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POLLEN MORPHOLOGY OF SOME *DELPHINIUM* L. (*RANUNCULACEAE*) TAXA IN TURKEY

Received 16.07.2003

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Abstract:

This study seeks to explain the pollen morphology of some *Delphinum* L. (*Ranunculaceae*) taxa in Turkey. Palynological observations have covered 21 taxa. Pollen morphology of 21 *Delphinium* L. (*Ranunculaceae*) taxa has been examined by light microscopy. Pollen grains are 3-colpate; oblate-sphaeroidal, prolate-sphaeroidal, subprolate and prolate. intersubangular or interangular in polar view. Nexine is equal to sexine or thicker than sexine, and ornemantation is micro-echinate. Colpi membrane with many granüles. The pollen grains show stenopalynous features. The aim of the article is to obtain palynological data of some *Delphinium* L. taxa.

Key Words: *Delphinium*, *Ranunculaceae*, Pollen morphology, Turkey.

Introduction

Taxonomic studies of the genus *Delphinium* L. (*Ranunculaceae*) in Turkey have been carried out by Mısırdalı et al. (1) and Ilarslan (2).

The genus *Delphinium* L. belongs to the family *Ranunculaceae* and 31 taxa of *Delphinium* L. were found in Turkey (2).

The morphological features of the pollen in some genus of *Ranunculaceae* have been treated by several authors (3-19).

Erdtman has provided a short description of pollen grains of *Ranunculaceae*. Pollen grains of *Ranunculaceae* have pore or colpi. Pollen grains of *Ranunculus arvensis* L., *Ranunculus liyalli* L., *Paoenia* L. and *Souliea vaginata* Franch. were examined by Erdtman (3). Pollen grains of many ranunculaceous taxa were studied and the large size of pantoporate *Ranunculus arvensis* L. type was introduced by Erdtman et al. (4 -5).

A palynological study of the tribe *Ranunculaceae* was made by Santisuk (6).

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Chester and Raine prepared a pollen key including pollen grains of *Ranunculaceae* family (7).

Pollen morphology of *Delphinium peregrium* L., *Consolida raveyi* Boiss., *Consolida hellespontica* Boiss. was determined by Bozdoğan and Pehlivan (8).

Palynological characters of *Delphinium elatum* L. were shown in a palynological database by Oberschneider et al. (9).

Pollen morphology of *Ranunculus constantinopolitanus* Urv. was described by Aytuğ (10).

Pollen morphology of *Helleborus* L. belongs to the family *Ranunculaceae* was examined by Nowicke and Skvarla (11).

Pollen morphologies of many *Ranunculaceae* taxa and *Delphinium verdunense* were studied by Clarke et al. (12).

To determine the taxonomic identities of Korean Adonis, the pollen morphology of 18 taxa were studied by Lee et al. (13).

Pollen grains of *Hydrastis* L., *Paeonia* L. and *Trollius* L. were investigated by Lee and Blackmore (14).

Pollen grains of Indian specimens were studied by Nair (15).

Pollen morphology of Australian *Anemone* L. was examined by Huynh (16)

Pollen morphology of the Bulgarian represantatives of the family *Ranuncillaceae-Adonis* L. was studied by Petrov et al. (17).

Morphological features of the pollen of 62 species of *Aconitum* L. was investigated and pollen characteristics among the species were evaluated by XI (18).

Exine structure of *Nigella* L. was examined by Nowicke and Skvarla. According to this study pollen grains of *Nigella* L. have unusual exine stratification (19).

This investigation was initiated to describe pollen morphology of some *Delphinium* L. taxa, occuring in Turkey.

Materials and Methods

All the pollen samples were taken from herbarium specimens at Ankara University, Faculty of Science (ANK) and Hacettepe University, Faculty of Science (HUB). In this study, collection areas, date of collections and collectors of *Delphinium* L. taxa were given in Table 1.

The pollen slides for light microscopy were prepared using the method described by Erdtman (20).

Morphological examinations of pollen grains were done with a binocular James Swift Light microscope (Ocular X 10, Objective X 100). Measurements of pollen grains belonging to each taxa were done until the Gaussian curve was obtained. Means of measurements (M),

standart deviations of measurements (S), variations obtained with the formulas of Sokal and Rholf (21)

SPSS packet programme was used for drawning graphs. Datas concerning size are based upon the measurements of a minumum 100 pollen grains for each taxa. Orthomatw device which was bounded to Life Phan Photo microscope was used for photographing. Light micrographs of pollen grains were taken considering optic cross section and ornemantation at high focus, in polar view and equatorial view. Terminology mainly follows Erdtman (4).

Table 1. Collection areas, date of collection, collectors, and herbaria of *Delphinium* L. taxa.

Taxa	Collection Areas	Date	Collectors and Herbaria
D. peregrinum	B-7 Malatya, Doğanşehir, 30 km to Malatya, Hampınar Plantation Forestry Area, 1150 m.	08.07.1986	R. İlarslan 1664-ANK
D. virgatum	C-3 Antalya, Termessos National Park, Scrubs and stony slopes, 450-500 m.	18.07.1987	R. İlarslan 1645-ANK
D. venulosum	C-5 Niğde, Çaykavak Pass, Hasan Hill, Calceroous slopes, 1600 m.	28.07.1984	R. İlarslan 1613-ANK
D. davisii	A-4 Zonguldak, Karabük, Eğriova, GüneyörenTown, Rocky places, 850 m.	07.08.1984	R. İlarslan 1618-ANK
D. staphisagria	C-3 Antalya, Kumluca, Above the Adrasan Bay, Calceroous area, North Slope, 120 m.	08.06.1979	H. Peşmen 4410-HUB
D. albiflorum	A-9 Kars, Sarıkamış, Kemaltepe, Sarıkamış-Karakurt Road, from Sarıkamış 10 km, Çıplak Mount, Protected area, 2050 m.	27.07.1986	R. İlarslan 1657-ANK
D. dasystachyum	B-7 Erzincan, Üzümlü (Cimin), Keşiş Mountain, Büyük Mezire, under the Orta yol, Çifte Dereler, 2200-2300 m.	19.08.1984	R. İlarslan 1605-ANK
D. schmalhausenii	A-9 Kars, Ardahan, Yalnızçam, Bağdaşan Town, Rum Stream, Near stream, 2400 m.	27.08.1984	R. İlarslan 1620-ANK
D. ilgazense	A-4 Kastamonu, Ilgaz, National Park Area, Kuşkayası Place, northwest slopes, Rocky places, 1600-1700 m.	16.08.1984	R. İlarslan 1611-ANK
D. fissum subsp. *anatolicum*	A-4 Ankara, Çubuk, Karagöl, Rocky slopes.	15.08.1973	S. Erik 349-HUB
D. kitianum	C-4 Konya, Ermenek, Kazancı Town, Cevizli Tarla, Above the Büyükyer Place, Northwest slope, 1300 m.	10.07.1985	R. İlarslan 1639-ANK
D. carduchorum	B-9 Van, Başkale, 80 km, Güzeldere Pass, 2680 m.	04.07.1985	R. İlarslan 1633-ANK
D. munzianum	A-8 Erzurum, Tortum-Oltu, From Tortum 20 km, Calcereous stony slopes, 1900-2000 m.	17.07.1996	R. İlarslan 1663-ANK
D. nydeggeri	C-5 Niğde, Çamardı, Demirkazık Village, Cınbarın Ağzı, South slopes, 1600 m.	27.07.1984	R. İlarslan 1616-ANK
D. gueneri	C-3 Isparta, Eğirdir, Aksu Town, Yaka Village, Kapız, Stream, Derin Valley, 1350-1400 m.	12.07.1985	R. İlarslan 1641-ANK
D. petrodavisianum	C-4 Konya, Ermenek, Hadım Road, 5 km, Rocky slopes, 1300-1400 m.	11.07.1985	R. İlarslan 1642-ANK
D. vanense	B-9 Bitlis/Van, Above the Kuzgunkıran Pass, Stony mount slopes, 2400-2650 m.	04.07.1986	R. İlarslan 1635-ANK
D. macrostachyum	C-8 Mardin, Bakırkırı, Rocky slopes, 1500-1600 m.	05.07.1985	R. İlarslan 1631-ANK
D. buschianum	A-9 Kars, Ardahan, Tunçoğlu (Panik) town, Kısır Mount, Rocky of Ramis, 2400-2500 m.	27.08.1984	R. İlarslan 1621-ANK
D. formosum	A-8 Rize, Çamlıhemşin, between Çat-Hisarcık, Mixed forest, Deep metamorfical valley, 1200-1600 m.	08.08.1981	Güner 4023-HUB
D. flexuosum	A-9 Artvin, Ardanuç, Kutul yaylası, Karanlık Quercus Forest, Müezzinler Town, Under the plateau, 2150-2200 m.	28.08.1984	R. İlarslan 1603-ANK

Results

General Pollen Morphology of *Delphinium* L. Genus

Pollen grains of taxa belonging to *Delphinium* L. genus are tricolpate. Average polar axis diameter of pollen grains of taxa ranging from 21.15-37.81 μm, average equatorial diameter ranging from 19.88-27.97 μm. The shape of the pollen grains is oblate-sphaeroidal, prolate-sphaeroidal, subprolate and prolate. Apocolpia is between 2.51-9.92 μm. Ornemantation of exine is microechinate. Exine thickness is between 0.62-1.98 μm at mesocolpia. Nexine is equal to sexine or thicker than sexine. The pollen grains have long or short colpi, with acute apices. Colpi membrane with many granüles.

Pollen Description

Section : ***Delphinium*** DC.

Delphinium peregrinum L. (Figure 1)

Pollen grains 3-colpate, subprolate. Polar axis 24.26 μm. Equatorial diameter 20.50 μm. Amb interangular or intersubangular. Apocolpia 3.16 μm. Exine 1.86 μm thick at mesocolpia. Sexine 0.62 μm, nexine 1.24 μm. Ornemantation of exine is micro-echinate. Colpi 19.84 μm long with acute apices. Wideness of colpi 3.72 μm. Colpi membrane with many granüles.

Delphinium virgatum Poir. (Figure 2)

Pollen grains 3-colpate, oblate-sphaeroidal. Polar axis 21.71 μm. Equatorial diameter 22.88 μm. Amb intersubangular. Apocolpia 3.51 μm. Exine 1.75 μm thick at mesocolpia. Sexine 0.62 μm, nexine 1.13 μm. Ornemantation of exine is micro -echinate. Colpi 23.43 μm long with acute apices. Wideness of colpi 3.78 μm. Colpi membrane with many granüles.

Delphinium venulosum Boiss. (Figure 3)

Pollen grains 3-colpate, prolate-sphaeroidal. Polar axis 25.88 μm. Equatorial diameter 23.07 μm. Amb intersubangular. Apocolpia 2.66 μm. Exine 1.96 μm thick at mesocolpia. Sexine 0.98 μm, nexine 0.98 μm. Ornemantation of exine is micro-echinate. Colpi 19.77 μm long with acute apices. Wideness of colpi 3.72 μm. Colpi membrane with many granüles.

Delphinium davisii Munz (Figure 4)

Pollen grains 3-colpate, subprolate. Polar axis 31.99 μm. Equatorial diameter 24.53 μm. Amb intersubangular. Apocolpia 6.20 μm. Exine 1.24 μm thick at mesocolpia. Sexine 0.62 μm, nexine 0.62 μm. Ornemantation of exine is micro-echinate. Colpi 25.42 μm long with acute apices. Wideness of colpi 2.85 μm. Colpi membrane with many granüles.

Section : **Staphisagria** DC.

Delphinium staphisagria L. (Figure 5)

Pollen grains 3-colpate, prolate. Polar axis 37.81 μm. Equatorial diameter 27.54 μm. Amb intersubangular. Apocolpia 4.96 μm. Exine 1.82 μm thick at mesocolpia. Sexine 1.20 μm, nexine 0.62 μm. Ornemantation of exine is micro-echinate. Colpi 28.21 μm long with acute apices. Wideness of colpi 5.89 μm. Colpi membrane with many granüles.

Section : **Oligophyllon** DC.

Delphinium albiflorum DC. (Figure 6)

Pollen grains 3-colpate, prolate. Polar axis 27.94 μm. Equatorial diameter 20.32 μm. Amb intersubangular. Apocolpia 6.20 μm. Exine 1.24 μm thick at mesocolpia. Sexine 0.62 μm, nexine 0.62 μm. Ornemantation of exine is micro-echinate. Colpi 26.66 μm long with acute apices. Wideness of colpi 5.58 μm. Colpi membrane with many granüles.

Delphinium dasystachyum Boiss. & Bal. (Figure 7)

Pollen grains 3-colpate, subprolate. Polar axis 24.29 μm. Equatorial diameter 20.77 μm. Amb interangular or intersubangular. Apocolpia 2.97 μm. Exine1.25 μm thick at mesocolpia. Sexine 0.47 μm, nexine 0.78 μm. Ornemantation of exine is micro-echinate. Colpi 18.71 μm long with acute apices. Wideness of colpi 3.96 μm. Colpi membrane with many granüles.

Delphinium schmalhausenii Albov (Figure 8)

Pollen grains 3-colpate, subprolate. Polar axis 25.67 μm. Equatorial diameter 19.88 μm. Amb intersubangular. Apocolpia 4.96 μm. Exine 1.44 μm thick at mesocolpia. Sexine 0.51 μm, nexine 0.93 μm. Ornemantation of exine is micro-echinate. Colpi 21.26 μm long with acute apices. Wideness of colpi 3.97 μm. Colpi membrane with many granüles.

Delphinium ilgazense P. H. Davis (Figure 9)

Pollen grains 3-colpate, prolate. Polar axis 29.36 μm. Equatorial diameter 21.57 μm. Amb intersubangular. Apocolpia 8.86 μm. Exine 0.93 μm thick at mesocolpia. Sexine 0.31 μm, nexine 0.62 μm. Ornemantation of exine is micro-echinate. Colpi 22.70 μm long with acute apices. Wideness of colpi 3.10 μm. Colpi membrane with many granüles.

Delphinium fissum subsp. ***anatolicum*** Chowdhuri & P. H. Davis (Figure 10)

Pollen grains 3-colpate, prolate-sphaeroidal. Polar axis 24.68 μm. Equatorial diameter 21.99 μm. Amb intersubangular. Apocolpia 8.51 μm Exine 1.25 μm thick at mesocolpia. Sexine 0.48 μm, nexine 0.77 μm. Ornemantation of exine is micro-echinate. Colpi 23.43 μm long with acute apices. Wideness of colpi 3.88 μm. Colpi membrane with many granüles.

Delphinium kitianum İlarslan (Figure 11)

Pollen grains 3-colpate, subprolate. Polar axis 27.71 μm. Equatorial diameter 22.88 μm. Amb intersubangular. Apocolpia, 8.62 μm. Exine 1.47 μm thick at mesocolpia. Sexine 0.78 μm, nexine 0.69 μm. Ornemantation of exine is micro-echinate. Colpi, 23.31 μm long with acute apices. Wideness of colpi 4.40 μm. Colpi membrane with many granüles.

Delphinium carduchorum Chowdhuri & P. H. Davis (Figure 12)

Pollen grains 3-colpate, subprolate. Polar axis 30.80 μm. Equatorial diameter 24.26 μm. Amb intersubangular. Apocolpia, 9.92 μm. Exine 0.62 μm thick at mesocolpia. Sexine 0.31 μm, nexine 0.31 μm. Ornemantation of exine is micro-echinate. Colpi 25.42 μm long with acute apices. Wideness of colpi 4.71 μm. Colpi membrane with many granüles.

Delphinium munzianum P. H. Davis & Kit Tan (Figure 13)

Pollen grains 3-colpate, subprolate. Polar axis 26.87 μm. Equatorial diameter 22.19 μm. Amb interangular or intersubangular. Apocolpia, 3.16 μm. Exine 1.24 μm thick at mesocolpia. Sexine 0.62 μm, nexine 0.62 μm. Ornemantation of exine is micro-echinate. Colpi 24.67 μm long with acute apices. Wideness of colpi 4.15 μm. Colpi membrane with many granüles.

Delphinium nydeggeri Hub.-Mor. (Figure 14)

Pollen grains 3-colpate, subprolate. Polar axis 27.88 μm. Equatorial diameter 23.60 μm. Amb interangular or intersubangular. Apocolpia 3.90 μm. Exine 1.45 μm thick at mesocolpia. Sexine 0.83 μm, nexine 0.62 μm. Ornemantation of exine is micro-echinate. Colpi 23.25 μm long with acute apices. Wideness of colpi 4.27 μm. Colpi membrane with many granüles.

Delphinium gueneri P. H. Davis (Figure 15)

Pollen grains 3-colpate, subprolate. Polar axis 27.97 μm. Equatorial diameter 22.30 μm. Amb interangular or intersubangular. Apocolpia 6.20 μm. Exine1.24 μm thick at mesocolpia. Sexine 0.62 μm, nexine 0.62 μm. Ornemantation of exine is micro-echinate. Colpi 24.64 μm long with acute apices. Wideness of colpi 4.57μm. Colpi membrane with many granüles.

Delphinium petrodavisianum Ilarslan (Figure 16)

Pollen grains 3-colpate, subprolate. Polar axis 26.80 μm. Equatorial diameter 22.81 μm. Amb intersubangular. Apocolpia, 2.48 μm. Exine 1.30 μm thick at mesocolpia. Sexine 0.63 μm, nexine 0.67 μm. Ornemantation of exine is micro-echinate. Colpi 21.26 μm long with acute apices. Wideness of colpi 3.97 μm. Colpi membrane with many granüles.

Delphinium vanense Rech. (Figure 17)

Pollen grains 3-colpate, oblate-sphaeroidal. Polar axis 21.15 μm. Equatorial diameter 27.97 μm. Amb interangular or intersubangular. Apocolpia 2.60 μm. Exine 1.65 μm thick at mesocolpia. Sexine 0.65 μm, nexine 1.00 μm. Ornemantation of exine is micro-echinate. Colpi 23.25 μm long with acute apices. Wideness of colpi 3.96 μm. Colpi membrane with many granüles.

Delphinium macrostachyum Boiss. ex Huth (Figure 18)

Pollen grains 3-colpate, subprolate. Polar axis 25.63 μm. Equatorial diameter 21.30 μm. Amb intersubangular. Apocolpia, 3.16 μm. Exine 1.30 μm thick at mesocolpia. Sexine 0.68

μm, nexine 0.62 μm. Ornemantation of exine is micro-echinate. Colpi, 21.08 μm long with acute apices. Wideness of colpi 3.84 μm. Colpi membrane with many granüles.

Section: *Pogonanthus* W.T. Wang

Delphinium buschianum Grossh. (Figure 19)

Pollen grains 3-colpate, prolate-sphaeroidal. Polar axis 23.15 μm. Equatorial diameter 21.15 μm. Amb interangular or intersubangular. Apocolpia, 6.20 μm. Exine 1.30 μm thick at mesocolpia. Sexine 0.43 μm, nexine 0.87 μm. Ornemantation of exine is micro-echinate. Colpi 18.41 μm long with acute apices. Wideness of colpi 4.34 μm. Colpi membrane with many granüles.

Delphinium formosum Boiss. & Huet (Figure 20)

Pollen grains 3-colpate, subprolate. Polar axis 28.62 μm. Equatorial diameter 24 μm. Amb interangular or intersubangular. Apocolpia 5.58 μm. Exine 1.24 μm thick at mesocolpia. Sexine 0.62 μm, nexine 0.62 μm. Ornemantation of exine is micro-echinate. Colpi 23.00 μm long with acute apices. Wideness of colpi 4.58 μm. Colpi membrane with many granüles.

Delphinium flexuosum M. Bieb. (Figure 21)

Pollen grains 3-colpate, prolate-sphaeroidal. Polar axis 23.64 μm. Equatorial diameter 20.78 μm. Amb interangular or intersubangular. Apocolpia 3.10 μm. Exine 1.64 μm thick at mesocolpia. Sexine 0.62 μm, nexine 1.02 μm. Ornemantation of exine is micro-echinate. Colpi 20.95 μm long with acute apices. Wideness of colpi 3.72 μm. Colpi membrane with many granüles.

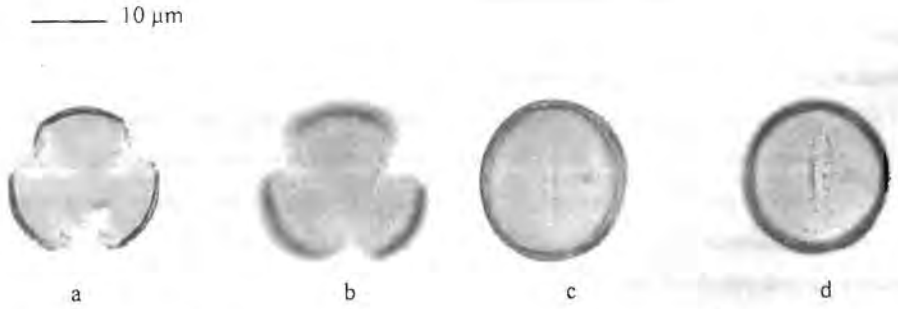

Figure 1. D. peregrinum

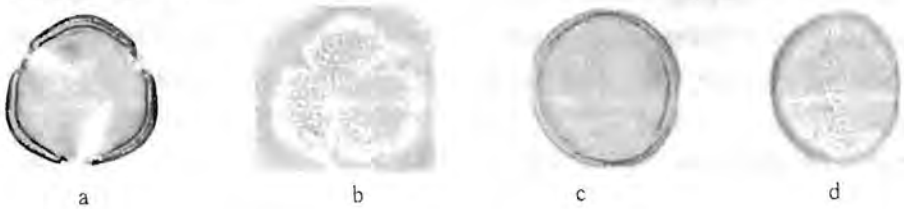

Figure 2. D. virgatum

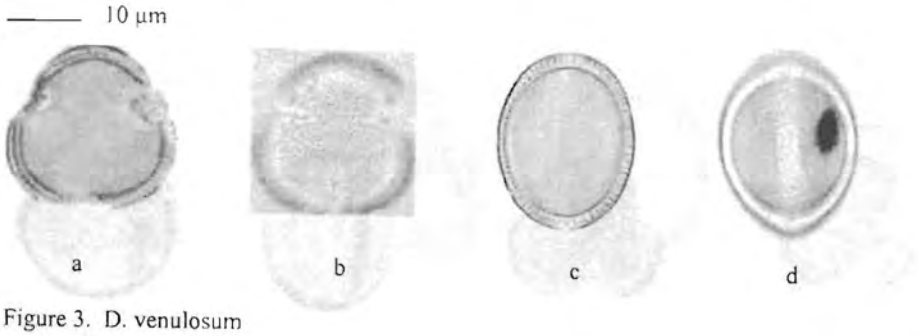

Figure 3. D. venulosum

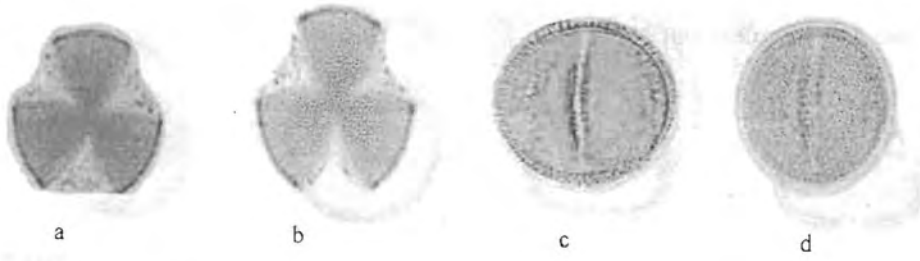

Figure 4. D. davisii

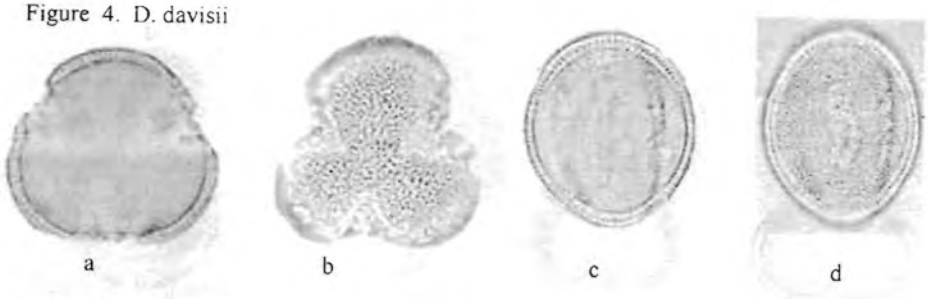

Figure 5. D. staphisagria

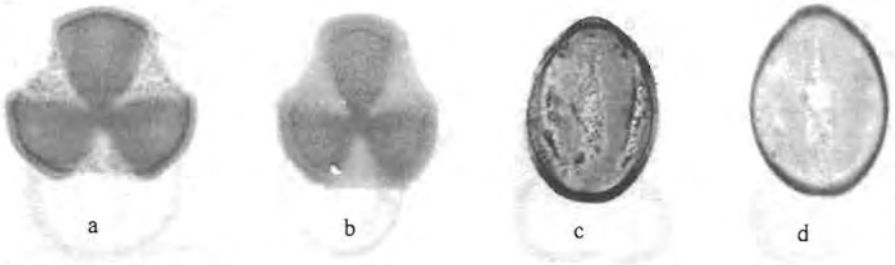

Figure 6. D. albiflorum

Figure 7. D. dasystachyum

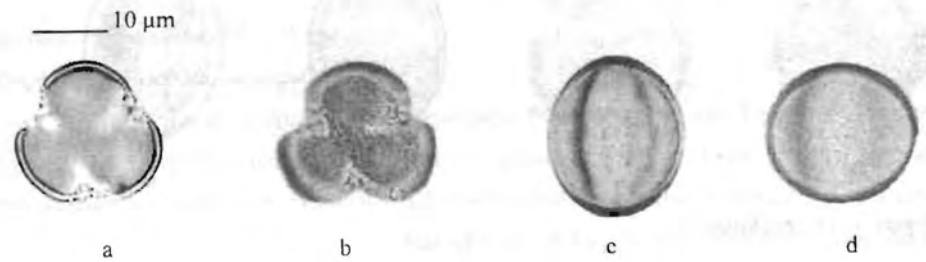

Figure 8. D. schmalhausenii

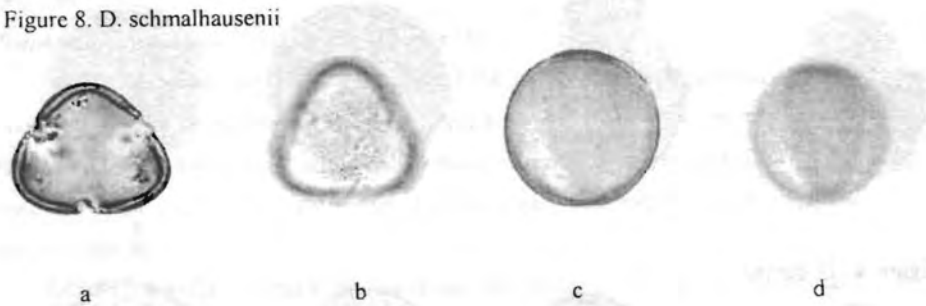

Figure 9. D. ilgazense

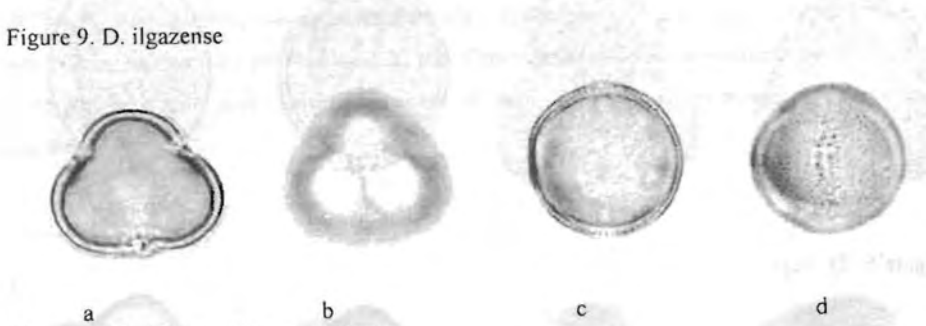

Figure 10. D. fissum subsp. anatolicum

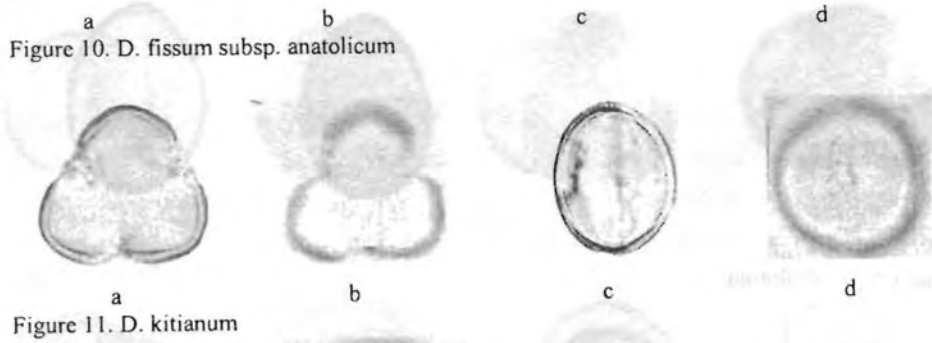

Figure 11. D. kitianum

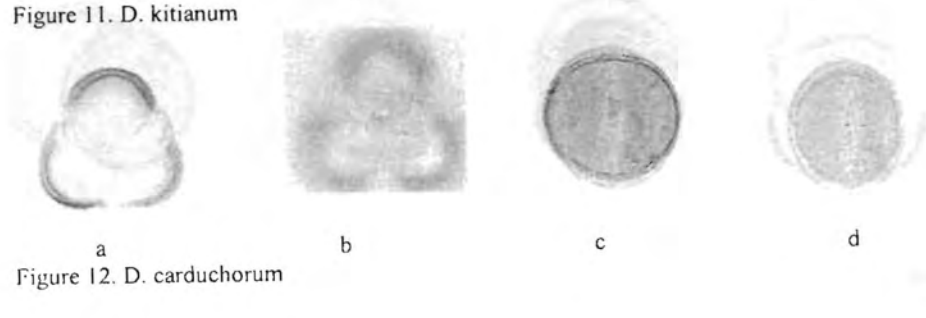

Figure 12. D. carduchorum

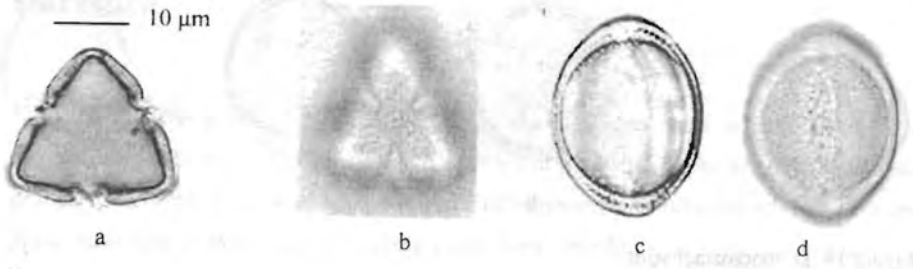

Figure 13. D. munzianum

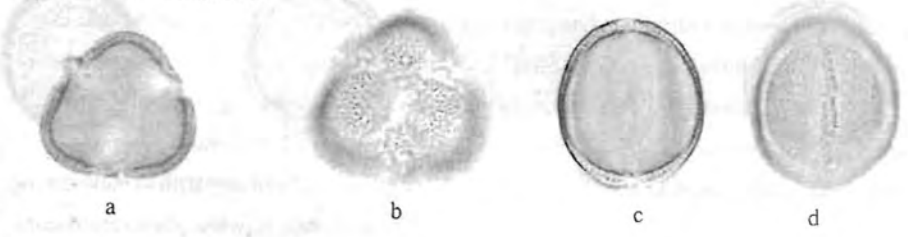

Figure 14. D. nydeggeri

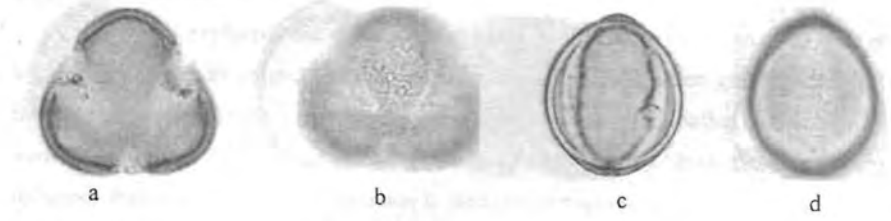

Figure 15. D. gueneri

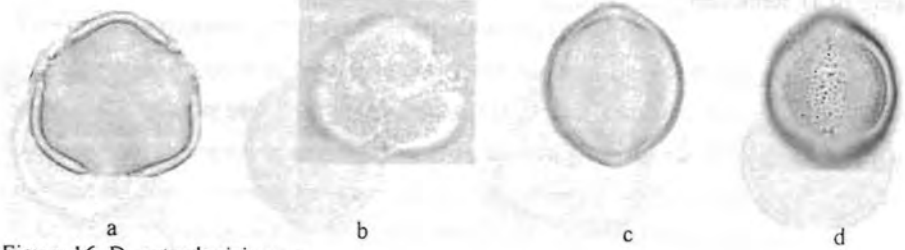

Figure 16. D. petrodavisianum

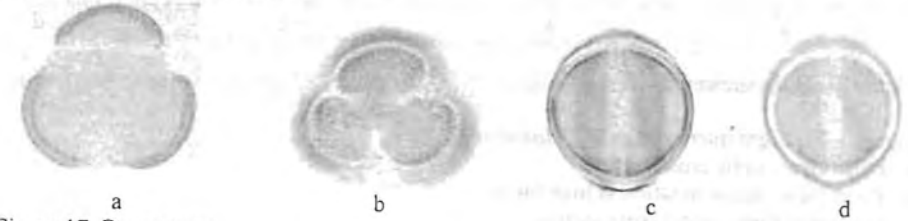

Figure 17. D. vanense

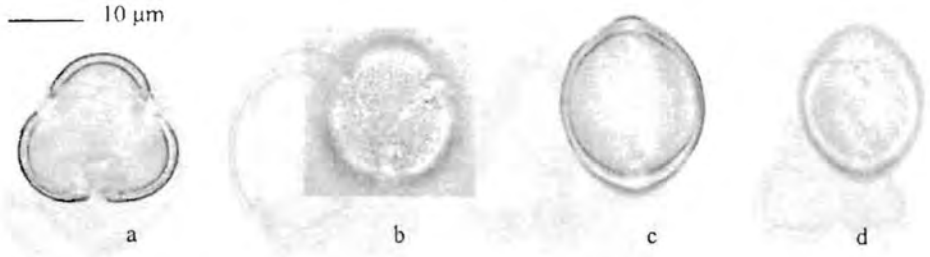

Figure 18. D. macrostachyum

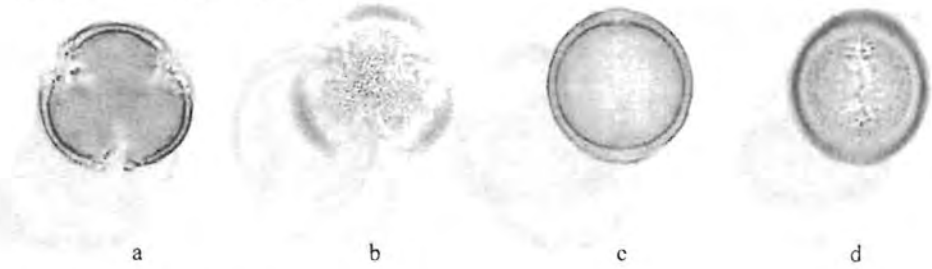

Figure 19. D. buschianum

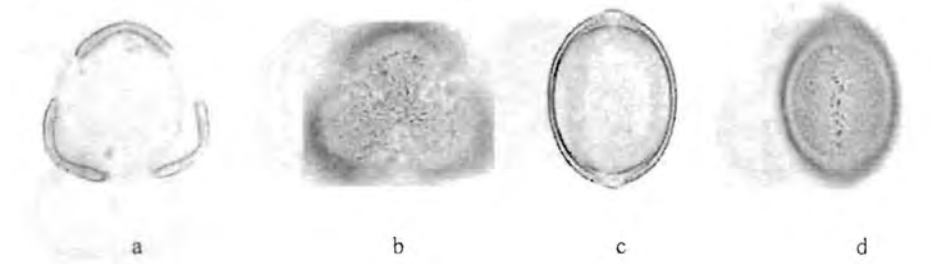

Figure 20. D. formosum

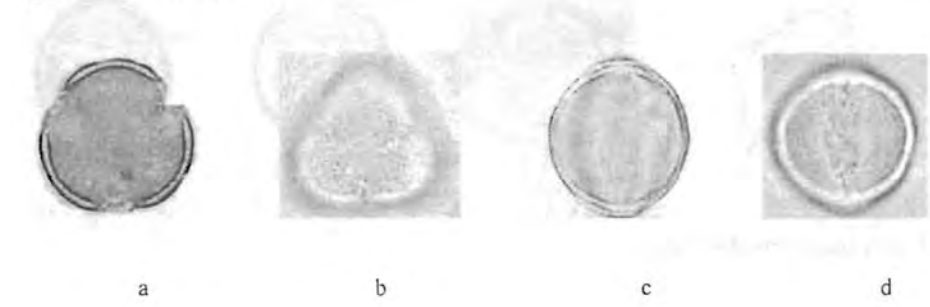

Figure 21. D. flexuosum

Figure 1-21. Light micrographs of *Delphinium* L. taxa
a - Polar view ; optic cross section
b - Polar view ; ornemantation at high focus
c - Equatorial view ; optic cross section
d - Equatorial view ; ornemantation at high focus

Discussion

The pollen grains of belonging to *Delphinium* L. genus are 3-colpate. AMB shape is İntersubangular or angular in polar view. The shape of the pollen grains are oblate-sphaeroidal, prolate-sphaeroidal, subprolate and prolate. Exine ornemantation is micro-echinate. The pollen grains have long or short colpi, with acute apices. Colpi membrane with many granüles.

The pollen grains of taxa belonging to *Delphinium* L. taxa show stenopalynous features.

According to SPSS graphs, there are variations and similarities among the pollen grains of taxa existing in the same section (Figure 22-24). According to palynological data, polar axis diameter, equatorial diameter and AMB shape of all pollen grains are not equal.

D. *peregrinum* and D. *virgatum* exist in the *Delphinium* DC. section. Morphologically D. *peregrinum* is different from D. *virgatum*. D. *peregrinum* L. has obovate petals, upper leaves are linear lanceolata, claw is aproximately equal to lamina (2). D. *virgatum*, D. *venulosum*, D. *davisii* pollen grains have aproximately same equatorial diameter and polar axis and exist in the *Delphinium* DC section.

Davis (22) explained that the most of the taxa belonging to *Delphinium* L. were probably hybrid. According to palynogical knowledge, when abnormal pollen grains amount is more than % 50, pollen grains are hybrid. (23) Diameters of hybrid pollen grains can be rather various. In this study, pollen grain diameters of 21 *Delphinium* L. taxa diameters are not very different. Pollen grains of 21 *Delphinium* L. taxa are homogenous.

İlarslan described *Delphinium* L. taxa occuring in Turkey and he found variations among the taxa. The pollen grains of the genus *Delphinium* L. show variation between the species that sometimes corresponds with the morphologic features (2).

Pollen characteristics can use to distinguish Staphisagria section. *D. staphisagria* L. was seperated from other taxa distincly by İlarslan (2). *D. staphisagria* L. has long, hairly stem and swollen fruit. Diameters of pollen grains of *D. staphisagria* L. are wider than the other species too. But the pollen characteristics can not used to distinguish all the *Delphinium* L. sections and subsections. The variations can be used to distinguish between some closely related species.

Our observations with light microscobe have revealed the taxa of the *Delphinium* L. genus.

The investigated taxa are homogenous with respect to their pollen morphology, but a closer studies carried out by SEM and TEM will reveal new data for a better grouping.

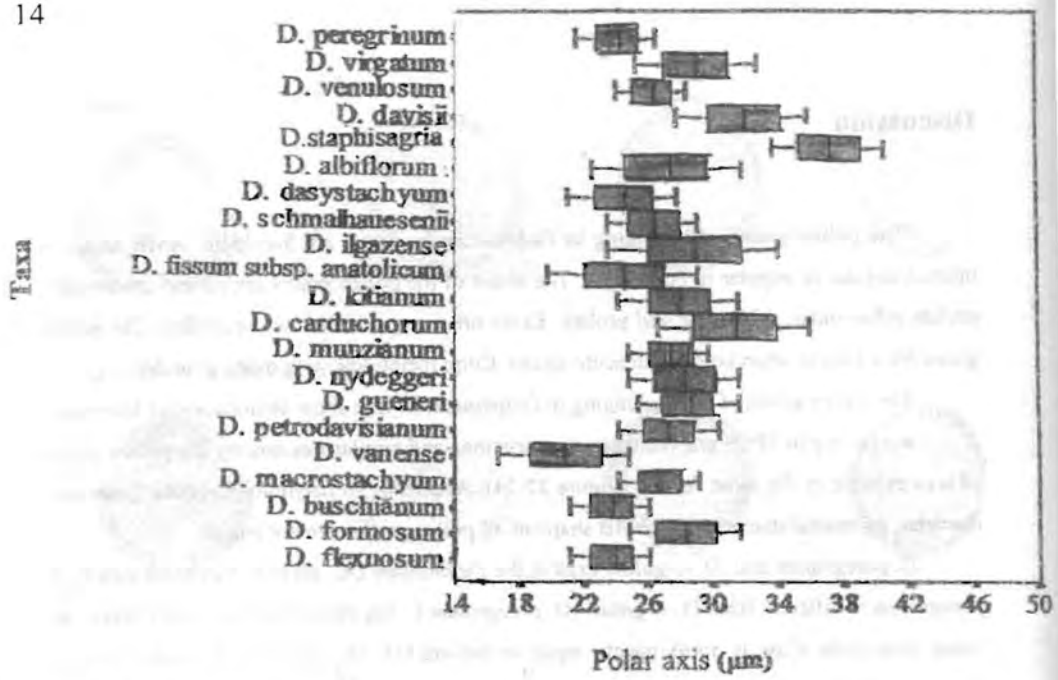

Figure 22. Polar axis diameters of *Delphinium* L. taxa

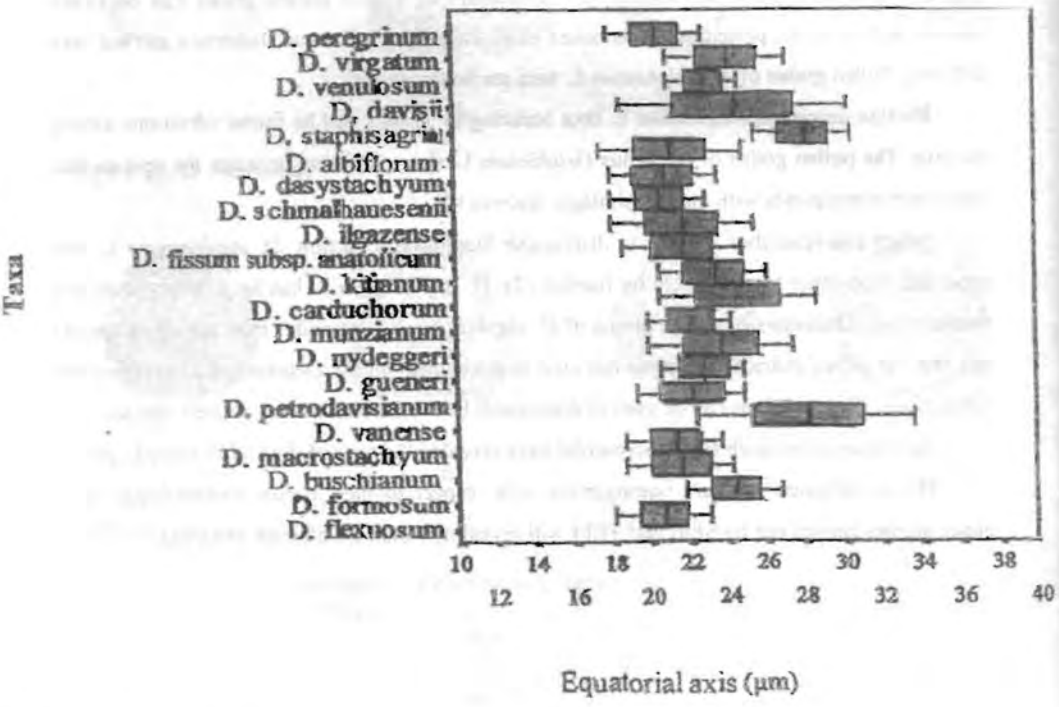

Figure 23. Equatorial axis diameters of *Delphinium* L. taxa

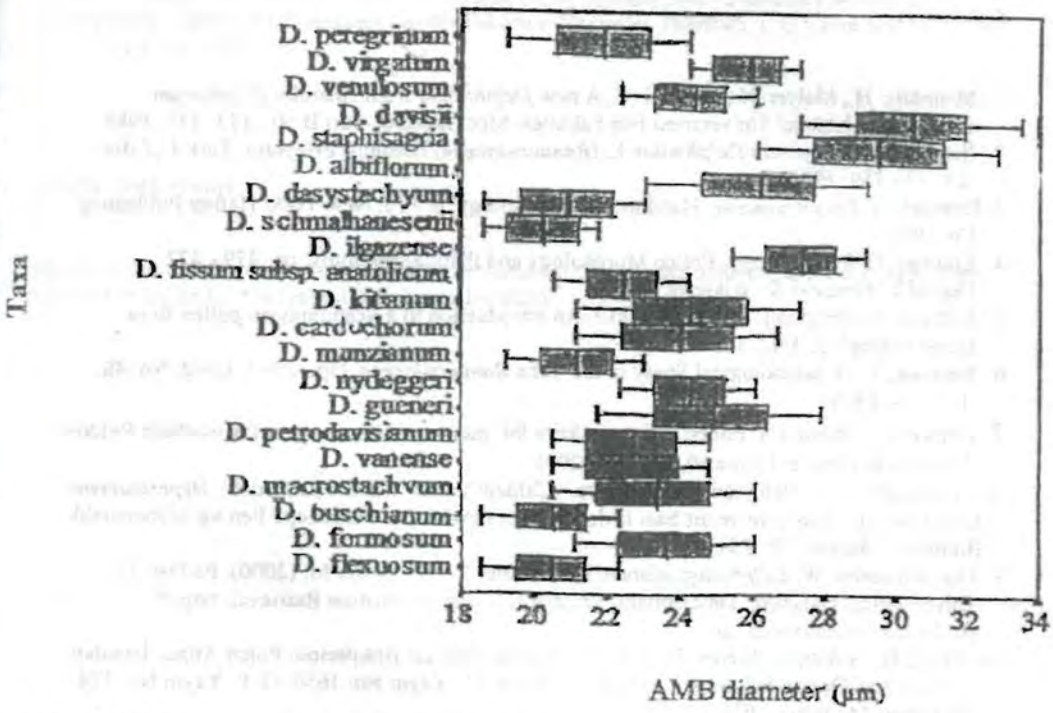

Figure 24. AMB diameters of *Delphinium* L. taxa

Variation

Min — Max

Average Value

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PLACENTAL STRUCTURE IN THE RATS: HISTOLOGICAL AND IMMUNOHISTOCHEMICAL STUDIES

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Abstract

The aim of this paper is to review the current knowledge of the localization and function of TN (Tenascin) and type IV collagen and EGF (Epidermal Growth Factor) and EGFR (Epidermal Growth Factor Receptor) in normal rat placenta formation. In this study, immunolocalization of ECM (Extracellular matrix) components such as TN and type IV collagen and EGF and its receptor in the placenta of Wistar albino rats were determined. Therefore, the samples of placenta obtained from rats on days 10, 12, 14, 16, 18, 20 and 21 of gestation were evaluated morphologically; fixed and stained with H&E for histology method for immunohistochemistry. Besides, during the experiment, body weights of all rats were recorded.

Morphological and histological investigation showed that there were changes in placental histology with placental development. Time- and region dependent changes in the immunolocalization of TN, type IV collagen, EGF and EGFR were determined in the placentas of rats during their gestational period.

Key Words: Placenta, type IV collagen, TN, EGF, EGFR

Introduction

The placenta is a temporary organ and is the site of physiologic exchanges between the mother and the fetus. The placenta is cluster of cells derived from two distinct individuals,

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mother and fetus. Rat placenta is histologically classified as the hemochorial type.

ECM is a complex mixture of structural and functional proteins, glycoproteins, and proteoglycans arranged in a unique tissue specific three dimensional ultrastructure. These proteins serve many functions including the provision of structural support, attachment sites for cell surface receptors, cell migration, cell proliferation, orientation, immune responsiveness, and tissue and organ morphogenesis (1). The biochemical composition of the ECM varies between tissues. Variation in biochemical composition of the ECM is correlated with variation in its structure and organization, which is reflected in the functional characteristics of different tissue.

Type IV collagen and TN are two of the main components of ECM. Type IV collagen is mainly a single protein species with M.W. of 550.000-600.000 dalton and consisting of two different peptide chains, α1(IV) and α2 (IV), assembled into a triple-stranded molecule (2). TN has six large, identical subunits which are disulfide-linked to a "hexabrachion" structure. TN plays a morphoregulatory role during development, tissue remodeling, wound healing, tumorogenesis, and in disease by regulating the cell adhesive and signaling properties of neural and non-neural cells (3).

The ECM and its components and growth factors play important roles in modulating trophoblast functions, implantation, decidualization, and placentation processes in mouse, rat and human.

EGF, a single chain polypeptide with a molecular weight of approximately 6000 dalton, stimulates proliferation in many tissues (4). EGF has been shown in tissue culture to regulate the growth of a variety of ectoderm-, mesoderm-, and endoderm- derived cells (5). EGF may play a significant role in the induction and regulation of the differentiated function of trophoblasts (6).

EGF acts via the EGF receptor. EGF binds to the cell surface EGFR (7). EGFR has intrinsic tyrosine kinase activity which plays a role in signal transduction. EGFR activation may also stimulate DNA synthesis, cell replication and transformation.

ECM proteins interact directly with cell surface receptors to initiate signal transduction pathways and to modulate those triggered by differentiation and growth factors. The ECM also controls the activity and presentation of a wide range of growth factors. Growth factors can modulate both synthesis and the degration of ECM through their action on structural component, matrix-degrading proteases, and protease inhibitors (8).

Much like tumor invasion trophoblast invasion of the uterus is a multi-step process involving: attachment of trophoblast cells to ECM components; degradation of ECM; and migration through the ECM. At the same time, during implantation a large number of growth factors and their receptors have been identified at the fetal-maternal interface at various gestational ages (9). Trophoblast proliferation, migration and invasiveness are regulated in an independent or interdependent manner by a wide variety of molecules at the fetal-maternal interface. They include growth factors and growth factor-binding proteins, proteoglycans, proteolytic enzymes and their inhibitors, as well as components of the ECM (10).

The aim of this paper is to determine the localization and function of TN and type IV collagen and EGF and EGFR in normal rat placentation and their role in the placentation and placental histology. Furthermore, the size, shape and histological organization of normal rat placenta were also investigated.

Materials & Methods

Animals and Experimental Design

Wistar albino virgin female rats aged 8 and 12 weeks used in this study were raised in our laboratory (Hacettepe University, Faculty of Science, Department of Biology). The lab condition was maintained at 25.47 ± 0.36 °C, with 68.76 ± 0.73 % humidity, and a 12 : 12 h light : dark cycle. The rats were bred each night between 17.00 and 09.00 h until the female was determined sperm positive by vaginal swabbing. Conception was assumed to have taken place if the vaginal swab, taken the following morning was found to be sperm positive. This day was designated day 1 of gestation. There were 3 animals in each gestational day group.

The animals were fed with standard pellet chow and *ad libitum* throughout the experiment. The body weights were recorded daily during the experiment. At the same time, on days 16, 18, 20 and 21 of gestation, placental index (pi = placental weight / fetus weight) was calculated.

Histological study

On days 10, 12, 14, 16, 18, 20 and 21 of gestation, the pregnant rats in each experimental group were sacrificed by decapitation. The uterus was opened and the placentas were removed, weighed and immediately fixed in Bouin's fluid. The fixed tissues were processed in the usual manner and embedded in paraffin and then 6 μm tissue sections were cut. Sections were stained with Hematoxylin & Eosin (H&E) and then examined with light microscopy.

Immunohistochemistry

The antibodies that specifically recognized mouse EGF and human tenascin (Sigma); human type IV collagen (ICN); goat EGFR (Santa Cruz Biotechnology) were obtained commercially.

The Bouin-fixed tissue samples were embedded in paraffin wax. 5 μm tissue sections were cut and mounted on glass slides coated with PLL (poly L lysine) solution (Sigma). Immunuhistochemistry was carried out on deparaffinized tissue sections using the PAP (unlabelled peroxidase-antiperoxsidase) method. All following steps were performed at room temperature. The tissue sections used for type IV collagen antibody were treated with trypsin (Sigma) for 6 min at 37 °C. Endogenous peroxidase activity was quenched by immersing the tissue sections in freshly prepared 5 % H_2O_2 in methanol for 30 min to eliminate endogenous peroxidase activity. The sections were rinsed in PBS and then treated with 10 % normal goat serum for 45 min. The deparaffinized tissue sections treated separately for 60 min with the following antibodies: rabbit anti-mouse EGF (1:500 dilution), mouse anti-goat EGFR (1:50 dilution), rabbit anti-human collagen type IV (1:50 dilution) or monoclonal anti-human tenascin (1:1000 dilution) in phosphate buffered saline (PBS). Tissues that were

previously known to stain positively for each respective antibody were used as a positive control. Negative control sections were treated without any primary antibodies. The specimens were rinsed in PBS and then exposed to goat-anti rabbit IgG (1:300 dilution, Sigma) for 60 min. The specimens were then rinsed in PBS and exposed for 60 min to PAP solution (1:300 dilution, Sigma). They were rinsed thoroughly with PBS and incubated in freshly prepared 3, 3'-diaminobenzidine tetrahydrochlorid (DAB, Sigma) solution for 5 min and rinsed distillated water. Following the completion of the immunohistochemical staining procedure, the sections were counterstained with Haematoxylin, and mounted. The sections of placentas were evaluated by two different observations by light microscopic level. The labeling intensity defined as (-) negative; (±) faint; (+) weakly positive; (++) positive; (+++) clearly positive were evaluated.

Statistical Analysis

Data were analyzed by analysis of variance (ANOVA), with least significant difference post hoc comparisons (Games-Howell, Hochberg GT 2) being used to identify sources of significant main effects. The statistical significance was assigned at the 0.05 level.

Results

Histological study

Table 1 shows weight gain at last pregnancy and placental index (pi) data of the rats in experimental groups.

Decidua, trophoblastic giant cells, spongiotrophoblast and labyrinth regions in the placental sections of rats in experiment were stained with H&E.

When the histological structure of placentas of the rats in experiment was examined, it was seen that the ectoplacental cone had formed by day 10 of gestation of rats. This region is composed of proliferating trophoblast cells and was presenting a layer of trophoblastic giant cells in this region. Two different regions existed in placentas of rats between the GD 12

and GD 16: the labyrinth and spongiotrophoblasts. The labyrinth region and basal zone were shown absolute increase with advancing pregnancy (Figure 1).

Table 1. The data of morphological studies in the experiment

GD	n	n_{rf}	Initial weight (g)	Final weight (g)	Weight gain (g)	Weight gain (%)	Placenta weight (g)	Fetus weight (g)	Placental index
10	42		167 ± 0.01	205 ± 0.03	38	22.75			
12	39		165 ± 0.02	206 ± 0.06	41	24.84			
14	39	1	155 ± 0.02	171 ± 0.06	36	23.22			
16	36		160 ± 0.04	216 ± 0.05	56	35.00	0.32 ± 0.01	0.32 ± 0.007	0.98 ± 0.030
18	32		160 ± 0.03	222 ± 0.04	62	38.75	0.32 ± 0.01	0.79 ± 0.010	0.40 ± 0.008
20	33		174 ± 0.02	260 ± 0.03	86	49.42	0.37 ± 0.01	1.42 ± 0.020	0.25 ± 0.010
21	30		175 ± 0.01	270 ± 0.03	95	54.28	0.48 ± 0.02	2.25 ± 0.040	0.21 ± 0.010

There are 3 animals in each gestation day group. Values are given as mean ± SE.
Placental index: Placenta weight (g) / fetus weight (g), n: Fetus number, n_{rf}: Resorption fetus number, GD: Gestation day

Immunohistochemistry

The immunolocalization of type IV collagen, TN, EGF and EGFR were evaluated in different placenta regions of rats in different gestation days. Table 2 shows the immunolocalization of type IV collagen, TN, EGF and EGFR in the placenta of rats of different gestation days. The relative differences in labeling intensity for all antibodies were qualified.

At 10 days of gestation, the intensity for TN was present in decidua and uterus. But, this intensity for TN was decreased in decidua and uterus at 12 and 14 days of gestation. Trophoblastic giant cells, spongiotrophoblasts and labyrinth trophoblasts were showed positive staining for TN at 12 days of gestation. However, it has to be emphasized that not every cell showed similar TN immunreactivity. At day 14 of gestation, positive staining for

TN in trophoblastic giant cells and some spongiotrophoblast were observed (Figure 2). There were increases for TN immunreactivity in the spongiotrophoblasts and labyrinth trophoblasts of placenta in rats from GD 12 to GD 20 (Figure 3). No immunreactivity of TN is present in the Reichert's membrane at GD 18 and GD 20 (Table 2).

A fibrillar pattern of staining was seen in the intercellular areas and was especially prominent in sections stained for type IV collagen (Table 2). The expression of type IV collagen, an important component of the ECM, was intense within ECM of the uterus, decidua, and trophoblastic giant cells at GD 12 and GD14 (Figure 4). At day 16 of gestation, clearly positive staining for type IV collagen in some spongiotrophoblast was observed (Figure 5). Type IV collagen immunreactivity in blood vessels and trophoblasts of labyrinth was some what heterogeneous, in some areas was weakly positive and positive while others were clearly positive throughout pregnancy in rats. The Reichert's membrane on days 18 and 20 of gestation exhibited intensely expression of type IV collagen.

Data for intensity of EGF staining in different placenta regions at different gestation days of pregnant rats are also summarized in Table 2. Positive staining for EGF was seen in the uterus and decidua between the GD 10 - GD 14 of gestation (Figure 6). In GD 14, no positive reaction or faint staining for EGF was seen in the spongiotrophoblasts. The positive staining for EGF was observed in trophoblastic giant cells at GD 20 (Figure 7). However, there was no immunreactivity in Reichert's membrane at GD 18 and GD 20.

The intensity of the staining for EGFR in the placenta sections varied during the pregnancy. EGFR immunreactivity was seen negative or faint in the uterus of rats in early pregnancy (GD 10), while decidua was seen clearly positive staining for EGFR at the same period (Figure 8). The intensity of the staining for EGFR in the trophoblastic giant cells and spongiotrophoblasts showed different levels between the GD 12 – GD 16 (Figure 9). But the intensity for EGFR was reduced in the trophoblastic giant cells and spongiotrophoblasts at GD 18. The Reichert's membrane showed no positive reaction for EGFR at GD 18 and GD 20 (Table 2).

Table 2: The immunolocalization of TN, type IV collagen, EGF and EGFR in the placenta of rats in different gestation day[a]

Antibody	GD	Implantation region	Uterus	Decidua		Trophoblastic giant cell	Spongio-trophoblast	Labyrinth	Reichert's membran
TN	10	++	+++	++					
				DB	*DC*				
	12		+	+	++	+/++	+/++	+/++	
	14		±	+		++	++	++	
	16					+	++	+++	
	18					±	+++	+++	±
	20					++	+++	+++	±
TypeIV collagen	10	++	++	++					
	12		+++	+++	+++	++/+++	++	+	
	14		++	+++		+++	++	++	
	16					+	+++	++/+++	
	18					±	++	+	+++
	20					+/++	+/++	±/+	+++
EGF	10	++	++	++					
	12		++	++	++	+	++	±/+	
	14		+		++	++	-/±	+	
	16					+	+	+	
	18					++	+/++	+/++	-
	20					++	+/++	+	-
EGFR	10	++	-/±	+++					
	12		+	++	++	++	++	+	
	14		+		++	+/++	+/++	+	
	16					+	+/+++	++	
	18					±	±	+	-
	20					++	++	++	-

[a]Intensity of labelling defined as (-), negative; (±), faint; (+), weakly positive; (++), positive; (+++), clearly positive. DB: Decidua basalis, DC: Decidua capsülaris

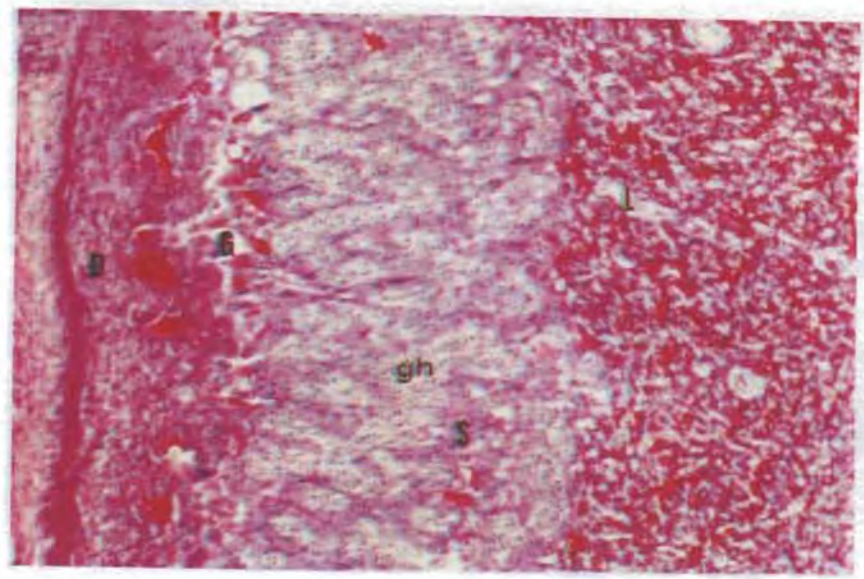

Figure 1. Histological structure of the rat placenta at GD 20. D, decidua; G, trophoblastic giant cell; S, spongiotrophoblast; gh, glycogen cell; L, labyrinth. H&E, x80

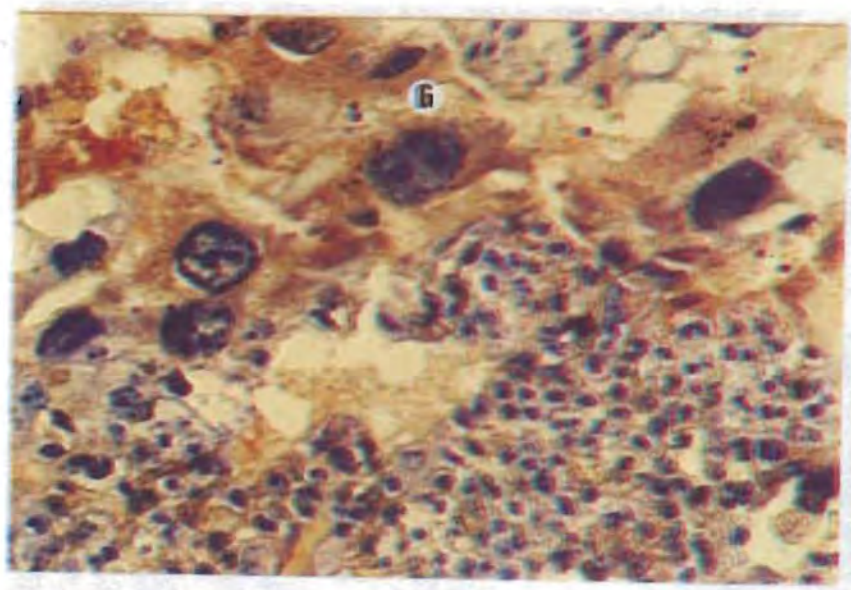

Figure 2. Immunohistochemical localization of TN at GD 14 placenta. Immunreactivity is found in the trophoblastic giant cell (G). x450

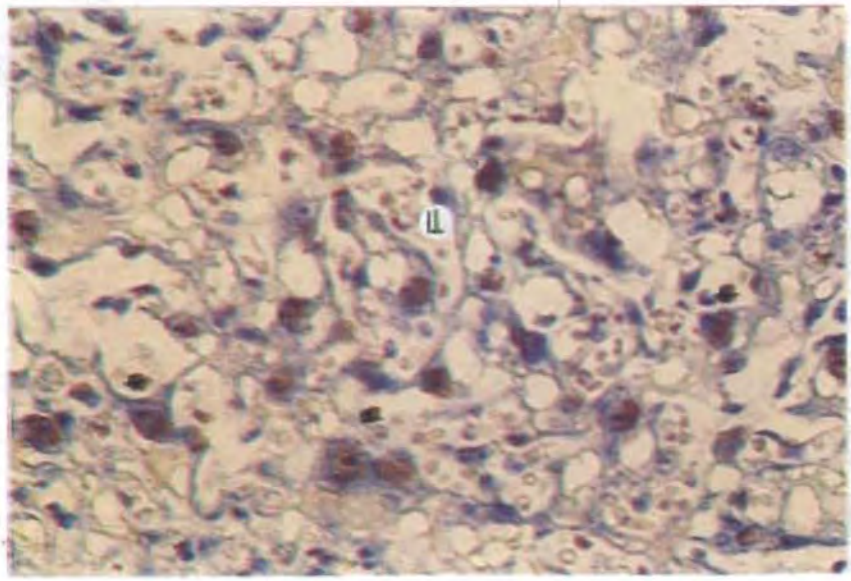

Figure 3. Immunohistochemical localization of TN at GD 20 placenta. Immunreactivity is found in the some labyrinth (L) trophoblasts (arrowheads). x450

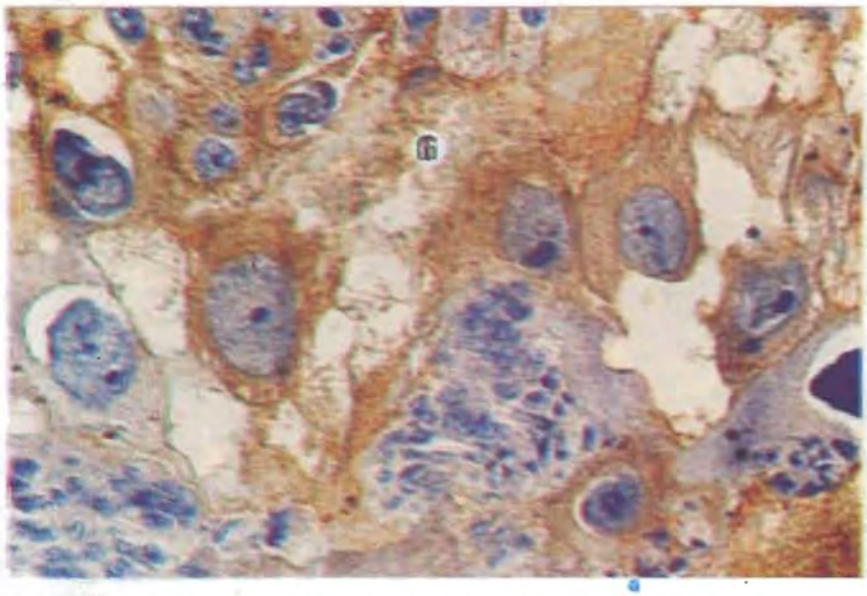

Figure 4. Immunohistochemical localization of type IV collagen at GD 14 placenta. Immunreactivity is found in the trophoblastic giant cells (G). x450

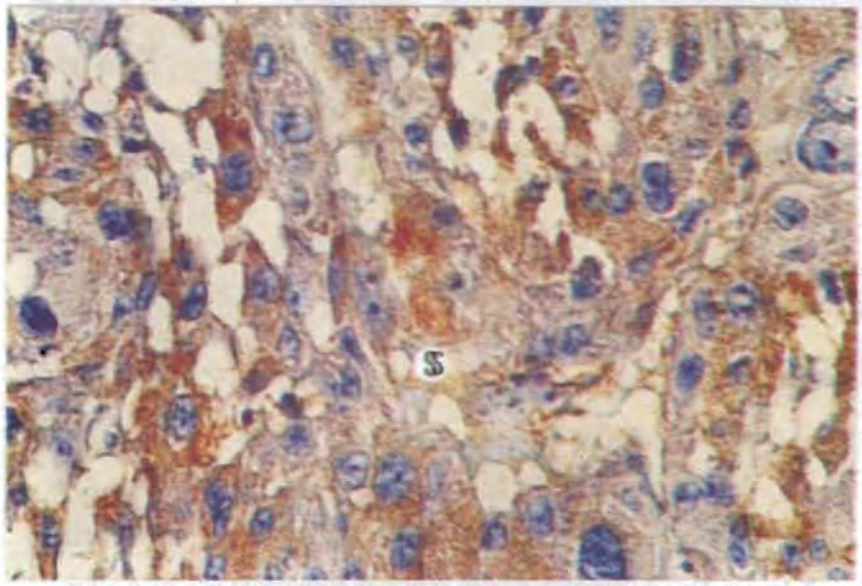

Figure 5. Immunohistochemical localization of type IV collagen at GD 16 placenta. Immunreactivity is found in the some spongiotrophoblasts (S). x450

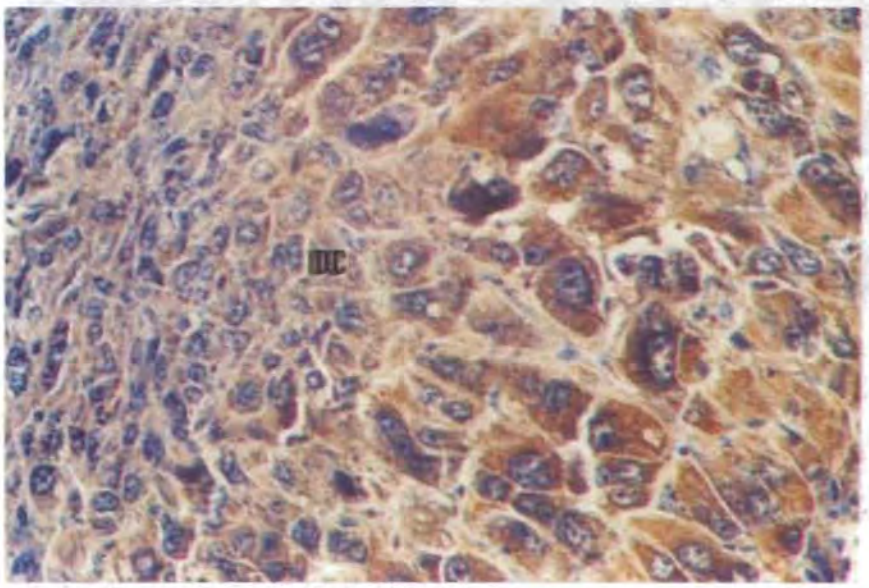

Figure 6. Immunohistochemical localization of EGF in decidua cells at GD 12. x450

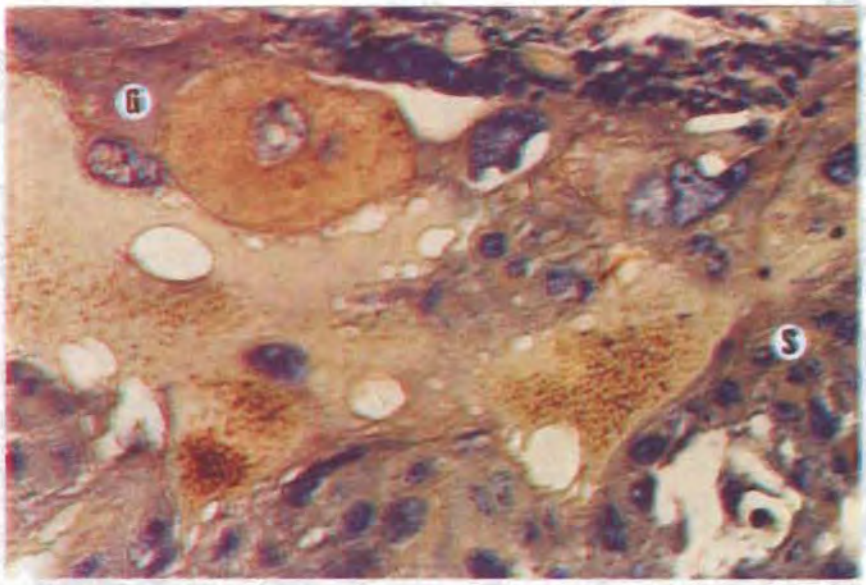

Figure 7. Immunohistochemical localization of EGF in the trophoblastic giant cell (G) at GD 20 placenta. (S) spongiotrophoblast. x450

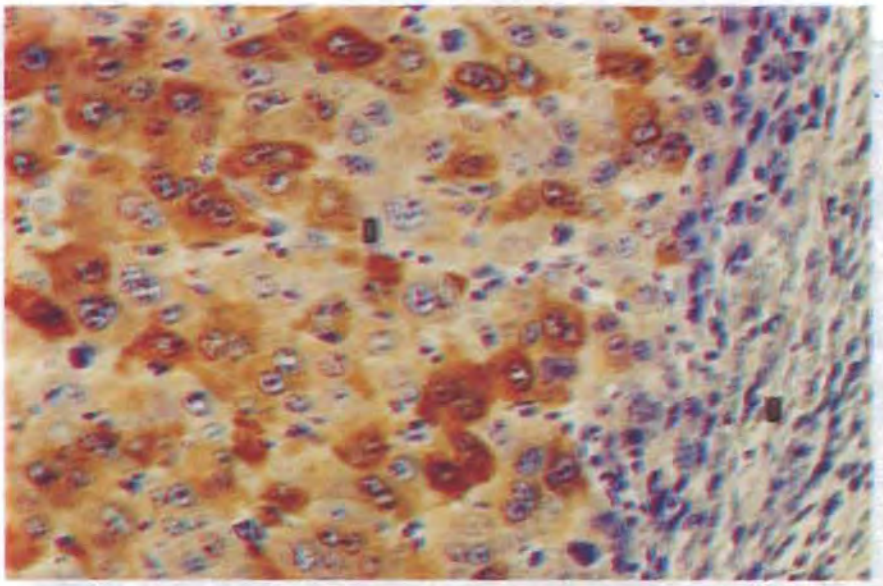

Figure 8. Immunohistochemical localization of EGFR at GD 10. Immunreactivity is found in the decidual cells (D), but no EGFR immunreactivity is found in uterus (U). x450

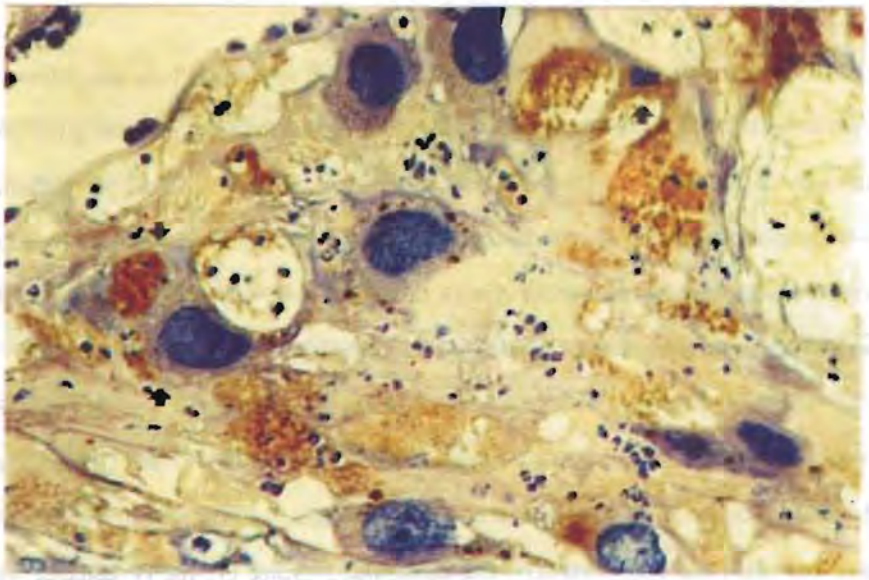

Figure 9. Immunohistochemical localization of EGFR at GD 16 placenta. Trophoblastic giant cell is found immunostaining for EGFR (arrowheads). x450

Discussion

This study was designed to evaluate the placentation, the immunolocalization and the roles of a growth factor and its receptor and some components of ECM in the placentation in Wistar albino rats at different gestational day that has not been studied in this experimental design beforehand. In this study, the weight gain, placenta and fetus weight and placental index were examined during the gestation; these parameters were suited to the preceding studies (11-13). The histological changes in the placenta during the placentation in rats show similarities to the findings reported by Bridgman (14); Davies and Glasser (15); Knudsen et al (16); Doğruman and Dağlıoğlu (17) and Bulmer and Peel (18).

In our study, the expression of type IV collagen was intense within ECM of the uterus, decidua, and trophoblastic giant cells at GD 12 and GD14. Type IV collagen immunreactivity in blood vessels and trophoblasts of labyrinth was heterogeneous throughout pregnancy. The Reichert's membrane on days 18 and 20 of gestation exhibited intensely expression of type IV collagen.

Forsberg et al. (13) reported that type IV collagen immunreactivity was found in Reichert's membrane, labyrinth, basal zone and walls of vessels in the rat placenta at GD 20. In the study of Kitaoka et al. (19), it was concluded that at GD 9.5 normal rat placenta exhibited positive staining in decidua, cytotrophoblasts and maternal arters. In another study, there were weakly positive staining for type IV in endometrium, miyometrium and decidua whereas clearly positive staining for type IV collagen in the labyrinth and trophoblasts (20).

Possibly a basement membrane matrix containing high levels of type IV collagen and lower levels of TN is permissive for trophoblast migration at GD 12 and GD 14. The synthesis and secretion of large quantities of type IV collagen for may provide trophoblast invasion regulation and promotion (21). The diminished presence of type IV collagen we observed in labyrinth at GD 16 may correspond to a reduced rate of cell migration in the labyrinth at this time. In the present study, we show that TN and type IV collagen are highly synthesized by trophoblastic giant cell early in their differentiation.

At 10 days of gestation, the intensity for TN were present in decidua and uterus. There are increases for TN immunreactivity in the spongiotrophoblasts and labyrinth trophoblasts of placenta in rats from GD 12 to GD 20. Castellucci et al. (22) reported that TN expressed during the development of the placenta, particularly in the mesenchymal villi, cell islands and cell columns in human, and in the human placenta. TN expression can be correlated with villous growth, cell proliferation, and fibrinoid deposition, TN is a peculiar component of the ECM, particularly expressed during embryonic and tumor development, wound healing, cell proliferation.

In our study, the positive staining for EGF was seen in the uterus and decidua between the GD 10 - GD 14 of gestation. The intensity of the staining for EGFR in the placentas sections varied during the pregnancy. Decidua was seen clearly positive staining for EGFR at the GD 10. The intensity for EGFR was reduced in the trophoblastic giant cells and spongiotrophoblasts at GD 18.

EGFR was showed increasing immunoreactivity along the highly differentiated syncytiotrophoblast plasma membranes with advancing pregnancy. EGFR expression is strongly related to the proliferative trophoblast in humans (23).

Ladines-Llave et al. (24) reported that in the very early placenta obtained at 4 to 5 weeks, EGF and EGFR were almost exclusively localized to cytotrophoblasts, whereas EGF and EGFR in early placentas obtained between 6 and 12 weeks predominantly localized to syncytiotrophoblasts. The dynamic change in cytological localization of EGF and EGFR in developing human placentas may reflect the change in a possible role of EGF in the course of fetoplacental development.

Immunoreactivity for EGF and EGFR in the placenta was found cell specific in our study. In the placenta, the level of proliferative activity may vary between GD 14-GD 18. In early gestation, there was a prominent role of EGF for trophoblast growth and for differentiation functions in the late phases of gestation (25). In our study, the results of immunostaining for EGF and EGFR were seen that the synthesis of these molecules closely related with gestation day in rats. These data accorded with Arnholdt and colleagues's (25) results.

Although various studies have been done on the changes in the human placental EGFR, their results have not been consistent. The present findings are to some extent consistent with those reported for the different rat strain. The fact that both EGF and EGFR in GD 10-GD 21 placentas were localized to mitotically active placental cells suggest that, in placentation, EGF and EGFR may be lined to the proliferation or differentiation of these cells.

Interaction between ECM components by the trophoblast cells and those produced by the decidua may be important in controlling the direction and extent of cell migration and also in determining the structural organization of the placenta. While several studies have investigated the role of some ECM components and growth factors in the placentation at the early and late pregnancy in rats (26, 27), there were no studies have compared the roles of these components in our studied days. The results of this study will be useful to evaluate of significance of the changes in composition of the ECM and some growth factors playing a

part in the proliferation, migration and differentiation of cells during the rat placentation. The results presented in this paper showed that during the establishment of the rat placenta, TN and type IV collagen polypeptides were not coordinately expressed.

The results presented here, in addition to those of previous reports of other researchers, indicated that the different cells of rat placenta showed different immunreactivity for TN, type IV collagen, EGF and EGFR between the GD 10 - GD 21. Growth factors can modulate both the synthesis and the degration of ECM through their action on structural components, matrix-degrading proteases, and protease inhibitors. These data will be useful for explanation of significant changes in composition ECM and the synthesis of growth factors and their receptors and the interactions between the growth factors and ECM components during the rat placental development.

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THE CULTURE OF BACILLUS SPP. FROM COMB FOUNDATION

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Abstract

Beeswax is processed in small factories the most important problems of beekeeping. Any pathogen bacteria found in comb foundations improves the spreading of honeybee diseases, especially American Foulbrood (AFB). Each of 22 samples from 22 firms were collected. Colonies of *Bacillus* spp. were identified. As a result in 9 of the 13 firm's infected comb foundations, *Bacillus alvei* was found. In the other 4 firms's samples, *Paenibacillus larvae* which causes AFB were observed. In conclusion, non-hygenic beewax is the main affect for the spreading of the diseases.

Key Words: Pacnibacillus larvae, Bacillus alvei, beewax, comb foundation

Introduction

Beeswax is an important bee product which returns partly into the hive in the form of comb foundations, but comb foundations have to be sterillized before use. When beeswax is processed in smaller factories producing foundations, cleaning and disinfection usually necessitate 0.5% H_2SO_4 and temperature of 80-90 ^{0}C for 30 minute (1).

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This procedure is sufficient for liquidation of vegetative stage of bacteria. The main bacterial diseases, American foulbrood (AFB) which is caused by *Paenibacillus larvae* and European foulbrood (EFB) which is caused by *Melissococcus pluton*, are very big problem in beekeeping industry (2). *Bacillus alvei;* also causes secondary infections in EFB diseases and is commonly found in infected brood samples (3). So, this temperature does not destroy *P. larvae* spores, their heat resistance can even be increased utilising these temperatures (4).

It is recommended that a total temperature of 121^{0}C at a pressure of 1 atm for 20-30 minute remove *P. larvae* spores from beeswax (5-7). Savov and Arsenov (8) investigated the resistance of *P. larvae* spores to temperature without increased pressure and noted that dry heat destroys the spores at a temperature of 110 ^{0}C in 2-3 h, and at 140 ^{0}C in 60-90 min. Kostecki and Orlowski (9) and Kostecki and Jelinski (10) elaborated a method for determining *P. larvae* spores in wax using a solvent and subsequent centrifugation. Isolation of spores from wax with subsequent cultivation is likewise found in a study by Hansen and Rasmussen (11) and modified to the comb foundation.

Acid resistance of spores is known (12), and it was examined that the number of *P. larvae* spores were not reduced by 0.5% H_2SO_4 in wax for 37.5 h (1).
In the comb foundations, any pathogen bacteria should not be found in order to prevent spreading of the honey bee diseases especially AFB. In this study the comb foundation is examined for being sterile truely. Thus, it can be observed the spreading of major bacterial diseases because of non-sterillized comb foundations.

Materials and Methods

The study with comb foundations involved the isolation of *Bacillus* spp. spores according to the method of Rose (13) and Wilson (14). The 220 samples which are traded comb foundations of twenty-two different firms in different regions of Turkey were collected and examined in the laboratory. One gr mixed of ten foundations were dissolved in 9 ml chloroform. The suspension was centrifuged at 3000 rpm for 30 min in order to settle down any spores. After that, 8 ml supernatants discarded, one ml remaining solution and pellets were hand-rolled. This solution was inoculated into 9 ml nutrient broth. It was incubated at 37^{0}C for 12 h and later submitted to 70^{0}C for 30 min. as the same. Incubation and heating were replicated 3 times. Thus, all non-sporulating microorganisms and vegetative cells germinated from spores capable of growing in nutrient broth were eliminated. The viability of *Bacillus* spp. spores was determined by transferring of implantations 1 ml of the sample prepared in this way on the BHI medium and BHI with 3 µg/ml nalidixic acid (2). Grown

colonies of *Bacillus* spp. were identified under the microscope with Gram stain and using biochemical tests (15).

Biochemical tests: Bacterial cultures isolated from comb foundations were stained Gram reaction and tested for catalase production, Voges-Proskauer, starch hydrolysis, decomposition of gelatin, use of citrate, acid production from mannitol and growth in nutrient broth (16,17).

Results and Discussion

A method of *Bacillus* spp. spores isolation from comb foundation was tested and verified based on studies of Rose (13) and Wilson (14) firstly, and secondly Machova (1).
In thirteen of the twenty-two cultures, Gram(+) and vegetative bacil formes of bacteria and spore formes were observed. These were identified as *Bacillus* spp. microscopically.
According to Alippi (17), *P. larvae* strains were Gram positive and they were catalase and Voges-Proskauer negative. They liquifed gelatine, but did not hydrolyse starch. Citrate was not used and variable results were obtained from mannitol. *B. alvei* cultures were Gram positive, Gram negative or Gram variable. Citrate was not used and no acid was produced under aerob condition from mannitol. They liquifed gelatin and hydrolysed starch and were positive for catalase and Voges-Proskauer.

The results of biochemical tests showed that in nine of the thirteen cultures, these were fit *B. alvei* which causes secondary infection in EFB diseases in all biochemical tests results. Biochemical tests results of the remain four samples also fit *P. larvae* which causes the major bacterial brood diseases, AFB. These results were compared with each other in Table 1.

Table 1. The results of biochemical tests compared with according to Alippi (1991).

	Bacteria	Catalase	VogPros.	Gelatine	Starch H.	Mannitol	Citrate	Gram
Alippi's Results	***P. larvae***	-	-	+	-	+,-	-	+
	B. alvei	+	+	+	+	-	-	+,-
Our Results	*Firm 1*	+	+	+	+	-	-	+
	Firm 2	-	-	+	-	+	-	+
	Firm 3	+	+	+	+	-	-	+
	Firm 4	+	+	+	+	-	-	+
	Firm 5	-	-	+	-	+	-	+
	Firm 6	+	+	+	+	-	-	+
	Firm 7	+	+	+	+	-	-	+
	Firm 8	-	-	+	-	-	-	+
	Firm 9	-	-	+	-	-	-	+
	Firm 10	+	+	+	+	-	-	-
	Firm 11	+	+	+	+	-	-	+,-
	Firm 12	+	+	+	+	-	-	+
	Firm 13	+	+	+	+	-	-	+

In this respect, the occurence of *Bacillus* spp. in comb foundations threats Turkish beekeeping day by day. This event is the main affect for spreading of the diseases. So, the comb foundations have to be controlled for the sterilization in routine. In Turkey, Hacettepe University will inspect these firms for the goverment.

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EFFECTS OF PHOTOPERIOD ON TESTIS DEVELOPMENT OF INSECTICIDE RESISTANT AND SUSCEPTIBLE POPULATIONS OF THE HOUSE FLY, *Musca domestica* L. (Diptera: Muscidae)

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Abstract

The present study was conducted to examine the influences of different photoperiod regimes on selected testis development parameters in relation to insecticide resistance and susceptible strains of the house fly, *Musca domestica* L. (Diptera: Muscidae), under laboratory conditions. The control stocks were bred under 12/12 hr L:D (hours light:dark) photoperiod condition, and experimental stocks were bred under 0/24 hr, 6/18 hr, 12/12 hr and 18/6 hr L:D photoperiod conditions. The effect of photoperiod was evaluated by measuring the length, width and volume of the testis of both the resistant and susceptible strain.

Key Words: Diptera, house fly, *Musca domestica*, testis development, photoperiod

Introduction

The house fly is a pest well known. Its association with household garbage and other organic disposals also makes it a potential vector for many diseases which can affect public health. Therefore efforts to control this organism have been and still are an important research subject. The house flies natural ability to adapt to various habitats has made it a cosmopolite insect well distributed in most parts of the world. The house flies high reproduction rate and its ability to adapt to various environments makes control efforts very difficult therefore information of its capacity to increase under different environmental conditions is of high importance (2).

The anatomy of the external and internal reproductive organs of certain Diptera and their reproduction has been the topic of many scientific papers. Lachance *et al.* (3) gave a detailed description of the effects of chemosterilants on spermatogenic stages in the house fly. French and Hoopingarmer (1) gave a description of gametogenesis in the house fly.

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Insecticide resistant and susceptible house fly populations show differences in terms of various biological activities. Şişli *et al.* (4 and 5) and Çağlar (6 and 7) studied the life table of house fly and showed that the maturing period of embryos, larvae and pupae of resistant populations and the mean generation lenght (T) were longer than those of susceptible populations when reared under the same conditions; however, the innate capacity for increase (r_m) for susceptible populations was higher than that for resistant populations.

In addition, insecticide resistance rate in any population shows seasonal and annual changes. Photoperiod is a reliable indicator of seasonal change, and can provide the insect with a vital opportunity to prepare physiologically and behaviorally for inclemency's. The efficacy of photoperiod as an anticipatory cue in the life history of an individual insect is dependent on the life stages during which the insect is capable of perceiving the requisite photoperiodic changes, and the extent to which these stages overlap with the critical photoperiodic shifts (8).

The present study was conducted to examine the influences of different photoperiod regimes on selected testis development parameters in relation to insecticide resistance and susceptible strains of the house fly, *M. domestica*, under laboratory conditions.

Material and Methods

The insecticide resistant (ANT) and World Health Organization standard susceptible populations (WHO) of house fly were used in this study. The insecticide resistant strain was collected from the Antalya municipal refuse site and reared under laboratory conditions. This population had acquired a high level of resistance to most of the pyrethroid and organophosphate insecticides used in the municipal garbage dump of Antalya (9). The standard-susceptible population was obtained from Danish Pest Infestation Laboratory where it was bred for 83 generations without contact to any chemicals.

Prior to this study both stocks were maintained under the following conditions: 12/12 hr L: D (hours light: dark) photoperiod, 75±5% RH relative humidity and 25±1°C temperature. Experimental stocks were set up from these stocks and were transferred to 0/24 hr, 6/18 hr, 12/12 hr and 18/6 hr L:D photoperiod conditions for rearing.

Ten adult males were collected from each experimental condition on a daily basis from the same cohort. Collected males were dissected in Ringer's Solution under 10X of a binocular microscope [Leica Zoom 2000 Model, Germany]. The dissected testes were fixed 10 min in a 1/3 25% glacial acetic acid: 96% ethanol solution, followed by a solution [(6) methanol: (3) chloroform: (2) propionic acid] (10), and stained 5 min with aceto-carmine (11). The dissected testes were embedded in glycerin and slide mounted with enthellan. The

status of testis development was estimated according to French and Hoopingarner (1). The prepared testes were measured for the following parameters; length, width and volume. Measuring of the parameters was done by using a standard light microscope (16 x 10/0.25) [Prior A 216 Model, England] fitted with a micrometer (1 unit= 30 micron). The volume of the testis was calculated by using the volume formulae [$\pi/6$ x (length x width)^2] based on the assumption that the testis is an ellipsoid (12).

The relative effects of photoperiod on testis development time were evaluated by one-way ANOVA for equal sample sizes. Differences in testis length between insecticide resistant and susceptible strains were evaluated with two-way ANOVA for equal sample sizes (13)

Results

The effect of photoperiod was evaluated by measuring the length, width and volume of the testis in both insecticide resistant and susceptible house fly strains. The minimum, maximum, mean and standard deviations of the selected parameters were calculated and are presented in Table 1.

12/12 hr L:D photoperiod: The maximum length, in the susceptible strain was reached in the fourth and fifth days and in the resistant strain was reached in the fourth day. Overall growth in the resistant strain was higher than the susceptible strain. Testis width of both strains reached the highest mean values in the second day and no significant difference between the resistant and susceptible strains was recorded. Testis volume of both strains reached the highest mean values in the second day. It was again noted that overall growth of the resistant strain was higher than the susceptible strain. For the given photoperiod condition the two peaks seen in all of the parameters (length, width, volume) seems to indicate that sperm production continues up to the fifth day and two ejaculations occurred during the six day period.

6/18 hr L:D photoperiod: The maximum length, in the susceptible and resistant strain were determined in the fifth and sixth respectively. Overall growth in the resistant strain was higher than the susceptible strain. The highest mean value of testis width in the susceptible strain was detected in the fourth and fifth days and in the resistant strain was detected in the sixth day. The highest mean values of testis volume in the susceptible and resistant strains were determined in the sixth and second day respectively.

Table I. Daily testis development of the susceptible (WHO) and resistant (ANT) populations bred under different photoperiod conditions.

			Photoperiod conditions (Hour L:D)							
			Susceptible (WHO)				Resistant (ANT)			
Day	Parameters		0/24	6/18	12/12	18/6	0/24	6/18	12/12	18/6
	Length (μ)	min	800	869	800	864	1024	992	800	960
		max	1184	1248	1216	1344	1504	1440	1312	1408
		x ± sx	1002±131	1104.8±125	1001.6±108	1064.5±129	1204.7±149	1189.1±120	1104±137	1144±116
	Width (μ)	min	544	640	640	544	640	640	608	576
1		max	960	1152	1152	864	1024	928	1056	1152
		x ± sx	754±126	901±152	820.8±122	697.6±90	784.9±109	776.4±78	812.4±118	793.6±146
	Volume (μ^3)	min	99,1	172,1	176,4	142,7	247,8	247,8	123,8	160
		max	605	973,6	640,1	426,9	944,6	702	1004,6	1228,1
		x ± sx	323.3±153	563.6±248	369.7±145	299.6±108	488.2±202	457.4±138	459.2±230	475.9±282
	Length (μ)	min	800	763	800	800	1152	992	992	1056
		max	1440	1344	1504	1248	1696	1568	1504	1472
		x ± sx	1168.8±177	1133.2±179	1146.9±150	992±133	1337.3±130	1289.6±149	1248±146	1245.9±123
	Width (μ)	min	608	672	640	512	608	800	800	800
2		max	1056	1152	1536	896	1120	1344	1312	1088
		x ± sx	858.9±144	882.8±145	966.7±184	754.6±94	858.9±135	1009.6±158	1005.5±159	904.5±74
	Volume (μ^3)	min	123,8	167,2	151,3	95	333	490,1	329,6	454,4
		max	1087,9	1254,5	2792,9	456,4	1888,3	2037,7	2037,7	1342,3
		x ± sx	593.8±323	586.9±345	729.2±545	300.9±111	744.1±400	927.9±426	899.9±468	688.2±241
	Lenght (μ)	min	928	960	864	800	1088	1088	1024	800
		max	1344	1504	1248	1344	1440	1536	1536	1408
		x ± sx	1121.4±88	1171.5±148	1098.1±106	1143.1±141	1273.6±98	1325.5±148	1285.3±152	1230.2±145
	Width (μ)	min	704	640	704	640	608	448	512	576
3		max	1088	1280	1552	896	992	1120	1376	960
		x ± sx	914.5±109	890.6±179	875.8±110	787.5±74	812.8±113	864±147	842.6±234	785.7±93
	Volume (μ^3)	min	335,6	300,5	250	137,2	317	139,4	162,4	214,4
		max	1054,2	1939,5	921,7	654,4	975,1	1548,8	1964,4	814,1
		x ± sx	569.6±196	641±453	503±175	441.2±140	578.5±198	748.2±355	725.6±532	499.1±153
	Lenght (μ)	min	960	928	544	960	1056	1120	1024	1024
		max	1440	1280	1568	1312	1440	1472	1600	1504
		x ± sx	1209.6±124	1113.6±102	1185.6±226	1171.2±88	1216±93	1321.8±104	1277.7±176	1270±135
	Width (μ)	min	768	704	448	544	704	608	736	480
4		max	1056	1120	1120	1024	992	992	1344	736
		x ± sx	921.6±87	868.3±124	904±161	788.8±114	869.1±85	825.3±99	912±185	656±69
	Volume (μ^3)	min	373,5	223,4	31,1	205,5	373,5	317	323,7	224,7
		max	1105	1075,6	1523,1	843,8	934,5	1115,9	2420	491,1
		x ± sx	671±215	509.9±206	699.3±417	463.3±166	596.5±159	641.5±200	825.4±657	365.9±89
	Lenght (μ)	min	992	640	1120	1152	1056	1248	1248	1120
		max	1472	1536	1568	1504	1504	1568	1504	1440
		x ± sx	1185.6±136	1160.4±215	1267.5±115	1288.5±111	1294.4±100	1385.3±99	1378±70	1281.6±97
	Width (μ)	min	544	512	736	704	704	640	480	640
5		max	1056	1120	1088	1056	992	1088	1024	1120
		x ± sx	833.6±129	828.6±137	892.4±92	836.3±115	811.2±68	796.2±113	820±140	787.2±114
	Volume (μ^3)	min	194,3	56,2	355,6	397,4	425	369	239	296,5
		max	1264,5	1548,8	1523,1	1054,2	843,8	964,9	1241,3	913,2
		x ± sx	553.9±291	551.5±338	708.9±311	614.8±176	581.9±125	646.9±178	688.6±246	548.3±181
	Lenght (μ)	min	1056	1216	1120	1120	1088	1088	992	896
		max	1504	1440	1248	1440	1600	1728	1536	1504
		x ± sx	1217.7±152	1320.4±89	1168.8±42	1268.8±98	1315.4±126	1396.8±155	1313.6±143	1253.1±156
	Width (μ)	min	640	736	736	704	640	672	576	576
6		max	992	1024	1024	1216	832	928	1088	896
		x ± sx	801.7±88	837.1±76	853.9±79	894.4±133	724.2±69	788.8±77	811.2±125	764.6±107
	Volume (μ^3)	min	268,9	419,2	387,2	383,5	284,5	307	344,2	170,9
		max	975,1	1137,9	688,4	1604,6	857,4	1200,4	1401,3	795,5
		x ± sx	522±208	654.3±186	523.1±86	707.5±318	492±168	667.9±258	620.2±267	493.5±164

Overall growth in the resistant strain was higher than the susceptible strain. During the study period testis length increased continuously in both of the strains. Growth in width and volume of the testes however showed only one peak value indicating that sperm production decreased after day two and only one ejaculation occurred.

18/6 hr L:D photoperiod condition: The maximum length of testis, in the susceptible strain was recorded at day 5 and in the resistant strain was recorded at day 6. However the overall growth of the testis in the resistant strain was higher than the susceptible strain. The highest mean value of testis width in the resistant and susceptible strain were determined in day one and six respectively. The highest mean value of testis volume in the susceptible strain was reached in the sixth day and in the resistant strain was reached in the second day, but overall volume growth was again higher in the resistant strain. For the populations reared in this photoperiod a continuous increase in length was noted. The two peaks seen in width and volume growth indicate that sperm production was continuous and two ejaculations occurred.

0/24 hr L:D photoperiod condition: The maximum length, in the susceptible strain was reached in the sixth day and in the resistant strain was reached in the second day. Overall growth of the testis in the resistant strain was higher than the susceptible strain. The highest mean value of testis width in the both strain was reached in the second day. The highest mean value of testis volume in the susceptible strain was reached in the fourth day and in the resistant strain was reached in the second day, but overall volume growth was higher in the resistant strain. For the given photoperiod condition the two peaks seen in all of the parameters indicates that sperm production was continuous and two ejaculations occurred.

Statistical evaluation of the data, shows that the daily differences in length of the testis for the different photoperiod conditions were of significant importance ($p<0.05$). Differences in the length of the testis at different photoperiod regimes were evaluated separately for the two strains. In the susceptible strain it was observed that the differences between 0/24 hr L:D and 6/18 hr, 12/12 hr L:D photoperiod condition and differences between the 6/18 hr L:D and 12/12 hr, 18/6 hr L:D photoperiod conditions are of significant importance ($p<0.05$). In the resistant strain it was observed that the differences between 6/18 hr L:D and 18/6 hr L:D photoperiod condition and the differences between the 18/6 hr L:D and 12/12 hr L:D photoperiod condition is of significant importance ($p<0.05$).

The daily variation in width of the testis for both strains for the given photoperiod conditions were statistically evaluated. The daily differences in width of the testis for the different photoperiod conditions were of significant importance ($p< 0.05$), except for the 6/18

hr L:D photoperiod condition. In the resistant strain daily differences in width of the testis were of significant importance ($p< 0.05$) between all photoperiod conditions. Differences in the length of the testis at different photoperiod regimes were evaluated separately for the two strains. In the susceptible strain differences in mean testis length between photoperiod conditions was not significant ($p>0.05$). In the resistant strain significant differences in testis length ($p<0.05$) was observed t between the 6/18 hr L:D and 18/6 hr L:D photoperiod condition and the 6/18 hr and 12/12 hr L:D photoperiod condition.

In the susceptible strain, statistical evaluation of the data for the daily differences in volume of the testis for the different photoperiod conditions, showed the differences between photoperiods to be of significant importance ($p<0.05$), except for the 6/18 hr L:D photoperiod condition. In the resistant strain the daily differences in volume of the testis was determined to be significant between all photoperiod conditions ($p<0.05$). Differences in the volume of the testis at different photoperiod regimes were evaluated separately for the two strains. In the susceptible strain it was observed that the differences between 0/24 hr L:D and 6/18 hr, 12/12 hr, 18/6 hr L:D photoperiod conditions are of significant importance ($p<0.05$). In the resistant strain it was observed that the differences between 18/6 hr L:D and 6/18 hr, 12/12 hr L:D photoperiod conditions are of significant importance ($p<0.05$).

Discussion

The house fly, *M. domestica*, is a cosmopolitan species that often occurs in and around landfills, where larvae and adults are potentially exposed to numerous toxic chemicals and apparently prospers under these conditions. This species has also been prolific in developing resistance to a variety of insecticides, involving a number of different detoxification mechanisms (14; 15). Species can show different reactions to various environmental conditions because of genetic variation. For examples, Elvin and Krafsur (16) determined that a laboratory strain of *M. domestica* matured faster under different temperature conditions than a wild strain. Insecticide resistant and susceptible strains of *M. domestica* could be expected to have developmental differences due to underlying genetic differences. Şişli *et al.* (5) and Çağlar (6 and 7) reported that larval and pupal maturation time of a susceptible strain of *M. domestica* was longer than a resistant strain. In general, resistant strains of an organism will have a higher survival rate in an environment with the causal agent present. Because population losses during development is lower, population increases are possible with lower sperm production rates. Lower energy and resource expenditures on sperm production would leave and increased amount of energy for use in other life functions, such as growth or activity, potentially increasing survival chances. Increases in T value and life span also support this concept.

Photoperiod is often used by insects as a measure of seasonal change and as cues for activities like reproduction or diapause. Diapause is induced mainly by short photoperiods (17) which also act to maintain it (18). The finding of a peak of ecdysteroids in prediapause adults of both sexes by Briers and De Loof (19) has led to the suggestion that diapause induction results from a change in hormonal balance between juvenile hormone and ecdysteroids rather than from a depletion of juvenile hormone alone. Tauber and Tauber (20) showed that the urinary organs of diapause females in *Chrysopa carnea* were active in a 12/12 hr L:D photoperiod. However, there was a sharp decrease in fecundity in a 18/6 hr L:D photoperiod. Colorado potato beetle, *Leptinotarsa decemliniata* reared under a long-day photo regime (18 hr) from egg entered the reproductive phase 5 days after adult emergence. Under short-day conditions (10 hr) the adults did not show reproductive activity and entered diapause 10-12 days after adult emergence (21).

Our results are consistent with those in the present study. According to the evaluated data, we can state that the resistant strains always showed a higher capacity for growth than the susceptible strains in all of the photoperiod conditions examined. Apart from this when the effects of photoperiod on testis development is assessed it can be seen that the best growth occurs in the 12/12 hr L:D photoperiod condition. The 18/6 hr L:D photoperiod condition is also worth mentioning. In this condition the resistant strain showed better growth during the first two days but in the following days the susceptible strain showed better growth. Although growth in the 18/6 hr L:D photoperiod condition for the susceptible strain can clearly be seen it is not certain if sperm ejaculation can take place with normal success in this photoperiod.

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FIELD TRIALS OF *Bacillus thuringiensis* subsp. *israelensis* DE BARJAC FORMULATION (VECTOBAC® 12 AS) FOR CONTROL OF *Culex* (*Culex*) *tritaeniorhynchus* GILES (DIPTERA: CULICIDAE) IN BELEK, TURKEY

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Abstract

In this study, the efficiency and residual activity of aqueous suspension of *Bti* formulation, Vectobac® 12 AS which has a potency of 1200 International Toxicity Units per mg (1200 ITUs/mg), was evaluated for control of *Culex* (*Culex*) *tritaeniorhynchus* Giles (Diptera: Culicidae). Field trials were conducted in eight small sized plots in Belek. 250 ml/ha, 500 ml/ha, 750 ml/ha and 1000 ml/ha dosages were used in pasture breeding habitat with high vegetation cover. In addition to the former trials, 500 ml/ha and 1000 ml/ha dosages were tested in marshy breeding habitat with semi vegetation cover. Mean larval density of pretreatment and posttreatment were compared and the 24-h posttreatment efficiency and residual activity of Vectobac® 12 AS were evaluated. The 81.16% reduction in total mean larval densities obtained at 24-h posttreatment with 500 l/ha dosage of Vectobac® 12 AS in marshy habitat started to drop consistently after the 2nd day and it was found to be 37.80% at the 6th day.

Key Words: *Culex* (*Culex*) *tritaeniorhynchus*, *Bacillus thuringiensis* subsp. *israelensis*, Vectobac® 12 AS, Biological control.

Introduction

Culex (*Culex*) *tritaeniorhynchus* Giles is distributed throughout eastern Asia, the Oriental Region, southwestern Asia, and Africa (1). Generally, it is a common species in Mediterranean and Aegean region of Turkey (2, 3, 4, 5, 6, 7, 8, 9, 10). The larval stages of *Cx. tritaeniorhynchus* are found in rice fields, floodwaters, and marshy areas with floating or emergent vegetation. Other breeding habitats of the species are ponds, swamps, stream margins, springs, irrigation ditches, grassy pools, seepages, and animal footprints (1, 11).

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Females generally feed on birds, pigs and other domestic animals and are also attract to humans, both indoor and outdoor during any time at night (8, 12). *Culex tritaeniorhynchus* is a vector or potential vector of pathogens that cause human diseases. It is the primary vector of Japanese encephalitis in the areas of southern Asia, and it has been found infected with dengue, Rift Valley Fever, Sindbis, Getah and Tembusu viruses, and microfilariae of both *Brugia malayi* and *Wuchereria bancrofti* in many areas of eastern and southeastern Asia (1, 12). Moreover, the transmission efficiency for West Nile virus in the laboratory demonstrates that it is an excellent potential vector for this pathogen in Pakistan (13).

Bacillus thuringiensis subsp. *israelensis* de Barjac (*Bti*) has became important for the control of mosquito larvae because of concern over the potential adverse health effects from environmental contamination due to application of conventional insecticides and the development of resistance in target populations (14, 15). *Bti* is gram-positive sporulating bacteria, which produce protoxin crystals during sporulation, which can be ingested by mosquito larvae and are highly toxic to susceptible individuals (16). In present, *Bti* is used in all continents. Worldwide usage of *Bti* is steadily increasing and the extensive laboratory studies, coupled with no reported cases of human or animal disease after more than 25 years of widespread use, clearly argue for the safety of this active microbial bio-control agent (17, 18). Due to its high specificity, *Bti* is remarkably safe for the environment; since it poses no threat to non-target organisms (except for a few other nematocerous Diptera when exposed to much higher than recommended rates of application) (18, 19, 20).

Up to date no research on the vectorial capacity of Turkish *Cx. tritaeniorhynchus* has been conducted but since the species is generally distributed throughout important tourism regions and is found frequently near international airports, it can be a major threat as a potential vector of many pathogens. Therefore it is extremely important to use suitable control agents and develop effective control methods.

In this study, the efficiency and residual activity of aqueous suspension of *Bti* formulation, Vectobac® 12 AS (1200 ITUs/mg), for control of *Cx. tritaeniorhynchus* was evaluated by field trials conducted in eight small sized plots in Belek.

Material and Methods

Study area:

Located between 36° 51' 00" and 36° 51' 19" N and 31° 04' 53" and 31° 65' 38" E, Belek is part of a 29 km long national reserve situated between the Aksu and Köprüçay rivers in

the Serik District of Antalya Province, where tourism is of high economic importance. Annual rainfall is 908 mm and is heaviest in winter and spring. Annual average temperature and relative humidity are 18°C and 65% RH, respectively (9).

Field trials;

Trials were conducted in two different breeding habitats of *Cx. tritaeniorhynchus* in Belek. The first breeding habitat might be characterized as a pasture habitat with high vegetation coverage and mean water depth of 5 cm. The other breeding habitat, might be characterized as a marshy habitat with semi vegetation coverage and mean water depth of 20 cm. Prior to treatment of formulation, pH, water temperature (Model WTW Typ-U-455) and conductivity (Model WTW LF 90) of breeding habitats were recorded. The values obtained from habitats were 9.19, 25.2 °C, 0.44 mS/cm and 8.76, 29.2 °C, 0.71 mS/cm, respectively. Efficiency trials for Vectobac® 12 AS were conducted in pasture habitat while residual activity trials were conducted in the marshy habitat. Efficiency tests of Vectobac® 12 AS at 24-h period posttreatment, at 250 ml/ha, 500 l/ha, 750 ml/ha and 1000 ml/ha dosages were conducted in five 25 m^2 plots (1 control; 4 treatment) within the pasture habitat. Residual activity of the formulation at 500 ml/ha (dosage recommended for clean and vegetation free habitats) and 1000 ml/ha (dosage recommended for polluted habitats with high vegetation coverage) dosages were conducted in the marshy habitat in three 50 m^2 plots (1 control; 2 treatment). In order to determine the larval population density in both control and treatment plots, 20-dip samples were taken randomly using a long-handled dipper. Larval populations were categorized into early (1^{st} and 2^{nd} instars) and late (3^{rd} and 4^{th} instars) instars, than were counted and recorded as mean number of larvae per plots. Counting larvae put back into their own plots. The pupae, if any, were excluded from counting since no feeding activity occurs in the pupal stage. Afterwards the formulation was sprayed evenly on to the entire water surface of the treatment plots by a 10-liter stainless steel pressurized hand sprayer. For determining the efficiency of the formulation, larval sampling was conducted at 24-h posttreatment. For residual activity, larval sampling was conducted daily, for six days following treatment. The reduction in larval densities was calculated for both trials by using values obtained from the posttreatment samplings. Percentage reduction in larval densities was calculated using the formula of Mulla et al. (21), which takes into account the natural changes in the mosquito larval populations and data were analyzed with SAS statistics program (22).

$$\text{Percentage Reduction} = 100-[(C1 \times T2/T1 \times C2)] \times 100$$

Where C1 = mean number of larvae in control groups before treatment, T1 = mean number of larvae in treatment groups before treatment, T2 = mean number of larvae in treatment groups after treatment, and C2 = mean number of larvae in control groups after treatment.

Results and Discussion

The 24-h posttreatment efficiency values for Vectobac® 12 AS at 250 ml/ha, 500 l/ha, 750 ml/ha and 1000 ml/ha dosages are given in Table 1.

Table 1. Efficiency of Vectobac® 12 AS formulation on densities of early and late instars *Cx. tritaeniorhynchus* and the percentage of reduction in field trials at 24-h posttreatment with varying concentrations in pasture breeding habitat.

Formulation	Dosages ml/ha	Mean no. of larvae / 20 dips				% Reduction	
		Pretreatment		Posttreatment			
		Larval instars				Larval instars	
		early	late	early	late	early	late
Vectobac® 12 AS	1000	22.30	7.10	0.95	0.85	95.61	90.10
	750	23.20	9.50	1.05	2.30	95.34	79.98
	500	25.70	7.05	5.35	3.75	78.55	56.01
	250	21.00	7.70	9.75	6.85	52.17	26.42
Control plot		22.20	8.85	21.55	10.70		

There was no significant difference in mean number of early and late instars between pretreatmant and 24-h posttreatment in control plots ($F= 0.43$, $df = 1$, $P= 0.52$).

After the application of Vectobac® 12 AS, it was observed that the level of reduction in the density was relatively high in early instars when compared with the late instars. 1000 ml/ha dosage of Vectobac® 12 AS caused 95.61% reduction in early instars while causing 90.10% reduction for late instars. The larvicidal effect of the formulation on both the early and late instars was found to decrease according to decreasing dosages. 52.17% and 26.42% reduction in densities of early and late larval instars were recorded at the 250 ml/ha dosage (Table 1). When the efficiency of formulation was evaluated for the pasture habitat, the dosages 250 ml/ha and 500 ml/ha gave very low results. This result was in agreement with the recommended dosage (1000 ml/ha) for application in breeding habitats with high vegetation cover and in highly polluted breeding habitats. These results also pointed out the importance of *Bti* usage in control of mosquito larvae according to the characteristics of the breeding habitat.

Larvicidal activity of formulations tends to decrease after treatment due to various factors (20). Therefore, formulations or application dosages providing 50-60% reduction values at 24-h posttreatment in any breeding habitat cannot automatically be accepted as effective formulations for those habitats. Because of the high reproductive capacity of mosquitoes the

efficiency of the applied larvicides must be high and the period of their residual activity must be relatively long.

The residual activity values of Vectobac® 12 AS at dosages 500 ml/ha and 1000 ml/ha in the marshy habitat are given in Table 2. Although there was an increase in mean larval densities in the control groups after application, there was no significant difference between pretreatment and posttreatment in the mean number of early, late and total instars in the control plot ($F= 1.18$, $df= 6$, $P= 0.32$). On the other hand, the mean larval densities in plots which were subjected to 500 ml/ha and 1000 ml/ha dosages of Vectobac® 12 AS decreased after treatment and reduction in daily larval densities was found to be statistically significant (500 l/ha; $F= 45.99$, $df= 6$, $P<0.001$) (1000 ml/ha; $F= 138.71$, $df= 6$, $P<0.001$).

Table 2. Pretreatment and posttreatment larval densities and % reduction values in treatment and control plot in marshy breeding habitat.

Formulation	Dosage ml/ha	Mean no. of larvae / 20 dips							% reduction		
		Pretreatment			Posttreatment						
		Larval instars			days	Larval instars			Larval instars		
		early	late	total		early	late	total	early	late	total
Vectobac® 12 AS	500	25.20	8.65	33.65	1.	6.95	1.20	8.15	72.88	48.38	81.16
					2.	8.05	2.60	10.65	70.62	79.93	73.15
					3.	5.60	5.85	11.45	75.36	72.55	72.78
					4.	8.60	3.90	12.50	42.35	85.30	67.53
					5.	7.25	8.50	15.75	52.47	65.45	57.62
					6.	11.00	13.85	24.85	40.50	42.15	37.80
	1000	32.10	8.45	40.55	1.	1.45	0.30	1.75	95.56	96.21	95.68
					2.	2.90	0.75	3.65	91.69	94.07	92.36
					3.	6.40	1.30	7.20	77.89	93.76	85.80
					4.	8.25	2.65	10.90	56.59	89.78	76.50
					5.	7.80	5.55	13.35	59.86	76.90	70.19
					6.	9.85	14.80	23.75	58.17	36.72	50.67
Control plot		41.30	11.85	53.15	1.	42.00	11.10	53.10			
					2.	44.90	17.75	62.65			
					3.	37.25	29.20	66.45			
					4.	24.45	36.35	60.80			
					5.	25.00	33.70	58.70			
					6.	30.30	32.80	63.10			

As mentioned above, the reduction in mean larval densities in days following treatment does not indicate that the formulation or the dosage has a residual activity. The 81.16% reduction in total mean larval densities obtained at 24-h posttreatment with 500 l/ha dosage of Vectobac® 12 AS in marshy habitat started to drop consistently after the 2nd day and it was found to be 37.80% at the

6th day. Although the decrease in mean larval densities was statistically significant the high decrease in larvicidal efficiency after the 2nd and 3rd day and the 73.15% and 72.78% reduction values in total mean larval densities in these days, indicates that the 500 ml/ha dosage of the formulation does not have sufficient residual activity in order to be effective in *Cx. tritaeniorhynchus* control programs. On the other hand, the percent reduction in the plot treated with 1000 m/ha dosage of the formulation in the 1st, 2nd and 3rd day was 95.68%, 92.36%, and 85.80%, respectively. After the 3rd day, larvicidal efficiency decreased rapidly and at the 6th day only 50.67% reduction was obtained. These results indicate that 1000 ml/ha dosage of the formulation is effective for only 3-4 days, so it is recommended that Vectobac® 12 AS should be applied at 3-4 day intervals against *Cx. tritaeniorhynchus*. The acceptable efficiency values of *Bti* based formulations can change significantly depending on breeding habitat, application dosage and type of formulation but usually a high percent reduction value (80%-100%) is desired (21, 22, 23). Amakraj *et al.* (24) accepts the period where the percent reduction in mean larval densities is over 80% as the period where residual activity of Vectobac® 12 AS continues.

Romi *et al.* (25) obtained complete control of mean larvae/dip densities of *Anopheles arabiensis* in early period rice field with 600 ml/ha dosage of Vectobac® 12 AS at 1st and 2nd day posttreatment but determined that the larvicidal activity was lost after the 5th day. The field trial conducted with Vectobac® 12 AS (2000 ml/ha) in Zaire showed that the larvicidal activity was lost after 48 h against *Culex quinquefasciatus* in polluted gutter water (26). Amalraj *et al.* (24) determined that 1200 ml/ha dosage of Vectobac® 12 AS suppressed the immature density of *Cx. quinquefasciatus* by more than 80% for 1-3 days in cesspits in India. Aldemir, (27) conducted control trials for *Culex theileri*, *Culex pipiens*, *Anopheles sacharovi* and *Anopheles maculipennis s.l.* in marshy and pasture breeding habitats in Gölbaşı with 750 ml/ha and 1000 ml/ha dosages of Vectobac® 12 AS and determined 92.3%-100% larval mortality at 24-h posttreatment but larval population densities started to increase at the 3rd day of posttreatment.

Results obtained from the trials are consistent with the works mentioned above although they were conducted with different species in different breeding habitats and indicate that Vectobac® 12 AS had a high efficiency yet its residual activity was short. In order to establish successful control programs for species like *Cx. tritaeniorhynchus* which prefer breeding habitats with high vegetation cover, choosing the right formulation according to breeding habitat features, and choosing the right dosage which has minimal effect on non-target organisms is of vital importance.

Generally control programs for mosquito species using rice fields, pastures and marshes as breeding habitats require comprehensive time, cost and labor. Therefore together with

environmental effects, larvicidal efficiency and residual activity of formulations are also important in determining the proper agents for control. The larvicidal efficiency of Vectobac® 12 AS for control of *Cx. tritaeniorhynchus* was notably high but the fact that the formulation had to be applied every 3-4 days even in high dosage rates like 1000 ml/ha indicates that problems might arise when limitation due to time, cost and labor are considered.

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CHEMICAL COMPOSITION AND ANTIBACTERIAL ACTIVITIES OF PROPOLIS COLLECTED FROM DIFFERENT REGIONS OF TURKEY

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Abstract

The aim of the present work is to study the chemical composition and antibacterial activities of Turkish propolis collected different regions of Turkey such as Adapazarı, Burdur, Bursa, Kemaliye-Erzincan, Ordu and Trabzon provinces. Ethanol extracts of propolis (EEP) were prepared for chemical analysis. Gas chromatography coupled with mass spectrometry (GC-MS) was used for the chemical analysis in the organic compound base. *Escherichia coli*, *Salmonella typhi*, Proteus sp., *Staphylococcus aureus*, *Bacillus subtilis* and Beta Hemolytic Streptococcus bacteria were used to determine the antibacterial activity of propolis samples. All propolis samples have different chemical composition, however antibacterial activities of the propolis samples showed similar antibacterial properties. It was observed that all propolis samples affected to the growth of the gram positive bacteria. The activity against gram negative bacteria was weak.

Key words: Turkish Propolis, Chemical Composition, Antibacterial Activity

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Introduction

Propolis is a kind of resinous bee product, collected by worker honeybees from the buds and leaves of the plants, trunk wounds and trees (eg, *Castanea sativa*, Populus spp., *Aesculus hippocastanum*, Pinus spp and Betula spp.). The bees attach the propolis on their hind legs, and carry it back to their colony (1,2), where it is combined with bee wax and used by worker "hive" bees to seal and sterilize the colony nest (3).

Propolis is used in various folkloric and medical applications for its antiseptic (4), antimycotic (5), anti-inflammatory (6) and other beneficial properties in many part of the world (7). Use of propolis by humans has a long history, predated only by the discovery of honey. Propolis contains 50-70 % resins and 10 % essential oils, coming from the trees, mixed with 30-50 % wax for proper consistency and 5-10 % pollen (8). Chemical composition of propolis is very complex and contains many different organic compounds (eg, aliphatic and aromatic acids and their esters, alcohols, ketones, aldehydes, chalcones, flavanoids, amino acids, sugar, inorganic metal ions, and the other compounds) (9). Chemical composition of propolis is changed by different parameters such as climate condition, location and years. Since the different chemical compounds in propolis may affect different biological reactions in living organisms, determination of chemical composition and amount of the each component is important for the use.

Some chemical compounds in different group in propolis are effective inhibitor to some enzymes and showing antibacterial activity (10). In the propolis sample, there are many different chemical compounds to show antibacterial activity to the different bacteria (11, 12). But so far, it has not known very well that which compound is showing antibacterial activity to which bacteria. To examine the activity of the chemical compounds to different bacteria, TLC and HPLC chromatographic techniques were used for the fractionation of the propolis samples (13, 14). Collected fractions were tested for the antibacterial activity of some chemical compounds. The antiviral and antimicrobial activities, the free radical scavenging effect, and antitumor activity of propolis samples were also examined (15,16) . For the antimicrobial activity, propolis samples were studied seasonal effect on the survival curves of *Staphylococcus aureus* and *Escherichia coli*. After incubation with propolis, it has been observed that there was no significant difference on the antibacterial activity of the propolis samples (17). Antibacterial activity of propolis samples collected wide range area in the world were examined using different bacteria. It has been found that propolis samples showed antibacterial activity to GTFs (10), Infectious Bursal Disease Virus and Reo-Virus (15), *Staphylococcus aureus* (16,18), *Escherichia coli* (15, 16), *Candida albicans* (15), *Aertemia salina nauplii* (11) and *Helicobacter pylori* (19).

In this study antibacterial activity of Turkish propolis collected wide range area in Turkey was examined using different gram(+) and gram(-) bacteria and made correlation between the chemical composition of the propolis samples with the activity of those.

Materials and methods

Sampling

All group samples collected from different regions in Turkey belonged to Apis mellifera colonies. The hives were located at Bursa province in West, at Adapazari province in North-West, at Burdur province in South-West of Anatolia, at Ordu province in North, at Trabzon province in North-East, and at Kemaliye-Erzincan province in East in Turkey (Fig 1).

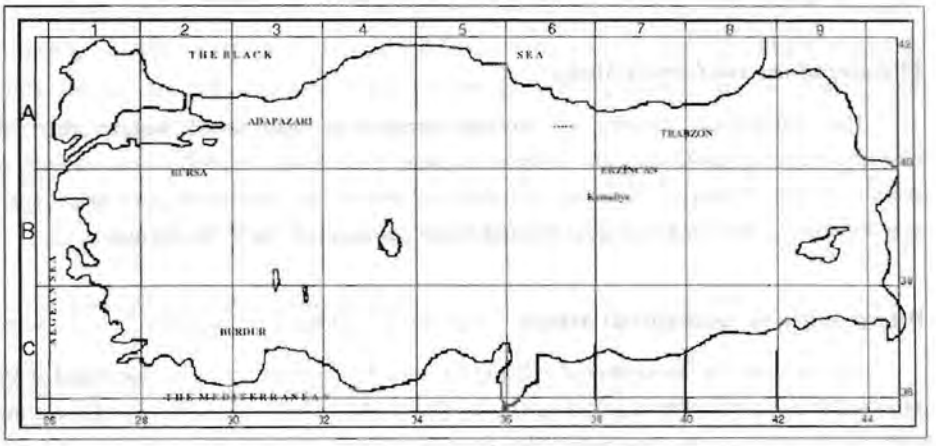

Fig.1.Various locations where propolis samples were collected from Turkey.

The samples were obtained by scraping the walls, frames and other hive parts. Also samples were collected using a propolis trap.

Preparation of Ethanol Extracts of Propolis (EEP)

The hardened propolis was ground and 100 g of sample were dissolved in 300 ml ethanol (% 96) This mixture was preserved for two weeks in a bottle closed tightly and kept in the incubator at 30 °C. After incubation procedure, supernatant was filtered twice with Whatman No: 4 and No:1 filter paper. The final filtered concentrated solution (1:10, w/v) called Ethanol Extracts of Propolis (EEP) was evaporated to dryness. About 5 mg of residue were mixed with 75 µl of dry pyridine and 50 µl bis(trimethylsilyl)trifluoroacetamide (BSTFA), heated at 80 °C for 20 min and then final supernatant was analyzed by GC-MS.

GC-MS System

A gas chromatography (GC 5890, Hewlett-Packard, Palo Alto, CA, USA) coupled with mass detector (MS 5972, Hewlett-Packard, Palo Alto, CA, USA) was used for the analysis of ethanol extracts of propolis samples. Experimental conditions of GC-MS system was as follows: HP-1 column (25m X 0.2 mm and 0.02 μm of film tickness) was used and flow rate of mobile phase (He) was set at 1.0 ml/min. In the gas chromatography part, temperature was kept for 1 min at 50 °C, then increased to 200 °C with 15 °C /min heating ramp. After this period, temperature was kept at 200 °C for 5 min. Finally, temperature was increased to 280 with 25 °C / min heating ramp and then kept at 280 °C for 10 min.

Microorganisms and Culture Media

The antibacterial activity of isolated compounds was tested against the following microorganisms: *Escherichia coli*, *Salmonella tyhpi*, Proteus sp. *Staphylococcus aureus*, *Bacillus subtilis* and Beta hemolytic Streptococcus. Bacterial strains were maintained on nutrient agar, blood agar and eosine methylene blue agar. All media were sterilized at 120 °C for 20 min.

Determination of Antibacterial Activity

The cultures were sub-cultured in liquid medium (Brain Heart Infusion) incubated at 35 °C for 24 h, and then used for the test. The optical density at 550 nm (OD_{550}) of the culture was measured with an UV-VIS spectrophotometer (Beckman 650, USA) (an OD_{550} equal to 1 corresponds to approximately 10^6 cell/ml). These suspensions were prepared immediately before the test was carried out.

The minimal inhibitory concentrations (MICs) were determined. The MIC was defined as the lowest concentration at which no bacterial growth was observed after incubation at 35 °C for 24 h.

Results and Discussion

1μl of EEP extract was injected to the GC-MS system to screen the sample and identify the compounds that were available in each propolis sample. Compounds, such as amino acids, aliphatic acids and their esters, aromatic acids and their esters, alcohols, aldehydes, flavones, flavavones, hydrocarbons, ketones and terpenoids, and other compounds, in each sample were identified by computer search using reference Willey Library (HP commercial library) and mass spectra patterns. It was found that all six samples could be divided into four main sample groups. In the first group, there were three samples collected from Trabzon, Ordu and Kemaliye-Erzincan provinces. First group samples mainly contain high content of alcohols and aromatic acid esters, and low content

of aromatic acids (Table 1). In the second group sample that was collected from Bursa city, the content of flavones, aromatic acids and ketones are really high compared the other propolis groups (Table 1). There was only one sample in the third group collected at Adapazari region. Aromatic acids were the main component in this sample and vitamin A content of this sample was the highest in all propolis samples. However, ketone content of this propolis sample is the lowest compared to the all other propolis samples. In the fourth group propolis, there is only one propolis sample that was collected from Burdur region. This propolis sample is enormously different than the first three propolis samples because of the different origin of this propolis. Only this propolis sample was collected from south part of Turkey. Alcohol content of this sample is extremely lower than the others, aromatic acid esters are the highest, aromatic acids are the lowest, flavonoids are the lowest, and ketones are the highest in the all propolis samples.

Table 1. Chemical composition of propolis collected from different regions of Turkey.

Compound (%)	**Bursa**	**Erzincan**	**Burdur**	**Ordu**	**Adapazarı**	**Trabzon**
Alcohols	4.40	12.20	1.71	14.20	6.34	15.03
Aliphatic acids	2.75	1.30	2.22	2.70	6.41	3.1
Amino acids	Very low	Very low	Very low	-	Very low	-
Aromatic acid esters	Very low	5.70	13.14	6.80	3.10	5.12
Aromatic acids	7.54	2.35	1.52	4.35	18.15	3.35
Aromatic aldehyde	3.91	0.95	-	1.60	1.86	0.95
Flavonoids	47.40	49.40	31.8	35.80	37.55	43.55
Ketones	11.20	10.50	24.74	22.90	6.95	21.30
Vitamin A	-	1.20	-	0.25	1.84	-
Terpenoids	1.92	2.70	4.50	0.45	7.70	very low
Others	20.88	13.70	20.37	10.95	10.10	7.60

The climatic condition of Bursa, Trabzon, Ordu, Kemaliye-Erzincan, Adapazari provinces are quite different from each other. Flora of first five provinces namely Bursa, Trabzon, Ordu, Adapazari and Kemaliye-Erzincan match with the flora of Euro-Sibiria. The other flora, Burdur, shows the properties of step flora. The main observations of four different propolis groups could be concluded as follows:

Flavonoids content of propolis that were collected Bursa and Kemaliye-Erzincan region are really high; Castanea sativa and Populus alba were widely growth at districts of Bursa where propolis samples are collected whereas Populus spp ve Quercus spp were growth widely at districts of Erzincan where propolis samples are collected.

Propolis from Burdur region showed different pattern from all other samples. This propolis has highest content of ketone, alcohols and aromatic acids and the lowest content of terpenoids. Population of trees was negligibly low at districts of Burdur where Propolis samples were collected. That's why chemical composition of Burdur province propolis sample is extremely different than the other propolis samples.

From the literatures, it is well known that propolis samples or some specific compounds in the propolis samples have been shown antibacterial activity to different type of bacteria (12,20,21). Antibacterial activity of propolis has been attributed to phenolic compounds, especially flavonoids, phenolic acids and their esters. Ethanol extract of the propolis collected different region in Turkey were examined for the antibacterial activity of six different bacteria namely *Escherichia coli*, *Salmonella typhi*, Proteus sp., *Staphylococcus aureus*, *Bacillus subtilis*, and Beta hemolytic Streptococcus. These bacteria associated with a variety of infectious diseases.

Antibacterial activity of all propolis samples are similar each other even if they have different chemical composition. However, effective activity of the different propolis sample is changing by the chemical composition changing of propolis samples. In this study, it was observed that all different propolis samples showed antibacterial activity to the gram positive bacteria such as *Staphylococcus aureus*, *Bacillus subtilis*, and Beta hemolytic Streptococcus. Propolis possesses antibacterial activity especially against gram positive bacteria. This activity is reported to be due to flavonoids, aromatic acids and esters present in the resin but the relationship between the structure and antibacterial activity of propolis constituents is unknown. It is reported that the mechanism of antimicrobial activity is complex and could be attributed to a synergism between phenolic and other compounds in the resin. The propolis samples showed very low antibacterial activity to the gram negative bacteria used in this study. All of these results obtained in this study are similar to the literature values (12,17,22,23) This is a confirmation that bees are able to find plant sources that provide a good defense against infections in any ecosystem they inhabit. The antibacterial and effective activity of the propolis samples to different gram positive and gram negative bacteria are given in Table 2. These results showed that antimicrobial activity is increased in the order of the studied propolis samples; Ordu > Bursa > Trabzon > Kemaliye-Erzincan > Burdur > Adapazari. Propolis sample collected Ordu city showed the highest antibacterial activity to gram positive bacteria because of the high content of the alcohols and ketones (1). Second highest antibacterial activity was observed for the propolis sample collected from Adapazari city. This propolis sample contains also high aromatic acids and

alcohols. However, Burdur propolis sample showed lowest antibacterial activity to the bacteria because of the lowest content of the alcohols and aromatic acids. Apart from these results, we concluded that some kind of alcohols or aromatic acids in the propolis samples showed good effect against gram positive bacteria which are *Staphylococcus aureus*, *Bacillus subtilis* and Beta hemolytic Streptococcus.

Table 2. Antimicrobial activity of propolis samples collected from different regions in Turkey.*

Propolis Region	Amount of active propolis sample (mg/ml)	Type of tested bacteria	Minimal Inhibitory Concentration (MIC) (µg/ml)
Adapazari	92	*E. coli*	920
		Salmonella typhi	920
		Proteus sp.	920
		Staphylococcus aureus	92
		Bacillus subtilis	92
		Beta hem.Streptococcus	92
Burdur	650	*E. coli*	6500
		Salmonella typhi	6500
		Proteus sp.	6500
		Staphylococcus aureus	65
		Bacillus subtilis	65
		Beta hem.Streptococcus	65
Bursa	100	*E. coli*	1000
		Salmonella typhi	1000
		Proteus sp.	1000
		Staphylococcus aureus	10
		Bacillus subtilis	10
		Beta hem.Streptococcus	10
Kemaliye-Erzincan	320	*E. coli*	3200
		Salmonella typhi	3200
		Proteus sp.	3200
		Staphylococcus aureus	32
		Bacillus subtilis	32
		Beta hem.Streptococcus	32
Ordu	74	*E. coli*	740
		Salmonella typhi	740
		Proteus sp.	740
		Staphylococcus aureus	7.4
		Bacillus subtilis	7.4
		Beta hemolytic Streptococcus	7.4

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WINTER DIET OF A LONG-EARED OWL POPULATION IN ANKARA, BEYTEPE

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Abstract

In this study, the pellets of Long-eared owl (*Asio otus*) population that lived in Ankara, Beytepe between 2002 and 2003 has been analyzed and the diet components of that species have been examined. This species migrates to higher altitudes of the same region in Beytepe during winter and stays there for approximately two months. The data obtained from the examination of 346 pellets indicated that the mammal species are the main diet component of that species. Among those mammal species, the rodents have the highest proportion with 69.4%. According to the results of the research bird species are the second feeding group of that species with a proportion 26.85%. The findings are in general agreement with similar research elsewhere.

Key Words: Long-eared Owl, winter diet, Beytepe, Turkey

Introduction

Long-eared owl is one of the 10 owl species that has been recorded in Turkey (12). This species of owl is a middle sized, nocturnal and sometimes crepuscular species (6). Since the insects and rodents are among its diet, it is one of the significant bird species for agriculture and forestry. (15, 16 and 25).

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The Long-eared Owls are territorial during the breeding season. In the winter period, they form common dormitories together with other individuals of the same species. These dormitories can host a variable number of individuals, from a few to hundreds of specimens (6). Many studies has been carried out in North America, Eurasia, and North Africa regarding the reproduction and feeding of Long-eared owl, the nocturnal bird of prey which is under conservation (1,2,8,10,11,22,26,28,31,34). In most of those studies the pellets of this species had been analyzed. As known, the Long-eared Owl swallows its prey as a whole and then it spits out the food residues such as hair, feather and bones as a piece of nub that is called "pellet". The aim of this study is to look into the existence of small mammals in the diet of the Long-eared Owl with the using of the analysis of pellets gathered in an area that is quite little known from the mammalogy and ornithological point of view (6).

Material and Methods

The research area is in the location of Beytepe, Ankara and covers a surface of about 2400 ha. Its coordinates are as follows: 39°53'21" N and 32°45'11" E. The elevation of the location is 1070 m NN. The dormitory was located in a reforestation area of coniferous trees. Black pine (*Pinus nigra*) and Cedar (*Cedrus libani*) trees are prevalent in the area. The observations on this species had been carried out following the arrival of the Long-eared owl species to the observation area in 2002 and 2003. The pellets of those species had been regularly collected. A total of 346 pellets had been numerated and dated and kept in plastic bags. The pellets after having been collected, were left to dry to a constant weight. The hair, feather and bone pieces had been separated. The determination of the samples was carried out with the help of a binocular microscope. Pellet analysis had been realized in conformance (4,9,13,18,21,24 and 33).

Results

Long-eared owl is a bird species that has been observed in Beytepe, Ankara region. According to the findings of the observation carried out by the author of this paper in 1987-1988, it was found out that this species breeds in deserted Magpie (*Pica pica*) nests which are in the north and relatively lower places. It is also observed that individuals of Long-eared owls regularly come in the beginning of November to the Cedar Plantation, which is in higher altitudes and a winter place for the species and stay there approximately two months. Both in 1987 and 1988, the species preferred the same trees. 11 individuals came to the observation area in 14 December 2002 and left the area in January 21. In the observations carried out in 2003 it was observed that 11 individuals came to the observation area in 6

November and left the area in 10 January. The Cedar trees in the observation area are 30 years old and very healthy. The Long-eared Owl individuals generally prefer the trees that are at the border of the plantation. The reason for this preference might be related to their predation behavior.

During observations it is seen that they only hunt during the night. It is seen that they fly from the trees they perch all day to open areas. When they fly, they lift from the surface 1, 5-2m.

Totaly 346 pellets were gathered from only one site of the research area in 2002 and 2003. The weights of pellets had been measured and their minimum, maximum and average weights had been determined. It is observed that the average weight of the samples collected in 2003 is slightly higher than that of the samples collected in 2002. The weight variables regarding the pellets collected in 2002 and 2003 have been presented in Table 1. Besides, the measure values of bones and feather found in the pellets have been presented in the same Table. In the research carried out in 2002 and 2003 on 346 pellets, traces of 213 animals from different species had been found. The contents that have been examined and classified are presented in the Table 2.

The examination of the pellets has indicated that the main food source of Long-eared owl species living in the research area is mammal species. Among those species, the rodents are in the first place. The traces in the pellets indicate that the second feeding preference of Long-eared Owl is birds most of which are passerines. The species determined by pellet analysis and their numerical distribution have been presented in Table 2.

As seen from Table 2, the main preferred food component of Long-eared Owl in Beytepe is rodents (69.4%). Among those, Common Vole (*Microtus* sp.) whose main habitat is woodlands is in the first place (44.4%). It is followed by Long-tailed Field Mouse (*Apodemus* sp.) (25%). Another prey of the Long-eared owl is Bicoloured white-toothed shrew (*Crocidura leucodon*) which is an insectivorous mammal species. Its proportion in the above mentioned species diet composition is 3.7%. It is also observed that birds are also important components of diet composition of the Long-eared Owl species (26.85%). The pellets that were collected in Beytepe, where they only prefer during winter had been analyzed and it was observed that House Sparrows (*Passer domesticus*) are in the first place (6.94%) It is followed by Great Tit (*Parus major*) (5.9%), Chaffinch (*Fringilla coelebs*) and Coal Tit (*Parus ater*) (3.24%). Tree Sparrow (*Passer montanus*) is in the last line (2.31%) of Long-eared Owl's food spectrum.

DISCUSSION

By the help of pellet analysis, it is observed that the most significant food component of Long-eared Owl individuals living in Beytepe, is mammals. In most of the studies carried out in Europe where Long-eared Owl is widely seen, similar results were obtained. (1,9,11,12,21,25,26 and 28). For example, the proportion of mammals in the diet is 69.4% in Beytepe, whereas this proportion is 97% in Yugoslavia (28). The difference can be explained by the periods that the researches were carried out. It is also emphasized that the Common Vole has the most significant place among the mammal species (51%). In a research carried out in Germany, this proportion is 65.4% (3). The proportion for the same species in Beytepe is 44.4 %. It was stated in a study carried out in Israel that the most dominant species among 279 pellets is Field Vole (*Microtus socialis*), a similar species (34). In a study carried out in Romania, it was found that Common Vole and *Apodemus sylvaticus* are among the first three species on the diet of long-eared owl (8). The proportion of *Apodemus sylvaticus* in the diet of Long-eared Owl in Beytepe is 25%. This proportion was in South Italy 24.06% (4). In one study in Romania, it is mentioned that the bird species House Sparrow, Tree Sparrow and Chaffinch, that are included in the diet of Long-eared Owl living in Beytepe are also on the diet composition of Long-eared Owl living in Romania. The same bird species were found also in the pellets gathered in south Italy (4).

The research on the pellets collected each year in Beytepe lasted approximately two months, whereas the other research lasted a whole year. Apart from mammal species, birds are also included in the diet of this species. The House Sparrow, Tree Sparrow and Chaffinch which were determined by examining the feathers in the pellets are among bird species that can be seen in the research area year round. Those species are not preyed only by Long-eared Owl, but also by Tengmalm's Owl (*Aegolius funereus*) which is another nocturnal birds of prey species (24). The same author mentions that those species also prey Snow Finch (*Montifringilla nivalis).* Even though this mentioned species is found in Ankara, it was not registered in Beytepe.

This study indicates that the proportion of bird species that are included in the diet is 26.85% whereas the amount is 48% out of 671 pellets in Italy (16). Despite all those results, it is not possible to define Long-eared Owl as a "bird hunter". Even though it prefers small mammals, its feeding preference may change 50% depending on the feeding conditions (4).

It is determined that the weights of pellet samples that were collected in two years time might be different from each other. A similar situation was emphasized by (28) and it was stated that the content and the weight of the pellets might be changed by climate.

Many researches indicate that Long-eared Owl preys also on insects, frogs and snakes (1,9,12,14,20,24,26,31,33 and 34). Since there is not any stream in the observation area and the species comes to the area in December and January, no traces of amphibian and reptilian were found within the collected pellets.

There are many types of owls; the Long-eared Owl may be the most interesting of them all. Especially, in terms of their feeding ecology. For example, diets of Buzzard (*Buteo buteo*) and this species overlapped by 70%, the diets of Buzzard and another owl species Tawny owl (*Strix aluco*) by 58%, but the diets of these two owl species by only 31% (31).

As it known, many birds of prey species have been killed for hunting not only in our country but also all around the world. Even though there is a restriction in preying those species by the help of legal measures and increasing environmental consciousness, it could not be completely uprooted. The same situation is valid for our country as well. As known, all birds of prey species are under protection in Turkey and this should be perpetuated more efficiently (27). The main reason for this strict protection is the decrease in the number of individual species (29 and 30). Another reason for protecting those species is their place in food chain. Those birds of prey species which are significant rings of chain are predators of many animals that are considered predators and they keep these predators number under control. They also play a significant role in providing continued existence of those populations by preying their weak and overpopulated individuals.

One of the characteristics of owls is that they are nocturnal species. Therefore, they may prey many of the predators that are active during night time. On the other hand, in our country, this species is regarded as ominous. To eliminate this prejudice is very important for the future of this species in our country. In addition to legal measures it is also a must to explain the importance of this species to the society

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Table 1. The weight measurements of the Long-eared Owl's pellets, collected in the years 2002 and 2003 in Ankara-Beytepe (n=346).

Years	Number of the pellets	weight of the respective pellets (g)			Weight of the portions of bones (g)			Other material (g)		
		Min	Max	On average	Min	Max	On average	Min	Max	On average
2002	154	0,78	4,1	3,18	1,7	3,2	1,88	0,56	3,1	1,91
2003	192	0,94	5,35	2,95	1,3	3,66	2,41	1,2	3,96	2,86
On average	173	0,86	4,72	3,06	1,5	3,43	2,14	0,88	3,53	2,38

Table 2. Animal species and their proportions determined in the pellet samples.

Species			**Number of prey**			
English	**Latin**					
BIRDS		**Status in Turkey*)**	**2002**	**2003**	**Total**	**%**
House Sparoow	*Passer domesticus*	Resident	7	8	15	6,94
Great Tit	*Parus major*	Resident	5	6	11	5,09
Chaffinch	*Fringilla coelebs*	Resident	4	3	7	3,24
Coal Tit	*Parus ater*	Resident	3	4	7	3,24
Tree Sparrow	*Passer montanus*	Resident	2	3	5	2,31
Unidentified birds			6	7	13	6,01
	Total 5 Species		**27**	**31**	**58**	**26,85**
MAMMALS						
Common Vole	*Microtus* sp.		45	51	96	44,4
Long-tailed Field Mouse	*Apodemus* sp.		24	30	54	25
Bicoloured white-toothed shrew	*Crocidura* sp.		5	3	8	3,7
	Total 3 Species		**74**	**84**	**158**	**73,15**
Total	8 Species		**101**	**115**	**216**	**100**

*) according to 9

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HELMINTH FAUNA OF TWO CYPRINID FISH SPECIES (*Chalcalburnus chalcoides* Güldenstadt 1972, *Rutilus rutilus* L.) FROM LAKE ULUABAT, TURKEY

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Abstract

This study was investigated presence of helminth fauna of two fish species (*C. chalcoides, R. rutilus*) in Lake Uluabat Turkey, between July-1998 and June-1999. Six parasite species were identified: *Dactylogyrus crucifer* on gills of *R. rutilus*, *D. chalcalburni* on gills of *C. chalcoides*. *D. sphyrna* and *Paradiplozoon homoion* on the same habitat both of host fish species. *Caryophyllaeus laticeps* in intestine of both fish species and *AsymphIodora markewitschi* were found in intestine of *C. chalcoides*.

Key Words: *Caryophyllaeus*, *Dactylogyrus, Paradiplozoon*, parasite, Uluabat

Introduction

Lake Uluabat, one of the largest lakes in the Marmara region of Turkey, is significant in terms of fishing. Several studies have been conducted on the helminth fauna of the lake: Oğuz & Öztürk (1) was identified two parasite species, *Asymphlodora markewitschi* and *Rhabdocona* sp. in rudd (*Scardinius erythropthalmus* L.). In pike (*Esox lucius* L.) by Öztürk et al.'s (2) who revealed six parasite species; *Argulus foliaceus*, *Tetraonchus monenteron*, *Rhipidocotyle fennica*, *Diplodiscus subclavatus*, *Raphidascaris acus* and *Acanthocephalus anguillae*. In sand goby (*Gobius fluviatilis* L.) by Öztürk et al. (3), who find out four parasite species: *Gyrodactylus gobii,*

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Bothriocephalus acheilognathi, Ligula pavlovsk and Eustrongylides excsius. In addition to those mentioned above, Öztürk (4) also examined that tench (*Tinca tinca*) and identified five parasite species: *Dactylogyrus macracanthus, Asymphlodora tincae, Acanthocephalus lucii, Ergasilus sieboldi, Argulus foliaceus* and *Piscicola geometra.*

While several studies have been conducted on parasite fauna for various cyprinid fish species (5, 6, 7, 8), none of observations on helminth fauna of the two cyprinid fish species, *Chalcalburnus chalcoides* and *Rutilus rutilus* in Lake Uluabat. The present study aims to increase of helminth fauna of the host fish species. The data of parasite specimens were analysed; prevalence, mean intensity seasonal variation and relation host size of the parasites.

Material and Methods

Study area

Lake Uluabat, is in north-west Anatolia, geographically located at 41° 11' N, 29° 04'E. It is a eutrophic lake with a surface of 156±2.13 km^2 and mean depth 3.31±0.27 metres (9).

Ichthyohelminthologic Techniques

The number of 97 *Chalcalburnus chalcoides* and 86 *Rutilus rutilus* specimens were collected monthly or bimonthly from July-1998 to June-1999. They were placed in to plastic containers containing lake water and transferred to the research laboratory and examined immediately. During the examination, the method of the helminthological autopsy and lately used for the ichthyohelminthologic techniques (10). All possible sites of infection were examined for the occurrence of parasites with a stereo microscope using transmitted light at a magnification varying between x12 and x50. Parasite specimens were fixed by 4% formalin and mounted in glycerine-jelly. All the species identified using the reference keys of (11).

Statistical Analyses

The total number of parasites was determined directly by numerical count. Spearman's test was used to measure correlation between the density of each parasite species and fish size. Kruskal-Wallis analysis of variance was used to determine significance of differences in parasite mean intensities. Minimum and maximum values are given in table, with the arithmetic mean and standard deviation in parentheses (Table 1-5). All statistical analyses were performed using the statistical program SPSS 10.0.

Results

A total of 6 parasite species found 2 fish species belonging to cyprinidae, *Chalcalburnus chalcoides* and *Rutilus rutilus*. They are listed according to the systematic applied by (Bykhowskaya-Pavlovskaya et al. (11): *Dactylogyrus sphyrna* Linstow, 1878; *Dactylogyrus crucifer* Wagener, 1857; *Dactylogyrus chalcalburni* Dogiel ve Bychowsky, 1934; *Paradiplozoon homoion* Bychowsky ve Nagibina, 1959; *Asymphlodora markewitschi* Kulkowskaya, 1947; *Caryophyllaeus laticeps* (Pallas,1781). Basic data on the findings are provided in Table 1.

Throughout the entire research period, *Dactylogyrus crucifer* was found on 78 of 86 host fish specimens (90.6% prevalence). The highest mean density (69.6±53.6) was recorded in the whole period of the study for the parasite species (Table 1). Density of the parasite species was sharply increased from summer to autumn. When the evaluation was performed in terms of the host fish size, the parasite species was found more commonly in big fish size of host fish than small and middle-size specimens (Table 5). The second parasite species, *Dactylogyrus chalcalburni* had a higher prevalence (31.9% prevalence, 12.7±7.9 parasite/fish). As shown in Table 2, density of *D. chalcalburni* increased in spring with warming of water. If evaluated in terms of the size of the host fish, *D. chalcalburni* was found on the host fish, and the mean density of infection varied between 8.0 and 16.1, and prevalence had values between 24.3 to 60.0 (Table 4).

Asypmhlodora markewitshi was observed to the intestines of *C. chalcoides*. Maximum density of the parasite was counted in summer (19.0±4.1 parasites fish). The difference in mean number of parasites was more significant than the percentage of parasites between the spring and summer. Detailed data related to seasonal infections are given in Table 2. On the other hand, the parasite was found on small and medium-size specimens of the host fish, remained between 0.1 and 10.8% when evaluated in terms of the host fish size. This shows an increasing trend in the number of the parasites according to length of host fish. Similarly, parasite density, with respect to both rate of infection and mean parasite number, reached maximum level on the biggest host fish size (Table 4).

Three parasitic species, *Dactylogyrus sphyrna*, *Paradiplozoon homoion*, *Caryophyllaeus laticeps* in both of the host fish species (Table 1). *D. sphyrna* was found in spring, summer and autumn for *C. chalcoides*, especially small host fish specimens. For *R. rutilus* when seasonally viewed, rate of the infection of *D. sphyrna* increased steadily from spring and it was appeared in summer and autumn. The infeciton was regularly decreased to infection in winter when the water temperature was decreased (Table 3). *P. homoion* was shown in all season except winter and it is not difference compared to that of spring to autumn for *R. rutilus*. *P. homoion* was found on the all *R. rutilus* fish specimens, especially occurred in small host fish specimens. The prevalence of the parasite clearly higher in bigger than smaller fish specimens for *C. chalcoides* (Table 4), and it was found only in summer (Table 2). *C. laticeps* was found in spring and autumn for *R. rutilus* (4.6% prevalence, 1.2±0.5 parasite/fish). It was found in *C. chalcoides* in spring (6.1%, 2.6 1.2). The infection rate and mean density were low (Table 2, 3). The density of the parasite changed from 1 to 2, and the highest infection rate was observed in autumn for *R.rutilus* (Table 3). On the other hand, the parasite was found in small and medium-size host fish specimens for *R. rutilus*. Its percentage and density changed between 3 to 7%, and 1 to 2 parasite/fish (Table 5). However the parasite density with respect to both rate

of infection and mean parasite number reached the maximum level in the biggest host fish specimens for *C. chalcoides* (21.5% prevalence, 3.0 ± 1.4 parasite/fish) (Table 4).

Discussion

Total of six parasite species was identified on two fish species in cyprinidae at Lake Uluabat in this study. The parasite species are all helminth species (Table 1). In comparison with the findings of the other studies (1, 2, 3, 4, 12) carried out in the same lake on various fish species such as *Esox lucius*, *Cyprinus carpio*, *Scardinius erythropthalmus*, *Tinca tinca*. This study found different parasite species on *Chalcalburnus chalcoides* and *Rutilus rutilus*. This result justifies the suggestion presented by researchers. Such as Dogiel (13) indicated that the abundance of the host fish with complexity of its parasite fauna. Granat & Esch (14) show that parasite species may exhibit considerable differences in variety between different fish.

In the present study, *Paradiplozoon homoion* was found low density (10.4%, 1.3 parasite/fish) on gills of *R. rutilus* and (6.1%, 1.0) on gills of *C. chalcoides*. The findings of the study for *P. homoion* was in close agreement with Chubb (15) and Öztürk & Altunel (16) whose data was given above says that, density of diplozoid species was very low (e.g. 1, 2, or 4 specimens/fish). Stranock (17) says that it may be related to the water quality, and the author's study result shows that the parasite species was shown in clear water basins but it was not recorded polluted water basin. As the same, the present study area is eutrophic features and the lake is also polluted (9), which may be the main reason for the low level infection.

Temperature is commonly regarded as one of the most important abiotic factors in determining the occurrence of parasite infection and its seasonal variations in the previous studies (18, 19, 20). Seasonal changes in percentage and intensity of infection of the two host fish species investigated were noted during the one year of collection. These results were suggested at

the present study data. As shown in Table 2, 3 two of parasitic species, *P. homoion* and *A. markewitshi* were reached the maximum infection level in summer period when the water temperature was the maximum level (Table 6). The other species, *C. laticeps* had the low density among the parasite species during the entire study (Table 1). The parasite species was recorded in spring. Agreeing with Kritscher (21) findings, density of *C. laticeps* reached the peak level in spring and summer. Similarly, Kennedy (22) found maximum level in spring, and the infection decreased in summer and autumn.

Conversely, the seasonal abundance of dactylogyrids is sometimes more influenced by other abiotic and biotic factors than by temperature; e.g. *Dactylogyrus solidus* is very sensitive to oxygen depletion as Bauer (23). Gonzales-Lanza and Alvarez-Pellitero (24) was described that the relationship of the *Dactylogyrus legionensis* to temperature is not clear, and that other abiotic (light, pH, oxygen and salinity) have more influence than previously believed. In line with the present study data, the infection rate and mean intensity for *Dactylogyrus chalcalburni, D. crucifer, D. sphyrna* had already began risen in spring, when the water temperature had only just begun to rise above the winter temperature. There was a decrease in the intensities for summer, although at least the water temperature remained stable at that time (Table 6). Thus, in the present case it can be concluded that the high dactylogyrid intensities in spring and autumn were apparently influenced by the oxygen depletion or low water temperature of the host fish species, agree with Gonzales-Lanza and Alvarez-Pellitero (24) and Öztürk & Altunel (16).

The relationship between the level of monogenean infection and the age of the fish has been studied by several researchers. One of them, Gonzales-Lanza and Alvarez-Pellitero (24) found that the prevalence of a *Dactylogyrus* species on the host fish aged 2-7 years increased significantly with the increase in length of the host fish. Koskivaara et al. (25) studied was not found monogenean parasites on roach aged 3 years and after. Öztürk et al. (2) was found that the monogenetic species on pike, in which the mean

density increase parallel with the age of the host fish species. Inconsistant results with several other studies (3, 24) were found in terms of infection of monogenetic parasites. In this study a significant difference was detected among the sizes of the host fish and the parasite species, *D. chalcalburni, D. crucifer, D. sphyrna* were observed on all fish sizes as Samman (26). The other parasites, *A. markevitschi* and *C. laticeps* were acquired by younger fish, and as fish size larger, its intensity had an increasing trend. In other words, there exists a positive correlation between the increase in intensity and rate of infection for the parasite species and the size of host fish. On the other hand, the number of *C. laticeps* did not depend on host fish size. However, Karanis and Taraschewski (27) recorded high infection rate in bigger host fish specimens than smaller, while Eslami and Anwar (28) and Kulakowskaya (29) find out highest infection rate in small host fish specimens.

In conclusion, variance of infection rates has been assessed in terms of seasons and size of host fish. It is beyond the scope of this paper to postulate on how useful parasites may be in the future in helping to understand the fish and fisheries in the study area.

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Table 1. Helminth species of *C. chalcoides* and *R. rutilus* examined from Lake Uluabat. Examined fish number (N), infected fish number (In) and rate (%), minimum-maximum (M) and mean parasite number (X±S.D.)

N	In	(%)	Identified Parasite Species	M & (X±S.D.)
	78	(90.6)	*D. crucifer* on *R. rutilus*	6-300 (69.6±53.6)
	47	(54.6)	*D. sphyrna* on *R. rutilus*	2-18 (8.0±3.8)
86	9	(10.4)	*P. homoion* on *R. rutilus*	1-2 (1.3±0.5)
	4	(4.6)	*C. laticeps* in *R. rutilus*	1-2 (1.2±0.5)
	31	(31.9)	*D. chalcalburni* on *C. chalcoides*	4-36 (12.7±7.9)
	43	(44.3)	*D. sphyrna* on *C. chalcoides*	2-20 (6.8±3.9)
97	6	(6.1)	*P. homoion* on *C. chalcoides*	1 (1.0±0.0)
	6	(6.1)	*C. laticeps* in *C. chalcoides*	2-5 (2.6±1.2)
	7	(7.2)	*A. markewitschi* in *C. chalcoides*	3-26 (16.7±7.1)

Table 2. Seasonal changes of parasite species (Dc: *D. chalcalburni*, Ds: *D. sphyrna*, Ph: *P. homoion*, Cl: *C. laticeps*, Am: *A. markewitschi*) for *C. chalcoides* in Lake Uluabat. Examined fish number (N), seasons (S), infected fish number (In) and rate (%), minimum -maximum (M) and mean (X±S.D.) parasite number

		In & (%)				
N	S	Dc	Ds	Ph	Cl	Am
30	Spring	18 (60.0)	18 (60.0)	0 (0.0)	6 (20.0)	1 (3.3)
44	Summer	0 (0.0)	12 (27.2)	6 (13.6)	0 (0.0)	6 (13.6)
13	Autumn	13 (100)	13 (100)	0 (0.0)	0 (0.0)	0 (0.0)
10	Winter	0	0	0	0	0

M & (X±S.D.)				
Dc	Ds	Ph	Cl	Am
4-36 (15.7±8.9)	2-12 (5.7±2.8)	0 (0±0.0)	2-5 (2.6±1.2)	3 (3.0±0.0)
0 (0±0.0)	2-10 (5.2±3.1)	1 (1.0±0.0)	0 (0±0.0)	14-26 (19.0±4.1)
4-14 (8.4±2.9)	4-20 (9.9±4.2)	0 (0±0.0)	0 (0±0.0)	0 (0±0.0)
0	0	0	0	0

Table 3. Seasonal changes of parasite species (Df: *D. crucifer*, Ds: *D. sphyrna*, Ph: *P. homoion*, Cl: *C. laticeps*) for *R. rutilus* in Lake Uluabat. Examined fish number (N), seasons (S), infected fish number (In) and rate (%), minimum-maximum (M) and mean (X±S.D.) parasite number

		In & (%)			
N	S	Df	Ds	Ph	Cl
27	Spring	25 (92.5)	19 (70.3)	4 (14.8)	2 (7.4)
14	Summer	8 (57.1)	6 (42.8)	3 (21.4)	0 (0.0)
18	Autumn	18 (100)	10 (55.5)	2 (11.1)	2 (11.1)
27	Winter	27 (100)	12 (44.4)	0 (0.0)	0 (0.0)

M & (X±S.D.)			
Df	Ds	Ph	Cl
6-180 (61.0±50.4)	4-18 (9.8±4.0)	1 (1.0±0.0)	1 (1.0±0.0)
24-60 (41.2±12.2)	4-12 (8.0±2.8)	1-2 (1.6±0.5)	0 (0±0.0)
10-140 (52.8±34.5)	4-14 (9.0±2.8)	1-2 (1.5±0.7)	1-2 (1.5±0.7)
46-300 (97.1±63.6)	2-8 (4.2±1.7)	0 (0±0.0)	0 (0±0.0)

Table 4. Relationships between the parasite species (Dc: *D. chalcalburni*, Ds: *D. sphyrna*, Ph: *P. homoion*, Cl: *C. laticeps*, Am: *A. markewitschi*) and host fish size groups of *C. chalcoides* in Lake Uluabat. Examined fish number (N), fish size (L, cm), infected fish number (In) and rate (%), minimum-maximum (M) and mean parasite number (X±S.D.)

	In & (%)				
N (L)	Dc	Ds	Ph	Cl	Am
18 (6-7)	10 (55.5)	10 (55.5)	0 (0.0)	0 (0.0)	0 (0.0)
5 (8-9)	3 (60.0)	3 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
18 (14-15)	0 (0.0)	6 (33.3)	1 (5.5)	0 (0.0)	2 (1.1)
37 (16-17)	9 (24.3)	16 (43.2)	3 (8.1)	2 (5.4)	4 (10.8)
19 (18-19)	9 (47.3)	8 (42.1)	2 (10.5)	4 (21.5)	1 (5.2)

	M & (X±S.D.)			
Dc	Ds	Ph	Cl	Am
4-14 (8.0±3.1)	4-20 (9.8±4.3)	0 (0±0.0)	0 (0±0.0)	0 (0±0.0)
8-12 (10.0±2.0)	6-15 (10.3±6.1)	0 (0±0.0)	0 (0±0.0)	0 (0±0.0)
0 (0±0.0)	2-10 (5.5±3.5)	1 (1.0±0.0)	0 (0±0.0)	14-20 (17.0±4.2)
6-25 (16.1±6.8)	2-12 (5.6±3.0)	1 (1.0±0.0)	2 (2.0±0.0)	3-20 (14.2±7.6)
4-36 (15.4±11.1)	2-10 (5.3±2.6)	1 (1.0±0.0)	2-5 (3.0±1.4)	26 (26.0±0.0)

Table 5. Relationships between the number of parasite species (Df: *D. crucifer*, Ds: *D. sphyrna*, Ph: *P. homoion*, Cl: *C. laticeps*) and host fish size groups of *R. rutilus* in Lake Uluabat. Examined fish number (N), fish size (L, cm), infected fish number (In), rate (%), minimum-maximum (M) and mean parasite number (X±S.D.)

	In & (%)			
N (L)	Df	Ds	Ph	Cl
30 (14-16)	26 (86.6)	12 (40.0)	2 (6.6)	1 (3.3)
36 (17-18)	32 (88.8)	21 (58.3)	4 (11.1)	2 (5.5)
7 (19-21)	7 (100)	4 (57.1)	2 (28.5)	0 (0.0)
13 (21-22)	13 (100)	10 (76.9)	1 (7.6)	1 (7.6)

	M & (X±S.D.)		
Df	Ds	Ph	Cl
20-140 (70.1±33.3)	4-16 (8.8±3.3)	1-2 (1.5±0.7)	1 (1.0±0.0)
6-180 (57.9±42.0)	4-18 (8.2±3.9)	1-2 (1.5±0.5)	1 (1.0±0.0)
2-130 (67.0±35.9)	2-14 (7.2±5.1)	1 (1.0±0.0)	0 (0±0.0)
10-300 (98.6±98.2)	2-14 (6.7±4.1)	1 (1.0±0.0)	2 (2.0±0.0)

Table 6. The value of water temperature (WT), PH and dissolved Oxygen (DO) of Lake Uluabat during the study period (surface)

Months	WT (^{0}C)	PH	DO
VII-98	27,8	8,62	7,17
VIII-98	27,3	8,47	5,76
IX-98	21,3	8,52	7,13
X-98	17,0	8,94	9,44
XI-98	11,7	8,66	8,70
XII-98	4,8	8,43	9,99
I-99	7,6	7,68	9,22
II-99	11,7	7,56	9,32
III-99	10,6	8,63	10,35
IV-99	23,2	8,50	7,76
V-99	22,1	8,54	6,33
VI-99	27,7	8,90	6,38

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THE ROTIFERA FAUNA OF EUPHRATES RIVER BASIN (TURKEY)

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Abstract

In Turkey, limnological studies were begun by Vavra in Sarı Lake in 1905, and 3 rotifer, 1 copepod and 3 cladoceran species were identified. Later, Dumont and Ridder 1987, were observed 78 rotifer species in 19 different localities and Segers et al 1992, observed 91 rotifer species in 21 different localities.

Although a lot of water bodies were located in the Euphrates river basin, a few study is available on the rotifer fauna. This study was carried out 17 different localities from Euphrates river basin between 2001-2002 on a quarterly basis and rotifer composition, zoogeographic distribution was discussed.

Key Words: Rotifera, Taxonomy, Zoogeography, Euphrates river Basin

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Introduction

Until now a lot of publications dealing with the fauna of Turkish Rotifera are available only a few lists have been given about the rivers. Emir (1991) was given 11 species of rotifers from Euphrates river, Segers et al. (1992) recorded 91 rotifer species from different localities in Turkey. Studies by Ustaoğlu & Balık (1987), Emir (1989), Ustaoğlu & Balık (1990), Saler (Emiroğlu), et al (2000), Saler (Emiroğlu) and Şen (2001); Altındağ & Yiğit (2002) were studied on the rotifer species. Akbulut (2001) and Altındağ & Yiğit (2001), were added species as new for Turkey. and a check list was prepared for zooplankton of Turkish Inland waters by Ustaoğlu (2004) and as a result of the studies the number of the rotifer species are 229 in Turkey.

Materials and methods

Samples were collected during 2001-2002 on (2.11.2001, 15.1.2002, 14.2.2002, 25.4.2002, 5.5.2002, 4.7.2002, 26.11.2002) from different localities using a standard plankton net (mesh diameter 44 μm) and fixed with formaldehyde (Yıldız et al., 2003). The species were identified according to Emir (1994); Koste (1978); Kolisko (1974); Segers (1995); Nogrady & Paurriot (1995).

The following wetlands were studied see in the Figure 1. and the occurrence of the rotifer species were given in the Tables 2 and 3.

Results and Disscussion

Samples from 17 freshwater sites in Euphrates river basin yielded 65 different species of Rotifera, 9 of which are new to the Turkish fauna. In the following list, taxa new to Turkish fauna are showed with an asterisk. The numbers following the species names refer to the localities above (Table 1.).

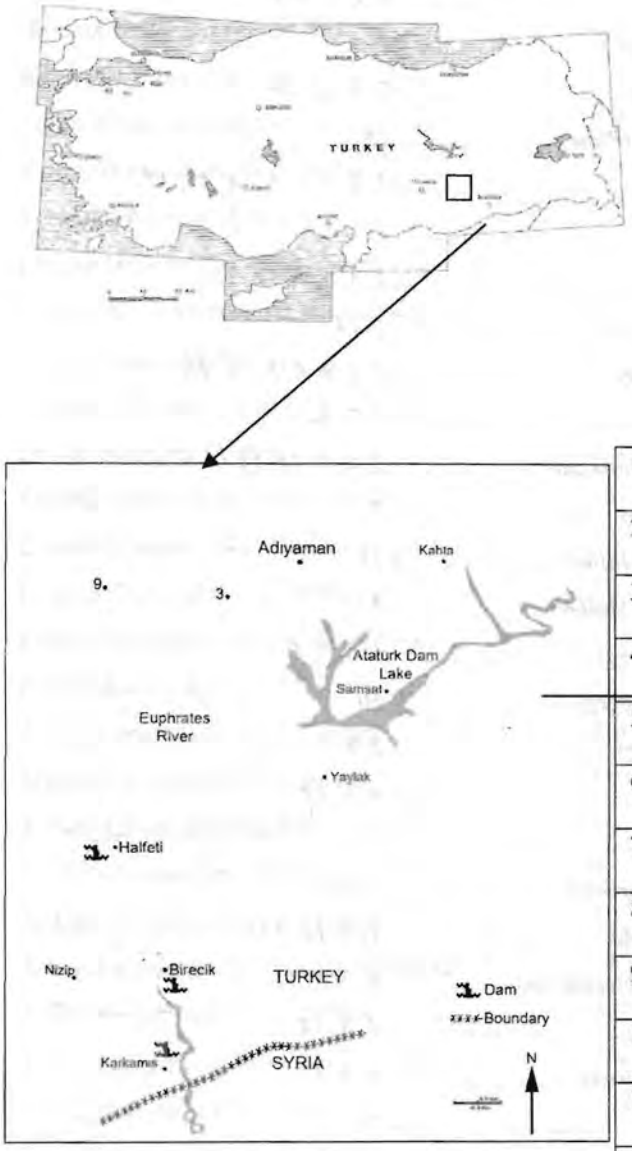

Fig. 1. Study Area (Modified from Segers at all., 1992 and Garmin Map Source ver. 6.9).

1- Atatürk Dam Lake
2- Karababa
3- Eğriçay
4- Adıyaman Lake
5- Çakal Stream
6- Halfeti Dam Lake
7- Çam Stream
8- Karkamış Dam Lake
9- Göksu Stream
10- Kalburcu Stream
11- Mizar Köprü Altı
12- Cendere Stream
13- Çaylarbaşı Lake
14- Adıyaman (Ata. Dam)
15- Hacıhıdır
16- Çaylarbaşı
17- Birecik Dam Lake

Table 1. Identified rotifer species from Euphrates River basin

Anuraeopsis fissa (Gosse) *1, 2, 3, 5, 8*

**Anuraeopsis navicula Rousselet* *7, 5*

Ascomorpha saltans Barthsch *2, 7, 12, 16*

**Ascomorphella volvocicola (Plate)* *15*

Asplanchna priodonta Gosse *1, 2, 4, 5, 6, 14, 15, 17*

Asplanchna sieboldi (Leydig) *11*

Brachionus angularis Gosse *2, 3, 5, 7, 12, 15*

Brachionus bidentata Anderson *1, 14*

Brachionus calyciflorus Pallas *1, 2, 4, 6, 8, 14, 15*

Brachionus leydigii Cohn *5, 7, 14*

Brachionus quadridentatus Hermann *2, 4, 7, 14, 15*

Brachionus falcatus Zacharias *7*

Brachionus urceolaris (O.F.Müller) *15*

Cephalodella catellina (O.F. Müller) *7*

Cephalodella gibba (Ehrenberg) *1, 5, 7, 8*

Cephalodella gracilis (Ehrenberg) *10*

Colurella colurus (Ehrenberg) *2, 8*

Colurella obtusa (Gosse) *4, 5, 14*

Conochilus natans (Seligo) *7*

**Conochilus coenobasis (Skorikov)* *7, 15*

Conochilus unicornis Rousselet *1, 7, 14, 15*

Dicranophorus hauerianus Wiszniewski *5, 7, 8, 11*

**Encentrum putorius Wulf.* *1, 8, 14*

Encentrum saundersiae (Hudson) *4, 8, 17*

**Euchlanis deflexa (Gosse)* *8*

Euchlanis dilatata Ehrenberg *1, 5, 7, 8, 17*

Euchlanis meneta Myers *5*

Testudinella incisa (Ternetz) *13*
Testudinella patina (Hermann) *12, 16*
Trichocerca cylindrica (Imhof.) *8, 5*
Trichocerca elongata (Gosse) *5, 8*
Trichocerca rattus (O.F.Müller) *6, 8*
Trichocerca weberi Jennings *5, 8*
Trichotria pocillum (O.F.Müller) *1, 8*
Trichotria tetractis (Ehrenberg) *1, 5, 8*

The distribution of the rotifer species were changed according to the habitats (Tables 2-3). Totally 50 species were recorded from the lake habitats while totally 40 species were recorded from the river systems. Of the species identified from the Euphrates river basin, most belong to the family Brachionidae (10 spp.) especially genera *Brachionus* (6 spp.), *Lecane* (6 spp.), *Synchatidae* (7 spp.).

In Turkey most of the studies on rotifers mostly from the lakes, the littoral and periphytic habitats has been studied. Segers at al. (1992) recorded 91 different species of rotifers and identified many littoral species of Lecane.

In this study 40 species were recorded from the river system. Among them *Brachionus falcatus* was the new record from the Çam Stream; *Trichocerca elongata* and *Euchlanis meneta* were new record from the Çakal Stream; *Anuraeopsis navicula* from the Kalburlu Stream.

Totally 50 species were recorded from the stagnant waters, among them *Encentrum putorius* and *Synchaeta longipes* from Adıyaman; *Trichocerca elongata, T. cylindrica, Lecane stichaea, Heterolepadella ehrenbergii* and *Euchlanis deflexa* were firstly recorded from the Karkamış Dam lake.

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Table 2. The distribution of the rotifer species in lentic habitats

Sampling locality	Atatürk Dam Lake							Adıyaman							Halfeti Dam Lake							Hacıdır Dam Lake							Karkamış Dam Lake						
Sampling time	11	1	2	4	5	7	9	11	1	2	4	5	7	9	11	1	2	4	5	7	9	11	1	2	4	5	7	9	11	1	2	4	5	7	9
Anuraeopsis fissa	*																														*					
Asplanchna priodonta	*				*			*			*				*				*	*		*													
Ascomorphella volvocicola																									*										
Brachionus angularis																						*				*	*								
B. bidentata								*																											
B. calyciflorus							*				*								*			*					*						*		
B. leydigii													*																						
B.urceolaris																						*			*	*	*								
Brachionus calyciflorus											*																								
Brachionus quadridentatus								*		*	*		*									*					*								
Cephalodella gibba				*																											*		*		
Cephalodella sterea																																*			
C.gracilis																											*								
Colurella obtusa													*										*												
Colurella colurus																																*			
Conochilus unicornis								*																	*	*									
C. coenabasis																									*	*									

Table 2 (cont.)

Dicranophorus hauerianus																										*					
Encentrum putorius								*																				*			
Encentrum saundersiae												*																		*	
Euchlanis deflexa																															
Euchlanis dilatata								*				*														*		*	*	*	
Gastropus stylifer	*	*																													
Heterolepadella ehrenbergi																														*	
Hexarthra fennica							*			*																					
Keratella tropica																			*												
Keratella quadrata																			*												
K. Cochlearis																								*							
Lepadella patella																														*	
Lecane bulla		*										*																*		*	*
Lecane closterocerca																														*	
Lecane luna					*																					*			*	*	*
Lecane lunaris																										*				*	
Lecane stichaea																														*	*
Lophocharis naias																										*					
Mytilina mucronata													*													*		*			*

Table 2 (cont.).

Mytilina ventralis																												*			*		*	
Notholca caudata																																	*	
Notholca squamula																															*			
Polyarthra dolichoptera		*		*							*																	*						
Polyarthra remata											*																					*		
Polyarthra vulgaris				*				*																							*			
Synchaeta longipes								*																										
Synchaeta oblonga		*																																
Synchaeta littoralis																												*						
Synchaeta pectinata								*							*																*			
Trichocerca cylindrica																												*						
Trichocerca elongata																												*			*			
Trichotria pocillum																												*			*		*	
Trichotria tatractis				*																								*			*	*		

Table 3. The distribution of the rotifer species in lotic habitats.

Sampling locality	Karababa							Eğri Stream							Çakal Stream							Çam Stream							Kalburlu Stram						
Sampling time	11	1	2	4	5	7	9	11	1	2	4	5	7	9	11	1	2	4	5	7	9	11	1	2	4	5	7	9	11	1	2	4	5	7	9
Anuraeopsis fissa			*					*							*													*								
A. navicula																																			*
Asplanchna priodonta		*	*												*																				
Ascomorpha saltans			*																									*							
Brachionus angularis								*							*			*				*						*							
B. calyciflorus		*																																	
B. leydigii															*										*										
B. falcatus																												*							
Brachionus calyciflorus						*																													
B. quadridentatus				*																		*			*		*								
Cephalodella gibba															*		*								*										
Cephalodella catellina																									*										
C. gracilis																															*				
Colurella obtusa															*																				
Colurella colurus				*																															
Conochilus unicornis																									*			*							
C. coenabasis																									*										
C. natans																									*										
Dicranophorus hauerianus																				*								*							

Table 3 (cont.)

Encentrum putorius																										*								
Encentrum saundersiae																																		
Euchlanis dilatata															*		*							*									
E. meneta																		*															
Gastropus stylifer		*																															
Hexarthra fennica		*																															
Lepadella patella																								*									
L. quadricarinata								*																									*
Lecane bulla																										*							
Lecane closterocerca							*											*								*							
Lecane luna						*																											
Lecane lunaris			*																														
Lecane scutata																																	*
Polyarthra dolichoptera		*				*																											
Polyarthra remata		*																															
Polyarthra vulgaris																										*							
Synchaeta longipes																																	
Synchaeta oblonga															*																		
Synchaeta pectinata								*																									
Trichocerca elongata															*																		
Trichotria tatractis															*																		

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EFFECTS OF SALINITY AND TEMPERATURE ON GERMINATION OF *Kalidiopsis wagenitzii* Aellen (CHENOPODIACEAE)

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Abstract

In this study, effects of salinity and temperature on germination of *Kalidiopsis wagenitzii* Aellen seeds grown as an endemic in Salt Lake, Turkey were investigated. Seeds were germinated for seven days at the different NaCl concentrations (control, 50, 100, 200, 400 and 600 mM) and temperatures (5, 10, 15, 20, 25 and 30 °C) in the growth cabinets. The optimum germination temperature was found 25 °C and the percentage germination was 60%, 59% and 53% in control, 50 and 100 mM NaCl respectively. The present study showed that if the seed is protected from salinity and temperature stress, *Kalidiopsis wagenitzii* could germinate during early spring.

Key Words: Germination, *Kalidiopsis wagenitzii*, salinity, temperature

Introduction

Salinization of the soil is a serious problem in arid and semiarid regions (1). Saline sites suffer variations in surface salinity, available soil water, temperature, soil crust strength and other factors which can influence germination and establish (2). Adaptation of the species

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to salinity during germination is crucial for establishment of species in saline environments (3, 4). Halophytes differ from glycophytes in point of view of its salt tolerances. However, halophyte seeds behave confronted with salt stress: the start of germination is delayed, germination is reduced and many seeds remain dormant due to low water potentials under high salinity levels (5). One of the most important stages in the halophyte life period is germination in like other plants. Salinity and temperature interact in their control of seed germination (6), with the greatest inhibition due to salinity usually found at the minimum or maximum limits of tolerance to temperature (7). While it was found that seeds of some halophytes germinated in the high salt concentrations, some halophytes gave response like glycophytes to salinity in the germination stage (5, 8, 9).

Turkey has a rich flora point of view of endemic species. *Kalidiopsis wagenitzii*, one of endemic species, was collected from south of Salt Lake 40-50 years ago and introduced to science. Now, it is considered an endangered species by IUCN categories (10).

Although *Kalidiopsis wagenitzii* has been described as a halophyte, physiology of species has not been studied yet. Because of these interesting features, it would be useful to study some physiological parameters from the beginning of the germination.

Our aim was to investigate the effects of salinity and temperature on germination of *Kalidiopsis wagenitzii* Aellen seeds.

Material and Methods

Seed material

Kalidiopsis wagenitzii Aellen was collected from Eskil-Yenikent south of Salt Lake in 1999. Seeds were separated using sieves from plants to study in the laboratory. Seeds selected carefully were used in the germination experiments.

Experimental conditions

In the experiment, six different salt concentrations and temperature regimes were used. Temperature regimes were used as 5, 10, 15, 20, 25 and 30 °C. Salinized culture solutions were prepared by adding NaCl to the ½ strength Hoagland culture solution. Concentrations of NaCl were control (½ strength Hoagland), 50, 100, 200, 400 and 600 mM. Three 50-seeds replicates were placed directly Petri dishes and submerged 5 ml related culture solutions. Dishes were placed in the growth cabinets at the different temperature regimes for 7 days. Seeds were observed daily and considered germinated when the radicle emerged 1 mm from the seed. All conditions were maintained constant during experiments.

Statistical analysis

Germination data was transformed (arcsine) before statistical analysis. These data were analyzed using Statistica Programme. Duncan's Multiple Range test was used to determine significant differences of means at a 5 % level.

Results

Germination percentage of *K. wagenitzii* seeds significantly decreased with an increase in salinity at all temperatures (Table 1). The highest germination percentage was obtained control and 50 mM NaCl. The germination percentage increased with increasing temperature, and the highest percentage of germination was observed at the 25 °C. Germination percentages at 25 °C in the control and 50 mM NaCl were around 60%. The percentage was also high at 30 °C that the highest temperature degree, but it was lower than 25 °C. The lowest germination percentage occurred at 5 °C that the lowest temperature degree. These results showed that any further increases or decreases in temperature inhibited germination at all salinities.

Discussion

Environmental factors have important effects on germination either glycophyte and halophyte seeds. Salinity and temperature may be concerned among factors that affect germination significantly. Our results have supported this. Although some halophyte species indicated high germination percentage in the high salt concentration (1, 11, 12, 13, 14) *K. wagenitzii* showed lower germination percentage higher than 50 mM NaCl concentration.

Germination is inhibited by temperatures above and below the optimum. Inhibition is clear especially at low temperatures.

A negative effect of salinity on germination has been reported for several halophyte species (11, 15). Researchers have suggested that salinity is inhibitory to the germination of halophyte seeds in two ways: 1) causing a complete inhibition of the germination process at salinities beyond the tolerance limits of a species and 2) delaying the germination of seeds at salinities that cause some stress to seeds but do not prevent germination (16, 17). The germination responses of seeds of annual halophytes to salinity are highly variable and species specific (16).

Temperature has an effect on germination and salt tolerance. Khan et al. (17) found that the cooler thermoperiods inhibited germination considerably at all salinities. Naidoo and

Table 1. The effects of salinity and temperature on germination of *Kalidiopsis wagenitzii* Aellen. All means followed by the same letter are not significantly different at the 5% probability level.

Temperature (°C)	NaCl (mM)					
	Control	50	100	200	400	600
5	*E6,95±2,45a**	E4,92±2,92a	D4,07±2,34a	C4,07±2,34a	B0±0b	B0±0b
10	D11,35±1,24a	E6,10±2,34b	D4,92±2,92bc	C4,07±2,34bc	AB2,03±2,03cd	B0±0d
15	C21,04±3,00a	D17,94±0,50ab	C15,47±2,25b	C4,92±2,92c	AB2,89±2,88cd	B0±0d
20	C22,49±2,38a	C20,43±2,19a	BC17,89±0,92b	B11,16±1,74c	A4,07±2,34d	AB2,03±2,03d
25	A60,50±3,79a	A59,96±2,90a	A53,56±2,74b	A28,96±1,02c	A4,07±2,34d	A4,07±2,34d
30	B29,28±3,42a	B26,39±2,12a	B19,36±0,53b	BC10,19±4,00c	B0±0d	B0±0d

* Values followed by different capital letter in column represent difference of temperature levels.

** Values followed by different letter in line represent difference of salinity levels.

Naicker (18) also determined that warmer thermoperiods were more stimulatory to germination in comparison with cooler thermoperiods. Similar results were reported by some researchers (7, 13, 19, 20).

It is suggested that salinity and temperature have a synergistic effect on germination. The study with *Hordeum jubatum* has confirmed the theory. While the germination of *H. jubatum* was inhibited by salinity, this inhibition in the germination was increased by warmer temperatures (7). Temperature is the predominant environmental factor that delays the germination of *H. jubatum* (7). However, appropriate germination responses of halophytic species to environmental parameters determine their distribution in saline environments (21).

High salinity-induced dormancy in most halophyte seeds is controlled by inhibiting compounds which regulate germination activity (19, 22). Species adapted to high saline environments bind ions to cytoplasmic proteins.

Several studies have showed that seeds of glycophyte and halophyte respond in a similar manner to increased salinity stress in relation to both a reduction in the total number of seeds germinating and a delay in the initiation of the germination process and that seeds of many halophytes remain dormant due to low water potentials (4, 23). A reduction in the salinity of the surface layers of the soil is a prerequisite for successful germination (24). Seed germination in saline environments usually occurs during spring or in a season with high precipitation, when soil salinity levels are reduced (7, 11, 17, 24, 25).

The results from our study are supported by other studies. The greatest germination of *K. wagenitzii* is in the low salinity levels or nonsaline conditions and at the spring temperature of 25 °C.

As a result, *K. wagenitzii*, grown as an endemic in Turkey, achieves germination stage the most sensitive to stress in spring that rainy and temperature is optimum 25 °C. It alleviates summer having high salinity and temperatures with more tolerant developmental stage. Though it is more sensitive to stress during the germination, how could it continues its viability by dealing with stress at the rest of the developmental stage? The answer of this question may be that: *K. wagenitzii* is a perennial shrub and thick branched plant. It exhibits a tolerant development to stress and produces a large number of seeds. Tolerant and strong seeds germinate under suitable environmental conditions and attain other developmental stages. It may improve some adaptations for stress tolerance at these stages. This hypothesis needs to be investigated. Because responses to salinity of *K. wagenitzii* at the different

developmental stages indicate variety, it can be used as a model plant to determine plant stress tolerances.

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