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**Original article (Orijinal araştırma)**

**Toxicity and repellency of different insecticides to *Odontotermes obesus* (Rambur, 1842) (Blattodea: Termitidae: Macrotermitinae)<sup>1</sup>**

*Odontotermes obesus* (Rambur, 1842) (Blattodea: Termitidae: Macrotermitinae)'a karşı farklı insektisitlerin zehir ve kaçırıcı özellikleri

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**Abstract**

Fungus-growing termites are important pests for buildings and agriculture in Asia and Africa. This study assessed four insecticides (fipronil 5% SC, imidacloprid 20 SL, thiamethoxam 25 WG and chlorfenapyr 360 SC) for their toxicity and repellency to the fungus-growing termite, *Odontotermes obesus* (Rambur, 1842) (Blattodea: Termitidae: Macrotermitinae). The study was conducted at Ghazi University, Dera Ghazi Khan, Pakistan from September-October, 2016 and was undertaken to identify effective termiticides along with their optimal concentrations for future use in baits and localized treatments. The chemicals assessed were differed significantly in their toxicity. Chlorfenapyr and thiamethoxam were more toxic and faster acting with lower LC<sub>50</sub> and LT<sub>50</sub> values than imidacloprid and fipronil. The four chemicals were statistically similar at each concentration. *Odontotermes obesus* was not repelled by 0-20 mg/l chlorfenapyr, 0-40 mg/l fipronil, 0-80 mg/l imidacloprid or 0-20 mg/l thiamethoxam. These results suggest that chlorfenapyr, imidacloprid and thiamethoxam may be used as soil termiticides, whereas fipronil can be used both as soil termiticide and in termite baiting programs.

**Keywords:** Fungus-growing termites, neurotoxins, soil treatment, termite baiting

**Öz**

Mantar yetiştiren termitler, Asya ve Afrika'daki tarımsal bölgelerin ve binaların önemli zararlılarıdır. Bu çalışmada, dört farklı insektisit (fipronil 5% SC, imidacloprid 20 SL, thiamethoxam 25 WG ve chlorfenapyr 360 SC) mantar yetiştirici termit, *Odontotermes obesus* (Rambur, 1842) (Blattodea: Termitidae: Macrotermitinae) üzerindeki zehir ve kaçırıcı etkileri değerlendirilmiştir. Çalışma, yemlerin ve yerel uygulamaların gelecekteki kullanımları için etkili termitisitlerin belirlenmesi ve bunların optimal dozlarının saptanması amacıyla Eylül-Ekim 2016'da Pakistan'ın Dera Ghazi Khan şehrindeki Ghazi Üniversitesi'nde yürütülmüştür. Değerlendirilen kimyasalların zehirliliklerinde önemli farklılıklar görülmüştür. Chlorfenapyr ve thiamethoxam daha düşük LC<sub>50</sub> ve LT<sub>50</sub> değerleri ile daha fazla zehirli ve daha etkili olurken; imidacloprid ve fipronil daha yüksek LC<sub>50</sub> ve LT<sub>50</sub> değerleri ile nispeten daha az zehirli ve daha az etkili olarak tespit edilmiştir. Dört kimyasal da istatistiksel olarak her bir dozda birbirleriyle eşit miktarda bulunmuştur. *Odontotermes obesus* için kaçırıcı olmayan veya çok az kaçırıcılık gösteren chlorfenapyrin 0-20 mg/l dozları, fipronilin 0-40 mg/l dozları, imidacloprid 0-80 mg/l dozları ve thiamethoxamın 0-20 mg/l dozlarında herhangi bir tercih edilmeme durumu görülmemiştir. Bu sonuçlar, Chlorfenapyr, imidacloprid ve thiamethoxam toprak termitisitleri olarak ve fipronilin ise hem toprak termitisiti olarak hem de termit yem programlarında kullanılabileceğini göstermektedir.

**Anahtar sözcükler:** Mantar yetiştirici termitler, sinir zehirleri, toprak uygulaması, termit yemi

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## Introduction

Termites are important pests of timber, wood products, trees and agricultural crops in tropical and subtropical and warm temperate areas of the world (Su & Scheffrahn, 2000; Scholz et al., 2010; Rouland-Lefèvre, 2011). Their infestation may be prevented or controlled by various methods in different countries (Howick & Staunton, 2017). Until the 1950s, this was mainly achieved by using wood preservatives, by chemical dusting and fumigation but all these control methods were mostly replaced by the application of soil termiticides with the advent of cyclodienes after World War II (Ware, 1999). The low cost and ease of use made soil termiticides a dominant management technique, especially in buildings (e.g., Findlay, 1962; Hickin, 1971). The cyclodienes provided effective control of termites for decades until their use was prohibited by the US Environmental Protection Agency (EPA) in the 1980s, mainly due to perceptions of their harmful effect on the environment and public health (Walker & Newton, 1998). After their suspension, cyclodienes were replaced by organophosphates and synthetic pyrethroids but these insecticides were not ideal as soil termiticides due to their short residual activity and their minimal impact on termite populations (Grace et al., 1993; Forschler, 1994). To address such problems, chitin synthesis inhibitors (CSIs, e.g., hexaflumuron) and newer neurotoxins (e.g., imidacloprid, fipronil) came into the market.

Current termite management methods for buildings include selective soil treatments (interior and/or exterior perimeter treatment or spot treatments) and baiting with slow-acting and non-repellent chemicals (Curl, 2004; Web, 2017; Iqbal & Evans, 2018). In these applications, foraging termites absorb a lethal dose when they pass through treated zones and/or consume a bait matrix, thereby transferring it to the colony members during trophallaxis and mutual grooming processes (Esenther & Gray, 1968; Su, 1994). The aim of both techniques is to use only small amounts of active ingredients with a consequent reduction in environmental contamination.

The scavenging termite, *Odontotermes obesus* (Rambur, 1842) (Blattodea: Termitidae: Macrotermitinae), is a fungus-growing higher termite. This species is highly problematic in agriculture, forestry and buildings (Akhtar & Kausar, 1991; Uys, 2002). There are some laboratory and field studies that have been conducted against this particular species involving different insecticides but with different objectives and methodologies (Kumawat, 2001; Ahmed et al., 2007; Bhagawati et al. 2017; Rasib et al. 2018). Previous studies aimed to determine if a repellent concentration(s) of a specific insecticides or plant extract for creating soil barrier treatment would keep the termites away from the structure or building thus killing only a small fraction of termite individuals. Consequently, the aim of this study was to assess the four insecticides, chlorfenapyr, imidacloprid, fipronil and thiamethoxam, for their suitability either as spot/soil barrier treatments or bait active ingredients against *O. obesus*.

The aim was to determine the optimal concentrations of a suitable insecticide with a delayed toxicity and non-repellency, this property is necessary for acquiring and transfer of insecticide either through feeding of bait or passing through a treated zone. The ultimate goal of this study was to identify the active ingredient and a concentration for use in the future for soil treatments or baiting programs against *O. obesus* and related pest termite species, and thereby provide a new environmentally benign tool for termite management.

## Materials and Methods

### Collection of *Odontotermes obesus*

Workers of *O. obesus* were collected from monitoring stations installed at the Airport Campus, Ghazi University Dera Ghazi Khan, Pakistan as a bait station preference experiment. Three types of aggregation stations were installed, viz., small (0.5 L), medium (3 L) and large stations (8 L) during May 2016. The stations were plastic containers filled with wood (*Bombax* sp.). The termites were collected weekly during September-October 2016 and were immediately placed in plastic boxes (14 × 8.5 × 6 cm) containing a moist paper towel. The boxes were brought to the laboratory (located at the Airport Campus, Ghazi University, Dera Ghazi Khan, Pakistan) with a great care to avoid injuries to individuals and workers then they were separated from the debris following the method of Gay et al. (1955) with little modification. The apparatus consists of a rectangular container (75 × 60 × 15 cm) that held a glass sheet (48 × 32 cm) with

glass rods supporting it from below. The workers of *O. obesus* were released on the top of glass sheet and allowed to travel. The healthy traveling workers fell down into the rectangular container and were collected in glass Petri dishes for use in the experiments.

### **Insecticides**

Fipronil (Regent 5% SC, Bayer CropScience, Leverkusen, Germany), imidacloprid (Confidor 20 SL, Bayer CropScience), chlorfenapyr (Squadron 360 SC, FMC United, Philadelphia, PA, USA), thiamethoxam (Actara 25 WG, Syngenta, Basel, Switzerland) were purchased from a commercial source for the toxicity and repellency studies.

### **Lethal concentration and lethal time estimation**

No-choice feeding bioassays were performed to determine the trends in mortality of *O. obesus*. Five concentrations (causing between 0 and 100% mortality) were prepared by serial dilution for each of the insecticides. Two ml of concentration was spread on Whatman No. 1 filter paper which was placed on the base of a rectangular plastic box (14 × 8.5 × 6 cm). Filter paper moistened with the same quantity of distilled water was used as a control. Filter papers were dried in a fume hood for 24 h, then 150 healthy workers of at least the third instar plus 10 soldiers were introduced into the box. Each concentration was replicated four times. There were four control boxes for each insecticide. The boxes were then maintained at 25±2°C and 70±5% RH, and a filter paper beneath the cover of the box was dampened with distilled water daily during treatment. Workers mortality was recorded after 12, 24, 36 and 48 h of exposure. Workers were considered dead when they showed no movement when probed with a fine brush. From this data, lethal time was also calculated.

### **Laboratory repellency test**

Repellency of different concentrations of the insecticides to *O. obesus* was tested following the methods of Iqbal and Evans (2017). The following concentrations were prepared in distilled water: chlorfenapyr 0, 1.25, 2.5, 5, 10 and 20 mg/l; fipronil 0, 2.5, 5, 10, 20 and 40 mg/l; imidacloprid 0, 5, 10, 20, 40 and 80 mg/l, and thiamethoxam 0, 1.25, 2.5, 5, 10 and 20 mg/l.

For the repellency study, filter paper pieces (4.5 cm<sup>2</sup>) were cut and placed on a glass sheet. Then 0.5 ml of each insecticide concentration was applied to the filter paper with a micropipette and replicated three times. The treated filter papers were allowed to dry overnight and were then randomly placed in rectangular plastic boxes (14 × 8.5 × 6 cm). Each box contained all the concentrations of a single insecticide. Then a total of 150 healthy workers and five soldiers of *O. obesus* were released into the middle of each plastic box with each receiving workers and soldiers from separate colonies. These plastic boxes were placed into dark conditions in a large plastic tray along with moist tissue paper. The workers were allowed to settle on any treated filter paper of their choices for 30 min and then examined at 30 min intervals for 150 min. The total numbers of workers that settled on each treated paper and those not on any paper were recorded by photographs taken at each observation.

### **Data analysis**

The mortality data were corrected using Abbott's formula (Abbott, 1925), if the mortality rate in the control was more than 5%. Median lethal concentrations (LC<sub>50</sub>) and lethal times (LT<sub>50</sub>) were determined by probit analysis using SPSS software (IBM SPSS Statistics for Windows, Version 23.0, IBM Corp, Armonk, NY, USA).

LC<sub>50</sub> and LT<sub>50</sub> values were considered to be significantly different based on non-overlapping of 95% confidence limits. For the repellency experiment, the mean numbers of workers at each concentration of insecticide were calculated and subjected to Friedman's two-way nonparametric analysis of variance using Statistix 8.1 (Statistix, Tallahassee, FL, USA) for comparison.

## Results and Discussion

### Lethal concentration estimation (LC<sub>50</sub>)

Based on concentration lethal 50% of treated workers of *O. obesus* (LC<sub>50</sub>) after 12, 24, 36 and 48 h, the toxicity of the insecticides differed significantly as indicated by non-overlapping 95% confidence limits. Chlorfenapyr was the most toxic insecticide with the lowest LC<sub>50</sub> followed by thiamethoxam, fipronil and imidacloprid. The LC<sub>50</sub> of chlorfenapyr were 11.2, 2.3 and 1.03 after 12, 24 and 36 h respectively. All workers were dead after 48 h at almost all of the concentrations of chlorfenapyr. The raw LC<sub>50</sub> of imidacloprid after 24-48 h ranged from 6.9 to 39.8 mg/l (Table 1).

Table 1. Toxicity (LC<sub>50</sub>) of four insecticides against workers of *Odontotermes obesus* at different exposure time (h) on treated filter paper

Insecticide	Time (h)	LC <sub>50</sub> <sup>a</sup> (mg/l) (95% CL <sup>b</sup> ) <sup>*</sup>	df	χ <sup>2</sup> <sup>c</sup>	P	N <sup>d</sup>
Chlorfenapyr	12	11.2 (10.30-12.40) A	3	3.20	0.35	2279
Fipronil	12	39.8 (30.10-57.60) C	3	2.87	0.42	1752
Imidacloprid	12	39.0 (33.00-48.10) C	2	3.39	0.18	1466
Thiamethoxam	12	19.4 (15.10-27.20) B	3	5.06	0.16	1828
Chlorfenapyr	24	2.3 (2.00-2.60) A	3	3.48	0.32	2279
Fipronil	24	6.8 (6.30-7.50) C	3	4.49	0.21	1752
Imidacloprid	24	15.2 (13.90-16.60) D	2	3.72	0.11	1466
Thiamethoxam	24	4.9 (4.10-5.80) B	3	1.45	0.69	1828
Chlorfenapyr	36	1.03 (1.02-1.39) A	3	3.89	0.27	2279
Fipronil	36	3.4 (2.70-4.10) C	3	5.44	0.14	1752
Imidacloprid	36	9.7 (8.90-10.50) D	2	3.70	0.15	1466
Thiamethoxam	36	2.0 (1.60-2.40) B	3	0.98	0.81	1828
Chlorfenapyr	48	-	-	-	-	-
Fipronil	48	2.1 (1.50-2.60) B	3	6.58	0.08	1752
Imidacloprid	48	6.9 (4.40-9.10) C	2	4.96	0.08	1466
Thiamethoxam	48	1.3 (1.00-1.50) A	3	0.42	0.93	1828

\* Confidence limits followed by the same letter are overlapping so the LC<sub>50</sub> are not statistically different;

<sup>a</sup> LC<sub>50</sub>, concentration lethal to 50% of the population; <sup>b</sup> CL, confidence limits; <sup>c</sup> Chi-square; <sup>d</sup> number of workers exposed.

### Lethal time estimation

The insecticides differed significantly in terms of LT<sub>50</sub> values at all concentrations on the basis of non-overlapping confidence limits. The LT<sub>50</sub> values decreased with the increase in concentrations of all insecticides with minimum values recorded for chlorfenapyr (24.2 h at 2.5 mg/l and 9.2 h at 20 mg/l) followed by thiamethoxam (31.1 h at 2.5 mg/l and 12.5 h at 20 mg/l), fipronil (42.9 h at 2.5 mg/l and 14.8 h at 20 mg/l) and imidacloprid (193 h at 2.5 mg/l and 20.0 h at 20 mg/l) (Table 2).

Table 2. Time mortality response (LT<sub>50</sub>) of *Odontotermes obesus* to different insecticides

Insecticide	Concentration (mg/l)	LT <sub>50</sub> <sup>a</sup> (h) (95% CL <sup>b</sup> )*	df	χ <sup>2</sup> <sup>c</sup>	P	N <sup>d</sup>
Chlorfenapyr	2.5	24.2 (23.1-25.5) A	1	1.26	0.26	390
Fipronil	2.5	42.9 (34.4-66.7) BC	2	5.27	0.07	283
Imidacloprid	2.5	193.0 (118.0-527.0) D	2	3.31	0.19	255
Thiamethoxam	2.5	31.1 (28.2-34.6) B	2	2.56	0.27	289
Chlorfenapyr	5	17.2 (16.2-18.1) A	1	1.62	0.2	380
Fipronil	5	29.3 (27.6-31.3) C	2	0.61	0.74	298
Imidacloprid	5	70.8 (59.4-91.4) D	2	1.77	0.41	297
Thiamethoxam	5	21.2 (19.1-23.2) B	2	0.17	0.92	299
Chlorfenapyr	10	13.0 (12.0-13.9) A	1	0.78	0.37	415
Fipronil	10	20.4 (19.4-28.2) C	2	2.09	0.35	290
Imidacloprid	10	35.4 (32.6-39.0) D	2	3.09	0.21	343
Thiamethoxam	10	17.4 (15.7-19.0) B	2	2.08	0.35	310
Chlorfenapyr	20	9.23 (8.24-10.1) A	1	0.86	0.35	449
Fipronil	20	14.8 (13.9-15.6) BC	2	0.14	0.92	330
Imidacloprid	20	20.0 (18.5-21.4) D	2	2.72	0.32	301
Thiamethoxam	20	12.5 (10.7-14.0) B	2	3.55	0.16	305

\* Confidence limits followed by the same letter are overlapping so the LT<sub>50</sub> are not statistically different;

<sup>a</sup> LT<sub>50</sub>, time for 50% of the population to be killed; <sup>b</sup> CL, confidence limits; <sup>c</sup> Chi-square; <sup>d</sup> number of workers exposed.

## Laboratory repellency study

### Repellency of chlorfenapyr

Most workers of *O. obesus* had settled on different concentrations of chlorfenapyr after 60 min. The numbers of workers on different concentrations after 60, 90, 120 and 150 min were not significantly different (Friedman's statistic = 6.43; df = 6; P-value, χ<sup>2</sup> approximation = 0.376) (Figure 1a). However, after 60 min, maximum numbers of workers were found on filter paper pieces treated with 5 mg/l (27.88) and 20 mg/l (26.98). At 90, 120 and 150 min, maximum numbers of workers were observed on filter paper pieces treated with 0 mg/l (28.6), 20 mg/l (29.2) and 20 mg/l (32.3) of chlorfenapyr, respectively (Figure 2a).

### Repellency of fipronil

The movement of workers in the treatment and control boxes was minimal after 60 min. The numbers of workers settled on 0-40 mg/l treated filter paper after 60, 90, 120 and 150 min were not statistically different (Friedman statistic = 4.07; df = 6; P-value, χ<sup>2</sup> approximation = 0.667) (Figure 1b). The maximum numbers of workers were recorded on 20 mg/l at 90 min (39.7) followed by 10 mg/l at 120 min (34.1) and 60 min (32.7), and 2.5 mg/l at 150 min (31.9) (Figure 2b).

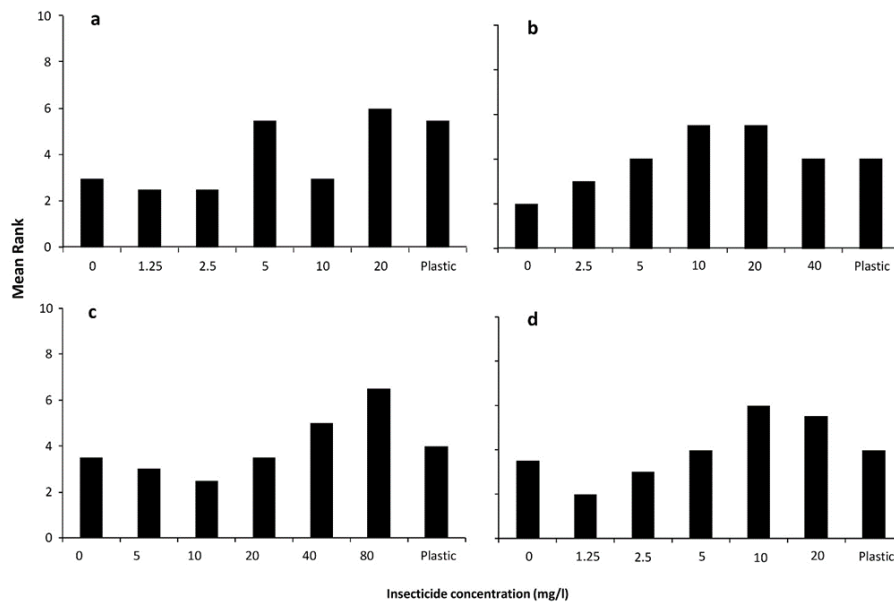


Figure 1. Friedman's test indicating mean ranks of numbers of workers of *Odontotermes obesus* on filter paper pieces treated with different concentrations of chlorfenapyr (a), fipronil (b), imidacloprid (c) and thiamethoxam (d) after 60, 90, 120 and 150 min of exposure in repellency tests. "Plastic" indicates termites not settling on any treated paper.

### Repellency of imidacloprid

The workers of *O. obesus* became stable and there was little movement in the imidacloprid treated boxes after 60 min. The numbers of workers settled on different concentrations after 60, 90, 120 and 150 min and did not differ significantly (Friedman's statistic = 4.71; df = 6; P-value,  $\chi^2$  approximation = 0.581) (Figure 1c). The repellency of *O. obesus* to different concentrations of imidacloprid showed maximum numbers on 80 mg/l followed by 5 mg/l, 40 mg/l after 60, 90, 120, and 150 min (Figure 2c).

### Repellency of thiamethoxam

After 60 min the workers had settled in the thiamethoxam-treated boxes with no significant difference in numbers of workers settling on the filter paper treated with different concentrations of thiamethoxam (Friedman's statistic = 4.92; df = 6; P-value,  $\chi^2$  approximation = 0.553) (Figure 1d). The maximum numbers of workers of *O. obesus* were observed at the 10 mg/l after 60 min, 120 min and 150 min. However, at 90 min, maximum workers were recorded on 0 mg/l (Figure 2d).

The efficacy of newer insecticides either in baits or in soil treatments is determined by their slow-acting and non-repellent properties (Su et al., 1987; Saran & Rust, 2007; Vargo & Parman, 2012). If the insecticide kills the foraging populations quickly the exposed workers will die in the tunnel before returning to their colony (Saran & Rust 2007). Whereas, slow-acting insecticides allow cross contamination from exposed to unexposed termites in the colony causing significant impact (Rust & Saran, 2006).



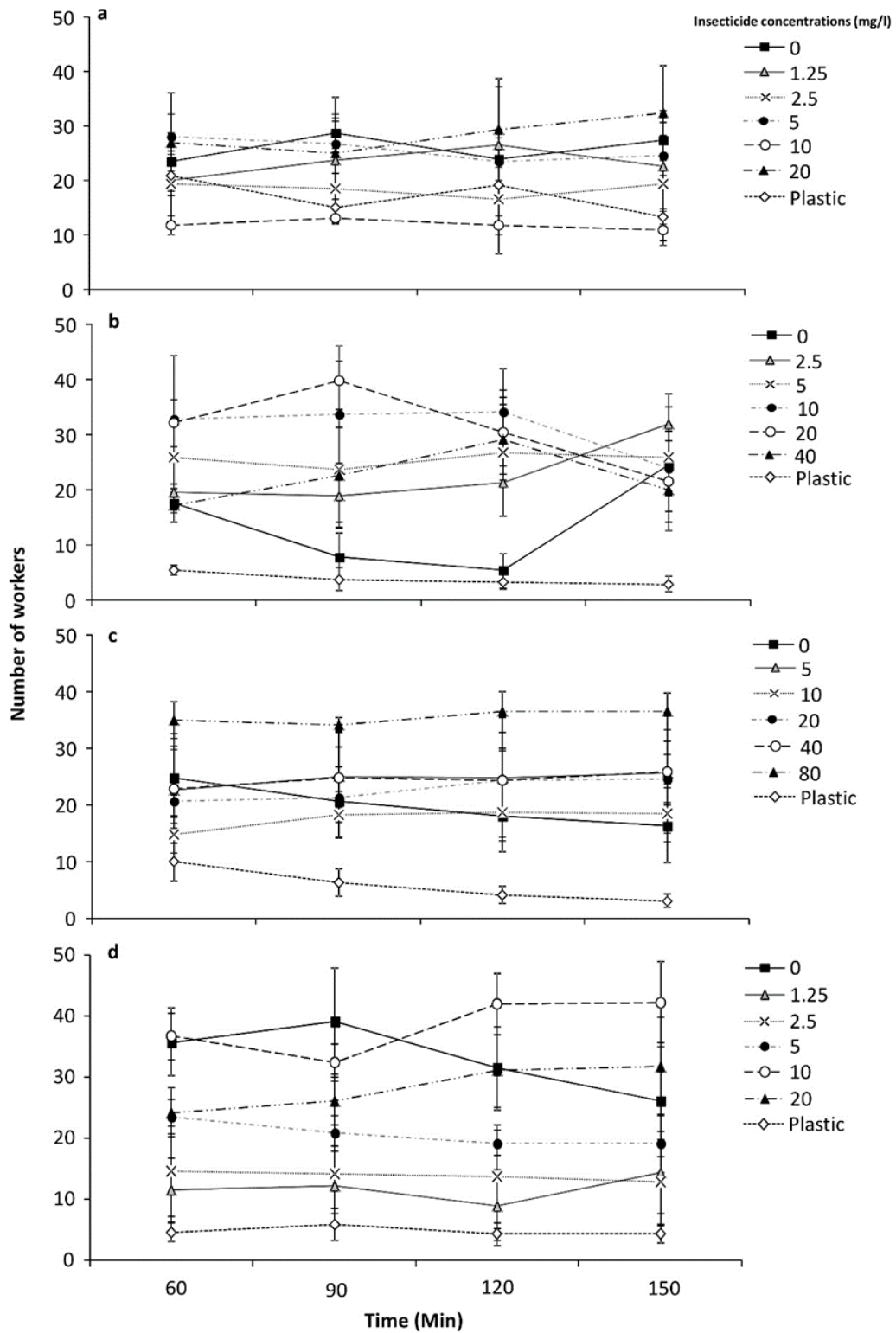


Figure 2. Mean ( $\pm$ SE) numbers of *Odontotermes obesus* workers on filter paper treated with chlorfenapyr (a), fipronil (b), imidacloprid (c) and thiamethoxam (d) over time in a laboratory repellency experiment. "Plastic" indicates termites not settling on any treated paper.

Although, four newer insecticides were evaluated against *O. obesus* to determine their slow-action and non-repellent properties under laboratory conditions, the use of termites in laboratory bioassays is not as straightforward as some researchers might like to think (Lenz, 2009). Reported here are preliminary findings showing that chlorfenapyr and thiamethoxam caused high mortalities and were fast-acting, whereas fipronil and imidacloprid were comparatively less toxic and slower-acting. Workers of *O. obesus* were not repelled by any concentration of chlorfenapyr, fipronil, imidacloprid or thiamethoxam.

All concentrations of chlorfenapyr (0-20 mg/l) were non-repellent to the workers of *O. obesus* but the mortality of the workers was very fast in comparison with the other three insecticides. Workers remained exposed to treated filter papers until the end of the experiment. However, in the two previous studies using *Reticulitermes flavipes* (Kollar, 1837) and *Reticulitermes hesperus* Banks, 1920 (Blattodea: Rhinotermitidae), chlorfenapyr was reported as being slow-acting and non-repellent. This could be due to differences in the methodology. Rust and Saran (2006) reported that chlorfenapyr was transferred from exposed to unexposed workers of *R. hesperus* but the mortality rate was dependent upon the concentration and exposure time, with faster action with increased concentration and exposure time (Shelton et al., 2006; Rust & Saran, 2006). The rapid death of exposed workers limits the horizontal transfer of chlorfenapyr. Moreover, it was suggested that a lethal concentration of chlorfenapyr is rapidly acquired by the workers due to non-repellency and that it negatively affects the foraging behavior of the exposed workers within 4 h (Rust & Saran, 2006). This will result in fewer workers returning to the colony for horizontal transfer, thus chlorfenapyr is not suitable for perimeter treatment and baiting to cause colony elimination at the tested concentrations.

The results presented here suggest that fipronil is slow-acting and non-repellent to *O. obesus* at 0-20 mg/l. These results are similar to many previous studies (Gautam et al., 2012; Li et al., 2016) and confirms that it has the potential to be transferred to unexposed workers due to non-repellent and delayed toxicity (Bagnères et al., 2009; Li et al., 2016). Moreover, Saran & Rust (2007) reported no changes in behavior of *R. hesperus* during the first 8 h in both the brief and continuous exposure studies. Henderson (2003) also showed similar results, indicating normal tunneling activity for up to 9 h after exposure to low concentrations of fipronil in soil. Remmen & Su (2005a) reported non-repellency of fipronil against *Coptotermes formosanus* Shiraki, 1909 (Blattodea: Rhinotermitidae) and *R. flavipes*. The efficacy of fipronil as a bait active ingredient has also been tested against three fungus-growing termitid termites, *Macrotermes gilvus* (Hagen, 1858) (Blattodea: Termitidae) in Singapore (Iqbal & Evans, 2018), *Microtermes mycophagus* (Desnoux, 1906) (Blattodea: Termitidae) in Pakistan (Iqbal & Saeed, 2013) and *Odontotermes formosanus* Holmgren, 1912 (Blattodea: Termitidae) in China (Huang et al., 2006), as well as one non-fungus-growing *Reticulitermes* species in the USA (Forschler & Jenkins, 2000). In all four field-baiting studies, fipronil baits successfully eliminated termite colonies. Based on all of these studies, fipronil can be used in soil treatments (both spot and barrier) as well as in baits, using even higher concentrations (20-40 mg/l) to eliminate termite colonies.

Imidacloprid has been found to be slow-acting and non-repellent to various termite species across the globe (Thorne & Breisch 2001; Luo, 2010; Manzoor et al., 2014). Although it may appear to be suitable for baiting on the basis of those assessments, in practice imidacloprid baits have failed to eliminate termite colonies (Iqbal & Evans, 2018). Imidacloprid has been reported to cause a cessation of termite feeding on baits, trophallaxis and mutual grooming (Boucias et al., 1996; Tomalski & Vargo, 2004; Iqbal & Evans, 2018). Feeding inhibition caused by imidacloprid could result in its slow action in termites. This is because imidacloprid has been reported to cause minimal toxicity when administered through acute dermal and inhalation routes as compared to oral administration (Sheets, 2010). Several other studies have reported confused and erratic movement of workers after exposure to this chemical (Thorne & Breisch, 2001; Quarcoo et al., 2010, 2012). In other studies, workers became immobile or showed decreased movement when exposed to even small quantities of imidacloprid (Henderson, 2003; Luo, 2010). Similar patterns were also observed in imidacloprid-treated boxes. The reduced bait-feeding and the negative impact of imidacloprid on termite movement make it unsuitable for termite baiting. However, it could be an effective soil treatment at 40-80 mg/l and provide residual control for 5-10 years against a wide range of termite species (Reid et al., 2002).

Based on the results present here, thiamethoxam was faster-acting than chlorfenapyr but showed non-repellency to the workers of *O. obesus*. In previous studies, thiamethoxam caused fast mortality of *Coptotermes gestroi* (Wasmann, 1896) (Blattodea: Rhinotermitidae) workers at 50 mg/l while it showed delayed transfer toxicity 0.25-25 mg/l at higher concentrations in a laboratory trial (Acda, 2014).

The results presented here are similar to the studies of Remmen & Su (2005a, b) who reported thiamethoxam as fast-acting active ingredient than fipronil against *C. formosanus* and *R. flavipes* with no repellency to the workers. Although this was not tested in the present study, it is suspected that thiamethoxam, being a neonicotinoid insecticide, would also affect the foraging activity of workers in the field, resulting in an inability of sufficient workers to return back to the colony for horizontal transfer of this termiticide to other workers. Like imidacloprid, thiamethoxam has also been reported to reduce feeding intensity in higher termites (Delgarde & Lefevre, 2002), making it unsuitable for use in termite baits. However, it can be used effectively as a soil barrier treatment.

This study indicates that fipronil and imidacloprid are slow-acting and non-repellent to *O. obesus*, whereas, chlorfenapyr and thiamethoxam are fast-acting and non-repellent. Based on previous studies in Pakistan and other countries, it is concluded that fipronil can be effectively used in baits and soil treatments for *O. obesus* and other fungus-growing termites. However, chlorfenapyr, thiamethoxam and imidacloprid should only be used as soil termiticides that will kill termites through direct contact. However, further laboratory and field studies are required to test the suitability of some other concentrations of these active ingredients for baiting as were reported by Aihetasham & Iqbal (2012) for wood feeding preference to *Microcerotermes championi* (Snyder, 1933) (Blattodea: Termitidae).

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**Original article (Orijinal araştırma)**

**Modeling of development and water consumption of mealworm, *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) larvae using nonlinear growth curves and polynomial functions**

Büyüme eğrileri ve polinomial fonksiyonlar kullanılarak unkurdu, *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) larvalarının gelişim ve su tüketimlerinin modellenmesi

**Abdullah Nuri ÖZSOY<sup>1\*</sup>**

**Abstract**

The water needs of *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) have to be met in either in-vitro culture or mass production. In this study, water needs of larvae were met directly by using a purpose-built water diffuser. The efficacy of larvae grown with the water diffuser (W) was tested against the control group (CONT). Various growth models were used to test their appropriateness to describe the experimental data. This research was conducted in the Population Genetic Laboratory of Animal Science Department of Isparta University of Applied Sciences in 2018. The highest larval weights were 138 mg in W and 144 mg in CONT treatments. The larvae in W entered the pupal period 2 weeks before the larvae in CONT. The growth of larvae in both groups was successively modeled with Gompertz and logistic growth curve models, and quadratic and cubic polynomial functions. The mean weekly water consumption of the W larvae was found to be between 58.4-129 mg. The water consumption of larvae can be described by polynomial functions. There were significant correlation coefficients for larval age, larval weight and water consumption. Consequently, using the diffuser instead of fresh vegetables or fruits is more suitable to meet the water requirement of the larvae.

**Keywords:** Growth curves, polynomial functions, *Tenebrio molitor*, water consumption

**Öz**

*Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae)'un laboratuvar ortamında yetiştirilmesinde ve kitlesel üretimde su ihtiyaçlarının karşılanması gerekmektedir. Yapılan bu çalışmada larvaların su ihtiyaçları yeni bir yöntem kullanılarak doğrudan karşılanmıştır. Yöntemin (W) etkinliği oluşturulan kontrol grubuna (CONT) karşı test edilmiştir. Farklı büyüme modellerinin deneysel veri setini tanımlamadaki uygunluğu da test edilmiştir. Araştırma 2018 yılında, Isparta Uygulamalı Bilimler Üniversitesi Tarım Bilimleri ve Teknolojileri Fakültesi Zootečni Bölümü Popülasyon Genetiği Laboratuvarında yapılmıştır. Araştırmada W grubunda en yüksek larva ağırlığı 137.6 mg, CONT grubunda ise 144.2 mg bulunmuştur. Su uygulaması yapılan gruptaki larvalar, kontrol grubundaki larvalardan iki hafta önce pupa evresine girmişlerdir. Her iki grupta larvaların büyümesi, Gompertz, logistic büyüme eğrisi modelleri ve quadratik ve kubik polinomial fonksiyonları ile başarılı bir şekilde modellenmiştir. W grubu larvaların ortalama haftalık su tüketimleri 58.4 ile 128.8 mg aralığında bulunmuştur. Larvaların su tüketimleri polinomial fonksiyonlar kullanılarak modellenebilmektedir. Araştırmada larva yaşı, larva ağırlığı ve su tüketimi parametreleri arasında önemli korelasyonlar belirlenmiştir. Tüm bunlar dikkate alındığında *T. molitor* larvalarının su ihtiyacının ortama verilen taze sebze veya meyveler yerine, diffuzör aracılığı ile karşılanmasının daha uygun bir yöntem olduğu sonucuna varılmıştır.

**Anahtar sözcükler:** Büyüme eğrileri, polinomial fonksiyonlar, *Tenebrio molitor*, su tüketimi

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## Introduction

Mealworm, *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) is a good model organism due to its short life cycle and being easy to grow (Pölkki et al., 2012; Simon et al., 2013; Grau et al., 2017). In addition, *T. molitor* can be a good alternative to currently available protein sources for aquaculture (Ng et al., 2001; Gasco et al., 2016; Choi et al., 2018) and poultry production (Ramos-Elorduy et al., 2002; De Marco, 2015; Bovera et al., 2016) due to its high-quality lipid and high protein content (Finke, 2002; Ramos-Elorduy et al., 2002; Siemianowska et al., 2013; Hervé et al., 2014; Jin et al., 2016; Özsoy et al., 2017). *Tenebrio molitor* needs minimal physical space, has high conversion efficiency and can convert organic waste into usable animal feed. However, it has restricted use in poultry feed because of high production costs (Ramos-Elorduy et al., 2002). In the mass production of *T. molitor*, feed and water expenses constitute a significant part of the production costs. Wheat flour, bran, oatmeal, corn and corn flakes are used as the basic food in experimental *T. molitor* production. Similar to insect species such as *Ephestia kuehniella* Zeller, 1879 and *Sitotrepa panicea* L. 1758, *T. molitor* carbohydrate requirements are very high and carbohydrates in their diet need to be 80% or higher (Fraenkel et al., 1950). Well performance of *T. molitor* larvae requires meeting their feed and as well as water needs without causing any stress. The water needs of *T. molitor* in the laboratory and mass production can be met by variety of methods or mechanisms: i) absorbance of water through the cuticle in microclimate with high humidity (Fraenkel et al., 1950; Murray, 1968; Punzo & Rosen, 1984), ii) ingestion with feed of high moisture content (Aguilar-Miranda et al., 2002; Gholy & Alkoaik, 2009; Ravanzaadi et al., 2012; Siemiauska et al., 2013), and iii) free-choice water supplement (Urs & Hopkins, 1973; Morales-Ramos et al., 2012). However, it has been reported that direct water supplement has advantages over other water intake mechanisms for speed of larval growth and early maturity with relatively higher weight, decreased mortality at any growth stage and reproductive success. Increasing humidity, supplying fresh fruits and/or vegetables and free-choice water given on cotton pads often stimulate growth of secondary organisms and cause physical degradation of the feed. Therefore, there is a need to overcome these deleterious effects of water supplement methods with a method not interfering with the feed while maintaining advantages of free-choice supplement.

Growth curve models and polynomial mathematical functions are used to express the change in the time dependent-weights of living organisms. Gompertz and logistic growth models are frequently used nonlinear regressions to describe the growth patterns of living organisms (Akbulut et al., 2004; Nariç et al., 2009; Aytikin & Zülkadir, 2013).

In this study, a new method was used to meet the water requirements of *T. molitor* larvae, which could be an alternative to the use of fresh vegetable pieces in the growth medium. The water needs of the larvae were met directly by using a purpose-built water diffuser. The water consumption or requirement of the larvae up to the pupal period was therefore determined at weekly intervals. In addition, the larvae grown with the water diffuser (W) and control larvae (CONT) were modeled using growth and water consumption growth curves (Gompertz and logistic) and polynomial functions (quadratic and cubic functions). In this way, the changes in water requirements of the larvae can be monitored by age.

## Materials and Methods

### Materials

A population of *T. molitor* was cultured at the Population Genetics Laboratory, Department of Animal Science, Faculty of Agricultural Sciences and Technologies, Isparta University of Applied Sciences in 2018. The gender identification was made at pupal period (Sokoloff, 1977), and then male and female pupae were transferred to separate growth boxes to develop to adults.



## Methods

The mature insects were taken into the mating box that supplemented with nutrient for 48 h. Adult insects were removed from the nutrient medium at the end of the mating period and the eggs were kept in an incubation cabinet adjusted to 28°C and 60% RH in feed media for 7 d. The hatched larvae were kept together at 28°C for seven additional days and fed with 70% semolina, 20% wheat bran and 10% yeast mixture. Then, 100 randomly selected larvae from this population were placed in the 350 mL plastic boxes for each trial unit after precisely measuring their initial weight with a balance sensitive to 0.01 mg. The weekly weight gains were precisely measured till formation of pupae.

Four experimental groups were formed in the study. Two of these groups were control groups (CONT1 and CONT2, water requirement of the larvae was supplied fresh potatoes at 3-d intervals) and the other two were water-supplied groups (W1 and W2, water supplied directly with the water diffuser). The ration and water given to the groups are given in Table 1.

Table 1. Ration and water supplied to the experimental groups

Experimental groups	Rations	Water
W1- W2	70% semolina, 20% wheat bran, 10% yeast	Water diffuser
CONT1- CONT2	70% semolina, 20% wheat bran, 10% yeast	Fresh potatoes at 3-d interval

Water requirement of insects in W1 and W2 treatments was given to the larvae by a purpose-built water diffuser. It was assembled by combining a water container and a diffuser stick (Figure 1). Precisely measured water was consumed by the larvae through the diffuser. The larval weights in the experimental groups were measured at weekly intervals in groups of 10 using an analytical balance with 0.01 mg sensitivity (Radwag® AS 110.R2, Radom, Poland). Weighing results were recorded in milligrams. The insects in W groups consumed water ad libitum.



Figure 1. Water-diffuser design and water supplement to the larvae.

## Data analysis and modeling

Larval ratio, pupal ratio, mortality ratio and individual water consumption were determined in this study using the following equations.

Larval ratio = number of alive larvae / number of individuals in the control treatment x 100,

Pupal ratio = number of alive pupae / number of individuals in the control treatment x 100,

Mortality ratio = number of dead individuals / number of individuals in the control treatment x 100, and

Water consumption of Individual = water consumption / number of alive larvae.

Gompertz and logistic growth models, and the mathematical models such as quadratic and cubic forms of polynomial functions used in the research and their parameters are given in Table 2.

The data set were subjected to one-way ANOVA procedure to separate treatment means. The statistical analysis such as ANOVA, growth curve, estimates of polynomial function parameters and correlations were performed in Minitab (Minitab, 2018) package program.

Table 2. Growth models and polynomial functions and their parameters

Growth models	
Gompertz growth model	Logistic growth model
$GGM(y) = \exp(-\exp(\theta_2 - \theta_3 t))$	$LGM(y) = \frac{\theta_1 + (\theta_2 - \theta_1)}{(1 + \exp((t - \theta_3)/\theta_4))}$
$\theta_1$ ; asymptote	$\theta_1$ ; asymptote
$\theta_2$ ; y - intercept	$\theta_2$ ; y - intercept
$\theta_3$ ; scale	$\theta_3, \theta_4$ ; scale
t; time	t; time
Polynomial functions	
Quadratic function	Cubic function
$QF(y) = \theta_1 + \theta_2 t + \theta_3 t^2$	$CF(y) = \theta_1 + \theta_2 t + \theta_3 t^2 + \theta_4 t^3$
$\theta_1, \theta_2, \theta_3$ ; scale	$\theta_1, \theta_2, \theta_3, \theta_4$ ; scale
t; time	t; time

## Results and Discussion

The weekly mean larval weight, pupae weight, larval ratio, pupal ratio, mortality ratio of the larvae is presented given in Table 3.

The highest larval weight was determined in the water-supplied group (W) 138 mg and in the control group (CONT) 144 mg. These maximums were obtained in week 12 in the W group and in week 11 in the CONT group. The larvae started to enter the pupal period starting from the week 9 in the CONT group and week 7 in the W group. The mean weight of larvae of the experimental groups started to differ from the week 2 onwards in favor of the water-supplied group. This superiority of water-supplied group continued up to week 9. Larval weights at weeks 9 and 10 of the two groups were not statistically different ( $p > 0.05$ ). In the subsequent weeks, the larvae in the control group weighed more than the water-supplied group ( $p < 0.01$ ). However, the highest larval weight for both treatments were obtained in week 9 and subsequent weeks. In both experimental groups, the differences in weight were negligible at weeks 1, 2 and 3, but they differed at weeks 4, 5, 6, 7 and 8. Weekly mean larval weights in the study were higher than those reported by Ramos-Erorduy et al. (2002); lower than those of Kim et al. (2016a) and Ghaly & Alkoaik (2009) and similar to those of Özsoy et al. (2017) and Kim et al. (2016b). The differences between the values reported in the literature and this study is likely to be due to the nutrient value of the rations, genetic differences of the populations and experimental methods used.

Table 3. Time-dependent variations of the measured parameters in the experimental groups

Time (week)	Group	LR <sup>1</sup> (%)	Weekly larval weight, LW <sup>2</sup> (mg)	PR <sup>3</sup> (%)	PW <sup>4</sup> (mg)	MR <sup>5</sup> (%)	Weekly LW means of groups (mg) <sup>6</sup>	
							W	CONT
1	W	100.0	0.39 ± 0.27 a	0.0	-	0.0	0.39 ± 0.27 f	0.39 ± 0.41 g
	CONT	100.0	0.39 ± 0.41 a	0.0	-	0.0		
2	W	99.0	1.26 ± 0.09 a	0.0	-	1.0	1.26 ± 0.09 f	1.00 ± 0.12 g
	CONT	97.0	1.00 ± 0.12 b	0.0	-	3.0		
3	W	97.0	5.53 ± 1.66 a	0.0	-	3.0	5.53 ± 1.66 f	2.90 ± 0.34 g
	CONT	95.5	2.90 ± 0.34 b	0.0	-	4.5		
4	W	97.0	22.1 ± 4.73 a	0.0	-	3.0	22.1 ± 4.73 e	8.92 ± 1.70 f
	CONT	94.5	8.92 ± 1.70 b	0.0	-	5.5		
5	W	97.0	50.5 ± 4.82 a	0.0	-	3.0	50.5 ± 4.82 d	26.8 ± 4.46 e
	CONT	94.5	26.8 ± 4.46 b	0.0	-	5.5		
6	W	97.0	87.1 ± 7.52 a	0.0	-	3.0	87.1 ± 7.52 c	62.7 ± 9.48 d
	CONT	92.5	62.7 ± 9.48 b	0.0	-	7.5		
7	W	94.0	116.2 ± 6.31 a	1.0	90.4	5.0	116.2 ± 6.31b	97.1 ± 8.07 c
	CONT	91.5	97.1 ± 8.07 b	0.0	-	8.5		
8	W	86.5	131.0 ± 8.46 a	7.5	101.7 ± 13.1	6.0	131.0 ± 8.46 a	122.3 ± 9.48 b
	CONT	89.5	122.3 ± 9.48 b	0.0	-	10.5		
9	W	60.0	134.8 ± 2.99 a	30.0	110.0 ± 14.8	10.0	134.8 ± 2.99 a	136.7 <sup>a</sup> ± 9.25 a
	CONT	83.0	136.7 ± 9.25 a	5.5	107.7 ± 10.9	11.5		
10	W	53.5	136.8 ± 8.85 a	36.5	120.8 ± 15.2	10.0	136.8 ± 8.85 a	140.1 ± 9.05 a
	CONT	75.5	140.1 ± 9.05 a	12.0	112.6 ± 15.2	12.5		
11	W	44.0	133.4 ± 9.81 b	45.0	130.3 ± 30.9	11.0	133.4 ± 9.81 a	144.4 ± 8.05 a
	CONT	69.5	144.4 ± 8.05 a	18.0	121.0 ± 20.9	12.5		
12	W	39.5	137.6 ± 9.11 b	49.5	128.3 ± 13.0	11.0	137.6 ± 9.11 a	144.2 ± 5.07 a
	CONT	59.0	144.2 ± 5.07 a	28.0	125.7 ± 15.4	13.0		

<sup>1</sup> Larval ratio, <sup>2</sup> Results of variance analysis of weekly larval weight means between experimental groups, <sup>3</sup> Pupal ratio, <sup>4</sup> Pupal weight, <sup>5</sup> Mortality ratio, <sup>6</sup> Results of variance analysis of larval weight means within the experimental groups. The difference between the weights of larvae having the same letters is not important (p < 0.05).

The growth of larvae in W and CONT groups was modeled by using Gompertz and logistic growth models, and polynomial functions (quadratic and cubic) as given in Table 2. The model and function equations obtained from W group are given in Table 4 and the curves created using these models are given in Figure 2.

Table 4. General model of growth curve and polynomial functions of larvae in water-supplied group (W)

Model	Equations	$R^2$
Gompertz	$GM(y) = 139.4exp(-exp(3.75 - 0.76t))$	90.0
Logistic	$LM(y) = \frac{136.8 + (-1.13 - 136.8)}{(1 + exp((t - 5.46)/0.88))}$	90.5
Quadratic	$QM(y) = -51.5 - 0.99t^2 + 28.4t$	93.0
Cubic	$CM(y) = 4.78 - 0.41t^3 + 7.03t^2 + 15.0t$	98.0

All of the equations obtained for the water-supplied group (Table 4) have a coefficient of determination of 90% and greater. In addition,  $R^2$  values for each model (Table 4) were similar. In the case of water-supplied larvae, the development of larvae can be defined to a large extent by Gompertz, logistic growth model and polynomial functions. Also, the plots of the growth curves and polynomial functions (Figure 2) are largely consistent with observed values.

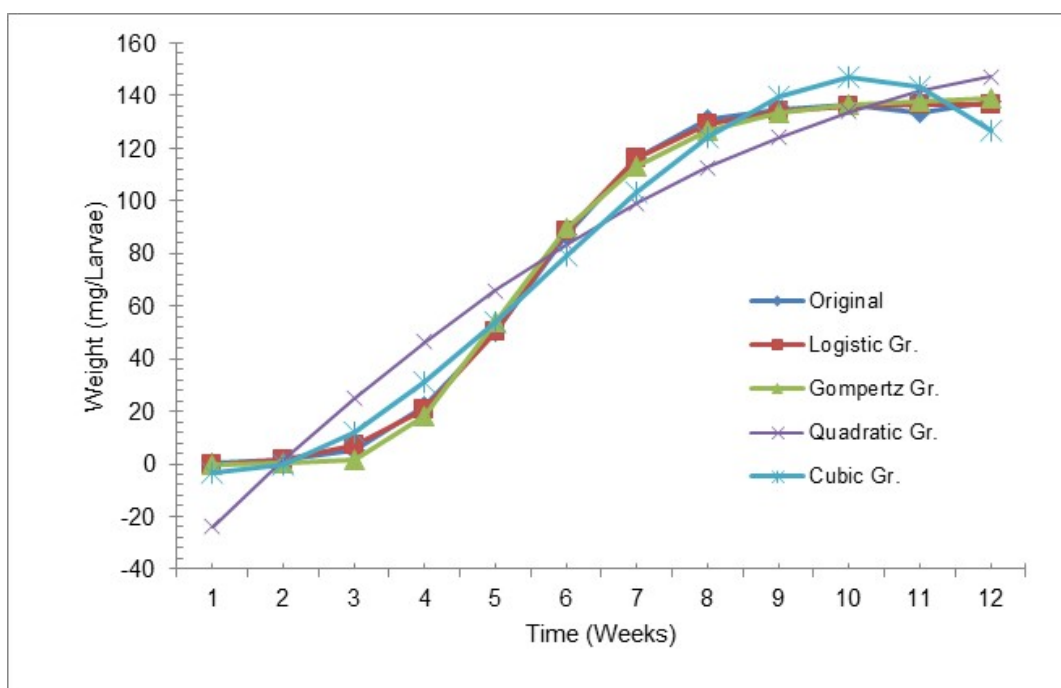


Figure 2. Growth curves of water applied larvae for different functions.

The equations of the growth curves and polynomial functions for the CONT group are given in Table 5. Accordingly, all of the  $R^2$  values for the equations were high (92.5-99.0%). This indicated that the actual growth of *T. molitor* populations can be described to a large extent by the growth curve models and functions used.

The graphs of the general model equations and polynomial functions for the control group presented in Table 5 are shown in Figure 3. The observed values were close to the values predicted by growth curve and polynomial functions. Thus, any of these equations or functions can be successively used to describe the growth of the larval population. However, the quadratic model was less efficient for describing the growth in weeks 1, 3-5, 8, 9 and 12.

Table 5. General model of growth curve and polynomial functions of larvae in control group

Models	Equations	R <sup>2</sup>
Gompertz	$GM(y) = 148.0 \exp(-\exp(4.05 - 0.70t))$	92.5
Logistic	$LM(y) = \frac{144.1 + (-1.16 - 144.1)}{(1 + \exp((t - 6.29)/0.94))}$	92.6
Quadratic	$QM(y) = -40.0 - 0.17t^2 + 18.9t$	93.0
Cubic	$CM(y) = 27.5 - 0.49t^3 + 9.47t^2 + 33.2t$	99.0

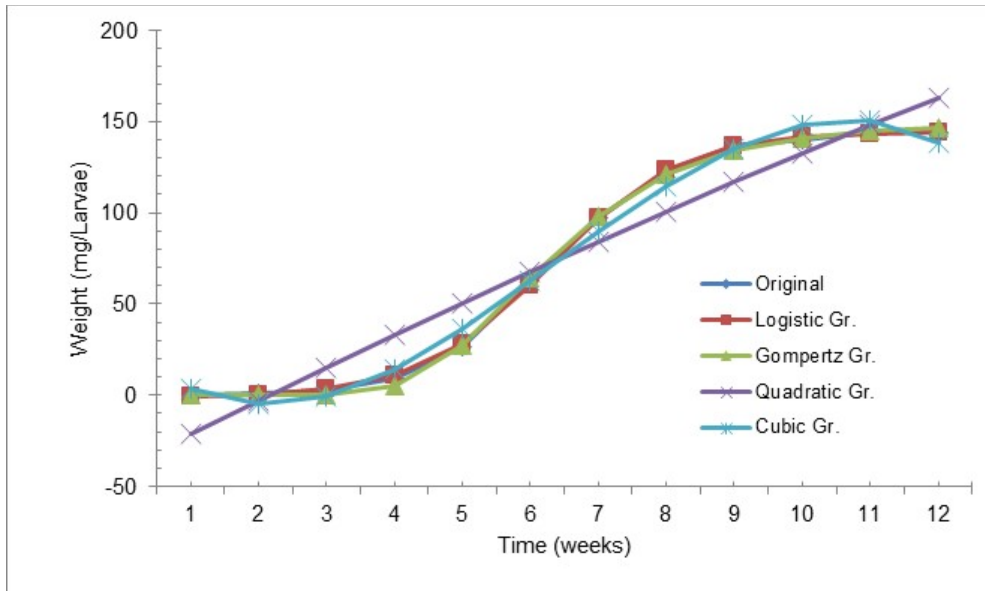


Figure 3. Growth curves of larvae in control treatment for different functions.

The individual weekly water consumption of the larvae found in the two groups are given in Table 6. Also, the differences between the weekly water consumption of larvae are presented in Table 6. There was no significant difference between the water consumption of the larvae from week 2 to 11<sup>th</sup> week. However, the mean weight at week 12 (129 mg/larva) was clearly higher than in the preceding weeks. The fluctuation in the weekly mean water consumption of larvae may have due to various reasons. One reason could be the variation in the larval age. The 48-h mating period might have resulted in considerable variation in hatching time. Another variation source is the lack of synchrony in the molting of each individual. Given that larvae do not consume water during molting, this can result in significant differences in water consumption over the entire period of the experimental.

Water consumption estimations using quadratic and cubic polynomial functions were obtained from the time dependent individual water consumption data (Table 6). Water consumption estimation plots are given in Figure 4. As with the weight curves, quadratic and cubic polynomial functions had very high determination coefficients of 88.6 and 96.4%, respectively, in predicting actual water consumption of the *T. molitor*. This shows that quadratic and cubic polynomial functions can be used to predict the water consumption in in-vitro studies. The cubic function can reliably predict the water requirement of *T. molitor* until 7 weeks old, which was final growth stage before pupation. However, there were relatively higher errors in the preceding weeks due possibly to asynchrony in molting within the population.

However, the correlations between larvae age, water consumption and weights can give us different information. Correlation coefficient matrix for these parameters and water consumption is given in Table 7.

Table 6. The weekly individual water consumption of larvae in the water-supplied group

Group	Time (Week)										
	2	3	4	5	6	7	8	9	10	11	12
W1	64.9	64.8	77.5	75.0	59.1	64.0	56.1	64.5	76.2	94.9	134.2
W2	62.9	56.0	67.6	61.1	57.7	67.4	73.1	72.8	97.1	94.0	123.3
Average*	63.9 c	60.4 c	72.5 bc	68.1 bc	58.4 c	65.7 bc	64.6 bc	68.6 bc	86.6 bc	94.4 b	128.8 a

\* Means followed by the same letter are not significantly different ( $p < 0.05$ ).

Table 7. Pearson's correlation coefficients between larval age, weight and individual water consumption

Parameters	Larval age (Weeks)	Larval weight (mg)
Larval age	1	
Larval weight	0.72**	1
Water consumption	0.93**	0.45*

\* and \*\* indicate significance at  $p < 0.05$  and \*\* at  $p < 0.01$ , respectively.

The correlation coefficients clearly revealed that there were linear relationships among larvae age, larvae weight and water consumption. There was a similar type of relation between larvae weight and water consumption.

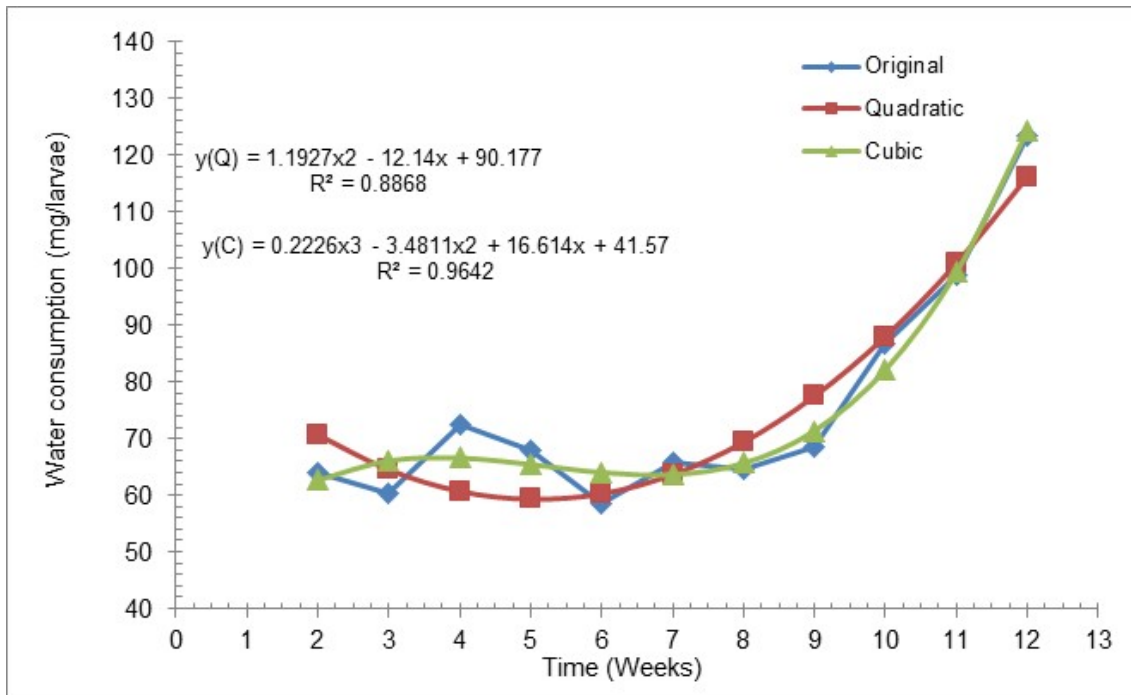


Figure 4. Weekly water consumption plots of quadratic and cubic polynomial functions.

## Conclusions

Gompertz and logistic growth curves, and quadratic and cubic polynomial functions can be successfully used to describe the growth performance of mealworms in both experimental groups. The water requirements of *T. molitor* ranged from 54.4 to 94.4 mg per week with a weekly mean of 70.3 mg. Water consumptions is likely to be linearly related to larvae weight and age.

Using a water diffuser to meet the water needs of *T. molitor* larvae, which is a model organism and can be an alternative protein source in animal feed, can advance pupal development by up to 2 weeks in mealworm cultivation. This means an earlier larval harvest or shorter cultivation period, which would have economic benefits for commercial production. Direct supplementation of water requirement of mealworm larvae by means of a water diffuser can result in a shorter intergenerational period than the control group. This is an important feature for such model organisms. The usage of water diffuser can reduce labor costs as well as enable supplementation of mealworm diets with a variety of water-soluble nutrients, such as amino acids and carbohydrates.

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**Original article (Orijinal araştırma)**

**Geometric morphometric analysis of pronotum shape in two isolated populations of *Dorcadion anatolicum* Pic, 1900 (Coleoptera: Cerambycidae) in Turkey<sup>1</sup>**

*Dorcadion anatolicum* Pic, 1900 (Coleoptera: Cerambycidae)' un Türkiye'deki izole popülasyonlarında pronotum şekil değişiminin geometrik morfometri analizleri

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**Abstract**

The genus *Dorcadion* Dalman, 1817 (Coleoptera: Cerambycidae) comprises species living at ground surface and having lost the ability to fly. Hence the populations of the species are easily isolated from each other. These biological features are considered to be important factors for the acceleration of speciation in this genus. The effects of population isolation can be measured through the morphological characters of samples. The morphological characters that enabled the taxonomists to identify this genus are mainly subjective. This situation causes some systematic problems. *Dorcadion anatolicum* Pic, 1900 (Coleoptera: Cerambycidae) is an endemic species in Turkey and the subspecies status of some populations is debated. The aim of this study was to determine pronotum shape variation via geometric morphometrics from two isolated localities and to contribute to knowledge of the taxonomic and evolutionary status of *D. anatolicum*. The samples were collected from two different localities of Turkey (Kahramanmaraş and Konya Provinces) in March-April 2018. Results of morphometric analysis revealed that the pronotum shape variations of the samples allowed morphological discriminations of populations.

**Keywords:** Coleoptera, *Dorcadion anatolicum*, geometric morphometrics, landmark, pronotum

**Öz**

*Dorcadion* Dalman, 1817 (Coleoptera: Cerambycidae) cinsi türleri toprak yüzeyinde yaşayan ve uçuş kabiliyetleri olmayan türlerdir. Ancak bu türlerin popülasyonları birbirlerinden kolayca izole olabilirler. Bu tarz biyolojik özellikler türleşme sürecini hızlandıran önemli faktörler olarak düşünülebilir. İzole popülasyon en etkileri örneklerin morfolojik karakterleri üzerinden ölçülebilir. Taksonomistlerin cinsin tanımlamasında kullandığı morfolojik karakterler genel olarak öznedir. Bu durum bazı sistematik problemlere neden olmaktadır. Türkiye'ye endemik olan *Dorcadion anatolicum* Pic, 1900 (Coleoptera: Cerambycidae) türünün bazı popülasyonlarının alttür statüsü tartışmalıdır. Bu çalışmanın amacı, iki izole lokaliteden alınan örneklerin pronotum şekil farklılıklarını geometrik morfometri analizleri ile belirlemek ve *D. anatolicum*'un taksonomik ve evrimsel durumuna katkıda bulunmaktır. Çalışmada kullanılan örnekler Türkiye'nin iki farklı popülasyonundan (Kahramanmaraş ve Konya) 2018 yılı mart ve nisan aylarında toplanmıştır. Morfometrik analiz sonuçları örneklerin pronotum şekil değişikliklerinin, popülasyonların morfolojik anlamda ayırımına izin verdiğini ortaya koymuştur.

**Anahtar sözcükler:** Coleoptera, *Dorcadion anatolicum*, geometrik morfometri, homolog referans noktası, pronotum

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## Introduction

Isolated populations are good models for the study of the process of speciation. Studies can focus on genetic, ecological and behavioral variation, but the morphological approach still remains essential because morphology is used to discriminate species. Geometric morphometrics (Bookstein, 1991; Rohlf, 1993; Rohlf & Marcus, 1993) allows quantification of geometric variation of the anatomical structures and the visualization of morphological variation among samples of organisms. The main advantage of geometric morphometrics is that it captures geometry of analyzed objects by landmark coordinates, and preserves this information throughout the analysis (Bookstein, 1996). Unlike earlier techniques, geometric morphometrics has the ability to show shape changes like deviation of displacement vectors from the mean value or deformation grids in original sample space on each of the landmarks. Visualized shape variations can help to characterize populations within species or sexes.

Beetle bodies (or a body part such as head, pronotum, femur and elytra) have been the subject of geometric morphometric analysis in the past (Pizzo et al., 2006; Benitez, 2013; Qubaiova et al., 2015; Zuniga-Reinoso & Benitez, 2015). External shape morphology evolution in two polymorphic sister species of the genus *Onthophagus* Latreille, 1802 (Coleoptera: Scarabaeidae) were analyzed (Pizzo et al., 2006). Body morphometrics can help to characterize populations within species and sexes, as shown by the analysis of *Ceroglossus* Solier, 1848 (Coleoptera: Carabidae) (Benitez, 2013). Body shape variation has also been used for cryptic species of *Nyctelia* Berthold, 1827 (Coleoptera: Tenebrionidae) to enable identification (Zuniga-Reinoso & Benitez, 2015). There are also two remarkable studies of genus *Oreoderus* Burmeister, 1842 (Coleoptera: Scarabaeidae) (Li et al., 2016) and *Ablattaria* Reitter, 1884 (Coleoptera: Silphidae) (Qubaiova et al., 2015) using geometric morphometrics.

With six genera, and 17 subgenera, Dorcadionini is a tribe belonging to the subfamily Lamiinae and includes a total of 278 species of which 227 are endemic in Turkey (Özdikmen, 2016). The members of this genus generally cannot fly due to the atrophy of the flight wings. The larvae of *Dorcadion* appear at the end of May or June and feed on the grass roots. They became pupae after about 13-14 weeks after wintering as mature larvae. The adults emerge after 2-3 weeks and crawl on meadow vegetation (Baur et al., 2002; Kumral et al., 2012).

*Dorcadion anatolicum* Pic, 1900 (Coleoptera: Cerambycidae) is endemic to Central and Southeastern Anatolian Regions of Turkey (Özdikmen, 2010). This species has three subspecies: *Dorcadion anatolicum seydisehirense* Breuning, 1946; *Dorcadion anatolicum brignolii* Breuning, 1946; *Dorcadion anatolicum postapertum* Breuning, 1946 (Sama, 1982). However, these subspecies have not been used recently. According to Özdikmen (2010), the subspecific structure of *D. anatolicum* needs to be clarified.

Considering the biology of *Dorcadion*, isolated populations of various species belonging to this genus may be the results of anthropogenic and environmental effects. Over time, the reflection of isolated gene pools and different environmental interactions on individuals belonging to these isolated populations can be observed quantitatively and qualitatively. It is also important to understand the process of evolution of this species. Thus, we used landmark-based geometric morphometrics method to analyze pronotum shape morphology in two distant localities of *D. anatolicum*.

## Materials and Methods

Samples of the *Dorcadion anatolicum* were collected from two different localities of Turkey (Kahramanmaraş and Konya Provinces) on March-April 2018 (Figure 1).

Sexes of samples were distinguished by the shape and size of the fore tarsus and confirm by using gonads. The study was evaluated on only male individuals to eliminate variations that may arise from sexual dimorphism. A total of 73 specimens (35 from Kahramanmaraş and 38 from Konya) were used in this study. A single image was taken by a camera attached to Leica EZ4HD microscope for each specimen of pronotum.



Figure 1. The localities from where samples were collected. Locality 1: Kahramanmaraş Province (Göksun-Kayseri Road, around Mehmetbey Town, 38°6'36" N, 36°28'17" E); Locality 2: Konya Province (Taşkent District, Avşar Town, Feslekan Plateau, 36°51'9" N, 32°30'44" E) (Anonymous, 2019).

Landmark-based morphometric methods were chosen as they are the most effective technique in learning about the shape information of an organism and eligibility to use powerful statistical methods for testing differences in shape. In this study, 10 landmarks on the pronotum were digitized on photographs using tpsDig 2.17 (Rohlf, 2013). The position of landmarks is given in Figure 2.

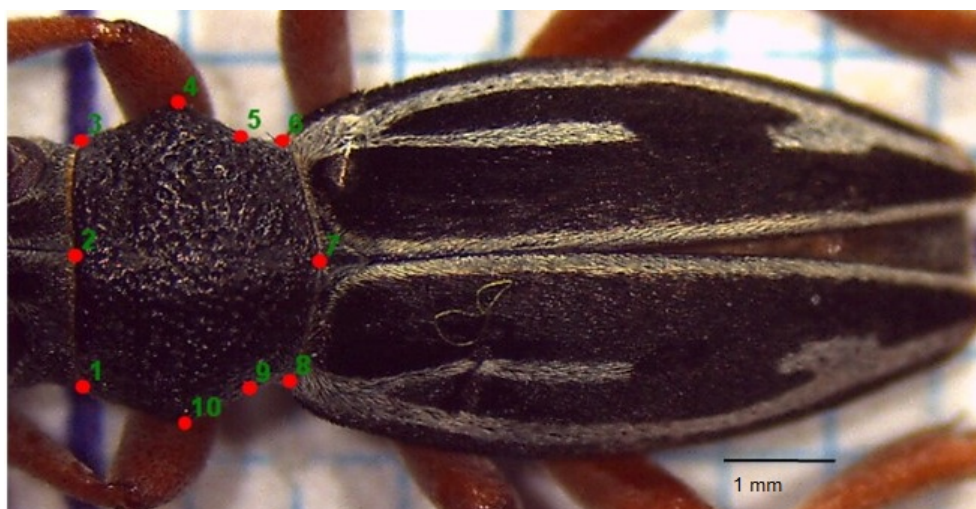


Figure 2. Selected landmarks on *Dorcadion anatolicum* male, representing the dorsal side of the pronotum: 1) Anterior margin left edge; 2) middle of anterior margin; 3) anterior margin right edge; 4) right spine apex; 5) right protuberance posterior limit; 6) posterior right edge; 7) middle of posterior margin; 8) posterior margin left edge; 9) left protuberance posterior limit; and 10) left spine apex.

A generalized Procrustes analysis (GPA) has been developed to superimposition of landmark configurations and to eliminate the effects of translation, rotation and scale (Rohlf, 1999). GPA, multivariate descriptions of the shape variables, relative warp analysis (principal component analysis of the partial warp scores) and visualization of transformation grids allowed us to describe shape variations. Principal components analysis (PCA) performed using the covariance matrix of the Procrustes shape coordinates to summarize multivariate data by building linear combinations of the original variables that are uncorrelated and maximize the sample total amount of variance explained (Viscosi & Cardini, 2011). We used PCA of partial warps using MorphoJ. PC scores were used as dependent shape variables and MANOVA were performed using IBM SPSS 25 to compare the variation of the pronotum shape between the localities.

The discriminant analysis (DA) is probably one of the most widely used statistical method for investigating taxonomic differences and is generally used when only two groups are compared (Viscosi & Cardini, 2011). Discriminant analysis was conducted on the PC scores of pronotum to obtain a classification matrix based on shape variation using IBM SPSS 25. We used the percentages of correct classification to evaluate the discrimination of pronotum shape between populations. To compare overall pronotum size among populations, the centroid size (the square root of the sum of the square distances between each landmark and the centroid) (Bookstein, 1996) was computed for each population and tested by independent samples t-test. Regression analysis were used to explore how shape varies with size. Size correction using log-transformed centroid size effects on shape were tested using MorphoJ (Klingenberg, 2011).

## Results and Discussion

PCA of all specimens explained 46.1% of shape variation within samples by the two first PC axes extracted from the variance-covariance matrix (PC1 explains 26.6% and PC2, 19.5%). A total of up to nine axes were required to cover more than 90% of the shape variation. In the PCA plots, individuals of the two populations were mixed and did not form any distinct cluster (Figure 3).

The multivariate analysis of variance (MANOVA) of pronotum shape showed a significant difference between the two populations (Hotelling's Trace = 0.614,  $F = 4.24$ ,  $p = 0.000$ ). Discriminant function was performed using the first nine PCs to determine the degree of morphological separation between the two groups. The DA conducted on the PC scores of pronotum evidenced that 94.7% of Konya population and 88.86% of Kahramanmaraş population were correctly classified. The percentage of correct classifications were high for all leave-out-one cross-validated groups (Konya 71.1%; Kahramanmaraş 71.4%) (Figure 4). The DA found significant differences between means in Procrustes distances ( $P < 0.0001$ ) for the two populations. Pronotum shape variation measurements has allowed to separate the samples from the two different habitats. The pronotum of Konya samples were observed to be shorter than the Kahramanmaraş samples in anterior and posterior directions and less pointed in lateral edges (revealed with landmarks 2 and 7).

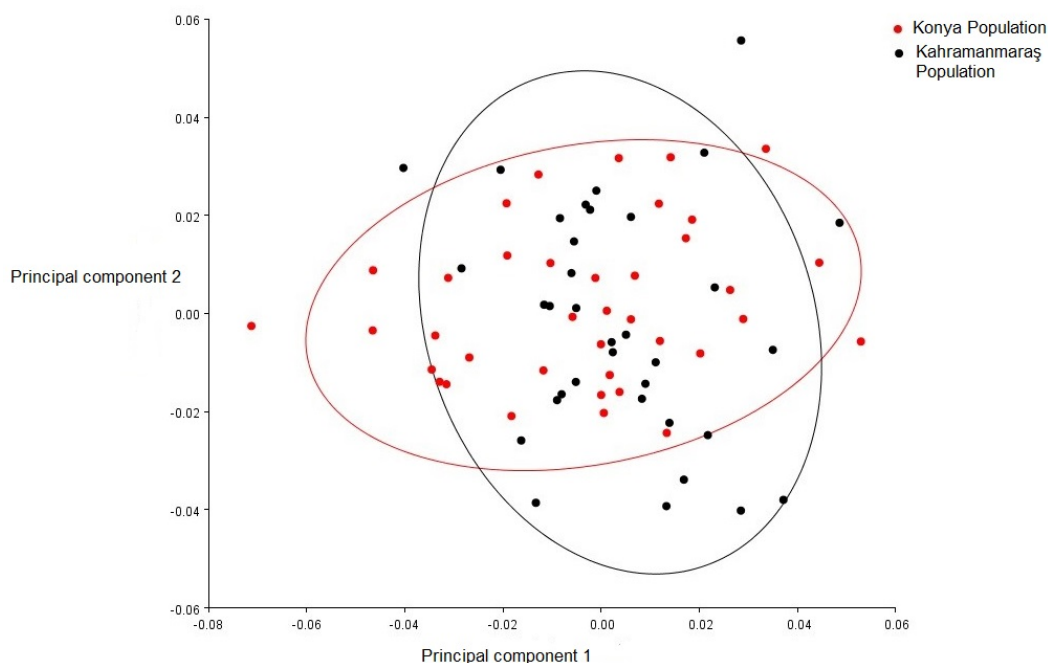


Figure 3. Shape differences between populations, Konya (red) and Kahramanmaraş (black).

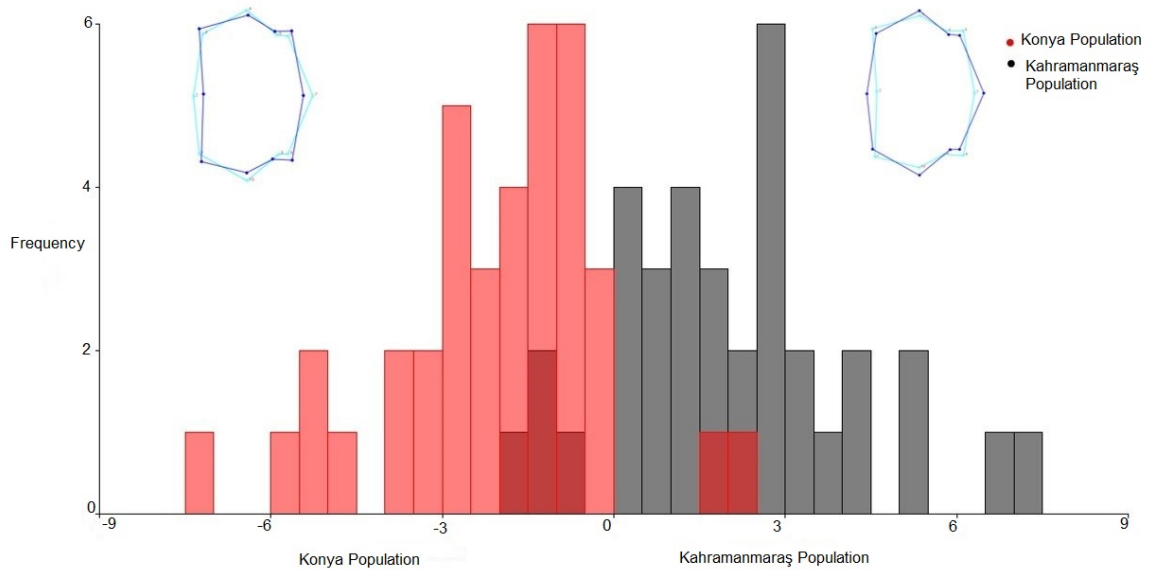


Figure 4. Cross validation scores of shape variables of Konya and Kahramanmaraş populations of the different groups. The violet lines show the extreme shape change in positive and negative direction. Light blue lines are the mean shape and violet lines show the shape change of the pronotum (scales are -5.0 and +5.0, respectively).

An independent samples t-test of centroid sizes of pronotum did not show statistically significant differences between populations ( $t = 1.75$ ,  $p = 0.085$ ) (Figure 5).

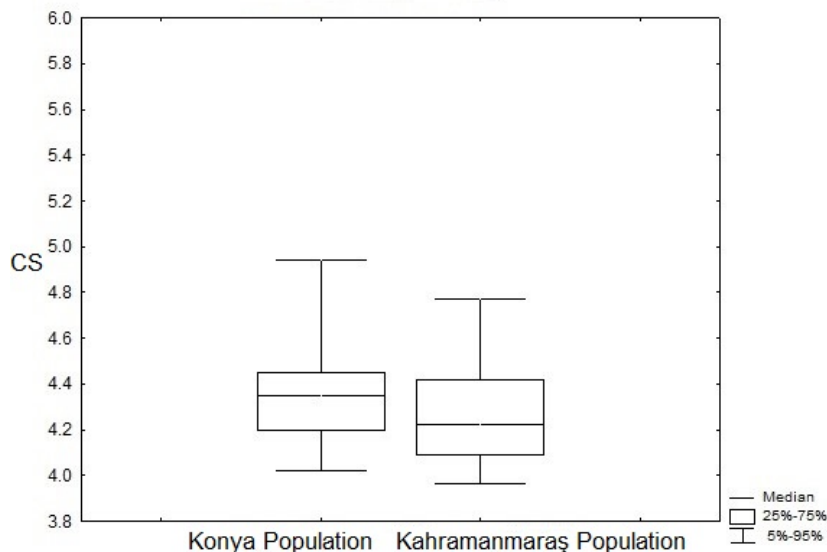


Figure 5. Boxplot of centroid sizes of Konya and Kahramanmaraş populations.

Multivariate regression of the shape variables versus log-transformed centroid sizes were statistically significant with permutation test ( $P = 0.017$ ), but only 3.45% of variance was explained. This test as well as the large overlap between populations in the scatterplot of regression scores versus size (Figure 6) suggests that the effect of size on shape, although weak, is very similar in the two populations.

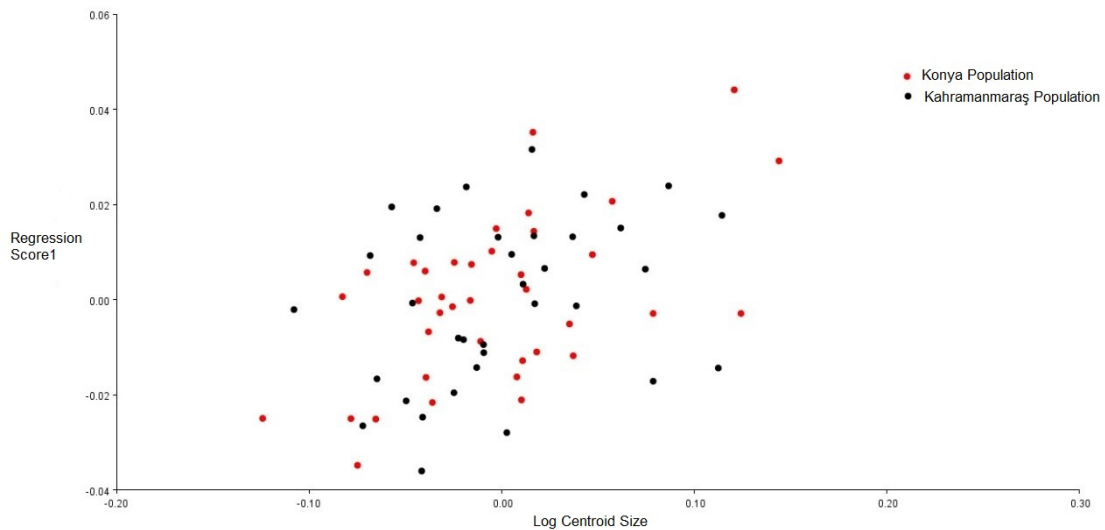


Figure 6. Regression of shape onto log-centroid size pooling within the two analyzed populations.

*Dorcadion anatolicum* is an endemic species in Turkey and its subspecific structure is doubtful (Özdikmen, 2010). The taxonomic studies of the genus *Dorcadion* are generally based on external morphological characters. Identification of species and subspecies are based on similarities and differences in these characters. These characters generally do not have a quantitative basis; therefore, the studies are sometimes based on ambiguous definitions. Compared to other external morphological characters, pronotum shape variations are important taxonomic characters more commonly and safely used in the *Dorcadion* classification (Önalp, 1990).

These two isolated habitats are located on the Taurus Mountain range and have similar geographic characteristics due to the influence of the Mediterranean climate (Figure 1). This situation may be insufficient to observe the selective pressure caused by geographical differences. However, it may be sufficient to evaluate the genetic isolation of these populations (e.g., genetic drift and founder effect). Generally, separated populations of a species can show variation in different taxonomic characters over time. When these differences are found to be sufficient by taxonomists, the populations may be considered as different subspecies. Even if this does not lead to any systematic category for distinction, it is expected that separate populations contain small or large variations to observe the evolutionary dynamic of the species (Rieseberg et al., 2004; Butlin et al., 2008).

Considering the distance between the two localities and the biological characteristics such as mobility and phenology of *Dorcadion* species, we thought that the effects of isolation between habitats could be quantified by measuring the specimens from the two populations. Therefore, geometric morphometrics was applied and the variation of pronotum shape in *Dorcadion* populations was clearly showed by this technique. Despite the fact that centroid size of the two populations were not significantly different ( $t = 1.75$ ,  $p = 0.085$ ) (Figure 5) the information provided by the analysis of shape variables was significantly different between Konya and Kahramanmaraş populations (Hotelling's Trace = 0.614,  $F = 4.24$ ,  $p = 0.000$ ). As suggested by discriminant function analysis 94.7% of Konya population and 88.9% of Kahramanmaraş population were correctly classified. Geometric variation between populations located in landmarks are 2, 4, 7 and 10 respectively. The Kahramanmaraş population showed a different direction of shape variation by forming a prominent pointed structure at the lateral edges. Differences in landmarks 4 and 10 of Kahramanmaraş samples lead to the presence of rose thorn shaped structure on the both lateral edges. Compared to Konya samples, pronotum variation in landmarks 2 and 7 of the Kahramanmaraş

samples gave a clearer ledge in the anterior and posterior media. Therefore, the pronotum produces a triangular recess towards sides of elytra and head. Evaluation of all the pronotum variation, indicated that the pronotum of Konya samples had smoother median points in four planes compared to Kahramanmaraş samples (Figure 4).

Although there many studies have found significant differences in pronotum shape in Coleoptera (Pizzo et al., 2006; Ober & Connolly, 2015; Eldred et al., 2016; Li et al., 2016), geometric morphometrics was applied here to *Dorcadion* for the first time. Combining pronotal shape morphology with phylogenetic analysis Ober & Connolly (2015) showed that the pronotum shape generally reflects phylogenetic relationships, and may be the most important morphological trait for recognizing distinct populations of *Scaphinotus petersi* Roeschke, 1907 (Coleoptera: Carabidae) in the Arizona Sky Islands. Dascălu & Fusu (2012) applied ordinary morphometry analysis to two subspecies of *Dorcadion axillare* Küster, 1847 (Coleoptera: Cerambycidae). Their study showed that all the univariate measures (pronotal length, pronotal width measured at base, maximum elytral length, maximum elytral width and total body length) for the different populations are largely overlapping, even though the differences between the mean values were statistically significant. Based on the results presented here, pronotum shape is an important morphological trait for recognizing distinct populations of *Dorcadion*. It could be said that we observed measurable shape variations of the same magnitude.

Our results also show that geometric morphometric analyses are useful to determine of variations of these habitats at species level. These different shape variations of pronotum can be interpreted as the first observable effects of isolation. While a variation in shape reflects the genetic constitution, the diversity in size of morphological characters between populations usually depends on environmental conditions (Alibert et al., 2001). We also consider that the variation of pronotum shape between these isolated populations occurs as differences in their genetic pools rather than selective pressure. Considering epigenetic effects of the emergence of phenotypes, these quantitative variations can be useful for taxonomical and evolutionary studies. The evaluation of other morphological characters via similar methods and the molecular analysis of genetic structure of these populations may lead to significant outcomes.

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**Original article (Orijinal araştırma)**

## **Life table parameters of cotton mealybug, *Phenacoccus solenopsis* Tinsley, 1898 (Hemiptera: Pseudococcidae) on four different plants**

Pamuk unlubiti, *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae)'in dört farklı bitkide yaşam çizelgesi parametreleri

**Asime Filiz ÇALIŞKAN KEÇE<sup>1</sup>**

### **Abstract**

Cotton mealybug is one of the most widespread invasive mealybug species and causes economically serious damage to vegetables, ornamentals and other agricultural crops. This study was conducted between 2018-2019 in Çukurova University, Faculty of Agriculture, Department of Plant Protection, Nedim Uygun Biological Control laboratory for the determination life table parameters of *Phenacoccus solenopsis* Tinsley, 1898 (Hemiptera: Pseudococcidae) on four host plants (cotton, eggplant, pepper and tomato). This study conducted in climate cabinets at 25±2°C, 60±10% RH and 16:8 h L:D photoperiod. Thirty replicates (individual insects) were used for each host plant. Petri dishes (6 cm diameter) were used for these experiments. Eggplant was determined as the most suitable host plant, with highest values of life table parameters ( $R_0=184$  nymphs/female,  $r_m=0.269/d$ ,  $\lambda=1.31/d$ ,  $GRR=264$  nymphs/female) were obtained with eggplant.

**Keywords:** Cotton mealybug, life table, *Phenacoccus solenopsis*, vegetables

### **Öz**

Pamuk unlubiti sebzeler, süs bitkileri ve diğer ürünlerde ekonomik olarak ciddi zararlara neden olan en yaygın istilacı unlubit türlerinden birisidir. Bu çalışma 2018-2019 yılları arasında Çukurova Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü, Nedim Uygun Biyolojik Mücadele Laboratuvarında, *Phenacoccus solenopsis* Tinsley, 1898 (Hemiptera: Pseudococcidae)'in dört farklı konukçu bitki (pamuk patlıcan, biber ve domates) üzerinde yaşam çizelgesi parametrelerinin hesaplanması amacıyla yapılmıştır. Çalışma 25±2°C, 60±10% RH and 16:8 h L:D gün aydınlatmalı iklim kabinlerinde, 30 tekerrürlü olarak kurulmuştur. Bu denemeler için 6 cm'lik petri kapları kullanılmıştır. En yüksek yaşam çizelgesi parametrelerine ( $R_0=184$  nimf/dişi,  $r_m=0.269/d$ ,  $\lambda=1.31/d$ ,  $GRR=264$  nimf/dişi) sahip olan patlıcan en uygun konukçu bitki olarak belirlenmiştir.

**Anahtar sözcükler:** Pamuk unlubiti, yaşam çizelgesi, *Phenacoccus solenopsis*, sebzeler

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## Introduction

*Phenacoccus solenopsis* Tinsley, 1898 (Hemiptera: Pseudococcidae) (cotton mealybug), was first described by Tinsley (1898) from specimens collected in New Mexico, USA. Cotton mealybug has been found in Cyprus, Egypt, Iran, Israel, and Turkey in the Palearctic Region (Kaydan et al., 2013; García Morales et al., 2016). According to Fand & Suroshe, (2015), this pest has caused damage to 202 host plant species from 55 families. In Turkey, *P. solenopsis* has been detected in the whole Eastern Mediterranean Region on 72 host plants species in 55 families (Çalışkan-Keçe & Ulusoy, 2018). In addition, *P. solenopsis* has caused more the 60% economic losses in cotton (*Gossypium hirsutum* L.) between 2005-2009. *Phenacoccus solenopsis* is known as one of the most a harmful pest of vegetables and ornamental and other agricultural crops plants (Fand & Suroshe, 2015) and is one of the most widespread invasive mealybug species.

*Phenacoccus solenopsis* can spread rapidly to uninfested areas, via international trade (Fand & Suroshe, 2015). Due to the high reproductive ratio of *P. solenopsis* and inefficient control methods in new regions, the cotton mealybug can reach unexpectedly high populations and cause serious damage. As for other mealybug species, *P. solenopsis* is also covered with white powdery wax, and this negatively affects the control strategies. Therefore, chemical control is not an effective solution (Joshi et al., 2010). Detailed information about the life cycle of *P. solenopsis* can help to determine the best timing for application of insecticides and biological control agents.

A number of studies of the biology of *P. solenopsis* have been conducted in recent times (Fand & Suroshe, 2015). The biology of *P. solenopsis* has been studied under different temperature conditions by several research groups (Kumar & Kontodimas, 2012; Prasad et al., 2012; Kumar et al., 2013). In addition, host plant suitability has been studied (Çalışkan et al., 2016; Dogar et al., 2018; Nagrare et al., 2018). Nevertheless, for the reasons given above, comprehensive studies of the life table parameters of *P. solenopsis* on different vegetable crops should be undertaken to help develop control strategies in agriculture areas.

This study aimed to determine biological characteristics (developmental time, longevity, preoviposition, oviposition and postoviposition period) and the life table parameters of *P. solenopsis* on four plant species, cotton, eggplant (*Solanum melongena* L.), pepper (*Capsicum annuum* L.) and tomato (*Solanum lycopersicum* L.) under laboratory conditions.

## Materials and Methods

### Host plant culture

The four host plants (cotton, eggplant, pepper and tomato) used were obtained from Çukurova University, Faculty of Agriculture, Plant Protection Department, Nedim Uygun Biological Control Laboratory between 2018-2019. The plants were cultivated in a climate room (25±2°C, 60±10% RH and 16:8 h L:D photoperiod) without any insecticide application.

### Mealybug culture

*Phenacoccus solenopsis* was cultured on sprouted potatoes under laboratory conditions (25±2°C, 60±10% RH and 16:8 h L:D photoperiod).

### Experiments

The experiments were conducted in a climate room (25±2°C, 60±10% RH and 16:8 h L:D photoperiod). Thirty replicates (individual insects) were used for each host plant.

Petri dishes (6 cm diameter) were used for these experiments. Preoviposition, oviposition and postoviposition stages of the females, and the survival parameters for both sexes were recorded daily.

### Statistical analysis

One-way ANOVA and Duncans test ( $p \leq 0.05$ ) were used for analysis of data. Statistical analysis was performed by using IBM SPSS STATISTICS 23.

Population growth parameters of *P. solenopsis* on the four plant species (cotton, eggplant, pepper and tomato) were analyzed with an age-stage, two-sex life table (Chi & Liu, 1985; Chi, 1988). TWOSEX-MS Chart (Chi 2014) was used to analyze data of life table.

## Result and Discussion

The cotton mealybug completed its life cycle on cotton, eggplant, pepper and tomato. Mean developmental periods of preadult stages were found between 15.2 and 27.7 d for females and between 16.7 and 23.8 d for males (Table 1). The shortest female developmental time obtained was  $15.2 \pm 0.27$  d on eggplant and  $16.7 \pm 0.20$  d for males on cotton. Female and male developmental times (preadult) on eggplant and cotton was longer than when mealybug reared on tomato and pepper. Significant differences were found between plant species for developmental time of preadult stages ( $p < 0.05$ ).

Table 1. Developmental time (mean $\pm$ SE) of *Phenacoccus solenopsis* individuals on four host plants

Host plants	First nymphal stage		Second nymphal stage		Third nymphal stage		Total preadult	
	Female	Male	Female	Male	Female	Male	Female	Male
Cotton	6.8 $\pm$ 0.26 a (n=16)	6.8 $\pm$ 0.26 a (n=13)	4.5 $\pm$ 0.26 a (n=16)	4.1 $\pm$ 0.29 a (n=13)	4.6 $\pm$ 0.22 a (n=16)	5.9 $\pm$ 0.10 a (n=13)	15.6 $\pm$ 0.40 a (n=16)	16.7 $\pm$ 0.20 a (n=13)
Eggplant	6.4 $\pm$ 0.14 a* (n=18)	7.0 $\pm$ 0.39 a (n=12)	4.5 $\pm$ 0.25 a (n=18)	4.7 $\pm$ 0.43 a (n=12)	4.3 $\pm$ 0.11 a (n=18)	6.0 $\pm$ 0.17 a (n=12)	15.2 $\pm$ 0.27 a (n=18)	17.6 $\pm$ 0.56 a (n=12)
Pepper	6.6 $\pm$ 0.27 a (n=15)	6.3 $\pm$ 0.33 b (n=15)	3.7 $\pm$ 0.19 b (n=15)	5.5 $\pm$ 0.35 b (n=15)	5.3 $\pm$ 0.25 b (n=15)	7.7 $\pm$ 0.26 b (n=15)	15.9 $\pm$ 0.42a (n=15)	19.6 $\pm$ 0.51 b (n=15)
Tomato	8.9 $\pm$ 0.65 b (n=14)	8.6 $\pm$ 0.63 c (n=16)	10.4 $\pm$ 1.26 c (n=14)	9.2 $\pm$ 1.15c (n=16)	8.5 $\pm$ 0.94 c (n=14)	6.0 $\pm$ 0.43 a (n=16)	27.7 $\pm$ 1.81 b (n=14)	23.7 $\pm$ 1.09 c (n=16)

\* Columns followed by the same letters are not statistically different according to the Duncan (5%) test.

Figure 1 show survival rates of *P. solenopsis* on different host plants. This figure helps to interpret each stage of *P. solenopsis* in terms of survival rates. According to Figure 1, eggplant and cotton were better hosts than pepper and tomato. Life table parameters are given in Figure 2.

Host species affected preoviposition, oviposition and postoviposition periods of *P. solenopsis* ( $p < 0.05$ ) (Table 2). The longest oviposition period was found on eggplant ( $15.9 \pm 1.18$  d) and the shortest on tomato ( $8.31 \pm 1.61$  d). In addition, fecundity of *P. solenopsis* differed according to host ( $p < 0.05$ ) (Table 2). The highest fecundity was obtained on eggplant ( $307 \pm 23.1$ ) and the lowest on tomato ( $95.1 \pm 18.8$ ). In addition, significant difference was found for longevity of females and males on different hosts ( $p < 0.05$ ) (Table 3).

The maximum values for life table results were obtained on eggplant ( $r_m = 0.269/d$ ,  $\lambda = 1.31/d$ ,  $R_0 = 184$  nymphs/female and  $GRR = 264$  nymphs/female) (Table 2).

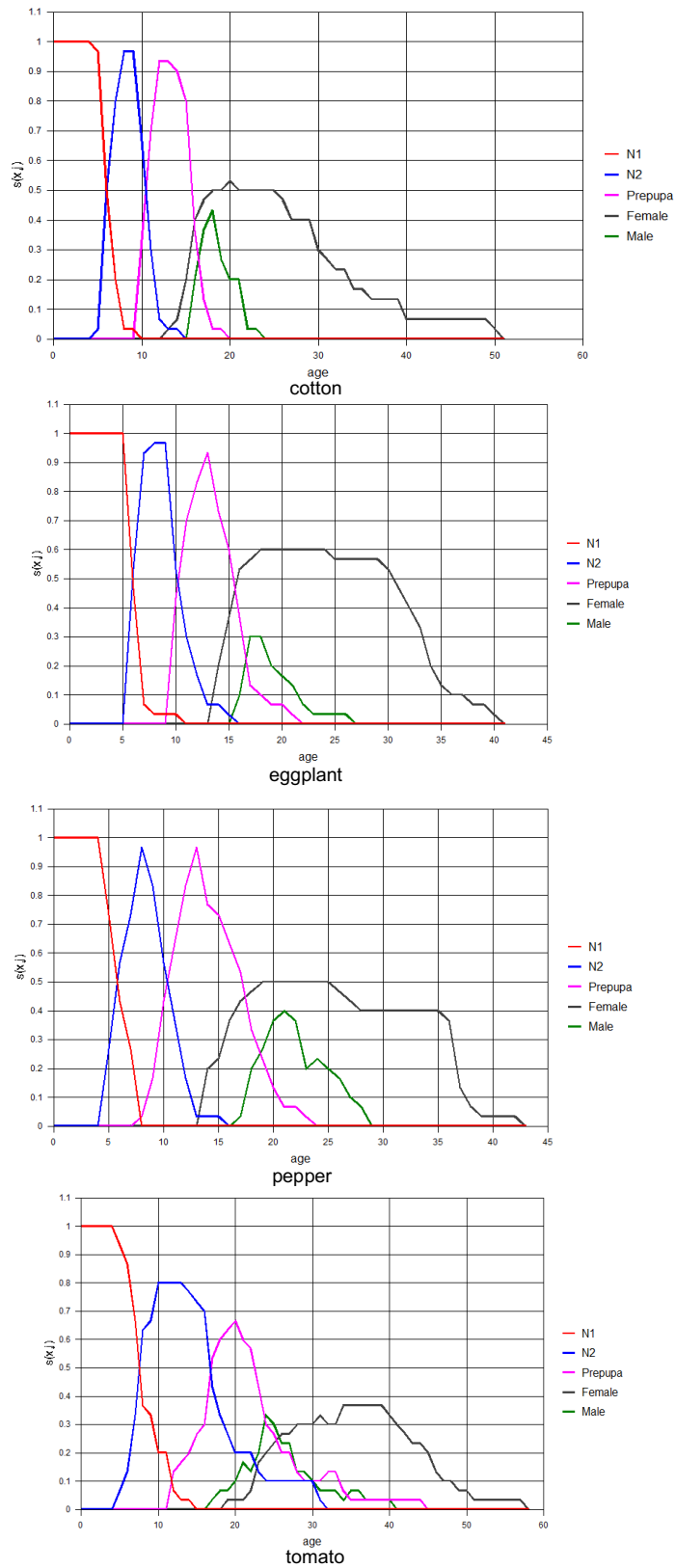


Figure 1. Survival ratio of *Phenacoccus solenopsis* for each stage on four host plants.

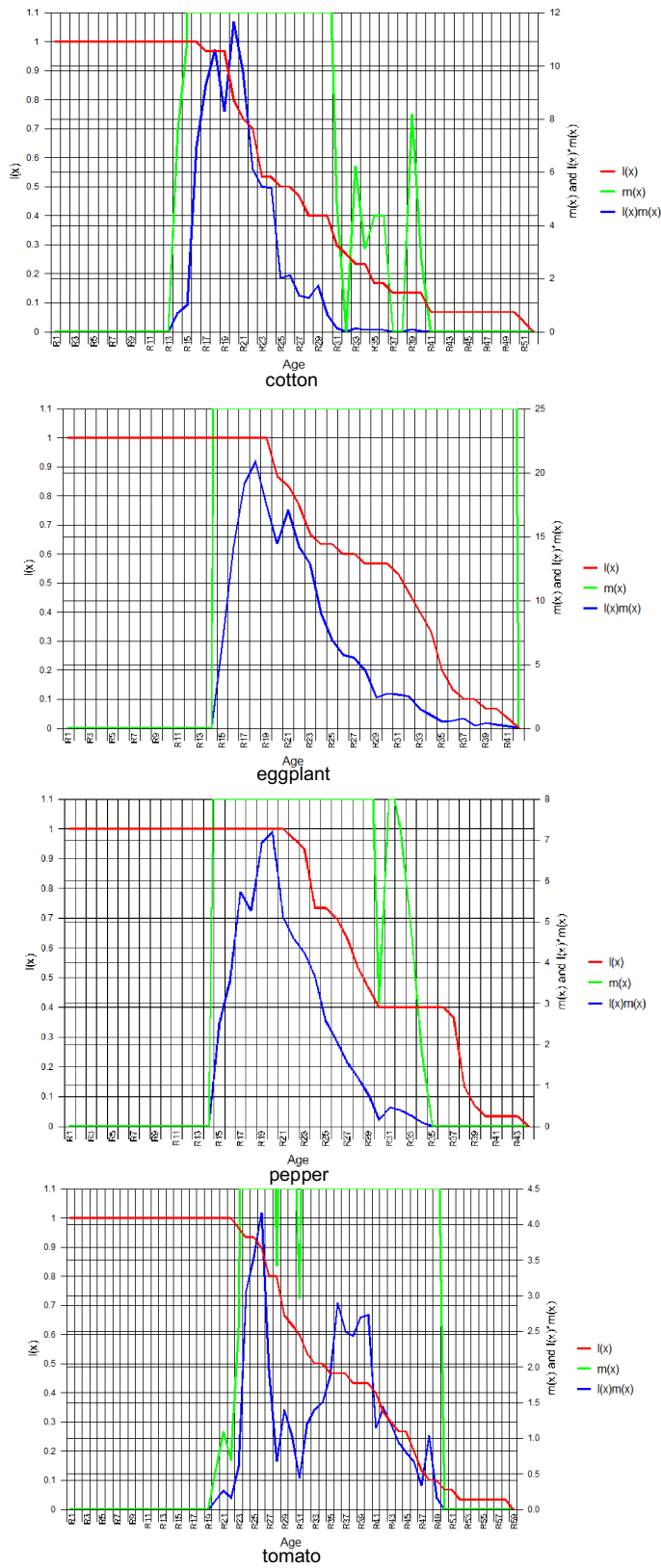


Figure 2. Age specific life table graphics ( $l_x$ ,  $m_x$ ,  $l_x m_x$ ) of *Phenacoccus solenopsis* on four host plants.

Table 2. The life table parameters of *Phenacoccus solenopsis* on four host plants (n =30, mean±SE)

Host plant	$r_m$	$\lambda$	$R_0$	T	GRR
Cotton	0.230±0.13 c	1.26±0.58 b	85±9.0 c	19.4±1.48 a	118±18.3 c
Eggplant	0.269±0.23 d	1.31±0.25 c	184±14.6 d	19.3±4.36 a	264±14.2 d
Pepper	0.207±0.19 b	1.23±0.22 b	58±10.6 b	19.6±0.93 a	67±3.0 a
Tomato	0.123±0.10 a	1.13±0.33 a	44±5.7 a	30.9±1.70 b	98±2.9 b

\*Within columns means followed by the same letter are not statistically different according to the Duncans test (5%).

Table 3. Reproduction and survival parameters of *Phenacoccus solenopsis* on four host plants (n=30, mean±SE)

Host plant	Pre-oviposition	Oviposition	Post-oviposition	Fecundity	Longevity	
					Female	Male
Cotton	15.9± 0.42 a	9.8±0.98 c	7.6±2.28 b	159±29.8 c	17.8±1.90 b	4.0±0.44 b
Eggplant	15.2±0.27 a	15.9±1.18 d	2.8±0.57 a	307±23.1 d	18.6±0.82 b	3.5±0.34 a
Pepper	15.6±0.46 a	8.7±1.37 b	11.1±1.8 6 d	117±24.3 b	20.0 ±1.29 c	5.2±0.30 c
Tomato	28.1±1.92 b	8.3±1.61 a	8.6±1.73 c	95±18.8 a	16.3±1.20 a	5.1±0.31 c

\*Within columns means followed by the same letter are not statistically different according to the Duncans test (5%).

According to results of this study, biological features of *P. solenopsis* changed significantly with different host plant species. Eggplant was the most suitable host in this study. Development time of cotton mealybug on eggplant (15.2 d) and cotton (15.6 d) were superior to pepper and tomato. Sana-Ullah et al. (2011) found that females of *P. solenopsis* developed in 17 d on *Hibiscus rosa-sinensis* L. at 25°C and 65% RH. Also, Dogar et al. (2018) reported that, *P. solenopsis* developed faster on *H. rosa-sinensis* than other hosts. In addition, Nagrare et al. (2018) found that cotton is one of the most suitable host plants for cotton mealybug because it completed its development in 16.6 d.

Pre-oviposition, oviposition and postoviposition durations of cotton mealybug were affected by host species. The highest fecundity and longest longevity were found on eggplant (307 nymphs/female, 33.8 d), followed by cotton. Dogar et al. (2018) found that the highest fecundity of *P. solenopsis* on cotton. Çalışkan et al. (2016) found that *Hibiscus syriacus* L. and *H. rosa-sinensis* were particularly suitable host plants for cotton mealybug fecundity.

Each host plant species was different for *P. solenopsis* population parameters. Eggplant was the best host in this study. The intrinsic rate of increase ( $r_m$ ) and net reproduction rate ( $R_0$ ) are one of the most important parameters for determining the population increase of insects (Goundoudaki et al., 2003). The data presented here had the highest values of  $r_m$  and  $R_0$  with eggplant and cotton. Whereas, the lowest values were on tomato and pepper. Therefore, eggplant and cotton are better hosts than pepper and tomato for *P. solenopsis*.

Various other studies have determined life table parameters of *P. solenopsis* on different host plants under laboratory conditions (Fand et al., 2010; Guan et al., 2012; Kedar et al., 2013; Kumar et al., 2013; Çalışkan et al., 2016; Dogar et al., 2018; Nagrare et al., 2018). Nagrare et al. (2018) found that the highest net reproductive rate was on cotton (284 females/female/generation) and the lowest value was obtained on tomato. According to Kumar et al. (2013), the highest  $r_m$  and  $R_0$  values were on cotton (0.215/d and 141 nymphs/female). In addition, Çalışkan et al. (2016) found that the highest  $r_m$  and  $R_0$  values were on *H. syriacus* and *H. rosa-sinensis*. Moreover, Dogar et al. (2018) showed that the highest  $r_m$  values were on *H. rosa-sinensis*.

According to the results of this study, *P. solenopsis* has potential to cause economically serious damage to vegetable and cotton crops. If natural enemies are not sufficient, *P. solenopsis* will seriously damage vegetable and cotton crops. Owing to broad host range of *P. solenopsis*, this mealybug can spread rapidly within and between agricultural areas.

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## Original article (Orijinal araştırma)

# Efficacy of entomopathogenic nematodes against neonate larvae of *Capnodis tenebrionis* (L., 1758) (Coleoptera: Buprestidae)<sup>1</sup>

*Capnodis tenebrionis* (L., 1758) (Coleoptera: Buprestidae)'in ilk dönem larvalarına karşı entomopatojen nematodların etkinliği

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## Abstract

Entomopathogenic nematodes (EPN) have a high potential for control of pests living in isolated places such as underground or galleries. In this study, mortality rates of *Capnodis tenebrionis* (L., 1758) (Coleoptera: Buprestidae) larvae from four EPN species *Steinernema affine* Boviën, 1937, *Steinernema carpocapsae* Weiser, 1934, *Steinernema feltiae* Filipjev, 1934 (Rhabditida: Steinernematidae) and *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) collected from Turkey under controlled conditions were determined. EPN used in the study were cultured on *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae). Adults of *C. tenebrionis* were collected from the orchards of Çanakkale Province and, eggs and larvae were cultured under controlled conditions. Three densities of EPN species, viz. 50, 500 and 1000 infective juveniles/*C. tenebrionis*, were applied in 12-well plates. Cherry saplings were planted into pots with sterilized soil mixture and 10 neonate larvae of *C. tenebrionis* added to each pot. To each pot, 40,000 infective juveniles were applied for each EPN species in 10 ml of water. Mortalities of *C. tenebrionis* larvae were determined 1, 3, 5 and 7 d after application. In the plates, mortality of *C. tenebrionis* larvae increased with time after EPN application. For all application rates, mortality of *C. tenebrionis* larvae was 100% by day 5. Mortality of *C. tenebrionis* larvae ranged between 50 and 90% depending on species and time in pots. Efficacy studies were conducted in 2016 in Çanakkale. Research on the efficacy of EPN species that have a high mortality under controlled conditions is important to determine their potential to control the target pest.

**Keywords:** *Capnodis tenebrionis*, *Heterorhabditis bacteriophora*, *Steinernema affine*, *Steinernema carpocapsae*, *Steinernema feltiae*

## Öz

Toprak altı ve galeriler gibi izole alanlarda yaşayan zararlıların mücadelesinde entomopatojen nematodlar (EPN) yüksek bir potansiyele sahiptir. Bu çalışmada Türkiye'den elde edilen dört entomopatojen nematod (EPN) türünün *Steinernema affine* Boviën, 1937, *Steinernema carpocapsae* Weiser, 1955, *Steinernema feltiae* Filipjev, 1934 (Rhabditida: Steinernematidae) ve *Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae) kontrollü koşullarda *Capnodis tenebrionis* (L., 1758) (Coleoptera: Buprestidae) larvalarında meydana getirdikleri ölüm oranları belirlenmiştir. Çalışmada kullanılan EPN'ler laboratuvarında *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae) üzerinde üretilmiştir. *Capnodis tenebrionis* erginleri Çanakkale ili meyve bahçelerinden toplanmış ve kontrollü koşullarda yumurta ve larvaları üretilmiştir. EPN türlerinin 50, 500 ve 1000 infeksiif juvenil/*C. tenebrionis* olmak üzere 3 farklı yoğunluğu 12 hücreli kuyucuklarda uygulanmıştır. Saksı denemelerinde, sterilize edilmiş toprak karışım içeren saksılara kiraz fidanları dikilmiş ve her saksıya 10'ar adet 1. dönem *C. tenebrionis* larvası bulaştırılmıştır. Kiraz fidanları sterilize toprak karışımı içeren saksılara dikilmiş ve her saksıya 10 adet 1. dönem *C. tenebrionis* larvası aktarılmıştır. Her saksıya her bir EPN türü için 40.000 infeksiif juvenil 10 ml su içerisinde uygulanmıştır. *C. tenebrionis* larvalarının ölüm oranları uygulamadan 1, 3, 5 ve 7 gün sonra belirlenmiştir. Tüm uygulama oranlarında *C. tenebrionis* larvalarının ölüm oranı 5. günde %100'dür. *Capnodis tenebrionis* larvalarının ölüm oranları türe ve zamana bağlı olarak %50-90 arasında değişiklik göstermiştir. Etkinlik çalışmaları 2016 yılında Çanakkale ilinde gerçekleştirilmiştir. Kontrollü koşullar altında yüksek ölüm oranına sahip olan EPN türlerinin etkinliklerinin araştırılması hedef zararlının kontrolü için potansiyellerini belirlemek için önemlidir.

**Anahtar sözcükler:** *Capnodis tenebrionis*, *Heterorhabditis bacteriophora*, *Steinernema affine*, *Steinernema carpocapsae*, *Steinernema feltiae*

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## Introduction

*Capnodis tenebrionis* (L., 1758) (Coleoptera: Buprestidae) is an important pest in stone fruit orchards. Larvae of the pest can cause the death of trees and yield loss by burrowing to form galleries in the trunk of trees. This pest has been reported in Spain, Italy, Turkey, Iran, Syria, North Africa, Israel, France and Palestine (David'yan, 2003, Abu Jbara, 2005, Bonsignore et al., 2008; Şahin & Gözel, 2017).

Chemical control is effective only against the adult stage, as neonate larvae of the pest are under the soil and following larval stages feed under the bark of the tree. Consequently, chemical control of the adults is not an effective control method, so alternative methods such as entomopathogenic nematodes (EPN) are needed. EPN kill their hosts with the help of symbiotic bacteria species *Xenorhabdus* spp. Thomas & Poinar, 1979 and *Photorhabdus* spp. (Boemare et al. 1993) (Bacteria: Enterobacteriales) (Akhurst, 1993). Generally, each EPN species is associated with only one bacterial species, except some *Steinernema* spp. (Steinernematidae: Rhabditida), which share the same *Xenorhabdus* bacteria (Akhurst, 1993). EPN reproduce in the cadaver of their insect host under suitable conditions created by the symbiotic bacteria. Several EPN generations can be completed in a single host. Infective juveniles (IJ), the only life stage of EPN that they can move freely in the soil, are produced in the event of food depletion and are released from the cadaver (Grewal et al., 1997).

There are several studies using EPN against *C. tenebrionis*, such as the study of Marannino et al. (2003) in which they reported 100% mortality of *C. tenebrionis* larvae from *Steinernema carpocapsae* Weiser, 1955 and *Heterorhabditis bacteriophora* Poinar, 1976. Also, Garcia del Pino & Morton (2005) has reported that the mortality of *C. tenebrionis* larvae caused by *Steinernema arenarium* Artyukhovsky, 1967 was 90%, which was significantly higher than *Steinernema feltiae* Filipjev, 1934 (76%), *H. bacteriophora* (76%) and *S. carpocapsae* (59%). Other studies have obtained similar results (Hourieh et al., 2008; Martinez de Altube et al., 2008; Morton & Garcia del Pino, 2008, 2009; Yiğit et al., 2015; Şahin et al., 2018a, b).

EPN have an important place in biological control of underground pests due to their ability to survive for long periods and their active behavior in searching for hosts in soil. Also, according to Bedding et al. (1983) and Kaya (1985) EPN can be effective against pests that live in sheltered habitats like galleries.

In this study, four native EPN isolates collected from Turkey were studied at different application rates to determine their efficacy against *C. tenebrionis* in 12-well plates and potted saplings under controlled conditions.

## Materials and Methods

### EPN mass rearing

In this study, four native species of nematodes; *Steinernema affine* Boviën, 1937 (isolate 47), *S. carpocapsae* (isolate 1133), *S. feltiae* (isolate 96) and *H. bacteriophora* (isolate 1144) were used against neonate larvae of *C. tenebrionis*. All isolates were reared in the last instar of *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae), which is the most commonly used insect host of EPN (Bedding & Akhurst, 1975; Kaya & Stock, 1997). Before using the nematodes, their viability and numbers were checked under the stereomicroscope Leica DM1000.

### Rearing of *Capnodis tenebrionis* larvae

Adults of *C. tenebrionis* were collected from neglected cherry nurseries in Çanakkale Province. These adults were transferred to the laboratory in sampling boxes with fresh apricot shoots.

Rearing technique of *C. tenebrionis* was modified from the method developed by Garrido et al. (1987). *Capnodis tenebrionis* adults were placed into insect rearing cages with young apricot shoots from chemically-untreated orchards for adult feeding.

Sterilized (121°C, 12 h) and screened (1 mm) sand was used as the egg laying medium for females by spreading the sand at 2 cm deep on the bottom of cages. Sand was checked daily for eggs and was screened with a sieve. Particles remaining on the sieve were controlled under binocular microscope and

eggs were placed into Petri dishes with a soft brush. These Petri dishes were placed into a climate chamber (25°C, 60-70% RH, 16:8 h L:D photoperiod) for incubation. Hatched neonate larvae were transferred to another Petri dish to be used in EPN experiments with daily controls.

### EPN efficacy experiments

#### EPN inoculation of *Capnodis tenebrionis* in the laboratory

The method of Garcia del Pino and Morton (2005) was used in the experiment. Efficacy of EPN on *C. tenebrionis* was investigated in plates with 12 wells (3 cm diameter). Each well (3 x 4 cm) in the plates was filled with 6 cm<sup>3</sup> sterilized sand with one 2-d-old *C. tenebrionis* neonate larva added. Each EPN isolate was applied in three application rates, 50, 500 and 1000 IJ/*C. tenebrionis*, in 100 µl distilled water with 12 replicates. The experiment was repeated two times on different days. Distilled water was used as a control treatment.

To determine the efficacy of the EPN, mortality rates of the larvae were calculated by examining individuals 1, 3, 5 and 7 d after establishment of the experiment to observe the change in mortality related to time, according to Morton and Garcia del Pino (2009). Dead individuals were transferred to White traps with a soft tipped brush to verify that death of the larvae was caused by EPN (White, 1927). Efficacy tests of EPN were conducted in at 23±2°C in the dark. IJ emerging from cadavers were photographed.

#### EPN inoculation of *Capnodis tenebrionis* in cherry saplings

Cherry cv. Regina saplings (grafted on cv. Maxima rootstocks) were planted into pots (30 x 30 cm) containing a sterilized (120°C, 12 h) soil mixture. The soil mixture was prepared with sand and soil, and 750 g soil mixture was placed into each pot and the pots were watered. Two d after planting, the saplings, 10 *C. tenebrionis* neonate larvae were transferred to the soil surface near the root collar of each sapling. The saplings were stored in a climate chamber at 23±2°C and 12:12 h L:D photoperiod for a day. Then the EPN were applied at 25 IJ/cm<sup>2</sup>, which is a typical rate used for releasing EPN (Shields, 2015), with a total of 40,000 IJ per pot in 10 ml water. This rate was calculated based on the soil quantity in order to achieve homogeneous dispersal of the EPN. Given that too much water can kill *C. tenebrionis* larvae, soil surface was just dampened every 2 d to ensure EPN survival. Saplings were uprooted 1, 3, 5 and 7 d after EPN application and the number of living and dead *C. tenebrionis* larvae in the soil counted.

Dead larvae were transferred to White traps to verify that death of the larvae was caused by EPN. Also, the damage on the roots of the sapling and the number of larvae inside the roots were noted and photographed. Efficacy studies were conducted in 2016 in Çanakkale.

### Statistical analysis

Data from the study was analyzed with repeated measures ANOVA using SPSS® 23 software. The Tukey's multiple comparison test ( $P < 0.01$ ) was used to determine the differences between days, rates and species in MSTATC®.

## Results

#### *Capnodis tenebrionis* larval mortality in the laboratory

Mortality rates of *C. tenebrionis* larvae caused by the different EPN isolates on different days after EPN application are given in Table 1. Dead larvae were not observed until day 7 in all control treatments. By day 5, all EPN isolates at all application rates had killed 100% of the neonate larvae of *C. tenebrionis*. Although larval mortality of *S. feltiae*, *S. carpocapsae* and *H. bacteriophora* reached 100% by day 5, 100% mortality of *S. affine* with 50 IJ had occurred by day 3. With 500 and 1000 IJ, 100% of *C. tenebrionis* neonate larvae of *S. feltiae* were dead by day 1. *Steinernema affine* reached 100% mortality by day 3, but it took 5 d for *S. carpocapsae* and *H. bacteriophora* at both rates.

Table 1. Mortality of *Capnodis tenebrionis* larvae with different entomopathogenic nematodes at different application rates and days after application (mean±se)

Entomopathogenic nematode	Day	50 IJ	500 IJ	1000 IJ
<i>Steinernema feltiae</i>	1	33.3±6.8 B a III*	100.0±0.0 A a I	87.5±8.0 A a I
	3	66.7±6.8 A b II	100.0±0.0 A a I	100.0±0.0 A a I
	5	100.0±0.0 A a I	100.0±0.0 A a I	100.0±0.0 A a I
	7	100.0±0.0 A a I	100.0±0.0 A a I	100.0±0.0 A a I
<i>Steinernema carpocapsae</i>	1	20.8±4.2 A ab III	20.8±4.2 A c I	25.0±4.8 A b II
	3	70.8±8.0 A b II	83.3±6.8 A a I - II	83.3±11.8 A ab I - II
	5	100.0±0.0 A a I	100.0±0.0 A a I	100.0±0.0 A a I
	7	100.0±0.0 A a I	100.0±0.0 A a I	100.0±0.0 A a I
<i>Steinernema affine</i>	1	20.8±4.2 B ab II	66.7±11.8 AB b II	87.5±8.0 A a I
	3	100.0±0.0 A a I	100.0±0.0 A a I	100.0±0.0 A a I
	5	100.0±0.0 A a I	100.0±0.0 A a I	100.0±0.0 A a I
	7	100.0±0.0 A a I	100.0±0.0 A a I	100.0±0.0 A a I
<i>Heterorhabditis bacteriophora</i>	1	8.3±4.8 A b III	16.7±0.0 A c II	16.7±6.8 A b III
	3	66.7±9.6 A b II	83.3±6.8 A a I - II	66.7±15.2 A b II
	5	100.0±0.0 A a I	100.0±0.0 A a I	100.0±0.0 A a I
	7	100.0±0.0 A a I	100.0±0.0 A a I	100.0±0.0 A a I

\* Means followed by the same uppercase letter for the same entomopathogenic nematode (EPN) and day are not significantly different ( $P \leq 0.01$ ); means followed by the same lowercase letter for the same EPN application rate and day are not significantly different ( $P \leq 0.01$ ); means followed by the same roman letter in the same EPN application rate and eEPN are not significant different ( $P \leq 0.01$ ).

After 1 d, the lowest mortality was with 50 IJ of *H. bacteriophora* with 8.3% and the highest mortality was 100% with 500 IJ of *S. feltiae*. Only this application rate of *S. feltiae* was able to kill 100% of the *C. tenebrionis* larvae in 1 d. With 50 IJ, the mortality caused by *S. feltiae* was significantly higher than the other isolates by day 1 ( $F=24.8$ ,  $P=0.000$ ,  $df=9$ ). The difference between the mortalities from *S. carpocapsae*, *S. affine* and *H. bacteriophora* was not statistically significant. By day 3 with this application rate, the highest mortality was with *S. affine*, but with the other isolates there was no statistically significant increase in mortality. By day 5, the differences between mortalities with isolates as they all have reached 100%.

After 1 d with 500 IJ, larval mortality with *S. feltiae* had reached to 100%, while it was 20.8% with *S. carpocapsae*, 66.7% with *S. affine* and 16.7% with *H. bacteriophora*. The mortality caused by *S. feltiae* was

significantly higher than with the other isolates, and mortality with *S. carpocapsae* and *H. bacteriophora* was significantly lower than the others. With 500 IJ, 100% larval mortality was reached with *S. affine* by day 3 and with *S. carpocapsae* and *H. bacteriophora* by day 5 ( $F=5.81$ ,  $P=0.000$ ,  $df=6$ ).

After 1 d with 1000 IJ, the lowest larval mortality was with *H. bacteriophora* at 16.7%, while the highest was with *S. feltiae* and *S. affine*, both at 87.5%. Mortality with *S. carpocapsae* was 25.0%, which was significantly lower than with both *S. feltiae* and *S. affine* ( $F=13.3$ ,  $P=0.000$ ,  $df=6$ ). There was no significant difference between *S. carpocapsae* and *H. bacteriophora*. Larval mortality of 100% was reached by day 3 with *S. feltiae* and *S. affine*, but by day 5 with *S. carpocapsae* and *H. bacteriophora*.

### ***Capnodis tenebrionis* larval mortality in cherry saplings**

Mortality of *C. tenebrionis* larvae caused by different EPN at different days after application are given in Table 2. After 1 d, larval mortalities were 62.5, 52.5, 70.0, 50.0 and 0.00% with *S. feltiae*, *S. carpocapsae*, *S. affine*, *H. bacteriophora* and control, respectively. There was no significant difference between the EPN species on day 1 ( $F=1.90$ ,  $df=3$ ,  $p=0.271$ ) and 7 ( $F=0.73$ ,  $df=3$ ,  $p=0.584$ ). After 3 d, mortalities with *S. feltiae*, *S. carpocapsae* and *S. affine* were not significantly different, however, mortality with *H. bacteriophora* was significantly lower than *S. feltiae* and *S. carpocapsae* ( $F=0.90$ ,  $df=3$ ,  $p=0.031$ ). Similarly, there was no significant difference with *S. feltiae*, *S. carpocapsae* and *S. affine* on day 5, however, mortality with *H. bacteriophora* was significantly lower than *S. carpocapsae* but not from *S. feltiae* and *S. affine* ( $F=0.44$ ,  $df=3$ ,  $p=0.027$ ). Mortality was 5% in control treatment by day 7, with no mortality observed on the other days.

Table 2. Mortality of *Capnodis tenebrionis* 1, 3, 5 and 7 d after inoculation with entomopathogenic nematodes in cherry saplings (mean±se)

Entomopathogenic nematode	Day 1	Day 3	Day 5	Day 7
<i>Steinernema feltiae</i>	62.5±2.5 B a*	75.0±5.0 AB a	80.0±5.0 AB ab	92.5±2.5 A a
<i>Steinernema carpocapsae</i>	52.5±7.5 B a	77.5±2.5 AB a	85.0±5.0 A a	92.5±2.5 A a
<i>Steinernema affine</i>	70.0±10.0 A a	72.50±12.5 A ab	80.0±10.0 A ab	87.5±7.5 A a
<i>Heterorhabditis bacteriophora</i>	50.0±5.0 B a	57.5±2.5 B b	75.0±0.0 A b	82.5±7.5 A a

\* Means followed by the same uppercase letter in the same row are not significantly different ( $P \leq 0.05$ ); means followed by the same lowercase letter in the same column are not significantly different ( $P \leq 0.05$ ).

## **Discussion**

Results of the laboratory study show that, all four EPN isolates were capable of killing neonate larvae of *C. tenebrionis* to varying degrees. Similarly, Garcia del Pino & Morton (2005) reported 95% mortality of *C. tenebrionis* with *S. carpocapsae*, *S. feltiae* and *H. bacteriophora* after 5 d. Marannino et al. (2003) reported that, the mortality of *S. carpocapsae* with *H. bacteriophora* reached 100%.

Mortality of *C. tenebrionis* caused by different EPN changed with time after application. Especially mortality with *S. feltiae* was observed to reach to 100% in 1 or 3 d, while it took 5 d with *H. bacteriophora*. Similar results were recorded in the study by Morton & Garcia del Pino (2009), with 100% mortality after 1 and 3 d with *S. feltiae* and *S. carpocapsae*, and 100% mortality after 5 d with *H. bacteriophora*. Therefore, we concluded that *S. feltiae* is faster at infecting the host than other EPN isolates even at higher application rates.

Generally, the mortality with different application rates of the EPN did not differ significantly between the EPN on the same day of assessment. Only, the mortalities with 50 IJ of *S. feltiae* and *S. affine* on day 1 and *H. bacteriophora* on day 3 were significantly different from the other application rates. Also, all the

EPN were able to kill 100% of the neonate larvae after 5 d. Accordingly, it can be said that application of 50 IJ is potentially enough to kill neonate larvae of *C. tenebrionis* under controlled conditions. Marannino et al. (2003) also reported that 50 IJ of *S. carpocapsae* and *H. bacteriophora* in 2 ml tap water were able to kill 100% of the *C. tenebrionis* neonate larvae in a plate experiment. We also know that all EPN species can be effective in killing their hosts with the help of their symbiotic bacteria in 24-48 h, depending on the temperature and humidity.

In the cherry sapling experiment, mortality of *C. tenebrionis* larvae ranged between 50 to 92.5% over the assessment period and EPN applied. These results were lower than those of Marannino et al. (2003), who reported mortality of *C. tenebrionis* larvae in plants at 100% with *S. carpocapsae* and 98.9% for *H. bacteriophora*.

Results of our study support the idea that EPN are effective in controlling *C. tenebrionis* and the mortality caused by EPN is higher than insecticide treatments. Marannino et al. (2003) and Sanna-Passino & Delrio (2001) report mortality of 67.3 and 83.3% with diazinon (banned in Turkey in 2009 because it causes lung cancer) and chlorpyrifos, respectively. Also, EPN do not have the unwanted effects of insecticides, such as insecticide residues and pest resistance. Ben-Yehuda et al. (2000) tested nine chemical compounds and three application methods to improve the chemical control of *C. tenebrionis* and *Capnodis carbonaria* (Klug, 1829) (Coleoptera: Buprestidae), and have found that *C. tenebrionis* is more resistant insecticides than *C. carbonaria*.

Using local EPN species and biopesticides for the biological control of *C. tenebrionis*, which is an important pest of stone-fruits in Turkey, are important strategies for its successful control. In this study, our local isolates of EPN were effective against *C. tenebrionis* neonate larvae. In the light of these results, we think any application method with water would be highly effective for control of neonate larvae of *C. tenebrionis*, because of the EPN need moisture to remain infective. With the prevalence of drip irrigation in fruit orchards of Turkey, we suggest farmers could use their drip irrigation systems or surface systems to easily applying EPN to soil. Also, efficacy of EPN is generally higher than chemical control against underground pests and the ability of EPN to reproduce on other underground insect species contributes to their survival for the long term, thus increases their persistence in soil. Research on the efficacy of these EPN against *C. tenebrionis* under field conditions would be an important component of future studies.

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**Original article (Orijinal araştırma)**

**Response of eggplant genotypes to avirulent and virulent populations of *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae)<sup>1</sup>**

*Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae)'nın virüent ve avirüent popülasyonlarına patlıcan genotiplerinin tepkisi

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**Abstract**

Eggplant is widely grown throughout the world. However, some eggplant genotypes are susceptible to *Meloidogyne* spp., so *Solanum torvum* (Sw.) is commonly used as a resistant rootstock for root-knot nematodes. Further investigations of resistant sources to root-knot nematodes are still necessary for breeding programs. In this study, a total of 60 eggplant genotypes, including wild sources, wild rootstocks, wild × wild eggplant rootstocks, wild × cultivated eggplant rootstocks, cultivated eggplant rootstocks, pure lines, standard commercial cultivars and commercial hybrids, were tested with avirulent S6 and *Mi-1* virulent V14 populations of *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae) under controlled conditions. The study was conducted in 2016-2017. The seedlings were inoculated with 1000 second-stage juveniles of *M. incognita*. Plants were uprooted 8 weeks after nematode inoculation, and the numbers of egg masses and galls on the roots and juveniles in the soil of pots were counted. *Solanum torvum* (Y28) was found to be resistant to S6 and V14 populations of *M. incognita*. The remaining genotypes were susceptible to both populations. These results could be used for breeding and management purposes for the control of root-knot nematode.

**Keywords:** Eggplant, *Meloidogyne incognita*, resistance, *Solanum torvum*

**Öz**

Patlıcan dünyada yaygın bir şekilde yetiştirilmektedir. Bununla birlikte bazı patlıcan genotipleri kök-ur nematodlarına (*Meloidogyne* spp.) karşı duyarlıdır. Bu nedenle *Solanum torvum* (Sw.) dünyada kök-ur nematodlarına karşı dayanıklı anaç olarak yaygın bir şekilde kullanılmaktadır. Kök-ur nematodlarına dayanıklı yeni patlıcan genetik kaynaklarının araştırılması ıslah için gereklidir. Bu çalışmada yabancı kaynaklar, yabancı anaçlar, yabancı x yabancı anaçlar, yabancı x kültür formu patlıcan anaçları, kültür formu anaçlar, saf hatlar, standart ticari çeşitler ve ticari hibritler olmak üzere toplam 60 genotip *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 (Tylenchida: Meloidogynidae)'nın avirüent S6 ve *Mi-1* virüent V14 popülasyonu ile kontrollü koşullar altında testlenmiştir. Çalışma 2016-2017 yıllarında yürütülmüştür. Patlıcan fideleri *M. incognita*'nın 1000 ikinci dönem larvası ile inokulasyon yapılmış ve bitkiler inokulasyondan 8 hafta sonra sökülüştür. Köklerdeki yumurta ve ur sayıları ile topraktaki larva sayıları sayılmıştır. *Solanum torvum* (Y28)'un *M. incognita*'nın S6 ve V14 popülasyonlarına dayanıklı, diğer genotiplerin tümünün ise her iki popülasyona duyarlı olduğu belirlenmiştir. Bu sonuçlar kök-ur nematodlarının kontrolü için yapılacak olan ıslah ve mücadele çalışmalarında kullanılabilir.

**Anahtar sözcükler:** Patlıcan, *Meloidogyne incognita*, dayanıklılık, *Solanum torvum*

<sup>1</sup> This study represents first author's master thesis.

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## Introduction

Eggplant is belonging to the Solanaceae and its fruits have enormous diversity in shape, color and size (Collonnier et al., 2001; Sadilova et al., 2006). First cultivated in India and China (Lester & Hasan, 1991; Doğanlar et al., 2002), eggplant is a good source of minerals and vitamins (Russo, 1996; Sadilova et al., 2006). In addition, the related some species of eggplant have been used as valuable genetic resources for eggplant breeding and rootstocks (Bletsos et al., 1998; Johnson et al., 2014; Petran & Hoover, 2014). Worldwide, eggplant is grown on 1.7 Mha, with a total production of 51 Mt. Turkey is the world's fourth eggplant producer, after China, India and Egypt, with an annual production of 0.8 Mt (FAO, 2016).

Eggplant production is adversely affected by *Meloidogyne* spp. Root-knot nematodes (RKNs) induce the formation of specialized feeding sites (galls) in the roots of infected plants (Di Vito et al., 1986; Khan & Haider, 1991). Severe infestations cause considerable yield losses of eggplant crops and can also affect consumer acceptance of the produce. RKNs are soil borne pathogens (Starr et al., 1989; Manzanilla-López & Starr, 2009) and have a wide range of hosts (Hussey, 1985; Khurma et al., 2008; Jones et al., 2013); consequently, their management is difficult. RKN management strategies include the use of nematicides and resistant cultivars and rootstocks (Devran et al., 2010). However, the use of some nematicides has been limited because of health and environmental problems (Devran et al., 2008; Moens et al., 2009; Devran et al., 2013). In contrast, resistant plants can serve as environmentally and eco-friendly alternatives for management of RKNs (Boerma & Hussey, 1992; Rahman et al., 2002; Devran et al., 2013).

Eggplants cultivated are susceptible to RKNs; however, some wild eggplant species are resistant to some RKN species (Daunay & Dalmaso, 1985; Hebert, 1985; Ali et al., 1992; Boiteux & Charchar, 1996; Rahman et al., 2002; Uehara et al., 2016; 2017; Öçal et al., 2018). At present, *Solanum torvum* (Sw.) is commonly used as a rootstock (Uehara et al., 2017). This species also shows resistance to high-salinity soils and several serious soilborne pathogens, such as *Ralstonia solanacearum* (Smith) (Burkholderiales: Burkholderiaceae), *Fusarium oxysporum* Schlechtendal (Hypocreales: Nectriaceae) and *Verticillium dahlia* Klebahn (Hypocreales: Hypocreaceae) (Stravato & Cappelli, 2000; Collonnier et al., 2001; Gousset et al., 2005; Zhang et al., 2015). However, *S. torvum* has a long germination time (Liu et al., 2009), which causes problems in grafting and seedling production. Therefore, the investigation of new genotypes that are resistant to RKNs is critical for eggplant breeding. Here, we investigated the responses of 60 eggplant genotypes to avirulent and virulent populations of *M. incognita* under controlled conditions.

## Materials and Methods

### Plant material

The eggplant genotypes used in this study are listed in Table 1. In the experiments, *Solanum torvum* cv. Hawk (Solanales: Solanaceae) (Vilmorin, France) and *Solanum melongena* L. (Solanales: Solanaceae), the commercial eggplant cv. Faselis F<sub>1</sub> (Semini, MO, USA) were used as resistant and susceptible entries, respectively.

Table 1. Eggplants genotypes assessed in this study

Plant Code	Genotype	Property	Species
Y1	S-IN-F-11	Wild rootstock	<i>Solanum integrifolium</i>
Y2	Eggplant Rootstock-4	Wild x wild eggplant rootstock	<i>S. integrifolium</i> x <i>S. incanum</i>
Y4	LS2436	Pure lines	<i>Solanum melongena</i>
Y5	Eggplant Rootstock -1	Wild rootstock	<i>Solanum incanum</i>
Y6	Eggplant Rootstock -2	Wild rootstock	<i>Solanum incanum</i>
Y7	Eggplant Rootstock -3	Wild rootstock	<i>Solanum integrifolium</i>
Y8	P-1	Wild genotype	<i>Solanum integrifolium</i>
Y9	P-2	Standard commercial cultivars	<i>Solanum melongena</i>
Y10	P-3	Standard commercial cultivars	<i>Solanum melongena</i>
Y11	P-4	Standard commercial cultivars	<i>Solanum melongena</i>
Y12	P-5	Standard commercial cultivars	<i>Solanum melongena</i>
Y13	P-6	Standard commercial cultivars	<i>Solanum melongena</i>
Y14	12 T 233	Wild genotype	<i>Solanum aethiopicum</i>
Y15	11-T-235	Wild genotype	<i>Solanum incanum</i>
Y16	Genotype-78	Wild genotype	<i>Solanum incanum</i>
Y17	Ls2436 x S00019	Cultivated x wild eggplant rootstock	<i>S. melongena</i> x <i>S. aethiopicum</i>
Y18	P-AN-33872 x ls2436	Wild x cultivated eggplant rootstock	<i>S. aethiopicum</i> x <i>S. melongena</i>
Y19	09-T-82	Pure line	<i>Solanum melongena</i>
Y20	11-T-331-12	Pure line	<i>Solanum melongena</i>
Y21	S-0002 x LS-2436	Cultivated x wild eggplant rootstock	<i>S. melongena</i> x <i>S. aethiopicum</i>
Y22	SS-PL-2 x Genotype 78	Cultivated x wild eggplant rootstock	<i>S. melongena</i> x <i>S. incanum</i>
Y23	LS2436 x S00830	Wild x cultivated eggplant rootstock	<i>S. aethiopicum</i> x <i>S. melongena</i>
Y24	P-AN-33871 x ls2436	Wild x cultivated eggplant rootstock	<i>S. aethiopicum</i> x <i>S. melongena</i>
Y25	Genotype x Genotip 78	Wild x wild eggplant rootstock	<i>S. aethiopicum</i> x <i>S. incanum</i>
Y26	09 T 80	Pure line	<i>Solanum melongena</i>
Y27	11 T 295	Pure line	<i>Solanum melongena</i>
Y28	Hawk	Wild rootstock	<i>Solanum torvum</i>
Y29	Köksal Rootstok	Wild x cultivated eggplant rootstock	<i>S. melongena</i> x <i>S. incanum</i>
Y30	P-AN33873 wild	Wild genotype	<i>Solanum aethiopicum</i>
Y31	<i>S. integrifolium</i>	Wild genotype	<i>Solanum integrifolium</i>
Y32	Cultivated Rootstok	Cultivated eggplant rootstock	<i>Solanum melongena</i>
Y33	MM195006T44 x <i>S. integrifolium</i>	Wild x wild eggplant rootstock	<i>S. integrifolium</i> x <i>S. integrifolium</i>
M1	Faselis F1	Commercial hybrids	<i>Solanum melongena</i>
M2	Anamur F1	Commercial hybrids	<i>Solanum melongena</i>
M3	Sicilia F1	Commercial hybrids	<i>Solanum melongena</i>
M4	Brigitte F1	Commercial hybrids	<i>Solanum melongena</i>
M5	Darko F1	Commercial hybrids	<i>Solanum melongena</i>
M6	Karaok F1	Commercial hybrids	<i>Solanum melongena</i>
M7	Karanta F1	Commercial hybrids	<i>Solanum melongena</i>
M8	Aykara F1	Commercial hybrids	<i>Solanum melongena</i>
M9	Karnaz F1	Commercial hybrids	<i>Solanum melongena</i>
M10	Oriental F1	Commercial hybrids	<i>Solanum melongena</i>
M11	Doyran Karası F1	Commercial hybrids	<i>Solanum melongena</i>
M12	Me39 F1	Commercial hybrids	<i>Solanum melongena</i>
M13	Volta F1	Commercial hybrids	<i>Solanum melongena</i>
M14	Aydın Siyahı	Standard commercial cultivars	<i>Solanum melongena</i>
M15	Pala Yalova 49	Standard commercial cultivars	<i>Solanum melongena</i>
M16	Kemer 27	Standard commercial cultivars	<i>Solanum melongena</i>
M17	Yamula Patlıcanı	Standard commercial cultivars	<i>Solanum melongena</i>
M18	Korkuteli Söğüt	Standard commercial cultivars	<i>Solanum melongena</i>
M19	Topan 374	Standard commercial cultivars	<i>Solanum melongena</i>
M20	Bursa Topan	Standard commercial cultivars	<i>Solanum melongena</i>
M21	AGR 703	Cultivated eggplant rootstocks	<i>Solanum melongena</i>
M22	Ahtapot F1	Wild x wild eggplant rootstocks	<i>S. incanum</i> x <i>S. aethiopicum</i>
M23	Vista F1	Wild x cultivated eggplant rootstocks	<i>S. melongena</i> x <i>S. incanum</i>
M24	16SP3143	Wild rootstocks	Unknown
M25	16SP3144	Wild rootstocks	Unknown
M26	16SP3145	Wild rootstocks	Unknown
M-27	Wild Eggplant 4	Wild rootstocks	Unknown
M-28	Kumluca Patlıcan	Pure lines	<i>Solanum melongena</i>

### **Nematode culture**

Avirulent S6 and *Mi-1* virulent V14 populations of *M. incognita* were used in this study. The S6 population were identified in previous studies (Devran & Söğüt, 2009, 2010, 2011) and V14 has been used as laboratory culture since 2015 (unpublished data). Each RKN isolate was established as a single mass for pure cultures according to previous studies (Mıstanoğlu et al., 2016; Özalp & Devran, 2018).

### **Nematode inoculation and evaluation**

The study was conducted at the Nematology Laboratory of the Department of Plant Protection, Faculty of Agriculture, Akdeniz University in 2016-2017. Eggplant seedlings at the two true-leaf stage were transplanted into 250 ml plastic pots, containing sterilized sandy. One thousand J2s were inoculated into holes surrounding the root. Five plants for each genotype were tested with each nematode population. The pots were incubated in a growth chamber at 25±0.5°C, 65% RH and 8:16 h L:D photoperiod. The seedlings were uprooted 8 weeks after nematode inoculation and evaluated according to Özalp & Devran (2018).

The J2s from the soil of each pot were extracted using a modified Baermann funnel technique (Hooper 1986). The reproduction factor (Rf) was calculated by the formula,  $Rf = Pf/Pi$ , where Pf = final *M. incognita* population and Pi = initial *M. incognita* population (Ferris, 1985).

The number of egg masses and galls on each plant root was counted and assessed on a 0-5 scale, according to Hartman and Sasser (1985).

### **Statistical analyses**

The entries were separated into eight groups for statistical analysis, since eggplant genotypes have very different genetic backgrounds. The data were log transformed [ $\log_{10}(x+1)$ ] and analyzed by ANOVA. The statistical analyses were conducted with the general linear model procedure (PROC GLM) of the statistical package SAS (v. 9.0 for Windows; SAS Institute, Inc., Cary, NC, USA). Significant differences with in treatments were tested using Duncan's test.

## **Results**

Sixty eggplant genotypes, including wild source, wild rootstocks, wild × wild eggplant rootstocks, wild × cultivated eggplant rootstocks, cultivated eggplant rootstocks, pure lines, standard commercial cultivars and commercial hybrids were tested with avirulent S6 and *Mi-1* virulent V14 populations of *M. incognita*. At the end of the experiments, the numbers of juveniles (J2s), egg masses and galls were evaluated in all plants.

### **Wild genotypes (Group 1)**

Six wild eggplant genotypes, Y8, Y14, Y15, Y16, Y30 and Y31, were tested with the S6 and V14 populations of *M. incognita* (Table 2). The S6 population of *M. incognita* produced a few egg masses and galls on the Y8 genotype, whereas, the V14 population of *M. incognita* multiplied very well on the Y8 genotype. The Rf value of the S6 population of *M. incognita* on Y8 was <1, whereas the Rf value of the V14 population of *M. incognita* on Y8 was >1. The Y8 genotype was only resistant to the S6 population of *M. incognita*, based on the egg mass index. However, the Y8 genotype was susceptible according to the gall index (Hartman & Sasser, 1985) (Table 2). The Y14, Y15, Y16 Y30 and Y31 genotypes were susceptible to both the V14 and S6 populations of *M. incognita* (Table 2). Although the Y15 genotype was susceptible to the V14 population, the Rf <1. Significant differences were noted among some wild genotypes based on the numbers of egg masses and galls on the roots, juveniles in the soil and the 0-5 scale scores (Table 2).

Table 2. Number of egg masses, galls and Rf values in wild genotypes against avirulent S6 and virulent V14 populations of *M. incognita*

Plant Code	<i>M. incognita</i> avirulent S6 population					
	Egg Mass	Egg Mass Index*	Gall	Gall Index*	Rf	
Y8	14.80 d	2.80 c	23.60 d	3.20 c	0.404 c	
Y14	113.40 a	4.60 a	370.00 a	5.00 a	2.590 a	
Y15	40.75 bc	3.50 b	61.00 c	4.00 b	3.074 a	
Y16	97.00 a	4.60 a	219.20 b	5.00 a	2.982 a	
Y30	30.00 c	3.50 b	210.70 b	5.00 a	1.042 bc	
Y31	52.60 b	3.80 b	435.00 a	5.00 a	1.486 ab	
Plant Code	<i>M. incognita</i> virulent V14 population					
	Egg Mass	Egg Mass Index*	Gall	Gall Index*	Rf	
Y8	63.20 b	4.00 b	111.80 a	5.00 a	5.340 a	
Y14	85.00 b	4.40 ab	128.00 a	5.00 a	3.620 a	
Y15	19.25 c	3.00 c	22.00 b	3.00 c	0.990 b	
Y16	193.80 a	4.80 a	128.40 a	4.60 b	3.270 a	
Y30	104.50 ab	4.75 a	162.50 a	4.80 a	4.750 a	
Y31	74.80 b	4.00 b	178.80 a	5.00 a	1.090 b	

\* 0-5 Scale (Hartman & Sasser 1985). 0-2: Resistance, 3-5: Susceptible. Rf: Reproduction factor. Means in columns followed by the same letter are not significantly different ( $P \leq 0.05$ ) according to Duncan's test.

### Wild rootstocks (Group 2)

Nine wild eggplant rootstocks, Y1, Y5, Y6, Y7, Y28, M24, M25, M26 and M27, were tested with the S6 and V14 populations of *M. incognita*. Both *M. incognita* populations produced a few egg masses and galls on Y28. The Rf values of the V14 and S6 populations of *M. incognita* on Y28 were <1. The V14 population of *M. incognita* produced a few egg masses on the Y7 genotype, but produced many galls on Y7. The Y7 genotype was resistant to the V14 population of *M. incognita* based on the egg mass index; however, this genotype was susceptible according to the gall index (Hartman and Sasser 1985) (Table 3). In addition, the Rf value of the V14 population of *M. incognita* on Y7 was <1 (Table 3). Nevertheless, Y7 was susceptible to the S6 population of *M. incognita* according to the gall index, egg mass index and Rf value. The other rootstocks were susceptible to the V14 and S6 populations of *M. incognita* (Table 3). Although the M24, M25, M26 and M27 genotypes were susceptible to the S6 populations, with Rf <1, results showed that Y28 was resistant according to the 0-5 scale score (Hartman & Sasser, 1985) (Table 3). Significant differences were observed among the wild rootstocks with respect to egg masses, galls, juveniles in the soil and the 0-5 scale scores (Table 3).

### Wild x wild eggplant rootstocks (Group 3)

Three eggplant rootstocks (Y2, Y33 and M22) obtained from wild x wild eggplant rootstocks crosses were tested with the S6 and V14 populations of *M. incognita*. Both populations produced many egg masses and galls on the roots of all plants. The Rf value of the S6 population on M22 was <1. However, the Rf values of both populations on the other plants were >1. All rootstocks were susceptible to both populations of *M. incognita* according to the 0-5 scale scores (Hartman & Sasser, 1985) (Table 4). Significant differences were noted among the wild rootstocks with respect to egg masses, galls, juveniles in the soil and the 0-5 scale scores (Table 4).

Table 3. Number of egg masses, galls and Rf values in wild rootstocks against avirulent S6 and virulent V14 populations of *M. incognita*

Plant Code	<i>M. incognita</i> avirulent S6 population					
	Egg Mass	Egg Mass Index*	Gall	Gall Index*	Rf	
Y1	104.20 a	4.80 a	271.60 b	5.00 a	6.206 a	
Y5	20.60 c	3.00 c	49.60 e	4.00 c	2.412 b	
Y6	54.00 b	4.00 b	191.20 c	5.00 a	2.744 ab	
Y7	41.00 b	4.00 b	68.80 e	4.00 c	3.154 ab	
Y28	2.40 d	1.20 d	12.20 f	2.60 d	0.242 d	
M24	109.80 a	4.80 a	259.60 b	5.00 a	0.470 cd	
M25	93.50 a	4.25 ab	384.20 a	5.00 a	0.302 c	
M26	39.80 b	3.80 b	173.00 c	5.00 a	0.216 d	
M27	49.20 b	3.60 b	124.20 d	4.60 b	0.764 c	

Plant Code	<i>M. incognita</i> virulent V14 population					
	Egg Mass	Egg Mass Index*	Gall	Gall Index*	Rf	
Y5	21.20 d	3.00 b	25.60 f	3.20 d	2.230 c	
Y6	209.60 a	5.00 a	233.20 a	5.00 a	15.720 a	
Y7	7.25 d	2.25 c	41.75 e	4.00 c	0.610 cd	
Y28	1.20 d	0.60 d	1.60 g	0.80 e	0.034 d	
M24	159.80 b	5.00 a	79.20 d	4.20 bc	8.016 b	
M25	117.50 c	5.00 a	99.20 c	4.75 ab	2.170 c	
M26	135.20 bc	4.80 a	152.60 b	5.00 a	10.140 ab	

\* 0-5 Scale (Hartman & Sasser 1985). 0-2: Resistance, 3-5: Susceptible. Rf: Reproduction factor. Means in columns followed by the same letter are not significantly different ( $P \leq 0.05$ ) according to Duncan's test.

Table 4. Number of egg masses, galls and Rf values in wild x wild eggplant rootstocks against avirulent S6 and virulent V14 populations of *M. incognita*

Plant Code	<i>M. incognita</i> avirulent S6 population					
	Egg Mass	Egg Mass Index*	Gall	Gall Index*	Rf	
Y2	56.20 b	4.00 b	335.00 b	5.00 a	1.840 b	
Y33	113.80 a	4.80 a	193.60 c	5.00 a	7.770 a	
M22	107.20 a	4.60 a	562.80 a	5.00 a	0.450 c	

Plant Code	<i>M. incognita</i> virulent V14 population					
	Egg Mass	Egg Mass Index*	Gall	Gall Index*	Rf	
Y2	80.60 a	4.00 b	75.00 b	4.00 b	1.074 c	
Y33	96.33 a	4.60 a	81.60 b	4.00 b	9.003 a	
M22	58.20 b	4.00 b	110.80 a	4.60 a	4.746 b	

\* 0-5 Scale (Hartman & Sasser 1985). 0-2: Resistance, 3-5: Susceptible. Rf: Reproduction factor. Means in columns followed by the same letter are not significantly different ( $P \leq 0.05$ ) according to Duncan's test.

### Wild x cultivated eggplant rootstocks (Group 4)

Nine eggplant rootstocks (Y17, Y18, Y21, Y22, Y23, Y24, Y25, Y29 and M23) obtained from wild x cultivated eggplants crosses were tested with the S6 and V14 populations of *M. incognita*. Both populations multiplied very well on all rootstocks. The Rf values of two populations on seven rootstocks except for M23 and Y22 were >1 (Table 5). However, Rf value of S6 population on M23 and V14 population on Y22 were <1 and (Table 5). Results showed that all rootstocks were susceptible to two populations of *M. incognita* according to scale score (Hartman & Sasser, 1985) (Table 5). Significant differences were observed among rootstocks with respect to egg masses, galls, juveniles in the soil and 0-5 scale scores (Table 5).

Table 5. Number of egg masses, galls and Rf values in wild x cultivated eggplant rootstocks against avirulent S6 and virulent V14 populations of *M. incognita*

Plant Code	<i>M. incognita</i> avirulent S6 population					
	Egg Mass	Egg Mass Index*	Gall	Gall Index*	Rf	
Y17	149.40 b	5.0 a	293.8 d	5.00 a	2.660 bc	
Y18	303.20 a	5.0 a	396.2 c	5.00 a	3.810 b	
Y21	100.40 c	4.60 b	325.20 d	5.00 a	3.340 b	
Y22	71.40 d	4.00 c	215.20 e	5.00 a	1.190 d	
Y23	65.40 d	4.00 c	158.40 f	5.00 a	2.830 bc	
Y24	141.80 b	5.00 a	405.60 c	5.00 a	4.220 b	
Y25	68.20 d	4.00 c	515.20 a	5.00 a	1.690 cd	
Y29	72.80 d	4.20 c	241.40 e	5.00 a	9.250 a	
M23	113.40 bc	4.80 ab	457.00 b	5.00 a	0.440 e	

Plant Code	<i>M. incognita</i> virulent V14 population					
	Egg Mass	Egg Mass Index*	Gall	Gall Index*	Rf	
Y17	149.40 bc	4.80 a	123.00 b	5.00 a	4.650 a	
Y18	125.00 abc	4.80 a	123.40 b	4.80 a	3.640 a	
Y21	142.80 ab	4.80 a	155.40 ab	4.80 a	2.020 ab	
Y22	145.80 ab	5.00 a	159.80 ab	5.00 a	0.780 b	
Y23	87.50 c	4.25 b	120.25 b	5.00 a	2.010 ab	
Y24	96.40 c	4.20 b	163.20 ab	5.00 a	2.280 ab	
Y25	57.50 d	4.00 b	114.25 b	5.00 a	1.810 ab	
Y29	52.00 d	3.75 b	138.50 ab	4.75 a	2.400 ab	

\* 0-5 Scale (Hartman & Sasser 1985). 0-2: Resistance, 3-5: Susceptible. Rf: Reproduction factor. Means in columns followed by the same letter are not significantly different ( $P \leq 0.05$ ) according to Duncan's test.

### Cultivated eggplant rootstocks (Group 5)

Two eggplant rootstocks Y32 and M21 were tested with the S6 and V14 populations of *M. incognita*. Both populations produced many egg masses and galls on Y32 and M21. Rf values of two populations on Y32 and M21 were >1 (Table 6). Results indicated that two rootstocks were susceptible to two populations of *M. incognita* according to 0-5 scale scores (Hartman & Sasser, 1985) (Table 6). Significant differences were noted among cultivated eggplant rootstocks with respect to egg masses, galls, juveniles in the soil and 0-5 scale values (Table 6).

Table 6. Number of egg masses, galls and Rf values in cultivated eggplant rootstocks against avirulent S6 and virulent V14 populations of *M. incognita*

Plant Code	<i>M. incognita</i> avirulent S6 population					
	Egg Mass	Egg Mass Index*	Gall	Gall Index*	Rf	
Y32	80.00 b	4.20 a	144.60 b	4.80 a	1.448 b	
M21	219.20 a	5.00 a	263.60 a	5.00 a	7.014 a	

Plant Code	<i>M. incognita</i> virulent V14 population					
	Egg Mass	Egg Mass Index*	Gall	Gall Index*	Rf	
Y32	172.20 a	5.00 a	145.60 a	5.00 a	5.118 a	
M21	144.40 b	4.60 a	129.20 b	5.00 a	4.234 a	

\* 0-5 Scale (Hartman & Sasser 1985). 0-2: Resistance, 3-5: Susceptible. Rf: Reproduction factor. Means in columns followed by the same letter are not significantly different ( $P \leq 0.05$ ) according to Duncan's test.

### Pure lines (Group 6)

Six pure lines eggplants (Y4, Y19, Y20, Y26, Y27 and M28) were tested with the S6 and V14 populations of *M. incognita*. Both populations multiplied very well on all lines. Rf values of the S6 population on all genotypes except Y27 were  $>1$  (Table 7). In addition, Rf values of S6 and V14 populations on M28 were not counted. All pure lines were susceptible to two populations of *M. incognita* according to 0-5 scale (Hartman & Sasser, 1985) (Table 7). Significant differences were observed among some pure lines with respect to egg masses, galls, juveniles in the soil and 0-5 scale values (Table 7).

Table 7. Number of egg masses, galls and Rf values in pure lines against avirulent S6 and virulent V14 populations of *M. incognita*

Plant Code	<i>M. incognita</i> avirulent S6 population					
	Egg Mass	Egg Mass Index*	Gall	Gall Index*	Rf	
Y4	75.80 a	4.00 a	230.60 a	5.00 a	2.634 a	
Y19	67.00 a	4.00 a	229.60 a	5.00 a	2.564 a	
Y20	67.00 a	4.00 a	230.80 a	5.00 a	1.970 a	
Y26	91.30 a	4.00 a	81.30 a	4.30 b	2.176 ab	
Y27	35.50 b	3.75 b	73.50 b	4.00 c	0.598 b	
M28	97.40 a	4.60 a	244.00 a	5.00 a	-	

Plant Code	<i>M. incognita</i> virulent V14 population					
	Egg Mass	Egg Mass Index*	Gall	Gall Index*	Rf	
Y4	127.20 ab	5.00 a	107.60 cd	4.80 a	2.852 b	
Y19	134.00 ab	4.75 a	141.50 abc	4.75 a	4.238 b	
Y20	79.60 c	4.20 b	80.60 d	4.20 b	1.392 c	
Y26	116.60 b	4.80 a	130.80 bc	5.00 a	13.820 a	
Y27	114.80 b	5.00 a	163.60 ab	5.00 a	14.230 a	
M28	184.80 a	5.00 a	179.60 a	5.00 a	-	

\* 0-5 Scale (Hartman & Sasser 1985). 0-2: Resistance, 3-5: Susceptible. Rf: Reproduction factor. Means in columns followed by the same letter are not significantly different ( $P \leq 0.05$ ) according to Duncan's test. - indicates no nematode test.

### Standard commercial cultivars (Group 7)

Twelve standard commercial cultivars (Y9, Y10, Y11, Y12, Y13, M14, M15, M16, M17, M18, M19 and M20) were tested with the S6 and V14 populations of *M. incognita*. The S6 population multiplied on all



plants and produced many egg masses and galls. Rf values of S6 on all cultivars except Y11 were >1. Results showed that all pure lines were susceptible to the S6 population of *M. incognita* according to 0-5 scale (Hartman & Sasser, 1985) (Table 8). Significant differences were noted among some standard commercial cultivars with respect to egg masses, galls, juveniles in the soil and 0-5 scale values (Table 8).

Table 8. Number of egg masses, galls and Rf values in standard commercial cultivars against avirulent S6 and virulent V14 populations of *M. incognita*

Plant Code	<i>M. incognita</i> avirulent S6 population									
	Egg Mass		Egg Mass Index*		Gall		Gall Index*		Rf	
Y9	125.00	ab	4.80	a	434.80	a	5.00	a	2.854	a
Y10	94.50	bc	4.50	a	300.70	bc	5.00	a	1.580	abcd
Y11	95.00	bc	4.40	a	313.20	b	5.00	a	0.910	d
Y12	78.40	bc	4.20	a	279.60	bc	5.00	a	2.392	ab
Y13	66.40	c	4.20	a	261.00	bcd	5.00	a	1.872	abc
M14	157.80	a	4.80	a	252.40	bcd	5.00	a	2.254	ab
M15	65.00	c	4.20	a	152.75	f	5.00	a	2.208	ab
M16	94.00	bc	4.40	a	128.80	f	5.00	a	2.406	ab
M17	125.00	ab	4.60	a	333.30	b	5.00	a	8.633	cd
M18	127.40	bc	4.40	a	205.80	de	5.00	a	2.598	ab
M19	100.60	bc	4.20	a	208.80	cde	5.00	a	1.376	bcd
M20	90.80	bc	4.20	a	159.60	ef	5.00	a	1.270	bcd

Plant Code	<i>M. incognita</i> virulent V14 population									
	Egg Mass		Egg Mass Index*		Gall		Gall Index*		Rf	
Y9	169.80	a	5.00	a	226.8	a	5.00	a	13.490	a
Y10	113.60	abc	4.80	a	139.0	b	4.80	ab	12.580	ab
Y11	106.00	abc	4.60	ab	64.40	c	4.00	c	8.440	bc
Y12	1.20	d	0.80	c	16.00	d	3.00	d	0.240	f
Y13	89.40	bc	4.40	ab	111.80	b	4.80	ab	5.680	c
M14	161.60	a	5.00	a	154.60	b	5.00	a	8.470	abc
M15	96.40	bc	4.40	ab	74.80	c	4.40	bc	4.670	de
M16	104.60	abc	4.60	ab	62.60	c	4.00	c	2.250	de
M18	158.50	ab	4.75	a	160.25	b	4.75	ab	6.670	c
M19	78.25	c	4.00	b	79.00	c	4.25	c	2.540	e
M20	144.40	ab	5.00	a	129.20	b	5.00	a	2.660	de

\* 0-5 Scale (Hartman & Sasser 1985). 0-2: Resistance, 3-5: Susceptible. Rf: Reproduction factor. Means in columns followed by the same letter are not significantly different (P ≤ 0.05) according to Duncan's test.

V14 population multiplied and produced many egg masses all genotypes except Y12. Rf values of V14 populations of *M. incognita* on all genotypes except Y12 were >1 (Table 8). All genotypes except Y12 were susceptible to the V14 population. Y12 was resistance to according to egg mass index, but it was susceptible to according to gall index (Hartman & Sasser, 1985) (Table 8). Significant differences were observed among standard commercial cultivars according to egg masses, galls, juveniles in the soil and 0-5 scale values (Table 8).

### Commercial hybrids (Group 8)

Thirteen commercial hybrids M1, M2, M3, M4, M5, M6, M7, M8, M9, M10, M11, M12 and M13 were tested with the S6 and V14 populations of *M. incognita*. Both populations produced many egg masses and galls on roots of all hybrids. Rf values of two populations on all hybrids except M10 were >1 (Table 9). Only Rf value of S6 population on M10 <1. All hybrids were susceptible to two populations of *M. incognita* according to 0-5 scale (Hartman & Sasser, 1985) (Table 9).

Table 9. Number of egg masses, galls and Rf values in commercial hybrids against avirulent S6 and virulent V14 populations of *M. incognita*

Plant Code	<i>M. incognita</i> avirulent S6 population					
	Egg Mass	Egg Mass Index*	Gall	Gall Index*	Rf	
M1	90.80 d	4.30 b	159.60 ef	5.00 a	1.266 cde	
M2	103.00 cd	4.80 ab	222.20 cd	5.00 a	3.112 ab	
M3	175.20 ab	4.80 ab	339.20 a	5.00 a	2.880 ab	
M4	94.40 d	4.60 ab	241.80 cd	5.00 a	2.834 ab	
M5	107.20 cd	4.80 ab	265.00 bc	5.00 a	1.284 de	
M6	189.80 ab	5.00 a	255.40 bcd	5.00 a	2.680 bc	
M7	155.20 abc	4.60 ab	205.20 cd	5.00 a	2.302 b	
M8	97.40 d	4.40 ab	132.40 f	5.00 a	2.196 bcd	
M9	200.40 a	5.00 a	197.00 de	5.00 a	1.322 cde	
M10	142.40 abc	5.00 a	308.00 ab	5.00 a	0.806 e	
M11	195.60 a	4.80 ab	307.60 ab	5.00 a	1.962 bcd	
M12	131.50 bcd	5.00 a	225.20 cd	5.00 a	2.165 bcd	
M13	178.00 ab	5.00 a	235.40 cd	5.00 a	4.906 a	

Plant Code	<i>M. incognita</i> virulent V14 population					
	Egg Mass	Egg Mass Index*	Gall	Gall Index*	Rf	
M1	210.60 ab	5.00 a	213.20 ab	5.00 a	4.478 c	
M2	87.00 c	4.40 b	88.80 d	4.40 b	4.210 c	
M3	250.80 a	5.00 a	230.20 a	5.00 a	9.934 abc	
M4	256.60 a	5.00 a	208.80 abc	5.00 a	14.940 ab	
M5	169.00 b	4.80 a	174.00 bc	5.00 a	12.760 ab	
M7	241.40 a	5.00 a	190.40 abc	5.00 a	11.700 ab	
M8	205.40 ab	5.00 a	179.20 abc	5.00 a	9.914 abc	
M9	196.00 ab	5.00 a	157.60 c	4.80 a	7.902 bc	
M10	168.20 b	5.00 a	192.40 abc	5.00 a	18.160 a	
M11	168.00 b	5.00 a	189.60 abc	5.00 a	13.120 ab	
M12	214.40 ab	5.00 a	181.60 abc	5.00 a	14.430 ab	
M13	237.20 ab	5.00 a	225.40 ab	5.00 a	14.540 ab	

\* 0-5 Scale (Hartman & Sasser 1985). 0-2: Resistance, 3-5: Susceptible. Rf: Reproduction factor. Means in columns followed by the same letter are not significantly different ( $P \leq 0.05$ ) according to Duncan's test.

## Discussion

Global eggplant production has increased in recent years (FAO, 2016); however, RKNs continue to pose a significant threat to eggplant growth in infested areas. Sikora & Fernandez (2005) reported that root nematodes cause 17-20% product losses in eggplant. Therefore, the use of resistant eggplant genotypes is required for management of RKN. In the present study, 60 eggplant genotypes with different genetic backgrounds were tested with avirulent S6 and *Mi-1* virulent V14 populations of *M. incognita*.

*Solanum integrifolium* Poir. Y8 and Y7 genotypes were resistant to S6 and V14 according to the egg mass index but were susceptible according to the gall index. Several studies have shown that *S. integrifolium* is susceptible to *M. incognita* (Daunay & Dalmasso, 1985; Ali et al., 1992; Rahman et al., 2002; Uehara et al., 2016). In the present study, *Solanum aethiopicum* L. Y14 and Y30 genotypes were susceptible to both the avirulent and virulent populations of *M. incognita*. Hebert (1985) previously reported that *S. aethiopicum* genotypes were resistant to *M. incognita*, although other studies have reported that *S. aethiopicum* genotypes were susceptible or moderately resistant to *M. incognita* (Gisbert et al., 2011; Dhivya et al., 2014). In the present study, *S. torvum* was resistant to both the avirulent S6 and virulent V14 populations of *M. incognita*, in agreement with previous studies that showed resistance of *S. torvum* to *M. incognita* populations (Daunay & Dalmasso, 1985; Hebert, 1985; Ali et al., 1992; Rahman et al., 2002; Dhivya et al., 2014). Gonzalez et al. (2010) found that *S. torvum* was resistant to both *M. incognita* and *M. arenaria*, while other studies demonstrated that the *S. torvum* cvs Tonashimu, Torero and Torvum Vigor were resistant to populations of *M. incognita* (Uehara et al., 2016, 2017). Recent work has shown that *S. torvum* was resistant to both avirulent and virulent populations of *M. incognita* (Öçal et al., 2018). The present findings agree with these previous studies.

In this study, all *S. incanum* genotypes were susceptible to the S6 and V14 populations of *M. incognita*, in agreement with the findings of Gisbert et al. (2011), who showed susceptibility of a *Solanum incanum* L. genotype to a population of *M. incognita*. In other studies, the *S. incanum* genotype was found resistant or moderately resistant to a population of *M. incognita* (Hebert, 1985; Dhivya et al., 2014). These different responses may reflect differences in the genetic backgrounds of the studied plants. In the present study, the eggplant cross combinations showed differences in susceptibility to RKN populations. For example, *S. integrifolium* × *S. incanum* (Y2), *S. integrifolium* × *S. integrifolium* (Y33) and *S. aethiopicum* × *S. incanum* (M22) were susceptible to both the S6 and V14 populations of *M. incognita*, as were the *S. melongena* × *S. aethiopicum* combinations Y17, Y18, Y21, Y22, Y23, Y24 and Y25 and the *S. melongena* × *S. incanum* genotypes Y29 and M23. Gisbert et al. (2011) reported that *S. melongena* × *S. aethiopicum* and *S. melongena* × *S. incanum* combinations were susceptible in fields infested with *M. incognita*. Similarly, Ali et al. (1992) showed that cultivar eggplant × wild eggplant genotype crosses were susceptible to a population of *M. incognita*.

In this study, a total of 32 of 33 *S. melongena* genotypes, including cultivated eggplant rootstocks, pure lines, standard commercial cultivars and commercial hybrids, were susceptible to the *Mi-1* virulent V14 and avirulent S6 populations of *M. incognita*. Only the Y12 genotype was resistant to the *Mi-1* virulent V14 population of *M. incognita*, according to the egg mass numbers. Gisbert et al. (2011) reported that rootstock AGR 703 F<sub>1</sub> was susceptible to a population of *M. incognita*. In previous studies, *S. melongena* genotypes were reported to be either susceptible or resistant to populations of *M. incognita* (Ullah et al., 2011; Nayak & Sharma 2013; Begum et al., 2014; Nayak & Pandey, 2015). Local genotypes ANS6 and ASIS1 were susceptible, but IVIA371 and PI263727 were resistant (Gisbert et al., 2011). The cultivated eggplant cv. Senryo 2 gou was susceptible to populations of *M. incognita* (Uehara et al., 2016; 2017), while the rootstock cultivar Daitaro was susceptible to the virulent *M. incognita* Chiba and Niigata populations (Uehara et al., 2016). In another study, *S. melongena* cultivars, including Pusa Purple Long, Purple Cluster and Purple Round, were susceptible to *M. incognita* (Alam et al., 1974; Dhawan & Sethi, 1976; Ravichandra et al., 1988; Nayak & Sharma, 2013).

In the present study, the Rf values were calculated for the two populations of *M. incognita* on all genotypes, and all Rf values of the populations on resistant genotypes were <1. However, although the Y15 and Y22 genotypes were susceptible to the V14 population of *M. incognita*, their Rf values were <1. Similarly, although the M10, M22, M23, M24, M25, M26, M27, Y11 and Y27 genotypes were susceptible to the S6 population of *M. incognita*, their Rf values were <1. These differences may reflect the life cycle of the nematodes, the plant-nematode interaction and/or the root structures of the plants.

In conclusion, many commercial eggplant cultivars are grown throughout the world, but none are resistant to RKNs. *Solanum torvum* is widely employed commercially as a rootstock to protect against RKNs (Lee, 1994). Recently, the *SacMi* gene from *Solanum aculeatissimum* Jacq., which has been reported to confer resistance to *M. incognita*, has been cloned and characterized (Zhou et al., 2018). The investigation of new resistant sources, such as *S. aculeatissimum*, is needed for management in fields infested with RKNs. A more in-depth knowledge of the responses of different eggplant genotypes to RKNs would be valuable, so future research should test resistant genotypes against different RKN species to establish better integrated management practices. The findings could then be used in RKN breeding and management approaches.

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**Original article (Orijinal araştırma)**

**Ichneumonidae (Hymenoptera) fauna of Kovada Lake National Park, Isparta, Turkey<sup>1</sup>**

Kovada Gölü Milli Parkı (Isparta, Türkiye) Ichneumonidae (Hymenoptera) faunası

**Ayşegül ÖZDAN<sup>2\*</sup>**

**Mehmet Faruk GÜRBÜZ<sup>2</sup>**

**Abstract**

This study examined the Ichneumonidae (Hymenoptera) fauna of Kovada Lake National Park, Isparta, Turkey. Ichneumonidae specimens were collected between April 2010 and October 2014 by sweep net and malaise traps at six stations. In total, 455 individual Ichneumonidae within 10 subfamilies were collected. Among these, 22 genera and 31 species were identified. Six species are new records for Turkey, *Lissonota frontalis* (Desvignes, 1856), *Bathythrix strigosa* (Thomson, 1884), *Hemiteles similis* (Gmelin, 1790), *Diadromus albinotatus* (Gravenhorst, 1829), *Chorinaeus scrobipalpa* Aeschlimann, 1983 and *Trieceles bellulus* Kusigemati, 1984.

**Keywords:** Hymenoptera, Ichneumonidae, Isparta, Kovada Lake National Park

**Öz**

Bu çalışma, Isparta İli Kovada Gölü Milli Parkı Ichneumonidae (Hymenoptera) faunası incelenmiştir. Ichneumonidae örnekleri Nisan 2010 ile Ekim 2014 yılları arasında atrap ve malaise tuzağı ile toplanmıştır. Toplam 10 altfamilyaya ait 455 birey toplanmıştır. Bunlardan 22 cins ve 31 tür teşhis edilmiştir. Teşhis edilen türlerden 6 tanesi Türkiye için yeni kayıttır. Bu türler; *Lissonota frontalis* (Desvignes, 1856), *Bathythrix strigosa* (Thomson, 1884), *Hemiteles similis* (Gmelin, 1790), *Diadromus albinotatus* (Gravenhorst, 1829), *Chorinaeus scrobipalpa* Aeschlimann, 1983, *Trieceles bellulus* Kusigemati, 1984.

**Anahtar sözcükler:** Hymenoptera, Ichneumonidae, Isparta, Kovada Gölü Milli Parkı

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## Introduction

Parasitic Hymenoptera, a large insect group having a wide significance in terrestrial ecosystems (Shaw & Hochberg, 2001), constitute a specialized group of Hymenoptera, one of the mega insect orders (Stevens et al., 2007). The family Ichneumonidae with about 25,285 described species is the most diverse family of Hymenoptera (Yu et al., 2016). This family consist of 39 subfamilies (Quicke, 2015). Despite their abundance and importance in ecosystems as biological pest control, the taxonomy, ecology, and distribution of many groups of Ichneumonidae is still unknown (Riedel & Turrisi, 2013).

The number of Ichneumonidae species in Turkey has been recorded as 1,293 species in 57 genera (Yu et al., 2016). As a result of many studies (Özgen et al., 2010; Okyar et al., 2012; Çoruh & Özbek, 2013; Çoruh & Kolarov, 2013, 2016; Çoruh et al., 2013, 2014a, b, 2016, 2018, 2019; Riedel et al., 2014; Kolarov et al., 2014a, b, 2015, 2016, 2018; Çoruh & Çalmaşur, 2016; Özdan & Gürbüz, 2016; Riedel 2018a, b; Sarı & Çoruh, 2018) many new specimens have been added and the numbers of ichneumonid fauna of Turkey have now reached to about 1,268 species.

Kovada Lake, is located within the boundaries of the city of Isparta in southern Turkey. Kovada Lake and its surrounding were declared as Kovada Lake National Park in 1970 (Alkan, 2009). In 1992, Kovada Lake National Park was defined as a first level protected area. The area is 6,534 ha including its surroundings (Aslan & Karaca, 2012). The national park is located in the Mediterranean phytogeographic region. Kovada Lake National Park has a rich diversity of flora and fauna.

Although many studies have been concluded on Kovada Lake National Park, there have been no studies conducted on the Ichneumonidae fauna. Therefore, the aim of this study was to survey the Ichneumonidae fauna of the Kovada Lake National Park.

## Materials and Methods

### Sampling and collection

This study is based on Ichneumonidae specimens gathered from April to October in 2010-2014. Specimens were collected by sweep net and malaise traps from six stations in Kovada Lake National Park (KLNP) in Isparta Province (Figure 1). The plastic pot of the malaise trap was half full of 95% ethyl alcohol and the samples were collected every 15 d. One trap was placed in each site. The ichneumonid specimens which were taken from the malaise traps separated from other insects under the Ziess Discovery V8 microscope. All the specimens are labeled and deposited at the Biology Department of Süleyman Demirel University, Isparta.

### Study sites

Station I (37°38.861' N, 30°52.213' E, 909 m): It is at the lowest altitude of the selected areas. The vegetation is characterized by *Anthemis* sp. (Asteraceae), *Astragalus* sp. (Fabaceae), *Avena* sp. (Poaceae), *Cirsium sintonisii* Freyn. (Asteraceae), *Malva sylvestris* L. (Malvaceae), *Rubus* sp. (Rosaceae), *Salix* sp. (Salicaceae), *Scorzonera suberosa* C. Koch. (Asteraceae), *Tamarix* sp. (Tamaricaceae), *Triticum* sp. (Poaceae), and *Veronica* sp. (Scrophulariaceae). *Pinus nigra* Arnold (Pinaceae) and *Platanus orientalis* L. (Platanaceae) surround this area. Depending on the spraying of existing orchards around the station, it is an area exposed to chemicals. In addition, vegetation is damaged due to anthropogenic effects.

Station II (37°37.392' N, 30°52.414' E, 914 m): The vegetation is characterized by *Alkanna tinctoria* Tausch. (Boraginaceae), *Lamium* sp. (Lamiaceae), *Muscari* sp. (Hyacinthaceae), *Paliurus spina-christi* Miller (Rhamnaceae), *Quercus coccifera* L. (Fagaceae), *Trifolium stellatum* L. (Fabaceae), and *Veronica* sp. (Plantaginaceae). This area is exposed to chemicals due to the spraying of nearby cherry and apple orchards.



Station III (37°38.626' N, 30°52.137' E, 932 m): The dominant plant species are *Pinus nigra* Arnold. (Pinaceae), *Quercus coccifera* L. (Fagaceae) and *Styrax officinalis* L. (Styracaceae).

Station IV (37°37.846' N, 30°52.130' E, 956 m): The vegetation consist of *Cedrus libani* A.Rich (Pinaceae), *Daphne sericea* Wahl (Thymelaeaceae), *Eryngium kotschy* Boiss. (Apiaceae), *Euphorbia* sp. (Euphorbiaceae), *Muscari* sp. (Liliaceae), *Ornithogalum* sp. (Liliaceae), *Juniperus* sp. (Cupressaceae), *Paliurus spina-christi* L. (Rhamnaceae), *Pinus nigra* Arnold (Pinaceae), *Pistacia terebinthus* L. (Anacardiaceae), *Quercus coccifera* L. (Fagaceae) and *Vicia cracca* L. (Fabaceae).

Station V (37°37.243' N, 30°52.086' E, 985 m): *Astragalus* sp. (Fabaceae), *Euphorbia* sp. (Euphorbiaceae), *Ornithogalum* sp. (Liliaceae), *Pinus nigra* Arnold (Pinaceae), *Quercus coccifera* L. (Fagaceae), *Quercus cerris* L. (Fagaceae), *Silene* sp. (Caryophyllaceae), *Styrax officinalis* L. (Styracaceae), *Verbascum* sp. (Scrophulariaceae) and *Vicia cracca* L. (Fabaceae) are the dominant plants.

Station VI (37°36.331' N, 30°53.717' E, 909 m): This area is a cherry orchard and exposed to insecticide in May-July. Other notable plant species are *Anthemis* sp. (Asteraceae), *Avena* sp. (Poaceae), *Convolvulus arvensis* L. (Convolvulaceae), *Malva sylvestris* L. (Malvaceae), *Taraxacum* sp. (Asteraceae), *Verbascum* sp. (Scrophulariaceae) and *Vicia* sp. (Fabaceae).

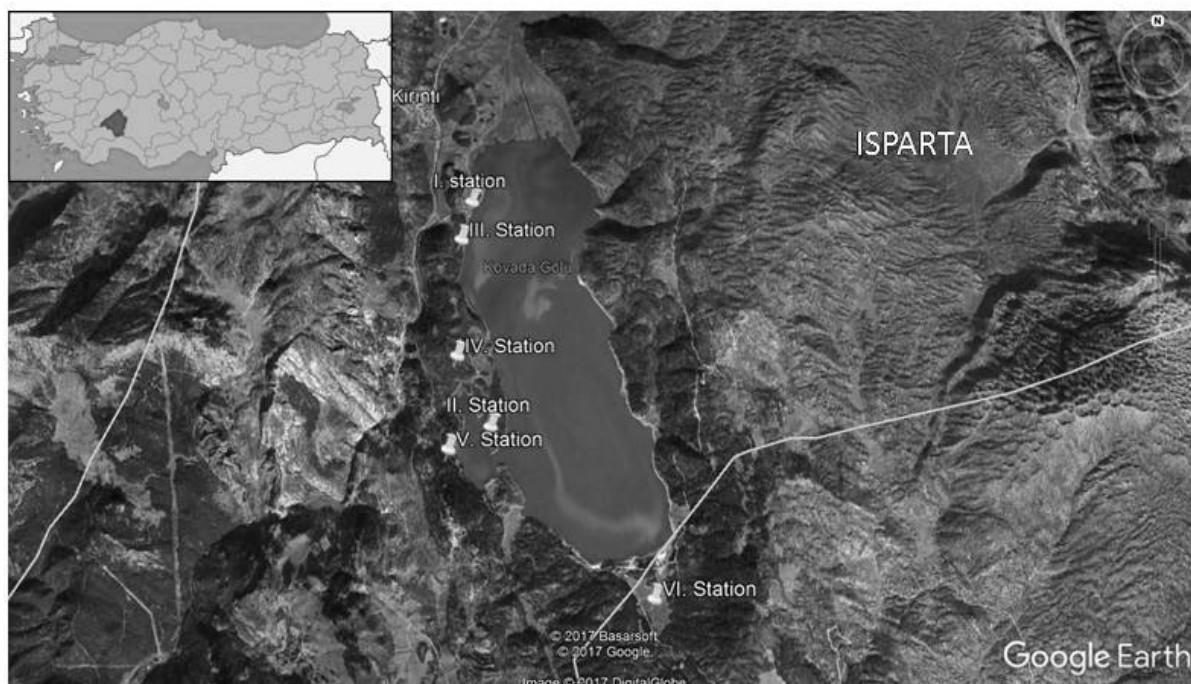


Figure 1. Sampling sites (stations) of Kovada Lake National Park (Anonymous, 2019).

## Results and Discussion

In total 31 species in 22 genera were identified from the study areas. Of these six species are new records for the fauna of Turkey. With this study the number of Ichneumonidae species in Turkey increased to 1274.

The collection data, locations and Turkish distribution are given for each species. The newly recorded species is marked with by an asterisk (\*) in the text below.

### **Subfamily Anomaloninae Viereck, 1918**

#### ***Anomalon cruentatum* (Geoffroy, 1785)**

Material examined: KLNP, Station II, 18.IX.2010, 1♀; 10.VI.2012, 1♀; 17.VI.2013, 2♂♂; 25.IX.2014, 1♀.

Distribution in Turkey: Adana, Gaziantep, Adiyaman, İçel, Antalya, Kırklareli, Edirne, Tekirdağ, İstanbul (Kolarov et al., 1994); Çanakkale (Kolarov et al., 1997a) Afyon, Muğla (Kolarov et al., 2002); Isparta (Gürbüz, 2004); Antalya, Bayburt, Bingöl, Diyarbakır, Erzincan, Erzurum, Iğdır, Kahramanmaraş, Kars (Çoruh et al., 2004), Adiyaman, Batman, Diyarbakır, Elazığ, Malatya, Mardin (Akkaya, 2005), Bolu, Zonguldak, Kastamonu (Okyar & Yurtcan 2007); Isparta (Gürbüz et al., 2009a, b; Birol, 2010; Özdan & Gürbüz, 2016), Erzurum, Tunceli (Kolarov et al., 2014a), Bayburt, Erzurum, Kars (Çoruh & Kolarov, 2016), Tekirdağ (Beyarslan et al., 2006); Erzurum (Çoruh et al., 2018; Sarı & Çoruh, 2018).

General Distribution: Eastern Palearctic, Europe, Oriental, Western Palearctic (Yu et al., 2016).

### **Subfamily Banchinae Wesmael, 1845**

#### ***Exetastes adpressorius* (Thunberg, 1824)**

Material examined: KLNP, Station II, 17.VI.2010, 1♀.

Distribution in Turkey: Edirne (Kolarov & Beyarslan, 1994b); Ankara, Kırıkkale, Kırşehir (Özdemir, 1996); Bayburt, Erzurum (Pekel, 1999); Tunceli (Kolarov et al., 2014a); Erzurum (Çoruh & Çalmaşur, 2016; Çoruh et al., 2018).

General Distribution: Eastern Palearctic, Europe, Nearctic, Western Palearctic (Yu et al., 2016).

#### ***Lissonota (Lissonota) culiciformis* Gravenhorst, 1829**

Material examined: KLNP, Station II, 18.IX.2010, 1♀.

Distribution in Turkey: Erzincan (Pekel et al., 2000); Isparta, Burdur (Kolarov & Gürbüz, 2006); Isparta (Gürbüz et al., 2009b); Erzincan (Çoruh et al., 2014b).

General Distribution: Eastern Palearctic, Europe, Nearctic, Western Palearctic (Yu et al., 2016).

#### **\**Lissonota (Lissonota) frontalis* (Desvignes, 1856)**

Material examined: KLNP, Station II, 17.VI.2010, 1♀.

General Distribution: Eastern Palearctic, Europe, Western Palearctic (Yu et al., 2016).

#### ***Lissonota (Lissonota) fundator* (Thunberg, 1824)**

Material examined: KLNP, Station III, 17.VI.2010, 1♀; 25.V.2014, 1♀.

Distribution in Turkey: Isparta, Burdur (Kolarov & Gürbüz, 2006); Hatay (Gürbüz et al., 2008); Isparta (Gürbüz et al., 2009a); Hatay (Gürbüz et al., 2011).

General Distribution: Eastern Palearctic, Europe, Nearctic, Western Palearctic (Yu et al., 2016).

#### ***Lissonota (Loxonota) histrio* (Fabricius, 1798)**

Material examined: KLNP, Station II, 18.IX.2010, 1♀.

Distribution in Turkey: Erzurum (Pekel & Özbek, 2000); Diyarbakır, Elazığ, Mardin (Akkaya, 2005); Isparta (Gürbüz et al., 2009a); Ordu (Kolarov et al., 2016); Erzurum, Rize (Kolarov et al., 2017).

General Distribution: Eastern Palearctic, Europe, Nearctic, Western Palearctic (Yu et al., 2016).

*Lissonota (Lissonota) proxima* Fonscolombe, 1854

Material examined: KLNP, Station V, 16.XI.2010, 1♀.

Distribution in Turkey: Isparta (Özdan & Gürbüz, 2016).

General Distribution: Eastern Palearctic, Europe, Western Palearctic (Yu et al., 2016).

*Lissonota uncinata* Holmgren, 1860

Material examined: KLNP, Station III, 16.XI.2012, 1♀.

Distribution in Turkey: Adana (Kolarov & Beyarslan, 1994b).

General Distribution: Europe, Western Palearctic (Yu et al., 2016).

#### **Subfamily Campopleginae Forster, 1869**

*Dusona intelligator* Aubert, 1966

Material examined: KLNP, Station II, 10.VI.2012, 1♀.

Distribution in Turkey: Toros (Kolarov, 1995).

General Distribution: Europe, Western Palearctic (Yu et al., 2016).

#### **Subfamily Cremastinae Forster, 1869**

*Pristomerus luridus* Kokujev, 1905

Material examined: KLNP, Station IV, 1♀.

Distribution in Turkey: Erzurum (Pekel & Özbek, 2000; Çoruh et al., 2014b).

General Distribution: Eastern Palearctic, Europe, Western Palearctic (Yu et al., 2016).

*Temelucha schoenobia* (Thomson, 1890)

Material examined: KLNP, Station I, 4.IX.2010, 1♂.

Distribution in Turkey: Antalya (Kolarov & Beyarslan, 1999); Adana, Adıyaman, Antalya, Aydın, Gaziantep, Hatay, İzmir (Kolarov, 2016).

General Distribution: Eastern Palearctic, Europe, Western Palearctic (Yu et al., 2016).

*Temelucha discoidalis* (Szépligeti, 1899)

Material examined: KLNP, Station V, 24.VI.2012, 1♀.

Distribution in Turkey: Erzurum (Pekel & Özbek, 2000); Ankara (Kolarov & Yurtcan, 2009), Hatay (Çoruh et al., 2013)

General Distribution: Eastern Palearctic, Europe, Western Palearctic (Yu et al., 2016).

#### **Subfamily Cryptinae Kirby, 1837**

*Aritranis longicauda* (Kriechbaumer, 1873)

Material examined: KLNP, Station II, 24.VI.2012, 1♀.

Distribution in Turkey: Isparta (Gürbüz & Kolarov, 2008; Gürbüz et al., 2009b).

General Distribution: Europe, Western Palearctic (Yu et al., 2016).

*Mesostenus albinotatus* Gravenhorst, 1829

Material examined: KLNP, Station I, 07.VIII.2010, 1♂.

Distribution in Turkey: Turkey (Sedivy, 1959); Erzurum (Çoruh & Çoruh, 2008); Isparta (Gürbüz & Kolarov, 2008; Gürbüz et al., 2009b); Rize (Çoruh et al., 2014a); Erzurum (Çoruh et al., 2014b; Kolarov et al., 2016).

General Distribution: Eastern Palearctic, Europe, Nearctic, Western Palearctic (Yu et al., 2016).

\* *Bathythrix strigosa* (Thomson, 1884)

Material examined: KLNP, Station V, 17-22.V.2011, 1♀.

Hosts: *Diprion pini* Linnaeus, 1758 (Hymenoptera: Tenthredinoidea), *Taleporia tubulosa* (Retzius, 1783) (Lepidoptera: Psychidae) (Yu et al., 2016).

General Distribution: Europe, Western Palearctic (Yu et al., 2016).

*Dichrogaster saharator* (Aubert, 1964)

Material examined: KLNP, Station III, 16.10.2010, 1♀.

Distribution in Turkey: Çanakkale (Kolarov et al., 1997a); Isparta (Kolarov & Gürbüz, 2007).

General Distribution: Eastern Palearctic, Europe, Western Palearctic (Yu et al., 2016).

*Dichrogaster schimitscheki* (Fahringer, 1935)

Material examined: KLNP, Station V, 07.X.2012, 1♀.

Distribution in Turkey: Isparta (Kolarov & Gürbüz, 2007; Gürbüz et al., 2009b).

General Distribution: Europe, Nearctic, Western Palearctic (Yu et al., 2016).

*Eudelus simillimus* (Taschenberg, 1865)

Material examined: KLNP, Station III, 04.IX.2010, 1♀.

Host: *Tortrix viridana* (Linnaeus, 1758) (Lepidoptera: Tortricidae).

Distribution in Turkey: Turkey (Sedivy, 1959); Ankara (Kolarov, 1995).

General Distribution: Europe, Western Palearctic (Yu et al., 2016).

\**Hemiteles similis* (Gmelin, 1790)

Material examined: KLNP, Station II, 24.VI.2012, 1♀.

General Distribution: Eastern Palearctic, Europe, Nearctic, Western Palearctic (Yu et al., 2016).

### **Subfamily Ichneumoninae Latreille, 1802**

*Heterischnus truncator* (Fabricius, 1798)

Material examined: KLNP, Station VI, 16.XI.2010, 1♀.

Distribution in Turkey: Istanbul (Kolarov, 1995); Çanakkale (Kolarov et al., 1997a); Istanbul (Yurtcan et al., 1999); Erzurum (Özbek et al., 2003); Giresun, Trabzon (Kolarov et al., 2014b); Trabzon (Çoruh et al., 2019).

General Distribution: Palearctic, Europe (Yu et al., 2016).

\**Diadromus albinotatus* (Gravenhorst, 1829)

Material examined: KLNP, Station II, 24.VI.2012, 1♀.

General Distribution: Eastern Palearctic, Europe, Western Palearctic (Yu et al., 2016).

#### **Subfamily Mesochorinae Forster, 1869**

*Mesochorus fulgurans* Curtis, 1833

Material examined: KLNP, Station I, 07.VII.2010, 1♀; 23.VI.2014, 1♀.

Distribution in Turkey: Rize (Çoruh et al., 2014b; Riedel et al., 2014), Isparta (Özdan & Gürbüz; 2016).

General Distribution: Eastern Palearctic, Europe, Oriental, Western Palearctic (Yu et al., 2016).

#### **Subfamily Metopiinae Forster, 1869**

*Exochus flavifrons* Boheman, 1863

Material examined: KLNP, Station II, 18.IX.2010, 2♂♂.

Distribution in Turkey: Erzurum (Çoruh & Kolarov, 2012; Çoruh et al., 2014b); Rize (Çoruh et al., 2014a).

General Distribution: Europe, Western Palearctic (Yu et al., 2016).

*Exochus thomsoni* Schmiedeknecht, 1924

Material examined: KLNP, Station II, 18.IX.2010. 3♀♀; Station VI, 1♀; 05.X.2013, 1♀.

Distribution in Turkey: Erzurum (Çoruh & Kolarov, 2012; Çoruh et al., 2014b); Tunceli (Kolarov et al., 2014a), Erzurum, Rize (Kolarov et al., 2017).

General Distribution: Eastern Palearctic, Europe, Western Palearctic (Yu et al., 2016).

*Exochus erythronotus* (Gravenhorst, 1820)

Material examined: KLNP, Station II, 18.IX.2010, 1♀.

Distribution in Turkey: Aydın (Kolarov et al., 2009), Kars (Çoruh & Kolarov, 2012).

General Distribution: Europe, Western Palearctic (Yu et al., 2016).

\* *Chorinaeus scrobipalpa* Aeschlimann, 1983

Material examined: KLNP, Station II, 10.VI.2012, 1♀.

Hosts: *Scrobipalpa nitentella* (Lepidoptera: Gelechiidae) (Yu et al., 2016).

General Distribution: Eastern Palearctic, Europe, Western Palearctic (Yu et al., 2016).

\**Trieces bellulus* Kusigemati, 1984

Material examined: KLNP, Station III, 10.VI.2012, 1♀.

General Distribution: Eastern Palearctic (Yu et al., 2016).

#### **Subfamily Pimplinae Wesmael, 1845**

*Pimpla artemonis* Kasparyan, 1973

Material examined: KLNP, Station II, 17.VI.2010, 1♂; 05.V.2013, 1♀; 01.VI.2014, 1♂.

Distribution in Turkey: Edirne, İstanbul (Yurtcan & Beyarslan, 2005); Bayburt, Erzurum, Kars, Rize (Çoruh & Özbek, 2008); Artvin, Erzurum, Isparta, Kars (Çoruh & Kolarov, 2010; Çoruh et al., 2014b).

General Distribution: Europe, Western Palearctic (Yu et al., 2016).

*Clistopyga rufator* Holmgren, 1856

Material examined: KLNP, Station II, 22.V.2010, 2♀♀; 11.XI.2012, 1♀; Station V, 11.XI.2014, 1♀.

Distribution in Turkey: Edirne (Yurtcan, 2004), Kırklareli (Yurtcan, 2007), Adana (Buncukçu, 2008), Hatay (Gürbüz et al., 2008); Erzurum, Kars (Çoruh & Özbek, 2008); Erzurum (Çoruh, 2010); Kars (Çoruh & Kolarov, 2010).

General Distribution: Eastern Palearctic, Europe, Western Palearctic (Yu et al., 2016).

*Zatypota bohemani* (Holmgren, 1860)

Material examined: KLNP, Station II, 15.VII.2012, 1♀.

Distribution in Turkey: İstanbul (Kolarov, 1987); Elazığ, İçel, Osmaniye (Kolarov & Beyarslan, 1994a); Edirne (Yurtcan & Beyarslan, 2005), Erzurum, Kars (Çoruh & Özbek, 2008; Çoruh et al., 2014b) Erzurum (Çoruh & Kolarov, 2010); Adana, Hatay (Gürbüz et al., 2008).

General Distribution: Eastern Palearctic, Europe, Nearctic, Western Palearctic (Yu et al., 2016).

### **Subfamily Tryphoninae Shuckard, 1840**

*Acrotomus succinctus* (Gravenhorst, 1829)

Material examined: KLNP, Station I, 01.V.2010, 1♀; 5.V.2013, 1♀.

Distribution in Turkey: Edirne (Kolarov & Beyarslan, 1994a); Bilecik, Çanakkale (Kolarov et al., 1997b); Tekirdağ (Beyarslan et al., 2006); İzmir (Yurtcan et al., 2006), Isparta (Gürbüz et al., 2009b); Erzurum (Kolarov & Çalmaşur, 2011); Rize (Çoruh et al., 2014a); Elazığ, Sivas (Yaman, 2014).

General Distribution: Europe, Nearctic, Oriental, Palearctic (Yu et al., 2016).

Kovada Lake National Park which covering on area of 6,534 ha has very rich flora and fauna. Considerable research has been conducted on different subjects around the lake. This study focused only on the Ichneumonidae fauna on the western side of the lake. Although KLNP is under threat because of using insecticide in agricultural area, it contains new records for Turkey. Precautions should be taken as soon as possible for protecting these insect populations.

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**Original article (Orijinal araştırma)**

## **Taxonomic and biogeographic evaluations of the subfamily Cryptinae (Hymenoptera: Ichneumonidae)**

Türkiye Cryptinae (Hymenoptera: Ichneumonidae) altfamilyası üzerinde taksonomik ve biocoğrafik değerlendirmeler

**Saliha ÇORUH<sup>1\*</sup>**

### **Abstract**

The taxonomic and biogeographic data of specimens belonging to the subfamily Cryptinae (Hymenoptera: Ichneumonidae) collected from different regions in Turkey between 1990 and 2018 were studied. An additional 13 samples collected before 1990 were also included. Three tribes, 61 genera and 187 species were identified. Most of samples were collected during last 25 years or recorded in this time from seven different regions of Turkey by researchers. Among the species listed, *Agrothereutes tiloidalis* Kolarov & Beyaslan, 1994, *Stilpnus adanaensis* Kolarov & Beyaslan, 1994 and *Aptesis cavigena* Kolarov & Gürbüz, 2009 were described from Turkey. Also, these species are endemic to Anatolia. Detailed composition, biogeographic and zoogeographic data, vertical distribution, seasonal dynamics, individual diversity, available host data and plants visited by adults are given.

**Keywords:** Cryptinae, Hymenoptera, Ichneumonidae, Turkey

### **Öz**

Türkiye'nin farklı bölgelerinden 1990 ve 2018 yılları arasında toplanan Cryptinae (Hymenoptera: Ichneumonidae) altfamilyasına ait türleri içeren bu çalışma, taksonomik ve biocoğrafik değerlendirmeleri amaçlamıştır. Buna ek olarak 1990 yılından önce toplanmış olan 13 türü de içermektedir. Sonuçlar değerlendirildiğinde, üç tribus ve 61 cinse bağlı 187 tür teşhis edilmiştir. Türlerin çoğu son 25 yıl süresince toplanmış, *Agrothereutes tiloidalis* Kolarov & Beyaslan, *Stilpnus adanaensis* Kolarov & Beyaslan ve *Aptesis cavigena* Kolarov & Gürbüz türleri ilk kez ülkemizden bilim dünyasına kazandırılmıştır. Bu türler endemic durumdadır. Çalışmada her bir tür için tür kompozisyonu, biocoğrafik ve zoocoğrafik veriler, dikey dağılımlar, sezonal aktiviteler, konukçu ve ziyaret edilen bitkiler de verilmiştir.

**Anahtar sözcükler:** Cryptinae, Hymenoptera, Ichneumonidae, Türkiye

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## Introduction

The order Hymenoptera includes well-known species, including bees, sawflies, wasps and ants, which are among the most common animals on earth. The order contains about 8% of all described species (Davis et al., 2010). Parasitic Hymenoptera have often been used for biological control and these programs demonstrate the great impact that they can have on host populations (Sharkey, 2007).

The Ichneumonidae Latreille, 1802 includes 45 subfamilies, 1601 genera and 25,285 described species (Yu et al., 2016). According to recent studies, number of Ichneumonidae of Turkey is 1257 species in 287 genera (Sarı & Çoruh, 2018).

The subfamily Cryptinae (Figure 1) is the largest subfamily of Ichneumonidae and can be encountered in virtually all terrestrial habitats. The nomenclature of this group is complex, also using the names Phygadeuontinae and Gelinae (Townes, 1969). The most common feature to distinguish a cryptine is the sternaulus. The second recurrent vein is always present in almost all species. First abdominal segment slender, or sometime of moderates proportion. Glymma always lacking (Azura & Idris, 2002).



Figure 1. Cryptinae species: a) *Meringopus calescens* (Gravenhorst, 1829) (from Rudow, 1886); b) *Acroricnus seductor* (Scopoli, 1786) (from Tixier-Inrep, 2015).

Almost all Cryptinae have been described as idiobiont ectoparasitoids. The most common hosts of Cryptinae are endopterygote pupae or prepupae enclosed in cocoons or plant tissue. There are also some endoparasitic species in the Hedycryptina, Phygadeuontina and Stilpnina. A few species are koinobionts. Furthermore, some species parasitize the egg sacs of Pseudoscorpionida and Araneae and many can develop as secondary parasitoids (Goulet & Huber, 1993). Although there is considerable information on the host relationships of some Cryptinae, virtually nothing is known of their biology. Furthermore, as a consequence of the large size of this subfamily, it is structurally very diverse (Gauld & Gaston, 1995).

Lately, Santos (2017) restricted Cryptinae to the tribes Aptesini and Cryptini and elevated the Phygadeuontini and Ateleutina to subfamily status.

Worldwide the subfamily comprises about 403 genera and 5,080 species (Yu et al., 2016). In this case, Cryptinae has the most species in the Ichneumonidae. The catalog of Ichneumonidae of Turkey (Kolarov, 1995) listed 66 Cryptinae species. Since 1995, the number of cryptine fauna of Turkey has reached 187 species (Kolarov et al., 1997a, b; Jussila, 2001; Kolarov et al., 2002; Schwarz, 2005, 2007; Çoruh & Özbek, 2005; Kolarov & Bordera, 2007; Kolarov & Gürbüz, 2007; Kırtay, 2008; Çoruh & Çoruh, 2008; Çoruh & Kesdek, 2008; Gürbüz & Kolarov, 2008; Kolarov & Yurtcan, 2008; Kolarov & Gürbüz, 2009; Özdemir & Güler, 2009; Gürbüz et al., 2009a, b; Quicke et al., 2009; Çoruh & Özbek, 2011; Eroğlu et al., 2011; Çoruh & Çoruh, 2012; Özdan, 2014; Çoruh et al., 2014a, b; Kolarov et al., 2014; Çoruh & Çalmaşur, 2016; Çoruh & Kolarov, 2016; Özdan & Gürbüz, 2016; Çoruh et al., 2016; Kolarov et al., 2016; Sarı & Çoruh, 2018; Çoruh et al., 2018).

The present study aimed to provide detailed information on the subfamily Cryptinae species in Turkey.

## Materials and Methods

Samples were collected from 48 of the 81 provinces of Turkey in seven regions (Figure 2) of Anatolia. Adults were collected with an entomological sweep net (40 cm in diameter), aspirator, malaise and light trap. They were preserved in 75% alcohol in insect envelopes in the field and then pinned before drying. Some of the samples are also reared from host insects under laboratory conditions.

Plants visited by insects were also identified, pressed and stored in recent studies.

Fauna lists usually contain localities, altitude, collecting date, number and sex of each specimen examined. Information on world distribution for each species listed is based on Yu et al. (2016).



Figure 2. The geographical region of cryptine collected.

## Results and Discussion

A total of 187 species belonging to subfamily Cryptinae are discussed with different evaluations.

### Faunistic evaluations

A total of 187 species in 61 genera in three tribes of Cryptinae have been recorded in Turkey (Table 1). The total number of samples was 1485. However, the number of samples for 31 species is unclear.

Table 1. Data of collected species: Individual numbers (IN), vertical distribution (VD), seasonal dynamics (SD), geographical regions (GR), zoogeographic regions (ZR), host records (HR), plant visited records (PVR), first record of Turkey (FRT) of specimens

Names of Taxa	IN		VD (m.)	SD	GR	ZR	HR	PVR	FRT
	♂	♀							
<b>TRIBE CRYPTINI KIRBY, 1837</b>									
<b>Genus <i>Acroncinus</i> Ratzeburg, 1852</b>									
<i>Acroncinus seductor</i> (Scopoli, 1786)	1		F	Jul	EAR, MiR	E, EP, WP			Fahringer & Friese, 1921
<i>Acroncinus seductor elegans</i> Mocsary, 1883	1		F	Jul	EAR	E, WP			Çoruh & Özbek, 2011
<i>Acroncinus seductor syriacus</i> (Mocsary, 1883)	1		D	Jul	MiR	E, WP			Gürbüz & Kolarov, 2008
<i>Acroncinus stylator</i> (Thunberg, 1822)	2	3	A,E	Jul&Aug	MR, EAR	E, EP, NEAR, WP			Kolarov, 1987
<b>Genus <i>Agrothereutes</i> Förster, 1850</b>									
<i>Agrothereutes abbreviator</i> (Fabricius, 1793)	1	2	B,H,F	Jul,Sep	BSR, EAR, MiR	E, WP	X		Fahringer, 1921
<i>Agrothereutes bombycis</i> (Boudier, 1836)	2		A	Apr	MR	E, WP			Beyarslan & Kolarov, 1994
<i>Agrothereutes fumipennis</i> (Gravenhorst, 1829)	4	6	C,D,F,H	Ap-May-Jun,Jul-Aug-Sep	BSR, EAR, MiR	E, EP, WP	X		Çoruh & Özbek, 2005
<i>Agrothereutes grossus</i> (Gravenhorst, 1829)	3		A	Jun	MR	E, WP			Beyarslan & Kolarov, 1994
<i>Agrothereutes hospes</i> (Tschek, 1871)	2	2	A,C,D,F	Jun-Jul&Oct	BSR, EAR, MiR	E, EP, WP	X		Beyarslan & Kolarov, 1994
<i>Agrothereutes leucorhaeus</i> (Donovan, 1810)	?	?	?	?	?	E, WP			Kolarov & Bordera, 2007

Table 1. Continued

Names of Taxa	IN		VD (m.)	SD	GR	ZR	HR	PVR	FRT
	♂	♀							
<i>Agrothereutes parvulus</i> (Habermehl, 1926)	3		A,D	Jun	BSR, MtR	E, EP, WP			Gürbüz & Kolarov, 2008
<i>Agrothereutes tiloidalis</i> Kolarov & Beyarslan, 1994	1	2	A	Apr,Aug	MR, MtR	WP			Kolarov & Beyarslan, 1994
<b>Genus <i>Aritranis</i> Förster, 1869</b>									
<i>Aritranis buccatus</i> (Tschek, 1872)	?	?	A	Aug	MtR	E, WP			Sedivy, 1959
<i>Aritranis claviventris</i> (Kriechbaumer, 1894)	1	1	D	May	MtR	E, EP, WP			Beyarslan & Kolarov, 1994
<i>Aritranis coxator</i> Tschek, 1871		1	A	Aug	MR	E, EP, WP			Kolarov et al., 1997a
<i>Aritranis director</i> (Thunberg, 1822)	71	6	C,D,E,F	May-Jun-Jul	BSR, EAR, MtR	E, EP, NEAR, WP			Gürbüz & Kolarov, 2008
<i>Aritranis femoralis</i> Gravenhorst, 1829	1	2	A	May	EAR, MR	E, EP, WP		X	Beyarslan & Kolarov, 1994
<i>Aritranis graefei</i> Thomson, 1896	?	?	A	?	AR	E, WP	X	X	Öncüer, 1991
<i>Aritranis heliophilus</i> (Tschek, 1871)	2	2	A	Jun-Jul-Aug-Sep	MR, MtR	E, EP, WP			Beyarslan & Kolarov, 1994
<i>Aritranis longicauda</i> (Kriechbaumer, 1873)	34	11	C,D,E	Apr-May-Jun	MtR	E, WP			Gürbüz & Kolarov, 2008
<i>Aritranis nigrifemur</i> (Szepligetli, 1916)	2	2	D,E	Jun-Jul	Anatolia, MtR	E, EP, WP			Sedivy, 1959
<i>Aritranis nigripes</i> (Gravenhorst, 1829)	?	?	B	Aug	MtR	E, EP, WP			Sedivy, 1959
<i>Aritranis occisor</i> (Gravenhorst, 1829)	1		C	Jun	Anatolia, MtR	E, EP, WP			Schwarz, 2005
<i>Aritranis quadriguttata</i> (Gravenhorst, 1829)	1		B	Aug	MR	E, EP, WP			Kolarov et al., 1997a
<i>Aritranis signatoria</i> Fabricius, 1793	1	1	A	Aug	MR	E, WP			Kolarov et al., 1997a
<b>Genus <i>Buathra</i> Cameron, 1903</b>									
<i>Buathra laborator</i> (Thunberg, 1824)	9	11	D,G,H	May-Jun&Aug	BSR, EAR, MtR	E, EP, NEAR, WP	X	X	Gürbüz & Kolarov, 2008
<i>Buathra tarsoleucos</i> (Schrank, 1781)	2	3	D,G	May&Jul&Aug	EAR, MR, MtR	E, EP, WP			Fahringer, 1922
<b>Genus <i>Caenocryptus</i> Thomson, 1873</b>									
<i>Caenocryptus rufiventris</i> (Gravenhorst, 1829)	?	?	A	Jun	MR	E, EP, WP			Sedivy, 1959
<b>Genus <i>Cryptus</i> Fabricius, 1804</b>									
<i>Cryptus amator</i> Fabricius, 1804	?	?	D	Jul	Anatolia	E, EP, WP		X	Fahringer, 1922
<i>Cryptus diana</i> Gravenhorst, 1829		3	D	May-Jun-Jul	MtR	E, EP, WP			Gürbüz & Kolarov, 2008
<i>Cryptus leucocheir</i> (Ratzeburg, 1844)	?	?	D	?	CAR	E, EP, WP			Kolarov, 1995
<i>Cryptus minor</i> Gravenhorst, 1829	?	?	C	?	AR	E, WP	X		Kolarov, 1987
<i>Cryptus moschator</i> (Fabricius, 1787)	1		C	May	EAR	E, NEAR, WP			Kolarov et al., 2014a
<i>Cryptus spinosus</i> Gravenhorst, 1829	3	1	D	Jun&Aug	MtR	E, EP, WP			Sedivy, 1959
<i>Cryptus spiralis</i> (Geoffroy, 1785)	10	12	E,F,H	May-Jun-Jul&Sep	CAR, EAR, MtR	E, EP, WP		X	Çoruh & Özbek, 2005
<i>Cryptus subspinosus</i> Smith van Burgst, 1913		1	D	May	Anatolia, MtR	E, EP, WP			Schwarz, 2005
<i>Cryptus triguttatus</i> Gravenhorst, 1829	4	4	A,D	May-Jun-Jul-Aug	AR; MR	E, EP, WP			Beyarslan & Kolarov, 1994
<i>Cryptus tuberculatus</i> Gravenhorst, 1829	1	2	B,E	May-Jun-Jul	MtR, MR	E, EP, WP			Sedivy, 1959
<i>Cryptus viduatorius</i> Fabricius, 1804	76	30	A,E,F,G	Apr-May-Jun-Jul-Aug	EAR, BSR, MtR, MR	E, EP, WP		X	Kolarov, 1987
<b>Genus <i>Enclisis</i> Townes, 1970</b>									
<i>Enclisis omatoiceps</i> (Thomson, 1885)	?	?	?	?	Anatolia	E, WP			Schwarz, 1989
<b>Genus <i>Gambrus</i> Förster, 1869</b>									
<i>Gambrus camifex</i> (Gravenhorst, 1829)	1	7	A,C,D	Mar&Jun-Jul	MtR	E, EP, WP			Beyarslan & Kolarov, 1994
<i>Gambrus incubitor</i> (Linnaeus, 1758)	3	1	A,D,E	Mar&May-Jun	BSR, MtR	AFR, E, EP, WP			Beyarslan & Kolarov, 1994
<i>Gambrus inferus</i> Thomson, 1896	1	5	A	Apr&Aug	MtR, MR	E, WP			Kolarov, 1987
<i>Gambrus opacus</i> Szepligetli, 1916		4	G,H	Jun-Jul	EAR	E, WP	X		Çoruh & Özbek, 2005
<i>Gambrus omatulus</i> (Thomson, 1873)	3		A	Sep	MtR	E, EP, WP			Kolarov et al., 1997a
<i>Gambrus tricolor</i> (Gravenhorst, 1829)		2	B,D	Jun&Sep	BSR	E, WP			Kolarov & Yurtcan, 2008

Table 1. Continued

Names of Taxa	IN		VD (m.)	SD	GR	ZR	HR	PVR	FRT
	♂	♀							
<b>Genus <i>Hidryta</i> Förster, 1869</b>									
<i>Hidryta frater</i> (Cresson, 1864)	1		A	Sep	MR	E, NEAR, WP			Kolarov et al., 1997a
<i>Hidryta sortida</i> (Tschek, 1871)	3		D	May	MtR	E, EP, WP			Gürbüz & Kolarov, 2008
<b>Genus <i>Hoplocryptus</i> Thomson, 1873</b>									
<i>Hoplocryptus confector</i> (Gravenhorst, 1829)	1		D	Jun	Anatolia, MtR	E, EP, WP			Schwarz, 2007
<i>Hoplocryptus femoralis</i> (Gravenhorst, 1829)	8	6	D,E,F	May-Jun&Aug	Anatolia, BSR, EAR	E, EP, WP			Schwarz, 2007
<i>Hoplocryptus fugitivus</i> (Gravenhorst, 1829)	1	6	F,G	Jun	EAR, MtR	E, WP			Çoruh & Özbek, 2005
<i>Hoplocryptus murarius</i> (Bömer, 1782)	2		A,B	Jun	Anatolia, BSR	E, EP, WP			Schwarz, 2007
<i>Hoplocryptus odoriferator</i> (Dufour & Perris, 1840)	1		E	May	MtR	E, WP			Schwarz, 2007
<i>Hoplocryptus quadriguttatus</i> (Gravenhorst, 1829)	2		E	Jun	MtR	E, EP, WP			Schwarz, 2007
<b>Genus <i>Idiolispa</i> Förster, 1869</b>									
<i>Idiolispa analis</i> (Gravenhorst, 1807)	11		B,C,D,F	May&Jul	EAR, MtR, SAR	E, EP, NEAR, ORR, WP			Beyarslan & Kolarov, 1994
<b>Genus <i>Ischnus</i> Gravenhorst, 1829</b>									
<i>Ischnus agitator</i> (Oliver, 1792)	5	6	A,C,D,G	May-Jun-Jul-Aug	AR, EAR, MtR	E, EP, WP		X	Gürbüz & Kolarov, 2008
<i>Ischnus alternator</i> Gravenhorst, 1829	2	3	A,D	Jun&Aug	BSR, MR	E, EP, WP			Kolarov et al., 1997a
<i>Ischnus inquisitorius</i> (Müller, 1776)	2		E	Aug-Sep	BSR, EAR	E, EP, NEAR, NTR, WP			Kolarov & Yurtcan, 2008
<i>Ischnus migrator</i> (Fabricius, 1775)	4		B,E	Jun- ul	MtR	E, EP, WP		X	Fahringer, 1922
<i>Ischnus minutiorius</i> (Fabricius, 1804)		7	A	May-Jun	MtR	E, WP			Beyarslan & Kolarov, 1994
<b>Genus <i>Latibulus</i> Gistel, 1848</b>									
<i>Latibulus argiolus</i> (Rossi, 1790)	1	2	B,E	May-Jun-Jul-Aug	CAR, EAR	E, EP, WP		X	Fahringer, 1922
<b>Genus <i>Listrocryptus</i> Brauns, 1905</b>									
<i>Listrocryptus spatulatus</i> Brauns, 1905	1		A	Jun	MtR	E, WP			Beyarslan & Kolarov, 1994
<b>Genus <i>Listrognathus</i> Tschek 1871</b>									
<i>Listrognathus (Listrognathus) furax</i> (Tschek, 1871)	4		D	May	MtR	E, EP, WP			Gürbüz & Kolarov, 2008
<i>Listrognathus ligator</i> Gravenhorst 1829	?	?	?	?	Anatolia	E, WP			Horstmann, 1990
<i>Listrognathus obnoxius</i> (Gravenhorst, 1829)	4		A	May	MR	E, WP			Beyarslan & Kolarov, 1994
<b>Genus <i>Meringopus</i> Förster, 1869</b>									
<i>Meringopus calescens</i> (Gravenhorst, 1829)	73	226	D,G,H	Jul	AR, EAR	E, EP, NEAR, ORR, WP		X	Beyarslan & Kolarov, 1994
<i>Meringopus calescens calescens</i> (Gravenhorst, 1829)	3		H	Jun	EAR	E, EP, NEAR, ORR, WP		X	Kolarov et al., 2016
<i>Meringopus calescens persicus</i> Heinrich, 1937	4	1	D,H	Jun-Jul	EAR	WP			Kolarov & Yurtcan, 2008
<i>Meringopus cyanator</i> (Gravenhorst, 1829)	21	30	G,H	Jun-Jul	EAR	E, EP, WP	X	X	Çoruh & Özbek 2005
<i>Meringopus nigerrimus</i> (Fonscolombe, 1850)	1		G	Jul	EAR	E, EP, NEAR, WP			Çoruh & Özbek 2005
<i>Meringopus pseudonymus</i> (Tschek, 1872)	6		C,D	May-Jun&Aug	EAR, MR, MtR	E, EP, WP		X	Kolarov, 1987
<i>Meringopus titillator</i> (Linnaeus, 1758)	24	2	E,F,G,H	May-Jun-Jul&Aug	CAR, EAR, MtR	E, EP, WP		X	Szepligeti, 1916
<i>Meringopus titillator rhodius</i> (Dalla Torre, 1902)	2	4	D,H	Sep	EAR, MtR	E, EP, WP		X	Gürbüz & Kolarov, 2008
<b>Genus <i>Mesostenus</i> Gravenhorst, 1829</b>									
<i>Mesostenus albinotatus</i> Gravenhorst, 1829	11	14	B,E,F,H	Jun-Jul-Aug	BSR, EAR, MtR	E, EP, NEAR, WP		X	Sedivy, 1959
<i>Mesostenus grammicus</i> (Gravenhorst, 1829)	3	7	A,D,E	Jun-Jul&Sep	EAR, MR, MtR	E, EP, WP			Kolarov, 1987
<i>Mesostenus transfuga</i> Gravenhorst, 1829	13	11	A,E,F,H	May-Jun-Jul-Aug	AR, MR, MtR, EAR	E, EP, OCC, WP	X	X	Beyarslan & Kolarov, 1994
<b>Genus <i>Mymeleonostenus</i> Uchida 1936</b>									
<i>Mymeleonostenus italicus</i> (Gravenhorst, 1829)	4	14	A,C,D,F	May-Jun-Jul	BSR, EAR, MR, MtR	E, EP, WP		X	van Rossem, 1969

Table 1. Continued

Names of Taxa	IN		VD (m.)	SD	GR	ZR	HR	PVR	FRT
	♂	♀							
<b>Genus <i>Nematopodius</i> Gravenhorst, 1829</b>									
<i>Nematopodius formosus</i> Gravenhorst, 1829		1	D	Jul	BSR	E, WP			Çoruh et al., 2016
<b>Genus <i>Polytribax</i> Förster, 1869</b>									
<i>Polytribax perspicillator</i> (Gravenhorst, 1807)	7	2	A	May&Aug	MtR	E, EP, WP			Beyarslan & Kolarov, 1994
<b>Genus <i>Pycnocryptus</i> Thomson, 1873</b>									
<i>Pycnocryptus claviventris</i> Krichbaumer, 1894		1	D	Jun	MtR, SAR	E, EP, WP			Beyarslan & Kolarov, 1994
<i>Pycnocryptus director</i> (Thunberg, 1822)	5	3	A,D	May-Jun-Jul	MtR, MR	E, EP, NEAR, WP			Beyarslan & Kolarov, 1994
<i>Pycnocryptus rarus</i> (Hebemehl, 1920)	?	?	A	Jun	BSR	E, WP			Sedivy, 1959
<b>Genus <i>Pycnocryptodes</i> Aubert, 1971</b>									
<i>Pycnocryptodes reticulator</i> Aubert, 1971		1	C	Jul	MtR	EP, WP			Gürbüz & Kolarov, 2008
<b>Genus <i>Schreineria</i> Schreiner, 1905</b>									
<i>Schreineria populnea</i> (Giraud, 1872)		2	A,D	Jul	BSR	E, EP, WP			Çoruh et al., 2014a
<b>Genus <i>Stenarella</i> Szépligeti, 1916</b>									
<i>Stenarella domator</i> (Pado, 1761)	1	3	D	May&Jul	MR, MtR	E, EP, WP		X	Fahringer, 1922
<b>Genus <i>Synechocryptus</i> Schmiedeknecht, 1904</b>									
<i>Synechocryptus mactator</i> (Tschek, 1870)		3	C	May	AR, MtR	E, EP, WP			Kolarov, 1987
<b>Genus <i>Thrybius</i> Townes, 1965</b>									
<i>Thrybius praedator</i> (Rossi, 1792)	?	?	A	Jul	MtR	E, EP, WP			Fahringer, 1922
<b>Genus <i>Trychosis</i> Förster 1869</b>									
<i>Trychosis atripes</i> (Gravenhorst, 1829)		2	A,D	Jun-Jul	MR, MtR	E, EP, WP			Beyarslan & Kolarov, 1994
<i>Trychosis legator</i> (Thunberg, 1822)	28	12	A,B,C,D	Apr-May-Jun-Jul-Aug	BSR, EAR, MR, MR, SAR	E, EP, WP			Kolarov, 1987
<i>Trychosis neglecta</i> (Tschek, 1870)		3	D,E	Jun & Aug	MR, MtR	E, EP, WP		X	Fahringer, 1922
<i>Trychosis mesocastana</i> (Tschek, 1871)		1	A	Jul	MtR	E, WP			Kolarov et al., 1997b
<i>Trychosis pauper</i> (Tschek, 1871)	13	2	D	May-Jun-Jul-Aug	EAR, MR, MR	E, EP, WP			Kolarov et al., 1997b
<i>Trychosis priesneri</i> Rossem, 1971	1	1	D,E	M	CAR, MtR	E, EP, WP			van Rossem, 1971
<i>Trychosis timenda</i> Rossem, 1990		8	A	May&Aug	MtR, MR	E, WP			Beyarslan & Kolarov, 1994
<i>Trychosis tristator</i> (Tschek, 1871)		8	A,C	May-Jun-Jul-Aug	EAR, MR, MR	E, EP, WP			Beyarslan & Kolarov, 1994
<b>Genus <i>Xylophrurus</i> Förster, 1869</b>									
<i>Xylophrurus augustus</i> (Dalman, 1823)	1	9	C,E	Apr-May-Jun	AR, CAR, EAR, MtR	E, WP			Özdemir & Güler, 2009
<i>Xylophrurus lancifer</i> (Gravenhorst, 1829)		1	G	Jun	EAR	E, EP, WP			Kolarov et al., 2016
<b>TRIBE HEMIGASTERINI ASHMEAD, 1900</b>									
<b>Genus <i>Aptesis</i> Förster, 1850</b>									
<i>Aptesis assimilis</i> (Gravenhorst, 1829)	7	2	E,H	Jun-Jul	EAR	E, WP			Kolarov et al., 2016
<i>Aptesis cavigena</i> Kolarov & Gürbüz, 2009		1	E	Jun	MtR	WP			Kolarov & Gürbüz, 2009
<i>Aptesis cretata</i> (Gravenhorst, 1829)		1	B	Aug	MtR	E, WP			Kolarov et al., 1997a
<i>Aptesis nigrocineta</i> (Gravenhorst, 1815)		1	D	Jul	EAR	E, EP, WP			Kolarov et al., 2014a
<i>Aptesis senicula</i> (Krichbaumer, 1893)	5	1	C,D,E,F	May	BSR, EAR, MR	E, WP			Beyarslan & Kolarov, 1994
<b>Genus <i>Giraudia</i> Foerster, 1869</b>									
<i>Giraudia gyrationa</i> (Thunberg, 1824)	?	?	B	Aug	MtR	E, EP, WP		X	Fahringer, 1922



Table 1. Continued

Names of Taxa	IN		VD (m.)	SD	GR	ZR	HR	PVR	FRT	
	♂	♀								
<b>Genus <i>Parmortha</i> Townes, 1962</b>										
<i>Parmortha pleuralis</i> (Thomson, 1873)	2		A	Aug	MtR	E, EP, NEAR, WP			Kolarov et al., 1997a	
<i>Pleolophus brachypterus</i> (Gravenhorst, 1815)	1		E	Jul-Aug	EAR, MtR	E, EP, WP		X	Fahringer, 1922	
<b>Genus <i>Polytribax</i> Förster, 1869</b>										
<i>Polytribax rufipes</i> (Gravenhorst, 1829)	?	?	C	Jul-Aug	AR	E, WP		X	Fahringer, 1921	
<b>TRIBE PHYGADEUONTINI FORSTER, 1869</b>										
<b>Genus <i>Aclastus</i> Förster, 1869</b>										
<i>Aclastus gracilis</i> (Thomson, 1884)	21	3	D,E	May-Jun-Jul&Sep	MR, MtR	E, EP, NEAR, WP			Kolarov et al., 1997b	
<i>Aclastus micator</i> (Gravenhorst, 1807)	19	5	A,D	May-Jun-Jul&Sep	AR, MtR	E, EP, NEAR, WP			Beyarslan & Kolarov, 1994	
<i>Aclastus solutus</i> (Thomson, 1984)	3	3	A,D	May-Jun-Jul	AR, MtR	E, EP, WP			Beyarslan & Kolarov, 1994	
<i>Aclastus transversalis</i> Horstman, 1980	1		D	May	MtR	E, WP			Kolarov & Gürbüz, 2007	
<b>Genus <i>Acrolyta</i> Förster, 1869</b>										
<i>Acrolyta distincta</i> (Bridgman, 1883)	1	3	A	Aug	MR	E, WP			Kolarov et al., 1997a	
<i>Acrolyta semistrigosa</i> (Schmiedeknecht, 1897)	1		D	Jun	MtR	E, WP			Kolarov & Gürbüz, 2007	
<b>Genus <i>Atractodes</i> Gravenhorst, 1829</b>										
<b>Subgenus <i>Atractodes (Asyncrita)</i> Förster, 1876</b>										
<i>Atractodes (Asyncrita) assimilis</i> Förster, 1876	2		D,F	May&Sep	MtR, SAR	E, WP			Beyarslan & Kolarov, 1994	
<i>Atractodes (Asyncrita) foveolatus</i> (Gravenhorst, 1829)	1		H	Aug	BSR	EP, E, WP			Beyarslan & Kolarov, 1994	
<b>Subgenus <i>Atractodes (Atractodes)</i> Gravenhorst, 1829</b>										
<i>Atractodes (Atractodes) fumatus</i> Haliday, 1838	1		D	Jul	MtR	EP, E, NEAR, WP			Beyarslan & Kolarov, 1994	
<i>Atractodes (Atractodes) pusillus</i> Förster, 1876	1		E	Oct	MtR	EP, E, NEAR, WP			Beyarslan & Kolarov, 1994	
<b>Genus <i>Bathythrix</i> Förster, 1869</b>										
<i>Bathythrix claviger</i> (Taschenberg, 1865)	?	?	A	May	MR	EP, E, NEAR, ORR WP		X	Schimitschek, 1944	
<i>Bathythrix collaris</i> (Thomson, 1896)	4		D	Jul	BSR	E, WP			Çoruh et al., 2016	
<i>Bathythrix decipiens</i> (Gravenhorst, 1829)	2		D	May&Sep	BSR, MtR	E, WP			Kolarov & Gürbüz, 2007	
<i>Bathythrix fragilis</i> (Gravenhorst, 1829)	1		A	Jul	BSR	E, WP			Çoruh et al., 2016	
<i>Bathythrix lamina</i> (Thomson 1884)	5	5	A,C,F	Jun-Jul&Sep	BSR, MR, MtR	E, WP			Kolarov et al., 1997a	
<i>Bathythrix linearis</i> (Gravenhorst, 1829)	1		B	Jun	BSR	EP, E, WP			Çoruh et al., 2014a	
<i>Bathythrix pellucidator</i> (Gravenhorst, 1829)	4		A	Jun	BSR	EP, E, WP			Çoruh et al., 2014a	
<b>Genus <i>Blapsidotes</i> Förster, 1869</b>										
<i>Blapsidotes vicinus</i> (Gravenhorst 1829)	10	1	C,D	May-Jun-Jul&Sep	BSR, MtR	EP, E, WP			Kolarov & Gürbüz, 2007	
<b>Genus <i>Ceratophygadeuon</i> Viereck, 1924</b>										
<i>Ceratophygadeuon anurus</i> (Thomson, 1884)	?	?	F	Aug	EAR	E, WP			Horstmann, 1993	
<b>Genus <i>Chirotica</i> Förster, 1869</b>										
<i>Chirotica decorator</i> (Villers, 1789)	?	?	A	Jun	MtR	EP, E, WP			Kolarov, 1987	
<i>Chirotica insignis</i> (Gravenhorst 1829)	1		E	Aug	EAR	E, WP			Çoruh & Kolarov, 2016	
<i>Chirotica orientalis</i> Horstmann, 1983	1		B	Apr	SAR	WP		X	Kolarov & Erkin, 1987	
<i>Chirotica ruficeps</i> Horstmann, 1983	?	?	H	Aug	EAR	E, WP			Horstmann, 1993	
<i>Chirotica terebrator</i> Horstmann, 1983	1		B	May	SAR	EP, E, WP		X	X	Horstmann, 1993
<b>Genus <i>Diaglyptellana</i> Horstmann, 1976</b>										
<i>Diaglyptellana punctatus</i> (Holmgren, 1857)	?	?	C	Jul	CAR	E, WP			Sedivy, 1959	

Table 1. Continued

Names of Taxa	IN		VD (m.)	SD	GR	ZR	HR	PVR	FRT
	♂	♀							
<b>Genus <i>Diaglyptellodes</i> Aubert, 1993</b>									
<i>Diaglyptellodes sculpturator</i> (Aubert, 1977)	1		D	May	Anatolia, MtR	EP, E, WP			Aubert, 1977
<b>Genus <i>Dichrogaster</i> Doumerc, 1855</b>									
<i>Dichrogaster aestivalis</i> (Gravenhorst, 1829)	36	13	A,B,D,F	May-Jun-Jul	CAR, EAR, MtR, MR, SAR	EP, E, WP			Beyarslan & Kolarov, 1994
<i>Dichrogaster diatropus</i> Townes, 1983	1	1	A	May&Sep	CAR, MtR	E, NEAR, WP			Townes, 1983
<i>Dichrogaster liostylus</i> Thomson, 1885	3		B	Jun	BSR	EP, E, ORR, WP		X	Kolarov, 1995
<i>Dichrogaster longicauda</i> (Thomson 1885)	21	1	D,E,G	May-Jul	EAR, CAR, MtR	EP, E, NEAR WP		X	Townes, 1983
<i>Dichrogaster modesta</i> (Gravenhorst, 1829)	6		E,F	Sep	BSR, MtR	E, WP		X	Kolarov et al., 1997a
<i>Dichrogaster perlae</i> (Dounmerc, 1855)	1		D	Jun	MtR	E, WP			Kolarov & Gürbüz, 2007
<i>Dichrogaster saharator</i> (Aubert, 1964)		2	D	May	MR, MtR	EP, E, WP			Kolarov et al., 1997b
<i>Dichrogaster schimitscheki</i> (Fahringer, 1935)	8	3	D,E	May-Jun	MtR	E, NEAR, WP			Kolarov & Gürbüz, 2007
<b>Genus <i>Echthrus</i> Gravenhorst, 1829</b>									
<i>Echthrus reluctator</i> (Linnaeus, 1758)	?	?	A	Aug	MtR	EP, E, WP		X X	Fahringer, 1922
<b>Genus <i>Encrateola</i> Strand, 1917</b>									
<i>Encrateola laevigata</i> (Ratzeburg, 1848)		2	A,G	Jun-Jul	BSR, EAR, MtR	AFR, EP, E, NEAR, WP		X	Beyarslan & Kolarov, 1994
<b>Genus <i>Endasys</i> Förster, 1869</b>									
<i>Endasys brevis</i> (Gravenhorst, 1829)	2		D,E	May	Anatolia, MtR	EP, E, WP			Sawoniewicz, & Luhman, 1992
<i>Endasys erythrogaster</i> (Gravenhorst, 1829)	?	?	C	May	CAR	EP, E, WP		X	Kolarov, 1987
<i>Endasys femoralis</i> (Habermehl 1912)	1		D	Jul	MtR	E, WP			Kolarov & Gürbüz, 2007
<i>Endasys minutulus</i> (Thomson 1883)	8		E	Jun	MtR	E, NEAR, WP			Kolarov & Gürbüz, 2007
<i>Endasys parviventris</i> (Gravenhorst, 1929)	?	?	?	?	Anatolia	EP, E, ORR, WP			Sawoniewicz, & Luhman, 1992
<i>Endasys plagiator</i> (Gravenhorst, 1829)	5		E,G,H	Jun	Anatolia, EAR, MtR	E, WP			Sawoniewicz, & Luhman, 1992
<i>Endasys rubricator</i> (Thunberg, 1822)	?	?	D	May	CAR	E, WP			Kolarov, 1987
<i>Endasys senilis</i> (Gmelin, 1790)	1		D	Jun	MtR	E, WP			Kolarov & Gürbüz, 2007
<b>Genus <i>Eudelus</i> Förster, 1869</b>									
<i>Eudelus similimus</i> Taschenberg, 1865	?	?	D	Jul	CAR	E, WP			Sedivy, 1959
<b>Genus <i>Gelis</i> Thunberg, 1827</b>									
<i>Gelis agilis</i> (Fabricius, 1775)	3		A,G	Jun-Jul	Anatolia, BSR, EAR	EP, E, WP			Fahringer, 1922
<i>Gelis cursitans</i> (Fabricius, 1775)	1		A	Jun	BSR	E, WP			Çoruh et al., 2014a
<i>Gelis cyanurus</i> (Förster, 1851)	?	?	E	Apr	Anatolia, CAR	E, WP			Diller, 1969
<i>Gelis exareolatus</i> (Förster, 1850)	?	?	D	Jun	CAR	EP, E, WP			Kolarov, 1987
<i>Gelis fomicarius</i> (Linnaeus, 1758)	1		B	Jul	BSR	EP, E, WP			Çoruh et al., 2014a
<i>Gelis instabilis</i> (Förster, 1851)	8		A,D	May-Jun-Jul-Aug	Anatolia, EAR, MtR, MR	EP, E, WP			Fahringer, 1922
<i>Gelis micurus</i> (Förster, 1850)		1	A	Jul	MtR	E, WP			Beyarslan & Kolarov, 1994
<i>Gelis mutillatus</i> (Gmelin, 1790)	1		G	Jun	EAR	EP, E, WP			Çoruh et al., 2014a
<i>Gelis rufipes</i> (Förster, 1876)	2	2	B,C,F	May-Jun-Jul	AR, SAR	E, WP			Beyarslan & Kolarov, 1994
<i>Gelis sculpturator</i> Aubert, 1977	?	?	B	Mar&Jun	CAR	EP, E, WP			Aubert, 1977
<i>Gelis trux</i> (Förster, 1850)	3		A,G	Jun	BSR, EAR	EP, E, WP			Çoruh et al., 2014a

Table 1. Continued

Names of Taxa	IN		VD (m.)	SD	GR	ZR	HR	PVR	FRT
	♂	♀							
<b>Genus <i>Grasseiteles</i> Aubert, 1965</b>									
<i>Grasseiteles ciliator</i> Aubert, 1968	?	?	B	Jun	MtR	EP, E, WP	X		Aubert, 1968
<b>Genus <i>Glyphicnemis</i> Förster, 1869</b>									
<i>Glyphicnemis profligator</i> (Fabricius, 1775)	13	2	A,B,D,G	May-Jun-Jul	BSR, EAR, MTR	EP, E, WP			Çoruh & Özbek, 2005
<i>Glyphicnemis vagabunda</i> (Gravenhorst 1829)	49	28	D,E,G,H	Apr&Jun-Jul-Aug	EAR, MtR, MR	EP, E, WP		X	Sawoniewicz, 1985
<b>Genus <i>Helcostizus</i> Förster, 1869</b>									
<i>Helcostizus restaurator</i> (Fabricius, 1775)	?	?	A	Mar	MtR	EP, E, NEAR, WP	X		Schimitschek, 1944
<b>Genus <i>Isadelphus</i> Förster, 1869</b>									
<i>Isadelphus armatus</i> (Gravenhorst, 1829)	1		D	Jun	MtR	E, WP			Kolarov & Gürbüz, 2007
<b>Genus <i>Lochetica</i> Kriechbaumer, 1892</b>									
<i>Lochetica westoni</i> (Bridgman, 1880)	1		D	May	MtR	EP, E, WP			Kolarov & Gürbüz, 2007
<b>Genus <i>Lysibia</i> Förster, 1869</b>									
<i>Lysibia nana</i> (Gravenhorst, 1829)	6	6	A,F,E	Apr&Jun-Jul-Aug	AR, MtR, MR	EP, E, NEAR, OCC, ORR, WP	X	X	Fahringer, 1922
<b>Genus <i>Mesoleptus</i> Gravenhorst, 1829</b>									
<i>Mesoleptus filicomis</i> (Thomson, 1884)	2		A, D	Aug	Anatolia, MtR	EP, E, WP			Kohl, 1905
<i>Mesoleptus incessor</i> (Haliday, 1838)	?		?	?	Anatolia				Jussila, 2010
<i>Mesoleptus laevigatus</i> (Gravenhorst, 1820)	2	2	G	Aug	Anatolia, EAR	EP, E, WP			Fahringer, 1922
<i>Mesoleptus laticinctus</i> (Walker, 1874)	1	1	A,B	Jun	Anatolia, BSR	EP, E, ORR, WP			Kolarov, 1987
<i>Mesoleptus marginatus</i> (Thomson, 1884)	4		A,F	May&Jul-Aug-Sep	MtR, MR	E, WP		X	Kolarov, 1987
<i>Mesoleptus scrutator</i> (Haliday, 1838)	8	7	A,D	May-Jun-Jul-Aug	AR, MtR, MR	EP, E, WP			Beyarslan & Kolarov, 1994
<i>Mesoleptus transversor</i> Thunberg, 1822	2		B	Aug	MR	E, WP			Kolarov et al., 1997a
<b>Genus <i>Phygadeuon</i> Gravenhorst, 1829</b>									
<i>Phygadeuon trichops</i> Thomson, 1884	1		E	April	MtR	EP, E, WP			Kolarov & Gürbüz, 2007
<i>Phygadeuon vexator</i> (Thunberg, 1822)	2		E	May	MtR	E, WP			Kolarov & Gürbüz, 2007
<b>Genus <i>Rhembobius</i> Förster, 1869</b>									
<i>Rhembobius perscrutator</i> (Thunberg, 1822)	1		H	Ju	EAR	EP, E, WP			Çoruh et al., 2016
<i>Rhembobius quadrispinus</i> (Gravenhorst, 1829)	3		A,D	May-Jun-Jul	BSR, MtR, MR	E, WP			Kolarov et al., 1997b
<b>Genus <i>Stilpnus</i> Gravenhorst, 1829</b>									
<i>Stilpnus adanaensis</i> Kolarov & Beyarslan, 1994	1		A	May	MR	WP			Kolarov & Beyarslan, 1994
<i>Stilpnus gagates</i> (Gravenhorst, 1807)	3	1	A,E	Jun&Sep	AR	EP, E, NEAR, NTR, OCC, WP			Beyarslan & Kolarov, 1994
<b>Genus <i>Thaumatogelis</i> Schwarz, 1995</b>									
<i>Thaumatogelis femoralis</i> (Brischke, 1881)	2		G	Jul	EAR	E, WP		X	Çoruh et al., 2016
<b>Genus <i>Theroscopus</i> Förster, 1850</b>									
<i>Theroscopus hemipterus</i> (Fabricius, 1793)	?	?	G	Sep	AR	E, ORR, WP			Sedivy, 1959
<i>Theroscopus subzonatus</i> (Gravenhorst, 1829)	?	?	C	Jul	CAR	E, WP			Sedivy, 1959
<b>Genus <i>Zoophthorus</i> Förster 1869</b>									
<i>Zoophthorus australis</i> (Thomson, 1885)	1		D	May	MtR	E, WP			Kolarov & Gürbüz, 2007
<i>Zoophthorus graculus</i> (Gravenhorst, 1829)	21	1	A	Jun	MR	EP, E, NEAR, NTR, WP			Kolarov & Beyarslan, 1994

Vertical distribution (VD) (m): A: 0-500 m, B: 501-750 m, C: 751-1000 m, D: 1001-1250 m, E: 1251-1500 m, F: 1501-1750 m, G: 1751-2000 m, H: 2001-2500 m. Seasonal dynamics (SD): March: March; Ap: April, M: May, J: June, Jl: July, A: August, S: September, O: October. Geographical regions (GR): AR: Aegean Region, BSR: Black Sea Region, CAR: Central Anatolia Region, EAR: Eastern Anatolia Region, MR: Marmara Region, MtR: Mediterranean Region, SAR: Southeastern Anatolia. Zoogeographic regions (ZR): AFR: Afrotropical Region, E: Europe, EP: Eastern Palearctic, NEAR: Nearctic Region, NTR: Neotropical, ORR: Oriental, WP: Western Palearctic.

Table 2. Provinces and references of species collected in Turkey

Taxa	Distribution in Turkey	References
<b>TRIBE CRYPTINI KIRBY, 1837</b>		
<i>Acroricnus seductor</i> (Scopoli, 1786)	Erzurum, Isparta	Fahringer & Friese, 1921; Fahringer, 1922; Schimilschek, 1944; Schmidt, 1954; Sedivy, 1959; Kolarov, 1995; Schwarz, 2005; Gürbüz & Kolarov, 2008; Çoruh & Özbek, 2011; Çoruh et al., 2014b
<i>Acroricnus seductor elegans</i> Mocsary, 1883	Erzurum	Çoruh & Özbek, 2011; Çoruh et al., 2014b
<i>Acroricnus seductor syriacus</i> (Mocsary, 1883)	Isparta	Gürbüz & Kolarov, 2008
<i>Acroricnus stylator</i> (Thunberg, 1822)	Istanbul, Erzurum	Kolarov, 1987; Öncüer, 1991; Kolarov, 1995; Çoruh et al., 2018
<i>Agrothereutes abbreviator</i> (Fabricius, 1793)	Erzurum, Hatay, Kastamonu	Fahringer, 1921; Kolarov & Beyarslan, 1994; Kolarov, 1995; Kolarov & Yurtcan, 2008
<i>Agrothereutes bombycis</i> (Boudier, 1836)	Edirne	Beyarslan & Kolarov, 1994
<i>Agrothereutes fumipennis</i> (Gravenhorst, 1829)	Erzurum, Isparta, Kastamonu	Çoruh & Özbek, 2005; Gürbüz & Kolarov, 2008; Kolarov & Yurtcan, 2008; Gürbüz et al., 2009a; Çoruh et al., 2014b
<i>Agrothereutes grossus</i> (Gravenhorst, 1829)	Kırklareli	Beyarslan & Kolarov, 1994
<i>Agrothereutes hospes</i> (Tschek, 1871)	Isparta, Giresun, Van	Beyarslan & Kolarov, 1994; Gürbüz et al., 2006; Gürbüz & Kolarov, 2008; Çoruh et al., 2014a
<i>Agrothereutes leucorhaeus</i> (Donovan, 1810)	Anatolia	Kolarov & Bordera, 2007
<i>Agrothereutes parvulus</i> (Habermehl, 1926)	Isparta, Giresun, Ordu	Gürbüz & Kolarov, 2008; Çoruh et al., 2014b
<i>Agrothereutes tiloidalis</i> Kolarov & Beyarslan, 1994	Antalya, Edirne	Kolarov & Beyarslan, 1994; Kolarov, 1995
<i>Aritranis buccatus</i> (Tschek, 1872)	Adana	Sedivy, 1959; Öncüer, 1991; Kolarov, 1995
<i>Aritranis claviventris</i> (Kriechbaumer, 1894)	Adana, Antalya	Beyarslan & Kolarov, 1994; Gürbüz & Kolarov, 2008
<i>Aritranis coxator</i> Tschek, 1871	Bilecik	Kolarov et al., 1997a
<i>Aritranis director</i> (Thunberg, 1822)	Antalya, Burdur, Erzurum, Isparta, Trabzon, Rize	Gürbüz & Kolarov, 2008; Gürbüz et al., 2009a; Çoruh et al., 2014a, Özdan, 2014; Sarı & Çoruh, 2018; Çoruh et al., 2018
<i>Aritranis femoralis</i> (Gravenhorst, 1829)	Balıkesir, Erzurum	Beyarslan & Kolarov, 1994; Schwarz, 2007; Çoruh & Çoruh, 2008; Çoruh et al., 2014b
<i>Aritranis graefei</i> Thomson, 1896	İzmir	Öncüer, 1991; Kolarov, 1995
<i>Aritranis heliophilus</i> (Tschek, 1871)	Bursa, Edirne, Hatay, Kırklareli	Beyarslan & Kolarov, 1994; Kolarov et al., 1997a, Schwarz, 2007
<i>Aritranis longicauda</i> (Kriechbaumer, 1873)	Isparta	Gürbüz & Kolarov, 2008; Gürbüz et al., 2009a,b; Özdan, 2014
<i>Aritranis nigrifemur</i> (Szepligetli, 1916)	Anatolia, Isparta	Sedivy, 1959; Gürbüz & Kolarov, 2008; Gürbüz et al., 2009a
<i>Aritranis nigripes</i> (Gravenhorst, 1829)	Adana	Sedivy, 1959; Öncüer, 1991; Kolarov, 1995; Schwarz, 2005
<i>Aritranis occisor</i> (Gravenhorst, 1829)	Anatolia, Isparta	Schwarz, 2005; Gürbüz & Kolarov, 2008
<i>Aritranis quadriguttata</i> (Gravenhorst, 1829)	Bursa	Kolarov et al., 1997a; Schwarz, 2007
<i>Aritranis signatoria</i> Fabricius, 1793	Bursa	Kolarov et al., 1997a
<i>Buathra laborator</i> (Thunberg, 1824)	Burdur, Erzurum, Isparta, Trabzon	Gürbüz & Kolarov, 2008; Çoruh & Çoruh, 2012; Çoruh et al., 2014a; Çoruh & Çalmaşur, 2016; Çoruh et al., 2016; Kolarov et al., 2016
<i>Buathra tarsoleucos</i> (Schrank, 1781)	Bursa, Isparta, Erzurum	Fahringer, 1922; Kolarov, 1995; Gürbüz & Kolarov, 2008; Özdan 2014; Çoruh et al., 2014b, Kolarov et al., 2014
<i>Caenocryptus rufiventris</i> (Gravenhorst, 1829)	Edirne	Sedivy, 1959; Kolarov, 1995
<i>Cryptus armator</i> Fabricius, 1804	Anatolia	Fahringer, 1922; Kolarov, 1995
<i>Cryptus diana</i> Gravenhorst, 1829	Isparta	Gürbüz & Kolarov, 2008
<i>Cryptus leucocheir</i> (Ratzeburg, 1844)	Konya	Kolarov, 1995; Schwarz, 2015

Table 2. Continued

Taxa	Distribution in Turkey	References
<i>Cryptus minator</i> Gravenhorst, 1829	Kütahya	Kolarov, 1987; Öncüer, 1991; Kolarov, 1995
<i>Cryptus moschator</i> (Fabricius, 1787)	Tunceli	Kolarov et al., 2014; Çoruh et al., 2014b
<i>Cryptus spinosus</i> Gravenhorst, 1829	Adana, Isparta	Sedivy, 1959; Öncüer, 1991; Kolarov, 1995; Gürbüz & Kolarov, 2008; Eroğlu et al., 2011
<i>Cryptus spiralis</i> (Geoffroy, 1785)	Erzurum, Isparta, Karabük, Kars	Çoruh & Özbek, 2005; Çoruh & Çoruh 2008; Gürbüz & Kolarov, 2008; Gürbüz et al., 2009a; Çoruh et al., 2014b
<i>Cryptus subspinosus</i> Smith van Burgst, 1913	Anatolia, Isparta	Schwarz, 2005; Gürbüz & Kolarov, 2008
<i>Cryptus triguttatus</i> Gravenhorst, 1829	Afyon, Bursa, Edirne, Muğla	Beyarslan & Kolarov, 1994; Kolarov et al., 1997a; Kolarov et al., 2002; Schwarz, 2015
<i>Cryptus tuberculatus</i> Gravenhorst, 1829	Edirne, Isparta, Tekirdağ	Sedivy, 1959; Kolarov, 1995; Kolarov & Yurtcan 2008; Özdan 2014; Schwarz, 2015
<i>Cryptus viduatorius</i> Fabricius, 1804	Bilecik, Bursa, Erzurum, Isparta, Içel, Kırklareli, Rize, Trabzon	Kolarov, 1987; Beyarslan & Kolarov, 1994; Kolarov, 1995; Kolarov et al., 1997a; Çoruh & Çoruh, 2008; Gürbüz & Kolarov, 2008; Gürbüz et al., 2009b; Çoruh & Çoruh, 2012; Özdan, 2014; Çoruh et al., 2014a, b; Çoruh & Kolarov, 2016; Özdan & Gürbüz, 2016; Çoruh et al., 2016; Kolarov et al., 2016, San & Çoruh, 2018; Çoruh et al., 2018
<i>Enclisis ornaticeps</i> (Thomson, 1885)	Anatolia	Schwarz, 1989; Kolarov, 1995; Kolarov & Bordera, 2007
<i>Gambrus carnifex</i> (Gravenhorst, 1829)	Adana, Afyon, Denizli	Beyarslan & Kolarov, 1994; Kolarov et al., 2002
<i>Gambrus incubitor</i> (Linnaeus, 1758)	Isparta, Rize, Kahramanmaraş	Beyarslan & Kolarov, 1994; Gürbüz & Kolarov, 2008; Gürbüz et al., 2009a; Çoruh et al., 2014a
<i>Gambrus inferus</i> Thomson, 1896	Antalya, Balıkesir, Bilecik, İstanbul	Kolarov, 1987; Kolarov, 1995; Öncüer, 1991; Beyarslan & Kolarov 1994; Kolarov et al., 1997a
<i>Gambrus opacus</i> Szepligeti, 1916	Erzurum	Çoruh & Özbek, 2005; Çoruh et al., 2014b
<i>Gambrus ornatulus</i> (Thomson, 1873)	Bilecik, Bursa	Kolarov et al., 1997a
<i>Gambrus tricolor</i> (Gravenhorst, 1829)	Kastamonu, Rize	Çoruh & Özbek, 2005; Kolarov & Yurtcan, 2008; Çoruh et al., 2014a,b
<i>Hidryta frater</i> (Cresson, 1864)	Çanakkale	Kolarov et al., 1997a
<i>Hidryta sordida</i> (Tschek, 1871)	Isparta	Gürbüz & Kolarov, 2008; Gürbüz et al., 2009a
<i>Hoplocryptus confector</i> (Gravenhorst, 1829)	Anatolia, Isparta	Schwarz, 2007; Gürbüz & Kolarov, 2008
<i>Hoplocryptus femoralis</i> (Gravenhorst, 1829)	Anatolia, Artvin, Erzurum, Tunceli	Schwarz, 2007; Çoruh & Özbek, 2011; Kolarov et al., 2014; Çoruh et al., 2014b
<i>Hoplocryptus fugitivus</i> (Gravenhorst, 1829)	Erzurum, Isparta	Çoruh & Özbek, 2005; Gürbüz & Kolarov, 2008; Çoruh et al., 2014b
<i>Hoplocryptus murarius</i> (Börner, 1782)	Anatolia, Rize	Schwarz, 2007; Çoruh et al., 2014a,b
<i>Hoplocryptus odoriferator</i> (Dufour & Perris, 1840)	Isparta	Schwarz, 2007; Gürbüz & Kolarov, 2008
<i>Hoplocryptus quadriguttatus</i> (Gravenhorst, 1829)	Isparta	Schwarz, 2007; Gürbüz & Kolarov, 2008; Gürbüz et al., 2009a
<i>Idiolispa analis</i> (Gravenhorst, 1807)	Isparta, Gaziantep, Tunceli	Beyarslan & Kolarov, 1994; Gürbüz & Kolarov, 2008; Çoruh et al., 2014b; Kolarov et al., 2014
<i>Ischnus agitator</i> (Oliver, 1792)	Afyon, Denizli, Erzurum, Isparta, İzmir, Uşak	Kolarov et al., 2002; Gürbüz & Kolarov, 2008; Çoruh et al., 2016
<i>Ischnus alternator</i> Gravenhorst, 1829	Bursa, Giresun, Ordu, Rize, Trabzon	Kolarov et al., 1997a, Çoruh et al., 2014a, Kolarov et al., 2016
<i>Ischnus inquisitorius</i> (Müller, 1776)	Sinop, Tunceli	Kolarov & Yurtcan, 2008; Çoruh et al., 2014b, Kolarov et al., 2014
<i>Ischnus migrator</i> (Fabricius, 1775)	Adana, Isparta	Fähringer, 1922; Kolarov, 1995; Gürbüz & Kolarov, 2008; Gürbüz et al., 2009a
<i>Ischnus minorius</i> (Fabricius, 1804)	Balıkesir, Edirne, Kırklareli	Beyarslan & Kolarov, 1994
<i>Latibulus argiolus</i> (Rossi, 1790)	Amasya, Ankara, Erzincan, Erzurum, Konya	Fähringer, 1922, Kolarov, 1995; Kolarov & Çalmaşur, 2011; Kolarov & Yurtcan, 2008; Çoruh et al., 2014b, Kolarov et al., 2014

Table 2. Continued

Taxa	Distribution in Turkey	References
<i>Listrocyptus spatulatus</i> Brauns, 1905	Tekirdağ	Beyarslan & Kolarov, 1994
<i>Listrognathus (Listrognathus) furax</i> (Tschek, 1871)	Isparta	Gürbüz & Kolarov, 2008
<i>Listrognathus ligator</i> Gravenhorst, 1829	Anatolia	Horstmann, 1990; Kolarov, 1995
<i>Listrognathus obnoxius</i> (Gravenhorst, 1829)	Kırklareli	Beyarslan & Kolarov, 1994
<i>Meringopus calescens</i> (Gravenhorst, 1829)	Erzurum, Izmir, Van	Beyarslan & Kolarov, 1994; Schwarz, 2005; Anlaş et al., 2009; Çoruh & Çoruh, 2008; Çoruh & Çoruh, 2012; Çoruh et al., 2014b
<i>Meringopus calescens calescens</i> (Gravenhorst, 1829)	Erzurum	Kolarov et al., 2016
<i>Meringopus calescens persicus</i> Heinrich, 1937	Erzurum	Kolarov & Yurtcan, 2008; Çoruh & Özbek, 2011; Çoruh et al., 2014b, Kolarov et al., 2016
<i>Meringopus cyanator</i> (Gravenhorst, 1829)	Erzurum	Çoruh & Özbek, 2005; Çoruh & Kesdek, 2008; Çoruh & Çoruh, 2008; Çoruh et al., 2014b
<i>Meringopus nigerrimus</i> (Fonscolombe, 1850)	Erzurum	Çoruh & Özbek, 2005, Çoruh et al., 2014b
<i>Meringopus pseudonymus</i> (Tschek, 1872)	Istanbul, Isparta, Erzurum, Tunceli	Kolarov, 1987; Kolarov, 1995; Gürbüz & Kolarov, 2008; Çoruh & Çoruh, 2012; Çoruh et al., 2014b, Kolarov et al., 2014
<i>Meringopus titillator</i> (Linnaeus, 1758)	Antalya, Erzurum, Isparta, Karaman, Kars	Szepligeli, 1916; Kolarov, 1995; Kolarov & Gürbüz, 2007; Çoruh & Çoruh 2002; Çoruh & Özbek, 2011; Çoruh et al., 2014b, Kolarov et al., 2016
<i>Meringopus titillator rhodius</i> (Dalla Torre, 1902)	Erzurum, Isparta	Gürbüz & Kolarov, 2008, Çoruh & Çoruh, 2012; Gürbüz et al., 2009a
<i>Mesostenus albinotatus</i> Gravenhorst, 1829	Adana, Elazığ, Erzurum, Isparta, Rize	Sediv, 1959; Aubert, 1972; Kolarov 1995; Gürbüz & Kolarov, 2008; Çoruh & Çoruh 2008; Gürbüz et al., 2009a; Çoruh et al., 2014a, Kolarov et al., 2016
<i>Mesostenus grammicus</i> Gravenhorst, 1829	Çanakkale, Elazığ, Erzurum, Isparta, İstanbul, Kırklareli	Kolarov, 1987; Kolarov & Beyarslan, 1994; Kolarov, 1995; Kolarov et al., 1997b; Gürbüz & Kolarov, 2008; Gürbüz et al., 2009a, Çoruh et al., 2018
<i>Mesostenus transfuga</i> Gravenhorst, 1829	Adana, Antalya, Aydın, Burdur, Bursa, Edirne, Erzurum, Hatay, Isparta, Kırklareli, Mersin, Tekirdağ	Kolarov & Beyarslan, 1994; Kolarov, 1995; Kolarov et al., 1997a; Çoruh & Çoruh, 2008; Gürbüz & Kolarov, 2008; Gürbüz et al., 2009a
<i>Myrmeleonostenus italicus</i> (Gravenhorst, 1829)	Antalya, Erzincan, Isparta, Kırklareli, Tunceli, Zonguldak	van Rossem, 1969, Beyarslan & Kolarov, 1994; Kolarov, 1995; Gürbüz & Kolarov, 2008; Gürbüz et al., 2009a; Özdan 2014; Çoruh et al., 2014b; Kolarov et al., 2014; Çoruh et al., 2016
<i>Nematopodius formosus</i> Gravenhorst, 1829	Rize	Çoruh et al., 2016
<i>Polytribax perspicillator</i> (Gravenhorst, 1807)	Balıkesir, Edirne	Beyarslan & Kolarov, 1994
<i>Pycnocryptus claviventris</i> Krichbaumer, 1894	Adana, Urfa	Beyarslan & Kolarov, 1994; Kolarov, 1995
<i>Pycnocryptus director</i> (Thunberg, 1822)	Edirne, Isparta, Kırklareli, Tekirdağ	Beyarslan & Kolarov, 1994; Gürbüz et al., 2009a
<i>Pycnocryptus rarus</i> (Hebermehl, 1920)	Bolu	Sediv, 1959, Öncüer, 1991; Kolarov, 1995
<i>Pycnocryptodes reticulator</i> Aubert, 1971	Isparta	Gürbüz & Kolarov, 2008
<i>Schreineria populnea</i> (Giraud, 1872)	Giresun, Rize	Çoruh et al., 2014a; Kolarov et al., 2016
<i>Stenarella domator</i> (Pado, 1761)	Istanbul, Isparta	Fähringer, 1922, Kolarov, 1995; Gürbüz & Kolarov, 2008, Gürbüz et al., 2009a; Özdan, 2014
<i>Synechocryptus mactator</i> (Tschek, 1870)	Afyon, İstanbul	Kolarov, 1987; Kolarov, 1995, Öncüer, 1991; Schwarz, 1997; Özdemir & Güler, 2009
<i>Thybius praedator</i> (Rossi, 1792)	Istanbul	Fähringer, 1922; Kolarov, 1995
<i>Trychosis atripes</i> (Gravenhorst, 1829)	Isparta, Kırklareli	Beyarslan & Kolarov, 1994; Gürbüz & Kolarov, 2008
<i>Trychosis legator</i> (Thunberg, 1822)	Adana, Burdur, Çanakkale, Edirne, Erzurum, Gaziantep, Gümüşhane, Isparta, Kırklareli, Tekirdağ, Tunceli, Rize	Kolarov, 1987; Beyarslan & Kolarov, 1994; Kolarov et al., 1997b; Gürbüz & Kolarov, 2008; Çoruh et al., 2014b, Kolarov et al., 2014; Çoruh et al., 2016

Table 2. Continued

Taxa	Distribution in Turkey	References
<i>Trychosis neglecta</i> (Tschek, 1870)	Adana, İstanbul, Isparta	Fahringer, 1922; Sedivy, 1959; Öncüer, 1991; Gürbüz & Kolarov, 2008; Gürbüz et al., 2009a
<i>Trychosis mesocastana</i> (Tschek, 1871)	Çanakkale	Kolarov et al., 1997b
<i>Trychosis pauper</i> (Tschek, 1871)	Çanakkale, Erzurum, Isparta, Tunceli	Kolarov et al., 1997b; Gürbüz & Kolarov, 2008; Gürbüz et al., 2009a; Çoruh et al., 2014b; Kolarov et al., 2014a
<i>Trychosis priesneri</i> Rossem, 1971	Antalya, Konya, Isparta	van Rossem, 1971; Kolarov, 1995; Gürbüz & Kolarov, 2008
<i>Trychosis timenda</i> Rossem, 1990	Adana, Antalya, Edirne, Tekirdağ	Beyarslan & Kolarov, 1994
<i>Trychosis tristator</i> (Tschek, 1871)	Çanakkale, Edirne, Isparta, Kırklareli, Tunceli	Beyarslan & Kolarov, 1994; Kolarov et al., 1997b; Gürbüz & Kolarov, 2008; Gürbüz et al., 2009a; Çoruh et al., 2014b; Kolarov et al., 2014
<i>Xylophrurus augustus</i> (Dalman, 1823)	Afyon, Isparta, Konya, Tunceli, Erzurum	Özdemir & Güler, 2009; Özdan, 2014; Çoruh et al., 2014b; Kolarov et al., 2014
<i>Xylophrurus lancifer</i> (Gravenhorst, 1829)	Erzurum	Kolarov et al., 2016
<b>TRIBE HEMIGASTERINI ASHMEAD, 1900</b>		
<i>Aptesis assimilis</i> (Gravenhorst, 1829)	Erzurum	Kolarov et al., 2016; Çoruh et al., 2018
<i>Aptesis cavigena</i> Kolarov & Gürbüz, 2009	Isparta	Kolarov & Gürbüz, 2009
<i>Aptesis cretata</i> (Gravenhorst, 1829)	Bilecik	Kolarov et al., 1997a
<i>Aptesis nigrocincta</i> (Gravenhorst, 1815)	Tunceli	Kolarov et al., 2014; Çoruh et al., 2014b
<i>Aptesis senicula</i> (Kriechbaumer, 1893)	Adana, Mersin, Tunceli, Rize	Beyarslan & Kolarov, 1994; Kolarov et al., 2014; Çoruh et al., 2014b; Kolarov et al., 2016
<i>Giraudia gyratoria</i> (Thunberg, 1824)	İstanbul	Fahringer, 1922; Kolarov, 1995
<i>Parmortha pleuralis</i> (Thomson, 1873)	Bilecik	Kolarov et al., 1997a
<i>Pleolophus brachypterus</i> (Gravenhorst, 1815)	İstanbul, Tunceli	Fahringer, 1922; Kolarov, 1995; Çoruh et al., 2014b; Kolarov et al., 2014
<i>Polytribax rufipes</i> (Gravenhorst, 1829)	İzmir	Fahringer, 1921; Kolarov, 1995
<b>TRIBE PHYGADEUONTINI FORSTER, 1869</b>		
<i>Aclastus gracilis</i> (Thomson, 1884)	Bilecik, Çanakkale, Isparta	Kolarov et al., 1997a, b; Kolarov & Gürbüz, 2007; Gürbüz et al., 2009a
<i>Aclastus micator</i> (Gravenhorst, 1807)	Adana, Afyon, Antalya, Hatay, Isparta, Muğla	Beyarslan & Kolarov, 1994; Kolarov et al., 2002
<i>Aclastus solutus</i> (Thomson, 1984)	Adana, Afyon, Muğla	Beyarslan & Kolarov, 1994; Kolarov et al., 2002
<i>Aclastus transversalis</i> Horstman, 1980	Isparta	Kolarov & Gürbüz, 2007
<i>Acrolyta distincta</i> Bridgman, 1883	Bilecik, Bursa	Kolarov et al., 1997a
<i>Acrolyta semistrigosa</i> (Schmiedeknecht 1897)	Isparta	Kolarov & Gürbüz, 2007
<i>Atractodes (Asyncrita) assimilis</i> Förster, 1876	Adana, Kahramanmaraş	Beyarslan & Kolarov, 1994; Jussila, 2001
<i>Atractodes (Asyncrita) foveolatus</i> (Gravenhorst, 1829)	Rize	Beyarslan & Kolarov, 1994; Jussila, 2001
<i>Atractodes (Atractodes) fumatus</i> Haliday, 1838	Antalya	Beyarslan & Kolarov, 1994
<i>Atractodes (Atractodes) pusillus</i> Förster, 1876	Adana	Beyarslan & Kolarov, 1994; Jussila, 2001
<i>Bathythrix claviger</i> (Taschenberg, 1865)	İstanbul	Schimitschek, 1944; Kolarov, 1995
<i>Bathythrix collaris</i> (Thomson, 1896)	Rize	Çoruh et al., 2016
<i>Bathythrix decipiens</i> (Gravenhorst, 1829)	Isparta, Sinop	Kolarov & Gürbüz, 2007; Kolarov & Yurtcan, 2008
<i>Bathythrix fragilis</i> (Gravenhorst, 1829)	Ordu	Çoruh et al., 2016

Table 2. Continued

Taxa	Distribution in Turkey	References
<i>Bathythrix lamina</i> (Thomson, 1884)	Çanakkale, Isparta, Kastamonu, Rize	Kolarov et al., 1997a; Kolarov & Gürbüz 2007; Kolarov & Yurtcan 2008; Gürbüz et al., 2009a; Çoruh et al., 2014a
<i>Bathythrix linearis</i> (Gravenhorst, 1829)	Rize	Çoruh et al., 2014a
<i>Bathythrix pellucidator</i> (Gravenhorst, 1829)	Rize, Ordu	Çoruh et al., 2014a
<i>Blapsidotes vicinus</i> (Gravenhorst 1829)	Antalya, Burdur, Kastamonu, Isparta	Kolarov & Gürbüz, 2007; Kolarov & Yurtcan, 2008
<i>Ceratophygadeuon anurus</i> (Thomson, 1884)	Van	Horstmann, 1993; Kolarov, 1995
<i>Chirotica decorator</i> (Villers, 1789)	Istanbul	Kolarov, 1987; Kolarov, 1995
<i>Chirotica insignis</i> (Gravenhorst, 1829)	Kars	Çoruh & Kolarov, 2016
<i>Chirotica orientalis</i> Horstmann, 1983	Diyarbakır	Kolarov & Erkin, 1987; Kolarov, 1995
<i>Chirotica ruficeps</i> Horstmann, 1983	Kars	Horstmann, 1993; Kolarov, 1995
<i>Chirotica terebrator</i> Horstmann, 1983	Diyarbakır	Horstmann, 1983; Kolarov, 1995
<i>Diaglyptellana punctatus</i> (Holmgren, 1857)	Ankara	Sedivy, 1959; Kolarov, 1995
<i>Diaglyptellodes sculpturator</i> (Aubert, 1977)	Anatolia, Isparta	Aubert, 1977; Schwarz, 2003; Kolarov & Gürbüz, 2007
<i>Dichrogaster aestivalis</i> (Gravenhorst, 1829)	Adana, Afyon, Antalya, Burdur, Çanakkale, Denizli, Edirne, Elazığ, Gaziantep, Isparta, Kahramanmaraş, Şanlıurfa, Tekirdağ	Beyarslan & Kolarov, 1994; Kolarov et al., 1997b, Kolarov et al., 2002; Kolarov & Gürbüz 2007; Gürbüz et al., 2009a; Kolarov et al., 2002
<i>Dichrogaster diatropus</i> Townes, 1983	Bursa, Çanakkale, Konya	Townes, 1983; Kolarov, 1995; Kolarov et al., 1997a
<i>Dichrogaster liostylus</i> Thomson, 1885	Samsun, Rize	Kolarov, 1995; Çoruh et al., 2014a
<i>Dichrogaster longicaudata</i> (Thomson 1884)	Erzurum, Eskişehir, Isparta	Townes, 1983; Kolarov & Gürbüz, 2007; Kırtay, 2008; Gürbüz et al., 2009a; Quike et al., 2009; Eroglu et al., 2011; Çoruh et al., 2016
<i>Dichrogaster modesta</i> (Gravenhorst, 1829)	Bursa, Kastamonu, Sinop	Kolarov et al., 1997a; Kolarov & Yurtcan 2008
<i>Dichrogaster perlae</i> (Dounmerc, 1855)	Isparta	Kolarov & Gürbüz, 2007
<i>Dichrogaster saharator</i> (Aubert, 1964)	Çanakkale, Isparta	Kolarov et al., 1997b; Kolarov & Gürbüz 2007
<i>Dichrogaster schimitscheki</i> (Fahringer, 1935)	Isparta	Kolarov & Gürbüz, 2007; Gürbüz et al., 2009a
<i>Echthrus reluctator</i> (Linnaeus, 1758)	Istanbul	Fahringer, 1922; Kolarov, 1995
<i>Encrateola laevigata</i> (Ratzeburg, 1848)	Adana, Giresun, Erzurum, Hatay	Beyarslan & Kolarov 1994; Çoruh et al., 2014a, Çoruh et al., 2016
<i>Endasys brevis</i> (Gravenhorst, 1829)	Anatolia, Isparta	Sawoniewicz, & Luhman, 1992; Kolarov & Gürbüz 2007; Gürbüz et al., 2009a
<i>Endasys erythrogaster</i> (Gravenhorst, 1829)	Ankara	Kolarov, 1987; Kolarov, 1995
<i>Endasys femoralis</i> (Habermehl 1912)	Isparta	Kolarov & Gürbüz 2007; Gürbüz et al., 2009a
<i>Endasys minutulus</i> (Thomson 1883)	Isparta	Kolarov & Gürbüz 2007; Gürbüz et al., 2009a
<i>Endasys parviventris</i> (Gravenhorst, 1929)	Anatolia	Sawoniewicz, & Luhman, 1992; Kolarov, 1995
<i>Endasys plagiator</i> (Gravenhorst, 1829)	Anatolia, Erzurum, Isparta	Sawoniewicz, & Luhman, 1992; Kolarov, 1995; Kolarov & Bordera, 2007; Kolarov & Gürbüz, 2007; Gürbüz et al., 2009a; Çoruh et al., 2014a, Kolarov et al., 2016
<i>Endasys rubricator</i> (Thunberg, 1822)	Ankara	Kolarov, 1987; Kolarov, 1995
<i>Endasys senilis</i> (Gmelin 1790)	Isparta	Kolarov & Gürbüz, 2007
<i>Eudelus simillimus</i> Taschenberg, 1865	Ankara	Sedivy, 1959; Kolarov, 1995
<i>Gelis agilis</i> (Fabricius, 1775)	Anatolia, Erzincan, Giresun, Trabzon	Fahringer, 1922; Kolarov et al., 2016
<i>Gelis cursitans</i> (Fabricius, 1775)	Rize	Çoruh et al., 2014a
<i>Gelis cyanurus</i> (Förster, 1851)	Anatolia, Akşehir	Diller, 1969; Kolarov, 1995; Schwarz, 1998



Table 2. Continued

Taxa	Distribution in Turkey	References
<i>Gelis exareolatus</i> (Förster, 1851)	Ankara	Kolarov, 1987; Öncüer, 1991; Kolarov, 1995
<i>Gelis formicarius</i> (Linnaeus, 1758)	Rize	Çoruh et al., 2014a
<i>Gelis instabilis</i> (Foerster, 1851)	Anatolia, Adana, Antalya, Burdur, Çanakkale, Edirne, Elazığ, Kırklareli	Fähringer, 1922, Kolarov, 1995, Beyarslan & Kolarov 1994; Kolarov et al., 1997b
<i>Gelis micrurus</i> (Förster, 1850)	Antalya	Beyarslan & Kolarov, 1994
<i>Gelis mutilatus</i> (Gmelin, 1790)	Erzurum	Çoruh et al., 2014a
<i>Gelis rufipes</i> (Förster, 1876)	Afyon, Denizli, Kahramanmaraş	Beyarslan & Kolarov, 1994; Kolarov et al., 2002
<i>Gelis sculpturator</i> Aubert, 1977	Ankara, Kırkkale	Aubert, 1977; Kolarov, 1995
<i>Gelis trux</i> (Förster, 1850)	Erzurum, Rize	Çoruh et al., 2014a
<i>Grasseiteles ciliator</i> Aubert, 1968	Adana, Hatay	Aubert, 1968; Kolarov, 1995
<i>Glyphicnemis profligator</i> (Fabricius, 1775)	Isparta, Erzurum, Trabzon	Çoruh & Özbek, 2005; Kolarov & Gürbüz 2007; Çoruh et al., 2014a,b; Kolarov et al., 2016
<i>Glyphicnemis vagabunda</i> (Gravenhorst, 1829)	Adana, Edirne, Erzurum, Isparta	Sawoniewicz, 1985; Beyarslan & Kolarov, 1994; Kolarov & Gürbüz 2007, Kolarov & Bordera, 2007; Çoruh & Çoruh, 2008; Çoruh et al., 2014a; Kolarov et al., 2016; Çoruh et al., 2018
<i>Helcostizus restaurator</i> (Fabricius, 1775)	Istanbul	Schimitschek, 1944; Kolarov, 1995
<i>Isadelphus armatus</i> (Gravenhorst, 1829)	Isparta	Kolarov & Gürbüz, 2007
<i>Lochetica westoni</i> (Bridgman, 1880)	Antalya	Kolarov & Gürbüz, 2007
<i>Lysibia nana</i> (Gravenhorst, 1829)	Adana, Aydın, Balıkesir, Bursa, Edirne, Isparta, İstanbul, İzmir	Fähringer, 1922; Kolarov & Beyarslan, 1994; Kolarov, 1995; Kolarov et al., 1997a; Kolarov et al., 2002; Kolarov & Gürbüz 2007; Çoruh et al., 2014b
<i>Mesoleptus filicornis</i> (Thomson, 1884)	Antalya, Hatay	Kohl, 1905; Beyarslan & Kolarov, 1994
<i>Mesoleptus incessor</i> (Haliday, 1838)	Anatolia	Jussila, 2010
<i>Mesoleptus laevigatus</i> (Gravenhorst, 1820)	Anatolia, Erzurum	Fähringer, 1922; Kolarov et al., 2014; Çoruh et al., 2014b
<i>Mesoleptus laticinctus</i> (Walker, 1874)	Anatolia, Rize	Kolarov, 1987; Çoruh et al., 2014a
<i>Mesoleptus marginatus</i> (Thomson, 1884)	Edirne, Hatay, İstanbul, Tekirdağ	Kolarov, 1987; Beyarslan & Kolarov, 1994
<i>Mesoleptus scrutator</i> (Haliday, 1838)	Afyon, Antalya, Balıkesir, Denizli, Isparta, İzmir, Uşak	Beyarslan & Kolarov, 1994; Kolarov et al., 2002
<i>Mesoleptus transversor</i> Thunberg, 1822	Bilecik	Kolarov et al., 1997a
<i>Phygadeuon trichops</i> Thomson, 1884	Isparta	Kolarov & Gürbüz, 2007
<i>Phygadeuon vexator</i> (Thunberg, 1822)	Isparta	Kolarov & Gürbüz, 2007; Gürbüz et al., 2009a
<i>Rhembobius perscrutator</i> (Thunberg, 1822)	Erzurum	Çoruh et al., 2016
<i>Rhembobius quadrispinus</i> (Gravenhorst, 1829)	Çanakkale, Giresun, Isparta	Kolarov et al., 1997b; Kolarov & Gürbüz, 2007; Kolarov et al., 2016
<i>Stilpnus adanaensis</i> Kolarov & Beyarslan, 1994	Adana	Kolarov & Beyarslan, 1994; Kolarov, 1995
<i>Stilpnus gagates</i> (Gravenhorst, 1807)	Mersin	Beyarslan & Kolarov, 1994
<i>Thaumatogelis femoralis</i> (Brischke, 1881)	Erzincan, Erzurum	Çoruh et al., 2016
<i>Theroscopus hemipterus</i> (Fabricius, 1793)	Afyonkarahisar	Sedivy, 1959; Kolarov, 1995
<i>Theroscopus subzonatus</i> (Gravenhorst, 1829)	Ankara	Sedivy, 1959; Kolarov, 1995
<i>Zoophthorus australis</i> (Thomson, 1885)	Isparta	Kolarov & Gürbüz; 2007
<i>Zoophthorus graculus</i> (Gravenhorst, 1829)	Çanakkale, Edirne, Kırklareli	Kolarov & Beyarslan, 1994; Kolarov et al., 1997b

Table 3. Parasitoids Cryptinae species reared from different hosts in Turkey

Names of Taxa	Hosts Name	Order and Family of Hosts	Reference (s)
<b>TRIBE CRYPTINI KIRBY, 1837</b>			
<i>Agrothereutes hospes</i> (Tschek, 1871)	<i>Galleria mellonella</i> (L.)	Lepidoptera: Pyralida	Gürbüz et al., 2006
<i>Aritranis graefei</i> Thomson, 1896	<i>Agapantia villasoviridescens</i> Deg.	Coleoptera: Cerambycidae.	Öncüer, 1991
<i>Buathra laborator</i> (Thunberg, 1824)	<i>Malacosoma neustria</i> L.	Lepidoptera: Lasiocampidae	Çoruh & Çalmaşur, 2016
<i>Cryptus minor</i> Gravenhorst, 1829	<i>Tarpa</i> sp.	Lepidoptera	Kolarov, 1987
<i>Gambrus opacus</i> Szepliget, 1916	<i>Malacosoma neustria</i> L.	Lepidoptera: Lasiocampidae	Çoruh & Özbek, 2005
<i>Meringopus cyanator</i> (Gravenhorst, 1829)	<i>Lymantria dispar</i> L.	Lepidoptera: Lymantriidae	Çoruh & Özbek, 2005
	<i>Malacosoma neustria</i> L.	Lepidoptera: Lasiocampidae	
<i>Mesostenus transfuga</i> Gravenhorst, 1829	<i>Cadra cautella</i> Walk	Lepidoptera: Crambidae	Kolarov, 1995
	<i>Plodia interpunctella</i> Hb.	Lepidoptera: Pyralidae	
<b>TRIBE PHYGADEUONTINI FORSTER, 1869</b>			
<i>Bathythrix claviger</i> (Taschenberg, 1865)	<i>Phymatodes alni</i> L.	Coleoptera: Cerambycidae	Kolarov, 1995
<i>Chirotica orientalis</i> Horstmann, 1983	<i>Psychida</i> sp.	Lepidoptera: Psychidae	Kolarov, 1995
<i>Chirotica terebrator</i> Horstmann, 1983	<i>Amicta oberthuri</i> Hey.	Lepidoptera: Psychidae	Kolarov, 1995
<i>Echthrus reluctator</i> (Linnaeus, 1758)	<i>Ergates faber</i> (L.)	Coleoptera: Cerambycidae	Kolarov, 1995
<i>Endasys erythrogaster</i> (Gravenhorst, 1829)	<i>Socieras pyricola</i> Wocke	Lepidoptera: Nepticulidae	Kolarov, 1995
<i>Grasseiteles ciliator</i> Aubert, 1968	<i>Aonidiella auranti</i> (Maskell)	Hemiptera: Diaspididae	Kolarov, 1995
<i>Helcostizus restaurator</i> (Fabricius, 1775)	<i>Phymatodes pusillus</i> var. <i>humeralis</i> Com.	Coleoptera: Cerambycidae	Kolarov, 1995
	<i>Rhopalopus clavipes</i> (F.)		
<i>Lysibia nana</i> (Gravenhorst, 1829)	<i>Vanessa</i> sp.	Lepidoptera: Nymphalidae	Kolarov, 1995
	<i>Apanteles glomeratus</i> L.	Hymenoptera: Braconidae	

Table 4. Plants visited by Cryptinae species in Turkey

Names of Taxa	Hosts Name	Order and Family of Hosts	Reference (s)
<b>TRIBE CRYPTINI KIRBY, 1837</b>			
<i>Agrothereutes abbreviator</i> (Fabricius, 1793)	<i>Zea mays</i> L.	Family: Poaceae	Kolarov & Yurtcan, 2008
	<i>Beta vulgaris</i> L.	Family: Chenopodiaceae	
<i>Agrothereutes fumipennis</i> (Gravenhorst, 1829)	<i>Zea mays</i> L.	Family: Poaceae	Kolarov & Yurtcan, 2008
	<i>Beta vulgaris</i> L.	Family: Chenopodiaceae	
<i>Aritranis femoralis</i> (Gravenhorst, 1829)	<i>Carum carvi</i> L.	Family: Apiaceae	Çoruh & Çoruh, 2008
<i>Aritranis graefei</i> Thomson, 1896	<i>Cynara</i> sp.	Family: Asteraceae	Öncüer, 1991
<i>Buathra laborator</i> (Thunberg, 1824)	<i>Phragmites australis</i> (Cav.) Steud.	Family: Poaceae	Çoruh & Çoruh, 2012
	<i>Polygonum bistorta</i> L. Samp.	Family: Polygonaceae	
	<i>Mentha longifolia</i> (L.) Huds.	Family: Lamiaceae	
	<i>Medicago sativa</i> L.	Family: Fabaceae	
	<i>Elaeagnus angustifolia</i> L.	Family: Elaeagnaceae	
<i>Cryptus armator</i> Fabricius, 1804	<i>Eryngium campestre</i> L.	Family: Apiaceae	Kolarov, 1995

Table 4. Continued

Names of Taxa	Hosts Name	Order and Family of Hosts	Reference (s)
<i>Cryptus spiralis</i> (Geoffroy, 1785)	<i>Daucus carota</i> L.	Family: Apiaceae	Çoruh & Çoruh, 2008
	<i>Ferula communis</i> L.		
<i>Cryptus viduatorius</i> Fabricius, 1804	<i>Daucus carota</i> L.	Family: Apiaceae	Çoruh & Çoruh, 2008
	<i>Ferula communis</i> L.		
	<i>Mentha longifolia</i> (L.)	Family: Lamiaceae	Çoruh & Çoruh, 2012
	<i>Daucus carota</i> L.	Family: Apiaceae	Çoruh & Kolarov, 2016
	<i>Medicago sativa</i> L.	Family: Fabaceae	Çoruh et al., 2016
	<i>Ferula orientalis</i> L.	Family: Apiaceae	Kolarov et al., 2016
<i>Ischnus agitator</i> (Oliver, 1792)	<i>Medicago sativa</i> L.	Family: Fabaceae	Çoruh et al., 2016
<i>Ischnus migrator</i> (Fabricius, 1775)	<i>Styrax officinalis</i> L.	Family: Styracaceae	Kolarov, 1995
<i>Latibulus argiolus</i> (Rossi, 1790)	<i>Achillea micrantha</i> Th.	Family: Asteraceae	Kolarov, 1995
<i>Meringopus calescens</i> (Gravenhorst, 1829)	<i>Carum carvi</i> L.	Family: Apiaceae	Çoruh & Çoruh, 2008
	<i>Phragmites australis</i> (Cav.) Trin.ex Steudel.	Family: Poaceae	
	<i>Polygonum bistorta</i> L.	Family: Polygonaceae	Çoruh & Çoruh, 2012
	<i>Mentha longifolia</i> (L.) Hudson	Family: Lamiaceae	
	<i>Myrica germanica</i> (L.) Desv.	Family: Tamaricaceae	
	<i>Salix triandra</i> L. (Salicaceae)	Family: Salicaceae	
<i>Meringopus calescens calescens</i> (Gravenhorst, 1829)	<i>Ferula communis</i> L.	Family: Apiaceae	Kolarov et al., 2016
<i>Meringopus cyanator</i> (Gravenhorst, 1829)	<i>Carum carvi</i> L.	Family: Apiaceae	Çoruh & Çoruh, 2008
<i>Meringopus pseudonymus</i> (Tschek, 1872)	<i>Polygonum bistorta</i> L.	Family: Polygonaceae	Çoruh & Çoruh, 2012
<i>Meringopus titillator</i> (Linnaeus, 1758)	<i>Carum carvi</i> L.	Family: Apiaceae	Çoruh & Çoruh, 2008
	<i>Seselis libanotis</i> (L.) W. Koch		
	<i>Ferula orientalis</i> L.	Family: Apiaceae	Kolarov et al., 2016
<i>Meringopus titillator rhodius</i> (Dalla Torre, 1902)	<i>Mentha longifolia</i> (L.) Hudson	Family: Lamiaceae	Çoruh & Çoruh, 2012
<i>Mesostenus albinotatus</i> Gravenhorst, 1829	<i>Pimpinella tragiium</i> Vill.	Family: Apiaceae	Çoruh & Çoruh, 2008
	<i>Euphorbia stricta</i> L.	Family: Euphorbiaceae	Kolarov et al., 2016
<i>Mesostenus transfuga</i> Gravenhorst, 1829	<i>Pimpinella tragiium</i> Vill.	Family: Apiaceae	Çoruh & Çoruh, 2008
	<i>Seselis libanotis</i> (L.) W. Koch		
<i>Myrmeleonostenus italicus</i> (Gravenhorst, 1829)	<i>Medicago sativa</i> L.	Family: Fabaceae	Çoruh et al., 2016
<i>Stenarella domator</i> (Pado, 1761)	<i>Sambucus ebulus</i> L.	Family: Adoxaceae	Kolarov, 1995
<i>Trychosis neglecta</i> (Tschek, 1870)	<i>Hypericum rhodopaeum</i> Friv.	Family: Clusiaceae	Kolarov, 1995
<b>TRIBE HEMIGASTERINI ASHMEAD, 1900</b>			
<i>Giraudia gyratoria</i> (Thunberg, 1824)	<i>Heracleum platytenium</i> Boiss.	Family: Apiaceae	Kolarov, 1995
<i>Pleolophus brachypterus</i> (Gravenhorst, 1815)	<i>Heracleum platytenium</i> Boiss.	Family: Apiaceae	Kolarov, 1995
<i>Polytribax rufipes</i> (Gravenhorst, 1829)	<i>Achillea santolonia</i> L.	Family: Asteraceae	Kolarov, 1995

Table 4. Continued

Names of Taxa	Hosts Name	Order and Family of Hosts	Reference (s)
<b>TRIBE PHYGADEUONTINI FORSTER, 1869</b>			
<i>Chirotica terebrator</i> Horstmann, 1983	<i>Lens esculenta</i> Moench	Family: Fabaceae	Kolarov, 1995
<i>Dichrogaster liostylus</i> Thomson, 1885	<i>Coryllus avellana</i> L.	Family: Betulaceae	Kolarov, 1995
<i>Dichrogaster longicaudata</i> (Thomson 1885)	<i>Medicago sativa</i> L.	Family: Fabaceae	Çoruh et al., 2016
<i>Dichrogaster modesta</i> (Gravenhorst, 1829)	<i>Zea mays</i> L.	Family: Poaceae	Kolarov & Yurtcan, 2008
	<i>Beta vulgaris</i> L.	Family: Chenopodiaceae	
<i>Echthrus reluctator</i> (Linnaeus, 1758)	<i>Pinus brutia</i> Ten.	Family: Pinaceae	Kolarov, 1995
<i>Encrateola laevigata</i> (Ratzeburg, 1848)	<i>Medicago sativa</i> L.	Family: Fabaceae	Çoruh et al., 2016
<i>Glyphicnemis vagabunda</i> (Gravenhorst, 1829)	<i>Carum carvi</i> L.	Family: Apiaceae	Çoruh & Çoruh, 2008 Kolarov et al., 2016
	<i>Seselis libanotis</i> (L.) W. Koch	Family: Apiaceae	
<i>Lysibia nana</i> (Gravenhorst, 1829)	<i>Cynara</i> sp.	Family: Asteraceae	Kolarov, 1995
<i>Mesoleptus marginatus</i> (Thomson, 1884)	<i>Sambucus ebulus</i> L.	Family: Adoxaceae	Kolarov, 1995
<i>Thaumatogelis femoralis</i> (Brischke, 1881)	<i>Medicago sativa</i> L.	Family: Fabaceae	Çoruh et al., 2016

From Table 1 it can be seen that 97 species and 28 genera belonging to tribe Cryptini; nine species and five genera tribe Hemigasterini; 81 species and 28 genera tribe Phygadeuontini were recorded. Cryptini had the greatest number of species (Figure 3a).

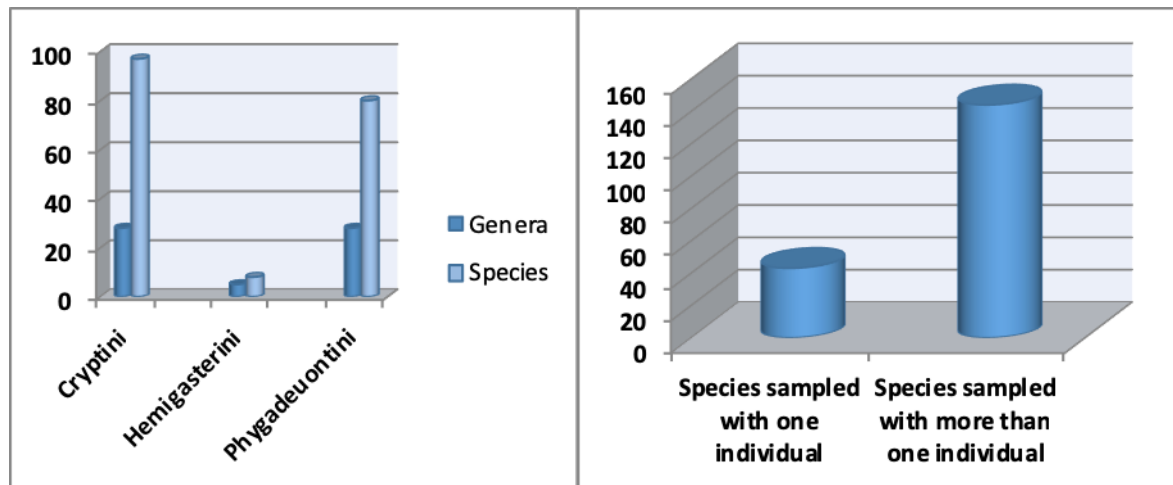


Figure 3. Number of species: a) according to per tribe; b) according to individuals.

In the Cryptini, *Meringopus calescens* (Figure 1a) was the most abundant species, with 299 individuals. This is followed by *Cryptus viduatorius* (106), *Aritranis director* (77), *Glyphicnemis vagabunda* (77) and *Dichrogaster aestivalis* (49), respectively.

Despite all this, many species were collected as a single individuals in the study area. These species were *Acroricnus seductor*, (Figure 1b) *A. seductor elegans*, *A. seductor syriacus*, *Aptesis cavigena*, *Aritranis occisor*, *A. quadriguttata*, *Cryptus moschator*, *C. subspinosus*, *Hidryta frater*, *Hoplocryptus confector*, *H. odoriferator*, *Listrocryptus spatulatus*, *Meringopus nigerrimus*, *Nematopodius formosus*, *Pycnocryptus claviventris*, *Pycnocryptodes reticulator*, *Trychosis mesocastana*, *Xylophrurus lancifer*,

*Aptesis cretata*, *A. nigrocincta*, *Pleolophus brachypterus*, *Acrolyta semistrigosa*, *A. (Ansyrtia) foveolatus*, *Atractodes (A.) fumatus*, *A. (A.) pusillus*, *Bathythrix fragilis*, *Chirotica insignis*, *C. orientalis*, *C. terebrator*, *Diaglyptellodes sculpturator*, *Dichrogaster perlae*, *Endasys femoralis*, *E. senilis*, *Gelis cursitans*, *G. formicarius*, *G. micrurus*, *G. mutillatus*, *Isadelphus armatus*, *Lochetica westoni*, *Phygadeuon trichops*, *Rhembobius perscrutator*, *Stilpnus adanaensis* and *Zoophthorus australis* (Figure 3b).

Additionally, Table 2 shows distribution of each species according to province in the seven different regions.

### Ecological evaluations

Numerous physical parameters that influence insect physiology vary substantially with altitude, including temperature, air density and oxygen partial pressure (Dillon et al., 2006).

Samples were collected eight altitude ranges (Table 1) with 68 species collected between 0-500 m (A), 25 species between 501-750 (B), 25 species between 751-1000 (C), 75 species between 1001-1250 (D), 40 species between 1251-1500 (E), 24 species between 1501-1750 (F), 22 species between 1751-2000 (G), 19 species between 2001-2500 (H) (Figure 4a).

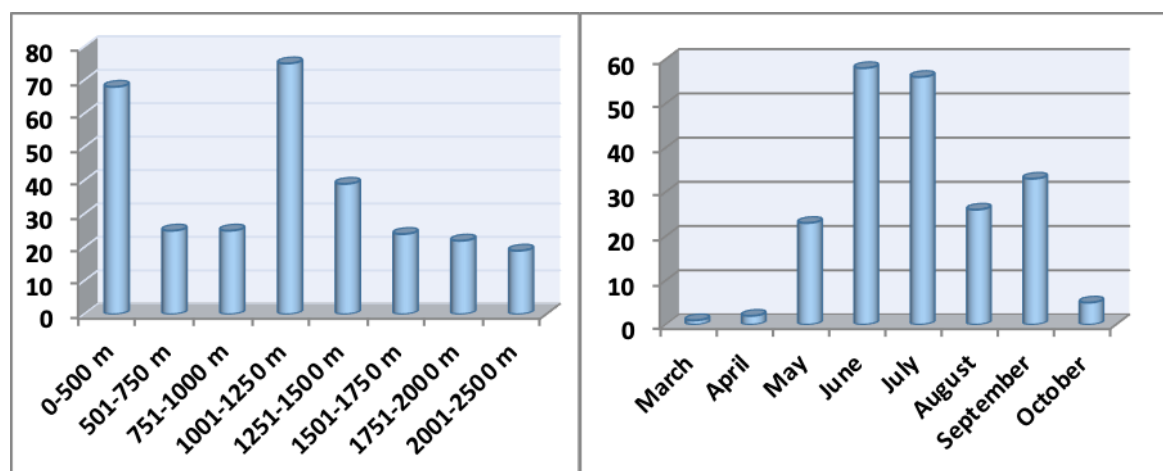


Figure 4. Number of species: a) according to altitude range; b) according to months.

Figure 4a shows that most (40.1%) of the insects were collected between 1001-1250 m, and least (10.2%) samples were collected between 2001-2500 m. Also, 108 species were found one altitude range, and only 13 species were determined from four different range. Altitude was an important factor in species distribution.

Seasonal climatic conditions can exert a strong influence on insect abundance and activity (Vasconcellos et al., 2010). Figure 4b show that the insects were collected in 8 months of the year.

The most insect were collected in June, but on a few were collected in the first month of spring and the last month of autumn (March and October) (Figure 4b). *Agrothereutes fumipennis* was collected in six months, *Cryptus viduatorius* and *Trychosis legator* in five months (Table 1). Also 103 species were collected in only one month.

### Zoogeographic evaluations

Geographic distribution is one of the major characteristics of any animal taxon, be it species, genus or family. A general comprehension of geographic distributions of major taxa is essential to understand natural environments, to recognize species diversity patterns and to plan conservation strategies (Gaston, 2000; Myers et al., 2000; Lamoreux et al., 2006).

The study area consisted of seven geographic different regions in Turkey. Most of the samples (110) were collected from the Mediterranean Region. Only eight species were collected from South East Anatolia

(Figure 5a). *Trychosis legator* and *Dichrogaster aestivalis* were collected five regions. However, 52% of species were collected from only a single region.

The Mediterranean Region is dominant region for the cryptine species, followed by Eastern Anatolia (57) and Marmara region (45).

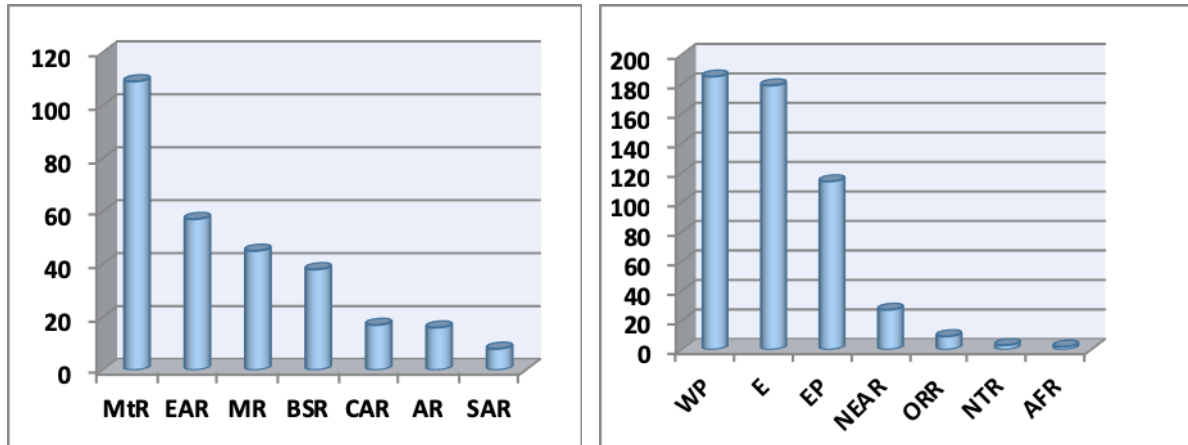


Figure 5. Number of species: a) according to geographic regions; b) according to zoogeographic region.

There are seven global regions for zoogeographic distribution. The regional distribution of the species listed in Table 1 was 186 species Western Palearctic (99.4%), 179 species European (95.7%), 114 species Eastern Palearctic (60.9%), 27 species Nearctic (14.4%), nine species Oriental (4.8%), three species Neotropical (1.6%), two species Afrotropical (1.0%). In conclusion, Western Palearctic and European have the highest numbers of species (Figure 5b). All species were distributed in the Western Palearctic Region. Of this species, *Lysibia nana* and *Stilpnus gagates* were found six zoogeographic regions. Notably, while *Stilpnus gagates* has been found to be have a wide global distribution, it was only found in one region of Turkey. Similarly, *Xylophrurus augustus* was found five different geographic regions of Turkey, but is only common in Europe and Western Palearctic Regions.

Some important observations are also given in Table 1 is examined. For example, although *A. seductor elegans* has a wide global distribution, only one specimen of it has been reported (Schimitschek, 1944) in Turkey and since 1944, this species has not been found in Turkey. Another example is *Agrothereutes tiloidalis*, which has only been found in Turkey. *Agrothereutes tiloidalis* is endemic to Turkey. It is notable that *Chirotica orientalis* is present only Israel, Syria and Turkey.

#### Evaluations of hosts and plants visited by adults

Cryptinae can be found in mostl kinds of habitats globally. Typically they are parasitic in cocoons of the Lepidoptera, sawflies, braconids, ichneumonids and Neuroptera. Some of them attack egg cocoons of spiders and pupae of Diptera (Azura & Idris, 2002).

In this study, a total of 15 Cryptinae (Figure 6a) species came from 17 different hosts (Table 3). At the same time, four species have got two different hosts. The order Lepidoptera were the most numerous of the hosts (Figure 6b).

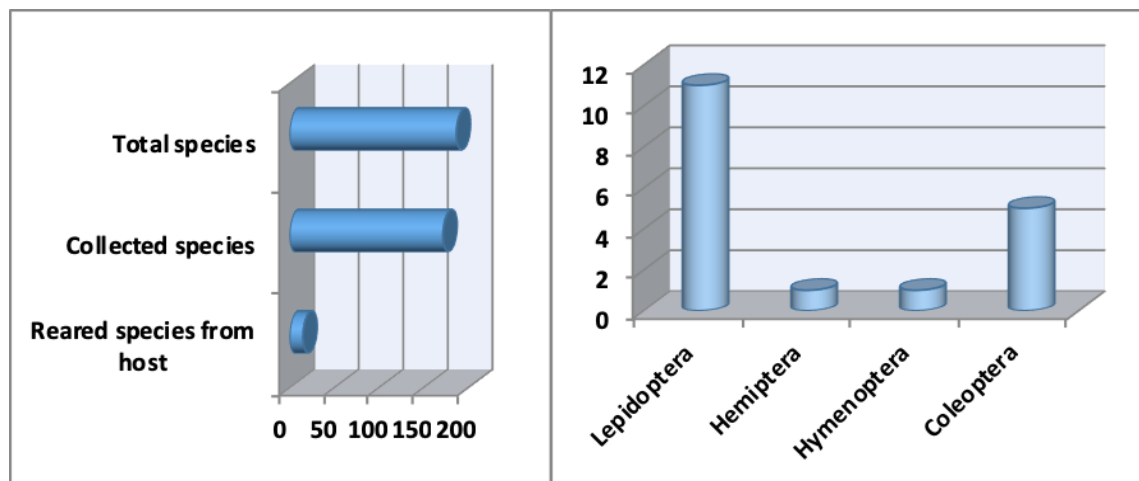


Figure 6. Number of species: a) according to reared from different hosts; b) according to order.

*Malacosoma neustria* was a host of three different species. Of these, *Buathra laborator* was reared from *M. neustria* feeding on *Elaeagnus angustifolia* in Erzurum. *Malacosoma neustria* was recorded a new host for this species in Turkey (Çoruh & Çalmaşur, 2016). This species was previously reared from *M. neustria* (Meyer, 1929).

Moreover, *Meringopus cyanator* and *Gambrus opacus* reared from *M. neustria* as a result of this work also (Çoruh & Özbek, 2005). *Gambrus opacus* is only known to have one host anywhere in the world (Yu et al., 2016).

In addition, plant-insect relationships are of great importance in ecosystem (Petanidou & Lamborn, 2005). Table 4 shows that there were 27 species of plants visited by the 35 cryptine species (Figure 7a), with *Medicago sativa* being the most visited plant (Figure 7b).

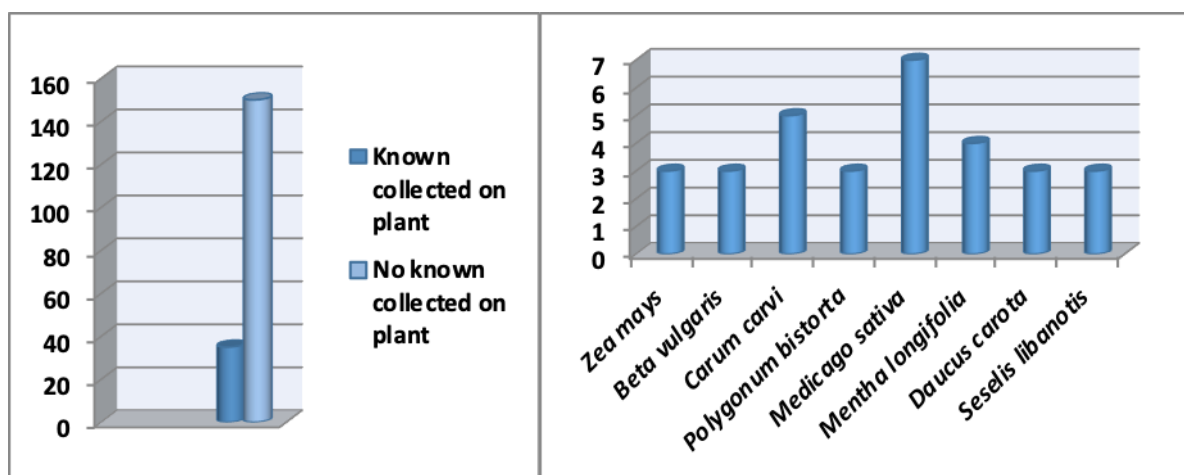


Figure 7. Number of species: a) according to collected plants; b) according to collected plants species.

The data presented here will help in the design of future studies and will assist taxonomists who are working on the subfamily Cyrtinae. These results will help to more comprehensively identify research needs and speed up the advancement of knowledge for this group of important insects.

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## Original article (Orijinal araştırma)

# Toxic and in vitro anti-acetylcholinesterase and anti-carboxylesterase effects of various plant extracts on *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae)<sup>1</sup>

Bazı bitki ekstraktlarının *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) üzerine toksik in vitro anti-asetilkolinesteraz ve anti-karboksil esteraz enzim etkisi

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## Abstract

The significance of discovering new active substances that are environment friendly when compared to pesticides, agriculturally sustainable, plant-based, and in the status of GRAS (generally regarded as safe) has been increasing every day. For this purpose, leaves of *Daphne odora* L., *Dieffenbachia amoena* L., *Eucalyptus camaldulensis* L., *Ficus carica* L., *Lantana camara* L., *Matricaria chamomilla* L., *Mentha pulegium* L. and *Nerium oleander* L. were collected from Adana in 2018. Toxic and in vitro anti-acetylcholinesterase (AChE) and anti-carboxylesterase (CE) activities of aqueous leaf extracts of these species on the important polyphagous pest, *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae), were determined after 24 and 72 h. The fastest and greatest toxic effect was obtained with 20% *F. carica* extract giving 75.6% mortality. This was followed by *N. oleander* with 71.6% and *D. odora* with 62.1% mortality. *Aphis gossypii* in vitro anti-AChE and anti-CE activities were highest at 10% concentration of the plant extracts and inhibition levels were 51.8-82.5% with *F. carica* extract, 40.9-54.9% with *D. odora* extract and 40.2-82.5% with *E. camaldulensis* extract. In conclusion, *D. odora*, *E. camaldulensis*, *F. carica* and *N. oleander* extracts gave promising results for future studies on the discovery of potential xenobiotics against *A. gossypii* and for pest control.

**Keywords:** Anti-acetylcholinesterase, anti-carboxylesterase, *Aphis gossypii*, plant extract

## Öz

Pestisitlerle karşılaştırıldığında çevre dostu, tarımsal olarak sürdürülebilir, bitki orjinli ve GRAS statüsünde yeni aktif maddelerin keşfedilmesinin önemi her geçen gün artmaktadır. Bu amaçla, Adana ilinden 2018 yılında *Daphne odora* L., *Dieffenbachia amoena* L., *Eucalyptus camaldulensis* L., *Ficus carica* L., *Lantana camara* L., *Matricaria chamomilla* L., *Mentha pulegium* L. ve *Nerium oleander* L. bitkileri toplanmıştır. Bu bitkilere ait yaprakların sulu ekstraktlarının tarımsal alanlarda önemli bir polifag zararlı olan *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) üzerinde toksik, in vitro anti-asetilkolinesteraz (AChE) ve anti-karboksilesteraz (CE) enzim aktiviteleri incelenmiştir. En hızlı ve en yüksek toksik etki %75.6 ölüm oranı ile 3. günde *F. carica* bitkisinin %20 konsantrasyonunda gözlemlenmiştir. Bunu %71.6 ölüm oranıyla *N. oleander*, %62.1 ölüm oranıyla *D. odora* bitkisinde gözlemlenmiştir. *Aphis gossypii* in vitro anti-AChE ve anti-CE aktiviteleri en yüksek test edilen bitki ekstraktlarının %10 konsantrasyonunda görülürken, engelleme seviyeleri sırasıyla *F. carica* için %51.8-82.5, *D. odora* için %40.9-54.9, *E. camaldulensis* için %40.2-82.5 olarak belirlenmiştir. Sonuç olarak, *D. odora*, *E. camaldulensis*, *F. carica* ve *N. oleander* bitki ekstraktları *A. gossypii* mücadelesinde ve zararlılarla mücadelede potansiyel ksenobiyotiklerin keşfinde yapılacak sonraki çalışmalar için ümit var sonuçlar ortaya koymaktadır.

**Anahtar sözcükler:** Anti-asetilkolinesteraz, anti-karboksilesteraz, *Aphis gossypii*, bitki ekstraktı

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## Introduction

Cotton aphid, *Aphis gossypii* Glover, 1877 (Hemiptera: Aphididae) is one of the main pests among aphids that cause economic losses to various agricultural crops. *Aphis gossypii* is a polyphagous species with a wide range of hosts; the pest causes severe damage to cotton and Cucurbitaceae plants in Turkey and many other countries around the world (Ozgun & Sekeroglu, 1986; Tomizawa & Casida, 2005). Today, the chemical control of *A. gossypii* is an important issue in agriculture as this pest can severely decrease yield and quality in cultivated cotton. Although there are various natural enemies of *A. gossypii* in cotton cultivation, chemical control is the most preferred method by producers (Godfrey et al., 1997).

As a result of the increase in insecticide use throughout the world in the last century, different resistance levels of 586 insect pest species against 325 active substances have been reported (Sparks & Nauen, 2015). Widespread and inappropriate use of insecticides affects environment, human health and non-target organisms. Insecticides used against insects and arthropods have a broad-spectrum impact; they cause oxygen deficiency, paralysis as a result of inactivity, and eventually cause death with inhibition or reduction of respiration due to neuro-inhibition in the nervous system (Scharf et al., 2003). Resistance mechanism in organisms included enhanced metabolic enzyme activities and a decrease in the level of sensitivity towards xenobiotics as a result of mutations in target proteins (Nauen, 2007). In the process of xenobiotic detoxification, enzymes have a multigene family that is transcribed in living organisms such as several esterases, oxidases and glutathione S transferase. (Field et al., 1999; Bass & Field, 2011).

Acetylcholinesterase (AChE) and carboxylesterases (CE) are in phase I metabolic enzyme group and they can metabolize various internal and external substrates in pests; this metabolic enzyme group is made of broad-spectrum enzymes that are capable of metabolizing chemical insecticides such as organophosphate, carbamate or pyrethroid (Hollingworth & Dong, 2008). Increase or decrease in the amount of these enzymes leads to the loss of efficiency in insecticides; thus, agents with new and different action mechanisms should be developed in insect control. In the last decade, the demand for biodegradable substances, which are considered as an alternative to synthetic pesticides and could be used in integrated control programs, has significantly increased. Plant-based pesticides are the center of attention since they are eco-friendly and conform with integrated control approaches due to GRAS (generally regarded as safe) status in terms of environmental and human health. Plant-based secondary metabolites (e.g., alkaloids, carotenoids, fats, gums, phenols, resin acids, sterols, suberins, tannins and terpenes) have active role in ensuring self-protection against microbial pathogens and invertebrate pests (Gottlieb, 1990; Wink & Schimmer, 1999). Before the discovery of modern pesticides, plant-based nicotine and pyrethrin extracts were commonly used as insecticides in agriculture. Pyrethroids, which are derived from the leaves and flowers of chrysanthemum species, are important toxins which may cause death and paralysis of the nervous system. This toxin was specifically developed in order to obtain the most successful commercial pesticide (Raffa & Priester, 1985; Gershenson & Croteau, 1991). Given that the structure of these compounds is quite complex when compared to artificial pesticides, either development of resistance is delayed or resistance in organisms becomes completely impossible (Völlinger, 1987). In laboratory studies, although exposed to neem oil for 42 generations, resistance development was not observed in a species such as *Plutella xylostella* (L.), which normally develops resistance to all synthetic pesticides within a short period of time. This was due to the complex mechanism of action of the plant metabolite components (Völlinger, 1987; Schmutterer, 1988). There is an increasing need for new active substances that are less harmful or completely harmless, economically feasible, highly efficient, ecologically sustainable and that can be used instead of synthetic chemicals used in pest control.

For this purpose, toxic and anti-AChE and anti-CE effects of aqueous extracts of eight plants on *A. gossypii*, a polyphagous agricultural pest, are investigated and analyzed in order to determine ecological bioactive substances affective under detoxification mechanisms.

## Materials and Methods

The test insect, *A. gossypii*, was collected from the cotton cultivation by field surveys in 2017 and cultured under greenhouse conditions at 22°C, 65±5% RH and 16:8 h L:D photoperiod. Previously labeled plant materials used in the experiment were *Daphne odora* L. (Malvales: Daphne), *Dieffenbachia amoena* L. (Alismatales: Thymelaeaceae), *Eucalyptus camaldulensis* L. (Myrtales: Myrtaceae), *Ficus carica* L. (Rosales: Moraceae), *Lantana camara* L. (Lamiales: Verbenaceae), *Matricaria chamomilla* L. (Asterales: Asteraceae), *Mentha pulegium* L. (Lamiales: Lamiaceae) and *Nerium oleander* L. (Gentianales: Apocynoideae); plants were obtained from campus area of Adana Biological Control Research Institute (37°00'38.0" N, 35°20'23.7" E). The treatment with the leaf extracts were compared to the insecticide, with malathion active ingredient (25% w/w malathion), treatments in order to determine toxicity and inhibition effect on the enzymes to *A. gossypii*.

### Preparation of plant extracts

The leaves of the plants were separated and dried at 40°C for 72 h in an incubator (Nüve incubator). The dried leaves were then removed from the incubator and crushed in a mill (IKA, homogenisator). Following this step, they were separated and weighed as 200 g/L of sterile purified water. They were allowed to infuse on a magnetic mixer in Erlenmeyer flask (250 ml) for 24 h. The prepared aqueous solutions were filtered by coarse filter paper and stored in the refrigerator in light-proof bottles until used for the experiments.

### Toxicity of plant extracts on *Aphis gossypii* individuals

Firstly, the bioassay experiment was established for the correct concentration of malathion. Leaf samples taken from cotton plants were cut into 4 cm diameter discs. The leaves were dipped in the insecticide solutions for 10 s, dried and then placed in Petri dishes containing 1.5% agar. Three replicates of about six different rates, excluding a control, were tested. The field collected populations were tested against 1-750 ppm for malathion insecticides. Distilled water was used as the control. About 30 adult aphids were transferred to each Petri dish. After the Petri dishes were covered with Parafilm, they were placed in a controlled environment at 22±1°C, 70% RH and 16:8 h L:D photoperiod. Mortality was assessed after 72 h. Rate-response regressions were computed using Polo-Plus computer program (LeOra Software, Berkeley, CA, USA). And it was calculated LC<sub>50</sub> (lethal concentration to kill 50%) of the test population.

Fresh cotton leaf samples taken from cotton plants were cut into 4 cm diameter discs. These were dipped in aqueous plant extracts at 10 and 20% concentrations and 175 ppm insecticide (malathion) for 30 s for four replicates. Then, the cotton leaves left to dry on the metal grid and placed with the lower surface facing upwards in plastic Petri dishes (4 cm diameter with ventilation pore) containing 1.5% agar solution. Cotton leaves dipped in pure water were included as a control. Thirty *A. gossypii* apterous individuals were transferred to the extract treated leaves in each Petri dish, and alive and dead individuals were counted after 24 to 72 h after. During counting process, aphids were gently touched with a fine-tipped art brush to determine their vitality. According to the following equation the Henderson-Tilton formula (Henderson & Tilton, 1955) was used to calculate the toxic effect level of each treatment.

$$\text{Corrected \%} = \left( 1 - \frac{n(\text{Control before treatment}) \times n(\text{treated after treatment})}{n(\text{Control after treatment}) \times n(\text{treated before treatment})} \right) \times 100$$

### Inhibitory effect of plant extracts on carboxylesterase and acetylcholinesterase

Enzyme activity was determined by the method of Nauen et al., (2002). Twenty aphids were homogenized in 100 µl sodium phosphate buffer (0.1 M, pH 7.5), then centrifuged at 10,000 g for 4 min at 4°C and the supernatant used as the enzyme source. Supernatant was diluted 10 times and 25 µl diluted

supernatant was used in an enzyme analysis. The substrate solution was prepared with 0.06 mg/ml fast blue RR salt and 500  $\mu$ l 100 mM  $\alpha$ -naphthyl acetate sodium phosphate (0.2 M, pH 6.0). Plant extracts were used for enzyme analyses by diluting them in sodium phosphate (0.2 M, pH 6.0) buffer to 1, 3 and 10%. Two hundred  $\mu$ l substrate solution, 25  $\mu$ l enzyme and 25  $\mu$ l plant extract were used for each reaction for three replicates. Pure water was used as a control to replace the enzymes that were individually prepared for each extract. Enzyme activity was read for 10 min with a microplate spectrophotometer at 23°C at 450 nm. Mean levels of CE activity were based on protein content and  $\alpha$ -naphthol standard curves.

AChE was determined with the method developed by Stumpf & Nauen (2001). Fifty *A. gossypii* individuals were homogenized with a plastic crusher in phosphate buffer (0.1 M, pH: 7.5) with 500  $\mu$ l 0.1% Triton X-100 in an Eppendorf tube. The homogenate was used as supernatant enzyme source after centrifugation at 10,000 g, at 4°C for 5 min. One hundred  $\mu$ l (0.5 mM) acetylcholine iodide, 100  $\mu$ l 5.5-dithiobis (2-nitrobenzoic acid) and 70  $\mu$ l enzyme solution and 30  $\mu$ l plant extract were added to the microplate wells in order to measure AChE activity. Plant extracts were used for enzyme analyses by diluting them in sodium phosphate buffer (0.2 M, pH 6.0) at 1, 3 and 10%. AChE activity was measured at 23°C for 10 min at 412 nm in the kinetic microplate reader for three replicates. Control cells were read without homogenate.

All protein content was determined by the method of Bradford (1976) using bovine serum albumin as the standard. Comparative activity levels were calculated as percentages according to the following equation. The control that did not contain any extract and inhibition effect.

$$\text{Inhibition activity percentage (\%)} = 100 - \left\{ \frac{\text{Control sample activity} - \text{Inhibition sample activity}}{\text{Control sample activity}} \times 100 \right\}$$

### Statistical analysis

One-way ANOVA and Duncan's multiple range test have been done by IBM SPSS Statistics 23.

## Results and Discussion

When the toxic effects of aqueous extracts treatments on *A. gossypii* were observed, it was determined that the fastest and greatest toxic effect following the insecticide was with *F. carica* extract with 33.7% (Day 1) and 75.7% mortality (Day 3) at 20% (Table 1). The lowest mortality rate was observed with *M. chamomilla* extract at 10 and 20% with 1.3 and 3.9% mortality, respectively. After 24 h, the highest toxic effect following *F. carica* was with *M. pulegium* extract giving 29.9% mortality at 20% and *D. odora* extract giving 22.8% mortality at 20%. After 72 h, the most effective plant extracts following *F. carica* were *N. oleander* giving 71.6% mortality at 20% and *D. odora* giving 62.2% mortality at 20%. The malathion LC<sub>50</sub> value was computed as 152 ppm with a confidence interval (50.2-191). Mortality with the insecticide used as a control was 85.1% after 72 h (Table 1). There are several published studies on the toxic and repellent effects of plant extracts on *A. gossypii*. Dadel & Saleh (2017) reported 79% mortality of *A. gossypii* 48 h after the treatment with *N. oleander* chloroform extract. In the same study, 71.6% mortality was reported 72 h after the treatment with *N. oleander* aqueous extract. Singh et al. (2012) reported that *E. globulus* plant extract had a repellent effect of 96% against *A. gossypii*; and in the current study it was found that there was 54% insecticidal effect of *E. camaldulensis* on aphids after 72 h.

In similar studies, it was reported that different concentrations of *Acalypha indica* L., *Cassia angustifolia* M. Vahl., *Cascabela thevetia* (L.) Lippold, *Ocimum basilicum* L. and *Schinus molle* L. aqueous extracts had different repellent effects on *A. gossypii* (Bayhan et al., 2006; Singh et al., 2012; Pinto et al., 2013; Birgücü et al., 2015).



In studies conducted with other plants, it was observed that plant oils obtained from plants such as *Azadirachta indica* A. Juss., *Achillea millefolium* L. and *Cannabis sativa* L., and aqueous extracts of *Lycopersicon esculentum* and *Nicotiana tabacum* had insecticidal effects on *A. gossypii* (Özger et al., 2013; Yankova et al., 2014; Ghosh, 2015; Dadel & Saleh 2017; Ghada et al., 2017).

Table 1. Mean percentage toxic effects of aqueous plant extracts on *Aphis gossypii*

Plant species	Mortality (%±SE)					
	10%			20%		
	24 h	72 h		24 h	72 h	
<i>Daphne odora</i>	3.9±1.7 b	43.2±3.3 bc		22.1±3.9 bcd	62.2±2.8 abc	
<i>Dieffenbachia amoena</i>	1.3±4.3 b	27.1±1.3 cd		14.3±7.7 bcd	36.5±6.2 c	
<i>Eucalyptus camaldulensis</i>	3.9±1.0 b	52.7±1.4 bc		6.5±2.5 cd	54.0±4.0 bc	
<i>Ficus carica</i>	14.3±3.4 b	64.9±3.1 ab		33.8±2.7 ab	75.7±1.3 ab	
<i>Lantana camara</i>	2.6±1.3 b	48.7±2.0 bc		6.5±3.1 cd	60.8±1.5 abc	
<i>Matricaria chamomilla</i>	1.3±2.8 b	35.1±5.7 bc		3.9±4.3 d	37.8±5.2 c	
<i>Mentha pulegium</i>	16.9±4.1 ab	47.3±2.5 bc		29.8±1.3 abc	56.8±1.4 bc	
<i>Nerium oleander</i>	5.2±1.0 b	48.6±3.6 bc		10.4±3.3 bcd	71.6±2.2 ab	
Malathion	48.1±2.7 a			85.1±1.4 a		

Means follow by the same letter are not significantly different according to Duncan's multiple range test (p < 0.05).

Analysis of the inhibitory effect of AChE and CE activities of the aqueous extracts indicated that the most effective extract was *F. carica* (all concentrations) on both enzymes (Table 2). *Ficus carica* extract had high inhibitory effect on AChE (51.9% inhibition) and CE (82.5% inhibition) at 10%. The lowest inhibitory effect at 10% was with *D. amoena* extract on AChE (20.9% inhibition) and with *L. camara* extract on CE (28.7% inhibition) (Table 2). The most effective plant extracts following with *F. carica* were *D. odora* (41.0% inhibition) and *E. camaldulensis* (40.3% inhibition) on AChE, and *E. camaldulensis* (82.5% inhibition) and *M. pulegium* (79.5% inhibition) on CE.

Table 2. Inhibition effects of aqueous plant extracts on acetylcholinesterase and carboxylesterase from *Aphis gossypii*

Plant species	Mean inhibition (%±SE)					
	Acetylcholinesterase			Carboxylesterase		
	1%	3%	10%	1%	3%	10%
<i>Daphne odora</i>	33.0±1.1 e	37.6±4.1 d	41.0±1.0 c	23.5±1.5 c	35.2±0.8 d	55.0±2.0 c
<i>Dieffenbachia amoena</i>	12.8±2.5 b	18.1±3.1 a	20.9±3.1 a	15.2±1.7 b	19.9±2.6 b	36.0±1.0 b
<i>Eucalyptus camaldulensis</i>	29.1±1.8 de	39.2±2.0 d	40.3±2.8 c	29.1±0.8 d	63.4±2.5 f	82.5±2.5 ef
<i>Ficus carica</i>	38.8±3.4 f	40.6±2.0 d	51.9±3.4 db	64.8±1.4 g	77.6±1.5 h	83.9±1.8 f
<i>Lantana camara</i>	7.6±1.3 a	17.6±1.2 a	26.1±2.1 b	2.5±1.0 a	13.5±2.0 a	28.7±0.3 a
<i>Matricaria chamomilla</i>	22.3±2.5 c	23.6±0.1 b	27.3±3.3 b	16.8±1.4 b	28.0±3.0 c	38.7±3.1 b
<i>Mentha pulegium</i>	30.6±2.7 e	36.7±1.3 d	39.4±2.1 d	42.3±1.3 f	51.3±2.1 e	79.5±1.9 de
<i>Nerium oleander</i>	25.3±2.7 cd	28.7±2.3 c	30.3±3.0 b	35.9±1.1 e	69.5±5.5 g	77.8±1.0 d
M/min/mg protein		13.28±0.77			9.34±0.51	

Means follow by the same letter are not significantly different according to Duncan's multiple range test (p < 0.05).

There are only a few in vitro studies on *A. gossypii* anti-AChE and anti-CE; however, several studies about inhibition of different enzymes have been reported. In other studies, it was reported that *Artemisia annua* L. extract and 4% azadirachtin of *Periplaneta americana* L. (40%) had an inhibitory effect on AChE (Zibae et al., 2010), and 80% neem oil had an inhibitory effect on AChE (Singh & Singh, 2005; Shafeek et al., 2004). It was reported that 25 g/L distilled water extracts of *Artemisia absinthium* L., *Punica granatum* L. and *Thymus vulgaris* L. significantly inhibited AChE activity (Korayem et al., 1993). Senthil et al. (2008)

observed that LC<sub>50</sub> azadirachtin concentration significantly inhibited AChE activity when compared to the control. In this study, analysis of both toxic and enzyme inhibitory effects of tested plant extracts showed that there are anti-AChE and anti-CE activities of *F. carica*, *E. camaldulensis* and *M. pulegium* plants in parallel with their toxic effects.

AChE and CE inhibition is an important target for insecticides and for several plant metabolites in insects (Houghton et al., 2006). Esterase enzymes are especially important and responsible for detoxification which hydrolyzes ester bonds in synthetic chemicals (Hemingway & Karunaratne, 1998). AChE inhibitors are used effectively in the fields of pharmacology and pesticides for controlling insects, other arthropods and some vertebrates. Different degrees of toxic or enzyme inhibition effects of plant extracts can result from substances such as flavonoids, terpenes phenols, alkaloids, sterols, waxes, fats, tannins, sugars, gums, suberins, resin acids and carotenoids, and concentration levels of the components in an organism (Wink & Schimmer, 1999). Thus, this study was undertaken to determine the potential plant extract activities and to provide data for future studies. It was reported that there may be increases in insecticidal and enzyme inhibition periods in parallel with the increase in plant extract concentrations (Junqing et al., 2011; Hansson et al., 2012). In the literature, it is mentioned that plant metabolite alkaloids and terpenes can have significant insecticide effects; however, they are not economic or safe in addition to the fact that they are difficult to isolate (Rattan, 2010). Thus, in this study it was determined that an efficiency of up to 80% with aqueous plant extracts can be obtained more easily, so is promising in terms of potential use as insecticides. Considering the negative effect of synthetic pesticides against non-target organisms, it can be concluded that plant metabolites are better products with GRAS status (Scott et al., 2003). Furthermore, plant metabolites could affect more than one target area in insect metabolism with little or no resistance development. In conclusion, it was determined that aqueous *D. odora*, *E. camaldulensis*, *F. carica* and *M. pulegium* leaf extracts have significant bioinsecticide effect and in vitro anti-AChE and anti-CE activities on *A. gossypii*. It was also determined that these plant extracts can be used as bioinsecticides for *A. gossypii* control. Especially in organic agriculture and integrated farming practices, alternative methods have increasingly gained significance for pest control. Further detailed studies about the extension of encapsulation, shelf life and expiration date of these metabolites could enable the use of these pesticides widely and more practically.

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## Original article (Orijinal araştırma)

# Insecticidal efficacy of local diatomaceous earths against adult and larvae of *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae)<sup>1</sup>

Yerli diyatom topraklarının *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae)'ün ergin ve larvalarına karşı insektisidal etkinliği

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## Abstract

In this study, insecticidal activity of diatomaceous earths (DE) of different particle size (Turco 000, 004 and 020) obtained from domestic sources in Turkey were tested against *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) larvae and adults under laboratory conditions. DE were tested against larvae and adults of *T. molitor* at four different rates (0, 0.001, 0.002, 0.003 and 0.004 mg/cm<sup>2</sup>), and LC<sub>50</sub> and LC<sub>90</sub> values were calculated. Turco 000 grade DE with the smallest particle size had 100% efficacy at all rates against the adults at 60 h, and after 48 h, LC<sub>50</sub> and LC<sub>90</sub> values were 0.006 and 0.019 g/cm<sup>2</sup>, respectively. After 48 h treatment, the LC<sub>50</sub> and LC<sub>90</sub> values for Turco 004 were 0.013 and 0.022 g/cm<sup>2</sup>, respectively, whereas they were 0.022 and 0.041 g/cm<sup>2</sup> with Turco 020, respectively. The DE applied to the larvae had activity in varying proportions. LC<sub>50</sub> values were 0.014, 0.034 and 0.032 g/cm<sup>2</sup> after 72 h for Turco 000, 004 and 020, respectively. LC<sub>90</sub> values were 0.053, 0.089 and 0.075 g/cm<sup>2</sup>, respectively. The results obtained in this study are promising for control of this pest with local DE.

**Keywords:** Lethal toxicity, diatomaceous earth, particle size, *Tenebrio molitor*

## Öz

Bu çalışmada Türkiye'de yerli kaynaklardan elde edilen farklı tanecik boyutuna sahip diyatom topraklarının (Turco 000, 004 ve Turco 020) *Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae) larva ve erginlerine karşı insektisidal etkinliği laboratuvar koşullarında test edilmiştir. Bu amaçla diyatom toprakları dört farklı dozda (0.001, 0.002, 0.003 ve 0.004 g/cm<sup>2</sup>) zararlının larva ve erginlerine karşı denenmiş, LC<sub>50</sub> ve LC<sub>90</sub> değerleri hesaplanmıştır. En küçük parçacık boyutuna sahip Turco 000 kodlu diyatom toprağı zararlının erginlerine karşı 60. saat uygulama yapılan dozlarda %100 ölüme neden olmuş ve 48. saat sonunda LC<sub>50</sub> ve LC<sub>90</sub> değerleri sırasıyla 0.006 ve 0.019g/cm<sup>2</sup> olarak hesaplanmıştır. 48 saatlik uygulama süresi sonunda Turco 004 için LC<sub>50</sub> ve LC<sub>90</sub> değerleri sırasıyla 0.013 g/cm<sup>2</sup>, 0.022g/cm<sup>2</sup> olarak hesaplanmıştır. Aynı zaman dilimi içerisinde Turco 020 kodlu diyatom toprağı için LC<sub>50</sub> ve LC<sub>90</sub> değerleri ise sırasıyla 0.022 ve 0.041 g/cm<sup>2</sup> olmuştur. Zararlının larvaları için uygulama yapılan diyatom toprakları değişen oranlarda aktiviteye sahip olmuştur. Turco 000, 004 ve 020 kodlu diyatom toprakları için 72. saat sonunda LC<sub>50</sub> değerleri sırasıyla 0.014, 0.034 ve 0.032 g/cm<sup>2</sup> olarak hesaplanırken LC<sub>90</sub> değerleri sırasıyla 0.053, 0.089 ve 0.075 g/cm<sup>2</sup> olarak hesaplanmıştır. Bu çalışmada elde edilen sonuçlar yerel diyatom topraklarının bu zararlının mücadelesinde kullanımı açısından ümit var sonuçlar içermektedir.

**Anahtar sözcükler:** Letal toksisite, yerel diyatom toprağı, tanecik büyüklüğü, *Tenebrio molitor*

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## Introduction

*Tenebrio molitor* L., 1758 (Coleoptera: Tenebrionidae), yellow mealworm, is an important pest of durable and stored products and commodities all over the world (Vainikka et al., 2006; Sallam, 2013). Larvae are very voracious and can feed on a wide variety of postharvest products from grains to flour, tobacco and foodstuffs (Sallam, 2013). *Tenebrio molitor* causes losses of up to 15% of grain and flour production worldwide (Dunkel, 1992; Flinn et al., 2003; Neethirajan et al., 2007). *Tenebrio molitor* adults are dark brown or black, 14-17 mm long and elytra have the longitudinal thin line on top. The larvae have a yellowish and segmented appearance, and are 25-30 mm long. Females deposit up to 400-500 eggs in total into the food where they feed. The larvae go through 17-18 d larvae period and then turn into pupae in the same environment and have one generation per year (Hill, 2002). Besides the active consumption of grain/food material, *T. molitor* deposits their sticky eggs and frass in flour, which turns the flour lumpy and smelling of mold. In addition, they cause loss of quality with dirt and cause residues.

For the control of storage pests, fumigation is the preferred chemical control method worldwide, due to its rapid penetration, ease of use and low cost. Methyl bromide (MeBr) was the most widely used fumigant. However, MeBr was banned except in quarantine and pre-shipment uses, under the Montreal Protocol (Protocol No: 26369, 1987) due to its ozone-depleting properties. The other important fumigant is phosphine. Widespread phosphine gas usage has led to resistant insect populations and concerns about risks to human safety associated with its application (Annis, 2016).

The residue of some insecticides applied directly to protect stored grains from insect pests may cause acute or chronic toxicity to the consumer at significant levels. Also, the development of resistance in the pests also causes practical problems, such as ineffectiveness of the active ingredient. Resistance to some important stored product pests against many effective substances used in storage, such as chlorpyrifos-methyl, etrimfos, fenitrothion, malathion, pirimiphos-methyl and the like, has been reported (Arthur, 1996). Diatomaceous earth (DE) treatment is one of the alternatives to chemical control.

Diatoms, either solitary or colonial, are microscopic photosynthesizing algae that have a siliceous skeleton, called the frustule and are found in almost every aquatic environment ranging from freshwater to marine. In fact, they are found virtually anywhere there is enough moisture. They have both benthic and planktonic forms that are both restricted to the photic zone, since they are all strictly autotrophic (water depths down to about 200 m depending on the water clarity). Diatoms have a variety of different diameter or length such as 20-200 microns, some even reach up to 2 mm in length. They are recorded in geological records since the Cretaceous period. Diatoms may occur in such large amounts and be well preserved enough to form sediments composed almost entirely of diatom frustules, these are called diatomites, or if only partly of frustules, then they are called DE. In both cases, these are economic deposits that can be used in a number of applications including agriculture, filters, paints, toothpaste and many others (Finkel et al., 2009). The chemical composition of raw DE is mostly silicon, as well as aluminum, carbon, iron, magnesium, manganese, nickel, phosphorus, sodium, sulfur, zinc and other elements (Subramanyam, 1993).

In insects, the cuticle acts as an exoskeleton and provides protection and support for internal organs. The main barrier to prevent water loss from an insect is the epicuticular lipids. In insect morphology, epicuticular lipids act as a platform for the semiochemicals and also have an important role like retention of water in the body, protection from the body external corrosive and toxic substances (Howard & Blomquist, 2005). DE absorbs the cuticular lipids and it also abrades the cuticles of insects, and causing death by desiccation (Ebeling, 1971; Rigaux et al., 2001). DE can be successfully incorporated into the IPM programs as they have proven to be very effective against insect pest species with low mammalian toxicity, long-lasting efficacy and are natural insecticides.

In this study, the aim was to determine the efficacy of local DE obtained from central Anatolia around Ankara Province as protectants against *T. molitor*, which is an important pest of stored products all over the world. Also, the present study was designed to assess the effect of particle size and behavioral effects of the DE on the insects under laboratory conditions.

## Materials and Methods

### Insect rearing

*Tenebrio molitor* was reared in 25 x 16 x 11 cm storage containers in laboratory conditions (25±2°C and 60-70% RH). The rearing diet consisted of a mixture of 0.5% flour and 95% wheat bran placed into production container up to two-thirds of the volume, and the top of the container was covered with tulle for ventilation. Egg cartons were placed in the container for egg deposition by the females and water-soaked cotton was placed to meet their water requirements.

### Bioassays

Local Turco 000, 004 and 020 DEs were used at four different rates (0.001, 0.002, 0.003 and 0.004 g/cm<sup>2</sup>) and untreated control placed into 16 cm<sup>2</sup> glass bottles (Hosseini et al., 2014). Five *T. molitor* adults with 7-d-old or five *T. molitor* larvae with 45-50-d-old were placed in each glass bottle containing 0.11 g bran as the food and the mouth covered with tulle. The bottles were incubated at 25±2°C and 60-70% RH. After 12, 24, 36, 48, 60 and 72 h, counts were made and live and dead adults or larvae were recorded. Trials were set up to randomized block design with 18 replicates. The trials were conducted under laboratory conditions in 2018.

### Diatomaceous earth

DE used in this study were acquired from a local company operating in Ankara-Kazan and Beypazarı Districts (Beg-Tuğ Mineral Corp., Turkey). The particle sizes of the DE ranged from 1-10, 10-30 and 43-65 µm for Turco 000, 004 and 020, respectively. Local DE mainly composes of SiO<sub>2</sub> in the range of 83 to 95% and other minerals are present in oxidized forms of aluminum, calcium and iron in small amounts.

### Statistical analysis

Rate-response test results were analyzed with the help of Polo-PC probit package program (LeOra, 2002) and LC<sub>50</sub> and LC<sub>90</sub> values and their confidence intervals were determined. All percentage mortality data were subjected to arcsine transformation [ $n' = \arcsin(\sqrt{n})$ ] to obtain normal distribution, and then treated by GLM (general linear model) ANOVA procedures using package program of MINITAB 16 (Mckenzie & Goldman, 2005) to determine the interaction between the factors and it was determined in this way whether there was any interaction.

## Results and Discussion

It was determined that DEs with different particle size have different insecticidal activity against *T. molitor* adults (Table 1). Turco 000, 004 and 020 DEs did not result in any mortality of the adults after 12 h exposure and therefore, LC<sub>50</sub> and LC<sub>90</sub> values could not be calculated. After 24 h exposure, LC<sub>50</sub> and LC<sub>90</sub> values for Turco 000 DE were 0.049 and 0.099 g/cm<sup>2</sup>, respectively. As expected, the LC<sub>50</sub> and LC<sub>90</sub> values decreased with increasing exposure time and after 36 h exposure, these values were determined as 0.017 and 0.040 g/cm<sup>2</sup>, respectively. For Turco 004, LC<sub>50</sub> values for 24, 36, 48 and 72 h were 0.054, 0.021, 0.013 and 0.008 g/cm<sup>2</sup>, respectively, and LC<sub>90</sub> values were 0.132, 0.036, 0.022 and 0.017 g/cm<sup>2</sup>, respectively. LC<sub>50</sub> and LC<sub>90</sub> values for Turco 000 and 004 could not be calculated for 12 h DE exposure since no mortality was observed in any of the DE treatments, and also no probit estimations were provided in 60 and 72 h for

Turco 000 and 72 h for Turco 004 due to 100% mortality in all application rates. LC<sub>50</sub> values for 24, 36, 48, 60 and 72 h exposure for Turco 020 DE were 0.071, 0.034, 0.022, 0.012 and 0.010 g/cm<sup>2</sup>, respectively, and the LC<sub>90</sub> values were 0.121, 0.064, 0.041, 0.026 and 0.020 g/cm<sup>2</sup>, respectively. LC<sub>50</sub> and LC<sub>90</sub> values after 12 h DE exposure could not be calculated because there was no mortality at any DE rate.

Table 1. Insecticidal activity of local diatomaceous earth (DE) against *Tenebrio molitor* adults

DE	HAT	Slope±SE	LC <sub>50</sub> (g/cm <sup>2</sup> )	99% confidence interval	LC <sub>90</sub> (g/cm <sup>2</sup> )	99% confidence interval	Heterogeneity
Turco 000	12	*	*	*	*	*	*
	24	4.23±0.46	0.049	0.045-0.055	0.099	0.083-0.130	1.14
	36	3.38±0.41	0.017	0.014-0.019	0.040	0.035-0.047	0.87
	48	2.63±0.66	0.006	0.002-0.010	0.019	0.013-0.023	0.82
	60	**	**	**	**	**	**
	72	**	**	**	**	**	**
Turco 004	12	*	*	*	*	*	*
	24	3.31±0.42	0.054	0.049-0.062	0.132	0.104-0.195	0.61
	36	5.32±0.52	0.021	0.019-0.023	0.036	0.033-0.041	0.82
	48	6.16±1.27	0.013	0.011-0.015	0.022	0.020-0.026	0.53
	60	4.05±1.20	0.008	0.003-0.011	0.017	0.013-0.021	0.53
	72	**	**	**	**	**	**
Turco 020	12	*	*	*	*	*	*
	24	5.44±0.91	0.071	0.064-0.084	0.121	0.098-0.185	0.61
	36	4.67±0.42	0.034	0.031-0.037	0.064	0.057-0.075	1.07
	48	4.76±0.44	0.022	0.020-0.024	0.041	0.037-0.046	1.01
	60	3.72±0.58	0.012	0.009-0.014	0.026	0.023-0.031	0.67
	72	4.04±0.93	0.010	0.005-0.012	0.020	0.017-0.024	0.63

HAT: hours after treatment;

\* LC values could not be calculated because there was no mortality;

\*\* LC values could not be calculated because there was 100% mortality.

Similarly, to *T. molitor* adults, the insecticidal efficacy of local DE larvae varied with particle size and exposure time (Table 2). LC<sub>50</sub> values for Turco 000 DE were 0.314, 0.053, 0.031, 0.021, 0.017 and 0.014 g/cm<sup>2</sup> for 12, 24, 36, 48, 60 and 72 h exposure, respectively. The LC<sub>90</sub> values were 1.853, 0.152, 0.076, 0.067, 0.059 and 0.053 g/cm<sup>2</sup>, respectively. LC<sub>50</sub> and LC<sub>90</sub> values could not be calculated since Turco 004 DE had no insecticidal activity at any application rates after 12 h exposure. LC<sub>50</sub> values between 24 and 72 h exposure were 0.095, 0.058, 0.048, 0.039 and 0.034 g/cm<sup>2</sup>. The LC<sub>90</sub> values were 0.242, 0.139, 0.112, 0.095 and 0.089 g/cm<sup>2</sup>, respectively. While the LC<sub>50</sub> values between 24 and 72 h exposure for the Turco 020 DE were 0.094, 0.058, 0.043, 0.035 and 0.032 g/cm<sup>2</sup>, respectively, The LC<sub>90</sub> values were 0.276, 0.177, 0.106, 0.087 and 0.075 g/cm<sup>2</sup>, respectively.

DE was found to be significant in terms of time and rate interactions in statistical analysis. Both these treatments and DE by time, DE by rate and DE by time by rate interactions were statistically significant. However, DE by time by rate interactions were statistically insignificant for larvae (Table 3).



Table 2. Insecticidal activity of local diatomaceous earth (DE) against *Tenebrio molitor* larvae

DE	HAT	Slope±SE	LC <sub>50</sub> (g/cm <sup>2</sup> )	99% confidence interval	LC <sub>90</sub> (g/cm <sup>2</sup> )	99% confidence interval	Heterogeneity
Turco 000	12	1.66±0.58	0.314	0.138-0.448	1.853	0.403-2.145	0.75
	24	2.80±0.37	0.053	0.046-0.063	0.152	0.112-0.254	0.72
	36	3.30±0.36	0.031	0.028-0.035	0.076	0.064-0.098	1.01
	48	2.52±0.35	0.021	0.016-0.024	0.067	0.055-0.092	0.91
	60	2.35±0.37	0.017	0.011-0.021	0.059	0.048-0.084	1.09
	72	2.16±0.38	0.014	0.008-0.018	0.053	0.043-0.076	1.03
Turco 004	12	*	*	*	*	*	*
	24	3.16±0.59	0.095	0.073-0.180	0.242	0.142-0.987	1.53
	36	3.41±0.45	0.058	0.051-0.070	0.139	0.105-0.226	1.22
	48	3.45±0.40	0.048	0.043-0.054	0.112	0.089-0.163	1.27
	60	3.36±0.37	0.039	0.035-0.044	0.095	0.077-0.133	1.38
	72	3.01±0.34	0.034	0.030-0.038	0.089	0.073-0.122	1.09
Turco 020	12	*	*	*	*	*	*
	24	2.74±0.51	0.094	0.074-0.154	0.276	0.164-0.878	1.09
	36	2.66±0.38	0.058	0.051-0.071	0.177	0.126-0.319	0.94
	48	3.24±0.37	0.043	0.038-0.048	0.106	0.086-0.147	1.05
	60	3.23±0.35	0.035	0.031-0.039	0.087	0.072-0.114	1.07
	72	3.45±0.35	0.032	0.028-0.035	0.075	0.064-0.093	1.02

HAT: hours after treatment;

\* LC values could not be calculated because there was no mortality.

Table 3. ANOVA parameters for main effects and interactions for mortality of *Tenebrio molitor* larvae and adults

Source	Adult						Larvae					
	DF	Seq SS	Adj SS	Adj MS	F	P	DF	Seq SS	Adj SS	Adj	F	P
DE	2	37073	37073	18536	198	0	2	76136	76136	38068	112	0
Rate	3	140923	140923	46974	501	0	3	268642	268642	89547	263	0
Time	5	1380885	1380885	276177	2950	0	5	399273	399273	79855	235	0
DE by rate	6	13784	13784	2297	24.5	0	6	10438	10438	1740	5.11	0
DE by time	10	17553	17553	1755	18.7	0	10	15539	15539	1554	4.57	0
Rate by time	15	89026	89026	5935	63.3	0	15	57462	57462	3831	11.3	0
DE by rate by	30	19754	19754	658	7.03	0	30	9940	9940	331	0.97	0.51
Error	12	114717	114717	94			1368	465610	465610	340		
Total	12	1813715					1439	1303041				

DEs with different particle size have been observed to have varying efficacy against *T. molitor* larvae. According to the results obtained from this study, the insecticidal activity of Turco 000 grade DE with the smallest particle size was higher than that of the other DE grades. In a previous study with the DE product Fossil Shield® with a particle size of 5-30 µm applied to plywood plates at 0, 2 and 4 g/m<sup>2</sup>, significant activity was reported against *Tribolium confusum* du Val., 1863, *T. molitor*, *Sitophilus granarius* (L., 1758) and *Plodia interpunctella* (Hübner, 1813) (Mewis & Ulrichs, 2001). In present study, LC<sub>90</sub> value after 72 h DE exposure for Turco 000 with the smallest particle size was 0.053 g/cm<sup>2</sup>. The particle size of the Fossil Shield® used in the study varies between 5-30 µm, while the local DE with the smallest particle size used in this study is between 1-10 µm. It is also known that the chemical composition of the DE has as important role in its insecticidal activity as particle size (Korunic et al., 1998). Japp (2008) revealed that, the DE samples collected from different regions of Argentina at 63 g/m<sup>2</sup> showed the mortality for the lesser mealworm (*Alphitobius diaperinus* Panzer, 1797 [Coleoptera: Tenebrionidae] between 7-98%. Oliveira et al., (2017), reported that for the elimination of the *A. diaperinus* from the poultry house with 280 g/m<sup>2</sup> DE. It has been suggested that the differences between these studies are due to particle size and chemical composition of the DEs. DEs with small particle size can be more effective than DEs with large particles. This is especially important in active moving insects. Depending on the intensity of movement and activity of the insects, the lethal effect of DEs increases. Many studies have been conducted on the use of DEs against stored product pests (Vayias et al., 2006; Vayias & Stephou, 2009; Eroglu et al., 2019), vegetable pests (Llewellyn & Eivaz, 1979; Ulrichs et al., 2001; El-Wakeil & Saleh, 2009; Wakil et al., 2012) and many other pests affecting public health (Faulde et al., 2006; Hosseini et al., 2014). The number of studies on the use of environmentally-friendly inputs that have the potential to replace synthetic pesticides have increased recently. There are many studies on the use of DEs alone, or in combination with different materials to control insect pests. Combining DEs with entomopathogen fungi, plant-based essential oils and extracts are the major topics being studied (Athanassiou et al., 2006; Yang et al., 2010; Riasat et al., 2011; Wakil et al., 2011; Ashraf et al., 2017).

DE, by physically abrading cuticular layer, damages to epicuticular lipids and causes desiccation that leads to death of the insect (Ebeling, 1971; Korunic, 1998; Rigaux et al., 2001). Insect susceptibility to DEs depends on their morphology and physiology (Korunic, 1998). One of the factors of efficacy of DE in insects is the thickness of the epicuticular lipid layer. Increased thickness of this layer is considered to reduce the efficacy of DE because of reduced water loss. Mewis & Ulrichs (2001) reported that weight loss and death did not occur in *T. molitor* larvae after they were treated with Fossil Shield®, a commercially available DE, since DE did not cause desiccation. In this study, for 72 h exposure to *T. molitor* larvae, the LC<sub>90</sub> value of Turco 000 was found to be 0.053 g/cm<sup>2</sup>. Otitodun et al. (2015) revealed that, the mortalities with a 14-d treatment were 69 and 98% for *Rhyzopertha dominica* (F., 1792) and *S. granarius* adults, respectively.

In our experiments, the LC<sub>50</sub> value after 48 h exposure was 0.006 g/cm<sup>2</sup> for Turco 000 with the smallest particle size. In insects, the different reactions of larvae and adult stages to DE can be explained by the natural differences occurring in cutaneous compounds between biological phases. The variation of mortality rates between species can be attributed to the origin of DEs (fresh or marine) as well as their physical and chemical properties, and environmental factors such as temperature and humidity and physiological and morphological characteristics of insects.

In conclusion, the results obtained in this study are promising for the control of *T. molitor* with local DEs. It is also considered that present study will become more significant with the help of other disciplines, which enable different formulations of DEs. That will definitely help further development of the DEs by the pesticide industry.

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