ISSN 1010-6960
E-ISSN 2536-491X

Türkiye Entomoloji Dergisi

Turkish Journal of Entomology

Cilt (Vol.): 44 Sayı (No.): 3 2020

Türkiye Entomoloji Dergisi
(Turkish Journal of Entomology)

Cilt (Vol.) **44** **Sayı** (No.) **3** **Eylül** (September) **2020**

Sahibi (Owner)
Ferit TURANLI

Sorumlu Müdür (Editor in Chief)
Galip KAŞKAVALCI

Dil Editörü (Linguistic Editor)
Ian Timothy RILEY

Turkish Journal of Entomology

The Turkish Journal of Entomology is a quarterly journal which has been published by the Entomological Society of Turkey. It accepts original research articles in the fields of entomology and agricultural zoology in Turkish or English.
Abstracted/Indexed in Biological Abstracts, BIOSIS Previews, CAB Abstracts, 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 a single 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

Basım Yeri: META Basım 0.232 343 64 54 Bornova - İzmir

Türkiye Entomoloji Dergisi
(Turkish Journal of Entomology)

Cilt (Vol.) **44** **Sayı** (No.) **3** **Eylül** (September) **2020**

İçindekiler (Contents)

Orijinal araştırmalar (Original articles)

Türk. entomol. derg., 2020, 44 (3): 285-294
DOI: http://dx.doi.org/10.16970/entoted.651503

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Detection and monitoring of *Thrips meridionalis* (Priesner, 1926) (Thysanoptera: Thripidae) with the colored sticky traps[1]

Thrips meridionalis (Priesner, 1926) (Thysanoptera: Thripidae)'in renkli tuzaklar ile tespiti ve izlenmesi

Gökhan AYDIN[2*] **Martin ŠLACHTA[3,4]** **İsmail KARACA[5]**

Abstract

This study was conducted to determine the capture rates of *Thrips meridionalis* (Priesner, 1926) (Thysanoptera: Thripidae) adults on different colored traps between 2017 and 2019 in Isparta Province of Turkey. Three locations were selected for investigation: (1) various orchards with apple trees, (2) various orchards with cherry trees, and (3) various orchards with walnut. The active period of the insect was determined to be between 15 March and 1 April over the three years. Ghost-white traps captured the most *T. meridionalis* and the differences between this color trap and other colored traps were found significantly differed according to a Tukey's HSD test in all orchards and in all years ($P<0.001$). It is suggested that an important technique in integrated pest management for monitoring and controlling of *T. meridionalis* adults could be to use ghost-white sticky traps.

Keywords: Apple, cherry, colored traps, peach thrips, walnut

Öz

Bu çalışma *Thrips meridionalis* (Priesner, 1926) (Thysanoptera: Thripidae) erginlerinin farklı renk tuzakları ile yakalanma oranlarının belirlenmesi amacı ile 2017-2019 tarihleri arasında Isparta ilinde yapılmıştır. Denemeler 3 farklı lokasyonda yapılmıştır: (1) elma ağırlıklı meyve bahçesi, (2) kiraz ağırlıklı meyve bahçesi ve (3) ceviz ağırlıklı meyve bahçesi. Zararlının aktif uçuş zamanının üç yıllık çalışma verilerine dayanarak 15 Mart-01 Nisan tarihleri aralığında olduğu tespit edilmiştir. Çalışma sonunda en fazla *T. meridionalis* bireyi cezbeden renk, kirli beyaz olarak belirlenmiş ve Tukey HSD testi sonuçları sözü edilen rengin çekicilik oranının diğer tüm renklerin çekicilik oranına göre tüm meyve bahçelerinde ve tüm deneme boyunca farklı olduğunu göstermiştir ($P<0.001$). *Thrips meridionalis* erginlerinin izlenmesi ve kontrolü için kirli beyaz renk tuzaklarının entegre mücadelede kullanılabilirliğinin etkili bir yöntem olabileceği düşünülmektedir.

Anahtar sözcükler: Elma, kiraz, renkli tuzaklar, şeftali thripsi, ceviz

---

[1] This study is presented orally and published as summary at the International Conferences on Science and Technology (26-30 August, 2019) in Kosovo. Part of this work was supported by Global Change Research Institute of the Czech Academy of Sciences.
[2] Isparta University of Applied Sciences, Atabey Vocational High School, Department of Plant and Animal Breeding, 32670 Atabey, Isparta, Turkey
[3] University of South Bohemia, Faculty of Economics, Studentská 13, 37005 České Budějovice, Czech Republic
[4] Global Change Research Institute of the Czech Academy of Sciences, 37005 České Budějovice, Czech Republic
[5] Isparta University of Applied Sciences, Faculty of Agriculture, Department of Plant Protection, 32260, Isparta, Turkey
* Corresponding author (Sorumlu yazar) e-mail: gokhanaydin@isparta.edu.tr
Received (Alınış): 27.11.2019 Accepted (Kabul ediliş): 18.03.2020 Published Online (Çevrimiçi Yayın Tarihi): 12.05.2020

Introduction

Thrips (Thysanoptera), minute and slender insect, are widespread throughout the world in many temperate zones, with even a few species living in arctic regions. Their minute size makes them difficult to detect. Thrips have fringed wings and are blown by the wind which assists their natural ability to disperse widely (Grasselly et al., 1995). About 5,000 species have been described and only a few hundred are crop pests, however, these can cause serious direct damage or transmit pathogens to growing crops and harvestable produce (Grasselly et al., 1995). Therefore, thrips are a major economic threat to many plant industries (Sathe et al., 2015). Although colored sticky traps can accidentally capture of beneficial insects, controlling and monitoring of thrips has been commonly done with colored sticky traps (Martin et al., 2011; Atakan & Pehlivan, 2015).

Thrips meridionalis (Priesner, 1926) (Thysanoptera: Thripidae), peach thrips, is a species widely distributed in the warmer parts of Europe (Alford, 2007). Adult females and males look similar each other but males have a paler color. Their body color is brownish-black. Antenna of adults have eight segments, including two segmented styli (Alford, 2007). Peach thrips can be found in the flowers of almond, apple, apricot, cherry, grapevine, nectarine, pear, peach, plum and other species in the Rosaceae (Alford, 2007; Hazır et al., 2011; Uzun et al., 2015; Kaplan et al., 2016).

In southern Europe, *T. meridionalis* has three overlapping generations per year. Adults overwinter under dead leaves. They become active with the rise of temperature depending on the region, the earliest in mid-February through to late-May. They initially feed on early-flowering hosts, such as an almond. When the other host plants begin to flower, peach thrips then migrate to these hosts (Alford, 2007; Uzun et al., 2015; Kaplan et al., 2016).

During the ovulation period, eggs are deposited in the flowers over several weeks. Adults emerge over about one month. Females of the second generation deposit their eggs in leaves or fruit. The third-generation mates during autumn and fertilized females overwinter (Lacasa et al., 1991; Alford, 2007).

Thrips infestation of flowers can reduce fruit set owing to damage to the stamens and may cause development of necrotic patches on fruitlets due to damage to the ovaries. Sometimes very young fruitlets can be damaged and develop necrosis. These damaged areas enlarge while the fruitlets are growing. The damaged areas can split, allowing resin to exude from the cracks. Damaged fruits may wither and die without reaching maturity (Grasselly et al., 1995; Alford, 2007).

The capturing efficiency of colored sticky traps has been tested for many thrips species, especially *Frankliniella occidentalis* (Pergande, 1895) (Thysanoptera: Thripidae), and their predators, and *Orius niger* (Wolff, 1811) (Hemiptera: Anthocoridae). These traps are also used to determine the spatial distribution of both the pests and their natural enemies (Atakan & Canhilal, 2004; Atakan, 2010; Atakan & Bayram, 2011; Elekcioglu, 2013). However, such studies have not been conducted for *T. meridionalis* in Turkey.

Although *T. meridionalis* has been detected in low population densities in some areas of Turkey (Hazır et al., 2011), its population density is high in Atabey District, Isparta, due to presence of suitable hosts and climate. Therefore, the possibility of using colored sticky traps for *T. meridionalis* was investigated in this study.

Materials and Methods

Selection of study area

The study was conducted in three years, 2017, 2018 and 2019. Colored sticky traps were set up between 1 March and 15 April in each year. The active period of adults was determined to be 15 March to 27 March in 2017, 19-30 March in 2018 and 23 March to 1 April in 2019. The three locations were selected

for investigation: (1) various orchards with apple trees (37°56’33.71" N, 30°37’31.96" E), (2) various orchards with cherry trees (37°56'24.06"N, 30°37’29.97"E), and (3) various orchards with walnut (37°56’36.51" N, 30°37’32.44" E) in Atabey District, Isparta Province.

Sampling method

Eleven different colors (RGB hexadecimal color codes given in parentheses) were tested for capture of *T. meridionalis*: ghost-white (F8F8FF), floral-white (FFFAF0), silver (C0C0C0), dodger-blue (1E90FF), deep-sky-blue (00BFFF), sky-blue (87CEEB), dark-red (8B0000), dark-olive-green (556B2F), coral (FF7F50), hot-pink (FF69B4) and yellow (FFFF00). Colored paper (21 x 30 cm), glued to a 0.6 mm thick cardboard backing and attached vertically to 2-m high posts in the three locations.

Three replicate blocks, each with a single trap of each color, were established in each orchard, giving 33 traps in each location. The colors were distributed randomly within each block. Traps were placed 1 m apart and blocks 50 m apart. Based on the findings of previous studies, the traps were placed 2 m above the soil surface in sunny places. The traps were checked each day during the study. Some of the traps were replaced with new ones at different times of the day when they were found with a high density of *T. meridionalis*. At the end of each year, all traps were collected and captured specimens counted under a binocular microscope in a laboratory. Selected specimens were placed in 70% ethanol for later identification.

Thrips identification

Adult females of *T. meridionalis*: body brownish black; 8-segmented antenna, third antenna segment light yellowish. Adult males: similar to female but paler in color. Adult females of *Thrips tabaci* Lindeman, 1889 (Thysanoptera: Thripidae): pale yellow to dark brown; antenna 7-segmented, segment I light yellow, yellowish gray; II darker grayish brown; bases of II-IV and V usually pale with apices brown. About 1.3% of the individuals were determined as *T. tabaci*.

Climatic Data

Climatic data devices (HOBO Data Logger, Onset Computer Corporation4, Bourne, MA, USA) were set up for measurement of ambient temperature at each of the three locations. The average climatic data across the three locations are shown in Figure 1.

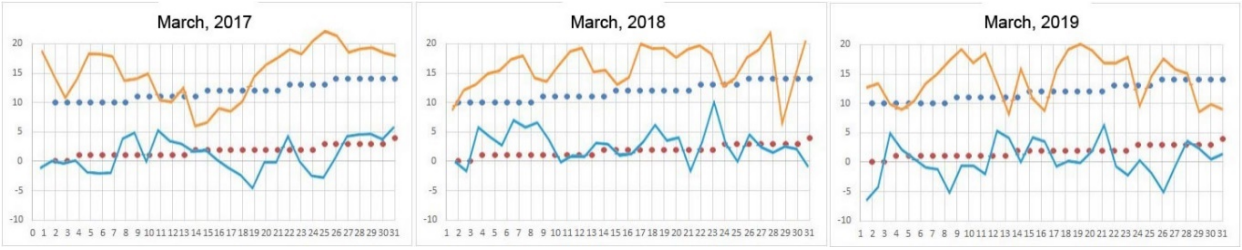

Figure 1. Average daily minimum (lower solid line) and maximum (upper solid lines) temperatures recorded in the three experimental locations. The dotted lines indicate the daily average of temperatures. X-axis: days; y-axis: temperature values.

Data analysis

Repeated measures analysis of variance (ANOVA) (SPSS version 10.1, IBM, Armonk, NY, USA) was performed to determine the effect of three factors (color, location and year) and their interactions on capture rates of *T. meridionalis.* The number of *T. meridionalis* capture with the different colors was the dependent variable. Data for each of the three years were treated as repeated measure and repeated measurements applied to the factor’s levels of the year and color. Tukey’s post hoc tests (multiple comparisons) were performed for evaluate the differences between the level averages of the factors ($P<0.05$). An ANOVA

(Tukey's honest significant difference test; HSD) was calculated to examine the differences of number of adults captured on the different colored sticky traps ($P<0.05$).

No *T. meridionalis* were captured on some colors (coral, dark-olive-green, dark-red, hot-pink and silver) during the study, so these colors were dropped from the analysis.

The numbers captured on the different colors were also examined by cluster analysis. Similarity analyses were calculated with the Multi-Variate Statistical Package (MVSP) 3.11c (Kovach, 1999). Similarity coefficients were compared by percent similarity. The average linkage between two groups is considered as the average distance between all pairs of cases and one number from each group. The percent similarity equation was:

$$PS_{A,B} = 100 \sum_{i=1}^{n} \min(P_{iA}, P_{iB})$$

where $P_{A,B}$ is the percent similarity of color trap A and color trap B, P_{iA} is the proportion of individuals of color trap A in the i^{th} (relating to total number of individuals of color trap A), P_{iB} is the proportion of individuals of color trap B in the i^{th}, and n is the total number of color trap.

Dendrograms were produced according to the unweighted pair-group mean arithmetic method using MVSP software.

Results

Thrips meridionalis was only captured on deep-sky-blue, dodger-blue, floral-white, ghost-white, sky-blue and yellow colored traps.

A total of 23,226 thrips were captured on the traps across the locations; 7,998, 8,111 and 7,117 in 2017, 2018 and 2019, respectively. The most (2,863) were captured at Location 3 in 2017, and the least (2225) at Location 1 in 2019. The was no correlation between temperature and capture rate.

The effect of year by location, year by color, location by color, and year by location by color interaction on population of *T. meridionalis* performed by repeated measures-ANOVA is shown Table 1.

Table 1. Repeated measures ANOVA for *Thrips meridionalis* captured on colored sticky traps

Interaction	Df	Mean Square	F	P*
Year x Location	4	1405	1.85	0.184
Year x Color	10	2475	3.76	**0.001**
Location x Color	10	1656	2.28	**0.040**
Year x Location x Color	20	876	1.33	0.196

* Significant effects are indicated in bold.

Repeated measures ANOVA showed statistically significant year by color ($F=3.76$, df=10; 60, $P=0.001$) and location by color ($F=2.28$, df=10; 30, $P=0.040$) interactions, but year by location and year by color by location interactions were not statistically significant (Tables 2 & 3).

The effect of year by color ($F=3.76$, df=10; 60, $P=0.001$) and location by color ($F=2.28$, df=10; 30, $P=0.040$) interactions may have resulted from different capture rates of traps in different years. However, the differences in capture rate with ghost-white compared to other colors was statistically significant for both year by color and location by color interactions (Tables 2 & 3).

Ghost-white traps had significantly higher numbers of captured *T. meridionalis* than the other colored traps and represented the majority of the capture over all locations and years (Figure 2). Floral-white traps the second highest captures (mean captured *T. meridionalis* is given in Table 4).

Table 2. Mean number of *Thrips meridionalis* caught by the different colored sticky traps during 2017-2019

	2017			2018			2019		
Color	T	M±SE		T	M±SE		T	M±SE	
Y	50	5.6±0.51	Da	105	11.7±0.74	Ca	49	5.4±0.59	Ca
DSB	286	31.8±0.74	Ca	290	32.2±1.99	Ca	209	23.2±2.68	Ca
DB	165	18.3±1.00	CDa	124	13.8±0.48	Ca	58	6.4±0.68	Ca
GW	6673	741.4±24.20	Aa	6674	741.6±14.24	Aa	5999	666.6±23.95	Aa
FW	581	64.6±1.61	Ba	675	75.0±1.41	Ba	602	66.9±1.35	Ba
SB	243	27.0±1.65	Ca	243	27.0±1.51	Ca	200	22.2±0.58	Ca

Data were pooled over locations. T, total number; M, mean; SE, standard error of the mean; Y, yellow; DSB, deep-sky-blue; DB, dodger-blue; GW, ghost-white; FW, floral-white; SB, sky-blue; Means in the same column followed by the same letter are not statistically different, Tukey's post hoc test. Capital letters show the difference between within years, and lower letters between years.

Table 3. Results of repeated measures-ANOVA for *Thrips meridionalis*

	Location I		Location II		Location III	
Color	M±SE		M±SE		M±SE	
Y	9.3±0.72	Da	7.0±0.72	Ca	6.3±0.72	Ca
DSB	37.0±1.38	Ca	28.6±1.38	Ca	21.7±1.38	Ca
DB	15.2±0.46	CDa	11.7±0.46	Ca	11.7±0.46	Ca
GW	690.9±21.98	Aa	708.8±22.0	Aa	749.9±21.98	Aa
FW	68.0±0.56	Ba	70.9±0.56	Ba	67.6±0.56	Ba
SB	25.1±1.56	CDa	25.7±1.56	Ca	25.4±1.56	Ca

Data were pooled over sampling years. T, total number; M, mean; SE, standard error of the mean; Y, yellow; DSB, deep-sky-blue; DB, dodger-blue; GW, ghost-white; FW, floral-white; SB, sky-blue; Means in the same column followed by the same letter are not statistically different, Tukey's post hoc test. Capital letters show the difference between within location, and lower letters between locations.

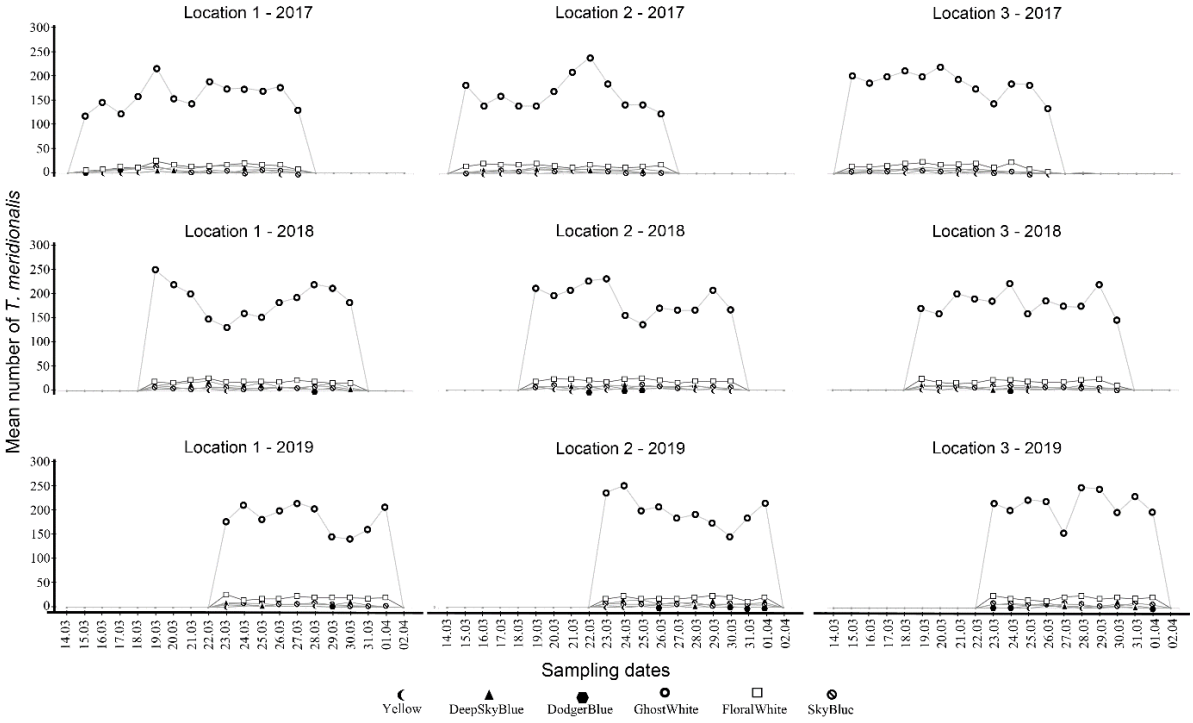

Figure 2. Daily capture of *T. meridionalis* in six colored sticky traps in three locations from 2017 to 2019.

Ghost-white traps captured most *T. meridionalis* and this was significantly different from all other colors according to a Tukey's HSD test in all locations and years. There were significant differences in the capture rates between most of the colors in each location and year, especially for ghost-white*s* (F=2950, df=5; 30, P<0.001).

In 2017, the most thrips were captured on ghost-white color at Location 3 (2438) between 15 and 27 March (Figure 2). This was followed by Locations 2 and 1 (2133 and 2102). Differences between capture rates with floral-white and deep-sky-blue were statistically different from yellow whereas individual capture rates of sky-blue and dodger-blue were not statistically different from yellow at Location 1. Also, significant differences were found between floral-white color yellow. However, differences in capture rates with yellow, deep-sky-blue, dodger-blue and sky-blue were not significant at both Locations 2 and 3 (Table 4).

Table 4. Mean number of adults of *Thrips meridionalis* caught by the different colored sticky traps at three locations from 2017 to 2019

2017	Location I			Location II			Location III		
Color	T	M±SE		T	M±SE		T	M±SE	
Y	13	2.2±0.60	c	14	2.0±0.69	c	23	3.3±0.71	c
DSB	128	9.9±1.01	b	86	6.6±1.03	bc	72	6.6±1.17	bc
DB	65	5.4±1.09	bc	50	4.2±0.71	bc	50	4.2±0.71	bc
GW	2102	161.7±7.9	a	2133	164.1±9.29	a	2438	187.5±6.81	a
FW	184	14.2±1.60	b	202	15.5±0.85	b	195	15.0±1.76	b
SB	81	6.2±1.15	bc	77	6.4±0.78	bc	85	6.1±0.74	bc
2018	Location I			Location II			Location III		
Color	T	M±SE		T	M±SE		T	M±SE	
Y	50	4.6±0.61	c	34	0.7±2.21	c	21	1.9±0.41	c
DSB	144	12.0±1.38	b	77	6.4±2.91	bc	69	6.3±1.20	bc
DB	51	4.6±0.68	c	36	3.6±2.12	bc	37	4.1±6.80	bc
GW	2260	188.3±10.13	a	2246	187.2±30.45	a	2168	180.7±0.92	a
FW	226	18.8±0.89	b	239	19.9±3.15	b	210	17.5±0.63	b
SB	81	6.8±0.74	bc	83	6.9±1.98	bc	79	6.6±0.41	bc
2019	Location I			Location II			Location III		
Color	T	M±SE		T	M±SE		T	M±SE	
Y	21	2.1±0.41	c	15	2.5±0.81	c	13	2.2±0.54	c
DSB	61	6.1±0.91	bc	94	9.4±1.06	bc	54	6.0±0.78	bc
DB	21	2.3±0.55	c	19	2.7±0.64	c	18	4.5±0.96	bc
GW	1856	185.6±8.82	a	2000	200.0±9.72	a	2143	214.3±8.86	a
FW	202	20.2±1.02	b	197	19.7±1.09	b	203	20.3±1.16	b
SB	64	6.4±0.96	bc	71	7.1±0.59	bc	65	6.5±0.75	bc

T, total number; M, mean; SE, standard error of the mean; Y, yellow; DSB, deep-sky-blue; DB, dodger-blue; GW, ghost-white; FW, floral-white; SB, sky-blue; Means in the same column followed by the same letter are not statistically different by Tukey's HSD test (P<0.05).

In 2018, the most thrips were captured on ghost-white color at Location 1 (2260) between 19 and 30 March (Figure 2). This was followed by Locations 2 and 3 (2246 and 2168). Significant differences (P<0.05) were found between ghost-white and the other colors in all years. Whereas differences between deep-sky-blue, dodger-blue, floral-white and sky-blue were not significant but these were statistically different from yellow at Locations 1 and 2. Similar were found at Location 3 (Table 4).

In 2019, the most thrips were captured on ghost-white Location 3 (2143) between 23 March and 1 April (Figure 2). This was followed by Locations 2 and 1 (2000 and 1856). Ghost-white capture rate was statistically significant from the other colors at all locations. Yellow had the lowest capture rate and this was

statistically different from all other colors at Location 1. Dodger-blue had the same capture rate as yellow at Locations 2 and 3. There was no significant difference between deep-sky-blue, dodger-blue, floral-white and sky-blue at any locations (Table 4).

The dendrogram (Figure 3) shows that ghost-white was the most dissimilar color and it differed significantly from all other colors in all years and at all localities.

Sky-blue and dodger-blue were found to be similar to each other (61%) and as the most similar at Location 1 in 2017. However, the most similar colors at Location 2 were dodger-blue and deep-sky-blue (59%) and at Location 3 it was sky-blue and deep-sky-blue w (63%) in 2017 (Figure 3).

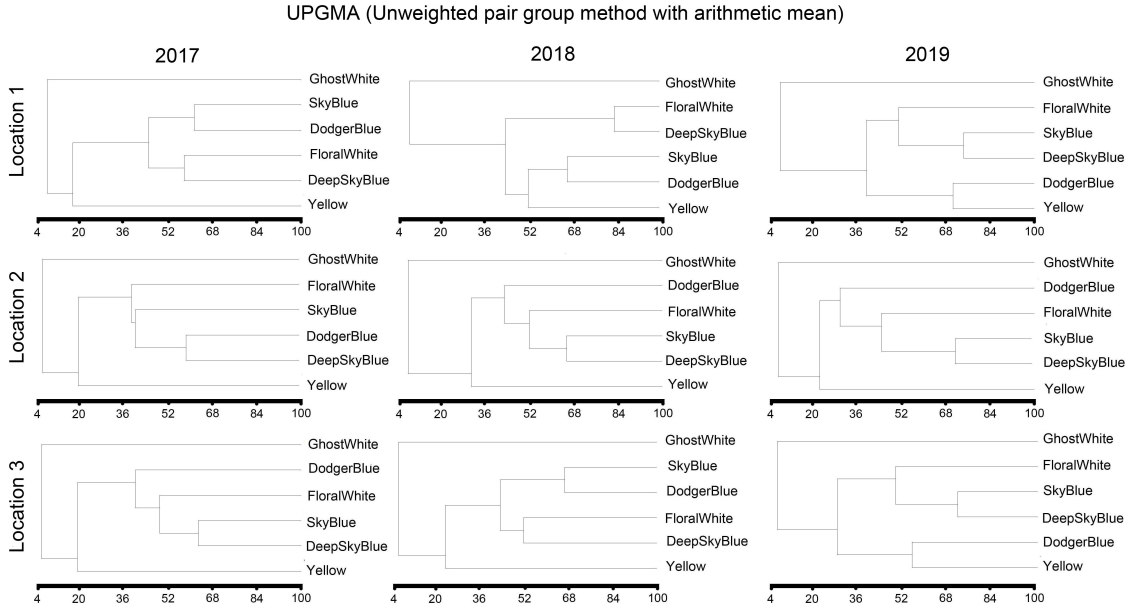

Figure 3. Dendrogram showing the capture similarities for *Thrips meridionalis* with different colored sticky traps based on the analysis of Euclidean data.

In 2018, floral-white and deep-sky-blue were the most similar (84%). Sky-blue and dodger-blue in were the second most similar (67%). Sky-blue and deep-sky-blue were the most similar (65%) at Location 2. Sky-blue and dodger-blue were similar (67%) and the second most similar (52%) were floral-white and deep-sky-blue at Location 3 (Figure 3).

In 2019, the most similar were sky-blue and deep-sky-blue (74%) and dodger-blue and yellow (71%) at Location 1. Sky-blue and deep-sky-blue were the most similar (71%) and floral-white color was similar to these (45%) at Location 2 (Figure 3).

Sky-blue and deep-sky-blue color were the most similar (71%) and dodger-blue and yellow were the second most similar (55%) at Location 3.

Discussion

According to study of Uzun & Tezcan (2015), *T. meridionalis* was an abundant species (48%) in cherry orchards along the *T. tabaci* (48%), *Taeniothrips inconsequens* (Uzel, 1895) (48%), *Haplothrips reuteri* (Karny, 1907) (41%), *Aeolothrips intermedius* Bagnall, 1934 (14%) and *F. occidentalis* (14%) in Isparta Province. In contrast, Kaplan et al. (2016) reported that *T. meridionalis* was a rare species (0.8%) in Mardin Province, Turkey.

In our study, *T. meridionalis* was found at high population density although it appears to be active only for a short period each year. This might be attributed to host flowering and the other ecological factors. The hosts of *T. meridionalis* are recorded as almond, apple, apricot, cherry, grapevine, nectarine, pear, peach, plum and other species in the Rosaceae (Alford, 2007; Hazır et al., 2011; Uzun et al., 2015; Kaplan et al., 2016). Flowering periods of these hosts will be important for the feeding of *T. meridionalis*. Although the colored sticky traps were in position for about 1.5 months, the number of days in which thrips were captured was quite limited. The emergence of the insect at high population density for only a certain short period followed by a sudden decline is similar to many ecological events. Alford (2007) affirmed that the peach thrips has three overlapping generations annually in Southern Europe and adult individuals overwinter under the dead leaves. They become active with the rise in temperature, depending on the region, the earliest in mid-February through to late-May. They initially feed on early-flowering hosts, such as almond. Result of the present study confirmed those of Alford (2007). We think that the shortness of peach thrips activity period depends on the duration of the flowering period of almonds. In the present study, there were almond trees growing naturally around the selected locations where apples, cherries and walnuts were most cultivated. It was observed that the flowering period of almond and the flight of the thrips were almost the same. The cherry flowering period begins at the end of the almond flowering period. No *T. meridionalis* were captured on any colored sticky traps during that period. This might indicate that thrips might have migrated to cherry flower. This agrees with Alford (2007), Uzun et al. (2015), and Kaplan et al. (2016) who reported that when the other host plants reach the flowering stage, peach thrips migrate to other hosts from the early-flowering hosts. However, the reason for thrips not being caught in cherry orchards could also be in part due to other biotic or abiotic factors. One reason why the thrips were not captured after March might be due to the fact that cherry flowers were more attractive than the colored sticky traps.

Although there have been no studies on the color preferences of *T. meridionalis,* attractiveness of colors has been investigated for many other thrips species (Demirel & Yildirim, 2008; Elekcioglu, 2013; Atakan et al., 2014, 2016; Atakan & Pehlivan, 2015). Thrips have been shown to be most attracted to either white, yellow or blue sticky traps. Raymond (2009) reported that the main technique used to monitoring for western flower thrips, *F. occidentalis,* is to use either blue or yellow traps. In the study of Blumthal et al. (2005) four different colors were tested for *F. occidentalis* and a significantly higher number of *F. occidentalis* was found on yellow colored sticky traps matching *Gerbera jamesonii* Bolus ex Adlam (Asterales: Asteraceae) and *Chrysanthemum* L. (Asterales: Asteraceae). Similar results were found with yellow being the most attractive color for avocado thrips, *Scirtothrips perseae* Nakahara, 1997 (Thysanoptera: Thripidae) and white for predatory thrips, *Franklinothrips orizabensis* Johansen, 1974 (Thysanoptera: Thripidae) and *F. occidentalis* (Yee et al., 1999; Hoddle et al., 2002). Other studies also showed that yellow is the best color for monitoring and controlling *F. occidentalis* (Moreno et al., 1984; Samways, 1986; Blumthal et al., 2005). Some other studies showed that the most attractive color for *F. occidentalis* and *T. tabaci* was blue in lemon and orange orchards (Chen et al., 2004; Kaas, 2005; Elekcioglu, 2013) and blue with UV reflection appears to be an important component of trap efficacy (Ranamukhaarachchi & Wickhramarachchi, 2007). Elimem & Chermiti (2013) reported that the white-cream rose cv. Ociane was more attractive to *F. occidentalis* than the red cv. First-red in greenhouses; 29.5 and 39.9 thrips/flower on Ociane but only 12.4 and 29.6 thrips/flower on First-red, respectively. Rodriguez-Saona et al. (2010) showed that the white colored sticky traps were more attractive to *Scirtothrips ruthveni* Shull, 1909 (Thysanoptera: Thripidae) and *Frankliniella tritici* (Fitch, 1855) (Thysanoptera: Thripidae) compared to yellow or blue traps. However, Natwick et al. (2007) showed that blue traps consistently captured more adult thrips of both *F. occidentalis* and *T. tabaci* compared to yellow. There were no significant differences in numbers of adult *T. tabaci* caught on white and blue colors although blue traps caught significantly more onion thrips, *T. tabaci* than white traps (Liu & Chu, 2004). Walker (1974) reported that white was the most attractive to thrips. Given the better reflection of light compared to other colors, such as blue and yellow, the majority of thrips species, especially flower thrips, are attracted to white traps (Hoddle et al., 2002).

As there is still some disagreement about which color is the most attractive to thrips species, further studies are needed. In our study, capture rates obtained with ghost-white for *T. meridionalis* were significantly ($P<0.001$) greater than all other colors tested.

These variable results for different thrips species, even at the same species, suggest that in addition to the color as observed by the human eye, other ecological factors could be important. Also, the temperature and photoperiod may influence the responses of the thrips (Bournier, 1983). Therefore, other biotic and abiotic ecological factors should be investigated in relation to color attractiveness in order to identify ecological factors involved.

Conclusions

According to the Tukey's HSD test ($P<0.01$) the largest number of *T. meridionalis* adults were captured on ghost-white sticky traps at all locations during this study. The cluster analysis Euclidean distance for all locations confirmed that ghost-white color traps were more attractive for *T. meridionalis* adults than any of the other colors tested. Therefore, it is concluded that ghost-white sticky traps are a particularly useful way to monitor and control *T. meridionalis* adults.

Acknowledgements

Part of this work was supported by Global Change Research Institute of the Czech Academy of Sciences. We would like to thank Prof. Dr. Ekrem Atakan (Çukurova University, Agriculture Faculty, Plant Protection Department, Adana, Turkey) for identifying the thrips species. We would also like to thank Assoc. Prof. Dr. Özgür Koşkan (Isparta University of Applied Science, Agriculture Faculty, Isparta, Turkey) for helpful advice on the statistical analysis. This study was presented orally and published as summary at the International Conferences on Science and Technology in Kosovo, 2019.

References

Alford, D. V., 2007. Pests of Fruit Crops: A Color Handbook-A Color Handbook. Academic Press, 461 pp.

Atakan, E., 2010. Influence of weedy field margins on abundance patterns of the predatory bugs *Orius* spp. and their prey, the western flower thrips (*Frankliniella occidentalis*), on faba bean. Phytoparasitica, 38 (4): 313-325.

Atakan, E. & A. Bayram, 2011. Distributions of western flower thrips (Thysanoptera: Thripidae) and its predatory bug *Orius niger* (Hemiptera: Anthocoridae) assessed by coloured sticky traps and plant samplings in cotton. Archives of Phytopathology and Plant Protection, 44 (16): 1595-1608.

Atakan, E. & R. Canhilal, 2004. Evaluation of yellow sticky traps at various heights for monitoring cotton insect pests. Journal of Agricultural and Urban Entomology, 21 (1): 15-24.

Atakan, E. & S. Pehlivan, 2015. Attractiveness of various colored sticky traps to some pollinating insects in apple. Turkish Journal of Zoology, 39 (3): 474-481.

Atakan, E., S. Pehlivan & M. Ölçülü, 2014. Attraction of colored sticky traps to Thrips major Uzel (Thysanoptera: Thripidae) in nectarine. Turkish Journal of Entomology, 38 (1): 51-60.

Atakan, E., S. Pehlivan & S. Satar, 2016. Response of some beneficial insect species to colored sticky traps in citrus. Turkish Journal of Entomology, 40 (4): 385-396.

Blumthal, M., R. A. Cloyd, L. A. Spomer & D. F. Warnock, 2005. Flower color preferences of Western Flower Thrips. HortTechnology, 15 (4): 846-853.

Bournier, A., 1983. Les Thrips. Biologie, Importance Agronomique. INRA, Paris, 128 pp.

Chen, T. Y., C. C. Chu, G. Fitzgerald, E. T. Natwick & T. J. Henneberry, 2004. Trap evaluations for Thrips (Thysanoptera: Thripidae) and Hoverflies (Diptera: Syrphidae). Environmental Entomology, 33 (5): 1446-1420.

Demirel, N. & A. Yildirim, 2008. Attraction of various sticky color traps to *Thrips tabaci* Lindeman (Thysanoptera: Thripidae) and *Empoasca decipiens* Paoli (Homoptera:Cicadellidae) in cotton. Journal of Entomology, 56 (6): 389-394.

Elekcioglu, Z. E., 2013. Color preference, distribution and damage of Thrips associated with lemon and orange in Adana, Turkey. Pakistan Journal of Zoology, 45 (6):1705-1714.

Elimem, M. & B. Chermiti, 2013. Color preference of *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae) and *Orius* sp (Hemiptera; Anthocorridae) populations on two rose varieties. Floculture and Ornamental Biotechology, 7 (1): 94-98.

Grasselly, D., G. Perron & E. Navarro, 1995. "Western Flower Thrips in Peach Orchards in France, 389-392". In: Thrips Biology and Management (Eds. B. L. Parker, M. Skinner & T. Lewis). NATO ASI Series Book Series (NSSA, Volume 276), Springer, Boston, MA, USA, 636 pp.

Hazır, A., M. R. Ulusoy & E. Atakan, 2011. Adana ve Mersin illeri nektarin bahçelerinde saptanan Thysanoptera türleri ve zarar oranı üzerine araştırmalar. Turkish Journal of Entomology, 35 (1): 133-144.

Hoddle, M. S., L. Robinson & D. Morgan, 2002. Attraction of Thrips (Thysanoptera: Thiripidae and Aeolthiripidae) to colored sticky cards in a California avocado orchard. Crop Protection, 21: 383-388.

Kaas, J. P., 2005. Vertical distribution of thrips and whitefly in greenhouses and relative efficiency of commercially available sticky traps for population monitoring. Proceedings of the Netherlands Entomological Society Meeting, 16: 109-115.

Kaplan, M., E. Bayhan & E. Atakan, 2016. Determination of Thysanoptera species, their seasonal abundance and distribution in vineyard areas of Mardin Province. Turkish Bulletin of Entomology, 6 (2): 161-168.

Kovach, W. L., 1999. MVSP-a multivariate statistical package for Windows, ver. 3.1 Kovach Computing Services. Pentraeth, Wales, Great Britain.

Lacasa, A., M. Martinez, C. Torres & J. Aliaga, 1991. Datos preliminares sobre *Frankliniella occidentalis* como plaga de la nectarina y el melocotonero en el sureste Espanol. Fruticultura Profesional, 36: 39-46.

Liu, T. X. & C. C. Chu, 2004. Comparison of absolute estimates of *Thrips tabaci* (Thysanoptera: Thripidae) with field visual counting and sticky traps in onion field in South Texas. Southwestern Entomologist, 29 (2): 83-89.

Martin, J. L., A. Gonzales, A. Conese, I. Porraz, J. Manera & J. Martinez-Nicolas, 2011. Coloured sticky trap influence in accidental capture of beneficial insect in pepper crop under greenhouse. Óptica Pura y Aplicada, 44 (1): 155-161.

Moreno, D. S., W. A. Gregory & L. K. Tanigoshi, 1984. Flight response of *Aphytis melinus* (Hymenoptera: Aphelinidae) and *Scirtothrips citri* (Thysanoptera: Thripidae) to trap color, size, and shape. Environmental Entomology, 13 (4): 935-940.

Natwick, E. T., J. A. Byers, C. C. Chu, M. Lopez, & T. J. Henneberry, 2007. Early detection and mass trapping of *Frankliniella occidentalis* and *Thrips tabaci* in vegetable crops. Southwestern Entomologist, 32 (4): 229-238.

Ranamukhaarachchi, S. L. & K. S. Wickramarachchi, 2017. Color preference and sticky traps for field management of *Thrips ceratothripoides claratris* (Shumsher) (Thysanoptera: Thripdae) in tomato in Central Thailand. International Journal of Agriculture & Biology, 9 (6): 839-844.

Raymond, A. C., 2009. Western Flower Thrips (*Frankliniella occidentalis*) management on ornamental crops grown in greenhouses: Have we reached an impasse? Pest Technology, 3 (1): 1-9.

Rodriguez-Saona, C. R., S. Polavarapu, J. D. Barry, D. Polk, R. Jörnsten, P. V. Oudemans & O. E. Liburd, 2010. Color preference, seasonality, spatial distribution and species composition of thrips (Thysanoptera: Thripidae) in northern highbush blueberries. Crop Protection, 29 (11): 1331-1340.

Samways, M. J., 1986. Spatial distribution of *Scirtothrips aurantii* Fuare (Thysanoptera: Thripidae) and threshold level for one per cent damage on citrus fruit based on trapping with fluorescent yellow sticky traps. Bulletin of Entomological Research, 76 (4): 649-659.

Sathe, T. V., M. Pranoti, S. S. Patil & A. S. Desai, 2015. Diversity, economic importance and control strategies of thrips (Thysanoptera) on crop plants. Indian Journal of Applied Research, 5 (8): 761-763.

Uzun, A., S. Tezcan & O. Demirözer, 2015. Thrips (Thysanoptera) species occurring in cherry orchards in Isparta Province of Western Turkey. Linzer Biologische Beiträge, 47 (1): 963-968.

Walker, F. W., 1974. Response of selected Thysanoptera to colored surfaces. Environmental Entomology, 3 (2): 295-304.

Yee, W. L., P. A. Phillips, M. S. Hoddle & J. G. Morse, 1999. Further progress on Avocado Thrips biology and management. California Avocado Society 1999 Yearbook, 83: 105-125.

Türk. entomol. derg., 2020, 44 (3): 295-304
DOI: http://dx.doi.org/10.16970/entoted.679739

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

A new species and additional records of genus *Drusilla* Leach, 1819 (Coleoptera: Staphylinidae: Aleocharinae) from Turkey

Türkiye'den *Drusilla* Leach, 1819 (Coleoptera: Staphylinidae: Aleocharinae) cinsine bağlı yeni bir tür ve ek kayıtlar

Semih ÖRGEL[1*]

Abstract

A study was conducted on the fauna of the genus *Drusilla* Leach, 1819 (Coleoptera: Staphylinidae: Aleocharinae) in Anatolia. The aim of this study was to contribute to biodiversity research by describing a new species, also, to provide a better understanding of the distributions of known species. The field trips were made in different provinces of Turkey between 2010-2019. Specimens were collected by hand and pitfall traps. *Drusilla anlasi* sp. n. from East Menteşe Mountains in Muğla Province, western Anatolia, is originally described. Additional records are given for six of the nine known species from Anatolia. The distributions of the known species are discussed and distribution maps are given. The diagnostic characters of the new species and sexual characters of the *Drusilla lydica* Assing, 2007, *Drusilla recta* Assing, 2005 and *Drusilla canaliculata* (Fabricius, 1787) are illustrated. A key to the *Drusilla* species of Anatolia is presented.

Keywords: Anatolia, distribution, *Drusilla*, rove beetle, taxonomy

Öz

Anadolu'da *Drusilla* Leach,1819 (Coleoptera: Staphylinidae: Aleocharinae) cinsinin faunası üzerine bir çalışma yapılmıştır. Bu çalışmanın amacı, yeni bir tür tanımlayarak biyoçeşitlilik araştırmalarına katkıda bulunmak, ayrıca, bilinen türlerin dağılımlarının daha iyi anlaşılmasını sağlamaktır. Arazi çalışmaları 2010-2019 yılları arasında Türkiye'nin farklı illerinde yapılmıştır. Örnekler elle ve çukur tuzaklarla toplanmıştır. Batı Anadolu'nun Muğla İli'ndeki Doğu Menteşe Dağları'ndan, *Drusilla anlasi* sp. n. türünün orijinal deskripsiyonu yapılmıştır. Anadolu'dan bilinen dokuz türden altısı için ek kayıtlar verilmiştir. Bilinen türlerin dağılımı tartışılmış ve dağılım haritaları verilmiştir. Yeni türün ayırıcı karakterleri ve *Drusilla lydica* Assing, 2007, *Drusilla recta* Assing, 2005 ve *Drusilla canaliculata* (Fabricius, 1787) türlerinin eşeysel karakterleri şekillendirilmiştir. Anadolu'daki *Drusilla* türleri için bir tanı anahtarı sunulmuştur.

Anahtar sözcükler: Anadolu, yayılış, *Drusilla*, cepkenli böcekler, taksonomi

[1] Manisa Celal Bayar University, Alaşehir Vocational School, 45600, Alaşehir, Manisa, Turkey
* Corresponding author (Sorumlu yazar) e-mail orgelsemih@gmail.com
Received (Alınış): 24.01.2020 Accepted (Kabul ediliş): 24.03.2020 Published Online (Çevrimiçi Yayın Tarihi): 12.05.2020

Introduction

Schülke & Smetana (2015) listed 58 species for the genus *Drusilla* Leach, 1819 in the Palearctic Region catalogue. After this, three new species were described (Assing, 2015, 2017). Thus, a total of 61 species are now known in the Palearctic Region. Nine of these species are distributed in Anatolia (Assing, 2005, 2007; Anlaş, 2009). Until 2005, only *Drusilla canaliculata* (Fabricius, 1787), the most widespread species in the Palearctic Region, was known from Anatolia (Coiffait, 1978). Then, Assing (2005) described five new species from Anatolia. The last described species belonging to genus *Drusilla* in Anatolia is *Drusilla lydica* Assing, 2007 (Assing, 2007). Anlaş (2009) listed the Anatolian *Drusilla* species and their distributions. Subsequent records were provided by Assing (2009, 2010, 2013, 2015). In this study, samples were collected in several regions of Turkey and this study aims to be better understanding the distributions of species. Also, the aim of this study is contributing to biodiversity research by describing new species.

In Anatolia, the vast majority of *Drusilla* species is known from the Taurus Mountains range and south of these mountains. In particular, four species from the east of the Central Taurus Mountains were described. Only one species is known from the southwest of Anatolia. However, this number has increased to two with this study. *Drusilla canaliculata* is the most common species of genus *Drusilla* in Anatolia.

In the subfamily Aleocharinae Fleming, 1821 (Coleoptera: Staphylinidae), morphology and internal structures of aedeagus, chaetotaxy of paramere and external morphology are usually taxonomic characters of high importance for the separation of the species. But these characters are highly similar and not reliable for the identification of *Drusilla* species. Assing (2005) indicated that the spermatheca is the most important and reliable character for the determination of western Palearctic *Drusilla* species.

Materials and Methods

The field trips of this study were made in different provinces of Turkey between 2010 and 2019 (Figure 1). Specimens were collected by hand and pitfall traps. Photographs of the primary and secondary characters were taken with a Zeiss Axiocam ERC5s digital camera connected to a Stemi 508 (Zeiss Germany) stereomicroscope. Helicon Focus 6.0.18 image stacking software was used to combine photographs which were taken in different focal planes. CorelDRAW Graphics Suite X7 was used to create the plates and edit photographic images. Google Earth Pro was used to create the maps.

Figure 1. Locations in Anatolia in which the material used in this study were collected.

Hanley & Ashe (2003) were followed for dissection techniques. Terminology for the body parts, aedeagus and spermatheca structures follows Assing (2005). The material is deposited in AZMM (Alaşehir Zoological Museum, Manisa, Turkey) of Manisa Celal Bayar University.

The following abbreviations are used for the measurements: AL, length of antenna; AW, maximal width of abdomen; EL, length of elytra from apex of scutellum to posterior margin at suture; EW, width of elytra; HL, head length from anterior margin of clypeus to posterior margin of head; HW, 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; TaL, length of metatarsus; TiL, length of metatibia; and TL, total length.

Results

Additional records are given for six of the nine known species from Turkey and apart from these species, *Drusilla anlasi* sp. n. is described from Muğla. Now the species number of the genus *Drusilla* has risen to 10 in Turkey.

***Drusilla anlasi* sp. n. (Figures 2a-d, 3a-h and 5)**

Type material. Holotype. TURKEY, ♂ "TR - Muğla Province, 7 km NW Özlüce, East Menteşe Mountains, 1605 m, 37°15'56" N, 28°26'37" E, 22.III.2014, leg. Anlaş & Örgel / Holotypus ♂ *Drusilla anlasi* sp. n. det. S. Örgel, 2019" (AZMM). Paratypes. TURKEY, 2♂♂, 4♀♀, same data as holotype (AZMM).

Etymology: The species is dedicated to Dr. Sinan Anlaş (Manisa, Turkey), a specialist of the family Staphylinidae, who has actualized significant entomological research in Turkey and adjacent countries and collected the type specimens with the author.

Description. Measurements (in mm) and ratios (n = 7): AL: 1.63-1.86, 1.74; HL: 0.35-0.39, 0.37; HW: 0.44-0.49, 0.47; PW: 0.48-0.54, 0.52; PL: 0.56-0.61, 0.59; EL: 0.38-0.43, 0.41; EW: 0.68-0.72, 0.70; AW: 0.81-0.87, 0.84; TiL: 0.86-1.04, 0.95; TaL: 0.73-0.85, 0.77; ML: 0.61-0.63, 0.62 (n = 3); TL: 4.07-4.97, 4.43; HL/HW: 0.79; PW/HW: 1.11; PL/PW: 1.13; EL/PL: 0.69; EW/PW: 1.35; AW/EW: 1.20; TiL/TaL: 1.23.

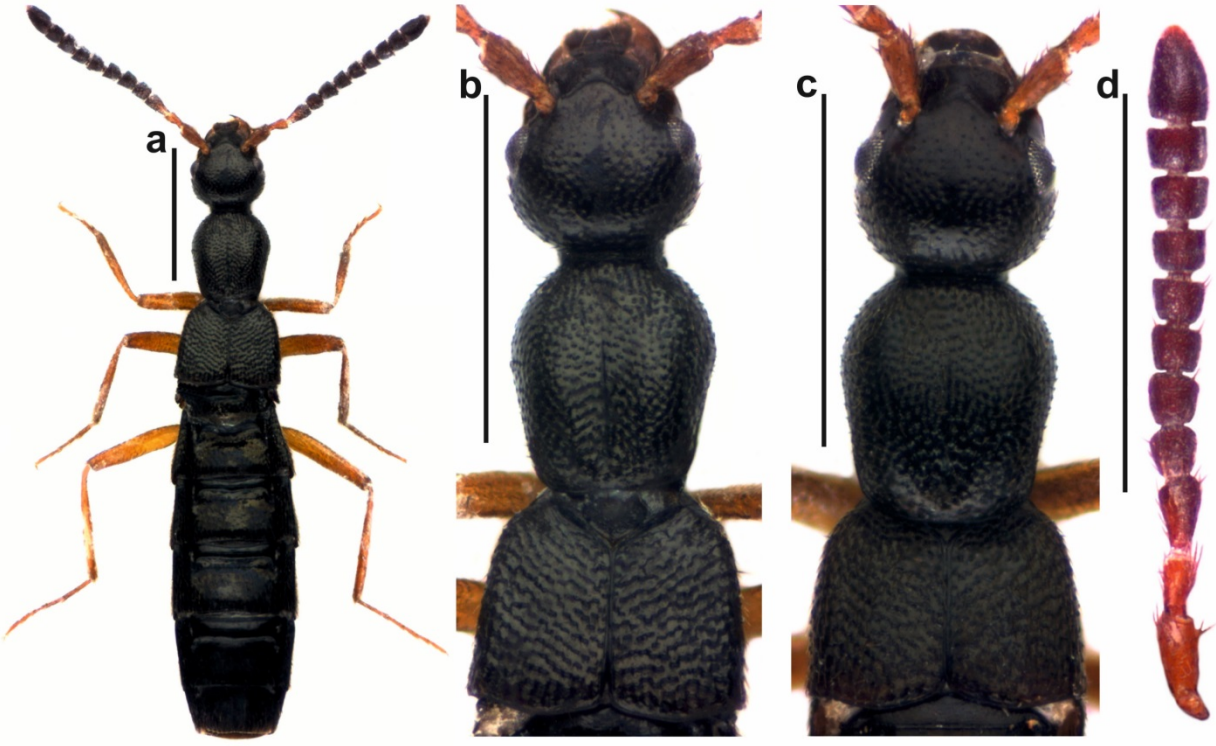

Figure 2. *Drusilla anlasi* sp. n. (from Muğla): a) ♂ habitus; b) ♂ forebody; c) ♀ forebody; d) Antenna (Scale bars: 1 mm for Figure 2a-d).

Habitus as in Figure 2a. Body uniformly blackish; legs reddish brown; antennae blackish brown with the I., II. and half of III. antennomeres distinctly paler.

Head. Slightly transverse (see ratio HL/HW and Figure 2a-c); eyes of medium size, 0.80 times as long as postocular region in lateral view, moderately convex in dorsal view; puncturation coarse and sparse; microsculpture absent; antennomere I-III distinctly and IV, V weakly oblong, III 1.83 times as long as wide, VI-X transverse, XI 1.08 times as long as the total length of IX and X.

Pronotum. Distinctly oblong and wider than head (see ratios PL/PW, PW/HW and Figure 2a-c); widest at anterior, distinctly narrowed posteriorly; puncturation similar to that of head, but much denser and distinct; microsculpture absent.

Elytra. Distinctly transverse; shorter and wider than pronotum (see ratios EL/PL, EW/PW and Figure 2a-c); lateral margins subparallel in dorsal view; punctation much denser than that of head and distinctly granulose; microsculpture absent; hind wings reduced; metatarsus shorter than metatibia (see ratio TiL/TaL); metatarsomere I as long as the total length of II and III.

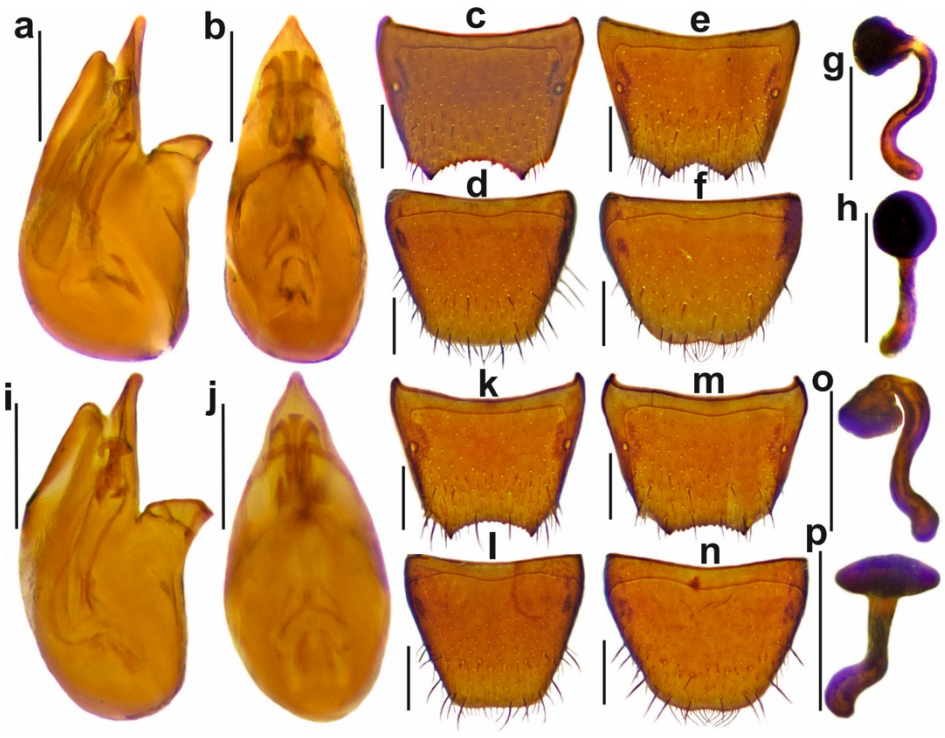

Figure 3. Sexual characters of *Drusilla anlasi* sp. n. (from Muğla) (a-h) and *D. lydica* Assing (from Aydın) (i-p): a, i) median lobe of aedeagus in lateral view; b, j) median lobe of aedeagus in ventral view; c, k) ♂ tergite VIII; d, l) ♂ sternite VIII; e, m) ♀ tergite VIII; f, n) ♀ sternite VIII; g, h, o, p) spermatheca in different aspects (Scale bars: 0.2 mm for Figure 3a-p).

Abdomen. Broadest at segment V and clearly wider than elytra (see ratio AW/EW); punctation fine and sparse, sparser posteriorly; microsculpture absent.

♂: Pronotum with narrow and deep impression along midline; posterior incision of tergite VIII wide, deep and with 8-11 serrates; sternite VIII unmodified, posterior margin truncate or weakly convex; apex of median lobe of aedeagus in lateral view straight (lateral margin on the side facing the ventral) (Figure 3a-d).

♀: Pronotum with wide and shallow impression along midline; posterior incision of tergite VIII narrower than male and with 8-11 serrates; sternite VIII modified, concave posterior margin with very short and thin setae and with longer than these setae; bulbus distalis of spermatheca in longitudinal section circular (Figure 3e-h).

Distribution and Bionomics. Specimens were collected under stones in meadows at elevations of 1605 m from Eastern Menteşe Mountains and are probably endemic to these mountains (Figure 5).

Differential diagnosis. *Drusilla anlasi* sp. n. mainly differs from all known *Drusilla* by its different shape of the median lobe of aedeagus, spermatheca and posterior margin of male and female tergite VIII. This new species is the most similar to *D. lydica* from Aydın and İzmir (Turkey) but differs in straight lateral margin on the side facing the ventral in apex of median lobe of aedeagus in lateral view (in *Drusilla lydica,* apex of median lobe of aedeagus is bent ventrally in lateral view (Figure 3i) and circular bulbus distalis of spermatheca in longitudinal section (in *D. lydica,* bulbus distalis of spermatheca is dilated transversely in longitudinal section (Figure 3p). If the distribution of these two species is examined, *D. lydica* is known from Aydın Mountains and *D. anlasi* is known from Menteşe Mountains and these two mountain ranges are separated by Büyük Menderes Plain and are isolated from each other. Besides, Menteşe Mountains are separated by valley of Dalaman Stream with Western Taurus in south. Therefore, these two species are probably endemic to the mountains in which they are located. *Drusilla anlasi* sp. n. can be separated with *Drusilla recta* Assing, 2005, which has convex lateral margin on the side facing the ventral in apex of median lobe of aedeagus in lateral view and bent ventrally (Figure 4a). Also, in *D. recta,* male and female tergite VIII are very weakly serrate or without serrate in posterior margin (Figure 4c,e) and *D. anlasi* separated by distinctly serrate in posterior margin of male and female tergite VIII. Bulbus distalis of spermatheca is also smaller than *D. anlasi* sp. n. (Figure 4g). *Drusilla canaliculata*, which is one of the most common species in the genus *Drusilla*, distinctly differs from *D. anlasi* sp. n. with thin and strongly bent ventrally in apex of median lobe of aedeagus in lateral view (Figure 4h). Duct of spermatheca is also longer than *D. anlasi* sp. n. (Figure 4n).

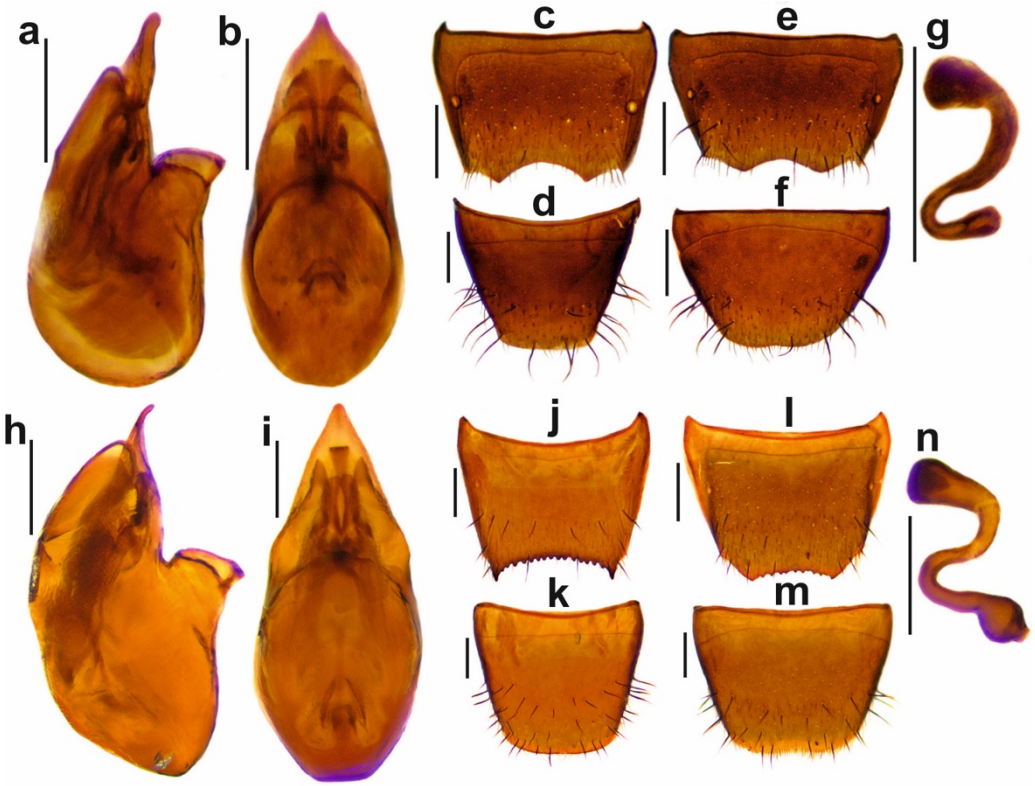

Figure 4. Sexual characters of *Drusilla recta* Assing (from Karaman) (a-g) and *D. canaliculata* (Fabricius) (from Kars) (h-n): a, h) median lobe of aedeagus in lateral view; b, i) median lobe of aedeagus in ventral view; c, j) ♂ tergite VIII; d, k) ♂ sternite VIII; e, l) ♀ tergite VIII; f, m) ♀ sternite VIII; g, n) spermatheca (Scale bars: 0.2 mm for Figure 4a-n).

Additional Records

***Drusilla anceps* Assing, 2005 (Figure 5)**

Material. Karaman: Sertavul Pass, 36°54'58" N, 33°16'08" E, 1648 m, 24.IV.2013, ♀, leg. Koç.

Distribution. This species is described from Sertavul Pass where is the border of Western and Central Taurus Mountains (Assing, 2005) and recorded for the first time since its description (Figure 5). It is most probably endemic to Sertavul Pass.

***Drusilla denigrata* Assing, 2005 (Figure 5)**

Material. Hatay: Samandağ, İkizköprü, 36°10'39" N, 35°59'28" E, 523 m, 29.VII.2010, ♂, leg. Yağmur.

Distribution. This species is known from around the southern part of the Amanos Mountains (Figure 5) and is probably endemic to this area (Assing, 2005, 2010, 2015).

***Drusilla limata* Assing, 2005 (Figure 5)**

Material. Isparta: Sütçüler, 9 km E Kesme, 37°28'24" N, 31°19'46" E, 1760 m, 14.IV.2016, 2♂♂, ♀, leg. Örgel, Sütçüler, 8 km S Aşağıkartoz, 37°28'14" N, 31°19'48" E, 1770 m, 14.IV.2016, ♂, leg. Örgel.

Distribution. It is known from the Bey Mountains in the Western Taurus, Davraz Mountain and west of the Dim Valley in Geyik Mountains (Assing, 2005, 2007, 2013). Assign (2009) also recorded from the east of the Dim Valley (Figure 5). He examined a female from Karaman Province and remarked that in this female, differences in spermatheca morphology and antennae coloration than is usually the case in *D. limata*. He also noted that “more material is needed to decide if it is indeed conspecific with *D. limata* or if it represent a different species”. Given the Dim Valley is an important barrier for many insect species, the female in Karaman is most probably a different species, as Assing (2009) is suspected.

Figure 5. Distribution of *Drusilla anlasi* sp. n. (small square □), *D. lydica* (small circles ○), *D. limata* (triangles △), *D. recta* (pentagons ⬠), *D. anceps* (stars ☆), *D. sinuosa* (inverse triangles ▽), *D. denigrata* (complex stars ✪), *D. pallidicornis* (large square □) and *D. cernens* (large circle ○) in Turkey (Records of Assing (2005, 2006, 2007, 2008, 2009, 2010, 2013, 2015) are included with present paper records).

***Drusilla lydica* Assing, 2007 (Figures 3i-p and 5)**

Material. Aydın: Central province, 4 km N Karaköy, 37°56'41" N, 27°53'46" E, 1430 m, 24.III.2014, 9♂♂, 6♀♀, leg. Anlaş & Örgel; Manisa: Alaşehir, Kestanederesi, 38°16'05" N, 28°23'06" E, 12.IV.2014, ♂, ♀, leg. Yağmur & Örgel.

Distribution. This species is described from Aydın Mountains (Assing, 2007) and has been recorded for the first time since its description. Moreover, it was recorded for the first time from the Bozdağ Mountains (Figure 5). These two mountain ranges reaching parallel to each other are separated by Küçük Menderes

Plain, but they contact each other in the eastern parts. Therefore, the isolation between the two mountains is not complete and many species can spread in these two mountains. This species is most probably endemic to these mountains.

***Drusilla recta* Assing, 2005 (Figures 3a-g and 5)**

Material. Karaman: Sarıveliler, 5 km SW Çevrekavak, 36°37'11" N, 32°35'54" E, 1702 m, 25.V.2016, 2♂♂, ♀, leg. Örgel & Yaman, same data but 15.XI.2016, ♂, 2♀♀, pitfall trap, Sarıveliler, 5 km S Civler, 36°37'16" N, 32°34'31" E, 1717 m, 25.V.2016, 2♂♂, ♀, leg. Örgel & Yaman.

Distribution. It is known from the area between the east of the Dim Valley in the Geyik Mountains and the west of the Bolkar Mountains in the Central Taurus (Figure 5) (Assing, 2005, 2006, 2008, 2009). This species is probably endemic to this area.

***Drusilla canaliculata* (Fabricius, 1787) (Figures 4h-n and 6)**

Material. Aksaray: Ekecik Mountain, radar road, 38°38'36" N, 34°01'30" E, 1704 m, 23.III.2018, ♂, leg. Örgel & Yaman, Ihlara Valley, 38°15'12" N, 34°18'06" E, 1300 m, 22.III.2018, ♂, leg. Örgel & Yaman; Ankara: Ayaş 10 km SE, Abdülselam Mountain, 39°56'40" N, 32°22'25" E, 1395 m, 11.III.2018, ♂, ♀, leg. Örgel & Yaman, Kızılcahamam, 40°43'43" N, 32°41'15" E, 1647 m, 18.V.2018, 4♀♀, leg. Örgel & Yaman, Kızılcahamam 2 km NE, Eğerlibaş, 40°35'24" N, 32°48'52" E, 1857 m, 18.V.2018, ♂, ♀, leg. Örgel & Yaman, Kızılcahamam, 5 km W Ortaköy, 40°36'15" N, 32°49'56" E, 1750 m, 18.V.2018, ♂, 4♀♀, leg. Örgel & Yaman; Antalya: Akseki 12 km S, 36°58'16" N, 31°44'45" E, 789 m, 10.VII.2011, ♂, leg. Yağmur; Ardahan: Hanak, Sulakçayır, pitfall trap, 11.VII-13.VIII.2012, 3♂♂, 6♀♀, leg. Altın, Hanak, Damal road, pitfall trap, 11.VII-03.IX.2012, 2♂♂, leg. Altın, Çıldır Lake, 41°05'09" N, 43°10'35" E, 1377 m, 13.VII.2012, ♂, leg. Anlaş; Bursa: Karacabey, Langoz Forests, 01.V.2017, 4♂♂, 3♀♀, leg. Yağmur & Kaya; Çankırı: Bayramören, 2 km N Yazıören, 40°57'36" N, 33°06'21" E, 1572 m, 19.V.2018, ♂, leg. Örgel & Yaman, Çerkeş, 7 km S Kısaç, 40°39'40" N, 32°51'18" E, 1690 m, 18.V.2018, 5♂♂, 2♀♀, leg. Örgel & Yaman, Korgun, 3 km SW Çukurören, 40°38'55" N, 33°22'05" E, 1390 m, 20.V.2018, ♀, leg. Örgel & Yaman, Ilgaz Mountains, 41°00'28" N, 33°37'00" E, 1841 m, 27.VI.2016, ♂, 2♀♀, leg. Örgel & Yaman, Ilgaz Mountains, 41°00'37" N, 33°36'14" E, 1750 m, 21.V.2018, ♂, ♀, leg. Örgel & Yaman, Ilgaz Mountains, 41°02'49" N, 33°42'46" E, 1926 m, 21.V.2018, 3♀♀, leg. Örgel & Yaman; Eskişehir: Türkmen Mountain, 39°26'22" N, 30°22'22" E, 1660 m, 27.III.2018, ♀, leg. Örgel & Yaman; Erzincan: Üzümlü, Küçüksarıkaya, 39°42'05" N, 39°50'02" E, 1713 m, 18.V.2011, ♂, 2♀♀, leg. Anlaş; Erzurum: Oltu, Kırdağ, 40°30'20" N, 42°05'09" E, 2332 m, 15.VII.2012, ♀, leg. Yağmur; Gümüşhane: Karaca Cave 2 km NE, 40°33'04" N, 39°24'40" E, 1307 m, 15.V.2011, ♂, leg. Anlaş, Kelkit, Çimenli, 39°58'06" N, 39°22'48" E, 1689 m, 16.V.2011, ♂, 3♀♀, leg. Anlaş, Cehennem Valley, Monastery road, 15.V.2011, ♂, 2♀♀, leg. Anlaş; Kars: Sarıkamış, Soğanlı Mountain, 40°17'23" N, 42°25'46" E, 2330 m, 17.VII.2012, ♀, leg. Altın, Sarıkamış, Bozat 4 km SE, 40°20'37" N, 42°44'59" E, 04.V.2017, 2♂♂, 7♀♀, leg. Altın, Sarıkamış, Soğanlı Martyrdom, 40°22'31" N, 42°29'40" E, 01.V.2017, 8♂♂, ♀, leg. Altın, Sarıkamış, 40°24'44" N, 42°28'12" E, 01.X.2014, ♂, leg. Altın, Sarıkamış, Yağbasan 2 km SW, 40°21'20" N, 42°37'40" E, 05.V.2017, 4♂♂, 2♀♀, leg. Altın; Kayseri: Pınarbaşı, Kaynar, 38°54'18" N, 36°26'44" E, 1593 m, 09.IV.2018, 4♂♂, ♀, leg. Örgel & Yaman, Tuzla Lake, 39°00'55" N, 35°47'12" E, 1168 m, 08.IV.2018, ♀, leg. Örgel & Yaman; Kastamonu: Tosya, Kilkuyu, 40°56'26" N, 34°13'53" E, 1608 m, 10.IV.2017, 4♂♂, 2♀♀, leg. Anlaş, Yağmur & Örgel; Kırıkkale: Bahşili, Sarıkayalar, 39°44'12" N, 33°17'13" E, 1307 m, 07.III.2018, 2♀♀, leg. Örgel & Yaman; Kırşehir: 1 km NW Yağmurluarmutlu, 39°14'30" N, 33°57'04" E, 1300 m, 27.III.2019, 3♂♂, leg. Örgel & Köksal; Konya: Seydişehir, 3 km E Oğlakçı, 37°34'38" N, 32°00'20" E, 1510 m, 06.IV.2019, 2♂♂, leg. Örgel & Köksal, Seydişehir, 5 km NW Dikilitaş, 37°34'40" N, 32°00'21" E, 1554 m, 20.V.2016, ♂, leg. Örgel & Yaman, Seydişehir, 6 km NE Oğlakçı, 37°35'48" N, 32°01'36" E, 1700 m, 06.IV.2019, ♂, ♀, leg. Örgel & Köksal; Nevşehir: Hacıbektaş, 38°56'22" N, 34°37'40" E, 1210 m, 26.III.2018, 2♂♂, leg. Örgel & Yaman; Niğde: Central province, 3 km S Tepeköy, 38°03'03" N, 34°37'19" E, 1798 m, 31.V.2016, ♀, leg. Örgel & Yaman;

Sinop: 41°53'45" N, 34°34'41" E, 94 m, 01.V.2014, ♀, leg. Koç; Sivas: Doğanşar, 40°08'09" N, 37°24'39" E, 1380 m, 14.IV.2019, ♀, leg. Anlaş, Örgel & Köksal, Hafik-Doğanşar road, 40°00'43" N, 37°26'34" E, 1490 m, 14.IV.2019, ♂, leg. Anlaş, Örgel & Köksal, Hafik, Toraç Mountain, 40°12'48" N, 37°18'28" E, 1996 m, 15.IV.2018, 6♂♂, ♀, leg. Anlaş, Örgel & Yaman, Koyulhisar, 3 km E Subaşı, 40°20'39" N, 37°49'37" E, 1790 m, 16.IV.2018, ♂, leg. Anlaş, Örgel & Yaman, Yıldızeli, 39°57'02" N, 36°41'43" E, 1560 m, 12.IV.2019, ♂, ♀, leg. Anlaş, Örgel & Köksal, Yıldızeli, 39°56'58" N, 36°41'29" E, 12.IV.2019, ♂, ♀, leg. Anlaş, Örgel & Köksal, Yıldız Mountain, 40°08'18" N, 36°56'18" E, 1730 m, 13.IV.2019, 5♂♂, 4♀♀, leg. Anlaş, Örgel & Köksal, Zara-Suşehri road, 14.IV.2019, ♂, leg. Anlaş, Örgel & Köksal; Yozgat: Akdağmadeni Mountains, 39°26'38" N, 35°49'53" E, 1800 m, 07.IV.2018, 3♂♂, leg. Örgel & Yaman, Çayıralan, Akdağmadeni Mountains, 39°24'54" N, 35°48'34" E, 1825 m, 07.IV.2018, 2♂♂, leg. Örgel & Yaman, Kadışehri, 40°04'58" N, 35°53'58" E, 1670 m, 11.IV.2019, ♂, ♀, leg. Yağmur, Örgel & Yaman, Kadışehri, 40°04'46" N, 35°54'25" E, 1550 m, 11.IV.2019, ♀, leg. Örgel & Yaman.

Distribution. This species is widespread in Anatolia except for the south of Taurus Mountains and southwestern Anatolia. This species has not been recorded in southwestern Anatolia and is rare in the south of the Taurus Mountains (Anlaş, 2009; Assing, 2009, 2010, 2015; Japoshvili & Anlaş, 2011; Sert et al., 2015) (Figure 6).

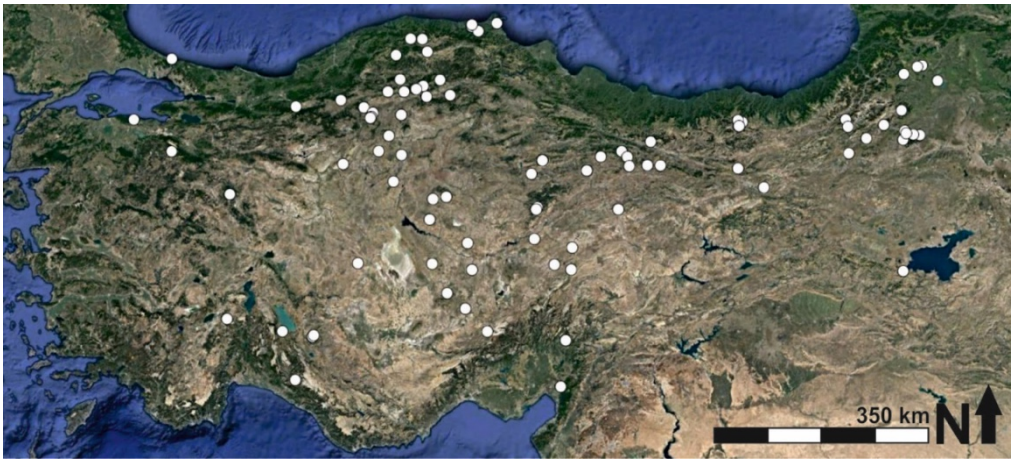

Figure 6. Distribution of *Drusilla canaliculata* in Turkey (Records of Coiffait (1978), Assing (2005, 2006, 2008, 2009, 2010, 2015), Japoshvili & Anlaş (2011) and Sert et al. (2015) are included with present paper records).

Key to the Turkish species of *Drusilla*

The key in Assing (2005, 2007) is modified as follows:

1. Pronotum, elytra and anterior of abdomen usually reddish; larger species. Widespread in Anatolia, except that south and southwestern ……..……..... *D. canaliculata* (Fabricius, 1787)

- Body distinctly darker in most species; smaller species ……..……..……..… 2.

2. Puncturation less densely and more finely in pronotum ……..…….....…….…… 3.

- Puncturation densely and granulosely in pronotum ……..……..…….....…. 9.

3. Elytral suture approximately 0.65 times as long as pronotum. East of Central Amanos Mountains (Hatay) ……..……..…….....…. *D. cernens* Assing, 2005

- Elytral suture most about 0.60 times as long as pronotum ……..…..……..……..…… 4.

4. Antennae apically darkened; elytra usually without impressions ……..……..……... 5.

- Antennae uniformly yellowish or reddish; elytra usually with impressions ……..……..…..……..… 6.

5. Femora of legs usually infuscate ……..…..…… 7.

- Legs uniformly yellowish brown; posterior margin of tergite VIII in both sexes serrate and concave; apex of median lobe of aedeagus bent ventrally in lateral view; bulbus distalis of spermatheca large, duct medium length. West of North Amanos Mountains and a locality of Southeastern Anatolia Region (Gaziantep, Kahramanmaraş, Mardin, Osmaniye) ……..…….. *D. sinuosa* Assing, 2005

6. Antennomere I not infuscate; posterior margin of ♂ tergite VIII without serrate and wide but not deep concave; posterior margin of ♀ tergite VIII with weak serrate and narrower concave than ♂; bulbus distalis of spermatheca larger than all Turkish *Drusilla* species, duct thinner than *D. sinuosa*. Amik Plain, where is east of Amanos Mountains (Hatay) ……..…….....…….…… *D. pallidicornis* Assing, 2005

- Antennomere I infuscate ……..…….…..…… 8.

7. Posterior margin of tergite VIII in both sexes shallow concave and weak serrate; lateral margin on the side facing the ventral in apex of median lobe of aedeagus straight in lateral view; bulbus distalis of spermatheca small, duct short. Sertavul Pass, where is border of Western and Central Taurus Mountains (Karaman, Mersin) ……..……...…….…..…… *D. anceps* Assing, 2005

- Posterior margin of tergite VIII in both sexes deeper concave and stronger serrate; lateral margin on the side facing the ventral in apex of median lobe of aedeagus bent ventrally in lateral view; bulbus distalis of spermatheca bigger, duct taller. Around the southern part of the Amanos Mountains (Hatay) ……..….. *D. denigrata* Assing, 2005

8. Posterior margin of tergite VIII in both sexes very shallow concave and weak serrate; lateral margin on the side facing the ventral in apex of median lobe of aedeagus straight in lateral view; bulbus distalis of spermatheca almost the same width as the duct, duct tall. Bey Mountains in the Western Taurus, Davraz Mountain and west of the Dim Valley in Geyik Mountains (Antalya, Isparta, Karaman) ……..…… *D. limata* Assing, 2005

- Posterior margin of tergite VIII in both sexes very deeper concave and weak or not serrate; lateral margin on the side facing the ventral in apex of median lobe of aedeagus convex in lateral view and bent ventrally; bulbus distalis of spermatheca almost the wider than the duct, duct shorter. East of the Dim Valley in the Geyik Mountains and the west of the Bolkar Mountains in the Central Taurus. (Antalya, Karaman, Mersin) ……..…….. *D. recta* Assing, 2005

9. Apex of median lobe of aedeagus weak bent ventrally in lateral view; bulbus distalis of spermatheca dilated transversely in longitudinal section. Aydın and Bozdağ Mountains (Aydın, İzmir, Manisa) ..…..……..…….……..……..……..……..……. *D. lydica* Assing, 2007

- Lateral margin on the side facing the ventral in apex of median lobe of aedeagus straight in lateral view; bulbus distalis of spermatheca circular in longitudinal section. East Menteşe Mountains (Muğla) ……..…..…… *D. anlasi* sp. n.

Acknowledgments

I am most grateful to Dr. Sinan Anlaş (Manisa, Turkey) for his helpful comments on the scientific content of the manuscript, also, Dr. Ersen Aydın Yağmur (Manisa, Turkey) and Dr. Halil Koç (Sinop, Turkey) for their gift to staphylinid samples collected during their field trips, in addition to, Serkan Yaman (Manisa, Turkey) and Doruk Köksal (Aydın, Turkey) for their help in my field trips.

References

Anlaş, S., 2009. Distributional checklist of the Staphylinidae (Coleoptera) of Turkey, with new and additional records. Linzer Biologische Beiträge, 41 (1): 215-342.

Assing, V., 2005. On the western Palaearctic species of *Drusilla* Leach, with special reference to the species of the eastern Mediterranean (Coleoptera: Staphylinidae, Aleocharinae). Koleopterologische Rundschau, 75: 111-149.

Assing, V., 2006. New species and records of Staphylinidae from Turkey IV, with six new synonymies (Coleoptera: Staphylinidae). Koleopterologische Rundschau, 76: 223-276.

Assing, V., 2007. New species and additional records of Staphylinidae from Turkey V (Coleoptera). Stuttgarter Beiträge zur Naturkunde, Serie A (Biologie) 700: 1-64.

Assing, V., 2008. A revision of the Western Palaearctic and Middle Asian species of *Drusilla* Leach. IV. A new species from Iran and additional records (Coleoptera: Staphylinidae, Aleocharinae, Lomechusini). Entomologische Blätter, 103/104: 51-58.

Assing, V., 2009. New species and additional records of Lomechusini from the Palaearctic region (Coleoptera: Staphylinidae: Aleocharinae). Stuttgarter Beiträge zur Naturkunde, Serie A Neue Serie 2: 201-226.

Assing, V., 2010. Two new species and additional records of Lomechusini from the Palaearctic region (Coleoptera: Staphylinidae: Aleocharinae). Linzer Biologische Beiträge, 42 (2): 1093-1104.

Assing, V., 2013. On the Staphylinidae (Coleoptera) of Turkey IX. Five new species, a new synonymy, and additional records. Stuttgarter Beiträge zur Naturkunde, A Neue Serie 6: 103-125.

Assing, V., 2015. New species and additional records of Lomechusini from the Palaearctic region, primarily from China (Coleoptera: Staphylinidae: Aleocharinae). Contributions to Entomology, 65 (2): 243-262.

Assing, V., 2017. On the Lomechusini fauna of the East Palaearctic and Oriental regions, with a focus on the genera *Orphnebius* and *Amaurodera* (Coleoptera: Staphylinidae: Aleocharinae). Contributions to Entomology, 67 (1): 63-106.

Coiffait, H., 1978. Staphylinides récoltés par T. Deuve en Anatolie septentrionale (Col. Staph.). Nouvelle Revue d'Entomologie, 8 (2): 163-175.

Hanley, R. S. & J. S. Ashe, 2003. Techniques for dissecting adult aleocharine beetles (Coleoptera: Staphylinidae). Bulletin of Entomological Research, 93 (1): 11-18.

Japoshvili, G. & S. Anlaş, 2011. Notes on the family Staphylinidae (Coleoptera) collected by pitfall traps in Gölcük, Isparta province of Turkey. Journal of Entomological Research Society, 13 (1): 41-48.

Schülke, M. & A. Smetana, 2015. "Staphylinidae, 304-1134". In: Catalogue of Palaearctic Coleoptera. Volume 2. Hydrophiloidea - Staphylinoidea Revised and Updated (Eds. I. Löbl & D. Löbl). Brill, Boston, USA, 1702 pp.

Sert, O., Y. Turan, S. Fırat & B. Şabanoğlu, 2015. Faunistic composition, ecological properties and zoogeographical composition of the Subfamily Aleocharinae (Coleoptera: Staphylinidae) of the Central Anatolian Region of Turkey. Transactions of the American Entomological Society, 141 (1): 197-221.

Türk. entomol. derg., 2020, 44 (3): 305-318
DOI: http://dx.doi.org/10.16970/entoted.702157

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

A laboratory study of the acaricidal, repellent and oviposition deterrent effects of three botanical oils on *Tetranychus urticae* (Koch, 1836) (Acari: Tetranychidae)[1]

Tetranychus urticae (Koch, 1836) (Acari: Tetranychidae)'ye karşı üç bitkisel yağın akarisit, uzaklaştırıcı ve yumurta bırakma engelleyici özellikleri üzerine laboratuvar çalışması

Gizem KESKİN[2] **Nabi Alper KUMRAL[3]** **Oya KAÇAR[4]**

Abstract

The biological activities of essential oil obtained from water distillation process of basil leaves [*Ocimum basilicum* L. (Lamiales: Lamiaceae)] cv Round Midnight and crude oil obtained from the cold-pressed process of chinaberry tree seeds [*Melia azedarach* L. (Sapindales: Meliaceae)] and a commercial neem oil product (Nimbecidine) [*Azadirachta indica* (A. Juss, 1830) (Sapindales: Meliaceae)] were assessed against two-spotted spider mites, *Tetranychus urticae* (Koch, 1836) (Acari: Tetranychidae) using a residual method on leaf disc under laboratory conditions at Bursa Uludağ University during 2018-2019. The lethal concentrations (LC_{50} and LC_{90}) of Nimbecidine, chinaberry and basil oils were estimated as 0.8 and 1.8 mg/L, 4.0 and 6.9%, 5.4 and 11.7%, respectively, 72 h after treatment. The lethal times (LT_{50} and LT_{90}) of Nimbecidine (1 mg/L), chinaberry (6%) and the basil (8.4%) were 64 and 107 h, 41 and 73 h, 65 and 110 h, respectively. The females had a strong aversion to bean leaf surfaces sprayed with the sublethal concentrations of Nimbecidine (0.125-0.75 mg/L), chinaberry (0.75-3%) and basil (0.7-1.4%) oils. Significant decreases were recorded in the number of eggs laid on bean leaves sprayed with the sublethal concentrations for Nimbecidine (0.031-0.5 mg/L), chinaberry (0.75-3%) and the basil (1.4-5.6%) oils compared with unsprayed bean leaves. The study showed that the assessed concentrations of the oils obtained from the basil and chinaberry compared to the commercial botanical product (Nimbecidine) have similar biological effects on *T. urticae.*

Keywords: Acaricide, biological effects, chinaberry, basil, neem, spider mite

Öz

Mor reyhan [*Ocimum basilicum* L. 1753 'Round Midnight' (Lamiales: Lamiaceae)] yapraklarından su distilasyonuyla ve tesbih ağacı [*Melia azedarach* L., 1753 (Sapindales: Meliaceae)] tohumlarından soğuk presleme yöntemi ile elde edilen yağların ve karşılaştırma materyali olarak ticari bir neem yağı formülasyonun (Nimbecidine) [*Azadirachta indica* (A. Juss, 1830) (Sapindales: Meliaceae)], İkinoktalı kırmızıörümcek *Tetranychus urticae* (Koch, 1836) (Acari: Tetranychidae) üzerindeki biyolojik etkileri, yaprak diski üzerinde kuru kalıntı yöntemi kullanılarak Bursa Uludağ Üniversitesi'nin laboratuvar koşullarında 2018-2019 yıllarında değerlendirilmiştir. Uygulamadan 72 saat sonra, Nimbecidine, tesbih ağacı ve mor reyhanın öldürücü konsantrasyonları (LC_{50} ve LC_{90}) sırasıyla 0.8 ve 1.8 mg/L, %4.0 ve 6.9, %5.4 ve 11.7 olarak tespit edilmiştir. Nimbecidine (1 mg/L), tesbih ağacı (%6) ve mor reyhanın (%8.4) belirlenen konsantrasyonlarının öldürücü zamanları (LT_{50} ve LT_{90}) 64 ve 107 s, 41 ve 73 s, 65 ve 110 s olarak belirlenmiştir. Nimbecidine (0.125-0.75 mg/L), tesbih ağacı (%0.75-3) ve mor reyhanın (%0.7-1.4) öldürücü altı dozlarıyla ilaçlanan fasulye yaprak yüzeylerinden *T. urticae* dişilerinin güçlü bir şekilde kaçtıklarını göstermiştir. Nimbecidine (0.031-0.5 mg/L), tesbih ağacı (%0.75-3) ve mor reyhanın (%1.4-5.6) sublethal dozlarının uygulandığı yapraklarda akarların yumurta bırakma sayıları kontrole göre önemli şekilde azalmıştır. Bu çalışma tesbih ağacı ve mor reyhandan elde edilen yağların belirlenen konsantrasyonlarının neemin ticari formülasyonu olan Nimbecidine gibi biyolojik etkiler gösterebileceğini ortaya koymuştur.

Anahtar sözcükler: Akarisit, biyolojik etkiler, tesbih ağacı, mor reyhan, neem, kırmızıörümcek

[1] This study is a part of Gizem Keskin's MSc research at the University of Bursa Uludağ, Graduate School of Natural and Applied Science, Entomology Department.

[2] Bursa Uludağ University, Institute of Natural and Applied Science, Department of Entomology, 16059, Bursa, Turkey
[3] Bursa Uludağ University, Agricultural Faculty, Department of Plant Protection, 16059, Bursa, Turkey
[4] Bursa Uludağ University, Agricultural Faculty, Department of Field Crops, 16059, Bursa, Turkey
* Corresponding author (Sorumlu yazar) e-mail: akumral@uludag.edu.tr
Received (Alınış): 13.03.2020 Accepted (Kabul ediliş): 25.04.2020 Published Online (Çevrimiçi Yayın Tarihi): 26.05.2020

Introduction

The two-spotted spider mite, *Tetranychus urticae* (Koch, 1836) (Acari: Tetranychidae), is a common critical pest in several kinds of cultivated crops (Helle & Sabelis, 1985). The high population of *T. urticae* causes spotty yellowing, curling also drying in leaves and thus decreases the productivity. Various synthetic acaricides are used to control the mite in greenhouses and fields. The purpose of the application of these chemicals is to reduce the mite population, but this strategy is not sustainable for agriculture, because, the widespread use of acaricides can cause some ecological problems: (1) destroying non-target beneficial organisms, (2) threaten human health with their residues on food, and (3) developing resistance to these chemicals by pests (Nauen et al., 2001; Kim et al., 2004; Simon, 2014; Salman & Keskin, 2019; Kumral et al., 2020). Due to these negative impacts, researchers focused on searching for environmentally safe natural pesticides with low toxicity to non-target organisms (Nicoletti et al., 2012; Fernandes et al., 2019). For example, neem oil, derived from the seed of the neem tree [*Azadirachta indica* (A. Juss) (Sapindales: Meliaceae)], has specific biological effects, such as lethality, insect growth disrupter, repellency, feeding and oviposition deterrent, on mite and insect pests, because it contains various biologically active limonoids, such as azadirachtin, nimbin, salannin, azadirachtol, nimbidin and gedunin (Martinez-Villar et al., 2005; Isman, 2006; Nicoletti et al., 2012). Given that neem has multiple biological but low side effects, various formulations with different trademarks (e.g., Nemazal, Nimbecidine, Nimiks, Suhulet, Ozoneem) have been registered and used against spider mites, mouths, thrips, flies and aphids in Turkey and many countries (Immaraju, 1998; Anonymous, 2020).

In this study, as an alternative to neem oil, the biological activities of the essential oils of basil leaves [*Ocimum basilicum* L.t (Lamiales: Lamiaceae) cv. Round Midnight] and crude oil of chinaberry tree seeds [*Melia azedarach* L. (Sapindales: Meliaceae)] were investigated against *T. urticae*. Chinaberry tree is often confused with *A. indica*, also known as neem tree belonging to the same plant family. However, *A. indica* has some limitations being adapted to tropical and subtropical regions and altitudes not higher than 1000 m (Forim et al., 2010). Whereas, chinaberry trees can grow in subtropical regions in eastern parts of Australia and Asia, and are common in the Mediterranean basin of Turkey. This species is often planted in parks, public gardens, and along stream banks, footpaths and roadsides as an ornamental tree because of its dense canopy, fragrant lilac flowers and yellow fruits (Erdem, 2019). Like neem, limonoids, triterpenoids and steroids obtained from *M. azedarach*, have antifeedant and insecticidal effects on some arthropod pests (Nakatani et al., 1995; Castiglioni et al., 2002; Banchio et al., 2003; El-Sawi, 2008; Sharma et al., 2010; Attia et al., 2011; Yanar et al., 2011a, b; Akihisa et al., 2013; Elkertati et al., 2013). Although there have been some toxicological studies on *T. urticae* with extracts from different parts of *M. azedarach*, none were performed with the oil obtained from cold-pressed seeds or investigated the oils repellence and oviposition deterrent effects.

The genus *Ocimum* (Lamiaceae) has 65 species naturally distributed across Asia, Africa and Central America (Paton et al., 1999). The morphological features of *O. basilicum* and their chemical contents have wide variation (Marotti et al., 1996; Simon et al., 1999; Vieira & Simon, 2000; Labra et al., 2004). It is widely used in spices, pharmaceuticals, food, and perfumery industries due to its valuable essential oil and fragrance. Also, essential oil of basil has increasing importance due to the biological effects, such as antimicrobial, antifungal, insecticide, and antioxidant (Deshpande & Tipnis, 1977; Prasad et al., 1986; Bassiouny et al., 1990; Marotti et al., 1996; Zollo et al., 1998). Also, basil cv. Round Midnight is an essential source of anthocyanins for the food industry (Simon et al., 1999). Although a few toxicological studies of other basil cultivars on *T. urticae* and stored product pests, the toxic, oviposition deterrent and repellent effects on *T. urticae* have not been previously investigated (Keita et al., 2000; Aslan et al., 2004; Martins et al., 2016; Kasap & Kök, 2019). In this study, acaricidal and adverse biological effects of *M. azedarach* (chinaberry) and *O. basilicum* (basil cv. Round Midnight) on *T. urticae* were compared with a commercial neem oil product (Nimbecidine) under laboratory conditions.

Materials and Methods

Spider mite culture

A laboratory colony of *T. urticae* was used in this experiment. The colony was maintained without exposure to any chemicals for 10 years. Mites were reared on bean plants [*Phaseolus vulgaris* L. (Fabales: Fabaceae)] cv. Magnum from MayAgro Seed Corp., Bursa, Turkey, at 27±1ºC, 65% RH and 16:8 h L:D photoperiod in a controlled environment room.

Botanical test materials

Basil cv. Round Midnight used in this study was obtained from Medicinal and Aromatic Plants Research Area, Department of Field Crops, Agricultural Faculty, University of Bursa Uludağ during the growing season of 2018. Commercial seeds of this cultivar were obtained from a private company (Anadolu Seed Production and Marketing Inc., Turkey). The seeds were sown at the beginning of March in a greenhouse and when the seedlings had reached 10 cm high, they were transferred to the field (4 May 2018) on a 30 × 25 cm grid. During the vegetation period, plots were irrigated as needed. The dark violet leaves together stems were harvested in August at 50% flowering. The leaves were air-dried in the dark, then the leaves and stems were separated. The extraction of the essential oils from 50 g of dry leaves was done by hydro distillation with using a modified Clevenger-type apparatus for 2 h. The oil was stored in dark glass bottles at 4ºC (Marotti et al., 1996). The essential oil content was determined by the volumetric method (v/w) and expressed as a percentage (Wichtl, 1971). Mean essential oil content of dry leaf was 0.4-0.5%. Previous studies have shown that this genotype has a linalool chemotype (Kaçar et al., 2009). The fruits of chinaberry tree (*M. azedarach*) were collected by Greenza Company (Bursa, Turkey) from an ornamental planting of Adana (Turkey) in October 2018 during the ripening period. The fruits were dried indoors and seeds separated from the kernel with a knife. Paste from these seeds was obtained using a cold-pressed machine (under 40ºC) developed by Greenza Company and then pure oil was obtained from this paste by filtering. As a commercial neem oil (*A. indica*) product, Nimbecidine (0.3 g/L azadirachtin) was provided from Manufacturer Company (VitaLonga, AgroBest Grup, Izmir, Turkey).

Toxicity tests

To determine toxicity, bioassays were performed under laboratory conditions using a leaf disc in Petri dish (120 mm diameter) method following Keskin & Kumral (2015). Briefly, each bean leaf disc (90 mm diameter) was put on warm agar solutions (2%) poured into a Petri dish. The lid of Petri dish was pierced with a steel needle ensured the ventilation. A range of concentrations of the chinaberry and basil oils were prepared in 25% ethanol. Nimbecidine was diluted with distilled water. Two ml of different concentrations have applied the underside of the leaf disc with spray tower adjusted to 1.5 kg/cm^2 working pressure and 3-s delivery (Potter precision, Burkard Manufacturing Co. Ltd., Rickmansworth, UK). Leaf discs were then dried at room condition for 15-30 min (Potter, 1952). In each bioassay, six concentrations, three replicates and one control (25% ethanol for chinaberry and basil oils; distilled water for Nimbecidine) were used. The concentrations caused 10-90% mortality was used as an acaricide. Female *T. urticae* were collected from a synchronized colony reared from same-aged eggs. Twenty newly emerged females were transferred on sprayed leaf discs with a brush. To prevent the escape of the mites, the dishes were sealed with Parafilm. The Petri dishes were kept at 27±1ºC, 65% RH and 16:8 h L:D photoperiod in a controlled environment room. The Petri dish was checked after 24, 48, 72 and 96 h under a stereomicroscope. Mites unable to move when touched with a brush were considered dead. To determine the lethal times (LT_{50} and LT_{90}), nearly LC_{90} concentration (within confidence limits) at 96 h (1 mg/L of Nimbecidine, 8.4% of basil and 6% of chinaberry) were sprayed as described above, and mortality results were determined every 12 h.

Oviposition deterrent tests

The methodology using Petri dishes was similar to the toxicity test, except, based on toxicity test, sublethal and lethal concentrations were used in this test: Nimbecidine (0.031, 0.125 and 0.5 mg/L), basil (1.4, 2.8 and 5.6%) and chinaberry (0.75, 1.5 and 3%). After applying the oils, a teliochrysalid female and an adult male obtained from a synchronized colony were placed onto a leaf disc (90 mm diameter). For each concentration, 12 females were monitored until 20% of the females had died (Simon, 2014). The number of eggs laid by females was determined daily and removed. The females on leaves sprayed with distilled water for Nimbecidine and with 25% ethanol for others were used as control treatments. The Petri dishes were placed with the same conditions as above.

Repellency test

Repellency bioassays were performed using a two-choice leaf disc in the Petri dish method modified by van den Boom et al. (2003). Briefly, one bean leaf disc with the same diameter within plastic Petri dishes (120 mm diameter) was then sprayed with different concentrations of Nimbecidine (0.25, 0.5 and 0.75 mg/L), basil (0.7 and 1.4%) and chinaberry (0.75, 1.5 and 3%) under the Potter precision spray tower at same settings as the toxicity test. The other Petri dish was treated with distilled water for Nimbecidine and 25% ethanol for others. After spraying, the Petri dishes were dried for 15-30 min at room temperature. Then, the two Petri dishes were connected with a silicon tube (length of each side: 5 cm, width: 1 cm). The outlet of silicon tube was in contact with the leaves so the mites could walk on to the leaves easily. Twenty newly emerged females were individually transferred into an opening in the middle of the tube and the opening was sealed with Parafilm. If the mite moved from the tube to either leaf and started feeding, the chosen leaf was recorded as the preference of the mites, 24, 48 and 72 h after spraying. The experiment was repeated three times on different days.

Statistical Analysis

Mortality percentages and fecundity decreasing percentages were corrected using control percentages with Abbott's formula (Abbott, 1925). Lethal concentrations (LC_{50} and LC_{90} values), lethal times (LT_{50} and LT_{90}) and fecundity decreasing concentrations (EC_{50} and EC_{90}) of each test materials were estimated by Probit analysis (Finney, 1971) with SPPS software (version 23, IBM Corporation, Armonk, NY, USA). Then, the logistic regression analysis was performed for 72-h exposure data. The response variable for an individual mite was then either 1 if dead, or 0 if not (JMP, version 7.0.2, SAS Institute, NC, USA). For the oviposition deterrent test, differences in egg-laying per female were tested by repeated measured variance analysis (MANOVA) followed by a post hoc Tukey's HSD test (JMP, version 7.0.2). The normality of the means was tested with the Shapiro-Wilk test using a SPPS software. Non-normal data were log-transformed before applying the MANOVA. Pearson's X^2 test was used in repellency tests. In the test, it was expressed as the null hypothesis if the number of females exhibited a 50:50 distribution across the oil and solvent sprayed surfaces in each replicate (van den Boom et al., 2002).

Results

Acute toxicity effects

Basil, chinaberry oils and Nimbecidine killed *T. urticae* females at different concentrations and in different times (Table 1). Twenty-four h after applying chinaberry oil, LC_{50} and LC_{90} were 6.29 and 9.11%, respectively. The median and high lethal concentrations were decreased significantly in both 72 and 96 h after treatment. In Nimbecidine bioassays, the LC_{50} and LC_{90} were 4.26 and 7.79 mg/L, respectively, determined 24 h after spraying. Similarly, after 48 h and 96 h, a significant decrease was recorded in these lethal concentrations. After 96 h, the LC_{50} and LC_{90} were 0.41 and 0.97 mg/L, respectively. In the basil tests, the LC_{50} and LC_{90} were 8.16 and 13.8%, respectively, 24 h after applying the oil. This lethal

concentration significantly decreased by 72 h after treatment. Eventually, after 96 h, the LC_{50} and LC_{90} were 3.19 and 9.62%, respectively. Based on simple logistic regression analyses, all test materials significantly increased the death rate of female mites compared with the control (Figure 1). Moreover, these toxic effects depended on the concentration (chinaberry X^2=238, P<0.01; Nimbecidine X^2=123, P<0.01; the basil X^2=53.5, P<0.01).

Table 1. Lethal concentrations and lethal times of chinaberry (*Melia azedarach),* Nimbecidine (*Azadirachta indica*) and basil cv. Round Midnight (*Ocimum bacillium*) on *Tetranychus urticae* females

Chinaberry n[8]	Time (hour) 24		48		72		96	
	% concentration (confidential limits)							
[1]LC_{50}	6.29 (5.65-7.24)	a[7]	5.20 (4.65-5.95)	a	4.04 (3.59-4.59)	b	2.34 (1.91-2.62)	c
[2]LC_{90}	9.11 (7.98-11.06)	a	8.31 (7.31-9.85)	a	6.94 (6.14-8.09)	a	4.65 (4.03-5.58)	b
[3]EC_{50}	1.90 (1.31-2.52)	a	0.96 (0.43-1.55)	ab	0.71 (0.01-1.28)	b	0.63 (0.43-0.83)	b
[4]EC_{90}	4.66 (3.77-6.35)	a	1.89 (1.38-3.83)	ab	1.97 (1.38-4.03)	ab	1.17 (0.95-1.57)	b
	hour (confidential limits)							
[5]LT_{50}	41.44 (37.47-45.68)							
[6]LT_{90}	72.68 (66.25-81.33)							
Nimbecidine n[8]	Time (hour) 24		48		72		96	
	mg/L (confidential limits)							
[1]LC_{50}	4.26 (2.49-27.44)	a	1.15 (0.99-1.35)	b	0.79 (0.57-1.21)	b	0.41 (0.32-0.52)	c
[2]LC_{90}	7.79 (4.40-2.71)	a	2.14 (1.84-2.59)	b	1.83 (1.35-3.05)	b	0.97 (0.79-1.28)	c
[3]EC_{50}	0.44 (0.17-0.70)	a	0.11 (0.06-0.16)	b	0.08 (0.06-0.11)	b	0.07 (0.02-0.16)	b
[4]EC_{90}	1.04 (0.76-1.77)	a	0.34 (0.26-0.51)	b	0.18 (0.14-0.27)	b	0.22 (0.14-1.35)	b
	hour (confidential limits)							
[5]LT_{50}	63.74 (57.48-70.33)							
[6]LT_{90}	107.37 (96.63-124.18)							
Midnight basil n[8]	Time (hour) 24		48		72		96	
	% concentration (confidential limits)							
[1]LC_{50}	8.16 (7.56-10.03)	a	7.61 (6.66-8.88)	a	5.40 (4.77-6.16)	b	3.19 (2.37-4.05)	c
[2]LC_{90}	13.82 (11.99-16.73)	a	13.7 (11.79-16.40)	a	11.7 (10.37-13.57)	a	9.62 (8.07-12.16)	a
[3]EC_{50}	1.77 (1.29-2.30)	a	1.28 (0.51-1.95)	ab	0.96 (0.21-1.74)	ab	0.97 (0.59-1.28)	b
[4]EC_{90}	3.93 (3.18-5.37)	a	3.56 (2.69-5.69)	ab	2.69 (1.88-5.21)	ab	2.15 (1.73-2.87)	b
	hour (confidential limits)							
[5]LT_{50}	64.53 (59.25-70.17)							
[6]LT_{90}	109.53 (100.94-120.73)							

[1] EC: Effective concentration; concentration that killed 50% of mite population; [2] Concentration that killed 90% of mite population;
[3] LC: Lethal concentration; concentration decreasing 50% of mite oviposition; [4] Concentration decreasing 90% of mite oviposition;
[5] LT: Lethal time; time in which 50% of mite population was killed with 6% of chinaberry, 1 mg/L of Nimbecidine and %8.4 of basil;
[6] Time in which 90% of mite population were killed with 6% of chinaberry, 1 mg/L of Nimbecidine and %8.4 of basil;
[7] The same letter within a row indicates no statistical differences based on confidence limits; [8] n (number of individuals)=360.

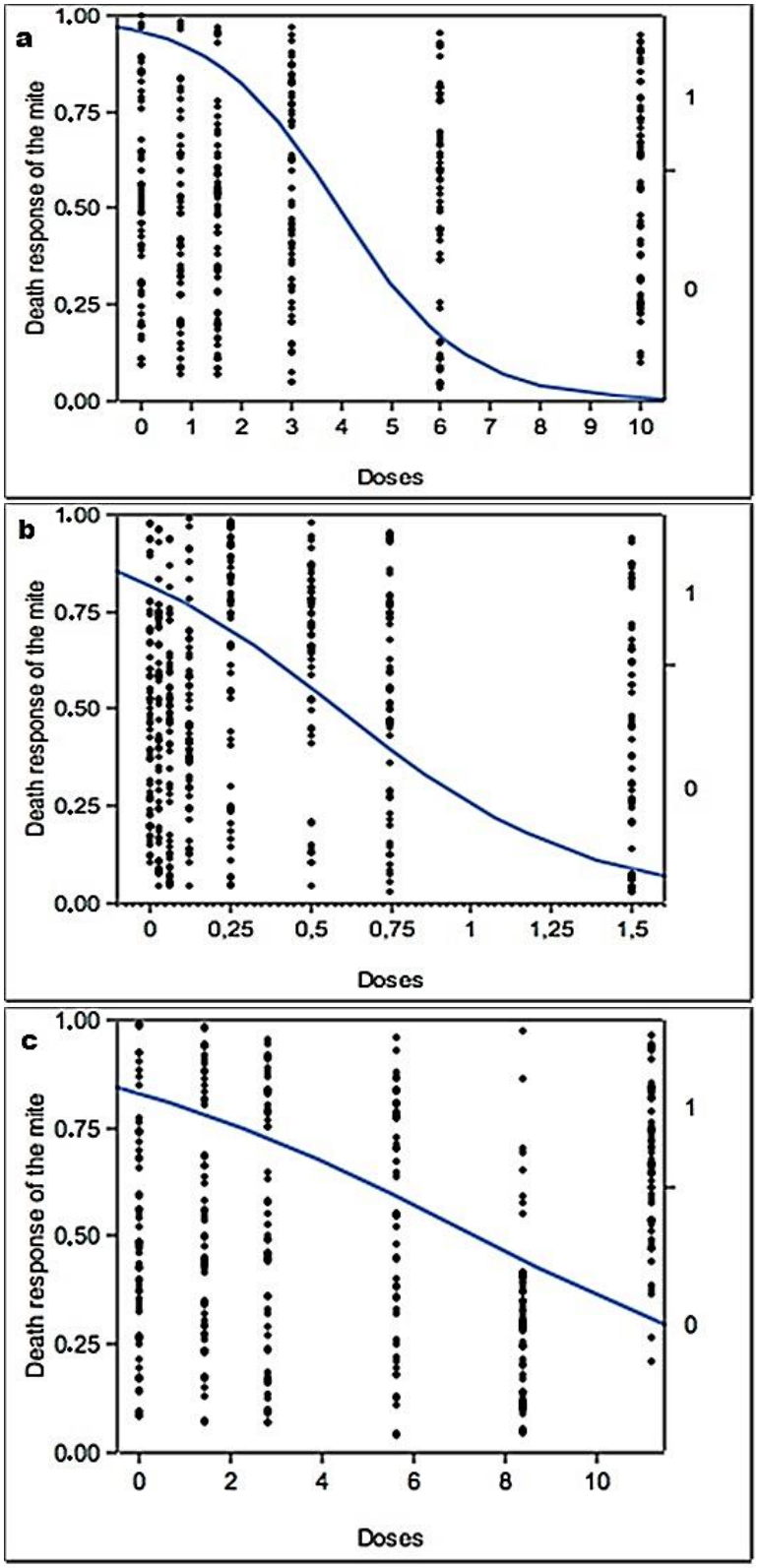

Figure 1. The logistic fit of the death response of *Tetranychus urticae* females by concentrations of a) chinaberry (*Melia azedarach*), b) Nimbecidine (*Azadirachta indica*) and c) basil cv. Round Midnight (*Ocimum bacillium*) at 72 h. "1" and "0" indicated alive and dead mites indicated, respectively.

The lethal times for determining concentrations of chinaberry, Nimbecidine and basil are summarized in Table 1. The median lethal times (LT_{50}) for chinaberry, Nimbecidine and the basil were 41.4, 63.7 and 64.5 h, respectively. The concentrations of chinaberry, Nimbecidine and basil killed 90% of the mite population after 72.7, 107 and 110 h, respectively.

Oviposition deterrent effects

The average numbers of eggs laid by females on leaves sprayed with different concentrations of the botanical oils and solvents are shown in Figure 2. After 24 h, a significant decrease was observed in the oviposition of females exposed to 3% chinaberry compared with ethanol (control). Similar significant reductions in egg-laying were recorded for females exposed to 1.5% of chinaberry, after 48 h ($F_{19,40}$=44.0, P<0.01) (Figure 2). Also, the exposure time was negatively related to the fecundity of the mite exposed to 0.75, 1.5 and 3% chinaberry (time $F_{7,7}$=40.7, P<0.01; concentration $F_{3,3}$=74.5, P<0.01). The effective concentrations (EC_{50} and EC_{90}) were estimated as 0.63 and 1.17%, respectively, 96 h after spraying chinaberry. Based on the confidence limits, the concentrations were decreased significantly after 48 h (Table 1). In Nimbecidine bioassays, significant differences between the water-treated control females and exposed to 0.125 and 0.5 mg/L Nimbecidine treated females after 24 h ($F_{24,50}$=28.3, P<0.01) (Figure 2). Additionally, exposure time for the concentrations (0.125 and 0.5 mg/L) was reduced significantly for egg-laying (time $F_{7,7}$=19.9, P<0.01; concentration $F_{3,3}$=80.4, P<0.01). The EC_{50} and EC_{90} at 96 h were 0.07 and 0.22%, respectively. Similarly, a significant decrease in the oviposition deterrent concentrations was determined after 48 h (Table 1). Similar significant differences between mean egg-laying of females on ethanol-treated and basil oil-treated leaves were found after 24 h ($F_{19,40}$=74.7, P<0.01) (Figure 2). Similar to other compounds, the exposure time for all tested concentrations was significant in terms of reducing the egg-laying (time $F_{7,7}$=16.3, P<0.01; concentration $F_{3,3}$=93.5, P<0.01). Also, EC_{50} and EC_{90} for basil at 96 h were 0.97 and 2.15%, respectively. Similarly, the oviposition deterrent concentrations were significantly lower after 48 h (Table 1).

Repellency effects

The repellency test results for different concentrations of chinaberry, Nimbecidine and basil are shown in Figures 3, 4 and 5. For each replicate, the two concentrations of chinaberry (1.5 and 3%) showed significant repellent activity at 24, 48 and 72 h (for 3% X^2=38.4, 56.6, 19.8, 33.6, 40.5, 27.4, 31.4, 42.5 and 44.4, P<0.01; for 1.5% X^2=39.1, 21.8, 16.0, 8.16, 18.4, 25.0 and 7.84; P<0.01). While two of the replicates at the 0.75% concentration displayed high avoidance of chinaberry (X^2=14.4, 23.4, 7.8, 25.0, 7.8 and 24.0; P<0.01), one showed no significant effect (Figure 3). Nimbecidine avoidance was significant for all replicates of all test concentrations (for 0.125 mg/L X^2=51.8, 70.6, 33.6, 43.6, 70.6, 36, 43.6, 70.6 and 29.2; P<0.01; for 0.5 mg/L X^2=40.9, 11.6, 21.2, 12.9, 4.8, 16.0 and 9.0, P<0.01; for 0.75 mg/L X^2=31.4, 60.8, 38.4, 21.2, 25.0, 31.4, 17.6, 25.0 and 25.0, P<0.01;), except one replicate in 0.5 mg/L concentration at 48 h (Figure 4). Avoidance rates for both concentrations of basil were significant for all replicates and times (for 0.7% X^2=100, 31.4, 11.6, 51.8, 21.2, 51.8, 11.6, 25.0 and 31.4, P<0.01; for 1.4% X^2=100, 54.8, 84.6, 100, 16.0, 60.8, 64.0, 64.0 and 81.0, P<0.01) (Figure 5).

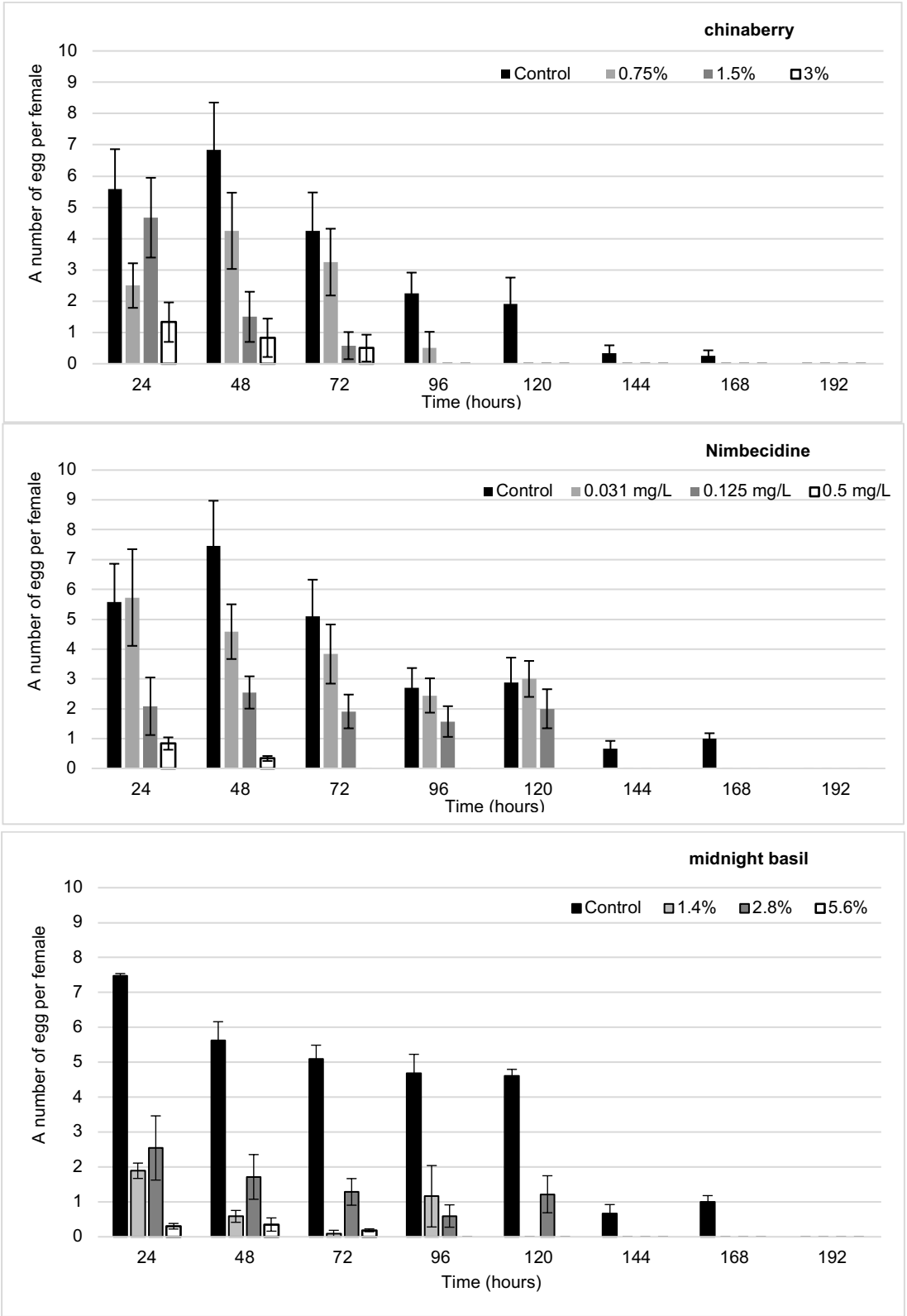

Figure 2. The egg-laying activity of *Tetranychus urticae* females at different concentrations of chinaberry (*Melia azedarach),* Nimbecidine (*Azadirachta indica*) and basil cv. Round Midnight (*Ocimum bacillium*).

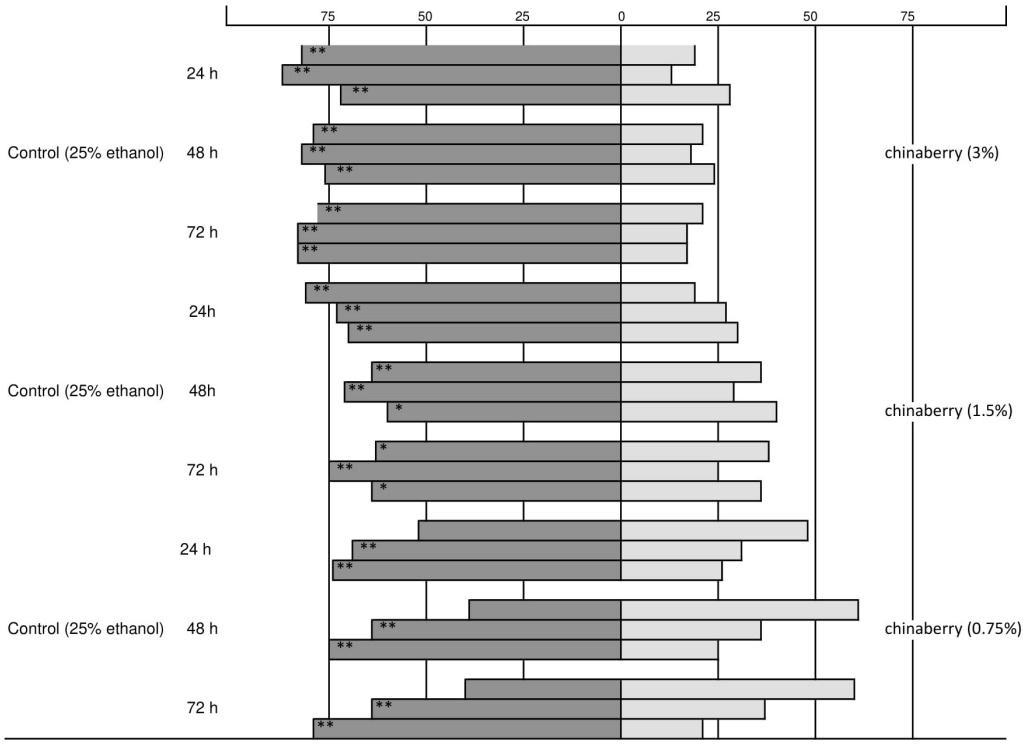

Figure 3. Percentage avoidance of the area sprayed with chinaberry (*Melia azedarach*) by *Tetranychus urticae* after 24, 48, 72 h (* $P<0.05$, ** $P<0.01$). Each column in each 24 h shows replicates for each concentration.

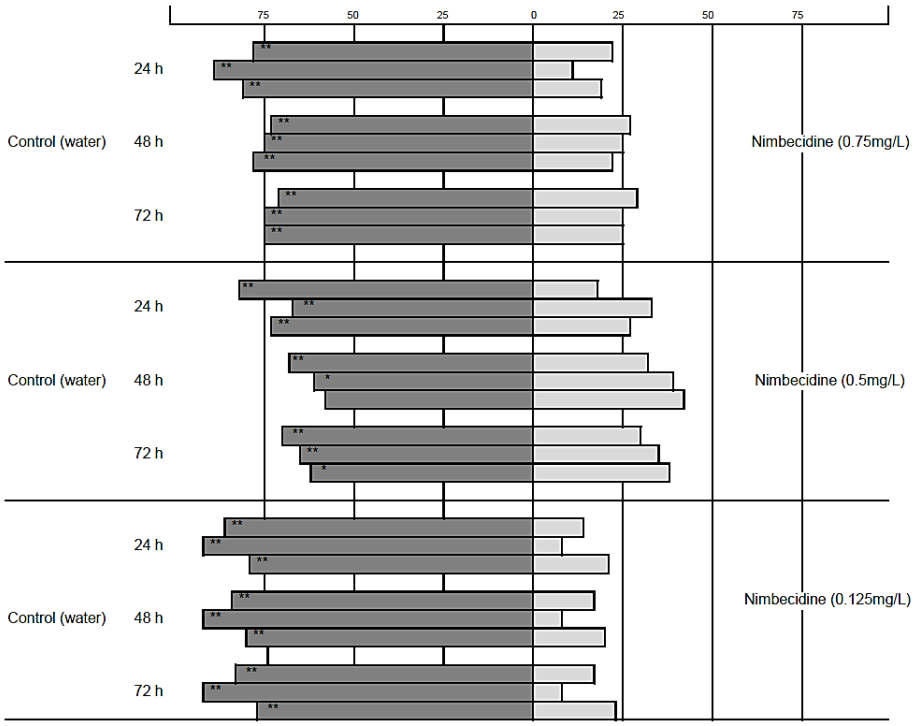

Figure 4. Percentage avoidance of the area sprayed with Nimbecidine (*Azadirachta indica*) by *Tetranychus urticae* after 24, 48, 72 h (* $P<0.05$, ** $P<0.01$). Each column in each 24 h shows replicates for each concentration.

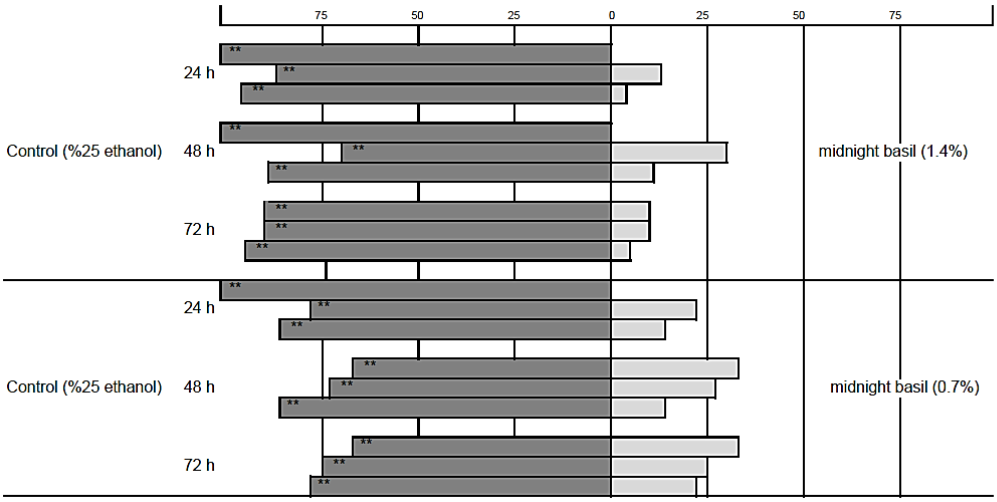

Figure 5. Percentage avoidance of the area sprayed with basil (*Ocimum bacillium*) cv. Round Midnight by *Tetranychus urticae* after 24, 48 and 72 h (* P<0.05, ** P<0.01). Each column in each 24 h shows replicates for each concentration.

Discussion

This showed that all test materials had lethal effects on *T. urticae* females depending on concentration and time. With the same conclusion, Elkertati et al. (2013) demonstrated that dichloromethane and ethanol extracts of oil obtained from chinaberry seeds were highly toxic to *T. urticae.* Similar acaricidal effects on *T. urticae* were reported by El-Sawi (2008) for an extract obtained from chinaberry leaves. An aqueous seed extract of chinaberry was found highly toxic to adults of tea red spider mite [*Oligonychus coffeae* (Nietner, 1861) (Acari: Tetranychidae)] with 56-96% mortality (Roy & Mukhopadhyay, 2012). The study demonstrated that the concentration (1.8 mg/L azadirachtin a.i.) of Nimbecidine killed 90% of *T. urticae* females 72 h after treatment. The recommended concentration (0.5 mg/L azadirachtin a.i.) of the formulation for other pests in Turkey killed 90% of *T. urticae* females at 107 h after application. With the similar results, the acaricidal effects of different formulations containing neem on *T. urticae* have also reported by numerous researchers (Sundaram & Sloane, 1995; Martínez-Villar et al., 2005; Pavela, 2009; Deka et al., 2011; Tehri & Gulati, 2014). In the present study, 9.6% basil oil killed 90% of the females within 100-121 h. Similarly, some researchers found that essential oils extracted from different cultivars of the basil had acute toxicity to *T. urticae* under greenhouse conditions (Refaat & Momen, 2002; Mateeva et al., 2003; Aslan et al., 2004; Pavela et al., 2016; Traka et al., 2018). Refaat & Momen (2002) reported that 0.5 and 2% basil oil effected *T. urticae* survival with rates of 80-100%. The main compounds contributing to significant mortality of *T. urticae* were determined to be linalool (66.5%), eugenol (18.9%) and eucalyptol (7.1%) (Traka et al., 2018).

The present study showed that all compounds had oviposition deterrent effects on *T. urticae* females depending on concentration and time. Moreover, the sublethal doses of all three compounds reduced the lifespan of *T. urticae* females compared to the control. Similarly, some other researchers have reported that seed extracts of chinaberry had deterrent effects on the fecundity of *T. urticae* (El-Sawi, 2008, Yanar et al., 2011a, b). In accordance with our results, commercial formulations of *A. indica* significantly reduced the oviposition of *T. urticae* (Sundaram & Sloane, 1995; Martínez-Villar, 2005). Our results are similar to those of some authors, who reported the negative impact of a sweet basil cultivar on the oviposition activity of the spider mite (Refaat & Momen, 2002; Pavela et al., 2016).

The two-choice experiments with *T. urticae* showed that females preferred non-oil treated surfaces over the solvent-only treated areas with sublethal concentrations of chinaberry, Nimbecidine and basil. Our results are in accordance with those of El-Sawi (2008), who showed that the extracts of the chinaberry leaves prepared with the various solvents had a repellent effect on *T. urticae*. Additionally, *A. indica* and/or its formulations were found to have repellent and antifeedant properties against some mite species, such as *T. urticae, Tetranychus cinnabarinus* (Boisd., 1867) (Acari: Tetranychidae) and *Euseius alatus* De Leon, 1966 (Acari: Phytoseiidae) (Sundaram & Sloane, 1995; Brito et al., 2006; Hummel et al., 2012). Similarly, Refaat & Momen (2002) showed that a sweet basil cultivar had repellency effects on *T. urticae*. Also, two studies showed minimal *T. urticae* preference to sweet basil volatiles, probably due to the dominant occurrence of the oxygenated hydrocarbon compounds camphor, caryophyllene oxide, cineole, methyl eugenol, limonene, myrcene, and thymol, all known insect repellents (Chokechaijaroenporn et al., 1994; Refaat & Momen, 2002).

The toxic, oviposition deterrent and repellency effects of all test materials were showed for *T. urticae* in this study. These effects could be due to the presence of certain alkaloids, terpenoids, flavonoids, other oxygenated hydrocarbon compounds that are responsible for many of the insecticidal and/or acaricidal properties of plants (Lee et al. 1991; Chokechaijaroenporn et al., 1994; Refaat & Momen, 2002; Maciel et al., 2006; López et al., 2008; Zheljazkov et al., 2008; Chiffelle et al., 2009; Elkertati et al., 2013; Martins et al., 2016; Pavela et al., 2016; Sharopov et al., 2016). Thus, the results of this study showed that the assessed concentrations of oils obtained from the basil and chinaberry compared to a commercial botanical product (Nimbecidine) have similar biological effects on *T. urticae*. The rapid degradation potential of botanical pesticides encourages the use of these acaricides for plant protection. In the future, field studies should be conducted to investigate their potential acaricidal effects under natural conditions.

Acknowledgements

We thank Agrobest Grup (Izmir, Turkey) for supply Nimbecidine materials and Greenza (Bursa, Turkey) for supply chinaberry material and provide the oil extractions.

References

Abbott, W. S., 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18 (2): 265-267.

Akihisa, T., X. Pan, Y. Nakamura, T. Kikuchi, N. Takahashi, M. Matsumoto, E. Ogihara, M. Fukatsu, K. Koike & H. Tokuda, 2013. Limonoids from the fruits of *Melia azedarach* and their cytotoxic activities. Phytochemistry, 89 (1): 59-70.

Anonymous, 2020. Republic of Turkey, Ministry of Food, Agriculture and Livestock, General Directorate of Food and Control, Department of Plant Protection Products. Plant Protection Products Database. (Web page: https://bku.tarim.gov.tr/Arama/Index) (Date accessed: March 2020).

Aslan, I., H. Özbek, Ö. Çalmaşur & F. Şahin, 2004. Toxicity of essential oil vapours to two greenhouse pests, *Tetranychus urticae* Koch and *Bemisia tabaci* Genn. Industrial Crops and Products, 19 (2): 167-173.

Attia, S., K. L. Grissa, G. G. Zeineb, A. C. Mailleux, G. Lognay & T. Hance, 2011. Assessment of the acaricidal activity of several plant extracts on the phytophagous mite *Tetranychus urticae* (Tetranychidae) in Tunisian citrus orchards. Bulletin de la Société Royale Belge d'Entomologie= Bulletin van de Koninklijke Belgische Vereniging voor Entomologie, 147 (1): 71-79.

Banchio, E., G. Valladares, M. Defago, S. Palacios & C. Carpinella, 2003. Effects of *Melia azedarach*, (Meliaceae) fruit extracts on the leafminer *Liriomyza huidobrensis*, (Diptera, Agromyzidae): Assessment in laboratory and field experiments. Annals of Applied Biology, 143 (2): 187-193.

Bassiouny, S. S., F. R. Hassanien, A. Faer & S. M. El-Kayati, 1990. Efficiency of antioxidants from natural sources in bakery products. Food Chemistry, 37 (4): 297-305.

Brito, H. M., J. M. G. Gondim, J. V. D. Oliveira & C. A. da Câmara, 2006. Toxicidade de formulações de nim (*Azadirachta indica* A. Juss.) ao ácaro-rajado ea *Euseius alatus* De Leon e *Phytoseiulus macropilis* (Banks) (Acari: Phytoseiidae). Neotropical Entomology, 35 (4): 500-505.

Castiglioni, E., J. D. Vendramim & M. A. Tamai, 2002. Evaluación del efecto tóxico de extractos acuosos y derivados de meliáceas sobre *Tetranychus urticae* (Koch) (Acari, Tetranychidae). Agrociencia-Sitio en Reparación, 6 (2): 75-82.

Chiffelle, I., F. A. Huerta & R. D. Lizana, 2009. Physical and chemical characterization of *Melia azedarach* L. fruit and leaf for use as botanical insecticide. Chilean Journal of Agricultural Research, 69 (1): 38-45.

Chokechaijaroenporn, O, N. Bunyapraphatsara & S. Kongchuensin, 1994. Mosquito repellent activities of *Ocimum* volatile oils. Phytomedicine, 1 (2): 135-139.

Deka, S., R. K. Tanwar, R. Sumitha, N. Sabir, O. M. Bambawale & B. Singh, 2011. Relative efficacy of agricultural spray oil and azadirachtin against two-spotted spider mite (*Tetranychus urticae*) on cucumber (*Cucumis sativus*) under greenhouse and laboratory conditions. Indian Journal of Agricultural Sciences, 81 (2): 158-162.

Deshpande, R. S. & H. P. Tipnis, 1977. Insecticidal activity of *Ocimum basilicum* Linn. Pesticides, 11 (5): 11-12.

El-Sawi, S. A., 2008. Toxicity and bioactivity of *Melia azedarach* L. on the two spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae), and its predacious mite, *Euseius scutalis* (Athais-Henriot) (Acari: Phytoseiidae). Egyptian Journal of Biological Pest Control, 18 (2): 289-295.

Elkertati, M., A. Blenzar, A. B. Jotei, I. Belkoura & B. Tazi, 2013. Acaricide effect of some extracts and fractions on *Tetranychus urticae* Koch (Acari: Tetranychidae). African Journal of Agricultural Research, 8 (23): 2970-2976.

Erdem, T., 2019. Some chemical properties of infrared dried Neem fruit in Turkey. Turkish Journal of Agriculture-Food Science and Technology, 7 (7): 958-962.

Fernandes, S. R., L. Barreiros, R. F. Oliveira, A. Cruz, C. Prudêncio, A. I. Oliveira, C. Pinho, N. Santos & J. Morgado, 2019. Chemistry, bioactivities, extraction and analysis of azadirachtin: State-of-the-art. Fitoterapia, 134: 141-150.

Finney, D. J., 1971. Probit Analysis, 3rd Edition. Cambridge University Press, London, UK, 333 pp.

Forim, M. R., V. E. Cornélio, M. F. das GF da Silva, E. Rodrigues-Filho, J. B. Fernandes, P. C. Vieira, S. S. Matinez, M. P. Napolitano & R. A. Yost, 2010. Chemical characterization of *Azadirachta indica* grafted on *Melia azedarach* and analyses of azadirachtin by HPLC-MS-MS (SRM) and meliatoxins by MALDI-MS. Phytochemical Analysis, 21 (4): 363-373.

Helle, W. & M. W. Sabelis, 1985. Spider Mites: Their Biology, Natural Enemies and Control B. World Crop Pests 1B. Elsevier, Amsterdam, 458 pp.

Hummel, H. E., D. F. Hein & H. Schmutterer, 2012. The coming of age of azadirachtins and related tetranortriterpenoids. Journal of Biopesticides, 5 (S): 82-87.

Immaraju, J. A., 1998. The commercial use of azadirachtin and its integration into vial pest control programmes. Pest Science, 54 (3): 285-289.

Isman, M. B., 2006. Botanical insecticides, deterrents, and repellents in modern agricultural and an increasingly regulated world. Annual Review of Entomology, 51 (1): 45-56.

Kaçar, O., E. Göksu & N. Azkan, 2009. Agronomic properties and essential oil composition of basil varieties and landraces (*Ocimum basilicum* L.) in Turkey. Asian Journal of Chemistry, 21 (4): 3151-3160.

Kasap, İ. & Ş. Kök, 2019. Determination of the insecticide effect of some plant extracts on two spotted spider mite, *Tetranychus urticae* Koch. COMU Journal of Agricultural Faculty, 7 (1): 137-144.

Keita, S. M., C. Vincent, J. P. Schmit, S. Ramaswamy & A. Bélanger, 2000. Effect of various essential oils on *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Journal of Stored Products Research, 36 (4): 355-364.

Keskin, N. & N. A. Kumral, 2015. Screening tomato varietal resistance against the two-spotted spider mite [*Tetranychus urticae* (Koch)]. International Journal of Acarology, 41 (4): 300-309.

Kim, Y. J., S. H. Lee, S. W. Lee & Y. J. Ahn, 2004. Fenpyroximate resistance in *Tetranychus urticae* (Acari: Tetranychidae): cross-resistance and biochemical resistance mechanisms. Pest Management Science, 60 (10): 1001-1006.

Kumral, A. Y., N. A. Kumral & O. Gürbüz, 2020. Chlorpyrifos and deltamethrin degradation potentials of two *Lactobacillus plantarum* (Orla-Jensen, 1919) (Lactobacillales: Lactobacillaceae) strains. Turkish Journal of Entomology, 44 (2): 165-176.

Labra, M., M. Miele, B. Ledda, F. Grassi, M. Mazzei & F. Sala, 2004. Morphological characterization, essential oil composition and DNA genotyping of *Ocimum basilicum* L. cultivars. Plant Science, 167 (4): 725-731.

Lee, S. M., J. A. Klocke, M. A. Barnby, R. B. Yamasaki & M. F. Balandrin, 1991. "Insecticidal Constituents of *Azadirachta indica* and *Melia azedarach* (Meliaceae), 293-304". In: Naturally Occurring Pest Bioregulators (Ed. P. Heddin). ACS Publications, American Chemical Society, USA, 434 pp.

López, M. D., M. J. Jordán & M. J. Pascual-Villalobos, 2008. Toxic compounds in essential oils of coriander, caraway and basil active against stored rice pests. Journal of Stored Products Research, 44 (3): 273-278.

Maciel, M. V., S. M. Morais, C. M. L. Bevilaqua, A. L. F. Camurça-Vasconcelos, C. T. C. Costa & C. M. S. Castro, 2006. Ovicidal and larvicidal activity of *Melia azedarach* extracts on *Haemonchus contortus*. Veterinary Parasitology, 140 (1-2): 98-104.

Marotti, M., R. Piccaglia & E. Giovanelli, 1996. Differences in essential oil composition of basil (*Ocimum basilicum* L.) Italian cultivars related to morphological characteristics. Journal of Agriculture and Food Chemistry, 44 (12): 3926-3929.

Martinez-Villar, E., F. J. Saenz-De-Cabezon, F. Moreno-Grijalba, V. Marco & I. P. Moreno, 2005. Effects of azadirachtin on the two-spotted spider mite, *Tetranychus urticae* (Acari: Tetranychidae). Experimental and Applied Acarology, 35 (3): 215-222.

Martins, M. I. G., A. E. G. Sant'Ana, F. M. T. Vasconcelos, W. L. Silva, L. M. Lima, R. Carvalho, P. A. Melo Filho & R. C. Santos, 2016. Bioactivity of basil (*Ocimum basicilum* L.) on control of the spider mite (*Tetranychus urticae* Koch.) in peanut. African Journal of Biotechnology, 15 (30): 1597-1607.

Mateeva, A. A., C. Christov, S. Stratieva & N. Palagatscheva, 2003. "Alternative plant protection means against *Tetranychus urticae* Koch., 259-26". Second International Symposium on Plant Health in Urban Horticulture (27-29 August 2003, Berlin, Germany), 304 pp.

Nakatani, M., R. C. Huang, H. Okamura, T. Iwagawa, K. Tadera & H. Naoki, 1995. Three new antifeeding meliacarpinins from Chinese *Melia azedarach* Linn. Tetrahedron, 51 (43): 11731-11736.

Nauen, J., N. Stumpf, A. Elbert, C. P. W. Zebitz & W. Kraus, 2001. Acaricide toxicity and resistance in larvae of different strains of *Tetranychus urticae* and *Panonychus ulmi* (Acari: Tetranychidae). Pest Management Science, 57 (3): 253-261.

Nicoletti, M., S. Mariani, O. Maccioni, T. Coccioletti & K. Murugan, 2012. Neem cake: chemical composition and larvicidal activity on Asian tiger mosquito. Parasitology Research, 111 (1): 205-213.

Paton, A., R. M. Harley & M. M. Harley, 1999. "*Ocimum*: An Overview of Classification and Relationships, 11-46". In Basil: The Genus *Ocimum* (Eds. R. Hiltunen & Y. Holm), Harwood Academic Publishers, Netherlands, 160 pp.

Pavela, R., 2009. Effectiveness of some botanical insecticides against *Spodoptera littoralis* Boisduvala (Lepidoptera: Noctudiae), *Myzus persicae* Sulzer (Hemiptera: Aphididae) and *Tetranychus urticae* Koch (Acari: Tetranychidae). Plant Protection Science, 45 (4): 161-167.

Pavela, R., E. Stepanycheva, A. Shchenikova, T. Chermenskaya & M. Petrova, 2016. Essential oils as prospective fumigants against *Tetranychus urticae* Koch. Industrial Crops and Products, 94: 755-761.

Potter, C., 1952. An improved apparatus for applying direct sprays and surface films with data on the electrostatic charge on atomized spray fluids. Annals of Applied Biolology, 39 (1): 1-28.

Prasad, G., A. Kuman, A. K. Singh, A. K. Bhattacharya, K. Singh & V. D. Sharma, 1986. Antimicrobial activity of essential oils of some *Ocimum* species and clove oil. Fitoterapia, 57 (6): 429-432.

Refaat, M. & F. M. Momen, S. A. A. Amer, 2002. Acaricidal activity of sweet basil and French lavender essential oils against two species of mites of the family Tetranychidae (Acari: Tetranychidae). Acta Phytopathologica et Entomologica Hungarica, 37 (1-3): 287-298.

Roy, S. & A. Mukhopadhyay, 2012. Bioefficacy assessment of *Melia azedarach* (L.) seed extract on tea red spider mite, *Oligonychus coffeae* (Nietner) (Acari: Tetranychidae). International Journal of Acarology, 38 (1): 79-86.

Salman, S. Y. & C. Keskin, 2019. Contact toxicity of pine, laurel and juniper essential oils to spirodiclofen-resistant and-susceptible *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) populations. Turkish Journal of Entomology, 43 (1): 97-105.

Sharma, A., U. Chauhan & P. R. Gupta, 2010. Bioefficacy of some biopesticides and its effect on various biological parameters of two spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychydiae) on sweet pepper *Capsicum annum*. Journal of Entomological Research, 34 (3): 195-202.

Sharopov, F. S., P. Satyal, N. A. Ali, S. Pokharel, H. Zhang, M. Wink, M. A. Kukaniev & W. N. Setzer, 2016. The essential oil compositions of *Ocimum basilicum* from three different regions: Nepal, Tajikistan, and Yemen. Chemistry and Biodiversity, 13 (2): 241-248.

Simon, J. E., M. R. Morales, W. B. Phippen, R. F. Vieira & Z. Hao, 1999. Basil: a source of aroma compounds and a popular culinary and ornamental herb. Perspectives on New Crops and New Uses, 16: 499-505.

Simon, Y. J., 2014. The Toxicology and Biochemistry of Insecticides. CRC press, London, UK, 276 pp.

Sundaram, K. M. S. & L. Sloane, 1995. Effects of pure and formulated azadirachtin, a neem-based biopesticide, on the phytophagous spider mite, *Tetranychus urticae* Koch. Journal of Environmental Science & Health Part B, 30 (6): 801-814.

Tehri, K. & R. Gulati, 2014. Field efficacy of some biorationals against the two spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae). Journal of Applied and Natural Science, 6 (1): 62-67.

Traka, C. K., E. A. Petrakis, A. C. Kimbaris, M. G. Polissiou & D. C. Perdikis, 2018. Effects of *Ocimum basilicum* and *Ruta chalepensis* hydrosols on *Aphis gossypii* and *Tetranychus urticae*. Journal of Applied Entomology, 142 (4): 413-420.

van den Boom, C. E., T. A. Van Beek & M. Dicke, 2002. Attraction of *Phytoseiulus persimilis* (Acari: Phytoseiidae) towards volatiles from various *Tetranychus urticae*-infested plant species. Bulletin of Entomological Research, 92 (6): 539-546.

van den Boom, C. E., T. A. Van Beek & M. Dicke, 2003. Differences among plant species in acceptance by the spider mite *Tetranychus urticae* Koch. Journal of Applied Entomology, 127 (3): 177-183.

Vieira, R. F. & J. E. Simon, 2000. Chemical characterization of basil (*Ocimum* spp.) found in the markets and used in traditional medicine in Brazil. Economic Botany, 54 (2): 207-216.

Wichtl, M., 1971. Die Pharmakognostisch-Chemische Analyse Akad. Verlagsgesellschaft, Frankfurt, Germany, 479 pp.

Yanar, D., I. Kadioglu & A. Gökçe, 2011a. Acaricidal effects of different plant parts extracts on two-spotted spider mite (*Tetranychus urticae* Koch). African Journal of Biotechnology, 10 (55): 11745-11750.

Yanar, D., I. Kadıoglu & A. Gökce, 2011b. Ovicidal activity of different plant extracts on two-spotted spider mite (*Tetranychus urticae* Koch) (Acari: Tetranychidae). Scientific Research and Essays, 6 (14): 3041-3044.

Zheljazkov, V. D., C. L. Cantrell, W. B. Evans, M. W. Ebelhar & C. Coker, 2008. Yield and composition of *Ocimum basilicum* L. and *Ocimum sanctum* L. grown at four locations. HortScience, 43 (3): 737-741.

Zollo, P. H. A., L. Biyiti, F. Tchoumbougnang, C. Menut, G. Lamaty & P. Bouchet, 1998. Aromatic plants of tropical Central Africa. Part XXXII. Chemical composition and antifungal activity of thirteen essential oils from aromatic plants of Cameroon. Flavour and Fragrance Journal, 13 (2): 107-114.

Türk. entomol. derg., 2020, 44 (3): 319-331
DOI: http://dx.doi.org/10.16970/entoted.650742

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Neonicotinoid resistance in adults and nymphs of *Bemisia tabaci* (Genn., 1889) (Hemiptera: Aleyrodidae) populations in tomato fields from Tokat, Turkey[1]

Tokat (Türkiye) domates alanlarındaki *Bemisia tabaci* (Genn., 1889) (Hemiptera: Aleyrodidae) ergin ve nimf popülasyonlarında neonikotinoid direnci

Tarık BALKAN[2*] **Kenan KARA[2]**

Abstract

Bemisia tabaci (Genn., 1889) (Hemiptera: Aleyrodidae) is one of the most important agricultural pests in Turkey and in the world. This polyphagous pest is a highly efficient vectors of plant viruses and has the ability to rapidly develop resistance to diverse range of insecticides, hence controlling this pest is problematic. In this study, bioassays and biochemical tests were conducted to determine resistance to neonicotinoid in *B. tabaci* populations collected in 2017-2018 from Tokat (Turkey). According to the adult test results, resistance ratios for acetamiprid, imidacloprid and thiamethoxam were 5.64-16.8, 10.0-30.9 and 4.01-14.9, respectively. The highest resistance ratio for acetamiprid and thiamethoxam in the Pazar population were 16.8 and 14.9, respectively. The highest resistance ratio to imidacloprid was 30.9 in the TOGU campus population. According to the nymph test results, resistance ratios for acetamiprid, imidacloprid and thiamethoxam were 2.96-8.60; 4.29-8.74 and 2.48-4.88, respectively. Enzyme analysis revealed statistically higher metabolic resistance. Maximum enzyme activities were 4.37 and 3.79 pmol/min/mg protein for cytochrome P450 monooxygenase in TOGU campus and Pazar populations, respectively.

Keywords: Acetamiprid, *Bemisia tabaci*, imidacloprid, insecticide resistance, thiamethoxam

Öz

Bemisia tabaci (Genn., 1889) (Hemiptera: Aleyrodidae) Türkiye ve dünyadaki en önemli tarım zararlılarından biridir. Bu polifag zararlı oldukça etkili bir bitki virüs vektörüdür ve çeşitli insektisitlere karşı hızla direnç geliştirme kabiliyetine sahiptir, bu yüzden zararlıyı kontrol etmek zordur. Bu çalışma 2017-2018 yıllarında Tokat (Türkiye)'tan toplanan farklı *B. tabaci* popülasyonlarının neonikotinoid grubu insektisitlere karşı direnç durumunu biyoassay ve biyokimyasal yöntemlerle belirlemek amacıyla yapılmıştır. Ergin testlerinde ortaya çıkan sonuçlara göre acetamiprid, imidacloprid ve thiamethoxam için direnç oranları, sırasıyla 5.64-16.8; 10.0-30.9 ve 4.01-14.9 arasındadır. Acetamiprid ve thiamethoxam için en yüksek direnç oranı, Pazar popülasyonunda sırasıyla 16.8 ve 14.9 'dur. Imidacloprid'e en yüksek direnç ise TOGU kampüs popülasyonunda 30.9'dur. Nimf testlerinde ortaya çıkan sonuçlara göre acetamiprid, imidacloprid ve thiamethoxam için direnç oranları, sırasıyla 2.96-8.60; 4.29-8.74 ve 2.48-4.88 arasındadır. Enzim analizi istatistiki anlamda yüksek metabolik direnci ortaya çıkarmıştır. Her üç insektisit içinde, TOGU kampüs ve Pazar popülasyonlarında en yüksek monooksigenaz P450 enzim aktivitesi sırasıyla 4.37 ve 3.79 pmol/dk/mg protein bulunmuştur.

Anahtar sözcükler: Acetamiprid, *Bemisia tabaci*, imidacloprid, insektisit direnci, thiamethoxam

[1] This study was a part of the PhD thesis of the first author and supported by Tokat Gaziosmanpasa University, Scientific Research Unit, Grant Project No: 2016/45. This study was presented as an oral presentation at the II. International Agriculture, Environment and Health Congress (ICEAH) (18-19 October 2019, Aydın, Turkey).
[2] Tokat Gaziosmanpaşa University, Faculty of Agriculture, Department of Plant Protection, 60250, Tokat, Turkey
* Corresponding author (Sorumlu yazar) e-mail: tarik.balkan@gop.edu.tr
Received (Alınış): 25.11.2019 Accepted (Kabul ediliş): 06.05.2020 Published Online (Çevrimiçi Yayın Tarihi): 08.06.2020

Introduction

Bemisia tabaci (Genn., 1889) (Hemiptera: Aleyrodidae) is one of the most important pests worldwide (Anonymous, 2019a). This species was initially described by Gennadius in 1889 as *Aleyrodes tabaci* (Thomas, 2001). *Bemisia tabaci* was first recorded in Turkey in 1928 (Ulusoy et al., 1996; Ulusoy, 2001). It damages more than 600 host plants belonging to 63 families worldwide (Taylor, 2011).

Both adults and nymphs of *B. tabaci* suck the plant sap and severely reduce plant growth and health. In addition, during feeding, the honeydew that forms a sticky film on the leaves after a time supports sooty mold growth. This reduces the quality of the product and its market value. More importantly, *B. tabaci* is an important virus vector of more than 300 plant viruses that cause serious economic damage and major crop losses (Bedford et al., 1993, 1994; Markham et al., 1994; Paul et al., 2011; Gilbertson et al., 2015).

Bemisia tabaci has been recognized as highly cryptic species complex and recorded 24 biotypes which differ in host range, host plant adaptability, induction of phytotoxic reactions, insecticide resistance and virus-transmission capabilities among biotypes. However, biotype B and Q, two common biotypes, are particularly important plant pests (Boykin, 2014). B and Q biotypes have been identified in studies in Turkey (Bayhan et al., 2006; Ulusoy et al., 2007; Topakcı & Göçmen, 2011; Karut et al., 2012, 2014; Satar & Ulusoy, 2016).

This pest has an extraordinary potential to develop resistance to different insecticides (Denholm et al., 1998). Six hundred and thirty-one records of resistance in *B. tabaci* have been reported in the world, 250 of which are related to neonicotinoid group chemicals.There are 59 active ingredients in these records (APRD, 2019).

Neonicotinoids are the most widely used insecticides in the world. This group includes acetamiprid, clothianidin, dinotefuran, imidacloprid, nitenpyram, thiacloprid and thiamethoxam. They have reached a share of around 25% in the global pesticide market with a monetary value of around 2.63 billion USD (Jeschke et al., 2011).

Neonicotinoids are highly effective insecticides that control many important pests (Nauen et al., 2008; Jeschke et al., 2011). These have been used effectively against various kinds of insect pests by different treatments in more than 120 countries for 25 years (Nauen et al., 2008; Bass et al., 2015). These chemicals target (nAChRs) in the insect central nervous system and are effective against a wide range of target species (Anonymous, 2019b). Neonicotinoids are selective agonists of the nicotinic acetyl choline receptors in the central nervous system of insects (Jesche et al., 2011). The mode of action classification scheme of the Insecticide Resistance Action Committee (IRAC) lists seven commercial neonicotinoids in Group 4A (nAChR agonists) (Bass et al., 2015).

There are two major resistance mechanisms to insecticides in insect pests such as whiteflies. These are target site resistance and metabolic resistance. It has been determined that especially monooxygenase activity (P450) is caused by neonicotinoid resistance (Karunker et al., 2008; Roditakis et al., 2011; Nauen et al., 2015; Bass et al., 2015; Satar et al., 2018).

Continuous use of neonicotinoids has led to resistance in white flies. The resistance in *B. tabaci* has become a serious problem in various regions of the USA, European countries, China, Israel, Pakistan, including in Iran and Turkey over the last 25 years (Cahill et al., 1996; Elbert & Nauen, 2000; Nauen et al., 2002, 2008; Byrne et al., 2003; Horowitz et al., 2004; Roditakis et al., 2005; Feng et al., 2010; Luo et al., 2010; Schuster et al., 2010; Wang et al., 2010; Bahşi et al., 2012; Basit et al., 2013; Smith & Nagle, 2014; Basij et al., 2017; Naveen et al., 2017; Şahin & İkten, 2017; Satar et al., 2018). In agriculture, repeated insecticide applications lead to the development of resistance. It also increases the dependence on chemicals, increases the cost of production significantly and causes concerns in scientific communities (Naranjo & Ellsworth, 2009).

An increasing number of studies on neonicotinoid resistance in *B. tabaci* have been published. However, there is no study that determined the sensitivity of *B. tabaci* populations in tomato grown areas in Tokat Province, Turkey. Tomato is the most commonly produced product in this region and it is grown in 37.8% (~6000 ha) of vegetable production areas (Anonymous, 2019c). Although acetamiprid, imidacloprid and thiamethoxam are licensed against *B. tabaci* nymphs and adults, most of the studies to date have been performed on adult *B. tabaci* individuals (Nauen et al., 2008). In this study, nymph resistance was examined in addition to adults. For this reason, the aim was to determine the level of resistance to acetamiprid, imidacloprid and thiamethoxam in *B. tabaci* nymph and adult populations which are both harmful in tomato cultivation in Tokat Province.

Materials and Methods

Bemisia tabaci populations

Bemisia tabaci populations were collected from tomato production areas in Tokat. Populations were collected in July 2017 and August 2018 (Table 1). *Bemisia tabaci* were collected from at least 10 points in each tomato production area and brought to the laboratory in a cooler box within a few hours. The samples were identified using the keys of Martin et al. (2000).

Table 1. The collection places and dates of *Bemisia tabaci*

Location	Date	Coordinates
Yayladali (Susceptible)	24 July 2017	40.374527, 36.592487
TOGU campus (greenhouse)	25 July 2017	40.332352, 36.474065
Erbaa	26 July 2017	40.733764, 36.465677
Turhal	27 July 2017	40.311277, 36.282048
Zile	28 July 2017	40.215354, 35.651539
Pazar	4 August 2018	40.269830, 36.232960
Central	8 August 2018	40.340024, 36.414255
Niksar	17 August 2018	40.529501, 36.908518
Guryildiz	27 August 2018	40.341306, 36.363476

Insecticides and chemicals

Insecticides and chemicals

In this study, three neonicotinoid insecticides were selected. Active ingredients, commercial names and modes of action of insecticides used in this investigation are detailed in Table 2. 1,4-Dithioerythritol (DTT) (>98%), 1-chloro-2,4-dinitrochlorobenzene (CDNB) (99%), 7-ethoxycoumarin (99%), bovine serum albumin, ethylene diamine tetraacetic acid (EDTA) (>99%), fast blue RR salt, glutathione reductase, NADPH (97%) (tetrasodium salt), oxidized glutathione (≥98%), reduced glutathione (GSH) (≥98%), sucrose (≥99.5%), Tris-HCL buffer, Triton X-100, Trizma base (≥99.9%), and α-Naphthyl acetate (α-NA) (98%) were purchased from Sigma-Aldrich (St. Louis, MO, USA).

Table 2. Active ingredients and commercial names for neonicotinoids and their mode of action

Active ingredient (a.i)	Commercial name	IRAC mode of action*
Acetamiprid	Mospilan 20 SL, Nippon Soda Co.	Nerve action, Nicotinic acetylcholine receptor (nAChR) competitive modulators (4A)
Imidacloprid	Confidor SC 350, Bayer CropScience	Nerve action, Nicotinic acetylcholine receptor (nAChR) competitive modulators (4A)
Thiamethoxam	Actara 240SC, Sygenta	Nerve action, Nicotinic acetylcholine receptor (nAChR) competitive modulators (4A)

*(IRAC, 2020).

Rearing of *Bemisia tabaci*

Bemisia tabaci populations was reared in a laboratory. The adults were reared on tomato in net-covered cages (50 x 50 x 60 cm) at 25±1ºC, 65±5% RH and 16:8 h L:D photoperiod. The tomato plants were produced at 25±1ºC and 16:8 h L:D photoperiod in a controlled climate room. In 2017, a *B. tabaci* population was collected from Yayladali (Tokat, central) that has not been exposed to insecticide applications. This populations were maintained in a controlled climate room and used as susceptible reference in bioassay.

Bioassays Methods

Adult bioassay

To determine the resistance status of *B. tabaci*, LC_{50} values were determined by modifying the IRAC 008 method (IRAC, 2016a). In this method, the three-leaf tomato plants were dipped in concentrations in six doses (5, 10, 25, 50, 100, 200 mg a.i./L) prepared for each active ingredient and in distilled water (as a control) for 5 s. The material was dried and then placed in glass containers with a bottom drilled diameter of 2-3 mm. In this way, with the help of aspirator, 20 adults were transferred into polystyrene container for bioassay and then the top of the containers were closed with a tulle cloth. In order to prevent the death of the plants in the cups, water was added in a second glass container with holes and these containers were placed in an insectarium at 25±1ºC, 60-70%RH and 16:8 h L:D photoperiod. Bioassays were performed in three replicates. Mortality was recorded after 72 h.

Nymph bioassay

In order to determine the resistance status of *B. tabaci* nymphs, LC_{50} values were determined by modifying the IRAC 016 method (IRAC, 2016b). Each of the leaves of the tomato plant in the same stage was cut into a rectangular shape about 4 x 6 cm in order to form a certain area and placed in empty cabins. Adult whiteflies were collected using the aspirator from the cages, and about 50 insects per leaf were left on plants whose leaves were cut into a rectangular shape. Adult whiteflies were left in cages until they laid eggs (24 h) and then all adults were removed from the cages. The leaves of the plants which were kept for 9 d were taken together with the nymphs and leaf dipping method was applied for 5 s. The rectangular leaves were dipped in six concentrations (5, 10, 25, 50, 100, 200 mg a.i./L) prepared for each active ingredient and in distilled water (as a control) for 5 s, then dried and placed into polystyrene containers drilled to the bottom with a diameter of 2-3 mm. The cups were covered with a thin tulle curtain and left to the insectarium at 25±1ºC and 65±5% RH and 16:8 h L:D photoperiod. Bioassays were performed with three replicates. Nymphal mortality rates (adults were considered alive) were determined seven days after pesticide applications.

Biochemical assays

Bemisia tabaci populations collected from tomato production areas were placed in ice boxes and brought to the laboratory and stored at -80ºC for enzyme analysis. The total protein amounts of *B. tabaci* individuals were determined according to the Bradford (1976) method, in which bovine serum albumin was used as a standard.

Determination of esterase activity

Twenty *B. tabaci* individuals were homogenized by pressing with plastic pestle in Eppendorf tubes containing 100 µl sodium phosphate buffer (0.1 M, pH 7.5) and 0.1% Triton X-100. This homogenate was used as an enzyme source after centrifugation at 10000 g at 4ºC for 5 min. The supernatant taken from the upper portion of the Eppendorf tube as the enzyme source was diluted tenfold with distilled water. Twenty-five µl of supernatant and 25 µl phosphate buffer (0.2 M, pH 6) were added to the microplate cells. In the

study, 30 mg of fast blue RR salt was dissolved in 50 ml of 0.2 M sodium phosphate buffer and 500 µl of 100 mM α-naphtyl acetate was added to this mixture. The substrate solution obtained was added 200 µl to the microplate cells. Enzyme activity was determined with Infinite P200 Pro (Tecan) microplate reader at 23ºC for 10 min at 450 nm (Stumpf & Nauen, 2002). Enzyme readings were made at three-times.

Determination of glutathione S-transferase activity

Glutathione S-transferase (GST) activity was determined using CDNB and GSH as substrate. Thirty *B. tabaci* adults were homogenized in 300 µl of Tris-HCL buffer (0.05 M, pH 7.5). The total reaction volume of each cell of the 96-cell plate with flat bottom was adjusted as 300 µl. As a result, the reaction consisted of 100 µl of supernatant, CDNB in buffer (containing 0.1% v/v ethanol) and reduced GSH (final concentration of 0.4 mM CDNB and 4 mM GSH). The change in absorbance was measured kinetically at 20ºC and 340 nm for 5 min. The non-enzymatic reaction of CDNB and GSH was measured without homogenate as control (Rauch & Nauen, 2003). Enzyme assays were performed in three replicates.

Determination of cytochrome P450 monooxygenase activity

Monooxygenase enzyme activity which is dependent on Cytochrome-P450 was determined by O-deethylation of 7-ethoxycoumarin. Ten mg of *B. tabaci* frozen at -80ºC were homogenized in Na/K phosphate buffer (0.1 M, pH 7.6, 1 mM EDTA, 1 mM DTT, 200 mM sucrose). The homogenate was centrifuged at 5000 g at 4ºC for 5 min, and the obtained liquid fraction was centrifuged at 15,000 g for 15 min, then at 100,000 g for 60 min. The microsomal pellet remaining at the bottom of the Eppendorf tube was remixed in 300 µl buffer and used as an enzyme source. 50 µl of the microsomal fraction and 40 µl of Na/K phosphate buffer (0.1 M, pH 7.6, containing 1 µl of 40 mM 7-ethoxycoumarin in acetone) were placed in cells of 96-cell black plates. The reaction was initiated by adding 10 µl of watery NADPH to each cell. The final concentration was consisted of 1 mM NADPH and 0.4 mM 7-ethoxycoumarin. The plate was shaken and incubated at 30ºC for 30 min. The NADPH which has fluorescence feature was removed by addition of 10 µl of oxidized glutathione (30 mM in water) and 10 of glutathione reductase (0.5 U). The reaction was stopped with 120 µl of 50% acetonitrile in Trizma base buffer (0.05 M, pH 10) after 10 min. The amount of 7-hydroxycoumarin released during the incubation was measured spectrofluorometer (Tecan) (390 extension and final 465 nm). The standard curve of 7-hydroxycoumarin was used to convert the optical density to pmol of product form. For each population, applications were repeated two times and non-microsomal pelleted cells were used as control (Rauch & Nauen, 2003).

Statistical analysis

Probit analyses of the concentration-dependent mortality data were calculated using PoloPlus (LeOra software, Berkley, CA, USA). Resistance ratios (RRs) were obtained by dividing LC_{50} values by the corresponding value for the susceptible population. Data of enzyme activities were subjected to one-way ANOVA, and the means were compared using Tukey's HSD test ($P < 0.05$) (SPSS version 22.0, IBM Corp., Armonk, NY, USA).

Results

LC_{50} and resistance ratios are given in Tables 3 and 4. The reference population was always the most susceptible population.

Resistance of adults

Acetamiprid resistance ratios were determined from 5.64 to 16.8. The most susceptible population was Guryildiz and the most resistant was the Pazar population. Slope values are between 1.42 and 2.51. Erbaa population is considered the most heterogeneous population since it shows the least slope of the regression line.

Imidacloprid resistance ratios ranged from 10.0 to 30.9, while the most susceptible Zile population was found to be the most resistant TOGU campus population. Slope values are between 1.41 and 2.56. The TOGU campus population, which showed the highest RR_{50} (30.9) among all test populations, displayed the slope of the lowest regression line (1.41).

Thiamethoxam resistance ratios were between 4.01 and 14.9, while it was the most susceptible Guryildiz population and the most resistant Pazar population. Slope values are between 1.41 and 2.47. Turhal population is considered the most heterogeneous population since it gave the least slope of the regression line.

Table 3. Log-dose probit mortality results for *Bemisia tabaci* adult populations tested with acetamiprid, imidacloprid, thiamethoxam

Insecticide	Population	n	Slope±SE	LC_{50} mg(a.i.)/L (95% CL)	LC_{90} mg(a.i.)/L (95% CL)	RR_{50}
	Susceptible	420	1.79±0.18	12.1 (9.4-4.9)	63.0 (47.8-91.5)	1.00
	Guryildiz	420	2.51±0.24	68.1 (58.1-80.4)	220.7 (172.0-311.4)	5.64
	Erbaa	420	1.42±0.16	90.4 (70.0-124.0)	722.3 (423.2-1630.2)	7.49
	Central	420	1.75±0.19	96.8 (77.9-125.9)	525.2 (342.1-995.7)	8.02
Acetamiprid	Zile	420	1.66±0.19	122.1 (95.6-167.7)	728.3 (444.8-581.9)	10.12
	Turhal	420	1.72±0.20	123.7 (97.4-168.5)	689.2 (425.6-447.0)	10.25
	Niksar	420	1.76±0.21	135.5 (106.4-186.9)	728.3 (444.8-1581.9)	11.23
	TOGU campus	420	1.99±0.28	187.1 (145.2-272.8)	826.9 (491.8-2015.0)	15.51
	Pazar	420	2.11±0.31	202.9 (157.3-299.9)	819.5 (489.5-2025.3)	16.82
	Susceptible	420	1.58±0.18	8.6 (6.1-11.1)	55.7 (41.2-84.9)	1.00
	Zile	420	2.20±0.24	85.8 (71.2-107.1)	327.3 (232.2-544.0)	10.02
	Turhal	420	1.57±0.20	118.4 (90.1-172.2)	772.2 (437.6-1913.8)	13.83
	Guryildiz	420	2.56±0.31	123.7 (104.3-152.3)	391.4 (284.5-640.7)	14.44
Imidacloprid	Niksar	420	1.89±0.24	136.8 (106.4-194.2)	652.2 (394.5-1473.9)	15.98
	Erbaa	420	1.97±0.25	146.9 (117.1-199.4)	655.9 (415.9-1355.3)	17.15
	Central	420	1.73±0.24	158.2 (118.8-241.8)	869.4 (483.0-2345.0)	18.48
	Pazar	420	2.15±0.35	206.1 (154.9-331.7)	814.0 (460.2-2377.6)	24.07
	TOGU campus	420	1.41±0.22	264.8 (178.5-507.7)	2139.4 (941.6-9452.4)	30.93
	Susceptible	420	1.63±0.16	15.6 (12.2-19.4)	95.3 (69.9-145.6)	1.00
	Guryildiz	420	2.47±0.23	62.5 (53.2-73.7)	206.4 (161.3-289.0)	4.01
	Niksar	420	1.57±0.18	105.7 (82.8-144.0)	695.24 (421.3-1489.0)	6.79
	Central	420	1.60±0.19	113.7 (89.0-155.5)	713.7 (431.8-1545.8)	7.30
Thiamethoxam	Zile	420	1.70±0.20	120.5 (95.0-163.7)	681.5 (421.1-1426.8)	7.74
	Erbaa	420	1.71±0.20	122.1 (96.2-166.1)	685.4 (423.4-1437.2)	7.84
	TOGU campus	420	1.40±0.17	122.7 (92.4-179.7)	1014.4 (553.0-2639.2)	7.88
	Turhal	420	1.41±0.17	124.9 (94.3-182.3)	1004.1 (550.0-2605.6)	8.02
	Pazar	420	1.77±0.27	232.5 (169.8-384.0)	1227.9 (647.4-3842.1)	14.94

n: Number of whiteflies tested; SE: Standard Error; LC: Lethal Concentration; CL: Confidence Limits;
RR: Resistance Ratio calculated as (LC_{50} of field population) / (LC_{50} of Susceptible population)

Resistance of nymphs

Acetamiprid resistance ratios were determined from 2.96 to 8.60. While the most susceptible population was Guryildiz, the most resistant was found TOGU campus. Slope values are between 1.12 and 1.97. Pazar population is considered the most heterogeneous population since it gave the least slope of the regression line.

Imidacloprid resistance ratios ranged from 4.29 to 8.74. Erbaa population was the most susceptible and Central population was the most resistant. Slope values are between 1.10 and 1.97. The TOGU campus population is considered the most heterogeneous population since it gave the least slope of the regression line.

Thiamethoxam resistance ratios were determined between 2.48 and 4.88. The most susceptible population was determined in Niksar and the most resistant population was found in Pazar. Slope values are between 1.19 and 2.07. Niksar population is considered the most heterogeneous population since it gave the least slope of the regression line.

Table 4. Log-dose probit mortality results for *B. tabaci* nymph populations tested with acetamiprid, imidacloprid, thiamethoxam

Insecticide	Population	n	Slope±SE	LC_{50} mg(a.i.)/L (95% CL)	RF_{50}
Acetamiprid	Susceptible	1056	1.44±0.16	6.2 (4.0-8.4)	1.00
	Guryildiz	1077	1.31±0.14	18.3 (13.6-23.6)	2.96
	Erbaa	1073	1.70±0.16	21.1 (16.8-26.1)	3.42
	Turhal	1122	1.69±0.14	29.2 (23.9-35.4)	4.73
	Zile	1128	1.63±0.14	31.7 (25.8-38.8)	5.13
	Pazar	1108	1.12±0.13	33.8 (25.2-45.0)	5.47
	Central	1120	1.97±0.16	35.8 (29.9-42.8)	5.79
	Niksar	1131	1.80±0.14	36.7 (30.7-43.9)	5.94
	TOGU campus	1137	1.83±0.15	53.1 (44.4-64.3)	8.60
Imidacloprid	Susceptible	1004	1.17±0.16	4.1 (2.0-6.4)	1.00
	Erbaa	1086	1.41±0.14	17.6 (13.3-22.3)	4.29
	Turhal	1073	1.57±0.14	19.6 (15.5-24.1)	4.78
	Zile	1115	1.77±0.14	21.3 (17.5-25.7)	5.21
	TOGU campus	1119	1.10±0.12	27.1 (20.1-35.8)	6.63
	Pazar	1148	1.33±0.14	28.6 (22.2-36.7)	6.99
	Guryildiz	1099	1.42±0.14	28.7 (22.5-36.2)	7.01
	Niksar	1159	1.25±0.13	35.5 (27.3-46.2)	8.67
	Central	1100	1.97±0.16	35.8 (29.9-42.8)	8.74
Thiamethoxam	Susceptible	1086	1.76±0.16	8.1 (6.0-10.2)	1.00
	Niksar	1123	1.19±0.13	20.0 (14.8-26.1)	2.48
	Guryildiz	1081	1.87±0.15	20.2 (16.5-24.2)	2.50
	Erbaa	1114	1.60±0.14	20.6 (16.4-25.3)	2.55
	Zile	1117	1.80±0.14	23.0 (18.9-27.6)	2.85
	TOGU campus	1097	1.37±0.13	26.2 (20.4-32.9)	3.23
	Turhal	1036	1.96±0.15	31.3 (26.3-37.1)	3.88
	Central	1105	1.77±0.15	33.8 (27.7-41.0)	4.18
	Pazar	1141	2.07±0.16	39.4 (33.2-46.7)	4.88

n: Number of whiteflies tested; SE: Standard Error; LC: Lethal Concentration; CL: Confidence Limits;
RR: Resistance Ratio calculated as (LC_{50} of field population) / (LC_{50} of Susceptible population).

Enzyme activity levels in populations

The results from the biochemical assays enzyme activities for the *B. tabaci* adult populations are given in Table 5. There was no statistical difference between populations in terms of GST and EST enzyme activities. For P450, the lowest enzyme activities ratios (1.68 and 1.65) were detected in Niksar and Central. The highest activity ratio was 4.20 in the TOGU campus population.

Table 5. Esterase (EST), glutathione S-transferase (GTS), cytochrome P450 monooxygenase (P450) activities for *B. tabaci* populations from Tokat

Population	EST (mOD/min/mgprotein)	GST (mOD/min/mgprotein)	P450 (pmol/min/mgprotein)*		P450 Ratio
Susceptible	0.8563	0.0070	1.0413	f	1.00
Erbaa	0.9652	0.0074	2.2498	cd	2.16
Guryildiz	1.1638	0.0090	2.3163	cd	2.22
Central	1.1845	0.0125	1.7553	e	1.68
Niksar	1.0570	0.0091	1.7185	e	1.65
Pazar	1.1275	0.0093	3.7933	b	3.64
TOGU Campus	1.3329	0.0087	4.3715	a	4.20
Turhal	1.2621	0.0079	2.4399	c	2.34
Zile	1.0520	0.0087	2.0549	de	1.97

*Values followed by the different letters are significantly different (P< 0.05) after Tukey's HSD test.

Discussion

In terms of population sampling region, adult resistance bioassay revealed different levels of resistance. In general, a high proportion of imidacloprid and acetamiprid resistance was found in almost all populations. In addition, it was concluded that there was moderate resistance for thiamethoxam in all populations.

It was determined that the LC_{50} values obtained for the three insecticides for the susceptible population were lower than the LC_{50} values of all other field populations. According to this result, the population was accepted as sensitive**.**

The Pazar population had the highest RR_{50} for three neonicotinoid group insecticides and showed a high resistance (Table 3). It was concluded that there is a high level of resistance due to the intensive cultivation, the presence of other pests in this region in addition to *B. tabaci* and the common use of neonicotinoid group preparations. Therefore, it is obvious that it will be useful to use different insecticide groups in the control of whiteflies in Pazar.

Different resistance levels have been determined in the studies of *B. tabaci* adults and neonicotinoid insecticides around the world. Schuster et al. (2006), Rao et al. (2012), Castle et al. (2013), Gnankine et al. (2013), Wang et al. (2016), Basij et al. (2017), Naveen et al. (2017), Hajjar et al. (2020) and Taquet et al. (2020) have worked on neonicotinoid resistance against *B. tabaci* in different countries and on different host plants. They have determined that *B. tabaci* has developed resistance at different rates.

In Turkey, Bahşi et al. (2012), investigated resistance levels and the potential of resistance development of acetamiprid, chlorpyrifos-ethyl and cypermethrin in *B. tabaci* populations collected from Antalya district. Resistance levels for acetamiprid, chlorpyrifos and cypermethrin were determined as 6-299, 2-16 and 1-22, respectively. In addition, 18 and 4 times increases in resistance levels of the populations selected with acetamiprid and chlorpyrifos-ethyl were determined. According to these results, Antalya populations of *B. tabaci* showed significant resistance to acetamiprid, chlorpyrifos and cypermethrin. Şahin & İkten (2017) studied the resistance of different *B. tabaci* populations collected from Antalya against to neonicotinoid group insecticides. They observed that LC_{50} resistance ratios were between 4.4-30.4 relative to a susceptible population for acetamiprid. Similarly, they found that durability for thiamethoxam ranged from 8.6 to 31.8 times compared to the susceptible population. Satar et al. (2018), showed that whiteflies were resistant to all neonicotinoids tested when their susceptible SUD-S strain and *B. tabaci* populations were compared. They reported that the highest resistance factor was 2060 for imidacloprid in Kumluca and 5.36 times for thiamethoxam in Samandağ.

Different levels of resistance have been determined. It can be said that there is moderate resistance to imidacloprid and low resistance to thiamethoxam in all populations when nymph resistance bioassay results are evaluated on the basis of population sampling regions. In addition, low resistance to acetamiprid was found in three populations and moderate resistance was found in five populations (Table 4). Compared to adult bioassay results with nymph resistance bioassay results, all populations were found to be more susceptible to three effective agents. This is thought to be due to incomplete body development in nymphs.

There are only a few reported studies on *B. tabaci* nymphs and neonicotinoid insecticides. Jones et al. (2011) applied imidacloprid against adults and nymphs in three *B. tabaci* populations and found that nymphs were more susceptible in all three populations. Nauen et al. (2008) evaluated age-specific resistance of *B. tabaci* to neonicotinoid insecticides. The highest resistance rate was 13 times in prepupa period and 580 times in adult stage. The findings of the current study (Table 4) were similar with the studies performed by the above authors, and it was confirmed that the nymphs are more susceptible than the adults of whiteflies.

In the current study esterase activity was determined, but no statistical difference was found between populations. Jeschke & Nauen (2005), reported that the difference in esterase activity is not related to neonicotinoide resistance but to organic phosphates. In the current study, similarly, low EST activity was detected in comparison with susceptible and resistant populations.

There was no statistical difference between the populations in GST activity. Neonicotinoid resistance is the result of monoxygenase enzyme activity rather than GST activity. Vontas et al. (2000) and Rauch & Nauen (2003) reported that the activity of this enzyme is generally associated with insecticide resistance of organic chlorinated and chlorinated hydrocarbon groups. Rauch & Nauen (2003) found that the highest GST activity was in the susceptible race USA-B and found no higher GST activity in any resistant population. Feng et al. (2010) did not observe any difference in terms of GST between two *B. tabaci* races. Basij et al. (2017) reported that susceptible *B. tabaci* race had higher GST activity than resistant ones. In the present study, low GST activity was found to be similar when the susceptible population was compared to resistant populations.

The most susceptible one of the nine populations used in the studies, was found to have the lowest P450 activity. The TOGU campus population was found to have 4.19 times more enzyme activity than the susceptible population. It had 3.64 times more P450 enzyme activity in the Pazar population. Cytochrome P450 is an enzyme that is effective in gaining resistance to neonicotinoid group preparations in insects. In the current study, cytochrome P450 enzyme activity paralleled the bioassay findings in terms of resistance to neonicotinoid group insecticide. As a result of this research, TOGU campus and Pazar cytochrome P450 activities, which are the highest resistant populations, were found to be higher than the susceptible populations. In this respect, it can be said that whiteflies develop resistance to these pesticides because neonicotinoid pesticides are commonly used in the areas where populations are collected. Nauen et al. (2002) and Rauch & Nauen (2003) found that neonicotinoid group resistance in B and Q biotypes collected from Spain, Germany and Israel was due to increased cytochrome P450-dependent monooxygenase activity. An important relationship between cytochrome P450-dependent monooxygenase activity and imidacloprid resistance level was also observed in Q biotypes of *B. tabaci* populations collected from Crete (Roditakis et al., 2009). Karunker et al. (2008) *B. tabaci* B and Q biotypes related to the high imidacloprid resistance to the cytochrome P450 gene CYP6CM1 in their study carried out, the most important resistance mechanism in all populations found that increased cytochrome P450 monooxygenase enzyme detoxification. Wang et al. (2009) applied imidacloprid to *B. tabaci* s NJ (B biotype) population. They applied this process for 30 generations and obtained the NJ-Imi population. It was 490 times more resistance to imidacloprid. They found that the cause of resistance in the NJ-Imi population was related to the overproduction of cytochrome P450 monooxygenase enzyme. Feng et al. (2010) reported that cytochrome P450 monooxygenase activities increased by 1.21 and 1.68 times, respectively, as a result of biochemical analyzes of two populations. Rao et al. (2012) reported that resistance in biotype strains collected from China was caused by overexpression of cytochrome P450 monooxygenase gene CYP6CM1. Basij et al. (2017) studied the sensitivity of imidacloprid and acetamiprid of nine *B. tabaci* populations collected from different regions of Iran. They reported that the resistance ratio of the populations was between 9.72 and 205 for imidacloprid and 6.38 and 175 for acetamiprid. They found that cytochrome P450 monooxygenase enzyme activity was associated with imidacloprid and acetamiprid resistance. Therefore, they reported that cytochrome P450 monooxygenase is the only enzyme system responsible for neonicotinoid resistance in nine populations of *B. tabaci*.

Conclusions

In the current study, it was determined that *B. tabaci* had developed resistance to acetamiprid, imidacloprid and thiamethoxam. The LC_{90} values of susceptible population for imidacloprid and thiamethoxam (55.7 and 95.3 mg a.i./L) were much lower than the recommended rates of those insecticides (350 and 240 mg a.i./L). The application of imidacloprid and thiamethoxam are prohibited by Ministry of Agriculture and Forestry, General Directorate of Food and Control in open agricultural open areas because of toxicity to bees. As a result of the current study, acetamiprid, which is not included in the ban, has been found to have moderate resistance.

The LC_{90} values of the susceptible population for acetamiprid (62.99 mg a.i./L) are almost equal to the recommended rate of this insecticide (60 mg a.i./L). This indicates that sensitive *B. tabaci* can still be controlled under field conditions. However, in order to prevent the medium level *B. tabaci* resistance to rising to higher levels, insecticides, which have different mode of action, should be used in rotation. In order to fully understand the acetamiprid resistance, it is useful to perform multiple resistance studies and synergistic studies related to cytochrome P450 monooxygenase with other insecticides commonly used in the region.

According to these results, it is concluded that nymphs are more sensitive than adults. Therefore, it is thought that targeting nymphal stages will increase the success and prevent the development of resistance. In addition, insecticides should be used at an appropriate dose, the frequency of application should be reduced, and the control studies should be managed in a more sustainable manner by not using insecticides which have the same mode of action in a row. Continuous use of pesticides with the same mode of action in *B. tabaci* pest management leads to the elimination of susceptible populations and can also contribute to the development of cross-resistance. In this regard, resistance mechanisms should be studied in more detail. Defining resistance mechanisms helps overcome resistance management problems. Besides such studies, cultural, biological, biotechnical and other control measures should be used intensively.

Acknowledgments

We are grateful to Tokat Gaziosmanpaşa University Scientific Research Projects Coordination Unit (project number 2016/45) for financial aid.

References

Anonymous, 2019a. *Bemisia tabaci*. (Web page: https://gd.eppo.int/taxon/BEMITA/distribution) (Data accessed: January 2019).

Anonymous, 2019b. Neonicotinoids. (Web page: http://www.pan-uk.org/about_neonicotinoids) (Data accessed: December 2019).

Anonymous, 2019c. Tomato production in Tokat. (Web page: http://www.oka.org.tr/Documents/TOKAT_Tarim_ve_Kirsal_Kalkinma_Eylem_Plani.pdf) (Data accessed: May 2019).

APRD, 2019. *Bemisia tabaci*. (Web page: https://www.pesticideresistance.org/ display.php?page=species&arId=505) (Data accessed: August 2019).

Bahşi, Ş. Ü., F. Dağlı, C. İkten & H. Göçmen, 2012. Antalya ve ilçelerinden toplanan *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) populasyonlarının Acetamiprid, Chlorpyrifos-ethyl ve Cypermethrin'e karşı duyarlılık düzeyleri. Akdeniz Üniversitesi Ziraat Fakültesi Dergisi, 25 (1): 17-22.

Basij, M., K. Talebi, M. Ghadamyari, V. Hosseininaveh & S. A. Salami, 2017. Status of resistance of *Bemisia tabaci* (Hemiptera: Aleyrodidae) to neonicotinoids in Iran and detoxification by cytochrome P450-dependent monooxygenases. Neotropical Entomology, 46 (1):115-124.

Basit, M., S. Saeed, M. A. Saleem, I. Denholm & M. Shah, 2013. Detection of resistance, cross-resistance, and stability of resistance to new chemistry insecticides in *Bemisia tabaci* (Homoptera: Aleyrodidae). Entomological Society of America, 106 (3): 1414-1422.

Bass, C., I. Denholm, M. S. Williamson & R. Nauen. 2015. The global status of insect resistance to neonicotinoid insecticides. Pesticide Biochemistry and Physiology, 121: 78-87.

Bayhan, E., M. R. Ulusoy & J. K. Brown, 2006. Host range, distribution, and natural enemies of *Bemisia tabaci* 'B biotype' (Hemiptera: Aleyrodidae) in Turkey. Journal of Pest Science, 79 (4): 233-240.

Bedford, I. D., R. W. Briddon, J. K. Brown, R. C. Rosell & P. G. Markham, 1994. Geminivirus transmission and biological characterisation of *Bemisia tabaci* (Gennadius) biotypes from different geographic regions. Annals of Applied Biology, 125 (2): 311-325.

Bedford, I. D., R. W. Briddon, P. G. Markham, J. K. Brown & R. C. Rossell, 1993. A new species of *Bemisia* or biotype of *Bemisia tabaci* (Gennadius), as a future pest of European agriculture. Plant Health and the European Single Market, BCPC Monograph, 54: 381-386.

Boykin, L. M., 2014. *Bemisia tabaci* nomenclature: lessons learned. Pest Management Science, 70 (10): 1454-1459.

Bradford, M. M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein dye binding. Analytical Biochemistry, 72 (1-2): 248-254.

Byrne, F. J., S. Castle, N. Prabhaker & N. C. Toscano, 2003. Biochemical study of resistance to imidacloprid in B biotype *Bemisia tabaci* from Guatemala. Pest Management Science, 59 (3): 347-352.

Cahill, M., K. Gorman, S. Day & I. Denholm. 1996. Baseline determination and detection of resistance to imidacloprid in B*emisia tabaci* (Homoptera: Aleyrodidae). Bulletin of Entomological Research, 86 (4): 343-349.

Castle, S. J. & N. Prabhaker, 2013. Monitoring changes in *Bemisia tabaci* (Hemiptera: Aleyrodidae) susceptibility to neonicotinoid insecticides in Arizona and California. Journal of Economic Entomology, 106 (3): 1404-1413.

Denholm, I., M. Cahill, T. Dennehy & A. R. Horowitz, 1998. Challenges with managing insecticide resistance in agricultural pests, exemplified by the whitefly *Bemisia tabaci*. Philosophical Transactions of the Royal Society B, 353 (1376): 1757-1767.

Elbert, N. & R. Nauen, 2000. Resistance of *Bemisia tabaci* (Homoptera: Aleyrodidae) to insecticides in southern Spain with special reference to neonicotinoids. Pest Management Science, 56 (1): 60-64.

Feng, Y., Q. Wu, S. Wang, X. Chang, W. Xie, B. Xu, & Y. Zhang, 2010. Cross-resistance study and biochemical mechanisms of thiamethoxam resistance in B-biotype *Bemisia tabaci* (Hemiptera: Aleyrodidae). Pest Management Science, 66 (3): 313-318.

Gilbertson, R. L., O. Batuman, C. G. Webster & S. Adkins, 2015. Role of the insect supervectors *Bemisia tabaci* and *Frankliniella occidentalis* in the emergence and global spread of plant viruses. Annual Review of Virology, 2 (1): 67-93.

Gnankiné, O., L. Mouton, A. Savadogo, T. Martin , A. Sanon , R. K. Dabire, F. Vavre & F. Fleury, 2013. Biotype status and resistance to neonicotinoids and carbosulfan in *Bemisia tabaci* (Hemiptera: Aleyrodidae) in Burkina Faso, West Africa. International Journal of Pest Management, 59 (2): 95-102.

Hajjar, M. J., I. Almarzouk, & K. Alhudaib, 2020. Biotype and status of insecticide resistance of whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) in Alhassa oasis, Eastern Province of Saudi Arabia. Entomological Research, 50 (2): 74-81.

Horowitz, A. R., S. Kontsedalov & I. Ishaaya, 2004. Dynamics of Resistance to the Neonicotinoids Acetamiprid and Thiamethoxam in *Bemisia tabaci* (Homoptera: Aleyrodidae). Journal of Economic Entomology, 97 (6): 2051-2056.

IRAC, 2016a. *Bemisia tabaci* adult biyoassay method. (Web page: http://www.irac-online.org/methods/bemisia-tabaci-adults) (Data accessed: February 2019).

IRAC, 2016b. *Bemisia tabaci* nymphs biyoassay method. (Web page: http://www.irac-online.org/methods/trialeurodes-vaporariorum-bemisia-tabaci-nymphs) (Data accessed: February 2019).

IRAC, 2020. Mode of Action Classification Scheme, Version 9.4. (Web page: https://www.irac-online.org/ documents /moaclassification) (Data accessed: March 2020).

Jeschke, P. & R. Nauen, 2005. "Neonicotinoid Insecticides, 53-105". In: Comprehensive Molecular Insect Science (Eds. L. I. Gilbert, L. Iatrou & S. S. Gill). Elsevier, Oxford, UK, 459 pp.

Jeschke, P., R. Nauen, M. Schindler & A. Elbert, 2011. Overview of the status and global strategy for neonicotinoids, Journal of Agricultural and Food Chemistry, 59 (7): 2897-2908.

Jones, C. M., M. Daniels, M. Andrews, R. Slater, R. J. Lind, K. Gorman, M. S. Williamson & I. Denholm, 2011. Age-specific expression of a P450 monooxygenase (CYP6CM1) correlates with neonicotinoid resistance in *Bemisia tabaci*. Pesticide Biochemistry and Physiology, 101 (1): 53-58.

Karunker, I., B. Juergen, L. Bettina, P. Tanja, R. Nauen, R. Emmanouil, V. John, G. Kevin, D. Ian & M. Shai, 2008. Overexpression of cytochrome P450 CYP6CM1 is associated with high resistance to imidacloprid in the B and Q biotypes of *Bemisia tabaci* (Hemiptera: Aleyrodidae). Insect Biochemistry and Molecular Biology, 38 (6): 634-644.

Karut, K., M. Kaydan, S. Castle, C. Kazak & M. Ulusoy, 2014. Çukurova'da pamukta bulunan *Bemisia tabaci* (Gennadius, 1889) (Hemiptera: Aleyrodidae)'nin tür kompleksi üzerine çalışmalar. Turkish Journal of Entomology, 38 (1): 43-50.

Karut, K., A. Malik, C. Kazak, M. Kamberoğlu & M. Ulusoy, 2012. Determination of biotypes of *Bemisia tabaci* Gennadius 1889 (Hemiptera: Aleyrodidae) on different host plant in Adana (Balcalı) by using two different molecular methods. Turkish Journal of Entomology, 36 (1): 93-100.

Luo, C., C. M. Jones, G. Devine, F. Zhang, I. Denholm & K. Gorman, 2010. Insecticide resistance in *Bemisia tabaci* biotype Q (Hemiptera: Aleyrodidae) from China. Crop Protection, 29 (5): 429-434.

Markham, P. G., I. D. Bedford, S. Liu & M. S. Pinner, 1994. The transmission of geminiviruses by *Bemisia tabaci*. Pesticide Science, 42 (2): 123-128.

Martin, J. H., D. Mifsud & C. Rapisarda, 2000. The whiteflies (Hemiptera: Aleyrodidae) of Europe and the Mediterranean Basin. Bulletin of Entomological Research, 90 (5): 407-448.

Naranjo, S. E. & P. C. Ellsworth, 2009. Fifty years of the integrated control concept: Moving the model and implementation forward in Arizona. Pest Management Science, 65 (12): 1267-1286.

Nauen, R., P. Bielza, I. Denholm & K. Gorman, 2008. Age-specific expression of resistance to a neonicotinoid insecticide in the whitefly *Bemisia tabaci.* Pest Management Science, 64 (11): 1106-1110.

Nauen, R., N. Stumpf & A. Elbert, 2002. Toxicological and mechanistic studies on neonicotinoid cross resistance in Q-type *Bemisia tabaci* (Hemiptera: Aleyrodidae). Pest Management Science, 58 (9): 868-875.

Nauen R., K. Wolfel, B. Lueke, A. Myridakis, D. Tsakireli, E. Roditakis, A. Tsagkarakou, E. Stephanou & J. Vontas, 2015. Development of a lateral flow test to detect metabolic resistance in *Bemisia tabaci* mediated by CYP6CM1, a cytochrome P450 with broad spectrum catalytic efficiency. Pesticide Biochemistry and Physiology, 121: 3-11.

Naveen, N. C., R. Chaubey, D. Kumar, K. B. Rebijith, R. Rajagopal, B. Subrahmanyam & S. Subramanian, 2017. Insecticide resistance status in the whitefly, *Bemisia tabaci* genetic groups asia-I, asia-II-1 and asia-II-7 on the indian subcontinent. Scientific Reports (Nature Publisher Group), 7: 40634.

Paul, J. B., L. Shu-Sheng, M. B. Laura & B. D. Adam, 2011. *Bemisia tabaci*: A statement of species status. Annual Review of Entomology, 56 (1): 1-19.

Rao, Q., Y. Xu, C. Luo, H. Zhang, C. M. Jones, G. J. Devine, K. Gorman & I. Denholm, 2012. Characterisation of Neonicotinoid and Pymetrozine Resistance in Strains of *Bemisia tabaci* (Hemiptera: Aleyrodidae) from China, Journal of Integrative Agriculture, 11 (2): 321-326.

Rauch, N. & R. Nauen, 2003. Identification of biochemical markers linked to neonicotinoid cross resistance in *Bemisia tabaci* (Hemiptera: Aleyrodidae). Archives of Insect Biochemistry and Physiology, 54 (4): 165-176.

Roditakis, E., N. E. Roditakis & A. Tsagkarakou, 2005. Insecticide resistance in *Bemisisa tabaci* (Homoptera: Aleyrodidae) populations from Crete. Pest Management Science, 61 (6): 577-582.

Roditakis, E., M. Grispou, E. Morou, J. B. Kristoffersen, N. Roditakis, R. Nauen, J. Vontas & A. Tsagkarakou, 2009. Current status of insecticide resistance in Q biotype *Bemisia tabaci* populations from Crete. Pest Management Science, 65 (3): 313-322.

Roditakis, E., E. Morou, A. Tsagkarakou, M. Riga, R. Nauen, M. Paine & J. Vontas, 2011. Assessment of the *Bemisia tabaci* CYP6CM1vQ transcript and protein levels in laboratory and field-derived imidacloprid resistant insects and cross-metabolism potential of the recombinant enzyme. Insect Science, 18 (1): 23-29.

Şahin, İ. & C. İkten, 2017. Neonicotinoid resistance in *Bemisia tabaci* (Genn., 1889) (Hemiptera: Aleyrodidae) populations from Antalya, Turkey. Turkish Journal of Entomology, 41 (2): 169-175.

Satar, G. & M. R. Ulusoy, 2016. Akdeniz Bölgesi'nden toplanan *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) popülasyonlarının biyotiplerinin belirlenmesi. Türkiye Entomoloji Bülteni, 6 (3): 205-212.

Satar, G., M. R. Ulusoy, R. Nauen & K. Dong, 2018. Neonicotinoid insecticide resistance among populations of *Bemisia tabaci* in the Mediterranean region of Turkey. Bulletin of Insectology, 71 (2): 171-177.

Schuster, D. J., R. S. Mann, M. Toapanta, R. Cordero, S. Thompson, S. Cyman, A. Shurtleff & R. F. II. Morris, 2010. Monitoring neonicotinoid resistance in biotype B of *Bemisia tabaci* in Florida. Pest Management Science, 66 (2): 186-195.

Schuster, D. J., R. S. Mann, M. Toapanta, R. Cordero, S. Thompson & R. F. Morris, 2006. Monitoring of imidacloprid resistance in biotype B of *Bemisia tabaci.* Florida Fourth International Bemisia Workshop International Whitefly Genomics Workshop. Journal of Insect Science, 8 (4): 41-42.

Smith, H. A. & C. A. Nagle, 2014. "Susceptibility of *Bemisia tabaci* to Group 4 Insecticides, 27-28". The Florida Tomato Proceedings (3 September 2014, Florida, USA), 60 pp.

Stumpf, N. & R. Nauen, 2002. Biochemical markers linked to abamectin resistance in *Tetranychus urticae* (Acari: Tetranychidae). Pesticide Biochemistry and Physiology, 72 (2): 111-121.

Taquet, A., H. Delatte, B. Barrès, C. Simiand, M. Grondin & H. Jourdan-Pineau, 2020. Insecticide resistance and fitness cost in *Bemisia tabaci* (Hemiptera: Aleyrodidae) invasive and resident species in La Réunion Island. Pest Management Science, 76 (4): 1235-1244.

Taylor, J. E., 2011. The Distribution of Relationship Between, and Factors Influencing the Abundance of *Bemisia tabaci* and the Incidence of *Tomato Yellow Leaf Curl Virus* in Southern Florida Tomato. University of Florida (Unpublished) Phd Thesis, 206 pp.

Thomas, M. P., 2001. The *Bemisia tabaci* species complex. Crop Protection, 20 (9): 725-737.

Topakcı, N. & H. Göçmen, 2011. A research on the morphological characters of B and Q biotypes of *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) using scanning electron microscopy. Turkish Journal of Entomology, 35 (3): 495-508.

Ulusoy, M. R., 2001. Türkiye Beyazsinek Faunası. Baki Kitabevi Yayımları, Adana, 98 s.

Ulusoy, M. R., K. Karut, M. A. Kanberoğlu & Z. Akdağcık, 2007. Doğu Akdeniz Bölgesi'nde *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae) Biyotiplerinin Belirlenmesi: Biyotiplerin Biyolojileri, Doğal Düşmanları, Konukçu Bitki Tercihleri ile Virüs Vektör Özelliklerinin Araştırılması. TÜBİTAK-TOVAG, 105O173 Nolu Proje Raporu, 46 s.

Ulusoy, M. R., A. Sarı, C. Can & N. Uygun, 1996. "Pamuk beyazsineği, *Bemisia tabaci* (Gennadius)'nin farklı kültür bitkileri üzerindeki gelişmesinin saptanması, 186-191". Türkiye 3. Entomoloji Kongresi (24-28 Eylül 1996, Ankara) Bildirileri, 716 pp.

Vontas, J. G., A. A. Enayati, G. J. Small & J. Hemingway, 2000. A simple biochemical assay for glutathione S-transferase activity and its possible field application for screening glutathione S-transferase-based insecticide resistance. Pesticide Biochemistry and Physiology, 68 (3): 184-192.

Wang, Z., H. Yan, Y. Yang & Y. Wu, 2010. Biotype and insecticide resistance status of the whitefly *Bemisia tabaci* from China. Pest Management Science, 66 (12): 1360-1366.

Wang, R., H. Zheng, C. Qu, Z. Wang, Z. Kong & C. Luo, 2016. Lethal and sublethal effects of a novel cis-nitromethylene neonicotinoid insecticide, cycloxaprid, on *Bemisia tabaci*. Crop Protection, 83: 15-19.

Wang, Z., M. Yao & Y. Wu, 2009. Cross-resistance, inheritance and biochemical mechanisms of imidacloprid resistance in B-biotype *Bemisia tabaci.* Pest Managment Science, 65 (11): 1189-1194.

Türk. entomol. derg., 2020, 44 (3): 333-346
DOI: http://dx.doi.org/10.16970/entoted.678808

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Current genetic status of honey bees in Anatolia in terms of thirty polymorphic microsatellite markers[1]

Anadolu'da bulunan bal arılarının otuz polimorfik mikrosatellit belirteçleri açısından güncel genetik durumları

Kemal KARABAĞ[2*] Rahşan İVGİN TUNCA[3] Emel TÜTEN[2] Taylan DOĞAROĞLU[3]

Abstract

Turkey, having three phytogeographical floristic regions, is a natural bridge among three continents. A lot of subspecies and ecotypes of honey bees have been reported within Turkey. However, hybridization due to informal cultivation and uncontrolled migratory beekeeping practices are thought to affect the genetic diversity of local honey bee populations, and this may result the loss of allele combinations resulting from long evolutionary processes. Numerous identification and conservation studies on honey bee subspecies have been conducted in many countries to determine the loss of genetic variability. On this basis, genetic causes and phylogenetic relationships of four common honey bee subspecies [*Apis mellifera anatoliaca* Maa, 1953, *Apis mellifera* carnica Pollmann, 1879, *Apis mellifera caucasica* Pollmann, 1889, *Apis mellifera syriaca* Skorikov, 1829 (Hymenoptera: Apidae)] from five provinces (Artvin, Düzce, Hatay, Kırklareli and Muğla) selected based on their importance in apicultural activities were studied using 30 microsatellite loci in 2018. The genetic distances of populations ranged from 0.30 to 0.70. Genetic variation was 8.96% among the populations, 44.9% among the individuals within the populations and 46.1% for all individuals. Further genetic researches on the honey bee populations will be of advantage for anticipating potential future problems.

Keywords: *Apis mellifera*, bottleneck, genetic variation, microsatellite

Öz

Üç fitocoğrafik floristik bölgeye sahip olan Türkiye, üç kıta arasında doğal bir köprüdür. Bugüne kadar Türkiye sınırları içerisinde birçok bal arısı alt türü ve ekotipi bildirilmiştir. Ancak, kayıt dışı yetiştiricilik ve kontrolsüz göçer arıcılık uygulamalarına bağlı melezleşmenin yerel bal arısı popülasyonlarının genetik çeşitliliğini etkilediği düşünülmektedir ve bu, uzun evrimsel süreçlerden kaynaklanan allel kombinasyonlarının kaybıyla sonuçlanabilir. Bal arısı alttürleri üzerinde genetik değişkenliğin kaybını önlemek amacıyla birçok ülkede tanımlama ve koruma çalışmaları yapılmıştır. Bu temelde, 2018'de arıcılık faaliyetlerindeki öneminden dolayı seçilen beş ilden (Artvin, Düzce, Hatay, Kırklareli ve Muğla) dört yaygın bal arısı ırkının [*Apis mellifera anatoliaca* Maa, 1953, *Apis mellifera* carnica Pollmann,1879, *Apis mellifera caucasica* Pollmann, 1889, *Apis mellifera syriaca* Skorikov, 1829 (Hymenoptera: Apidae)] genetik açıdan durumları ve filogenetik ilişkileri otuz mikrosatellit lokusu kullanılarak güncellenmeye çalışılmıştır. Popülasyonlar arası genetik mesafe 0.30 ile 0.70 arasında değişmiştir. Genetik varyasyonlar, popülasyonlar arasında %8.96, popülasyonlardaki bireyler arasında %44.9 ve tüm bireyler arasında %46.1 olarak hesaplanmıştır. Bal arısı ile ilgili daha fazla genetik araştırma, gelecekteki potansiyel sorunlardan kaçınmak için avantajlı olacaktır.

Anahtar sözcükler: *Apis mellifera*, darboğaz, genetik varyasyon, mikrosatelit

[1] This study was supported by Republic of Turkey Ministry of Agriculture and Forestry General Directorate of Agricultural Research and Policies, Turkey, Grant Project No: TAGEM-15/ARGE/19.
[2] Akdeniz University, Faculty of Agriculture, Department of Agricultural Biotechnology, 07058, Antalya, Turkey
[3] Muğla Sıtkı Koçman University, Ula Ali Koçman Vocational School, Department of Plant and Animal Production, Beekeeping Program, 48000, Muğla, Turkey
* Corresponding author (Sorumlu yazar) e-mail: karabag@akdeniz.edu.tr
Received (Alınış): 22.01.2020 Accepted (Kabul ediliş): 11.05.2020 Published Online (Çevrimiçi Yayın Tarihi): 12.06.2020

Introduction

Honey bee (*Apis mellifera* L., 1758) belonging to (Apidae) Hymenoptera are (essential pollinators in both nature and agriculture (Ryabov et al., 2014; Tantillo et al., 2015; Amakpe et al., 2018; Ellis et al., 2018; McMenamin et al., 2018) for producing of food (Poposka et al., 2018; Sforcin et al., 2017). Honey bees are also a model organism for neurobiology, development, social behavior and epigenomics (Güder et al., 2017). Honey bees adapt to many environmental conditions all over the world (Agra et al., 2018; Nawrocka et al., 2018) and they have 29 subspecies (De la Rua et al., 2003; Bouga et al., 2011; Chahbar et al., 2013; Oleksa & Tofilski, 2015; Ilyasov et al., 2016). According to Ruttner (1988), the classification and distinction of the *A. mellifera* subspecies using morphometric analyses suggested that the honey bee originally evolved in Africa and Europe, but also speciated in the Middle East (Ruttner, 1988). Also, recent analysis of single nucleotide polymorphisms strongly supported the hypothesis that honey bees originated in Africa (Whitfield et al., 2006). The history of spread and isolation of subpopulations resulted in notable variation in morphological traits (Nawrocka et al., 2018) and early morphometric analyses classified these into M, A, C and O lineages, which owe their origin to the glacial history of Europe (Ellis et al., 2018).

Turkey is at the junction of Africa, Asia and Europe where different honey bee subspecies scattered and adapted to those different climatic and floristic conditions (Kekeçoğlu & Soysal, 2010) that cover three phytogeographic regions: Euro-Syberian, Mediterranean and Iran-Turanian (Bouga et al., 2011). Subspecies [*Apis mellifera anatoliaca* Maa, 1953, *Apis mellifera carnica* Pollmann,1879, *Apis mellifera caucasica* Pollmann, 1889, *Apis mellifera meda* Skorikov, 1829, *Apis mellifera syriaca* Skorikov, 1829 (Hymenoptera: Apidae)] and ecotypes of the subspecies within Turkeys have been reported (Ruttner, 1988, Smith et al., 1997; Kandemir et al., 2000, 2006; Palmer et al., 2000; Bodur et al., 2007; Özdil et al., 2009; Fontana et al., 2018). There are important distinct ecotypes of *A. mellifera anatoliaca* such as Muğla and Düzce (Yığılca) bees in Turkey. Muğla honey bee is the well-known ecotype of *A. mellifera anatoliaca* spreading around the Aegean Sea coast in the west of Turkey (Ivgin Tunca & Kence, 2011; Akyol et al., 2014). Also, Düzce Province seems to have maintained characteristics of *A. mellifera anatoliaca.* Caucasian bee is in the northeastern region of Turkey (Kambur & Kekeçoğlu, 2018), especially in Artvin (Kence et al., 2009), there are also in neighboring countries, Georgia and Russia (Nikolova et al., 2015; Ghassemi-Khademi et al., 2018). According to Ruttner (1988), honey bee from Croatia is *A. mellifera carnica*, nonetheless there are *A. mellifera carnica* in many European and Balkan countries (Bouga et al., 2011). Many colonies from Kırklareli-Thrace have been reported to show similar morphometric and allozyme properties with *A. mellifera carnica (*Kandemir et al., 2000, 2005). The investigation of the genetic origin of Thrace honey bees in Turkey is ongoing (Ünal & Özdil, 2018). *Apis mellifera syriaca* from northern Syria is spatially in distinct from *A. mellifera anatoliaca* in Hatay-Turkey, and question remains concerning the level of introgression between them (Alburaki et al., 2013). In this respect, Anatolia has nearly 20% of the global honey bee genetic diversity. Hybridization and introgression because of commercial beekeeping manipulations affects the genetic variability of local honey bee populations, hence it can lead to the loss of combinations of alleles that have resulted from long periods of adaptive evolution (Bouga et al., 2011; Ellis et al., 2018). To counteract this process, numerous conservation efforts for the protection of native honey bees are being established across Europe. Honey bee subspecies have been routinely identified (Parejo et al., 2018). At first, identification was based on morphometrics and this method had been practiced for a long time. Molecular techniques have begun to be used with the developments in molecular technology as well as morphometric methods in the identification of honey bee subspecies (Bouga et al., 2011; Meixner et al., 2013). Although, numerous molecular markers such as RFLP, mitochondrial DNA analysis and allozyme analysis have been used in the studies of honey bee population genetics (Kandemir & Kence, 1995; Smith et al., 1997; Kekeçoğlu et al., 2009; Özdil et al., 2009), nowadays, the SSR (simple sequence repeat) loci have been widely used (Bodur et al., 2007; Liu et al., 2016; Rahimi et al., 2016; Haddad et al., 2018; Hassett et al., 2018; Yu et al., 2019).

Considering the increasing importance of the conservation of local honey bee genetics, this work aimed to determine the current genetic status and phylogenetic relationships among common honey bee subspecies in Turkey using microsatellites.

Materials and Methods

Sample collection

The colonies were obtained from different beekeepers in 2018 from five provinces selected based on their significance in apicultural activities in Turkey. A representative sampling of each province was randomly performed, resulting in 5 to 15 apiaries sampled per province. So, 30 colonies were determined for each of the five locations where the most common of the four-known species of honey bees in Turkey (Table 1). All samples were stored in collection tubes with pure ethanol at +4ºC until used for DNA extraction.

Table 1. Coordinates and altitudes of the locations where samples collected

Locations	Coordinate	Altitude (m)
Muğla (*A. mellifera anatoliaca)*	37º12′N 28º21′E	656
Hatay *(A. mellifera syriaca)*	36º12′N 36º9′E	85
Kırklareli (*A. mellifera carnica)*	41º44′N 27º13′E	203
Artvin (*A. mellifera caucasica)*	41º10′N 41º49′E	240
Düzce (*A. mellifera anatoliaca)*	40º50′N 31º9′E	146

DNA extraction and microsatellite amplification

Bees were taken from storage in alcohol using sterile tweezers were kept in bi-distilled water for 5 min to remove the alcohol, then dried on the blotting paper for 3 h. The body segments of the bees were carefully separated and the thorax of the bees taken from the same hives was collected in the same 5 ml tubes. Cold nitrogen was poured over the thorax samples and very quickly crushed with sterile glass rods. A little manipulated CTAB method described by Doyle (1990) was used for total DNA extractions from the thorax of worker bees. Quantities and qualities of DNAs were determined using BioDrop spectrophotometer. Also, DNA molecules were checked whether they were in one piece (broken or not broken) in a 2% agarose gel. DNAs were stored at −20ºC until needed.

Thirty microsatellite loci (described previously by Solignac et al., 2003) were used in the study PCR assays were conducted with 2 µl of each template DNA in a total reaction volume of 40 µl.The PCR reaction mix contained 0.25 mM dNTP mix, 2.0 mM $MgCl_2$, 1.5 units of Taq DNA polymerase and 0.075 mM each primers. The thermal cycling for PCR were 94ºC for an initial denaturation for 5 min; 35 cycles of 94ºC for 30 s, 30 s at the primer specific annealing temperature, and 72ºC for 45 s; and a final 72ºC for 5 min. The PCR products were detected on 2% agarose gel and were evaluated using fragment analysis in AATI fragment analyzer to determine the repeat sequence lengths of the microsatellite loci precisely.

Statistical analyses

The lengths of fragments obtained were scored with PROSize2.0. N, number of loci; N_P, number of polymorphic loci; N_A, number of observed alleles; N_E, number of effective alleles; H_E, expected heterozygosis; H_O, observed heterozygosis; F_{Is}, coefficient of inbreeding; HW, Hardy Weinberg equilibrium; and F-statistics were calculated by Popgene v.1.32 (Yeh et al., 1997). PIC values that give the information about the usefulness of a marker were determined by using a microsatellite toolkit (Park, 2001). Also, diagnostic alleles which are described by Garnery et al. (1998) as the allele occurred in relatively high proportions in a population and are either absent or in very low frequencies in all others were determined

for the bee colonies studied. Null alleles, also known as non-amplifying alleles, which are commonly encountered in population genetic studies, were estimated using ML-NullFreq (Kalinowski & Taper, 2006). Theta (H), G-W index (M value) (Garza & Williamson, 2001), allelic size range (ASR), and total heterozygosis, analysis of molecular variance (AMOVA) which are to determine the percentages of variation sources (Excoffier et al., 1992) were performed by using Arlequin v.3.11 (Excoffier et al., 2007). In addition, Bottleneck 1.2.02 (Piry et al., 1999) was used by comparing the decline in allele number versus heterozygosity to estimate the signatures of mutation-drift equilibrium. Because of more than 20 polymorphic loci and the high number of individuals, two-phase mutation model (TPM) was chosen with sing rank test (to calculate how many loci with heterozygosity deficiency or heterozygosity excess), standardized differences test (for the genetic signature of bottlenecks in the honey bee populations studied), and also Wilcoxon test (to determine whether heterozygosity deficiency or excess). The allele frequency distribution was established to see whether it is approximately L-shaped (as expected under mutation-drift equilibrium) or not (recent bottlenecks provoke a mode shift). Microsatellite alleles were classified into 10 frequency classes, which allowed us to check whether distribution followed normal L-shaped form where alleles with low frequencies (0.01 to 0.1) are the most abundant (Luikart et al.,1998).

Identification of the genetic structure of the populations was obtained by the clustering method of the software Structure v. 2.3.4. (Pritchard et al., 2000). This method assumes that there are K populations, each of which is characterized by allele frequencies at each locus. In the analysis, a burn-in of 100,000 iterations and an MCMC (Markov Chain Monte Carlo algorithm) of 500,000 iterations was applied for $2 \leq K \leq 8$ to estimate the most probable number of clusters. The most optimum number of clusters (ΔK) was determined in the Structure Harvester (Earl & vonHoldt, 2012) by calculating the distribution of the ΔK statistic as described by Evanno et al. (2005). Genetix v.4.05 (Belkhir et al., 1996-2004) was used for factorial correspondence analysis (FCA). FCA is based on the principle of examining genotypic data in a three-dimensional plane to reveal the relationships among individuals in the populations studied. In this analysis, the logic of linear transformation of the genotypes of each individual is used to draw a diagram in multiple dimensions. Thanks to the drawn diagram, each individual was compared to another individual and it was ensured that individuals were grouped together by forming a common class.

Results and Discussion

The descriptive statistics of genetic polymorphism showed that the 30 microsatellite loci used in this study were suitable for revealing heterozygosis. It is evident from the data presented in Table 2 that the means of the total heterozygosity, number of alleles and allele size range detected by the loci are quite high. The highest and lowest total heterozygosity was found at loci ap001 (0.92) and ap068 (0.62), respectively. Although, the locus with the highest number of alleles (22) was ap243, the highest value (41) for allele size range was found at loci ap001 (Table 2). When all loci in the populations are evaluated together, based on high theta (H) values, the changes in the repeat number of the microsatellite loci are high.

Table 2. Some descriptive statistics of genetic variations of 30 microsatellite loci in the honey bee populations studied

Locus	H_T	N_A	ASR	Theta (H)
ac006	0.80	12	13	2.41±1.03
ap238	0.86	18	21	3.09±1.34
a007	0.85	12	19	3.40±0.30
ap001	0.92	21	41	4.83±3.09
ac306	0.88	18	28	4.15±1.51
ap243	0.89	22	27	4.19±1.30
ab024	0.71	6	5	1.86±0.16
ap289	0.84	13	16	2.70±0.84
ap273	0.67	4	5	1.58±0.07
a088	0.73	5	8	1.98±0.14
ap218	0.73	10	9	1.81±0.61
ap068	0.62	5	5	1.90±0.26
ap226	0.85	11	18	2.63±0.93
ap049	0.74	10	12	1.74±0.44
ap223	0.82	17	21	2.95±0.85
ap249	0.81	8	10	2.31±0.43
ap288	0.71	12	16	1.73±0.28
hbc1605	0.90	21	33	3.39±1.08
ab124	0.78	17	32	2.51±1.11
hbc1601	0.73	8	12	2.03±0.34
a028	0.78	12	16	2.12±0.67
hbc1602	0.90	19	22	4.65±1.41
ap043	0.49	9	14	1.63±0.10
a113	0.81	13	29	2.52±0.42
a107	0.86	8	7	3.56±0.35
a014	0.86	8	7	3.77±0.31
a079	0.66	4	3	1.74±0.06
ap019	0.88	10	9	4.24±0.24
a076	0.88	10	9	4.22±0.55
a043	0.88	9	8	4,27±0,31
Mean	0.79±0.10	11.73±5.14	15.83±9.58	2.12±0.36

H_T, Total heterozygosis; ASR, allelic size range; N_A, number of observed alleles; and Theta (H), molecular diversity indices.

All microsatellite loci were found polymorphic in this study, and also the values of genetic polymorphism indicators, N_P, N_A, N_E, ASR, PIC, H_E, G-W, theta (H) and F_{IS}, were generally high in Anatolian honey bee subspecies (Table 3). The highest values of the N_A, N_E, ASR, PIC, H_E and theta (H) were found in Kırklareli but the lowest in Düzce. The observed heterozygosity in the Artvin population was higher than in the other populations. The highest intragroup variations (F_{IS}) was found in the Muğla honey bee population and the lowest in the Artvin honey bee population. Purely one diagnostic allele that 117 bp allelic size for Ap288 was observed in only the Kırklareli population. Also, numerous null alleles were calculated in all populations.

Table 3. Main diversity parameters for all honey bee populations according to studied SSR loci

	Muğla	Hatay	Kırklareli	Artvin	Düzce	Mean
N	30	30	30	30	30	30
N_P	30	30	30	30	30	30
N_A	8.13±3.80	7.97±4.07	9.57±3.87	7.77±3.71	7.23±2.57	8.13±3.60
N_E	6.78	6.05	7.93	6.10	4.43	8.56
ASR	10.50±7.52	11.50±8.87	13.87±8.70	10.67±7.10	11.10±6.80	11.52±7.79
PIC	0.79	0.74	0.83	0.75	0.68	0.75
H_E	0.76±0.14	0.70±0.18	0.80±0.12	0.73±0.17	0.66±0.18	0.73±0.16
H_O	0.33±0.28	0.33±0.27	0.40±0.20	0.44±0.26	0.37±0.22	0.37±0.25
GW	0.82±0.21	0.75±0.22	0.74±0.22	0.75±0.20	0.71±0.25	0.75±0.22
Theta (H)	2.28	1.90	2.66	2.03	1.72	2.12±0.36
F_{is}	0.59	0.58	0.52	0.40	0.47	0.52
HW ($p<0.01$)	0.00	0.00	0.00	0.00	0.00	0.00

N, loci number; N_P, number of polymorphic loci N_A, number of observed alleles; N_E, effective allele number; ASR, allelic size range; PIC, polymorphic information content; H_E; expected heterozygosis; H_O; observed heterozygosis; G-W, Garza-Williamson index; Theta (H), molecular diversity indices; F_{is}, coefficient of inbreeding; and HW, Hardy Weinberg equilibrium.

Although none of the populations studied was genetically bottleneck according to the GW index (Table 3) and the normal L-shaped distribution (Figure 1), which is a typical property of a population in equilibrium, none of the populations studied was found in HW balance (Table 3). However, differential results were estimated using the Bottleneck program. Namely, the sign test showed that there were statistical differences between expected heterozygosity excess and observed heterozygosity excess in the Muğla (0.04 to <0.05) and Düzce (0.01 to <0.05) populations. Statistically important heterozygosity deficiency in the Düzce population and heterozygosity excess in the Muğla and Artvin populations were estimated using by Wilcoxon's signed-rank test. Moreover, the Muğla, Hatay and Düzce populations were to be genetic bottleneck according to standardized differences test (Table 4).

Table 4. Bottleneck analysis using standardized differences test at two-phase mutation model

		TPM				
Statistical tests		Muğla	Hatay	Kırklareli	Artvin	Düzce
Sign test	EHE	18.03	18.06	17.87	17.85	17.79
	HD	7.00	14.00	10.00	10.00	19.00
	HE	23.00	16.00	20.00	20.00	11.00
	P	0.04	0.28	0.28	0.27	0.01
Standardized differences test	T2	2.83	-1.89	1.44	1.05	-6.62
	P	0.00	0.03	0.08	0.15	0.00
Wilcoxon's signed rank test	HD (*P*)	0.99	0.56	0.94	0.95	0.02
	HE (*P*)	0.00	0.44	0.07	0.05	0.99
	HDE (*P*)	0.00	0.89	0.13	0.10	0.03

TPM, two-phase mutation 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; and T2: standardized differences test. Positive values of the 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 (unique) alleles in the population.

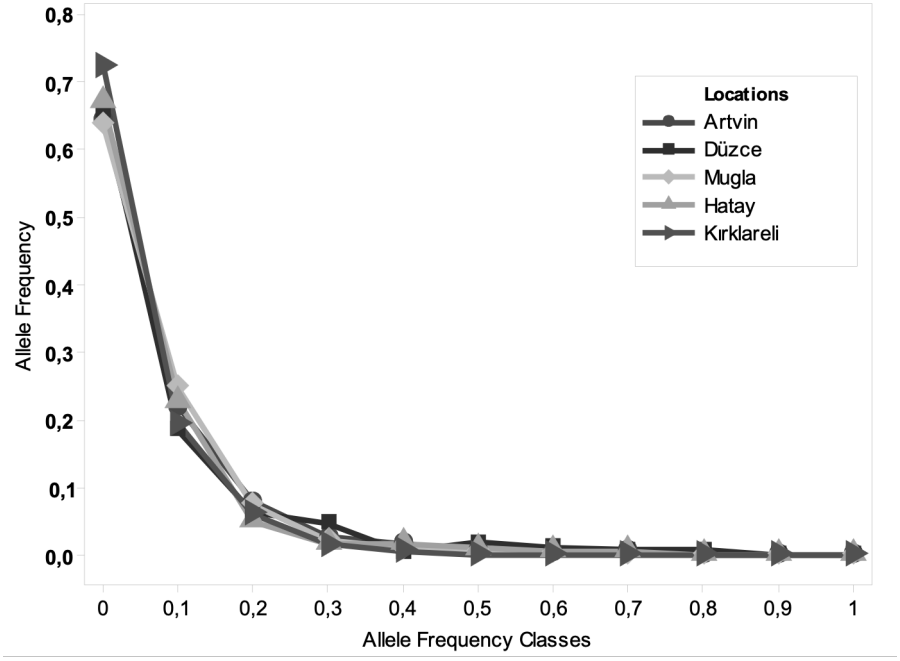

Figure 1. L-shaped mode-shift graph showing lack of recent genetic bottleneck in honey bee subspecies.

Genetic differences

The pairwise F_{ST} values developed by Weir & Cockerman (1984) were calculated to determine the genetic differences among populations (Table 5). Also, Nei's original measures of genetic identity and genetic distance were estimated (Table 6). The population pairwise F_{ST} values for the honey bee populations studied ranged from 0.04 to 0.16. The lowest and the highest pairwise F_{ST} value were determined between Kırklareli and Artvin (0.04) and, Hatay and Düzce (0.16), respectively. The genetic distances among populations (Nei, 1972) were ranged from 0.70 (Hatay and Muğla) to 0.30 (Kırklareli and Artvin).

Table 5. Pairwise F_{ST} values for honey bee populations studied

	Muğla	Hatay	Kırklareli	Artvin	Düzce
Muğla	*				
Hatay	0.12	*			
Kırklareli	0.06	0.07	*		
Artvin	0.10	0.09	0.04	*	
Düzce	0.13	0.16	0.10	0.10	*

Table 6. Nei's original measures of genetic identity (above diagonal) and genetic distance data (below diagonal) (Nei, 1972)

	Muğla	Hatay	Kırklareli	Artvin	Düzce
Muğla	*	0.49	0.66	0.52	0.57
Hatay	0.69	*	0.69	0.64	0.50
Kırklareli	0.41	0.35	*	0.74	0.64
Artvin	0.63	0.44	0.29	*	0.58
Düzce	0.55	0.68	0.44	0.52	*

AMOVA revealed the distribution of genetic among populations and within populations. Sources of total genetic variation were 8.96% among the populations, 44.9% among the individuals within the populations and 46.1% for all individuals. Also, the statistical significance of the differences between the populations was tested by permutation test. It was determined that among individuals (F_{It} 0.54), among the populations (F_{St} 0.09) and within populations (F_{Is} 0.49) genetic differences were significant ($p < 0.05$). Only a small part of the total genetic diversity was caused by the differences among the populations (9.00%) but this was statistically significant. These results coincided with the pairwise F_{ST} values (Table 5).

Clustering Analysis

The genetic structures of the honey bee populations were determined based on Bayesian clustering analysis by using Structure v. 2.3.4. According to the result of this analysis, ΔK value was calculated by the Structure Harvester and found to be four (ΔK = 4). These results indicated the phylogenetic relationships were best expressed in four clusters according to the 30 microsatellite markers (Figure 2) in the five populations studied. The honey bee populations in Muğla, Hatay and Düzce were clearly separated from each other. Interestingly, two populations (Kırklareli and Artvin) which were the most far to each other geographically were clustered in the almost same color. The honey bee population in Kırklareli Province was more heterogeneous than the other populations, and share almost all colors reflecting the common genetic similarities with the other bee populations.

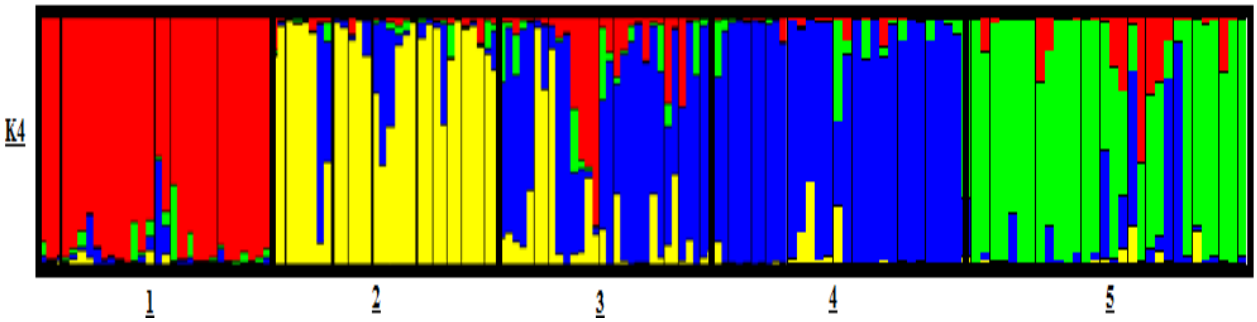

Figure 2. Genetic structure analysis of the examined five honey bee populations. K is the number of groups (populations: 1, Muğla; 2, Hatay; 3, Kırklareli; 4, Artvin; and 5, Düzce). Each color corresponds to one cluster, and the length of the colored segment represents the individual's membership coefficient in the cluster according to cluster analysis.

Factorial correspondence analysis revealed the phylogenetic relationships among populations on a three-dimensional plane (Figure 3). Figures 2 and 3 illustrated similar clustering. The results of FCA showed that honey bee populations were in four main groups: the first was Muğla (western Anatolia), the second was Hatay (southeast Anatolia), the third was Kırklareli (north western Anatolia, Thrace Region) and Artvin (northeast Anatolia), and the fourth was Düzce (central Anatolia). The Hatay population was clearly separated from the other natural populations and have the highest proportional differences (32.6%) on the x-axis. The populations of Muğla and Düzce, which are the ecotypes of the Anatolian bee, were found closer to each other.

Nearly two decades ago, microsatellite studies on honey bee populations generally focused on European and African honey bee subspecies (Franck et al., 1998, 2001), and then studies published for island populations and Mediterranean honey bee populations (Franck et al., 2001; Bodur et al., 2007; Dall'Olio et al., 2007). Previous studies using different methods showed the presence of five honey bee subspecies and different ecotypes in Turkey (Ruttner, 1988; Smith et al., 1997; Kandemir et al., 2000, 2006; Palmer et al., 2000; Bodur et al., 2007; Özdil et al., 2009; Güder et al., 2017). More recently, microsatellites have been used for determining the genetic structure of honey bee populations in many different regions of the world (Liu et al., 2016; Hassett et al., 2018).

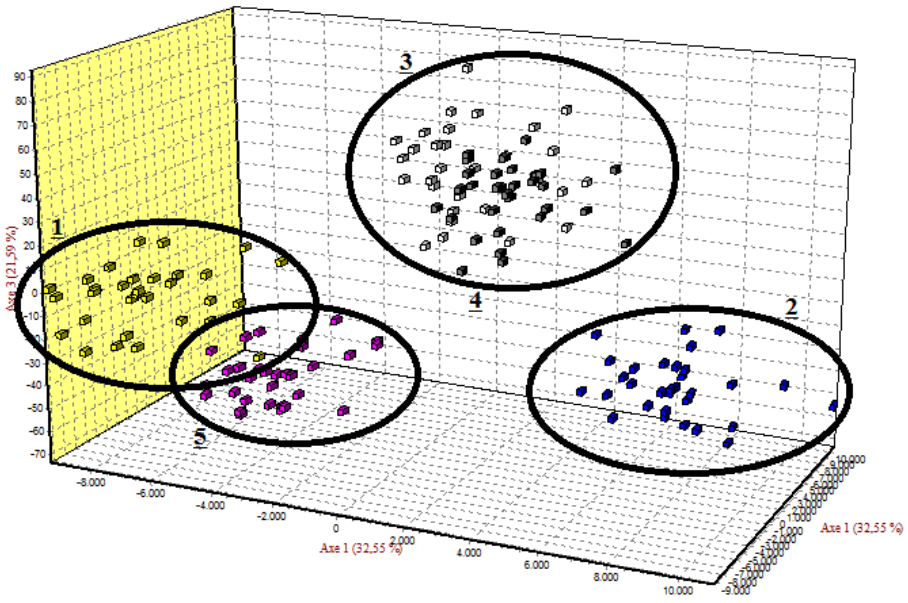

Figure 3. Factorial Correspondence Analysis of the honey bee populations studied (populations: 1, Muğla; 2, Hatay; 3, Kırklareli; 4, Artvin; and 5, Düzce).

Different numbers of microsatellite loci have been used in many studies in both Turkey and other countries (Bodur et al., 2007; Cánovas et al., 2011; Alburaki et al., 2013; Ilyasov et al., 2016; Ghassemi-Khademi et al., 2018; Hassett et al., 2018). In the current study, we used 30 microsatellite loci in order to determinate of the current status of the honey bee populations and all loci studied were suitable.

The allele number is an indicator for the adequacy of sample size to measure of genetic variation (Mielnik-Sikorska et al., 2013). The number of observed alleles present at each locus and in each population were more variable in this study. Average number of alleles the honey bee populations ranged from 7.23 (Düzce) to 9.57 (Kırklareli). The mean number of alleles considering all loci for all of the honey bee populations studied was estimated as 8.13, which is higher than the estimated values from previous microsatellite studies (Ivgin Tunca, 2009; Bodur et al., 2007).

In the current study, the gene diversity value ranged from 0.66 to 0.80. These values indicate the gene diversity among the honey bee populations of Turkey and the results are very close to Middle East, North and West Mediterranean honey bee populations. Lebanon honey bees including Middle Eastern honey bee populations were studied and the gene diversity for those populations was estimated to be 0.65 (Franck et al., 2000a). Also, Bodur et al. (2007) and Ivgin Tunca (2009) found gene diversity values between 0.54, 0.68 and 0.59, similar to Middle East and North Mediterranean honey bee populations. Also, Mediterranean honey bee gene diversity was reported ranging from 0.39 to 0.68 (Franck et al., 2000b). Dall'Olio et al. (2007) studied the genetic variability of *Apis mellifera ligustica* Spinola, 1806 (Hymenoptera: Apidae) at eight polymorphic microsatellite loci and reported the gene diversity for North Mediterranean honey bees ranged from 0.53 to 0.64.

The pairwise F_{ST} values for the populations studied ranged from 0.04 to 0.16. Bodur et al. (2007) estimated pairwise F_{ST} values ranged from 0.00 to 0.18 for Turkish honey bee populations using nine different microsatellite loci. Also, Ivgin Tunca (2009) was found F_{ST} ranged from -0.07 to 0.35 for 18 populations. F_{ST} values were determined for lineages by many studies (Franck et al., 2000a, 2001; Garnery et al., 1998; Dall'Olio et al., 2007). This study suggested that the moderate level of genetic differentiation was observed in Turkish honey bee populations considering the wide range of pairwise F_{ST} values.

The population from Kırklareli had the highest level of polymorphism with N_A, N_E, PIC, H_E and H_O, whereas the values were the lowest in Düzce (Table 3). Intragroup variation (0.59) in Muğla was considered to be the highest because of having large number of honey bees then that of the other locations. (Table 3). According to gene diversity (H_E) and effective allele numbers for the Kırklareli honey bee population, the higher values were observed among populations that showed the allelic richness. According to previous SNP results, Thrace honey bee (Kırklareli and Edirne) and Anatolian honey bee had different genetic composition. They were clustered separately compared to samples from other Anatolia Region and also, they were closer to European honey bees (Ivgin Tunca et al., 2012; Kence et al., 2012).

According to the results of our research, there are alterations in the genetic structures of the populations in the provinces where *A. mellifera caucasica* queen bees have been intensively sold. *Apis mellifera caucasica* queen bees, produced in the province of Artvin, are especially preferred in areas with cooler climates such as Kırklareli. The fact that the results for Kırklareli province and Artvin Province were clustered together, indicate the negative effects of this commercial queen bee trade. This situation can be seen as a good example of the genetic effect of commercial Caucasian queen bees produced in Artvin and sold to regions especially in the cold climate zone.

The deviations from HWE were determined for all population levels, all of them showed significant deviations ($p < 0.01$) in favor of homozygotes for the Artvin population (F_{IS}). Heterozygote deficiency is thought to result from the negative effects of the breeding model in a protected and closed area (Ministry of Agriculture and Rural Affairs of Turkey, Official Gazette 2004/25668).

Numerous colonies brought to Muğla Province by migratory beekeepers during pine honey production periods increases the bee populations of this province. Therefore, increased intragroup variation can occur, which leads to an increase in the genetic diversity. A migratory apiculture model poses a risk for genetic resources. However, as this activity usually does not occur in the swarming seasons, its effects are less than that of commercial queen breeding. Although the genetic effects of migratory beekeeping activity are not yet felt, the risk associated with the activity should be considered. According to our results, the reason for the clustering of the Muğla and Düzce populations close to each other was that they both consist of Anatolian bees. Since Anatolian bees are used extensively in these two provinces, the negative effects of commercial queen breeding are not yet felt. The consequences of the Hatay Province indicate that the bee populations there are quite different from the other provinces in our study. Syrian bees that dominate this region are very different from our other working groups in terms of genetic origin.

Genetic diversity needs to be taken into consideration during the planning of conservation programs if these are to be successful. Decreasing genetic diversity, which is one of the main causes of honey bee colony losses worldwide, clearly demonstrates the importance of conserving gene resources. With unique allele frequencies of honey bees in Anatolia, the region has the potential to be a source of honey bee genetic diversity for the world. As a transition zone between Africa, Asia and Europe, Turkey is a country that needs to be emphasized in terms of conservation of genetic resources.

There have been many studies on the genetic structure of honey bee populations. The results of the current study parallel the scientific literature and our results support the increased genetic variation in honey bee populations in recent years in Turkey. Ecological factors and geographic features that can vary widely in Anatolia, constitute the infrastructure of observed genetic diversity in honey bee populations in Turkey. The enormous diversity of floral sources resulting from these characteristics is highly effective in maintaining genetic diversity. However, adversities such as the migratory beekeeping model, improper breeding programs and uncontrolled commercial queen bee distribution pose a risk to the genetic diversity and sustainability of local honey bee ecotypes.

Our results on the status of honey bee populations using microsatellite markers in major beekeeping regions of Turkey should be taken into consideration during conservation, honey bee breeding and queen breeding programs. Realization of national beekeeping activities according to the results of scientific researches will be highly effective in minimizing the problems experienced in beekeeping evident in recent years. Further scientific investigation on the genetic structure of the honey bee populations will be of great advantage for avoiding potential future problems. In addition, commercial queen breeding and other beekeeping activities in the area should be conducted in accordance with sustainable ecological models. Increasing and supporting the scientific studies on these issues will accelerate the success to be achieved. The measures mentioned above should be implemented as soon as possible for maintaining the sustainability of honey bee genetic resources in Anatolia.

Acknowledgement

We thank to Agriculture Ministry of Turkey. The project was supported by TAGEM-15/ARGE/19**.**

References

Agra, M. N., C. A. Conte, P. M. Corva, J. L. Cladera, S. B. Lanzavecchia & M. A. Palacio, 2018. Molecular characterization of *Apis mellifera* colonies from Argentina: genotypic admixture associated with ecoclimatic regions and apicultural activities. Entomologia Experimentalis et Applicata, 166 (9): 724-738.

Akyol, E., A. Unalan, H. Yeninar, D. Ozkok & C. Ozturk, 2014. Comparison of colony performances of Anatolian, Caucasian and Carniolan honeybee (*Apis mellifera* L.) genotypes in temperate climate conditions. Italian Journal of Animal Science, 13 (3): 637-640.

Alburaki, M., B. Bertrand, H. Legout, S. Moulin, A. Alburaki, W. S. Sheppard & L. Garnery, 2013. A fifth major genetic group among honeybees revealed in Syria. BMC Genetics, 14 (1): 117-127.

Amakpe, F., L. De Smet, M. Brunain, F. J. Jacobs, B. Sinsin & D. C. de Graaf, 2018. Characterization of Native Honey Bee Subspecies in Republic of Benin Using Morphometric and Genetic Tools. Journal of Apicultural Science, 62 (1): 47-60.

Belkhir, K., P. Borsa, L. Chikhi, N. Raufaste & F. Bonhomme, 1996-2004. GENETIX 4.05, logiciel sous Windows pour la ge´ne´tique des populations. Laboratoire Ge´nome, Populations, Interactions,CNRS UMR 5000, Universite´de Montpellier II, Montpellier (France).

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.

Bouga, M., C. Alaux, M. Bienkowska, R. Büchler, N. L. Carreck, E. Cauia & A. Gregorc, 2011. A review of methods for discrimination of honey bee populations as applied to European beekeeping. Journal of Apicultural Research, 50 (1): 51-84.

Cánovas, F., P. De la Rua, J. Serrano & J. Galián, 2011. Microsatellite variability reveals beekeeping influences on Iberian honeybee populations. Apidologie, 42 (3): 235-251.

Chahbar, N., I. Munoz, R. Dall'Olio, P. De la Rúa, J. Serrano & S. Doumandji, 2013. Population structure of North African honey bees is influenced by both biological and anthropogenic factors. Journal of Insect Conservation, 17 (2): 385-392.

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.

De la Rúa, P., J. Galián, J. Serrano & R. F. Moritz, 2003. Genetic structure of Balearic honeybee populations based on microsatellite polymorphism. Genetics Selection Evolution, 35 (3): 339-350.

Doyle, J. J., 1990. Isolation of plant DNA from fresh tissue. Focus, 12: 13-15.

Earl, D. A. & B. M. vonHoldt, 2012. Structure harvester: A website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources, 4: 359-361.

Ellis, J. S., G. Soland-Reckeweg, V. G. Buswell, J. V. Huml, A. Brown & M. E. Knight, 2018. Introgression in native populations of *Apis mellifera mellifera* L: implications for conservation. Journal of Insect Conservation, 22 (4): 377-390.

Evanno, G., S. Regnaut & J. Goudet, 2005. Detecting the number of cluster of individuals using the software STRUCTURE: a simulation study. Molecular Ecology, 14 (8): 2611-2620.

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.

Fontana, P., C. Costa, G. Di Prisco, E. Ruzzier, D. Annoscia, A. Battisti & R. Dall'Olio, 2018. Appeal for biodiversity protection of native honey bee subspecies of *Apis mellifera* in Italy (San Michele all'Adige declaration). Bulletin of Insectology, 71 (2): 257-271.

Franck P., L. Garnery, G. Celebrano, M. Solignac & J. M. Cornuet, 2000a. 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.

Franck, P., L. Garnery, M. Solignac & J. M. Cornuet, 1998. The origin of west European subspecies of honeybees (*Apis mellifera*): New insights from microsatellite and mitochondrial data. Evolution, 52 (4): 1119-1134.

Franck, P., L. Garnery, M. Solignac & J. M. Cornuet, 2000b. Molecular confirmation of a fourth lineage in honeybees from the Near East. Apidologie, 31 (2): 167-180.

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.

Garza, J. C. & E. G. Williamson, 2001. Detection of reduction in population size using data from microsatellite loci. Molecular Ecology, 10 (2): 305-318.

Ghassemi-Khademi, T., H. Rajabi-Maham & S. Pashaei-Rad, 2018. Genetic diversity evaluation of Persian honeybees (*Apis mellifera meda*) in North West of Iran, using microsatellite markers. Journal of Wildlife and Biodiversity, 2 (2): 37-46.

Güder, A., R. Işık & F. Özdil, 2017. Analysis of mtDNA 16S rDNA and ND5 Genes in Thracen Honey Bees of Turkey (*Apis mellifera L.*). Journal of Animal Production, 58 (2): 7-14.

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.

Ilyasov, R. A., A. V. Poskryakov, A. V. Petukhov & A. G. Nikolenko, 2016. Molecular genetic analysis of five extant reserves of black honeybee *Apis melifera melifera* in the Urals and the Volga region. Russian Journal of Genetics, 52 (8): 828-839.

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.

Ivgin Tunca, R. & M. Kence, 2011. Genetic diversity of honey bee (*Apis mellifera* L.: Hymenoptera: Apidae) populations in Turkey revealed by RAPD markers. African Journal of Agricultural Research, 6 (29): 6217-6225.

Ivgin Tunca, R., M. Kence, A. Galindo, T. Giray & A. Kence, 2012. "SNP (Single Nucleotide Polymorphism) analysis on the honeybees of Turkey, 138". 5th European Conference of Apidology, (03-07.09.2012, Halle an der Saale, Germany), Martin-Luther-University, 296 pp.

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.

Kambur, M. & M. Kekeçoğlu, 2018. The loss of genetic diversity on native Turkish honey bee (*Apis mellifera* L.) subspecies. Anadolu Journal of Agricultural Sciences, 33 (1): 73-84.

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, İ., M. Kence & A. Kence, 2005. Morphometric and electrophoretic variation in different honeybee (*Apis mellifera* L.) populations. Turkish Journal of Veterinary and Animal Sciences, 29 (3): 885-890.

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.

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.

Kekeçoğlu, M. & M. I. Soysal, 2010. Genetic diversity of bee ecotypes in Turkey and evidence for geographical differences. Romanian Biotechnological Letters, 15 (5): 5646-5653.

Kence, M., H. J. Farhoud & R. I. Tunca, 2009. Morphometric and genetic variability of honey bee (*Apis mellifera* L.) populations from northern Iran. Journal of Apicultural Research, 48 (4): 247-255.

Kence, A., R. Ivgin Tunca, M. Kence & T. Giray, 2012. "Studies on the characterization and conservation of Kırklareli Honey Bees, 28." 1st International Symposium on the Carniolan honey bee, (16 March 2012, Celje, Slovenia).

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.

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.

McMenamin, A. J., K. F. Daughenbaugh, F. Parekh, M. C. Pizzorno & M. L. Flenniken, 2018. Honey bee and bumble bee antiviral defense. Viruses, 10 (8): 395.

Meixner, M. D., M. A. Pinto, M. Bouga, P. Kryger, E. Ivanova & S. Fuchs, 2013. Standard methods for characterizing subspecies and ecotypes of *Apis mellifera*. Journal of Apicultural Research, 52 (4): 1-28.

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.

Nawrocka, A., İ. 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.

Nikolova, S. R., M. Bienkowska, D. Gerula & E. N. Ivanova, 2015. Microsatellite DNA polymorphism in selectively controlled *Apis mellifera carnica* and *Apis mellifera caucasica* populations from Poland. Archives of Biological Sciences, 67 (3): 889-894.

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., 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.

Palmer, M. R., D. R. Smith & O. Kaftanoglu, 2000. Turkish honey bees: Genetic variation and evidence for a fourth lineage of *Apis mellifera* mtDNA. Journal of Heredity, 91 (1): 42-46.

Parejo, M., D. Henriques, M. A. Pinto, G. Soland-Reckeweg & M. Neuditschko, 2018. Empirical comparison of microsatellite and SNP markers to estimate introgression in *Apis mellifera mellifera*. Journal of Apicultural Research, 57 (4): 504-506.

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.

Poposka D., L. Dimitrov, M. Golubovski, A. Uzunov, R. Brodschneider & S. Andonov, 2018. Pioneer efforts for estimation of the economic impact of honey bee colony losses in the Republic of Macedonia. Monitoring Workshop: (7-8 February 2018, Slovakia).

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.

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

Ryabov, E. V., G. R. Wood, J. M. Fannon, J. D. Moore, J. C. Bull, D. Chandler & D. J. Evans, 2014. A virulent strain of deformed wing virus (DWV) of honeybees (*Apis mellifera*) prevails after *Varroa destructor*-mediated, or in vitro, transmission. PLoS Pathogens, 10 (6): e1004230.

Sforcin, J. M., V. Bankova & A. K. Kuropatnicki, 2017. Medical benefits of honeybee products. Evidence-Based Complementary and Alternative Medicine, Special Issue: 1-2.

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., 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.

Tantillo, G., M. Bottaro, A. Di Pinto, V. Martella, P. Di Pinto & V. Terio, 2015. Virus infections of honeybees *Apis Mellifera*. Italian Journal of Food Safety, 4 (3): 157-168.

Ünal, G. & F. Özdil, 2018. Genetic characterization of Thrace honey bee populations of Turkey: restriction and sequencing of inter cytochrome C oxidase I-II (CoxI-CoxII) genes. Journal of Apicultural Research, 57 (2): 213-218.

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

Whitfield, C. W., S. K. Behura, S. H. Berlocher, A. G. Clark, J. S. Johnston, W. S. Sheppard & N. D. Tsutsui, 2006. Thrice out of Africa: ancient and recent expansions of the honey bee, *Apis mellifera*. Science, 314 (5799): 642-645.

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.

Türk. entomol. derg., 2020, 44 (3): 347-354
DOI: http://dx.doi.org/10.16970/entoted.669236

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Redescription of a rare flesh fly species, *Sarcophaga* (*Latistyla*) *czernyi* Böttcher, 1912 (Diptera: Sarcophagidae), with the ultrastructure of the male terminalia[1]

Nadir bir et sineği türü, *Sarcophaga* (*Latistyla*) *czernyi* Böttcher, 1912 (Diptera: Sarcophagidae)'nin erkek terminalyası ultrastrüktürüyle yeniden tanımlanması

Gamze PEKBEY[2*]

Abstract

Sarcophaga (*Latistyla*) *czernyi* Böttcher, 1912 (Diptera: Sarcophagidae) is a poorly known Palearctic species, which is here newly recorded from Turkey based on a single male specimen collected from Antalya Province in 2016. The species has an interesting and unique structure of the phallus with large, tubular styli with recurving teeth and a lobate and hook-shaped vesica. This study redescribed of the species with the external and genital morphology documented with photography and the first data on the ultrastructure of male terminalia using SEM. The taxonomic status, synonymy and subgeneric inclusion of the species are discussed.

Keywords: *Macabiella*, new record, phallus, SEM, synonymy

Öz

Sarcophaga (*Latistyla*) *czernyi* Böttcher, 1912 (Diptera: Sarcophagidae) az bilinen ve Türkiye'de ilk kez kaydedilen Palearktik bir türdür. Bu çalışmanın incelenen materyali, 2016 yılında Antalya İli'nden toplanan bir erkek örneğine dayanmaktadır. Tür, halka şeklinde dişlere sahip boru şeklindeki styli ve lob benzeri kanca şeklindeki vesica ile oldukça ilginç ve benzersiz bir aedegal yapıya sahiptir. Bu çalışma, SEM kullanılarak erkek terminalya yapısı hakkındaki ilk verilerle türü yeniden tanımlanmaktadır. Dış ve genital morfolojiler, kameraya monte edilmiş bir stereo mikroskop ile tarif edilmiş ve fotoğraflanmıştır. Türün taksonomik durumu, sinonimi ve altcinse dahil edilmesi de tartışılmıştır.

Anahtar sözcükler: *Macabiella*, yeni kayıt, phallus, SEM, sinonim

[1] This study was funded by Yozgat Bozok University Scientific Research Project Unit (BAP, Project No: 6602c-ZF/19-245).
[2] Yozgat Bozok University, Faculty of Agriculture, Department of Plant Protection, 66100, Yozgat, Turkey
* Corresponding author (Sorumlu yazar) e-mail: gamze.pekbey@bozok.edu.tr
Received (Alınış): 02.01.2020 Accepted (Kabul ediliş): 17.05.2020 Published Online (Çevrimiçi Yayın Tarihi): 01.06.2020

Introduction

Sarcophagidae is the second-most diverse family of Oestroidea with about 3,000 valid species worldwide (Pape et al., 2011). *Sarcophaga* Meigen, 1826 comprises more than 850 valid species separated into numerous subgenera and is thereby the largest genus in the family (Whitmore et al., 2018; Pape, 2019).

As in the most of the sarcophagids, *Sarcophaga* spp. also share a uniform external morphology such as gray body coloration, three longitudinal black stripes dorsally on thorax, and checkered patterns on abdomen (Mello-Patiu, 2016). Therefore, an accurate identification of the species strongly depends on a detailed examination of the terminalia of both sexes, but in particular of the male (Pape, 1996; Whitmore et al., 2018). Although the species have a wide range of larval feeding habits, the species are generally characterized as necrophagous, saprophagous or predatory on various terrestrial invertebrates (Mello-Patiu, 2016; Whitmore et al., 2018).

Sarcophaga (*Latistyla*) *czernyi* Böttcher, 1912 has a narrow distribution and has so far only been recorded from Croatia, Greece, Israel and Lebanon (Lehrer, 2006; Xue et al., 2011; Krčmar et al., 2019), to which can now be added Turkey. The species was initially described by Böttcher (1912) as *Sarcophaga czernyi* based on two males collected by L. Czerny in Croatia. For a long time, the taxonomic status, particularly the generic and subgeneric rank, was unstable (Enderlein, 1928; Rohdendorf, 1937; Strukan, 1970; Verves, 1986, 1990; Lehrer, 1994; Pape, 1996). In the most recent studies, *S. czernyi* was placed in *Latistyla* Strukan, 1970 at genus (Xue et al., 2011) or subgenus level (Krčmar et al., 2019).

Ever since the publication of the first Turkish checklist with 81 species by Kara & Pape (2002), the number of flesh flies known from Turkey has gradually increased (Whitmore et al., 2018; Verves et al., 2018; Pekbey, 2019), and it has now risen to 157 with the addition of this new record.

Materials and Methods

The material is represented by a single male of *Sarcophaga* (*Latistyla*) *czernyi*, deposited in the entomology collection of Yozgat Bozok University of Turkey and collected in Turkey, Antalya Province, Burmahan village, 37°11'06" N, 31°16'28" E, 700 m, 08.VII.2016.

For humidification, the dry specimen was kept for 24 h in a moisture chamber, and the terminalia were extended from the abdomen using fine insect pins (Figure 1a). The epandrial complex was subsequently removed from the protandrial segment under a Leica S8APO stereomicroscope and examined without maceration. Photographs were taken with a Leica DFC 450 camera mounted on a Leica M125 stereomicroscope, and series of photographs with different focal depths were stacked by Helicon Focus Pro version 7.5.4 (Helicon Soft Ltd, Kharkiv, Ukraine) to obtain a composite image. SEM images of gold-coated terminalia were taken with a FEI Quanta 450 FEG scanning electron microscope (Thermo Fisher Scientific, Waltham, MA, USA) at the Science and Technology Application and Research Centre of Yozgat Bozok University (BILTEM). The extracted terminalia were stored in glycerine contained in a micro-vial pinned beneath the specimen.

Identification was done using Böttcher (1912) and Lehrer (1994); nomenclature follows Krčmar et al. (2019); terminology of external morphology and terminal structures follow Whitmore et al. (2018); synonymy and distribution follow Pape (1996), Lehrer (2006) and Xue et al. (2011).

Results

Sarcophaga (*Latistyla*) *czernyi* Böttcher, 1912

Sarcophaga czernyi Böttcher, 1912: 731. Syntypes, two males: Croatia, Dalmatia, Dubrovnik (as "Ragusa"), in Senckenberg Deutsches Entomologisches Institut, Müncheberg, Germany.

Parasarcophaga paularnaudi Lehrer, 1981: 185. Lebanon, 70 miles SE of Beirut, Koura (as "El Coura"), Bterram. Holotype male: California Academy of Sciences, San Francisco, USA.

Chresonyms. *Thyrsocnema czernyi* Enderlein 1928: 43; *Parasarcophaga* (?*Rosellea*) *czernyi* Rohdendorf, 1937: 246-247; *Sarcophaga czernyi* Séguy, 1941: 96; *Parasarcophaga* (*Latistyla*) *czernyi* Strukan, 1970: 96; *Parasarcophaga* (*Rosellea*) *czernyi* Verves, 1986: 172; *Rosellea czernyi* Verves, 1990: 542; *Sarcophaga* (*Macabiella*) *czernyi* Pape, 1996: 360; *Latistyla czernyi* Xue et al., 2011: 314; *Sarcophaga* (*Latistyla*) *czernyi* Krčmar et al., 2019: 134; *Parasarcophaga* (*Rosellea*) *paularnaudi* Verves, 1986: 172; *Macabiella paularnaudi* Lehrer, 1994: 15; 1998: 43; 2006: 18; *Rosellea paularnaudi* Verves, 1990: 542.

Description

Male. Body length: 12.7 mm.

Head. Eye bare, red. Parafacial plate and fronto orbital plate black, with silvery pollinosity. Frontal vitta dark gray. Frontal bristles nine pairs, stronger anteriorly. Fronto orbital plate with dispersed short and black setulae. Occiput densely gray pollinose. Antennae black, postpedicel with brownish pollinosity. Arista brown, thickened on basal 2/3, plumose. Postpedicel 3.51 times as long as pedicel. Frons narrow, at vertex 0.25 times, at level of antennal base 0.35 times as wide as head, and at narrowest point 0.57 times as wide as an eye in dorsal view. Frontal vitta 0.45 times as wide as frons and slightly widening anteriorly to antennal insertion. Width of parafacial plate at level of antennal base 0.51 and gena 0.32 times eye height. Gena with dispersed short and black setulae slightly longer towards posterior half. Postgenal setae long and blonde. Vibrissa stout. Lower facial margin hardly visible in lateral view. Two rows of black postoccipital setae. İnner vertical seta long and strong, outer vertical seta absent. Ocellar bristles of medium length. One pair of reclinate fronto orbital setae. A row of erect bristles along facial ridge. Palpus and prementum brownish.

Thorax. Black with silvery pollinosity and three dorsal longitudinal black stripes. The median one reaching to scutellum. Chaetotaxy: acrostichals 0+1, dorsocentrals 4-5+4, intraalars 0+2, supraalars 3, posthumerals 2, notopleurals 4 (2 primer+2 subprimer), katepisternals 3, postalar wall setulose, propleuron bare, scutellum with one pair of basal and two pairs of subapical setae.

Legs. Black. All femora with a row of distinct and long bristly setae on dorsal and ventral surfaces and with hair-like setae. Hind femur with a row of strong anteroventral setae. Mid tibia with three posteroventral and one anterodorsal, hind tibia with two posterodorsal and 1 anterodorsal setae, and with ventral villosity. All tarsi as long as tibiae. Pretarsus slightly swollen towards the tip (Figure 1a).

Wing. Hyaline. Costal spine absent. Tegula black. Basicosta yellow. Cell r_{4+5} open at wing margin. Wing vein R_1 bare. R_{4+5} with short setulae at base. M right-angled. Cross vein dm-cu nearly straight. Second costal section 1.4 times as long as fourth costal section. Calypter whitish. Halter brownish yellow (Figure 1b).

Abdomen. Black in ground color with gray pollinosity and silvery checkered pattern. Protandrial segment black with gray pollinosity. Syntergite 1+2 and tergite 3 without median marginals. Tergite 4 with a pair of median marginals. Tergite 5 with a complete row of marginal bristles.

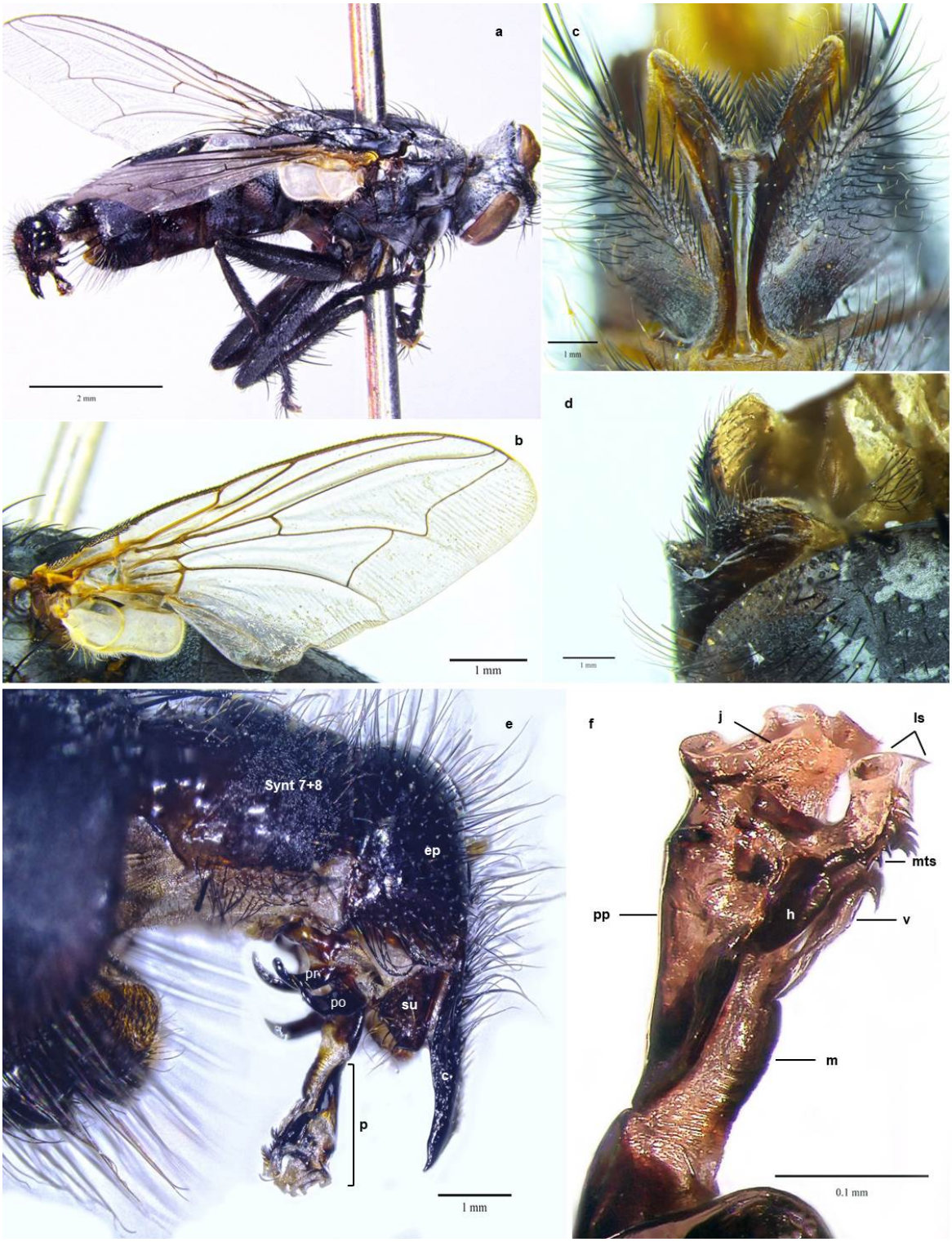

Figure 1. Male of *Sarcophaga* (*Latistyla*) *czernyi* Böttcher, 1912: a) habitus, right lateral view; b) wing, ventral view; c) abdominal sternite 5, ventral view; d) abdominal sternite 5, left lateral view flipped vertically; e) terminalia, right lateral view; and f) phallus, right lateral view, flipped vertically. Abbreviations: c, cercus; ep, epandrium; h, harpes; j, juxta; ls, lateral stylus; m, membrane; mts, marginal teeth of stylus; p, phallus; po, postgonite; pp, paraphallus; pr, pregonite; su, surstylus; Synt 7+8, syntergite 7+8; and v, vesica.

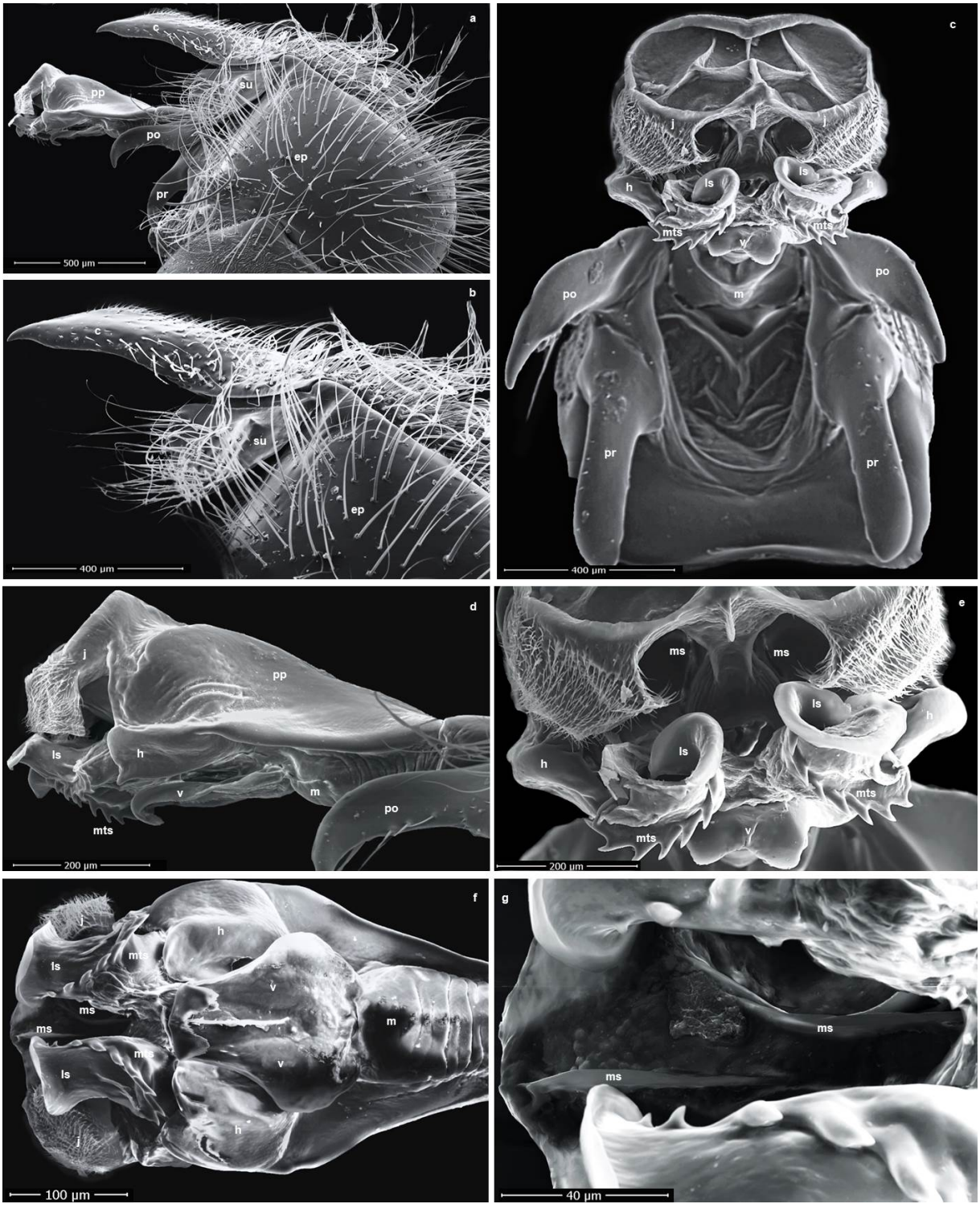

Figure 2. Ultrastructure of male terminalia of *Sarcophaga* (*Latistyla*) *czernyi* Böttcher, 1912: a) terminalia, left lateral view; b) cercus and surstylus left lateral view; c) distiphallus and gonites, apical view; d) distiphallus, left lateral view; e) lateral styli and vesica, apical view; f) distiphallus, ventral view; and g) median stylus, ventral view. Abbreviations: c, cercus; ep, epandrium; h, harpes; j, juxta; ls, lateral stylus; m, membrane; ms, median stylus; mts, marginal teeth of styli; po, postgonite; pp, paraphallus; pr, pregonite; su, surstylus; and v, vesica.

Terminalia. Sternite 5 Y-shaped, basal part straight with a sharply protruding longitudinal keel in lateral view, each arm with short and thick inner median setae (Figure 1c-d). Syntergosternite 7+8 not extended and without marginal bristles, only with scattered and weak setulae on dorsal surface (Figure 1e). Epandrium shiny black, subrectangular. Cerci black, cercal prongs deeply carved beyond midline in dorsal view, concave in profile, convex at the edges and slightly notched at apex, on posterior surface with a slightly protruding longitudinal groove. Surstyli dark brown, robust and subtriangular with a tuft of dense and long setae apically (Figures 1e, 2a-b). Gonites dark brown, similar in shape, slightly curved in outline, blunt at the tip. Pregonites a bit longer than postgonites (Figures 1e, 2a-d). Phallus dark brown. Basiphallus shortened and about 1/2 length of distiphallus. Distiphallus broader in lateral view (Figures 1e, 2a-d). Juxta composed of two membranous lobes fused into a single and rounded wide plate in profile, with numerous microtrichia on apical half; perpendicular to longitudinal axis of distiphallus and curved inwards (Figures 1f, 2c-d, f). The distal part of paraphallus not articulated with the basal part and shown only by a pair of long, wide sclerites in lateral view. Vesica well developed, welded into a plate with two vertices curved ventrally, hook-like (Figures 1f, 2c-f). Harpes flattened and slightly subrectangular, with rounded edges and notched apicoventrally in lateral view. Lateral styli very large and tubular, with strong and well-sclerotized marginal teeth (Figures 1f, 2c-f). Median styli filamentous, not protruding beyond juxta (Figure 2e-g).

Female. Unknown.

Distribution. Palearctic-Croatia, Greece, Israel, Lebanon, Turkey (Pape, 1996; Lehrer, 2006; Xue et al., 2011; Krčmar et al., 2019;).

Biology. Unknown.

Discussion

When Böttcher (1912) described *Sarcophaga czernyi*, he observed that the general shape of the phallus was reminiscent of different types of the *aratrix* and *tuberosa* groups. Rohdendorf (1937) erected the subgenus *Rosellea* and also mentioned that various appendages of the phallus of *czernyi* showed affinities to *aratrix* (*Rosellea*) and *tuberosa* (*Liosarcophaga*). Particular characteristics were the paired appendages (= membranous processes; in that paper styli used instead), directed distally and covered with numerous denticles along their anterior margin. However, Rohdendorf likened the right-angled juxta to *S.* (*Robineauella*) *caerulescens* Zetterstedt, 1838 (as *S. scoparia* Pandellé, 1896). Eventually, he tentatively placed it into *Parasarcophaga* (?*Rosellea*), pointing out that this species was unknown to him and possibly it was a representative of a new, yet undescribed subgenus, as indicated by its distinct structure of the membranal processes of distiphallus.

Enderlein (1928) assigned *S. czernyi* to the genus *Thyrsocnema*. Séguy (1941) followed Böttcher's classification of a broad *Sarcophaga* without subgenera. Strukan (1970) was the first author to describe a new subgenus (*Latistyla*, placed within *Parasarcophaga* Johnston & Tiegs, 1921) specifically designating *S. czernyi* as type species. Lehrer (1981) described *Parasarcophaga paularnaudi* Lehrer, 1981 based on a male from Lebanon, with detailed illustration of the phallus. Verves (1986) accepted *paularnaudi* and *czernyi* as separate species and introduced an affiliation within *Parasarcophaga* (*Rosellea*). In the early classifications, *Parasarcophaga* was a quite large and heterogeneous genus of Sarcophaginae, and many subdivisions into subgenera were used by some authors (Rohdendorf, 1937, 1965; Verves, 1986). Contrarily, Pape (1987) preferred to keep the species as *aratrix* (Pandellé, 1896), *uliginosa* (Kramer, 1908), *caerulescens* Zetterstedt, 1838 and *Liosarcophaga* spp. solely in *Parasarcophaga* for the Danish and Fennoscandian species.

Lehrer (1994) erected *Macabiella* as an independent genus and assigned *P. paularnaudi* as type species. This species was synonymized with *czernyi* by Pape (1996), who placed it in *Sarcophaga*, subgenus *Macabiella*.

Lastly, Xue et al. (2011) synonymized *Macabiella* Lehrer, 1994 with *Latistyla* Strukan, 1970 and placed *L. czernyi* and *L. paularnaudi* in the subtribe Boettcheriscina. Krčmar et al. (2019) listed the species as *Sarcophaga* (*Latistyla*) *czernyi* in their checklist of Croatian flesh flies.

Although the holotype of *L. paularnaudi* (Lehrer, 1994) could not be examined in the present study, it is conclusively seen that the original description of Lehrer (1994, p. 15, Figure 1b) is in line with the data presented here and in previous studies (Bötcher, 1912; Rohdendorf, 1937). The localities of the collected specimens are bordering the Mediterranean Sea, and the geographical and morphological affinities of these two nominal species strengthen their synonymy. Consequently, in the present study *L. paularnaudi* is accepted as a synonym of *S.* (*L.*) *czernyi* as proposed by Pape (1996).

References

Böttcher, G., 1912. Die männlichen Begattungswerkzeuge bei dem Genus Sarcophaga Meig. und ihre Bedeutung für die Abgrenzung der Arten. Deutsche Entomologische Zeitschrift, 6: 705-736.

Enderlein, G., 1928. Klassifikation der Sarcophagiden. Sarcophagiden- Studien I. Archiv für Klassifikatorische und Phylogenetische Entomologie, 1 (1): 1-56.

Kara, K. & T. Pape, 2002. Check list of Turkish Sarcophagidae (Insecta, Diptera) with new records. Mitteilungen aus dem Museum für Naturkunde in Berlin Deutsche Entomologische Zeitchrift, 49 (2): 291-295.

Krčmar, S., D. Whitmore, T. Pape & E. Buenaventura, 2019. Checklist of the Sarcophagidae (Diptera) of Croatia, with new records from Croatia and other Mediterranean countries. ZooKeys, 831: 95-155.

Lehrer, A. Z., 1981. *Parasarcophaga paularnaudi* n. sp. nouvelle Sarcophagine du Proche-Orient (Diptera, Sarcophagidae). Bulletin et Annales de la Société Royale Entomologique de Belgique, 117 (7-9): 185-188.

Lehrer, A. Z., 1994. Deux nouveaux genres paléarctiques de parasarcophages et la réhabilitation du genre *Varirosellea* Xue (Insecta: Diptera: Sarcophagidae). Revue roumaine de biologie. Série de biologie animale, 39 (1): 13-18.

Lehrer, A. Z., 1998. Le présence de *Macabiella paularnaudi* (Lehrer, 1981) dans la faune d'Israel (Diptera, Sarcophagidae). Bulletin de la Société entomologique de Mulhouse (Juillet-Septembre), 43-45.

Lehrer, A. Z., 2006. Liste des Sarcophaginae et Paramacronychiinae du Proche Orient, identifiés dans les collections de TAU (Diptera, Sarcophagidae). Fragmenta Dipterologica, 3: 14-22.

Mello-Patiu, C. A., 2016. Family Sarcophagidae. Zootaxa, 4122 (1): 884-903.

Pape, T., 1987. The Sarcophagidae (Diptera) of Fennoscandia and Denmark. Fauna entomologica scandinavica, E. J. Brill-Scandinavian Science Press Ltd., Leiden, Copenhagen, 203 pp.

Pape, T., 1996. Catalogue of the Sarcophagidae of the World (Insecta: Diptera). Memoirs on Entomology International, Associated Publishers, Florida, 8: 558 pp.

Pape, T., 2019. Flesh Flies (Diptera: Sarcophagidae). (Web page: www.sarcophagidae.myspecies.info) (Date accessed: December 2019).

Pape, T., V. Blagoderov & M. B. Mostovski, 2011. "Order Diptera Linneaus, 1758, 222-229". In Animal Biodiversity: An Outline of Higher-Level Classification and Survey of Taxonomic Richness (Ed. Z. Q. Zhang) Magnolia Press, Auckland, New Zealand, 229 pp.

Pekbey, G., 2019. A preliminary study on determination of small carrion visitor Sarcophagidae (Diptera) species from Yozgat (Turkey), with Two New Records. Türk Tarım ve Doğa Bilimleri Dergisi, 6 (3): 362-354.

Rohdendorf, B. B., 1937. Sem. Sarcophagidae. Chast' I. [Fam. Sarcophagidae. Part I.]. Fauna SSSR. Nasekomye Dvukrylye, Faune de l'URSS. Insectes diptéres, Akademiya Nauk SSSR, Moscou-Leningrad, 19 (1): XV+51pp.

Rohdendorf, B. B., 1965. Sostav triby Sarcophagini (Diptera, Sarcophagidae) Evrazii [Composition of the tribe Sarcophagini (Diptera, Sarcophagidae) of Eurasia]. Entomologicheskoye Obozreniye, 44 (3): 676-695.

Séguy, E., 1941. Études sur les mouches parasites. Tome 2. Calliphorines (suite), Sarcophagines et Rhinophorides de l'Europe occidentale et meridionale. Recherches sur la morphologie et la distribution géographique des Diptères à larves parasites. Encyclopédie entomologique Sér. A. 21: 1-436.

Strukan, D., 1970. Parasarcophagina Jugoslavije (Sarcophagidae-Diptera). Zbornik za prirodne nauke, Matica Srpska, 38: 91-114.

Verves, Y. G., 1986. “Family Sarcophagidae, 172-250”. In: Catalogue of Palaearctic Diptera. Vol. 12. Calliphoridae - Sarcophagidae. (Eds. A. Soos & L. Papp). Academy Press, Budapest, Amsterdam, New York, 265 pp.

Verves, Y. G., 1990. Prof. Hugo de Souza Lopes and the modern system of Sarcophagidae (Diptera). Memórias do Instituto Oswaldo Cruz, 84 (Suppl. 4): 529-545.

Verves, Y., M. Barták & S. Kubík, 2018. Checklist of flesh flies of Turkey (Diptera, Sarcophagidae). ZooKeys, 743: 95-136.

Whitmore, D., E. Buenaventura & T. Pape, 2018. Odd, outsized, and obscure: *Sarcophaga* (*Hadroxena*) *karakoncolos* sp. n. (Diptera: Sarcophagidae) from Turkey. Zootaxa, 4422 (3): 385-394.

Xue, W. Q., Y. G. Verves & D. Jing, 2011. A review of subtribe Boettcheriscina Verves 1990 (Diptera: Sarcophagidae), with descriptions of a new species and genus from China, Annales de la Société Entomologique de France, 47 (3-4): 303-329.

Türk. entomol. derg., 2020, 44 (3): 355-364
DOI: http://dx.doi.org/10.16970/entoted.612447

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

A SEM study of the aedeagus and spermatheca of *Cassida viridis* Linnaeus, 1758 (Coleoptera: Chrysomelidae: Cassidinae) from Turkey

Türkiye'den *Cassida viridis* Linnaeus, 1758 (Coleoptera: Chrysomelidae: Cassidinae)'in aedeagus ve spermatekaları üzerine bir SEM çalışması

Neslihan BAL[1*]

Abstract

It is accepted male genitalia are not diagnostic, but spermathecae are partly diagnostic within the genus *Cassida* Linnaeus, 1758 (Coleoptera: Chrysomelidae: Cassidinae). However, studies on genitalia have been based only on stereomicroscopic examination. The ultrastructure of genitalia has mostly not been studied. The aim of this study was to determine whether this ultrastructures could be diagnostic. Aedeagi and spermathecae of 63 specimens of *Cassida viridis* Linnaeus, 1758 were collected from Çankırı, Hatay, Kayseri, Osmaniye and Zonguldak Provinces in Turkey in 2003, 2007 and 2015, and examined for the first time. Thus, new diagnostic characters were obtained and it revealed that it was diagnostic to either species or subgenus. Photos of aedeagus and spermatheca taken with both SEM and stereomicroscope are given.

Keywords: Aedeagus, *Cassida viridis,* SEM, spermatheca, Turkey

Öz

Cassida Linnaeus, 1758 (Coleoptera: Chrysomelidae: Cassidinae) cinsi içerisinde genel olarak erkek genitalinin ayırt edici olmadığı, ancak spermatekanın kısmen ayırt edici olduğu kabul edilmektedir. Bununla birlikte şimdiye kadar yapılan genital çalışmalarının sadece stereo mikroskoba dayalı olduğu görülmektedir. Genital yapılarının ince yapısı çoğunlukla çalışılmamıştır. Bu çalışmanın amacı, bu altyapının tanısal olup olmayacağını belirlenmesiydi. Bu çalışmada Çankırı, Hatay, Kayseri, Osmaniye ve Zonguldak (Türkiye) illerinden 2003, 2007 ve 2015 yıllarında toplanan toplam 63 *Cassida viridis* Linnaeus, 1758 örneğine ait aedeagus ve spermateka yapıları ilk defa olarak incelenmiştir. Bu sayede yeni ayırt edici karakterler elde edilmiş ve diğer bir alt cinsteki daha önce çalışmış türlerden ayırt edici olduğu ortaya konulmuştur. Aedeagus ve spermatekanın hem SEM hem de stereo mikroskop fotoğrafları da çalışma içerisinde sunulmuştur.

Anahtar sözcükler: Aedeagus, *Cassida viridis*, SEM, spermateka, Türkiye

[1] Gazi University, Faculty of Science, Department of Biology, 06560, Ankara, Turkey
* Corresponding author (Sorumlu yazar) e-mail neslihansilkin@gmail.com
Received (Alınış): 28.08.2019 Accepted (Kabul ediliş): 23.05.2020 Published Online (Çevrimiçi Yayın Tarihi): 05.06.2020

Introduction

Cassida viridis Linnaeus, 1758 (Coleoptera: Chrysomelidae: Cassidinae) is in the subgenus *Odontionycha* Weise, 1891 of the genus *Cassida* Linnaeus, 1758. The Cassidinae fauna of Turkey includes 51 species of six genera and the genus *Cassida* Linnaeus, 1758 includes 41 species in Turkey (Ekiz et al., 2013; Özdikmen et al., 2014; Özdikmen & Kaya, 2014).

The subgenus *Odontionycha* was established by Weise (1891) without designation of type species. *Cassida viridis* Linnaeus, 1758 was designated by Hincks (1952) as type species of the subgenus. According to Borowiec (2007a), *Odontionycha* Weise, 1891 was completely artificial and species included by Weise (1891) in the group spread across five subgenera or genera. The group is coherent morphologically and biologically, and represents a monophyletic group. The subgenus includes six species with the type species *C. viridis* distributed in Palearctic region, *Cassida gansuica* Chen & Zia, 1964, *Cassida inflate* Gressitt, 1952, *Cassida semipunctata* Chen & Zia, 1964, *Cassida tsinlinica* Chen & Zia, 1964 that are distributed only in China and *Cassida innotata* Boheman, 1854 that is distributed only in Afrotropical region (Borowiec, 2007a). Consequently, the subgenus *Odontionycha* is represented only by the type species *C. viridis* in Turkey (Borowiec & Sekerka, 2010; Warchalowski, 2010; Ekiz et al., 2013; Özdikmen et al., 2014; Özdikmen & Kaya, 2014).

According to Bordy & Doguet (1987), Borowiec & Świętojańska (2001) and Borowiec (2007a) male genitalia are not diagnostic within the genus *Cassida*. Spermathecae in the genus *Cassida* are partly diagnostic. However, studies on genitalia have been based on only stereomicroscope examination. Ultrastructures of genitalia have mostly not been studied (Ataş et al., 2019a, b). For this reason, ultrastructural investigations of aedeagi and spermathecae are very important in the genus *Cassida.*

The aim of this work was to examine the ultrastructure of aedeagus and spermatheca of *C.* (*Odontionycha*) *viridis* via SEM from Turkey for the first time.

Materials and Methods

The available specimens (63 in total) for the study were collected from Çankırı, Hatay, Kayseri, Osmaniye and Zonguldak Provinces in Turkey in 2003, 2007 and 2015 (Figure 1). The specimens were deposited at Gazi University (Turkey, Ankara). The spermathecae and aedeagi were dissected from abdomen, remaining tissue were removed with fine tweezers. For dissecting microscopic examination after cleaning, the samples were placed 70% ethanol and examined with Olympus SZX7 stereomicroscope. For scanning electron microscopy (SEM), cleaned samples were dehydrated by using an ascending series of ethanol (70, 80, 90 and 100%) and then they were air dried. After that, the specimens were mounted onto SEM stubs by using a double-sided adhesive tape, coated with gold by using a Polaron SC 502 Sputter Coater, and examined with a JEOL JSM 6060 SEM at 5 kV and 10 kV.

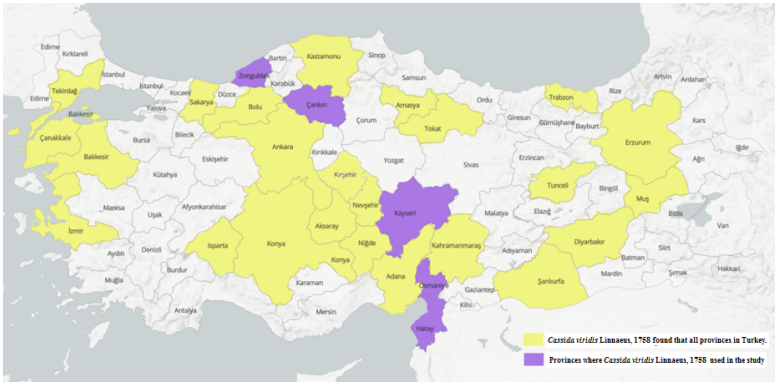

Figure 1. *Cassida viridis* Linnaeus, 1758 in Turkey and the provinces where the samples used in the study were collected.

Results and Discussion

***Cassida viridis* Linnaeus, 1758** (Figure 2)

Cassida viridis Linnaeus, 1758 (Coleoptera: Chrysomelidae: Cassidinae) has a Palearctic chorotype. It is distributed in most of Europe, Asia (China, eastern Russia, Japan, Kazakhistan, Korea, Siberia, Syria, Turkey, Uzbekistan) and North Africa (Morocco). It is widely distributed in Turkey. It has been recorded by various authors in many provinces from all Turkish regions. It is known from Adana, Aksaray, Amasya, Ankara, Balıkesir, Bolu, Çanakkale, Çankırı, Diyarbakır, Erzurum, Hatay, Isparta, İzmir, Kahramanmaraş, Kastamonu, Kayseri, Kırşehir, Konya, Muş, Nevşehir, Niğde, Osmaniye, Sakarya, Şanlıurfa, Tokat, Trabzon, Tunceli and Zonguldak Provinces in Asian Turkey (Anatolia); İstanbul and Tekirdağ Provinces in European Turkey (Figure 1) (Günther, 1954; Gül-Zümreoğlu, 1972; Kısmalı & Sassi, 1994; Gök & Çilbiroğlu, 2003; Borowiec, 2007b; Özdikmen, 2011; Ekiz et al., 2013; Özdikmen & Kaya, 2014; Dikmen & Özuluğ, 2018).

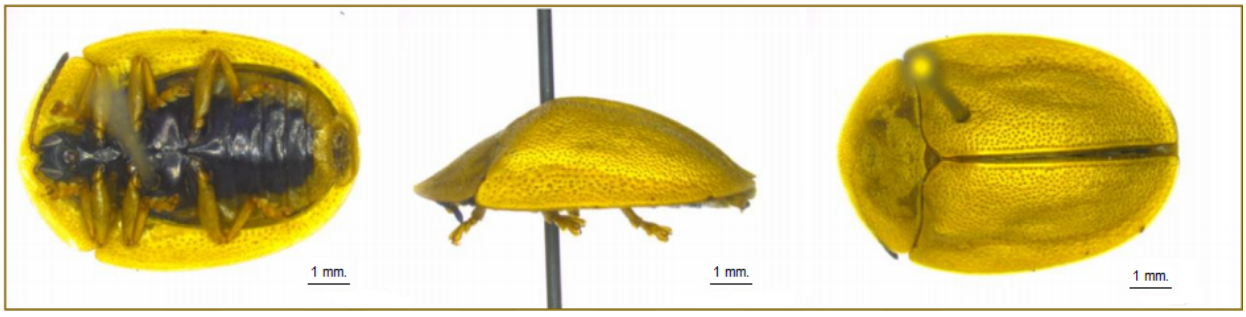

Figure 2. *Cassida viridis* Linnaeus, 1758, ventral view, lateral view and dorsal view in stereomicroscope.

Material examined. Turkey, Çankırı Province. Center, Aşağıçavuş Village, 40°41’ N 33°36’ E, 27.IV.2015, 833 m, 1 specimen; Ilgaz, between Kırşlar-Okçular, 40°56’ N 33°29’ E, 29.V.2015, 1059 m, 1 specimen; Ilgaz, exit of Kırşlar Village, 40°56’ N 33°29’ E, 17.VI.2015, 914 m, 2 specimens; Hatay Province. Serinyol, 36°21'57" N 36°13'02" E, 30.III.2007, 115 m, 19 specimens; between Harbiye-Yayladağı, 36°07'42" N 36°08'41" E, 30.III.2007, 275 m, 1 specimen; Erzin, Gökgül, 36°57'45" N 36°17'22" E, 04.VI.2007, 600 m, 1 specimen; Kayseri Province. Sarız, Yedioluk, 10.VI.2015, 1740 m, 1 specimen; Sarız, Yedioluk, 38°34'24" N 36°25'59" E, 10.VI.2015, 1740 m, 2 specimens; Pınarbaşı, Kerimoğlu, 38°43'11" N 36°24'49" E, 30. V. 2018, 1522 m, 1 specimen; İncesu, Karakoyunlu, 38°37'53" N 35°09'28" E, 28.V.2018, 1114 m, 1 specimen; Pınarbaşı, Uzun Plateau, 38°57'41"N 36°40'15"E, 05.VI.2018, 1623 m, 4 specimens; Pınarbaşı, Aşağıbey Meadow, 38°39'25"N 36°27'01"E, 05.VI.2018, 1622m, 1 specimen; Pınarbaşı, Aşağıbey Meadow, 38°39'29"N 36°27'04"E, 05.VII.2018, 1623 m, 11 specimens; Felahiye, Cumhuriyet, 39°05'11"N 35°33'28"E, 06.VII.2018, 1 specimen; Pınarbaşı, Gümüşgün, 38°44'48"N 36°26'35"E, 31.VII.2018, 1515 m, 1 specimen; Pınarbaşı, Aşağıkızılçevlik village, 38°50'31"N 36°32'24"E, 31.VII.2018, 1622 m, 7 specimens; Osmaniye Province. Issızca, 17.V.2007, 1 specimen; Zorkunfenk Plateau, 36°59'66" N 36°20'64" E, 04.VI.2007, 1049 m, 1 specimen; Zorkun, 5 km to Erzin, 36°58'56" N 36°17'47" E, 04.VI.2007, 989 m, 1 specimen; Zonguldak Province. Yedigöller 35 km to Devraklar, 15.V.2003, 205 m, 5 specimens.

Aedeagus and spermatheca of *C. viridis* were examined with both stereomicroscope and SEM for the first time. There is only one species in Turkey in this subgenus, so it was not possible to compare it with species. Observations on the ultrastructures of the aedeagus and spermatheca were as follows:

Aedeagus

Median lobe completely light brown.

In lateral view, median lobe distinctly and almost regularly (elliptically or semicircularly) curved from median foramen to apex in general. Median lobe gradually, but slightly narrowed from the base to the apex. The apex of median lobe right and pointed with triangular apex (Figures 3, 4).

In dorsal view, lateral margins of the median lobe almost parallel (Figure 4). Apex of median lobe more or less prolonged (Figures 3, 4, 6-10). Upper and lateral margins of orifice more or less rounded (Figures 3, 9, 10). Dorsal plate distinct and covered two-thirds of the orifice (Figures 3, 9, 10). Median lobe in lateral and fore parts of orifice thickened. Thickening in lateral parts smaller than the fore part. Median lobe behind the orifice more or less flattened. Flattened part and basally V-shaped (Figures 3, 8, 9). Median lobe especially in anterior half scattered, irregular and sparsely ultrastructural pits (Figures 6, 7, 9, 10). The pits on ventral parts of median lobe much more than on dorsal parts (Figures 5, 6, 8, 9). The pits located only in lateral parts of terminal part of median lobe (Figures 9, 10). Dorsal plate and flattened area behind it without ultrastructural pits. The terminal area from upper margin of orifice to aedeagal apex also without ultrastructural pits (Figures 9, 10).

In ventral view, lateral margins of the median lobe almost parallel (Figures 6, 7). Apex of median lobe more or less prolonged (Figures 6-10). Ventral surface of median lobe only in apical part with a median, it has a flattened area (Figures 6, 7).

Here, the aedeagi of *C. viridis* have been described from SEM images for the first time. Bordy (2009) gave a stereomicroscopic drawing of aedeagus of this species. The stereomicroscopic results obtained in this study are in agreement with the results of Bordy (2009). Moreover, many new diagnostic characters that were previously unknown based on the ultrastructures of aedeagi were revealed by this SEM investigation.

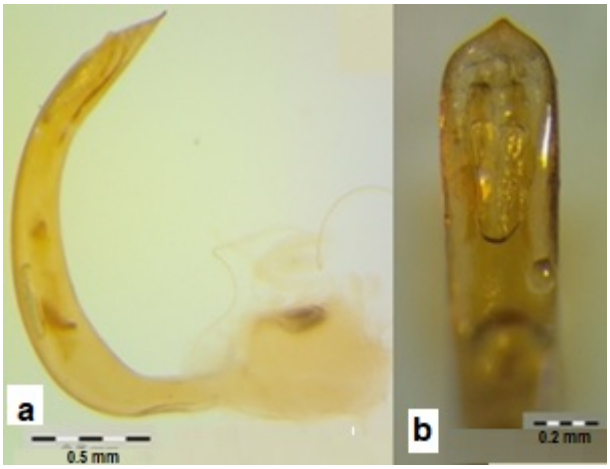

Figure 3. Aedeagus of *Cassida viridis* Linnaeus, 1758, in stereomicroscope, a) lateral view, b) dorsal view.

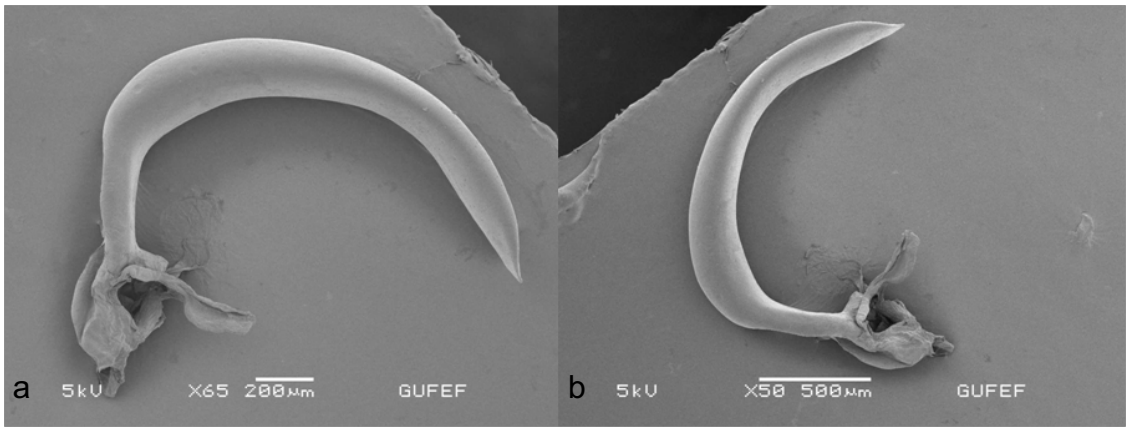

Figure 4. a, b) Aedeagus of *Cassida viridis* Linnaeus, 1758, in SEM, lateral view.

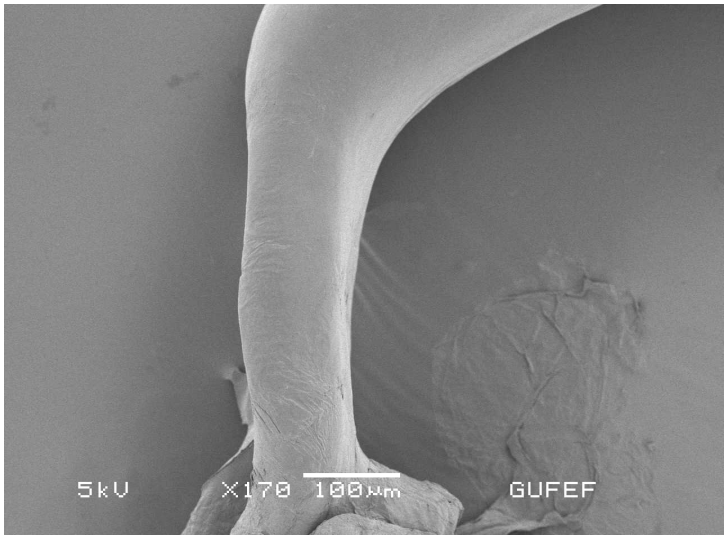

Figure 5. Aedeagus of *Cassida viridis* Linnaeus, 1758, in SEM, median foramen in lateral view.

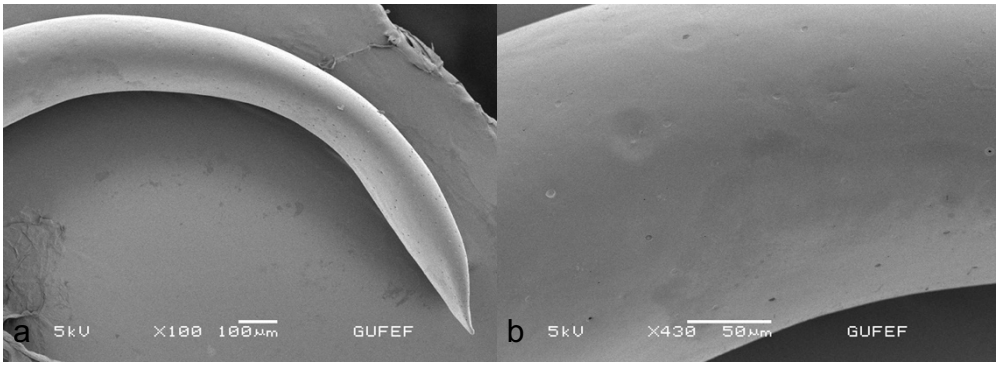

Figure 6. Aedeagus of *Cassida viridis* Linnaeus, 1758, in SEM, a) anterior half of median lobe in lateral view, b) the pits on basal part of median lobe in ventrolateral view.

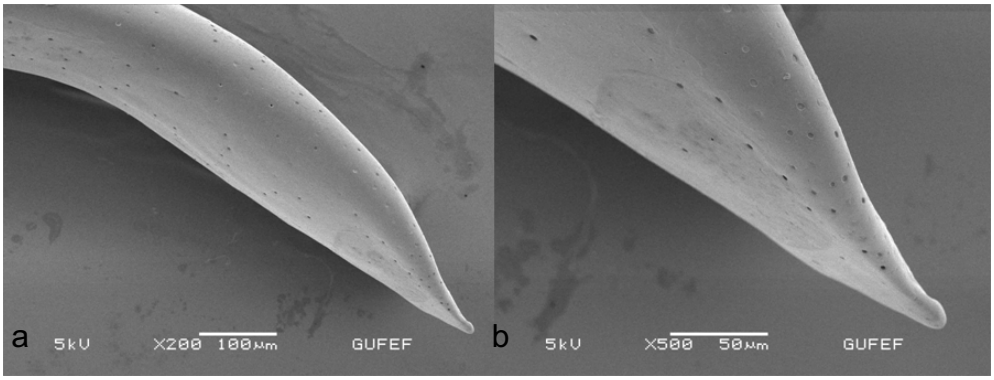

Figure 7. a, b) Aedeagus of *Cassida viridis* Linnaeus, 1758, in SEM, the pits on anterior half and apical part of median lobe in ventrolateral view.

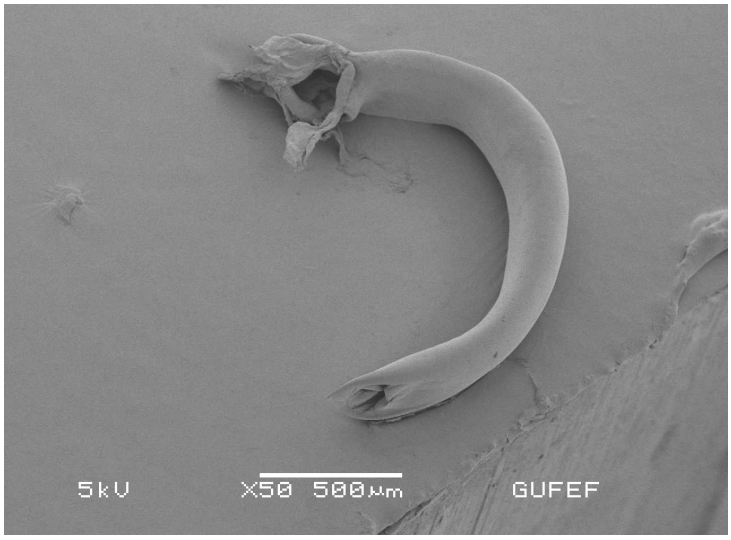

Figure 8. Aedeagus of *Cassida viridis* Linnaeus, 1758 in SEM, dorsolateral view.

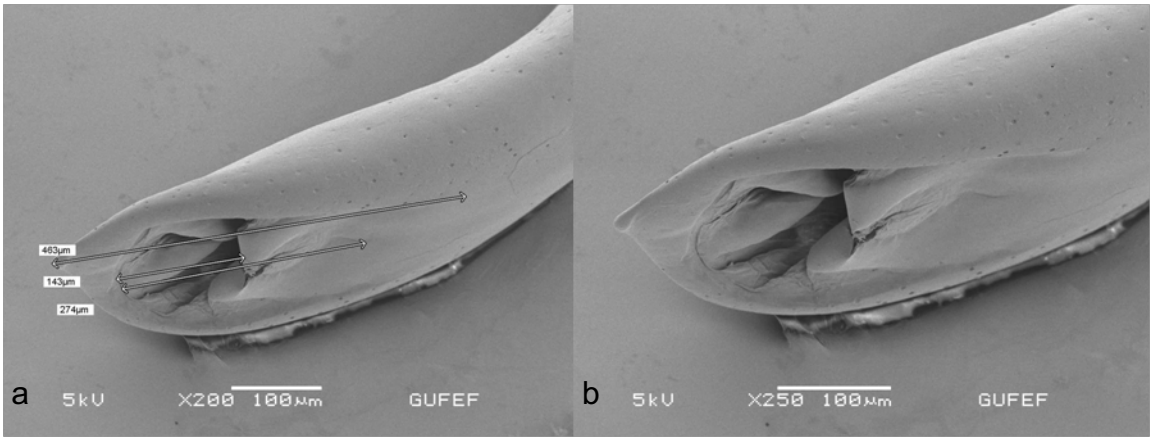

Figure 9. a, b) Aedeagus of *Cassida viridis* Linnaeus, 1758, in SEM, the pits on terminal part of median lobe in dorsolateral view.

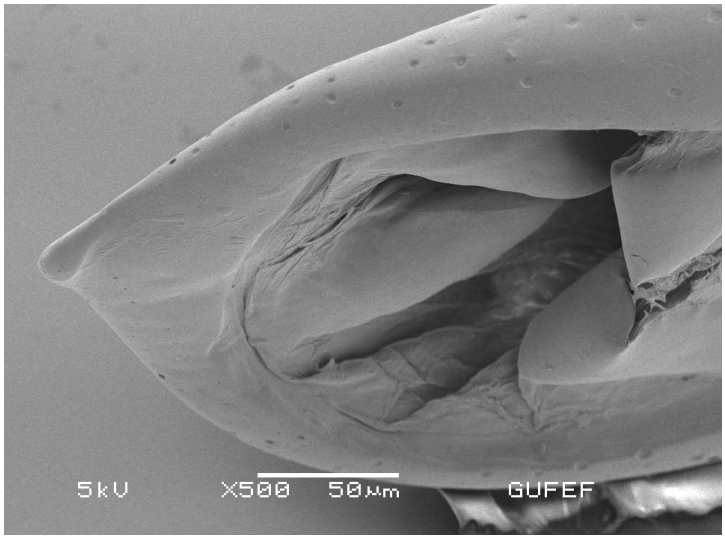

Figure 10. Aedeagus of *Cassida viridis* Linnaeus, 1758, in SEM, the pits on apical part of median lobe in dorsal view.

Spermatheca

General view of spermatheca symmetrical C-shaped, distinctly curved (Figures 11, 12). Nodulus almost parallel, not swollen. Nodulus with an integument on internal surface basally (Figures 12, 13). Cornu not swollen. Apex of cornu rounded. Cornu with an appendix (an integument) in front of the apex (Figures 12, 13). Ampulla like a mushroom or doorknob in general. Ampulla joined to external surface of nodulus beyond the basal end of nodulus and so spermathecae symmetrical C-shaped (Figures 11-13). Collum like a peduncle, thinner than the basal part of nodulus (Figures 11-13). Ramus distinctly swollen, like a rounded knob (Figures 11-13). Spermathecal gland broken, joined to ramus just the median part of top surface of ramus (Figures 12-14). Ductus spermatheca joined to ampulla just the area between collum and ramus (Figures 11-14). Ductus spermatheca rather long, thin and distinctly spiral and the diameter of last part almost equal with first part (Figures 11-16). Only collum scattered, irregular and sparsely ultrastructural pits. Ductus spermatheca without ultrastructural pits (Figures 14-16).

Figure 11. Spermatheca of *Cassida viridis* Linnaeus, 1758, in stereomicroscope, lateral view.

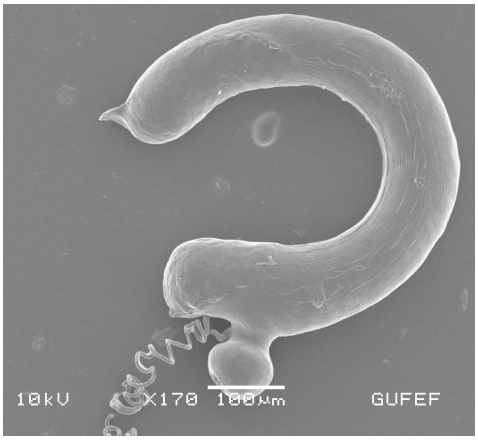

Figure 12. Spermatheca of *Cassida viridis* Linnaeus, 1758, in SEM, lateral view.

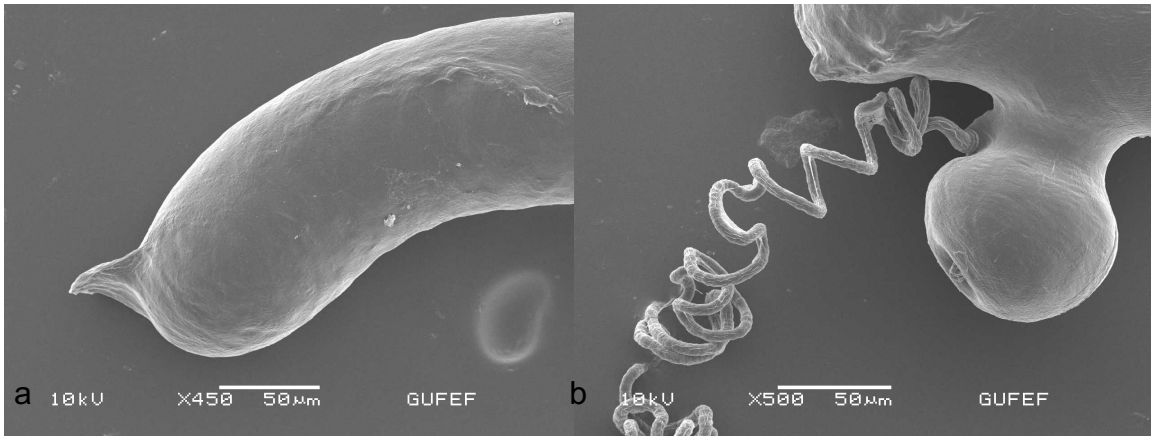

Figure 13. Spermatheca of *Cassida viridis* Linnaeus, 1758, in SEM, a) apex of cornu in lateral view, b) ampulla (collum and ramus) and ductus spermatheca in lateral view.

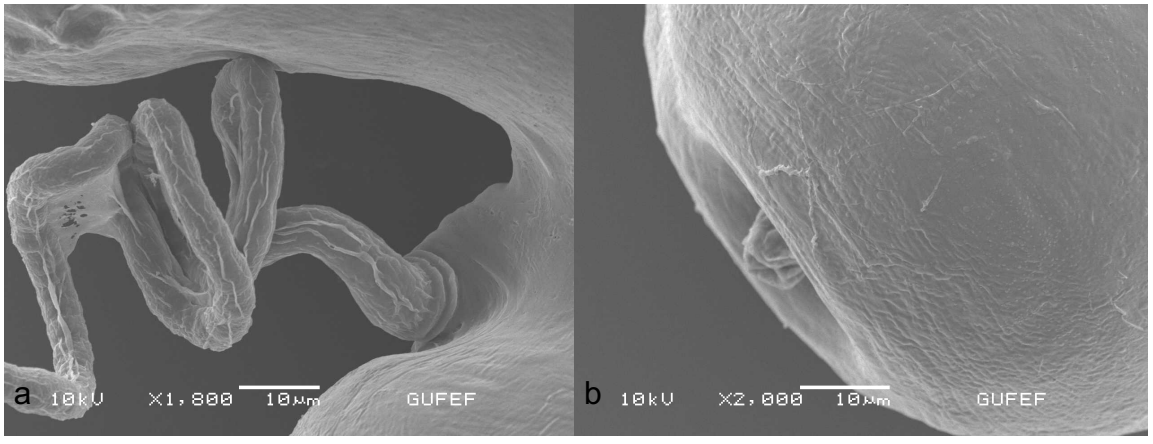

Figure 14. Spermatheca of *Cassida viridis* Linnaeus, 1758, in SEM, a) proximal part of ductus spermatheca in lateral view, b) broken spermathecal gland on top surface of ramus in lateral view.

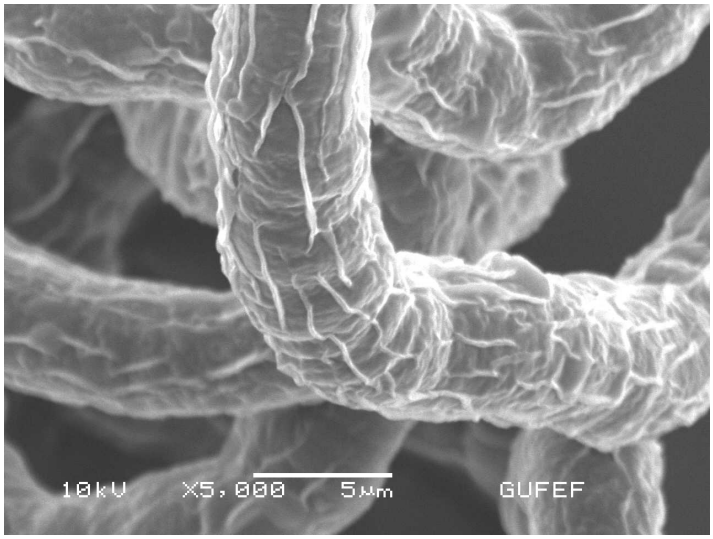

Figure 15. Spermatheca of *Cassida viridis* Linnaeus, 1758, in SEM, ductus spermatheca.

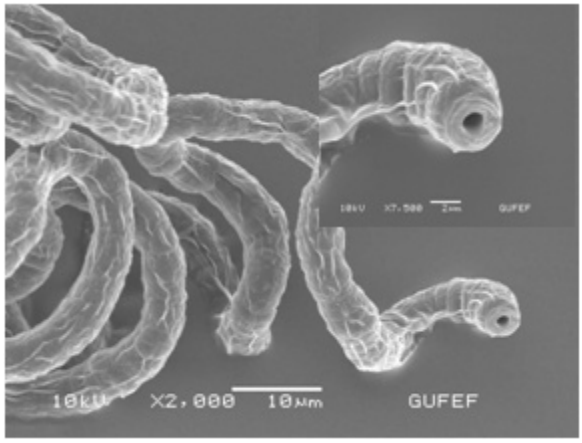

Figure 16. Spermatheca of *Cassida viridis* Linnaeus, 1758, in SEM, basal part of ductus spermatheca.

Here, the spermathecae of *C.viridis* have been described from SEM images for the first time. Bordy (2009) gave a stereomicroscopic drawing of spermathecal of this species. The stereomicroscopic results obtained in this study are in agreement with the results of Bordy (2009). Moreover, many new diagnostic characters that were previously unknown based on the ultrastructures of spermathecae were revealed by this SEM investigation.

Acknowledgement

I thank Prof. Dr. Zekiye Suludere (Gazi University Faculty of Science, Developmental Biology Department) for her tremendous support in the process of photographing the study material with a scanning electron microscope. I also thank Prof. Dr. Hüseyin Özdikmen (Gazi University Faculty of Science, Zoology Department) for his guidance during my studies on matters taxonomic and academic.

References

Ataş, F., H. Özdikmen, N. Bal, D. Amutkan Mutlu & Z. Suludere, 2019a. A SEM study on aedeagus and spermatheca of *Cassida seraphina* Ménétries, 1836 (Coleoptera: Chrysomelidae: Cassidinae) from Turkey. Munis Entomology & Zoology, 14 (2): 395-411.

Ataş, F., H. Özdikmen, N. Bal, D. Amutkan Mutlu & Z. Suludere, 2019b. A SEM study on aedeagus and spermatheca of *Cassida hablitziae* Motschulsky, 1838 (Coleoptera: Chrysomelidae: Cassidinae) from Turkey. Munis Entomology & Zoology, 14 (2): 519-529.

Bordy, B., 2009. Coléopteres Chrysomelidae. Volume 3. Hispinae et Cassidinae. Faune de France, 250 pp.

Bordy, B. & S. Doguet, 1987. Contribution à la connaissancedes Cassidinae de France. Étude de leur spermathèque (Coleoptera, Chrysomelidae). Nouvelle Revue d'Entomologie (N.S.), 4 (2): 161-176.

Borowiec, L., 2007a. Two new species of *Cassida* Linnaeus, 1758 (Coleoptera: Chrysomelidae: Cassidinae) from Madagascar and notes on subgenera of the genus *Cassida*. Zootaxa, 1586: 47-58.

Borowiec, L., 2007b. Cassidinae (Coleoptera: Chrysomelidae) collected in Turkey during expeditions of the Upper Silesian Museum, Bytom. Annals of the Upper Silesian Museum in Bytom-Entomology, 14-15: 7-12.

Borowiec, L. & L. Sekerka, 2010. "Cassidinae, 368-390". In: Catalogue of Palaearctic Coleoptera, Vol. 6. Chrysomeloidea (Eds. I. Löbl & A. Smetana). Apollo Books, Stenstrup, 924 pp.

Borowiec, L. & J. Świętojańska, 2001. Revision of *Cassida litigiosa* group from Southern Africa (Coleoptera: Chrysomelidae: Cassidinae). Annales Zoologici Warszawa, 51: 153-184.

Dikmen, F. & O. Özuluğ, 2018. Insect (Coleoptera and Orthoptera) species of İstanbul in the Zoology Collection of Istanbul University. Turkish Journal of Bioscience and Collections, 2 (1): 27-43.

Ekiz, A. N., İ. Şen, E. G. Aslan & A. Gök, 2013. Checklist of leaf beetles (Coleoptera: Chrysomelidae) of Turkey, excluding Bruchinae. Journal of Natural History, 47 (33-34): 2213-2287.

Gök, A. & E. G. Çilbiroğlu, 2003. The Chrysomelidae fauna of Kovada Stream Arboretum (Eğirdir-Isparta, Turkey). Nouvelle Revue d'Entomologie (NS), 20: 61-73.

Gül-Zümreoğlu, S., 1972. İzmir Bölge Zirai Mücadele Araştırma Enstitüsü Böcek ve Genel Zararlılar Kataloğu, 1928-1969, 1. Kısım. T. C. Tarım Bakanlığı Zirai Mücadele ve Zirai Karantina Genel Müdürlüğü Yayınları, İzmir, 52 s.

Günther, V., 1954. Ergebnisse der Zoologischen expeditiondes National museums in Pragnach der Turkei. Acta Entomologica Musei Nationalis Pragae, 29: 159-162.

Hincks, W. D., 1952 The genera of the Cassidinae (Coleoptera: Chrysomelidae). Transactions of the Royal Entomological Society of London, 103 (10): 327-358.

Kısmalı, S. & D. Sassi, 1994. Preliminary list of Chrysomelidae with notes on distribution and importance of species in Turkey. II. Subfamily Cassidinae Spaeth. Turkish Journal of Entomology, 18 (3): 141-156.

Özdikmen, H., 2011. A comprehensive contribution for leaf beetles of Turkey with a zoogeographical evaluation for all Turkish fauna (Coleoptera: Chrysomelidae). Munis Entomology & Zoology, 6 (2): 540-638.

Özdikmen, H. & G. Kaya, 2014. Chorotype identification for Turkish Chrysomeloidea (Coleoptera) Part I-Chrysomelidae: Hispinae and Cassidinae. Munis Entomology & Zoology, 9 (1): 58-70.

Özdikmen, H., N. Mercan, N. Cihan, G. Kaya, N. N. Topcu & M. Kavak, 2014. The importance of superfamily Chrysomeloidea for Turkish biodiversity (Coleoptera). Munis Entomology & Zoology, 9 (1): 17-45.

Warchalowski, A., 2010. The Palaearctic Chrysomelidae. Identification Keys. Vol. 1 & 2. Natura Optima Dux Foundation, Warszawa, 1212 pp.

Weise, J., 1891. Bemerkungen zur Gattung *Cassida*. Wiener Entomologische Zeitung, 10: 203-205.

Türk. entomol. derg., 2020, 44 (3): 365-373
DOI: http://dx.doi.org/10.16970/entoted.700189

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Insecticidal activities of wild type and recombinant invertebrate iridescent viruses on five common pests

Yaban tip ve rekombinant omurgasız iridesan virüslerinin beş yaygın zararlı üzerindeki böcek öldürücü aktiviteleri

Dönüş GENÇER[1,2] Aydın YEŞİLYURT[1] Mustafa GÜLLÜ[3]
İsmail DEMİR[1] Remziye NALÇACIOĞLU[1*]

Abstract

Invertebrate iridescent virus 6 (IIV6) can infect a broad range of pest insect species. Viruses with new features created by recombinant DNA technology can be used effectively as biological control agents. Previously, recombinants have been constructed: IIVs harboring *green fluorescent protein* gene (*gfp*) in place of IIV6 157L ORF (rCIV-Δ157L-gfp) and a scorpion *Androctonus australis* (Linnaeus, 1758) insect toxin gene (*AaIT*) fused with *gfp* (rCIV-Δ157L/gfp-AaIT). In this study, wild type IIV6 and the two recombinants, were evaluated for their ability to cause infections on *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae), *Spodoptera littoralis* (Boisduval, 1883) (Lepidoptera: Noctuidae), *Lymantria dispar* (Linnaeus, 1758) (Lepidoptera: Erebidae), *Euproctis chrysorrhoea* (Linnaeus, 1758) (Lepidoptera: Erebidae) and *Tenebrio molitor* (Linnaeus, 1758) (Coleoptera: Tenebrionidae) larvae. This study was performed at Karadeniz Technical University, Department of Biology during 2018 and 2019. Five different concentrations (10^3, 10^4, 10^5, 10^6 and 10^7 $TCID_{50}$/ml) of viruses were used to inject each insect larvae. All larvae, infected with rCIV-Δ157L/gfp-AaIT, became paralyzed, except *S. littoralis.* The LC_{50} of insect larvae infected by rCIV-Δ157L/gfp-AaIT were determined as 0.3 x 10^7, 0.7 x 10^5, 0.2 x 10^5, 0.15 x 10^5, 0.7 x 10^4 $TCID_{50}$/ml on *S. littoralis, T. molitor, L. dispar, H. armigera* and *E. chrysorrhoea*, respectively. LT_{50} values, calculated according to the highest virus concentrations, were found as 10.5, 6.2, 4.7, 7.5 and 5 d on *S. littoralis, T. molitor, L. dispar, H. armigera* and *E. chrysorrhoea*, respectively, for rCIV-Δ157L/gfp-AaIT. This study showed that recombinant IIV6 has increased pathogenicity on some insects from Lepidoptera and Coleoptera.

Keywords: Invertebrate iridescent virus, insecticidal activity, recombinant virus

Öz

Omurgasız iridesan virüs 6 (IIV6), çeşitli zararlı böcek türlerini düşük oranda enfekte edebilir. Rekombinant DNA teknolojisi ile oluşturulan yeni özelliklere sahip virüsler biyolojik kontrol ajanları olarak etkin bir şekilde kullanılabilir. Daha önce IIV6 157L ORF'si yerine birisi yeşil floresan protein geni (rCIV-Δ157L-gfp) ve diğeri de gfp ile birleştirilmiş bir akrep *Androctonus australis* (Linnaeus, 1758) böcek toksin geni (rCIV-Δ157L/gfp-AaIT) ihtiva eden iki rekombinant IIV6 inşa edilmişti. Bu çalışmada, yaban tip IIV6 ve iki rekombinant virüs, *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae), *Spodoptera littoralis* (Boisduval, 1883) (Lepidoptera: Noctuidae), *Lymantria dispar* (Linnaeus, 1758) (Lepidoptera: Erebidae), *Euproctis chrysorrhoea* (Linnaeus, 1758) (Lepidoptera: Erebidae) ve *Tenebrio molitor* (Linnaeus, 1758) (Coleoptera: Tenebrionidae) larvalarında enfeksiyon oluşturma yeteneği açısından değerlendirildi. Çalışma 2018 ve 2019 yılları arasında Karadeniz Teknik Üniversitesi Biyoloji Bölümü'nde gerçekleştirildi. Her bir böcek larvasını enjekte etmek için virüslerin beş farklı konsantrasyonu (10^3, 10^4, 10^5, 10^6 ve10^7 $TCID_{50}$/ml) kullanıldı. rCIV-Δ157L/gfp-AaIT ile enfekte olmuş *S. littoralis* dışında tüm larvalar felç oldu. rCIV-Δ157L/gfp-AaIT ile enfekte olmuş böcek larvalarının LC_{50}'si sırasıyla, *S. littoralis, T. molitor, L. dispar, H. armigera* ve *E. chrysorrhoea* üzerinde 0.3 x 10^7, 0.7 x 10^5, 0.2 x 10^5, 0.15 x 10^5, 0.7 x 10^4 $TCID_{50}$/ml olarak belirlenmiştir. En yüksek virüs konsantrasyonlarına göre hesaplanan LT_{50} değerleri, rCIV-Δ157L/gfp-AaIT için *S. littoralis, T. molitor, L. dispar, H. armigera* ve *E. chrysorrhoea*'da sırasıyla 10.5, 6.2, 4.7, 7.5 ve 5 gün olarak bulundu. Bu çalışma, rekombinant IIV6'nın Lepidoptera ve Coleoptera takımlarına ait bazı böceklerde patojeniteyi artırdığını göstermiştir.

Anahtar sözcükler: Omurgasız iridesan virüs, insektisidal aktivite, rekombinant virüs

[1] Karadeniz Technical University, Faculty of Science, Department of Biology, 61080, Trabzon, Turkey
[2] Trabzon University, Salpazarı Vocational School, Department of Property Protection and Security, 61670, Trabzon, Turkey
[3] Bingöl University, Faculty of Agriculture, Department of Plant Protection, 12000, Bingöl, Turkey
* Corresponding author (Sorumlu yazar) e-mail: remziye@ktu.edu.tr
Received (Alınış): 09.03.2020 Accepted (Kabul ediliş): 09.06.2020 Published Online (Çevrimiçi Yayın Tarihi): 21.07.2020

Introduction

A large portion of the economy is derived from agricultural products in many developing countries. Lepidoptera and Coleoptera larvae constitute the majority of pest insects damaging a wide variety of food, fiber, oilseed, forage and horticultural plants. Due to the emergence of insect populations resistant to currently used products, the demand for new insecticides is increasing.

The Iridoviridae family comprises large, icosahedral, double-stranded DNA viruses that infect a wide range of both vertebrate and invertebrate hosts. The family includes two subfamilies: Alphairidovirinae and Betairidovirinae. Alphairidovirinae comprises *Megalocytivirus*, *Ranavirus* and *Lymphocystivirus* genera whose members infect vertebrates. Betairidovirinae comprises *Chloriridovirus* and *Iridovirus* genera whose members infect invertebrates (Chinchar et al., 2017). The members of the *Iridovirus* genus had been described in insects and crustaceans. The type species of the *Iridovirus* genus is invertebrate iridescent virus 6 (IIV6) which can infect numerous insect species with agricultural importance (Henderson et al., 2001; Jakob et al., 2002; Jenkins et al., 2011). IIV6, as well as the other invertebrate iridescent viruses (IIVs), can produce either covert or patent infections. Mild infections are not lethal and not obvious to the naked eye but may cause extended development time, reduced fecundity and longevity (Marina et al., 2003). Patent infections are almost invariably lethal. The color of patently infected insects turns into an obvious iridescent color that typically ranges from violet, blue, green or orange. However, IIVs often cause a low prevalence of patent infections in nature.

IIV6 has very low *per os* infectivity but is highly infectious by injection. The range of insect species naturally infected by IIV6 includes a few members from Lepidoptera, and Orthoptera orders (Williams, 2008). However, the range of insect species that can be infected with IIV6 by injection is extensive (Mitsuhashi, 1967; Fukuda, 1971; Jensen et al., 1972; Ohba, 1975; Henderson et al., 2001). Virus transmission in nature may be through cannibalism, endoparasitic wasps or parasitic nematodes, which all do not occur commonly for all insects. Low infectivity and difficulties in transmitting the virus to susceptible hosts in the nature limit the usage of IIV6 as a biological control agent (Williams et al., 2005). Currently there are very few data available about the usage of iridoviruses in biocontrol studies. In a study performed by Hunter et al. (2003) the root weevil *Diaprepes abbreviates* (Linnaeus, 1758) (Coleoptera: Curculionidae) was infected with IIV6, in order to evaluate its potential in biocontrol studies. IIV6 infection in *D. abbreviates* caused both patent and sublethal infections in both larvae and adults. In another study, IIV6 infected *Phyllophaga vandinei* (Smyth, 1916) (Coleoptera: Scarabaeidae), scarab beetle, larvae or adults showed 30% mortality. Moreover, covert IIV6 infection changed feeding and mating behavior in *P. vandinei* adults (Jenkins et al., 2011).

The infectivity of the viruses that cause covert infections can be enhanced through bioengineering. A recombinant IIV6 (rCIV-Δ157L/gfp-AaIT) harboring a scorpion *Androctonus australis* (Linnaeus, 1758) (Scorpiones: Buthidae) insect toxin (*AaIT*) gene was previously constructed (Ozgen et al., 2014; Nalcacioglu et al., 2016). The gene was fused with the *green fluorescent protein* gene (*gfp*) and replaced with the IIV6 *157L* open reading frame (ORF) locus. Generated recombinant IIV6 produced enhanced insecticidal activity against *Galleria mellonella* (Linnaeus, 1758) (Lepidoptera: Pyralidae). In this study, wild type IIV6 (wt-CIV) and its two recombinants (rCIV-Δ157L-gfp and rCIV-Δ157L/gfp-AaIT) were tested on *Helicoverpa armigera* (Hübner, 1805) (Lepidoptera: Noctuidae)*, Spodoptera littoralis* (Boisduval, 1883) (Lepidoptera: Noctuidae)*, Lymantria dispar* (Linnaeus, 1758) (Lepidoptera: Erebidae), *Euproctis chrysorrhoea* (Linnaeus, 1758) (Lepidoptera: Erebidae) and *Tenebrio molitor* (Linnaeus, 1758) (Coleoptera: Tenebrionidae) larvae by injection in the laboratory to determine concentration-mortality responses for the different hosts.

Materials and Methods

Cell line, viruses and insects

Spodoptera frugiperda 9 (Sf9) cells (obtained from Laboratory of Virology, Wageningen University, The Netherlands) were maintained in Sf-900 II SFM (Gibco, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 5% fetal bovine serum (FBS, Sigma-Aldrich, St. Louis, MO, USA) at 28°C as monolayer in flasks.

Wild type IIV6 (wt-CIV) and its two recombinants (rCIV-Δ157L-gfp and rCIV-Δ157L/gfp-AaIT) previously constructed by Ozgen et al. (2014) and Nalcacioglu et al. (2016) were provided from Karadeniz Technical University, Microbiology Laboratory. Virus titers were determined by using the 50% tissue culture infectious dose ($TCID_{50}$) (Hierholzer & Killington, 1996).

Lab-reared cultures of *H. armigera, S. littoralis, T. molitor* and field collected *L. dispar* and *E. chrysorrhoea* larvae were used in bioassays. *Helicoverpa armigera* and *S. littoralis* larvae were maintained on artificial diet (266 g wet beans, 4 g ascorbic acid, 1.25 g sorbic acid, 2.5 g methyl 1-4 hydroxybenzoate, 3 g wheat germ, 14 g agar-agar, 35 g yeast and 800 ml distilled water) under laboratory conditions (Bergomaz & Boppré, 1986). *Tenebrio molitor* larvae were maintained on artificial medium (400 g wheat bran, 100 g wheat flour, 100 g corn flour, small pieces of carrot) (Kim et al., 2017). *Lymantria dispar* and *E. chrysorrhoea* larvae were fed with oak leaves which are collected from the nature. The leaves were firstly cleaned with 0.05% sodium hypochlorite solution for 2 min, then rinsed three times in distilled water and dried on filter papers (Gencer et al., 2018). All insect larvae were incubated at 28°C, 60-70% RH and 16:8 h L:D photoperiod.

Bioassays

For each insect species and each virus concentration, bioassays were performed by using 30 larvae in triplicate. wt-CIV, rCIV-Δ157L-gfp and rCIV-Δ157L/gfp-AaIT stock concentrations were calculated as 1.27×10^8, 1.70×10^8 and 3.85×10^8 $TCID_{50}$/ml, respectively. Five different dilutions (1×10^7, 1×10^6, 1×10^5, 1×10^4 and 1×10^3 $TCID_{50}$/ml) were prepared from each virus. All insect larvae were kept on ice until their movements slowed. Then, 5 µl of each dilution were injected to insect larvae with a plastic syringe and a 0.30 mm x 8 mm gauge needle. Injections for lepidopteran larvae were made from the right side of the abdomen, about a quarter above the anus. Coleopteran larvae were injected between the epidermis and cuticle of an abdominal sternite. The control group of insects were injected with only water. The larvae were individually kept in 40 ml plastic boxes with 16:8 light/dark periods at 28°C and 60% RH. Starting from the first day following injections, mortality, physical and behavioral changes of the larvae were observed daily until 21 d post infection (Kalha et al., 2014).

Mortality data were corrected using Abbott's formula (Abbott, 1925), LC_{50} and LT_{50} values were calculated by probit analysis using MS Excel (Finney, 1952).

Confirmation of dead larvae

To prove that insect death was a result of iridovirus infection, total DNA isolation was performed from dead larvae by using a DNA isolation kit (DNeasy Blood & Tissue Kit, Qiagen, Hilden, Germany) according to the manufacturer's instructions. The presence of iridoviruses was confirmed by polymerase chain reaction (PCR) using primers for *mcp* (*major capsid protein*) gene of IIV6 (forward primer: 5'-ATGTCTATTTCTTCGTCAAATGTAAC-3' and reverse primer: 5'-TTGGAAATTAATTCTCATTTCATTGTAT-3'). PCR amplification was performed in 0.2-ml PCR tubes, with 25 µl volumes containing 10-60 ng of DNA, 0.2 mM of each primer and each dNTP, 0.5 unit of Taq DNA polymerase and 2.5 µl of 10X reaction buffer (Promega) and 1.5 mM of $MgCl_2$. PCR conditions were adjusted as follows: one cycle for 3 min at 95°C was followed by 35 cycles of 30 s at 95°C, 30 s at 50°C and 60 s at 72°C. The amplification was completed with a final extension step of 10 min at 72°C. PCR products were visualized on 0.1% agarose gel with ethidium bromide.

Results

The mortalities of all insects infected with wt-CIV, rCIV-Δ157L-gfp and rCIV-Δ157L/gfp-AaIT viruses are shown in Figure 4. The highest mortalities of rCIV-Δ157L/gfp-AaIT were 100% and 98% of *E. chrysorrhoea* and *L. dispar*, respectively, for 10^7 $TCID_{50}$/ml concentration (Figure 4). *Euproctis chrysorrhoea* and *L. dispar* larvae had the highest mortalities as a result of virus injections, but they did not produce any iridescent color. A few *H. armigera* larvae infected with wt-CIV developed iridescent color that can be observed at patent iridovirus infections (Figure 1). The mortality of *H. armigera* was over 80% with rCIV-Δ157L/gfp-AaIT infections except for 10^3 and 10^4 $TCID_{50}$/ml concentrations (Figure 4). Even though the mortality was similar between wt-CIV and rCIV-Δ157L-gfp infections, the mortality of recombinant virus (rCIV-Δ157L/gfp-AaIT) infections were higher than the other viruses tested.

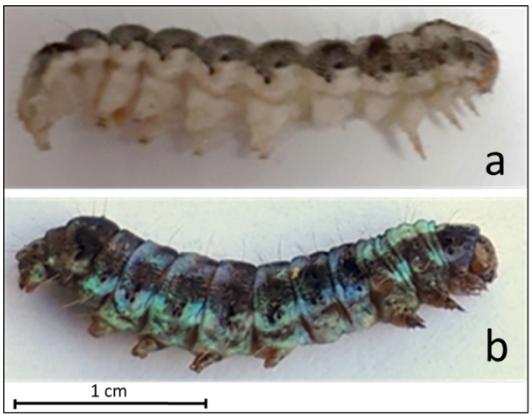

Figure 1. Patent infection of invertebrate iridescent virus 6 (IIV6) on *Helicoverpa armigera* larvae: a) uninfected larvae; b) infected larvae.

Spodoptera littoralis larvae were the least affected of the species used in this study. However, those infected larvae were not able to emerge properly from the pupae. The emerged *S. littoralis* adults, infected previously with wt-CIV, all had physical defects and were not able to fly (Figure 2). Malformed pupae appeared from the larvae infected with rCIV-Δ157L/gfp-AaIT and could not develop to adults properly. Mortalities reached a maximum of 60% with the recombinant iridoviruses infections on *S. littoralis* larvae (Figure 4). On the other hand, the mortality of *S. littoralis* larvae infected with wt-CIV remained below 40%.

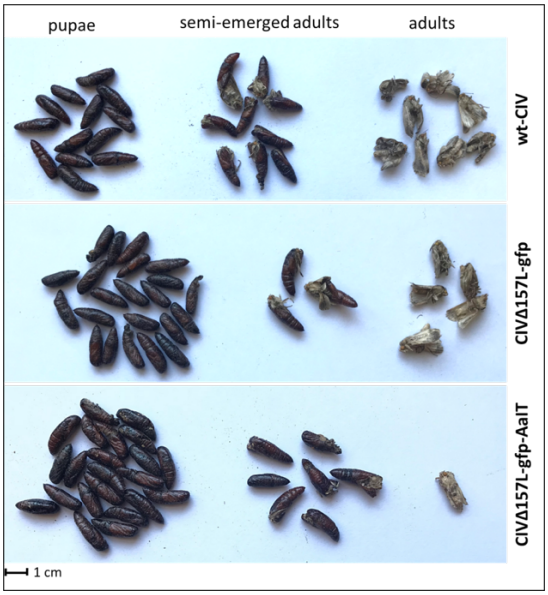

Figure 2. Defects in *Spodoptera littoralis* pupae and adults as a result of iridovirus infection.

Viruses expressing toxin genes caused paralysis of all larvae except *S. littoralis* at different levels. However, in *T. molitor* larvae, paralysis was clearly seen 3 d after virus infections with 10^6 and 10^7 $TCID_{50}$/ml concentrations. Paralyzed larvae could not move their bodies, except their legs. Additionally, these insects died a few days after they became paralyzed. The color of the dead *T. molitor* larvae infected with each type of iridoviruses used in this study appeared to be darkened (Figure 3).

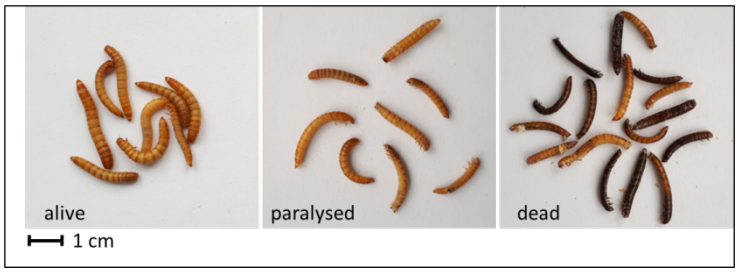

Figure 3. Effects of recombinant iridovirus expressing toxin gene on *Tenebrio molitor* larvae.

The highest concentrations of wt-CIV, rCIV-Δ157L-gfp and rCIV-Δ157L/gfp-AaIT caused 57, 76 and 83% mortality of *T. molitor* larvae, respectively (Figure 4).

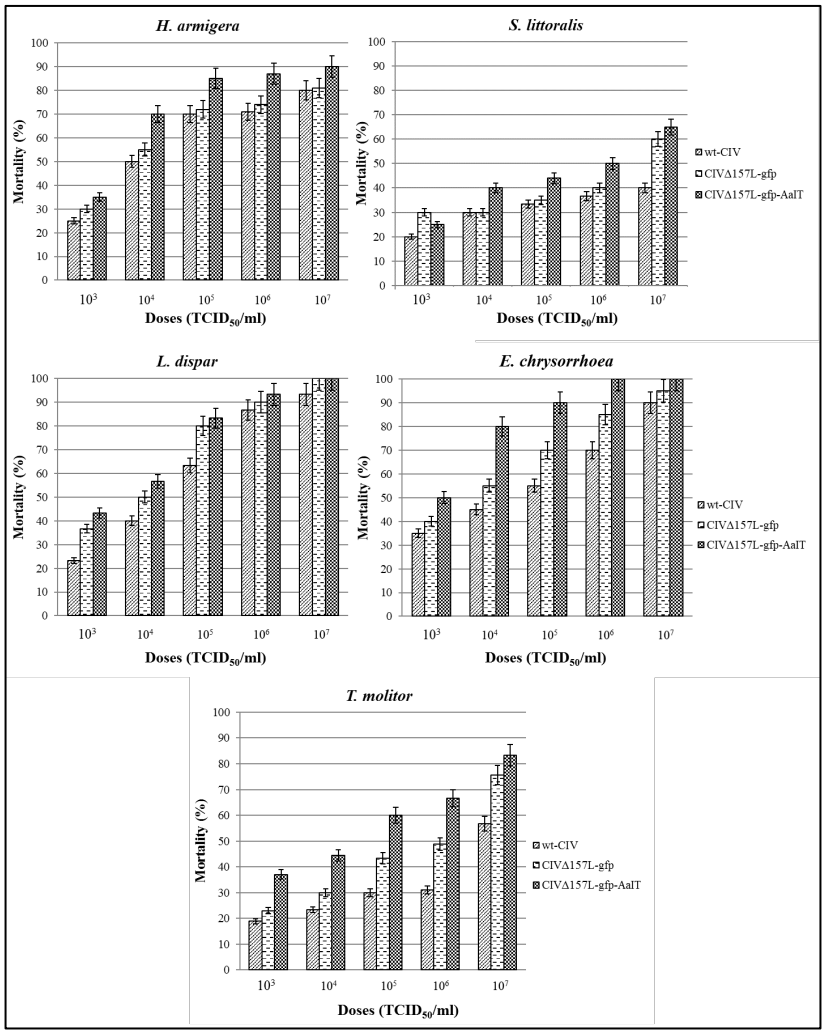

Figure 4. Mortality of insect larvae resulting from iridovirus infections. Bars show standard errors.

Insect deaths as a result of virus infections were confirmed by PCR (Figure 5). Control groups of each insect successfully completed metamorphosis process during the bioassay period.

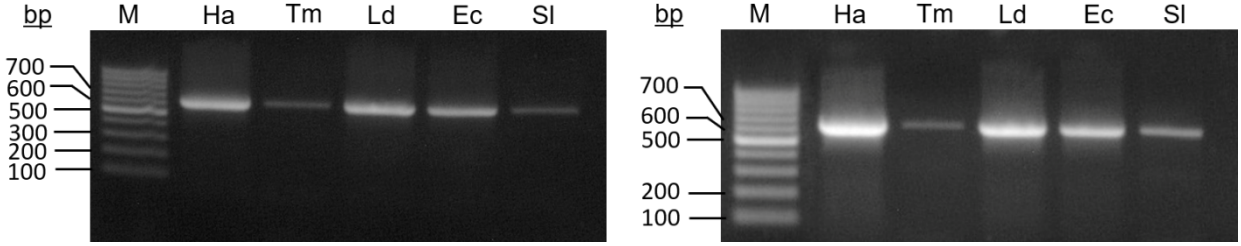

Figure 5. Verification that insect larvae have died due to virus infection. PCR fragments (630 bp) amplified from DNA isolated from wt-CIV (a) and rCIV-Δ157L/gfp-AaIT (b) infected insect larvae with *mcp*-specific primers. Ha, *Helicoverpa armigera;* Tm, *Tenebrio molitor;* Ld, *Lymantria dispar;* Ec, *Euproctis chrysorrhoea;* Sl, *Spodoptera littoralis*; and M, marker (100 bp).

The LC_{50} values for wt-CIV infections were calculated as 0.1 x 10^6, 0.2 x 10^6, 0.2 x 10^6, 0.9 x 10^7 and 3.3 x 10^9 $TCID_{50}$/ml on *E. chrysorrhoea*, *H. armigera*, *L. dispar*, *T. molitor* and *S. littoralis* larvae, respectively. The three lowest LC_{50} values of rCIV-Δ157L-gfp were determined as 0.4 x 10^5, 0.5 x 10^5 and 0.9 x 10^5 $TCID_{50}$/ml on *E. chrysorrhoea*, *L. dispar* and *H. armigera,* respectively. The LC_{50} values of the rCIV-Δ157L/gfp-AaIT virus were lower than that of the other two viruses. The best LC_{50} values were demonstrated on *E. chrysorrhoea* and *H. armigera* larvae as follows; 0.7 x 10^4 and 0.1 x 10^5 $TCID_{50}$/ml respectively. LT_{50} values were calculated for the highest virus concentrations. The shortest days, necessary for the death of the 50% of the larvae, were obtained with of rCIV-Δ157L-gfp. All LC_{50} and LT_{50} values are shown in Table 1 for each insect and virus.

Table 1. Lethal concentrations (LC_{50}) and lethal times (LT_{50}) with wt-CIV, rCIVΔ157L-gfp and rCIVΔ157L-gfp-AaIT virus infections of five common pests. LT_{50} values were calculated for 10^7 $TCID_{50}$/ml virus concentrations

	Viruses	LC_{50} ($TCID_{50}$/ml)	Slope±SE	X^2	LT_{50}(day) (95% CL)
Helicoverpa armigera	wt-CIV	0.2x10^6 (0.01-2.1)	0.359±0.522	0.574	0.2x10^6 (0.01-2.1)
	rCIVΔ157L-gfp	0.9x10^5 (0.07-12)	0.332±0.565	0.666	0.9x10^5 (0.07-12)
	rCIVΔ157L-gfp-AaIT	0.15x10^5 (0.01-1.53)	0.394±0.514	0.527	0.15x10^5 (0.01-1.53)
Spodoptera littoralis	wt-CIV	3.36x10^9 (0.07-1605)	0.136±1.367	0.896	3.36x10^9 (0.07-1605)
	rCIVΔ157L-gfp	3.7x10^7 (0.04-336.6)	0.183±0.996	0.648	3.7x10^7 (0.04-336.6)
	rCIVΔ157L-gfp-AaIT	0.3x10^7 (0.01-12)	0.237±0.765	0.883	0.3x10^7 (0.01-12)
Lymantria dispar	wt-CIV	0.2x10^6 (0.4-1)	0.582±0.348	0.980	0.2x10^6 (0.4-1)
	rCIVΔ157L-gfp	0.5x10^5 (0.9-2.7)	0.571±0.369	0.787	0.5x10^5 (0.9-2.7)
	rCIVΔ157L-gfp-AaIT	0.2x10^5 (0.5-1.5)	0.581±0.377	0.820	0.2x10^5 (0.5-1.5)
Euproctis chrysorrhoea	wt-CIV	0.1x10^6 (0.2-1.6)	0.398±0.476	0.839	0.1x10^6 (0.2-1.6)
	rCIVΔ157L-gfp	0.4x10^5 (0.7-3.4)	0.471±0.428	0.982	0.4x10^5 (0.7-3.4)
	rCIVΔ157L-gfp-AaIT	0.7x10^4 (0.1-4.4)	0.641± 0.384	0.734	0.7x10^4 (0.1-4.4)
Tenebrio molitor	wt-CIV	0.9x10^7 (0.07-11.4)	0.335±0.559	0.335	0.9x10^7 (0.07-11.4)
	rCIVΔ157L-gfp	0.8x10^6 (0.1-4.97)	0.484±0.397	0.475	0.8x10^6 (0.1-4.97)
	rCIVΔ157L-gfp-AaIT	0.7x10^5 (0.08-7.7)	0.380±0.504	0.472	0.7x10^5 (0.08-7.7)

Discussion

Invertebrate iridoviruses (IIVs) infect agriculturally and medically important insect species. However, these viruses have attracted little interest due to the often-low prevalence of lethal infections in host populations (Williams, 2008).

Insect viruses that do not produce high mortality on the target insect pest are often thought of as ineffective biocontrol agents. Enhancement of virus infectivity, through genetic engineering, is a method to enhance their effectiveness as biocontrol agents. A recombinant iridovirus harboring an insect specific neurotoxin has been constructed previously. This recombinant iridovirus showed increased speed of kill in *G. mellonella* larvae (Nalcacioglu et al., 2016). In this study, we examined the infectivity of wild type and recombinant iridoviruses (rCIV-Δ157L-gfp and rCIV-Δ157L/gfp-AaIT) on five different species of insect larvae. IIV6 infection was done by injection because IIVs are poorly infectious when applied orally but are highly infectious by injection (Williams et al., 2005).

In a study performed by Jakob et al. (2002) peroral infection of IIV6 was investigated on a few Lepidoptera larvae including *G. mellonella*, *Spodoptera exigua* (Hubner, 1808) (Lepidoptera: Noctuidae), *S. littoralis, H. armigera* and *Agrotis segetum* (Denis & Schiffermüller, 1775) (Lepidoptera: Noctuidae). Their results showed that only *G. mellonella* became orally infected with the virus. In the same study, *L. dispar* larvae was tested for peroral infection of cricket iridovirus (CrIV) which was considered to be a variant and/or a novel strain of IIV6. However, no CrIV infection was observed in *L. dispar* larvae. Invertebrate iridescent virus 29 infection was reported in a laboratory colony of *T. molitor* larvae previously (Kelly et al., 1979). *E. chrysorrhoea* and *L. dispar* have been tested first time for IIV6 infection in the present study. These insects are remarkably sensitive to IIV6 according to the current biotest results. Bioassay results on *H. armigera* larvae showed that IIV6 was highly effective on this insect. Iridescent color changes were clearly detected in heavily infected *H. armigera* larvae.

The lowest mortality was recorded with *S. littoralis* larvae in this study. Mortality results were not statistically significant, but infected *S. littoralis* larvae entered the pupal stage in a shorter time than uninfected ones. This result is opposite for the other infected insect larvae that entered to the pupal stage later than those that were not infected. The pupae of the infected *S. littoralis* did not development to healthy adults. Developmental stages were observed in three different styles; non-emerging pupae, semi-emerged adults and developed into flightless adults with deformed wings.

An insect-selective neurotoxic polypeptide derived from scorpion venom (AaIT), has recently been used to engineer different organisms such as viruses, fungi, bacteria and plants against insect pests (Deng et al., 2019). Many types of different baculoviruses including *AaIT* gene have been constructed to enhance the insecticidal activity against lepidopteran larvae (Treacy & All, 1996; Yao et al., 1996; Elazar et al., 2001). A recombinant baculovirus containing a scorpion toxin gene (AcMNPV contains AaIT) produced a significant decrease in the time to kill (LT_{50} 88 h) *Heliothis virescens* (Fabricius, 1777*)* (Lepidoptera: Noctuidae) larvae compared to wild type AcMNPV (LT_{50} 125 h) (McCutchen et al., 1991). Similarly, a recombinant baculovirus Bombyx mori nuclear polyhedrosis virus (BmNPV) carrying a synthetic *AaIT* gene has been assayed in *Bombyx mori* (Linnaeus, 1758) (Lepidoptera: Bombycidae) larvae. Those results indicate that baculoviruses, carrying toxin genes, can be used to reduce insect feeding damage and can result with increased mortality (Maeda et al., 1991). Although iridoviruses kill insect larvae later than baculoviruses, the results of this study have shown that recombinant iridovirus containing the *neurotoxin* gene caused death in a shorter time than the wild type virus.

rCIV-Δ157L-gfp was highly insecticidal activity on all used insect larvae compared to the wild type IIV6. This result may be relevant with deleted ORF 157L in its structure. ORF 157L shows homology to the iap (inhibitor of apoptosis) gene of a baculovirus (Razvi & Welsh, 1995). Although it is shown that 157L

does not directly inhibit apoptosis (İnce et al., 2008), it may have an indirect function at apoptosis inhibition of the virus. Deletion of this gene can cause insect cells to go into apoptosis and eventually insect death. This may be the reason for high insecticidal activity of rCIV-Δ157L-gfp.

In conclusion, considering all insects used in this study, lethal effects were determined on *H. armigera, L. dispar* and *E. chrysorrhoea* larvae when they were injected with IIV6. *Spodoptera littoralis* was the most resistant species to IIV6 infection. *T. molitor* larvae survived longer, even if they were paralyzed. We conclude that the paralyzed beetle larvae survived longer than lepidopteran larvae without access to food.

In this study, the lethal effects of wild type and recombinant iridoviruses on various hosts were investigated by injection and these viruses were found to be lethal to Coleoptera and Lepidoptera orders. Virus infection of insects in nature through hemolymph can occur by rare mechanisms. The virus may infect insect larvae through wounds or parasites and parasitoids may introduce these viruses to the susceptible hosts (Williams et al., 2005). However, since these mechanisms do not occur commonly in the nature, another way need to be found for an effective infection of insects with iridoviruses. Encapsulation may be a good choice for iridoviruses. Encapsulation of the virus by a protective substance and infecting the insects via the oral route will be our next study on iridoviruses.

References

Abbott, W. S., 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18: 265-267.

Bergomaz, R. & M. Boppré, 1986. A simple instant diet for rearing Arctiidae and other moths. Journal of the Lepidopterists' Society, 40 (3): 131-137.

Chinchar, V. G., P. Hick, I. A. Ince, J. K. Jancovich, R. Marschang, Q. Qin, K. Subramaniam, T. B. Waltzek, R. Whittington, T. Williams & Q. Y. Zhang, 2017. ICTV virus taxonomy profile: Iridoviridae. Journal of General Virology, 98 (5): 890-891.

Deng, S. Q., J. T. Chen, W. W. Li, M. Chen & H. J. Peng, 2019. Application of the scorpion neurotoxin AaIT against insect pests. International Journal of Molecular Sciences, 20 (14): 3467.

Elazar, M., R. Levi & E. Zlotkin, 2001. Targeting of an expressed neurotoxin by its recombinant baculovirus. Journal of Experimental Biology, 204 (15): 2637-2645.

Finney, D. J., 1952. Probit Analysis: A Statistical Treatment of the Sigmoid Response Curve. Cambridge University Press, Cambridge, England, 256 pp.

Fukuda, T., 1971. Per os transmission of Chilo iridescent virus to mosquitoes. Journal of Invertebrate Pathology, 18 (1): 152-153.

Gencer, D., Z. Bayramoglu, R. Nalcacioglu, R. G. Kleespies, Z. Demirbag & I. Demir, 2018. Characterisation of three Alphabaculovirus isolates from the gypsy moth, *Lymantria dispar dispar* (Lepidoptera: Erebidae), in Turkey. Biocontrol Science and Technology, 28 (2): 107-121.

Henderson, C. W., C. L. Johnson, S. A. Lodhi & S. L. Bilimoria, 2001. Replication of Chilo iridescent virus in the cotton boll weevil, *Anthonomus grandis*, and development of an infectivity assay. Archives of Virology, 146 (4): 767-775.

Hierholzer, J. C. & R. A. Killington, 1996. “Virus Isolation and Quantitation, 25-46” In: Virology Methods Manual (Eds. B. W. Mahy & H. I. Kangro), Virology Methods Manual Academic Press, London, 374 pp.

Hunter, W. B., S. L. Lapointe, X. H. Sinisterra, D. S. Achor & C. J. Funk, 2003. Iridovirus in the root weevil *Diaprepes abbreviatus*. Journal of Insect Science, 3 (9): 1-6.

İnce, İ. A., M. Westenberg, J. M. Vlak, Z. Demirbağ, R. Nalçacioğlu & M. M. van Oers, 2008. Open reading frame 193R of Chilo iridescent virus encodes a functional inhibitor of apoptosis (IAP). Virology, 376 (1): 124-131.

Jakob, N. J., R. G. Kleespies, C. A. Tidona, K. Müller, H. R. Gelderblom & G. Darai, 2002. Comparative analysis of the genome and host range characteristics of two insect iridoviruses: Chilo iridescent virus and a cricket iridovirus isolate. Journal of General Virology, 83 (2): 463-470.

Jenkins, D., W. Hunter & R. Goenaga, 2011. Effects of Invertebrate Iridescent Virus 6 in *Phyllophaga vandinei* and its potential as a biocontrol delivery system. Journal of Insect Science, 11 (44): 1-10.

Jensen, D. D., T. Hukuhara & Y. Tanada, 1972. Lethality of Chilo iridescent virus to *Colladonus montanus* leafhoppers. Journal of Invertebrate Pathology, 19 (2): 276-278.

Kalha, C. S., P. P. Singh, S. S. Kang, M. S. Hunjan, V. Gupta & R. Sharma, 2014. ''Entomopathogenic Viruses and Bacteria for Insect-Pest Control, 225-244''. In: Integrated Pest Management: Current Concepts and Ecological Perspective (Ed. D. P. Abrol). Academic Press, San Diego, 576 pp.

Kelly, D. C., M. D. Ayres & T. Lescott, 1979. A small iridescent virus (type 29) isolated from *Tenebrio molitor*: a comparison of its proteins and antigens with six other iridescent viruses. Journal of General Virology, 42 (1): 95-105.

Kim, S. Y., H. G. Kim, H. J. Yoon, K. Y. Lee & N. J. Kim, 2017. Nutritional analysis of alternative feed ingredients and their effects on the larval growth of *Tenebrio molitor* (Coleoptera: Tenebrionidae). Entomological Research, 47 (3): 194-202.

Maeda, S., S. L. Volrath, T. N. Hanzlik, S. A. Harper, K. Majima, D. W. Maddox, B. D. Hammock & E. Fowler, 1991. Insecticidal effects of an insect-specific neurotoxin expressed by a recombinant baculovirus. Virology, 184 (2): 777-780.

Marina, C. F., J. I. J. E. Arredondo-Jimenez, J. E. Ibarra, I. Fernandez-Salas & T. Williams, 2003. Effects of an optical brightener and an abrasive on iridescent virus infection and development of *Aedes aegypti*. Entomologia Experimentalis et Applicata, 109 (2): 155-161.

McCutchen, B. F., P. V. Choudary, R. Crenshaw, D. Maddox, S. G. Kamita, N. Palekar, S. Volrath, E. Fowler, B. D. Hammock & S. Maeda, 1991. Development of a recombinant baculovirus expressing an insect-selective neurotoxin: Potential for pest control. Bio/Technology, 9 (9): 848-852.

Mitsuhashi, J., 1967. Infection of leafhopper and its tissues cultivated in vitro with Chilo iridescent virus. Journal of Invertebrate Pathology, 9 (3): 432-434.

Nalcacioglu, R., H. Muratoglu, A. Yesilyurt, M. M. van Oers, J. M. Vlak & Z. Demirbag, 2016. Enhanced insecticidal activity of Chilo iridescent virus expressing an insect specific neurotoxin. Journal of Invertebrate Pathology, 138: 104-111.

Ohba, M., 1975. Studies on the parthogenesis of Chilo iridescent virus 3. Multiplication of CIV in the silkworm *Bombyx mori* L. and field insects. Scientific Bulletin of the Faculty of Agriculture Kyushu University, 30: 71-81.

Ozgen, A., H. Muratoglu, Z. Demirbag, J. M. Vlak, M. M. van Oers & R. Nalcacioglu, 2014. Construction and characterization of a recombinant invertebrate iridovirus. Virus Research, 189: 286-292.

Razvi, E. S. & R. M. Welsh, 1995. Apoptosis in viral infections. Advanced Virus Research, 45: 1-60.

Treacy, M. F. & J. N. All, 1996. Impact of insect-specific AaHIT gene insertion on inherent bioactivity of baculoviruses against tobacco budworm, *Heliothis virescens*, and cabbage looper, *Trichoplusia ni*. Beltwide Cotton Conferences, 2: 911-917.

Williams, T., 2008. Natural invertebrate hosts of iridovirases (iridoviridae). Neotropical Entomology, 37 (6): 615-632.

Williams, T., V. Barbosa-Solomieu & V. G. Chinchar, 2005. A decade of advances in iridovirus research. Advances in Virus Research, 65: 173-248.

Yao, B., Y. Pang, Y. Fan, R. Zhao, Y. Yang & T. Wang, 1996. Construction of an insecticidal baculovirus expressing insect-specific neurotoxin AaIT. Science China Life Sciences, 39 (2): 199-206.

Türk. entomol. derg., 2020, 44 (3): 375-384
DOI: http://dx.doi.org/10.16970/entoted.663690

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Fungal pathogens of *Amphimallon solstitiale* Linnaeus, 1758 (Coleoptera: Scarabaeidae)

Amphimallon solstitiale Linnaeus, 1758 (Coleoptera: Scarabaeidae)'nin fungal patojenleri

Seda BİRYOL[1] **Davut EFE[2]** **Ardahan ESKİ[3]**
Zihni DEMİRBAĞ[1] **İsmail DEMİR[1*]**

Abstract

European June beetle, *Amphimallon solstitiale* Linnaeus, 1758 (Coleoptera: Scarabaeidae) is one of the most important soilborne pests in many parts of Turkey and the world. Entomopathogenic fungi are important microbial control agents that can be used to control soilborne pests, and it is desirable to obtain them from local insect populations. The study was conducted at Karadeniz Technical University, Faculty of Science, Microbiology Laboratory in 2017. In this study, fungal pathogens of *A. solstitiale* were investigated to find an effective microbial control agent. Fungi were isolated from infected larvae and morphological-molecular characterization of the isolates showed that all isolates were *Metarhizium flavoviride* Gams & Roszypal (Deuteromycotina: Hyphomycetes). Using phylogenetic analysis and pathogenicity tests, the isolates were found to be different genotypes of *M. flavoviride.* All isolates gave more than 80% mortality at a concentration of 10^6 conidia/ml, with one isolate (As2) causing 96% mortality. Therefore, dose-mortality experiments were conducted with As2, and the median lethal concentration was determined to be 3.87 x 10^3 conidia/ml. This study demonstrated that *M. flavoviride* As2 is an effective microbial control agent that can be used for biological control of *A. solstitiale.*

Keywords: *Amphimallon solstitiale,* biological control, entomopathogenic fungi, *Metarhizium flavoviride*

Öz

Avrupa Haziran böceği, *Amphimallon solstitiale* Linnaeus, 1758 (Coleoptera: Scarabaeidae) Türkiye'de ve dünyanın birçok bölgesinde önemli toprakaltı zararlılarından biridir. Entomopatojen funguslar bu zararlıların mücadelesinde kullanılabilecek önemli mikrobiyal mücadele ajanlarıdır ve bunların lokal böcek popülasyonlarından elde edilmesi tercih edilir. Çalışma 2017 yılında Karadeniz Teknik Üniversitesi, Fen Fakültesi, Mikrobiyoloji Laboratuvarında gerçekleştirilmiştir. Bu çalışmada, zararlıya karşı etkili bir mikrobiyal mücadele ajanı bulmak için zararlının fungal patojenleri araştırılmıştır. Bulaşık larvalardan fungus izolasyonu yapılmış ve morfolojik-moleküler karakterizasyonu sonucu izolatlar *Metarhizium flavoviride* Gams & Roszypal, (Deuteromycotina: Hyphomycetes) olarak tanımlanmıştır. Filogenetik analizler ve patojenite çalışmaları sonucunda izolatların *M. flavoviride*'nin farklı genotipleri olduğu belirlendi. En yüksek ölüm ise %96 ile As2 izolatında görülmüş olup, tüm izolatlar 10^6 konidya/ml konsantrasyonda %80'in üzerinde ölüme neden olmuştur. As2 izolatı ile doz denemeleri sonucunda, izolatın ortalama öldürücü konsantrasyonu 3.87 x 10^3 konidya/ml olarak belirlenmiştir. Bu çalışmalar, *M. flavoviride* As2 izolatının *A. solstitiale*'nin biyolojik mücadelesinde kullanılabilecek etkili bir mikrobiyal mücadele etmeni olduğunu göstermektedir.

Anahtar sözcükler: *Amphimallon solstitiale,* biyolojik mücadele, entomopatojen fungus, *Metarhizium flavoviride*

[1] Karadeniz Technical University, Faculty of Science, Department of Biology, 61080, Trabzon, Turkey
[2] Bingöl University, Faculty of Agriculture, Department of Plant Protection, 12000, Bingöl, Turkey
[3] Bilecik Şeyh Edebali University, Vocational School, Program of Biomedical Equipment Technology, 11230, Bilecik, Turkey
* Corresponding author (Sorumlu yazar) e-mail: idemir@ktu.edu.tr
Received (Alınış): 23.12.2019 Accepted (Kabul ediliş): 10.06.2020 Published Online (Çevrimiçi Yayın Tarihi): 21.07.2020

Introduction

The larvae of scarab beetles (Coleoptera: Scarabaeidae), also known as white grubs, cause significant economic loses by feeding on the roots of several agricultural plants in many parts of the world. One of these insects, the European June beetle, *Amphimallon solstitiale* Linnaeus, 1758 (Coleoptera: Scarabaeidae), is a major soilborne pest of a wide range of crops throughout Europe and Turkey. It is also a serious pest of hazelnuts cultivated in the Black Sea Region of Turkey (Allen, 1995; de Goffau, 1996). The adults of the pest mostly feed on leaves whereas the subterranean larvae cause damage directly through feeding on plant roots and indirectly by creating wounds which allow entry of opportunistic plant pathogens. Larvae feeding on roots can cause drying, wilting and death of the plants.

Chemical pesticides are usually the first choice to control noxious insects. However, excess use of these has led to insecticide resistance, environmental pollution, and adverse effects on human, non-target fauna and flora (Ffrench-Constant et al., 2004). Therefore, scientists have focused on developing alternative methods for pest management (Wilson & Tisdell, 2001; Keller & Zimmermann, 2005; Danismazoglu et al., 2012; Gokce et al., 2013; Sevim et al., 2013). The use of microbial control agents such as viruses, bacteria, nematodes and fungi are the best alternative to chemical pesticides and the development of new commercial products an important area of research (Sevim et al., 2012; Kocacevik et al., 2016; Eski et al., 2017). Of these beneficial microorganisms, the mode of infection of entomopathogenic fungi (EPF) differs from other entomopathogens. There is no need to ingest EPF because they can infect their host directly through the cuticle (Shah & Pell, 2003; Ortiz-Urquiza & Keyhani, 2013). Therefore, EPF can infect non-feeding stages such as eggs and pupae (Anand et al., 2009). Due to their mode of action and high mortality rate of the pest, interest in EPF has increased significantly (Humber, 2008). Therefore, considerable efforts have been focused on the development and utilization of EPF, as they have the potential to be a key tool in sustainable pest management programs.

Although, some bacteria have been isolated from *A. solstitiale* and tested for biological control of this pest (Sezen et al., 2005), no study has investigated fungal pathogens as potential microbial control agents. In this study, isolation and characterization of EPF from *A. solstitiale* were performed, and their insecticidal effect were tested on *A. solstitiale* larvae.

Materials and Methods

Isolation of fungi

Amphimallon solstitiale larvae were collected from soil samples at different localities in Trabzon, Turkey between May and June 2016 and 2017, incubated in ventilated plastic boxes (30 ml) at 28ºC and 16:8 h L:D photoperiod and fed on hazelnut roots. The plastic boxes were checked daily. The infected larvae were removed, surface sterilized according to Mohammadyani et al. (2016) and incubated in the moist chamber for sporulation. After 4 d, fungi development of cadavers inoculated onto potato dextrose agar plus 1% yeast extract medium (PDAY) and 50 µg/ml ampicillin, the latter to avoid bacterial contamination (AppliChem, Darmstadt, Germany) and incubated 2 weeks at 28ºC, 65% RH and 16:8 h L:D photoperiod. A single colony was subculture from each isolate to obtain pure cultures. One hundred µl of conidial suspension of 1 x 10^6 conidia/ml pure culture was transferred to PDAY and incubated at 28ºC for 7 d under 16:8 h L:D photoperiod. After incubation, the growing colonies were transferred to the new medium and allowed to sporulated under appropriate conditions (28ºC for 2-3 weeks), and then stock cultures were prepared in 20% glycerol.

Morphological characterization

The macroscopic characterizations of isolates were determined by examining growth of the fungal colony and color of conidia. Based on these features, the initial identification of fungal isolates was

performed according to the fungal identification key (Humber, 2012) and confirmed by Dr. Humber (ARSEF Collection of Entomopathogenic Fungal Cultures, US Department of Agriculture, Agricultural Research Service, Washington DC, USA).

Molecular characterization

Hundred µl of conidial suspension of each isolate was spread on PDAY medium and incubated at 25ºC for 14 d to select colonies originated from single conidia, and each colony was subculture onto fresh medium. DNA was extracted from the mycelium of each isolate using the Quick-DNA Fungal/Bacterial MiniPrep Kit (Zymo Research, Irvine, CA, USA) following the manufacturer's instructions. The isolated DNA was stored at -20ºC until used. In order to determine the molecular characteristics of fungal isolates, the partial sequences of ITS1-5.8S-ITS2*,* RPB1 (RNA polymerase II largest subunit), *β-tubulin* and *pr1* genes were amplified by polymerase chain reaction (PCR) using primer pairs listed in Table 1.

Table 1. Primer pairs used for molecular characterization of fungal isolates

Primers	Primer sequence	Product size (bp)	Reference
ITS5	5'-GGAAGTAAAATCGTAACAAGG-3'	600	White et al., 1990
ITS4	5'-TCCTCCGCTTATTGATATGC-3'		
RPB1Af	5'-GARTGYCCDGGDCAYTTYGG-3'	800	Stiller & Hall, 1997
RPB1C	5'-CCNGCDATNTCRTTRTCCATRTA-3'		
T1	5'-AACATGCGTGAGATTGTAAGT-3'	1350	O'Donnell & Cigelnik,1997
T2	5'-TCTGGATGTTGTTGGGAATCC-3'		
METPR1	5 'CACTCTTCTCCCAGCCGTTC 3'	1200	Leal et al., 1997
METPR4	5'GTAGCTCAACTTCTGCACTC 3'		
METPR2	5'AGGTAGGCAGCCAGACCGGC 3'		
METPR5	5'TGCCACTATTGGCCGGCGCG 3'		

All targets except *pr1* were amplified with standard polymerase chain reactions using T100 thermal cycler (Bio-Rad, Watford, Hertfordshire, UK). Reaction mixture (50 µl total volume) contained 50 ng of DNA template, 10 µl 5X Phusion HF reaction buffer, 200 µM of each dNTPs, 1 µl (50 pmol) each primer, and 1-unit Phusion-DNA polymerase. PCR was performed under the following conditions: 98ºC for 30 s, followed by 98ºC for 10 s, 55ºC for 15 s, 72ºC for 1 min for 35 cycles with a final extension at 72ºC for 10 min.

Two successive nested-PCR amplifications of the *pr1A* gene were performed by using two primer pairs. In the first PCR, DNA from the samples as template and the outer pair of primers (METPR1 and METPR4) were used. In the second PCR, an aliquot of the first PCR as template and the inner pair of primers (METPR2 and METPR5) were used. PCR were applied as described above.

PCR products were analyzed by electrophoresis in 0.7% agarose gels and then visualized under UV light. The amplified products were purified and cleaned up using the NucleoSpin gel and PCR clean-up kit (Macherey-Nagel, Düren, Germany) following the manufacturer's instructions, quantified using NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA), and addressed to sequencing (Macrogen, Amsterdam, the Netherlands).

Phylogenetic analysis

The sequences of the fungal isolates were aligned with the sequences in the NCBI GenBank. (www.ncbi.nlm.nih.gov/BLAST). The phylogenetic evolutionary analyses were performed using the neighbor-joining (NJ) method with MEGA X software (Kumar et al., 2018). Bootstrap tests were conducted with 1000 replicates in the NJ analysis.

Screening experiments

The conidial suspension used in the bioassay was prepared from 1-month-old fungus cultures, and fungal spores were harvested from agar surface using sterile 0.01% Tween 80 (AppliChem). The spore suspension was counted using a Neubauer hemocytometer, and the concentration was adjusted to 1 x 10^6 conidia/ml. The larvae of *A. solstitiale* were collected from agricultural fields in the vicinity of Trabzon, Turkey for bioassay, kept in plastic boxes (30 m^3), fed on hazelnut roots and allowed one week to adapt to the laboratory conditions. Ten healthy larvae were used in each bioassay, and the tests were replicated three times for each application. A dipping method was used for bioassay experiment. Larvae were dipped in 10 ml of 1 x 10^6 conidia/ml suspension for 5 s then put into plastic boxes (10 x 5 cm) containing sterile soil and hazelnut root pieces as food. Control larvae were exposed to 0.01% aqueous Tween 80. Experiments were performed at 20ºC under 16:8 h L:D photoperiod at 15 d. After the experiment, the dead larvae were removed from the boxes and surface sterilized and placed in incubator for mycosis.

Dose-response experiments

Metarhizium flavoviride Gams & Roszypal (Deuteromycotina: Hyphomycetes) isolate As2 (obtained during this study) was used in dose-response experiments as it had the highest virulence based on the screening experiments. Healthy larvae were collected from the farmland and used for dose-response experiments. They were treated with five conidial concentrations (10^8, 10^7, 10^6, 10^5 and 10^4 conidia/ml). The control group was treated only with 0.01% Tween 80. Bioassays were conducted as described for the screening test.

Temperature sensitivity and UV resistance

Temperature and UV tolerance of *M. flavoviride* isolate As2, shown the highest mortality on *A. solstitiale* were determined. Fungus was grown on PDA medium (Difco, Laboratories, Detroit, MI, USA) at 28ºC and under a 16:8 h L:D photoperiod. The spores were harvested from Petri dishes by adding 10 ml of sterile 0.01% Tween 80 onto the 4-weeks-old cultures. The prepared conidial suspension was filtered into 50 ml plastic universal bottle by a sterile cheesecloth to remove mycelium and vortexed for 5 min to obtain homogeneous suspension. Subsequently the concentration of conidial suspension was adjusted to 10^5 conidia/ml using a Neubauer hemocytometer. The experiment was designed according to the method of Bidochka et al. (2002). Ninety-six-well cell culture plates were filled with 100 µl of PDA and inoculated with 10^5 conidial suspension. Evaluation of the fungal growth was performed at 8, 15, 25 and 37ºC. Criteria from Bidochka et al. (2002) were adapted for assessment of positive growth; at 8ºC and OD_{630}>0.15 after 14 d; at 16ºC and OD_{630}>0.50 after 5 d; at 25ºC and OD_{630}>0.50 after 2 d; and at 37ºC and OD_{630}>0.25 after 3 d. UV tolerance of the fungal isolates was tested by exposing fungal conidia to UV radiation (306 nm) for 30 and 60 min. The cell culture plates were then incubated at 25ºC and OD_{630}>0.25 after 2 d was chosen as indicative of UV tolerance.

Statistical analysis

The control mortalities were corrected using Abbott's formula (Abbott, 1925). Statistical differences between the isolates were evaluated by one-way ANOVA, followed by Tukey's post-hoc test ($p < 0.05$). Each fungal isolate and control group were compared for mortality and mycosis. The LC_{50} value of *M. flavoviride* isolate As2 was estimated by probit analysis (Finney, 1971). Chi-square test was used to analyze growth of isolate As2 isolate in response to different temperatures and UV exposure using SPSS v 22.0 (IBM Corp., Armonk, NY, USA).

Results

Isolation and characterization of fungi

The specimens were classified as infected if mycelia growth was evident on the outside of cadavers. Four fungal isolates were obtained from *A. solstitiale* larvae. The isolates were identified based on the shape and size of conidia when grown on PDAY according to Humber (2012). In this way, the four isolates were determined to be *Metarhizium* sp. Sorokin, 1879. One isolate (As2) was differed from the others in colony morphology producing a darker green color 10 d after inoculation.

After amplification for ITS1-5.8S-ITS2, RPB1, *β-tubulin* and *pr1,* 600, 800, 1350 and 1200 bp amplicons were visualized in agarose gels, respectively. The partial sequences of ITS1-5.8S-ITS2, RPB1, and *β-tubulin* and *pr1* were used to construct phylogenetic trees. All isolates were determined to be identical to *M. flavoviride* as described by Bischoff et al. (2009). The four isolates As1 and As19, and As2 and As18 formed distinct two group on the phylogenetic trees (Figures 1 & 2). The sequences were deposited in the GenBank database under the accession numbers KY327805, KY348739, KY348740 and KY348741.

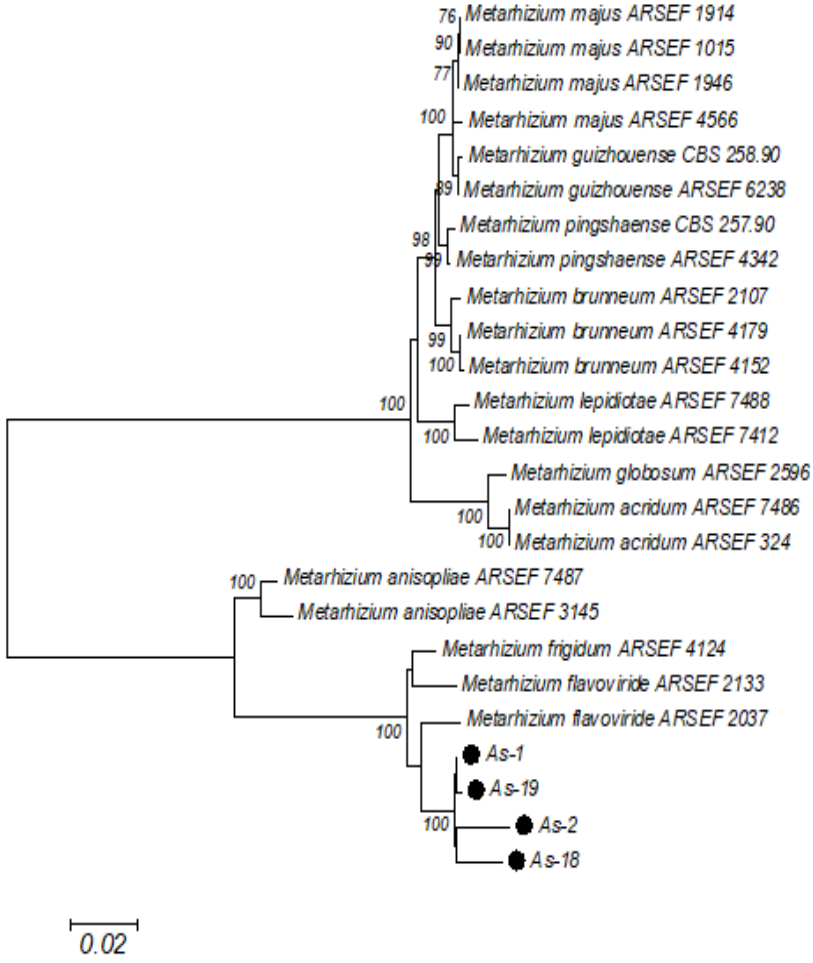

Figure 1. Taxonomic position of *Metarhizium flavoviride* As1, As2, As18 and As19 within the genus *Metarhizium* based on the combined data from ITS1-5.8S-ITS2, RPB1*, β-Tubulin* sequences. The reference isolates were taken from the study of Bischoff et al. (2009). The dendrogram was constructed by using the neighbor-joining analysis with *p*-distance model in MEGA X. Bootstrap values C ≥ 70% are labeled.

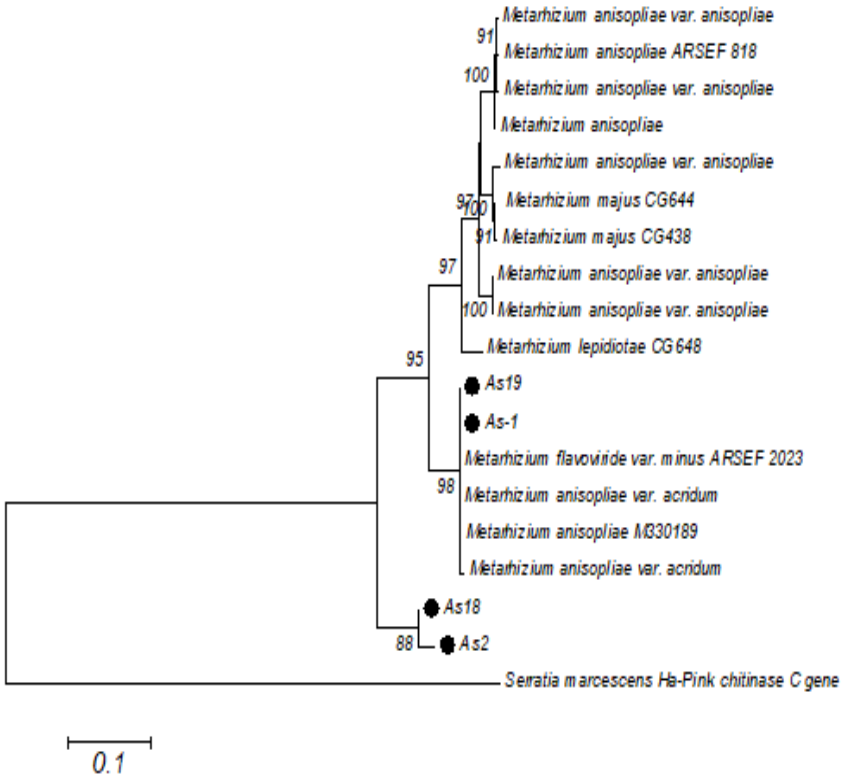

Figure 2. Taxonomic position of *Metarhizium flavoviride* As1, As2, As18 and As19 within the genus *Metarhizium* based on the combined sequence of *pr1 (*protease-type subtilisin) gene. The dendrogram was constructed by using the neighbor-joining analysis with *p*-distance model in MEGA X. Bootstrap values C ≥ 70% are labeled. The reference isolates were taken from the study of Bischoff et al. (2009). *Serratia marcescens* Ha-Pink chitinase C gene was used as an outgroup.

Screening test and dose-response experiments

The four isolates of *M. flavoviride* isolates (As1, As2, As18 and As19) gave high pathogenicity to *A. solstitiale* larvae 15 d post inoculation. Mortalities ranged from 86 to 97%, with isolates having different mycosis rates. Isolates As2 and As18 gave the highest mortality (93 to 96%, $p < 0.05$; Table 2). Although the mortality caused by these two isolates was equivalent, with isolate As2 all cadavers exhibited mycosis.

Table 2. Screening the pathogenicity test of locally isolated fungi against *Amphimallon solstitiale*

Fungal treatment at 10^6 conidia/ml	Mortality (%)±SE			
	5 DAT	10 DAT	15 DAT	Mycosis
As1	26±3.2 d	43±2.0 c	83±2.1 c	97±2.0 a
As2	53±3.5 b	83±3.1 a	96±3.8 a	100±0.0 a
As18	41±4.2 c	51±2.5 b	93±2.0 ab	98±2.1 a
As19	64±3.0 a	80±1.5 a	88±2.5 bc	97±2.1 a

Each value is mean of three replicates; Data analyzed by one-way ANOVA and Tukey's test. Values in a column followed by the same letter are not significantly different (p < 0.05); SE, standard error; and DAT, days after treatment.

In the dose-response experiments, *M. flavoviride* isolate As2 gave complete mortality of *A. solstitiale* larvae within 15 d after treatment with a conidia suspension of 1 x 10^8 conidia/ml (Figure 3). The LC_{50} of the isolate was estimated as 3.87 x 10^3 conidia/ml (Table 3). All concentrations caused a significantly higher mortality than control ($p < 0.05$).

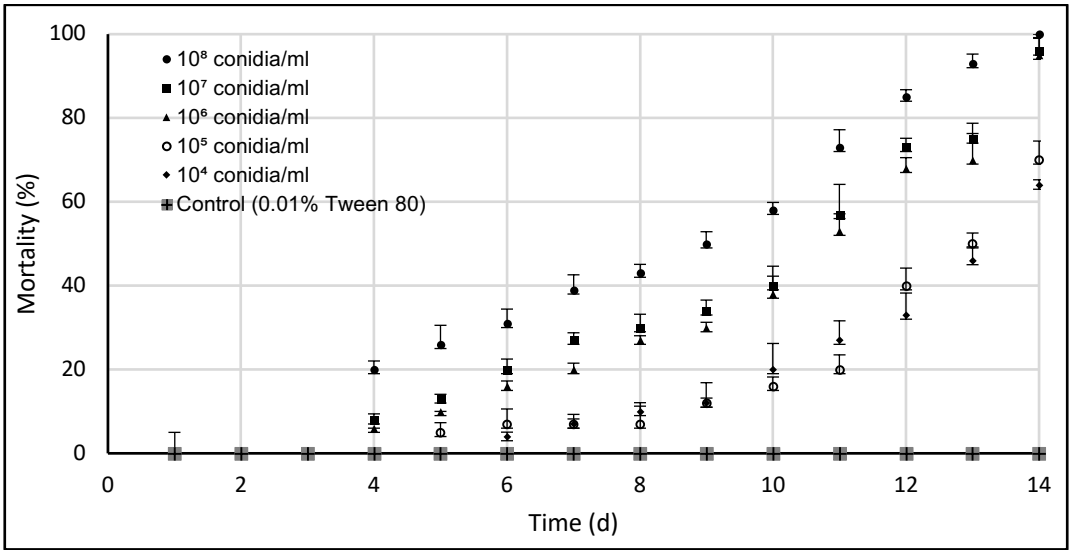

Figure 3. Cumulative mortality of *Amphimallon solstitiale* larvae after application of five different doses of the spore concentration of *Metarhizium flavoviride* strain As2.

Table 3. Median lethal concentration (LC_{50}) of *Metarhizium flavoviride* strain As2 against *Amphimallon solstitiale* larvae in the laboratory conditions

Isolate	LC_{50} (conidia/ml) (*FL*, %95)	Slope±*SE*	LC_{95} (conidia/ml) (*FL*, %95)	*df*	X^2
As2	3.87×10^3 ($1.7 - 2.77 \times 10^4$)	0.53± 0.071	3.38×10^6	3	8.32

FL: Fiducial limit, *SE*: Standard error, *df*: Degree of freedom, X^2: Chi square.

Effects of temperature and UV exposure on the growth of *Metarhizium flavoviride* isolate As2

The effect of temperature and UV exposure on *M. flavoviride* isolate As2 were compared using Chi-square test and there were not significant effects measured (p>0.05).

Discussion

Synthetic chemical insecticides are still the most commonly-used control strategy for white grubs. However, the use of chemicals to eliminate harmful insects have negative impacts on parasitoids of the grubs, evolution of insect resistance, contamination of environment and harmful effects on human. Entomopathogenic fungi represents a better alternative for eco-friendly management of white grubs.

In this study, isolation and identification of EPF from *A. solstitiale* was conducted and, four *M. flavoviride* isolates (As1, As2, As18 and As19) were obtained. The predominantly entomopathogenic fungal genus *Metarhizium* has a global distribution and includes several common species such as *Metarhizium anisopliae* Metschn.*, M. flavoviride* and *M. brunneum* Petch (Keller et al., 2003; Zimmermann, 2007; Kepler et al., 2014; Alkhaibari et al., 2016). In recent years, the taxonomy of the genus *Metarhizium* has been revised with the inclusion of other genera. In addition, some *M. flavoviride* variants were raised to species level (Kepler et al., 2014). Kepler & Rehner (2013) reported that additional genomic regions are needed to determine the identity to species level. In order to ensure the full recognition of the diversity of the genus belonging to *Metarhizium*, biological and distribution studies should be supported by molecular-based studies. Rarely all isolates of an EPF from a pest will belong to same genus. Sevim et al. (2010) characterized five *Beauveria* Vuillemin (Hyphomycetes: Moniliales) isolates from *Thaumetopea pityocampa* Denis & Schiffermüller, 1775 (Lepidoptera: Notodontidae) with all having distant taxonomic positions according to ITS and *EF1α* sequences. In the present study, the four *Metarhizium* isolates had different

taxonomic positions according to phylogenetic analysis. Also, the phylogenetic tree base on *pr1* gene encoding serine-like protease indicated that isolates were different genotypes of *M. flavoviride.* Studies on *Galleria mellonella* larvae indicated that the *pr1* gene is upregulated during pathogenesis and enhances virulence (Small & Bidochka, 2005). Presence of *pr1A* gene in our isolates may be indicative of enhanced virulence. Cito et al. (2014) tested the efficacy of various *Metarhizium* isolates against *Rhynchophorus ferrugineus* Olivier, 1790 (Coleoptera: Dryophthoridae) and found that mortality caused by isolates with high Pr1 enzymatic activity was greater.

There have not been any reports of EPF isolated from *A. solstitiale*. However, Sezen et al. (2005) determined the bacteria associated with *A. solstitiale* and showed that one *Bacillus cereus* Frankland & Frankland, 1887 strain gave 90% mortality of *A. solstitiale.* In addition, they tested a mixture of another *B. cereus* strain and a *Bacillus thuringiensis* strain isolated from *Melolontha melolontha* Fabricius, 1775 (Coleoptera: Scarabaeidae) and obtained complete mortality of *A. solstitiale* under laboratory conditions. However, bacteria have a low efficacy against soilborne insects under field conditions because bacterial agents must be ingested to be effective and it is too difficult to deliver bacterial agents and products to soilborne pests such as *A. solstitiale*.

Use of entomopathogenic fungi against similar pests provided several additional advantages such as continuity in natural environments and horizontal transportation in pest populations. All isolated *Metarhizium* showed significant insecticidal activity against the pest. Also, the pathogenicity of *M. flavoviride* on *A. solstitiale* was determined in the present study. According to the screening tests, the highest mortality of *A. solstitiale* larvae was 97% within 15 d with isolate As2. All cadavers also exhibited mycosis in a humidity camber (Table 2). At the end of the dose-response tests conducted with this isolate, mortality reached 100% within 14 d (Figure 3). Several other studies on the insecticidal potential of *M. flavoviride* isolated from soil and insects have been conducted against harmful insects (Moore et al., 1992; Seyoum et al., 1994; Thomas & Jenkins, 1997). Magalhaes et al. (1997) tested *M. flavoviride* CG 423 (Brazilian isolate) and CG 291 (Australian isolate) against *Rhammatocerus schistocercoides* Rehn, 1906 (Orthoptera: Acrididae) and observed high mortality (>85%) at 10^7 conidia/ml 8 d post inoculation. Similarly, Li et al. (2012) noted that *M. flavoviride* Mf82 had the highest virulence against *Nilaparvata lugens* (Stål, 1854) (Hemiptera: Delphacidae) with 83.5% mortality within 10 d post inoculation. An *M. flavoviride* isolate obtained from soil sample using *Tenebrio*-bait method was tested against *Riptortus pedestris* Fabricius 1775 (Hemiptera: Alydidae), *Plutella xylostella* Linnaeus, 1758 (Lepidoptera: Plutellidae) and *Tenebrio molitor* Linnaeus, 1758 (Tenebrionidae: Coleoptera) and caused 15, 100 and 95% mortality, respectively (Kim et al., 2018). These studies show that geographic conditions where the EPF were isolated, the genus of the applied insect and, bioassay method affects the virulence. In addition, fungal pathogens show higher insecticidal effect against their hosts compared to other insects (Tanyeli et al., 2010; Sönmez et al., 2016). Sevim et al. (2010) showed that a *Beauveria bassiana* isolate from *Thaumetopoea pityocampa* Denis & Schiffermüller, 1775 (Lepidoptera: Thaumetopoeidae) could cause complete mortality its larvae. Similarly, *M. flavoviride* isolated from *A. solstitiale* gave complete mortality on its larvae.

In conclusion, EPF of the European June beetle were isolated for the first time and their insecticidal potential determined. The results indicate that the isolates obtained are strong candidates for microbial control of this pest. In particular, *M. flavoviride* isolate As2, which gave the highest mortality at low conidial concentration was determined to be the most effective isolate. In the further studies, this isolate should be formulated as a mycoinsecticide to protect it from adverse environmental conditions and tested under the field conditions against the *A. solstitiale* and the other root-feeding white grubs.

Acknowledgments

We thank Dr. Richard Humber for help with the morphological characterization of fungi. Dr. I. T. Riley is thanked for advice during preparation of this manuscript.

References

Abbott, W. S., 1925. A method of computing the effectiveness of an insecticide. Journal of Economic Entomology, 18 (2): 265-267.

Alkhaibari, A. M., A. T. Carolino, S. I. Yavasoglu, T. Maffeis, T. C. Mattoso, J. C. Bull, R. I. Samuels & T. M. Butt, 2016. *Metarhizium brunneum* blastospore pathogenesis in *Aedes aegypti* larvae: Attack on several fronts accelerates mortality. PLOS Pathogens, 12 (7): e1005715.

Allen, A. A., 1995. Examples of antennal and fore-limb teratology in Coleoptera. Entomologist's Monthly Magazine, 131: 1568-1571.

Anand, R., B. Prasad & B. N. Tiwary, 2009. Relative susceptibility of *Spodoptera litura* pupae to selected entomopathogenic fungi. BioControl, 54 (1): 85-92.

Bidochka, M. J., F. V. Menzies & A. M. Kamp, 2002. Genetic groups of the insect-pathogenic fungus *Beauveria bassiana* are associated with habitat and thermal growth preferences. Archieves of Microbiology, 178 (6): 531-537.

Bischoff, J. F., S. A. Rehner & R. A. Humber, 2009. A multilocus phylogeny of the *Metarhizium anisopliae* lineage. Mycologia, 101 (4): 512-530.

Cito, A., G. Mazza, A. Strangi, C. Benvenuti, G. P. Barzanti, E. Dreassi, T. Turchetti, V. Francardi & P. F. Roversi, 2014. Characterization and comparison of *Metarhizium* strains isolated from *Rhynchophorus ferrugineus.* FEMS Microbiology Letters, 355 (2): 108-115.

Danismazoglu, M., I. Demir, A. Sevim, Z. Demirbag & R. Nalcacioglu, 2012. An investigation on the bacterial flora of *Agriotes lineatus* (Coleoptera: Elateridae) and pathogenicity of the flora members. Crop Protection, 40: 1-7.

de Goffau, L. J. W., 1996. Population development and dispersal of Melolontha and other Scarabaeidae in the Netherlands during the past ten years. Bulletin OILB SROP (France), 19 (2): 9-14.

Eski, A., İ. Demir, K. Sezen & Z. Demirbağ, 2017. A new biopesticide from a local *Bacillus thuringiensis* var. *tenebrionis* (Xd3) against alder leaf beetle (Coleoptera: Chrysomelidae). World Journal of Microbiology & Biotechnology, 33 (95): 1-9.

Ffrench-Constant, R. H., P. J. Daborn & G. Le Goff, 2004. The genetics and genomics of insecticide resistance. Trends in Genetics, 20 (3): 163-170.

Finney, D. J., 1971. Probit Analysis. Wiley Subscription Services, Inc., A Wiley Company, London, UK, 272 pp.

Gokce, C., H. Yilmaz, Z. Erbas, Z. Demirbag & I. Demir, 2013. First record of *Steinernema kraussei* (Rhabditida: Steinernematidae) from Turkey and its virulence against *Agrotis segetum* (Lepidoptera: Noctuidae). Journal of Nematology, 45 (4): 253-259.

Humber, R. A., 2008. Evolution of entomopathogenicity in fungi. Journal of Invertebrate Pathology, 98 (3): 262-266.

Humber, R. A., 2012. "Identification of Entomopathogenic Fungi, 151-186". In: Manual of Techniques in Insect Pathology (Ed. L. A. Lacey). Academic Press, London, UK, 484 pp.

Keller, S., P. Kessler & C. Schweizer, 2003. Distribution of insect pathogenic soil fungi in Switzerland with special reference to *Beauveria brongniartii* and *Metharhizium anisopliae.* BioControl, 48 (3): 307-319.

Keller, S. & G. Zimmermann, 2005. Scarabs and other soil pests in Europe: Situation, perspectives and control strategies. Insect Pathogens and Insect Parasitic Nematodes: Melolontha, 28 (2): 9-12.

Kepler, R. M. & S. A. Rehner, 2013. Genome-assisted development of nuclear intergenic sequence markers for entomopathogenic fungi of the *Metarhizium anisopliae* species complex. Molecular Ecology Resources, 13 (2): 210-217.

Kepler, R. M., R. A. Humber, J. F. Bischoff & S. A. Rehner, 2014. Clarification of generic and species boundaries for *Metarhizium* and related fungi through multigene phylogenetics. Mycologia, 106 (4): 811-829.

Kim, J. C., M. R. Lee, S. Kim, S. J. Lee, S. E. Park, Y. S. Nai, G. S. Lee, T. Y. Shin & J. S. Kim, 2018. *Tenebrio molitor*-mediated entomopathogenic fungal library construction for pest management. Journal of Asia-Pacific Entomology, 21 (1): 196-204.

Kocacevik, S., A. Sevim, M. Eroglu, Z. Demirbag & I. Demir, 2016. Virulence and horizontal transmission of *Beauveria pseudobassiana* S.A. Rehner & Humber on *Ips sexdentatus* and *Ips typographus* (Coleoptera: Curculionidae). Turkish Journal of Agriculture and Forestry, 40 (2): 241-248.

Kumar, S., G. Stecher, M. Li, C. Knyaz & K. Tamura, 2018. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. Molecular Biology & Evoluation, 35 (6): 1547-1549.

Leal, S. C. M., D. J. Bertioli, T. M. Butt, J. H. Carder, P. R. Burrows & J. F. Peberdy, 1997. Amplification and restriction endonuclease digestion of the *Pr1* gene for the detection and characterization of *Metarhizium* strains. Mycological Research, 101 (3): 257-265.

Li, M. Y., H. F. Lin, S. G. Li & L. Jin, 2012. Virulence of *Metarhizium flavoviride* 82 to different developmental stages of the brown planthopper, *Nilaparvata lugens* (Hemiptera: Delphacidae). Acta Entomologica Sinica, 55 (3): 316-323.

Magalhaes, B. P., M. R. Faria & M. S. Tigano, 1997. Characterization and virulence of a Brazilian isolate of *Metarhizium flavoviride* Gams and Rozsypal (Hyphomycetes). The Memoirs of the Entomological Society of Canada, 129 (s171): 313-321.

Mohammadyani, M., J. Karimi, P. Taheri, H. Sadeghi & R. Zare, 2016. Entomopathogenic fungi as promising biocontrol agents for the rosaceous longhorn beetle, *Osphranteria coerulescens*. BioControl, 61 (5): 579-590.

Moore, D., M. Reed, G. Le Patourel, Y. J. Abraham & C. Prior, 1992. Reduction of feeding by the desert locust, *Schistocerca gregaria*, after infection with *Metarhizium flavoviride*. Journal of Invertebrate Pathology, 60 (3): 304-307.

O'Donnell, K. & E. Cigelnik, 1997. Two divergent intra genomic rDNA ITS2 types within a monophyletic lineage of the fungus Fusar- ium are non-orthologous. Molecular Phylogenetics & Evoluation, 7 (1): 103-116.

Ortiz-Urquiza, A. & N. O. Keyhani, 2013. Action on the surface: Entomopathogenic fungi versus the insect cuticle. Insects, 4 (3): 357-374.

Sevim, A., I. Demir & Z. Demirbağ, 2010. Molecular characterization and virulence of Beauveria spp. from the Pine processionary moth, *Thaumetopoea pityocampa* (Lepidoptera: Thaumetopoeidae). Mycopathologia, 170 (4): 269-277.

Sevim, A., I. Demir, E. Sonmez, S. Kocacevik & Z. Demirbag, 2013. Evaluation of entomopathogenic fungi against the Sycamore lace bug, *Corythucha ciliate* (Say) (Hemiptera: Tingidae). Turkish Journal of Agriculture and Forestry, 37 (5): 595-603.

Sevim, A., E. Eryuzlu, Z. Demirbag & I. Demir, 2012. A Novel cry2Ab gene from the indigenous isolate *Bacillus thuringiensis* subsp. *kurstaki*. Journal of Microbiology and Biotechnology, 22 (1): 137-144.

Seyoum, E., D. Moore & A. K. Charnley, 1994. Reduction in flight activity and food consumption by the desert locust, *Schistocerca gregaria* Forskål (Orth., Cyrtacanthacrinae), after infection with *Metarhizium flavoviride*. Journal of Applied Entomology, 118 (3): 310-315.

Sezen, K., I. Demir, H. Kati & Z. Demirbag, 2005. Investigations on bacteria as a potential biological control agent of summer chafer, *Amphimallon solstitiale* L. (Coleoptera: Scarabaeidae). Journal of Microbiology, 43 (5): 463-468.

Shah, P. & J. Pell, 2003. Entomopathogenic fungi as biological control agents. Applied Microbiology & Biotechnology, 61 (5-6): 413-423.

Small, C. L. N. & M. J. Bidochka, 2005. Up-regulation of Prl, a subtilisin-like protease, during conidiation in the insect pathogen *Metarhizium anisopliae*. Mycological Research, 109 (3): 307-313.

Sönmez, E., A. Sevim, Z. Demirbağ & I. Demir, 2016. Isolation, characterization and virulence of entomopathogenic fungi from *Gryllotalpa gryllotalpa* (Orthoptera: Gryllotalpidae). Applied Entomology & Zoology, 51 (2): 213-223.

Stiller, J. W. B. & D. Hall, 1997. The origin of red algae: implications for plastid evolution. Proceedings of the National Academy of Sciences of the United States of America, 94 (9): 4520-4525.

Tanyeli, E., A. Sevim, Z. Demirbag, M. Eroglu & I. Demir, 2010. Isolation and virulence of entomopathogenic fungi against the great spruce bark beetle, *Dendroctonus micans* (Kugelann) (Coleoptera: Scolytidae). Biocontrol. Science & Technology, 20 (7): 695-701.

Thomas, M. B. & N. E. Jenkins, 1997. Effects of temperature on growth of *Metarhizium flavoviride* and virulence to the variegated grasshopper, *Zonocerus variegatus*. Mycological Research, 101 (12): 1469-1474.

White, T. J., T. Bruns, S. Lee & J. Taylor, 1990. "Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics, 315-322". In: PCR Protocols: A Guide to Methods and Applications (Eds. M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White). Academic Press, San Diego, 482 pp.

Wilson, C. & C. Tisdell, 2001. Why farmers continue to use pesticides despite environmental, health and sustainability costs. Ecological. Economics, 39 (3): 449-462.

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

Türk. entomol. derg., 2020, 44 (3): 385-397
DOI: http://dx.doi.org/10.16970/entoted.740223

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

Identification, distribution and genetic diversity of *Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959 (Tylenchida: Heteroderidae) populations in Turkey

Globodera rostochiensis (Wollenweber, 1923) Skarbilovich, 1959 (Tylenchida: Heteroderidae)'in Türkiye popülasyonlarının tanımlanması, yaygınlık ve genetik çeşitliliği

Ahmet ALTAŞ[1] **Emre EVLİCE[2]** **Göksel ÖZER[1]**
Abdelfattah DABABAT[3] **Mustafa İMREN[1*]**

Abstract

The golden nematode, *Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959 (Tylenchida: Heteroderidae) is one of the most economically important pests of potato in the world. Tests for this nematode are routinely performed for outbreaks and densities in potato growing areas. The morphological and molecular analyses for precise determination of the nematode are employed to establish appropriate management strategies. This study showed that 96% of soil samples obtained from Bozdağ and Ödemiş Districts of İzmir Province, during 2017 and 2018 potato growing seasons, were positive for *G. rostochiensis*. The mean number of cysts ranged from 0.01 to 3.70 cysts g^{-1} soil in the fields examined. The examination of the morphological and morphometric features of the second-stage juveniles and cysts of the *G. rostochiensis* revealed slight differences among the populations obtained from Bozdağ and Ödemis. To assess the accuracy of the identification, partial sequences of ribosomal DNA for all populations were amplified, sequenced, and deposited in GenBank. The comparisons of the sequences with those of corresponding *G. rostochiensis* populations available in GenBank showed 99.89-100% nucleotide similarity. The results of this study will help to better understand the physiology, ecology and biology of the nematode to quarantine this pest more effectively.

Keywords: *Globodera rostochiensis*, ITS, İzmir, morphology, phylogeny

Öz

Altın nematod, *Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959 (Tylenchida: Heteroderidae) dünyada ekonomik açıdan en önemli patates zararlılarından birisidir. Genellikle patates yetiştirilen alanlarındaki dağılımları ve yoğunluklarını belirlemek amacıyla bu nematod için rutin olarak testler gerçekleştirilmektedir. Morfolojik ve moleküler analizler bu nematodun kesin tanısını yapmak ve mücadele stratejilerini oluşturmak amacıyla kullanılmaktadır. Bu çalışma, İzmir İli'nin Bozdağ ve Ödemiş ilçelerinden 2017 ve 2018 yılları patates yetiştiriciliği sezonlarında alınan toprak örneklerinin %96'sının *G. rostochiensis* ile enfekte olduğunu göstermiştir. Bulaşık alanlardaki ortalama kist sayısı, 0.01 ila 3.70 kist g^{-1} toprak arasında değişmiştir. *Globodera rostochiensis*'in ikinci dönem larva ve kistlerinin morfolojik ve morfometrik karakterlerinin incelenmesi, Bozdağ ve Ödemiş'ten elde edilen popülasyonlarda arasındaki hafif farklılıkları ortaya koymuştur. Tanımlamanın doğruluğunu değerlendirmek için, tüm popülasyonlar için kısmi ribozomal DNA sekansları amplifiye edilmiş, sekanslanmış ve GenBank veritabanına kaydedilmiştir. Morfolojik ölçümler ve filogenetik analizler sonucunda Bozdağ ve Ödemiş'ten elde edilen popülasyonlar arasında küçük farklılıkların olduğu belirlenmiştir. Sekansların GenBank'ta mevcut karşılık gelen *G. rostochiensis* popülasyonlarıyla karşılaştırılması sonucunda nükleotid benzerliği %99.89-100 oranında görülmüştür. Bu çalışmanın sonuçları, bu zararlıyı daha etkili bir şekilde karantinaya almak için nematodun fizyolojisini, ekolojisini ve biyolojisini daha iyi anlamaya yardımcı olacaktır.

Anahtar sözcükler: *Globodera rostochiensis*, ITS, İzmir, morfoloji, filogenetik

[1] Bolu Abant Izzet Baysal University, Faculty of Agriculture and Natural Sciences, Department of Plant Protection, 14030, Bolu, Turkey
[2] Plant Protection Central Research Institute, 06172, Yenimahalle, Ankara, Turkey
[3] International Maize and Wheat Improvement Center (CIMMYT), 06511, Emek, Ankara, Turkey
* Corresponding author (Sorumlu yazar) e-mail: mustafaimren@ibu.edu.tr
Received (Alınış): 21.05.2020 Accepted (Kabul ediliş): 17.06.2020 Published Online (Çevrimiçi Yayın Tarihi): 30.07.2020

Introduction

Potato (*Solanum tuberosum* L. subsp*. tuberosum*) is the most cultivated tuber crop and an important staple food for over one billion people in the world. It ranks as the fourth for its importance among food crops worldwide; following rice, wheat, and maize (FAOSTAT, 2020). Turkey is in the top ten potato producer countries in Europe with a production area of about 136 000 ha. Turkey annually produces about 34 t ha^{-1}, which is still below the average yield potential and the rest (about 40 t ha^{-1}) is being imported from developed countries (FAOSTAT, 2020). Potato yield reduction is attributed to several biotic and abiotic factors, including pests and pathogens (Subbotin et al., 2010). Plant-parasitic nematodes cause an annual loss of 12% in potato production worldwide (Chitwood, 2003). In tropical and subtropical climates, losses associated with nematodes are estimated at 14.6% compared to 8.8% in temperate countries (Sasser & Freckman, 1987). Among the top 10 plant-parasitic nematodes causing severe economic damage to crops around the world, six genera are resulting yield reduction in potato (Jones et al., 2013). Also, eight species from the seventeen quarantine nematodes declared by the European and Mediterranean Plant Protection Organization (EPPO, 2013) for the Euro-Mediterranean region, are major parasites of potato. Despite their considerable importance, nematodes of potato are not well studied in Turkey (Kepenekci, 2012).

Potato cyst nematodes (PCN), *Globodera* spp. are obligate parasites resulting in economic damage to potato around the world (Subbotin et al., 2010). These nematodes are quarantined internationally and subjected to strict regulatory measures (Fleming & Powers, 1998). These nematodes have already been found in 75 countries from Africa, Asia, Europe, North America, South America and Oceania (Ibrahim et al., 2000; Indarti et al., 2004; Andres et al., 2006; Gitty & Tanha Maafi, 2010). Species of PCN include golden potato cyst nematode, *Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959, and pale potato cyst nematode, *Globodera pallida* Stone, 1973 (Tylenchida: Heteroderidae) are considered harmful quarantine organisms, described in European Union Directives 2000/29/EC and 2009/7/EC and are also part of EPPO A2 List (quarantine species already present in the EPPO region, A2/125 and A2/124, respectively) (EPPO, 2013). These species are regulated by the European Directive 2007/33/EC on the control of PCNs and are subject to stringent regulatory measures when detected singly or in combination (EPPO, 2013). Specific identification of these species is just possible by observation of the female color at the appropriate stage of development, either a change from white to yellow in *G. rostochiensis* or prolonged white (slightly cream but no yellow phase) in *G. pallida*.

The golden nematode, *G. rostochiensis*, is a regulated pathogen of potato and a threat to the potato industry in several countries (Scurrah et al., 2005). EPPO has recognized the nematode as plant health quarantine species in the A2 list, which shows the local presence of the pathogen within the Euro-Mediterranean region. The losses caused by the nematode mainly occurs in temperate regions, in Mediterranean countries, where the host plants are grown from mid-autumn to spring (Mugniéry, 1989). In Turkey, *G. rostochiensis* was recorded for the first time in 1985 (Enneli & Öztürk, 1996), following the importing of seed potato from European countries (Baldwin & Mundo-Ocampo, 1991). The number of infested potato-producing areas in Turkey has significantly increased in the last few years (Kepenekci, 2012). Therefore, the crop protection services observed and reported these nematodes in many potato growing areas in the country (Ulutaş et al., 2012; Imren, 2018; Özarslandan et al., 2019; Toktay et al., 2020).

The pathogenic variability within potato cyst nematode, *G. rostochiensis* populations is determined by a set of differential host genotypes, and populations are classified into five pathotypes designated as Ro1 to Ro5 (Subbotin et al., 2010). The generation rates of nematode populations on solanaceous plants containing resistance genes can be used to differentiate pathotypes (Kort et al., 1977). The identification of nematode species as well as their pathotype provides crucial information to select appropriate and efficient management strategies (Ganguly & Rao, 2003). The morphological discrimination of *Globodera* spp. is conducted via microscopic examination of the structures of cysts and infective juveniles (Golden,

1990; Siddiqi, 2000; Subbotin et al., 2010). The increasing number of species in the genus *Globodera* caused difficulties in obtaining sufficient criteria for the differentiation of species and requires highly specialized taxonomists due to the minor morphological and morphometric differences within its species (Subbotin et al., 1999, 2003). Molecular diagnostic techniques based on polymorphism of certain DNA fragments provide fundamental clues to overcome these taxonomic bottlenecks about morphological identification (Szalanski et al., 1997; Al-Banna et al., 2004; Subbotin et al., 2010). The sequences of the ribosomal DNA region including the ITS1, ITS2 and ribosomal genes facilitate reliable and rapid identification of *Globodera* spp. and differentiate them from other closely related cyst nematode species (Ferris et al., 1995; Subbotin et al., 2000; Madani et al., 2005, 2008; Skantar et al., 2007). The ribosomal DNA region can also be used as excellent genetic markers for diagnostics and the evaluation of phylogenetic relationships due to a large number of copies in individual cells, the lack of recombination, and strict maternal inheritance.

We conducted analyses to understand genetic structures of *G. rostochiensis* populations from two districts (Bozdağ and Ödemiş) of İzmir Province, Turkey. The objectives of the current study were (1) determine the distribution of the *G. rostochiensis* in Izmir Province, (2) to describe and evaluate the morphology and taxonomic features of local *G. rostochiensis* populations, and (3) to assess phylogenetic relationships of the populations based on the partial of ribosomal DNA sequences.

Materials and Methods

Nematode populations

A comprehensive survey was conducted during 2017 and 2018 potato growing seasons to collect soil samples from fields located in Bozdağ and Ödemiş Districts of Izmir Province, Turkey. Samples were taken prior to the potato harvest, between the end of September and the beginning of November (Table 1). Cyst extraction from soil samples was performed using a standard flotation and sieving technique (Southey, 1986). Extracted cysts were firstly categorized to genus level under a V20 model stereo-binocular microscope (Zeiss, Jena, Germany). At least 20 full cysts were selected and handpicked with a needle from each sample and stored at 4°C for further use in the morphological and molecular analysis. Additionally, the density of nematodes g^{-1} of soil was estimated.

Table 1. Location and density of *Globodera rostochiensis* sampled in this study

No	District	Location	Latitude	Longitude	Density (Cyst)	Accession Numbers
1	Bozdağ	Koca çayır	38º32’87 N	28º05’82 E	1.60	MT193688
2	Bozdağ	Yukarıçayır	38º32’90 N	28º06’12 E	0.50	
3	Bozdağ	Yukarıçayır	38º33’03 N	28º06’08 E	0.50	
4	Bozdağ	Tekke	38º33’42 N	28º06’22 E	1.10	MT193689
5	Bozdağ	Nazır	38º34’68 N	28º06’16 E	0.70	
6	Bozdağ	Burunucu	38º35’35 N	28º06’43 E	1.20	MT193690
7	Bozdağ	Kocaçayırlık	38º36’22 N	28º07’21 E	1.30	MT193691
8	Bozdağ	Çavdar	38º33’95 N	28º11’48 E	2.00	MT193692
9	Bozdağ	Çavdar	38º34’15 N	28º11’27 E	0.70	
10	Bozdağ	Büyük çavdar	38º34’55 N	28º11’71 E	0.50	
11	Bozdağ	Gündalan	38º35’53 N	28º10’95 E	1.20	MT193693
12	Bozdağ	Gündalan	38º34’84 N	28º10’47 E	3.70	MT193694
13	Bozdağ	Gündalan	38º35’87 N	28º10’01 E	2.60	
14	Bozdağ	Taşlıharım	38º32’52 N	28º06’07 E	1.50	MT193695

Table 1. Continued

No	District	Location	Latitude	Longitude	Density (Cyst)	Accession Numbers
14	Bozdağ	Taşlıharım	38º32’52 N	28º06’07 E	1.50	MT193695
15	Bozdağ	Taşlıharım	38º32’02 N	28º05’82 E	3.10	MT193696
16	Bozdağ	Ovacık	38º31’09 N	28º05’43 E	1.10	MT193697
17	Bozdağ	Ovacık	38º30’64 N	28º05’17 E	1.70	MT193698
18	Bozdağ	Gölcük Adabaşı	38º30’50 N	28º02’42 E	0.50	
19	Bozdağ	Karşıyaka	38º32’11 N	28º03’53 E	0.20	
20	Bozdağ	Gölcük	38º33’38 N	28º03’35 E	1.40	MT193699
21	Bozdağ	Boğaz	38º34’09 N	28º03’58 E	0.40	
22	Bozdağ	Çayırağzı	38º34’00 N	28º03’75 E	2.30	MT193700
23	Bozdağ	Aşağı Boğaz	38º35’13 N	28º03’47 E	1.20	MT193701
24	Bozdağ	Örselli yolu	38º31’41 N	28º02’11 E	0.40	
25	Bozdağ	Subatan	38º31’80 N	27º98’78 E	2.60	MT193702
26	Bozdağ	Subatan	38º32’33 N	27º99’22 E	0.80	
27	Bozdağ	Subatan	38º33’74 N	27º99’07 E	0.10	
28	Bozdağ	Kireçocağı	38º46’53 N	27º98’52 E	1.10	MT193703
29	Ödemiş	Küçükavulcuk	38º24’14 N	28º03’47 E	0.40	
30	Ödemiş	Küçükavulcuk	38º23’76 N	28º03’72 E	1.50	MT193704
31	Ödemiş	Küçükavulcuk	38º22’86 N	28º02’49 E	0.00	
32	Ödemiş	Küçükavulcuk	38º24’32 N	28º02’96 E	0.70	
33	Ödemiş	Büyükavulcuk	38º23’62 N	28º23’16 E	2.50	MT193705
34	Ödemiş	Gerçekli-Topçukuyu	38º22’39 N	28º06’29 E	2.10	MT193706
35	Ödemiş	Ocaklı-Türbe	38º22’88 N	28º01’36 E	1.50	MT193707
36	Ödemiş	Yolüstü-Beylikkırı	38º20’60 N	28º03’37 E	0.60	
37	Ödemiş	Gerekli-Petrol	38º19’86 N	28º04’26 E	0.20	
38	Ödemiş	Gerekli-Canigırı	38º21’81 N	28º05’60 E	2.10	MT193708
39	Ödemiş	Alidereli	38º21’46 N	27º99’91 E	0.20	
40	Ödemiş	Konaklı-Millik	38º12’95 N	27º99’74 E	1.30	MT193709
41	Ödemiş	Konaklı-Köy civarı	38º40’60 N	27º36’45 E	2.10	MT193710
42	Ödemiş	Yeniköy-Karadoğan	38º23’00 N	27º91’40 E	0.06	
43	Ödemiş	Karadoğan-Kırarası	38º23’85 N	27º91’52 E	0.40	
44	Ödemiş	Karadoğan Değirmen	38º24’48 N	27º92’22 E	1.20	MT193711
45	Ödemiş	Ortaköy-Yumurtacı	38º24’54 N	27º93’35 E	1.40	MT193712
46	Ödemiş	Ortaköy-Yumurtacı	38º24’57 N	27º93’17 E	0.08	
47	Ödemiş	Yeniköy-Köygirişi	38º23’91 N	27º95’06 E	0.00	
48	Ödemiş	Günlüce Altı	38º24’92 N	27º97’14 E	0.08	
49	Ödemiş	Günlüce at çiftliği	38º26’21 N	27º96’80 E	1.30	MT193713
50	Ödemiş	Günlüce Riga Çiftlik	38º25’38 N	27º97’05 E	0.04	
51	Ödemiş	Yeniköy-Karapınar	38º25’13 N	27º94’43 E	0.01	
52	Ödemiş	Seviköy Küsküt Mh.	38º20’21 N	27º86’86 E	1.10	MT193714
53	Ödemiş	Seviköy Akçagün	38º21’43 N	27º85’13 E	0.04	
54	Ödemiş	Yusufdere-Yolkıyı	38º22’11 N	27º84’18 E	0.02	
55	Ödemiş	Kayaköy-Alabaşı	38º18’51 N	27º84’47 E	2.10	MT193715
56	Ödemiş	Kayaköy-Alabaşı	38º19’06 N	27º83’99 E	2.10	MT193716
57	Ödemiş	Kayaköy köyaltı	38º20’04 N	27º82’93 E	3.40	MT193717

Morphological identification

The identity of a newly discovered population of potato cyst nematodes, *Globodera* spp., was first performed based on morphological characteristics of second-stage juvenile (J2) (length of body, stylet and tail, and hyaline portion) and cyst (distance fenestra to anus, fenestra diameter, Granek's ratio, and numbers of cuticular ridges between anus and vulva). Vulva cones were excised from cysts and mounted in Canada balsam (Hooper, 1970). Juveniles were treated by gentle heating (60°C) and fixed in a TAF solution (triethanolamine, formalin and ultrapure water at a ratio of 2:7:91) and processed to glycerin. TAF-fixed specimens were examined under an Axio Lab. A1 model light microscope (Carl Zeiss AG, Oberkochen, Germany). Measurements were estimated using ZEN Lite software with the support of an Axiocam ERc5s digital camera (Carl Zeiss AG, Oberkochen, Germany). The observed features of the cysts and second-stage juveniles were compared with those of reference materials and the description of the neotypes in the literature (Golden & Ellington, 1972; Manduric et al., 2004).

The data were normalized using the Shapiro-Wilk normality test before they were analyzed using analysis of variance (ANOVA) (Shapiro & Wilk, 1965). Significant differences among characters were determined using protected least significant differences using SPSS statistical software V 17.0 (IBM Corp., Armonk, NY, USA) at $P < 0.001$. The standard test of means was conducted to detect the significant variance between populations ($P \leq 0.05$).

Molecular identification

DNA was extracted from each population using the Worm Lysis Buffer Method (WLB) (Waeyenberge et al., 2000). A single J2 from a single cyst was handpicked and transferred into a 0.2 ml tube containing 10 µl sterile ultrapure water. Then 8 µl of lysis buffer (100 mM Tris-Cl, 500 mM KCl; 15 mM $MgCl_2$, 10 mM dithiothreitol, 0.1% gelatin, and 4.5% Tween 20) and 2 µl of proteinase K (Cat No./ID: 19131 Qiagen GmbH, Hilden, Germany) at 600 µg/ml were added to the mix in the tube. The microtube was heated to 65°C in a DB-100 dry-block heater (Techne, Cambridge, UK) for 1 h, and consecutively to 95°C for 5 min to inactivate the proteinase K (Holterman et al., 2006). The lysate was centrifuged at 16,000 g for 5 min and the supernatant was transferred into a new 0.2 ml tube and stored at -20°C for further use. F194 (5′-CGTAACAAGGTAGCTGTAG-3′) and F195 (5′-TCCTCCGCTAAATGATATG-3′) primers developed by Ferris et al. (1993) were used for amplification of the ITS for all samples. PCR reactions were carried out in a total volume of 50 µl containing 1 µl of nematode lysate, 1 × Ammonium Buffer, 1 µM each of primers, 0.2 mM dNTPs and 1-unit Ampliqon TEMPase Hot Start DNA polymerase (Berntsen, Rødovre, Denmark). Three PCR reactions lacking DNA (no template control) were also performed. PCR amplification was conducted in a T100 thermal cycler (Bio-Rad Laboratories, CA, USA) programmed as follows: initial denaturation at 94°C for 15 min followed by 30 cycles of 30 s at 94°C, 30 s at 58°C, 45 s at 72°C and a final extension at 72°C for 5 min. PCR products were detected by electrophoresis on 1.2% agarose gels and visualized by staining with ethidium bromide. The DNA banding patterns were visualized and documented by a G:Box F3 gel documentation system (Syngene, UK). PCR products were subjected to bidirectional sequencing by a commercial company (Macrogen Inc., Seoul, Korea) after purification with Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA) according to the manufacturer instructions.

Phylogenetic analysis

A BLAST search for ITS sequences was performed in the GenBank database to determine the closest available reference sequences in the complete nucleotide collection of the National Center for Biotechnological Information (NCBI; http://blast.ncbi.nlm.nih.gov). All sequences of the populations obtained in the present study were deposited in the GenBank database under accession numbers from MT193688 to MT193717 (Table 1). The sequence with those of corresponding *G. rostochiensis* population from different countries available in the GenBank nucleotide database (EF622522 from Australia,

FJ212167 from Canada; KR057953 from Serbia, DQ847117 from the USA, JF907550 from Poland and MT193701 from Japan) were aligned with Clustal W (Thompson et al., 1994) and edited manually using MEGAX (Kumar et al., 2018). The maximum likelihood method was performed to construct a phylogenetic tree from the sequence data using MEGAX software based on the General Time Reversible model (Tamura & Nei, 1993). The confidence of phylogenetic tree topologies was confirmed by bootstrap analysis from 1,000 replicates (Felsenstein, 1985). The sequence of *G. pallida* population from the USA (EF153837) was included as an outgroup to root the phylogenetic tree.

Results and Discussion

The morphological and molecular identification confirmed the presence of the golden nematode, *G. rostochiensis*, in the surveyed areas. *Globodera rostochiensis* was detected in potato fields located both in Bozdag, with the mountain slopes at an altitude of about 1,000 m, and in flat areas in Ödemiş. Overall, 55 of 57 soil samples (96%) obtained from Bozdağ and Ödemiş Districts were found being infected with *G. rostochiensis*. Two locations in Ödemiş were found free of potato cyst nematode (Table 1). In Turkey, first report of the presence of *G. rostochiensis* was declared in 1996 from a field of seed potatoes in Dörtdivan District of Bolu Province (Enneli & Öztürk, 1996). Although, strict regulatory measures have been enforced to prevent further spread of nematode; it was not possible to completely prevent it from contaminating important potato producing areas in Turkey (Kepenekci, 2012). Ulutaş et al. (2012) reported that *G. rostochiensis* was found in 17 and 62% of the fields investigated in the Aegean Region and Ödemis District, respectively. Since then, a remarkable increase has occurred in the incidence of infestation of potato cultivation areas in İzmir Province. The presence of the nematode has recently been confirmed by Demirbaş Pehlivan et al. (2020). This phenomenon requires the extension services and quarantine departments to establish a control method to minimize the population of *G. rostochiensis* in the infested areas.

In this study, the mean number of cysts in all the infested fields was 0.14 cysts g^{-1} of soil; however, the infestation levels of *G. rostochiensis* varied between the surveyed locations. The fields with less than 0.20 cysts g^{-1} of soil represented about 65% of the total number of infested potato fields. The mean number of cysts of 0.21-0.50 represented 19% of infested fields, and the average of more than 0.5 cysts represented only 4.2% of the surveyed fields (Table 1). The highest density of cysts (3.70 g^{-1} of soil) was found in Gündalan in Bozdağ (sample 57) and the other higher densities were 3.40 and 3.10 cysts g^{-1} of soil in Kayaköy-Köyaltı location of Ödemiş (sample 15) and in Taşlıharım location Bozdağ (sample 12), respectively. The lowest cyst density (0.01 g^{-1} of soil) was found in Yeniköy-Karapınar (sample 51) and the other lower densities were 0.02 and 0.04 cysts g^{-1} of soil found in Yusufdere Yolkıyı (sample 54) and Günlüce-Riga Çiftlik (sample 50) location of Ödemiş, respectively. Similarly, Özarslandan et al. (2019) reported that the mean cysts number of *G. rostochiensis* in the soil was found 0.24 cysts g^{-1} of soil in Nevşehir Province in the Central Anatolia Region, Turkey. The occurrence and density of *G. rostochiensis* in potato growing areas in Izmir Province would ultimately reach the economic threshold levels. Greco et al. (1982) reported that the loss threshold for *G. rostochiensis* and *G. pallida* in Italian potato growing areas was between 1.4 and 2.1 eggs g^{-1} of soil, respectively. Also, the economic threshold for *G. rostochiensis* in the UK is calculated as 15 eggs g^{-1} of soil (Dale, 1988). Therefore, it is recommended to establish a strategy of combining cultural control methods including crop rotation with non-host crops and the use of resistant potato cultivars. The application of chemicals should be limited to avoid environmental pollution or deterioration of the physical properties of the soil.

Second-stage juveniles of *G. rostochiensis* had cylindrical bodies, tapering at both extremities, mostly posterior (Figure 1a). The tail was shortened, and the head was slightly offset with prominent cephalic sclerotization and rounded. The stylet was visible and anteriorly flattened to rounded knobs. The median bulb was ellipsoidal with a prominent valve. Esophageal glands extended ventrally for about 35

percent of body length (Figure 1b). Genital primordium located about 60 percent of body length. Tail tapered to a finely rounded terminus (Figure 1c).

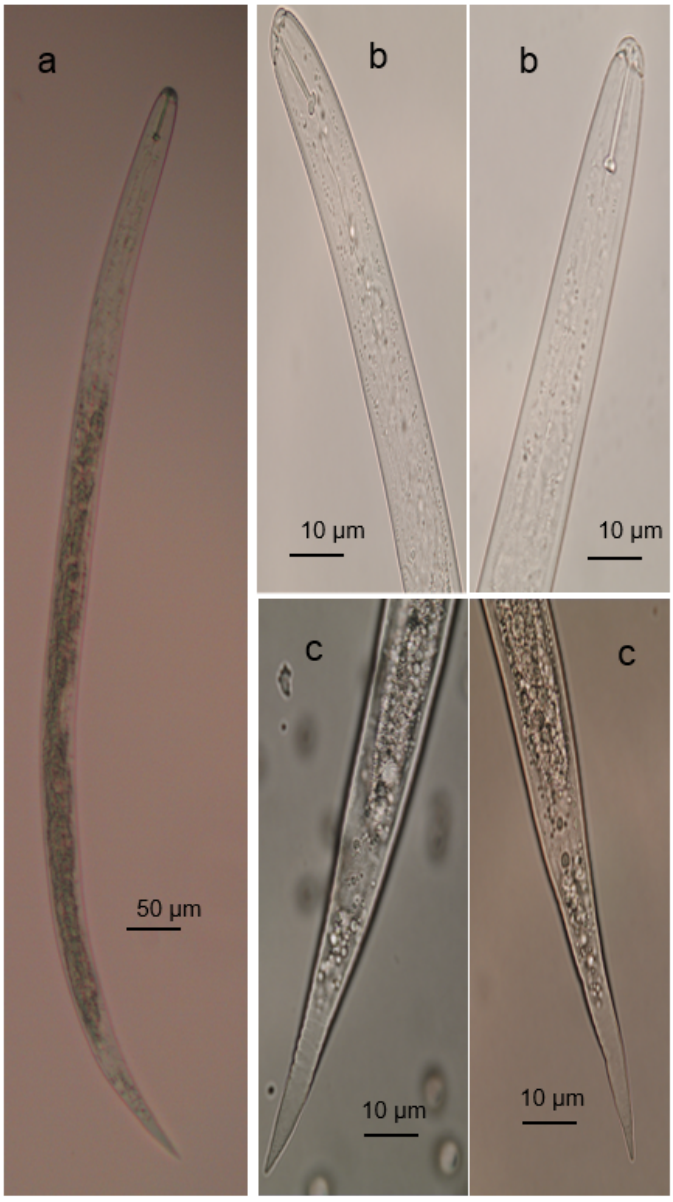

Figure 1. Second-stage juveniles of *Globodera rostochiensis* from İzmir. Specimens from İzmir: (a) body, (b) heads, and (c) tails.

In the Bozdağ populations, the body length of juveniles varied from 399 to 496 µm, and stylet length was 19 to 22 µm with rounded, slightly backward sloping stylet knobs (Table 2). Tail length and hyaline length were 38 to 46 µm and 17 to 28 µm, respectively. Among Ödemiş populations, the body length varied from 418 to 550 µm, and stylet length was 19-23 µm with anteriorly indented stylet knobs. Tail length and hyaline length were 32 to 56 µm and 21 to 30 µm, respectively (Table 2). Similarly, Wouts & Baldwin (1998) and Siddiqi (2000) stated that body length ranged from 445 to 510 µm, stylet length was 18 to 29 µm, tail length was 37 to 55 µm and the hyaline tail part was 21 to 31 µm for the second stages juveniles of *G. rostochiensis*, which was in accordance with our results.

Table 2. Morphometrics (in µm) of second-stage juveniles of *Globodera rostochiensis* from Izmir Province, Turkey

Characters	Bozdağ population			Ödemiş population		
	Range	Mean	SD	Range	Mean	SD
Body length	399-496	452.00	5.00	418-550	470.00	7.00
Stylet length	19-22	19.80	0.20	19-23	20.90	0.70
Tail length	38-46	41.40	1.12	32-56	47.26	2.30
Hyaline tail terminal length	17-28	20.64	1.14	21-30	27.80	1.55

The average values of morphological characters of second-stage juveniles were larger in populations of *G. rostochiensis* from Ödemiş than those from Bozdag (Table 2). However, the differences in body length and tail length between Ödemiş and Bozdağ populations were not significant ($P \leq 0.5$). The morphological average values of the J2 of *G. rostochiensis* were consistent with the range described in previous studies (Fleming & Powers, 1998; EPPO, 2013). For all 30 populations, the morphological characters of J2s, especially body length and tail length, were consistent with Wouts and Baldwin (1998). However, the measurements of the J2s slightly differed from those estimated by Golden (1986), who measured a longer tail length (42 to 67 µm) and a longer hyaline length (20 to 36 µm). According to their round shape and light-brown color cysts, populations were considered as *G. rostochiensis*.

The cysts were brown or yellow, spherical or subspherical in shape, with protruding necks and lacking a terminal cone. Cysts had a small projecting neck and with tanned brown skin. The cuticle surface had a zigzag pattern of ridges and a distinct D-layer was present. The perineal area consisted of a single circumfenestration around the vulval slit in vulval basin; underbridge and bullae were rarely present (Figure 2). Morphometric data for larvae also indicates similarity to *G. rostochiensis* (Table 3). Morphometrics of cyst perineal region *G. rostochiensis* (Table 3) provided clear differences between populations of Ödemiş and Bozdağ. The populations from Ödemiş had the largest values of vulva-anus distance, the number of cuticular ridges between vulva and anus, and Granek's ratio, fitting well within ranges of *G. rostochiensis* whereas populations from Bozdağı had small mean values of Granek's ratio and the number of cuticular ridges overlapping of *G. rostochiensis* (Subbotin et al. 2010).

Table 3. Morphometrics (in µm) of second-stage juveniles of *Globodera rostochiensis* from Izmir Province, Turkey

Characters	Bozdağ population			Ödemiş population		
	Range	Mean	SD	Range	Mean	SD
Body length excluding neck	423-592	521	7	469-712	587	8
Body with	389-545	472	12	446-610	534	10
Distance from anus to nearest edge of fenestra (anus-vulva)	52-68	58.4	1.4	56-71	66.6	2
Fenestra length	17-23	18.6	0.2	18-26	21.6	1.2
Number of cuticular ridges between vulva-anus	18-21	19	0.2	19-24	22	0.4
Granek's ratio	2.63-4.0	3	0.3	2.9-4.2	3.7	0.6

The cyst length of *G. rostochiensis* for Bozdağ populations varied from 423 to 592 µm, and body width was 389 to 545 µm with a spherical shape, with a short neck and no terminal cone. The anus to vulva and fenestra length were 52 to 68 µm and 17 to 23 µm, respectively. The number of cuticular ridges between vulva-anus and Granek's ratio were 18 to 21 and 2.63 to 4.00 (Table 3). The cyst length of *G. rostochiensis* for Ödemiş populations ranged from 469 to 712 µm and body width varied from 446 to 610 µm with a spherical shape, small projecting neck, lacking terminal cone. The anus to vulva and fenestra length were 56 to 71 µm and 18 to 26 µm, respectively. The number of cuticular ridges between vulva-anus and Granek's ratio were 19 to 24 µm and 2.9 to 4.2, respectively. Likewise, Wouts & Baldwin (1998), Siddiqi (2000) reported that cyst length without neck and width were 423 to 592 µm and 469 to 712 µm. The neck

length and mean fenestral diameter were 104±19 µm and 19.0±2.0 µm. The fenestral length and Granek's ratio were 20 µm and 3.0, respectively.

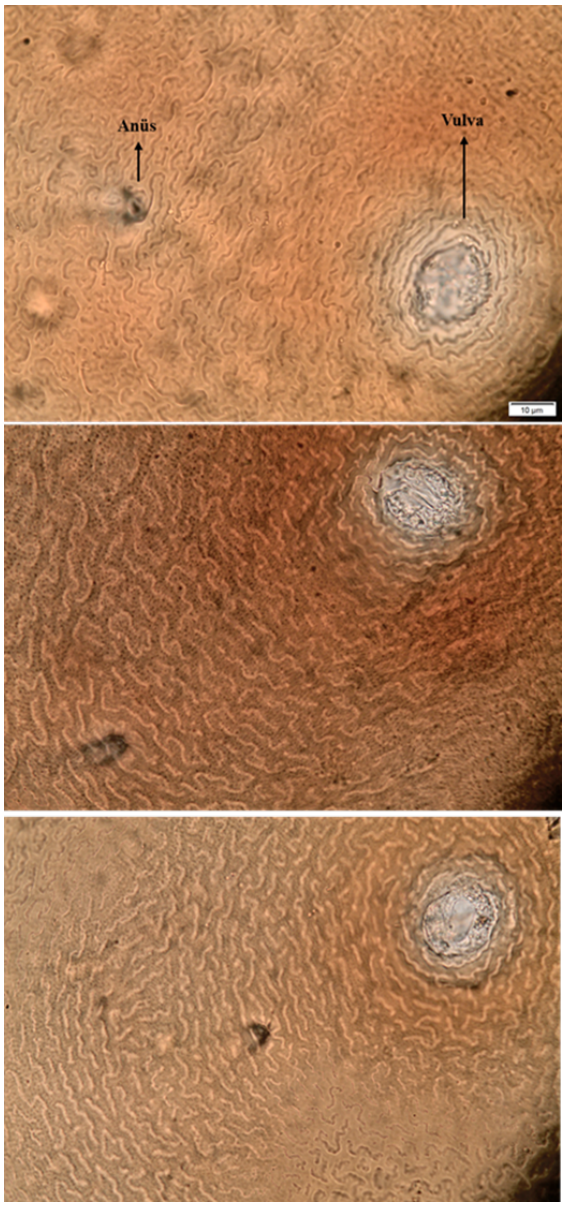

Figure 2. Photomicrographs of the anal-vulval regions of *Globodera rostochiensis* cysts from İzmir Province, Turkey.

The mean morphological and morphometric values of cysts were higher for Ödemiş populations than those of Bozdağ populations (Table 3). However, the differences between Ödemiş and Bozdağ populations were not significant ($P \leq 0.5$) for number of ridges and anus-vulva distance. Also, the average values associated with the morphological and morphometric features of the cysts were within the range described in previous studies (Fleming & Powers, 1998; EPPO, 2013). For all populations, the overlap was high in cysts morphological and morphometric characters especially fenestral length and Granek's ratio with Wouts & Baldwin (1998), but these values were slightly differed clearly from other Golden (1986) by slightly higher mean in the distance from anus to the nearest edge of fenestra 58.4 (52-68 µm).

All samples produced a single fragment of about 950 bp using F194 and F195 primers. The amplified sequences were used as BLAST queries against the NCBI database and had 99.89-100% nucleotide similarity with those of corresponding species recorded in GenBank. The result of the study, 30 cyst populations from Bozdağ and Ödemis locations compared with closely related cyst samples in GenBank and were all identified as *G. rostochiensis* based on their ITS sequences. The *G. rostochiensis* populations in this study were compared molecularly with international genotypes from different countries (Figure 3). The analysis involved 37 nucleotide sequences. The clustering of the populations on the phylogenetic tree that occurred according to their species levels based on genetic distance was constructed from the ITS sequence alignment (Figure 3).

Figure 3. Maximum likelihood tree was generated using the ITS sequences of populations of *Globodera rostochiensis* and reference populations from GenBank including their accession numbers and strain numbers. Numbers on the branches represent bootstrap values obtained from 1,000 bootstrap replications.

However, Madani et al. (2010) reported that *G. rostochiensis* populations from different locations in Canada were clustered in one group within the phylogenetic tree. Knoetze et al. (2013) reported that the ITS sequence alignment of *G. rostochiensis* populations from South Africa clustered together with a high bootstrap value similarity using the minimum evolution method. Different cyst nematode species were thought to be phylogenetically examined based on ITS sequences and this region was thought to be useful in identifying species (Subbotin et al., 2000). Therefore, the slight distances among *G. rostochiensis* population can be explained by high gene flow among potato cyst nematode populations.

Population diversity within the genus *Globodera* has often been used successfully for diagnostic purposes to discriminate *G. rostochiensis* populations from non-quarantine species (Baldwin & Mundo-Ocampo, 1991; Fleming & Powers, 1998; Manduric et al., 2004). This study is the first to provide a comprehensive molecular analysis of *G. rostochiensis* populations from Izmir and is complementary to other studies of Turkish potato cyst nematodes populations. This study emphasized the agroecological distribution of the potato cyst nematode species *G. rostochiensis* in Bozdağ and Ödemiş Districts in İzmir. The differences determined by morphological and morphometric techniques also demonstrated a variation among *G. rostochiensis* populations, indicating that climatic conditions of the mountainous area could influence variability. It is be concluded that the results of this study will help to investigate more of pathotypes of golden nematode and to gain a more complete understanding of the physiology, ecology and biology of the genus *Globodera* as agricultural pests for an effective management.

Acknowledgements

The authors would like to thank the Doğal Pesticides and Chemicals Industry Trade S.A. for providing the funding for this study. For technical assistance, we also thank Nancy De Sutter from the Flanders Research Institute for Agriculture, Fisheries and Food, Belgium.

References

Al-Banna, L., A. T. Ploeg, V. M. Williamson & I. Kaloshian, 2004. Discrimination of six *Pratylenchus* species using PCR and species-specific primers. Journal of Nematology, 36 (2): 142-146.

Andres, M. F., R. Alonso & A. Alemany, 2006. First report of *Globodera rostochiensis* in Mallorca Island, Spain. Plant Disease, 90 (9): 1262-1262.

Baldwin, J. G. & M. Mundo-Ocampo, 1991. “Heteroderinae, Cyst and Non-Cyst Forming Nematodes, 275-362”. In: Manual of Agricultural Nematology (Eds. W. R. Nickle & M. Dekker). CRC Press, 1035 pp.

Chitwood, D. J., 2003. Research on plant-parasitic nematode biology conducted by the United States Department of Agriculture-Agricultural Research Service. Pest Management Science (formerly Pesticide Science), 59 (6-7): 748-753.

Dale, M. F. B., 1988. Breeding for tolerance to potato cyst nematode. Aspects of Applied Biology, 17 (1): 95-101.

Demirbaş Pehlivan, H., G. Kaşkavalcı, E. B. K. Uludamar, H. Toktay & H. Elekcioğlu, 2020. Identification and prevalence of potato cyst nematodes and root-knot nematodes in the potato production areas of İzmir Province, Turkey. Turkish Journal of Entomology, 44 (2): 259-272.

Enneli, S. & G. Öztürk, 1996.” Important Plant parasitic nematodes damaging potatoes in Central Anatolia Region in Turkey, 396-403”. Proceedings of the Third Turkish National Congress of Entomology (24-28 September 1996, Ankara, Turkey) Ankara University Printing House, 716 pp.

EPPO, 2013. European and Mediterranean Plant Protection Organization. EPPO Bulletin, 43: 471-495.

FAOSTAT, 2020. Food and Agriculture Organization Statistical Database. (Web page: http://www.fao.org/faostat/en/#data/QC) (Date accessed: 15 March 2020).

Felsenstein, J., 1985. Confidence limits on phylogenies: An approach using the bootstrap. Evolution, 39 (4): 783-791.

Ferris, V. R., J. M. Ferris & J. Faghihi, 1993. Variations in spacer ribosomal DNA in some cyst-forming species of plant parasitic nematodes. Fundamental and Applied Journal of Nematology, 16 (2): 177-184.

Ferris, V. R., L. I. Miller, J. Faghihi & J. M. Ferris, 1995. Ribosomal DNA comparisons of *Globodera* from two continents. Journal of Nematology, 27 (3): 273-283.

Fleming, C. C. & T. O. Powers, 1998. "Potato Cyst Nematodes: Species, Pathotypes and Virulence Concepts, 51-57". In: Potato Cyst Nematodes Biology, Distribution and Control (Eds. R. J. Marksand & B. B. Brodie). CAB International, 424 pp.

Ganguly, A. K. & U. Rao, 2003. "Application of Molecular Biology in Nematology, 312-317". In: Advances in Nematology (Ed. P. C. Trivedi). Scientific Publishers, 317 pp.

Gitty, M. & Z. Tanha Maafi, 2010. First report of a potato cyst nematode, *Globodera rostochiensis*, on potato in Iran. Plant Pathology, 59 (2): 412.

Golden, A. M., 1986. "Morphology and Identification of Cyst Nematodes, 23-79". In: Cyst Nematodes (Eds. F. Lamberti & C. E. Taylor). Plenum Press, 478 pp.

Golden, A. M., 1990. "Preparation and Mounting Nematodes for Microscopic Observation, 197-205". In: Plant Nematology Laboratory Manual (Eds. B. M. Zuckerman, W. F. Mai & L.R. Krusberg). Amherst MA Press, 115 pp.

Golden, A. M. & D. M. S. Ellington, 1972. Redescription of *Heterodera rostochiensis* (Nematoda: Heteroderidae) with a key and notes on closely related species. Proceedings of the Helminthological Society of Washington, 39 (1): 64-78.

Greco, N., M. Di Vito, A. Brandonisio, I. Giordano & G. De Marinis, 1982. The effect of *Globodera pallida* and *G. rostochiensis* on potato yield. Nematologica, 28 (4): 379-386.

Holterman, M., A. Van Der Wurff & S. Van Den Elsen, 2006. Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. Molecular Biology and Evolution, 23 (9): 1792-1800.

Hooper, D. J., 1970. Handling, fixing, staining and mounting nematodes. Ministry of Agriculture, Fisheries and Food's Technical Bulletin, 5 (2): 39-54.

Ibrahim, I. K. A., Z. A. Handoo & A. A. El-Sherbiny, 2000. A survey of phytoparasitic nematodes on cultivated and non-cultivated plants in Northwestern Egypt. Journal of Nematology, 32 (4S): 478- 485.

Imren, M., 2018. Determination of plant parasitic nematodes in potato growing areas in Bolu Province. International Journal of Agriculture and Wildlife Science, 4 (2): 26-32.

Indarti, S., B. Mulyadi & B. Triman, 2004. First record of potato cyst nematode *Globodera rostochiensis* in Indonesia. Australasian Plant Pathology, 33 (2): 325-326.

Jones, J. T., A. Haegeman, E. Danchin, H. Gaur, J. Helder, M. G. Jones, T. Kikuchi, R. Manzanilla-López, J. E. Palomares-Rius, W. M. Wesemael & R. N. Perry, 2013. Top 10 plant-parasitic nematodes in molecular plant pathology. Molecular Plant Pathology, 14 (9): 946-961.

Kepenekci, İ., 2012. Nematology (Plant Parasitic and Entomopathogen Nematodes). Education Directorate of Publications and Publications, Agricultural Science Series, 1155 pp.

Knoetze, R., A. Swart & L. R. Tiedt, 2013. Description of *Globodera capensis* n. sp. (Nematoda: Heteroderidae) from South Africa. Nematology, 15 (2): 233-250.

Kort, J., H. Ross, H. J. Rumpenhorst & A. R. Stone, 1977. An international scheme for identifying and classifying pathotypes of potato cyst nematodes *Globodera rostochiensis* and *G. pallida*. Nematologica, 23 (3): 333-339.

Kumar, S., G. Stecher, M. Li, C. Knyaz & K. Tamura, 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. Molecular Biology and Evolution, 35 (6): 1547-1549.

Madani, M., S. A. Subbotin & M. Moens, 2005. Quantitative detection of the potato cyst nematode, *Globodera pallida*, and the beet cyst nematode, *Heterodera schachtii*, using real-time PCR with SYBR green I dye. Molecular and Cellular Probes, 19 (2): 81-86.

Madani, M., S. A. Subbotin, L. J. Ward, X. Li & S. H. De Boer, 2010. Molecular characterization of Canadian populations of potato cyst nematodes, *Globodera rostochiensis* and *G. pallida* using ribosomal nuclear RNA and cytochrome b genes. Canadian Journal of Plant Pathology, 32 (2): 252-263.

Madani, M., L. J. War & S. H. De Boer, 2008. Multiplex real-time polymerase chain reaction for identifying potato cyst nematodes, *Globodera pallida* and *Globodera rostochiensis*, and the tobacco cyst nematode, *Globodera tabacum*. Canadian Journal of Plant Pathology, 30 (4): 554-564.

Manduric, S., E. Olsson, J. E. Englund & S. Andersson, 2004. Separation of *Globodera rostochiensis* and *G. pallida* (Tylenchida:Heteroderidae) using morphology and morphometrics. Nematology, 6 (2): 171-181.

Mugniéry, D., M. S. Phillips, H. J. Rumpenhorst, A. R. Stone, A. Treur & D. L. Trudgill, 1989. Assessment of partial resistance of potato to, and pathotype and virulence differences in, potato cyst nematodes. EPPO Bulletin, 19 (1): 7-25.

Özarslandan, A., D. Dinçer, R. Bozbuga, M. İmren & İ. H. Elekçioğlu, 2019. Study on the presence of potato cyst nematodes: *Globodera rostochiensis* (Wollenweber, 1923) Skarbilovich, 1959 and *Globodera pallida* Stone 1973 (Tylenchida: Heteroderidae) in Nevşehir Province, Turkey. Turkish Journal of Agriculture and Natural Sciences, 6 (2): 125-129.

Sasser, J. N. & D. W. Freckman, 1987. "A World Perspective on Nematology: The Role of the Society, 7-14". In: Vistas on Nematology (Eds. A. Veech & D. W.Dickerson). Journal of Society of Nematologists, 509 pp.

Scurrah, M. I., B. Niere & J. Bridge, 2005. "Nematode Parasites of Solanum and Sweet Potatoes, 193-219". In: Plant Parasitic Nematodes in Subtropical and Tropical Agriculture (Eds. M. Luc, R. A. Sikora & J. Bridge). CABI Publishing, 841 pp.

Shapiro, S. S. & M. B. Wilk, 1965. An analysis of variance test for normality (complete samples). Biometrika, 52 (3/4): 591-611.

Siddiqi, M. R., 2000. Tylenchida, Parasites of Plants and Insects. CABI Publishing, 848 pp.

Skantar, A. M., Z. A. Handoo, L. K. Carta & D. J. Chitwood, 2007. Morphological and molecular identification of *Globodera pallida* associated with potato in Idaho. Journal of Nematology, 39 (2): 133-144.

Southey, J. F., 1986. Laboratory Methods for Work with Plant and Soil Nematodes. London: CABI Publishing, 202 pp.

Subbotin, S. A., A. Mundo-Ocampo & J. G. Baldwin, 2010 Systematics of Cyst Nematodes (Nematode: Heteroderinae). Nematology Monographs and Perspectives, 8A. Brill, 364 pp.

Subbotin, S., R. Perry, A. Warry & P. Halford, 2000. Variations in ribosomal DNA sequences and phylogeny of *Globodera* parasitizing solanaceous plants. Nematology, 2 (6): 591-604.

Subbotin, S. A., D. Sturhan, H. J. Rumpenhorst & M. Moens, 2003. Molecular and morphological characterization of the *Heterodera avenae* species complex (Tylenchida: Heteroderidae). Nematology, 5 (4): 515-538.

Subbotin, S. A., L. Waeyenberge, I. A. Molokanova & M. Moens, 1999. Identification of *Heterodera avenae* group species by morphometrics and rDNA-RFLPs. Nematology, 1 (2): 195-207.

Szalanski, A. L., D. D. Sui, T. S. Harris & T. O. Powers, 1997. Identification of cyst nematodes of agronomic and regulatory concern with PCR-RFLP of ITS1. Journal of Nematology, 29 (3): 255-267.

Tamura, K. & M. Nei, 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. Molecular Biology Evolution, 10 (3): 512-526.

Thompson, J. D., D. G. Higgins & T. J. Gibson, 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Research, 22 (22): 4673-4680.

Toktay, H., E. Evlice, M. Imren, G. Özer, M. A. Ali & A. Dababat, 2020. Characterization of potato golden cyst nematode populations (*Globodera rostochiensis*) in Turkey. International Journal of Agriculture and Biology, 23 (6): 1095-1100.

Ulutaş, E., A. Özarslandan, G. Kaşkavalcı & İ. H. Elekcioglu, 2012. Ege Bölgesi patates alanlarında *Globodera rostochiensis* Wollenweber, 1923' in moleküler yöntemlerle saptanması. Türkiye Entomoloji Dergisi, 36 (1): 155-160.

Waeyenberge, L., A. Ryss, M. Moens, J. Pinochet & T. Vrain, 2000. Molecular characterisation of 18 *Pratylenchus* species using rDNA restriction fragment length polymorphism. Nematology, 2 (2): 135-142.

Wouts, W. M. & J. G. Baldwin, 1998. "Taxonomy and Identification, 83-122". In: The Cyst Nematodes (Ed. S. B. Sharma). Dordrecht, Kluwer, 452 pp.

Türk. entomol. derg., 2020, 44 (3): 399-412
DOI: http://dx.doi.org/10.16970/entoted.657190

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

A chemotaxonomic approach to fatty acid composition of the genera *Helochares* Mulsant, 1844 and *Coelostoma* Brullé, 1835 (Coleoptera: Hydrophilidae)[1]

Helochares Mulsant, 1844 ve *Coelostoma* Brullé, 1835 (Coleoptera: Hydrophilidae) cinslerinin yağ asiti kompozisyonuna taksonomik bir yaklaşım

Fatma CAF[2*] Günay YILDIZ[3] Nurgül SEN ÖZDEMİR[2] Abdullah MART[4]

Abstract

In this study, total lipid and fatty acid composition of insects belonging to the genera *Helochares* Mulsant, 1844 and *Coelostoma* Brullé, 1835 (*Coelostoma orbiculare* Fabricius, 1775; *Coelostoma transcaspicum* Reitter, 1906; *Helochares obscurus* Müller, 1776; *Helochares lividus* Forster, 1771) of Hydrophilidae (Coleoptera) family collected from Bingöl Province were determined. Specimens were collected in 2015. Fatty acid component of the insects was determined by mass-gas chromatography (GC-MS). Total saturated fatty acids (ΣSFA) were between 23.9-40.8%, total monounsaturated fatty acids (ΣMUFA) were 21.6-53.2% and total polyunsaturated fatty acids (ΣPUFA) were 14.3-27.4%. Myristic acid (14:0), pentadecanoic acid (15:0) palmitic acid (16:0), heptadecanoic acid (17:0) from SFA, palmitoleic acid (16:1n-7), oleic acid (18:1n-9) from MUFA; linoleic acid (18:2n-6), linolenic acid (18:3n-3), arachidonic acid (ARA, 20: 4n-6), eicosapentaenoic acid (EPA, 20: 5n-3) were the most important fatty acids. ANOSIM results showed that only the difference among the species was significant (R=0.63); the difference among subfamilies (R=0.17) and among the genera (R=0.17) were partially significant and that the difference among the families (R=0.08) was not significant.

Keywords: Bingöl, chemotaxonomic approach, *Coelostoma,* fatty acids, *Helochares*

Öz

Bu çalışmada, Bingöl İli'nden toplanan Hydrophilidae (Coleoptera) familyasının *Helochares* Mulsant, 1844 ve *Coelostoma* Brullé, 1835 cinsine (*Coelostoma orbiculare* Fabricius, 1775; *Coelostoma transcaspicum* Reitter, 1906; *Helochares obscurus* Müller, 1776; *Helochares lividus* Forster, 1771) ait böceklerin toplam lipit ve yağ asiti kompozisyonu belirlenmiştir. Örnekler 2015 yılında toplanmıştır. Böceklerin yağ asiti bileşeni gaz kromatografisi (GC-MS) ile belirlenmiştir. Toplam doymuş yağ asitleri (ΣSFA) %23.9-40.8, toplam tekli doymamış yağ asitleri (ΣMUFA) %21.6-53.2 ve toplam çoklu doymamış yağ asitleri (ΣPUFA) %14.3-27.4 arasında değişim göstermiştir. SFA' dan miristik asit (14: 0), pentadekanoik asit (15: 0) palmitik asit (16: 0), heptadekanoik asit (17: 0); MUFA'dan palmitoleik asit (16: 1n-7), oleik asit (18: 1n-9); ve PUFA' dan linoleik asit (18: 2n-6), linolenik asit (18: 3n-3), araşidonik asit (ARA, 20: 4n-6), eikosapentaenoik asit (EPA, 20: 5n-3) en önemli yağ asitleri olarak saptanmıştır. Hydrophilidae familyası bireylerinde sadece türler arasındaki farkın nispeten önemli olduğu (R=0.63), alt familyalar (R=0.17) ve cinsler (R=0.17) arasındaki farkın kısmen önemli olduğu ve familyalar (R=0.08) arasındaki farkın önemli olmadığı ANOSIM sonuçlarıyla ortaya konulmuştur.

Anahtar sözcükler: Bingöl, kemotaksonomik yaklaşım, *Coelostoma,* yağ asitleri, *Helochares*

[1] This study was supported by Bingöl University (Turkey), Scientific Research Unit, Grant Project No: BAP-281-265-2015.
[2] Bingöl University, Vocational School of Technical Sciences, Department of Veterinary Medicine, 12000, Bingöl, Turkey
[3] Bingöl University, Faculty of Arts and Sciences, Department of Biology, 12000, Bingöl, Turkey
[4] Osmaniye Korkut Ata University, Faculty of Arts and Sciences, Department of Biology, 80010, Osmaniye, Turkey
* Corresponding author (Sorumlu yazar) e-mail: fcaf@bingol.edu.tr
Received (Alınış): 10.12.2019 Accepted (Kabul ediliş): 23.06.2020 Published Online (Çevrimiçi Yayın Tarihi): 26.07.2020

Introduction

The great majority of Hyrdophilidae are aquatic, but there are also species living in semiaquatic and terrestrial habitats. Although they are found in many different habitats, many members of the Hydrophilidae family live among the plants and mosses in the shallow parts of rivers, lakes, ponds, canals, pools, trenches, streams and temporary water deposits (Hansen, 1987). It is known that the semiaquatic members are found in soil close to a water source or under rubble, such as plants and straws, which start to rot, whereas terrestrial members are known to be found inside or under feces of vertebrate animals such as goats and cows, in places where there is a lot of vegetative decay, and even in bird nests (Cercyon) (Fikåček, 2006). They are caught with instruments such as fine sieves and light traps, and they are generally black, brown or yellowish color (Angus, 1992). Adults generally feed with watery plants, mosses and vegetative detritus, and rarely consume food of animal origin. Fish and water birds love eating them. The larvae are carnivorous (Demirsoy, 1997). Three genera and 58 species of Hydrophilidae have been recorded (Gentili, 2000; Gentili & Whitehead, 2000; İncekara et al., 2005; Darılmaz & Kıyak, 2006a, b; Kıyak et al., 2006) and 13 new species reported by Mart (2009) in Turkey.

Lipids important in the human and animal diets are a group of compounds consisting of glycerol and fatty acids (Gilby, 1965). Fatty acids are fairly important in that they are the main component of fats, and they also participate in the structure of the cell membrane and are the precursors of bioactive metabolites. Fatty acids stored in the form of triacylglycerol are vital for insects as they serve as the main energy source in times when they do not find food and during long flights (Downer & Matthews, 1976; Beenakkers et al., 1985).

Some of the fat reserves of insects are met by assimilation of vegetable oils and partly by synthesis from carbohydrates (Stanley-Samuelson et al., 1988). Lipids are important in insect biochemistry as energy sources, hormones and structural compounds. Lipids have also been identified as the major source of energy in insect embryogenesis (Gilbert, 1967). As an energy source, nutritional lipids are more important than nutritional proteins. Fatty acids, which have important functions in all organisms, such as energy storage, transport, mobilization, and being structural components of bio membranes, have a few specific functions in insects. Fatty acids are also important as precursors in the biosynthesis of waxes, pheromones and eicosanoids. However, it is also known that they components of protection secretions (Wakayama et al., 1980; Stanley-Samuelson et al., 1988; Başhan, 1996).

The first known physiological role of eicosanoids has been discovered in insects (Stanley, 2000). Eicosanoids are an important intermediate substance in the biology of invertebrates in many areas such as production, ion transport (Stanley, 2000), hormone signal transduction system (Keeley et al., 1996) and immune system (Stanley, 2000). Eicosanoids also have a function in animal populations where there is a hunter-prey relationship or a host-parasite relationship (Stanley, 2000). Insects can synthesize saturated and monounsaturated fatty acids, like highly structured animals, through similar synthesis processes (Thompson, 1979). In addition, biological factors such as age and gender of the insects, temperature, adult nutrition and duration of activity affect fatty acid composition (Cohen, 1990). Canavoso et al. (2001) suggested that lipid and fatty acid contents vary depending on the species, gender and stage of development, and therefore fatty acid profile should be examined with respect to gender and developmental stages. We used insect groups living both in terrestrial and aquatic environments. In this study, intergenus and interspecies total lipid, and within and between species fatty acid compositions of Hydrophilidae (Coleoptera) family (*Helochares* and *Coelostoma*) collected from Bingöl Province were determined. In addition, PRIMER-version-7 statistical software was used to demonstrate chemotaxonomic similarities.

Materials and Methods

This study was conducted in two stages, namely field and laboratory studies.

Field studies

Materials used in this study were collected from Bingöl Province central, districts and various villages within these districts. Samples were collected between May and June 2015 by sifting between plants and moss in the shallow sections of various streams, springs, creeks, sediments and hot water sources. The species investigated were *Helochares lividus* (Forster, 1771)*, Helochares obscurus* (Müller, 1776)*, Coelostoma orbiculare* (Fabricius, 1775) and *Coelostoma transcaspicum* Reitter, 1906 because only two the species could be identified in the research area. Identification of the species was made according Mart (2005). Mouth aspirator, stereomicroscope, numbered insect needles and glassware were used to collect these species. Collected insects were brought to the laboratory in storage jars without being killed. No chemicals were used during the collection of samples because samples used in fatty acid analysis. The samples brought to the laboratory were stored in the freezer at -80°C for use in studies after the species were diagnosed.

Material examined. *Helochares lividus,* Bingöl (central), Ağaçeli Village, 28.VI.2015, 14 ♂♂, 16 ♀♀; *H. obscurus,* Bingöl, Solhan Yüzenada, 21.VI.2015, 9 ♂♂, 8 ♀♀; *C. transcaspicum,* Bingöl, Ilıcalar, 21.V.2015, 14 ♂♂ 13 ♀♀; and *C. orbiculare*, Bingöl, Ilıcalar, 21.V.2015, 13 ♂♂ 15 ♀♀.

Helochares (Mulsant, 1844) species is within the Acidocerinae subfamily, whereas *Coelostoma* (Brullé, 1835) is within the Sphaeridiinae subfamily (Short & Fikácek, 2013).

Helochares obscurus has a body length of 5.0-5.9 mm and a width of 2.6-2.8 mm. Pronotum is 1.2-1.4 mm high and 2.1-2.2 mm wide.

Helochares lividus has a body length of 5.4-5.8 mm and a width of 2.4-2.7 mm. Pronotum is 1.3-1.4 mm high and 2.0-2.1 mm wide.

Coelostoma orbiculare is 4.3-4.9 mm tall and 2.3-2.7 mm wide. Pronotum is 1.0 mm high and 2.2 mm wide.

Coelostoma transcaspicum has a body length of 5.6-5.9 mm and a width of 3.0-3.2 mm. Pronotum is black, short, wide and has a smooth back edge.

Laboratory studies

Extraction of lipids from the samples was carried out by the method of Hara & Radin (1978) using a mixture of 3:2 (v/v) hexane and isopropanol. For this, the tissues were degraded with 10 mL of hexane isopropanol at 3:2 (v/v) in the homogenizer at 11,000 rpm for 30 s. They were then centrifuged for 10 min at 6,000 rpm, and the supernatant from the tissue samples were taken and put into capped test tubes.

Preparation of fatty acid methyl esters

In order to perform gas chromatography analysis on the fatty acids contained in the lipids, they were converted to derivatives such as methyl esters having a non-polar, volatile and stable structure. Although there are different methods for converting fatty acids in lipids to methyl ester derivatives, the acid-catalyzed esterification method described by Christie (1992), which is easy to use and highly efficient, was used.

According to this method; to prepare the methyl esters, the lipid extract in the hexane isopropanol phase was taken up into the 30 ml non-leaking screw cap test tubes. Five ml of 2% methanolic sulfuric acid was added and mixed thoroughly with the vortex. The mixture was left to methylate in an incubator at 50°C for 15 h. After 15 h, the tubes were removed from the incubator, cooled to room temperature, and

mixed thoroughly after adding 5 mL of 5% NaCl. The fatty acid methyl esters formed in the tubes were extracted with 5 ml of hexane and the hexane phase at the top was removed with a pipetted and treated with 5 ml of 2% $KHCO_3$ and left for 4 h to separate the phases. The solvent in the mixture containing methyl esters was then evaporated at 45°C and under nitrogen flow, and the fatty acids at the bottom of the test tubes were dissolved in 1 mL of hexane and transferred into closed autosampler vials for gas chromatography analysis.

After this methylation process, samples were analyzed for fatty acids by Agilent 5975 C model GC/MS gas chromatography device. For this analysis, a Machery-Nagel (Germany) capillary column (30 m x 0.25 mm, 0.25 µm) was used. During the analysis, the column temperature was 140-220ºC, the injection temperature was 250ºC and the detector temperature was 260ºC. Column temperature program was set between 140ºC and 250ºC. Helium (0.5 ml/min) was used as carrier gas. Prior to the analysis of fatty acid methyl esters of the samples, mixtures of standard fatty acid methyl esters (Supelco 37 Component FAME Mix) were injected and the retention times of each fatty acid were determined. After this procedure, necessary programming was done and mixtures of fatty acid methyl esters of the samples were analyzed.

Statistical analyses

Multivariate statistical analyzes were used to reveal the differences between fatty acids in the samples. Plymouth Routines in Multivariate Ecological Research (PRIMER version 7) software was used to establish the relationship between fatty acids and lipid classes. This software is used in the evaluation of ecological data. Multivariate statistical analyzes were performed as analysis of similarities (ANOSIM), evaluation of proportional similarities of non-parametric data (MDS) and percentage of similarities (SIMPER). Global R values obtained from ANOSIM results were used to test the differences between species, subfamilies and genera. Pethybridge et al. (2011) characterized global R values >0.75 as indicating well separated groups, while those <0.25 as barely separated groups. We characterized global R values >0.75 as indicating well separated groups-no difference, <0.25 as barely separated groups-partial difference and <0.1 as indicating unseparated groups-no difference.

Results

Between and in species fatty acid compositions of the samples

Fatty acid analysis was performed on males and females of *Helochares lividus* (n=30)*, Helochares obscurus* (n=17)*, Coelostoma orbiculare* (n=27) *and Coelostoma transcaspicum* (n=28) collected from the sampling area. A total of 29 fatty acids and 10 common fatty acids were detected in the samples. The most important fatty acids in the samples were 14:0 (myristic acid), 16:0 (palmitic acid), 22:1n-11, 20:1n-9, 20:5n-3 (eicosapentaenoic acid, EPA), polyunsaturated fatty acids: PUFAs, monounsaturated fatty acids: MUFAs, and omega 3 (ω-3) fatty acids (Tables 1 & 2).

Table 1. Fatty acid compositions of *Helochares lividus* and *Helochares obscurus* (%)

Fatty acids (Common Name) (%)	*H. lividus* ♀ (n=16)	*H. lividus* ♂ (n=14)	*H. obscurus* ♀ (n=8)	*H. obscurus* ♂ (n=9)
14:0 (myristic acid)	2.93	2.03	2.43	2.62
i15:0 (pentadecanoic acid isomer)	0.57	0.45	-	-
15:0 (pentadecanoic acid)	0.71	0.38	2.36	1.97
i16:0 (palmitic acid isomer)	2.11	-	2.94	3.42
16:0 (palmitic acid)	22.76	24.79	17.06	17.14
i17:0 (heptadecanoic acid isomer)	0.33	-	-	1.36
17:0 (heptadecanoic acid)	0.57	0.43	1.33	1.51
18:0 (stearic acid)	6.75	12.71	6.58	8.20
20:0 (arachidic acid)	0.35	0.45	-	1.33
Σ SFA (Total Saturated Fatty Acids)	37.08	41.24	32.70	37.55
14:1 (myristoleic acid)	0.38	0.28	-	-
15:1(pentadecaenoic acid)	0.25	-	1.19	-
17:1 (heptadecanoic acid)	0.60	-	-	-
16:1 n-11 (palmitoleic acid)	0.70	2.11	1.14	-
16:1 n-9 (palmitoleic acid)	19.69	11.78	13.12	11.34
18:1n-11 (vaccenic acid)	-	0.91	-	-
18:1n-9 (oleic acid)	13.95	13.6	19.88	19.86
18:1n-7 (vaccenic acid)	1.48	0.67	3.08	3.61
18:1n-6 (vaccenic acid)	-	1.40	-	-
22:1w9 (erucic acid)	1.55	-	-	2.22
Σ MUFA (Total Monounsaturated Fatty Acids)	39.2	30.75	38.41	37.03
16:2n-4 (hecsadecadienoic acid)	1.47	1.22	-	-
18:2 (linoleic acid)	0.91	-	-	-
18:2n-6 (linoleic acid)	9.87	12.3	13.55	13.75
18:2n-4 (linoleic acid)	0.44	1.08	2.78	3.19
18:3n-6 (gamma-linolenic acid)	0.79	0.55	-	-
18:3n-3 (alpha-linolenic acid)	5.14	5.46	5.30	1.46
20:4n-6 (arachidonic acid)	2.18	2.62	2.88	3.52
20:3n-3 (eicosatrienoic acid)	-	-	1.29	-
20:5n-3 (eicosapentaenoic acid)	3.53	3.59	3.06	3.47
Σ PUFA (Total Polyunsaturated Fatty Acids)	24.33	26.82	28.86	25.39
Omega 3 (ω 3)	8.67	9.59	9.66	4.93
Bacterial Fatty acids	5.13	2.91	7.83	8.27
Terrestial Fatty acids (18:3n3+18:2n6)	15.01	17.75	18.85	15.21

Table 2. Fatty acid compositions of *Coelostoma transcaspicum* and *Coelostoma orbiculare* (%)

Fatty acids (%)	*C. transcaspicum* ♀ (n=13)	*C. transcaspicum* ♂ (n=14)	*C. orbiculare* ♀ (n=15)	*C. orbiculare* ♂ (n=13)
14:0 (myristic acid)	3.16	0.25	2.9	2.8
i15:0 (pentadecanoic acid isomer)	-	0.29	0.43	-
15:0 (pentadecanoic acid)	-	0.38	0.43	0.41
i16:0 (palmitic acid isomer)	2.93	2.83	2.04	1.67
16:0 (palmitic acid)	24.81	22.14	16.70	19.22
i17:0 (heptadecanoic acid isomer)	-	-	2.52	-
17:0 (heptadecanoic acid)	0.37	0.54	0.65	0.64
18:0 (stearic acid)	5.37	-	7,77	7,76
20:0 (arachidic acid)	0,34	0,56	0,45	0,51
Σ SFA (Total Saturated Fatty Acids)	36,98	26,99	33,89	33,01
14: 1 (myristoleic acid)	0,36	3,85	0,35	0,34
17: 1 (pentadecacanoic acid)	-	0,24	0,28	0,24
16:1 n-11 (heptadecanoic acid)	0,78	0,74	0,73	-
16:1 n-9 (palmitoleic acid)	17,94	18,87	16,98	21,06
16:1 n-7 (vaccenic acid)	-	-	-	0,23
18:1n-11 (vaccenic acid)	0,59	-	20,79	-
18:1n-9 (oleic acid)	24.86	22.96	9.51	16.39
18:1n-7 (vaccenic acid)	2.88	0.73	2.14	0.72
18:1n-6 (vaccenic acid)	-	2.84	0.73	1.61
22:1w9 (erucic acid)	-	2.98	-	-
ΣMUFA (Total Monounsaturated Fatty Acids)	47.41	53.21	51.51	40.59
16:2n-4 (hecsadecadienoic acid)	-	-	-	0.83
18: 2 (linoleic acid)	-	0.31	-	0.97
18:2n-6 (linoleic acid))	4.56	4.65	1.28	12.27
18:2n-4 (alpha-linoleic acid)	0.53	0.77	-	0.25
18:3n-6 (gamma-linoleic acid)	0.48	0.76	0.92	1.15
18:3n-3 (alpha-linoleic acid)	3.50	4.63	3.24	2.01
18:3n-4 (octadecatrienoic acid)	0.40	0.48	0.36	-
20:4n-6 (arachidonic acid)	2.44	4.45	4.12	4.31
20:5n-3 (eicosapentaenoic acid)	2.43	3.34	4.37	3.70
Σ PUFA (Total Polyunsaturated Fatty Acids)	14.34	19.39	14.29	25.49
Omega 3 (ω 3)	5.93	7.97	7.62	5.71
Bacterial fatty Acids	3.30	7.13	7.37	4.58
Terrestial Fatty Acids (18:3n3+18:2n:6)	8.06	9.28	4.52	14.28

According to PERMANOVA results, average similarities of fatty acids, within and between species are shown in Table 3. The SIMPER shows the fatty acid similarity in terms of species, genus, gender and subfamily and the contribution on the similarity of each fatty acid in each group.

According to SIMPER, the fatty acid similarity within the Acidocerinae subfamily was 78%. The highest contribution to this similarity was made by palmitic acid (29%), followed by oleic acid (19%) and palmitoleic acid (19%). Fatty acids 18:3n-4, 18:2, and 17:1 made the least contribution to the similarity. The similarity within the Sphaeridinae subfamily was 75%. Palmitic acid (22%), oleic acid (18%) and palmitoleic

acid (17%) made the highest contribution to the fatty acid similarity within the subfamily, whereas the fatty acids with the least contribution were 16:1n-11, 14:1 and 17:1. EPA amount was found to be 4.4%. When we compared the subfamilies of Acidocerinae and Sphaeridinae, the fatty acid similarity ratio was found to be 74%. The fatty acids that contributed most to this similarity were oleic acid (12%), palmitic acid (11%), and linoleic acid (11%), respectively.

Table 3. Fatty acid similarity percentages according to SIMPER and ANOSIM results in the samples ($p < 0.01$)

	Average similarity within/between species %				*R*
	H. lividus	*C. transcaspicum*	*C. orbiculare*	*H. obscurus*	*C. transcaspicum*
Helochares lividus	83.10				1.0
Coelostema transcaspicum	76.04	82.98			
Coelostema orbiculare	75.57	71.24	70.99		0.5
Helochares obscurus	77.29	73.42	72.30	90.34	1.0

Figure 1 shows a two-dimensional configuration plot of and MDS analysis of a resemblance matrix of fatty acid data. Multivariate analyses of FA were identified in both of male and female sample means. The samples plotted were factored by species. Stress is called as a degree to which to dimensional configuration plot distorts the sample relationship. If the stress value 0.1, it gives a potentially useful representation. The value must to below 0.05 for an excellent representation (Clarke & Warwick, 2001). Stress value is 0.07 in Figure 1. The value shows a potentially useful representation. The importance of some fatty acids in species gender of the samples. For example, i17:0, 16:1w11, 18:1w7 were more important in female of *C. orbiculare* than the others. Fatty acids 20:2a and 14:1 were more important in male of *C. transcaspicum* than the others.

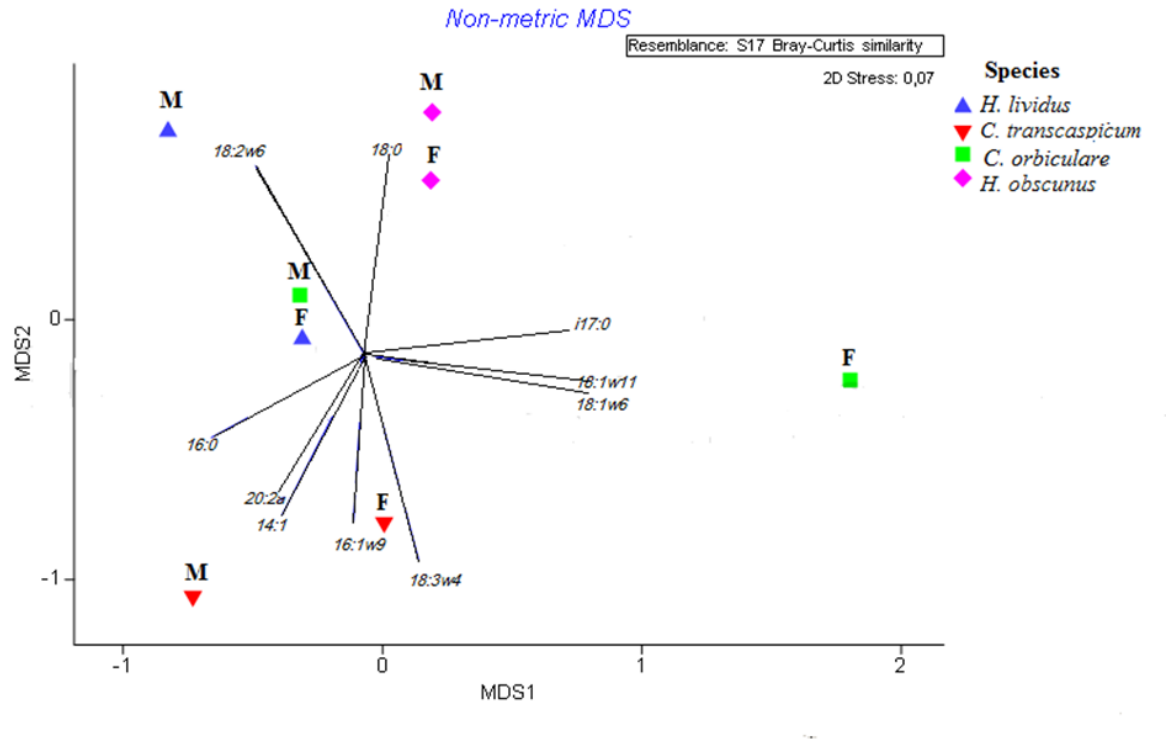

Figure 1. Proportions of fatty acid of the insect samples in a MDS (Multi Dimensional Configuration) plot. Pearson correlations, >0.65, 2D stress 0.07; F: female; M: male.

We found that the average similarity in fatty acid of all female samples in all the species was 74% within gender. The fatty acids that contributed most to this similarity were palmitic acid (24%), palmitoleic acid (20%) and oleic acid (17%), whereas the fatty acids with the least contribution were i17:0, 17:0 and 15:1. Fatty acid similarity rate of all the males in all the species was found to be 75% within the gender. The fatty acids that contributed most to this similarity were palmitic acid (24%), oleic acid (20%) and palmitoleic acid (16%), whereas the fatty acids with the least contribution were 20:2, 20:3n-3 and 22:1n-11. EPA amount in the females was 3.82% whereas that of the males was 4.58%.

Similarity of males and females was found as 76% between genders. Oleic acid (12%), linoleic acid (11%) and 18:1n-11 (11%) had the highest effect on this similarity, whereas 20:1n-9 had the least effect. ANOSIM global R value showed that gender is not statistically significant in fatty acid composition ($R=0.08$, $p < 0.01$).

Average similarity of *Helochares* was found to be 78% within the genus. Palmitic acid (29%), oleic acid (19%), and palmitoleic acid (19%) had the highest effect on this similarity, whereas 18:3n-4, 18:2, 17:1 fatty acid had the least effect. Fatty acid similarity of *Coelostoma* was found to be 75% within the genus. Palmitic acid (22%), oleic acid (18%), and palmitoleic acid (17%) had the highest effect on the similarity, whereas 16:1n-11, myristoleic acid and 17:1 had the least effect.

Average similarity in fatty acids of *Helochares* and *Coelostoma* genera was 74% between the species. ANOSIM Global R value revealed that there was a partial difference between these species ($R=0.17$, $p < 0.05$).

Palmitic acid, oleic acid and palmitoleic acid contributed the highest on the similarity in *H. lividus, C. transcaspicum* and *C. orbiculare*. However, linoleic acid replaced of palmitoleic acid different from the others with a contribution of 15% in *H. obscurus*.

When we compared to fatty acid composition of *H. lividus* and *C. transcaspicum*, the average similarity between oleic acid and linoleic acid compositions of these species was 73% and the fatty acids with the highest contribution were oleic acid (21%) and stearic acid (15%).

The average similarity was 76% between *H. lividus* and *C. orbiculare* with palmitic acid (12%) and 18:1n-11 (21%) making the highest contribution. Palmitic acid and oleic acid had a 15% and 13% contribution to fatty acid composition.

It was found that 18:1n-9 and 18:1n-11 fatty acids made a 19 and 18% contribution to fatty acid composition similarity between *C. transcaspicum* and *C. orbiculare*, respectively.

When we compared to fatty acid composition of *C. transcaspicum* and *H. obscurus*, linoleic acid had the highest contribution with 17%, followed by palmitic acid (12%) and palmitoleic acid (12%).

When were compared, oleic acid had highest contribution with 19%, followed by linoleic acid (12%) and palmitoleic acid (12%) in *H. obscurus* and *C. orbiculare*. ANOSIM global R values revealed that the difference between species was more significant compared to other factor groups ($R=0.63$, $p < 0.01$).

In all species, terrestrial fatty acids predominate. However, it is observed that they are in a higher percentage in Acidocerinae subfamily species than in other species. Fatty acids of terrestrial origin are found in *H. lividus* and *H. obscurus* from the Acidocerinae subfamily at a rate of 17.0 and 16.4%, respectively. However, it is determined that they are also at significant levels in the Sphaeridinae subfamily species. In female *C. transcaspicum* and *C. orbiculare* samples of the Sphaeridinae subfamily, this rate was found to be 4.52 and 8.06%, respectively. When total lipid amounts were compared between species, rates ranging from 12.1 to 48.4% were observed. When *H. lividus* was compared with *H. obscurus*, the highest lipid content was found in *H. lividus*, whereas when male and female samples were compared, the

highest lipid content was again found in males of *H. lividus* with 48%. When *C. transcaspicum* was compared with *C. orbiculare*, the total lipid amount was found to be highest in *C. orbiculare* with 32.3%, and when male and female samples were compared, the highest lipid amount was found in females of *C. orbiculare* with 19.9% (Table 4).

Table 4. Total lipid amount of species according to gender (*Helochares*: 23♂, 24♀; *Coelostoma*: 27♂, 28♀)

	Total lipid (%)	
	♀	♂
Helochares lividus	12.1	48.4
Helochares obscurus	-	-
Coelostema transcaspicum	28.4	32.0
Coelostema orbiculare	19.9	32.3

Discussion

In this study, total lipid and fatty acid composition among (*Helochares* and *Coelostoma)* genera and species of Hydrophilidae (Coleoptera) family collected from Bingöl Province. Our findings indicate that percentage of total lipids differs between genders. This difference is lowest in the males and females of C*. transcaspicum*, and highest in males and females of *H. lividus*. Çakmak et al. (2007) found that total lipid was higher in males than in females. The reason of total lipid levels in female specimens the lower than male may be that females use lipids as a substitute food source for eggs during oogenesis (Ziegler & Antwerpen, 2006). Also, it may be an adaptation mechanism of male insects against low temperature since female insects are reported to be more resistant to low temperatures (Uçkan & Gülel, 2001). In insects, metabolic activities (fatty acid and total fat) vary according to species, gender, nutrition and the habitat (Nurullahoğlu et al., 2004; Çakmak et al., 2007).

A total of 25 fatty acids, consisting of nine saturated, eight monounsaturated and eight polyunsaturated fatty acids, were identified in the females of *H. lividus*. In the males of this species, seven saturated, seven monounsaturated, seven polyunsaturated and a total 21 fatty acids were identified. In the females of *H. obscurus*, a total of 17 fatty acids, consisting of six saturated, five monounsaturated, and six polyunsaturated fatty acids were identified.

High rates of palmitic, palmitoleic, stearic, oleic, arachidonic, linoleic and linolenic acids were detected in the specimens. These findings are consistent with the results obtained for other insect groups (Stanley-Samuelson& Dadd, 1983; Thompson, 1973). Based on our analyses, it was found that the major fatty acids were palmitic acid (16.7-24.8%) and oleic acid (9.51-24.9%). Oleic acid is used in growth and as an energy source in insect groups (Dadd, 1973). Therefore, its levels are generally high in all insects.

Hoback et al. (1999) indicated that adult males and females *of Magicicada septendecim* (Linnaeus, 1758) had similar fatty acid profiles, and both saturated and unsaturated C16 and C18 fatty acids were dominant. In our study, fatty acid composition of males and females did not show statistically any significant difference. Only, in our study, the minor differences in oleic and palmitic acid ratios depending gender, which are the dominant fatty acids, may be due to differences in metabolic activities of these fatty acids in males and females. Oleic acid has been shown to be important in egg production in female insects by demonstrating that oleic acid levels in the ovarium increase in *T. molitor* females during sexual maturity (Khebbeb et al., 1997). The difference in the amount of oleic acid between the genders is thought to be caused by the use of oleic acid for egg formation in females or by the conversion of oleic acid to linoleic acid for this purpose (Nurullahoğlu et al., 2004). We think that the clear difference of oleic acid levels between males and females of *C. orbiculare* in our study could be due to the fact that this species was in

the egg laying period and oleic acid was being used as a substitute food or converted into linoleic acid. In the other species, there was no significant difference in oleic acid levels depending on the genders. The females of *Coelostoma* do not carry the egg sac on the abdomen. However, *Helochares* carries the egg sac on the abdomen (Hansen, 1987). According to our results, this situation did not change the fatty acid results in the insects. In our findings, palmitoleic acid (16:1 n-9) was a predominant unsaturated fatty acid. It was at higher levels in the females of *Helochares* whereas it was higher in the males of *Coelostoma*. The highest percentage (21.1%) was detected in the males of *Coelostoma orbiculare*. It has an important role in pheromone synthesis in many insects (Stanley-Samuelson et al., 1988). In various studies on fatty acids in insects, it has been reported that oleic acid is the most abundant fatty acid in all stages (Nurullahoğlu, 2003; Nurullahoğlu et al., 2004; Seven, 2004; Khani et al., 2007; Üstüner et al., 2010).

Studies with vertebrate and invertebrate revealed that C16:1 n-7 fatty acid is generally in low abundance. The fatty acid has been reported to be at high levels in dipterans (Thompson, 1973), some heteropterans (Spike et al., 1991) and diatoms (Kharlamenko et al., 1995). In our study, C16:1 n-7 was low levels. We think that the Hydrophilidae family can obtain this compound from the algae that constitute their food and from the 16:0 fatty acid. Linoleic acid was significantly higher in *Helochares* than in *Coelostoma*. However, it was significantly lower in *Coelostoma* than in *Helochares*. The fatty acid has been reported to have beneficial effects on human health, such as lipid lowering effects, boosting the immune system, anticarcinogenic, antidiabetogenic and antiatherogenic properties.

Invertebrate and vertebrates synthesize fatty acids up to 18:1n-9, and take 18:2n-6 essential fatty acids with two double bonds and 18:3n-3 essential fatty acids with three double bonds from external nutrients. They synthesize arachidonic acid (ARA, 20:4n-6) and eicosopentanoic acid (EPA, 20:5n-3) from these essential components taken from diets. Especially, prostaglandins members of 20:4n-6 to 20:5n-3 fatty acids eicosanoids have important functions such as reproduction and nodulation in insects (Stanley & Howard, 1998).

Fatty acids 18:2n-6 and 18:3n-3 and their metabolites are precursors of eicosanoids, which are important for insect physiology. Reproduction, cellular immunity and thermoregulation are known to be affected by these eicosanoids (Bozkuş, 2003; Stanley, 2006). Insects can convert 18C polyunsaturated fatty acids to 20C polyunsaturated fatty acids by biochemical processes as all living groups. ARA and EPA are 20C polyunsaturated fatty acids and have a biological importance. They are presented in higher levels in aquatic insects (Stanley-Samuelson et al., 1988). ARA and EPA are presented in aquatic insects higher levels than terrestrial insects. The situation is a result of adaptation mechanism to aquatic environments (Stanley-Samuelson et al., 1988). In this study, the amount of ARA and EPA were high (3.21 and 3.43%, respectively). It was thought that these fatty acids are responsible for immunity against infections and balancing of body temperature. Furthermore, ARA that is synthesized by the elongation and desaturation system from linoleic acid and linoleic acid taken from diets. It was reported that ARA is presented little amounts in some higher terrestrial plants (Shinmen et al., 1991; Shanab et al., 2018) and the major supply of ARA is aquatic organisms (Suloma et al., 2007) especially lower aquatic plants-microalgae (Shanab et al., 2018). Even fishes only heap them by the intake of PUFA-rich microalgae through food-chain (Sayanova & Napier, 2011). Mammals including humans cannot synthesize ARA directly due do the genetic absence of some of its biosynthesis enzymes (Ouyang et al., 2013). The presence of ARA in insects indicates that the insect can synthesize arachidonic acid (Çakmak et al., 2005). However, we think that ARA is of dietary origin because it is found in both the aquatic and terrestrial insects. Çakmak et al. (2005) studied terrestrial insects, so they obtained a different result. Additionally, in our study, it was observed that ARA was present in similar levels in both *Helochares* and *Coelostoma*.

It was observed that the percent distribution of linolenic (18:3-n3) acid, the precursor of eicosanoids and prostaglandins, ranged from 1.46 to 5.46%. This fatty acid is less than 1% in omnivorous insects (Baldus & Mutchmor, 1988). This ratio was found to be higher than 1% in our study, suggesting that although these insect groups are omnivorous, they mainly feed phytophagous, or it may also be related to the fact that these are aquatic insects (Stanley-Samuelson et al., 1990, 1991). In addition, the presence of terrestrial fatty acids in all insect species examined in this study may also indicate that these insects also feed on diets with terrestrial origin. Furthermore, fatty acid results also indicate that bacterial feeding is also at a considerable level (Table 2). Although the majority of Hydrophilidae are aquatic, there are also species living in semiaquatic and terrestrial habitats (Hansen, 1987).

Saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) composition of *Helochares* were found to be similar. It was also found to be a good PUFA source. In the adults of *Coelostoma*, MUFA percentage was higher than SFA. Kalyoncu & Özge (2014), reported similar results showing that MUFA percentage in adult insects was higher than that of SFA. In addition, PUFA percentage in *Coelostoma* was higher in male insects.

In conclusion, within the factor groups (genus, family, subfamily, gender and species) analyzed in the individuals of the Hydrophilidae family in terms of fatty acid composition. ANOSIM results revealed that only gender difference was found to be insignificant for fatty acid composition (R=0.08), whereas differences in subfamilies (R=0.17) and genera (R=0.17) were partially significant, and the difference between species was more significant compared to other factor groups (R=0.63). Furthermore, it was determined that fatty acids cannot be an important biochemical parameter for identifying species in the taxonomy of the Hydophilidae family. This results not valid for all Hydophilidae family. The results include only the studied species of the family. It was determined that the fatty acid composition showed over 70% similarity at the level of these genera and species. The partial difference found in different genders of the same species can be attributed to the regulation of total lipid and fatty acid metabolism according to changing needs.

Acknowledgments

This research was supported by the BAP-281-265-2015 project of Bingöl University.

References

Angus, R. B., 1992. Süsswasser Fauna von Mitteleuropa (Insecta: Coleoptera: Hydrophilidae: Helophorinae). Gustav Fischer Verlag, Jena, New York, 144 pp.

Baldus, T. J. & J. A. Mutchmor, 1988. The effects of acclimation and post-treatment temperature on the toxicity of allethrin to the American cockroach, *Periplaneta americana*. Comparative Biochemistry and Physiology Part C: Comparative Pharmacology, 89 (2): 403-407.

Başhan, M., 1996. Effect of various diets on the total lipit compositions the black cricket *Melanogrillus desertus* Pall. Turkish Journal of Zoology, 20 (4): 375-379.

Beenakkers, A. M. T., D. J. Van Der Horst & J. A. Van Marrewijk, 1985. Insect lipids and lipoproteins and their role in physiological processes. Progress in Lipid Research, 24 (1): 19-67.

Bozkuş, K., 2003. Phospholipid and triacylgliserol fatty acid compositions from various development stages of *Melanogryllus desertus*. Turkish Journal of Biology, 27 (2): 73-78.

Çakmak, Ö., M. Başhan & H. Bolu, 2005. *Monosteira lobulifera* Reut. (Heteroptera: Tingidae)' nın fosfolipid ve triaçilgliserol fraksiyonundaki yağ asiti bileşimi. Fırat Üniversitesi Fen ve Mühendislik Bilimleri Dergisi, 17 (4): 637-643.

Çakmak, Ö., M. Başhan & A. Satar, 2007. Total fatty acid composition of *Lertha sheppardi* (Neuroptera: Nemopteridae) during its main life stages. Biologia, 62 (6): 774-780.

Canavoso, L. E., Z. E. Jouni, K. J. Karnas, J. E. Pennington & M. A. Wells, 2001. Fat metabolism in insects. Annual Review of Nutrition, 21 (1): 23-46.

Christie, W. W., 1992. Preparation of fatty acid methyl esters. Inform, 3 (9): 1031-1034.

Clarke, K. R. & R. M. Warwick, 2001. Change in Marine Communities: An Approach to Statistical Analysis and Interpretation, 2nd Edition. PRIMER-E, Plymouth, 151 pp.

Cohen, A. C., 1990. Fatty acid distributions as related to adult age, sex and diet in the phytophagous Heteropteran, *Lygus hesperus* (Heteroptera: Miridae). Journal of Entomological Science, 25 (1): 75-84.

Dadd, R. H., 1973. Insect nutrition: Current development and metabolic implications. Annual Review of Entomology, 18: 381-420.

Darılmaz, M. & S. Kıyak, 2006a. *Helochares lividus*: New Distributional Records from Turkey (Coleoptera: Hydrophilidae). Entomological Problems, 36 (1): 79.

Darılmaz, M. & S. Kıyak, 2006b. A contribution to the knowledge of the Turkish water beetles fauna (Coleoptera). Munis Entomology and Zoology, 1 (1): 129-144.

Demirsoy, A., 1997. Yaşamın Temel Kuralları, Omurgasızlar/Böcekler, Entomoloji Cilt II/Kısım II, Beşinci Baskı. Meteksan Matbaacılık, Ankara, 941 s.

Downer, R. G. H. & J. R. Matthews, 1976. Patterns of lipid distribution and utilization in insects. American Zoologist, 16 (6): 733-745.

Fikáček, M., 2006. Taxonomic status of *Cercyon alpinus, C. exorabilis, C. strandi* and *C. tatricus* and notes on their biology (Coleoptera: Hydrophilidae: Sphaeridiinae). Annalen des Naturhistorischen Museums in Wien, 107B: 145-164.

Gentili, E., 2000. Distibuzione del genere *Laccobius* (Coleoptera: Hydrophilidae) in Anatolia e Problemi Relativi. Biogeographia-The Journal of Integrative Biogeography, 21 (1): 173-214.

Gentili, E. & P. E. Whitehead, 2000. A new species of *Laccobius* (Col., Hydrophilidae) from Lycia, Turkey. The Entomologist's Montly Magazine, 136: 73-76.

Gilbert, L. I., 1967. Lipid metabolism and function in insect. Advances in Insect Physiology, 4: 69-211.

Gilby, A. R., 1965. Lipids and their metabolism in insects. Annual Review of Entomology, 10: 141-160.

Hansen, M., 1987. The Hydrophilidae (Coleoptera) of Fennoscandia and Denmark. Fauna Entomologica Scandinavica, 18: 1-253.

Hara, A. & N. S. Radin, 1978. Lipid exctraction of tissues with a low-toxicity solvent. Analytical Biochemistry, 90 (1): 420-426.

Hoback, W. W., R. L. Rana & D. W. Stanley, 1999. Fatty acid compositions of phospholipids and triacylglycerols of selected tissues, and fatty acid biosynthesis in adult periodical cicadas, *Magicicada septendecim*. Comparative Biochemistry and Physiology Part A, 122 (3): 355-362.

İncekara, Ü., A. Mart & O. Erman, 2005. Some Notes on two newly recorded aquatic Coleoptera (Hydrophilidae, Helophoridae) species from Turkey. Fırat Üniversitesi Fen ve Mühendislik Bilimleri Dergisi, 17 (2): 449-454.

Kalyoncu, L. & S. Özge, 2014. *Plodia interpunctella* (Hubner) (Lepidoptera: Pyralidae)'nın farklı gelişim evrelerinin yağ asiti bileşimi. Selçuk Üniversitesi Fen Fakültesi Fen Dergisi, 38: 10-18.

Keeley, L. L., J. H. Park, K. H. Lu & J. Y. Bradfield, 1996. Neurohormone signal transduction for dual regulation of metabolism and gene expression in insects: hypertrehalosemic hormone as a model. Archives Insect Biochemistry Physiology, 33: 283-301.

Khani, A., S. Moharramipour, M. Barzegar & H. Naderi-Manesh, 2007. Comparison of fatty acids composition in total lipid of diapause and non-diapause larvae of *Cydia pomonella* (Lepidoptera: Tortricidae). Insect Science, 14 (2): 125-131.

Kharlamenko, V. I., N. V. Zhukova, S. V. Khotimchenko, V. I. Svetashev & G. M. Kamenev, 1995. Fatty acids as markers of food sources in a shallow water hydrothermal ecosystem (Kraternaya Bight, Yankich Island, Kurile Islands). Marine Ecology Progress Series, 120: 231-241.

Khebbeb, M. E. H., J. Delachambre & N. Soltani, 1997. Lipid metabolism during the sexual maturation of the mealworm (*Tenebrio molitor*): effect of ingested diflubenzuron. Pesticide Biochemistry and Physiology, 58 (3): 209-217.

Kıyak, S., S. Canbulat, A. Salur & M. Darılmaz, 2006. Additional notes on aquatic Coleoptera fauna of Turkey with a new record (Helophoridae: Hydrophilidae). Munis Entomology and Zoology, 1 (2): 273-278.

Mart, A., 2005. Bingöl İli Helophoridae, Hydrophilidae ve Hydrochidae (Coleoptera) Türleri Üzerine Sistematik Araştırmalar, (Basılmamış) Doktora Tezi, Atatürk Üniversitesi Fen Bilimleri Enstitüsü, Erzurum, Türkiye, 165 s.

Mart, A., 2009. Water scavenger beetles (Coleoptera: Hydrophilidae) provinces of Central Black Sea Region of Turkey. Journal of the Entomological Research Society, 11 (1): 47-70.

Nurullahoğlu, Z. Ü., 2003. *Achroia grisella* F. (Lepidoptera: Pyralidae) larva ve pupunun yağ asiti bileşimi. Selçuk Üniversitesi Fen-Edebiyat Fakültesi Fen Dergisi, 21: 75-78.

Nurullahoğlu, Z. Ü., F. Uçkan, O. Sak & E. Ergin, 2004. Total lipid and fatty acid composition *of Apanteles galleria* and its parasitized Host. Annals of the Entomological Society of America, 97 (5): 1000-1006.

Ouyang, L. L., S. H. Chen, Y. Li & Z. G. Zhou, 2013. Transcriptome analysis reveals unique C4- like photosynthesis and oil body formation in an arachidonic acid-rich microalga *Myrmecia incisa* Reisigl H4301. BMC Genomics, 14 (1): 1-13.

Pethybridge, H., R. K. Daley & P. D. Nichols, 2011. Diet of demersal sharks and chimaeras inferred by fatty acid profiles and stomach content analysis. Journal of Experimental Marine Biology and Ecology, 409 (2): 290-299.

Sayanova, O. & J. A. Napier, 2011. Transgenic oilseed crops as an alternative to fish oils. Prostaglandins Leukot Essent Fatty Acids, 85 (5): 253-260.

Seven, E., 2004. *Plodia interpunctella* (Lepidoptera: Pyralidae) Larva ve Pupunun Total Lipid, Total Yağ Asiti Ve Yağ Asiti Bileşimi. Selçuk Üniversitesi Fen Bilimleri Enstitüsü, (Basılmamış) Yüksek Lisans Tezi, Konya, 25 s.

Shanab, S. M. M., R. M. Hafez & A. S. Fouad, 2018. A review on algae and plants as potential source of arachidonic acid. Journal Advanced Research, 11: 3-13.

Shinmen, Y., K. Katoh, S. Shimizu, S. Jareonkitmongkol & H. Yamada, 1991. Production of arachidonic acid and eicosapentaenoic acids by *Marchantia polymorpha* in cell culture. Phytochemistry, 30 (10): 3255-3260.

Short, A. E. Z. & M. Fikácek, 2013. Molecular phylogeny, evolution and classification of the Hydrophilidae (Coleoptera). Systematic Entomology, 38: 723-752.

Spike, B. P., R. J. Wright, S. D. Danielson & D. W. Stanley-Samuelson, 1991. The fatty acid compositions of phospholipids and triacylglycerols, from two chinch bug species *Blissus leucopterus leucopterus* and *B. iowensis* (Insecta; Hemiptera; Lygaeidae) are similar to the characteristic dipteran pattern. Comparative Biochemistry and Physiology, 99 (4): 799-802.

Stanley-Samuelson, D. W. & R. H. Dadd, 1983. Long-chain polyunsaturated fatty acids: Patterns of occurrence in insects. Insect Biochemistry, 13 (5): 549-558.

Stanley, D. W. & R. W. Howard, 1998. The biology of prostaglandins and related eicosanoids in invertebrates: Cellular organismal and ecological actions. American Zoologist, 38 (2): 369-381.

Stanley-Samuelson, D. W., R. W. Howard & E. C. Toolson, 1990. Phospholipid fatty acid composition and arachidonic acid uptake and metabolism by the cicada *Tibicen dealbatus* (Homoptera: Cicadidae). Comparative Biochemistry and Physiology, 97 (2): 285-289.

Stanley-Samuelson, D. W., E. Jenson, K. W. Nickerson, K. Tiebel, C. L. Ogg & R. W. Howard, 1991. Insect immune response to bacterial infection is mediated by eicosanoids. Proceedings of the National Academy of Sciences of the United States of America, 88 (3): 1064-1068.

Stanley-Samuelson, D. W., R. A. Jurenka, C. Cripps, G. J. Blomquist & M. Derenobles, 1988. Fatty acids in insects: composition, metabolism, and biological significance. Archives of Insect Biochemistry and Physiology, 9 (1): 1-33.

Stanley, D. W., 2000. Eicosanoids in invertebrate signal transduction systems. Princeton University Press, Princeton, N.J., USA, 277 pp.

Stanley, D., 2006. Prostaglandins and other eicosanoids in insects: Biological significance. Annual Review of Entomology, 51 (1): 25-44.

Suloma, A., H. Y. Ogata, H. Furuita, E. S. Garibay & D. R. Chavez, 2007. "Arachidonic Acid Distribution in Seaweed, Seagrass, İnvertebrates and Dugong in Coral Reef Areas in The Philippines, 107-111". In: Sustainable Production Systems of Aquatic Animals in Brackish Mangrove Areas (Ed. K Nakamura). Japan International Research Center for Agricultural Sciences Working Report Tsukuba, Ibaraki, Japan, 151 pp.

Thompson, S. N., 1973. A review and comparative characterization of the fatty acid compositions of seven insect orders. Comparative Biochemistry and Physiology, 45 (2): 467-482.

Thompson, S. N., 1979. The effect of dietary carbohyrate on larval development and lipogenesis in the parasite, *Exeristes robarator* (Fabricius (Hymenoptera: Ichneumonidae. Journal of Parasitology, 65 (6): 849-854.

Uçkan, F. & A. Gülel, 2001. The effects of cold storage on the adult longevity, fecundity and sex ratio of *Apanteles galleria* Wilkinson (Hym: Braconidae). Turkish Journal of Zoology, 25 (3): 187-191.

Üstüner, P., L. Kalyoncu & A. Aktümsek, 2010. Besinin *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) larva ve pupunun toplam lipid, yağ asiti oranlarına ve yağ asiti bileşimine etkileri. Süleyman Demirel Üniversitesi Fen Dergisi, 5 (1): 29-37.

Wakayama, E. J., J. E. Dillwith & G. J. Blomquist, 1980. In vitro biosynthesis of prostaglandins in the reproductive tissues of the male house fly *Musca domestica* (L.). American Zoologist, 20: 904.

Ziegler, R. & R. V. Antwerpen, 2006. Lipid uptake by insect oocytes. Insect Biochemistry and Molecular Biology, 36 (4): 264-272.

Türk. entomol. derg., 2020, 44 (3): 413-422
DOI: http://dx.doi.org/10.16970/entoted.715181

ISSN 1010-6960
E-ISSN 2536-491X

Original article (Orijinal araştırma)

New records and some new distribution data for the Turkish Nematinae (Hymenoptera: Symphyta: Tenthredinidae) fauna

Türkiye Nematinae (Hymenoptera: Symphyta: Tenthredinidae) faunası için yeni kayıtlar ve bazı yeni dağılımlar

Önder ÇALMAŞUR[1*]

Abstract

Ten species of the subfamily Nematinae of the Tenthredinidae (Hymenoptera: Symphyta) are recorded for the first time from Turkey: *Cladius compressicornis* (Fabricius, 1804), *Craesus latipes* (Villaret, 1832), *Dineura testaceipes* (Klug, 1816), *Euura annulata* (Gimmerthal, 1834), *Euura leucapsis* (Tischbein, 1846), *Euura obducta* (Hartig, 1837), *Euura oligospila* Förster, 1854, *Nematus crassus* Eschscholtz, 1822, *Pristiphora pallidiventris* (Fallen, 1808), and *Pseudodineura fuscula* (Klug, 1816). New distribution data are given for a further six species. The specimens were collected during 2000-2017 in Northeast Anatolia Region by the author. The number of species in the Turkish Symphyta fauna has risen to 370 with these new records.

Keywords: Fauna, Hymenoptera, new record, Tenthredinidae, Turkey

Öz

Nematinae (Hymenoptera: Symphyta: Tenthredinidae) altfamilyasına ait 10 tür: *Cladius compressicornis* (Fabricius, 1804), *Craesus latipes* (Villaret, 1832), *Dineura testaceipes* (Klug, 1816), *Euura annulata* (Gimmerthal, 1834), *Euura leucapsis* (Tischbein, 1846), *Euura obducta* (Hartig, 1837), *Euura oligospila* Förster, 1854, *Nematus crassus* Eschscholtz, 1822, *Pristiphora pallidiventris* (Fallen, 1808) ve *Pseudodineura fuscula* (Klug, 1816) Türkiye için yeni kayıt olarak belirlenmiştir. Altı tür için de yeni dağılım bilgileri verilmiştir. Örnekler 2000-2017 yılları süresince yazar tarafından Kuzey Doğu Anadolu Bölgesi'nden toplanmıştır. Bu türlerle birlikte Türkiye Symphyta faunası 370 türe ulaşmıştır.

Anahtar sözcükler: Fauna, Hymenoptera, yeni kayıt, Tenthredinidae, Türkiye

[1] Atatürk University, Faculty of Agriculture, Department of Plant Protection, 25240, Erzurum, Turkey
* Corresponding author (Sorumlu yazar) e-mail: calmasur@atauni.edu.tr
Received (Alınış): 06.04.2020 Accepted (Kabul ediliş): 23.06.2020 Published Online (Çevrimiçi Yayın Tarihi): 30.07.2020

Introduction

Turkey has a rich and varied fauna and flora because of its diverse topography and climatic conditions. Turkey's location at the junction of the Euro-Siberian, Mediterranean and Turanian zoogeographic regions, and the fact that it is a crossroad between the continents of Europe, Asia and Africa, only adds to this richness. One of the most remarkable regions of the world is characterized by the major extension of biodiversity areas such as the Caucasus, Irano-Anatolian and Mediterranean Basin into Turkey (Myers et al., 2000).

The order Hymenoptera is one of the most diverse and speciose groups of insects and includes about 153,000 species world-wide according to Aguiar et al. (2013), or more than 250,000 described species according to Gullan & Cranston (2010).

The Symphyta (Hymenoptera) are structurally primitive. Most families include relatively few taxa, with a total of 812 genera and 8630 species in the world (Taeger et al., 2018). There have been 360 determined symphytan species in Turkey (Çalmaşur, 2019). Symphytans may be recognized by the absence of a marked constriction between the first abdominal segment and second, and by the existence of at least one closed anal cell in the forewing. The larvae have a well-developed head capsule and most have thoracic legs; the majority of free-feeding larvae are caterpillar-like with abdominal prolegs. The larvae of Orussidae are not phytophagous (Gauld & Bolton, 1988; Goulet & Huber, 1993). The Tenthredinoidea superfamily is the most well-known of Symphyta, containing 7614 species in the nine families (Argidae, Blasticotomidae, Cimbicidae, Diprionidae, Electrotomidae, Pergidae, Tenthredinidae, Xyelidae and Xyelotomidae) (Taeger et al., 2018). The Tenthredinidae is the best represented family in the northern temperate regions; species wealth tends to increase towards the north. There are more than 6000 species in 360 genera in temperate regions of the Northern Hemisphere (Liston, 1995; Goulet & Huber, 1993; Taeger & Blank, 1998; Lacourt, 1999). The Tenthredinidae are commonly called sawflies; it is the biggest family of the nine (Comstock, 1964), and it includes 415 genera, 5721 species (Taeger et al., 2018).

The first extensive work on Turkey's sawfly fauna was achieved by Guichard & Harvey (1967). Benson (1966, 1968) studied the fauna of Turkish Symphyta, and described new taxa and constructed keys to some genera and species take place in Turkey. The first more recent contributions to data on Turkish sawflies fauna were presented by Çalmaşur & Özbek (2004a, b).

In recent years, several studies have been published on Turkish sawflies and a number of new records have been reported (Çalmaşur, 2006, 2011, 2019; Çalmaşur & Özbek, 2004c, 2006a, b; Hastaoğlu Örgen & Başıbüyük, 2006; Korkmaz et al., 2010a, b).

In this study are reported ten species of sawflies for the first time from Turkey. These species records combined with data from the literature reveal that the total number of symphytan species is well above 370 in Turkey.

Materials and Methods

Materials

Specimens included were collected during 2000-2017 in various localities of Turkey by the author. Although the specimens have been collected in different parts of the country, the majority of the sawfly samples were collected from eastern Turkey. Material collected by insect net. Samples are deposited in EMET (Entomology Museum Erzurum, Turkey).

Methods

For determination of the material, Benson (1952) and Zhelochovtsev (1988) were used. Undetermined specimens were identified and others confirmed by Dr. David R. Smith. Images of samples were taken with a Leica MZ 16 A with computer. The genera and species are listed in alphabetic order and global distribution data organized according to Taeger et al. (2018). All records are arranged in the following way at material examined, province, town, local place, geographic name and altitude (if available), date of collecting, number of individuals as male and female. New records of species are marked by an asterisk (*).

Results

Nematinae

******Cladius (=Priophorus) compressicornis* **(Fabricius, 1804)**

Mouthparts normal; maxillary palp longer than cardo with stipes, and articles 3 and 5 the longest. Labial palps about twice as long as ligula. Clypeus subtruncate in front, very narrow. Malar space only about half as long as distance between antennal sockets. Inter-antennal crest very prominent, medially carinate. Antenna in female subcylindrical and about as long as costa and stigma of forewing; in male laterally compressed (so that third segment is scarcely four times longer than broad) with each segment produced at the apex beneath, as long as body with head. Wing venation as in *Pristiphora*. Claws with swollen basal lobe and an inner tooth, well separated from end tooth and longer than it. Body length: 5-8 mm (Figures 1a, 2a, 3a).

Global distribution: Canada, China, Europe, Japan, Russia, USA.

Distribution in Turkey: new for Turkish fauna.

Material examined: Rize, Çamlıhemşin, Ayder, 1200-1550 m, 30.VII.2000, ♀.

Host plants: unknown.

Cladius (=Trichiocampus) grandis **(Serville, 1823)**

Antenna brownish; head black; labial and maxillary palpi orange. Thorax orange with pectus, cervical sklerites, median portion of pronotum, and all of mesonotum and metanotum black. Abdomen and legs entirely orange. Wings hyaline; costa orange; stigma black; veins brownish. Average length: 8-9 mm (Figures1b, 2b, 3b).

Global distribution: Canada, Europe, Russia, Turkey, USA.

Distribution in Turkey: Antalya, Bursa, Edirne, Erzurum, Eskişehir, İstanbul, İzmit, Karabük, Yozgat (Liston, 1995; Lacourt, 1999).

Material examined: Erzurum, 4. Kuyu, 1850 m, 31.V.2011, ♀.

Host plants: *Populus balsamifera* L.*, P. nigra* L.*, P. tremula* L.*, Salix caprea* L. (Taeger & Blank, 1998).

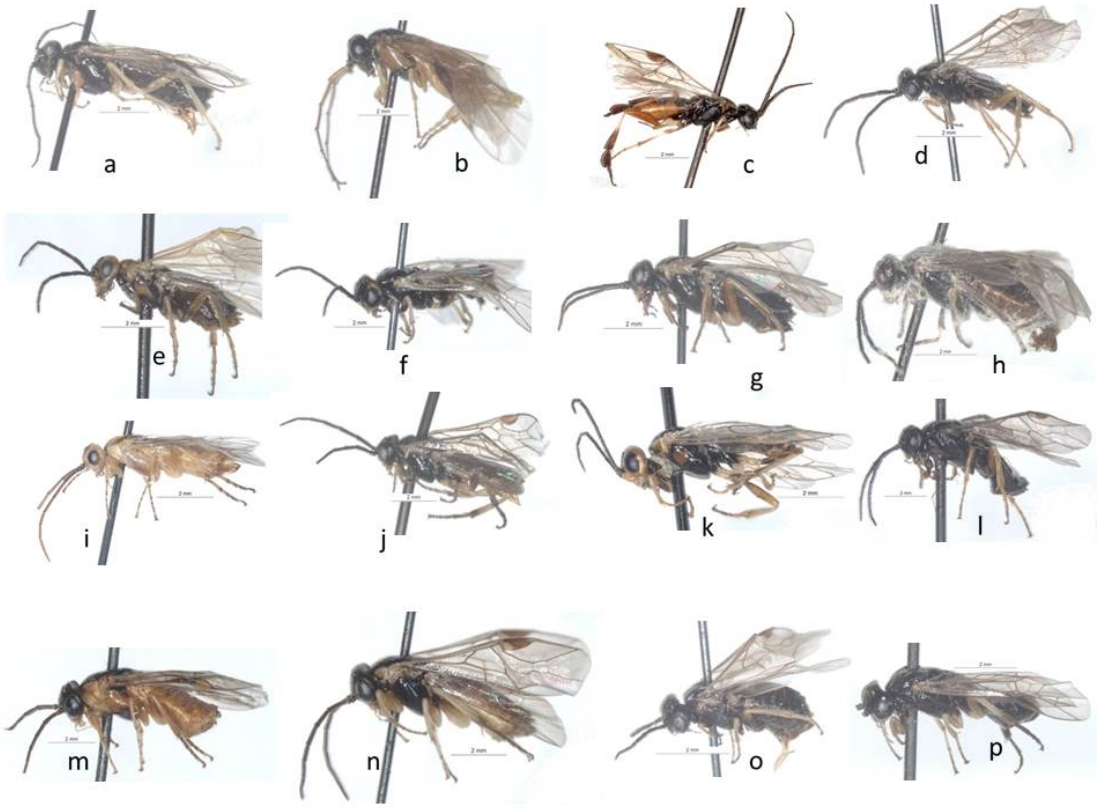

Figure 1. Lateral view of Sawfly species: a) *Cladius compressicornis*; b) *Cl. grandis*; c) *Craesus latipes*; d) *Dineura testaceipes*; e) *Euura annulata*; f) *E. leucapsis*; g) *E. leucosticta*; h) *E. obducta*; i) *E. oligospila*; j) *E. vaga*; k) *Nematus crassus*; l) *Pristiphora araratensis*; m) *Pr. conjugata*; n) *Pr. pallidiventris*; o) *Pseudodineura fuscula*; p) *Stauronematus platycerus.*

******Craesus latipes* **(Villaret, 1832)**

Adults are similar to *C. septentrionalis* (L.) but slightly smaller (7.5-10 mm), with the cloudy apex of the fore wings less extensive and the hind femora of females reddish. Mesopleura wrinkled, without distinct punctation. POL equal or more than OCL (10:0.9-10) (Figures 1c, 2c, 3c).

Larvae black or brownish black, with the legs and underside of the last few abdominal segments pale yellowish in *C. latipes*. Head black; body yellow to blue-green, variably marked with orange-yellow, and with prominent black along the sides in *C. septentrionalis*.

Global distribution: Europe, Russia.

Distribution in Turkey: new for Turkish fauna.

Material examined: Bayburt, Çalıdere, 1850 m, 16.VI.2010, 2 ♂♂.

Host plants: *Betula alba* L.*, B. pendula* L. (Lacourt, 1999).

******Dineura testaceipes* **(Klug, 1816)**

Head black, except for pale labrum, sometimes whole clypeus, and more or less supraclypeal area; antenna from completely black to completely pale. Thorax black: darkest specimens with only tegula and upper posterior corner of pronotum pale; palest specimens with tegula, whole pronotum, broad margins of mesoscutal lobes, anterior of mesoscutellum, and mesepisternum except for ventral quarter pale. Abdomen from nearly completely black, except for pale hypopygium, valvifer 2, and cerci, to extensively pale on all

sterna and downturned lateral parts of terga, with terga 8-10 completely pale; dorsum of terga 1-7 always mainly black. Body length: ♀ 5.0-6.5 mm, ♂ 4.2-5.5 mm (Figures 1d, 2d, 3d).

Global distribution: Europe, Russia.

Distribution in Turkey: new for Turkish fauna.

Material examined: Erzurum, Konaklı Dam Basin, 2100 m, 06.VI.2017, 2 ♂♂.

Host plants: *Crataegus* spp., *Sorbus* spp., *Cotoneaster* spp. (Taeger & Blank, 1998).

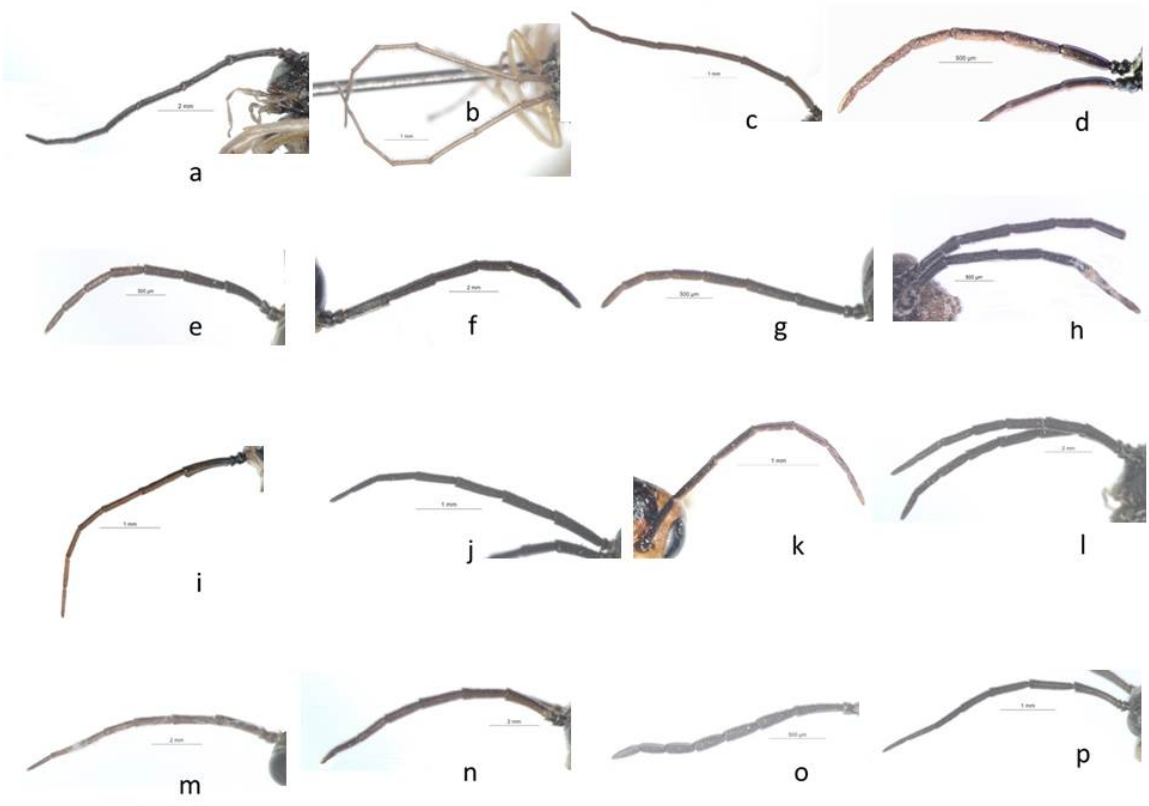

Figure 2. Antenna of Sawfly species: a) *Cladius compressicornis*; b) *Cl. grandis*; c) *Craesus latipes*; d) *Dineura testaceipes*; e) *Euura annulata*; f) *E. leucapsis*; g) *E. leucosticta*; h) *E. obducta*; i) *E. oligospila*; j) *E. vaga*; k) *Nematus crassus*; l) *Pristiphora araratensis*; m) *Pr. conjugata*; n) *Pr. pallidiventris*; o) *Pseudodineura fuscula*; p) *Stauronematus platycerus.*

******Euura annulata* **(=***Pachynematus annulatus)* **(Gimmerthal, 1834)**

On head, only temporoparietal spots ocherous. Pronotal angles usually black. Saw with 3-4 readily noticeable denticles. Third antennal segment shorter than larger ocular diameter (in female 10:12-13). Body length: 3.5-5 mm (Figures 1e, 2e, 3e).

Global distribution: Canada, Europe, Russia, USA.

Distribution in Turkey: new for Turkish fauna.

Material examined: Erzurum, 4. Kuyu, 1850 m, 11.VI.2016, 2 ♀♀.

Host plants: *Rumex obtusifolius* L. (Taeger & Blank, 1998).

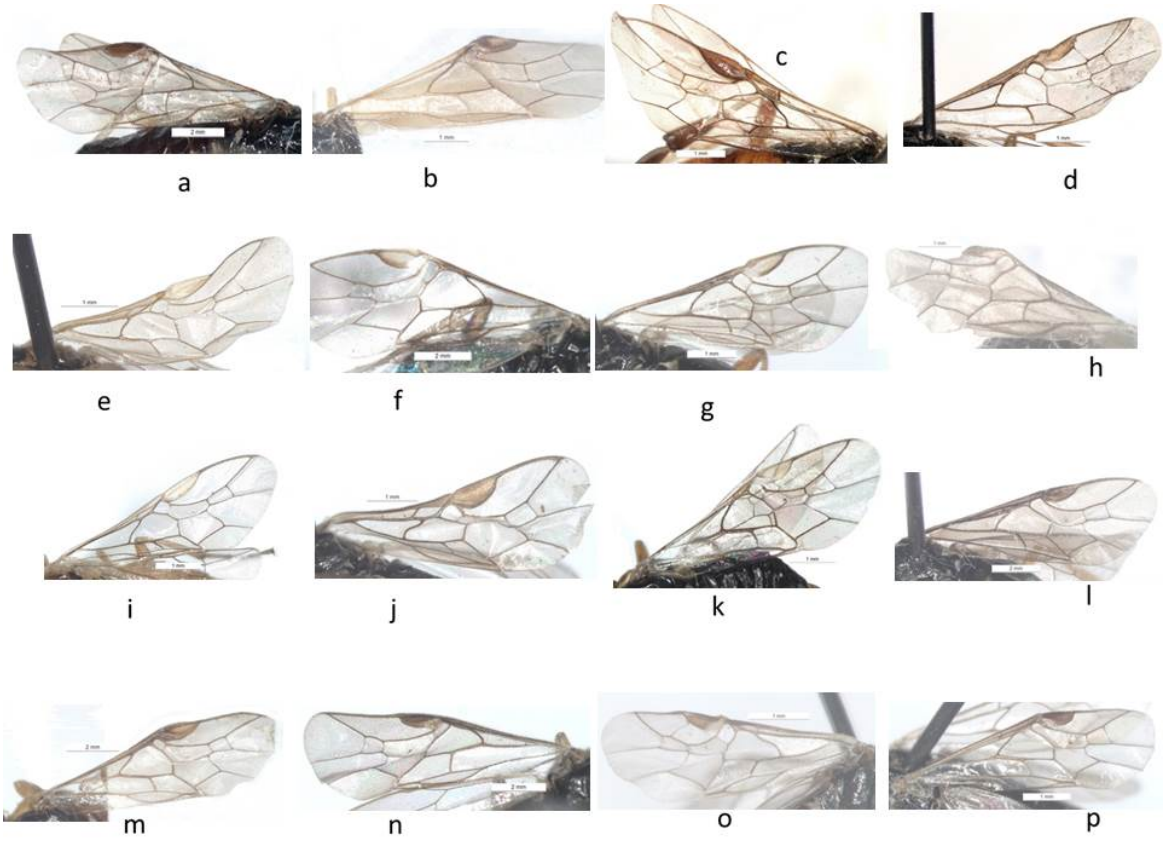

Figure 3. Wing of Sawfly species: a) *Cladius compressicornis*; b) *Cl. grandis*; c) *Craesus latipes*; d) *Dineura testaceipes*; e) *Euura annulata*; f) *E. leucapsis*; g) *E. leucosticta*; h). *E. obducta*; i) *E. oligospila*; j) *E. vaga*; k) *Nematus crassus* l) *Pristiphora araratensis*; m) *Pr. conjugata*; n) *Pr. pallidiventris*; o) *Pseudodineura fuscula*; p) *Stauronematus platycerus.*

******Euura* **(=***Phyllocolpa) leucapsis* **(Tischbein, 1846)**

Head and thorax black with light colored pattern. Antennae in male shorter than C and stigma. Mesopleura black or with small yellow spots on pronotum, only its angles light colored. Hind femora largely black. Body length: 3.5-5 mm (Figures 1f, 2f, 3f).

Global distribution: Europe, Russia.

Distribution in Turkey: new for Turkish fauna.

Material examined: Erzurum, Köprüköy, Örentaş, 1900-2100 m, 12.VI.2001, 2 ♀♀; Oltu, Çamlıbel, 1700 m, 19.VI.2013, 2 ♀♀.

Host plants: *Salix cinerea* L. (Kopelke, 2007).

***Euura leucosticta* (=*Phyllocolpa leucosticta)* (Hartig, 1837)**

Body entirely black, tegulae light colored. Scutellum flat, without punctation. Antennal socket with hairs, matte, inner lines of antennal segments brownish. Inner spur of hind tibia curved. Stigma and legs entirely light colored. Body length: 4.5-5.5 mm (Figures 1g, 2g, 3g).

Global distribution: Algeria, Europe, Russia.

Distribution in Turkey: Gümüşhane (Benson, 1968).

Material examined: Erzurum, Aşkale, Kandilli, Ortabahçe, 1850 m, 04.VI.2015, 10 ♀♀, ♂.

Host plants: *Salix acuminata* Schleich, *S. atrocinerea* Brot.*, S. aurita* L.*, S. caprea* L.*, S. cinerea* L.*, S. pedicellata* Desf. (Taeger & Blank, 1998).

******Euura obducta (=Pachynematus obductus)* **(Hartig, 1837)**

Clypeus like entire head black (but clypeal anterior margin sometimes white), thorax black, only corners of pronotum may be white. Stigma black or dark brown. Hind tarsus shorter than hind tibia. Sheath dark brown to black, apically rounded, process on abdominal tergite VIII. in male markedly projecting, pointed. Body length: 5-6 mm (Figures 1h, 2h, 3h).

Global distribution: Canada, Europe, USA.

Distribution in Turkey: new for Turkish fauna

Material examined: Erzurum, 4. Kuyu, 1850 m, 30.V.2001, ♀.

Host plants: *Carex* sp., *Festuca* sp., *Poa* sp. (Taeger & Blank, 1998).

******Euura oligospila(=Nematus oligospilus)* **(Förster, 1854)**

Body entirely yellowish. Antenna pale yellow, scape and pedicel black, upper surface of flagellum brown. Head with black spot posterior to postocellar area. Mesoscutum with three black stripes, metapostnotum with black medial spots Wings hyaline, costa and stigma yellow. Other veins brownish. Body length: 5-7 mm (Figures 1i, 2i, 3i).

Global distribution: Argentina, Australia, Canada, Chile, Europe, Lesotho, New Zealand, Pakistan, Russia, South Africa, USA.

Distribution in Turkey: new for Turkish fauna.

Material examined: Erzurum, Atatürk University Campus, 1850 m, 19.IX.2003, ♀, 25.VI.2015, ♀; Aziziye, Atlıkonak, 1850 m, 11.VI.2018, ♀; Köprüköy, Örentaş, 2000 m, 12.VI.2001, ♀.

Host plants: *Salix alba* L.*, S. caprea* L.*, S. fragilis* L.*, S. hastata* L.*, S. pentandra* L. (Taeger & Blank, 1998).

***Euura vaga* (=*Pachynematus vagus)* (Fabricius, 1781)**

Process on abdominal tergite VIII in male not large; Clypeus in female black, weakly notched. Body length: 7mm (Figures 1j, 2j, 3j).

Global distribution: Canada, China, Europe, Turkey, USA.

Distribution in Turkey: Sivas (Taeger et al., 2018).

Material examined: Rize, İkizdere, Ovit, 1600-2400 m, 29.VII.2000, ♀.

Host plants: Grasses, *Carex nigra* (L.) (Taeger & Blank, 1998).

******Nematus crassus* **Eschscholtz, 1822 (Det.: by L. Zombori)**

Body thick, head black in the middle, of a nut brown on the sides, parts of the mouth yellow; antennae longer than the half of the body, filiform, black, border of the thorax brown; two longitudinal lines on the thorax, the greater part of the thorax chestnut brown; abdomen convex, shining black; wings longer than the body; legs yellow. Body length: 8-9 mm (Figures 1k, 2k, 3k).

Global distribution: Canada, Europe, Japan, Russia.

Nearctic region (Taeger et al., 2018).

Distribution in Turkey: new for Turkish fauna.

Material examined: Erzurum, Köprüköy, Örentaş, 2000 m,12.VI.2001, ♀.

Host plants: *Populus* sp., *Rumex obtusifolius* L., *Salix fragilis* L. (Taeger & Blank, 1998).

***Pristiphora araratensis* Haris, 2006**

Female. Black, including antennae, mouthparts and palpi. All coxae and trochanters, basal fifth of hind femur, basal third of middle and fore femora black. Apical three segments of tarsi darkened. Other parts of legs: the remaining parts of femora, entire tibiae, first and second segments of tarsi ochraceous. Venation black. Costa, subcosta and stigma dark brown. Wings dark infuscate (darkest in the genus *Pristiphora).* Head moderately densely and moderately deeply punctured, shiny. Frontal area not marked. Distance of the hind ocellus from the hind margin of the head about as long as the diameter of an ocellus. Mesonotum, mesoscutellum, mesoscutellar appendage and mesopleuron strongly shiny, hardly, sporadically, superficially and shallowly punctured. Claws with small inner tooth. Sawsheath emarginated in dorsal view. Body length: 6.0 mm (Figures 1l, 2l, 3l).

Holotype: female, Turkey, Kars, Mts. Ararat, 04. 06. 1989, leg. Podlussanyi. The holotype is deposited in the Hungarian Natural History Museum, Budapest.

Global distribution: Turkey.

Distribution in Turkey: Ağrı (Ağrı Mt) (Haris, 2006).

Material examined: Bingöl, Çirişli Pass, 2000 m, 11.VI.2002, ♂. Erzurum: Atlıkonak, 2000 m, 11.VI.2016, ♀, Hasankale, 1700 m, 11.VI.2001, ♂, Umudum, 2000 m, 26.VI.2003-30.VI.2017, 6 ♀♀, 5 ♂♂. Kars: Karakurt, 1500 m, 04.V.2003, ♀, 2 ♂♂.

Host plants: unknown.

***Pristiphora conjugata* (Dahlbom, 1835)**

Stigma light colored, more so than C. Antennae basally yellow or brown. Lower part of face light colored. Mesopleura yellow, abdomen with wide medial black stripe. Body length: 6-7.5 mm (Figures 1m, 2m, 3m).

Global distribution: China, Europe, Japan, Russia, Turkey.

Distribution in Turkey: Ankara (Taeger at al., 2018).

Material examined: Erzurum, 12.VII.2002, 1850 m, 2♀♀.

Host plants: *Populus nigra* L., *P. tremula* L.*, Salix caprea* L.*, S. fragilis* L. (Taeger & Blank, 1998).

******Pristiphora pallidiventris* **(Fallen, 1808)**

On hind legs femoral and tibial apices and entire tarsi black. Stigma darker than C; abdominal tergite IX in female apically black. Claw with denticle. Antennae longer than head and thorax together. Abdomen in male ventrally completely light colored. Body length: 5-6 mm (Figures 1n, 2n, 3n).

Global distribution: Canada, Europe, Japan, Russia.

Distribution in Turkey: new for Turkish fauna.

Material examined: Artvin, Oruçlu, 21.V.2006, ♀. Erzurum: Tortum, Esendurak, 1500 m, 11.IX.2001, ♀; Uzundere, 950 m, 04.V.2017, ♀.

Host plants: Rosaceae, *Rubus ideaus* L. (Taeger & Blank, 1998).

***Pseudodineura fuscula* (Klug, 1816)**

Sheath short, apically thick and obtuse. Femora black, apically somewhat ocherous. Pronotal angles black. Length of eight antennal segment 2.5 times its width. (Figures 1o, 2o, 3o).

Global distribution: Europe, Russia, USA.

Distribution in Turkey: New for Turkish fauna

Material examined: Erzurum, 4. Kuyu, 1850 m, 11.V.2003, 2 ♀♀; 23.V.2016, ♀.

Host plants: *Ranunculus cassubicus* L.*, R. repens* L.*, R. platanifolius* L. (Taeger & Blank, 1998).

***Stauronematus (=Pristiphora) platycerus* (Hartig, 1840)**

Pronotum completely black, or only extreme upper an rear edges brown. Female head in dorsal view subparallel behind eyes, male only slightly contracted. Abdomen entirely black, mesepisternum more densely pubescent above than below but usually without entirely glabrous area on lower half. Body length: 5-6.5 mm (Figures 1p, 2p, 3p).

Global distribution: China, Europe, Iran, Russia,Turkey.

Distribution in Turkey: Sivas (Taeger et al., 2018).

Material examined: Erzurum, 1850 m, 02.VIII.2016, 3 ♀♀; Uzundere, 1000 m, 12.VI.2002, 2 ♀♀.

Host plants: *Alnus* sp., *Populus* sp, *Salix* sp. (Liston, 2007).

References

Aguiar, A. P., A. R. Deans, M. S. Engel, M. Forshage, J. T. Huber, J. T. Jennings, N. F. Johnson, A. S. Lelej, J. T. Longiona, V. Lohrmann, I. Mikó, M. O. C. Rasmussen, A. Taeger & D. S. K. Yu, 2013. "Order Hymenoptera, 51-62". In: Animal Biodiversity: An Outline of Higher-level Classification and Survey of Taxonomic Richness (Ed. Z. Q. Zhang). Zootaxa, 3703 pp.

Benson, R. B., 1952. Hymenoptera (Symphyta). Handbooks for the Identification of British Insects, 6 (2b): 51-138.

Benson, R. B., 1966. A new genus of the Lycaotini (Blennocampinae) in Turkey (Hymenoptera, Tenthredinidae). Proceedings of the Royal Entomological Society of London, (B) 35: 75-77.

Benson, R. B., 1968. Hymenoptera from Turkey. Symphyta. Bulletin of the British Museum (N.H.) Entomology, 22 (4): 4-207.

Çalmaşur, Ö., 2006. Four new records for the Turkish Tenthredinidae (Hymenoptera) fauna. Turkish Journal of Entomology, 30 (3): 33-41.

Çalmasur, Ö., 2011. Check-list of the family Cimbicidae (Hymenoptera: Symphyta) of Turkey and some biological observations. Munis Entomology & Zoology, 6 (2): 779-784.

Çalmaşur, Ö., 2019. New records and some new distribution data to the Turkish Allantinae, Blennocampinae, Heterarthrinae and Selandrinae (Tenthredınidae, Symphyta, Hymenoptera) fauna. Munis Entomology & Zoology, 14 (1): 96-103.

Çalmaşur, Ö. & H. Özbek, 2004a. A contribution to the knowledge of the Tenthredinidae {Symphyta, Hymenoptera) Fauna of Turkey Part I: The Subfamily Tenthredininae. Turkish Journal of Zoology, 28 (1): 37-54.

Çalmaşur, Ö. & H. Özbek, 2004b. A contribution to the knowledge of the Tenthredinidae (Symphyta, Hymenoptera) fauna of Turkey Part II: The Subfamilies Blennocampinae, Dolerinae, Nematinae and Selandriinae. Turkish Journal of Zoology, 28 (1): 55-71.

Çalmaşur, Ö. & H. Özbek, 2004c. *Heterarthrus ochropoda* (Klug) (Hymenoptera: Tenthredinidae), a new record and a new pest of *Populus* spp. (Salicaceae) in Turkey. Proceedings of the Entomological Washington, 106 (3): 717-721.

Çalmaşur, Ö. & H. Özbek, 2006a. A willow sawfly, *Nematus salicis* (Linnaeus) (Hymenoptera: Tenthredinidae), a new record and new pest of *Salix* spp. in Turkey. Proceedings of the Entomological Society of Washington, 108 (1): 139-144.

Çalmaşur, Ö. & H. Özbek, 2006b. Check-list of the Argidae fauna (Hymenoptera: Sympyta) of Turkey. Zoology in the Middle East, 39 (1): 89-96.

Comstock, C. H., 1964. An Introduction to Entomology. Comstock Publishing Comp., Binghampton, N.Y., USA, 1064 pp.

Gauld, I. & B. Bolton, 1988. The Hymenoptera. Oxford University Press, 332 pp.

Goulet, H. & J. T. Huber, 1993. Hymenoptera of The World: An Identification Guide to Families. Canada Communication Group-Publishing Ottawa, Canada KIA 0S9, 669 pp.

Guichard, K. M. & D. H. Harvey, 1967. Collecting in Turkey 1959, 1960 and 1962. Bulletin of The British Museum (Natural History) Entomology, 19 (4): 223-250.

Gullan, P. J. & P. S. Cranston, 2010. The Insect-An Outline of Entomology, Wiley-Blackwell Publishing, Fourth Edition. 584 pp.

Haris, A., 2006. Study on the Palaearctic *Pristiphora* species (Hymenoptera: Tenthredinidae). Natura Somogyiensis, Kaposvár, 9 : 201-277.

Hastaoğlu Örgen, S. & H. H. Başıbüyük, 2006. "Members of Sawfly Family Argidae (Hymenoptera: Insecta) from Turkey, 393-396". In: Recent Sawfly Research Synthesis and Prospects (Eds. S. Blank, S. Schmidt & A. Taeger). Goecke & Evers, Keltern, 702 pp.

Kopelke, J. P., 2007. The European species of genus *Phyllocolpa,* part II: the leucapsis-group (Insecta, Hymenoptera, Tenthredinidae, Nematinae). Senckenbergiana Biologica, 87 (2): 149-161.

Korkmaz, E. M., M. Budak, S. Hastaoğlu Örgen, E. Bağda, L. Gençer, S. Ülgentürk & H. H. Başıbüyük, 2010a. New records and a checklist of Cephidae (Hymenoptera: Insecta) of Turkey with a short biogeographical consideration. Turkish Journal of Zoology, 34 (2): 419-447.

Korkmaz, E. M., S. Hastaoğlu Örgen, L. Gençer, S. Ülgentürk & H. H. Başıbüyük, 2010b. Orta Anadolu Bölgesi buğday tarlalarındaki bazı ekin zararlıları ve parazitoitlerinin saptanması. Türkiye Entomoloji Dergisi, 34 (3): 361-377.

Lacourt, P. J., 1999. Repertoire des Tenthredinidae Ouest-Palearctiques (Hymenoptera, Symphyta). Memories de la SEF, NO: 3, Societe Entomologique de FR, Juillet, Paris, 432 pp.

Liston, A. D., 1995. Compendium of European Sawflies. Chalastos Forestry, Daibersdorf 6, D-84177 Gottfrieding, Germany, 200 pp.

Liston, A. D., 2007. Revision of *Stauronematus* Benson, 1953 and additions to the sawfly fauna of Corsica and Sardinia (Hymenoptera, Tenthredinidae). Beitrage Entomology, 57 (1): 135-150.

Myers, N., R. A. Mittermeier, C. G. Mittermeier, G. A. B. da Fonseca & J. Kent, 2000. Biodiversity hotspots for conservation priorities. Nature, 403 (6772): 853-858.

Taeger, A. & S. M. Blank, 1998. Pflanzenwespen Deutschlands (Hymenoptera, Symphyta). Goecke und Evers, 364 pp.

Taeger, A., A. D. Liston, M. Prous, E. K. Groll, T. Gehroldt & S. M. Blank, 2018. ECatSym. Electronic World Catalog of Symphyta (Insecta, Hymenoptera). Program version 5.0 (19 Dec 2018), data version 40 (23 Sep 2018). Senckenberg Deutsches Entomologisches Institut (SDEI), Müncheberg. (Web page: https://sdei.de/ecatsym/) (Date accessed: 21 May 2019).

Zhelochovtsev, A. N., 1988. "Symphyta, 7-234". In: Oprendeliatel Nasekomykyh Evropeiskoi Chasti SSSR. III. Perepondhatokrylye 6. Opredeliteli po faune SSSR 158. Nauka Leningrad. (1994, English translation, 27, Order Hymenoptera, Suborder Symphyta (Chalastogastra). Keys to the Insects of the European Part of the USSR. (Ed. G. S. Medvedjev). Amerind Publishing Co., Pvt. Ltd., New Delhi, 387 pp.

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 araştırmalar yayımlanır.
Makale Özetleri, Biological Abstracts, BIOSIS Previews, CAB Abstracts, FAO AGRIS, Elsevier Scopus, Global Health, Information Reference Library, Review of Agricultural Entomology, SCI-E, TÜBİTAK/ULAKBİM, VINITI, 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