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Original article (Orijinal araştırma)

Lethal and sublethal effects of mixtures of entomopathogenic fungi and synthetic insecticides on biological aspects of *Musca domestica* L.

Entomopatojen fungus ve sentetik insektisit karışımlarının *Musca domestica* L.'nın biyolojik dönemleri üzerinde lethal ve sublethal etkileri

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Summary

The current study was performed to assess the effectiveness of the entomopathogenic fungi *Metarhizium anisopliae* var. *anisopliae* (Metschnikof) Sorokin and *Isaria fumosorosea* (Wize) Brown&Smith applied in combination with some synthetic insecticides against the house fly, *Musca domestica* L. (Diptera: Muscidae) at Department of Entomology, Bahauddin Zakariya University, Multan, Pakistan during 2015. An extensive, typically dose-dependent range of responses was shown by flies towards fungus-synthetic insecticide combinations using a bait method. The insecticides acetamiprid, emamectin benzoate, imidacloprid and lufenuron in combination with insect pathogenic fungi showed higher mortality than expected with significant synergistic interactions. In addition, the mixtures were also assessed for sublethal effects on biological parameters of *M. domestica*, namely adult longevity, fecundity, egg hatching, larval duration, percent pupation, pupal weight, pupal duration, adult emergence and sex ratio. The results showed a considerable impact of fungi and synthetic insecticide mixtures on all biological parameters of *M. domestica* ($P<0.05$) except adult emergence. A noteworthy reduction in adult longevity, fecundity, egg hatching, percent pupation and pupal weight was observed, while larval and pupal durations were prolonged. The results highlight the potential of combined use of entomopathogenic fungi and synthetic insecticides for the control of *M. domestica*. Moreover, the combination of entomopathogenic fungi and synthetic insecticides can reduce the concentrations of the active ingredient required to achieve the same result as when these are applied separately. However, field trials are required to validate the effects of entomopathogenic fungi and synthetic insecticides mixtures before they can be recommended as elements of an integrated pest management program for *M. domestica*.

Keywords: Biological parameters*, Isaria fumosorosea, Metarhizium anisopliae* var. *anisopliae*, *Musca domestica*

Özet

Bu çalışma, *Metarhizium anisopliae* var. *anisopliae* (Metschnikof) Sorokin ve *Isaria fumosorosea* (Wize) Brown&Smith entomopatojen funguslarının bazı sentetik insektisitler ile birlikte kullanımıyla *Musca domestica* L. (Diptera: Muscidae)'ya karşı etkinliğini değerlendirmek amacı ile 2015 yılında Bahauddin Zakariya Üniversitesi, department of Entomoloji Bölümü (Multan, Pakistan)'nde yapılmıştır. Kapsamlı, tipik bir doza bağlı tepki aralığı, sineklerde fungus ve sentetik insektisit kombinasyonlarına karşı bir tuzak yem metodu kullanılarak gösterilmiştir. Acetamiprid, emamectin benzoate, imidacloprid ve lufenuron gibi insektisitlerin böcek patojeni funguslar ile birlikte kombinasyonu, önemli düzeyde sinerjistik etkileşimlerle beklenenden daha yüksek ölüm göstermiştir. Ayrıca, karışımların *Musca domestica*'nın, ömür, doğurganlık, yumurta açılımı, larva dönemleri süresi, pupa oluşturma yüzdesi, pupa ağırlığı, pupa süresi, ergin çıkışı ve eşey oranı gibi biyolojik parametreleri üzerinde subletal etkileri de değerlendirilmiştir. Sonuçlar, ergin çıkışı dışında *M. domestica* tüm biyolojik parametreleri üzerinde fungus ve sentetik insektisit karışımlarının önemli bir etkisi ($P <0.05$) olduğunu göstermiştir. Ömür, doğurganlık, yumurtadan açılımı, pupa oluşturma yüzdesi ve pupa ağırlığında dikkate değer bir azalma gözlenirken, larva ve pupa dönemlerinin süreleri uzamıştır. Sonuçlar *M. domestica* kontrolü için entomopatojen fungus ve sentetik insektisitlerin kombine kullanımının potansiyelini vurgulamaktadır. Bundan başka, entomopatojen fungus ve sentetik insektisit kombinasyonu, aynı sonuca ulaşmak için gerekli olan aktif bileşen konsantrasyonları ayrı olarak uygulandığına göre daha azaltılabilir. Ancak, *M. domestica* için entegre zararlı yönetimi programının bir bileşeni olarak tavsiye edilmeden önce, entomopatojen fungus ve sentetik insektisit karışımlarının etkilerini doğrulamak için arazi denemeleri de yapılmalıdır.

Anahtar sözcükler: Biyolojik parametreler, *Isaria fumosorosea, Metarhizium anisopliae* var. *anisopliae*, *Musca domestica*

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Introduction

The house fly, *Musca domestica* L. (Diptera: Muscidae), is one of the most important insect pests of dairy and poultry farms and due to its pestiferous nature, and it is responsible for transmitting pathogens causing serious diseases such as cholera, diarrhea, dysentery, gastroenteritis and peptic ulcer in both man and animals (Li & Sutzenberger, 2000; Khan et al., 2012). Being a potential danger to human and livestock health, the control of *M. domestica* is essential (Mansour et al., 2011). The chief method used worldwide for the control of *M. domestica* is the utilization of insecticides belonging to various groups i.e., carbamates, organophosphates, pyrethroids and new chemicals (Cao et al., 2006; Shi et al., 2011). Unfortunately, the extensive use of insecticides has caused serious problems such as resistance in target insects and residual effects in the atmosphere (Phillip et al., 2001; Akiner & Caglar, 2006). In Pakistan, *M. domestica* resistance to organophosphates, pyrethroids and new chemistry insecticides has been documented in earlier studies (Khan et al., 2013; Abbas et al., 2015), making it necessary to seek alternative measures for controlling *M. domestica*. Biological control may be a promising and environment friendly alternative for *M. domestica* management (Mishra et al., 2011). *Metarhizium anisopliae* (Metschnikof) Sorokin and *Isaria fumosorosea* (Wize) Brown & Smith have been reported to have high infection rates in insect populations (Kaufman et al., 2005; Sharififard et al., 2011). Compared to many synthetic insecticides, the speed of kill associated with many entomopathogenic fungi is slow, thus often limiting their use. However, the combined use of entomopathogenic fungi with synthetic insecticides may have additive effects (Serebrov et al., 2005; Purwar & Sachan, 2006; Ericsson et al., 2007; Kassab et al., 2014) and as such, promote the application of insecticide at lower doses resulting in reduced environmental pollution and decreased risk of resistance development.

A study was undertaken to determine the effect of different concentrations of two isolates of insect pathogenic fungi, *M. anisopliae* var. *anisopliae* and one isolate of *I. fumosorosea* alone and in combination with corresponding doses of six synthetic insecticides against *M. domestica*. In addition, the sublethal effects of each fungus-insecticide combination were investigated on different biological parameters of *M. domestica*.

Materials and Methods

Musca domestica rearing

Adults of *M. domestica* were reared in the Laboratory of Insect Microbiology and Biotechnology, Department of Entomology, Bahauddin Zakariya University, Multan, Pakistan during 2015. The flies were kept in a rearing cage of 30×30×30 cm with mesh screen and cloth sleeve at the front. The rearing conditions were maintained at 25±2°C, relative humidity of 65±2% and 12L:12D h photoperiod. Sugar and powdered milk in a ratio of 3:1 was provided as food for the adult flies. Medium containing wheat bran, rice meal, yeast, sugar and dry milk powder in a ratio of 40:10:3:3:1 by weight as water based paste (Bell et al., 2010) was also provided in cages as an egg laying substrate and food for hatched larvae. The diet was changed after 2-4 d depending upon the number of larvae.

Entomopathogenic fungi culture and synthetic insecticides

Two isolates of *M. anisopliae* var. *anisopliae* and one isolate *I. fumosorosea* already available in Laboratory of Insect Microbiology and Biotechnology, Department of Entomology, Bahauddin Zakariya University, Multan, Pakistan (Table 1) were selected based on maximum mortalities caused in the preliminary experimentation. Fungal spores used as the infectious propagules were cultured on PDA (potato dextrose agar media) (Freed et al., 2011a, b). Briefly, the fungi were inoculated on PDA and cultured at 25°C for 14 d. The conidia were then scraped from the plates and mixed with a sterile solution of Tween-80 (0.05%). Conidial concentrations were estimated by using a hemocytometer. LC_{10}, LC_{30} and LC_{50} for each fungal isolate were measured separately (data unpublished).

Six synthetic insecticides (Table 1) were included for toxicity bioassay. Preliminary experiments determined lethal concentrations (LC_{10}, LC_{30} and LC_{50}) for each chemical insecticide separately (Table 1).

Table 1. Concentrations of entomopathogenic fungi and synthetic insecticides assessed against *Musca domestica* in this study

Fungal Species	Source	LC_{10} (spores ml^{-1})	LC_{30} (spores ml^{-1})	LC_{50} (spores ml^{-1})
Metarhizium anisopliae var.	cotton field (Multan)	8.78×10^{6}	1.64×10^{7}	3.38×10^{7}
M. anisopliae var. *anisopliae*	maize field (Mansehra)	9.21×10^{6}	4.56×10^{6}	1.30×10^{6}
Isaria fumosorosea (If-03)	cotton field (Multan)	9.70×10^{7}	1.20×10^{8}	1.41×10^{8}
Insecticides	Manufacturer	$LC_{10\ (ppm)}$	$LC_{30\ (ppm)}$	$LC_{50\ (ppm)}$
Acetamiprid	Arysta LifeScience	0.03	0.14	0.39
Bifenthrin	FMC United	0.02	0.08	0.22
Emamectin benzoate	Syngenta	0.00002	0.0002	0.001
Fipronil	Bayer CropScience	0.00003	0.0004	0.002
Imidacloprid	Bayer CropScience	0.022	0.09	0.27
Lufenuron	Syngenta	0.00002	0.0002	0.001

Lethal effects of entomopathogenic fungi and synthetic insecticides combinations

Suspensions of fungi and synthetic insecticides were mixed in corresponding concentrations (LC_{10}, LC_{30} and LC_{50}) and incorporated into the adult diet, and an insecticide- and fungus-free diet was provided in control treatments.

For each fungus-insecticide combination, there were three treatment doses, LC_{10} of fungus+ LC_{10} of insecticide (low dose), LC_{30} of fungus+ LC_{30} of insecticide (intermediate dose) and LC_{50} of fungus+ LC_{50} of insecticide (high dose). Each treatment combination was replicated four times with ten adults (2-3 day old, male to female ratio 50:50) per replication placed in plastic jars (15×6×6 cm).

Musca domestica mortality was recorded every 24 h for 7 d. For effective comparison, following the same procedure as described above, the LC_{10}, LC_{30} and LC_{50} of fungi and LC_{10}, LC_{30} and LC_{50} of synthetic insecticides were applied individually.

Sublethal effects

In addition to mortality caused by the fungus-insecticide combinations, the sublethal effects of these mixtures were also assessed on different biological parameters of the surviving *M. domestica* population, namely adult longevity, fecundity, egg hatching, larval duration, percent pupation, pupal weight, pupal duration, adult emergence and sex ratio. The adult flies were provided with egg laying medium as described above, while, male and female longevity was recorded separately as described by Fletcher et al. (1990). The egg laying substrate was examined daily for eggs count and changed every 2 d. If eggs were present, they were counted and left in same medium for hatching and larval development. Fecundity was calculated by dividing the total number of eggs laid to the total number of surviving females over the entire experiment. Following egg hatching, the larvae were counted and percent hatching was calculated. The larvae were provided with food and kept until pupation to estimate the duration of the larval period. Once pupation had occurred, the pupae were separated from the medium, cleaned, counted and weighed to calculate percent pupation and determine pupal weight. The pupae were placed in petri dishes and kept separately in plastic jars and observed until adult emergence from which percent emergence was measured according to Khazanie (1979) and the sex ratio was determined by counting males and females separately. Each experiment, including the controls, was repeated twice. The data was pooled for analysis.

Data analysis

Mortality data was corrected with the help of Abbott's formula to account for natural mortality recorded in the control treatment (Abbott, 1925). The statistical program POLO-PC (LeOra Software, Berkeley, CA, USA) was used to determine the different concentrations (LC_{10}, LC_{30} and LC_{50}) for each fungal isolate and different doses (LC_{10}, LC_{30} and LC_{50}) for synthetic insecticides separately. The synergistic effect of mortality was analyzed by comparing mortality rates induced by mixtures of fungi and synthetic insecticides (observed) with the sum of mortalities by fungi and synthetic insecticides separately (expected). For measurement of expected mortality (M_e), the following formula was used:

$$M_e = M_f + M_i(1 - M_f/100)$$

Where, M_f and M_i were the observed percent mortalities caused by the fungus and the chemical insecticide separately (Farenhorst et al., 2010). Paired samples t-test was used for pair-wise comparisons between each treatment and to eliminate potential treatment variations i.e., differences between fungi and synthetic insecticides effectiveness by using Statistix 8.1 (Analytical Software, Tallahassee, USA). Positive M_{fi}-M_e values were considered synergistic (Koppenhöfer & Kaya, 1998). The means for longevity, fecundity and other parameters were added and the average was taken from both repetitions. Any differences amongst fungus-insecticide combinations on the sublethal parameters were assessed, and analyzed with the analysis of variance coupled with the LSD separation of means at the 5% level of significance.

Results

Mortality bioassays

Mortality of *M. domestica* after application of insect pathogenic fungi and different synthetic insecticides individually are presented in Table 2. The expected and observed mortality rates were also recorded for mixtures of entomopathogenic fungi and synthetic insecticides at different doses (Table 3). The insecticides in combination with fungi affected the survival rate of *M. domestica*. The mortality rate increased at higher concentrations of fungi and synthetic insecticides. The maximum mortality percentage (97.2±1.7) was observed at the high dose of Ma-4.1 (1.30×10^6 spores ml^{-1}) with acetamiprid (0.3 ppm) followed by Ma-4.1 with emamectin benzoate (high dose) (86.9±1.8). Moreover, significant synergetic interactions were observed between pathogenic fungi and synthetic insecticides especially, when both were applied at the highest dose. All synthetic insecticides, except bifenthrin and fipronil, showed higher mortality than expected and effects seemed to be synergistic on all three doses of fungal isolates.

Table 2. Percentage mortality (±SE) caused by *Metarhizium anisopliae* var. *anisopliae* (Ma-2.3 and Ma-4.1), *Isaria fumosorosea* (If-03) and synthetic insecticides applied individually against the *Musca domestica* at each of the three tested doses

	Concentrations		
Treatment	LC_{10}	LC_{30}	LC_{50}
Ma-2.3	9.20±0.43	20.65±1.21	43.80±1.56
Ma-4.1	11.32±0.54	26.70±0.67	46.70±2.10
If-03	10.10±0.32	20.30±1.23	41.20±2.12
Acetamiprid	11.30±0.14	31.40±0.46	53.20±0.32
Bifenthrin	13.30±0.20	31.40± 0.12	51.60±0.25
Emamectin	15.80± 0.25	33.61±0.23	55.40±0.68
Fipronil	17.60± 0.48	34.30±0.25	56.30±0.61
Imidacloprid	11.62±0.68	28.53±0.61	49.50±0.47
Lufenuron	16.17±0.21	31.60±0.78	51.30±0.12
Control	3.45±0.54	2.43±0.23	2.49±0.31

Table 3. Effect of combinations of *Metarhizium anisopliae* var. *anisopliae* (Ma-2.3 and Ma-4.1), *Isaria fumosorosea* (If-03) and synthetic insecticides on percent mortality (±SE) of *Musca domestica*

	Low dose (LC_{10})														
	Ma-2.3					Ma-4.1					IF-03				
	EXP.	OBS.	T-test	P-value	M_{fi}-M_e	EXP.	OBS.	T-test	P-value	M_{fi}-M_e	EXP.	OBS.	T-test	P-value	M_{fi}-M_e
Acetamiprid	19.2±0.5	27.8±0.8	3.31	0.04*	8.6	21.6±0.4	32.3±0.8	4.21	0.02*	10.9	20.4±0.3	26.5±0.5	1.56	0.01*	6.35
Bifenthrin	20.7±0.5	18.4±1.9	1.21	0.21	-2.4	22.9±0.9	21.7±0.7	1.23	0.71	-1.20	21.6±1.2	19.5±1.0	0.82	0.53	-2.18
Emamectin benzoate	22.5±1.2	24.2±1.4	2.12	0.13	1.7	24.6±1.2	28.2±1.4	3.45	0.03*	3.61	23.4±0.3	23.7±1.4	2.14	0.11	0.29
Fipronil	23.9±0.5	16.3±0.5	0.67	0.31	-7.4	25.0±0.7	17.9±1.3	0.91	0.35	-7.97	24.8±0.7	16.3±0.6	0.76	0.75	-8.27
Imidacloprid	19.5±0.3	20.8±0.3	1.02	0.12	1.4	21.6±0.5	21.7±0.7	2.02	0.13	0.09	20.4±1.2	21.4±0.7	1.87	0.21	1.01
Lufenuron	22.8±0.7	22.9±0.7	1.13	0.23	0.18	24.9±0.5	31.3±0.6	4.32	0.02*	6.45	23.7±0.9	23.9±0.9	1.1	0.24	0.24
	Intermediate dose (LC_{30})														
	Ma-2.3					Ma-4.1					IF-03				
	EXP.	OBS.	T-test	P-value	M_{fi}-M_e	EXP.	OBS.	T-test	P-value	M_{fi}-M_e	EXP.	OBS.	T-test	P-value	M_{fi}-M_e
Acetamiprid	42.1±0.9	50.9±1.4	5.43	0.01*	8.76	48.1±1.3	61.2±1.8	6.21	0.03*	12.95	41.7±1.2	49.7±1.6	4.78	0.04*	7.83
Bifenthrin	42.5±2.1	29.3±3.2	0.71	0.43	-12.89	48.5±2.2	35.3±2.6	0.31	0.28	-12.91	41.1±1.7	29.2±2.6	0.42	0.12	-12.66
Emamectin benzoate	42.6±1.3	43.6±1.6	2.32	0.13	0.67	49.0±0.4	60.8±0.4	3.27	0.04	11.78	42.3±0.8	42.75±0.9	1.78	0.21	0.13
Fipronil	43.4±1.5	30.1±2.1	0.21	0.71	-13.08	49.5±1.9	30.8±1.4	0.62	0.71	-18.41	42.1±0.7	31.9±0.8	0.61	0.51	-10.98
Imidacloprid	41.0±0.3	41.5±0.6	1.02	0.12	0.41	47.1±1.2	47.5±0.9	1.34	0.32	0.35	40.7±0.9	40.9±0.2	1.82	0.42	0.21
Lufenuron	42.3±2.1	45.9±3.9	2.11	0.04*	3.72	48.3±1.3	54.1±0.9	3.41	0.02*	5.76	41.9±3.2	44.1±4.5	2.51	0.01*	2.17
	High dose (LC_{50})														
	Ma-2.3					Ma-4.1					IF-03				
	EXP.	OBS.	T-test	P-value	M_{fi}-M_e	EXP.	OBS.	T-test	P-value	M_{fi}-M_e	EXP.	OBS.	T-test	P-value	M_{fi}-M_e
Acetamiprid	67.1±0.9	86.3±0.8	9.21	0.02*	17.63	69.9±1.3	97.2±1.7	9.72	0.04*	25.60	64.5±1.2	83.8±1.1	8.31	0.02*	17.65
Bifenthrin	66.7±1.9	40.3±2.9	0.51	0.32	-28.51	69.6±0.8	53.3±1.0	0.43	0.52	-18.42	64.1±1.8	38.7±2.6	0.69	0.43	-27.49
Emamectin benzoate	68.4±1.1	80.3±1.4	5.14	0.01*	11.76	71.3±0.6	86.9±1.8	5.31	0.03*	15.44	65.8±0.6	79.4±0.9	4.81	0.021*	13.52
Fipronil	68.2±2.5	48.2±5.0	0.89	0.46	-20.20	71.1±1.4	49.8±2.6	0.13	0.61	-21.55	65.6±3.2	46.9±5.2	0.74	0.81	-18.87
Imidacloprid	68.4±2.1	69.3±1.8	0.71	0.04	0.45	71.3±0.3	73.2±1.2	1.01	0.04*	1.50	65.8±3.2	71.3±1.1	3.45	0.03*	5.13
Lufenuron	68.8±2.1	72.1±2.4	1.53	0.03	3.29	71.7±1.5	76.3±0.5	1.56	0.03*	4.56	66.2±1.8	73.4±1.1	4.35	0.02*	7.24

Exp= expected mortality; Obs= observed mortality; M_{fi} = observed mortality of mixture; ^Expected mortality Me= Mf+ Mi(1 - Mf/100) with Mf and Mi observed mortalities caused by fungus and synthetic insecticides alone respectively; *Results show significant paired samplest-test comparisons for both observed and expected mortality rates (means ±SE); LC_{10}, LC_{30} and LC_{50}= fungus and insecticide dose.

Sublethal effects on *Musca domestica* progeny

Adult longevity

A decreasing trend was found for male longevity of *M. domestica* in response to fungi and synthetic insecticides combinations (Table 4). Ma-4.1 with acetamiprid followed by Ma-4.1 with emamectin benzoate at the high dose, caused significant reduction in the male longevity of *M. domestica* in comparison to all the other treatments and the control ($F = 6.64$; $df = 6,12$; $P < 0.0001$). A similar trend was observed for female longevity, in which the application of Ma-4.1 with acetamiprid (high dose) caused significant reduction in the female longevity of *M. domestica* from 20.8 to 8.5 d followed by 8.6±0.3 due to the application of Ma-4.1 with emamectin benzoate (high dose) as compared to all other treatments ($F = 3.32$; $df = 6,12$; $P = 0.001$) (Table 4).

Fecundity and hatching percentage

A broad range of variation in fecundity of *M. domestica* was observed in all treatments (Table 5). A significant difference was observed for mixtures of different doses of fungi and synthetic insecticides especially at the high dose. The least number of eggs (118±4.1, 121±4.6 and 123±4.4) were recorded for treatments Ma-4.1 with lufenuron (high dose), Ma-4.1 with acetamiprid (high dose), Ma-4.1 with emamectin benzoate (high dose), respectively. Hatching percentage also varied significantly among the treatments (Table 5). The lowest egg hatching percentages, 67.9±1.0, 70.0±0.7 and 70.1±0.5, was recorded for Ma-4.1 with emamectin benzoate (high dose), If-03 with lufenuron (high dose) and Ma-4.1 with lufenuron (high dose) ($F = 3.81$; $df = 6,12$; $P = 0.0003$), respectively.

Larval period, pupation percentage, pupal weight and pupal duration

The larval period ranged from 6.3-9.1 d which showed significant prolongation among all treatments. If-03 with imidacloprid (high dose) (9.1±0.1), Ma-2.3 with imidacloprid (high dose) (9.1±0.1) and If-03 with emamectin benzoate (high dose) (8.9±0.1) ($F = 2.35$; $df = 6,12$; $P < 0.01$) caused maximum prolongation of the larval period (Table 6).

A significant reduction in the pupation percentage was recorded with increasing concentrations of fungus-insecticide mixtures (intermediate and high dose). The lowest pupation percentage was recorded for If-03 with acetamiprid (high dose) (60.0±2.4), Ma-4.1 with emamectin benzoate (high dose) (60.2±2.2) and Ma-2.3 with acetamiprid (high dose) (60.8±1.5) ($F = 2.18$; $df = 6,12$; $P = 0.03$) (Table 6).

A similar inverse trend was observed for pupal weight (mg), decreasing as the fungus-insecticide concentration combinations increased. The minimum pupal weight was recorded for Ma-4.1 with imidacloprid (high dose) (10.3±0.3), Ma-2.3 with acetamiprid (high dose) (10.8 ±0.1) and If-03 with acetamiprid (high dose) (11.0 ±0.2) ($F=3.46$; $df=6,12$; $P=0.0008$) (Table 7). Moreover, significant prolongation was observed in the pupal period in all treatments compared to the control. The treatment of Ma-4.1 with lufenuron (high dose) (8.4±0.1), Ma-4.1 with imidacloprid (high dose) (8.0±0.1) and Ma-4.1 with acetamiprid (high dose) (7.8±0.1) ($F = 3.81$; $df = 6,12$; $P = 0.0003$) (Table 7) caused maximum prolongation in pupal period.

Table 4. Sublethal effects of *Metarhizium anisopliae* var. *anisopliae* (Ma-2.3 and Ma-4.1), *Isaria fumosorosea* (If-03) and synthetic insecticide mixture on longevity of adults of *Musca domestica*

	MALE LONGEVITY (days) (±SE)								
	Low dose (LC_{10})			Intermediate dose (LC_{30})			High dose (LC_{50})		
Insecticide/fungus	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03
Acetamiprid	16.1±0.3	14.8±0.3	16.1±0.3	13.9±0.3cd	11.6±0.4g	14.1±0.1bcd	10.6±0.4fgh	7.9±0.1j	10.9±0.5efgh
Bifenthrin	14.4±0.5	16.1±0.2	14.8±0.3	12.6±0.2efg	14.3±0.5bc	12.9±0.2def	10.6±0.3fgh	12.1±0.5de	11.3±0.2defg
Emamectin benzoate	14.1±0.3	15.0±0.3	14.3±0.1	12.2±0.5fg	12.9±0.3def	12.2±0.5fg	9.1±0.5ij	8.1±0.3j	9.9±0.0hi
Fipronil	15.6±0.1	16.1±0.3	15.8±0.1	14.9±0.1bc	13.9±0.7cd	15.3±0.2b	14.5±0.3b	13.4±0.4bc	14.6±0.2b
Imidacloprid	16.5±0.8	15.9±0.2	16.8±0.6	13.9±0.3cd	13.8±0.2cde	14.2±0.1bcd	12.1±0.2de	10.2±0.4ghi	12.3±0.1cd
Lufenuron	15.7±0.8	14.9±0.3	15.8±0.7	14.7±0.8bc	12.4±0.5fg	14.3±1.1bc	12.4±0.9cd	8.5±0.4j	11.9±0.8def
Control	19.2±0.2	19.4±0.5	19.5±0.3	19.5±0.3a	20.3±0.7a	20.0±0.4a	19.6±0.2a	19.9±0.5a	19.8±0.1a
P		0.06			0.0005			<0.0001	
F		1.88			3.62			6.64	
LSD value		ns			1.31			1.33	
	FEMALE LONGEVITY (days) (±SE)								
	Low dose (LC_{10})			Intermediate dose (LC_{30})			High dose (LC_{50})		
Insecticide/fungus	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03
Acetamiprid	16.5±0.2	15.8±0.3	16.6±0.3	13.0±0.4hij	12.8±0.3ij	13.3±0.1efghi	11.9±0.5cdef	8.5±0.3h	12.0±0.5 bcdef
Bifenthrin	15.9±0.4	15.6±0.3	15.9±0.3	13.2±0.4ghi	13.0±0.2hij	13.4±0.1efghi	12.0±0.6 bcdef	10.3±0.7g	12.3±0.4bcde
Emamectin benzoate	16.2±0.5	15.2±0.1	16.4±0.4	14.2±0.4bcd	12.4±0.1j	14.4±0.1b	10.9±0.5fg	8.6±0.3h	11.1±0.5efg
Fipronil	14.8±0.3	15.3±0.5	14.8±0.3	13.5±0.2defgh	13.2±0.0fghi	13.5±0.1defgh	12.7±0.6bc	13.1±0.2b	12.7±0.6bc
Imidacloprid	16.1±1.0	15.9±0.2	15.5±0.9	13.7±0.3cdefgh	14.2±0.1bc	13.9±0.2bcdef	11.7±0.3cdef	11.3±0.3efg	11.4±0.3defg
Lufenuron	15.5±0.7	16.9±0.3	15.3±0.5	13.9±0.3bcde	13.2±0.0fghi	13.8±0.3bcdefg	12.5±0.5bcd	11.3±0.5 defg	12.2±0.4bcde
Control	20.7±0.2	20.4±0.4	20.9±0.1	20.3±0.1a	20.9±0.1a	20.6±0.1a	20.6±0.1a	20.5±0.5a	20.8±0.4a
P		0.21			0.0004			0.001	
F		1.36			3.73			3.32	
LSD value		ns			0.68			1.20	

Means in rows and columns followed by same letters are not statistically different; LSD, P<0.05; ns = not significant, LC_{10}, LC_{30} and LC_{50}=fungus and insecticide dose.

Table 5. Effects of binary mixture of *Metarhizium anisopliae* var. *anisopliae* (Ma-2.3 and Ma-4.1), *Isaria fumosorosea* (If-03) and synthetic insecticide on fecundity and percent hatching of *Musca domestica* progeny

FECUNDITY (±SE)									
	Low dose (LC_{10})			Intermediate dose (LC_{30})			High dose (LC_{50})		
Insecticide/fungus	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03
Acetamiprid	234±4.7fg	239±7.3efg	237±2.6efg	182±3.1efg	165±10.2gh	180±2.1efg	142±9.6cd	121±4.6ef	136±5.1de
Bifenthrin	247±6.0def	233±13.9fg	250±3.3de	184±2.1efg	174±10.6fgh	178±8.1efgh	157±7.0c	137±3.5de	157±7.0c
Emamectin benzoate	232±4.6fg	206±3.0h	232±4.3fg	174±4.1fgh	158±6.2h	169±4.3fgh	129±9.5def	123±4.4ef	142±2.5cd
Fipronil	273±3.3bc	231±7.1g	273±3.0bc	247±6.3b	196±9.1de	249±5.3b	194±9.5b	190±5.7b	196±10.5b
Imidacloprid	280±6.5b	262±2.2cd	277±4.3bc	224±6.7c	189±8.2ef	224±6.7c	185±2.5b	145±9.0cd	188±0.6b
Lufenuron	267±6.9bc	238±6.5efg	272±2.1bc	217±5.6cd	175±11.9fgh	219±5.8c	198±1.8b	118±4.0f	201±0.8b
Control	382±3.9a	380±5.5a	382±2.6a	375±8.2a	377±7.8a	381±5.5a	383±3.6a	380±5.6a	384±3.2a
P		0.003			0.02			<0.0001	
F		2.96			2.33			6.42	
LSD value		15.88			20.96			17.48	

PERCENT HATCHING (±SE)									
	Low dose (LC_{10})			Intermediate dose (LC_{30})			High dose (LC_{50})		
Insecticide/fungus	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03
Acetamiprid	82.6±0.5d	85.4±1.0 bc	84.0±0.6 cd	81.1±0.4bc	83.3±0.4b	81.1±0.4bc	79.4±0.7bc	77.5±0.7cd	80.3±0.5b
Bifenthrin	85.8±0.5bc	84.6±0.6 c	86.9±0.5b	83.2±0.7b	81.9±0.3bc	82.9±0.3b	78.6±0.7bc	75.9±1.3de	79.5±1.6bc
Emamectin benzoate	84.9±0.8 bc	83.4±0.8 c	84.7±0.9 bcd	80.8±1.2bc	77.9±2.0e	80.8±1.3c	74.1±0.5e	67.9±1.0g	74.4±0.4e
Fipronil	80.6±0.2def	78.9±0.7f	79.6±0.6f	77.5±0.5e	76.0±0.9f	77.8±0.7e	75.8±0.4de	75.5±0.6de	75.7±0.6de
Imidacloprid	82.3±0.7 cd	86.2±0.6b	83.0±0.3 cd	74.9±1.4fg	79.8±0.8c	76.4±1.4f	74.7±1.9e	77.8±0.6bcd	73.9±2.0e
Lufenuron	81.4±0.9de	82.2±0.6d	81.6±0.9de	72.8±0.4g	78.0±1.6cd	73.9±1.1fg	70.7±0.3f	70.1±0.5fg	70.0±0.7fg
Control	95.7±0.9a	96.2±0.8a	95.9±0.9a	93.5±0.2a	95.3±1.2a	93.9±0.68a	95.2±0.8a	94.7±0.8a	94.5±0.9a
P		0.002			0.008			0.0003	
F		3.10			2.61			3.81	
LSD value		2.04			2.82			2.59	

Means in rows and columns followed by same letters are not statistically different; LSD, $P<0.05$; ns =not significance at 5% level; LC_{10}, LC_{30} and LC_{50}=fungus and insecticide dose.

Table 6. Effects of *Metarhizium anisopliae* var. *anisopliae* (Ma-2.3 and Ma-4.1), *Isaria fumosorosea* (If-03) and synthetic insecticide mixture on larval duration and percent pupation of *Musca domestica* progeny

LARVAL DURATION (days) (±SE)									
	Low dose (LC_{10})			Intermediate dose (LC_{30})			High dose (LC_{50})		
Insecticide/fungus	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03
Acetamiprid	7.4±0.3cd	7.5±0.1cd	7.5±0.2cd	7.6±0.2 gh	7.7±0.1 gh	7.7±0.2 gh	8.1±0.1h	8.1±0.7h	8.2±0.1gh
Bifenthrin	7.5±0.1cd	7.3±0.1de	7.5±0.1cd	7.6±0.10gh	7.6±0.2h	7.7±0.4 gh	8.5±0.1efg	8.3±0.1fgh	8.5±0.1 cdef
Emamectin benzoate	7.7±0.1bcd	7.3±0.1de	7.8±0.1bcd	8.4±0.03bcd	7.8±0.7gh	8.4±0.1bc	8.8±0.1abc	8.5±0.3 cdef	8.9±0.1ab
Fipronil	8.1±0.2ab	7.3±0.1de	7.9±0.4abc	8.4±0.04bcd	7.9±0.1fg	8.3±0.1cde	8.6±0.1 bcdef	8.5±0.1defg	8.8±0.1abcd
Imidacloprid	8.4±0.2a	7.4±0.1cd	7.8±0.4abc	8.8±0.1a	7.7±0.1 gh	8.6±0.2ab	9.1±0.1a	8.4±0.2fg	9.1±0.1a
Lufenuron	7.6±0.1bcd	7.6±0.1bcd	7.6±0.1cd	8.1±0.1ef	8.1±0.2def	8.1±0.1def	8.6±0.2cdef	8.8±0.2bcde	8.5±0.2 cdef
Control	6.3±0.2f	6.8±0.1ef	6.4±0.2f	6.5±0.3i	6.6±0.3i	6.5±0.3i	6.7±0.4i	6.7±0.4i	6.4±0.40i
P		0.007			<0.0001			0.01	
F		2.66			5.59			2.35	
LSD value		0.53			0.26			0.31	

PERCENT PUPATION(±SE)									
	Low dose (LC_{10})			Intermediate dose (LC_{30})			High dose (LC_{50})		
Insecticide/fungus	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03
Acetamiprid	76.2±0.5	74.6±0.9	74.2±1.5	65.2±1.3ij	68.6±1.0hi	64.4±0.5j	60.8±1.5g	61.2±2.2fg	60.0±2.4g
Bifenthrin	79.1±1.0	78.3±1.2	81.0±0.2	71.8±2.4efgh	70.3±1.1fgh	74.7±1.8de	62.5±0.9efg	64.5±1.4 defg	63.3±1.5 defg
Emamectin benzoate	82.5±0.5	82.6±0.9	82.9±0.4	72.7±0.9efg	75.2±1.0cde	73.9±1.4def	62.6±1.2efg	60.2±2.2g	64.5±2.0 defg
Fipronil	81.1±0.7	80.1±0.4	81.4±0.5	79.7±0.6b	76.6±1.2bcd	78.2±1.5bc	73.2±0.8b	66.9±1.1 cde	74.8±2.6b
Imidacloprid	79.9±1.3	81.5±1.2	83.2±1.2	68.6±1.5h	75.0±1.9cde	71.4±0.6efgh	67.8±0.4cd	71.1±0.5bc	65.8±2.0def
Lufenuron	76.4±1.0	74.2±0.9	77.6±0.8	69.9±0.6gh	70.4±0.9fgh	70.7±0.7fgh	66.2±1.3 cde	63.8±1.2 defg	67.4±1.4cde
Control	92.7±3.2	94.9±3.9	94.0±3.1	94.0±4.6a	93.5±4.4a	93.4±4.3a	93.0±5.2a	94.2±5.8a	93.2±5.0a
P		0.06			0.003			0.03	
F		1.86			2.95			2.18	
LSD value		ns			3.87			4.97	

Means in rows and columns followed by same letters are not statistically different; LSD, $P<0.05$;ns =not significant;LC_{10}, LC_{30} and LC_{50}=fungus and insecticide dose.

Table 7. Effects of *Metarhizium anisopliae* var. *anisopliae* (Ma-2.3 and Ma-4.1), *Isaria fumosorosea* (If-03) and synthetic insecticide mixture on pupal weight and pupal duration of *Musca domestica* progeny

PUPAL WEIGHT (mg)(±SE)									
	Low dose (LC_{10})			Intermediate dose (LC_{30})			High dose (LC_{50})		
Insecticide/fungus	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03
Acetamiprid	15.7±0.1c	15.9±0.2c	15.6±0.4cd	13.8±0.3d	14.7±0.3cd	13.7±0.3de	10.8±0.1g	12.6±0.3ef	11.0±0.2fg
Bifenthrin	16.9±0.3bc	16.7±0.4bc	17.1±0.3b	13.9±0.3d	14.8±0.3cd	14.2±0.1cd	11.4±0.6f	12.9±0.5f	11.4±0.6f
Emamectin benzoate	15.8±0.2c	17.2±0.3ab	16.1±0.5bc	13.9±0.6d	16.1±0.2b	14.0±0.6c	12.6±0.3d	11.2±0.4f	13.1±0.5c
Fipronil	16.1±0.4bc	15.4±0.2cd	16.4±0.6bc	15.7±0.2c	15.1±0.4cd	15.9±0.3bc	14.6±0.7b	12.7±1.0e	14.9±0.6b
Imidacloprid	16.5±0.2bc	15.7±0.2c	16.5±0.3bc	14.8±0.4c	13.9±0.5de	15.5±0.2bc	12.1±0.4ef	10.3±0.3g	12.1±0.4ef
Lufenuron	15.9±0.4c	16.2±0.4bc	16.1±0.4bc	16.2±0.1b	15.9±0.3bc	16.1±0.2b	13.4±0.5c	11.3±0.4fg	14.3±0.1b
Control	19.1±0.3a	19.2±0.2a	19.4±0.2a	18.9±0.2a	19.3±0.2a	19.2±0.1a	19.1±0.2a	18.9±0.4a	19.1±0.2a
P		0.02			0.0001			0.0008	
F		2.35			4.15			3.46	
LSD value		1.14			1.03			1.36	

PUPAL DURATION (days)(±SE)									
	Low dose (LC_{10})			Intermediate dose (LC_{30})			High dose (LC_{50})		
Insecticide/fungus	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03
Acetamiprid	6.1±0.1	6.1±0.1	6.0±0.1	7.1±0.1cd	7.4±0.1abc	6.8±0.2de	7.5±0.1bcde	7.8±0.1bc	7.3±0.1cdefg
Bifenthrin	6.1±0.2	6.2±0.4	6.1±0.2	7.3±0.1abc	7.5±0.6ab	7.4±0.2abc	7.6±0.1bcd	7.2±0.5defg	7.4±0.1bcde
Emamectin benzoate	6.2±0.1	5.9±0.3	6.2±0.1	7.3±0.1abc	6.6±0.1e	7.2±0.1abc	7.7±0.1bcd	7.6±0.2bcd	7.5±0.3bcd
Fipronil	6.3±0.1	6.3±0.1	6.2±0.1	7.4±0.1abc	7.5±0.1ab	7.2±0.1bc	7.5±0.3bcde	7.8±0.5bcd	7.3±0.1cdef
Imidacloprid	6.2±0.1	6.0±0.1	6.0±0.2	6.5±0.1e	7.3±0.8abc	6.7±0.2e	6.8±0.6g	8.0±0.1ab	6.9±0.1efg
Lufenuron	6.3±0.2	6.4±0.1	6.3±0.2	6.7±0.2e	7.6±0.6a	6.8±0.2de	6.8±0.3fg	8.4±0.1a	6.9±0.1efg
Control	5.4±0.1	5.4±0.2	5.3±0.1	5.6±0.2f	5.7±0.2f	5.6±0.1f	5.2±0.5h	5.5±0.2h	5.3±0.1h
P		0.97			<0.0001			0.0003	
F		0.36			5.01			3.81	
LSD value		ns			0.38			0.60	

Means in rows and columns followed by same letters are not statistically different; LSD, $P<0.05$;ns = not significant; LC_{10}, LC_{30} and LC_{50}=fungus and insecticide dose.

Percent adult emergence and sex ratio

The data regarding fungi and synthetic insecticides mixtures showed no significant differences at any of three tested concentrations (Table 8). Moreover, there was no difference in sex ratio except when fungi and synthetic insecticides were applied simultaneously at the low dose. The lowest male percentage (47.0±0.8) was observed in treatment If-03 with lufenuron (low dose), while minimum female emergence (46.7±0.6) was observed in Ma-2.3 with bifenthrin (low dose) (F = 2.31; df = 6,12; P = 0.02) (Table 9).

Table 8. Effects of binary mixture of *Metarhizium anisopliae* var. *anisopliae* (Ma-2.3 and Ma-4.1), *Isaria fumosorosea* (If-03) and synthetic insecticides on adult emergence (±SE) of *Musca domestica* progeny

	Low dose (LC_{10})			Intermediate dose (LC_{30})			High dose (LC_{50})		
Insecticide/fungus	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03
Acetamiprid	76.4±0.4	75.7±0.5	76.4±0.4	71.9±0.4	72.4±0.5	71.9±0.4	68.6±0.5	66.2±1.0	68.7±0.7
Bifenthrin	74.1±0.6	73.6±1.1	75.1±0.4	71.1±0.5	70.7±0.8	71.6±0.3	65.6±0.7	69.3±1.7	65.9±0.5
Emamectin benzoate	75.9±0.9	76.4±0.8	75.8±0.9	70.4±0.9	70.3±0.3	70.4±0.7	64.2±1.4	66.9±0.6	63.7±1.4
Fipronil	71.8±0.7	74.5±1.2	73.1±1.1	68.7±0.5	69.7±1.2	69.5±1.0	62.4±0.5	64.6±1.6	64.3±1.3
Imidacloprid	72.6±0.6	76.3±1.7	75.6±1.8	65.3±1.6	69.2±0.7	68.4±0.5	60.9±0.7	62.2±1.0	62.2±1.0
Lufenuron	75.9±1.0	74.6±1.5	77.5±2.0	72.1±0.4	70.3±0.5	71.7±0.3	66.2±1.3	67.4±0.9	65.9±0.8
Control	92.5±1.1	94.1±1.2	93.0±1.5	94.1±1.1	94.5±0.9	93.2±0.8	94.1±0.8	94.4±1.1	93.3±0.5
P		0.52			0.11			0.22	
F		0.94			1.61			1.34	
LSD value		ns			ns			ns	

Means in rows and columns followed by same letters are not statistically different; LSD, P<0.05; ns =not significant; LC_{10}, LC_{30} and LC_{50}=fungus and insecticide dose.

Discussion

Keeping in mind the short life cycle and high reproduction potential of *M. domestica*, it would be beneficial to find an approach for increasing pest mortality and reducing the lethal time using entomopathogenic fungi. In the current study, synthetic insecticides were applied in mixtures with entomopathogenic fungi. A synergistic effect was observed for all three fungal isolates and the synthetic insecticides, acetamiprid, emamectin benzoate, imidacloprid and lufenuron, at the three doses by comparison of observed and expected mortality. Synthetic insecticides have been mixed fungi in previous studies (Pachamuthu & Kamble, 2000; Ericsson et al. 2007; Asi et al., 2010; Sharififard et al., 2011; Archana & Ramaswamy, 2012; Kassab et al., 2014). These studies mainly focused on the mortality of the pest whereas the current study also examined sublethal doses, LC_{10} and LC_{30}. The comparison of the percent mortality of binary mixture and single products was evaluated and significant differences were observed in the mortality of binary mixture as compared to single products. The insecticides, acetamiprid, emamectin benzoate, imidacloprid and lufenuron, increased pest mortality and showed potential against *M. domestica* when combined with entomopathogenic fungi.

In cases where fungi and synthetic insecticides are applied alone, the difference in effectiveness is due to different mode of action. Although, the mechanisms for effects of fungi and synthetic insecticides mixtures are unclear, synthetic insecticides may influence the insect cuticle and facilitate penetration for fungal spores, or possibly restrain immune response and facilitate fungal infection process (Hiromori & Nishigaki, 2001). As indicated by the $LC_{10,}$ the most toxic synthetic insecticide (fipronil) was much less efficient when combined with entomopathogenic fungi, which highlights the influence of insecticide on the pathogenic fungi and make it is less compatible as compared to other synthetic insecticides.

Table 9. Effects of *Metarhizium anisopliae* var. *anisopliae* (Ma-2.3 and Ma-4.1), *Isaria fumosorosea* (If-03) and synthetic insecticides mixture on sex ratio of *Musca domestica* progeny

MALE RATIO (±SE)									
	Low dose (LC_{10})			Intermediate dose (LC_{30})			High dose (LC_{50})		
Insecticide/fungus	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03
Acetamiprid	51.0±1.2ab	53.0±0.7a	51.0±1.5ab	51.0±0.7	53.3±0.9	52.5±0.5	53.0±0.4	58.0±2.5	54.0±0.7
Bifenthrin	53.3±0.6a	48.3±2.1bcd	50.8±0.5ab	48.8±1.8	51.0±1.8	48.8±1.8	53.3±1.1	52.5±0.5	52.8±1.1
Emamectin benzoate	52.0±1.7a	50.8±0.9ab	53.0±0.8a	51.0±1.5	49.8±2.3	50.8±1.7	54.8±0.8	51.5±2.6	54.3±0.6
Fipronil	51.9±0.4a	50.3±1.1abc	51.7±0.5a	52.8±0.6	51.9±0.7	51.0±1.3	49.5±3.2	51.0±1.0	52.5±2.2
Imidacloprid	53.2±1.3a	50.3±1.1abc	51.9±0.7a	49.8±1.4	51.5±0.2	51.5±1.4	50.1±2.7	50.6±1.7	53.3±1.1
Lufenuron	47.3±2.1cd	51.5±0.5a	47.0±0.8da	48.0±2.3	49.7±1.0	49.8±0.8	53.1±1.2	46.5±1.6	50.4±0.6
Control	50.8±0.5ab	50.8±0.5ab	50.3±0.3abc	50.5±0.3	50.9±0.4	50.9±0.5	51.8±0.5	50.5±0.3	51.1±0.4
P		0.02			0.87			0.05	
F		2.31			0.55			1.93	
LSD value		3.12			ns			ns	
FEMALE RATIO (±SE)									
	Low dose (LC_{10})			Intermediate dose (LC_{30})			High dose (LC_{50})		
Insecticide/fungus	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03	Ma-2.3	Ma-4.1	If-03
Acetamiprid	49.0±1.2cd	47.0±0.7d	49.0±1.5cd	49.0±0.7	46.7±0.9	47.5±0.5	47.0±0.4	42.0±2.5	46.0±0.7
Bifenthrin	46.7±0.6d	51.7±2.1abc	49.2±0.5cd	51.2±1.8	49.0±1.8	51.2±1.8	46.7±1.1	47.3±0.5	47.2±1.1
Emamectin benzoate	48.0±1.7d	49.2±0.9cd	47.0±0.8d	49.0±1.5	50.2±2.3	49.2±1.7	45.2±0.8	49.5 ±2.6	46.7±0.6
Fipronil	48.1±0.44d	49.7±1.1bcd	48.3±0.5d	47.2±0.6	48.1±0.7	49.0±1.3	50.5±3.2	49.0±1.0	47.5±2.2
Imidacloprid	46.8±1.3d	49.7±1.1bcd	48.1±0.7d	50.2±1.4	48.5±0.2	48.5±1.4	49.9±2.7	49.4±1.7	46.7±1.0
Lufenuron	52.7±2.1ab	48.5±0.5d	53.0±0.8a	52.0±2.3	50.3±1.0	50.1±0.8	46.9±1.2	53.5±1.6	49.6±0.6
Control	49.2±0.5cd	49.2±0.5cd	50.7±0.3bcd	49.5±0.3	49.1±0.4	49.1±0.5	48.2±0.5	50.5±0.3	48.9±0.4
P		0.02			0.87			0.05	
F		2.31			0.55			1.93	
LSD value		3.12			ns			ns	

Means in rows and columns followed by same letters are not statistically different; LSD, $P<0.05$;ns= not significant; LC_{10}, LC_{30} and LC_{50}=fungus and insecticide dose.

In addition to lethal effects, binary mixtures of pathogenic fungi and synthetic insecticides significantly reduced the adult longevity in comparison to the normal adult life. The shortened life of females affected the number of eggs laid. A similar trend was observed by Flores et al. (2004) and Pelizza et al. (2013) who reported decreased survival and reduced fecundity in *Aedes aegypti* L. (Diptera: Culicidae) as a sublethal effect of entomopathogenic fungi. In addition, females of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), *Ceratitis fasciventris* (Bezzi) (Diptera: Tephritidae) and *Ceratitis cosyra* (Walker) (Diptera: Tephritidae) suffered 82, 73 and 37% reduction in fecundity, respectively, following infection by *M. anisopliae* (Dimbi et al., 2013). The percent hatch was reduced at higher doses of insect pathogenic fungi and synthetic insecticide mixture in comparison with control, which is consistent with an earlier study in which percent hatch of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) was reduced compared to the control when treated with LC_{25} concentration of methoxyfenozide (Enríquez et al., 2010). The insect pathogenic fungi and synthetic insecticide mixtures significantly influenced and prolonged the time to pupation in *M. domestica* at high doses. Similarly, *Muntingia calabura* L. prolonged the larval duration of diamondback moth, *Plutella xylostella* L. (Lepidoptera: Plutellidae) (Bandeira et al., 2013).

In the current study, percent pupation of *M. domestica* at the high dose was significantly different from the control. Similar results were observed when *Anopheles stephensi* L. (Diptera: Culicidae) was tested against *Beauveria bassiana* (Prasad & Veerwal, 2012). Also, pupal weight was negatively affected by application of fungi and insecticide mixtures, which is similar to findings of Ruiu et al. (2006) where pupal weight reduced after larvae of *M. domestica* were fed on diet containing sublethal doses of *Brevibacillus laterosporus* (Laubach). The current study found prolonged pupal duration which is consistent with the study of Malarvannan et al. (2010), where *Spodoptera litura* Fabricius (Lepidoptera: Noctuidae) had prolonged pupal duration when treated with fungi in comparison to the control.

No significant differences in adult emergence were found in any of the treatments. This result is consistent with the study of El-Razik et al. (2013), where adult emergence in *Callosobruchus maculatus* (F) (Coleoptera: Chrysomelidae) was reduced by a synthetic insecticide and oil mixture. Moreover, significant differences in the sex ratio was observed only in the low dose treatment, which was similar to the study of Shaalan et al. (2005), where disturbance in sex ratio was noted after the binary application of *Callitris glaucophylla* (Thompson and Johnson) (Callitris: Cupressaceae) extracts and synthetic insecticides.

The present study of *M. anisopliae* var. *anisopliae*, *I. fumosorosea* and chemical insecticide mixtures and their sublethal effects on life parameters of *M. domestica* revealed that normal developmental stages and time periods were affected. The mixture of insect pathogenic fungi and synthetic insecticides can significantly alter the average development of *M. domestica*. However, field studies are important to validate the efficacy of mixtures of entomopathogenic fungi and synthetic insecticides observed to have high mortality and reduced lethal time against *M. domestica* in laboratory studies. In conclusion, entomopathogenic fungi and synthetic insecticides mixture, if validated under field conditions, might become a key element of integrated pest management for *M. domestica*.

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Original article (Orijinal araştırma)

Efficacy of indoxacarb and chlorfenapyr against Subterranean termite *Heterotermes indicola* (Wasmann) (Isoptera: Rhinotermitidae) in the laboratory

Toprakaltı termiti *Heterotermes indicola* (Wasmann) (Isoptera: Rhinotermitidae)'ya karşı indoxacarb ve chlorfenapyr'in laboratuvar koşullarında etkisi

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Summary

Efficacy, feeding deterrence and transfer of indoxacarb and chlorfenapyr by the subterranean termite, *Heterotermes indicola* (Wasmann), were evaluated in laboratory tests at Nuclear Institute for Food and Agriculture (NIFA), Peshawar, Pakistan during 2013-2014. Chemical concentrations tested ranged from 1 to 100 ppm (wt/wt) of indoxacarb and 1 to 7 ppm (wt/wt) of chlorfenapyr. Observations revealed that indoxacarb caused rapid mortality at doses > 50 ppm. At 10 ppm, mortality was relatively slower and ELT50 and ELT90 (effective lethal exposure times for 50 and 90% mortality) were recorded as 6.7 and 25.3 d, respectively. At concentrations below 10 ppm, it took longer for indoxacarb to cause 100% mortality. In comparison, chlorfenapyr caused rapid mortality at all tested concentrations except the lowest concentration of 1 ppm, and 100% mortality occurred at 9 d, ELT50 and ELT90 calculated as 2.7 and 8.6 d, respectively. Various concentrations of both termiticides ranging from 1 to 100 ppm (wt/wt) were evaluated in feeding deterrence and transfer studies. The results showed that indoxacarb did not deter feeding of *H. indicola* at any concentration, and only consumption of filter paper treated with 100 ppm indoxacarb resulted in 100% mortality. Chlorfenapyr did not deter feeding at concentrations below 100 ppm. Mortality remained low regardless of concentration and did not exceed 60% in the feeding deterrence tests. In transfer studies, indoxacarb was successfully transferred from donors to recipients at concentrations of 70 and 100 ppm. Chlorfenapyr transfer generally caused low recipient mortality and transfer from donors to recipients was only evident at 1 ppm where recipient mortality exceeded 80%.

Keywords: Chlorfenapyr, deterrence, *Heterotermes indicola*, indoxacarb, toxicity, transfer

Özet

Indoxacarb ve chlorfenapyrin'in, Toprakaltı termiti, *Heterotermes indicola* (Wasmann)'da beslenme engelleyici ve bireyler arasında taşınma etkileri 2013-2014 yıllarında Gıda ve Tarım Nükleer Enstitüsü (NIFA) (Peshawar, Pakistan)'nde laboratuvar testleri ile değerlendirilmiştir. Test edilen kimyasal konsantrasyonları indoxacarb için 1-100 ppm (ağırlık/ağırlık) ve chlorfenapyrin 1 ile 7 ppm (ağırlık/ağırlık) arasında değişmiştir. Gözlemler indoxacarb'ın 50 ppm üzerindeki dozlarda hızlı ölüme sebep olduğunu ortaya koymuştur. 10 ppm'de, ölüm nispeten daha yavaş gerçekleşmiş olup, ELT50 ve ELT90 (%50 ve %90 öldürücü etkili maruz kalma süresi) sırasıyla 6.7 ve 25.3 gün olarak kaydedilmiştir. 10 ppm altındaki konsantrasyonlarda, indoxacarb için %100 ölüm daha uzun sürede gerçekleşmiştir. Buna karşılık chlorfenapyrin, 1 ppm'lik en düşük konsantrasyon hariç, test edilen tüm konsantrasyonlarda hızlı bir ölüm oranına sebep olarak %100 ölüm oranı 9 günde saptanmış, ELT50 ve ELT90 ise sırasıyla 2,7 ve 8,6 gün şekilde hesaplanmıştır. Her iki termit öldürücünün 1 ila 100 ppm (ağırlık/ağırlık) aralığındaki çeşitli konsantrasyonları, beslenme engelleyici ve bireyler arasında taşınma çalışmaları için denenmiştir. Sonuçlar, indoxacarb'ın hiçbir konsantrasyonun, *H. indicola*'nın beslenmesini engellemediğini göstermiş ve sadece 100 ppm indoxacarb uygulanmış filtre kağıdının tüketimi %100 ölümle sonuçlanmıştır. Chlorfenapyrin 100 ppm altındaki konsantrasyonlarda beslenme engelleyici özellik göstermemiştir. Ölüm, konsantrasyon ne olursa olsun düşük kalmış ve beslenme engelleme testlerinde %60'ı aşmamıştır. Bireyler arasında taşınma çalışmalarda, indoxacarb taşıyıcılardan alıcılara 70 ve 100 ppm konsantrasyonlarda başarıyla taşınmıştır. Chlorfenapyrin taşınması genellikle düşük alıcı ölümüne sebep olmuş ve taşıyıcılardan alıcılara aktarımı, sadece 1 ppm için %80'in üzerinde olmuştur.

Anahtar sözcükler: Chlorfenapyr, caydırıcılık, *Heterotermes indicola*, indoxacarb, toksisite, transfer

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Introduction

Heterotermes is a genus of subterranean termites which is endemic to most parts of the world and considered as one of the most important economic termite pests (Baker & Carriere, 2011). The genus includes many pest species globally, and includes desert species from the southwest of the USA. These are ranked in the top three major termite pests (Baker & Bellamy, 2006). In Brazil and other parts of South America, *Heterotermes* is reported to cause severe economic loss, damaging forest trees like eucalypts and pines in addition to cash crops like cotton, rice, coffee and cassava. This termite genus is a widely distributed pest in sugarcane crops in Brazil and has been reported to cause crop losses of more than 10 t/ha annually (Batista-Pereira et al., 2004; Jenkins, 2006). In Pakistan, *Heterotermes indicola* (Wasmann) remains active throughout the year as being one of the most persistent subterranean termite species (Manzoor & Mir, 2010). It has been reported as a major pest of crops like sugarcane, plum and apricot in northern Pakistan (Badshah et al., 2004). In addition, this termite has been a significant crop pest in the deserts of India bordering Pakistan (Gera & Kumar, 2011). It has the capacity to destroy standing trees by hollowing them out from the inside, without generating external signs of injury (Balachander et al., 2013).

Current methods of termite control include the application of repellent insecticides, which prevent the entry of termites by creating a continuous chemical barrier (Su, 2005). These repellent insecticides kill only a few termites that come in contact with the chemical and the others are repelled. Thus, there is always a chance of re-infestation since the colony remains viable and active (Su & Scheffrahn, 1988). The surviving colony has the potential to continue to grow and termite problems may increase over time (Su, 2003).

In recent times, the focus of termite research has shifted from work on repellent termiticides to non-repellent termiticides. Unlike repellent termiticides, the non-repellent insecticides do not inhibit termite invasion just by repelling, but rather they rely on termites foraging in treated areas to achieve maximum lethal contact (Su & Scheffrahn, 2000). In general, non-repellent insecticides cause delayed mortality and allow the foraging termites to disseminate the acquired toxicant within the colony through social grooming and trophallaxis. Thus the termite colony is impacted and significantly reduced or eliminated (Thorne & Breisch, 2001). In recent years, novel termiticides are being developed with non-repellent characteristics, for example, chlorfenapyr, indoxacarb, fipronil and imidacloprid (Gahlhoff & Koehler, 2001; Shelton & Grace, 2003; Hu, 2005).

In this study, we evaluated the efficacy of indoxacarb and chlorfenapyr against the subterranean termite *H. indicola*. Indoxacarb belongs to the oxadiazine chemical family, which have low ecotoxicological risks. Indoxacarb acts selectively towards insects, and higher animals quickly degrade it into inactive metabolites. This rapid metabolic degradation is a crucial factor for the safety of higher non-target animals including humans (Wing et al., 2000). In contrast, chlorfenapyr is an aryl-substituted cyanopyrrole with broad-spectrum activity against insects and mites. It is basically a pro-insecticide which becomes activated by the oxidative removal of the N-ethoxymethyl group (Treacy et al., 1994). It also has high binding capacity to soil which is a useful characteristic for a termiticide, resulting in very low leaching rates (Rust & Saran, 2006).

Both insecticides have been under evaluation for the last few years against different subterranean termites. Hu (2005) tested the efficacy and non-repellency of indoxacarb treated soil against, *Reticulitermes flavipes* (Kollar) and *Coptotermes formosanus* Shiraki, and Spomer et al. (2011) investigated the penetration of subterranean termite species *R. flavipes* in various kinds of indoxacarb treated soils at different depths. Hu et al. (2005) also investigated transfer of indoxacarb among the workers of *C. formosanus.* Rust & Saran (2006) tested chlorfenapyr for its toxicity, and transfer, against the western subterranean termite, *Reticulitermes hesperus* Banks, and Shelton et al. (2006) investigated the toxicity of chlorfenapyr and its transfer among the workers of *R. flavipes*. Therefore, the main objective of this study was to determine the efficacy of both insecticides against *H. indicola* considering the degree of impact, deterrence and horizontal transfer.

Materials and Methods

Detection and collection of termites

Termites were detected and collected using methods described by Farid et al. (2014). A total of 300 detection stakes (4 × 2.5 × 28 cm) made of poplar wood were placed 2.5 cm apart and 25 cm deep in soil at different locations of Peshawar, Pakistan. Of these, 25 stakes infested by termites were later replaced by same number of NIFA TERMAPS (termite collection traps) made of PVC pipe (17 × 25 cm), containing bundles of five poplar wood slices (15 × 8 × 1 cm) wrapped in strips of filter paper (Whatman® No. 42), held together using a rubber band (Salihah et al., 1993). Traps were examined fortnightly and infested bundles, with significant numbers of termites, were brought to the laboratory. The termites were separated from the trap materials and kept at 27 ± 2°C, 80% RH in glass Petri dishes (14 × 3 cm) containing two pieces of round filter paper, moistened with distilled water. After acclimatization, the termites were used in experiments within 14 d.

Termiticides

Formulated indoxacarb (50 g a.i. per L, Steward®) provided by DuPont® and chlorfenapyr (360 g a.i. per L, Pirate®) provided by BASF® corporation were used to make stock solutions. The tested concentrations were prepared by serial dilutions. Formulated termiticides were used instead of technical grade insecticides because they are easy to mix and apply in soil and they are the actual products used for the control of termites.

Toxicity tests

Concentrations (w/w) ranging from 1 to 100 ppm of indoxacarb and 1 to 7 ppm of chlorfenapyr were prepared in 500 ml glass jars. A suitable range of concentrations was determined by performing preliminary experiments. Round filter paper sheets weighing 0.21 g and measuring 9 cm in diameter were dipped for 5 s in the insecticide solutions, to achieve the required concentration (weight of active ingredient/weight of filter paper). The amount of insecticide solution that 0.21 g filter paper can absorb was determined by Farid et al. (2014). Treated filter papers were dried at room temperature for at least 8 h and two pieces were placed in each glass Petri dish (9.0 × 1.5 cm). In total 100 termite workers and 3 soldiers were transferred to each dish for 24 h, then removed and placed in the same sized dishes containing untreated filter papers. Daily mortality was recorded. Each insecticide concentration was considered a treatment and dishes were replicated four times. Control termites were exposed for 24 h to filter paper dipped in distilled water. Mortality was recorded daily and all experimental units were kept at 27 ± 2°C and 80% RH. Probit analysis was done to calculate the ELT50 and ELT90 (effective lethal exposure times for kill 50 and 90% mortality) of the termites (Su et al., 1987).

Feeding deterrence test

Feeding deterrence caused by indoxacarb and chlorfenapyr was tested using two rectangular pieces (3 × 2 cm) of filter paper held horizontally, 3 cm apart from each other in plastic Petri dishes (9 × 1.5 cm), with bottom papers were roughened with sand paper to facilitate movement of the termites. The dishes were then filled with 25 g of 60 - mesh size sterilized sand, moistened with 20% (w/v) distilled water. One of the two pieces of filter paper was treated by dipping in a prepared termiticide solution. Seven different indoxacarb and eight chlorfenapyr concentrations (weight of active ingredient/weight of filter paper) ranging from 1 to 100 ppm were used, while the second piece of filter paper was left untreated and dipped in distilled water to serve as a control. Dry weights of both the pieces were determined before the experiment by drying them in an oven at 120°C for 6 h. Two hundred workers and 10 soldiers of *H. indicola* were released in each dish, and maintained as described above. Eight dishes were prepared for each concentration (indoxacarb 1, 5, 10, 20, 50, 70 and 100 ppm; chlorfenapyr 1, 3, 5, 7, 10, 25, 50 and 100 ppm) and four dishes disassembled during destructive sampling after 1 and 2 weeks. Used filter papers were cleaned and oven dried as described above. Consumption of both the treated and untreated filter paper for each concentration were determined by subtracting the final weight from initial weight, and compared using paired sample t-test and means were separated by using Tukey's HSD test. Termite mortality was also recorded after 1 and 2 weeks. Termites were considered dead if they showed no movement when probed with a mounted needle (Su & Scheffrahn, 1993).

Transfer test

Toxicant transfer studies used termite workers divided into donors and recipients. The donor termites were exposed to filter paper treated with seven concentrations ranging from 1 to 100 ppm of indoxacarb and eight concentrations ranging from 1 to 100 ppm of chlorfenapyr for 24 h. Recipients were not exposed to treated filter paper, but were fed on filter paper moistened with 0.2 % Nile Blue A (The BDH Ltd, Poole, UK) for 3 d to color them blue to distinguish them from the creamy white donor termites. Donors and recipients were then released together in an equal ratio (1:1) in Petri dishes containing untreated filter paper (9 cm diameter) moistened with 5 ml distilled water. In total, 50 workers were introduced, 25 donors and 25 recipients. Each concentration was analyzed as a treatment and plates replicated four times. The dishes were kept at 27 ± 2°C and 80% RH in desiccators. Live donors and recipients were counted to estimate the respective mortalities after 10 d. Dead termites including those moribund or partially consumed were not removed from the Petri dishes during the experimental period. Percent donor mortality, recipient mortality and dead donors missing (assumed to be consumed by fellow termites) were recorded and subjected to one way ANOVA and means separated using SNK (Student-Newman-Keuls) posterior test. Statistical analysis was performed using SPSS version 16.0 (SPSS, 2007).

Results

Toxicity test

Toxicity tests showed an increase in mortality with increasing insecticide concentrations. At higher concentrations of 50 to 100 ppm of indoxacarb, 100% mortality was achieved within 2 - 3 d, but at lower concentrations of 1 to 20 ppm significant mortality did not occur even after 19 d. However, cumulative percent mortality was higher than control mortality for all concentrations of indoxacarb (Figure 1).

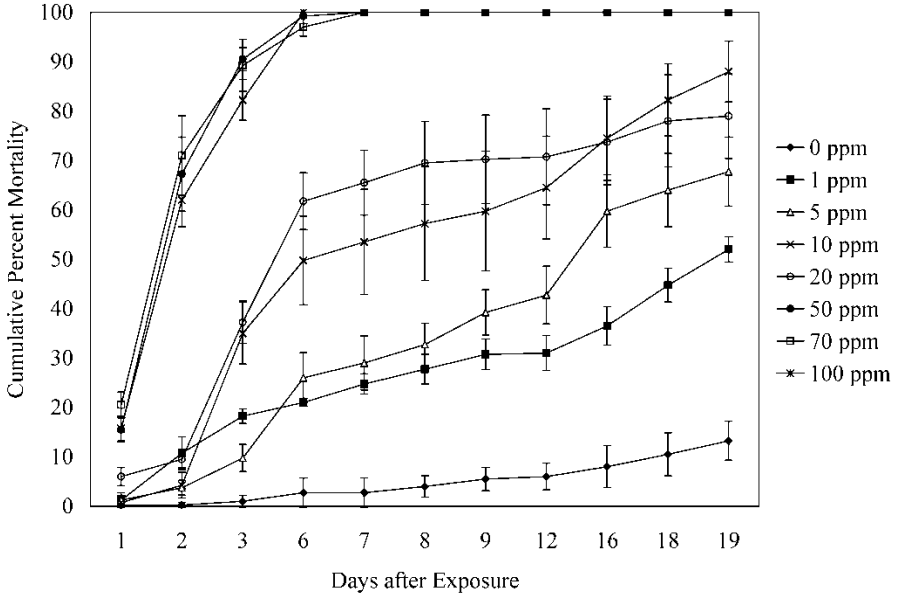

Figure 1. Cumulative percent mortality of *H. indicola* at various intervals after exposure to various concentrations of indoxacarb.

The ELT50 was 23.3 d for 1 ppm and 12.5 d for 5 ppm of indoxacarb. At 10 and 20 ppm of indoxacarb, the same mortality level was achieved in less than 7 and 6 d, respectively. Concentrations greater than 50 ppm was killed 50% of termites in less than 2 d. The ELT90 was projected to be between a few months to over a year (168 to 373 d) for 1 ppm, whereas for 5 and 10 ppm ELT90 was determined as 49.3 and 25.3 d respectively. The higher concentrations of 50, 70 and 100 ppm caused 90% mortality in a much shorter time i.e. 2.8 to 3.5 d (Table 1).

Table 1. Estimated lethal time (d) required for 50 and 90% mortality (ELT50, ELT90), with 95% confidence limits (CI) of *Heterotermes indicola* after exposure to various concentrations of indoxacarb

Dose (ppm)	ELT50 (d)	95% CI	EL 90 (d)	95% CI	Probit Model
1	23.3	23.3 - 27.4	239.0	167.8 - 373.2	ELT = -1.73 + 1.26 × dose
5	12.5	11.8 - 13.2	49.3	43.0 - 57.9	ELT = -2.35 + 2.15 × dose
10	6.7	6.1 - 7.4	25.3	21.3 - 31.6	ELT = -1.85 + 2.23 × dose
20	5.5	4.8 - 6.1	27.4	24.0 - 38.6	ELT = -1.36 + 1.83 × dose
50	1.6	1.5 - 1.7	2.9	2.8 - 3.2	ELT = -1.00 + 4.80 × dose
70	1.5	1.4 - 1.6	3.2	2.9 - 3.4	ELT = -0.74 + 4.03 × dose
100	1.7	1.6 - 1.8	3.3	3.1 - 3.5	ELT = -1.00 + 4.40 × dose

Concentrations of chlorfenapyr (1 to 7 ppm) were assessed in the same manner. At 7 ppm, 100% mortality of exposed workers occurred within 2 to 3 d. At 3 ppm, 100% mortality occurred at 6 d, and the concentration of 1 ppm was the only dose of chlorfenapyr, which took 9 d for 100% mortality to occur (Figure 2).

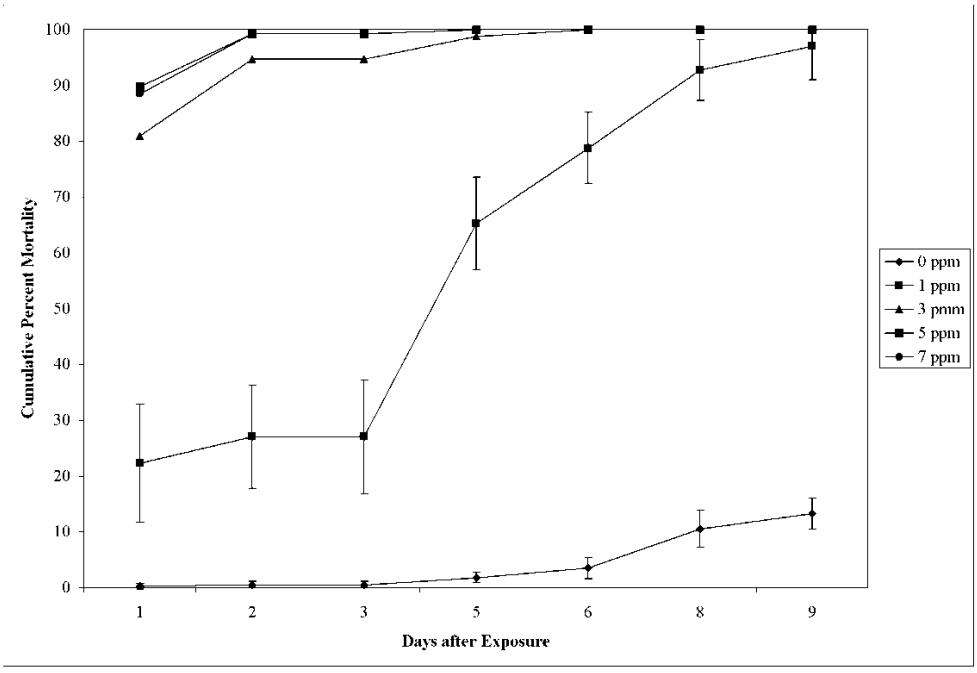

Figure 2. Cumulative percent mortality of *H. indicola* at various intervals after exposure to various concentrations of chlorfenapyr.

The ELT50 and ELT90 recorded was 2.7 and 8.6 d respectively, for 1 pm of chlorfenapyr, whereas for concentrations 3 to 7 ppm, the ELT50 was less than 1 d and ELT90 ranged between 1 to 1.4 d (Table 2).

Table 2. Estimated lethal time (d) required for 50% and 90% mortality (ELT50, ELT90, along with 95% CI) of *Heterotermes indicola* after exposure to various concentrations of chlorfenapyr

Dose (ppm)	ELT50 (d)	95% CI	ELT90 (d)	95% CI	Probit Model
1	2.7	1.9 - 3.4	8.6	6.3 - 14.5	ELT = -1.09 + 2.53 × dose
3	0.4	0.3 - 0.6	1.4	1.2 - 1.7	ELT = 0.87 + 2.40 × dose
5	0.4	0.2 - 05	1.0	0.8 - 1.1	ELT = 1.26 + 3.89 × dose
7	0.5	0.3 - 0.6	1.0	0.9 - 1.1	ELT = 1.20 + 4.11 × dose

Feeding deterrence test

In feeding deterrence tests with indoxacarb, the termites did not distinguish between treated and untreated filter paper at any of the concentrations tested. The consumption of treated and untreated filter paper was not significantly different after one week (p = 0.06 to 0.44) or two weeks (p = 0.11 to 0.62) for concentrations ranging from 1 to 100 ppm. The consumption of both treated and untreated filter paper was comparatively more at lower concentrations after one or two weeks (Table 3 & 4).

Table 3. Difference in filter paper consumption by *Heterotermes indicola* between untreated paper and those treated with different concentrations of indoxacarb after one week

Dose (ppm)	Consumption (mg) Mean ± SE		t statistics (p value)
	Untreated	Treated	
1	17.6 ± 0.46a	16.1 ± 0.97a	1.09 (0.38)
5	16.7 ± 0.78ab	14.9 ± 0.43ab	2.93 (0.10)
10	16.2 ± 0.67ab	13.9 ± 0.76abc	3.17 (0.09)
20	14.7 ± 0.29bc	11.1 ± 1.0bcd	3.47 (0.07)
50	12.4 ± 0.61c	10.6 ± 1.3cd	1.40 (0.30)
70	12.5 ± 0.63c	08.4 ± 0.51de	3.83 (0.06)
100	07.2 ± 0.44d	06.2 ± 0.5e	0.94 (0.44)

*Means followed by same letters in a column are not significantly different at p = 0.05 using Tukey's HSD test.

Table 4. Difference in filter paper consumption by *Heterotermes indicola* between untreated paper and those treated with various concentrations of indoxacarb after 2 weeks

Dose (ppm)	Consumption (mg) Mean ± SE		t statistics (p value)
	Untreated	Treated	
1	32.9 ± 0.80a	32.7 ± 0.95a	0.57 (0.62)
5	32.3 ± 0.76a	30.8 ± 0.12a	1.86 (0.20)
10	24.2 ± 0.89b	21.8 ± 0.99b	3.94 (0.59)
20	22.6 ± 0.81bc	18.4 ± 0.80bc	2.74 (0.11)
50	19.3 ± 0.52c	16.5 ± 0.78cd	2.13 (0.16)
70	14.4 ± 0.62d	11.8 ± 0.77de	2.06 (0.17)
100	09.8 ± 0.49e	9.2 ± 0.55e	0.57 (0.62)

*Means followed by same letters in a column are not significant different at p = 0.05 using Tukey's HSD test.

When total consumption (treated plus untreated filter paper) and termite mortality were compared for indoxacarb concentrations, it was observed that consumption decreased as the insecticide concentration increased while morality increased. Effective concentrations that resulted in close to 100% *H. indicola* mortality after two weeks in deterrence tests were 70 and 100 ppm. Although total consumption was higher at the lower concentrations of 1 to 50 ppm, mortality did not exceed 40% (even after two weeks). An increase in mortality was observed at both 70 and 100 ppm, over 1 and 2 weeks. After one week, mortality at both concentrations was less than 50% which increased significantly, and reached near 100% by the end of the second week (Figure 3).

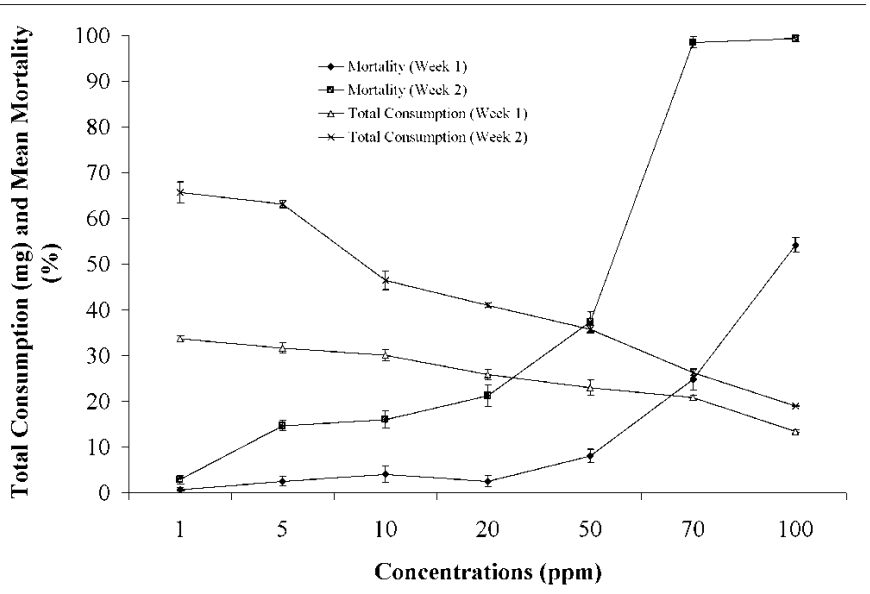

Figure 3. Total consumption of blotting paper (treated + untreated) and percent mortality caused at different concentrations of indoxacarb after week 1 and 2.

Chlorfenapyr proved to be a non-deterrent termiticide at all concentrations for up to one week. At 1 ppm, consumption of untreated filter paper was 22.1 ± 0.83 mg, whereas the consumption of treated filter paper was 20.0 ± 0.94 mg and the difference in consumption was non-significant ($p = 0.28$). The p values calculated were 0.10, 0.40, 0.60, 0.30, 0.46, 0.86 and 0.34 for 3, 5, 7, 10, 25, 50 and 100 ppm of chlorfenapyr, respectively. It is obvious from non-significant p values ($p > 0.05$) that there was no significant difference between the consumption of treated and untreated filter paper at these concentrations. As far as consumption of only treated paper was concerned, it was almost the same at concentrations 3 to 25 ppm after one week. The highest consumption of treated filter paper was recorded at 1 ppm and lowest consumption at 100 ppm (Table 5).

Similarly all the tested concentrations of chlorfenapyr remained non-deterrent ($p = 0.21$ to 0.98) after two weeks, except at 100 ppm where there was significantly ($p < 0.02$) less consumption of treated paper, compared with untreated paper. Highest consumption was recorded at 1 ppm followed by 3 ppm. At concentrations ranging from 5 to 50 ppm consumption was non-significantly different, but higher than 100 ppm and lesser than consumption at 1 and 3 ppm (Table 6).

Table 5. Difference in filter paper consumption by *Heterotermes indicola* between untreated paper and those treated with various concentrations of chlorfenapyr after one week

Dose (ppm)	Consumption (mg) Mean ± SE		t statistics (p value)
	Untreated	Treated	
1	22.1 ± 0.83a	20.0 ± 0.94a	1.45 (0.28)
3	17.0 ± 0.44bc	18.9 ± 0.72ab	-2.88 (0.10)
5	20.6 ± 0.71ab	18.9 ± 0.86ab	1.04 (0.40)
7	16.9 ± 0.98bc	16.2 ± 0.55bc	0.60 (0.60)
10	14.3 ± 0.96cd	16.5 ± 0.66bc	-1.38 (0.30)
25	14.4 ± 063cd	15.5 ± 0.66bc	-0.9 (0.46)
50	13.4 ± 0.78cd	13.6 ± 0.64cd	-0.18 (0.86)
100	12.9 ± 0.80d	11.4 ± 0.48d	1.22 (0.34)

*Means followed by same letters in column are not significantly different at p = 0.05 using Tukey's HSD test.

Table 6. Difference in filter paper consumption by *Heterotermes indicola* between untreated paper and those treated with various concentrations of chlorfenapyr after 2 weeks

Dose (ppm)	Consumption (mg) Mean ± SE		t statistics (p value)
	Untreated	Treated	
1	50.9 ± 1.02a	48.2 ± 0.70a	1.79 (0.21)
3	32.0 ± 1.17b	31.3 ± 1.17b	0.33 (0.76)
5	26.3 ± 0.72c	24.1 ± 1.2c	1.32 (0.31)
7	26.3 ± 1.3c	25.0 ± 0.78c	0.70 (0.55)
10	26.2 ± 0.8c	25.5 ±0.69c	0.67 (0.56)
25	20.7 ± 1.29d	21.9 ± 0.78c	-0.58 (0.61)
50	22.0 ± 1.03cd	21.9 ± 0.93c	0.01 (0.98)
100	21.3 ± 1.23cd	13.6 ± 0.29d	5.78 (0.02)

*Means followed by same letters in column are not significantly different at p = 0.05 using Tukey's HSD test.

Mortality recorded in the deterrence tests did not exceed 25 and 60% after one and two weeks respectively, even at the highest tested concentration of 100 ppm chlorfenapyr (Figure 4). Total consumption of untreated and treated filter paper decreased with increasing concentrations. Maximum total consumption (101 mg) occurred at 1 ppm, but mortality was almost negligible even after two weeks. Regardless of consumption, mortality did not exceeded more than 40% for all concentrations up to 50 ppm, even after two weeks.

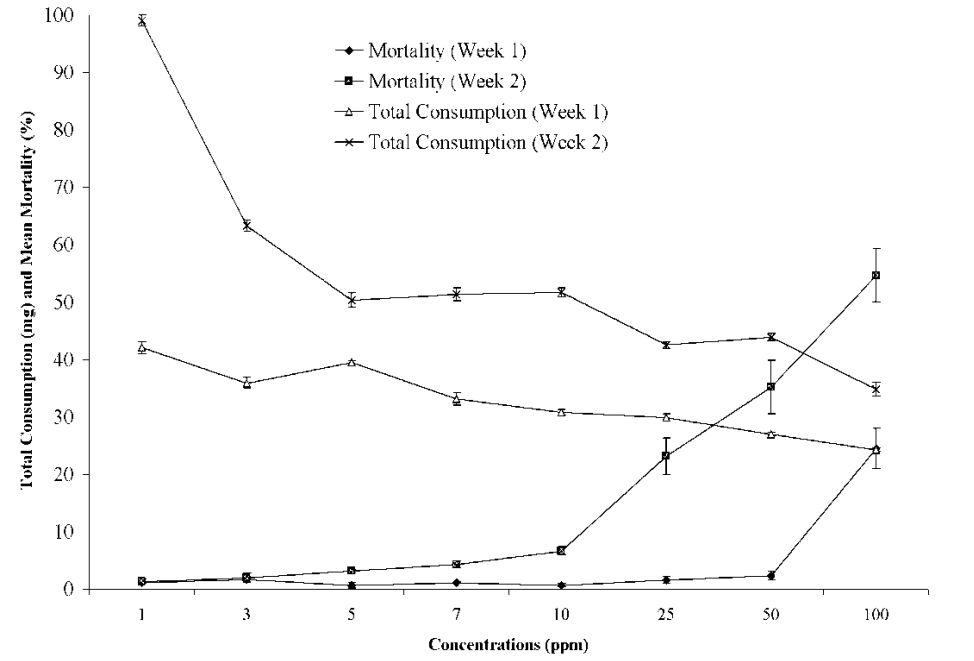

Figure 4. Total consumption of blotting paper (treated + untreated) and percent mortality caused at different concentrations of chlorfenapyr after 1 and 2 weeks.

Transfer test

Transfer tests involving indoxacarb revealed that donor mortality was more than 50% when termites were exposed to concentrations equal to or higher than 5 ppm. At 50 ppm, donor mortality was 97%, whereas 100% mortality was recorded in donor termites exposed to 70 and 100 ppm of indoxacarb. This was significantly higher than the 5% control mortality (0 ppm; $p < 0.0001$). Although high donor mortalities were recorded at most concentrations of indoxacarb, recipient mortality remained less than 50% for all the concentrations equal to or less than 50 ppm. At 70 and 100 ppm recipient mortalities were 93 and 99%, respectively, indicating a significant transfer of indoxacarb from donors to recipients. Whereas, 97% donor mortality at 50 ppm did not result in significant recipient mortality, with only 48% of recipients found dead. At 0 to 20 ppm, the recipient mortality ranged from 5 to 33%, which was significantly lower than mortality at effective concentrations of 70 and 100 ppm of indoxacarb ($p< 0.0001$).

Variability occurred in missing (presumed dead) termite donors, assumed to be consumed by recipient workers. The percentage of missing termite donors significantly decreased with the increase of concentrations ($p < 0.0001$). The lowest number of missing dead donors (14%) was recorded at 100 ppm indoxacarb compared to the highest (75%) within the controls (Table 7).

Results of transfer studies of chlorfenapyr revealed that 100% of donors were killed within 10 d of exposure to concentrations ranging from 3 to 10 ppm. Similarly, at 1 ppm the mortality recorded was 98%, whereas at 0 ppm (control), donor mortality was significantly lower (6%; $p < 0.0001$). In comparison, recipient mortality was low at all but the lowest concentration, indicating a low transfer of chlorfenapyr. At 1 ppm, mortality reached 82% after 10 d, indicating significant transfer of the toxicant. Recipient mortality ranged from 5 to 11% when they were released with the donors exposed to concentrations ranging from 3 to 10 ppm ($p < 0.0001$). Only 1 ppm seemed to be an effective concentration with significant transfer results from donors to recipients.

Table 7. Mean cumulative percent mortality of donors and recipients of *Heterotermes indicola* in 10 d after mixing the donors treated with various concentrations of indoxacarb

Dose (ppm)	Donor mortality ± SE (%)	Recipient mortality ± SE (%)	Dead donor missing (%)
0	6.0 ± 1.1a	5.0 ± 1.0a	75.0 ± 14.4a
1	30.0 ± 4.1b	5.0 ± 2.5a	47.5 ± 4.8b
5	55.0 ± 5.0c	6.0 ± 1.1a	53.2 ± 3.1bc
10	64.0 ± 2.8d	24.0 ± 3.6b	43.3 ±5.1bc
20	76.0 ± 3.6e	33.0 ± 4.4c	45.7± 4.1bc
50	97.0 ± 1.9f	48.0 ± 4.3d	25.7± 1.6cd
70	100.0 ± 0.0f	93.0 ± 3.4e	20.0 ± 1.6d
100	100.0 ± 0.0f	99.0 ± 1.0e	14.0 ± 2.5d

*Means followed by same letters in column are not significantly different at p = 0.05 using SNK test.

The number of missing termite donors, assumed to be consumed by other fellow recipients, was not significantly different (30 to 36%) in all but the lowest concentration of chlorfenapyr. Only at 0 ppm (control) were the number of missing dead donors recorded significantly higher (75 ± 14), than all the other concentrations ($p < 0.001$) (Table 8).

Table 8. Mean cumulative percent donors and recipient *Heterotermes indicola* mortality after 10 d of releasing recipients with donors treated with various concentrations of chlorfenapyr

Dose (ppm)	Donor mortality ± SE (%)	Recipient mortality ± SE (%)	Dead donor missing (%)
0	06.0 ± 1.1a	05.0 ± 1.0a	75.0 ± 14.4a
1	98.0 ± 1.1b	82.0 ± 2.5b	31.6 ± 3.1b
3	100.0 ± 0.0b	11.0 ± 1.9a	30.0 ± 2.5b
5	100.0 ± 0.0b	06.0 ± 2.0a	32.0 ± 3.6b
7	100.0 ± 0.0b	06.0 ± 1.1a	32.0 ± 4.3b
10	100.0 ± 0.0b	7.0 ± 1.9a	36.0 ± 3.6b

*Means followed by same letters in column are not significantly different at p = 0.05 using SNK test.

Discussion

Toxicity test results of indoxacarb showed that concentrations greater than 20 ppm killed 90% of exposed termites rapidly within the span of 2 to 3 d, while lower concentrations of 1 and 5 ppm did not kill the same number of termites even after several weeks. However, at 10 ppm indoxacarb the projected mortality was greater than 90% at 3 weeks. This showed that the contact toxicity of indoxacarb was dose dependent. Exposed termite workers, if not killed instantly, have the potential to disseminate a toxicant to the whole colony through the process of trophallaxis and social grooming (Shelton et al., 2006). In our study, 10 ppm seems to be an appropriate dose at which indoxacarb showed the characteristics of a slow acting toxicant. Whereas concentrations greater than 10 ppm did not provide the opportunity for termites to socialize with other nest mates as they died too rapidly. At the lowest concentrations, toxicant transfer to other unexposed termites was not enough to cause secondary mortality. Hu et al. (2005), also confirmed that indoxacarb was slow acting, and reported that at some concentrations, the required mortality was achieved after about 3 weeks. This would support the concept of colony decline caused by a slower acting termiticide, which is a very important aspect of control. Iqbal & Saeed (2013) evaluated

indoxacarb against *Microtermes mycophagus* (Desneux) and Mao et al. (2011) tested it on *R. flavipes* and *C. formosanus* along with other insecticides. Both studies confirmed a low toxicity and delayed time of action for indoxacarb. Relatively new technologies and most of the non-repellent termiticides are slow acting in nature, and need greater time to cause lethal effects (Su et al., 1987). Based on our findings and supported by previous studies, indoxacarb could be used as a slow acting toxicant, with the potential of transference among conspecific individuals of *H. indicola* through social grooming or physical contact with exposed coworkers, or via a treated medium.

Results of toxicity tests comparing chlorfenapyr and indoxacarb revealed that chlorfenapyr was comparatively more toxic to the termites. All concentrations of chlorfenapyr caused rapid mortality, except 1 ppm, which took more than one week to kill 100% of the workers of *H. indicola*. Mortality of 100% at the low concentration of 1 ppm showed that chlorfenapyr has high contact toxicity against *H. indicola*, and killed termite workers relatively rapidly. Manzoor et al. (2012), similarly reported that chlorfenapyr is highly toxic to *H. indicola* in laboratory bioassays, where 97% mortality was achieved in about 8 h of exposure. Yeoh & Lee (2007) also found that chlorfenapyr and fipronil are highly toxic against the Asian subterranean termite Coptotermes gestroi (Wasmann) even at very low doses. The fast kill rate of chlorfenapyr, along with its non-repellency to *H. indicola* makes it a promising candidate for soil barrier treatments around structures, where quick knockdown of termites is required. Rust & Saran (2006) similarly suggested that chlorfenapyr can be used in soil as an effective chemical barrier because of its non-repellency and high mortality rate. They confirmed that 1 h exposure to 75 ppm chlorfenapyr resulted in 88% mortality of the western subterranean termite, *R. hesperus*. However, in our experiment chlorfenapyr at 1 ppm or less showed relatively slow mortality compared to higher concentrations, and if termites are exposed to concentrations lower than 1 ppm, they die slower and therefore have more time to transfer the toxicant to other nest mates through trophallaxis, social grooming and cannibalism. But ideally, if quick mortality of *H. indicola* is required around a structure, chlorfenapyr could be effective at concentrations more than 1 ppm, applied to media surrounding buildings. Field based research is required to confirm results in the built environment.

Feeding deterrence test results showed that indoxacarb did not deter feeding by *H. indicola* at any of the tested concentrations. This showed that indoxacarb is not only non-repellent but also non-deterrent to termite workers of *H. indicola*. Higher mortalities at 70 and 100 ppm might be due to the combined effect of contact and oral toxicity of indoxacarb. There was an overall decrease in consumption of filter paper at these concentrations, and this could be due to intoxication of termite workers after contact with treated filter paper. It was observed that termites did not avoid contact with treated filter paper and also consumed it regardless of concentration, which indicated the non-repellency and non-deterrence of indoxacarb for *H. indicola*. Yeoh & Lee (2007) confirmed that indoxacarb was a non-deterrent termiticide when tested against *C. gestroi* at the concentrations of 1, 10, 50 and 100 ppm. Spomer et al. (2011) studied the efficacy of indoxacarb and chlorantraniliprole, and also confirmed that subterranean termites showed no deterrence towards indoxacarb. Termites maintained contact and feeding on filter paper treated with 70 to 100 ppm of indoxacarb, which caused high mortalities after 2 weeks, making it a promising candidate to be used as a slow acting toxicant bait against *H. indicola*.

In feeding deterrence tests involving chlorfenapyr, treated filter paper was offered as a food substrate along with untreated filter paper. Termites fed on it during the first week irrespective of the concentration used, showing that chlorfenapyr was a non-deterrent during the first week, but during the second week at 100 ppm a deterrent effect was observed. The consumption of filter paper treated with 100 ppm was 13.6 ± 0.29 mg, which was significantly less than that of untreated filter paper at 21.3 ± 1.23 mg. Termite workers avoiding feeding on 100 ppm treated filter paper, may be due to a learnt behavior after a sublethal exposure according to Su et al. (1995). Termites consuming a sublethal dose during the first week, may have started avoiding the 100-ppm treated filter paper in the second week. Yeoh & Lee (2007) reported that the deterrence properties of chlorfenapyr at concentrations of 1, 10, 50 and 100 ppm (w/w) were mainly concentration dependent, i.e. no deterrence at low concentrations, but at higher concentrations chlorfenapyr became deterrent to termites and consumption was significantly reduced.

Termite mortality due to chlorfenapyr at concentrations of 1 to 50 ppm remained less than 25%, even after 2 weeks. Low mortality at these concentrations suggested that the amount of toxicant acquired by termite workers was not enough to cause high mortality. Even at the highest concentration of 100 ppm, mortality recorded was only 60% after 2 weeks, which was low compared to the mortality caused by indoxacarb at the same dose. This difference in mortality indicated that chlorfenapyr was less effective when fed orally compared to its contact toxicity to *H. indicola*. Low mortality at 100 ppm could be due to lower consumption resulting from avoidance of treated filter paper. Additionally, a high concentration of a non-repellent termiticide acts like a fast-acting termiticide and it has been reported that corpses near treatment areas could repel healthy termites (Su et al., 1995). It is likely that the 60% mortality was due to the combination of contact and feeding toxicity. Shelton et al. (2006) reported rapid contact mortality of *R. flavipes* when exposed to 50, 100, 250 and 500 ppm of chlorfenapyr, all the exposed workers dying within 5 d of treatment, supporting our findings that chlorfenapyr has more contact toxicity than oral toxicity against subterranean termites. However, our results showed that chlorfenapyr became a feeding deterrent at 100 ppm. Rust & Saran (2006) reported that chlorfenapyr did not deter *R. hesperus* even at the higher concentration of 300 ppm. This is likely due to differences in the subterranean termite species tested. Overall our results from feeding deterrence tests suggest that chlorfenapyr was not effective as a feeding toxicant against termite workers of *H. indicola*. At 100 ppm or higher it became a feeding deterrent, while at lower concentrations it did not cause the desired mortality. However, it could be used as a chemical barrier when applied in soil due to its non-repellency and high toxicity to *H. indicola*.

The horizontal transfer of non-repellent insecticides from exposed individuals to unexposed nest mates is a very important process and often considered essential for the successful management of subterranean termites. Our transfer studies on indoxacarb showed more than 50% mortality of donors that were exposed to concentrations equal or greater than 5 ppm, but recipient mortality remained below 50% at most of the concentrations tested except 70 and 100 ppm. The low mortality of recipients at concentrations ranging from 1 to 50 ppm might be because termites failed to acquire sufficient toxicant from donors to cause death. Higher recipient mortalities of 93 and 99% occurred when donors were exposed to 70 and 100 ppm of indoxacarb, respectively. Increased doses and exposure usually causes greater effects on subterranean termites (Hoi, 2007). Hu et al. (2005) investigated horizontal transfer of indoxacarb in subterranean termites *C. formosanus*, and also reported that higher doses caused greater recipient mortality compared to lower doses, and the highest dose of 200 ng/donor resulted in 100% donor and recipient death, in less than 3 weeks.

It was observed that mortality in donors was not instant. Delayed mortality showed the slow acting characteristic of indoxacarb, which ultimately helped in transfer of toxicant from donors to recipients. Neoh et al. (2012) investigated the effectiveness of various non-repellent insecticides including indoxacarb against *C. gestroi* and stated that the amount of toxicant taken up by donor termites and transferred to recipients termites was concentration based. Buczkowski et al. (2012) also studied the horizontal transfer mechanism in subterranean termites, *R. flavipes*. Termite workers were exposed to 5 to 100 ppm of chlorantraniliprole, they found it highly efficient in transferring at concentrations of 25 and 50 ppm. Both concentrations caused 100% mortality in donors and recipients after 3 weeks of releasing them together. High donor mortalities at higher concentrations and subsequent high recipient mortality might be due to their social behavior i.e. grooming, trophallaxis and care of intoxicated (donor) termite workers by toxin-free (recipient) termite workers. The intoxicated or dying termites usually receive extra care and grooming from other active nest mates and they have never been removed or isolated. Healthy termite workers have been observed trying to remove toxicants attached to intoxicated termites (Hu et al., 2006). Another additional effect which could result in higher mortalities, is the missing numbers of dead donors which are assumed to have been eaten by recipients (cannibalism). Kubota et al. (2008) also explained the transfer of toxicant from donors to recipients through trophallaxis, cannibalism and social grooming when they investigated horizontal transmission, and lethal dose of bistrifluron in *C. formosanus*. They confirmed that certain toxicants taken up by donors remained in their body for several weeks, and continued transferring mates through trophallaxis, while some toxicants stuck to the donor bodies and transferred through social grooming.

Results of horizontal transfer of chlorfenapyr among workers of *H. indicola* showed that 98 to 100% of donor termites were killed when exposed to filter paper treated with concentrations ranging from 1 to 10 ppm, but recipient mortality remained at 5 to 11%, except at 1 ppm, when 86% recipient mortality was recorded. The high recipient mortality at 1 ppm was evidence of successful transfer of chlorfenapyr from donors to recipients. The higher recipient mortality at 1 ppm might be due to delayed mortality of donors whereas at other higher concentrations (3 to 10 ppm) donors were killed rapidly before transfer of the toxicant could occur. At 1 ppm the donors were intoxicated and they had sufficient time to acquire an effective amount of toxicant to cause high mortality in untreated workers. Rust & Saran (2006) also reported 100% donor mortality and 96% recipient mortality during their study of horizontal transfer of chlorfenapyr by *R. hesperus*. Hoi (2007) also investigated six insecticides, including chlorfenapyr, for horizontal transfer in *C. gestroi* and reinforced the fact that recipient mortality varied significantly when they were released together with donors exposed to different doses of different insecticides. Recipient mortality mostly depends upon the dose to which donors were exposed. The number of missing dead donors ranged non-significantly between 30 and 36% at concentration ranges of 1 to 10 ppm of chlorfenapyr, indicating that cannibalism was not a factor involved in the transfer of chlorfenapyr. Instead, most of the transfer occurred through social grooming. Shelton et al. (2006), reported only 1 ppm of chlorfenapyr is required per donor to cause high recipient mortality (80%).

Conclusion

Toxicity of both indoxacarb and chlorfenapyr was dose dependent but chlorfenapyr was found comparatively more toxic than indoxacarb. Indoxacarb proved to be non-deterrent to *H. indicola* workers feeding at all concentrations tested, whereas, chlorfenapyr became a feeding deterrent at higher concentrations. In addition, indoxacarb showed potential use as slow acting toxicant bait because at certain doses it caused delayed mortality, while chlorfenapyr caused rapid contact mortality at all concentrations tested. Therefore, chlorfenapyr has potential as a soil barrier application. Indoxacarb and chlorfenapyr were both transferred from donors to recipients, and transfer was dose dependent.

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Original article (Orijinal araştırma)

Flea beetles collected from olive trees of Antalya Province, including the first record of the monotypic genus *Lythraria* Bedel, 1897 (Coleoptera: Chrysomelidae) for Turkey[1]

Monotipik cins *Lythraria* Bedel, 1897'nın Türkiye için ilk kaydı ile birlikte Antalya ilindeki zeytin ağaçlarından toplanan yaprak pire böcekleri

Ebru Gül ASLAN[2*] **Medine BAŞAR[3]**

Summary

Lythraria Bedel is a monotypic genus of leaf beetles in the tribe Alticini (Chrysomelidae: Galerucinae), with its unique species *Lythraria salicariae* (Paykull, 1800) distributed across the Palearctic ecozone. *Lythraria salicariae* was recorded for the first time from Turkey during field sampling conducted in olive grove areas of various regions in the Antalya Province. A total of 26 flea beetle species classified in 10 genera were collected by beating from olive trees, including *L. salicariae*. This contribution adds taxonomic and zoogeographic knowledge about *L. salicariae*, and brings the actual number of flea beetle species reported in Turkey to 345 across 23 genera.

Keywords: Alticini, Antalya, *Lythraria*, new record, olive trees, Turkey

Özet

Yaprak böceklerinin Alticini (Chrysomelidae: Galerucinae) tribusuna ait monotipik bir cins olan *Lythraria* Bedel, Palearktik bölgede yayılış gösteren tek bir türe, *L. salicariae* (Paykull, 1800), sahiptir. Antalya ilinin farklı bölgelerindeki zeytin bahçelerinde gerçekleştirilen örneklemeler sırasında, *Lythraria salicariae* Türkiye için ilk kez kaydedilmiştir. *Lythraria* ile birlikte toplam 10 cinse ait 26 yaprak pire böceği türü zeytin ağaçlarından darbe yöntemiyle toplanmıştır. Bu çalışmayla *Lythraria salicariae*'nın taksonomik ve zoocoğrafik verilerine yeni katılımlar sağlanmış, ayrıca Türkiye'den rapor edilen toplam yaprak pire böceği tür sayısı 23 cinse ait 345 tür olarak güncellenmiştir.

Anahtar sözcükler: Alticini, Antalya, *Lythraria*, yeni kayıt, zeytin ağaçları, Türkiye

[1] This study includes some data from the second author's doctorate thesis

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Introduction

Alticini, known as flea beetles, is a very large and diverse tribe of leaf beetles within the subfamily Galerucinae according to the current classification of Chrysomelidae (Bouchard et al., 2011), with about 8,000 recognized species placed in more than 500 genera (Biondi & D'Alessandro, 2012; Nadein, 2015).This tribe of beetles (closely related to Galerucini) is distributed worldwide, mainly occurring in the tropical regions of Africa, Asia and South America (Konstantinov & Vandenberg, 1996; Santiago-Blay, 2004). They are mostly small, well known as phytophagous, whose thickened hind femora are generally used by taxonomists to distinguish this group from others. Most of the species are mono- or oligophagous, and the entire genera are more or less specialized as feeders on stems, leaves and roots of most higher plant families. Many members are harmful agricultural pests while several species are beneficial as biological control agents of weeds (Jolivet & Hawkeswood, 1995; Jolivet & Verma, 2002). Consequently, this group is commonly studied in different regions of the world using different collecting methods. Currently, 344 species of Alticini from 22 genera are known to occur in Turkey (Aslan & Alkan, 2015; Bayram & Aslan, 2015) of which about 11% are endemic.

This report arose from an ongoing agricultural study carried out in some olive orchards of Antalya province in order to determine harmful and beneficial insect species, and to search the population fluctuations of the important ones. Among the collected insects, chrysomelids were identified by the first author and unexpectedly one of them, *Lythraria salicariae* (Paykull, 1800), was determined as new record for Turkish fauna.

Lythraria Bedel 1897 is a monotypic genus of flea beetles in the tribe Alticini (Chrysomelidae: Galerucinae) containing *Lythraria salicariae* (Paykull 1800) found across the Palearctic ecozone (Europe, Caucasus, Siberia, Russian Far East, Japan) (Konstantinov & Vandenberg, 1996). A list of flea beetles collected by beating from olive trees is provided here including the first record of the genus *Lythraria* from Turkey. This contribution brings the current number of Turkish Alticini fauna to 345 species in 23 genera.

Materials and Methods

Flea beetle specimens were gathered from different localities in Antalya Province, Turkey during 2013-2015. The specimens were collected from olive trees by beating on a sheet with a stick. They were then taken to the laboratory for further analysis and dissection. The specimens were identified to species by the first author under an Olympus SZ61 stereomicroscope using the taxonomic keys and figures given by Konstantinov (1998), Čížek & Doguet (2008), Warchalowski (2010). Female genitalia and habitus of the new record were photographed with a digital camera attached to Leica Z16 APO stereomicroscope. All specimens are deposited in the personal collection of the first author at Department of Biology, Süleyman Demirel University, Turkey.

Results

A total of 108 individuals belonging to 26 species of Alticini in 10 genera, including a new genus record for the Turkish fauna, were identified. The species are listed in Table 1, including collection date, location, and number of individuals. General information on the new record is provided below.

Table 1. Twenty six species of Alticini (Chrysomelidae: Galerucinae) collected by beating from olive grove areas in Antalya Province, Turkey

Species	Collection date and locality	Number of specimens
Altica lythri Aubé, 1843	27.04.2013, Gazipaşa	2
Aphthona fuentei Reitter, 1901	06.03.2014, Aksu	2
A. nigriceps (Redtenbacher, 1842)	01.05.2013, Serik; 23.04.2015, Aksu	6
A. pygmaea (Kutschera, 1861)	14.04.2014, Aksu; 30.04.2015, Serik	5
A. warchalowskii Fritzlar, 2001	13.10.2013, Manavgat	2
Chaetocnema tibialis (Illiger, 1807)	11.10.2013, Döşemealtı	3
Epitrix hirtipennis (Melsheimer, 1847)	28.09.2013, Akseki	4
Hermaeophaga ruficollis (Lucas, 1849)	06.04.2013; 13.05.2014; 13.09.2014, Aksu; 18.05.2013, Kumluca; 08.06.2013, Döşemealtı; 28.09.2013, Gündoğmuş; 13.10.2013, Manavgat	16
Longitarsus albineus (Foudras, 1860)	16.06.2013, Gazipaşa	3
L. luridus (Scopoli, 1763)	06.03.2014; 28.03.2014, Aksu	4
L. lycopi (Foudras, 1860)	27.04.2013, Alanya	2
L. nanus (Foudras, 1860)	10.10.2013, Gebiz	2
L. nimrodi Furth, 1979	26.05.2014, Aksu	1
L. ochroleucus (Marsham, 1802)	11.06.2014, Aksu	2
L. parvulus (Paykull, 1799)	28.09.2013, Akseki	1
L. pellucidus (Foudras, 1860)	03.05.2013; 04.06.2014, Aksu; 13.10.2013, Manavgat	9
L. succineus (Foudras, 1860)	10.10.2013, Gebiz; 28.03.2014; 04.06.2014, Aksu	5
Lythraria salicariae (Paykull,1800)	16.06.2013, Alanya	2
Podagrica malvae (Illiger, 1807)	10.10.2013, Serik; 13.05.2014, Aksu	3
Phyllotreta variipennis (Boieldieu, 1859)	01.09.2013, Kumluca	2
Psylliodes anatolica Gök & Çilbiroğlu, 2004	01.05.2013, Serik; 03.05.2013, Aksu; 10.10.2013, Gebiz; 11.10.2013, Döşemealtı	10
P. cuprea (Koch, 1803)	21.04.2014; 28.04.2014, Aksu	7
P. isatidis Heikertinger, 1912	28.04.2014; 25.07.2014; 30.04.2015, Aksu	9
P. pallidicolor Pic, 1903	06.03.2014, Aksu	1
P. tricolor Weise, 1888	05.05.2014; 24.06.2014, Aksu	3
P. wrasei Leonardi & Arnold, 1995	13.05.2014, Aksu	2

***Lythraria salicariae* (Paykull, 1800) (Coleoptera: Chrysomelidae)**

Material examined. Dim Çayı, Alanya, Antalya Province (36º 32' 14" N, 32º 05' 18" E), 14 m, 16.VI.2013, 2 ♀♀, leg. M. Başar.

Known distribution. *Europe*: Armenia, Austria, Belgium, Bosnia and Herzegovina, Bulgaria, Belarus, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Great Britain, Germany, Greece, Hungary, Italy, Latvia, Lithuania, Luxembourg, The Netherlands, Norway, Poland, Romania, Russia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Ukraine, Serbia and Montenegro. *Asia*: East Siberia, Iran, Iraq, Japan, Kazakhstan, Mongolia, Korea. *North Africa*: not reported (Löbl & Smetana, 2010).

Distribution in Turkey. Antalya (new record from Turkey).

Diagnostic notes. Completely yellowish-brown; about 2.2-2.3 mm in length, upper sides finely and shallowly punctuate, elytral suture dark, punctures arranged in striae disappearing towards apical; spermatheca quite typical, especially in the structure and morphology of the ductus (Fig. 1).

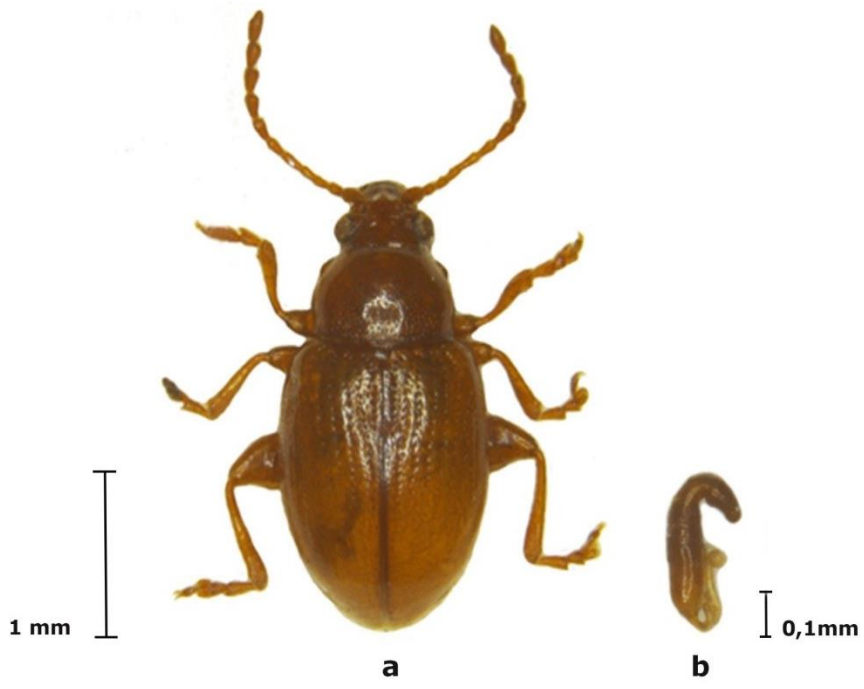

Figure 1. *Lythraria salicariae* (Paykull); a) habitus b) spermatheca.

Host plant information. Beetles were collected from an olive tree by beating method. The habitat was a neglected orchard along a roadside including olive and citrus fruit trees. It is not possible to conclude that olive trees were the host plant, as feeding and ovipositing were not observed. However, *Lysimachia vulgaris* L., *Lysimachia punctata* L. (Primulaceae) and *Lythrum salicaria* L. (Lythraceae) are reported as hosts for this species (Chatenet, 2002; Čížek & Doguet, 2008; Bukejs, 2009). Dolgovskaya et al. (2004) searched for potential biocontrol agents of purple loosestrife (*L. salicaria*), and listed four flea beetle species in their study including *L. salicariae*.

Discussion

Specimens of Alticini are generally collected either by sweep netting or by hand, but different collecting methods of this group have been applied in some recent studies (e.g. Flowers & Hanson, 2003; Furth et al., 2003; Linzmeier & Ribeiro-Costa, 2008; 2009; Aslan et al., 2012), especially those on the diversity of a particular area. Their direct association with herbaceous plants (and sometimes shrubs) makes the sweep netting more effective. However, it seems that different collecting methods such as beating and Malaise trapping clearly provide interesting catches for Alticini. It is not possible to conclude that olive trees were the actual host plants of the collected species. Most of them were collected in low numbers, and feeding activity was not recorded. It is possible that some were searching for temporary favorable environmental conditions, or were compelled to feed in a stress situation. For example, some species of *Psylliodes* have been observed on *Quercus* spp. (Fagaceae) without indication of feeding; only seeking for milder microhabitats (Aslan & Gök, 2006). Further studies with detailed observations are needed to understand presence of these beetles on olive trees, and the relationship between them. In a recent study conducted by Bayram & Aslan (2015) in the Aegean Region of Turkey, Alticini species composition, richness and abundance were studied comparatively in different habitats including also two different olive grove areas. However, because samplings were made from undergrowth vegetation using sweep-net, the authors did not record any species associated with olive trees.

As the present results show, alternative methods can be productive, and similar fieldwork using different collecting methods may give different results for species of Alticini. As a complement to sweep netting, it is likely that additional methods (as beating) can increase the efficiency of collection and species diversity of these beetles.

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Original article (Orijinal araştırma)

Occurrence of entomopathogenic fungi on insect pests of stored wheat and maize in Central and South Anatolia in Turkey

Türkiye'nin Orta ve Güney Anadolu Bölgesi'nde bulunan buğday ve mısır depolarındaki zararlılarda tespit edilen entomopatojen funguslar

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Summary

Naturally occurring fungal pathogens of stored-grain insect pests were identified and quantified using different insect sampling techniques in wheat and maize storage facilities in Central and South Anatolia in Turkey. Storage facilities were sampled by probing, trapping and visual inspection in five cities (Şanlıurfa, Kahramanmaraş, Adana, Mersin and Konya) from June to November 2013. Entomopathogenic fungi recovered from dead insects were identified to species level by sequencing the ITS1-5.8S-ITS2 region of the genomic DNA. Of the three species isolated, the majority were *Beauveria bassiana* (97 isolates), followed by *Purpureocillium lilacinum* (20 isolates). The third species, *Beauveria varroae* (9 isolates), is the first record on stored-product pest insects. Thirty-five isolates were from *Tribolium* spp., 29 from *Sitophilus* spp., 24 from *Cryptolestes ferrugineus,* 22 from *Rhyzopertha dominica,* 8 from *Oryzaephilus surinamensis,* 4 from *Trogoderma granarium,* 3 from *Latheticus oryzae* and 1 from a species of Cryptophagidae. The fungal infection of stored-grain pests did not vary significantly according to the time of sampling. A higher frequency of occurrence was recorded for Adana than the other cities and for *Tribolium* species than the other hosts. Grain samples taken by probing resulted in a higher frequency of fungal infection, but commodity type did not have a significant effect. The results demonstrated that (1) entomopathogenic fungi occurred at a low frequency, and (2) location, together with sampling technique, can affect their recovery. Further exploration of this ecosystem could yield important information for improving their use for management of stored-grain pests.

Keywords: Coleoptera, Hypocreales, microbial control, stored-product pests

Özet

Türkiye'nin Orta ve Güney Anadolu Bölgesi'ndeki buğday ve mısır depolarında tahıl zararlılarının doğal olarak bulunan fungal patojenleri, çeşitli böcek örnekleme yöntemleri kullanılarak belirlenmiş ve teşhis edilmiştir. 2013 yılı haziran – kasım aylarında beş ilde (Şanlıurfa, Kahramanmaraş, Adana, Mersin ve Konya) depolar, sonda kullanarak, tuzak yerleştirerek ve gözlemle örneklenmiştir. Ölü böceklerden elde edilen entomopatojen funguslar genomik DNAnın ITS1-5.8S-ITS2 bölgesi sekanslanarak tür seviyesinde teşhis edilmiştir. İzole edilen üç türün büyük çoğunluğu *Beauveria bassiana* (97 izolat) olup bunu *Purpureocillium lilacinum* (20 izolat) izlemiştir. Üçüncü tür ise *Beauveria varroae* (9 izolat)'dir ve depo zararlılarından ilk kayıttır. Tüm izolatların 35'i *Tribolium* spp., 29'u *Sitophilus* spp., 24'ü *Cryptolestes ferrugineus,* 22'si *Rhyzopertha dominica,* 8'i *Oryzaephilus surinamensis,* 4'ü *Trogoderma granarium,* 3'ü *Latheticus oryzae* ve birisi bir Cryptophagidae türünden izole edilmiştir. Depolanmış tahıl zararlılarında fungal enfeksiyonlar örnekleme zamanına göre önemli ölçüde değişmemiş, en yoğun olarak iller arasında Adana'da ve türler arasında da *Tribolium* türlerinde bulunmuştur. Sonda ile alınan tahıl örneklerinde fungal enfeksiyon daha yoğun tespit edilmiştir, ancak ürün cinsinin önemli bir etkisi belirlenmemiştir. Sonuçlar, entomopatojen fungusların düşük yoğunlukla dağılım gösterdiğini, ve lokasyon ile örnekleme tekniğinin fungus izolasyonunu etkileyebileceğini ortaya koymuştur. Bu ekosisteminde daha fazla çalışma yapılarak depolanmış tahıl zararlılarının mücadelesindeki kullanımlarını geliştirmek için önemli bilgilere ulaşılabilecektir.

Anahtar sözcükler: Coleoptera, Hypocreales, mikrobiyal mücadele, depo zararlıları

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Introduction

Cereals are important sources of nutrition for both humans and livestock throughout the world. These commodities are typically stored for various durations of time and require protection against insect and mite pests. Due to pest damage, unprotected grain storage usually leads to reduction in the weight and value of grain along with the germination decline of seeds (Moino et al., 1998; Padin et al., 2002; Haq et al., 2005; Stejskal et al., 2015). Although the use of synthetic insecticides to control stored-grain pest populations has been widespread (Athanassiou & Palyvos, 2006), the practice has been challenged due to various undesirable consequences including pest resistance to the chemicals (Arthur, 1996), residue accumulation in grain (Ferizli et al., 2005), and detrimental effects on humans and the environment (Michalaki et al., 2007). Therefore, efforts have been directed at evaluating nontoxic and environmentally-friendly techniques to control stored-grain pests. Entomopathogenic fungi have been considered as an alternative or complementary treatment (Moino et al., 1998; Michalaki et al., 2007; Sewify et al., 2014; Wakil & Schmitt, 2014) because of their natural occurrence, and low hazard towards human and the environment (Moore et al., 2000). Numerous studies have established the potential of entomopathogenic fungi as bioinsecticides against various insect pests of stored products (Cherry et al., 2005; Wakil & Ghazanfar, 2010; Shams et al., 2011; Barra et al., 2013; Khashaveh & Chelav, 2013; Sewify et al., 2014). Several other studies have also shown the potential of entomopathogenic fungi in combination with diatomaceous earth (Athanassiou & Steenberg, 2007; Athanassiou et al., 2008; Wakil et al., 2011; Riasat et al., 2011, 2013; Shafighi et al., 2014). The majority of research examining entomopathogenic fungi and stored-grain pests has been conducted with fungi isolated from sources other than the stored-grain pests themselves. Although the existence of entomopathogenic fungi in nature is well known, the extent and the manner of their distribution in stored-grain pest populations have received little attention. Odour et al. (2000) conducted a survey of *Beauveria bassiana* (Bals.-Criv.) Vuill. infections in pests of stored maize in Kenya, and Wakil et al. (2014) searched for entomopathogenic fungi infecting stored-grain insects in Pakistan. In the Mycopest Project, eight *B. bassiana* isolates were collected from UK grain stores (Wakefield et al., 2005). A better understanding of the natural occurrence of these fungal pathogens in their intended ultimate application ecosystem, grain stores, can be valuable in developing a fungus-based control strategy against stored-grain pests. To meet this objective, in the current study, naturally occurring fungal pathogens of stored-grain pests were identified and quantified from wheat (*Triticum aestivum* L. and *Triticum durum* Desf.) and maize (*Zea mays* L.) bulk stores in five cities in Turkey using different sampling techniques.

Materials and Methods

Sampling stored-grain insects

Insects were sampled from stored wheat and maize in five cities (Şanlıurfa, Kahramanmaraş, Adana, Mersin and Konya) in Central and South Anatolia, Turkey monthly from June to November 2013. In each month, a minimum of ten storage facilities in each city were sampled using three sampling techniques; (1) probing with a 2 m long metal grain probe in various sites of the bulk grains, yielding a total of 5 kg of grain, (2) trapping with five probe pitfall traps (Storgard WB Probe® II, Trécé Inc., Salinas, California, USA) in grain bulk, and (3) visual inspection of the facilities. Insect samples collected by trapping and visual inspection were placed in about 1 kg of grain stored in the facilities from which the samples were taken. Samples were put in plastic containers for transport back to the laboratory. Insects present in grain samples were separated by sieving (10, 18 and 35 mesh metal sieves, Retsch, Haan, Germany). Insect cadavers were taken and live insects were returned to the grain for incubation at 26±2°C, 65±5% RH in darkness for 1 month. Thereafter, the grain samples were sieved again to collect cadavers of those that had died during the incubation period. Following each examination, the insect cadavers were stored at 4°C until processed.

Isolation of fungi

Collected insects were identified according to taxonomic keys published by Gorham (1991) and Rees (2004). The collected cadavers were surface sterilized according to the procedure of Lacey & Brooks (1997) before incubation in humid chambers (sealed sterile Petri dishes lined with sterile damp filter paper) to promote fungal growth and sporulation on the surface of the cadavers. The chambers were kept at 26±2°C with a 16L:8D h photoperiod. The cadavers were checked daily and isolations were performed from those with fungal sporulation. Potato dextrose agar (PDA, Merck 1.10130, Darmstadt, Germany) supplemented with 0.6 g/L streptomycin sulfate and 10^5 IU/L penicillin was used for isolation

and PDA alone for subcultures. Once the purity of the cultures was ensured, lyophilized samples were deposited in the entomopathogenic fungal culture collection of the Department of Plant Protection, University of Kahramanmaraş Sütçü İmam, Turkey.

Identification of fungi

Morphological characteristics of fungi in cultures and on slides were combined with molecular techniques for precise identification to species (Humber, 1997; Luangsa-ard et al., 2011; Rehner et al., 2011). Fungal mycelia were grown in 1/4 strength Sabouraud dextrose broth plus yeast extract (0.5%) at 26±2°C on an orbital shaker for 4-6 days and subsequently harvested by filtration. The mycelial mat was lyophilized and 50 mg was ground into powder using a mortar and pestle. A CTAB procedure (Sirohi et al., 2013) with some modifications was followed to obtain genomic DNA. The powder was transferred to 1 ml CTAB buffer (10 ml 1M Tris HCL, pH 8.0; 28 ml 5 M NaCl; 4 ml 0.5 M EDTA; 2 g CTAB; completed to 100 ml with distilled deionized H_2O) and kept at 70°C for 75 min. After addition of 500 µl chloroform, the sample was further incubated for 7-8 min at 70°C, followed with 10 min centrifugation at 10,000 g. A 500 µl aliquot of the supernatant with 300 µl of isopropanol added was chilled at -20°C for 30 min before centrifuging for 10 min at 10,000 g. The precipitated DNA was washed using 200 µl of 70% ethanol, dried and resuspended in 50 µl of TE buffer (1 ml 1 M Tris HCl pH 8.0; 0.2 ml 0.5 M EDTA; completed to 100 ml with distilled deionized H_2O), and placed at -20°C until further processing. ITS1-5.8S-ITS2 sequences of the isolates were amplified using primers ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990). The PCR reaction mixture included 1 µl of each primer (20 pmol), 1 µl dNTP (1 mM), 0.5 µl Taq DNA Polimeraz (5 U/µl), 4 µl Taq buffer (10X), 32 µl distilled deionized H_2O. The DNA was denatured at 95°C for 5 min, followed by 35 cycles of amplification: 30 s at 95°C, 30 s at 50°C and 1 min at 72°C with final extension for 10 min at 72°C by using ABI Veriti Thermal Cycler 9902 (Applied Biosystems, Foster City, CA, USA). The PCR products were sent to MedSanTek (İstanbul, Turkey) for forward and reverse sequencing.

Statistical analysis

Forward and reverse sequences of each isolate were combined using BioEdit (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Isolates from the same species were aligned and those with the same sequences were grouped in clades. One isolate from each clade was chosen to represent the clade in the subsequent analyses. The Mega 6.06 (http://www.megasoftware.net) was used to align isolated sequences with representative sequences described by Rehner et al. (2011) for *Beauveria* and Luangsa-ard et al. (2011) for *Purpureocillium*. To determine the taxonomic positions of the fungal isolates, maximum likelihood and bootstrapping analyses were conducted using Mega 6.06. The trees were rooted using isolates from other genera of entomopathogenic fungi. Frequency of fungal infection was calculated for each host insect. Using Minitab16 (http://www.minitab.com/en-us/), contingency table analyses were employed to examine the effects of insect sampling time, site, host insects, sampling technique, examination time of the samples, and the commodities, from which samples were taken, on the variation of fungal infection. In order to avoid cells having expected frequencies <1, in the contingency table for host insects, *Sitophilus* and *Tribolium* spp. were pooled separately to genera, and the smallest two groups (*Latheticus oryzae* and Cryptophagidae) were excluded.

Results

A total of 126 fungi were isolated from 85,155 cadavers sampled from stored bulk grain from 5 cities and 87 sampling sites (61 wheat and 26 maize). Amplification of ITS1-5.8S-ITS2 region of genomic DNAs of *B. bassiana* (Bals.-Criv.) Vuill*., Beauveria varroae* Rehner & Humberand and *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson isolates produced 593, 594 and 620 bp sequences, respectively. Each *Beauveria* species had two sequences with only one nucleotide difference (Figures 1, 2) and thus each species was grouped into two clades. One isolate from each clade was chosen as a representative sample for further analyses. All of the *P. lilacinum* sequences were identical and one isolate was used as the representative in further phylogenetic analyses. Figures 3 and 4 illustrate the taxonomic positions within related genera. The sequences of representing isolates were deposited in NCBI GenBank with the following accession numbers; NCBI GenBank accession numbers for *B. bassiana* 151138, 54276, *B. varroae* 35727, 16787 and *P. lilacinum* 135233 were KU687110, KU687111, KU687112, KU687113, KU687114, respectively.

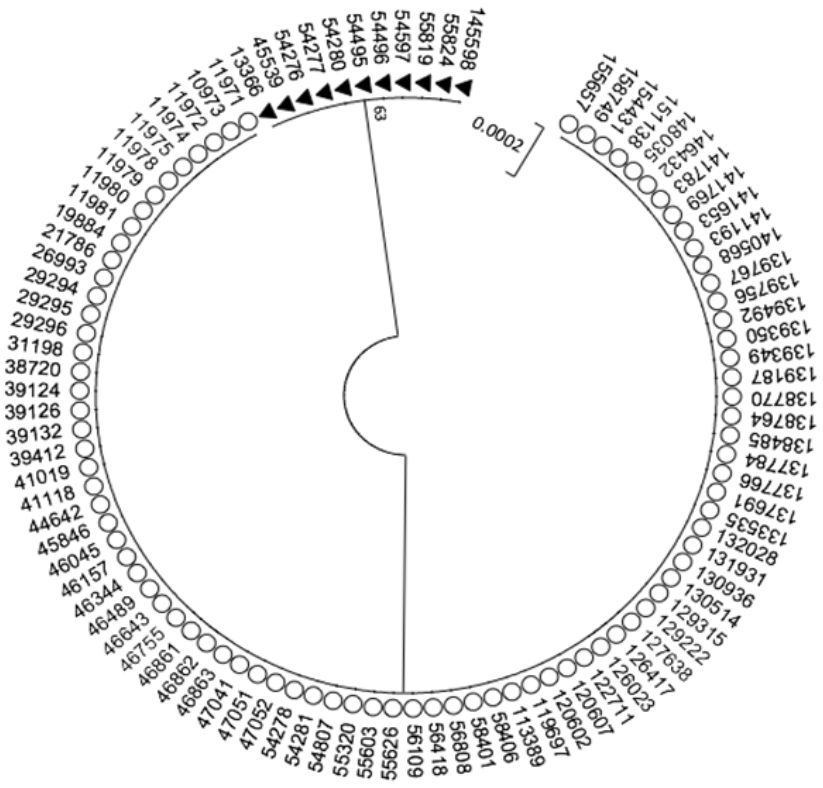

Figure 1. Two clades of *Beauveria bassiana* isolates based on ITS1-5.8S-ITS2 sequences; isolates marked with circles form clade Bb1 and those with triangles form clade Bb2.

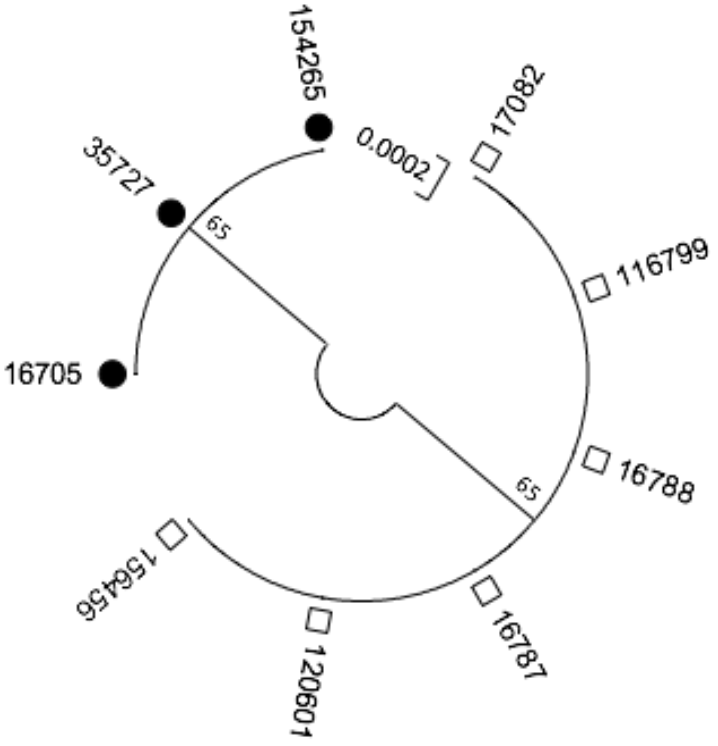

Figure 2. Two clades of *Beauveria varroae* isolates based on ITS1-5.8S-ITS2 sequences; isolates marked with filled circles form clade Bv1 and those marked with squares form clade Bv2.

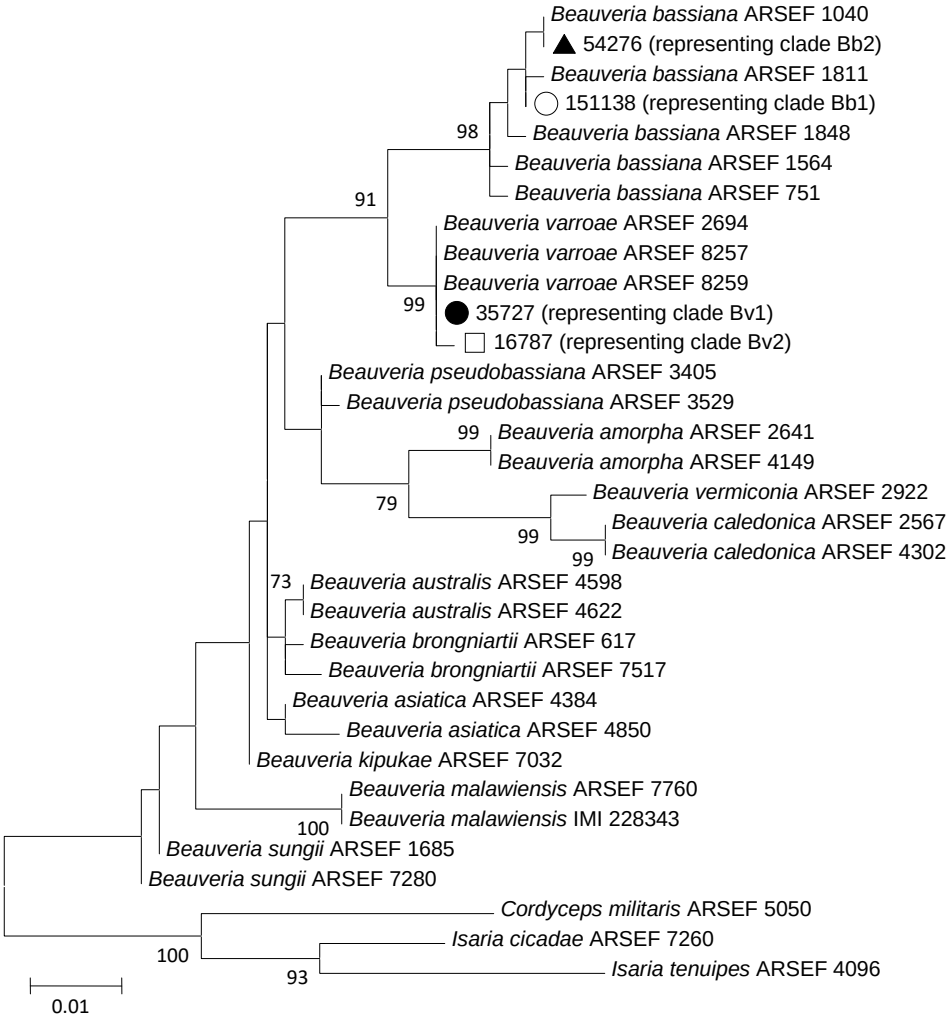

Figure 3. Phylogenetic position of isolates belonging to the genus *Beauveria* based on ITS1-5.8S-ITS2 sequences. Bootstrap values ≥70 are labeled.

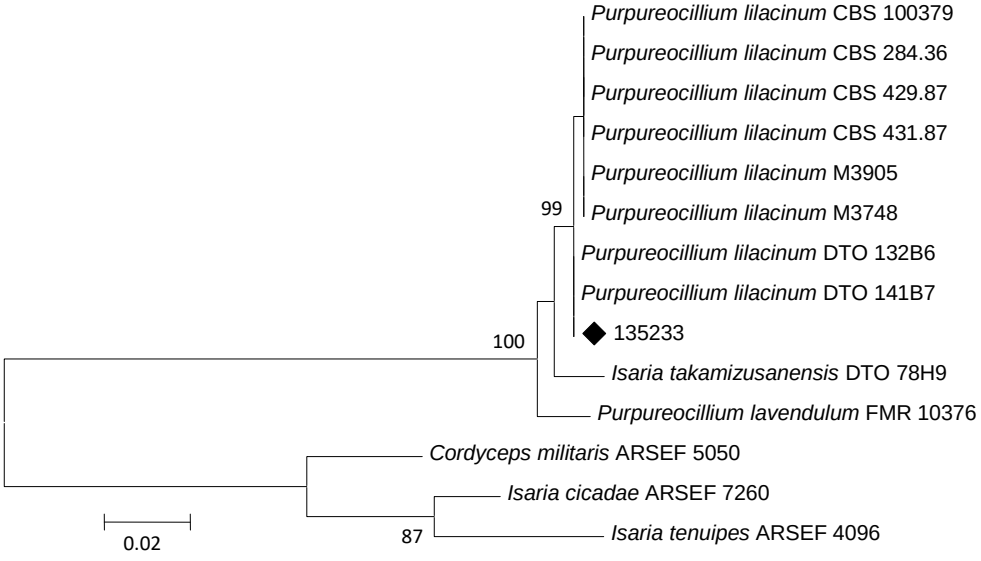

Figure 4. Phylogenetic position of isolates belonging to the genus *Purpureocillium* based on ITS1-5.8S-ITS2 sequences (Isolate 135233 represents all *Purpureocillium* isolates, which have the same sequences). Bootstrap values ≥70 are labeled.

Sampling parameters including fungal isolate identification, insect host, grain type, location and time of isolation are given in Table 1. The majority of isolates (97) were identified as *B. bassiana*, followed by *P. lilacinum* with 20 isolates and *B. varroae* with 9 isolates (Table 2). According to the insect host infection distribution, the highest frequency of *B. bassiana* isolation was from *Tribolium* spp., followed by *Sitophilus* spp., *Cryptolestes ferrugineus* and *Rhyzopertha dominica*. *Beauveria varroae* isolates were obtained only from *C. ferrugineus*, *R. dominica*, *Sitophilus oryzae* and *Trogoderma* specimens. The highest infection frequency of *P. lilacinum* was in *C. ferrugineus* followed by *R. dominica* (Table 2).

Table 1. Details of entomopathogenic fungal isolates recovered from collected stored-grain pests

Isolate number	Fungus species	Host insect species	Grain type	City	Sampling date
0973	*Beauveria bassiana*	*Tribolium castaneum*	wheat	Konya	18.06.2013
11971	*B. bassiana*	*T. castaneum*	wheat	Mersin	19.06.2013
11972	*B. bassiana*	*Sitophilus oryzae*	wheat	Mersin	19.06.2013
11974	*B. bassiana*	*S. oryzae*	wheat	Mersin	19.06.2013
11975	*B. bassiana*	*S. oryzae*	wheat	Mersin	19.06.2013
11978	*B. bassiana*	*T. castaneum*	wheat	Mersin	19.06.2013
11979	*B. bassiana*	*Rhyzopertha dominica*	wheat	Mersin	19.06.2013
11980	*B. bassiana*	*R. dominica*	wheat	Mersin	19.06.2013
11981	*B. bassiana*	*T. castaneum*	wheat	Mersin	19.06.2013
13366	*B. bassiana*	*S. oryzae*	wheat	Adana	20.06.2013
16705	*Beauveria varroae*	*Trogoderma* sp.	wheat	Konya	16.07.2013
16787	*B. varroae*	*Cryptolestes ferrugineus*	wheat	Konya	16.07.2013
16788	*B. varroae*	*R. dominica*	wheat	Konya	16.07.2013
17082	*B. varroae*	*Trogoderma granarium*	wheat	Konya	16.07.2013
19884	*B. bassiana*	*T. castaneum*	wheat	Mersin	18.07.2013
21786	*B. bassiana*	*C. ferrugineus*	maize	Adana	19.07.2013
26993	*B. bassiana*	*T. castaneum*	wheat	Konya	21.08.2013
29294	*B. bassiana*	*C. ferrugineus*	wheat	Adana	23.08.2013
29295	*B. bassiana*	*S. oryzae*	wheat	Adana	23.08.2013
29296	*B. bassiana*	*Oryzaephilus surinamensis*	wheat	Adana	23.08.2013
31198	*B. bassiana*	*T. castaneum*	maize	K.Maraş	27.08.2013
31304	*Purpureocillium lilacinum*	*S. oryzae*	mixed	K.Maraş	27.08.2013
35727	*B. varroae*	*R. dominica*	wheat	Konya	18.09.2013
35937	*P. lilacinum*	*C. ferrugineus*	wheat	Konya	18.09.2013
38720	*B. bassiana*	*T. castaneum*	wheat	Adana	20.09.2013
38813	*P. lilacinum*	Cryptophagidae	wheat	Adana	20.09.2013
39124	*B. bassiana*	*R. dominica*	wheat	Adana	20.09.2013

Table 1. (Continued)

Isolate number	Fungus species	Host insect species	Grain type	City	Sampling date
39126	*B. bassiana*	*S. oryzae*	wheat	Adana	20.09.2013
39132	*B. bassiana*	*T. castaneum*	wheat	Adana	20.09.2013
39412	*B. bassiana*	*R. dominica*	wheat	Adana	20.09.2013
41019	*B. bassiana*	*T. castaneum*	wheat	Şanlıurfa	27.09.2013
41118	*B. bassiana*	*C. ferrugineus*	wheat	Şanlıurfa	27.09.2013
41121	*P. lilacinum*	*T. castaneum*	wheat	Şanlıurfa	27.09.2013
44642	*B. bassiana*	*Sitophilus* sp.	wheat	Mersin	12.10.2013
45539	*B. bassiana*	*T. castaneum*	maize	Mersin	12.10.2013
45846	*B. bassiana*	*T. castaneum*	wheat	Adana	25.10.2013
46045	*B. bassiana*	*C. ferrugineus*	wheat	Adana	25.10.2013
46157	*B. bassiana*	*T. castaneum*	wheat	Adana	25.10.2013
46344	*B. bassiana*	*O. surinamensis*	wheat	Adana	25.10.2013
46489	*B. bassiana*	*C. ferrugineus*	wheat	Adana	25.10.2013
46643	*B. bassiana*	*T. castaneum*	wheat	Adana	25.10.2013
46755	*B. bassiana*	*S. oryzae*	wheat	Adana	25.10.2013
46861	*B. bassiana*	*C. ferrugineus*	wheat	Adana	25.10.2013
46862	*B. bassiana*	*C. ferrugineus*	wheat	Adana	25.10.2013
46863	*B. bassiana*	*T. castaneum*	wheat	Adana	25.10.2013
47041	*B. bassiana*	*Tribolium* sp.	wheat	Adana	25.10.2013
47051	*B. bassiana*	*S. oryzae*	wheat	Adana	25.10.2013
47052	*B. bassiana*	*S. oryzae*	wheat	Adana	25.10.2013
48423	*P. lilacinum*	*T. castaneum*	wheat	K.Maraş	20.10.2013
54276	*B. bassiana*	*T. castaneum*	wheat	Mersin	09.11.2013
54277	*B. bassiana*	*T. castaneum*	wheat	Mersin	09.11.2013
54278	*B. bassiana*	*T. castaneum*	wheat	Mersin	09.11.2013
54280	*B. bassiana*	*C. ferrugineus*	wheat	Mersin	09.11.2013
54281	*B. bassiana*	*S. oryzae*	wheat	Mersin	09.11.2013
54495	*B. bassiana*	*T. castaneum*	wheat	Mersin	09.11.2013
54496	*B. bassiana*	*S. oryzae*	wheat	Mersin	09.11.2013
54597	*B. bassiana*	*T. castaneum*	wheat	Mersin	10.11.2013
54807	*B. bassiana*	*Sitophilus* sp.	wheat	Mersin	10.11.2013
55320	*B. bassiana*	*C. ferrugineus*	wheat	Mersin	10.11.2013

Table 1. (Continued)

Isolate number	Fungus species	Host insect species	Grain type	City	Sampling date
55321	*P. lilacinum*	*C. ferrugineus*	wheat	Mersin	10.11.2013
55322	*P. lilacinum*	*C. ferrugineus*	wheat	Mersin	10.11.2013
55603	*B. bassiana*	*Latheticus oryzae*	wheat	Adana	23.11.2013
55604	*P. lilacinum*	*L. oryzae*	wheat	Adana	23.11.2013
55610	*P. lilacinum*	*O. surinamensis*	wheat	Adana	23.11.2013
55615	*P. lilacinum*	*C. ferrugineus*	wheat	Adana	23.11.2013
55626	*B. bassiana*	*R. dominica*	wheat	Adana	23.11.2013
55627	*P. lilacinum*	*R. dominica*	wheat	Adana	23.11.2013
55819	*B. bassiana*	*T. castaneum*	maize	Adana	23.11.2013
55824	*B. bassiana*	*S. oryzae*	maize	Adana	23.11.2013
56109	*B. bassiana*	*R. dominica*	wheat	Adana	24.11.2013
56418	*B. bassiana*	*C. ferrugineus*	wheat	Adana	24.11.2013
56717	*P. lilacinum*	*S. oryzae*	wheat	Adana	24.11.2013
56808	*B. bassiana*	*O. surinamensis*	wheat	Adana	24.11.2013
57202	*P. lilacinum*	*R. dominica*	wheat	Adana	23.11.2013
58401	*B. bassiana*	*R. dominica*	wheat	K.Maraş	16.11.2013
58406	*B. bassiana*	*T. castaneum*	wheat	K.Maraş	16.11.2013
59552	*P. lilacinum*	*Sitophilus* sp.	wheat	K.Maraş	16.11.2013
60778	*P. lilacinum*	*T. granarium*	wheat	Şanlıurfa	03.11.2013
113389	*B. bassiana*	*S. oryzae*	wheat	Adana	20.06.2013
116799	*B. varroae*	*T. granarium*	wheat	Konya	16.07.2013
119697	*B. bassiana*	*T. castaneum*	wheat	Mersin	18.07.2013
120601	*B. varroae*	*C. ferrugineus*	wheat	Adana	19.07.2013
120602	*B. bassiana*	*C. ferrugineus*	wheat	Adana	19.07.2013
120607	*B. bassiana*	*O. surinamensis*	wheat	Adana	19.07.2013
122711	*B. bassiana*	*Tribolium confusum*	wheat	K.Maraş	25.07.2013
126023	*B. bassiana*	*C. ferrugineus*	wheat	Konya	21.08.2013
126417	*B. bassiana*	*T. castaneum*	wheat	Konya	21.08.2013
127638	*B. bassiana*	*S. oryzae*	wheat	Mersin	22.08.2013
129216	*P. lilacinum*	*C. ferrugineus*	wheat	Adana	23.08.2013
129222	*B. bassiana*	*R. dominica*	wheat	Adana	23.08.2013
129315	*B. bassiana*	*S. oryzae*	wheat	Adana	23.08.2013

Table 1. (Continued)

Isolate number	Fungus species	Host insect species	Grain type	City	Sampling date
130514	*B. bassiana*	*T. castaneum*	wheat	Adana	23.08.2013
130936	*B. bassiana*	*R. dominica*	wheat	K.Maraş	27.08.2013
131931	*B. bassiana*	*S. oryzae*	wheat	K.Maraş	27.08.2013
132028	*B. bassiana*	*R. dominica*	wheat	K.Maraş	27.08.2013
133535	*B. bassiana*	*S. oryzae*	wheat	K.Maraş	27.08.2013
133630	*P. lilacinum*	*O. surinamensis*	wheat	K.Maraş	27.08.2013
135233	*P. lilacinum*	*R. dominica*	wheat	Şanlıurfa	28.08.2013
137691	*B. bassiana*	*R. dominica*	wheat	Mersin	19.09.2013
137766	*B. bassiana*	*S. oryzae*	wheat	Mersin	19.09.2013
137784	*B. bassiana*	*C. ferrugineus*	wheat	Mersin	19.09.2013
138485	*B. bassiana*	*O. surinamensis*	wheat	Mersin	19.09.2013
138764	*B. bassiana*	*C. ferrugineus*	wheat	Adana	20.09.2013
138770	*B. bassiana*	*Sitophilus* sp.	wheat	Adana	20.09.2013
139187	*B. bassiana*	*O. surinamensis*	wheat	Adana	20.09.2013
139349	*B. bassiana*	*R. dominica*	wheat	Adana	20.09.2013
139350	*B. bassiana*	*R. dominica*	wheat	Adana	20.09.2013
139492	*B. bassiana*	*C. ferrugineus*	wheat	Adana	20.09.2013
139756	*B. bassiana*	*C. ferrugineus*	wheat	Adana	20.09.2013
139767	*B. bassiana*	*T. castaneum*	wheat	Adana	20.09.2013
139988	*P. lilacinum*	*Sitophilus* sp.	wheat	Adana	20.09.2013
140568	*B. bassiana*	*C. ferrugineus*	wheat	Şanlıurfa	27.09.2013
141193	*B. bassiana*	*L. oryzae*	wheat	Şanlıurfa	27.09.2013
141653	*B. bassiana*	*R. dominica*	wheat	K.Maraş	20.09.2013
141769	*B. bassiana*	*Sitophilus granarius*	wheat	K.Maraş	20.09.2013
141783	*B. bassiana*	*R. dominica*	wheat	K.Maraş	20.09.2013
145598	*B. bassiana*	*T. castaneum*	maize	Mersin	12.10.2013
146432	*B. bassiana*	*S. oryzae*	wheat	Adana	25.10.2013
148035	*B. bassiana*	*T. castaneum*	wheat	K.Maraş	20.10.2013
151138	*B. bassiana*	*R. dominica*	wheat	Şanlıurfa	06.10.2013
154265	*B. varroae*	*S. oryzae*	wheat	Mersin	09.11.2013
154431	*B. bassiana*	*T. castaneum*	wheat	Mersin	09.11.2013
155657	*B. bassiana*	*T. castaneum*	wheat	Adana	23.11.2013
156456	*B. varroae*	*S. oryzae*	wheat	Adana	24.11.2013
158749	*B. bassiana*	*T. castaneum*	maize	K.Maraş	17.11.2013
160466	*P. lilacinum*	*R. dominica*	wheat	Şanlıurfa	02.11.2013

Table 2. Total number of insects examined and frequency of insects infected by entomopathogenic fungi

Host insects	Number of insects examined	*Beauveria bassiana* infections		*Beauveria varroae* infections		*Purpureocillium lilacinum* infections		Total fungal infections	
		Number of insects	Frequency (%)	Number of insects	Frequency (%)	Number of insects	Frequency (%)	Number of insects	Frequency (%)
Cryptolestes ferrugineus	18427	17	17.5	2	22.2	5	25.0	24	19.0
Oryzaephilus surinamensis	5954	6	6.2	0	0.0	2	10.0	8	6.3
Rhyzopertha dominica	16138	16	16.5	2	22.2	4	20.0	22	17.5
Sitophilus granarius	2069	1	1.0	0	0.0	0	0.0	1	0.8
Sitophilus oryzae	24144	18	18.6	2	22.2	2	10.0	23	18.3
Sitophilus spp.	826	4	4.1	0	0.0	2	10.0	5	4.0
Tribolium castaneum	11731	31	32.0	0	0.0	2	10.0	33	26.2
Tribolium confusum	2641	1	1.0	0	0.0	0	0.0	1	0.8
Tribolium spp.	62	1	1.0	0	0.0	0	0.0	1	0.8
Trogoderma granarium	1404	0	0.0	2	22.2	1	5.0	2	1.6
Trogoderma spp.	280	0	0.0	1	11.1	0	0.0	2	1.6
Latheticus oryzae	603	2	2.1	0	0.0	1	5.0	3	2.4
Cryptophagidae	76	0	0.0	0	0.0	1	5.0	1	0.8

Contingency table analyses revealed that fungal infection of stored-grain pests did not vary significantly with time of sampling within individual grain storage sites (Table 3). The occurrence of fungal infection, however, differed significantly within sampling sites, with the highest variation seen in Adana (X^2=7.11) where actual occurrence of infection was higher than the expected frequency (Table 4). In the follow-up chi-square test excluding Adana (X^2=2.22 and p=0.528) the variation was not significant between the other sites. There was also significant variation between fungal infection within host insect (Table 5), with the main source of variation came from *Tribolium* spp. (X^2=9.27) in which a higher than expected frequency was recorded. The follow-up chi-square test after elimination of *Tribolium* spp. indicated that the variation within the other host insects was not significant (X^2=2.62 and p=0.623). Insect sampling technique was another factor resulting in significant variation in the total observed fungal infections and in those recorded from the cadavers collected before and after incubation (Table 6). In all cases, the major source of variability was probing technique for which the frequency of fungus-infected insects was much higher than the expected. X^2 and p values of follow-up chi-square tests (probing eliminated) were 0.611 and 0.434 for cadavers collected before incubation, 1.260 and 0.262 after incubation, 1.850 and 0.174 for total infections, respectively. Fungus isolation from the cadavers separated from grain before incubation did not significantly differ from those cadavers obtained after incubation period regardless of sampling technique (Table 7). Similarly, the frequency of fungal infection in pests collected from wheat and maize did not vary significantly, regardless of the time of cadavers were collected from the grain samples (Table 8).

Table 3 Contingency table for the variation of fungal infections due to insect sampling dates (expected values are given in parentheses)

Sampling date	FI**	NI**	N_i	X^2
June	11 (8)	5063	5074	1.626
July	12 (13)	8558	8570	0.037
August	19 (23)	15195	15214	0.549
September	29 (31)	20849	20878	0.116
October	20 (24)	16103	16123	0.624
November	35 (29)	19261	19296	1.458
N_i	126	85029	85155	
X^2	4.404	0.006		4.411*

*p=0,492; **FI: individuals from which entomopathogenic fungi were isolated; NI: individuals not infected by entomopathogenic fungi.

Table 4 Contingency table for the variation of fungal infections due to insect collection sites (expected values are given in parentheses)

Collection site	FI**	NI**	N_i	X^2
Adana	57 (40)	27054	27111	7.112
Konya	11 (13)	8461	8472	0.189
Kahramanmaraş	17 (27)	17942	17959	3.456
Mersin	32 (33)	22576	22608	0.063
Şanlıurfa	9 (13)	8983	8992	1.396
N_i	126	85016	85142	
X^2	12.198	0.018		12.216*

*p=0,016; **FI: individuals from which entomopathogenic fungi were isolated; NI: individuals not infected by entomopathogenic fungi.

Table 5 Contingency table for the variation of fungal infections due to host insect species (expected values are given in parentheses)

Host insects	FI**	NI**	N_i	X^2
Cryptolestes ferrugineus	24 (27)	18403	18427	0.306
Oryzaephilus surinamensis	8 (9)	5946	5954	0.053
Rhyzopertha dominica	22 (24)	16116	16138	0.099
Sitophilus spp.	29 (39)	27011	27040	2.760
Tribolium spp.	35 (21)	14401	14436	9.265
Trogoderma granarium	4 (2)	1681	1685	0.971
N_i	122	83558	83680	
X^2	13.435	0.019		13.454*

*p=0,019; **FI: individuals from which entomopathogenic fungi were isolated; NI: individuals not infected by entomopathogenic fungi.

Table 6. Contingency table for the variation of fungal infections due to insect sampling technique, which were examined before and after one month of incubation (expected values are given in parentheses)

	Individuals obtained before incubation				Individuals obtained after incubation				Total individuals examined			
Sampling technique	FI**	NI**	N_i	X^2	FI**	NI**	N_i	X^2	FI**	NI**	N_i	X^2
Probe	23 (6)	4435	4458	43.919	10 (3)	1698	1708	19.802	33 (9)	6133	6166	62.578
Visual	44 (55)	38507	38551	2.121	36 (39)	24947	24983	0.302	80 (94)	63454	63534	2.09
Trap	9 (15)	10476	10485	2.337	4 (8)	4966	4970	1.89	13 (23)	15442	15455	4.264
N_i	76	53418	53494		50	31611	31661		126	85029	85155	
X^2	48.309	0.068		48.377*	21.96	0.034		21.996*	68.83	0.102		68.993*

*p<0,001; **FI: individuals from which entomopathogenic fungi were isolated; NI: individuals not infected by entomopathogenic fungi.

Table 7. Contingency table for the variation of fungal infections due to examination time of the grain samples taken by three different sampling techniques (expected values are given in parentheses)

	Probe sampling				Visual sampling				Trap sampling				Total (Probe+Visual+Trap)			
Time of examination	FI**	NI**	N_i	X^2	FI**	NI**	N_i	X^2	FI**	NI**	N_i	X^2	FI**	NI**	N_i	X^2
Before incubation	23 (24)	4435	4458	0.03	44 (49)	38507	38551	0.43	9 (9)	10476	10485	0.004	76 (79)	53418	53494	0.126
After incubation	10 (9)	1698	1708	0.08	36 (31)	24947	24983	0.66	4 (4)	4966	4970	0.008	50 (47)	31611	31661	0.212
N_i	33	6133	6166		80	63454	63534		13	15442	15455		126	85029	85155	
X^2	0.11	0.00		0.11*		1.08	0.00	1.08*		0.012	0.000	0.012*	0.338	0.000		0.338*

*respectively p=0.738, p=0.298, p=0.915, p=0.561; **FI: individuals from which entomopathogenic fungi were isolated; NI: individuals not infected by entomopathogenic fungi

Table 8. Contingency table for the variation of fungal infections due to type of sampled grains from which insects were collected before and after one month of incubation (expected values are given in parentheses)

	Individuals obtained before incubation				Individuals obtained after incubation				Total individuals examined			
Grain	FI**	NI**	N_i	X^2	FI**	NI**	N_i	X^2	FI**	NI**	N_i	X^2
Maize	5 (7)	5106	5111	0.705	2 (4)	2332	2334	0.772	7 (11)	7438	7445	1.466
Wheat	71 (69)	48312	48383	0.074	48 (46)	29279	29327	0.061	119 (115)	77591	77710	0.14
N_i	76	53418	53494		50	31611	31661		126	85029	85155	
X^2	0.778	0.001		0.78*	0.832	0.001		0.834*	1.604	0.002		1.607*

*respectively p=0.377, p=0.361, p=0.205; **FI: individuals from which entomopathogenic fungi were isolated; NI: individuals not infected by entomopathogenic fungi.

Discussion

The occurrence of entomopathogenic fungi in soil and in populations of pest insects from cropping areas has been examined in many studies; however, fungal pathogens of stored-product pest populations have received little attention particularly within the grain storage facilities themselves. Odour et al. (2000) recovered 26 *B. bassiana* isolates from insect pests of stored maize, Wakefield et al. (2005) obtained 8 *B. bassiana* isolates and Wakil et al. (2014) 26 fungal pathogens from stored-grain insects. This rather low occurrence is comparable to our 126 isolates, considering the high number of insects processed (frequency < 0.0015). These results, together with our observation that none of the insects collected from storage facilities showed any external evidence of fungal growth, suggest that entomopathogenic fungi occur at low frequency within stored-grain pest populations. This could be due to abiotic stress on the fungi, particularly low humidity, which could limit the ability of the fungus to persist in the stored grain, and/or a consequence of microbial defensive systems employed by stored-grain pests (Ortiz-Urquiza & Keyhani, 2013, 2015). The most commonly encountered fungal species was *B. bassiana* in both our study and that of Wakil et al. (2014). In addition, the frequency of host insect species from which pathogenic fungi were isolated was similar in our study and Wakil (2014). *Tribolium castaneum* was found to be the most frequently infected insect species. Thus although *T. castaneum* represented about 13.8% of the total insects examined, the frequency of fungal isolation from this insect accounted for about 24% of the total (31/126). *Tribolium* spp. are known to be difficult to kill with entomopathogenic fungi in laboratory bioassays (Rice & Cogburn, 1999; Padin et al., 2002; Akbar et al., 2004; Wakefield et al., 2005; Wakefield, 2006; Michalaki et al., 2006, 2007). This was recently shown to be due in part to the production of cuticular defensive secretions that inhibit growth of *B. bassiana* and other microbes (Pedrini et al, 2015). Wakil et al. (2014) also found differences in the number of fungal isolates with respect to the location where they had been sampled and a similar variation was noted in our study, especially in sites sampled in the city of Adana, which contributed most of the observed variation. Our work has provided a set of entomopathogenic fungi derived from the stored-grain pests directly. Future experiments comparing these isolates to wild types may provide clues as to whether these isolates have adapted to the stored grain environment and/or to the insects themselves or whether they represent low residual opportunistic infections. These data could have implications in developing isolates that may be more useful in stored grain pest control applications.

To the best of our knowledge, this is the first record of *B. varroae* infection in stored-product pest insects. This could be partially due to the technique used in previous studies to identify isolated *Beauveria* species, as some species cannot be easily distinguished according to their morphological features (Rehner et al., 2011). Another reason could be that there has been insufficient scrutiny of naturally occurring fungal pathogens of stored-product pests, indicating the importance of further investigation.

Odour et al. (2000) recommended using dead insects when collected rather than those that died during incubation in the laboratory. However, according to our results, isolates could also be obtained from insects that died during the one month of incubation. Incubation conditions may also alter the outcome, as Odour et al. (2000) incubated the samples under room conditions, whereas in this study the samples were incubated at 26±2°C, 65±5% RH in darkness. It is not clear if some live insects were already infected at collection or if they were infected during incubation. However, our data suggest a potential for increased isolation of resident fungal insect pathogens with incubation after sampling.

Sampling time did not affect the frequency of isolation of fungal pathogens, but the sampling techniques employed had a statistically significant effect. Collection of fungal infected insects by probing was more effective than the other techniques used. Assuming the insects that have died from fungal infection are distributed evenly in the bulk grain, probing can potentially allow their retrieval in a more representative manner, whereas the other techniques concentrated more on active insects and may therefore cause bias.

This and previous work have shown that stored-grain insects are naturally infected by entomopathogenic fungi in storage facilities but occur at low frequencies in the insect populations. A better understanding of this ecosystem for entomopathogenic fungi and their activities, together with a complete list of naturally existing insect pathogenic fungal species, may have important implications for developing microbial control strategies based on these fungi.

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Original article (Orijinal araştırma)

A study of Ichneumonidae (Hymenoptera) from northeastern Anatolia II, with new records

Kuzeydoğu Anadolu'dan Ichneumonidlerle ilgili bir çalışma ve yeni kayıtlar

Saliha ÇORUH[1*] Janko KOLAROV[2] İrfan ÇORUH[1]

Summary

In total, forty five taxa belonging to Brachycyrtinae, Campopleginae, Cryptinae and Ichneumoninae subfamilies (Ichneumonidae: Hymenoptera) have been reported to occur in northeastern Anatolia. A study was conducted in July 2015 in this area and 20 ichneumonid taxa were recorded for the first time for the Turkish fauna. These species are: *Campoletis excavata* (Smits van Burgst, 1914), *Olesicampe fulviventris* (Gmelin, 1790), *Olesicampe proterva* (Brischke, 1880), *O. sternella* (Thomson, 1887), *Bathythrix collaris* (Thomson, 1896), *B. fragilis* (Gravenhorst, 1829), *Nematopodius formosus* Gravenhorst, 1829, *Rhembobius perscrutator* (Thunberg, 1822), *Thaumatogelis femoralis* (Brischke, 1881), *Apaeleticus inimicus* (Gravenhorst, 1820), *Dirophanes fulvitarsis* (Wesmael, 1845), *Heterischnus excavatus* (Constantineanu, 1959), *Ichneumon deliratorius* (Linnaeus, 1758), *Nematomicrus tenellus* Wesmael, 1845, *Oronotus binotatus* (Gravenhorst, 1829), *Phaeogenes curator* (Thunberg, 1822), *P. heterogonus* Holmgren, 1890, *P. semivulpinus* (Gravenhorst, 1829), *Stenodontus biguttatus* (Gravenhorst, 1829) and *Tycherus impiger* (Wesmael, 1845). For each species discussed, new locations are added and zoogeographical characterization is given.

Key words: Fauna, Ichneumonidae, Northeastern Anatolia, zoogeographical characterization

Özet

Kuzeydoğu Anadolu'dan Brachycyrtinae, Campopleginae, Cryptinae ve Ichneumoninae (Hymenoptera: Ichneumonidae) altfamilyalarına ait 45 tür belirlenmiştir. Çalışma, 2015 yılı Temmuz ayında yapılmıştır. 20 ichneumonid türü Türkiye faunası için ilk kez kaydedilmiştir. Bu türler: *Campoletis excavata* (Smits van Burgst, 1914), *Olesicampe fulviventris* (Gmelin, 1790), *O. proterva* (Brischke, 1880), *O. sternella* (Thomson, 1887), *Bathythrix collaris* (Thomson, 1896), *B. fragilis* (Gravenhorst, 1829), *Nematopodius formosus* Gravenhorst, 1829, *Rhembobius perscrutator* (Thunberg, 1822), *Thaumatogelis femoralis* (Brischke, 1881), *Apaeleticus inimicus* (Gravenhorst, 1820), *Dirophanes fulvitarsis* (Wesmael, 1845), *Heterischnus excavatus* (Constantineanu, 1959), *Ichneumon deliratorius* (Linnaeus, 1758), *Nematomicrus tenellus* Wesmael, 1845, *Oronotus binotatus* (Gravenhorst, 1829), *Phaeogenes curator* (Thunberg, 1822), *P. heterogonus* Holmgren, 1890, *P. semivulpinus* (Gravenhorst, 1829), *Stenodontus biguttatus* (Gravenhorst, 1829) ve *Tycherus impiger* (Wesmael, 1845)'dir. Bilinen türler için yeni lokaliteler eklenirken, her bir tür için de zoocoğrafik notlar verilmiştir.

Anahtar sözcükler: Fauna, Ichneumonidae, Kuzeydoğu Anadolu, zoocoğrafik notlar

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Introduction

Ichneumonidae is the largest family within the order Hymenoptera and may be among the largest insect families on earth (Gauld, 1991). It consists of at least 24.268 described species (Yu et al., 2012). The overall distribution of Ichneumonidae is as follows: Afrotropical: 4.611; Australasian: 2.349; Eastern Palaearctic: 9.255; Europe: 10.469; Nearctic: 7.707; Neotropical: 7.413; Oceanic: 816; Oriental: 7.942; Western Palaearctic: 11.275 species (Yu et al., 2012). The established number of species from Turkey is approximately 1.200 species. Most species occurring in the tropical countries are unknown.

The ichneumonids are widely used for biological control programs against insect pests all over the world (Narendran, 1998). Therefore this family is of a great importance.

Faunistic research on the family Ichneumonidae in Turkey started in the 19th century. In the catalogue of Kolarov (1995), 383 ichneumonid species are listed. There has been a remarkable intensity of studies on this family over the last three years (Çoruh & Kolarov, 2013; Çoruh & Özbek, 2013; Çoruh et al., 2013; Çoruh et al., 2014a, 2014b, 2014c; Kolarov et al., 2014a, 2014b, Özdan, 2014; Riedel et al., 2014; Yaman, 2014; Kolarov et al., 2015; Yurtcan & Kolarov, 2015; Kolarov et al., 2016) and consequently the number of Ichneumonidae species in Turkey increased to 1181. With the 20 new records reported in this paper, the number is now 1201.

The Turkey ichneumonid fauna is rich and diverse, but very few areas have been intensively and extensively explored. Faunistic work has continued in northeastern Anatolia (Rize, Giresun, Ordu, Trabzon, Erzurum, Erzincan, Gümüşhane) for 3 years (Kolarov et al., 2014a, 2014b, Çoruh et al., 2014a, 2014b; Kolarov et al., 2015; Kolarov et al., 2016). The aim of the study is to investigate the ichneumonid biodiversity northeastern Anatolia.

Material and methods

Sampling and collection method

Insects were collected in two different regions, the East Black Sea Region (Rize, Giresun, Ordu, Trabzon) and the Eastern Anatolia Region (Erzurum, Erzincan, Gümüşhane) (Figure 1) during July 2015. The samples were collected by sweep net on different flowering plants by the first two authors. The nomenclature and general distribution follows Yu et al. (2012). Faunistic data are listed in the following order: country, locality, coordinates, date of collection, altitude, and number and sex of the specimens. The specimens are currently deposited in the Collection of University of Plovdiv (Bulgaria). The identification of the material was made by the second author.

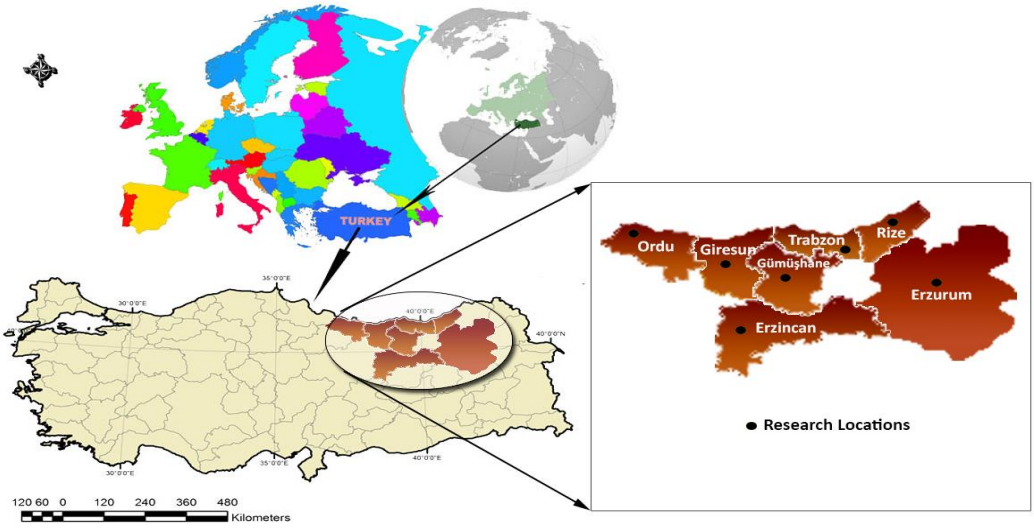

Figure 1. Map of study areas.

The plant species visited by Ichneumonid species were collected by hand, pressed and deposited in Herbarium of Plant Protection Department (Erzurum). Plant specimens were identified according to Davis (1965-1988) and Herbarium of Atatürk University, Faculty of Agriculture, Department of Plant Protection by third author.

Study area

Habitat and vegetation data for each location for collected insects are given below.

Kandilli (Erzurum):

Habitat data: roadside, meadow area near water channel, dominant plant *Medicago sativa.*

Vegetation: *Anchusa leptophylla*, *Astrodaucus orientalis*, *Bromus japonicus*, *Bupleurum rotundifolium*, *Carum carvi*, *Centaurea pseudoscabiosa*, *Cirsium arvense*, *C. echinus*, *Convolvulus arvensis*, *Cynodon dactylon*, *Dactylis glomerata*, *Eryngium billardieri*, *Festuca ovina*, *Galium verum*, *Hypericum elongatum*, *Inula oculus-christi*, *Isatis* sp., *Melampyrum arvense*, *Melilotus officinalis*, *Mentha longifolia*, *Lactuca serriola*, *Leontodon crispus*, *Linaria kurdica*, *Phlomis pungens*, *Poa bulbosa*, *Rhinanthus angustifolius*, *Rumex crispus*, *Sonchus arvensis*, *Teucrium orientale*, *Turgenia latifolia*, *Verbascum speciosum*, *Vicia cracca*, *Xeranthemum annuum.*

Gelinkaya (Erzurum):

Habitat data: roadside, dominant plants *Salix triandra*, *Populus tremula* and *Medicago sativa.*

Vegetation: *Achillea millefolium*, *Astragalus lineatus*, *Bromus arvensis*, *Campanula stevenii*, *Cephalaria procera*, *Cichorium intybus*, *Cirsium arvense*, *Coronilla varia*, *Dactylis glomerata*, *Eryngium giganteum*, *Euphorbia virgata*, *Filipendula vulgaris*, *Galium verum*, *Geranium tuberosum*, *Heracleum pastinacifolium*, *Lactuca serriola*, *Lapsana communis*, *Lathyrus pratensis*, *Lolium perenne*, *Lotus corniculatus*, *Medicago sativa*, *Phleum pratense*, *Phragmites australis*, *Populus tremula*, *Rhinanthus angustifolius*, *Rumex crispus, Salix triandra*, *Senecio vulgaris*, *Seseli libanotis*, *Tragopogon dubius*, *Trifolium pratense*, *Vicia cracca.*

Tepebaşı Valley (Aşkale, Erzurum):

Habitat data: roadside, dry land, pasture, dominant plant *Quercus petraea.*

Vegetation: *Acantholimon caryophyllaceum*, *Allium rotundum*, *Anthemis tinctoria*, *Astragalus lagurus*, *A. microcephalus*, *Bupleurum rotundifolium*, *Carum carvi*, *Cephalaria procera*, *Cirsium arvense*, *Coronilla varia*, *Crataegus orientalis*, *Crepis armena*, *Dactylis glomerata*, *Daucus carota*, *Delphinium cyphoplectrum*, *Eryngium billardieri*, *Euphorbia stricta*, *Festuca ovina*, *Globularia trichosantha*, *Hypericum elongatum*, *Melampyrum arvense*, *Melilotus officinalis*, *Muscari comosum*, *Onobrychis altissima*, *Phlomis pungens*, *Potentilla argentea*, *Quercus petraea*, *Salvia candidissima*, *Sanguisorba minor*, *Scabiosa argentea*, *Seseli libanotis*, *Silene vulgaris*, *Stipa pulcherrima*, *Trifolium montanum*, *Verbascum cheiranthifolium*, *Xeranthemum annuum.*

Ahmetli (Erzincan):

Habitat data: roadside, semi-wet mown pasture.

Vegetation: *Achillea millefolium*, *Alchemilla caucasica*, *Cardaria draba*, *Carduus nutans*, *Carex panicea*, *Cephalaria procera*, *Cerinthe minor*, *Cichorium intybus*, *Cirsium arvense*, *Convolvulus arvensis*, *Crepis armena*, *Dactylis glomerata*, *Equisetum ramosissimum*, *Euphorbia petrophila*, *Festuca ovina*, *Galium verum*, *Grammosciadium daucoides*, *Hypericum elongatum*, *Marrubium parviflorum*, *Mentha longifolia*, *Ononis spinosa*, *Papaver dubium*, *Phleum pratense*, *Plantago atrata*, *Poa bulbosa*, *P. nemoralis*, *Rhinanthus angustifolius*, *Rumex acetosella*, *Sanguisorba minor*, *Senecio paucilobus*, *S. vernalis*, *Silene vulgaris*, *Tanacetum balsamita*, *Taraxacum crepidiforme*, *Verbascum oreophilum.*

Avcılar (Erzincan):

Habitat data: hillside, dominant plant *Medicago sativa.*

Vegetation: *Achillea biebersteinii*, *Asperula orientalis*, *Centaurea solstitialis*, *Cerinthe minor*, *Cichorium intybus*, *Daucus carota*, *Digitaria sanguinalis*, *Echium italicum*, *Eryngium billardieri*, *E. campestre*, *Euphorbia stricta*, *Lactuca serriola*, *Medicago sativa*, *Melilotus alba*, *Poa bulbosa*, *Sanguisorba minor*, *Scabiosa argentea*, *Silene vulgaris*, *Xeranthemum annuum.*

Pöske Mountain (Erzincan):

Habitat data: roadside, oak area (red soil).

Vegetation: *Allium armenum*, *Anchusa leptophylla*, *Artemisia austriaca*, *Bromus japonicus*, *Bupleurum falcatum*, *Centaurea virgata*, *Chondrilla juncea*, *Cichorium intybus*, *Crepis armena*, *Cynanchum acutum*, *Echinops galaticus*, *Euphorbia petrophila*, *E. virgata*, *Festuca callieri*, *F. ovina*, *Galium verum*, *Gladiolus atroviolaceus*, *Helichrysum arenarium*, *Hypericum elongatum*, *Isatis* sp., *Koeleria cristata*, *Potentilla argentea*, *Salix pentandra*, *Salvia candidissima*, *S. sclarea*, *Sanguisorba minor*, *Scabiosa caucasica*, *Verbascum cheiranthifolium*, *V. oreophilum*, *Xeranthemum annuum.*

Kekiktepe (Eynesil, Giresun):

Habitat data: seaside, hazelnut garden on highest peak.

Vegetation: *Agrimonia eupatoria*, *Amaranthus retroflexus*, *Calystegia sepium*, *Chenopodium album*, *Cichorium intybus*, *Commelina communis*, *Conyza canadensis*, *Corylus avellana Daucus carota*, *Epilobium angustifolium*, *Hypericum perforatum*, *Leontodon hispidus*, *Paspalum dilatatum*, *Plantago lanceolata*, *P. major*, *Poa longifolia*, *Prunella vulgaris*, *Pteridium aquilinum*, *Rorippa sylvestris*, *Rubus discolor*, *Solanum nigrum*, *Sonchus oleraceus*, *Trifolium pratense*, *Urtica dioica*, *Vicia cracca*.

Yolağzı (Keşap, Giresun):

Habitat data: roadside, half shade, semi-wet, hazelnut garden.

Vegetation: *Arctium minus*, *Artemisia vulgaris*, *Conyza canadensis*, *Corylus avellana*, *Epilobium angustifolium*, *Erigeron acer*, *Euphorbia peplus*, *Holcus lanatus*, *Hypericum perforatum*, *Lactuca serriola*, *Lapsana communis*, *Nasturtium officinale*, *Plantago major*, *Polygonum persicaria*, *Pteridium aquilinum*, *Rubus hirtus*, *Rumex acetosella*, *Senecio nemorensis*, *Sorghum halepense*, *Stachys sylvatica*, *Telekia speciosa*, *Vicia cracca.*

Köycük (Kelkit, Gümüşhane):

Habitat data: roadside, garden areas.

Vegetation: *Allium atroviolaceum*, *Bupleurum rotundifolium*, *Cardaria draba*, *Centaurea solstitialis*, *Chenopodium vulvaria*, *Cirsium arvense*, *C. echinus*, *Convolvulus arvensis*, *C. galaticus*, *Coronilla varia*, *Dactylis glomerata*, *Daucus carota*, *Descurainia sophia*, *Echinops pungens*, *Echium vulgare*, *Eryngium billardieri*, *Euphorbia stricta*, *Falcaria vulgaris*, *Hypericum elongatum*, *Isatis* sp., *Lactuca serriola*, *Lapsana communis*, *Onopordum acanthium*, *Papaver tauricola*, *Plantago lanceolata*, *Salvia verticillata*, *Verbascum cheiranthifolium*, *Xanthium strumarium*, *Xeranthemum annuum.*

Çamlık (İkizdere, Rize):

Habitat data: mown and unmown pasture, dominant plant *Ulmus glabra.*

Vegetation: *Alchemilla sintenisii*, *Arctium minus*, *Campanula rapunculoides*, *Digitalis ferruginea*, *Geranium pyrenaicum*, *G. sanguineum*, *Lapsana communis*, *Leontodon hispidus*, *Mentha longifolia*, *Origanum vulgare*, *Pilosella hoppeana*, *Plantago lanceolata*, *P. major*, *Poa pratensis*, *Prunella vulgaris*, *Pteridium aquilinum*, *Ranunculus kotschyi*, *Rhinanthus angustifolius*, *Salvia verticillata*, *Sonchus oleraceus*, *Taraxacum crepidiforme*, *Trifolium pratense*, *T. repens*, *Ulmus glabra, Urtica dioica.*

Turnasuyu (Ordu):

Habitat data: roadside, mown pasture, shade, hazelnut garden.

Vegetation: *Agrimonia eupatoria*, *Alyssum repens*, *Anthemis tinctoria*, *Artemisia vulgaris*, *Cerastium glomeratum*, *Cichorium intybus*, *Conyza canadensis*, *Corylus avellana*, *Crepis vesicaria*, *Epilobium angustifolium*, *Euphorbia stricta*, *Geranium asphodeloides*, *Holcus lanatus*, *Hypericum perforatum*, *Juncus acutus*, *Lolium perenne*, *Plantago major*, *Poa pratense*, *Polygonum persicaria*, *Prunella vulgaris*, *Rorippa sylvestris*, *Sambucus ebulus*, *Senecio vulgaris*, *Trifolium pratense*, *Urtica dioica*, *Xanthium strumarium.*

Yomra (Trabzon):

Habitat data: roadside, 20 m above the road, shade, hazelnut garden with cut herbs.

Vegetation: *Agrimonia eupatoria, Calystegia sepium, Corylus avellana, Erigeron acer, Holcus lanatus, Medicago lupulina, Paspalum dilatatum, Poa pratensis, Pteridium aquilinum, Rubus discolor, Tanacetum macrophyllum, Trifolium pratense, Trifolium repens, Vicia cracca.*

Results

In the present paper 45 species from the subfamilies Brachycyrtinae, Campopleginae, Cryptinae and Ichneumoninae are listed. Of these, 20 species (marked in the text by an asterisk) are new records for Turkish fauna.

Subfamily Brachycyrtinae Viereck, 1919

Brachycyrtus Kriechbaumer, 1880

Brachycyrtus ornatus Kriechbaumer, 1880

Material examined: Erzurum: Gelinkaya (40° 01.741′ N, 40° 54.855′ E), 1803 m, 26.VII.2015, 1 ♀.

Distribution in Turkey: Karabük (Kolarov & Yurtcan, 2002).

Distribution in World: Holarctic and Neotropical region.

Associated plants: *Picea* sp., *Pinus virginiana.*

Remark: this species was collected on *Medicago sativa* while feeding.

Subfamily Campopleginae Förster, 1869

Alcima Förster, 1869

Alcima orbitale (Gravenhorst, 1829)

Material examined: Erzurum: Aşkale, Tepebaşı Valley (39° 51.741′ N, 40° 37.454′ E) (Figure 2), 2009 m, 23.VII.2015, 1 ♀.

Distribution in Turkey: Erzurum and Tunceli (Çoruh et al., 2014c; Kolarov et al., 2014c).

Distribution in World: Palaearctic region.

Associated plants: *Angelica sylvestris*, *Heracleum sphondylium*, *Peucedanum oreoselinum*.

Campoletis Förster 1869

Campoletis crassicornis (Tschek, 1871)

Material examined: Giresun: Eynesil, Kekiktepe (41° 02.723′ N, 39° 05.641′ E), 4 m, 25.VII.2015, 4 ♂♂, 2 ♀♀.

Distribution in Turkey: Adana and Burdur (Kolarov & Beyarslan, 1995; Çoruh et al., 2013).

Distribution in World: Iceland, Europe, Azerbaijan and Turkey.

Associated plants: *Peucedanum oreoselinum.*

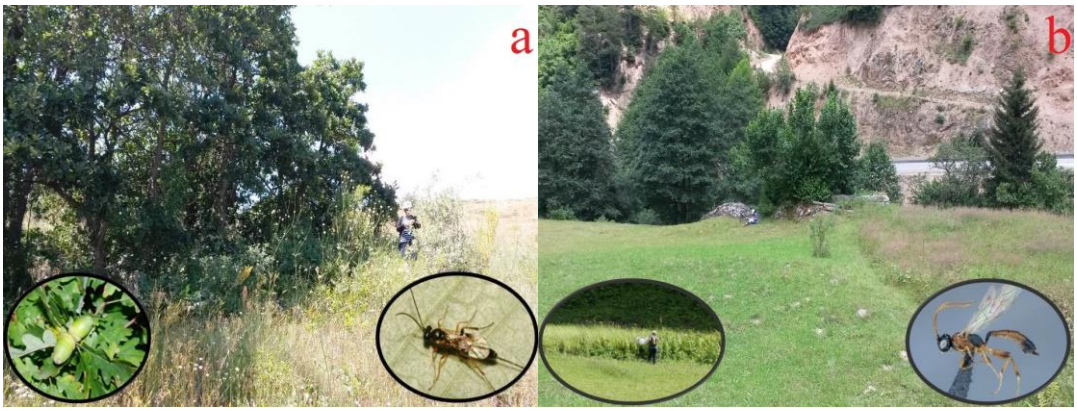

Figure 2. Same localities collect insects (a: Tepebaşı Valley; b:Çamlık).

**Campoletis excavata* (Smits van Burgst, 1914)

Material examined: Erzurum: Gelinkaya (40° 01.741′ N, 40° 54.855′ E), 1803 m, 26.VII.2015, 6 ♂♂.

Distribution in World: Europe and Azerbaijan.

Remark: this species was collected on *Medicago sativa* while feeding.

Campoletis latrator (Gravenhorst, 1829)

Material examined: Erzurum: Aşkale, Tepebaşı Valley (39° 51.741′ N, 40° 37.454′ E), 2009 m, 23.VII.2015, 4 ♀♀, 2 ♂♂, Gelinkaya (40° 01.741′ N, 40° 54.855′ E) 1803 m, 26.VII.2015, 8 ♀♀, 2 ♂♂; Giresun: Keşap, Yolağzı (40° 55.998′ N, 38° 34.578′ E), 1 m, 25.VII.2015, 1 ♂; Gümüşhane: Kelkit, Köycük (40° 08.584′ N, 39° 25.354′ E) 1393 m, 23.VII.2015, 1 ♂.

Distribution in Turkey: Adana, Edirne, Elazığ, Erzurum, Gaziantep, Isparta, Rize and Tunceli (Kolarov & Beyarslan, 1995; Çoruh et al., 2013; Kolarov et al., 2014c; Kolarov et al., 2016).

Distribution in World: Europe and Turkey.

Remark: this species was collected on *Medicago sativa* while feeding in Gelinkaya.

Casinaria Holmgren, 1859

Casinaria tenuiventris (Gravenhorst, 1829)

Material examined: Giresun: Eynesil, Kekiktepe (41° 02.723′ N, 39° 05.641′ E) 4 m, 25.VII.2015, 2 ♀♀.

Distribution in Turkey: Anatolia (Kolarov, 1995; Kolarov, 2008).

Distribution in World: Europe, Azerbaijan, Turkey, Israel, Egypt, Iran, Turkmenistan and China (Heilongjiang, Jilin and Liaoning).

Associated plants: *Chaerophyllum aromaticum*, *Daucus carota*, *Heracleum sphondylium*, *Peucedanum oreoselinum*, *Rubus fruticosus*.

Chromoplex Horstmann 1987

Chromoplex picticollis (Thomson, 1887)

Material examined: Gümüşhane: Kelkit, Köycük (40° 08.584′ N, 39° 25.354′ E), 1393 m, 23.VII.2015, 2 ♀♀.

Distribution in Turkey: Çanakkale, Trabzon, Isparta, Izmir, Muğla and Hatay (Kolarov et al., 1997; Özbek et al., 2000; Kolarov et al., 2002; Gürbüz, 2005; Çoruh et al., 2013).

Distribution in World: Europe, Turkey, Israel and Egypt.

Diadegma Förster, 1869

Diadegma mediterraneum (Constantineanu, 1930)

Material examined: Erzincan: Avcılar (39° 36.899′ N, 39° 49.328′ E), 1221 m, 23.VII.2015, 1 ♀.

Distribution in Turkey: Erzurum (Çoruh et al., 2005).

Distribution in World: Europe, Turkey and Jordan.

Remark: this species was collected on *Medicago sativa* while feeding.

Dusona Cameron, 1901

Dusona leptogaster (Holmgren, 1860)

Material examined: Rize: İkizdere, Çamlık (40° 42.979′ N, 40° 37.017′ E), 1099 m, 26.VII.2015, 1 ♂.

Distribution in Turkey: Anatolia (Horstmann, 2011).

Distribution in World: Palaearctic region.

Meloboris Holmgren, 1859

Meloboris collector (Thunberg, 1822)

Material examined: Gümüşhane: Kelkit, Köycük (40° 08.584′ N, 39° 25.354′ E) 1393 m, 23.VII.2015, 1 ♀; Rize: İkizdere, Çamlık (40° 42.979′ N, 40° 37.017′ E), 1099 m, 26.VII.2015, 1 ♀.

Distribution in Turkey: Adana, Ankara, Erzurum, Gaziantep, Hatay, Isparta and Trabzon (Kolarov, 1995; Kolarov & Beyarslan, 1995; Kolarov et al., 2014c; Kolarov et al., 2016).

Distribution in World: Iceland, Azores, Canary and Madeira islands, Europe, Azerbaijan, Turkey, Israel, Afghanistan, China (Qinghai) and South Africa.

Associated plants: *Angelica sylvestris*, *Peucedanum oreoselinum*, *Picea abies*.

Olesicampe Förster, 1869

**Olesicampe fulviventris* (Gmelin, 1790)

Material examined: Erzurum: Aşkale, Tepebaşı Valley (39° 51.741′ N, 40° 37.454′ E), 2009 m, 23.VII.2015, 1 ♂.

Distribution in World: Europe.

**Olesicampe proterva* (Brischke, 1880)

Material examined: Ordu: Turnasuyu (40° 58.572′ N, 37° 58.577′ E) 1 m, 24.VII.2015, 1 ♀.

Distribution in World: Europe.

**Olesicampe sternella* (Thomson, 1887)

Material examined: Erzurum: Aşkale, Tepebaşı Valley (39° 51.741′ N, 40° 37.454′ E), 2009 m, 23.VII.2015, 1 ♀.

Distribution in World: Europe.

Subfamily Cryptinae Kirby, 1837

Bathythrix Förster, 1869

**Bathythrix collaris* (Thomson, 1896)

Material examined: Rize: İkizdere, Çamlık (40° 42.979′ N, 40° 37.017′ E), 1099 m, 26.VII.2015, 4 ♂♂.

Distribution in World: Europe.

**Bathythrix fragilis* (Gravenhorst, 1829)

Material examined: Ordu: Turnasuyu (40° 58.572′ N, 37° 58.577′ E) 1 m, 24.VII.2015, 1 ♀.

Distribution in World: Europe and Azerbaijan.

Buathra Cameron, 1903

Buathra laborator (Thunberg, 1822)

Material examined: Erzurum: Gelinkaya (40° 01.741′ N, 40° 54.855′ E), 1803 m, 26.VII.2015, 1 ♀,1 ♂.

Distribution in Turkey: Burdur, Erzurum, Isparta and Trabzon (Gürbüz & Kolarov, 2008; Çoruh & Çoruh, 2012; Çoruh et al., 2014a; Kolarov et al., 2016; Çoruh & Çalmaşur, 2016).

Distribution in World: Holarctic region.

Associated plants: *Peucedanum oreoselinum*.

Remark: this species was collected on *Medicago sativa* while feeding.

Cryptus Fabricius, 1804

Cryptus viduatorius Fabricius, 1804

Material examined: Erzurum: Gelinkaya (40° 01.741′ N, 40° 54.855′ E), 1803 m, 26.VII.2015, 22 ♂♂.

Distribution in Turkey: Erzurum, Kırklareli, İçel, Isparta, Istanbul and Rize (Beyarslan & Kolarov, 1994; Kolarov, 1995; Çoruh & Çoruh, 2008; Çoruh et al., 2014a; Çoruh et al., 2014c; Özdan, 2014; Kolarov et al., 2016).

Distribution in World: Europe, Georgia, Azerbaijan, Cyprus, Turkey, Iran, Tajikistan and Siberia.

Associated plants: *Anethum graveolens*, *Angelica sylvestris*, *Daucus carota*, *Daucus carota* subsp. *sativus*, *Euphorbia* sp., *Euphorbia nicaeensis*, *E. virgata*, *Ferula communis*, *Heracleum sphondylium*, *Medicago sativa*, *Peucedanum oreoselinum.*

Remark: this species was collected on *Medicago sativa* while feeding.

Dichrogaster Doumerc, 1855

Dichrogaster longicaudata (Thomson, 1884)

Material examined: Erzurum: Kandilli, 6 km from Aşkale (39° 57′ N, 40° 52′ E), 1904 m, 22.VII.2015, 1 ♂.

Distribution in Turkey: Eskişehir and Isparta (Kolarov & Gürbüz, 2007; Kırtay, 2008; Eroğlu et al., 2011).

Distribution in World: Western Palaearctic and Nearctic region.

Associated plants: *Bauhinia* sp., *Oryza sativa*.

Remark: this species was collected on *Medicago sativa* while feeding.

Encrateola Strand, 1917

Encrateola laevigata (Ratzeburg, 1848)

Material examined. Erzurum: Gelinkaya (40° 01.741′ N, 40° 54.855′ E), 1803 m, 26.VII.2015, 1 ♀.

Distribution in Turkey: Anatolia, Adana, Giresun and Hatay (Beyarslan & Kolarov, 1994; Kolarov, 2008; Çoruh et al., 2014a).

Distribution in World: Europe, Turkey, Afghanistan, North America and South Africa.

Associated plants: *Angelica sylvestris*, *Cornus* sp., *Foeniculum vulgare*, *Prunus domestica*, *Vaccinium uliginosum.*

Remark: this species was collected on *Medicago sativa* while feeding.

Gelis Thunberg, 1827

Gelis agilis (Fabricius, 1775)

Material examined: Erzincan: Ahmetli (39° 53.481′ N, 39° 21.197′ E), 1988 m, 23.VII.2015, 1 ♂; Giresun: Eynesil, Kekiktepe (41° 02.723′ N, 39° 05.641′ E) 4 m, 25.VII.2015, 1 ♂.

Distribution in Turkey: Anatolia and Trabzon (Kolarov, 2008; Kolarov et al., 2016).

Distribution in World: Iceland, Europe, Azerbaijan and Turkey.

Associated plants: *Lonicera* sp., *Mentha longifolia*, *Picea abies*, *Prunus* sp., *Quercus robur*, *Salix* sp.

Ischnus Gravenhorst, 1829

Ischnus agitator (Olivier, 1792)

Material examined: Erzurum: Gelinkaya (40° 01.741′ N, 40° 54.855′ E), 1803 m, 26.VII.2015, 1 ♂.

Distribution in Turkey: Isparta (Gürbüz & Kolarov, 2008).

Distribution in World: Tunisia, Europe, Azerbaijan, Turkey and Iran.

Associated plants: *Daphne gnidium*, *Juniperus* sp., *Quercus ilex*.

Remark: this species was collected on *Medicago sativa* while feeding.

Myrmeleonostenus Uchida, 1936

Myrmeleonostenus italicus (Gravenhorst, 1829)

Material examined: Erzincan: Avcılar (39° 36.899′ N, 39° 49.328′ E), 1221 m, 23.VII.2015, 1 ♂.

Distribution in Turkey: Antalya, Isparta, Kırklareli, Tunceli and Zonguldak (Beyarslan & Kolarov, 1994; Kolarov, 1995; Gürbüz & Kolarov, 2008; Gürbüz et al., 2009a,b; Çoruh et al., 2014c; Kolarov et al., 2014c).

Distribution in World: Algeria, Tunisia, Europe, Azerbaijan, Turkey, Iran and Tajikistan.

Associated plants: *Fraxinus* sp.

Remark: this species was collected on *Medicago sativa* while feeding.

Nematopodius Gravenhorst, 1829

**Nematopodius formosus* Gravenhorst, 1829

Material examined: Rize: İkizdere, Çamlık (40° 42.979′ N, 40° 37.017′ E), 1099 m, 26.VII.2015, 1 ♀.

Distribution in World: Europe and Georgia.

Associated plants: *Alnus glutinosa, Picea* sp., *Pteridium aquilinum.*

Rhembobius Förster, 1869

Rhembobius perscrutator (Thunberg, 1822)

Material examined: Erzurum: Aşkale, Tepebaşı Valley (39° 51.741′ N, 40° 37.454′ E), 2019 m, 23.VII.2015, 1 ♀.

Distribution in World: Palaearctic region.

Associated plants: *Armoracia rusticana*, *Carpinus betulus*.

Thaumatogelis Schwarz, 1995

**Thaumatogelis femoralis* (Brischke, 1881)

Material examined: Erzurum: Gelinkaya (40° 01.741′ N, 40° 54.855′ E), 1803 m, 26.VII.2015, 1 ♂; Erzincan: Ahmetli (39° 53.481′ N, 39° 21.197′ E), 1988 m, 23.VII.2015, 1 ♂.

Distribution in World: Europe.

Remark: in Gelinkaya this species was collected on *Medicago sativa* while feeding.

Trychosis legator (Thunberg, 1822)

Material examined: Gümüşhane: Kelkit, Köycük (40° 08.584′ N, 39° 25.354′ E), 1393 m, 23.VII.2015, 1 ♂; Rize: İkizdere, Çamlık (40° 42.979′ N, 40° 37.017′ E) 1099 m, 26.VII.2015, 2 ♂♂.

Distribution in Turkey: Adana, Burdur, Edirne, Erzurum, Gaziantep, Isparta, Kırklareli, Tekirdağ, and Tunceli (Beyarslan & Kolarov, 1994; Gürbüz & Kolarov, 2008; Kolarov et al., 2014c).

Distribution in World: Palaearctic region.

Subfamily: Ichneumoninae Latreille,1802

Aethecerus Wesmael, 1844

Aethecerus nitidus Wesmael, 1845

Material examined: Erzurum: Gelinkaya (40° 01.741′ N, 40° 54.855′ E), 1803 m, 26.VII.2015, 1 ♀.

Distribution in Turkey: Anatolia (Kolarov, 1995).

Distribution in World: Europe, Turkey, Iran and Siberia.

Associated plants: *Anethum graveolens*, *Daucus carota* subsp. *sativus*, *Listera ovata*.

Remark: this species was collected on *Medicago sativa* while feeding.

Apaeleticus Wesmael, 1844

**Apaeleticus inimicus* (Gravenhorst, 1820)

Material examined: Erzurum: Gelinkaya (40° 01.741′ N, 40° 54.855′ E), 1803 m, 26.VII.2015, 1 ♀.

Distribution in World: Madeira islands and Europe.

Associated plants: *Angelica sylvestris*, *Chaerophyllum aromaticum*, *Daucus carota*, *Heracleum sphondylium*, *Medicago sativa*, *Peucedanum oreoselinum*, *Quercus ilex* subsp. *rotundifolia*.

Remark: this species was collected on *Medicago sativa* while feeding.

Centeterus Wesmael, 1844

Centeterus nigricornis Thomson, 1891

Material examined: Erzurum: Gelinkaya (40° 01.741′ N, 40° 54.855′ E), 1803 m, 26.VII.2015, 1 ♀, 1 ♂.

Distribution in Turkey: Nevşehir (Özdemir, 1996).

Distribution in World: Europe and Turkey.

Associated plants: *Angelica sylvestris*, *Rubus fruticosus*.

Remark: this species was collected on *Medicago sativa* while feeding.

Colpognathus Wesmael, 1844

Colpognathus celerator (Gravenhorst, 1807)

Material examined: Erzurum: Gelinkaya (40° 01.741′ N, 40° 54.855′ E), 1803 m, 26.VII.2015, 1 ♀; Giresun: Keşap, Yolağzı (40° 55.998′ N, 38° 34.578′ E), 1 m, 25.VII.2015, 1 ♀.

Distribution in Turkey: Giresun, Ordu and Trabzon (Kolarov et al., 2014b).

Distribution in World: Palaearctic region.

Associated plants: *Anthriscus sylvestris*, *Chaerophyllum aromaticum*, *Cornus mas*, *Corylus avellana*, *Daucus carota*, *Ferulago sylvatica*, *Fraxinus excelsior*, *Heracleum sphondylium*, *Oryza sativa*, *Peucedanum oreoselinum*, *Picea abies*.

Remark: in Gelinkaya this species was collected on *Medicago sativa* while feeding.

Phaeogenes Wesmael, 1845

**Dirophanes fulvitarsis* (Wesmael, 1845)

Material examined: Erzincan: Pöske Mt. (39° 51.160′ N, 39° 21.515′ E), 1838 m, 23.VII.2015, 1 ♂.

Distribution in World: Palaearctic region.

Associated plants: *Angelica sylvestris*, *Peucedanum oreoselinum*.

Dirophanes invisor (Thunberg, 1822)

Material examined: Erzincan: Ahmetli (39° 53.481′ N, 39° 21.197′ E), 1988 m, 23.VII.2015, 1 ♂.

Distribution in Turkey: Ankara (Özdemir, 1996).

Distribution in World: Algeria, Europe, Azerbaijan, Turkey and Iran.

Associated plants: *Dirophanes invisor.*

Diadromus Wesmael, 1845

Diadromus collaris (Gravenhorst, 1829)

Material examined: Rize: İkizdere, Çamlık (40° 42.979′ N, 40° 37.017′ E), 1099 m, 26.VII.2015, 2 ♂♂; Erzurum: Tepebaşı Valley, (39° 51.741′ N, 40° 37.454′ E), 2009 m, 23.VII.2015, 1 ♀.

Distribution in Turkey: Ankara, Erzurum, Kırşehir and Konya (Avcı & Özbek, 1990; Özdemir, 1996; Özbek et al., 2003).

Distribution in World: Palaearctic, Oriental, Australasian, Oceanic, Afrotropical and Neotropical regions.

Associated plants: *Anthriscus sylvestris*, *Chaerophyllum aromaticum*, *Chaerophyllum bulbosum*, *Daucus carota*, *Heracleum sphondylium*, *Oryza sativa*, *Peucedanum oreoselinum*.

Herpestomus Wesmael, 1845

Herpestomus brunnicornis (Gravenhorst, 1829)

Material examined: Erzincan: Avcılar (39° 36.899′ N, 39° 49.328′ E), 1221 m, 23.VII.2015, 1 ♂.

Distribution in Turkey: Ankara, Eskişehir, Nevşehir, Kayseri, Kırşehir and Sivas (Özdemir, 1996; Gencer, 2003).

Distribution in World: Palaearctic region, introduced into North America.

Associated plants: *Heracleum sphondylium*, *Pinus sylvestris*, *Rubus* sp., *Rubus fruticosus*.

Remark: this species was collected on *Medicago sativa* while feeding.

Heterischnus Wesmael, 1859

**Heterischnus excavatus* (Constantineanu, 1959)

Material examined: Erzurum: Aşkale, Tepebaşı Valley (39° 51.741′ N, 40° 37.454′ E), 2009 m, 23.VII.2015, 1 ♂.

Distribution in World: Europe.

Associated plants: *Angelica sylvestris*, *Laserpitium latifolium*.

Heterischnus truncator (Fabricius, 1798)

Material examined: Erzurum: Aşkale, Tepebaşı Valley (39° 51.741′ N, 40° 37.454′ E), 2009 m, 23.VII.2015, 1 ♀.

Distribution in Turkey: Erzurum, Giresun, İstanbul and Trabzon (Kolarov, 1995; Özbek et al., 2003; Çoruh et al., 2014c; Kolarov et al., 2014b).

Distribution in World: Europe, Azerbaijan, Turkey and Iran.

Associated plants: *Anethum graveolens*, *Daucus carota*, *Daucus carota* subsp. *sativus*, *Mentha* sp., *Oryza sativa*, *Rubus fruticosus*, *Rubus idaeus*, *Setaria glauca*.

Ichneumon Linnaeus, 1758

**Ichneumon deliratorius* (Linnaeus, 1758)

Material examined: Erzurum: Gelinkaya (40° 01.741′ N, 40° 54.855′ E), 1803 m, 26.VII.2015, 1 ♂. Distribution in World: Holarctic region.

Associated plants: *Anthriscus sylvestris*, *Chaerophyllum aromaticum, Daucus carota, Heracleum sphondylium*, *Laserpitium latifolium*, *Oryza sativa*, *Picia abies*, *Pteridium aquilinum*, *Quercus petraea*, *Rubus fruticosus*.

Nematomicrus Wesmael, 1845

**Nematomicrus tenellus* Wesmael, 1845

Material examined: Erzurum: Aşkale, Tepebaşı Valley (39° 51.741′ N, 40° 37.454′ E), 2009 m, 23.VII.2015, 1 ♂.

Distribution in World: Europe.

Oronotus Wesmael, 1844

**Oronotus binotatus* (Gravenhorst, 1829)

Material examined: Ordu: Turnasuyu (40° 58.572′ N, 37° 58.577′ E), 1 m, 24.VII.2015, 17 ♂♂; Trabzon: Yomra (40° 56.365′ N, 39° 52.131′ E), 20 m, 25.VII.2015, 2 ♂♂; Giresun: Keşap, Yolağzı (40° 55.998′ N, 38° 34.578′ E), 1 m, 25.VII.2015, 2 ♂♂.

Distribution in World: Europe and Georgia.

Phaeogenes Wesmael, 1845

**Phaeogenes curator* (Thunberg, 1822)

Material examined: Rize: İkizdere, Çamlık (40° 42.979′ N, 40° 37.017′ E), 1099 m, 26.VII.2015, 1 ♂.

Distribution in World: Europe and Mongolia.

Associated plants: *Heracleum sphondylium*.

**Phaeogenes heterogonus* Holmgren, 1890

Material examined: Erzurum: Aşkale, Tepebaşı Valley (39° 51.741′ N, 40° 37.454′ E), 2009 m, 23.VII.2015, 2 ♂♂.

Distribution in World: Europe, Azerbaijan and Iran.

**Phaeogenes semivulpinus* (Gravenhorst, 1829)

Material examined: Erzurum: Aşkale, Tepebaşı Valley (39° 51.741′ N, 40° 37.454′ E), 2009 m, 23.VII.2015, 1 ♂.

Distribution: Europe and Kazakhstan.

Stenodontus Berthomieu, 1896

**Stenodontus biguttatus* (Gravenhorst, 1829)

Material examined: Erzurum: Gelinkaya (40° 01.741′ N, 40° 54.855′ E), 1803 m, 26.VII.2015, 1 ♂.

Distribution in World: Algeria, Europe and Israel.

Associated plants: *Chaerophyllum aromaticum*, *Daucus carota* subsp. *sativus*, *Heracleum sphondylium*.

Tycherus Förster, 1869

**Tycherus impiger* (Wesmael, 1845)

Material examined: Erzincan: Pöske Mt. (39° 51.160′ N, 39° 21.515′ E), 1838 m, 23.VII.2015, 1 ♂.

Distribution in World: Europe.

Associated plants: *Alnus glutinosa*, *Frangula alnus*, *Symphytum officinale*.

Vulgichneumon Heinrich, 1962

Vulgichneumon saturatorius (Linnaeus, 1758)

Material examined: Giresun: Keşap, Yolağzı (40° 55.998′ N, 38° 34.578′ E), 1 m, 25.VII.2015, 1 ♂.

Distribution in Turkey: Ardahan, Erzurum, Giresun, Rize (Riedel et al., 2010; Çoruh et al., 2014c; Kolarov et al., 2014b).

Distribution in World: Palaearctic and Oriental region.

Associated plants: *Acer campestre*, *Angelica sylvestris*, *Anthriscus cerefolium, A. trichosperma, Chaerophyllum aromaticum, Daucus carota, Heracleum sphondylium, Listera ovata, Pinus mugo, Rubus idaeus.*

Zoogeographical characterization

The zoogeographical characterization follows mainly the chorotype classification of the Near East fauna proposed by Vigna Taglianti et al. (1999). After investigation of the recent geographic distribution of the species, listed above, they can be divided into the following groups:

1. Almost cosmopolitan distribution: *Diadromus collaris*.

2. Ranges in two zoogeographical regions: *Brachycyrtus ornatus*, distributed in Holarctic and Neotropical regions; *Meloboris collector* and *Encrateola laevigata*, distributed in Palaearctic/Holarctic and Afrotropical regions; *Vulgichneumon saturatorius*, distributed in Palaearctic and Oriental regions.

3. Holarctic distributions: *Buathra laborator*, *Dichrogaster longicaudata* and *Ichneumon deliratorius*.

4. With Palaearctic ranges: *Alcima orbitale*, *Dusona leptogaster*, *Rhembobius perscrutator*, *Trychosis legator*, *Colpognathus celerator*, *Dirophanes fulvitarsis* and *Herpestomus brunnicornis*.

5. Sibero-European distributions: *Cryptus viduatorius* and *Aethecerus nitidus*.

6. Asiatic-European range: *Casinaria tenuiventris*.

7. Central-Asiatic-European distributions: *Phaeogenes curator* and *Ph. semivulpinus*.

8. Turano-Europeo-Mediterranean: *Ischnus agitator*, *Dirophanes invisor* and *Myrmeleonostenus italicus*.

9. Turano-European distributions: *Heterishnus truncator.*

10. Europeo-Mediterranean ranges: *Chromoplex picticollis*, *Diadegma mediterraneum* and *Stenodontus biguttatus*.

11. Most numerous are the species with European distributions, namely *Campoletis crassicornis*, *C. excavata*, *C. latrator*, *Olesicampe fulviventris*, *O. proterva*, *O. sternella*, *Bathythrix collaris*, *B. fragilis*, *Gelis agilis*, *Nematopodius formosus*, *Thaumatogelis femoralis*, *Apaeleticus inimicus*, *Centeterus nigricornis*, *Heterishnus excavatus*, *Nematomicrus tenellus*, *Oronotus binotatus* and *Tycherus impiger*.

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Original article (Orijinal araştırma)

Caterpillar (Lepidoptera) communities on oak (*Quercus pubescens*) in Ankara Province (Turkey)[1]

Ankara ilinde (Türkiye) meşe üstündeki (*Quercus pubescens*) tırtıl (Lepidoptera) kommuniteleri

Özge TORUN[2] **Selma Seven ÇALIŞKAN[2]***

Summary

Lepidopteran larval communities feeding on *Quercus pubescens* (Willdenow) in Turkey were examined. A total of 190 larval specimens were collected from trees within Ankara Province between the months April and September in both 2013 and 2014 and were further fed under laboratory conditions. Twenty-five taxa belonging to 14 families were identified as follows; seven Geometridae, four Noctuidae, two Tortricidae, two Thyatiridae and one species for each of the Gelechiidae, Arctiidae, Gracillariidae, Lasiocampidae, Lymantriidae, Oecophoridae, Pyralidae, Yponomeutidae, Pterophoridae and Lycaenidae families. Four species are new records for Ankara Province and two species are new for the fauna of Turkey. The Geometridae family was the most represented family feeding on oak trees. *Tortrix viridana* (L., 1758) (Tortricidae) was the most abundant species with 37 individuals. Feeding activity of both *Eupithecia dodoneata* Guenée, 1858 and *Cosmia trapezina* L., 1758 at the larval stage on *Q. pubescens* is a new finding.

Key words: Larva, Lepidoptera, *Quercus*, new record, Turkey

Özet

Çalışmada *Quercus pubescens* (Willdenow, 1796)'te beslenen lepidoptera takımına ait larva kommuniteleri incelenmiştir. Ankara il sınırları içinde bulunan meşe ağaçları üstünden 190 larva örneği 2013 ve 2014 yılları Nisan-Eylül ayları arasında toplanmış ve laboratuvar ortamında beslenmiştir. 14 familyaya ait 25 taksa şu şekilde teşhis edilmiştir: Geometridae 7, Noctuidae 4, Tortricidae ve Thyatiridae 2, Gelechiidae, Arctiidae, Gracillariidae, Lasiocampidae, Lymantriidae, Oecophoridae, Pyralidae, Yponomeutidae, Pterophoridae ve Lycaenidae'den ise 1'er tür olmak üzere toplam 25 taksa tespit edilmiştir. 4 tür Ankara, 2 tür ise Türkiye faunası için yeni kayıttır. Geometridae familyası tür sayısı ile meşede beslenen en kalabalık familya olarak belirlenmiştir. Familyalara ait birey sayıları değerlendirildiğinde ise Tortricidae familyasında yer alan *Tortrix viridana* (L., 1758) 37 birey ile ilk sırada yer alır. *Eupithecia dodoneata* Guenée, 1858 ve *Cosmia trapezina* L., 1758 larvalarının *Q. pubescens* ile beslenmesi yeni bir bulgudur.

Anahtar sözcükler: Larva, Lepidoptera, *Quercus*, yeni kayıt, Türkiye

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Introduction

Food plants for butterfly larvae are important in the biology of the species since spatial and temporal distribution, abundance and sometimes the color pattern of adults are directly dependent on food plants. Thus, one of the keys to understanding the biology of butterflies is the precise identification of larval food plants (Oakley et al., 1969).

Oaks belong to the woody plants that host the richest insect assemblages in Central Europe (Patočka et al., 1999). Lepidoptera larvae have been shown to be the most important group of oak defoliators (Patočka et al., 1962, 1999; Milka & Medarevic, 2010). About 308 Lepidoptera species are known to damage the leaves of oaks in Europe (Patočka, 1954, 1980; Patočka et al., 1999; Reiprich, 2001; Csóka & Szabóky, 2005). Lepidoptera fauna of some oak species in Central Europe have been adequately studied (Patočka et al., 1962, 1999; Csóka, 1991, 1998; Kulfan, 1992, 1997, 2002; Kulfan et al., 1997, 2006; Kulfan & Degma, 1999; Harapin & Jurc, 2000; Turčáni et al., 2009; Kalapanida & Petrakis, 2012; Parák et al., 2012). In Bulgaria, Zlatanov (1971) discovered 67 lepidopteran species feeding on *Quercus robur* L., 49 species feeding on *Quercus cerris* L. and 29 species feeding on *Quercus rubra* L. In a study of forest trees in Israel, 236 Lepidoptera species were determined and *Quercus* was established as the most preferred food plant for 91 Lepidoptera species (Halperin & Sauter, 1992).

Studies focusing on Lepidoptera that damage oak forests are limited in Turkey (Kansu, 1963; Baş, 1980; Avcı, 1997; Çanakçıoğlu & Mol, 1998). In studies conducted by Şimşek & Özdemir (2000) and Şimşek (2002), Lepidoptera species in mixed forest areas in Çankırı were studied and six Lepidoptera species were shown to be damaging to oak. A study on damaging species of oak in Kahramanmaraş indicated that seven out of 20 harmful insects belonged to Lepidoptera (Kanat & Akbulut, 2005). Kemal & Koçak (2008) recorded *Orthosia rubricosa* (Esper) in an oak woodland.

The main aim of this paper is to investigate the structure of communities of lepidopteran larvae on oak, *Quercus pubescens* (Willdenow), in Ankara province.

Material and Methods

Field studies were conducted in oak forests in Şereflikoçhisar, Kızılcahamam, Güdül, Kazan, Beypazarı counties and localities with oak trees in Etimesgut, Yenimahalle, Keçiören counties in Ankara Province, between the months April and September in both 2013 and 2014. Larva specimens were collected from trees with clamps or by hand together with plant samples. A shaking method was also used (Kıyak, 2000). Specimens were fed with nutritional plants to obtain pupa and imago stages under laboratory conditions. Larva and pupal stage samples were photographed with a Leica Z16 APO microscope and imago samples were photographed with a Canon EOS 550D camera. Larva, pupa and imago photos of species are given in Figures 1 to 3. Dispersion data for the species was based on Koçak & Kemal (2009).

Larvae identification was based on the keys of Blaschke (1914), Meyer (1919), Gerasimov (1952), Patočka (1954, 1980), Koch (1984), Treadwell (1996), Patočka et al. (1999), Beck (2002) and Turčáni et al. (2009).

Results

In this study, 56 imago specimens were obtained from 190 larvae collected when feeding on *Q. pubescens*. Twenty-five taxa from 14 families were determined (Table 1). *Anacampsis timidella* (Wocke, 1887), *Eupithecia dodoneata* Guenée, 1858, *Operophtera brumata* (L., 1758) and *Conobathra tumidana* (Denis & Schiffermüller, 1775) are new records for the Ankara and *Diurnea lipsiella* (Denis & Schiffermüller, 1775) and *Polyploca ridens* (Fabricius, 1787) are new records for the fauna of Turkey.

Figure 1. Larval stages of the Lepidoptera species feeding on *Quercus pubescens* in the Ankara Province, Turkey.

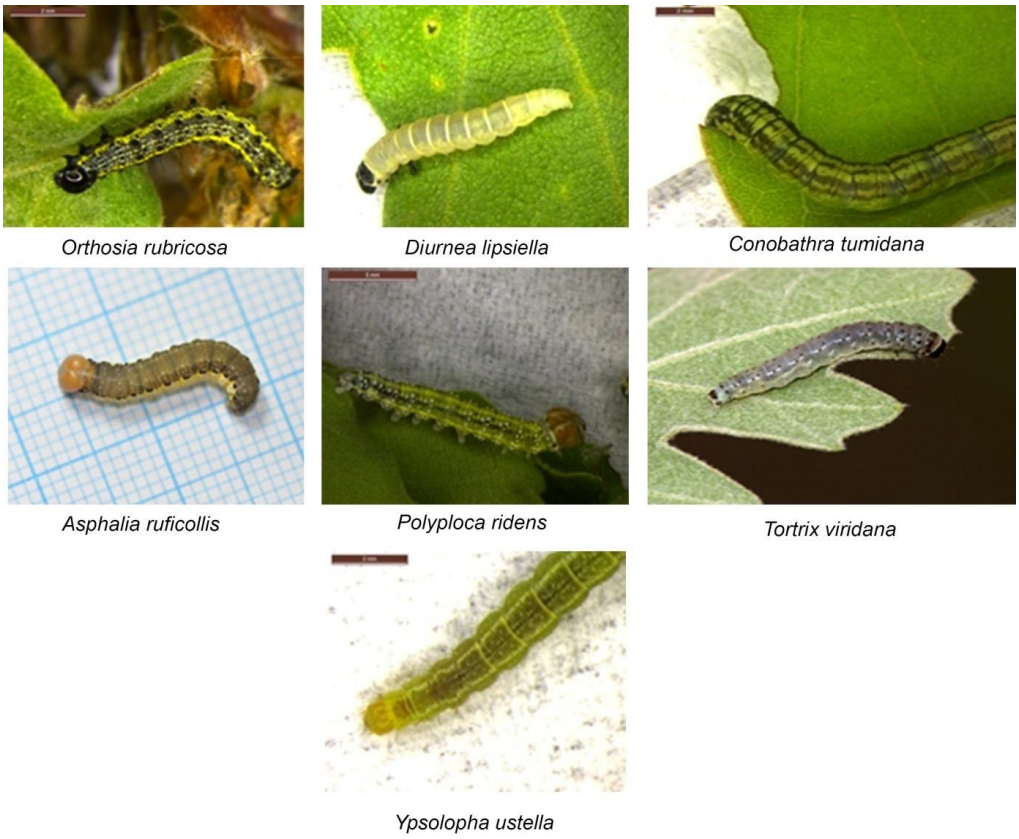

Figure 1 (continue). Larval stages of the Lepidoptera species feeding on *Quercus pubescens* in the Ankara Province, Turkey.

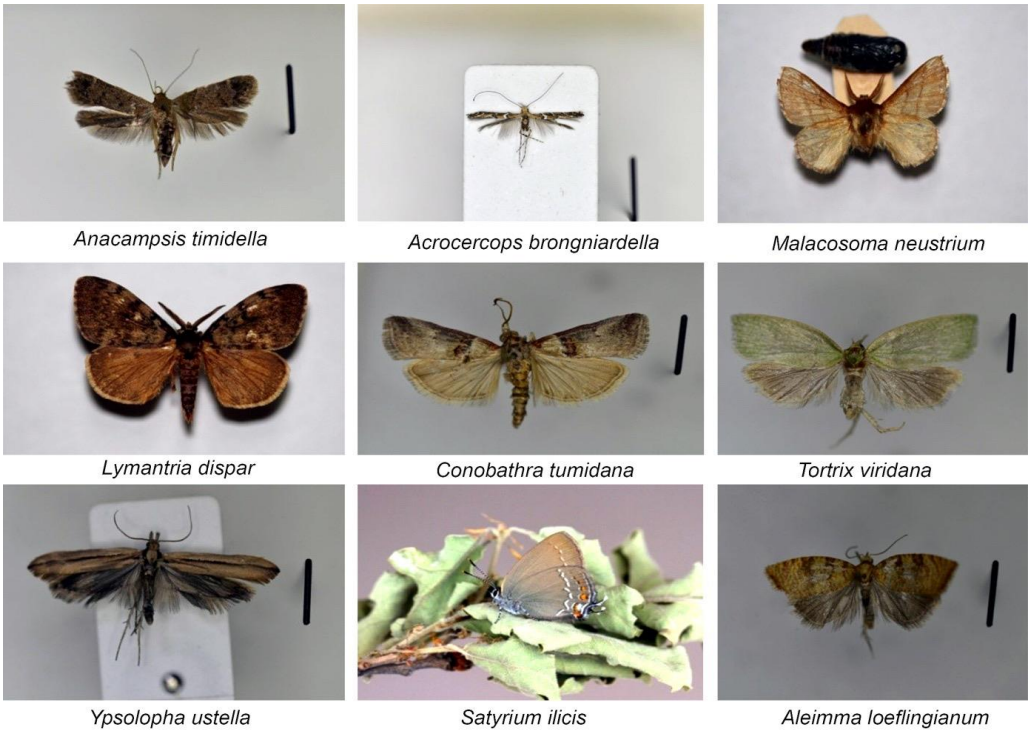

Figure 2. Imagos of the Lepidoptera species collected on *Quercus pubescens* in the Ankara Province, Turkey.

Figure 3. Pupal stages of the Lepidoptera species collected on *Quercus pubescens* in the Ankara Province, Turkey.

Table 1. Caterpillar (Lepidoptera) communities collected on *Quercus pubescens* in the Ankara Province, Turkey. PSD: pupa starting date, AED: adolescence ending dates

Family	Taxa	Larvae	Images	Location and date of sampling	PSD	AED
Lycaenidae	*Satyrium ilicis* (Esper, 1779)	1	1	Güdül, 01.05.2014	07.05.2014	18.05.2014
Arctiidae	*Eilema complana* (L., 1758)	1	-	Kızılcahamam, Kargasekmez, 01.06.2013	-	-
Gelechiidae	*Anacampsis timidella* (Wocke, 1887)	5	3	Beypazarı, 03.05.2014	11-14.05.2014	24-25.05.2014
Geometridae	*Alsophila hirsutaria* (Fab., 1781)	5	-	Kızılcahamam, Kurtboğazı, 07.05.2013	12-14.05.2013	-
	Apocheima hispidarium (Denis & Schiffermüller, 1775)	1	-	Şereflikoçhisar, 01.05.2013	20.05.2013	-
	Colotois pennaria (L., 1761)	1	-	Kızılcahamam- Güdül, 07.05.2014	29.05.2014	-
	Erannis defoliaria (L., 1761)	1	-	Kızılcahamam- Güdül, 07.05.2014	20.05.2014	-
	Eupithecia dodoneata Guenée, 1858	3	-	Kurtboğazı, 07.05.2013, Beypazarı, 03.05.2014, Kızılcahamam-Güdül, 08.05.2014	-	-
	Operophtera brumata (L., 1758)	17	-	Beypazarı, 03.05.2014, Kızılcahamam-Güdül, 07.05.2014, Güdül,01.05.2014, Kazan,04.05.2014	10-15.05.2014	-
	Phigalia pedaria (Fab., 1787)	1	-	Şereflikoçhisar, 01.05.2013	-	-
Gracillariidae	*Acrocercops brongniardella* (Fab., 1798)	3	1	Beypazarı, 03.05.2014	13.05.2014	27.05.2014
Lasiocampidae	*Malacosoma neustrium* (L., 1758)	4	3	Kızılcahamam, Soğuksu, 01.06.2013	15-16.06.2013	26-27.06.2013
Lymantriidae	*Lymantria dispar* (L., 1758)	13	6	Kızılcahamam, Kargasekmez, 01.06.2013, Beypazarı, 03.05.2014, Kızılcahamam- Güdül, 07.05.2014	09-11.06.2014	20-21.06.2014
Noctuidae	*Conistra* sp.	2	-	Beypazarı, 03.05.2014	-	-
	Cosmia trapezina (L., 1758)	1	-	Kızılcahamam, Kurtboğazı, 07.05.2013	-	-
	Orthosia pulverulenta (Esper, 1786)	7	1	Kızılcahamam, Kurtboğazı, 07.05.2013	16- 17.05.2013	15.02.2014
	Orthosia rubricosa (Esper, 1786)	20	-	Kızılcahamam- Güdül, 08.05.2014	-	-

Table 1. (continued)

Family	Taxa	Larvae	Images	Location and date of sampling	PSD	AED
Oecophoridae	*Diurnea lipsiella* (Denis & Schiffermüller, 1775)	2	-	Güdül, 01.05.2014	-	-
Pterophoridae	*Agdistis* sp.	1	1	Kızılcahamam, Kurtboğazı, 1♂, 07.05.2013	-	-
Pyralidae	*Conobathra tumidana* (Denis & Schiffermüller, 1775)	13	11	Kazan, 03.05.2014	13-14.05.2014	07-12.06.2014
Thyatiridae	*Asphalia ruficollis* (Fab., 1787)	6	-	Kızılcahamam, Kurtboğazı, 07.05.2013	11.05.2013	
	Polyploca ridens (Fab., 1787)	2	2	Beypazarı, 03.05.2014	24.05.2014	20.04.2015
Tortricidae	*Aleimma loeflingianum* (L., 1758)	4	2	Kızılcahamam, Kurtboğazı, 07.05.2013, Beypazarı, 03.05.2014	09.05.2014	16.05.2014
	Tortrix viridana (L., 1758)	37	20	Şereflikoçhisar, 01.05.2013, Kızılcahamam, Kurtboğazı, 07.05.2013, Güdül, 01-08.05.2014, Beypazarı, 03.05.2014, Kazan, 04.05.2014.	12-15.05.2014	20-25.05.2014
Yponomeutidae	*Ypsolopha ustella* (Clerck, 1759)	8	7	Beypazarı, 03.05.2014	05-15.05.2014	20-30.05.2014

Geometridae was found to be the most represented family feeding on oak trees with seven species (29% of the total number of species collected) (Figure 4). Depending on the population density of this family, it is considered to be a potentially harmful for *Q. pubescens*.

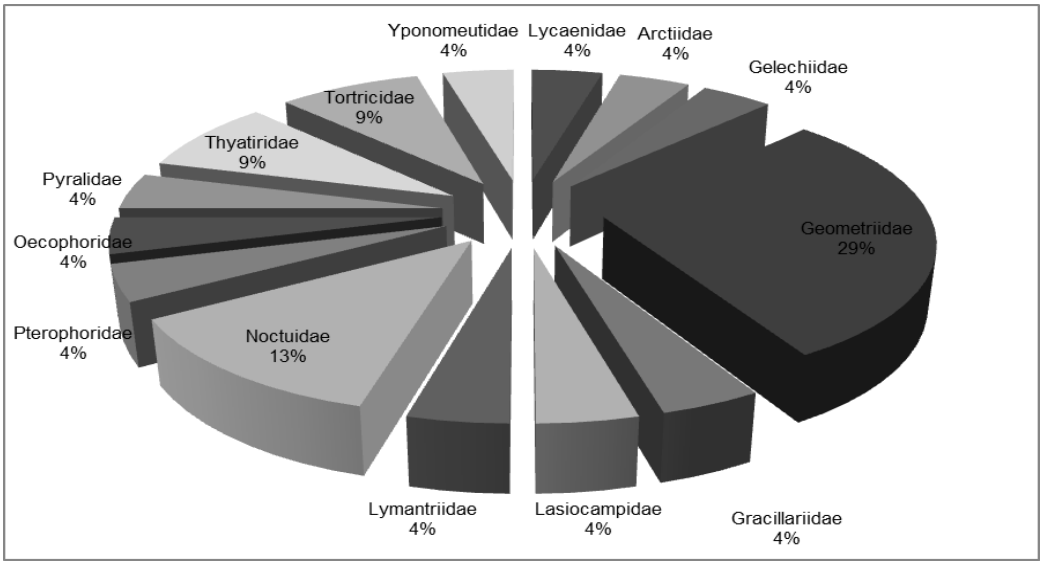

Figure 4. Relative proportions of the Lepidoptera families identified in caterpillar communities feeding on *Quercus pubescens*, in the Ankara Province, Turkey.

Discussion

Ovcharov et al. (2000), studied the preferences of some important oak-damaging Lepidoptera species and determined that *Q. cerris* was more durable than *Quercus petraea* (Mattuschka) Liebl., which was mostly damaged by Geometrids, and than *Quersus frainetto* Ten., which was damaged by Tortricids. Our results were consistent with these findings, as we found that Geometrids were the most diverse group on *Q. pubescens* in Ankara Province. We also found that the Noctuidae family had the second highest prevalence with four species (13% of the total number of species collected), which was consistent with the findings of Kulfan et al. (2013) for *Quercus dalechampii* Ten.

According to Patočka et al. (1999), *O. brumata* and *Tortrix viridana* L. are adapted to xerothermic habitats and prefer *Q. cerris* over other oak species. Consistent with the findings of Parák et al. (2012), our study confirmed that these two species had the highest larval densities.

Eupithecia dodoneata Guenée, 1858 has commonly been reported to feed on *Q. robur* and *Quercus ilex* L. during the larval stage (Petersen, 1909; Allan, 1949; Kulfan, 1997), as well as *C. trapezina* on different oak species (Csóka, 1991; Kulfan, 1997; Kulfan et al., 2006). Our study provides the first report of larval herbivory by these two species on *Q. pubescens.* Parák et al. (2012) reported collection of *D. lipsiella* larvae from *Q. pubescens*. Larvae of this species were also discovered on oak in our study, which represents a new record for the fauna of Turkey.

In Turkey, *Acrocercops brongniardella* (Fabricius, 1798), is only known from Ankara and Bursa, and was obtained from galleries in oak leaves (Koçak & Kemal, 2009). In a study conducted in Croatia, this species was discovered on oak seedlings and found to affect photosynthesis of oak leaves, when it occurred in high density (Matošević et al., 2008). In this study, they were found on *Q. pubescens* and observed with low population density.

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Original article (Orijinal araştırma)

Insecticidal activity of weed plants, *Euphorbia prostrata* and *Chenopodiastrum murale* against stored grain insect pest *Trogoderma granarium* Everts, 1898 (Coleoptera: Dermestidae)

Yabani bitkiler *Euphorbia prostrata* ve *Chenopodiastrum murale*'nin depolanmış tahıl zararlısı *Trogoderma granarium* Everts, 1898 (Coleoptera: Dermestidae)'a karşı insektisidal aktivitesi

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Summary

The efficiency of petroleum ether extracts of weed plants, *Euphorbia prostrata* and *Chenopodiastrum murale,* for the control of *Trogoderma granarium* was investigated. The extracts were prepared in petroleum ether using the whole plants. Diet incorporation was used for mortality bioassay and area preference was used for repellency against third instar larvae of *T*. *granarium*. The results showed relatively high rate of larval mortality after 6 days with extract concentrations of 10, 20 and 30%. At 30% the corresponding mortality rates induced by *E. prostrata* and *C. murale* were 20 and 25%, respectively. Low larval mortalities were obtained for both plant extracts at 10%. Similarly, repellency assay at 30% extracts found the maximum proportion of larvae that moved away from the treated region of the filter paper to be 88 and 87% for *E. prostrata* and *C. murale* extracts, respectively. The repellency of both plant extracts had a positive relationship applied dosage but was negatively correlated with exposure time. The lowest mean number of larvae in F_1 generation was found with 30% *E. prostrata* and *C. murale* extracts (60 and 53, respectively) as compared to the control (149). Overall the results indicated that the *C. murale* extract had a higher insecticidal activity against *T*. *granarium* than the *E. prostrata* extract.

Keywords: Mortality, progeny reduction, repellency, *Trogoderma granarium*, weed plants

Özet

Trogoderma granarium'un mücadelesinde, *Euphorbia prostrata* ve *Chenopodium murale* yabani bitkilerinin petrol eteri ekstraktlarının verimliliği araştırılmıştır. Ekstraktlar tüm bitki kullanılarak, petrol eteri içinde hazırlanmıştır. *rogoderma granarium*'un üçüncü dönem larvalarında, ölüm denemeleri için diyet karışımı ve kaçırıcı etki için alan tercihi kullanılmıştır. Sonuçlar, %10, 20 ve 30 ekstrakt konsantrasyonlarında 6 gün sonra nispeten yüksek larva ölüm oranı göstermiştir. %30 konsantrasyona karşılık gelen ölüm oranları *E. prostrata* ve *C murale* için sırasıyla, %20 ve 25 olmuştur. Düşük larva ölümleri her iki bitkinin %10'luk ekstraktında elde edilmiştir. Benzer şekilde,%30 ekstraktıyla yapılan kaçırıcı etki denemelerinde filtre kağıdının uygulama yapılmış bölgesinden daha uzağa hareket eden larvaların maksimum oranı, *E. prostrata* ve *C. murale* için sırasıyla %88 ve 87 olarak bulunmuştur. Her iki bitki ekstraktının kaçırıcı etkisinde uygulanma dozlarında pozitif bir ilişki varken maruz kalma süresi için olumsuz korelasyon bulunmuştur. F1 neslinde en düşük ortalama larva sayısı kontrol (149) ile karşılaştırıldığında *E. prostrata* ve *C. murale* için (sırasıyla 60 ve 53) %30 konsantrasyonda bulunmuştur. Tüm sonuçlar, *C. murale* ekstraktının *T. granarium*'a karşı *E. prostrata* ekstraktından daha yüksek bir insektisidal aktiviteye sahip olduğunu göstermiştir.

Anahtar sözcükler: Ölüm, döl azalma, kaçırıcı etki, *Trogoderma granarium,* yabani bitkiler

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Introduction

The khapra beetle, *Trogoderma granarium* Everts, 1898 (Coleoptera: Dermestidae) is the most serious storage pest of dried plant and animal matter, especially wheat (Hinton, 1945; Szito, 2006). It reduces the quantity, as well as the quality, of stored grain. Severe infestation may cause 5 to 30% weight loss which may increase to 70%, if the beetle is left undisturbed (USDA-APHIS-PPQ, 1983). *Trogoderma granarium* seriously deteriorates the nutritional quality of stored wheat. Infested grain suffers from reduction in proteins, gluten, crude fat, ash, starch, reducing and non-reducing sugars, and mineral components (Girish et al., 1975; Jood et al., 1992; Mason, 2002). Heavily infested grain contains the barbed hairs of larvae, which cause dermal and gastric problems in people handling such grain (Morison, 1925; Pruthi & Singh, 1950; Mason, 2002; Stibick, 2007). Consumption of infested grain can cause vomiting, diarrhea and food refusal especially in the young children (Anonymous, 2001).

The management of stored insect pests mostly relies on the use of synthetic insecticides such as deltamethrin, permethrin, pirimiphos-methyl, chlorpyrifos-methyl and fumigation with methyl bromide or phosphine (White & Leesch, 1995). Such treatments are simple and inexpensive, and even a single application of fumigation can control insect infestation for a substantial period (Daglish & Bengston, 1991; Alsarar et al., 2014). However, widespread use of these chemicals can lead to development of insect resistance (Waiss et al., 1981; Zettler & Cuperus, 1990; White, 1995; Tapondjou et al., 2002), health and environment hazards (Taylor, 1994; Prakash & Rao, 2006; Rahman et al., 2009), toxicity to non-target organisms, and pesticide residues inducing mutagenic and carcinogenic effects on human health (Lee et al., 2004; Isman, 2006). Also, methyl bromide is now banned due to its ozone depleting ability (Taylor, 1994).

Recently, there has been growing interest in the use of botanicals which contain chemicals produced by plants that are repellent, feeding deterrents and disrupting to insect behavior and physiology, and toxic to a number of stored grain insect pests (Hiremath et al., 1997; Verma & Dubey, 1999; Isman et al., 2001; Wheeler & Isman, 2001; Isikber et al., 2006; Isman, 2006; Moreira et al., 2007; Srinivasan, 2008). Plants are a rich source of bioactive chemicals. Both primary as well as secondary plant metabolites can be evaluated against the target pests (Salunke et al., 2009), have insecticidal activity (Dev & Koul, 1997) and are used throughout the world due to their environment friendly nature (Belmain et al., 2001).

During the last few years, weeds are being increasingly investigated for their phytochemical, pharmological and biological properties (Naqvi & Parveen, 1991; Ahmad et al., 2003 a, b). Weeds are generally considered as unwanted plants and crop pests; however, they have shown insecticidal properties for many insects (Sagheer et al., 2013; Alkan et al., 2015; Vázquez-Covarrubias et al., 2015). Some weeds are poisonous (Shamsuddin, 2001); for example, *Euphorbia prostrate* Aiton (Euphorbiaceae) is a prostrate annual herb found all over India (Singla & Pathak, 1989; Chen et al., 1992), which is used for the treatment of bleeding hemorrhoids, chronic fevers and abdominal diseases as a nerve tonic and blood purifier (Qureshi et al., 2009). It is also used as an antidote for bites of venomous insects (such as wasps and scorpions) and to fight against infertility and painful menstruation to avoid the miscarriage (Schmelzer & Gurib-Fakim, 2008). Likewise, *Chenopodiastrum murale* (L.) S. Fuentes, Uotila & Borsch (Amaranthaceae) is an annual, widespread herbaceous noxious weed about 20-70 cm long, found in more than 43 countries (Zohary, 1966). Nanoparticles synthesized from *C. murale* have been reported to have antioxidant and antibacterial activities (Abdel-Aziz et al., 2014). Moreover, chemical constituents extracted from *C. murale* including essential oils, flavonoids, sterols, alkaloids and coumarins shown antibacterial, antifungal, phytotoxic and insecticidal activities (Naqvi & Parveen, 1991; Ahmad et al., 2003a).

According to the literature, very little work has been carried out to investigate the insecticidal potential of these weed plants on the stored grain insect pests (Moreira et al., 2007). Thus, the present study was designed to evaluate the insecticidal and repellent potential of two weed plants; *E. prostrata* and *C. murale* at different concentrations and exposure intervals for control of stored grain insect pest, *T. granarium*.

Materials and Methods

Bioassays were performed in the Entomology Laboratory, Government College University, Faisalabad to investigate the insecticidal effects of the selected weed plants against larvae of *T. granarium*.

Mass rearing of *Trogoderma granarium*

Trogoderma granarium were reared on healthy wheat grain apparently free from insect infestation in sterilized plastic jars (1 kg capacity) under optimum conditions of temperature and relative humidity, 30±2ºC and 65±5%, respectively. Whole common wheat (*Triticum aestivum* L., cv. Nela, 14% moisture), was used as the culture media. The larvae were sieved through a 2.0 mm aperture seive. The larvae were counted with the aid of magnifying lens. One hundred beetles were released into labeled 500 ml glass jars having 200 g of sterilized whole wheat and covered with muslin to insect prevent entry or escape. Adults were allowed to mate and lay eggs with the incubator. Homogeneous population was achieved after a time period of 28-35 days. Third instar larvae were then used for the assays (Sagheer et al., 2013).

Preparation of plant extracts

Euphorbia prostrata and *C. murale* were collected from the vicinity of Faisalabad and identified by the Department of Botany, Government College University Faisalabad. Whole plants were cleaned by washing in water and then dried in the shade (Alkan et al., 2015). A grinder was used to crush the plant material into fine powder. The extraction was made by mixing 100 g of ground sieved sample and 300 ml of petroleum ether (40-60%) in the ratio of 1:3 (w/v) and shaking for 24 h using a rotary shaker at 220 rpm. After 24 h, the extract was filtered through Whatman No. 1 filter paper. After filtration, the extracts were stored in clean and airtight bottles at 4ºC until used. Concentrations of 10, 20 and 30% (v/v) were prepared using petroleum ether from the stock solutions of each plant (Sagheer et al., 2013; Alkan et al., 2015).

Mortality bioassay (diet incorporation method)

A bioassay was performed to observe the toxic effect of the plant extracts on the larvae of *T. granarium*. The three extract concentrations in petroleum ether were applied on 50 g wheat (5 ml crude plant extract on 50 g wheat @ 0.1 ml/g). For the control, the wheat was only treated with petroleum ether, which was air dried to evaporate the petroleum ether and then poured into 250 ml sterilized plastic jars. Thirty larvae were released in each jar and the jar covered with muslin secured with a rubber band. These jars were placed in incubator at 30±2ºC and 65± 5% RH (Moreira et al., 2007). Each treatment was replicated three times in a completely randomized design. The insects were confirmed dead when there was no response to probing the abdomen with sharp pin. Percentage mortality of the larvae was recorded 2, 4 and 6 days after treatment. Mortality in controls was used to correct the mortality according to Abbot's formula (Abbot, 1925).

Repellency bioassay

In another bioassay, the repellent effect of the plant extracts was checked against *T. granarium* larvae by using a modification of the area preference method described by McDonald et al. (1970). For repellency test, 80 mm diameter Whatman No. 1 filter paper was cut into two equal halves. The three extract concentrations in petroleum ether were applied separately to one half of the filter paper placed in a Petri dish (100 x 15 mm) using 10 µl micropipette and petroleum ether alone was applied to the other half (10 µl/cm^2 on treated area). After air-drying for 10 min, each treated half of the filter paper was attached lengthwise to untreated half using adhesive tape and placed in a Petri dish. Twenty third-instar larvae of *T. granarium* were released separately at the center of both halves in each petri dish. Petri dishes were covered with a lid to prevent the escape of test insects and kept under controlled conditions (30±2ºC and 65±5% RH). Each treatment was replicated three times and counts of the larvae on each filter paper disk were made after 24, 48 and 72 h. Wheat grain (0.5 g) were also provided on both sides in order to avoid the mortality due to starvation.

Percent repellency (RP) was calculated by using the following formula:

$$PR= [(NC-NT) / (NC+NT)] \times 100$$

Where, NC= number of larvae present on control half and NT= number of larvae present on treated half.

Growth regulatory effect of plant extracts on the larvae of *Trogoderma granarium*

The larval emergence and inhibition of *T. granarium* in F_1 generation were recorded in order to investigate the growth regulatory effect of the plant extracts on *T. granarium*. The wheat grains were sterilized and the three extract concentrations in petroleum ether were applied by spraying and mixing

(0.1 ml/g) on wheat grain including a control treatment. The solvent was allowed to evaporate and 50 g treated grain was placed separate plastic jars (250 ml). Thirty third-instars were released into each jar and then incubated under optimum conditions (30±2ºC and 65±5% RH). The mean emergence and percent inhibition of F_1 larvae was recorded after 35 days (Sagheer et al*.,* 2013; Alkan et al., 2015).

Statistical analysis

The data of corrected mortality, repellency and growth regulation was subjected to ANOVA using Statistica 13.0 for Windows. The means were separated using Tukey's HSD test with $P < 0.05$ considered statistically significant (Tapondjou et al., 2002; Sagheer et al*.*, 2013; Pandir & Bas, 2016).

Results and Discussion

Mortality of *Trogoderma granarium* larvae

Mortalities of *T. granarium* larvae were observed at various exposure time and concentrations of both *E. prostrata* and *C*. *murale* extracts in petroleum ether. The comparison of mean mortality rates of the third instar larvae of *T. granarium* induced by various concentrations of *E. prostrata* extract during 6 days of exposure period is shown in Table 1. There were significant differences in percent mortality of *T. granarium* larvae at the three concentrations of *E. prostrata* extract after 6 days ($F=3.65$; $P<0.05$). The highest mean mortality (20) was found at 30% *E. prostrata* extract, followed by mortality of 16 and 9% at 20 and 10% concentrations, respectively. The 30% concentration of *E. prostrata* extract resulted in significantly higher mortality of *T. granarium* larvae than at 10%, while the mortality at 20 and 30% *E. prostrata* extract were not statistically different. These results indicated that the larval mortality increased with increasing of extract concentration.

Table 1. Mean percent mortalities of *Trogoderma granarium* larvae exposed to different concentrations of *Euphorbia prostrata* extract for 6 days exposure time

Concentration (%)	Mortality (%) ± SE
10	9 ± 4.9 b*
20	16 ± 6.2 ab
30	20 ± 7.1 a

* According to Tukey's HSD test, means with same lowercase letter are not significantly different at P=0.05.

Table 2 shows that exposure time had a significant effect on the mortality of *T. granarium* larvae exposed to 30% *E. prostrata* extract ($F=39.3$; $P<0.05$). The highest mortality (35%) was found after 6 while the lowest mortality (0.7%) was observed after 2 days. The larval mortality after 6 days was significantly higher than after 2 and 4 days, but there was no significant difference between mortalities after 2 and 4 days. The results indicated that the larval mortality increased with increasing of exposure time.

Table 2. Mean percent larval mortalities of *Trogoderma granarium* exposed to 30% concentration of *Euphorbia prostrata* extract for different exposure intervals

Exposure interval (days)	Mortality (%) ± SE
2	0.7 ± 0.49 b*
4	9 ± 2.8 b
6	35 ± 4.9 a

* According to Tukey's HSD test, means with same lowercase letter are not significantly different at P=0.05.

The mean mortality rates of *T. granarium* larvae exposed to *C. murale* extract at the three concentrations are shown in Table 3. The highest mean mortality (25%) was observed at 30% concentration and the lowest at 10% (14%). Extract concentration had significant effect on mortality of *T. granarius* larvae ($F=6.85$; $P<0.05$). The mean mortality at 30% concentration was significantly higher than at 10%. However, the mortalities at 20 and 30% *C. murale* extract were not statistically different.

Table 3. Mean percent larval mortalities of *Trogoderma granarium* exposed to different concentrations of *Chenopodiastrum murale* extract for 6 days exposure time

Concentration (%)	Mortality (%) ± SE
10	14 ± 4.6 b*
20	20 ± 6.0 ab
30	25 ± 7.4 a

* According to Tukey's HSD test, means with same lowercase letter are not significantly different at P=0.05.

Mean percent mortality of *T*. *granarium* larvae exposed to 30% *C. murale* extract for different times are given in Table 4. The larval mortalities were 0, 19 and 40% at 2, 4 and 6 days after treatment, respectively. There were significant differences in larval mortalities between the exposure periods (F=85.39; P<0.05). The larval mortality after 6 days was significantly higher than those after 2 and 4 days. The results showed that the insecticidal potential of *C. murale* extract increased with the increased exposure time. It is concluded that increase in both concentration of *C. murale* extract and exposure period resulted in the increase of mortality of *T. granarium* larvae.

Table 4. Mean percent mortalities of *Trogoderma granarium* larvae exposed to 30% concentration of *Chenopodiastrum murale* extract for different exposure times

Exposure interval (days)	Mortality (%) ± SE
2	12 ± 0.87 c*
4	19 ± 2.5 b
6	40 ± 4.1 a

* According to Tukey's HSD test, means with same lower-case letter(s) are not significantly different at P=0.05.

Repellence of *Trogoderma granarium* larvae

The repellency of different concentrations of *E. prostrata* extract against *T*. *granarium* larvae is shown in Table 5. *Euphorbia prostrate* extracts exhibited significant repellent effect at all treatment concentrations. At 10% extract, only 39% repellency was observed, whereas at 20 and 30% extracts gave 68% and 88% repellency, respectively. The statistical analysis indicated 30% extract resulted in higher percentage of larval repellency than the two lower extract concentrations (F=23.68; P<0.05; Table 5).

Table 5. Mean percent larval repellency in *Trogoderma granarium* exposed to different concentrations of *Euphorbia prostrata* extract for 72 h

Concentration (%)	Repellency (%) ± SE
10	39 ± 8.2 c*
20	68 ± 6.2 b
30	88 ± 4.0 a

* According to Tukey's HSD test, means with same lowercase letter(s) are not significantly different at P=0.05.

The repellence of *T. granarium* exposed to *E. prostrate* extract at various exposure intervals at 30% concentration is shown in Table 6. The highest mean repellency was observed (80%) after 24 h exposure, followed by 66 and 49% after 48 and 72 h, respectively. The repellency at all exposure times were significantly different from each other (F=9.5; P<0.05). The repellency after 24 h was significantly higher than after 72 h. Overall the results indicated that larval repellency decreased with increasing exposure period.

Table 6. Mean percent larval repellency in *Trogoderma granarium* exposed to 30% concentration of *Euphorbia prostrata* extract for different exposure intervals

Exposure interval (hours)	Repellency (%) ± SE
24	80 ± 7.6 a*
48	66 ± 8.2 ab
72	49 ± 9.3 b

* According to Tukey's HSD test, means with same lower-case letter(s) are not significantly different at P=0.05.

The repellency of *C. murale* extract applied at different concentrations on *T*. *granarium* larvae is summarized in Table 7. At 10% extract, 40% exposed larvae were repelled, which it increased to 61% with 20% extract. There was no difference in repellency for10% and 20% extracts. However, 30% extract gave a significantly higher repellency (87%) for 10 and 20% ($F=13.14$; $P<0.05$).

Table 7. Mean percent larval repellency in *Trogoderma granarium* exposed to different concentration of *Chenopodiastrum murale* extract for 72 h

Concentration (%)	Repellency (%) ± SE
10	40 ± 7.1 b*
20	61 ± 8.4 b
30	87 ± 5.3 a

* According to Tukey's HSD test, means with same lowercase letter are not significantly different at P=0.05.

Repellence of *T. granarium* larvae exposed to *C*. *murale* extract at 30% concentration for different exposure intervals is shown in Table 8. After 24 h, the highest repellency was observed (76%), followed by 66 and 47% repellency after 48 and 72 h, respectively. Statistical analysis indicated that the exposure time had significant effect on repellency of *T. granarium* larvae ($F=5.18$; $P<0.05$). Repellency after 24 h was significantly higher than after 72 h. The results indicated the time dependent efficacy of *C. murale* extract, showing decreased repellency with the increase of exposure duration.

Table 8. Mean percent larval repellency in *Trogoderma granarium* exposed to 30% concentration of *Chenopodiastrum murale* extract for different exposure intervals

Exposure interval (hours)	Repellency (%) ± SE
24	76 ± 7.7 a*
48	66 ± 9.4 ab
72	47 ± 9.1 b

* According to Tukey's HSD test, means with same lowercase letter are not significantly different at P=0.05.

Effect of extracts of *Euphorbia prostrata* and *Chenopodiastrum murale* on progeny of *Trogoderma granarium*

The mean larval emergence and inhibition of *T. granarium* in F_1 generation at various concentrations of *E. prostrata* and *C. murale* were recorded after 35 days of infestation (Table 9). The results indicated that lower number of larvae emerged in F_1 generation at three concentrations of both plant extracts as compared to the control. Moreover, there was considerable difference observed in the number of larvae in F_1 generation of both extracts. The lowest mean number of larvae (53) and highest larval inhibition (64%) in F_1 generation was observed in 30% *C. murale* extract treatment followed by *E. prostrata* (60), also at 30%, as compared to 101 and 112 larvae with 10% *C. murale* and *E. prostrata* extracts, which induced 32 and 24% larval inhibition, respectively. The highest number of larvae (149) in F_1 generation was obtained when the wheat grains were left untreated for 35 days (Table 9).

Table 9. Mean number of emerged larvae and their percent inhibition in F1 progeny of *Trogoderma granarium* following treatment with *Euphorbia prostrata* and *Chenopodiastrum murale* whole plant extracts at different concentrations

Concentration (%)	F1 progeny ± SE			
	Euphorbia prostrata		*Chenopodiastrum murale*	
	Mean no. of larvae	Percent larval inhibition	Mean no. of larvae	Percent larval inhibition
10%	112 ± 5.9 b*	24 ± 4.0 b	101 ± 5.2 b	32 ± 3.5 b
20%	94 ± 4.7 b	37 ± 3.2 b	83 ± 7.5 b	44 ± 5.1 b
30%	60 ± 3.1 c	60 ± 2.1 c	53 ± 3.3 c	65 ± 2.2 c
Control	149 ± 7.8 a		149 ± 7.8 a	

* According to Tukey's HSD test, means with same lowercase letter are not significantly different at P=0.05.

Table 9 also shows inhibition of *T. granarium* at the three concentrations of both extracts. The numbers of emerged larvae at the higher concentrations were significantly lower than at the lower concentrations and, also the inhibition increased with increased concentration of both *C. murale* and *E. prostrata* extracts. The statistical analysis indicated that both *C. murale* ($F=19.19$; $P<0.05$) and *E. prostrata* ($F=31.84$; $P<0.05$) extracts had significant effects on emergence and inhibition in F_1 generation of *T. granarium*.

Trogoderma granarium has been considered the worst insect pest of stored grain because it causes huge quantitative and qualitative losses to stored commodities. Synthetic pesticides have been the main control strategy. However, due to their adverse effects on humans and the environment, there is a need to find a safe and environmentally friendly method of control. Thus, many investigations are being carried out around the world to evaluate the pesticidal and repellent properties of plant extracts. The present research work is a continuation to this research to discover the insecticidal potential of weeds, which are commonly considered as unwanted, and pests of land. The results obtained indicated that *C. murale* extract was most effective resulting in the highest mortality (25%) of *T. granarium* larvae after 6 days of exposure, followed by 20% larval mortality (*E. prostrata*) at 30% concentration. The lowest percent mortality (9%) was obtained after 2 days with 10% *E. prostrate* extract. It was clear that larval mortality is directly related to applied concentration and exposure time. Similarly the highest larval repellency was obtained at the 30% *E. prostrata* (88%) and *C. murale* (87%) extracts compared to 39 and 40% at the lowest concentrations, respectively, showing no difference in the efficacy of both plants. In relation to treatment time, *E. prostrata* and *C. murale* repelled higher numbers of larvae after 24 h, 80 and 76%, respectively. The repellency of *E. prostrata* and *C. murale* extracts was decreased with the increase of exposure time. These repellency results revealed no difference in the efficacy of the two plants, giving almost same results. However, a negative relationship between the larval repellency and exposure interval was found.

This pattern is in line with the previous findings (Odeyemi & Ashamo, 2005) that the plant extracts become more toxic increased dose and exposure time. The efficacy of various plant extracts in reducing reproduction and increasing mortality of adult stored grain insects was evaluated by Tapondjou et al. (2002). They used powders and essential oils from leaves of *Dysphania ambrosioides* (L.) Mosyakin & Clemants against six stored grain insect pests; *Callosobruchus chinensis* L., 1758, *Callosobruchus maculatus* Fab., 1775, *Acanthoscelides obtectus* (Say, 1831), *Sitophilus granaries* L., 1758, *Sitophilus zeamais* Motschulsky, 1855 and *Prostephanus truncates* (Horn, 1878). The powdered dry leaves were mixed with grains at different rates ranging from 0.05 to 6.4% (w/w). A dosage of 0.4% killed more than 60% of *C. chinensis* and *C. maculatus* two days after treatment, while a dosage of 6.4% caused total mortality of *S. granarius* and *S. zeamais* within the same exposure time. Moreover, all the concentrations inhibited F1 progeny production and adult emergence of the tested insects. A concentration of 0.2 µl/cm^2 of the essential oil almost killed all the tested insects (80-100%). Consistently, 10µl/cm^2 of extracts was used instead of using essential oils and gave up to 60% progeny inhibition. The insecticidal efficacy of essential oil from *Foeniculum vulgare* Mill.,

Teucrium polium L. and *Satureja hortensis* L. was studied by Heydarzade & Moravvej (2012) against adult *C. maculatus* using a contact toxicity assay. They found essential oil from *S. hortensis* as highly persistent, while *T. polium* showed lower persistency. An increase in mortality with an increase in concentration was observed, which is consistent to the present study. Subsequently, Hasan et al. (2014) used essential oils of four plants, viz., *Azadirachta indica* A. Juss., *Curcuma longa* L., *Nigella sativa* L. and *Piper nigrum* L., for repellency and toxicity studies on *T. granarium*. Concentrations of 5, 10, 15 and 20% were used to test the insecticidal potential to protect stored wheat. The highest mean repellency (90%) was obtained with *A. indica* at 20% and the lowest repellency (26%) with *P. nigrum* at 5%. The highest mortality (31%) was recorded with *A. indica,* whereas lowest mortality (15%) was obtained after 30 days at 20%. The study indicated significant *T. granarium* mortality and feeding deterrence in larvae feeding on wheat grain indicating that these essential oils could be used to develop new botanical insecticides.

Derbalah (2012) tested seven plant extracts [*Argyranthemum frutescens* (L.) Sch.Bip., *Bauhinia purpurea* L., *Caesalpinia gilliesii* (Wallich ex Hook.) Wallich ex D. Dietr., *Cassia fistula* L., *Euonymus japonicus* L., *Senna alexandrina* Mill. and *Thespesia populnea* var. *acutiloba* Bak.] against *T. granarium* and found that all the extracts induced significant mortality and reduced the F_1 progeny emergence. However, *S. alexandrina* was found to be the most effective of these botanical extracts. In other work, Pacual-Villalobos (1998) reported 14% larval mortality in *T. granarium* treated with Neem extract (*A. indica*) compared to 7% with *P. nigrum* extract. Recently, Pandir & Bas (2016) tested the essential oils from basil (*Ocimum basilicum* L.), paprika (*Capsicum annuum* L.), peppermint (*Mentha* x *piperita* L.) and rosemary (*Rosmarinus officinalis* L.) at the concentration of 0.1, 1, 5, 10, 20, 50 and 100 µl/L for the control of different stages of *Ephestia kuehniella* Zeller, 1879. Among different life stages of *E. kuehniella,* the larval stage was found to be the most tolerant to essential oils. Overall it was concluded that insecticidal potential of the essential oils of these plants increased with increased application concentration.

Similarly, Al-Moajel (2004) reported the dose dependent mortality and progeny reduction in *T. granarium* while testing the botanical powders from eleven different plants [*Albizia lebbeck* (L.) Benth.*, Allium cepa* L.*, Allium cepa* var. *ascalonicum* Don.*, Capsicum frutescens* L.*, Carthamus tinctorius L., Delonix regia* (Boj. ex Hook.) Raf.*, Eruca sativa* Mill.*, Lawsonia inermis L., Mesua ferrea* L.*, Raphanus sativus* L. and *Vachellia farnesiana* (L.) Wight & Arn.]. *Capsicum frutescens* induced the highest mortality (77-85%) of *T. granarium,* while F1 progeny was significantly reduced by *R. sativus* (71%), *C. tinctorius* (67%), *C. frutescens* (59%), *A. cepa* var. *ascalonicum* (53%) and *L. inermis* (47%) powders. It was concluded that *C. frutescens* and *L. inermis* showed significant effect on mortality of both adults and larvae of *T. granarium* and also significantly reduced the F1 progeny. Sagheer et al. (2013) also evaluated the repellent potential of four medicinal plants extracted in acetone against *T. granarium* larvae, resulting in the 55, 52, 51 and 47% larval repellency from *Nicotiana tobaccum* L.*, Peganum harmala* L.*, Salsola baryosma* (Schult.) Dandy and *Saussurea costus* (Falc.) Lipsch. extracts at 20% concentration, respectively. They also concluded that the repellent effect of plant extracts was increased with the increase in application concentration.

Alkan et al. (2015) also investigated the antifeedant activity and growth inhibition effects of *Achillea millefolium* L., *Heracleum platytaenium* Boiss. and *Humulus lupulus* L. extracts against third instar larvae of Colorado potato beetle (*Leptinotarsa decemlineata* Say, 1824). They found that a concentration of 50 g/L of all plant extracts induced antifeedant activity. It was suggested that *H. platytaenium* and *H. lupulus* extracts were excellent antifeedants and larval growth inhibitors. Although, there are many studies reporting the use of plant extracts against stored grain insect pests, the plant extracts used in the present study has not previously been evaluated for the control of *T. granarium* larvae.

Conclusion

The results demonstrate that extracts of *E. prostrata* and *C. murale* have insecticidal, repellent and progeny reduction potential against *T. granarium*. Overall, the results indicated that petroleum ether extract of *C. murale* had higher insecticidal activity than the extract of *E. prostrata.* More research work on weeds, so called the pest of crops, is needed for their use in stored grain insect pest management programs.

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Original article (Orijinal araştırma)

First report of sugar beet nematode, *Heterodera schachtii* Schmidt, 1871 (Nemata: Heteroderidae) in sugar beet growing areas of Şanlıurfa, Turkey

Şanlıurfa ili Şeker pancarı üretim alanlarında yeni bir zararlı; Şeker pancarı kist nematodu, *Heterodera schachtii* Schmidt (Nemata: Heteroderidae)

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Summary

The sugar beet nematode, *Heterodera schachtii*, is the major pest of sugar beet and causes serious yield losses of about 10-70%. *Heterodera schachtii* occurs in more than 50 countries and regions, however, there has be limited investigation of *H. schachtii* in Turkey. Therefore, a survey of *H. schachtii* in the sugar beet producing regions of Şanlıurfa was conducted in 2014 and 2015 growing seasons. Using morphological and molecular methods, 12 samples collected from three districts, Bozova, Karaköprü and Siverek, in Şanlıurfa Province, Turkey, were identified as *H. schachtii*. In pathogenicity test, the seedling emergence was delayed and reduced, the seedlings were stunted and necrotic, and the white females of *H. schachtii* were evident 25 days after inoculation. Phylogenetic analyses were also conducted. The 12 *H. schachtii* populations from Şanlıurfa Province clustered together with populations from Europe and Morocco at the value of 99%. Sugar beet is the second largest crop in Turkey with the annual production of more 16 Mt. To our best knowledge, this is the first report of *H. schachtii* in Şanlıurfa Province of Turkey.

Keywords: Cyst nematode, identification, phylogenetic analyses, sugar beet

Özet

Şeker pancarı kist nematodu, *Heterodera schachtii* Schmidt (Nemata: Heteroderidae), şeker pancarı üretim alanlarında önemli bir zararlı olup, epidemi yaptığında %10-70 arasında bir ürün kaybına neden olabilmektedir. Günümüzde *H. schachtii* 50'den fazla ülkede yaygın olarak bulunmakla birlikte, Türkiye'de *H. schachtii* araştırmaları oldukça sınırlıdır. Bu çalışmada, 2014 ve 2015 üretim sezonunda Şanlıurfa ili şekerpancarı üretim alanlarında *H. schachtii*'nin surveyi ve teşhisi yapılmıştır. Araştırma sonucunda, Şanlıurfa ilinde üç farklı lokasyonda; Bozova, Karaköprü ve Siverek ilçelerinden alınan on iki örnek, morfolojik ve moleküler yöntemlerle *H. schachtii* olarak tanımlanmıştır. Koch postülat testinde, beyaz *H. schachtii* dişileri inokülasyondan sonra 25. günde kist oluşturmuş, çimlenmede gecikme ve azalma olup, filizlenmede nekroz ve bodurlaşma görülmüştür. Ayrıca, çalışmada, filogenetik analiz yapılmış, Şanlıurfa ilinden toplanan *H. schachtii* popülasyonlarının Avrupa ve Fas popülasyonları ile %99 oranında benzerlik gösterdiği saptanmıştır. Şekerpancarı, yıllık 16 milyon tondan fazla üretimi ile Türkiye'de ikinci en fazla üretilen ürün olma özelliğinde olup, bu çalışma, Türkiye'de Şanlıurfa ili şekerpancarı üretim alanlarında şekerpancarı kist nematodu, *H. schachtii* için ilk kayıt niteliğindedir.

Anahtar sözcükler: Kist nematodu, teşhis, filogenetik analiz, şekerpancarı

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Introduction

The sugar beet nematode, *Heterodera schachtii* Schmidt, 1871, is a major pathogen on sugar beet (*Beta vulgaris* L. ssp. *vulgaris* var. *altissima*), and is the most serious nematode pest recognized in sugar beet (Cooke, 1987). *Heterodera schachtii* is widely distributed throughout the world, especially in Europe, North America, Australia, the Middle East, Africa and South America (Evans & Rowe, 1998). *Heterodera schachtii* has a wide range of other host species, including over 200 plant species within 95 genera from 23 different plant families; most hosts are found in the Chenopodiaceae and Cruciferae (Steele, 1965). In Europe, the annual yield loss associated with the sugar beet nematode was estimated at 90 million EUR (Muller, 1999).

Heterodera schachtii, Schmidt, 1871, *Heterodera betae* Wouts, Rumpenhorst & Sturhan, 2001 and *Heterodera trifolii* Goffart, 1932 belong to the *H. schachtii sensu stricto* group and can be distinguished by their morphology (Subbotin et al., 2000). Identifications based on morphological characters of cysts and J2s is difficult and needs skillful nematologists, so it can more accurate by the use of molecular techniques (Subbotin et al., 1999). During the last fifteen years, researchers have collected and characterized more than 40 species of nematodes from the genus *Heterodera* (Waeyenberge et al., 2009). The SCAR-PCR (sequence characterized amplified region) amplification with species-specific primers has been used for the identification of several cyst nematodes such as *Heterodera avenae* Wollenweber, 1924, *Heterodera filipjevi* Madzhidov, 1981, *Heterodera latipons* Franklin, 1969, *Heterodera glycines* Goffart, 1936 and *H. schachtii* without requiring the subsequent RFLP step (Yan et al., 2013). *Heterodera schachtii* was reported for the first time from sugar beet growing areas in Kırklareli Province in Turkey by Diker (1959). Later on, it has been increasingly reported from other areas and has become recognized as a damaging pathogen of sugar beet producing areas in Eskişehir and Adapazarı Provinces in Central Anatolian Region of Turkey (Susurluk & Ökten, 1999; Osmanoğlu, 1999). The southeast region of Turkey has been the main area for the growing of crops from the Chenopodiaceae, Cruciferae and Leguminosae (Albayrak et al., 2010; Bagdatli & Acar, 2009). However, no previous survey has been conducted to detect *H. schachtii* in Şanlıurfa Province of Southeast Anatolian Region of Turkey.

Here we report the occurrence of the *H. schachtii* in Şanlıurfa Province of Southeast Anatolian Region of Turkey through morphological, molecular and phylogenetic identification. The objectives of this study were to 1) survey sugar beet growing areas in the Southeast Anatolian Region of Turkey to extend knowledge of the distribution of *H. schachtii*, 2) identify *H. schachtii* specimens to the species level using molecular and morphological tools, and 3) verify the pathogenicity of detected populations. Above all, this survey was designed to determine the current status of species distribution as a basis for developing SCN-management strategies.

Materials and Methods

Sampling and DNA extraction

In 2014 and 2015, a cyst nematode survey was conducted in sugar beet fields in southeast regions of Turkey. A total of 1 kg of soil was collected from each field by sampling five random points taken along a zigzag transect. In total, 130 points were sampled in the southeast region of Turkey (Table 1). From each soil sample, 100-ml subsamples were taken for extraction of cysts by wet sieving and sucrose flotation centrifugation procedures (Riggs et al., 2000). Cysts from each sample were hand-picked with a brush under a stereomicroscope. Cysts were randomly selected from each subsample, sterilized in 0.5% NaClO for 5 min and rinsed 3 times in sterile distilled water, and transferred to 24-well culture plates at 20°C for hatching (Griffin, 1981). The hatched invasive second-stage juveniles (J2s) were collected for pathogenicity testing, morphological observation and molecular identification.

Nematode genomic DNA was extracted as described by Qi et al. (2012). The cyst sample in the microtube was frozen in liquid nitrogen and melted in 37°C water bath, and this process was repeated four times. Successively, samples were frozen at -80°C for 1.5 h and then incubated at 65°C for 1.5 h followed by 10 min at 95°C in PCR thermo-cycler. Afterwards, the samples were centrifuged at 1000 revs/min at a temperature of 4°C for 1.5 min. Then the supernatant was transferred into a new microtube and stored at -20°C for later use. At least three replicates of each nematode population detected were tested.

Table 1. Origin and occurrence of populations of *Heterodera schachtii* in The South Anatolia Region, Turkey, and the identification of *Heterodera schachtii* species within specific SCAR primers

Province	Valid samples[a]	Cysts in 100 ml soil	Location	Results
Diyarbakir	6 (15)	5	Oğuzlar	-
Diyarbakir	5 (7)	6	Alabal	-
Gaziantep	6 (10)	11	Karkamış	-
Gaziantep	5 (13)	7	Oğuzeli	-
Kilis	3 (5)	11	Elbeyli	-
Kilis	2 (6)	6	Üçdamlar	-
Kilis	6 (9)	10	Yavuzlar	-
Kilis	3 (5)	4	Kızıltepe	-
Kilis	2 (5)	8	Havuzluçam	-
Mardin	7 (10)	15	Şanlı	-
Mardin	6 (8)	11	Şenyuva	-
Mardin	4 (5)	7	Toprakkale	-
Mardin	6 (9)	6	Akıncılar	-
Mardin	7 (9)	8	Ortaköy	-
Şanlıurfa	5 (5)	7	Siverek	+
Şanlıurfa	3 (3)	9	Bozova	+
Şanlıurfa	4 (6)	12	Karaköprü	+

[a] Numbers in brackets are the total number of samples. [b] "-" negative result, "+" positive result.

SCAR-PCR identification

Extracted DNA (2.5 µl) was added into an Eppendorf tube containing: 12.5 µl 2×Taq PCR StarMix buffer (GenStar, Beijing, China), 1µl of 10 µM of primers and ddH_2O was added to make a final volume of 25 µl. SCAR-PCR primers for *H. schachtii* (SHF6 and rDNA2) were used to PCR-amplify the specific fragments (Amiri et al., 2002). The PCR program consisted of 4 min at 94°C, 10 cycles of 30 s at 94°C, 40 s at 45°C and 1 min at 72°C for elongation; 20 cycles of 30 s at 94°C, 40 s at 55°C and 1 min at 72°C for elongation. The reaction was terminated by a final extension cycle at 72°C for 10 min. After PCR amplification, 5 µl of each PCR products was separated on a 1.5% agarose gel.

Morphological observation

For morphological observation and species identification, mature cysts containing eggs and fully developed juveniles were selected randomly. Vulva cone from mature cysts were mounted (Zhang, 1988). Slides of J2s were prepared as described by Lin (1991) and Duan & Chen (2006) with minor adaptations, fixed with 10% formalin overnight and transferred into the glass cavity block and filled with 3% glycerol (3

ml glycerol and 95 ml sterile water) and covered with a glass lid at room temperature. Three weeks later, the nematode specimens were transferred to a clean drop of anhydrous glycerol on a glass slide and the mounted specimens were covered with a cover slip and sealed using clear nail polish. These slides were viewed under a microscope (Leica DM2500) attached with a digital camera connected to a computer for processing and storing the images. The distance measurement function of the image analysis software was first calibrated using a stage micrometer for each of the objectives before taking measurements of the specimen according to the manufacturer's instructions. All measurements were recorded and for each of the samples, the data was summarized by calculating the averages, range and standard error.

Inoculation for pathogenicity test

To prove a causal relationship between the *H. schachtii* and the disease, hatched J2s were collected and used to infect sugar beet (*B. vulgaris* KWS2320) roots, three-day-old sugar beet roots were inoculated with 200 pre-parasitic J2s. The infected plants were planted in a sterile mixture of sand and loam (1:1, v/v) and kept in a glasshouse at 24°C for observation (Griffin, 1981). Plants were grown under normal plant growing conditions with irrigation, fertilization, and disease and insect control as needed.

rDNA-ITS amplification and phylogenetic analyses

The primers TW81 and AB28 described by Joyce et al. (1994) were used to amplify the ITS-rDNA region. PCR program consisted of an initial denaturation step at 95°C for 4 min followed by 35 cycles of 30 s at 94°C (denaturation), 45 s at 56°C, and at 72°C for 1 min for elongation. The reaction was terminated at a final extension cycle at 72°C for 10 min. ITS-PCR products of the populations were purified using the TIAN gel Midi Purification Kit (Tiangen Biotech, Beijing, China) per the manufacturer's instructions. The purified products were cloned into the pGEM®-T Easy Vector and transformed into DH5α Competent Cells (Tiangen Biotech). The clones of each population were isolated using blue and white selection, and subjected to PCR for confirmation.

The positive clones were then sequenced. All sequences of ITS-rDNA obtained were submitted to GenBank (GenBank Accession No. KT874516 - KT874527) and a database search performed using BLAST. Twelve new sequences, along with 16 sequences of *Heterodera* genera as the in-group taxa and one *Globodera* genera as outgroup taxa were download from the NCBI, where subjected to phylogenetic analysis (Table 2). The sequences were edited and analyzed with MEGA 5.05 (Center for Evolutionary Medicine and Informatics, Biodesign Institute, Tempe, AZ, USA) (Tamura et al., 2011). To determine statistical consistency of the classification, the phylogeny reconstructions statistical method using maximum likelihood and bootstrap analysis with 1000 bootstrapped data sets was used. Gaps were treated as a missing data. A tree clustering the populations at different levels based on genetic distance was constructed from the ITS sequence alignment with MEGA 5.05 (Tamura & Nei, 1993).

Results

SCAR-PCR identification and pathogenicity testing

In this survey, cysts were detected in 62% of the 130 samples (Table 1). Four to 15 cysts were checked from every 100-ml subsample. Twelve specimens, collected from three districts Siverek, Karaköprü and Bozova of Şanlıurfa Province were identified as *H. schachtii* by SCAR-PCR (Table 2). The size of SCAR-PCR products was 255 bp for single juveniles from these *H. schachtii* populations, same to the positive control (Figure 1). The density of *H. schachtii* in Siverek, Karaköprü and Bozova was 11.0, 18.1 and 37.5 eggs/g soil, respectively. The results of the pathogenicity test indicated that the growth of *B. vulgaris* KWS2320 seedlings was delayed by the infection of with *H. schachtii*. Ten days after inoculation, the inoculated plants were stunted, and by 20 days, yellowing and necrosis of the foliage and white females in the roots were evident. After 35 days brown cysts had been formed. The pathogen was confirmed to be *H. schachtii*.

Table 2. Nematode species and populations used in the phylogenetic analyses

Species	Population	Country	Accession number	Source of data
H. arenaria	Linconshire	UK	AF274396.1	Subbotin et al., 2001
H. aucklandica	Zaaren	Belgium	AY148379.1	Subbotin et al., 2003
H. australis	York	Australia	AY148395.1	Subbotin et al., 2003
H. avenae	Cukurova	Turkey	AY148364.1	Subbotin et al., 2003
H. filipjevi	Selcuklu	Turkey	AY148398.1	Subbotin et al., 2003
H. latipons	Kilis	Turkey	KM199826.1	Imren et al., 2014
H. mani	Hamminkeln	Germany	AY148377.1	Subbotin et al., 2003
H. pratensis	Otterndorf	Germany	AY148383.1	Subbotin et al., 2003
H. ripae	Ussurijskii	Russia	AF393840.1	Eroshenko et al., 2001
H. ustinovi	unknown	Belgium	AY148407.1	Subbotin et al., 2003
H. schachtii	Sanliurfa	Turkey	KT874516	Present study
H. schachtii	Sanliurfa	Turkey	KT874517	Present study
H. schachtii	Sanliurfa	Turkey	KT874518	Present study
H. schachtii	Sanliurfa	Turkey	KT874519	Present study
H. schachtii	Sanliurfa	Turkey	KT874520	Present study
H. schachtii	Sanliurfa	Turkey	KT874521	Present study
H. schachtii	Sanliurfa	Turkey	KT874522	Present study
H. schachtii	Sanliurfa	Turkey	KT874523	Present study
H. schachtii	Sanliurfa	Turkey	KT874524	Present study
H. schachtii	Sanliurfa	Turkey	KT874525	Present study
H. schachtii	Sanliurfa	Turkey	KT874526	Present study
H. schachtii	Sanliurfa	Turkey	KT874527	Present study
H. schachtii	Aisne	France	EF611103	Madani et al., 2007
H. schachtii	Herme	Belgium	EF611107	Madani et al., 2007
H. schachtii	Mouloya	Morocco	EF611118	Madani et al., 2007
H. schachtii	Munster	Australia	EF611123	Madani et al., 2007
H. betae	unknown	Germany	EF611122	Madani et al., 2007
H. trifolii	Hokkaido	Japan	LC030417	Kushida et al. unpub
G. rostochiensis	British	Canada	FJ212167.1	Madani et al., unpubl

Morphological identification

The cysts were lemon shaped, and light to dark brown in color. The vulval cone was ambifenestrate with dark brown, molar-shaped bullae positioned underneath the vulval bridge (Figure 2). The key morphometrics of cysts (n = 12) were: body length (excluding neck) from 610 to 783 µm; body

width from 395 to 530 µm; length/width ratio from 1.44 to 1.57 µm; underbridge from 98 to 120 µm; and vulval slit from 40.7 to 45 µm (Table 3). The J2s were cylindrical in shape, stylet moderately heavy with prominent, forward directed knobs, tail acutely conical with rounded tip and a hyaline region in the tail terminus. The key morphometrics of J2 (n = 20) were: mean body length from 426 to 510 µm; mean body width from 21 to 23 µm; mean stylet length from 24 to 25 µm, mean tail length from 55 to 62 µm; and mean length of the hyaline tail region tail from 31 to 38 µm (Table 3).

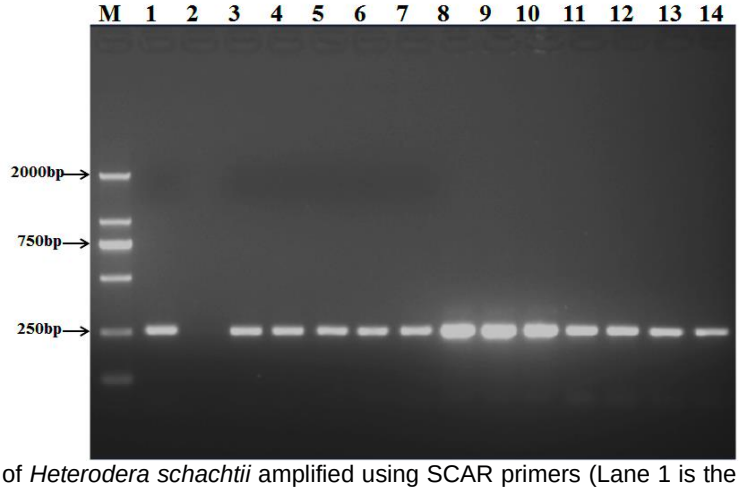

Figure 1. PCR patterns of *Heterodera schachtii* amplified using SCAR primers (Lane 1 is the positive control; Lane 2 is the negative control; Lanes 3-14 are *Heterodera schachtii* populations collected in Turkey; and M is a 2000 bp DNA ladder).

Table 3. Morphometrics of cysts and J2s of *Heterodera schachtii* population in Şanlıurfa, Turkey (all measurements in µm)

Traits	*Heterodera schachtii* population		
	Siverek	Bozova	Karaköprü
	Mean ± SD (Range)	Mean ± SD (Range)	Mean ± SD (Range)
Cysts (n=12)			
Body length excluding	692.0±57.0 (638-755)	700.3±80.1 (610-783)	703.2±61.5 (628-765)
Body width (W)	453.8±31.5 (425-490)	459.8±52.2 (395-505)	473.2±49.6 (410-530)
L/W	1.52±0.02 (1.50-1.54)	1.52±0.03 (1.48-1.56)	1.49±0.04 (1.44-1.53)
Underbridge length	117.0±2.9 (114-120)	113.3±10.4 (98-120)	115.5±4.79 (110-120)
Vulva slit length	44.8±0.1 (44.7-44.9)	43.9±1.9 (41-45)	44.6±0.2 (44.5-44.9)
Second-stage juveniles (J2s) (n=20)			
Body length	461.2±15.6 (442-488)	470.8±23.7 (426-490)	479.0±11.7 (455-489)
Midbody width	21.1±0.4 (21-22)	21.3±0.8 (21-23)	21.4±0.5 (21-22)
Stylet length (S)	24.2±0.4 (24-25)	24.3±0.5 (24-25)	24.7±0.4 (24-25)
Tail length (T)	46.7±1.0 (45-48)	47.0±1.4 (45-49)	47.6±1.0 (47-49)
Hyaline tail length (H)	24.3±0.51 (24-25)	24.0±0.6 (23-25)	24.6±1.1 (23-26)
H/S	1.01±0.03 (0.96-1.04)	0.99±0.02 (0.96-1.00)	0.99±0.04 (0.92-1.04)
T/H	1.92±0.06 (1.80-1.95)	1.96±0.07 (1.88-2.02)	1.94±0.08 (1.85-2.08)

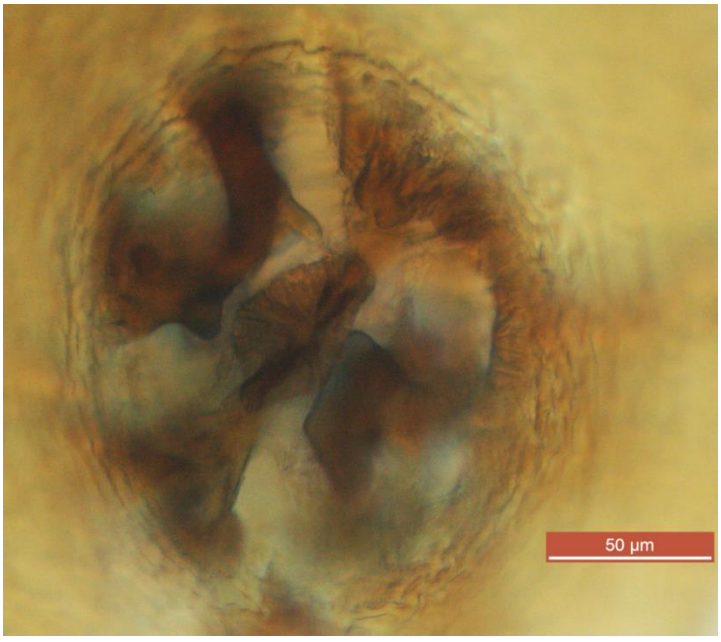

Figure 2. Vulval cone of *Heterodera schachtii* from Şanlıurfa, Turkey, showing the identity of the internal structures (fenestra, vulva slit, underbridge and bullae).

ITS-rDNA sequences and phylogenetic analyses

Ribosomal DNA (rDNA) from the ITS regions was PCR-amplified from each confirmed population of *H. schachtii* and then sequenced. Twenty-eight *Heterodera* populations were clustered, with a *Globodera rostochiensis* Wollenweber, 1923, population as an outgroup. A phylogenetic tree of the *Heterodera* populations was constructed based on the ITS1-5.8S-ITS2 rDNA sequences. All bootstrap values were larger than 60%. Species with bootstrap values of over 70% were placed in clades. The populations of the same species and same sites clustered together. All the groups formed were distinguished from each other in the *Heterodera* sequences, supported by a moderate to high bootstrap value. *Heterodera schachtii sensu stricto* clustered together as group A with a value of 100%, distinctly different from the group B, *H. avenae* complex populations and the outgroup *Globodera rostochiensis* population (Figure 3). *Heterodera schachtii* group included the 12 *H. schachtii* populations collected from Şanlıurfa, *H. schachtii* populations from Australia (EF611123.1), Morocco (EF611118.1), Belgium (EF611107.1), France (EF611103.1), *H. trifolii* from Japan (LC030417.1), and *H. betae* from Germany (EF611122.1). *Heterodera schachtii* populations from Belgium (EF611107.1) and France (EF611103.1) clustered as a sub-branch with a value of 95%. *Heterodera trifolii* from Japan (LC030417.1), *H. betae* from Germany (EF611122.1) and *H. schachtii* populations from Morocco (EF611118.1) clustered as sub-branch with a value of 87%. *Heterodera avenae* complex populations included *H. ripae* (AF393840.1), *H. ustinovi* (AY148407.1), *H. pratensis* (AY148383.1), *H. australis* (AY148395.), *H. mani* (AY148377.1), *H. arenaria* (AF274396.1), *H. aucklandica* (AY148379.1) and the populations of *H. avenae* (AY148364.1), *H. filipjevi* (AY148398.1), *H. latipons* (KM199826.1) from Turkey. *Heterodera avenae* complex populations were clustered in one branch at a value of 99%.

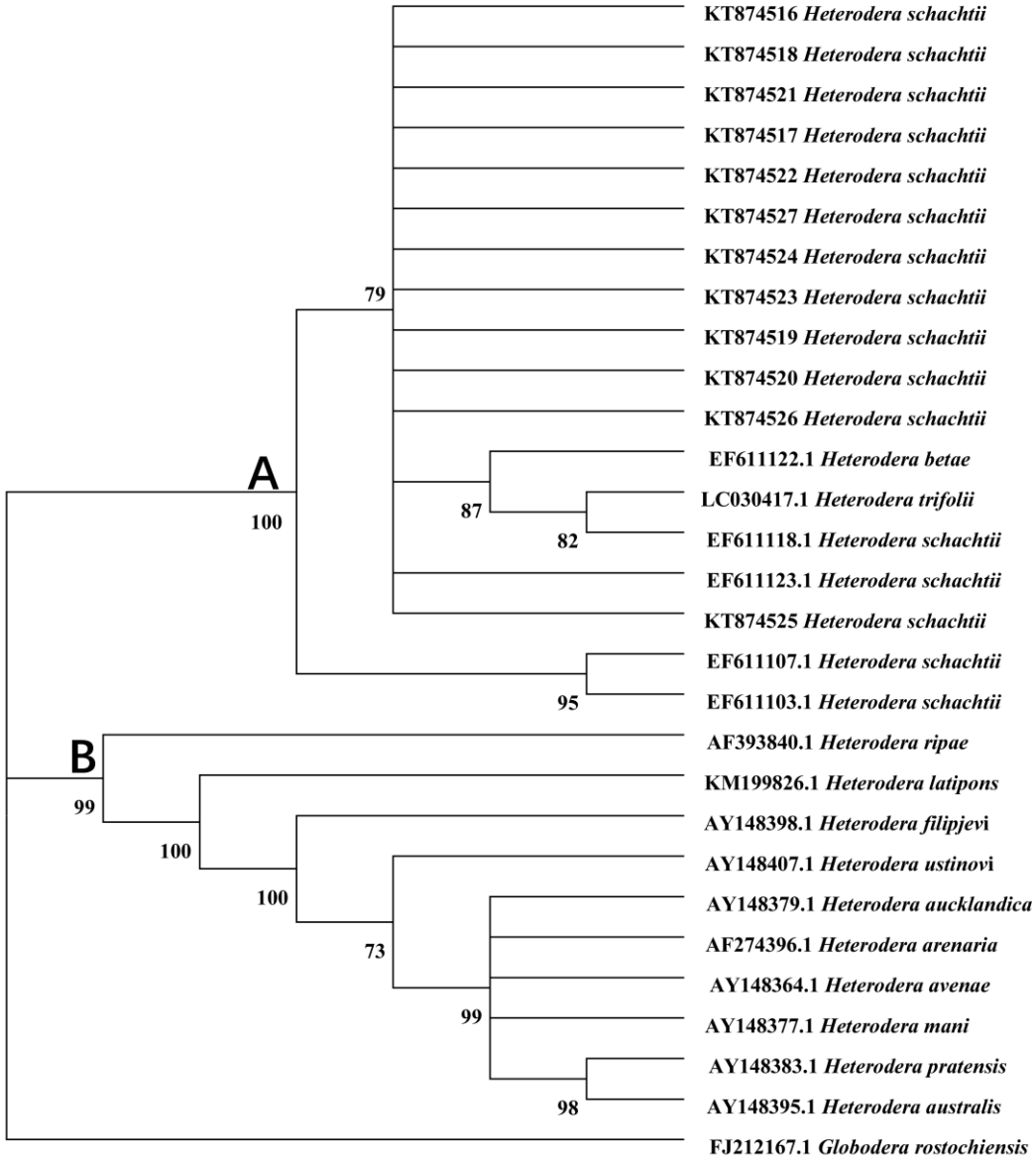

Figure 3. Molecular phylogenetic analysis (maximum likelihood) constructed through the ITS sequences alignment for 29 cyst-forming nematodes. Bootstrap values (more than 70%) are given in the appropriate clades.

Discussion

Yield loss in sugar beet caused by the plant parasitic nematodes is estimated to be about 10%, of which nearly 90% is associated with the cyst nematode, *H. schactii* (Rahmani et al., 2013). In Turkey, sugar beet is the crop with the second largest production, with the annual yield of more than 16 Mt (FAO, 2016). In Turkey, 80% of the agricultural holdings (about 500,000) that produce sugar beet are smaller than 1 ha (Albayrak et al., 2010). The fields surveyed in 2014 to 2015 were of limited size making it very to for growers to control sugar beet nematode systematically. The eggs are the survival stage and can stay viable for several years in soil. A small percentage of eggs within cysts have been reported to survive under fallow conditions for over 12 years (Hafez, 1997). Initial population (Pi) level at of 9, 18, 34 and 68 eggs and larvae per g soil resulted in deceased marketable yield of table beets of 23, 25, 42 and 54%, respectively. Marketable head weight of direct-seeded cabbage was also decreased by 21, 28, 46 and 54%, and that of transplanted cabbage by 25, 31, 34 and 42%, respectively (Abawi & Mai, 1980).

The density of *H. schachtii* in Siverek, Karaköprü, Bozova was 11.0, 18.1 and 37.5 eggs per g soil, respectively, which are potentially damaging based on the findings of Abawi & Mai (1980). However, there have been few studies focusing on *H. schachtii*. These results indicate the need to monitor *H. schachtii* population in Turkey to prevent significant economic loss in sugar beet.

Heterodera schachtii was reported for the first time from sugar beet fields in Kırklareli Province in Turkey (Diker, 1959). Later on, it was also detected many part of sugar beet areas in Kırklareli Province (Tokmakoğlu, 1974). More recently, Susurluk & Ökten (1999) and Osmanoğlu (1999) reported it from Eskişehir and Adapazarı Provinces in the Central Anatolian Region of Turkey, and it has been increasingly detected and has become recognized as a damaging pathogen of sugar beet producing areas in in those areas. The first symptom of *H. schachtii* is yellowing and stunting of plant growth (Polychronopoulos & Lownsbery, 1968). These symptoms are consistent with our pathogenicity test: after sugar beet seedlings were inoculated with *H. schachtii* J2s, the seedling emergence was delayed and reduced, plants were stunted and necrotic, and the infected roots had a whiskered appearance with more lateral roots. The differences between *H. schachtii* and the *H. avenae* group were: *H. avenae* had no obvious underbridge and more bullae, *H. filipjevi* had a slight underbridge with a few bullae, and *H. latipons* had a heavy underbridge with no bullae. Additionally, there was a heavy underbridge with bullae of *H. schachtii*. For the J2s, the main diagnostic features were the stylet and tail lengths followed by body length. *Heterodera schachtii*, *H. betae* and *H. trifolii* belongs to the Schachtii group, *H. betae* is a member of the *H. trifolii* species complex, and is distinguished from closely related species by a combination of morphological and morphometric characteristics. *Heterodera schachtii with a* terminal vulval slit about as long as vulval bridge differs from *H. trifolii* by a shorter average fenestral length and from *H. betae* by shorter average cyst body length. *Heterodera schachtii* J2s have the shortest average tail length and the shortest average length of the hyaline part of the tail. The data showed the morphology of the cysts and juveniles were consistent with those reports of *H. schachtii* (Subbotin et al., 2010).

ITS-rDNA within *H. schachtii* is complex and heterogeneous (Amiri et al., 2002). Amiri et al. (2002) designed primers, using the available ITS-rDNA sequence information, which is specific for species of the *H. schachtii sensu stricto* group. This method of identification of *H. schachtii* is highly sensitive, with amplification obtained even when a single J2 or a single cyst was mixed with other nematode species (Amiri et al., 2002). The different levels of intraspecific variation of the ITS-rDNA sequence have an effect on the evolution and cluster analysis (Madani et al., 2007). The analyses of phylogenetic relationships of the ITS sequences showed that the *H. schachtii* populations from Turkey were clustered together with populations from Europe, Australia and Morocco. The 12 new *H. schachtii* populations clustered with populations from Australia (EF611123.1) and Morocco (EF611118.1) in one sub-branch with a bootstrap value of 79%, the *H. schachtii* populations from Belgium (EF611107.1) and France (EF611103.1) clustered closer to another sub-branch with a bootstrap value of 95%. Relative genera and species of *H. schachtii*, the *H. trifolii* and *H. betae* were clustered together in one branch as the Schachtii group, and separate from the *H. avenae* complex group. ITS-rDNA sequences did not group isolates according to their geographical origin or taxonomic grouping. Concerted evolution has not homogenized all rDNA variants within individual populations. When two ITS clone variants were sequenced from the same population these sequences did not clustered together in Maximum Parsimony trees (Madani et al., 2007). Mostly, Maximum likelihood and Bayesian outperformed Neighbor Joining, Maximum Parsimony and Parsimony in terms of tree reconstruction accuracy (Hall, 2005; Ogden & Rosenberg, 2006). The results presented here are consistent with the report of Madani (2007) but differ from those of Tanha Maafi (2003) for *H. trifolii*. Ogden & Rosenberg (2006) indicated that as the length of the branch and of the neighboring branches increase, alignment accuracy decreases, and the length of the neighboring branches is the major factor in topological accuracy. Thus, multiple-sequence alignment can be an important factor in downstream effects on topological reconstruction (Ogden & Rosenberg, 2006). Additionally, we found the *H. schachtii* population from Morocco (EF611118.1) was more closely related to *H. trifolii* from Japan (LC030417.1) and clustered as a sub-branch with a value of 87%, and *H. betae* from Germany (EF611122.1) clustered together with *H. schachtii* (EF611118.1) and *H. trifolii* (LC030417.1) as a subgroup with a value of 87% within the Schachtii group. *Heterodera betae* may *have*

originated from interspecific hybridization of two diploid amphimictic species, with *H. schachtii* suggested as one of the parent species, or *H. betae* represents a polyploid parthenogenetic form that has evolved from autopolyploidization of diploid amphimictic *H. schachtii* or another very closely related species (Madani et al., 2007). This hypothesis could explain the origin and evolution relationship of *H. trifolii*, *H. betae* and other polyploid species from the Schachtii group.

Heterodera schachtii has been recognized as a serious problem for sugar beet production and is an important quarantine nematodes in many countries due to its devastating damage to sugar beet (Peng et al., 2015). Sugar beet nematode has spread extensively in many regions of sugar beet cultivation, and therefore efforts have been made to reduce its effects on sugar and root yield. The most appropriate control method has been the release of resistant cultivars (Rahmani et al., 2013) with resistance genes introduced from wild *Beta* species (Kleine et al., 1998). The annual rate of decline of viable eggs and larvae in fields after removal of sugar beet or another host crop can vary from 40 to 50% depending on the type of soil, soil temperature, soil moisture, history of pesticide use (including herbicides), susceptibility and availability of host plants (including weeds), and the presence of predators and parasites (Hafez et al., 1997). Furthermore, their combined effects should aim to decrease and maintain *H. schachtii* population densities below the damage threshold.

Acknowledgments

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Original article (Orijinal araştırma)

A study on determination of Cerambycidae (Coleoptera) fauna of Isparta Province (Turkey)[1]

Isparta ili Cerambycidae (Coleoptera) faunasının belirlenmesi üzerine bir çalışma

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Summary

The aims of this study were to understand the faunistic composition and some ecological properties (vertical distributions and seasonality) of Cerambycidae (Coleoptera) of Isparta Province, Turkey. The study was conducted in 2009 and 2013. A total of 53 longhorn beetle species belonging to three subfamilies were collected. The great majority of the cerambycid fauna of the province is in the subfamily Lamiinae. The results showed that longhorn beetle species richness peaked in May and July. Also, the results of the study indicate that longhorn beetles have a clear vertical distribution patterns.

Keywords: Cerambycidae, Coleoptera, fauna, Isparta, Turkey

Özet

Bu çalışmada, Isparta ili Cerambycidae (Coleoptera) faunasının faunistik kompozisyonunun ve belirlenen türlerin bazı ekolojik özelliklerinin (dikey dağılışları ve mevsimsel tercihleri) belirlenmesi amaçlanmıştır. Çalışma 2009-2013 yılları arasında gerçekleştirilmiştir. Çalışma sonucunda, üç altfamilyaya ait toplam 53 teke böceği türü toplanmıştır. Tespit edilen türlerin büyük çoğunluğunu Lamiinae altfamilyasına ait türler oluşturmaktadır. Sonuçlar teke böceği tür zenginliğinin mayıs ve temmuz aylarında en yüksek olduğunu göstermiştir. Ayrıca, çalışmanın sonuçları teke böceği türlerinin belirgin bir dikey dağılış örüntüsüne sahip olduklarını ortaya koymuştur.

Anahtar sözcükler: Cerambycidae, Coleoptera, fauna, Isparta, Türkiye

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Introduction

The beetle family, Cerambycidae, is one of the largest groups of insects, with more than 35,000 species worldwide (Lawrence, 1982; Grimaldi & Engel, 2005; Švácha & Lawrence, 2014). The longhorn beetle fauna of Turkey has been studied intensively over the past several decades (Tezcan & Rejzek, 2002). According to Löbl & Smetana (2010) about 650 species and subspecies were known from Turkey.

Longhorn beetles vary greatly in body size (2.5-17 cm), morphology, coloration, and natural history (Bílý & Mehl, 1989; Bense, 1995; Linsley & Chemsak, 1997). Adult cerambycid species are phytophagous or xylophagous (Booth et al., 1990). Larvae of most longhorn beetle species are xylophagous (e.g. feeding inside living, moribund, or even decomposing wood), while for the rest of the species, larvae feed in stems or roots of some herbaceous plants (Linsley, 1959; Susana, 2009; Gnjatovic & Zikic, 2010). Some species are important pests, damaging and even killing trees and woody crops in managed and natural landscapes (Solomon, 1995; Ocete et al., 2002, 2010). Fruit and nut trees, grapes, coffee, cacao, and vegetable and field crops are all attacked by cerambycids (Linsley, 1959). In addition to their economic importance, longhorn beetles might potentially be excellent indicator species of the health of the wood decomposer community because of their habitat specificities, and also of the changes in a variety of ecological processes because of their diverse adult-feeding behaviors (such as feeding on sap, twigs, pollen, nectar and leaves) (Speight, 1989, Vance et al., 2003). It is important to detect the Cerambycidae fauna of a region because the family could potentially be used as an indicator group of the future environmental monitoring studies.

The cerambycid fauna of Turkey is still poorly known although knowledge about Turkish longhorn beetles has increased considerably in the last few decades (Breuning, 1962, 1978; Demelt & Alkan, 1962; Demelt, 1963; Acatay, 1971; Gül-Zümreoğlu, 1975; Erdem & Çanakçıoğlu, 1977; Sama, 1982; Çanakçıoğlu, 1983; Adlbauer, 1988, 1992; Önalp, 1990; Lodos, 1998; Tezcan & Rejzek 2002; Tozlu et al., 2002; Özdikmen & Çağlar, 2004; Özdikmen & Hasbenli, 2004; Özdikmen & Demirel, 2005; Özdikmen et al., 2005; Malmusi & Saltani, 2005; Danilevsky, 2010). Detailed studies of the longhorn beetle fauna have not been conducted for all provinces, including Isparta. Isparta Province is situated in southwestern Turkey between Irano-Anatolian and Mediterranean Basin biodiversity hotspots (Sargın & Okudum, 2014; Conservation International, 2016). The province is bordered by the provinces of Afyonkarahisar to the north, Konya to the east, Antalya to the south and Burdur to the southwest.

During faunistic surveys of the beetles of Isparta Province between 2009-2013, beetles were collected from different localities including specimens of longhorn beetles. Additional species of Cerambycidae, among these specimens, were determined for Isparta Province. The main aim of this study was to make contributions to the longhorn beetle fauna of Isparta Province. Another aim was to make contributions to some ecological properties, such as vertical distribution and seasonality of the species collected.

Material and Methods

Study area

Isparta Province (Figure 1) is located in the transition region between the Mediterranean and Central Anatolian climates, so features of both climates occur. However, high temperatures and precipitation characteristic for the Mediterranean coastline and relatively lower temperatures and precipitation characteristic for the Central Anatolia climate are not completely effective in the study area. In the lower-lying area in the south of the Isparta Province, the Mediterranean climate is effective, while in the north of the Isparta Province the Central Anatolian climate is effective. During winter, the latter area is colder and has lower participation than the coastal area (Sargın & Okudum, 2014). Mean annual precipitation is 508 mm and the mean annual temperature 12.2°C, with July and August being the warmest months (Turkish General Directorate of State Meteorology, 2014). The province contains mountainous coniferous forests (*Pinus brutia* Ten., *Pinus nigra* spp. *pallasiana* (Lamb.) Asch. & Graebn., *Cedrus libani* A. Rich., *Abies cilicica* spp. *isaurica* (Ant. & Kotschy) Carr., *Juniperus excelsa* M. Bieb.,

Juniperus foetidissima Willd. and *Juniperus oxycedrus* Linnaeus), vast cultivated plains and a few large freshwater lakes. Apart from extensive areas of maquis vegetation, some mountain slopes and valleys are covered by scattered oak forest stands (*Quercus cerris* Linnaeus, *Quercus coccifera* Linnaeus, and *Quercus vulcanica* Boiss. & Heldr. ex Kotschy), both abandoned coppices and wood pastures currently grazed by domestic goats (Bergner et al., 2015; Güngör et al., 2015).

Figure 1. The study area (Anonymous, 2016).

Field methods

Longhorn beetles (Cerambycidae) were collected from various habitats in Isparta Province between 2009 and 2013. Beetles were collected from the herb layer by sweep netting and from the shrub and tree layers by beating branches over an umbrella. The beetles were killed using ethyl acetate. Information, including collection dates, geodetic coordinates, altitudes and vegetation types, were recorded. For evaluating altitude differentiation, sampling areas were determined as A: 750-1000 m B: 1001-1250 m, C: 1251-1500 m, D: 1501-1750 m.

Specimens were sorted and pinned. All pinned specimens were identified to species level using the keys in Bense (1995), Harde (1966) and Breuning (1962). Beetle samples were deposited at the Biology Department of Süleyman Demirel University, Isparta, Turkey.

New records from Isparta are marked with an asterisk after the species names.

Results and Discussion

In this study, a total of 53 species and subspecies belonging to 31 genera included in Cerambycidae were recorded from Isparta Province. Species collected during the study are given below. The number of individuals examined is shown in parentheses after the date of collection.

Subfamily: Lepturinae

Tribe: Lepturinii

Genus: *Anastrangalia* Casey, 1924

1. *Anastrangalia montana* (Mulsant et Rey, 1863)*

Material examined: Isparta: Dedegöl Mountain, 37°41'13" N, 31°22'35" E, 1197 m, 17.VII.2011 (1), Leg. Ö. D. Kaya.

Genus: *Etorofus* Matshushita, 1933

2. *Etorofus pubescens* (Fabricius, 1787)*

Material examined: Isparta: Dedegöl Mountain, 37°41'13" N, 31°22'35" E, 1197 m, 17.VII.2011 (1), 37°39'06" N, 31°21'45" E, 1284 m, 17.VII.2011, (1), 37°42'38" N, 31°20'21" E, 1413 m, 17.VII.2011, (1), Leg. Ö. D. Kaya.

Genus: *Pedostrangalia* Sokolov, 1897

3. *Pedostrangalia verticenigra* (Pic, 1892)*

Material examined: Isparta: Dedegöl Mountain, 37°41'13" N, 31°22'35" E, 1197 m, 17.VII.2011 (2), Leg. Ö. D. Kaya.

Genus: *Pseudovadonia* Lobanov, Danilevsky & Murzin, 1981

4. *Pseudovadonia livida* (Fabricius, 1776)

Material examined: Isparta: Davraz, 37°48'29" N, 30°46'48" E, 1603 m, 20.VI.2010 (2); Kızıldağ National Park, 38°02'08" N, 31°22'18" E, 1476 m, 26.V.2010 (1), Kovada Lake National Park, 37°37'38.99" N, 30°52'4.58" E, 926 m, 16.V.2010 (1), Leg. Ö. D. Kaya.

Genus: *Rutpela* Nakane & K. Ohbayashi, 1957

5. *Rutpela maculata* (Poda, 1761)*

Material examined: Isparta: Dedegöl Mountain, 37°42'38" N, 31°20'21" E, 1413 m, 17.VII.2011 (2), Leg. Ö. D. Kaya.

Genus: *Stenurella* Villiers, 1974

6. *Stenurella bifasciata* (Müller, 1776)*

Material examined: Isparta: Davraz, 37°48'29" N, 30°46'48" E, 1603 m, 20.VI.2010 (2); Kızıldağ National Park, 38°02'6.49" N, 31°22'26.20" E, 1487 m, 22.V.2010 (1); Kızıldağ National Park, 38°02'08" N, 31°22'18" E, 1476 m, 26.V.2010 (1). Leg. Ö. D. Kaya.

Genus: *Stictoleptura* Casey, 1924

7. *Stictoleptura fulva* (De Geer, 1775)

Material examined: Isparta: Dedegöl Mountain, 37°41'13" N, 31°22'35" E, 1197 m, 17.VII.2011 (4), 37°39'06" N, 31°21'45" E, 1284 m, 17.VII.2011 (1), 37°42'38" N, 31°20'21" E, 1413 m, 17.VII.2011, (2), Leg. Ö. D. Kaya.

Genus: *Vadonia* Mulsant, 1863

8. *Vadonia unipunctata* (Fabricius, 1787)

Material examined: Isparta; Kızıldağ National Park, 38°02’08" N, 31°22’18" E, 1476 m, 26.V.2010 (1), 38°02’09" N, 31°22’44" E, 1375 m, 15.V.2009 (1), 38°2’01" N, 31°22’26" E, 13.VI.2010 (1) Leg. İ. Şen

Tribe: Rhagiini

Genus: *Cortodera* Mulsant, 1863

9. *Cortodera flavimana* (Waltl, 1838)

Material examined: Isparta: Kovada Lake National Park, 37°36’59" N, 30°52’26" E, 931 m, 25.IV.2010 (2), Leg. Ö. D. Kaya.

Subfamily: Cerambycinae

Tribe: Callichromatini

Genus: *Aromia* Audinet-Serville, 1834

10. *Aromia moschata* (Linnaeus, 1758)

Material examined: Isparta: Eğirdir, 37°50’43.98" N, 30°53'27.77" E, 983 m, 08.VII.2011 (2), Leg. Ö. D. Kaya.

Tribe: Callidiini

Genus: *Phymatodes* Mulsant, 1839

11. *Phymatodes testaceus* (Linnaeus, 1758)*

Material examined: Kızıldağ National Park, 38° 2'6.49" N, 31°22'26.20" E, 1487 m, 22.V.2010 (2) Leg. Ö. D. Kaya.

Tribe: Cerambycini

Genus: *Cerambyx* Linnaeus, 1758

12. *Cerambyx dux* (Faldermann, 1837)

Material examined: Eğirdir, 37°50'43.98" N, 30°53'27.77" E, 983 m, 08.VII.2011 (2), Leg. Ö. D. Kaya.

Tribe: Certallini

Genus: *Certallum* Dejean, 1821

13. *Certallum ebulinum* (Linnaeus, 1767)

Material examined: Isparta: Kızıldağ National Park, 38°01'55" N, 31°22'42" E, 1363 m, 26.VI.2010 (2), 38°2'01" N, 31°22'26" E, 1427 m, 13.VI.2010 (1) Leg. Ö. D. Kaya.

Tribe: Clytini

Genus: *Chlorophorus* Chevrolat, 1863

14. *Chlorophorus sartor* (Fabricius, 1781)

Material examined: Isparta: Kızıldağ National Park, 38°02'08" N, 31°22'18" E, 1476 m, 26.V.2010 (2); Kovada Lake National Park, 37°36'51" N, 30°52'41" E, 913 m, 2.V.2010 (1); 37°37'43" N, 30°52'22" E, 913 m, 11.VII.2010 (1), Leg. Ö. D. Kaya.

15. *Chlorophorus trifasciatus* (Fabricius, 1781)*

Material examined: Isparta: Kovada Lake National Park, 37°36'51" N, 30°52'41" E, 913 m, 16.VI.2010 (1); Kızıldağ, 38°01'55" N, 31°22'42" E, 1363 m, 24.VII.2010 (1), Leg. Ö. D. Kaya.

16. *Chlorophorus varius* (Müller, 1766)

Material examined: Isparta: Kovada Lake National Park, 37°36'31" N, 30°53'35" E, 910 m, 25.VII.2010 (3), Leg. Ö. D. Kaya.

Genus: *Plagionotus* Mulsant, 1842

17. *Plagionotus bobelayei* (Brullé, 1832)

Material examined: Isparta: Kovada Lake National Park, 37°36'51" N, 30°52'41" E, 913 m, 5.VI.2010, (1), Leg. Ö. D. Kaya.

18. *Plagionotus floralis* (Pallas, 1773)

Material examined: Isparta: Davraz, 37°48'29" N, 30°46'48" E, 1603 m, 20.VI.2010 (1); Kızıldağ National Park, 38°01'52" N, 31°22'27" E, 1441 m, 26.VI.2010 (4); Kovada Lake National Park, 37°36'51" N, 30°52'41" E, 913 m, 16.VI.2010 (3), 37°36'33.47" N, 30°53'45.21" E, 914m, 9.V.2010 (2), Leg. Ö. D. Kaya.

Tribe: Graciliini

Genus: *Penichroa* Stephens, 1839

19. *Penichroa fasciata* (Stephens, 1831)*

Material examined: Isparta: Kovada Lake National Park, 37°36'31" N, 30°53'35" E, 910 m, 25.VII.2010, (3), Leg. Ö. D. Kaya.

Tribe: Hesperophanini

Genus: *Trichoferus* Wollaston, 1854

20. *Trichoferus fasciculatus* (Faldermann, 1837)*

Material examined: Isparta: Kovada Lake National Park, 37°37'32" N, 30°52'06" E, 927 m,

7-14.VIII.2010, (1), Leg. Ö. D. Kaya.

21. *Trichoferus kotschyi* Ganglbauer, 1883*

Material examined: Isparta: Kovada Lake National Park, 37°37'32" N, 30°52'06" E, 927 m,

7-14.VIII.2010, (1), Leg. Ö. D. Kaya.

Tribe: Hylotrupini

Genus: *Hylotrupes* Audinet-Serville, 1834

22. *Hylotrupes bajulus* (Linnaeus, 1758)

Material examined: Isparta: 37°45'48.59" N, 30°30'23.71" E, 1172m, 3.VII.1999, (3), Süleyman Demirel University Campus, 37°49'51.04" N, 30°31'17.77" E, 1061 m, 20.VIII.2012 (2), Leg. A. Gök, Leg. Ö. D. Kaya.

Tribe: Purpuricenini

Genus: *Calchaenesthes* Kraatz, 1863*

23. *Calchaenesthes oblongomaculata* (Guérin-Meneville, 1844)

Material examined: Isparta: Kovada Lake National Park, 37°36'51" N, 30°52'41" E, 913 m, 30.V.2010 (1), Leg. Ö. D. Kaya.

Genus: *Purpuricenus* Dejean, 1821

24. *Purpuricenus budensis* (Götz, 1783)

Material examined: Isparta: Süleyman Demirel University Campus, 37°49'59.93" N, 30°32'57.23" E, 1088 m, 08.VII.2011 (3); Kovada Lake National Park, 37°37'43" N, 30°52'22" E, 913 m, 11.VII.2010 (1), Leg. Ö. D. Kaya.

25. *Purpuricenus dalmatinus* Sturm, 1843*

Material examined: Isparta: Kovada Lake National Park, 37°37'44" N, 30°52'22" E, 914 m, 23.V.2010, (4), 30.V.2010 (1); Süleyman Demirel University Campus37°49'59.93" N, 30°32'57.23" E, 1088 m, 08.VII.2011 (1) Leg. Ö. D. Kaya.

26. *Purpuricenus desfontainii* (Fabricius, 1792)*

Material examined: Isparta: Süleyman Demirel University Campus, 37°49'59.93" N, 30°32'57.23" E, 1088 m, 08.VII.2011 (2) Leg. Ö. D. Kaya.

Subfamily: Lamiinae

Tribe: Acanthoderini

Genus: *Acanthoderes* Audinet-Serville, 1835

27. *Acanthoderes clavipes* (Schrank, 1781)*

Material examined: Isparta: 37°51'23" N, 30°28'34" E, 1102 m, 18.VI.2013, (1), Leg. Ö. D. Kaya.

Tribe: Agapanthiini

Genus: *Agapanthia* Audinet-Serville, 1835

28. *Agapanthia kirbyi* (Gyllenhal, 1817)

Material examined: Isparta: Davraz, 37°48'42"K, 30°45'13"D, 1541 m, 20.VI.2010 (1), Leg. Ö. D. Kaya.

29. *Agapanthia lateralis* Ganglbauer, 1884

Material examined: Isparta:Süleyman Demirel University Campus, 37°49'59.93" N, 30°32'57.23" E, 1088 m, 8.VIII.2011 (5), Leg. Ö. D. Kaya.

30. *Agapanthia violacea* (Fabricius, 1775)

Material examined: Isparta: Kızıldağ National Park, 38°01'37" N, 31°23'10" E, 1299 m, 1.V.2010 (3) Leg. Ö. D. Kaya.

Genus: *Calamobius* Guerin-Meneville, 1847

31. *Calamobius filum* (Rossi, 1790)

Material examined: Isparta: Davraz, 37°48'42"K, 30°45'13"D, 1541 m, 20.VI.2010 (1); Kızıldağ National Park, 38°01'37" N, 31°23'10" E,1299 m, 1.V.2010 (2); Kovada Lake National Park, 37°36'51" N, 30°52'41" E, 913 m, 1.V.2010 (4); 2.V.2010 (1) Leg. Ö. D. Kaya.

Genus: *Theophilea* Pic, 1895

32. *Theophilea cylindricollis* Pic, 1895*

Material examined: Isparta: Kovada Lake National Park, 913 m, 10.V.2011 (30); Leg. Ö. D. Kaya.

Tribe: Dorcadiini

Genus: *Dorcadion* Dalman, 1817

33. *Dorcadion anatolicum* Pic, 1900

Material examined: Isparta; Davraz 3, 3.VII.2010 (3), Davraz, 37°48'29" N, 30°46'48" E, 1603 m, 20.VI.2010 (1), Leg. Ö. D. Kaya.

34. *Dorcadion mniszechi* Kraatz, 1873*

Material examined: Isparta; Davraz 7, 3.VII.2010 (1), Leg. Ö. D. Kaya.

35. *Dorcadion smyrnense* (Linnaeus, 1757)*

Material examined: Isparta; Davraz 1, 6.VI.2010 (2), 23.V.2010, (3), Kızıldağ National Park, 38°2'6.49" N, 31°22'26.20" E, 1487 m, 22.V.2010 (1), Leg. Ö. D. Kaya.

Tribe: Lamiini

Genus: *Morimus* Brullé, 1832

36. *Morimus asper* (Sulzer, 1776)*

Material examined: Isparta: Dedegöl Mountain, 37°41'01" N, 31°21'21" E, 1277 m, 8.VII.2011 (2) Leg. Ö. D. Kaya.

37. *Morimus orientalis* Reitter, 1894*

Material examined: : Isparta: Dedegöl Mountain, 37°41'01" N, 31°21'21" E, 1277 m, 8.VII.2011 (1) Leg. Ö. D. Kaya.

Tribe: Monochamini

Genus: *Monochamus* Dejean, 1821

38. *Monochamus galloprovincialis* (Olivier, 1795)

Material examined: Isparta: Dedegöl Mountain, 37°40'01" N, 31°21'25" E, 1305 m, 8.VII.2011 (1) Leg. Ö. D. Kaya.

Tribe: Phytoeciini

Genus: *Oberea* Dejean, 1835

39. *Oberea erythrocephala* (Schrank, 1776)*

Material examined: Isparta: Davraz, 37°48'29" N, 30°46'48" E, 1603 m, 20.VI.2010 (3); Leg. İ. Şen.

40. *Oberea oculata* (Linnaeus, 1758)

Material examined: Isparta: Dedegöl Mountain, 37°42'38" N, 31°20'21" E, 1413 m, 17.VII.2011, (1), Leg. Ö. D. Kaya.

Genus: *Opsilia* Mulsant, 1863

41. *Opsilia coerulescens* (Scopoli, 1763)

Material examined: Isparta: Kovada Lake National Park, 37°36'51" N, 30°52'41" E, 913 m, 12.VII.2010, (2), Kızıldağ National Park, 38° 2'6.49" N, 31°22'26.20" E, 1487 m, 22.V.2010, (1) Leg. Ö. D. Kaya.

Genus: *Phytoecia* Dejean, 1835

42. *Phytoecia (Blepisanis) vittipennis* (Reiche, 1877)*

Material examined: Isparta: Kızıldağ National Park, 38°1'59.72" N, 31°22'35.81" E, 1383 m, 08.VIII.2010 (5), Leg. Ö. D. Kaya.

43. *Phytoecia (Helledia) armeniaca* Frivaldszky, 1878*

Material examined: Isparta: Sermet, 37°45'50" N, 30°34'03" E, 1036 m, 16.VI.2010 (1), Leg. İ. Şen.

44. *Phytoecia (Helladia) humeralis* (Waltl, 1828)

Material examined: Isparta: Kızıldağ National Park, 38° 1'59.72" N, 31°22'35.81" E, 1383 m, 08.VIII.2010 (2), 18.V.2010 (2); Leg. Ö. D. Kaya.

45. *Phytoecia (Helladia) praetextata* (Steven, 1817)*

Material examined: Isparta: Kızıldağ National Park, 37°36'59" N, 30°52'26" E, 931 m, 25.IV.2009 (1), Leg. İ. Şen.

46. *Phytoecia (Helladia) millefolii* (Adams, 1817)*

Material examined: Isparta: Kovada Lake National Park, 37°36'51" N, 30°52'41" E, 913 m, 30.V.2010 (1), Leg. Ö. D. Kaya.

47. *Phytoecia (Phytoecia) caerulea* spp. *baccueti* Brullé, 1832

Material examined: Isparta: Kızıldağ National Park, 18.V.2010 (2); Sivritepe, 37°49'49" N, 30°28'33" E, 1237 m, 23.IV.11 (1); Kızıldağ National Park, 38°01'51" N, 31°22'02" E, 26.IV.09 1614m (1); 38°01'37" N, 31°23'10" E, 1299 m, 1.V.2010 (1), 29.V.2010 (2), Leg. Ö. D. Kaya.

48. *Phytoecia (Phytoecia) caerulea* spp. *caerulea* Scopoli, 1772

Material examined: Isparta: Kızıldağ National Park, 38° 2'6.49" N, 31°22'26.20" E, 1487 m, 18.V.2010 (3) Leg. Ö. D. Kaya.

49. *Phytoecia (Phytoecia) cylindrica* (Linnaeus, 1758)*

Material examined: Isparta: Kovada Lake National Park, 37°36'59" N, 30°52'26" E, 931 m, 25.IV.2010 (2); Leg. Ö. D. Kaya.

50. *Phytoecia (Phytoecia) geniculata* Mulsant, 1863*

Material examined: Isparta: Sivritepe, 37°49'37.24" N, 30°27'49.13" E, 1411 m, 20.IV.2011 (1), Leg. Ö. D. Kaya.

51. *Phytoecia (Phytoecia) manicata* Reiche & Saulcy, 1858

Material examined: Isparta: Kızıldağ National Park, 38° 1'59.72" N, 31°22'35.81" E, 1383 m, 08.VIII.2010 (2), Leg. Ö. D. Kaya.

52. *Phytoecia (Phytoecia) virgula* (Charpentier, 1825)

Material examined: Isparta: Kızıldağ National Park, 38°01'37" N, 31°23'10" E, 1299 m, 1.V.2010 (2); Kovada Lake National Park, 37°36'51" N, 30°52'41" E, 913 m, 30.V.2010 (1), Leg. Ö. D. Kaya.

Tribe: Saperdini

Genus: *Saperda* Fabricius, 1775

53. *Saperda populnea* (Linnaeus, 1758)*

Material examined: Isparta: 37°51'23" N, 30°28'34" E, 1102 m, 18.VI.2013 (1), Leg. Ö. D. Kaya.

It was observed that the vast majority of Cerambycidae fauna in the research area consists of species belonging to three subfamilies, Lamiinae (51%, Figures 2 and 3), Cerambycinae (32%, Figures 2 and 4) and Lepturinae (17%, Figures 2 and 5). *Phytoecia* was represented with the highest number of species (11 species). This genus was followed by *Agapanthia*, *Chlorophorus*, *Purpuricenus* and *Dorcadion* each with three species, *Plagionotus*, *Trichoferus, Morimus* and *Oberea* each with two species, *Anastrangalia, Etorufus, Pedostrangalia, Pseudovadonia, Rutpela, Stenurella, Stictoleptura*, *Vadonia*, *Cortodera*, *Cerambyx*, *Certallum*, *Penichroa, Aromia, Hylotrupes, Calchaenestes, Saperda, Acanthoderes, Monochamus, Opsilia, Calamobius* and *Theophilea* genera each with a single species.

Cerambycidae fauna of the Mediterranean Region of Turkey (including Antalya, Burdur, Isparta, İçel, Adana, Osmaniye, Hatay, Kahramanmaraş and Kilis Provinces) was reviewed by Özdikmen (2011), which included 57 cerambycid species for Isparta Province. Sama et al. (2012) added 20 more species to the list for the province. In total, 77 longhorn beetle species had previously been recorded from Isparta Province. In this study, 53 cerambycid species belonging to 31 genera included in three subfamilies (Lepturinae, Cerambycinae and Lamiinae) were detected. Then number of species detected for each genera previously recorded in Turkey (Löbl & Smetana, 2010; Özdikmen, 2012; Sama et al., 2012) was as follows (detected/recorded): Acanthoderes (1/2), Agapanthia (3/30), Anastrangalia (1/3), Aromia (1/1), Calamobius (1/1), Calchaenesthes (1/2), Cerambyx (1/8), Certallum (1/2), Chlorophorus (3/13), Cortodera (1/23), Etorofus (1/1), Hylotrupes (1/1), Monochamus (1/2), Morimus (2/2), Oberea (2/6), Opsilia (1/1), Pedostrangalia (1/7), Penichroa (1/1), Phymatodes (1/9), Phytoecia (11/65), Plagionotus (2/6), Pseudovadonia (1/1), Purpuricenus (3/10), Rutpela (1/1), Saperda (1/7), Stenurella (1/7), Stictoleptura (1/16), Theophilea (1/1), Trichoferus (2/10), Vadonia (1/5). Among these species, 26 of them were new records, so the longhorn beetles fauna of Isparta Province has been increased to 103 species and the distribution of previously known species broadened.

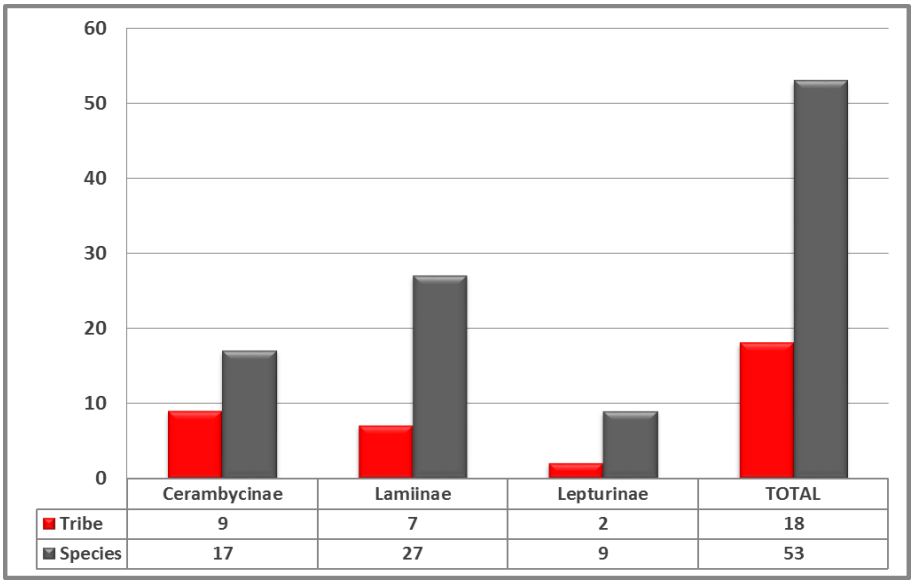

Figure 2. Number of tribes and species in the subfamilies of Cerambycidae in Isparta Province, Turkey.

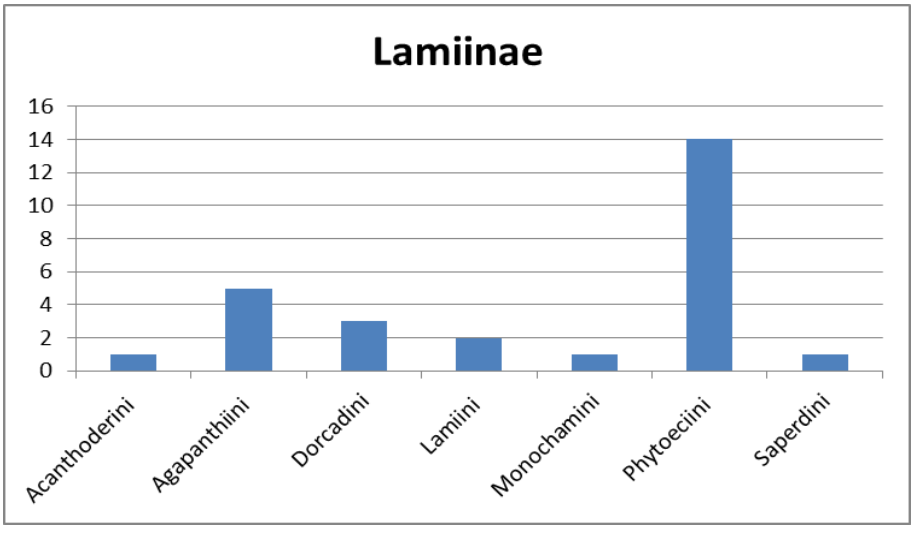

Figure 3. Number of species in tribes of the Cerambycidae tribe, Lamiinae, in Isparta Province, Turkey.

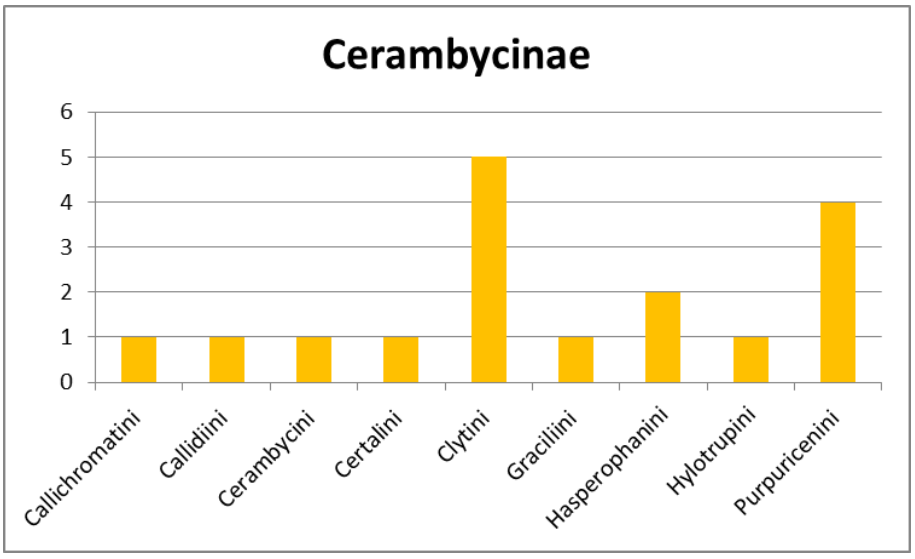

Figure 4. Number of species in tribes of the Cerambycidae tribe, Cerambycinae, in Isparta Province, Turkey.

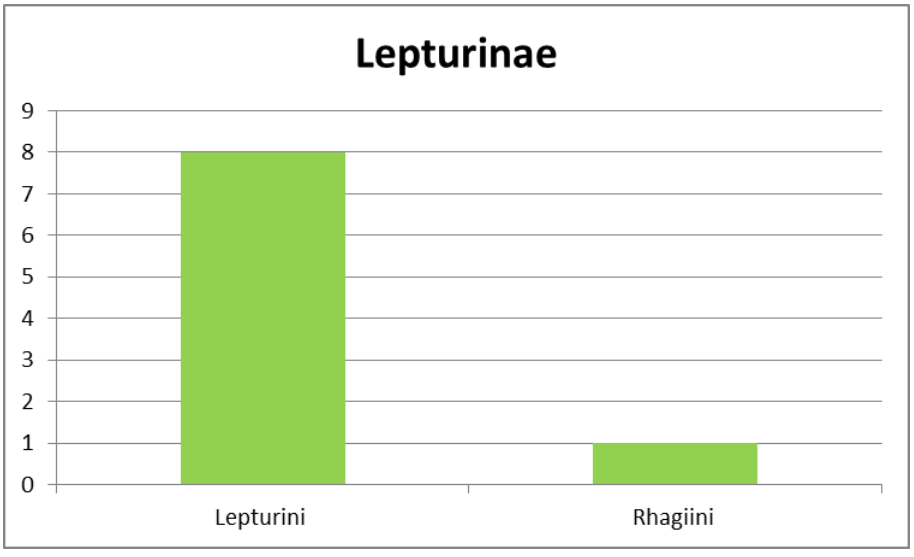

Figure 5. Number of species in tribes of the Cerambycidae tribe, Lepturinae, in Isparta Province, Turkey.

Theophilea cylindricollis had the highest number of individuals detected (30). This species was followed by *C. filum* (8), *P. caerulea baccueti*, *S. fulva* (7), *D. smyrnense*, *P. dalmatinus* (6) and *H. bajulus*, *P. vittipennis* (5).

Examination of the month of collection in the field over the two years, as well as those already in the collection of the Biology Department, Suleyman Demirel University revealed that six species had been collected 6 in April, 18 in May, 13 in June, 22 in July and 6 in August (Figures 6 and 7). It is can be seen that Cerambycidae species are most active in the May and July, followed by June. April and August had the least collections. Cerambycidae species can be monophagous, oligophagous or polyphagous in many different tree species. Adaptation to either conifers or broadleaf trees is evident in most cases (Bense, 1995). Cerambycidae is a highly plant dependent group and its development is directly influenced by factors such as temperature and humidity. Therefore climatic factors in the study area are likely to have influenced the species present in the samples.

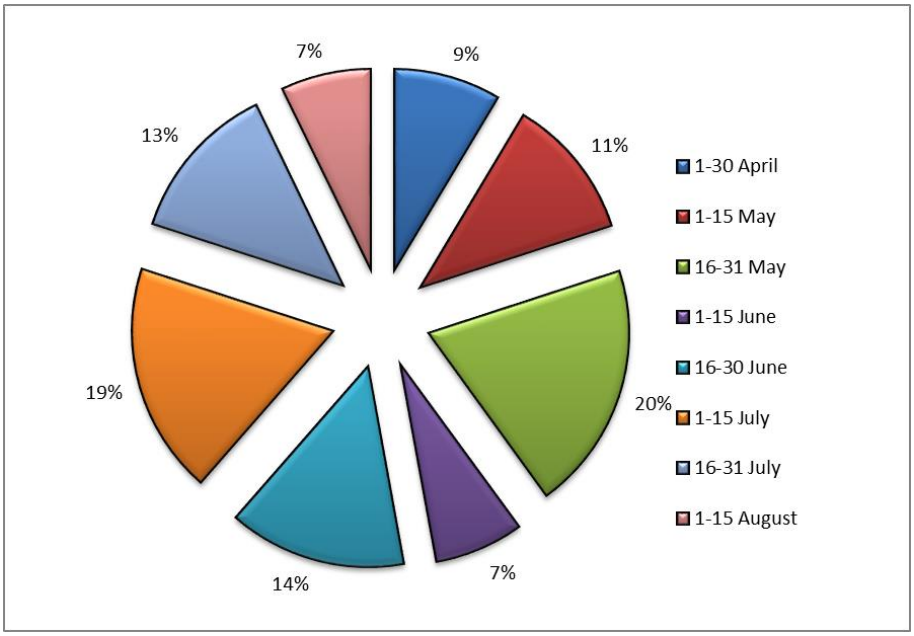

Figure 6. Percentage of Cerambycidae specimens collected from April and August in Isparta Province, Turkey.

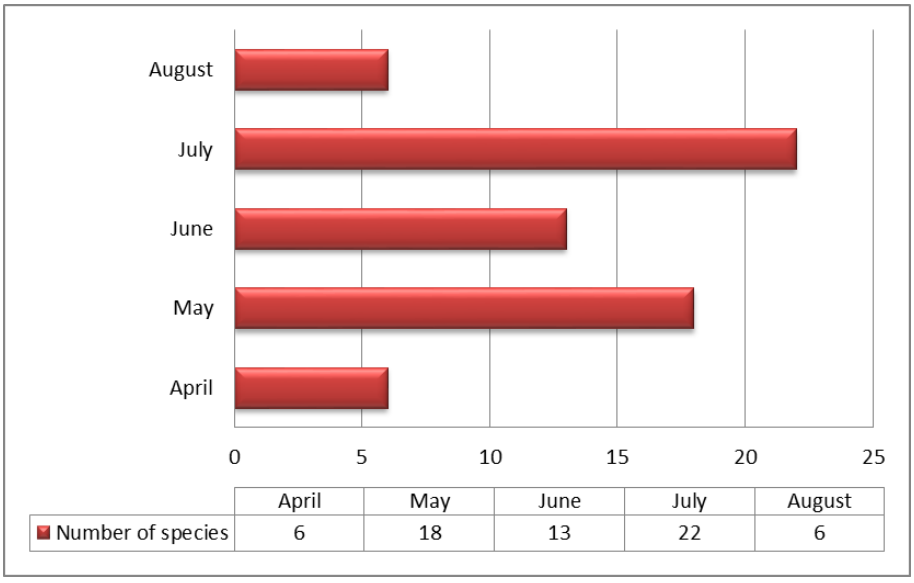

Figure 7. Number of Cerambycidae species collected from April to August in Isparta Province, Turkey.

Specimens were collected from different altitudes and vertical distributions of species exhibited differences. Evaluation of the results showed that there was differences number of species collected in each altitude range (Figure 8).

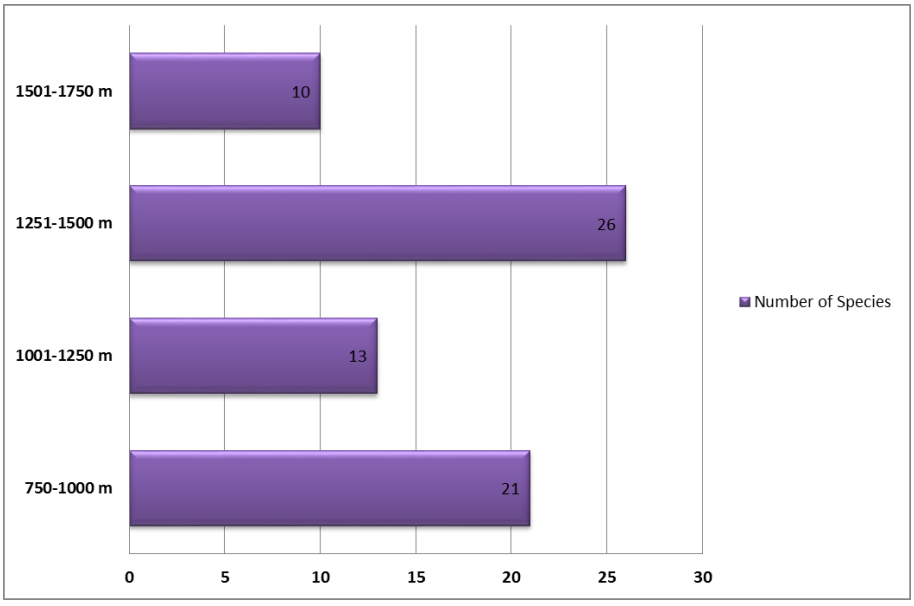

Figure 8. Number of Cerambycidae species collected in four altitude ranges in Isparta Province, Turkey.

Among the species collected, *Pedostrangalia verticenigra* (Pic, 1892) is notable because of its area of distribution. According to the distribution records, the species was previously only known from the East Black Sea Region (Artvin, Erzurum and Rize Provinces) of Turkey. It was considered a local endemic for those provinces until it was recorded on Samos Island, Greece by Dauber (2004). Although, Samos Island is close to Western Anatolia, the known distribution areas of the species are quite distant from each other. The present record shows that the species may be distributed more widely in western Turkey, but additional surveys are needed to confirm this possibility.

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Original article (Orijinal araştırma)

İzmir ve Manisa illeri kestane ağaçlarında *Parthenolecanium rufulum* (Cockerell) (Hemiptera: Coccidae)'un yayılışı biyolojisi ve doğal düşmanlarının belirlenmesi

Determination of the distribution, biology and natural enemies of *Parthenolecanium rufulum* (Cockerell) (Hemiptera: Coccidae) on chestnut trees in İzmir and Manisa provinces in Turkey

Cevdet KAPLAN[1*] **Tevfik TURANLI[2]**

Summary

This study was conducted to determine the distribution, biology, parasitoids and predators of *Parthenolecanium rufulum* (Cockerell) (Hemiptera: Coccidae), which is harmful on chestnut areas in İzmir and Manisa provinces between the years of 2012 to 2014. The study was carried out with weekly and biweekly periods from February to November. The natural enemies of the pest were investigated by visual observations, examining laboratory culture, and using strike method. As a result, it was determined that the *P. rufulum* distributed only in districts of Kemalpaşa and Turgutlu. *P. rufulum* has one generation per year and in overwinters as the 2nd instar nymph. Molting to adult female occurred the last days of April. Egg hatching occurred in late June and ended nearly two weeks later. Second instar nymphs were observed in late August. The second instar nymph's migration from leaves to twigs started at mid-October and the whole population was settled on branches from mid-November to overwinter. Total parasitism ratio of adult with and without egg was 3.8-18.6%. *Pachyneuron aphidis* Bouche and *Pachyneuron muscarum* (L.) (Hymenoptera: Pteromalidae), *Cheiloneurus* sp. and *Microterys* sp. (Hymenoptera: Encyrtidae), *Colesterocerum* sp. (Hymenoptera: Eulopidae) were parasitoid species and, *Adalia bipunctata* (L.), *Adalia fasciatopunctata revelierei* Mulsant, *Chilocorus bipustulatus* (L.), *Coccinella septempunctata* (L.), *Oenopia (Synharmonia) conglobate* (L.) (Coleoptera: Coccinellidae) and *Chrysopa* sp. (Neuroptera: Chrysopidae) were predator species that were determined as natural enemies of the pest.

Key words: Biology, chestnut, distribution, natural enemy, *Parthenolecanium rufulum*

Özet

Bu çalışma, 2012-2014 yıllarında kestane alanlarında zararlı *Parthenolecanium rufulum* (Cockerell) (Hemiptera: Coccidae)'un İzmir ve Manisa illerinde yayılışı, biyolojisi, parazitoit ve predatörlerinin belirlenmesi amacıyla yürütülmüştür. Çalışma, şubat ve kasım aylarında haftalık ve iki haftalık periyotlarla yapılan arazi çıkışlarıyla gerçekleştirilmiştir. Zararlının doğal düşmanlarının tespiti kestaneliklerde gözle kontrol, darbe metodu ve laboratuvarda kültüre alınan zararlının gözlemlenmesiyle belirlenmiştir. Çalışma sonucunda, *P. rufulum*'un sadece Kemalpaşa ve Turgutlu ilçelerindeki kestaneliklerde yayılış gösterdiği belirlenmiştir. *P. rufulum*'un yılda bir döl verdiği ve kışı 2. nimf döneminde 1-2 yıllık sürgünlerde geçirdiği saptanmıştır. Ergin dişiler nisan ayının sonuna doğru oluşmaktadır. Yumurta açılımı haziran ayının sonunda başlamakta ve yaklaşık iki hafta sonra bitmektedir. İkinci dönem nimflere ağustos sonunda rastlanmıştır. İkinci dönem nimflerin ekim ayının ortasından itibaren yapraklardan sürgünlere geçmeye başladığı ve tüm nimflerin kışlamak üzere kasım ortasına kadar sürgünlere yerleştikleri belirlenmiştir. Yumurtalı ve yumurtasız ergin dişilerde toplam parazitlenme oranının %3.8-18.6 olduğu belirlenmiştir. *Pachyneuron aphidis* Bouché ve *Pachyneuron muscarum* (L.) (Hymenoptera: Pteromalidae), *Microterys* sp. ve *Cheiloneurus* sp. (Hymenoptera: Encyrtidae), *Colesterocerum* sp. (Hymenoptera: Eulopidae) zararlının parazitoitleri olarak; *Adalia bipunctata* (L.), *Adalia fasciatopunctata revelierei* Mulsant, *Chilocorus bipustulatus* (L.), *Coccinella septempunctata* (L.), *Oenopia* (*Synharmonia*) *conglobata* (L.) (Coleoptera: Coccinellidae) ve *Chrysopa* sp. (Neuroptera: Chrysopidae) ise predatörleri olarak tespit edilmiştir.

Anahtar sözcükler: Biyoloji, kestane, yayılış, doğal düşmanlar, *Parthenolecanium rufulum*

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Giriş

Kestane (*Castanea sativa* Mill.), Türkiye'de Marmara ve Kuzey Anadolu'da özellikle meşe, gürgen, kayın, ıhlamur gibi yapraklı bitki türleriyle karışık halde bulunmaktadır. Ege ve Akdeniz bölgesinde yerel olarak doğal yayılış göstermekle birlikte, daha çok kültürü de yapılmaktadır. Kestane, gıda ve ahşap sanayinde kereste olarak ve ayrıca ekolojik ve peyzaj amaçlı olarak da Akdeniz Bölgesi'nde ekonomik öneme sahip önemli bir ağaç türüdür (Santos et al., 2014). Dünyada yaklaşık 2 milyon ton kestane meyvesi üretimi yapılmaktadır (Anonymous, 2016a). Türkiye, 60 bin ton kestane üretimi ile en büyük üretici konumundaki Çin'den sonra dünyada ikinci sırada yer almakta ve dünya kestane üretiminin yaklaşık %3'ünü karşılamaktadır. Türkiye'deki kestane ağacı varlığı 2.373 bin adet ağaç olup bunun %61.88'ini ve kestane üretiminin %63.28'ini Ege Bölgesi karşılamaktadır. İzmir ve Manisa illeri ise Türkiye'deki kestane ağaç varlığının %20.67'ünü ve kestane üretiminin ise %21.36'sını sağlamaktadır (Anonymous, 2016b). Kestane özellikle İzmir'in Beydağ ve Ödemiş ilçelerinde üreticilerin temel geçim kaynağını oluşturmaktadır.

Türkiye'de son yıllarda kestane ağaçlarında meydana gelen kurumaların kestane kanseri (*Cryphonectria parasitica*)'nin yanı sıra, gövde ve dallarda zarar yapan zararlılardan da kaynaklandığı bildirilmiştir (Karagöz & Gençsoylu, 2004; Çeliker & Onoğur, 2011; Kaplan & Turanlı, 2011). Orman Genel Müdürlüğü tarafından hazırlanan 2013-2017 yıllarını kapsayan Kestane Eylem Plan Raporu ile öngörülen faaliyetler neticesinde, kestane alanlarının genişletilmesi, iyileştirilmesi, hastalık ve zararlıları ile mücadele edilmesi, mevcut kestane sahalarından azami seviyede faydalanılması hedeflenmektedir (Anonymous, 2014).

Ege Bölgesi'nde kestane ağaçlarında varlığı daha önce bilinmeyen *Parthenolecanium rufulum* (Cockerell) (Hemiptera: Coccidae), üreticilerden gelen şikâyetler üzerine yapılan surveylerde tespit edilmiştir. Bir Coccid olan bu zararlı yüksek popülasyon yoğunluklarında önemli zararlara neden olabilmektedir. Bu türlerin birçoğu meyve ağaçlarında, bağlarda, orman ağaçlarında, park ve süs bitkilerinde beslenmekte ve önemli zararlar oluşturmaktadır (Kaydan et al., 2013). Coccid türleri bitkileri sokup emerek beslenmeleri sonucu, bitki gelişiminde durgunluk, yapraklarda sararma ve zamanından önce dökülmeler görülmekte, meyvelerde kalite ve verim düşmektedir. Bu türlerin, beslenmeleri sonucu bitkiler üzerinde oluşan ballı madde üzerinde saprofit mantarların gelişmesiyle karaballık veya fumajin meydana gelmektedir. Fumajin, bitki yüzeyini kaplayarak, fotosentez yapımını engellemekte ve böylece bitkilerin gördüğü zarar artmaktadır.

Parthenolecanium rufulum'un bulunduğu bazı ülkelerde yapılan araştırmalarda türün tanımı, konukçuları, biyolojisi ve popülasyon yoğunluğu hakkında bilgilere rastlanmaktadır (Kosztarab & Kozar, 1988; Rainato & Pellizzari, 2009). Türkiye'de *P. rufulum* ile ilgili detaylı bir çalışma bulunmamaktadır. Sadece dar bir alanı kapsayan tespit çalışmaları mevcuttur. Türkiye'de *P. rufulum* Ankara, Amasra, Bartın, Balıkesir, Bursa, Giresun, İstanbul, Kastamonu, Muğla, Ordu ve Trabzon'da çoğunlukla fındık ve meşe ağaçlarında bulunduğu kaydedilmiştir (Bodenheimer, 1953,1958; Erdem, 1968; Kurt,1982; Ecevit et al., 1987; Toros & Hancıoğlu,1997; Zeki et al., 2004; Kaydan et al., 2007, 2014; Ülgentürk & Toros, 1999; Ülgentürk et al., 2008, 2013).

Kestanede gövde, dal ve sürgünlerde zarar yapan türler önemlidir. Sürgün ve dallarda zarar yapan türler içerisinde Coccid türleri zaman zaman önemli oranda zarara yol açmaktadır. Ancak bunlarla beslenen farklı cinslere bağlı çok sayıda parazitoit ve predatör türler de bulunmaktadır. İzmir ve Manisa illerinde daha önce kestanede zararlı Coccid türler ve doğal düşmanları üzerinde herhangi bir çalışma yürütülmemiştir. Bu çalışma ile *P. rufulum*'un İzmir ve Manisa illerindeki kestane alanlarındaki yayılışı, bulaşma oranı, bazı biyolojik özellikleri ile parazitoit ve predatörlerin belirlenmesi ile ileride yapılacak mücadele programları ve hazırlanacak teknik talimatlar için veri oluşturacaktır.

Materyal ve Yöntem

Parthenolecanium rufulum'un yayılışının ve yoğunluğunun belirlenmesi

İzmir ve Manisa illeri kestane alanlarında bulunan *P. rufulum'*un yayılış alanlarını belirlemek amacıyla 2012-2014 yılları arasında survey yapılmıştır. Surveyler, kestane yetiştiriciliğinin yaygın olarak yapıldığı İzmir'in Beydağ, Kemalpaşa ve Ödemiş ilçeleri ile Manisa'nın Turgutlu ve Sarıgöl ilçelerinde yapılmıştır İzmir ve Manisa illeri kestane alanları, üretim miktarı ve ağaç varlıkları Çizelge 1'de verilmiştir.

Çizelge 1. İzmir ve Manisa illeri kestane alanları, üretim miktarı ve ağaç sayıları (Anonymous, 2016b)

İl	Alan	Üretim (ton)	Meyve veren yaşta ağaç sayısı	Meyve vermeyen yaşta ağaç sayısı	Toplam ağaç sayısı
İzmir	25.257	9.742	374.300	48.050	422.350
Manisa	3.880	2.482	57.375	20.995	63.370

Survey çalışmaları sırasında seçilen bahçelerde incelenen ağaç sayısı Lazarov & Grigorov (1958)'un survey metoduna göre yapılmıştır.

1-20 ağaç olan bahçenin tüm ağaçları
21-70 ağaç olan bahçeden 10-30 ağaç
71-150 ağaç olan bahçeden 31-40 ağaç
151-500 ağaç olan bahçeden 41-80 ağaç
501-1000 ağaç olan bahçenin % 15'i
1000'denfazla ağaç olan bahçenin ise %5'i incelenmiştir

Survey için seçilen kestaneliklerde her iki köşegen boyunca yürünerek bahçeyi temsil edecek nitelikte ve sayıda ağaçta gözlem ve inceleme yapılmıştır. Örnekleme için seçilen her ağaç 4 yönden göz ile incelenmiş ve genel durum dikkate alınarak *P. rufulum* varlığı ve popülasyon yoğunluğu değerlendirilmiştir (Özgen & Bolu, 2009). İzmir ilinde kestane ağaçlarının %0,49'u Manisa ilinde ise kestane ağaçların %0,19'u kontrol edilmiştir.

Parthenolecanium rufulum' un sürgünlerdeki yoğunluğunu belirlemek için mart ayında dal üzerindeki 2. dönem nimf ve haziran ayında ise ergin dişi sayımı yapılarak sürgündeki nimf ve ergin yoğunluğu belirlenmeye çalışılmıştır. Bunun için Kemalpaşa ve Turgutlu'daki bulaşık bahçelerden bahçeleri temsil edecek nitelikte 5 ağaçtan 1-2 yıllık 20 cm uzunlukta, toplam 20 dal parçası kesilmiştir ve bunlar önce kese kâğıdı, daha sonra polietilen torbalara yerleştirilerek buz kutusu ile laboratuvara getirilmiştir. Laboratuvara getirilen dalların üzerindeki *P. rufulum* bireyleri sayılarak sürgün veya dal başına ortalama birey sayısı belirlenmiştir (Anonymous, 2011). Laboratuvara getirilen bulaşık dal ve sürgünler, içerisinde su bulunan kavanozlarda bekletilmiş ve bunlar üzerindeki bireyler teşhis için uygun olgunluğa geldikten sonra % 70'lik alkole aktarılmış ve konunun uzmanına gönderilmiştir.

***Parthenolecanium rufulum'un* biyolojisi**

Parthenolecanium rufulum'un kışı nerede, hangi dönemde geçirdiği ve yumurtalarının ne zaman açıldığı, kaç nimf dönemi geçirdiği, nimflerin sürgünler geçiş zamanı ile ilgili gözlemler 2013 ve 2014 yıllarında özellikleri Çizelge 2'de belirtilen Kemalpaşa ve Turgutlu'daki zararlı ile bulaşık iki bahçede yürütülmüştür. Biyolojik dönemlerin tespiti 100 birey üzerinde yapılmış ve yıl içerisinde her biyolojik dönemin bulunduğu periyotlar grafik halinde gösterilmiştir. Nimf dönemlerinin ayrımından Rainato & Pellizzari (2009)'den yararlanılmıştır. Bahçelerdeki gözlemler kritik dönemlerde (mart- temmuz) haftalık diğer dönemlerde ise 2-3 haftalık aralıklarla yapılmıştır. Ayrıca iklim odasında (25±1ºC ve % 65±5 orantılı nem ve 16:8 saat aydınlanma koşulları) 20 adet ergin dişi birey üzerinde yumurta verimi ve yumurta açılımı takip edilmiştir.

İklim verileri (sıcaklık ve orantılı nem) Kemalpaşa ilçesinde en yakın Meteoroloji istasyonundan, Turgutlu ilçesinde ise çalışmaların yürütüldüğü bahçeye konulan "HOBO On Set Data Logger" kayıt cihazından alınmış ve değerlendirilmiştir. HOBO kayıt cihazında bazı aylarda kayıt alınamamıştır.

***Parthenolecanium rufulum*'un parazitoit ve predatörleri ile parazitlenme oranın belirlenmesi**

Bu çalışmalar, *P. rufulum* ile bulaşık olan Kemalpaşa ve Turgutlu'daki bahçelerde 2013 ve 2014 yıllarında yapılmıştır. Zararlının parazitoitlerini ve parazitlenme oranını belirlemek için mayıs ve haziran aylarında *P. rufulum* ile bulaşık bahçelerde (Çizelge 2) bahçeyi temsil edecek nitelikte 5 ağacın her birinden 20 cm uzunlukta, 1-3 yıllık daldan her bahçeden toplam 20 dal parçası kesilmiştir. Kesilen dal örnekleri önce kese kâğıdına, sonra polietilen torbalara yerleştirilerek buz kutusu içinde laboratuvara getirilmiştir. Dal örnekleri *P. rufulum* dışındaki türler temizlendikten sonra içi su dolu küçük kavanozlara yerleştirilmiştir. Bu

şekilde hazırlanan örnekler etrafı karartılmış daha büyük boydaki plastik kavanozlara konulmuştur. Buradan çıkan parazitoitleri toplamak amacıyla açık tarafı kavanozun içine gelecek şekilde her bir kavanoza bir cam tüp yerleştirilmiştir. Kavanozlara konulan zararlı ile bulaşık dal örnekleri iklim odasında (25±1ºC ve % 65±5 orantılı nem ve 16:8 saat aydınlanma koşulları) kültüre alınmış ve tüpler günlük olarak kontrol edilmiştir. Tüp içinde toplanan parazitoit erginleri alınarak morfolojik özelliklerine göre birbirinden ayrılmış ve tanı için hazırlanmıştır (Özgen & Bolu,2009). Parazitoit çıkışı sona erdikten sonra tüm dal ve sürgünlerdeki parazitli olan ve olmayan bireyler sayılarak parazitlenme oranı ve çıkış yapan parazitoit türler belirlenmiştir. Parazitlenme oranı (%) ise Parazitli bireyx100/Toplam birey sayısı formülü ile hesaplanmıştır.

Çizelge 2. İzmir ve Manisa illeri kestaneliklerinde zararlı *Parthenolecanium rufulum* (Cockerell)'un biyolojisi ile ilgili gözlemlerin yapıldığı kestanelikler ve özellikleri

İl	İlçe	Köy	Bahçedeki ağaç sayısı (adet)	İncelenen ağaç sayısı (adet)	Rakım	Açıklama
İzmir	Kemalpaşa	Ovacık	116	42	38*22'30.9''K, 27*40'31.5''D, yükseklik 726 m	Kimyasal mücadele yapılmayan meşe ormanı kenarında
Manisa	Turgutlu	Hacıisalar	90	35	38*21'53,87''K, 27*49'20.49'D, yükseklik 687 m	Kimyasal mücadele yapılmayan meşe ormanı kenarında

Predatörlerin belirlenmesinde Steiner (1962)'nin önerdiği darbe yöntemi uygulanmıştır. Zararlı ile bulaşık olan bahçelerde rastgele seçilen 20-25 ağacın 4 farklı noktasına olmak üzere toplam 100 darbe yapılarak örnekler toplanmıştır. Toplanan örnekler öldürme şişelerinde etil asetat yardımıyla öldürülerek laboratuvara getirilmiştir. Saptanan predatör türler morfolojik özelliklerine göre ayrılmış ve tanı için hazırlanarak konu uzmanına gönderilmiştir. Predatör türlerde erkek ve dişi birey ayrımı yapılmadan aynı tür bireyler bir arada sayılıp aynı türün toplam bireyi üzerinden değerlendirilmiştir. Tespit edilen türlerin, *P. rufulum*'un predatörleri olup olmadığına bahçelerde yapılan gözlemler ile literatür bildirişlerine göre karar verilmiştir.

Araştırma Sonuçları ve Tartışma

Parthenolecanium rufulum'un yayılışının ve yoğunluğunun belirlenmesi

İzmir ve Manisa illerinde 2012-2014 yıllarında Çizelge 2'de belirtilen toplam 52 kestane bahçesinde survey yapılmıştır. Orman alanlarına yakın olan Kemalpaşa ve Turgutlu'daki üç kestane bahçesinde *P. rufulum* belirlenmiştir. Bu bahçelerde bulaşık ağaç sayısının %12-28 arasında değiştiği belirlenmiştir (Çizelge 3).

Çizelge 3. İzmir ve Manisa illerinde 2012-2014 yıllarında survey yapılan ilçeler, köyler, incelenen bahçe sayısı ve bulaşık bahçe ve ağaç oranları (%)

İl	İlçe	Köy	İncelenen bahçe sayısı	İncelen ağaç sayısı	Bulaşık bahçe sayısı	Bulaşık bahçe oranı (%)	Bulaşık ağaç oranı (%)
İzmir	Beydağ	Adaküre	2	110	0	0	0
		Çamlık	3	130	0	0	0
		Çomaklar	7	436	0	0	0
		Eğridere	6	216	0	0	0
		Erikli	13	358	0	0	0
		Halıköy	6	395	0	0	0
	Ödemiş	Bıçakçı	4	190	0	0	0
		Pirinççi	4	126	0	0	0
	Kemalpaşa	Ovacık	3	112	2	66.60	28
Manisa	Turgutlu	Hacıisalar	2	70	1	50.00	12
	Sarıgöl	Karacaali	2	52	0	0	0
Toplam			52	2195	3	5.66	

Zararlının nimf ve ergin dişi yoğunluğu yıllara ve bahçelere göre farklılık göstermiştir. Kemalpaşa'da birinci bahçedeki 2013 ve 2014 yıllarındaki nimf ve ergin dişi yoğunluğunun sırasıyla 21.80 (3-48) nimf/20 cm ve 28.00 (12-59) nimf/20 cm saptanırken, ergin yoğunluğu 18.40 (3-29) ergin dişi/20 cm ve 19.00 (7-25) ergin dişi/20 cm olarak belirlenmiştir. İkinci bahçede ise nimf yoğunluğunun sırasıyla 20.60 (5-38) nimf/20 cm ve 24,00 (16-32) nimf/20 cm, ergin yoğunluğu ise 16.00 (3-22) ergin dişi/20 cm ve 14.40 (8-20) ergin dişi/20 cm belirlenmiştir.

Kemalpaşa'daki iki bahçe birlikte değerlendirildiğinde; *P. rufulum''*un 2013 ve 2014 yıllarındaki nimf ve ergin dişi yoğunluğunun sırasıyla 21.20 (3-48) nimf/20 cm ve 26.00 (12-59) nimf/20 cm, ergin yoğunluğu ise 16.20 (3-29) ergin dişi/20 cm ve 16.70 (7-25) ergin dişi/20 cm olarak belirlenmiştir.

Turgutlu'daki bahçede 2013 ve 2014 yıllarındaki nimf ve ergin dişi yoğunluğunun sırasıyla 14.40 (6-24) nimf/20 cm ve 15.15 (5-36) nimf/20 cm iken ergin yoğunluğu ise 12.24 (5-22) ergin dişi/20 cm ve 14.00 (4-26) ergin dişi/20 cm olduğu görülmüştür.

İki yılık çalışma birlikte değerlendirildiğinde; zararlının yoğunluğunun bahçelere ve yıllara göre değiştiği görülmüştür. Sürgünlerdeki nimf yoğunluğunun 14.40-26.00 nimf/20 cm iken ergin yoğunluğunun ise 12.24-16.70 ergin dişi/20 cm olduğu belirlenmiştir. İtalya'da Yaz meşesi (*Quercus robur* L.) üzerinde kışlayan 2. dönem nimf yoğunluğunun 30 nimf/m, ergin dişi yoğunluğun ise 11 dişi/m olduğu belirlemiştir (Rainato & Pellizzari 2009). Bulgularımızdaki farklılığın ortamda bulunan parazitoit ve predatörlerin tür ve sayısı, zararlının beslendiği konukçu türünden kaynaklanabileceği tahmin edilmektedir.

***Parthenolecanium rufulum*'un biyolojisi**

Parthenolecanium rufulum'un biyolojik dönemlerinin 2013-2014 yıllarında Kemalpaşa ve Turgutlu ilçelerinde kestane alanlarında yıl içerisinde bulunma dönemleri Şekil 1'de iklim verileri ise Şekil 2'de verilmiştir. Kemalpaşa ve Turgutlu ilçelerinde 2013 yılında şubat-nisan aylarında sürgünlerde *P. rufulum*'un ikinci nimf döneminde olduğu, Kemalpaşa ilçesinde ilk ergin dişi 25 Nisan 2013, Turgutlu'da ise 22 Nisan 2013 tarihlerinde belirlenmiştir. İlk yumurtalı ergine Kemalpaşa ve Turgutlu'da 14 Mayıs 2013, yumurta açılımı her iki ilçede 26 Haziran 2013 tarihlerinde tespit edilmiştir (Şekil 1). Birinci dönem nimflere Kemalpaşa'da en son 6 Eylül 2013, Turgutlu'da ise 13 Eylül 2013 tarihinde rastlanmıştır. İkinci dönem nimflerin sürgünlere geçişi Kemalpaşa'da 18 Ekim 2013, Turgutlu'da ise 14 Ekim 2013 tarihinde belirlenmiş ve geçişin her iki ilçede 20 Kasım 2013 tarihine kadar devam etmiştir (Şekil 1).

Parthenolecanium rufulum'un ergin dişileri 2014 yılında ilk olarak Kemalpaşa'da 22 Nisan 2014, Turgutlu'da ise 25 Nisan 2013 tarihlerinde görülmüştür. İlk yumurtalı ergine her iki bahçede 12 Mayıs 2014 tarihinde rastlanmıştır. Yumurta açılımı iki bahçede de 28 Haziran 2014 tarihinde başlamış ve 18 Temmuz 2014 tarihine kadar devam etmiştir. Birinci dönem nimflere Kemalpaşa'da 28 Haziran 2014-19 Eylül 2014, Turgutlu'da ise 28 Haziran 2014-16 Eylül 2014 tarihleri arasında rastlanmıştır. İkinci dönem nimfler Kemalpaşa'da 19 Eylül 2014, Turgutlu'da ise 16 Eylül 2014 tarihinden itibaren görülmüş ve bu nimflerin sürgünlere geçişi her iki bahçede 10 Ekim 2014 tarihinden itibaren başladığı görülmüştür (Şekil 1).

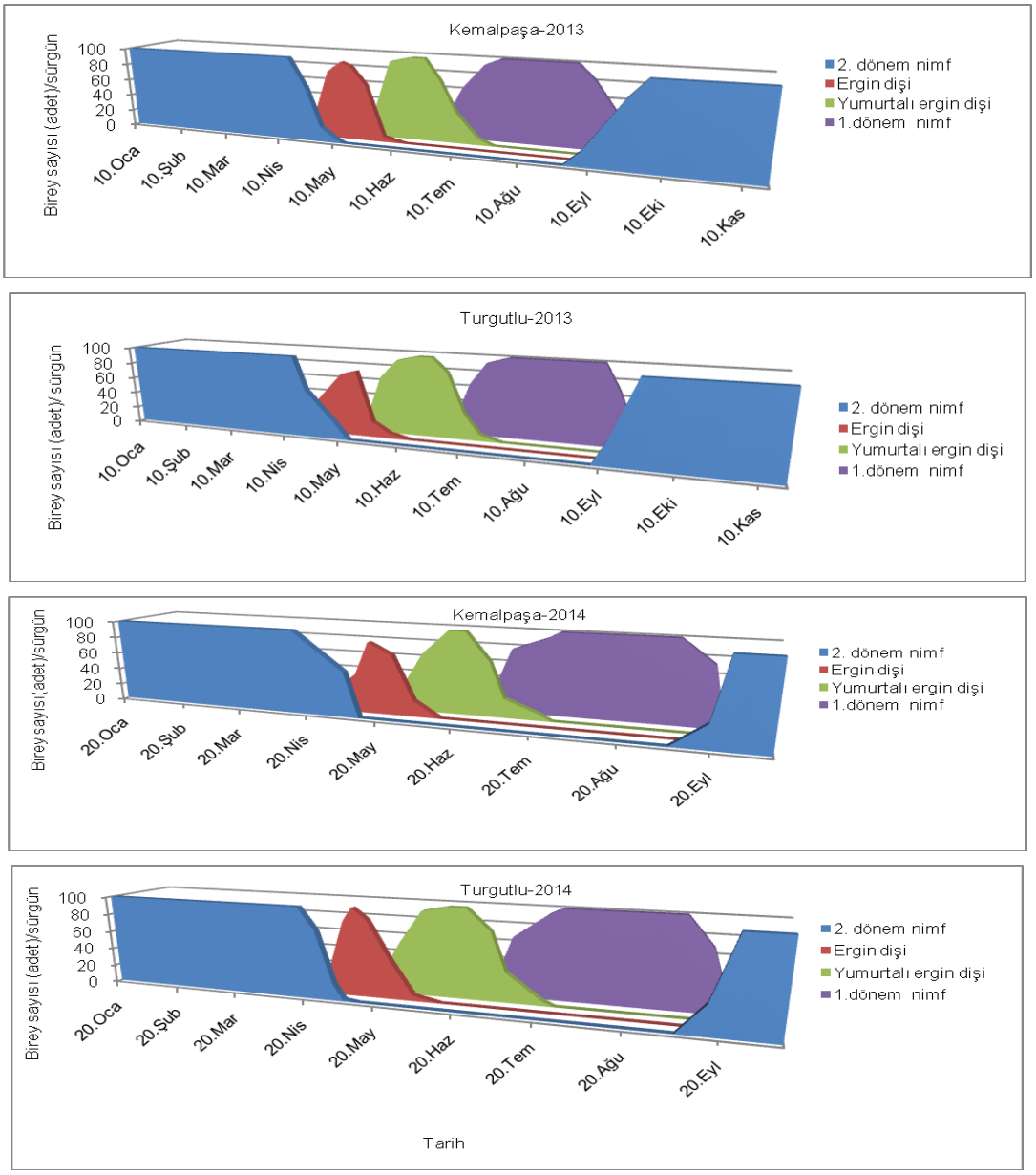
İki yıllık çalışma sonucunda *P. rufulum*'un erkek bireylerine rastlanılmamıştır. Zararlının kışı ikinci dönem nimf halinde 1-2 yıllık sürgünler üzerinde geçirdiği ve yılda 1 döl verdiği belirlenmiştir.

Parthenolecanium rufulum'un mücadeleye esas doğadaki bazı biyolojik özelliklerini belirlemeye yönelik iki yıllık çalışma sonucunda değerlendirildiğinde; *P. rufulum*'un kışı ikinci nimf döneminde sürgünler üzerinde geçirdiği ve yılda 1 döl verdiği belirlenmiştir. Yıllara ve bahçelere göre değişmek üzere ergin dişilere nisan ayı sonlarında rastlanıldığı ve yumurta açılımının haziran ayının son haftası içinde başladığı ve temmuz ortasına kadar devam ettiği, yumurtadan çıkış yapan birinci dönem nimflerin yaprakların alt kısmına geçerek yaprak damarları boyunca yerleştikleri görülmüştür. İkinci dönem nimflerin eylül ortalarında görüldüğü ve bu nimflerin yapraklardan sürgünlere geçişinin ekim ortalarına doğru kestane hasadı döneminde başlayıp yaprak dökümüne kadar devam ettiği belirlenmiştir. Biyolojik dönemlerin iklim verileri olan aylık ortalama sıcaklık ve orantılı nem ile birlikte değerlendirildiğinde iki yılık iklim verilerinin birbirine yakın olduğu ve bunun biyolojik dönemlerin süresi ve başlangıç dönemlerinin benzerlik gösterdiği görülmektedir (Şekil 1). *P. rufulum* nimflerinin nisan ayı sonunda ortalama sıcaklığın 15-20ºC, orantılı nemin %60-65 olduğu dönemde ergin olmaya başladığı saptanmıştır. Koşnillerin mücadelesi için kritik dönem olan yumurtaların açılımı ise haziran ayı sonu ile temmuz ortasında ortalama

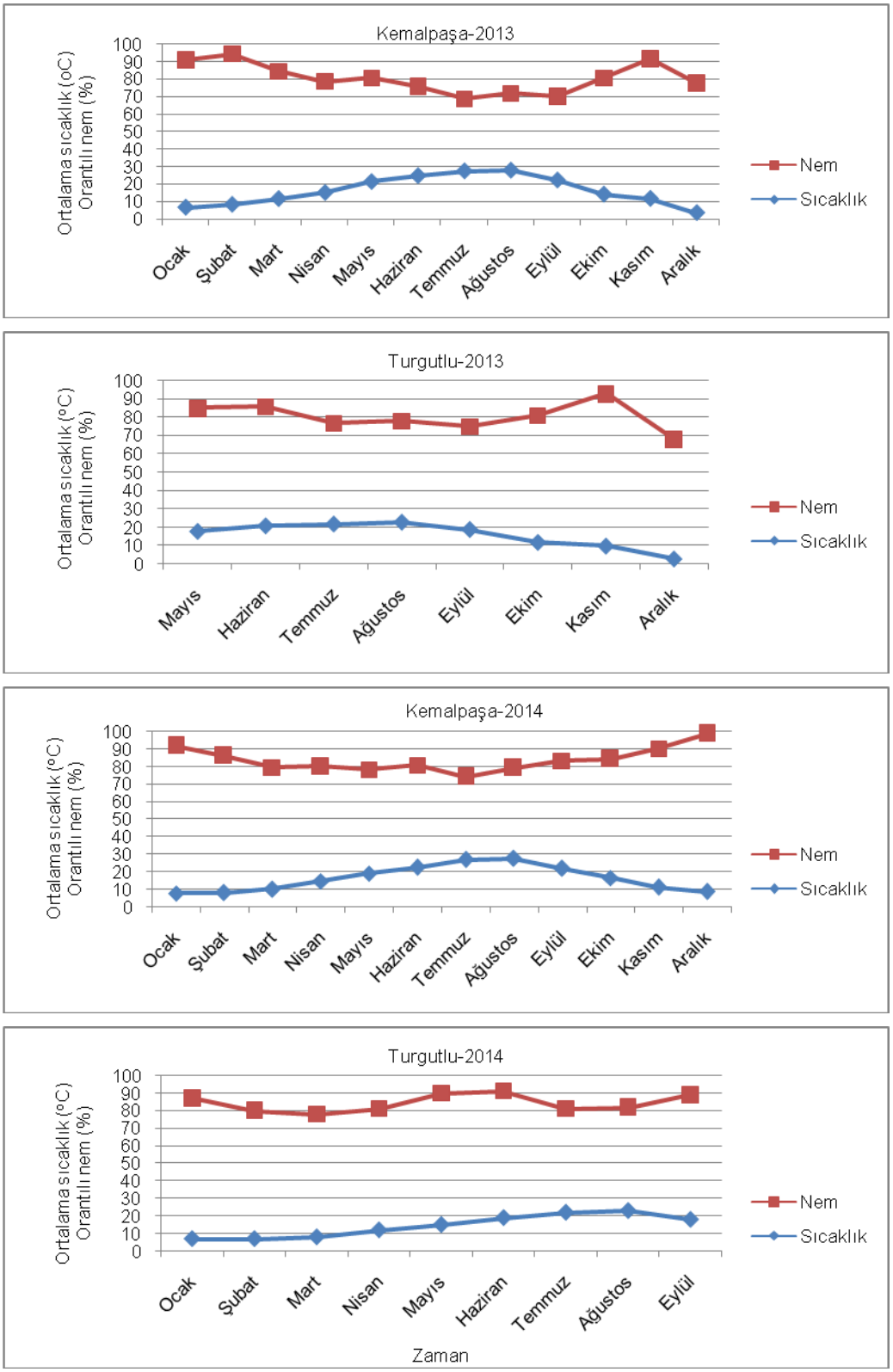
sıcaklığın 20-25ºC, orantılı nemin ise %60-65 olduğu dönemde olduğu görülmüştür. İkinci dönem nimflerin yapraklardan sürgüne geçişi ekim ve kasım ayı içerisinde olduğu bu dönemde ortalama sıcaklığın 10-15ºC ve orantılı nemi ise %70-85 civarında olduğu belirlenmiştir (Şekil 1,2).

Laboratuvarda 20 dişi bireyde yapılan yumurta sayımında bir dişinin yumurta veriminin ortalama 1676 yumurta/dişi (min:486; max:2116 yumurta/dişi) olduğu saptanmıştır.

Ecevit et al. (1987), Karadeniz bölgesinde fındıkta *P. rufulum*'un *P. corni* ile aynı gelişme seyrini gösterdiğini, *P. rufulum*'da yumurta açılımının haziran sonlarında başladığını, ikinci nimf dönemine geçişin eylül ve ekim sonlarında olduğu yumurtasız erginler mart ortalarından itibaren yaklaşık 42 gün sürdüğü, yumurtalı erginler nisan sonu, mayıs ortalarında başlayıp 58 gün devam ettiğini saptamışlardır.



Şekil 1. Kemalpaşa ve Turgutlu ilçelerinde *Parthenolecanium rufulum*'un 2013-2014 yıllarındaki biyolojik dönemleri ve süresi.



Şekil 2. Kemalpaşa ve Turgutlu ilçelerinin 2013-2014 yılları iklim verileri.

Rainato & Pellizzari (2009), İtalya'da 2006-2008 yıllarında *Quercus robur* üzerinde *P. rufulum*'un biyolojisi çalışmalarında; *P. rufulum*'un yılda bir döl verdiğini, kışı ikinci. nimf döneminde geçirdiğini, ergin dişilerin nisan ortasında görülmeye başladığını, dişilerin nisan sonu ve mayıs sonu arasında yumurta bıraktığı, yumurta açılımının mayıs sonunda olduğunu ve ağustos ayında ikinci dönem nimflerin görüldüğünü eylül ortasından itibaren kademeli olarak yapraklardan sürgünlere göçün olduğunu ve bu göçün aralık aynın ilk on gününde tamamladığını belirtmektedirler. Camacho (2015), ABD'de yapılan çalışmada meşe ağaçlarında zararlı olan ve karışık popülasyonlar halinde bulunan *Parthenolecanium corni* (Bouche) ve *Parthenolecanium quercifex* (Fitch) (Hemiptera:Coccidae)'in gelişimleri tahmin etmek için gün derece ve fenolojik gözlemler üzerinde çalışmıştır. Bu türlerin yumurtalarının nisan ortası ve haziran ayı başında açıldığı, ikinci dönem nimflerin ekim ayında oluştuğu, üçüncü dönem nimflerin ve erginlerin ise mart ortası ile nisan ayı başlarında saptandıkları bildirilmektedir. Daha önce yapılan bu çalışmalardan Ecevit et al. (1987) tarafından Karadeniz bölgesinde yapılan çalışmada elde edilen sonuçlar bulgularımızı desteklemektedir.

Parazitoit ve predatörler

Çalışma sonucunda *P. rufulum*'dan belirlenen parazitoit ve predatör türler Çizelge 4'te verilmiştir. Belirlenen parazitoitler Encyrtidae, Eulopidae ve Pteromolidae (Hymenoptera) familyalarına bağlı türler oluşturmuştur. Parazitoit türler; *Pachyneuron aphidis* Bouché ve *Pachyneuron muscarum* (L.) (Pteromalidae), *Microterys* sp. ve *Cheiloneurus* sp. (Encyrtidae), *Colesterocerum* sp. (Eulopidae) (Hymenoptera) mayıs, haziran ve temmuz aylarında kültüre alınan ergin dişi örneklerden çıkış yapmışlardır.

Örnekleme yapılan kestane bahçelerinde çok sayıda avcı böcek belirlenmiştir. Ancak *P. rufulum* ile bulaşık bahçelerde özellikle zararlının birinci ve ikinci nimf dönemlerinin yoğun olduğu temmuz ve ağustos aylarında *Adalia fasciatopunctata revelierei* Mulsant *Adalia bipunctata* (L.), *Chilocorus bipustulatus* (L.), *Coccinella septempunctata* (L.), *Oenopia (Synharmonia) conglobata* (L.) (Coccinellidae) ve *Chrysoperla* sp.(Chrysopidae) predatör türleri saptanmıştır.

Parthenolecanium rufulum'dan tespit edilen doğal düşmanları değerlendirildiğinde; parazitoit (Hymenoptera) türlerin daha yoğun olduğu gözlemlenmiştir. Türkiye'de bu zararlının doğal düşmanlarının belirlenmesine yönelik araştırmalar çok sınırlıdır. Ülgentürk et al. (2004), Ankara ilinde *Quercus* sp. ve *Corylus* sp. de zararlı olan *P. rufulum* üzerinde *Coccophagus lycimnia* (Walker) (Aphelinidae) *Microterys* sp., *Microterys nr bellae* Tryapitzin (Hymenoptera: Encyrtidae)'yi belirlemiştir. Japoshvili & Karaca (2007) bazı araştırıcılara atfen; *Cheiloneurus claviger* Thomson, *Cheiloneurus paralia* (Walker) ve *Microterys sylvius* (Dalman) (Hymenoptera: Encyrtidae) türlerinin konukçuları arasında *P. rufulum*'un olduğunu bildirmişlerdir.

Parthenolecanium rufulum'dan belirlemiş olduğumuz parazitoit türleri Türkiye'de ve yurtdışında birçok araştırıcı tarafından başta Coccid türler olmak üzere değişik böcek türlerinde saptamışlardır. Türkiye'de *P. aphidis* ilk olarak Manisa Turgutlu'da *Aphis fabae* Scop ve *Aphis prunica* Passerini (Hemiptera: Aphididae) ve İzmir'de *Hylopterus pruni* Geoffroy (Hemiptera: Aphididae)'den saptanmıştır (Soydanbay, 1976). Japoshvili & Karaca (2002), Isparta ilinde bulunan Coccid türler ve bunların Türkiye ve Gürcistan'daki parazitoitleri çalışmasında; *Parthenolecanium corni* Bouche (Hemiptera: Coccidae)'nin parazitoiti olarak *Microterys lunatus* (Dalman), *Microterys duplicatus* (Nees), *M. sylvius*, *C. claviger*, *P. muscarum*'u, *Rhodococcus perornatus* (Cockerell & Parrott) (Hemiptera: Coccidae)'un parazitoiti olarak *M. nr bellae*'yi, *Sphaerolecanium prunastri* (Hemiptera: Coccidae)'nin parazitoiti olarak *P. muscarum* ve *Microterys hortulnalus* Erdös'i belirlemiştir. Muştu et al. (2010), *P. aphidis* ve *P. muscarum*'u Ankara'da coccinellid türlerin parazitoitleri, Soydanbay (1976), *P. muscarum*'u Manisa'da *A. fabae*'den, Balıkesir ve İzmir'de *Sphaerolcanium prunastri* Fonscolombe (Hemiptera: Coccidae)'de, İzmir'de *Filippia oleae* Costa (Hemiptera: Coccidae), ve Şanlıurfa'da *Didesmicoccus* sp. (Hemiptera: Coccidae)'den elde edildiği, *P. muscarum*'un genel olarak Coccidae türlerinin paraziti olduğunu bazen aynı ortamda bulunan aphid predatörü coccinellid pupalarını ve psillid nimflerini de parazitlediğini bildirilmektedir (Grahman, 1969; Boucek; 1970,1977). Bazı araştırıcılar bu türün hyperparazit olduğunu, Türkiye'de yapılan diğer bazı çalışmalarda ise bu türün *Coccus pseudomagnolirum* (Klow)'un ve *Ceraplastes rusci* L. ve *S. prunastri* (Hemiptera: Coccidae)'nin parazitoiti olduğu bildirmiştir (Öncüer,1974, 1977).

Çizelge 4. *Parthenolecanium rufulum* (Cockerell)'un İzmir ve Manisa illeri Kestane alanlarında belirlenen doğal düşmanları ve yayılışları

Takım	Familya	Tür	Yayılışı
Hymenoptera	Encyrtidae	*Microterys* sp.	Kemalpaşa, 20.VI.2013, 1♂ ve 1♀, Turgutlu, 26.VI.2013, 2♀, Kemalpaşa, 07.VII.2014, 1♂ ve 1♀, Turgutlu, 20.VII.2014, 1♂ ve 1♀
		Cheiloneurus sp.	Kemalpaşa, 20.VI.2013, 1♂, Kemalpaşa, 07.VII.2014, 1♂ ve 2♀, Turgutlu, 07.VII.2014, 1♂
	Pteromolidae	*Pachyneuron aphidis* Bouche	Kemalpaşa, 20.IV.2013, 1♂ ve 2♀, Turgutlu, 26.VI.2013, 1♀, Kemalpaşa, 24.VI.2014, 2♀, Kemalpaşa, 07.VII.2014, 1♂ ve 2♀, Turgutlu, 07.VII.2014, 1♂ ve 3♀
		Pachyneuron muscarum (L.)	Kemalpaşa, 20.VI.2013, 1♂, Turgutlu, 26.VI.2013, 1♂ ve 3♀, Kemalpaşa 07.VII.2014, 2♂ ve 2♀, Kemalpaşa, 20.VII.2014, 1♂, Turgutlu, 07.VII.2014, 1♂ ve 1♀, Turgutlu, 20.VII.2014, 2♂ ve 1♀
	Eulopidae	*Colesterocerum* sp.	Kemalpaşa, 20.VI.2013, 1♂ ve 1♀, Turgutlu, 26.VI.2013, 1♀, Kemalpaşa, 24.06.2014, 1♀, Kemalpaşa, 07.VII.2014, 2♂ ve 1♀, Turgutlu, 07.VII.2014, 1♂ ve 1♀
Coleoptera	Coccinellidae	*Adalia bipunctata* (L.)	Beydağ, Adaküre, 06.VI.2013 (2); Ödemiş, Bıçakçı, 06.VI.2013 (1); Kemalpaşa, Ovacık, 29.VII.2013 (3); Turgutlu, Hacıisalar 29.VII:2013 (2); Beydağ, Eğridere, 26.VII.2013 (3); Beydağ, Çomaklar, 26.VII.2013 (5); Beydağ, Erikli, 26.VII.2013 (3); Beydağ, Adaküre, 01.VIII.2013 (3); Beydağ, Halıköy, 01.VIII.2013 (7); Ödemiş, Pirinççi, 01.VIII.2013 (4); Beydağ, Eğridere, 15.VIII.2013 (1); Beydağ, Çamlık, 15.VIII.2013 (3); Beydağ, Çomaklar, 15.VIII.2013(2); Beydağ, Erikli, 15.VIII.2013 (7); Beydağ, Halıköy, 23.VIII.2013 (3); Ödemiş, Bıçakçı, 23.VIII.2013 (3); Ödemiş, Pirinççi, 23.VIII.2013 (1); Kemalpaşa, Ovacık, 07.VII.2014 (5); Beydağ, Çomaklar, 10.VII.2014 (1); Beydağ, Halıköy Ovacık, 08.VII.2014 (5); Beydağ, Halıköy, 10.VII.2014 (2); Kemalpaşa, Ovacık, 05.VIII.2014 (2); Beydağ, Halıköy, 07.VIII.2014 (1); Beydağ, Halıköy, 21.VIII.2014 (6); Beydağ, Halıköy, 09.IX.2014 (2); Beydağ, Çomaklar, 25.IX.2014 (1); Beydağ, Halıköy, 25.IX.2014 (1)
		Adalia fasciatopunctata revelierei Mulsant	Beydağ, Adaküre, 06.VI.2013 (3); Beydağ, Halıköy, 06.VI.2013 (11); Ödemiş, Bıçakçı, 06. VI.2013 (8); Kemalpaşa, Ovacık, 29.VII.2013 (13); Turgutlu, Hacıisalar 29.VII:2013 (5); Sarıgöl, Karacaali, 29.VII:2013 (3); Beydağ, Eğridere, 26.VII.2013 (3); Beydağ, Çamlık, 26.VII.2013 (7); Beydağ, Çomaklar, 26.VII.2013 (4); Beydağ, Erikli, 26.VII.2013 (17); Beydağ, Halıköy, 01.VIII.2013 (7); Ödemiş, Bıçakçı, 01.VIII.2013 (2); Ödemiş, Pirinççi, 01.VIII.2013 (1); Beydağ, Eğridere, 15.VIII.2013 (2); Beydağ, Çamlık, 15.VIII.2013(1); Beydağ, Çomaklar, 15.VIII.2013 (3); Beydağ, Halıköy, 23.VIII.2013 (3); Beydağ, Çomaklar, 10.IX.2013 (1); Beydağ, Halıköy, 18.IX.2013 (5); Kemalpaşa, Ovacık, 25.IX.2013 (2); Kemalpaşa, Ovacık, 07.VII.2014 (8); Beydağ, Halıköy, 10.VII.2014 (10); Beydağ, Çomaklar, 10.VII.2014 (1); Beydağ, Halıköy, 09.IX.2014 (1) Kemalpaşa, Ovacık, 05.VIII.2014 (3); Beydağ, Halıköy, 07.VIII.2014 (2); Beydağ, Halıköy, 07.VIII.2014 (2); Ödemiş, Bıçakçı, 21.VIII.2014 (1); Beydağ, Halıköy, 25.IX.2014 (3)
		Chilocorus bipustulatus (L.)	Kemalpaşa, Ovacık, 04.VI.2013 (8); Turgutlu, Hacıisalar, 04.VI.2013 (5); Kemalpaşa, Ovacık, 29.VII.2013 (13); Turgutlu, Hacıisalar, 04.VI.2013 (7); Kemalpaşa, Ovacık, 8.VII.2014 (11); Beydağ, Halıköy-2, 10.VII.2014 (2); Beydağ, Halıköy, 07.VIII.2014 (2); Beydağ, Halıköy, 25.IX.2014 (2)
		Coccinella septempunctata (L.)	Kemalpaşa, Ovacık 24.IV.2013 (2); Turgutlu, Hacıisalar, 24.IV.2013 (3); Kemalpaşa, Ovacık, 30.V.2013 (8); Turgutlu, Hacıisalar, 30.IV.2013 (4); Kemalpaşa, Ovacık, 04.VI.2013 (5); Kemalpaşa, Ovacık 29.VII.2013 (7); Turgutlu, Hacıisalar, 29.VII:2013 (3); Sarıgöl, Karacaali, 29.VII.2013 (2); Beydağ, Adaküre, 06.VI.2013 (2); Beydağ, Halıköy, 06.VI.2013 (3); Ödemiş, Bıçakçı, 06.VI.2013 (5); Ödemiş, Pirinççi, 06.VI.2013 (2); Kemalpaşa, Ovacık, 18.VI.2014 (2); Beydağ, Çomaklar, 20.VI.2014 (2); Beydağ, Halıköy, 07.VIII.2014 (1) Ödemiş, Bıçakçı, 07.VIII.2014 (3); Ödemiş, Pirinççi, 07.VIII.2014 (2)
		Oenopia (Synharmonia conglobata (L.)	Kemalpaşa, Ovacık, 29.VII.2013 (3); Turgutlu, Hacıisalar 29.VII:2013 (2); Beydağ, Çomaklar, 26.VII.2013 (1); Beydağ, Erikli, 26. VII.2013 (3); Beydağ, Halıköy, 01.VIII.2013 (5); Ödemiş, Bıçakçı, 01.VIII.2013 (2); Beydağ, Çomaklar, 15.VIII.2013 (3); Beydağ, Halıköy, 23.VIII.2013 (6); Ödemiş, Bıçakçı, 23.VIII.2013 (1); Ödemiş, Pırınçı, 23.VIII.2013 (2); Kemalpaşa, Ovacık, 07.VII.2014 (1); Beydağ, Halıköy, 21.VIII.2014 (6)
Neuroptera	Chrysopidae	*Chrysoperla* sp.	Kemalpaşa, Ovacık, 30.V.2013 (2); Turgutlu, Kemalpaşa, Ovacık, 04.VI.2013 (3); Kemalpaşa, Ovacık 29.VII.2013 (2); Turgutlu, Hacıisalar, 29.VII:2013 (3); Beydağ, Adaküre, 06. VI.2013 (1); Beydağ, Halıköy, 06. VI.2013 (4); Ödemiş, Bıçakçı, 06. VI.2013 (2; Kemalpaşa, Ovacık, 18.VI.2014 (1); Beydağ, Çomaklar, 20.VI.2014 (2); Beydağ, Halıköy, 07.VIII.2014 (3) Ödemiş, Bıçakçı, 07.VIII.2014 (2); Ödemiş, Pirinççi, 07.VIII.2014 (1); Kemalpaşa, Ovacık, 01.IX.2014 (2); Turgutlu, Hacıisalar, 01.IX.2014 (1); Beydağ, Halıköy, 09.IX.2014 (6); Kemalpaşa, Ovacık, 22.IX.2014 (1); Turgutlu, Hacıisalar, 22.IX.2014 (2); Beydağ, Halıköy, 25.IX.2014 (2); Beydağ, Çomaklar, 25.IX.2014 (1)

Pachyneuron muscarum'un çok sayıda takım ve familyaya ait türlerde birincil parazitoit ve hiperparazitoit olduğu belirtilmektedir (Anonymous, 2015), Doğanlar (1986), Türkiye'de *Pachyneuron* Walker (Hymenoptera: Pteromolidae) türleri üzerinde yaptığı çalışmada; *Pachyneuron* cinse giren sekiz türün bulunduğunu, bu türler *Pachyneuron muscarum* (L), *Pachyneuron groenlandicum* (Holmgren), *Pachyneuron aeneum* (Masi), *Pac. aphidis*, *Pachyneuron formosum* Walker*, Pachyneuron ahlaense* Mani & Saravwat ve *Pachyneuron erzurumicum* Doğanlar olduğunu, Ankara ilinde süs bitkilerinde zararlı *S. prunastri*'nin doğal düşmanları arasında *Pac. muscarum* türünü de belirlemiştir (Ülgentürk, 2001).

Ülgentürk et al. (2004), Ankara, Afyon, Burdur ve Isparta illerinde meyve ağaçlarından ve kenar bitkilerde bulunan Coccidlerin doğal düşmanlarının tespiti çalışmasında predatör türler olarak *C. bipustulatus* ve *Chrysopa* sp. (Neuroptera: Chrysopidae)'yi; Özgen & Bolu (2009), Malatya ilinde kayısı ağaçlarında zararlı *S. prunastri* ile beslenen predatör tür olarak *Exochomus quadripustulatus* (L.) ve *Chrysopa* sp.'yi belirlemişlerdir.

Bu çalışmada belirlenen predatör türlerden *A. fasciatopunctata revelierei*, *A. bipunctata*, *C. bipustulatus*, *C. septempunctata*, *O. (Synharmonia) conglobata* türlerinin kabuklubit ve yaprakbitlerinin predatörleri oldukları önceki çalışmalarda bildirilmektedir (Düzgüneş et al., 1981; Öncüer, 1991; Uygun, 1981).

Parazitlenme oranı

Yapılan çalışmada yumurtalı ve yumurtasız erginlerde parazitlenme oranları; 2013 ve 2014 yıllarında Kemalpaşa'daki bahçede %15.71 ve %18.60 iken, Turgutlu'daki bahçede %3.80 ve %9.43 olduğu saptanmıştır (Çizelge 5). İki yıllık çalışma birlikte değerlendirildiğinde *P. rufulum* ergin dişi bireylerinde parazitlenme oranının bahçelere ve yıllarla göre değişmekle birlikte % 3.80-18.60 arasında değiştiği belirlenmiştir. Parazitoitler içerisinde en yaygın ve yoğun olan tür *P. muscarum* (L.), (% 30), en az olan tür ise *Cheiloneurus* sp. (%10) belirlenmiştir.

Çizelge 5. İzmir ve Manisa illerinde kestane alanlarında zararlı *Parthenolecanium rufulum* (Cockerell)'un 2013-2014 yıllarındaki parazitlenme oranı (%)

İl	İlçe	Parazitlenme oranı (%)	
		2013	2014
İzmir	Kemalpaşa	15.71	18.6
Manisa	Turgutlu	3.8	9.43

Ecevit et al., (1987), Giresun, Ordu ve Trabzon illerinde yaptıkları çalışmada; *P. corni* ve *P. rufulum'*da 1. dönem larvalarda %36.50, 2. dönemde %7.03, 3. dönemde %12.40, yumurtasız erginlerde %13.40 ve yumurtalı erginlerde %20.47 oranında parazitlenme tespit etmiştir. Japoshvili (2001), Gürcistan'da bitki zararlısı Coccoidler ve parazitlenme durumları ile ilgili çalışmasında; *P. rufulum*'un potansiyel zararlılar grubunda yer aldığını, parazitleme oranın %3 olduğunu, parazitoitlerinin ise *Blastothrix longipennis* Howard ve *Coccophagus lycimina* Walker olduğunu bildirmektedir.

Sonuç olarak; İzmir ve Manisa illeri kestane alanlarında sınırlı alanlarda bulunan *P. rufulum'*un, meşe ormanlarına yakın 687-752 m rakımda bulunan kestane bahçelerinde belirlenmiştir. Zararlının kışı ikinci nimf döneminde 1-2 yıllık sürgünlerde geçirdiği, nimflerin nisan ayı sonunda ortalama sıcaklığın 15-20ºC, orantılı nemin %60-65 olduğu dönemde ergin olmaya başladığı saptanmıştır. Coccidlerin mücadelesi için kritik dönem olan yumurtaların açılımı ise haziran ayı sonu ile temmuz ortasında ortalama sıcaklığın 20-25ºC, orantılı nemin ise %60-65 olduğu dönemde olduğu görülmüştür. İleride zararlı ile ilgili oluşturacak mücadele programlarında ve teknik talimatında bu kritik dönemler göz önünde bulundurulmalıdır. Zararlının doğada çok sayıda doğal düşmanı da bulunmaktadır. Zararlının sürekli olarak gözlem altında tutulması, bulaşık alanlarda çoğaltma materyalleri olarak aşı kalemi ve bulaşık fidanların alınmaması, bu konuda kestane üreticileri ve uygulama kuruluşlarındaki teknik elemanların bilgilendirerek zararlının izlenmesi gerekmektedir.

Teşekkür

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