	75 (145-70)	66 (156-90)		
Cemetery area per person (m ²)/person	6.2 (90000/14500)	Current status: 5.95 (26000/4369)		
Cemetery area required for 6 m ² per grave	87000 (14500x6)	15600 (93600/6)		
Number of graves required (no)	_	11266 (67600/6)		

Table 1. Data obtained in relation to Çanakkale city cemetery (Anonymous, 2019a)

Per grave, 6 m² is the required standard area (Anonymous, 2011)

There should be 1 fountain for every 75-100 graves (Özkan et al., 1996)

In the current empty area of the New City Cemetery, there is potential for 11266 more graves.

There is a need for 75 more fountains for Çanakkale Municipality Cemetery and 66 more fountains for the New City Cemetery.



Figure 4. Views from Çanakkale Municipality City Cemetery (Former Cemetery).
Discussion

In Currently it is not very accurate to say that cemeteries are spaces only designed for the dead. This is because if funeral procedures and mourning activities are assumed to be integral to cemeteries, the necessity to evaluate these areas as social spaces is revealed. As a result, it is necessary to shape these areas from multiple aspects and with different criteria, led by grave planning and design but incorporating laws and directives, regional climate characteristics, dominant wind, soil structure, topography, geographic, geologic and geophysical features, aspect and elevation. Apart from this, when landscape infrastructure and furnishings are considered together, it will be possible for the area to serve more rationally. The study by Lehrer (1974) emphasized that in terms of solution recommendations to save cemeteries from derelict structure and unmaintained form, demands for use in line with planning for recreational or social activities will be a gain in terms of society and the town. Among his proposed uses, there were alternatives including design of grave cities and many bodies in shrine models in multistory residences. In this way, the excess amount of open space in cemeteries was considered. Here the proportion of open and green spaces in the urban texture, and as a result amount of green space per person, naturally increases. In this framework, an increase in the working area for urban planners, especially landscape architects may be ensured. Afla and Reza (2012) dealt with investigation of current problems with Malay cemeteries and the equipment and uses of Muslim cemeteries in the Kuala Lumpur metropolitan region. In conclusion, they developed applicable design recommendations. Üçışık and Rushbrook (2001) investigated the mixing of gases and products resulting from physiologic and chemical decomposition of cemetery burials with groundwater. The study results developed recommendations about improving these waters and locations and design of future grave sites. There is a need to deal with cemetery planning with the same sensitivity as any other urban uses in the structure of developed cities and to structure planning and design (Capels and Senville, 2006). Cemeteries in rural America from the 1850s are counted as strong symbols of the period. The esthetic and teaching qualities of these strong structures in relation to this period come to the fore. A thesis study by Buckley (2013) attempted to develop opinions and recommendations to guide development of these areas. The study by Özkan et al. (1996) emphasized that in planning cemeteries, slope of 15% at most may be appropriate; however, the use of flat or close to flat areas as cemeteries may cause a drainage problem and not be appropriate. Additionally, the same study stated that main access roads should be 12-18 m, while side roads should be 2-4 m (Cetiner, 1991; Güçlü et al., 1996; Anonymous, 2011; 2019a; b). According to these criteria, when the current status of the cemeteries included in this study are examined, the Old Cemetery is located on a flat/close to flat area and is distant from planning norms. The New Cemetery has appropriate qualities in terms of the slope factor. The cemetery entrances and side roads within the cemeteries appear to be close to standard in both cemeteries. Anonymous (2019b) stated the need to surround the cemeteries with 2 m walls according to the Municipal Cemetery Regulations. This is the situation in the Old Cemetery. However, it is clear this is not very rational because this structure traps air within the cemetery and odors that may spread may reach uncomfortable dimensions. At the same time, this is not a very attractive aspect in terms of visitors to the cemetery. In fact, this may lead to shorter visits by relatives of the dead, and even be a repellent force in relation to this topic. If these areas are surrounded by more open boundary elements, supported by esthetic and functional landscape infrastructure and furnishings, they will become more attractive and may even gain use for recreational aims, as in other countries.

According to Erkan (1983) emphasized the necessity to calculate 7 m² area for each grave in a cemetery. The same area was stated as 6 m² by Anonymous (2011; 2019a; b). With the calculation by Erkan (1983), Akbulut and Önder (2011) found 1.7 m² area per person in a study of Aksaray city cemeteries, while Güçlü et al. (1996) found area of 1.2 m² grave area per person in a study of the Contemporary and New Cemetery located in Erzurum urban area. According to the same calculation,

Aksaray was identified to need 894,401 m^2 cemetery area, while Erzurum required 146.44 ha additional cemetery area. The study by Özhancı and Aklıbaşında, (2017) found 254 ha total cemetery area with 2.7 m^2 grave area per person. According to the standard, 405 ha additional area was required. It was revealed that Çanakkale city cemeteries have values close to the standard for area measurements.

In this study, physical dimensions of "Çanakkale Municipality City Cemetery" and "New City Cemetery" were dealt with and the following recommendations are made in relation to variation and development.

- The use of higher walls as boundary elements will have cold and repellent force in addition to trapping bad odors and will unavoidably have limiting effects on visitors and surroundings. As a result, boundaries should use functional and esthetic green plant cover.
- Especially road tree planting was fully planned in Çanakkale Municipality City Cemetery, while it was included in the planning for the New City Cemetery but does not have sufficient dimensions. Additionally, it is not possible to say the planting design has homogeneous distribution in the area in general. As a result, the planting design in the New City Cemetery should have equal distribution and use esthetic/functional plant types.
- Planting work should address the area in general, the planning should not both grave and burial sections, with cemetery planting in line with functional and esthetic principles and of sufficient amounts
- Circulation within the cemetery should be developed
- Open and enclosed mourning and resting spaces should be constructed
- Entrances should be organized and developed for esthetic and functional aspects
- Circulation within the cemeteries should be developed and collection-rest areas should be created
- Structural units to meet administrative, religious, individual/social needs (flowers, stones/marble, etc.) at the entrance should be included
- Car park planning developed in line with capacity and security
- Increased landscape furnishings like resting/seating groups, waste containers, information panels/signs, fountains
- Development of security and lighting facilities
- Studies about planning the area by developing psychologically healing designs should be performed.

In conclusion, though the areal dimensions of Çanakkale city cemeteries are sufficient, it appears the landscape infrastructure and furnishings are insufficient. The study mentions the sufficient and insufficient criteria of the cemeteries and reveals the necessity to abide by standards and criteria in order to achieve contemporary cemetery planning. Currently it is necessary to ensure the use of cemeteries by individuals for recreational purposes as open and green spaces in the city (Odabaş et al., 1994). For this, important tasks await municipalities, initially, along with other local administrators.

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Seven years of arboreal pollen monitoring in the İğneada waterlogged forests (NW Turkey)

Nurgül KARLIOĞLU KILIÇ

Istanbul University-Cerrahpaşa, Faculty of Forestry, Department of Forest Botany, 34473, Bahçeköy, Istanbul, Turkey. Corresponding author: <u>nurgulk@istanbul.edu.tr</u>

Abstract

This study was carried out in the İğneada Waterlogged Forests (Kırklareli) located downhill of Istranca mountains at 20 km distance to Bulgaria border line and had a shore to Black Sea. These forests have different vegetation types such as waterlogged forest, peat, bog and sand dunes plant communities, sand zone and wetlands. The main arboreal species of these forests are Acer campestre, Acer trautvetteri, Alnus glutinosa, Carpinus betulus, Carpinus orientalis, Fraxinus angustifolia, Fraxinus ornus, Juglans regia, Quercus cerris, Quercus frainetto, Quercus petraea, Quercus robur, Ulmus leavis and Ulmus minor. Pollen monitoring results were obtained from six different sampling sites in the waterlogged forests around Mert and Saka Lake. The purpose of this study is to determine the modern pollen distribution of these forests and to create a basic calibration scheme for the fossil pollen studies. For this purpose, seven years of modern pollen distribution was monitored between September 2009 and 2016 using Tauber pollen traps which were placed at 6 different points in this study area. These traps were changed once a year in the field and transferred to the Palynology Laboratory of Istanbul University-Cerrahpaşa. The protocol of European Pollen Monitoring Programme (EPMP) was followed in the laboratory methodology. The majority of the pollen influx obtained from the Tauber pollen traps in the İğneada Waterlogged forests belongs to Fraxinus and Carpinus from 2009 to 2016. As a result of this study, the annual arboreal pollen influxes of Fraxinus, Carpinus, Quercus, Acer, Corylus, Ulmus, Hedera helix and Alnus was higher respectively in the years of 2009-2016. Seven-year pollen influx data of these pollen traps reflects the current vegetation.

Keywords: European Pollen Monitoring Programme, Tauber pollen trap, İğneada.

Özet

Bu çalışma, Bulgaristan sınırına 20 km uzaklıkta İstranca Dağları'nın aşağı yamaçlarında bulunan ve Karadeniz'e kıyısı olan İğneada Longoz Ormanları'nda gerçekleştirilmiştir. Bu ormanlar; subasar orman, turba, bataklık ve kumul bitki toplulukları, kumsal ve sulak alanlar gibi farklı vejetasyon tiplerine sahiptir. Bu ormanların önemli odunsu taksonları Acer campestre, Acer trautvetteri, Alnus glutinosa, Carpinus betulus, Carpinus orientalis, Fraxinus angustifolia, Fraxinus ornus, Juglans regia, Quercus cerris, Quercus frainetto, Quercus petraea, Quercus robur, Ulmus leavis ve Ulmus minor'dür. Polen izleme sonucları, Mert ve Saka gölü etrafında bulunan subasar ormanlarının icindeki altı farklı örnek alanından elde edilmiştir. Bu çalışmanın amacı, bu ormanların güncel polen dağılımını belirlemek ve fosil polen çalışmaları için temel bir kalibrasyon şeması oluşturmaktır. Bu amaçla, çalışma alanında 6 farklı noktaya yerleştirilen Tauber polen tuzakları kullanılarak Eylül 2009 ve 2016 yılları arasında yedi yıllık güncel polen birikimi izlenmiştir. Bu tuzaklar yılda bir kez arazide değiştirildikten sonra İstanbul Üniversitesi-Cerrahpaşa Palinoloji Laboratuvarı'na getirilmiştir. Laboratuvar metodolojisinde Avrupa Polen İzleme Programı (European Pollen Monitoring Programme: EPMP) protokolü takip edilmiştir. İğneada Longoz Ormanlarındaki tuzaklardan 2009-2016 yılları arasında elde edilen polen yoğunluğunun çoğunluğu Fraxinus ve Carpinus'a aittir. Bu çalışmanın sonucu olarak, 2009-2016 yılları arasındaki yıllık odunsu bitki polen yoğunluğu sırasıyla Fraxinus, Carpinus, Quercus, Acer, Corylus, Ulmus, Hedera helix ve Alnus'ta fazla cıkmıştır. Polen tuzaklarına ait 7 yıllık polen yoğunluğu verileri güncel vejetasyonu yanşıtmaktadır. Anahtar kelimeler: Avrupa Polen İzleme Programı, Tauber polen tuzağı, İğneada.

Introduction

Monitoring the modern pollen influx for many years is very important in order to determine the paleovegetation and climate of Quaternary based on the fossil pollen studies. There is little knowledge about the distribution and sedimentation processes of the modern pollen grains and how they represent the current vegetation at the regional and local scale. In accordance with this purpose, European Pollen Monitoring Programme (EPMP) was established in 1996 and modern pollen-monitoring stations have been created in many European countries during the last 23 years. Pollen influx data has been obtained by monitoring the pollen accumulation at varying vegetation points by using Tauber pollen traps properly according to the protocol of EPMP (Hicks et al., 1996; Hicks, 2001; Van der Knaap et al., 2001; Pidek, 2004; Tonkov et al., 2001; Atanassova, 2007; Filipova-Marinova et al., 2007; Giesecke et al., 2010; Tonkov et al., 2016).

The modern palynological studies conducted in Turkey are mostly about pollen allergy, pollen analysis in the atmosphere and airborne pollen concentration. In these studies; Hirst Spore, Burkard and Lanzoni style traps were used at a certain height and a lot of pollen calendars belong to many provinces of Turkey were created with this method (Aytuğ et al., 1974; İnce and Pehlivan, 1990; Pehlivan and Bütev, 1994; İnceoğlu et al., 1994; Kaya and Aras, 2004; Bıçakçı, 2006; Öneş et al., 2008; Erkan et al., 2011; Bıçakçı and Tosunoğlu, 2019).

Among these studies, the first pollen monitoring study in Turkey was conducted as a doctorate thesis in the context of EPMP, which aims to find out the relationship between meteorological parameters and monthly pollen influx in Belgrad Forest and İğneada Longoz Forest between 2007 and 2009 (Karlıoğlu, 2011; Karlıoğlu and Akkemik 2012, Karlıoğlu et al. 2014, Karlıoğlu et al. 2015). EPMP studies have been continued in different regions of Turkey in accordance with this protocol (Şenkul et al. 2018a, Şenkul et al. 2018b; Şenkul et al. 2018c; Karlıoğlu Kılıç et al., 2019; Şenkul and Karlıoğlu Kılıç, 2019).

The aim of this study is to investigate the seven years of arboreal pollen monitoring in the different sample areas of İğneada Waterlogged Forests in accordance with the EPMPP. This study will be a basic calibration scheme in order to better interpret the quantitative reconstructions of previous fossil pollen diagrams.

Material and Methods

Study Site

İğneada Waterlogged (Longoz) Forests belong to Kırklareli-Demirköy province locates downhill of Istranca mountains at 20 km distance to Bulgaria border line and has shore to Black Sea. According to Davis' (1965-1985) grid system locates in A1 (E) square. The altitudinal profile of İğneada waterlogged forests is between 0-20 m (Fig.1). These forests have different vegetation types such as waterlogged forest, peat, bog and sand dunes plant communities and sandy meadows at river side banks, sand zone and wetlands. They are accepted as one of the 122 Important Plant Areas of Turkey (Özhatay et al 2005). The dominant arboreal plant species in the area are *Acer campestre* L. (Common Maple), *Alnus glutinosa* (L.) Gaertn. (Common Alder), *Fraxinus angustifolia* Vahl. (Narrow-leafed Ash), *Quercus cerris* L. (Turkish Oak), *Quercus frainetto* Ten. (Hungarian Oak), *Quercus robur* L. (Pedunculate Oak), *Quercus petraea* (Mattuschka) Liebl. (Sessile Oak), *Fagus orientalis* Lipsky. (Oriental Beech), *Carpinus betulus* L. (Common Hornbeam), *Carpinus orientalis* (Hornbeam), *Ulmus minor* Miller (Common Elm) and *Ulmus leavis* Pall. (European White Elm). *Fraxinus angustifolia* is the most widespread tree species in the İğneada Waterlogged Forests. Another tree species, which has a wide spread like ash, is common maple. There is a rich ecological diversity in this forest, which has a total area of approximately 1600 ha (Kavgacı, 2007; Karlıoğlu, 2011).



Figure 1. Location map of the study area and position of the Tauber pollen traps in the sample areas ("red circles" show the location of the pollen traps in the field)

Modern Pollen Analysis

Tauber pollen traps were placed in six sample areas (TMF1, TMF2, TMF3, TSF1, TSF2 and TSF3) of İğneada Waterlogged Forests where the vegetation changed in 2007 for the first time and these traps were changed monthly between September 2007 and 2009 as a doctorate thesis (Figure 1). Then, these same traps were changed annually between 2009 and 2016. Each Tauber pollen trap (Tauber, 1974) was brought from the field to the Palynology Laboratory of Istanbul University-Cerrahpaşa. For every 1-year period, the mixture in the each trap was used for the modern pollen analysis according to the protocol of EPMP (Hicks et al., 1996). Every mixture in each trap was filtered by using a sieve of 250 mµ and removed from animal and plant remains (Hicks et al., 1996). Two *Lycopodium* spore tablets (Stockmarr 1971) were added into this mixture,

and this mixture was centrifuged (10 minutes at 3000-3500 rpm) until reaching the sediment. Then 10 ml acetolysis mixture was added to the resulting sediment. After acetolysis process, pollen slides were prepared by adding 2 ml of glycerin. Pollen grains and *Lycopodium* spores were counted by Leica DM 750 light microscope with x40 and x100 immersion objective and x10 ocular. Reference slides and illustrated pollen keys were used for the identification of pollen grains (Aytuğ, 1967; Aytuğ et al., 1971; Wodehouse, 1935; Erdtman, 1952; 1957; Hyde and Adams, 1958; Faegri and Iversen, 1964; Moore et al., 1991; Hesse et al., 2009; Iwanami et al., 1988). Also, arboreal plants around the traps (0-10.5 m) were identified using the vegetation ring.

Results

Annual pollen influxes (cm²/year) of the arboreal plants around the traps have been determined between 2009 and 2016 in the six sample areas of İğneada Waterlogged Forests (Figure 2; Figure 3). Also, the seven years of arboreal pollen monitoring diagram was drawn in TILIA (Figure 4).

Annual Pollen Influx in the Mert Lake Waterlogged Forest between 2009-2016

The pollen trap in the open area (TMF1) was missing between 2009 and 2010 in the Mert Lake Waterlogged Forest. *Carpinus* and *Fraxinus* have the highest annual pollen influx (cm²/year) between 2009 and 2011 in TMF3 (in the Forest). The pollen trap in the forest area was missing from 2011 to 2012. Annual pollen influxes of *Fraxinus, Carpinus* and *Quercus* were higher in the forest area (TMF3) between 2011 and 2012. In the years of 2012-2013, the annual pollen influx of *Carpinus, Fraxinus and Acer* was higher in all sample areas of Mert Lake Waterlogged Forest. The pollen trap in the open area was missing between 2013 and 2014. Annual pollen influxes of *Carpinus, Fraxinus, Corylus* and *Hedera helix* were higher in the forest and at the forest edge in the years of 2013-2014. The pollen trap in TMF3 was missing from 2014 to 2016. Annual pollen influx was very low from 2014 to 2015. In this year, the highest annual pollen influxes belong to *Fraxinus* and *Carpinus* in TMF1 and TMF2. When the annual arboreal pollen influx in the years of 2015-2017, pollen influxes of *Ulmus, Fraxinus, Carpinus, Acer* and *Alnus* were higher in TMF1 and TMF2. In 2016-2017, pollen influx of *Ulmus* was very high when it was compared with the other years (Figure 2; Figure 4).

3.2. Annual Pollen Influx in the Saka Lake Waterlogged Forest between 2009-2016

The pollen trap in the forest area (TSF3) was missing between 2009 and 2010 in the Saka Waterlogged Forests. Carpinus, Fraxinus and Quercus have the highest annual pollen influx (cm²/year) between 2009 and 2010 in TSF2 (forest edge) and in the open area (TSF1). The pollen traps in TSF1 and TSF2 were missing from 2010 to 2011. When the annual arboreal pollen influx in the years of 2010-2011 was examined in TSF3, the pollen influxes of Fraxinus, Corylus, Acer and Carpinus were higher in this sample area. The pollen trap in TSF2 was missing between 2011-2012. Annual pollen influxes of Carpinus, Fraxinus, Ouercus and Corylus were higher in the forest area (TSF3) and open area (TSF1) from 2011 to 2012. All the traps were missing in the years of 2012-2013. The traps in TSF1 and TSF2 were also missing between 2013 and 2014. When the annual arboreal pollen influx in the years of 2013-2014 was examined in TSF3, the pollen influxes of Fraxinus, Corylus, Acer, Hedera helix and Quercus were higher in this sample area. In the years of 2014-2015, the pollen trap in TSF2 was missing. Annual pollen influxes of Fraxinus, Corylus, Carpinus Hedera helix and Alnus were higher in the forest area (TSF3) and open area (TSF1) from 2014 to 2015. The pollen trap in TSF2 was missing between 2015 and 2016 again. When the annual arboreal pollen influx in the years of 2015-2016 was examined, the pollen influxes of Corylus, Carpinus, Fraxinus, Quercus and Alnus were higher in TSF1 and TSF3. Total annual pollen influx in the years of 2015-2016 was very higher than the other years (Figure 3; Figure 4).



Figure 2. Annual pollen influx (cm²/year) of arboreal plants in Mert Lake Waterlogged Forest between 2009-2016.(TMF1: Open areaTMF2: Forest edgeTMF3:In the Forest area)





Figure 4. Seven years of arboreal pollen monitoring in İğneada Waterlogged Forests between 2009 and 2016.

The arboreal plant species around the Tauber pollen traps

The identification of the arboreal plant species around the Tauber pollen traps is very important to show how far the pollen grains are transported in the sample areas. Therefore, all woody plant taxa around the pollen traps were listed in Table 1.

TMF1	Table 1. The arboreal plant species around pollen traps surrounding circle with the radius 10.5 m Acer campestre, Crataegus monogyna, Fraxinus angustifolia, Prunus x domestica, Rubus sanctus, Ulmus minor.
TMF2	Acer campestre, Crataegus monogyna, Fraxinus angustifolia, Hedera helix, Prunus x domestica, Rosa canina, Ruscus aculeatus, Smilax excelsa, Ulmus minor.
TMF3	Acer campestre, Carpinus betulus, Corylus avellana, Fagus orientalis, Fraxinus angustifolia, Hedera helix, Rubus sanctus, Ruscus aculeatus, Smilax excelsa, Sorbus torminalis, Tilia tomentosa.
TSF1	Fraxinus angustifolia, Pyrus elaeagnifolia, Rosa canina.
TSF2	Acer campestre, Crataegus monogyna, Fraxinus angustifolia, Hedera helix, Malus sylvestris, Quercus robur, Rosa canina, Ruscus aculeatus, Smilax excelsa.
TSF3	Acer campestre, Carpinus betulus, Cornus mas, Corylus avellana, Crataegus monogyna Fraxinus angustifolia, Hedera helix, Quercus robur, Ruscus aculeatus, Smilax excelsa.

Discussion

According to the results of pollen analysis in the pollen traps, the annual pollen influxes (cm²/year) of *Fraxinus, Carpinus, Quercus, Acer, Corylus, Ulmus, Hedera helix* and *Alnus* was higher respectively between 2009 and 2016 in the İğneada Waterlogged Forests. Total annual pollen influx between 2015 and 2016 was higher than the other years. Seven-year arboreal pollen influx data of pollen traps reflect the current vegetation in this forest. Karlıoğlu and Akkemik (2012), Karlıoğlu et al. (2015), Şenkul et al. (2018a; 2018b), and Karlıoğlu Kılıç et al. (2019) found that annual pollen influx (cm²/year) is very related to the modern vegetation in the different regions of Turkey.

Pinus sp. does not exist in all sample areas of İğneada Waterlogged Forests, but it has pollen influx in the all traps. Pollen grains of *Pinus* have 2 air-filled bladders (sacci), and these air sacs increase surface area, but don't increase the pollen mass. Thereby, the pollen grains of *Pinus* can be easily transported to long distances by wind (Schwendemann et al., 2007; Szczepanek et al., 2017).

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Growth and yield models for uneven-aged forest stands managed under a selection system in northern Iran

Siavash Kalbi^{1,*}, Asghr Fallah¹, Shaban Shataee³, Pete Bettinger³, Rassoul Yousefpour⁵

^{1,*)} Sari Agriculture and Natural Resource University, Sari, Iran

³Gorgan Agriculture and Natural Resource University, Golestan, Iran

⁴⁾ Warnell School of Forestry and Natural Resources, University of Georgia, Athens, USA.

⁵⁾ Chair of Forestry Economics and Forest Planning, Faculty of Environment and Natural Resources, University of

Freiburg, Freiburg, DE, Germany Corresponding author: s.kalbi63@gmail.com

Abstract

Predicting future forest growth and yield is a key element of sustainable forest management. Hyrcanian forests are the most valuable forests in the north of Iran, and industrial harvesting occurs only in this area of the country. While uneven-aged Hyrcanian forests are one of the most important vegetated areas, and the only commercial forests in Iran, there is a lack of growth and yield models for management and planning purposes. The aim of this study is to develop distance-independent individual tree growth and yield models for uneven-aged forests in northern Iran managed under selection systems. A distance-independent diameter growth model, a static height model, an ingrowth model, and a survival model for uneven-aged stands of *Fagus orientalis* Lipsky were developed using measurements from Sangdeh, within the Mazandaran providence in Iran. The models are based on 130 permanent sample plots established in 2009 and remeasured in 2014. For modeling diameter and height growth, we employed a mixed effect regression. For modeling survival, we used binary logistic regression analysis. Ingrowth was modeled using multilinear regression. Results showed the best growth and yield model had relative RMSE and bias values, respectively, that were 31.9% and 6.3% for the diameter growth model, 11.3% and 0.17% for the height model, and 22% and 0.14% for the ingrowth model. Wald tests and other model evolution parameters showed that the parameter estimates for tree mortality were statistically significant. Overall results indicated that growth and yield model performance was consistent with expectations, and that the general fit to the validation data was acceptable.

Keywords: Individual-tree model, mixed-effect regression, diameter and height growth, tree mortality, Sangdeh.

Introduction

Uneven-aged forestry is a popular and acceptable method of forest management in the Hyrcanian forest of northern Iran. Forests are long-lived dynamic biological systems that are continuously changing (Kimmins 1990). Moreover, growth models may play an important role in managing forests and in formulating the forest policy. Forest managers often need to project forest conditions into the future in order to make sound decisions (Peng 2000), because management decisions are often made based on knowledge of present and future resource conditions. Inventories collected at a single instant in time can provide data on current wood volumes and associated statistics. Models are needed to predict future forest growth and yield under different management scenarios, and thus they are a key element of sustainable forest management (Kimmins 1990, 1997). Progress in developing useful models for predicting forest growth is needed for managing these types of forests in Iran. Growth and yield models, which are based on functions to measurement data from an of the forest population of interest, are the tools that have mostly been utilized

for providing decision support and fundamental operational needs (Mohren et al. 2004). There have been many studies involving growth and yield modeling (e.g., Biging 1985; Lappi 1986; Gregoire 1987; Budhathoki et al. 2008; Uzoh & Oliver 2008, Adame et al. 2008, Crecente-Campo et al. 2008, Pukkala et al. 2009; Subedi and Sharma 2011, Lhotka and Loewenstein 2011 (individual diameter growth); Fridman and Ståhl 2001; Shifley et al. 2006; Yao et al. 2001; Yang et al. 2003 (tree mortality); Moser 1972; Ek 1974; Curtis et al. 1981; Curtis et al. 1982; Bravo et al. 2008) and these provide guidance for our efforts.

Hyrcanian forests are the most valuable forests in the north of Iran and industrial harvesting occurs only in this area of the country. In general, Oriental beech (Fagus orientalis Lipsky) is the main species in these forests. Oriental beech is not only is economically important for producing timber, but also for soil and water conservation. Though uneven-aged stands generally exist in Iran, growth and yield models of unevenaged forest management for this region of the world are rare. Only one previous study (Bayat et al. 2013) illustrated the development of growth and yield models for uneven-aged forests in northern Iran. Growth and yield models can be categorized as whole stand, individual tree, diameter distribution, gap models and others. The advantage of individual-tree models is the possibility of describing a stand much more thoroughly and simulating numerous treatments more simply comparing with other models (Pukkala; Kolström 1988). De Groot et al. (2004) and Pukkala et al. (2009) recommended that individual-tree models may be the best types of models for describing the dynamics of uneven-aged stands. According to Vanclay (1994), the essential components would capture increment, mortality, and recruitment of trees. Developing models for uneven-aged forestry has also become important because of demands on forests by the public for more close to nature forest management and more forest diversity (Lähde et al. 1999). This research is therefore aimed at developing a system that facilitates distance-independent individual tree growth and vield for uneven-aged Oriental beech forests. The system contains a diameter increment model, a height model, an ingrowth model, and a survival model.

Buongiorno and Michie (1980) included ingrowth in the matrix growth model to deal with the problem of the exponential growth of the number of trees in each size class. The model is based on the probabilities of the transition of trees between diameter classes and ingrowth of new trees in the lowest diameter class (Kant 1990). Peng (2000) reviewed the literature regarding growth and yield models for uneven-aged stands, discusses basic types of models and their merits, and reports recent progress in modeling the growth and dynamics of uneven-aged stands

Ling (2010) used a matrix of stand growth model for managing uneven aged boreal forest in the south and central Alaska. This model was a simulator for a 300-year period with a cutting cycle of 40 years. Accurate predictions of tree growth and yield are needed for determining optimize timber harvesting operations, and to evaluate how stand and tree parameters change over time, and in response to silvicultural interventions. Some researchers believe that the use of growth models in the implementation of uneven management is difficult in Hyrcanian forests (Heshmatol Vaezin et al. 2008) and others consider this method to be feasible and applicable in these forests (Mohammadi-Limaei 2008; Bayat et al. 2013). In view of the importance of Hyrcanian forests, there is a need for a reliable system of growth and yield predictions that, with appropriate economic parameters and ecological models, will support multifunctional forest management and planning. Traditionally, the prediction of forest growth and yield in Iran has been mainly based on historical records or experience developed for specific forestry conditions. However, these approaches may not be sufficient when developing sound management plans for complex forest systems (mixed and any-aged stands). Although uneven-aged and mixed stands commonly exist in Iran, systematic uneven-aged for sufficient management is rare (Bayat et al. 2013). There is only one study in growth and yield models for uneven-

aged stands that can be used in simulation and numerical optimization (Bayat 2012) that this study is carreid out in a limited area of Hyrcanian forest (934 ha) and cannot be considered for the whole of the Caspian forests. Therefore, due to the high heterogeneity in forest type, initial and secondary topography characteristics and climate of the Hyrcanian forests and for achieved to accurate results, this study should be done in different parts of these forests. The aim of this study is to develop a set models including individual tree diameter growth, individual tree height growth, survival (mortality) and ingrowth in order to make proper decisions on the economic utilization of these renewable and valuable assets as well as exploit other benefits of these natural treasures in uneven-aged forests in northern Iran.

Methods

Study area

The research was conducted for forests located in the Hyrcanian forest of Iran, specifically District 3 of the Sangdeh's forests, in the northern part of the country (Fig. 1). The management plan for District 3 of Sangdeh's forest suggests that the total area is about 2,709 ha. The study area is comprised of uneven-aged forests that are dominated by Oriental beech (90 percent of the forest region). The elevation of the forest areas ranges from 320 to 1,350 m above sea level.



Fig. 1- Location of the research region and geographical distribution of the inventory sample plots.

The forest inventory includes 130 circular, 0.1 ha fixed area plots systematically located on a rectangular grid of 150×200 m, and remeasured every 5 years. All trees with a diameter at breast height (DBH) at least 12.5 cm were measured before the 2009 growing season and again following the 2014 growing season. Five-year diameter growth was determined as the difference between the two measurements. Every plot

center was recorded using with GPS. Diameters were measured in a uniform direction in both measurements (2009 and 2014). Moreover, new ingrowth trees (trees that surpassed the 12.5 cm DBH threshold) were also measured in 2014. In addition, we recorded the status of each tree (living or dead).

For every plot, we computed the following stand or tree parameters: stand basal area (BA), number of trees per hectare (N), and basal area in the plot of all trees larger than the subject tree (BAL). Summary statistics of plot-level variables for model calibration are presented in Table 1.

Variable	Ν	Mean	Min	Max	SD
Dbh (cm)	130	33.46	16.43	110.00	17.18
Basal area (m ² /ha)	130	26.12	7.59	54.09	10.46
Density (trees/ha)	130	350	70	650	110.00
BAL (m^2/ha)	130	25.66	7.33	53.89	10.24

Table 1 Summary statistics corresponding to plot-level variables for modelling.

Growth and yield modeling

According to results Vanclay (1994), The more detailed approaches of forest stand modelling are not based on the overall growth of a forest stand, but need to discriminate several growth components in order to model these processes effectively (Vanclay 1994). In natural, mixed and uneven-even aged forestry, the following set of models are needed to support individual tree growth methods (e.g., Vanclay 1994; Trasobares et al. 2004):

- Individual tree diameter increment
- Individual tree survival
- Individual tree height increment
- Ingrowth estimation

The potential parameters for to the diameter increment model include: (1) tree size (DBH), (2) competition (BA, BAL, and its transformations ln (BAL), BAL/G, 1_BAL/G (see e.g., Wykoff, 1990; Vanclay 1994). Diverse types of diameter growth were considered for the dependent variable: diameter increment (DBH₂₀₁₄ – DBH₂₀₀₉), 5-year diameter growth rate ($Drate = [(DBH_{2014} - DBH_{2009}) / DBH_{2009})]$ and log transformed diameter growth. Linear mixed effect regression was used to model diameter increment following Calama and Montero (2005), Adame et al. (2008) and Lhotkaa and Loewenstein (2011).

The linear mixed model, incorporating the plot as a random variable, was (as described by West et al. 2007).

$Y_i = X_i\beta + Z_ju_j + \varepsilon_{ij}$	(1)
$\mu_{j} \sim N \; (0, \delta^2_{plot})$	(2)
$\mathcal{E}_{ij} \sim N\left(0, \delta^2\right)$	(3)

Where Yi is the vector corresponding to diameter increments for the ith tree. Xi represents the design matrix and coefficients of the fixed effects explaining tree size, competitive position and species composition. β is $p \times 1$ vector of fixed effects. Zjuj is the design matrix and coefficients corresponding to the random plot impacts of the jth plot. $\delta 2$ is the residual error variance. Eij is an $n \times 1$ vector of random errors. The model covariance structure considers residuals are not correlated and have a constant variance.

In this study, we used a binary logistic model to predict the possibility of tree survival. The explanatory variables were tree size (DBH and different derivatives) and competition factors (BA, BAL, N). There are numerous possibilities for transforming this variable that they have been used. Only significant variables (p < 0.05) with VIF (Variance inflation factor) less than 10 were chosen (eq. 5).

5

$$P_{i} = \frac{1}{1 + e^{-[b(0) + b(1) \cdot x(1) + b(2) \cdot x(2) + \dots + b(n) \cdot x(n)]}}$$

Where P_i represents the possibility of tree mortality, b_0-b_n are parameters to be estimated and x(1)-x (n) are descriptive variables.

The performance of this model was evaluated using tree criteria include area under the receiving Operation Characteristic (AUC), the Chi-square value and the Nagelkerke R2 statistics.

In this study, static height models associated with the second measurement period were developed due to errors in the height measurements associated with the first inventory. Consequently, height growth could not be estimated, and therefore we used the relationship between diameter and the height to model height. For modeling heights, we used a nonlinear mixed effect model:

$$H = 1.3 + a \times (1 - exp(-b \times d))^{(c)}$$

where H represents tree height (m) and d is the DBH (cm). a, b and c are parameters to be estimated.

For ingrowth, a linear model was prepared that had the ability to predict the trees per hectare entering the first DBH class (12.5 cm) over a growth period of 5 years.

$$IN = a_0 + a_1Ba + a_2BAL + a_3 \ln(Ba) + a_4 \ln(BAL) + a_5 (N)$$

where IN is the ingrowth (number of trees per hectare).

a0 to a4 are estimating the parameters of models. BAL is the basal area in the plot of all trees larger than the subject tree. Ba is the basal area (m^2/ha).

Model evaluation

With 30% of the data of plots, statistics were computed to assess bias, relative bias (B%), root mean square error (RMSE), and relative root mean square error (RMSE%). The definition of these statistics are as follows:

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n} (est_i - obs_i)^2}{n}}$$
(8)

Relative RMSE = $100 \times$ (RMSE/mean observation value) est_i is an estimated value. obs_i is observation value.

$$BIAS = \frac{\sum_{i=1}^{n} (est_i - obs_i)}{n}$$
(10)

325

(9)

6

7

Relative bias = $100 \times (Bias/mean observation value)$

(11)

Where: est_i represents the i^{th} predicted value; obs_i refers to the i^{th} experimental value and n is the number of observations.

For evaluating the binary logistic model, we used the following evaluation methods: the area under the receiver operating characteristic curve (ROC) to assess model fitness, Chi-squared values to assess the bias between diameter classes, and Pearson Chi-squared statistics to test the deviations between estimated and experimental values (Agresti, 1996).

Results

After summarizing the differences in plot growth between the first and second inventory measurements of the 130 plots, descriptive characteristics of the individual tree variables were developed (Table 2).

Table 2 Modeling results (Model columns represent outcomes from the modeling process. Evaluation columns represent used for Model evaluation).

Variable	Diameter growth (cm y ⁻¹)		Ingrow	th (N ha ⁻¹ y ⁻¹)	Survival (N ha ⁻¹ y ⁻¹)		
	Model	Evaluation	Model	Evaluation	Model	Evaluation	
Mean	0.48	0.47	3.57	2.71	2.40	2.50	
Min	0.24	0.28	0	0	0	0	
Max	0.76	0.80	12	12	20	20	
SD	0.11	0.12	2.60	2.80	3.28	2.14	

Different types of dependent variables were assessed for the diameter increment model. The natural logarithm corresponding to DBH increment plus a constant amount of one (log ($DBH_{2014}-DBH_{2009}+1$)) was chosen, which resulted in a linear relation with the predictor variables, and normally distributed residuals. The following equation describes the model of the 5-year diameter increment:

 $log (DBH_{2014 ij} - DBH_{2009 ij} + 1) = -0.943873 + u_{j} + 0.79424 log(DBH_{2009 ij}) - 0.08518 (BAij) - (12)$ $0.08678 (BAL) + 0.02648 (Fagus) + 0.04449 (Carpinus) + \varepsilon_{ij}$

where DBH_{2009 ij} and DBH_{2014 ij} are tree diameters (cm) of the *i*th tree on the *j*th plot in the year of 2009 and 2014, BAL equals the total basal area of trees larger than the subject tree, BA refers to the basal area of the tree, $u_j * N(0, \delta^2_e)$ represents a random plot parameter and \mathcal{E}_{ij} refers to the model residual of the *i*th tree on the *j*th plot. *Fagus* and *Carpinus* are indicator variables of different species (e.g., *Fagus* = 1 if species is Oriental beech and 0 otherwise). Results of the evaluation model and variance components for diameter growth model shown in Table 3. Findings indicated the most appropriate model has *RMSE* equal to 0.149 cm y⁻¹.

Table 3. Results of evaluating the model for individual diameter tree growth

Characteristic	Statisti	cal model fit	ted			Variance con	nponents
	\mathbb{R}^2	RMSE	RMSE%	Bias	Bias%	δ^2 plot	δ^2 residual

Diameter growth (cm	0.61	0.149	31.9	0.029	6.3	0.00815	0.08144
v ⁻¹)							

The height model was developed by modifying the Chapman-Richards model:

$$H = 1.3 + (a + u) \times (1 - \exp(-b \times DBH)^{(c + v)}$$
(13)

Where u and v are random impacts. Where H represents tree height (m) and d is the DBH (cm). The Tab. 4 shows the height model results for the best model developed. Results indicate that the best model has a relative RMSE and relative bias of 11.30% and 0.17%, respectively.

Table 4 Parameters and results for the tree height model.

Characteristic	а	и	b	С	v	RMSE	RMSE%	Bias	Bias%
Height (m)	35.46	-0.827	0.027	-1.1	-0.995	3.24	11.30	0.10	0.17
The location from the model of the complete structure dense dense dense of									

The logistic function for the probability of survival we developed was:

$$P_{ij} = \frac{1}{1 + \exp\left[-(a_1 + a_2 \ln \text{DBH}_{ij} + a_3 \frac{\text{BAL}_{ij}}{\text{BA}_{ij}} + a_4 \text{BA}_{ij} + a_5 \text{Carpinus}\right]}$$
(14)

Where P_{ij} represents the probability that tree *i* of plot *j* survives for a period of 5 years. The coefficients of the best model for survival can be found in Table 5.

Variable	Coefficients	Standard deviation	Z value	Pr(> z)
a_1	103.00	10.570	9.74	< 2e-16 ***
a_2	-3.78	1.891	-1.99	< 2e-16 ***
a ₃	-109.14	10.890	-10.35	< 2e-16 ***
a_4	-0.23	0.015	15.22	< 2e-16 ***
a_5	1.08	0.028	3.92	8.53e-05***

Table 5 The statistical analysis outcomes for equations corresponding to individual tree mortality model.

* (p < 0.05), ** (p < 0.01), *** (p < 0.001).

Results of the assessment of the individual tree survival model are found in Table 6. Result indicate that the AUC value of the mortality model was 0.80. Additionally, the Chi-square value was 120.70 and the Nagelkerke R^2 statistics was 0.23 which suggests that the model had a reasonable fit to the data well.

Table of Results for the best model for the mortanty	Т	able	6]	Results	for	the	best	model	for	tree	mortal	lity
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test	Value	р
Chi-Square value	120.70	0.000
AUC	0.80	0.000
Hosmer and Lemeshow Chi-square test	15.38	0.000
Nagelkerke R ²	0.23	
AIC	795.25	

Fig. 2 illustrates the ROC curve on the basis of the sensitivity, where the area under the ROC curve (AUC) was 0.80.



ROC curve of the mortality model.

Fig. 3 displays the results of comparing the estimated mortality possibility of reference trees with their experimental mortality possibility, and standardized residuals with predicted values for the most appropriate logistic model.



Fig 3- The estimated mortality possibilities for the most appropriate logistic model versus experimental and standardized residual mortality.

2-

The ingrowth model estimates the number per unit area that grow into the 12.5 cm DBH class over the period of 5-year. The best model form for ingrowth was:

$$IN = 69.08 - 0.552930(BA) - 11.99400 (\ln(BAL))$$
 15

Where *In* is the number of ingrowth trees per hectare. The model for ingrowth produced a coefficient of determination (R^2) of 0.86 (Tab. 7).

Table 7 Results of the best model for tree ingrowth.

Variable	\mathbb{R}^2	RMSE	RMSE (%)	Bias	Bias (%)
Ingrowth	0.86	3	22	0.02	0.14
(trees ha ⁻¹ y ⁻¹)					

The QQ plot, residual plot, and histogram (Fig. 4) associated with this model showed that distribution of residuals was normal without clear evidence of heteroscedasticity. The fitted values were completely in accordance with the modeled values.



Analysis of residuals and response values achieved by applying the application of the linear regression model.

Discussion

Hyrcanian forests are located in the northern region of Iran and across the south coast of the Caspian Sea, where they are also named Caspian forests. These forests generally contain mixtures uneven-aged stands of deciduous forests dominated by Oriental beech. There is expanding willingness of researchers, forest

managers, and society towards the management of these forests without clear-felling, following continuous cover management practices. However, this endeavor has been delayed somewhat as a result of the absence of growth and yield models, and no instructions have been issued to manage the uneven-aged forests in this area so far (Bayat et al. 2013). Therefore, growth and yield models are needed for forest management planning to provide a reliable method for examining the impacts of silvicultural and harvesting choices (Vanclay 1994; Trasobares et al. 2004). The current research study showed that reasonably valid, individual tree, distance independent models for diameter increment, height growth, survival, and ingrowth of unevenaged *Fagus orientalis* can be developed based on measurements of permanent sample plots. The predictor variables for all models are correlated with tree size and tree competition.

Distance independent, individual tree diameter growth models normally contain indicators of competitive position and/or stand density to account for the impact of tree competition when the tree positions are not known (Vanclay 1994). In prior research, tree diameter growth has commonly been expressed with deterministic linear or non-linear equations. Ordinary linear (OLS) and non-linear (ONLS) least squares regression methods have been extensively utilized for fitting these functions (Calama and Montero 2004). The absence of independence between tree measurement observations can lead to biased estimates when ordinary least squares regression methods are applied (Searle et al. 1992). Consequently, due to potential spatial correlation among observations from the same measurement plots (Fox et al. 2001), we utilized a mixed effects model for predicting five-year diameter increment in trees. Other diameter growth research considered these same issues (Biging 1985; Lappi 1986;, Gregoire 1987; Budhathoki et al. 2008; Uzoh and Oliver 2008; Pukkala et al. 2009).

Outcomes corresponding to the individual tree diameter growth model suggest that larger values of DBH caused greater annual growth increment, while larger values of the other variables (BA and BAL) caused smaller annual growth increment. The findings suggest that larger trees and trees located on better sites, and demonstrating greater vigor, had greater annual diameter growth increment. Increases in BAL suggest that competition causes a decrease in diameter growth increment, as the tree in question will be much smaller than others around it as BAL increases. BAL has been suggested as a proxy for the ability of trees to compete for light (Schwinning and Weiner 1998). Moreover, BA also impacts individual tree growth, as a reduction in the growth rate of individual trees was observed here as a measurement plot became more crowded or dense. These findings are in agreement with outcomes of other individual tree diameter growth models (Adame et al. 2008; Crecente-Campo et al. 2008; Subedi and Sharma 2011; Lhotka & Loewenstein 2011). Other studies have posited that competitive position, determined using BAL or modifications of BAL, may be the strongest predictors of diameter growth in both even-aged (Adame et al. 2008, Uzoh and Oliver 2008) and uneven-aged stands (Pukkala et al. 2009).

The R² and RMSE values for the best individual tree diameter growth model, using a mixed effect regression process, was 0.61 and 0.149 cm ha⁻¹y⁻¹, respectively. These are higher than those obtained in other studies (0.06 to 0.14 by Trasobares et al. (2004); 0.40 to 0.56 by Pukkala et al. (2009); 0.25 to 0.57 by Lhotka and Loewenstein (2011); 0.38 to 0.50 by Øyen et al. (2011)), therefore the fitness of the model seems sufficient. Furthermore, the RMSE we obtained achieved in this research was lower than previous studies (0.26 to 0.36 (cm ha⁻¹y⁻¹) in Lhotka & Loewenstein (2011) and 0.62 to 0.72 (cm ha⁻¹y⁻¹) in Trasobares et al. (2004)), suggesting model outcomes are reasonable.Data concerning heights of trees is inhibited through the complexity of its measurement in closed-canopy forests, and the associated time and

cost required may be prohibitive. The predicted mixed effect regression height models from our work express tree height as a function of DBH; the best model we produced resulted in a RMSE value equal to 11.3%, which was noticeably lower in the present study than reported by Trasobares et al. (2004), where it ranged from 21 to 24%. The reason for these favorable results can be related to environmental conditions and forest structure. Moreover, the simulated stand dynamics does not seem to be affected by the use of static height models, as height never appears as a predictor in the diameter increment, ingrowth, and survival models. Height models are needed for predicting tree volume, and probable errors in height models can produce bias in volume predictions, yet they do not invalidate conclusions about, for example, the sustainability of diverse management plans.

Mortality is most suitably simulated through multiplying the frequencies corresponding of trees by their survival possibility (Vanclay 1994). If individual trees are considered, a decision must to be made whether a tree survives over the next few years (the model's time step) or not. In the current study, the possibility of a tree surviving for 5 years was best described using the BAL, the BA, and ln(DBH) for each tree. Wald tests indicated that the predictions of survival are significant (p < 0.05). Eid and Tuhus (2001) determined that the possibility of survival increased as DBH increased, and as BAL declined, which makes sense (larger trees, less competition). Trasobares et al. (2004) showed that the basal area at breast height played a major role in regeneration modeling. In another study, Fridman and Ståhl (2001) suggested that DBH, BAL, altitude, and BA were appropriate explanatory variables for survival models, which is similar to the outcomes of our work. Shifley et al. (2006) utilized crown class, BA of trees, and DBH as predictors of future mortality. Increasing competition (as expressed by BAL) reduces survival, and therefore BAL and the ratio of basal area of individual trees and their stand has been considered as variables for capturing competition for light sources. These have been used as a proxy for one-sided competition (Yao et al. 2001), while BA and number of trees have been used as a proxy for two-sided competition (Yang et al. 2003). In the current study, the Nagelkerke R² statistics was 0.23, and the AUC was 0.80, which suggests fair suitability of the proposed survival model. This value represents the extent to which the model can predict the dependent variable correctly; this value was between 0.5 and 1 in this study. The value of 0.5 represents the randomness of the model; the value of 0.7 represents a good accuracy of the model and a value more than 0.9 indicates the high accuracy of the model (Lei et al. 2004).

Generally, ingrowth is not considered in growth and yield models (Curtis et al., 1981; Curtis et al., 1982; Bravo et al. 2008) or, if it is taken in account, the obtained prediction is weak. For this study, the R² associated with our ingrowth model was 86%. By comparison, Trasobares et al. (2004a and 2004b) achieved lower fitness results for ingrowth models of *Pinus sylvestris* (R² = 0.11), *Pinus nigra* (R² = 0.11), and *Pinus halepensis* (R² = 0.04) in a study of Mediterranean forests. On the other hands, several others have developed ingrowth models with greater power. For example, Moser (1972) and Ek (1974) reported R² values over 0.70 for northern hardwoods forests of North America. Our results therefore seem robust for Hyrcanian forests in the northern region of Iran.

Conclusions

In this study we developed a set of empirical prediction models for periodic diameter growth, height growth, ingrowth, and mortality for uneven-aged beech (*Fagus orientalis*) stand types located in northern Iran. The model specifications were developed empirically from 130 permanent plots that were measured twice, then

modeled using mixed effects regression procedures. Model selection was based on performance metrics. Results showed the best growth and yield model had relative RMSE and bias values, respectively, that were 31.9% and 6.3% for the diameter growth model, 11.3% and 0.17% for the height model, and 22% and 0.14% for the ingrowth model. Model evolution parameters showed that the parameter estimates for tree mortality were statistically significant. Overall results indicated that growth and yield model performance was consistent with expectations, and that the general fit to the validation data was acceptable. The results indicated that the empirical models performed relatively well in terms of amount of the variation explained. Therefore, they seem suitable for predicting tree growth in uneven-aged hardwood stand types in the region where the study was situated.

Authors' Contributions

Siavah Kalbi wrote the manuscript and conducted the analysis of data. Asghar Fallah contributed to the research design and data collection along with technical assistance and interpretation of data. Pete Bettinger, Shaban Shatee and Rassoul Yousfpour conducted an interpretation of data, and assisted in the writing and editing of the manuscript.

Competing interests

The authors declare that they have no competing interests.

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