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Original article (Orijinal araştırma)

Repellency of three plant essential oils against red flour beetle *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae)

Un biti, *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae)'a karşı üç bitki esansiyel yağının kaçırıcı etkileri

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Summary

Tribolium castaneum (Herbst, 1797) is an insect pest found in stored products. To control this pest, it is necessary to develop safe alternatives to replace hazardous fumigants. This research aimed to determine the repellency of essential oils from three different plants, *Hypericum hemsleyanum*, *Mentha haplocalyx* and *Stemona japonica*, against *T. castaneum* adults under laboratory conditions. The repellency of essential oils was determined by area preference method at a concentration of 31.5 µg/cm^2. Filter paper disks were placed in petri dishes, one half was treated with essential oil and other half served as control. Twenty *T. castaneum* adults were placed in the center of each paper disk. Insects were counted in treated and control areas at 12, 24, 48, and 72 h after insect release. *Hypericum hemsleyanum* and *M. haplocalyx* showed the strong repellency at all assessment times, with values of 94, 71, 69 and 70%, and 91, 65, 73 and 83% at 12, 24, 48 and 72 h, respectively, followed by *S. japonica*. This research showed that these oils are strong repellents and can potentially be used to repel *T. castaneum* in stored products.

Keywords: Ethanol extracted, *Hypericum hemsleyanum*, *Mentha haplocalyx*, red flour beetle, repellence, *Stemona japonica*

Özet

Tribolium castaneum (Herbst, 1797) depolanmış ürünlerde bulunan zararlı bir böcek türüdür. Bu zararlıyı kontrol etmek için, tehlikeli fumigantlar yerine güvenli alternatiflerin geliştirilmesi gerekmektedir. Bu araştırma laboratuvar koşullarında *T. castaneum* erginlerine karşı üç farklı bitki, *Hypericum hemsleyanum, Mentha haplocalyx* ve *Stemona japonica* esansiyel yağların kaçırıcı etkilerini belirlemek amacıyla yapılmıştır. Uçucu yağların kaçırıcı özelliği, alan tercihi yöntemi ile 31.5 µg/cm^2'lik bir konsantrasyonda belirlenmiştir. Filtre kağıdı diskleri petri kabına yerleştirilmiş ve bir yarısı uçucu yağ ile muamele edilmiş, diğer yarısı ise kontrol olarak kullanılmıştır. Yirmi adet *T. castaneum* ergini her bir kağıt diskin ortasına yerleştirilmiştir. Böceklerin sayımı, kontrol ve uygulama alanında böcek salınmasından 12, 24, 48 ve 72 saat sonra yapılmıştır. *Hypericum hemsleyanum* ve *M. haplocalyx*, tüm değerlendirme zamanlarında en güçlü kaçırıcı etkiyi göstermiş; 12, 24, 48 ve 72 saat sonra sırasıyla %94, 71, 69, 70 ve %91, 65, 73, 83 değerleri elde edilmiş olup; ve *S. japonica* bunları takip etmiştir. Bu araştırma, bu yağların güçlü kaçırıcılar olduğunu ve depolanmış ürünlerde *T. castaneum*'u uzaklaştırmak için potansiyel olarak kullanılabilir olduğunu göstermiştir.

Anahtar sözcükler: Ethanol ekstraktı, *Hypericum hemsleyanum*, *Mentha haplocalyx,* un biti, kaçırıcı etki, *Stemona japonica*

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Introduction

In stored products worldwide, insect pest infestation may cause up to 40% damage (Matthews, 1993). *Tribolium castaneum* (Herbst, 1797) (Coleoptera: Tenebrionidae) is known as the most common insect pest in stored food for human and animal consumption. It affects a wide range of products, including beans, cacao, dried, flour, fruits, grain, nuts, peas and spices. The presence of both adults and larvae in stored food directly affects the quality and quantity of products (Campbell & Runnion, 2003). Insects may cause damage to seed embryos, resulting in decreased germination (Baier & Webster, 1992; Moino et al., 1998). Therefore, control of stored-product pests is necessary to provide a stable and safe food supply at affordable prices (Nadeem et al., 2012; Ukeh et al., 2012; Jahromi et al., 2014). Control of pests in stored products relies on gaseous fumigants, including hazardous chemicals, such as methyl bromide or phosphine. There is a global concern about the negative effects of these chemicals, including direct toxicity to users, increasing cost of application, environmental pollution, resistance to pesticides and pest resurgence, and toxicity to non-target organisms (Lee et al., 2004; Isman, 2006).

Considering the damage caused by *T. castaneum*, there is a need to develop and commercialize safe alternatives to replace hazardous insecticides. To protect the environment and avoid bad ecological effects, researchers have focused on the new ways of carrying out insect pest management in grain stores; moreover, they have diverted their attention toward the use of organic products as pesticides, such as plant extracts (Rajendran & Sriranjini, 2008). Plant essential oils are complex mixtures of a large number of chemical constituents in variable proportions (Van Zyl et al., 2006), they have bioactivities against bacteria fungi, insects, nematodes and viruses (Negahban et al., 2007; Kotan et al., 2008; Park et al., 2008; Razzaghi-Abyaneh et al., 2008). Plant essential oils are environmentally friendly and biodegradable; furthermore, they do not persist in soil and water and are easily extractable (Isman, 2000; 2006).

In the current study, the repellency of essential oils from three different plants, *Hypericum hemsleyanum* H. Lév. & Vaniot, *Mentha haplocalyx* Briq., *Stemona japonica* (Blume) Miquel against *T. castaneum* adults was determined under laboratory conditions. The results will provide data useful for the development of new repellants for stored-product pests.

Materials and Methods

Insects

Tribolium castaneum was reared on wheat flour in the Hubei Insect Resource Utilization and Sustainable Pest Management Key Laboratory of Huazhong Agricultural University, China. *Tribolium castaneum* adults were maintained in glass jars (250 mL) containing wheat flour and 5% yeast; jars were covered with black cloth to provide air and darkness at the top. The insects were reared in the laboratory at 25°±2°C and 50±5% RH with a 14 L:10 D photoperiod.

Ethanol-extracted botanical oils

Plant materials were bought from a franchised outlet of Beijing Tongrentang Group, China. The plant materials, foliage of *M. haplocalyx*, and roots of *H. hemsleyanum* and *S. japonica,* for essential oil extraction were dried in the sunlight then stored at 25°C. Samples were extracted according to the methods of Su et al. (2009) and Yao et al. (2011). Before extraction, plant parts were dried in an oven at 45°C for up to 3 days. Plant parts were crushed into a powder then passed through a 0.425 mm aperture sieve. Samples (1 g) were then suspended in 95% ethanol (5 mL) and incubated in dark at 20±5°C for 7 days, mixing with a vortex mixer twice per day. The solvent was removed with a Buchner funnel, the filtrate was stored at room temperature and solid residue re-extracted in 2.5 mL of 95% ethanol using the same procedure. Filtrates from the first and second extraction were combined and concentrated to dryness with rotary evaporator. The extracts were then weighed and stored in brown collection bottles and stored at 4°C. Crude oils were dissolved in dimethyl sulfoxide (0.05 g oil + 0.3 mL dimethyl sulfoxide + 1% Tween-20), then distilled water added to a final volume of 50 mL, to produce the working solution of essential oil.

Area preference test

Area preference tests were performed using the area preference method of Tapondjou et al. (2005) with modifications. Working solution (0.1 mL) of essential oil was uniformly applied to half a filter paper disk to a final concentration of 31.5 µg/cm^2. The same volume of the solute without essential oil was applied to the other half to serve as a control. Paper disks were placed in 90-mm petri dishes and the solvent allowed drying. One hour after the application, 20 adults of *T. castaneum* were placed in the center of each paper disk. The dishes were covered with black plastic to provide darkness and placed in the same environmental conditions as for rearing. Insects were counted in treated and control areas at 12, 24, 48 and 72 h after insect release. With 20 insects per dish and 8 replicate dishes of each essential oil, a total of 480 insects were used.

Gas chromatography and mass spectrometry

The essential oil components were separated and identified by gas chromatography mass spectrometry (GC-MS) on a Varian 450-GC/320-MS (Varian Medical Systems, Inc. Palo Alto, CA, USA) according to Karina *et al*. (2014). The constituents were identified from the gas chromatography using MANLIB, REPLIB, PMWTox3N and Wiley (NIST, 2011).

Data analysis

The following equation was used to calculate the percent repellency: PR (%) = [(C-T) / (C+T)] × 100 (Liu et al., 2013). Analysis of variance (ANOVA) and Tukey's post hoc tests were used to compare the mean percentage of repellencies (PRs) between essential oils. Paired t-tests were used to compare the mean number of insects in the treated and untreated areas of the filter paper disk. Statistical analysis was performed using SPSS version 20 (IBM Corp. Armonk, NY, USA), with a significance level of $p < 0.05$. The percentage data were arcsine square root transformed, and all count data were square root (x+1) or log10(x+1) transformed before analysis. The untransformed means are presented in the results.

Results

Repellency of essential oils

The results of the *t*-test show that *M. haplocalyx* oil maintained strong repellency during the entire assessment period. Its repellency was significantly higher than the control at all assessment times. It was the least repellant at 12 h (F=30.92, df=7, *P*<0.01) and 24 h (F=24.32, df=7, *P*<0.01) after insect release, but its repellency to best at 48 h (F=29, df=7, *P*<0.01) and 72 h (F=33, df=7, *P*<0.01) (Figure 1). The repellency of *H. hemsleyanum* oil was significantly higher than the control during the entire assessment period. *Hypericum hemsleyanum* oil had the strongest repellency of *T. castaneum* adults at 12 h (F=51.23, df=7, *P*<0.01) and 24 h (F=31.45, df=7, *P*<0.01), but its repellency decreased to second at 48 h (F=23.30, df=7, *P*<0.01) and 72 h (F=26.19, df=7, *P*<0.01) (Figure 1). Essential oil from *S. japonica* was significantly different from the control; its repellency was second at 12 h (F=51.23, df=7, *P*<0.01) and 24 h (F=25.18, df=7, *P*<0.01). However, at 48 and 72 h, its repellency had declined, and it was no longer significantly different from the control (Figure 1).

Duration of repellency

The essential oils of *M. haplocalyx*, *H. hemsleyanum* and *S. japonica* tested against *T. castaneum* exhibited high repellency at 12 h (F=0.53, df=4, *P* > 0.01), 24 h (F=50.02, df=4, *P*<0.01), 48 h (F=272.24, df=4, *P*<0.01) and 72 h (F=619.81, df=2, *P*<0.01).

Mentha haplocalyx acted as a strong repellent against *T. castaneum* adults at 12, 24, 48 and 72 h after insect release, with mean percentages of 91, 65, 73, and 83, respectively (Figure 2). However, *H. hemsleyanum* had second repellency; it was the strongest repellent at 12 and 24 h, with values of 94 and 71%, respectively, and second repellent at 48 and 72 h with values of 69 and 70%, respectively (Figure 2). Finally, *S. japonica* exhibited 94 and 66% repellency at 12 and 24 h after insect release, but it had lost all repellency by 48 and 72 h (Figure 2).

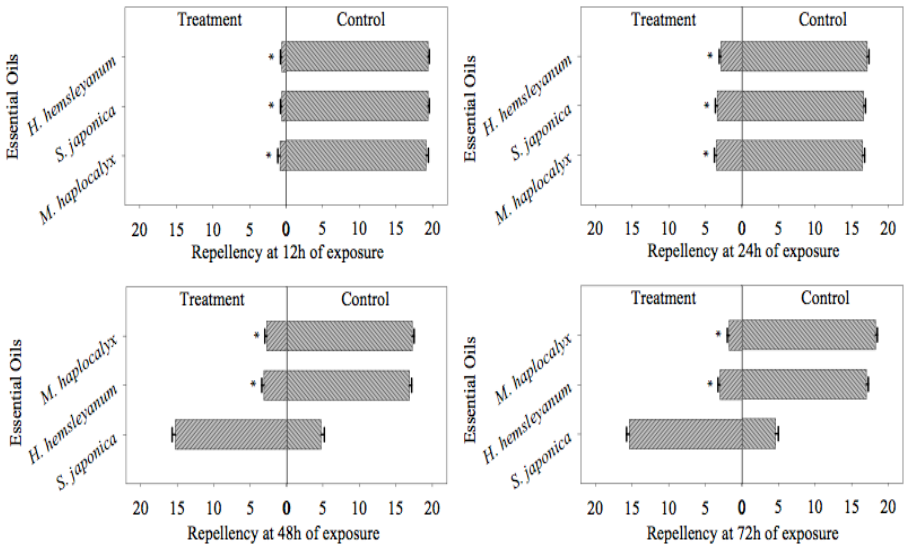

Figure 1. Mean number and SE of repellency at 12, 24 48 and 72 h of *Tribolium castaneum* adults' release. Values are means of 8 replicates (20 insects/replicate). The mean numbers of adults in the treated and control were analyzed by paired t-test at significance level of $P < 0.05$.

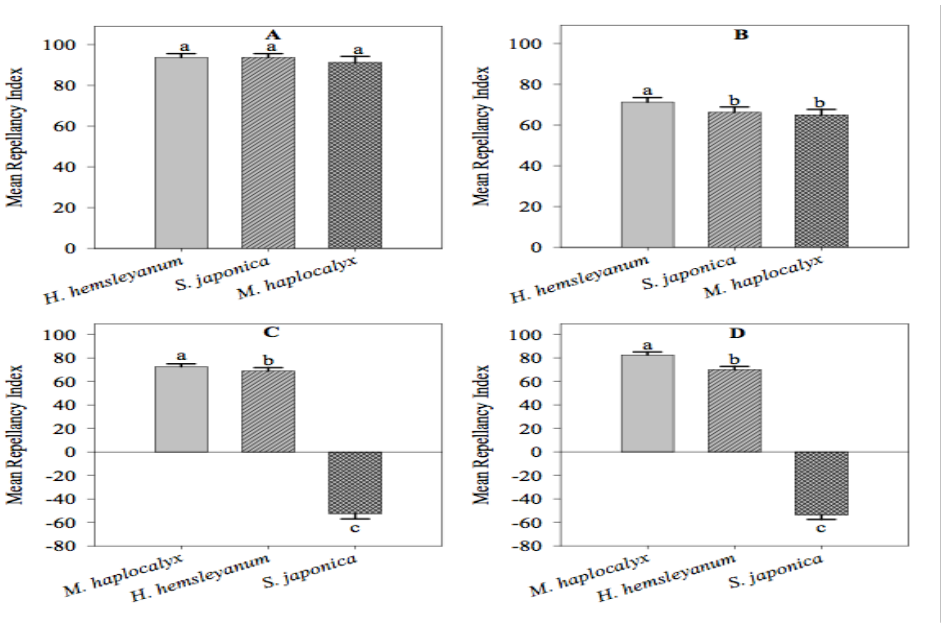

Figure 2. Percentage (mean ± SE) of repellency of *Tribolium castaneum*: A: 12 h after insect release, B: 24 h after insect release, C: 48 h after insect release, D: 72 h after insect release. Values are means of 8 replicates. The mean numbers of adults were analyzed by one-way ANOVA, using a Tukey HSD post-hoc test at significance level of $P < 0.05$.

Gas chromatography and mass spectrometry

After recording strong repellency for the essential oils tested, the oils were analyzed by GC-MS. The results revealed complex mixtures of chemical constituents with nine major components identified in each oil. The primary chemicals identified from *H. hemsleyanum*, *M. haplocalyx* and *S. japonica* oils are presented in Table 1.

Table 1. Chemical components of essential oils based on GC-MS assay

Components	Retention Time (minutes)	Percent of Total (%)
Hypericum hemsleyanum oil		
Phenol, 3-methyl	7.26	2.4
Palmitic acid	12.64	2.6
Hexadecanoic acid, ethyl ester	12.73	1.0
2H-1-benzopyran	13.45	0.7
9,12-Octadecadienoic acid (Z,Z)	13.49	5.1
Linoleic acid ethyl ester	13.55	1.9
Osthole	13.61	35.6
Lomatin acetate	14.47	0.3
1,2-dihydrocyclobuta[b]anthracen-1-one	15.05	6.7
Mentha haplocalyx oil		
L-(-)-menthol	7.85	8.3
Cyclohexanol, 5-methyl-2-(1-methylethyl)	7.89	2.0
2-Hexadecen-1-ol, 3,7,11,15-tetramethyl	11.94	1.2
Hexadecanoic acid	12.64	4.4
Hexadecanoic acid, ethyl ester	12.74	1.2
Phytol	13.33	1.3
9,12-Octadecadienoic acid (Z,Z)	13.49	1.6
9,12,15-Octadecatrienoic acid, (Z,Z,Z)	13.52	4.5
24(Z)-Methyl-25-homocholesterol	23.88	1.4
Stemona japonica oil		
4-Vinylphenol	8.40	0.3
2-Furancarboxaldehyde, 5-(hydroxymethyl)	8.53	0.7
dl-Stenine	14.17	0.9
9,10-Anthracenedicarbonitrile	16.26	0.4
1-Tert-butyl-5-methoxy-2,2-dimethylindan	17.59	7.6
Benzo[a]naphthacene	21.13	1.6
Methyl 4,5,7-trimethoxy-2-naphthoate	21.55	5.7
Stemonine	22.33	31.2
Syn-7-benzhydrylbicyclo[2.2.1]heptan-2-one	23.28	3.3

Discussion

In this study the repellency of the three essential oils, *M. haplocalyx* oil was the most repellent for the targeted insect species, and its repellency was maintained throughout the assessment period. Previous studies showed repellency effects from *Mentha* sp. against many insect and non-insect pests. El-Seedi et al. (2012) investigated the oils of *Mentha* sp. which showed strong repellency (93.2% using a 15 μg/cm^2 concentration in a lab test and 59.4% using 6.5 μg/cm^2 on test cloths in the field) against ticks, *Ixodes ricinus* (L., 1758). A 14-d experiment was conducted in Ebeling choice boxes to determine the toxicity and repellency of *Mentha* oil to American cockroaches (*Periplaneta americana* (L., 1758)) and German cockroaches (*Blattella germanica* (L., 1767)); it showed 100% repellency to both species during each day of the experiment (Appel et al. 2001). Ren et al. (2007) reported that *M. haplocalyx* oil showed bioactivity, such as repellency, insecticidal properties, and growth and reproduction regulation, against numerous insect pests. *Mentha haplocalyx* contains the active components of hexadecanoic acid ethyl ester, menthol and phytol. Hexadecanoic acid ethyl ester (palmitic acid ester) and linoleic acid ethyl ester possess antioxidant, pesticide, and nematicide properties (Jananie et al., 2011). Menthol blocks voltage-sensitive sodium channels, reducing neural activity that may stimulate muscles (Haeseler et al., 2002). Phytol chemical is a natural bioactive compound in plants that acts in a defense systems against diseases (Krishnaiah et al., 2009). At 12 and 24 h *M. haplocalyx* oil had a slightly lower repellency index compared to the other two oils; however, its repellency persisted for a longer time and showed maximum repellency on the repellency index at 48 and 72 h. So it is concluded that *M. haplocalyx* oil is a strong and persistent repellent against the target insect. The components of *M. haplocalyx* essential oil, which are active against T. castaneum, need to be studied further.

Hypericum hemsleyanum oil was the second most repellent to *T. castaneum* adults. Previous studies have also shown its repellency and toxicity against some insect pests. Moore & Debboun (2007) reported that most of the *Heracleum* species suppress the growth of some flies and mosquitoes, because they contain furanocoumarins. However, aqueous and aqueous-alcohol extracts from the *Hypericum* sp. were not repellent to *Sitophilus oryzae* (L., 1763), but rather they were attractants for this stored-product pest (Ciepielewska et al., 2005). Major chemical components of *H. hemsleyanum* were identified with GC-MS analysis in the current study included hexadecanoic acid ethyl ester, linoleic acid ethyl ester, methyl phenol and osthole. Jegadeeswari et al. (2012) demonstrated that hexadecanoic acid has antioxidant activity. Hexadecanoic acid ethyl ester (palmitic acid ester) and linoleic acid ethyl ester possess antioxidant, pesticide and nematicidal characteristics (Jananie et al., 2011). In our GC-MS result, osthole and methyl phenol were the major chemical components of *H. hemsleyanum* essential oil, probably these chemicals are important for repellency against *T. castaneum*, but there are no previous reports on bioactivity of osthole and methyl phenol.

Stemona japonica showed repellency up to 24 h. However, by 48 h its repellency was lost. So it had no long-lasting repellency against the target pest. Extracts of roots and leaves of *Stemona* sp. have been shown to have repellent, antifeedant, and insect toxicity activities against larvae of *Spodoptera littoralis* (Boisduval, 1833) (Brem et al., 2002). In the GC-MS result, the dominate chemical components of *S. japonica* were 1-tert-butyl-5-methoxy-2,2-dimethylindan, stemonine, and methyl 4,5,7-trimethoxy-2-naphthoate. Brem et al. (2002) demonstrated that tuberostemonine has outstanding repellent activity against larvae of *S. littoralis.* There is no published research on the bioactivity of 1-tert-butyl-5-methoxy-2,2-dimethylindan and methyl 4,5,7-trimethoxy-2-naphthoate. This is the first report of repellent activity of *S. japonica* oil against *T. castaneum*.

Conclusion

The results of our research on the repellency of essential oils proved that these oils are strong repellents that can be effectively used to repel adults of *T. castaneum*. *Mentha haplocalyx* and *H. hemsleyanum* showed repellency throughout the duration of the experiment. However, the repellency of *S. japonica* was lost with 48 h. These results could be helpful for the management of pests in stored products. Further research is required to determine the individual bioactivity of the chemical components

of these essential oils against store-product pests, including the fumigant and contact toxicity of these essential oils and their chemical components against larval stage of *T. castaneum*.

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References

Appel, A. G., J. G. Michael & J. T. Marla, 2001. Repellency and toxicity of mint oil to American and German cockroaches (Dictyoptera: Blattidae and Blattellidae). Journal of Agriculture and Urban Entomology, 18: 149-156.

Baier, A. H. & B. D. Webster, 1992. Control of *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) in *Phaseolus vulgaris* L. seed stored on small farms. II. Germination and cooking time. Journal of Stored Products Research, 28: 295-299.

Brem, B., C. Seger, T. Pacher, O. Hofer, S. Vajrodaya & H. Greger, 2002. Feeding deterrence and contact toxicity of *Stemona* alkaloids ― A source of potent natural insecticides. Journal of Agricultural and Food Chemistry, 50: 6383-6388.

Campbell, J. F. & C. Runnion, 2003. Patch exploitation by female red flour beetles, *Tribolium castaneum*. Journal of Insect Science, 3: 1-8.

Ciepielewska, D., K. Bozena & N. Mariusz, 2005. Effect of plant extract on some stored-product insect pests. Polish Journal of Natural Sciences, 18: 7-14.

El-Seedi, H. R., S. K. Nasr, A. Muhammad, A. T. Eman, G. Ulf, P. Katinka & B. K. Anna-Karin, 2012. Chemical composition and repellency of essential oils from four medicinal plants against *Ixodes ricinus* nymphs (Acari: Ixodidae). Journal of Medical Entomology, 49: 1067-1075.

Haeseler, G., D. Maue, J. Grosskreutz, J. Bufler, B. Nentwig, S. Piepenbrock, R. Dengler & M. Leuwer, 2002. Voltage-dependent block of neuronal and skeletal muscle sodium channels by thymol and menthol. European Journal of Anaesthesiology, 19: 571-579.

Isman, M. B., 2000. Plant essential oils for pest and disease management. Crop Protection, 19: 603-608.

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

Jahromi, M. G., A. A. Pourmirza & M. H. Safaralizadeh, 2014. Repellent effect of sirinol (garlic emulsion) against *Lasioderma serricorne* (Coleoptera: Anobiidae) and *Tribolium castaneum* (Coleoptera: Tenebrionidae) by three laboratory methods. African Journal of Biotechnology, 11: 280-288.

Jananie, R. K., V. Priya & K. Vijayalakshmi, 2011. Determination of bioactive components of *Cynodon dactylon* by GC-MS analysis. New York Science Journal, 4: 16-20.

Jegadeeswari, P., A. Nishanthini, S. Muthukumaraswamy & V. R. Mohan, 2012. GC-MS analysis of bioactive components of *Aristolochia krysagathra* (Aristolochiaceae). Journal of Current Chemical and Pharmaceucal Science, 2: 226-236.

Karina, C. G., P. B. Nayive, P. C. Nerlis, S. Elena & O. V. Jesus, 2014. Plants cultivated in Choco, Colombia, as source of repellents against *Tribolium castaneum* (Herbst). Journal of Asia-Pacific Entomology, 17: 753-759.

Kotan, R., S. Kordali, A. Cadir, M. Kesdek, Y. Kaya & H. Kilic, 2008. Antimicrobial and insecticidal activities of essential oil isolated from Turkish *Salvia hydrangea* DC. ex Benth. Biochemical Systematics and Ecology, 36: 360-368.

Krishnaiah, D., T. Devi, A. Bono & R. Sarbatly, 2009. Studies on phytochemical constituents of six Malaysian medicinal plants. Journal of Medicinal Plant Research, 3: 67-72.

Lee, B. H., P. C. Annis, F. Tumaalii & W. S. Choi, 2004. Fumigant toxicity of essential oils from the Myrtaceae family and 1,8-cineol against 3 major stored-grain insects. Journal of Stored Products Research, 40: 553-564.

Liu, X. C., L. G. Zhou, Z. L. Liu & S. S. Du, 2013. Identification of insecticidal constituents of the essential oil of *Acorus calamus* Rhizomes against *Liposcelis bostrychophila* Badonnel. Molecules, 18: 5684-5696.

Matthews, G. A., 1993. “Insecticide Application in Stores, 305-315”. In: Application Technology for Crop Protection (Eds. G. A. Matthews & E. C. Hislop), CAB, London, 368 pp.

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

Moore, S. J. & M. Debboun, 2007. "The History of Insect Repellents, 3-30". In: Insect Repellents: Principles, Methods, and Use. (Eds. M. Debboun, S. P. Frances & D. Strickman), CRC Press, Boca Raton, FL, 495 pp.

Nadeem, M., J. Iqbal, M. K. Khattak & M. A. Shahzad, 2012. Management of *Tribolium castaneum* (Hbst.) (Coleoptera: Tenebrionidae) using Neem (*Azadirachta indica* A. Juss) and Tumha (*Citrullus colocynthis*) (L.). Pakistan Journal of Zoology, 44: 325-1331.

Negahban, M., S. Moharramipour & F. Sefidkon, 2007. Fumigant toxicity of essential oil from *Artemisia sieberi* Besser against three stored-product insects. Journal of Stored Products Research, 43: 123-128.

NIST/EPA/NIH Mass Spectral Database (NIST 11) and NIST Mass Spectral Search Program (Version 2.0g), 2011. U.S. Department of Commerce National Institute of Standards and Technology Standard Reference Data Program Gaithersburg, MD 20899. (Web page: http://www.nist.gov/srd/upload/NIST1a11Ver2-0Man.pdf (Accessed date: January 2016).

Park, I. K., J. N. Kim, Y. S. Lee, S. G. Lee, J. Young, Y. A. Ahn & S. C. Shin, 2008. Toxicity of plant essential oils and their components against *Lycoriella ingénue* (Diptera: Sciaridae). Journal of Economic Entomology, 101: 139-144.

Rajendran, S. & V. Sriranjini, 2008. Plant products as fumigants for stored product insect control. Journal of Stored Products Research, 44: 126-135.

Razzaghi-Abyaneh, M., M. Shams-Ghahfarokhi, T. Yoshinari, M. B. Rezaee, K. Jaimand, H. Nagasawa & S. Sakuda, 2008. Inhibitory effects of *Satureja hortensis* L. essential oil on growth and aflatoxin production by *Aspergillus parasiticus*. International Journal of Food Microbiology, 123: 228-233.

Ren, W., H. Liao & L. F. Du, 2007. Inhibitory effect of trypsin inhibitor from *Cassia obtusifolia* seeds against *Pieris rapae* Larvae. Sichuan Journal of Zoology, 26: 635-637. doi: 10.1007/s10529-006-9281-6.

Su, Y. P., C. J. Yang, H. X. Hua, W. L. Cai & Y. J. Lin, 2009. Bioactivities of ethanol extracts from thirteen plants against *Nilaparvata lugens* (Stal). Chinese Agricultural Science Bulletin, 25: 198-202.

Tapondjou, L. A., C. Adler, D. A. Fontem, H. Bouda & C. H. Reichmuth, 2005. Bioactivities of cymol and essential oils of *Cupressuss empervirens* and *Eucalyptus saligna* against *Sitophilus zeamais* Motschulsky and *Tribolium confusum* du Val. Journal of Stored Products Research, 41: 91-102.

Ukeh, D. A., E. E. Oku, I. A. Udo, A. I. Nta & J. A. Ukeh, 2012. Insecticidal effect of fruit extracts from *Xylopia aethiopica* and *Dennettia tripetala* (Annonaceae) against *Sitophilus oryzae* (Coleoptera: Curculionidae). Chilean Journal of Agricultural Research, 72: 195-200.

Van-Zyl, R. L., S. T. Seatlholo, S. F. Van-Vuuren & A. M. Viljoen, 2006. The biological activities of 20 nature identical essential oil constituents. Journal of Essential Oil Research, 18: 129-133.

Yao, Y. J., Y. Y. Liang, L. Q. Wang, W. Zhang, C. J. Yang, Y. J. Lin & H. X. Hua, 2011. Control effect of extract and compound of *Acorus gramineus* against *Nilaparvata lugens*. Chinese Journal of Applied Entomology, 48: 463-467.

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Original article (Orijinal araştırma)

Meloidogyne species infesting tomatoes, cucumbers and eggplants grown in Kahramanmaraş Province, Turkey[1]

Kahramanmaraş bölgesinde tarımı yapılan domates, hıyar ve patlıcan bitkilerinde mevcut *Meloidogyne* türleri

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Summary

In 2009 and 2010, 107 samples of roots and associated soil were collected from vegetable-growing fields in Andırın, Narlı, Pazarcık and Türkoğlu of Kahramanmaraş Province, Turkey. Populations of root-knot nematode were found in tomatoes, eggplants and cucumbers and the species identified using isozymes analysis, mainly esterase resolved by PAGE (Polyacrylamide Gel Electrophoresis) and perineal patterns of nematode females. A single species of root-knot nematode, *Meloidogyne incognita* was identified from the samples collected. Two esterase phenotypes, one with a single band (I1) and the other with double bands (I2) were found at proportions of 8.5 and 15.8% of total samples, respectively. About 24% of the fields were found to be infested.

Keywords: Esterase phenotypes, identification, *Meloidogyne incognita*, perineal patterns

Özet

Kahramanmaraş'ın Andırın, Narlı, Pazarcık ve Türkoğlu bölgelerinden 2009-2010 yıllarında sebze alanlarından toplam 107 topraklı bitki kök örneği toplanmıştır. Domates, patlıcan ve hıyar bitkilerinden elde edilen nematodlar PAGE (Poliakrilamid Jel Elektroforez) belirlenen esteraz enzim fenotipleri ve perineal (anal) kesit yöntemi ile tür teşhisleri yapılmıştır. Örneklerde mevcut olan nematodların *Meloidogyne incognita* tek türüne ait olduğu bulunmuştur. Bu nematoda ait tek ve çift bantlı olmak üzere iki ayrı esteraz fenotipi olan I1 ve I2 belirlenmiş olup, bunların tüm örnekler içinde sırası ile %8.5 ve 15.8 oranında olduğu görülmüştür. Örneklenen tüm Kahramanmaraş bölgesindeki sebze alanlarının yaklaşık % 24'ünün ilgili kök-ur nematodu ile bulaşık olduğu tespit edilmiştir.

Anahtar sözcükler: Esteraz fenotipleri, teşhis, *Meloidogyne incognita*, anal kesit

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Introduction

Meloidogyne is one of the most widespread and economically important plant- parasitic nematode genera and damages a wide variety of plants across the world (Sasser & Carter, 1985). Plant parasitic nematodes can cause a loss of 78 (Barker et al., 1994) to 100 billion USD (Sasser, 1987) annually. *Meloidogyne* spp. are believed to be a major contributor of this loss. More than 90 root-knot nematode species (*Meloidogyne* spp.) are reported across the world (Hunt & Handoo, 2009). The survey conducted in the International *Meloidogyne* Project in 75 countries found that *Meloidogyne incognita* Kofoid & White, 1919, *Meloidogyne javanica* Treub, 1885, *Meloidogyne arenaria* Neal, 1889 and *Meloidogyne hapla* Chitwood, 1949 were among the most common and economically important species of root-knot nematode in agricultural soils (Netscher & Sikora, 1990). Root-knot nematodes can be found in different regions of the world; however, crop losses caused by these nematodes are greatest in tropical regions (Johnson & Fassuliotis, 1984; Mai, 1985). *Meloidogyne incognita* and *M. javanica* are commonly found in tropical regions, whereas *M. hapla* is better adapted to temperate areas (Taylor & Sasser, 1978). *Meloidogyne incognita* has the widest geographic distribution of all the species described, followed closely by *M. javanica* and *M. arenaria* (Nestscher & Sikora, 1990). *Meloidogyne incognita* along with *Meloidogyne javanica* has been found infesting about 3000 plant species around the world.

Until 2014, eight species of root-knot nematodes (*Meloidogyne arenaria*, *M. artiellia*, *M. chitwoodi*, *M. ethiopica*, *M. exigua*, *M. hapla*, *M. incognita*, and *M. javanica*) have been detected in various agricultural areas of Turkey (Yüksel, 1974; Di Vito et al., 1994; Elekçioğlu & N. Uygun, 1994; Kaşkavalcı & Öncüer, 1999; Söğüt & Elekçioğlu, 2000; Devran et al., 2009; Devran & Söğüt, 2009; Özarslandan et al., 2009; Özarslandan & Elekçioğlu, 2010; Akyazı & Ecevit, 2011; Aydınlı et al., 2013; Kepenekçi et al., 2014). However, only five root-knot nematode species; *M. arenaria*, *M. hapla, M. incognita*, *M. javanica* and *M. thamesi* and have been reported infesting vegetables. Nonetheless, *M. arenaria* and *M. hapla* were found to be widespread but relatively rare (Yüksel, 1974; Elekçioğlu & Uygun, 1994).

Deciding on an effective and successful nematode control method depends directly on accurate problem recognition and knowledge of nematode species involved. Accurate identification of *Meloidogyne* spp. is very critical and important for designing and implementing control strategies such as integrated pest management, crop rotation, use of resistant cultivars, and plant breeding and regulatory programs (Roberts, 1992; Young, 1992; Sijmons et al., 1994; Gheysen et al., 1996; Tytgat et al., 2000; Coyne et al., 2009).

Identification of root-knot nematode species has mostly been performed by four procedures; isozymes, morphology of selected characters, molecular methods and host differential tests. The first two of these methods were applied in the current study. Isozyme phenotypes of nematode females, although long established, are still a useful method in nematology (Esbenshade & Triantaphyllou, 1985). Esterase (EST) and malate dehydrogenase (MDH) isozyme phenotypes (Dickson et al., 1970; Esbenshade & Triantaphyllou, 1985) resolved in polyacrylamide gel electrophoresis were found very effective, and fast in the identification and differentiation of root-knot nematodes species. These enzymes have been used successfully to identify different root-knot nematode species by many scientists from different countries across to the world (Fargette, 1987; Pais & Abrantes, 1989; Carneiro & Almeida, 2001; Castro et al., 2003; Cetintas et al., 2003, Cofcewicz et al., 2004, 2005; Brito et al., 2008). Furthermore, mitochondrial haplotypes used to identify some tropical species of *Meloidogyne*, was in total agreement with esterase analysis (Janssen et al., 2016).

Identification of *Meloidogyne* spp. by morphology, to some degree, is time-consuming and difficult (Taylor & Sasser, 1978; Jepson, 1987). Nevertheless, perineal patterns of females are one of the most frequently used tools for the morphological identification of root-knot nematodes, particularly for differentiating the four commonly found species, *M. arenaria*, *M. hapla, M. javanica* and *M. incognita* (Jepson, 1987; Hunt & Handoo, 2009). However, the method can sometimes be inconclusive for closely related *Meloidogyne* spp., since individuals within the same population often vary considerably (Zijlstra et al., 2000).

The objectives of this study were to determine the prevalence and geographical distribution of *Meloidogyne* spp. in Kahramanmaraş Province, South Mediterranean Region, Turkey, and to identify and characterize root-knot nematode species found infesting three vegetable crops (tomato, eggplant and cucumber) in this area.

Materials and Methods

One hundred and seven samples of roots and associated soil were collected from tomato, eggplant and cucumber fields in the districts of Andırın, Narlı, Pazarcık and Türkoğlu of Kahramanmaraş Province, Turkey, in 2009 and 2010 (Table 1). To enable detection of gall formed by root-knot nematodes, the survey was conducted when plants were at least three months old after planting. This sampling period provided samples with the greatest amount of visible root-knot nematode galling, thus aiding with the recognition of root-knot nematode infested plants. Among five tomato cultivars grown in the region, Joker F1 and Servet F1 is intermediate resistance, and remaining cultivars, Bulanık, Pembe and Tokat F1 are susceptible to *M. incognita*. Additionally, two cucumber cultivars; Toros F1 and Başak F1, and six eggplant cultivars; Karnaz F1, Adana F1, Adana Dolmalık, Pala 49, Kemer and Anamur Karası are also susceptible to *M. incognita*. Consequently, most of host cultivars grown in the region are susceptible to the root-knot nematode. Also, given that most of the fields sampled were not routinely treated nematicides as this is not a common practice in the province, the samples provide a reliable indicator of root-knot nematode incidence in the area sampled.

Table 1. Sampling locations and detection of Meloidogyne in tomato, cucumber and eggplant crops in Kahramanmaraş Province, Turkey

District	Location	Host plant	Samples collected	Infested samples	Incidence (%) and phenotype
Andırın	Merkez	Tomato	4	0	0
Andırın	Merkez	Cucumber	2	0	0
Andırın	Merkez	Eggplant	3	0	0
Kahramanmaraş	Akçakoyun Köyü	Tomato	1	0	0
Kahramanmaraş	Çiğli Köyü	Tomato	5	5	100, I2
Kahramanmaraş	Hasancıklı Köyü	Tomato	2	0	0
Kahramanmaraş	Hasancıklı Köyü	Cucumber	1	0	0
Kahramanmaraş	Hasancıklı Köyü	Eggplant	2	0	0
Kahramanmaraş	Kılılı Beldesi	Tomato	2	0	0
Narlı	Karabıyık Köyü	Tomato	1	0	0
Narlı	Narlı Yol Ayrımı	Tomato	2	0	0
Narlı	Narlı Yol Ayrımı	Cucumber	1	0	0
Narlı	Narlı Yol Ayrımı	Eggplant	1	0	0
Pazarcık	Salmanıpak Köyü	Tomato	3	0	0
Pazarcık	Salmanıpak Köyü	Cucumber	3	0	0
Pazarcık	Salmanıpak Köyü	Eggplant	4	0	0
Pazarcık	Kabarobası Köyü	Tomato	4	0	0
Pazarcık	Kabarobası Köyü	Cucumber	4	0	0
Pazarcık	Kabarobası Köyü	Eggplant	4	0	0
Pazarcık	Ulubahçe Köyü	Tomato	1	0	0
Pazarcık	Ulubahçe Köyü	Cucumber	1	0	0
Pazarcık	Ufacıklı Köyü	Cucumber	1	0	0

Table 1. (Continued)

District	Location	Host plant	Samples collected	Infested samples	Incidence (%) and phenotype
Pazarcık	Kurtdere Köyü	Tomato	4	1	25, I2
Pazarcık	Kurtdere Köyü	Cucumber	3	0	0
Pazarcık	Kurtdere Köyü	Eggplant	3	0	0
Türkoğlu	Aydın Kavak Köyü	Tomato	8	8	100, I2
Türkoğlu	Aydın Kavak Köyü	Cucumber	3	3	100, I2
Türkoğlu	Aydın Kavak Köyü	Eggplant	9	9	100, I1
Türkoğlu	Balık Alanı Köyü	Tomato	4	0	0
Türkoğlu	Balık Alanı Köyü	Cucumber	3	0	0
Türkoğlu	Beyoğlu Kasabası	Tomato	7	0	0
Türkoğlu	Beyoğlu Kasabası	Cucumber	3	0	0
Türkoğlu	Beyoğlu Kasabası	Eggplant	5	0	0
Türkoğlu	Çakallı Köyü	Tomato	1	0	0
Türkoğlu	Merkez	Tomato	1	0	0
Türkoğlu	Çoban Tepe Köyü	Tomato	1	0	0

Plant roots with symptoms of root-knot nematode from each crop in each location was individually collected in polyethylene bags and kept in a cooler for further evaluation. The roots were washed gently in tap and individual root-knot nematode females were collected under light microscope with 40X magnification. Root samples with low infestation or with females not suitable for analysis, were cut into approximately 2 cm lengths, mixed and transferred to 16 cm diameter clay pots containing field soil to increase the population of nematodes. Root-knot nematode susceptible tomato (*Solanum lycopersicum* Mill. cv. SC 2121; Pinaper Tohumculuk, Adana, Turkey) seedlings were transplanted into the pots and maintained in a greenhouse. The pots were watered daily and fertilized as needed. Sixty days after transplanting, infested roots containing adult females were washed and nematode females recovered for study.

Sample preparation, loading and electrophoresis

Eight females were extracted from the roots of each plant for esterase study. The females were individually preserved (one female per tube) in 10 µl of extraction buffer (56% deionized water, 12% 0.5 M Tris-HCl, pH 6.8, 30% glycerol, 2% of 0.5% [w/v] bromophenol blue; BioRad, Hercules, CA, USA) in conical 50-µl microfuge tubes and frozen at -5°C. PAGE (polyacrylamide gel electrophoresis) were performed on a total of 512 young egg-laying females using a Bio-Rad mini-PROTEIN II (Bio-Rad) electrophoresis unit with 10 wells per gel. Before electrophoresis, the females were thawed and individually homogenized in a microhaematocrite plastic tube in 10 µl of extraction buffer, and then each was loaded into a well of a polyacrylamide gel consisting of a 4% stacking (pH 6.8) and a 8% separating (pH 8.8) sections in Tris-glycine buffer. Two females from a greenhouse isolate of *M. javanica* were individually extracted and stored in extraction buffer. These were used as standards in each gel. The standard *M. javanica* female extract was placed into wells 1 and 10. The voltage was maintained at 80 v for the first 15 min and increased to 200 v for the remainder of the run. Following electrophoresis, the gels were removed and placed in a staining solution to determine esterase activity (Harris & Hopkinson, 1976; Esbenshade & Triantaphyllou, 1985). Gels were stained in dark for about 30 min, and transferred to a fixative solution consisted of 10% glycerol, 20% ethyl alcohol and 70% distilled H_2O. Esterase phenotype bands were observed and gel photographs were taken under UV or white light (Figure 2). In addition, relative mobility of band(s) was calculated (Esbenshade & Triantaphyllou, 1985; Fargette 1987) and phenotype designations were assigned according to Esbenshade &Triantaphyllou (1985) (Figure 1).

Perineal patterns

Females dissected from plant roots were selected randomly and fixed in TAF (7 ml 40% formaldehyde, 2 ml triethanolamine, 91 ml distilled water) solution until used. Overall, 387 of 567 females dissected from tomato, cucumber and eggplant were processed for examination of perineal patterns (Table 2). Females were cut in 45% lactic acid and mounted in glycerin (Taylor & Netscher, 1974; Hartman & Sasser, 1985). Perineal patterns examined under a light microscope as described by Eisenback et al. (1980, 1981) and Jepson (1987). Examination and photography of perineal patterns were completed within 12 h following slide preparation (Figure 3).

Table 2. The number of females and perineal patterns examined from tomato, cucumbers and eggplants sampled in Kahramanmaraş Province, Turkey

Sample ID	Plant Source	Number of females collected	Number of perineal patterns examined
09-TAKK1	Tomato	19	14
09-TAKK2	Tomato	30	26
09-TAKK3	Tomato	18	10
10-TAKK4	Tomato	20	16
10-TAKK5	Tomato	23	18
10-TAKK6	Tomato	21	13
10-TAKK7	Tomato	20	15
10-TAKK8	Tomato	25	17
09-TCI1	Tomato	17	13
09-TCI2	Tomato	11	9
09-TCI3	Tomato	19	15
10-TCI4	Tomato	13	8
10-TCI5	Tomato	15	14
10-TKU1	Tomato	5	3
09-CAKK1	Cucumber	13	9
10-CAKK2	Cucumber	10	6
10-CAKK3	Cucumber	8	5
09-EAKK1	Eggplant	25	18
09-EAKK2	Eggplant	19	10
09-EAKK3	Eggplant	30	16
09-EAKK4	Eggplant	29	13
10-EAKK5	Eggplant	32	19
10-EAKK6	Eggplant	17	14
10-EAKK7	Eggplant	26	17
10-EAKK8	Eggplant	38	21
10-EAKK9	Eggplant	35	23
10-EAKK10	Eggplant	29	25

Results and Discussion

The survey revealed that about 24% of the fields sampled were infested with *Meloidogyne* populations. All populations were identified as *M. incognita*. Infected tomato, eggplant and cucumber fields were found in Aydın Kavak in Türkoğlu District of Kahramanmaraş Province. In Çiğli and Kurtepe villages in Pazarcık District of Kahramanmaraş Province infected tomato fields were found (Table 1). In Turkey, the use of esterase phenotypes for the identification of *Meloidogyne* species has been reported previously (Mennan et al., 2011; Aydınlı & Mennan, 2011, 2016; Çetintaş & Çakmak, 2011). The current study is the broadest study to determine the root-knot nematode incidence and to identify *Meloidogyne* species occurring in Kahramanmaraş Province by these two methods.

Two esterase phenotypes (Est = I1 and Est = I2) were detected among the populations of *M. incognita* (Figures 1 and 2). Relative migrations of the bands for phenotypes I1 and I2 were 49, and 49 and 51, respectively (Figure 1).

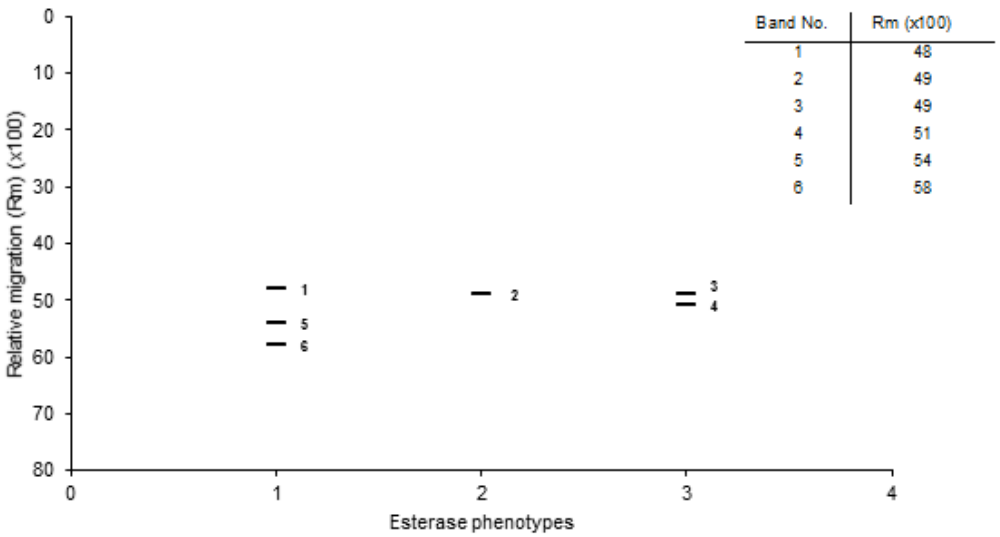

Figure 1. Schematic representation of esterase phenotypes of *Meloidogyne* populations infesting different crops in Kahramanmaraş Province, Turkey. 1: *M. javanica* (Est = J3); 2: *M. incognita* (Est = I1), and 3: *M. incognita* (Est = I2).

It appeared that the nine infested eggplant samples (8.5% of all samples) collected from Aydın Kavak (Türkoğlu) were phenotype I1, and remaining 17 infested tomato and cucumber samples (15.8% of all samples) collected from Aydın Kavak, Çiğli and Kurtepe were phenotype I2. The phenotypes, I1 and I2, are species specific and have proved to be of high diagnostic value for distinguishing *M. incognita* from other *Meloidogyne* spp. (Esbenshade & Triantaphyllou, 1985; Carneiro & Almeida, 2001; Carneiro et al., 2004; Cofcewicz et al., 2004; 2005; Brito et al., 2008). The phenotype I1 is the most common phenotype and has been observed in many populations of *M. incognita* infesting many different crops around the world (Esbenshade & Triantaphyllou, 1985; Fargette, 1987; Castro et al., 2003; Carneiro et al. 2004, Cofcewicz et al., 2004; 2005; Brito et al., 2008). Nonetheless, the phenotype I2 were also isolated from populations of *M. incognita* infesting several crops, including vegetables, fruit trees and agronomic crops in several countries (Castro et al., 2003; Carneiro et al. 2004; Cofcewicz et al., 2004; 2005; Brito et al., 2008). In our study phenotype I2 was found to be the dominant phenotype.

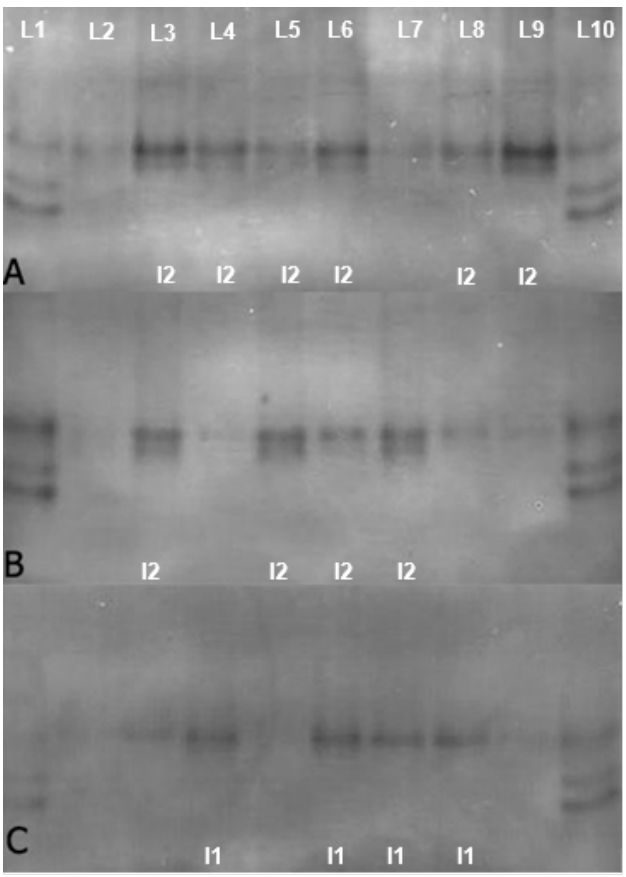

Figure 2. Esterase bands resolved from individual root-knot nematode females following electrophoresis on polyacrylamide slab gels. Lanes and 10 - standard controls, *Meloidogyne javanica* (esterase phenotype J3). Lanes 2 to 9 - *Meloidogyne incognita* from each of root-sampled vegetable hosts A) tomato (esterase phenotype I2), B) cucumber (esterase phenotype I2), and C) eggplant (esterase phenotype I1) sampled in Kahramanmaraş Province, Turkey.

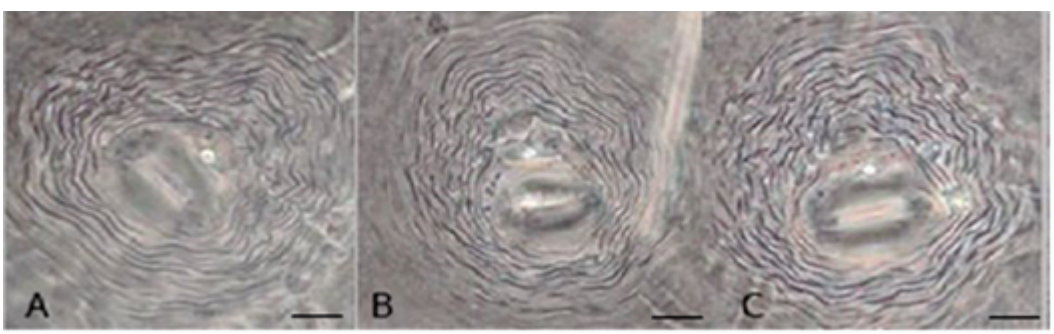

Figure 3. Perineal patterns of *Meloidogyne incognita* from A) tomato, B) cucumber, and C) eggplant sampled in Kahramanmaraş Province, Turkey (bar=20 µm).

Perineal patterns in this study were typically of *M. incognita*. *Meloidogyne incognita* perineal patterns had a moderately high squarish dorsal arch with a distinct whorl in the tail terminal area. The distinct lateral lines were absent (Figure 3).

It is difficult to distinguish all *Meloidogyne* species using perineal patterns alone due to considerable morphological variations between and within populations. However, perineal patterns are a valuable tool for supporting for other methods, such as biochemical analysis (Carneiro et al., 2004; Hernandez et al., 2004). This study also founded that the perineal patterns were valuable and corroborated the findings of the PAGE analysis.

It is believed that the excessive monocultures of pepper (*Capsicum annuum* L.) over many years has resulted the dominance of a single species of nematode (*M. incognita*) in the area sampled. It has been reported that *M. javanica* has three host races (parasitic to pepper or peanut, or non-parasitic to both) (Rammah & Hirschmann, 1990). Although there are no early reports about of *M. javanica* in this predominately pepper-growing area, we postulate that existence of non-parasitic races of *M. javanica* lead to their decline to an undetectable level. Consequently, this situation allowed *M. incognita* to dominate as a single species. Root-knot nematode races do not show any major and minor morphological differences and they can be determined only by a host test. Since race determination is not possible by morphological, cytological, biochemical criteria, it is evident that host tests need to be carried out before choosing crop rotations in a given agricultural area (Rammah & Hirschmann, 1990). Given that root-knot nematodes induce similar above ground symptoms to those caused by other pathogens and plant nutrient deficiencies, farmers should be actively informed about this pest and its impact. Before implementing chemical control, some of other management practices, including proper rotation programs, cultural control and use of resistant varieties should be implemented. Growing non-host plants, such as peanut and strawberries, or vegetable cultivars with the *Mi-1* resistant gene could reduce the pathogen incidence in this area. Solarization is also one of the important management practices that can reduce the number of nematode eggs and second stage juveniles in soil. With the root-knot nematodes found in this study occurring mostly in the vegetable-growing areas with inappropriate irrigation systems, drip or sprinkler irrigation systems should be adopted to avoid or prevent further dispersal of existing plant parasitic nematode species, especially *Meloidogyne* spp.

References

Akyazı, F. & O. Ecevit, 2011. Identification and distribution root knot nematode species (*Meloidogyne* spp.) in vegetable fields in Tokat Province. Anadolu Journal of Agricultural Science, 26: 1-9.

Aydınlı, G. & S. Mennan, 2011. Atypical esterase phenotype from root-knot nematodes in vegetable greenhouse in Samsun. Proceeding of the Fourth Plant Protection Congress of Turkey, 28-30 June 2011, Kahramanmaraş, Turkey, 236 pp.

Aydınlı, G. & S. Mennan, 2016. Identification of root-knot nematodes (*Meloidogyne* spp.) from greenhouses in the Middle Black Sea Region of Turkey. Turkish Journal of Zoology, (Accepted paper) DOI: 10.3906/zoo-1508-19.

Aydınlı, G., S. Mennan, Z. Devran, S. Sirca & G. Urek, 2013. First report of the root-knot nematode *Meloidogyne ethiopica* on tomato and cucumber in Turkey. Plant Diseases, 97: 1262.

Barker, K. R., R. S. Hussey, L. R. Krusberg, G. W. Bird, R. A. Dvnn, H. Ferris, V. R. Ferris, D. W. Freckman, C. J. Gabriel, P. S. Grewal, A. E. Macguidwin, D. L. Riddle, P. A. Roberts & D. P. Schmitt, 1994. Plant and soil nematodes: Societal impact and focus for the future. Journal of Nematology, 26: 127-137.

Brito, J. A., R. Kaur, R. Cetintas, J. D. Stanley, M. L. Mendes, E. J. McAvoy, T. O. Powers & D. W. Dickson. 2008. Identification and isozyme characterization of *Meloidogyne* spp. infecting horticultural and agronomic crops, and weed plants in Florida. Nematology, 10: 757-766.

Carneiro, R. M. D. G. & M. R. A. Almeida, 2001. Técnica de eletroforese usada no estudo de enzimas dos nematóides de galhas para identificação de espécies. Nematologia Brasileira, 25: 555-560.

Carneiro, R. M. D. G., M. S. Tigano, O. Randig, M. R. A. Almeida & J. L. Sarah, 2004. Identification and genetic diversity of *Meloidogyne* spp. (Tylenchida: Meloidogynidae) on coffee from Brazil, Central America and Hawaii. Nematology, 6: 287-298.

Castro, J. M. C., R. D. Lima, & R. M. D. G. Carneiro, 2003. Variabilidade isoenzimática de populações de *Meloidogyne* spp. provenientes de regiões Brasileiras produtoras de soja. Nematologia Brasileira, 27: 1-12.

Cetintas, R., R. D. Lima, M. L. Mendes, J. A. Brito & D. W. Dickson, 2003. *Meloidogyne javanica* on peanut in Florida. Journal of Nematology, 35 (4): 433-436.

Cofcewicz, E. T., R. M. D. G. Carneiro, P. Castagnone-Sereno & P. Quénéhervé, 2004. Enzyme phenotypes and genetic diversity of root-knot nematodes parasitizing Musa in Brazil. Nematology, 6: 85-95.

Cofcewicz, E. T., R. M. D. G. Carneiro, O. Randig, C. Chabrier, & P. Quénéhervé, 2005. Diversity of *Meloidogyne* spp. on Musa in Martinique, Guadeloupe, and French Guiana. Journal of Nematology, 37: 313-322.

Coyne, D. L., H. H. Fourie & M. Moens, 2009. "Current and future management strategies in resource-poor farming, 444-475". In: Root-Knot Nematodes (Eds. R. N. Perry, M. Moens & J. L. Starr). 1st Ed. Wallingford, UK: CAB International, 520 pp.

Çetintas, R. & B. Çakmak, 2011. Diagnosis of *Meloidogyne* species found on tomatoes, eggplants and cucumbers grown in Kahramanmaraş province by PAGE (Polyacrylamide Gel Elektrophoresis) and perineal patterns. Proceeding of the Fourth Plant Protection Congress of Turkey, 28-30 June 2011, Kahramanmaraş, Turkey, 48.

Devran, Z. N. Mutlu, A. Özarslandan & I. H. Elekçioğlu, 2009. Identification and genetic diversity of *Meloidogyne chitwoodi* in potato production areas of Turkey. Nematropica 39: 75-83.

Devran, Z. & M. A. Söğüt, 2009. Distribution and identification of root-knot nematodes from Turkey. Journal of Nematology, 41: 128-133.

Dickson, D. W., J. N. Sasser & D. Huisingh, 1970. Comparative disc-electrophoretic protein analyses of selected *Meloidogyne*, *Ditylenchus*, *Heterodera* and *Aphelenchus* spp. Journal of Nematology, 2 (4): 286-293.

Di Vito, M., N. Greco, G. Oreste, M.C. Saxena, K. B Singh & I. Kusmenoglu, 1994. Plant parasitic nematodes of legumes in Turkey. Nematologia Mediterranea, 22: 245-251.

Eisenback, J. D., H. Hirschmann & A. C. Triantaphyllou, 1980. Morphological comparison of *Meloidogyne* female head structures, perineal patterns, and stylets. Journal of Nematology, 12: 300-313.

Eisenback, J. D., H. Hirschmann, J. N. Sasser & A. C. Triantaphyllou, 1981. A Guide to the Four Most Common Species of Root-knot Nematodes (*Meloidogyne* species) with A Pictorial Key. North Carolina, USA: A cooperative Publication of the Departments of Plant Pathology and Genetics, North Carolina State University and the United States Agency for International Development, Raleigh, 48 pp.

Elekçioğlu, I. H. & N. Uygun, 1994. Occurrence and distribution of plant parasitic nematodes in cash crop in eastern Mediterranean Region of Türkiye. Proceedings of the 9th Congress of the Mediterranean Phytopathological. Union-Kuşadası-Aydın-Türkiye. 409-410.

Esbenshade, P. R. & A. C. Triantaphyllou, 1985. Use of enzyme phenotypes for identification of *Meloidogyne* species (Nematoda: Tylenchida). Journal of Nematology, 17: 6-20.

Fargette, M., 1987. Use of the esterase phenotype in the taxonomy of the genus *Meloidogyne*. 1. Stability of the esterase phenotype. Revue Nématologie, 10: 39-43.

Gheysen, G., W. Van Der Eycken, N. Barthels,. M. Karimi, & M. Van Montagu, 1996. The exploitation of nematode-responsive plant genes in novel nematod control methods. Pesticide Science, 47: 95-101.

Harris, H. & D. A. Hopkinson, 1976. Handbook of Enzyme Electrophoresis in Human Genetics. New York: North-Holland Publications, 92 pp.

Hartman, K. M. & J. N. Sasser. 1985. "Identification of *Meloidogyne* Species on the Basis of Different Host Test and Perineal Pattern Morphoplogy, 69-77". An Advanced treatise on *Meloidogyne*, Vol. 2. Methodology, (Eds. K. R. Barker, C. C. Carter & J. N. Sasser) North Carolina State University Graphics. Raleigh, North Carolina, 223 pp.

Hernandez, A., M. Fargette & J. L. Sarah, 2004. Characterisation of *Meloidogyne* spp. (Tylenchida: Meloidogynidae) from coffee plantations in Central America and Brazil. Nematology, 6: 193-204.

Hunt, D. J. & Z. A. Handoo. 2009. "Taxonomy, Identification and Principal Species, 55-97". Root-knot Nematodes, (Eds. R. N. Perry, M. Moens & J. L. Starr). 1st Ed. Wallingford, UK: CAB International, 97 pp.

Janssen, T., G. Karssen, M. Verhaeven, D. Coyne & W. Bert, 2016. Mitochondrial coding genome analysis of tropical root-knot nematodes (*Meloidoyne*) supports haplotype based diagnostics and reveals evidence of reticulate evolution. Scientific Reports, 6: 22591; DOI 10.1038/srep22591.

Jepson, S. B., 1987. Identification of Root-Knot Nematodes (*Meloidogyne* species). CAB International Institute of Parasitology. Wallingford, Oxon, UK. 265 pp.

Johnson, A. W. & G. Fassuliotis, 1984. "Nematodes Parasites of Vegetable Crops, 323-372". In: Plant and Insect Nematodes (Ed. W. R. Nickle) Marcel Dekker Inc., New York, NY, USA, 713 pp.

Kaşkavalcı, G. & C. Öncüer, 1999. Investigations on distribution and economic importance of *Meloidogyne* Goeldi, 1887 (Tylenchida: Meloidogynidae) species found in the major areas of hot climate vegetables in Aydın province. Turkish Journal of Entomology, 23 (2): 149-160.

Kepenekci İ., E. Evlice & G. Öztürk, 2014. Taxonomic characteristics of *Meloidogyne exigua* Goeldi which is a new root-knot nematodes for Turkey and other root-knot nematode species. Turkish Bulletin of Entomology, 54: 1-9.

Mai, W. F., 1985. "Plant Parasitic Nematodes: Their Threat to The Agriculture, 1-17". In: An Advanced Treatise on *Meloidogyne*, Vol. I. Biology and Control (Eds. J. N. Sasser & C. C. Carter). North Carolina State University Graphics, Raleigh, North Carolina, 422 pp.

Mennan, S., G. Aydınlı & T. Katı, 2011. First report of root-knot nematode (*Meloidogyne arenaria*) infecting parsley in Turkey. Journal of Phytopathology, 159: 694-696.

Netscher, C. & R. A. Sikora, 1990. "Nematode Parasites on Vegetables, 237-283". In: Plant Parasitic Nematodes in Subtropical and Tropical Agriculture (Eds: M. Luc, R. A. Sikora & J. Bridge). Commonwealth Agricultural Bureaux International Institute of Parasitology, Wallingford, 817 pp.

Özarslandan, A. & İ. H. Elekçioğlu, 2010. Identification of the Root-knot nematode species (*Meloidogyne* spp.) (Nemata: Meloidogynidae) collected from different parts of Turkey by moleculer and morphological methods. Turkish Journal of Entomology, 34: 323-335.

Özarslandan, A., Z. Devran, N. Mutlu & I. H. Elekcioğlu, 2009. First report of Columbia Root-Knot nematode (*Meloidogyne chitwoodi*) in potato in Turkey. Plant Disease, 93: 316.

Pais, C. S. & I. M. O. Abrantes, 1989. Esterase and malate dehydrogenase phenotypes in Portuguese populations of *Meloidogyne* species. Journal of Nematology, 21: 342-346.

Rammah, A. & H. Hirschmann, 1990. Morphological comparison of three host races of *Meloidogyne javanica*. Journal of Nematology, 22: 56–68.

Roberts, P. A., 1992. Current status of the availability, development, and use of host plant resistance to nematodes. Journal of Nematology, 24 (2): 213-227.

Sasser, J. N. & C. C. Carter, 1985. "Overview of the international *Meloidogyne* project 1975-1984, 19-24". In: An Advanced Treatise on *Meloidogyne*, Vol. I. Biology and Control (Eds. J. N. Sasser & C. C. Carter). North Carolina State University Graphics, Raleigh, North Carolina, 422 pp.

Sasser J. N. A., 1987. Perspective on Nematode Problems Worldwide, 1–12". Nematode Parasitic to Cereals and Legumes in Temperate Semi-Arid Regions (Eds. M. C. Saxena, R. A. Sikora & J. P. Sarivastava). Proceedings of a Workshop Held at Larnaca, Cyprus, 222 pp.

Sijmons, P. C., H. J. Atkinson & U. Wyss, 1994. Parasitic strategies of root nematodes and associated host cell responses. Annual Review of Phytopathology, 32: 235-259.

Söğüt, M. A & I. H. Elekçioğlu, 2000. Determination of *Meloidogyne* Goeldi, 1892 (Nemata: Heteroderidae) species races found in vegetable growing areas of the Mediterranean region of Turkey. Turkish Journal of Entomology, 24: 33-40.

Taylor, D. P. & C. C. Netscher, 1974. An improved technique for preparing perineal patterns of *Meloidogyne* spp. Nematologica, 20: 268-269.

Taylor, A. l. & J. N. Sasser, 1978. Identification and Control of Root-knot Nematodes (*Meloidogyne* spp.). Department of Plant Pathology and Crop Physiology, North Carolina State University and U.S. Agency International Development. Raliegh, N.C. 111 pp.

Tytgat, T. J. D. Meutter, G. Gheysen & A. Coomans, 2000. Sedentary endoparasitic nematodes as a model for other plant parasitic nematodes. Nematology, 2(1): 113-121.

Young, L. D., 1992. Problems and strategies associated with long-term use of nematod resistant cultivars. Journal of Nematology, 24 (2): 228-233.

Yüksel, H., 1974. Kök-ur nematodlarının (*Meloidogyne* spp.) Türkiye'deki durumu ve bunların populasyon problemleri üzerinde düşünceler. Atatürk Üniversitesi Ziraat Fakültesi Ziraat Dergisi, 5 (1): 83-105.

Zijlstra, C., D. T. H. M. Donkers-Venne & M. Fargette, 2000. Identification of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* using sequence characterised amplified region (SCAR) based PCR assays. Nematology, 2: 847-853.

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Original article (Orijinal araştırma)

Bioactivities of *cry* gene positive *Bacillus thuringiensis* (Berliner) (Bacillales: Bacillaceae) strains on *Ephestia kuehniella* Zeller, 1879 and *Plodia interpunctella* (Hübner, 1813) (Lepidoptera: Pyralidae)[1,2]

cry gene pozitif *Bacillus thuringiensis* (Berliner) (Bacillales: Bacillaceae) izolatlarının *Ephestia kuehniella* Zeller, 1879 ve *Plodia interpunctella* (Hübner, 1813) (Lepidoptera: Pyralidae) üzerindeki biyoaktiviteleri

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Summary

Bacillus thuringiensis is the bacterium most commonly used for biopesticide production due to parasporal crystal formation during its growth cycle. As a consequence of repeated use, *B. thuringiensis* biopesticides may cause the development of resistance in the pests. Therefore, it is necessary to explore new *B. thuringiensis* strains with a certain degree of bioactivity. In this study (2012-2013), the bioactivity of native *B. thuringiensis* strains from the Aegean Region of Turkey were tested against second instar larvae of *Ephestia kuehniella* and *Plodia interpunctella*. The bioactivity of 21 *B. thuringiensis* strains with *cry1*, *cry2* or *cry9* gene was determined as percent mortality according to Abbott's formula**.** The highest mortality rates were 42 and 63% in *E. kuehniella* and *P. interpunctella*, respectively. These mortality rates were equal to or 1.8 times greater than that of *B. thuringiensis* subsp. *kurstaki*. In addition, plasmid profiles of *B. thuringiensis* strains changed between 5-18 kb**.** Moreover, SDS-PAGE analysis of the most toxic strains indicated the presence of Cry1 and Cry2 proteins**.** Two different *cry2* gene profiles containing either *cry2Aa1* or combination of *cry2Aa1* and *cry2Ab2* genes were detected by PCR analysis**.** In addition, partial DNA sequence analysis of *cry2A* genes indicated phylogenetic differences among the toxic strains and *B. thuringiensis* subsp. *kurstaki*. As a result, these *B. thuringiensis* strains may be used to control both *E. kuehniella* and *P. interpunctella* as alternative biopesticides in cases of insect resistance to currently used *B. thuringiensis* preparations.

Keywords: *Bacillus thuringiensis*, bioactivity, *Ephestia kuehniella*, *Plodia interpunctella*

Özet

Bacillus thuringiensis üreme döngüsü sırasında kristal oluşturması nedeniyle biyopestisit üretimi için en çok kullanılan bakteridir. *Bacillus thuringiensis* biyopestisitlerinin tekrarlayan kullanımları zararlılarda direnç gelişimine neden olabileceğinden, belirli düzeyde biyoakiviteye sahip yeni *B. thuringiensis* izolatlarının araştırılmasına ihtiyaç vardır. Bu çalışmada, Ege Bölgesin'den elde edilen doğal *B. thuringiensis* izolatlarının biyoaktivitesi *Ephestia kuehniella* ve *Plodia interpunctella*'nın ikinci dönem larvalarına karşı 2012-2013 yıllarında araştırılmıştır. *cry1*, *cry2* ya da *cry9* geni taşıyan 21 *B. thuringiensis* izolatının biyoaktivitesi Abbott formülüne göre yüzde ölüm olarak belirlenmiştir. En yüksek ölüm oranları, *E. kuehniella* ve *P. interpunctella*' ya karşı sırasıyla %42 ve %63 bulunmuştur. Bu ölüm oranları, *B. thuringiensis* subsp. *kurstaki*'ninkine eşit veya *B. thuringiensis* subsp. *kurstaki*'ninkinden 1.8 kat daha yüksektir. Buna ek olarak, *B. thuringiensis* izolatlarının plazmit profilleri 5-18 kb arasında değişmiştir. Ayrıca, en toksik izolatların SDS-PAGE analizi Cry1 ve Cry2 proteinlerinin varlığını göstermiştir. PCR analizi ile ya *cry2Aa1* veya *cry2Aa1* ve *cry2Ab2* genlerinin kombinasyonunu içeren iki farklı *cry2* gen profili belirlenmiştir. Ayrıca, *cry2A* genlerinin kısmi DNA sekans analizi, toksik izolatlar ve *B. thuringiensis* subsp. *kurstaki* arasındaki filogenetik farklılıkları göstermiştir. Sonuç olarak, bu *B. thuringiensis* izolatları bilinen *B. thuringiensis* preperasyonlarına karşı böcek direnci olması durumunda alternatif biyopestisitler olarak hem *E. kuehniella* hem de *P. interpunctella*'yı kontrol etmek için kullanılabilir.

Anahtar sözcükler: *Bacillus thuringiensis*, biyoaktivite, *Ephestia kuehniella*, *Plodia interpunctella*

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Introduction

Bacillus thuringiensis (Berliner) (Bacillales: Bacillaceae) is a Gram positive, spore-forming bacterium that produces parasporal crystal inclusions during sporulation (Rowe et al., 1987; Schnepf et al., 1998). These parasporal crystals, also known as delta-endotoxins or insecticidal crystal proteins (ICPs), are mostly composed of one or more crystal (Cry) proteins and cytolytic proteins (Cyt) (Crickmore et al., 1998; Schnepf et al., 1998). *Bacillus thuringiensis* has being used for controlling insect pests from the different orders including Lepidoptera, Diptera, Coleoptera (Höfte & Whiteley, 1989), Hemiptera, Hymenoptera, Homoptera and Mallophaga (Schnepf et al., 1998). Also, it has been reported that some strains of *B. thuringiensis* exhibit activity against nematodes, acari and protozoa as well (Feitelson et al., 1992; Schnepf et al., 1998).

Given their high specificity and environmental safety, the spore-crystal mixtures of *B. thuringiensis* have been successfully used as bioinsecticides (Höfte & Whiteley, 1989; Schnepf et al., 1998) and approximately 2% of total insecticidal market consists of *B. thuringiensis* as a biological control agent (Bravo et al., 2011). Insecticidal Cry proteins have been used in preparations of novel insect formulations and transgenic plants have been constructed using genes encoding Cry proteins (Sanchis, 2011). Over 700 *cry* gene sequences have been identified and they are usually located on large plasmids (reviewed by Palma et al., 2014). As a result of the useful applications of insecticidal proteins there has been an intense search for new *B. thuringiensis* strains from the diverse habitats with different specificities.

Ephestia kuehniella Zeller, 1879 and *Plodia interpunctella* (Hübner, 1813) (Lepidoptera: Pyralidae) are major lepidopteran pests of stored plant products (Sedlacek et al.,1995). These pests feeding on stored grain and crops can cause loss of weight and decreased quality. The aim of this study was to investigate the toxic effects of native *B. thuringiensis* strains on *E. kuehniella* and *P. interpunctella,* and to identify the plasmid profiles and *cry2*-type genes of the most toxic strains against both target pests.

Materials and methods

Bacterial strains

Twenty-one native *B. thuringiensis* strains from the *B. thuringiensis* collection of H. Gunes Molecular Biology Laboratory were selected based on their *cry1*, *cry2* or *cry9* gene content. The *B. thuringiensis* strains were originally obtained from fig-growing areas in Aegean Region of Turkey in an earlier study (Alper et al., 2014). The reference strain, *B. thuringiensis* subsp. *kurstaki* (BGSC 4D1), was kindly provided by Prof. Dr. Zeigler (*Bacillus* Genetic Stock Center, Columbus, Ohio, USA).

Bioassay

Bioassays were carried out with spore-crystal mixtures of all native strains and the reference strain of *B. thuringiensis*. Each *B. thuringiensis* strain was cultured in 100 ml of nutrient broth medium for 3 days at 28°C in order to obtain the spore-crystal mixtures. After spinning the bacterial culture for 15 min at 4°C and 6000 rpm, the pellet was washed twice with ice-cold 1 M NaCl and three times with sterile distilled water. The pellet was dried overnight at 37-40°C and stored as powder at -20°C until used (Bravo et al., 1998).

Bioactivity of *B. thuringiensis* strains were investigated against second instar larvae of both *E. kuehniella* and *P. interpunctella* reared in 2012-2013 by the method of Ozkan (2006). Distilled water containing 0.1% Tween 80 was used to suspend the spore-crystal powder. A diet that included whole meal and wheat powder (3:1) was combined with suspension at 500 ppm (i.e., 500 µg spore-crystal mixture in 1 g compost) and dried. Diet containing spore-crystal mixture of *B. thuringiensis* subsp. *kurstaki* or diet without toxin was used as positive and negative control, respectively. Bioassays were performed using 20 larvae per toxin with three replicates at 25°C, 70% RH and a 16L:8D h photoperiod. Larval mortality was determined after 7 days. Corrected mortality data were calculated by using Abbott's formula (Abbott, 1925).

Plasmid pattern

After growing *B. thuringiensis* strains on nutrient agar overnight at 37°C, bacterial colonies were scraped gently with sterile distilled water and plasmid DNA was extracted according to the method of O'Sullivan & Klaenhammer (1993). Plasmid patterns were obtained by running undigested total plasmid DNA on an agarose gel at 80V and visualized in a gel documentation system (Vilber Lourmat, France). DNA ladder, SM1163 (Fermentas, St. Leon-Rot, Germany) was used as DNA weight marker.

Determination of *cry2*-type gene

Gene specific primers for the *cry2Aa1* and *cry2Ab2* genes as described by Salehi Jouzani et al. (2008) along with PCR were used to identify *cry2* gene content of *cry2* positive strains displaying bioactivity against the pests. Genomic DNA isolation was carried out according to the method of Ausebel et al. (2003). PCR mixture contained 500 ng of genomic DNA, 200 µM dNTP, 0.2-0.5 µM each of forward and reverse primer, 1.5 mM $MgCl_2$ and 2 U of *Taq* DNA polymerase (Fermentas) in a volume of 50 µl. The amplification reaction was carried out under following conditions: denaturation at 95°C for 1 min., annealing at 54°C for *cry2Aa1* and 60°C for *cry2Ab2* genes for 1 min., and extension at 72°C for 1 min. for total of 35 cycles and a final extension at 72°C for 10 min. Advanced Primus 96 Thermal Cycler (PeqLab, Erlangen, Germany) was used for amplifications. A total of 10 µl of each amplified PCR product was analyzed on 1% agarose-ethidium bromide gel in TAE buffer and DNA bands were visualized in a gel documentation system.

Table 1. The specific primers for identification of *cry2*-type genes

Target gene	Sequence	Product size	Reference
cry2Aa1	F-5'- CGGATAAAATAATCTGGGAAATAGT -3'	498 bp	Salehi Jouzani et al., 2008
	R-5'- GAGATTAGTCGCCCCTATGAG -3'		
cry2Ab2	F-5'- CGGATAAAATAATCTGGGAAATAGT -3'	546 bp	Salehi Jouzani et al., 2008
	R-5'- TGGCGTTAACAATGGGGGGAGAAAT -3'		
cry2	F-5'-CGGATAAAATAATCTGGGAAATAGT- 3'	1556 bp	Sauka et al., 2005
	R-5'-TGGCGTTAACAATGGGGGGAGAAAT-3'		

SDS-PAGE analysis

In order to determine protein profiles of the six most toxic *B. thuringiensis* strains, they were inoculated in 5 ml nutrient broth and allowed to sporulate for 3-4 days at 30°C by shaking at 200 rpm. Preparation of spore-crystal mixtures and electrophoresis were done as described in Alper et al. (2014). For electrophoresis, 5 µg spore-crystal mixture determined by Bradford assay (Bradford, 1976) was loaded per lane along with protein weight marker, SM0661 (Fermentas). The gels were stained with Comassie Brilliant Blue R250 (Sigma-Aldrich, St. Louis, MS, USA).

Partial DNA sequence analysis for *cry2* gene of toxic *Bacillus thuringiensis* strains

In order to determine if toxic *B. thuringiensis* strains posses new *cry*-type genes, genomic DNA of the strains was amplified with *cry2A* forward and *cry2A* reverse universal primers as described by Sauka et al. (2005). This primer pair results in a PCR product at around 1556 bp. The PCR products were purified from the agarose gel using silica bead DNA gel extraction kit (Fermentas) and cloned in pJET1.2/blunt cloning vector (Fermentas). Transformation into competent *Escherichia coli* DH10B strain was carried out according to the manufacturer's protocol. The presence of inserts in recombinant colonies were verified using forward sequencing and reverse sequencing primers of the vector pJET1.2.

Afterwards, plasmid DNA was isolated using the GeneJET plasmid miniprep kit (Fermentas). Nucleotide sequence of cloned insert was performed by Refgen Inc. (Ankara, Turkey) and the sequences were combined with contig analysis of the BioEdit program 7.1 (http://bioedit.software.informer.com). After confirming the quality of the nucleotide peaks, DNA sequences were compared with known sequences using BLASTN database (http://www.ncbi.nlm.nih.gov). Sequence alignments were generated with the CLUSTAL W and analyzed using MEGA-4 program (Tamura et al., 2007). Phylogenetic tree was built with neighbor joining methods (Saitou & Nei, 1987).

Statistical analysis

The data obtained from the bioassays were subjected to analysis of variance (one-way ANOVA) for comparing the toxicity of strains. After analysis, significance of differences between applications of different spore-crystal mixtures was determined by Duncan's multiple range test at significance levels of $P \leq 0.05$. All statistical analysis were carried out by SPSS Statistics (Version 10, 2003, IBM, Armonk, NY, USA).

Results

Profiles of *cry* genes of *Bacillus thuringiensis* strains

Given that Cry1, Cry2 and Cry9 proteins were toxic to lepidopteran insects (Bravo et al., 1998), we selected twenty-one native *B. thuringiensis* strains from our *B. thuringiensis* collection with *cry* genes encoding these Cry proteins. Profiles of *cry* genes of the strains were identified previously (Alper et al., 2014) and are presented in Table 2.

Table 2. Content of *cry* genes of *Bacillus thuringiensis* strains

cry gene(s)	*Bacillus thuringiensis* strains and reference strain
cry 1	1T, 2T, 7T, 113T, 5MY, 75MY, 76MY
cry 9	3T
cry 1, cry 2	5T, 8T, 9T, 11T, 176T, 43MY, 44MY
cry 1, cry 2, cry 3	107T, 13MY, 42MY
cry 1, cry 2, cry 9	6T, 10T
cry 1, cry 2, cry 3, cry 9	4T
cry1, *cry2*	*B. thuringiensis* subsp. *kurstaki* (BGSC 4D1)

Bioactivity of *Bacillus thuringiensis* strains against *Ephestia kuehniella* and *Plodia interpunctella*

The bioassays against *E. kuehniella* and *P. interpunctella* revealed differences between the toxicities of the strains and their effect on the two pests. Against the larvae of *E. kuehniella*, three strains (2T, 3T 5MY) caused mortality less than 15%; 14 strains displayed mortality between 15 and 28%; and finally four strains (10T, 107T, 13MY and 42MY) exhibited mortality between 30 and 42% (Table 3). The highest mortality rates (41 and 42%) were obtained from the strains 42MY and 13MY, respectively. The reference strain *B. thuringiensis* subsp. *kurstaki* exhibited mortality similar to those strains. There was no significant difference among 13MY, 42MY and *B. thuringiensis* subsp. *kurstaki* in terms of toxicity on larvae of *E. kuehniella*. However, the toxic effects of these strains and the reference strain were statistically significant compared to negative control and the other strains (Table 3).

Table 3. Effects of native *Bacillus thuringiensis* strains on larvae of *Ephestia kuehniella* and *Plodia interpunctella* (Mean ± SE) with mortality range

	Ephestia kuehniella		*Plodia interpunctella*	
Strain	Alive larvae*	% Mortality	Alive larvae*	% Mortality
1T	17,57 ± 0,41 defghi	19	15,04 ± 0,70 ef	32
2T	18,90 ± 0,22 ij	10	16,66 ± 0,66 fg	25
3T	18,95 ± 0,26 ij	9	17,85 ± 0,67 ghijk	17
4T	18,04 ± 0,40 fghi	15	16,90 ± 0,73 fgh	23
5T	16,14 ± 0,57 bcde	26	17,00 ± 0,61 gh	22
6T	18,04 ± 0,40 fghi	15	16,90 ± 0,63 fgh	23
7T	16,14 ± 0,57 bcde	26	16,95 ± 0,64 fgh	23
8T	17,09 ± 0,56 bcdefgh	22	13,00 ± 0,70 cd	41
9T	16,52 ± 0,38 bcdef	25	17,47 ± 0,55 ghi	20
10T	15,46 ± 0,45 bc	30	17,66 ± 0,40 ghij	18
11T	18,04 ± 0,42 fghi	15	16,52 ± 0,67 fg	25
107T	15,33 ± 0,60 b	32	15,04 ± 0,70 ef	32
113T	16,23 ± 0,62 bcdef	25	17,57 ± 0,46 ghij	18
176T	17,85 ± 0,57 efghi	18	18,71 ± 0,26 hijkl	12
5MY	18,38 ± 0,27 ghij	13	19,76 ± 0,09 kl	3
13MY	13,19 ± 0,90 a	42	11,14 ± 1,08 b	53
42MY	13,33 ± 1,01 a	41	9,04 ± 1,04 a	63
43MY	15,85 ± 0,64 bcd	28	15,04 ± 0,67 ef	32
44MY	16,38 ± 0,49 bcdef	25	11,38 ± 0,82 bc	48
75MY	16,80 ± 0,58 bcdefg	23	19,28 ± 0,20 ijkl	6
76MY	17,28 ± 0,31 cdefghi	20	19,47 ± 0,16 jkl	5
4D1	13,14 ± 0,86 a	42	14,33 ± 0,65 de	34
Negative control(NC)	20,00 ± 0,00 j	0	20,00 ± 0,00 l	0

*Means with the same letter in the column are not different statistically (P>0.05).

When spore-crystal mixture of *B. thuringiensis* strains was applied to *P. interpunctella* larvae, different degree of mortality was observed. Mortality range of the most of the strains was between 20 and 50% (Table 3). Four strains 8T, 13MY, 42MY and 44MY were found to be the most toxic strains with their toxicity higher than that of the reference strain *B. thuringiensis* subsp. *kurstaki*. In other words, the mortality rates obtained from the native strains 42MY, 13MY and 44MY and 8T were respectively 1.8, 1.5, 1.4 and 1.2 times higher than that of the reference strain. In short, the toxic effects of 13MY, 42MY, 44MY and 8T were determined to be statistically significant compared to negative controls, *B. thuringiensis* subsp. *kurstaki* and the other strains (P< 0.05) (Table 3).

Plasmid pattern

Since *cry* genes are generally detected in several circular or linear plasmids in *B. thuringiensis* (Carlson et al., 1996), plasmid patterns of toxic strains were identified. Agarose gel electrophoresis of plasmid preparations indicated the presence of major plasmid band around 18 kb in all strains. Molecular weight of the plasmid bands for 13MY, 42 MY, 10T and 107T changed between 5 and 18 kb (Figure 1). In addition, the number of plasmid bands ranged from 1 to 6.

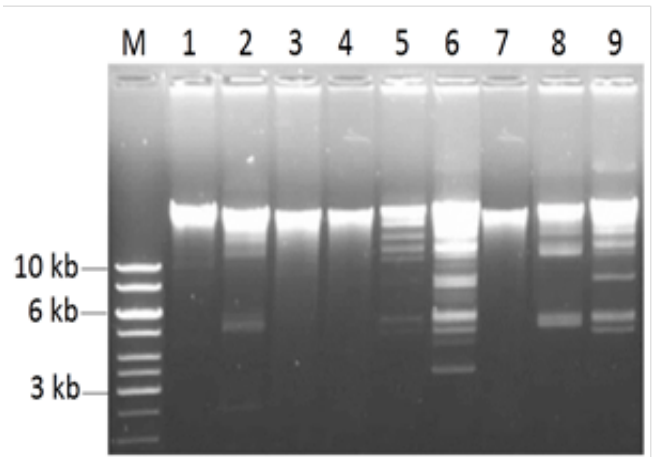

Figure 1. Plasmid profiles of *Bacillus thuringiensis* strains. M: DNA Marker, 1: 8T, 2: 10T, 3: 43MY, 4: 44MY, 5: 45MY, 6: 107T, 7: *B. thuringiensis* subsp. *kurstaki*, 8: 13MY, 9: 42MY.

Identification of *cry2*-type gene composition by PCR

A total of six strains, which are the most effective strains 10T, 107T, 13MY and 42MY against *E. kuehniella*; and 8T, 13MY, 42MY and 44MY against *P. interpunctella* were further characterized for their specific *cry2*-type genes using gene specific primers for *cry2Aa1* and *cry2Ab2* genes. PCR analysis indicated two different *cry2* gene profiles. The gene *cry2Aa1* was determined in all strains. Although two strains showed amplification with only *cry2Aa1*, four strains showed amplification with *cry2Aa1* and *cry2Ab2* genes together (Table 4).

Table 4. Profiles of the *cry2*-type gene of *Bacillus thuringiensis* strains

Profile	*cry2* gene content	*Bacillus thuringiensis* strains
1	*cry2Aa1*	8T, 10T
2	*cry2Aa1, cry2Ab2*	107T, 13MY, 42MY, 44MY

Cry protein profiles

The most toxic *B. thuringiensis* strains to *E. kuehniella* and *P. interpunctella* were subjected to SDS-PAGE analysis in order to determine their Cry protein contents (Figure 2). All profiles seem to be similar and density of two bands around 130 and 65 kDa were stronger than that the others. These results indicate that *cry1* and *cry2* genes were expressed in the toxic strains as well as in *B. thuringiensis* subsp. *kurstaki*. However, expression levels of Cry1 and Cry2 proteins from 8T and 10T seemed to be less than that of other *B. thuringiensis* strains.

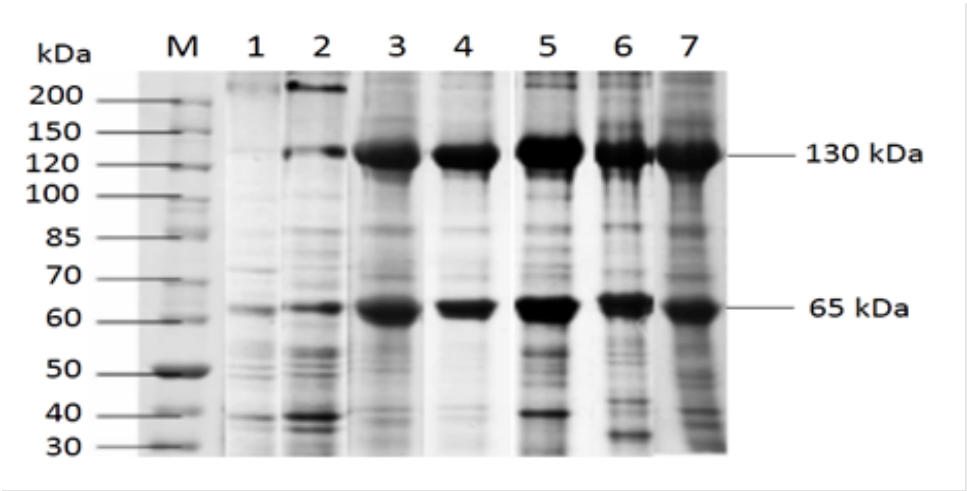

Figure 2. SDS-PAGE analysis of Cry Protein profiles from toxic *Bacillus thuringiensis* strains. M: Protein marker, 1: 8T, 2: 10T, 3: 13MY, 4: 42MY, 5: 44MY, 6: 107T, 7: *B. thuringiensis* subsp. *kurstaki*.

Partial DNA sequence analysis of *cry2A* gene

An amplicon of about 1556 bp was obtained for the *cry2A* gene (Figure 3). After cloning the PCR products of 13MY, 42MY, 44MY and 107T into pJET1.2/blunt vector, DH10B competent cells were transformed with the plasmid constructs. PCR products in recombinant colonies were verified and subjected to sequence analysis. DNA sequences of the *cry2A* genes from *B. thuringiensis* strains were aligned and compared with the reported sequences from GenBank. Analysis of the partial 471 bp *cry2* gene sequences of the strains revealed 94-95% matching to *cry2Aa* and *cry2Ab* genes. Given that 449 bp sequence belonged to endotoxin mid domain, more sequence differences may be present in domains I and III. The phylogenetic tree has two clusters with two and 15 sequences in each cluster (Figure 4). Based on this phylogenetic analysis, *cry2A* gene of strains 13MY, 107T, 42MY and 44MY appear to have diverged in different a lineage compared to *B. thuringiensis* subsp. *kurstaki,* which indicate that they could be distinct *cry2A* genes.

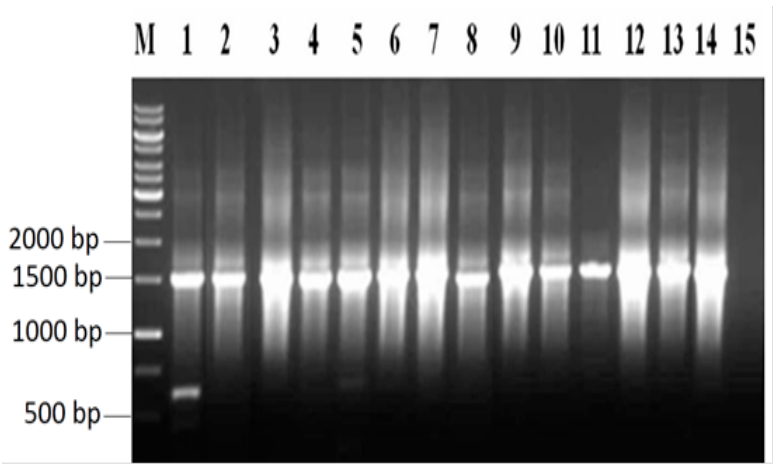

Figure 3. Amplification of *cry2A* gene in the *Bacillus thuringiensis* strains. M: Marker 1 kb DNA Ladder, 1: 8T, 2: 10T, 3: 23T, 4: 69T, 5: 71T, 6: 106T, 7: 107T, 8: 153T, 9: 13MY, 10: 42MY, 11: 44MY, 12: 45MY, 13: 51MY, 14: *B. thuringiensis* subsp. *kurstaki*, 15: negative control.

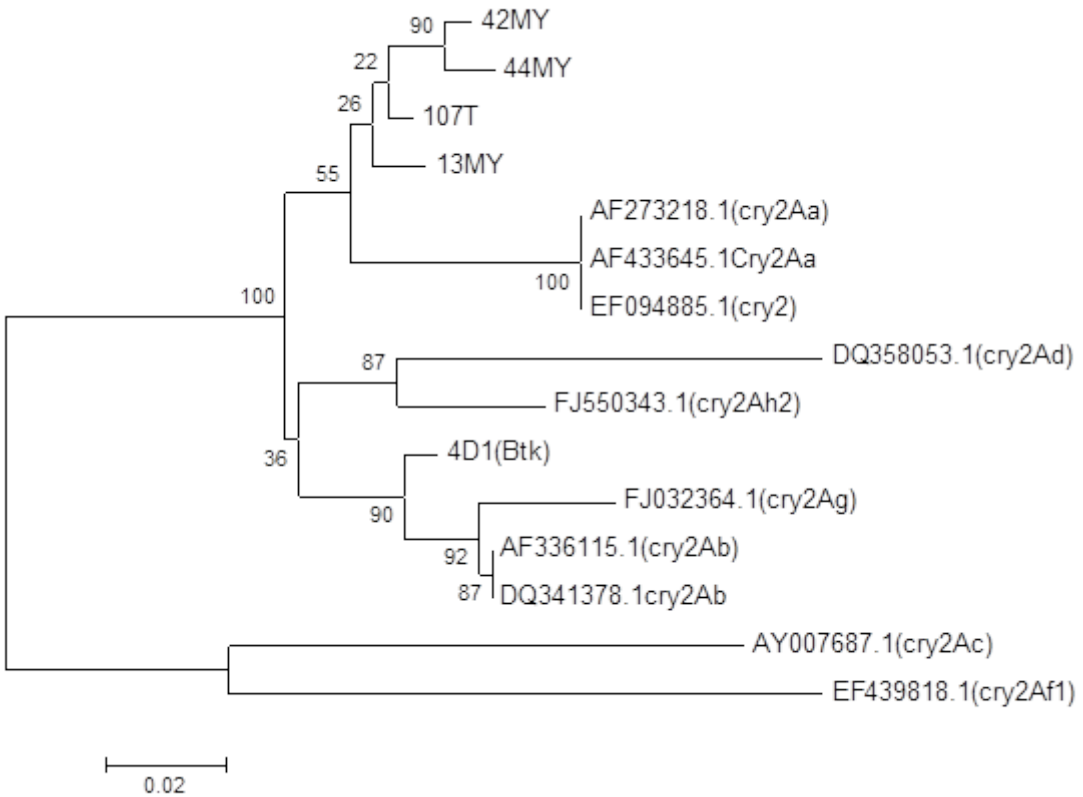

Figure 4. Phylogenetic tree obtained from the alignment of *cry2* gene sequences along with the *cry2A* genes of the native *Bacillus thuringiensis* strains as described in material and methods.

Discussion

In this study, bioactivities of native *B. thuringiensis* strains against stored product pests *E. kuehniella* and *P. interpunctella* were investigated. In addition, plasmid patterns, protein profiles and *cry2*-type genes of the most toxic strains were identified. Substantial evidence indicates that *B. thuringiensis* from different regions produce genetically and functionally different toxins (Palma et al., 2014) and a single amino acid changes in insecticidal Cry proteins can significantly alter their toxicity (Udayasuriyan et al., 1994; Rajamohan et al., 1995). The fact that some insects develop resistance against *B. thuringiensis* toxins after repeated exposure (Tabashnik, 1994; Ferre et al., 1995) makes it necessary to detect and identify new *B. thuringiensis* strains for the management of resistant insect populations. Therefore, screening of *B. thuringiensis* strains with certain bioactivities against various pests is being undertaken worldwide.

Here, we applied spore and crystal mixtures to stored product pests during bioassays because the mixture is more effective on mortality compared to crystals or spores alone (Crickmore, 2006). The highest toxicity value of four strains changed between 30-42% for *E. kuehniella* larvae while two strains exhibited 53-63% toxicity to *P. interpunctella* larvae at a spore-crystal concentration of 500 ppm. The strains 13MY and 42MY were found to be the most toxic strains for both *E. kuehniella* and *P. interpunctella* larvae. The toxic effects of 13MY and 42MY on *E. kuehniella* larvae were similar to that of *B. thuringiensis* subsp. *kurstaki*. However, the toxic effects of 13MY and 42MY on *P. interpunctella* larvae were found to be 1.5 and 1.8 times higher than that of *B. thuringiensis* subsp. *kurstaki*, respectively.

These results are partially consistent with other studies. For example, Chilcott & Wigley (1993) reported that the mortality rates of *B. thuringiensis* strains in lepidopteran larvae ranged from 37 to 88%. Azizoglu et al. (2011) showed 40 and 47% mortality to *E. kuehniella* larvae while 50 and 54% mortality to *P. interpunctella* larvae with *B. thuringiensis* strains U14.1 and U6.6. In addition, Hongyu et al. (2000) reported more than 60% mortality to *P. interpunctella* larvae. Moreover, *B. thuringiensis* serovar *morrisoni* strain 85PPb with both *cry1* and *cry2* genes retarded larval growth of *E. kuehniella* by 84% (Apaydin et al., 2008). Furthermore, Bozlağan et al. (2010) showed that insecticidal activities of the most effective strains against *E. kuehniella* larvae and *P. interpunctella* larvae were 1.8 and 5.2 times higher than that of *B. thuringiensis* subsp. *kurstaki*, respectively. Finally, the mortality rates of spore-crystal mixture from a novel *B. thuringiensis* strain SY49-1 at concentration of 100 µg/g were 70% on *P. interpunctella* and 90% on *E. kuehniella* larvae (Azizoğlu et al., 2016). The discrepancy among these results might be due to *cry* gene content of the strains, expression of related genes at the protein level, susceptibility of target insects, and differences in methodology for bioassay or a combination of these factors.

One of the factors that affect the toxicity to pests is *cry* gene content of the strains. Therefore, identification of *cry* genes in a *B. thuringiensis* strain is important to estimate its insecticidal potential. Given that Cry1, Cry2 and Cry9 proteins are effective on lepidopteran larvae, we used *B. thuringiensis* strains positive for *cry1*, *cry2* and/or *cry9* genes. *Bacillus thuringiensis* subsp. *kurstaki* was used as a reference strain because it has both *cry1* and *cry2* genes. Bioassay results indicated that the native *B. thuringiensis* strains exhibited different level of toxicity to the same pest. This difference in toxicity may be most probably due to the difference in the level of expression of these proteins or type of *cry1* or *cry2* genes. In fact, we showed that the strains 13 MY and 42 MY contain *cry1* gene different from that of *B. thuringiensis* subsp. *kurstaki* in our previous study (Alper et al., 2014). Similarly, partial DNA sequence analysis of *cry2A* genes was different from that of the reference strain according to phylogenetic tree in this present study. Verification of sequence differences using Taq polymerase enzyme with proof reading activity will indicate that variations in nucleotide sequence of *cry2* gene among strains may be the reason for different bioactivity level of each Bt strain.

The *cry2* gene encode 65 kDa proteins in many *B. thuringiensis* strains (Höfte & Whiteley, 1989) and Cry2 group proteins were known to be toxic to both lepidopteran and dipteran insects (Bravo et al., 1998). Even though Cry2Aa protein is toxic to both lepidopteran and dipteran larvae, Cry2Ab protein is only toxic to lepidopteran insects. In the present study, we examined *cry2*-type gene of the most toxic strains using specific primers. We found two different profiles of *cry2* gene (Table 4). All of these strains carried *cry2Aa1*; however, two strains contained only *cry2Aa1* and other four strains had both *cry2Aa1* and *cry2Ab2* together. Similar to our result, Sauka et al. (2005) showed that among the native *B. thuringiensis* strains, 94.9% of them had *cry2Aa/cry2Ab* gene combination whereas 3.4% and 1.7% of them contained *cry2Ab* or *cry2Aa*, respectively. In addition, Liang et al. (2011) reported that *cry2Aa/cry2Ab* gene combination was the most frequent (90.4%), whereas percentages of *cry2Aa* and *cry2Ab* positive genes in their *B. thuringiensis* strains were 6.8 and 2.5, respectively. In other words, occurrence of *cry2Aa/cry2Ab* gene combination was the most frequent compared to that of individual cry2-type genes in *B. thuringiensis* strains. Given that the toxicity spectrum of Cry2A type proteins is wider than that of currently used Cry1A proteins, the toxic strains with *cry2*-type genes will be promising strains for management of the insect resistance.

In conclusion, the *B. thuringiensis* strains 13MY and 42MY were found to be the most toxic to the larvae of both *E. kuehniella* and of *P. interpunctella*. In addition, toxicity of the strains 42MY, 13MY, 44MY and 8T to *P. interpunctella* was 1.8, 1.5, 1.4 and 1.2 times higher than that of the reference strain of *B. thuringiensis* subsp. *kurstaki*. In the case of development of resistance due to repeated exposure to a certain kind of *B. thuringiensis* toxin, these *B. thuringiensis* strains will serve as an alternative resource of toxins and toxin genes for control of these pests. Future research arising from this study will be the cloning of *cry2*-type genes for recombinant production of the toxin.

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References

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

Alper, M., H. Güneş, A. Tatlıpinar, B. Çöl, H. S. Civelek, C. Özkan & B. Poyraz, 2014. Distribution, occurrence of *cry* genes, and lepidopteran toxicity of native *Bacillus thuringiensis* isolated from fig tree environments in Aydın Province. Turkish Journal of Agriculture and Forestry, 38: 898-907.

Apaydin, Ö., Ç. Çınar, F. Turanli, Ş. Harsa & H. Güneş, 2008. Identification and bioactivity of native strains of Bacillus thuringiensis from grain-related habitats in Turkey. Biological Control, 45 (1): 21-28.

Ausebel, F. M., R. Brent, R. E. Kingston, D. D. Moore, J. G. Seidman, J. A. Smith & K. Struhl, 2003. "Preparation and Analysis of DNA, (2.0.1-2.1.5) 177-184". In: Current Protocols in Molecular Biology. John Wiley & Sons Inc., New York, (A.5.45) 4648 pp.

Azizoglu, U., S. Yılmaz, A. Ayvaz, S. Karabörklü & M. Akbulut, 2011. Characterization of local *Bacillus thuringiensis* isolates and their toxicity to *Ephestia kuehniella* (Zeller) and *Plodia interpunctella* (Hübner) larvae. Egyptian Journal of Biological Pest Control, 21 (2): 143-150.

Azizoglu, U., A. Ayvaz, S. Yılmaz, S. Karabörklü & R. Temizgul, 2016. Expression of *cry1Ab* gene from a novel Bacillus thuringiensis strain SY49-1 active on pest insects. Brazilian Journal of Microbiology, 47: 597-602.

Bozlağan, İ., A. Ayvaz, F. Öztürk, L. Açık, M. Akbulut & S. Yılmaz, 2010. Detection of *cry1* gene in *Bacillus thuringiensis* isolates from agricultural fields and their bioactivity against two stored product moth larvae. Turkish Journal of Agriculture and Forestry, 34: 145-154.

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

Bravo, A., S. Sarabia, L. Lopez, H. Ontiveros, C. Abarca, A. Ortiz, M. Ortiz, L. Lina, F. J. Villalobos, G. Peña, M-E. Nuñez-Valdez, M. Soberon & R. Quintero, 1998. Characterization of *cry* genes in a Mexican *Bacillus thuringiensis* strain collection. Applied and Environmental Microbiology, 64 (12): 4965-4972.

Bravo, A., S. Likitvivatanavong, S. S. Gill & M. Soberón, 2011. *Bacillus thuringiensis*: A story of a successful bioinsecticide. Insect Biochemistry and Molecular Biology, 41: 423-431.

Carlson, C. R., T. Johensen & A. B. Kolsto, 1996. The choromosome map of *Bacillus thuringiensis* subsp. canadensis HD224 is highly similar to that of *Bacillus cereus* type strain ATCC14579. FEMS Microbiology Letters, 141 (2-3): 163-167.

Chilcott, C. N & P. J. Wigley, 1993. Isolation and toxicity of *Bacillus thuringiensis* from soil and insect habitats in New Zealand. Journal of Invertebrate Pathology, 61 (3): 244-247.

Cricmore, N., D. R. Zeigler, J. Feitelson, E. Schnepf, J. Van Rie, D. Lereclus, J. Baum & D. H. Dean, 1998. Revision of the nomenclature for the *Bacillus thuringiensis* pesticidal crystal proteins. Microbiology and Molecular Biology Reviews, 62 (3): 807-813.

Crickmore, N., 2006. Beyond the spore-past and future developments of *Bacillus thuringiensis* as a biopesticide. Journal of Applied Microbiology, 101 (3): 616-619.

Feitelson, J. S., J. Payne & L. Kim, 1992. *Bacillus thuringiensis*: insects and beyond. Bio/Tecnology, 10: 271-275.

Ferre, J., B. Escriche, Y. Bel & J. Van Rie, 1995. Biochemistry and genetics of insect resistance to *Bacillus thuringiensis* insecticidal crystal proteins. FEMS Microbiology Letters, 132 (1-2): 1-7.

Hongyu, Z., Y. Ziniu & D. Wangxi, 2000. Isolation, distribution and toxicity of *Bacillus thuringiensis* from warehouses in China. Crop Protection, 19 (7): 449-454.

Höfte, H. & H. R. Whiteley, 1989. Insecticidal crystal proteins of *Bacillus thuringiensis*. Microbiological Reviews, 53 (2): 242–255.

Liang, H., Y. Liu, J. Zhu, P. Guan, S. Li, S. Wang, A. Zheng, H. Liu & P. Li, 2011. Characterization of *cry2*-type genes of *Bacillus thuringiensis* strains from soil-isolated of Sichuan Basin, China. Brazilian Journal of Microbiology, 42 (1): 140-146.

O`Sullivan, D. J. & T. R. Klaenhammer, 1993. Rapid mini-prep ısolation of high-quality plasmid DNA from *Lactococcus* and *Lactobacillus* spp. Applied and Environmental Microbiology, 59 (8): 2730-2733.

Ozkan, C., 2006. Laboratory rearing of the solitary egg-larval parasitoid, *Chelonus oculator* Panzer (Hymenoptera: Braconidae) on a new recorded factitious host *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae). Journal of Pest Science. 79: 27-29

Palma, L., D. Munoz, C. Berry, J. Murillo & P. Cabellero, 2014. *Bacillus thuringiensis*: An overview of their biological activity. Toxins, 6 (12): 3296-3325.

Rajamohan, F., E. Alcantara, M. K. Lee, X. J. Chen, A. Curtiss & D. H. Dean, 1995. Single amino acid changes in domain II of *Bacillus thuringiensis* CryIAb delta-endotoxin affect irreversible binding to *Manduca sexta* midgut membrane vesicles. Journal of Bacteriology, 177 (9): 2276-2282.

Rowe, G. E., A. Margaritis & H. T. Dulmage, 1987. Bioprocess developments in the production of bioinsecticides by *Bacillus thuringiensis*. Critical Reviews in Biotechnology, 6 (1): 87-127.

Saitou, N. & M. Nei, 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution, 4: 406-425.

Salehi Jouzani, G. R., A. P. Abad, A. Seifinejad, R. Marzban, K. Kariman & B. Maleki, 2008. Distribution and diversity of Dipteran-specific *cry* and *cyt* genes in native *Bacillus thuringiensis* strains obtained from different ecosystems of Iran. Journal of Industrial Microbiology & Biotechnology, 35 (2): 83-94.

Sanchis, V., 2011. From microbial sprays to insect-resistant transgenic plants: history of the biospesticide *Bacillus thuringiensis*. A review. Agronomy for Sustainable Development, 31 (1): 217-231.

Sauka, D. H., J. G. Cozzi & G. B. Benintende, 2005. Screening of *cry2* genes in *Bacillus thuringiensis* isolates from Argentina. Antonie van Leeuwenhoek, 88 (2): 163-165.

Schnepf, E., N. Crickmore, J. Van Rie, D. Lereclus, J. Baum, J. Feitelson, D.R. Zeigler & D.H. Dean, 1998. *Bacillus thuringiensis* and its pesticidal crystal proteins. Microbiology and Molecular Biology Reviews, 62 (3): 775–806.

Sedlacek, J. D., P. A. Weston & R. J. Barney, 1995. “Lepidoptera and Psocoptera, 41-70”. In: Integrated Management of Insect in Stored Products (Eds. B. Subramanyam & D. W. Hagstrum) Marcel-Dekker, Inc., New York, USA, 432 pp.

SPSS, 2001. SPSS Version 10.0.SPSS Inc, 233 S.Wacker Drive, Chicago, Illinois.

Tabashnik, B. E., 1994. Evolution of resistance to *Bacillus thuringiensis*. Annual Reviews of Entomology, 39: 47-79.

Tamura, K., J. Dudley, M. Nei & S. Kumar, 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution, 24 (8): 1596-1599.

Udayasuriyan, V., A. Nakamura, H. Mori, H. Masaki & T. Uozumi, 1994. Cloning of a new cryIA(a) gene from *Bacillus thuringiensis* strain FU-2-7 and analysis of chimaeric CryIA(a) proteins for toxicity. Bioscience, Biotechnology, and Biochemistry, 58 (5): 830-835.

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Original article (Orijinal araştırma)

Response of tomato seedlings with different number of true leaves to *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949

Farklı sayıda gerçek yapraklı döneme sahip domates fidelerinin *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949'ya tepkileri

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Summary

Tomato is one of the most important vegetables grown throughout the world. Root-knot nematodes cause significant economic yield losses in tomato. Development of tomato cultivars which are resistant to root-knot nematodes is the most effective management method. In tomato, resistance to root-knot nematodes is determined by bioassay and molecular markers. Molecular markers are commonly used to screen for resistance genes in breeding programs. However, bioassays are also required for identification of the performance of resistance genes. Different parameters such as stage of seedling, soil temperature, nematode quantity and nematode virulence effect performance of bioassays. In the present study, the response of tomato seedlings with different numbers of true leaves to *Meloidogyne incognita* isolate S6 was compared under controlled conditions. Seedlings showed different reactions to *M. incognita* inoculation. The results indicated that stage of tomato seedlings can be important for bioassay and that tomato seedlings with four true leaves are best for nematode testing. These results will help in the optimization of root-knot nematode tests used in tomato breeding.

Keywords: Bioassay, *Meloidogyne*, tomato seedling, reaction

Özet

Domates dünyada yetiştiriciliği yapılan en önemli sebzelerden birisidir. Kök-ur nematodları domateste önemli düzeyde ekonomik kayıplara neden olmaktadır. Kök-ur nematodlarına dayanıklı domates çeşitlerinin geliştirilmesi en önemli mücadele yöntemidir. Domateste kök-ur nematodlarına dayanıklılık biyolojik testler ve moleküler markörler tarafından belirlenebilmektedir. Moleküler markörler ıslah programlarında dayanıklılık geninin tespitinde yaygın şekilde kullanılmaktadır. Bununla birlikte dayanıklılık geninin performansının belirlenmesi için biyolojik testler gereklidir. Nematod virülensliği, nematod sayısı, toprak sıcaklığı ve fidenin dönemi gibi farklı parametreler biyolojik testin performansını etkilemektedir. Bu çalışmada, farklı sayıda gerçek yaprak dönemine sahip domates fidelerinin *M. incognita*' nın S6 izolatına tepkisi kontrollü koşullar altında karşılaştırılmıştır. Fideler *M. incognita* inokulasyonuna farklı farklı tepkiler göstermiştir. Sonuçlar, biyolojik test için domates fidesinin döneminin önemli olduğunu ve nematod testi için dördüncü gerçek yapraklı döneme sahip fidelerin en uygun olduğunu göstermiştir. Bu sonuçlar, domates ıslahında kullanılacak olan kök-ur nematodlar testlerinin optimizasyonuna yardım edecektir.

Anahtar sözcükler: Biyolojik test, *Meloidogyne*, domates fidesi, reaksiyon

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Introduction

Root-knot nematodes are one of the most important pathogens attacking cultivated plants. The second-stage juveniles of the root-knot nematodes move intercellularly after penetrating the root (Williamson, 1998). They secrete substances through their stylet. These secretions emanate from two subventral and one dorsal esophageal gland cells, and are crucial for infection and for the formation of host feeding cells (Bird, 1967; Hussey & Mims, 1990). They establish a permanent feeding site in the differentiation zone of the root. Root-knot nematodes cause nuclear division without cytokinesis in host cells because of feeding. This process cause large multinucleate cells, known as giant cells (Huang, 1985). Galled roots impair the ability of the plants to take up water and nutrients, resulting in reduced translocation of minerals and photosynthesis (Abad et al., 2003). Affected plants often show symptoms of stunting, wilting or chlorosis (Karssen & Moens, 2006; Schomaker & Been, 2006). In addition, root-knot nematodes interact with soil-borne plant pathogens, resulting in increased damage from other diseases (Williamson, 1998; Karssen & Moens, 2006). Therefore, root-knot nematodes cause significant economic yield losses alone or in combination with other biotic and abiotic factors in crop fields (Schomaker & Been, 2006).

Tomato is one of the most important vegetables grown around the world. Root-knot nematodes are considered to be a major pest of tomato. Managing nematode problems can be difficult in tomato growing fields. Chemical treatments have been used for controlling root-knot nematodes. However, environmental effects and legal regulations have limited their use (Wesemael et al., 2011). Therefore, alternative management methods are required. Resistance breeding is obviously the most effective method to control of root-knot nematode. Genetic resistance to root-knot nematodes has been shown to reduce nematode populations, and thereby decrease the need for pesticides (Williamson, 1999). Therefore, development of the tomato cultivars resistant to root-knot nematodes is one of the most important strategies for controlling root-knot nematodes (Devran et al., 2013). Resistance to root-knot nematodes in tomato plants is determined by bioassays and molecular methods. Bioassays are required for determining the performance of resistant genes in plant-nematode interactions. Bioassays also give reliable and logical information about the resistance of plants. These assays are carried out under controlled conditions in a growth chamber and different parameters, such as stage of seedling, soil temperature and nematode quantity, are important (Devran et al., 2013). Also, knowing the virulence of the nematode species or race is essential. In tomato, resistance assays for root-knot nematodes are actively carried out by researcher focusing nematode-host interactions (Ramsay et al., 2004; Melillo et al., 2006). However, there is no detailed information about the response of tomato seedlings with different number of true leaves to nematode infection. Also, there has been no assessment of the effect of tomato seedling stage on bioassays performance. Therefore, in this study, response of tomato seedlings with different number of true leaf stages to *Meloidogyne incognita* (Kofoid & White, 1919) Chitwood, 1949 was evaluated under controlled conditions.

Material and Methods

Plant material

The susceptible tomato cv. Tueza F_1 seeds were provided by Multi Seed (Antalya, Turkey). The seeds were sown in seedling trays in facilities of Multi Seed. Seedlings were transferred singly to 250-ml plastic pots containing steam-sterilized sandy soil (75% sand, 15% silt, and 10% clay) five weeks after sowing.

Nematode isolate

Meloidogyne incognita race 2 isolate S6 was used in this study. The isolate was identified using the molecular methods and host reaction tests described in previous studies (Devran & Söğüt, 2009; Devran & Söğüt, 2011).

Nematode culture

Egg masses of *M. incognita* were collected from roots of infected tomato plants using a needle and incubated in a petri dish at room temperate. Second-stage juveniles that hatched from the egg masses were collected, placed in a refrigerator at 4^{o}C and used within 1 day. Number of juveniles were counted under microscope.

Nematode inoculation

Tomato seedlings with two, three, four, five, six, seven and eight true leaves were inoculated with 1000 second-stage juveniles each. The juveniles were injected into a 2-cm deep hole close to the stem of the plants. Five replicates for each true leave stage seedlings were laid out in a randomized block design in a growth chamber (16-h photoperiod, 25±0.5°C and 65% RH). The plants were harvested 8 weeks after inoculation. They were gently uprooted and roots of plants were washed under tap water before scoring of egg masses and galls.

DNA isolation

Plant genomic DNA was extracted from young leaf tissue using the Wizard Magnetic Kit (Promega, Madison, WI, USA) following the manufacturer's instructions. Nematode DNA was also isolated from more than ten second-stage juveniles with the DNAeasy Tissue and Blood Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

PCR amplification

Meloidogyne incognita was checked using the species-specific primers MincF/MincR (unpublished data). The other nematode species, *M. javanica*, *M. arenaria* and *M. ethiopica* were also used as negative control. The absence of the *Mi-1* gene in Tueza F1 was checked using the Mi23 marker (Seah et al., 2007). Browny F_1 and Seval F_1 were used as homozygous resistant and heterozygous resistant for *Mi-1* gene, respectively. The PCR reaction was performed in a total volume of 25 µL with 2.5 µL of DNA, 2 mM $MgCl_2$, 200 µM dNTPs, 0.4 µM of each primer, 2.5 µL 10X PCR buffer and 1 U Taq DNA polymerase (Fermentas, Vilnius, Lithuania). Amplification was performed in a thermal cycler (Veriti 96-Well, Applied Biosystems, Foster City, CA, USA) using the following conditions: 3 min at 94^{o}C, 35 cycles at 94^{o}C for 30 s, 56^{o}C for 30 s and 72^{o}C for 1 min with a final extension at 72^{o}C for 7 min. Amplified products were analyzed on a 2% agarose gel in 1X TAE buffer and visualized by ethidium bromide staining.

Data collection and analysis

Egg masses and galls on roots of tomato seedlings were counted. Second-stage juveniles from 100 g soil per pot were extracted using a modified Baermann funnel technique (Hooper, 1986) and counted under microscope. These data were analyzed by ANOVA using the statistical package SAS (v. 9.0 for Windows; SAS Institute Inc., Cary, NC, USA). Significant differences within treatments were tested using least significant difference (LSD).

Results and Discussion

Molecular identification

Meloidogyne incognita was confirmed using species-specific primers MincF/MincR. The primer pairs produced an approximately 150 bp amplicon in *M. incognita* samples, and did not yield any PCR product in another nematode species, *Meloidogyne javanica*, *Meloidogyne arenaria* and *Meloidogyne ethiopica* as expected (Figure 1). Our findings were in accordance with an earlier study (unpublished data). These results indicated that the *M. incognita* isolate S6 was a pure culture.

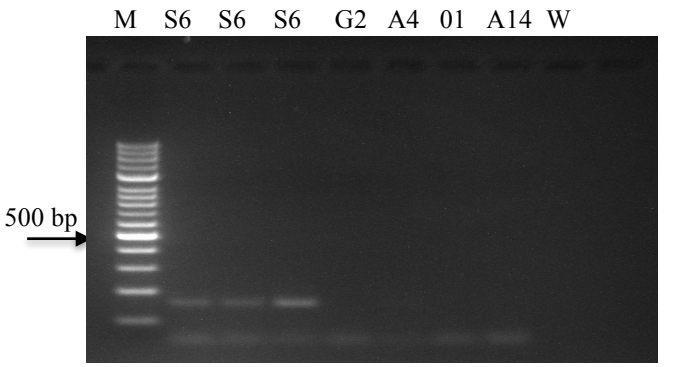

Figure 1. PCR products of MincF and MincR primer sets. M: Molecular marker (100 bp DNA ladder, GeneAll), S6: *Meloidogyne incognita*, G2 and A4: *Meloidogyne javanica*, O1: *Meloidogyne arenaria*, A14: *Meloidogyne ethiopica*: W: Water.

The absence of the *Mi-1* gene in the tomato seedling was verified with molecular marker Mi23. Mi23 primer pairs yielded 380 bp and 430 bp fragments in homozygous resistant and susceptible plants, respectively. Heterozygous plants produced 380 and 430 bp fragments (Figure 2). Marker analysis showed that Tueza F_1 was susceptible as expected. Our results were consistent with earlier studies (Devran et al., 2013; Devran & Söğüt, 2014).

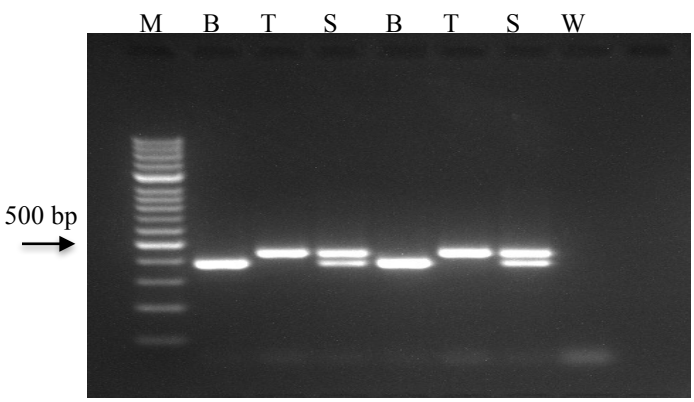

Figure 2. PCR products of Mi23F and Mi23R primer sets. M: Molecular marker (100 bp DNA ladder, GeneAll), B: Browny F_1 (homozygous resistant), T: Tueza F_1 (susceptible), S: Seval F_1 (heterozygous resistant), W: water.

Bioassay of seedlings

Meloidogyne incognita isolate S6 showed different reactions according to the stage of tomato seedlings inoculated. There were significant differences in the number of egg masses produced (Table 1). *Meloidogyne incognita* isolate S6 produced the highest number of egg masses on seedlings with four, five and six true leaves and the lowest number on seedlings the two true leaves. There were no statistically significant differences among seedlings with four, five and six true leaves, between those with seven and eight true leaves.

Table 1. Number of egg masses on the roots of tomato seedlings with different numbers of true leaves

Number of true leaves	Number of egg masses on roots
2	91 ± 68 c
3	151 ± 64 bc
4	314 ± 74 a
5	294 ± 31 a
6	298 ± 77 a
7	250 ± 88 ab
8	233 ± 33 ab

Means in columns followed by the same letter are not significantly different (P ≤ 0.05) according to LSD test.

Tomato seedlings with four true leaves stages had the highest number of gall on their roots (Table 2). Seedlings with two and three true leaves had the lowest number of gall. The number of galls was not statistically significant among seedlings with two and three true leaves, those with five and six true leaves or those with seven and eight true leaves.

Table 2. Number of galls on roots of tomato seedlings with different numbers of true leaves

Number of true leaves	The number of gall on roots
2	100 ± 40 c
3	131 ± 43 c
4	330 ± 33 a
5	280 ± 41 ab
6	276 ± 30 ab
7	261 ± 42 b
8	257 ± 40 b

Means in columns followed by the same letter are not significantly different (P ≤ 0.05) according to to LSD test.

The number of second-stage juveniles was not statistically significant in seedlings with two, three and six true leaves (Table 3). Tomato seedlings with five true leaves had the highest number of second-stage juveniles. Seedlings with two and tree true leaves had the lowest number of second-stage juveniles. There was no correlation number of second-stage juveniles according to stages of true leaves. Therefore, number of second-stage juveniles is not useful unless supported by assessment of number of egg masses and galls.

Table 3. Number of second-stage juveniles in soil from the pots with tomato seedlings with different number of true leaves

Number of true leaves	Number of second-stage juveniles into pots (100 g soil from per pot)
2	15220 ± 13890 c
3	11652 ± 11007 c
4	41470 ± 13498 bc
5	86436 ± 21809 a
6	37920 ± 32282 c
7	83124 ± 37629 ab
8	43050 ± 27940 bc

Means in columns followed by the same letter are not significantly different (P≤0.05) according to to LSD test.

Our findings showed that the stage of tomato seedling is important for nematode inoculation. Therefore, tomato seedlings with same number of true leaves should be inoculated with root-knot nematodes for consistent results in bioassay tests. The seedlings with four, five and six true leaves had higher number of egg masses and galls on their roots than seedling at other stages. Our results indicated that tomato seedlings with four leaves are the best for root-knot nematode testing according to the number of egg masses and galls on root. In the previous studies (Khan et al., 2000; Wasemael et al., 2006), the number of egg masses on roots of young plants was higher than the number of egg masses on roots of old plants. However, our results showed that the number of egg masses and galls on roots of seedlings with two true leaves was lower than other stages. In this study, *M. incognita* isolate S6 affected to tomato seedlings with two and three true leaves more than old plants. Therefore, roots and shoots of these did not grow effectively. Also, the number of egg masses and galls on the seedlings with two and three true leaves were the lowest. This may be because of weak development of root system or stunting of roots. Accordingly, Shane & Barker (1986) reported that plant development can be adversely affected when young seedlings are inoculated with nematode. Consequently, results can be used for optimizing root-knot nematode testing in tomato breeding programs.

References

Abad, P., B. Favery, M. N. Rosso & P. Castagnone-Sereno, 2003. Root-knot nematode parasitism and host response: Molecular basis of a sophisticated interaction. Molecular Plant Pathology, 4 (4): 217–224.

Bird, A. F., 1967. Changes associated with parasitism in nematodes. I. Morphology and physiology of preparasitic and parasitic larvae of *Meloidogyne javanica*. The Journal of Parasitology, 768-776.

Devran, Z. & M. A. Söğüt, 2009. Distribution and identification of root-knot nematodes from Turkey. Journal of Nematology, 41: 128-133.

Devran, Z. & M. A. Söğüt, 2011. Characterizing races of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* in the West Mediterranean region of Turkey. Crop Protection, 30: 451-455.

Devran, Z., B. Başköylü, A. Taner & F. Doğan, 2013. Comparison of PCR-based molecular markers for identification of Mi gene. Acta Agriculturae Scandinavica, Section B - Soil & Plant Science, 45: 395-402.

Devran, Z. & M. A. Söğüt, 2014. Response of heat-stable tomato genotypes to Mi-1 virulent root-knot nematode populations. Turkish Journal of Entomology, 38: 229-238.

Hooper D. J., 1986. “Handling, Fixing, Staining and Mounting Nematodes, 58-80”. In: Laboratory Methods for Work with Plant and Soil Nematodes, (Eds: J.F. Southey) Her Majesty’s Stationery Office, London. 202 pp.

Huang, C. S., 1985. “Formation, Anatomy and Physiology of Giant Cells Induced by Root-Knot Nematodes, 155-164”. In: An Advanced Treatise on Meloidogyne, Vol. 1, (Eds: J. N. Sasser & C. C. Carter), Raleigh: North Carolina State University Graphics, 223 pp.

Hussey, R. S. & C. W. Mims., 1990. Ultrastructure of esophageal glands and their secretory granules in root-knot nematode *Meloidogyne incognita*. Protoplasma, 156: 9-18.

Karssen, G. & M. Moens., 2006. “Root-Knot Nematodes. 59-90” In: Plant Nematology, (Eds: R. N. Perry & M. Moens), CABI, London, 447 pp.

Khan, H., R. Ahmad, A. S. Akhtar, A. Mahmood, T. Basit, & T. Niaz, 2000. Effect of inoculum density of *Meloidogyne incognita* and plant age on the severity of root-knot disease in tomato. International Journal of Agriculture & Biology, 2000 (02): 360–363.

Melillo, M. T., P. Leonetti, M. Bongiovanni, P. Castagnone-Sereno & T. Bleve-Zacheo, 2006. Modulation of reactive oxygen species activities and H_2O_2 accumulation during compatible and incompatible tomato–root-knot nematode interactions. New Phytologist, 170 (3): 501-512.

Ramsay, K., Z. Wang & M. G. K. Jones, 2004. Using laser capture microdissection to study gene expression in early stages of giant cells induced by Root-Knot Nematodes. Molecular Plant Pathology, 5 (6): 587-592.

Schomaker, C. H. & T. H. Been, 2006. “Plant Growth and Population Dynamics, 275-301”. In: Plant Nematology, (Eds: R.N. Perry & M. Moens), British Library, London, UK, 447 pp.

Seah, S., V. M. Williamson, B. E. Garcia, L. Mejía, M. S. Salus, C. T. Martin & D. P. Maxwell, 2007. Evaluation of a co-dominant SCAR marker for detection of the *Mi-1* locus for resistance to root-knot nematode in tomato germ plasm. Tomato Genetic Cooperative Report, 57: 37–40.

Shane, W. W. & K. R. Barker, 1986. Effects of temperature, plant age, soil texture, and *Meloidogyne incognita* on early growth of soybean. Journal of Nematology, 18 (3): 320-327.

Wesemael, W. M. L., N. Viaene & M. Moens, 2006. The influence of root diffusate and host age on hatching of the root-knot nematodes, *Meloidogyne chitwoodi* and *M. fallax.* Nematology, 8 (6): 895-902.

Wesemael, W. M. L., R. N. Perry & M. Moens, 2011. Root-knot nematodes (*Meloidogyne* spp.) in Europe. Nematology, 13 (1): 3-16.

Williamson, V. M., 1998. Root-knot nematode resistance genes in tomato and their potential for future use. Annual Review of Phytopathology, 36: 277-293

Williamson, V. M., 1999. Plant nematode resistance genes. Plant Biology, 2: 327–331.

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Original article (Orijinal araştırma)

Response of some beneficial insect species to colored sticky traps in citrus

Turunçgilde faydalı böceklerin yapışkan renkli tuzaklara tepkileri

Ekrem ATAKAN[1*] Serkan PEHLİVAN[1] Serdar SATAR[1]

Summary

Colored sticky traps have been commonly used for the sampling of detrimental insect species worldwide. However, there is no information about the catches of non-target insects such as predaceous and pollinating insects by colored traps. In this study, trials with colored sticky traps (white, yellow, blue and green) in mandarin cv Okitsu (Satsuma) trees were carried out during 2011 and 2012 in Adana Province, Turkey. Colored plates were hung, at about 1.7 m above ground level, from the exterior canopy of the selected trees and were positioned to the four cardinal directions. A total of 15 beneficial insect species were captured on various colored plates. Yellow and green traps were more attractive to the coccinellid, *Oenopia conglobata* (L., 1758), than the other colored traps. Significantly more of the predaceous hoverfly, *Episyrphus balteatus* (De Geer, 1776) and the honey bee, *Apis mellifera* L., 1758 were obtained on white traps. Green and blue traps were the least attractive to *A. mellifera*. The yellow, blue and green traps hung on the west side of the trees captured significantly more beneficial insects, but white traps hung on the south sides of outer branches trapped considerable numbers of *E. balteatus* and *O. conglobata*. White and yellow sticky traps may provide more ecological data for beneficial insects.

Keywords: *Apis mellifera*, citrus, *Episyrphus balteatus*, *Oenopia conglobata,* sticky traps

Özet

Renkli yapışkan tuzaklar dünyada zararlı böcekleri örneklemek için yaygın bir şekilde kullanılmaktadır. Bununla birlikte renkli tuzakların avcı ve polinatör böcek gibi hedef dışı böcekleri yakalamasıyla ilgili bilgiler bulunmamaktadır. Renkli yapışkan tuzak (beyaz, sarı, mavi ve yeşil) denemeleri 2011-2012 yıllarında Adana ilinde Okitsu (Satsuma) mandalinada yürütülmüştür. Renkli plakalar seçilen ağaçların dış dallarına yerden 1.7 m yüksekliğe ve dört ana yöne doğru asılmıştır. Tuzaklarda toplam 15 faydalı böcek türü yakalanmıştır. Sarı ve yeşil tuzaklar coccinellid *Oenopia conglobata* (L., 1758)'ya diğer tuzaklardan daha çekici olmuşlardır. Beyaz tuzaklar avcı çiçek sineği *Episyrphus balteatus* (De Geer, 1776) ve balarısı *Apis mellifera* L., 1758' nı önemli ölçüde yakalamıştır. Mavi ve yeşil tuzaklar *A. mellifera* için en az derecede çekici olmuştur. Sarı, mavi ve yeşil tuzaklar ağaçların batı tarafına asıldığında daha fazla sayıda faydalı böcek yakalamıştır, fakat beyaz tuzaklar güneydeki dış dallara asıldığında dikkate değer sayıda *E. balteatus* ve *O. conglobata* yakalamışlardır. Beyaz ve sarı yapışkan tuzaklar faydalı böcekler için daha fazla ekolojik veri sağlayabilirler.

Anahtar sözcükler: *Apis mellifera*, turunçgil, *Episyrphus balteatus*, *Oenopia conglobata,* yapışkan tuzaklar

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Introduction

Citrus are one of the fruit crops grown in the temperate Mediterranean, Aegean and Black Sea Regions of Turkey. In 2015, 987,000 t of citrus fruit were produced in Adana Province, in the eastern Mediterranean Region of Turkey (TUİK, 2015).

In Turkey, various problems are encountered in citrus cultivation, including management of pest insects and acarines colonizing citrus trees. Citrus growers commonly use chemical pesticides against pest species. Controlling pests by using pesticides is often considered a unique the only option for pest management of citrus orchards. However, the use of pesticides has negative impacts on the natural balance by eliminating natural enemies of harmful insects and non-targeted organisms, and causing secondary pest outbreaks, pollution of the environment, and development of resistance to pesticides. Integrated pest management (IPM) strategies including trapping to properly estimate pest insect densities may help reduce the adverse effects of pesticide use.

Sticky traps have commonly been used to sample harmful and beneficial insects in wild and cultivated plants worldwide. Colored sticky traps are a simple and low-cost method for determining the abundance of pest insects and their natural enemies in orchards (Wallis & Shaw, 2008). Yellow sticky traps, as a sampling tool, were used to monitor the abundance of pest insects, such as thrips and leafhoppers, visiting citrus trees in Adana Province, Turkey (Başpınar & Uygun, 1994). Yellow sticky traps combined with lures have also been recommended for capturing the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann, 1824) (Diptera: Tephritidae), in citrus orchards in this region. Colored traps have been applied more commonly in integrated pest management programs of various crops because of the response of insects to colors (Gerling & Horowitz, 1984; Chandler, 1985; Meyerdirk & Oldfield, 1985; Elekçioğlu, 2013). The efficacy of sticky traps may depend on when the traps are installed relative to crop phenology (Ladd et al., 1984; Meyerdirk & Moreno, 1984; Chandler, 1985; Bryne et al., 1986).

Surveys of harmful and beneficial insect species and their population dynamics and prey-predator relationships in IPM programs of citrus in Turkey have been well documented (Uygun & Şekeroğlu, 1984). In Turkey, biological control is the principal component of integrated control of citrus pests, and it has been successfully applied in field practices (Soylu & Ürel, 1977; Kansu & Uygun, 1980). Studies associated with citrus in the region show that the citrus orchards in the Mediterranean Region of Turkey have a rich fauna of beneficial insects (Uygun & Şekeroğlu, 1984; Yiğit & Canhilal, 2005). In these studies, various methods, such as visual observation of trees, tapping of shoots or branches, removing plant parts, such as flowers and leaves, and use of baited and pheromone traps, have been commonly used to detect citrus pests.

Despite the use of colored traps for capturing detrimental insect species, capturing of non-target insects, such as predators and pollinating insects, have been ignored. Data of color attractiveness to predators or pollinators may be helpful in selecting trap color and placement in fruit orchards in the region. In this study, we determined the efficacy of 4 colors of traps for trapping three common beneficial insect species on citrus mandarin cv Okitsu (Satsuma) between blooming and the first formation of young fruits. We also investigated efficacy of cardinal directions for each trap color and interaction between trap color and cardinal directions. Marmalade hoverfly, *Episyrphus balteatus* (De Geer, 1776) (Diptera: Syrphidae), is one of the most common and distinctive hoverflies in the UK and throughout the Palearctic ecozone , which includes Europe, North Asia, and North Africa. Marmalade hoverfly larvae normally feed on aphids (Schneider, 1969). *Oenopia* (*Synharmonia) conglobata* (L.,1758) (Coleoptera: Coccinellidae) is an inhabitant of fruit orchards and well-known as a common and effective predator of aphids and coccids in Turkey (Uygun, 1981). The honey bee*, Apis mellifera* (L., 1758) (Hymenoptera: Apidae), is the most important beneficial hymenopteran species because it pollinates many crops and wild plants, and is well known as honey makers worldwide (Robinson et al., 1989). Determined data may be useful for evaluation in IPM program in citrus orchards of Turkey or other countries sharing similar ecological areas.

Materials and Methods

Experimental design

The study was conducted in the Research and Practice Area of the Plant Protection Department, Faculty of Agriculture, Çukurova University, Adana Province, Turkey, in 2011 and 2012. Trials were established during the blossoming period of mandarin when pollinators were more active. The experimental mandarin cv. Okitsu (Satsuma) area covered almost 0.04 ha with trees of nearly 6 years old planted at 5 × 5 m. There were a total of 10 rows and each row had 14 trees. Two middle rows were selected for the experiment. Colored sticky traps (15 × 20 × 0.3 cm) with four colors were hung on the two middle trees 10 m apart in each row. Sixteen traps (four each of white, yellow, blue and green plexiglas plates) coated with the commercial adhesive, Stickem Special (Kapar Organik Tarım, Ankara, Turkey) were installed on the exterior of the canopy of otherwise untreated trees at about 1.7 m above ground. One trap of each color was positioned at each the four cardinal directions in each tree. Therefore each combination of color and cardinal direction was replicated four times.

Sampling of insects on traps

Sampling was conducted during the blooming stage of trees from 6 March to 19 April 2011 (14 consecutive sampling dates) and from 2 March to 30 April 2012 (15 sampling dates). Traps were replaced with new ones every 3-4 days at about 09:50 h. The traps were taken to the laboratory for processing. Insects on the traps were removed with kerosene, washed with ethanol (96%) and kept until the specimens cleared.

Harmful and beneficial insects were also examined from one inflorescence or young shoot from each cardinal directions and phenological status of trees recorded during the sampling period. During the two years of the study, no plant protection products were applied to control harmful organisms.

Insect identification

Genitalia preparations of predatory bugs of the genus *Orius* were made, and the specimens were identified by a key (Péricart, 1972). The predatory big-eyed bug, *Piocoris luridus* Fieb., was identified by using the key to predatory Hemiptera (Lygaeidae; Geocorinae) by Çakır & Önder (1990). Hoverflies (syrphids) were identified by Prof. Dr. A. Faruk Özgür (Adana Province, Turkey), and coccinellid predators were identified by Prof. Dr. Nedim Uygun (Adana Province, Turkey). Predatory thrips were identified by the first author. All identified specimens were lodged in the collection of the Industrial Plant Pests Laboratory, Plant Protection Department, University of Çukurova, Adana, Turkey.

Data analysis

ANOVA was performed at a significance level of $P < 0.05$ by using the SPSS statistical package (SPSS, 2006). A general linear model (GLM-ANOVA) procedure was used to measure the effects of color, cardinal direction, and interactions between color and cardinal direction. Data were pooled over sampling dates because of low numbers of insects in each taxon on the traps for some sampling dates. Comparison of means was done by Tukey's Honestly Significant Difference (HSD) test.

We did not evaluate the numbers of winged aphids captured on the traps, because too few of them were caught on the colored traps in either year.

Results

Catch of beneficial insect species on colored traps

Fifteen beneficial insect species were identified in both years (Table 1). In general, white and yellow traps were more attractive to beneficial insects. Traps of these two colors caught both higher numbers of common insect species and numbers of insect species. A small numbers tiny wasps on the yellow sticky traps but these were not identified.

Oenopia conglobata, *E. balteatus,* and *A. mellifera* were regularly captured on the traps of all colors in both years. Most of the other coccinellids were trapped by yellow traps and to a lesser extent by green traps. Other identified generalist predators, such as *Chrysoperla carnea* (Stephens), were infrequently captured on the traps. Overall, white traps captured 48.4 and 41.7% of a total adult population in 2011 and 2012, respectively. Yellow traps were second, accounting for 21.3 and 27.5% in 2011 and 2012, respectively.

Monthly abundance of beneficial insects on traps

No individual adults of *A. mellifera* and *E. balteatus* were captured on any colored trap in April 2011 (Table 2). Only *O. conglobata* was captured on traps in April 2011. Densities of *A. mellifera* and *E. balteatus* on white traps were higher than on the other traps in May 2011. Numbers *of O. conglobata* on white traps were less than the numbers of the other two beneficial insects in May. Mean densities of *O. conglobata* were consistently greater on yellow and green traps in 2011. Few *A. mellifera* and *E. balteatus* were captured on yellow or blue traps in April 2012. Mean numbers of *A. mellifera* and *E. balteatus* in May 2012 were greater, with means of 2.43 ± 0.61 and 2.06 ± 0.64 individuals per trap, compared to data of the previous year. High numbers of *O. conglobata*, with a mean of 2.12 ± 0.77 individuals per trap, were captured on green traps in 2012.

Response of three beneficial insects to trap color

Seasonal mean numbers of the three beneficial insect species captured on the different colored traps are given in Figure 1. Trap color was a significant factor in catches of these three predators in both years (Table 3). Significantly greater numbers of *A. mellifera* with a mean of 4.25 ± 0.52 insects per trap in 2011 ($F = 13.606$, $df = 3, 60$; $P < 0.0001$) and of 1.37 ± 0.19 in 2012 ($F = 37.391$, $df = 3, 124$; $P < 0.0001$) were caught on white traps compared to yellow, green and blue traps in both years (Figure 1a, b). Mean densities of *E. balteatus* on white and blue traps were similar but significantly greater than for other colored traps in 2011 and 2012 (in 2011: $F = 4.186$, $df = 3, 60$; $P = 0.009$; in 2012: $F = 6.446$, $df = 3, 124$; $P < 0.0001$; Figure 1a, b). Yellow was the least attractive to *E. balteatus* adults in both years. Mean numbers of *O. conglobata* captured by blue and white traps were significantly fewer than those captured by yellow and green traps in 2011 and 2012 (in 2011: $F = 4.793$, $df = 3, 60$; $P = 0.005$; in 2012: $F = 5.929$, $df = 3, 124$; $P = 0.001$, respectively; Figure 1a, b).

Effect of cardinal direction on catches of three beneficial insects

Cardinal direction and cardinal direction by color were important components for catch of the three beneficial insect species in both years (Table 3). The effects of cardinal direction on the catches of seasonal mean numbers of the three beneficial insect species are given in Figure 2.

The effect of cardinal direction on the catches of *A. mellifera* was significantly different on yellow traps facing east ($F = 81.000$, $df = 3, 12$; $P < 0.0001$) in 2011 (Figure 2a). White traps facing east or south had significantly greater numbers of *A. mellifera* in 2011 ($F = 7.40$, $df = 3, 12$; $P = 0.005$). Yellow and blue traps for each direction caught generally similar numbers of this insect. Similar results were also obtained in 2012. Cardinal direction did not have a significant effect on *A. mellifera* numbers by trap color, except for white traps, in 2012. White traps facing south had a significant mean number of *A. mellifera*, with 2.12 ± 0.35 adults per trap ($F = 4.808$, $df = 3, 28$; $P = 0.008$) in 2012. Yellow, blue and green traps facing west caught significantly more *E. balteatus* individuals ($F = 9.566$, $df = 3, 12$; $P = 0.002$; $F = 16.342$, $df = 3, 12$; $P < 0.0001$; $F = 8.120$, $df = 3, 12$; $P = 0.005$, respectively; Figure 2b) in 2011.

Table 1. Beneficial insects captured on various colored sticky traps suspended in mandarin trees during 2011 - 2012 in Adana Province, Turkey

	2011					2012				
Order / Family Species	W	Y	B	G	Total	W	Y	B	G	Total
Coleoptera / Coccinellidae										
Chilocorus bipustulatus (L., 1758)	6	6	2	0	14	0	5	0	1	6
Oenopia (Synharmonia) conglobata (L., 1758)	9	19	3	20	51	15	24	5	35	79
Scymnus subvillosus (Goeze, 1777)	0	0	0	0	0	11	28	0	12	51
Stethorus gilvifrons (Mulsant, 1850)	2	0	0	0	0	0	0	0	0	0
Diptera / Syrphidae										
Episyrphus balteatus (De Geer, 1776)	69	33	51	15	168	33	15	32	5	85
Eristalis (*Eristalomia*) *tenax* (L., 1758)	0	1	0	0	1	0	0	0	0	0
Metasyrphus corollae (Fabricius, 1794	4	1	2	0	7	0	1	0	1	2
Spaerophoria scripta (L, 1758)	8	1	0	0	9	4	0	0	0	4
Volucella sp.	0	2	0	0	2	0	0	0	0	0
Hemiptera / Lygaeidae										
Piocoris luridus (Fieber, 1844)	0	1	0	0	1	0	0	0	0	0
Hemiptera / Miridae										
Orius niger Wolff, 1811	1	0	0	0	1	0	1	0	0	1
Hymenoptera / Apidae										
Apis mellifera L., 1758	68	9	3	3	83	45	5	1	2	53
Neuroptera /Chrysopidae										
Chrysoperla carnea (Stephens, 1836)	1	1	5	1	8	4	9	3	8	24
Thysanoptera /Aeolothripidae										
Aeolothrips spp. *(Aeolothrips collaris* Priesner, 1919 + *Aeolothrips intermedius* Bagnall, 1934)	0	0	0	0	0	32	7	0	1	40
Total	168	74	66	39	347	144	95	41	65	345
%	48.4	21.3	19.0	11.2	100	41.7	27.5	11.8	18.8	100

W: White, Y: Yellow, B: Blue, G: Green

Table 2. Monthly numbers of three beneficial insect species captured on the colored sticky traps suspended in mandarin trees during 2011 - 2012 in Adana Province, Turkey

			April		May	
Year	Insect species	Trap color	Total no	Per sample	Total no	Per sample
2011	*Apis mellifera*	White	0	0.00 ± 0.00	68	4.25 ± 0.78
		Yellow	0	0.00 ± 0.00	9	0.56 ± 0.40
		Blue	0	0.00 ± 0.00	3	0.18 ± 0.13
		Green	0	0.00 ± 0.00	3	0.18 ± 0.13
	Episyrphus balteatus	White	0	0.00 ± 0.00	69	4.31 ± 0.97
		Yellow	0	0.00 ± 0.00	33	2.06 ± 0.62
		Blue	0	0.00 ± 0.00	51	3.18 ± 0.74
		Green	0	0.00 ± 0.00	15	0.93 ± 0.35
	Oenopia conglobata	White	0	0.00 ± 0.00	9	0.56 ± 0.24
		Yellow	0	0.00 ± 0.00	17	1.06 ± 0.24
		Blue	0	0.00 ± 0.00	3	0.18 ± 0.10
		Green	3	0.18 ± 0.18	17	1.06 ± 0.33
2012	*Apis mellifera*	White	6	0.37 ± 0.22	39	2.43 ± 0.61
		Yellow	1	0.06 ± 0.06	4	0.25 ± 0.19
		Blue	0	0.00 ± 0.00	1	0.06 ± 0.06
		Green	0	0.00 ± 0.00	2	0.12 ± 0.08
	Episyrphus balteatus	White	0	0.00 ± 0.00	33	2.06 ± 0.64
		Yellow	0	0.00 ± 0.00	15	0.93 ± 0.29
		Blue	0	0.12 ± 0.85	30	1.87 ± 0.63
		Green	0	0.00 ± 0.00	5	0.31 ± 0.15
	Oenopia conglobata	White	13	0.81 ± 0.27	2	0.12 ± 0.12
		Yellow	16	1.00 ± 0.38	10	0.62 ± 0.27
		Blue	4	0.25 ± 0.11	1	0.06 ± 0.06
		Green	34	2.12 ± 0.77	1	0.06 ± 0.06

Table 3. Effects of color, cardinal direction and their interaction on catches of insects on colored traps in mandarin trees during 2011 - 2012 in Adana Province, Turkey

	2011				2012			
Sources	df	MS	F	P	df	MS	F	P
Apis mellifera								
Color	3	62.516	94.512	0.000	3	13.437	49.752	0.000
Direction	3	5.266	7.961	0.000	3	0.771	2.854	0.040
Color x direction	9	4.918	7.436	0.000	9	1.333	4.937	0.000
Error	48				112			
Episyrphus balteatus								
Color	3	33.750	6.750	0.001	3	5.799	7.982	0.000
Direction	3	25.588	5.157	0.004	3	2.716	3.758	0.013
Color x direction	9	18.556	3.771	0.001	9	1.737	2.391	0.016
Error	48				112			
Oenopia conglobata								
Color	3	2.896	*6.178*	0.001	3	5.112	7.400	0.000
Direction	3	0.437	*0.933*	0.432	3	1.008	1.459	0.230
Color x direction	9	1.382	*2.948*	0.007	9	2.945	4.263	0.000
Error	48				112			

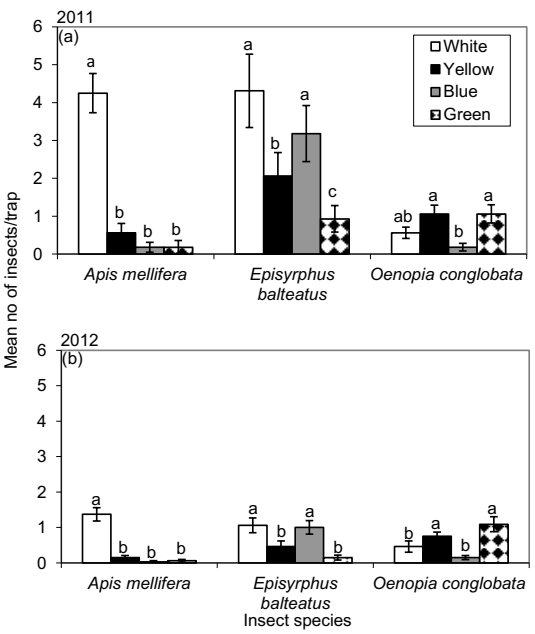

Figure 1. Seasonal mean numbers (± SEM) of three beneficial insect species captured on variously colored sticky traps suspended in mandarin trees during (a) 2011 and (b) 2012 in Adana Province, Turkey. Same letters on the bars are not statistically different according to Tukey's HSD test ($P < 0.05$).

White traps facing south trapped significant numbers of *E. balteatus* adults in 2011 (F = 12.250, df = 3, 12; P = 0.001). White traps facing east and south trapped significantly more *E. balteatus* adults (F = 4.591, df = 3, 28; P = 0.010) in 2012. White traps facing south and green facing north had significant numbers of *O. conglobate* (F = 4.636, df = 3, 12; P = 0.022; F = 5.560, df = 3, 12; P = 0.013; respectively; Figure 2c) in 2011. White traps facing south and green facing north had significant numbers of *O. conglobata* (F = 4.636, df = 3, 12; P = 0.022; F = 5.560, df = 3, 12; P = 0.013; respectively; Figure 2c) in 2011. The effect of cardinal direction on the capture of this predatory insect was not significantly different for other colors in 2012. Green traps facing the west side of trees captured more numbers of *O. conglobata* adults, with a mean of 2.25 ± 0.36 in 2012 (F = 7.279, df = 3, 28; P = 0.001; Figure 2c). The effect of cardinal direction on the capture of this predatory insect was not significantly different for other colors in 2012.

Discussion

In spring, mostly predatory coccinellids and syrphids were detected on the colored traps. Other insects identified were captured sporadically on the traps and their numbers were few during the trials in both years. We investigated efficacy of the colored traps in catching of beneficial insects during a limited period (in two spring months), we can postulate that beneficial species numbers captured on the different colored traps would also be high if a study was conducted for the whole season in this region. Rich beneficial insect fauna in citrus ecosystem in the Mediterranean region of Turkey have been reported by the researchers (Uygun et al., 2001; Uygun & Satar, 2007).

Adults of *E. balteatus* were not caught on the colored traps placed in trees during the blossoming period. Syrphid adults caught on the traps when the young outer fresh shoots and leaves were infested by the aphids, suggesting that syrphid females lay eggs on leaves infested with the aphid colonies (*Aphis gossypii* Glov. and *Aphis spiraecola* Patch). Larval syrphids are often recognized as beneficial, preying on aphids, lepidopterous larvae and insects in the Aleyrodidae, Psyllidae, and Coccidae families (Clausen, 1972; Resendiz-Ruiz, 1993). We also noted that predacious syrphid larvae were feeding on aphids. *Oenopia conglobata* was the first predatory insect captured on the traps. Relatively high abundance of this predaceous insect is likely to relate to the dense colonization of the aphid species on the trees from late April to early May in both years. *Oenopia conglobata* is one of the most common aphid feeders in citrus orchards in the region. However, few larval coccinellids were preying on aphids on the plant during the trapping period in this study.

Population densities of the hoverflies and the honey bees captured on the traps were lower in 2012 (Table 3). The reason for no or few syrphids and honey bees on traps during the blooming period may have been that dense numbers of thrips belonging to *Thrips tabaci* Lindeman, 1889 and *Thrips major* Uzel, 1895 (Thysanoptera: Thripidae) were on the surfaces of the traps; thus, the thrips might have prevented catches of these large beneficial insect adults in April.

Blue and green traps captured fewer honey bees, but white traps were the most attractive to them. Rodriguez-Saona et al. (2012) similarly reported that honey bees were trapped mostly by the white traps, and were less attracted to or repelled by green, yellow and red sticky traps hung in cranberry marshes. In the current study, the greater numbers of honey bees caught by the white traps may related to flower color of the citrus (white), which is more attractive to the honey bees and the other insects visiting blooms (Lunau & Maier, 1995). We found fewer honey bees on the green and blue traps. Knight & Miliczky (2003) reported similar results: green and red colored sticky traps captured the lowest numbers, and white was the most attractive to honey bees. Perhaps, the bees recognize that the white flowers are crucial sources of pollen and nectar.

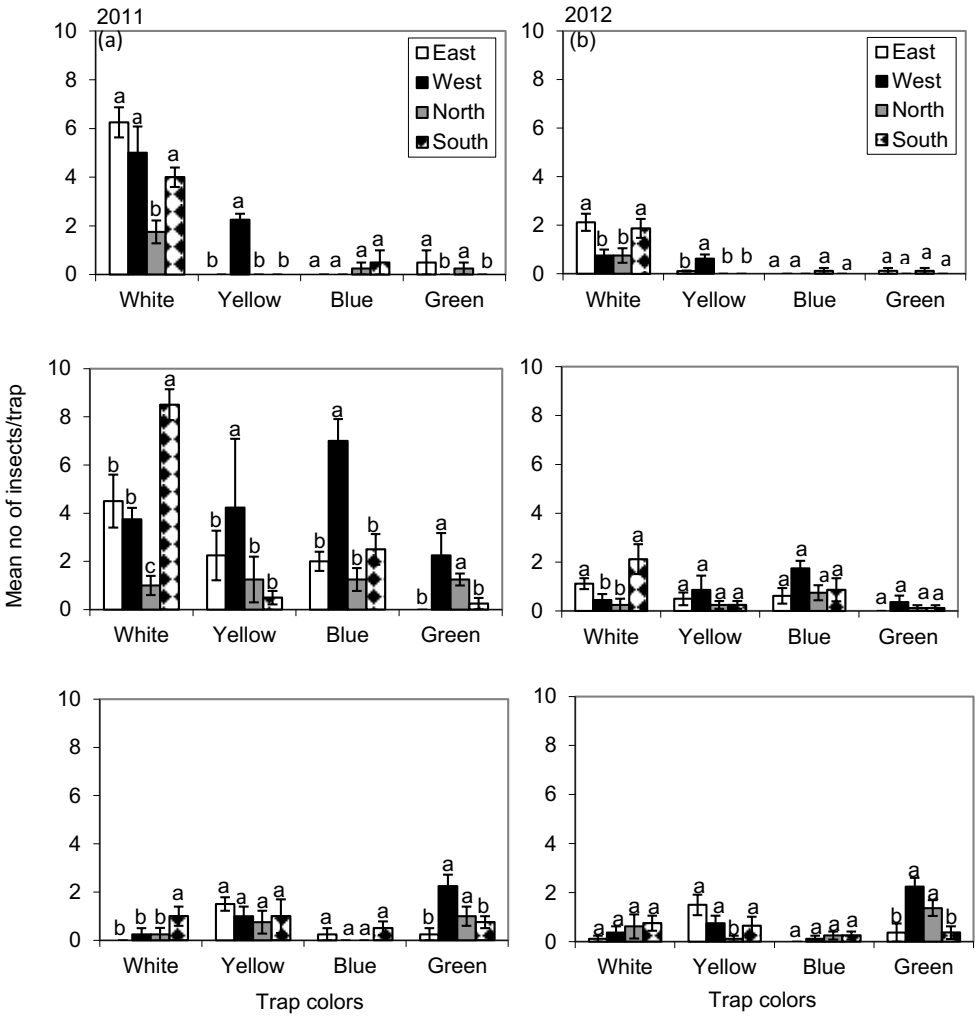

Figure 2. Effects of cardinal directions on catches of mean numbers (± SEM) of 3 beneficial insect species (a) *Apis mellifera*, (b) *Episyrphus balteatus*; (c) *Oenopia conglobata* by colored sticky traps suspended in citrus trees during 2011-2012. Same letters on the bars are not statistically different according to Tukey's HSD test ($P < 0.05$).

The hoverflies were more active mainly when infestation of the aphids occurred in the orchard during May, and the hoverflies were often detected on white and blue traps in both years. Green was less attractive to the hoverfly adults. A hoverfly species, *Toxomerus marginatus* (Say), was more common during the flowering period, and blue traps were the most attractive to the adults, followed by white traps, in cranberry marshes in New Jersey, USA (Rodriguez-Saona et al., 2012). The study of Hoback et al. (1999) also revealed that blue sticky traps captured greater numbers of the hoverfly, *Allograpta obliqua* (Say), in broccoli fields in Maricopa, USA. Similar results were obtained in a laboratory study using blue, yellow and white sticky cards for *A. obliqua* (Chen et al., 2004).

The reflectance (%) of the four colors in current study was not measured; a higher reflectance of white when compared with yellow, blue or green wavelengths (Teulon & Penman 1992; Hoddle et al., 2002) may affect the attractiveness of the white traps to syrphids and honey bee adults. Rodriguez-Saona et al. (2012) found that hoverflies and honey bees were the most attracted to blue and white traps; these colors are commonly for flowers and are often attractive to bees and other flower visitors (Lunau & Maier, 1995). These insects might recognize that blue and white floral colors are associated with nectar and pollen sources during their searching, and consequently were not attracted to other colors, such as green and red. The use of white traps, which catch considerable numbers of syrphid and honey bee adults,

would be appropriate for sampling. Yellow traps which were less attractive to both beneficial insect species may be used to monitor some sucking-pest insects, such as aphids and thrips, especially during April, the flowering period of citrus in this region. Yellow is known to be strongly attractive, especially to sucking pests worldwide.

Green and yellow traps were more attractive to the predaceous coccinellid, *O. conglobata,* than were other colored traps. Although the coccinellid species trapped were different, our results agree with the findings of previous studies of coccinellids sampled by variously colored traps. For example, Rodriguez-Saona et al. (2012) revealed that lady beetles identified as *Coleomegilla maculata* (De Geer, 1775), *Coccinella septempunctata* (L., 1758) and *Hippodamia convergens* Guérin-Méneville, 1842 were trapped mostly on yellow traps in cranberry marshes in New Jersey, USA. Maredia et al. (1992) reported that *C. septempunctata* was strongly attracted to yellow traps. We frequently observed fewer alate aphids on yellow traps on most sampling dates in both years. Therefore, yellow traps can be used to investigate prey-predator associations between coccinellids and citrus aphids in spring time in this region.

The effect of cardinal direction on the capture of beneficial insects was statistically important. Honey bees were caught mostly on white traps facing east and south (Figure 2). This may indicate that honey bees visit commonly sides of the trees becoming warmer and sunnier. White traps positioned to the south captured more syrphids, but yellow, blue and green traps facing west trapped greater numbers of individuals in 2011 (Figure 2). This might be because the spectral reflectance of the colors used in this study (Teulon & Penman, 1992; Hoddle et al., 2002); spectral reflectance of the different colored trap affects the attraction of diurnal insect species (Childers & Brecht, 1996). Sunlight on the southern sides of the trees in spring might have greater reflectance with white traps than for other colors, resulting in high numbers of syrphid adults. The effect of cardinal direction on the capture of the three beneficial insect species by the colored traps was unclear in 2012 (Figure 2). This may be due to lower abundance of the captured and identified insect species, compared with the abundance in 2011 (Figures 1 and 2).

The use of white and blue traps for the syrphids, and yellow traps for coccinellids, may provide more ecological knowledge on these beneficial insects in citrus orchards in this region and also in other geographical areas having similar ecological conditions. Catches on white traps could especially be a valuable indicator of the activities of pollinating insects in citrus.

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References

Başpınar, H. & N. Uygun, 1994. Studies on the determination of Cicadellidae species in citrus orchards in east Mediterranean region of Turkey, and monitoring their population changes by different sampling methods, and food plants, and relationships between stubborn disease and its vector cicadellid. Turkish Journal of Agriculture and Forestry, 18**:** 9-20.

Bryne, D. N., P. Von Bretzel & C. J. Hoffman,1986. Impact of trap design and placement when monitoring for the sweet potato whitefly (Homoptera: Aleyrodidae). Environmental Entomology, 15 (2)**:** 300-304.

Chandler, L. D.,1985. Flight activity of *Liriomyza trifolii* (Diptera: Agromyzidae) in relationship to placement of yellow traps in bell pepper. Journal of Economic Entomology, 78 (4)**:** 825-828.

Chen, T. Y., C. C. Chu, T. J. Henneberry & K. Umeda, 2004. Monitoring and trapping insects on poinsettia with yellow sticky card trap equipped with light-emitting diodes. HortTechnology,14 (3): 337–341.

Childers, C. C. & J. K. Brecht, 1996. Colored sticky traps for monitoring *Frankliniella bispinosa* (Morgan) (Thysanoptera : Thripidae) during flowering cycle in citrus. Journal of Economic Entomology, 89 (5): 1240–1249.

Clausen, C. P., 1972. Entomophagous Insects. Hafner Publishing, Newyork, X+688pp (Reprint).

Çakır, S. & F. Önder, 1990. Türkiye Geocorinae (Het.:Lygaeidae) altfamilyası üzerinde sistematik ve faunistik araştırmalar. Türkiye Entomoloji Dergisi, 14 (1): 37-52.

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

Gerling, D. & A. R. Horowitz, 1984. Yellow traps for evaluating the population levels and dispersal patterns of *Bemisia tabaci* (Gennadius) (Homoptera: Aleyrodidae). Annals of the Entomological Society of America, 77 (6): 753-759.

Hoback, W. W., T. M. Svatos, S. M. Spomer & L. G. Higley, 1999. Trap color and placement affects estimates of insect family-level abundance and diversity in a Nebraska salt marsh. Entomologia Experimentalis et Applicata, 91 (3): 393-402.

Hoddle, M. S., L. Robinson & D. Morgan, 2002. Attraction of thrips (Thysanoptera: Thripidae and Aeolothripidae) to colored sticky cards in California avocado orchard. Crop Protection, 21 (5): 383–388.

Kansu, A. & N. Uygun, 1980. Doğu Akdeniz Bölgesinde Turunçgil Zararlıları İle Tüm Savaş Olanaklarının Araştırılması. Çukurova Üniversitesi Ziraat Fakültesi Yayınları 141, Bilimsel Araştırma ve İncelemeler 33, Adana, 63 s.

Knight, A. L. & E. Miliczky, 2003. Influence of trap colour on the capture of codling moth (Lepidoptera: Tortricidae), honeybees, and non-target flies. Journal of Entomological Society of British Columbia, 100: 65-70.

Ladd, T. L., B. R. Stinner & H. R. Krueger, 1984. Influence of color and height of eugenol baited sticky traps on attractiveness to northern corn rootworm beetles (Coleoptera: Chrysomelidae). Journal of Economic Entomology, 77 (3): 652-654.

Lunau, K. & E. J. Maier, 1995. Innate colour preference of flower visitors. Journal of Comparative Physiology, 177 (1): 1-19.

Maredia, K. M., S. H. Gage, D. D. Landis & T. M. Wirth, 1992. Visual response of *Coccinella septempunctata* (L.) *Hippodamia parenthesis* (Say) (Coleoptera: Coccinellidae) and *Chrysoperla carnea* (Stephend) (Neuroptera: Chrysopidae) to colors. Biological Control, 2 (3): 253-256.

Meyerdirk, D. E. & D. S. Moreno, 1984. Flight and color-trap preference of *Parabemisia myricae* (Kuwana) (Homoptera: Aleyrodidae) in a citrus orchard. Environmental Entomology, 13 (1): 167-170.

Meyerdirk, D. E. & G. Oldfield, 1985. Evaluation of trap color and height placement for monitoring *Circulifer tenellus* (Baker) (Homoptera: Cicadellidae). The Canadian Entomologist, 117 (4): 505-511.

Péricart, J., 1972. Hémiptères Anthocoridae, Cimicidae et Microphysidae de l'ouest-paléarctique. Masson et Cie Editeurs, Paris, France.

Resendiz-Ruiz, M. E., 1993. A new predator on the whitefly. Southwestern Entomologist, 18(2): 147–148.

Robinson, W. S., R. Nowogrodski & R. A. Mors, 1989. The value of honey bees as pollinators of US crops. American Bee Journal, 129 (6): 411-423.

Rodriguez-Saona C. S., J. A. Byers & D. Schiffhauer, 2012. Effect of trap color and height on capture of blunt-nosed and sharp-nosed leafhoppers (Hemiptera: Cicadellidae) and non-targeted arthropods in cranberry bogs. Crop Protection, 40: 132-144.

Schneider, F., 1969. Bionomics and physiology of aphidophagous Syrphidae. Annual Review of Entomology, 14: 103-124.

Soylu O. Z. & N. Urel, 1977. Güney Anadolu Bölgesi turunçgillerinde zararlı böceklerin parazit ve predatörlerinin tesbiti üzerine araştırmalar. Türkiye Entomoloji Bülteni, 17: 77-104.

SPSS,2006. SPSS Base 15.0 User's Guide, Chicago: Prentice Hall.

Teulon, D. J. & D. R. Penman, 1992. Colour preferences of New Zealand thrips (Terebrantia: Thysanoptera). New Zealand Entomologists 15 (1): 8-13.

TUİK, 2015. Republic of Turkey Ministry of Food, Agriculture and 6 Livestock. (Web page: http://www.tuikapp.tuik.gov.tr/bitkiselapp/bitkisel.zul 7), (Acces Date: May, 2016).

Uygun, N., 1981. Türkiye Coccinellidae (Coleoptera) Faunası Üzerinde Taksonomik Araştırmalar. Çukurova Üniversitesi Ziraat Fakültesi Yayınları, No: 57, Adana.

Uygun, N. & E. Şekeroğlu, 1984. Integrated pest management studies in newly established citrus orchard. Türkiye Bitki Koruma Dergisi, 8: 169-175.

Uygun, N., İ. Karaca, M. R. Ulusoy & D. Şenal, 2001 Turunçgil Zararlıları ve Entegre Mücadelesi (Türkiye Turunçgil Bahçelerinde Entegre Mücadele, Editor, N. Uygun), TÜBİTAK, TARP, 157 s.

Uygun, N. & S. Satar, 2007. The current situation of citrus pest and their control methods in Turkey. Integrated control in citrus fruit crops. IOBC/WPRS, 38: 2-9.

Wallis, D. R. & P. W. Shaw, 2008. Evaluation of coloured sticky traps for monitoring beneficial insects in apple orchards. New Zealand Plant Protection, 61**:** 328-332.

Yiğit, A. & R. Canhilal, 2005. Establishment and dispersal of *Serangium parcesetosum* Sicard (Coleoptera, Coccinellidae), a predatory beetle of citrus whitefly, *Dialeurodes citri* Ashm. (Homoptera, Aleyrodidae) in the East Mediterranean region of Turkey. Journal of Plant Diseases and Protection, 112 (3): 268–275.

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Original article (Orijinal araştırma)

Aphid (Hemiptera: Aphididae) species determined in Çanakkale Province with a new record for the aphid fauna of Turkey[1]

Türkiye yaprakbiti faunası için yeni bir kayıt ile birlikte Çanakkale ilinde belirlenen yaprakbiti (Hemiptera: Aphididae) türleri

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Summary

As a result of a study conducted to determine aphid species on herbaceous plants, shrubs and trees in cultivated and uncultivated areas from 2013 to 2015 in Çanakkale Province, Turkey, 39 species and one subspecies in 24 genera of Aphididae family were determined. Of these species, *Aphis sedi* Kaltenbach, 1843 collected from *Lampranthus* sp. (Aizoaceae) was new record for the aphid fauna of Turkey. Also, *Helianthus annuus* L. (Asteraceae) and *Pimpinella saxifraga* L. (Apiaceae) were determined as new host records for *Aulacorthum solani* (Kaltenbach, 1843) and *Hyadaphis foeniculi* (Passerini, 1860) in Turkey, respectively. The present study and other current studies indicated that local studies are important for the aphid fauna of Turkey.

Keywords: Aphid, aphid fauna, Çanakkale, Turkey

Özet

Çanakkale ilinde 2013-2015 yılları arasında tarım ve tarım dışı alanlarda bulunan otsu bitki, çalı ve ağaçlar üzerinde bulunan yaprakbitlerini belirlemek için gerçekleştirilen bu çalışmada Aphididae familyasından 24 cinse ait 39 tür ve bir alttür belirlenmiştir. Bu türlerden *Lampranthus* sp. (Aizoaceae) üzerinden toplanan *Aphis sedi* Kaltenbach, 1843 Türkiye yaprakbiti faunası için yeni kayıttır. Ayrıca *Helianthus annuus* L. (Asteraceae) ve *Pimpinella saxifraga* L. (Apiaceae) sırasıyla *Aulacorthum solani* (Kaltenbach, 1843) ve *Hyadaphis foeniculi* (Passerini, 1860) için ülkemizde yeni konukçu kaydı olarak belirlenmiştir. Mevcut çalışma ve yapılan diğer güncel çalışmalar bölgesel çalışmaların Türkiye yaprakbiti faunası için önemini göstermiştir.

Anahtar sözcükler: Yaprakbiti, yaprakbiti faunası, Çanakkale, Türkiye

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Introduction

Aphids (Hemiptera: Aphididae) are important pests feeding on herbaceous plants, shrubs and trees throughout the world. While approximately 40% of the aphid fauna of the world live on trees, the other 55% live on herbaceous plants and shrubs (the host plants of the remaining 5% are unknown). Many factors such as complicated life cycle, host plants, humidity, polymorphism, stress and temperature have significant effect on morphology of aphids. Therefore, diagnosis of aphids is quite complicated (Blackman & Eastop, 2006).

Aphids cause direct damage by sucking plant sap and also excrete honeydew on plant leaves. Moreover, they cause indirect damage by transferring phytopathogenic virus diseases. Despite using different control methods against aphids, their damage to cultivated and uncultivated plants increases day by day. In developed countries, such as the USA, crop losses caused by aphids were estimated at about 30% per annum. Also, losses were estimated at about 50% per year in developing countries, including Turkey (Ruberson, 1999). For example, the invasive grapevine aphid (*Aphis illinoisensis* Shimer, 1866) is an important pest causing damage in vineyards in Turkey, Greece, Tunisia and Algeria (Remaudière et al., 2003; Tsitsipis et al., 2005; Kamel-Ben Halima & Mdellel 2010; Laamari & Coeur d'Acier, 2010).

The known world aphid fauna recently reached about 5000 species belonging to 510 genera (Blackman & Eastop, 2016). The first studies of the aphid fauna of Turkey were carried conducted by Trotter (1903), Fahringer (1922) and Houard (1922). Then Bodenheimer & Swirski (1957) listed the Mediterranean aphids including 90 aphids species from Turkey. The *Aphidoidea* of Turkey listed 258 aphid species collected by Çanakçıoğlu (1975) and is the most comprehensive study up to the 21st century in Turkey. In the 2000s, there was a significantly increase in studies of the aphid fauna of Turkey. These studies were conducted by Toros et al. (1996), Görür (2002), Toros et al. (2002), Aslan & Uygun (2005), Özdemir et al. (2005), Özdemir et al. (2006), Remaudière et al. (2006), Geneci & Görür (2007), Toper Kaygın et al. (2008), Akyürek et al. (2010), Akyıldırım et al. (2011), Barjadze et al. (2011), Görür et al. (2012), Barjadze & Özdemir (2014), Barjadze et al. (2014a), Güçlü et al. (2015) and Şenol et al. (2015a). With new records these added, the aphid fauna of Turkey reached to 532 species belonging to 142 genera (Şenol et al., 2015b).

Although these studies of the aphid fauna of Turkey were conducted in different areas, the known this aphid fauna is still limited. Also, there were no detailed studies focusing on Çanakkale Province. The aim of this study was to determine the aphid species and their host plants in cultivated and uncultivated areas of Çanakkale Province, Turkey.

Material and Methods

Aphid species were collected from herbaceous plants, shrubs and trees from 2013 to 2015 in Çanakkale Province, Turkey. Both apterous and alate aphid specimens were collected in cultivated and uncultivated areas from their host plants using a soft brush and put into an Eppendorf tube contained 70% alcohol. Collection and preparation of aphid specimens followed to the method of Hille Ris Lambers (1950).

Identification of aphid species, was conducted according to Bodenheimer & Swirski (1957), Heie (1986), Blackman & Eastop (2006, 2016). Host plants of aphids were arranged according to Holman (2009). The World distribution and taxonomic statutes of aphid species were checked according to Fauna Europaea (http://www.faunaeur.org) (Nieto Nafria, 2016). All of aphid specimens in this study were collected by Şahin Kök and voucher specimens were deposited in the Department of Plant Protection of Agricultural Faculty, Çanakkale Onsekiz Mart University, Turkey.

Results and Discussion

In this study conducted in Çanakkale Province, 39 species and one aphid subspecies belonging to six subfamilies (Anoeciinae, Aphidinae, Calaphidinae, Chaitophorinae, Eriosomatinae and Lachninae) in 24 genera of the family, Aphididae, were identified on host plants collected from cultivated and uncultivated areas. The sampling was done on herbaceous plants, shrubs and trees. Among identified aphid species, *Aphis sedi* Kaltenbach, 1843 collected from *Lampranthus* sp. (Aizoaceae) was determined as new record for the aphid fauna of Turkey. *Helianthus annuus* L. (Asteraceae) and *Pimpinella saxifraga* L. (Apiaceae) were determined as new host records for *Aulacorthum solani* (Kaltenbach, 1843) and *Hyadaphis foeniculi* (Passerini, 1860) in Turkey, respectively. Also, *Aphis craccivora* Koch, 1854, *Aphis fabae* Scopoli, 1763 and *Aphis gossypii* Glover, 1877 were determined as the most common aphid species. Distribution map (Figure 1), taxonomy, locality, coordinates, host plants and collected dates of identified aphid species are given below.

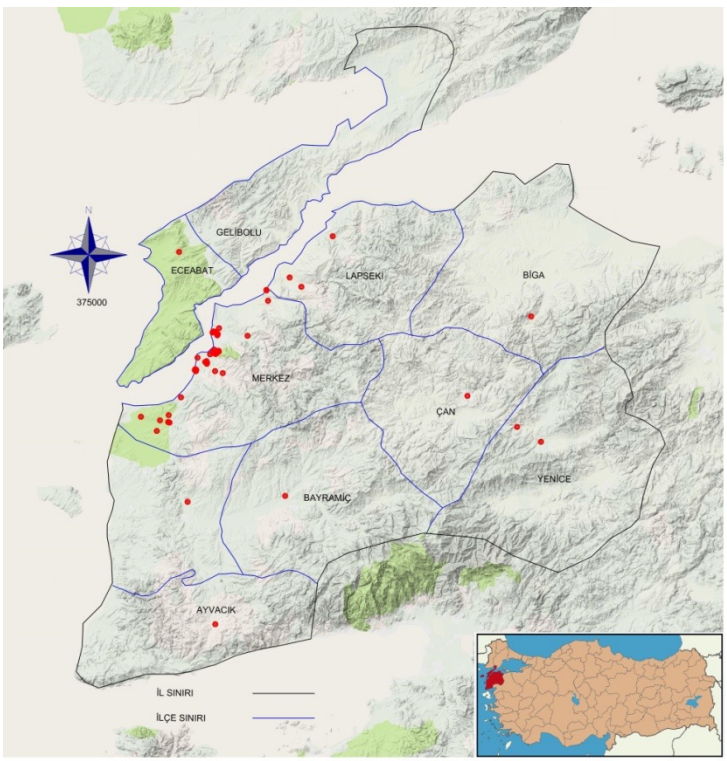

Figure 1. Location of sites sampled for aphid species in Çanakkale, Province,Turkey.

Family Aphididae

Subfamily Anoeciinae

Anoecia corni (Fabricius, 1775)

Material examined: Çanakkale, Halileli, 39°58'27.9" N 26°17'40.9" E, *Avena* sp. (Poaceae), 09.X.2013.

World distributions: Worldwide except Australian region (Nieto Nafria, 2016).

Turkish distributions: *A. corni* was recorded from Tekirdağ, Mersin, Diyarbakır, Isparta, Trabzon (Tuatay & Remaudière, 1964; Toros et al., 2002; Ölmez Bayhan et al., 2003; Akyıldırım et al., 2014; Barjadze et al., 2014b).

Subfamily Aphidinae

Tribe Aphidini

Aphis craccivora Koch, 1854

Material examined: Çanakkale, Kepez-TOKİ, 40°06'25.8" N 26°24'46.6" E, *Lycopersicum esculentum* L. (Solanaceae), *Phaseolus vulgaris* L. (Fabaceae) and *Solanum melongena* L. (Solanaceae), 28.XI.2013; Çanakkale, university campus, 40°06'31.1" N 26°24'39.0" E, *Trigonella* sp. (Fabaceae), 15.V.2014; Çanakkale, Kepez, 40°06'23.3" N 26°23'56.9" E, *Acacia* sp. (Fabaceae), 25.V.2014; Çanakkale, Yapıldak, 40°13'56.1" N 26°32'25.4" E:, *Portulaca oleracea* L. (Portulacaceae), 06.VI.2015; Çanakkale, university campus, 40°06'41.0" N 26°25'00.4" E, *Robinia pseudoacacia* L. (Fabaceae), 06.VI.2015.

World distribution: Worldwide (Nieto Nafria, 2016).

Turkish distribution: *A. craccivor*a was recorded from Adana, Aksaray, Ankara, Artvin, Balıkesir, Bartın, Çanakkale, Denizli, Diyarbakır, Hatay, İzmir, Kahramanmaraş, Kastamonu, Mersin, Niğde, Samsun, Trabzon and Van (Bodenheimer & Swirski, 1957; Tuatay, 1993; Toros et al., 1996; Toros et al., 2002; Ölmez Bayhan et al., 2003; Görür, 2004; Aslan & Uygun, 2005; Ünal & Özcan, 2005; Ayyıldız & Atlıhan, 2006; Özdemir et al., 2006; Geneci & Görür, 2007; Çıraklı et al., 2008; Eser et al., 2009; Toper Kaygın et al., 2009; Akyürek et al., 2012; Akyıldırım et al., 2014).

There are different remarks about *A. craccivora* on *Robinia* (Fabaceae) and other host plants. Takahashi (1966) indicated that a long-haired variant of *A. craccivora* on *Robinia* (Fabaceae) has been separated as a subspecies. Mehrparvar et al. (2012) reported that Iranian populations of *A. craccivora* associated with *R. pseudoacacia* are different from other population and their populations are not homogeneous morphological entities. Blackman & Eastop (2016) reported that *A. craccivora* populations on *Robinia* should be identified as *Aphis robiniae* Macchiati, 1885. Despite these opinions of different authors, we believe that morphological and molecular differences of *A. craccivora* populations on both *Robinia* and other host plants should be further investigated.

Aphis fabae Scopoli, 1763

Material examined: Çanakkale, İzmir Road, 40°05'17.1" N 26°23'28.0" E, *Vicia faba* L. (Fabaceae), 14.III.2013; Çanakkale, Halileli, 39°59'16.3" N 26°17'45.2" E, *Sonchus* sp. (Asteraceae), 21.III.2013; Çanakkale, Kepez, Dardanos Campus, 40°04'32.9" N 26°21'38.9" E, *Artemisia* sp. (Asteraceae), 18.IV.2013; Çanakkale, city center, 40°09'02.2" N 26°24'23.4" E, *Hedera helix* L. (Araliaceae), 21.IV.2013; Çanakkale, city center, 40°08'45.2" N 26°25'06.2" E, *Viburnum* sp. (Adoxaceae), 21.IV.2013; Çanakkale, Ezine, Akköy, 39°49'11.1" N 26°20'42.4" E, *Onopordum* sp. (Asteraceae), 25.IV.2013; Çanakkale, Güzelyalı, 40°01'21.6" N 26°19'35.3" E, *Cistus* sp. (Cistaceae), 02.V.2013; Çanakkale, Kumkale, 39°59'03.2" N 26°13'34.0" E, *Rumex* sp. (Polygonaceae), 02.V.2013; Çanakkale, Çan Road, 40°08'34.7" N 26°29'36.4" E, *Phaseolus vulgaris* L. (Fabaceae), 04.IX.2013; Çanakkale, university campus, 40°06'37.3" N 26°24'58.6" E, *Viburnum opulus* L. (Adoxaceae), 04.IV.2014; Çanakkale, Kepez, 40°05'29.6" N 26°23'12.5" E, *Cirsium arvense* (L.) Scop. (Asteraceae), 25.IV.2014; Çanakkale, university campus, 40°06'43.1" N 26°25'16.0" E, *Spartium junceum* L. (Fabaceae), 23.V.2014; Çanakkale, Yapıldak, 40°13'56.1" N 26°32'25.4" E, *Vitis* sp. (Vitaceae), 10.VIII.2014; Çanakkale, university campus, 40°06'41.0" N 26°25'00.4" E, *Robinia pseudoacacia* L. (Fabaceae), 06.VI.2015.

World distribution: Worldwide except Australian region (Nieto Nafria, 2016).

Turkish distribution: *A. fabae* was recorded from Adana, Aksaray, Ankara, Artvin, Balıkesir, Bartın, Çanakkale, Denizli, Diyarbakır, Hatay, İstanbul, İzmir, Kahramanmaraş, Konya, Mersin, Niğde, Osmaniye, Rize, Trabzon and Van (Bodenheimer & Swirski, 1957; Tuatay, 1993; Toros et al., 1996; Toros et al., 2002; Ölmez Bayhan et al., 2003; Aslan & Uygun, 2005; Ayyıldız & Atlıhan, 2006; Geneci & Görür, 2007; Çıraklı et al., 2008; Eser et al., 2009; Toper Kaygın et al., 2009; Akyıldırım et al., 2014).

Aphis fabae solanella Theobald, 1914

Material examined: Çanakkale, Ayvacık, Assos Road, 39°34'56.5" N 26°25'00.1" E, *Solanum nigrum* L. (Solanaceae), 10.IX.2013.

World distribution: Worldwide except Australian and Nearctic regions (Nieto Nafria, 2016).

Turkish distribution: *A. fabae solanella* was recorded from Adana, Ankara, Bartın, Diyarbakır, Hatay, Kahramanmaraş, Mersin, Osmaniye and Van (Toros et al., 1996; Toros et al., 2002; Ölmez Bayhan et al., 2003; Aslan & Uygun, 2005; Özdemir et al., 2006; Toper Kaygın et al., 2009).

Aphis gossypii Glover, 1877

Material examined: Çanakkale, Çan, Yenice Road, 39°58'00.9" N 27°10'24.1" E, *Capsicum* sp. (Solanaceae), 04.IX.2013; Çanakkale, İzmir Road, 39°58'24.7" N 26°17'53.4" E, *Gossypium hirsutum* L. (Malvaceae), 24.IX.2013; Çanakkale, Kepez, Dardanos Campus, 40°04'32.5" N 26°21'44.4" E, *Viburnum* sp., (Adoxaceae) 02.V.2014; Çanakkale, university campus, 40°06'38.9" N 26°24'43.9" E, *Malva* sp. (Malvaceae), 23.V.2014; Çanakkale, Yapıldak, 40°13'56.1" N 26°32'25.4" E, *Abelmoschus esculentus* (L.) Moench (Malvaceae), 06.VI.2015.

World distribution: Worldwide (Nieto Nafria, 2016).

Turkish distribution: *A. gossypii* was recorded from Adana, Aksaray, Ankara, Artvin, Balıkesir, Bartın, Çanakkale, Denizli, Diyarbakır, Hatay, İzmir, Kahramanmaraş, Mersin, Niğde, Rize, Samsun, Trabzon and Van (Tuatay, 1993; Toros et al., 1996; Toros et al., 2002; Ölmez Bayhan et al., 2003; Görür, 2004; Aslan & Uygun, 2005; Ayyıldız & Atlıhan, 2006; Özdemir et al., 2006; Geneci & Görür, 2007; Çıraklı et al., 2008; Eser et al., 2009; Toper Kaygın et al., 2009; Akyürek et al., 2012; Akyıldırım et al., 2014).

Aphis hederae Kaltenbach, 1843

Material examined: Çanakkale, city center, 40°09'02.2" N 26°24'23.4" E, *Hedera helix* L. (Araliaceae), 21.IV.2013.

World distribution: Afrotropical region, Nearctic region, Near East, North Africa and Neotropical region (Nieto Nafria, 2016).

Turkish distribution: *A. hederae* was recorded from Mersin (Toros et al., 2002).

Aphis nerii Boyer de Fonscolombe, 1841

Material examined: Çanakkale, city center, 40°08'56.4" N 26°24'17.9" E, *Nerium* sp. (Apocynaceae), 11.VII.2014.

World distribution: Worldwide (Nieto Nafria, 2016).

Turkish distribution: *A. nerii* was recorded from Adana, Ankara, Bartın, Diyarbakır, İzmir, Kahramanmaraş, Mersin and Samsun (Bodenheimer & Swirski, 1957; Toros et al., 2002; Ölmez Bayhan et al., 2003; Aslan & Uygun, 2005; Eser et al., 2009; Toper Kaygın et al., 2009; Akyürek et al., 2012).

Aphis pomi de Geer, 1773

Material examined: Çanakkale, Kalabaklı, 40°04'12.9" N 26°25'53.1" E, *Malus domestica* Borkh. (Rosaceae), 22.X.2013.

World distribution: Worldwide except Afrotropical region (Nieto Nafria, 2016).

Turkish distribution: *A. pomi* was recorded Adana, Ankara, Artvin, Bartın, Çanakkale, Diyarbakır, Hatay, Kahramanmaraş, Mersin, Niğde, Samsun and Van (Bodenheimer & Swirski, 1957; Tuatay, 1993; Toros et al., 1996; Toros et al., 2002; Ölmez Bayhan et al., 2003; Görür, 2004; Aslan & Uygun, 2005; Toper Kaygın et al., 2009; Akyürek et al., 2012; Akyıldırım et al., 2014).

Aphis punicae Passerini, 1863

Material examined: Çanakkale, Yapıldak, 40°13'56.1" N 26°32'25.4" E, *Punica granatum* L. (Lythraceae), 06.VI.2015.

World distribution: Afrotropical region, North Africa, Near East and Oriental region (Nieto Nafria, 2016).

Turkish distribution: *A. punicae* was recorded from Adana, Antalya, Bartın, Çanakkale, Denizli, Diyarbakır Hatay, İzmir, Kahramanmaraş and Mersin (Bodenheimer & Swirski, 1957; Tuatay, 1993; Toros et al., 2002; Ölmez Bayhan et al., 2003; Aslan & Uygun, 2005; Çıraklı et al., 2008; Eser et al., 2009; Toper Kaygın et al., 2009).

Aphis rumicis Linnaeus, 1758

Material examined: Çanakkale, Kepez, Dardanos Campus, 40°04'32.9" N 26°21'38.9" E, *Rumex* sp. (Polygonaceae), 18.IV.2013.

World distribution: Worldwide except Australian and Afrotropical region (Nieto Nafria, 2016).

Turkish distribution: *A. rumicis* was recorded from Adana, Ankara (Bodenheimer & Swirski, 1957; Toros et al., 2002; Özdemir et al., 2006).

Aphis sedi Kaltenbach, 1843

Material examined: Çanakkale, Kepez, 40°06'44.7" N 26°24'22.9" E, *Lampranthus* sp. (Aizoaceae), 14.V.2014.

World distribution: Australia, Europe, Kazakhstan, New Zealand, North Korea, South Africa, North and South America, (Blackman & Eastop, 2016).

Aphis sedi is new species for the Turkish aphid fauna (Figure 2).

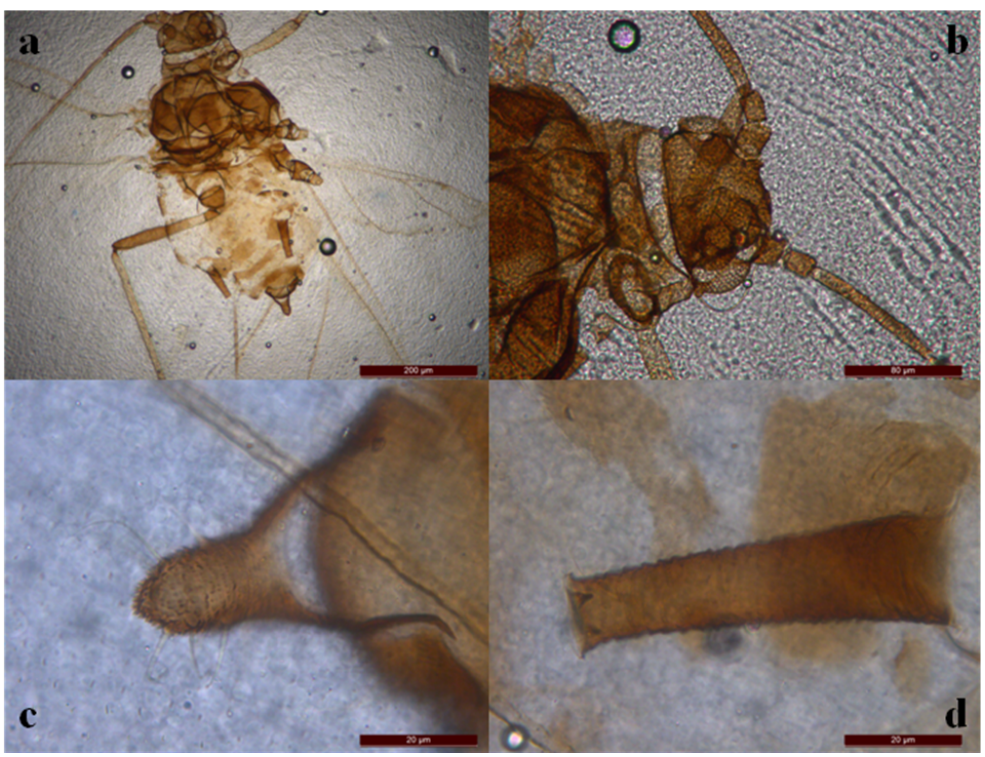

Figure 2. *Aphis sedi* Kaltenbach, 1843 (a) alate adult, (b) head, (c) cauda, (d) siphunculus.

Aphis spiraecola Patch, 1914

Material examined: Çanakkale, university campus, 40°06'34.0" N 26°25'00.8" E, *Citrus limon* L. (Rutaceae), 09.IV.2013.

World distribution: Worldwide except Australian and east Palearctic regions (Nieto Nafria, 2016).

Turkish distribution: *A. spiraecola* was recorded from Adana, Artvin, Bartın, Denizli, Diyarbakır, Hatay, İzmir, Kahramanmaraş, Mersin, Samsun and Trabzon (Toros et al., 2002; Ölmez Bayhan et al., 2003; Aslan & Uygun, 2005; Çıraklı et al., 2008; Eser et al., 2009; Toper Kaygın et al., 2009; Akyürek et al., 2012; Akyıldırım et al., 2014).

Aphis umbrella (Börner, 1950)

Material examined: Çanakkale, Kepez, 40°05'35.7" N 26°23'25.3" E, *Malva* sp. (Malvaceae), 25.IV.2014.

World distribution: East Palearctic, Near East, North Africa and Nearctic region (Nieto Nafria, 2016).

Turkish distribution: *A. umbrella* was recorded from Adana, Ankara (Bodenheimer & Swirski, 1957; Toros et al., 2002; Özdemir et al., 2006).

Hyalopterus pruni (Geoffroy, 1762)

Material examined: Çanakkale-Eceabat, Küçükanafarta, 40°18'17.6" N 26°19'08.2" E, *Prunus domestica* L. (Rosaceae), 03.V.2013; Çanakkale, Çan city center, 40°01'38.1" N 27°02'54.2" E, *Prunus persica* L. Batsch (Rosaceae), 07.VI.2013; Çanakkale, Kepez, 40°06'31.9" N 26°24'31.8" E, *Typha* sp. (Typhaceae), 25.IV.2014.

World distribution: Worldwide (Nieto Nafria, 2016).

Turkish distribution: *H. pruni* was recorded from Adana, Aksaray, Ankara, Denizli, Diyarbakır, Gaziantep, Hatay, İzmir, Kahramanmaraş, Mersin, Niğde and Van (Bodenheimer & Swirski, 1957; Toros et al., 1996; Toros et al., 2002; Ölmez Bayhan et al., 2003; Aslan & Uygun, 2005; Geneci & Görür, 2007; Çıraklı et al., 2008; Eser et al., 2009).

Tribe Macrosiphini

Acyrthosiphon gossypii Mordvilko, 1914

Material examined: Çanakkale, Biga, Eybekli, 40°10'54.0" N 27°12'33.8" E, *Sonchus* sp. (Asteraceae), 02.IV.2013.

World distribution: Worldwide except Australian, Nearctic and Neotropical regions (Nieto Nafria, 2016).

Turkish distribution: *A. gossypii* was recorded from Adana, Aydın, Diyarbakır, İzmir, Hatay and Siirt (Tuatay et al., 1972; Tuatay, 1988; Toros et al., 2002).

Aulacorthum solani (Kaltenbach, 1843)

Material examined: Çanakkale, Kepez, 40°06'55.5" N 26°24'37.2" E, *Helianthus annuus* L. (Asteraceae), 15.V.2015.

World distribution: Worldwide (Nieto Nafria, 2016).

Turkish distribution: *A. solani* was recorded from Aydın, Diyarbakır, Erzican, Eskişehir, İstanbul, İzmir, Mersin, Niğde, Osmaniye, Samsun and Van (Tuatay, 1988; Toros et al., 2002; Ölmez Bayhan et al., 2003; Görür, 2004; Eser et al., 2009; Akyürek et al., 2012).

Host plants in Turkey: *Antirrhinum* sp. (Plantaginaceae)*, Cydonia oblonga* Mill. (Rosaceae)*, Dianthus anatolicus* Boiss. (Caryophyllaceae)*, Hydrangea macrophylla* (Thunb.) Ser. (Hydrangeaceae), *Lycopersicum esculentum* L. (Solanaceae)*, Taraxacum scaturiginosum* G. Hagl. (Asteraceae)*, Tulipa gesneriana* L. (Liliaceae), *Veronica anagalloides* Guss. (Scrophulariaceae) (Tuatay, 1988; Toros et al., 2002; Ölmez Bayhan et al., 2003; Görür, 2004; Eser et al., 2009; Akyürek et al., 2012).

Aulacorthum solani adults were determined from colonizes on *H. annuus*. Both winged and wingless adults of *A. solani* were collected from the host plant. In this study, *H. annuus* was determined as new host record for *A. solani* in Turkey.

Brachycaudus helichrysi (Kaltenbach, 1843)

Material examined: Çanakkale, Kepez, 40°05'29.6" N 26°23'12.5" E, *Cirsium arvense* (L.) Scop. (Asteraceae), 25.IV.2014; Çanakkale, Kepez, 40°06'55.5" N 26°24'37.2" E, *Helianthus annuus* L. (Asteraceae), 15.V.2015; Çanakkale, city center, 40°08'36.5" N 26°25'01.4" E, *Anthemis sp.* (Asteraceae), 31.V.2015.

World distribution: Worldwide (Nieto Nafria, 2016).

Turkish distribution: *B. helichrysi* was recorded from Adana, Adapazarı, Aksaray, Ankara, Antalya, Artvin, Bartın, Burdur, Denizli, Diyarbakır, Erzurum, Eskişehir, Gaziantep, Gümüşhane, Hatay, Isparta, İstanbul, İzmir, Kahramanmaraş, Rize, Samsun, Trabzon and Van (Tuatay et al., 1972; Tuatay, 1988; Toros et al., 2002; Ölmez Bayhan et al., 2003; Aslan & Uygun, 2005; Özdemir et al., 2006; Geneci & Görür, 2007; Çıraklı et al., 2008; Eser et al., 2009; Toper Kaygın et al., 2009; Akyıldırım et al., 2014).

Brachycaudus (Prunaphis) cardui (Linnaeus, 1758)

Material examined: Çanakkale, Kepez, 40°05'29.6" N 26°23'12.5" E, *Cirsium arvense* (L.) Scop. (Asteraceae), 25.IV.2014;

World distribution: Worldwide except Australian region (Nieto Nafria, 2016).

Turkish distribution: *B. cardui* was recorded from Adana, Ankara, Antalya, Artvin, Aydın, Bartın, Bitlis, Bolu, Denizli, Diyarbakır, Erzurum, Isparta, İstanbul, İzmir, Kahramanmaraş, Mersin, Rize, Samsun, Trabzon and Van (Düzgüneş & Tuatay, 1956; Tuatay & Remaudière, 1964; Giray, 1974; Çanakçıoğlu, 1975; Tuatay, 1988; Toros et al., 1996; Toros et al., 2002; Ölmez Bayhan et al., 2003; Aslan & Uygun, 2005; Özdemir et al., 2006; Çıraklı et al., 2008; Eser et al., 2009; Toper Kaygın et al., 2009; Akyürek et al., 2012; Akyıldırım et al., 2014; Barjadze et al., 2014b).

Brevicoryne brassicae (Linnaeus, 1758)

Material examined: Çanakkale, Halileli, 39°58'39.3" N 26°16'27.1" E, 21.III.2013; Çanakkale, Umurbey, 40°15'24.1" N 26°35'56.5" E, 31.III.2013; Çanakkale, Lapseki, 40°20'13.2" N 26°42'27.4" E, 31.III.2013; Çanakkale, Çıplak 39°57'25.2" N 26°15'58.5" E, 19.IX.2013, *Brassica oleracea* L. (Brassicaceae); Çanakkale, Kepez, 40°04'26.4" N 26°24'43.1" E, *Sinapis arvensis* L. (Brassicaceae), 02.V.2014.

World distribution: Worldwide (Nieto Nafria, 2016).

Turkish distribution: *B. brassicae* was recorded from Adana, Aksaray, Amasya, Ankara, Antalya, Diyarbakır, Hatay, İzmir, Kahramanmaraş, Mersin, Ordu, Samsun, Sinop, Şanlıurfa and Van (Bodenheimer & Swirski, 1957; Tuatay et al., 1972; Tuatay, 1988; Toros et al., 1996; Toros et al., 2002; Ölmez Bayhan et al., 2003; Aslan & Uygun, 2005; Özdemir et al., 2006; Geneci & Görür, 2007; Eser et al., 2009; Akyürek et al., 2012).

Cavariella aegopodii (Scopoli, 1763)

Material examined: Çanakkale, university campus, 40°06'48.9" N 26°25'14.2" E, *Salix* sp. (Salicaceae), 24.IV.2015.

World distribution: Worldwide (Nieto Nafria, 2016).

Turkish distribution: *C. aegopodii* was recorded from Ankara, Bartın, Burdur, Çankırı, Erzurum, Eskişehir, İstanbul, İzmir and Van (Bodenheimer & Swirski, 1957; Giray, 1974; Tuatay, 1988; Toper Kaygın et al., 2009).

Dysaphis devecta (Walker, 1849)

Material examined: Çanakkale, Yapıldak, 40°13'52.9" N 26°32'26.7" E, *Malus domestica* Borkh. (Rosaceae), 20.V.2015.

World distribution: East Palearctic, Near East and Nearctic region (Nieto Nafria, 2016).

Turkish distribution: *D. devecta* was recorded from Adana, Aksaray, Ankara, Burdur, Diyarbakır, Kahramanmaraş, Malatya, Mersin, Niğde and Van (Tuatay & Remaudière, 1964; Tuatay, 1990; Toros et al., 1996; Toros et al., 2002; Ölmez Bayhan et al., 2003; Görür, 2004; Aslan & Uygun, 2005; Geneci & Görür, 2007).

Dysaphis (Pomaphis) plantaginea (Passerini, 1860)

Material examined: Çanakkale, Yapıldak, 40°13'52.9" N 26°32'26.7" E, *Malus domestica* Borkh. (Rosaceae), 20.V.2015.

World distribution: Worldwide except Australian region (Nieto Nafria, 2016).

Turkish distribution: *D. plantaginea* was recorded from Adana, Ankara, Antalya, Burdur, Çanakkale, Diyarbakır, Elazığ, Gaziantep, Giresun, Gümüşhane, Hatay, Isparta, Kahramanmaraş, Kayseri, Mersin, Niğde, Samsun and Şanlıurfa (Bodenheimer & Swirski, 1957; Tuatay, 1990; Toros et al., 2002; Ölmez Bayhan et al., 2003; Görür, 2004; Aslan & Uygun, 2005; Akyürek et al., 2012; Barjadze et al., 2014b).

Hyadaphis foeniculi (Passerini, 1860)

Material examined: Çanakkale, city center, 40°09'26.6" N 26°25'16.4" E, *Pimpinella saxifraga* L. (Apiaceae), 21.IV.2013.

World distribution: Worldwide (Nieto Nafria, 2016).

Turkish distribution: *H. foeniculi* was recorded from Adana, Ankara, Bartın, Bitlis, Isparta, İstanbul, and İzmir (Bodenheimer & Swirski, 1957; Tuatay & Remaudière, 1964; Çanakçıoğlu, 1966; Giray, 1974; Toros et al., 2002; Özdemir et al., 2006; Toper Kaygın et al., 2009; Barjadze et al., 2014b).

Host plants in Turkey: *Chenopodium album* L. (Amaranthaceae), *Daucus* sp. (Apiaceae), *Foeniculum vulgare* Mill. (Apiaceae), *Lonicera caerulea* L. (Caprifoliaceae), *Lonicera* sp. (Caprifoliaceae), *Pastinaca sativa* L. (Apiaceae), *Petroselinum* sp. (Apiaceae), *Pimpinella anisum* L. (Apiaceae), *Robinia pseudoacacia* L. (Fabaceae) (Bodenheimer & Swirski, 1957; Giray, 1974; Düzgüneş et al., 1982; Tuatay, 1990; Toros et al., 2002; Özdemir et al., 2006; Toper Kaygın et al., 2009; Barjadze et al., 2014b).

Hyadaphis foeniculi adults were determined from colonizes on *P. saxifraga*. Both winged and wingless adults of *H. foeniculi* were collected from the host plant. In this study, *P. saxifraga* was determined as new host record for *H. foeniculi* in Turkey.

Hyperomyzus lactucae (Linnaeus, 1758)

Material examined: Çanakkale, university campus, 40°06'34.8" N 26°24'52.1" E, 11.IV.2013; Çanakkale, Kepez, Dardanos Campus, 40°04'39.9" N 26°21'43.9" E, 18.IV.2013, *Sonchus* sp. (Asteraceae).

World distribution: Worldwide (Nieto Nafria, 2016).

Turkish distribution: *H. lactucae* was recorded from Adana, Ankara, Antalya, Aydın, Diyarbakır, Eskişehir, İstanbul, Kahramanmaraş, Mersin, Muğla and Sakarya (Tuatay & Remaudière, 1964; Tuatay, 1990; Toros et al., 2002; Ölmez Bayhan et al., 2003; Aslan & Uygun, 2005; Özdemir et al., 2006).

Liosomaphis berberidis (Kaltenbach, 1843)

Material examined: Çanakkale, university campus, 40°06'39.8" N 26°24'59.5" E, *Berberis* sp. (Berberidaceae), 27.VI.2015.

World distribution: Australian, east Palearctic, Nearctic and Near East regions (Nieto Nafria, 2016).

Turkish distribution: *L. berberidis* was recorded from Ankara, Bitlis, Çankırı, Giresun, Isparta, İstanbul and Konya (Tuatay & Remaudière, 1964; Tuatay, 1990; Barjadze et al., 2014b).

Macrosiphum euphorbiae (Thomas, 1878)

Material examined: Çanakkale, city center, 40°08'45.2" N 26°25'01.6" E, *Rosa* sp. (Rosaceae), 21.IV.2013.

World distribution: Worldwide (Nieto Nafria, 2016).

Turkish distribution: *M. euphorbiae* was recorded from Adana, Aksaray, Amasya, Ankara, Balıkesir, Bartın, Denizli, Erzurum, Hatay, İstanbul, İzmir, Mersin, Sakarya and Samsun (Tuatay & Remaudière, 1964; Giray, 1974; Tuatay, 1990; Toros et al., 2002; Ayyıldız & Atlıhan, 2006; Özdemir et al., 2006; Geneci & Görür, 2007; Çıraklı et al., 2008; Eser et al., 2009; Toper Kaygın et al., 2009; Akyürek et al., 2012).

Macrosiphum rosae (Linnaeus, 1758)

Material examined: Çanakkale, city center, 40°09'02.9" N 26°24'29.3" E, 09.IV.2013; Çanakkale, Kepez, 40°05'59.6" N 26°22'03.3" E, 05.VI.2015, *Rosa* sp. (Rosaceae).

World distribution: Worldwide except east Palearctic (Nieto Nafria, 2016).

Turkish distribution: *M. rosae* was recorded from Adana, Ankara, Antalya, Bartın, Bolu, Burdur, Çankırı, Denizli, Diyarbakır, Giresun, Hatay, Isparta, İstanbul, İzmir, Kahramanmaraş, Mersin, Samsun and Van (Düzgüneş & Tuatay, 1956; Bodenheimer & Swirski, 1957; Giray, 1974; Tuatay, 1990; Toros et al., 1996; Toros et al., 2002; Ölmez Bayhan et al., 2003; Aslan & Uygun, 2005; Çıraklı et al., 2008; Eser et al., 2009; Toper Kaygın et al., 2009; Akyürek et al., 2012; Barjadze et al., 2014b).

Myzus cerasi (Fabricius, 1775)

Material examined: Çanakkale, Umurbey, 40°14'19.2" N 26°37'43.5" E, 30.IV.2013; Çanakkale, Yapıldak, 40°13'56.1" N 26°32'25.4" E, 15.V.2015, *Prunus avium* L. (Rosaceae).

World distribution: Worldwide except Afrotropical and Neotropical regions (Nieto Nafria, 2016).

Turkish distribution: *M. cerasi* was recorded from Adana, Diyarbakır, Isparta, Kahramanmaraş, Kocaeli, Mersin, Samsun, Trabzon and Van (Bodenheimer & Swirski, 1957; Toros et al., 1996; Toros et al., 2002; Ölmez Bayhan et al., 2003; Aslan & Uygun, 2005; Akyürek et al., 2012; Akyıldırım et al., 2014; Barjadze et al., 2014b).

Myzus (Nectarosiphon) persicae Sulzer, 1776

Material examined: Çanakkale, Yenice, 39°56'16.8" N 27°14'00.2" E, *Capsicum* sp. (Solanaceae), 04.IX.2013.

World distribution: Worldwide (Nieto Nafria, 2016).

Turkish distribution: *M. persicae* was recorded from Adana, Aksaray, Ankara, Balıkesir, Bartın, Çanakkale, Diyarbakır, İstanbul, İzmir, Kahramanmaraş, Mersin, Samsun and Van (Bodenheimer & Swirski, 1957; Tuatay, 1991; Toros et al., 1996; Toros et al., 2002; Ölmez Bayhan et al., 2003; Aslan & Uygun, 2005; Özdemir et al., 2006; Ayyıldız & Atlıhan, 2006; Geneci & Görür, 2007; Eser et al., 2009; Toper Kaygın et al., 2009; Akyürek et al., 2012).

Sitobion avenae (Fabricius, 1775)

Material examined: Çanakkale, Kepez, 40°05'25.4" N 26°23'19.3" E, 25.IV.2013; Çanakkale, Yapıldak, 40°12'40.5" N 26°32'40.7" E, 27.IV.2014, *Triticum* sp. (Poaceae); Çanakkale, Kepez, Dardanos Campus, 40°04'34.0" N 26°21'52.5" E, *Elymus* sp. (Poaceae), 23.V.2014.

World distribution: Worldwide (Nieto Nafria, 2016).

Turkish distribution: *S. avenae* was recorded from Adana, Çanakkale, Diyarbakır, Hatay, İzmir, Kahramanmaraş, Mersin, Samsun and Van (Tuatay, 1991; Toros et al., 1996; Toros et al., 2002; Ölmez Bayhan et al., 2003; Aslan & Uygun, 2005; Eser et al., 2009; Akyürek et al., 2012).

Uroleucon sonchi (Linnaeus, 1767)

Material examined: Çanakkale, Kepez, 40°05'24.0" N 26°23'31.1" E, *Sonchus oleraceus* L. (Asteraceae), 14.III.2013; Çanakkale, Kepez, Dardanos Campus, 40°04'25.1" N 26°21'48.8" E, 18.IV.2013; Çanakkale, Bayramiç, Doğancı, 39°49'56.6" N 26°35'24.8" E, 25.IV.2013, *Senecio vernalis* Waldst. & Kit. (Asteraceae).

World distribution: Worldwide (Nieto Nafria, 2016).

Turkish distribution: *U. sonchi* was recorded from Adana, Ankara, Çanakkale, Diyarbakır and Mersin (Tuatay, 1991; Toros et al., 2002; Ölmez Bayhan et al., 2003; Özdemir et al., 2006).

Subfamily Calaphidinae

Tribe Panaphini

Chromaphis juglandicola (Kaltenbach, 1843)

Material examined: Çanakkale, Kepez, 40°06'38.1" N 26°24'23.6" E, *Juglans regia* L. (Juglandaceae), 31.V.2015.

World distribution: East Palearctic, Near East, Nearctic, North Africa and Oriental regions (Nieto Nafria, 2016).

Turkish distribution: *C. juglandicola* was recorded from Adana, Diyarbakır, Hatay, Mersin, Van and Kahramanmaraş (Toros et al., 1996; Toros et al., 2002; Ölmez Bayhan et al., 2003; Aslan & Uygun, 2005).

Panaphis juglandis (Goeze, 1778)

Material examined: Çanakkale, Kepez, 40°06'38.1" N 26°24'23.6" E, *Juglans regia* L. (Juglandaceae), 31.V.2015.

World distribution: East Palearctic, Near East, Nearctic and Oriental region (Nieto Nafria, 2016).

Turkish distribution: *P. juglandis* was recorded from Adana, Diyarbakır, Kahramanmaraş, Kastamonu, Mersin and Van (Toros et al., 1996; Toros et al., 2002; Ölmez Bayhan et al., 2003; Aslan & Uygun, 2005; Ünal & Özcan, 2005).

Tinocallis (Sarucallis) kahawaluokalani (Kirkaldy, 1906)

Material examined: Çanakkale, university campus, 40°06'37.1" N 26°24'47.1" E, *Lagerstroemia indica* L. Pers. (Lythraceae), 27.VI.2015.

World distribution: Afrotropical, east Palearctic, Nearctic, Neotropical and Oriental regions (Nieto Nafria, 2016).

Turkish distribution: *T. kahawaluokalani* was recorded from Adana (Remaudière et al., 2006).

Tinocallis (Sappocallis) saltans (Nevsky, 1929)

Material examined: Çanakkale, city center, 40°09'03.8" N 26°24'26.3" E, *Ulmus* sp. (Ulmaceae), 20.VI.2015.

World distribution: East Palearctic, Near East, Nearctic, Neotropical and Oriental regions (Nieto Nafria, 2016).

Turkish distribution: *T. saltans* was recorded from forest trees by Çanakçıoğlu (1966).

Eucallipterus tiliae (Linnaeus, 1758)

Material examined: Çanakkale, university campus, 40°06'36.4" N 26°24'49.5" E, *Tilia* sp. (Malvaceae), 06.VI.2015.

World distribution: Worldwide except Afrotropical and Oriental regions (Nieto Nafria, 2016).

Turkish distribution: *E. tiliae* was recorded from Bartın, Isparta, İzmir and Kastamonu (Ünal & Özcan, 2005; Toper Kaygın et al., 2008; Eser et al., 2009; Demirözer et al., 2015).

Subfamily Chaitophorinae

Tribe Chaitophorini

Chaitophorus leucomelas Koch, 1854

Material examined: Çanakkale, university campus, 40°06'32.9" N 26°24'59.3" E, *Populus* sp. (Salicaceae), 11.IV.2013.

World distribution: Worldwide except Australian and Oriental regions (Nieto Nafria, 2016).

Turkish distribution: *C. leucomelas* was recorded from Adana, Ankara, Diyarbakır, Kahramanmaraş, Konya, Mersin and Van (Bodenheimer & Swirski, 1957; Toros et al., 1996; Toros et al., 2002; Ölmez Bayhan et al., 2003; Aslan & Uygun, 2005; Uysal et al., 2006).

Subfamily Eriosomatinae

Tribe Pemphigini

Prociphilus fraxini (Fabricius, 1777)

Material examined: Çanakkale, university campus, 40°06'41.6" N 26°25'01.4" E, *Fraxinus excelsior* L. (Oleaceae), 13.V.2015.

World distribution: Near East and Oriental regions (Nieto Nafria, 2016).

Turkish distribution: *P. fraxini* was recorded from İstanbul and Kastamonu (Çanakçıoğlu, 1970; Ünal & Özcan, 2005).

Subfamily Lachninae

Tribe Eulachnini

Cinara pini (Linnaeus, 1758)

Material examined: Çanakkale, Kepez, 40°06'37.7" N 26°24'37.1" E, *Pinus* sp. (Pinaceae), 14.V.2014.

World distribution: East Palearctic, Near East, Nearctic and Oriental regions (Nieto Nafria, 2016).

Turkish distribution: *C. pini* was recorded from Bolu, Antalya, Çanakkale, Çankırı, İzmir, Kahramanmaraş, Kars, Muğla, Samsun and Trabzon (Çanakçıoğlu, 1966; Tuatay, 1999; Aslan & Uygun, 2005; Akyürek et al., 2012; Akyıldırım et al., 2014).

Tribe Lachnini

Pterochloroides persicae (Cholodkovsky, 1899)

Material examined: Çanakkale, city center, 40°09'02.2" N 26°24'36.7" E, *Prunus* sp. (Rosaceae), 17.VI.2015.

World distribution: Worldwide except Australian and Nearctic regions (Nieto Nafria, 2016).

Turkish distribution: *P. persicae* was recorded from Adana, Aksaray, Ankara, Artvin, Denizli, Hatay, Kahramanmaraş, Mersin, Niğde and Van (Bodenheimer & Swirski, 1957; Toros et al., 1996; Toros et al., 2002; Görür, 2004; Aslan & Uygun, 2005; Geneci & Görür, 2007; Akyıldırım et al., 2014).

Local studies are very important to determine the aphid fauna in any country. Although biodiversity in Turkey is more rich than its neighboring countries, the aphid fauna recorded for Turkey is so far quite limited. For example, while the aphid fauna of Greece and Italy consist of 364 and 760 species respectively, the aphid fauna of Turkey had only reached 532 species (Patti & Barbagallo, 1998; Tsitsipis et al., 1998; Şenol et al., 2015b). Likewise, Iran is located in the same biogeographical region as Turkey. The aphid fauna of Iran is represented by 485 species based on recent local studies (Mortazavi et al., 2015). Owing to the fact that many local areas have been sampled only sporadically, both the aphid fauna of Turkey and Iran are still quite limited. The results of both in the current study and other recent studies (Akyürek at al., 2010; Görür et al., 2011; Barjadze & Özdemir, 2014a; Şenol et al., 2015a) show that Turkey has a rich aphid fauna and the number of species will increase as local studies are conducted in different areas. Therefore, it is recommended that local aphid fauna studies be conducted so that the aphid species diversity and richness of Turkey is more fully known.

References

Akyıldırım, H., İ. Tepecik & G. Görür, 2011. "Aphid species (Hemiptera: Aphidoidea) damage to plants in Büyükada (İstanbul) district, 195". IVth Turkey Plant Protection Congress (June, 28-30, Kahramanmaraş, Turkey) Proceedings, 496 pp.

Akyıldırım, H., Ö. Şenol, G. Görür, N. Aktaç, & E. Demirtaş, 2014. Determined aphid and ant associations from Trabzon, Rize and Artvin provinces of the Turkey. Journal of the Entomological Research Society, 16 (2): 29-37.

Akyürek, B., Ü. Zeybekoğlu & G. Görür, 2010. The determination of Aphid (Homoptera: Aphididae) fauna in Kurupelit campus in Ondokuz Mayıs University. Turkish Journal of Zoology, 34 (2): 421-424.

Akyürek, B., Ü. Zeybekoğlu & G. Görür, 2012. Ondokuz Mayıs Üniversitesi Kurupelit Yerleşkesi (Samsun)'nin yaprakbiti (Hemiptera: Aphididae) türleri ve konukçu bitkileri. Türkiye Entomoloji Bülteni, 2 (2): 91-108.

Aslan, M. M. & N. Uygun, 2005. Aphids (Homoptera: Aphididae) of Kahramanmaraş Province. Turkish Journal of Zoology, 29: 201-209.

Ayyıldız, Y. & R. Atlıhan, 2006. Balıkesir ili sebze alanlarında görülen yaprakbiti türleri ve doğal düşmanları. Yüzüncü Yıl Üniversitesi Ziraat Fakültesi Tarım Bilimleri Dergisi, 16 (1): 1-5.

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

Barjadze, S. & I. Özdemir, 2014. A new genus of Macrosiphini Wilson, (1910) 1887 (Hemiptera: Aphididae) from Rhododendron in Turkey. Zootaxa, 3835 (1): 121-126.

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

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

Blackman, R. L. & V. F. Eastop, 2006. Aphids on the World's Herbaceous Plants and Shrubs. John Wiley & Sons Ltd., Naturel History Museum, London. 1439 pp.

Blackman, R. L. & V. F. Eastop, 2016. Aphids on the World's Plants an Online Identification and Information Guide, (Web page: http://www.aphidsonworldsplants.info) (Date accessed: June 2016)

Bodenheimer, F. S. & E. Swirski, 1957. The Aphidoidea of the Middle East. Weizmann Science Press of Israel, Jerusalem, 378 pp.

Çanakçıoğlu, H. 1970. Orman ağaçlarına arız olan bazı aphidlere karşı yapılan kimyasal mücadele denemeleri ve neticeleri. İstanbul Üniversitesi Orman Fakültesi Dergisi A, 20 (1): 94-113.

Çanakçıoğlu, H., 1966. Türkiye'de orman ağaçlarına arız olan bitki bitleri (Aphidoidea) üzerinde araştırmalar. İstanbul Üniversitesi Orman Fakültesi Dergisi, 16 (2): 131-190.

Çanakçıoğlu, H., 1975. The Aphidoidea of Turkey. İstanbul Üniversitesi Orman Fakültesi Yayınları. Yayın No: 189, 309 s.

Çıraklı, A., G. Görür & M. Işık, 2008. Denizli il merkezinde belirlenen afit (Hemiptera: Aphididae) türleri. Selçuk Üniversitesi Ziraat Fakültesi Dergisi, 22 (44): 12-18.

Demirözer, O., A. Uzun & D. Şenal, 2015. Isparta il merkezinde bulunan ıhlamur ağaçları üzerinde saptanan trips ve yaprakbiti türleri. Türkiye Entomoloji Bülteni, 5 (1): 21-28.

Düzgünes, Z. & N. Tuatay, 1956. Türkiye Aphid'leri. Ankara Ziraat Mücadele Enstitüsü Müdürlügü Sayi, 4: 1-63.

Düzgüneş, Z., S. Toros, N. Kılınçer & B. Kovancı, 1982. Ankara İlinde Bulunan Aphidoidea Türlerinin Parazit ve Predatörlerinin Tespiti. Tarım ve Orman Bakanlığı, Zirai Mücadele ve Zirai Karantina Genel Müdürlüğü Yayınları, 251 s.

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

Fahringer, J., 1922. Eine Rhynchotenausbeute aus der Türkei, Kleinasien und den benachbarten Gebieten. Konovia, 1: 296-307.

Geneci, E. & G. Görür, 2007. Aphid (Homoptera: Aphididae) species of the Central Aksaray. International Journal of Natural of Engineering Sciences, 1: 19-21.

Giray, H., 1974. İzmir ili çevresinde Aphididae (Homoptera) familyası türlerine ait ilk liste ile bunların konukçu ve zarar şekilleri hakkında notlar. Ege Üniversitesi Ziraat Fakültesi Dergisi, 11 (1): 39-69.

Görür, G., 2002. New records for Turkish aphid fauna (Homoptera : Aphididae). Zoology in the Middle East, 25: 67-69.

Görür, G., 2004. Aphid (Homoptera : Aphididae) species on pome fruit trees in Nigde Province of Turkey. Turkiye Entomoloji Dergisi, 28 (1): 21-26.

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

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

Güçlü, Ş., H. Kavaz, C. Güçlü & I. Özdemir, 2015. Aphids (Hemiptera: Aphididae) and their parasitoids on ornamental trees and shrubs in Erzurum, Turkey. Turkish Journal of Entomology, 39 (1): 3-9.

Heie, O. E., 1986. The Aphidoidea (Hemiptera) of Fennoscandia and Denmark (III), Family Aphididae: Subfamily Pterocommatinae & Tribe Aphidini of Subfamily Aphidinae. E. J. Brill/Scandinavian Science Press Ltd., Leiden-Copenhagen, 314 pp.

Hille Ris Lambers, D., 1950. On mounting Aphids and other soft skinned insects. Entomologische Berichten, XIII: 55-58.

Holman, J., 2009. Host Plant Catalog of Aphids, Palaearctic Region. Springer, Branisovska, ISBN: 978-1-4020-8285-6, 1216 pp.

Houard, C., 1922. Les Zoocécidies des Plantes d'Afrique, d'Asie et d'Océanie. Tome premier. Cryptogames, Gymnospermes, Monocotylédones, Dicotylédones (1re partie). Librairie scientifique Jules Hermann, Paris, 496 pp.

Kamel-Ben Halima, M. & L. Mdellel, 2010. First record of the grapevine aphid, *Aphis illinoisensis* Shimer, in Tunisia. EPPO Bulletin, 40: 191–192.

Laamari, M. & A. Coeur d'Acier, 2010. Le puceron de la vigne *Aphis illinoisensis* arrive en Algérie. EPPO Bulletin, 40: 167–168.

Mehrparvar, M., S. M. Madjdzadeh, N. M. Arab, M. Esmaeılbeygı & E. Ebrahımpour, 2012. Morphometric discrimination of black legume aphid, *Aphis craccivora* Koch (Hemiptera: Aphididae), populations associated with different host plants. North-Western Journal of Zoology, 8 (1): 172-180.

Mortazavi, Z. S., H. Sadeghi, N. Aktaç, L. Depa & L. Fekrat, 2015. Ants (Hymenoptera: Formicidae) and their aphid partners (Homoptera: Aphididae) in Mashhad region, Razavi Khorasan Province, with new records of aphids and ant species for Fauna of Iran. Halteres, 6: 4-12.

Nieto Nafria, J. M., 2016. Fauna Europaea: Hemiptera: Aphidoidea. Fauna Europaea version 2.6, (Web page: http://www.faunaeur.org) (Date accessed: July 2016)

Ölmez Bayhan, S., M. R. Ulusoy & S. Toros, 2003. Determination of Aphididae (Homoptera) fauna of Diyarbakir province of Turkey. Türkiye Entomoloji Dergisi, 27 (4): 253-268.

Özdemir, I., G. Remaudière, S. Toros & N. Kılınçer, 2005. New aphid records from Turkey including the description of a new *Lachnus* Species (Hemiptera: Aphididae). Revue Française d'Entomologie, 27 (3): 97-102.

Özdemir, I., S. Toros, A. N. Kılınçer & M. O. Gürkan, 2006. A survey of Aphididae (Homoptera) on wild plants in Ankara, Turkey. Ekoloji, 15 (58): 38-41.

Patti, I. & S. Barbagallo, 1998. "An Approach to the Knowledge on the Italian Aphid Fauna, 397-405". In: Aphids in Natural and Managed Ecosystems (Eds. J. M. Nieto Nafria & A. F. G. Dixon) Universidad de Leon, Leon, Spain, 688 pp.

Remaudière, G., E. Sertkaya & I. Özdemir, 2003. Alerte! Découverte en Turquie du puceron américain *Aphis illinoisensis* nuisible à la vigne. Revue Française d'Entomologie (NS), 25: 170.

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

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

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

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

Takahashi, R., 1966. Descriptions of some new and little-known species of *Aphis* of Japan, with a key to species. Transactions of the American Entomological Society, 92: 519-556.

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

Toper Kaygın, A., G. Görür & F. Çota Sade, 2009. Aphid (Hemiptera: Aphididae) species determined on herbaceous and shrub plants in Bartin Province in Western Blacksea Region of Turkey. African Journal of Biotechnology, 8 (12): 2893-2897.

Toros, S., B. Yaşar, M. S. Özgökçe & İ. Kasap, 1996. "Van ilinde Aphidoidea (Homoptera) üstfamilyasına bağlı türlerin saptanması üzerine çalışmalar, 549". The Third Turkish National Congress of Entomology Proceedings (September, 24-28, Ankara, Turkey), 716 pp.

Toros, S., N. Uygun, R. Ulusoy, S. Satar & I. Özdemir, 2002. Doğu Akdeniz Bölgesi Aphidoidea Türleri. Tarım ve Köyişleri Bakanlığı Tarımsal Araştırmalar Genel Müdürlüğü, Ankara. 108 s.

Trotter, A., 1903. Galle della Peninsola Balkanica e Asia Minore. Nuovo Giornale botanico italiano, 10: 6-54.

Tsitsipis, J. A., D. Lykouressis, N. Katis, A. D. Avgelis, J. Gargalianou, A. Papapanayotou & G. M. Kokinis, 1998. "Aphid Species Diversity Demonstrated by Suction Trap Captures in Different Areas in Greece, 495-501". In: Aphids in Natural and Managed Ecosystems (Eds. J. M. Nieto Nafria & A. F. G. Dixon) Universidad de Leon, Leon, Spain, 688 pp.

Tsitsipis, J. A., E. Angelakis, J. T. Margaritopoulos, K. Tsamandali & K. D. Zarpas, 2005. First record of the grapevine aphid *Aphis illinoisensis* in the island Kriti, Greece. OEPP/EPPO Bulletin, 35: 541–542.

Tuatay, 1999. Türkiye yaprakbitleri (Homoptera: Aphididae): V. Chaitophinae, Lachninae ve Thelaxinae. Bitki Koruma Bülteni, 39 (1-2): 1-21.

Tuatay, N. & G. Remaudière, 1964. Première contribution au catalogue des Aphididae (Hom.) de la Turquie. Revue de Pathologie Végétale et d' Entomologie Agricole de France, 43: 243-278.

Tuatay, N. 1993. Aphids of Turkey (Homoptera: Aphididae) IV. Aphidinae: Macrosiphini Part IV. Bulletin of Plant Protection, 33 (1-2): 83-105.

Tuatay, N., 1988. Türkiye yaprakbitleri (Homoptera: Aphididae) I. Aphidinae Macropsophini (I. Kısım). Bitki Koruma Bülteni, 28 (1-2): 1-28.

Tuatay, N., 1990. Türkiye yaprakbitleri (Homoptera: Aphididae) II. Aphidinae Macropsophini (II. Kısım). Bitki Koruma Bülteni, 30 (1-4): 29-44.

Tuatay, N., 1991. Türkiye yaprakbitleri (Homoptera: Aphididae) III. Aphidinae: Macropsophini (III. Kısım). Bitki Koruma Bülteni, 31 (1-4): 3-18.

Tuatay, N., 1993. Türkiye yaprakbitleri (Homoptera: Aphididae) IV. Aphidinae: Aphidini (I. Kısım). Bitki Koruma Bülteni, 33 (3-4): 83-106.

Tuatay, N., A. Kalkandelen & N. (Çağatay) Aysev, 1972. Nebat Koruma Müzesi Böcek Katalogu (1961-1971). Tarım Bakanlığı Zirai Mücadele ve Zirai Karantina Genel Müdürlüğü Yayınları, Yenigün Matbaası, Ankara,119 s.

Uysal, M., A. Şahbaz & I. Özdemir, 2006. Konya ilinde kavaklarda beslenen yaprakbiti (Homoptera: Aphididae) türleri. Selçuk Üniversitesi Ziraat Fakültesi Dergisi, 20 (38): 143-149.

Ünal, S. & E. Özcan, 2005. Kastamonu yöresi Aphididae (Homoptera) türleri. Süleyman Demirel Üniversitesi Orman Fakültesi Dergisi, A (1): 76-83.

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Original article (Orijinal araştırma)

Toxicological and physiological effects of ethephon on the model organism, *Galleria mellonella* L. 1758 (Lepidoptera: Pyralidae)[1]

Etefonun model organizma *Galleria mellonella* L. 1758 (Lepidoptera: Pyralidae) üzerine toksikolojik ve fizyolojik etkileri

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Summary

Ethephon (ETF) has been used in agriculture as an ethylene releaser type of plant growth regulator. The aim of this work was to determine the ecotoxicological effects of ETF on the survival and the antioxidant metabolism of the insects using a model organism *Galleria mellonella* L. 1758. A toxicity test was performed to determine the lethal doses of ETF on larvae. According to probit assay, the LD_{50} and LD_{99} values for force fed larvae were 344 and 419 µg/5 µl, respectively, 30 d after treatment. Analyses performed with 10 doses ≤LD_{50} at 24 and 48 h upon feeding larvae revealed that the malondialdehyde level increased at 300 and 330 µg/5 µl doses, whereas glutathione-S-transferase activity increased only with a 360 µg/5µl dose of ETF at 24 h. However, an increase in glutathione-S-transferase activity was evident at all ETF doses at 48 h. An increase in glutathione peroxidases activity was determined at 250, 300 and 330 µg/5µl at 24 and 48 h. All ETF doses caused an important increase in catalase activity at 24 h but remained unchanged at 48 h. Superoxide dismutase activity also elevated at doses >250 µg/5µl at 24 h when compared to the control. Same changes in superoxide dismutase activity were also observed at all doses of ETF except for 360 µg/5µl at 48 h. These results showed that ETF induced oxidative stress resulted in toxic effects that affected on the survival of model organism *G. mellonella*.

Keywords: Antioxidant enzymes, ethephon, *Galleria mellonella*, malondialdehyde, toxicology

Özet

Etilen salınımına neden olan Etefon (ETF), bir bitki büyüme düzenleyicisi olarak tarımda kullanılmaktadır. Bu çalışmada bir model organizma olan *Galleria mellonella* L. 1758 (Lepidoptera: Pyralidae) türü kullanılarak, ETF'nin böceklerin antioksidan metabolizması ve canlılığı üzerindeki ekotoksikolojik etkilerinin araştırılması amaçlanmıştır. Larval dönemde ETF'nin letal dozunun belirlemesi amacıyla toksitite testi yapılmıştır. Larvalara zorla besleme (ağızdan besleme) yöntemi ile uygulanan ETF dozlarına göre, 30 günlük süreç içinde belirlenen LD_{50} ve LD_{99} değerleri sırasıyla 344 ve 419 µg/5µl olarak belirlenmiştir. LD_{50} ve daha düşük ETF dozlarıyla yapılan toksikolojik analizlerde ise iki zaman dilimi (24. ve 48. saat) tercih edilmiştir. 24. saatte, larval hemolenfteki malondialdehit seviyesi, 300 ve 330 µg/5µl ETF dozlarında artarken, glutatyon-S-transferaz aktivitesi sadece 360 µg/5µ'lik dozda yükselmiştir. Ancak 48. saatte kontrol ve tüm dozlarda glutatyon-S-transferaz aktivitesi yükselmiştir. Glutatyon peroksidaz aktivitesi ise hem 24. hem de 48. saatte, 330 ve 360 µg/5µl ETF dozlarında artmıştır. Tüm ETF dozları 24. saatte katalaz aktivitesinde artışa neden olurken, bu artış 48. saatte de aynı kalmıştır. Süperoksit dismutaz aktivitesi ise 24. saatte, 250 µg/5µl ve daha yüksek dozlarda yükselmiştir. 48. saatteki süperoksit dismutaz aktivitesinde de benzer değişimler meydana gelirken, 360 µg/5µl dozunda azalma belirlenmiştir. Bu sonuçlar, ETF'nin oksidatif stresi teşvik etmesi sonucunda model organizma *G. mellonella*'nın canlılığı üzerinde toksik etkisi olduğunu göstermiştir.

Anahtar sözcükler: Antioksidan enzimler, etefon, *Galleria mellonella*, malondialdehit, toksikoloji

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Introduction

The plant hormones that regulate plant growth are collectively known as the growth hormones or plant growth regulators (PGRs). The commercial forms of PGRs are widely used for increasing agricultural productivity. However, several studies have recently reported that various PGRs also have toxic effects on insects causing survival, developmental, reproductive and biochemical disturbances (Kaur & Rup, 2003; Gupta et al., 2009; Uçkan et al., 2011a, b, 2014, 2015) and induce oxidative stress (Altuntaş, 2015a). For a considerable amount of time, entomologists have been investigating the overall effects of some PGRs on insects. Their results provide reliable data on the biological and biochemical effects of gibberellic acid (GA_3), and indol-3-acetic acid which belong to two major classes of PGRs; the gibberellins and auxins, respectively (Uçkan et al., 2008, 2011a, b, 2014, 2015; Altuntaş et al., 2012, 2014; Altuntaş, 2015a). Altuntaş (2015a) also reported that dietary GA_3 induced oxidative stress in *G. mellonella* larvae, in particularly, exposure of different doses of GA_3 into larval diet activated important antioxidant enzymes in animals.

Ethephon [(2-chloroethyl) phosphonic acid, ETF] is a synthetic growth regulator, and belongs to ethylene releasers, an important class of the PGRs. ETF is used in agricultural systems for promoting fruit ripening, abscission and flower induction by releasing ethylene gas, a natural plant hormone (Zhang et al., 2012; Bhadoria et al., 2015; Hussain et al., 2015). Several dietary studies have been conducted on ETF toxicity to rats, birds, and marine or freshwater invertebrates (Haux et al., 2000, 2002; Al-Twaty, 2006; Abd El Raouf & Girgis, 2011; Anant & Avinash, 2012). Previous studies revealed that ETF not only acts as a plant growth regulator agent but also has mutagenic, teratogenic and biochemical effects on higher animals, since it is an organophosphorus pesticide. Ethephon is also an eye and skin irritant, but not a skin sensitizer, and classified by International Agency for Research on Cancer as group D (not carcinogenic to humans) (Bui, 2007). Acute oral studies using rats have shown that ETF is slightly toxic to mammals (Haux et al., 2002).

Studies on the negative effects of various PGRs, including ETF, on antioxidant mechanism were largely related to higher animals. It has been observed that abscisic acid and GA_3 cause lipid peroxidation in some tissues of rats and they change the activities of the enzymes in the antioxidant defense system. ETF has been found to be an inhibitor of plasma cholinesterase in humans, dogs, rats and mice (Haux et al., 2000, 2002; Tuluce & Çelik, 2006). It is well known that lipid peroxidation of cell membranes, damage to DNA and proteins, and activation of enzymes are regulated by antioxidants (Felton & Summers, 1995). The effects of non-lethal doses of pesticides like ETF may induce defense mechanisms to protect the insect against environmental stressors, because the antioxidant mechanism is a metabolic process for detoxification of environmental pollutants and chemicals (Büyükgüzel et al., 2010, 2013; Aslantürk et al., 2011; Emre et al., 2013; Erdem & Büyükgüzel, 2015; Altuntaş, 2015a, b; Dere et al., 2015). However, the effect of ETF on the antioxidant system of insects is currently unknown. Therefore, this work will provide further information about the ecotoxicological characteristics of ETF on insects, using *Galleria mellonella* L. 1758 (Lepidoptera: Pyralidae) as a model organism for entomological studies.

Galleria mellonella is a well-known model organism and system for ecotoxicological, ecophysiological and immunological investigations (Uçkan et al., 2008, 2011a, b; Altuntaş et al., 2012; Büyükgüzel et al., 2010, 2013; Maguire et al., 2016). It is also known that *G. mellonella* is an excellent model organism which can be used instead of mammalian species for *in vivo* toxicity of environmental pollutants and pathogenicity studies (Maguire et al., 2016). In comparison to other mammalian model organism and invertebrate models, rearing *G. mellonella* larvae in the laboratory is easier and faster

(Cook & McArthur, 2013; Maguire et al., 2016). In addition, large hemolymph sample volumes can be obtained from *G. mellonella* larvae for the measure of the physiological state of the internal environment of the insect. Any changes in the activity of antioxidant enzymes in the larval hemolymph profile resulting from ETF exposure would give us valuable information about insect physiology and biochemistry (Altuntaş et al., 2012, 2015a, b; Büyükgüzel et al., 2010, 2013). Thus, here we aimed to determine the ecotoxicological and ecophysiological effects of ETF on insects, using *G. mellonella* as a model organism, which is of great important for the risk assessment and management of ETF compounds. For this purpose, we determined if ETF had any toxicity affecting the survival of larvae and any effects on malondialdehyde (MDA) concentration and the activity of antioxidant enzymes, glutathione-S-transferase (GST), glutathione peroxidases (GPx), catalase (CAT) and superoxide dismutase (SOD), in the hemolymph of *G. mellonella* last instars.

Materials and Methods

Insects

Laboratory cultures of *G. mellonella* were maintained by feeding the insects with a modified Bronskill (1961) artificial diet including dark honeycomb (100 g), pollen (20 g), bran (340 g), glycerol (150 ml), honey (75 ml) and distilled water (75 ml). Colonies were kept at 25 ± 2°C, 60 ± 5% RH, and a photoperiod of 12L:12D h in Anadolu University, Eskişehir, Turkey.

Toxicity of ETF

ETF (Sigma, St. Louis, MO, USA) was dissolved in distilled water to prepare stock solution (1 mg/ml). ETF doses (250, 270, 300, 330, 350, 360, 370, 380, 400 and 430 µg/5 µl/larva) were prepared from the stock solution to determine the acute toxicity on larvae. Larvae of an approximate similar weight (0.14 ± 0.01 g) were selected from the insect culture for the force feeding treatment. Selected larvae were starved for 3 h, and then force fed with 5 µl of the ETF solution containing different doses or distilled water with a Hamilton syringe (22 gauges) (Dere et al., 2015). Each larva was exposed to a 2 g diet and observed daily to determine larval mortality in 30 d after treatment. Both experimental and control assays were performed with a total of 60 larvae (20 larvae in each of three replicates) for each dose. The lethal doses (LD_{10}, LD_{20}, LD_{30}, LD_{40}, LD_{50}, LD_{95} and LD_{99}) of ETF application were determined by probit analysis using the SPSS software (IBM IBM, Armonk, NY, USA) at 95% confidence levels.

Sample preparation

MDA concentration and antioxidant enzyme activities in hemolymph of last instars were carried out with doses below the upper limit (95% confidence levels) of LD_{50} that were determined for ETF (0, 250, 300, 330 and 360 µg/5 µl/larva) in toxicological studies. To collect hemolymph from force fed larvae exposed to different doses of ETF or distilled water at 24 and 48 h after treatment, 10 larvae (0.16 ± 0.01 g) were used in each analysis and 10 µl of hemolymph was collected. Each larvae was kept on ice for 5 min for anesthesia and were sterilized with a cotton ball containing 70% ethanol, subsequently hemolymph samples were collected into micro centrifuge tubes (0.5 ml) containing 1 µg 1-phenyl-2-thiourea and cold homogenization buffer (1:2 v/v) by removing the third pair proleg. All samples were frozen at -80°C until used. Before all analyses, samples were homogenized according to Altuntaş (2015a). All assays were repeated four times.

Assays of malondialdehyde concdentration and antioxidant enzyme activities

MDA analysis was performed according to kit protocol based on measuring the reaction of MDA with thiobarbituric acid at 95°C (Cayman Chemical, Ann Arbor, MI, USA) and this acidic reaction was monitored at 530 nm using a microtiter plate reader (Spectra Max M2). The concentration of MDA was calculated as the nmol/mg protein using the extinction coefficient 1.56 x 10^5 M^{-1} cm^{-1}.

Total GST activity in larval hemolymph was assayed using kit protocol (Cayman Chemical) by following the principle of 1-chloro-2,4-dinitrobenzene (CDNB), a substrate, and glutathione (GSH) conjugate formation. The increase in absorbance activity was monitored at 340 nm for 5 min with a microtiter plate reader and specific activity was defined as conjugated 1 nmol CDNB with reduced GSH/min/mg protein at 25°C according to the extinction coefficient of 0.00503 μM^{-1} cm^{-1}.

The activity of GPx was determined using a commercially kit protocol (Cayman Chemical). This kit measures the oxidation rate of nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm for 5 min in a microtiter plate reader. Specific activity of GPx was calculated as nmol/min/mg protein according to 0.00622 μM^{-1} cm^{-1} extinction coefficient value.

The assay of CAT activity is based on determining the H_2O_2 decomposition at 240 nm for 3 min (Chance & Maehly, 1995). Decreasing absorbance was recorded in ultraviolet-visible spectrophotometer (Shimadzu UV-1601, Tokyo, Japan) and results were expressed as hydrolysis of 1 mmol H_2O_2/minute/mg protein using e240 = 0.0394 $mM^{-1}cm^{-1}$.

SOD assay was performed by 2-(4-iodophenyl)-3-(4-nitrophenyl)-5-phenyltetrazolium chloride reacting with superoxide radicals, using xanthine and xanthine oxidase at 450 nm (Cayman Chemical). One unit of total activity of SOD (U/mg protein) was calculated by the quantity of enzyme needed to cause 50% inhibition of the superoxide radicals in one mg protein.

To determine MDA amount and antioxidant enzyme activities, Bradford (1976) method was performed to measure protein concentration in homogenates. Bovine serum albumin was also used to prepare the standard curve. All analyses were repeated four times using 10 larvae per treatment.

Statistics

All data was normally distributed. Therefore, one-way analysis of variance tests was performed to compare the normally distributed means for MDA level and antioxidant enzyme activities. To define significant differences among means, Tukey's Honestly Significant Difference (HSD) post hoc tests were used. Furthermore, time related changes in enzyme activities and MDA level (24 and 48 h) were determined with t-tests. SPSS program was carried out for all statistical analyses (SPSS, 2010). Means were considered statistically significant when $P \leq 0.05$.

Results

Toxicity of ETF

The ETF-treated larvae of *G. mellonella* exhibited toxic symptoms in a dose-dependent manner (Table 1). The ETF-treatment significantly decreased larval survival ≥50% beyond 360 µg/5 µl. LD_{50} and LD_{90} doses of ETF-treated larvae were determined as 344 (95% confidence limits, 331-361) and 419 (95% confidence limits, 392-458) µg/5 µl/larva, respectively (X^2 = 24.1, df = 8, P = 0.002). A 100% mortality was recorded at the highest concentration tested of 430 µg/5 µl. According to probability doses of ETF obtained from probit analysis, we used the doses ≤LD_{50} (250, 300, 330 and 360 µg/5 µl/larva) for analysis of the MDA level and antioxidant enzyme activities in larval hemolymph.

Table 1. Mortality and lethal doses of ETF (µg/5 µl/larva) on force fed *Galleria mellonella* larvae

ETF Doses (µg/5 µl)	*No. of exposed larvae (n=60)	No. of dead larvae	Lethal Doses (µg/5 µl/larva)			
Control	60	0	Lethal Doses	Probability Doses	95 % Confidence limits**	
250	60	2			Upper	Lower
270	60	6				
300	60	14	LD_{10}	283	257	300
330	60	20	LD_{20}	303	281	317
350	60	24	LD_{30}	318	300	331
360	60	30	LD_{40}	331	316	344
370	60	36	LD_{50}	344	331	361
380	60	50	LD_{70}	373	359	393
400	60	54	LD_{90}	419	397	458
430	60	60	LD_{99}	443	415	495

***** All assays were designed with a total of 60 larvae (20 larvae in each of three replicates) for each dose.
****** Values are displayed with lower and upper confidence limits, Probit = -38.294 + 15.095 X doses (doses are transformed using the base 10 logarithm).

Effects on MDA level

MDA levels in larval hemolymph of the ETF force fed larvae (doses ≤LD_{50}) differed depending on dose and time (Figure 1). MDA levels of controls were 2.77 and 2.39 nmol/mg protein at 24 and 48 h, respectively. The ETF treatment had the most significant effect on MDA level with more than 80% increase in doses >250 µg/5 µl when compared to the control at 24 h ($F = 28.9$; $df = 4, 15$; $P < 0.001$). Similar changes were also detected at 300 and 330 µg/5 µl except for 360 µg/5 µl at 48 h following treatments ($F = 26.3$; $df = 4, 15$; $P < 0.001$). The exposure to ETF in diet did not significantly change the level of MDA in larval hemolymph at doses 250 and 300 µg/5 µl (t-test, $P>0.05$). However, it decreased considerably from 24 to 48 h at doses of 330 and 360 µg/5 µl (t-test, $P<0.05$).

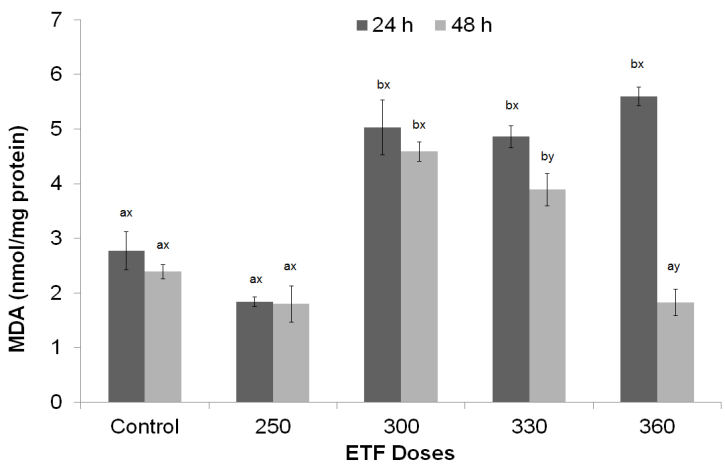

Figure 1. MDA content in the hemolymph of control and ETF-treated last instars. Vertical bars represent the mean ± standard error per replicate (n = 40). Statistically significant differences are indicated with letters a-b among groups at 24 or 48 h ($P < 0.05$, Tukey-HSD test) and x-y between two time points at the same dose ($P < 0.05$, t-test).

Effects on antioxidant enzyme activities

GST activity was 4.55 (24 h) and 5.31 (48 h) nmol/min/mg protein in larval hemolymph of the control group. GST activities in hemolymph of larvae did not change in tested doses of ETF except for 360 µg/5 µl 24 h when compared to the control (F= 51.5; df= 4, 15; P<0.001). On the other hand, GST activity increased nearly two-fold at all ETF doses at 48 h after treatment when compared to the control (F = 19.759; df = 4, 15; P < 0.001). Data indicated that GST activity in control and all assay groups were significantly higher at 48 h than at 24 h, except for 360 µg/5 µl (t-test, P < 0.05, Figure 2).

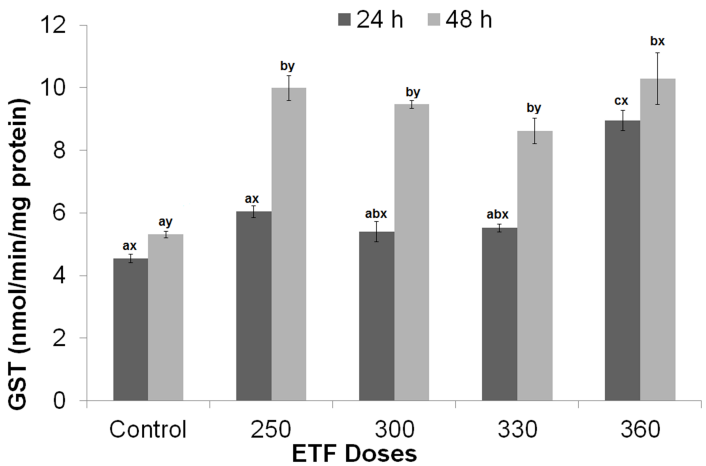

Figure 2. GST activity of larval hemolymph of *Galleria mellonella* force fed with different concentrations of ETF. Vertical bars represent the mean ± standard error per replicate (n = 40). Statistically significant differences are indicated with letters a-c among groups at 24 or 48 h (P < 0.05, Tukey-HSD test) and x-y between two time points at the same dose (P < 0.05, t-test).

EFT exposure significantly increased GPx activity in larval hemolymph at 330 and 360 µg/5 µl doses at 24 (F = 7.69; df = 4, 15; P<0.001) and 48 h (F = 7.88; df = 4, 15; P < 0.001) after treatment when compared to the control and other doses (Figure 3). In particular, the highest activity occurred at 330 µg/5 µl ETF (0.96 and 0.57 nmol/mg protein/min, respectively) at 24 and 48 h. Furthermore, GPx activities of all groups nearly halved at 48 h compared to the activities at 24 h after treatment (t tests, P < 0.05, Figure3).

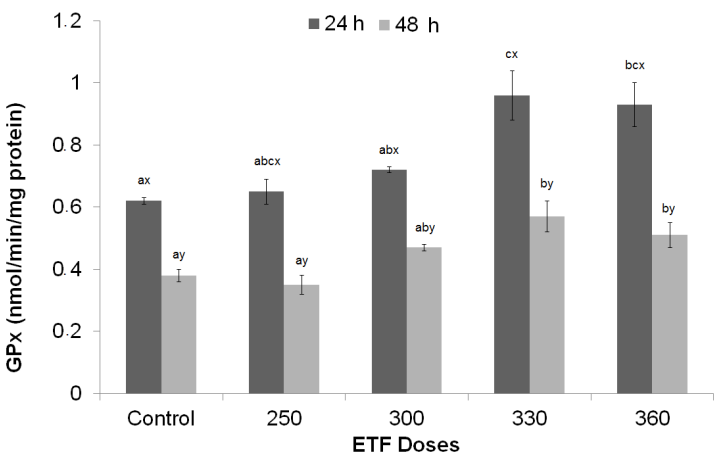

Figure 3. GPx activity of larval hemolymph of *G. mellonella* force fed with different concentrations of ETF. Vertical bars represent the mean ± standard error per replicate (n=40). Statistically significant differences are indicated with letters a-c among groups at 24 h or 48 h (P<0.05, Tukey-HSD test) and x-y between two time points at the same dose (P<0.05, t-test).

CAT activity in control was 0.45 and 0.49 mmol/min/mg protein at 24 and 48 h, respectively, however exposure to ETF caused nearly twice the activity of CAT in comparison with that of control at 24 ($F = 13.3$; $df = 4, 15$; $P < 0.001$) and 48 h ($F = 46.5$; $df = 4, 15$; $P < 0.001$). Time dependent changes between 24 and 48 h in CAT activities were not significant in the control and all experimental groups, except for 250 µg/5 µl (t-tests, $P > 0.05$, Figure 4).

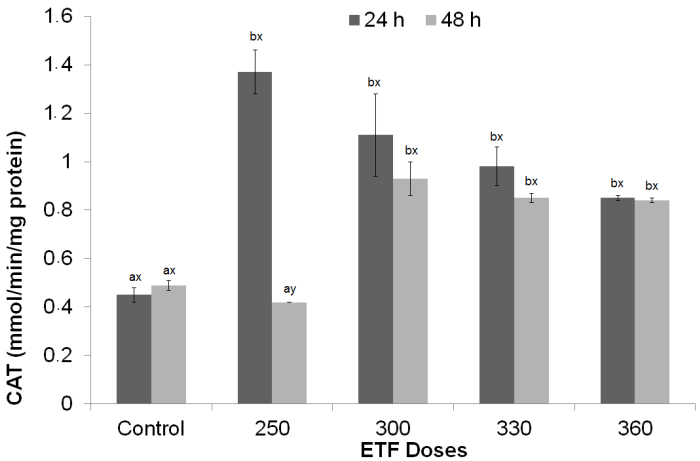

Figure 4. CAT activity of larval hemolymph of *Galleria mellonella* force fed with different concentrations of ETF. Vertical bars represent the mean ± standard error of per replicate (n = 40). Statistically significant differences are indicated with letters a-b among groups at 24 or 48 h ($P < 0.05$, Tukey-HSD test) and x-y between two time points at the same dose ($P < 0.05$, t-test).

SOD activities of control were 0.54 and 0.15 U/mg protein at 24 and 48 h, respectively. Force feeding with ETF increased the activity of SOD five-fold at 250 µg/5 µl at 24 h compared to the control. Significant elevations were also evident at doses >250 µg/5 µl ($F = 44.6$; $df = 4, 15$; $P < 0.001$). Similarly, a three-fold elevation of SOD activity in larval hemolymph at 300 µg/5 µl ETF exposure was recorded at 48 h compared to the control ($F = 142$; $df = 4, 15$; $P < 0.001$). However, these increased activities in all ETF doses did not exhibit a dose-dependent response at 24 and 48 h, because SOD activity decreased again at higher doses of ETF compared to other doses. SOD activities in larval hemolymph were considerably lower for both untreated and ETF-treated groups at 48 h after treatment compared to those at 24 h (t tests, $P < 0.05$, Figure 5).

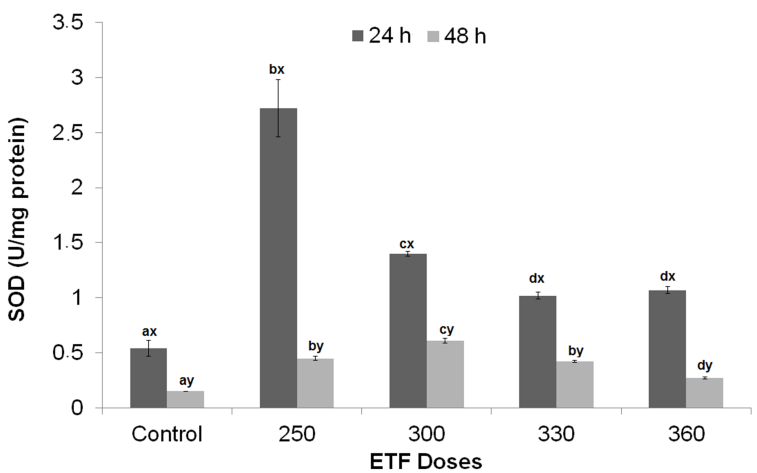

Figure 5. SOD activity of larval hemolymph of *Galleria mellonella* force fed with different concentrations of ETF. Vertical bars represent the mean ± standard error per replicate (n = 40). Statistically significant differences are indicated with letters a-d among groups at 24 or 48 h ($P < 0.05$, Tukey-HSD test) and x-y between two time points at the same dose ($P < 0.05$, t-test).

Discussion

Several studies have reported that exposure of ETF to plants indirectly induces plant defense responses and increase resistance or tolerance of plants against insect pests (Henneberry et al., 1988; Stotz et al., 2000). ETF has been shown to be nontoxic to shrimp, and slightly toxic to estuarine/marine mollusks. It is also practically nontoxic to cold water fish, and nontoxic to slightly toxic to warm water fish and freshwater invertebrates. Other studies on rats showed that ETF has slight acute toxicity to mammals when applied orally (Haux et al., 2000, 2002; Al-Twaty, 2006; Abd El Raouf & Girgis, 2011; Anant & Avinash, 2012). Wang et al. (2011) reported that acute toxicity of ETF to the *Daphnia magna* Straus, 1820 embryos had an EC_{50} range of 125-130.5 mg/l. Despite previous studies, the results presented here are the first detailed report of the toxicological effects of ETF on insects. Mortality data, obtained from the toxicity test and probit analysis showed that ETF has considerable acute toxicity to *G. mellonella* larvae. Due to the high insecticidal potential (LD_{50} dose = 72 µg/µl) of ETF, we investigated the potential effects of ETF on the fundamental physiological processes of *G. mellonella.* It is of great importance to discover the effects of environmental chemicals, such as ETF, on the biochemical and physiological response mechanisms of insects. It is also known that activities of antioxidant enzymes can be stimulated by oxidative stress state and these adaptation mechanisms are more important for organophosphate pesticide or xenobiotic-induced stress conditions in insects (İçen et al., 2005; Dere et al., 2015; Erdem & Büyükgüzel, 2015).

We consider that exposure to doses of ETF ≤LD_{50} caused an oxidative stress in force fed larvae because MDA level in hemolymph of last instars increased at all doses of ETF except for 360 µg/5 µl at 48 h (Figure 1). It is also known that MDA is a lipid peroxidation product and used for as biological indicator of oxidative stress in insects (Ahmad, 1995; Hyrsl et al., 2007). On the other hand, the MDA level decreased to control level again at higher doses of ETF after 48 h. In addition, a considerable decrease was recorded between 24 and 48 h at 330 and 360 µg/5 µl. This decrease may be associated with the increasing activity of GST with time and ETF doses (Figure 2), because, GST, a phase II detoxifying enzyme, has an important role in the cellular detoxification of stressors in insects (Hyrsl et al., 2007; Oruc, 2011; Erdem & Büyükgüzel, 2015; Altuntaş, 2015a). Therefore, the increase in GST activity may be related to an inhibition of the lipid peroxidation process and physiological response mechanism against ETF toxicity for cellular detoxification. These results are consistent with the findings of Altuntaş (2015a). In that study, the author reported that GST activity in hemolymph of *G. mellonella* larvae increased at low doses of GA_3 treatment. Similar results have also been reported with GST activity by organophosphate insecticides and other PGRs in vertebrate animals and insects (Yu, 2004; Hyrsl et al., 2007; Oruc, 2011; Tuluce & Çelik, 2006). We assume that GST activity can be used as a biomarker to evaluate ecotoxicological properties of ETF for insects.

Considerable elevation in the activity of GPx at 330 and 360 µg/5 µl (LD_{50}) of ETF at both times tested (Figure 3) may be an attempt to counteract the elevation of MDA level as a defense mechanism against the accumulation of lipid peroxidation products in the cells (Hemming & Lindroth, 2000; Fahmy, 2012). This is because, GPx regulates hydrogen peroxides and lipid hydroperoxides using reduced glutathione (Peric-Mataruga et al., 1997). However, previous studies revealed low GPx activity in insects including lepidopteran species (Ahmad et al., 2005; Erdem & Büyükgüzel, 2015). Furthermore, it is reported that this deficiency in the activity of GPx is supplemented by peroxidase activity (GSTPx) of GST (Peric-Mataruga et al., 1997) and higher CAT activity. Therefore, these previous findings are consistent with our data on to this higher activity at GST and CAT.

In this study, the important finding was a substantial increase in CAT and SOD activities in the hemolymph of last instars at all ETF doses at 24 h after treatment (Figures 4 and 5). This result was also similar to that of 48 h except at the lower doses of ETF for CAT activity. However, no change was detected in CAT activity at 24 and 48 h while SOD activity decreased in control and at all doses of ETF at 48 h. In particular, an important decline was observed at LD_{50} dose at 48 h in respect of other doses. These substantial changes in the activities of SOD and CAT, the primary enzymes against ROS-mediated toxicity in all living organisms, may be attributed to several reasons. Firstly, the elevated CAT activity may be associated with scavenging of hydrogen peroxides by CAT. The results obtained are in agreement

with previous findings reported for some lepidopteran species treated with various pesticides and PGRs (Krishnan & Kodrik, 2006; Aslantürk et al., 2011; Büyükgüzel et al., 2013; Altuntaş, 2015a, b). The second reason may be that the increased SOD activity in ETF treated larvae at 24 h caused an increase in hydrogen peroxide concentration and a further elevation in CAT activity in response. It has been found that CAT activity is normally higher in insects than mammals (Ahmad & Pardini, 1990). Therefore, the third assumption is that the relative low levels of SOD increase with respect to treatment time and higher activity of GPx at high doses of EFT may affect hydrogen peroxide concentration. Collectively, the results presented here indicated that the elevated SOD activity at lower doses of ETF treatment may be derived from increasing hydrogen peroxide concentration, and as a result, CAT activity increased in larval hemolymph. Altuntaş (2015b) also determined that the total lipid, protein and glucose amount in hemolymph of *G. mellonella* last instars decreased following ETF treatment. ETF-induced stress conditions may cause the lipids and glucose to be used for cell repair, lipoprotein formation (Riberio et al., 2001) and the increased protein catabolism. This is because protein catabolism may be stimulated due to high energy demand under stress conditions (Sancho et al., 1998). Therefore, the current study demonstrated that treatment of larvae with $\leq LD_{50}$ ETF doses caused peroxidation of cellular lipids and increases in the activities of antioxidant enzymes.

In conclusion, this study showed that ETF has toxic effects on insects via biochemical and physiological alterations in a dose and time dependent manner when received orally. These results obtained from model organism *G. mellonella,* provide reliable data which can be used as an index of the ecotoxicological and physiological significance of ETF in the context for insects. Consequently, consideration is needed to avoid the reckless use of this type of chemicals at high concentrations without evaluating technical procedures as such use might cause disruption of the ecological balance.

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References

Abd El Raouf, A. & S. M. Girgis, 2011. Mutagenic, teratogenic and biochemical effects of ethephon on pregnant mice and their fetuses. Global Veterinaria, 6 (3): 251-257.

Ahmad, S. & R. S. Pardini, 1990. Mechanisms for regulating oxygen toxicity in phytophagous insects. Free Radical Biology and Medicine, 8: 401-413.

Ahmad, S., 1995. Oxidative stress from environmental pollutants. Archives of Insect Biochemistry and Physiology. 29: 135-157.

Ahmad, S., C. A. Pritsos, S. M. Bowen, C. R. Heisler, G. J. Blomquist & R. S. Pardini, 2005. Subcellular distrubition and activities of superoxide dismutase, catalase, glutathione peroxidase, and glutathione reductase in the southern armyworm, *Spodoptera eridania*. Archives of Insect Biochemistry and Physiology, 7: 173-18.

Altuntaş, H., A. Y. Kılıç, F. Uçkan & E. Ergin, 2012. Effects of gibberellic acid on hemocytes of *Galleria mellonella* L. (Lepidoptera: Pyralidae). Environmental Entomology, 41 (3): 688-696.

Altuntaş, H., F. Uçkan, A. Y. Kılıç, & E. Ergin, 2014. Effects of gibberellic acid on hemolymph free amino acids of *Galleria mellonella* (Lepidoptera: Pyralidae) and endoparasitoid *Pimpla turionellae* (Hymenoptera: Ichneumonidae). Annals of the Entomology Society of America, 107 (5): 1000-1009.

Altuntaş, H., 2015a. Determination of gibberellic acid (GA_3)-induced oxidative stress in a model organism *Galleria mellonella* L. (Lepidoptera: Pyralidae). Environmental Entomology (Physiology), 44 (1): 100-105, DOI: 10.1093/ee/nvu020.

Altuntaş, H., 2015b. Effects of ethephon on the hemolymph metabolites of the greater wax moth *Galleria mellonella* L. (Lepidoptera: Pyralidae). Acta Physica Polonica A, 128: 182-183, DOI: 10. 12693/AphysPolA.128.B-182.

Al-Twaty, N. H. A., 2006. Mutagenic effects of ethephon on albino mice. Journal of Biological Sciences, 6 (6): 1041-1046.

Anant, J. D. & B. G. Avinash, 2012. Modulation in serum biochemicals in European rabbit, *Oryctolagus cuniculus* (Linn.) exposed to ethephon. European Journal of Experimental Biology, 2 (3): 794-799.

Aslantürk, A., S. Kalender, M. Uzunhisarcıklı & Y. Kalender, 2011. Effects of methidathion on antioxidant enzyme activities and malondialdehyde level in midgut tissues of *Lymantria dispar* (Lepidoptera) larvae. Journal of the Entomological Research Society, 13 (3): 27-38.

Bhadoria, P., M. Mahindra, V. Bahrioke & A. S. Bhadoria, 2015. Effect of ethephon on the liver in albino rats: a histomorphometric study. Biomedical Journal, 38: 421-427.

Bui, Q. Q., 2007. Ethephon and jackfruit, Palo Alto, California. 231pp.

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

Bronskill, J. F., 1961. A cage to simplify the rearing of thegreater wax moth, *Galleria mellonella* (Pyralidae). Journal of Lepidopterists' Society, 15: 102-104.

Büyükgüzel, E., P. Hyrsl & K. Büyükgüzel, 2010. Eicosanoids mediate hemolymph oxidative and antioxidative response in larvae of *Galleria mellonella* L. Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 156: 176-83.

Büyükgüzel, E., K. Büyükgüzel, M. Snela, M. Erdem, K. Radtke, K. Ziemnicki, & Z. Adamski, 2013. Effect of boric acid on antioxidant enzyme activity, lipid peroxidation and ultrastructure of midgut and fat body of *Galleria mellonella*. Cell Biology and Toxicology, 29: 117-129.

Chance, B. & A. C. Maehly, 1955. Assay of catalase and peroxidases. Methods Enzymology, 2: 764-775.

Cook, S. M. & J. D. McArthur, 2013. Developing Galleria mellonella as amodel host for human pathogens. Virulence, 4 (5): 350–353.

Dere, B., H. Altuntaş, & Z. U. Nurullahoğlu, 2015. Insecticidal and oxidative effects of azadirachtin on the model organism *Galleria mellonella* l. (Lepidoptera: Pyralidae). Archives of Insect Biochemistry and Physiology, DOI:10.1002/arch.21231.

Emre, İ., T. Kayış, M. Coşkun, O. Dursun & H. Y. Cogun, 2013. Changes in antioxidative enzyme activity, glycogen, lipid, protein, and malondialdehyde content in cadmium-treated *Galleria mellonella* larvae. Annals of the Entomology Society of America, 106 (3): 371-377.

Erdem, M. & E. Büyükgüzel, 2015. The Effects of xanthotoxin on the biology and biochemistry of *Galleria mellonella* L. (Lepidoptera: Pyralidae). Archives of Insect Biochemistry and Physiology, DOI: 10.1002/arch.21236.

Fahmy, N. M., 2012. Impact of two insect growth regulators on the enhancement of oxidative stress and antioxidant efficiency of the cotton leaf worm, *Spodoptera littoralis* (Biosd.). Egyptian Academic Journal of Biological Sciences, 5 (1): 137-149.

Felton, G. W. & C. B. Summers, 1995. Antioxidant systems in insects. Archives of Insect Biochemistry and Physiology, 29: 187-97.

Gupta, G., S. R. Yadav & A. K. Bhattacharya, 2009. Influence of synthetic plant growth substances on the survivorship and developmental parameters of *Spilarctia obliqua* Walker (Lepidoptera: Arctiidae). Journal of Pesticide Science, 82: 41-46.

Haux, J. E., G. B. Quistad & J. E. Casida, 2000. Phosphobutyrylcholinesterase: Phosphorylation of the Esteratic Site of Butyrylcholinesterase by Ethephon [(2- Chloroethyl) phosphonic acid] Dianion. Chemical Research in Toxicology, 13: 646-651.

Haux, J. E., O. Lockridge & J. E. Casida, 2002. Specificity of ethephon as a butyrylcholinesterase inhibitor and phosphorylating agent. Chemical Research in Toxicology, 15: 1527-1533.

Hemming, J. D. C. & R. Lindroth, 2000. Effects of phenolic glycosides and protein on gypsy moth (Lepidoptera : Lymantriidae) and forest tent caterpillar (Lepidoptera : Lasiocampidae) performance and detoxification activities. Environmental Entomology, 2: 1108-1115.

Henneberry, T. J., T. Meng, W. D. Hutchison, L. A. Bariola & B. Deeter, 1988. Effects of ethephon on boll weevil (Coleoptera: Curculionidae) population development, cotton fruiting, and boll opening. Journal of Economic Entomology, 81 (2): 628-633.

Hussain, I., A. Saeed, A. Muhammad & A. Rashid, 2015. Ethephon application at kimri stage accelerates the fruit maturation period and improves phytonutrients status (Hillawi and Khadrawi (c.v.)) of date palm fruit. Pakistan Journal of Agricultural Sciences, 52 (2): 415−423.

Hyrsl, P., E. Büyükgüzel & K. Büyükgüzel, 2007. The effects of boric acid-induced oxidative stress on antioxidant enzymes and survivorship in *Galleria mellonella*. Archives of Insect Biochemistry and Physiology, 66: 23-31.

İçen, E., F. Armutçu, K. Büyükgüzel & A. Gürel, 2005. Biochemical stress indicators of greater wax moth exposure to organophosphorus insecticides. Journal of Economic Entomology, 98: 358-366.

Kaur, R. & P. J. Rup, 2003. Influence of four plant growth regulators on development of the melon fruit fly, *Bactrocera cucurbitae* (Coquillett). Insect Science and Its Application, 23: 121-125.

Krishnan, N. & D. Kodrik, 2006. Antioxidant enzymes in *Spodoptera littoralis* (Boisduval): Are they enhanced to protect gut tissues during oxidative stress? Journal of Insect Physiology, 52: 11-20.

Maguire, R., O. Duggan, & K. Kavanagh, 2016. Evaluation of *Galleria mellonella* larvae as an in vivo model for assessing the relative toxicity of food preservative agents. Cell Biology and Toxicology, DOI: 10.1007/s10565-016-9329-x.

Oruc, E., 2011. Effects of diazinon on antioxidant defense system and lipid peroxidation in the liver of *Cyprinus carpio* (L.). Environmental Toxicology, 26: 571-578.

Peric´-Mataruga, V., D. Blagojevic, M.B. Spasic, J. Ivanovic & M. Jankovic-Hladni, 1997. Effect of the host plant on the antioxidative defence in the midgut of *Lymantria dispar* L. caterpillars of different population origins. Journal of Insect Physiology, 43: 101-106.

Riberio, S., J. P. Sousa, J. A. A. Nogueira & A. M. V. M. Soares, 2001. Effect of endosulfan and parathion on energy reserves and physiological parameters of the terrestrial isopod *Porcellio dilatatus*. Ecotoxicology and Environmental Safety, 49: 131-138.

Sancho, E., M. D. Ferrando, C. Fernandez & E. Andreu, 1998. Liver energy metabolism of *Anguilla anguilla* after exposure to fenitrothion. Ecotoxicology and Environmental Safety, 41: 68-175.

SPSS, Inc., SPSS 18.0 Statistics. SPSS, Chicago, IL (2010).

Stotz, H. U., B. R. Pittendrigh, J. Kroymann, K. Weniger, J. Fritsche, A. Bauke & T. Mitchell-Olds, 2000. Induced plant defense responses against chewing insects ethylene signaling reduces resistance of arabidopsis against egyptian cotton worm but not diamondback moth. Plant Physiology, 124: 1007-1017.

Tuluce, Y. & I. Celik, 2006. Influence of subacute and subchronic treatment of abcisic acid and gibberellic acid on serum marker enzymes and erythrocyte and tissue antioxidant defense systems and lipid peroxidation in rats. Pesticide Biochemistry and Physiology, 86: 85-92.

Uçkan, F., A. Tüven, A. Er & E. Ergin, 2008. Effects of gibberellic acid on biological parameters of the larval endoparasitoid *Apanteles galleriae* (Hymenoptera: Braconidae). Annals of the Entomology Society of America, 101: 593-597.

Uçkan, F., İ. Haftacı & E. Ergin, 2011a. Effects of indol-3-acetic acid on biological parameters of the larval endoparasitoid *Apanteles galleriae* (Hymenoptera: Braconidae). Annals of the Entomology Society of America, 104 (1): 77-82.

Uçkan, F., Z. Öztürk, H. Altuntaş & E. Ergin, 2011b. Effects of gibberellic acid (GA_3) on biological parameters and hemolymph metabolites of the pupal endoparasitoid *Pimpla turionellae* (Hymenoptera: Ichneumonidae) and its host *Galleria mellonella* (Lepidoptera: Pyralidae). Journal of the Entomological Research Society, 13: 1-14.

Uçkan, F., H. K. Soydabaş & R. Özbek, 2014. Effect of indol-3 acetic acid on the biochemical parameters of *Achoria grisella* hemolymph and *Apanteles galleriae* larva. Pakistan Journal of Biotechnology, 11 (2): 163-171.

Uçkan, F., R. Özbek & E. Ergin, 2015. Effects of indol-3-acetic acid on the biology of *Galleria mellonella* and its endoparasitoid *Pimpla turionellae*. Belgian Journal of Zoology, 145 (1): 49-58.

Wang, K. S., C. Y. Lu & S. H. Chang, 2011. Evaluation of acute toxicity and teratogenic effects of plant growth regulators by *Daphnia magna* embryo assay. Journal of Hazardous Materials, 190: 520-528, DOI:10.1016/j.jhazmat.2011.03.068.

Yu, S. J., 2004. Induction of detoxification enzymes by triazine herbicides in the fall armyworm, *Spodoptera frugiperda*. Pesticide Biochemistry and Physiology, 80: 113-122.

Zhang, L., S. Li, X. Liu, C. Song & X. Liu, 2012. Effects of ethephon on physicochemical and quality properties of kiwifruit during ripening. Postharvest Biology and Technology, 65: 69−75.

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Ichneumonidae (Hymenoptera) fauna of Gelincik Mountain Natural Park (Isparta, Turkey)[1]

Gelincik Dağı Tabiat Parkı (Isparta, Turkey) Ichneumonidae (Hymenoptera) faunası

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Summary

This study was carried out to determine the Ichneumonidae (Hymenoptera) fauna of Gelincik Mountain Natural Park. Six stations were chosen with different altitude and floristic structure and were conducted between April 2010 and October 2012. Specimens were collected via sweeping net and malaise traps. A total of 145 samples were 47 species belonging to 43 genera were identified. Of these, 14 are new records for Ichneumonidae fauna for Turkey. These species are: *Arotrephes perfusor* (Gravenhorst, 1829)*, Barichneumon peregrinator* (Linnaeus, 1758), *Charitopes gastricus* (Holmgren, 1868), *Dicaelotus pumilus* (Gravenhorst, 1829), *Diphyus trifasciatus* (Gravenhorst, 1829), *Gelis gallicator (*Aubert, 1971)*, Herpestomus arridens* (Gravenhorst, 1829), *Ichneumon caloscelis* Wesmael, 1845, *Lissonota proxima* Fonscolombe, 1854, *Mesoleius melanoleucus* (Gravenhorst, 1829), *Odontocolon quercinus* (Thomson, 1877), *Olesicampe fulcrans* (Thomson, 1887)*, Symplecis invisitata* Rossem, 1981 and *Tropistes falcatus* (Thomson, 1884). Also, three genera, *Arotrephes*, *Charitopes* and *Tropistes* are new records for Turkey.

Keywords: Fauna, Gelincik Mountain Natural Park, Hymenoptera, Ichneumonidae, new records

Özet

Bu çalışma Gelincik Dağı Tabiat Parkı Ichneumonidae faunasını araştırmak üzere gerçekleştirilmiştir. Farklı yükseklik ve floristik yapıya sahip altı istasyon seçilmiş ve çalışmalar Nisan 2010-Ekim 2012 tarihleri arasında yapılmıştır. Örnekler atrap ve malaise tuzağı ile toplanmıştır. Toplam 145 birey toplanmıştır ve 43 cinse bağlı 47 tür teşhis edilmiştir. Bu türler arasından 14 tür Türkiye Ichneumonidae faunası için yeni kayıttır. Bu türler: *Arotrephes perfusor* (Gravenhorst, 1829)*, Barichneumon peregrinator* (Linnaeus, 1758), *Charitopes gastricus* (Holmgren, 1868), *Dicaelotus pumilus* (Gravenhorst, 1829), *Diphyus trifasciatus* (Gravenhorst, 1829), *Gelis gallicator (*Aubert, 1971)*, Herpestomus arridens* (Gravenhorst, 1829), *Ichneumon caloscelis* Wesmael, 1845, *Lissonota proxima* Fonscolombe, 1854, *Mesoleius melanoleucus* (Gravenhorst, 1829), *Odontocolon quercinus* (Thomson, 1877), *Olesicampe fulcrans* (Thomson, 1887)*, Symplecis invisitata* Rossem, 1981 ve *Tropistes falcatus* (Thomson, 1884). *Arotrephes*, *Charitopes* ve *Tropistes* cinsleri de Türkiye için yeni kayıttır.

Anahtar sözcükler: Fauna, Gelincik Dağı Tabiat Parkı, Hymenoptera, Ichneumonidae, yeni kayıtlar

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Introduction

Ichneumonidae is the largest hymenopteran family with 51 generally recognized subfamilies, 1 579 genera and 24 281 described species globally (Yu et al., 2012). The estimated total number of species varies from 60 000 (Townes, 1969; Wahl & Sharkey, 1993) to more than 100 000. Ichneumonids are distributed worldwide and play a significant role in natural and agricultural ecosystems as parasitoids of arthropods (Kopylov & Zhang, 2015).

Ichneumonids have been used successfully as biocontrol agents and given the largely undocumented fauna there is huge potential for their utilization in managed biocontrol programs (Ghahari et al., 2012).

Faunistic research on the family Ichneumonidae in Turkey started in the 19th century (Çoruh et al., 2016). In the catalog of Kolarov (1995) only 383 species are listed. The last decade new records have been added to this fauna. With this study, the number of Ichneumonidae species in Turkey increased to 1234.

Gelincik Mountain National Park is located in Isparta, Turkey, has a great potential in recreational terms with its rich natural and cultural resources. This area has a rich flora important both for its biodiversity as well as for including many ornamental, medicinal and aromatic plants with 24.6% endemism. Similarly, the area is important for reproduction, nutrition and survival for different animal species (Erduran & Cırık, 2011). To further our knowledge of this valuable natural resource, the aim of this study was to survey the Ichneumonidae fauna of Gelincik Mountain Natural Park.

Material and Methods

Sampling

Ichneumonidae samples were collected from April to October between 2010 and 2012. Six stations which have different altitude and floristic structure were chosen in Gelincik Mountain Natural Park in Isparta Province (Figure 1).

Figure 1. Sampling sites (stations) in Gelincik Mountain Natural Park.

Station I (38° 06.584' N 30° 44.837' E; 1100-1150 m): The vegetation is characterized by *Quercus coccifera.* Other plant species commonly encountered in the area are *Achillea* sp. L., *Aegilops geniculata* Roth., *Centaurea iberica* Trev. Ex Sprengel., *Juniperus oxycedrus* L., *Lamium maculatum* L. and *Trifolium repens* L.

Station II (38° 06.020' N 30° 44.112' E; 1250-1300 m): The vegetation consists of *Cedrus libani* A. Rich, *Lepidium draba* L., *Centaurea depressa* M. Bieberstein, *Convolvulus arvensis* L., *Crataegus orientalis* Pallas ex Bieb., *Geranium tuberosum* L*., Pinus nigra* Arnold, *Ranunculus ficaria* L., *Ranunculus cuneatus* Boiss. and *Trifolium davisii* Hossain.

Station III (38° 05.916' N 30° 43.776' E; 1300-1350 m): *Cedrus libani* A. Rich, *Pinus nigra* Arnold, and *Poa* sp. L. are dominant plants in the area. Other notable plant species are *Cirsium* sp. Miller, *Crataegus orientalis* Pallas ex Bieb., *Malva* sp. L., *Scutellaria* sp. L., *Sideritis* sp. L., *Stachys cretica* L. and *Vicia* sp. L.

Station IV (38° 05.869' N 30° 44.985' E; 1450-1500 m): The vegetation is characterized by *Cedrus libani* A. Rich, *Crataegus orientalis* Pallas ex Bieb., *Jasminum* sp. L. *Pinus nigra* Arnold, *Rosa dumalis* Bechst. and *Stachys cretica* L.

Station V (38° 05.604' N 30° 41.957' E; 1543-1550 m): The vegetation consists of *Achillea* sp. L. *Centaurea* sp. L., *Centaurea iberica* L., *Cotoneaster* sp. Medik., *Potentilla* sp. L. and *Sedum* sp. L.

Station VI (38 5,488' N 30 41,422 E; 1520 m): The vegetation is characterized by *Cedrus libani* A. Rich. and *Pinus nigra* Arnold.

Sampling and collection

Ichneumonidae specimens were sampled using malaise traps and sweep net. Samples were taken from malaise traps about every fifteen days. Ichneumonid species were separated from the other insects under a stereomicroscope in the laboratory. The samples collected by sweep net were taken into 70% ethanol solution in the plastic containers. Collected ichneumonids were taken to the laboratory to be mounted and labeled. All of the samples were lodged in the collection of the Department of Biology, Faculty of Arts and Sciences at Süleyman Demirel University, Isparta, Turkey.

Results and Discussion

Forty-seven species belonging to 43 genera were identified with 14 of these new records for Turkey. With this new records, the number of Ichneumonidae species in Turkey increased to 1234.

A list of the species is given below along with the collection date, locations, specimen numbers of each sex and distribution in Turkey. For newly recorded species, hosts, associated plants, general distributions are also given according to Yu et al*.,* 2012. Newly recorded species are indicated with an asterisk.

List of the species

Subfamily Anomaloninae

***Anomalon cruentatum* Geoffroy, 1785**

Material examined: GDTP, II. Station, 3.VII.2010, ♀, I. Station, 3.VII.2010, ♀, III. Station, 29.V.2010, ♀, VI. Station, 17.VI.2012, 2♀♀, 24.VI.2012, V. Station, ♀.

Distribution in Turkey: Afyon, Muğla (Kolarov et al., 2002) Isparta-Merkez-Kirazlıdere-Gönen (Gürbüz, 2004); Antalya, Bayburt, Bingöl, Diyarbakır, Erzincan, Erzurum, Iğdır, Kahramanmaraş, Kars (Çoruh et al., 2004), Adıyaman-Merkez, Batman-Hasankeyf, Diyarbakır-Çermik-Merkez, Elazığ- Yemişlik, Malatya-Yeşilyurt-Doğanyurt, Mardin-Savur (Akkaya, 2005), Isparta-Gölcük-Çünür (Buncukçu, 2008), Isparta-Kasnak Meşesi Tabiatı Koruma Alanı (Kırtay, 2008), Isparta-Davraz (Birol, 2010), Erzurum, Tunceli (Kolarov et al., 2014a), Bayburt, Kars, Erzurum (Çoruh & Kolarov, 2016).

***Barylypa uniguttata* (Gravenhorst, 1829)**

Material examined: GDTP, II. Station, 24.IV.2010, 2♂♂, III. Station, 7-14.V.2011, 3♀♀, 5♂♂, II. Station, 16-30.V.2011, 2♀♀, 2♂♂, IV. Station, 17.VI.2012, ♀.

Distribution in Turkey: Çanakkale (Kolarov et al., 1994), Isparta (Gürbüz et al., 2009a).

Subfamily Banchinae

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

Material examined: GDTP, II Station, 9.X.2010, 4♂♂, 24.XI.2010, ♀.

Distribution in Turkey: Erzurum (Pekel & Özbek, 2000); Diyarbakır-Merkez, Elazığ-Hazar, Mardin-Savur (Akkaya, 2005), Ordu (Kolarov et al., 2016).

****Lissonota (Lissonota) proxima* Fonscolombe, 1854**

Material examined: GDTP, II. Station, 9.X.2010, ♂, III. Station, 15.X.2012, ♀.

Diagnosis: First flagellomere segment four times as long as wide (Figure 2b). Mesosoma black. Legs red, trochanters black. Tarsal claws not pectinate (Figure 2c). Tegulae brown. Fore wing with areolet (Figure 2d). Radial vein (R3) beyond areolet straight or weakly curved. Second and third metasomal tergites completely reddish and punctated. Propodeum and hind coxae densely punctated (Figure 2e). Metasoma with red pattern (Figure 2a). Coxa, femur, tibia red.

Associated with plant: *Angelica sylvestris* L. (Apiales: Apiaceae), *Chaerophyllum aromaticum* L. (Apiales: Apiaceae).

General Distribution: Palearctic.

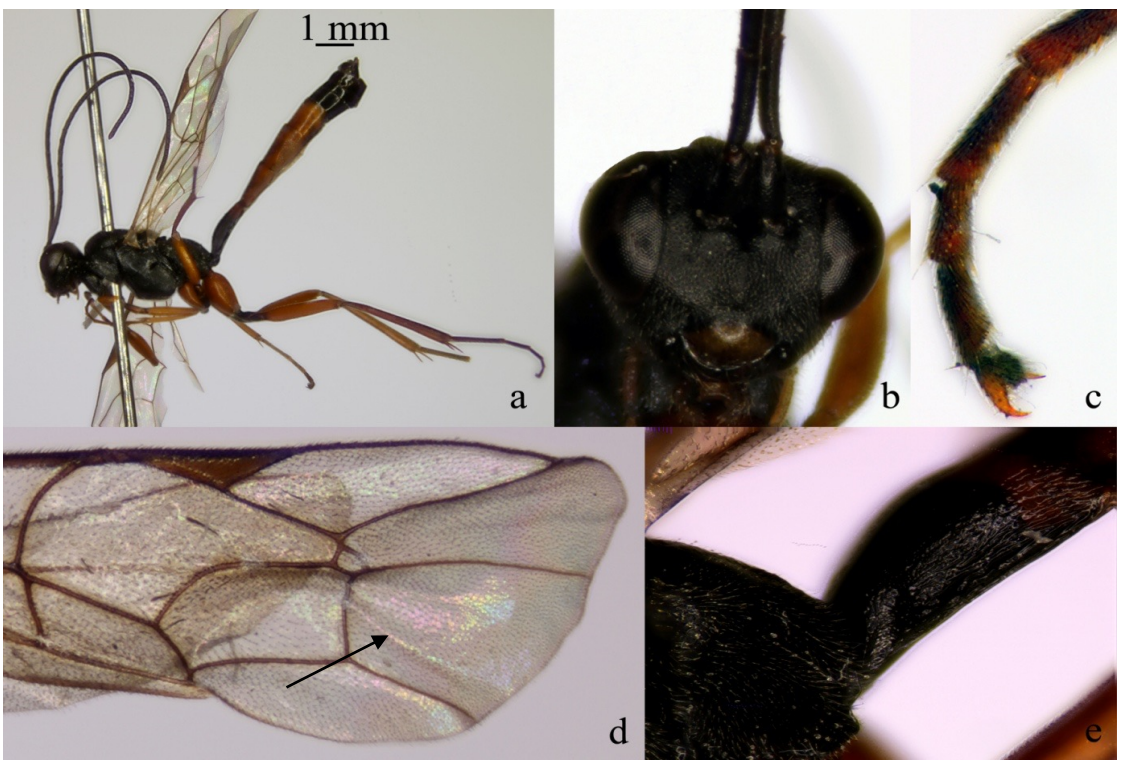

Figure 2. *Lissonota* (*Lissonota*) *proxima* a. habitus, lateral view, b. head, facial view, c. tarsus and tarsal claws, d. forewing with areolet and e. propodeum, first metasomal tergite.

Subfamily Campopleginae

***Campoletis viennensis* (Gravenhorst, 1829)**

Material examined: GDTP, III. Station, 3.VII.2010, ♀.

Distribution in Turkey: Adana (Kolarov & Beyaslan, 1995), Bayburt (Özbek et al., 2000, Çoruh et al., 2014b), Hatay (Çoruh et al., 2013), Bayburt (Çoruh & Kolarov, 2016).

***Cymodusa australis* (Smits van Burgst, 1913)**

Material examined: GDTP, II. Station, 27.V.2012, ♀.

Distribution in Turkey: Edirne (Kolarov & Beyarslan, 1995).

****Olesicampe fulcrans* (Thomson, 1887)**

Material examined: GDTP, III. Station, 24.IV.2010, 8♂♂, ♀, 19.V.2010, ♂.

Diagnosis: Eyes with inner margins not converging strongly ventrally (Figure 3b). Temple long and swollen. The lower tooth of mandible often longer than the upper. Base of mandible and scape yellow. Fore wing with areolet. Propodeal spiracles circular or elongate (Figure 3c). Metatrochanter conspicuous (Figure 3d). Metasomal tergites III-V red, femur and tibia red (Figure 3a).

General Distribution: Palearctic.

Figure 3. *Olesicampe fulcrans* a. habitus, lateral view, b. head, facial view, c. propodeum and first tergite and d. metatrochanter, lateral view.

Subfamily Cremastinae

***Pristomerus pallidus* (Kriechbaumer, 1884)**

Material examined: GDTP, I. Station, 27.V.2012, ♀.

Distribution in Turkey: Erzurum (Pekel & Özbek, 2000).

Subfamily Cryptinae

***Aritranis director* (Thunberg, 1824)**

Material examined: GDTP, II. Station , 29.V.2011, ♂, GDTP, III. Station, 19. V.2010, ♂, 29.V.2010, 2♂♂, 13.VI.2010, 18♂♂.

Distribution in Turkey: Isparta, Egirdir, Yalvaç, Burdur, Antalya (Gürbüz & Kolarov, 2008); Kasnak Meşesi Tabiatı Koruma Alanı-Isparta (Gürbüz et al., 2009b).

***Buathra tarsoleucos* (Schrank, 1781)**

Material examined: GDTP, II. Station, 12.VIII.2012, ♀.

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

***Cryptus tuberculatus* Gravenhorst, 1829**

Material examined: GDTP, III. Station, 29.V.2010 ♀, 13.VI.2010, ♀, I. Station, 13.VI.2010, ♂.

Distribution in Turkey: Locality not given (Sevidy, 1959), Tekirdağ (Kolarov & Yurtcan, 2008).

***Cryptus viduatorius* Fabricius, 1804**

Material examined: GDTP, III. Station, 24.IV.2010, 2♀♀, 3.VII.2010, ♀.

Distribution in Turkey: İstanbul (Kolarov, 1995); Isparta, Egirdir (Gürbüz & Kolarov, 2008); Kasnak Meşesi Tabiatı Koruma Alanı-Isparta (Gürbüz et al., 2009b), Erzurum (Çoruh & Çoruh, 2008; Çoruh et al., 2014b), Rize (Çoruh et al., 2014a), Erzurum (Çoruh & Kolarov, 2016; Çoruh et al., 2016).

***Myrmeleonostenus italicus* (Gravenhorst, 1829)**

Material examined: GDTP, III. Station, 29.V.2010, ♀.

Distribution in Turkey: Isparta, Antalya (Gürbüz & Kolarov, 2008); Kasnak Meşesi Tabiatı Koruma Alanı-Isparta (Gürbüz et al., 2009b); Erzincan (Çoruh et al., 2016).

***Stenarella domator* (Poda, 1761)**

Material examined: GDTP, III. Station, 31.VII.2010, ♀, II. Station, 12.VII.2012, ♂.

Distribution in Turkey: Belgrat Ormanları (Kolarov, 1995); Isparta, Egirdir (Gürbüz & Kolarov, 2008); Kasnak Meşesi Tabiatı Koruma Alanı-Isparta (Gürbüz et al., 2009b).

***Xylophrurus apum* (Thomson, 1873)**

Material examined: GDTP, II. Station, 13.VI.2010, ♀.

Distribution in Turkey: Afyon-Sultan Dağı (Özdemir & Güler, 2009).

**** Arotrephes perfusor* (Gravenhorst, 1829)**

Material examined: GDTP, II. Station, 1.VII.2012, ♀.

Diagnosis: Face shiny, Clypeus convex and black (Figure 4d). Antenna reddish brown. Legs red (Figure 4a). Notaulus not reaching to center of mesoscutum (Figure 4b). Tegula brownish. Wings normal. Areolet present. Second intercubitus (3rs-m) distinct. Second recurrent vein (2m-Cu) with two bullae (Figure 4c). Postpectal carina incomplete. Center of pronotum without carina. Second and third metasomal tergites black. Body black.

General Distribution: Western Palearctic.

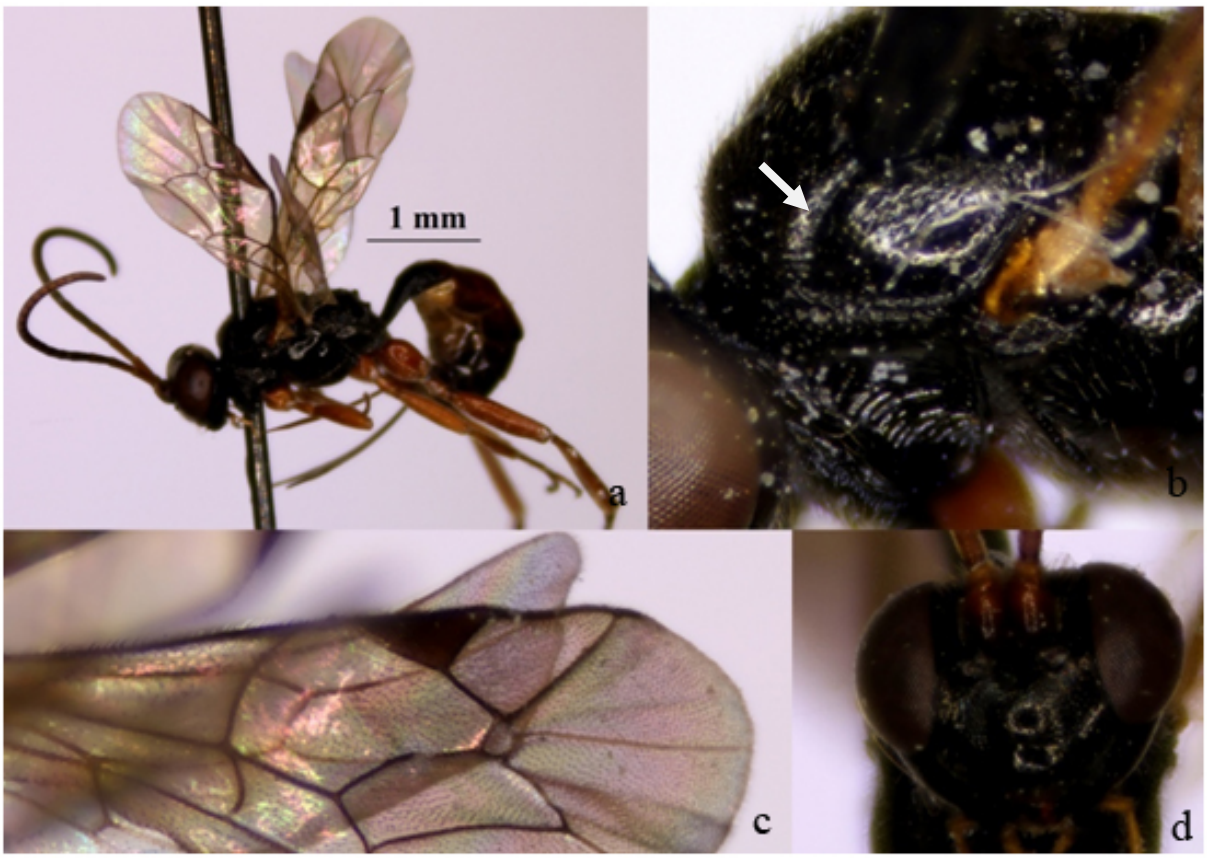

Figure 4. *Arotrephes perfusor* a. habitus, lateral view, b. view of Notaulus, c. forewing and d. head, facial view.

***Charitopes gastricus* (Holmgren, 1868)**

Material examined: GDTP, III. Station, 14.V.2011, ♀.

Diagnosis: Eyes without hairs. Antenna red-brown. Fifth flagellomere 1.7-1.9 times as long as wide (Figure 5b). 2. recurrent vein (2m-cu) complete, inclivous and with two bullae (Figure 5c). Body prolonged (Figure 5a). Mesoscutum mat. Tergites polished, red-brown (Figure 5d). Ovipositor sheath about 0.35-0.65 times as long as forewing. Ovipositor sheath about 1.1 times as long as metatibia.

General Distribution: Palearctic, Nearctic, Neotropical.

***Gelis areator* (Panzer, 1804)**

Material examined: GDTP, II. Station, 17.VI.2012, 2♀♀.

Distribution in Turkey: Edirne (Okyar et al., 2012).

**** Gelis gallicator* (Aubert, 1971)**

Material examined: GDTP, II. Station, 1.VII.2012, ♀.

Diagnosis: Head, antenna red (Figure 6b). Clypeus convex. Mesosoma red. Epomia short or absent. Mesosoma mat (Figure 6c). Areolet absent. Pterostigma brown. Fore wing with single brown band (Figure 6d). Ovipositor sheath shorter than metatibia (Figure 6a).

General Distribution: Western Palearctic.

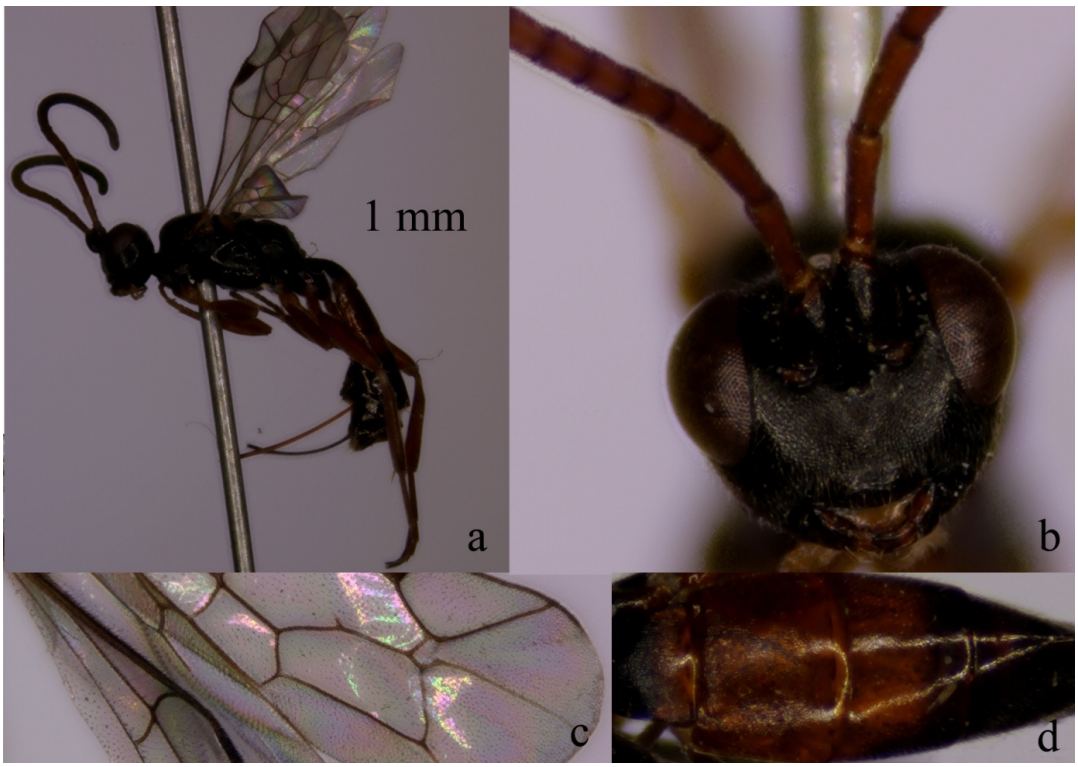

Figure 5. *Charitopes gastricus* a. habitus, lateral view, b. head, facial view, c. forewing and d. metasoma dorsal view.

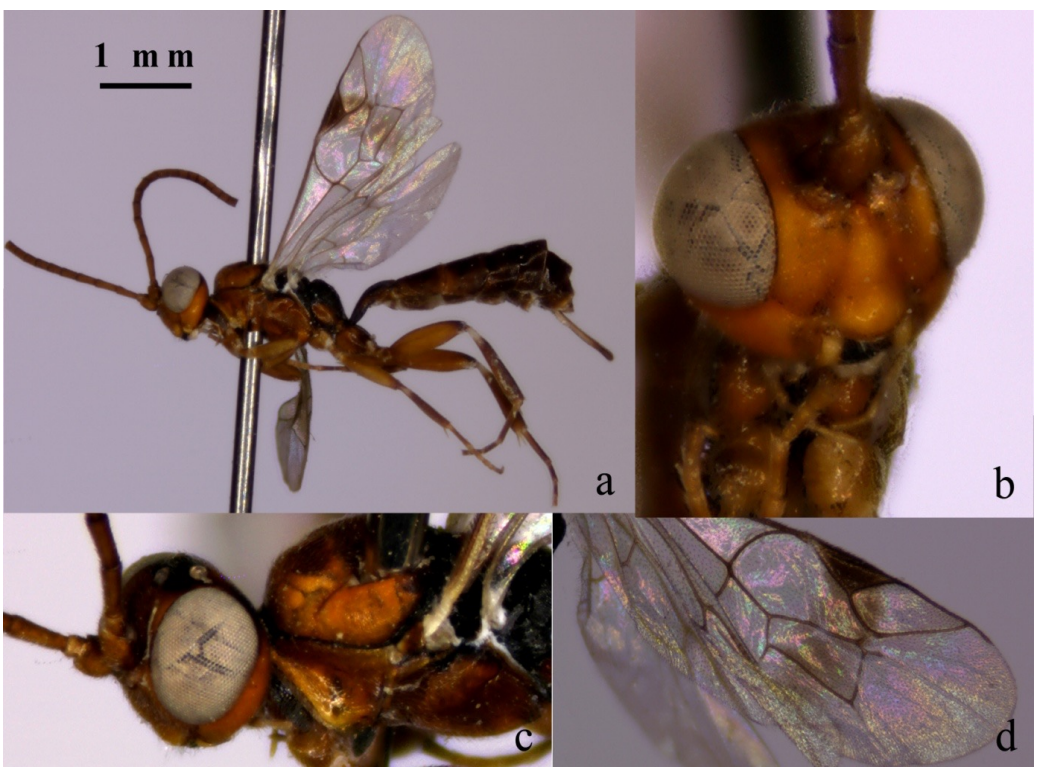

Figure 6. *Gelis gallicator* a. habitus, lateral view, b. head, facial view, c. head and mesosoma, lateral view and d. forewing.

***Tropistes falcatus* (Thomson, 1884)**

Material examined: GDTP, II. Station, 1.VII.2012, ♀.

Diagnosis: Clypeus and mandible black (Figure 7b). Mesosoma laterally compressed (Figure 7a). Wings fully complete. Areolet present. Vein 3rs-m incomplete or little complete. Nervellus slightly vertical or inclivous (Figure 7c). Notaulus not reaching to center of mesoscutum (Figure 7d). Postpectal carina incomplete. Second epipleural tergite separated with fold. Legs red. Second metasomal tergite red and with black punctated. Base of third metasomal tergite red.

General Distribution: Palearctic.

Subfamily Ctenopelmatinae

***Mesoleius melanoleucus* (Gravenhorst, 1829)**

Material examined: GDTP, III. Station, 13.VI.2010, ♀.

Diagnosis: Face black, Clypeus and mandible yellow (Figure 8b). Lower tooth of mandible not longer than upper tooth. Flagellum red. Antennae with 38-45 flagellomeres. Prepectal carina reaching to anterior mesopleuron. Mesopleuron distinctly punctated (Figure 8c). Areolet absent. Nervellus intercepted about at its middle or below middle. Legs yellow (Figure 8a). Tarsal claws not pectinate. Glymma present. Epipleura of II and III metasomal segments separate with ledge. Second metasomal tergite without longitudinal carina. Ovipositor with subapical dorsal notch.

General Distribution: Western Palearctic

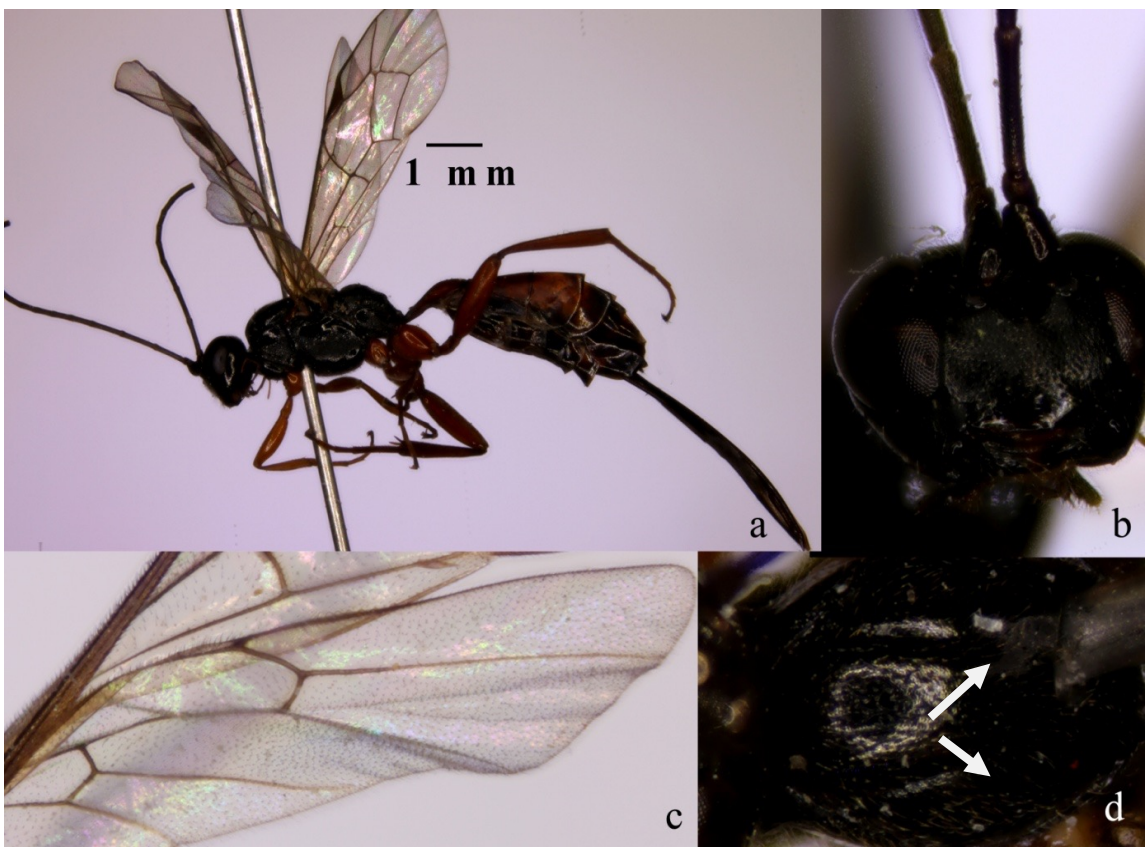

Figure 7. *Tropistes falcatus* a. habitus, lateral view, b. head, facial view, c. hindwing and d. view of notaulus.

Figure 8. *Mesoleius melanoleucus* a. habitus, lateral view , b. head, facial view and c. mesopleuron, lateral view.

Subfamily Diplazontinae

***Diplazon tibiatorius* (Thunberg, 1822)**

Material examined: GDTP, III. Station, 19.V.2010, ♀.

Distribution in Turkey: Ankara (Kolarov, 1995); Ankara (Özdemir, 2001); Tekirdağ (Yurtcan et al., 1999).

Subfamily Ichneumoninae

***Anisobas cingulatellus* Horstmann, 1997**

Material examined: GDTP, II. Station, 25.IX.2010, ♀.

Distribution in Turkey: Edirne, Tekirdağ (Yurtcan et al., 1999); Erzurum (Riedel et al., 2010).

***Colpognathus celerator* (Gravenhorst, 1807)**

Material examined: GDTP, 1.VII.2012, VI. Station, ♂.

Distribution in Turkey: Erzurum (Çoruh & Özbek, 2008), Giresun, Ordu, Trabzon (Kolarov et al., 2014b), Giresun (Çoruh et al., 2016).

***Dicaelotus pumilus* (Gravenhorst, 1829)**

Material examined: GDTP, III. Station, 19.V.2010, ♂.

Diagnosis: Head black, not bulbous, frons slightly convex, with distinct punctuation, clypeus clearly separate from face, upper mandibular tooth longer than lower tooth. Lower tooth of mandible strong (Figure 9b). Antennae reddish brown. Scape black. Postanellus shorter than second flagellomere. Frontal orbit not yellow. Metasoma partly black with red pattern (Figure 9a). Pterostigma brown. Second and third metasomal tergites punctated (Figure 9c). Thyridia absent.

General Distribution: Palearctic.

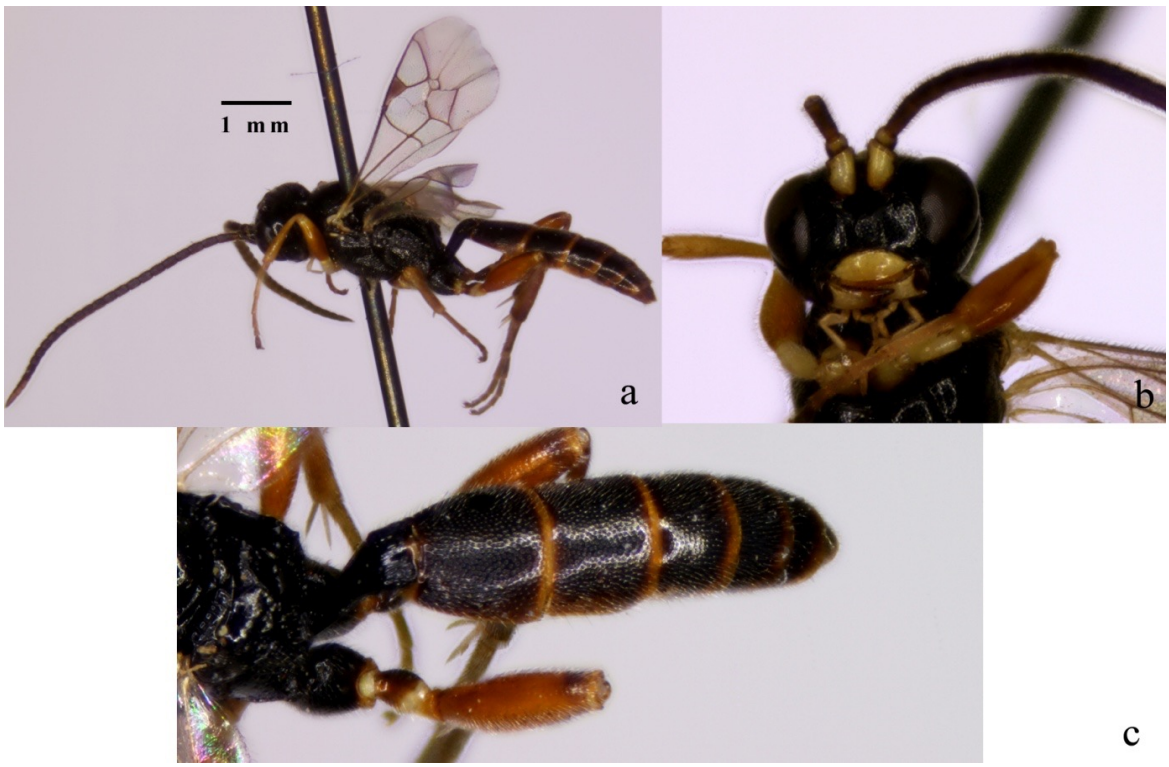

Figure 9. *Dicaelotus pumilus* a.habitus, lateral view, b. head, facial view and c. metasoma, dorsolateral view.

***Herpestomus arridens* (Gravenhorst, 1829)**

Material examined: GDTP, II. Station, 29.V.2010, ♀, V. Station, 2.VII.2012, ♀, II. Station, ♀.

Diagnosis: Face, Clypeus and frontal orbit red (Figure 10b). Antenna red. Mesosoma laterally compressed. Corner of pronotum and Tegula yellow (Figure 10c). Legs red. Vein Cu antefurcal (Figure 10a). Postpetiol scarce punctated or female straight (Figure 10d).

General Distribution: Palearctic.

***Diadromus albinotatus* (Gravenhorst, 1829)**

Material examined: GDTP, III. Station, 5.XI.2011, ♀.

**** Diphyus trifasciatus* (Gravenhorst, 1829)**

Material examined: GDTP, IV. Station, 17.VII.2010**,** 2♂♂, II. Station 9.X.2010, 2♂♂.

Diagnosis: Lower tooth of mandible shorter than upper tooth and different color (Figure 11b). Third and fourth flagellomeres prolonged. Facial orbit large and yellow. Metasoma densely yellow (Figure 11c). Corner of pronotum and Tegula yellow. Mesoscutum weakly punctate. Scutellum quite large (Figure 11d). Meta tibia yellow (Figure 11a). Seventh of metasomal tergit quite short.

General Distribution: Palearctic.

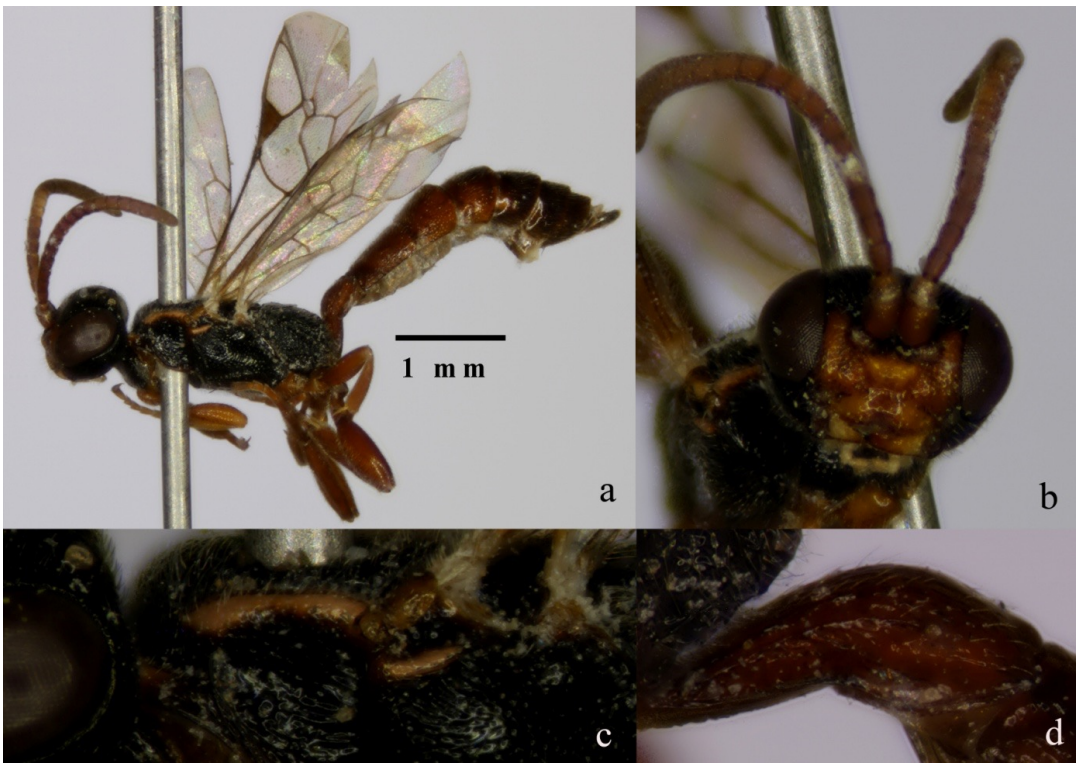

Figure 10. *Herpestomus arridens* a. habitus, lateral view, b. head, facial view, c. pronotum, and mesopleuron, lateral view and d. first tergite of metasoma.

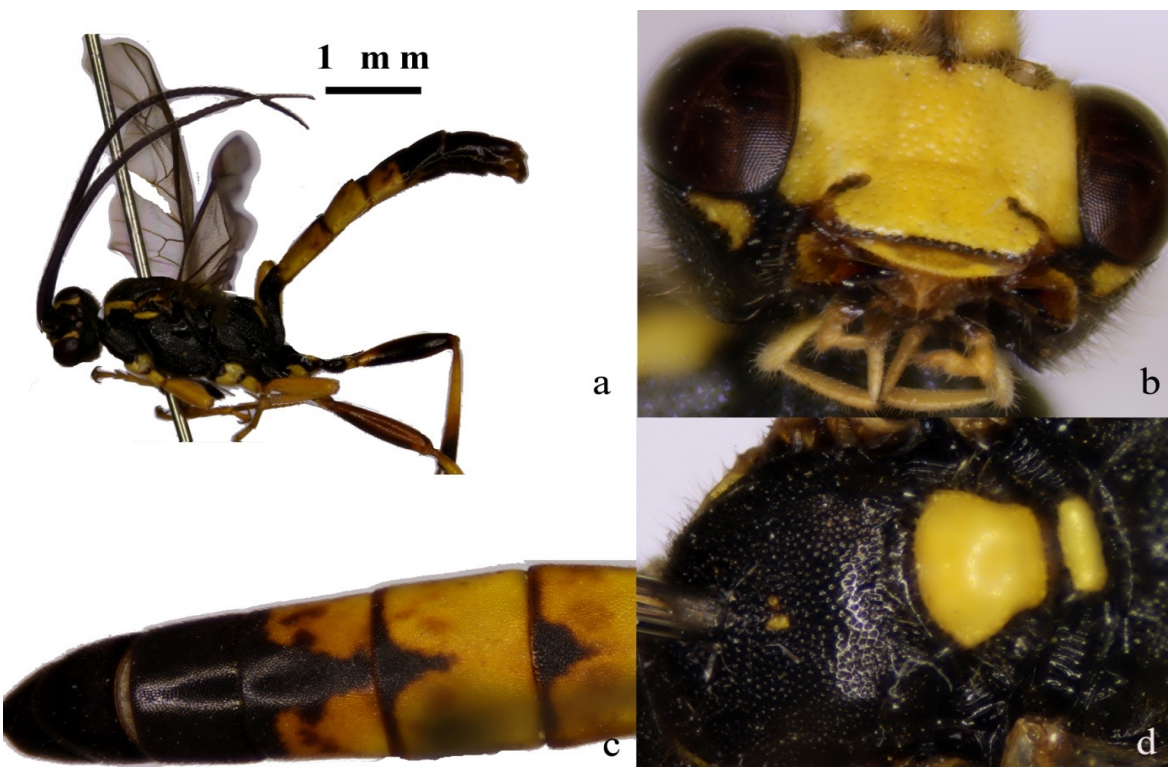

Figure 11. *Diphyus trifasciatus* a. habitus, lateral view, b. head, facial view, c. metasoma, dorsal view and d. mesoscutum and scutellum, dorsal view.

***Ichneumon caloscelis* Wesmael, 1845**

Material examined: GDTP, II. Station, 9.X.2010, 2♂♂.

Diagnosis: Lower tooth of mandible distinct (Figure 12b). Antenna with 32 flagellomeres. First flagellomere segment elongate. Temple behind eyes narrowed. Propodeum without tooth, densely punctated (Figure 12c). Scutellum yellow, mesoscutum almost black (Figure 12d). Area supermedia transverse or square. Metatibia yellow with black apex (Figure 12a).

General Distribution: Palearctic.

***Spilichneumon occisorius* (Fabricius, 1793)**

Material examined: GDTP, II. Station, 9.X.2010, ♂, GDTP, II. Station, 24.XI.2010, 8♂♂.

Distribution in Turkey: Konya, Eskişehir (Özdemir, 1996).

***Virgichneumon digrammus* (Gravenhorst, 1820)**

Material examined: GDTP, IV. Station, 17.VII.2010**,** ♀, 16.VII.2011, ♂, ♀.

Distribution in Turkey: Erzurum (Riedel et al., 2010).

***Barichneumon peregrinator* (Linnaeus, 1758)**

Material examined: GDTP, III. Station, 5.XI.2011, ♀.

Diagnosis: Face black, Frontal orbit white (Figure 13b). Flagellomeres small, fifth flagellomeres square. Occipital carina joining hypostomal carina behind the mandibles base. Scutellum white (Figure 13c). Metatibia red-black (Figure 13a). Metafemur red and infuscate. Second metasomal tergite quite punctate. Thyridia small (Figure 13d). Metasomal tergites II-V red.

General Distribution: Palearctic.

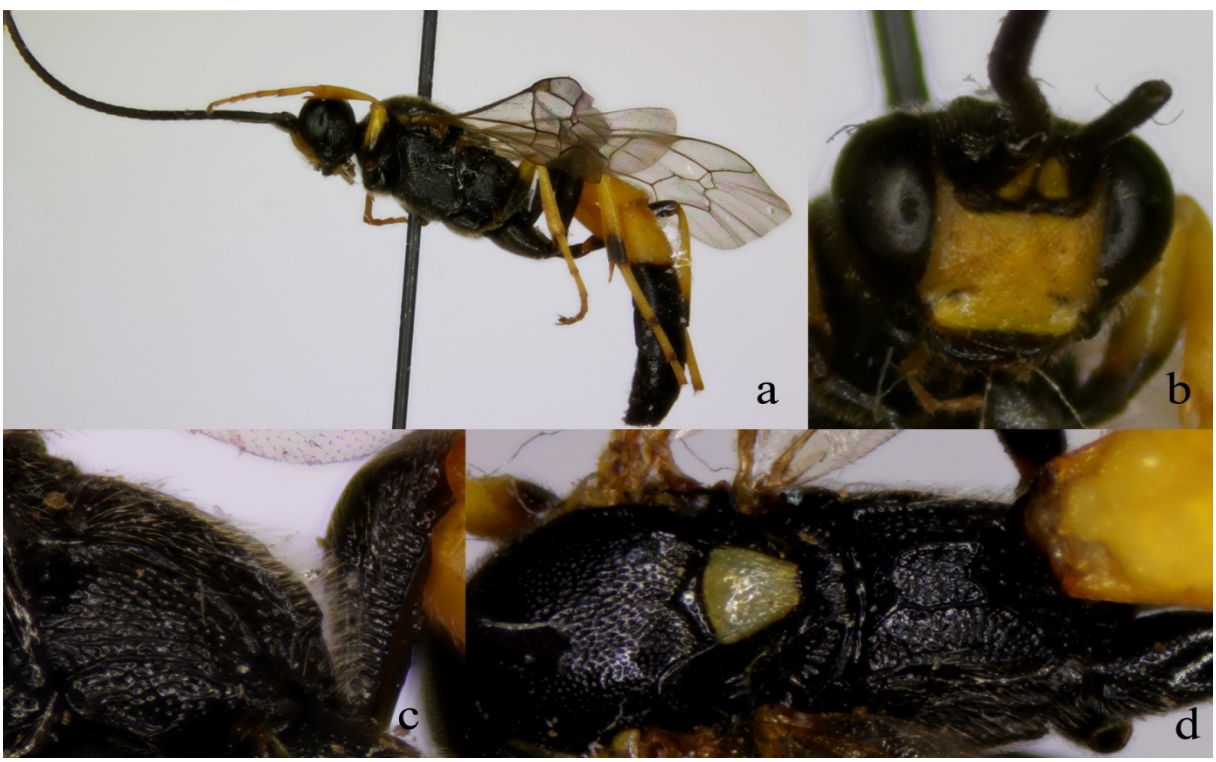

Figure 12. *Ichneumon caloscelis* a. habitus, lateral view, b. head, facial view, c. metapleuron and propodeum, lateral view and d. mesosoma and first metasomal segment, dorsal view.

Figure 13. *Barichneumon peregrinator* a. habitus, lateral view, b. head, facial view c. propodeum, metacoxa, first metasomal tergite, dorsal view and d. propodeum, metacoxa, first and second metasomal tergites and Thyridia of metasoma, dorsal view.

Subfamily Mesochorinae

***Mesochorus arenarius* (Haliday, 1838)**

Material examined: GDTP, III. Station, 24.IV.2010, ♂, 2♀♀, 19.V.2010, ♀.

Distribution in Turkey: Turkey (Kolarov, 1989).

***Mesochorus fulgurans* Curtis, 1833**

Material examined: GDTP, III. Station, 13.VI.2010, ♀.

Subfamily Metopiinae

***Exochus vafer* Holmgren, 1873**

Material examined: GDTP, II. Station, 19.V.2010, ♂.

Distribution in Turkey: Isparta, İzmir (Kolarov et al., 2009); Erzincan (Çoruh & Kolarov, 2012).

Subfamily Orthocentrinae

***Symplecis invisitata* Rossem, 1981**

Material examined: GDTP, II. Station, 5.XI.2011, ♀.

Diagnosis: Labium, labial palpi and maxillary palpi white. Mandibles yellow, teeth of the same length. Eyes strongly converging, malar space very narrow (Figure 14b). Face polished, brown. Pronotum polished, brown. Epomia present. Pleuron 1 brown. Mesopleuron highly polished, speculum smoother and shiny (Figure 14c). Mesoscutum coriaceous, notauli short but well developed anteriorly. Scutellum polished. Forewing without areolet. Nervellus inclivous, intercepted below the middle but discoidella absent. Tegula and wing base white. Front coxae whitish, middle and hind coxae more brown.

Propodeum with all carina and with rather long widely placed hairs (Figure 14d). Mesopleuron highly polished. First, second, third and fourth metasomal sternit white, with widely placed with hairs, following sternite brown (Figure 14a).

General Distribution: Palearctic, Nearctic.

Subfamily Ophioninae

***Ophion obscuratus* Fabricius, 1798**

Material examined: GDTP, III. Station, 13.VI.2010, 2♀♀, ♂.

Distribution in Turkey: Erzurum, Muş (Kolarov et al., 2000), Tunceli (Kolarov et al., 2014a), Erzurum (Çoruh & Çalmaşur, 2016).

Subfamily Pimplinae

***Perithous scurra* (Panzer, 1804)**

Material examined: GDTP, II. Station, 1.VI.2012, ♀.

Distribution in Turkey: Ankara (Kolarov, 1995); Erzurum (Çoruh, 2005).

***Pimpla turionellae* (Linnaeus, 1758)**

Material examined: GDTP, V. Station, 16.IX.2012, ♀.

Distribution in Turkey: Ankara, Eskişehir, Konya, Nevşehir (Özdemir & Kılınçer, 1990), Kırklareli (Yurtcan, 2004), Isparta (Gürbüz, 2004), Erzurum (Çoruh, 2005), Osmaniye (Gürbüz et al., 2008), Kasnak Meşesi Tabiatı Koruma Alanı (Kırtay, 2008), Davraz Dağı (Birol, 2010).

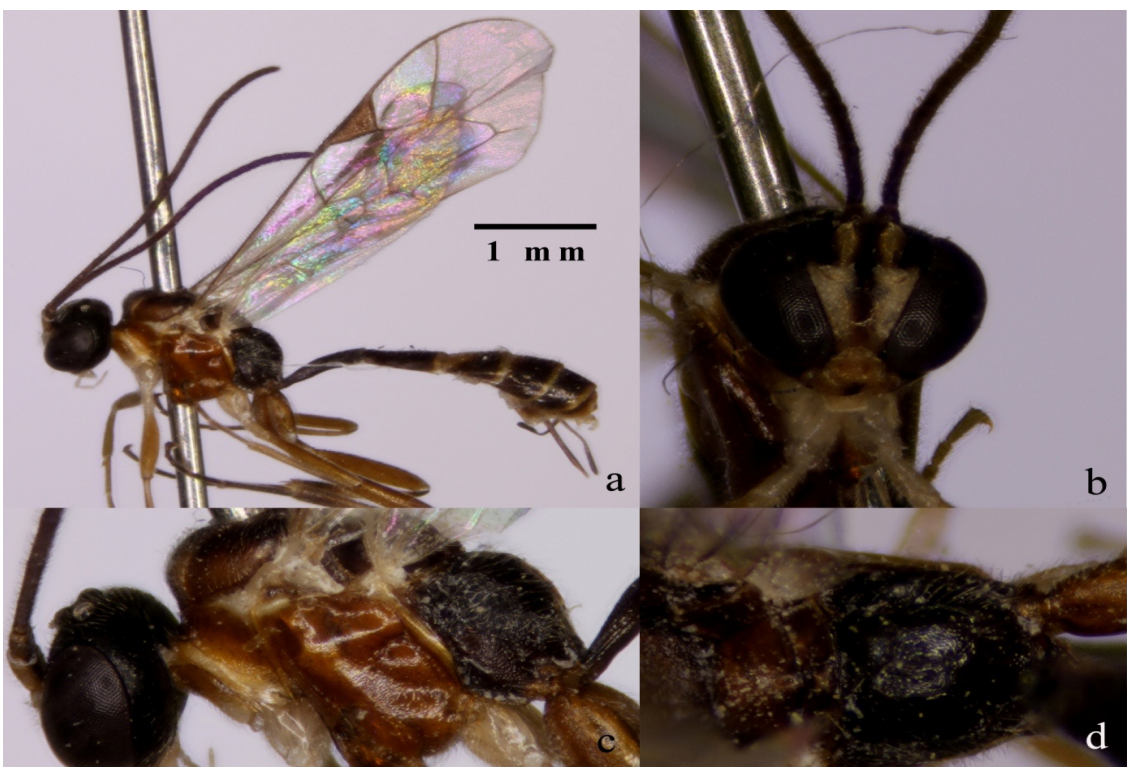

Figure 14. *Symplecis invisitata* a. habitus, lateral view, b. head, facial view, c. mesosoma, lateral view and d. propodeum and scutellum, dorsal view.

***Clistopyga rufator* Holmgren, 1856**

Material examined: GDTP, II. Station, 14.V. 2011, ♀, 22.V.2011, ♀, IV. Station, 16.VI.2012, ♀.

Distribution in Turkey: Edirne (Yurtcan, 2004), Kırklareli (Yurtcan, 2007), Adana (Buncukçu, 2008), Hatay (Gürbüz et al., 2008).

***Iseropus stercorator* (Fabricius, 1793)**

Material examined: GDTP, II. Station, 1.VII.2012, 6♀♀, 12.VI.2012, II. Station, ♀, 17.VI.2012, ♂, 22.VI.2012, ♂.

Distribution in Turkey: Erzurum (Çoruh &Özbek, 2008; Çoruh, 2005).

Subfamily Tersilochinae

***Gelanes fusculus* Holmgren, 1860**

Material examined: GDTP, III. Station, 17.VI.2010, ♂.

Distribution in Turkey: Habib-i Neccar, Hatay (Gürbüz et al., 2008).

***Probles anatolicus* Horstmann, 1981**

Material examined: GDTP, II. Station, 24.IV.2010, ♀, II. Station, 9.X.2010, 2♂♂, I. Station, 27.V.2012 3♀♀, ♂.

Distribution in Turkey: Unknown (Horstmann, 1981).

Subfamily Tryphoninae

***Exenterus abruptorius* (Thunberg, 1824)**

Material examined: GDTP, II. Station, 17.VI.2012, ♂.

Distribution in Turkey: Konya (Özdemir, 2001).

***Monoblastus brachyacanthus* (Gmelin, 1790**)

Material examined: GDTP, III. Station, 19.V.2010, ♀.

Distribution in Turkey: Erzurum, Tekirdağ (Kolarov & Beyarslan, 1994), Edirne, Kırklareli (Yurtcan & Beyarslan, 2002), Isparta, Eğirdir, Uluborlu, Burdur (Gürbüz & Kolarov, 2006).

***Netelia dilatata* (Thomson, 1888)**

Material examined: GDTP, II. Station, 22.V.2011, ♀, 3♂♂.

Distribution in Turkey: Erzurum (Kolarov et al., 1999); Ankara, Konya (Özdemir, 2001); Isparta (Gürbüz & Kolarov, 2006), Elazığ, Eskişehir, Malatya, Sivas (Yaman, 2014), Erzurum (Çoruh & Kolarov, 2016).

Subfamily Xoridinae

****Odontocolon quercinus* (Thomson,1877)**

Material examined: GDTP, III. Station, 16.IX.2012, ♀.

Diagnosis: Face black (Figure 15b), mandibles with double teeth. Flagellomere I not longer than flagellomere II. Mesosoma completely black (Figure 15a) and laterally compressed.. Propodeum smooth and shiny. Epomia absent. Metafemur with a large tooth laterally (Figure 15c). Metatibia includes both short and long hairs. Metafemur red color. Pterostigma black-brown. Second metasomal tergite quite spotted (Figure 15d).

Hosts: *Cynips quercusfolii* Linnaeus 1758 (Hymenoptera:Cyniipidae), *Hylotrupes bajulus* (Linnaeus 1758) (Coleoptera: Cerambycidae), *Monochamus galloprovincialis* Oliver (Coleoptera: Cerambycidae).

General Distribution: Western Palearctic, Europe.

Figure 15. *Odontocolon quercinus* a. habitus, lateral view , b. head, facial view, c. view of metafemur and d. first and second metasomal tergites, dorsal view.

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References

Akkaya, A., 2005. Güneydoğu ve Doğu Anadolu Bölgesi'nde Anomaloninae, Banchinae, Collyriinae, Ophioninae ve Pimplinae (Hymenoptera: Ichneumonidae) Türlerinin Sistematik Yönden İncelenmesi. Dicle Üniversitesi Fen Bilimleri Enstitüsü, (Basılmamış) Doktora Tezi, Diyarbakır, 98 s.

Birol, O., 2010. Isparta İli Davraz Dağı Ichneumonidae (Hymenoptera) Faunası Üzerine Bir Araştırma. Süleyman Demirel Üniversitesi, Fen Bilimleri Enstitüsü, (Basılmamış) Yüksek Lisans Tezi, Isparta, 71 s.

Buncukçu, A., 2008. Isparta İli Merkez ve Adana, Yumurtalık İlçesi-Halep Çamlığı Ichneumonidae Türlerinin Tespiti Ve Kültüre Edilebilen Türlerin Biyolojilerinin Araştırılması, Süleyman Demirel Üniversitesi, Fen Bilimleri Enstitüsü, (Basılmamış) Yüksek Lisans, Isparta, 74 s.

Çoruh, S., 2005. Erzurum ve Çevre İllerdeki Pimplinae (Hymenoptera : Ichneumonidae) Türleri Üzerinde Faunistik, Sistematik ve Ekolojik Çalışmalar. Atatürk Üniversitesi, Fen Bilimleri Enstitüsü, (Basılmamış) Doktora tezi, Erzurum, 211 s.

Çoruh, İ. & S. Çoruh, 2008. Ichneumonidae (Hymenoptera) Species Associated with Some Umbelliferae Plants Occurring in Palandöken Mountains of Erzurum, Turkey. Turkish Journal of Zoology, 32, 121–124.

Çoruh, S. & H. Özbek, 2008. New and rare Ichneumonidae (Hymenoptera) species from Turkey. Zoology in the Middle East, 43: 114–116.

Çoruh, S. & J. Kolarov, 2012. Description of the male of *Ophion internigrans* Kokujev, 1906 (Hymenoptera: Ichneumonidae: Ophioninae) with a key to the Turkish *Ophion* Fabricius, 1798 Species. Journal Entomological Research Society, 14 (2): 55-60.

Çoruh, S. & J. Kolarov, 2016. Faunistic notes on the Ichneumonidae (Hymenoptera) of Turkey with a new record. Acta entomologica serbica, 21 (1): inpress.

Çoruh, S. & Ö. Çalmaşur, 2016. A new and additional records of the Ichneumonidae (Hymenoptera) from Turkey. Turkish Journal of Zoology, 40: 625-629.

Çoruh, S. H. Özbek. & J. Kolarov, 2004. New and little known Anomaloninae (Hymenoptera, Ichneumonidae) from Turkey. Linzer Biologische Beitrage, 36: 1199–1204.

Çoruh, S., M. F. Gürbüz, J. Kolarov, M. Yurtcan & A. Buncukçu, 2013. New and little known species of Ichneumonidae (Hymenoptera) for the Turkish Fauna. Journal of the Entomological Research Society, 15: 71–83.

Çoruh, S., J. Kolarov & İ. Çoruh, 2014a. Ichneumonidae (Hymenoptera) from Anatolia. II. Turkish Journal of Entomology, 38: 279–290.

Çoruh, S., J. Kolarov & H. Özbek, 2014b. The fauna of Ichneumonidae (Hymenoptera) of eastern Turkey with zoogeographical remarks and host data. Journal of Insect Biodiversity, 2: 1–21.

Çoruh, S., J. Kolarov, İ. Çoruh, 2016. A study of Ichneumonidae (Hymenoptera) from northeastern Anatolia II, with new records. Türkiye Entomoloji Dergisi, 40 (3): 265-280.

Erduran, F. & U. Cırık, 2011. Gelincik Dağı Tabiat Parkı'nın rekreasyonel peyzaj değerlerinin belirlenmesi. Journal of Agricultural Faculty of Atatürk University, 42 (1): 63-77.

Ghahari, H., M. Fischer & R. Jussila, 2012. Braconid and ichneumonid wasps (Hymenoptera, Ichneumonoidea) as the parasitoids of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae) in Iran. Entomofauna, 33 (18): 281-288.

Gürbüz, M. F., 2004. Isparta İli Ichneumonidae (Hymenoptera) Familyası Türleri Üzerine Faunistik ve Sistematik Araştırmalar. Süleyman Demirel Üniversitesi, Fen Bilimleri Enstitüsü, (Basılmamış) Doktora Tezi, Isparta, 68 s.

Gürbüz, M. F. & J. Kolarov, 2006. A study of Turkish Ichneumonidae (Hymenooptera) II. Tryphoninae. The Gazi Entomological Research Society, 8: 1.

Gürbüz, M. F. & J. Kolarov, 2008. A study of the Ichneumonidae (Hymenoptera). IV. Cryptinae, Cryptini. Turkish Journal of Zoology, 32: 373–377.

Gürbüz, M. F., T. Ljubomirov, J. Kolarov, M. Yurtcan, M. A. Tabur, S. Çoruh & A. Buncukçu, 2008. Investigation of the Ichneumonidae, Ampulicidae, Crabronidae and Sphecidae (Hymenoptera, Insect) Fauna in Natural Protection Zones of East Mediteranean Region in Turkey. Tübitak TBAGU/ 168 (106T189) No'lu Proje, 127 s.

Gürbüz, M. F., M. Y. Aksoylar & A. Buncukçu, 2009a. A faunistic study on Ichneumonidae (Hymenoptera) in Isparta, Turkey. Linzer Biologische Beiträge, 41 (2): 1969–1984.

Gürbüz, M. F., H. Kırtay & O. Birol, 2009b. A study of Ichneumonidae (Hymenoptera) of Kasnak Oak Forest nature reserve in Turkey with new records. Linzer Biologische Beiträge. 41 (2): 1985–2003.

Horstmann, K., 1981. Revision der Europäischen Tersilochinen II (Hymenoptera, Ichneumonidae). Spixiana Supplement, 4: 1-76.

Kırtay, H., 2008. Isparta Kasnak Meşesi (*Quercus vulcanıca* Boiss. and Heldr. ex Kotschy) Ormanı Tabiatı Koruma Alanı Ichneumonidae (Hymenoptera) Faunası Üzerine Bir Araştırma. Süleyman Demirel Üniversitesi, Fen Bilimleri Enstitüsü, (Basılmamış) Yüksek Lisans Tezi, Isparta, 77 s.

Kolarov, J., 1989. Taxonomic and faunistic study on Bulgarian Cremastinae (II). Faunistische Abhandlungen, 16 (13): 149–154.

Kolarov, J., 1995. A cataloque of the Turkish Ichneumonidae (Hymenoptera). Entomofauna, Zeitschrift für Entomologie, 7: 137–188.

Kolarov, J. & A. Beyarslan, 1994. Investigations on the Ichneumonidae (Hym.) fauna of Turkey. 1. Pimplinae and Tryphoninae. Türkiye Entomoloji Dergisi 18 (3): 133-140.

Kolarov, J. & A. Beyarslan, 1995. "New and little known Turkish Campopleginae (Hymenoptera: Ichneumonidae) 18-21". III. National Scientific Conference of Entomology (18-20 September, Sofia, Bulgaria) Proceedings.

Kolarov, J. & M. Yurtcan, 2008. A study of the Ichneumonidae (Hymenoptera) of the North Anatolia (Turkey) I. Brachycyrtinae, Cryptinae and Xoridinae. Acta entomologica serbica, 13 (1/2): 89-91.

Kolarov, J., A. Beyarslan & M. Yurtcan, 1994. "Yeni ve az bilinen Türkiye Anomaloninae türleri (Hymenoptera: Ichneumonidae), 248-251". XII. Ulusal Biyoloji Kongresi (6-8 Temmuz 1994, Edirne, Türkiye) Bildirileri.

Kolarov, J., H. Özbek & E. Yıldırım, 1999. New distributional data of the Turkish Ichneumonidae (Hymenoptera). I. Pimplinae and Tryponinae. Journal of the Entomological Research Society, 1 (2): 9–15.

Kolarov, J., S. Pekel, H. Özbek, E. Yıldırım & Ö. Çalmaşur, 2000. "New distributional data of the Turkish Ichneumonidae (Hymenoptera) III. The subfamily Ophioninae, 349-356". Türkiye 4. Entomoloji Kongresi (12-15 Eylül 2000, Aydın, Türkiye) Bildirileri.

Kolarov, J., M. Yurtcan & A. Beyarslan, 2002. "Ichneumonidae species of the Turkish Aegean Region. Parasitic wasps: Evolution, systematics, biodiversity and biological control, 299-305". International Symposium (14–17 May 2001, Agroinform, Koszeg-Hungary) Proceedings.

Kolarov, J., S. Çoruh, M. Yurtcan & M. F. Gürbüz, 2009. A study of Metopiinae from Turkey with description of a new species (Hymenoptera: Ichneumonidae). Zoology İn The Middle East, 46: 75–82.

Kolarov, J., E. Yıldırım, S. Çoruh & M. Yüksel, 2014a. Contribution to the knowledge of the Ichneumonidae (Hymenoptera) fauna of Turkey. Zoology in the Middle East, 60: 154-161.

Kolarov, J., S. Çoruh & I. Çoruh, 2014b. Ichneumonidae (Hymenoptera) from Anatolia. III. Turkish Journal of Entomology, 38: 377-388.

Kolarov, J., S. Çoruh & İ. Çoruh, 2016. Contribution to the knowledge of the Ichneumonidae (Hymenoptera) fauna of Turkey from northeastern Anatolia, Part I. Turkish Journal of Zoology, 40: 40-56.

Kopylov, D. S. & H. Zhang, 2015. New ichneumonids (Insecta: Hymenoptera: Ichneumonidae) from the Lower Cretaceous of north China. Cretaceous Research, 52: 591-604.

Okyar, Z, M. Yurtcan, A. Beyarslan & N. Aktaş, 2012. The Parasitoid complex of White-spotted Pinion *Cosmia diffinis* (Linnaeus, 1767) (Lepidoptera: Noctuidae) on *Ulmus minor* Miller (Ulmaceae) in Edirne Province (European Turkey). Journal of the Kansas Entomological Society, 85 (2): 91-96.

Özbek, H., S. Pekel & J. Kolarov, 2000. New distributional data of the Turkish Ichneumonidae (Hymenoptera) II. Ctenopelmatinae and Campopleginae. Journal of the Entomological Research Society, 2: 17-24.

Özdemir, Y., 1996. İç Anadolu Bölgesinde tespit edilen Banchinae ve Ichneumoninae (Hymenoptera: Ichneumonidae) türleri. Bitki Koruma Bülteni, 36 (3–4): 91–104.

Özdemir, Y., 2001. İç Anadolu Bölgesinde saptanan Diplazontinae ve Tryphoninae (Hymenoptera: Ichneumonidae) türleri. Türkiye Entomoloji Dergisi, 25 (3): 183–191.

Özdemir, Y. & N. Kılınçer, 1990. "İç Anadolu Bölgesinde saptanan Pimplinae ve Ophioninae (Hymenoptera: Ichneumonidae) türleri, 309-318". Türkiye II. Biyolojik Mücadele Kongresi (26–29 Eylül 1990, Ankara, Türkiye) Bildirileri.

Özdemir, Y. & Güler Y., 2009. Sultandağı Havzası kiraz bahçelerinde tespit edilen ichneumonidae (Hymenoptera) türleri. Bitki Koruma Bülteni, 49 (3): 135-143.

Pekel, S. & H. Özbek, 2000. Erzurum ili Cremastinae (Hymenoptera: Ichneumonidae) altfamilyası üzerinde faunistik ve sistematik bir çalışma. Türkiye Entomoloji Dergisi, 24 (3): 215–228.

Riedel, M., S. Çoruh & H. Özbek, 2010. Contribution to the Ichneumoninae Hymenoptera, Ichneumonidae) fauna of Turkey, with description of three new species. Türkiye Entomoloji Dergisi, 34 (2): 133-156.

Sedivy, J., 1959. Wissenschaftliche Ergebnisse der zoologischen Expedition des National-Museums in Prag nach der Tuerkei. 26. Hymenoptera, Ichneumonidae. Acta Faunistica Entomologica Musei Nationalis Pragae, 33: 107–116.

Townes, H. K., 1969. The genera of Ichneumonidae: 1. Ephialtinae to Agriotypinae. Memoirs of the American Entomological Institute, 11: 1–300.

Wahl, D. B. & M. J. Sharkey, 1993. "Superfamily Ichneumonoidae, 358-449". In: The Hymenoptera of the World, An Identification Guide to Families (Eds. H. Goulet & J. Huber), Canada Agriculture, Canada, 668 pp.

Yaman, G., 2014. Türkiye Tryphoninae (Hymenoptera: Ichneumonidae) Türlerinin Kontrol Listesi. Trakya Üniversitesi, Fen Bilimleri Enstitüsü, (Basılmamış) Yüksek Lisanas Tezi, Edirne, 88 s.

Yu, D., C. Van Achterberg & K. Horstmann, 2012. Taxapad 2012, Ichneumonoidae 2011. Database on flash-drive. www.taxapad.com, Ottawa, Ontario, Canada.

Yurtcan, M., 2004. Trakya Bölgesi Pimplinae (Hymenoptera: Ichneumonidae) Faunasının Taksonomik ve Faunistik Yönden Araştırılması. Trakya Üniversitesi Fen Bilimleri Enstitüsü (Basılmamış) Doktora Tezi, Edirne, 110 s.

Yurtcan, M., 2007. Ephialtini tribe (Hymenoptera, Ichneumonidae, Pimplinae) of Turkish Thrace region, Entomofauna, 28: 389-404.

Yurtcan, M., A. Beyarslan & J. Kolarov, 1999. Investigations on the Ichneumonidae (Hymenoptera) fauna of Turkey. V. Diplazontinae and Ichneumoninae. Acta Zoologica Bulgarica, 1: 36.

Yurtcan, M. & A. Beyarslan, 2002. The species of Tryphoninae (Hymenoptera: Ichneumonidae) in Turkish Thrace. Turkish Journal of Zoology, 26: 77-95.

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The insecticidal potential of botanical extracts for management of Peach fruit fly, *Bactrocera zonata* Saunders, 1842 (Diptera: Tephritidae)

Şeftali meyve sineği, *Bactrocera zonata* Saunders, 1842 (Diptera: Tephritidae) mücadelesinde bitkisel ekstraktların insektisidal potansiyeli

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Summary

Methanolic extracts of *Isodon rugosus* Wall. ex Benth (Labiatae), *Boenninghausenia albiflora* (Hook.) Rchb. ex Meisn. (Rutaceae), *Calotropis procera* Aiton (Dryand) (Apocynaceae), *Daphne mucronata* Royle (Thymelaeaceae), *Tagetes minuta* L. (Asteraceae), *Cinnamomum camphora* (L.) J. Presl (Lauraceae) and *Eucalyptus sideroxylon* A. Cunn. ex Woolls (Myrtaceae), grown in lower Himalayan regions of Pakistan were evaluated at 2% concentration against Peach fruit fly *Bactrocera zonata* Saunders, 1842 (Diptera: Tephritidae). *Tagetes minuta* extract showed maximum of 73% mortality against male fruit flies and in case of female maximum mortality shown by *C. camphora* and *I. rugosus* was only 16.6%. *Boenninghausenia albiflora* extract had maximum repellence of 44.4% followed by 42% by *D. mucronata* extract and the minimum number of flies were settled on these two plant extracts as compared to the others. The lowest number of pupae 3.3% as collected from guavas treated with *T. minuta* extract; this was significantly lower than the 62.6% pupae recovered from untreated guavas. The lowest numbers of adults (0.33%) were recovered from guavas treated with *T. minuta* extract compared to 45.3% adults from untreated guavas. The pupae inhibition was highest (94.6%) for *T. minuta* extract. Inhibition of adult emergence was highest i.e. 99.2% for *T. minuta* extract. *Tagetes minuta* can be exploited as a potent source of pesticide against fruit fly, *B. zonata*, due to maximum pesticidal potential as compared to all other plant extracts applied. The results are discussed in relation with potential benefits of incorporating plant based insecticides in integrated pest management strategies against *B. zonata*.

Keywords: *Bactrocera zonata*, botanical insecticides, insecticidal activity

Özet

Pakistan'da Himalaya'nın düşük rakımlı bölgelerinde yetişen, *Isodon rugosus* Wall. ex Benth (Labiatae), *Boenninghausenia albiflora* (Hook.) Rchb. ex Meisn.(Rutaceae), *Calotropis procera* Aiton (Dryand) (Apocynaceae), *Daphne mucronata* Royle (Thymelaeaceae), *Tagetes minuta* L. (Asteraceae), *Cinnamomum camphora* (L.) J. Presl (Lauraceae) ve *Eucalyptus sideroxylon* A. Cunn. ex Woolls (Myrtaceae) bitkilerinin metanolik ekstraktlarının %2'lik konsantrasyonlarının Şeftali meyve sineği *Bactrocera zonata* Saunders, 1842 (Diptera: Tephritidae)'ne karşı etkileri değerlendirilmiştir. *Tagetes minuta* ekstraktı, erkek meyve sineklerine karşı maksimum %73 ölüm oranı gösterirken, dişilerde en fazla ölüm oranı sadece %16.6 ile *C. camphora* ve *I. rugosus* ekstraktlarından elde edilmiştir. Maksimum uzaklaştırıcı etkiye %44.4 ile *B. albiflora*, bunu takiben %42 ile *D. mucronata* ekstraktları sahip olurken en az sayıda sinek diğerlerine kıyasla bu iki bitki ekstraktında saptanmıştır. En düşük pupa sayısı %3.3 ile *T. minuta* ekstraktı uygulanan guava meyvelerinden toplanırken; bu oran uygulama yapılmamış olanlardan elde edilen %62.6'lık pupa oranına göre önemli derecede düşük olarak değerlendirilmiştir. Erginlerin en düşük sayıları (%0.33), *T. minuta* ekstraktı uygulanan guava meyvelerinden elde edilirken, uygulama yapılmamış olanlardan %45.3 oranında ergin elde edilmiştir. Pupaların engellenmesi oranı, en yüksek (%94.6) *T. minuta* ekstraktından elde edilmiştir. Erginlerin ortaya çıkışının engellenmesi %99.2 ile *T. minuta* ekstraktı için en yüksek olmuştur. Uygulanan diğer bitki ekstraktlarına kıyasla maksimum insektisit potansiyeline sahip olduğu için, *T. minuta* meyve sineği *B. zonata*' ya karşı etkili bir pestisit kaynağı olarak kullanılabilir. Sonuçlar bitki bazlı insektisitlerin *B. zonata*'ya karşı entegre zararlı mücadele stratejilerine dahil edilmesinin potansiyel faydaları ile ilişkili olarak tartışılmıştır.

Anahtar sözcükler: *Bactrocera zonata*, bitkisel insektisitler, insektisidal aktivitesi

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Introduction

The Peach fruit fly, *Bactrocera zonata* Saunders, 1842 (Diptera: Tephritidae), is one of the most economically important insect pests that causes economic loss by damaging fruit and by interfering international horticultural trade (Shehata et al., 2008). It is native to Asia where it causes severe damage to over 50 species of fruit crop. Its most preferred host is guava, in which losses may reach 50%, if effective control measures are not adopted (Awad et al., 2014)

Like many other species in the genus *Bactrocera*, males of *B. zonata* show strong attraction to a phenylpropanoid compound, methyl eugenol (ME; 1,2-dimethoxy-4-(2-propenyl) benzene), which occurs naturally in many plant species (Tan & Nishida, 2012). ME is used to monitor and suppress populations of fruit flies by a male lure and kill approach (male annihilation technique; MAT) (Steiner et al., 1970). The MAT relies on attracting males from the field population in devices containing ME and insecticides (Vargas et al., 2010). To suppress female populations in conjunction with MAT, sprays of protein baits containing insecticides (bait application technique; BAT) can also be used. As components of integrated pest management, both MAT and BAT are more efficient when used on an area-wide basis (AW-IMP). In addition to these control measures, farmers routinely apply synthetic insecticides. However, synthetic insecticide application is undesirable because of adverse effects to the environment, poisonous residues in fruit and issues for international trade (El-Aw et al., 2008).

Due to the limitations of each control strategy as a stand-alone technique, it is recommended that an IPM approach be adopted (Vargas et al., 2015). Although MAT and BAT are components of IPM and these are environmentally benign techniques, overuse of baits with synthetic insecticides deposits a huge quantity of insecticides into the environment, so to protect the environment safer insecticides i.e. Spinosad are incorporated into baits. Therefore, exploring plant based insecticides may lead to the discovery of safer insecticides either for direct application or for incorporation into the baits. Plant extracts can potentially be eco-friendly alternatives to synthetic insecticides in IPM of fruit fly populations.

Different plant extracts have been used effectively against a wide range of insect pests (Isman, 2006). Extracts from some plants, e.g. *Acorus calamus* L., *Azadirachta indica* A. Juss, *Curcuma longa* L., *Peganum harmala* L., *Saussurea lappa* (Decne.) C. B. Clarke and *Valeriana jatamansi* Jones, have shown repellence and growth inhibition of *B. zonata* (Akhtar et al., 2004). More recently, Siddiqi et al. (2006) analyzed turmeric plant extracts in solvents such as petroleum ether, acetone and ethanol, and found that acetone extract gave the highest repellence and growth inhibition of *B. zonata*. An advantage of plant based insecticides is that they contain many substances and are capable of showing higher efficacy against target pests. For example, *A. indica* extract shows anti-feeding effects, repellence, toxicity and anti-oviposition effects on the oriental fruit fly, *Bactrocera dorsalis* Hendel, 1912 and melon fly, *Bactrocera cucurbitae* Coquillett, 1849 (Shivendra & Singh, 1998).

The main objective of this study was to evaluate the insecticidal potential of seven plant species for mortality, repellence and oviposition deterrence of *B. zonata*. This was achieved by analyzing the toxic effects of crude methanolic extracts of the plants on *B. zonata* in laboratory bioassays. The selected plants have been long known for their folk or ethnobotanical uses in northern Pakistan. Exploring the scientific base of traditional use in order to transform local knowledge into commercial use was the major and long term goal of this study.

Materials and Methods

Plant material

The leaves of *Cinnamomum camphora* (L.) J. Presl (Lauraceae) and *Eucalyptus sideroxylon* A. Cunn. ex Woolls *(*Myrtaceae), and aerial parts of *Isodon rugosus* Wall. ex Benth (Labiatae), *Boenninghausenia albiflora* (Hook.) Rchb. ex Meisn. (Rutaceae), *Calotropis procera* Aiton (Dryand) (Apocynaceae), *Daphne mucronata* Royle (Thymelaeaceae) and *Tagetes minuta* L. (Asteraceae) were collected from northern Pakistan (34.1558° N, 73.2194° E). These plants are commonly grown in Pakistan and their taxonomic identification was done by Dr. Zafar Jamal, Chairman Botany Department, Abbottabad Government College, Pakistan.

Rearing of fruit fly, *Bactrocera zonata*

Pupae of *B. zonata* were collected from the laboratory colony maintained on an artificial larval diet at the Nuclear Institute of Agriculture, Tandojam, Pakistan. Three to 4 d before eclosion of pupae, the pupae were received at the Insect Pest Management Program, National Agricultural Research Centre Islamabad, Pakistan. After emergence, the flies were kept in 30x30x45 cm screened cages and maintained at 26±1°C and 60±5% RH with a 10L:14Dh photoperiod, and fed *ad libitum* with a protein diet containing hydrolyzed yeast (MP Biomedicals Inc.; www.mpbio.com) and sugar in a 1:3 ratio by weight, and water. On the first day of emergence flies were sexed and kept in separated cages having different food regimes. Male and female flies were identified on the basis of morphological characteristics; the female flies have long pointed ovipositor at the end of their abdomen. Female flies were separated into two groups. One group of females was reared on protein diet that contained both yeast and sugar and the other group was reared on sugar only until they were transferred to experimental cages. Females reared on protein diet were used to assess oviposition deterrence and those on sugar only to assess toxicity. The reason for keeping flies deprived of protein was that protein is critical for producing fertile eggs and protein deprived females will show attraction to protein. Therefore, for assessment of toxicity, females were initially maintained on sugar only and switched to a diet containing protein at the onset of their sexual maturity. Males were in one group and reared on protein diet from emergence onwards because males showed strong attraction to methyl eugenol and there was no need to have different food regimes for their attraction purpose.

Preparation of plant extracts

Plants were dried in the shade for three months. Dried plant material was ground to powder using an electric grinder. Metabolites were extracted by a maceration method using organic solvent methanol at room temperature (Padin et al., 2013). After 2 d the solvent layer was filtered with Watman No.1 filter paper and the process repeated three times. The filtrate was concentrated using a rotary evaporator at 35°C and resulting extracts stored at 4°C.

Bioassays

Adult male fruit fly toxicity bioassay

After 14 d of emergence *B. zonata* males from large cage were shifted to experimental screened cages 15x15x20 cm and kept them for 1 h before bioassay. Laboratory adopted males reached sexual maturity at 14 d. Studies of *B. zonata* male age response to ME have not been undertaken, however, Shelly et al. (2010) reported that many of the ME responsive males are responsive at the beginning of their sexual maturity. Therefore, sexually mature males were selected for toxicity bioassay by mixing plant extracts with ME. For this bioassay, crude methanolic plant extracts were tested against adult male fruit fly. Four mg each of plant extract was mixed with 200 µL ME and 50 µL added to single filter papers in three replicate Petri dishes. These Petri dishes (without lids) were then placed in experimental cages having male flies at 10:00 h and removed after 24 h. Thirty males (10 males in each replication) were exposed to each treatment. Three controls were included; ME (a negative control), untreated filter paper (a negative control) and organophosphate synthetic insecticide i.e. 2,2-dichlorovinyl dimethyl phosphate (DDVP; the positive control). In the positive control,4 µL of DDVP was mixed with 200 µL of ME. Mortality was observed after 24 and 48 h.

Adult female fruit fly toxicity bioassay

From emergence, female flies were provided with sugar only and after 14 d they were transferred to experimental cages, starved for 8 h and switched to protein diet. Feeding toxicity bioassay was used to analyze the toxicity of plant extracts by mixing each plant extract into the diet of the flies (Shakunthala & Thomas, 2001b). For this purpose, each crude methanolic plant extract was mixed at 2% into the adult diet and placed on filter paper in Petri dishes. Four mg of each plant extract was first dissolved in 200 µL of methanol and then mixed with diet containing 2 mg of sugar and 2 mg of hydrolyzed yeast and placed in experimental cages. In each treatment total of 30 flies were exposed in three replicates of 10 flies. Three control treatments were included; methanol only (a negative control), food containing hydrolyzed yeast and sugar (a negative control) and commercial protein bait containing Spinosad (GF 120; positive control). In the positive control, 12 mg of GF 120 was mixed with 90 µL of water as recommended by the manufacture. Mortality was observed after 24 and 48 h.

Repellence and oviposition deterrence bioassay

Fifty each of virgin male and female fruit flies, maintained on protein diet for 14 d, were combined for copulation 90 min before sunset in a 45x45x45 cm plexiglass screen cage. Fruit fly couples were collected in plastic vials, transferred to separate cages and left to continue copulation. Next morning female flies were transferred to experimental cages and provide a protein diet and water *ad libitum*. Fifteen flies (five per replicate) were taken for each treatment for evaluation. Next day at 10:00 h, the females were provided access to guava fruit treated with 2% crude methanolic plant extracts. The fruit used were of uniform size, cold treated at 4°C for 22 d in order to eliminate any larvae from wild flies and kept at room temperature for 24 h before exposure for oviposition. In each treatment, 60 mg of plant extract was mixed with 3 mL of methanol. An aliquot of 1 mL was applied on each guava by pipette while continuously rotating the guava to ensure uniform distribution over the fruit. Three guavas were used for each treatment (1 guava per replication). After treatment, the guavas were allowed to dry for 2 h, then exposed to flies for 48 h. For repellence bioassay, settled or repelled females from treated guava in each treatment were counted every 2 h. For oviposition, deterrent effect bioassay, female flies were removed after 48 h and guavas were placed in sawdust for 15 d so that larvae could pupate in sawdust. Number of pupae and emerged adults were counted. Two negative control treatments were included; methanol only and untreated guavas.

Data analysis

Percent repellence was calculated by using the formula (Rehman et al., 2009):

$$\%R = [1/2\ (A\text{-}B)/A] \times 100$$

Where R represents repellence, a represent half of the number of flies settled on both treated and untreated guavas and B represents number of flies settled on treated guava. Differences in mortality, repellence and oviposition deterrence caused by different plant extracts were analyzed by one-way analysis of variance (ANOVA) Complementary pairwise comparisons of means were performed by Tukey's test. All analyses were performed with SPSS version 16.

Results

Adult mortality

Male mortality between treatments was significantly different (F= 5.79, *P*>0.001). Five treatments, DDVP and *T. minuta*, *I. rugosus*, *E. sideroxylon* and *D. mucronata* extracts, gave similar but significantly higher mortality than all other treatments (Table1). Female mortality was significantly higher with GF 120 treatment than all other treatments (Table1).

Table1. Mean percentage mortality of males and females of *Bactrocera zonata* exposed tomethanolic extracts of different plants in female protein baits and male lures under laboratory conditions

Pesticide/Plant extracts	Mortality (female)	Mortality (male)
GF 120*	100% a	-
DDVP*	-	100% a
Tagetes minuta	6.6% b	73.3% a
Isodon rugosus	16.6% b	53.3% a
Daphne mucronata	13.3% b	50% a
Eucalyptus sideroxylon	3.3% b	50% a
Calotropis procera	0% b	43.3% b
Cinnamonum camphora	16.6% b	40% b
Boenninghausenia albiflora	10% b	26.6% b
Methanol*	0% b	-
Methyl eugenol*	-	13.3% b
Untreated	0%b	20% b

*GF 120, positive control for female toxicity bioassay; DDVP, positive control for male toxicity bioassay; methanol, negative control for female toxicity bioassay; methyl eugenol, negative control for male toxicity bioassay. Means followed by the same letter within a column are not significantly different (Tukey's test, P< 0.05).

Effect of treatments on settlement and repellence behavior of females

Mean number of females settling on untreated guavas was greater than on treated guavas (F= 5.79, *P*>0.001). The minimum number of females (3.3 of 15) that settled on any treated guavas was on those treated with *D. mucronata* extract, followed by those treated with *B. albiflora* (3.7 of 15), *I. rugosus* (4 of 15) and *C. camphora* (4.7 of 15) extracts. Both methanol and *C. procera* extract treated guavas had 5 of 15 females settle. With *E. sideroxylon* and *T. minuta* extracts 5.3 and 6.3 of the 15 females settled, which was also less than the 8.3 females that settled on untreated guavas.

In the repellence bioassay, different treatments showed significantly different repellence (F= 3.06, *P*>0.023). *Boenninghausenia albiflora, D. mucronata, I. rugosus* and *C. camphora* extracts gave similar but significantly higher repellence than *C. procera and E. sideroxylon* extracts, and methanol. *Tagetes minuta* extract showed the least repellence, which was similar to that of untreated guavas (Figure1).

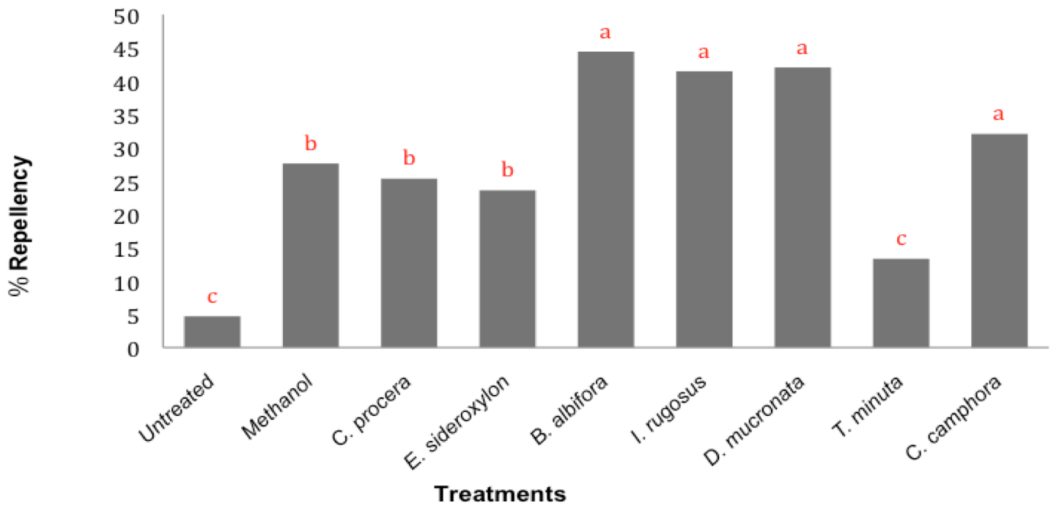

Figure1. Mean percent repellence (%) caused by selected plant extracts at 2% concentration against fruit flies, *Bactrocera zonata,* under laboratory conditions. Means followed by the same letter within a column are not significantly different (Tukey's test, P< 0.05).

Oviposition deterrence

Effect of plant extracts on recovery of pupae

There were significant treatment effects on number of pupae recovered from guavas. (F= 5.15, *P*= 0.001) (Table 2). The lowest number of pupae (3.3) was obtained from guavas treated with *T. minuta* extract, followed by those treated with *D. mucronata* and *E. sideroxylon*. Treatment with *B. albiflora*, *C. camphora*, *C. procera* and *I. rugosus* extracts resulted in recovery of about 30 to 40 pupae. The highest number of pupae were recovered from untreated guavas.

Effect of plant extracts on adult emergence

Paralleling the results for pupae, the lowest number of adults emerged was for guavas treated with *T. minuta* extract, followed those treated with *D. mucronata* and *E. sideroxylon* extracts (Table 2).Guavas treated with *B. albiflora*, *C. camphora, C. procera* and *I. rugosus* extracts had greater numbers, but the highest number of adults that emerged was for untreated guavas.

Table 2. Mean number of *Bactrocera zonata* pupae recovered and adults emerging for guava fruit treated with various plant extracts and exposed for oviposition for 48 h

Plants Extracts	Pupae Count	Adult Emergence
Tagetes minuta	3.3 ± 6.16 a*	0.3 ± 5.39 a
Daphne mucronata	26.7 ± 4.55 b	7.0 ± 4.95 a
Eucalyptus sideroxylon	28.3 ± 4.62 b	13.0 ± 4.71 b
Boenninghausenia albiflora	31.7 ± 4.69 c	13.3 ± 4.73 b
Cinnamomum camphora	35.0 ± 8.38 c	16.7± 4.44 b
Calotropis procera	35.7 ± 9.58 c	27.3 ± 3.67 c
Isodon rugosus	39.7 ± 9.78 c	29.7 ± 4.16 c
Untreated	62.70 d	45.30 d

*Means followed by the same letters within each column are not significantly different (Tukey's test, *P*< 0.05).

Discussion

The plant species selected for study are common in Pakistan, so are readily available. Apart from *I. rugosus* and *D. mucronata,* the other species have all been reported to have insecticidal effects on a range of insect pests, so this is the first report of the insecticidal potential of *I. rugosus* and *D. mucronata*.

The plant extracts were assayed for their effect as toxicants against *B. zonata* males and females, their repellence effect on females and oviposition deterrence effect. Extracts of *T. minuta* were found to give the highest male mortality and oviposition deterrence, whereas *B. albiflora* extract showed the strongest repellence. Female mortality with these plant extracts was less than for males, which may have been due to differences in the mode of application for males and females. The highest mortality of males with *T. minuta* extract was about 73% (Table 1), but against females, the highest mortality was only 16% with *I. rugosus* extract, which was not significantly different from the results with the other plant extracts. The lower mortality in females compared to males may have been due to the plant extracts being mixed into the diet and females may have consumed less toxicants by restricting their intake, whereas the males may have been unable to restrict their intake of plant extracts mixed with ME (Haq et al. 2014).

Currently farmers use synthetic insecticides to control fruit flies. In a summer crop of guava, 5 to 7 insecticide sprays are applied, in mango, 2 to 3 sprays and in plums, peaches, apricot and pear, sprays are applied every 10 to 15 days. Ten percent of total synthetic insecticides applied in Pakistan are for fruit flies (Stonehouse et al., 1998; Siddiqi et al., 2006). Due to unacceptable levels of insecticide residues in fruit and vegetables, exports of these are adversely affected.

As plants contain a rich source of bioactive compounds, they may give an alternative solution to synthetic insecticides for control plant pests and diseases (Ghosh et al., 2012; Pino et al., 2013). According to different reports, plant extracts showed strong pesticidal properties and have additional advantages as these chemicals can be specific for targeted pests, biodegradable to nontoxic products and therefore, considered as appropriate to apply in integrated pest management programs (Tare et al., 2004).

In this study, we assayed the insecticidal effects of different plants by applying extracts in ME and protein baits that ensured the ingestion of these extracts. Among the plant extracts assayed, *T. minuta* extracts had the highest toxicity for males. The studies of Shivendra & Singh (1998), Shakunthala & Thomas (2001a) and Tewari (2001) revealed the insecticidal properties of *A. calamus* and *A. indica* against fruit flies. Assessing the efficacy of neem extracts by applying them along with food, Van Randen & Roitberg (1998) reported an inverse effect on adult survival and on the development of eggs of Western fruit fly. Van Randen & Roitberg (1998) also reported that the artificial diet containing neem based insecticides has negative effect on pupae formation and adult emergence of Western fruit fly. Shakunthala & Thomas (2001b) indicated the significant changes in the appearance of reproductive organs of adult *B. cucurbitae* when the flies were fed with a diet containing *A. calamus* extract. However, the plant extracts assayed in this study did not cause high female mortality. In addition to plant extracts causing mortality of adult fruit flies, they can repel fruit fly females and deter their oviposition. These effects encourage their incorporation in integrated pest management strategies against fruit flies. This study recorded the highest repellence with *B. albiflora* and *D. mucronata* extracts and in was accordance with Walter (1999) and Jimenez et al. (2000), who reported repellence of a number of botanical pesticides against *B. zonata* on guava. Similarly, Singh et al. (2007) reported the repellent effect of neem products as biopesticide against *B. zonata* and *B. dorsalis*. Later studies by Rehman et al. (2009) indicated the effectiveness of petroleum ether, ethanol and acetone extracts of *A. calamus, Citrullus colocynthis* (L.) Schrad.*, C. longa, P. harmala, S. lappa, V. jatamansi* and for repellence and oviposition deterrence of *B. zonata* and reported promising effects of these plant based pesticides. Solangi et al. (2011) reported that botanical pesticides, such as neem oil, neem seed powder solution, tobacco leaf solution and solution prepared from Eucalyptus leaves, have repellent effects on *B. zonata.*

Khattak et al. (2006) demonstrated the repellence and growth inhibition caused by petroleum ether, acetone and ethanol extracts of *P. harmala, S. lappa* and *Valariana officinalis* L. of *B. zonata*. These results are in concurrence with the studies of Akhtar et al. (2004), reporting that three plants, sweet flag,

neem seed and turmeric rhizomes had repellent effects on *B. zonata* and that turmeric extract had pronounced effect on suppression of egg laying and emergence of pupae and adults. Siddiqi et al. (2006) indicated the pesticidal effect of acetone, petroleum ether and ethanol extracts of turmeric on *B. zonata* settling response and fecundity, and reported promising results. Studies on foraging and oviposition behavior of different *Bactrocera* spp. found that these species have a non-resource based mating system (Kuba & Koyama, 1985; Iwahashi & Majima, 1986) and adult flies engaged in mating during dusk time at the surrounding vegetation of the main host fruits. This behavior of flies suggests that such control strategies should be useful as an area-wide integrated pest management (AW-IPM) approach. The systemic insecticides are not the preferred choice for fruit flies control in guava fruit and, the insecticides having contact action remained insufficient to give successful control of fruit flies, unless targeting the fruit fly adults in abandoned areas and vegetation. Therefore, plant extract formulations giving oviposition deterrence effects have an added advantage over synthetic insecticides and can be included in integrated pest management programs for the control of fruit flies (Khattak et al., 2006).

Conclusion

The results of this study demonstrated broad-spectrum toxic effects of the tested plant extracts against *B. zonata*. The noteworthy results are the efficiency of the extracts against fruit fly as toxicants, repellents and oviposition deterrents. These actions can be exploited for the control of *B. zonata* by developing proper delivery strategies. Further investigations are required to separate and identify active compounds present in these active extracts through chromatographic techniques and by different spectroscopic analysis. Such compounds may also have profound effects on hormonal balance and reproductive physiology of other insect pests. This information can be helpful in developing some competent formulations for commercial use against *B. zonata*.

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References

Akhtar, N., G. Jilani, R. Mahmood, M. Ashfaque & J. Iqbal, 2004. Effects of plant derivatives on settling response and fecundity of peach fruit fly *Bactrocera zonata* (Saunders). Sarhad Journal of Agriculture, 20: 269-274.

Awad, A. A., N. A. Ali & H. O. Mohamed, 2014. Ultrastructure of the antennal sensillae of male and female peach fruit fly, *Bactrocera zonata*. Journal of Insect Science, 14 (45): 1-15.

El-Aw, M. A. M, K. A. A. Draz, A. G. Hashem & I. R. El-Gendy, 2008. Mortality comparison among Spinosad-, Actara-, Malathion-, and Methomyl-containing baits against peach fruit fly, *Bactrocera zonata* (Saunders) (Diptera: Tephritidae) under laboratory conditions. Journal of Applied Science and Research, 4: 216–223.

Ghosh, A., N. Chowdhury & G. Chandra, 2012. Plant extracts as potential mosquito larvicides. The Indian Journal of Medical Research,135 (5): 581–598.

Haq, I., Vreysen, M. J. B., Cáceres, C., Shelly, T. E. & Hendrichs, J., 2014. Methyl eugenol aromatherapy enhances the mating competitiveness of male *Bactrocera carambolae* Drew & Hancock (Diptera: Tephritidae). Journal of Insect Physiology, 68: 1-6.

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

Iwahashi, O. & T. Majima, 1986. Lek formation and male-male competition in the melon fly, *Dacus cucurbitae* Coquillett (Diptera: Tephritidae), Applied Entomology and Zoology, 21: 70-75.

Jimenez, V. J. A., M. A. Hoy & F. S. Davies, 2000. Integrated pest management-compatible pesticides for the guava fruit flies *Bactrocera zonata*. Journal of Economic Entomology, 93: 357–367.

Khattak, M. K., M. F. Shahzad & G. Jilani, 2006. Effect of different extracts of Harmal, *Peganum harmala* L. rhizomes of Kuth, *Saussurea lappa* C. B. Clarke and Balchar, *Valariana officianalis* L. on the settling and growth of peach fruit fly, *Bactrocera zonata* Saunders. Pakistan Journal of Entomology, 28 (1): 129-132.

Kuba, H. & J. Koyama, 1985. Mating behavior of wild melon flies, *Dacus cucurbitae* Coquillett (Diptera: Tephritidae) in a field cage: Courtship behaviour. Applied Entomology and Zoology, 20: 365-372.

Padin, S. B., C. Fuse, M. I. Urrutia & G. M. Dal Bello, 2013. Toxicity and repellency of nine medicinal plants against *Tribolium castaneum* in stored wheat. Bulletin of Insectology, 66 (1): 45-49.

Pino, O., Y. Sánchez & M. M. Rojas, 2013. Metabolitos secundarios de origen botánico como una alternativa en el manejo de plagas. I: Antecedentes, enfoques de investigación y tendencias. Revista de Protección Vegetal, 28 (2): 81-94.

Rehman, J., G. Jilani, M. A. Khan, R. Masih & S. Kanvil, 2009. Repellent and oviposition deterrent effects of indigenous plant extracts to peach fruit fly, *Bactrocera zonata* Saunders (Diptera: Tephritidae). Pakistan Journal of Zoology, 41: 101-108.

Shakunthala, N. & J. Thomas, 2001a. Oviposition deterrence of *Acorus calamus* L. extracts on melon fly, *Bactrocera cucurbitae*. Journal of Tropical Agricultural Science, 39: 149-151.

Shakunthala, N. & J. Thomas, 2001b. Evaluation of chemosterilent effect of *Acorus calamus* L. extracts on melon fly, *Bactrocera cucurbitae* Coq. Journal of Tropical Agricultural Science, 39: 145-148.

Shehata, N. F., M. W. F. Younes & Y. A. Mahmoud, 2008. Biological studies on the peach fruit fly, *Bactrocera zonata* (Saunders) in Egypt. Journal of Applied Science and Research, 4: 1103–1106.

Shelly, T. E., J. Edu & D. O. McInnis, 2010. Pre-release consumption of methyl eugenol increases the mating competitiveness of sterile males of the Oriental fruit fly, *Bactrocera dorsalis*, in large field enclosures. Journal of Insect Science, 10 (8): 1-16.

Shivendra, S. & R. P. Singh, 1998. Neem (*Azadirachta indica*) seed kernel extracts and azadirachtin as oviposition deterrents against the melon fly (*Bactrocera cucurbitae*) and the oriental fruit fly (*Bactrocera dorsalis*). Phytoparasitica, 26: 191-197.

Siddiqi, A. R., G. Jilani, J. U. Rehman & S. Kanvil, 2006. Effect of turmeric extracts on settling response and fecundity of peach fruit fly (Diptera: Tephritidae). Pakistan Journal of Zoology, 38: 131-135.

Singh, M., D. Gupta, R. Gupta & S. D. Kashyap, 2007. Population dynamics of fruit flies, *Bactrocera* spp. (Diptera: Tephritidae). Himachal Journal of Agricultural Research, 33 (2): 292-294.

Solangi, B. K., R. Sultana, M. S. Wagan & N. Ahmed, 2011. Repellent action of botanical pesticides against fruit fly, *Bactrocera zonata* Saunders in laboratory. Pakistan Journal of Entomology, Karachi, 26 (1): 41-45.

Steiner, L. F., W. G. Hart, E. J. Harris, R. T. Cunningham, K. Ohinata & D. C. Kamakahi, 1970. Eradication of the oriental fruit fly from the Mariana Islands by the methods of male annihilation and sterile insect release. Journal of Economic Entomology, 63:131-135.

Stonehouse, J. M., J. D. Mumford & G. Mustafa, 1998. Economic losses to tephritid fruit flies (Diptera: Tephritidae) in Pakistan. Crop Protection, 17 (2): 159-164.

Tan, K. H. & R. Nishida, 2012. Methyl eugenol: its occurrence, distribution, and role in nature, especially in relation to insect behaviour and pollination. Journal of Insect Science,12 (56): 1-74.

Tare, V., S. Deshpandem & R. Sharma, 2004. Susceptibility of two different strains of *Aedes aegypti* (Diptera: Culicidae) to plant oils. Journal of Economic Entomology, 97: 1734–1736.

Tewari, K. K., 2001. Effect of plant extracts spray on fruit fly transmission of cucumber mosaic virus. Journal of Physical Research, 14: 207-208.

Van Randen, E. J & B. D. Roitberg, 1998. The effect of neem (*Azadirachta indica*) based insecticides on the survival and development of Juvenile Western cherry fruit fly (*Rhagolelis indifferens*) (Diptera: Tephritidae), Canadian Entomologist, 130: 869-876.

Vargas, R. I., J. C. Piñero, R. F. Mau, E. B. Jang, L. M. Klungness, D. O. McInnis, E. B. Harris, G. T. Mc Quate, R. C. Bautista & L. Wong, 2010. Area-wide suppression of the Mediterranean fruit fly, *Ceratitis capitata*, and the Oriental fruit fly, *Bactrocera dorsalis*, in Kamuela, Hawaii. Journal of Insect Science, 10 (135): 1-17.

Vargas, R. I., J. C. Piñero & L. Leblanc, 2015. An overview of pest species of *Bactrocera* fruit flies (Diptera: Tephritidae) and the integration of biopesticides with other biological approaches for their management with a focus on the pacific region. Insects, 6 (2): 297-318.

Walter, J. F., 1999. "Commercial Experience with Plant Products in Relation to Control Fruit Flies, 155-170". In: Biopesticides: Use and Delivery (Eds. F. R. Hall & J. J. Menn). Humana Press, Totowa, New Jersey.

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Original article (Orijinal araştırma)

Rosmarinus officinalis L. (Lamiales: Lamiaceae) uçucu yağının *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Chrysomelidae)'un ergin öncesi dönemlerine karşı fumigant toksisitesi[1]

Fumigant toxicity of *Rosmarinus officinalis* L. (Lamiales: Lamiaceae) essential oil against immature stages of *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Chrysomelidae)

Melek GÜDEK[2*] **Hüseyin ÇETİN[3]**

Summary

The cowpea beetle, *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Chrysomelidae) is a major pest of stored legumes seeds such as cowpea, chickpea, lentil, soybean, bean in both Turkey and many other countries. In this study, the fumigant effect of the essential oil obtained from rosemary [*Rosmarinus officinalis* L. (Lamiales: Lamiaceae)] plant was investigated against eggs, first and last-instar larvae and pupae of *C. maculatus* in chickpea. The study was conducted at Selcuk University, Faculty of Agriculture, Department of Plant Protection Entomology Laboratory under conditions at 28±2°C temperature, 55±5% relative humidity and fully dark conditions in 2013. As parallel to the increase of exposure time and applied dose, an increase occurred in mortality of immature stages of *C. maculatus*. First instar larvae and eggs were the most susceptible stages to rosemary oil while the pupae and last-instar larvae were the most tolerance stages. LC_{50} values for eggs, first and last instar larvae and pupae were found as 34.57, 27.64, 60.39 and 60.34 µl/l for 24 h exposure time of rosemary essential oil vapor, respectively. At 50 µl/l air dose of rosemary oil and exposure time of 24 h, mortality rates of eggs, first instar larvae, last instar larvae and pupae were determined 100, 91.33, 56 and 46%, respectively. In conclusion, rosemary essential oil showed high fumigant toxicity against the immature stages of *C. maculatus*.

Key words: *Rosmarinus officinalis*, essential oil, fumigant toxicity, *Callosobruchus maculatus,* immature stages

Özet

Türkiye'de ve birçok ülkede Börülce tohum böceği, *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Chrysomelidae) börülce, nohut, mercimek, soya fasulyesi, kuru fasulye gibi depolanan baklagil tohumlarında önemli bir zararlıdır. Bu çalışmada, nohutta Börülce tohum böceğinin yumurtalarına, dane içerisindeki ilk ve son dönem larvalarına ve pupalarına karşı biberiye [*Rosmarinus officinalis* L. (Lamiales: Lamiaceae)] uçucu yağının fumigant toksisitesi araştırılmıştır. Çalışma, 2013 yılında Selçuk Üniversitesi Ziraat Fakültesi Bitki Koruma Bölümü Entomoloji Laboratuvarında 28±2ºC sıcaklık, %55±5 orantılı nem ve tamamen karanlık ortamda yürütülmüştür. Doz ve maruz bırakma süresinin artışına paralel olarak *C. maculatus*'un ergin öncesi dönemlerinin ölüm oranlarının arttığı belirlenmiştir. Ergin öncesi dönemler içerisinde en hassas dönemlerin yumurta ve birinci larva dönemi olurken, en dayanıklı dönemlerin ise pupa ve son dönem larvaların olduğu tespit edilmiştir. 24 saat süreyle biberiye uçucu yağının gaz haline maruz bırakılan yumurtaların, birinci dönem larvaların, son dönem larvaların ve pupaların LC_{50} değerleri sırasıyla 34.57, 27.64, 60.39 ve 60.34 µl/l hava olarak belirlenmiştir. Biberiye uçucu yağın 50 µl/l hava konsantrasyonuna24 saat süreyle maruz bırakılan yumurta, birinci dönem larva, son dönem larva ve pupalarda sırasıyla, %100, 91, 56 ve 46'ya varan ölüm oranları tespit edilmiştir. Sonuç olarak biberiye uçucu yağının *C. maculatus*'un ergin öncesi dönemlerine karşı yüksek fumigant toksisiteye sahip olduğu tespit edilmiştir.

Anahtar sözcükler. *Rosmarinus officinalis*, uçucu yağ, fumigant toksisite, *Callosobruchus maculatus*, ergin öncesi dönemler

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Giriş

Mercimek, nohut, fasulye, bezelye, bakla ve börülceyi içine alan yemeklik dane baklagiller yüksek miktarlarda protein içermelerinden dolayı insan ve hayvan beslenmesinde oldukça önemli bir yere sahiptir. Kullanılabilir tarım alanları nüfus artışına paralel olarak artmamakta aksine her geçen gün tarım yapılan alanlar daralmaktadır. Bu nedenle birim alandan elde edilen ürün miktarının arttırılması birinci derecede önemli olmakla beraber üretimden tüketime kadar ürünün uygun bir şekilde korunması da büyük önem taşımaktadır (Anonymous, 2014).

Baklagil tohum böceklerinden Börülce tohum böceği, *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Chrysomelidae) tropik ve subtropik bölgelerde börülce, nohut, mercimek, soya fasulyesi ve fasulyenin en önemli zararlılarındandır. *Callosobruchus maculatus,* hasattan önce börülcelere bulaşmakta ve depoda ayda bir nesil vererek çok hızlı bir şekilde çoğalmakta ve depolanmış baklagillerdeki bulaşmalar 3-4 ay içinde %50'nin üzerine çıkmaktadır (Baidoo et al., 2010). Bulaşmış oldukları üründe beslenmeleri sonucu, üründe ağırlık kayıplarına, tohumluk özelliklerinin düşmesine, kalite ve besin değerlerinde olumsuz değişmelere yol açarak ticari değerin düşmesine neden olmaktadır (Boxall, 2001; Ofuya et al., 2010). Bunlarla mücadelede sentetik insektisitler yoğun bir şekilde kullanılmaktadır.

Depolarda kullanılmakta olan malathion, chlorpyrifos –methyl, fenitrothion, primiphos-methyl, etrimfos vb. birçok etkili maddeye karşı bazı ülkelerde önemli depolanmış ürün zararlıların direnç geliştirdiği bildirilmektedir (Ferizli & Emekçi, 2010). Nitekim, Nijerya'da 3 coğrafik bölgeden toplanılan *C. maculatus*'un dayanıklılığı üzerinde iklimin etkisinin araştırıldığı farklı bir çalışmada; *C. maculatus* ile bulaşık börülce tohumları 20 lokasyondan toplanmış ve laboratuvarda primiphos methyl'in 5 farklı dozu uygulanmıştır. Bir günlük erginlerin yedi günlük erginlerden daha hassas olduğunu, dayanıklılığın Mangrov ormanlarından alınan örneklerde kademeli olarak bir artışa sebep olurken, Savana ormanlarına doğru kademeli olarak azalmadan önce yağmur ormanlarının kenarlarında zirveye ulaştığını, bunun sebebinin de sıcak ve nemli bölgelerde çoğalmanın daha hızlı olmasına dolayısıyla canlıların daha toleranslı olmasına bağlanırken, sıcak ve daha kurak yerlerde ani hava değişiminin canlıları daha çok etkilemesine bağlanmıştır (Odeyemi et al., 2006).

Fosfinle fumigasyonda belli düzeydeki gaz konsantrasyonun belli süreyle ortamda tutulması oldukça önemlidir. Ülkemizde özellikle fumigasyonda gaz sızdırmaz ortamın yetersizliği ve gaz konsantrasyonlarının uygulama süresince ölçülmesi konusunda çok büyük eksiklikler bulunmaktadır. Bu durumlar özellikle fosfinde direnci tetikleyen koşul olarak karşımıza çıkmaktadır (Ferizli & Emekçi, 2010). Fosfinle fumigasyonda dünyada 45'den fazla ülkede depo zararlılarının fosfine karşı dayanıklılık geliştirdikleri tespit edilmiştir. Pakistan'da buğday ve pirinç depolarından toplanılan *Tribolium castaneum* (Herbst, 1797), *Rhyzoperta dominica* (Fabricius, 1792) ve *Trogoderma granarium* Everts, 1898 türlerinde fosfininin neden olduğu dayanıklılık seviyesi değişkenlik göstermiş ve *T. castaneum*'da 80 kat daha fazla dayanıklılığa sebep olduğu bildirilmiştir. (Alam et al., 1999). Fas'da *R. dominica, T.castaneum* ve *Stophilus oryzae* (Linnaeus, 1763) üzerinde yapılan bir çalışmada *S. oryzae*'nin bir populasyonu hariç test edilen tüm örneklerde fosfine dayanıklık saptanmıştır (Benhalima et al., 2004).

Uzun yıllardan beri devam eden ve özellikle son yıllarda yoğunlaşan sentetik pestisitlerin kullanımı ekolojik dengeyi bozarak insan sağlığını tehdit eder duruma gelmiştir. Sürekli ve yoğun bir şekilde sentetik kimyasalların kullanımı hedef zararlıların direnç geliştirmesine (Zettler, 1982; Tripathi et al., 2001), hedef olmayan canlıların (parazitler, predatörler, parazitoitler, tozlayıcı böcekler) etkilenmesine çevrede ve üründe kalıntıya ve bitkilerde fitotoksisiteye neden olmuştur (Ferizli & Emekçi, 2000; Isman, 2000; Mahfuz & Khalequzzaman, 2007; Khani & Asghari, 2012). Bu durum tarımsal alanda alternatif mücadele arayışını hızlandırmış ve sentetik pestisitlere alternatif olarak bitkilerdeki sekonder bileşikler ve uçucu yağların pestisit olarak kullanımını daha önemli hale getirmiştir. Son yıllarda uçucu yağların ve bileşenlerinin, önemli depo zararlılarından baklagil tohum böceklerinin erginlerine fumigant etkisi üzerindeki çalışmalar oldukça hız kazanmıştır (Raja et al., 2001; Karakoç et al.,2006; Al-Sarar et al., 2014; Çetin et al., 2014; Selimoğlu et al., 2015).

Özellikle daha önce uçucu yağlarla yapılan çalışmaların çoğunluğu, baklagil tohum böceklerinin erginleri üzerinde yürütülmüştür. Ergin öncesi dönemlere karşı çok az çalışma bulunmaktadır. Halbuki depoya ergin öncesi dönemlerle bulaşık olarak getirilen üründeki bu bulaşmalar ergin çıkış delikleri gözlemleninceye kadar fark edilmemekte ve zararlı uygun koşullarda hızlı bir şekilde gelişmesini sürdürmektedir. Dolayısıyla fumigant olarak kullanılacak ürünün ergin öncesi dönemlere karşıda toksik etki göstermesi oldukça önemlidir. Bu çalışmada da biberiye uçucu yağının börülce tohum böceğinin ergin öncesi dönemlerine (yumurta, larva ve pupa) karşı fumigant toksisitesi araştırılmıştır. Böylece biberiye uçucu yağının *C. maculatus* ile mücadelesinde biyo-fumigant olarak kullanılabilme potansiyeli ortaya konulmaya çalışılmıştır.

Materyal ve Yöntem

Callosobruchus maculatus (Fabricius, 1775)'un yetiştirilmesi

Deneme, laboratuvar koşullarında 2013 yılında, Selçuk Üniversitesi Ziraat Fakültesi Bitki Koruma Bölümü Entomoloji Laboratuvarı'nda yürütülmüştür. Denemede kullanılan *C. maculatus*, 28±2ºC sıcaklık, %55±5 nispi nemde ve tamamen karanlık koşullarda çalışan iklim kabininde (Nüve Klimatik Test Kabini TK 120) bulunan stok kültürden elde edilmiştir. Stok kültürden elenerek alınan erginler içerisinde nemi %12 olarak ölçülen yaklaşık 250 g temiz nohut (*Cicer arietinum* L.) (o yıl hasat edilmiş Cevdet Bey çeşidi) bulunan 1 l'lik cam kavanozlara aktarılmıştır. Bu kavanozlarda 4-5 gün süreyle yumurta bırakmalarına izin verilen erginler tekrar elenerek ortamdan alınıp stok kültür kavanozlarına aktarılmıştır.

Günlük olarak kavanozlar içerisindeki çıkışlar takip edilmiş ilk ergin çıkışlarını takip eden 3-4 gün sonra ortamdaki erginler alınarak stok kültür kavanozuna aktarılmış ve ertesi gün daha çok sayıda çıkan erginlerden (1 günlük) 50 dişi, 50 erkek içerisinde (dişiler daha büyük ve pygidiumunun kenarları koyu renkte ortası açık renkte iken erkekler daha küçük ve bunların pygidiumu tamamen homojen beyazımsı renktedir) 200 tane nohut bulunan kavanoza aktarılmış ve homojen bir şekilde aynı yaşta yumurta elde etmek için ertesi gün erginler hafif bir şekilde elenerek alınmıştır. Böylece aynı yaştaki yumurtalarla bulaşık nohutlar, ergin öncesi dönemlere fumigant toksisite çalışmalarında kullanılmıştır.

Zararlının ergin öncesi dönemlerin saptanması

Ön denemelerde ergin bir dişi ve bir erkek, içerisinde 10 tane nohut bulunan cam petri kaplarına konulmuş ve bir gün boyunca çiftleşip yumurta bırakması sağlanmıştır. Ertesi gün petriden erginler uzaklaştırılmış ve yumurtalar günlük olarak stereo mikroskop altında iğne yardımıyla kazınarak yumurtanın altında kalan larva giriş delikleri kontrol edilmiştir. Böylece 5. günde ilk giriş deliklerinin görülmesiyle yumurtadan ilk larva çıkışları tespit edilmiştir. Nohut tanelerinde çıkış kapaklarının arkasında kahverengileşmenin görülmesiyle 20. günde pupa dönemine girdiği ve 3-4 gün sonra erginlerin çıktığı belirlenmiştir.

Biberiye (*Rosmarinus officinalis* L.) bitkisinden uçucu yağının elde edilişi

Biberiye uçucu yağının elde edilmesinde Topuz & Madanlar (2011)'ın bildirdiği yöntem kullanılmıştır. Biberiye, Antalya'nın Geyikbayır köyünden (755 m rakımından 36.876º enlem 30.457º boylama sahip koordinatlarından) getirtilmiştir. Biberiye bitkisinin teşhisi, Selçuk Üniversitesi Fen Fakültesi Biyoloji Bölümü Botanik Anabilim Dalı öğretim üyelerinden Prof. Dr. Yavuz BAĞCI tarafından yapılmıştır. Bitki gölge, havadar laboratuvar ortamında kurutulup yapraklarını dallarından ayırdıktan sonra öğütülmüştür. Daha sonra öğütülmüş biberiyeden 100 g tartılıp Clevenger düzeneğinde 1:10 oranında su ile karıştırılmış ve 2-3 saat su destilasyonuna tabi tutularak uçucu yağ elde edilmiştir. Elde edilen uçucu yağ denemede kullanılmak üzere +4'deki buzdolabında muhafaza edilmiştir.

Konsantrasyonların hazırlanması

Denemede kullanılan konsantrasyonlar, biberiye uçucu yağı ile asetonun (Merck, KN: 100014.2500) 1/1 oranında seyreltilmesiyle elde edilmiştir. Ön denemeler sonucunda %5-99 arasında ölüme neden olan 10, 20, 30, 40 ve 50 µl/l hava konsantrasyonları belirlenmiştir. Uygulamalar esnasında asetonun uçması için 40 sn bekletildikten (Çetin et al., 2009) sonra kavanoz kapakları kapatılmıştır.

Pozitif kontrollerde saf aseton (50 µl/l hava) kullanılmıştır. Negatif kontrollerde hiçbir şey uygulanmamış, denemeler her bir maruz bırakma süresi için ayrı ayrı kurulmuş ve uçucu yağa maruz bırakma süreleri kadar kavanoz kapakları kapalı tutulmuştur. Daha sonra kapaklar açılmış ve ağızları tülbentle kapatılmıştır. Ergin çıkışları sona erene kadar 28±2ºC sıcaklık, %55±5 nispi nemde ve tamamen karanlık koşullarda çalışan iklim kabininde bekletilmiş ve sayımları yapılmıştır.

Fumigant toksisite çalışmaları

Yumurtaya fumigant toksisite

Dane nem oranı %12 olarak ölçülen eşit büyüklükteki 200 adet nohut,1 litrelik cam kavanozlara konulmuş ve üzerlerine stok kültürden elenen farklı yaşlardaki 50 dişi ve 50 erkek ergin bırakılarak tülbentle kapatılmıştır. Bir gün sonra nohutlar elenerek erginler uzaklaştırılmıştır. Stereo mikroskop altında, nohutlar üzerindeki 1 günlük yumurtalar iğne yardımıyla kazınarak her danedeki yumurta sayısı 5'e düşürülmüştür. Bu nohutlardan 10 tanesi, 7 cm yüksekliğinde, 3 cm çapındaki plastik tüpler içerisine yerleştirilmiş ve tüplerin ağzı bir tülbent ile kapatılmıştır. Bu şekildeki plastik tüplerden 3'ü 1 l'lik cam kavanozlara yerleştirilmiştir. Kavanoz kapaklarının iç kısmına 2X2 cm ölçülerinde kesilmiş kurutma kağıtları yapıştırılmış ve üzerine 1:1 oranında seyreltilmiş uçucu yağ konsantrasyonları mikropipet yardımıyla kurutma kağıtlarına emdirilmiştir. Asetonun buharlaşması için 40 sn bekletildikten sonra kapaklar kapatılmış ve 24, 48,72 ve 96 saat boyunca zararlının yumurtaları biberiye uçucu yağına maruz bırakılmıştır. Maruz bırakma sürelerinin sonunda kavanoz kapakları açılmış, sonrasında tülbentle kapatılarak uçucu yağ kokusu geçinceye kadar havalandırılmış ve ergin çıkışları tamamlanana kadar 28±2ºC sıcaklık, %55±5 nispi nemde ve tamamen karanlık koşullarda çalışan iklim kabininde bekletilmiştir. Ergin çıkışları tamamlandıktan sonra çıkış yapan ve yapamayan ergin sayıları kaydedilmiştir. Biberiye uçucu yağının yumurtaya karşı toksisitesi ergin çıkışlarına göre belirlenmiştir. Pozitif kontrollerde aseton uygulanmış, negatif kontrollerde hiçbir şey uygulanmamıştır. Çalışma tesadüf parselleri deneme deseninde 3 tekerrürlü olarak yürütülmüştür.

Birinci ve son dönem larva ile pupalara fumigant toksisite

Yumurtaya fumigant toksisite denemesinde olduğu gibi üzerinde 5 yumurta bulunan 10 tane nohut, tüplere yerleştirilmiş, ağızları tülbentle kapatılmıştır. Tüplerin 3'ü 1'lik cam kavanoza yerleştirilerek onların ağzı da tülbentle kapatılmıştır. Bu şekilde hazırlanan kavanozlara genç larvalar için 6. gün yaşlı larvalar için 16. ve pupalar için 20. güne kadar hiçbir şey uygulanmamış olup 28±2ºC sıcaklık, %55±5 nispi nemde ve tamamen karanlık koşullarda çalışan iklim kabininde bekletilmiştir. Bu günler sonunda uçucu yağın larvalara 10, 20, 30, 40 ve 50 µl/l hava konsantrasyonları; pupalara 20, 30, 40, 50 ve 60 µl/l hava konsantrasyonları uygulanıp kapakları kapatılmış ve 24, 48, 72 ve 96 saat maruz bırakma süreleri yumurtalara uygulandığı şekilde uygulanmıştır. Maruz bırakma sürelerinin sonunda kavanoz kapakları açılmış, tülbentle kapatılarak ergin çıkışları gerçekleşene kadar bekletilmiş ve ergin çıkışlarının tamamlanmasından sonra çıkış yapan ve yapamayan ergin sayıları kaydedilmiştir.

Yumurta, genç ve yaşlı larvaya ve pupaya karşı toksisite, başlangıçtaki yumurta sayıları ve çıkış yapan ergin sayıları karşılaştırılarak ölüm oranları tespit edilmiştir. Denemeler 3 tekerrürlü olarak ve her tekerrürde 50 birey olacak şekilde yürütülmüştür.

İstatistiksel analizler

Biberiye uçucu yağının *C. maculatus*'a fumigant etki çalışmalarından elde edilen % ölüm değerlerine ilk olarak ARCSIN transformasyonu uygulanmış, daha sonra SPSS 17 versiyon (Statistical Package for Social Sciences) yazılım paketi kullanılarak çift yönlü varyans analizi (ANAVO) yapılmış (SPSS, 2008), farkın önemli olduğu tespit edilen değerlere %5 önem seviyesinde DUNCAN testi yapılarak ortalamalar arasındaki farklar tespit edilmiştir. LC_{50} ve LC_{90} değerlerinin tespiti POLO PLUS (LeOra Software, 1987) programında probit analizine tabi tutularak yapılmıştır. Probit analizinde ölüm değerleri girilmeden önce aseton ve uçucu yağdaki ölümlerden doğal ölümler çıkarılmış ve elde edilen değerler girilerek LC_{50} ve LC_{90} değerleri tespit edilmiştir.

Araştırma Sonuçları ve Tartışma

Biberiye uçucu yağının *C. maculatus*'un ergin öncesi dönemleriyle (yumurta, birinci ve son dönem larva, pupa) bulaşık nohut tanelerine fumigant toksisitesini tespit etmek amacıyla yürütülen bu çalışmada; biberiye uçucu yağının oldukça yüksek fumigant toksisite gösterdiği, ölüm oranlarında istatistiksel olarak önemli farklılıkların ($P<0.05$) olduğu, uygulanan dozların ve maruz bırakma sürelerinin artışına bağlı olarak ölümlerin önemli derecede arttığı tespit edilmiştir.

Yumurtalardaki ölüm oranları incelendiğinde, uygulanan uçucu yağ dozları içerisinde %100 ölüm oranı 50 µl/l hava konsantrasyonunda 24 saat maruz bırakma süresinde tespit edilmiştir. 24 saat maruz bırakma süresinde 10, 20, 30, 40 ve 50 µl/l konsantrasyondaki ölüm oranları sırasıyla %32.67, 42.00, 54.67, 78.67, 100.00 olarak saptanmıştır. Yumurtalara karşı uygulanan konsantrasyonlar ile maruz bırakılan süreler arasındaki interaksiyonun önemli olduğu ($P<0.001$, $F=12.498$, s.d.=18) belirlenmiştir (Çizelge 1).

Çizelge 1. Farklı sürelerde biberiye uçucu yağının farklı uygulama dozlarına maruz bırakılan *Callosobruchus maculatus* yumurtalarının ölüm oranları (%)

	Ölüm Oranı (%) ± Standart Hata			
Doz (µl/l hava)	24 saat	48 saat	72 saat	96 saat
10	32.67±0.67 e*C**	36.00±1.15dB	37.33±0.67dAB	40.00±1.15 dA
20	42.00±1.16 dB	46.00±1.15 dB	72.00± 2.31 cA	77.33±2.91 cA
30	54.67±1.76 cD	67.33±3.71 cC	79.33±2.91 bB	92.67±1.76 bA
40	78.67±4.05 bB	92.00± 5.29 bA	100.00±0.00 aA	100.00±0,00 aA
50	100.00±0.00 aA	100.00±0.00 aA	100.00±0.00 aA	100.00±0.00 aA
Pozitif kontrol (aseton)	36.67±0.67deA	36.67±0.67 dA	37.33±0.67 dA	37.33±0.67deA
Negatif kontrol (doğal ölümler)	32.00±1.15 eA	32.00±1.15 dA	32.00±1.15 dA	32.00±1.15eA

* Küçük harfler aynı sütundaki dozlar arasındaki istatistiksel farkı;
** Büyük harfler aynı satırdaki maruz bırakma süreleri arasındaki istatistiksel farkı göstermektedir.
Aynı satırda ve sütunda bulunan harfler aynı ise istatistiksel olarak ($P>0.05$) bir farklılık yoktur.

LC_{50} ve LC_{90} değerlerinin maruz bırakma sürelerinin artışıyla düştüğü 24 saat maruz bırakma süresinde sırasıyla 34 ve 45 µl/l, 96 saat maruz bırakma süresinde sırasıyla18 ve 28 µl/l olduğu belirlenmiştir (Çizelge 2).

Çizelge 2. Biberiye uçucu yağının *Callosobruchus maculatus*'un yumurtalarına karşı farklı maruz bırakma sürelerindeki LC_{50} ve LC_{90} değerleri

Maruz bırakma süresi (saat)	n[a]	Eğim±SH	LC_{50} (µl/l hava) (Alt-üst güven aralığı)[b]	LC_{90} (µl/l hava) (Alt-üst güven aralığı)	λ^{2C}	SD	Heterojenite
24	612	10.71±1.66	34.57 (30.16-37.08)	45.54 (42.05-54.72)	27.23	13	2.10
48	612	8.36±0.94	28.93 (25.70-31.36)	41.18 (37.85-46.83)	22.68	13	1.74
72	612	5.95±0.60	20.88 (17.43-23.57)	34.28 (30.38-41.01)	28.88	13	2.22
96	612	6.47±0.72	18.08 (16.16-19.68)	28.54 (26.44-31.37)	27.98	13	0.61

[a] Toplam test edilen birey sayısı
[b] Alt üst güven aralığı (%95 önem seviyesinde)
[c] Ki-kare değeri
SD: Serbestlik derecesi

Genç larvalardaki ölüm oranları incelendiğinde biberiye uçucu yağının larvalara karşı toksik etki gösterdiği görülmüştür. Yumurtalardaki fumigant toksisiteye benzer olarak genç larvalarda da dozların ve maruz bırakma sürelerinin artışıyla ölümlerin arttığı tespit edilmiş ve %100 ölüm ilk olarak 50 µl/l hava konsantrasyonda 48 saat maruz bırakma süresinde saptanmıştır. 24 saat maruz bırakma süresinde 10,

20, 30, 40 ve 50 µl/l hava konsantrasyonundaki oranları sırasıyla %37.33, 41.33, 78.00, 84.67, 91.33 olarak tespit edilmiştir. Hiçbir şey uygulamayan kontrollerimizde yaklaşık olarak % 30-32 civarında doğal ölümler olmuştur. Aseton uygulanan pozitif kontrollerde ise yaklaşık %36-32 arasında ölümler meydana gelmiş dolayısıyla doğal ölümleri bunlardan çıkarttığımızda pozitif kontrollerde %0-6 arasında ölümlerin olduğu tespit edilmiştir (Çizelge 3). Nitekim Çağırgan, (2010), 17 farklı nohut çeşidinin *C. maculatus*'a karşı dayanıklılığının belirlenmesi amacıyla yürüttüğü çalışmada, *C. maculatus*'un ergin çıkış oranlarının çeşitlere göre farklılık gösterdiğini en fazla ergin çıkışının Canıtez çeşidinde görüldüğünü ve %16 ölüm olduğunu, en az ergin çıkışının Cevdet Bey çeşidinde %22 ölüm olduğunu tespit etmiştir. *Callosobruchus maculatus'*un yumurtadan ergin döneme gelinceye kadarki dönemde doğal ölümlerin olduğu bu çalışmada da görülmüştür.

Genç larvalara uygulanan biberiye uçucu yağının konsantrasyonları ile maruz bırakma süreleri arasındaki interaksiyonun önemli olduğu ($P<0.001$, $F=14.966$, s.d.=18) saptanmıştır.

Çizelge 3. Farklı sürelerde biberiye uçucu yağının farklı uygulama dozlarına maruz bırakılan *Callosobruchus maculatus*'un birinci dönem larvalarının ölüm oranları (%)

	Ölüm Oranı (%) ± Standart Hata			
Doz (µl/l hava)	24 saat	48 saat	72 saat	96 saat
10	37.33±1.76e*C**	41.33±1.33 eC	54.67±1.76 dB	62.67±2.91 cA
20	47.33±2.40 dC	54.67±4.05 dC	64.67±2.40 cB	75.33±1.76 bA
30	78.00±1.15 cD	84.67±1.33 cC	92.67±0,67 bB	100.00±0.00 aA
40	84.67±1.76 bC	95.33±2.40 bB	100.00±0.00 aA	100.00±0.00 aA
50	91.33±1.76 aB	100.00±0.00 aA	100.00±0.00 aA	100.00±0.00 aA
Pozitif kontrol (aseton)	34.67±0.67 eB	35.33±0.67eAB	36.67±0.67eAB	37.33±0.67 dA
Negatif kontrol (doğal ölümler)	32.00±1.15 eA	32.00±1.15 eA	32.00±1.15 fA	32.00±1.15 eA

* Küçük harfler aynı sütundaki dozlar arasındaki istatistiksel farkı;
** Büyük harfler aynı satırdaki maruz bırakma süreleri arasındaki istatistiksel farkı göstermektedir.
Aynı satırda ve sütunda bulunan harfler aynı ise istatistiksel olarak ($P>0.05$) bir farklılık yoktur.

Biberiye uçucu yağının birinci dönem larvalarda gösterdiği fumigant toksisitesi, maruz bırakma sürelerinin artışına paralel olarak artmıştır. Maruz bırakma sürelerinin artışına karşılık LC_{50} ve LC_{90} değerlerinde ciddi düzeyde düşüş görülmüştür. LC_{50} ve LC_{90} değerleri 24 saat maruz bırakma süresinde sırasıyla 27. 64 ve 51.65 µl/l hava iken 96 saat maruz bırakma süresinde sırasıyla 13.17 ve 25.75 µl/l hava olarak tespit edilmiştir (Çizelge 4).

Çizelge 4. Biberiye uçucu yağının *Callosobruchus maculatus*'un birinci dönem larvalarına karşı farklı maruz bırakma sürelerindeki LC_{50} ve LC_{90} değerleri

Maruz bırakma süresi (saat)	n[a]	Eğim±SH	LC_{50} (µl/l hava) (Alt-üst güven aralığı)[b]	LC_{90} (µl/l hava) (Alt-üst güven aralığı)[b]	λ^{2C}	SD[d]	Heterojenite
24	612	4.72±0.54	27.64 (25.00-29.90)	51.65 (46.59-59.93)	12.40	13	0.95
48	612	7.31± 0.8	24.32 (21.89-26.29)	36.41 (33.58-40.76)	15.95	13	1.23
72	612	9.28±1.25	22.20 (18.97-24.48)	30.51 (27.62-36.05)	25.44	13	1.96
96	612	4.40±0.45	13.17 (10.20-15.40)	25.75 (21.64-33.15)	32.67	13	2.51

[a] Toplam test edilen birey sayısı
[b] Alt üst güven aralığı (%95 önem seviyesinde)
[c] Ki-kare değeri
SD: Serbestlik derecesi

Biberiye uçucu yağının son dönem larvalara karşı fumigant toksisitesi birinci dönem larvalardan daha düşük olmuştur. Son dönem larvaların, birinci dönem larvalardan biberiye uçucu yağına daha dayanıklı olduğu tespit edilmiştir. %100'e varan ölümlere ancak en yüksek dozda (50 µl/l hava) ve 96 saat maruz bırakma süresinde ulaşılmıştır. 24 saat maruz bırakma süresinde, 10, 20, 30, 40 ve 50 µl/l hava konsantrasyonlardaki ölüm oranları sırasıyla %31.33, 36.00, 39.33, 54.00 ve 56.00 olarak belirlenmiştir (Çizelge 5). Konsantrasyonlar ile maruz bırakma süreleri arasındaki interaksiyonun da istatistiksel olarak önemli olduğu ($P<0.001$, $F= 36.678$, s.d.=18) saptanmıştır.

Çizelge 5. Farklı sürelerde biberiye uçucu yağının farklı uygulama dozlarına maruz bırakılan *Callosobruchus maculatus*'un son dönem larvalarının ölüm oranları (%)

	Ölüm Oranı (%) ± Standart Hata			
Doz (µl/l hava)	24 saat	48 saat	72 saat	96 saat
10	31.33±0.67 c*B**	34.00±1.15 eB	36.00±1.15 eA	38.00±1.15eA
20	36.00±1.15 bcD	41.33±0.67 dC	47.33±1.76 dB	54.00±2.31 dA
30	39.33±1.76 bC	54.67±1.33 cB	59.33±2.40cB	66.67±1.33 cA
40	54.00±2.00 aC	88.00±2.31 bB	93.33±0.67 bAB	95.33±1.76 bA
50	56.00±3.05 aD	91.33±0.67 aC	98.00±1.15 aB	100.00±0.00 aA
Pozitif kontrol (aseton)	33.33±0.67 cB	34.67±0.67 eAB	34.67±0.67 eAB	36.67±0.67 eA
Negatif kontrol (doğal ölümler)	32.00±0.71 cA	32.00±1.15eA	32.00±1.15eA	32.00±1.15 eA

* Küçük harfler aynı sütundaki dozlar arasındaki istatistiksel farkı;
** Büyük harfler aynı satırdaki maruz bırakma süreleri arasındaki istatistiksel farkı göstermektedir.
Aynı satırda ve sütunda bulunan harfler aynı ise istatistiksel olarak ($P>0.05$) bir farklılık yoktur.

Son dönem larvaların lethal konsantrasyon değerleri incelendiğinde LC_{50} ve LC_{90} değerleri birinci dönem larvalara oranla daha yüksek bulunmuştur. LC_{50} ve LC_{90} değerleri, 24 saat maruz bırakma süresinde sırasıyla 60.39 ve 134.46 µl/l hava iken 96 saat maruz bırakma süresinde sırasıyla 27.23 ve 40.58 µl/l hava olarak tespit edilmiştir (Çizelge 6).

Çizelge 6. Biberiye uçucu yağının *Callosobruchus maculatus*'un son dönem larvalarına karşı farklı maruz bırakma sürelerindeki LC_{50} ve LC_{90} değerleri

Maruz bırakma süresi(saat)	n[a]	Eğim±SH	LC_{50} (µl/l hava) (Alt-üst güven aralığı)[b]	LC_{90} (µl/l hava) (Alt-üst güven aralığı)[b]	λ^{2C}	SD	Heterojenite
24	612	3.69±0.59	60.39 (52.59-76.41)	134.46 (98.66-240.03)	10.78	13	0.83
48	612	6.79±0.69	32.68 (30.65-34.54)	50.47 (46.83-55.93)	12.93	13	0.99
72	612	7.61±0.85	29.64 (26.41-32.14)	43.69 (40.02-49.97)	21.05	13	1.62
96	612	7.39±0.82	27.23 (23.97-29.75)	40.58 (36.98-46.67)	21.95	13	1.69

[a] Toplam test edilen birey sayısı
[b] Alt üst güven aralığı (%95 önem seviyesinde)
[c] Ki-kare değeri
SD: Serbestlik derecesi

Pupa dönemi zararlının biyolojik dönemleri içerisinde biberiye uçucu yağına en dayanıklı dönem olarak saptanmıştır. Dolayısıyla en düşük doz 20 µl/l hava, en yüksek doz 60 µl/l hava uygulanmıştır. En yüksek ölüme (%100) 60 µl/l hava dozunda 72 saat maruz bırakma süresinde ulaşılmıştır. 24 saat maruz bırakma sonunda 20, 30, 40, 50 ve 60 µl/l hava konsantrasyonlarında ölüm oranları sırasıyla %32.67, 32.67, 33.33, 46.00 ve 63.33 olarak belirlenmiştir (Çizelge 7).

Pupalara uygulanan biberiye uçucu yağının konsantrasyonları ile maruz bırakma süreleri arasındaki interaksiyonun önemli olduğu ($P<0.001$, $F=59.10$, s.d.=12) saptanmıştır.

Çizelge 8'deki pupalara ait LC_{50} ve LC_{90} değerleri incelendiğinde 24 saat maruz bırakma süresindeki LC_{50} ve LC_{90} değerlerinin son dönem larvalarınkiyle hemen hemen aynı olurken 48 ve 72 saat maruz bırakma sürelerinde daha düşük olduğu tespit edilmiştir. Ayrıca 24 ve 48 saat maruz bırakma sürelerindeki LC_{50} ve LC_{90}değerleri birbirine oldukça yakın (sırasıyla 60.34 ve 76.70; 52.61 ve 73.23) olurken, 72 saat maruz bırakma süresinde LC_{50} ve LC_{90} değerlerinde ani bir düşüş (sırasıyla 37.44 ve 46.50) olduğu saptanmıştır.

Çizelge 7. Farklı sürelerde biberiye uçucu yağının farklı uygulama dozlarına maruz bırakılan *Callosobruchus maculatus*'un pupalarının ölüm oranları (%)

	Ölüm Oranı (%) ± Standart Hata		
Doz (µl/l hava)	24 saat	48 saat	72 saat
20	32.67±0.67 c*A **	32.67±0,67 dA	33.33±0.67 dA
30	32.67±0.67 cB	33.33±0,67 dB	36.67±1.76 dA
40	33.33±0.67 cC	46.00±2.00 cB	83.33±2.40 cA
50	46.00±2.31 bC	58.67±2.91 bB	94.00±1.15 bA
60	63.33±1.76 aC	79.33±5.69 aB	100.00±0.00 aA
Pozitif kontrol (aseton)	32.00±0.00 cA	32.00±0.00 dA	32.67±0.67 dA
Negatif kontrol (doğal ölümler)	32.00±1.15 cA	32.00±1.15 dA	32.00±1.15 dA

* Küçük harfler aynı sütundaki dozlar arasındaki istatistiksel farkı;
** Büyük harfler aynı satırdaki maruz bırakma süreleri arasındaki istatistiksel farkı göstermektedir.
Aynı satırda ve sütunda bulunan harfler aynı ise istatistiksel olarak (P>0.05) bir farklılık yoktur.

Çizelge 8. Biberiye uçucu yağının *Callosobruchus maculatus*'un pupalarına karşı farklı maruz bırakma sürelerindeki LC_{50} ve LC_{90} değerleri

Maruz bırakma süresi (saat)	n[a]	Eğim±SH	LC_{50} (µl/l hava) (Alt-üst güven aralığı)[b]	LC_{90} (µl/l hava) (Alt-üst güven aralığı)[b]	λ^{2C}	SD	Heterojenite
24	612	12.30±1.73	60.34 (58.07-63.79)	76.70 (70.78-87.94)	8.54	13	0.66
48	612	8.93±0.91	52.61 (50.17-55.67)	73.23 (55.67-66.94)	6.07	13	1.24
72	612	13.62±1.17	37.44 (35.81-38.96)	46.50 (44.35-49.53)	7.20	13	1.32

[a] Toplam test edilen birey sayısı
[b] Alt üst güven aralığı (%95 önem seviyesinde)
[c] Ki-kare değeri
SD: Serbestlik derecesi

Biberiye uçucu yağının *C. maculatus*'un ergin öncesi dönemlerine fumigant toksisitesini araştırdığımız çalışmada biberiye uçucu yağının zararlının ergin öncesi dönemlerine karşı toksisite gösterdiği saptanmıştır. Ergin öncesi dönemler içerisinde en hassas dönemin birinci dönem larva ve yumurta, en dayanıklı dönemin ise pupa ve son dönem larva olduğu tespit edilmiştir.

24 saat maruz bırakma süresinde ve 50 µl/l hava dozunda, yumurtalarda, birinci dönem, son dönem larvalarda ve pupalarda ölüm oranlarının sırasıyla %100, 91, 56, 46 olduğu tespit edilmiştir. Negatif (hiçbir kimyasal uygulanmayan sadece uçucu yağa maruz bırakma süresi kadar kavanoz kapaklarının kapalı tutulduğu uygulamalarda) %30 doğal ölümlerin olduğu tespit edilmiştir. Pozitif kontrollerde (aseton uygulanmış) ise negatif kontrol (doğal ölümler) ile karşılaştırıldığında %10'un altında ölümlerin gerçekleştiği saptanmıştır.

LC_{50} ve LC_{90} değerleri, maruz bırakma sürelerinin artışına bağlı olarak azalmıştır. 24 saat maruz bırakma sürelerinde yumurta, birinci dönem, son dönem larva ve pupalardaki LC_{50} ve LC_{90} değerleri sırasıyla 34.57 ve 45.54, 27.64 ve 51.65, 60.39 ve 134.46, 60.34 ve 76.70 µl/l hava olarak belirlenmiştir.

Ketoh et al. (2005), *Cymbopogon schoenanthus* uçucu yağının, *C. maculatus*'un börülce tohumları üzerine yeni bırakılmış yumurtalarının (yumurta bırakılmasından 1 gün sonra) ve yumurtadan yeni çıkmış henüz tohum içerisine giriş yapmamış larvalarının (yumurta bırakıldıktan 3 gün sonra) gelişimini 33.3 µl/l hava konsantrasyonunda 24 saat maruz bırakma süresinde %100 engellediğini, aynı dozda 48 saat maruz bırakma süresinde yumurta bırakıldıktan sonra 5. gündeki tohum içerisindeki ilk dönem ve ikinci dönem larvaların %100'nü öldürdüğünü, 10. gündeki üçüncü dönem larvaların %68'ni, 15. gündeki dördüncü dönem larvaların ve pupaların %45'ni öldürdüğünü tespit etmişlerdir. Papachristos & Stamopoulos (2002), *Acanthoscelides obtectus* (Say, 1831) larva ve pupalarına karşı en fazla toksisite gösteren uçucu yağın *Lavandula hybrida* L. olduğunu, ilk dönem larvaların ilerleyen larva dönemlerine göre daha duyarlı olduğunu bununla birlikte tüm larva dönemlerinin pupalardan da daha duyarlı olduğunu, biberiye uçucu yağının 48 saat maruz bırakma süresindeki LC_{50} değerlerinin ilk dönem larvalarda 1.1 µl/l hava iken ikinci dönem larvalarda 2.2 µl/l hava ve 3-4. dönem larvalarda 10.6 µl/l hava, pupalarda ise 62.7 µl/l hava olarak tespit etmişlerdir. Papachristos & Stamopoulos (2004) başka bir çalışmalarında da *A. obtectus*'un yumurtalarına karşı *L. hybrida, Rosmarinus officinalis* L. ve *Eucalyptus globulus* Labill uçucu yağlarının 24 saat maruz bırakma süresindeki fumigant toksisitelerinin yumurta yaşına ve uçucu yağa bağlı olarak değiştiğini, 3 gün ve daha az yaşındaki yumurtaların çıkışlarında en yüksek dozda (250 µl/l hava) bile kayda değer bir azalış olmadığını, 4 ile 6 gün yaş arasındaki yumurtaların çıkışlarında ise önemli derecede azalma olduğunu tespit etmişlerdir. *L. hybrida* ve *R. officinalis* uçucu yağlarının benzer toksik etkiyi ve *E. globulus* uçucu yağından daha fazla toksik etki gösterdiğini saptamışlardır. 24 saat boyunca *R. officinalis* uçucu yağına maruz bırakılan 0-3 gün yaşındaki yumurtalarda LC_{50} değerinin >250 µl/l hava'dan büyük olmasına karşın 4, 5 ve 6 gün yaşındaki yumurtaların LC_{50} değerlerinin sırasıyla 14.9, 3.7, 1.3 µl/l hava olduğunu belirlemişlerdir. Embriyonik gelişmenin ilerledikçe duyarlılığın daha fazla arttığını, 8. günde yumurtalarının açılmaya başladığı *A. obtectus* yumurtalarında, uçucu yağ buharının etkilediği zamanın yumurtanın bırakıldığı andan itibaren 4. günün (yumurta içerisinde embriyonun gözlenebildiği) kritik nokta olduğunu çünkü uçucu yağ ve monoterpenoid gibi bileşiklerin nörotoksin gibi işlev görebilmesi için hedef sinir sisteminin gelişmeye başladığında ancak ovicidal etkinin meydana gelebileceğini, ayrıca chorion ve vitellin zarının geçirgenliğinin embriyonun gelişmesi boyunca değiştiğinden dolayı ileri yaşlardaki yumurtalara uçucu yağ buharı difüzyonun kolaylaşacağını, fiziksel ve biyokimyasal süreçlerin daha fazla etkilenebileceğinin mümkün olabileceğini ifade etmişlerdir. Risha et al. (1990) *Acorus calamus* bitkisinin rizomlarından elde ettikleri uçucu yağın depolanmış ürün zararlılarından *Sitophilus granarius* (Linnaeus, 1758), *S. oryzae*, *Tribolium confusum* Jacquelin du Val, 1863 ve *Callosobruchus chinensis* Linnaeus, 1758 türlerinin ergin öncesi dönemlerine karşı toksik etkilerini inceledikleri çalışmada en fazla duyarlılığa sahip olan türün *C. chinensis* yumurtalarında olduğunu bunu *S. granarius* ve *S. oryzae*'nın takip ettiğini fakat *T. confusum* yumurtalarını hiç etkilemediğini tüm durumlarda genç embriyonik dönemlerin ileriki dönemlerden daha duyarlı olduğunu larva ve pupa dönemlerinde fark edilebilir hiç bir duyarlılık olmadığını tespit etmişlerdir. Saraç & Tunç (1995) konsantrasyonlar ve maruz bırakma süreleri açısından değerlendirdiklerinde *Ephestia kuehniella* Zeller, 1879 yumurtalarının son dönem larvalardan daha az tolerans gösterdiğini, *T. confusum* yumurtalarının erginlerden daha fazla tolerans gösterdiğini tespit etmişlerdir.

El-Nahal et al. (1989), *C. chinensis*'in genç embriyonik dönemlerinin uçucu yağ buharına ileriki dönemlerden daha duyarlı olduğunu belirtmişlerdir. Çetin et al. (2014), 18 tane tıbbi ve aromatik bitkinin uçucu yağını *A. obtectus* erginlerine karşı uygulamışlar. *R. officinalis* ve *Salvia fruticosa* Mill. uçucu yağların en etkili yağlar olduğunu ve bu uçucu yağların 10 µl/l hava sabit dozda 24 saat maruz bırakma süresinde *A. obtectus* erginlerinde %100 ölüm meydana getirdiğini saptamışlardır.

Isikber et al. (2006), biberiye (*R. officinalis*) ve defne (*Laurus nobilis* L.) uçucu yağların gaz halinin *T. confusum*'un tüm gelişme dönemlerine karşı toksik etki gösterdiğini ve LT_{90} değerleri göz önüne alındığında biberiye ve defne uçucu yağlarına karşı *T. confusum*'un tüm gelişme dönemlerinin toleransı büyükten küçüğe doğru sırasıyla, pupa>larva>ergin ve larva>ergin>yumurta>pupa olduğunu bildirmişlerdir. Konsantrasyon x süre (C x T) (g h l^{-1}) değerlerine bakıldığında, biberiye uçucu yağ defne uçucu yağına göre *T. confusum*'un erginlerine ve larvalarına karşı daha toksik olurken, defne uçucu

yağının ise biberiye uçucu yağına göre *T. confusum*'un yumurtalarına ve pupalarına karşı daha toksik olduğunu saptamışlardır.

Araştırmamızda biberiye uçucu yağının ergin öncesi tüm gelişme dönemlerine fumigant toksisite gösterdiği bu toksisitenin böceğin gelişme dönemlerine göre farklılık gösterdiği saptanmıştır. Gelişme dönemlerinin biberiye uçucu yağı buharına karşı hassasiyet sıralamasının birinci dönem larva> yumurta>son dönem larva > pupa şeklinde olduğundan Ketoh et al. (2005), El- Nahal et al. (1989) ve Papachristos & Stamopoulos (2002)'un çalışma, sonuçlarıyla benzerlik göstermiştir. Biberiye uçucu yağının depolara bulaşık olarak getirilmiş ürünün mücadelesinde kullanma potansiyelinin olduğu tespit edilmiştir. Sonuçların, Papachristos & Stamopoulos (2004), Isikber et al., (2006), çalışmalarından farklı olması zararlının farklı bir tür ve kullanılan uçucu yağın farklı olmasından kaynaklandığı düşünülmektedir.

Genel olarak uçucu yağlar ham yağlardan daha zor ve oldukça az miktarlarda elde edilmektedir. Biberiye uçucu yağı verimi %1 olarak ifade edilse de bu miktar bitkinin hangi dönemde toplandığına, çevre şartlarına, toprak tipine ve kullanılan kısmına (yaprak, sap) göre %0.10 ile %0.78 arasında değiştiği tespit edilmiştir (Başkaya et al., 2016). Bu çalışmada da biberiye bitkisinin yapraklarından elde edilen uçucu yağ verimi ortalama %0.3'tür. İnsektisitler kadar ucuz olmasa da uçucu yağların çevre ve insan sağlığına olumsuz etkisinin olmaması, zararlılar üzerinde birçok yönde etki gösterdiği için direnç geliştirmesinin daha güç olmasından dolayı organik üretimde kullanılması mümkün olabilir. Az miktarda elde edilen uçucu yağın kullanım şekli de oldukça önemlidir. Çünkü uçucu yağlar hızlı şekilde gaz haline dönüşebilmektedir. Bal arılarında varroa parazitine karşı timol etken maddeli sünger tabletlerin kullanıldığı gibi biberiye uçucu yağının da tablet haline getirilmesi mümkün olabilir. Dolayısıyla bu yöndeki çalışmalara ağırlık verildiği takdirde hem daha ucuz hem de daha sağlıklı ürünler elde edilmesi mümkün olacaktır.

Yararlanılan Kaynaklar

Alam, M. S., S. S. Shaukat, M. Ahmed, S. Igbal & A. Ahmad, 1999. A survey of resistance to phosphine in some Coleopterous pests of stored wheat and rice grain in Pakistan. Pakistan Journal of Biological Sciences, 2 (3): 623-626.

Al-Sarar, A. S., H. I. Hussein, Y. Abobakr, A. E. Bayoumi & M. T. Al-Otaibi, 2014. Fumigant toxicity and antiacetylcholineterase activity of saudi *Mentha longifolia* and *Lavandula dentata* species against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae). Türkiye Entomoloji Dergisi, 38 (1): 11-18.

Anonymous, 2014. Yemeklik tane baklagiller. (Web page:www.ziraattube.com/m/1402/yemeklik tane baklagiller.html), (Erişim tarihi: 10.04.2014)

Baidoo, P. K., M. B. Mochiah & M. O. Akyaw, 2010. The effect of time of harvest on the damage caused by the cowpea weevil *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae). Journal of Stored Products and Postharvest Research, 1 (3): 24-28.

Başkaya, Ş., F. Ayanoğlu & N. P. Bahadırlı, 2016. Biberiye (*Rosmarinus officinalis* L.) bitkisinin uçucu yağ oranı, uçucu yağ bileşenler ve antioksidan içeriğinde morfogenetik ve ontogenetik varyabilite. Mustafa Kemal Üniversitesi Ziraat Fakültesi Dergisi, 21 (1): 12-20.

Benhalima, H., M. Q. Chaudhry, K. A. Mills & N. R. Price, 2004. Phosphine resistance in stored product insects collected from various grain storage facilities in Morocco. Journal of Stored Products Research, 40: 241-249.

Boxall, R. A., 2001. Post-harvest losses to insect-a world overview. International Biodeterioration and Biodegration, 48: 137-152.

Çağırgan, O., 2010. Farklı Nohut Çeşitlerinin Börülce Tohum Böceği (*Callosobruchus maculatus*F.) (Coleoptera: Bruchidae)'ne Karşı Dayanıklılığının Belirlenmesi. Selçuk Üniversitesi Fen Bilimleri Enstitüsü (Basılmamış) Yüksek Lisans Tezi, Selçuklu, Konya, 38s.

Çetin, H., M. Uysal, Ö. Alaoğlu & A. Şahbaz, 2009. Asetonun fasülye tohum böceği [*Acanthoscelides obtectus* Say (Coleoptera: Bruchidae)]'ne fumigant etkisi. Türkiye Entomoloji Dergisi, 33 (1): 23-30.

Çetin, H., M. Uysal, A. Şahbaz, Ö. Alaoğlu, A. Akgül & M. Özcan, 2014. Tıbbi ve aromatik bitki uçucu yağlarının fasulye tohum böceği (*Acanthoscelides obtectus* Say) (Coleoptera: Bruchidae) erginlerine fumigant etkileri. Selçuk Tarım ve Gıda Bilimleri Dergisi, 1 (1): 6-11.

El- Nahal, A. K. M., G. H. Schmidt & E. M. Riska, 1989. Vapours of *Acorus calamus* oil- a space treatment for stored-product insects. Journal of Stored Products Research*,* 25: 211-216.

Ferizli, A. G. & M. Emekçi, 2000. "Carbon dioxide fumigation as a Methyl bromide alternative for the dried fig industry,81-91". Annual International Research Conference on Methyl bromide Alternatives and Emissions Reductions, (6-9, November, 2000, Orlando, Florida) Proceedings.

Ferizli, A. G. & M. Emekçi, 2010. "Depolanmış ürün zararlılarıyla savaşım, sorunlar ve çözüm yolları, 579-587". TMMOB Ziraat Mühendisleri Odası Ziraat Mühendisliği VII. Teknik Kongresi (11-15 Ocak 2010 Ankara) Bildiriler Kitabı, 2, 1300 s.

Isikber, A. A., M. H. Alma, M. Kanat & A. Karci, 2006. Fumigation toxicity of essential oils from *Laurus nobilis* and *Rosmarinus officinalis* against all life stages of *Tribolium confusum*. Phytoparasitica*,* 34: 167-177.

Isman, M. B., 2000. Plant essential oils for pest and disease manegement. Crop Protection, 19: 603-608.

Karakoç, Ö. C., A. Gökçe & İ. Telci, 2006. Bazı bitki uçucu yağlarının *Sitophilus oryzae* L., *Sitophilus granarius* L. (Col.: Curculionidae) ve *Acanthoscelides obtectus* Say. (Col.: Bruchidae)'a karşı fumigant etkileri. Türkiye Entomoloji Dergisi, 30 (2): 123-135.

Ketoh, G. K., H. K. Koumaglo & I. A. Glitho, 2005. Inhibition *Callosabruchus maculatus* (F.) (Coleoptera: Bruchidae) development with essential oil exracted from *Cymbopogon schoenanthus* L. Spreng. (Poaceae), and the wasp *Dinarmus basalis* (Rondani) (Hymenoptera: Pteromalidae). Journal of Stored Products Research, 41: 363-371.

Khani, A. & J. Asghari, 2012. Insecticide activity of essential oils of *Mentha longifolia, Pulicaria gnaphalodes* and *Achiilea wilhelmsii* against two stored product pests, the flour beetle, *Tribolium castaneum* and the cowpea weevil, *Callosobruchus maculatus*. Journal of Insect Science, 12 (73): 1-10.

LeOra Software, 1994. Polo-PC a User's Guide to Probit or Logit Analysis, 1119 Shattuck Avenue, Berkeley, CA, 94707.

Mahfuz, I. & M. Khalequzzaman, 2007. Contact and fumigant toxicity of essential oils against *Callosobruchus maculatus.* University Journal of Zoology, Rajshahi University, 26: 63-66.

Odeyemi, O. O., O. A. Gbaye & O. Akeju, 2006. "Resistance of *Callosobruchus maculatus* (Fab.) to primiphos methyl in three zones in Nigeria, 324-329". Proceedings of the 9th International Working Conference on Stored Product Protection (15-18 October 2006, Sao Paulo, Brazil), 1351 pp.

Ofuya, T. I., O. F. Olotah & O. J. Ogunsolo, 2010. Fumigant toxicity of crushed bulbs of two *Allium* species to *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae). Chilean Journal of Agricultural. Research, 70: 510-514.

Papachristos, D. P. & D. C. Stamopoulos, 2002. Toxicity of vapours of three essential oils to the immature tages of *Acanthoscelides obtectus* (Say) (Coleoptera: Bruchidae). Journal of Stored Products Research*,* 38 (4): 365-373.

Papachristos, D. P. & D. C. Stamopoulos, 2004. Fumigant toxicity of three essential oils on the eggs of *Acanthoscelides obtectus* (say) (Coleoptera: Bruchidae). Journal of Stored Products Research*,* 40 (5): 517-525.

Raja, N., S. Albert, S. Ignacimuthu & S. Dorn, 2001. Effect of plant volatile oils in protecting stored cowpea *Vigna unguiculata* (L.) Walpers against *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) infestation. Journal of Stored Products Research, 37: 127-132.

Risha, E. M., A. K. M. El-Nahal & G. H. Schmidt, 1990. Toxicity of vapours of *Acorus calamus* L. oil to the immature stages of some stored- product Coleoptera. Journal of Stored Products Research, 26 (3): 133-137.

Saraç, A. & I. Tunç, 1995. Toxicity of essential oil vapours to stored product insects. Zeitschrift Fuer Pflanzenkrankheiten und Pflanzenschutz, 102: 69-74.

SPSS, 2008. SPSS Statistics for Windows, Version 17.0. Chicago: SPSS Inc.

Selimoğlu, T., A. Gökçe & D. Yanar, 2015. Bazı bitki uçucu yağlarının *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) üzerindeki fumigant toksisiteleri. Türkiye Entomoloji Dergisi, 39 (1): 109-118.

Topuz, E. & N. Madanlar, 2011. Bazı bitkisel kökenli uçucu yağların *Tetranychus cinnabarinus* (Boisduval, 1867) (Acari: Tetranychidae) üzerine kontakt ve repellent etkileri. Türkiye Entomoloji Bülteni, 1 (2): 99-107.

Tripathi, A. K., V. Prajapati, K. K. Aggarwal & S. Kumar, 2001. Insecticidal and ovicidal activity of essential oil of *Anethum sowa* Kurz against *Callosabruchus maculatus* F. (Coleoptera: Bruchidae), International Journal of Tropical Insect Science, 21 (1): 61-66.

Zettler, J. L., 1982. Insecticide resistance in selected stored product insects infesting peanuts in the South- Eastern United States. Journal Economic Entomology, 75: 359-362.