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Original article (Orijinal araştırma)

The toxic effects of some acaricides on the tomato russet mite and its predator *Amblyseius swirskii* Athias-Henriot, 1962 (Acari: Phytoseiidae)[1]

Bazı akarisitlerin domates pas akarı ve avcısı *Amblyseius swirskii* Athias-Henriot, 1962 (Acari: Phytoseiidae)'ye toksik etkileri

Ayşenur KOLCU[2] **Nabi Alper KUMRAL[3*]**

Abstract

The tomato russet mite, *Aculops lycopersici* (Massee, 1937) (Acari: Eriophyidae) is a common pest of tomatoes. The predatory mite, *Amblyseius swirskii* Athias-Henriot, 1962 (Acari: Phytoseiidae), can control *A. lycopersici* populations. To integrate biological and chemical control of *A. lycopersici*, side effects of the lethal concentrations of acaricides, as a predator, on *A. swirskii* should be considered. The lethal concentrations of 14 acaricides for *A. lycopersici* were determined under laboratory conditions at Bursa Uludağ University during 2017-2018. To understand the toxic impacts of the acaricides on juveniles and females of *A. swirskii*, the LC_{99} values for *A. lycopersici* of each acaricide were applied to *A. swirskii*. The reproduction reduction effects of the LC_{99} values were also assessed. Quite low concentrations of abamectin, milbemectin, pyridaben, azadirachtin and sulphur were found to be toxic for *A. lycopersici*. Based on the side effect scale, the LC_{99} values of abamectin, acequinocyl, bifenazate, fenproximate, fenbutatin oxide, hexythiazox, milbemectin and sulphur that killed *A. lycopersici* were found to be slightly toxic to both females and juveniles of *A. swirskii*. The results of this comparative toxicological study have showed that more field studies should be conducted to evaluate the effectiveness of using low concentrations of acaricides with *A. swirskii* in combination for controlling *A. lycopersici*.

Keywords: Acaricide, biological control, phytoseiids, side-effect, tomato russet mite, toxicology

Öz

Domates pas akarı, *Aculops lycopersici* (Massee, 1937) (Acari: Eriophyidae) domatesin ana zararlılarından biridir. Avcı akar *Amblyseius swirskii* Athias-Henriot, 1962 (Acari: Phytoseiidae), *A. lycopersici* popülasyonlarını baskı altında tutabilmektedir. Ancak, *A. lycopersici*'nin mücadelesinde biyolojik ve kimyasal yöntemleri birbiriyle entegre edebilmek için *A. swirskii*'ye akarisitlerin yan etkilerinin dikkate alınması gerekmektedir. Bu nedenle, öncelikle *A. lycopersici*'ye 14 farklı akarisitin öldürücü konsantrasyonları 2017-2018 yılları arasında Bursa Uludağ Üniversitesi'nde laboratuvar koşullarında belirlenmiştir. Bu akarisitlerin *A. swirskii* üzerindeki yan etkilerini anlamak için, *A. lycopersici* için belirlenen LC_{99} değerleri *A. swirskii*'nin hem ergin öncesi hem de dişi dönemlerine uygulanmıştır. Ayrıca, bu LC_{99} değerlerinin avcının üremesini azaltıcı etkileri değerlendirilmiştir. Çalışmada, abamectin, milbemectin, pyridaben, azadirachtin ve kükürtün çok düşük konsantrasyonları dahi *A. lycopersici* için oldukça zehirli bulunmuştur. Yan etki skalasına göre, abamectin, acequinocyl, bifenazate, fenproximate, fenbutatin oxide, hexythiazox, milbemectin ve kükürtün *A. lycopersici* için bulunan LC_{99} değerleri *A. swirskii*'nin ergin öncesi ve dişi dönemleri için hafif zehirli bulunmuştur. Bu karşılaştırmalı toksikoloji çalışmaya göre, *A. lycopersici*'nin mücadelesinde *A. swirskii*'nin birlikte kullanımı için akarisitlerin düşük konsantrasyonlarının kullanımının ileride yapılacak saha çalışmaları ile değerlendirilmelidir.

Anahtar sözcükler: Akarisit, biyolojik mücadele, phytoseiidler, yan etki, domates pas akarı, toksikoloji

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Introduction

The tomato russet mite, *Aculops lycopersici* (Massee, 1937) (Acari: Eriophyidae) is one of the main pests of tomatoes all over the world (Abou-Awad, 1979; Şekeroğlu & Özgür, 1984; Lindquist et al., 1996; Duso et al., 2010; Çobanoğlu & Kumral, 2014; Aysan & Kumral, 2018; Vervaet et al., 2021). Since the first visible symptoms of *A. lycopersici* on tomato leaves are similar to those of microelement deficiencies, most farmers are not able to recognize the early mite infestations. When the mite cannot be controlled, high populations can develop quickly and become a serious threat to tomato production (Abou-Awad, 1979; Royalty & Perring, 1987; Duso et al., 2010; Kumral et al., 2014). Therefore, the detection of *A. lycopersici* populations and the correct timing of the acaricide application are often problematic (Duso et al., 2010; Vervaet et al., 2021). In terms of practical control of *A. lycopersici*, the only feasible method is the chemical control. Abamectin, milbemectin and pyridaben are registered acaricides against *A. lycopersici* in Turkey (BKU, 2021). The effectiveness of these acaricides on *A. lycopersici* was demonstrated in previous studies (Vervaet et al., 2021). Nevertheless, the biological effects of several acaricides/insecticides, to *A. lycopersici* are not known.

The European Union Directive 2009/128/EC encourages member and associated countries to use more environmentally friendly alternatives to synthetic pesticides, such as biological control methods. Various biological control methods have recently been used as part of this effort. For instance, spider mites and eriophyid mites have been controlled successfully by members of the Phytoseiidae family (Gerson et al., 2003). *Amblyseius swirskii* Athias-Henriot, 1962 (Acari: Phytoseiidae) is commercially used as a biological control agent against whiteflies, spider mites and thrips in more than 50 countries (Calvo et al., 2015). This mite is also an effective predator of *A. lycopersici* (Van Houten et al., 2013; Kumral et al., 2022, 2023). The ecological solution for the control of *A. lycopersici* is to prefer acaricides that have no side effect for non-target organisms such as predatory mites. To date, the recommended field concentrations of the registered pesticides are found as very toxic to some predatory mites (Fiedler & Sosnowska, 2012; Döker & Kazak, 2019; Kumral et al., 2021).

The integration of biological control agents with pesticide applications can be successful, only in cases when these agents are not affected negatively (Overmeer & Van Zon, 1982; Blümel et al., 1999). Therefore, side effect studies on the female biological control agents should focus on acute toxic effects including the effects on their reproductions (Overmeer & Van Zon, 1982). As well as examining their juvenile stages. This is because biological control agents are in general more susceptible to insecticide/acaricides during their juvenile stages compared to adults. The survival of juveniles is greatly important for maintaining the population of the predator (van Zon & Wysoki, 1978). One of the aims of this study was to determine the lethal concentrations of 14 acaricides having different modes of action (abamectin, acequinocyl, azadirachtin, bifenazate, bifenthrin, fenbutatin-oxide, fenpyroximate, hexythiazox, sulphur, milbemectin, pyridaben, spiromesifen, spirodiclofen and tebufenpyrad) for *A. lycopersici.* The second aim was to establish the side-effects of these lethal concentrations (LC_{99} values determined for *A. lycopersici*) on juveniles and adult females of *A. swirskii* under laboratory conditions.

Materials and Methods

Chemicals

The 14 commercially used acaricide formulations belonging to different chemical groups and modes of action were tested: abamectin, acequinocyl, azadirachtin, bifenazate, bifenthrin, fenbutatin-oxide, fenpyroximate, hexythiazox, sulphur, milbemectin, pyridaben, spiromesifen, spirodiclofen and tebufenpyrad (Table 1).

Table 1. The information about tested acaricides

Active substance	Chemical group[1]	Mode of Action[1]	Mode of action classes[1]	Commercial name	Company	Formulation type[2]	Rate of active substance (g/L)[2]	HRC (mg/L)[2]
Abamectin	Avermectins	Glutamate-gated chloride channel (GluCl) allosteric modulators	6	Algamek	Agrobest	EC	18	4.5
Milbemectin				Milbeknock	SumiAgro	EC	9.3	9.3
Bifenthrin	Synthetic pyretroids	Sodium channel modulators	3	Bifenstar	Koruma	EC	100	70
Sulphur	Minerals	Compounds of unknown or multible MoA	UN	Power sulphur'H	Safa Tarım	WP	80%	3200
Azadirachtin	Botanical acaricides			Nimbecidine	Agrobest	SC	0.3	1.5
Acequinocyl	Unclassified	Mitochondrial complex III electron transport inhibitors	20A	Kanemite	SumiAgro	SC	156	195
Bifenazate	Hydrazine carboxylate		20D	Fluramite	Hektaş	SC	240	144
Fenbutatine oxide	Organometal	Inhibitors of mitochondrial ATP synthase	12B	Quiz	Hektaş	SC	550	330
Pyridaben	Pyridazinone	Mitochondrial complex I electron transport inhibitors	21A	Sanmite	SumiAgro	WP	20%	150
Fenproximate	Pyrazolium			Raincall	Koruma	SC	50	37.5
Tebufenpyrad				Croshe	Hektaş	WP	20%	150
Hexythiazox	Carboxamide	Mite growth inhibitors	10A	Nissuron	SumiAgro	SC	50	50
Spridomesifen	Tetronic& Tetramic acid derivates	Inhibitors of acetyl CoA carboxylase	23	Oberon	Bayer	SC	240	120
Spirodiclofen				Smach	Hektaş	SC	240	60

[1] The data were obtained from mode of action database of Insecticide Resistance Action Committee (IRAC, 2022).
[2] HRC, highest recommended concentration. The data were provided from Turkish Agricultural Ministry Pesticide Registration Database (BKU, 2021).

Mite populations

The *A. lycopersici* population was collected from the tomato fields of the Gorukle Campus (Nilufer, Turkey) in 2012. The species was identified by Edward Ueckermann (Pretoria, South Africa) based on the photos that were taken by using scanning electron microscopy techniques (Kumral et al., 2014). The mite population was mass reared for numerous generations on potted tomato plants in a climate room at 27 ± 1°C, 70 ± 5% relative humidity and a photoperiod of 16 h light: 8 h dark.

The Turkish native field population of *A. swirskii* was obtained from the Acarology laboratory of Ankara University. The species was collected from an orange orchard of Adana (Turkey) in 2015 and identified by Sultan Çobanoğlu (Ankara, Turkey) based on identification keys by Swirski et al. (1998) and Chant & McMurtry (2007). The predatory mite population was mass reared on bean leaves placed adaxial-side down on water saturated cotton wools in plastic boxes (15 x 10 x 5 cm) with air holes, in the same climate conditions described earlier. The predator was fed with *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) and *Typha latifolia* pollen (Overmeer, 1985). The tested mites were fed on *A. lycopersici* and pollen for two subsequent generations before the bioassays. Newly laid eggs were collected from culture boxes and transferred to the new rearing boxes to obtain a group of individuals at the same age for the experiments.

Bioassays on *Aculops lycopersici*

The acute toxic effects of fresh acaricide residues on *A. lycopersici* adults were evaluated using a modified leaf disc method described by Keskin & Kumral (2015) and Abou-Awad & El-Banhawy (1985) under laboratory conditions. Briefly, each tomato leaf disc (30 mm diameter) prepared by a metal leaf cutter was placed on warm Agar-agar solutions (2%) then poured into a Petri dish (55 mm diameter). Five or more different (max. 10) acaricide concentrations resulting in 10-90% mortality in newly emerged *A. lycopersici* female and male adults were used for the bioassays (Table 2). For each bioassay, three replicates were performed for both concentrations and controls (distilled water). Each replication of a bioassay was performed at a different time. Two ml of different acaricide concentrations were applied on the abaxial-side of the leaf disc for 3 s with spray tower resulting in a deposition of 1.5 mg/cm^2 (Potter precision, Burkard Manufacturing Co. Ltd. Rickmansworth U.K.). The sprayed tomato leaf discs were then dried at room temperature for 30 min (Potter, 1952). Forty *A. lycopersici* females (only protonymphs for the mite growth inhibitors acaricide) were transferred onto the leaf discs using a paintbrush. Then, Petri lids having many ventilation holes (>0.1 µm diameter) opened with hot needles were closed. Finally, the Petri dishes were put in the above-mentioned climate conditions. The survival of mites was checked once every 24 h after the acaricide application. The mites were checked using a brush, and the mites unable to walk were considered as dead. The mortality rates in control trials were lower than 7.5% (Table 2).

Bioassays on *Amblyseius swirskii*

The acute toxic effect of fresh acaricide residues on *A. swirskii* was assessed using a modification of the standardized method described by Overmeer & Van Zon (1982) under laboratory conditions (Kumral et al. 2021). Briefly, 2 mL of LC_{99} values (determined for *A. lycopersici*) for each acaricide were applied on the undersurface of the tomato leaves with the spray tower for 3 s resulting in a deposition of 1.5 mg liquid per cm^2. Following that, the tomato leaf discs were dried during 30 min under room conditions. The tomato leaves sprayed with distilled water were used as a control trial (Potter, 1952). The test tool, Plexiglas Munger cells, which have 8 x 10 x 1 cm dimensions with a circular hole of diameter of 5 cm in the center were used (Overmeer, 1982; Kumral et al., 2021). Sprayed tomato leaf and a filter paper were put between Plexiglasses with a circular hole and without a hole. A piece of filter paper was slightly soaked with distilled water.

To evaluate acute toxic effects to *A. swirskii* females, an equal amount of prey (30 mites) and pollen were put into the cell, followed by the same age phytoseiid females (~5 days old) obtained from same age population. For the juvenile test, matured eggs (1.5-2 days old) were used. A total of twenty predatory mites or eggs were used for each replicate. For each acaricide, three replicates were performed at different times. Munger cells were closed by placing a top Plexiglas plate onto the whole fragment of the Munger cell, which was held together with the aid of four binder clips. The cells were put in a climate room at the same conditions. The numbers of death female and juvenile mites were recorded under a stereomicroscope. Juveniles and females of *A. swirskii* were considered as dead after 4 days if any larva stage did not reach adult stage and no movement for mites were observed when a gentle touch was applied by a brush.

The effects of acaricides on the reproduction of *A. swirskii* females during the lifespan were investigated by using the method described by Overmeer & Van Zon (1982). The LC_{99} values (determined for *A. lycopersici*) were applied to tomato leaves with the same bioassay method. Following the application, a mated *A. swirskii* female (each newly emerged female was paired with a male adult for 12 h) were introduced on the surface of each tomato leaf disc put into a Munger cell. The tomato leaves treated with distilled water only served as control. Ten females were treated for each replicate and each treatment comprised of three replicates. The mortality and number of eggs were recorded once per 24 h until all of the females died naturally.

Data analysis

Mortality percentages for *A. lycopersici* and *A. swirskii* were corrected using control percentages with Abbott's formula (Abbott, 1925). The SPSS 23.0 program was used for the probit analysis of the concentration–response data for generating the LC_{50} and LC_{99} values (Finney, 1971). A one-way ANOVA was conducted to determine differences in mortality rates of juvenile stages and females of *A. swirskii* among different acaricide treatments. Before the analyses, corrected mortality data were transformed by using arcsin transformation. Means obtained in all ANOVAs were separated using Tukey's HSD post-hoc test (α = 0.05). Moreover, the combined total side effect (*E*) of the acaricides on *A. swirskii* was calculated using the following formula as suggested by Overmeer & Van Zon (1982):

$$E= 100- [(100-M) \times R]$$

where *E* is the coefficient of toxicity; *M* shows corrected mortality effects using Abbott formula on *A. swirskii* juvenile stages or females of LC_{99} values calculated for *A. lycopersici; M* was calculated separately for both juvenile stages (*JM*) and females (*FM*); *R* is the ratio between the mean number of eggs laid by *A. swirskii* females treated LC_{99} of acaricides and the mean number of eggs produced by the females exposed to distilled water (control group). If the reproduction reduction rate (R) is found as "1", the reproduction of females treated by any acaricide is not affected when compared with those of the control females (Overmeer & Van Zon, 1982). The concentrations of each acaricides were classified using these *E* results: class I (<30% = harmless), class II (30-79% = slightly harmful), class III (80-99% = moderately harmful), class IV (>99 % = harmful) (Sterk et al., 1999).

Results

Effects on *Aculops lycopersici*

Table 2 shows the toxicity results of 14 acaricides to *A. lycopersici*. The estimated LC_{50} and LC_{99} values showed differences among acaricides. Some acaricides such as pyridaben, azadirachtin, abamectin and milbemectin, respectively, have higher toxic effect to *A. lycopersici* at low concentrations compared with those of other acaricides. The LC_{99} value of each acaricide was used as the side effect studies of *A. swirskii* for further experiments.

Table 2. The bioassay and probit analysis results for *Aculops lycopersici*

Active substance	n[a]	C[b]	T[c] (h)	MC[d] (%)	Slope±SE	LC_{50} (a.i. mg/L)	95% confidental limits		LC_{99} (a.i. mg/L)	95% confidental limits		X^2	P[e]
							Lowest conc.	Highest conc.		Lowest conc.	Highest conc.		
Abamectin	960	7	24	3.3	13.51±1.04	0.059	0.05	0.07	0.23	0.21	0.26	15.52	0.69
Milbemectin	960	7	48	5.0	13.71±1.11	0.095	0.09	0.11	0.26	0.24	0.30	30.97	0.19
Bifenthrin	1320	10	48	2.5	0.29±0.02	3.30	2.69	4.13	11.14	9.09	14.69	139.88	<0.01
Sulphur	716	5	48	3.3	3.26±0.26	5.61	4.77	6.65	28.99	19.13	61.93	41.68	<0.01
Azadirachtin	1080	8	72	3.4	1.55±0.01	0.03	0.02	0.06	0.17	0.12	0.35	154.66	<0.01
Acequinocyl	1080	8	72	3.3	13.51±1.04	32.98	30.97	35.25	79.08	73.06	85.59	25.01	0.46
Bifenazate	1200	9	72	7.5	0.01±0.00	141.45	130.48	152.59	410.56	380.77	447.40	31.36	0.30
Fenbutatin oxide	1080	8	72	0.0	0.02±0.01	47.63	34.99	64.19	180.44	142.32	253.3	57.62	<0.01
Pyridaben	840	6	24	0.0	0.97±0.66	0.004	0.003	0.005	0.12	0.01	0.016	80.78	<0.01
Fenproximate	1080	8	72	5.9	0.09±0.01	13.64	12.59	14.79	41.15	37.71	45.47	23.10	0.57
Tebufenpyrad	1200	9	72	3.3	0.06±0.01	16.43	13.53	20.39	53.43	44.29	68.12	95.04	<0.01
Hexythiazox	1080	8	96	2.5	0.04±0.01	29.99	27.54	32.48	92.87	86.38	100.70	32.17	0.15
Spridomesifen	1080	8	96	6.7	0.02±0.01	17.19	4.69	29.81	128.75	93.27	218.2	189.27	<0.01
Spirodiclofen	960	7	96	6.7	0.076±0.01	12.07	9.74	15.00	42.54	35.13	54.91	56.02	<0.01

[a] number of tested individuals; [b] number of tested concentrations for each acaricides; [c] Observation time for each acaricides; [d] Mortality rate of control individuals; [e] Probability.

Side effects for *Ambylesius swirskii*

The side effects of LC_{99} values (for *A. lycopersici*) of 14 acaricides on juveniles and females of *A. swirskii* are given in Table 3. The corrected mortality rates (JM) differed significantly in juveniles after the exposure of different acaricides ($F_{13,50}$= 11.72; P<0.01). Significantly low JM values (5.48 to 31.68%) were detected for juveniles treated with acequinocyl, milbemectin, bifenazate, abamectin, bifenthrin and fenproximate. Moderate JM values (35.72 to 53.23%) were observed for hexythiazox, fenbutatin oxide, pyridaben and sulphur. Based on JM values, the highly toxic acaricides for juveniles were tebufenpyrad (89.06%), azadirachtin (87.29%), spirodiclofen (82.04%) and spiromesifen (80.14%).

The LC_{99} values of the acaricides significantly reduced the survival of *A. swirskii* females (Table 3). Sulphur (5.55%), fenpyroximate (12.50%), abamectin (13.89%) and pyridaben (15.81%) were much less toxic to *A. swirskii* females compared with other acaricides ($F_{13,50}$=6.69; P<0.01). Moderate mortality rates (24.44 to 54.17%) of females (FM) were observed after milbemectin, spirodiclofen, fenbutatin-oxide, hexythiazox, azadirachtin, bifenazate, acequinocyl and spiromesifen applications. Significantly high FM values were determined in the females by exposure to tebufenpyrad (59.38%) and bifenthrin (77.50%).

Table 3. The side effects on *Amblyseius swirskii* of 14 acaricides

Active substance	C[a] (a.i. mg/L)	N[b]	JM[c] (%)	FM[d] (%)	R[e]	E[f] (%)	Juvenile toxicity scale[g]	E[h] (%)	Female toxicity scale[g]
Abamectin	0.23	60	23.70 cd[i]	13.89 d[i]	0.67	48.88	II	42.29	II
Milbemectin	0.26	60	15.23 cd	24.44 b-d	0.34	71.18	II	74.31	II
Bifenthrin	11.14	60	28.38 cd	77.50 a	0.70	49.87	II	84.25	III
Sulphur	28.99	60	53.23 bc	5.55 d	0.51	76.15	II	51.83	II
Azadirachtin	0.17	60	87.29 a	40.63 b-d	0.21	97.33	III	87.53	III
Acequinocyl	79.08	60	5.48 d	47.50 a-d	0.41	61.25	II	78.48	II
Bifenazate	410.56	60	22.81 cd	40.63 b-d	0.52	59.86	II	69.13	II
Fenbutatin oxide	180.44	60	39.95 b-d	30.55 b-d	0.47	71.78	II	67.36	II
Pyridaben	0.12	60	51.80 b-d	15.81 d	0.40	80.72	III	66.32	II
Fenproximate	41.15	60	31.68 cd	12.50 d	0.51	65.16	II	55.38	II
Tebufenpyrad	53.43	60	89.06 a	59.38 ab	0.40	95.62	III	83.75	III
Hexythiazox	92.87	60	35.72 cd	34.38 b-d	0.41	73.64	II	73.09	II
Spridomesifen	128.75	60	80.14 ab	54.17 a-c	0.52	89.63	III	76.17	II
Spirodiclofen	42.54	60	82.04 ab	27.50 b-d	0.49	91.19	III	64.48	II

[a], Applied concentrations (LC_{99} value for *Aculops lycopersici*) for each acaricides
[b], A number of tested individual, 60 females or 60 juveniles (20 x 3 replicates) also used in the control spraying water. The mortality rates were not exceed 20% in the bioassays.
[c], The corrected mortality rates of juveniles (larvae or nymphs)
[d], The corrected mortality rates of females
[e], Reproduction reduction rate of treated females compared with untreated ones
[f], Total side effect according to juvenile deaths= 100-[(100-JM)) x R]
[g], Total side effect according to female deaths= 100-[(100-FM)) x R]
[h], The side effect scale [I = harmless (<%30), II = slightly harmful (30–79%), III = moderately harmful (80–99%), IV = harmful (>99)] (Sterk et al., 1999)
[i], Means followed by a different letter differ significantly in same column (<0.05)

Compared with untreated control, the decreases in fecundity of *A. swirskii* females (R) were determined by exposure to the LC_{99} values of the acaricides (Table 3). The R ratios were much less in treated females with azadirachtin (0.21) and milbemectin (0.34). Moderate R ratios (0.40 to 0.52) were observed in females treated with tebufenpyrad, pyridaben, acequinocyl, hexythiazox, fenbutatin-oxide, spirodiclofen, sulphur, fenpyroximate, bifenazate and spiromesifen, low to high, respectively. Significant high R ratios were found in females exposed to abamectin (0.67) and bifenthrin (0.70) (Table 3).

Based on the side effect scale, the concentrations that killed *A. lycopersici* for abamectin, acequinocyl, bifenazate, fenproximate, fenbutatin oxide, hexythiazox, milbemectin and sulphur were slightly harmful for both juveniles and females (Table 3). The concentrations of pyridaben, spiromesifen and spirodiclofen were found to be slightly harmful for females but moderately harmful for juveniles. The other acaricides (tebufenpyrad and azadirachtin) were observed as moderately harmful for both juveniles and females. None of the acaricide concentrations was found either harmless or harmful against *A. swirskii.*

Discussion

In this study, the concentrations (lower than their HRCs registered in Turkey) of abamectin, milbemectin, bifenthrin, pyridaben, sulfur and azadirachtin were found to be toxic to *A. lycopersici*. Similarly, previous studies showed that, low concentrations of abamectin, milbemectin, pyridaben and bifenthrin were effective against *A. lycopersici* (Royalty & Perring, 1987; Silva et al., 1988; Arbabi, 2013; Spasov et al., 2014; Fischer & Klötzli, 2015) and some other eriophyid mites such as *Epitrimerus pyri* Nalepa,1898, *Aculus schlechtendali* (Nalepa, 1890) and *Eriophyes dioscoridis* Soliman & Abou-Awad, 1977 (Acari: Eriophyidae) (Van Leeuwen et al. 2010). Furthermore, several laboratory and field studies showed that sulphur was highly toxic to *A. lycopersici* (Cermelli et al., 1982; Silva et al., 1988; Baradan-Anakari & Daneshvar, 1992; Hıncal et al., 2002; Fischer & Klötzli, 2015). Additionally, Kashyap et al. (2015) demonstrated that azadirachtin at a concentration of 0.25% was successful (99% of mite population) for the control of *A. lycopersici* populations in field conditions.

Acequinocyl, fenbutatin oxide, fenpyroximate, tebufenpyrad, spridomesifen and spirodiclofen concentrations proximate to HRCs were found toxic to *A. lycopersici*. Previous studies reported that high or moderate concentrations of fenbutatin oxide, propargite, fenpyroximate, spridomesifen and spirodiclofen caused toxic effects on *A. lycopersici* (Cermelli et al., 1982; Ky & Shepherd, 1988; Atanasov, 1995; Elbert et al., 2005; Spasov et al., 2014). Results of the current study showed that LC_{99} values of bifenazate and hexythiazox (the only mite growth regulator) were found to be higher than their HRC. Lethal concentrations of acequinocyl, tebufenpyrad and bifenazate against *A. lycopersici* were determined for the first time in this study. Van Leeuwen et al. (2010) noted that the effects of bifenazate and acequinocyl which are new acaricidal compounds, are not known against many rust and gall mites, yet. In the same way, some authors have reported that the mite growth regulator acaricide, hexythiazox, are effective against *A. lycopersici* (Arbabi, 2013; Fischer & Klötzli, 2015), but hexythiazox effectiveness was lower compared with the HRCs for other mite pests. The discrepancy may be due to species differences (BKUtarim, 2020).

This study also gave us information about the acute toxic and egg laying reducing effects (side effects) of acaricides at their LC_{99} concentrations (determined for *A. lycopersici*) on *A. swirskii*. According to JM and FM values, the most toxic acaricides were tebufenpyrad, azadirachtin, spirodiclofen and spiromesifen for juveniles and, tebufenpyrad and bifenthrin for females. Based on R ratios, azadirachtin and milbemectin slightly reduced the fecundity of females. According to the side effect scale, tebufenpyrad and azadirachtin were moderately harmful for both juveniles and females. Pyridaben, spiromesifen and spirodiclofen are more detrimental against juveniles compared with females. In agreement with our findings, Momen et al. (1997) showed that two concentrations (0.2 and 0.05%) of a product formulated from Neem seeds decreased the fecundity and increased the mortality of *A. swirskii* females. On the contrary, Audenaert et al. (2014) demonstrated that azadirachtin did not cause mortality on *A. swirskii* under field conditions and it was safe

to use it in combination with this predatory mite. These variations may be a result of different formulations, concentrations and test conditions. Additionally, the quick degradation of azadirachtin in field conditions might be taken into consideration. Similar to our study, several authors reported that tebufenpyrad, pyridaben and pyrethroid acaricides were highly toxic to different strains of *A. swirskii* including an organophosphorus resistant strain (El-Banhawy et al., 2007; Fiedler & Sosnowska, 2012; Fernandez et al., 2017a, b).

Consistent with the findings of Alinejad et al. (2016) who previously reported that HRC of spirodiclofen was very toxic to *A. swirskii*, it was found in this study that only sub-lethal concentrations had fewer side effects on its development and reproduction. In contrast, some authors found that HRC and sublethal concentrations of spirodiclofen and spiromesifen were harmless under the laboratory or field conditions, despite reduced oviposition and life-span of *A. swirskii* females (Audenaert et al., 2014; Fernandez et al., 2017a, b; Döker & Kazak, 2019).

The differences between our findings and the literature records could have arisen from the use of different side effect formulas and scales. In the current study, the side effect value was calculated and evaluated by considering both mortality rates and negative impact on fecundity, whereas other studies included only mortality rates. Since the sensitivity of the formula increases, the value obtained for the negative impact on fecundity is added to the side effect calculation as a multiplier (Fernandez et al*.,* 2017b).

The present study indicated that the LC_{99} values of abamectin, acequinocyl, bifenazate, fenproximate, fenbutatin oxide, hexythiazox, milbemectin and sulphur for *A. lycopersici* were slightly toxic to both females and juveniles of *A. swirskii*. While reducing effects on fecundity of *A. swirskii* were relatively limited for those of abamectin and bifenthrin, the effects of the rest acaricides remained at moderate level. Based on the side effect scale, the concentrations of abamectin, acequinocyl, bifenazate, fenproximate, fenbutatin oxide, hexythiazox, milbemectin and sulphur were found to be slightly harmful. But, among these acaricides, only the concentrations of bifenazate and fenproximate were close to or slightly higher than their HRCs registered for other mite pests in Turkey. Similar to our results, some authors showed the compatibility of acequinocyl, bifenazate, fenproximate and hexythiazox with *A. swirskii* in different agricultural ecosystems (Fiedler & Sosnowska, 2012; Audenaert et al., 2014; Lopez et al., 2015). Although Masui et al. (2014) noted that acequinocyl did not affect the population of *A. swirskii* in field studies in melon fields, the one third of acequinocyl HRC was found as slightly harmful for *A. swirskii* in our study. The reason for discrepancy may be the differences between laboratory and field conditions. Whenever, a lower concentration of abamectin was found to be harmless during this study, the use of the HRC of this acaricide shows a really high toxic effect to *A. swirskii* (Trottin-Caudal et al., 2008; Gradish et al., 2011; Cuthbertson et al., 2012; Fernandez et al., 2017b). Some studies showed that limited side effects on *A. swirskii* were observed from evaporation and dusts arising from application of sulphur (Gazquez et al., 2011; Pijnakker & Ramakers, 2009). However, our experimental concentration for sulphur was quite lower than its HRC. Among acaricides allowed to use in organic farming, the lower concentrations of sulphur and azadirachtin were favorable towards *A. lycopersici*. The determined concentration of azadirachtin was shown to be unfavorable for *A. swirskii* due to its negative effects on the survival and fecundity of the predator. The information about the side effects of the rest of acaricides such as tebufenpyrad, fenbutatin oxide, milbemectin, against *A. swirskii* is limited in the literature.

Consequently, eriophyid mites are tiny arthropods that are very sensitive to pesticides. As in this study, even very low concentrations of some acaricides were able to kill them easily. The results of this comparative toxicological study showed that the use in combination of low concentrations of some acaricides with *A. swirskii* in the control of *A. lycopersici* have potential. Practically, when *A. lycopersici* reaches high populations in a field, the acaricides can be applied, and then the phytoseiid could be released. Conserving the population of the predatory mite, this strategy might be used on tomatoes, but the hypothesis must be confirmed in field conditions in the future.

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Original article (Orijinal araştırma)

A faunistic study of Ichneumonidae (Hymenoptera) from Northeastern Anatolia Region (Erzurum: Yakutiye and Uzundere) of Türkiye[1]

Türkiye'nin Kuzey Doğu Anadolu Bölgesi (Erzurum: Yakutiye ve Uzundere) Ichneumonidae (Hymenoptera) türleri üzerine faunistik bir araştırma

Gülnur BARİK[2] **Saliha ÇORUH[2*]**

Abstract

This faunistic study was conducted to determine the species of Ichneumonidae collected by insect net from Erzurum, Yakutiye and Uzundere districts of Türkiye between June and November in 2020-2021. As a result of this research, 232 specimens representing 17 species from 14 genera belonging to subfamilies Cremastinae, Cryptinae, Ichneumoninae and Pimplinae were distinguished. Among them, *Mesoleptus vigilatorius* (Förster, 1876), *Mesostenus funebris* Gravenhorst, 1829 and *Cubocephalus associator* (Thunberg, 1822) are new to the Turkish fauna. The collecting area, date of collection, altitude of collection, specimens and sex number, visited plants, their distribution in Türkiye and general geographic distribution of species have been presented.

Keywords: Erzurum, Hymenoptera, Ichneumonidae, new records, Türkiye

Öz

Bu faunistik çalışma, 2020-2021 yıllarının, Haziran ve Kasım ayları arasında Erzurum'un Yakutiye ve Uzundere ilçelerinden atrapla toplanan Ichneumonidae türlerini tespit etmek amacıyla yapılmıştır. Araştırma sonucunda, Cremastinae, Cryptinae, Ichneumoninae ve Pimplinae altfamilyalarına ait 14 cinse bağlı 17 türden 232 örnek ayırt edilmiştir. Teşhis edilen türlerden, *Mesoleptus vigilatorius* (Förster, 1876), *Mesostenus funebris* Gravenhorst, 1829 ve *Cubocephalus associator* (Thunberg, 1822) Türkiye faunası için yeni kayıt durumundadır. Ayrıca türlerin toplama alanları, toplanma tarihi, toplandığı rakım, birey sayısı ve cinsiyeti, ziyaret ettiği bitkiler, Türkiye ve genel coğrafi dağılımları da verilmiştir.

Anahtar sözcükler: Erzurum, Hymenoptera, Ichneumonidae, yeni kayıtlar, Türkiye

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Introduction

With about 25 000 described species (Yu et al., 2016) and between 60 000 and 100 000 estimated (Townes, 1969; Rasnitsyn, 1978), Ichneumonidae or “Darwin wasps” (Klopfstein et al., 2019) constitute one of the most diverse animal groups today and the largest family of parasitoid wasps. The family is divided into more than 40 subfamilies (Broad et al., 2018). Biology of Darwin wasps is extremely diverse. Ichneumonids mostly parasitize the immature stages of the Holometabola, and are frequently associated with Lepidoptera and sawflies (Hymenoptera). Therefore, some species of this family have been intentionally introduced for biological control (Rasplus 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).

“A cataloque of the Turkish Ichneumonidae (Hymenoptera)” was the first comprehensive study that summarized all the published data on the Turkish Ichneumonidae (Kolarov, 1995). In this study, 383 species belonging to 19 subfamilies were listed. In the past 27 years, the number of species has reached about 1439, with most valuable contributions by Çaylak & Çoruh (2020a, b), Kıraç & Gürbüz (2020), Kolarov et al. (2020, 2021), Schwarz (2020), Teymuroğlu & Çoruh (2021), Yurtcan et al. (2021), Bulak Korkmaz & Çoruh (2022), Çoruh (2022), Çoruh et al. (2022a, b), Çoruh & Riedel (2022), Doğru (2022), İneciklioğlu (2022), Kaplan & Riedel (2022) and Kolarov & Çoruh (2022). Despite their ecological importance, Darwin wasps are still among the most poorly studied groups of organisms.

In this study, Ichneumonidae material from Erzurum, Yakutiye and Uzundere districts was examined, and new faunistic data is provided.

Materials and Methods

Ichneumonidae species were collected by insect net from natural gardens, orchards, agricultural fields and different weeds in 10 localities (Table 1) of Erzurum (Yakutiye and Uzundere) (Figure 1) in Türkiye (Anatolia), during the 2020-2021 summer. All examined material was collected by the first author and determined by the second author and Janko Kolarov (Bulgaria). After identifying, each species was photographed by the digital shooting unit (Canon EOS 1100 D, Canon EF 100 mm, f/2.8L Macro lens, Kaiser digital), and partially focused images were combined using Helicon focus 6.7.1. software. All the material is deposited in the Entomology Museum Erzurum, Türkiye (EMET). The species recorded from Türkiye for the first time are marked by an asterisk (*). General distributions and associated plants were taken from Yu et al. (2016).

Table 1. Data of collected species

Region	Locality	Altitude (m)	Number of specimens
Yakutiye	Atatürk University Campus	1876	60
	Güzelova	1700	14
Uzundere	Bağbaşı	1000	2
	Erikli	1420	14
	Engüzek kapı	1150	1
	Center	1000	44
	Pehlivanlı	900	84
	Sapaca	1200	2
	Yukarı Serdarlı	1681	11

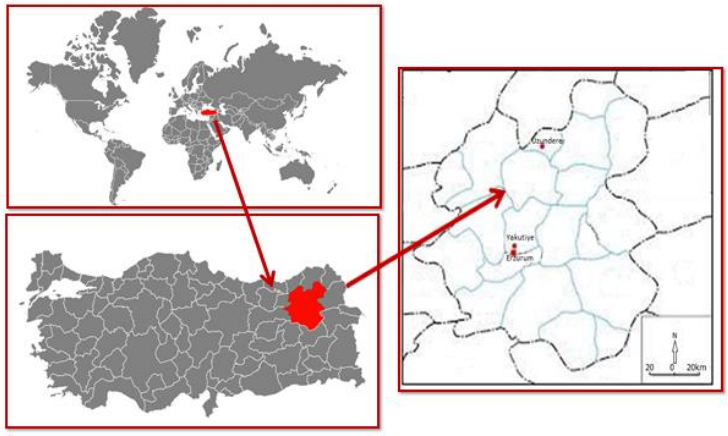


Figure 1. Map of study area.

Study area

Erzurum is a province located in the northeastern Anatolia and at an altitude about 1900 m from sea level. It has 10 districts, two of which are central district. Specimens for this study were collected in Yakutiye (Central district) and Uzundere districts of Erzurum. Uzundere is quite different from other districts with its microclimate features. Contrary to the continental climate, the Black Sea climate is dominant in the district. In addition to all these, Tortum Lake and Waterfall are very important geographical riches of the district**.**

Results

In this study, we report 232 specimens belonging to 14 genera for Erzurum (Yakutiye and Uzundere). Among them, three species, *Mesoleptus vigilatorius* (Förster, 1876), *Mesostenus funebris* Gravenhorst, 1829 and *Cubocephalus associator* (Thunberg, 1822) are new to the Turkish fauna.

Subfamily Cremastinae Förster, 1869

Dimophora nitens (Gravenhorst, 1829)

Material examined. Uzundere: Pehlivanlı, 40°29’40”N, 41°30’07”E, 900 m, 08.IX.2020, 4♀♀, 40°28’37”N, 41°27’39”E, 1000 m, 21.VI.2021, 2♂♂, 3♀♀.

Distribution. Australia and Palearctic. This species is known from Ankara, Çanakkale and Isparta provinces in Türkiye.

References. Kolarov (1997), Kolarov & Beyarslan (1999), Gürbüz (2005) and Çoruh et al. (2014b) (Figure 3a).

Associated plant. *Peucedanum oreoselinum* (L.) (Apiaceae)

Remarks. New records for East Anatolia and Erzurum.

Subfamily Cryptinae Kirby, 1837

Aptesis senicula (Kriechbaumer, 1893)

Material examined. Uzundere: Erikli, 40°32’07”N, 41°33’39”E, 1420 m, 21.VII.2021, 5♀♀; Pehlivanlı, 40°29’40”N, 41°30’07”E, 900 m, 23.IX.2020, ♂, 40°28’37”N, 41°27’39”E, 1000 m, 03.VI.2021, 2♀♀, 21.06.2021, 2♀♀; Yukarı Serdarlı, 40°28’22”N, 41°18’50”E, 1681 m, 21.VI.2021, 3♂♂, 4♀♀.

Distribution. Europe and West Palearctic. This species is known from Adana, Bursa, Mersin, Tunceli and Rize provinces in Türkiye.

References. Beyarslan & Kolarov (1994), Çoruh et al. (2014b), Kolarov et al. (2014c, 2016), Çoruh (2019), Çaylak (2019) and Çaylak & Çoruh (2020b) (Figure 3b).

Remarks. New records for East Anatolia and Erzurum.

Cryptus dianae Gravenhorst, 1829

Material examined. Yakutiye: Güzelova, 40°02'46"N, 41°20'23"E, 1700 m, 26.VII.2020, 2♀♀.

Distribution. Palearctic. This species is known from Isparta province in Türkiye.

References. Gürbüz & Kolarov (2008) and Çoruh (2019) (Figure 3c).

Associated plant. *Angelica sylvestris* L. (Apiaceae), *Euphorbia nicaeensis* All., *Euphorbia segueriana* Neck (Euphorbiaceae), *Peucedanum oreoselinum* (L.) (Apiaceae), *Quercus* spp. (Fagaceae).

Remarks. New records for East Anatolia and Erzurum.

Cryptus viduatorius Fabricius, 1804

Material examined. Uzundere: Erikli, 40°32'07"N, 41°33'39"E, 21.VI.2021, 1420 m, 2♀♀; Center, 41°31'31"N, 41°32'26"E, 21.VII.2021, 1000 m, 7♂♂, 2♀♀. Yakutiye: Atatürk University Campus, 39°53'58"N, 41°14'50"E, 1876 m, 16.VI.2021, 2♀♀; Güzelova, 40°02'46"N, 41°20'23"E, 1700 m, 26.VI.2020, 3♂♂.

Distribution. Palearctic. This species is known from Bilecik, Bursa, Erzurum, Isparta, Içel, Istanbul and Kırklareli provinces in Türkiye.

References. Kolarov (1987), Beyarslan & Kolarov (1994), Kolarov (1995), Kolarov et al. (1997a, 2016), Gürbüz & Kolarov (2008), Çoruh & Çoruh (2008, 2012), Gürbüz et al. (2009a), Çoruh et al. (2014a, b, 2016, 2018), Sarı & Çoruh (2018), Çoruh (2019) and Yılmaz (2020) (Figure 3d).

Associated plants. *Anethum graveolens* L. (Apieae), *Angelica sylvestris* L., *Daucus carota* L. (Apiaceae), *Euphorbia nicaeensis* All., *Euphorbia virgata* Waldst. & Kit. (Euphorbiaceae), *Ferula communis* L., *Heracleum sphondylium* L. (Apiaceae), *Medicago sativa* L. (Fabaceae), *Peucedanum oreoselinum* (L.) (Apiaceae).

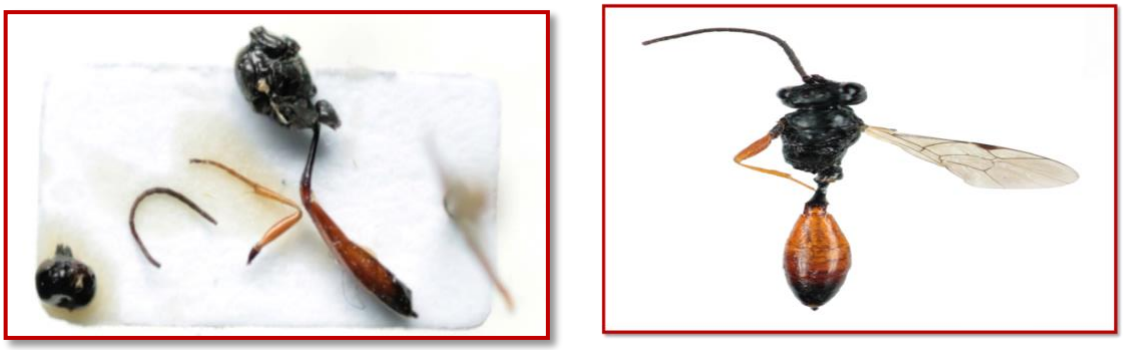


Figure 2. New species: *Mesoleptus vigilatorius.*

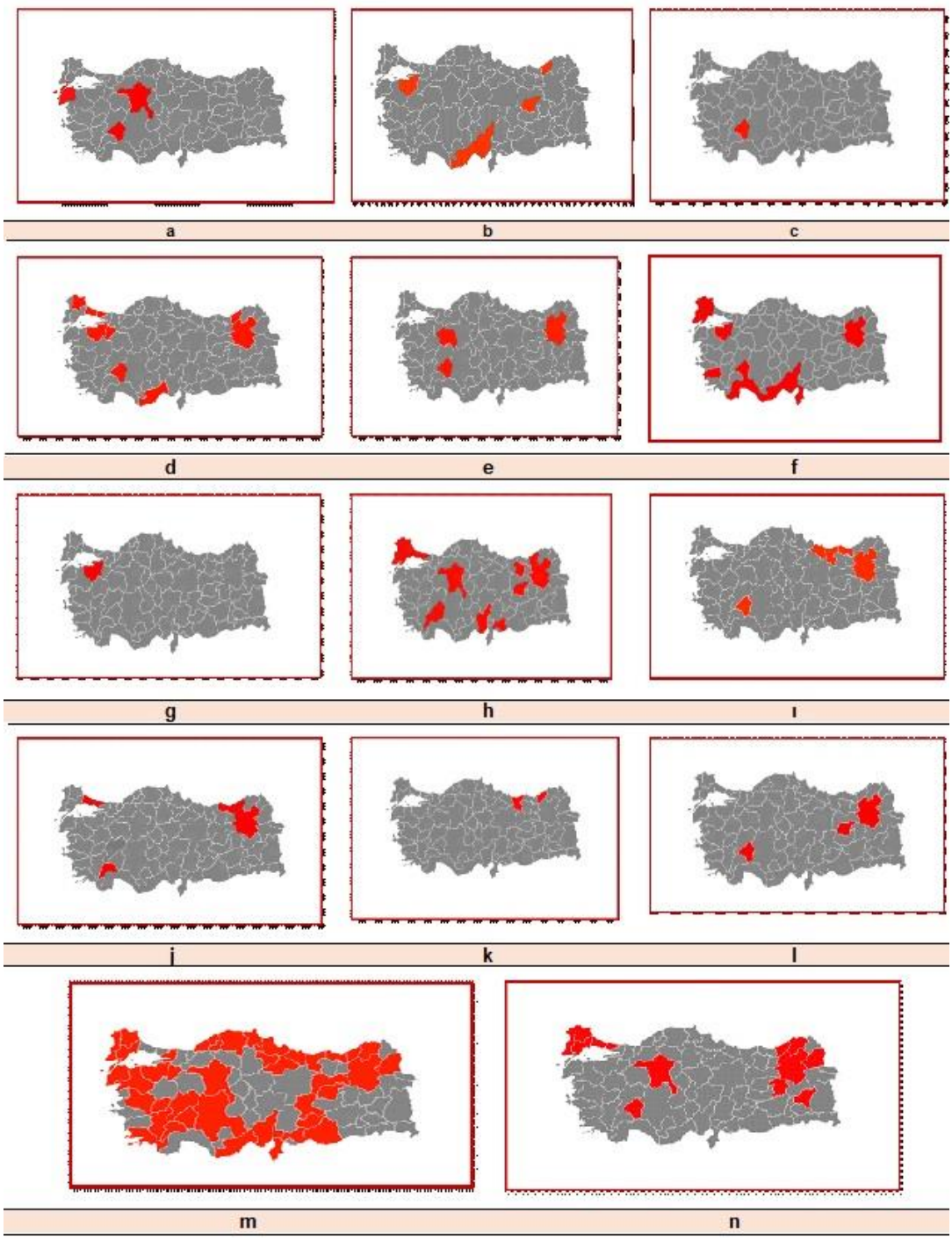


Figure 3. Distribution of species in Türkiye: a) *Dimophora nitens*, *b) Aptesis senicula*, c) *Cryptus dianae*, d) *Cryptus viduatorius*, e) *Dichrogaster longicaudata*, f) *Mesostenus transfuga*, g) *Phygadeuon nitidus*, h) *Trychosis legato,* ı) *Colpognathus celerator,* j) *Heterischnus truncator*, k) *Heterischnus excavates,* l) *Perithous septemcinctorius,* m) *Pimpla spuria,* n) *Scambus brevicornis.*

**Cubocephalus associator* (Thunberg, 1822)

Material examined. Uzundere: Yukarı Serdarlı, 40°28’22”N, 41°18’50”E, 1682 m, 27.VII.2021, 2♂♂.

Distribution. Europe and West Palearctic.

Associated plant. *Angelica* spp. (Apiaceae)

Dichrogaster longicaudata (Thomson, 1884)

Material examined. Uzundere: Center, 41°31’31”N, 41°32’26”E, 21.VI.2021, 1000 m, 2♂♂, 2♀♀. Yakutiye: Güzelova, 40°02’46”N, 41°20’23”E, 1700 m, 19.VII.2020,♂.

Distribution. Holarctic. This species is known from Erzurum, Eskişehir and Isparta provinces in Türkiye.

References. Kolarov & Gürbüz (2007), Kırtay (2008), Gürbüz et al. (2009a), Eroğlu et al. (2011), Çoruh et al. (2016) and Çoruh (2019) (Figure 3e).

Associated plants. *Bauhinia* sp. (Fabaceae), *Oryza sativa* L. (Poaceae)

**Mesoleptus vigilatorius* (Förster, 1876) (Figure 2)

Material examined. Pehlivanlı, 40°03’29”N, 41°20’54”E, 900 m, 19.VIII.2020,♀.

Distribution. Palearctic.

**Mesostenus funebris* Gravenhorst, 1829

Material examined. Uzundere: Center 41°31’31”N, 41°32’26”E, 1000 m, 21.VI.2021, 3♂♂, 2♀♀; Yukarı Serdarlı, 40°28’22”N, 41°18’50”E, 1682 m, 27.VII. 2021, 2♀♀.

Distribution. Palearctic.

Mesostenus transfuga (Gravenhorst, 1829)

Material examined. Uzundere: Bağbaşı, 40°30’32”N, 41°27’38”E, 1000 m, 21.VI.2021, 2♂♂; Pehlivanlı, 40°03’29”N, 41°20’54”E, 900 m, 08.IX.2020, ♀, 40°28’37”N, 41°27’39”E, 1000 m, 21.VI.2021, 3♂♂. Yakutiye: Atatürk University Campus, 39°53’58”N, 41°14’50”E, 1876 m, 16.VI.2021, 2♂♂.

Distribution. Oceanic and Palearctic. This species is known from Adana, Antalya, Aydın, Bursa, Edirne, Erzurum, Hatay, Isparta, Kırklareli, Mersin and Tekirdağ provinces in Türkiye.

References. Soydanbay (1976), Öncüer (1991), Kolarov (1995), Beyarslan & Kolarov (1994), Kolarov et al. (1997a), Çoruh & Çoruh (2008), Gürbüz & Kolarov (2008), Gürbüz et al. (2009b) and Çoruh (2019) (Figure 3f).

Associated plants. *Euphorbia segueriana* Kunst, *E. virgata* Waldst. & Kit. (Euphorbiaceae), *Fraxinus excelsior* L. (Oleaceae), *Pimpinella tragium* Vill. (Apiaceae), *Seseli libanotis* (L.) W. D. J.Koch. (Apiaceae).

Phygadeuon nitidus Gravenhorst, 1829

Material examined. Uzundere: Pehlivanlı, 900 m, 40°29’40”N, 41°30’07”E, 23.IX.2020, 4♂♂.

Distribution. Europe and West Palearctic. This species is known from Bursa province in Türkiye.

References. Çaylak & Çoruh (2020b) (Figure 3g).

Remarks. New record for East Anatolia and Erzurum.

Trychosis legator (Thunberg, 1822)

Material examined. Uzundere: Pehlivanlı, 40°29’40”N, 41°30’07”E, 900 m, 26.VII.2020, 5♀♀, 40°28’37”N, 41°27’39”E, 1000 m, 21.VI.2021, 3♀♀. Yakutiye: Güzelova, 40°02’46”N, 41°20’23”E, 1700 m, 19.VIII.2020, 6♂♂.

Distribution. Palearctic. This species is known from Adana, Ankara, Burdur, Çanakkale, Edirne, Erzurum, Gaziantep, Gümüşhane, Isparta, Istanbul, Kırklareli, Tekirdağ, Tunceli and Rize provinces in Türkiye.

References. Kolarov (1987), Kolarov & Beyarslan (1994a), Kolarov et al. (1997b, 2014c), Gürbüz & Kolarov (2008), Çoruh et al. (2014b, 2016) and Çoruh (2019) (Figure 3h).

Associated plants. *Anethum graveolens* L., *Chaerophyllum bulbosum* L. (Apiaceae), *Cornus sanguinea* L. (Cornaceae), *Daucus carota* L. (Apiaceae), *Euphorbia cyparissias* L., *E. nicaeensis* All., *E. segueriana* Kunst, *E. virgata* Waldst. & Kit. (Euphorbiaceae), *Fraxinus excelsior* L. (Oleaceae), *Heracleum sphondylium* L., *Pastinaca* spp., *Peucedanum oreoselinum* (L.) (Apiaceae), *Quercus* spp. (Fagaceae).

Subfamily Ichneumoninae, Latreille 1802

Colpognathus celerator (Gravenhorst, 1807)

Material examined. Uzundere: Center, 41°31’31”N, 41°32’26”E, 21.VI.2021, 1000 m, 3♂♂, 2♀♀; Pehlivanlı, 40°29’40”N, 41°30’07”E, 900 m, 24.VI.2020, 6♂♂,♀; 26.VII.2020,♂; Sapaca, 40°33’02", 41°34’45”E, 1200 m, 16.VII.2021, 2♀♀. Yakutiye: Atatürk University Campus, 39°53’58”N, 41°14’50”E, 1876 m, 16.VI.2021, 4♂♂, 3♀♀.

Distribution. Palearctic. This species is known from Erzurum, Giresun, Isparta, Ordu and Trabzon provinces in Türkiye.

References. Çoruh & Özbek (2008), Kolarov et al. (2014a), Çoruh et al. (2016), Çoruh (2017) and Özdan & Gürbüz (2019) (Figure 3ı).

Associated plants. *Anthriscus sylvestris* (L.) Hoffm., *Chaerophyllum aromaticum* L. (Apiaceae), *Cornus mas* L. (Cornaceae), *Corylus avellana* L. (Betulaceae), *Daucus carota* L., *Ferulago sylvatica* (Besser) Rchb. (Apiaceae), *Fraxinus excelsior* L. (Oleaceae), *Heracleum sphondylium* L. (Apiaceae), *Oryza sativa* L. (Poaceae), *Peucedanum oreoselinum* (L.) (Apiaceae), *Picea excelsa* Engelm. (Pinaceae).

Remarks. This species was collected while feeding on *Medicago sativa* L.

Heterischnus truncator (Fabricius, 1798)

Material examined. Uzundere: Pehlivanlı, 40°29’40”N, 41°30’07”E, 900 m, 24.VI.2020,♂. Yakutiye: Atatürk University Campus, 39°53’58”N, 41°14’50”E, 1876 m, 16.VI.2021, 3♂♂, 7♀♀.

Distribution. Palearctic. This species is known from Erzurum, Giresun, Isparta, Istanbul and Trabzon provinces in Türkiye.

References. Kolarov (1989, 1995), Yurtcan et al. (1999), Özbek et al. (2003), Çoruh et al. (2014b, 2016), Kolarov et al. (2014b) and Özdan & Gürbüz (2019) (Figure 3j).

Associated plants. *Anethum graveolens* L., *Daucus carota* L. (Apiaceae), *Mentha* spp. (Lamiaceae), *Oryza sativa* L. (Poaceae), *Rubus fruticosus* L., *R. idaeus* L. (Rosaceae), *Setaria glauca* (L.) (Poaceae).

Heterischnus excavatus (Constantineanu, 1959)

Material examined. Yakutiye: Atatürk University Campus, 39°53'58"N, 41°14'50"E, 1876 m, 16.VI.2021, 2♂♂, 2♀♀; Güzelova, 40°03'29"N, 41°20'54"E, 1700 m, 19.VIII.2020,♂.

Distribution. Europe and West Palearctic. This species is known from Giresun and Rize provinces in Türkiye.

References. Kolarov et al. (2014b) (Figure 3k)

Associated plants. *Angelica sylvestris* L., *Laserpitium latifolium* L. (Apiaceae).

Subfamily Pimplinae Wesmael, 1845

Perithous septemcinctorius (Thunberg, 1822)

Material examined. Uzundere: Engüzek kapı, 40°30'36"N, 41°31'20"E, 1150 m, 16.VII.2021,♀; Pehlivanlı, 40°29'40"N, 41°30'07"E, 900 m, 19.VIII.2020, ♀, 40°28'37"N, 41°27'39"E, 1000 m, 03.VI.2021, 2♀♀.

Distribution. Holarctic. This species is known from Erzurum, Isparta and Tunceli provinces in Türkiye.

References. Kolarov & Gürbüz (2004), Çoruh & Kolarov (2010), Kolarov et al. (2014c) and Çoruh (2016) (Figure 3l).

Associated plants. *Ampelopsis hederacea* Ehrh. (Vitaceae), *Carpinus* spp. (Betulaceae), *Chaerophyllum bulbosum* L. (Apiaceae), *Prunus domestica* L., *P. domestica insititia* (L.) Bonnier & Layens., *Pyrus communis* L. (Rosaceae).

Pimpla spuria Gravenhorst 1829

Material examined. Uzundere: Erikli, 40°32'07"K, 41°33'39"E, 1420 m, 21.VII.2021, 7♀♀; Pehlivanlı, 40°29'40"N, 41°30'07"E, 900 m, 26.VII.2020, 4♂♂, 40°28'37"N, 41°27'39"E, 1000 m, 03.VI.2021, 11♂♂, 13♀♀. Yakutiye: Atatürk University Campus, 39°53'58"N, 41°14'50"E, 1876 m, 16.VI.2021, 3♂♂, 11♀♀.

Distribution. Oriental and Palearctic. This species is known from Adıyaman, Adana, Afyon, Ankara, Artvin, Balıkesir, Bilecik, Burdur, Bursa, Çanakkale, Denizli, Edirne, Erzincan, Erzurum, Eskişehir, Gaziantep, Giresun, Hatay, Isparta, Istanbul, Kars, Kırklareli, Kocaeli, Konya, Manisa, Mersin, Muğla, Osmaniye, Ordu, Rize, Şanlıurfa, Tekirdağ, Trabzon, Tunceli, Uşak and Yalova provinces in Türkiye.

References. Özdemir & Kılınçer (1990), Öncüer (1991), Kolarov & Beyarslan (1994b), Kolarov (1995), Kolarov et al. (1997a, b, 1999, 2002, 2014c, 2016), Gürbüz (2004), Kolarov & Gürbüz (2004) Yurtcan (2004), Yurtcan & Beyarslan (2005), Çoruh (2005, 2016), Buncukçu (2008), Çoruh & Özbek (2008), Çoruh & Kolarov (2010), Eroğlu et al. (2011), Çoruh et al. (2014a, b), Sarı & Çoruh (2018), Teymuroğlu (2021) and Yurtcan et al. (2021) (Figure 3m).

Associated plants. *Acer campestre* L. (Aceracea), *Anethum graveolens* L. (Apiaceae), *Chaerophyllum bulbosum* L. (Apiaceae), *Daucus carota* L. (Apiaceae), *Euphorbia nicaeensis*, All. (Euphorbiaceae), *Heracleum sphondylium* L. (Apiaceae), *Tamarix* spp. (Tamaricaceae).

Scambus brevicornis (Gravenhorst 1829)

Material examined. Uzundere: Center 41°31'31"N, 41°32'26"E, 1000 m, 21.VI.2021, 9♂♂, 12♀♀; Pehlivanlı, 40°28'37"N, 41°27'39"E, 1000 m, 21.VI.2021, 13♂♂. Yakutiye: Atatürk University Campus, 39°53'58"N, 41°14'50"E, 1876 m, 16.VI.2021, 14♂♂, 7♀♀; Güzelova, 40°02'46"N, 41°20'23"E, 1700 m, 16.VI.2020, ♂.

Distribution. Holarctic. This species is known from Ankara, Artvin, Bingöl, Bitlis, Edirne, Erzurum, Isparta, Istanbul, Kars, Kırklareli, Rize and Tekirdağ provinces in Türkiye.

References. Özdemir & Kılınçer (1990), Kolarov & Beyarslan (1994b), Kolarov (1995), Kolarov et al. (1999, 2020), Özdemir & Özdemir (2002), Kolarov & Gürbüz (2004), Çoruh (2005), Çoruh et al. (2007), Yurtcan (2007) and Çoruh & Kolarov (2010) (Figure 3n.)

Associated plants. *Alnus glutinosa* (L.) Gaertn (Betulaceae), *Anethum graveolens* L., *Angelica sylvestris* L. (Apiaceae), *Aster tripolium* L., *Cirsium arvense* (L)., *Cirsium vulgare* (Savi) Ten. (Asteraceae), *Cnicus paluster* (L.) Willd. (Asteraceae), *Daucus carota* L. (Apiaceae), *Fraxinus excelsior* L. (Oleaceae), *Heracleum* spp. (Apiaceae), *Larix europaea* DC., *Larix polonica* Rac. (Pinaceae), *Peucedanum oreoselinum* (L). (Apiaceae), *Populus tremula* L. (Salicaceae), *Salvia sylvesris* L. (Lamiaceae), *Suaeda maritima* (L.) Domort. (Amaranthaceae), *Vincetoxicum officinale* Medik (Apocynaceae).

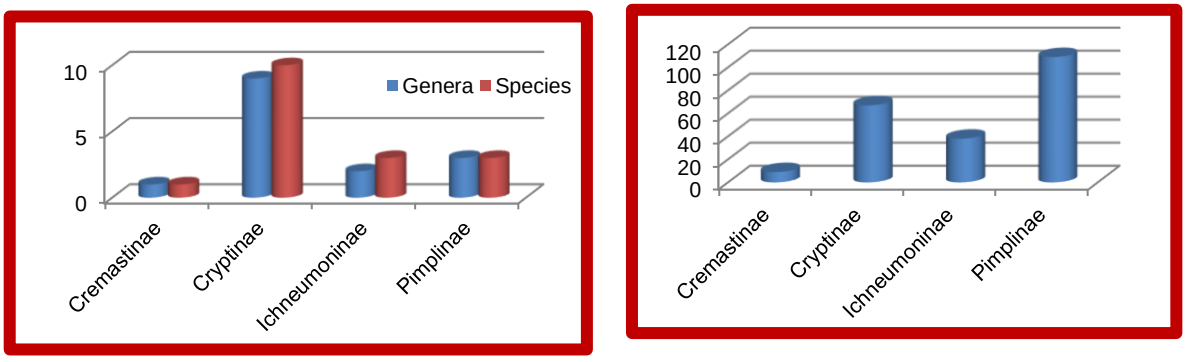
Discussion

The study aimed to reveal the Ichneumonidae (Hymenoptera) fauna in Erzurum, Yakutiye, and Uzundere, and the findings are presented here. The ichneumonids specimens were collected from various altitudes in different months. In total, 17 species of 14 genera belonging to subfamilies Cremastinae, Cryptinae, Ichneumoninae and Pimplinae were identified.

As the study findings have revealed, one species and one genus belonging to subfamily Cremastinae, 10 species and nine genera subfamily Cryptinae, three species and two genera subfamily Ichneumoninae, three species and three genera subfamily Pimplinae were recorded (Figure 4a). Of these, *Mesoleptus vigilatorius*, *Mesostenus funebris* and *Cubocephalus associator* were recorded for the first time for the fauna of Türkiye.

It was noticed that the Pimplinae shows density in terms of the specimen numbers (Figure 4b). Of these, *Scambus brevicornis* and *Pimpla spuria are* the most abundant species, with 56 and 49 specimens, respectively. *Mesoleptus vigilatorius* and *Mesostenus transfuga* are collected as a single specimen in the study area.

All the samples were collected in five different altitude ranges in this study. As can be seen in Table 1, 12 species collected from between 750 and1000 m (A), one species between 1001 and1250 (B), five species between 1251 and1500 m (C), nine species between 1501and1750 (D), six species between 1751 and-2000 m (E) (Figure 5a).



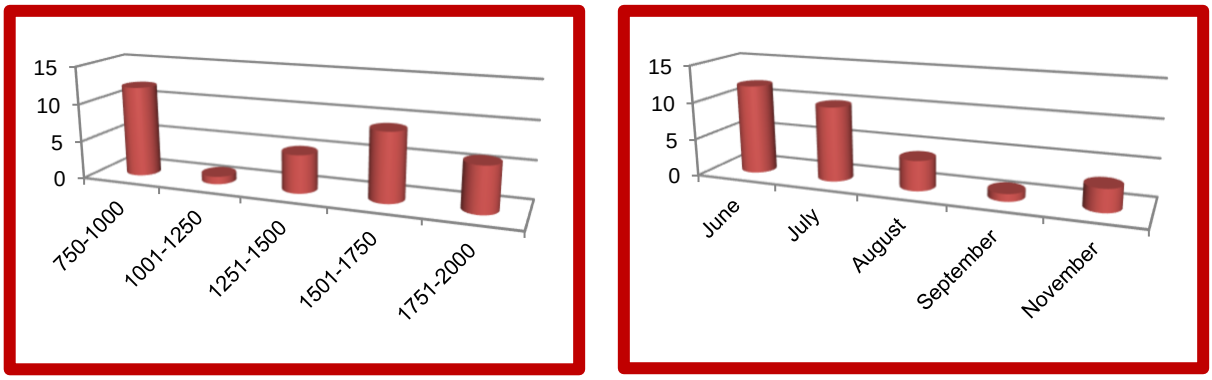
a b

Figure 4. Number of species: a) according to genera and species per subfamily, b) according to specimen numbers.

Table 2. Data of collected species: Specimen numbers (SN), vertical distribution (VD), seasonal dynamics (SD), geographical regions (GR), zoogeographical regions (ZR), associated plant (AP), new record of Türkiye (NRT) of specimens

Name of taxa	SN	VD	SD	GR	ZR	AP	NRT
ORDER: HYMENOPTERA							
FAMILY: ICHNEUMONIDAE Latreille, 1802							
SUBFAMILY: CREMASTINAE Forster, 1869							
Genus *Dimophora* Förster, 1869							
Dimophora nitens	9	A	J, S	CAR, MR, MtR	AUS, WP	x	Kolarov, 1997
SUBFAMILY: CRYPTINAE Kirby, 1837							
Genus *Aptesis* Förster, 1850							
Aptesis senicula	17	A, C, D	J, Jl, N	BSR, EAR, MR, MtR	E, WP		Beyarslan & Kolarov, 1994
Genus *Cryptus* Fabricius, 1804							
Cryptus dianae	2	D	Jl	MtR	WP	x	Gürbüz & Kolarov, 2008
Cryptus viduatorius	16	C, D, E	J, Jl	BSR, EAR, MR, MtR	WP	x	Kolarov, 1987
Genus *Cubocephalus* Ratzeburg, 1848							
Cubocephalus associator	2	D	Jl	*	E, WP	x	New record
Genus *Mesostenus* Gravenhorst, 1829							
**Mesostenus funebris*	7	C, D	J, Jl	*	WP	x	New record
Mesostenus transfuga	8	A	J, N	AR, EAR, MR, MtR	OCE, WP	x	Soydanbay, 1976
Genus *Dichrogaster* Doumerc, 1855							
Dichrogaster longicaudata	5	A, D	J, Jl	CAR, EAR, MtR	HOL	x	Kolarov & Gürbüz, 2007
Genus *Mesoleptus* Gravenhost, 1829							
**Mesoleptus vigilatorius*	1	A	A	*	WP		New record
Genus *Phygadeuon* Gravenhorst, 1829							
Phygadeuon nitidus	4	A	N	MR	E, WP		Çaylak & Çoruh, 2020
Genus *Trychosis* Förster, 1869							
Trychosis legator	14	A, D	Jl, A	BSR, CAR, EAR, MR, MtR, SAR	WP	x	Kolarov, 1987
SUBFAMILY: ICHNEUMONINAE Latreille, 1802							
Genus *Colpognathus* Wesmael, 1845							
Colpognathus celerator	22	A, C, E	J, Jl	BSR, EAR; MtR	WP	x	Çoruh & Özbek, 2008
Genus *Heterischnus* Wesmael, 1859							
Heterischnus truncator	11	A, E	J	BSR, EAR, MtR, MR	WP	x	Kolarov, 1989
Heterischnus excavatus	5	D, E	J, A	BSR	E, WP	x	Kolarov et al., 2014b
SUBFAMILY: PIMPLINAE Wesmael, 1845							
Genus *Perithous* Holmgren, 1859							
Perithous septemcinctorius	4	A, B	J, Jl, A	EAR, MtR	HOL	x	Çoruh & Kolarov, 2010
Genus *Pimpla* Fabricius, 1804							
Pimpla spuria	49	A, C, E	J, Jl	AR, BSR, CAR, EAR, MR, MtR	ORR, WP	x	Özdemir, 1981
Genus *Scambus* Hartig, 1838							
Scambus brevicornis	56	A, D, E	J	CAR, BSR, EAR, MR, MtR	HOL	x	Özdemir & Kılınçer, 1990

Vertical distribution (VD) (meter): A: 750-1000 m., B: 1001-1250 m., C: 1251-1500 m., D: 1501-1750 m., E: 1751-2000 m., Seasonal dynamics (SD): J: June, Jl: July, A: August, S: September, N: November. 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. Zoogeographical regions (ZR): AUS: Australian, E: Europe, HOL: Holarctic Region, OCE: Oceanic Region, ORR: Oriental, WP: Western Palearctic. AS: Associated Plant.



a b

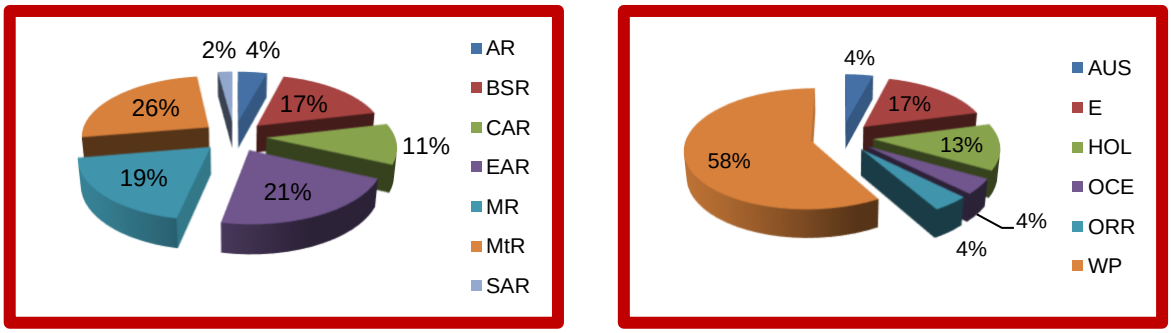
Figure 5. Number of species: a) according to altitude range, b) according to months.

Table 1 reads that most of our insects were collected from an altitude range between 750 and1000 m, but least specimens were collected between 1001 and1250 m with of percentage of 1, 72. The number of species collected was directly proportional to the frequency of visits to the study locality. Despite all this, among the existing species, five species were collected from three different localities, which means that these species were able to live in a wide range of altitudes.

Ichneumon samples were collected mainly in June, July and August as well as in September and November. While a single species entered in insect net in September, most species were collected in June and July (Figure 5b). According to the results, *Aptesis senicula* and *Perithous septemcinctorius* were collected in three different months. Also, six species were collected in only one month. These temporal variations might be because climatic conditions can exert a strong influence on insect abundance and activity (Vasconcellos et al., 2010). Accordingly, we see that, the species identified in the study are active in 5 months a year.

Species were also analyzed for their geographic distributions in Türkiye. Based on this analysis, 12 species were identified from the Mediterranean Region, while 10 were collected from the Eastern Region, previously. The regions where the species were least distributed were the Aegean Region with two samples, and the Southeastern Region with one sample (Figure 6a).

In Table 2, we report that *Pimpla spuria* (35 provinces) was collected in six different regions of Türkiye, and *Trychosis legator* and *Scambus brevicornis* were collected in five different regions. Among the existing species, *Cryptus dianae* (one province), *Phygadeuon nitidus* (one province) and *Heterischnus excavatus* (two provinces) were in only one region.



a b

Figure 6. Number of species: a) according to geographic regions, b) according to zoogeographic region.

There are six main zoogeographic regions in the world for animal distribution (Sclater, 1858). When we examine Table 2, we can see the zoogeographic distribution of the species as follows: 14 species have Western Palearctic distribution, four species European, three species Holarctic, one species Australian, Oceanic and Oriental. In conclusion, Western Palearctic has the highest number of species (Figure 6b).

Cubocephalus associator was found in Europe and West Palaearctic Regions. Although this species is likely to be found in Türkiye, it has only now been discovered. Similarly, *Trychosis legator* was found in six different geographic regions in our country while it had only been commonly seen in West Palearctic Regions in the world before.

Among the existing species, *Cryptus dianae* (Figure 7a) and *Phygadeuon nitidus* (Figure 7b) are rare species for Türkiye, and based on the findings from this study, Erzurum was found to be a second locality for these species.

Figure 7. Rare species for Türkiye: a) *Cryptus dianae,* b) *Phygadeuon nitidus.*

Overall, it is thought that the ecological and faunistic information given about all the above-mentioned species will guide future studies and will be a source for science volunteers to work on this subject. In addition, as in this study, it is predicted that the number of existing species will increase much more with the comprehensive studies to be carried out in many areas that have not been visited in Türkiye.

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Original article (Orijinal araştırma)

New records of erythraeoid mites (Acari: Erythraeoidea) from northeastern Türkiye[1]

Türkiye'nin kuzeydoğusundan erythraeoid akarların (Acari: Erythraeoidea) yeni kayıtları

İbrahim KARAKURT[2*]

Abstract

In this survey, soil samples obtained from Erzincan and Bayburt provinces (Türkiye) between 2013 and 2022 were evaluated. Eleven species of the superfamily Erythraeoidea Robineau-Desvoidy, 1828 are reported for the first time for the Turkish mite fauna. While 9 of the detected species [*Abrolophus artemisiae* (Schrank, 1803); *Abrolophus miniatus* (Hermann, 1804); *Abrolophus quisquiliarus* (Hermann, 1804); *Abrolophus rhopalicus* (Koch, 1837); *Abrolophus strojnyi* Gabryś, 1992; *Balaustium murorum* (Hermann, 1804); *Moldoustium haitlingeri* Noei, Saboori & Šundić, 2013; *Erythraeus* (*Erythraeus*) *cinereus* (Dugès, 1834) and *Erythraeus* (*Zaracarus*) *rupestris* (L., 1758)] belong to the family Erythraeidae Robineau-Desvoidy, 1828, and two species [*Fessonia papillosa* (Hermann, 1804) and *Smaris squamata* (Hermann, 1804)] belong to the family Smarididae Vitzthum, 1929. In addition, the diagnosis of the genus *Moldoustium* Haitlinger, 2008 is re-presented. Also, erythraeoid mites recorded from Türkiye so far are listed.

Keywords: Checklist, Erythraeidae, habitat, Parasitengona, Smarididae

Öz

Bu çalışmada 2013 ile 2022 yılları arasında Erzincan ve Bayburt illerinden (Türkiye) alınan toprak örnekleri değerlendirilmiştir. Türkiye akar faunası için, Erythraeoidea Robineau-Desvoidy, 1828 üstfamilyasına dahil onbir tür ilk kez kaydedilmektedir. Tespit edilen türlerden 9 tanesi [*Abrolophus artemisiae* (Schrank, 1803); *Abrolophus miniatus* (Hermann, 1804); *Abrolophus quisquiliarus* (Hermann, 1804); *Abrolophus rhopalicus* (Koch, 1837); *Abrolophus strojnyi* Gabryś, 1992; *Balaustium murorum* (Hermann, 1804); *Moldoustium haitlingeri* Noei, Saboori & Šundić, 2013; *Erythraeus* (*Erythraeus*) *cinereus* (Dugès, 1834) ve *Erythraeus* (*Zaracarus*) *rupestris* (L., 1758)] Erythraeidae Robineau-Desvoidy, 1828 familyasına dahil iken, ikisi [*Fessonia papillosa* (Hermann, 1804) ve *Smaris squamata* (Hermann, 1804)] Smarididae Vitzthum, 1929 familyasına aittir. Ek olarak, *Moldoustium* Haitlinger, 2008 cinsinin teşhis bilgileri yeniden sunulmuştur. Ayrıca, Türkiye'den şu ana kadar kaydedilmiş erythraeoid akarlar listelenmiştir.

Anahtar sözcükler: Kontrol listesi, Erythraeidae, habitat, Parasitengona, Smarididae

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Introduction

The terrestrial Parasitengona (Trombidia) is represented by about 2000 species (excluding chigger mites). Three active stages were generally observed in the life cycle of Trombidia; larva, deutonymph, and adult. The superfamily Erythraeoidea is represented by over 900 species (Beron, 2017) and consists of the families Erythraeidae and Smarididae, which are distributed worldwide except in Antarctica (Mąkol & Wohltmann, 2012, 2013; Sevsay, 2017). Free-living active postlarval instars of Erythraeoidea feed as predators, whereas heteromorphic larvae of this taxon are usually ectoparasites on various arthropods. The fauna of Erythraeoidea is poorly known in Türkiye. Until now, while thirty-two species of Erythraeidae and only two species of Smarididae have been collected from Türkiye (Mąkol & Wohltmann, 2012; Noei et al., 2017, 2019; Sevsay, 2017; Pamuk & Sevsay, 2020; Karakurt, 2021; Karakurt & Sevsay, 2021; Oner et al., 2021; Elverici et al., 2022; Karakurt, 2022). The present work, which aims to contribute to the Turkish mite fauna, contains 9 species of Erythraeidae and 2 species of Smarididae newly recorded from Türkiye. In addition, the diagnosis of the genus *Moldoustium* is re-presented. Also, in this paper, a list of erythraeoid mites of Türkiye is presented.

Materials and Methods

Mites were extracted by Berlese funnels from soil samples between May 2013 and June 2015 in Bayburt Province, Türkiye, and between October 2021 and June 2022 in Erzincan Province, Türkiye. Mite samples were preserved in 70% ethyl alcohol and mounted on microscope slides using Hoyer's medium (Walter & Krantz, 2009). Photographs were taken and measurements (given in micrometers) were calculated using an Olympus BX63 microscope. The morphological terminology follows Wohltmann et al. (2007). Measurements are given in micrometers (µm).

For comparison of species of *Moldoustium*, type species of this genus in Poland and Iran were re-examined by Prof. Ryszard Haitlinger and Prof. Alireza Saboori, respectively.

Specimens examined are deposited in Acarology Laboratory of Erzincan Binali Yıldırım University, Türkiye (EBYU).

Results and Discussion

Erythraeidae

Abrolophus artemisiae (Schrank, 1803)

Material examined. Bayburt, 40°21'N, 39°51'E, 2455 m a.s.l. (low humidity litter under the *Astragalus* sp. (Fabaceae)) 05 May 2014, 2♀♀; 40°34'N, 40°24'E, 1740 m a.s.l. (low humidity litter under the *Astragalus* sp.) 29 October 2014, ♀♂.

Remarks (Figures 1 a-c). Gabryś (2016) divided the active postlarval of *Abrolophus* species in Poland into two groups according to the shapes of the anus (except for *A*. *tardus* (Halbert, 1915)); '*norvegicus-passerinii-strojnyi*' group and '*quisquiliarus-miniatus-crassitarsus-rhopalicus-artemisiae*' group (op. cit. Figures 54-55). *Abrolophus artemisiae* belongs to the latter group. This species, distributed in Europe (Mąkol & Wohltmann, 2012; Roland & Gabryś, 2021), has been recorded for the first time from Türkiye.

Abrolophus miniatus (Hermann, 1804)

Material examined. Erzincan, Kemah, 39°47'N, 38°52'E, 1250 m a.s.l. (low humidity litter under the *Populus* sp. (Salicaceae)) 28 May 2022, ♀.

Remarks (Figures 1 d-f). According to Gabryś (2016) this species belongs to the '*quisquiliarus-miniatus-crassitarsus-rhopalicus-artemisiae*' group.

This species, distributed in Azerbaijan, Europe and Siberia (Russia) (Beron, 2008; Mąkol & Wohltmann, 2012; Stålstedt et al., 2019; Alizade, 2020; Roland & Gabryś, 2021), has been recorded for the first time from Türkiye.

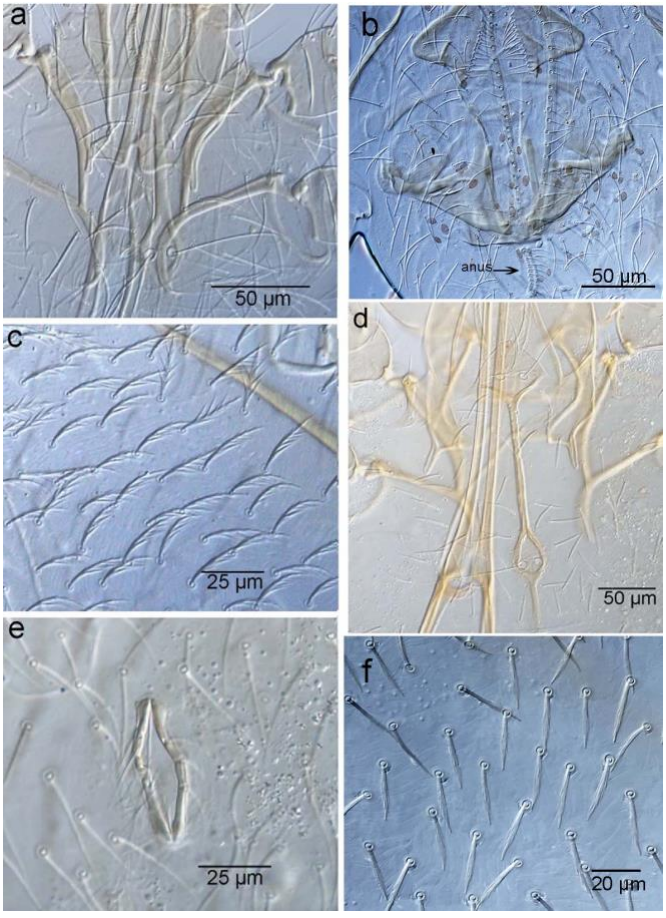

Figure 1. *Abrolophus artemisiae* (Schrank, 1803), adult, photomicrographs a) crista metopica, b) male genital sclerite and anus, c) dorsal setae; *Abrolophus miniatus* (Hermann, 1804), female, photomicrographs d) crista metopica, e) anus, f) dorsal setae.

Abrolophus quisquiliarus (Hermann, 1804)

Material examined. Bayburt, 40°07'N, 40°13'E, 1770 m a.s.l., (moist litter under the *Populus* sp.) 25 April 2015, 2 larvae.

Remarks (Figure 2 a). Łaydanowicz & Mąkol (2008) obtained a larva of *Abrolophus quisquiliarus* by rearing from its female and provided information on the taxonomy of this species. This species, distributed in Azerbaijan, Europe, Russia and Ukraine (Beron, 2008; Mąkol & Wohltmann, 2012; Roland et al., 2015; Stålstedt et al., 2019; Alizade, 2020; Roland & Gabryś, 2021), has been recorded for the first time from Türkiye.

Abrolophus rhopalicus (Koch, 1837)

Material examined. Bayburt, 40°09'N, 39°52'E, 1676 m a.s.l. (moist moss and grassland), 01 May 2014, ♀; 40°16'N, 39°52'E, 1694 m a.s.l. (moist moss), 05 May 2014, ♀♂; 40°07'N, 40°13'E, 1772 m. a.s.l. (low humidity litter under the *Astragalus* sp.), 25 April 2015, ♂.

Remarks (Figures 2 b-e). This species, distributed in Europe and Azerbaijan (Mąkol & Wohltmann, 2012; Stålstedt et al., 2019; Alizade, 2020), has been recorded for the first time from Türkiye.

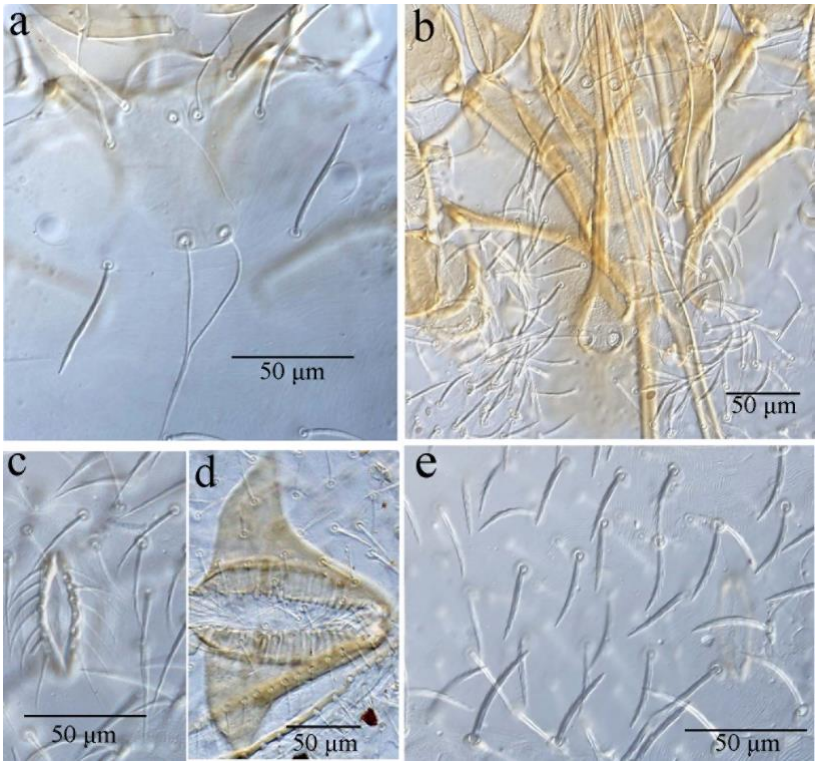

Figure 2. *Abrolophus quisquiliarus* (Hermann, 1804), larva, photomicrograph a) scutum; *Abrolophus rhopalicus* (Koch, 1837), adult, photomicrographs b) crista metopica, c) anus, d) anterior part of male genital sclerite, e) dorsal setae.

Abrolophus strojnyi Gabryś, 1992

Material examined. Bayburt, 40°11'N, 40°18'E, 1596 m a.s.l. (moist litter under the *Populus* sp.), 18 April 2014, ♂.

Remarks (Figures 3 a-c). Based on Gabryś (2016), this species belongs to '*norvegicus-passerinii-strojnyi*' group. This species, distributed in Azerbaijan, Hungary and Poland (Gabryś, 1992; Alizade, 2020), has been recorded for the first time from Türkiye.

Balaustium murorum (Hermann, 1804)

Material examined. Bayburt, Aygır lake, 40°39'N, 40°23'E, 2850 m a.s.l. (moist moss) 22 June 2013, 2 larvae.

Remarks (Figure 3 d). Mąkol (2010) redescribed *Balaustium murorum* and stated *bs* and *cs* setae of the larva of this species bear tiny setules. These setae also bear tiny setules in the examined species. This species, distributed in Japan, Tunisia and Western Palaearctic (Mąkol, 2010; Mąkol & Wohltmann, 2012; Roland et al., 2015; Roland & Gabryś, 2021), has been recorded for the first time from Türkiye.

Moldoustium Haitlinger, 2008

Diagnosis (After Haitlinger, 2008). Palp trochanter without seta. Palp femur and genu with two setae. Coxal setal formula: 2 (3 in *M. baltiensis*)-1-1. Scutum, weakly marked or absent. A pair of eyes with a single lens, located laterally at the level slightly above the posterior sensillary area. All tarsi with two claws and a slender normal empodium.

Distribution. Hungary, Iran, Moldova, Montenegro, Ukraine (Haitlinger, 2008; Noei et al., 2013; Haitlinger & Šundić, 2019) and Türkiye.

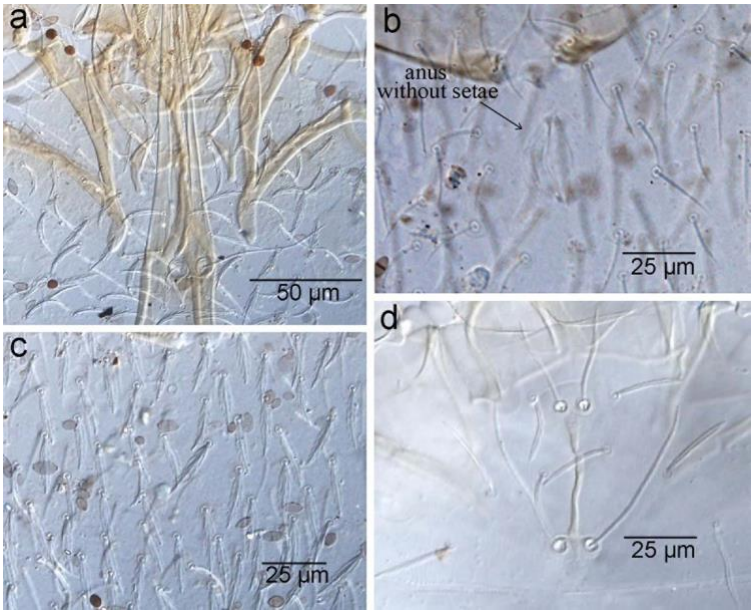

Figure 3. *Abrolophus strojnyi* Gabryś, 1992, male, photomicrographs a) crista metopica, b) anus, c) dorsal setae; *Balaustium murorum* (Hermann, 1804), larva, photomicrograph d) scutum.

Moldoustium haitlingeri Noei, Saboori & Šundić, 2013

Material examined. Bayburt, Aygır lake 40°39'N, 40°23'E, 2850 m a.s.l. (moist moss) 22 June 2013, larva.

Remarks (Figures 4 a-c). The genus *Moldoustium*, only known from the larval stage, is represented by two species: *Moldoustium baltiensis* Haitlinger, 2008 and *Moldoustium haitlingeri* Noei, Saboori & Šundić, 2013. The absence of seta on palp trochanter of its members supports this taxon to remain at the genus level (Fuentes Quintero et al., 2014). Noei et al. (2013) stated coxae I of this species have three setae. However, the examination of Iranian and Turkish samples reveals that the coxa I of *M*. *haitlingeri* bears two setae instead of three (a third seta is present between the coxal plates I and II). This species, distributed in Iran and Montenegro (Noei et al., 2013), has been recorded for the first time from Türkiye.

Erythraeus (Erythraeus) cinereus (Dugès, 1834)

Material examined. Erzincan, Refahiye, 39°50'N, 38°49'E, 1850 m a.s.l. (humidity litter under the *Pinus* sp. (Pinaceae)) 15 October 2021, ♂; 39°47'N, 38°52'E, 1580 m a.s.l. (low humidity litter under the *Populus* sp.), 18 June 2022, ♀.

Remarks (Figures 4 d-e). Stålstedt et al. (2016) redescribed *Erythraeus* (*Erythraeus*) *cinereus* and provided neotype designation of this species. This species, distributed in Western Palaearctic (Stålstedt et al., 2016), has been recorded for the first time from Türkiye.

Erythraeus (Zaracarus) rupestris (L., 1758)

Material examined. Erzincan, Refahiye 39°42'N, 39°35'E, 1100 m a.s.l. (low humidity litter under the *Populus* sp.), 28 May 2022, larva.

Remarks (Figure 4 f). Karakurt et al. (2022) provided the first correlation between larval *Erythraeus* (*Zaracarus*) *rupestris* and its postlarval instars by laboratory rearing. This species, distributed in Western Palaearctic (Mąkol & Wohltmann, 2012; Karakurt et al., 2022), has been recorded for the first time from Türkiye.

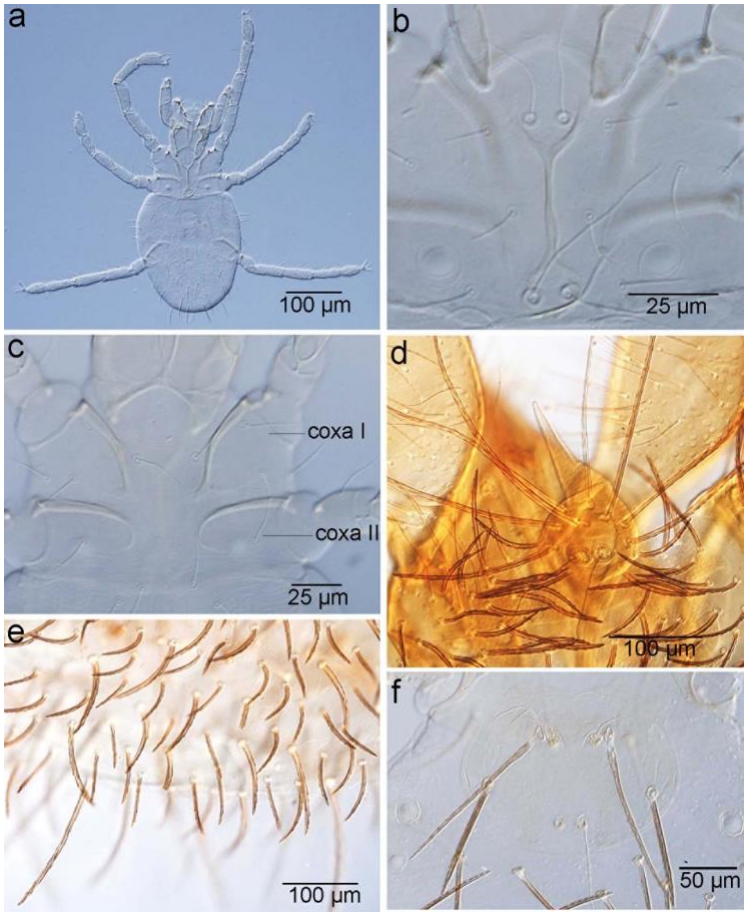

Figure 4. *Moldoustium haitlingeri* Noei, Saboori & Šundić, 2013, larva, photomicrographs a) general view, b) scutum, c) coxae I-II; *Erythraeus (Erythraeus) cinereus* (Dugès, 1834), male, photomicrographs d) anterior sensillary area, e) dorsal setae; *Erythraeus* (*Zaracarus*) *rupestris* (L., 1758), larva, photomicrograph f) scutum.

Smarididae

Fessonia papillosa (Hermann, 1804)

Material examined. Erzincan, Refahiye, 39°47'N, 38°52'E, 1580 m a.s.l. (low humidity litter under the *Populus* sp.), 18 June 2022, ♀.

Remarks (Figures 5 a-c). Wohltmann (2010) provided the first correlation between larval *Fessonia papillosa* and its postlarval instars by laboratory rearing. This species, distributed in Europe (Croatia, France, Germany, Greece, Hungary, Italy) and Iran (Beron, 2008; Wohltmann, 2010; Noei et al., 2013), has been recorded for the first time from Türkiye.

Smaris squamata (Hermann, 1804)

Material examined. Bayburt, 40°24'N, 40°07'E, 2014 m a.s.l. (low humidity litter under the *Astragalus* sp.), 05 April 2014, ♀; 40°29'N, 40°15'E, 1848 m a.s.l. (moist moss and grassland) 09 May 2015, ♀.

Remarks (Figures 5 d-f). This species, distributed in Europe (Algeria, Bulgaria, France, Germany, Greece, Italy, Monaco, Romania, Spain) (Mąkol & Wohltmann, 2012), has been recorded for the first time from Türkiye.

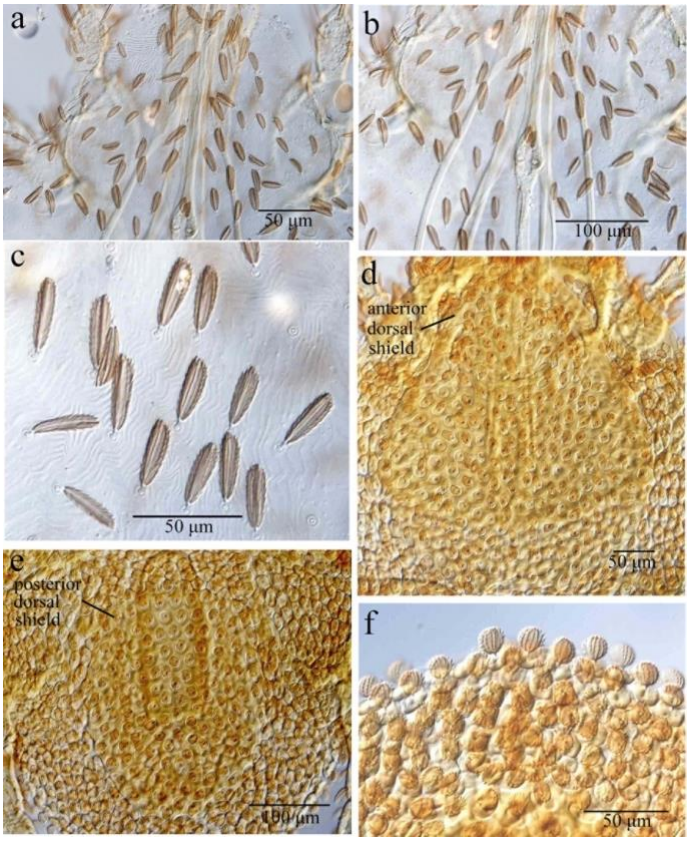

Figure 5. *Fessonia papillosa* (Hermann, 1804), adult, photomicrographs a) anterior part of crista, b) anterior sensillary area, c) dorsal setae; *Smaris squamata* (Hermann, 1804), female, photomicrographs d) anterior dorsal shield, e) posterior dorsal shield, f) dorsal setae.

Conclusions

Members of Erythraeoidea are known to adapt to usually xeric environments (Wohltmann, 2000). Likewise, the habitats of the species recorded in the present study have xeric and meso-xeric conditions. The superfamily of Erythraeoidea is distributed worldwide except for Antarctica (Mąkol & Wohltmann, 2012, 2013). The number of recorded erythraeoid mites has reached 45 in Türkiye (see Table 1) with the present paper.

Table 1. List of erythraeoid mites recorded from Türkiye (After Sevsay, 2017)

Taxa	References
Erythraeidae Robineau-Desvoidy, 1828	
Abrolophinae Witte, 1995	
Abrolophus Berlese, 1891	
Abrolophus artemisiae (Schrank, 1803)	Present paper
Abrolophus miniatus (Hermann, 1804)	Present paper
Abrolophus quisquiliarus (Hermann, 1804)	Present paper
Abrolophus rhopalicus (Koch, 1837)	Present paper
Abrolophus silesiacus (Haitlinger, 1986)	Haitlinger (2010) (as *Hauptmannia amilberti*); synonymized by Haitlinger & Łupicki (2015)

Table 1. Continued

Taxa	References
Abrolophus strojnyi Gabryś, 1992	Present paper
Abrolophus viburnicolus (Fain & Çobanoğlu, 1998)	Fain & Çobanoğlu (1998) (as *Hauptmannia viburnicola*); synonymized by Mąkol & Wohltmann (2012)
Abrolophus viticolus (Fain & Çobanoğlu, 1998)	Fain & Çobanoğlu (1998) (as *Hauptmannia viticola*); synonymized by Mąkol & Wohltmann (2012)
Marantelophus Haitlinger, 2011	
Marantelophus rudaensis (Haitlinger, 1986)	Fain & Çobanoğlu (1998) (as *Hauptmannia viticola*); Haitlinger (2000) (as *Rudaemannia rudaensis*); Mąkol & Wohltmann (2012) (as *Abrolophus rudaensis*); synonymized by Haitlinger & Šundić (2014) Note: *Hauptmannia viticola* was synonymized by Haitlinger (2000) with *Rudaemannia rudaensis* without any evidence. Because of this uncertainty, Mąkol & Wohltmann (2012) listed both species separately.
Marantelophus emanueli (Haitlinger, 2010)	Haitlinger (2010) (as *Grandjeanella emanueli*); Mąkol & Wohltmann (2012) (as *Grandjeanella emanueli*); synonymized by Kamran & Altawi (2015)
Marantelophus multisetosus (Zhang & Goldarazena, 1996)	Goldarazena et al. (2000) (as *Grandjeanella multisetosa*); synonymized by Haitlinger (2011); Mąkol & Wohltmann (2012)
Nagoricanella Haitlinger, 2009	
Nagoricanella bella (Zhang, 1996)	Saboori & Çobanoğlu (2010) (as *Grandjeanella bella*); Mąkol & Wohltmann (2012) (as *Marantelophus bella*); synonymized by Saboori et al. (2016); Maral (2021) (as *Grandjeanella bella*)
Balaustiinae Grandjean, 1947	
Balaustium von Heyden, 1826	
Balaustium akramii Noei, 2017	Noei et al. (2017)
Balaustium izmirensis Noei & Ersin, 2019	Noei et al. (2019)
Balaustium murorum (Hermann, 1804)	Present paper
Bursaustium Haitlinger, 2000	
Bursaustium gaspari Haitlinger, 2000	Haitlinger (2000); Mąkol & Wohltmann (2012)
Moldoustium Haitlinger, 2008	
Moldoustium haitlingeri Noei, Saboori & Šundić, 2013	Present paper
Callidosomatinae Southcott, 1957	
Charletonia Oudemans, 1910	
Charletonia cardinalis (Koch, 1837)	Haitlinger (2000); Beron (2008); Mąkol & Wohltmann (2012)
Charletonia cilissa (Cooreman, 1955)	Cooreman (1955) (as *Cavannea cilissa*); synonymized by Beron (2008); Mąkol & Wohltmann (2012)
Charletonia krendowskyi (Feider, 1954)	Elverici et al. (2022)
Erythraeinae Robineau-Desvoidy, 1828	
Curteria Southcott, 1961	
Curteria duzgunesae (Saboori, Çobanoğlu & Bayram, 2007)	Saboori et al. (2007) (as *Zhangiella duzgunesae*); synonymized by Saboori et al. (2009); Mąkol & Wohltmann (2012)
Curteria curticristata (Willmann, 1951)	Karakurt (2021)

Table 1. Continued

Taxa	References
Eatoniana Cambridge, 1898	
Eatoniana plumipes (Koch, 1856)	Mąkol & Sevsay (2015)
Erythraeus Latreille, 1806	
Erythraeus (***Erythraeus*****)** Latreille, 1806	
Erythraeus (*Erythraeus*) *adanaensis* Saboori & Çobanoğlu, 2010	Saboori & Çobanoğlu (2010); Mąkol & Wohltmann (2012)
Erythraeus (*Erythraeus*) *ankaraicus* Saboori, Çobanoğlu & Bayram, 2004	Saboori, Çobanoğolu, et al. (2004); Bayram & Çobanoğlu (2005); Gencsoylu (2007); Mąkol & Wohltmann (2012); Oner et al. (2021)
Erythraeus (*Erythraeus*) *elmalicus* Haitlinger, 2010	Haitlinger (2010); Mąkol & Wohltmann (2012)
Erythraeus (*Erythraeus*) *hilariae* Haitlinger, 2010	Haitlinger (2010); Mąkol & Wohltmann (2012)
Erythraeus (*Erythraeus*) *kresnensis* Beron, 1982	Haitlinger (2000); Mąkol & Wohltmann (2012); Haitlinger (2016)
Erythraeus (*Erythraeus*) *cinereus* (Dugès, 1834)	Present paper
Erythraeus (*Erythraeus*) *phalangoides* (De Geer, 1778)	Oner et al. (2021)
Erythraeus (*Erythraeus*) *uhadi* Kamran & Alatawi, 2014	Oner et al. (2021)
Erythraeus (*Erythraeus*) *sifi* Haitlinger, 2000	Haitlinger (2000); Mąkol & Wohltmann (2012)
Erythraeus (***Zaracarus*****)** Southcott, 1995	
Erythraeus (*Zaracarus*) *aydinicus* Saboori, Çakmak & Nouri-Gonbalani, 2004	Saboori et al. (2004); Mąkol & Wohltmann (2012)
Erythraeus (*Zaracarus*) *budapestensis* Fain & Ripka, 1998	Haitlinger (2010); Mąkol & Wohltmann (2012); Karakurt et al. (2022)
Erythraeus (*Zaracarus*) *coleopterus* Mortazavi, Hajiqanbar & Saboori, 2012	Oner et al. (2021)
Erythraeus (*Zaracarus*) *kurdistaniensis* Khanjani & Ueckermann, 2005	Oner et al. (2021)
Erythraeus (*Zaracarus*) *passidonicus* Haitlinger, 2006	Haitlinger (2010); Mąkol & Wohltmann (2012)
Erythraeus (*Zaracarus*) *rupestris* (L., 1758)	Present paper
Leptinae Billberg, 1820	
Leptus Latreille, 1796	
Leptus (***Leptus*****)** Latreille, 1796	
Leptus (Leptus) *esmailii* Saboori & Ostovan, 2000	Pamuk & Sevsay (2020)
Leptus (Leptus) *molochinus* (Koch, 1837)	Karakurt & Sevsay (2021)
Leptus (Leptus) *rosellae* Haitlinger, 1999	Haitlinger (1999); Mąkol & Wohltmann (2012)
Smarididae Vitzthum, 1929	
Hirstiosomatinae Southcott, 1946	
Hirstiosoma Womersley, 1934	
Hirstiosoma latreillei (Grandjean, 1947)	Karakurt & Sevsay (2021) (as *Hirstiosoma ampulligera*)
Hirstiosoma ampulligera (Berlese, 1887)	Karakurt (2022)

Table 1. Continued

Taxa	References
Smaridinae Vitzthum, 1929	
Fessonia von Heyden, 1826	
Fessonia papillosa (Hermann, 1804)	Present paper
Smaris Latreille, 1796	
Smaris squamata (Hermann, 1804)	Present paper

Considering the number of recorded species from Türkiye for this taxon, it can be expressed that the potential of species or records of erythraeoid mites in this country is high. Future systematic or taxonomic studies will contribute to the evaluation of this potential.

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Original article (Orijinal araştırma)

Two new species of *Hilara* Meigen, 1822 (Diptera: Empididae) from Mount Nemrut (Bitlis, Türkiye)

Nemrut Dağı'ndan (Bitlis, Türkiye) iki yeni *Hilara* Meigen, 1822 (Diptera: Empididae) türü

Mustafa Cemal ÇİFTÇİ[1*]

Abstract

Hilara Meigen, 1822 (Diptera: Empididae) is a very difficult genus to identify in the Empididae, however, several researchers have divided the genus *Hilara* into fourteen species groups for convenience. The current study identified two new species of *Hilara* Meigen, 1822. The materials used in this study were collected in the vicinity of Nemrut Mountain Crater Lake from Bitlis Province in May 2021 by using a collection net. *Hilara nemrutica* spec. nov. (*Hilara maura*-group) and *Hilara derenae* spec. nov. (*Hilara interstincta*-group) from Bitlis province, Türkiye, are described, male genitalia and forelegs are illustrated, and distinguished from closely related congeners.

Keywords: Bitlis, Empididae, fauna, *Hilara*, new species

Öz

Hilara Meigen, 1822 (Diptera: Empididae) cinsi Empididae familyasındaki tür teşhisi oldukça zor bir cinstir, buna rağmen birkaç araştırmacı *Hilara* cinsini sistematik olarak kolaylık sağlaması için on dört tür grubuna bölmüşlerdir. Bu çalışmada kullanılan örnekler Bitlis İlinde bulunan Nemrut Dağı Krater gölü çevresinden Mayıs 2021'de, toplama ağı kullanılarak toplanmıştır. *Hilara nemrutica* spec. nov. (*Hilara maura*-grup) ve *Hilara derenae* spec. nov. (*Hilara interstincta*-grup) Türkiye'nin Bitlis ilinden tanımlanmış, erkek genitalleri ve ön bacakların çizimleri yapılmış ve yakından ilişkili akraba türler ile ayrımları verilmiştir.

Anahtar sözcükler: Bitlis, Empididae, fauna, *Hilara*, yeni türler

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Introduction

Hilara Meigen, 1822 (Diptera: Empididae) is a rather difficult genus of Empididae to identify, with very similar general appearances of species lacking distinctive characteristics (Chvála, 2005). Despite this difficulty, several researchers have tried to classify the genus *Hilara* into species groups and currently fourteen morphologically distinct species groups are recognized (Collin, 1961; Chvála, 2005, 2008; Chvála & Merz, 2009).

In this study, one new species from the *Hilara maura*-group and one new species from the *Hilara interstincta*-group were described. The *Hilara maura*-group was initially identified by Collin (1961) with eight species and revised later with new species added by few researchers and all together there were twenty-one species known from the Middle East and Europe (Chvála, 1996, 2008; Çiftçi et al., 2012, 2020; Kustov et al., 2013). Chvála (1996) revised the European *H. maura*-group and placed the species into four complexes: maura complex, clypeata complex, nitidula-femorella complex and media complex.

The *Hilara interstincta*-group is defined by Chvála (2005), with eight species from Europe and characterized by a large, robust and strong bristled body. With later revisions by Chvála (2008) and Chvála & Merz (2009), this species group now includes twenty-four species from Europe and the Middle East. With additional studies by Çiftçi & Hasbenli (2011) and Çiftçi et al. (2020), two new species have been added to the group and the final number of this species-group has reached twenty-six.

These two species groups are represented by very few species from Türkiye. Only nine species from these two groups are known to be in Türkiye, including *Hilara elifae* Çiftçi & D. Çiftçi, 2020, *Hilara freidbergi* Chvála, 2008, *Hilara fusitibia* Strobl, 1899, *Hilara hasankoci* Çiftçi & Hasbenli, 2011, *Hilara megalochira* Collin, 1937, and *Hilara turcica* Chvála, 2008 from the *H. interstincta*-group, and *Hilara metinaktasi* Çiftçi, Hasbenli & Özgül, 2012, *Hilara barlasi* Çiftçi, Hasbenli & Özgül, 2012 and *Hilara hasbenlii* Çiftçi, 2020 within the *H. clypeata* complex from the *H. maura*-group (Chvála, 2008; Çiftçi & Hasbenli, 2011; Çiftçi et al., 2012, 2020; Çiftçi, 2021).

Materials and Methods

In this study, forty-four male and seventeen female specimens were used, and all the specimens were captured by collecting nets. The specimens were collected in the vicinity of Nemrut Crater Lake in Bitlis province in May 2021.

The morphological studies were conducted using Nikon SMZ445 and Nikon SMZ800N stereo microscopes. All the figures were drawn by using Nikon SMZ800N microscope with drawing tube attachment. For the morphological terms used in the current study, the morphological nomenclature by the McAlpine (1981), Stuckenberg (1999) and Sinclair (2000) was followed, with a few modifications.

The material used in this study is deposited in the author's private collection.

Results

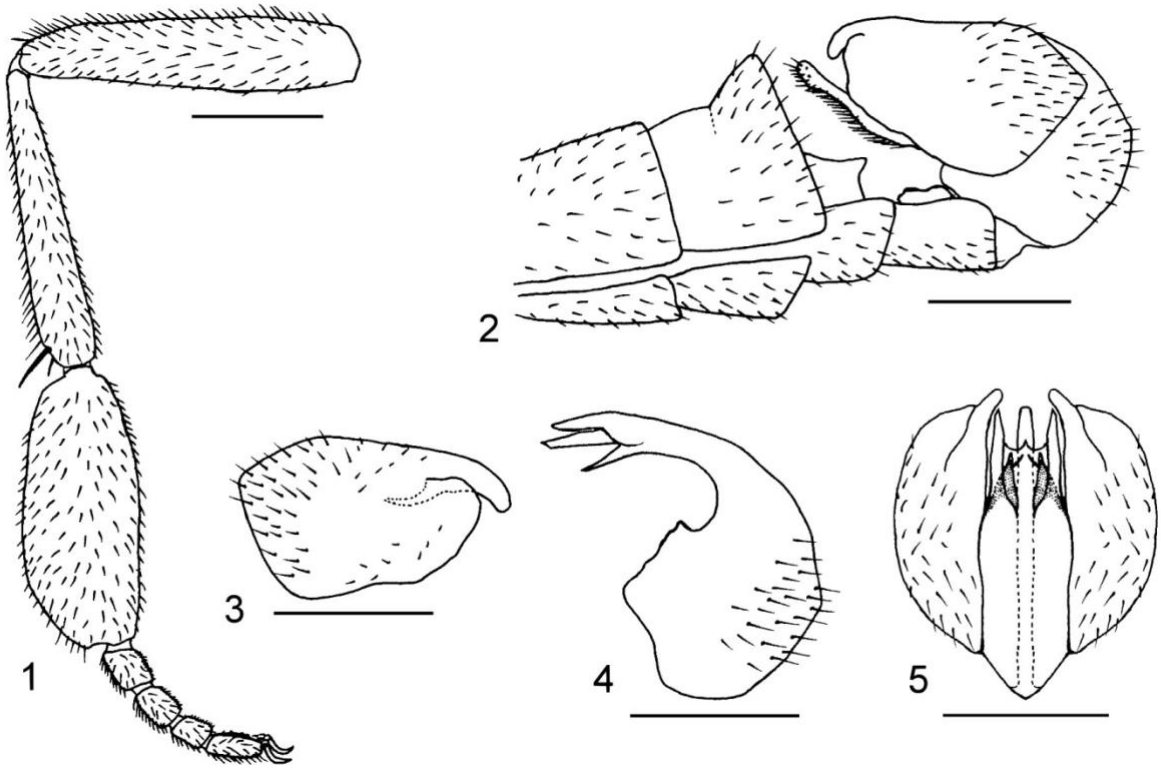
***Hilara nemrutica* spec. nov. (Figures 1-5)**

Type material. Holotype ♂. Türkiye. Bitlis: Tatvan, Nemrut Mountain, Nemrut crater, 38°38'N, 42°14'E, 2300 m, 19.V.2021, leg. M.C. Çiftçi. Paratypes. 36 ♂♂, 17 ♀♀, same data as holotype.

Etymology. The name of the new species refers to its type locality, Nemrut Mountain.

Diagnosis. Large species, body length between 4,5 and 5 mm, thorax and abdomen subshining black, both with slightly grey dusting. Scutum brownish dusting with slightly visible three black stripes from anterior view. Prothoracic collar without a bristle on sides, prothoracic sensorial pit without hairs, acrostichal bristles four-serial, dorsocentral bristles two- to three-serial, all black and very short. Legs black, scarcely

subshining, “knees” (base of tibiae and distal end of femora) faintly yellowish. Fore basitarsus in male thickened and very long, hind femur and female hind tibia slender, abdominal hairs yellow.



Figures 1-5. *Hilara nemrutica* spec. nov. 1. Fore leg; 2. Postabdomen; 3. Epandrial lamella; 4. Hypandrium; 5. Tip of hypandrium and epandrial lamella in dorsal view. Scale: 0,3 mm.

Description. Male. Head black, occiput and frons almost velvety, very small part of frons above antennae with grey dusting, face grey dusting and wider than frons. Ocellar bristles as long as postpedicel without style and fine, frontal bristles finer and shorter, slightly longer than antennal style. Occipital bristly hairs black and shorter than antennal style on the upper half of head, longer, finer and yellowish white on the lower half. Palpus black with grey dusting, ventrally with dense brownish hairs and two longer bristly hairs at tip. Labrum almost as long as half-length of head.

Thorax subshining black with slightly grey dusting. Lower half of pleura densely grey dusting as all coxae. Scutum brownish dusting with slightly visible three black stripes from anterior view. Scutum and scutellum subshining and slightly brownish dusting from dorsal and posterior view and stripes invisible. All thoracic hairs and bristly hairs black, only humeri with finer yellowish hairs, humeral bristle absent. Acrostichal and dorsocentral bristles equally long, as long as pedicel, acrostichal bristles four-serial, dorsocentral bristles two- to three-serial and ends with three pairs of long and fine bristles. Bristles on scutum and scutellum short and fine, only two notopleurals, one short supra-alar, one longer postalar and four to six scutellar bristles present, posthumeral and intrahumeral bristles absent. Prothoracic collar without a bristle on sides, only with short yellow hairs nearly as long as antennal style. Proepisternum and sides of prosternum with yellow short hairs. Prothoracic spiracle black and prothoracic sensorial pit without hairs.

Wings slightly brownish, veins black, pterostigma long and dark brown. Costal bristle short, nearly as long as antennal style. Radial fork normally shapes, not acute. Squama black with pale fringes, halter completely black.

Legs subshining black scarcely grey dusting, “knees” (base of tibiae and distal end of femora) faintly yellowish especially on fore legs. All coxae densely grey dusting as lower part of pleura. All hairs and bristles on legs mostly black, anteriorly tip of fore coxa and laterally mid and hind coxae with black bristles, other hairs on all coxae fine and yellow. All femora slender, only with short hairs. All tibiae dorsally with bristly hairs as long as depth of tibiae, ventrally with densely very short hairs, fore tibia posteroventrally with longer and finer hairs on apical half, no distinct bristles on all tibiae only with short apical circlet of bristles. All tarsomeres with short hairs, fore basitarsus swollen (Figure 1), very long, nearly as long as fore tibia.

Abdomen subshining black with slightly grey dusting. Abdominal hairs short and yellow, first two segments and sides of all terga with longer yellow hairs. Hind marginal bristles on first five terga absent, tergum six with black short and fine hind marginal bristles as long as antennal style. Genitalia large (Figures 2-5), epandrial lamella also enlarged, convex and with finger-like process, tip of hypandrium with two pairs of horn-shaped projections, one pair above, one pair below and the pair above is longer than the pair below.

Body length: 4.3-5.2 mm, wing length: 4.8-5.4 mm.

Female. They are quite similar to males except for the sexual differences. General appearance almost the same, only the pleura completely grey dusting. All hairs and bristly hairs finer and shorter than in male. Legs slender, apical circlet of bristles on all tibiae shorter, hind tibia slender and simple, fore basitarsus not thickened and half the length of the fore tibia. Abdomen with yellow hairs as in males, but shorter, hind marginal bristles absent.

Body length: 4.5-5.5 mm, wing length: 4.8-5.7 mm.

Remarks: *Hilara nemrutica* spec. nov. is a species of *Hilara maura*-group and assigned to the *Hilara clypeata* complex by its simple and slender hind femur in both sexes and unguarded prothoracic sensorial pit. *Hilara nemrutica* resembles North European species *Hilara submaura* Collin, 1927 and East Mediterranean species *Hilara alboclypeata* Chvála, 2008 and *Hilara barlasi* Çiftçi, Hasbenli & Özgül, 2012 by slightly brownish clouded wing, faintly yellowish “knees” (base of tibiae and distal end of femora) and yellowish abdominal hairs. The differential features of all species are given in Table 1.

Table 1. The differential features of *Hilara nemrutica* spec. nov. and its closely related congeners

Features	*Hilara submaura* Collin	*Hilara alboclypeata* Chvála	*Hilara barlasi* Çiftçi, Hasbenli & Özgül	*Hilara nemrutica* spec. nov.
Dusting of scutum in anterior view	grey dusting	grey dusting	brownish dusting	brownish dusting
Humeral bristle	inconspicuous	absent	short and fine	absent
Bristles on mid femur	with a pale bristle at base	without any bristles or bristly hairs, only with short hairs	anteriorly with bristly hair at base	without any bristle or bristly hairs
Dorsal bristly hairs on tibiae	present on hind tibia	absent	present on hind tibia	present on all tibiae
Epandrial lamella	with quite adjacent small and slender process	with rather short and apically narrowed process	oval and with thin, long, slightly bent upwards and hook-like process	enlarged, convex and with finger-like process
Tip of hypandrium	simple and apically slightly narrowed	long, slender and simple	slightly broad, apically narrowed and without any projection	with two pairs of horn-shaped projections

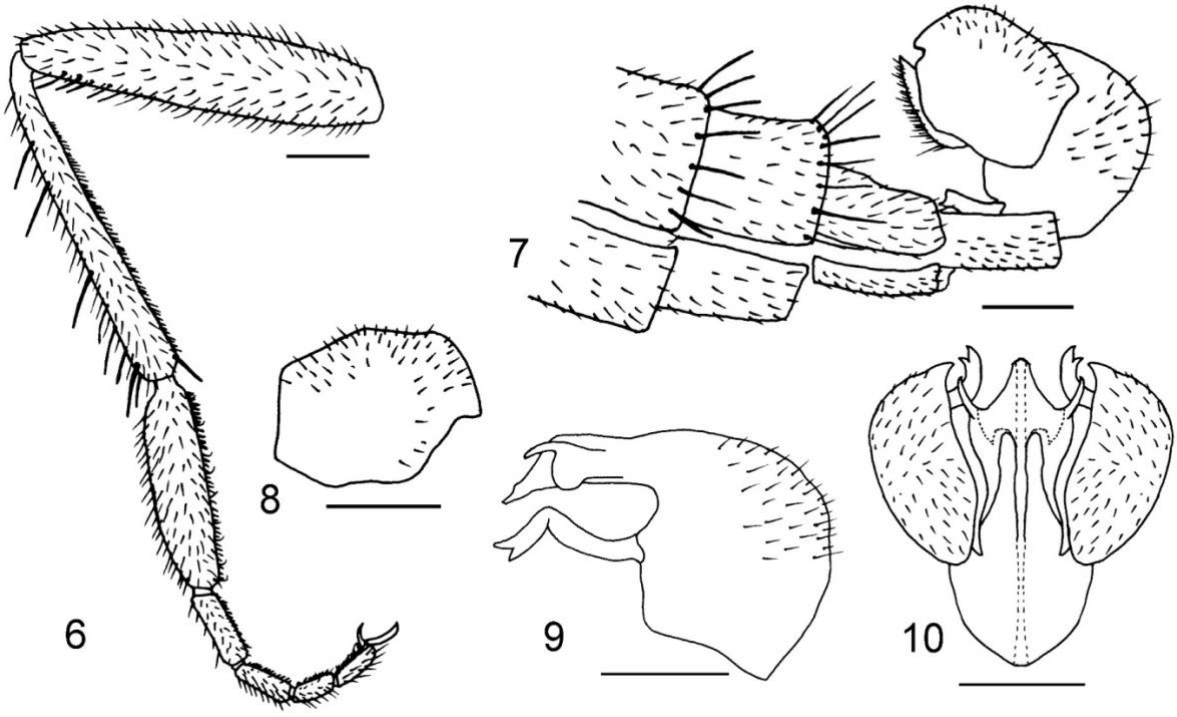
All three species and *H. nemrutica* with three blackish stripes on scutum at least visible in anterior view but *H. submaura* and *H. alboclypeata* with grey dusting scutum while *H. barlasi* and *H. nemrutica* with brownish dusting scutum in anterior view. *Hilara barlasi* closely resembles *H. nemrutica* by the general appearance but clearly distinguished by short and fine humeral bristle and mid femur anteriorly with bristly hair at base, in *H. nemrutica* humeral bristle absent and all femura without any bristle or bristly hairs. Moreover, the genital structures of the above-mentioned species, especially the epandrial lamella and tip of hypandrium, clearly distinguish them from *H. nemrutica*.

***Hilara derenae* spec. nov. (Figures 6-10)**

Type material. Holotype ♂. Türkiye. Bitlis: Tatvan, Nemrut Mountain, Nemrut crater, 38°38'N, 42°14'E, 2300 m, 19.V.2021, leg. M.C. Çiftçi. Paratypes. 6 ♂♂, same data as holotype.

Etymology. This species is dedicated to author's first daughter Meryem Deren Çiftçi who really like insects.

Diagnosis. Very large, black species with slightly grey dusting and brownish wings. Body length 7 mm. Body and legs strongly black bristled. Occiput dull black, labrum long, strong and shiny black Scutum with three black stripes slightly visible from laterodorsal and anterodorsal view, acrostichal bristles two-serial. All femora ventrally spinose and all tibiae ventrally with fine short hairs. Fore basitarsus slightly wider than tip of fore tibia.



Figures 6-10. *Hilara derenae* spec. nov. 6. Fore leg; 7. Postabdomen; 8. Epandrial lamella; 9. Hypandrium; 10. Tip of hypandrium and epandrial lamella in dorsal view. Scale: 0,3 mm.

Description. Male. Head dull black, frons black, face grey dusting and wider than frons. Occiput densely long black bristled longer than antennal style. Ocellar and frontal bristles same length, nearly as long as postpedicel with style. Antennae black, antennal style thick, as long as postpedicel. Palpus black with grey dusting, ventrally with dense black hairs and three strong very long bristles. Labrum shiny black, slightly shorter than height of head.

Thorax black with slightly grey dusting, in laterodorsal and anterodorsal view scutum with three slightly visible black stripes and clearly visible only in posterior view. Lower half of pleura with densely grey dusting as coxae and upper half of pleura and edge of scutum scarcely subshining. Hairs and bristles on thorax long and dense. Acrostichal bristles two-serial, nearly as long as antennal style, dorsocentral bristles one-serial, slightly longer than acrostichal bristles, longer on posterior part and ends with three pairs of rather long bristles. Large bristle on scutum and scutellum strong and nearly as long as postpedicel with style: one humeral, one fine intrahumeral, one posthumeral, three notopleurals, anterior one finer and shorter, anterior part of notopleural depression with fine short hairs, three supra-alars, middle one finer and shorter, one postalar and four pairs of scutellar bristles, outer pair fine. Prothoracic collar with short fine hairs between two long lateral bristles as long as postpedicel. Proepisternum with few short hairs and two or three bristle-like black hairs longer than postpedicel without style, sides of prosternum with short and fine black hairs.

Wings brownish, veins black and pterostigma slightly visible and dark. Two long costal bristles as long as postalar bristle, anal vein faint and not reaching wing margin. Squama black, fringes brownish, halter black with paler stem.

Legs black, only base of tibiae and distal end of femora yellow, also trochanters partly yellow, coxae densely grey dusting, femora grey dusting, tibiae and tarsomeres slightly grey dusting. Hairs and bristles on legs short and black. Fore femur ventrally on apical half with three or four bristle-like hairs, mid femur ventrally with two rows of bristles as long as depth of mid femur and at apical half becoming thicker and shorter and also mid femur anteriorly with two bristles at base. Hind femur ventrally with strong bristles as long as depth of hind femur and mid and hind femora anteriorly with one strong bristle at tip. Apical circlets of bristles on all tibiae distinct and strong and all tibiae ventrally with short and dense pubescence. Fore and hind tibiae dorsally with long bristles, longer than depth of fore and hind tibiae, mid tibia with only one dorsal bristle at base. All tarsal segments longer than depth of tarsal segments, all hairs fine and short. Fore basitarsus cylindrical, as long as two thirds of fore tibia and not much wider than tip of fore tibia (Figure 6).

Abdomen black, densely grey dusting. All hairs black, hairs on terga slightly longer than antennal style, hairs on sterna very short and pale. Hind marginal bristles long and distinct, fine on basal segments, very strong on tergum five and six. Genitalia large (Figures 7-10), slightly grey dusting. Epandrial lamella convex with no prominent process, only a small beak-shaped projection. Tip of hypandrium wide and very enlarged with a pair of horn-shaped projections on the sides and tip of postgonite bifurcated.

Body length: 6.6-7.3 mm, wing length: 6.9-7.5 mm.

Female. Unknown.

Remarks. *Hilara derenae* spec. nov. belongs to *Hilara interstincta* group by the following characters: very large, robust and strongly bristled body; numerous long hairs and strong long bristles on palpus; long and strong labrum; strong and long legs and apical circlets of strong bristles on all tibiae; hind femur anteriorly with a strong spine-like bristle at tip. *Hilara derenae* resembles *Hilara interstincta* (Fallén, 1816) and *Hilara lugubris* (Zetterstedt, 1819) by its large and strongly black bristled body, two row of ventral bristles on mid femur and dorsal bristles on fore tibia. The differential features of all species are given in Table 2. *Hilara derenae* is easily distinguished from *H. interstincta* by the yellowish base of tibiae and distal end of femora, two-serial acrostichal bristles, long humeral and intrahumeral bristles, and the different shape of the epandrial lamella and the tip of the hypandrium. In *H. interstincta*, base of tibiae and distal end of femora black, acrostichal bristles four-serial, humeral bristle fine, intrahumeral bristle short, epandrial lamella with finger-shaped process and tip of hypandrium simple. *Hilara derenae* much more similar to *H. lugubris* because of brownish wings, three striped scutum, ventrally strong bristled hind femur, yellowish

knees and apically bifurcated postgonite. *Hilara derenae* can be easily distinguished from *H. lugubris* by its two-serial acrostichal bristles, shape of epandrial lamella and enlarged tip of hypandrium.

Table 2. The differential features of *Hilara derenae* spec. nov. and its closely related congeners

Features	*Hilara lugubris* (Zetterstedt)	*Hilara interstincta* (Fallén)	*Hilara derenae* spec. nov.
Acrostichal bristles	two to three serials	four serials	two serial
Humeral and Intrahumeral bristles	small and fine	Humeral bristle fine, intrahumeral bristle shorter	long, nearly as long as postpedicel with style
Bristles on mid tibia	with a row of four-five posterodorsal spine-like bristles	apically with 2-3 anteroventral and posteroventral shorter bristles	with only one dorsal bristle at base
Color of base of tibiae and distal end of femora	yellow	black	yellow
Epandrial lamella	with short process	with finger-like process	with no prominent process
Tip of hypandrium	simple	simple	wide and very enlarged with a pair of horn-shaped projections on the sides

In this study, *Hilara nemrutica* spec. nov. from the *Hilara maura*-group and *Hilara derenae* spec. nov. from the *Hilara interstincta*-group were identified. Before this study, only three species from the *Hilara maura*-group were known from Türkiye, these species are *Hilara metinaktasi* Çiftçi, Hasbenli & Özgül, 2012 from Adana province, *Hilara barlasi* Çiftçi, Hasbenli & Özgül, 2012 from Uşak province and *Hilara hasbenlii* Çiftçi, 2020 from Sivas province (Çiftçi et al., 2012, 2020). With *Hilara nemrutica* spec. nov. identified from Bitlis province (Mount Nemrut), the number of species of this group in Türkiye has increased to four. Six species from the *Hilara interstincta*-group were previously known from Türkiye, these species are *Hilara elifae* Çiftçi & D. Çiftçi, 2020, from Ardahan province, *Hilara freidbergi* Chvála, 2008 and *Hilara fusitibia* Strobl, 1899 from Siirt province, *Hilara hasankoci* Çiftçi & Hasbenli, 2011 from Çanakkale and İstanbul provinces, *Hilara megalochira* Collin, 1937 from Adıyaman province, and *Hilara turcica* Chvála, 2008 from Hatay province (Chvála, 2008; Çiftçi, 2021; Çiftçi & Hasbenli, 2011; Çiftçi et al., 2020). With *Hilara derenae* spec. nov. identified from Bitlis province (Mount Nemrut), the number of species of this group in Türkiye has increased to seven. Thus, the number of known species of these two species groups from Türkiye has reached eleven.

Hilara metinaktasi, *H. barlasi* and *H. hasbenlii* from *H. maura*-group and *H. elifae*, *H. hasankoci* and *H. turcica* from *H. interstincta*-group are endemic to Türkiye. The Eastern Mediterranean species, *H. freidbergi* is known from Israel and Türkiye, while *H. megalochira* is known from Cyprus, Israel, Syria and Türkiye, and *H. fusitibia*, which has a distinctly Mediterranean distribution, is known from Spain, Morocco, Tunisia, Greece and Türkiye (Chvála, 2008; Çiftçi, 2021).

When we look at the endemic species in Türkiye, it is seen that their distribution is in one or two provinces.This is not because their distribution is local, but because of the scarcity of studies in Türkiye. With future studies, the distribution of these species may expand.

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Original article (Orijinal araştırma)

Response of *Rattus norvegicus* (Berkenhout, 1769) (Rodentia: Muridae) to entomopathogenic bacteria infected insect cadavers[1]

Rattus norvegicus (Berkenhout, 1769) (Rodentia: Muridae)'un entomopatojen bakteriler ile enfekte böcek kadavralarına tepkisinin belirlenmesi

Derya ULUĞ[2*]

Abstract

Xenorhabdus Thomas & Poinar (Enterobacterales: Morganellaceae) and *Photorhabdus* Thomas & Poinar (Enterobacterales: Morganellaceae) bacteria are mutualistically associated with *Steinernema* Travassos, 1927 (Rhabditida: Steinernematidae) and *Heterorhabditis* Poinar, 1976 (Rhabditida: Heterorhabditidae) nematodes, respectively, and are known to produce several secondary metabolites that protect nematode-killed insects from different competitors. One of these compounds called "the scavenger deterrent factor" (SDF) is known to deter different arthropod, bird, and fish species from feeding on insects killed by *Xenorhabdus* or *Photorhabdus* bacteria. The effects of SDF from five different *Xenorhabdus* and one *Photorhabdus* species against the Norway rat, *Rattus norvegicus* (Berkenhout, 1769) (Rodentia: Muridae) were investigated using either a one-choice or two-choice experimental design during 2019-2020 in Aydın Adnan Menderes University. Rats were given four-day-old bacteria-killed *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae) larvae and feeding behavior was observed and recorded. The results demonstrate that the Norway rat is deterred from feeding on insects killed by certain *Xenorhabdus* and *Photorhabdus* species and it is likely due to the distastefulness of these cadavers. Ecologically, the data suggest that insects killed by the entomopathogenic nematode/bacterium complex in nature may be protected from attack from insectivorous mammals, especially those that feed on soil-dwelling insects.

Keywords: *Photorhabdus*, scavenger deterrent factor, *Xenorhabdus*

Öz

Xenorhabdus Thomas & Poinar (Enterobacteriales: Morganellaceae) ve *Photorhabdus* Thomas & Poinar (Enterobacteriales: Morganellaceae) cinslerine ait bakteriler sırasıyla *Steinernema* Travassos, 1927 (Rhabditida: Steinernematidae) ve *Heterorhabditis* Poinar, 1976 (Rhabditida: Heterorhabditidae) cinslerine ait enotomopatojen nematodlarla mutualistik ilişki içerisindedirler. Bu bakterilerin nematodla enfekte kadavraları rekabetçi organizmalardan korumak amacıyla pek çok sekonder metabolit ürettiği bilinmektedir. Bu sekonder metabolitlerden bir tanesi olan yağmacı uzaklaştırıcı faktörün farklı eklembacaklı, kuş ve balık türlerine karşı uzaklaştırıcı etki gösterdiği bilinmektedir. 2019-2020 yılları arasında Aydın Adnan Menderes Üniversitesi'nde yürütülen bu çalışmada farklı *Xenorhabdus* ve *Photorhabdus* türleri ile enfekte kadavraların *Rattus norvegicus* (Berkenhout, 1769) (Rodentia: Muridae)'a karşı uzaklaştırıcı etkisi tek ya da ikili besin tercihi deneyleri ile test edilmiştir. Ratlara bakteriler ile enfekte edilmiş 4 günlük enfekte *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae) kadavraları verilmiş ve ratların beslenme davranışları gözlemlenerek kaydedilmiştir. Yapılan çalışma sonucunda bazı *Xenorhabdus* ve *Photorhabdus* türleri ile enfekte kadavraların ratlara karşı uzaklaştırıcı etki gösterdiği belirlenmiştir. Bu etkinin büyük olasılıkla larvalarda oluşan kötü tattan kaynaklandığı düşünülmektedir. Ekolojik olarak, veriler entomopatojen nematod/bakteri kompleksinin doğada böceklerle beslenen memelilerin saldırılarına karşı korunabileceğini göstermiştir.

Anahtar sözcükler: *Photorhabdus*, yağmacı uzaklaştırıcı faktör, *Xenorhabdus*

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Introduction

Entomoptahogenic bacteria in the genera *Xenorhabdus* Thomas & Poinar, 1979 (Enterobacterales: Morganellaceae) and *Photorhabdus* Thomas & Poinar, 1979 (Enterobacterales: Morganellaceae) are mutualistically asssociated with entomopathogenic nematodes (EPNs) in the genus *Steinernema* Travassos, 1927 (Rhabditida: Steinernematidae) and *Heterorhabditis* Poinar, 1976 (Rhabditida: Heterorhabditidae), respectively (Boemare, 2002; Gülcü et al., 2017). In their life cycle, the only free-living stage of these nematodes is the third-stage juvenile called the “infective juvenile” (IJ). The IJ carries bacterial cells in its intestine. When the IJs locate an insect host, they enter it through natural openings (mouth, anus, spiracles) and then penetrate into the insect hemocoel and release their mutualistic bacterium (Gaugler & Kaya, 1990). The bacterium multiplies and releases numerous toxins and enzymes which kill the host within 48 h (Bode, 2009). The IJs initiate their development and the nematodes mature by feeding on both the insect tissues and the multiplying mutualistic bacterium. When the food sources in the cadaver are depleted, the second stage nematodes reacquire their mutualistic bacterial cells and develop into the IJ stage. These new IJs exit the cadaver and enter the soil to search for new hosts (Hazır et al., 2003). The nematode’s developmental time depends on a number of factors such as host size, EPN species, and environmental factors (i.e., temperature and moisture). For example, a given EPN species may have 1-3 generations depending on its host size. Generally, the developmental time from the IJs entering the host until the new generation of IJs exits the insect cadavers usually takes 7-20 days at 20°C to 25°C. During this period, it is crucial that the host cadaver remains intact because the developing EPNs in the cadaver require the intact host for food and protection from the environmental soil conditions (e.g., desiccation, excessive moisture, other microorganisms) (Kaya & Stock, 1997). In addition, the cadavers with the developing nematodes are at risk of being consumed by different foraging omnivores and scavengers before IJ emergence is completed. Thus, *Xenorhabdus* spp. and *Photorhabdus* spp. not only help the EPNs by killing their insect hosts and serving as a food resource for them but also by protecting the cadavers from invasion by opportunistic bacterial and fungal competitors in addition to opportunistic omnivores and scavengers by producing one or more secondary deterrent metabolites (Çimen et al., 2021).

In terms of scavenging arthropods, numerous studies have shown that certain ant species did not feed on EPN-killed insects (Baur et al., 1998; Kaya et al., 1998; Zhou et al., 2002; Gülcü et al., 2018). Interestingly, these ants tended to consume less insect cadavers containing the heterorhabditid/*Photorhabdus* complex compared to cadavers containing the steinernematid/*Xenorhabdus* complex. This avoidance behavior was linked to the presence of a compound(s) produced by the mutualistic bacteria and was called the ‘ant deterrent factor(s)’ (ADF) (Zhou et al., 2002). This compound(s) was renamed as “scavenger deterrent factor” (SDF) as it also deters other scavengers such as wasps and crickets from feeding on cadavers with the mutualistic bacteria (Gülcü et al., 2012). Subsequently, different scavenging and omnivorous arthropod species (i.e., earwigs, cockroaches, beetles, and collembolans) have been reported to be deterred from feeding on insects killed by the EPN/bacterium complex (Uluğ et al., 2014; Mertz et al., 2015; Jones et al., 2016). Besides invertebrate animals, two avian species, *Erithacus rubecula* (L., 1758) (Passeriformes: Muscicapidae) and *Parus major* (L., 1758) (Passeriformes: Paridae) (Fenton et al., 2011; Jones et al., 2017) and two cyprinid fish species *Devario aequipinnatus* (McClelland, 1839) (Cypriniformes: Cyprinidae) and *Alburnoides bipunctatus* (Bloch, 1782) (Cypriniformes: Cyprinidae) (Raja et al., 2017) have also been deterred from feeding on insects killed by the EPN/bacterium complex or by the mutualistic bacterium alone. The objective of this study was to determine if SDF produced by different *Xenorhabdus* and *Photorhabdus* species will have a similar deterrent effect on mammal omnivores/scavengers as it did on birds and fishes. Accordingly, the feeding behavior of the Norway rat, *Rattus norvegicus* (Berkenhout, 1769) (Rodentia: Muridae) exposed to insect cadavers containing *Xenorhabdus* and *Photorhabdus* was tested.

Materials and Methods

Rats

Female *R. norvegicus* (Sprague-Dawley strain) were obtained from Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Laboratory Animals Production and Research Center and rats at ca. 6 months of age were used. These rats were housed in Eurostandard Type IV cages (61X43.5X21.5 cm) with wood shavings as bedding and were given ad libitum access to standard laboratory fodder and water and maintained at 12 h light/12 h dark and 24°C (Gomez et al., 2004).

Bacteria

The *Xenorhabdus* and *Photorhabdus* species were obtained from Helge B. Bode (Max Planck Institute, Marburg, Germany) and their associated EPN species are given in Table 1. They were kept in 20% glycerol at −80°C until they were subcultured and used in the experiments (Hazır et al., 2016).

Table 1. Species of *Xenorhabdus* and *Photorhabdus* used in experiments and their associated entomopathogenic nematode species

Bacteria Species	Associated Entomopathogenic Nematode Species
Xenorhabdus nematophila ATCC 19061 Thomas and Poinar, 1979 (Enterobacterales: Morganellaceae)	*Steinernema carpocapsae* (Weiser, 1955) (Rhabditida: Steinernematidae)
Xenorhabdus cabanillasii JM26-1 Tailliez et al., 2006 (Enterobacterales: Morganellaceae)	*Steinernema riobrave* Cabanillas, Poinar & Raulston, 1994 (Rhabditida: Steinernematidae)
Xenorhabdus kozodoii DSMZ 17907Tailliez et al., 2006 (Enterobacterales: Morganellaceae)	*Steinernema arenarium* (Artyukhovsky, 1967) (Rhabditida: Steinernematidae)
Xenorhabdus ehlersii DSMZ 16337 Lengyel et al., 2005 (Enterobacterales: Morganellaceae)	*Steinernema serratum* Shen & Wang, 1992 (Rhabditida: Steinernematidae)
Xenorhabdus ishibashii DSMZ 22670 Kuwata et al., 2013 (Enterobacterales: Morganellaceae)	*Steinernema aciari* Qiu et al., 2005 (Rhabditida: Steinernematidae)
Photorhabdus kayaii DSMZ 1519Tailliez et al., 2006 (Enterobacterales: Morganellaceae)	*Heterorhabditis bacteriophora* Poinar, 1976 (Rhabditida: Heterorhabditidae)

Each bacterium from the stock cultures was inoculated onto Luria-Bertani (LB) agar (Merck, Darmstadt-Germany) and incubated at 30°C for 24 h. Then, a loopful of each bacterial species was inoculated into their own flasks containing 10 ml LB broth and incubated at 30°C to obtain an overnight culture. *Bacillus thuringiensis* subsp. *kurstaki* Bulla et al. 1979 (Bacillales: Bacillaceae) (Btk) (Rebound Bioinsecticide® WP, Hektaş), a biological control agent for lepidopterous insect pests, was used as an entomopathogenic bacterial control to compare the effects of *Xenorhabdus* and *Photorhabdus* on the rats.

Obtaining bacteria-killed insects

Each overnight *Xenorhabdus* and *Photorhabdus* bacterial culture was centrifuged at 10000 rpm for 2 minutes. After removing the supernatant, each bacterial pellet was suspended in sterile 0.9% phosphate buffered saline (PBS) solution. A spectrophotometer (Shimadzu UV1280) was used to adjust the optical densities (OD) of each bacterial suspension to OD_{600}=1 (Çimen et al., 2021). A last instar larva of the wax moth *Galleria mellonella* (L., 1758) (Lepidoptera: Pyralidae) was injected with 10 µl of a given bacterial suspension using a 1 ml sterile syringe. The bacterial suspension was injected into the larva through the second or third proleg. All infected larvae were kept at room temperature (23-24°C) for 4 days before the dead insects were use in the experiments.

A Btk commercial formulation was suspended in distilled water at the rate of 1.5 g/L and mixed into the *G. mellonella* diet (Han & Ehlers, 2000) to obtain the Btk-killed larvae. Last instar larvae were added to the treated diet, kept at room temperature, and the 4-day-old Btk-killed larvae were removed from the diet and used in the experiment. Healthy last instar larvae were placed at -20°C for 4 h and these freeze-killed larvae were used as negative controls. Freeze-killed larvae were kept at room temperature for at least 1 h before the experiments.

Experimental design

The randomly chosen female rats were individually transferred into 48X26.5X21 cm cages and starved for 12 h before the experiments. In the one-choice experiments, only one larva (4-day-old larva killed by *Xenorhabdus* or *Photorhabdus* or Btk-infected or freeze-killed larva) was given to a rat. In the two-choice experiments, each rat was introduced simultaneously to 4-day-old larva killed by *Xenorhabdus* or *Photorhabdus* and Btk-infected or freeze-killed larva (Aydın Adnan Menderes University Ethics Committee Approval Number: 64583101/2019/026) (Table 2). *Galleria mellonella* larvae were not introduced to the rats prior to experiments. Each rat was given 10 minutes to interact with larvae and replaced with another rat if not. The rat's response and consumption of the insect cadaver were taped. The insect cadavers were recorded as "consumed" (if they were entirely consumed) or "not consumed" (if the rats took a bite but did not continue feeding on larvae or no bites). Each set of experiments had 5 replicates and conducted 3 times on different dates. For each replicate, the rats were only used once. All the experiments were conducted at Aydın Adnan Menderes University, Faculty of Veterinary Medicine, Laboratory Animals Production and Research Center.

Table 2. Experimental design for two-choice tests with the rat with five different *Xenorhabdus* and a *Photorhabdus* species-killed *Galleria mellonella* larvae and control groups (freeze-killed or *Bacillus thuringiensis kurstaki* (Btk)-killed larvae)*

Experiment Number	Two choice tests	
1	*X. nematophila*-killed	Freeze-killed
2	*X. nematophila*-killed	Btk-killed
3	*X. cabanillasii*-killed	Freeze-killed
4	*X. cabanillasii*-killed	Btk-killed
5	*X. kozodoii*-killed	Freeze-killed
6	*X. kozodoii*-killed	Btk-killed
7	*X.ishibashii*-killed	Freeze-killed
8	*X.ishibashii*-killed	Btk-killed
9	*X.ehlersii*-killed	Freeze-killed
10	*X.ehlersii*-killed	Btk-killed
11	*P.kayaii*-killed	Freeze-killed
12	*P.kayaii*-killed	Btk-killed

* The *Xenorhabdus* and *Photorhabdus*-killed larvae were injected with 10 µl of the respective bacterial species and kept at room temperature (23-24°C) for 4 days before exposed to the rat. The Btk-killed larvae were fed food treated with Btk and collected 4 days later. The freeze-killed larvae were held at -20ºC for 4 h and these freeze-killed larvae were kept at room temperature for at least 1 h before the experiments.

Statistical Analysis

The feeding behavior of the rats (consumed vs non-consumed) was analyzed with Chi Square Test of Independence to determine the role of scavenger deterrent factor ($\alpha = 0.05$). Chi Square Test was used to analyze the rat response in the two-choice experiments. (SPSS 22.0 IBM Corp., Chicago, IL, US).

Results and Discussion

In the one-choice experiments, each rat fully consumed freeze-killed, Btk-killed, *Xenorhabdus kozodoii*-, *Xenorhabdus ishibashii*- or *Xenorhabdus ehlersii*-killed larva but took a bite from *Xenorhabdus nematophila*-, *Xenorhabdus cabanillasii*- or *Photorhabdus kayaii*-killed larva and then stopped feeding on each of them (Table 3) (Supplementary videos 1 and 2). The statistical analysis showed that there was a significant difference in the response of the rats to different bacteria-infected larvae and control groups (X^2 = 40, p<.001, α = 0.05). In the two-choice experiments, when the rats were offered *X. nematophila*-, *X. cabanillasii*- or *P. kayaii*- larva and freeze-killed or Btk-killed larvae, they only consumed the freeze-killed and Btk-killed larvae (Figure 1, 2). On the other hand, the rats consumed all the 4-day-old *X. kozodoii*-, *X. ishibashii*- and *X. ehlersii*-killed larvae as well as the freeze-killed or Btk-killed larvae in the two-choice tests. There was a significant difference in the response of the rats to the different treatments offered in the two-choice experiments (X^2 = 36, p<.001, α = 0.05).

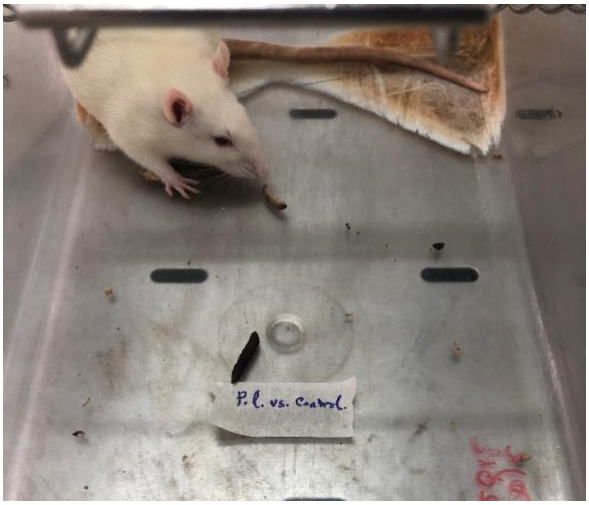

Figure 1. Rat feeding on a freeze-killed larva (control) in the two-choice experiments with *Photorhabdus kayaii*-killed larva.

Table 3. Consumption of *Xenorhabdus*- and *Photorhabdus*-killed *Galleria mellonella* larvae by *Rattus norvegicus* in one-choice experiments

Treatments	Consumed	Not Consumed
X. nematophila-killed		+
X. cabanillasii-killed		+
X. kozodoii-killed	+	
X. ishibashii-killed	+	
X. ehlersii-killed	+	
P. kayaii-killed		+
Freeze-killed	+	
Btk-killed	+	

The response of an omnivore mammalian species to SDF produced by *Xenorhabdus* spp. and *Photorhabdus* spp. was demonstrated for the first time with this study. On several occasions, the rats were observed to approach and attempt to feed on *X. nematophila*-, *X. cabanillasii*- or *P. kayaii*-killed larva, but upon taking a bite, the rats immediately rejected these larvae, whereas both freeze-killed and Btk-killed control groups and other tested *Xenorhabdus*-killed larvae were consumed. After taking a bite from the *X. nematophila*-, *X. cabanillasii*- or *P. kayaii*-killed larva, it was observed that the rats tried to clean their mouths (Supplementary video 2) which appeared to be a response to a distasteful substance.

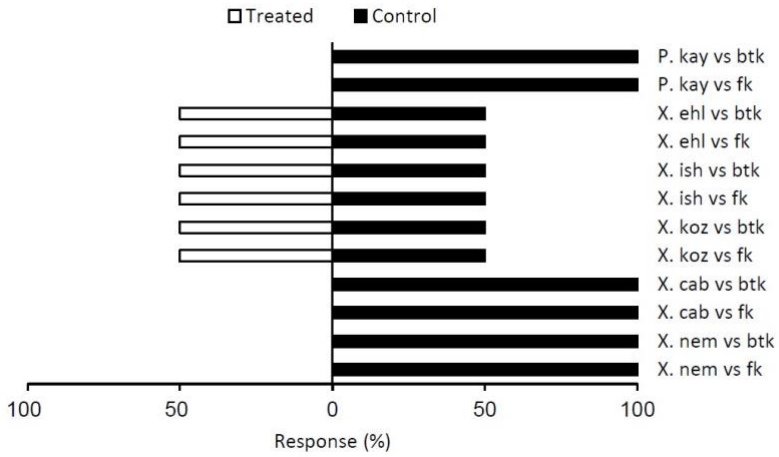

Figure 2. Response of rats in two choice experiments (P.kay=*P.kayaii*; X.ehl=*X.ehlersii*; X.ish=*X.ishibashii*; X.koz=*X.kozodoii*; X.cab=*X. cabanillasii*; X.nem=*X. nematophila*; btk= *Bacillus thuringiensis*-killed; fk= freeze-killed).

Multiple defense mechanisms or the combination of different mechanisms such as color and odor, increases the chances of survival of the EPN-bacterium complex in the insect cadavers. Previous studies with avian predators have suggested that color, especially with *Photorhabdus*-killed insects, and odor can both play a role in the protection of the *Heterorhabditis/Photorhabdus* killed insects at different stages of the infection against scavenger attacks (Fenton et al., 2011; Jones et al., 2017). *Photorhabdus*-killed larvae generally turn red 2 to 4 days after death, bioluminesce and produce an odor (Ffrench-Constant & Bowen, 2000). The color change in the dead larvae containing the *Photorhabdus* is most likely an indication of the presence of a distasteful chemical. Avian foragers usually rely on visual cues when they encounter a new diet. After their brief aversion (neophobia), in some cases, they completely reject this diet, which is called dietary conservatism (Marples et al., 1998, 1999). This behavior recently has also been shown to be present in some individuals of different fish species (Thomas et al., 2010; Richards, 2014).

Depending on the species, *Xenorhabdus*-killed insects generally turn ochre, brown or black (Hazır et al., 2022). There has been no study where color or odor plays a role in feeding deterrence of *Xenorhabdus*-killed insects by omnivores and scavengers. In this study, *Photorhabdus*- and *Xenorhabdus*-killed larvae were attacked almost every time (data not shown-personnel observation) indicating that the color change in *Xenorhabdus*- or *Photorhabdus*-killed larvae did not play a role in deterring the rats from feeding on the cadaver. In this experimental design, the control groups and bacteria-killed larvae were placed closely together in the cages, so it is highly unlikely that odor itself had a significant effect on selection by the rat to feed on a given larva. Based on the feeding behavior of rats, the main reason why the rats rejected the *P. kayaii*-, *X. nematophila*-, and *X. cabanillasii*-killed larvae appears to be due to the distastefulness of the cadavers (Supplementary video 1). The rats reject these cadavers only after taking one or two bites and exhibited cleaning behaviors of attempting to get rid of cadaver material from their mouths (Supplementary video 2).

Interestingly, not all EPN-associated bacteria had SDF activity on the rats. It is known that different *Xenorhabdus* and *Photorhabdus* species or even strains produce different secondary metabolites (Bode 2009), and these metabolites probably act differently against different scavengers. Recently, two volatile compounds (hexadecanal and 2-heptadecanone) isolated from *Steinernema feltiae* (Filipjev, 1934) (Rhabditida: Steinernematidae)-infected larvae were found to be highly deterrent to the ant species *Lasius niger* (L., 1758) (Hymenoptera: Formicidae) (Jaffuel et al., 2021). These two compounds were highly active when the ants were very close to the bait area (“touching”).

EPNs sold as biological control agents are usually mass produced in vivo or in vitro and the IJs are applied in an aqueous suspension for the control of insect pests. However, recent studies have showed that they can be also successfully applied as “infected cadavers” (i.e., larvae killed by an EPN species and the IJs allowed to remain in the cadaver) with a superior infectivity, persistence and pest control (Gulzar et al., 2020; Perez et al., 2003; Shapiro-Ilan et al., 1999, 2003). “Infected cadavers” protect the IJs from desiccation and UV inactivation. Ecologically, soil insects naturally infected with the EPN/bacterium complex are important in allowing the IJs to persist the soil. However, consumptive and destructive actions of ground foraging omnivores and scavengers can have a top-down impact on EPN populations in soil and can reduce the success of biological control applications as well as natural biological control of pest insects. It will be interesting to determine whether insectivorous mammals such as shrews, moles, raccoons, skunks, etc. that feed on soil insects are adversely affected by SDF and assess their impact on natural biological control of insect pests. In addition, future studies should endeavor to identify whether there is one or a complex of deterrent compound(s) that affects the feeding behavior of scavengers and omnivores.

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Original article (Orijinal araştırma)

A study on morphological variations of male *Helophorus* (*Helophorus*) *aquaticus* (L., 1758) (Coleoptera: Helophoridae) in Türkiye[1]

Türkiye'deki erkek *Helophorus* (*Helophorus*) *aquaticus* (L., 1758) (Coleoptera: Helophoridae)'un morfolojik varyasyonları üzerine bir araştırma

Ahmet POLAT[2*]

Abstract

The geographic structure of Türkiye is well-suited for determining species diversity and interspecific variation as Türkiye has many regions with different altitudes and ecological characteristics. A large number of specimens belonging to the Helophoridae family belonging to the order Coleoptera were collected from different regions of Turkey between 2007-2021, examined and kept as museum material. Among the museum materials evaluated in terms of variation, *Helophorus* (*Helophorus*) *aquaticus* (L., 1758) species were seen to be both more common and more diverse in each region. In this study, male specimens varying in color, size, pronotum, tarsi, and genital structure were selected among the samples collected from different regions and geographic locations within the same locality. A total of 17 different morphological variations were identified and listed by examining the pronotum, elytra, tarsi, and aedeagophore structures. The relationships of these variations with altitude have been evaluated. As a result of this study, it has been determined that variations of the same species can coexist in the same locality, as well as similar variations in localities in different geographical regions. In addition, it was determined that the size of the insects increased as the altitude increased.

Keywords: Altitude, Coleoptera, Helophoridae, *Helophorus aquaticus*, variation

Öz

Türkiye'nin coğrafi yapısı tür çeşitliliğini ve türler arası varyasyonu belirlemeye oldukça elverişlidir ve Türkiye'nin farklı rakımlara ve farklı ekolojik özelliklere sahip birçok bölgesi vardır. Coleoptera takımı Helophoridae familyasına ait çok sayıda örnek 2007-2021 yılları arasında Türkiye'nin farklı yörelerinden toplanmış, incelenmiş ve müze materyali olarak saklanmıştır. Varyasyon açısından değerlendirilen müze materyalleri arasında, *Helophorus* (*Helophorus*) *aquaticus* (L., 1758) türlerinin her bölgede hem daha yaygın hem de daha çeşitli olduğu görülmüştür. Bu çalışmada, farklı bölgelerden ve farklı coğrafi konumlardan toplanan ve aynı lokasyonu paylaşan erkek örnekler arasından farklı renk, boyut, pronotum, tarsi ve genital yapılara sahip bireyler seçilmiştir. Pronotum, elytra, tarsi ve aedeagophore yapıları incelenerek toplam 17 farklı morfolojik varyasyon tanımlanmış ve listelenmiştir. Bu varyasyonların rakım ile ilişkileri değerlendirilmiştir. Bu çalışma sonucunda, aynı türün varyasyonlarının aynı lokalitede bir arada bulunabileceği gibi, farklı coğrafi bölgelerdeki lokalitelerde de benzer varyasyonların olabileceği tespit edilmiştir. Ayrıca rakım arttıkça böceklerin boyutlarının da arttığı tespit edilmiştir.

Anahtar sözcükler: Rakım, kınkanatlılar, Helophoridae, *Helophorus aquaticus*, varyasyon

[1] This study was carried out using museum materials from Atatürk University, Faculty of Science, Department of Biology, Zoology Laboratory.

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Introduction

Türkiye is located in the heart of a continent, called as the 'world island', which is known as one of the most diverse and resource-rich region extending over Euroasia and Africa mass land (Mackinder, 2004) This region function like a bridge connecting Türkiye to the Mediterranean, Black Sea, Balkans, Caucasus, and the Middle East Türkiye is a peninsula surrounded by seas on three sides, and differs in climates due to the variations in altitude ranging from 0-5137 m. Different ecological features and climates have also led to an increase in species diversity.

Taxa that are crowded with several species are older and have more diversity (McPeek & Brown, 2007). Diversification rate differences can result from both intrinsic factors (e.g., key innovations) and extrinsic factors (e.g., habitat shifts), or ecological limits (density-dependence) on clade diversity (Rabosky, 2009, 2010). It has been determined that the variations seen in insects are related to the environment (Daly, 1985; Palmer, 1994; Cepeda-Pizarro et al., 1996, 2003; Krasnov et al., 1996; Williams, 2001). There are many morphological characters (size of the hind limbs, structure of the elytra, form of the subelytral space, etc.) that are considered indicative of differentiation of populations (Lestrel, 1997; Møller & Zamora-Munoz, 1997; Shiokawa & Iwahashi, 2000; Bonacci et al., 2006; Garnier et al., 2006; Talarico et al., 2007).

Despite the morphological differences found in some Coleoptera families, there is a correlation between the size of their body parts. The body size in animals of the same species is an integral feature that affects both their physiology and behavior (Chown & Gaston, 2010). On the other hand, studies conducted in recent years have shown that changes in altitude also cause changes in the size of insects.

Size differences in widely distributed taxonomic clades have attracted attention of many scientists. Bergmann (1847), who defined this subject first, stated that larger populations and species were found in colder environments, while smaller ones were found in warmer environments. This theorem is called the “Bergmann rule”. A similar theorem, called “Allen's rule”, was used by Allen (1877) who argued that because animals living in cold climates must conserve as much heat as possible, they must have developed relatively low surface area-to-volume ratios to minimize the surface area from which heat radiates. He also noted in his study that the limbs of the species changed accordingly.

Although it is an established ecological principle, empirical support for Allen's rule is weak. Support for Allen's rule stems mainly from studies on a single species, and studies on different species have alternative adaptations that run counter to the predictions of the Bergmann and Allen rules (Nudds & Oswald, 2007).

There are literatures that address the relationships between body size and community structure for arthropods, specifically Coleoptera. While variability of body size is recorded in geographic gradients in many insect species, direction of the relationship may be different. Some species have an increase in size in the latitude direction, while we see a decrease in that of others (Blanckenhorn & Demont, 2004). In the latitudinal slope, length of light period, air temperature and duration of the growing season tend to decrease due to regional, climatic and seasonal conditions. In particular, the temperature changes constantly depending on latitude and altitude.

In a study on *Teleogryllus emma* (Ohmachi & Matsuura, 1951) (Orthoptera: Gryllidae) populations, it was observed that the body size of the Emma field cricket, represented by head width, tended to be smaller from south to north and from low to high, reaching larger sizes in warm regions than in cold regions (Masaki, 1967). In another study on *Carabus dehaanii* subsp. *tosanus* (Nakane & Iga&Ueno, 1953) (Coleoptera: Carabidae) in Japan, it was determined that the body lengths of insects grown at high temperatures in the setup in the laboratory were greater than those grown at low temperature (Tsuchiya et al., 2012).

Although there are many studies parallel to the Bergman & Allen rules, there are many exceptions to both rules. Body size at the intraspecific level may increase, decrease, or not change significantly towards higher latitudes (Blanckenhorn & Demont, 2004; Shelomi, 2012). In addition, clinical body size variation in arthropods generally follows the opposite of the Bergmann's rule (Mousseau, 1997).

In another study conducted with the carabid beetles, it was determined that the sizes of these insects showed an adaptation contrary to the Bergmann's rule (Park, 1949). On the Japanese islands, the elytra length of *Phyllotreta striolata* (Fabricius, 1803) (Coleoptera: Chrysomelidae) has again been found to contradict this rule (Hukushima, 1960). The length and width of the elytra, pronotum and head were measured in the selected specimens of *Pterostichus montanus* (Motschulsky, 1844) (Coleoptera, Carabidae). Based on the results obtained, it was noted that the species living in the coastal region were the smallest, and the species living in the low mountains were the largest (Sukhodolskaya et al., 2021).

Perhaps many other examples can be found that contradict Bergmann's rule. For some species, it is possible that there are different factors other than temperature and latitude that can affect the dimensions. In a study with carabids, the larger *Pterostichus* and *Carabus* species are known to prefer less disturbed habitats (Blake et al., 1994). In addition, it has been determined that there is a relationship between body size and development time, and that development time causes variation in body size (Roff, 1980). In addition, it has been reported that availability of food, especially during the growth period, has an effect on size (Yom-Tov & Geffen, 2011; Brandmayr & Pizzolotto, 2016). The morphometric analyses (Novotny & Wison, 1997) performed on species which contain the Auchenorrhyncha group including foam beetles, revealed that there is a significant relationship between body size and Hemipter, which uses phloem and xylem sap as food. Larger beetles have also been reported to be more resistant to famine, drought and temperature extremes and winter (Kingsolver & Huey, 2008).

The colors of insects and the variations in these colors are quite remarkable. It has been observed that coloration of small insects is related to the ambient temperature, and the heat absorption in these animals can be affected by their coloration, since dark colored forms absorb solar radiation faster. In this respect, dark forms in many polymorphic species tend to be seen at a higher frequency in cold habitats than in open habitats that are directly exposed to the sun. Melanism due to thermal selection has been shown to be an adaptive value for Colias butterflies in the alpine cold habitats. Thermal melanism has been discussed as an important component of evolutionary adaptation in many species such as foam beetles, grasshoppers, and spiders (Yurtsever, et al., 2005). However, in the study of *Hologymnetis argenteola* (Bates, 1889) and *Hologymnetis cinerea* (Gory & Percheron, 1833) (Coleoptera: Scarabaeidae: Cetoniinae) species, the dorsal color and degree of punctuation of insects varied significantly with no apparent relationship to geography or altitude (Ratcliffe & Deloya, 1992).

In this 15-year-long study, it was determined that the Helophoridae family was denser than other species among the aquatic insects collected from different cities and different altitudes of Türkiye. We have determined that this family has a high adhesion ability at different altitudes. Among the analyzed samples, it was observed that *Helophorus* (*Helophorus*) *aquaticus* (L., 1758) showed both morphological variation and different sizes.

The current study discusses the effects of altitude on size and variations of *H. aquaticus* obtained from different regions of Türkiye and observed in localities with different ecological characteristics between 2007 and 2021.

Colors, body lengths, aedeagophore structures, and legs of the samples were photographed and length measurements were recorded. Comparisons of the measured parameters were evaluated according to the altitude at which the samples were collected. It was aimed to investigate whether the altitude affects color, height, and aedeagophore lengths. The results were evaluated separately for each parameter.

Materials and Methods

Helophorus aquaticus selected for the research has a very wide distribution in the world. They spread from sea level to the peaks of high mountains (Angus, 1988). They are usually found in stagnant fresh waters, small, shallow and muddy ponds, temporary puddles, shallow parts of standing waters and streams, and prefer shady waters on swampy ground. Normally in spring, they lay cocoons, which contain about 15 eggs, into the mud at the water's edge. The larvae do not emerge until the next spring (or early summer). Larval development takes about three weeks. Adults can usually be seen from spring to autumn (Hansen, 1987). It is known that they live in almost every region of Turkey. It has records from approximately 40 provinces (Polat et al., 2021).

This study was conducted to compare in terms of variation the male *H. aquacus* species belonging to the Helophoridae family collected from various regions of Turkey within 15 years and to examine whether altitude causes morphological changes in individuals belonging to the same species. The localities are lettered alphabetically, and the number of individuals, their coordinates, annual averages of temperature and altitudes of the provinces where they are gathered are presented in Table 1. The map of Turkey where the localities are marked is given in Figure 1.

The samples were collected from the shore of marshes, geological formations and lakes, through slow-flowing water, grassy areas where aquatic insects can live, and places where vegetative decay is high. Collected samples were taken to the laboratory after treatment with ethyl acetate.

Table.1. Localities where samples were collected

Sample	Number of samples	Locality	Annual average temperature (°C)	Altitude (m)
a	3♂♂	Ordu, Ünye: Çatalpınar, 41°06'06K 37°14'06D, 28.V.2007	14.5	41
b	3♂♂	Amasya, Merkez: Doğantepe, 40°34’37K 35°36’43D, 03.VI.2008	13.6	491
c	4♂♂	Tokat, Erbaa: İverönü, 40°36’30K 36°36’03D, 29.V.2007	12.5	531
d	4♂♂	Tokat, Yeşilyurt: 40°00’30K 36°11’59D, 01.VI.2007	12.5	1065
e	4♂♂	Amasya, Hamamözü: Yeniköy, 40°48’01K 35°09’00D, 03.VI.2008	13.6	1081
f	4♂♂	Kayseri, Develi: Karapınar, 38°24’11K 35°19’29D, 30.V.2009	10.7	1084
g	3♂♂	Kahramanmaraş, Elbistan: Kuşkayası, 38°18'22K 37°05'48D, 09.IX.2011	16.7	1155
h	4♂♂	Tokat, Zile: Yünlü, 40°23’14K 35°51’48D, 03.VI.2008	14.5	1212
i	4♂♂	Giresun, Dereli: Bektaş plateau, 40°37'08K 38°16'36D, 24.V.2007	14.6	1354
j	4♂♂	Kahramanmaraş, Göksun: Soğukpınar, 38°03'15K 36°34'37D, 26.VI.2012	16.7	1361
k	3♂♂	Kayseri, Yahyalı: Avlağı, 38°00’05K 35°32’41D, 25.VI.2010	10.7	1483
l	3♂♂	Kayseri, Pınarbaşı: 38°43’11K 36°23’00D, 25.VI.2011	10.7	1507
m	5♂♂	Erzurum, Erzurum Marshes 40°01'09"K 41°18'52"D, 1756m, 19.06.2017	5.7	1756
n	3♂♂	Van, Özalp: Çalımlı, 38°39'50K 43°47'55D, 1909m 2021	9.4	1909
o	3♂♂	Kayseri, Hisarcık: Erciyes Mountain 38°35’26K 35°30’18D, 25.V.2010	10.7	1972
p	4♂♂	Muş, Varto:Hamurpet Lake, 39°08'03"K 41°42'14"D, 21.06.2017	9.8	2169
q	3♂♂	Erzurum, Hınıs: Erzurum Geological formations, 39°24'04”K 41°26'08”D, 01.07.2017	5.7	2661

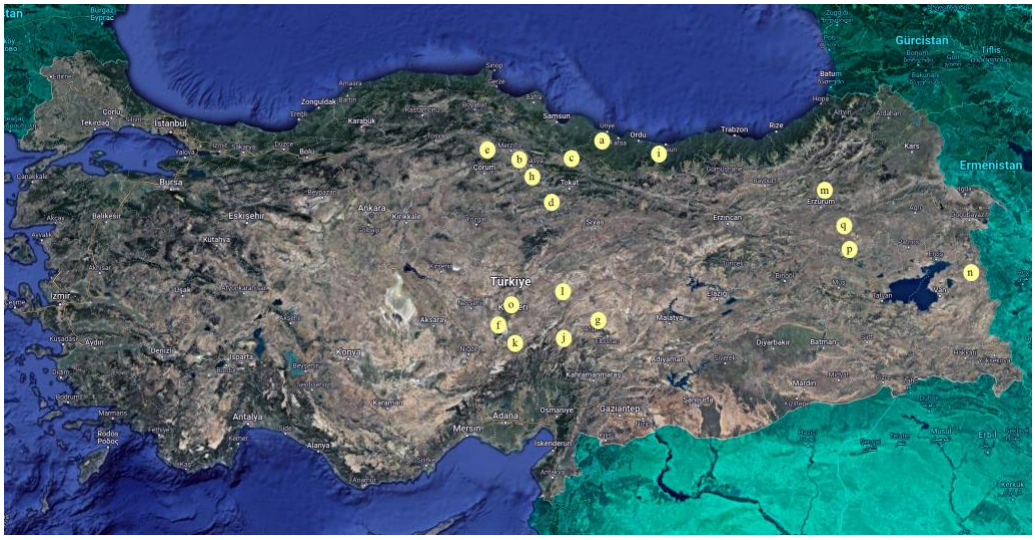

Figure 1. Türkiye map with localities where samples were collected.

Species were identified by examining the morphological characters of the samples brought to the laboratory. Male *H. aquaticus* species belonging to the Helophoridae family were selected among the identified species to determine their morphological variations.

Aedeagophores were soaked in 10% KOH solution for 1-2 hours to separate the muscle tissue around the chitin structure. They were then placed on a slide with a drop of glycerin. Measurements were made by drawing aedeagophore shapes on Nikon SMZ1500 stereomicroscope. The common and distinctive features of the species were photographed with the Leica DFC295 brand macroscope. +5 light setting and 40% iris setting were used in all photo shoots. Color samples of body parts were taken from photographs for each species using the 31x31 average scale in Adobe Photoshop CS6 program and color determinations were made by comparing them with the Pantone color library.

Body measurements were taken using a micrometric eyepiece. Body lengths and widths were recorded by measuring the longest and widest parts of the body and regional coloration conditions in the body were examined. The structures and dimensions of the pronotum were compared. The coloration of the legs and their dimensions compared to the body were evaluated. Paramere structures and dimensional differences were evaluated by comparing aedeagophore structures.

Statistical analysis

Statistical analyses of the study were performed with SPSS 20.0 (IBM Inc, Chicago, IL, USA) program. Descriptive statistics were presented as numerical variables using the mean and standard deviation (mean±SD) and categorical data as frequency (percentage ratio). ANOVA was used to compare morphometric measurements according to the types of samples. Tukey HSD post-hoc test was preferred for pairwise comparisons of the results. The correlation values between the altitude of the species and all morphometric measurements were calculated by Pearson's correlation analysis. Simple linear regression models were established to determine the effect levels of elevation on the measurements. A $p<0.05$ value was considered statistically significant for the type-I error rate of 5% in the analyses.

Results and Discussion

The morphological variations of the samples were examined by comparing the data obtained after the analysis of the morphometric characters Detected morphological variations are presented under the headings. A total of 62 samples collected from 17 different elevations were included in the study. 3 or 4

samples were collected foreach elevation. Descriptive information about the collected samples are presented in Table 1. In the correlational analysis, most of the measurements and altitude were found to have a positive and significant relationship. Only Elytra width and leg height were not associated with elevation. The highest correlation values with the elevations of the samples were found between body height (r=0.880; p<0.001) and pronotum width (r=0.822; p<0.001) (Table 5). Morfometric measurements of all samples were compared. It was observed that all the values differed significantly between samples (p<0.001). Measurements did not differ significantly between species collected from the same locality, but significant differences were observed between species collected from different locations for each morphometric measurement (Tables 3 & 4).

Since there were significant relationships between altitude and other measurements, simple linear regression models were created to determine the effect of altitude on the measurements and to create a prediction model. Significance and explanatory coefficient information of the models are presented in the table (Table 6). In parallel with the correlation values, the best models explaining the elevation were trunk height, pronotum height and pronotum width. Regression models including elytra width and leg length as predictors were not significant.

Color

In our study, samples collected in different years and preserved as museum material were used. While transforming the samples into museum material, no chemicals were used that would cause color change or impair pigmentation.

Our samples were examined using the Nikon SMZ1500 stereomicroscope. It has been observed that the colors of individuals collected from the same locality are very close to each other. For this reason, the most intact body parts were selected among the samples for photographing. Selected specimens were photographed using a Leica DFC295 macroscope at +5 light setting and 40% iris setting. Photographs of the samples were uploaded to Adobe Photoshop CS6 program one by one; color samples were taken from the body parts with an average scale of 31x31 and compared with the colors in the Pantone color library.

The head is generally dark brown to black. The golden yellow and black tubercles are scattered all over the surface. Antennas are brown, maxillary palps are dark brown (Figure 2). Pronotum can be black or dark brown. Sternite appears dark brown or black (Figure 3). Elytra is brown and pale spots are sparsely distributed throughout the elite. Elytral speckles and a dark speck of "Λ" are evident (Figure 2). When viewed from the ventral; the last segment of the tarsi and the nails are dark black in color, while the other parts range from light brown to dark brown (Figure 5).

Among our samples, head, pronotum, elytra, and leg colors were examined. It was determined that the colors of the head, pronotum, and elytra generally varied between brown and black. On the legs, when viewed from the ventral side, the last segment of the tarsi and the claws were dark black, and the other parts were light brown to dark brown.

Although a study done by Ratcliffe & Deloya (1992) stated that the coloration was not related to geography or altitude, it was observed in this research that the colors of our samples were darker depending on the increase in altitude. Considering that the temperature decreases at higher altitudes, the idea that insects need to be darker in color to better cool the solar radiation seems more likely. Similarly, Yurtsever et al. (2005) emphasized that those living in cold habitats are darker in many polymorphic species. However, this may show different results for different groups.

The colors of the body parts of the samples observed at different altitudes are given in the Table 2. It is noteworthy that the samples collected from higher altitudes were darker in color.

Figure 2. Dorsal views of the samples.

Table 2. The colors of the body parts at different altitudes

Sample	Altitude (m)	Head			Pronotum			Elytra		
		Black	Dark brown	Brown	Black	Dark brown	Brown	Black	Dark brown	Brown
a	41	X			X			X		
b	491		X				X			X
c	531			X			X		X	
d	1065		X				X			X
e	1081	X			X				X	
f	1084			X			X			X
g	1155	X					X			X
h	1212	X			X			X		
i	1354	X			X				X	
j	1361	X				X			X	
k	1483	X				X			X	
l	1507		X				X			X
m	1756	X				X			X	
n	1909	X			X			X		
o	1972		X			X			X	
p	2169	X			X			X		
q	2661		X			X			X	

The sizes of the body and body parts

The sizes of the body

The measurements of the body and body parts of our samples were made morphometrically. The full length and width ratios of the body, the length and width of the pronotum and elytra, and the lengths of the legs and aedeagophore parts were recorded. Measurements were made from the longest and widest parts of the body or body parts. The result of these measurements is presented in the Tables 3 & 4. Scatter plots and regression lines were drawn for each parameter (Figure 6).

The data presented in Tables 3&4 show that there are differences between the groups. The letters next to each measured parameter indicate this difference. The letters given to the examples were chosen according to the altitude. The sample collected from the lowest altitude is given the letter “a” and the sample collected from the highest altitude is given the letter “q”. Thus, as a result of the test, the letter "a" was assigned to the largest sized sample, and the letter "q" to the smallest sized sample. When the results are examined, it is seen that there is an altitude/size relationship in all parameters, but the largest species are found at the highest altitude and the smallest species at the lowest altitude.

Table 3. The size of body parts depending on altitude (The letters next to each measured parameter indicate the difference)

Sample	Altitude (m)	Body height (mm)	Body width (mm)	Elytra height (mm)	Elytra width (mm)	Pronotum height (mm)	Pronotum width (mm)
a	41	4.46±0.049 j	2.20±0.018 ab	3.66±0.036 bc	2.16±0.019 efg	0.88±0.007 h	1.55±0.012 j
b	491	4.53±0.075 ij	1.98±0.008 bc	3.37±0.047 c	2.02±0.013 hi	0.89±0.009 h	1.45±0.017 l
c	531	4.69±0.049 j	1.95±0.024 bc	3.11±0.027 c	1.94±0.009 i	0.81±0.010 j	1.46±0.009 l
d	1065	4.66±0.061 j	1.98±0.023 bc	3.24±0.045 c	1.96±0.017 i	0.92±0.011 g	1.49±0.014 k
e	1081	5.11±0.080 fgh	2.15±0.035 abc	2.13±0.022 d	3.39±0.040 a	0.95±0.011 f	1.69±0.010 d
f	1084	5.10±0.043 fgh	2.19±0.022 ab	3.45±0.044 c	2.23±0.025 def	0.92±0.009 g	1.63±0.025 h
g	1155	5.27±0.008 cdef	2.32±0.023 c	3.76±0.024 bc	2.36±0.020 c	0.98±0.010 de	1.69±0.021 d
h	1212	5.30±0.108 cde	2.10±0.094 abc	3.40±0.055 c	2.14±0.112 fg	0.99±0.051 d	1.70±0.055 d
i	1354	5.05±0.051 gh	2.17±0.018 abc	3.50±0.036 c	2.20±0.011 efg	0.84±0.008 i	1.59±0.015 i
j	1361	5.14±0.028 efgh	2.12±0.028 abc	3.49±0.036 c	2.19±0.018 efg	0.92±0.010 g	1.65±0.011 gh
k	1483	5.35±0.041 cd	2.09±0.024 abc	3.42±0.011 c	2.13±0.029 fg	0.99±0.008 d	1.68±0.020 de
l	1507	5.19±0.034 defg	2.08±0.002 abc	3.42±0.016 c	2.05±0.022 ef	0.97±0.008 e	1.66±0.020 fg
m	1756	5.00±0.069 h	2.09±0.075 abc	3.26±0.128 c	2.08±0.051 gh	0.94±0.076 f	1.67±0.063 ef
n	1909	5.42±0.079 c	2.15±0.017 abc	3.55±0.019 c	2.16±0.020 efg	0.95±0.010 f	1.69±0.017 d
o	1972	5.73±0.044 b	2.31±0.037 ab	3.60±0.022 a	2.27±0.022 cde	1.03±0.011 c	1.75±0.022 c
p	2169	5.74±0.038 b	2.31±0.037 ab	3.77±0.028 bc	2.33±0.016 cd	1.08±0.011 b	1.85±0.012 b
q	2661	6.48±0.061 a	2.61±0.020 a	4.35±0.044 ab	2.59±0.013 b	1.21±0.008 a	1.92±0.029 a
	Sign.	*<0.001**	*<0.001**	*<0.001**	*<0.001**	*<0.001**	*<0.001**

* Significant at 0.05 level according to ANOVA test, the pairwise comparison was performed by Tukey HSD, Sign.: significant level p.

Many studies reporting the presence of large species in high altitudes suggest that various reasons may cause this situation. Blake et al. 1994 attributed this to the disturbance of habitats, Novotny & Wison (1997), Yom-Tov & Geffen (2011) and Brandmayr & Pizzolotto (2016) attributed this to an increase in the amount of nutrients, and Roff (1980) to an increase in development time due to a decrease in temperature. Kingsolver & Huey (2008) stated that larger insects are more resistant to famine, drought and extreme temperatures and winter. All these assumptions are likely to be true.

Table 4. The size of aedeagophore parts and leg height depending on altitude (The letters next to each measured parameter indicate the difference)

Sample	Altitude (m)	Basal-aedeagophore tip (mm)	Basal-paramere tip (mm)	Basal part (mm)	Top width (mm)	Leg height (mm)
a	41	0.76±0.008 fg	0.79±0.005 ef	0.23±0.001 def	0.23±0.001 cdef	2.09±0.014 g
b	491	0.72±0.009 i	0.77±0.008 gh	0.24±0.002 def	0.24±0.002 fg	2.01±0.020 i
c	531	0.84±0.011 c	0.88±0.004 bc	0.25±0.002 d	0.25±0.002 bc	2.25±0.024 b
d	1065	0.79±0.008 e	0.78±0.002 ef	0.25±0.002 cd	0.25±0.002 a	2.25±0.027 b
e	1081	0.73±0.008 i	0.76±0.008 h	0.25±0.002 cd	0.25±0.002 defg	1.95±0.022 k
f	1084	0.74±0.008 h	0.80±0.004 de	0.23±0.004 ef	0.23±0.004 fg	2.22±0.025 c
g	1155	0.77±0.008 ef	0.79±0.003 def	0.24±0.003 de	0.24±0.003 fg	2.18±0.025 e
h	1212	0.75±0.009 gh	0.75±0.087 fg	0.25±0.077 d	0.25±0.077 ab	1.99±0.105 j
i	1354	0.87±0.011 b	0.87±0.012 bc	0.24±0.002 de	0.24±0.002 efg	2.25±0.024 b
j	1361	0.76±0.004 fg	0.80±0.007 def	0.22±0.002 f	0.22±0.002 g	1.89±0.014 l
k	1483	0.77±0.006 ef	0.79±0.007 def	0.23±0.002 def	0.23±0.002 cde	2.22±0.028 c
l	1507	0.77±0.003 ef	0.81±0.010 d	0.24±0.001 de	0.24±0.001 efg	2.01±0.023 i
m	1756	0.78±0.006 ef	0.81±0.007 d	0.25±0.088 cd	0.25±0.088 cdef	2.28±0.068 a
n	1909	0.76±0.010 fgh	0.80±0.009 de	0.24±0.002 def	0.24±0.002 defg	2.20±0.016 d
o	1972	0.84±0.003 c	0.87±0.007 bc	0.27±0.002 bc	0.27±0.002 ab	2.06±0.014 h
p	2169	0.94±0.008 a	0.96±0.012 a	0.28±0.003 ab	0.28±0.003 ab	2.21±0.024 cd
q	2661	0.81±0.008 d	0.89±0.006 b	0.29±0.002 a	0.29±0.002 cd	2.15±0.033 f
	Sign.	*<0.001**	*<0.001**	*<0.001**	*<0.001**	*<0.001**

* Significant at 0.05 level according to ANOVA test, the pairwise comparison was performed by Tukey HSD, Sign.: significant level p.

Pronotum

As seen in the Table 3 pronotum is 0.8-1.2 mm long and 1.4-1.9 mm wide. It can be black or dark brown. The tubercles are smaller than those in the head and are spread over the whole area. Sternite appears dark brown or black. The part between the cocca and the head is short, straight looking, and slightly raised. Variations were also detected in the pronotum of our samples. Some have greater depths of grooves (g, h, p, q). In some of the samples, the margins of the pronotum are sharper (f, l, p) (Figure 3).

Head and pronotum are frequently used in body measurement comparisons, especially in insects. In our study, the most significant results were obtained from the pronotum measurements. ANOVA showed that pronotum width and length were significantly affected by altitude. ANOVA data showing that *H. aquaticus* species have larger and wider pronotum at high altitudes and making the differences according to the lettering given to the samples are presented in Table 3.

Aedeagophore

Aedeagophore of samples are 7.2-9.6 mm long and the base piece is longer than the parameres. Parameres approach each other towards the tip. The middle lobe is shorter and thicker than parameres. The pedestal arms are curved and their loose ends converge (Figure 4).

In some of our samples, the parameres are wider and end by tapering (a and n), some of them have slight indentations on the paramere edges (e and m), in some, the struts are more curved (d, p, and o), in some, the struts are shorter and it ends at the tip of the basal part (d, h and m), in some, the basal part ends with sharper lines (c, f, and k) (Figure 4).

While making aedeagophore measurements, 4 parameters (Basal-aedeagophore type, Basal-paramere type, Basal part, Top width) were taken as basis. When the obtained values were compared with the altitude, it was seen that the 4 parameters increased in direct proportion to the altitude. ANOVA showed that each parameter was significant and reliable (Table 4).

Figure 3. Pronotum views of samples.

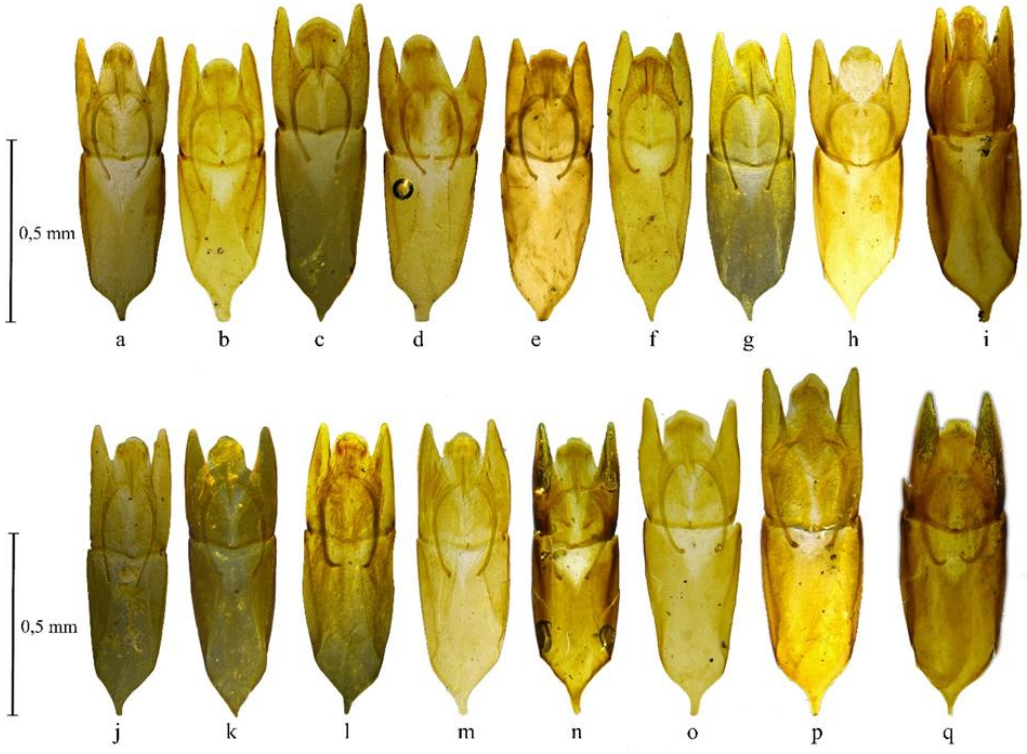

Figure 4. Aedeagophore views of samples.

Leg

From the ventral view, the legs are thick and long. The last segment of the tarsi and the nails are dark black in color, while the color of other parts range from light brown to dark brown. The denticles on the last part of the seventh abdominal sternites are small. In some of the samples, the legs are yellowish-brown (g, k, m, q), some are blackish (f, j), others are dark brown (Figure 5).

The leg lengths of each sample were measured and its relationship with height was tried to be determined. However, the results were not found to be significant since no parallelism could be found with the correlation values.

Figure 5. Legs views of samples.

Table 5. The correlation between altitude and other morphological measurements

Altitude (m)	Body height (mm)		Body width (mm)		Elytra height (mm)		Elytra width (mm)		Pronotum height (mm)		Pronotum width (mm)	
r; *p*	0.880	<0.001*	0.616	<0.001*	0.415	0.001*	0.134	0.300	0.756	<0.001*	0.822	<0.001*
	Basal-aedeagophore tip (mm)		Basal-paramere tip (mm)		Basal part (mm)		Top width (mm)		Leg height (mm)			
r; *p*	0.380	0.002*	0.400	0.001*	0.325	0.010*	0.325	0.010*	0.161	0.210		

**: Significant at 0.05 level according to Pearson Correlation Analysis; r: correlation coefficient; p: significant level.*

Table 6. The regression models of morphological measurements on altitude

	R^2	F	*p*	Model
Body height (mm)	0.774	205.016	*<0.001**	4.271+0.001*altitude
Body width (mm)	0.379	36.658	*<0.001*	1.948+0.001*altitude
Elytra height (mm)	0.172	12.492	*<0.001*	2.967+0.001*altitude
Elytra width (mm)	0.018	1.095	*0.300*	Not significant
Pronotum height (mm)	0.571	79.923	*<0.001**	0.8+0.001*altitude
Pronotum width (mm)	0.675	124.70	*<0.001**	1.43+0.001*altitude
Basal-aedeagophore tip (mm)	0.144	10.127	*0.002**	0.742+3.5E-05*altitude
Basal-paramere tip (mm)	0160	11.449	*0.001**	0.755+4.39E-05*altitude
Basal part (mm)	0.105	7.075	*0.010**	0.222+1.8E-05*altitude
Top width (mm)	0.105	7.075	*0.010**	0.222+1.8E-05*altitude
Leg height (mm)	0.026	1.605	*0.210*	Not significant

**: Significant at 0.05 level according to Linear Regression, R^2: Coefficient of determination*

In the present study, the morphological examination of the specimens belonging to the Helophoridae family living in Türkiye was conducted. Among the samples examined, species of *H. aquaticus* that showed variation among the collected samples from the same locality were selected, the differences were evaluated morphometrically. The morphological differences of the species were stated by making comparisons among themselves. Seventeen morphological variations have been identified in terms of color, size, and structure (aedeagophore, pronotum, elytra, and leg). Differences were noted. It is believed that the results of this study will now enable accurate and faster identification of *H. aquaticus* species. In addition, by giving the heights of the locations where the samples were collected, the relationship between morphological differences and altitude was determined.

It has been determined that there are serious relationships between altitude and size in many insect species. In some, the size increased with altitude, while in others it decreased. In this study, 11 parameters (body height, body width, elytra height, elytra width, pronotum height, pronotum width, basal-aedeagophore type, basal-paramere type, basal part, top width, leg height) were measured in order to understand the effect of altitude. The ANOVA analysis performed in the study using all these parameters indicated a statistically significant effect of altitude on size, at $p<0.05$. Specifically, all the data show that small specimens of *H. aquaticus* live at low altitudes, while large specimens live at higher altitudes. Our results contradict the Bergmann and Allen rules. being in line with many other studies in literature (Park, 1949; Hukushima, 1960; Mousseau, 1997; Novotny & Wison, 1997; Blanckenhorn & Demont, 2004; Shelomi, 2012; Sukhodolskaya et al., 2021). With this study, it has been shown that the Bergmann and Allen rules are not inclusive for all species. In addition, this study confirmed that the variations are directly related to the environment.

Statistical analyses performed in this study show that the size of all body parts of our samples increased with height, except for elytra width and leg length. In parallel with the correlation values, the best models explaining the elevation were trunk height, pronotum height and pronotum width. Of course, it may not be possible for these results to be a common rule for all species. However, it has been observed that the relationship between body and elytra lengths of *H. aquaticus* and altitude results in the opposite direction of Bergman and Allen's rules, so that both rules cannot be generalized to all species. There may be many factors affecting the size of coleopters, but this study has revealed that one of them is altitude.

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Original article (Orijinal araştırma)

Determination of insecticide residues in "Bayramiç Beyazı" nectarines and their risk analysis for consumers[1]

"Bayramiç Beyazı" nektarinlerde insektisit kalıntılarının belirlenmesi ve tüketiciler için risk analizi

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Abstract

In this study, insecticide residues on "Bayramiç Beyazı" nectarines were investigated with the use of QuEChERS method and LC-MS/MS analysis. Analytical method was verified through SANTE 11312/2021 Guidelines. The limit of quantification were below the MRLs for 12 insecticides. Method recovery was identified as 89.6%. Such a value was within the SANTE recovery (60-140%) limits. Nectarine samples were collected from Çanakkale open markets between 15 June-30 September, 2022 and analyzed at ÇOMÜ Agriculture Faculty-Pesticide Laboratory (Çanakkale-Türkiye). Abamectin, acetamiprid, deltamethrin, etoxazole, novaluron, pyriproxyfen, spirodiclofen, tetramethrin and thiacloprid residue levels were below the MRLs. On the other hand, dimethoate, imidacloprid and omethoate residues exceeded their MRLs only in one sample each. The maximum residues of acetamiprid, deltamethrin, etoxazole and novaluron were about 1/2, 1/5, 1/10, and 1/70 of the MRLs in one sample, respectively. Risk assessments revealed that exposure levels for adults were low (hazard quotient, HQ ≤ 1), with the exception of omehoate residues. Omethoate posed a chronic risk to human health through consumption of nectarines. For the remaining 11 insecticides, there was no risk for human health. However, the highest acute HQ were found for dimethoate even though its HQ was less than or equal to 1 The use of dimethoate is in the process of being banned in Türkiye, while omethoate (metabolite of dimethoate) is already banned. Presence of omethoate residue may be due to the degradation product of dimethoate.

Keywords: Acute and chronic risk assessment, Bayramiç Beyazı, insecticide residues, nectarin, QuEChERS

Öz

Bu çalışmada QuEChERS yöntemi ile "Bayramiç Beyazı" nektarinlerde insektisit kalıntıları araştırılmıştır. Analiz metodu SANTE 11312/2021'e göre doğrulanmıştır. 12 adet insektisitin LOQ limiti, MRL değerlerinin altında bulunmuştur. Metodun geri kazanımı %89.6 olmuştur. Bu rakamlar SANTE geri alımları (%60-140) ile uyumludur. Nektarin örnekleri 15 Haziran-30 Eylül 2022 arasında Çanakkale pazarından toplanmış ve ÇOMÜ Ziraat Fakültesi-Pestisit Laboratuvarı(Çanakkale-Türkiye)'nda analizleri yapılmıştır. Abamectin, acetamiprid, deltamethrin, etoxazole, novaluron, pyriproxyfen, spirodiclofen, tetramethrin ve thiacloprid kalıntı seviyeleri MRL değerlerinin altındadır. Öte yandan, sadece birer örnekte dimethoate, imidacloprid ve omethoate kalıntıları MRL değerlerini aşmıştır. Acetamiprid, deltamethrin, etoxazole ve novaluronun kalıntıları, birer örnekde MRL'lerin sırasıyla 1/2, 1/5, 1/10 ve 1/70'i olarak bulunmuştur. Risk değerlendirmeleri, yetişkinler için, omehoate hariç, maruz kalma düzeylerinin düşük olduğunu ortaya çıkarmıştır (tehlike katsayısı, HQ ≤ 1). Nektarin tüketiminde insan sağlığı açısından omethoate riskli bulunmuştur. Geri kalan 11 insektisit için insan sağlığı açısından herhangi bir risk bulunmamıştır. Bununla birlikte, HQ≤1 olmasına rağmen en yüksek akut HQ dimethoate için bulunmuştur. Zaten Türkiye'de dimethoate yasaklanma sürecindedir, dimethoate'ın metaboliti olan omethoate kullanımı ise yasaklanmıştır, Omethoate kalıntısı bulunması dimethoate'ın parçalanma ürünü olmasından kaynaklanabilir.

Anahtar sözcükler: Akut ve kronik risk değerlendirmesi, Bayramiç Beyazı, insektisit kalıntıları, nektarin, QuEChERS

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Introduction

The “Bayramiç Beyazı” nectarine has been grown as an endemic species for years in Bayramiç District (located in Kazdağları region) of Çanakkale province. With a unique color, taste, smell, aroma and long shelf life, it differs from the other nectarine varieties. The geographical registration approval of the “Bayramiç Beyazı” nectarine has been published in the Official Journal of the European Union (EU) on April 16, 2021. It is the 1st product of Çanakkale with an EU-geographical indication. This fruit is produced in an area of 5500 da with 250 thousand trees. An average of 13 to 15 kt of nectarine is produced annually. In recent years, its cultivation has reached an important level because it can be marketed at high prices in big cities such as İstanbul and İzmir (Anonymous, 2022). Pests including *Grapholita molesta* (Busck, 1916) (Lepidoptera: Torticidaae), *Anarsia lineatella* (Zeller, 1839) (Lepidoptera: Gelechiidae)*, Myzus persicae* (Sulzer, 1776) (Hemiptera: Aphididae), *Sphaerolecanium prunastri* (Boyer de Fonscolombe, 1834) (Hemiptera: Coccidae)*, Nilotaspis halli* (Green, 1923) (Homoptera: Diaspididae) are the most common harmful insects in nectarine cultivation. While farmers want to protect nectarines against these harmful pests, they prefer chemical control as it gives fast and effective results. So acetamiprid, cyantraniliprole, emamectin benzoate, malathion pyriproxyfen and spinosad pesticides are used against these pests (BKÜ, 2023a). However, ignorant spraying is very harmful to the environment and human health (Ambrus et al., 2023). It is possible to receive alerts about these pesticides from the Rapid Alert System for Food and Feed portal (RASFF, 2023).

The QuEChERS (Quick-Easy-Cheap-Efficient-Rugged-Safe) method, developed by Anastassiades et al. (2003), is generally used for pesticide residue analyses of vegetable and fruits (Lehotay et al., 2005; AOAC, 2007; Polat & Tiryaki, 2019; Polat, 2021; Çatak & Tiryaki, 2020; Balkan & Yılmaz, 2022a). However, a further verification of the method should be conducted if it is to be used by local laboratories (Omeroglu et al., 2012).

Dülger & Tiryaki (2021) used QuEChERS method to investigate boscalid, chlorpyrifos and tebuconazole residues on nectarine and peach samples collected from Çanakkale markets. The overall recovery was determined as 113.5% with a relative standard deviation (RSD) of 17.3% for peach samples and 113.61% with an RSD of 11.4% for nectarine samples. These values were a fit for the recovery (60-140%) and repeatability (RSD ≤ 20%) limits of SANTE. Researchers reported that residue levels did not exceed maximum residue limits (MRLs) in any samples. Chronic exposure levels were low and pesticide residues did not pose any health risks.

Camara et al. (2020) investigated pesticide residues in various fruit juices and assessed dietary risk exposure. Long term chronic risk assessment indicated that potential consumer risk for the imidacloprid pesticide was practically negligible for human health, with the risk Quotients (RQ) of 0.044 and 0.000 for unprocessed peach and peach juice, respectively.

Chatzicharisis et al. (2012) investigated insecticide residue levels on peach and nectarine samples. Decreasing residue levels were seen over time following application. Bupirimate, chlorpyrifos, fenoxycarb, iprodione and pirimicarb residue levels on peach samples were lower than relevant EU-MRLs while chlorothalonil residues were below the quantification limit (LOQ).

Pesticide residues were investigated in peach and nectarine samples imported into the United Arab Emirates. Residues above MRL were found in half of the analyzed samples. The pesticides reported were dimethoate and omethoate from Organophosphorus Class (Osaili et al., 2022). In another study conducted in China, 18 pesticide residues were found in peaches, and acetamprid residues exceeding the EU-MRL were found in 2 samples. Imidacloprid, pyriproxyfen and spirodiclofen residues were less than EU-MRL. Acute and chronic dietary exposure assessment indicated that potential dietary risk induced by the pesticides was not significant for Chinese consumers (Zhang et al., 2021).

Galietta et al. (2011) conducted a study in Uruguay about dissipation curves of pesticide in peach samples. Recovery rates of azoxystrobin, acetamiprid and thiacloprid were observed as 95.3, 98.6 and 80.6%, respectively. Dissipation curves revealed that the time required for insecticide residues to go below MRL was 10-12 days for thiacloprid and 25 days for acetamiprid. Kaya & Tuna (2019) reported that chlorantraniliprole, deltamethrin, phosmet and spirodiclofen residue levels in peach samples were less than the corresponding MRL. However, Ersoy et al. (2011) reported that chlorpyrifos residue levels on peach samples were greater than the MRL. Choi et al. (2011) conducted a study on chlorfluazuron residues in peach samples, which indicated that residues were lower than the MRL in all samples.

To control the safe and efficient use of pesticides, their residues should regularly be monitored in food and environmental samples. The samples should be taken randomly and dietary risk assessment should be performed (Ambrus et al., 2023). On the other hand, improper use of insecticides may result in serious risks on human health. Extended periods of exposure to insecticides may cause cancers, headaches, nausea and endocrine disorders (Yousefi et al., 2022). Therefore, dietary risk assessment of insecticides has recently gained a great attention (Gebara et al., 2011; Marete et al., 2020; Chen et al., 2021). For dietary risk assessments, both acute and chronic risks to the consumer health are evaluated. Dietary risk assessments are performed based on daily food consumption and detected pesticide residue data on foodstuffs. For short-term acute diatery risk assessments, acute reference dose (ARfD, mg/kg bw/day) values are used. Then, estimated short-term intake (ESTI, mg/ kg bw/day) and acute hazard quotient (HQ) are calculated. For long-term chronic dietary risk assessments, acceptable daily intake (ADI, mg/kg bw/day) values are used. Then, estimated daily intake (EDI, mg/ kg bw/day) and chronic HQ values are calculated. HQ values of >1 indicate a potential risk for human health (EFSA, 2007; Balkan & Yılmaz, 2022b).

In this study, insecticide residues on/in “Bayramiç Beyazı” nectarines sampled from Çanakkale open markets were investigated with the QuEChERS method. The method was verified through SANTE (Directorate-General for Health and Food Safety) Guidelines. Total 377 pesticides were analysed in the LC-MS/MS system (located in Çanakkale Food Control Directorate) and insecticides residues above LOQ, were evaluated. Consumer acute and chronic risk assessment for insecticides were also performed.

Materials and Methods

Chemicals and Reagents and Insecticide solutions

Standards for insecticides were supplied from Dr. Ehrenstorfer GmbH (Wesel, Germany) and Chem Service (West Chester, PA, USA). QuEChERS extraction [6 g anhydrous magnesium sulfa ($MgSO_4$); 1.5 g anhydrous sodium acetate (NaOAC)] and clean-up kits [1.2 g $MgSO_4$, 400 mg primary and secondary amines (PSA, 40 µm particle size) and 400 mg C_{18}] were used. The other solvents and reagents including acetonitrile (MeCN) and acetic acid (HAc) were at analytical grade. Stock solution of insecticides (400 µg/mL) were used to prepare working solutions (1.0 µg/mL) through series of dilutions. Calibration (matrix match standards) was performed on blank nectarines. Calibration solutions of matrix-matched (MC) were prepared with MeCN (1-1000 pg/µL) (Poole, 2007). Spiking solutions corresponding to 1 x LOQ and 10 x LOQ were prepared. For MC and quantifications, representative apple matrix was used (CAC, 2003; SANTE, 2021).

Instruments

An LC-MS device was used for chromatographic analyses (Waters I Class Plus UPLC + Xevo TQ-S micro MS Detector; ESI + mode). The device is connected with Acquity UPLC BEH C_{18} column (1.7 mm, 100 x 2.1 mm). Flow rate, injection volume and total run time were 0.35 mL/min, 1 µL and 15 minutes, respectively. A gradient program including 10 mM ammonium acetate ($NH_4CH_3CO_2$) in methanol (B) and 10 mM $NH_4CH_3CO_2$ in water of pH 5 (A) was used. Insecticide retention times (tR), precursor ion and fragment ions are given in Table 1.

The other materials used in the present study included precise balance (± 0.0001 g) (Shimadzu ATX224), centrifuge (Hettich EBA 280, 4500 rpm), vortex (VELP scientifica), centrifuge tubes, Agilent GC vials (1.5 mL), blender and N_2 stream.

Verification of QuEChERS-AOAC Official Method 2007.01

Method verification was performed in accordance with verification parameters of SANTE, such as linearity, recovery, precision and LOQ parameters (SANTE, 2021). Blank nectarine samples of 1 kg were homogenized with a blender. For recovery tests, 15 g blank nectarine samples were spiked with 100 µL of insecticide spike solutions (in MeCN) corresponding 1 x LOQ and 10 x LOQ level of insecticides. Tests were conducted in five replicates (five replicate analytical portions). Resultant mixtures were vortexed for 30 seconds and left standing for 15 minutes for interaction of insecticides with the sample. Figure 1 presents the further analytical steps taken in analyses. MC calibration curve was used to quantify insecticides. The rates of recovery were calculated as the ratio of measured concentration to spiked concentration. Recovery and precision of the method were assessed based on SANTE European Guidelines (SANTE, 2021). Linearity of the method was checked for the range 1-1000 pg/µL.

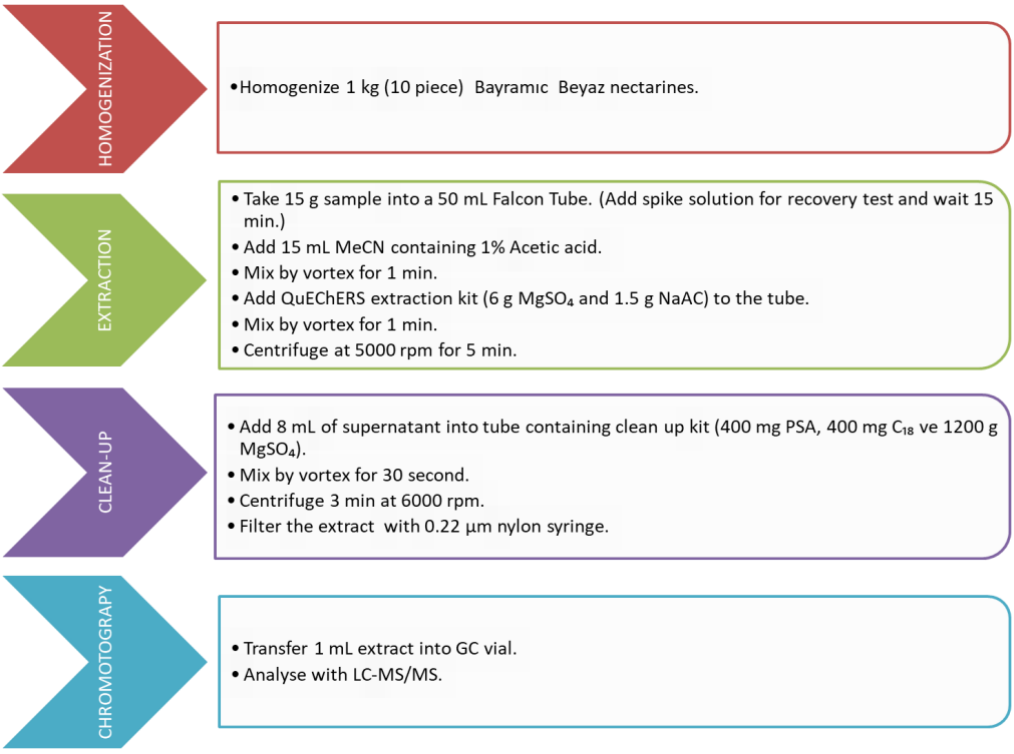

Figure 1. Analytical ssteps QuEChERS-AOAC Official Method 2007.01.

Collecting samples and analyses

Nectarines were taken from different stands in the open markets of Çanakkale province for 14 weeks between 15 June-30 September, then the analyses were performed. The nectarine samples of about 1 kg were homogenized and 15 g of analytical portions (in triplicates) were taken. Analytical steps taken are presented in Figure 1. Total 210 analyses (5 stands/week x 14 weeks x 3 analytical portions) were performed. The analyses of the spiked samples and collected samples from the market were performed with the use QuEChERS method (AOAC, 2007). Chromatographic analyses of 377 pesticides were performed in the LC-MS/MS system and insecticide residues above LOQ were evaluated in the study.

Methodology for assessing dietary intake of insecticides

Acute and chronic risks to consumer health were estimated based on the previous studies (Chen et al., 2011; Soydan et al., 2021). Annual nectarine consumption per person was taken as 7.3 kg (i.e., 0.02 kg of nectarine per day) in Türkiye (TSI, 2022). The average body weight of an adult is taken as 60 kg in toxicological research (EFSA, 2019; WHO, 2021; Calderon et al., 2022). ADI (mg/kg bw/day) and ARfD (mg/kg bw/day) values of insecticides were taken from IUPAC Pesticide Properties DataBase (PPDB, 2022). ARfD values of insecticides were used for short-term acute dietary risk assessments (Liu et al., 2016; Malhat et al., 2021). ESTI (mg/ kg bw/day) and acute HQ values were calculated with the use of the following equations.

$$ESTI, mg/kg\ bw\ /\ day = \frac{Daily\ nectarine\ consumption, kg/day * high\ residue, mg/kg}{Body\ weight} x100 \quad (1)$$

$$Acute\ HQ = \frac{ESTI, mg/kg\ bw\ /\ day}{ARfD, mg/kg\ bw\ /\ day} x100 \quad (2)$$

Similar to acute risk assessments, ADI values were used for long-term chronic dietary risk assessments. EDI (mg/ kg bw/day) and chronic HQ values were calculated with the use of the following equations.

$$EDI, mg/kg\ bw\ /\ day = \frac{Daily\ nectarine\ consumption, kg/day * mean\ residue, mg/kg}{Body\ weight} x100 \quad (3)$$

$$Chronic\ HQ = \frac{EDI, mg/kg\ bw/\ day}{ADI, mg/kg\ bw\ /\ day} x100 \quad (4)$$

The level of concern for HQ value was set as 1. Therefore, HQ values of ≥ 1 represents a risk for human health and HQ values of < 1.0 presents non-potential risk for human health.

Results and Discussion

Method verification

Matrix-matched calibration curves of 12 insecticide standards were linear over the 1-1000 pg/µL concentration ranges with various determination coefficient (R^2 ≤0.999). R^2, retention times (tR, min) (ranged between 4.49-11.55 min) and MC line equations (4-point level) of all insecticides are also provided in Table 1. For quantification of the insecticides, regression equations of matrix-matched calibration curves (analytical function) were used. LOQs and MRLs of all insecticides are provided in Table 2. These LOQ values were quite lower than the MRLs.

Trueness and precision of the method are assessed as recovery (Q %) and repeatability (RSD %), respectively (Tiryaki, 2016; TURKAK, 2022). Percent recovery values together with standard deviation (SD) and RSD for Bayramiç Beyazı nectarine samples are given in Table 2. Individual recovery (mean recovery of 1 x LOQ and 10 x LOQ spiking levels with 5 replicate analyses) of each pesticide and their RSDs were provided in the table. Insecticide recoveries from nectarine samples varied between 65.2-115.3% with relative standard deviations (RSDs) of between 2.35-7.3%. The number of recovery data (n) was 10 for each insecticide. The overall method recovery was identified as 89.6% with a RSD of 11.8% (n=120). Present LOQ values (Table 2) also revealed that the method could detect insecticide residues lower than the MRL (Table 3) set by the EU (2022).

Present recovery values comply with method verification parameteres (SANTE, 2021; EURACHEM, 2014). Dülger & Tiryaki (2021) identified mean recovery of boscalid, chlorpyrifos and tebuconazole as 113.6% with an RSD of 11.4 % for nectarine and 113.5% with an RSD of 17.3% for peach. Galietta et al. (2011) measured the mean recovery of azoxystrobin, thiacloprid and acetamiprid in peaches as 95.3, 80.6, 98.6%, respectively.

These findings revealed that QuEChERS method may offer an accurate and rapid tool in detection of insecticide residues in Bayramiç Beyazı nectarine samples.

Table 1. Retention times (tR), calibration ranges, calibration curve equations, determination coefficients (R^2) and selected ion groups of the analyzed insecticides

İnsecticide	tR*, min	Calibration range, pg/µL	Calibration equation y=a+bx	Determination co-efficient, R^2	Precursor ion, m/z (CE**)	Fragment ion, m/z (CE)
Abamectin	11.5	5-1000	y=1030.1+813.014x	0.99955	890.4 > 305.2 (13)	890.4 > 567.3 (25)
Acetamiprid	4.9	1-100	y=7256.9+127041x	0.99996	223.1 > 125.9 (21)	223.1 > 55.9 (15)
Deltamethrin	11.3	3-600	y=813.5 +3904.8x	0.99987	523.0 > 280.9 (15)	523.0 > 506.0 (9)
Dimethoate	4.8	1-100	y=7458.5+113468x	0.99948	230.0 > 198.9 (9)	230.0 > 124.9 (20)
Etoxazole	11.0	1-100	y=9280.6+201567x	0.99991	360.2 > 140.9 (48)	360.2 > 112.9 (60)
Imidacloprid	4.4	1-100	y=1659.5+20360.1x	0.99874	256.0 > 209.0 (15)	256.0 > 175.0 (20)
Novaluron	10.3	1-200	y=-22.5 +7292.69x	0.99992	493.0 > 158.0 (18)	4 93.0 > 141.0 (48)
Omethoate	2.7	1-200	y=7881 + 107889x	0.99980	214.0 > 124.9 (20)	214.0 > 182.9 (10)
Pyriproxyfen	10.7	1-100	y= 56099+270369x	0.99831	322.3 > 95.4 (16)	322.1 > 227.1 (15)
Spirodiclofen	11.2	1-200	y=-05.91+ 11752.9x	0.99992	411.4 > 313.2 (11)	411.4 > 71.3 (18)
Tetramethrin	10.5	1-100	y=253436+58830.9x	0.99975	332.2 > 164.1 (25)	332.2 > 135.1 (16)
Thiacloprid	5.4	1-100	y=3346.4+176582x	0.99999	253.0 > 125.6 (20)	253.0 > 89.9 (39)

*tR, retention time (min); *** CE, Collision Energy (V)

Table 2. Spiking levels and recovery (including SD and RSD) values of insecticides obtained in method verification studies

İnsecticide	Spike level, µg/kg	Found , µg/kg	Recovery, %	Mean Recovery, % (As a tool for truness)	SD	RSD,% (As a tool for precision)
Abamectin	5	4.5	89.4	86.2	5.6	6.5
	50	41.5	83.0			
Acetamiprid	1	0.8	86.0	81.5	6.7	8.2
	10	7.7	76.9			
Deltamethrin	3	3.1	104.9	99.9	7.3	7.3
	30	28.5	94.9			
Dimethoate	1	0.9	93.4	83.5	11.4	13.6
	10	7.4	73.6			
Etoxazole	1	1.0	99.6	93.7	7.8	8.3
	10	8.8	87.7			
Imidacloprid	1	0.99	98.2	97.9	5.5	5.6
	10	9.8	96.8			
Novaluron	1	0.8	77.6	72.9	6.0	8.2
	10	6.8	68.2			
Omethoate	1	0.9	97.4	94.0	5.7	6.0
	10	9.1	90.6			
Pyriproxyfen	1	0.9	91.4	87.1	6.3	7.2
	10	8.3	82.8			
Spirodiclofen	1	1.1	109.8	101.1	9.6	9.5
	10	9.2	92.5			
Tetramethrin	1	0.8	85.8	83.9	4.4	5.2
	10	8.2	82.1			
Thiacloprid	1	0.9	99.0	94.4	6.1	6.4
	10	8.9	89.7			

Recovery range: 65.2-115.3; RSD range: 2.3 - 7.3%; Overall recovery (Accuracy): 89.6% with an RSD of 11.8% (n=120).

Residues in the “Bayramiç Beyazı” nectarines

Totally, 210 analytical portions, [70 samples (14-week x 5-stand, coded as A, B, C, D and E) and 3 replicates] were analyzed. A total of 12 insecticides, namely abamectin, acetamiprid, deltamethrin, dimethoate, etoxazole, imidacloprid, novaluron, omethoate, pyriproxyfen, spirodiclofen, tetramethrin, thiacloprid were detected in ”Bayramiç Beyazı” nectarine samples.

The detected insecticide residues were below their MRLs, except for dimethoate, imidacloprid and omethoate. The insecticide (totally 8) residues detected only in a few samples and their details are given

in Table 3. Abamectin, imidacloprid and spirodiclofen residues (in one sample each) were found to be above the LOQs (below the MRLs) with the residue levels of 6.1, 5.0 and 32.0 µg/kg, respectively. Pyriproxyfen and thiacloprid residues (in two sample each) were found to be above the LOQs. Tetramethrin residues (above the LOQ) were found (106.6 µg/kg) only in one sample (12th week, Stand D). There is no specified MRL value for tetramethrin in nectarine. In one sample (2nd week, Stand E), dimehoate residue was found approximately 10 times (97.8 µg/kg) of the MRL value. Imidacloprid residues were found approximately 2 times (17.7 µg/kg) of the MRL in one sample (6th week, Stand B). Omethoate residue was found slightly over (10.2 µg/kg) the MRL values in one sample (2nd week, Stand A).

Omethoate and thiacloprid were banned in Türkiye on 31 August 2012 and 30 June 2022, respectively. Dimethoate is also in the process of being banned(BKÜ, 2023b).

Table 3. Insecticide residues observed in ”Bayramiç Beyazı” nectarines and comparison with LOQ and MRL values

Insecticide	Below the MRL Residue, µg/kg (Number of sample detected)	MRL-exceeding Residue, µg/kg (Number of sample detected)	LOQ, µg/kg	MRL , µg/kg
Abamectin	6.1 (1)	-	5	20
Dimehoate	-	97.8 (1)	1	10
Imidacloprid	5.0 (1)	17.7 (1)	1	10
Omethoate	-	10.2 (1)	1	10
Pyriproxyfen	8.1 (1) 7.1 (1)	-	1	500
Spirodiclofen	32 (1)	-	1	2000
Tetramethrin	106.6 (1)*		1	-
Thiacloprid	85.5 (1) 26.5 (1)	-	1	500

*There is no specified MRL value for nectarine.

Residue levels of the remaining 4 insecticides, which were below the MRLs, are presented in Figure 2-5. Maximum acetamiprid residue (102.4 µg/kg, half of the MRL) was detected in the 1st week of Stand-C (Figure 2). Maximum deltamethrin (34.6 µg/kg, about 1/5 of the MRL, Figure 3) residue was detected in the 10th week of Stand-D, etoxazole (9.7 µg/kg, about 1/10 of the MRL, Figure 4) in the 12th week of Stand-D and novaluron (28.3 µg/kg, about 1/70 of the MRL, Figure 5) in the 9th week of Stand-C samples. Novaluron was banned in Türkiye on 30 June 2022.

Dülger & Tiryaki (2021) reported that boscalid, chlorpyrifos and tebuconazole residues in peach and nectarine samples were below the corresponded MRLs. Maximum boscalid residue in peach samples was measured as 566.8 µg/kg and maximum boscalid residue in nectarine samples was measured as 322.1 µg/kg. Maximum values for tebuconazole were 47.5 and 56.9 µg/kg, respectively. Chlorpyrifos residue levels were all below LOQ. In another study, chlorpyrifos residue levels greater than the corresponding MRL were reported for peach samples (Ersoy et al., 2011). In a previous study, Galietta et al. (2011) indicated that 25 and 12 days were required to pass after application for acetamiprid and thiacloprid residues below MRL in peach samples. Soydan et al. (2021) investigated pesticide residues in fruits and analyzed total 92 samples. Of the analyzed samples, 23.9% had residues below the LOQ, 57.6% had residues exceeding MRL and 18.4% had residues below the MRL. Osaili et al. (2022) found omehoate and dimethoate residues above the MRL in half of the nectarine samples they analyzed. In another study, 18 pesticide residues were found in peaches. Acatamprid residues exceeding the EU-MRL were found in 2 samples. Imidacloprid, pyriproxyfen and spirodiclofen residues were less than EU-MRL (Zhang et al., 2021).

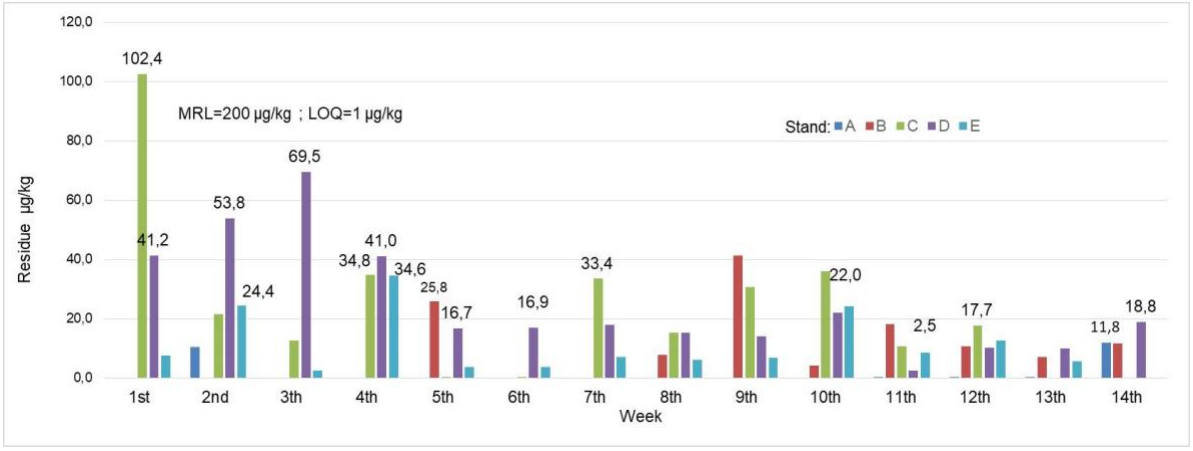

Figure 2. Stand and week-based acetamiprid residues in “Bayramiç Beyazı” nectarines.

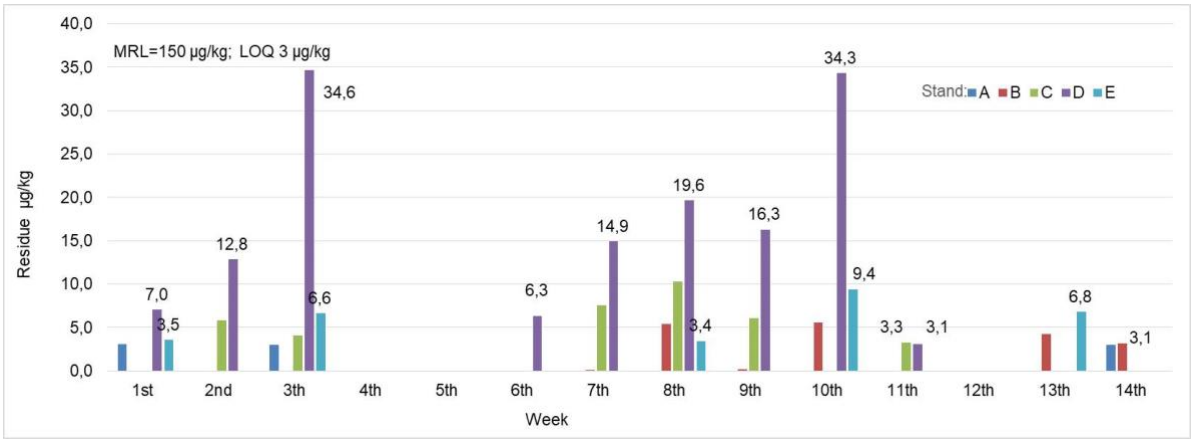

Figure 3. Stand and week-based deltamethrin residues in “Bayramiç Beyazı” nectarines.

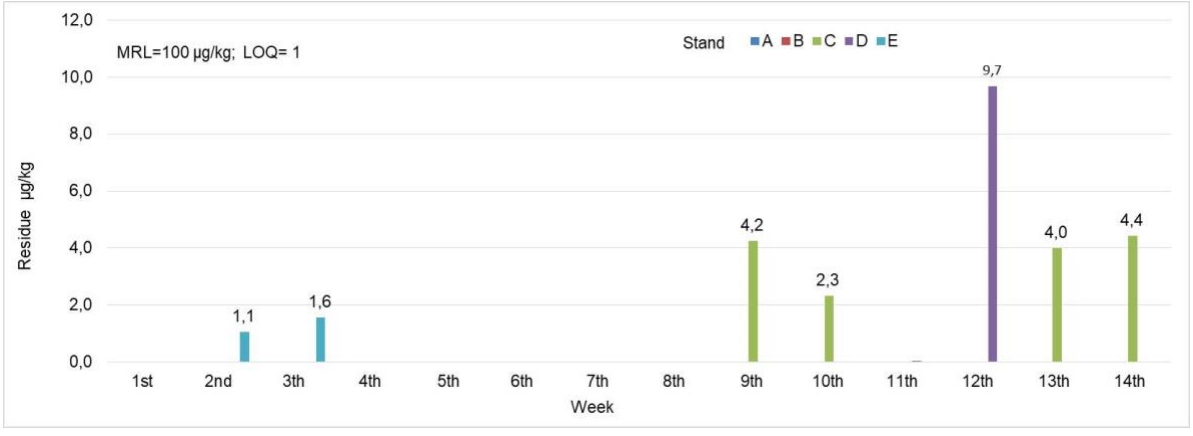

Figure 4. Stand and week-based etoxazole residues in “Bayramiç Beyazı” nectarines.

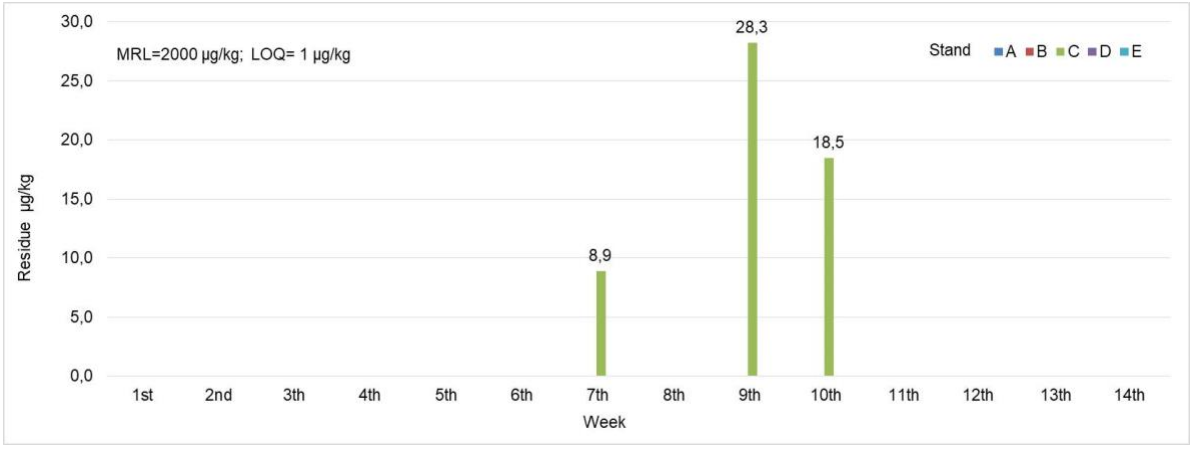

Figure 5. Stand and week-based novaluron residues in "Bayramiç Beyazı" nectarines.

Risk assessment for dietary intake of insecticides

For acute and chronic risk assessments, ESTI (mg/kg bw/day) and acute HQ were used for short-term risk and EDI (mg/kg bw/day) and chronic HQ were used for long-term risks. Resultant values are provided in Table 4. For all the insecticides, the number of residue figures and residue ranges (including mean residue) were also given in the table.

The ESTI values (calculated with Equation 1) for short-term risk ranged from 0.21E-05 to 3.45E-05. The acute HQ (calculated with Equation 2) of insecticides ranged between 0.0077 to 0.3449. The highest values of acute exposure (HQ_{acute}) were found for dimethoate (0.3449). This value was followed by Omethoate, acetamiprid, thiacloprid and deltamethrin insecticides with the HQ_{acute} values of 0.1742, 0.1386, 0.0989 and 0.0513, respectively. The EDI values (calculated with Equation 3) for long-term risk ranged from 0.09E-05 to 0.65E-05. The chronic HQ (calculated with Equation 4) of insecticides ranged between 0.0025 to 1.1285. The highest values of chronic exposure ($HQ_{chronic}$) were found for omethoate (1.1285). Therefore, with a $HQ_{chronic}$ value of ≥ 1, omethoate represents a risk for human health. This value was followed by abamectin, novaluron, thiacloprid, dimethoate, and deltamethrin insecticides with the HQ values of 0.0813, 0.0618, 0.0511, 0.0474, and 0.0327, respectively (Table 4). Deltamethrin, dimethoate and thiacloprid are moderately hazardous (Classs II), while omethoate and abamectin are highly hazardous (Class Ib) insecticides (WHO, 2019).

Both acute and chronic risk of insecticides with no ARfD and ADI values in PPDB Database (tetramethrin) were not assessed. Similarly, acute risk assessments of insecticides with no ARfD values in PPDB Database (etoxazole, novaluron, pyriproxyfen, spirodiclofen) were not performed. Although MRL- exceeded residues were found for dimethoate and imidacloprid, risk assessment revealed that there was no consumer acute and chronic health risk of all the insecticides because HQ values of all the insecticides (except omethoate) were less than 1.0. Even if their HQs were below 1, the highest acute risk was found for dimethoate with acute HQ of 0.3449. There is a risk for human health for omethoate since HQ values of ≥ 1. The lowest acute HQ and chronic HQ values belonged to imidacloprid and pyriproxyfen residues, respectively (Table 4).

Balkan & Yılmaz (2022b) performed dietary risk assessments for 260 compound residues of leafy vegetables. Pesticide residues were detected in 57.6% of samples. Of the samples, five had residue levels of above MRLs, however they posed no short and long-term risks on consumer health. The greatest risk was detected for acetamiprid with HQ_{acute} of 0.97% and for cypermethrin with $HQ_{chronic}$ of 0.29%. Our findings were in agreement with Kanbolat et al. (2023)'s findings, indicating omethoate and dimethoate cause acute and chronic toxicity for consumers.

Soydan et al. (2021) performed chronic health risk assessments for pesticide residues of vegetable and fruits. The lowest EDI values ranged from 357E-5 to 898000E-5. Lower HQ values were observed in strawberry, grape and dried apricot with a value of 0.01, although HQ value of 32 out of 62 insecticides tested was almost 0.

Dülger & Tiryaki (2021) performed consumer dietary chronic risk assessments for tebuconazole boscalid and chlorpyrifos residues on nectarine and peach matrices. Pestice residues did not exceed the MRLs in any samples. Risk assessments based on WHO method revealed that chronic exposure levels of insecticides were low and there was no risk to human health.

Chronic risk assessment for imidacloprid residues in peach and peach juice was carried out by Camara et al. (2020). The potential consumer risk for the imidacloprid pesticide was negligible for human health. In another study, acute and chronic dietary exposure assessment for peach indicated that potential dietary risk induced by the acetamprid, imidacloprid, pyriproxyfen and spirodiclofen pesticides were not signifcant for Chinese consumers (Zhang et al., 2021)

Table 4. Chronic and acute risk assessments of insecticides for “Bayramiç Beyazı” nectarine

Compound	Number of residue data	Residue range (mean resdue). ug/kg	Short term Acute dietary risk			Long-term Chronic Dietary risk		
			ARfD**	ESTI**	HQ $_{Acute}$	ADI**	EDI**	$HQ_{Chronic}$
Abamectin*	3	5.79-6.4 (6.1)	0.005	0.21E-05	0.0425	0.0025	0.20E-05	0.0813
Acetamiprid	147	1.6-103.9 (19.62)	0.025	3.47E-05	0.1386	0.0250	0.65E-05	0.0262
Deltamethrin	72	3.0-38.5 (9.8)	0.025	1.28E-05	0.0513	0.0100	0.33E-05	0.0327
Dimethoate	24	1.0-103.5 (14.2)	0.010	3.45E-05	0.3449	0.0010	0.47E-05	0.0474
Etoxazole	20	1.0-10.7 (4.05)	NL***	-		0.0400	0.13E-05	0.0034
Imidacloprid	6	4.9-18.6 (11.3)	0.080	0.62E-5	0.0077	0.0600	0.38E-05	0.0063
Novaluron	9	8.4-29.2(18.5)	NL	-	-	0.0100	0.62E-05	0.0618
Omethoate	3	9.7-10.4(10.1)	0.002	0.35E-5	0.1742	0.0003	0.34E-05	1.1285
Pyriproxyfen	6	6.5-8.5(7.6)	NL	-		0.1000	0.25E-05	0.0025
Spirodiclofen	81	1.1-36.7(2.6)	NL	-		0.0150	0.09E-05	0.0058
Tetramethrin	3	96.0-115.5(106.6)	NL	-		NL	-	
Thiacloprid	27	1.0-89.0(15.3)	0.030	2.97E-05	0.0989	0.0100	0.51E-05	0.0511

* Abamectin ARfD and ADI values were taken from EFSA (2020).
** The unit of ARfD, ESTI, ADI and EDI is “mg/kg bw/day”.
*** NL: not listed; there was no specified ARfD and/or ADI in PPDB (2022).

Conclusion

Usage of insecticides is an important component of agricultural activities and significantly reduce labor costs for pest control. However, these chemical substances pose important risks on environment and human health. This study was conducted to investigate abamectin, acetamiprid, deltamethrin, dimethoate, etoxazole, imidacloprid, novaluron, omethoate, pyriproxyfen, spirodiclofen, tetramethrin, thiacloprid residues in “Bayramiç Beyazı” nectarines sampled from Çanakkale-Türkiye open market. The QuEChERS AOAC 2007.01 was efficiently used for the analyses of 12 insecticide residues on nectarine sample matrix. Method validation criteria were met. Residue levels of 9 insecticides were below the MRLs, whereas, in one sample each, dimethoate (approximately 10 times of MRL), imidacloprid (approximately 2 times of MRL) and omethoate (slightly over MRL) residues exceeded their MRLs. Diatery risk assessments revealed that present insecticide (except omethoate) concentrations did not pose any risks on human health. Omethoate was found to pose a chronic risk for human health. The highest acute HQ values were found for dimethoate, even if their HQ was ≤ 1. These two insecticides belong to the Organophosphate Class and should be taken into consideration. The use of dimethoate is in the process of being banned in Türkiye, while omethoate (metabolite of dimethoate) is already banned. Omethoate can cause residue as a degradation product of dimethoate.

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Original article (Orijinal araştırma)

Histochemical and ultrastructural analysis of macromolecules in trophocytes of the Oriental cockroach, *Blatta orientalis* (L., 1758) (Blattodea: Blattidae)[1]

Doğu hamam böceği *Blatta orientalis* (L., 1758) (Blattodea: Blattidae)'in trofositlerindeki makromoleküllerin histokimyasal ve ince yapı analizi

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Abstract

The fat body is a major storage area for glycogen, lipid and protein. The trophocyte is the main cell of fat body and stores these macromolecules. The fat body consists of two parts; peripheral and perivisceral. Peripheral fat body is located below the integument while perivisceral fat body is around the digestive tract. The study was conducted in EGEMIKAL Analysis Laboratory and Histology Laboratory of Ege University between 2018 and 2020. The fat body contents of insects at all stages were examined comparatively in three selected sections through histochemical and ultrastructural studies. We identified macromolecules stored in the trophocytes. Both the granular form of proteins and asterisk structure of glycogen localized around the lipid droplets were observed clearly. It was found that accumulation of protein continued in the trophocytes, but glycogen accumulation decreased considerably in adults compared to all nymphal stages. We also found that larger lipid droplets were stored in the PF fat body, while glycogen and protein accumulation was much higher in the PV fat body. These results may contribute to understanding of the mechanisms underlying activities such as amino acid, nitrogen, lipid and carbohydrate metabolism and protein synthesis in insects.

Keywords: *Blatta orientalis*, fat body, histochemistry, TEM, trophocyte

Öz

Böcek yağ dokusu glikojen, lipid ve protein için başlıca depolama alanıdır. Trofosit yağ dokusunun temel hücresidir ve bu makro molekülleri depolamaktadır. Yağ dokusu integümentin hemen altında yer alan periferal ve sindirim kanalının etrafında yer alan perivisseral yağ dokusu olmak üzere iki kısımdan oluşmaktadır. Bu çalışma 2018-2020 yılları arasında Ege Üniversitesi EGEMİKAL Analiz Laboratuvarı ve Histoloji Laboratuvarı'nda yürütülmüştür. Böceklerin tüm dönemlerine ait seçilen üç bölgesindeki yağ dokusu karşılaştırmalı olarak histokimyasal ve ince yapı çalışmalarıyla incelenmiştir. Trofositlerde depolanan makromoleküller belirlenmiştir. Hem granüler protein hem de lipid damlalarının etrafında yerleşim gösteren yıldız şeklindeki glikojen yapıları net bir şekilde gözlenmiştir. Tüm nimfal evrelerle erginler karşılaştırıldığında trofositlerde protein birikiminin devam ettiği, ancak glikojen birikiminin önemli ölçüde azaldığı belirlenmiştir. Ayrıca, periferal yağ dokusunda daha iri lipid damlaları depo edilirken, perivisseral yağ dokusunda ise daha fazla glikojen ve protein birikiminin olduğu tespit edilmiştir. Bu bulgular, böceklerde amino asit, nitrojen, lipid ve karbonhidrat metabolizmaları ve protein sentezi gibi faaliyetlerin altında yatan mekanizmaların anlaşılmasına katkı sağlayabilecektir.

Anahtar sözcükler: *Blatta orientalis*, böcek yağ dokusu, histokimya, TEM, trofosit

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Introduction

Insect fat body is considered as a tissue equivalent to that of the liver in vertebrates (Sobotnik et al., 2006; Liu et al., 2009; Gullan & Cransto, 2014). The fat body participates in numerous metabolic functions during the life cycle of insects. It is a major storage area for glycogen, lipid and protein (Haunerland & Shirk, 1995; Lipovsek & Novak, 2016) and the target organ of hormones such as neural, juvenile and ecdysone hormones (Hoshizaki, 2005; Roma et al., 2010; Lipovsek & Novak, 2016). It is also responsible for many activities such as amino acid, nitrogen, lipid and carbohydrate metabolism and protein synthesis (Liu et al., 2009; Arrese & Soulages, 2010; Li et al., 2019).

The fat body is divided into two regions in many insects. These are peripheral (PF) fat body located below the integument and perivisceral (PV) fat body, observed around the digestive tract (Resh & Carde, 2003; Lipovsek & Novak, 2016). These two regions can have different lipid, glycogen, and protein densities (Carvalho et al., 2013). It has been suggested that lipid synthesis and storage occur mainly in PF fat body, whereas PV fat body is associated with protein synthesis and storage in various insects (Dean et al., 1985; Roma et al., 2010).

The primary cell of fat body is the trophocyte. It differentiates to form other cell types; mycetocyte, urocyte, chromotocyte, and hemoglobin cells (Roma et al., 2010; Park et al., 2013; Toprak et al., 2020). A trophocyte is a major amorphous cell with a central nucleus. In its cytoplasm, there is an intense lipid, protein, and glycogen storage. In some species, lipids can be stored in the form of small droplets, while in others as large droplets that cover most of the cytoplasm. In some cases, proteins crystallize or form granules as they accumulate while carbohydrates are stored as glycogen (Paes-de-Oliveira & Cruz-Landim, 2003; Lipovsek et al., 2011; Vaca et al., 2019).

Cockroaches are thought to be harmful insects due to several diseases (dysentery, typhoid, cholera) they may transmit. Also, long-term contact with cockroaches may cause allergic rhinitis (Stankus et al., 1990) and asthma (Sohn & Kim, 2012; Bassirpour & Zoratti, 2014) in humans. Especially, *Blatta orientalis* (L., 1758) (Blattodea: Blattidae), *Blattella germanica* (L., 1767) (Blattodea: Blattellidae) and *Periplaneta americana* (L., 1758) (Blattodea: Blattidae) species are known as important household pests (Cornwell, 1968; Resh & Carde, 2003). Many forms of insecticides have been developed for these harmful insects, but due to their long-term use and toxicity to humans and animals, environmental contamination, and toxicity to non-target insects, these insects have started to show resistance to chemicals, like cyclodiene, deltamethrin, fipronil, imidacloprid, organophosphate, carbamate, pyrethroid insecticides (Thompson et al., 1993; Dehkordi et al., 2017; Nasirian & Salehzadeh, 2019). For this reason, newly created insecticides are focused on tissues such as fat body which was not considered as a convenient target before (Cornwell, 1968; Resh & Carde, 2003). For example, plants are of great interest for novel botanical insecticides (neem oils, pongom oils, essential oils from some aromatic plants) for insect pest- controlling agents without environmental contamination and toxicity (Pavela, 2007; Grdiša & Gršić, 2013). Therefore, it is very important to know the structure and contents of trophocytes in these insects throughout their development. Although trophocytes have been determined in the species of Blattodea orders (Park et al., 2013; Makki et al., 2014), there are not enough studies related to the *B. orientalis*. Our study focuses on macromolecules of trophocytes of cockroaches, *B. orientalis* (Oriental cockroach) at different developmental stages (6 nymphal stages and adult) under optimum conditions and employs different histochemical methods to analyze the macromolecules apart from light and electron microscopy observations.

Materials and Methods

Insect rearing and sampling

The study material, *B. orientalis* specimens were reared and monitored in Ege University, Faculty of Science-EGEMIKAL Analysis Laboratory, Insect Group Biological Activity Laboratory. The laboratory

conditions were kept constant at 25°C, 60-70% humidity, 12 hours night/12 hours day during the whole growing period. Further applications were carried out in the Histology Laboratory of Ege University between 2018 and 2020. Sampling was done at the beginning of each nymphal (6 in total) and adult stages as described by Zülfikaroğlu et al. (2022). Approximately 10 individuals at each stage were used for histology and histochemistry (240 in total) while 30 individuals were used for electron microscopy in total.

Histology and histochemistry

For histological analyses, the insects, which were divided into two parts, were fixed in Bouin's solution (saturated picric acid, formaldehyde, and acetic acid, 15:5:1) for 24 hours. Then, the fixative was washed away with 70% alcohol solution. After the dehydration (70-100%) and clarification (xylol), they were embedded in paraffin (Merck, 107337), and were cut into 5 µm thickness sections. Mayer's Hematoxylin-Eosin (H&E) (Merck, 109249; CI. 75290) was used for staining.

For histochemical analyses, two staining methods were applied: (1) Samples were fixed in Saint-Marie solution (95% ethanol, glacial acetic acid, 99:1) and dehydrated directly without washing. After the clarification, the tissues were embedded in paraffin and they were sectioned. These sections were stained with alcian blue 8GX (Merck, 105234; CI. 74240)-Periodic Acid Schiff (PAS) (Merck, 109033; CI 42500) for the determination of glycogen storages (2). They were fixed in 10% formalin solution and washed under running tap water for as long as the fixation period. After the routine histological preparation steps, the sections were stained with mercuric bromophenol blue (MBB) (saturated acetic acid, mercuric chloride, bromophenol blue (B8026, Sigma, 40:24:1) for the determination of protein storages (Humason, 1962; Presnell & Schreibman, 1997). Since the previously mentioned fixatives were insufficient for penetrating the enlarged body of the growing insects, the last nymphal and adult stages were fixed in Carnoy solution (60% ethanol, 30% chloroform, 10% glacial acetic acid) for both histological and histochemical analyses. This fixative was preferred because of its compatibility with the stains used in this study. All samples were kept in fixatives for at least 24 hours. Finally, the slides were photographed through ZEN image analysis software using a Zeiss Axio Scope A1 microscope.

Transmission electron microscopy

At the last nymphal stage, female and male cockroaches were dissected. The fat bodies obtained from the cockroaches were placed in Eppendorf tubes with a Karnovsky fixative (Karnovsky, 1965) (0.2M cacodyl tampon, 25% glutaraldehyde, 8% paraformaldehyde and distillated water). The samples were washed with buffer after the overnight incubation at +4ºC. Then, the second fixative, 1% osmium tetroxide (Millonig, 1961), was applied. After that, semi-thin and thin sections were cut and stained as in Zülfikaroğlu et al. (2022). Finally, both section types were examined and photographed under a Zeiss Axio Scope A1 light microscope and a Transmission Electron Microscope (TEM) Geol 100C accordingly.

Statistical analysis

The diameters of lipid droplets in semi-thin sections from the 6th nymph, female and male cockroaches, which showed the most difference between stages, were measured with the help of ZEN image analysis software. The measurements were carried out in three regions (thorax, beginning, and end of abdomen) in a selected area of 500 μm^2. Measurements were conducted 3 times for 10 individuals per stage (last nymph, female and male). Considering the diameter variable, the three stages were compared firstly in terms of each region, and then the three regions were compared across the stages. In order to statistically perform these comparisons, One Way ANOVA method was planned to be applied, but since the assumptions for normality and homogeneity of variances required for this method were not ensured, Kruskal-Wallis test was applied. All analyses were performed using the statistical software-IBM SPPS 25.

Results

To show the general structure and morphology of the trophocytes, H&E staining was used. Trophocytes are generally large and their nucleus is centrally located. In the study, the lipid droplets in the cytoplasm appeared on the foreground with their transparent appearance following the H&E staining. These cells are separated from one another by a basal lamina. Two types of fat body were detected for all the stages. While the cell boundaries of trophocytes were more distinct and compact for the early stages their boundaries were transformed into a spongy structure at the 6th nymphal and adult stages, especially in the PV fat body (Figure 1).

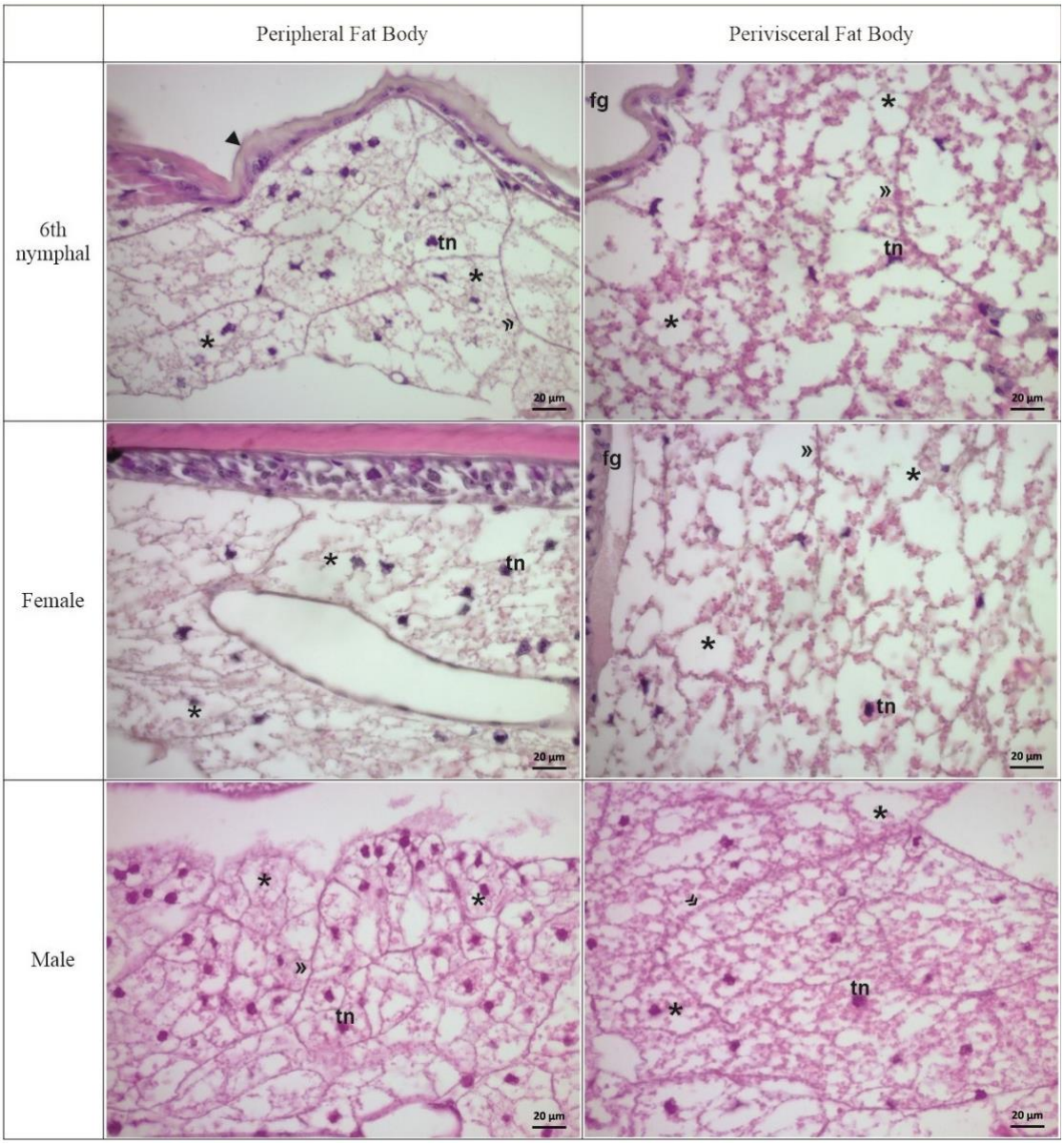

Figure 1. Peripheral and perivisceral fat bodies in the thorax regions of various stages stained with Mayer H&E. fg: foregut; tn: nucleus of trophocyte; *: trophocyte; ►: integument; »: basal lamina.

PAS staining was used to show glycogen accumulation in the fat bodies of *B. orientalis*. In the developmental stages, the thorax, the beginning and the end of the abdomen revealed lipid droplets in the middle of trophocytes, with pink-purple colored glycogen accumulation around them. In all three regions, it was observed that the density of glycogen deposits accumulated in the trophocytes of the PF fat body was less than that of the PV fat body (Figures 2&3). Additionally, the amount of glycogen stored in trophocytes decreased considerably in adults compared with all the nymphal stages (Figure 3).

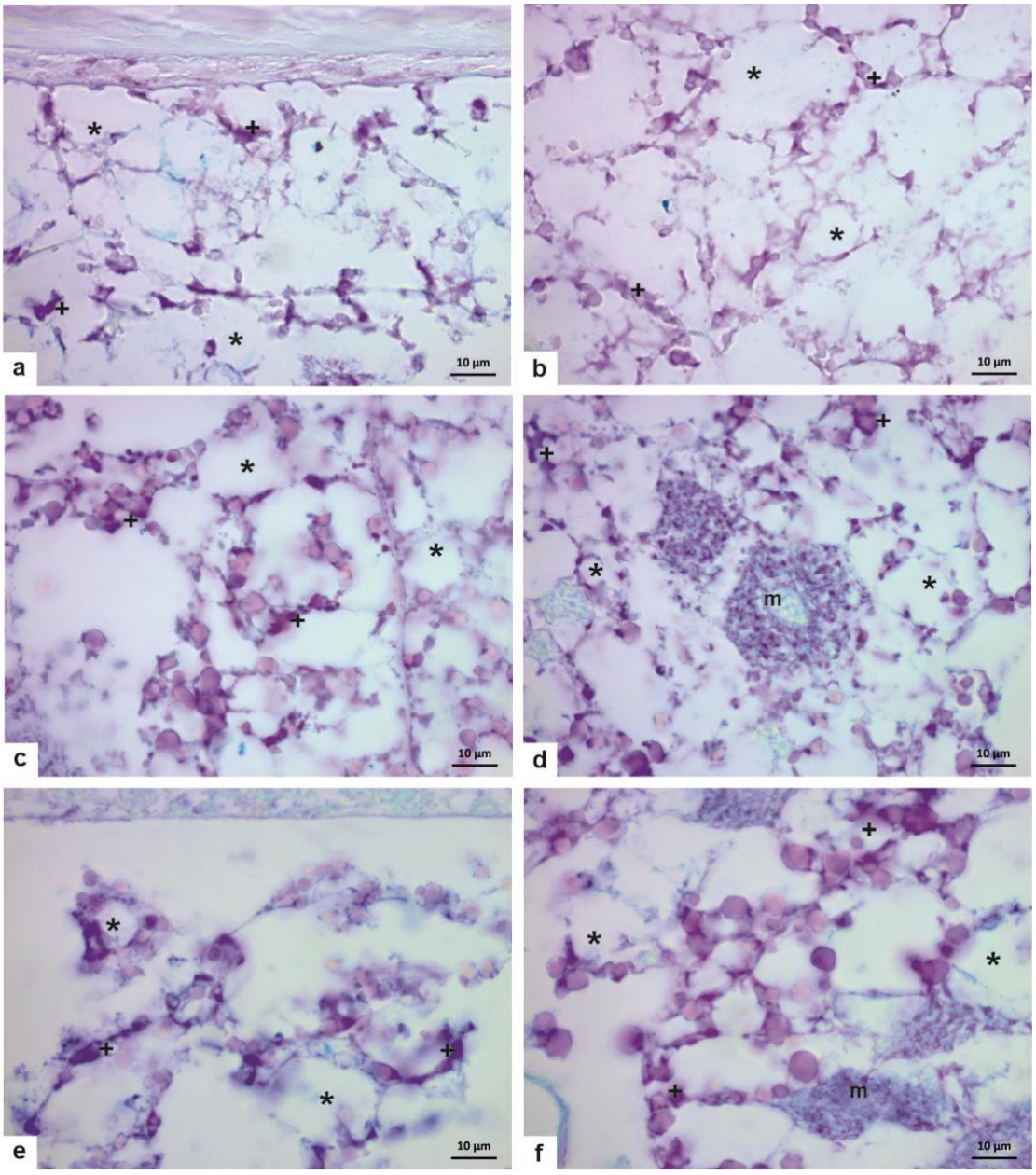

Figure 2. Accumulation of glycogens at the 6th nymphal stage stained with PAS: a) thorax PF fat body; b) thorax PV fat body; c) beginning of abdomen PF fat body; d) beginning of abdomen PV fat body; e) end of abdomen PF fat body; f) end of abdomen PV fat body. m: mycetocytes; *: trophocyte; +: glycogen.

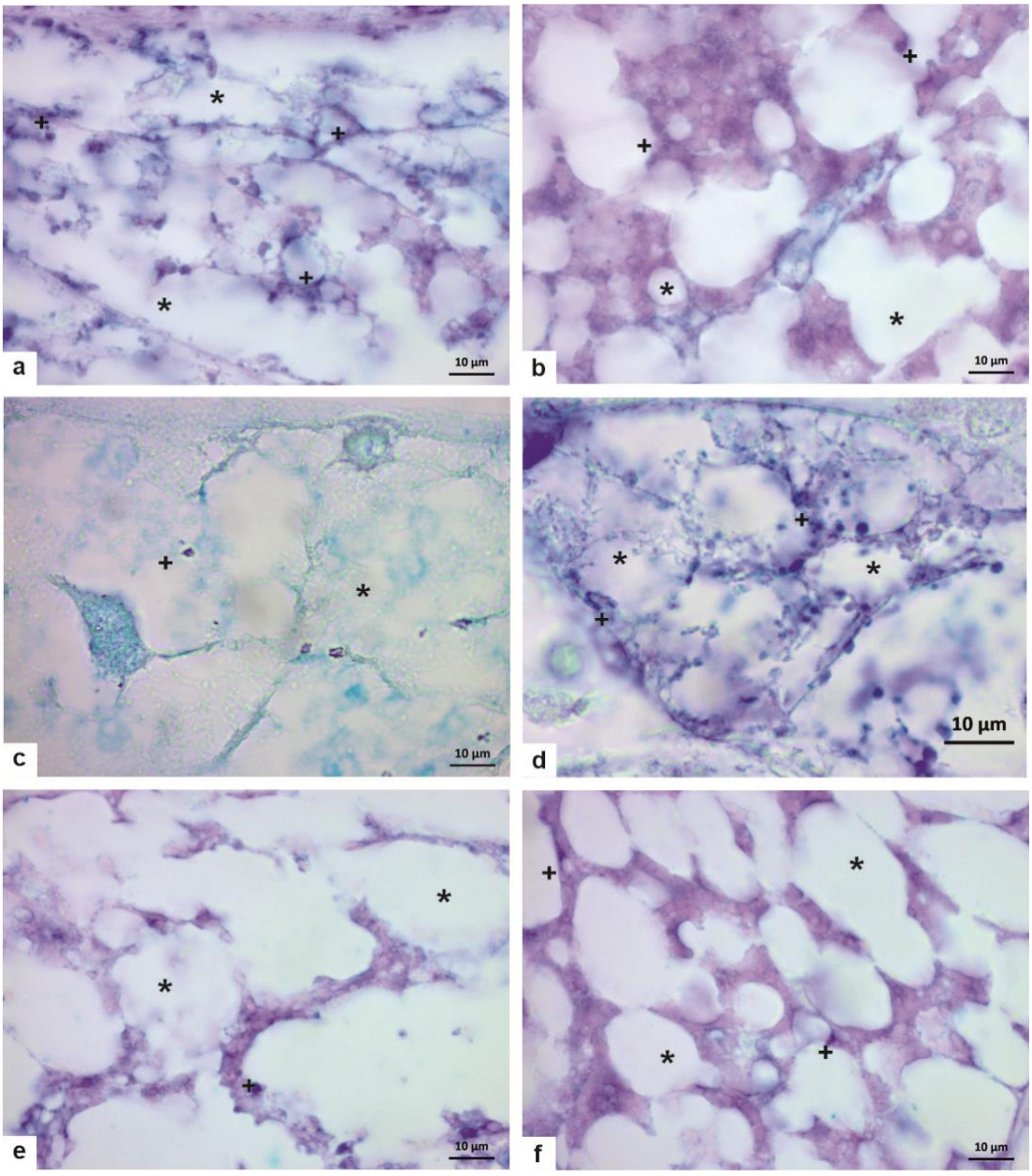

Figure 3. Accumulation of glycogens in the adult stage stained with PAS: a) thorax PF fat body; b) thorax PV fat body; c) beginning of abdomen PF fat body; d) beginning of abdomen PV fat body; e) end of abdomen PF fat body; f) end of abdomen PV fat body. *: trophocyte; +: glycogen.

Proteins accumulating in the trophocytes were stained with MBB. Protein granules were blue colored and like glycogen; they were positioned around lipid droplets (Figures 4&5). Throughout the development of the nymph, there was a notable increase in the accumulation of protein granules. Examination of the PF and PV fat bodies in each region revealed that the accumulation of protein granules in the PF fat body was less significant than in the PV fat body (Figures 4&5).

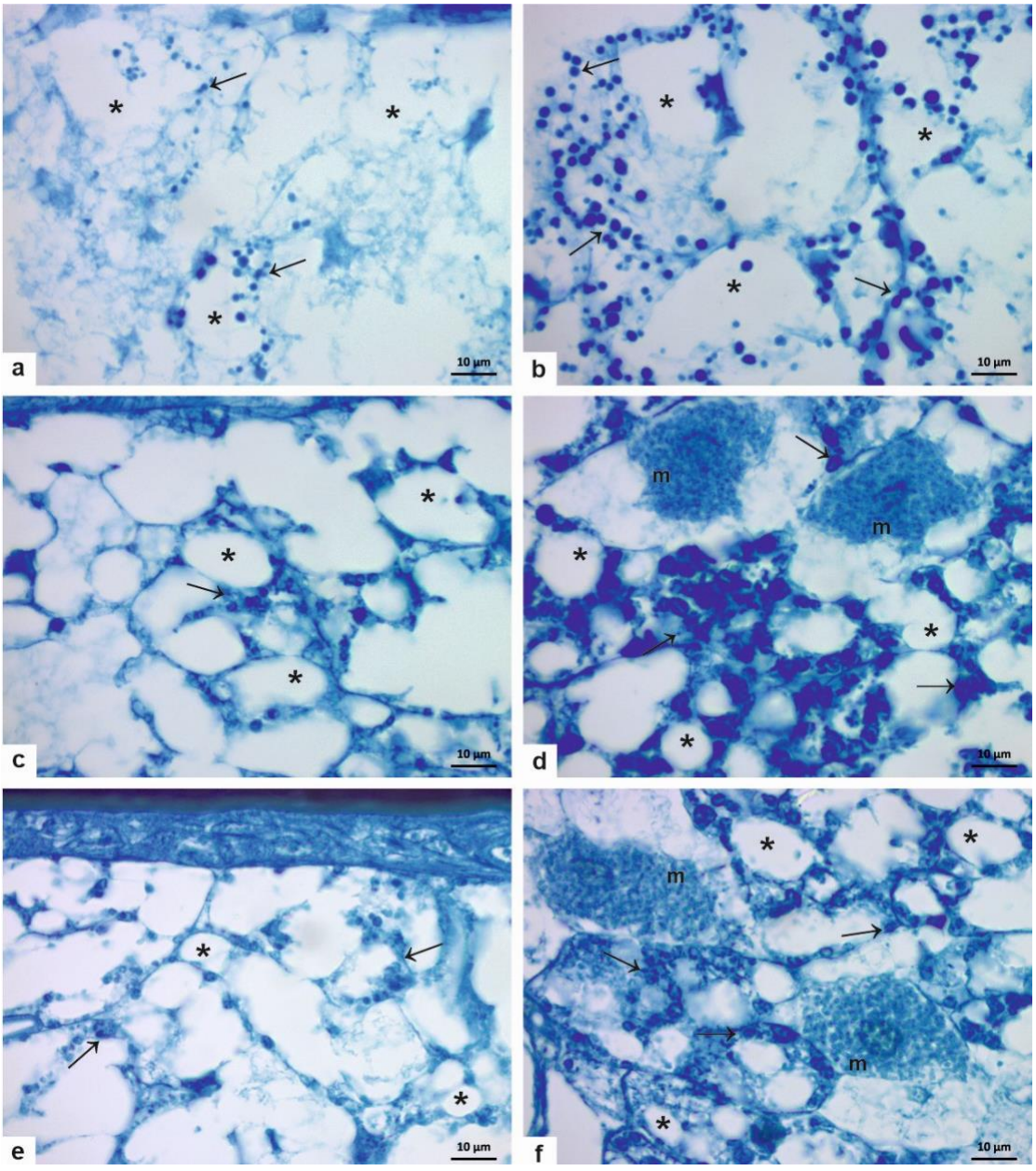

Figure 4. The accumulation of proteins at the 6th nymphal stage stained with MBB: a) thorax PF fat body; b) thorax PV fat body; c) beginning of abdomen PF fat body; d) beginning of abdomen PV fat body; e) end of abdomen PF fat body; f) end of abdomen PV fat body. m: mycetocytes; *: trophocyte; →: protein granules.

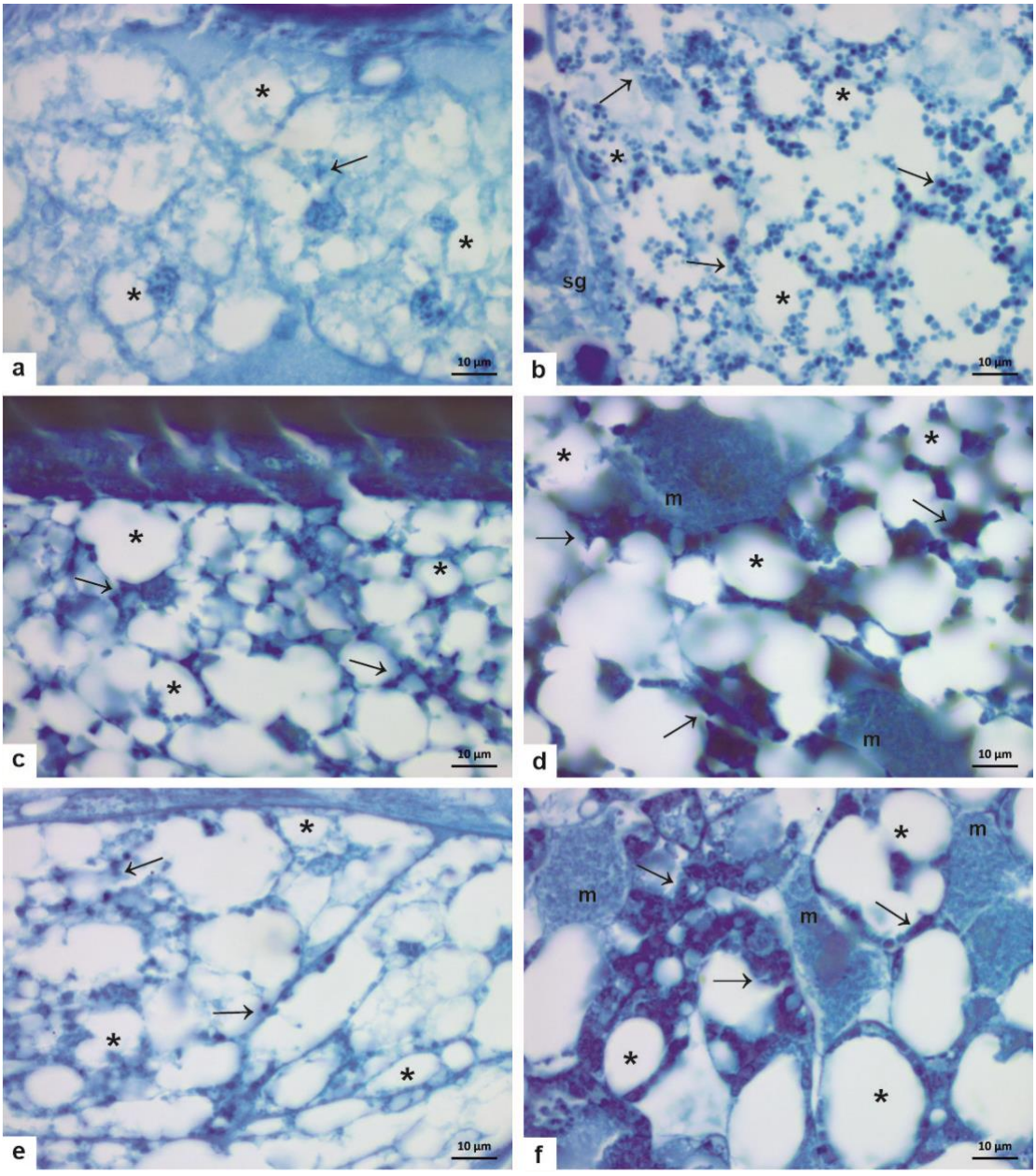

Figure 5. The accumulation of proteins in the adult stained with MBB: a) thorax PF fat body; b) thorax PV fat body; c) beginning of abdomen PF fat body; d) beginning of abdomen PV fat body; e) end of abdomen PF fat body; f) end of abdomen PV fat body. m: mycetocytes; sg: salivary gland; *: trophocyte; →: protein granules.

The semi-thin sections of the 6th nymphal stage and adult (female and male) cockroaches, stained with toluidine blue, showed the lipid droplet boundaries clearly in the cytoplasm of trophocytes (Figure 6). Thus, these sections were used for the statistical analyses. Furthermore, the thin sections of these stages revealed electron-lucent lipid droplets (Figure 7). Additionally, the other macromolecules were easily distinguished around the nucleus on electron microscopy images. While the glycogen deposits from the 6th nymphal and adult stages showed an asterisk shape structure scattered in the cytoplasm, the dark granular form of the storage proteins were also evident in these stages (Figure 7).

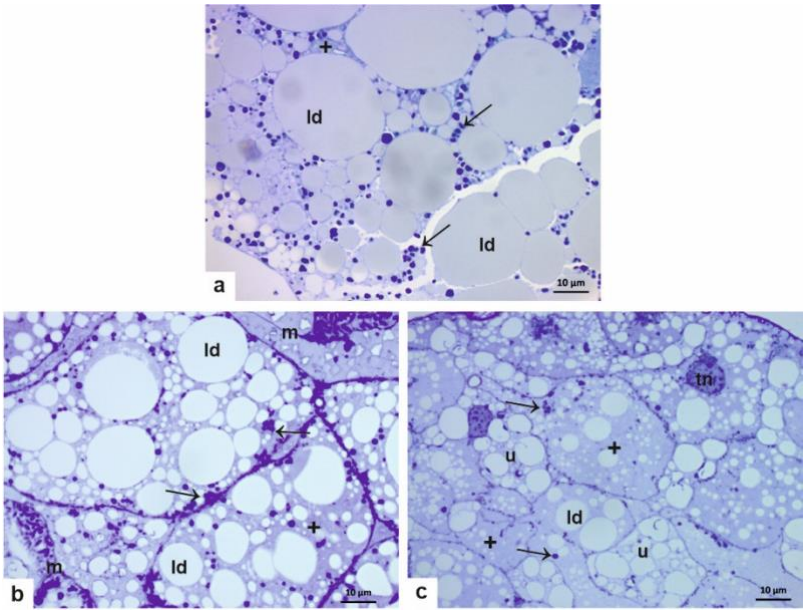

Figure 6. Demonstration of trophocytes stained with toluidine blue: a) 6th nymphal stage thorax; b) female end of abdomen; c) male end of abdomen. ld: lipid droplet; m: mycetocyte; tn: nucleus of trophocyte; u: urocyte; +: glycogen; →: protein granule.

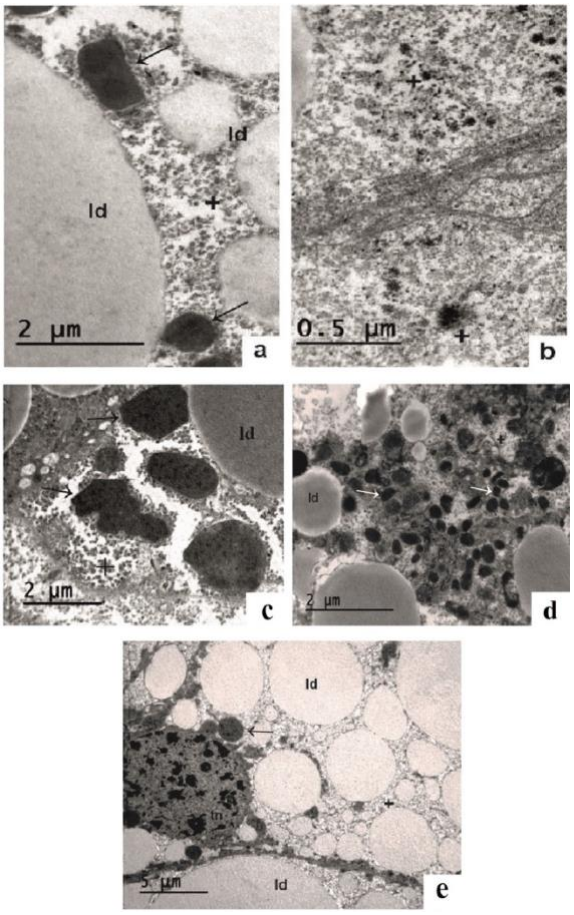

Figure 7. Ultrastructures of trophocytes: a) 6th nymphal thorax; b) Adult thorax; c) 6th nymphal stage, beginning of abdomen; d) adult, beginning of abdomen; e) adult, end of abdomen. ld: lipid droplet; tn: nucleus of trophocyte; +: glycogen; →: protein granule.

According to the Kruskal-Wallis test results, it can be said that there is statistically no significant difference in the diameters across all the three stages and three regions. p-Value>0.05 for all comparisons (Tables 1&2). Also, Figure 8 shows the mean diameter comparisons between stages and regions. Error bars are constructed using standard deviation.

Table 1. Kruskal-Wallis test results for comparisons between three stages while one region held constant

Null Hypothesis	Region	Kruskal-Wallis Test Statistic	p-Value
The distribution of "diameter" is the same across categories of "stage"	Thorax	1.910	0.385
	Beginning of abdomen	4.113	0.128
	End of abdomen	4.056	0.132

The significance level is 0.05.

Table 2. Kruskal-Wallis test results for comparisons between three regions while one stage held constant

Null Hypothesis	Stage	Kruskal-Wallis Test Statistic	p-Value
The distribution of "diameter" is the same across categories of "region"	6th nymph	0.923	0.630
	Female	0.534	0.766
	Male	0.083	0.959

The significance level is 0.05.

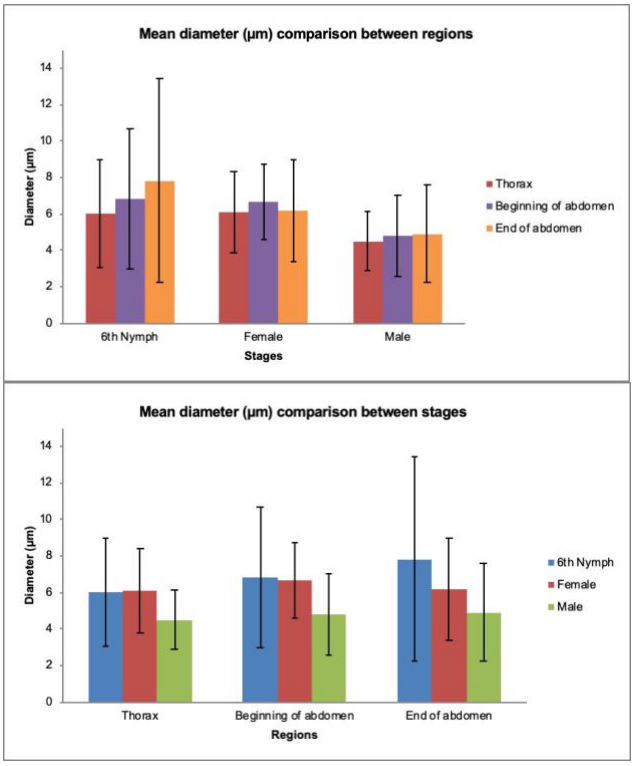

Figure 8. Mean diameter (µm) (±SD) comparisons. No significant differences observed among means ($p < 0.05$).

Discussion

There are numerous studies on the structure, composition, and function of fat body in insect species. For example, studies on *Leptinotarsa decemlineata* (Say, 1824) (Coleoptera: Chrysomelidae) and some Diptera species have shown that larger lipid droplets are stored in the PF fat body, while the PV fat body has smaller lipid droplets and contains more protein granules (Dean et al., 1985). The abundance of protein in the PV fat body of *Helicoverpa zea* Boddie, 1850 (Lepidoptera: Noctuidae) was also determined to be higher than that in the PF fat body (Haunerland & Shirk, 1995). In *Lutzomyia longipalpis* (Lutz & Neiva, 1912)

(Diptera: Psychodidae) and *Phlebotomus papatasi* (Scopoli, 1786) (Diptera: Psychodidae), it was observed that glycogen accumulation was higher in PV fat body cells after feeding the flies with sugar (Assis et al., 2014). Although the PF and PV fat bodies of the caterpillar *Anticarsia gemmatalis* Hübner, 1818 (Lepidoptera: Erebidae) have different roles, it has been stated that both of them were involved in the synthesis of lipids and other substances (Carvalho et al., 2013). In our study on *B. orientalis*, we observed that larger lipid droplets were stored in the PF fat body while more protein granules and glycogen were deposited in the PV fat body similar to the results reported by Dean et al. (1985) and Haunerland & Shirk (1995).

Trophocytes are the basic cells in the fat body and store lipids, proteins, and glycogens (Arrese & Soulages, 2010; Azeez et al., 2014; Lipovsek & Novak, 2016). We have not found any studies comparing the cell boundaries of trophocytes in PF and PV fat bodies in literature. there is a study on *A. gemmatalis*, in which cytoplasm of the trophocytes in the PF fat body was reported to be more acidophilic while that of PV was found to be more basophilic (Carvalho et al., 2013), which suggested that this situation was associated with trophocytes in PV containing a large amount of granular endoplasmic reticulum (GER). In our study on the Oriental cockroach, we have found that the cellular boundaries of both the PF and PV fat bodies were more distinct in the first nymphal stages, whereas in 6th nymphal and adult stages, especially in the PV fat body, their boundaries were deteriorated and transformed into a more reticular structure. This could be resulted from the increased accumulation of glycogen and protein in the PV fat body as the development proceeds.

Generally, studies show that trophocytes contain an irregular-shaped nucleus with dense chromatin and cytoplasm with various vesicular structures, mitochondria, GER and Golgi body (Paes-de-Oliveira & Cruz-Landim, 2003; Roma et al., 2010). These vesicular structures in the cytoplasm; are either digestive vacuoles (autophagic, heterophagic, multi-vesicular bodies) or storage vacuoles (protein, glycogen, lipid, urates) (Locke, 1984; Roma et al., 2010). In *B. orientalis*, we have also found that the trophocytes contained a nucleus with very dense chromatin and cytoplasm with accumulations of lipids, proteins and glycogens, as observed in *Aedes aegypti* (L., 1762) (Diptera: Culicidae) (Martins & Ramalho-Ortigao, 2012), *Melipona quadrifasciata* Lepeletier, 1836 (Hymenoptera: Apidae) (Furtado et al., 2013), *Pachycondyla villosa* (Fabricius, 1804) (Hymenoptera: Formicidae) (Zara & Caetano, 2004) and *P. americana* (Park et al., 2013).

Lipids, which constitute about 70% of the cytoplasm in trophocytes, are mostly in the form of triglycerides. When transported to hemolymph, they are converted into diglyceride. In fat bodies of different insects, lipids can be stored around the nucleus as small or large droplets (Paes-de-Oliveira & Cruz-Landim, 2003; Lipovsek et al., 2011). It has been shown that lipid accumulation is vital to meet the basic energy needs of flying muscles and other organs in the species of Diptera order (Assis et al., 2014), or during metamorphosis in *Calpodes ethlius* (Stoll, 1782) (Lepidoptera: Hesperiidae) (Haunerland & Shirk, 1995), and during foraging and oogenesis in the Attini tribe (Roma et al., 2010). While lipids needed in the cuticle are provided by oenocytes and trophocytes (Klowden, 2007), variations may be seen in the lipid storage capacity across males and females for some species due to different needs. For example, it has been stated that the lipid storage in male *Hyalophora cecropia* L., 1758 (Lepidoptera: Saturniidae) is higher compared to the female due to long flights during the search for mates (Dean et al., 1985). In our study on *B. orientalis*, the analysis of trophocytes' lipid content was done on individuals at the 6th nymphal stage and adults (female and male). The lipids were stored as small or large, white spherical droplets around the nucleus. We propose that the female stores lipid as an energy source at the 6th nymphal stage to create egg packages; whereas in males, lipid stores are used for foraging and mate-seeking. The analysis of the lipid droplet measurements revealed no statistically significant difference between individuals at the 6th nymphal and adult stages as well as among the regions (thorax, beginning, and end of abdomen). This may be due to the cockroach being a hemimetabolous insect, and it does not undergo a major change during metamorphosis.

Another macromolecule that meets the basic energy needs in addition to lipids in trophocytes is carbohydrate. Among insects, carbohydrates are stored as glycogen, similar to other animal groups (Lipovsek et al., 2011; Li et al., 2019; Vaca et al., 2019; Toprak et al., 2020). It was thought that the high number of glycogen stores detected in *Atta laevigata* (Smith, 1858) (Hymenoptera: Formicidae) and *Mycetarotes parallelus* (Emery, 1906) (Hymenoptera: Formicidae) are used for foraging and courtship display (Roma et al., 2010). Also, glycogen is mainly used in the formation of the cuticle (Klowden, 2007). In our study on *B. orientalis*, it has been determined that glycogen was deposited freely in the cytosol. In nymphal stages, glycogen accumulated intensely, especially in the PV fat body. However, after transitioning to the adult form, the amount of glycogen considerably decreased since it was used for cuticle formation and maturation of gonads. In the studies of other species, similar results have been found related to glycogen. For example, in *Rhodnius* genus, which is commonly known as kissing bugs, the glycogen stores are considerably reduced after the formation of the cuticle (Dean et al., 1985). In *Bombus terrestris* (L., 1758) (Hymenoptera: Apidae), *Calpodes ethlius* (Stoll, 1782) (Lepidoptera: Hesperiidae) and *P. villosa* (Zara & Caetano, 2004), it was observed that the trophocyte cytosol had glycogen stores accumulated freely as long as the feeding continued (Roma et al., 2010). Park et al. (2013) determined that lipid and glycogen stores were exhausted in *P. americana*, as in *Manduca sexta* L., 1763 (Lepidoptera: Sphingidae) in case of prolonged fasting. Then, they showed that the glycogen was converted rapidly into trehalose, the sugar form in the hemolymph, and the repositories were quickly filled after refeeding.

Storage of proteins in trophocytes may have various forms in different species. For example, in the ants of the Attini tribe, they are stored in granular form of various sizes; whereas in *Atta sexdens rubropilosa* Forel, 1908 (Hymenoptera: Formicidae) and *Monomorium pharaonis* (L., 1758) (Hymenoptera: Formicidae), they are accumulated in crystal form (Roma et al., 2010). Also, in some Lepidoptera, such as silkworm, s protein accumulation continues during the pupal stage and it is exhausted a few days after becoming an adult (Dean et al., 1985). In *H. zea*, when the larval stage is completed, the protein synthesis is stopped and they are stored in granular form in the trophocytes (Roma et al., 2010). However, in cockroaches such as *P. americana*, protein accumulation starts at the beginning of the nymphal stage and continues during adult life even if there are fluctuations due to metabolic functions. Furthermore, after the cuticle synthesis in *Rhodnius*, it was determined that the protein storage was not exhausted, unlike glycogen (Dean et al., 1985). As with *A. laevigata* and *M. parallelus* (Roma et al., 2010), intense protein accumulation was observed in *P. villosa* and *Scaptotrigona postica* (Latreille, 1807) (Hymenoptera: Apidae) (Zara & Caetano, 2004), and the irregular-shaped nucleus in their trophocytes was also thought to support the intense protein synthesis (Roma et al., 2010). In *B. orientalis*, we have observed a dense protein accumulation during the developmental stages, especially in the PV fat body. In parallel to the findings in the literature (Dean et al., 1985), the protein storage didn't decrease after the adult stage. We have demonstrated that proteins were stored in a granular form. The active protein synthesis and storage could be supported by the trophocytes with multilobed nuclei.

Literature information on the fat body structure in *B. orientalis* is currently inadequate. Majority of the studies used *Periplaneta* sp. as an organism, and some are focused on the effects of stress (Chowański et al., 2017) and starvation (Park et al., 2013). In studies using *B. germanica* (Patino-Navarrete et al., 2014) and *B. orientalis* species (Corsaro et al., 2007), only symbionts in mycetocytes were examined. In this study, histologically reliable comparisons were made by determining standard locations such as the thorax, the beginning of the abdomen and the end of the abdomen. Different fat body types in the first and last nymphal stages and adults were also included as peripheral and perivisceral fat body. Furthermore, the article was statistically enriched by measuring the diameters of lipid droplets in the trophocytes in the semi-thin sections of adults with the last nymphal stage. We also analyzed the macromolecules of trophocytes in *B. orientalis* at different developmental stages under optimum conditions for the insect, which can be used as a baseline for comparative analyses under stress conditions.

In conclusion; lipid, protein, and glycogen contents stored in the trophocytes of *B. orientalis* under optimum-rearing conditions were analyzed in this study. We hope that these results will contribute to understanding the mechanisms underlying the activities such as; amino acid, nitrogen, lipid and carbohydrate metabolism and protein synthesis in insects. We think that it would be more reasonable to apply the application in the nymphal stages of cockroaches in future insecticide studies. In this way, it will be more effective in insect control as there will be a decrease in the transition rate of the insect to adult. Both the PV fat body providing the energy required for the transition to adult, and the PF fat body being supportive in protecting against external factors (cold, impact, etc.) are affected. Hopefully, these data may provide new insights for pest control studies.

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Original article (Orijinal araştırma)

Susceptibility of different *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) populations to indigenous *Bacillus thuringiensis* strains[1]

Farklı *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) popülasyonlarının yerel *Bacillus thuringiensis* suşlarına duyarlılığı

Ardahan ESKİ[2]*

Abstract

Tomato leafminer, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) is one of the most important tomato pests worldwide and causes 100% product loss if not controlled. Chemical insecticides, which have been overused for many years, have induced resistance in the pests and made it difficult to control their populations in the field. The use of biological agents that express insecticidal proteins, such as *Bacillus thuringiensis*, is an alternative to conventional insecticides to suppress pest populations. In this study, to recover novel *B. thuringiensis* strains from soil samples, a survey was conducted in Bilecik province in 2021. Thirteen local *B. thuringiensis* strains were isolated and the susceptibility of three different field populations (Samsun, İzmir, and Bilecik) of *T. absoluta* to these strains was evaluated. *Bacillus thuringiensis* B3 (*Bt*-B3) strain, which contains lepidopteran-active toxin genes, was more virulent for all *T. absoluta* populations tested. In addition, Samsun population was more sensitive to the B3 strain than İzmir and Bilecik. The LC_{50} values of *Bt*-B3 were determined to be 13.28, 26.06 and 24.24 ppm for Samsun, İzmir and Bilecik populations, respectively. Sequencing of the 16S rRNA gene region confirmed that the isolate was *B. thuringiensis*, while electron microscopy revealed that the isolate produced bipyramidal, cubic and spherical insecticidal proteins. The results of this study indicate that the isolate *Bt*-B3 appears to be a promising biocontrol agent for integrated pest management of *T. absoluta* in Türkiye.

Keywords: *Bacillus thuringiensis*, biocontrol, *cry* genes, *Tuta absoluta*

Öz

Domates güvesi, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) dünya çapında en önemli domates zararlılarından biridir ve mücadele edilmediği takdirde %100 ürün kaybına neden olur. Uzun yıllardır aşırı kullanılan kimyasal insektisitler, zararlıda direnç oluşturmuş ve popülasyonlarını kontrol etmeyi zorlaştırmıştır. *Bacillus thuringiensis* gibi insektisidal proteinleri eksprese eden biyolojik savaş etmenlerinin kullanımı, zararlı popülasyonlarını baskılamak için geleneksel insektisitlere bir alternatiftir. Bu çalışmada, toprak örneklerinden yeni *B. thuringiensis* suşları elde etmek için 2021 yılında Bilecik ilinde bir sürvey yapıldı. On üç yerel *B. thuringiensis* suşu izole edilmiş ve üç farklı tarla popülasyonunun (Samsun, İzmir ve Bilecik) bu suşlara duyarlılığı değerlendirilmiştir. Lepidopteran-aktif toksin genleri içeren *B. thuringiensis* B3 (*Bt*-B3) suşu, test edilen tüm *T. absoluta* popülasyonlarında daha virülent bulundu. Ayrıca Samsun popülasyonu, B3 suşuna İzmir ve Bilecik popülasyonuna göre daha duyarlıydı. *Bt*-B3'ün LC_{50} değerleri Samsun, İzmir ve Bilecik popülasyonları için sırasıyla 13.28, 26.06 ve 24.24 ppm olarak belirlendi. İzolatın 16S rRNA gen bölgesinin sekanslanması, *B. thuringiensis* olduğunu doğrularken, elektron mikroskopisi izolatın bipiramidal, kübik ve küresel insektisidal proteinler ürettiğini ortaya koydu. Çalışma sonuçları, *Bt*-B3 izolatının Türkiye'de *T. absoluta*'nın entegre zararlı mücadelesi için umut verici bir biyolojik savaş etmeni olduğunu göstermektedir.

Anahtar sözcükler: *Bacillus thuringiensis*, biyolojik savaş, *cry* genleri, *Tuta absoluta*

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Introduction

The tomato leafminer, *Tuta absoluta* (Meyrick, 1917) (Lepidoptera: Gelechiidae) is known to be one of the most destructive pests affecting tomato production in the Mediterranean region. *Tuta absoluta* is an oligophagous pest that damages several species of Solanaceae plants such as potato, eggplant, tobacco, common bean, and various wild solanaceous plants (Desneux et al., 2011; Zhang et al., 2021).

The larvae can decimate both yield and fruit quality by feeding on leaves, stems and fruit. The pest has been responsible for losses of 80-100% in tomato plantations in the greenhouse and open field. Yield losses of up to 100% have been reported in severe outbreaks, and even with the use of conventional pesticides, losses can still exceed 5% (Terzidis et al., 2014). The main approaches currently used to control *T. absoluta* are based on synthetic chemical pesticides. Unfortunately, some of them do not provide the desired effect, as resistance develops due to intensive use. Resistance to avermectins, pyrethroids, diamides, benzoylureas, organophosphates, oxadiazines, semicarbazones and spinosyns have been reported worldwide (Siqueira et al., 2001; Silva et al., 2016; Langa et al., 2022). The pest may also develop resistance to other insecticides over time. In addition, the adverse effects of chemicals on non-target organisms limit the use of its predators, -as biological control strategies (Arnó & Gabarra, 2011). Therefore, integration with other control strategies such as biological and biotechnological methods is necessary instead of chemical insecticides.

An alternative to chemical insecticides is insect-pathogenic microorganisms such as *Bacillus thuringiensis* (*Bt*), which produce insecticidal crystal proteins during the sporulation phase. *Bt* strains may have one or more *cry* genes, providing the strains to express one or more Cry proteins. The crystal proteins shown to have lethal effect against different orders of insects such as Lepidoptera, Coleoptera, and Diptera. To date, local *Bt* isolates and *Bt*-based biopesticides have been tested to suppress *T. absoluta* populations under laboratory, greenhouse and open field conditions (Giustolin et al., 2001; Sandeep Kumar et al., 2020; Aynalem et al., 2021; Buragohain et al., 2021).

It is important to continue the search for novel *Bt* strains that are effective to lepidopteran insects such as *T. absoluta* because their virulence is not fully same in different ecological conditions, as there are insect populations that are resistant to *Bt*. Therefore, the aim of this study was to isolate novel *Bt* strains containing lepidopteran-active *cry* genes and to assess the susceptibility of different field populations of *T. absuluta*.

Materials and Methods

Isolation of *Bacillus thuringiensis* from soil samples

Soil samples were collected from different locations in Bilecik, Türkiye, during the summer of 2020. After scraping the surface material, the samples were collected from a depth of 15 cm below the surface using a soil corer and placed in an autoclaved glass jar (Eski & Gezgin, 2022). The samples were transferred to the laboratory for isolation of *B. thuringiensis*.

Bacillus thuringiensis isolation was conducted according to the procedure described by Santana et al. (2008). Five grams of samples were preheated in an incubator at 80°C for 3 hours. One gram of the soil sample was mixed in 10 ml of 0.85% NaCl by vigorous shaking for 2 minutes and serially diluted tenfold with phosphate saline buffer. Dilutions were then incubated at 80°C for 12 minutes and a 0.1 ml sample was spread on tryptic soy agar plates. After incubation at 30°C for 2 days, *Bt*-like colonies were picked up (cream-colored and looking like fried eggs on the plate) and purified by serial spreading.

Presence of insecticidal crystal proteins

Potential *Bt* isolates were grown in terrific broth medium (Estruch et al., 1996) at 200 rpm and 30°C until the complete lysis of cells. Then, spore and crystals were collected by centrifugation at 12000 × g for 5 min. The pellet containing spores and crystals was washed with cold NaCl (0.5 M) to remove extracellular components and finally resuspended in sterile distilled water. The spore-crystal mixtures of each isolate were stained with amido black and Ziehl's carbol fuchsin (Smirnoff, 1962) and checked for the presence of crystal under a phase contrast microscope. In addition, the spore-crystal mixtures were stored at 4°C to be used for screening tests.

Cry gene profile of isolates

Total DNA was extracted from 100 mg wet weight of bacteria (approximately 10^9 bacterial cells) using the Zymo DNA isolation kit (Zymo Research, Irvine, CA, USA). *Cry* genes were amplified by PCR using *cry* gene specific oligonucleotide primers (Table 1) (Jain et al., 2012). The 25 µl of PCR mixture contained 200 µM of each dNTP, 0.2 µM concentration of each primer, 0.4 U *Taq* DNA polymerase, 2.5 µl 10× PCR buffer, 1 µl total DNA template. Reactions were set for 30 cycles consisting of denaturation at 95°C for 1 min, annealing at 49-60°C (depended on each pair of primer) for 30 s and extension at 68°C for 30 s. PCR products were electrophoresed on a 1.5% agarose gel in TAE buffer and visualized under UV transilluminator.

Table 1. Oligonucleotide primers used in *cry* gene screening

Primer (product size)	Primer sequence (5' -> 3')	T_m (°C)
cry1 (277 bp)	CATGATTCATGCGGCAGATAAAC (f) TTGTGACACTTCTGCTTCCCATT (r)	55
cry2 (701 bp)	GTTATTCTTAATGCAGATGAATGGG (f) CGGATAAAATAATCTGGGAAATAGT (r)	52
cry3 (604bp)	CGTTATCGCAGAGAGATGACATTAAC (f) CATCTGTTGTTTCTGGAGGCAAT (r)	54
cry4 (439 bp)	GCATATGATGTAGCGAAACAAGCC (f) GCGTGACATACCCATTTCCAGGTCC (r)	59
cry5 (474 bp)	TTACGTAAATTGGTCAATCAAGCAAA (f) AAGACCAAATTCAATACCAGGGTT (r)	52
cry7 (420 bp)	AAGCAGTGAATGCCTTGTTTAC (f) CTTCTAAACCTTGACTACTT (r)	49
cry9 (359 bp)	CGGTGTTACTATTAGCGAGGGCGG (f) GTTTGAGCCGCTTCACAGCAATCC (r)	60
cry11 (305 bp)	TTCCAACCCAACTTTCAAGC (f) AGCTATGGCCTAAGGGGAAA (r)	51

f: forward primer, r: reverse primer.

Screening experiments

The spore-crystal mixture used in the bioassays was prepared as described above and dried using a freeze dryer according to the manufacturer's instructions. The freeze-dried spore-crystal mixtures were then resuspended in sterile distilled water at a concentration of 50 ppm and used in bioassays.

Susceptibility of three different field populations (Samsun, İzmir and Bilecik) of *T. absoluta* to spore crystal mixtures was evaluated using the leaf-dip bioassay as described in Insecticide Resistance Action Committee Test Method No. 22. Tomato leaf disks (3 cm) were immersed in the bacterial suspension and allowed to dry on a wire mesh. When the leaf surface was completely dry, these discs were placed in Petri dishes (60 mm) containing moistened cotton. Then, one second instar *T. absoluta* larva was placed in each Petri dish. At least thirty-two larvae were used for the bioassays, and the tests were replicated three times for each application. Sterile distilled water was used in the control group. The bioassay was performed at 25°C, 65% RH, and a photoperiod of 14:10 h (L:D) for 3 days. The mortality rate was recorded daily.

Concentration response experiments

As a result of the screening tests, *Bt*-B3 isolate, which had a high effect on all the three *T. absoluta* populations, was used in concentration response experiments. Six different spore-crystal suspensions of the isolate were prepared at concentrations of 60, 50, 40, 30, 20 and 10 ppm, and bioassays were performed as indicated in the screening tests.

Statistical analysis

Mortality data was corrected using the Abbott's formula (Abbott, 1925) and subjected to analysis of variance, followed by comparison of means with Tukey's test. Data normality and homogeneity of variance were checked using the Shapiro-Wilk test and Bartlett's test, respectively. Lethal concentrations needed to kill 50% and 90% of the larvae were calculated using Probit analysis. SPSS Statistics 24 software package (IBM, Armonk, NY, USA) was used as the statistical tool.

Detailed characterization of *Bt*-B3

The crystal structures of *Bt*-B3 isolate, which had the highest virulence in the tested populations and whose spore crystal presence was detected by phase contrast microscopy, were determined by electron microscopy, and molecular characterization was performed by amplification of the 16S rDNA gene region.

The morphology of insecticidal crystal proteins of *Bt*-B3 was examined under the scanning electron microscope. Twenty microliters of the spore crystal mixture were transferred on a stub and dried at 37°C for 24 h. The dried stub was coated with platinum dust using an automatic sputter coater (Quorum Technology SC7620-CF). The coated spore-crystal mixture was examined with a Zeiss Evo LS10 (Tokyo, Japan) and photographed at various magnifications.

Total DNA previously extracted from *Bt*-B3 was amplified by 16S rRNA gene specific primers (UNI16S-F and UNI16S-R) (Weisburg et al., 1991). The 25 µ of PCR mixture contained: 200 µM of each dNTP, 0.2 µM concentration of each primer, 0.5 U of *Taq* DNA polymerase (NEB,), 2.5 µl of 10× reaction buffer and 50 ng of DNA template. Amplification was performed using a thermal cycler, with the following program: 95°C for 30 s, 1 cycle; 95°C for 30 s, 55°C for 30 s, 68°C for 1 min, 30 cycles; 68°C for 5 min, 1 cycle. The PCR product was loaded on 1.0% agarose gel and visualized under UV light. The amplified fragment was sent for sequencing at Ficus Biotechnology (Ankara, Turkey).

The obtained sequence was compared with NCBI nucleotide database using BLAST tool and submitted to the GenBank database. The phylogenetic trees based on partial 16S rDNA sequences were inferred using the neighbor-joining (NJ) algorithm and 1000 bootstrap replicates in the MEGA X (Kumar et al., 2018).

Results

Isolation of *B. thuringiensis* isolates

Forty soil samples were collected from 24 different locations in Bilecik, Turkey, and a total of 64 *Bacillus*-like colonies were isolated. Spore crystal staining of the isolates revealed that 13 of them had insecticidal crystal proteins that distinguished *Bt* from all other sporulating bacteria. Since the *Bt* index was defined as the ratio between the number of *Bt* colonies identified and the total number of *Bacillus*-like colonies examined, the *Bt* index was 0.203. Phase-contrast microscopy examination revealed that M5, M6, P4, P6, Y5, B3, O5, O6, G4, G8, S3, S6, and S10 formed crystals.

PCR analysis was performed to identify *cry* genes encoding toxin proteins produced during the sporulation phase. Among the eight *cry* gene primers used in the present study, *cry1* and *cry2* were the predominant genes observed in seven *Bt* strains. The other *cry* genes such as *cry3* and *cry4* were observed in four strains each. However, the *cry5* gene was found in only one isolate (O5) (Table 2).

Table 2. *Cry* gene contents of indigenous *Bt* isolates

Bt isolates	*cry* genes							
	*cry*1	*cry*2	*cry*3	*cry*4	*cry*5	*cry*7	*cry*9	*cry*11
M5	+	+	-	-	-	-	+	-
M6	+	+	-	-	-	-	-	-
P4	-	-	+	-	-	-	-	-
P6	-	+	-	-	-	-	+	-
Y5	-	+	-	+	-	-	-	-
B3	+	+	-	+	-	+	-	+
O5	-	-	+	-	+	-	-	-
O6	-	-	+	-	-	-	-	+
G4	+	-	-	+	-	-	-	-
G8	+	-	-	+	-	-	-	-
S3	+	+	-	-	-	-	-	-
S6	-	-	+	-	-	+	+	-
S10	+	+	-	-	-	-	-	-
Frequency (%)	53.8	53.8	30.8	30.8	7.7	15.4	23	15.4

Screening test and concentration response experiments

The insecticidal activities of thirteen *Bt* isolates were tested on three different populations (İzmir, Bilecik and Samsun) of tomato leafminer. In the laboratory experiments, it was found that all *Bt* isolates showed pathogenicity on the Samsun population of the pest and their virulence ranged from 30% to 90% ($F=19.96$; $df=12,26$; $p < 0.05$). Accordingly, the most effective isolate was *Bt*-B3, which killed 90% of the larvae, while the least effective isolate was S6 with 30% (Figure 1A). It was also found that the virulence of the isolates in the İzmir and Bilecik populations was lower than the effect in the Samsun population. The virulence varied between 20-75% ($F=13.72$; $df=12,26$; $p < 0.05$) and 25-96% ($F=373.78$; $df=12,26$; $p < 0.05$) in the İzmir and Bilecik populations, respectively. B3 was the most effective isolate in both the İzmir (75%) and Bilecik (96%) populations (Figure 1B, C).

Concentration response experiments were performed with *Bt*-B3 isolate, which showed the highest effect in all the three populations. *Bt*-B3 at a concentration of 60 ppm showed mortality rates of 95%, 85%, and 81% in Samsun, İzmir and Bilecik populations of the pest, respectively. *Bt*-B3 had the highest toxicity in Samsun population with the lowest LC_{50} value ($F=147.8$; $df=2,6$; $p < 0.05$) (Table 3).

Table 3. Lethal concentrations (LC_{50} and LC_{90}) of *Bt*-B3 isolate against three different field populations of *T. absoluta*

Insect population	LC_{50} (FL, 95%)	Slope ± SE	LC_{90}	df	X^2	*p* value
İzmir	26.06 (17.95-34.73) b	2.5±0.21	100.28	4	12.29	0.015
Samsun	13.28 (2.52-20.77) a	2.5±0.22	60.06	4	19.41	0.001
Bilecik	24.24 (20.76-27.69) b	2.0±0.21	121.31	4	2.43	0.656

Lowercase letters represent statistical differences between LC_{50} values according to Tukey's multiple comparison test ($P<0.05$). FL: fiducial limit, SE: standard error, df: degree of freedom, X^2: Chi-square.

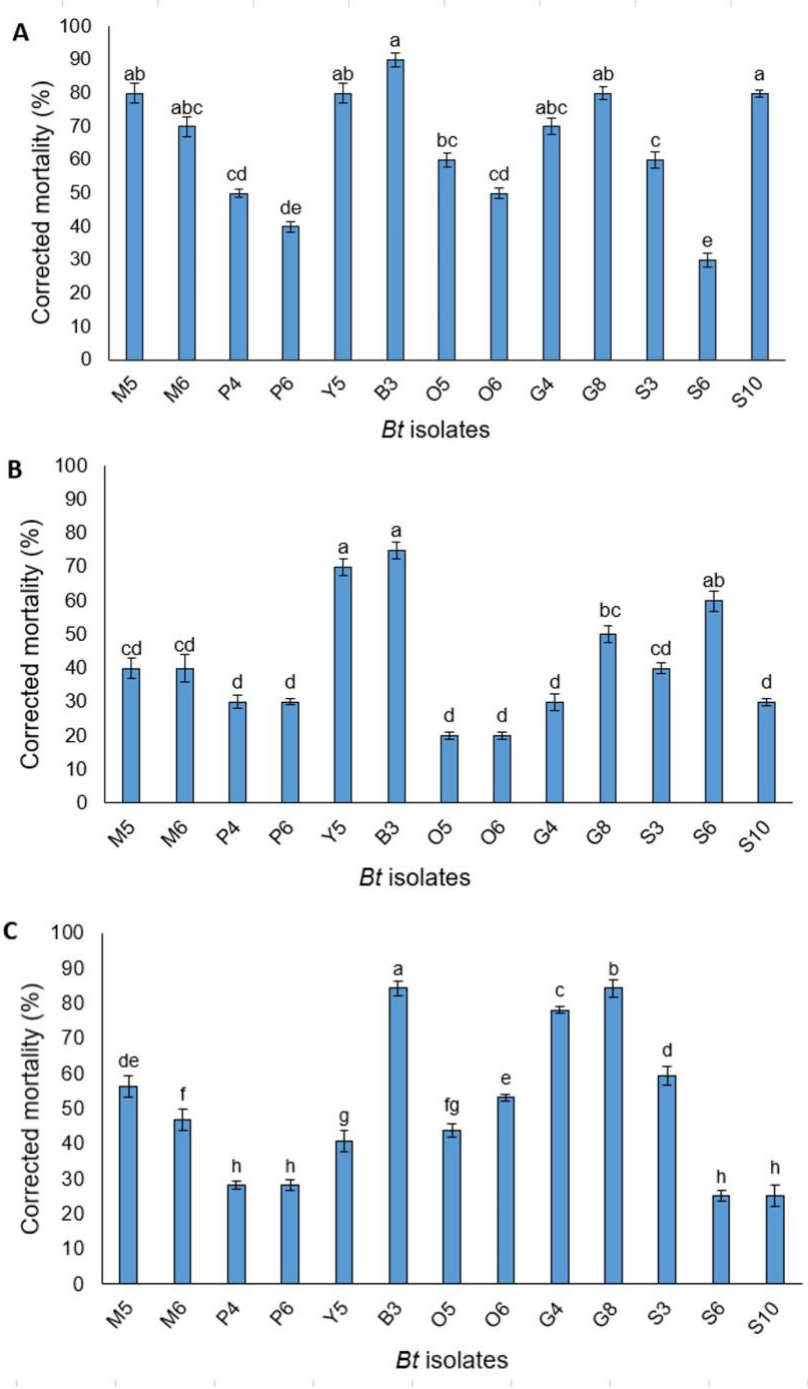

Figure 1. Corrected mortality rates of Samsun (A), İzmir (B), and Bilecik (C) populations of *T. absoluta* exposed to native *Bt* isolates with 50 ppm spore-crystal mixture 72 hours after treatment. Different letters represent statistically significant differences between mortality rates according to Tukey's multiple comparison test ($P<0.05$). Mortality indicates the mean of three replicates. The bars show the standard deviation of the mean values.

Detailed characterization of *Bt*-B3

The spore crystal morphology of *Bt*-Se13 isolates was characterized using SEM. The SEM images showed that bipyramidal, cubic, and spherical crystal proteins were present (Figure 2).

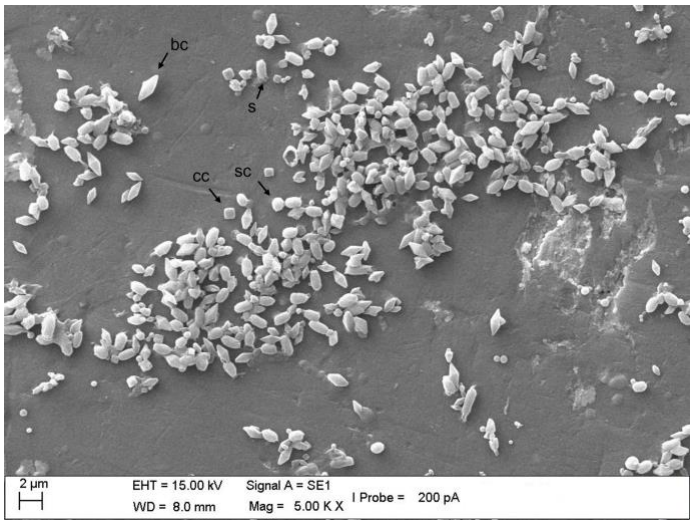

Figure 2. Scanning electron microscopy of *Bt*-B3 isolate. s: endospore; bc: bipyramidal crystal; cc:cubic crystal; sc:spherical crystal.

We also sequenced approximately 1.350 bp of the 16S rRNA gene of the *Bt*-B3 isolate for molecular identification and nucleotide sequence homology search was carried out using BLASTn (http://www.ncbi.nlm.nih.gov). The *Bt*-B3 isolate was found to have the highest homology (99%) with other known *B. thuringiensis* isolates. The sequence was submitted to the NCBI GenBank database with accession number OM732508. Phylogenetic analysis also showed that *Bt*-B3 isolate clustered with reference *B. thuringiensis* isolates (Figure 3).

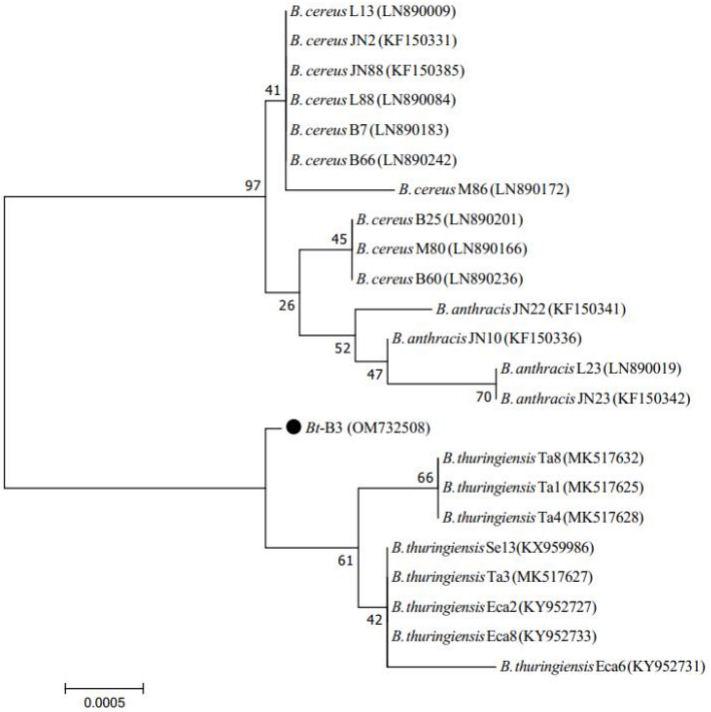

Figure 3. Phylogenetic analysis of *Bt*-B3 isolate inferred from neighbor-joining analysis with *p*-distance model of 16S rRNA sequence.

Discussion

Native *Bt* isolates have novel insecticidal genes with broader toxicity and might be more effective than exotic strains. Therefore, soil screening for novel and effective *Bt* strains is one of the global strategies for pest control. In this study, 13 indigenous *Bt* strains were isolated from forty soil samples (*Bt* index=0.325). Previous studies reported variable frequency of isolation of *Bt* from soil samples, ranging from 3 to 85% (Ramalakshmi & Udayasuriyan, 2010; Hassan et al., 2021). Abo-Bakr et al. (2020) studied 16 Egyptian soil samples and isolated 16 *Bt* strains from 56 *Bacillus*-like colonies (*Bt* index=0.28). On the other hand, Djenane et al. (2017) isolated 180 colonies, of which only 16 isolates were identified as *Bt* (*Bt* index=0.08). The variation of *Bt* index in soil samples could be related to the chemical properties of the soil, such as macro/micronutrients, soil moisture, and soil oxygenation, which may affect *Bt* growth and toxin production (Polanczyk et al., 2009).

Since the aim of this study was to determine the effective isolates against *T. absoluta*, the *cry1*, *cry2*, and *cry9* genes known to be effective against Lepidoptera pests were identified in the isolates (Rosas-García et al., 2008; Salama et al., 2015). In this study, *Bt*-B3 isolate was found to possess *cry1*, *cry2*, *cry9* and *cry11* genes. We also found a high frequency (53.8%) of *cry1* and *cry2* genes in our *Bt* collection, which was similar to that described in other reports (Bravo et al., 1998; Wang et al., 2003), while *cry5* gene had the lowest frequency (7.7%) (Table 2). Thammasittirong & Attathom (2008) found that 81.3% of strains harbored *cry1* genes, 80.6% *cry2* genes, and 37.3% *cry9* genes in Thailand collection. In another report, Rashki et al. (2021) found that *cry1* genes were more abundant in Iranian *Bt* strains. On the other hand, *cry9* genes were more abundant (47.8%) than *cry1* and *cry2* genes (6.5 and 2.1%) in Brazilian collection (Pinto & Fiuza, 2003). It is clear that the occurrence, distribution and diversity of *cry* genes are variable and depend on the geographical region.

The strong insecticidal activity of the *Bt* strains seems to be due to the combined properties of several Cry proteins that combine to form an inclusion body. In our study, scanning electron microscopy showed that *Bt*-B3, the most effective isolate in all the populations tested, had spherical, cubic, and bipyramidal insecticidal Cry proteins.

While *Bt*-B3 had the highest virulence in the Samsun population with 90%, the lowest virulence was observed in the İzmir population with 74%. It is believed that the difference in insecticidal efficacy between the populations is due to the development of resistance in insects. Izmir is the first region where the pest was seen in Turkey in 2009, and chemical insecticides have been used for control since then. Thus, the insecticides used to prevent losses from the pest may have led to the development of resistance over time. In the screening tests, isolate *Bt*-B3 had the highest effect on all populations tested among the isolates. Therefore, concentration experiments with *Bt*-B3 were performed on populations and LC_{50} values of 13.28, 26.06, and 24.24 µg/ml were determined for the Samsun, İzmir, and Bilecik populations, respectively. The concentration-response experiments showed once again that the Samsun population was more sensitive to the *Bt*-B3 isolate.

Several studies have been conducted on the efficacy of *Bt* or *Bt*-based biopesticides on populations of *T. absoluta*. Sandeep Kumar et al. (2020) tested the *Bt* strains (4D1, 4D4, 4G1) on the laboratory reared population and the LC_{50} values for the second larval stage were 6.10, 6.62 and 8.18 µg/ml, respectively. However, Sabbour & Soliman (2014) found that the LC_{50} values of the commercial product Dipel and the strains *Bt* kurstaki HD-73 and HD-234 were 140, 109, and 90 µg/ml, respectively, on the larvae of *T. absoluta* under laboratory conditions. The differences in LC_{50} values are directly related to the virulence of the isolates and the susceptibility of the insect population. Previous studies have investigated the susceptibility of some conventional insecticides such as indoxacarb, metaflumizone, spinosad, chlorantraniliprole, and λ-cyhalothrin to different field populations of the pest (Yalçın et al., 2015; Bala et al., 2019; Prasannakumar et al., 2021). However, there is no study on the susceptibility of different field populations of *T. absoluta* to local *Bt* strains.

Our results suggest that *Bt*-B3 containing lepidopteran active *cry* genes may be an effective biological control agent to be used in integrated control of the pest. To achieve high efficacy of the spore-crystal mixture under greenhouse and field conditions, the *Bt*-B3 isolate should be formulated in further studies to overcome the adverse effect of the environment such as UV radiation, rain, and temperature, and then tested against *T. absoluta* under greenhouse and field conditions.

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Original article (Orijinal araştırma)

Resistance of some Turkish garlic genotypes and landraces against stem and bulb nematode, *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936 (Rhabditida: Anguinidae)[1]

Bazı yerel sarımsak genotip ve köy çeşitlerinin soğan sak nematoduna, *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936 (Rhabditida: Anguinidae) karşı dayanıklılıklarının belirlenmesi

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Abstract

Ditylenchus dipsaci (Kühn, 1857) Filipjev, 1936 (Rhabditida: Anguinidae) is one of the destructive agents of garlic and reduces yield and market value. One of the most practical and eco-friendly methods for nematode management is using resistant varieties. In the study, two endemic garlic species, *Allium tuncelianum* (Kolman) Ozhatay, Mathew & Siraneci and *Allium macrochaetum* subsp. *macrochaetum* Boiss. & Hausskn. (Alliaceae: Amaryllidaceae), 10 mutant and 32 landraces garlic genotypes, *Allium sativum* L. (Alliaceae: Amaryllidaceae) were investigated for their resistance reactions to *D. dipsaci* and effect of *D. dipsaci* on some plant growth parameters. All experiments were conducted at Atatürk Horticultural Central Research Institute in 2019-2020. None of the genotypes was found resistant to *D. dipsaci,* and reproduction factors, which ranged from 2.6 to 12.7, were grouped from susceptible to highly susceptible. The Tunceli garlic genotype had the lowest reproduction factor (2.6), 36.6% less than the highly susceptible Muğla6 genotype. Alata1, Muğla1, Muğla7 and Kula genotypes had the lowest decrease rate with nematode treatment at least in one of the plant growth parameters. The genotypes that had lower nematode multiplication and displayed better development under nematode infestation in this study are recommended for the field infested with *D. dipsaci* as sources for garlic breeding.

Keywords: Endemic garlic species, garlic landraces, garlic mutant clones, plant parasitic nematode

Öz

Ditylenchus dipsaci (Kühn, 1857) Filipjev, 1936 (Rhabditida: Anguinidae) sarımsakta zarar yapan en önemli etmenlerden biri olup verimi ve pazar değerini düşürmektedir. Nematod mücadelesinde en pratik ve çevre dostu yöntemlerden biri dayanıklı çeşitlerin kullanılmasıdır. Bu çalışmada iki endemik sarımsak türü, *Allium tuncelianum* (Kolman) Özhatay, Matthew & Siraneci ve *Allium macrochaetum* subsp. *macrochaetum* Boiss. & Hausskn. (Alliaceae: Amaryllidaceae), 10 mutant ve 32 yerel sarımsak, *Allium sativum* L. (Alliaceae: Amaryllidaceae), genotipinin *D. dipsaci*'ye karşı dayanıklılık durumları ve *D. dipsaci*'nin bazı bitki büyüme parametreleri üzerine etkisi belirlenmiştir. Tüm denemeler 2019-2020 yıllarında Atatürk Bahçe Kültürleri Merkez Araştırma Enstitüsü'nde yürütülmüştür. Genotiplerin hiçbiri *D. dipsaci*'ye dayanıklı bulunmamış ve üreme faktörleri 2.6 ile 12.7 arasında değişerek duyarlıdan çok duyarlıya doğru gruplanmıştır. En düşük üreme faktörü (2.6), yüksek hassas Muğla6 genotipinden %36.6 daha az olarak Tunceli sarımsak genotipinde belirlenmiştir. Alata1, Muğla1, Muğla7 ve Kula yerel genotiplerinde soğan sak nematodu uygulaması sonucunda bitki büyüme parametrelerinin en az birinde en düşük etki belirlenmiştir. Bu çalışmada nematodun üreme faktörünün düşük tespit edildiği ve nematod zararı altında daha iyi gelişme gösteren genotipler, *D. dipsaci* ile bulaşık alanlar için sarımsak ıslah materyali olarak önerilmektedir.

Anahtar sözcükler: Yerel sarımsak genotipleri, endemik sarımsak türleri, mutant sarımsak klonları, bitki paraziti nematodlar

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Introduction

Garlic, *Allium sativum* L. (Alliaceae: Amaryllidaceae) has an important role in human nutrition and has high economic value as a medicinal and aromatic plant species. Most of the garlic production in the world is undertaken in China, which accounts for 78% of the world's garlic production. Although Türkiye's garlic yield is far below the world average, it is the 10th garlic producer in the world. The average yield of garlic in the world is 1.719 kg/da while Türkiye's average yield remained at 925 kg/da in 2020 (FAO, 2020).

Genetic and environmental influences such as garlic varieties, climate and soil conditions, as well as diseases and pests are the most important factors affecting garlic yield. One of the most important pests of garlic is the stem and bulb nematode, *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936 (Rhabditida: Anguinidae). *Ditylenchus dipsaci* has been detected in garlic-growing areas in 80 different countries in all the continents except Antarctica to date (EPPO, 2022). *Ditylenchus dipsaci* was found in the garlic growing areas of Tekirdağ and Kırklareli provinces in the Marmara Region; Kastamonu, Tokat and Amasya provinces in the Black Sea Region; Kahramanmaraş province in the Mediterranean Region; Balıkesir and Bursa provinces in the Aegean Region; Gaziantep, Hatay and Adıyaman provinces in the Southeastern Anatolia Region; Aksaray province in the Central Anatolia Region in Türkiye (Ates Sonmezoglu et al., 2020; Öcal, 2021).

Ditylenchus dipsaci feeds endo parasitically and degrades the middle lamella between plant cells in the bulbs and leaves of garlic (Duncan & Moens, 2006). As a result of the damage, garlic plants show stunting and chlorosis in the above-ground part, underdevelopment and discoloration and splitting of bulbs, basal plate damage and reduction in roots (Mollov et al., 2012; Testen et al., 2014). Even though the initial population density is low, a fast population increase of *D. dipsaci* can result in significant crop damage. It has been determined that it causes yield losses of up to 64.5% on the garlic plant in Türkiye (Mennan, 2001; Yavuzaslanoğlu et al., 2015).

Three years of rotation with non-host crops is the primary way of controlling *D. dipsaci*, but this method usually unsuccessful due to the morphologically indistinguishable host races with different host preferences (Marek et al., 2005; Dikici et al., 2014). The use of fumigants and nematicides is uneconomical to control *D. dipsaci* in most crops, except in some scenarios in nurseries where the planting material is grown (Duncan & Moens, 2006). However, the presence and use of resistant garlic varieties are an effective, practical, and eco-friendly method of control to keep nematode populations below the economic damage threshold.

So far, some varieties of clover, rye, bean, oat and onion that show resistance to different races of the stem and bulb nematode have been identified (Plowright et al., 2002; Yavuzaslanoğlu, 2019; Yavuzaslanoğlu & Ozsoy, 2020). However, no study has been reported about resistance in garlic to stem and bulb nematode.

Objectives of this study are to investigate: (1) the reactions of 44 garlic genotypes, which include two endemic garlic species, local populations and clones developed by mutation breeding method, to the garlic population of stem and bulb nematode, and (2) the effect of stem and bulb nematode on some growth parameters of the genotypes.

Materials and Methods

Garlic genotypes

In the experiment, two endemic garlic species, *Allium tuncelianum* (Kolman) Özhatay, Matthew, Siranecı, and *Allium macrochaetum* subsp. *macrochaetum* (Alliaceae: Amaryllidaceae), which were collected from nature and cultured, 10 mutant clones, and 32 landraces of garlic (*A. sativum*) (Table 1) were used for investigation of their resistance reactions to *D. dipsaci* and the effect of *D. dipsaci* on some plant growth parameters. Garlic genotypes used in the experiment were obtained the garlic breeding program of Atatürk Horticultural Central Research Institute (Yalova, Türkiye).

Nematode inoculums

KAS-9 population of *D. dipsaci* which was isolated from a garlic field in Kastamonu Province, Taşköprü District, Vakıfbelören Village (N: 41°30'07.37", E: 34°15'01.19", Elevation: 650 m) in Türkiye, and identified by morphological and molecular methods (Ates Sonmezoglu et al., 2020), was used in the study. KAS-9 population was multiplied on sterile carrot discs to obtain nematode inoculums. Nematodes were extracted from two months old carrot cultures by washing them with tap water. Nematode concentration was adjusted to 200 nematodes/10 µl in carboximetilcelilose solution (1% w/v) for inoculation to garlic plants (Kühnhold et al., 2006).

Experimental setup

All experiments were conducted at Atatürk Horticultural Central Research Institute in 2019-2020. The experiment was performed in a growth chamber at 23±2ºC with a 16:8 hour light: dark cycle. The garlic seeds belonging to 44 garlic genotypes were sown in 760 ml pots (12.5x20x12.5 cm) which were filled with an autoclaved (Smith & Onions, 1994) soil mixture (70% sand, 29% soil, 1% farm manure). One seed of each plant species was planted per pot. Four weeks after germination in all pots, plants which were in the 3-4 leaf stage were inoculated with a 10 µl 1% CMC (carboxymethyl cellulose) solution containing 200 nematodes (Pi) applied directly between the first two leaves (Kühnhold et al., 2006). Plants used as negative control were inoculated with only 10 µl of 1% CMC (carboxymethyl cellulose) solution. Each pot served as a replicate, and the studies used a completely randomized plot design with four replications separately for inoculated and non-inoculated treatments.

Determination of resistance reactions of garlic genotypes to *Ditylenchus dipsaci*

Six weeks after inoculation, nematodes were extracted from all garlic plants for each pot with Oostenbrink dish (Hallman & Viaene, 2013). After 24 h at room temperature, the extracted nematodes were counted under stereo microscope. The nematode reproduction factor (RF) was calculated as nematodes per plant (Pf) divided by initial inoculum density (Pi= 200). Resistance reactions (RR) of genotypes were designated according to their RF values. Genotypes were classified as resistant (R) (RF < 1), moderately susceptible (MS) (1 ≤ RF < 2), susceptible (S) (2 ≤ RF < 4), and highly susceptible (HS) (4 ≤ RF) (Hajihasani et al., 2016). Since there is no resistant and susceptible garlic genotype previously determined to the stem and bulb nematode, relative susceptibility (RS) of the genotypes was calculated according to the Muğla6 genotype, which is the HS to *D. dipsaci* in this study with a 4.1 reproduction factor. The number of nematodes on each genotype divided by the number of nematodes on Muğla6 was given a percentage to express the RS value (Mwaura et al., 2015).

Determination of the effect of *Ditylenchus dipsaci* on plant growth parameters of garlic genotypes

Several plant growth parameters were investigated for the determination of nematode damage on garlic genotypes, including whole plant length (cm), garlic head length (mm), head diameter (mm) and whole plant fresh and dry weight (g). Plant shoots and roots were dried in an oven for 48 hours at 70°C to estimate their dry weights (Mohammad et al., 2007).

Statistical analysis

One-way analysis of variance (ANOVA) was performed to determine differences in *D. dipsaci* RF values among garlic genotypes, using Tukey multiple comparison tests ($p < 0.05$). Nematode RS values of garlic genotypes were also investigated with Dunnett's test, according to HS genotype, and Muğla6 as the control in the experiment.

In order to determine the effect of *D. dipsaci* on plant growth parameters of garlic genotypes such as plant height (cm), the fresh and dry weights of shoots and roots (g) obtained from treatments with and without nematodes were analyzed by paired *t* test ($\alpha=0.05$). Reproduction factor (RF) of *D. dipsaci* on garlic genotypes and the data obtained from treatments with and without nematodes for each genotype were

analyzed using analysis of variance (ANOVA). When ANOVA showed significant effects, the means were separated by Fisher's LSD test (p<005). All statistical analyses were performed using JMP 13 (SAS Institute Inc., Cary, NC, USA).

Results

Resistance reactions of garlic genotypes to *Ditylenhus dipsaci*

The reproduction factors of *D. dipsaci* on the genotypes in the experiment ranged from 2.6 to 12.7 (Table 1). Resistant genotype against *D. dipsaci* was not detected (RF<1).

Table 1. Names and origins of 44 garlic genotypes used in the study, reproduction factor (RF) of *Ditylenhus dipsaci* on garlic genotypes, relative susceptibility (RS) of garlic genotypes according to Muğla6 genotype in the study and their resistance reaction (RR)

Accession number	Genotype Name	Origins	RF	RS	RR
1	2-39/6	Mutant clone	9.2±0.9b-d[1]	224.3*	HS
2	2-78/11	Mutant clone	7.5±0.9b-j	182.9	HS
3	2-01/6	Mutant clone	7.6±0.9b-ı	185.3	HS
4	1-27/6	Mutant clone	7.1±1.3c-k	173.1	HS
5	2-6/12	Mutant clone	5.9±1.5e-m	143.9	HS
6	2-20/4	Mutant clone	10.0±1.2ab	243.9*	HS
7	2-65/5	Mutant clone	9.4±2.4b-d	229.2*	HS
8	3-64/3	Mutant clone	8.0±2.8b-h	195.1	HS
9	2-34/9	Mutant clone	7.0±1.8c-l	170.7	HS
10	2-34/2	Mutant clone	6.7±1.1b-m	163.4	HS
11	Reis10	Landrace	8.5±0.6b-f	207.3	HS
12	Kütahya Beyazı	Landrace	3.2±0.4mn	78.0	S
13	Selection13	Landrace	5.6±0.7g-n	136.5	HS
14	Muğla6	Landrace	4.1±0.5k-n	100.0	HS
15	Muğla3	Landrace	5.8±0.5e-m	141.4	HS
16	Selection10	Landrace	7.1±0.4c-k	173.1	HS
17	Kahramanmaraş	Landrace	4.8±1.2ı-n	117.0	HS
18	Muğla1	Landrace	9.6±0.9bc	234.1*	HS
19	Germencik	Landrace	6.6±1.1d-l	160.9	HS
20	Sabahattin Ufuk	Landrace	5.5±0.4f-n	134.1	HS
21	Kütahya Pembesi	Landrace	3.2±0.9mn	78.0	S
22	Gaziantep/Araban	Landrace	7.7±0.9b-ı	187.8	HS
23	Muğla7	Landrace	4.1±0.8lmn	100.0	HS
24	Selection4	Landrace	5.7±0.4f-m	139.0	HS
25	Burgaz3	Landrace	4.3±0.8k-n	104.8	HS
26	Muğla4	Landrace	5.0±0.7h-n	121.9	HS
27	Adıyaman Beşir	Landrace	5.5±0.7g-n	134.1	HS
28	Selection11	Landrace	5.5±0.7g-n	134.1	HS
29	Selection137	Landrace	5.9±0.8e-m	143.9	HS
30	Selection8	Landrace	5.9±0.4e-m	143.9	HS
31	Selection3	Landrace	6.9±0.8c-l	168.2	HS
32	AdilAtay	Landrace	8.3±0.9b-g	202.4	HS
33	Selection40	Landrace	12.7±1.8a	309.7*	HS
34	Germiyan	Landrace	8.7±1.8b-e	212.1*	HS
35	K-6Taşköprü	Landrace	7.8±0.6b-h	190.2	HS
36	Muğla5	Landrace	4.4±0.4j-n	107.3	HS
37	Afyonkarahisar	Landrace	6.3±0.5d-m	153.6	HS
38	Balıkesir	Landrace	3.6±0.9mn	87.8	S
39	Selection63	Landrace	5.8±0.4e-m	141.4	HS
40	Kula	Landrace	8.9±0.9b-d	217.0*	HS
41	Taşköprü56	Landrace	9.6±0.4bc	234.1*	HS
42	Alata1	Landrace	4.3±0.3k-n	104.8	HS
43	Tunceli garlic (*Allium tuncelianum*)	Endemic genotip	2.6±0.3n	63.4	S
44	Kaya garlic (*Allium macrochaetum*)	Endemic genotip	8.4±1.1b-g	204.8	HS

[1] Different letter in the same column indicate that the means are statistically significantly different among genotypes (p < 0.05, LSD test);
* There is a statistically significant difference in the relative susceptibility (RS) of garlic genotypes based on Muğla6 genotype (p < 0.05, Dunnett's test).

However, a significant difference was found in the nematode reproduction factors of 44 garlic genotypes (p<0.05). On the other hand, although 36.6% less nematodes were obtained in the Tunceli garlic genotype, which was classified as susceptible with the lowest RF value of 2.6, compared to Muğla6 genotype, no significant difference was detected in terms of both Rf and RS values (p>0.05). The Tunceli garlic was followed by Kütahya Beyazı (RF=3.2), Kütahya Pembesi (RF=3.2), and Balıkesir (RF=3.6) genotypes which were also classified as susceptible (Table 1). The relative susceptibility values of these genotypes compared to Muğla6 were determined as 78, 78 and 87.8, respectively, but no significant difference was determined according to Dunnet's test (p>0.05).

The highest RF value was determined in the Selection40 genotype with 12.7. It had a 309%, a higher reproduction rate which was significantly different than Muğla6 according to Dunnet's test (p<0.05). In Selection40, approximately five times more *D. dipsaci* was obtained compared to Tunceli garlic, which was the lowest reproduction factor detected. Selection40 followed by 2-20/4, Taşköprü56, Muğla1, 2-65/5, 2-39/6, Kula, and Germiyan genotypes which were significantly different from Muğla6 with 10, 9.6, 9.6, 9.4, 9.2, 8.9, and 8.7 RF, respectively (p< 0.05).

Effect of *Ditylenchus dipsaci* on garlic plant growth parameters

Significant differences were determined between genotypes in terms of plant height, head height, head diameter, plant fresh and dry weight values in both nematode inoculated and non-inoculated plants (p<0.01). Although the effect of stem and bulb nematode on plant growth parameters varies according to genotypes, a significant decrease was detected due to nematodes in 97.7%, 70.5%, 77.3%, 75%, and 90.9% of genotypes in terms of plant height, head length, head diameter, fresh weight and dry weight, respectively, (p<0.05) (Tables 2 & 3).

The longest plant length was determined in Alata1 (75.5 cm; 77.8 cm), Kayagarlic (69.6 cm; 77.0 cm), and Gaziantep/Araban (59.4 cm; 66.6 cm) genotypes in both inoculated and non-inoculated plants, respectively. The shortest plant length for inoculated and non-inoculated plants was obtained in Selection 137 (36.7 cm; 44.3 cm), 2-6/1 (36.8 cm; 40.7 cm) and Selection 11 (36.9 cm; 42.2 cm), respectively (Table 2). A significant reduction in plant length was determined with nematode treatment in all genotypes except Alata1 landrace (P<0.05, Table 2). The highest decrease in plant height was determined in the Selection63 landrace with 18.7%, while the least decrease was determined in Alata1 with 3.0%.

The longest head length was determined in Kayagarlic (42.8 cm; 45.4 cm), Kula (41.8 cm; 45.3 cm), and Taşköprü56 (40.3 cm; 44.8 cm) genotypes in nematode inoculated and non-inoculated treatments, respectively. Kütahya Pembesi (22.1 cm; 28.3 cm) and Germiyan (24.0 cm; 28.0 cm) genotypes had the lowest head length in nematode inoculated and non-inoculated treatments. Significant differences were determined in head length in most of the genotypes (Table 2). The highest and lowest head length reduction with nematode treatment was recorded in Kütahya Pembesi (21.9%) and Muğla1 (2.9%) genotypes, respectively (Table 2).

The largest head diameter was found in Alata1 (46.2 cm; 50.0 cm), while the smallest head diameter was of Tunceli garlic (14.5 cm; 16.2 cm). A significant difference was determined between the inoculated and non-inoculated treatments of genotypes except for 10 genotypes (P < 0.05). With nematode treatment, the maximum decrease in head diameter was obtained in Kahramanmaraş (25.7%), and the least decrease was obtained in Muğla7 (5.4%) genotypes (Table 2).

The lowest fresh weight was determined in inoculated genotypes in Kahramanmaraş (7.7 g), Kütahya Pembesi (9.5 g), Selection4 (9.5 g), and in non-inoculated ones in Selection4 (11.4 g), Muğla3 (12.2 g), Selection137 (12.5 g) genotypes. A significant difference was determined between nematode inoculated and non-inoculated all genotypes except 8 genotypes (p < 0.05). With nematode treatment, the highest decrease in fresh weight was obtained in Kahramanmaraş (41.2%), and the least decrease was observed in Kula (10.0%) genotypes (Table 3).

Table 2. Plant length, head length, head diameter, fresh weight and dry weight values of genotypes with *Ditylenchus dipsaci* (N+) and without-*D. dipsaci* (N-) treatments and their % reduction with nematode treatment

N[1]	Plant length (cm)			Head length (cm)			Head diameter (cm)		
	N+	N-	%	N+	N-	%	N+	N-	%
1	41.9±0.8mn[2]	46.1±0.3 n-s	9.1*	35.9±1.2 b	40.5±1.6b-d	11.3*	34.7±0.6c-e	41.1±1.1cd	15.6*
2	46.7±0.9jk	52.0±1.2lm	10.2*	30.5±1.3 e-j	36.0±1.4d-ı	15.3*	38.9±1.7b	42.2±1.2bc	7.8
3	55.6±0.9e-g	59.4±0.8ef	6.4*	35.3±0.7b	38.7±0.6c-e	8.8*	35.2±0.4cd	41.9±0.7bc	15.9*
4	37.0±0.6q	40.9±0.7t	9.5*	31.7±0.6ef	34.9±0.6e-k	9.2*	30.1±0.7ı-l	34.9±0.7f-k	13.7*
5	36.9±0.6q	40.8±1.0t	9.5*	31.9±0.7d-f	35.6±1.1e-j	10.4*	33.0±0.9ef	36.7±0.9e-g	10.1*
6	51.6±0.9hı	58.9±1.5e-h	12.3*	34.9±0.8b	40.3±0.6c	13.4*	36.1±0.8c	40.7±1.2cd	11.3*
7	46.2±0.8jk	53.4±0.9j-l	13.5*	31.5±1.0e-g	36.8±1.2c-g	14.4*	30.6±0.7g-k	33.8±0.6h-m	9.5*
8	42.5±0.5mn	48.1±0.9n-p	11.6*	32.4±0.7c-e	34.8±0.6e-k	6.9*	33.3±0.6d-f	36.5±1.2e-g	8.7*
9	39.8±0.9op	45.3±0.7q-s	12.1*	34.8±1.5bc	38.0±1.1c-f	8.4	34.8±0.3c-e	38.0±1.5e	8.4
10	41.8±0.9m-o	49.0±0.9m-o	14.7*	34.9±0.9b-d	37.8±1.1c-g	7.7	34.9±0.6c-e	37.7±1.1d-f	7.4
11	55.5±0.8e-g	62.5±1.0cd	11.2*	31.2±0.5e-h	35.7±0.6e-ı	12.6*	32.3±0.4fg	36.1±0.6e-h	10.5*
12	57.6±0.6d	66.0±0.5b	12.7*	25.6±0.5m-o	29.3±0.7no	12.6*	28.8±0.6j-o	31.6±0.4l-o	8.8*
13	46.5±0.9jk	55.1±0.6 ı-k	15.6*	29.1±1.3g-k	32.1±0.9ı-o	9.3	29.2±1.0ı-n	32.8±0.6j-n	10.9*
14	46.0±0.7jk	55.8±1.1 ıj	17.6*	35.2±1.1b	38.4±1.1c-f	8.6	33.3±0.9d-f	36.7±1.1e-g	9.2*
15	56.8±0.3d-f	65.9±0.5 b	13.8*	29.2±0.6g-k	31.3±0.6k-o	6.7*	25.8±0.5p-s	28.2±0.5qr	8.5*
16	47.2±0.8j	54.9±0.7 ı-k	14.0*	29.7±0.5f-k	34.9±0.7e-k	14.9*	29.9±0.6ı-l	32.4±0.8k-n	7.7*
17	46.0±0.5jk	55.1±0.4 ı-k	16.5*	30.2±1.0e-j	32.1±0.6ı-o	5.9	21.1±0.7u	28.4±1.4qr	25.7*
18	55.9±0.5d-f	64.3±0.8 bc	13.0*	37.1±1.0b	38.2±0.8c-f	2.9	33.1±0.4d-f	35.5±0.4e-h	6.7*
19	55.0±0.7fg	61.5±0.9 de	10.6*	31.8±0.5 ef	35.9±0.6d-ı	11.4*	32.2±0.6f-h	35.4±0.8f-ı	9.0*
20	47.4±0.5j	54.8±0.7ı-l	13.5*	29.0±1.2g-k	33.9±0.5f-n	14.5*	28.4±0.8k-o	30.8±0.5m-r	7.8
21	57.3±0.6de	65.7±1.1b	12.7*	22.1±0.6p	28.3±1.2o	21.9*	30.9±0.8g-ı	34.7±0.5f-k	10.9*
22	59.5±0.9c	66.7±0.9ab	10.7*	28.7±0.7ı-k	30.9±0.7l-o	7.1*	29.2±0.5ı-n	32.1±0.7l-n	9.0*
23	42.9±0.7m	47.6±0.8n-q	9.9*	30.7±0.6e-ı	33.8±1.0g-l	9.2*	27.5±0.6n-p	29.1±0.7o-r	5.4
24	41.9±0.7mn	45.9±0.8o-r	8.7*	27.5±0.9k-m	30.2±0.8l-o	8.9	28.8±0.8j-o	30.9±0.5n-p	6.7
25	50.6±0.8ı	59.3±1.2e-g	14.7*	25.1±0.7no	28.7±0.7o	12.5*	30.7±0.8g-j	35.1±0.7f-j	12.5*
26	52.9±0.5h	59.5±1.2e	11.1*	31.2±1.2e-h	35.5±1.1e-j	12.1*	26.9±0.7o-r	29.4±0.7o-q	8.5*
27	47.6±0.5j	54.5±0.4ı-l	12.6*	29.0±1.5g-k	31.8±1.5j-o	8.8	24.7±1.1st	28.7±0.9p-r	13.9*
28	36.9±0.6q	44.3±0.8rs	16.7*	25.9±1.2l-o	29.8±1.3no	13.1	25.2±1.0q-t	28.4±1.2qr	11.3
29	36.8±0.5q	44.4±0.5rs	17.1*	29.2±0.6g-k	33.7±0.6g-m	13.3*	28.9±1.1ı-o	33.0±1.1ı-n	12.4*
30	40.9±0.6n-p	46.6±0.4n-r	12.3*	28.9±0.8h-k	32.8±0.8h-n	11.9*	29.5±0.7ı-m	34.1±0.7h-l	13.5*
31	39.0±0.5p	45.4±0.6q-s	14.1*	30.9±0.6e-ı	35.6±0.5e-j	13.2*	30.7±0.7g-j	35.3±0.7f-j	13.0*
32	39.3±1.1p	45.5±1.2p-s	13.6*	25.7±0.7l-o	29.6±0.4m-o	13.2*	27.4±0.4m-q	30.8±0.4n-q	11.0*
33	39.8±0.5op	43.3±1.0st	8.08*	28.0±0.5j-m	32.7±1.5ı-n	14.8*	28.9±0.7ı-o	33.8±0.7h-m	14.5*
34	42.4±0.9mn	49.1±0.4n	13.6*	24.1±0.7op	21.6±6.4p	-11.5	24.9±0.6r-t	27.9±0.9r	10.7*
35	45.1±0.7kl	52.7±2.5kl	14.4*	30.0±1.4e-j	36.6±1.4c-h	18.0*	30.8±0.7g-j	35.2±0.9f-j	12.5*
36	46.4±0.9jk	56.8±0.7g-ı	18.3*	32.3±0.8de	35.7±1.3e-ı	9.5	28.5±0.9l-o	32.8±0.9j-n	13.1*
37	53.6±0.9gh	60.9±0.8de	12.0*	29.6±0.4f-k	33.3±0.7g-n	11.1*	30.0±0.5h-l	34.1±0.4g-l	12.0*
38	50.9±0.6ı	56.9±0.6f-ı	10.5*	24.4±0.7op	30.1±0.6l-o	18.9*	23.3±0.9 t	28.8±0.5p-r	19.1*
39	46.1±0.6jk	56.7±0.5hı	18.7*	29.8±0.4f-k	33.7±0.9g-l	11.6*	29.4±0.6 ı-n	32.8±1.4j-n	10.0
40	46.9±0.4jk	55.9±1.5ıj	16.1*	41.9±0.8a	45.3±0.7a	7.5*	38.5±0.9b	42.5±1.0bc	9.4*
41	47.0±0.3j	56.8±0.7g-ı	17.3*	40.4±0.8a	44.8±0.5ab	9.8*	39.9±0.9b	44.4±0.9b	10.1*
42	75.5±0.5a	77.8±1.1a	3.0	34.9±0.7b	39.7±0.8cd	12.1*	46.2±0.4a	50.1±1.1a	7.8*
43	43.4±0.8lm	48.4±1.1no	10.3*	28.2±1.4jkl	30.8±1.9l-o	8.4	14.5±0.8v	16.2±1.4s	10.5
44	69.7±0.5b	77.1±0.6a	9.6*	42.9±1.4a	45.4±1.0a	5.5	39.5±0.8b	42.2±1.0bc	6.4

[1] N: Accession number; [2] Different letters in the same column indicate that the means are statistically significantly different among genotypes ($p< 0.05$, LSD test); * There is a statistically significant difference between nematode treatments of the genotype in the investigated plant growth parameter ($p< 0.05$, t test)

Table 3. Fresh weight and dry weight values of genotypes with *Ditylenchus dipsaci* (N+) and without-*D. dipsaci* (N-) treatments and their % reduction with nematode treatment

N[1]	Fresh weight (g)			Dry weight (g)		
	N+	N-	%	N+	N-	%
1	20.8±0.8c[2]	29.7±0.5cd	29.9*	7.8±0.3g-ı	11.5±0.4c-g	32.1*
2	19.6±0.4c-e	22.6±1.0fg	13.2*	7.9±0.6f-ı	10.8±0.7e-g	26.8*
3	20.3±0.3c	24.7±0.3f	17.8*	8.6±0.5e-h	11.9±0.4c-e	27.7*
4	16.5±0.7g	21.9±1.1 fg	24.6	7.9±0.7f-ı	11.8±0.6c-f	33.0*
5	16.1±0.7g	20.4±0.9gh	21.1*	7.9±0.7g-ı	11.6±0.8d-f	31.8*
6	19.5±1.1cd	24.7±1.6f	21.1*	9.1±0.2d-f	12.3±0.6c-e	26.0*
7	17.5±1.2d-g	24.9±3.6ef	29.7	8.4±0.7e-h	11.7±0.8c-f	28.2*
8	16.7±1.0fg	21.6±1.0g	22.6*	8.9±0.5d-g	11.7±0.7c-f	23.9*
9	19.0±0.9c-e	27.9±1.2de	31.8*	7.6±0.4hı	11.7±1.0c-f	35.0*
10	17.0±0.3d-g	22.8±0.5fg	25.4*	7.9±0.2f-ı	10.0±0.0f-h	21*
11	12.9±0.7hı	17.5±0.5h-k	26.3*	6.9±0.6ij	9.0±0.5hı	23.3*
12	10.5±0.8k-p	13.8±0.7l-p	23.9*	5.7±0.5kl	7.8±0.7ı-m	26.9*
13	12.5±0.9h-k	14.4±0.8l-p	13.1	5.5±0.5lm	7.1±0.3k-r	22.5*
14	12.2±1.2h-m	14.0±0.8l-p	12.8	5.3±0.6l-o	7.1±0.7k-r	25.3
15	10.9±0.6ı-p	12.2±0.3op	10.6	5.1±0.3l-o	7.3±0.3j-q	30.1*
16	11.5±0.8ı-p	14.8±0.7j-o	22.3*	5.2±0.2l-o	7.3±0.2j-r	28.7*
17	7.7±0.5q	13.1±0.6m-p	41.2*	4.2±0.3n-p	6.5±0.6k-s	35.4*
18	16.4±1.1g	18.6±1.1hı	11.8	7.5±0.5hı	9.9±0.7gh	24.2*
19	17.3±0.7e-g	21.9±0.9fg	21.0*	7.6±0.5hı	9.7±0.5gh	21.6*
20	10.6±0.4 j-p	13.2±1.2l-p	19.7	4.9±0.3l-p	6.2±0.7l-s	20.9
21	9.5±0.5 pq	14.7±0.5j-o	35.3*	4.1±0.5n-p	5.9±0.3q-s	30.5*
22	11.9±0.7 h-o	14.4±1.0l-p	17.3	4.8±0.3l-p	6.6±0.3k-s	27.3*
23	10.1±0.5 m-p	13.1±0.9m-p	22.9*	4.7±0.5l-p	6.3±0.6o-s	25.4
24	9.6±0.9 pq	11.5±0.9p	16.5	3.7±0.4p	5.1±0.4s	27.5*
25	11.6±0.5 ı-p	14.9±0.4j-o	22.1*	4.7±0.2l-p	6.3±0.2m-s	25.4*
26	11.5±0.8 ı-p	13.3±0.6l-p	13.5	5.7±0.3j-l	7.8±0.7ı-n	26.9*
27	9.9±0.4 op	13.9±0.9l-p	28.7*	4.2±0.2n-p	5.8±0.3rs	27.5*
28	10.2±0.5 l-p	12.9±0.7m-p	20.9*	4.2±0.2n-p	5.4±0.2s	22.2*
29	9.9±0.5 n-p	12.6±0.5n-p	21.4*	4.5±0.3l-p	6.3±0.4n-s	28.5*
30	10.2±0.5 m-p	14.9±0.5j-o	31.5*	4.4±0.4m-p	5.8±0.4rs	24.1*
31	10.9±0.6 ı-p	15.0±0.6j-o	27.3*	5.3±0.6l-n	7.9±0.8ı-l	32.9*
32	10.8±0.3 ı-p	14.3±0.7k-p	24.5*	5.3±0.5l-o	7.6±0.5ı-p	30.2*
33	18.8±0.5 c-f	23.3±0.7fg	19.3*	9.9±0.5b-d	13.0±0.9b-d	23.8*
34	12.8±0.7 h-j	16.1±0.6ı-m	20.4*	6.8±0.7ı-k	8.7±0.5h-j	21.8
35	25.4±0.9 b	34.8±1.8b	27.0*	9.5±0.3c-e	11.7±0.6c-f	18.8*
36	10.1±0.6 m-p	13.1±0.8m-p	22.9*	4.9±0.3l-p	6.6±0.3l-s	25.7*
37	12.5±0.9 h-l	16.1±0.7ı-m	22.3*	5.0±0.0m-o	6.6±0.2k-s	24.2*
38	9.9±0.9 op	13.9±0.9l-p	28.7*	4.1±0.3op	6.1±0.4p-s	32.8*
39	12.0±0.9 h-n	15.5±1.2j-n	22.5*	5.2±0.3l-o	8.1±0.2ı-k	35.8*
40	25.1±0.8 b	27.9±0.6d	10.0*	10.6±0.3bc	14.5±0.4b	26.8*
41	24.9±0.9 b	33.1±2.2bc	24.8*	10.7±0.7b	13.1±0.7bc	18.3*
42	37.2±0.9 a	47.0±0.9a	20.8*	15.9±0.6a	18.8±0.7a	15.4*
43	10.9±1.0 ı-p	12.6±0.9n-p	13.5	5.1±0.1l-o	7.6±0.3ı-p	32.9*
44	13.9±0.7 h	17.6±0.5h-j	21.0*	10.6±0.2 bc	17.7±0.6a	40.1*

[1] N: Accession number; [2] Different letters in the same column indicate that the means are statistically significantly different among genotypes ($p< 0.05$, LSD test); * There is a statistical difference between nematode treatments of the genotype in the investigated plant growth parameter ($p< 0.05$, t test)

The highest dry weight was found in Alata 1 (15.9; 18.8 g), Taşköprü56 (10.7; 13.1 g), Kula genotypes (10.6; 14.5 g) and the lowest was in Selection4 genotype (3.7; 5.1 g) in inoculated and non-inoculated treatments, respectively. A significant difference was determined between nematode treatments in all genotypes except four genotypes ($p < 0.05$). With nematode treatment, the highest decrease in dry weight was obtained in Selection63 (35.8%), and the least decrease was in Alata1 (15.4%) (Table 3).

Discussion

In the study, resistance reactions of total 44 garlic genotypes, including garlic breeding material, landraces and wild relatives, to stem and bulb nematode were revealed. Although a fully resistant genotype was not detected, a much lower nematode multiplication rate was detected in Tunceli garlic, Kütahya Beyazı, Kütahya Pembesi and Balıkesir genotypes compared to other genotypes. Similar to our results, a study conducted in Türkiye to determine the resistance of commercial and local onion cultivars to stem and onion nematodes reported no fully resistant onion cultivars, but low nematode growth (Yavuzaslanoğlu, 2019; Yavuzaslanoğlu & Özsoy, 2020). Being important genetic resources, the garlic genotypes which show lower nematode multiplication and tolerance can be directly recommended for cultivation in areas where the stem and bulb nematode is infested.

To evaluate onion yield, Pang et al. (2009) used plant dry weight and Ibrahim (2010) used plant length, number of leaves and tuber weight. Islam et al. (2007) reported that there was a positive correlation between tuber yield and plant growth parameters such as plant length, plant weight, number of leaves, and stated that all parameters could be used to determine the tolerance in greenhouse conditions. Parameters of plant length, head diameter, plant fresh and dry weight were used to determine the tolerance of garlic genotypes to *D. dipsaci* and a significant decrease was detected in most of the genotypes. When plant growth parameters like plant length, head length, head diameter, plant fresh and dry weight values are evaluated; Alata1, Muğla1, Muğla7 and Kula landraces had the lowest decrease due to the nematode in terms of at least one plant growth parameter. Although these genotypes do not decrease nematode reproduction, they show good growth in presence of *D. dipsaci*. Therefore, these genotypes can be recommended for cultivation in nematode-infested areas. Similar results were revealed in a study by Yavuzaslanoğlu (2019), where significant differences were detected in some genotypes for plant length and plant diameter, but no significant differences were found in plant weight.

Some varieties of oat, rye, bean, clover and onion have been reported to be resistant to races of stem and bulb nematode (Plowright et al., 2002; Yavuzaslanoğlu, 2019; Yavuzaslanoğlu & Ozsoy, 2020). However, to our knowledge**,** there are no studies conducted to determine the resistance of garlic plant varieties to stem and bulb nematode. This is the first study that broadens our knowledge about resistance to stem and bulb nematode in garlic genotypes. Based on the results, we conclude that genotypes which displayless nematode reproduction and also showed tolerance against *D. dipsaci* damage can be used in infested areas and also used as genetic resources for garlic breeding against *D. dipsaci*. It is also necessary to observe the reaction of these garlic genotypes against *D. dipsaci* under field conditions.

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