ISSN 1010-6960
E-ISSN 2536-491X

Türkiye Entomoloji Dergisi

Turkish Journal of Entomology

Cilt (Vol.): 47 Sayı (No.): 3 2023

Türkiye Entomoloji Dergisi
(Turkish Journal of Entomology)

Cilt (Vol.) **47** **Sayı** (No.) **3** **Eylül** (September) **2023**

Sahibi (Owner)
Ferit TURANLI

Sorumlu Müdür (Editor in Chief)
Galip KAŞKAVALCI

Dil Editörü (Linguistic Editor)
Ferah ŞENAYDIN

İstatistik Editörü (Statistic Editor)
Tufan Can ULU

Editör Kurulu (Editorial Board)

ANLAŞ, S., Manisa
ATAKAN, E., Adana
BALCI, H., İzmir
BİRGÜCÜ, A. K., Isparta
CAN, F., Hatay
ÇAKMAK, İ., Aydın
ELEKCİOĞLU, N. Z., Adana
ERSİN, F., İzmir
FENT, M., Edirne
GÜNCAN, A., Ordu
HOFFMANN, K. H., Germany
IORIATTI, C., Italy
İVGİN TUNCA, Rahşan, Muğla
KARUT, Kâmil, Adana
KAŞKAVALCI, G., İzmir
KAYDAN, M. B., Adana
KERSTING, U., Cyprus
KONSTANTINOV, A. S., USA
KOVANCI, O. B., Bursa
KUMRAL, N. A., Bursa
MOORES, G. D., UK
RILEY, I. T., Australia
SARIKAYA, O., Bursa
SEHNAL, F., Czech Republic
SUSURLUK, İ. A., Bursa
ŞENAYDIN, F., İzmir
ULU, T. C., Bilecik
TOPRAK, U., Ankara
TURANLI, D., İzmir
TURANLI, F., İzmir
UECKERMANN, E., South Africa

Turkish Journal of Entomology

The Turkish Journal of Entomology is a quarterly journal which has been published by the Entomological Society of Turkey. İt accepts original research articles in the fields of entomology and agricultural zoology in Turkish or English.

Abstracted/Indexed in Biological Abstracts, BIOSIS Previews, CABAbstracts, FAO AGRIS, Elsevier Scopus, Global Health, Information Reference Library, Review of Agricultural Entomology, SCI-E, TÜBİTAK/ULAKBİM, VINITI, Zoological Record.

Annual subscription price:€75 Price of asingle issue: €20

Corresponding address:
Turkish Journal of Entomology
Ege Üniversitesi Kampüsü PTT Şubesi, P.O. Box: 10, 35100 Bornova, İzmir, Turkey
e-mail: dergi@entomoloji.org.tr
web : http://www.entomoloji.org.tr

All rights to articles published in this Journal are reserved by the Entomological Society of Turkey. Permission must be obtained for reproduction in whole or in part any form.

Türkiye Entomoloji Dergisi
(Turkish Journal of Entomology)

Cilt (Vol.) **47** **Sayı** (No.) **3** **Eylül** (September) **2023**

İnceleme ve Değerlendirmede Bilimsel Olarak Katkıda Bulunanlar
(Scientific Advisory Board)

ALTUNSOY, Ferhat, Eskişehir
ALTUNTAŞ, Hülya, Eskişehir
ATAKAN, Ekrem, Adana
ATLIHAN, Remzi, Van
AY, Recep, Isparta
AYDINLI, Gökhan, Samsun
BALKAN, Tarık, Tokat
BAŞPINAR, Hüseyin, Aydın
CAN, İlyas, Tokat
CANHİLAL, Ramazan, Kayseri
ÇIKMAN, Emine, Şanlıurfa
DAĞLI, Fatih, Antalya
DEMİREL, Nihat, Hatay
DÖKER, İsmail, Adana
ERSİN, Firdevs, İzmir
ESKİ, Ardahan, Bilecik
EVLİCE, Emre, Sivas
GÖZEL, Çiğdem, Çanakkale
GÖZEL, Uğur, Çanakkale
GÜLPERÇİN, Nilay, İzmir
GÜNCAN, Ali, Ordu
GÜRBÜZ, Mehmet Faruk, Isparta
GÜVEN KALKAR, Özlem, Kahramanmaraş
HATİPOĞLU, Ahmet, İzmir
HERRON, Grant, Australia
IŞIK, Raziye, Tekirdağ
IŞIKBER, A. Arda, Kahramanmaraş
İMREN, Mustafa, Bolu
İNAK, Emre, Ankara
KABALAK, Mahmut, Ankara
KAHYA, Yasin, Ankara
KARABÖRKLÜ, Salih, Sakarya
KARANFİL, Ali, Çanakkale
KASAPOĞLU ULUDAMAR, Ece B., Adana
KAYA, Serhat, Çanakkale
KAYDAN, Mehmet Bora, Adana
KESKİN, Bekir, İzmir
KHALAİM, Andrey, Russia
KONDO, Takumasa, Republic of Colombia
KONUŞ, Metin, Van
KORKMAZ, Ertan Mahir, Sivas
KÖK, Şahin, Çanakkale
MAMAY, Mehmet, Şanlıurfa
NDİFON, Elias, Nigeria
NIMKINGRAT, Prakaijan, Thailand
NIU, Gengyun, China
OSKAY, Devrim, Tekirdağ
ÖZDEMİR, Esengül, Şırnak
ÖZGÖKÇE, Mehmet Salih, Van
ÖZGÖNEN ÖZKAYA, Hülya, Isparta
ÖZYURT KOÇAKOĞLU, Nurcan, Ankara
PEHLİVAN, Serkan, Adana
SABOORİ, Alireza, Iran
SAĞLAM, Özgür, Tekirdağ
SHAVRİN, AV Alexey, Latvia
SUSURLUK, Hilal, Bursa
STEKOLSHCHİKOV, Andrey V., Russia
TAN, Ayşe Nur, Sakarya
TARASCO, Eustachio, Italy
TEZCAN, Serdar, İzmir
TİRENG KARUT, Şebnem, Adana
TİRYAKİ, Osman, Çanakkale
TOKTAY, Halil, Niğde
TOPRAK, Umut, Ankara
TOZKAR, Özge, Van
TURAN, Yavuz, İstanbul
UÇAK KOÇ, Aytül, Aydın
UÇKAN, Fevzi, Kocaeli
ULU, Tufan Can, Bilecik
ULUBAŞ SERÇE, Çiğdem, Niğde
VARLI, Sakin Vural, Balıkesir
WATANABE, Reiya, Japan
YAĞCI, Mürşide, Ankara
YALÇIN, Melis, Aydın
YARAN, Mehmet, Gaziantep
YAVUZASLANOĞLU, Elif, Karaman
YAZICI, Gülten, Ankara
YOLCI ÖMEROĞLU, Perihan, Bursa
YOUSSEF, Rawa Muhsen, Syria
YÖRÜK, Emre, İstanbul

İçindekiler (Contents)

Orijinal araştırmalar (Original articles)

Effects of storage temperature on viability and virulence of entomopathogenic nematodes *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae), *Steinernema carpocapsae* Weiser, 1955 and *Steinernema feltiae* Filipjev, 1934 (Rhabditida: Steinernematidae)
Depolama sıcaklığının entomopatojen nematodlar *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae), *Steinernema carpocapsae* Weiser, 1955 ve *Steinernema feltiae* Filipjev, 1934 (Rhabditida: Steinernematidae)'nin canlılık ve virülenslikleri üzerine etkileri
Alperen Kaan BÜTÜNER, Merve İLKTAN, İsmail Alper SUSURLUK 247-257

Pathogenicity of some entomopathogenic fungi on wheat weevil *Sitophilus granarius* (L., 1758) (Coleoptera: Curculionidae)
Buğday biti *Sitophilus granarius* (L., 1758) (Coleoptera: Curculionidae) üzerinde bazı entomopatojen fungusların patojenisitesi
Mahmut Alper ALTINOK, Memiş KESDEK, Şaban KORDALİ, Ayşe USANMAZ BOZHÜYÜK, Hacer Handan ALTINOK 259-269

A study of whether the genetic variation decreased or not in the protected Caucasian bee, *Apis mellifera caucasica* Pollmann, 1889 (Hymenoptera: Apidae) population in isolated regions
İzole bölgelerde korunan Kafkas arısı, *Apis mellifera caucasica* Pollmann, 1889 (Hymenoptera: Apidae) popülasyonunda genetik varyasyonun azalıp azalmadığına dair bir çalışma
Berkant İsmail YILDIZ, Emel TÜTEN SEVİM, Sinan AYDIN, Yaprak KARADUMAN ASLAN, Ramazan ÇETİN, Erdem SUR, Kemal KARABAĞ 271-282

The nematode assemblages of a lake ecosystem (Lake Korugöl Natural Park, Düzce, West Black Sea Cost of Türkiye): ecology and biodiversity patterns with first reports of 10 genera to the Türkiye nematofauna
Bir göl ekosisteminin nematod toplulukları (Korugöl Gölü Tabiat Parkı, Düzce, Türkiye): Türkiye nematofaunası için 10 cinsin ilk raporları ile ekoloji ve biyoçeşitlilik modelleri
Taylan ÇAKMAK 283-294

The effects of altitude and rearing period on some characteristics of honey bee queens, *Apis mellifera caucasica* Gorbachev, 1916 (Hymenoptera: Apidae))
Rakım ve yetiştirme döneminin ana arıların, *Apis mellifera caucasica* Gorbachev, 1916 (Hymenoptera: Apidae) bazı özellikleri üzerine etkileri
Nazlı Pınar CANVERDİ, Gonca ÖZMEN ÖZBAKIR, Soner ÇANKAYA, Muzaffer DUMAN, Ümit KAYABOYNU, Fatih YILMAZ, Hasan ESE, Belgin GÜNBEY 295-306

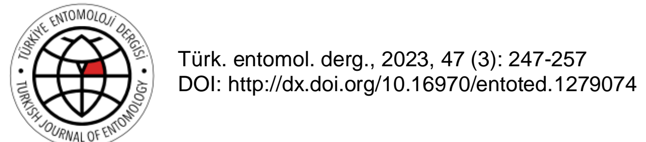
Identification of odorant receptor protein genes in two Cephid stem borers (Hymenoptera: Cephidae) by high-throughput sequencing
İki Cephid sap arısında (Hymenoptera: Cephidae) koku reseptör protein genlerinin yüksek verimli dizileme ile tanımlanması
Mahir BUDAK 307-316

Aphid species (Hemiptera: Aphidoidae) determined from Antalya, Karaman and Muğla with new aphid records
Antalya, Karaman ve Muğla'dan belirlenen yeni kayıt afit (Hemiptera: Aphidoidae) türleri
Hayal AKYILDIRIM BEĞEN, Gazi GÖRÜR, Özhan ŞENOL, Gizem BAŞER, Bengisu Venhar AKÇAY 317-328

On the genus *Lobrathium* Mulsant & Rey, 1878 (Coleoptera: Staphylinidae: Paederinae) of the Palaearctic region II: A new species from Türkiye and faunistic notes on some species
Palaearktik bölgedeki *Lobrathium* Mulsant & Rey, 1878 (Coleoptera: Staphylinidae: Paederinae) cinsi üzerine II: Türkiye'den bir yeni tür ve bazı türler üzerinde faunistik notlar
Sinan ANLAŞ 329-338

Effect of selected pesticides on the orientation of entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae)
Bazı pestisitlerin entomopatojen nematodların (Rhabditida: Heterorhabditidae ve Steinernematidae) yönelimleri üzerine etkisi
Tufan Can ULU 339-349

Efficacy of spore suspension and culture filtrate of *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycota: Hyphomycetes) isolates on *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) in different conditions
Beauveria bassiana (Balsamo) Vuillemin (Deuteromycota: Hyphomycetes) izolatlarının spor süspansiyonu ve kültür filtratının farklı koşullarda *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) üzerindeki etkinliği
Asiye UZUN YİĞİT 351-360



Türk. entomol. derg., 2023, 47 (3): 247-257
DOI: http://dx.doi.org/10.16970/entoted.1279074

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Effects of storage temperature on viability and virulence of entomopathogenic nematodes *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae)*,* *Steinernema carpocapsae* Weiser, 1955 and *Steinernema feltiae* Filipjev, 1934 (Rhabditida: Steinernematidae)[1]

Depolama sıcaklığının entomopatojen nematodlar *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae), *Steinernema carpocapsae* Weiser, 1955 ve *Steinernema feltiae* Filipjev, 1934 (Rhabditida: Steinernematidae)'nin canlılık ve virülenslikleri üzerine etkileri

Alperen Kaan BÜTÜNER[2] **Merve İLKTAN[2]** **İsmail Alper SUSURLUK[2*]**

Abstract

Entomopathogenic nematodes (EPN) are a widely used biological control agent. The aim of the study was to detect efficacy and mortalities of some EPN stored at different temperatures and periods. Three EPN species were used in the study. They were *Heterorhabditis bacteriophora* Poinar*,* 1976 (Rhabditida: Heterorhabditidae*)* HBH hybrid strain, *Steinernema carpocapsae* Weiser, 1955 TUR-S4 isolate and *Steinernema feltiae* Weiser, 1955 (Rhabditida: Steinernematidae) TUR-S3 isolate. The species were kept at 4, 15, 25 and 35°C for 7, 14 and 21 days. Subsequently, these species were applied at a dose of 15 Infective juveniles on *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) larvae. The study was carried out in laboratory conditions in 2023. As a result, HBH and TUR-S4 kept at 25°C for 14 days and TUR-S3 for 21 days showed the highest virulence as 93.33%. Mortality rates of the EPN species kept at the specified temperatures were also determined. The results have showed that the highest mortality rates for the HBH, TUR-S4 and TUR-S3 isolates were 11.96% on the 14th day at 35°C, 19.81% on the 21st day at 25°C and 7.39% on the 21st day at 35°C, respectively. This study is an important step in determining suitable temperature conditions for storing and transporting EPN.

Keywords: *Heterorhabditis bacteriophora, Steinernema carpocapsae, Steinernema feltiae,* storage, temperatures

Öz

Entomopatojen nematodlar (EPN) yaygın olarak kullanılan bir biyolojik mücadele ajanıdır. Bu çalışmanın amacı, farklı sıcaklık ve sürelerde depolanan bazı EPN'lerin etkinlik ve ölüm oranlarını belirlemektir. Çalışmada üç EPN türü kullanılmıştır. Bunlar*; Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) HBH hibrit ırkı, *Steinernema carpocapsae* Weiser, 1955 TUR-S4 izolatı ve *Steinernema feltia*e Weiser, 1955 (Rhabditida: Steinernematidae) TUR-S3 izolatıdır. Bu izolatlar 7, 14 ve 21 gün boyunca 4, 15, 25 ve 35°C'de depolandı. Daha sonra bu türler *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) larvaları üzerine 15 Infektif Juvenil dozunda uygulanmıştır. Çalışma, 2023 yılında laboratuvar koşullarında yapılmıştır. Sonuç olarak; HBH ve TUR-S4 25°C'de 14 gün, TUR-S3 ise 21 gün süreyle %93,33 ile en yüksek virülans göstermiştir. Ayrıca, aynı sıcaklıklarda tutulan EPN türlerinde ölüm oranları da belirlenmiştir. Sonuçlar, HBH, TUR-S4 ve TUR-S3 izolatları için en yüksek ölüm oranlarının sırasıyla 35°C'de 14. günde %11,96, 25°C'de 21. günde %19,81 ve 35°C'de 21. günde %7,39 olduğunu göstermiştir. Bu çalışma, EPN'nin depolanması ve nakliyesi sırasında uygun sıcaklık koşullarının belirlenmesinde önemli bir adımdır.

Anahtar sözcükler: *Heterorhabditis bacteriophora, Steinernema carpocapsae, Steinernema feltiae,* depolama, sıcaklık

---

[1] This study was supported by TÜBİTAK (The Scientific and Technological Research Council of Türkiye), Grant Project No: 219O370.
[2] Bursa Uludağ University, Faculty of Agriculture, Department of Plant Protection, 16059 Nilüfer-Bursa, Türkiye
* Corresponding author (Sorumlu yazar) e-mail: susurluk@uludag.edu.tr
Received (Alınış): 07.04.2023 Accepted (Kabul ediliş): 12.09.2023 Published Online (Çevrimiçi Yayın Tarihi): 24.09.2023

Introduction

Chemical control method in plant protection has been used for many years to reduce the impact of pests on agricultural productivity and to manage pests. However, in recent years, it has been revealed that pesticides used for chemical control have toxic effects on non-target organisms (Iyaniwura, 1991; Sánchez-Bayo, 2012; Rani et al., 2021). With the regulations being made as a result of these developments, the use of pesticides in agriculture has been restricted (Ewence et al., 2015; Gunnell et al., 2017; Robin & Marchand, 2019).

With the restriction on pesticides, which are commonly utilized for chemical control, new control methods are being promoted and brought to the forefront (Lu et al., 2012; Barzman et al., 2015). Biological control in agriculture is one of the alternatives to chemical control (Stern et al., 1959; Waage & Greathead, 1988; Baker et al., 2020). It is based on the principle of using living organisms to neutralize agricultural pests. Entomopathogenic nematodes (EPN) are among the most important organisms successfully used in biological control (Ehlers, 1996).

Entomopathogenic nematodes are endoparasitic organisms belonging to Secernentea class, Rhabditida order, Heterorhabditidae and Steinernematidae families (Ehlers, 1996, 2001; Shapiro-Ilan et al., 2006). These organisms living in the soil need to find a host and interact with it to survive (Smits, 1996; Susurluk & Ehlers, 2008; Ulu & Susurluk, 2014).

Infective Juveniles (IJs) are the third stage of the nematode juveniles, which can seek for hosts in the soil for months without any feeding (Boemare et al., 1996; Ehlers, 1996). For example, *Heterorhabditis bacteriophora* Poinar, 1976 has been observed to remain active in the soil for up to 22 months (Susurluk & Ehlers, 2008). In addition, EPN belonging to the families of Heterorhabditidae and Steinernematidae, *Photorhabdus* spp. and *Xenorhabdus* spp. establish a symbiotic relationship with the bacteria, respectively. However, although IJs can live in the soil for a long time (Ehlers, 1996), they can be affected by many abiotic factors, such as temperature, soil moisture and pesticides in the soil (Ehlers, 1996; Özdemir et al., 2021).

These factors directly affect the activity of IJs and may cause a decrease in their lethal activity on the host. Severe climatic conditions can drastically reduce the life span of EPN, which is one of the most serious threats (Glazer, 1996; Kurtz et al., 2007; Susurluk & Ehlers, 2008). Temperature, in particular, is one of the major abiotic factors with negative effects on IJs. Although IJs may live at specific temperatures, the period of exposure to these temperatures is crucial (Strauch et al., 2000; Susurluk & Ulu, 2015).

The main objective of the present study was to detect the virulence of *Heterorhabditis bacteriophora*, *Steinernema carpocapsae* and *Steinernema felitae* isolates on the larvae of *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) after storage at temperatures of 4, 15, 25, and 35°C for 7, 14, and 21 days. Moreover, it also aimed to assess the mortality rates (viability) in the used EPN isolates stored at the specified temperatures and durations.

Materials and Methods

Entomopathogenic nematode species

In the present study, the EPN utilized in this study included three species. One of them was a hybrid strain HBH of *Heterorhabditis bacteriophora* developed and patented (TPMK Patent No: TR 2013 06141 B) in Bursa Uludağ University, Faculty of Agriculture, Department of Plant Protection, Nematology Laboratory. Another EPN species was an isolate TUR-S4 of *Steinernema carpocapsae* isolated in Bursa-Turkey. The last EPN species was an isolate TUR-S3 of *Steinernema feltiae* isolated from Ankara-Turkey. These isolates used in this study were cultured on the last instar of *Galleria mellonella* L., 1758 (Lepidoptera: Pyralidae) larvae, then kept at 4°C (Kaya & Stock, 1997; Ulu & Susurluk, 2014) until use. Three-day-old isolates were used for the studies.

Experimental design

The used EPN species were stored in approximately 1000±20 IJs in 60 ml of Ringer's solution in a 250 ml capacity filter capped culture flask in the experiments. The 24-well tissue culture plates (each well measuring 1.5 cm in diameter x 3 cm deep) were filled with 10% moist alluvial soil, and the larvae of *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) were placed into them. Afterwards, the EPN taken from the flask were applied on the soil. A dose of 15 IJs was used in this study because of the more objective results of performing efficacy studies at a low dose. (Ulu & Susurluk, 2014; Dede et al., 2022).

The larvae in the EPN-treated 24-well tissue culture plates were incubated at 25°C for 3 days. Then, the numbers of both dead and living insect larvae in the wells were recorded and evaluated. The detected dead larvae were carefully dissected and examined for IJs of the species in order to observe whether their deaths were a result of dead by the EPN or not. This study was carried out with the EPN species used in the experiment at 4, 15, 25 and 35°C and for 7, 14 and 21-day periods. Storage time at each temperature was made on each indicated day. Normally, EPNs are stored at 4°C, as they can maintain their viability for a long time. For this reason, 4°C was considered as a control value in this study.

The larvae in the EPN-treated 24-well tissue culture plates were incubated at 25°C for 3 days (Kurtz et al., 2007; Susurluk & Ulu, 2015; El Khoury et al., 2018). This study was carried out in 3 replications with 10 insect larvae in each replication.

Determination of the mortality rates of *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, *Steinernema feltiae* at indicated periods

After the species were kept in the incubator at the trial temperatures for the indicated days, at the end of the days, the mortality rates of the IJs were evaluated by means of the solutions taken from tissue culture flasks. Dead IJs were counted from the samples taken from the flask, and then results were evaluated statistically. This stage of the study was conducted in 5 replications.

Statistical analyses

JMP®16 software was used to perform analysis of variance (ANOVA) on mortality data. Furthermore, the least significant difference test (LSD) ($p < 0.05$) was used to determine the difference between means.

Results

Determination of the efficiency of *Heterorhabditis bacteriophora* HBH on different temperature and days

According to the results, the mortality rate of the *T. molitor* larvae ranged between 76.67 to 90% at 15°C. There was no statistically significant difference on mortality rates at 15°C for 7, 14, and 21 days stored. Incubation at 25°C for 14 days, revealed, it was detected that the highest mortality rate of 93.33%. There was a statistically significant difference between 7 and 21 days, as well as 14 and 21 days ($F = 19.06$; $df = 11, 24$; $p < 0.0001$) when the mortality rates of the larvae were examined after the application of *H. bacteriophora* HBH stored at the temperature for 21 days. The mortality rate of *T. molitor* larvae varied between 23.33 and 33.33% at 35°C. No statistically significant difference was found among different days at that temperature. The mortality rate of the larvae was 83.33% at 7, 14, and 21 days at 4°C, which was the control (Table 1).

Determination of the efficiency of *Steinernema carpocapsae* TUR-S4 on different temperatures and days

The results showed that the highest mortality rate of *T. molitor* larvae was 90% at 15°C. A statistically significant difference was obtained at 15°C for 7, 14 and 21 days ($F = 11.66$; $df = 11,24$; $p < 0.0001$). The highest mortality rate in this experiment was obtained on the 14th day at 25°C. This value was found to be

93.33%. At 35°C, the lowest mortality rate was 56.67% on the 21st day. At this temperature, the highest mortality rate was 83.33% on 7th. There was a statistically difference between the mortality rates at this temperature for 7, 14 and 21 days. At 4°C, which was used as a control, the mortality rate was 83.33% on all days (Table 1).

Table 1. Percentage of mortality rates of *T. molitor* larvae caused by the used EPN isolates. The statistical analysis was performed for each species separately (Mean ± SE). Means in each isolate followed by the same letters are not significant different (<0.05)

EPN Species	Time (day)	Temperatures (°C)	Mortality rates (%)
Heterorhabditis bacteriophora HBH	7	4 (Control)	83.33 ± 3.33 a
		15	76.67 ± 3.33 a
		25	80.00 ± 0.00 a
		35	33.33 ± 12.02 b
	14	4 (Control)	83.33 ± 3.33 a
		15	83.33 ± 3.33 a
		25	93.33 ± 3.33 a
		35	20.00 ± 15.27 b
	21	4 (Control)	83.33 ± 3.33 a
		15	90.00 ± 5.77 a
		25	30.00 ± 5.77 b
		35	23.33 ± 3.33 b
Steinernema carpocapsae TUR-S4	7	4 (Control)	83.33 ± 3.33 bc
		15	83.33 ± 3.33 bc
		25	86.67 ± 3.33 ab
		35	83.33 ± 3.33 bc
	14	4 (Control)	83.33 ± 3.33 bc
		15	76.67 ± 3.33 cd
		25	93.33 ± 3.33 a
		35	70.00 ± 0.00 d
	21	4 (Control)	83.33 ± 3.33 bc
		15	90.00 ± 0.00 ab
		25	60.00 ± 5.77 e
		35	56.67 ± 3.33 e
Steinernema feltiae TUR-S3	7	4 (Control)	83.33 ± 3.33 bc
		15	76.67 ± 3.33 cd
		25	73.33 ± 3.33 d
		35	70.00 ± 0.00 d
	14	4 (Control)	83.33 ± 3.33 bc
		15	76.67 ± 3.33 cd
		25	90.00 ± 5.77 ab
		35	76.67 ± 3.33 cd
	21	4 (Control)	83.33 ± 3.33 bc
		15	73.33 ± 3.33 d
		25	93.33 ± 3.33 a
		35	50.00 ± 0.00 e

Determination of the efficiency of *Steinernema feltiae* TUR-S3 on different temperatures and days

The mortality rate of *T. molitor* larvae at 15°C varied between 73.33 to 76.67% at 25°C, the mortality rate was 90% on the 14th day. The highest mortality rate was 93.33% on the 21st day at 25°C. The mortality rates obtained on the 7th, 14th, and 21st days at 25°C ranged between 50 to 76.67%. Statistically significant differences were found between these mortality rates ($F = 11.11$; $df = 11,24$; $p < 0.0001$) (Table 1).

In addition to these mortality rates, all the species used in the study were subjected to a single statistic to assess the level of significance between them, and statistically significant differences were found ($F = 17.40$; $df = 35,72$; $p < 0.0001$) (Table 2).

Table 2. Percentage of mortality rates of *T. molitor* larvae caused by the used EPN isolates. The statistical analysis was performed for all species each other (Mean ± SE). Means followed by the same letters are not significant different (<0.05)

EPN Species	Time (day)	Temperatures(°C)	Mortality rates (%)
H. bacteriophora HBH	14	25	93.33 ± 3.33 a
S. feltiae TUR-S3	21	25	93.33 ± 3.33 a
S. carpocapsae TUR-S4	14	25	93.33 ± 3.33 a
S. carpocapsae TUR-S4	21	15	90.00 ± 0.00 ab
H. bacteriophora HBH	21	15	90.00 ± 5.77 ab
S. feltiae TUR-S3	14	25	90.00 ± 5.77 ab
S. carpocapsae TUR-S4	7	25	86.67 ± 3.33 abc
S. carpocapsae TUR-S4 (Control)	7	4	83.33 ± 3.33 abcd
S. feltiae TUR-S3 (Control)	7	4	83.33 ± 3.33 abcd
H. bacteriophora HBH (Control)	21	4	83.33 ± 3.33 abcd
S. carpocapsae TUR-S4	7	15	83.33 ± 3.33 abcd
S. carpocapsae TUR-S4 (Control)	21	4	83.33 ± 3.33 abcd
S. feltiae TUR-S3 (Control)	21	4	83.33 ± 3.33 abcd
S.carpocapsae TUR-S4	7	35	83.33 ± 3.33 abcd
H. bacteriophora HBH (Control)	14	4	83.33 ± 3.33 abcd
S.carpocapsae TUR-S4 (Control)	14	4	83.33 ± 3.33 abcd
S. feltiae TUR-S3 (Control)	14	4	83.33 ± 3.33 abcd
H. bacteriophora HBH	14	15	83.33 ± 3.33 abcd
H. bacteriophora HBH (Control)	7	4	83.33 ± 3.33 abcd
H. bacteriophora HBH	7	25	80.00 ± 0.00 bcde
H. bacteriophora HBH	7	15	76.67 ± 3.33 cde
S. feltiae TUR-S3	7	15	76.67 ± 3.33 cde
S. feltiae TUR-S3	14	35	76.67 ± 3.33 cde
S. feltiae TUR-S3	14	15	76.67 ± 3.33 cde
S. carpocapsae TUR-S4	14	15	76.67 ± 3.33 cde
S. feltiae TUR-S3	21	15	73.33 ± 3.33 de
S. feltiae TUR-S3	7	25	73.33 ± 3.33 de
S. carpocapsae TUR-S4	14	35	70.00 ± 0.00 ef
S. feltiae TUR-S3	7	35	70.00 ± 0.00 ef
S. carpocapsae TUR-S4	21	25	60.00 ± 5.77 fg
S. carpocapsae TUR-S4	21	35	56.67 ± 3.33 g
S. feltiae TUR-S3	21	35	50.00 ± 0.00 g
H. bacteriophora HBH	7	35	33.33 ± 12.02 h
H. bacteriophora HBH	21	25	30.00 ± 5.77 hi
H. bacteriophora HBH	21	35	23.33 ± 3.33 hi
H. bacteriophora HBH	14	35	20.00 ± 15.27 i

Determination of the mortality rates of *Heterorhabditis bacteriophora*, *Steinernema carpocapsae*, *Steinernema feltiae*

According to the results obtained, no mortality was detected on *H. bacteriophora* HBH after 7, 14 and 21 days at 15°C. A statistically significant difference was not found at this temperature value. At 25°C, the mortality rate was found to be 6.66% only on the 21st day. Mortality was not detected on the 7th and 14th days. The highest mortality rates were observed at 35°C. These mortality rates were between 4 to 11.96%. However, a statistically significant difference was not found when all HBH mortality rates were evaluated (F = 1.26; df = 11, 48; p = 0.2719). As a control, no mortality was observed on all days at 4°C. Although no mortality was detected on the 7th at 15°C for *S. carpocapsae* TUR-S4, mortality rates on the 14th and 21st days were 10.78 and 18.88%, respectively. The highest mortality rate for the isolate was determined on 21st days at 25°C. On the 14th day, this rate was 0.64%, while no mortality was detected on the 7th day. Also, a statistically significant difference was found between the days at 25°C (F = 12.02; df = 11, 48; p < 0.0001). At 35°C, the mortality was observed on all days. This value varied between 5.45 to 14.80%. There was not any mortality detected at 4°C. For *S. feltiae* TUR-S3, the highest mortality rate was found as 6.99% at 15°C. The mortality rate obtained at 25°C varies between 0 to 2.14%. The highest mortality rate for the isolate TUR-S3 was obtained as 7.39% on the 21st day at 35°C. A statistically significant difference was determined at that temperature value (F = 3.33; df = 11, 48; p = 0.0018) (Table 3).

Table 3. Percentage of mortality rates of the used EPN isolates during the experimental periods. The statistical analysis was performed for each species separately (Mean ± SE). Means in each isolate followed by the same letters are not significantly different (p<0.05)

EPN Species	Time (day)	Temperatures (°C)	Mortality rates (%)	
Heterorhabditis bacteriophora HBH	7	4 (Control)	0.00 ± 0.00	b
		15	0.00 ± 0.00	b
		25	0.00 ± 0.00	b
		35	4.00 ± 4.00	ab
	14	4 (Control)	0.00 ± 0.00	b
		15	0.00 ± 0.00	b
		25	0.00 ± 0.00	b
		35	11.96 ± 8.83	a
	21	4 (Control)	0.00 ± 0.00	b
		15	0.00 ± 0.00	b
		25	6.66 ± 6.66	ab
		35	8.33 ± 5.27	ab
Steinernema carpocapsae TUR-S4	7	4 (Control)	0.00 ± 0.00	d
		15	0.00 ± 0.00	d
		25	0.00 ± 0.00	d
		35	5.45 ± 3.63	cd
	14	4 (Control)	0.00 ± 0.00	d
		15	10.78 ± 2.18	bc
		25	0.64 ± 0.64	d
		35	7.66 ± 3.71	c
	21	4 (Control)	0.00 ± 0.00	d
		15	18.88 ± 3.48	a
		25	19.81 ± 3.55	a
		35	14.80 ± 1.85	ab

Table 3. Continued

EPN Species	Time (day)	Temperatures (°C)	Mortality rates (%)	
Steinernema feltiae TUR-S3	7	4 (Control)	0.00 ± 0.00	b
		15	0.00 ± 0.00	b
		25	0.00 ± 0.00	b
		35	0.00 ± 0.00	b
	14	4 (Control)	0.00 ± 0.00	b
		15	3.35 ± 2.07	ab
		25	0.62 ± 0.62	b
		35	2.22 ± 2.22	b
	21	4 (Control)	0.00 ± 0.00	b
		15	6.99 ± 1.77	a
		25	2.14 ± 1.43	b
		35	7.39 ± 3.46	a

In addition, mortality rates in the specified species were subjected to a single statistic to evaluate the level of significance between the species used in the study, and statistically significant differences were found ($F = 4.42$; $df = 35, 144$; $p < 0.0001$) (Table 4).

Table 4. Percentage of mortality rates of the used EPN isolates during the experimental periods. The statistical analysis was performed for all species each other (Mean ± SE). Means followed by the same letters are not significant different (<0.05)

EPN Species	Time (day)	Temperatures(°C)	Mortality rates (%)	
S. carpocapsae TUR-S4	21	25	19.81 ± 3.55	a
S. carpocapsae TUR-S4	21	15	18.88 ± 3.48	ab
S. carpocapsae TUR-S4	21	35	14.80 ± 1.85	abc
H. bacteriophora HBH	14	35	11.96 ± 8.83	bcd
S. carpocapsae TUR-S4	14	15	10.78 ± 2.18	cde
H. bacteriophora HBH	21	35	8.33 ± 5.27	cdef
S. carpocapsae TUR-S4	14	35	7.66 ± 3.71	cdefg
S. feltiae TUR-S3	21	35	7.39 ± 3.46	cdefgh
S. feltiae TUR-S3	21	15	6.99 ± 1.77	defgh
H. bacteriophora HBH	21	25	6.66 ± 6.66	defgh
S. carpocapsae TUR-S4	7	35	5.45 ± 3.63	defgh
H. bacteriophora HBH	7	35	4.00 ± 4.00	efgh
S. feltiae TUR-S3	14	15	3.35 ± 2.07	fgh
S. feltiae TUR-S3	14	35	2.22 ± 2.22	fgh
S. feltiae TUR-S3	21	25	2.14 ± 1.43	fgh
S. carpocapsae TUR-S4	14	25	0.64 ± 0.64	gh
S. feltiae TUR-S3	14	25	0.62 ± 0.62	gh
H. bacteriophora HBH	7	15	0.00 ± 0.00	h
H. bacteriophora HBH	7	25	0.00 ± 0.00	h
H. bacteriophora HBH	14	15	0.00 ± 0.00	h
H. bacteriophora HBH	14	25	0.00 ± 0.00	h
H. bacteriophora HBH	21	15	0.00 ± 0.00	h

Table 4. Continued

EPN Species	Time (day)	Temperatures(°C)	Mortality rates (%)
S. carpocapsae TUR-S4	7	15	0.00 ± 0.00 h
S. carpocapsae TUR-S4	7	25	0.00 ± 0.00 h
S. feltiae TUR-S3	7	15	0.00 ± 0.00 h
S. feltiae TUR-S3	7	25	0.00 ± 0.00 h
S. feltiae TUR-S3	7	35	0.00 ± 0.00 h
H. bacteriophora HBH (Control)	7	4	0.00 ± 0.00 h
H. bacteriophora HBH (Control)	14	4	0.00 ± 0.00 h
H. bacteriophora HBH (Control)	21	4	0.00 ± 0.00 h
S. carpocapsae TUR-S4 (Control)	7	4	0.00 ± 0.00 h
S. carpocapsae TUR-S4 (Control)	14	4	0.00 ± 0.00 h
S. carpocapsae TUR-S4 (Control)	21	4	0.00 ± 0.00 h
S. feltiae TUR-S3 (Control)	7	4	0.00 ± 0.00 h
S. feltiae TUR-S3 (Control)	14	4	0.00 ± 0.00 h
S. feltiae TUR-S3 (Control)	21	4	0.00 ± 0.00 h

Discussion

EPN are biological control agents used to control many insect pests. However, the survival of the EPN is directly related to some environmental factors such as temperature and humidity. One of the major limiting influences on the application of EPN under field conditions is temperature.

Nowadays, the effects of temperature on EPN have been investigated numerous times. These studies mostly focused on the virulence effects of EPN on different hosts at different temperatures (Nimkingrat et al., 2013; Ulu & Susurluk, 2014; Baimey et al., 2015; Yan et al., 2020). However, studies investigating the differences in the efficacy of EPN after storage at different temperatures for certain periods, as done in this study, are very few (Aryal et al., 2022). Similar to the present study is the study by Aryal et al. 2022, who reported using the EPN species isolated from Australia; *H. bacteriophora*, *H. zealandica, H. indica* and *S. felitae*. According to their results, *H. zealandica* had the highest virulence at 25 and 30°C. It is very important that EPN survive as long as possible under storage conditions and it is desirable that there is no decrease in the efficiency of the EPN that remain alive at the end of this period. In general, it is recommended that EPN be stored at low temperatures such as between 4 and 8°C (Burman & Pye, 1980; Georgis, 1992; Kurtz et al., 2007; Susurluk & Ehlers, 2008).

However, EPN can be exposed to higher temperatures, especially during transportations. This study was conducted to determine in more detail what lethal effects these undesirable conditions have on EPN and the change in the efficacy of survivors. The EPN species used in the study are commercially produced by firms. Knowing the reactions of these species in such conditions is very important both scientifically and commercially. In the study, it was detected that the mortality rate increased as the storage time of *H. bacteriophora* HBH increased. It was observed that the increase in temperature also increased this mortality rate. Similarly, Strauch et al. (2000) found that the survival rate of *H. bacteriophora* decreased at high temperatures. This result seems to be compatible with the present study.

In addition, in the present study, it was detected that there were some decreases in the virulence of the IJs that remained alive as the storage time extended. El Khoury et al. (2018) also found that increasing temperature decreased the pathogenicity of *H. bacteriophora.* Results from this study are in accordance with the present study. The results of the present study also indicate that as the storage time increased,

the mortality rate of *S. carpocapsae* TUR-S4 also increased. Temperature and length of storage time have significant effects on survival and efficacy of EPNs. Feng et al. (2006) observed an increase in mortality rate of *S. carpocapsae* as the storage period increased in different solutions. This result appears to be consistent with the findings of the present study.

Furthermore, it was found that an increase in temperature had a negative impact on the virulence of surviving IJs, more so than the storage period. Kung et al. (1991) found that an increase in temperature and longer storage period led to a decrease in the pathogenicity of *S. carpocapsae*. The findings of the present study are consistent with Kung et al. (1991). Similar to the other species used in the present study; the findings of this study indicated an increase in the mortality rate of *S. feltiae* TUR-S3 with an increase in storage time and temperature. Dunphy & Webster (1986) found that the mortality rate of *S. feltiae* increased at higher temperatures and longer storage periods. This result seems to be consistent with the present study. In *S. feltiae*, similar to the findings in other species, it was observed that an increase in temperature led to a decrease in the pathogenicity of the surviving IJs. This result seems to be compatible with Husin & Port (2021). In this study, a decrease in the virulence of *S. feltiae* was observed with increasing temperature, similar to the results of the present study.

In addition, the effect of a wider temperature range on the productivity and other biological activities of EPNs are still unclear. Therefore, further research on the relationship of EPN with temperature should be carried out. Consequently, the relationship of EPN with temperature is important to optimize their use in biological control, especially during transportation. Studies to date show that temperature has a significant impact on the efficiencies and survival of EPN (Ulu & Susurluk, 2014). How EPN species respond to possible high temperatures, especially during transportation, and the exposure time to these temperatures is very important in scientific and especially commercial terms. According to the present results, it is thought that providing new optimal conditions according to the response of EPN to temperatures on a species basis will increase the success of EPN in biological control.

Acknowledgements

This study was financially supported by the TÜBİTAK (The Scientific and Technological Research Council of Türkiye), Project number: 219O370. Moreover, we would like to express our heartfelt thanks to Assoc. Prof. Dr. Hilal SUSURLUK for providing valuable technical support.

References

Aryal, S., U. N. Nielsen, N. H. Sumaya, C. Wilson & M. Riegler, 2022. Effect of temperature on survival of Australian entomopathogenic nematodes and their virulence against the Queensland fruit fly, *Bactrocera tryoni*. BioControl, 67 (6): 617-628.

Baimey, H., L. Zadji, L. Afouda, M. Moens & W. Decraemer, 2015. Influence of pesticides, soil temperature and moisture on entomopathogenic nematodes from southern Benin and control of underground termite nest populations. Nematology, 17 (9): 1057-1069.

Baker, B. P., T. A. Green & A. J. Loker, 2020. Biological control and integrated pest management in organic and conventional systems. Biological Control, 140: 104095.

Barzman, M., P. Bàrberi, E. N. A. Birch, P. Boonekamp, S. Dachbrodt-Saaydeh, B. Graf, B. Hommel, J. E. Jensen, J. Kiss, P. Kudsk, J. R. Lamichhane, A. Messêan, A. C. Moonen, A. Ratnadass, P. Ricci, J. L. Sarah & M. Sattin, 2015. Eight principles of integrated pest management. Agronomy for Sustainable Development, 35 (4): 1199-1215.

Boemare, N., C. Laumond & H. Mauleon, 1996. The entomopathogenic nematode-bacterium complex: biology, life cycle and vertebrate safety. Biocontrol Science and Technology, 6 (3): 333-346.

Burman, M. & A. E. Pye, 1980. *Neoaplectana carpocapsae*: Movements of nematode populations on a thermal gradient. Experimental Parasitology, 49 (2): 258-265.

Dede, E., A. K. Bütüner, A. Susurluk, 2022. Biocontrol potential of *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) HBH hybrid strain against the beet webworm, *Loxostege sticticalis* L., 1761 (Lepidoptera: Pyralidae). Turkish Journal of Entomology, 46 (4): 399-405.

Dunphy, G. B. & J. M. Webster, 1986. Temperature effects on the growth and virulence of *Steinernema feltiae* strains and *Heterorhabditis heliothidis*. Journal of Nematology, 18 (2): 270-272.

Ehlers, R. U., 1996. Current and future use of nematodes in biocontrol: practice and commercial aspects with regard to regulatory policy issues. Biocontrol Science and Technology, 6 (3): 303-316.

Ehlers, R. U., 2001. Mass production of entomopathogenic nematodes for plant protection. Applied Microbiology and Biotechnology, 56 (5-6): 623-633.

El Khoury, Y., M. Oreste, E. Noujeim, N. Nemer & E. Tarasco, 2018. Effect of temperature on the pathogenicity of Mediterranean native entomopathogenic nematodes (Steinernematidae and Heterorhabditidae) from natural ecosystems. Redia, 101 (CI): 123-127.

Ewence, A., S. Brescia, I. Johnson & P. C. Rumsby, 2015. An approach to the identification and regulation of endocrine disrupting pesticides. Food and Chemical Toxicology, 78: 214-220.

Feng, S. P., R. C. Han, X. H. Qıu, L. I. Cao, J. H. Chen & G. H. Wang, 2006. Storage of osmotically treated entomopathogenic nematode *Steinernema carpocapsae*. Insect Science, 13 (4): 263-269.

Georgis, R., 1992. Present and future prospects for entomopathogenic nematode products. Biocontrol Science and Technology, 2 (2): 83-99.

Glazer, I., 1996. Survival mechanisms of entomopathogenic nematodes. Biocontrol Science and Technology, 6 (3): 373-378.

Gunnell, D., D. Knipe, S. S. Chang, M. Pearson, F. Konradsen, W. J. Lee & M. Eddleston, 2017. Prevention of suicide with regulations aimed at restricting access to highly hazardous pesticides: a systematic review of the international evidence. The Lancet Global Health, 5 (10): e1026-e1037.

Husin, T. O. B. & G. R. Port, 2021. Efficacy of entomopathogenic nematodes against *Tuta absoluta*. Biological Control, 160: 104699.

Iyaniwura, T. T., 1991. Non-target and environmental hazards of pesticides. Reviews on Environmental Health, 9 (3): 161-176.

Kaya, H. K. & P. Stock, 1997. “Techniques in Insect Nematology, 281-324”. In: Manual of Techniques in Insect Pathology (Ed. A. Lawrence), Academic Press, 409 pp.

Kung, S. P., R. Gaugler & H. K. Kaya, 1991. Effects of soil temperature, moisture, and relative humidity on entomopathogenic nematode persistence. Journal of Invertebrate Pathology, 57 (2): 242-249.

Kurtz, B., S. Toepfer, R. U. Ehlers & U. Kuhlmann, 2007. Assessment of establishment and persistence of entomopathogenic nematodes for biological control of western corn rootworm. Journal of Applied Entomology, 131 (6): 420-425.

Lu, Y., K. Wu, Y. Jiang, Y. Guo & N. Desneux, 2012. Widespread adoption of Bt cotton and insecticide decrease promotes biocontrol services. Nature, 487 (7407): 362-365.

Nimkingrat, P., S. Khanam, O. Strauch & R. U. Ehlers, 2013. Hybridisation and selective breeding for improvement of low temperature activity of the entomopathogenic nematode *Steinernema feltiae*. BioControl, 58 (3): 417-426.

Özdemir, E., E. Inak, E. Evlice, E. Yüksel, R. A. Delialioğlu & I. A. Susurluk, 2021. Effects of insecticides and synergistic chemicals on the efficacy of the entomopathogenic nematode *Steinernema feltiae* (Rhabditida: Steinernematidae) against *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Crop Protection, 144: 105605.

Rani, L., K. Thapa, N. Kanojia, N. Sharma, S. Singh, A. S. Grewal, A. L. Srivastav & J. Kaushal, 2021. An extensive review on the consequences of chemical pesticides on human health and environment. Journal of Cleaner Production, 283: 124657.

Robin, D. C. & P. A. Marchand, 2019. Evolution of the biocontrol active substances in the framework of the European Pesticide Regulation (EC) No. 1107/2009. Pest Management Science, 75 (4): 950-958.

Sánchez-Bayo, F., 2012. Insecticides mode of action in relation to their toxicity to non-target organisms. Journal of Environmental and Analytical Toxicology, S4: S4-002.

Shapiro-Ilan, D. I., D. H. Gouge, S. J. Piggott & J. P. Fife, 2006. Application technology and environmental considerations for use of entomopathogenic nematodes in biological control. Biological Control, 38 (1): 124-133.

Smits, P. H., 1996. Post-application persistence of entomopathogenic nematodes. Biocontrol Science and Technology, 6 (3): 379-388.

Stern, V. M. R. F., R. Smith, R. van den Bosch & K. Hagen, 1959. The integration of chemical and biological control of the spotted alfalfa aphid: the integrated control concept. Hilgardia, 29 (2): 81-101.

Strauch, O., I. Niemann, A. Neumann, A. J. Schmidt, A. Peters & R. U. Ehlers, 2000. Storage and formulation of the entomopathogenic nematodes *Heterorhabditis* indica and *H. bacteriophora*. BioControl, 45 (4): 483-500.

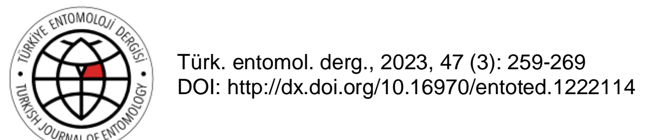
Susurluk, A., R. U. Ehlers, 2008. Field persistence of the entomopathogenic nematode *Heterorhabditis bacteriophora* in different crops. BioControl, 53 (4): 627-641.

Susurluk, I. A. & T.C. Ulu, 2015. Virulence comparisons of high-temperature-adapted *Heterorhabditis bacteriophora*, *Steinernema feltiae* and *S. carpocapsae.* Helminthologia, 52 (2): 118-122.

Ulu, T. C. & I. A. Susurluk, 2014. Heat and desiccation tolerances of *Heterorhabditis bacteriophora* strains and relationships between their tolerances and some bioecological characteristics. Invertebrate Survival Journal, 11 (1): 4-10.

Waage, J. K. & D. J. Greathead, 1988. Biological control: challenges and opportunities. Philosophical Transactions of the Royal Society of London. B, Biological Sciences, 318 (1189): 111-128.

Yan, X., M. Shahid Arain, Y. Lin, X. Gu, L. Zhang, J. Li & R. Han, 2020. Efficacy of entomopathogenic nematodes against the tobacco cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae). Journal of Economic Entomology, 113 (1): 64-72.


Türk. entomol. derg., 2023, 47 (3): 259-269
DOI: http://dx.doi.org/10.16970/entoted.1222114

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Pathogenicity of some entomopathogenic fungi on wheat weevil *Sitophilus granarius* (L., 1758) (Coleoptera: Curculionidae)

Buğday biti *Sitophilus granarius* (L., 1758) (Coleoptera: Curculionidae) üzerinde bazı entomopatojen fungusların patojenisitesi

Mahmut Alper ALTINOK[1*] **Memiş KESDEK[2]** **Şaban KORDALİ[3]**
Ayşe USANMAZ BOZHÜYÜK[4] **Hacer Handan ALTINOK[2]**

Abstract

In the present study, the toxicities of seven entomopathogenic fungal isolates, *Beauveria bassiana* (ARSEF-4984), *Isaria farinosa (*ARSEF-3580), *Isaria fumosorosea (*ARSEF-4501), *Lecanicillium muscarium* (ARSEF-5128), *L. muscarium (*ARSEF-972), *Lecanicillium lecanii* (TR38/11) and *L. muscarium* (Ve6), were tested against the adults of granary weevil, *Sitophilus granarius* (L., 1758) (Coleoptera: Curculionidae), under laboratory conditions (25±1°C, 75±5% RH and 14h light:10h dark). Studies were conducted in Atatürk University (Erzurum, Türkiye), in 2018. Fungal isolates were sprayed to adults at two different conidial concentrations (1×10^5 and 1×10^7 ml^{-1}). Mortality percentages were observed on the 2nd, 4th, 6th, 8th, and 10th days of treatment. A commercial isolate of *L. muscarium* were used as positive control and sterile water+0.25% Tween 20 used as negative control. The results demonstrated that the mortality rates of *S. granarius* adults treated with entomopathogenic fungi ranged from 1.01% to 98.9% across 10-day period. Higher concentration and longer exposure periods resulted in increasing virulence on the adult individuals. Among the strains tested, at 1×10^7 ml^{-1} concentration, *I. fumosorosea*, *L. muscarium* (ARFES-5128) and *L. lecanii* isolates displayed 97.85%, 94.62% and 93.58% cumulative mortalities respectively, on *S. granarius* adults by the 10th day of the experiment. These three isolates are regarded as highly promising biological control agents.

Keywords: Entomopathogen, fungi, stored grain

Öz

Bu çalışmada, yedi entomopatojen fungus izolatı *Beauveria bassiana*, *Isaria farinosa (*ARSEF-3580), *Isaria fumosorosea (*ARSEF-4501), *Lecanicillium muscarium* (ARSEF-5128), *L. muscarium (*ARSEF-972), *Lecanicillium lecanii* (TR38/11) ve ticari bir *L. muscarium* (Ve6), buğday biti, *Sitophilus granarius* (L., 1758) (Coleoptera: Curculionidae) erginlerine karşı laboratuvar şartlarında (25±1°C, 75±5% nisbi nem ve 14sa aydınlık:10sa karanlık) denenmiştir. Çalışma, Atatürk Üniversitesi'nde (Erzurum, Türkiye) 2018 yılında gerçekleştirilmiştir. Fungal izolatlar ergin bireylere iki farklı spor konsantrasyonunda ($1x10^5$ ve 1×10^7 ml^{-1}) spreylenmiştir. Yüzde ölümler uygulamadan sonraki 2., 4., 6., 8. ve 10. günlerde kaydedilmiştir. Pozitif kontrol olarak ticari bir *L. muscarium* izolatı ve negatif kontrol olarak da steril su+Tween20 (0,25%) kullanılmıştır. Sonuçlar, entomopatojen fungus ile uygulanan *S. granarius* erginlerinde, uygulamadan sonraki 10 gün süresince ölüm oranlarının %1,01 ile %98,9 arasında değiştiğini göstermiştir. Yüksek konsantrasyon ve daha uzun maruz kalma süreleri ergin bireylerde artan patojenisite ile sonuçlanmıştır. Uygulanan suşlar arasında, 1×10^7 ml^{-1} konsantrasyonda *I. fumosorosea*, *L. muscarium* (ARFES-5128) ve *L. lecanii* izolatları, denemenin 10. gününde *S. granarius* erginlerinde sırasıyla %97,85%, %94,62 ve %93,58 kümülatif ölüm oranlarına ulaşmıştır. Bu üç izolatın biyolojik mücadele kapsamında yüksek derecede ümitvar oldukları değerlendirilmiştir.

Anahtar sözcükler: Entomopatojen, fungus, depolanmış ürün

[1] Erciyes University, Faculty of Agriculture, Department of Plant Protection, 38039, Kayseri, Türkiye
[2] Muğla Sıtkı Koçman University, Faculty of Fethiye Agriculture, Department of Plant Production, Cumhuriyet Street, 48300 Karaçulha, Fethiye, Muğla, Türkiye
[3] Muğla Sıtkı Koçman University, Faculty of Fethiye Agriculture, Department of Plant Protection, 48000, Fethiye, Muğla, Türkiye
[4] Iğdır University, Vocational School of Technical Sciences, Department of Chemistry and Chemical Processing Technologies, 76100, Iğdır, Türkiye
* Corresponding author (Sorumlu yazar) e-mail: altinoka@erciyes.edu.tr
Received (Alınış): 20.12.2022 Accepted (Kabul ediliş): 25.09.2023 Published Online (Çevrimiçi Yayın Tarihi): 04.10.2023

Introduction

Pest infestations of stored grains may result in severe losses in quality, including loss of vigor of seeds, changes in natural color, providing habitat for insects and mites, toxic compounds from microbial pathogens and excretion and remainders of insects. There are about 130 species that were recorded, and majority of storage pests are from the Coleoptera and Lepidoptera families (Proctor, 1994; Hagstrum & Subramanyam, 2009; Morales-Quiros, 2019). Among these, *Sitophilus granarius* (L., 1758) (Coleoptera: Curculionidae), also called “wheat weevil, granary weevil or grain weevil”, is one of the most common pests of stored grains including wheat, barley, rye, corn, and rice worldwide. Female deposits about 50-400 eggs into cavities on grains and both adult and larvae feed on germ and endosperm of grains. Combining long term storage of grains with relatively short (30-60 days) development duration of the pest (Kirkpatrick & Wilbur, 1965; Campbell et al., 1976; Sinha & Sinha, 1991) and ability to avoid the grains contain their offspring (Danho et al., 2002; Woodbury, 2008), helps to explain the success of the pest.

This pest leads to large economic losses of stored wheat grains after harvesting in the tropical and subtropical regions of the world (Hare, 1980; Isman, 2006). The losses may vary from 10 to 40 percent, particularly in primitive grain stores (Saroukolai et al., 2000). Loss of grain weight and nutritional value are among quantitative and qualitative losses caused by this insect (Moina et al., 1998). Many chemical insecticides and fumigants have been tested to prevent the damage of this pest for years. Chemical insecticides generally provide an advantage as the rapid and efficient control method for *S. granarius* (Emsen et al., 2015; Kordalı et al., 2017). These chemicals present some serious risks such as environmental pollution because of their slow biodegradation, toxic effects on non-target organisms, food safety, pest resistance, direct toxicity to users, and cause depletion of ozone layer (Arthur, 1996; Isman, 2006; Santos et al., 2009; Kesdek et al., 2013; Kücükaydin et al., 2020).

Synthetic pesticide consumption is another disadvantage, considering the high cost-benefit ratio (Roy & Dreja, 1998). Therefore, authorities are hesitant to allow chemical insecticides on stored grains, primarily due to their residues (Thaung & Collins, 1986). Due to negative effects of synthetic insecticides, alternative control strategies are needed under storage conditions and one of the effective and environmentally friendly methods for control of storage pests is utilizing microbial biocontrol.

Entomopathogenic microorganisms generally belong to the groups of fungi, bacteria, protozoan, and viruses. They can cause diseases in infected arthropods, leading to a rapid decline in host arthropod populations. Entomopathogenic fungi are primary microorganisms used against harmful arthropods on culture plants since they are very effective on a wide range of insects, and development of resistance risk is low. They are also safe for non-target organisms and environment, and can adapt to various environmental conditions (Hajek & St. Leger, 1994; Lacey & Goettel, 1995; Inglis et al., 2001; Roy et al., 2001; Zimmermann, 2007). Their role is crucial in biological control of hazardous insects. They have gained the most interest in research for utilizing microbial pesticides. There are more than 100 genera and 700 species containing insect pathogens. They display low mammalian toxicity, being relatively safe for the environment (Roberts & Humber, 1981; Charnley, 1989; Cox & Wilkin, 1996). The common species of *Metarhizium, Beauveria*, *Isaria* and *Lecanicillium* and *Isaria* are quite amenable for mass production. At least 10 entomopathogenic fungi species have been widely used for biocontrol purposes (Hajek & St. Leger, 1994). The use of entomopathogenic fungi in the biological control against pests is an attractive alternative to the use of conventional pesticides, because these fungi are very strong control agents against a wide range of arthropod pests (Khetan, 2001; Sevim et al., 2013).

Entomopathogenic fungi enter the insect cuticle through both physical and enzymatic mechanisms (Erkılıç & Uygun, 1993). The process involves settling of fungus spores on the insect cuticle, followed by their germination and penetration into the cuticle by forming an appressorium (Clarkson & Chamley, 1996; Sevim et al., 2015). In most instances, insect mortality results from the presence of toxic substances rather than mycosis (Charnley, 2003).

There are many studies with the use of entomopathogenic fungi for control of stored product pests (Ferroni, 1977; Domsch et al., 1980; Serale & Doberski, 1984; Hidalgo et al., 1998; Padin et al., 2002; Cherry et al., 2005; Tunaz & Er, 2008; Barra et al., 2013; Emsen et al., 2015; Batta & Kavallieratos, 2017; Komaki et al., 2017; Usanmaz Bozhüyük et al., 2018; Kordali et al., 2021). Several strains were successfully tested on stored grains including maize (Barra et al., 2013), wheat (Batta & Kavallieratos, 2017), rice (Rice & Cogburn, 1999), sorghum (Ekesi et al., 2000), oat (Throne & Lord, 2004) and barley (Aregger, 1992).

The objective of the current investigation was to assess the effectiveness of seven entomopathogenic fungal isolates, namely *Beauveria bassiana* (Bals.-Criv.) Vuill. (ARSEF-4984), *Lecanicillium muscarium* Zare & Gams (ARSEF-972), *Lecanicillium lecanii* Zare & Gams (TR38/11), *Lecanicillium muscarium* (ARSEF-5128), *I. fumosorosea* Wize (ARSEF-4501), *Isaria farinosa* (Holmsk.) Fr. (ARSEF-3580), and *L. muscarium* Ve6 obtained from Mycotal®, for the purpose of controlling adult *S. granarius* in laboratory settings.

Materials and Methods

Insects

The insects tested in this study were collected from the private storage houses and flour mills in Fethiye (Muğla). Species identifications were conducted by Dr. Erol Yıldırım (Atatürk University, Erzurum, Turkey). Wheat grains (common wheat, *Triticum aestivum* L.) were purchased locally. They were stored in a freezer at −20°C to prevent deterioration and were inspected for two days to check for the presence of any arthropod pests prior to use for bioassays. Then, the grains were washed in tap water, allowed to dry, and heated to prevent pre-infestations that may occur prior to the experiments. *S. granarius* individuals were reared to adult stage in laboratory, at 25 ± 1°C, 75±5 relative humidity and L:D=14 h:10 h in the Department of Plant Protection, Atatürk University, Erzurum-Turkey. The adults obtained from rearing cultures were kept in cages containing enough amount of wheat grains. All experiments were implemented under identical conditions.

Fungal entomopathogens

A total of seven entomopathogenic fungi isolates were used in the study. Five of these were obtained from ARSEF collection (USA), *Beauveria bassiana (*4984), *Isaria farinosa (*3580), *I. fumosorosea (*4501), *Lecanicillium muscarium (*5128), *L. muscarium (*972). One isolate was a soil extraction from Kayseri province (*L. lecanii*; TR38/11). One additional isolate (Ve6; *L. muscarium* Ve6) was extracted from a commercial product (Mycotal, Koppert, NL) and used as positive control. Fungal isolates were allowed to grow on potato dextrose agar (PDA, Oxoid, CM0139) medium at 25°C for two weeks, before the spray treatments to *S. granarius* adults. Conidia were harvested from 14-day-old fungi cultures by gently scraping the surface of fungal growth uisng a sterilized glass micro spatula and mixed thoroughly in 3 ml distilled sterile water containing a surfactant, 0.25% (v/v) Tween 20, in screw capped bottles. Conidia viability of EPFs were tested with the following method: Conidia suspension of each isolate was spread onto water agar (15%) plates then incubated at 28°C in the dark for 18 h. The percentage of germinated conidia was examined by examining 50 conidia per plate. Germination capacity was defined as a conidium having a germ tube equal to the length of at least half the width of the conidium (Wyss et al., 2001). The suspensions were then mixed with tween 20 (0.25% v/v) and adjusted to two different (1×10^5 and 1×10^7 conidia ml^{-1}) concentrations by using a haemocytometer, under a light microscope, for subsequent use in the experiments.

Experiment design and application of entomopathogenic fungi

To test the pathogenicity of entomopathogenic fungi isolates, sterile plastic Petri dishes lined with sterile filter paper were used. In each Petri dish, 11 newly emerged of *S. granarius* adults (mixed males and females) were collected with an aspirator and fed with wheat grains (30 wheat grains/dish). The suspensions were sieved, adjusted to two different spore concentrations and 1 ml sprayed on each replicate

containing the insects, wheat grains and filter paper in Petri dishes, under a laminar flow hood and kept there until excess water evaporates. Then Petri dishes were incubated in 25±1°C with 75±5% RH, in 16:8 (L:D) photoperiod. Each assay was repeated three times for each of the doses and exposure time combination. Isolate extraction of *L. muscarium* was used as positive control and distilled sterile water containing 0.25% (v/v) Tween 20 was used as negative control in the study. The experiment was set up as completely randomized design with three replicates, each consisting of three Petri dishes with 11 *S. granarius* adults per Petri dish, as explained above. Alive and dead *S. granarius* adult individuals were counted every 48 h for 10 days, following the treatments. All the experiments were repeated at least twice.

Statistical analyses

Due to the initial uniform population structure, mortality rates from treatments were corrected according to Schneider-Orelli's formula (Puntener, 1981). Differences in virulence between the tested entomopathogenic fungi isolates were determined by analysis of variance using the SAS JMP v9.0 software package. Tukey's HSD test was used for comparison of means for each day and each isolate across the experiment, at $p \leq 0.05$ significance level. To prevent bias, angular transform applied to control-corrected mortality percentages, prior to statistical analyses.

Results

The isolates were tested against *S. granarius* at two different spore concentrations (1×10^5 and 1×10^7 conidia/ml) and results were corrected with control. Statistical tests were performed on control-corrected and transformed mean mortality values from each spore concentration level, individually. In lower concentration ($1\text{x}10^5$), means from the eight treatments (including positive control) were found statistically significant for each of the measurement days (10 days after treatment (DAT), $F_{(6,14)} = 8.16$, $p = 0.0006$) and separated to four to five mean groups in each measurement day, across the experiment, by Tukey's HSD test (Table 1). Best performing isolates were TR38/11 and (+) Control for most of the days, in lower spore concentration tests. In this experiment, over 75% control-corrected mortality rates were first achieved by (+) Control and TR38/11 isolates, on the 8th DAT. Highest mortality rates achieved on the 10th day by again the (+) Control isolate (98.9%), followed by TR38/11 (86.0%), 5128 (71.0%) and 4501 (64.5%) isolates (Figure 1). While the TR38/11 isolate scored a 37.5% mortality which was the highest for the beginning of the experiment, there were no statistical differences with (+) Control isolate (Table 1) on the 2nd DAT. The numbers were equalized around 50% about the 6th DAT and (+) Control performed better in the remaining days. The lowest mortality recorded by 3580 isolates (31.2%) on the10th DAT. The performance of 4501 and 4984 isolates were statistically on par, across the experiment (Table 1).

At a lower spore concentration of $1\text{x}10^5$, statistical significance was found for each of the measurement days (10 DAT, $F\ (6,14) = 8.16$, $p = 0.0006$) among the eight treatments (including the positive control). Tukey's HSD test separated these treatments into four to five mean groups for each measurement day throughout the experiment (Table 1).

The TR38/11 and (+) Control isolates performed best for most of the days in the lower spore concentration experiment, with over 75% control-corrected mortality rates first achieved by these isolates on the 8th DAT. The (+) Control isolate achieved the highest mortality rates on the 10th day (98.9%), followed by TR38/11 (86.0%), 5128 (71.0%), and 4501 (64.5%) isolates (Figure 1). While the TR38/11 isolate had the highest mortality rate at the beginning of the experiment (37.5%), there was no statistical difference with the (+) Control isolate on the 2nd DAT (Table 1). The mortality rates were centered around 50% on the 6th DAT, and the (+) Control isolate performed better in the remaining days. The 3580 isolate had the lowest mortality rate (31.2%) on the 10th DAT. The performance of the 4501 and 4984 isolates were statistically similar throughout the experiment (Table 1).

Table 1. Mean number of cumulative mortalities (±SE) in adults of *Sitophilus granarius* (Coleoptera: Curculionidae) 2-10 days after treatment with spore suspensions of seven entomopathogenic fungi (EPF) isolates adjusted to ($1x10^5$) spore concentration. Each treatment started with a total of 33 adult individuals

EPFs*	Days after treatments				
	2	4	6	8	10
TR38/11	13.0±1.0 aB**	15.7±2.3 aB	17.7±1.5 abB	22.0±1.0 abAB	28.7±3.8 abA
3580	0.0±0.0 dB	7.0±1.0 bcB	13.0±2.0 bcAB	18.0±2.7 bcAB	24.7±0.6 abcA
4501	4.3±3.2 cC	8.0±3.6 bcBC	15.7±4.0 bAB	18.7±2.1 bcA	22.0±2.0 bcA
4984	3.3±3.2 cD	5.3±3.2 cC	7.0±2.7 cBC	13.3±2.1 cAB	18.3±2.3 cA
5128	5.7±0.6 bcE	9.3±2.3 abcD	18.7±1.2 abC	23.0±2.0 abB	31.3±1.5 aA
972	7.7±2.1 abcC	12.3±2.5 abC	15.7±2.1 abB	18.0±1.7 bcB	22.0±1.0 bcA
(+) Control	10.7±0.6 abE	16.0±1.7 aD	22.0±1.7 aC	26.3±1.5 aB	30.3±0.6 abA
(-) Control	0.7±0.6	1.0±0.0	1.0±0.0	1.0±0.0	1.3±0.6

* ARSEF isolates are 3580 (*Isaria farinosa*), 4501 (*Isaria fumosorosea*), 4984 (*Beauveria bassiana*), 5128 (*Lecanicillium muscarium*), 972 (*Lecanicillium muscarium*). TR38/11 is a local isolate (*Lecanicillium lecanii*) extracted from Kayseri province soils (Turkey), and (+) control is *Lecanicillium muscarium* Ve6 isolate obtained from a commercial product (Mycotal; Koppert, NL).

** Mean mortality numbers followed by same lowercase letter (s) in each column, or same uppercase letter (s) in each row are statistically not different (Tukey's HSD, P≤0.05)

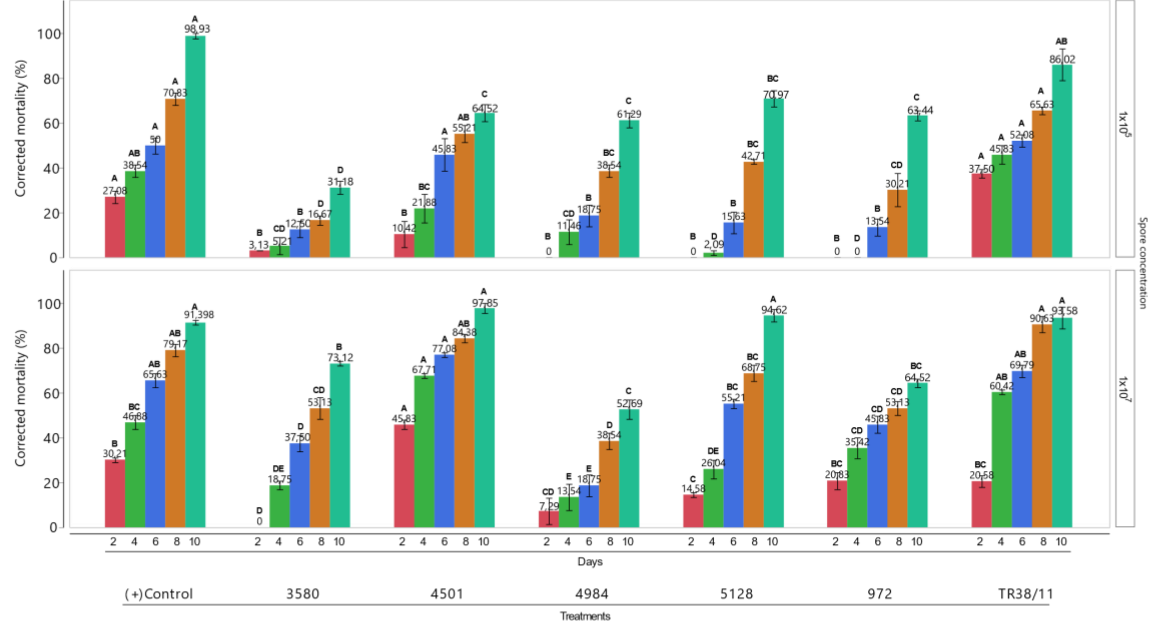


Figure 1. Cumulative mortality rates of seven entomopathogenic isolates (TR38/11: *Lecanicillium lecanii*; 3580: *Isaria farinosa*; 4501: *Isaria fumosorosea*; 4984: *Beauveria bassiana*; 5128: *Lecanicillium muscarium*; 972: *Lecanicillium muscarium*; (+) Control: commercial *Lecanicillium muscarium* Ve6) on *Sitophilus granarius* adults, 2-10 days after treatment (DAT). Different capital letters on same days after treatment (DAT) with each isolate indicates a statistically significant difference for the given DAT, among the isolates (Tukey's HSD, P≤0.05; Bars indicate standard error).

Same statistical tests were also performed for higher concentration ($1x10^7$) and results are presented in Table 2. Similarly, the difference between treatments at each of the measurement days was found statistically significant (10 DAT, $F_{(6,14)} = 28.62$, $p < 0.0001$). Contrary to lower concentration, the number of statistical groups among mean mortality numbers of isolates remained low, typically three to four mean groups formed in many of each measurement days, as seen in Table 2. There was an overall increase in mortalities in the higher concentration, as expected (Figure 1). Three treatments (TR38/11, 4501 and (+) Control) consistently outperformed the others, across the experiment. Mortalities from these three isolates were over 75%, by 8th DAT. Highest mortalities were again obtained by 10th DAT as seen in Figure 1, and isolate 4501 performed best with 97.85%, followed by 5128 (94.63%), TR38/11 (93.58) and (+) Control (91.40%).

Table 2. Mean number of cumulative mortalities (±SE) in adults of *Sitophilus granarius* (Coleoptera: Curculionidae) 2-10 days after treatment with spore suspensions of seven entomopathogenic fungi (EPF) isolates adjusted to ($1x10^7$) spore concentration. Each treatment started with a total of 33 adult individuals

EPFs*	Days after treatments				
	2	4	6	8	10
TR38/11	7.3±1.2 bD**	20.3±0.6 abC	23.3±1.5 abBC	30.0±2.0 aAB	31.0±2.7 aA
3580	2.0±0.0 cE	2.3±2.6 cD	5.0±2.0 cdC	6.3±1.2 dB	11.7±1.5 cA
4501	15.7±1.2 aD	22.7±0.6 aC	25.7±0.6 aBC	28.0±1.0 abB	32.3±1.2 aA
4984	0.3±0.6 dC	4.7±3.1 cBC	7.0±2.7 cBC	13.3±1.5 cAB	21.0±1.7 bcA
5128	0.0±0.0 dC	1.7±0.6 cC	6.0±2.7 cB	14.7±0.6 cB	24.0±2.0 bA
972	0.0±0.0 dD	1.0±0.0 cCD	5.3±2.1 cBC	10.7±4.0 cdAB	21.7±1.2 bA
(+) Control	9.7±1.6 bD	13.3±1.5 bCD	17.0±2.0 bC	23.7±1.5 bB	32.7±0.6 aA
(-) Control	0.7±0.6	1.0±0.0	1.0±0.0	1.0±0.0	1.3±0.6

* ARSEF isolates are 3580 (*Beauveria bassiana*), 4501 (*Isaria fumosorosea*), 4984 (*Beauveria bassiana*), 5128 (*Lecanicillium muscarium*), and 972 (*Lecanicillium muscarium*). TR38/11 is a local isolate (*Lecanicillium lecanii*) extracted from Kayseri province soils (Turkey), and (+) control is *Lecanicillium muscarium* (Ve6) obtained from a commercial product (Mycotal; Koppert, NL).

** Mean mortality numbers followed by same lowercase letter (s) in each column, or same uppercase letter (s) in each row are statistically not different (Tukey's HSD, P≤0.05).

Mean mortality by the isolates were initially compared for each of 2, 4, 6, 8 and 10th days after treatment (DAT) with Tukey's HSD, to find out if there are higher performing isolates in earlier days and whether their mortality efficiency persists throughout the experiment.

The last day of the experiment (10 DAT) was the one with highest mortality for all isolates and well separated from the other days for all isolates, with exception of isolate 4501 in 10^5 concentration tests. Comparing both concentration levels, none of the isolates drew a similar differentiation pattern across days, and mortalities from the same isolate were separated differently for each concentration, until the last day of the experiments.

Interactions between time and treatments were also statistically tested. In the individual tests for each of the concentrations, result for 10^5 spore concentration was, $F_{(34,70)} = 30.90$, $p < 0.0001$. The result for 10^7 concentration was, $F_{(34,70)} = 72.65$, $p < 0.0001$. These statistical tests based on control-corrected and post-transformed data indicated that the effect of time on the mortality of treatments were significant.

Discussion

In this study, the mortality rates of *S. granarius* adults were detected for seven isolates of five different entomopathogenic fungi species (*B. bassiana*, *L. lecanii*, *I. fumosorosea*, *I. farinosa*, *L. muscarium* (two isolates) and an isolate extract of commercial *L. muscarium* (as positive control)) across 10 days after the treatment. The pathogenicity tests were performed by spraying *S. granarius* adults at two different conidial concentrations (1×10^5 and 1×10^7) and constant laboratory conditions (at 25±1°C, 75±5% RH, 14h light:10h dark). The mortality rates of *S. granarius* adults were high, ranging from 52.7 to 97.9% among these entomopathogenic fungi isolates by the 10th day after treatments. Specifically, the adult mortalities for the same day at 1×10^5 conidial concentration were minimum 31.2% for *I. farinosa* (3580) and maximum 98.9% for *L. muscarium* ((+) Control). The adult mortalities at 1×10^7 conidial concentration was minimum 52.7% for *B. bassiana* (4984), and 97.9% for *I. fumosorosea* (4501). While the maximums of both concentrations appear equally pathogenic, considering overall increase in mortality rates in higher concentration, and having more than one isolate reaching over 90% mortality, results showed advantages of increased primary inoculum, as indicated in several other studies (Hidalgo et al., 1998; Dembilio et al., 2010).

Against *S. granarius*, many different entomopathogenic strains have been tested for pathogenicity by various researchers so far and majority of them have focused on *B. bassiana* strains, either alone, or

combined with some synergistic compounds, like diatomaceous earth formulations or traditional chemicals (Dal Bello et al., 2000; Athanassiou & Steenberg, 2007) with varying degrees of success. Mantzoukas et al. (2019) tested several strains of *B. bassiana* with $1x10^8$ concentration and mean mortality ranged between 3.3% and 100% among the isolates, as of the 14th day after treatments. In the same study, *I. fumosorosea* isolate reached 36.6% mortality for the same day. Kavallieratos et al. (2014), reported a 100% mortality with $2.11x10^7$ spore concentration of a *B. bassiana* isolate applied to *Sitophilus oryzae* (L., 1763) (Coleoptera: Curculionidae) adults, without food, 7 days after treatments. The *B. bassiana* isolate in our study performed poorly among others and reached only 56.7% mortality by the 10th day, under similar environmental and application conditions. The highest mortality rates achieved from the *I. fumosorosea* and two *Lecanicillium* isolates were over 90% by the 10th DAT in the present study. On the contrary, Ak (2019) reports that under 25°C and $1×10^8$ spore concentration, while *S. granarius* mortality sourced from *I. fumosorosea* isolate was 84.2%, *B. bassiana* and *L. muscarium* isolates were lower, 56.1% and 22.8%, respectively, by the seventh day after treatments. The same study also states highest mortality, with a *M. anisopliae* isolate.

Barra et al. (2013) recorded a 10% mortality on *Sitophilus zeamais* (Motschulsky, 1855) (Coleoptera: Curculionidae) (a pest of stored maize), by treatments of *Purpureocillium lilacinum* (Thom) Luangsa-Ard, Houbraken, Hywel-Jones & Samson, while 90% mortality was recorded with same isolate on another storage pest, *Tribolium confusum* Jacquelin du Val, 1863 (Coleoptera: Tenebrionidae).

Many studies on the mortality effects of entomopathogenic fungi against *S. granarius* adults were carried out by various researchers worldwide. Among these, *B. bassiana* is considered as one of the most successful entomopathogens. The use of *Beauveria bassiana* to control stored grain pests was studied by some researchers. Rice & Cogburn (1999) demonstrated that *B. bassiana* isolate (22292A) prevented mortalities of the three stored grain pests (*Sitophilus oryzae (*L.) (Coleoptera: Curculionidae), *Rhyzopertha dominica (*Fabricius, 1792) (Coleoptera: Bostrichidae) and *Tribolium castaneum (*Herbst, 1797) (Coleoptera: Tenebrionidae) adults ranging from 80~~%~~ to 100% on day 21 after treatment. Cherry et al. (2005) recorded that *B. bassiana* 0362 isolate caused mortality from 35.56~~%~~ to 100% against *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Chrysomelidae)) adults on the 6th day of treatment. Padin et al. (2002) studied the pesticidal effects of *B. bassiana* on *S. oryzae, T. castaneum* and *Acanthoscelides obtectus (*Say, 1831) (Coleoptera: Chrysomelidae) and adults for a long period. In their study, *B. bassiana* isolate caused significant mortality in *S. oryzae* adults but did not lead to significant mortality in *T. castaneum* and *A. obtectus* adults, after four months. Khashaveh et al. (2011) recorded up to 88.33% mortality effect of *B. bassiana* for *S. granarius* adults. Komaki et al. (2017) investigated that *B. bassiana* isolate resulted in mortalities between 69.3% and 100% at $1x10^5$ and $1x10^7$ doses, on the 2nd, 4th, 6th, 8th and 10th days of treatment on *T. confusum* adults. In our study, *B. bassiana (*4984) caused varying mortality rates at $1x10^5$ (ranging between 10.1-55.5% across the experiment) and $1x10^7$ (1.01- 63.6%) doses on *S. granarius* adults (Table 1).

Yang et al. (2009) stated that *I. farinosa* had mortality effect up to 88% on the *Pissodes punctatus* (Langor & Zhang in Langor, Situ & Zhang, 1999) (Coleoptera: Curculionidae) larvae, pupae, and adults. In another work, *I. farinosa* isolate caused very high mortalities in ovisacs, second larval stage, adult females, and first larval stage of *Planococcus citri* (Risso, 1813) (Hemiptera: Pseudococcidae) at 95% RH and at $1x10^8$ conidia ml^{-1} inoculum concentration, 89.39%, 84.07%, 84.53% and 78.71%, respectively (Demirci et al., 2011). Cabanillas & Jones (2013) indicated that *I. poprawskii* is pathogenic to the immature and adults of glassy-winged sharpshooter (*Homalodisca vitripennis* (Germar, 1821) (Hemiptera: Cicadellidae)). Also, in that study, pathogenicity, and virulence of *I. poprawskii* against *H. vitripennis* pointed out that this fungus could be a promising biocontrol agent to control this pest. It was specified that *Isaria fumosorosea* had a mortality effect on *S. oryzae* adults (Kavallieratos et al., 2014). Komaki et al. (2017) demonstrated that two *Isaria* fungi isolates (*Isaria fumosorosea (*ARSEF-4501 and *Isaria farinosa (*ARSEF-3580)) had mortality rates from 37.3 to 72.0% against *T. confusum* adults at $1×10^5$ and $1×10^7$ doses on the 10th day of treatment.

In the present study, *I. fumosorosea (*ISFUM-4501) and *I. farinosa (*IFA-3580) fungi isolates caused mortalities from 6.06 to 97.9% against *S. granarius* adults at 1×10^5 and 1×10^7 doses after 10 days of treatment. These two studies support each other. It is reported that the isolate of *Paecilomyces* sp. led to between 4.29% and 26.32% mortality against *S. zeamais* adults (Kassa et al., 2002). The same researchers demonstrated *Prostephanus truncatus* (Horn, 1878) (Coleoptera: Bostrichidae) to be more susceptible to the tested entomopathogenic fungi than *S. zeamais*. In this study, *L. lecanii (*TR38/11) fungus isolate caused the mortalities in the $1\text{x}10^5$ dose (from 39.3% to 86.8%) and $1\text{x}10^7$ dose (from 22.2% to 93.9%) on *S. granarius* adults between the 2nd and 10th of treatment (Table 1; Figure 1).

Although these differences may be connected to variances in the methods used, there may be a degree of variation in the virulence of different isolates which might be a contributing factor (Zettler, 1991). According to the findings of the study, all isolates acted better at higher concentration (except *I. farinosa (*IFA-3580) isolate) 10 days after treatment by resulting in mortality of over 60%. The tendency of increase observed in mortalities (except for *B. bassiana (*ARSEF-4984) and *I. farinosa* (ARSEF-3580)) throughout the treatment is also considered as an indicator of consistent pathogenicity. Among the isolates tested, *L. lecanii (*TR38/11) and *I. fumosorosea (*ISFUM-4501) particularly provided higher mortality rates, from the initial stages of the experiments. *L. muscarium (*ARSEF-5128) isolate gave a consistent increase in mortality and resulted in up to 94.9% mortality in *S. granarius* adults by 10 days after treatment.

Conclusions

Various chemical, biotechnical, and microbiological ways to control the entry of storage pests into storages were advised by the researchers, like treating the empty bins, and application of treatment to topmost layer of the stored products ("top-dress" or "cap off"), which helps to prevent emergence or new entries (Mason & Obermeyer, 2010). Aside from strain virulence which was considered as the primary factor for higher pest mortality, this shows the importance of treatment methodology for entomopathogenic fungi in stored conditions and points out the need for further research considering both pathogenicity and application methodology of fungal treatments, under actual storage conditions.

Based on higher virulence they provided, *L. lecanii (*TR38/11), *I. fumosorosea (*ARSEF-4501) and *L. muscarium (*ARSEF-5128) isolates are considered better candidates as biocontrol agents against adults of *S. granarius,* in stored wheat.

References

Ak, K., 2019. Efficacy of entomopathogenic fungi against the stored-grain pests, *Sitophilus granarius* L. and *S. oryzae* L. (Coleoptera: Curculionidae). Egyptian Journal of Biological Pest Control, 29 (12)**:** 1-7.

Aregger, E., 1992. Conidia production of the fungus *Beauveria brongniartii* on barley and quality evaluation during storage at 2°C. Journal of Invertebrate Pathology, 59 (1): 2-10.

Arthur, F. H., 1996. Grain protectants: current status and prospects for the future. Journal of Stored Product Research, 32 (4): 293-302.

Athanassiou, C. G. & T. Steenberg, 2007. Insecticidal effect of *Beauveria bassiana (*Balsamo) Vuillemin (Ascomycota: Hypocreales) in combination with three diatomaceous earth formulations against *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). Biological Control, 40 (3): 411-416.

Barra, P., L. Rosso, A. Nesci & M. Etcheverry, 2013. Isolation and identification of entomopathogenic fungi and their evaluation against *Tribolium confusum*, *Sitophilus zeamais*, and *Rhyzopertha dominica* in stored maize. Journal of Pest Science, 86 (2): 217-226.

Batta, Y. A. & N. G. Kavallieratos, 2018. The use of entomopathogenic fungi for the control of stored-grain insects. International Journal of Pest Management, 64 (1): 1-11.

Cabanillas, H. E. & W. A. Jones, 2013. Pathogenicity of *Isaria poprawskii* (Ascomycota: Hypocreales: Cordycipitaceae) against the glassy-winged sharpshooter, *Homalodisca vitripennis* (Hemiptera: Cicadellidae), under laboratory conditions. Crop Protection, 50: 46-52.

Campbell, A., N. B. Singh & R. N. Sinha, 1976. Bioenergetics of the granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). Canadian Journal of Zoology, 54 (5): 786-798.

Charnley, A. K., 2003. Fungal pathogens of insects: cuticle degrading enzymes and toxins. Advances in Botanical Research, 40: 241-321.

Charnley, A. K., 1989. "Mycoinsecticides: Present use and future prospects, 165-181". In: Proceedings of Progress and Prospects in Insect Control, (18th-20th September 1989, University of Reading, Farnham, Surrey, UK), BCPC Monograph No: 43, British Crop Protection Council, London, UK, 273 pp.

Cherry, A. J., P. Abalob & K. Hell, 2005. A laboratory assessment of the potential of different strains of the entomopathogenic fungi *Beauveria bassiana* (Balsamo) Vuillemin and *Metarhizium anisopliae* (Metschnikoff) to control *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in stored cowpea. Journal of Stored Products Research, 41 (3): 295-309.

Clarkson, J. M. & A. K. Chamley, 1996. New insights into the mechanisms of fungal pathogenesis in insects. Trends in Microbiology, 4 (5): 4-5.

Cox, P. D. & D. R. Wilkin, 1996. The Potential Use of Biological Control of Pests in Stored Grain. HGCA Research Review (United Kingdom), 53 pp.

Dal Bello, G., S. Padin, C. L. Lastra & M. Fabrizio, 2000. Laboratory evaluation of chemical-biological control of the rice weevil (*Sitophilus oryzae* L.) in stored grains. Journal of stored products research, 37 (1): 77-84.

Danho, M., C. Gaspar & E. Haubruge, 2002. The impact of grain quantity on the biology of *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae): oviposition, distribution of eggs, adult emergence, body weight and sex ratio. Journal of Stored Products Research, 38 (3): 259-266.

Dembilio, Ó., E. Quesada-Moraga, C. Santiago-Álvarez & J. A. Jacas, 2010. Potential of an indigenous strain of the entomopathogenic fungus *Beauveria bassiana* as a biological control agent against the Red Palm Weevil, *Rhynchophorus ferrugineus*. Journal of Invertebrate Pathology, 104 (3): 214-221.

Demirci, F., M. Muştu, M. B. Kaydan & S. Ülgentürk, 2011. Laboratory evaluation of the effectiveness of the entomopathogen; *Isaria farinosa*, on citrus mealybug, *Planococcus citri*. Journal of Pest Science, 84 (3): 337-342.

Domsch, K. H., W. Gams & T. H. Anderson, 1980. Compendium of Soil Fungi. Academic Press (London) LTD, 859 pp.

Ekesi, S., A. D. Akpa & I. Onu, 2000. Relative pathogenicity of different entomopathogenic fungi to *Sitotroga cerealella* in stored sorghum. Tropical Science, 40 (4): 206-210.

Emsen, B., E. Yıldırım & A. Aslan, 2015. Insecticidal activities of extracts of three lichen species on *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). Plant Protection Science, 51 (3): 155-161.

Erkılıç, L. & N. Uygun, 1993. Entomopatojen fungusların biyolojik mücadelede kullanılma olanakları (Possibilities of using entomopathogenic fungi in biological control). Turkish Journal of Entomology-Türkiye Entomoloji Dergisi, 17 (2): 117-128 (in Turkish with abstract in English).

Ferroni, P., 1977. Influence of relative humidity on the development of fungal infection caused by *Beauveria bassiana* in imagines of *Acanthoscelides obtectus.* Entomophaga, 22 (4): 393-396.

Hagstrum, D. W. & B. Subramanyam, 2009. A review of stored-product entomology information sources. American Entomologist, 55 (3): 174-183.

Hajek, A. E. & R.J. St. Leger, 1994. Interactions between fungal pathogens and insect hosts. Annual Review Entomology, 39 (1): 293-322.

Hare, J. D., 1980. Impact of defoliation by the Colorado potato beetle on potato yields, Journal of Economic Entomology, 73 (3): 369-373.

Hidalgo, E., D. Moore & G. Le Patourel, 1998. The effect of different formulations of *Beauveria bassiana* on *Sitophilus zeamais* in stored maize. Journal of Stored Production Research, 34 (2/3): 171-179.

Inglis, G. D., M. S. Goettel, T. M. Butt & H. Strasser, 2001. "Use of Hyphomycetous Fungi for Managing Insect Pests, 23-69". In: Fungi as Biocontrol Agents: Progress, Problems and Potential, Wallingford UK: CABI Publishing, 390 pp.

Isman, M. B., 2006. Botanical insecticides, deterrents, and repellents in modern agriculture and an increasingly regulated world. Annual Review of Entomology, 51 (1): 45-66.

Kassa, A., G. Zimmermann, D. Stephan & S. Vidal, 2002. Susceptibility of *Sitophilus zeamais* (Motsch.) (Coleoptera: Curculionidae) and *Prostephanus truncatus* (Horn) (Coleoptera: Bostrichidae) to Entomopathogenic Fungi from Ethiopia. Biocontrol Science and Technology, 12 (6): 727-736.

Kavallieratos, N. G., C. G. Athanassiou, M. M. Aountala & D. C. Kontodimas, 2014. Evaluation of the entomopathogenic fungi *Beauveria bassiana*, *Metarhizium anisopliae*, and *Isaria fumosorosea* for control of *Sitophilus oryzae*. Jouırnal of Food Protection, 77 (1): 4-17.

Kesdek, M., N. Bayrak, S. Kordali, A. Usanmaz, G. Contuk & S. Ercisli, 2013. Larvicidal effects of some essential oils against larvae of the Pine processionary moth, *Thaumetopoea pityocampa* (Denis & Schiffermüller) (Lepidoptera: Thaumetopoeidae). Egyptian Journal of Biological Pest Control, 23 (2): 201-207.

Khashaveh, A., Y. Ghosta, M. H. Safaralizadeh & M. Ziaee, 2011. The use of entomopathogenic fungus, *Beauveria bassiana* (Bals.) Vuill. in assays with storage grain beetles. Journal of Agricultural Science Technology, 13 (1): 35-43.

Khetan, S. K., 2001. Microbial Pest Control. Marcel Dekker, New York, 223 pp.

Kirkpatrick, R. L. & D. A. Wilbur, 1965. The development and habits of the granary weevil, *Sitophilus granarius* within the kernel of wheat. Journal of Economic Entomology, 58 (5): 979-985.

Komaki, M., Ş. Kordalı, A. Usanmaz Bozhüyük, H.H. Altınok, M. Kesdek, D. Şimşek & M.A. Altınok, 2017. Laboratory assessment for biological control of *Tribolium confusum* du Val., 1863 (Coleoptera: Tenebrionidae) by Entomopathogenic fungi. Journal of Turkish Entomology, 41 (1): 95-103.

Kordali, S., A. Usanmaz Bozhüyük, M. Kesdek, H.H. Altınok & M.A. Altınok, 2021. Efficacy of various entomopathogenic fungi strains as biocontrol agents for control of *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae). Journal of Agricultural Sciences (Tarim Bilimleri Dergisi), 27 (4): 454-459.

Kordalı, Ş., A. Usanmaz, N. Bayrak & A. Çakır, 2017. Fumigation of volatile monoterpenes and aromatic compounds against adults of *Sitophilus granarius* (L.) (Coleoptera: Curculionidae). Records of Natural Products, 11 (4): 362-373.

Kücükaydin, S., G. Tel-Cayan, M. E. Duru, M. Kesdek & M. Ozturk, 2020. Chemical composition and insecticidal activities of the essential oils and various extracts of two *Thymus* species: *Thymus cariensis* and *Thymus cilicicus*. Toxin Reviews, 40 (4): 1461-1471.

Lacey, L. A. & M. S. Goettel, 1995. Current developments in microbial control of insect pests and prospects for the early 21st century. Entomophaga, 40 (1-2): 3-27.

Lazzari, S. M. N. & F. A. Lazzari, 2012. “Insect Pests in Stored Grain, 417-450”. In: Insect Bioecology and Nutrition for Integrated Pest Management. Taylor & Francis Group, Boca Raton, Florida, USA, 750 pp.

Mantzoukas, S., A. Zikou, V. Triantafillou, I. Lagogiannis & P. A. Eliopoulos, 2019. Interactions between *Beauveria bassiana* and *Isaria fumosorosea* and their hosts *Sitophilus granarius* (L.) and *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae). Insects, 10 (10): 362.

Mason, L. J. & J. Obermeyer, 2010. Stored Product Pests: Stored Grain Insect Pest Management. Purdue Extension, Department of Entomology, Purdue University, 2 pp.

Moina, A., S. B. Alves & R. M. Pereira, 1998. Efficacy of *Beauveria bassiana* (Balsamo) Vuillemin isolates for control of stored grain pests. Journal of Applied Entomology, 122 (1-5): 301-305.

Morales-Quiros, A., C. Campabadal, D. E. Maier, S. M. Lazzari, F. A. Lazzari & T. W. Phillips, 2019. Chilled Aeration to control pests and maintain grain quality during summer storage of wheat in the north central region of Kansas. Applied Engineering in Agriculture, 35 (4): 657-688.

Padin, S., G. Dal Belloa & M. Fabrizio, 2002. Grain loss caused by *Tribolium castaneum*, *Sitophilus oryzae* and *Acanthoscelides obtectus* in stored durum wheat and bean treated with *Beauveria bassiana*. Journal of Stored Products Research, 38 (1): 69-74.

Proctor, D. L., 1994. “Insects of Stored Products, 285-307”. In: Practical Handbook of Warehousing (Eds. R. S. Smith and J. T. Dunwoody). Chapman & Hall, London, 595 pp.

Rice, W. C. & R. R. Cogburn, 1999. Activity of entomopathogenic fungus *Beauveria bassiana* (Deuteromycota: Hyphomycetes) against three coleopteran pests of stored grain. Journal of Economical Entomology, 92: 691-694.

Roberts, D. W. & R. A. Humber, 1981. “Entomogenous Fungi, 201-236”. In: Biology of Conidial Fungi, (Eds. G. T. Cole & B. Kendrick). Academic Press, New York, 484 pp.

Rodrigues, C. & D. Prassoli, 1990. Patogenicidade de *Beaureria brongniartii (*Sacc.) Petch. e *Metarhizium anisopliae (*Metsch.) Sorok. e seu efeito sobre o gorgulho do milho e caruncho do feijtio. Anais Sociedade Entomologica Brasil, 19 (2): 301-306.

Roy, H. E., J. K. Pell & P. G. Alderson, 2001. Targeted dispersal of the aphid pathogenic fungus *Erynia neoaphidis* by the aphid predator *Coccinella septempunctata*. Biocontrol Science and Technology, 11 (1): 99-110.

Roy, N. K. & P. Dureja, 1998. New ecofriendly pesticides for integrated pest management. Pesticides World, 3: 16-21.

Santos, J. C., L. R. D. A. Faroni, R. O Simões, M. A. G. Pimentel & A. H. Sousa, 2009. Toxicity of pyrethroids andorganophosphorus insecticides to Brazilian populations of *Sitophilus zeamais (*Coleoptera: Curculionidae). Bioscience Journal, 25 (6): 75-81.

Saroukolai, A. T., S. Moharramipour, M. H. Meshkatalsadat, 2000. Insecticidal properties of *Thymus persicus* essential oil against *Tribolium castaneum* and *Sitophilus oryzae*. Journal of Pest Science, 83 (1): 3-8.

Serale, T. & J. Doberski, 1984. An investigation of the entomogenous fungus *Beauveria bassiana* as a potential biological control agent for *Oryzaephilus surinamensis.* Journal of Stored Production Research, 20 (1): 17-24.

Sevim, A., İ. Demir, E. Sönmez, S. Kocaçevik & Z. Demirbağ, 2013. Evaluation of entomopathogenic fungi against the sycamore lace bug, *Corythucha ciliata (*Say) (Hemiptera: Tingidae). Turkish Journal of Agriculture and Forestry, 37 (5): 595-603.

Sevim, A., E. Sevim & Z. Demirbağ, 2015. General biology of entomopathogenic fungi and their potential to control pest species in Turkey (Entomopatojenik funguslerın genel biyolojileri ve Türkiye’de zararlı böceklerin mücadelesinde kullanılma potansiyelleri). Erzincan Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 8 (1): 115-147 (in Turkish with abstract in English).

Sinha, K. K. & A. K. Sinha, 1991. Effect of *Sitophilus oryzae* infestation on *Aspergillus flavus* infection and aflatoxin contamination in stored wheat. Journal of Stored Products Research, 27 (1): 65-68.

Thaung, N. & P. J. Collins, 1986. Joint effect of temperature and insecticides on mortality and fecundity of *Sitophilus oryzae (*Coleoptera: Curculinoidae) in wheat and maize. Journal of Economical Entomology, 79 (4): 909-914.

Throne, J. E. & J. C. Lord, 2004. Control of Sawtoothed Grain Beetles (Coleoptera: Silvanidae) in Stored Oats by Using an Entomopathogenic Fungus in Conjunction with Seed Resistance. Journal of Economic Entomology, 97 (5): 1765-1771.

Tunaz, H., C. Bengin & M. K. Er, 2008. Nodulation reaction to fungal infections in larvae of *Leptinotarsa decemlineata (*Say) (Coleoptera: Chrysomelidae) mediated by eicosanoids. Turkish Journal of Agricultural and Forestry, 32 (1): 11-18.

Usanmaz Bozhüyük, A., Ş. Kordali, M. Kesdek, D. Şimşek, M. A. Altınok, H. H. Altınok & A. Komaki, 2018. Mortality effects of six different entomopathogenic fungi strains on rice weevil, *Sitophilus oryzae (*L.) (Coleoptera: Curculionidae). Fresenius Environmental Bulletin 27 (6): 4373-4380.

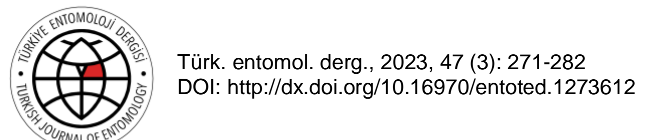
Woodbury, N., 2008. Infanticide avoidance by the granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae): the role of harbourage markers, oviposition markers, and egg-plugs. Journal of Insect Behavior, 21 (2): 55-62.

Wyss, G. S., R. Charudattan, & J. T. DeValerio, 2001. Evaluation of agar and grain media for mass production of conidia of *Dactylaria higginsii*. Plant Disease, 85 (11): 1165-1170.

Yang, S., H. Zhuang, Y. Li & R. Kuang, 2009. Insecticidal efficacy of *Isaria farinosa* in different life stages of *Pissodes punctatus* (Coleoptera: Curculionidae). Journal of Pest Science, 82 (4): 321-325.

Zettler, J. L., 1991. Pesticide resistance in *Tribolium castaneum* and *Tribolium confusum (*Coleoptera: Tenebrionidae) from flour mills in the United States. Journal of Economic Entomology, 84 (3): 763-767.

Zimmermann, G., 2007. Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. Biocontrol Science and Technology, 17 (6): 553-596.



Türk. entomol. derg., 2023, 47 (3): 271-282
DOI: http://dx.doi.org/10.16970/entoted.1273612

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

A study of whether the genetic variation decreased or not in the protected Caucasian bee, *Apis mellifera caucasica* Pollmann, 1889 (Hymenoptera: Apidae) population in isolated regions[1]

İzole bölgelerde korunan Kafkas arısı, *Apis mellifera caucasica* Pollmann, 1889 (Hymenoptera: Apidae) popülasyonunda genetik varyasyonun azalıp azalmadığına dair bir çalışma

Berkant İsmail YILDIZ[2] **Emel TÜTEN SEVİM[2]** **Sinan AYDIN[3]**
Yaprak KARADUMAN ASLAN[3] **Ramazan ÇETİN[3]** **Erdem SUR[3]**
Kemal KARABAĞ[2*]

Abstract

The Caucasian honeybee, *Apis mellifera caucasica* Pollmann, 1889 (Hymenoptera: Apidae), is one of the most productive bee subspecies. This subspecies, which has special importance for Türkiye, has been taken under protection in two isolated regions (Artvin and Ardahan) since 2000. To date, no study has been conducted on whether genetic diversity has decreased in these protected Caucasian honeybee colonies. Therefore in 2022, worker bees were collected from 100 different colonies in 15 different locations in these two regions and their genetic variations were examined using 30 microsatellite loci. The average number of alleles per locus was 13.57, and the loci had a high level of information content according to the PIC (0.7) value. F_{IS} (0.96) and F_{ST} (0.01) values showed low genetic diversity and high inbreeding in populations. Genetic variations were calculated as 0.77% among populations, 99.23% among individuals in populations, and 0% among all individuals. Also, populations deviated from the Hardy-Weinberg equilibrium (p<0.001). Significant bottleneck evidence was found for Artvin in the analysis results using the two-phase mutation model. These results provide important information that can be used as a guide for Caucasian bee breeding strategies and conservation programs.

Keywords: *Apis mellifera*, Caucasian bee, genetic variation, microsatellite, population structure

Öz

Kafkas arısı, *Apis mellifera caucasica* Pollmann, 1889 (Hymenoptera: Apidae), en verimli arı alt türlerinden biridir. Türkiye için özel bir öneme sahip olan bu alt tür, 2000 yılından itibaren iki izole bölgede (Artvin ve Ardahan) koruma altına alınmıştır. Bugüne kadar korunan Kafkas bal arısı kolonilerinde genetik çeşitliliğin azalıp azalmadığına dair bir çalışmaya rastlanmamıştır. Bu nedenle 2022 yılında, bu iki izole bölgede 15 farklı lokasyonda bulunan 100 farklı koloniden işçi arılar toplanmış ve 30 mikrosatelit lokusu kullanılarak genetik varyasyonlar incelenmiştir. Lokus başına ortalama allel sayısı 13.57 bulunmuştur ve PIC (0.7) değerine göre lokuslar yüksek düzeyde bilgi içeriğine sahiptir. F_{IS} (0.96) ve F_{ST} (0.01) değerleri popülasyonlarda düşük genetik çeşitlilik ve yüksek akrabalı yetiştirme olduğunu göstermiştir. Genetik varyasyonlar, popülasyonlar arasında %0.77, popülasyonlardaki bireyler arasında %99.23 ve tüm bireyler arasında %0 olarak hesaplanmıştır. Ayrıca, popülasyonlar Hardy-Weinberg dengesinden sapmıştır (P<0.001). İki fazlı mutasyon modeli kullanılarak yapılan analiz sonuçlarında Artvin için önemli bir darboğaz kanıtı bulunmuştur. Bu sonuçlar, Kafkas arısı ıslah stratejileri ve koruma programları için kılavuz olarak kullanılabilecek önemli bilgiler sağlamaktadır.

Anahtar sözcükler: *Apis mellifera*, kafkas arısı, genetik varyasyon, mikrosatelit, popülasyon yapısı

[1] The study was funded by The Republic of Türkiye Ministry of Agriculture with HAYGEM 2021A03-168656 grant no project.
[2] Akdeniz University, Faculty of Agriculture, Department of Agricultural Biotechnology, 07058, Konyaaltı, Antalya, Türkiye
[3] Republic of Türkiye Ministry of Agriculture, Caucasian Bee Production, Training and Gene Center Directorate, 75000, Central, Ardahan, Türkiye
* Corresponding author (Sorumlu yazar) e-mail: karabag@akdeniz.edu.tr
Received (Alınış): 30.03.2023 Accepted (Kabul ediliş): 25.09.2023 Published Online (Çevrimiçi Yayın Tarihi): 04.10.2023

Introduction

Apis mellifera, naturally spreads over a wide geographic range including Europe, Africa, West and Central Asia. Low migration capability and limited population size have resulted in presence of approximately 30 subspecies and high levels of genetic variation in this large geography (Ruttner, 1988). Within these subspecies, there are also ecotypes and breeding lines that are very important for researchers and beekeepers (Oleksa & Tofilski, 2015). Interactions between subspecies often occur through human activities. Commercially managed bee colonies are transported between remote areas for pollination services and food access. These activities affect genetic variability (Bouga et al., 2011). Although various revisions have been made in honey bees classification; genetic, morphometric and ethological studies have identified four major evolutionary lineages in the honey bee: African ancestry (A), Western and Northern European ancestry (M), Southeast European ancestry (C), and Middle Eastern ancestry (O) (Ruttner, 1988; Arias et al., 2006; Bouga et al., 2011; Nawrocka et al., 2018).

Many molecular markers such as restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD) and mitochondrial DNA (mtDNA) analyses have been used in honey bee population genetics studies, but for the last 20 years, microsatellite loci have been quite widely used due to their features such as high polymorphism, multiallelicity, abundance in the genome and easy scorable (Kandemir & Kence, 1995; Smith et al., 1997; Bodur et al., 2007; Kekeçoğlu et al., 2009; Özdil et al., 2009; Liu et al., 2016; Rahimi et al., 2016; Haddad et al., 2018; Hassett et al., 2018; Yu et al., 2019; Özdil et al., 2022). Latterly, single nucleotide polymorphisms (SNPs) have been used in population genetics studies, however, thanks to the advantages provided by microsatellites, they could not get ahead of microsatellites even in the genomic era (Zimmerman et al., 2020; Mukherjee et al., 2022; Wang et al., 2022). Genetic diversity and species richness in honey bee populations in Türkiye have been demonstrated by microsatellite and mtDNA studies (Kandemir et al., 2006; Bodur et al., 2007; Ivgin Tunca, 2009; Özdil et al., 2009). This extensive research has revealed the presence of five subspecies of honey bees; Anatolian bee (*Apis mellifera anatoliaca* Maa, 1953), Caucasian bee, *Apis mellifera caucasica* Pollmann, 1889, Iranian bee, *Apis mellifera meda* Skorikov, 1929, Syrian bee, *Apis mellifera syriaca* Skorikov, 1829) and Carniolan bee, *Apis mellifera carnica* Pollmann, 1879 (Hymenoptera: Apidae) (Kandemir et al., 2000), and it has about 20% of the honey bee subspecies in the world. Anatolia is a gene center for honey bees (Ruttner, 1988) and provides a great diversity in almost every characteristic of honey bees. Although many breeding programs have been implemented around the world, genetic variation has remained insufficient in the production of resistant stocks (Rinderer et al., 2010). At this point, this genetic diversity provides an advantage in possible breeding studies in our country.

Caucasian honey bee, which is one of the important gene resources in Anatolia, is the most preferred subspecies due to its superior characteristics in terms of honey production, its calm and docile behavior (Ruttner, 1988). The natural range of Caucasian honey bee has been extended by humans from the Caucasus to Bulgaria (Ivanova et al., 2007) along with a large number of hives brought to Ukraine, Germany and France (Ruttner, 1988). In Türkiye, it is located in the northeastern region, in neighboring provinces, especially in Ardahan, Artvin and Kars, but after the migratory beekeeping that started in the 1950s, the number of pure bee colonies in this region has gradually decreased (Kırpık et al., 2010). Likewise, Fıratlı and Budak (1994) reported that the genetic structure of honey bee populations in Türkiye have changed and confusion has increased in the populations. With the studies carried out by the Ministry of Agriculture and Forestry in 2000, Artvin and Ardahan provinces were declared as isolated regions for the *A.m caucasica* subspecies and tried to be taken under protection (Gül & Nergiz, 2022). The results of the studies conducted after the declaration have shown that there are still concerns about genetic diversity.

Understanding the genetic diversity that remains in natural populations is known to be crucial for conservation plans (Kuo & Jansen, 2004). Although the determination of genetic diversity in the Caucasian

honey bee colonies, which have been under protection in two isolated regions for 20 years, is of great importance, there is not enough information about the situation in literature. Therefore, we aimed to detect the genetic variations present in the Caucasian honey bee colonies in this study.

Materials and Methods

Sample collection

As material, a total of 100 worker bees were collected from 100 different colonies in 15 locations in two provinces (Artvin and Ardahan) in 2022 (Table 1).

Table 1. Geographical coordinates and altitudes of the colonies where bee samples were taken

	Locations	Coordinate	Altitude (m)
Artvin	City center	41º10′N 41º49′E	524
	Ardanuç	41º07′N 42º03′E	486
	Arhavi	41º21′N 41º18′E	7
	Borçka	41º21′N 41º40′E	124
	Hopa	41º23′N 41º25′E	8
	Kemalpaşa	41º28′N 41º31′E	15
	Murgul	41º15′N 41º39′E	952
	Şavşat	41º15′N 42º21′E	1108
	Yusufeli	40º49′N 41º32′E	601
Ardahan	City Center	41º06′N 42º42′E	1807
	Çıldır	41º07′N 43º07′E	1975
	Damal	41º20′N 42º50′E	2051
	Göle	40º47′N 42º36′E	2018
	Hanak	41º14′N 42º50′E	1818
	Posof	41º30′N 42º43′E	1545

DNA extraction and microsatellite amplification

Before DNA isolation, the samples were arranged on blotting paper one by one and kept at room temperature for 3 hours to remove the alcohol. The head and thorax of the bees were separated from the other body parts with the help of sterile tweezers and placed in numbered 1.5 ml microcentrifuge tubes. Liquid nitrogen was added to each microcentrifuge tube and the samples were homogenized with the help of sterile plastic rods. Total DNA extraction was performed using the High Pure PCR Template Preparation Kit (Roche, Basel, Switzerland). The quality and quantity of the DNAs, which were kept at +4°C overnight, were determined by BioDrop spectrophotometer. In addition, the quality of each individual's genomic DNA was verified by performing electrophoresis on a 1% agarose gel. DNA samples were stored at -20°C until used in the next step.

For the genetic characterization of the Caucasian bee, 30 microsatellite markers proposed by Solignac et al. (2003) were used. PCR amplifications were performed using Xpert Fast Hotstart Mastermix (Grisp, Porto, Portugal) with 2 µl of template DNA (50 ng/µl) in a total mixture volume of 25 µl. The thermal cycler program was 94°C for an initial denaturation for 5 min; 35 cycles of 94°C for 30 s, 30 s at the primer-specific annealing temperature, and 72°C for 45 s; and a final 72°C for 5 min. PCR products were electrophoresed on a 2% agarose gel in TBE buffer (25 mM Tris, 25 mM Boric acid, 50 mM EDTA, pH 8.0). Precise fragment lengths of the PCR products were determined on the AATI fragment analyzer.

Statistical analysis

The fragment lengths were manually scored with the PROSize2.0 software (Advanced Analytic Technologies Inc., Ankeny, IA, USA). Belonging to populations; number of loci (N), number of polymorphic loci (N_P), number of observed alleles (N_A), number of effective alleles (N_E), expected (H_E) and observed (H_O) heterozygous values, F-statistics and Hardy-Weinberg equilibrium (HWE) were calculated using Popgene v.1.32 software (Yeh et al., 1997). Microsatellite toolkit software was used to calculate the polymorphic information content (PIC) of microsatellite markers (Park, 2001). Null alleles, also known as non-amplified alleles, were predicted using ML-NullFreq software (Kalinowski & Taper, 2006). Molecular variance (AMOVA) (Excoffier et al., 1992) was calculated with Arlequin v.3.11 software (Excoffier et al., 2007). The bottleneck hypothesis was investigated using Bottleneck 1.2.02 software (Cornuet & Luikart, 1996). Due to the number of loci (>20) and samples; Two-phase Mutation Model (TPM), sign test (to calculate how many loci with heterozygosity deficiency or heterozygosity excess), Wilcoxon's signed rank test (to determine whether heterozygosity deficiency or excess), standardized differences test (for the genetic signature of bottlenecks in the honey bee populations studied) were used to determine the bottleneck.

The genetic structure and genetic admixture levels of the populations were estimated with the Structure v.2.3.3 software (Pritchard et al., 2000) using the Bayesian clustering algorithm. Factorial correspondence analysis (FCA) was performed using Genetix v.4.05 software (Belkhir et al., 1996-2004) to reveal relationships between individuals in populations and to examine genotypic data in a three-dimensional plane.

Results and Discussion

The various genetic parameters tested for the Caucasian honey bee populations are described in Table 2. When all microsatellite loci were examined, the mean N_A was calculated as 13.57. It is seen that this value is higher than previous studies (Bodur et al., 2007; Ivgin Tunca, 2009; Karabağ et al., 2020). The high N_A value indicates that the number of samples used in the study is sufficient to measure genetic diversity (Mielnik-Sikorska et al., 2013). While the mean N_E was 4.91, the highest and lowest N_E values were calculated as 13.84 (Ap256) and 1.78 (A028). Accordingly, it can be suggested that loci above this mean N_E value (Ap223, Ap238, A113, A(b)124, Ap256, Ac306, Ap033, Ap068, Ap001, Ap289) should be used in genetic characterization studies in honey bees. While the mean PIC value for all loci was found 0.71, the highest and lowest PIC values were found as 0.92 (Ap256) and 0.39 (Ag005a). PIC values were on average above 0.5, meaning that the selected loci had high information content and were suitable for genetic diversity study (Botstein et al., 1980).

The mean F_{IS} value for all loci was found as 0.96. Also, the lowest F_{IS} value was 0.39 (Ap001) and except for the Ap223, Ap238, Ap085, Ap001 loci, the F_{IS} value was found to be 1 in all loci. The F_{IS} value is used to determine the deviation from the Hardy-Weinberg equilibrium in a population and is one of the important indicators in determining conservation priorities for populations. Positive values obtained indicate a deficiency of heterozygosity (Bodur, 2005). According to Simon & Buchenauer (1993), homozygosity reaches a dangerous level when the F_{IS} value is greater than 0.40. Inbreeding is the major cause of heterozygous deficiency in isolated and relatively small areas (Castric et al., 2002). The fact that the genetic difference was found to be 0.01 in terms of F_{ST} also supports the increasing inbreeding in the Artvin and Ardahan isolated regions.

Table 2. Descriptive statistics for genetic diversity of all population over 30 microsatellite loci

Locus	N	N_A	N_E	PIC	F_{IS}	F_{ST}	H_O	H_E	Mean H
Ap223	170.00	24.00	8.98	0.88	0.97	0.01	0.02	0.89	0.88
Ap238	164.00	23.00	6.69	0.84	0.78	0.01	0.18	0.86	0.84
Ap273	172.00	13.00	4.15	0.74	1.00	0.02	0.00	0.76	0.74
Ap243	186.00	14.00	3.58	0.69	1.00	0.01	0.00	0.72	0.71
Ap085	186.00	16.00	3.18	0.65	0.90	0.03	0.06	0.69	0.66
Ac011	184.00	12.00	4.05	0.72	1.00	0.04	0.00	0.76	0.72
A113	176.00	16.00	7.55	0.85	1.00	0.01	0.00	0.87	0.86
At003	176.00	9.00	2.54	0.58	1.00	0.01	0.00	0.61	0.60
A028	176.00	9.00	2.02	0.48	1.00	0.00	0.00	0.51	0.50
Ap249	176.00	11.00	4.10	0.73	1.00	0.00	0.00	0.76	0.75
A(b)124	176.00	17.00	6.34	0.82	1.00	0.01	0.00	0.85	0.84
Ap307	176.00	5.00	1.87	0.41	1.00	0.04	0.00	0.47	0.44
Ap256	192.00	24.00	13.84	0.92	1.00	0.02	0.00	0.93	0.91
Ap207	194.00	10.00	4.09	0.73	1.00	0.02	0.00	0.76	0.74
Ap043	194.00	7.00	2.21	0.50	1.00	0.01	0.00	0.55	0.54
Ap015	188.00	12.00	4.14	0.73	1.00	0.00	0.00	0.76	0.75
A043	192.00	10.00	4.69	0.76	1.00	0.01	0.00	0.79	0.78
Ac306	190.00	18.00	8.11	0.86	1.00	0.02	0.00	0.88	0.86
Ap033	168.00	20.00	6.73	0.82	1.00	0.02	0.00	0.86	0.83
A(b)024	168.00	16.00	4.91	0.78	1.00	0.02	0.00	0.80	0.78
A079	188.00	13.00	4.62	0.75	1.00	0.01	0.00	0.79	0.77
Ap274	180.00	14.00	3.03	0.63	1.00	0.01	0.00	0.67	0.66
Ap068	176.00	16.00	7.08	0.84	1.00	0.01	0.00	0.86	0.85
Ap297	184.00	11.00	2.76	0.58	1.00	0.00	0.00	0.64	0.64
Ap218	192.00	9.00	4.09	0.71	1.00	0.01	0.00	0.76	0.75
Ap001	184.00	21.00	7.56	0.85	0.39	0.01	0.52	0.87	0.86
A008	186.00	10.00	4.60	0.75	1.00	0.01	0.00	0.79	0.77
Ap226	196.00	7.00	2.29	0.50	1.00	0.01	0.00	0.57	0.56
Ap289	190.00	13.00	5.75	0.80	1.00	0.02	0.00	0.83	0.81
Ag005a	196.00	7.00	1.78	0.39	1.00	0.00	0.00	0.44	0.44
Mean	183.00	13.57	4.91	0.71	0.96	0.01	0.03	0.74	0.73

* N, number of total alleles used in each locus; N_A, number of observed alleles; N_E, number of effective allele; PIC, polymorphic information content; F_{IS}, inbreeding coefficient; F_{ST}, genetic differentiation coefficient; H_O, observed heterozygosis; H_E, expected heterozygosis; Mean H, mean heterozygosity.

The mean H_O value over all loci was found as 0.03. While the highest H_O value was 0.52 (Ap001), H_O was found to be 0 in other loci except Ap223, Ap238, Ap085, Ap001. In terms of H_E, the mean value was 0.74, highest and lowest values were 0.93 for Ap256 and 0.44 for Ag005a. These values (H_O and H_E) show the gene diversity in the Caucasian honey bee. Bodur et al. (2007) found that the mean H_O ranged from 0.52 (Eskişehir) to 0.67 (Cyprus) and the mean H_E ranged from 0.54 (Eskişehir) to 0.68 (Kastamonu) in 12 populations in Türkiye. İvgin Tunca (2009) found that the mean H_O ranged from 0.68 (Kars) to 0.38 (Artvin) and the mean H_E ranged from 0.45 (Muğla) to 0.74 (Artvin) in the honey bee populations in Türkiye. In a study examining genetic variability at eight microsatellite loci in *Apis mellifera ligustica* Spinola, 1806

(Hymenoptera: Apidae), the mean H_O was reported between 0.38 and 0.61, the mean H_E between 0.53 and 0.64 (Dall'Olio et al., 2007). In another study conducted on Lebanon honey bees, mean gene diversity was estimated to be 0.65 (Franck et al., 2000). Calculated values in terms of heterozygosity in this study are generally similar to the results reported in the literature. However, the observed heterozygosity was lower than the expected heterozygosity. This suggests that inbreeding has increased in protected colonies in the isolated region.

The indicators of genetic polymorphism and HWE values are given in Table 3 below for each two isolated regions. Statistics calculated for colonies in both isolated regions were found to be quite close to each other. While the F_{IS} and PIC values were higher in Artvin, the N_A, N_E, H_E and H_O values were higher in Ardahan.

Table 3. Main diversity parameters for each population

	Artvin	Ardahan
N	50.00	50.00
N_P	30.00	30.00
N_A	9.37	10.37
N_E	4.53	4.86
PIC	0.70	0.69
F_{IS}	0.98	0.93
H_E	0.74	0.74
H_O	0.02	0.04
HWE	0.00	0.00

* N, loci number; N_P, number of polymorphic loci N_A, number of observed alleles; N_E, number of effective allele; PIC, polymorphic information content; F_{IS}, coefficient of inbreeding; H_E, expected heterozygosis; H_O, observed heterozygosis; HWE, Hardy-Weinberg equilibrium ($p<0.01$).

Genetic differences for the two provincial populations were calculated using Arlequin with pairwise F_{ST} values developed by Weir & Cockerham (1984). According to this calculation, the two provinces are quite similar with only a slight difference (F_{ST}: 0.02). This study suggested that low levels of genetic differentiation were observed in isolated colonies, given the wide range of binary F_{ST} values previously reported (Garnery et al., 1998; Franck et al., 2000, 2001; Dall'Olio et al., 2007). Also, Nei's (1972) original genetic identity and genetic distance measurements were estimated and the genetic distance between populations was found to be 0.08. AMOVA analysis was performed to determine whether genetic variation was due to differentiation between populations or from individuals within the population. According to the results, the source of genetic variation was found to be 3.46% among populations, 96.54% among individuals within the population, and 0% for all individuals. As evident from these findings, Caucasian honey bee colonies under protection have the ability to represent the same race purely.

The differentiation between populations was also examined by Bayesian cluster analysis in Structure software (Figure 1). When K = 3, where the highest ΔK value is obtained, it is seen that phylogenetic relationships are best expressed and the Artvin (1) and Ardahan (2) populations are not clearly separated. Although same color bars representing individuals are found in both populations, it is seen that red segments are slightly more intense in the Ardahan (2) population. The fact that the colonies in the Artvin and Ardahan regions can be distinguished from each other, even if slightly, suggests that there are differences in the populations of the two provinces within the same protection area. The queen bees of the colonies in the two provinces obtained from different sources may cause this. However, the reasons for the differentiation in the Caucasian honey bee populations under protection in an isolated region should be investigated.

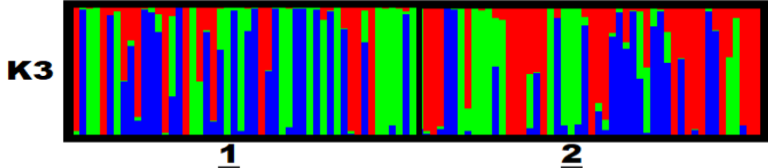


Figure 1. Genetic cluster analysis of Artvin and Ardahan populations. K represents the number of groups (1, Artvin; 2, Ardahan). The length of the colored bar represents the individual's membership coefficient in the cluster, according to cluster analysis.

As a result of the FCA analysis performed to reveal the phylogenetic relationships between the populations in three dimensions, the Artvin and Ardahan populations were separated from each other despite a pairwise F_{ST} value of 0.02 and interindividual variation was evident (Figure 2). The first axis explains 100% of the total variation.

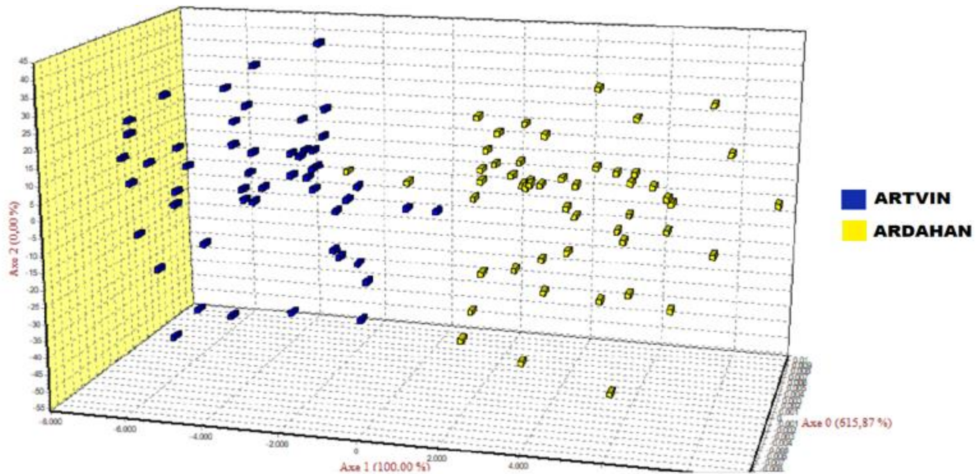


Figure 2. Factorial correspondence analysis of the Artvin and Ardahan populations on 30 polymorphic microsatellite loci. Each box represents a colony; the blue ones are Artvin and the yellow ones are Ardahan.

TPM is the most useful model, as mutations for microsatellite loci often do not yield consistent results with IAM or SMM (Dirienzo et al., 1994; Luikart et al., 1998; Piry et al., 1999; Fatima, 2006). TPM uses both IAM and SMM models together. Given that there are more than 20 loci, there appears to be a bottleneck in Caucasian honey bee populations according to Standardized differences test in the TPM model (Table 4) (Cornuet & Luikart, 1996). When the populations are evaluated separately as Artvin and Ardahan provinces, it is seen that the bottleneck is only present in Artvin (p<0.001), and there has not been a bottleneck in Ardahan recently (p>0.05).

The studied populations interestingly fit the GW index and the normal L-shaped distribution when the allele frequency distribution was analyzed with the qualitative graphical method defined by Luikart and Cornuet (1998) (Figure 3). However, the time and size of the bottleneck are effective in detecting a mode-shifted distribution. The fact that the bottleneck is not new or in the amount that can't be detected prevents a distorted distribution (Luikart et al., 1998; Wu et al., 2020). In addition, populations deviated from Hardy-Weinberg equilibrium (HWE) as a result of the disruption of genetic balance (p<0.01) (Table 3). Although deviation from HWE can be caused by many factors such as inbreeding, mutation and migration (Robertson & Hill, 1984), the deviation here supports the idea of the bottleneck brought about by inbreeding. The allele frequency distribution was analyzed graphically to determine whether it showed an L-shaped distribution. The microsatellite alleles were divided into 10 frequency classes, allowing us to determine whether the distribution shows the normal L-shaped form with an abundance of low-frequency alleles (0.01 to 0.1) (Luikart et al.,1998).

Table 4. Bottleneck analysis using two-phase mutation model

Statistical tests		TPM		
		Artvin population	Ardahan population	All populations
Sign test	EHE	17.75	17.74	17.63
	HD	18.00	16.00	25.00
	HE	12.00	14.00	5.00
	P	0.02658	0.11525	0.00000
Standardized differences test	T2	-3.130	-1.092	-7.683
	P	0.00087	0.13736	0.00000
Wilcoxon's signed rank test	HD (P)	0.02245	0.17461	0.00000
	HE (P)	0.97867	0.83063	1.00000
	HDE (P)	0.04491	0.34921	0.00001

* TPM, two-phased model; EHE, expected number of loci with heterozygosity excess; HD, one tail heterozygosity deficiency; HE, one tail heterozygosity excess; HDE, two tails for heterozygosity excess or deficiency; T2, standardized differences test. Positive values of the bottleneck statistic T2 are indicative of gene diversity excess caused by a recent reduction in effective population size, while negative values are consistent with a recent population expansion without immigration or immigration of some private alleles in the population.

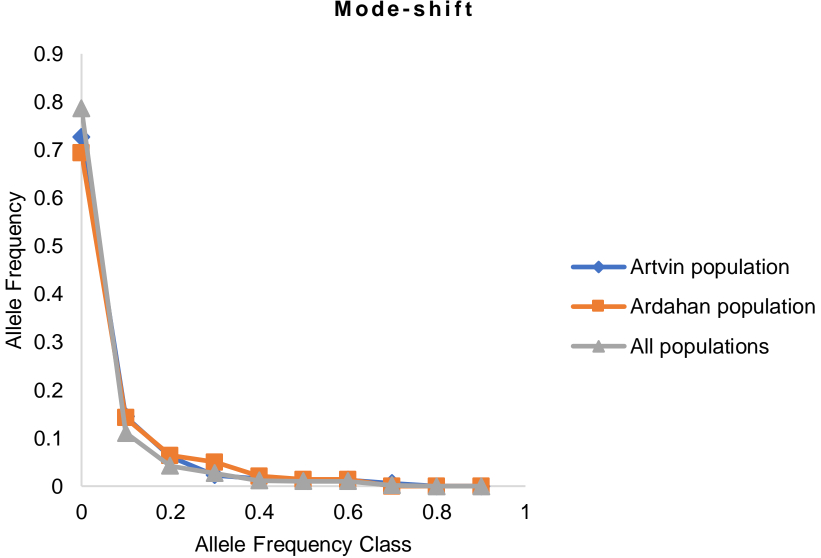


Figure 3. Allele frequency distribution of Artvin, Ardahan and all populations.

Such genetic bottlenecks are mostly associated with zoos, forest conditions, or relatively small and limited population size in isolated areas (Zhang et al., 2002; Luenser et al., 2005; Mukherjee et al., 2022). Although officially registered in Artvin and Ardahan provinces 138 362 hives (Gül & Nergiz, 2022); looking at the variation source in the Caucasian bee population, it was found that variation is insufficient and the variations come from among individuals, not among the populations. In the current situation, it would not be wrong to point out that there is no bee entry to the isolated area within the scope of the protection plan, that the queen bee is constantly supplied from the same source and that a bottleneck occurred after an inbreeding pressure caused by this. On the other hand, there are almost no studies on genetic factors, which are one of the most important factors affecting the quality of these queen bees, which are constantly supplied from the same source (Arslan et al., 2021). The founder effect, which is caused by the continuous use of queen bees from the same source, can also be shown as the cause of the bottleneck (Jamieson, 2011).

Bottlenecked populations have lost or are in danger of losing rare alleles, but there may still be some degree of heterozygosity (Luikart et al., 1998; Furlan et al., 2012; Ganapathi et al., 2012). Decreased genetic diversity and continued inbreeding can affect viability due to selection pressure as populations shrink in size (Al-Atiyat, 2008).

There are very few genetic characterization studies conducted on the Caucasian bee populations in Türkiye. Studies in the literature have reported that the Caucasian bee is in hybrid form in some regions and is in danger of losing its purity (Bodur, 2005; Kırpık et al., 2010). In this study, the genetic status of the Caucasian bee in the isolated regions of Artvin and Ardahan was determined. The results of the study show that the populations in the isolated region are generally Caucasian bees, but genetic diversity is beginning to be lost due to intensive inbreeding and there is evidence of genetic bottleneck in the Artvin population. However, it is understood from the Cluster and FCA analyses results that Artvin and Ardahan colonies differ in at least one locus. The reason for this may be that Artvin and Ardahan regions have different geographical and climatic environments and that the colonies here provide their queen bee needs from different sources. There is one queen bee production enterprise officially registered with the Ministry of Agriculture in the region, but there are also many unregistered practices that are not reported by beekeepers in the isolated region. Firstly, it is recommended to develop long-term strategies for programs that will reduce inbreeding and protect genetic diversity by increasing queen bee production enterprises in this isolated region. In addition, it is necessary to produce scientific outputs by ensuring that beekeeping activities in the region are wholly recorded and published regularly.

Acknowledgement

We thank The Republic of Türkiye Ministry of Agriculture. The project was supported by HAYGEM with project number 2021A03-168656.

References

Al-Atiyat, R. M., 2008. Extinction probabilities of Jordan indigenous cattle using population viability analysis. Livestock Science, 123 (2-3): 121-128.

Arias, M. C., T. E. Rinderer & W. S. Sheppard, 2006. Further characterization of honey bees from the Iberian Peninsula by allozyme, morphometric and mtDNA haplotype analyses. Journal of Apicultural Research, 45 (4): 188-196.

Arslan, S., M. M. Cengiz, A. Gül & S. Sayed, 2021. Evaluation of the standards compliance of the queen bees reared in the Mediterranean region in Turkey. Saudi Journal of Biological Sciences, 28 (5): 2686-2691.

Belkhir, K., P. Borsa, L. Chikhi, N. Raufaste & F. Bonhomme, 1996-2004. GENETIX, logiciel sous Windows™ pour la génétique des populations. Laboratoire Genome, Populations, Interactions, CNRS UMR 5000, Université de Montpellier II, Montpellier (France).

Bodur, C., 2005. Genetic Structure Analysis of Honeybee Populations based on Microsatellites. Middle East Technical University, (Unpublished) Ph.D. Thesis, Ankara, 116 pp.

Bodur, C., M. Kence & A. Kence, 2007. Genetic structure of honey bee, *Apis mellifera* L. (Hymenoptera: Apidae) populations of Turkey inferred from microsatellite analysis. Journal of Apicultural Research, 46 (1): 50-56.

Botstein, D., R. L. White, M. Skolnick & R. W. Davis, 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American journal of human genetics, 32 (3): 314-331.

Bouga, M., C. Alaux, M. Bienkowska, R. Büchler, N. L. Carreck, E. Cauia, R. Chlebo, B. Dahle, R. Dall'Olio, P. De la Rúa, A. Gregorc, E. Ivanova, A. Kence, M. Kence, N. Kezic, H. Kiprijanovska, P. Kozmus, P. Kryger, Y. Le Conte, M. Lodesani, A. M. Murilhas, A. Siceanu, G. Soland, A. Uzunov & J. Wilde, 2011. A review of methods for discrimination of honey bee populations as applied to European beekeeping. Journal of Apicultural Research, 50 (1): 51-84.

Castric, V., L. Bernatchez, K. Belkhir & F. Bonhomme, 2002. Heterozygote deficiencies in small lacustrine populations of brook charr Salvelinus fontinalis Mitchill (Pisces, Salmonidae): a test of alternative hypotheses. Heredity, 89 (1): 27-35.

Cornuet, J. M. & G. Luikart, 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics, 144 (4): 2001-2014.

Dall'Olio, R., A. Marino, M. Lodesani & R. F. A. Moritz, 2007. Genetic characterization of Italian honeybees, *Apis mellifera ligustica*, based on microsatellite DNA polymorphisms. Apidologie, 38 (2): 207-217.

Dirienzo, A., A. C. Peterson, J. C. Garza, A. M. Valdes, M. Slatkin & N. B. Freimer, 1994. Mutational processes of simple-sequence repeat loci in human-populations. Proceedings of National Academy of Sciences, 91 (8): 3166-3170.

Excoffier, L., G. Laval & S. Schneider, 2007. ARLEQUIN (version 3.0): an integrated software package for population genetics data analysis. Evolutionary Bioinformatics Online, 23 (1): 47-50.

Excoffier, L., P. E. Smousse & J. M. Quattro, 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics, 131 (2): 479-491.

Fatima, S., 2006. Study of Genetic Variability Among Gohilwadi, Surti and Zalawadi Goats Using Microsatellite Analysis. Anand Agriculture University, (Unpublished) Master Thesis, Anand, 120 pp.

Fıratlı, Ç. & E. Budak, 1994. Türkiye'de Çeşitli Kurumlarda Yetiştirilen Ana Arılar ile Oluşturulan Bal Arısı *Apis mellifera* L. Kolonilerinin Fizyolojik Morfolojik ve Davranış Özellikleri. Ankara Üniversitesi Ziraat Fakültesi, (Unpublished) Ph.D. Thesis, Ankara, 117 pp (in Turkish).

Franck P., L. Garnery, G. Celebrano, M. Solignac & J. M. Cornuet, 2000. Hybrid origins of honeybees from Italy (*Apis mellifera ligustica*) and Sicily (*A. m. sicula*). Molecular Ecology, 9 (7): 907-921.

Franck, P., L. Garnery & A. Loiseau, 2001. Genetic diversity of the honeybee in Africa: microsatellite and mitochondrial data. Heredity, 86 (4): 420-430.

Furlan, E., J. Stoklosa, J. Griffiths, N. Gust, R. Ellis, R. M. Huggins & A. R. Weeks, 2012. Small population size and extremely low levels of genetic diversity in island populations of the platypus, *Ornithorhynchus anatinus*. Ecology and evolution, 2 (4): 844-857.

Ganapathi, P., R. Rajendran & P. Kathiravan, 2012. Detection of occurrence of a recent genetic bottleneck event in Indian hill cattle breed Bargur using microsatellite markers. Tropical Animal Health and Production, 44 (8): 2007-2013.

Garnery, L., P. Franck, E. Baudry, D. Vautrin, J. M. Cornuet & M. Solignac, 1998. Genetic diversity of the west European honey bee (*Apis mellifera mellifera* and *A. m. iberica*) II. Microsatellite loci. Genetics Selection Evolution, 30 (1): 1-26.

Gül, A. & R. Nergiz, 2022. Kafkas Bal Arısı (*Apis mellifera caucasia*) gen merkezinin bozulmasına neden olan etmenler ve çözüm önerileri. Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Dergisi, 25 (2): 545-554 (in Turkish with abstract in English).

Haddad, N. J., N. Adjlane, D. Saini, A. Menon, V. Krishnamurthy, D. Jonklaas, J. P. Tomkins, W. L. Ayad & L. Horth, 2018. Whole genome sequencing of north African honey bee *Apis mellifera intermissa* to assess its beneficial traits. Entomological Research, 48 (3): 174-186.

Hassett, J., K. A. Browne, G. P. McCormack, E. Moore, N. I. H. B. Society, G. Soland & M. Geary, 2018. A significant pure population of the dark European honey bee (*Apis mellifera mellifera*) remains in Ireland. Journal of Apicultural Research, 57 (3): 337-350.

Ivanova, E. N., T. A. Staykova & M. Bouga, 2007. Allozyme variability in honey bee populations from some mountainous regions in the southwest of Bulgaria. Journal of Apicultural Research, 46 (1): 3-7.

Ivgin Tunca, R., 2009. Determination and Comparison of Genetic Variation in Honey Bee (*Apis mellifera* L.) Populations of Turkey by Random Amplified Polymorphic DNA and Microsatellite Analyses. Middle East Technical University, (Unpublished) Ph.D. Thesis, Ankara, 152 pp.

Jamieson, I. G., 2011. Founder effects, inbreeding, and loss of genetic diversity in four avian reintroduction programs. Conservation Biology, 25 (1): 115-123.

Kalinowski, S. T. & M. L. Taper, 2006. Maximum likelihood estimation of the frequency of null alleles at microsatellite loci. Conservation Genetics, 7 (6): 991-995.

Kandemir, I. & A. Kence, 1995. Allozyme variability in a central Anatolian honey bee (*Apis mellifera* L.) population. Apidologie, 26 (6): 503-510.

Kandemir, I., M. Kence & A. Kence, 2000. Genetic and morphometric variation in honeybee (*Apis mellifera* L.) populations of Turkey. Apidologie, 31 (3): 343-356.

Kandemir, I., M. Kence, W. S. Sheppard & A. Kence, 2006. Mitochondrial DNA variation in honey bee (*Apis mellifera* L.) populations from Turkey. Journal of Apicultural Research, 45 (1): 33-38.

Karabağ, K., R. I. Tunca, E. Tüten & T. Doğaroğlu, 2020. Current genetic status of honey bees in Anatolia in terms of thirty polymorphic microsatellite markers. Turkish Journal of Entomology, 44 (3): 333-346.

Kekeçoğlu, M., M. Bouga, P. Harizanis & M. I. Soysal, 2009. Genetic divergence and phylogenetic relationships of honey bee populations from Turkey using PCR-RFLP's analysis of two mtDNA segments. Bulgarian Journal of Agricultural Science, 15 (6): 589-597.

Kırpık, M. A., O. Bututaki & D. Tanrikulu, 2010. Determining the relative abundance of honey bee (*Apis mellifera* L.) races in Kars plateau and evaluating some of their characteristics. Kafkas Universitesi Veteriner Fakultesi Dergisi, 16 (1): 27-282.

Kuo, C. H. & F. J. Janzen, 2004. Genetic effects of a persistent bottleneck on a natural population of ornate box turtles (Terrapene ornata). Conservation Genetics, 5 (4): 425-437.

Liu, F., T. Shi, S. Huang, L. Yu & S. Bi, 2016. Genetic structure of Mount Huang honey bee (*Apis cerana*) populations: evidence from microsatellite polymorphism. Hereditas, 153 (8): 1-6.

Luenser, K., J. Fickel, A. Lehnen, S. Speck & A. Ludwig, 2005. Low level of genetic variability in European bisons (*Bison bonasus*) from the Bialowieza National Park in Poland. European Journal of Wildlife Research, 51 (2): 84-87.

Luikart, G., F. W. Allendorf, J. M. Cornuet & W. B. Sherwin, 1998. Distortion of allele frequency distributions provides a test for recent population bottlenecks. Journal of Heredity, 89 (3): 238-247.

Mielnik-Sikorska, M., P. Daca, B. Malyarchuk, M. Derenko, K. Skonieczna, M. Perkova, T. Dobosz & T. Grzybowski, 2013. The history of Slavs inferred from complete mitochondrial genome sequences. Plos One, 8 (1): e54360.

Mukherjee, S., A. Mukherjee, S. Kumar, H. Verma, S. Bhardwaj, O. Togla, S. N. Joardar, I. Longkumer, M. Mech, K. Khate, K. Vupru, M. H. Khan, S. Kumar & C. Rajkhowa, 2022. Genetic characterization of endangered Indian mithun (*Bos frontalis*), Indian bison/wild gaur (*Bos gaurus*) and tho-tho cattle (*Bos indicus*) populations using SSR markers reveals their diversity and unique phylogenetic status. Diversity, 14 (7): 548.

Nawrocka, A., I. Kandemir, S. Fuchs & A. Tofilski, 2018. Computer software for identification of honey bee subspecies and evolutionary lineages. Apidologie, 49 (2): 172-184.

Nei, M., 1972. Genetic distance between populations. The American Naturalist, 106 (949): 283-292.

Oleksa, A. & A. Tofilski, 2015. Wing geometric morphometrics and microsatellite analysis provide similar discrimination of honey bee subspecies. Apidologie, 46 (1): 49-60.

Özdil, F., D. Oskay, R. Işık, S. Yatkın, A. Aydın & A. Güler, 2022. Morphometric and genetic characterization of honey bees (L.) from thrace region of Turkey. Journal of Apicultural Science, 66 (1): 67-83.

Özdil, F., M. A. Yildiz & H. G. Hall, 2009. Molecular characterization of Turkish honey bee populations (*Apis mellifera* L.) inferred from mitochondrial DNA RFLP and sequence results. Apidologie, 40 (5): 570-576.

Park, S. D. E., 2001. The Excel Microsatellite-Toolkit. Animal Genomics Lab, University of College Dublin, Ireland.

Piry, S., G. Luikart & J. M. Cornuet, 1999. BOTTLENECK: A computer program for detecting recent reductions in the effective population size using allele frequency data. Journal of Heredity, 90 (4): 502-503.

Pritchard, J. K., M. Stephens & P. Donnelly, 2000. Inference of population structure using multilocus genotype data. Genetics, 155 (2): 945-959.

Rahimi, A., A. Mirmoayedi, D. Kahrizi, L. Zarei & S. Jamali, 2016. Genetic diversity of Iranian honey bee (*Apis mellifera* Skorikow, 1829) populations based on ISSR markers. Cellular Molecular Biology, 62 (4): 53-58.

Rinderer, T. E., J. W. Harris, G. J. Hunt & L. I. de Guzman, 2010. Breeding for resistance to *Varroa destructor* in north America. Apidologie, 41 (3): 409-424.

Robertson, A. & W. G. Hill, 1984. Deviations from Hardy-Weinberg proportions: sampling variances and use in estimation of inbreeding coefficients. Genetics, 107 (4): 703-718.

Ruttner, F., 1988. Biogeography and Taxonomy of Honey Bees. Springer-Verlag, Berlin, Germany, 284 pp.

Simon, D. L. & D. Buchenauer, 1993. Genetic Diversity of European Livestock Breeds. European Association for Animal Production Publication, Wageningen, Netherlands, 581 pp.

Smith, D. R., A. Slaymaker, M. Palmer & O. Kaftanoglu, 1997. Turkish honey bees belong to the east Mediterranean mitochondrial lineage. Apidologie, 28 (5): 269-274.

Solignac, M., D., A. Vautrin, A. Loiseau, F. Mougel, E. Baudry, A. Estoup, L. Garnery, M. Haberl & J. M. Cornuet, 2003. Five hundred and fifty microsatellite markers for the study of the honey bee (*Apis mellifera* L.) genome. Molecular Ecology Notes, 3 (2): 307-311.

Wang, H., S. Gao, Y. Liu, P. Wang, Z. Zhang & D. Chen, 2022. A pipeline for effectively developing highly polymorphic simple sequence repeats markers based on multi-sample genomic data. Ecology and Evolution, 12 (3): e8705.

Weir, B. S. & C. C. Cockerham, 1984. Estimating F-statistics for the analysis of population structure. Evolution, 38 (6): 1358-1370.

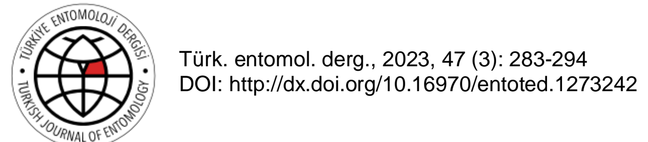
Wu, Q., F. Zang, Y. Ma, Y. Zheng & D. Zang, 2020. Analysis of genetic diversity and population structure in endangered *Populus wulianensis* based on 18 newly developed EST-SSR markers. Global Ecology and Conservation, 24 (7): e01329.

Yeh, F. C., R. C. Yang, T. B. J. Boyle, Z. H. Ye & J. X. Mao, 1997. POPGENE, the user-friendly Shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada.

Yu, Y., S. Zhou, X. Zhu, X. Xu, W. Wang, L. Zha, P. Wang, J. Wang, K. Lai, S. Wang, L. Hao & B. Zhou, 2019. Genetic differentiation of Eastern honey bee (*Apis cerana*) populations across Qinghai-Tibet Plateau-Valley Landforms. Frontiers in Genetics, 10 (483): 1-11.

Zhang, Y. P., X. X. Wang, O. A. Ryder, H. P. Li, H. M. Zhang, Y. Yong & P. Y. Wang, 2002. Genetic diversity and conservation of endangered animal species. Pure and Applied Chemistry, 74 (4): 575-584.

Zimmerman, S. J., C. L. Aldridge & S. J. Oyler-McCance, 2020. An empirical comparison of population genetic analyses using microsatellite and SNP data for a species of conservation concern. BMC Genomics, 21 (1): 1-16.


Türk. entomol. derg., 2023, 47 (3): 283-294
DOI: http://dx.doi.org/10.16970/entoted.1273242

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

The nematode assemblages of a lake ecosystem (Lake Korugöl Natural Park, Düzce, West Black Sea Cost of Türkiye): ecology and biodiversity patterns with first reports of 10 genera to the Türkiye nematofauna

Bir göl ekosisteminin nematod toplulukları (Korugöl Gölü Tabiat Parkı, Düzce, Türkiye): Türkiye nematofaunası için 10 cinsin ilk raporları ile ekoloji ve biyoçeşitlilik modelleri

Taylan ÇAKMAK[1*]

Abstract

This research was conducted to reveal the soil nematode fauna of Korugöl, Düzce and to contribute to the nematode diversity of the Western Black Sea Region of Türkiye. Field studies within the scope of the study were implemented in 2021. As a result of the study, 29 families, 45 genera and 46 nematode taxa were identified. 10 nematode taxon, namely Aporcelinus, Baladorylaimus, Dorylaimoides, Euteratocephalus, Labronemella, Laimydorus, Lindseyus, Metateratocephalus, Paractinolaimus and Tripylella are the first report for Türkiye's nematofauna. Classification of nematodes according to their feeding types were: 27.27% bacterivorous nematodes, 2.10% fungivoresnematodes, 1.51% herbivorous nematodes, 16.35% predator nematodes and 52.77% omnivorous nematodes.

Keywords: Biodiversity, coastal ecosystems, nematofauna, soil nematodes, Türkiye

Öz

Bu araştırma, Düzce Korugöl toprak nematod faunasını ortaya çıkarmak ve Türkiye'nin Batı Karadeniz Bölgesi nematod çeşitliliğine katkıda bulunmak amacıyla yapılmıştır. Çalışma kapsamında arazi çalışmaları 2021 yılında yapılmıştır. Çalışma sonucunda 29 familya ve 45 cinse ait 46 nematod taksonu tespit edilmiştir. Aporcelinus, Baladorylaimus, Dorylaimoides, Euteratocephalus, Labronemella, Laimydorus, Lindseyus, Metateratocephalus, Paractinolaimus ve Tripylella olmak üzere 10 nematod taksonu, Türkiye nematod faunası için ilk rapordur. Nematodların beslenme şekillerine göre sınıflandırılması: %27,27 bakteriovor, %2,10 fungivor, %1,51 herbivor, %16,35 predatör ve %52,77 omnivor nematodlar olarak tespit edilmiştir.

Anahtar sözcükler: Biyoçeşitlilik, kıyı ekosistemleri, nematofauna, toprak nematodları, Türkiye

[1] Düzce University, Faculty of Agriculture, Department of Agricultural Biotechnology, 81620, Düzce, Türkiye
* Corresponding author (Sorumlu yazar) e-mail: cakmaktaylan@gmail.com
Received (Alınış): 29.03.2023 Accepted (Kabul ediliş): 25.09.2023 Published Online (Çevrimiçi Yayın Tarihi): 04.10.2023

Introduction

Nematodes are animals with a wide variety of life strategies and feeding habits which comprise an important part of ecdysozoan. These varied life strategies of terrestrial nematodes have a critical impact on the food webs of the soil. To date, there are currently 25,000 to 30,000 identified species of nematodes, being the most diverse group with 1,000,000 estimated species number (Hugot et al., 2001). Free-living nematodes are the most abundant group of terrestrial habitats, which can be found in soils and freshwater sediments (Yeates et al., 1993). Parasitic species which are obligate to plants and animals (even man) cause worldwide damage and diseases (Lee, 2002). Their tremendous diversity, great abundance and survival ability give scientists a great chance to assess soil health via bio-indicator activity (Wilson & Kakouli-Duarte, 2009). Several biodiversity indices (Bongers, 1990; Bongers et al., 1997; Ferris & Bongers, 2009) have been used to monitor natural or human-impacted areas in terms of soil health (Bongers & Ferris, 1999). Korugöl is a mesotrophic lake, located in the Western Black Sea region of Anatolia within the borders of Düzce Province Merkez District and Kaynaşlı District, Türkiye, covering an area of 4.87 hectares at the altitude 480 m above sea level. It is first time that nematodes are being discovered from the zone with this contribution. This study was designed to characterize the soil nematode fauna and to monitor the dispersal of nematode groups that naturally occur in a lake ecosystem.

Materials and Methods

Sampling

A sampling survey was organized at Korugöl Natural Park, Düzce, Türkiye in September 2021. The sampling was done regarding to surrounding area of the lake including forestall and coastal soil habitats. A total of five different eco-habitats, namely marshland, meadow, *Quercus petraea* L., riverbed and *Fagus orientalis* L. trees (Figure 1, Table 1) were chosen as sampling sites.

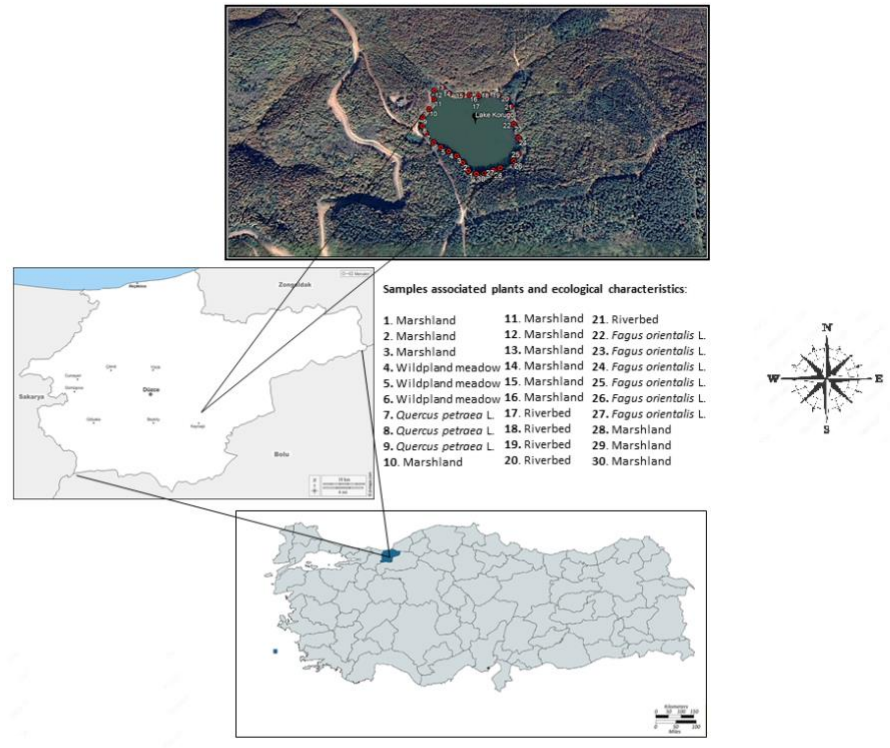


Figure 1. Location of Korugöl and sampling sites.

A total number of 30 samples were collected. Soil samples were collected from an area of 15 x 15 cm soil covering plots and digged up to 30 cm soil depth from each plot. Soil samples were stored into plastic bags, protected during the transport to the nematology laboratory with a thermo cooling bag and stored at +4°C. Then, the samples were prepared for the extraction of nematodes.

Table 1. The list of sampling sites studied in Korugöl Lake, Düzce, Türkiye

Sample Number	Habitat	UtmX	UtmY
1	Marshland	40°48'26.05"N	31°17'36.49"E
2	Marshland	40°48'26.74"N	31°17'35.94"E
3	Marshland	40°48'27.33"N	31°17'35.30"E
4	Wildpland meadow	40°48'27.75"N	31°17'34.50"E
5	Wildpland meadow	40°48'28.08"N	31°17'33.67"E
6	Wildpland meadow	40°48'28.53"N	31°17'32.91"E
7	*Quercus petraea* L.	40°48'29.35"N	31°17'32.04"E
8	*Quercus petraea* L.	40°48'30.04"N	31°17'31.61"E
9	*Quercus petraea* L.	40°48'30.91"N	31°17'31.56"E
10	Marshland	40°48'31.70"N	31°17'32.18"E
11	Marshland	40°48'32.70"N	31°17'32.63"E
12	Marshland	40°48'33.61"N	31°17'32.60"E
13	Marshland	40°48'33.98"N	31°17'33.49"E
14	Marshland	40°48'33.29"N	31°17'34.26"E
15	Marshland	40°48'33.10"N	31°17'35.65"E
16	Marshland	40°48'33.17"N	31°17'36.46"E
17	Riverbed	40°48'33.02"N	31°17'37.45"E
18	Riverbed	40°48'33.15"N	31°17'38.43"E
19	Riverbed	40°48'33.15"N	31°17'39.63"E
20	Riverbed	40°48'32.79"N	31°17'40.62"E
21	Riverbed	40°48'31.95"N	31°17'40.95"E
22	*Fagus orientalis* L.	40°48'31.15"N	31°17'40.75"E
23	*Fagus orientalis* L.	40°48'30.23"N	31°17'41.16"E
24	*Fagus orientalis* L.	40°48'28.93"N	31°17'41.58"E
25	*Fagus orientalis* L.	40°48'27.49"N	31°17'41.48"E
26	*Fagus orientalis* L.	40°48'26.87"N	31°17'40.98"E
27	*Fagus orientalis* L.	40°48'26.29"N	31°17'39.62"E
28	Marshland	40°48'26.09"N	31°17'38.95"E
29	Marshland	40°48'25.82"N	31°17'38.04"E
30	Marshland	40°48'25.78"N	31°17'37.27"E

Coastal ecosystems of Lake Korugöl

The studied area was a lake ecosystem. Vegetation varies widely from annuals to perennials around the lake. The flora around the lake consists of forest trees such as European black pine (*Pinus nigra* J.F. Arnold), beech (*F. orientalis*), oak (*Quercus robur* Asso) and hornbeam (*Carpinus betulus* L.) as the dominant tree species of Düzce province forests. Some of the forests around the lake are afforestation areas and coniferous trees have been planted. The terrestrial fauna around the lake is diverse in terms of forest structure. As it is known to be essential for soil biodiversity which depends on soil profile type, the soil type around the Korugöl lake has Class I alluvial soil. Alluvial soils are flat, nearly flat inclined, (A) C profile, azonal soils located at the bottoms of surface waters or on young sediments deposited by rivers (Keleş, 2006).

Nematode Extraction

Modified Baermann's (1917) funnel technique using petri dishes (12 cm ∅) was used during the extraction of nematodes. After separating rocks, a 100 g of fresh soil from each sampling site was evaluated. Plastic trays lined with paper towels were used for extraction of nematodes and were placed in

the nematology laboratory for 48 hours. Extracted vermiform terrestrial nematodes were collected after 48 hours. Nematode suspensions were heated up to 60°C for killing before fixation. Formalin solution of 4% was used for fixation and preservation of nematodes until preparing permanent glass slides. Extractions were labeled with the relevant sample number, transferred to plastic tubes, and stored at Düzce University Nematology Laboratory. The rest of the soil samples were also stored in the soil laboratory for having a backup requirement in case of future studies. The nematodes were identified immediately. The preparation of nematodes for permanent mounts were processed. Total of 855 nematodes were mounted. Collected nematode samples are stored in Düzce University Faculty of Agriculture Agricultural Biotechnology Nematode Collection.

Permanent glass slide preparation

Double distilled water was used to rinse nematodes and avoid soil debris after picking up nematodes from all samples. Each sample were put into staining blocks with 1.25 cm depth containing 96% ethanol with the extracted nematodes and placed in an incubator at 40°C. A few drops of glycerol: formalin (4%) (1:99) were added into staining block. A few drops of a solution of glycerol: ethanol (five parts glycerol and 95 parts of 96% ethanol) were added in the next morning, two-thirds of staining block's cavity was covered with a glass square. On day 2, a few drops of glycerol: ethanol (5:95) solution were added every two hours so that glycerol can transudate gradually for covering nematodes. The staining of nematodes process was continued by adding glycerol of two drops: ethanol (50:50) into the staining block at the end of the second day. One day later, all nematodes were found to be covered by glycerol (100%) before proceeding to make permanent glass slides preparation (Yoder et al., 2006).

Nematode Identification and analysis of ecological parameters

Nematodes were identified manually by using an Olympus CH microscope (Olympus Optical, Tokyo, Japan). Classification of nematodes were determined by the taxonomical key that was provided by De Ley & Blaxter (2005). Additional taxonomical data was included from Hodda et al. (2006) and Andrássy (2002, 2005, 2009). Nematodes were identified mostly down to genus level. Coloniser-persister classification of nematode life cycle properties (1-5) were obtained from Bongers (1990, 1999). Nematode feeding types classification was established according to Yeates et al. (1993). Structure index and Enrichment index were calculated according to Ferris et al. (2001) and Ferris & Bongers (2009) in order to obtain the maturity degree of nematode community composition in the ecosystem. Nematode Indicator Joint Analysis calculation system (Sieriebriennikov et al., 2014) was used to analyze food web structure, feeding type diagnostics and MI family indices. Shannon -Wiener index (Shannon & Weaver, 1949) was used for diversity index.

Results

The total number of identified nematodes have reached up to 855 individuals (number of females: 455; male: 83 and juvenile: 317). They belong to 46 species, 45 genera, 29 families, and 8 orders (Table 2).

Table 2. Nematode taxa collected in this study from Korugöl Lake, Düzce, Türkiye

Class	Order/Suborder	Number of Families	Number of Genera	Number of Specimen
Enoplea	Enoplida	2	3	52
	Mononchida	2	4	55
	Dorylaimida	11	16	293
	Triplonchida	2	2	119
Chromadorea	Monhysterida	1	2	43
	Plectida	5	6	197
	Rhabditina	2	2	15
Rhabditida	Tylenchina	4	10	81
Total	8	29	45	855

Nematodes were found at all sampling sites (480 m a.s.l. approx.). Besides, the total nematode abundance showed great variability among samples. The average number of nematodes per 100 gr of soil from the sampling sites were 10 to 56 individuals (Table 3).

Table 3. Abundance and occurrence rates of nematode genera at Korugöl Lake, Düzce, Türkiye

Genus Name	Family Name	Total Abundance	Relative Abundance (%)	Occurrence (%)	C-P Class	P-P Class	Feeding Type
Aporcelaimellus Heyns, 1965	Aporcelaimidae	135	15.79	86.67	5	0	Predators
Prismatolaimus Micoletzky, 1922	Prismatolaimidae	114	13.33	73.33	3	0	Bacterivores
Plectus Bastian, 1865	Plectidae	103	12.05	86.67	2	0	Bacterivores
Anaplectus Coninck & Schuurmans Stekhoven, 1933	Plectidae	87	10.18	86.67	2	0	Bacterivores
Tripylella Brzeski & Winiszewska-Slipinska, 1993	Tripylidae	51	5.96	36.67	3	0	Predators
Eudorylaimus Andrássy, 1959	Dorylaimidae	46	5.38	63.33	4	0	Predators
Tylencholaimus De Man, 1876	Tylencholaimidae	45	5.26	60.00	4	0	Fungivores
Geomonhystera Andrássy, 1981	Monhysteridae	39	4.56	53.33	2	0	Bacterivores
Clarkus Jairajpuri, 1970	Mononchidae	31	3.63	46.67	4	0	Predators
Coslenchus Siddiqi, 1978	Tylenchidae	29	3.39	33.33	0	2	Herbivores - epidermal/root hair feeders
Malenchus Andrassy, 1968	Tylenchidae	19	2.22	40.00	0	2	Herbivores - epidermal/root hair feeders
Mononchus Bastian, 1865	Mononchidae	18	2.11	33.33	4	0	Predators
Pungentus Thorne & Swanger, 1936	Nordiidae	17	1.99	36.67	4	0	Omnivores
Panagrolaimus Fuchs, 1930	Panagrolaimidae	13	1.52	26.67	1	0	Bacterivores
Nygolaimus Cobb, 1913	Nygolaimidae	12	1.40	16.67	5	0	Predators
Belondira Thorne, 1939	Belondiridae	9	1.05	23.33	0	5	Herbivores - ectoparasites
Filenchus Andrassy, 1954	Tylenchidae	9	1.05	16.67	2	0	Fungivores
Eucephalobus Steiner, 1936	Cephalobidae	8	0.94	23.33	2	0	Bacterivores
Tylencholaimellus Cobb, 1915	Tylencholaimellidae	8	0.94	10.00	4	0	Fungivores
Labronemella Andrássy, 1985	Qudsianematidae	6	0.70	3.33	4	0	Omnivores
Tobrilus De Man, 1879		5	0.58	16.67	3	0	Predators
Cephalobus Bastian, 1865	Cephalobidae	4	0.47	10.00	2	0	Bacterivores
Criconema Hofmanner & Menzel, 1914	Criconematidae	4	0.47	10.00	0	3	Herbivores - ectoparasites
Eumonhystera Andrássy, 1981	Monhysteridae	4	0.47	10.00	2	0	Bacterivores
Laimidorus Siddiqi, 1969	Dorylaimidae	4	0.47	13.33	4	0	Omnivores
Baladorylaimus Andrassy, 2001	Dorylaimidae	3	0.35	3.33	4	0	Predators
Hoplolaimus von Daday, 1905	Hoplolaimidae	3	0.35	6.67	0	3	Herbivores - semi-endoparasites
Metateratocephalus Eroshenko, 1973	Metateratocephalidae	3	0.35	6.67	3	0	Bacterivores
Mylonchulus Cobb, 1916	Mylonchulidae	3	0.35	6.67	4	0	Predators
Prionchulus (Cobb, 1916) Wu & Hoeppli, 1929	Mononchidae	3	0.35	10.00	4	0	Predators

Table 3. Continued

Genus Name	Family Name	Total Abundance	Relative Abundance (%)	Occurrence (%)	C-P Class	P-P Class	Feeding Type
Tylenchus Bastian, 1865	Tylenchidae	3	0.35	6.67	0	2	Herbivores - epidermal/root hair feeders
Anonchus Cobb, 1913	Aphanolaimidae	2	0.23	3.33	3	0	Bacterivores
Aporcelinus (Cobb, 1893) Andrassy, 2009	Aporcelaimidae	2	0.23	6.67	5	0	Omnivores
Dorylaimoides Thorne & Swanger, 1936	Mydonomidae	2	0.23	3.33	4	0	Omnivores
Rhabditis Dujardin, 1845	Rhabditidae	2	0.23	3.33	1	0	Bacterivores
Acrobeles von Linstow, 1877	Cephalobidae	1	0.12	3.33	2	0	Bacterivores
Alaimus de Man, 1880	Alaimidae	1	0.12	3.33	4	0	Bacterivores
Cervidellus Thorne, 1937	Cephalobidae	1	0.12	3.33	2	0	Bacterivores
Chronogaster Cobb, 1913	Chronogastridae	1	0.12	3.33	3	0	Bacterivores
Dorylaimellus Cobb, 1913	Belondiridae	1	0.12	3.33	0	5	Herbivores - ectoparasites
Euteratocephalus Andrassy, 1968	Metateratocephalidae	1	0.12	3.33	3	0	Bacterivores
Lindseyus Ferris & Ferris, 1973	Belondiridae	1	0.12	3.33	5	0	Predators
Mesodorylaimus Andrássy, 1959	Dorylaimidae	1	0.12	3.33	4	0	Omnivores
Paractinolaimus Meyl, 1957	Actinolaimidae	1	0.12	3.33	5	0	Predators
TOTAL		855					

Diversity and community analysis

Shannon-Wiener index was calculated for the30 sampling sites. The average value was 1.95±0.27. Obtained results from Shannon-Wiener index showed higher diversity between ecological characteristics of the sampling sites. The highest biodiversity was found at the five sampling points where a riverbed ecosystem is meeting the lake (2.09±0.35). After that, six samples associated with *F. orientalis* trees were found (2.02±0.30) to be the second most diverse nematode assemblages around the lake. Marshland related samples (13 samples) were found the third most diverse by the mean of Shannon-Wiener index (1.95±0.25). Three samples associated to meadow plants were found to be the fourth most diverse which was (1.94±0.38) followed by the three *Q. petraea*-associated samples (1.87±0.22) as it is the least value according to the terms of Shannon-Wiener index.

The average value of Maturity Index calculation for each sampling sites was 3.25±0.49. Nematode maturity indices (Maturity 2-5 & Sigma Maturity) were classified and found to have high values and showed variability at average of sampling plots (Average value of Sigma MI: 3.20±0.50; Maturity 2-5: 3.30±0.47). The average Enrichment Index value was 16.36±16,36. Overall, evaluated by means of ecological indices and soil maturity, soil characteristics were found at the highest value where habitat was occupied by *F. orientalis* and marshland plants (Table 4).

By the terms of plant parasitic nematodes, there were fifteen soil samples (Sample 2, 4, 6, 7, 8, 12, 17, 18, 19, 21, 22, 26, 28, 29, 30), associated with Marshland, Wildpland meadow, *Quercus petraea* L. trees, Riverbed and *Fagus orientalis* L. trees, dominated by Epidermal/root hair feeders such as Coslenchus, Malenchus and Tylenchus. Three samples (5, 9 and 11), associated with Wildpland meadow, *Quercus petraea* L and Marshland were dominated by ectoparasitic nematodes (Belondira, Criconema and Dorylaimellus). Samples 13 and 14, associated with Marshland, were dominated by semi endoparasitic nematodes (Hoplolaimus) (Figure 4).

Table 4. Values of Maturity, Maturity 2-5, Shannon Wiener, Sigma Maturity, Enrichment and Structure Indices values of all sampling sites in this study

Sample N° / Index name	Maturity	Maturity 2-5	Sigma Maturity	Shannon-Wiener	Enrichment	Structure
1	2.60	2.60	2.60	1.37	0.00	77.14
2	3.54	3.67	3.37	2.17	61.54	96.45
3	3.36	3.36	3.36	1.84	7.69	86.59
4	2.60	3.00	2.19	1.56	66.67	80.95
5	2.98	2.98	3.00	1.95	0.00	79.46
6	3.13	3.13	3.06	2.32	0.00	86.67
7	3.00	3.00	2.87	2.12	0.00	81.68
8	4.13	4.13	4.06	1.70	0.00	97.80
9	2.81	2.81	2.97	1.80	0.00	80.88
10	3.16	3.16	3.16	1.72	0.00	88.30
11	3.18	3.18	3.23	1.90	6.25	81.98
12	3.34	3.34	3.31	2.11	0.00	89.69
13	3.60	3.60	3.56	1.92	0.00	98.00
14	3.57	3.77	3.50	2.14	66.67	95.90
15	3.38	3.42	3.38	1.93	44.44	97.06
16	2.71	2.71	2.71	1.56	8.70	66.40
17	2.94	3.14	2.88	2.53	51.72	82.39
18	3.06	3.06	3.06	2.11	0.00	82.91
19	2.76	2.95	2.64	2.29	58.82	84.44
20	4.00	4.00	3.95	1.62	0.00	94.89
21	2.30	2.40	2.37	1.89	32.00	55.84
22	2.54	2.67	2.59	2.14	36.36	70.83
23	3.79	3.79	3.79	1.87	0.00	94.52
24	4.00	4.00	4.00	1.97	0.00	96.63
25	3.80	3.80	3.80	1.61	0.00	97.42
26	3.33	3.50	3.21	2.51	50.00	91.53
27	3.60	3.60	3.60	2.03	0.00	94.34
28	2.77	2.77	2.68	1.93	0.00	79.28
29	3.55	3.55	3.41	1.84	0.00	95.64
30	4.00	4.00	3.89	1.92	0.00	97.40
Average ± SD	3.25±0.49	3.30±0.47	3.20±0.50	1.95±0.27	16.36±16.36	86.76±10.40

Nematode composition by feeding types

Abundance of nematodes according to their feeding types is showed in Figures 2 & 3 for each soil sample. It is figured out that omnivore nematodes are dominating the community (52.77%) at all sampling sites. They are followed by bacterivorous nematodes (27.27%), predator nematodes (16.35%), fungivores nematodes (2.1%), and plant parasitic nematodes (herbivores) (1.51%). The high number of omnivore and predator nematodes occurrence was unusual compared to similar ecological characteristics. Plant parasitic nematodes were less abundantly present at all sampling zones. The relationship of density of nematodes is strongly related to the body size of nematodes. Omnivorous nematodes are known to be large in body size. The abundance of omnivorous nematodes might be explained as a result of less disturbance and balanced ecosystem around the Korugöl Lake (Traunspurger et al., 2006).

Within the plant-parasitic-nematodes, root hair feeders were found to be the most abundant group by 69.94% (Coslenchus, Malenchus and Tylenchus) followed by ectoparasitic nematodes (22.91%) (Belondira, Criconema and Dorylaimellus). Semi-endoparasitic plant-parasitic nematodes are the third common group with 7.14% (Hoplolaimus). Sedentary and Migratory endoparasites were not found at any sampling sites (Figure 3).

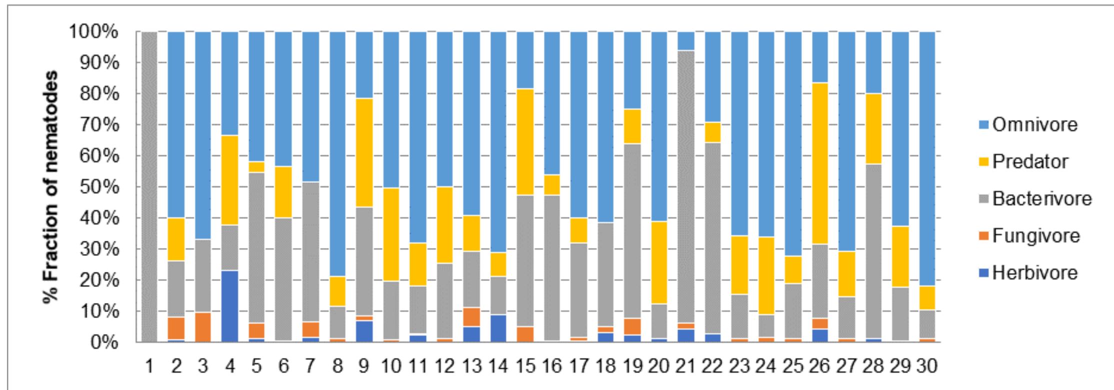


Figure 2. Feeding types and their abundance at the sampling sites.

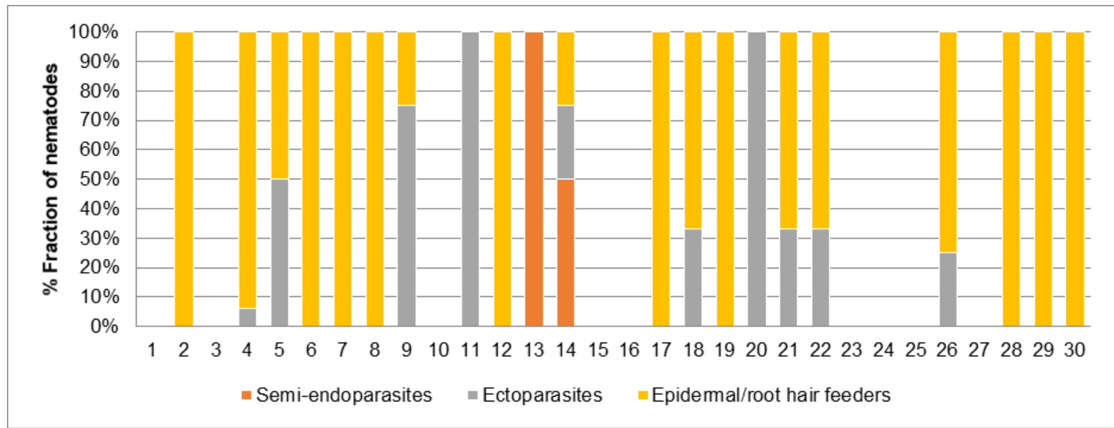


Figure 3. Distribution (%) of feeding types within the plant-parasitic nematodes from Korugöl Lake, Düzce, Türkiye.

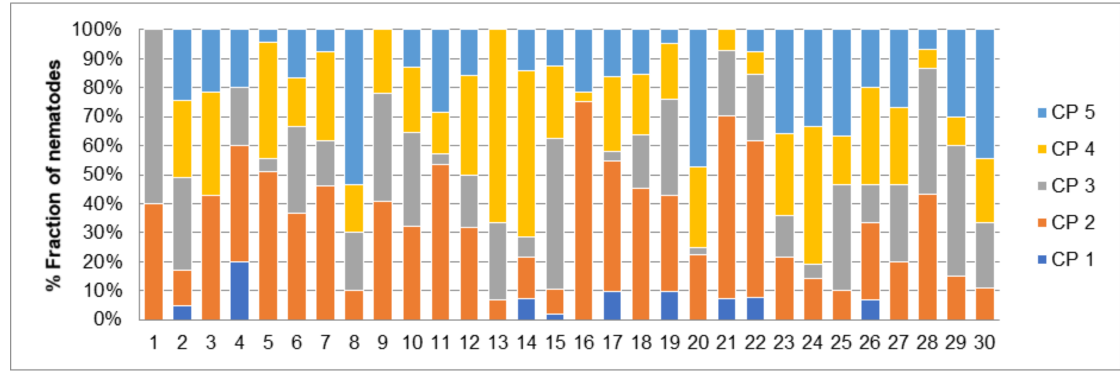


Figure 4. Free-living nematode c-p classification from Korugöl Lake, Düzce, Türkiye.

Community structure of free-living nematode assemblages

A classification within Free-living nematodes were provided from c-p 1 (colonizers, enrichment opportunists) to c-p 5 (persisters with long life cycle) based on their life cycles. The free-living nematode community at Lake Korugöl, did not show recognizable patterns but high average values of c-p classification. Colonizer nematodes with short life cycle (c-p 2 value) were found to be dominating the nematode assemblages by consisting of 32.23% of the sampling sites. On the other hand, enrichment opportunist nematodes with c-p value 4 were the second by 23.75% domination. 22.26% of the free living nematodes were appeared to be c-p 3 class, 19.26% of the community is at c-p 5 and the lowest group was detected 2.49% within c-p 1 nematodes (Figure 4).

Discussion

Faunistic

The rising interest of learning about nematode community behavior in soil food web allows us to monitor their role in the ecosystem. In Türkiye, the discovery of soil nematodes and especially diversity of free-living nematodes are still receiving very little interest from scientific community. The aim of this study is to determine soil nematode fauna in the West Blacksea region, Düzce Province with a holistic approach and to identify vermiform terrestrial nematodes up to genus level. As mentioned, (see results), ten taxa, namely, Aporcelinus ((Cobb) Andrassy), Baladorylaimus (Andrassy), Dorylaimoides (Thorne & Swanger), Euteratocephalus (Andrassy), Labronemella (Andrássy), Laimydorus (Siddiqi), Lindseyus (Ferris & Ferris), Metateratocephalus (Eroshenko), Paractinolaimus (Meyl) and Tripylella (Brzeski & Winiszewska-Slipinska) genera are first reports to the Türkiye nematofauna. Our contribution therefore not only significantly expand on what is known about nematode species in Türkiye, but also highlightsthe available knowledge regarding the geographic records in the West Black Sea Region of Türkiye.

Nematodes are biological indicators, and provide valuable information about soil health. The study is conducted in a natural park habitat where mostly rare animals and endemic plants are living. Therefore, it can be counted as a fragile ecosystem that any chemical contamination or disruption in soil will affect biodiversity drastically. On the other hand, lake ecosystems are rarely discovered in terms of terrestrial nematofauna in Türkiye. Unfortunately, there is no study to our knowledge, related to free-living nematode biodiversity in the West Black Sea region. The studies so far have investigated mostly the soil nematode communities from agricultural lands but not from natural habitats (Yildiz et al., 2021; Imren, 2015). These taxa are worldwide distributed; however, it is clear that most of the free-living nematode species are rarely known in Türkiye. Regarding the total diversity, this study makes a valuable contribution to faunistic studies of terrestrial nematode species.

Species distribution and nematode abundance

The distribution of species around a lake ecosystem was investigated with an integrative approach to the species relative abundance and occurrence patterns. This approach allowed us to detect species from a widespread group of nematodes that are occurring within the very abundant or abundant and very frequently or frequently around Lake Korugöl. These nematodes are listed as follows: *Aporcelaimellus* (Heyns), *Prismatolaimus* (Micoletzky), *Plectus* (Bastian), *Anaplectus* (Coninck & Schuurmans Stekhoven), *Tripylella* (Brzeski & Winiszewska-Slipinska). On the other hand, 19 genera are occurring scarce or very scarce and less constant or very less constant such as *Baladorylaimus* (Andrassy), *Hoplolaimus* (von Daday), *Metateratocephalus* (Eroshenko), *Mylonchulus* (Cobb), *Prionchulus* ((Cobb) Wu & Hoeppli), *Tylenchus* (Bastian), *Anonchus* (Cobb), *Aporcelinus* ((Cobb) Andrassy), *Dorylaimoides* (Thorne & Swanger), *Rhabditis* (Dujardin), *Acrobeles* (von Linstow), *Alaimus* (de Man), *Cervidellus* (Thorne), *Chronogaster* (Cobb), *Dorylaimellus* (Cobb), *Euteratocephalus* (Andrassy), *Lindseyus* (Ferris & Ferris), *Mesodorylaimus* (Andrássy) and *Paractinolaimus* (Meyl). When we look at the genera profile of nematodes, we found mostly omnivorous nematodes which prefer relatively undisturbed areas such as Forest - Boreal forests, tundra graslands, subtropical/tropical high altitude grasslands and temperate grasslands.

According to Yeates & Bird, 1994; Stamou et al., 2005, it is suggested that a nematode survey during the late summer season is optimal for the environmental variables in terms of soil humidity, high metabolic activity and temperature of soil. In our survey, which agrees with this contribution, the total nematode abundance was found minimum 10 to maximum 56 individuals per 100 gr of soil.

The nematode community

In general, nematode trophic groups did not show a detectable pattern. Apparently, omnivorous nematodes were found the most common group at all samples. Mostly the diverse group in terms of species richness was within the order Dorylaimida. Predatory nematodes as the most persistent group, were found at all sampling sites. The persistency of this group stems from their biology which also refers to occurrence at mature and fertile soils and having a long-life cycle. The total percentage of omnivorous and predator nematodes has reached to 69% of all nematode taxa which shows clues of soil maturity and an undisturbed ecosystem in Lake Korugöl. On the other hand, the abundance of bacterivorous, fungivores and plant parasitic nematodes had a little variation between the sampling sites. This shows clues about how dynamic can be a lake ecosystem as it has a unique cycle within itself. Maturity indices had similar values at all sites and did not differ significantly between sites. According to Schnürer et al. (1986) and Yeates (2007), the most important factors affecting the nematode community are that the environmental effect of regional and seasonal changes such as soil organic matter, texture, structure, chemical differences, and moisture along with environmental disturbances caused by humans. Some studies that are conducted in lake ecosystems shows a tendency of several patterns in respect with the seasonal fluctuations in the population dynamics of nematodes which have a short life cycle. Some authors noted significant annual density fluctuations (Fisher, 1968; Tudorancea & Zullini, 1989), whereas others found no such distinct changes of nematode abundance (Strayer, 1985). Previously similar nematode abundance and occurrence patterns were detected in high altitude nematode faunal survey in Mount Ağrı according to Cakmak et al. (2019) and Zhang et al. (2012). Overall, the ecological indices and impact of environmental changes by terms of nematode community did not show any consistent pattern at the surrounding soils of Lake Korugöl. It is recommended to monitor regularly the nematode community and the soil properties for further explanation of this matter.

This statement definitely once again discloses the critical position of terrestrial nematodes in the soil food web. The range of high tolerance may occur at different climatic conditions such as highly polluted habitats to mature soils which create habitat for tolerant species and sensitive species. Nematodes have low mobility and rapid responses to disturbance and enrichment changes. Life-cycle properties of nematodes ranging from 6 days to over 2 years provides wide opportunities, perspectives and practical tools to scientists not only for understanding environmental changes but also conservation of soil biodiversity.

Finally, this contribution indicates a study of the fauna of terrestrial nematodes at Lake Korugöl might give beneficial information on conservation of terrestrial nematode fauna of Türkiye and show how nematodes can be useful for soil monitoring as a rarely known approach.

Acknowledgment

I would like to thank Professor Reyes Peña Santiago from the University of Jaen for their kind help on the identification of the order Dorylaimida.

References

Andrássy, I., 2002. "Free-Living Nematodes from The Fertő-Hanság National Park, Hungary, 21-97". In: The Fauna of the Fertő-Hanság National Park, Budapest, Hungary (Eds. S. Mahunka), Hungarian Natural History Museum and Systematic Zoology Research Group of the Hungarian Academy of Science Budapest, Hungary, 829 pp.

Andrássy, I., 2005. Free-Living Nematodes of Hungary, I (Nematoda Errantia). Hungarian Natural History Museum and Systematic Zoology Research Group of the Hungarian Academy of Science, Budapest, Hungary, 518 pp.

Andrássy, I., 2009. Free-Living Nematodes of Hungary (Nematoda Errantia), Vol. III. Hungary, Hungarian Natural History Museum, 608 pp.

Baermann, G., 1917. Eine einfache methode zur auffindung von Ancylostomum (Nematoden) larven in Erdproben. Geneeskd Tijdschr Ned Indie, 57: 131-137 (in German).

Bongers, T. & H. Ferris, 1999. Nematode community structure as a bioindicator in environmental monitoring. Trends in Ecology & Evolution, 14 (6): 224-228.

Bongers, T., 1990. The maturity index: an ecological measure of environmental disturbance based on nematode species composition. Oecologia, 83 (1):14-19.

Bongers, T., H. van der Meulen & G. Korthals, 1997. Inverse relationship between the nematode maturity index and plant parasite index under enriched nutrient conditions. Applied Soil Ecology, 6 (2): 195-199.

De Ley, P. & M. Blaxter, 2005. A new system for Nematoda: combining morphological characters with molecular trees, and translating clades into ranks and taxa. Nematology Monographs & Perspectives, 2 (1): 633-653.

Ferris, H. & T. Bongers, 2009. "Indices for Analysis of Nematode Assemblages, 124-145". In: Nematodes as Environmental Bioindicators (Eds. M. Wilson & T. Kakouli-Duarte). CABI, Wallingford, USA, 352 pp.

Ferris, H., T. Bongers & R. G. de Goede, 2001. A framework for soil food web diagnostics: extension of the nematode faunal analysis concept. Applied Soil Ecology,18 (1): 13-29.

Fisher, K.D., 1968. Population patterns of nematodes in Lake Champlain. Nematologica 14 (1): 1-19.

Hodda, M., A. Ocana & W. Traunspurger, 2006. "Nematodes from Fresh Water Habitats, 179-211". In: Freshwater Nematodes: Ecology and Taxonomy (Eds. E. Abebe, I. Andrássy & W. Traunspurger). CABI, Wallingford, USA, 752 pp.

Hugot, J. P., P. Baujard & S. Morand, 2001. Biodiversity in helminths and nematodes as a field of study: an overview. Nematology, 3 (3): 199-208.

Imren, M., Ş. Yıldız, E. Kasapoğlu, H. Toktay, H. Kütük & A. A. Dababat, 2015. "The Plant-Parasitic Nematodes Associated with Cereal Crops in Bolu, Turkey, 131-140". Proceedings of the Nematodes of Small Grain Cereals: Current Status and Research, Fifth International Cereal Nematode Initiative Workshop (12-15 September 2015, Ankara, Turkey), (Eds. A. A. Dababat, H. Muminjanov & R. W. Smiley), Food and Agriculture Organization of United Nations, Ankara, Turkey, 384 pp.

Keleş, Z., 2006. Düzce İli Mikrobölgesel Zemin Parametrelerinin Haritalanması. Sakarya University (Unpublished) Doctoral Dissertation, Sakarya, Turkey, 87 pp (in Turkish with abstract in English).

Lee, D. L., 2002. The Biology of Nematodes. CRC Press, 338 pp.

Schnürer, J., M. Clarholm, S. Boström & T. Rosswall, 1986. Effects of moisture on soil microorganisms and nematodes: a field experiment. Microbial Ecology, 12 (2): 217-230.

Shannon, C. E. & W. Weaver, 1949. The mathematical theory of communication. University of Illinois Press, Urbana, 117 pp.

Sieriebriennikov, B., H. Ferris & R. G. M. de Goede, 2014. NINJA: An automated calculation system for nematode-based biological monitoring. European Journal of Soil Biology, 61 (1): 90-93.

Stamou, G. P., E. M. Papatheodorou, A. Hovardas & M. D. Argyropoulou, 2005. Some structural and functional characteristics of a soil nematode community from a Mediterranean grassland. Belgian Journal of Zoology, 135 (2): 253-259.

Strayer, D., 1985. The benthic micrometazoans of Mirror Lake, New Hampshire. Archiv für Hydrobiologie, 3 (Suppl. 72): 287-426.

Traunspurger, W. & Bergtold, M. 2006. "Patterns in the Size Structure of Freshwater Nematode Communities: The Cases of Lakes Königssee and Brunnsee, Germany, 132-143". In: Freshwater Nematodes: Ecology and Taxonomy. Wallingford UK: CABI Publishing, 736 pp.

Tudorancea, C. & A. Zullini, 1989. Associations and distribution of benthic nematodes in the Ethiopian Rift Valley lakes. Hydrobiologia, 179 (1): 81-96.

Turkish Ministry of Culture and Tourism, 2021. Kurugöl tabiat parkı. (Web page: https://www.kulturportali.gov.tr/turkiye/duzce/TurizmAktiviteleri/kurugol) (Date accessed: 06.07.2023) (in Turkish).

Wilson, M. J. & T. Khakouli-Duarte, 2009. Nematodes as Environmental Indicators. CABI, USA, 326 pp.

Yeates, G. W. & A. F. Bird, 1994. Some observations on the influence of agricultural practices on the nematode faunae of some South Australian soils. Fundamental & Applied Nematology, 17 (2): 133-145.

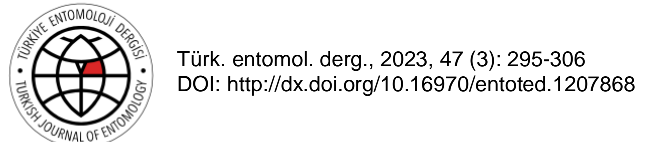
Yeates, G. W., T. Bongers, R. G. M. De Goede, D. W. Freckman & S. S. Georgieva, 1993. Feeding habits in soil nematode families and genera-an outline for soil ecologists. Journal of Nematology, 25 (3): 315-331.

Yeates, G., 2007. Abundance, diversity, and resilience of nematode assemblages in forest soils. Canadian Journal of Forest Research, 37 (2): 216-225.

Yıldız, Ş., G. Emine, Ö. Göksel & M. İmren, 2021. Investigations on soil nematode diversity in three contrasting habitat types in Bolu, Turkey. Turkish Journal of Entomology, 45 (4): 451-461.

Yoder, M., I. T. De Ley, I. Wm King, M. Mundo-Ocampo, J. Mann, M. Blaxter, L. Poiras & P. De Ley, 2006. DESS: a versatile solution for preserving morphology and extractable DNA of nematodes. Nematology, 8 (3): 367-376.

Zhang, M., W. J. Liang & X. K. Zhang, 2012. Soil nematode abundance and diversity in different forest types at Changbai Mountain, China. Zoological Studies, 51 (5): 619-626.



Türk. entomol. derg., 2023, 47 (3): 295-306
DOI: http://dx.doi.org/10.16970/entoted.1207868

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

The effects of altitude and rearing period on some characteristics of honey bee queens, *Apis mellifera caucasica* Gorbachev, 1916 (Hymenoptera: Apidae)[1]

Rakım ve yetiştirme döneminin ana arıların, *Apis mellifera caucasica* Gorbachev, 1916 (Hymenoptera: Apidae) bazı özellikleri üzerine etkileri

Nazlı Pınar CANVERDİ[2*] Gonca ÖZMEN ÖZBAKIR[3] Soner ÇANKAYA[4] Muzaffer DUMAN[5] Ümit KAYABOYNU[2] Fatih YILMAZ[6] Hasan ESE[7] Belgin GÜNBEY[8]

Abstract

In the study, some characteristics of honey bee queens, *Apis mellifera caucasica* Gorbachev, 1916 (Hymenoptera: Apidae), reared at two different altitudes (140 m vs 920 m) and three rearing seasons (May, June-I, June-II) in 2019-2020 were examined in Ordu. These are larvae grafting, length of the queen cell, weight of queens at various periods, and spermathecae parameters of queens. One-day old 50 larvae were grafted at each period and altitude. The effect of period and period*altitude interaction on the weight at emergence of queen was significant (p<0.001) while the effect of altitude and year was nonsignificant. Weights at emergence of queens were lower in May at high altitudes and in June-II at low altitudes. The effects of year, period and altitude were significant (p<0.001) in the terms of the diameter and volume of the spermathecae, and the number of spermatozoa in the spermathecae. When the two-year data was evaluated together, the number of spermatozoa in the spermathecae was higher in the queen bees reared in May. It is recommended to pay attention to the period and altitude when pollen and nectar flow is intense in queen rearing in Ordu and similar climatic conditions.

Keywords: Honeybee, queen, rearing period, spermathecae, number of spermatozoa

Öz

Çalışmada, Ordu ilinde, 2019-2020 yıllarında iki farklı rakım (140 m ile 920 m) ve üç yetiştirme döneminde (Mayıs, Haziran-I, Haziran-II) yetiştirilen ana arıların, *Apis mellifera caucasica* Gorbachev, 1916 (Hymenoptera: Apidae), bazı özellikleri incelenmiştir. Bu özellikler; larva kabul oranı, yüksük uzunluğu, ana arı çıkış ağırlığı, ana arının yumurtlama başlangıcındaki ağırlığı, yumurtlamaya başladıktan sonraki 3. gün ağırlığı, spermateka çapı ve hacmi ve spermatozoa sayısıdır. Her dönem ve rakımda bir günlük yaşta 50'şer adet larva aşılanmıştır. Ana arı çıkış ağırlığı üzerine dönem ve dönem*rakım interaksiyon etkisi önemli (p<0.001), rakım ve yıl etkisi önemsiz bulunmuştur. Yüksek rakımda Mayıs döneminde, düşük rakımda ise Haziran-II döneminde ana arı çıkış ağırlıkları daha düşüktür. Spermateka çapı, spermateka hacmi ve spermatozoa sayısı bakımından yıl, dönem ve rakımın etkisi önemli bulunmuştur (p<0.001). İki yıl verileri birlikte değerlendirildiğinde spermatozoa sayısı Mayıs dönemi yetiştirilen ana arılarda daha yüksektir. Ordu ve benzer iklim koşullarında ana arı yetiştiriciliğinde polen ve nektar akımının yoğun olduğu dönem ve rakıma dikkat edilmesi önerilmektedir.

Anahtar sözcükler: Bal arısı, ana arı, yetiştirme dönemi, sperm kesesi, sperm sayısı

[1] This study was supported by the General Directorate of Agricultural Research and Policies, Türkiye, Grant Project No: TAGEM/HAYSUD/B/19/A4/P5/01.
[2] Apiculture Research Institute, Honey Bee Breeding and Biotechnology, 52200, Ordu, Türkiye
[3] Harran University, Faculty of Agriculture, Department of Animal Science, 63300, Şanlıurfa, Türkiye
[4] Ondokuz Mayıs University, Yasar Doğu Faculty of Sports Sciences, 55139, Samsun, Türkiye
[5] Apiculture Research Institute, Honey Bee Rearing Technics, 52200, Ordu, Türkiye
[6] Apiculture Research Institute, Bee Health Department, 52200, Ordu, Türkiye
[7] Apiculture Research Institute, Assistant Administrative Manager, 52200, Ordu, Türkiye
[8] General Directorate of Agricultural Research and Policies, Department of Livestock and Aquaculture Research, 52200, Ordu, Türkiye
* Corresponding author (Sorumlu yazar) e-mail: nazlip10@gmail.com; nazlip10@hotmail.com
Received (Alınış): 21.10.2022 Accepted (Kabul ediliş): 30.09.2023 Published Online (Çevrimiçi Yayın Tarihi): 04.10.2023

Introduction

Beekeeping is an important animal breeding activity in terms of its role in economic, ecological, and rural development. In colony yield, environmental factors including climate, flora, and topography characteristics are effective together with the genetic structure of the colony and beekeeper practices. Furthermore, genetic capacity of the honey bee queen, *Apis mellifera* L., 1758 (Hymenoptera: Apidae) which is the most important individual of the colony, and genetic capacity of the drones with which it mates are the determinants of many physiological and behavioral characteristics that emerge in the colony. The queen bee, one of the individuals of the colony, plays a crucial role in ensuring the continuity of colonies. The low-quality queens will result in the honey yield and colony lifespan not being at the intended level, even if the other conditions are adequate. Quality of the queen is crucial for this reason. Morphological and physiological characteristics and health status of the queen are still being investigated by various studies (Delaney et al., 2011; Hatjina et al., 2014; Porporato et al., 2015; Amiri et al., 2017).

Rearing system, flora, season, larval age, condition, composition of the colonies used, feeding and characteristics of queen cells are seen more among the factors influencing the quality of queens (Woyke, 1971; Winston, 1987; Tarpy et al., 2011; Rangel et al., 2013; De Souza et al., 2019). The breeding quality of the queen and the drones which she mates with and whether a breeding program is implemented are the main factors. To explain the quality of the queen, the parameters such as the weight at emergence, live weight in various periods, the mating success and the frequency, the number of ovarioles, the ovary weight, the diameter of the spermathecae, the volume of the spermathecae and the number of spermatozoa in the spermathecae have been examined (Kahya et al., 2008; Büchler et al., 2013; Arslan et al., 2021; Facchini et al., 2021; Frost et al., 2021). It was reported that there were correlations between the weight at the emergence of a queen, its weight at different life stages and the condition of the colony, the various morphological and reproductive organ characteristics of the queen (Kahya et al., 2008; Delaney et al., 2011; Tarpy et al., 2012). It was found that the weight of the queen had a significant effect on the acceptance rate of the queen, starting to oviposition, rate of oviposition, diameter of the spermatheca, and the number of spermatozoa in the spermatheca (Akyol et al., 2008). Similarly, there is a correlation between the weight at the emergence of a queen, diameter of the spermathecae and amount of stored sperm (Dodoloğlu et al., 2004; Collins & Pettis 2013; Hatjina et al., 2014). A queen must have an efficient metabolism, a large spermatheca, and a large ovarium including more and longer ovarioles so that she can be considered as superior in terms of reproductive characteristics (Tarpy et al., 2000). The diameter of the spermatheca and volume of the spermatheca are affected by factors such as the genetic structure of the queen, rearing conditions, larval age, and season (Hatch et al., 1999; Güler & Alpay 2005; Uçak Koç & Karacaoğlu, 2011).

When the existence of colonies and amount of honey production are researched in our country, it is seen that the honey production per colony and production of other beekeeping products except honey are not at the level desired. Similarly, it is seen that it is insufficient when we compare the number of the queen breeders (143 ones) having production permits for rearing and commercial queens in our country and the queen production capacity of these enterprises (approximately 510 thousand units) with our colony existence (approximately 8.1 million colonies) and the annual estimated demand of queen (Burucu, 2021; Anonymous, 2022). The intensive rearing of queens starts primarily in the coastal provinces in the Mediterranean. On the other hand, the queen rearing continues to decrease by depending on the effects of season and flora in the inner and higher altitude areas.

The study aimed to examine the parameters of the acceptance rate of larvae, length of the queen cell, weight at the emergence of a queen, weight at the onset of oviposition, weight on the third day after oviposition, diameter of the spermatheca, volume of the spermatheca and the number of spermatozoa in the spermatheca of Caucasian queens *Apis mellifera caucasica*, Gorbachev, 1916 (Hymenoptera: Apidae), reared at two different altitudes and three rearing periods.

Materials and Methods

The study was conducted at a low altitude (Dedeli), which is at an altitude of 140 m (40°54'24'' N and 37°50'06'' E) and a high altitude (Sayaca), which is at an altitude of 920 m (40°53'24'' N and 37°41'55'' E) in Ordu province in two separate apiaries between 2019 and 2020. Ordu province is a region where the climate of the Black Sea is dominant. Although winters are cool and summers are warm, there is a possibility of precipitation in all months of the year. In the fields where this study was conducted in the province of Ordu, there are no predominant nectar and pollen sources during spring months. However, it is known that there are secondary or minor pollen sources. *Diospyros lotus* L. and *Trifolium repens* L. species are found in secondary amounts (Cınbırtoğlu, 2014). Additionally, different amounts of pollen and nectar source plants are found in the region during the spring and summer seasons. These include *Castanea sativa* Miller (Dominant-Dominant), *Laurocerasus officinalis* Roemer (Secondary-Iz), *Rhododendron ponticum* L. subsp. *ponticum* L. (Minor-Dominant), and *Vaccinium myrtillus* L. (Minor-Secondary) species (Cınbırtoğlu, 2021; Anonymous, 2023).

Queen rearing

Queen rearing was performed simultaneously in both apiaries during the periods of 10 May, 4 June (I) and 29 June (II). The standard procedure of rearing a queen was followed (Laidlaw, 1985). One-day old larvae were used in larvae grafting. 50 larvae in both apiaries for each period and a total of 600 larvae in two years were grafted. The queen cells prepared from beeswax and the frames with three-lathed were used in larvae grafting. A total of 65 honeybee colonies were used in this study, representing each season and altitude over a span of two years. As a source colony for larvae, one colony of Caucasian race *Apis mellifera caucasica*, Gorbachev, 1916 (Hymenoptera: Apidae) was used. For each altitude (140m and 920m) and season, one queenless starter colony (consisting of 2 frames with open brood, 4 frames with capped brood, and 2 frames with stored pollen and nectar), one queenright and double-storey finishing colony, five drone rearing colonies, and mating boxes were prepared. The mating nucleus was also prepared with young worker bees, which were shaken down equally every period, and they were distributed to the field in the trial apiary. Queen rearing colonies were regularly fed with sugar syrup at a rate of 1:1.

Methods

The acceptance rate of larvae (the number of larvae accepted / the number of larvae grafted *100) was determined 24 hours after larvae grafting. The accepted larvae were transferred to the finisher colonies. The length of the queen cell was measured with a digital caliper before placing it in the incubator (a day before the emergence of the queen). The emergence of the queens was observed in the incubator at each period (34ºC, 60% humidity). The larvae transfer success rate (The number of the queen at emergence/ The number of larvae grafted *100) was determined.

The electronic balance (WL-303L 0.001gr) was used to determine the weight at the emergence of a queen. The queens at the emergence were randomly distributed to the mating nuclei with a queen cage. The time to start oviposition of queens was determined by monitoring open-mated queens. The weight at the onset of the oviposition and weight on the third day after the oviposition of the queens were weighed. The characteristics of the spermatheca were determined in 15 queens randomly selected from among the queens reared in each period. The diameter of the spermatheca (mm) was measured under an ocular microscope with a micrometer (Olympus SZ61) without a tracheal net. The volume of spermathecae was calculated according to the measured diameter (calculated according to the formula of the sphere volume). After the spermathecae were discharged in a porcelain cup comprising 1 ml of 0.9% sodium chloride solution, the spermatozoa were dispersed by mixing it with a Pasteur pipette. Tap water was added to make up the mixture to 5 ml (Kaftanoğlu & Kumova, 1992; Genç, 1996; Carreck et al., 2013). The number of spermatozoa in the spermatheca was determined by the samples taken from this mixture under the microscope (Carl Zeiss Axio Scope A1) with the Thoma counting chamber (Kaftanoğlu & Kumova, 1992; Genç, 1996; Carreck et al., 2013).

Statistical analysis

The research was carried out in a factorial design (2*3*2) according to the plan of the randomized block design. Two-way ANOVA was used to analyze the data. The mathematical model of the design used in the study is;

$$Yijk=\mu+\alpha i+\beta j+(\alpha\beta ij)+\gamma k+eijk$$

these indicate

$Yijk$= measurement values,

μ= population mean,

αi= i-th altitude effect (A),

βj= j-th period effect (P),

$(\alpha\beta ij)$= altitude*period (A*P) interaction effect,

γk= k-th year effect (block effect),

$eijk$=P random error.

The differences between group means were determined by Duncan's multiple comparison test. In addition, whether the acceptance rate of larvae changed or not according to the periods was determined by the chi-square test of independence. The correlations among all parameters investigated such as the weight at the onset of the oviposition, the weight on the third day after oviposition, the diameter of the spermathecae, and the volume of the spermathecae were evaluated with the Pearson's correlation coefficient. The statistical package program of SPSS v.22.0 was used for all statistical calculations. It was considered that the research findings were significant at p<0.05 level by expressing as n, mean, and standard deviation.

Results and Discussion

The acceptance rate of larvae

The acceptance rate of larvae did not change according to altitude in May 2019. It was determined that acceptance rate of larvae changed according to the altitude in the June-I (χ^2_1 =6.002; p< 0.05) and June-II (χ^2_1=8.854; p< 0.05) periods. There was no difference in the acceptance rate of larvae according to the altitude for 2020 in all periods. Although there was no significant difference in the acceptance rate of larvae in June-I and June-II periods at low altitude in 2019 and 2020, it was found that there was a significant difference in the acceptance rate of the larvae in the 3rd rearing period in 2019-2020 (χ^2_1=6.529; p=0.011). The acceptance rate of larvae was higher in 2019 (86%) than in 2020 (64%). There was no statistical difference between the acceptance rate of larvae in 2019 and 2020 in all periods when queen rearing was conducted at high altitude. The acceptance rate of larvae (%) and the number of observation (n) for the length of the queen cell, weight at the emergence of the queens, weight at the onset of oviposition of the queen and weight on the third day after the oviposition of the queen were given in Table 1.

Table 1. The larvae acceptance rate

Periods	Acceptance rate of larvae (n, %) 2019		Acceptance rate of larvae (n, %) 2020	
	Low altitude (140 m)	High altitude (920 m)	Low altitude (140 m)	High altitude (920 m)
May	36 (72)	31 (62)	38 (76)	34 (68)
June-I	44 (88)	34 (68)	38 (76)	36 (72)
June-II	43 (86)	30 (60)	32 (64)	37 (74)

Güler & Alpay (2005) reported that there was no difference between the acceptance rate of larvae according to the queen genotypes, but the acceptance rate of larvae showed a significant difference according to the rearing periods in their study in Sivas province. The acceptance rate of larvae they reported for the genotype of Caucasian, *Apis mellifera caucasica*, Gorbachev, 1916 (Hymenoptera: Apidae) is similar to the values obtained in this study. The acceptance rate of larvae obtained from this study was similar to those reported by Gençer et al. (2000) at 73.47% and Cengiz et al. (2019) at 72.16%. Onk et al. (2016) found that there was a difference in the acceptance rate of larvae for the Caucasian queen according to the year and rearing period, and the values they obtained are higher than in this study. Dodoloğlu et al., (2004) reported that the acceptance rate of larvae was higher than in the current study.

The length of the queen cell

In the study, it was found that while the impact of the rearing period and year on the length of the queen cell was significant ($p < 0.001$), there was no interaction effect of altitude and period*altitude ($p > 0.05$). The length of the queen cell was higher in the measurements made in 2019 than in the ones in 2020. In both years, the length of the queen cell was high and similar in May (23.99 mm) and June-II (23.89 mm) periods and had the lowest average in the June-I (21.27 mm) period ($p < 0.05$). The average length of the queen cells was found 23.19±2.46 mm at low altitude and was found at 22.75±3.02 mm at high altitude ($p > 0.05$) (Table 2).

Table 2. The length of the queen cells by years and periods (mm)

Altitude	Year	Periods	n	The queen cell length (mm) (mean±SE)
Low	2019	May	35	23.12±1.62
		June-I	44	23.11±0.82
		June-II	43	24.70±2.61
	2020	May	35	25.04±1.63
		June-I	38	19.69±1.55
		June-II	32	23.51±1.79
	General	May	70	24.09 ± 1.88^{A}
		June-I	82	21.52 ± 2.10^{B}
		June-II	75	24.19 ± 2.35^{A}
High	2019	May	31	22.48±0.87
		June-I	34	22.58±0.93
		June-II	28	26.76±2.30
	2020	May	29	25.39±2.80
		June-I	31	19.13±1.07
		June-II	35	20.95±1.59
	General	May	60	23.89 ± 2.50^{A}
		June-I	65	20.94 ± 2.00^{B}
		June-II	63	23.53 ± 3.49^{A}

^{a,b} denote the significant differences between means in the same column ($p < 0.05$)

The weight at the emergence of the queens

In the study, it was found that the effect of period and period*altitude interaction upon weight at the emergence of the queen was significant ($p < 0.001$) while the impact of altitude and year was not significant ($p > 0.05$) (Table 3). The weight at the emergence of the queen obtained in the periods of May (218.4 mg) and June-I (222 mg) was higher than the period of June-II (213.5 mg) ($p < 0.05$). The mean weight at the

emergence of the queen was found 218.5±19.61 mg at low altitude and was found 218.0±22.47 mg at high altitude ($p>0.05$). However, if the interaction effect is taken into account, queens with low emergence weight are raised at high altitudes in the June-I period. On the other hand, the low weight at emergence was obtained at low altitude in the period of June-II.

Table 3. Queen weight at the emergence according to years and periods (mg)

Altitude	Year	Periods	n	Queen weight at the emergence (mg) (mean±SE)
		May	34	234.9±17.85
	2019	June-I	43	221.4±13.37
		June-II	42	200.4±14.61
		May	34	217.1±18.97
Low	2020	June-I	38	219.4±19.49
		June-II	31	221.1±17.07
		May	68	226.0±20.35^{A}
	General	June-I	81	220.5±16.41^{AB}
		June-II	73	209.3±18.68^{C}
		May	29	197.9±14.86
	2019	June-I	34	229.0±16.23
		June-II	28	216.7±18.18
		May	28	222.0±22.08
High	2020	June-I	30	221.2±30.65
		June-II	35	219.8±17.82
		May	57	209.6±22.15^{C}
	General	June-I	64	225.3±24.33^{AB}
		June-II	63	218.5±17.91^{B}

^{A,B,} denote the significant differences between means in the same columns ($p< 0.05$).

The weight at the onset of oviposition of the queen

While the effect of the period, altitude, and period*altitude interaction on the weight at the onset of oviposition of the queen were significant ($p< 0.001$), there was no effect of the year ($p>0.05$). In the rearing of the queen bee, the highest weight at the onset of oviposition was obtained in May at a low altitude. Although the weight at the onset of oviposition of the queen was highest in the period of June-II in high altitude, there was no difference between the periods (Table 4). The average weight at the onset of oviposition of the queen was found to be 221.9±24.52 mg at low altitude and 217.7±22.16 mg at high altitude according to general average of altitudes ($p< 0.05$). The weight at the onset of the oviposition of the queen was determined 225.6 mg in May, 216.1 mg in June-I, and 218.9 mg in June-II according to the general average of the rearing periods ($p< 0.05$). The correlations between various characteristics of queens were investigated. It was obtained that there was a low significant correlation between the weight at the emergence of the queens and their weight at the onset of the oviposition ($r = 0.167$; $p< 0.01$).

Table 4. The weight at the onset of oviposition and the weight on third day after oviposition of the queens (mg)

Altitude	Year	Periods	n	The queen weight at the onset of oviposition (mg) (mean±SE)	The queen weight on the third day after the oviposition (mg) (mean±SE)
Low	2019	May	33	245.6±12.53	234.9±14.87
		June-I	43	211.5±26.66	221.0±10.47
		June-II	41	213.7±21.67	214.0±12.26
	2020	May	33	223.3±25.22	231.8±13.57
		June-I	37	221.1±23.59	222.2±17.32
		June-II	31	220.8±18.46	232.0±18.52
	General	May	66	$234.5±22.75^{A}$	$233.3±14.21^{A}$
		June-I	80	$216.0±25.59^{B}$	$221.5±13.98^{BC}$
		June-II	72	$216.7±20.54^{B}$	$221.7±17.57^{BC}$
High	2019	May	28	206.8±32.00	208.8±35.19
		June-I	33	212.0±15.85	219.0±8.802
		June-II	28	219.1±18.02	225.5±20.75
	2020	May	27	224.1±14.58	228.8±12.27
		June-I	30	221.0±23.98	233.1±16.67
		June-II	34	223.5±19.73	255.6±13.32
	General	May	55	$215.2±26.41^{B}$	$218.4±28.32^{C}$
		June-I	63	$216.3±20.49^{B}$	$225.7±14.84^{B}$
		June-II	62	$221.6±18.97^{B}$	$225.5±16.87^{B}$

^{A,B,C} denote the significant differences between means in the same columns ($p< 0.05$).

The weight on the third day after the oviposition of the queen

While the interaction effect of the year and the period*altitude was significant ($p< 0.001$) on the weight on the third day after the oviposition of the queen, there were no effects of both altitude and period ($p> 0.05$). It was found that the weight on the third day after the oviposition of the queen in 2020 was higher than the one in 2019. In rearing queen bee, the highest weight on the third day after oviposition was obtained in the period of May in low altitude. The lowest weight on the third day after oviposition was found in May in high altitude (Table 4). It was found that there was a positive correlation between the weight at the emergence of the queen bees and weight on the third day after oviposition (r = 0.487; $p< 0.01$).

In the study, it was determined that the interaction of the rearing period and altitude had a significant effect when the results were examined in terms of weight at the emergence, weight at the onset of oviposition and weight on the third day after oviposition of the queen bees. The weight at the emergence of the queen is an important criterion related to both colony productivity and various morphological and physiological characteristics of the queens (Akyol et al., 2008; Collins & Pettis, 2013; Hatjina et al., 2014). Nevertheless, it was reported that there was a change in the body weight of queen from the weight at emergence to the weight at the onset of oviposition in many studies (Kahya et al., 2008). It was recommended that body weight of the Carniolan queens *Apis mellifera carnica*, Pollmann, 1879 (Hymenoptera: Apidae), should be determined when they reached the age of about 2 days (Skowrenek et al., 2004). Uçak Koç & Karacaoğlu (2011) reported that the live weight on the second day of the queens, the weight at the onset of oviposition and the preoviposition period of queen bees were affected by the period of rearing. The values obtained in terms of weight at the emergence of the queens in our study were found to be higher than the mean values which Dodologlu et al. (2004); Onk et al. (2016) and Cengiz et al. (2019) obtained in their studies.

When evaluated in terms of the periods (218.4±22.66 mg, 222.6±20.36 mg and 213.5±18.84 mg, respectively) and altitudes (low altitude 218.5±19.61 mg and high altitude 218.0±22.47 mg), the values in our study were higher than those reported by Tarpy et al. (2011), Oztokmak & Ozmen Ozbakır (2017) and Akyol et al. (2008). As a result of our study, the weight at onset of oviposition of queens (225.6±26.25 mg, 216.1±23.40 mg and 218.9±19.91 mg according to the periods; 221.9±24.2 mg and 217.7±22.16 mg according to the altitudes) was higher than the values Kahya et al. (2008), Kumar & Mall (2016) reported in their studies, but similar results were obtained with some periods in the studies of Uçak Koç & Karacaoğlu (2011). Kahya et al. (2008) stated that the weight on the third day after oviposition reached 220.9 mg in a study about determining the queen's live weight at different periods and reproductive characteristics of the queens. The values which Kahya et al. (2008) are found in their study were lower than the values found in this study (the period and the altitude values ranged from 223.3±20.84 mg to 226.5±23.04 mg).

The diameter of the spermathecae

While the effects of the year ($p<0.001$), period and altitude were significant in terms of the diameter of the spermatheca ($p<0.05$), the effect of the interaction was insignificant ($p>0.05$). The diameter of the spermathecae mean was determined 1.159^{AB} mm in May, 1.143^{B} mm in June-I, and 1.163^{A} mm in June-II according to the general average of the rearing periods ($p<0.05$). The diameter of the spermathecae mean was found to be high in 2020. While the diameter of the spermathecae mean was high in the periods of May and June-II in low altitude, it was low in June-I. On the other hand, the diameter of the spermathecae was high in the period of June-II at high altitude. The diameter of the spermathecae mean was determined 1.158±7.121 mm at low altitude and 1.151±5.938 mm at high altitude according to general average of altitudes ($p<0.05$) (Table 5).

Table 5. The spermathecae traits of queens*

Altitude	Year	Periods	n	The diameter of the spermathecae (mm) (mean±SE)	The volume of the spermathecae (mm^3) (mean±SE)	The number of spermatozoa (million) (mean±SE)
Low	2019	May	15	1.136±6.240	0.774±0.126	3.682±0.934
		June-I	15	1.110±6.022	0.722±0.117	4.062±0.987
		June-II	15	1.119±6.946	0.742±0.138	3.892±0.970
	2020	May	15	1.207±3.232	0.927±.0726	5.179±0.594
		June-I	15	1.182±5.995	0.871±0.127	4.331±0.749
		June-II	15	1.219±5.121	0.952±0.123	3.892±0.819
	General	May	30	1.172±6.096	0.848±0.127	4.431±1.083
		June-I	30	1.144±6.980	0.791±0.142	4.186±0.890
		June-II	30	1.162±7.918	0.832±0.168	3.883±0.903
High	2019	May	15	1.122±6.535	0.740±0.128	3.675±0.961
		June-I	15	1.113±5.667	0.727±0.111	3.198±0.687
		June-II	15	1.133±6.386	0.767±0.132	3.703±1.115
	2020	May	15	1.168±3.578	0.836±0.075	3.822±0.930
		June-I	15	1.174±5.112	0.852±0.111	3.876±0.916
		June-II	15	1.191±3.138	0.887±0.069	3.627±0.898
	General	May	30	1.144±5.766	0.786±0.116	3.746±0.941
		June-I	30	1.142±6.193	0.787±0.132	3.521±0.868
		June-II	30	1.165±5.640	0.834±0.118	3.661±0.898

* Since the interaction effect was insignificant ($p>0.05$), the lettering was not given in the table.

Gençer & Fıratlı (1999) reported that the diameter of the spermatheca of the queens raised from 1-day-old larvae was 1.063±0.077 mm in their study in which some internal and external characteristics of queen bees reared from 1- and 2-day-old larvae were compared. Dodoloğlu & Genç (1997) reported that the average spermatheca diameter of the queens mating naturally in Erzurum was 0.929 mm in their study while Uçak Koç & Karacaoğlu (2004) reported the average spermatheca diameter of the queens reared under the conditions in Aydın was 1.121 mm in his study. The values determined in this study were higher than those obtained by Gençer & Fıratlı (1999), Dodoloğlu & Genç (1997) and Uçak Koç & Karacaoğlu (2004) in their studies. Cengiz et al. (2019) stated that the diameter of the spermatheca of queens reared from 1-day-old larvae was 1.04±0.018 mm. On the other hand, Arslan & Cengiz (2020) reported that the average diameter of the spermatheca of queen bees was 1.015±0.007 mm in their studies where the quality criteria of queens taken from different enterprises were evaluated. Also, the mean spermatheca diameter was reported as 1.044±0.071 mm. in the study of Arslan et al. (2021), in which the quality characteristics of the queen bee were examined. Ozmen Ozbakır (2021) reported that the diameter of the spermatheca of the queen bees reared from emergency cells, one-day-old and two-day-old larvae were 1.14 mm, 1.21 mm and 1.16 mm, respectively. In our study, the diameter of the spermatheca of the queen bees was higher than the average diameter of the spermatheca which Kahya et al. (2008), Uçak Koç & Karacaoğlu (2005), Dodoloğlu et al. (2004), Cengiz et al. (2019), Arslan & Cengiz (2020) and Arslan et al. (2021) reported in their studies.

The volume of the spermathecae

In terms of the volume of the spermathecae, while the impacts of the year, the period and the altitude were found to be significant (p<0.05), the effect of the interaction was insignificant (p>0.05) (Table 5). The diameter of the spermathecae mean was determined 0.820^{A} mm^3 in May, 0.789^{B} mm^3 in June-I, and 0.832^{A} mm^3 in June-II according to the general average of the rearing periods (p<0.05). The volume of the spermathecae was higher in 2020. At low altitude, while the volume of the spermathecae was high in the periods of May and June-II, it was low in the June-I period. The volume of the spermathecae was higher in the period of June-II in high altitude. The volume of the spermathecae mean was determined 0.822±0.148 mm^3 at low altitude and 0.802±0.122 mm^3 at high altitude according to general average of altitudes (P< 0.05).

The volume of the spermathecae obtained in this study was higher than the values which Gençer & Fıratlı (1999); Kahya et al. (2008) and Arslan et al. (2021) reported in their studies. Al-Ghzawi and Zaitoun (2008) reported that the volume of the spermathecae (0.82 mm3) in Syrian queens *Apis mellifera syriaca*, Buttel-Reepen, 1906 (Hymenoptera: Apidae), was smaller than the volume of the spermathecae in Italian queens *Apis mellifera ligustica*, Spinola, 1806 (Hymenoptera: Apidae), (0.89 mm3). In this study, there were similar values in the mean volume of the spermathecae obtained at low altitude. Uçak Koç & Karacaoğlu (2011) reported that the period of queen rearing did not affect the volume of the spermathecae. Kahya et al. (2008) found that the volume of the spermathecae differed according to the weight of the queen and there was more volume of the spermatheca in the heavy queens. In this study, there were low insignificant correlations between the weight at emergence, diameter of the spermathecae and volume of the spermathecae. Furthermore, the effect of the rearing period on the volume of the spermathecae was significant, unlike the study of Uçak Koç & Karacaoğlu (2011).

The number of spermatozoa in the spermathecae

While the effect of the year, altitude (p<0.001), and period (p<0.05) were significant in terms of the number of spermatozoa, the interaction effect was insignificant (p>0.05). The number of spermatozoa mean was determined as 4.161 million at low altitude and 3.640 million at high altitude according to general average of altitudes (p<0.001). When considering 2019 and 2020 together in terms of the number of spermatozoa, the period of May was higher in both low altitude (4.431±1.083 million) and high altitude (3.746±0.941 million). The number of spermatozoa in 2020 was higher at low altitude (4.467 million) than the one at high altitude (3.775 million) (Table 5). The number of spermatozoa in the spermathecae mean

was determined as 4.115^{A} million in May, 3.892^{AB} million in June-I, and 3.781^{B} million in June-II according to the general average of the rearing periods ($p< 0.05$). There was no significant correlation between the weight at the emergence of the queen and characteristics of the spermatheca. A positive correlation was found between the diameter of the spermathecae and the volume of the spermathecae (r = 0.994; $p< 0.01$). There were low correlations between the number of spermatozoa in the spermathecae and the diameter of the spermathecae (r = 0.263; $p< 0.01$) and the volume of spermathecae (r = 0.265; $p< 0.01$). There were low correlations between the diameter of the spermathecae and the weight at the onset of oviposition (r = 0.116; $p< 0.05$), and there were also low correlations between the volume of spermathecae and the weight at the onset of oviposition (r = 0.117; $p< 0.05$).

Tarpy et al. (2011) noticed that the number of spermatozoa of the queens within the low-weight group was 2.80 million and the ones of the queens in the high weight group were 5.06 million while the average number of spermatozoa was 3.72 million in their study on the reproductive potentials of the queen and the mating success. While the values found in our study were partially similar to those of Tarpy et al. (2011), they were lower than the high-weight group of the queen in the same study. The number of spermatozoa was found to be 4.877 million in the study conducted by Kahya et al. (2008) on the determination of the live weights at different periods and reproductive characteristics of the queen. Cengiz et al. (2019) reported that the number of spermatozoa of the queens reared from one-day-old larvae was 4.44±0.429 million in their study. Dodologlu et al. (2004) determined the number of spermatozoa of the queens reared with the Doolittle method as 4.65±0.08 million in their study conducted by the Doolittle method and natural queen rearing. In addition, Arslan et al. (2021) reported that the mean number of spermatozoa was 4.454±0.177 million/queen in their study examined the quality characteristics of the queen. Dodologlu & Genç (1997) found the average number of spermatozoa of the queen bees mating naturally to be 4.625 million in their study in Erzurum. It is seen that the values in the studies carried out by other researchers are higher than the values found in our study. In the study in which Akyol et al. (2008) evaluated the queens as heavy, normal, and light according to their weights at emergence, the spermatozoa number of the queens was determined as 5.2 million/queen for the heavy group, 4.8 million/queen for the normal group and 4.2 million/queen for light group. The values found in our study were lower than those in the study of Akyol et al. (2008). The number of spermatozoa of the queen bees reared in the study of Uçak Koç & Karacaoğlu (2011) was higher than the number of spermatozoa obtained in this study.

Conclusion

As a result of the study, it was revealed that the rearing period and the altitude had effects separately and together on the quality parameters of Caucasian queens, *Apis mellifera caucasica*, Gorbachev, 1916 (Hymenoptera: Apidae). Especially in the conditions of Ordu province, it is recommended that the enterprises with commercial queen rearing and those engaged in beekeeping activities should pay attention to the altitude and the period when the flows of pollen and nectar are intense. In terms of the acceptance rate of larvae, June is recommended for the transfer of larvae in Ordu and the regions with similar conditions. In terms of the weight at the emergence of the queen, the weight at the onset of the oviposition, or the weight on the third day after oviposition, it is recommended that the transfer of larvae should be after 10 May at low altitudes, and at the beginning of June at higher altitudes. In the terms of the diameter of the spermathecae, while there were high and similar averages in May and June-II at low altitudes, high averages were obtained in June-II at high altitudes. In the terms of the characteristic of the number of spermatozoa, it is recommended that the transfer of larvae should be on a date after 10 May at both low and high altitudes.

References

Akyol, E., H. Yeninar & O. Kaftanoğlu, 2008. Live weight of queen honey bees (*Apis mellifera* L.) predicts reproductive characteristics. Journal of the Kansas Entomological Society, 81 (2): 92-100.

Al-Ghzawi, A. A. M. & S. Zaitoun, 2008. Origin and rearing season of honeybee queens affect some of their physiological and reproductive characteristics. Entomological Research, 38 (2): 139-148.

Amiri, E., M. K. Strand, O. Rueppell & D. R. Tarpy, 2017. Queen quality and the impact of honey bee diseases on queen health: Potential for interactions between two major threats to colony health. Insects, 8 (2): 48.

Anonymous, 2022. Businesses with queen bee production permit. (Web page https://www.tarimorman.gov.tr/Konular/Hayvancilik/Aricilik) (Date accessed: Oct 2022) (in Turkish).

Anonymous, 2023. Honey Forest action plan. 2013-2017. (Web page: https://kutuphane.tarimorman.gov.tr/vufind/Record/1205514) (Date accessed: Oct 2022) (in Turkish).

Arslan, S. & M. M. Cengiz, 2020. Evaluation of the quality criteria of queens produced in different provinces of Turkey in autumn. Uludag Bee Journal, 20 (1): 62-71.

Arslan, S., M. M. Cengiz, A. Gül & S. Sayed, 2021. Evaluation of the standards compliance of the queen bees reared in the Mediterranean region in Turkey. Saudi Journal of Biological Sciences, 28 (5): 2686-2691.

Büchler, R., S. Andonov, K. Bienefeld, C. Costa, F. Hatjina, N. Kezic, P. Kryger, M. Spivak, A. Uzunov & J. Wilde, 2013. Standard methods for rearing and selection of *Apis mellifera* queens. Journal of Apicultural Research 52 (1): 1-30.

Burucu, V., 2021. Product report beekeeping 2021. (Web page https://arastirma.tarimorman.gov.tr/tepge) (Date accessed: October 2022) (in Turkish).

Carreck, N. L., M. Andree, C S. Brent, D. Cox-Foster, H A. Dade, J D. Ellıs, F. Hatjına & D. van Engelsdorp, 2013. Standard methods for *Apis mellifera* anatomy and dissection. In: The COLOSS BEEBOOK, Volume I: standard methods for *Apis mellifera* research (Eds. V. Dietemann, J. D. Ellis & P. Neumann). Journal of Apicultural Research, 52 (4): http://dx.doi.org/10.3896/IBRA.1.52.4.03

Cengiz, M. M., K. Yazıcı & S. Arslan, 2019. The effect of the supplemental feeding of queen rearing colonies on the reproductive characteristics of queen bees (*Apis mellifera* L.) reared from egg and different old of larvae. Kafkas Üniversitesi Veteriner Fakültesi Dergisi, 25 (6): 849-855.

Cınbırtoğlu, Ş., 2014. Determination of some morphological and quality characteristics of pollen of preferred plant species by pollen collecting activity of honey bee (*Apis mellifera* L.) in spring period. Ordu University, Institute of Science and Technology, (Unpublished) Master Thesis, Ordu, 77 pp (in Turkish with abstract in English).

Cınbırtoğlu, Ş., 2021. Important Plants and Pollens of Flora of Turkey for Honey Bees (*Apis mellifera* L.). Tarım ve Orman Bakanlığı, Eğitim ve Yayın Dairesi Başkanlığı, Enstitü Yayın No: AAEM/38. ISBN: 978-625-8451-04-7, Ankara, 195 pp (in Turkish).

Collins, A. M. & J. S. Pettis, 2013. Correlation of queen size and spermathecal contents and effects of miticide exposure during development. Apidologie, 44 (3): 351-356.

De Souza, A. D., M. H. Huang & D. R. Tarpy, 2019. Experimental improvement of honey bee (*Apis mellifera*) queen quality through nutritional and hormonal supplementation. Apidologie, 50 (1): 14-27.

Delaney, D. A., J. J. Keller, J. R. Caren & D. R. Tarpy, 2011. The physical, insemination, and reproductive quality of honey bee queens (*Apis mellifera* L.). Apidologie, 42 (1): 1-13.

Dodoloğlu, A. & F. Genç, 1997. The effect of rearing and mating methods on some characteristics of queen honeybees (*Apis mellifera* L.). Turkish Journal of Veterinary and Animal Sciences, 21 (5): 379-385.

Dodoloğlu, A., B. Emsen & F. Genç, 2004. Comparison of some characteristics of queen honey bees (*Apis mellifera* L.) reared by using doolittle method and natural queen cells. Journal of Applied Animal Research, 26 (2): 113-115.

Facchini, E., M. G. De Iorio, F. Turri, F. Pizzi, D. Laurino, M. Porporato, R. Rizzi & G. Pagnacco, 2021. Investigating genetic and phenotypic variability of queen bees: Morphological and reproductive traits. Animals, 11 (11): 3054.

Frost, E. A., N. C. Chapman, R. G. Banks & S. Hermesch, 2021. "Breeding for Improved Fertility of Honey Bees, 97-109". In: Breeding Focus 2021-Improving Reproduction (Eds. S. Hermesch & S. Dominik), Animal Genetics and Breeding Unit University of New England, Armidale, NSW, Australia, 205 pp.

Gencer, H. V. & C. Fıratlı, 1999. Comparison of some internal and external structural characteristics of queen bees (*Apis mellifera* anatoliaca) reared from one and two day old larvae. Journal of Agricultural Sciences, 5 (3): 13-16 (in Turkish with abstract in English).

Genç, F., 1996. The effects of rearing months and larval transfer methods on the characteristics of queen bees (*Apis mellifera* L.) reared in Erzurum conditions. TÜBİTAK Project Final Report No. VHAG-1032.

Gençer, H. V., S. Q. Shah & Ç. Fıratlı, 2000. Effects of supplemental feeding of queen rearing colonies and larval age on the acceptance of grafted larvae and queen traits. Pakistan Journal of Biological Sciences, 3 (8): 1319-1322.

Guler, A. & H. Alpay, 2005. Reproductive characteristics of some honeybee (*Apis mellifera* L.) genotypes. Journal of Animal and Veterinary Advances, 4 (10): 864-870.

Hatch, S., D. R. Tarpy & D. J. C. Fletcher, 1999. Worker regulation of emergency queen rearing in honey bee colonies and the resultant variation in queen quality. Insectes Sociaux, 46 (4): 372-377.

Hatjina, F., M. Bieńkowska, L. Charistos, R. Chlebo, C. Costa, M. M. Dražić & J. Kopernicky, 2014. A review of methods used in some European countries for assessing the quality of honey bee queens through their physical characters and the performance of their colonies. Journal of Apicultural Research, 53 (3): 337-363.

Kaftanoglu, O. & U. Kumova, 1992. The effects of queen (*Apis mellifera* L.) breeding season on the quality of queen bees in Çukurova Region conditions, Journal of Veterinary and Animal Sciences, 16: 569-577.

Kahya, Y., H. V. Gençer & J. Woyke, 2008. Weight at emergence of honey bee (*Apis mellifera caucasica*) queens and its effect on live weights at the pre and post mating periods. Journal of Apicultural Research, 47 (2): 118-125.

Kumar, J. & P. Mall, 2016. Assessment of colony performance through brood and honey measurement in *Apis mellifera ligustica*. Journal of Experimental Zoology India, 19 (1): 1355-1358.

Laidlaw, H. H. Jr., 1985. Contemporary Queen Rearing. Hamilton, Illinois, USA, Dadant Publication, 199 pp.

Mackensen, O. & K. W. Tucker, 1970. Instrumental Insemination of Queen Bees. Agricultural Handbook (No. 390), US Agricultural Research Service, Washington, D.C., U.S.A, 28 pp.

Onk, K., M. M. Cengiz, K. Yazıcı & T. Kırmızıbayrak, 2016. Effects of rearing periods on some reproductive characteristics of Caucasian (*Apis mellifera caucasica*) queen bees. Atatürk Üniversitesi Veteriner Bilimleri Dergisi, 11 (3): 259-266.

Ozmen Ozbakır, G., 2021. Effects of rearing method on some morphological and reproductive characteristics of queen honey bees (*Apis mellifera* L.). Medycyna Weterynaryjna, 77 (2): 89-94.

Oztokmak, A. & G. Ozmen Ozbakır, 2017. Effects of queen emergence weight on some behavioral characteristics and colony performance parameters in Şanlıurfa local honey bees (*Apis mellifera* L.). Harran Journal of Agricultural and Food Sciences, 21 (4): 385-393.

Porporato, M., G. Grillone, A. Patetta, A. Manino & D. Laurino, 2015. Survey of the health status of some honey bee queens in Italy. Journal of Apicultural Science, 59 (2): 27-36.

Rangel, J., J. J. Keller & D. R. Tarpy, 2013. The effects of honey bee (*Apis mellifera* L.) queen reproductive potential on colony growth. Insect Sociaux, 60 (1): 65-73.

Skowronek, W., M. Bieñkowska & C. Kruk, 2004. Changes in body weight of honey bee queens during their maturation. Journal of Apicultural Science, 48 (2): 61-68.

Tarpy, D. R., J. J. Keller, J. R. Caren & D. A. Delaney, 2011. Experimentally induced variation in the physical reproductive potential and mating success in honey bee queens, Insectes Sociaux, 58 (4): 569-574.

Tarpy, D. R., J. J. Keller, J. R. Caren & D. A. Delaney, 2012. Assessing the mating 'health' of commercial honey bee queens. Journal of Economic Entomology, 105 (1): 20-25.

Tarpy, D. R., S. Hatch & D. J. C. Fletcher, 2000. The influence of queen age and quality during queen replacement in honey bee colonies. Animal Behaviour, 59 (1): 97-101.

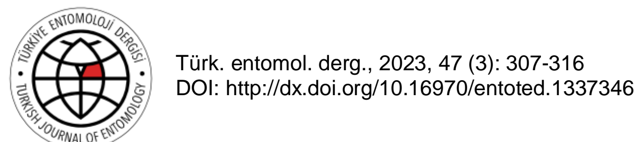
Uçak Koç, A. & M. Karacaoğlu, 2011. Effects of queen rearing period on reproductive features of Italian (*Apis mellifera ligustica*), Caucasian (*Apis mellifera caucasica*), and Aegean ecotype of Anatolian honey bee (*Apis mellifera anatoliaca*) queens. Turkish Journal of Veterinary & Animal Sciences, 35 (4): 271-276.

Uçak Koç, A. & M. Karacaoglu, 2004. Effects of rearing season on the quality of queen honeybees (*Apis mellifera* L.) raised under the conditions of Aegean region. Mellifera. 4 (7): 34-37.

Uçak Koç, A. & M. Karacaoglu, 2005. Anadolu arısı Ege ekotipi (*Apis mellifera anatolica*) ana arılarında üreme özellikleri. Adnan Menderes Üniversitesi Ziraat Fakültesi Dergisi, 2 (1): 73-77 (in Turkish with abstract in English).

Winston, M. L., 1987. The Biology of the Honey Bee. Harvard University Press, 281 pp.

Woyke, J., 1971. Correlations between the age at which honeybee brood was grafted, characteristics of the resultant queens and result of insemination. Journal Apicultural Research, 10 (1): 45-55.



Türk. entomol. derg., 2023, 47 (3): 307-316
DOI: http://dx.doi.org/10.16970/entoted.1337346

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Identification of odorant receptor protein genes in two Cephid stem borers (Hymenoptera: Cephidae) by high-throughput sequencing[1]

İki Cephid sap arısında (Hymenoptera: Cephidae) koku reseptör protein genlerinin yüksek verimli dizileme ile tanımlanması

Mahir BUDAK[2*]

Abstract

Insects are well adapted organisms to the terrestrial life on Earth. The evolution of the odorant receptor family is one of the causes underpinning this remarkable adaptation. Odorant receptors (ORs) sense aromas in the environment and cause the insect to respond. The ability of phytophagous insects to detect odor signals from their hosts is crucial for mating, oviposition, and feeding. The family of odorant receptor genes in Cephidae, pest on some economically important plants, is little understood. Bioinformatic tools were used to analyze the genomic data of the two pest species, *Syrista parreyssii* (Spinola, 1843) (Hymenoptera: Cephidae) (a rose pest) and *Pachycephus smyrnensis* J.P.E.F. Stein, 1876 (Hymenoptera: Cephidae), (a poppy pest), to determine their odorant receptors. The whole genome sequencing of *P. smyrnensis* collected in Sivas in 2020 was performed by next generation sequencing and short reads of *S. parreyssii* genome were obtained from previous studies. Following bioinformatic analyses, 67 and 82 putative odorant receptor genes were identified and annotated for *P. smyrnensis* and *S. parreyssii*, respectively. The ORs of these two species were found to be organized as repetitive genes in five separate clusters. No species-specific OR genes were identified in any of the investigated species. As a result, it was hypothesized that host specificity was acquired through the combined effect of multiple ORs.

Keywords: Cephini, next-generation sequencing, odorant receptors, repetitive genes, Sawflies

Öz

Böcekler, Dünya'daki karasal yaşama iyi uyum sağlamış organizmalardır. Koku reseptör ailesinin evrimi, bu olağanüstü adaptasyonun altında yatan nedenlerden biridir. Koku reseptörleri (OR'ler) çevredeki aromaları algılar ve böceğin tepki vermesine neden olur. Fitofag böceklerin konukçularından gelen koku sinyallerini algılama yeteneği çiftleşme, yumurtlama ve beslenme için çok önemlidir. Ekonomik açıdan önemli bazı bitkilerde zararlı olan Cephidae'deki koku reseptör genleri ailesi çok az anlaşılmıştır. Biyoinformatik araçlar, iki zararlı türün, *Syrista parreyssi* (Spinola, 1843) (Hymenoptera: Cephidae) (bir gül zararlısı) ve *Pachycephus smyrnensis* J.P.E.F. Stein, 1876 (Hymenoptera: Cephidae), (bir haşhaş zararlısı), koku reseptörlerini belirlemek amacıyla genomik verilerini analiz etmek için kullanılmıştır. Sivas'ta 2020 yılında toplanan *P. smyrnensis* tüm genom dizilemesi yeni nesil dizileme ile yapılmış ve *S. parreyssii* genomuna ait kısa okumalar ise önceki çalışmalardan elde edilmiştir. Analizler sonucunda *P. smyrnensis*'ten 67 olası koku reseptörü geni ve *S. parreyssii*'den 82 olası koku reseptörü geni tanımlandı ve açıklandı. Bu iki türün OR'lerinin beş ayrı kümede tekrarlayan genler olarak organize olduğu bulunmuştur. İncelenen türlerin hiçbirinde türe özgü OR genleri tespit edilmemiştir. Sonuç olarak, konakçı özgüllüğünün birden fazla OR'nin birleşik etkisi yoluyla kazanıldığı varsayılmıştır.

Anahtar sözcükler: Cephini, yeni nesil dizileme, koku almaçları, tekrarlayan genler, Testereli arılar

[1] This study was supported by Sivas Cumhuriyet University, Scientific Research Unit, Sivas, Türkiye, Grant Project No: F-508.
[2] Sivas Cumhuriyet University, Faculty of Science, Department of Molecular Biology and Genetics, 58140, Sivas, Türkiye
* Corresponding author (Sorumlu yazar) e-mail: mbudak@cumhuriyet.edu.tr
Received (Alınış): 03.08.2023 Accepted (Kabul ediliş): 30.09.2023 Published Online (Çevrimiçi Yayın Tarihi): 05.10.2023

Introduction

The Cephidae family is a member of the Cephoidea (Hymenoptera) superfamily. Fossil forms belonging to the Cephidae family were encountered in the Lower Cretaceous period (Gauld & Bolton, 1988). Cephidae contains approximately 160 species. The family is divided into three subfamilies: Athetocephinae, Australcephinae, and Cephinae. Cephinae is the most diverse and widespread subfamily among them. More than 40 of the species are distributed in Europe and the Mediterranean Region (Benson, 1951; Smith & Shinohara, 2002; Smith & Schmidt, 2009; Taeger et al., 2018). So far, twenty-eight species belonging to this family have been reported from Anatolia (Budak, 2012). All members of the Cephinae subfamily continue their life cycles as phytophagous in different plant groups. Several members of the Cephinae subfamily are responsible for substantial product losses on commercially relevant plants. *Pachycephus smyrnensis* J.P.E.F. Stein, 1876 (Hymenoptera: Cephidae), for example, causes harm to poppy plants (*Papaver somniferum* L., 1753 (Ranunculales: Papaveraceae)), whilst *Syrista parreyssi* (Spinola, 1843) (Hymenoptera: Cephidae) causes damage to roses by laying eggs on stems (Giray, 1985; Demirözer et al., 2011).

Pesticides are often used to control these insects; however, the chemicals are unsuccessful since the larvae develop in the stem (Altınayar, 1975; Li et al., 2015). Both to reduce product loss and to safeguard the environment, more environmentally friendly and effective pest control solutions must be developed. Insects use chemical sensors to identify host plants, which may be valuable for insect control (Venthur & Zhou, 2018). The chemosensory gene families are important members of insect genomes, which are involved in olfactory and gustatory functions (Sánchez-Gracia et al., 2009). Perception of chemicals in the environment enables insects to react. Therefore, the behaviors such as finding mates, hosts, food, oviposition sites and avoiding harmful situations are stimulated (Haverkamp et al., 2018). The olfactory system detects and recognizes many different volatile signal molecules belonging to various chemical classes (Keller & Vosshall, 2016). These molecules are detected by two gene families, odorant receptors (ORs), and ionotropic receptors (IRs). While ORs only detect volatile molecules, IRs can distinguish different types of molecules (Rimal & Lee, 2018). Research to date has shown that OR proteins cause stimulus-specific responses and that receptor structure provides a basis for detecting and identifying a wide range of chemicals (Hallem & Carlson, 2004; Hallem et al., 2004; Andersson et al., 2015).

The first insect ORs were identified in the fruit fly *Drosophila melanogaster* Meigen, 1830 (Diptera: Drosophilidae) at the end of the last century using a bioinformatics-based approach combined with the sequencing of an antenna cDNA library (Vosshall et al., 1999; Leal, 2013), and soon after, the data obtained by completing the genome sequence was expanded to complement 60 genes encoding 62 receptors (Vosshall et al., 2000; Robertson et al., 2003; Robertson, 2018). Since then, significant progress has been made in elucidating the roles of this ecologically essential gene family in insect biology. Based on a model of genetic evolution in *D. melanogaster*, Robertson et al. (2003) argued that the OR family may have evolved from the much larger (ancient) GR family, with the influence of terrestrial environment in insects, confirming the hypothesis of Scott et al. (2001). The origins of the GR family have been dates back to early animals, but ORs have to be a more recent gene family because they are not present in non-insect arthropods (Arthropods) (Robertson, 2015; Saina et al., 2015; Eyun et al., 2017). The appearance and spread of ORs among insects could be key to the insects' success (Yan et al., 2020). Also, it may have cleared the way for very distinct life strategies to develop over time.

Adapting to different hosts requires the development of specific odour receptors. Two large gene families, the odor-binding proteins (OBPs) and ORs, form the molecular basis of insect odor recognition (Hansson & Stensmyr, 2011). Great attempts have been made to reveal the evolutionary dynamics of the odorant receptor gene family since the initial identification of odorant receptors in insects (Clyne et al., 1999). Researchers have observed that the amount of OR genes and their diversity between insects can differ depending on the species (Missbach et al., 2014; Andersson et al., 2015). No correlation was found

between OR number and genome size. Genome size varies even between closely related Hymenoptera species but is more commonly associated with the insect's life history (Ardila-Garcia et al., 2010; Niu et al., 2022). Insect olfactory receptors function as heteromultimers consisting of at least one ligand-specific OR and the coreceptor Orco (Wicher et al., 2008). While the olfactory-related gene orco is substantially conserved among insect linages, other olfactory-related genes exhibit little sequence similarity, even among members of the Hymenoptera (Gress et al., 2013; Robertson et al., 2018). The number of OR genes vary from ten to six hundred in various insect genomes (Kirkness et al., 2010; McKenzie & Kronauer, 2018). Recent advances in the sequencing of genomes and transcriptomes have enabled the identification of putative OR genes of various insect species. Genes encoding ORs that are co-expressed in flies (*Drosophila*) and mosquitoes (*Anopheles*) are clustered together in the genome, as proven by transcriptome and genome studies involving OR genes (Ray et al., 2007). Then, expression analyses of clustered OR genes uncovered polycistronic RNA (Karner et al., 2015).

In this study, odorant receptor genes in two cephid pest species, *P. smyrnensis* and *S. parreyssii* were annotated by using next-generation sequencing and bioinformatics techniques. The gene annotation tools and were used to identify the sequences of odorant receptor gene families from *de novo* assembly of whole genome reads. Putative odorant receptor genes were then checked manually. On the basis of analyzed species, a gene family tree was constructed to infer the relationships between ORs across species. The main purpose of this study was to gain a better understanding of the evolutionary dynamics of the ORs in the Cephini subfamily.

Materials and Methods

The male specimens of *P. smyrnensis* were collected in Sivas (Türkiye), on flower of *Papaver rhoeas* L., 1753 (Ranunculales: Papaveraceae) in June 2020. Simple salting out protocol was used to recover whole genomic DNA from the specimen's hind leg (Miller et al., 1988). To verify the size distribution (>20 kbp), the isolated DNA was examined on a 0.8% agarose gel using a 1 kb extension ladder (Invitrogen). The libraries were constructed with Illumina DNA Prep with tagmentation and a 150bp pairwise short reads sequenced by Novagen (Beijing, China) by using HiSeq 2500 (Illumina, U.S.A.) Sequencing Platform. BioProject accession codes PRJNA816475 can be used to obtain short reads in GenBank and the SRA databases.

Genome assembly and annotation

The short reads produced in this study and other reads available in the SRA database (*P. smyrnensis*; SRX12828967, 164.3M paired reads and *S. parreyssii*; SRX12142183, 169.4M paired reads) were used for genome assembly analyses. The raw data was cleaned from adapter sequences and low-quality reads using fastp v 0.20.1. (Chen et al., 2018). Scaffolds construction was done using SPAdes v3.13.1 (Bankevich et al., 2012) with "-k 55, 87, 109, 121 --careful --cov-cutoff auto" options on TRUBA (TUBITAK ULAKBIM, High Performance and Grid Computing Center). Genome size (hence, GS) was estimated by Jellyfish version 1.1.11 (Marçais & Kingsford, 2011) and summary statistics for assemblies were calculated by a custom python script. The assembly quality was evaluated using BUSCO v.5.4.6 (Benchmarking Universal Single-Copy Orthologs) (Waterhouse et al., 2018) with the hymenoptera odb10 reference set.

Genome assembly of *Cephus cinctus* Norton, 1872 (Hymenoptera: Cephidae) (Acc.no: GCA_000341935.1) was included to analyses to test accuracy of genome annotations. Contigs over 1000 bp belonging to each specimen were analysed with Augustus v3.3.3 (Stanke et al., 2008) to predict coding sequences and saved as "gff" file. The amino acid sequences of each annotated gene were extracted from the "gff" file using the getAnnoFasta.pl script available in the Augustus (Stanke et al., 2006) and saved as a "fasta" file. InterProScan5 v54-87.0 (Jones et al., 2014; Blum et al., 2021) software was used to annotate the coding sequences of predicted genes identified in the previous analyses. The results were saved as "tsv" file, then sequences of ORs were pulled and saved separate files. The tblastn search was utilized to verify the

discovered sequences by the automated pipelines. The obtained results are available at https://doi.org/10.5281/zenodo.7540708.

Genome assembly and annotation

The OR sequences annotated from transcriptome analysis of antenna in the study of Robertson et al. (2018) was also included in the final data sets. OR protein alignments were produced with Mafft v7.453 (Katoh & Standley, 2013) with L-INS-i algorithm to find more accurately conserved regions. Gaps were trimmed using TrimAl v1.2 (Capella-Gutiérrez et al., 2009) with "-gt 0.7 -cons 60" options to remove gaps in 30% or more of the sequences. Gene tree inference was performed using RaxML v8.2.12 (Stamatakis, 2014) under the JTT + G substitution model, which was previously determined to be the most accurate model for OR gene trees (Brand & Ramrez, 2017) for 10 separate ML searches and 1000 bootstrap replicates.

Results and Discussion

The assembly of the genome of *P. smyrnensis* was accomplished by two pairs of short reads (193,287,106 in total), one from this study and one from the GenBank database. To facilitate genome assembly, we employed the haploid male *P. smyrnensis* to obtain NGS data. As a result, complexity associated with length polymorphisms between haplotypes were averted. The short reads used for *S. parreyssii* (169,365,520 reads) genome assembly were downloaded from GenBank. In their respective assemblies, the genomes of *P. smyrnensis* and *S. parreyssii* were found to have 95.6 (complete and single-copy BUSCOs: 95.4%, complete and duplicated BUSCOs: 0.2%) and 96.6 (complete and single-copy BUSCOs: 96.5%, complete and duplicated BUSCOs:0.1%) complete single-copy and duplicated genes, respectively. BUSCO is a powerful tool to assess the robustness and quality of genomic data based on expected gene content. For this purpose, BUSCO uses datasets of single copy orthologs derived from OrthoDB (Manni et al., 2021). Statistics for genome assemblies of *P. smyrnensis* and *S. parreyssii* were given in Table 1. The assembly sizes are quite similar to the in-silico genome size estimates (160.8 for *S. parreyssii* and 226.2 Mb for *P. smyrnensis*) determined by k-mer-based genome-size assessments (158 for *S. parreyssii* and 210.1 Mb *P. smyrnensis*). The NG50 and LG50 values are comparable with those for other hymenopterans in GenBank (Table 1).

Table 1. *Pachycephus. smyrnensis*, *Syrista parreyssi*, and *Cephus cinctus* genome assembly statistics

	Pachycephus smyrnensis	*Syrista parreyssi*	*Cephus cinctus*
Number of scaffolds	313,296	73,263	1,975
Contig length (max)	1,380,577	4,473,258	4,355,184
Number of scaffolds (>1K)	6366	1427	1,975
N50	113,745	733,566	622,163
L50	398	58	56
NG50	137,571	756,37	624,847
LG50	334	55	55
k-mer based estimated genome size	210.1M	158.0M	-
Genome size	226.2M	160.8 M	162.2M
k-mer based single copy region	149.1M	138.4M	-

Considering that *C. cinctus* and *S. parreyssii* are more recently evolved clades in Cephidae than *P. smyrnensis* (Budak, 2012), a decrease in genome size (GS) can be suggested. However, this reduction may have resulted from the insect-host relationship because insect-symbiont relationships are also known to play a role in genomic integration (Wernegreen, 2012). The k-mer-based estimates of single-copy regions are very close for both species (*S. parreyssii* and *P. smyrnensis*; Table 1). Therefore, this decrease in GS

can be mostly attributed to the reduction of repetitive regions. There may be a correlation between insect lifestyle and genome size, but this correlation is not always easy to demonstrate (Gregory, 2004). While the lifestyles of all three species studied here are similar, their host plants preferences are different. Although GS seems to evolve in response to host change, more research is needed to study the genomes of host plants and insects to elucidate this connection. According to Chak et al. (2021), eusocial organisms have larger genomes with the high proportion of repetitive sequences. Although several studies have attempted to explain the relationship between GS and insect lifestyles (Johnston et al., 2004; Tsutsui et al., 2008; Ardila-Garcia et al., 2010), additional studies are needed to shed light on genome size differences.

Selection of candidate OR genes

Augustus analysis projected 33,972 and 42,904 genes for the *S. parreyssii* and *P. smyrnensis* genomes, respectively (Table 2). It is a fact that this approach probably overestimates the actual number of genes present in the genome due to false positives (Saari et al., 2017). Genome annotation of non-model organisms is more challenging, but it is obvious that this obstacle will be addressed with high-throughput sequencing and improvements in bioinformatics. To identify the predicted genes obtained from the Augustus analysis, Pfam filtering and HMMER profiling were performed against the InterPro database (Blum et al., 2021) with interproseq5 software. Putative ORs were extracted from the identified genes using custom linux scripts. A total of 82, 67 and 56 ORs were found for *S. parreyssii*, *P. smyrnensis* and *C. cinctus*, respectively. To test the validity of the found OR genes, the amino acid sequences of the genes were compared with the GenBank database using the tblastn algorithm. One candidate gene identified in the *P. smyrnensis* genome did not match any OR available in the database (see supplementary material). OR genes annotated through bioinformatic analyses are named in the order in which they were discovered by the Augustus analysis. The suffixes (Cc for *C. cinctus*, Ps for *P. smyrnensis*, and Sp for *S. parreyssii*) were appended to the genes to help identify which ORs are related with which species on the tree (Figure 1).

Table 2. Number of predicted genes and ORs identified from insect genomes

	Pachycephus smyrnensis	*Syrista parreyssi*	*Cephus cinctus*
Number of predicted genes	42904	33972	36242
Number of predicted Ors	67	82	56
Ors in tandem repeats	27	45	24
Orco	1	1	1

Ten of the 72 CcinORs (CcinOR20, CcinOR23, CcinOR24, CcinOR36, CcinOR38, CcinOR41, CcinOR42, CcinOR53, CcinOR59 and CcinOR72) identified by Robertson et al. (2018) were not grouped with any of the annotated genes in the branches of the dendrogram. Five were found to be more closely linked to *S. parreyssii* OR genes (CcinOR5, CcinOR7P, CcinOR16, CcinOR34, and CcinOR43), while four were found to be more closely related to *P. smyrnensis* OR genes (CcinOR3p, CcinOR15, CcinOR28 and CcinOR71P). The remaining CcinORs were grouped with genes annotated from the *C. cintus* genome. Each of the three species tested had one conserved Orco gene as expected (g14373_Cc, g3653_Sp and g2593_Ps). Even in absence of transcriptome data, gene annotation success can be considered satisfactory. It is also necessary to correctly identify the repeating genes within a cluster to gain a better understanding of the evolution of ORs. The presence of repetitive sequences in the genome complicates genome assembly with short reads. The better way to circumvent this challenge is to use long reads in combination with short reads in genome assembly analyzes (Treangen & Salzberg, 2011; Claros et al., 2012).

Nearly half of the annotated ORs were in tandem-array (see Table 2, Figure 1), which was consistent with previous research but did not confirm the findings of Robertson et al. (2018). The phylogenetic tree using amino acid sequences of the identified ORS from these species revealed that the five clades were composed of tandem array genes (Figure 1). The existence of ORs in the genome as a tandem-array supports the

hypothesis of gene gain through duplication (Andersson et al., 2015). All three species have tandem-array genes in these five clades, however the cluster3 is dominated by *S. parreyssii* and *C.cinctus* ORs. Because *S. parreyssii* and *C. cinctus* are younger species (Budak et al., 2011), it is possible that *P. smyrnensis*' solitary OR (g28439_Ps, an orthologue of CcinOR25) at the clade's base gave rise to a new gene cluster.

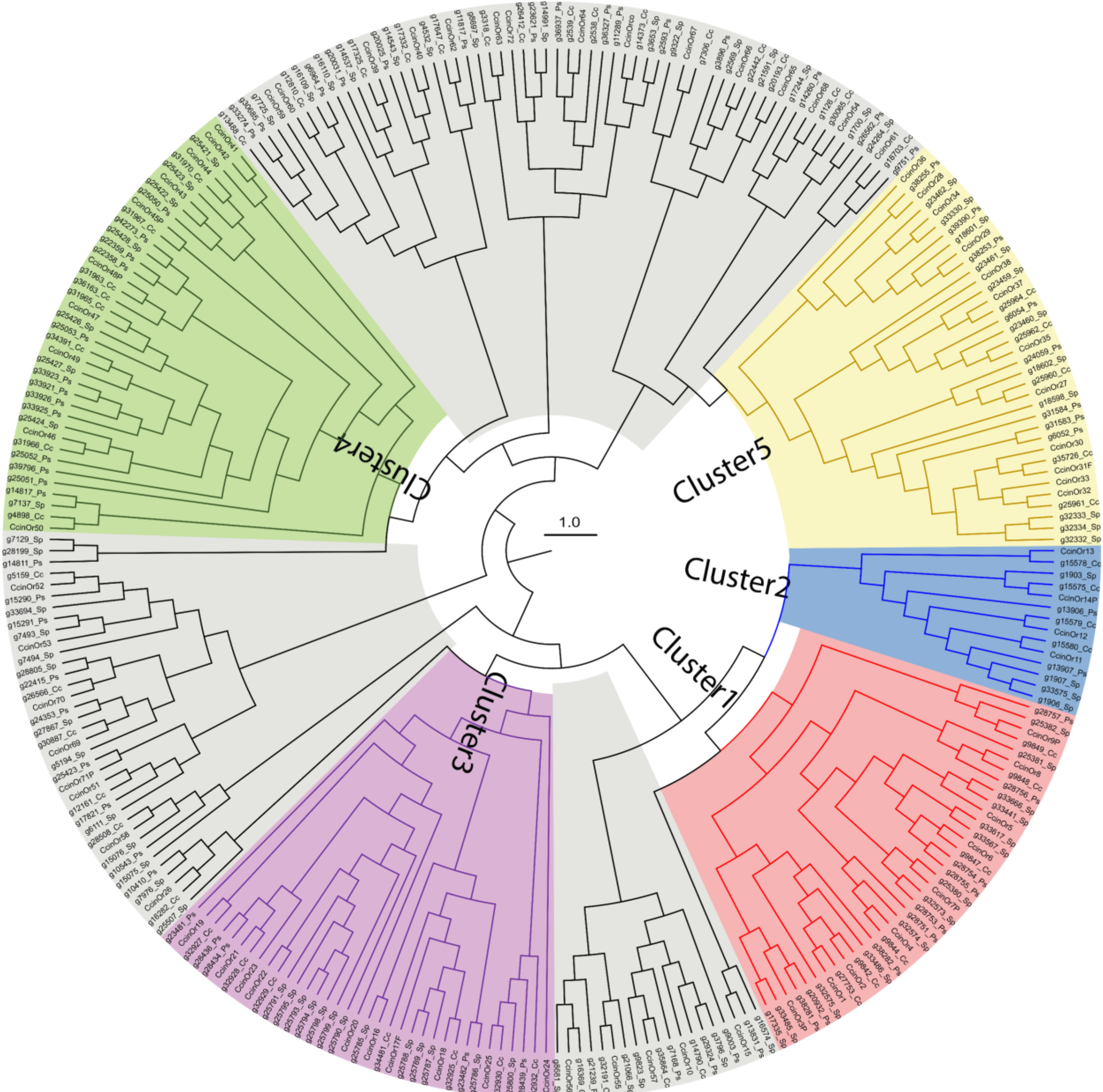


Figure 1. Phylogenetic relationships of the odorant receptor family in three cephid species. The branches are coloured by OR family clusters, red for cluster 1, blue for cluster 2, purple for cluster 3, green for cluster 4, and yellow for cluster 5. The scale bar indicates substitutions per site.

If ORs are the primary pathway for host recognition, it is reasonable to expect that organisms prefer different hosts will evolve specialized ORs for different odours. However, studies suggest that two strategies predominate for the processing and evaluation of odour signals. The first is combinatorial coding based on broadly tuned receptors: several different odours activate one type of receptor or, a particular odor can activate several types of receptors. The second is based on labelled lines: receptor types are narrowly

tuned for specific odours (Galizia, 2014; Wicher & Miazzi, 2021). There was no evidence of a species-specific OR gene family in any of the three species under investigation. It appears likely that it is more successful at host recognition when numerous receptors function in concert rather than when a single OR acts alone. The combined effect of mutations accumulated in different OR genes may have caused host shift in members of the Cephinae subfamily. On the other hand, it is likely that the plant generates a variety of odours, each of which is detected by different receptors, ensuring the insect's attraction to the host. Another notable finding is that while *S. parreyssii* has the fewest genes discovered as a result of gene annotations analysis, it has the highest number of ORs identified. This could also indicate that OR genes have evolved in concert within a clade, such as the observed evolutionary pattern in ribosomal genes. (Ganley & Kobayashi, 2007). Finally, it appears that there is no OR that is particular to host recognition among the ORs of these species. In order to better understand the host change mechanism in insects, it is important to examine a greater number of insect genomes as well as a greater number of gene interactions.

The functional characterization of insect ORs has been extensively studied in moths, flies, and mosquitoes, but has been neglected in other insect orders (Yuvaraj et al., 2021). The lack of functional data is a factor that severely limits our understanding of the molecular evolution of olfaction in symphyta. This challenge could be more easily addressed if more ORs for this group were identified and characterized. Studying the interactions between insect ORs and their ligands is important for understanding the molecular and functional evolution of insect OR families. This knowledge helps to find more effective OR agonists or antagonists for pest control. Future applications may lead to the development of more environmentally friendly strategies to replace chemicals in sawfly control.

Acknowledgements

This study is supported by the Scientific Research Project Fund of Sivas Cumhuriyet University under the project number F-508. The numerical calculations reported in this paper were partially performed at TÜBITAK ULAKBIM, High Performance and Grid Computing Center (TRUBA resources).

References

Altınayar, G., 1975. Ekin Sap Arıları (*Cephus pygmeus* (L.) ve *Trachelus tabidus* (F.) (Hymenoptera: Cephidae)'nin Konya Ilinde Biyo-Ekolojileri, Sebep Oldukları Ürün Kayıpları ve Savaş Yolları Üzerine Araştırmalar. Ankara Bölge Zirai Mücadele Araştırma Enstitüsü Yayınları, Araştırma Eserleri Serisi 36: 135 s (in Turkish).

Andersson, M. N., C. Löfstedt & R. D. Newcomb, 2015. Insect olfaction and the evolution of receptor tuning. Frontiers in Ecology and Evolution, 3: Article ID 53.

Ardila-Garcia, A. M., G. J. Umphrey & T. R. Gregory, 2010. An expansion of the genome size dataset for the insect order Hymenoptera, with a first test of parasitism and eusociality as possible constraints. Insect Molecular Biology, 19 (3): 337-346.

Bankevich, A., S. Nurk, D. Antipov, A. A. Gurevich, M. Dvorkin, A.S. Kulikov, V. M. Lesin, S. I. Nikolenko, S. Pham, A. D. Prjibelski, A. V. Pyshkin, A. V. Sirotkin, N. Vyahhi, G. Tesler, M. A. Alekseyev & P. A. Pevzner, 2012. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. Journal of Computational Biology, 19 (5): 455-477.

Benson, R. B., 1951. Hymenoptera: 2. Symphyta. Section (a). Handbooks for the identification of British insects. Royal Entomological Society of London, London, Vol. VI, Part 2 (a): 50 pp.

Blum, M., H. Y. Chang, S. Chuguransky, T. Grego, S. Kandasaamy, A. Mitchell, G. Nuka, T. Paysan-Lafosse, M. Qureshi, S. Raj, L. Richardson, G. A. Salazar, L. Williams, P. Bork, A. Bridge, J. Gough, D. H. Haft, I. Letunic, A. Marchler-Bauer, H. Mi, D.A. Natale, M. Necci, C. A. Orengo, A. P. Pandurangan, C. Rivoire, C. J. A Sigrist, I. Sillitoe, N. Thanki, P. D. Thomas, S. C. E Tosatto, C. H. Wu, A. Bateman & R. D. Finn, 2021. The InterPro protein families and domains database: 20 years on. Nucleic Acids Research, 49 (D1): 344-354.

Brand, P. & S. R. Ramírez, 2017. The evolutionary dynamics of the odorant receptor gene family in corbiculate bees. Genome Biology and Evolution, 9 (8): 2023-2036.

Budak, M., 2012. Systematics, Biogeography and Phylogeny of Cephidae (Hymenoptera: Insecta) Species of Turkey. Cumhuriyet Üniversitesi, Fen Bilimleri Ensititüsü, (Unpublished) PhD Thesis, Sivas, 201 pp (in Turkish with abstract in English).

Budak, M., E.M. Korkmaz & H.H. Basibuyuk, 2011. A molecular phylogeny of the Cephinae (Hymenoptera, Cephidae) based on mtDNA COI gene: a test of traditional classification. ZooKeys, 130: 363-378.

Capella-Gutiérrez, S., J. M. Silla-Martínez & T. Gabaldón, 2009. trimAl: A tool for automated alignment trimming in large-scale phylogenetic analyses. Bioinformatics, 25 (15): 1972-1973.

Chak, S. T. C., S. E. Harris, K. M. Hultgren, N. W. Jeffery & D. R. Rubenstein, 2021. Eusociality in snapping shrimps is associated with larger genomes and an accumulation of transposable elements. Proceedings of the National Academy of Sciences of the United States of America, 118 (24): e2025051118.

Chen, S., Y. Zhou, Y. Chen & J. Gu, 2018. Fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics, 34 (17): i884-i890.

Claros, M. G., R. Bautista, D. Guerrero-Fernández, H. Benzerki, P. Seoane & N. Fernández-Pozo, 2012. Why assembling plant genome sequences is so challenging. Biology, 1 (2): 439-459.

Clyne, P. J., G. C. Warr, M. R. Freeman, D. Lessing, J. Kim & J. R. Carlson, 1999. A novel family of divergent deven-transmembrane proteins: candidate odorant receptors in *Drosophila*. Neuron, 22 (2): 327-338.

Demirözer, O., I. Karaca & Y. Karsavuran, 2011. Population fluctuations of some important pests and natural enemies found in oil-bearing rose (Rosa damascena Miller) production areas in Isparta province (Turkey). Turkish Journal of Entomology, 35 (4): 539-558.

Eyun, S., H. Y. Soh, M. Posavi, J. B. Munro, D. S. T. Hughes, S. C. Murali, J. Qu, S. Dugan, S.L. Lee, H. Chao, H. Dinh, Y. Han, H. Doddapaneni, K. C. Worley, D. M. Muzny, E. Park, J. C. Silva, R. A. Gibbs, S. Richards & C. E. Lee, 2017. Evolutionary history of chemosensory-related gene families across the Arthropoda. Molecular Biology and Evolution, 34 (8): 1838-1862.

Galizia, C. G., 2014. Olfactory coding in the insect brain: data and conjectures. European Journal of Neuroscience, 39 (11): 1784-1795.

Ganley, A. R. D. & T. Kobayashi, 2007. Highly efficient concerted evolution in the ribosomal DNA repeats: Total rDNA repeat variation revealed by whole-genome shotgun sequence data. Genome Research, 17 (2): 184-191.

Gauld, I. D. & B. Bolton, 1988. The Hymenoptera. British Museum (Natural History) & Oxford University Press, London, 332 pp.

Giray, H., 1985. A list of pests of popy (*Papaver somniferum* L.) in Turkey, with notes on the types of damage of the important species. Türkiye Bitki Koruma Dergisi (Turkish Journal of Entomology), 9 (2): 109-124 (in Turkish with abstract in English).

Gregory, T. R., 2004. Insertion-deletion biases and the evolution of genome size. Gene, 324 (1): 15-34.

Gress, J. C., H. M. Robertson, D.K. Weaver, M. Dlakić & K. W. Wanner, 2013. Odorant receptors of a primitive hymenopteran pest, the wheat stem sawfly. Insect Molecular Biology, 22 (6): 659-667.

Hallem, E. A. & J. R. Carlson, 2004. Coding of odors by a receptor repertoire. Cell, 125 (1): 143-160.

Hallem, E. A., M. G. Ho & J. R. Carlson, 2004. The molecular basis of odor coding in the Drosophila antenna. Cell, 117 (7): 965-979.

Hansson, B. S. & M. C. Stensmyr, 2011. Evolution of Insect Olfaction. Neuron, 72 (5): 698-711.

Haverkamp, A., B. S. Hansson & M. Knaden, 2018. Combinatorial codes and labeled lines: How insects use olfactory cues to find and judge food, mates, and oviposition sites in complex environments. Frontiers in Physiology, 9: Article ID 49.

Johnston, J. S., L. D. Ross, L. Beani, D. P. Hughes & J. Kathirithamby, 2004. Tiny genomes and endoreduplication in Strepsiptera. Insect Molecular Biology, 13 (6): 581-585.

Jones P., D. Binns, H. Y. Chang, M. Fraser, W. Li, C. McAnulla, H. McWilliam, J. Maslen, A. Mitchell, G. Nuka, S. Pesseat, A. F. Quinn, A. Sangrador-Vegas, M. Scheremetjew, S. Yong, R. Lopez & S. Hunter, 2014. InterProScan 5: genome-scale protein function classification. Bioinformatics, 30 (9): 1236-1240.

Karner, T., I. Kellner, A. Schultze, H. Breer & J. Krieger, 2015. Co-expression of six tightly clustered odorant receptor genes in the antenna of the malaria mosquito *Anopheles gambiae*. Frontiers in Ecology and Evolution, 3: Article ID 26.

Katoh, K. & D. M. Standley, 2013. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Molecular Biology and Evolution, 30 (4): 772-780.

Keller, A. & L. B. Vosshall, 2016. Olfactory perception of chemically diverse molecules. BMC Neuroscience, 17: Article ID 55.

Kirkness, E. F., ,J. B. Haas, W. Sun, H. R. Braig, M. A. Perotti, J. M. Clark, S. H. Lee, H. M. Robertson, R. C. Kennedy, E. Elhaik, D. Gerlach, E. V. Kriventseva, C. G. Elsik, D. Graur, C. A. Hill, J. A. Veenstra, B. Walenz, J. M. C. Tubío, J. M. C. Ribeiro, J. Rozas, J. S. Johnston, J. T. Reese, A. Popadic, M. Tojo, D. Raoult, D. L. Reed, Y. Tomoyasu, E. Kraus, O. Mittapalli, V. M. Margam, H. Li, J. M. Meyer, R. M. Johnson, J. Romero-Severson, J. P. VanZee, D. Alvarez-Ponce, F. G. Vieira, M. Aguadé, S. Guirao-Rico, J. M. Anzola, K. S. Yoon, J. P. Strycharz, M. F. Unger, S. Christley, N. F. Lobo, M. J. Seufferheld, N. Wang, G. A. Dasch, C. J. Struchiner, G. Madey, L. I. Hannick, S. Bidwell, V. Joardar, E. Caler, R. Shao, S. C. Barker, S. Cameron, R. V. Bruggner, A. Regier, J. Johnson, L. Viswanathan, T. R. Utterback, G. G. Sutton, D. Lawson, R. M. Waterhouse, J. C. Venter, R. L. Strausberg, M. R. Berenbaum, F. H. Collins, E. M. Zdobnov & B. R. Pittendrigh, 2010. Genome sequences of the human body louse and its primary endosymbiont provide insights into the permanent parasitic lifestyle. Proceedings of the National Academy of Sciences, 107 (27): 12168-12173.

Leal, W. S., 2013. Odorant reception in insects: Roles of receptors, binding proteins, and degrading enzymes. Annual Review of Entomology, 58 (1): 373-391.

Li, H., R. Guan, H. Guo & X. Miao, 2015. New insights into an RNAi approach for plant defence against piercing-sucking and stem-borer insect pests. Plant, Cell & Environment, 38 (11): 2277-2285.

Marçais, G. & C. Kingsford, 2011. A fast, lock-free approach for efficient parallel counting of occurrences of k-mers. Bioinformatics. 27 (6): 764-770.

Manni, M., M. R. Berkeley, M. Seppey & E. M. Zdobnov, 2021. BUSCO: assessing genomic data quality and beyond. Current Protocols, 1 (12): e323.

McKenzie, S. K. & D. J. C. Kronauer, 2018. The genomic architecture and molecular evolution of ant odorant receptors. Genome Research, 28 (11): 1757-1765.

Miller, S. A., D. D. Dykes & H. F. Polesky, 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Research, 16 (3): Article ID 1215.

Missbach, C., H. K. M. Dweck, H. Vogel, A. Vilcinskas, M. C. Stensmyr, B. S. Hansson & E. Grosse-Wilde, 2014. Evolution of insect olfactory receptors. eLife, 3: e02115.

Niu, G., M. Budak, E. M. Korkmaz, Ö. Doğan, A. Nel, S. Wan, C. Cai, C. Jouault, M. Li & M. Wei, 2022. Phylogenomic analyses of the Tenthredinoidea support the familial rank of Athaliidae (Insecta, Tenthredinoidea). Insects, 13 (10): Article ID 858.

Ray, A., W. G. van Naters, T. Shiraiwa & J. R. Carlson, 2007. Mechanisms of odor receptor gene choice in *Drosophila*. Neuron, 53 (3): 353-369.

Rimal, S. & Y. Lee, 2018. The multidimensional ionotropic receptors of *Drosophila melanogaster*. Insect Molecular Biology, 27 (1): 1-7.

Robertson, H. M., 2015. The insect chemoreceptor superfamily is ancient in animals. Chemical Senses, 40: 609-614.

Robertson, H. M., C. G. Warr & J. R. Carlson, 2003. Molecular evolution of the insect chemoreceptor gene superfamily in *Drosophila melanogaster*. Proceedings of the National Academy of Sciences, 100 (2): 14537-14542.

Robertson, H. M., R. M. Waterhouse, K. K. O. Walden, L. Ruzzante, M. J. M. F. Reijnders, B. S. Coates, F. Legeai, J. C. Gress, S. Biyiklioglu, D. K. Weaver, K. W. Wanner & H. Budak, 2018. Genome sequence of the wheat stem sawfly, *Cephus cinctus*, representing an early-branching lineage of the Hymenoptera, illuminates evolution of hymenopteran chemoreceptors. Genome Biology and Evolution, 10 (11): 2997-3011.

Saari, T. W., A. L Schroeder, G. T. Ankley & D. L. Villeneuve, 2017. First-generation annotations for the fathead minnow (Pimephales promelas) genome. Environmental Toxicology and Chemistry, 36 (12): 3436-3442.

Saina, M., H. Busengdal, C. Sinigaglia, L. Petrone, P. Oliveri, F. Rentzsch & R. Benton, 2015. A cnidarian homologue of an insect gustatory receptor functions in developmental body patterning. Nature Communications, 6 (1): 6243.

Sánchez-Gracia, A., F. G. Vieira & J. Rozas, 2009. Molecular evolution of the major chemosensory gene families in insects. Heredity, 103 (3): 208-216.

Scott, K., R. Brady Jr., A. Cravchik, P. Morozov, A. Rzhetsky, C. Zuker & R. Axel, 2001. A Chemosensory Gene Family Encoding Candidate Gustatory and Olfactory Receptors in *Drosophila.* Cell, 104 (5): 661-673.

Smith, D. R. & A. Shinohara, 2002. A new genus and new species of Cephidae (Hymenoptera) from Sulawesi Utara, Indonesia. Proceedings of the Entomological Society of Washington, 104 (3): 624-628.

Smith, D. R. & S. Schmidt, 2009. A new subfamily, genus, and species of Cephidae (Hymenoptera) from Australia. Zootaxa, 2034 (1): 56-60.

Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics, 30 (9): 1312-1313.

Stanke, M., O. Keller, İ. Gunduz, A. Hayes, S. Waack & B. Morgenstern, 2006. AUGUSTUS: *ab initio* prediction of alternative transcripts. Nucleic Acids Research, 34 (2): W435-W439.

Stanke, M., M. Diekhans, R. Baertsch & D. Haussler, 2008. Using native and syntenically mapped cDNA alignments to improve de novo gene finding. Bioinformatics, 24 (5): 637-644.

Taeger, A., A. D. Liston, M. Prous, E. K. Groll, T. Gehroldt & S. M. Blank, 2018. ECatSym: electronic world catalog of symphyta. (Web page: https://www.sdei.de/ecatsym) (Date accessed: June 2023).

Treangen, T. J. & S. L. Salzberg, 2011. Repetitive DNA and next-generation sequencing: computational challenges and solutions. Nature Reviews Genetics, 13 (1): 36-46.

Tsutsui, N. D., A. V. Suarez, J. C. Spagna & J. S. Johnston, 2008. The evolution of genome size in ants. BMC Evolutionary Biology, 8 (1): Article ID 64.

Venthur, H. & J. J. Zhou, 2018. Odorant receptors and odorant-binding proteins as insect pest control targets: A comparative analysis. Frontiers in Physiology, 9: Article ID 1163.

Vosshall, L. B., A. M. Wong & R. Axel, 2000. An olfactory sensory map in the fly brain. Cell, 102 (2): 147-159.

Vosshall, L. B., H. Amrein, P. S. Morozov, A. Rzhetsky & R. Axel, 1999. A spatial map of olfactory receptor expression in the Drosophila antenna. Cell, 96 (5): 725-36.

Waterhouse, R. M., M. Seppey, F. A. Simão, M. Manni, P. Ioannidis, G. Klioutchnikov, E. V. Kriventseva & E. M. Zdobnov, 2018. BUSCO applications from quality assessments to gene prediction and phylogenomics. Molecular Biology and Evolution, 35 (3): 543-548.

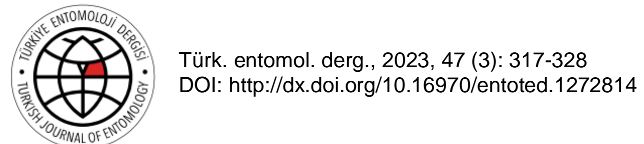
Wernegreen, J. J., 2012. Endosymbiosis. Current Biology, 22 (14): R555-R561.

Wicher, D. & F. Miazzi, 2021. Functional properties of insect olfactory receptors: ionotropic receptors and odorant receptors. Cell and Tissue Research, 383: 7-19.

Wicher, D., R. Schäfer, R. Bauernfeind, M. C. Stensmyr, R. Heller, S. H. Heinemann & B. S. Hansson, 2008. Drosophila odorant receptors are both ligand-gated and cyclic-nucleotide-activated cation channels. Nature, 452 (190): 1007-1011.

Yan, H., S. Jafari, G. Pask, X. Zhou, D. Reinberg & C. Desplan, 2020. Evolution, developmental expression and function of odorant receptors in insects. Journal of Experimental Biology, 223 (Suppl_1): jeb208215.

Yuvaraj, J. K., R. E. Roberts, Y. Sonntag, X. Q. Hou, E. Grosse-Wilde, A. Machara, D. Zhang, B. S. Hansson, U. Johanson, C. Löfstedt & M. N. Andersson, 2021. Putative ligand binding sites of two functionally characterized bark beetle odorant receptors. BMC Biology, 19: Article ID 16.



Türk. entomol. derg., 2023, 47 (3): 317-328
DOI: http://dx.doi.org/10.16970/entoted.1272814

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Aphid species (Hemiptera: Aphidoidae) determined from Antalya, Karaman and Muğla with new aphid records[1]

Antalya, Karaman ve Muğla'dan belirlenen yeni kayıt afit (Hemiptera: Aphidoidae) türleri

Hayal AKYILDIRIM BEĞEN[2*] **Gazi GÖRÜR[3]** **Özhan ŞENOL[3]**

Gizem BAŞER[3] **Bengisu Venhar AKÇAY[3]**

Abstract

Aphids (Hemiptera: Aphidoidae) are obligatory phytophagous and many of them highly polyphagous pests and distributed almost all parts of the earth, especially temperature zone. Türkiye has a potential area for aphids because of its rich plant diversity and different climatic region. Most of the aphid species is specific to their host plant and both cause direct and indirect damages. So far, 654 aphid species have been determined on numerous host plants in Türkiye. As a result of the study conducted in Antalya, Karaman and Muğla provinces in Türkiye between March 2020 and September 2021, 12 aphid species were added to Türkiye aphidofauna. These species are *Amphorophora urtica* Essig, 1942 on *Urtica dioica* L., *Aphis crepidis* (Börner, 1940) on *Crepis capillaris* L. (Wallr.), *Chaitophorus utahensis* (Knowlton, 1928) on *Salix* sp., *Monelliopsis caryae* (Monell, 1879) on *Juglans regia* L., *Doraphis populi* (Maskell, 1898) on *Populus* sp., *Eulachnus mediterraneus* Binazzi, 1983 on *Pinus* sp., *Metopolophium montanum* Hille Ris Lambers, 1966 on *Rosa* sp., *Protaphis echinopis* (Hille Ris Lambers, 1948) on *Echinops viscosus* DC., *Prociphilus erigeronensis* (Thomas, 1879) on *Cichorium* sp., *Sitobion leelamaniae* (David, 1958) on *Poa* sp. and *Triticum* sp., *Tiliaphis pseudoshinae* Quednau, 1979 on *Tilia* sp. and *Uroleucon iranicum* Holman, 1980 on *Gundelia tournefortii* (L.). Türkiye aphidofauna has reached 666 species with this study. Detailed information of locality of new records are also given.

Keywords: Aphid, Antalya, Karaman, Muğla, Türkiye

Öz

Afitler zorunlu fitofag ve birçoğu yüksek oranda polifag zararlıdırlar ve ılıman iklimler başta olmak üzere dünyanın hemen her yerine dağılmışlardır. Türkiye, zengin bitki çeşitliliği ve farklı iklim bölgeleri nedeniyle afitler için potansiyel bir alana sahiptir. Afit türlerinin çoğu konak bitkiye özgüdür ve bitki üzerinde hem doğrudan hem de dolaylı zararlara neden olur. Bugüne kadar, Türkiye'de çok sayıda konukçu bitki üzerinden, 654 yaprak biti türü tespit edilmiştir. Antalya, Karaman ve Muğla (Türkiye) illerinde, Mart 2020 ve Eylül 2021 yılları arasında yapılan çalışmalar neticesinde, Türkiye yaprak biti faunasına 12 yaprak biti türü eklenmiştir. Bu türler, *Urtica dioica* L. üzerinden *Amphorophora urtica* Essig, 1942, *Crepis capillaris* L. (Wallr.), üzerinden *Aphis crepidis* (Börner, 1940), *Salix* sp. üzerinden *Chaitophorus utahensis* (Knowlton, 1928), *Juglans regia* L. üzerinden *Monelliopsis carya* (Monell, 1879), *Populus* sp. üzerinden *Doraphis populi* (Maskell, 1898), *Pinus* sp. üzerinden *Eulachnus mediterraneus* Binazzi, 1983, *Rosa* sp. üzerinden *Metopolophium montanum* Hille Ris Lambers, 1966, *Echinops viscosus* DC. üzerinden *Protaphis echinopis* (Hille Ris Lambers, 1948), *Cichorium* sp. üzerinden *Prociphilus erigeronensis* (Thomas, 1879), *Poa* sp. ve *Triticum* sp. üzerinden *Sitobion leelamaniae* (David, 1958), *Tilia* sp. üzerinden *Tiliaphis pseudoshinae* Quednau, 1979 ve *Gundelia tournefortii* (L.) üzerinden *Uroleucon iranicum* Holman, 1980. Türkiye afit faunası sayısı bu çalışma ile 666'ya ulaşmıştır. Yeni kayıtların lokasyonlarına ilişkin detaylı bilgiler de verilmiştir.

Anahtar sözcükler: Afit, Antalya, Karaman, Muğla, Türkiye

---

[1] This study was supported by TÜBİTAK; Project Number 119Z250.
[2] Artvin Çoruh University, Health Services, 08000, Artvin, Türkiye
[3] Niğde Ömer Halisdemir University, Science and Art Faculty, Department of Biotechnology, 51000, Niğde, Türkiye
* Corresponding author (Sorumlu yazar) e-mail: h.akyildirim@artvin.edu.tr
Received (Alınış): 29.03.2023 Accepted (Kabul ediliş): 30.09.2023 Published Online (Çevrimiçi Yayın Tarihi): 05.10.2023

Introduction

Aphids are the most important pest group, distributed throughout the world especially subtropical zone because of plant richness. This small and sap-sucking insect group has high fecundity, cyclical parthenogenetic reproduction, telescoping generation and produce lots of individuals in a short time. Aphids are specific to host plant and close relationship with them and morphological characters are used in the identification of aphids. About 5600 aphid species are defined in the world (Blackman & Eastop, 2023; Favret, 2023) and 654 species listed for Türkiye aphid fauna (Şenol et al., 2014, 2017, 2021; Özdemir & Barjadze, 2015; Görür et al., 2017, 2023 a, b, c; Kök & Kasap, 2019; Kök, 2021; Kök & Özdemir, 2021; Akyürek et al., 2022; Görür, 2022; Başer et al., 2023). Aphids are also economically important because of damaging cultural, naturally growing ornamental plants by feeding on them and invading other plants or zoo-geographic areas easily. In the world, 300 of 5600 aphids are considered severe economic pests (Rodrigez et al., 2017; Blackman & Eastop, 2023; Favret, 2023). They damage the plant species and they are reported to cause about 40-45% yield loses in developing countries, and 30-35% yield loses in developed countries (Ruberson, 1999). Also, biological activity and reproduction of aphids are affected by global warming (Hulle et al., 2010). Therefore, aphids are perfect group for studying effects of global warming.

Türkiye is located at the transition zone between Europe and Asia and has 7 different regions, climatic conditions and floristic features of which change according to localities and geographies. Each region has variable and rich plant diversity and 31% of these plants is endemic (Güner et al., 2012). Türkiye's plant richness is nearly equal to Europe. Therefore, Türkiye is the most popular and interesting area for aphids, but there are many areas that need to be studied in Türkiye (Görür et al., 2019).

Studies on aphids and their damage to host plants in Türkiye started in the early 1900s. Türkiye aphid diversity and host plant interaction were partially researched with other faunistic studies for a long time. Then, first revision of Türkiye was conducted by Çanakçıoğlu (1975). In his book "Aphidoidae of Turkey", 258 aphid species were listed. Remaudiere (2006) summarized all the studies on Türkiye aphid fauna and 417 aphid species were listed. Recently, many researchers listed about 60 new aphid record of Türkiye aphid fauna (Toper Kaygın et al., 2008, 2010; Görür et al., 2009a, b, 2011a, b; Eser et al., 2009; Akyürek et al., 2010, 2011; Barjadze et al., 2011). Checklist of Türkiye aphidofauna was published by Görür et al. (2012) (480 species in 141 genera) and then Kök & Özdemir (2021) (591 species in 147 genera). From that day until today, the number of studies has increased with new aphid records. As a result of these studies, Türkiye aphid fauna number has reached 654 (Barjadze et al., 2014a, b; Şenol et al., 2014, 2015a, b, 2017, 2021; Özdemir & Barjadze, 2015; Görür et al., 2017, 2023 a, b, c; Kök & Kasap, 2019; Kök, 2021; Akyürek et al., 2022; Başer et al., 2023). However, since each region of Türkiye has not been studied, these aphid species numbers do not reflect the real data about Türkiye aphidofauna.

In this study, aphid species from Antalya, Karaman and Muğla, which have different geographic and climatic features, were studied, aphids which affect different plant species were determined and 12 new records were added to the Türkiye aphidofauna. Host plant species and morphological features on the host plant were described.

Materials and Methods

This survey was conducted in Antalya, Karaman and Muğla provinces monthly (about 10 days for each month) from March 2020 to September 2020 and from March 2021 to September 2021. Both aptera and alatae viviparous individuals were sampled with "0" number brush sensitively on all host plants and transferred to 96% ethanol. Permanent slides of the samples were conducted according to method proposed by Martin (1983). The aerial parts of unidentified plants, especially herbaceous species were sampled, dried between cardboard and transferred to laboratory for an identification. Plant species were determined with a distinguishing key, Flora of Turkey (Davis, 1965-85). The definition of the aphid samples

preserved in a permanent slide were performed based on the identification key offered by Blackman & Eastop (2023). General features, worldwide distribution, host plant range of the each identified species were controlled in consistency with the regularly updated web page provided by Blackman & Eastop (2023). The recent taxonomic status of all the determined species was checked according to Favret (2023). Mounted specimens were examined by a with Olympus BX51 microscope. Colony appearances, sampled host plant and detected features of each determined species were provided as follows. The voucher specimens were deposited at the Biotechnology Department of Niğde Ömer Halisdemir University.

Results

Aphid samples were collected from Antalya, Karaman and Muğla Provinces, and as a result of the analyses of the permanently prepared slides, 12 species were determined as new records for Türkiye aphid fauna. For each identified species, worldwide distribution and collection localities, collection dates and biological features are given (Figure 1-13).

Family: Aphididae

Subfamily: Aphidinae

Tribe: Aphidini

Subtribe: Aphidina

Genus: *Aphis* L., 1758

***Aphis* (*Aphis*) *crepidis* (Börner, 1940)**

Material examined. Muğla, Marmaris, National Park-Nimara cave, 36°48'47" N, 28°17'48" E, 134m, 24.IV.2021, apt. 5♀♀, *Crepis capillaris* (Asteraceae).

Description. Light green individuals (Figure 1), BL 1.70mm, ABD TERG 2-5 usually with dome-shaped MTu, have 3-7 secondary rhinaria on ANT III.

Distribution. Europe, Iran, Italy (Blackman & Eastop, 2023).

Figure 1. Morphological characters of *Aphis* (*Aphis*) *crepidis*: a) general view, b) rostrum, c) PT / base.

Genus: *Protaphis* Börner, 1952

***Protaphis echinopis* (Hille Ris Lambers, 1948)**

Material examined. Muğla, Menteşe, Muratlar, 37°15'40" N, 28°37'56" E, 616 m, 4.VII.2020, apt. 7♀♀, *Echinops viscosus* (Asteraceae).

Description. Blackish-green individuals, hairs on ANT III 14-18 μm long, BL 0,73 mm without secondary rhinaria (Figure 2).

Distribution. North-East Africa, Central Asia and Middle East (Blackman & Eastop, 2023).

Figure 2. Morphological characters of *Protaphis echinopis*: a) head, b) rostrum, c) rhinnaria on antenna.

Tribe: Macrosiphini

Genus: *Amphorophora* Buckton, 1876

***Amphorophora (Amphorophora) urtica* Essig, 1942**

Material examined. Muğla, Fethiye, 36°40'28" N, 29°07'44" E, 67 m, 26.IV.2021, apt. 15♀♀, *Urtica dioica* (Urticaceae) (Figure 3).

Description. Dark red aptera individuals (Figure 3), ANT III with 1-4 rhinaria, BL 3.4-3.5 mm, cauda with 9-14 hairs.

Distribution. Western North America (Blackman & Eastop, 2023).

Figure 3. *Amphorophora urtica* individuals on *Urtica dioica*.

Genus: *Metopolophium* Mordvilko, 1914

***Metopolophium* (*Metopolophium*) *montanum* Hille Ris Lambers, 1966**

Material examined. Muğla, Dalaman, 36°45'20" N, 28°48'04" E, 11 m, 22.VI.2021, apt. 8♀♀, *Rosa* sp. (Rosaceae) (Figure 4).

Description. Light green individuals, SIPH 2.0 × cauda, R IV+V 0.75-0.85, BL 1.7-1.8 mm.

Distribution. Switzerland, Austria, France and Spain (Blackman & Eastop, 2023).

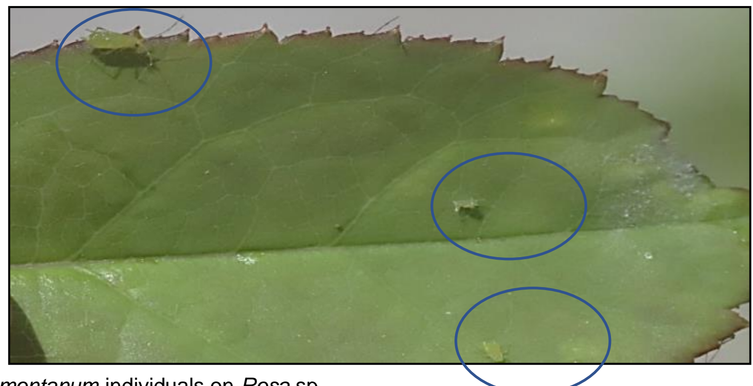


Figure 4. *Metopolophium montanum* individuals on *Rosa* sp.

Genus: *Sitobion* Mordvilko, 1914

***Sitobion* (*Sitobion*) *leelamaniae* (David, 1958) Banded Cereal Aphid**

Material examined. Muğla, Fethiye, 36°40'39" N, 29°10'09" E, 111 m, 30.V.2020, apt. 7♀♀, *Poa* sp. (Poaceae) (Figure 5): Antalya, Gündoğmuş, Ortaköy 36°50'41" N, 32°09'26" E, 1397 m, 15.III.2020, apt. 9♀♀, *Triticum* sp (Poaceae).

Description. Green individuals (Figure 5), antennae ringed with brown, siphunculi pale brown, darker towards apices, legs pale, BL 1.6-2.3 (2.45 mm) mm.

Distribution. South India, Sri Lanka and Southern Africa (Blackman & Eastop, 2023).

Figure 5. *Sitobion leelamaniae* individuals on *Poa* sp.

Genus: *Uroleucon* Mordvilko, 1914

***Uroleucon (Uroleucon) iranicum* Holman, 1980**

Material examined. Karaman, Ermenek, Aşağıakın village, 36°53'41" N, 33°01'30" E, 993 m, 30.V.2021, apt. 2♀♀, *Gundelia tournefortii* (Asteraceae).

Description. Green individuals, BL 2.35 mm, first tarsal segments are unusual for a *Uroleucon* in having only 3 hairs (Figure 6).

Distribution. Iran (Blackman & Eastop, 2023).

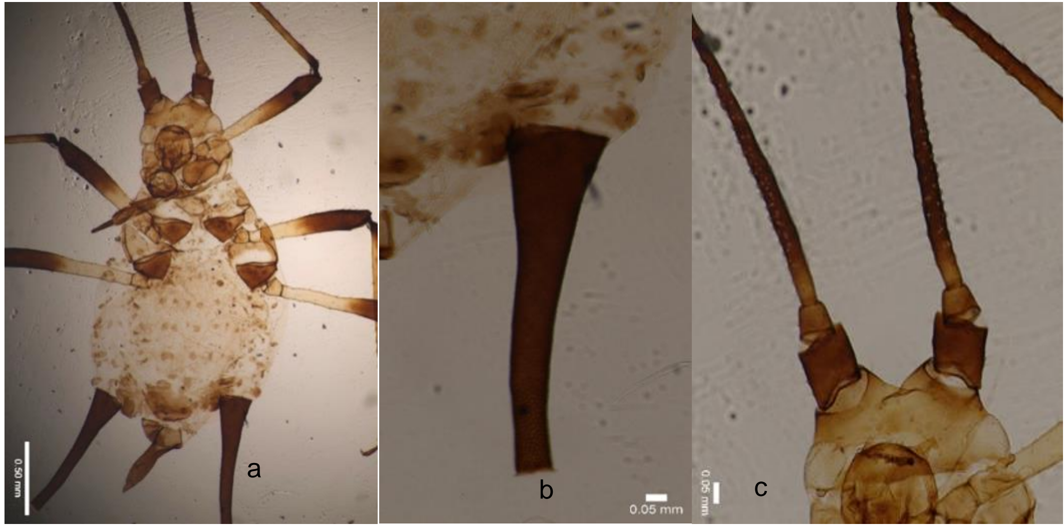


Figure 6. Morphological characters of *Uroleucon (Uroleucon) iranicum*: a) general view, b) siphinculi, c) antenna.

Subfamily: Chaitophorinae

Tribe: Chaitophorini

Genus: Chaitophorus Koch, 1854

***Chaitophorus utahensis (*Knowlton, 1928)**

Material examined. Muğla, Seydikemer-Karabel gateway, 36°47'12" N, 29°35'56" E, 1179 m, 23.VI.2021, apt. 20♀♀, *Salix* sp. (Salicaceae) (Figures 7 & 8).

Description. BL 1-2 mm, have dark wing veins and dark dorsal abdominal cross-bands, hind femora much darker than tergum, nymphs are brown, mature individuals are blackish colors (Figures 7 & 8).

Distribution. Western USA (Blackman & Eastop, 2023).

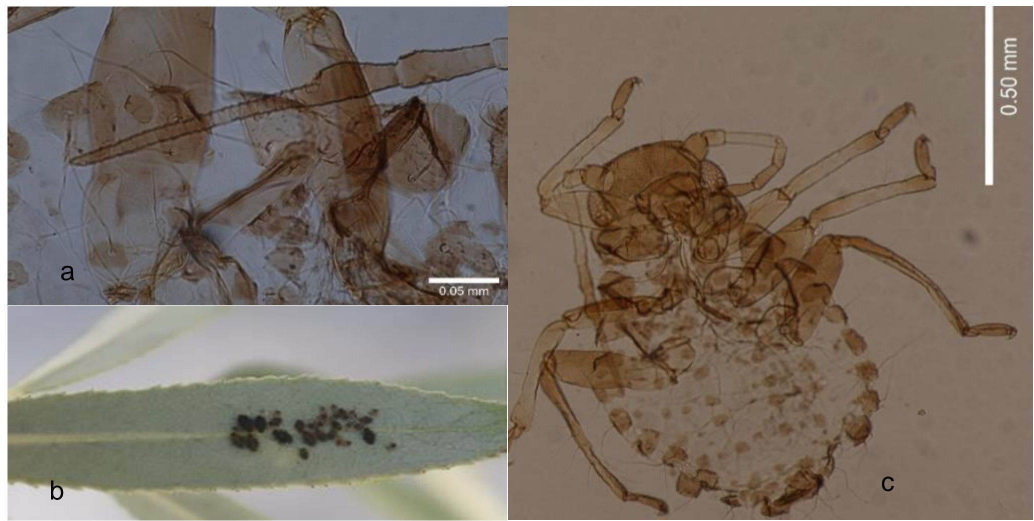


Figure 7. Morphological characters of *Chaitophorus utahensis*: a) antenna, b) individuals on plant, c) general view.

Figure 8. *Chaitophorus utahensis* individuals on *Salix* sp.

Subfamily: Calaphidinae

Tribe: Panaphidini

Genus: *Monelliopsis* Richards, 1965

***Monelliopsis caryae (*Monell, 1879)**

Material examined. Muğla: Ortaca, 36°49'41" N, 28°45'47" E, 25 m, 3.VI.2020, apt. 8♀♀, *Juglans regia* (Juglandaceae).

Description. Light yellow individuals, siphinculi brown, ANT PT/BASE 1.0-1.2, BL 1.0-1.2 mm (Figure 9).

Distribution. North America and Europe (Blackman & Eastop, 2023).

Figure 9. Dark siphinculi of *Monelliopsis caryae*.

Genus: *Tiliaphis* Takahashi, 1961

***Tiliaphis pseudoshinae* Quednau, 1979**

Material examined. Antalya: Korkuteli, 37°03'22" N, 31°13'08" E, 971 m, 7.VII.2020, apt. 10♀♀, *Tilia* sp. (Malvaceae).

Description. Dense green individuals, R IV+V less than 0.8× HT II, BL 1.65-2.1 mm.

Distribution. Korea and eastern Siberia (Blackman & Eastop, 2023).

Subfamily: Hormaphidinae

Tribe: Hormaphidini

Genus: *Doraphis* Matsumura and Hori, 1929

***Doraphis populi* (Maskell, 1898)**

Material examined. Karaman, Sarıveliler 36°36'25" N, 32°35'14" E, 1694 m, 1.IX.2021, apt. 15♀♀, *Populus* sp. (Salicaceae) (Figure 10).

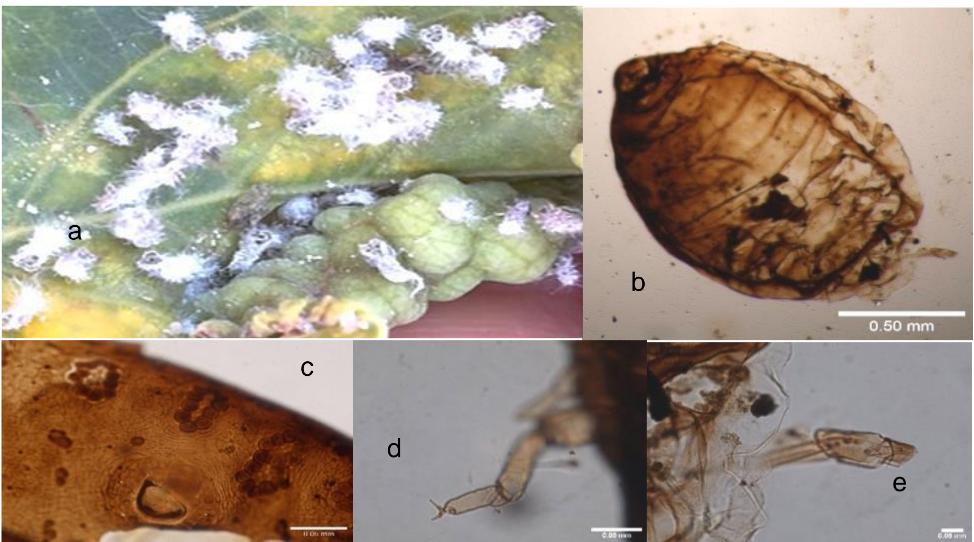


Figure 10. *Doraphis populi* individuals on *Populus* sp.: a) individuals on *Populus* sp., b) general view, c) siphunculi, d) hind tibia, e) rostrum.

Description. Blackish and powdery individuals, body either aleyrodiform, BL 1.5- 2.2 mm (Figure 10).

Distribution. China, Japan, India, Korea and Siberia (Blackman & Eastop, 2023).

Subfamily: Lachninae

Tribe: Eulachinini

Genus: *Eulachnus* Del Guercio, 1909

***Eulachnus mediterraneus* Binazzi, 1983**

Material examined. Muğla, Dokuzçam, Yılanlıdağ plateau, 37°23'12" N, 28°29'05" E, 1037 m, apt. 20.VI.2021, 10♀♀, *Pinus* sp. (Pinaceae).

Description. Grayish green individuals, BL 1.8-2.7 (2.40 mm) mm, longest hairs on ANT III 40-70 µm (Figure 11).

Distribution. Western Mediterranean (Blackman & Eastop, 2023).

Figure 11. Morphological characters of *Eulachnus mediterraneus*.: a) general view, b) antenna, c) rostrum, d) hind tibia and hind tarsus.

Subfamily: Eriosomatinae

Tribe: Pemphigini

Genus: *Prociphilus* Koch, 1857

***Prociphilus* (*Prociphilus*) *erigeronensis* (Thomas, 1879)**

Material examined. Muğla, Centre, 37°12'29" N, 28°22'07" E, 644 m, 20.VI.2021, apt. 15♀♀, *Cichorium* sp. (Asteraceae).

Description. Blackish individuals, BL 2.05-2.1 mm, R IV+V 0.13-0.15 mm (Figure 12).

Distribution. USA (Blackman & Eastop, 2023).

Figure 12. Morphological characters of *Prociphilus (Prociphilus) erigeronensis*: a) general view, b) last antenne segment, c) URS VI/V, d) hind tibia.

Discussion

Aphids cause intense damage to natural and exotic plant species in our country as well as all over the world. Damage levels can be high, especially in agriculture-dependent countries like ours. Türkiye aphid fauna has reached 654 with the recent studies (Kök, 2021; Şenol et al., 2021; Görür, 2022; Görür et al., 2023 a, b, c; Başer et al., 2023). It has been thought that under normal conditions there should be positive correlation between aphid species diversity and plant species diversity in any particular locality. Also, the influence of climate and geography is also great. For example, 68 aphid species were sampled from Artvin and 77 from Trabzon. Whereas there are 2727 plants in Artvin where 3 different climate types are seen, 2100 plants are distributed in Trabzon where the Black Sea climate prevails (Eminağaoğlu, 2015; Görür et al., 2019). In study area, Antalya, Muğla and Karaman, there are many plant species such as thistle, Rosary tree, Gum tree, Sycamore tree, Heather, Spruce, while there are special flowers and grain plants such as Tokuz grass, Gooseberry thistle, *Chrysanthemum, Asparagus*. Dense aphids were sampled from each plant, although not all new records were taken from these plants. As these areas have different geographical and climatic characteristics, it is usual that aphid diversity is high and new records are encountered.

12 new aphid records were given with this study and Türkiye's aphid fauna reached 666. *Prociphilus erigeronensis, Chaitophorus utahensis, Amphorophora urtica* species have been observed only in the USA so far and are given as a new record from Türkiye. Other new records are distributed in Europe and the Mediterranean (Blackman & Eastop, 2023). *Amphorophora* genus has a rich diversity in America, especially on the Pacific coast (Essig,1942). In our country, this species was sampled on *Urtica dioica*. *P. erigeronensis* individuals are determined on different genera (*Lactuca, Aster* and *Ambrosia,* (Asteraceae), *Poa, Triticum, Agrotis* (Poaceae), and *Arachis, Trifolium, Phaseolus* (Fabaceae), *Crataegus* (Rosaceae), and a variety of other ornamentals. *Monelliopsis caryae* (walnut aphid) species was given as a new record for Bulgaria in

2006 and widely distributed in North America (Tasheva-Terzieva et al., 2006). Walnut aphids reduce walnut yield by reducing nut quality, inhibiting the growth of seedlings and causing sooty mould to grow because of the abundant honeydew production. Since walnut trees have a wide distribution in our country, it is important to monitor these species and develop control methods.

Conclusion

As a result of this study, Türkiye aphid fauna has reached 666 species belonging to 160 genera. Because of climatic and geographic properties of study area and rich plant diversity, this number is expected to increase further. Results presented here is the part of an ongoing project, and thus it is possible to add more aphid species to Türkiye aphid fauna, and in turn strongly emphasize the importance of the conducting such studies to observe composition of Türkiye aphid fauna.

Acknowledgements

The authors thank to the Scientific and Technological Research Council of Türkiye (TÜBİTAK; Project Number 119Z250).

References

Akyürek, B., 2022. New additional aphid (Hemiptera: Aphidoidea: Aphididae) records for Samsun (Turkey) province. International Journal of Science Letters, 4 (1): 169-182.

Akyürek, B., Ü. Zeybekoğlu & G. Görür, 2010. New records of aphid species (Hemiptera: Aphidoidea) for the Turkish fauna from Samsun province. Turkish Journal of Zoology, 34 (2): 421-424.

Akyürek, B., Ü. Zeybekoğlu & G. Görür, 2011. Further contributions to the Türkiye Aphid (Hemiptera: Aphidoidea) fauna. Journal of the Entomological Research Society, 13 (3): 101-106.

Bahşi, N. & E. Çetin, 2020. Determining of agricultural credit impact on agricultural production value in Turkey. Ciência Rural, Santa Maria, 50 (11): e20200003.

Barjadze, S., G. Japoshvili, İ. Karaca & I. Özdemir, 2014b. Aphids (Hemiptera: Aphidoidea) of Gölcük Natural Park (Isparta Province, Turkey). Munis Entomology & Zoology Journal, 9 (1): 206-213.

Barjadze, S., İ. Karaca, B. Yaşar & G. Japoshvili, 2011. The yellow rose aphid *Rhodobium porosum*: a new pest of Damask Rose in Turkey. Phytoparasitica, 39 (1): 59-62.

Barjadze, S., I. Özdemir & R. Blackman, 2014a. Two new species of Aphidini *Latreille,* 1802 (Hemiptera: Aphididae) from Turkey. Zootaxa, 3873 (2): 197-194.

Başer, G., G. Görür, Ö. Şenol & H. Akyildirim Beğen, 2023. New contributions to Türkiye aphid fauna with invasive species. Munis Entomology & Zoology (In press).

Blackman, R. & V. F. Eastop, 2023. Aphids on the World's plants: An online identification and information guide. (Web page: http://www.aphidsonworldsplants.info) (Date accessed: June 2023)

Çanakçıoğlu, H., 1975. The Aphidoidea of Turkey. JFFIU İstanbul, 309 pp.

Eminağaoğlu, Ö., 2015. Artvin'in Doğal Bitkileri (Native Plants of Artvin). İstanbul: Promat 256 pp (???? in Turkish with abstract in English).

Eser, S. İ., G. Görür, İ. Tepecik & H. Akyıldırım, 2009. Aphid (Hemiptera: Aphidoidea) species of the Urla district of İzmir region. Journal of Applied Biological Sciences, 3 (1): 99-102.

Essig, E. O., 1942. College Entomology, The MacMillan Company, N.Y., 900 pp.

Favret, C., 2023. Aphid Species File. (Web page: http://aphid.speciesfile.org) (Date accessed: June 2021)

Görür, G., M. Işık, B. Akyürek & Ü. Zeybekoğlu, 2009a. New Records of Aphidoidea from Turkey. Journal of the Entomological Research Society, 11 (3): 1-5.

Görür, G., Ü. Zeybekoğlu, B. Akyürek, M. Işık & H. Akyıldırım, 2009b. Trabzon, Rize ve Artvin İllerinin Afit (Homoptera: Aphididae) Faunasının Belirlenmesi. Turkish Scientific Research Council (Unpublished) Project, Project Number 107T450 (in Turkish with abstract in English).

Görür, G., İ. Tepecik, H. Akyıldırım & G. Olcabey, 2011. Additions to the Turkish Aphid fauna (Hemiptera: Aphidoidea: Aphididae). North-Western Journal of Zoology, 7 (2): 318-321.

Görür, G., H. Akyıldırım, G. Olcabey & B. Akyürek, 2012. The aphid fauna of Turkey: An updated checklist. Archives of Biological Sciences, 64 (2): 675-692.

Görür, G., Ö. Şenol, G. Gezici, H. Akyıldırım Beğen & D. Parmaksız, 2017. New aphid (Hemiptera: Aphidoidea) records from South Eastern Parts of Turkey. Journal of Insect Biodiversity and Systematics, 3 (3): 257-264.

Görür, G., H. Akyıldırım Beğen & Ö. Şenol, 2019. Determined aphid-host plant relations from Eastern Black Sea regions of Turkey. Turkish Journal of Biodiversity, 2 (2): 34-38.

Görür, G., 2022. Contribution to the aphid fauna of the Ordu province with first record of an exotic aphid species, *Euceraphis gillettei* Davidson, 1915, in Turkey. Turkish Journal of Zoology, 46 (5): 418-422.

Görür, G., Ö. Şenol, H. Akyıldırım Beğen & B. Akyürek, 2023a. Turkish aphid. (Web page: www.turkishaphid.com) (Date accessed: July 2022).

Görür, G., G. Başer, H. Akyildirim Beğen, Ö. Şenol & B. Akyürek, 2023b. Sıcaklık Değişkenliğinin Afitlerin Yaşam Döngüsüne Etkileri: Dört Örnek Tür. Osmaniye Korkut Ata Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 6 (1): 68-77 (in Turkish with abstract in English).

Görür, G., H. Akyildirim Beğen, Ö. Şenol & G. Başer, 2023c. Novel contribution to the Türkiye aphid fauna with new host plant associations. North-Western Journal of Zoology, 19 (2): 257-264.

Güner, A., S. Aslan, T. Ekim, M. Vural & M.T. Babaç, 2012. Türkiye Bitkileri Listesi (Damarlı Bitkiler). Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırma Derneği Yayını, İstanbul, 1290 pp (in Turkish).

Hulle, M., A. Cœur d'Acier, S. Bankhead-Dronnet & R. Harrington, 2010. Aphids in the face of global changes. Comptes Rendus Biologies, 33: 497-503.

Kök, Ş. & İ. Kasap, 2019. Aphid (Hemiptera: Aphididae) species of the South Marmara Region of Turkey including the first record of *Dysaphis radicola meridialis* Shaposhnikov, 1964 for the aphid fauna of Turkey. Turkish Journal of Entomology, 43 (1): 63-78.

Kök, Ş., 2021. Diversity and plant interactions of aphids (Hemiptera: Aphidomorpha) adjacent to Çardak Lagoon with new aphid and host records for Turkey. Turkish Journal of Entomology, 45 (4): 425-439.

Kök, Ş. & I. Özdemir, 2021. Annotated Systematic Checklist of the Aphids (Hemiptera: Aphidomorpha) of Turkey. Zootaxa, 4925 (1): 1-74.

Martin, J. H., 1983. The identification of common aphid pests of tropical agriculture. Tropical Pest Management, 29 (4): 395-411.

Özdemir, I. & S. Barjadze, 2015. Some new records of aphid species (Hemiptera: Aphididae) from the Middle East and the Caucasus. Turkish Journal of Zoology, 39: 712-714.

Remaudière, G., S. Toros & I. Özdemir, 2006. New contribution to the aphid fauna of Turkey (Hemiptera, Aphidoidea). Revue Française d'Entomologie, 28 (2): 75-96.

Rodrigez, G.S., P.A. Brown, J. Ortego, S.L. Ciruelos & J.M. Nieto Nafria, 2017. Aphis species (Hemiptera, Aphididae) living on Mulinum (Apiaceae) in South America, with a description of a new species. Zootaxa, 4216 (1): 47-54.

Ruberson, J.R., 1999. Handbook of Pest Management. Published by Marcel Dekkar Inc., New York, 842 pp.

Şenol, Ö., H. Akyıldırım Beğen, G. Görür & E. Demirtaş, 2014. New records for the aphid fauna hemiptera aphidoidea of Turkey. Acta Zoologica Bulgarica, 66 (1): 133-136.

Şenol, Ö., H. Akyıldırım Beğen, G. Görür & E. Demirtaş, 2015a. New additions and invasive aphids for Turkey's aphidofauna (Hemiptera: Aphidoidea). Turkish Journal of Zoology, 39: 39-45.

Şenol, Ö., H. Akyıldırım Beğen, G. Görür & G. Gezici, 2015b. Some new aphid records for the Turkish aphidofauna (Hemiptera: Aphidoidae). Zoology in the Middle East, 61 (1): 90-92.

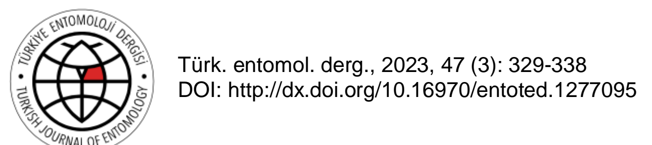
Şenol, Ö., G. Görür & H. Akyıldırım Beğen, 2017. Recent findings on aphid fauna from east and south eastern parts of Turkey. Biodiversity Conservation, 10 (3): 76-78.

Şenol, Ö., G. Görür, G. Başer & H. Akyıldırım Beğen, 2021. Contributions to the Turkish aphid fauna from Aegean and Mediterranean part of Turkey. Journal of Applied and Natural Science, 25 (3): 717-720.

Tasheva-Terzieva, E., P. B. Mirchev, G. T. Georgiev, P. S. Petkov & M. Todorov, 2006. The dew produce, *Monelliopsis caryae* (Monell ex Riley and Monell, 1879) (Hemiptera:Aphididae) - new species for aphidofauna of Bulgari*a.* Acta entomologica Bulgarica, 1 (2): 60-63.

Toper Kaygın, A., G. Görür & F. Çota, 2008. Contribution to the Aphid (Homoptera: Aphididae) species damaging on woody plants in Bartın, Türkiye. International Journal of Engineering Science and Application, 2 (1): 83-86.

Toper Kaygin, A., G. Gorur & F. Cota, 2010. New records of aphid fauna in Turkey. Journal of Insect Science, 10 (1): 1-5.


Türk. entomol. derg., 2023, 47 (3): 329-338
DOI: http://dx.doi.org/10.16970/entoted.1277095

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

On the genus *Lobrathium* Mulsant & Rey, 1878 (Coleoptera: Staphylinidae: Paederinae) of the Palaearctic region II: A new species from Türkiye and faunistic notes on some species[1]

Palaearktik bölgedeki *Lobrathium* Mulsant & Rey, 1878 (Coleoptera: Staphylinidae: Paederinae) cinsi üzerine II: Türkiye'den bir yeni tür ve bazı türler üzerinde faunistik notlar

Sinan ANLAŞ[2*]

Abstract

The genus *Lobrathium* Mulsant & Rey (Coleoptera: Staphylinidae: Paederinae) is represented in the Palaearctic region by 156 species. In the study, *Lobrathium* (s. str.) *thracicum* sp. n. (Kırklareli) from Türkiye is described, figured, and distinguished from geographically closest congeners. The genus is now known to occurin Türkiye by 12 species, 10 of which are endemic to this country. Faunistic notes on nine species of the genus *Lobrathium* in Afghanistan, Albania, Azerbaijan, Bosnia-Herzegovina, Cyprus, Czechia, Greece, France, Portugal, Russia, Slovenia, Spain, and Türkiye of the Palaearctic Region are presented. Two species that are taxonomically controversial, *Lobrathium apicale* (Baud di Selve, 1857) from Cyprus and *Lobrathium. afghanicum* Coiffait, 1979, synonym of *Lobrathium triste* (Cameron, 1924) from Afghanistan, are illustrated.

Keywords: Fauna, *Lobrathium*, new species, taxonomy, Thrace

Öz

Lobrathium Mulsant & Rey (Coleoptera: Staphylinidae: Paederinae) cinsi Palearktik Bölgede 156 tür temsil edilen bir cinstir. Bu çalışmada, Türkiye'den *Lobrathium* (s. str.) *thracicum* sp. n. (Kırklareli) türü tanımlanmış, şekillendirilmiş ve bu cinsin coğrafik olarak yakın türlerden farklılıkları gösterilmiştir. Böylece, bu cins Türkiye'de 12 türle bilinmekte olup bunların 10'u bu ülkeye endemiktir. Palaearktik Bölgedeki Afganistan, Arnavutluk, Azerbaycan, Bosna-Hersek, Kıbrıs, Çekya, Yunanistan, Fransa, Portekiz, Rusya, Slovenya, İspanya ve Türkiye'de bulunan dokuz *Lobrathium* türüne ait faunistik notlar sunulmuştur. Taksonomik olarak tartışmalı iki tür olan, Kıbrıs'tan *Lobrathium apicale* (Baud idi Selve, 1857) ve Afganistan'dan *Lobrathium triste* (Cameron, 1924) türünün sinonimi *Lobrathium afghanicum* Coiffait, 1979, türü şekillendirilmiştir.

Anahtar sözcükler: Fauna, *Lobrathium*, yeni tür, taksonomi, Trakya

---

[1] This study was partly supported by the Scientific and Technological Research Council of Turkey, Grant Project No: 119Z253).
[2] Manisa Celal Bayar University, Alaşehir Vocational School, 45600, Alaşehir, Manisa, Turkey
* Corresponding author (Sorumlu yazar) e-mail: sinan.anlas@gmail.com
Received (Alınış): 04.04.2023 Accepted (Kabul ediliş): 30.09.2023 Published Online (Çevrimiçi Yayın Tarihi): 05.10.2023

Introduction

The Holarctic genus *Lobrathium* Mulsant & Rey, 1878 is relatively better studied than most of the genera of the subfamily Paederinae (Staphylinidae) and is represented by 156 species in the Palaearctic region (Schülke & Smetana, 2015; Assing, 2019, 2021; Anlaş, 2020; Lin et al., 2022).

In the western Palaearctic region, the genus containing 31 species, only seven species is known from Middle Asia and Iran. Most of these species are distributed in Türkiye (11 species, nine of them are endemic), in Italy (six species, three of them are endemic), in Greece (five species, three of them are endemic), and in Caucasus (five species, four of them are endemic).

Lobrathium species are generally larger in size than other species of the subfamily Paederinae. Little is known about their biology, ecology and phenology. Members of the genus are mostly found near streams and rivers, around lakes and dams, in marshland and on damp grassland. They are most abundant in spring, and their distribution in an area is highly dependent on humidity. The species of *Lobrathium* are noctural and probably predators of small invertebrates.

In the study, a new *Lobrathium* species, *Lobrathium* (s. str.) *thracicum* sp. n., from Thrace in Türkiye is identified and described here together with new and additional records from Afghanistan, Albania, Azerbaijan, Bosnia-Herzegovina, Cyprus, Czechia, Greece, France, Portugal, Russia, Slovenia, Spain and Türkiye in the Palaearctic region. Including the species described herein, 32 *Lobrathium* species are currently known to occur in the western Palaearctic region.

Materials and Methods

Specimens were examined using Zeiss Stemi 508 stereomicroscopes. Aedeagus, tergites and sternites VII-VII were preserved in a microvial with pure glycerin, which were attached under pinned specimen. A digital camera (Axiocam 208, Zeiss) was used for the figures. All figures were modified using Helicon Focus v. 8, and CorelDraw Graphics Suite 2022 software.

The abbreviations are used for the measurements in below, which are given in millimeters:

AL—length of antenna;

AW—maximal width of abdomen;

EL—length of elytra from apex of scutellum to posterior margin;

EW—combined width of elytra;

HL—head length from base of labrum to posterior constriction along head midline;

HW—maximal head width (including eyes);

ML—length of aedeagus from apex of ventral process to base;

PL—length of pronotum along median line;

PW—maximal width of pronotum;

TL—body length from anterior margin of clypeus to apex of abdomen.

The material studied is preserved in:

AZMM—Alaşehir Zoological Museum, Manisa, Türkiye (S. Anlaş).

HNHM—Hungarian Natural History Museum, Budapest, Hungary (G. Makranczy, O. Merkl).

MHNG—Muséum d’Histoire Naturelle, Genève, Switzerland (G. Cuccodoro).

MNCN—Museo Nacional de Ciencias Naturales, Madrid, Spain (M. Paris).

MNHNP—Muséum National d'Histoire Naturelle, Paris, France (T. Deuve, A. Taghavian).

NHMW—Naturhistorisches Museum Wien, Austria (H. Schillhammer).

NMPC—National Museum, Praha, Czechia (M. Fikáček, Jiří Hájek).

ZIN—Zoological Institute of the Russian Academy of Sciences, St.-Petersburg, Russia (B. A. Korotyaev).

Results

In this study, 10 species were reported from Palaearctic region. A new species from Türkiye was described, figured, and distinguished from geographically closest congeners. Faunistic notes on nine species of the genus *Lobrathium* in the Palaearctic Region are presented.

Taxonomy

Description of new species

Lobrathium* (*Lobrathium*) *thracicum sp. n. (Figures 1a-l)

Type material. Holotype: Türkiye: ♂, "TR. Kırklareli, Pınarhisar, Yenice 5 km NE, Mahya Dağı, 866 m, 41°46'43''N, 27°37'32''E, 14.III.2021, leg. Anlaş, Örgel & Kacar. / Holotypus ♂, *Lobrathium* (*Lobrathium*) *thracicum* sp. n. det. S. Anlaş 2022" <red printed label> (AZMM). Paratypes: ♂, ♀, same data as holotype (AZMM). ♀, Kırklareli, Demirköy 7 km NW, 530 m, 41°47'02''N, 27°42'08''E, 13.III.2021, leg. Anlaş, Örgel & Kacar (AZMM). ♂, ♀, Demirköy, Sarpdere 4 km SW, Dupnisa Mağarası, 360 m, 41°50'26''N, 27°33'23''E, 15.III.2021, leg. Anlaş, Örgel & Kacar (AZMM). ♂, 2♀♀, Kırklareli, Demirköy, Balaban 5 km E, 632 m, 41°49'57''N, 27°37'15''E, 08.VI.2021, leg. Kacar & Çelik (AZMM). 2♀♀, Kırklareli, Demirköy, Balaban 4 km NE, 652 m, 41°48'41''N, 27°38'28''E, 08.VI.2021, leg. Kacar & Çelik (AZMM). ♀, Kırklareli, Armağan 2 km W, 175 m, 41°52'11''N, 27°26'44''E, 08.VI.2021, leg. Kacar & Çelik (AZMM). ♀, Kırklareli, Demirköy, Avcılar 6 km S, 175 m, 41°57'05''N, 27°50'40''E, 09.VI.2021, leg. Kacar & Çelik (AZMM). ♀, Kırklareli, Demirköy, İğneada Longoz Forests, 1 m, 41°50'59"N, 27°56'24"E, 26.IV.2022, leg. Kacar & Çelik (AZMM). ♂, 4♀♀, Kırklareli, Demirköy, İğneada Longoz Forests, 45 m, 41°50'30"N, 27°56'22"E, 28.IV.2022, leg. Kacar & Çelik. <all paratypes with red printed label> (AZMM).

Description of new species. Measurements (in millimetres) and ratios (range, n = 19): AL: 1.75-1.85, 1.81; HL: 0.60-0.65, 0.63; HW: 0.62-0.66, 0.64; PL: 0.74-0.85, 0.80; PW: 0.57-0.63, 0.60; EL: 0.60-0.68, 0.64; EW: 0.65-0.72, 0.69; AW: 0.72-0.84, 0.78; ML: 0.92-0.96, 0.95 (n=5); FL: 2.82-2.95, 2.89; TL: 6.2-6.6, 6.5; HL/HW: 0.97-0.99, 0.98; PW/HW: 0.92-0.95, 0.94; PW/PL: 0.74-0.77, 0.75; EL/PL: 0.80-0.81, 0.8; EW/PW: 1.15-1.15, 1.15; EL/EW: 0.92-0.94, 0.93; AW/EW: 1.11-1.17, 1.13.

Habitus as in Figure 1a. Body 6.2–6.6 mm long. Coloration: head dark brown, pronotum, elytra and abdominal segments VIII-X reddish, segment VII bicolored, with narrow posterior margin rufous, abdominal segments III-VI black; antennae and legs reddish brown.

Head approximately as wide as long, posterior angles rounded (Figures 1a–1b and see HL/HW); puncturation non-umbilicate, moderately coarse and dense; slightly denser and larger in lateral and posterior area than that in central dorsal area and near anterior margin of frons; microsculpture absent; pubescence reddish brown and sparse; eyes clearly visible in dorsal view and moderately projecting from lateral outline of head (Figs. 1a, b). Antennae long and slender (Figure 1a), approximately 1.8 mm long, antennomeres II and III distinctly oblong.

Pronotum distinctly longer than wide, 0.75 times as long as wide and 0.94 times as wide as head and slightly narrowed posteriorly (Figures 1a, b, and see PW/PL, PW/HW); puncturation similar to that of head, but weakly sparser and coarser, midline impunctate; microsculpture absent; pubescence reddish brown and also very sparse.

Elytra approximately 0.95 times as long as wide and, broader than pronotum, 1.15 times as wide as pronotum and widened posteriad (Figures 1a-1b and see EL/EW, EW/PW); punctation slightly granulose; coarser and larger than that of head and also pronotum, in non-regular series; microsculpture absent; pubescence similar to that of pronotum. Hind wings present but reduced.

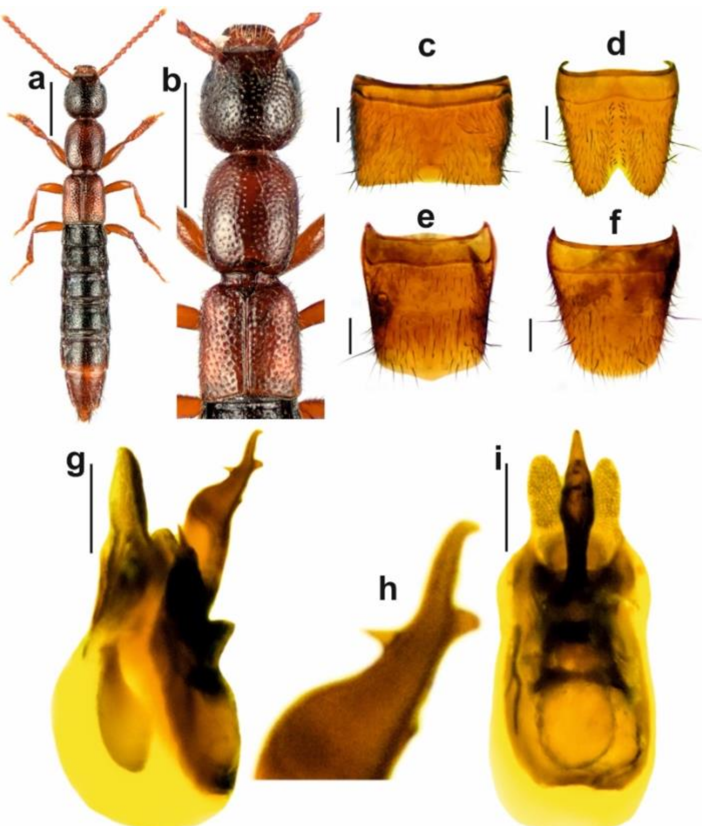


Figure 1. *Lobrathium thracicum* sp. nov. a) habitus; b) forebody; c) male sternite VII; d) male sternite VIII; e) female tergite VIII; f) female sternite VIII; g-h) aedeagus in lateral view; i) aedeagus in ventral view. Scale bars: 1.0 mm (Figs. a–b); 0.2 mm (Figs. c–i).

Abdomen wider than elytra, and approximately 1.15 times as wide as elytra (Figure 1a and see AW/EW), widest at segment VII; puncturation small, sparse and shallow; pubescence brown and sparse, microsculpture distinct, composed of dense and fine transverse striae and meshes; palisade fringe absent in posterior margin of tergite VII.

Male terminalia and genitalia. Sternite VII without modified setae and transverse, weakly concave posteriorly, median impression of the posterior margin of sternite present (Figure 1c); sternite VIII weakly oblong, with distinct median impression, the impression with approximately 30 modified short, black and stout setae, posterior incision of sternite VIII in the middle increasingly narrowed, moderately deep and V-shaped, less than 1/4 as long as length of the sternite (Figure 1d). Aedeagus large, approximately 0.95 mm long, ventral process moderately long, with two distinct ventral protrusions laterally, spine-shaped one on the left, and with a hook-shaped protrusion apically in lateral view (Figures 1g-1h), ventral process almost triangular, apically narrowed, and symmetric in ventral view (Figure 1i).

Female terminalia. Posterior margin of the female tergite VIII weakly angled in middle, without modified setae (Figure 1E); sternite VIII unmodified and without modified setae (Figure 1f).

Etymology. The name is derived from Thrace (Turkish name: Trakya), in the northwestern Türkiye, where the type localities are situated.

Differential Diagnosis. This new species can be distinguished from the geographically closest congeners (*L. rugipenne* (Hochhuth, 1851); *L. multipunctum* (Gravenhorst, 1802); *L. angulatum* Assing, 2005; *L. spinosum* Assing & Schülke, 2002; *L. bureschi* (Scheerpeltz, 1937) and *L. moreanum* Assing, 2019) by the different shapes of the male secondary and primary sexual characters. It differs from these species by the completely different morphology of the aedeagus and the following features:

- from *L. rugipenne* (widespread in the eastern Mediterranean region), and *L. multipunctum* (widespread in Europe and northwestern Africa), by the smaller body (*L. rugipenne*: on average 7.5-9.5 mm long; *L. multipunctum*: on average 6.5-8.5 mm long), by the different coloration of the body (*L. rugipenne*: head, pronotum and abdomen entirely blackish; elytra blackish, posteriorly with more or less distinctly reddish or reddish-yellow; *L. multipunctum*: whole body dark brown, reddish brown or brown, with elytra more or less lighter coloration), by the relatively smaller head, and smaller eyes, by the shorter and narrower elytra, and by the completely different appearance and chaetotaxy of the male sternite VII and sternite VIII.

- from *L. angulatum* (North Greece: Ipiros), by the smaller body (*L. angulatum*: 6.5-8 mm long); by the different coloration of the body (*L. angulatum*: Head dark brown to blackish brown; pronotum dark brown, with the anterior angles slightly lighter; eytra reddish; abdomen dark brown to blackish brown, with the posterior margin of segment VII and all of the following segments reddish), by the relatively broader head, and longer elytra, by the slightly denser puncturation of the abdomen and by the entirely different appearance and chaetotaxy of the male sternite VII and sternite VIII (*L. angulatum*: sternite VII posteriorly with large oval median impression, sternite VIII with distinctly U-shaped posterior excision, with transverse cluster of modified setae near the anterior margin of sternite VIII).

- from *L. spinosum* (southern Albania: Tomor Mountains), by the slightly larger body (*L. spinosum*: 5.6-6.4 mm long); by the different coloration of the body (*L. spinosum*: head and pronotum darkish brown to black; elytra brownish, abdomen black with abdominal segments VIII-X brown), by the much more transverse head, by the sparser puncturation of the abdomen and by the entirely different appearance and chaetotaxy of the male sternite VII and sternite VIII (*L. spinosum*: sternite VII posteriorly with large oval median impression, posterior margin of sternite VIII with deep and U-shaped incision).

- from *L. bureschi* (Bulgaria and Romania), by the slightly larger body (*L. bureschi*: 5.5-6.5 mm long); by the different coloration of the body (*L. bureschi*: body uniformly reddish brown), by the more oblong pronotum, by the relatively broader (in relation to head) pronotum, and by the entirely different appearance of the male sternite VII and sternite VIII (*L. bureschi*: sternite VII posteriorly with large oval median impression, sternite VIII with distinctly U-shaped posterior excision).

- from *L. moreanum* (Greece: Pelopónnisos), by the smaller body (*L. moreanum*: 8.7 mm long); by the different coloration of the body (*L. moreanum*: pronotum and head blackish; elytra blackish-brown and its posterior margin weakly paler; abdomen black with the posterior margin of tergite VII and abdominal segments VIII-X dark-reddish), by the much more transverse head; by the presence of a palisade fringe at the posterior margin of tergite VII; and by the entirely different appearance and chaetotaxy of the male sternite VII and VIII (*L. moreanum*: sternite VII anteriorly with a pair of distinct tubercles, sternite VIII extensively impressed along middle, with deeply U-shaped posterior excision, without setae).

For descriptions and illustrations of *L. angulatum*; *L. spinosum*; *L. bureschi* and *L. moreanum* see Scheerpeltz (1937), Assing & Schülke (2002) and Assing (2005, 2019).

Distribution and bionomics. This new species was collected on Istranca Mountains and Iğneada Longoz Forest, in Kırklareli province. The specimens were found under stones in varied pasture areas and sifted from leaf litter in mixed forest at altitudes of 1−866 m.

Faunistic records

Lobrathium anale (Lucas, 1846)

Material examined. France: ♂, VII.1974, Corse Ostriconi, leg. Löbl (MHNG). Italy: ♂, ♀, Sardinia, Tempio Krausse (HNHM).

Distribution. This species is known from France (Alpes-Maritimes, Corse), Italy (Sardegna, Sicilia), Portugal, Spain and the Islands of Canary, Algeria, Libya, Morocco and Tunisia (Assing, 2007; Schülke & Smetana, 2015; Anlaş, 2020).

Lobrathium angulatum Assing, 2005

Material examined. Greece: ♂, Grèce, Epire, Kato Kalentini, 230 m, 2.V.77, Löbl / Holotypus, *Lobrathium angulatum* sp. n. det. V. Assing 2004 (MHNG). 2♀♀, 13.V.2000, Epire, Vargiades environs, leg. Yunt (AZMM).

Distribution. The species was known only from Epire (Kato Kalentini and Polydroson) in Greece (Assing, 2005; Anlaş, 2020).

Lobrathium apicale (Baudi di Selve, 1857) (Figures 2a-f)

Material examined. Cyprus: ♀, 15. VII.(19)39, Cypr. Kambos, leg. Hakan Lindb., ex. coll. Scheerpeltz, Typus *Lathrobium lindbergi* Scheerpeltz, det. *Lobrathium apicale* (Baudi) V. Assing, 2001 (NHMW). ♂, 18-19.VII.(19)39, Cypr. Stavros, leg. Hakan Lindb., ex. coll. Scheerpeltz, Cotypus *Lathrobium lindbergi* Scheerpeltz, (NHMW). ♂, 19.VII.(19)39, Cypr. Paphos, Kannaviou, leg. P. H. Lindb., ex. coll. Scheerpeltz, Typus *Lathrobium lindbergi* Scheerpeltz (NHMW). 2♀♀, 12.III.2011, Lefkoşa, Değirmenlik, Yaylatepe 2 km S, Alevkayası, 820 m, 35°17´28´´N, 33°33´03´´E, leg. Anlaş (AZMM).

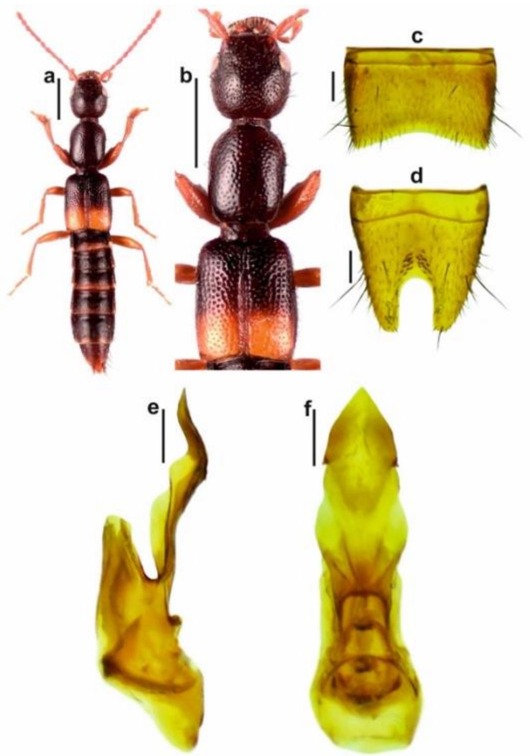


Figure 2. *Lobrathium apicale* (Baudi di Selve). a) habitus; b) forebody; c) male sternite VII; d) male sternite VIII; e) aedeagus in lateral view; f) aedeagus in ventral view. Scale bars: 1.0 mm (Figs. a–b); 0.2 mm (Figs. c-f).

Distribution. According to Schülke & Smetana (2015) *L. apicale* is only known from the Island of Cyprus.

Remarks. This species is also reported from Crete by Assing (2007). The record of this species from Crete is highly doubtful, and it should be confirmed. *L. apicale* is very similar to *L. candicum* Bordoni, 2009 (Crete: Kritsa/Lassithi). For that reason, the records of *L. apicale* from Crete most likely belong to *L. candicum*. The illustration of the aedeagus of *L. apicale* by Coiffait (1982) is inadequate for accurate identification. The male primary and secondary sexual characters and also habitus and forebody of *L. apicale* are figured (Figures 2a-f). For description and illustrations of *L. candicum* see Bordoni (2009).

Lobrathium multipunctum (Gravenhorst, 1802)

Material examined. Albania: ♂, Albania (without specific locality) (HNHM). Bosnia-Herzegovina: ♂, 2♀, 24.XI.1936, Bosnia, Sarajevo, Rijeka Miljacka, leg. Fodor (HNHM). Czechia: ♂, 26.III.1977, Chezch Republic, Pardubice (ZIN). France: ♂, Epaney, 18 Mars 1919 (MNHNP). Portugal: ♂, 30.IV.1957, Madere, Casinnas, St. Georges, coll. L. Levasseur, leg. Mateu (MNHNP). Slovenia: ♂, 25.VII.2007, Wocheim, Bohinj (HNHM). Spain: ♂, ♀, Badajoz (MNHNP). ♀, Ponferrada, Leon, leg. Paganetti, coll. J. Fodor (HNHM).

Distribution. *L. multipunctum* has a very wide distribution in Europe and Northwestern Africa, and is also distributed in the Nearctic Region (Assing, 2012; Schülke & Smetana, 2015; Anlaş, 2020).

Lobrathium moreanum Assing, 2019

Material examined. Greece: ♂, 3♀♀, 1935 Graecia, Pelopon Taygetos, (=Taÿgetos, Farida) leg. V. Purkyné (NMPC).

Distribution. This species is confined to Pelopónnisos, in Greece (Assing, 2019).

Lobrathium reitteri (Czwalina, 1889)

Material examined. ♂, Caucasus, Rost 1894, coll. Eppelsheim (NHMW).

Distribution: According to Solodovnikov (2001), this species occurs in the Northwestern Caucasus.

Lobrathium rugipenne (Hochhuth 1851)

Material examined. Azerbaijan: ♂, ♀, 16.VI.2007, Dashytuk and Apo environs, leg. Kasatkin (AZMM). Bulgaria: 2♂♂, ♀, 1928, Mts Rila, leg. Biró (HNHM). Russia: ♂, 16.VIII.1992, Krasnodar, Apsheronsky dist., Mezmay vill., leg. Khachikov (AZMM). Türkiye: ♂, ♀, Amasia (=Amasya), coll. F. Spelser (HNHM). 6♂♂, 8♀♀, 17.VI.2020, Amasya, Taşova, Borabay Lake 7 km SE, 40°51'17''N, 36°07'16''E, 1100 m, leg. Örgel & Kacar (AZMM). ♂, 17.VI.2020, Taşova, Borabay Lake 9 km E, 40°49'48''N, 36°04'42''E, 1700 m, leg. Örgel & Kacar (AZMM). 2♂♂, ♀, 13.VII.2020, Amasya, Taşova, Borabay Gölü 3 km W, 40°48'05''N, 36°07'07''E, 1370 m, leg. Örgel & Kacar (AZMM). ♂, 19.VI.2020, Amasya, Merzifon, Derealan 6 km W, 40°59'00''N, 35°20'09''E, 1500 m, leg. Örgel & Kacar (AZMM). ♀, 19.VI.2020, Amasya, Merzifon, Aşağibük 3 km S, 40°59'07''N, 35°20'16''E, 1460 m, leg. Kacar (AZMM). 4♂♂, 5♀♀, 24.IV.2021, Amasya, Taşova, Borabay 9 km SE, 40°49'52''N, 36°04'58''E,1720 m, leg. Anlaş, Kacar & Çelik (AZMM). 2♂♂, 2♀♀, 12.V.2011, Balıkesir, Edremit, Koşara Dağı, leg. Anlaş (AZMM). ♂, 10.VII.2020, Bartın, Ulus, Kerpiçli 4 km SW, 41°42'54''N, 32°53'36''E, 570 m, leg. Örgel & Kacar (AZMM). ♂, ♀, 10.VII.2020, Bartın, Ulus, Kerpiçli 3 km SW, 41°43'13''N, 32°53'57''E, 580 m, leg. Örgel & Kacar (AZMM). ♂, 27.VI.2012, Bartın, Amasra, Gergece Kaya Şelalesi leg. Danışman (AZMM). 2♂♂, 03.VII.2021, Bartın, Ulus, Kerpiçli 2 km NE, 41°43'14''N, 32°54'48''E, 590 m, leg. Örgel, Kacar & Çelik (AZMM). ♂, 05.VII.2020, Bolu, Mengen, Merkeşler 8 km N, 40°56'15''N, 31°47'49''E, 720 m, leg. Örgel & Kacar (AZMM). 2♂♂, 03.VII.2020, Bolu, Dereceören, 40°37'39''N, 31°22'28''E, 1135 m, leg. Örgel & Kacar (AZMM). ♂, 04.VII.2020, Bolu, Seben, Çökeler 10 km N, 40°32'37''N, 31°44'59''E, 1490 m, leg. Örgel & Kacar (AZMM). ♂, 13.IX.2012, Bolu, Abant, leg. Danışman (AZMM). ♂, 21.V.1978, Bolu, Ömerler, 800 m, leg. Besuchet & Löbl (MHNG). ♀,

Turquia, Bolu, Abant (MNCN). ♂, 11.XI.2020, Bursa, Uludağ, 40°08'33''N, 29°03'05''E, 935 m, leg. Örgel & Kacar (AZMM). 4♂♂, 2♀♀, 25.IX.2010, Bursa, Uludağ, 270 m, 40°02'53''N, 29°04'12''E, leg. Yağmur (AZMM). 3♂♂, 6♀♀, 26.VI.2022, Bursa, Kestel, Alaçam, Uludağ, 40°04'22''N, 29°15'46''E, 1984 m, leg. Anlaş, Kacar & Çelik (AZMM). ♀, Turquia, Bursa, Uludagh (as *Lobrathium multipunctatum*) (MNCN). 2♂♂, 8♀♀, 14.IV.2021, Çanakkale, Gökçeada, Dereköy 5 km N, 40°11'18''N, 25°45'30''E, 1 m, leg. Yağmur, Kacar & Çelik (AZMM). ♀, 14.IV.2021, Çanakkale, Gökçeada, Eşelek 2 km NW, 40°09'05''N, 25°58'43''E, 72 m, leg. Yağmur, Kacar & Çelik (AZMM). ♂, 3♀, 14.IV.2021, Çanakkale, Gökçeada, Dereköy 4 km N, Marmaras Şelalesi, 40°10'55''N, 25°45'50''E, 75 m, leg. Yağmur (AZMM), Kacar & Çelik. ♂, 14.IV.2021, Çanakkale, Gökçeada, Dereköy 1 km W, 40°09'26''N, 25°46'15''E, 226 m, leg. Yağmur, Kacar & Çelik (AZMM). ♂, 2♀♀, 16.IV.2021, Çanakkale, Lapseki, Taştepe 4 km S, 40°19'10''N, 26°50'40''E, 376 m, leg. Yağmur, Kacar & Çelik (AZMM). ♂, 16.IV.2021, Çanakkale, Lapseki, Taştepe 2 km S, 40°19'56''N, 26°50'16''E, 220 m, leg. Yağmur, Kacar & Çelik (AZMM). ♀, 05.XI.2021, Çanakkale, Gelibolu, Yeniköy 2 km NW, 40°29'26''N 26°35'24''E, 38 m, leg. Kacar & Çelik (AZMM). 2♀♀, 29.IV.2022, Çanakkale, Lapseki, Taştepe 3 km S, 40°19'52''N, 26°50'17''E, 156 m, leg. Kacar & Çelik (AZMM). 2♂♂, ♀, 19.V.2022, Çanakkale, Yenice, Örencik 3 km S, 39°48'20''N, 27°07'47''E, 300 m, leg. Kacar & Çelik (AZMM). 3♂♂, 3♀♀, 24.VI.2022, Çanakkale, Lapseki, Çavuşköy 5 km NE, 40°19'53''N, 26°50'10''E, 190 m, leg. Anlaş, Kacar & Çelik (AZMM). ♀, 01.V.2021, Çorum, Osmancık, Danişment 3 km S, 41°04'36''N, 34°55'48''E,1490 m, leg. Örgel, Kacar & Çelik (AZMM). 2♂♂, ♀, 01.V.2021, Çorum, Osmancık, Danişment 4 km SW, 41°04'23''N, 34°58'24''E,1400 m, leg. Örgel, Kacar & Çelik (AZMM). ♂, 3♀♀, 15.IV.2022, Çorum, Laloğlu 1 km E, 40°23'46''N, 34°32'45''E, 1030 m, leg. Kacar & Çelik (AZMM). 2♂♂, 09.V.2022, Çorum, Çağşak 5 km SE, 40°22'18''N, 34°31'43''E, 1416 m, leg. Kacar & Çelik (AZMM). ♂, 16.VI.2022, Düzce, Yığılca, Hocaköy 7 km NE, 41°01'38''N, 31°39'27''E, 1281 m, leg. Kacar & Çelik (AZMM). ♂, 27.VII.1969, Istanbul, Kilyos, leg. Besuchet (MHNG). ♂, Constantinapol (=Istanbul), Belgrader-wald, leg. Bodemeyer, coll. Rambousek (NMPC). ♂, ♀, Asia Minor, Sakarya, Alem Dagh, leg. Bodemeyer (HNHM).

Distribution. *Lobrathium rugipenne* is distributed in Albania, Armenia, Azerbaijan. Bulgaria, Georgia, Greece, South European Territory of Russia, former Yugoslavia, and Türkiye (Assing, 2007; Schülke & Smetana, 2015; Anlaş, 2020).

Lobrathium spinosum Assing & Schulke, 2002

Material examined. ALBANIA: ♂, 24.V.2004, Tomor Mts., Vodice Valley, 150-500 m, leg. Harmos & Murányi (HNHM).

Distribution: *Lobrathium spinosum* is only known from southern Albania (Tomor, Griba) (Assing & Schülke, 2002).

Lobrathium triste (Cameron, 1924) (Figures 3a-g)

Material examined. Afghanistan: ♂, Paghman (25 km NW Kabul, 2450 m, 15.VII.1960), A863, Voyage en Afghanistan, K. Lindberg, TYPUS *Lathrobium afghanicum* O. Scheerpeltz (NHMW). ♂, Paghman (25 km NW Kabul, 2450 m, 15.VII.1960), A863, Voyage en Afghanistan, K. Lindberg, COTYPUS *Lathrobium afghanicum* O. Scheerpeltz (NHMW). ♂, 09.V.1953, J. Klapperich, Bashgultal 1150 m, Nuristan, Afghanistan, *Lathrobium kashmiricum* Cam., ex. coll Scheerpeltz (NHMW). 2♂♂, ♀, 20.IV.1953, J. Klapperich, Bashgultal 1200 m, Nuristan, Afghanistan, *Lathrobium kashmiricum* Cam., ex. coll Scheerpeltz (NHMW). ♂, 12.X.1952, J. Klapperich, Aghelekan, 1900 m, Salangtal, Hindikusch, O-Afghanistan, *Lathrobium kashmiricum* Cam., ex. coll Scheerpeltz (NHMW). ♂, ♀, 30.X.1952, J. Klapperich, Tangi-Gharuh, 1600 m, am Kabulflu, Afghanistan, (NHMW). ♂, 10.V.1953, J. Klapperich, Kutiau, 1400 m, Nuristan, Afghanistan, *Lathrobium kashmiricum* Cam., ex. coll Scheerpeltz (NHMW).

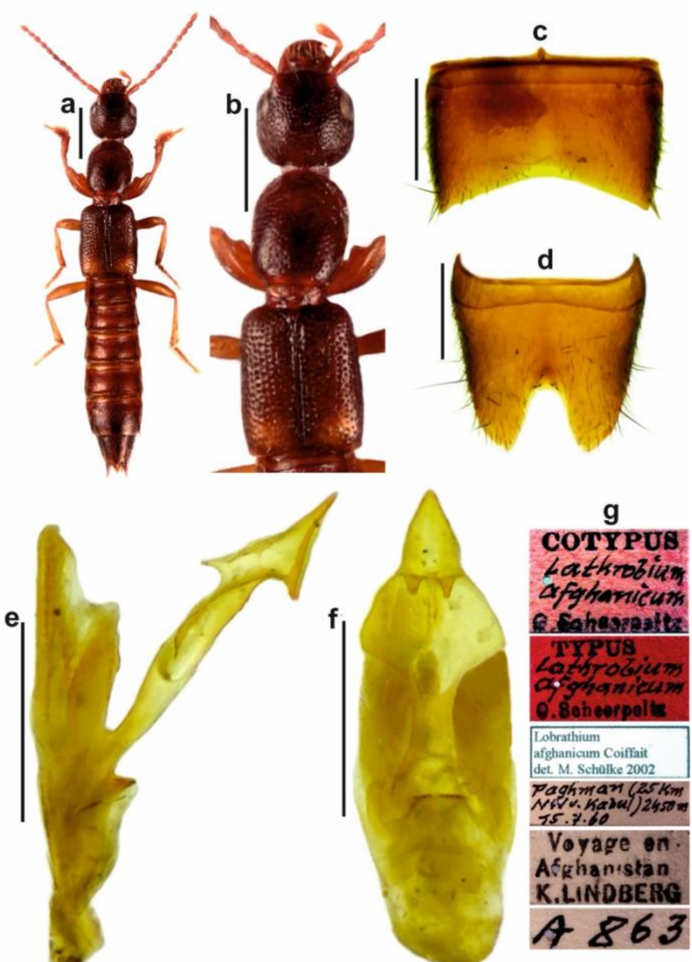


Figure 3. *Lobrathium afghanicum* Coiffait [=*L. triste* (Cameron)]. a) habitus; b) forebody; c) male sternite VII; d) male sternite VIII; e) aedeagus in lateral view; f) aedeagus in ventral view; g) labels. Scale bars: 1.0 mm (Figs. a-b); 0.5 mm (Figs. c-f).

Distribution. *Lathrobium triste* is known from Afghanistan, North India (Uttaranchal Pradesh, Kashmir), and Pakistan (Assing, 2012).

Remarks. *Lathrobium kashmiricum* Cameron, 1931; *Lobrathium afghanicum* Coiffait, 1979 and *L. nouristanicum* Coiffait, 1979 have been proposed as synonyms of *L. triste* by Assing (2012). But he was not examined the type material of *L. afghanicum*. I examined the type material of this species in the collections of the NHMW during my visit in 2021 (Figures 3a-g). An examination of the types of *L. afghanicum*, especially the aedeagus and sternite VIII, revealed that it is identical to *L. triste*. Therefore, I confirm that *L. afghanicum* is a synonym of the latter species.

Acknowledgements

I am most grateful to my colleagues for the loan of material and I would like to thank them for making their Staphylinid collections available to me. I also thank the anonymous reviewers for their valuable comments and suggestions. This study was partly supported by the Scientific and Technological Research Council of Turkey (TUBITAK Project Number 119Z253).

References

Anlaş, S., 2020. On the genus *Lobrathium* Mulsant & Rey, 1878 of the Palearctic Region: A new species from Turkey and new zoogeographic data (Coleoptera, Staphylinidae: Paederinae). Turkish Journal of Zoology, 44 (5): 446-454.

Assing, V. & M. Schülke, 2002. New species and records of *Lobrathium* Mulsant & Rey from Turkey, Albania, and Tajikistan (Coleoptera: Staphylinidae, Paederinae). Linzer biologische Beiträge, 34 (1): 277-287.

Assing, V., 2005. A new species of *Lobrathium* Mulsant & Rey from Greece (Coleoptera: Staphylinidae, Paederinae). Entomologische Blatter, 100 (3) 197-200.

Assing, V., 2007. A revision of Palearctic *Lobrathium* Mulsant & Rey. III. New species, new synonyms, and additional records (Coleoptera: Staphylinidae, Paederinae). Linzer biologische Beitrage, 39 (2): 731-755.

Assing, V., 2012. A revision of East Palearctic *Lobrathium* (Coleoptera: Staphylinidae: Paederinae). Bonn Zoological Bulletin, 61 (1): 49-128.

Assing, V., 2019. A revision of Palearctic *Lobrathium*. VII. A new species from Greece, a revalidation, and additional records (Coleoptera: Staphylinidae: Paederinae). Acta Musei Moraviae, Scientiae Biologicae, 104 (2): 109-115.

Assing, V., 2021. On the *Lathrobiina* of Taiwan II. A new species and an additional record of *Lobrathium* (Coleoptera: Staphylinidae: Paederinae). Contributions to Entomology, 71 (2): 233-236.

Bordoni, A., 2009. Palearctic Lathrobiina of the Museum of Zoology of Lund with description of *Lobrathium candicum* n. sp. from Crete (Coleoptera Staphylindae). Bollettino della Societa Entomologica Italiana, 141 (3): 139-142.

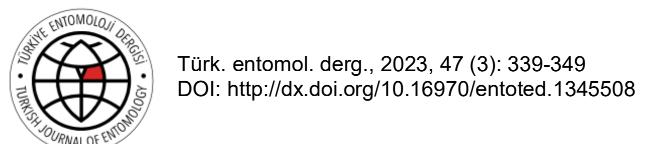
Coiffait, H., 1982. Coléoptères Staphylinidae de la région paléarctique occidentale. IV. Sous famille Paederinae. Tribu Paederini 1 (Paederi, Lathrobii). Supplément à la Nouvelle Revue d´Entomologie, 12 (4): 1-440.

Lin, X-B., X. Chen & Z. Peng, 2022. A new species and additional records of *Lobrathium* Mulsant & Rey (Coleoptera: Staphylinidae: Paederinae) from southern China. Zootaxa, 5133 (2): 241-246.

Scheerpeltz, O., 1937. Wissenschaftliche Ergebnisse einer von Herrn Hofrat F. Schubert, seinem Sohne Herrn cand. Phil. F. Schubert und Herrn Prof. Ing. K. Mandl im Sommer 1935 (1936) nach Bulgarien unternommenen Studienreise. Coleoptera: I. Staphylinidae. Mitteilungen aus den Koniglichen Naturwissenschaftlichen Instituten, 10: 185-246 (in German).

Schülke, M. & A. Smetana, 2015. “Staphylinidae, 304-1134”. In: Catalogue of Palaearctic Coleoptera. Volume 2. Hydrophiloidea - Staphylinoidea. (Ed. I. Löbl & D. Löbl). Revised and Updated Ed. Leiden, the Netherlands: Brill, 1702 pp.

Solodovnikov, A. Yu., 2001. Revision of the little-known apterous Paederinae (Coleoptera: Staphylinidae) from "Circassia" (North-Western Caucasus), with description of *Lathrobium* (*Lobrathium*) *bettae* sp. n. Russian Entomological Journal, 10 (1): 1-11.


Türk. entomol. derg., 2023, 47 (3): 339-349
DOI: http://dx.doi.org/10.16970/entoted.1345508

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Effect of selected pesticides on the orientation of entomopathogenic nematodes (Rhabditida: Heterorhabditidae and Steinernematidae)

Bazı pestisitlerin entomopatojen nematodların (Rhabditida: Heterorhabditidae ve Steinernematidae) yönelimleri üzerine etkisi

Tufan Can ULU[1*]

Abstract

Entomopathogenic nematodes (EPNs) play a crucial role in biological control, but they can be also applied together with pesticides. Therefore, the compatibility of pesticides with EPNs and the influence on their behavior significantly affect field success. This study investigated how selected pesticides (Deltamethrin, Imidacloprid, Pendimethalin, 2,4-D, and Boscalid + Pyraclostrobin) affect the orientation behavior of three commercial EPN species. Trials were conducted using steel olfactometers followed by the assessment of EPN dispersal ratios after 24 hours. The study was conducted in the Laboratory of the Plant Protection Department, Faculty of Agriculture and Natural Sciences, Bilecik Şeyh Edebali University between 2022 and 2023. According to the results, while nearly all pesticides exhibited a significant impact on the dispersal behavior of EPNs, the most notable effects were observed in the trials involving 2,4-D and Imidacloprid. These two pesticides demonstrated both repellent and attractive effects on different EPN species. The impact of other pesticides was comparatively negligible. All EPN species exhibited higher orientation towards larvae than the control application. The orientation behavior displayed variations depending on the pesticide type and the EPN species involved. It is expected that this study will contribute to our understanding of the relationship between EPNs and pesticides, and ultimately enhancing the efficacy of EPNs.

Keywords: Behavior, *Heterorhabditis*, pesticide, *Steinernema*, orientation

Öz

Entomopatojen nematodlar (EPN'ler) önemli biyolojik mücadele ajanı olmalarına karşın pestisitler ile birlikte de uygulanabilmektedir. Bu nedenle, pestisitlerin EPN'ler ile uyumluluğu ve EPN davranışı üzerindeki etkisi arazi başarısını etkilemektedir. Bu çalışmada, beş pestisitin (Deltamethrin, Imidacloprid, Pendimetalin, 2,4-D ve Boscalid + Pyraclostrobin) üç ticari EPN türünün yönelim davranışını nasıl etkilediği araştırılmıştır. Denemeler, çelik olfaktometrelerde gerçekleştirilmiş ve 24 saatlik inkübasyon sonrasında EPN yayılma oranları belirlenmiştir. Çalışma 2022-2023 yılları arasında Bilecik Şeyh Edebali Üniversitesi Ziraat ve Doğa Bilimleri Fakültesi Bitki Koruma Bölümü Laboratuvarında gerçekleştirilmiştir. Neredeyse tüm pestisitler EPN yayılımı üzerinde önemli etki göstermiş, en farklı sonuçlar 2,4-D ve Imidacloprid denemelerinde tespit edilmiştir. Her iki pestisit de EPN türüne göre hem itici hem de çekici etkide bulunmuştur. Diğer pestisitlerin etkileri daha sınırlı kalmıştır. Tüm EPN türleri, kontrol uygulamasına kıyasla larvalara daha yüksek yönelim göstermiştir. Sonuçlar, EPN türlerine ve pestisitlere bağlı olarak yayılma oranlarında farklılıklar olduğunu göstermiştir. Bu çalışmanın EPN'ler ve pestisitler arasındaki ilişkiyi anlamaya ve EPN uygulama etkinliğini artırmaya katkı sağlayacağı beklenmektedir.

Anahtar sözcükler: Davranış, *Heterorhabditis*, pestisit, *Steinernema*, yönelim

[1] Bilecik Seyh Edebali University, Faculty of Agriculture and Natural Sciences, Department of Plant Protection, 11100, Merkez, Bilecik, Türkiye
* Corresponding author (Sorumlu yazar) e-mail: tufan.ulu@bilecik.edu.tr
Received (Alınış): 18.08.2023 Accepted (Kabul ediliş): 30.09.2023 Published Online (Çevrimiçi Yayın Tarihi): 05.10.2023

Introduction

Entomopathogenic nematodes (EPNs) from the genera *Heterorhabditis* and *Steinernema* are obligate endoparasites on insects and they have found extensive use in biologically controlling agricultural pests. EPNs undergo a distinctive stationary phase known as the infective juvenile (IJ), which represents the sole free-living stage in their life cycle. Upon encountering a host, the IJs penetrate the host and release symbiotic bacterium into the host hemolymph. The bacterium proliferates within the host while simultaneously inducing host mortality through the secretion of an array of toxins and enzymes. The bacterium converts the host into a food source that EPNs feed on, facilitating their continued development. After undergoing 2-3 generations within the host, the available food becomes depleted, signaling the IJs to exit the cadaver and search for new hosts (Kaya & Gaugler, 1993).

Over the course of several years, EPNs have demonstrated remarkable efficacy in controlling agricultural pests (Koppenhöfer et al., 2020; Mokrini et al., 2020; Taşkesen et al., 2021). Their increasing adoption can be attributed to their ability for cost-effective mass production in bioreactors and their easy application through conventional spray equipment and drip irrigation systems (Wright et al., 2005; Susurluk & Ehlers, 2008; Shapiro-Ilan et al., 2012; Peters, 2013). Thanks to innovative formulation techniques and application methodologies, their efficacy extends not only below-ground but also in targeting insect pests above the soil (Şahin et al., 2018; Platt et al., 2020; Fallet et al., 2022). Despite the notable achievements in pest management, field applications of EPNs frequently yield inconsistent outcomes (Jaffuel et al., 2020). Furthermore, if not administered by professionals, the probability of success decreases due to possible inaccuracies in application. As a result, EPNs are often applied concurrently with conventional agricultural pesticides. An important benefit is the compatibility of EPNs with various pesticides, and in certain combinations, even synergistic effects have been observed (Amizadeh et al., 2019; Yüksel et al., 2019). Furthermore, simultaneous application also saves time and reduces application costs (Laznik & Trdan, 2017). Notably, this cooperative strategy of applying pesticides together with EPNs is particularly advantageous compared to predator insects, which are often susceptible to insecticides and thus incompatible for simultaneous application.

The interaction between EPNs and pesticides has long intrigued scientists. Determining whether a pesticide can be combined in a tank mixture or applied simultaneously with another pesticide carries significant importance. Early investigations explored the impacts of organophosphorus and carbamate compounds, which were commonly used pesticides during that period, on the development and viability of EPNs. These studies underscored the lack of compatibility between these pesticides and nematode species (Hara & Kaya, 1982, 1983). As the utilization of EPNs in agriculture continues to grow, numerous studies have examined the compatibility between various EPN species and pesticides. These investigations have unveiled a wide range of pesticides that are now recognized for their compatibility with EPNs, allowing for their simultaneous application (Ulu et al., 2016; Bajc et al., 2017; Laznik & Trdan, 2017; Aioub et al., 2021). While the majority of studies focused on insecticides, investigations into the effects of fungicides, acaricides, and herbicides have also been conducted (Koppenhöfer et al., 2003; Bajc et al., 2017; Laznik & Trdan, 2017; Sabino et al., 2019; Özdemir et al., 2020). It is important to emphasize that the compatibility is influenced by the specific pesticide and the EPN species (Laznik & Trdan, 2014). Moreover, prolonged exposure to pesticides intensifies their harmful effects.

While the majority of compatibility studies have mainly focused on EPN efficacy and viability, only a limited number of them have explored the changes in EPN behavior due to pesticide exposure. Numerous pesticides are recognized to induce paralysis or diminish mobility in EPNs, even potentially leading to their death (Hara & Kaya, 1983). Furthermore, certain pesticides have been documented to disrupt the locomotion patterns of EPNs and reduce the host-detection capabilities of infective juveniles (Gaugler & Campbell, 1991). Additionally, specific organic phosphorus and carbamate compounds trigger a behavior

known as nictation in some EPN species, simultaneously reducing their rate of movement (Ishibashi & Takii, 1993). Because of the scarcity of studies investigating the impact of pesticides on EPN behaviors, several aspects of this relationship remain unexplored. Given the frequent coexistence of pesticides and EPNs in nature, the influence of pesticides on EPN behavior holds significance for their overall effectiveness.

This study investigated the orientation behavior of three commonly found EPN species when exposed to selected pesticides, chosen for their common usage in Turkey and their high likelihood of encountering nematodes in their natural habitats. The study involved controlled trials using steel olfactometers to evaluate how these EPNs responded to different concentrations of pesticides. The objective was to uncover the reactions of EPNs in the presence of pesticides, a departure from previous research that primarily focused on compatibility and effectiveness evaluations of EPNs with pesticides. While significant insights have been gained into nematode behavior, certain aspects still remain unclear. Hence, this study aims to provide a deeper understanding of the interaction between pesticides and EPNs, which is crucial given their concurrent application.

Materials and Methods

Entomopathogenic nematodes and pesticides

The study involved three prevalent EPN species: *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae), *Steinernema feltiae* (Filipjev, 1934), and *Steinernema carpocapsae* (Weiser, 1955) (Rhabditida: Steinernematidae). These species are distributed globally and exhibit distinct host-seeking strategies (Susurluk et al., 2003). Additionally, all three species are recognized as registered biocontrol products in Turkey. They are labeled for managing many major pests, including mushroom gnats, western flower thrips, white grubs, and flathead woodborers. The commercial EPN products were supplied by Bioglobal A.Ş (Antalya, Türkiye), while the strains themselves were commercial strains obtained from e-nema GmbH (Schwentinental, Germany).

There were two insecticides, two herbicides, and one fungicide in the study. The selection of pesticides was based on active substances that are recognized to be compatible with EPNs (Chavan et al., 2018; Özdemir et al., 2021), suitable for application to soil or near-soil areas (or systemic), and likely to interact with EPNs in their natural environment. Further information regarding the pesticides employed in the study is presented in the Table 1.

Table 1. Details of the pesticides used in the study

Active Compound	Product Name (Formulation)	Pesticide Group	Chemical Class	Mode of Action
Imidacloprid	Insector® (SC)	Insecticide	Neonicotinoids	Acetylcholine mimic
Deltamethrin	Jetsis® (EC)	Insecticide	Pyrethroids	Sodium Channel Modulator
2,4-D	Ester H® (EC)	Herbicide	Phenoxy-carboxylates	Auxin mimics
Pendimethalin	Giant® (EC)	Herbicide	Dinitroanilines	Inhibition of Microtubule Assembly
Boscalid + Pyraclostrobin	Bellis® (WG)	Fungicide	Pyridine-carboxamides + Methoxy-carbamates	Succinate dehydrogenase inhibitors + Quinone outside Inhibitors

Olfactometer setup

The study utilized olfactometers that were originally produced for behavioral studies of EPNs, by the author. The stainless steel olfactometer comprises two primary components: the arm and the central part. While the design allows for up to three arms to be attached to each olfactometer (Ulu & Erdoğan, 2023; Ulu et al., 2023), this study employed a single arm configuration. The remaining two arm sockets were closed with a steel hex plug. The olfactometer has an approximate diameter of 2.5 cm and a length of about 10 cm from the central application hole to the arm's end. The Figure 1 illustrates the olfactometer's design and dimensions.

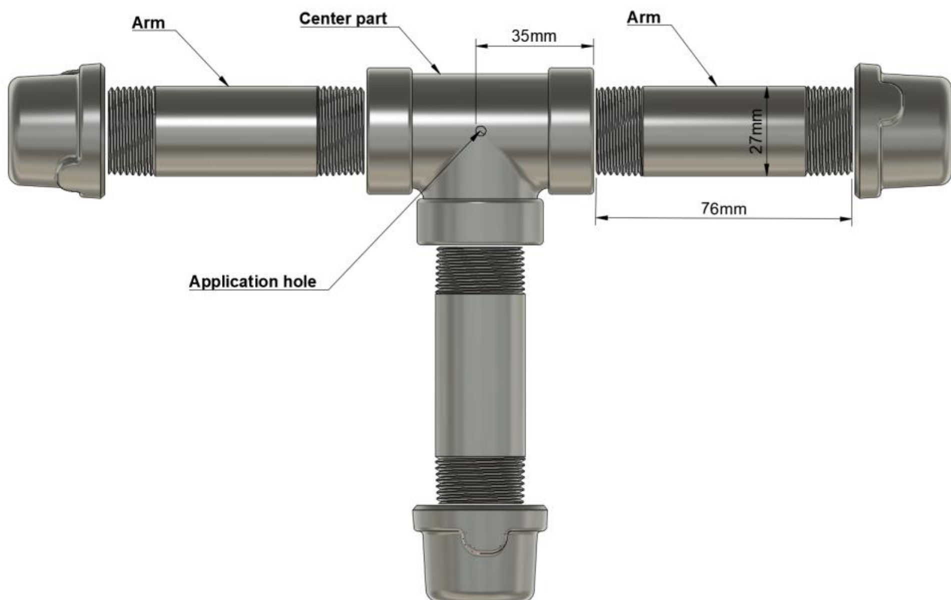


Figure 1. Illustration and dimensions of the olfactometer.

Experimental design

The study took place in the Laboratory of the Plant Protection Department, Faculty of Agriculture and Natural Sciences, Bilecik Şeyh Edebali University between 2022 and 2023. The olfactometers was filled with dust-free silica sand, a chemically inert substrate characterized by a particle diameter approximately 250 microns. Prior to filling, the sand in the center part was moistened with deionized water (DI), while the arm was moistened with pesticide solutions. The highest recommended field dose (X), half dose (0.5X), and double dose (2X) of each pesticide were prepared in glass beakers. The resulting pesticide solution was used to moisten the sand. The moisture content of the sand was adjusted to 10% for both parts. Subsequently, 5000 IJs were applied through the application hole with a pipette. The nematodes were applied using 50 microliters of DI water. The nematode cultures were no older than 1 week, and their mortality ratios were below 1%. Once the application was complete, the hole was sealed with parafilm, and the olfactometers were incubated at a controlled temperature of 24°C for 24 hours. The olfactometers are not airtight, but they are tight enough to preserve inner moisture for more than 24 h (<1% moisture loss during experiments), while allowing air exchange with the surrounding environment. The olfactometers were set up with the left arm, lower arm, and right arm interchangeably to mitigate directional effects and bias. Following the incubation period, the center part of the olfactometer was disassembled from its arm, and the sand within was gently washed in a container. The nematode suspension obtained was then filtered through a 25-micron mesh, and nematodes were collected in a beaker. The nematodes present in both the arm and center part were counted separately, and dispersal ratio was calculated according to the formula: Total number of infective juveniles (IJs) in the arm / Total number of IJs in the olfactometer. Each treatment was replicated using 5 olfactometers. Additionally, olfactometer setups were established at three distinct time points, resulting in a cumulative total of 2 + 2 + 1 olfactometers. These setups were performed using fresh populations of nematodes, comprising 5 technical replicates and 3 biological replicates in total. The negative control treatment consisted solely of DI water, while *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae) larvae were employed as the positive control treatment. The larva was placed at the end of the arm in a 3D printed mesh cage. It's worth noting that the larval treatment was not compared with pesticides, as its purpose was to observe the enhanced dispersal of IJs in the presence of an attractive cue. Mortality ratios of IJs were assessed during the counting procedure and were observed to be below 5% across all treatments.

Statistical analysis

All data were subjected to a normality test using the Shapiro-Wilk's method, and the homogeneity of variances was assessed using the Brown-Forsythe test. As all assumptions were met, a one-way ANOVA was employed to analyze the dispersal ratio of the treatments. Since each experiment had its corresponding control treatment, the Dunnett's comparison test was used to compare various pesticide doses with the negative control treatment. An unpaired t-test was applied to compare control and larva applications in the positive control. All analyses were conducted at a significance level of $p < 0.05$, and the graphs were generated using GraphPad v9.5.

Results and Discussion

Orientation of *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae)

In the positive control scenario, the IJs displayed statistically significant orientation towards the larval arm when compared to the control group (t (8)= 7.08, $p < 0.001$). In the control application, the proportion of IJs in the arm was 25.4%, which then increased to 43.4% in the presence of larvae. The dispersal ratio of *H. bacteriophora* varied based on the pesticide. No statistical change was observed in the orientation in Deltamethrin (F (3, 16) = 1.71, $p = 0.204$) and Boscalid + Pyraclostrobin (F (3, 16) = 0.64, $p = 0.598$) treatments. Pendimethalin, however, showed an increased orientation at higher doses. Although no statistical difference was noted in the 0.5X dose, an increased dispersal was observed in X and 2X doses compared to the control (F (3, 16) = 12.4, $p < 0.001$). The initial dispersal ratio in the control group was 23.2%, which then increased to 29.7% at the X dose. On the other hand, Imidacloprid displayed repellency at 0.5X and X doses, which diminished at 2X doses (F (3, 16) = 10.7, $p < 0.001$). Notably, among the pesticides tested, 2,4-D exhibited the most prominent effect, significantly reducing dispersal of IJs (F (3, 16) = 42.6, $p < 0.001$). A strong repellency was observed even at the 0.5X dose. The dispersal ratio, initially at 23.4% in the control group, declined to 8.7% at the 2X dose (Figure 2).

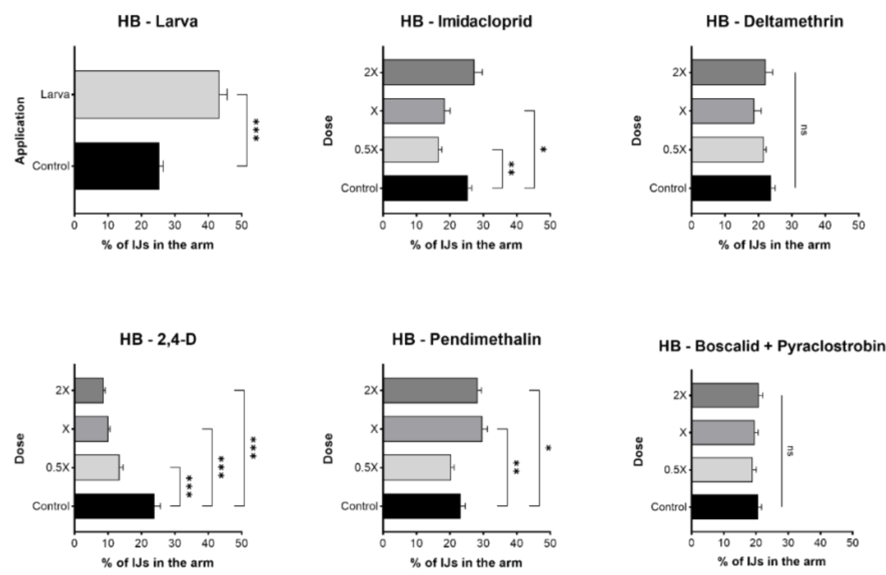


Figure 2. The dispersal ratios (X axis) of *Heterorhabditis bacteriophora* in the presence of a larva (first graph) and after a 24-hour exposure to selected pesticides. Error bars indicate standard error mean. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ indicate statistically significant differences compared to the corresponding control (represented by black bars), as determined by Dunnet's comparison test ($p < 0.05$) for pesticides, and unpaired t test ($p < 0.05$) for the larva application. Ns: not significant ($p > 0.05$).

Orientation of *Steinernema feltiae* (Filipjev, 1934) (Rhabditida: Steinernematidae)

In the positive control, *S. feltiae* exhibited a lesser orientation towards the larva in comparison to *H. bacteriophora*, yet a significant dispersal was observed when compared to the control application (t (8)= 3.56, p = 0.007). With a dispersal ratio of 17.2% in the control group, the presence of larva led to an increase to 24.5%. The dispersal of S. *feltiae* IJs was generally less influenced by pesticides in comparison to *H. bacteriophora*. Although there was a numerical decrease in the dispersal especially at 2X doses, no significant effect was observed for Imidacloprid (F (3, 16) = 1.21, p = 0.339), Deltamethrin (F (3, 16) = 2.50, p = 0.097), Pendimethalin (F (3, 16) = 3.05, p = 0.059), and Boscalid + Pyraclostrobin (F (3, 16) = 3.35, p = 0.045), compared to control. In contrast, unlike *H. bacteriophora*, the orientation of *S. feltiae* was increased at 0.5X and X doses of 2,4-D; however, this effect was not observed at the 2X dose (F (3, 16) = 6.20, p = 0.005). Starting at 19.3% in the control group, the dispersal ratio increased to 28.4% at the X dose (Figure 3).

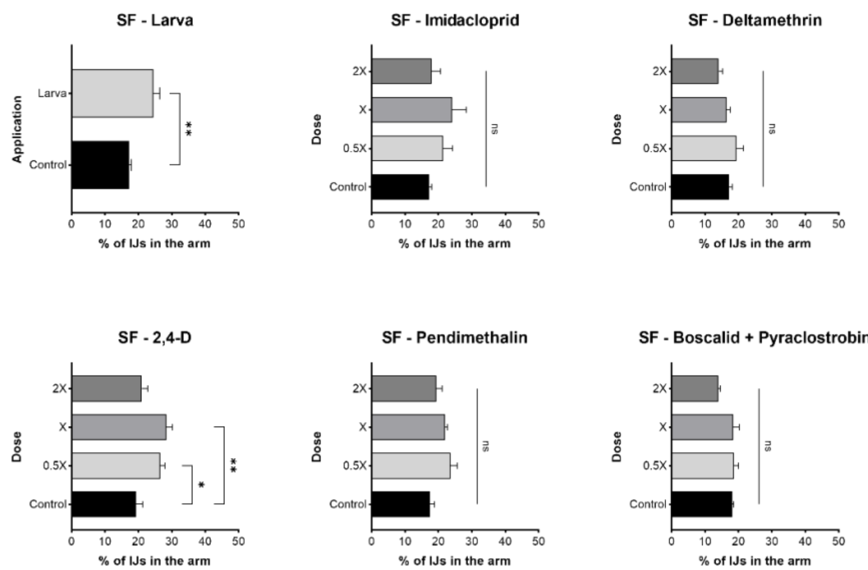


Figure 3. The dispersal ratios (X axis) of *Steinernema feltiae* in the presence of a larva (first graph) and after a 24-hour exposure to selected pesticides. Error bars indicate standard error mean. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ indicate statistically significant differences compared to the corresponding control (represented by black bars), as determined by Dunnet's comparison test ($p < 0.05$) for pesticides, and unpaired t test ($p < 0.05$) for the larva application. Ns: not significant ($p > 0.05$).

Orientation of *Steinernema carpocapsae* (Weiser, 1955) (Rhabditida: Steinernematidae)

As observed with the preceding two species, the orientation of *S. carpocapsae* towards the larvae displayed a significant enhancement when compared to the control group (t (8)= 7.24, $p < 0.001$). The dispersal ratio, which initially stood at 15.8% in the control group, increased to 25.4% in the larva application. Interestingly, while Deltamethrin had no impact on the dispersal of the other two species, it adversely affected *S. carpocapsae*, significantly reducing the dispersal ratio at the highest dosage (F (3, 16) = 7.61, p = 0.002). Specifically, the dispersal ratio, which was at 16.6% in the control group, reduced to 9.8% at the 2X dose. Similarly, there was a decline in dispersal ratios at higher doses in the case of Boscalid + Pyraclostrobin application (F (3, 16) = 5.35, p = 0.010). In contrast, Imidacloprid exhibited a notable increase in the orientation of *S. carpocapsae* (F (3, 16) = 9.69, $p < 0.001$). The dispersal ratio, initially measured at 15.7% in the control group, nearly doubled to 28.8% at the 2X dose. On the other hand, the application of 2,4-D (F (3, 16) = 2.22, p = 0.125) and Pendimethalin (F (3, 16) = 2.44, p = 0.102) showed no observable effect on the orientation of IJs (Figure 4).

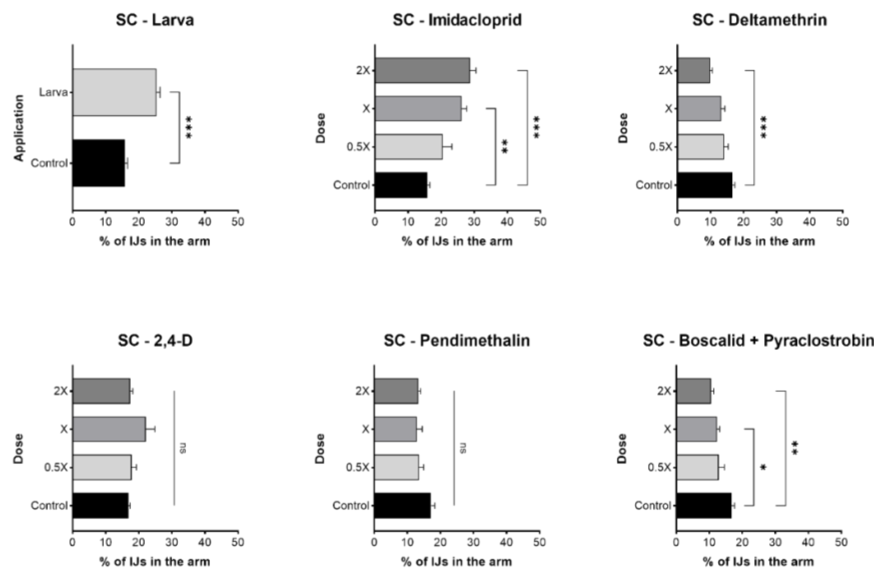


Figure 4. The dispersal ratios of *Steinernema carpocapsae* in the presence of a larva (first graph) and after a 24-hour exposure to selected pesticides. Error bars indicate standard error mean. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ indicate statistically significant differences compared to the corresponding control (represented by black bars), as determined by Dunnet's comparison test ($p < 0.05$) for pesticides, and unpaired t test ($p < 0.05$) for the larva application. Ns: not significant ($p > 0.05$).

The highest dispersal ratio among all pesticide applications was 29.4% at the 2X dose of *H. bacteriophora* Pendimethalin application. When considering the increase rate compared to the related control groups, the highest increase was observed with 1.82 times in the 2X dose of *S. carpocapsae* Imidacloprid application. The application of *H. bacteriophora* with larvae achieved the highest dispersal ratio among all applications, reaching 43.4%. On the other hand, the lowest dispersal ratio of 8.71% was determined at the 2X dose of *H. bacteriophora* 2,4-D application. In comparison to the control group, the dispersal decreased by a factor of 0.64.

The practice of applying EPNs alongside pesticides has become increasingly common in recent years. Thus, the present study focused on examining the effects of specific pesticides, which are commonly used and likely to intersect with EPNs in natural environments, on the orientation behavior of EPNs. The findings were varied and dependent on the EPN species and the pesticides, and the results were aligned with similar studies (García del Pino & Jové, 2005; Laznik & Trdan, 2017). For instance, *H. bacteriophora* demonstrated a repellent response to Imidacloprid and 2,4-D, while showing attraction towards Pendimethalin. On the other hand, *S. feltiae* exhibited attraction solely to 2,4-D, with no significant impact from other pesticides. Finally, *S. carpocapsae* was attracted by Imidacloprid and repelled by Deltamethrin and Boscalid + Pyraclostrobin. Among all EPN strains, there was a noteworthy orientation towards larvae in the positive control groups.

Previous reports have indicated the compatibility of Imidacloprid with many EPN species (Koppenhöfer et al., 2002; Atwa et al., 2013; Laznik & Trdan, 2014; Kwizera & Susurluk, 2017; Özdemir et al., 2020). The findings of this study revealed that *S. carpocapsae* demonstrated attraction towards Imidacloprid, which could contribute to their compatibility. On the other hand, it was observed that *H. bacteriophora* exhibited a certain level of repellency against Imidacloprid. Given that Imidacloprid is commonly used in drip irrigation for pest control, it becomes imperative to investigate the potential movement of *H. bacteriophora* away from the root zone in fields where Imidacloprid is applied. A similar concern can be said for Deltamethrin. While previous studies generally indicated Deltamethrin's compatibility with EPNs (Negrisoli et al., 2010), this study revealed its repellent effect on *S. carpocapsae*. Given the widespread and excessive usage of Deltamethrin,

investigating its interaction under field conditions becomes vital. Despite the limited number of studies focusing on 2,4-D, previous research findings have indicated both compatibility and incompatibility (Laznik & Trdan, 2017; Chavan et al., 2018). The current study aligns with this existing literature, as 2,4-D exhibited a strong repellent effect on *H. bacteriophora*, while it displayed a degree of attraction for *S. feltiae*. Unfortunately, arriving at a conclusive interpretation is challenging, as the remaining EPN-pesticide combinations exhibit minimal to no impact. However, even though it is hard to explain differential results (Radová, 2011), the inconsistent impact of pesticides and their doses on EPNs could be attributed to factors such as the specific active substances, formulation additives, mode of actions, nematode host-seeking strategies, or their interpretation of different stimuli.

Extensive research has explored the compatibility between EPNs and pesticides, revealing that numerous pesticides can be simultaneously applied well with EPNs (Radová, 2011; Bajc et al., 2017; Chavan et al., 2018; Özdemir et al., 2021). Nevertheless, investigations into how pesticides influence diverse behavioral traits of EPNs remain limited. Only a few studies have investigated the influence of pesticides on the behavior of EPNs. For instance, the impact of Oxamyl on the motility and host-seeking abilities of *H. bacteriophora* and *S. carpocapsae* has been observed, indicating a reduction in these capacities (Gaugler & Campbell, 1991). At elevated doses, paralysis entirely halts the movement of IJs. Conversely, Acephate, belonging to the same chemical group as Oxamyl, and Permethrin, a synthetic pyrethroid, have been indicated to enhance the nictation behavior of *S. carpocapsae*, and are considered suitable for combined application (Ishibashi & Takii, 1993). Furthermore, investigations have established that Fipronil does not adversely impact *H. bacteriophora* and *S. carpocapsae*, and even though the movement of *S. arenarium* was reduced, its efficacy remained unaffected (García del Pino & Jové, 2005). As evident from these studies, the absence of adverse effects of pesticides on the mortality or infectivity of EPNs does not necessarily imply the absence of changes in their behavior. Similar concerns have been brought up in the past as well (Zimmerman & Cranshaw, 1990). In addition, it has been indicated that insecticides targeting the nervous system can disrupt the sensory organs of nematodes, leading to a reduction in their host-finding capability (Patel & Wright, 1996). However, compatibility tests carried out in controlled laboratory conditions suggest that EPN efficiency remains high due to the ideal host encounter environment. This suggests that the high compatibility and efficacy results achieved under laboratory conditions are less likely to be reproduced in the field. Similarly, in this study, the post-application mortality ratio of EPNs remained below 5% based on the counts. From this viewpoint, it can be stated that the pesticides used in this study are compatible with EPNs, a conclusion supported by numerous studies (Rovesti et al., 1990; Negrisoli et al., 2010; Chavan et al., 2018; Özdemir et al., 2021). However, when exploring the effects of these chemicals on behavior, it becomes apparent that they can elicit both repellent and attractive responses. Given the extensive use of pesticides in agriculture, it's highly conceivable that potent chemicals in soil and plants can influence nematode behavior below ground. These responses could substantially shape the field effectiveness of EPNs within their natural environment. Hence, it is anticipated that studies conducted under field conditions will offer a more realistic perspective compared to efficacy tests conducted in ideal conditions within a limited area.

It's important to recognize that field efficiency may not directly mirror results obtained in controlled laboratory conditions. Controlled environments involve only a few stimuli, whereas the natural conditions involve the interaction of numerous stimuli. Our understanding of nematode orientation and dispersal highlights various factors that enhance or diminish these behaviors (Shapiro-Ilan et al., 2019). Attention to even the smallest details is crucial to enhance the success of biological control efforts. Understanding the behavior of EPNs and the idea of manipulating the behavior to increase efficiency is expected to be further explored in the future (Andaló et al., 2012). For instance, recent research indicates that pheromones contribute to enhanced nematode dispersion and host-seeking capacity, offering the potential to bolster field efficacy (Oliveira-Hofman et al., 2019; Kaplan et al., 2020). Certainly, the outcomes achieved in

controlled laboratory settings should be duplicated and validated within real-world field conditions. In addition, the evaluation of the behavioral effects of chemicals should not be conducted in isolation; rather, it should be considered in conjunction with their impact on the behavior, viability, and infectivity of EPNs.

In conclusion, the impact of chemical pesticides on the orientation behavior and dispersal of EPNs varies based on the specific pesticides and EPN species. Some pesticides are found to strongly repel certain species, while in other cases, they attract IJs. Given the limited number of studies on the impact of chemical pesticides on EPN behavior, generalizing the findings from this study is not straightforward. However, research on the compatibility of EPNs with pesticides has demonstrated varying outcomes depending on the specific chemicals and EPN types involved. Consequently, the results of this study suggest that the effects of chemicals on behavior are likely to exhibit similar variability. Simultaneously applying EPNs and chemicals proves to be a time and cost-effective approach. Considering the frequent coexistence of pesticides and EPNs in the natural environment, understanding their interactions becomes crucial for the effective implementation of biological control. Besides assessing how pesticides affect EPN efficacy, examining shifts in their behavior holds equal significance for effective pest management. Due to the varying results, it's advisable to continue conducting compatibility trials and consistently explore interaction between new EPN strains and chemical compounds. Thus, conducting a comprehensive investigation into the connection between pesticides and EPNs emerges as a pivotal step for future research.

Acknowledgements

I would like to thank Osman Sepetçi and Mehmet Çankaya for their support.

References

Aioub, A. A. A., R. M. El-Ashry, A. S. Hashem, A. E. Elesawy & A. E. A. Elsobki, 2021. Compatibility of entomopathogenic nematodes with insecticides against the cabbage white butterfly, *Pieris rapae* L. (Lepidoptera: Pieridae). Egyptian Journal of Biological Pest Control, 31 (1): 153.

Amizadeh, M., M. J. Hejazi, G. Niknam & G. Askari-Saryazdi, 2019. Interaction between the entomopathogenic nematode, *Steinernema feltiae* and selected chemical insecticides for management of the tomato leafminer, *Tuta absoluta*. BioControl, 64 (6): 709-721.

Andaló, V., V. Santos, G. F. Moreira, C. Moreira, M. Freire & A. Moino Jr., 2012. Movement of *Heterorhabditis amazonensis* and *Steinernema arenarium* in search of corn fall armyworm larvae in artificial conditions. Scientia Agricola, 69 (3): 226-230.

Atwa, A. A., M. M. Shamseldean & F. A. Yonis, 2013. The effect of different pesticides on reproduction of entomopathogenic nematodes. Turkish Journal of Entomology, 37 (4): 493-502.

Bajc, N., U. Držaj, S. Trdan & Ž. Laznik, 2017. Compatibility of acaricides with entomopathogenic nematodes (Steinernema and Heterorhabditis). Nematology, 19 (8): 891-898.

Chavan, S., N. Somasekhar & G. Katti, 2018. Compatibility of entomopathogenic nematode *Heterorhabditis indica* (Nematoda: Heterorhabditidae) with agrochemicals used in the rice ecosystem. Journal of Entomology & Zoology Studies, 6 (4): 527-532.

Fallet, P., D. Bazagwira, J. M. Guenat, C. Bustos-Segura, P. Karangwa, I. P. Mukundwa, J. Kajuga, T. Degen, S. Toepfer & T. C. J. Turlings, 2022. Laboratory and field trials reveal the potential of a gel formulation of entomopathogenic nematodes for the biological control of fall armyworm caterpillars (*Spodoptera frugiperda*). Biological Control, 176: 105086.

García del Pino, F. & M. Jové, 2005. Compatibility of entomopathogenic nematodes with fipronil. Journal of Helminthology, 79 (4): 333-337.

Gaugler, R. & J. F. Campbell, 1991. Behavioural response of the entomopathogenic nematodes *Steinernema carpocapsae* and *Heterorhabditis bacteriophora* to oxamyl. Annals of Applied Biology, 119 (1): 131-138.

Hara, A. H. & H. K. Kaya, 1982. Effects of selected insecticides and nematicides on the in vitro development of the entomogenous nematode *Neoaplectana carpocapsae*. Journal of Nematology, 14 (4): 486-91.

Hara, A. H. & H. K. Kaya, 1983. Toxicity of selected organophosphate and carbamate pesticides to infective juveniles of the entomogenous nematode *Neoaplectana carpocapsae* (Rhabditida: Steinernematidae). Environmental Entomology, 12 (2): 496-501.

Ishibashi, N. & S. Takii, 1993. Effects of insecticides on movement, nictation, and infectivity of *Steinernema carpocapsae*. Journal of Nematology, 25 (2): 204-213.

Jaffuel, G., I. Sbaiti & T. C. J. Turlings, 2020. Encapsulated entomopathogenic nematodes can protect maize plants from *Diabrotica balteata* larvae. Insects, 11 (1): 27.

Kaplan, F., A. Perret-Gentil, J. Giurintano, G. Stevens, H. Erdogan, K. C. Schiller, A. Mirti, E. Sampson, C. Torres, J. Sun, E. E. Lewis & D. Shapiro-Ilan, 2020. Conspecific and heterospecific pheromones stimulate dispersal of entomopathogenic nematodes during quiescence. Scientific Reports, 10 (1): 5738.

Kaya, H. K. & R. Gaugler, 1993. Entomopathogenic nematodes. Annual Review of Entomology, 38 (1): 181-206.

Koppenhöfer, A. M., D. I. Shapiro-Ilan & I. Hiltpold, 2020. Entomopathogenic nematodes in sustainable food production. Frontiers in Sustainable Food Systems, 4 (125): 1-14.

Koppenhöfer, A. M., R. S. Cowles, E. A. Cowles, E. M. Fuzy & H. K. Kaya, 2003. Effect of neonicotinoid synergists on entomopathogenic nematode fitness. Entomologia Experimentalis et Applicata, 106 (1): 7-18.

Koppenhöfer, A. M., R. S. Cowles, E. A. Cowles, E. M. Fuzy & L. Baumgartner, 2002. Comparison of neonicotinoid insecticides as synergists for entomopathogenic nematodes. Biological Control, 24 (1): 90-97.

Kwizera, V. & I. A. Susurluk, 2017. Evaluation of the effects of some insecticides based on neonicotinoids on entomopathogenic nematodes, *Steinernema feltiae* and *S. carpocapsae*. Invertebrate Survival Journal, 14 (1): 375-378.

Laznik, Ž. & S. Trdan, 2014. The influence of insecticides on the viability of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae) under laboratory conditions. Pest Management Science, 70 (5): 784-789.

Laznik, Ž. & S. Trdan, 2017. The influence of herbicides on the viability of entomopathogenic nematodes (Rhabditida: Steinernematidae and Heterorhabditidae). International Journal of Pest Management, 63 (2): 105-111.

Mokrini, F., S. E. Laasli, Y. Benseddik, A. B. Joutei, A. Blenzar, H. Lakhal, M. Sbaghi, M. Imren, G. Özer, T. Paulitz, R. Lahlali & A. A. Dababat, 2020. Potential of Moroccan entomopathogenic nematodes for the control of the Mediterranean fruit fly *Ceratitis capitata* Wiedemann (Diptera: Tephritidae). Scientific Reports, 10 (1): 19204.

Negrisoli, A. S., M. S. Garcia & C. R. C. Barbosa Negrisoli, 2010. Compatibility of entomopathogenic nematodes (Nematoda: Rhabditida) with registered insecticides for *Spodoptera frugiperda* (Smith, 1797) (Lepidoptera: Noctuidae) under laboratory conditions. Crop Protection, 29 (6): 545-549.

Oliveira-Hofman, C., F. Kaplan, G. Stevens, E. Lewis, S. Wu, H. T. Alborn, A. Perret-Gentil & D. I. Shapiro-Ilan, 2019. Pheromone extracts act as boosters for entomopathogenic nematodes efficacy. Journal of Invertebrate Pathology, 164: 38-42.

Özdemir, E., E. İnak, E. Evlice & Z. Laznik, 2020. Compatibility of entomopathogenic nematodes with pesticides registered in vegetable crops under laboratory conditions. Journal of Plant Diseases & Protection, 127 (4): 529-535.

Özdemir, E., E. İnak, E. Evlice, E. Yüksel, R. A. Delialioğlu & I. A. Susurluk, 2021. Effects of insecticides and synergistic chemicals on the efficacy of the entomopathogenic nematode *Steinernema feltiae (*Rhabditida: Steinernematidae) against *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae). Crop Protection, 144: 105605.

Patel, M. N. & D. J. Wright, 1996. The influence of neuroactive pesticides on the behaviour of entomopathogenic nematodes. Journal of Helminthology, 70 (1): 53-61.

Peters, A., 2013. Application and commercialization of nematodes. Applied Microbiology and Biotechnology, 97 (14): 6181-6188.

Platt, T., N. F. Stokwe & A. P. Malan, 2020. A review of the potential use of entomopathogenic nematodes to control above-ground insect pests in South Africa. South African Journal of Enology and Viticulture, 41 (1): 1-16.

Radová, Š., 2011. Effects of selected pesticides on survival and virulence of two nematode species. Polish Journal of Environmental Studies, 20 (1): 181-185.

Rovesti, L., T. Fiorini, G. Bettini, E. W. Heinzpeter & F. Tagliente, 1990. Compatibility of *Steinernema* spp. and *Heterorhabditis* spp. with pesticides. Informatore Fitopatologico, 40 (9): 55-61.

Sabino, P. H. S., A. S. Negrisoli, V. Andaló, C. C. Filgueiras, A. Moino & F. S. Sales, 2019. Combined application of entomopathogenic nematodes and insecticides in the control of leaf-miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on tomato. Neotropical Entomology, 48 (2): 314-322.

Şahin, Y. S., A. Bouhari, T. C. Ulu, B. Sadıç & İ. A. Susurluk, 2018. New application method for entomopathogenic nematode *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae) HBH strain against *Locusta migratoria* (Linnaeus, 1758) (Orthoptera: Acrididae). Turkish Journal of Entomology, 42 (4): 305-312.

Shapiro-Ilan, D. I., F. Kaplan, C. Oliveira-Hofman, P. Schliekelman, H. T. Alborn & E. E. Lewis, 2019. Conspecific pheromone extracts enhance entomopathogenic infectivity. Journal of Nematology, 51 (1): 1-5.

Shapiro-Ilan, D. I., R. Han & C. Dolinksi, 2012. Entomopathogenic nematode production and application technology. Journal of Nematology, 44 (2): 206-217.

Susurluk, A. I., I. Ünlü & I. Kepenekci, 2003. Host finding behavior of two different Turkish isolates of entomopathogenic nematode species, *Heterorhabditis bacteriophora*, Poinar 1976 (Rhabditida: Heterorhabditidae). Turkish Journal of Biology, 27 (4): 203-207.

Susurluk, A. & R. U. Ehlers, 2008. Field persistence of the entomopathogenic nematode *Heterorhabditis bacteriophora* in different crops. BioControl, 53 (4): 627-641.

Taşkesen, Y. E., E. Yüksel & R. Canhilal, 2021. Field performance of entomopathogenic nematodes against the larvae of *Zabrus* spp. Clairville, 1806 (Coleoptera: Carabidae). Uluslararası Tarım ve Yaban Hayatı Bilimleri Dergisi, 7 (3): 429-437.

Ulu, T. C. & H. Erdoğan, 2023. Field application of encapsulated entomopathogenic nematodes using a precision planter. Biological Control, 182: 105240.

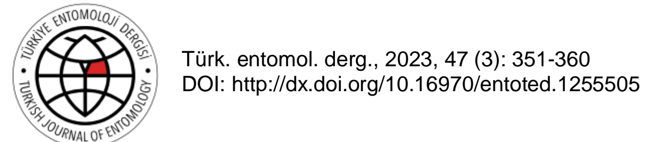
Ulu, T. C., B. Sadic & I. A. Susurluk, 2016. Effects of different pesticides on virulence and mortality of some entomopathogenic nematodes. Invertebrate Survival Journal, 13 (1): 111-115.

Ulu, T. C., H. Erdogan, K. R. Cruzado, B. Sadic & E. E. Lewis, 2022. “Conspecific and heterospecific dispersal effect of exometabolomes on some entomopathogenic nematode species, 130-130”. 61st Annual Meeting of Society of Nematologists, Anchorage, (26-29 September 2022, Alaska, USA), 143 pp.

Wright, D. J., A. Peters, S. Schroer & J. Fife, 2005. “Application Technology, 91-106”. In: Nematodes as Biocontrol Agents (Eds. P. S. Grewal, R. U. Ehlers & D. I. Shapiro-Ilan), Wallingford: CABI, 505 pp.

Yüksel, E., R. Canhilal & M. İmren, 2019. Azadirachtin ve spinosadın bazı yerel entomopatojen nematod izolatlarının canlılığı ve virülensliği üzerine etkileri. Uluslararası Tarım ve Yaban Hayatı Bilimleri Dergisi, 5 (2): 280-285 (in Turkish with abstract in English).

Zimmerman, R. J. & W. S. Cranshaw, 1990. Compatibility of three entomogenous nematodes (Rhabditida) in aqueous solutions of pesticides used in turfgrass maintenance. Journal of Economic Entomology, 83 (1): 97-100.


Türk. entomol. derg., 2023, 47 (3): 351-360
DOI: http://dx.doi.org/10.16970/entoted.1255505

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Efficacy of spore suspension and culture filtrate of *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycota: Hyphomycetes) isolates on *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) in different conditions

Beauveria bassiana (Balsamo) Vuillemin (Deuteromycota: Hyphomycetes) izolatlarının spor süspansiyonu ve kültür filtratının farklı koşullarda *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) üzerindeki etkinliği

Asiye UZUN YİĞİT[1*]

Abstract

In this study, the effectiveness of *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycota: Hyphomycetes) isolates on *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) was determined with Petri and pot experiments in laboratory and climate room conditions by applying spore suspension and culture filtrate in Isparta University of Applied Sciences of in 2022. In the Petri experiment, the spore suspensions ($1x10^6$, $1x10^7$, and $1x10^8$ spore/ml) and culture filtrates (1X, 5X, 10X) of BIM-001 and BY2 isolates of *B. bassiana* were sprayed in the form of mist for 10 seconds at a speed of 3 m/s onto leaf discs in Petri which included ten adult individuals. In the pot experiment, the same treatments were conducted with the same application method in the Petri experiment on single-leaf plants in pots. In the Petri and pot experiments, the difference between the mortality rates in the 10^8 spore/ml dose of BIM-001 and BY2 (64.00±4.52 and 42.00±2.49%, 58.00±2.91 and 41.00±2.77%, respectively) was significant on the 7th observation day. In the pure culture filtrate treatments, the mortality rates in the BIM-001 and BY2 were 73.00±2.13 - 68.00±3.59% and 60.00±3.65 - 57.00±5.17% respectively in the Petri and pot experiments. BIM-001 and BY2 culture filtrate in both Petri and pot experiments (1X) applications were not statistically significant. The BIM-001 isolate of *B. bassiana* was more effective than the BY2 isolate treatment against adult females of *T. urticae* both in vivo and in vitro. In conclusion, it is thought that the culture filtrate of *B. bassiana* BIM-001 isolate is considered to have potential for the control of two-spotted spider mites.

Keywords: Biocontrol, culture filtrate, entomopathogenic fungus, *Tetranychus urticae*

Öz

Bu çalışmada, 2022 yılında Isparta Uygulamalı Bilimler Üniversitesi'nde *Beauveria bassiana* (Balsamo) Vuillemin (Deuteromycota: Hyphomycetes) izolatlarının *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) üzerindeki etkinliği spor süspansiyonu ve kültür filtratı uygulanarak petri ve saksı denemeleri yoluyla laboratuvar ve iklim odası koşullarında belirlenmiştir. Petri denemesinde, *B. bassiana*'nın BIM-001 ve BY2 izolatlarının spor süspansiyonları ($1x10^6$, $1x10^7$ ve $1x10^8$ spor/ml) ve kültür filtratları (1X, 5X, 10X) petri içinde yaprak diskleri üzerinde bulunan ergin bireylere 3 m/s hızla 10 saniye mistleme şeklinde püskürtülmüştür. Saksı denemeleri petri denemelerindeki aynı uygulama yöntemiyle saksıdaki tek yapraklı bitkiler üzerinde gerçekleştirilmiştir. Petri ve saksı denemelerinde BIM-001 ve BY2'nin 10^8 spor/ml dozundaki ölüm oranları arasındaki fark (sırasıyla %64.00±4.52 ve %42.00±2.49, %58.00±2.91 ve %41.00±2.77) 7. gözlem gününde anlamlıydı. Saf kültür filtratı uygulamalarında BIM-001 ve BY2'deki ölüm oranları petri ve saksı denemelerinde sırasıyla %73.00±2.13 - 68.00±3.59 ve %60.00±3.65 - 57.00±5.17 olarak bulunmuştur. Hem petri hem de saksı denemelerinde BIM-001 ve BY2 kültür filtratı (1X) uygulamaları istatistiksel olarak anlamlı bulunmamıştır. *Beauveria bassiana* BIM-001 izolatı *T. urticae*'nin ergin dişilerine karşı hem in vivo hem de in vitro koşullarda BY2 izolatından daha etkili olmuştur. Sonuç olarak *B. bassiana* BIM-001 izolatının kültür filtratının iki noktalı kırmızı örümcek kontrolü için potansiyele sahip olduğu düşünülmektedir.

Anahtar sözcükler: Biyokontrol, kültür filtratı, entomopatojen fungus, *Tetranychus urticae*

---

[1] Isparta University of Applied Sciences, Faculty of Agriculture, Department of Plant Protection, 32000, Isparta, Türkiye
* Corresponding author (Sorumlu yazar) e-mail: asiyeuzun@isparta.edu.tr
Received (Alınış): 23.02.2023 Accepted (Kabul ediliş): 30.09.2023 Published Online (Çevrimiçi Yayın Tarihi): 05.10.2023

Introduction

The two-spotted spider mite (TSSM), *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) is a polyphagous and cosmopolitan agricultural pest causing economic losses worldwide (Migeon et al., 2010; Lagziri et al., 2015). It is known that it feeds on field plants, ornamental plants, annual and perennial plants (Sim et al., 2003; de Carvalho Ribeiroa et al., 2019; Assouguem et al., 2022). *Tetranychus urticae* has the ability to develop rapid pesticide resistance and is one of the most pesticide-resistant species among arthropods (Knowles, 1997; Van Leeuwen et al., 2008; Van Leeuwen et al., 2010). The rapid development and high fecundity of this species lead them to very quickly reach population levels that cause economic losses (Sato et al., 2005; El-Saiedy & Fahim, 2021). Entomopathogenic fungi can be preferred as an alternative to chemical insecticides. The microbial control agents have numerous advantages over conventional chemical insecticides. These can be listed as minimal adverse effects for humans and other non-target organisms, reduction of pesticide residues, and increased biodiversity in the ecosystem (Abd El-Ghany, 2015). Entomopathogenic fungi have an important role in the control of spider mite populations and they can be included in biological control programs as an alternative to synthetic acaricides currently in use (Maniania et al., 2008). There are thought to be about 750 fungi species that cause infections in insects or mites (Abd El-Ghany, 2015). *Beauveria* is one of the most widely recognized and encountered entomopathogenic fungi, due to its widespread distribution, and easy identification. *Beauveria*'s broad host range and wide variation in virulence against different hosts make it preferred for the biological control of pests (Rehner, 2005). Besides 707 insect species, 13 mite species have been reported among the hosts of *B. bassiana* (Balsamo) Vuillemin (Deuteromycota: Hyphomycetes) (Li, 1988; Goettel et al., 1990; Zimmermann, 2007). In order to decide the most virulent isolate, pathogenicity of different isolates against the target pest must be determined (Zimmermann, 2007). Recent studies have recorded the effectiveness of different isolates of *B. bassiana* against *T. urticae* (Chandler et al., 2005; Draganova & Simova, 2010; Örtücü & Albayrak İskender, 2017; Yucel, 2021). These studies investigated the lethal effect of different spore concentrations on *T. urticae*. Entomopathogenic fungi contain secondary metabolites that manipulate the host's immune mechanisms and cause death. This feature increases *B. bassiana*'s potential for pest control (Zibaee et al., 2011). About 33.9% of mycoinsecticide formulations were based on *B. bassiana* (Faria & Wraight, 2007). However, the killing speed needs to be increased, which is important limiting their use as mycoinsecticide (St Leger & Wang, 2010). It has been supported by different studies that culture filtrates of entomopathogenic fungi cause faster death than spore suspensions (Namara et al., 2017; Herlinda et al., 2020). As we mentioned before, *T. urticae* can reach devastating population levels very quickly due to its features. For this reason, it is thought that important to ensure the rapid death of this pest. In this context, this study aimed to determine the effects of *B. bassiana* isolates on *T. urticae* adults in culture filtrates as well as spore suspensions. In this study, the effect of culture filtrates and spore suspensions of local *B. bassiana* isolates on *T. urticae* was determined both in vivo and in vitro conditions.

Materials and Methods

Mite culture

As study materials bush bean (Atlantis, Arzuman), *Phaseolus vulgaris* L., 1753 (Fabales: Fabaceae), and the population of *T. urticae* were produced in the climate chamber (25±2ºC temperature, 65±5% humidity, and 16: 8 L: D photoperiod) in 2022. When plants have 3-4 leaves, two-spotted spider mites (red form) were transferred to these plant leaves. Then, bean plant leaves infected with mites are cut off and transferred to non-contaminated plants to ensure the continuity of the population. The *T. urticae* population was obtained from vegetable greenhouses in Antalya (Türkiye) in 2018. The identification of *T. urticae* was done according to Jeppson et al. (1975) and Bolland et al. (1998).

Fungal isolates

The other materials were *B. bassiana* BIM-001 isolated from *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) in potato areas Isparta/Center (37°50'16.77" N 30°32'17.61" E, 1017 m, 23.08.2018) and *B. bassiana* BY2 isolated from Phlaeothripidae (Thysanoptera) species in Burdur-Yeşilova wheat areas (37°30'52.3'' N 29°45'45'' E, 1191 m, 14.06.2017). These entomopathogenic fungi isolates were identified by Prof. Dr. Evrim Arıcı Şenkaynağı.

Preparation of culture filtrates of entomopathogenic fungus isolates

Two different entomopathogenic fungi isolates were produced in potato dextrose agar (PDA) for 14 days at 25°C. These isolates were incubated for two weeks. An agar disc (1 cm) from each isolate was transferred into potato dextrose water (PDB, 50 ml) and shaken at 25±1°C and 200 rpm for 10 days. Then, these prepared liquids were passed through Whatman filter paper, and culture filtrates were obtained (Kim et al., 2013).

Preparation of spore suspensions of entomopathogenic fungus isolates

Two different isolates of *B. bassiana* (BY2 and BIM-001) were cultured on potato dextrose agar (PDA) medium at 25±1°C for 15-30 days. At the end of the incubation period, 10 ml of sterile distilled water with 0.02% Tween 80 was poured into the Petri dishes, and spores were harvested with a glass spreader. The spore suspension was passed through sterile cheesecloth. Spore counts of these spore suspensions were performed under light microscope on Thoma slides and spore concentrations were prepared to 1×10^6, 1×10^7, and 1×10^8 spore/ml for each isolate (Polat et al., 2022).

Petri experiments

Leaf discs (4 cm diameter) placed upside down on a cotton layer in Petri dishes (9 cm diameter) with a few holes onto the lid for ventilation. A vaseline ring was formed around the leaf disc (Al-Azzazy et al., 2020). Ten adult females of *T. urticae* were transferred to leaf discs. Each petri dish was one replication and 10 replications were used for each spore suspension and culture filtrate. Petri dishes were transferred to an incubator (Siemens, Touch) at 25±2°C, 65±5% RH, and 16: 8 (L:D) photoperiod.

Spore suspension application

Three different spore suspensions, $1x10^6$, $1x10^7$, and $1x10^8$ of the isolates BIM-001 and BY2 of *B. bassiana* were sprayed on leaf discs with adult individuals (a replicate) (Draganova & Simova, 2010) in the form of mist for 10 seconds (0.25 ml) at a speed of 3 m/s (at 4 atm pressure) in Petri dishes. Entomopathogenic fungus spore suspensions and culture filtrate doses were applied by mounting glass material that sprays the liquid coming under pressure at the end of the motorized insect aspirator. Leaf discs were checked 24 hours, 3, 5, and 7 days after application (DAA), and the number of dead mites was recorded on all observation days.

Culture filtrate application

Three different concentrations, 1X (pure culture filtrate), 5X, and 10X of the isolates BIM-001 and BY2 of *B. bassiana* were sprayed on leaf discs with adult individuals (a replicate) (Shin et al., 2011) in the form of mist for 10 seconds (0.25 ml) at a speed of 3 m/s (at 4 atm pressure) in Petri dishes. Observations were made in the same way as in the spore suspension application.

Pot experiments

Bean plants grown in pots were left with one leaf (Doğan, 2016). Each plant was considered a replicate. A leaf disc (4 cm diameter) was created by making a vaseline ring on the leaf of each plant in the pots. In single-leaf plants, ten adult females of *T. urticae* were transferred into the vaseline ring on the leaves with the help of a brush, and then entomopathogenic fungus concentrations were applied at 4 atm pressure for

10 seconds (0.25 ml). The applications performed above in Petri dishes were also carried out on these plants for all three spore suspensions of each fungus isolate. Applications made in Petri dishes were also carried out on a single-leaf plant in vivo conditions for both spore suspension and culture filtrate application. Pot experiments were carried out at 25±1°C, 60±10% relative humidity, and 16: 8 [L: D] photoperiod.

Distilled water containing 0.02% Tween 80 was used in the control application in Petri and pot experiments. BIM-001 and BY2 isolates of *B. bassiana* were re-isolated from all dead individuals in both Petri and pot experiments (Meng et al., 2017).

Statistical analysis

The percentage of mortality was calculated using the raw data of the number of dead individuals obtained. The mortality rates calculated by counting the dead individuals in the applications on each observation day were first subjected to the homogeneity test (Levene's), and the Shapiro-Wilk's normality test was applied. The obtained mortality rates were transformed using angular transformation. Then, one-way analysis of variance (One-Way ANOVA) was performed on these data. Tukey's (HSD) multiple comparison test was applied to determine similar and different groups. Statistical analyses were performed with the SPSS® 20.0 package program. The median lethal time (LT50) values of the most effective applications were calculated using the Excel program. For each application, a regression equation was drawn in Excel, and LT50 values were calculated with the help of the regression coefficient.

Results and Discussion

Petri experiments

In the Petri experiment, there was no mortality in *T. urticae* adults 24 hours after the application of spore suspensions of *B. bassiana* BIM-001 and BY2. However, deaths were observed at the 3 DAA (day after application) and the death was significantly different from the control for doses of BIM-001 and 10^8 spore/ml dose of BY2. The highest mortality was detected in the 10^8 spore/ml dose of BIM-001 (16.00±3.40%) and determined to be significant from all other treatments ($F_{6,63}$= 6.071, $p< 0.001$). On the 5 DAA, 10^8 spore/ml dose of BIM-001 (46.00±4.00) were statistically different from each other ($F_{6,63}$= 28.924, $p< 0.001$). On the last observation (7 DAA), the difference between the mortality rate in 10^8 spore/ml of BIM-001 and the treatment 10^8 spore/ml of BY2 (64.00±4.52% and 42.00±2.49%, respectively) was statistically significant and the 10^8 spore/ml of BIM-001 was significant and different from other applications ($F_{6,63}$= 51.019, $p< 0.001$). Mortality rates in spore suspension applications were found to be significant and different from the control treatment at the 5 and 7 DAA (Table 1).

Table 1. Mortality rates caused by spore suspension treatments of BIM-001 and BY2 in the petri experiment

Treatments	Mortality rates (%)±Std. err.						
	1 DAA	3 DAA		5 DAA		7 DAA	
BIM-001-10^8	0.00	16.00±3.40	a*	46.00±4.00	a	64.00±4.52	a
BIM-001-10^7	0.00	12.00±2.49	ab	34.00±3.40	b	49.00±2.33	b
BIM-001-10^6	0.00	10.00±2.11	ab	21.00±2.33	c	32.00±1.33	cd
BY2 10^8	0.00	14.00±1.63	ab	28.00±2.49	bc	42.00±2.49	bc
BY2 10^7	0.00	9.00±2.33	abc	20.00±2.58	c	33.00±3.00	cd
BY2 10^6	0.00	6.00±1.63	bc	18.00±2.00	c	27.00±2.60	d
Control	0.00	0.00±0.00	c	0.00±0.00	d	2.00±1.33	e

* Different letters in the same column indicate significantly different mortality rates in the treatments ($P \leq 0.05$).

Twenty-four hours after application, the mortality was not observed in *T. urticae* adults in the treatments of culture filtrate. On the 3 DAA, the mortality rates were not different significantly for pure culture

filtrate treatment of BIM-001 and BY2 (30.00±2.58% and 28.00±3.89%, respectively), but these treatments were statistically different from other treatments ($F_{6,63}$= 13.849, p< 0.001). On the 5 DAA, the highest mortality rate was determined in the 1X culture filtrate treatment of BY2 (52.00±5.33%) ($F_{6,63}$= 27.913, p< 0.001). The mortality rates in the BIM-001 and BY2 1X culture filtrate (73.00±2.13% and 68.00±3.59%, respectively) treatments were found to be statistically different from the other treatments at the 7 DAA ($F_{6,63}$= 60.082, p< 0.001). In addition, mortality rates in both BIM-001 and BY2 treatments were significant and different from the control treatment on all observation days (Table 2).

Table 2. Mortality rates caused by culture filtrate treatments of BIM-001 and BY2 in the petri experiment

Treatments	Mortality rates (%)±Std. err.						
	1 DAA	3 DAA		5 DAA		7 DAA	
BIM-001-10^8	0.00	30.00±2.58	a*	49.00±2.33	ab	73.00±2.13	a
BIM-001-10^7	0.00	15.00±2.69	b	35.00±4.28	b	54.00±4.52	b
BIM-001-10^6	0.00	12.00±2.49	b	23.00±3.35	c	36.00±2.67	c
BY2 10^8	0.00	28.00±3.89	a	52.00±5.33	a	68.00±3.59	a
BY2 10^7	0.00	19.00±2.77	ab	32.00±2.91	c	40.00±3.33	c
BY2 10^6	0.00	12.00±3.27	b	21.00±2.77	c	30.00±3.33	c
Control	0.00	0.00±0.00	c	0.00±0.00	d	2.00±1.33	d

* Different letters in the same column indicate significantly different treatment mortality rates (P ≤ 0.05).

The 10^8 spore/ml dose of BIM-001 caused the highest mortality rate between the spore suspensions of BIM-001 and BY2 isolates, and the LT50 value was determined as 5.76 days (Figure 1a). In culture filtrate treatments of BIM-001 and BY2 isolates,1X culture filtrate of BIM-001 caused the highest mortality rate and the LT50 value was 5.09 days (Figure 1b).

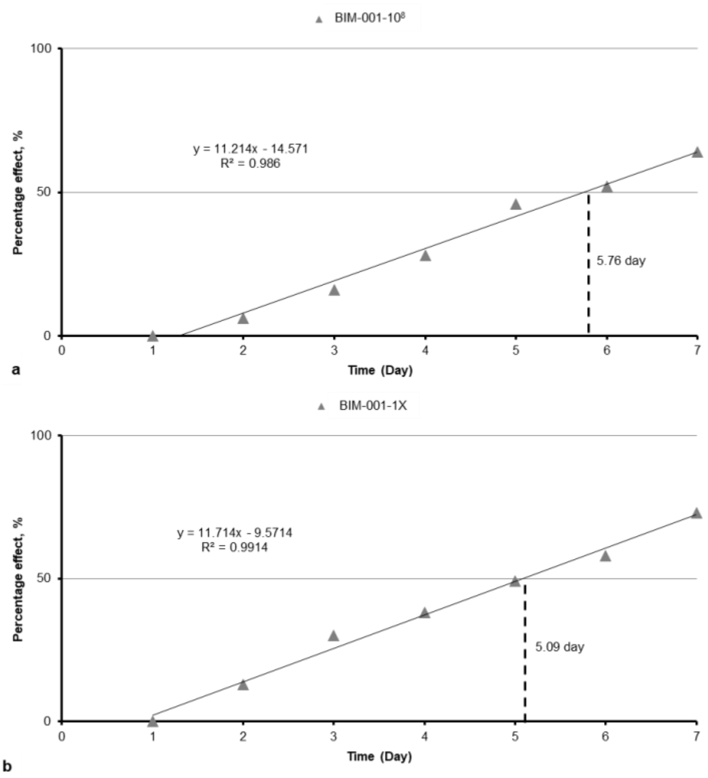


Figure 1. The median lethal time for a) 10^8 spore/ml and b) pure culture filtrate of *Beauveria bassiana* BIM-001 in the Petri experiment.

Pot experiments

In the pot experiment, the mortality in *T. urticae* adults was not detected in the treatments of different spore suspensions of BIM-001 and BY2 isolates 24 hours after application. While the highest mortality occurred in 10^8 spore/ml treatment of BY2 (9.00±1.80%) at the 3 DAA, it was not different from 10^8 spore/ml treatment of BIM-001 ($F_{6,63}$= 2.955, p= 0.013). On the 5 DAA, there was no statistical difference between the mortality rates of *T. urticae* in the10^8 spore/ml treatment of BIM-001 (32.00±2.91%) and BY2 isolates (24.00±1.63%) ($F_{6,63}$= 21.520, p< 0.001). On the 7 DAA, it was determined that the mortality rate in the 10^8 spore/ml treatment of BIM-001 (58.00±2.91%) was statistically different compared with the 10^8 spore/ml treatment of BY2 (41.00±2.77%). In addition, it was found that spore suspension treatments of both *B. bassiana* isolates were significant and different from the control treatment ($F_{6,63}$= 54.195, p< 0.001) (Table 3).

Table 3. Mortality rates caused by spore suspension treatments of BIM-001 and BY2 in the pot experiment

Treatments	Mortality rates (%)±Std. err.						
	1 DAA	3 DAA		5 DAA		7 DAA	
BIM-001-10^8	0.00	7.00±2.13	a*	32.00±2.91	a	58.00±2.91	a
BIM-001-10^7	0.00	6.00±2.21	ab	25.00±2.24	ab	42.00±3.27	b
BIM-001-10^6	0.00	4.00±1.63	ab	17.00±1.53	bc	29.00±2.33	cd
BY2 10^8	0.00	9.00±1.80	a	24.00±1.63	abc	41.00±2.77	b
BY2 10^7	0.00	8.00±2.00	a	21.00±3.14	bc	30.00±2.98	c
BY2 10^6	0.00	4.00±1.63	ab	15.00±2.24	c	19.00±1.80	d
Control	0.00	0.00±0.00	b	0.00±0.00	d	0.00±0.00	e

* Different letters in the same column indicate significantly different treatment mortality rates ($P \leq 0.05$).

In the pot experiment, the mortality was not observed 24 hours after the application of the culture filtrate treatments. On the 3 ($F_{6,63}$= 17.087, p< 0.001) and 5 DAA ($F_{6,63}$= 20.280, p< 0.001), the mortality rates in pure culture filtrate (1X) of BIM-001 were statistically significant from control and other applications. On the last observation day, it was determined that there was no significant difference between the mortality rates in the 1X culture filtrate of BIM-001 and BY2 (60.00±3.65% and 57.00±5.17%), and they were significant and different from other treatments. There was no significant difference in mortality rates between the treatments of the 10X culture filtrate (33%) and 10^8 spore/ml of BY2 (41%) ($F_{6,63}$= 29.386, p< 0.001). It was determined that mortality rates in culture filtrate treatments of BIM-001 and BY2 were significantly different from the control on the 3, 5, and 7 DAA (Table 4).

Table 4. Mortality rates caused by culture filtrate treatments of BIM-001 and BY2 in the pot experiment

Treatments	Mortality rates (%)±Std. err.						
	1 DAA	3 DAA		5 DAA		7 DAA	
BIM-001-10^8	0.00	29.00±2.33	a*	47.00±3.67	a	60.00±3.65	a
BIM-001-10^7	0.00	15.00±1.67	c	33.00±3.00	abc	41.00±4.82	b
BIM-001-10^6	0.00	10.00±2.11	cd	24.00±2.67	c	31.00±3.48	b
BY2 10^8	0.00	26.00±3.06	ab	39.00±3.14	ab	57.00±5.17	a
BY2 10^7	0.00	17.00±3.00	bc	31.00±4.82	bc	45.00±3.42	ab
BY2 10^6	0.00	12.00±2.91	c	22.00±3.89	c	33.00±3.00	b
Control	0.00	0.00±0.00	d	0.00±0.00	d	0.00±0.00	c

* Different letters in the same column indicate significantly different treatment mortality rates ($p \leq 0.05$).

The 10^8 spore/ml treatment of BIM-001 caused the highest mortality rate among the different spore suspensions of BIM-001 and BY2 isolates and the LT50 value was found as 6.70 days (Figure 2a). The pure culture filtrate (1X) application caused the highest mortality rate among culture filtrate treatments of BIM-001, and the LT50 value was determined as 5.68 days (Figure 2b).

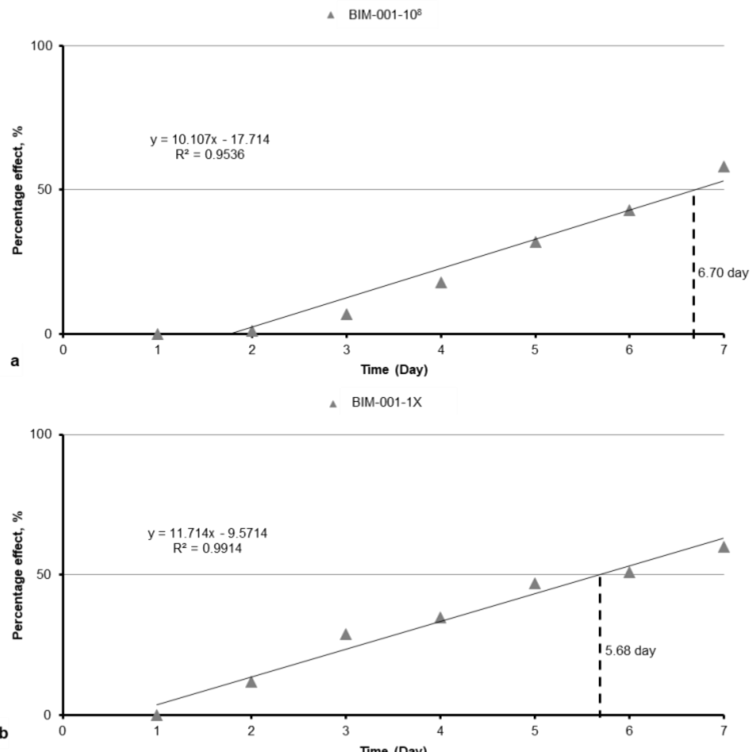


Figure 2. The median lethal time for a) pure culture filtrate and b) 10^8 spore/ml of *Beauveria bassiana* BIM-001 in the pot experiment.

The mode of action of entomopathogenic fungus is known to infect the insect body after the fungal spores are released. The fungal spores initially spread outside the host body and penetrate the host. Then, death occurs in the host in 4-7 days depending on the number of spores (Sharma & Sharma, 2021). Draganova and Simova (2010) investigated the effects of five isolates of *B. bassiana* on *T. urticae* by spraying conidial suspensions at 10^6, 10^7, and 10^8 conidia/ml concentrations. Isolates of *B. bassiana* caused mortality in the range of 80-100% at 5 days after the application. In a different study, Örtücü & İskender Albayrak (2017) determined the efficacy of *B. bassiana* isolates on *T. urticae* at three different doses ($1x10^6$, $1x10^7$ and $1x10^8$ conidi ml^{-1}) on *T. urticae*. PaF04 isolate was more effective than PaF09 and PaF76 isolates. For this isolate, 80.7±4.2%, 92±3.5%, and 100% mortality occurred in mites until the 5th day of $1x10^6$, $1x10^7$, and $1x10^8$ conidia ml^{-1} concentrations, respectively. In this study, BIM-001 and BY2 isolates of *B. bassiana* reached 18-46% mortality at 10^6, 10^7, and 10^8 spore/ml on the 5 DAA, while it remained in the range of 27-64% on the 7 DAA. Geroh et al. (2014) found that *B. bassiana* (strain ITCC-4668) isolate caused 63.31% and 59.49% mortality at $0.3×10^8$ and $0.3x10^7$ conidia ml^{-1} on the 7 DAA on *T. urticae*. Doğan (2016) determined that 80.3±3.8% mortality occurred 7 days after the application of $1x10^7$ conidiospore/ml dose of *B. bassiana* isolate to *T. urticae* adults under Petri conditions. Doğan (2016) found that the mortality rate was 45.8±1.4% on 7 days after the applied $1x10^7$ conidiospore/ml dose of *B. bassiana* isolate to *T. urticae* adults under pot conditions. Yeşilayer (2018) determined that mortality rates occurring 3, 5, and 7 days after $1x10^8$ conidi/ml dose of *B. bassiana* were applied 10.00±0.00%, 16.66±2.10% and 16.66±2.10%, respectively. Ali et al. (2020) reported that the mortality rates were 39.2% and 78.65% on

the *T. urticae* 7 days after *B. bassiana* B1 and B2 isolates ($1×10^8$ spore/ml) were applied. Yucel (2021) investigated the lethal effects of *B. bassiana* BGF14 and BCA32 isolates ($1×10^6$, $1×10^7$, and $1×10^8$ spore/ml) on *T. urticae*. On the 7 DAA, at 10^6, 10^7, and 10^8 spore/ml doses of BGF14 isolate occurred 47.10±2.94%, 44.95±3.62% and 76.98±3.32% mortality, and these mortality rates were 63.06±1.95%, 81.09±4.10%, and 87.27±2.76% for BCA32 isolate. In the current study, the mortality rates were observed at 49.00±2.33-64.00±4.52% and 33.00±3.00-42.00±2.49% at 10^7 and 10^8 spore/ml doses of BIM-001 and BY2 isolates on the 7 DAA in Petri treatments, these mortality rates were 42.00±3.27 -58.00±2.91% and 30.00±2.98-41.00±2.77% in pot treatments.

In culture filtrate applications, since the spores are removed, the spores do not come into contact with the host, and toxin production steps do not occur to kill the host. Therefore, direct host death and disease initiation occur more rapidly when culture filtrate is applied (Gustianingtyas et al., 2020; Herlinda et al., 2020). The enzymes such as chitinases, lipases, and proteases containing culture filtrates facilitate the infection process by disrupting the cuticle of the host (Hanan et al., 2020). Namara et al. (2017) reported that the spore-free culture filtrate renders the host more susceptible to a pathogen. Entomopathogenic fungi include secondary metabolites with antimicrobial, insecticidal, and cytotoxic activities (Gibson et al., 2014). Zibaee et al. (2011) reported that the secondary metabolites in the culture filtrate were toxic. *Beauveria bassiana* produces beauvericin, bassianolides, bassianin, and bascianolone as secondary metabolites (Kanaoka et al., 1978; Grove & Pople, 1980; Quesada-Moraga & Vey, 2004; Wang & Xu, 2012). The maximum efficacy on the pest is provided by the production of metabolites in the culture filtrate (Hanan et al., 2020). It is necessary to determine the effects of entomopathogenic fungi against *T. urticae* in order to reveal this activity. Yun et al. (2017) compared the effects of culture filtrates, air conidia, and blastospores of *B. bassiana* 2R-3-3-1 against *T. urticae*. On the 7 DAA, treatment with entomopathogenic fungus culture filtrate alone caused 83.3±2.7% mortality, while combined applications with air conidia (94.3±3.1%) or blastospores (98±2%) increased these mortality rates. However, the LT50 values of the treatments were determined as 3.96, 2.49, and 3.35 days for combined applications with entomopathogenic fungus culture filtrate alone, air conidia, or blastospores, respectively, and were not significantly different. In the present study, pure culture filtrates of BIM-001 and BY2 isolates caused 73.00±2.13% and 68.00±3.59% mortality in Petri dishes trials, while the mortality rates were detected as 60.00±3.65% and 57.00±5.17% in pot trials. The LT50 values in 10^8 spore/ml and 1X culture filtrate of BIM-001 were 5.76 and 5.09 days in Petri dishes, while it was 6.70 and 5.68 days in pot trials. As a result of this study, the BIM-001 isolate of *B. bassiana* was found the most effective treatment against *T. urticae* adult females both in vivo and in vitro conditions. These results suggest that the culture filtrate of *B. bassiana* BIM-001 isolate could have the potential for the control of two-spotted spider mites.

Zibaee et al. (2009) reported that secondary metabolites are more easily applicable in the field. The culture filtrate, which contains toxic secondary metabolites, has an important potential as a "mycoinsecticide active ingredient". The culture filtrate of entomopathogenic fungus has the advantages comparatively more stable than the culture containing conidia, longer storage time, and also there is no problem of nozzle clogging when the culture filtrate is sprayed (Soesanto et al., 2019). The results from this study support that culture filtrate applications of *B. bassiana* isolates are effective on *T. urticae* in both Petri dishes and pot experiments. In addition, it is thought to be important to evaluate the potential of achieving efficacy on also the different life stages of *T. urticae* in future studies.

Acknowledgments

The author thanks Prof. Dr. Ali Kemal Birgücü (Isparta University of Applied Sciences, Turkey) for assisting with the statistical analysis. Special thanks to Prof. Dr. Ozan Demirözer (Isparta University of Applied Sciences, Turkey) for all his contributions. The author is grateful to Prof. Dr. Evrim Arıcı Şenkaynağı (Isparta University of Applied Sciences, Turkey) for the identification of fungal isolates.

References

Al-Azzazy, M. M., A. S. Alsohim & C. E. Yoder, 2020. Biological effects of three bacterial species on *Tetranychus urticae* (Acari: Tetranychidae) infesting eggplant under laboratory and greenhouse conditions. Acarologia, 60 (3): 587-594.

Ali, S. S., E. N. S. Haron, M. A. Ahmed, A. A. Abas & M. E. A. Elshaier, 2020. Isolation of entomopathogenic fungi and efficacy as a biological control agent on red spider mite *Tetranychus urticae* (Acari: Tetranychidae). Egyptian Journal of Plant Protection Research Institute, 3 (2): 761-770.

Assouguem, A., M. Kara, H. Mechchate, Y. B. Korkmaz, S. Benmessaoud, A. Ramzi, K. R. Abdullah, O. M. Noman, A. Farah & A. Lazraq, 2022. Current Situation of *Tetranychus urticae* (Acari: Tetranychidae) in Northern Africa: The Sustainable Control Methods and Priorities for Future Research. Sustainability, 14 (4): 2395-2409.

Bolland, H. R., J. C. H. Gutierrez & W. Flechtmann, 1998. World Catalogue of the Spider Mite Family (Acari: Tetranychidae). BRILL, 392 pp.

Chandler, D., G. Davidson & R. J. Jacobson, 2005. Laboratory and glasshouse evaluation of entomopathogenic fungi against the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae), on tomato, *Lycopersicon esculentum*. Biocontrol Science and Technology, 15 (1): 37-54.

de Carvalho Ribeiro, N., C. A. G. da Camara, J. P. R. de Melo & M. M. de Moraes, 2019. Effect of the essential oil from the latex of the fruit *Mangifera indica* L. on *Tetranychus urticae* Koch (Acari, Tetranychidae). Acarologia, 59 (3): 335-347.

Doğan, Y. Ö., 2016. Entomopatojen Fungusların *Tetranychus urticae* (Acari: Tetranychidae)'ye Karşı Etkinliklerinin Belirlenmesi. Adnan Menderes Üniversitesi Fen Bilimleri Enstitüsü, (Unpublished) Yüksek Lisans Tezi, Aydın, 39 s (in Turkish with abstract in English).

Draganova, S. A. & S. A. Simova, 2010. Susceptibility of *Tetranychus urticae* Koch. (Acari: Tetranychidae) to Isolates of Entomopathogenic Fungus *Beauveria bassiana*. Phytomedicine, 25 (1): 51-57.

El-Saiedy, E. S. M. & S. F. Fahim, 2021. Evaluation of two predatory mites and acaricide to suppress *Tetranychus urticae* (Acari: Tetranychidae) on strawberry. Bulletin of the National Research Centre, 45 (1): 1-9.

Faria, M. R. & S. P. Wraight, 2007. Mycoinsecticides and Mycoacaricides: a comprehensive list with worldwide coverage and international classification of formulation types. Biological Control, 43 (3): 237-256.

Gustianingtyas, M., S. Herlinda, Suwandi, Suparman, H. Hamidson, Hasbi, A. Setiawan, M. Verawaty, Elfita & Arsi, 2020. Toxicity of entomopathogenic fungal culture filtrate of lowland and highland soil of South Sumatra (Indonesia) against *Spodoptera litura* larvae. Biodiversitas, 21 (5): 1839-1849.

Hanan, A., T. Nazir, A. Basit, S. Ahmad & D. Qiu, 2020. Potential of *Lecanicillium lecanii* (Zimm.) as a microbial control agent for green peach aphid, *Myzus persicae* (Sulzer) (Hemiptera: Aphididae). Pakistan Journal of Zoology, 52 (1): 131-137.

Herlinda, S., R. A. Efendi, R. Suharjo, H. A. Setiawan & E. M. Verawaty, 2020. New emerging entomopathogenic fungi isolated from soil in South Sumatra (Indonesia) and their filtrate and conidial insecticidal activity against *Spodoptera litura*. Biodiversitas, 21 (11): 5102-5113.

Jeppson, L. R., H. H. Keifer & E. W. Baker, 1975. Mites Injurious to Economic plants. University of California Press, Berkeley, 614 pp.

Kim, J. J., G. Jeong, J. H. Han & S. Lee, 2013. Biological control of aphid using fungal culture and culture filtrates of *Beauveria bassiana*. Mycobiology, 41 (4): 221-224.

Lagziri, M., M. Benicha, R. M'rabet & A. Amrani, 2015. El influence de l'usage préventif des pesticides sur les acariens *Tetranychus urticae* et *Phytoseiulus persimilis* (Acari: Tetranychidae, Phytoseiidae) présents en cultures de fraisiers du nord du maroc. Biotechnologie, Agronomie, Société et Environnement, 19 (4): 355-363 (in Freench with abstract in English).

Maniania, N. K., D. M. Bugeme, V. W. Wekesa, Jr. I. Delalibera & M. Knapp, 2008. Role of entomopathogenic fungi in the control of *Tetranychus evansi* and *Tetranychus urticae* (Acari: Tetranychidae), pests of horticultural crops. Experimental and Applied Acarology, 46 (1-4): 259-74.

Meng, X., J. Hu & G. Ouyang, 2017. The isolation and identification of pathogenic fungi from *Tessaratoma papillosa* Drury (Hemiptera: Tessaratomidae). Peerj, 5: 1-14.

Migeon, A., E. Nouguier & F. Dorkeld, 2010. "Spider Mites Web: A Comprehensive Database for the Tetranychidae, 557-560". In: Trends in Acarology, (Eds. M. W. Sabelis & J. Bruin). Dordrecht: Springer, 566 pp.

Namara, L. Mc., J. C. Carolan, C. T. Griffin, D. Fitzpatrick & K. Kavanagh, 2017. The effect of entomopathogenic fungal culture filtrate on the immune response of the greater wax moth, *Galleria mellonella*. Journal of Insect Physiology, 100: 82-92.

Örtücü, S. & N. Albayrak İskender, 2017. Determination of control potentials and enzyme activities of *Beauveria bassiana* (Bals.) Vull. isolates against *Tetranychus urticae* Koch (Acari: Tetranychidae). Trakya University Journal of Natural Sciences, 18 (1): 33-38.

Polat, İ., Y. Yanar & D. Yanar, 2022. Efficacy of local entomopathogenic fungi isolated from forestlands in Tokat Province (Türkiye) against the Colorado potato beetle, *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae). Türkiye Entomoloji Dergisi, 46 (2): 159-173.

Rehner, S. A., 2005. "Insect-Fungal Associations: Ecology and Evolution, 3-27". In: Phylogenetics of the Insect Pathogenic Genus *Beauveria* (Eds. F. E. Vega & M. Blackwell). Oxford University Press, New York, 336 pp.

Sato, M. E., M. Z. Da Silva, A. Raga & M. F. De Souza Filho, 2005. Abamectin Resistance in *Tetranychus urticae* Koch (Acari: Tetranychidae): Selection, Cross-Resistance and Stability of Resistance. Neotropical Entomology, 34 (6): 991-998.

Sharma, R. & P. Sharma, 2021. Fungal entomopathogens: a systematic review. Egyptian Journal of Biological Pest Control, 31 (57): 1-13.

Shin, T. Y., W. W. Lee, S. H. Ko, Z. Ji, D. H. Shin, K. H. Son, H. Y. Park & S. D. Woo, 2011. Preliminary evaluation of *Paecilomyces lilacinus* HY-4 to control *Tetranychus urticae*. Journal of Industrial Entomology, 22 (1): 25-28.

Sim, C., E. Seo & K. Cho, 2003. Life table and sensitivity analysis as fitness evaluation method of fenpyroximate and pyridaben resistant two spotted spider mite (*Tetranychus urticae* Koch). Journal of Asia-Pacific Entomology, 6 (2): 193-199.

Soesanto, L., E. Mugiastuti & A. Manan, 2019. Raw secondary metabolites application of two *Trichoderma harzianum* isolates towards vascular streak dieback on cocoa seedlings. Pelita Perkebunan, 35 (1): 22-32.

St Leger, R. J. & C. Wang, 2010. Genetic engineering of fungal biocontrol agents to achieve efficacy against insect pests. Applied Microbiology and Biotechnology, 85 (4): 901-907.

Van Leeuwen, T., B. Vanholme, S. Van Pottelberge, P. Van Nieuwenhuyse, R. Nauen, L. Tirry & I. Denholm, 2008. Mitochondrial heteroplasmy and the evolution of insecticide resistance: non-Mendelian inheritance in action. PNAS, 105 (16): 5980-5985.

Van Leeuwen, T., J. Vontas, A. Tsagkarakou, W. Dermauw & L. Tirry, 2010. Acaricide resistance mechanisms in the two-spotted spider mite *Tetranychus urticae* and other important Acari: a review. Biochemistry and Molecular Biology, 40 (8): 563-572.

Yeşilayer, A., 2018. Efficiency of two different entomopathogen fungi *Beauveria bassiana* and *Purpureocillium llacinum* Tr1 against *Tetranychus urticae*. Applied Ecology and Environmental Research, 16 (5): 6077-6086.

Yucel, C., 2021. Effects of local isolates of *Beauveria bassiana* (Balsamo) Vuillemin on the two spotted spider mite, *Tetranychus urticae* (Koch) (Acari: Tetranychidae). Egyptian Journal of Biological Pest Control, 31 (63): 1-7.

Yun, H. G., D. J. Kim, J. H. Lee, J. I. Ma, W. S. Gwak & S. D. Woo, 2017. Comparative evaluation of conidia, blastospores and culture filtrates from entomopathogenic fungi against *Tetranychus urticae*. International Journal of Industrial Entomology, 35 (1): 58-62.

Zibaee, A., A. R. Bandani & M. Tork, 2009. Effect of the entomopathogenic fungus, *Beauveria bassiana*, and its secondary metabolite on detoxifying enzyme activities and acetylcholinesterase (AChE) of the Sunn pest, *Eurygaster integriceps* (Heteroptera: Scutellaridae). Biocontrol Science and Technology, 19 (5): 485-498.

Zibaee, A., A. R. Bandani, R. Talaei-Hassanlouei & D. Malagoli, 2011. Cellular immune reactions of the sunn pest, *Eurygaster integriceps*, to the entomopathogenic fungus, *Beauveria bassiana* and its secondary metabolites. Journal of Insect Science, 11 (1): 1-16.

Zimmermann, G., 2007. Review on safety of the entomopathogenic fungi *Beauveria bassiana* and *Beauveria brongniartii*. Biocontrol Science and Technology, 17 (6): 553-596.

Türkiye Entomoloji Dergisi Yayın İlkeleri

Derginin yayın ilkeleri aşağıda özet olarak sunulmuştur. Ayrıntılar için web adresine (www.entomoloji.org.tr) bakınız.

1. Dergi, entomoloji ve tarımsal zooloji bilim dallarıyla ilişkili konulara açıktır.
2. Dergide Türkçe veya İngilizce yazılmış orijinal araştırmalar yayımlanır.
3. Yayımlanması istenilen eserlerin kısmen veya tamamen herhangi bir yerde yayınlanmamış veya yayımlanmayacak olması zorunludur.
4. Daha önce Kongre/Sempozyum vs. de sözlü/poster bildiri olarak sunulmuş ancak sadece kısa özet olarak basılmış eserler, dipnotta belirtilmesi koşuşuyla kabul edilir.
5. Lisansüstü tezleri veya TÜBİTAK, DPT, BAP gibi çeşitli kurumlarca desteklenen proje bulgularından kısımlar içeren eserler ilgililerinden gerekli izinler alındıktan sonra hazırlanmalı, ilgi durum dipnotta mutlaka belirtilmelidir.
6. Türkiye veya herhangi bir bölge için, başta karantina listesinde bulunan türler olmak üzere, yeni tür kayıtlarını içeren eserler gönderilmeden önce mutlaka ilgili kurumlara bilgi verilmiş olmalıdır.
7. Dergide yayımlanması istenilen eserler, web sayfasında sunulan "eser başvurusu" bölümünde açıklandığı gibi hazırlanarak, üst yazı, imzalı telif hakları formu ve başvuru ücreti dekontu ile dergi e-posta adresine gönderilmelidir.
8. Yayımlanması istenilen eserler web sayfasında sunulan "örnek makale taslağı" kullanılarak, gereksiz tekrar, şekil ve cetvellerden kaçınılarak, özden uzaklaşmayacak şekilde hazırlanmalı ve 16 sayfadan fazla olmamalıdır.
9. Yayın ilkelerine uygun olmayan eserler istenilen şekle göre yeniden düzenlenmek üzere yazara geri gönderilir. Detaylar için web sayfasında sunulan "eser değerlendirme süreci" ne bakınız.
10. Bir eser yayıma kabul edildiğinde, telif hakları formu tüm yazarlar tarafından imzalanıp dergimize gönderilmeden yayımlanmaz. Sorumlu yazara eserin pdf formatında hazırlanmış hali e-posta ile gönderilir, ayrıca telif ücreti ödenmez. Yayımlanan eserlere ait şekil dışı sorumluluklar yazarlarına aittir.

Türkiye Entomoloji Dergisi

Türkiye Entomoloji Dergisi, Türkiye Entomoloji Derneği tarafından yılda dört kez yayınlanır. Dergide, entomoloji ve tarımsal zooloji bilim dallarıyla ilişkili konularda, Türkçe veya İngilizce yazılmış orijinal araştırmalaryayımlanır.

Makale Özetleri, Biological Abstracts, BIOSIS Previews, CABAbstracts, FAOAGRIS, Elsevier Scopus, Global Health, Information Reference Library, Review of Agricultural Entomology, SCI-E, TÜBİTAK/ULAKBİM, VINİTİ, Zoological Record tarafından taranmaktadır.

Yıllık abone bedeli: 150 TL Tek sayı bedeli: 50 TL

Yazışma adresi:

Türkiye Entomoloji Dergisi
Ege Üniversitesi Kampüsü PTT Şubesi, PK. 10, 35100 Bornova, İzmir
e-posta: dergi@entomoloji.org.tr
web : http://www.entomoloji.org.tr

Bu dergide yayımlanan eserlerin tüm hakları Türkiye Entomoloji Derneği'ne aittir. Yayımlanan eserlerin herhangi bir şekilde kısmen veya tamamen çoğaltılması için izin alınması zorunludur.