Türkiye Entomoloji Dergisi
(Turkish Journal of Entomology)
Cilt (Vol.) **39** **Sayı** (No.) **4** **Aralık** (December) **2015**

İnceleme ve Değerlendirmede Bilimsel Olarak Katkıda Bulunanlar
(Scientific Advisory Board)

ADAMSKI, Zbigniew, Poland
AKBULUT, Mikail, Kayseri
AKİNER, Mustafa Muhammed, Rize
ATLIHAN, Remzi, Van
AYYAZ, Mahmood, Niğde
BARJADZE, Shalva, Georgia
BAŞHAN, Mehmet, Diyarbakır
BAYHAN, Selime Ölmez, Diyarbakir
BAYINDIR, Alime, Denizli
BEN-DOV, Yair, Israel
BÜYÜKGÜZEL, Ender, Zonguldak
BÜYÜKGÜZEL, Kemal, Zonguldak
CANHİLAL, Ramazan, Erciyes
CİVELEK, Hasan Sungur, Muğla
ÇETİNTAŞ, Ramazan, Kahramanmaraş
ÇIKMAN, Emine, Şanlıurfa
DAĞLI, Fatih, Antalya
DEVRAN, Zübeyir, Antalya
DOĞAN, Salih, Erzincan
DURSUN, Ahmet, Amasya
ELMA, Fatmanur, Konya
ELMALI, Meryem, Konya
FERİZLİ, Ahmet Güray, Ankara
GARONNA, A. P., Italy
GAUTAM, S. G., USA
GERMAIN, Jean-Francois, France
GÖKÇE, Ayhan, Niğde
HAMBÄCK, P., Sweden
HAUSMANN, Axel, Germany
HORTON, D. R., USA
HYRŠL, P., Czech Republic
IGNACIMUTHU, Savarimuthu, India
IŞIKBER, Ali Arda, Kahramanmaraş
KARUT, Kamil, Adana
KAŞKAVALCI, Galip, İzmir
KAYA, H. K., USA
KAYDAN, Bora, Adana
KAZAK, Cengiz, Adana
KAZAN, Kemal, Australia
KEPENEKÇİ, İlker, Ankara
KHANJANI, M., Iran
KNAAK, N., Brasil
KONDO, T., Colombia
KUBIK, Stepan, Germany
KUMRAL, Nabi Alper, Bursa
LIETTI, M. M., Argentina
MAAFI, Z., Iran
MÁCA, J., Czech Republic
MELAN, Kadir, Ankara
NAYAK, M. K., Australia
OKYAR, Zuhal, Edirne
OOSTERBROEK, P., Holland
ÖZDER, Nihal, Tekirdağ
ÖZKAN, Cem, Ankara
PARK, Youngjin Korea
PELLIZZARİ, G., Italy
PUTNAM, Sean, USA
RAHIM,Nor Abdul, Malaysia
RODITAKIS, E., Greece
SAĞLAM, Özgür, Tekirdağ
SHAPIRO-ILAN, D., USA
SÖĞÜT, Mehmet Ali, Isparta
STANLEY D. W., USA
SUSURLUK, Alper, Bursa
SYAHPUTRA, Edy, Indonesia
TEZCAN, Serdar, İzmir
TUNCA, Hilal, Ankara
TUNÇBİLEK, Aydın Şüzü, Kayseri
VELİOĞLU, Sibel, Ankara
WANI, A. A., India
YANAR Dürdane, Tokat
YAVUZASLANOĞLU, Elif, Karaman
ZEYBEKOĞLU, Ünal, Samsun

İçindekiler (Contents)

Orijinal araştırmalar (Original articles)

Türk. entomol. derg., 2015, 39 (4): 321-332 ISSN 1010-6960
DOI: http://dx.doi.org/10.16970/ted.56855

Orijinal araştırma (Original article)

Inhibitory effects of barley and wheat seed protein on digestive α-amylase and general protease activity of *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae)

Arpa ve buğday tohum proteininin *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae)'nın α-amilaz sindirimi ve genel proteaz aktivitesine inhibitör etkileri

Shabnam ASHOURI[1] Reza FARSHBAF POURABAD[1*]
Alireza BANDANI[2] Mehdi DASTRANJ[2]

Summary

In the current study, the effects of barley and wheat seed proteins on Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) certain digestive enzymes and biological parameters were investigated. Ammonium sulfate precipitation proteinaceous fractions 0-30% and 30-50% of both seeds produced the greatest inhibition on L_4 α-amylase but no significant inhibition on the protease activity. The inhibitory effects of fraction of 0-30% on the L_1, L_2, L_3, L_4 and adult's α-amylase activity of barley were 64, 53, 59, 49, and 56%, respectively, and of wheat were 70, 63, 63, 57, and 67%, respectively. While the inhibitory effects of the effective fraction of 30-50% on the L_1, L_2, L_3, L_4 and adult's α-amylase activity of barley were 45, 46, 52, 48, and 53%, respectively, and of wheat were 66, 59, 70, 56, and 68%, respectively. Type of inhibition in both cases was determined as partial mixed at the enzyme-inhibition kinetic tests. Zymograms confirmed the inhibition of insect α-amylase activity. Bioassays were conducted using four cultivars of potato leaves treated with barley extract. Weight of L_4 in Picasso and the percentage of L_4 evolution in all cultivars were reduced and the developmental durations were significantly increased at Marx and Picasso, in comparison with control. However, there was no significant effect on gut amylase activity of survived individuals.

Keywords: α-amylase, cereal, *Leptinotarsa decemlineata*, protease, seed protein

Özet

Bu çalışmada, arpa ve buğday tohum proteinlerinin, Patates Böceği, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae)'nın sindirim enzimleri ve biyolojik parametrelerine etkileri incelenmiştir. Her iki tohumdan elde edilen proteinlerin amonyum sülfatlı % 0-30 ve % 30-50'lik çözeltileri L4 lerin α-amilaz inhibasyonu üzerinde en yüksek etkiyi yaparken proteaz aktivitesi üzerindeki etkisi önemsiz bulunmuştur. Yüzde 0-30'luk protein çözeltilerinin L1, L2, L3, L4 ve erginlerin α-amilaz aktivitesine inhibasyon etkisi arpa proteinleri için sırasıyla % 64, 53, 59, 49 ve 56, buğday proteinleri için % 70, 63, 63, 57, ve 67 olarak bulunmuştur. Bununla birlikte % 30-50'lik protein çözeltilerinin L1, L2, L3, L4 ve erginlerin α-amilaz aktivitesine inhibasyon etkisi arpa ptoteinleri için sırasıyla % 45, 46, 52, 48, ve 53, buğday proteinleri için % 66, 59, 70, 56, ve 68 olarak tespit edilmiştir. Her iki durumda da enzim-inhibasyon kinetik testinde inhibasyonun kısmı karışık inhibasyon olduğu belirlenmişitir. Zymogram çalışmaları böceğin α-amilaz aktivasyonunun inhibe olduğunu onaylamıştır. Biyolojik denemeler arpa ekstraktlarının uygulandığı dört farklı patates çeşidinin yaprakları kullanılarak yapılmıştır. Picasso çeşidinde L4'lerin ağırlığı, tüm çeşitlerde gelişme yüzdesinin kontrole göre düştüğü, Marx ve Picasso çeşitlerinde gelişme süresinin kontrol ile karşılaştırıldığında belirgin şekilde uzadığı tespit edilmiştir. Bununla birlikte yaşayan bireylerin miğde amilaz aktiviteleri üzerinde belirgin bir etki bulunmamıştır.

Anahtar sözcükler: α-amilaz, tahıl, *Leptinotarsa decemlineata*, proteaz, tohum proteini

[1] University of Tabriz, Agricultural Faculty, Plant Protection Department, Tabriz, Iran
[2] Tehran University, College of Agriculture and Natural Resources, Plant Protection Department, Karaj, Iran
* Sorumlu yazar (Corresponding author) e-mail: rfpourabad@yahoo.com
Alınış (Received): 05.05.2015 Kabul ediliş (Accepted): 19.10.2015 Çevrimiçi Yayın Tarihi (Published Online): 04.11.2015

Introduction

The Colorado potato beetle (CPB), *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae), is the most destructive pest of solanaceous crops (Kondrak et al*.,* 2005; Lawrence et al., 2007). The maintenance of digestive tract integrity of insects has to be ensured at all costs because it is the main food entry way, otherwise, their life cycles may be interrupted. Hence, the gut is a potential target for controlling economically important crop pests (Becker-Ritt & Carlini, 2012). Proteolytic enzymes, also called proteases, catalyze the hydrolytic cleavage of specific peptide bonds in their target proteins (Carlini & Grossi-de-Sa, 2002; Habib & Fazili, 2007). Midguts of many insects contain large amounts of proteases which play a vital role in providing free amino acids essential for insect's normal growth and development (Wolfson & Murdock, 1990). Proteolysis as a key process in all living organisms must be extremely controlled; otherwise, it could be very hazardous to their natural environment (Carlini & Grossi-de-Sa, 2002). Alpha-amylases (α-1,4-glucan-4-glucanohydrolases, EC 3.2.1.1) constitute a family of endo-amylases that catalyze the hydrolysis of α-D-(1→4)-glucan linkages in starch components and other carbohydrates. This process is an important step towards transforming sugar polymers into smaller units that can be assimilated by the organism (Carlini & Grossi-de-Sa, 2002; Franco et al., 2002; Becker-Ritt & Carlini, 2012). When the action of the amylases is inhibited, nutrition of the organism is impaired causing shortness in energy (Carlini & Grossi-de-Sa, 2002).

Pest control in modern agriculture is increasingly moving away from reliance on exogenously applied pesticides, towards a more 'environmentally friendly' methods (Gatehouse et al., 2002). Plant resistance against insects is due to a set of defense mechanisms acquired by plants during evolution (Franco et al., 2002). On the other hand, one of the most important pest control mechanisms involves interaction of the enzymes with proteins that inhibit their activities. These compounds form less active or fully inactive complexes with their cognate enzymes, and are called enzyme inhibitors (Habib & Fazili, 2007). They act on key insect gut digestive hydrolases, the α-amylases and proteases. Several kinds of α-amylase and protease inhibitors, present in seeds and vegetative tissues especially in cereals, including wheat (*Triticum aestivum* L.), barley (*Hordeum vulgareum* L.), sorghum (*Sorghum bicolor* L.), rye (*Secale cereal* L.) and rice (*Oryza sativa* L.) act to regulate numbers of phytophagous insects (Franco et al*.,* 2002; Chen, 2008). The transgenic expression of insecticidal proteins such as α-amylase and protease inhibitors is also being evaluated as a potential protective strategy against insects (Macedo & Freire, 2011) and in most cases resulted in detrimental effects on insect growth and development (Chen, 2008).

Several extensive studies have been carried out to identify proteins with insecticidal properties against major economic pests (Karimi et al., 2010). The inhibitory effect of three proteinaceous inhibitors isolated from little millet (*Panicum sumatrense* Roth.) and finger millet (*Eleusine coracana* L.) (Poaceae) was tested on gut α-amylases of four stored grain and four phytophagous insect-pests (Sivakumar et al., 2006). Other studies were carried out using α-amylase inhibitors from different plant sources like beans (*Phaseolus* spp.) (Fabaceae) with inhibitory effect on α-amylase activities of *Tecia solanivora* Povolny (Lepidoptera: Gelechiidae) (Valencia-Jime´nez et al., 2008), Mungbean (*Vigna radiate* L.) (Fabaceae) on *Callosobruchus maculatus* F. (Coleoptera: Bruchidae) α-amylase activity (Wisessing et al., 2008), seeds of flamboyant (*Delonix regia* Bojer) (Fabaceae) tree on *C. maculatus*, *Anthonomus grandis* Boheman (Coleoptera: Curculionidae) and *Acanthoscelides obtectus* Say (Coleoptera: Bruchidae) α-amylase activity (Alves et al., 2009) and 24 species of Cerrado plants, against *Zabrotes subfasciatus* Bohemann (Coleoptera: Bruchidae) and *A. obtectus* α-amylase activity (Silva et al., 2009).

The purpose of this study was to investigate the effect of proteinaceous extract of the barley and wheat seeds on the α-amylase and protease activity and some biological parameters of the Colorado potato beetle.

Materials and Methods

Materials

Succinic acid disodium salt, bovine serum albumin (BSA), azocasein, ammonium persulfate for electrophoresis (APS) and dialysis bag (1 kDa cutoff, 28 mm) were purchased from Sigma (St Louis, MO, USA). Tris, phosphate buffer solution (pH: 7), 2-hydroxy-3,5-dinitrosalicylic acid (DNS), potassium sodium tartrate tetrahydrate, starch soluble, trichloroacetic acid (TCA), sodium hydroxide, ammonium sulfate, acrylamide, N,N′-methylene diacrylamide, dodecyl sulfate sodium salt (SDS), 2-morpholinoethanesulfonic acid (MES), sodium chloride, calcium chloride, phosphoric acid, glycerol, potassium iodide, iodine, coomassie brillant blue G 250, bromophenol blue, and N,N,N′,N′-tetramethyl ethylenediamine (Temed) were supplied by Merck (Darmstadt, Germany). Ethanol was from Arman Sina (Tehran, I.R.I); glysin from Scharlau (Barcelona, Spain) and Triton X-100 from Applichem (GmbH in Darmstadt, Germany). Spectrophotometric measurements were made using ELISA reader, BioTek® (Winooski, VT), ELx800.

Insect rearing

The colony of Colorado potato beetle was collected from potato farms of Ajabshir city, East Azarbayjan province, Iran. It was maintained on potato foliage cv. “Agria” at University of Tabriz, at 27±1 °C, 60±5% relative humidity, under 16:8 h (L:D) photoperiod and white fluorescent light. Insects were reared from egg hatch to adult in clear plastic dishes with fresh potato leaves.

Preparation of enzyme source

Enzyme source from guts of larvae and adults were prepared based on Bandani et al. (2009). Guts of adults, third and fourth instar larvae were excided by dissection in the ice-cold phosphate buffer solution (pH: 7). For first and second instar larvae, whole larvae were grounded according to Michaud et al. (1995). The samples were homogenized in cold distilled water (ddH_2O) and the mixtures were centrifuged at 13000 rpm for 30 min at 4 °C and stored at -20 °C before analysis. Protein concentration of the enzyme source was determined by protein assay and the protein content of the enzyme source was adjusted to 2 mg/ml for further use.

Preparation of seed protein extract

Seeds of barley (*H. vulgare* L. cv. Bahman) and wheat (*T. aestivum* L. cv. MV17) (Poaceae) were supplied by Seed and Plant Improvement Institute, Karaj, Iran. Proteinaceous seed extracts were extracted using the methods by Baker (1987), Melo et al. (1999) and Mehrabadi et al. (2010). Seed was grinded thoroughly, and 30 g was homogenized with 100 ml solution of 0.1 M NaCl for 2 h, followed by centrifugation at 10000 rpm for 30 min. Seed protein in the supernatant was extracted using a saturation of 0-30, 30-50, 50-70, 70-100% ammonium sulfate. A general fraction (0-70%) was prepared for preliminary inhibitory pH tests. After 45 min, the mixture of ammonium sulfate and precipitated proteins was centrifuged. At every fraction of the extraction, the supernatant was used for the next extraction, and the pellet was dissolved in a minimal volume of the Tris-HCl buffer (0.02 M and pH: 7). The pellet dialyzed against ddH_2O for 20 h with the dialysis water changed twice. Finally, the pellet was heated at 75 °C for 15 min to inactivate endogenous enzymes within extract and after centrifugation at 10000 rpm, before it was used as inhibitors in enzyme assays. Protein concentrations were achieved 0.6 and 1 mg/ml in barley and wheat protein extracts, respectively.

Amylase assay

The α-amylase activity was measured by the dinitrosalicylic acid (DNS) procedure (Bernfeld, 1955), using 1% soluble starch as substrate as described by Bandani et al. (2010). The enzyme source (10 µl) was incubated for 30 min at 37 °C with 65 µl universal buffer (0.02 M) containing succinic acid disodium salt, glycine and MES (pH: 5) and 25 µl soluble starch. The reaction was stopped by addition of 100 µl DNS and heated in a boiling water for 10 min. After cooling for 5 min, absorbance was read at 540 nm. Appropriate blanks (reaction without enzyme extract as control) were run for all investigations. Tests were performed in triplicate, and each of them was repeated three times.

Protease assay

General protease assay was conducted according to Elpidina et al. (2001) and Gatehouse et al. (1999), with slight modification, using 2% azocasein as substrate. The mixture contains 40 µl universal buffer (pH: 5), 10 µl enzyme source and 50 µl substrate solution. After 90 min incubation of reaction mixture at 37 °C, 100 µl of 30% trichloroacetic acid (TCA) was added, and the incubation mixture was kept at 4 °C for 30 min, centrifuged at 13000 rpm for 20 min to precipitate non-hydrolysis substrate. One hundred microliters of 1 M NaOH was added to 100 µl supernatant and the absorbance at 405 nm was recorded. Appropriate blanks (reaction without enzyme extract as control) were run for all assays. Tests were performed in triplicate, and each of them was repeated three times.

Enzyme inhibition assay

The inhibition assays followed procedure according to Mehrabadi et al. (2010). The enzyme extract was pre-incubated with proteinaceous seed extracts before addition of substrate for 30 min at 37 °C. Then the same procedure for the amylase and protease assays was conducted. In this study, pH dependence of the last instar larval gut enzymes inhibition by fraction 0-70% of two seed extracts, inhibitory activity of four proteinaceous fractions on the last instar larval α-amylase and the activity of the most effective fractions of both extracts on the developmental stages α-amylase activities were investigated. The inhibition percentage (%I) was calculated as follows;

% I=100[1−(absorbance at 540 nm experiment/absorbance at 540 nm control)]

Kinetic of enzyme inhibition

The inhibition was measured with different concentrations of substrates (mg/ml) and proteinaceous seed extracts (mg protein/ml) according to the description of Mehrabadi et al. (2010). The type of inhibition was determined by Lineweaver-Burk plot. The inhibitory constant (K_i), Michaelis constant (K_m) and total maximum velocity (V_{max}) values were determined. There were three replications.

Amylase zymogram

The visualization of amylase activity was carried out by semi-denaturing native polyacrylamide gel electrophoresis (PAGE) using the procedure described by Laemmli (1970) and Mehrabadi & Bandani (2010). Enzyme extract was incubated with inhibitors for 30 min at 37°C, and then the remaining amylase activity was determined. Electrophoresis was performed in 5 and 10% polyacrylamide for stacking and resolving gels, respectively, with a 1% starch solution as substrate, at 4 °C and a voltage of 120 V. The gel was rinsed with ddH_2O and washed by shaking gently with 1% (v/v) Triton X-100 for 15 min. Then, the gel was incubated in MES buffer (pH: 5) containing 2 mM $CaCl_2$ and 10 mM NaCl for 30 min. Consequently, after rinsing the gel with ddH_2O, it was soaked with a solution of 1.3% I_2 and 3% KI to stop the reaction and to stain the unreacted starch background. Zones of amylase activity appeared as a light band against the dark background of the gel.

Feeding trials

Newly hatched Colorado potato beetle larvae were reared on excised leaves of four potato cultivars (Marx, Picasso, Burren and Agria). Fifty newly emerged larvae were placed in aerated plastic arenas. The leaves were painted with barley proteinaceous extract containing 0.6 mg protein per ml and replaced daily throughout the experiment. The developmental period of first up to forth instar larvae (L_1-L_4) and the percentage of L_4 were recorded. Last instar larvae were weighed on the fourth day. The gut enzyme extracts were prepared as mentioned and their amylase activity was determined. Whole parameters were compared to that of insects fed with potato leaves painted with ddH_2O as control.

Estimation of protein concentration

The protein concentration of all samples was estimated according to the method of Bradford (1976), where bovine serum albumin (BSA) was used as a standard protein.

Statistical analysis

Analyses of Variance (ANOVA) were employed on the data using the MSTAT-C statistical package. Means of the three replicates were tested by Tukey's and Duncan's test for significant differences. Kinetic analysis was conducted using SigmaPlot 12.5 (Exploratory Enzyme Kinetics).

Results

in vitro analysis

pH influence on enzyme inhibition: The inhibition of *L. decemlineata* α-amylase and protease activities was pH dependent (Fig 1a and b). There were significant differences between pHs, except the inhibition of α-amylase activity with wheat seed extract. The highest inhibitory effect by both seed extracts on enzyme activities was observed at pH 6. The trend of both enzyme inhibitions in all cases was similar in pH 4-8.

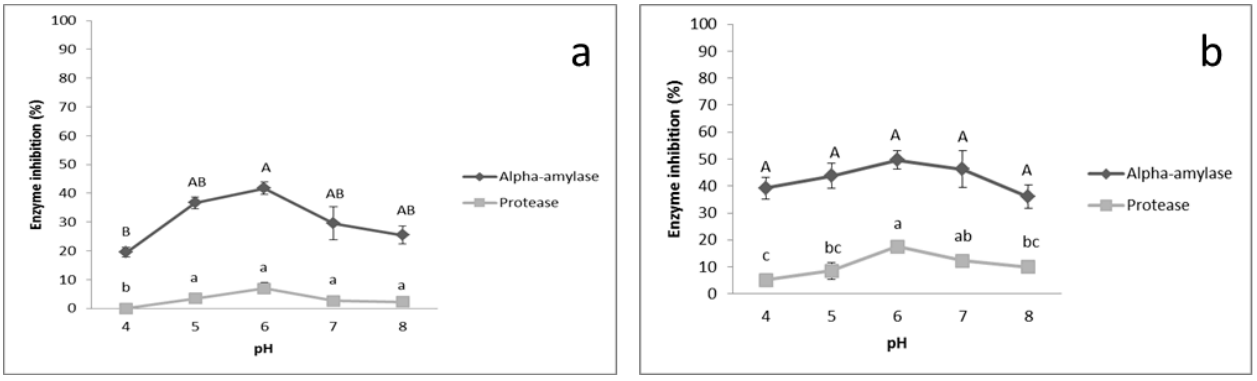

Figure 1. Influence of pH on the inhibition of the last instar larval digestive α-amylase and protease of *Leptinotarsa decemlineata* by barley (a) and wheat (b) proteinaceous seed extracts. Means followed by the different letters indicate significant differences ($p<0.05$) between data based on Tukey's test .

Enzyme inhibitory activity by proteinaceous fractions of seed extracts: Four ammonium sulfate precipitation proteinaceous fractions; 0-30, 30-50, 50-70, and 70-100% were used. These four fractions of barley seeds showed inhibitory effect with the percentage of 44, 41, 17, and 17% on the last instar larval α-amylase activity and 11, 29, 15, and 21% on the protease activity, respectively (Fig 2a). These inhibited values by wheat fractions were 50, 49, 21, and 21% on the α-amylase activity and 2, 19, 7, and 4% on the protease activity (Fig 2b). From the data, fractions from wheat seeds did not show significant inhibition on protease activity, while fractions from barley showed significant inhibition but the percentages were not substantial for further analysis. Since fractions 0-30 and 30-50% from both barley seeds and wheat produced the greatest inhibition on α-amylase activity, these fractions were used for further analysis.

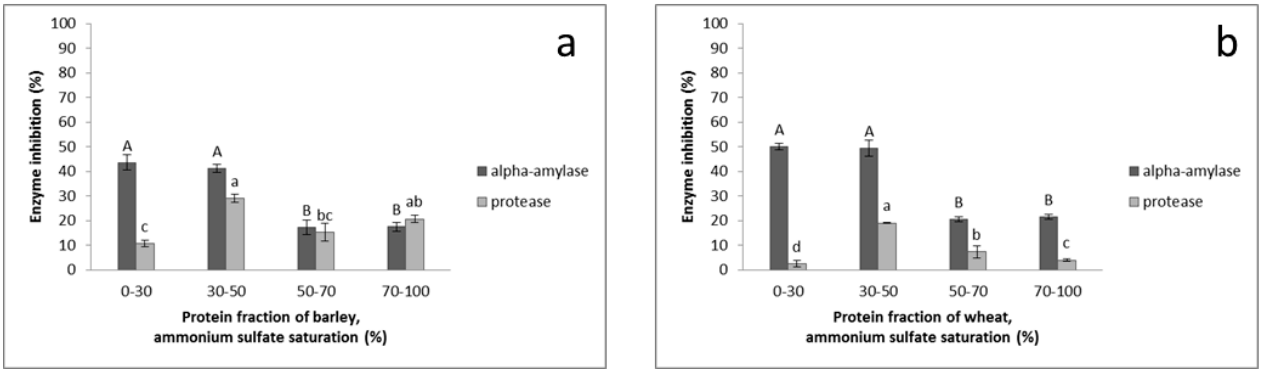

Figure 2. The effect of four ammonium sulfate precipitation proteinaceous fractions; 0-30, 30-50, 50-70, and 70-100% of barley (a) and wheat (b) on the inhibition of the last instar larval digestive α-amylase and protease of *Leptinotarsa decemlineata*. Means followed by the different letters indicate significant differences ($p<0.05$) between data based on Tukey's test .

Inhibition of α-amylase activity by effective proteinaceous fractions on CBP developmental stages: Differential inhibition of digestive α-amylase activity was seen in all developmental stages of CPB (L_1, L_2, L_3, L_4 and adults), by effective fractions of both seed extracts (Fig 3).

In barley, fraction 0-30% showed a significant inhibition on L_1 as compared to L_4 and fraction 30-50% did not show any significant differences ($p<0.05$) in inhibition of digestive α-amylase activity of all developmental stages (Fig 3a).

In wheat, the highest inhibition percentage was seen on L_1 at fraction 0-30%, and it is significant when compared to that of the L_4 (Fig 3b). A significantly high inhibitory activity of the second fraction of wheat was seen on L_3 and adults as compared to that L_2 and L_4 (Fig 3b).

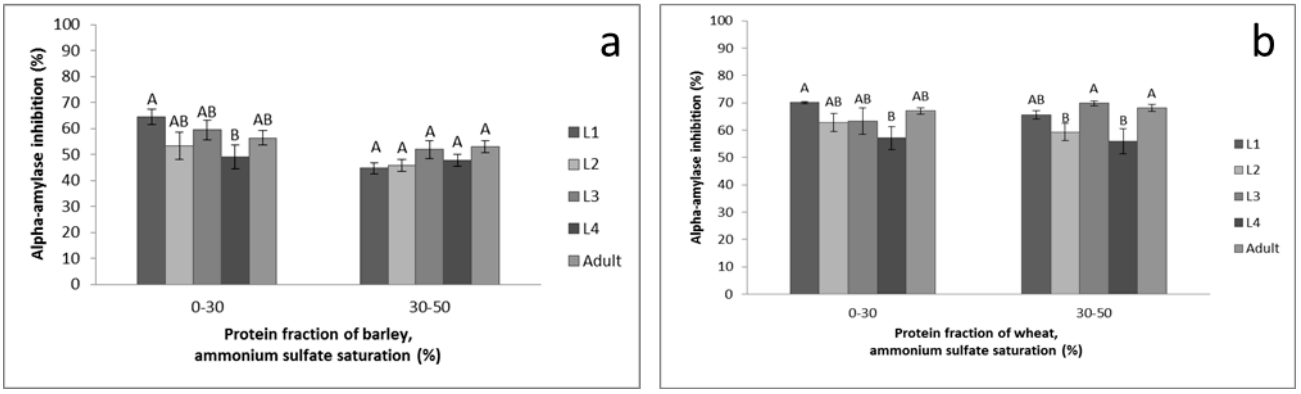

Figure 3. The inhibitory activities of effective proteinaceous fractions of barley (a) and wheat (b) on the digestive α-amylase of the first instar larvae (L1), second instar larvae (L2), third instar larvae (L3), fourth instar larvae (L4), and adults of *Leptinotarsa decemlineata*. Means followed by the different letters indicate significant differences ($p<0.05$) between data based on Tukey's test.

Kinetic of α-amylase inhibition: The detail of inhibition kinetic of the digestive α-amylase by the inhibitors was plotted using Lineweaver-Burk plots. As shown in these plots (Fig 4a and b), in the presence of inhibitors, α-amylase activity (V_{max}) was decreased with dose dependent manner; the highest velocity was recorded in the absence of inhibitors while minimum velocity was achieved in the presence of the highest dose of inhibitors. The lowest Ki value of wheat extract showed the highest potential of inhibitory activity. The interception of lines at a single point in the third quadrant ($\alpha<1$) indicates a mixed inhibition (competitive and uncompetitive inhibition) for barley (Fig 4a) and wheat extracts (Fig 4b). In both cases β value was in the interval $0<\beta<1$, that shows partial instead of full inhibition ($\beta\neq0$). These results were also supported by using "enzyme kinetics model comparison" of SigmaPlot software that compares the appropriate equations of inhibition mode by ranking R^2 and AICc (Akaike's Information Criterion), which draws median trajectory behavior plot known as direct linear plot (data not shown).

Amylase zymogram: For confirmation of the colorimetric inhibition assay results, the inhibition of incubated enzyme with first proteinaceous fraction of seeds was visualized in the semi-denaturing native PAGE (Fig 5). The gel inhibition assay confirmed that gut α-amylase of the adults was affected by the inhibitors. Gel assay using barley extract as the enzyme inhibitor showed that α-amylase activity was affected to some extent (Fig 5a). While for the wheat extract as the enzyme inhibitor, the intensity of the bands (two isozymes) were decreased completely, in comparison to the control (Fig 5b).

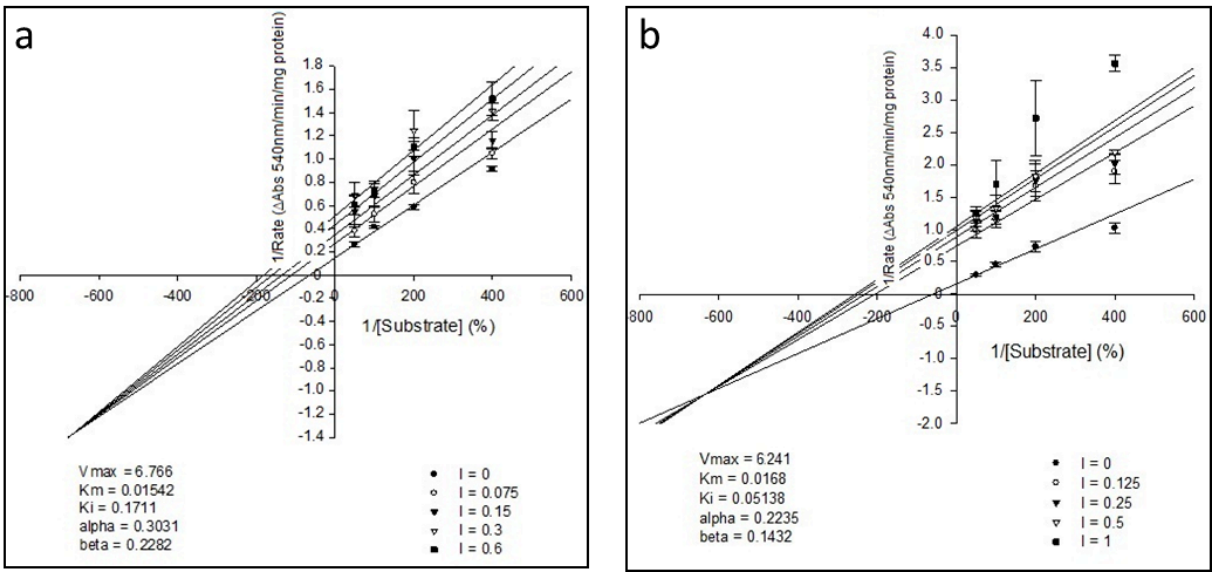

Figure 4. Lineweaver-Burk plot of *Leptinotarsa decemlineata* α-amylase in the presence of barley (a) and wheat (b) protein extracts, which give an estimation of K_i with variable concentrations of starch (0.25, 0.5, 1 and 2 %) and each crude enzyme inhibitors (I) (mg/ml).

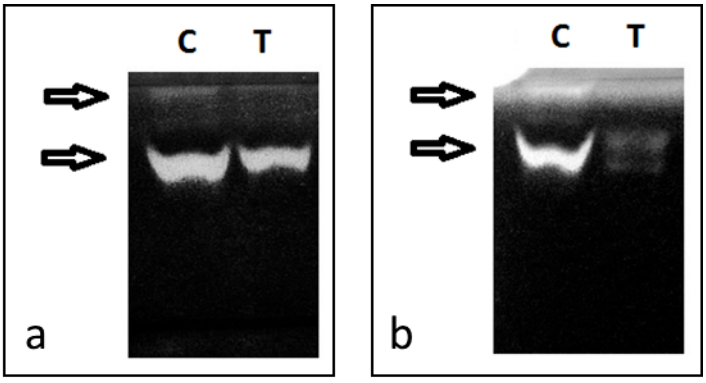

Figure 5. Gel inhibition assay of proteinaceous fraction 0-30% of barley (a) and wheat (b) on the adult's digestive α-amylase of *Leptinotarsa decemlineata*. Left columns showed controls α-amylase activity without seed extracts (C), and right columns to inhibited enzyme activity with seed extracts (T).

in vivo analysis

Diet bioassays: In order to understanding the effect of barley proteinaceous extract *in vivo*, insect feeding trials were conducted. Larvae of the Colorado potato beetle were reared from first instar on potato leaves of four cultivars (Marx, Picasso, Burren and Agria) as control and on leaves coated with extract. The developmental parameters of larvae were followed.

Weight of last instar larvae fed on treated leaves of Picasso cv. (103 mg) was lower than control (126 mg) significantly ($P<0.05$), and the L_1 to L_4 evaluation percentage in all cultivars were reduced significantly ($P<0.05$), as compared to the control (Table 1). Developmental durations up to L_4 molting (L_1 to L_4) were increased significantly when reared on Marx and Picasso ($P<0.05$), in comparison to the control. But there was no significant effect on gut α-amylase activity of survived individuals feeding on all cultivars (Table 1).

Table 1. The effects of barley proteinaceous extract on larval biological parameters and digestive α-amylase of *Leptinotarsa decemlineata* by using four different cultivars of potato leaves

Parameters	Treatment	Potato cultivars			
		Marx	Picasso	Burren	Agria
Larval weight (mg)	Control	122±5.3 ab	126±4.7 a	110.33±2.9 bc	112.33±2.3 bc
	Treated	116±4.5 abc	103.33±1.8 c	108.33±4.9 c	107.67±2.4 c
L_1 to L_4 evaluation (%)	Control	51.33±5.51 a	57.33±4.79 a	29.33±4.61 bc	42.67±4.35 ab
	Treated	16.67±1.043 cd	15.33±0.54 cd	10±1.11 d	23.33±1.61 cd
Duration up to L_4 molting (day)	Control	8.023±0.409 bc	7.333±0.086 c	8.906±0.774 abc	8.309±0.285 abc
	Treated	9.953±0.149 a	9.575±0.901 ab	9.267±0.371 ab	9.383±0.96 ab
L_4 gut α-amylase activity (U/mg protein)	Control	2.258±0.127 a	2.239±0.1 a	2.252±0.059 a	2.185±0.036 a
	Treated	2.32±0.051 a	2.248±0.121 a	2.173±0.089 a	2.223±0.057 a

Means followed by the different letters indicate significant differences ($p<0.05$) between data based on Duncan's test.

Discussion

Plants have evolved a wide array of defensive compounds, most of which are accumulate in the seed tissues constitutively or after induction, that confer resistance against phytophagous insects (Carlini & Grossi-de-Sa, 2002). Using naturally occurring plant enzyme inhibitors to target insect digestive enzymes has received serious consideration as a means of insect pest management (Zhu-Salzman et al., 2005). These inhibitors are the most extensively studied group of anti-insect chemicals (Chen, 2008).

It is considerable that *in vivo* conditions may crucially modulate α-amylase specificity. For example, the acidic optimum inhibitory pH may be responsible for their inhibition of amylases in coleoptera, whose intestinal contents are acidic (Franco et al., 2002). For this purpose, the inhibitory pH of proteinaceous extracts of barley and wheat on digestive α-amylase and protease of CPB was studied in current paper. In accordance with the optimum pH of enzymes activity, the maximum inhibitory effects of both extracts were observed at acidic pH (6 and 5). It was found that interaction between enzymes and seed extracts was also pH dependant. The accordance between gut lumen pH, amylase optimal pH, and pH optimum for amylase inhibition by plant amylase inhibitors has been described in other insect studies (Mehrabadi et al., 2010).

In preliminary tests, after achievement to optimum inhibitory pH with general fraction (0-70%) of ammonium sulfate precipitated protein of seeds, four other fractions (0-30%, 30-50%, 50-70% and 70-100%) were prepared and their inhibitory effects on both enzymes were compared. Results showed that digestive protease of the last instar larvae of CPB was less sensitive to these inhibitors, while starch hydrolysis was inhibited by fractions 0-30 and 30-50% of both seeds. When the sensitivity of digestive α-amylase activity of all developmental stages was compared, it was obvious that all stages were affected significantly. As a whole statement, the last instar larvae digestive α-amylase activity is less sensitive to be inhibited in most cases. Maybe this is due to the high activity of enzymes in the last instar larvae as compared to others. The hydrolytic activity of the amylase in the presence of different inhibitors and substrate concentrations was analyzed. To determine the mode of inhibition Lineweaver-Burk plots were drawn for the inhibited enzyme. In both seed extracts, the type of inhibition was found to be mixed (partial, not full inhibition), so the inhibitor can bind to enzyme or to the enzyme-substrate complex. According to the K_i values of both inhibitors, the wheat proteinaceous extract (K_i=0.05 mg/ml) had inhibitory activity higher than the barley (K_i=0.17 mg/ml). The gel assay showed two amylolytic bands in the insect gut, confirmed the inhibition of the adult's α-amylases by inhibitors and reduction in the bands intensity.

In the feeding assays, all studied biological parameters were affected slightly when larvae was fed with barley protein coated leaves of the four cultivars of potato. Several significant differences were shown in controls of different cultivars in larval weight and evaluation parameters values were in this order; Picasso>Marx>Agria> Burren. The best affected parameters were found in Picasso-inhibitor treatment but there was no significant reduction on gut α-amylase activity of survived individuals which suggest that they may be able to overcome α-amylase inhibitor. However, the reduction in enzyme activity of the survived larvae is difficult to be accessed *in vivo*.

In other literatures, Gutierrez et al. (1990) prepared crude α-amylase inhibitor from the endosperms of wheat and barley and found that 50µg of them causes 53% and 38% inhibition on Colorado potato beetle larval enzyme, respectively, which are in accordance with our findings. Feng et al. (1996) purified four α-amylase inhibitors from wheat flour that inhibited α-amylases of *Sitophilus oryzae* L. (Coleoptera: Curculionidae), *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), and *Tenebrio molitor* L. (Coleoptera: Tenebrionidae). They observed that the growth rate of *T. castaneum* larvae was slowed when purified inhibitor was included in the diet at a level of 10%. Franco et al. (2000) isolated five α-amylase inhibitors from wheat kernels and showed its inhibitory effect on the enzyme of *A. obtectus*. Warchalewski et al. (2002) showed that wheat proteinaceous α-amylase inhibitors in diets of adults of *Sitophilus granarius* L. (Coleoptera: Curculionidae), did not affect their survival, but it lengthened the development time of *T. confusum* Duval (Coleoptera: Tenebrionidae) larvae by 15 days. In their study, the wheat proteinaceous extract in a diet consisting of 50% crude inhibitors also caused *Ephesitia kuehniella* Zeller (Lepidoptera: Pyralidae) larvae failed to develop. However, in our results, barley proteinaceous extract had a limited influence on the developmental duration of CPB. It extended the duration of CPB molting on two cultivars of potato, Marx and Picasso, from 8.0 days in the control trial to 9.9 days in the treatment, and from 7.3 days to 9.6 days, respectively.

Piasecka-Kwiatkowska et al. (2007) studied inhibitory activities of three cereal grains; wheat, rye (*Secale cereale* L.) and triticale (X *Triticosecale wittmack*) against some stored-product pest α-amylases. They concluded that inhibitory effects were different and dependent on genus and cereal varieties. Priya et al. (2010) showed that wheat α-amylase inhibitors inhibits *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae) α-amylase and stated that this inhibitor showed mixed type of inhibition mainly competitive with some non-competitive behavior that is similar to our results. Mehrabadi et al. (2010) examined the effect of triticale extract on midgut amylases of *Eurygaster integriceps* Puton (Hemiptera: Scutelleridae) and showed that it had inhibitory effects on α-amylases, with dose dependent manner. Mehrabadi et al. (2011) studied the effect of seven plant species extracts including *Punica granatum* L. (Punicaceae), *Rheum officinale* B. (Polygonaceae), *Rhus coriaria* L. (Anacardiaceae), *Artemisia sieberi* B. (Compositae), *Peganum harmala* L. (Nitrariaceae) , *Datura stramonium* L. (Solanaceae) and *Thymus vulgaris* L. (Lamiaceae) on α-amylase activity of four stored insect pests including *C. maculatus*, *R. dominica*, *S. granarius*, and *Trogoderma granarium* E. (Coleoptera : Dermestidae) and showed that plant extracts can inhibit activity of insect α-amylases varying from nearly 4% to 95% inhibition. Khan (2011) extracted proteinaceous inhibitors from wheat, chick pea (*Cicer arietinum* L.) (Fabaceae), kidney bean (*Phaseolus vulgaris* L.) (Fabaceae), maize (*Zea mays* L.) (Poaceae), and millet (*Pennisetum typhoides* Burm) (Poaceae) and found that inhibitors isolated from millet (57.1%), wheat (75%), maize (82.1%) and kidney bean (67.8%) exhibited inhibitory activity against α-amylase from *T. castaneum*. Borzoei et al. (2013) showed 15.3% and 91.2% inhibition of α-amylase of *Plutella xylostella* L. (Lepidoptera: Plutellidae) in a low and high dose of the wheat seed extracts. In accordance with our findings, they found that interaction between the insect α-amylase and seed extracts is also pH dependant. They also stated that physiochemical environment of the insect gut affect interaction between the insect α-amylase and the inhibitors. Borzoui & Bandani (2013) found that the effect of wheat is greater than the triticale seed extract on both α-amylase and protease of *Ectomyelois ceratoniae* Zeller (Lepidoptera: Pyralidae). In their assays the lowest concentration of wheat and triticale seed extracts (0.106 mg protein/ml) inhibited 39% and 18% and the highest concentration (1.7 mg protein/ml) inhibited 82% and 75% of the amylase activity, respectively. Dastranj et al. (2013) stated that bean, and wheat cultivars; MV17, Aflak, Sivand, Saymon, and Zare inhibited the α-amylase activity of *T. molitor* with the percentage of 70.9, 58.3,56.2, 58.5, 57.2, and 48.5, respectively. Most of the results in current study were in accordance with mentioned literature.

Due to importance of α-amylases in insect survival, since larvae development would be suppressed by lower carbohydrate intake rates, consequently, reducing energy availability required for insect development, these enzymes can be good target candidates for bio-insecticides via their inhibitors from plant sources (Sivakumar et al., 2006; Silva et al., 2009). Searching for new enzyme inhibitors, has increasingly lead to more studies aimed at genetic modification techniques for the insertion of genes of resistance to insects in plant species, or for the development of specific bio-insecticides (Silva et al., 2009). If an increase in effectiveness of α-amylase inhibition achieves, further engineering of these proteins can be made to produce more effective inhibitor for application as bio-control agent instead of the currently employed insecticides (Wisessing et al., 2008).

These data revealed that wheat and barley seed extracts contain proteinaceous molecules that can interfere with digestive α-amylase and developmental parameters of the Colorado potato beetle, present an interesting potential for the development of insect-resistant transgenic plants that express heterologous α-amylase inhibitors. Many of these plants extract α-amylase inhibitors should be tested in field conditions and commercialized.

Acknowledgement

This project was funded by a grant from University of Tabriz. We would like to thank Seed and Plant Improvement Institute of Karaj (Iran) for providing wheat and barley seeds for this study.

References

Alves, D. T., I. M. Vasconcelos, J. T. A. Oliveira, L. R. Farias, S. C. Dias, M. D. Chiarello, S. Maria-Neto, & O. L. Franco, 2009. Identification of four novel members of Kunitz-like α-amylase inhibitors family from *Delonix regia* with activity toward Coleopteran insects. Pesticide Biochemistry and Physiology, 95: 166-172.

Baker, J. E., 1987. Purification of isoamylases from the rice weevil, *Sitophilus orizae* L. by HPLC and their interaction with partially purified amylase inhibitor from wheat. Insect Biochemistry, 17: 37-44.

Bandani, A. R., M. Kazzazi & M. Mehrabadi, 2009. Purification and characterization of midgut α-amylases of *Eurygaster integriceps*. Entomological Science, 12: 25-32.

Bandani, A. R., F. Maleki, S. Rahmani & M. Fazeli-Dinan, 2010. Characterization of α-amylase in the alimentary canal of *Naranga aenescens* Moore (Lepidoptera: Noctuidae), the Rice green caterpillar. Munis Entomology and Zoology, 5: 716-725.

Becker-Ritt, A. B. & C. R. Carlini, 2012. Fungitoxic and insecticidal plant polypeptides. Biopolymers (Peptide Science), 98: 367-384.

Bernfeld, P., 1955. Amylases, α and β. Method Enzyme, 1: 149-158.

Borzoui, E. & A. R. Bandani, 2013. Wheat and triticale proteinacious seed extracts inhibit gut α-amylase and protease of the carob moth, *Ectomyelois ceratoniae*. Molecular Entomology, 4: 13-21.

Borzoei, E., A. R. Bandani, M. Dastranj & M. Belbasi, 2013. Effect of proteinaceous extract of wheat seeds on α-amylase activity of diamondback moth, *Plutella xylustella* (L.) (Lepidoptera: Plutellidae). SOAJ Entomolgical Studies, 2: 1-10.

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.

Carlini, C. R. & M. F. Grossi-De-Sa, 2002. Plant toxic proteins with insecticidal properties: A review on their potentialities as bioinsecticides. Toxicon, 40: 1515-1539.

Chen, M. S., 2008. Inducible direct plant defense against insect herbivores: A review. Insect Science, 15: 101-114.

Dastranj, M., A. R. Bandani & M. Mehrabadi, 2013. Age-specific digestion of *Tenebrio molitor* (Coleoptera: Tenebrionidae) and inhibition of proteolytic and amylolytic activity by plant proteinaceous seed extracts. Journal of Asia-Pacific Entomology, 16: 309-315.

Elpidina, E. N., K. S. Vinokurov, V. A. Gromenko, Y. E. Rudenskaya & D. P. Zhuzhikov, 2001. Compartmentalization of proteinases and amylases in Nauphoeta cinerea midgut. Archive of Insect Biochemistry and Physiology, 48: 206-216.

Feng, G. H., M. Richardson, T. M. S. Chen, K. J. Kramer, T. D. Morgan & G. R. Reeck, 1996. α-Amylase inhibitors from wheat: Amino acid sequences and patterns of inhibition of insect and human α-amylases. Insect Biochemistry and Molecular Biology, 26: 419-426.

Franco, O. L., D. J. Rigden, F. R. Melo, C. B. Jr, C. P. Silva & M. F. Grossi-De-Sa, 2000. Activity of wheat α-amylase inhibitors towards bruchid α-amylases and structural explanation of observed specificities. European Journal of Biochemistry, 267: 2166-2173.

Franco, O. L., D. J. Rigden, F. R. Melo & M. F. Grossi-De-Sa, 2002. Plant alpha-amylase inhibitors and their interaction with insect alpha-amylases, structure, function and potential for crop protection. European Journal of Biochemistry, 269: 397-412.

Gatehouse, A. M. R., E. Norton, G. M. Davison, S. M. Babbe, C. A. Newell & J. A. Gatehouse, 1999. Digestive proteolytic activity in larvae of tomato moth, *Lacanobia oleracea*, effects of plant protease inhibitors *in vitro* and *in vivo*. Journal of Insect Physiology, 45: 545-558.

Gatehouse, L. N., J. T. Christeller, H. S. Gatehouse & X. Y. Zou, 2002. A strong inhibitor of chymotrypsin/elastase is highly antimetabolic to *Helicoverpa armigera* larvae. New Zealand Plant Protection, 55: 421-428.

Gutierrez, C., R. Sanchez-Monge, L. Gomez, M. Ruiz-Tapiador, P. Castafiera & G. Salcedo, 1990. α-Amylase activities of agricultural insect pests are specifically affected by different inhibitor preparations from wheat and barley endosperms. Plant Science, 72: 37-44.

Habib, H. & K. M. Fazili, 2007. Plant protease inhibitors: a defense strategy in plants. Biotechnology and Molecular Biology Reviews, 2: 68-85.

Karimi, J., E. Haubruge & F. Francis, 2010. Development of entomotoxic molecules as control agents: illustration of some protein potential uses and limits of lectins (Review). Biotechnology Agronomy Society and Environment, 14: 225-241.

Khan, N., 2011. In vitro effects of protienaceous alpha amylase inhibitors on red flour beetle, *Tribolium castaneum.* Science Research Reporter, 1: 101-104.

Kondrak, M., J. Kutas, B. Szenthe, A. Patthy, Z. Banfalvi, M. Nadasy, L. Graf & B. Asboth, 2005. Inhibition of Colorado potato beetle larvae by a locust proteinase inhibitor peptide expressed in potato. Biotechnology Letters, 27: 829-834.

Laemmli, U. K., 1970. Cleavage of structural proteins during the assembly of bacteriophage T4. Nature, 227: 680-685.

Lawrence, S. D., N. G. Novak & M. B. Blackburn, 2007. Inhibition of proteinase inhibitor transcripts by *Leptinotarsa decemlineata* regurgitant in *Solanum lycopersicum*. Journal of Chemical Ecology, 33: 1041-1048.

Macedo, M. L. R. & M. D. G. M. Freire, 2011. Insect digestive enzymes as a target for pest control. Invertebrate Survival Journal, 8: 190-198.

Mehrabadi, M. & A. R. Bandani, 2010. New approach toward α-amylase electrophoresis and isoamylase detection. Munis Entomology and Zoology, 5: 1085-1087.

Mehrabadi, M., A. R. Bandani & F. Saadati, 2010. Inhibition of Sunn pest, *Eurygaster integriceps*, α-amylases by α-amylase inhibitors (T- αAI) from Triticale. Journal of Insect Science, 10: 179-191.

Mehrabadi, M., A. R. Bandani, F. Saadati & M. Mahmudvand, 2011. Alpha-amylase activity of stored products insects and its inhibition by medicinal plant extracts. Journal of Agricultural Science and Technology, 13: 1173-1182.

Melo, F. R., M. P. Sales, L. S. Silva, O. L. Franco, J. R. C. Bloch & M. B. Ary, 1999. α-Amylase inhibitors from cowpea seeds. Protein and Peptide Letters, 6: 387-392.

Michaud, D., N. Bernier-Vadnais, S. Overney & S. Yelle, 1995. Constitutive expression of digestive cysteine proteinase forms during development of the Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae). Insect Biochemistry and Molecular Biology, 25: 1041-1048.

Piasecka-Kwiatkowska, D., D. Madaj & J. R. Warchalewski, 2007.The biological activity of wheat, rye and triticale varieties harvested in four consecutive years. Acta scientiarum Polonorum/Technologia alimentaria, 6: 55-65.

Priya, S., N. Kaur & A. K. Gupta, 2010. Purification, characterization and inhibition studies of a-amylase of *Rhyzopertha dominica*. Pesticide Biochemistry and Physiology, 98: 231-237.

Silva, E. M., A. Valencia, M. F. Grossi-de-Sa, T. L. Rocha, E. Freire, J. E. De Paula, & L. S. Espindola, 2009. Inhibitory action of cerrado plants against mammalian and insect α-amylases. Pesticide Biochemistry and Physiology, 95: 141-146.

Sivakumar, S., M. Mohan, O. L. Franco, & B. Thayumanavan, 2006. Inhibition of insect pest alpha-amylases by little and finger millet inhibitors. Pesticide Biochemistry and Physiology, 85: 155-160.

Valencia-Jime´nez, A., A. J. W. Arboleda, A. Lopez Avila, & M. F. Grossi-de-Sa, 2008. Digestive α-amylases from *Tecia solanivora* larvae (Lepidoptera: Gelechiidae): response to pH, temperature and plant amylase inhibitors. Bulletin of Entomological Research, 98: 575-579.

Warchalewski, J. R., J. Gralik, Z. Winiecki, J. Nawrot & D. Piasecka-Kwiatkowska, 2002. The effect of wheat a-amylase inhibitors incorporated into wheat-based artificial diets on development of *Sitophilus granarius* L., *Tribolium confusum* Duv., and *Ephestia kuehniella* Zell. Journal of Applied Entomology, 126: 161-168.

Wisessing, A., A. Engkagul, A. Wongpiyasatid, & K. Chuwongkomon, 2008. Proteomic and characterization of α-amylase inhibitor from Mungbean (*Vigna radiate*). Kasetsart Journal (Natural Science), 42: 245-250.

Wolfson, J. L. & L. L. Murdock, 1990. Diversity in digestive proteinase activity among insects. Journal of Chemical Ecology, 16: 1089-1102.

Zhu-Salzman, K., J. L. Bi & T. X. Liu, 2005. Molecular strategies of plant defense and insect counter-defense. Insect Science, 12: 3-15.

Türk. entomol. derg., 2015, 39 (4): 333-343 ISSN 1010-6960
DOI: http://dx.doi.org/10.16970/ted.92495

Orijinal araştırma (Original article)

The impact of the vegetation of apple orchard edges on quantity and quality structure of predatory hoverflies (Diptera: Syrphidae) communities

Elma bahçesi sınır vejetasyonlarının avcı çiçek sineği (Diptera: Syrphidae) komünitelerinin nicel ve nitel yapılarına etkisi

Hanna PIEKARSKA-BONIECKA[1] **Michał SIATKOWSKI**[2]
Paweł TRZCIŃSKI[1] **Idzi SIATKOWSKI**[3*]

Summary

The research on the impact of apple orchard edges vegetation on quantity and quality structure of predatory Syrphidae was carried out between 2008 and 2010 in the vicinity of Czempiń in the western part of Wielkopolska, Poland. Quality and quantity analyses of Syrphidae communities in the apple orchards and on their edges were conducted; the edges included fields, shrubberies and a road lined with trees and bushes. The total of 35 Syrphidae species were found, which constituted 8.8% of the national fauna of this family. In the orchards 24 species and on the edges 32 species were caught. The total number of specimens of the family was 5080, out of which 2338 were caught in the orchards and 2742 on the edges.. Apple orchards with developed edge vegetation yielded higher abundances of Syrphidae than the orchard bordering on the fields. All the orchards and their edges were dominated by two species, namely *Episyrphus balteatus* (De Geer, 1776) and *Eupeodes corollae* (Fabricius, 1794). It was found that the majority of Syrphidae flies communities inhabiting the orchards and their edges showed a high similarity in their quality and quality-quantity structures. The research showed that the occurrence of well-developed vegetation with diversified species on apple orchard edges positively influences the species richness and abundance of predatory Syrphidae occurring in the orchard habitat.

Keywords: Cluster method, diversity functions, hoverflies, plants of orchard edge, Poland, Syrphidae

Özet

Bu çalışma elma bahçesi sınır vejetasyonlarının bahçe içerisindeki avcı Syrphidae'lerin nicel ve nitel yapısı üzerindeki etkilerinin belirlenmesi için 2008 ve 2010 yılları arasında Wielkopolska (Polonya)'nın batı kısmında bulunan Czempin bölgesinde yürütülmüştür. Çalışmada elma bahçelerindeki ve bahçe sınır vejetasyonlarındaki Syrphidae komünitelerinin nicel ve nitel analizleri yapılmış olup sınır vejetasyonlarına bahçe kenarında bulunan çalılıklar, ağaçlar ve yol alanları dahil edilmiştir. Çalışma sonucunda toplamda ülke syrphid faunasının %8.8'ni oluşturan 35 Syrphidae türü saptanmıştır. Bahçe içerisinde 24, bahçe sınırlarında 32 syrphid türü belirlenmiştir. Çalışmada toplamda yakalanan birey sayısı 5080 olup bu bireylerden 2338 adeti bahçe içerisinden 2742 adeti bahçe kenarlarından yakalanmıştır. Çalışma sonucunda bahçe sınırlarının vejetasyon bakımından zengin olduğu bahçelerde tür zenginliğinin bahçe sınırlarının düzenlendiği ve vejetasyon bakımından zengin olmayan bahçelere oranla daha zengin olduğu belirlenmiştir. Vejetasyon bakımından daha zengin elma bahçeleri, bahçe kenarında bitkinin olmadığı elma bahçelerine nazaran daha baskın Syrphidae faunasına sahip bulunmuştur. Hem elma bahçeleri hem de bahçe sınır vejetasyonlarında en çok *Episyrphus balteatus* (De Geer, 1776) ve *Eupeodes corollae* (Fabricius, 1794) türlerine rastlanılmıştır. Bahçe içerisinde ve bahçe sınırlarında tespit edilen syrphid komünitelerinin nicel ve nitel bakımdan birbirlerine çok benzediği tespit edilmiştir. Çalışma sonucunda bahçe sınırlarının zenginleştirildiği elma bahçelerinde Syrphidae faunasının zenginleştirilmemiş elma bahçelerine nazaran daha zengin olduğu tespit edilmiştir.

Anahtar sözcükler: Kümeleme metodu, çeşitlilik faktörleri, çiçek sinekleri, bahçe sınırı bitkileri, Polonya, Syrphidae

[1] Department of Entomology and Environmental Protection, Poznań University of Life Sciences, Poznań, Poland
[2] Institute of Agricultural Engineering, Poznań University of Life Sciences, Poznań, Poland
[3] Department of Mathematical and Statistical Methods, Poznań University of Life Sciences, Poznań, Poland
* Sorumlu yazar (Corresponding author) e-mail: idzi@up.poznan.pl
Alınış (Received): 14.04.2015 Kabul ediliş (Accepted): 27.10.2015 Çevrimiçi Yayın Tarihi (Published Online): 02.12.2015

Introduction

The preservation of the existing uncultivated refuge habitats in agrocenoses, as well as enriching them with various advantageous elements, such as: shrubbery belts, road edges, tree groups of fragments of forest communities increases the diversity of their entomofauna, including predatory Syrphidae species. It was proved by numerous studies (Wnuk, 1972; Branquart & Hemptinne, 2000; Colley & Luna, 2000; Solomon et al., 2000; Carreck & Williams, 2002; Bostanian et al., 2004; Ambrosino et al., 2006; Rossi et al., 2006; Haenke et al., 2009). Refuge habitats stabilise the processes related to the functioning of ecosystems, and properly enriched habitats bordering directly on cultivated fields also shape the living conditions of beneficiary entomofauna of the Syrphidae family (MacLeod, 1999; Sutherland et al., 2001; Trzciński & Piekarska-Boniecka, 2009).

The larvae of Syrphidae reduce the population of aphids (Hemiptera, Aphidoidea), economically important pests in orchards. Adult Syrphidae play a significant role in biocenoses, as they pollinate plants. Therefore, flourishing vegetation may be a attractant factor for this insects to orchard.

The aim of the study was to define the impact of the vegetation of apple orchard edges in the form of fields, shrubberies and the road grown with trees and bushes on quality and quantity structure of the communities of predatory Syrphidae which occur in the orchard habitat.

Material and Methods

The study was conducted between 2008 and 2010 in three orchards located in the vicinity of Czempiń in Wielkopolska (Greater Poland). One orchard was located in Głuchowo and two orchards were in Gorzyczki. The orchard in Głuchowo was 15 km away from the orchards in Gorzyczki I, while the orchards in Gorzyczki I were 1 km away from each other, Gorzyczki II (Figure 1).

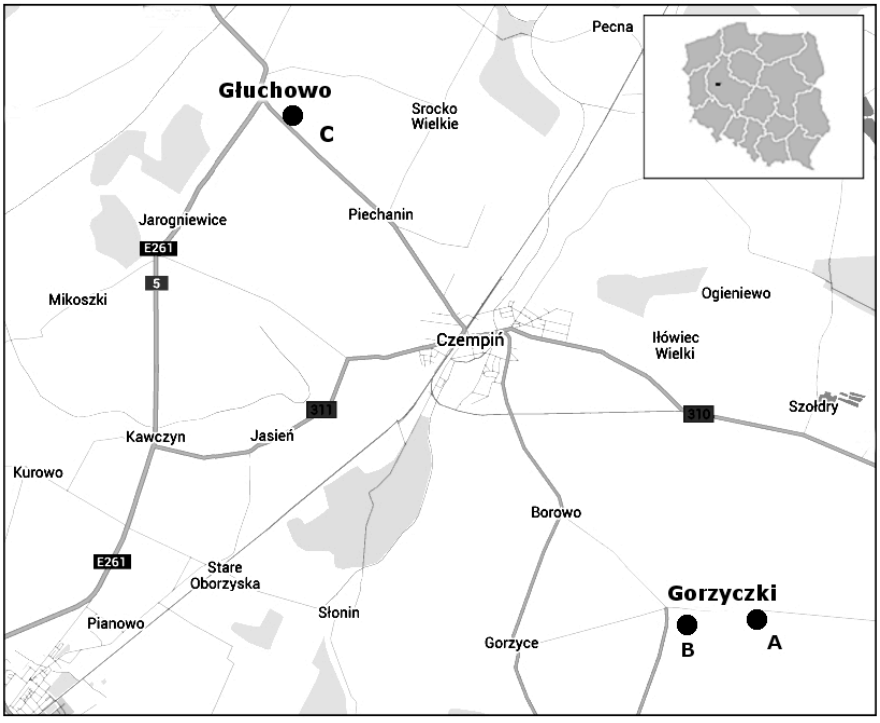

Figure 1. The location of apple orchards in the vicinity of Czempiń (Poland): A – the orchard in Głuchowo, B – orchard in Gorzyczki I, C – orchard in Gorzyczki II.

The study sites included:

A) Apple orchard, Głuchowo (UTM, XT18; 52.17466 °N, 16.71173 °E) of 40 ha surface area (A1 = Głuchowo orchard). The studies were conducted on 3-hectare plots with 15-year-old apple trees of the following cultivars: Gala, Ligol, Cortland, Paulared, Red Delicious and Golden Delicious. The apple tree plot was surrounded with cultivated fields (A2 = Głuchowo field), where sweet corn was grown in 2008, oats in 2009, and triticale in 2010.

B) Apple orchard, Gorzyczki I (UTM, XT27; 52.10106 °N, 16.81199 °E) 20 ha in area (B1 = Gorzyczki I orchard), where the studies covered 5-hectare plots with 15–year-old apple trees of: Paulared, Red Delicious, Golden Delicious and Jonagold cultivars. The apple tree plot was surrounded by shrubberies (B2 = Gorzyczki I shrubberies), namely thicket phytocenoses of *Euonymo-Prunetum spinosae* and *Querco-Ulmetum* forest, herbaceous communities and ruderal plant communities. Tree communities were formed mainly by: European elm (*Ulmus laevis* Pall.), sessile oak (*Quercus robur* L.), ash tree (*Fraxinus excelsior* L.), maple (*Acer platanoides* L.), boxelder maple (*Acer negundo* L.) and single apple trees (*Malus domestica* Borkh.) with hybrid black poplar (*Populus ×canadensis* Moench). Herbaceous plants were dominated by stinging nettle (*Urtica dioica* L.) and Canada thistle (*Cirsium arvense* (L.) Scop.). In the patches of ruderal shrubberies the following were recorded: elder (*Sambucus nigra* L.), common hawthorn (*Crataegus monogyna* Jacq.), matrimony vine (*Lycium barbarum* L.), dog rose (*Rosa canina* L.) and hazel (*Corylus avellana* L.).

C) Apple orchard in Gorzyczki II (UTM, XT27; 52.10208 °N, 16.81451 °E) 10 ha in area (C1 = Gorzyczki II orchard). The studies were conducted on 2-hectar plots with 20-year-old Golden Delicious apple trees. The orchard borders on a road (C2 = Gorzyczki II road) overgrown with plants typical of *Rhamno-Prunetea* class. The road was lined with walnut (*Juglans regia* L.), maples: boxelder (*Acer negundo* L.), common (*A. platanoides* L.), sycamore (*A. pseudoplatanus* L.) and sessile oak (*Quercus robur* L.), with some dog rose shrubs (*Rosa canina* L.), hawthorn (*Crataegus×media* Bechst.), hazel (*Corylus avellana* L.) and snoberry (*Symphoricarpos albus* Duhamel). Herbaceous plants were dominated by grass, stinging nettle (*Urtica dioica* L.), wormwood (*Artemisia absinthium* L.), yarrow (*Achillea millefolium* L.) and cleavers (*Galium aparine* L.).

In all the studied orchards apple trees were 1.4 m from each other in rows set 3 m apart. Between the trees fallow land was maintained and the rows of trees were divided by sward. The integrated fruit production policy was implemented in orchards. Apple protection program was also practised in the same terms against the diseases and pests in all the orchards. In each of the orchards 5 - 8 procedures against diseases and 6 - 8 procedures against pests were performed in the different years of study.

Method of study

The study used a common method of trapping Syrphidae imagines the yellow Moericke traps (Moericke, 1953). The trap was composed of a yellow plastic pan filled with water and glycol (preservative) and liquid lowering surface pressure, 18 cm in diameter and 11 cm deep. 20 pans were laid out on each site, 1-1.5 m above the ground. The traps were situated in the following manner: 10 of them in the orchard and the other 10 further away, several meters from the orchard's edge. The traps were placed up to 10 m apart from each other. Specimens were collected in ten-day intervals. Insects caught in one pan during ten days constituted one sample. The traps were placed in the orchard from April to October in each study year. The collected materials were stored in 75% ethyl alcohol and they were deposited in the Department of Entomology and Environmental Protection, the University of Life Sciences in Poznan.

Imagines of Syrphidae were determined based on the keys by van Veen (van Veen, 2004) and Speight and Sarthou (Speight & Sarthou, 2010).

Statistical analysis

The species richness data was pooled for each habitat and compared among study sites with dominance diversity curves. The similarity among the apple orchards and surrounding habitats was estimated with the abundance-based estimator for Jaccard and Sorensen classic index (Chao et al., 2005). An indvidual-based curve was calculated with 95 percent confidence intervals curve using Mao-Tau function (Colwell et al., 2004). In addition, expected species accumulation curves per individual were used to compare the taxonomical diversity in the samples of different sizes. The similarity was studied with cluster analysis, and the results were presented as a dendrogram. The abundance of predatory species found on each bait type and site were examined by applying principal components analysis. Statistical calculations were performed with Estimates (Colwell, 2011) and R software, version 3.2.1 (R Core Team, 2015).

Results

The total numbers of 3644 samples were caught between 2008 and 2010 in the orchard habitats near Czempiń, out of which 1818 samples were from the orchards and 1826 were from their edges. Overall 35 Syrphidae predatory species were reported, which constitutes 8.8% of the national fauna of this family (Soszyński, 2007) (Table 1). In the orchards 24 species occurred, while on the orchard edges 32 specimens were reported. The total number of the caught Syrphidae was 5080, with 2338 specimens from the orchards and slightly more, i.e. 2742, specimens from the edges.

In orchard habitats where orchards bordered on well-developed edge vegetation, i.e. shrubberies (B2) and a road lined with trees and bushes (C2), predatory Syrphidae species were found in higher abundances (Table 1).

In apple orchards, regardless of the abundance of the vegetation they bordered on, similar numbers of Syrphidae species were caught. In orchards with abundant vegetation (B1, C1) higher Syrphidae abundances were found than in the orchard (A1) which bordered on agricultural cultivations.

In all the orchards and their edges 8 (22.8%) common species were found, namely: *Episyrphus balteatus* (De Geer, 1776), *Eupeodes corollae* (Fabricius, 1794), *Melanostoma mellinum* (Linnaeus, 1758), *Scaeva pyrastri* (Linnaeus, 1758), *Sphaerophoria scripta* (Linnaeus, 1758), *Syrphus ribesii* (Linnaeus, 1758), *S. torvus* Osten-Sacken, 1875 and *S. vitripennis* Meigen, 1822. There were 2 species exclusive for the orchards (5.7%): *Sphaerophoria rueppellii* (Wiedemann, 1830) and *Triglyphus primus* Loew, 1840. In edge habitats 10 (28.6%) the following exclusive species were found: *Dasysyrphus tricinctus* (Fallen, 1817), *Epistrophe nitidicollis* (Meigen, 1822), *Melangyna lasiophthalma* (Zetterstedr, 1843), *M. quadrimaculata* Verrall, 1873, *M. umbellatarum* (Fabricius, 1794), *Parasyrphus punctulatus* (Verrall, 1873), *Pipizella viduata* (Linnaeus, 1758), *Platycheirus fulviventris* (Macquart, 1829), *Xanthogramma pedissequum* (Harris, 1776) and *X. stackelbergi* Violovitsh, 1975.

In all the apple orchards and on their edges (A1 – C2) one eudominant was found - this was *Episyrphus balteatus* with the share of 48.8 - 82.6%. Another abundant species was *Eupeodes corollae*, which was eudominant and dominant with 8.1 - 31.2% share in the orchard habitats (A1 - C2). In the apple orchard (A1) and the neighbouring fields (A2) one dominant was found: *Sphaerophoria scripta,* with shares 5.1% and 7.6%. Also in the shrubberies (B2) a dominant was caught: *Syrphus vitripennis* with 5.3% share. Other Syrphidae species were less abundant in particular habitats and they included mainly subrecedents and recedents (Table 1 and Figure 2).

Having analysed the number of species and abundances of Syrphidae in particular orchards and on their edges based on Mao-Tau function the most Syrphidae species and the highest abundances were found in the shrubberies (B2). The poorest species composition of those entomophages and the lowest abundance were found in the fields (A2) (Figure 3).

Table 1. The list of species and abundances of predatory Syrphidae caught in the orchard habitat near Czempiń in 2008 - 2010 (N – abundance, D - dominance index)

	Species	Habitats											
		Głuchowo				Gorzyczki I				Gorzyczki II			
		Orchard (A1)		Field (A2)		Orchard (B1)		Shrubberies (B2)		Orchard (C1)		Road (C2)	
		N	D	N	D	N	D	N	D	N	D	N	D
S1	*Baccha elongata* (Fabricius 1775)							3	0.2%	1	0.2%		
S2	*Dasysyrphus tricinctus* (Fallen, 1817)											1	0.1%
S3	*Epistrophe eligans* (Harris, 1780)							2	0.2%	1	0.2%	1	0.1%
S4	*Epistrophe nitidicollis* (Meigen, 1822)							1	0.1%				
S5	*Episyrphus balteatus* (De Geer, 1776)	384	64.9%	258	48.8%	720	64.1%	991	76.1%	501	80.5%	752	82.6%
S6	*Eupeodes corollae* (Fabricius, 1794)	100	16.9%	165	31.2%	205	18.2%	161	12.4%	70	11.3%	74	8.1%
S7	*Eupeodes latifasciatus* (Macquart, 1829)	1	0.2%										
S8	*Eupeodes luniger* (Meigen, 1822)					6	0.5%	9	0.7%	4	0.6%	1	0.1%
S9	*Melangyna lasiophthalma* (Zetterstedt, 1843)							1	0.1%				
S10	*Melangyna quadrimaculata* Verrall, 1873			1	0.2%			2	0.2%				
S11	*Melangyna umbellatarum* (Fabricius, 1794)											1	0.1%
S12	*Melanostoma mellinum* (Linnaeus, 1758)	23	3.9%	13	2.5%	37	3.3%	11	0.8%	4	0.6%	10	1.1%
S13	*Melanostoma scalane* (Fabricius, 1794)					11	1.0%	1	0.1%	2	0.3%	4	0.4%
S14	*Meligramma cincta* (Fallen, 1817)							1	0.1%		0.0%		
S15	*Meliscaeva auricollis* (Meigen, 1822)			1	0.2%					1	0.2%		
S16	*Parasyrphus punctulatus* (Verrall, 1873)							1	0.1%				
S17	*Pipizella viduata* (Linnaeus, 1758)							1	0.1%				
S18	*Platycheirus albimanus* (Fabricius, 1781)	1	0.2%			1	0.1%					1	0.1%
S19	*Platycheirus clypeatus* (Meigen, 1822)			3	0.6%	1	0.1%	1	0.1%	1	0.2%		

Table 1 (continued)

	Species	Habitats											
		Głuchowo				Gorzyczki I				Gorzyczki II			
		Orchard (A1)		Field (A2)		Orchard (B1)		Shrubberies (B2)		Orchard (C1)		Road (C2)	
		N	D	N	D	N	D	N	D	N	D	N	D
S20	*Platycheirus fulviventris* (Macquart, 1829)			1	0.2%								
S21	*Platycheirus peltatus* (Meigen, 1822)									1	0.2%	1	0.1%
S22	*Platycheirus scutatus* (Meigen, 1822)	1	0.2%			3	0.3%	1	0.1%	1	0.2%		
S23	*Scaeva pyrastri* (Linnaeus, 1758)	11	1.9%	20	3.8%	18	1.6%	5	0.4%	7	1.1%	5	0.5%
S24	*Scaeva selenitica* (Meigen, 1822)	1	0.2%	1	0.2%			1	0.1%	1	0.2%	2	0.2%
S25	*Sphaerophoria rueppellii* (Wiedemann, 1830)	1	0.2%										
S26	*Sphaerophoria scripta* (Linnaeus, 1758)	30	5.1%	40	7.6%	41	3.6%	20	1.5%	12	1.9%	14	1.5%
S27	*Sphaerophoria taeniata* (Meigen, 1822)	2	0.3%	2	0.4%	2	0.2%					2	0.2%
S28	*Syrphus ribesii* (Linnaeus, 1758)	10	1.7%	5	0.9%	9	0.8%	14	1.1%	1	0.2%	6	0.7%
S29	*Syrphus torvus* Osten-Sacken, 1875	2	0.3%	9	1.7%	8	0.7%	6	0.5%	1	0.2%	6	0.7%
S30	*Syrphus vitripennis* Meigen, 1822	21	3.5%	10	1.9%	50	4.4%	69	5.3%	12	1.9%	22	2.4%
S31	*Triglyphus primus* Loew, 1840					1	0.1%						0.0%
S32	*Volucella pellucens* (Linnaeus, 1758)									1	0.2%	1	0.1%
S33	*Xanthandrus comtus* (Harris, 1780)	4	0.7%			11	1.0%					4	0.4%
S34	*Xanthogramma pedissequum* (Harris, 1776)											1	0.1%
S35	*Xanthogramma stackelbergi* Violovitsh, 1975							1	0.1%			1	0.1%
	Total number of specimens	592	100%	529	100%	1124	100%	1303	100%	622	100%	910	100%
		1,121				2,427				1,532			
	Total number of species	15		14		16		22		18		21	
		19				26				25			

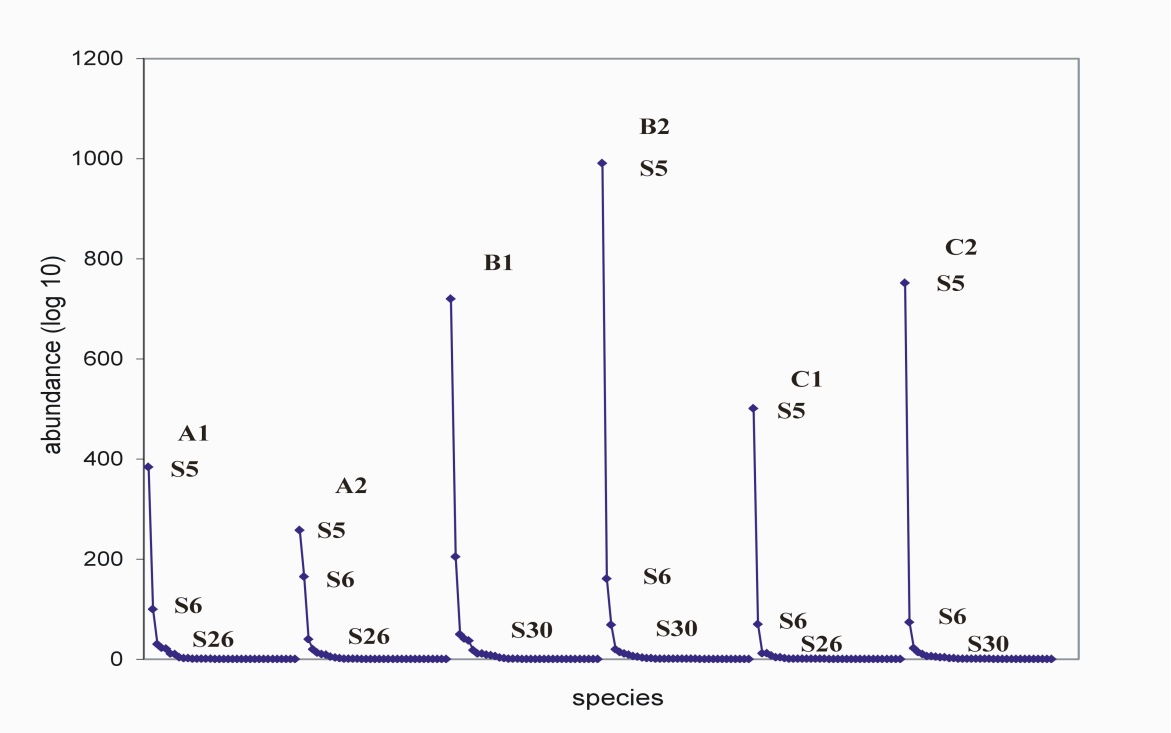

Figure 2. Dominance diversity curves based on the number of individuals (log 10 abundance) per species in each habitat. A1 = Głuchowo orchard, A2 = Głuchowo field, B1 = Gorzyczki I orchard, B2 = Gorzyczki I shrubberies, C1 = Gorzyczki II orchard, C2 = Gorzyczki II road, S5 = *Episyrphus balteatus*, S6 = *Eupeodes corollae*, S26 = *Sphaerophoria scripta*, S30 = *Syrphus vitripennis*. High quality figures are available online.

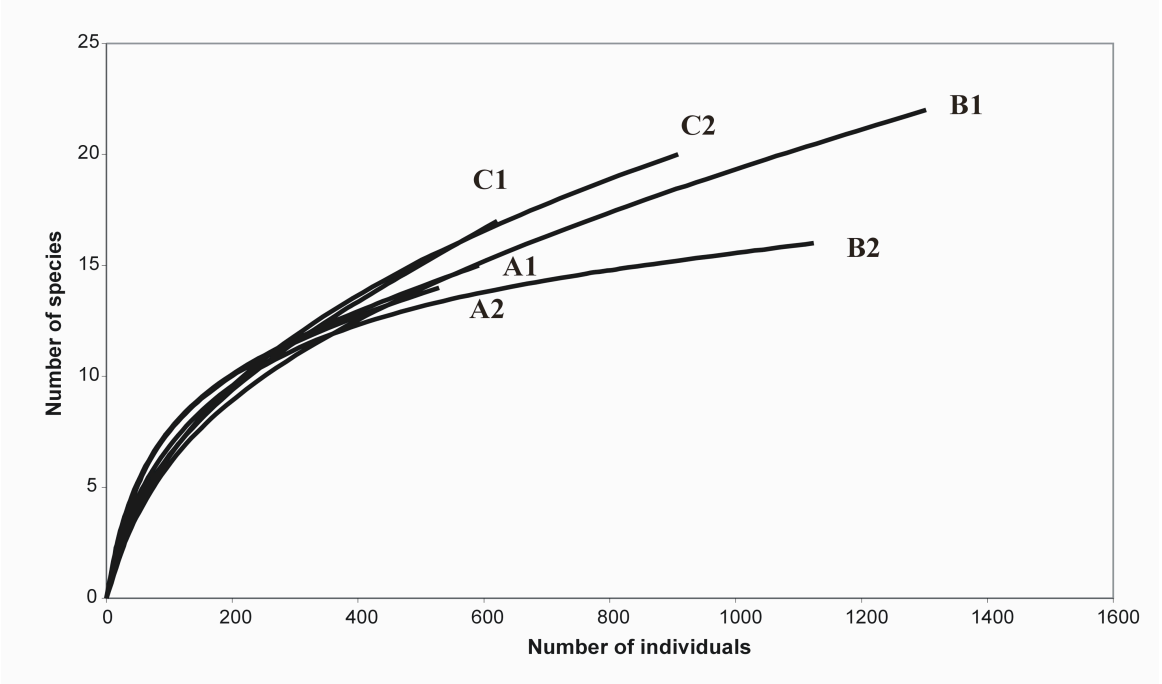

Figure 3. Expected species accumulation curves based on Mao-Tau function for all habitats. A1 = Głuchowo orchard, A2 = Głuchowo field, B1 = Gorzyczki I orchard, B2 = Gorzyczki I shrubberies, C1 = Gorzyczki II orchard, C2 = Gorzyczki II road. High quality figures are available online.

The communities of predatory Syrphidae caught in the apple orchards and on their edges were compared in quantity terms with Jaccard and Sorensen indices (Table 2). The communities of particular habitats were found to be mostly similar in over half of their species composition, except the communities caught in orchard in Gorzyczki II (C1) and shrubberies (B2). Those communities reached Jaccard index below 0.5.

Table 2. Compositional Jaccard and Sorensen classic index matrix for all the habitats: A1 = Głuchowo orchard, A2 = Głuchowo field, B1 = Gorzyczki I orchard, B2 = Gorzyczki I shrubberies, C1 = Gorzyczki II orchard, C2 = Gorzyczki II road

Jaccard Classic / Sorensen Classic	A1	A2	B1	B2	C1	C2
A1		0.520	0.542	0.500	0.444	0.500
A2	0.684		0.750	0.700	0.609	0.609
B1	0.703	0.857		0.650	0.636	0.636
B2	0.667	0.824	0.788		0.458	0.591
C1	0.615	0.757	0.788	0.629		0.583
C2	0.667	0.757	0.788	0.743	0.737	

The last task was to determine an estimated number of species in a particular habitat, specify 95% confidence interval and determine the standard deviation on the basis of species accumulation curve. Mao-Tau function was used to achieve this. It is known to show the average number of individuals per number of samples and is used in plotting a species accumulation curve. A detailed interpretation of Mao-Tau function was presented in Mao et al. (2005). An analysis showed that for the orchard habitats bordering on abundant edge vegetation (B1, C1) on the edges themselves (B2, C2) the mean number of Syrphidae caught in one sample was the highest. On the other hand, in the orchard bordering on fields (A1) one sample yielded the lowest number of these aphytophages (Table 3).

Table 3. Mao-Tau function for orchard and shrubberies communities (A1 = Głuchowo orchard, A2 = Głuchowo field, B1 = Gorzyczki I orchard, B2 = Gorzyczki I shrubberies, C1 = Gorzyczki II orchard, C2 = Gorzyczki II road)

Habitats	Mao -Tau	95% CI Lower Bound	95% CI Upper Bound	Standard Deviation
A1	17.5	12.56	22.44	2.52
A2	23.4	18.08	28.72	2.71
B1	27.35	21.83	32.87	2.81
B2	30.33	24.66	36.01	2.9
C1	32.83	26.93	38.73	3.01
C2	35	28.79	41.21	3.17

Syrphidae communities inhabiting apple orchards and their edges were compared in quantity and quality terms using order grouping with the cluster method (Figure 4). The caught Syrphidae were proved to create 2 groups of communities similar in quantity and quality structures. The following communities were similar: the communities of the apple orchard in Głuchowo (A1) and apple orchard in Gorzyczkach II (C1) as well as the community caught in the field (A2); the community of apple orchard in Gorzyczki I (B1) and that of the road lined with trees and shrubberies (C2).

Figure 4. Cluster analysis of habitats with group single linking as the clustering method (A1 = Głuchowo orchard, A2 = Głuchowo field, B1 = Gorzyczki I orchard, B2 = Gorzyczki I shrubberies, C1 = Gorzyczki II orchard, C2 = Gorzyczki II road). High quality figures are available online.

The Syrphidae community found in shrubberies (B2) definitely differed from other communities in its quality and quantity structure.

The analysis of the similarities in quantity and quality structures of Syrphidae communities found in particular habitats was completed with comparing their structures with principal components analysis method (Figure 5). The method allows to analyze the abundances of particular species. The results corroborated the similarity of the same communities as determined with the cluster method and a separate character of the community occurring in the shrubberies (B2). They also confirmed that the species *Episyrphus balteatus* (S5) and *Eupeodes corollae* (S6) were very abundant in comparison with others and at the same time they differed in abundance between each other.

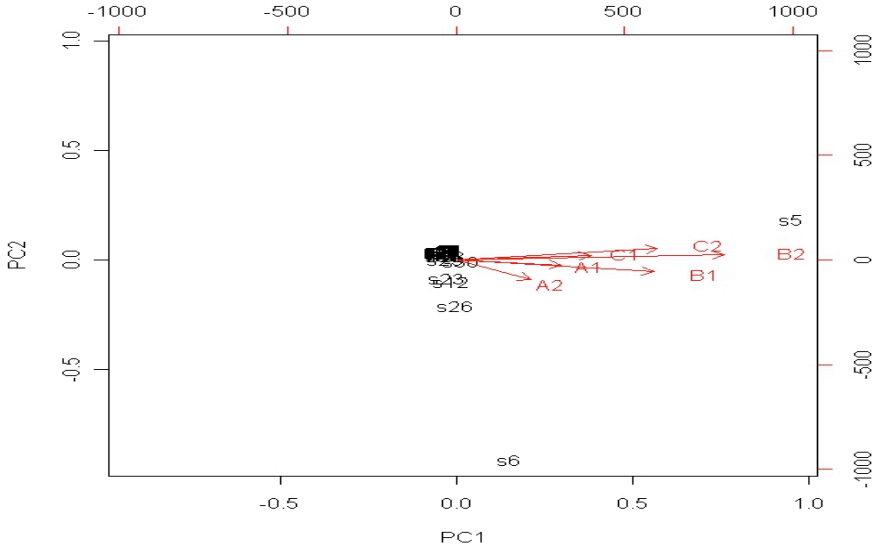

Figure 5. Plot of principal components analysis of habitats and species (A1 = Głuchowo orchard, A2 = Głuchowo field, B1 = Gorzyczki I orchard, B2 = Gorzyczki I shrubberies, C1 = Gorzyczki II orchard, C2 = Gorzyczki II road; the numbers denote the number of species as in Table 1). High quality figures are available online.

Discussion

The research conducted between the years 2008 and 2010 in orchard habitats near Czempiń clearly proved that in the apple orchards and on their edges which were made up of abundant in species and well-developed vegetation in the form of shrubberies and the road lined with trees and bushes, a high diversity of predatory Syrphidae species was found as well as their higher abundance than in orchard habitats composed of the orchards and neighbouring fields. The results confirmed a positive influence of wild vegetation in the vicinity of orchards onto their species richness and the increase of the abundance of predatory Syrphidae, as the research of aphytophages including Syrphidae, conducted by Wyss (1995) and Rossi et al. (2006) in apple orchards of Switzerland and Italy, proved such a relationship. Also earlier research by Trzciński & Piekarska-Boniecka (2009) concerning the occurrence of *Episyrphus balteatus* in apple orchards in the vicinity of Poznań indicated a positive impact of shrubberies near the orchard on the abundance of the species.

The results also showed that abundant vegetation of orchard edges constituted a more attractive habitat for predatory Syrphidae than the apple orchard biocenosis. This could have been caused by the influence of blooming flowers attracting the imagines of Syrphidae. Branquat & Hemptinne (2000), Ambrosino et al. (2006) and Kelm et al. (2009) indicated that it was the plant species of Apiaceae, Asteraceae, Lamiaceae, Ranunculaceae and Rosaceae which effectively attracted Syrphidae.

The research showed a definite dominance of *Episyrphus balteatus* and *Eupeodes corollae* (F.) in all the apple orchards and on their edges, thus it corroborated a high abundance of these species in the orchard habitat, as they had been previously indicated as dominant in this habitat by Wnuk (1972), Solomon et al. (2000), Miňarro & Dapena (2001), Rossi et al. (2006) and Trzciński & Piekarska-Boniecka (2009). The dominance of these species in all the orchards and on their edges indicates their migration between neighbouring biocenoses, which results in the controlling of the abundance of orchard-infesting aphids. *E. balteatus* is considered a very effective predator of *Aphis pomi* Deg., *Dysaphis devecta* (Walker) and *D. plantaginea* (Pass) and other species of aphids infesting fruit trees and bushes (Wnuk, 1972, 1977, 1979; Wyss, 1999; Wyss et al., 1999; Trandafirescus et al., 2004; Miňarro & Dapena, 2001). The migration of Syrphidae species between the orchard and edge vegetation is also supported by the fact that those communities in their majority showed a high similarity in species composition.

The research helped to establish that only the Syrphidae community inhabiting shrubberies definitely differed in its quality and quantity structure from those inhabiting the remaining habitats. The most Syrphidae species were caught there and the highest abundances were reported.

To sum up, the occurrence of well-developed and diversified vegetation of apple orchard edges positively influences the species richness and abundance of predatory Syrphidae occurring in orchards. Thus it helps to a natural control of the aphids infesting those orchards. Rich vegetation of orchard edges is a vital element of ecological structure, which should be used in designing orchards.

Acknowledgments

The authors thank the Editor-in-Chief and the anonymous referees for their invaluable comments and suggestions which helped improve this manuscript.

References

Ambrosino, M. D, J. M. Luna, P. C. Jepson, & S. D. Wratten, 2006. Relative frequencies of visits to selected insectary plants by predatory hoverflies (Diptera: Syrphidae), other beneficial insects, and herbivores. Environmental Entomology, 35: 394–400.

Bostanian, N. J., H. Goulet, J. O'Hara, L. Masner & G. Racette 2004. Towards insecticide free apple orchards: flowering plants to attract beneficial arthropods. Biocontrol Science and Technology, 14: 25–37.

Branquart, E. & J. L. Hemptinne, 2000. Selectivity in the exploitation of floral resources by hoverflies (Diptera: Syrphidae). Ecography, 23: 732–742.

Carreck, N. L. & I. H. Williams, 2002. Food for insects pollinators on farmland: insect visits to flowers of annual seed mixtures. Journal of Insect Conservation, 6: 13–23.

Chao, A., R. L. Chazdon, R. K. Colwell & T. Shen, 2005. A new statistical approach for assessing compositional similarity based on incidence and abundance data. Ecology Letters, 8: 148–159.

Colley, M. R. & J. M. Luna, 2000. Relative attractiveness of potential beneficial insectary plants to aphidophagous hoverflies (Diptera: Syrphidae). Environmental Entomology, 29: 1054–1059.

Colwell, R. K., 2011. Estimates: Statistical Estimation of Species Richness and Shared Species From Samples. Version 9. (Web page: http://viceroy.eeb.uconn.edu/estimates/) (Date accessed: Dec 2015)

Colwell, R. K., C. X. Mao & J. Chang, 2004. Interpolating, extrapolating, and comparing incidence-based species accumulation curve. Ecology, 85: 2717–2727.

Haenke, S., B. Scheid, M. Schaefer, T. Tscharntke & C. Thies, 2009. Increasing syrphid fly diversity and density in sown flower strips within simple vs. complex landscape. Journal Applied Ecology, 46: 1106–1114.

Kelm M., A. Biesiada, M. Krawczyk & M. Ciołek, 2009. Attractiveness of the flowers of selected herbal plants to the aphidovorous hoverflies (Diptera, Syrphidae). Zeszyty Problemowe Postępów Nauk Rolniczych, 539: 307–311.

MacLeod, A., 1999. Attraction and retention of *Episyrphus balteatus* DeGeer (Diptera: Syrphidae) at an arable field margin with rich and poor floral resources. Agriculture, Ecosystem and Environment, 73: 237–244.

Mao, C. X., R. K. Colwell & J. Chang, 2005. Estimating species accumulation curves using mixtures. Biometrics, 61: 433–41.

Miňarro, M. & E. Dapena, 2001. Predators of the rosy apple aphid, *Dysaphis plantaginea* (Pass.), in Asturian (NW Spain) apple orchards. Integrated Fruit Production, IOBC Bulletin, 24: 241–245.

Moericke, V., 1953. Wie finden geflügelte Blattläuse ihre Wirtspflanze? Mitteilungen aus der Biologischen Reichsanstalt, Berlin, 75: 90–97.

R Core Team, 2015. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Vienna, Austria. (Web page: https://www.R-project.org.), (Date accessed: Dec 2015)

Rossi, J., U. Gamba, M. Pinna, S. Spagnolo, C. Vissentin & A. Alma, 2006. Hoverlies in organic apple orchards in north-western Italy. Bulletin of Insectology, 59: 111–114.

Solomon, M. G., J. V. Cross, J. D. Fitzgerald, C. A. M. Campbell, R. L. Jolly, R. W. Olszak, E. Niemczyk & H. Vogt, 2000. Biocontrol of pests of apples and pears in Northen and Central Europe – 3. Predators. Biocontrol Science and Technology, 10: 91–128.

Soszyński, B., 2007. Hoverflies "Syrphidae" 102–105 In: Fauna of Poland – Characteristics and checklist of species, Vol. II. (Eds: W. Bogdanowicz, E. Chudzicka, I. Pilipiuk, E. Skibińska) MiLZ PAN, Warszawa: 505 pp.

Speight, M. C. D. & J. P. Sarthou, 2010. StN Keys For The Identification Of Adult Eutopean Syrphidae 2010. 1–108. (Web page: http://www.biodiversityireland.ie), (Date accessed: Dec 2015)

Sutherland, J. P., M. S. Sullivan & G. M. Poppy, 2001. Distribution and abundance of aphidophagous hoverflies (Diptera: Syrphidae) in wildflower patches and field margin habitats. Agricultural and Forest Entomology, 3: 57–64.

Trandafirescu M., I. Trandafirescu,, C. Gavat & V. Spita, 2004. Entomophagous complexes of some pests in apple and peach orchards in Southeastern Romania. Journal Fruit Ornamental Plant Research, 12: 235–261.

Trzciński, P. & H. Piekarska-Boniecka, 2009. Occurrence of marmelade fly *Episyrphus balteatus* (Deg.) (Diptera, Syrphidae) in apple orchards with integrated production of fruit and their surroundings. Progress in Plant Protection, 49: 1987–1990.

van Veen, M., 2004. Hoverflies of Northwest Europe: Identification Keys to the Syrphidae. KNNV Publishing, Utrecht, pp. 1–256.

Wnuk, A., 1972. Investigations on the species composition of predaceous Syrphidae (Diptera) occurring in the colonies of aphids on fruit trees and shrubs. Polish Journal of Entomology, 42: 235–247.

Wnuk, A., 1977. Evaluation of the effectiveness of *Episyrphus balteatus* (Deg.) (Diptera, Syrphidae) predation in the reduction *Aphis pomi* (Hom., Aphididae). Polish Journal of Entomology, 47: 755–760.

Wnuk, A., 1979. *Episyrphus balteatus* (De Geer) (Diptera, Syrphidae) as a predator of aphids (Homoptera, Aphidodea). Zeszyty Naukowe Akademii Rolniczej w Krakowie, pp. 1–72.

Wyss, E., 1995. The effect of weed strips on aphids and aphidophagous predators in an apple orchard. Entomologia Experimentalis et Applicata, 75: 43–49.

Wyss, E. 1999. Enhancement and release of predaceous arthropods to control aphids in organic apple orchards. IOBC Bulletin, 22: 47–51.

Wyss, E., M. Villiger & H. Muller-Scharer, 1999. The potential of three native insect predators to control the rosy apple aphid, *Dysaphis plantaginea*. Biocontrol, 44: 171–182.

Türk. entomol. derg., 2015, 39 (4): 345-353 ISSN 1010-6960
DOI: http://dx.doi.org/10.16970/ted.35600

Orijinal araştırma (Original article)

Antifeedant activity and growth inhibition effects of some plant extracts against larvae of Colorado potato beetle [*Leptinotarsa decemlineata* Say (Col: Chyrsomelidae)] under laboratory conditions [1]

Bazı bitki ekstraktlarının laboratuvar koşullarında Patates böceği [*Leptinotarsa decemlineata* Say (Col: Chyrsomelidae)] larvaları üzerine beslenme engelleyici ve larval gelişmeyi engelleyici etkileri

Mustafa ALKAN[2*] Ayhan GÖKÇE[3] Kenan KARA[4]

Summary

Antifeedant activity and growth inhibition effects of *Achillea millefolium* L. (Asteraceae), *Heracleum platytaenium* Boiss (Apiaceae), and *Humulus lupulus* L. (Cannabaceae) extracts were tested on the third instar larvae of Colorado potato beetle (*Leptinotarsa decemlineata* Say, Col: Chyrsomelidae) under laboratory conditions. Plant extracts were obtained treating "cones" of *H. lupulus*, leaves and stems of *H. platytaenium* and leaves, stems and flowers of *A. millefolium* with methanol using maceration technique. The plant extracts (50 g/L) were applied to potato leaflets and consumption area for individual insect was measure with a planimeter after 24, 48 and 72h. The single dose screening bioassay revealed that all tested plant extracts possessed antifeedant activity against the beetle larvae at 50 g/L concentration and average consumed leaf area were 0.04 cm^2, 0.05 cm^2 and 0.30 cm^2 for *H. lupulus, H. platytaenium* and *A. millefolium* respectively. In dose response bioassay, it was revealed that there was a negative correlation between the averages consumed leaflet area and the plant extract dose. The greatest antifeedant activity was seen in *H. lupulus* extract treated leaflets at 10 g/L concentration and the average consumed leaflet area was 0.68 cm^2 after 72 h. The *H. platytaenium* and *H. lupulus* extracts had similar effects on the larval growth indices. Both plants extracts had 1.2 growth index values at 30 g/L concentration. These results indicate that the *H. platytaenium* and *H. lupulus* extracts could have significant potential as antifeedant and larval growth inhibition agent in the control of larvae of Colorado potato beetle.

Keywords: Antifeedant effect, Colorado potato beetle, *Heracleum platytaenium, Humulus lupulus,* plant extract

Özet

Bu çalışmada *Achillea millefolium* L. (Asteraceae)*, Heracleum platytaenium* Boiss (Apiaceae), *Humulus lupulus* L. (Cannabaceae) ekstraktının Patates böceği (*Leptinotarsa decemlineata* Say, Col: Chyrsomelidae) üzerindeki beslenmeyi engelleyici ve larval gelişmeyi engelleyici etkileri laboratuvar şartlarında test edilmiştir. Bitki ekstraktlarının eldesinde *H. lupulus*'un kozalakları, *H. platytaenium*'un gövde ve yaprakları, *A. millefolium*'un ise çiçekleri kullanılmış ve maserasyon tekniği kullanılarak metanol ekstraktları elde edilmiştir. Bitki ekstraları (50 g/L) patates yapraklarına uygulanmış ve her bir böceğin tükettiği yaprak alanı 24, 48 ve 72 saat sonra planimetre ile ölçülmüştür. Tek doz tarama testlerinde tüm bitki ekstraktları 50 g/L konsantrasyonda beslenme engelleyici etkiye sahip olmuş ve ortalama yaprak tüketim alanı *H. lupulus*, *H. platytaenium*, *A. millefolium* için sırasıyla 0.04 cm^2, 0.05 cm^2 ve 0.30 cm^2 olarak hesaplanmıştır. Doz-etki denemelerin de ortalama yaprak tüketim alanı ile doz arasında negatif bir ilişki saptanmıştır. En yüksek beslenmeyi engelleyici etki 10 g/L konsantrasyonda *H. lupulus* ekstraktında 72. saat sonunda 0.68 cm^2 yaprak tüketim alanı ile görülmüştür. *H. lupulus* ve *H. platytaenium* ekstraktları larval gelişme indeksi üzerine benzer etkilere sahip olmuşlardır. Her iki bitki ekstraktı 30 g/L konsantrasyonda 1.2 gelişme indeks değerine sahip olmuştur. Bu sonuçlar *H. lupulus* ve *H. platytaenium* ekstraktlarının patates böceğine karşı beslenme engelleyici ve larval gelişmeyi engelleyici etkilerinin olduğunu ortaya koymaktadır.

Anahtar sözcükler: Beslenmeyi engelleyici etki, patates böceği, *Heracleum platytaenium, Humulus lupulus,* bitki ekstraktı

[1] This article has been drawn up from a certain part of the doctoral project of Mustafa Alkan which is promoted within the scope of the Project no. TAGEM-BS-12 / 04-04 / 01- 04 and this study was presented as a poster presentation at the International Conference on Biopesticide 7. 19-25 October 2014

[2] Plant Protection Central Research Institute, Ankara, Turkey
[3] Department of Plant Production and Technologies, Faculty of Agricultural Sciences and Technologies, Nigde University, Niğde, Turkey
[4] Department of Plant Protection, Faculty of Agriculture, Gaziosmanpasa University, Tokat, Turkey
* Sorumlu yazar (Corresponding author) e-mail: mustafa_alkan@ziraimucadele.gov.tr
Alınış (Received): 01.06.2015 Kabul ediliş (Accepted): 23.11.2015 Çevrimiçi Yayın Tarihi (Published Online): 04.12.2015

Introduction

The potato (*Solanum tuberosum* (L.) (Solanaceae) crop is attacked by many pests in the fields and stores, which may lead to significant qualitative and quantitative losses. One of the most important potato pests is Colorado potato beetle (CPB) (*Leptinotarsa decemlineata* Say, Coleoptera: Chyrsomelidae). The beetle feeds on all vegetative part of potato and sometimes causes 100 percent loss, unless insect control. The beetle is also vector of bacterial potato ring rot disease (Christie et al., 1991). Application of conventional insecticides is the effective control method against *L. decemlineata* but they have some limitations as well. The incremental and uncheck application of insecticides may cause phyto-toxicity and lead towards insecticide resistance development (Stewart et al., 1997; Mota-Sanchez et al., 2000; Whalon et al., 2011; Afzal et al., 2014).

Colorado potato beetle rapidly develops resistance to many conventional insecticides (Whalon et al., 2011). Integrated pest management program (IPM) is a classical approach to overcome and manage the resistance problem. However, the limited availability of natural enemies is one of the major constraints in effective biological control program against the CPB. Therefore, the contact, antifeedant and residual toxicity properties of plant extracts can be helpful as additional tool for effective control of this destructive pest (Gonzales-Coloma et al., 1998; Metsapul et al., 2001; Kutas & Nadasy, 2005; Szczepanik et al., 2005; Gokce et al., 2005, 2006, 2012). The ancient Egyptians are considered pioneer in using *Balanites* plant (Zygophyllaceae) extracted oil against dipteran species (Thacker, 2002). Before the introduction of chlorinated hydrocarbon and organic phosphorus pesticides, the plant based insecticides had been commonly used for insect pest management (Isman, 1997). The negative impact of pesticides on environmental and human health encouraged the use of biopesticides for insect pest management. Biopesticides include both microorganisms and plant extracts. The plant-based compounds are mainly plant secondary metabolites, which are produced by plants under stress conditions especially during pest attack, drought and stress conditions (Bourgaud, 2001). Some of these secondary metabolites have insecticidal, anti-fungal and anti-bacterial properties and their use can be helpful if incorporated in agricultural pest management programs (Charlwood & Rhodes, 1990). Neem oil provided successive and promising results against important agricultural pest species (Nitao, 1987; Pascual-Villalobos & Robledoet, 1999; Chiasson et al., 2004; Rehman et al., 2015). More than 2000 plant extracts have been reported to have insecticidal properties against various insect species (Dev & Koul, 1997; Koul, 2005). Beside this, the applicability of plant based pesticides is still very limited (Ahmed & Grainger, 1988; Parakash & Rao, 1996; Hassan & Gokce, 2014).

The objectives of the current study are to evaluate antifeedant activity of three different plant extracts on *L. decemlineata* larvae, and to examine growth inhibition effects of plant extracts on the larvae using different concentrations.

Materials and Methods

Collection of plant materials

The plants samples were collected as described by Çam et al. (2012). *Achillea millefolium* and *Humulus lupulus* were collected during the spring and summer seasons of 2010 in Tokat, Turkey and *Heracleum platytaenium* was collected during the summer season of 2009 in Trabzon, Turkey. The plant parts, used in the study, were separated from other parts of the plant, spread on blotting papers in a dark room and left to dry at 24±2 ºC for two weeks. After this process, the plant materials were well ground by using a mill (M 20 IKA Universal Mill, IKA Group, Wilmington, NC, USA). The samples were transferred into glass jars of 5 L and stored at 15±5 ˚C. The information about the plants, analyzed parts and location of collection is presented in Table 1.

Table1. Name of the plants species used in the study with analyzed parts and place of collection

Botanical Name	Family	Analyzed part	Location of the Collection	GPS location
Achillea millefolium L.	Asteraceae	Leaf, Stem, Flower	Tokat, Turkey.	40˚ 18' 55" N 36˚ 32' 11" E
Heracleum platytaenium Boiss.	Apiaceae	Leaf, Stem	Trabzon,	40˚ 38' 44" N 39˚ 20' 22" E
Humulus lupulus L.	Cannabaceae	"Cone"	Tokat, Turkey.	40˚ 21' 4" N 36˚ 30' 41" E

Preparation of plant extracts

Plant extracts were obtained through maceration method as reported by Alkan & Gökçe (2012). About 200 g of each plant powders was put into a glass jar. For obtaining *H. platytaenium* extracts, hexane, ethyl acetate, and methanol were added into jars respectively according to their polarity range. The plant powder in the glass jar was firstly treated with hexane for 48 hours; afterward the solvent plant suspension was filtered through filter paper and separated from plant materials. Thereafter, ethyl acetate was added to the remaining plant material, and again materials were left in the jars with this solvent for 48 hours. After this operation, ethyl acetate extract parts were filtered through filter paper. Lastly, methanol was added to the plant materials, and same procedure was repeated. *H. lupulus* and *A. millefolium* extracts were prepared with the maceration method but only methanol was used as solvent. The solvents in the existing suspension were evaporated by using the evaporator, and plant residues were obtained. Methanol extracts of plants were transferred into glass tubes, and were kept in 4±1 °C in the refrigerator. The extracts were diluted with 70 % acetone, and the concentrations used in single concentration tests and all other bioassays were prepared from these stock solutions.

Rearing of potato beetles

Colorado potato beetle larvae were continuously reared on potato plants (*Solanum tuberosum*, cultivar Granola) at Gaziosmanpaşa University Research Station in Tokat, Turkey during the study as described in Gökçe et al., 2006. The potato tubers were planted in a 0.2 ha potato field in the early spring of each year. CPB adults were released into the field when the potato plants were 3 to 5 leaves stage. The insects used in the studies were collected from the field colony. There was no pesticide application during the study.

Antifeedant activity of plant extracts against CPB larvae

Firstly, the single concentration experiments were conducted to evaluate the antifeedant activity of plants extracts (*A. millefolium, H. platytaenium, H. lupulus*) against the 3rd instar larvae of CPB. Before spraying with plant extracts, each leaflet area was measured using a digital planimeter (Placom KP-90N Digital Planimeter, Koizumi, Japan). The potato leaves were treated with the plant extracts at 5 % concentration (w/v) or 70 % acetone as control. The plant extracts were applied with a hand sprayer to develop a plant extract film layer on the treated leaf surface. Before releasing the larvae on leaves, the treated potato leaflets were left to dry at room temperature under a fume hood for 30 minutes. Then larvae were transferred individually onto treated leaflets and incubated at 26±1°C, 60 % RH (Relative Humidity) and a 16 light: 8 dark photo regimes. The leaflets that were treated as above were replaced every 24 hour for 7 days. The consumption area of each leaflet was measured after 24h using the digital planimeter and the results were recorded. Total 10 replications were done in a randomized complete block experimental design (RCBD).

Further studies of antifeedant activities of plant extracts were carried out at three different concentrations i.e. 0.1 %, 0.5 % and 1 % (w/v) to evaluate interaction between concentration of plant extracts and leaflet consumption area. The treatment of leaflets and incubation of larvae were performed as described above. Total four treatments including the control were carried out with ten replication for each treatment. The randomized complete block experimental design was used for testing activities.

Growth inhibition effects of plant extracts on 3rd instar CPB larvae

Totally three different concentrations i.e. 0.5 %, 1 % and 3 % of *H. platytaenium* and *H. lupulus* plant extract were used to test the impact of plant extracts on larval growth. The potato leaflets were treated with plant extracts or 70 % acetone as described above. The 3rd instar larvae were initially weighed using a digital balance (Kern & Sohn EG 2200-2NM, KERN & SOHN GmbH, Balingen, Germany) and the result were recorded. The larvae were individually transferred onto the treated leaflets and incubated at above conditions for 3 days. The larvae were shifted on fresh treated leaves after every 24 hours. The final weights of each larva were recorded at the end of three days. Ten larvae were used in per concentration of each plant extract and the control. The experiment was performed using the randomized complete block experimental design.

Statistical analysis

Antifeedant activity results were analyzed with paired t-test using Minitab® software (McKenzei & Goldman, 2005). Growth inhibition rate of CPB larvae was calculated using the below formula (Gökçe et al., 2012) that was modified from Abdelgaleil & Al-Aswad (2005). The calculated growth rates were subjected Kruskal- Wallis test.

GI=(CWC-TWC)/CWC

GI: Growth Index

CWC: Weight change in the control

TWC: Weight change in the treatment

Results and Discussion

Antifeedant activity of plant extracts against CPB larvae

The mean consumption areas of individual larvae in the single concentration test are presented in Table 2. The data showed that all treatments cause a reduction in mean consumed leaf area, except *H. platytaenium* after 24 hours. *H. lupulus* extract appear to be the most active extract with producing the minimum mean consumed leaflets area. The larvae showed avoiding feeding behavior on leaves treated with *H. lupulus* extract up to 48 hours but this behavior changed after 72 hours and average 0.04±0.01 cm^2 leaf area was consumed by each larva at that time interval (Table 2). The maximum leaf area among the treatments at all time interval was seen in *H. platytaenium* treated leaves (1.49 cm^2) after 24 hours. However, there was a reduction in this treatment after that time period and average 0.04 cm^2 and 0.05 cm^2 leaf areas were consumed after 48 hours and 72 hours respectively. Also the *A. millefolium* treatment significantly reduced the consumed leaf area over the tested time period (t=3.47; P<0.05).

Table 2. Antifeedant activity of a single concentration (5 %) of plant extracts on *Leptinotarsa decemlineata* larvae. Average consumed leaflets area (cm^2) per Colorado potato beetle larva

Post Treatment hours	Mean consumed leaf area (cm^2) ± Mean Standard Error					
	Heracleum platytaenium		*Humulus lupulus*		*Achillea millefolium*	
	Treatment	Control	Treatment	Control	Treatment	Control
24. hours	1.49±0.39	1.49±0.29	0.00±0.0*	2.79±0,63	0.37±0.37*	2.17±060
48. hours	0.04±0.29	2.30±0.40	0.00±0.0*	1.86±1,14	0.27±0.17*	2.07±046
72. hours	0.05±0.03*	3.29±0.81	0.04±0.1*	5.84±0,86	0.30±015*	2.95±055

* Indicates that the treatment is statistically different than control (paired test -t) (P <0.05).

The antifeedant activities of different concentrations of *H. platytaenium, H. lupulus* and *A. millefolium* plant extract are presented in Tables 3, 4 and 5 respectively. In all treatments, there was no significant difference between the treatment and the control group at 0.1 % concentration at all time intervals. The larvae consumed larger areas at 72 h in all treatments groups comparing with previous two time intervals.

In *H. platytaenium* extract treatments, the minimum leaf area (1.18 cm^2) was consumed at 1 % concentration treated leaflets after 24 hours as the maximum leaf area (2.07 cm^2) was consumed in the 0.5 % concentration treatment. As the incubation period was extended, the larvae consumed more leaflets area at 0.1 and 0.5 % concentrations. The maximum leaf area (4.79 cm^2) was consumed at 0.1 % concentration after 72 hours as the minimum was seen at 1 % concentration after 48h. Overall, the leaflets treated with 1 % *H. platytaenium* extract showed the greatest antifeedant activity against the 3rd instar larvae (Table 3) (t=5.86; P<0.05).

Table 3. Antifeedant activity of different concentrations of *H. platytaenium* methanol extract on Colorado potato beetle larvae over time period

Post Treatment hours	Leaf Feeding Area (cm^2) ± Mean Standard Error					
	0.1 %		0.5 %		1 %	
	Treatment	Control	Treatment	Control	Treatment	Control
24. hours	1.83±0.22	1.62±0.27	2.07±0.41	1.56±0.20	1.18±0.18	1.79±0.28
48. hours	1.37±0.22	2.02±0.34	1.30±0.20	1.03±0.44	0.65±0.28	1.90±0.58
72. hours	4.79±1.00	4.61±0.89	3.66±0.78	4.99±0.90	1.18±0.35*	5.18±0.58

* Indicates that the treatment is statistically different than control (paired test -t) (P <0.05).

Antifeedant activity of *A. millefolium* extract at different concentrations experiment revealed that this plant extract had a significant antifeedant activity when it was applied 1 % (w/v) concentration. At lower concentrations of this extract, the larvae consumed similar leaflets area. At 0.5 % (w/v) concentration, the leaflet areas consumed after 48 hours and 72 hours were 1.82 cm^2 and 1.58 cm^2 respectively (Table 4). While after 72 hours the maximum leaf area (3.57 cm^2) was consumed in the 0.1 % concentration treatment, the minimum consumption leaf area (0.81 cm^2) was recorded on the leaflets treated with 1 % *A. millefolium* extract after 72 hours (t=3.46; P>0.05).

Table 4. Antifeedant activity of different concentrations of *A. millefolium* methanol extract on Colorado potato beetle larvae at different time intervals

Post Treatment hours	Leaf Feeding Area (cm^2) ± Mean Standard Error					
	0.1 %		0.5 %		1 %	
	Treatment	Control	Treatment	Control	Treatment	Control
24. hours	0.82±0.11	1.13±0.22	0.87±0.20	1.75±0.23	0.50±0.08*	1.68±0.29
48. hours	1.50±0.37	1.54±0.22	1.82±0.50	1.51±0.49	0.22±0.76*	1.68±0.29
72. hours	3.57±0.98	3.26±0.41	1.58±0.47*	4.50±1.03	0.81±0.21*	2.59±0.51

* Indicates that the treatment is statistically different than control (paired test -t) (P <0.05).

Testing of different *H. lupulus* extract concentrations for antifeedant activity showed that this plant extract significant reduced the feeding of CPB larvae when it was tested at 1 % (w/v) concentration. The maximum leaf area was consumed at 0.1 % concentration with 1.07 cm^2 area after 24 hours, while it increased to 1.64 cm^2 and 7.32 cm^2 leaf areas after 48 and 72 hours (Table 5). This could be related with increasing size of larvae before molting to the next stage. The minimum consumption of leaflet area was observed at 1 % *H. lupulus* concentration i.e. 0.24, 0.48 and 0.68 cm^2 leaf area after 24, 48 and 72 hours respectively (t=6.56; P<0.05) (Table 5).

Table 5. Antifeedant effects of different concentrations of *H. lupulus* methanol extract on Colorado potato beetle larvae at different time intervals

Post Treatment hours	Leaf Feeding Area (cm^2) ± Mean Standard Error					
	0.1 %		0.5 %		1 %	
	Treatment	Control	Treatment	Control	Treatment	Control
24. hours	1.07±0.20	1.35±0.27	0.91±0.14	1.10±0.18	0.24±0.06*	1.87±0.22
48. hours	1.64±0.40	1.34±0.35	0.67±0.13	2.29±0.86	0.48±0.16*	1.84±0.34
72. hours	7.32±1.27	4.64±0.92	1.13±0.37*	4.37±1.07	0.68±0.19*	6.24±1.08

* Indicates that the treatment is statistically different than control (paired test-t) (P <0.05).

Natural products or botanical pesticides are an excellent alternative to synthetic pesticides as a means to reduce negative impacts on the human health and the environment (Mohan et al., 2011). The antifeedant studies of various plants extracts with promising results against CPB were also reported in previous studies (Gonzales-Coloma et al., 1998; Kutas & Nadasy, 2005; Szczepanik et al., 2005). Antifeedant activities of diterpenoid alkaloids isolated from *Delphinium cardiopetalums* DC. (Ranunculaceae) were tested against both *Spodoptera littoralis* Boisduval. (Noctuidae) and *Leptinotarsa decemlineata* and 15-acetylcardiopetamine was reported to be the most active alkaloids against CPB. Gokce et al. (2006) reported that *H. lupulus* extract showed promising results against 3rd instar *L. decemlineata* larvae as reducing consumed leaflet areas. In their study, they measured the dry weight of leaflets rather than measuring the leaflet areas so that their findings are not comparable with ours.

Growth inhibition effects of plant extracts on the 3rd instar CPB larvae

Growth inhibition effects of *H. platytaenium* and *H. lupulus* extracts against CPB larvae were examined on the 3rd instars by testing different concentrations. The results indicate that both plant extracts had a similar growth inhibition effect of at tested concentrations. The maximum effect was observed at 3 % concentration as the recorded indexes were 1.2 for the both plant extracts. There was a significant difference between the larval growth indexes that were recorded at different concentrations of *H. platytaenium* (H: 6.28; p<0.05). As pointed out above, the maximum growth inhibition index was recorded at 3 % concentration while it was 0.9 and 1.1 at 0.5 % and at 1 % concentrations respectively (Figure 1). These results showed that the larvae that were forced to feed on the plant extract treated leaflets lost weight during the experiment and showed no growth at all. Similar larval growth reduction of CPB was also reported by Gökçe et al., (2012) who tested different concentration of hops extract on CPB larvae and found that the growth rate was significantly affected at 40 mg/ml concentration.

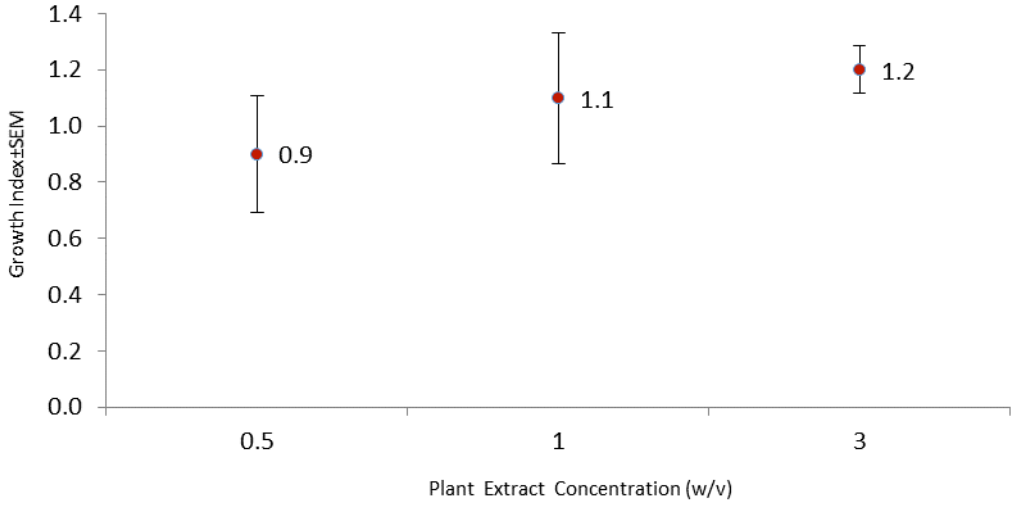

Figure 1. Effects of *Heracleum platytaenium* extracts on Colorado potato beetle larval growth.

Testing of different concentrations of *H. lupulus* plant extracts revealed that this plant extract significantly reduced the larval growth of potato beetle, except at 0.5 % concentration (H=8.01; P<0.05) (Figure 2). The larvae that were fed with 0.5 % of *H. lupulus* extract treated leaflet gained some weight and their body weights similar to the control group larvae. However, there was a weight loss in the larvae when they were forced to feed on the leaflets that were treated with 1 % and 3 % of *H. lupulus* extract concentration. Our results are in accordance with Gokce et al. (2012) who examined the effects of different doses of *H. lupulus* extract on CPB adults and larvae and they reported that the growth inhibition index was around 1 at the highest concentration of the plant extract.

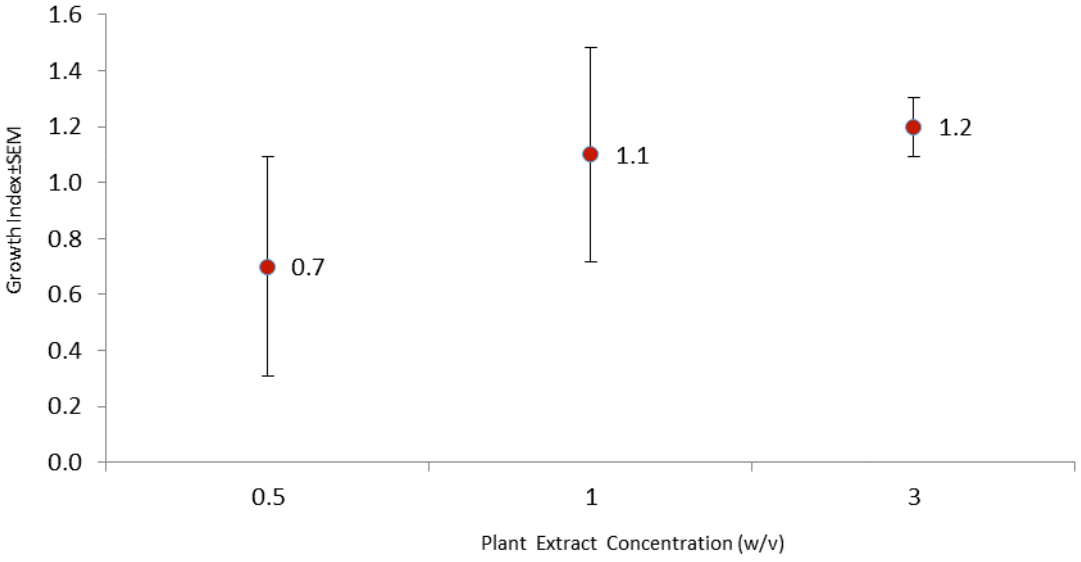

Figure 2. Effects of *Humulus lupulus* extract on Colorado potato beetle larval growth.

The results of the growth inhibition test showed that *H. lupulus* and *H. platytaenium* have strong activity against the 3rd instar larvae. Application of these plant extracts may lead to larval growth retardation and even death of larvae due to starvation. Inhibition of CPB larval growth seems to be related with antifeedant activity of the extracts as shown in this study. However, the plant extracts may cause some physiological activity in the larvae that lead to inhibition of growth. Therefore, further studies are needed to test whether the growth inhibition of larvae observed in this study is related with any physiological effect of plant extracts on the larvae. Additional studies should be carried out to explore full potential of these extracts. Especially extraction, purification and characterization of the active compound(s) may lead to development of new tools in the controlling of this destructive pest.

Acknowledgement

This study was supported by Turkish Republic Prime Minister State Planning Organization (27-DPT-01-07-01) and by the Ministry of Food, Agriculture and Livestock, General Directorate of Agricultural Research and Policy (TAGEM-BS-12 / 04-04 / 01- 04).

References

Abdelgaleil, S.A.M. & A.F. El-Aswad, 2005. Antifeedant and growth inhibitory effects of tetranortriterpenoids isolated from three meliaceous species on the Cotton Leafworm, *Spodoptera littoralis* (Boisd.). Journal of Applied Sciences Research, 1(2): 234-241.

Afzal, M.B.S, S.A. Shad, N. Abbas, M. Ayyaz & W.B Walker, 2014. Cross-resistance, the stability of acetamipridresistance and its effect on the biological parameters of cotton mealybug, *Phenacoccus solenopsis* (Homoptera: Pseudococcidae), in Pakistan. Pest Management Science, 71(1):151-58.

Ahmed, S. & M. Grainge, 1988. Handbook of Plants with Pest Control Properties. John Wiley&Sons Limited, 470pp.

Alkan, M. & A. Gökçe, 2012. Toxic and behavioural effects of *Tanacetum abrotanifolium* L. DRUCE (Asteraceae) stem and flower extracts on *Sitophilus granarius* and *Sitophilus oryzae* (Col., Curculionidae). Turkish Journal of Entomology, 36 (3): 377-389.

Bourgaud, F., A. Gravot, S. Milesi & E. Gontier, 2001. Production of plant secondary metabolites; a historical perspective. Plant Science , 161: 839-851.

Charlwood, B. V. & M. J. C. Rhodes, 1990. Secondary Products From Plant Tissue Culture, Oxford University Press, USA, 307pp.

Chiasson, H., C. Vincent & B. Bostanian, 2004. Insecticidal properties of a *Chenopodium* based botanical, Journal of Economic Entomology, 97:1378-1383.

Christie, R. D., A. C. Sumalde, J. T. Schutz & N. C. Gudmestad, 1991. Insect transmission of the bacterial ring rot pathogen. American Potato Journal, 68: 363-372.

Çam, H., A. Gökçe, İ. Kadıoğlu, Y. Yanar, N. Gören & M. E. Whalon, 2012. Bitki ekstraktlarının Patates böceği [*Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae)]'nin farklı dönemleri üzerine mide zehiri ve rezidüyel toksisite etkileri. Türkiye Entomoloji Dergisi, 36 (2): 249-254.

Dev, S. & O. Koul, 1997. Insecticides of Natural Origin. Harwood Academic Publishers, The Netherlands, 222 pp.

Gonzalez-Coloma, A., A. Guadano, C. Gutierrez, R. Cabrera, E. Pena, G. Fuente & M. Reina, 1998. Antifeedant *Delphinium* diterpenoid alkaloids. structure- activity relationships. Journal of Agricultural and Food Chemistry, 46: 286-290.

Gökçe, A., L. L. Stelenski & M. E. Whalon, 2005. Behavioral and electrophysiological responses of leafroller moths to selected plant extracts. Environmental Entomology, 34: 1426-1432.

Gökçe, A., M. E. Whalon, H. Çam, Y. Yanar, İ. Demirtaş & N. Gören, 2006. Plant extract contact toxicities to various developmental stages of Colorado potato beetles (Coleoptera: Chrysomelidae). Annals of Applied Biology, 149:197-202.

Gökçe, A., R. Isaacs & M. E. Whalon, 2012. Dose-response relationships for the antifeedant effects of *Humulus lupulus* extracts against larvae and adults of the Colorado potato beetle. Pest Management Science, 68: 476-481.

Hassan E. & A. Gökçe, 2014. "Production and Consumption of Biopesticides, 361-379pp." In: Advances in Plant Biopesticides (Ed: Singh Dwijendra) Springer, 401 p.

Isman, M. B., 1997. Neem and other biological insecticides: Barriers to commercialization. Phytoparasitica, 25(4): 239-249.

Koul, O., 2005. Insect Antifeedants. CRC Press, Florida, 1005pp

Kutas, J. & M. Nádasy, 2005. Antifeedant effects of several plant extracts on Colorado potato beetle larvae. Acta Phytopathologica et Entomologica Hungarica, 40: 355–365.

McKenzie, J.D. & R. Goldman, 2005. The student guide to MINITAB release 14. Pearson Education.

Metspalu, L., K. Hiiesaar, J. Jõudu & A. Kuusik, 2001. The effects of certain toxic plant extracts on the larvae of Colorado potato beetle, *Leptinotarsa decemlineata* (Say). Practice oriented results on the use of plant extracts and pheromones in pest control: Proceedings of the International Workshop, Tartu, Estonia, 24-25 January.

Mohan, M., Z. Haider, H. Andola & V.K. Purohit, 2011. Essential oils as green pesticides: for sustainable agriculture. Research Journal of Pharmaceutical, Biology and Chemical Sciences, 2: 100–106.

Mota-Sanchez, D., M. E. Whalon, E. Grafius & R. Hollingworth, 2000. Resistance of Colorado potato beetle to Imidacloprid. Resisitance Pest Management Newsletter, 11:31-34.

Nitao, J. K., 1987. Test for toxicity of Coniine to a polyphagous herbivore, *Heliothis zea* (Lepidoptera:Noctuidae), Environmantal Entomology, 16, 656-659.

Parakash, A. & J. Rao, 1996. Botanical Pesticides in Agriculture. CRC pres. LewisPublishers, pp 443.

Pascual-Villalobos, M. J. & A. Robledoet, 1999. Anti-insect activity of plant extracts from the wild flora in southeastern Spain. Biochemical Systematics and Ecology, 27: 1-10.

Rehman, H., M. Nadeem, M. Ayyaz & H.A. Begum, 2015. Comparative Efficacy of Neem Oil and Lambdacyhalothrin against Whitefly (*Bemisia tabaci*) and Jassid, (*Amrasca devastans* Dist.) in okra field. Russian Journal of Agircultural Sciences. 41: 138-145.

Stewart, J. G., G. G. Kennedy & A. V. Sturz, 1997. Incidence of insecticides resistance in population of Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae) on Prince Edward Island. Canadian Entomologist, 129: 21-26.

Szczepanik, M., I. Dams & C. Wawrzenczyk, 2005. Feding deterrent activity of terpenoid lactones with the p-menthane system against the Colorado potato beetle (Coleoptera: Chrysomelidae). Environmental Entomology, 34:1433-1440.

Thacker, J. M. R., 2002. An introduction to Arthropod Pest Control. Cambridge University Press, Cambridge, 343 pp.

Whalon, M. E., D. Mota-Sanchez, R. Hollingworth & L. Duynslager, 2011. Arthropod Pesticide Resisitance Database. (Web page: http://www. Pesticideresistance.com) (Accessed date: May 2014).

Türk. entomol. derg., 2015, 39 (4): 355-366 ISSN 1010-6960
DOI: http://dx.doi.org/10.16970/ted.94248

Orijinal araştırma (Original article)

Two new species of *Coccidohystrix* Lindinger (Hemiptera: Coccomorpha: Pseudococcidae) with notes on the related genus *Artemicoccus* Balachowsky

Coccidohystrix Lindinger (Hemiptera: Coccomorpha: Pseudococcidae) cinsine ait iki türün tanımlanması ve *Artemicoccus* cinsi üzerine sistematik araştırmalar

M. Bora KAYDAN[1] Giuseppina PELLIZZARI[2] Éva SZITA[3*]

Summary

Scale insect (Hemiptera: Coccomorpha: Pseudococcidae) samples were collected in Turkey (Hakkari-Yüksekova) and Bulgaria (Kresna Gorge) between 2007 and 2012. Two new *Coccidohystrix* Lindinger species, are described and illustrated. An identification key including the new species is also provided. Additionaly, the type material of *C. zangherii* was studied and the species is transferred into the genus *Artemicoccus* Balachowsky. A new identification key for species in the genus *Artemicoccus* is also provided.

Keywords: Phenacoccinae, lacking ostiole. Eriococcidae

Özet

Bu çalışmada 2007 ve 2012 yıllarında Türkiye (Hakkari-Yüksekova) ve Bulgaristan (Kresna Gorge)'dan toplanan unlubit (Hemiptera: Coccomorpha: Pseudococcidae) örnekleri incelenmiştir. Çalışma sonucunda *Coccidohystrix* Lindinger cinsine ait iki yeni tür tanımlanmış olup cinse ait yeni teşhis anahtarı oluşturulmuştur. Buna ek olarak *C. zangherii* Kozár & Pellizzari' nin tip materyalleri incelenerek *Artemicoccus* Balachowsky cinsine aktarılmış ve *Artemicoccus* cinsine ait yeni teşhis anahtarı oluşturulmuştur.

Anahtar sözcükler: Phenacoccinae, ostiole eksikliği, Eriococcidae

[1] Çukurova University, Imamoglu Vocational School, Adana, Turkey
[2] Department of Agronomy, Food, Natural Resources, Animals and Environment DAFNAE, University of Padova, Italy,
[3] Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, H-1022, Herman Ottó út 15, Budapest, Hungary
* Sorumlu yazar (Corresponding author) e-mail: szita.eva@agrar.mta.hu
Alınış (Received): 17.07.2015 Kabul ediliş (Accepted): 04.12.2015 Çevrimiçi Yayın Tarihi (Published Online): 18.12.2015

Introduction

More intensive collecting and the identification of new scale insect species can help to improve the knowledge on morphological features of these insects and often allow to clarify the systematic position of controversial taxa.

In recent years the genera *Artemicoccus* and *Coccidohystrix* have been in the scope of several papers in which the above reported taxa are considered as separate genera (Danzig et al., 2012) or as synonyms (Gavrilov-Zimin & Matile-Ferrero, 2014; Danzig & Gavrilov-Zimin, 2014).

Within the Phenacoccinae (Hemiptera: Pseudococcidae), five genera include species with dorsal cerarii, namely *Artemicoccus* Balachowsky (2 species), *Coccidohystrix* Lindinger (9 species), *Pedronia* Green (4 species), *Pedrococcus* Mamet (8 species) and *Synacanthococcus* Morrison (3 species) (Ben-Dov et al., 2015). The genus *Peliococcus* Borchsenius has also dorsal cerarii but the cerarian setae are narrowly conical setae whereas those of the five genera reported above are broadly conical (Williams, 2004). On the other hand in the genera *Artemicoccus* and *Coccidohystrix* both anterior and posterior ostioles are missing, while at least posterior ostioles occur in the other genera mentioned above. *Artemicoccus* and *Coccidohystrix* are considered as Palaearctic originated genera, *Pedronia*, *Pedrococcus* and *Synacanthococcus* are mainly from Oriental and Afrotropical regions (Williams, 2004).

The genus *Artemicoccus* was separated from the genus *Centrococcus* Borchsenius, (currently synonym of *Coccidohystrix* Lindinger) by Balachowsky (1953b) and includes two species: *C. bispinus* Borchsenius and *C. lubersaci* Balachowsky. Balachowsky (1953b) clarified the morphological differences between the two genera and added some important biological observation "*in Artemicoccus the adult females were enclosed in a felt eggsac, in Coccidohistryx the adult females were mobile and covered with powdery wax*". Moreover he considered that these mealybugs showed an affinity with Eriococcidae (Balachowsky 1953a, b).

Later Kozár & Pellizzari (1989) described the new species *C. zangherii* Kozár & Pellizzari in *Coccidohystrix* but admitted that it could be placed into the genus *Artemicoccus*; at that time they considered that the characters reported by Balachoswky were not sufficient to separate *Artemicoccus* from *Coccidohystrix* and concluded that a revision of these genera could led to the synonymy of *Artemicoccus* with *Coccidohystrix*.

Danzig et al. (2012) considered these two genera separately following Balachowsky (1953b) and Ben-Dov (1994) and provided a key for their differentiation. The main diagnostic morphological character between the two genera is the situation of dorsal conical setae: in *Coccidohystrix* the dorsal conical setae on dorsum are situated on elevated sclerotised prominences, while in *Artemicoccus* those are not on sclerotised prominences.

Gavrilov-Zimin & Matile-Ferrero (2014) regarded *Artemicoccus* a subjective synonym of *Coccidohystrix* and described further two new species, *C. maghribensis* Gavrilov-Zimin & Matile-Ferrero and *C. monicae* Gavrilov-Zimin & Matile-Ferrero. Moreover they transferred *Amonosterium echinatus* (Balachowsky) to the genus *Coccidohystrix* as *Coccidohystrix echinata* (Balachowsky) *comb. nov*.

Danzig & Gavrilov-Zimin (2014) considered *Artemicoccus* as a junior synonym of *Coccidohystrix* in their recent valuable volume on Pseudococcidae and indicated that the genus *Coccidohystrix* was very close to the family Eriococcidae.

Nine *Coccidohystrix sensu stricto* species are currently recognized (Ben-Dov et al., 2015); of which seven have a Palaearctic distribution (*C. artemisiae* (Kiritchenko), *C. burumandi* Moghaddam, *C. echinata* (Balachowsky), *C. insolita* (Green), *C. samui* Kozár & Konczné Benedicty, *C. splendens* (Goux), *C. zangherii* Kozár & Pellizzari Scaltriti), one is an Oriental species (*C. eleusines* Williams) and one an Afrotropical species (*C. madecassa* (Mamet)) (Ben-Dov et al., 2015) .

The collecting of two new species referred to *Artemicoccus* -*Coccidohystrix* gave us the opportunity to reconsider the morphology of these two controversial taxa, also on the basis of a recent phylogenetical study that resulted these two genera as separate ones in a very distinct clade (Kaydan et al., 2015)

In the present study the authors agree with Balachowsky (1953b), Ben-Dov (1994), Danzig et al. (2012) and regard *Coccidohystrix* and *Artemicoccus* as separate genera and described two new *Coccidohystrix* species, collected in Turkey and Bulgaria with new identification key of the genus. In addition the type material of *C. zangherii* was studied and the species transferred to the genus *Artemicoccus.* A new identification key for the genus *Artemicoccus* is also provided.

Materials and methods

Scale insect samples were collected in Turkey (Hakkari-Yüksekova) and Bulgaria (Kresna Gorge) between 2007 and 2012. Specimens were taken from wild plants in natural areas. Collecting data, province, locality, GIS coordinates, date of collection, collector, data of the host plant and the collection numbers are given.

Specimens were slide-mounted for light microscopy using the method of Kosztarab & Kozár (1988). Morphological terminology follows that of Kosztarab & Kozár (1988) and Williams (2004) for description of the new mealybug species. Measurements and counts of the new species were taken from all available material.

All type materials are deposited in the Scale Insect Collection of Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary (PPI).

Results

Genus Coccidohystrix Lindinger

Echinococcus Balachowsky, 1936. Type species: *Echinococcus echinatus* Balachowsky, by original designation. Homonym of *Echinococcus* Rudolphi, 1801, in Vermes.

Coccidohystrix Lindinger, 1943. Replacement name for *Echinococcus* Balachowsky.

Centrococcus Borchsenius, 1948. Unjustified replacement name for *Echinococcus*; discovered by Morrison & Morrison, 1966. Notes: Synonymy by community of type species.

Seabrina Neves, 1943: type species *Seabrina cistorum* Neves, 1943, by original designation and monotypy (synonymyzed by Danzig & Gavrilov-Zimin, 2014).

Type species: Echinococcus echinatus Balachowsky, by original designation

Generic diagnosis. Adult female. Female covered with white powdery wax or with few threads of wax (Balachowsky, 1953b; Marotta & Tranfaglia, 1994). Labium three segmented, slightly longer than wide. Posterior pair of spiracles always larger than anterior spiracles, spiracles associated with trilocular pores at opening of the atrium. Circulus absent. Legs well developed, claw with a denticle; tarsal digitules hair-like, not capitate; claw digitules knobbed, claw digitules broader than tarsal digitules. Both pairs of ostioles absent (except *C. insolita*, see comments). Anal lobes well developed. Anal ring oval, with 6 setae; with 1 inner row of pores and 1 or 2 outer rows of pores.

Dorsum. Antennae 7–9 segmented. Eyes oval, on a small basal cone. Number of cerari on margin varies between 16–18 pairs, each cerarius situated on an elevated sclerotised prominence bearing several (1–12) enlarged conical setae, several minute discoidal pores and 1 or 2 spine-like setae. These prominences form several rows along the body; the number of conical setae varies in different species. Dorsal body setae of various sizes (in *C. artemisiae*, *C. burumandi*, *C. eleusines* and *C. insolita*) or absent. Multilocular disc pores absent. Oral collar tubular ducts of one or two sizes or absent. Trilocular pores each 3–5 µm in diameter, often slightly larger than ventral trilocular pores, scattered over dorsum. Minute discoidal pores scattered.

Venter. Most ventral setae slender and hair-like, of various sizes. Oral collar tubular ducts of one or two sizes present around vulva or in transverse rows on body segments. Multilocular disc pores present on posterior abdominal segments, especially around vulva, sometimes also on thorax and head. Quinquelocular pores absent or present around median area of thorax or scattered on venter. Trilocular pores present, mostly near spiracles, each 2.5–5.0 µm in diameter. Minute discoidal pores scattered.

Comments. The genus *Coccidohystrix* can be distinguished by combination of the following characters (i) 7–9 segmented antennae; (ii) cerarii numbering 16–18 pairs, present on body margin, each cerarius formed of an elevated sclerotized prominence bearing several (1–12) enlarged conical setae, several minute discoidal pores and 1 or 2 spine-like setae, (iii) similar prominences distributed in longitudinal rows on dorsum, medial ones not divided; (iv) prominences and cerarii do not bear any trilocular pores (except in case of some population of *C. artemisiae* (Danzig et al., 2012).

The type material of *C. zangherii* was examined and consequently it was decided to transfer this species to the genus *Artemicoccus* as *A. zangherii* (Kozár & Pellizzari) *comb. nov.* Moreover, according to the drawings and descriptions of *C. echinata*, *C. maghribiensis* Gavrilov-Zimin & Matile-Ferrero and *C. monicae* Gavrilov-Zimin & Matile-Ferrero by Gavrilov-Zimin & Matile-Ferrero (2014) we regard these species as belonging to the genus *Artemicoccus* as *A. echinatus* (Balachowsky) *comb. nov.*, *A. maghribiensis* (Gavrilov-Zimin & Matile-Ferrero) *comb. nov.* and *A. monicae* (Gavrilov-Zimin & Matile-Ferrero) *comb. nov.*

Key to Palaearctic species of *Coccidohystrix*, adult females.

1 – Oral collar tubular ducts present on dorsum ..... *C. artemisiae* (Kiritchenko)

– Oral collar tubular ducts absent on dorsum 2

2 – Oral collar tubular ducts on venter very few, if present, only on posterior abdominal segment 3

– Oral collar tubular ducts on venter numerous ..... *C. insolita* (Green)

3 – Hind tibia with translucent pores 4

– Hind tibia without translucent pores 6

4 – Antennae 9 segmented; ventral quinquelocular pores scattered 5

– Antennae 7 segmented; ventral quinquelocular pores only on the posterior abdominal segment and near spiracles ..... *C. zsuzsannae* Kaydan *sp. nov.*

5 – Ventral trilocular pores present only on body submargin, with a few around spiracles .... *C. katiae* Kaydan & Szita *sp. nov.*

– Ventral trilocular pores scattered on body surface, numerous around spiracles .... *C. burumandi* Moghaddam

6 – Elevated sclerotised prominence on dorsum without small spine like setae; dorsal prominences in 6-7 rows, number of enlarged conical setae on dorsal cerarii maximum 8 ....*C. splendens* (Goux)

– Elevated sclerotised prominence on dorsum with small spine like setae; dorsal plates in 5 rows, number of enlarged conical setae on dorsal prominences maximum 5 ....*C. samui* Kozár & Konczné Benedicty

Coccidohystrix zsuzsannae Kaydan, *sp. nov.* (Fig. 1).

Material studied. *Holotype*. Female, Turkey, Hakkari-Yüksekove road, N: 37°40.966'; E: 044°06.227'; 1860 m; on plant from Asteraceae, 05.vii.2007, coll: M. Bora Kaydan, (KPCT: 3972).

Description. Adult female. Body elongate oval, 2.02 mm long, 1.32 mm wide. Eye marginal, 50–65 µm wide. Antenna 7 segmented, 400 µm long; apical segment 65 µm long, 20 µm wide, with apical setae 17.5 µm long plus three fleshy setae, each 20 µm long. Tentorium 170 µm long, 160 µm wide. Labium 140 µm long, 87.5 µm wide. Anterior spiracles 60 µm long, 25 µm wide across atrium; posterior spiracles 65 µm long, 25 µm wide across atrium. Legs well developed; data for first legs: coxa 145 µm, trochanter + femur 260 µm, tibia + tarsus 325 µm, claw 30 µm, tarsal digitules each 20 µm long, hair-like, claw digitules knobbed each 27.5 µm long; data for mid legs: coxa 130 µm, trochanter + femur 275 µm, tibia + tarsus 335 µm, claw 27.5 µm, tarsal digitules each 20 µm long, hair-like, claw digitules knobbed each 27.5 µm long; data for hind legs: coxa 140 µm, trochanter + femur 280 µm, tibia + tarsus 360 µm, claw 30 µm, tarsal digitules each 20 µm long, hair-like, claw digitules knobbed each 32.5 µm long. Anal ring 70 µm wide, with 6 setae, each setae 125–130 µm long. Cerarii 16 pairs, each cerarius formed of an elevated sclerotized prominence bearing several (5–8) enlarged conical setae, 3–6 minute discoidal pores and 1 or 2 spine-like setae each 4–5 µm long.

Dorsum.Enlarged conical setae, each 27–45 µm long on an elevated sclerotized prominence bearing 1–7 setae, 1–5 minute discoidal pores and 1–3 spine like setae. These prominences form five longitudinal rows along the body. Trilocular pores each 4–5 µm in diameter, a few on the surface; minute discoidal pores each 2–3 µm diameter, scattered. Multilocular disc pores, quinquelocular pores and oral collar tubular ducts absent.

Venter. Body setae hair-like setae, each 15.0–50.0 µm long, longest setae medially on head. Apical setae of anal lobe each 70 µm long. Multilocular disc pores absent, numbering of quinquelocular pores in rows on abdominal segments VIII + IX: 6; each pore 5 µm in diameter in pentagonal shape. Trilocular pores, each 3–4 µm in diameter, present on the submargin, numerous near spiracles, each spiracles with 61–75 associated trilocular pores. Minute discoidal pores scattered, each 2–3 µm in diameter. Oral collar tubular ducts concentrated on abdominal segments: V 2, VI 4, VII 4.

Comments. C. zsuzsannae sp. nov. can be distiguished from other *Coccidohystrix* species in having (i) antennae seven segmented (ii) oral collar tubular ducts concentrated on abdominal segments: V, VI, VII , (iii) a few quinquelocular pores only on abdominal segment VIII+IX; and (iv) a few trilocular pores sparse on dorsum and on ventral submargin. This species is very close to *C. splendens* (Goux) and *C. burumandi* Moghaddam, but differs from these two species in having seven-segmented antennae and much less quinquelocular pores on venter.

Etymology. This species is named after Zsuzsanna Konczné Benedicty (Plant Protection Institute, Centre for Agricultural Research, Hungarian Academy of Sciences, Budapest, Hungary) who made great studies on the scale insects.

Host plant. Asteraceae.

Distribution. Turkey.

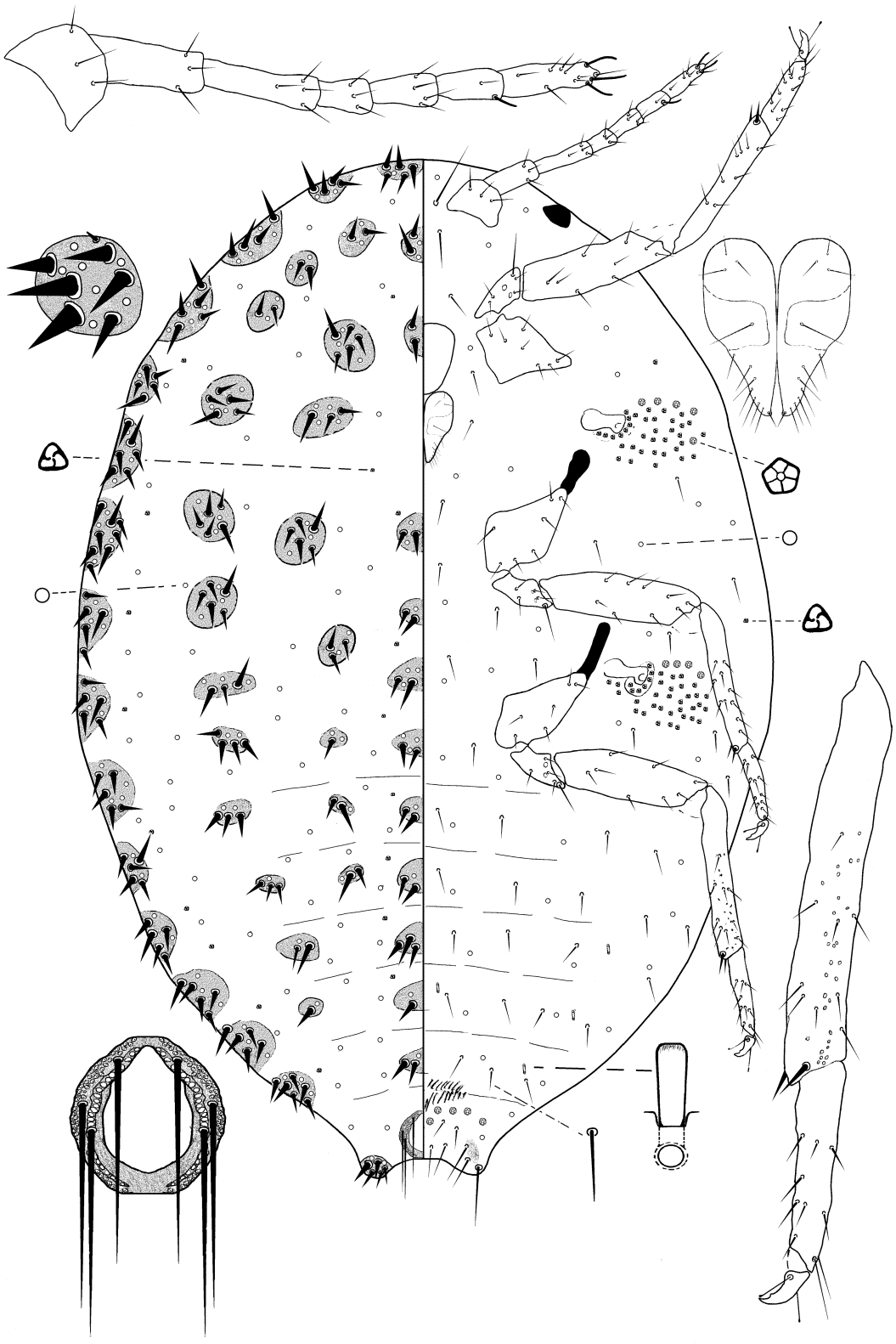

Figure 1. *Coccidohystrix zsuzsannae* Kaydan, *sp. nov.*, holotype female.

Coccidohystrix katiae Kaydan & Szita, *sp. nov.* (Fig. 2).

Material studied. *Holotype*. Female, Bulgaria, Kresna Gorge, *Thymus* sp. (Lamiaceae), 27.vi.2012, coll: K. Fetykó (PPI: 10517).

Description. Adult female. Body elongate oval, 1.66 mm long, 1.09 mm wide. Eye marginal, 45–50 µm wide. Antenna 9 segmented, 400 µm long; apical segment 55–60 µm long, 20.0 µm wide, with apical setae 15 µm long plus three fleshy setae, each 20.0–22.5 µm long. Tentorium 140 µm long, 125 µm wide. Labium 107.5 µm long, 95 µm wide. Anterior spiracles 55–60 µm long, 25 µm wide across atrium; posterior spiracles 62.5–65 µm long, 25.0–27.5 µm wide across atrium. Legs well developed; data for first legs: coxa 120–135 µm, trochanter + femur 207.5–215.0 µm, tibia + tarsus 247.5–250.0 µm, claw 32.5–35.0 µm, tarsal digitules each 22.5 µm long, hair-like, claw digitules knobbed each 25 µm long; data for mid legs: coxa 125 µm, trochanter + femur 215 µm, tibia + tarsus 255–260 µm, claw 30 µm, tarsal digitules each 20.0–22.5 µm long, hair-like, claw digitules knobbed each 25 µm long; data for hind legs: coxa 130 µm, trochanter + femur 220 µm, tibia+tarsus 292.5–300.0 µm, claw 27.5–32.5 µm, tarsal digitules each 25 µm long, hair-like, claw digitules knobbed each 20.0–22.5 µm long. Anal ring 70 µm wide, with 6 setae, each setae 110 µm long. Cerarii 17 pairs, each cerarius formed of an elevated sclerotized prominence bearing several (2–7) enlarged conical setae, 2–5 minute discoidal pores and 1 or 2 spine-like setae each 4–5 µm long.

Dorsum. Body setae enlarged, each 15–25 µm long on an elevated sclerotized prominence bearing 1–7 setae, 1-5 minute discoidal pores and 1-3 spine like setae. Trilocular pores each 3–4 µm in diameter, a few on the surface, minute discoidal pores each 2–3 µm diameter, scattered on dorsum. Multilocular disc pores, quinquelocular pores and oral collar tubular ducts absent.

Venter. Body setae hair-like slender setae, each 7.5–45.0 µm long, longest setae medially on head. Apical setae of anal lobe each 100 µm long. Multilocular disc pores absent, quinquelocular pores scattered on all surface, each pore 5–6 µm in diameter in pentagonal shape. Trilocular pores, each 3–4 µm in diameter, present on the submargin and associated to spiracles. Minute discoidal pores scattered over entire body, each 2–3 µm in diameter. Oral collar tubular ducts concentrated on abdominal segments VI 4, VII 2.

Comments. C. katiae can be distiguished from other *Coccidohystrix* species by (i) antennae nine segmented (ii) oral collar tubular ducts placed only anterior segments of vulva, (iii) quinquelocular pores scattered on venter; (iv) trilocular pores scattered on both venter and dorsum. This species is very close to *C. samui* Kozár & Konczné Benedicty but differs from this species in having translucent pores on tibia, (absent on *C. samui*).

Etymology. This species is named after Katia Trencheva (University of Forestry, Faculty of Agronomy, Plant Protection Department, Sofia, Bulgaria) who made great studies on the scale insects in Bulgaria.

Host plant. *Thymus* sp. (Lamiaceae).

Distribution. Bulgaria

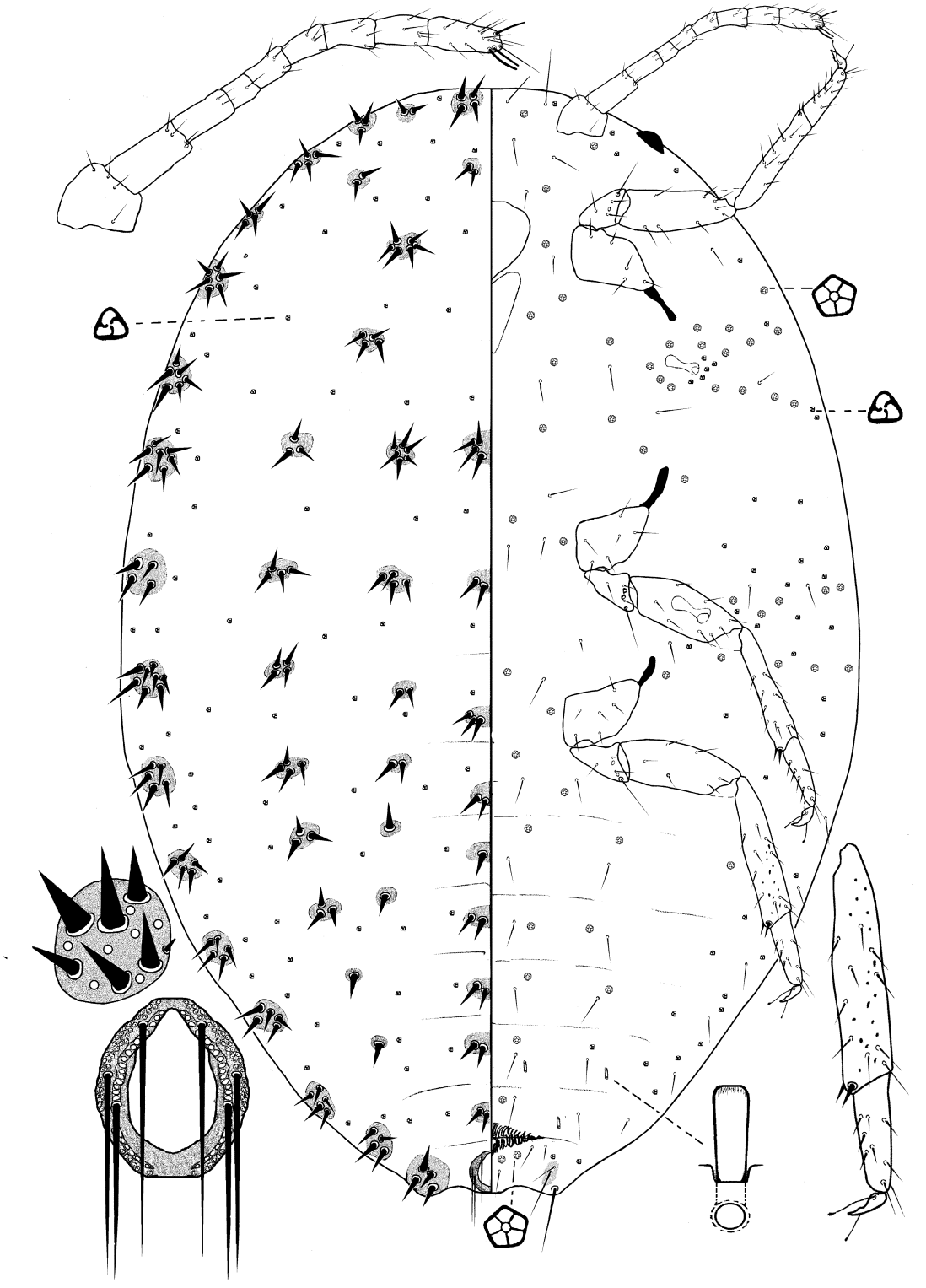

Figure 2. *Coccidohystrix katiae* Kaydan & Szita, *sp. nov.*, holotype female.

Genus *Artemicoccus* Balachowsky

Artemicoccus Balachowsky, 1953b: 146.

Type species: Centrococcus bispinus Borchsenius, 1949, by original designation.

Generic diagnosis. Adult female.

Body oval, yellowish, enclosed in a felt-like eggsac, similar to that of Eriococcidae. Labium three segmented, slightly longer than wide. Posterior pair of spiracles always larger than anterior spiracles, spiracles associated with trilocular pores at opening of the atrium. Circulus absent. Legs well developed, claw with a denticle; tarsal digitules hair-like, not capitate; claw digitules knobbed, claw digitules broader than tarsal digitules. Both pairs of ostioles absent. Anal lobes well developed. Anal ring oval, with 1 inner row of pores and 1 or 2 outer rows of pores plus with 6 setae.

Dorsum. Antennae 7 segmented. Eyes oval, on a small basal cone. Number of cerarii variable: 7–18 pairs present on body margin, each cerarius formed by 1–7 enlarged conical setae. Conical setae, similar to those of cerarii, singular or in groups of 2–5 also present on dorsum. Other dorsal body setae small cylindrical of various sizes. Multilocular disc pores absent. Oral collar tubular ducts of one or two sizes or absent. Trilocular pores each 3–5 μm in diameter, often slightly larger than ventral trilocular pores, scattered over dorsum. Minute discoidal pores scattered.

Venter. Most ventral setae slender and hair-like, of various sizes. Oral collar tubular ducts of one or two sizes present around vulva or in transverse rows on body segments. Multilocular disc pores present on posterior abdominal segments, especially around vulva, sometimes also on thorax and head. Quinquelocular pores absent or present around median area of thorax or scattered over surface. Trilocular pores each 2.5–5.0 μm in diameter, scattered over surface. Minute discoidal pores scattered.

After the detailed studies of Danzig et al. (2012) the borders between the genera *Artemicoccus* and *Coccidohystrix* become clearer, in having different combination of characters (characters for the genus *Coccidohystrix* in brackets): (i) enlarged conical setae situated on dorsum, not on elevated sclerotized plate (present on elevated sclerotized plate); (ii) dorsal spine-like setae (besides from conical enlarged setae) of different sizes (same size); (iii) anal lobes with three conical setae (more than three conical setae).

Artemicoccus zangherii (Kozár & Pellizzari), *comb. nova* (Figure 3)

Coccidohystrix zangherii Kozár & Pellizzari, 1989: 507. Holotype, female, ITALY: Campo Imperatore, Fonte Vetica, on *Juniperus nana* (deposited in DAFNAE). Paratype, female, same data as holotype.

Description: For detailes see (Kozár & Pellizzari) 1989.

Comments. It was mentioned by the authors in the original description that this species could have been placed into the genus *Artemicoccus* (Kozár & Pellizzari, 1989). They considered that *Artemicoccus* could be a possible synonym of *Coccidohystrix* but did not formally synonymized the two genera and described the new species in *Coccidohystrix.*

The type material of *C. zangherii* was examined and consequently, according to the genus concept reported above, this *Juniperus* feeding species is transferred from *Coccidohystrix* to *Artemicoccus* as *A. zangherii* (Kozár & Pellizzari) *comb. nov.*

Moreover, according to the drawings and descriptions of *C. echinata* (Balachowsky, 1930), *C. maghribiensis* Gavrilov-Zimin & Matile-Ferrero and *C. monicae* Gavrilov-Zimin & Matile-Ferrero we regard the above reported species as belonging to the genus *Artemicoccus* as *A. echinatus* (Balachowsky) *comb. nov.*, *A. maghribiensis* (Gavrilov-Zimin & Matile-Ferrero) *comb. nov.* and *A. monicae* (Gavrilov-Zimin & Matile-Ferrero) *comb. nov.*

Key to *Artemicoccus* species, adult female.

1 – Dorsal oral collar tubular ducts numerous 2

– Dorsal oral collar tubular ducts very few or absent ..... *A. lubersaci* (Balachowsky)

2 – Trilocular pores scattered on both venter and dorsum 3

– Trilocular pores present only on venter, near spiracles 5

3 – Groups of conical setae forming transverse rows on dorsum..... 4

– Groups of conical setae only on body margin; rarely a few conical setae placed on dorsum..... .....*A. echinatus* (Balachowsky)

4 – Dorsal oral collar tubular ducts of two sizes; conical enlarged dorsal setae up to seven for each setae group .....*A. zangherii* (Kozár & Pellizzari)

– Dorsal oral collar tubular ducts of only one size; conical enlarged dorsal setae up to three for each setae group ..... *A. bispinus* (Borchsenius)

5 – Groups of conical setae forming transverse rows on dorsum……..... .....*A. maghribiensis* Gavrilov-Zimin & Matile-Ferrero

– Groups of conical setae present only on body margin; rarely a few conical setae placed on dorsum .... *A. monicae* Gavrilov-Zimin & Matile-Ferrero

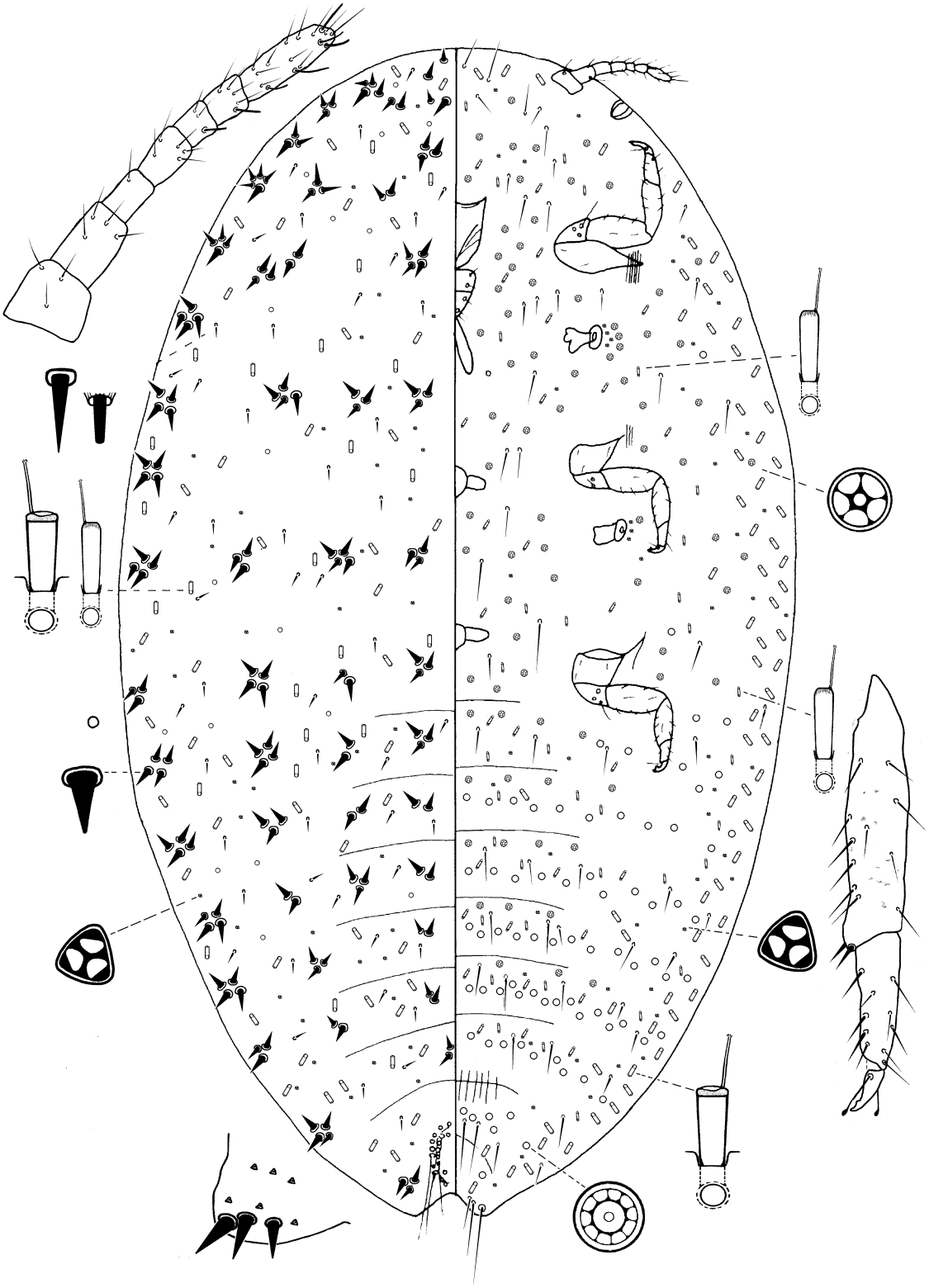

Figure 3. *Artemicoccus zangherii* (Kozár & Pellizzari), paratype female.

References

Balachowsky, A. S., 1936. Contribution à l'étude des coccides du Nord Africain (15me note). Sur deux remarquables Pseudococcinae découverts dans le Sahara Marocain par Ch. Rungs. Bulletin de la Société Entomologique de France, 41: 157-165.

Balachowsky, A.S., 1953 a. Communications. I. Sur un *Centrococcus* (Coccoidea-Pseudococcini) nouveau du Maroc Oriental II. Remarques sur les Pseudococcini spinuleux. Bulletin de la Société des Sciences Naturelles du Maroc, 31: 277-285.

Balachowsky, A.S., 1953 b. Remarques complementaires sur le statut des genres *Echinococcus* Balachw. et *Centrococcus* Borkhsenius (Coccoidea-Pseudococcini). Bulletin de la Société des Sciences Naturelles du Maroc, 33: 145-147.

Ben-Dov, Y., 1994. A Systematic Catalogue of the Mealybugs of the World (Insecta: Homoptera: Coccoidea: Pseudococcidae and Putoidae) with Data on Geographical Distribution, Host Plants, Biology and Economic Importance. Intercept Limited, Andover, UK, 686 pp.

Ben-Dov, Y., D.R. Miller & G.A.P. Gibson, 2015. ScaleNet: A Database of the Scale Insects of the World. (Web page: http://www.sel.barc.usda.gov/SCALENET/SCALENET.HTM.) (Accessed date: April 2015)

Borchsenius, N. S., 1948. Toward a revision of the genus *Phenacoccus* Ckll. (Insecta, Homoptera, Coccoidea). Doklady Akademii Nauk SSSR. Moscow (n.s.), 61: 953-956.

Borchsenius, N. S., 1949. Insects Homoptera. suborders mealybugs and scales (Coccoidea). Family mealybugs (Pseudococcidae). Vol. VII. Fauna SSSR. Zoologicheskii Institut Akademii Nauk SSSR. N.S., 38: 1-382.

Danzig, E.M. & I.A. Gavrilov, 2014. Fauna of Russia and Neighbouring Countries, New series, No. 148. Palaearctic Mealybugs (Homoptera: Coccinea: Pseudococcidae). Russian Academy of Sciences, Zoological Institute, St. Petersburg, 678 pp.

Danzig, E.M., M.B. Kaydan & I.A. Gavrilov-Zimin, 2012. A review of Palaearctic species of Artemicoccus and Coccidohistrix (Homoptera: Coccinea: Pseudococcidae). Zoosytematica Rossica, 21(2): 237-243.

Gavrilov-Zimin, I.A. & D. Matile-Ferrero, 2014. The genus *Coccidohystrix* Lindinger, 1943 in the Palaeartic region with description of two new species from Maghreb (Homoptera: Coccinea: Pseudococcidae). Zoosystematica Rossica, 23(1): 96-105.

Kaydan, M.B., F. Kozár & C. Hodgson, 2015. A review of the phylogeny of Palaearctic mealybugs (Hemiptera: Coccomorpha: Pseudococcidae). Arthropod Systematics and Phylogeny, 73 (1): 175-195.

Kosztarab, M. & F. Kozár, 1988. Scale Insects of Central Europe. Akademiai Kiadó, Budapest, Hungary, and Dr W. Junk Publishers, Dordrecht, The Netherlands, 456 pp.

Kozár, F. & G. Pellizzari Scaltriti, 1989. A new mealybug from Italy: *Coccidohystrix zangherii* sp. n. (Homoptera: Coccoidea, Pseudococcidae). Redia, 72 (2): 507-511.

Lindinger, L. 1943. Verzeichnis der Schildlaus-Gattungen, 1. Nachtrag. (Homoptera: Coccoidea). Zeitschrift der Wiener Entomologischen Gesellschaft 28: 205-265.

Marotta, S. & A.Tranfaglia, 1994. La coccidofauna dell'Appennino abruzzese (Homoptera Coccoidea). II contributo. Bollettino del Laboratorio di Entomologia Agraria 'Filippo Silvestri', 49: 99-116.

Morrison, H. & E.R. Morrison, 1966. An annotated list of generic names of the scale insects (Homoptera: Coccoidea). Miscellaneous Publication United States Department of Agriculture 1015: 1-206.

Neves, M., 1943. Sobre um género novo de "Kermidae" (On a new genus of "Kermidae"). Arquivos do Museu Bocage, Lisbon, 14: 1-10.

Williams, D.J., 2004. Mealybugs of Southern Asia. The Natural History Museum, Kuala Lumpur: Southdene SDN. BHD., 896 pp.

Türk. entomol. derg., 2015, 39 (4): 367-380 ISSN 1010-6960
DOI: http://dx.doi.org/10.16970/ted.49593

Orijinal araştırma (Original article)

Toxic and repellent effects of *Prunus laurocerasus* L. (Rosaceae) extracts against *Tetranychus urticae* Koch (Acari: Tetranychidae)[1]

Prunus laurocerasus L. (Rosaceae) ekstraktlarının *Tetranychus urticae* Koch (Acari: Tetranychidae)'ye karşı toksik ve repellent etkileri

Rana AKYAZI[2*] Mete SOYSAL[2] Errol HASSAN[3]

Summary

The toxic and repellent effects of leaves, flower and seed extracts of *Prunus laurocerasus* L. (Rosaceae) were investigated against *Tetranychus urticae* Koch (Acari: Tetranychidae) under laboratory conditions. Extracts at three different concentrations (1 %, 5 % (v/v), 10 % (w/v)) for ovicidal and repellent effects against eggs and adult females, respectively and at five different concentrations (1 %, 2.5 %, 5 %, 7.5 % (v/v), 10 % (w/v)) for the contact toxicity against female adults were evaluated. The results showed that seed extract was the most effective compared to flower and leaf extracts. It was found that at 5 % and 10 % concentrations of seed extract, the repellent effects were 92 % and 100 %, respectively within the first 72 hours. At 10% concentration of seed extract, the ovicidal and adulticidal effect were 96.56 % and 100 %, respectively. Moreover, further concentration response trials showed that LC_{50} and LC_{90} values for the contact toxicity of seed extract on eggs and adult females were LC_{50}=4.5 %, LC_{90}=9.4 % and LC_{50}=2.9 %, LC_{90}=9.1 %, respectively. The result indicated that seed extract of *P. laurocerasus* has good potential to be used to control *T. urticae*. However, the impact of these extracts on natural enemies of *T. urticae* should also be needed further studies.

Keywords: Acaricidal effect, cherry laurel, aqueous plant extracts, twospotted spider mite

Özet

Prunus laurocerasus L. (Rosaceae)'un yaprak, çiçek ve çekirdek ekstraktlarının *Tetranychus urticae* Koch (Acari: Tetranychidae)'ye karşı toksik ve repellent etkileri laboratuvar koşullarında araştırılmıştır. Ekstraktların yumurta ve ergin dişilere karşı sırası ile ovisidal ve repellent etkileri üç (% 1, % 5 (v/v), % 10 (w/v)), ergin dişilere karşı kontakt toksisiteleri ise beş (% 1, % 2.5, % 5, % 7.5 (v/v), % 10 (w/v)) farklı konsantrasyonda test edilmiştir. Sonuçlar çekirdek ekstraktının, çiçek ve yaprak ekstraktları ile kıyaslandığında en etkili ekstrakt olduğunu göstermiştir. Çekirdek ekstraktının 5 % ve 10 % konsantrasyonlarının ilk 72 saat içindeki repellent etkileri sırası ile % 92 ve % 100 olarak bulunmuştur. Çekirdek ekstraktının %10'luk konsatrasyonundaki ovisidal ve adultisidal etkileri ise sırası ile % 96.56 ve % 100 kadardır. Bununla birlikte, konsantrasyon tepki denemeleri çekirdek ekstraktının yumurta ve ergin dişi bireyler için LC_{50} ve LC_{90} değerlerinin sırası ile LC_{50}=4.5 %, LC_{90}=9.4 % ve LC_{50}=2.9 %, LC_{90}=9.1 %, olduğunu göstermiştir. Sonuçlar *P. laurocerasus*'un çekirdek ekstraktının *T. urticae* kontrolünde kullanılmak için iyi bir potansiyele sahip olduğunu işaret etmektedir. Fakat, ekstrakların *T. urticae*'nin doğal düşmanlarına etkilerinin belirlenmesi için çalışmaların devam etmesi gerekmektedir.

Anahtar sözcükler: Akarisidal etki, karayemiş, sulu bitki ekstraktları, iki noktalı kırmızı örümcek

[1] The part of this research was presented as poster on The Second International Persian Congress of Acarology (29- 31 August 2013, Iran) and The Fifth Plant Protection Congress of Turkey (3-5 February 2014, Antalya, Turkey) and published as abstract in the abstracts book.

[2] Plant Protection Department, Faculty of Agriculture, Ordu University, Ordu, Turkey
[3] The University of Queensland Gatton, School of Agriculture and Food Sciences, Gatton, Queensland, 4343, Australia
* Sorumlu yazar (Corresponding author) e-mail: ranaakyazi@odu.edu.tr
Alınış (Received): 08.06.2015 Kabul ediliş (Accepted): 17.12.2015 Çevrimiçi Yayın Tarihi (Published Online): 23.12.2015

Introduction

Tetranychus urticae Koch (Acari: Tetranychidae) is a phytophagous mite that feeds on a wide variety of plant families worldwide. Its control is still largely based on the use of pesticides in view of their easy of application and rapidity of action. However, due to short life cycle, abundant progeny and arrhenotokous reproduction of red spider mites, they are able to develop resistance against to pesticides very rapidly. Also, the use of synthetic pesticides poses harmful effects on environment, human safety and nontarget organisms such as natural enemies, honeybees and wildlife. Therefore, there is need to develop nontoxic natural products that have potential to replace synthetic pesticides for controlling this pest.

Plant extracts are one of nonchemical control options. Some of them, Neem (Sundaram & Sloane, 1995; Martinez-Villar et al., 2005), *Tanacetum vulgare* L. and *Artemisia absinthium* L. (Asteraceae) (Chiasson et al., 2001), *Satoreja hortensis* L. (Lamiaceae) (Aslan et al., 2004), *Calotropis porcera* (Ait.) (Asclepiadecae), *Nerium oleander* L. (Apocynacae) (Islam et al., 2008), were reported as botanical acaricides (Derbalah et al., 2013). They contain secondary metabolites known from their repellent, antifeedant, ovicidal and killing action against arthropod pests (Smith, 1989; Tomczyk & Suszko, 2011).

Many investigations have been performed on the ovicidal, toxic and repellency effects of some plant extracts on *T. urticae.* Yanar et al. (2011a) investigated the ovicidal activity of methanol extracts of nine plant species against *T. urticae* under laboratory conditions. They found that some of these plant extracts have a potential for ovicidal activity on *T. urticae* eggs. The extracts of different parts of 12 plant species were also evaluated for their acaricidal activity on *T. urticae* by Yanar et al. (2011b). Erdoğan et al. (2012) determined efficacy of pesticides extracted from *Allium sativum* L. (Alliaceae), *Rhododendron luteum* S. (Ericaceae), *Helichrysum arenarium* L. (Asteraceae), *Veratrum album* L. (Liliaceae) and *Tanacetum parthenium* L. (Asteraceae)] against this mite. Ghaderi et al. (2013) investigated the ovicidal activities of methanolic extracts of *Anisosciadium orientale* DC. (Apiaceae), *Scaligeria meifolia* Boiss. (Apiaceae), *Trigonella elliptica* Boiss. (Leguminosae) and *Ptelea viscosa* L. (Sapindaceae) against *T. urticae* under laboratory conditions. Derbalah et al. (2013) found that *Nigella sativa* Linn. (Ranunculaceae) (seeds) and *Artemisia cina* L. (leaf) (Asteraceae) extracts were toxic to eggs of *T. urticae* with LC_{50} values of 1850.92 and 2740.42 ppm. Additionally, aqueous extracts from *Sinapsis alba* L. (Brassicaceae), *A. sativum*, *Urtica dioica* L. (Urticaceae) (Dabrowski & Seredynska, 2007), water extract of *Artemisia judaica* L. (Asteraceae) (El-Sharabasy, 2010), aqueous solvent of *Nicotiana tabacum* L. (Solanaceae) and *Pegunum harmala* L. (Nitrariaceae) (Almansour & Akbar, 2013) were investigated for toxic effect on *T. utricae*. It found that all of them had toxic effects against *T. urticae* adult females too. The lethal effect of dichloromethane and ethanol extracts of *Melia azedarach* L. (Meliaceae), *P. harmala*, *N. sativa* and *Trigonella foenumgraecum* L. (Fabaceae) seeds was investigated on adult *T. urticae* under laboratory conditions by Elkertati et al. (2013).

At the same time, investigations show that several *Artemisia* spp. (Asteraceae) (Saber, 2004; (Dabrowski & Seredynska, 2007; El-Sharabasy, 2010), some *Capsicum* spp. (Solanaceae) (Antonious et al., 2006), wild tomato (Antonious & Snyder, 2006), *U. dioica* (Dabrowski & Seredynska, 2007), *Datura stramonium* L. (Solanaceae) (Kumral et al., 2010), *Mentha longifolia* L. (Lamiaceae), *Salvia officinalis* L. (Lamiaceae) and *Myrtus communis* L. (Myrtaceae) (Motazedian et al., 2012) have potential repellent action against *T. urticae* too.

Prunus laurocerasus is an evergreen species of cherry known insecticidal activity on arthropods (Rattan & Sharma, 2011). Thomas (2004) defined that the leaves of *P. laurocerasus* could be used as a pesticide. Furthermore, the toxicity effects of *P. laurocerasus* on *Plutella xylostella* L. (Insecta: Lepidoptera: Plutellidae) was determined as 50 % within seven days by Ertürk et al. (2004). But, there is no further research about effect of *P. laurocerasus* extracts on the phytophagous mites. The aim of this study was to identify the repellent effect of leaves, flower and seed extracts of *P. laurocerasus* against adults of *T. urticae* as well as the toxicity against eggs and adults of *T. urticae* under laboratory conditions.

Material and Method

Plant and mite rearing

Pinto bean plants, *Phaseoulus vulgaris* L. (Fabaceae), were used as a host plant for *T. urticae* and served as the test substrate. Plants were cultivated in a mixture of vermiculite and soil in plastic pots (26 x 14 cm) in the laboratory. Seeds were planted every two days in order to provide plants with primary leaves two or three days old for leaf discs. To reduce problems of leaf disc deterioration, young but fully expanded leaves were selected.

Tetranychus urticae was obtained from a stock colony maintained in the laboratory (Plant Protection Department, Ordu University, Ordu, Turkey) at 25 ± 2 °C and 70-80% relative humidity. The *T. urticae* colony was reared continuously on pinto bean plants by laying cut foliage containing abundant *T. urticae* on new plants at the 3-6 leaf stage. As the cut foliage dried, *T. urticae* moved to the fresh plants.

Plants and preparation of extracts

Leaves (pre-flowering) flowers and seeds of *P. laurocerasus* were collected during the spring and summer of 2011 and 2012.

Each plant material was dried under shade and powdered using a grinder. For extraction, powdered plant materials (50 g) were placed in an erlenmeyer flask, distilled water (500 ml) was added, and then it was shaken for 48 h in a horizontal shaker at 125 rpm in room temperature (25±2 ºC). The extracts were separated using fine muslin cloth and then filtered through Whatman No. 1 filter paper. This was called stock solution (% 10 w/v). The stock solution was dissolved in distilled water to obtain solutiones at 1 %, 2.5 %, 5 % and 7.5 % (v/v).

Experimental design for repellency

Extracts at three different concentrations (1 %, 5 % and 10 %) were evaluated for repellency. Experiments were performed using bean leaf discs 3 cm in diameter. Half of every disc was immersed for 5 seconds in each concentration of extract and after drying at room temperature; the other half was immersed for 5 seconds in distilled water. The treated leaf discs were placed underside up on water-soaked cotton in a plastic tray (15x11 cm). The wet cotton prevented escape and maintained leaf freshness. Adult female mites were released in the center of each disc to see where the red spider mites sattle down. The results were controlled after 2, 6, 24, 48, 72 and 96 hours by counting the number of adults present on each half of the leaf discs. Each treatment was replicated five times each with 10 adult mites.

Experimental design for ovicidal efficiency

Extracts at three different concentrations (1 %, 5 % and 10 %) were evaluated for ovicidal efficiency. Experiments were performed using 3 cm diameter bean leaf discs. Ten adult females of *T. urticae* were introduced on bean leaf discs for oviposition and kept overnight. After 24 hours the introduced mites were removed with the help of a fine brush. The eggs laid on leaf discs were counted. The leaf discs containing 25 eggs were used for assesment. For this purpose, some eggs were removed from discs to have 25 eggs on every one. The leaf discs were dipped for 5 seconds in extracts and allowed to dry at room temperature. Once dried, the treated leaf discs were placed underside up on water-soaked cotton on a plastic tray (15 x 11cm). The egg number on each disc was counted again. The discs that did not have 25 eggs were removed from the trial. The control discs were treated with distilled water. There were five replications for each concentration of each plant extract. Hatchability was determined for a period of 10 days after treatment. Those eggs that did not hatch after this period were regarded as non-viable.

Experimental design for contact toxicity

Tests were conducted using the standard slide-dip method to compare the acaricidal toxicity of the *P. laurocerasus* extracts on *T. urticae* engorged female adults in the laboratory (FAO, 2004). Extracts at five different concentrations (1 %, 2.5 %, 5 %, 7.5 % and 10 %) were evaluated for contact toxicity. The adult female mites, which were uniform in size, color, and brightness, were selected using a zero size brush. Double-sided adhesives were cut into 2 cm-long pieces and stuck to one end of the slides. The dorsums of 10 mites were fastened onto double stick adhesive tape. Each treatment consisted of 5 slides. Each slide was checked under a microscope. Inactive or injured mites were removed. The slides were then dipped for 5 seconds in the extracts. The control slides were treated with distilled water. The slides were shaken gently to remove excess solution around the body of mites after dipping (Kovach & Gorsuch, 1986; Wang et al., 2012). Furthermore, any droplet of extract solutions remaining on the slides after dipping was removed with blotting paper. The mite number on each slide was counted again. The slides that did not have 10 adult females after dipping were removed from the trial. Mortality counts were made 1, 24, 48, 72 hours after the application. Mites which failed to respond with leg movements after being proded lightly with a fine brush were considered to be dead.

Statistical analysis

The repellent effect was calculated by using the following formula (% Repellent effect index) developed by Obeng-Ofori et al. (1997).

RI (%) = [NC-NT/(NC+NT)]×100

NC = The number of mites on control diet.

NT = The number of mites on treated diet.

RI = Repellent effect index

Datas were corrected for mortality in the controls using Abbott's formula (Abbott, 1925). The Kolmogorov-Smirnov and Levene's tests were applied to test normality and homogeneity of variance, respectively. Contact and repellent effects were analysed by three-way repeated measures ANOVA (between-subjects factors: dose and extract; within-subjects factors: time). Ovicidal effects were analysed by two-way ANOVA. The means compared with Tukey post-hoc test and the results were displayed in the form of letters. Variables were displayed as mean with 95% confidence interval (CI). The statistical analysis was performed using Minitab SPSS 23 and Minitab 17 statistical package program.

Dose response models, allowing for control were fitted using the SAS Probit procedure. Lethal concentrations (LC_{50} and LC_{90}) were estimated, along with 95% confidence. The alpha level was set at 5%.

Results and Discussion

The repellect effect of *Prunus laurocerasus* on *Tetranychus urticae* adult females

According to the analysis of variance, two-way interaction between dose and exposure time (Fig. 4) and main effect of extract (Fig. 5) were significant ($P< 0.001$), while three-way interaction between dose, exposure time and extract was not significant. The repellent effects of leaf, flower and seed extracts of *P. laurocerasus* on adult females of *T. urticae* were given in Figs 1-5. As shown in Fig. 1, at the lowest concentration (1%), all extracts had repellency rate ranging between 64 %- 88 % within the first 48 h and 28 % - 39 % after this time. At a concentration of 5 % (Fig. 2), leaf and flower extracts had repellency rate ranging between 84 %- 88 % and 76 %-88 % within the first 24 h, respectively and 64%- 68% and 60%-64% at 48 h-72 h, respectively. But, repellency of leaf and flower extracts was 44% at 96 h. Seed extract had a repellency rates of 92%- 100% within the first 72 h and 68% at 96 h. All extracts had high repellent effect at concentrations of 10% (Fig 3) especially within the first 72 h, and repellency rate of leaf, flower and seed extracts ranged between 68% and 100%. After this time, although repellency of leaf and flower extracts was 64% and 44%, respectively, repellency rate of seed extract was 84% at 96h. In agreement with Zhang et al., (2013), as processing time increased, the repellent activity gradually decreased. Additionaly, an increase in extract concentration could probably increase its repellent activity (Fig. 4), as was observed by Momen et al., (1997). When all data were compared over the repellency rates, seed extract was the most effective of all extracts (Figs 5-6).

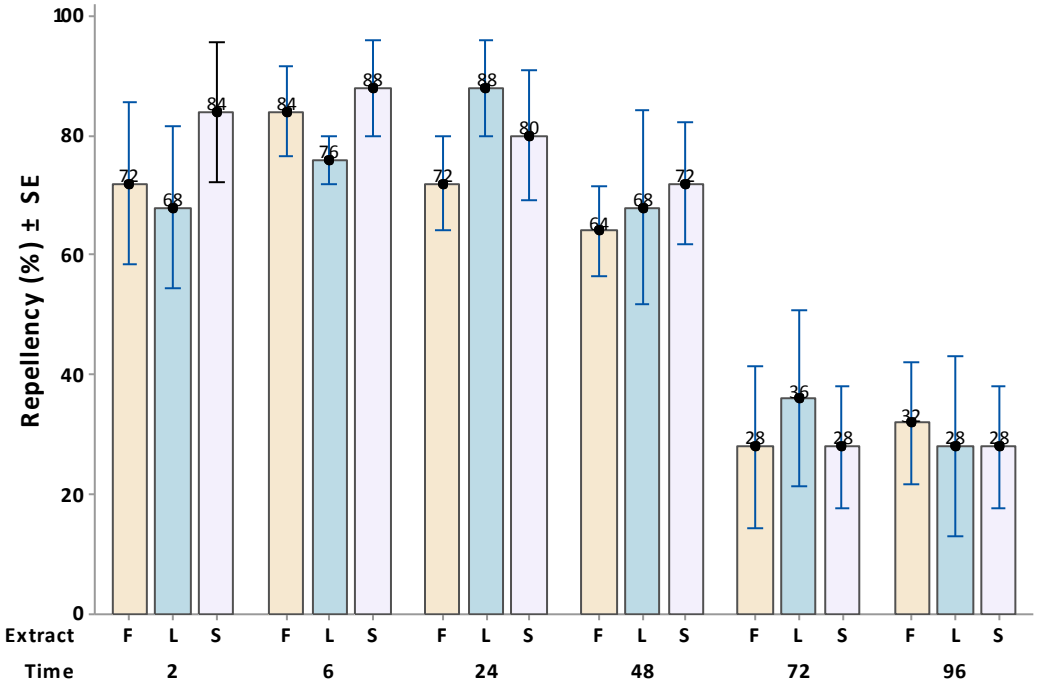

Figure 1. The repellent effects (Mean ±SE) of leaf (L), flower (F) and seed (S) extracts of *Prunus laurocerasus* against *Tetranychus urticae* adult females at 1% concentration at different counting times (hour).

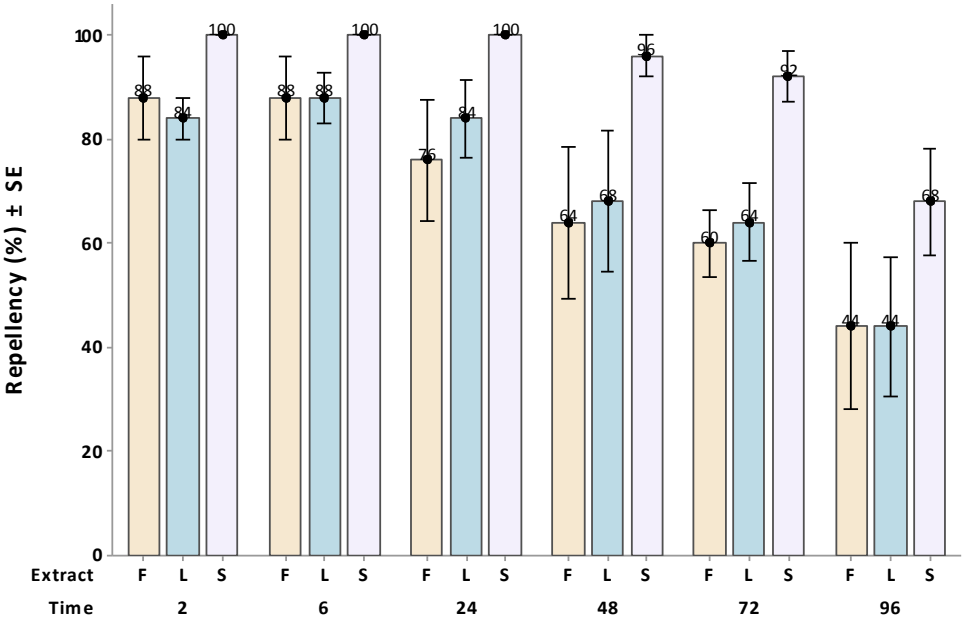

Figure 2. The repellent effects (Mean ±SE) of leaf (L), flower (F) and seed (S) extracts of *Prunus laurocerasus* against *Tetranychus urticae* adult females at 5% concentration at different counting times (hour).

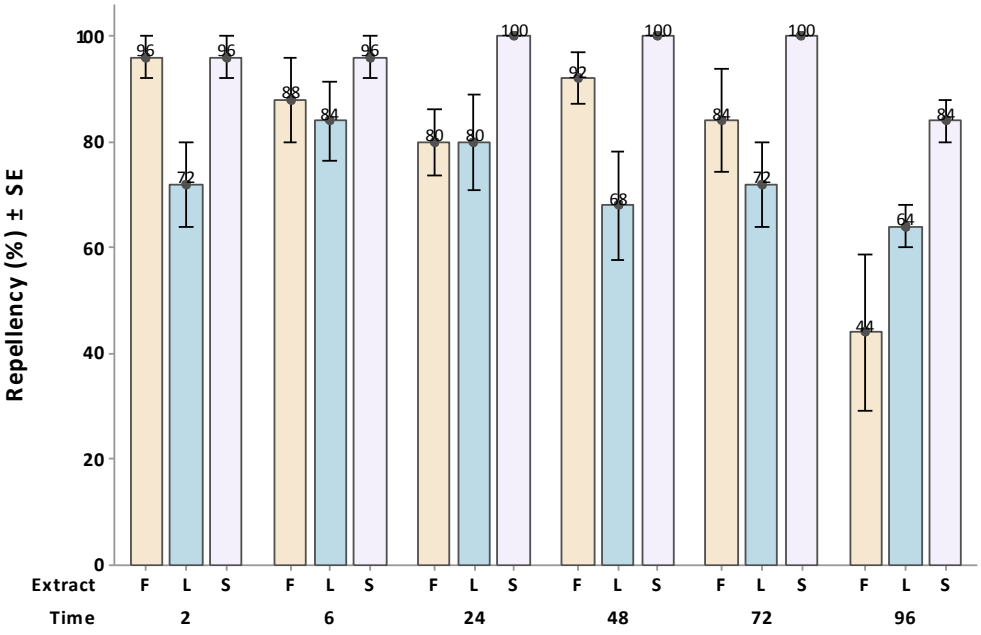

Figure 3. The repellent effects (Mean ±SE) of leaf (L), flower (F) and seed (S) extracts of *Prunus laurocerasus* against *Tetranychus urticae* adult females at 10 % concentration at different counting times (hour).

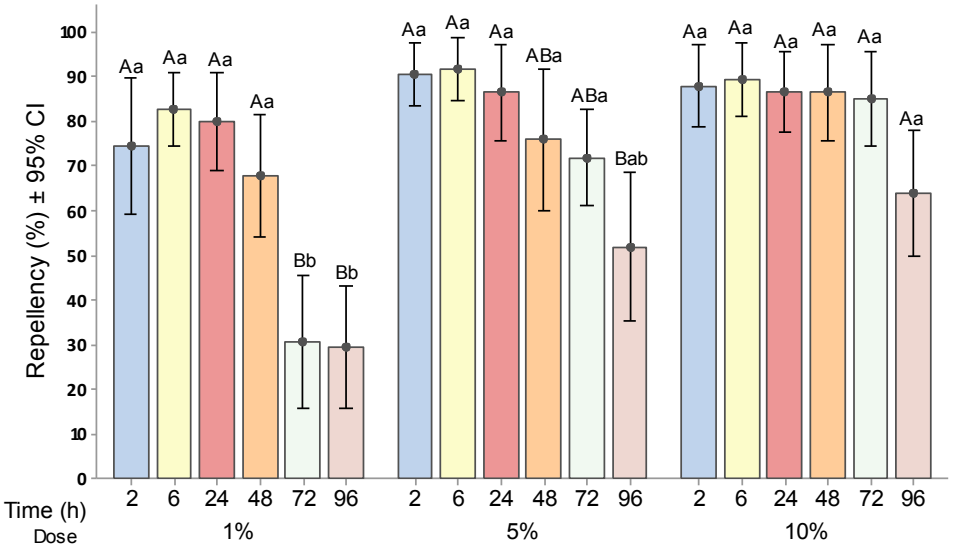

Figure 4. The repellent effects (Mean ± 95 % CI) of the extracts of different parts of *Prunus laurocerasus* against *Tetranychus urticae* adult females at different counting times and concentrations. Different upper letters represent statistically differences between times in the same dose and different lower letters represent statistically significant differences between doses in the same time according to Tukey's test (P<0.05) (CI: Confidence Interval).

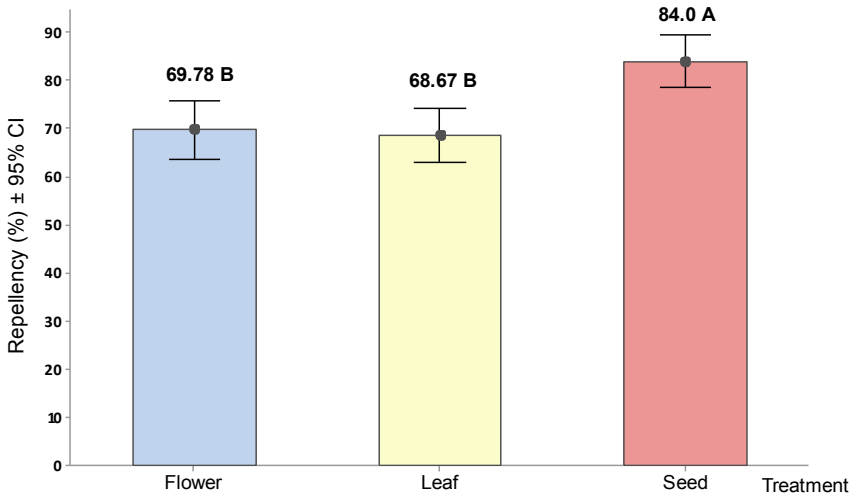

Figure 5. The repellent effects (Mean ± 95 % CI) of leaf, flower and seed extracts of *Prunus laurocerasus* against *Tetranychus urticae* adult females. Different upper letters represent statistically differences between treatment according to Tukey's test (P<0.05) (CI: Confidence Interval).

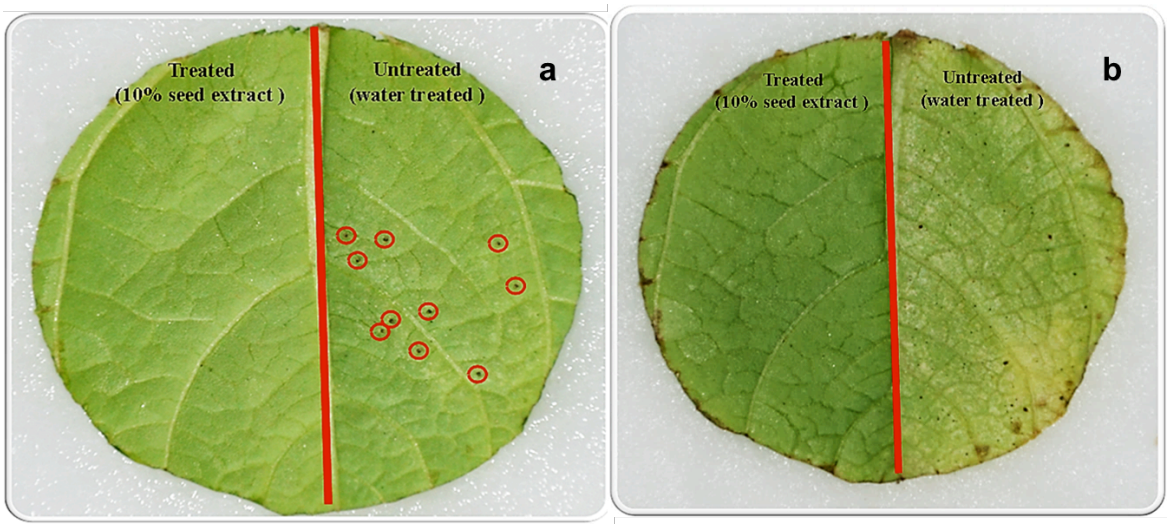

Figure 6. The repellent effect of a 10% *Prunus laurocerasus* seed extract on adult females of *Tetranychus urticae* (shown in circles) after 72 hours (a) (There is no mites on half of the leaf disc treated with a 10% seed extract), and feeding damage only on the untreated half of the leaf disc after 6 days (b).

High repellent effect of *P. laurocerasus* may be explained by the cyanide content of the plant based on EMEA (2000) and Dursun (2010) giving the content of this plant. According to Selmar (2010), some repellent effects were detected for a few intact cyanogenic glucosides; in most cases, the repellent effect for herbivores was due to the HCN liberated from cyanogenic glucosides. Moreover, carbonyl compounds produced during cyanogenesis, e.g. benzaldehyde, were also deterrents. Consequently, the repellent effect of cyanogenic plant is attributed mainly to the process of cynogenesis and formation of the decomposition products of cyanogenic glucosides (Selmar, 2010).

Although there is no research for repellent effect of *P. laurocerasus* extracts on phytophagous mites, some researchers investigated repellent efficacy of other plant extracts. Saber (2004) found that *Artemisia monosperma* Del. (Asteraceae) had repellency effects against females of *T. urticae*. The investigation of Antonious et al. (2006) suggests that methanolic extracts from accessions PI-596057

(*Capsicum baccatum* L.), PI-195299 (*C. annuum* L.), and Grif- 9270 (*C. annuum*) (Solanaceae) may have a great potential for repelling *T. urticae*. Antonious & Snyder (2006) found that the hexane leaf extracts of the *Lycopersicon hirsutum f. glabratum* Mueller, C.H. (Solanaceae) accessions (PI-251304, PI-134417, PI-134418, and PI-126449) exhibited strong repellency. Dabrowski & Seredynska (2007) showed the repellent action by water extract from *A. sativum*, *U. dioica*. and *S. alba* for *T. urticae*. Kumral et al. (2010) found repellent activities of the ethanol extracts obtained from both leaf and seed in the *D. stramonium* against adult *T. urticae*. El-Sharabasy (2010) evaluated the potential of crude extracts of *A. judaica* L. for repellent effect against adult females and immature stage of *T. urticae.* They found ethanolic leaf extraction was more effective as repellent effect against adult females and immature stage of *T. urticae*, followed by acetone, petroleum ether and aqueous extraction. Motazedian et al. (2012) found that *M. longifolia*, *S. officinalis* and *M. communis* essential oils have repellency effect against *T. urticae*.

The results also showed that at concentrations of 1%, the ovicidal effects of leaf and flower extracts were 12.59 % and 13.77 %, respectively (Table 1). The ovicidal effect was 24.37 % at the same concentration of seed extract. At concentrations of 5 % of the leaf, flower and seed extracts, the ovicidal effect was 24.41 %, 28.01 % and 40.37 %, respectively. Increasing the extract concentration by 10 % in leaf, flower and seed extracts increased their ovicidal effects to 55.57 %, 79.22 % and 96.56 %, respectively. We have demonstrated that the significantly high levels of egg mortality (96.56%) were caused by the seed extract of *P. laurocerasus* at 10 % concentration. During a 10 day observation period after treatment, the unhatched eggs treated with seed extract (5 % and especially 10 %) lose their original shape, then became orange in color (Fig. 7).

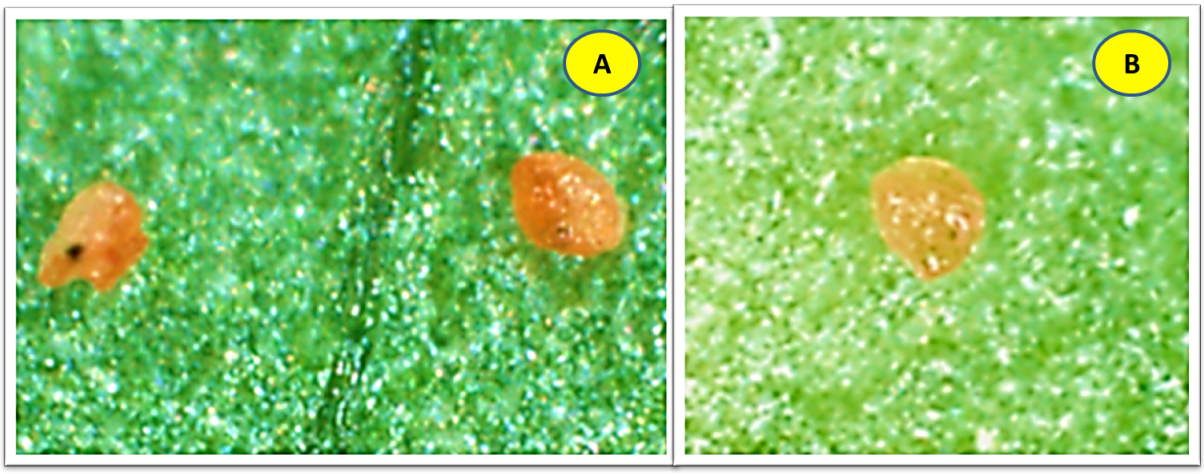

Figure 7. Ten days after treatment, unhatched *Tetranychus urticae* eggs treated with seed extracts at 10% (A) and %5 (B) concentrations.

The tests also showed that the seed extract is the most potent giving a LC_{50} and a LC_{90} of 4.5 % and 9.4 %, respectively followed by the flower extract with a LC_{50} and a LC_{90} of 6.6 % and 12.2 %, respectively and finally by the leaf extract with a LC_{50} and a LC_{90} of 8.9 % and 17.3 %, respectively against *T. urticae* eggs (Table 3).

Although there is no research about ovicidal effect of *P. laurocerasus* extracts on mites, Gençsoylu (2007) showed that leaf and root extracts from *Asphodelus aestivus* Brot. (Asphodelaceae) inhibit the egg hatching of *Tetranychus cinnabarinus* Boisduval (Prostigmata: Tetranychidae). Methanol extracts of nine plant species were evaluated for their ovicidal activity against *T. urticae* in a bioassay under laboratory conditions by Yanar et al. (2011b). They found that leaf and flower bud extracts of *Eucalyptus camaldulensis* Dehnh. (Myrtaceae) exhibited 63.26 % and 43.46 % mortality, respectively on eggs of two-spotted spider mite at 10 % extract concentration. *Xanthium strumarium* L. (Asteraceae) fruit and leaf extract caused 59.64 % and 57.45 % egg mortality, respectively in the same study. Also, Ghaderi et al. (2013) observed that the ovicidal activity of methanolic extracts of *S. meifolia*, *A. orientale*, *T. elliptica* and *P. viscosa* against *T. urticae* eggs were 45.84 %, 41.40 %, 40.11 % and 37.66 %, respectively.

Table 1. Ovicidal effect of different concentration of leaf, flower and seed extracts of *Prunus lausocerasus* against *Tetranychus urticae* eggs after a 10 days

Treatment	Dose (%)	Mean Ovicidal Effect (%)		±SE
Flower	1	13.77	aBC	8.29
	5	28.01	aB	5.49
	10	79.22	abA	2.71
	Control	7.20	aC	0.09
Leaf	1	12.59	aBC	6.55
	5	24.41	aB	6.07
	10	55.57	bA	9.05
	Control	7.20	aC	0.09
Seed	1	24.37	aBC	7.09
	5	40.37	aB	4.43
	10	96.56	aA	0.86
	Control	7.20	aC	0.09

Different lower letters represent statistically differences between extracts in the same dose and different upper letters represent statistically differences between doses in the same extract according to Tukey's test ($P<0.05$) (SE: Standard Error)

In agreement with Salman et al. (2013), it was observed that the percentage effect values of the extracts on *T. urticae* adults increased depending on the increases of counting time and concentration (Table 2). The significantly highest mortality rate (100%) occured at the 10 % concentration of the seed extract after 72 hours in all extracts. The effect of the flower and leaf extract were 72.50 % and 37.50 %, respectively at same dose and time. As a result, it can be said that the especially seed and flower extracts were seen to be more effective than the leaf extracts against *T. urticae* adult females.

LC_{50} values at 1, 24, 48 and 72 h exposure of to the seed extract were lower than flower and leaf extract (Table 3). At the same time, LC_{90} values at 1, 24, 48 and 72 h exposure to the seed extract were lower than flower and leaf extract too (Table 3). The datas obtained showed that the seed extracts of *P. laurocerasus* were most toxic against adult females.

In parallel with the result of our study, some aqueous extracts have acaricidal effect on *T. urticae.* Aqueous extracts from three plant species (*S. alba*, *A. sativum*, *U. dioica*) were tested against *T. urticae* by Dabrowski & Seredynska (2007). The high and significant mortality of *T. urticae* females were observed on leaves treated by *U. dioica.* After 6 days, mortality increased to 87 % and 96 %, at the 0.3 % and 0.5 % concentrations respectively. *Alium sativum* extracts caused only 48-57 % mite mortality and only between 34-41 % by *S. alba* extracts. El-Sharabasy (2010) evaluated the potential of water extract of *A. judaica* L. for toxic effect against adult females of *T. urticae.* They found that LC_{50} value was 103.2 gm/ml, after 72 hours. Almansour & Akbar (2013) investigated the toxic effect of aqueous solvent of *N. tabacum* and *P. harmala* on larval, nymphal and adult stages of *T. utricae*. The mortality rate varied among 20.1 - 88.2, 18.2 - 83.3, 17.7 - 82.3 % in *N. tabacum* while, in *P. harmala* it varied among 12.7 - 77, 13.7 - 75.8, 11.8 - 73.5 % in larval, nymphal and adult stages, respectively.

Table 2. The contact toxicity of different concentrations of leaf, flower and seed extracts of *Prunus laurocerasus* on *Tetranychus urticae* adult females at different counting times

		Contact Toxicity								
Time (h)	Dose (%)	Flower			Leaf			Seed		
		Mean (%)		±SE	Mean (%)		±SE	Mean (%)		±SE
1	0	0.00	Aa ii	0.00	0.00	Aa ii	0.00	0.00	Ba ii	0.00
	1	0.00	Aa ii	0.00	0.00	Aa iii	0.00	0.00	Ba ii	0.00
	2.5	2.00	Aa iii	2.00	4.00	Aa ii	2.45	2.00	Ba ii	2.00
	5	2.00	Aa iii	2.00	4.00	Aa ii	2.45	2.00	Ba iii	2.00
	7.5	6.00	Aa iii	4.00	12.00	Aa ii	5.83	24.00	Ba iii	6.78
	10	16.00	Ab iii	5.10	12.00	Ab ii	2.45	82.00	Aa i	5.83
24	0	2.00	Ba ii	2.00	2.00	Aa ii	2.00	2.00	Ca ii	2.00
	1	4.00	Ba ii	2.45	2.22	Aa iii	2.22	8.00	BCa i,ii	2.00
	2.5	8.00	Aba ii,iii	3.74	4.00	Aa ii	2.45	8.00	BCa ii	3.74
	5	10.22	Aba ii,iii	3.17	8.22	Aa ii	2.07	12.22	BCa ii,iii	1.96
	7.5	14.22	Aba iii	5.06	18.22	Aa i,ii	3.63	30.20	Ba ii,iii	11.3
	10	28.22	Ab ii,iii	5.66	18.44	Ab ii	5.89	87.78	Aa i,ii	1.96
48	0	8.00	Ba i,ii	2.00	8.00	Aa i,ii	2.00	8.00	Ca i,ii	2.00
	1	12.67	Aba ii	5.89	6.22	Aa ii	4.06	17.78	Ca ii	5.67
	2.5	17.33	ABab ii	2.57	2.22	Ab ii	2.22	23.78	BCa i	3.84
	5	17.33	Aba ii	2.57	4.44	Aa ii	2.72	16.89	Ca ii	5.78
	7.5	30.44	ABab ii	4.12	15.11	Ab ii	4.30	43.56	Ba ii	6.15
	10	34.67	Ab ii	3.76	24.00	Ab i,ii	4.24	93.56	Aa i,ii	2.64
72	0	18.00	Ba i	5.83	18.00	Aai	5.83	18.00	Da i	5.83
	1	28.61	Bab i	6.33	19.72	Abi	4.90	43.06	Ca i	3.78
	2.5	37.50	Ba i	6.43	21.39	Aa i	3.50	37.22	CDa i	7.11
	5	68.33	Aa i	3.89	23.61	Ab i	5.52	69.40	Ba i	10.10
	7.5	68.06	Aa i	2.56	30.6	Ab i	12.8	69.44	Ba i	5.27
	10	72.50	Ab i	3.30	37.50	Ac i	6.43	100.00	Aa i	0.00

Different upper letters represent statistically differences between doses in the same time and the same extract, different lower letters represent statistically differences between extracts in the same time and the same dose and different Roman Numerals (i, ii, iii) represent statistically differences between times in the same dose and the same extract according to Tukey's test ($p<0.05$)
Three-way interaction between dose: extract: time is significant ($p<0.05$) (SE: Standard Error)

Table 3. LC_{50} and LC_{90} values (with 95 % CI) of flower, leaf and seed extracts of *Prunus laurocerasus* against *Tetranychus urticae* adult females after 1, 24, 48, 72 h and *Tetranychus urticae* eggs after a 10 days

Activity	Treatment	LC_{50} (%)	95% CI for LC_{50}	LC_{90} (%)	95% CI for LC_{90}	Slope ± SE	χ^2
Ovicidal	Flower	6.6	(0.059-0.073)	12.2	(0.110-0.140)	22.86 ± 2.55	80.08
	Leaf	8.9	(0.073-0.117)	17.3	(0.137-0.263)	15.27 ± 3.18	23.01
	Seed	4.5	(0.036-0.054)	9.4	(0.081-0.114)	26.47 ± 3.47	58.08
Contact	Treatment	LC_{50} (%)	95% CI for LC_{50}	LC_{90} (%)	95% CI for LC_{90}	Slope ± SE	χ^2
1 h	Flower	15.7	(0.125-0.277)	22.8	(0.170-0.450)	18.03 ± 5.45	10.95
	Leaf	18.7	(0.136-0.460)	29.2	(0.199-0.804)	12.17 ± 4.31	7.96
	Seed	8.5	(0.074-0.100)	11.1	(0.097-0.149)	50.36 ±13.14	14.70
24 h	Flower	17.9	(0.128-0.503)	30.8	(0.203-0.990)	9.97 ± 3.75	7.07
	Leaf	14.9	(0.119-0.268)	22.7	(0.168-0.467)	16.53 ± 5.16	10.25
	Seed	7.8	(0.071-0.086)	11.8	(0.106-0.142)	31.88 ± 5.40	34.85
48 h	Flower	14.5	(0.104-0.444)	29.9	(0.193-1.126)	8.35 ± 3.30	6.38
	Leaf	13.9	(-)	20.9	(-)	18.13 ± 9.86	3.38
	Seed	6.7	(0.058-0.076)	11.9	(0.104-0.146)	24.58 ± 3.93	39.09
72 h	Flower	4.0	(0.018-0.055)	13.9	(0.109-0.214)	12.95 ± 3.03	18.33
	Leaf	12.8	(0.093-0.406)	26.5	(0.172-1.114)	9.37 ± 3.86	5.89
	Seed	2.9	(0.014-0.039)	9.1	(0.077-0.117)	20.69 ± 3.61	32.90

SE: Standard Error CI: Confidence Interval h: Hour

In the present study, it was determined over 50 % ovicidal activity at 10% concentrations of leaf, flower and seed extracts. Additionaly, at the same concentrations of the leaf, flower and seed extracts, the toxic effects on *T. urticae* adult females were 37.50 %, 72.50 % and 100%, respectively. The high acaricidal effect of *P. laurocerasus* may be explained by high concentrations of cyanogenic glycosides such as amygdalin, prunasin based on Poulton & Li (1994). EMEA (2000), Dursun (2010) and Wishhart & Media (2014) giving informations about the content of this plant. Acording to EMEA (2000) the leaves of *P. laurocerasus* contain cyanogenic glycosides. Poulton & Li (1994) indicated that *Prunus* seeds contain the cyanogenic diglucoside (R)-amygdalin. Whereas there is no research about cyanogenic glycosides content of *P. laurocerasus* flowers, Wishhart & Media (2014) reported that all parts of the plant contain hydrogen cyanide, also known as prussic acid. The toxicity of the plant is attributed to hydrocyanic acid, a poison that gives almonds their characteristic flavor, liberated from cyanogenic glycosides (EMEA, 2000; Ubalua, 2010). Acording to Robertson (2014), this toxin is found mainly in the leaves and seeds. When the leaves and seeds of the plant distilled with water, as it was done in the present study, yield a distillate of hydrocyanic acid (Felter & Lloyd, 1898).

Seed extract had the highest contact and ovicidal effect in the extracts. We believe that the differences among the effects of the leaf, flower and seed extracts of *P. laurocerasus* may be attributed to the difference in their chemical composition. Our hypothesis is supported by Dursun (2010) who reported that while mean amygdalin, HCN contents in the leaves of *P. laurocerasus* were 0.28, 0.32 g/kg respectively, mean amygdalin and HCN contents in its seeds were 94.35 and 5.64g/kg, respectively.

When all data were compared, it can be concluded that the ovicidal effect of seed extract and the contact toxicity of flower and especially seed extracts of *P. laurocerasus* at 10 % concentration has potential to be used in the control of two-spotted spider mite eggs and adults. Additionally, the repellent effect of seed extracts of *P. laurocerasus* was found to be promising for practical application.

Acknowledgements

We are thankful to Allan LISLE (School of Agriculture and Food Sciences, University of Queensland, Gatton, Australia) and Yeliz KASKO ARICI (Department of Biometry and Genetics, Ordu University, Ordu, Turkey) for their advice and guidence provided for the satistical analyses. Also, we would like to thank the anonymous reviewers for their constructive comments.

References

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

Almansour, N. A. & M. M. Akbar, 2013. The effect of some plant extracts in biology of *Tetranychus urticae* (Acarina: Tetranychidae). Journal of Purity, Utility Reaction and Environment, 2 (6): 153-159.

Antonious, G. F. & J. C. Snyder, 2006. Natural products: Repellency and toxicity of wild tomato leaf extracts to the two-spotted spider mite, *Tetranychus urticae* Koch. Journal of Environmental Science and Health, 41: 43-55.

Antonious, G. F., J. E. Meyer & J. C. Snyder, 2006. Toxicity and repellency of hot pepper extracts to spider mite, *Tetranychus urticae* Koch. Journal of Environmental Science and Health, 41: 1383-1391.

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

Chiasson, H., A. Be´langer, N. Bostanian, C. Vincent & A. Poliquin, 2001. Acaricidal properties of *Artemisia absinthium* and *Tanacetum vulgare*n (Asteraceae) essential oils obtained by different methods of extraction. Journal of Economic Entomology, 94: 167-171.

Dabrowski, T. Z & U. S. Seredynska, 2007. Characterisation of the two-spotted spider mite (*Tetranychus urticae* Koch, Acari: Tetranychidae) response to aqueous extracts from selected plant species. Journal of Plant Protection Research, 47: 113–124.

Derbalah, A. S., A. Y. Keratrum, M. E. El-Dewy & E. H. El-Shamy, 2013. Efficacy of some insecticides and plant extracts against *Tetranychus urticae* under laboratory conditions. Egyptian Journal of Plant Protection Research, 1 (3): 47-69.

Dursun, S., 2010. Determination of Cynanogenic Compounds (Amiygdaline and Prunasin) in Cherry laurel (*Prunus laurocerasus* L.). Ordu University, (Unpublished) Master's Thesis, Ordu, 44 pp.

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

El-Sharabasy, H. M., 2010. Acaricidal activities of *Artemisia judaica* L. extracts against *Tetranychus urticae* Koch and its predator *Phytoseiulus persimilis* Athias-Henriot (Tetranychidae: Phytoseiidae). Journal of Biopesticides, 3 (2): 514-519.

EMEA, 2000. *Prunus laurocerasus* summary report. (Web page: http://www.ema.europa.eu/docs/en_ GB/document_library/Maximum_Residue_Limits_-_Report/2009/11/WC500015796.pdf) (Date accessed: May 2015).

Erdoğan, P., A. Yıldırım & B. Sever, 2012. Investigations on the effects of five different plant extracts on the Two-Spotted Mite, *Tetranychus urticae* Koch. (Arachnida: Tetranychidae). Psyche, 2012: 1-5.

Ertürk, Ö., Ö. Kara, E. Sezer & G. Şan, 2004. Toxicity effect of some plant extracts on development of larvae of *Plutella xylostella* (L.) (Lepidoptera; Plutellidae). Ecology, 13 (50): 18-22.

FAO, 2004. Resistance management and integrated para- sites control in ruminants-guidelines. Module 1: Ticks: Acaricide Resistance, Diagnosis, Management and Pre- vention, Food and Agriculture Organization, Animal Pro- duction and Health Division, Rome, 25 pp.

Felter, H. W. & J. U. Lloyd, 1898. The leaves of *Prunus laurocerasus* Linne (*Cerasus laurocerasus*, Lois). (Web page: http://www.henriettes-herb.com/eclectic/kings/prunus-laur.html) (Date accessed: May 2015).

Gençsoylu, I., 2007. Effect of *Asphedolus aestivus* Brot. as a botanical acaricide against *Tetranychus cinnabarinus* Boisd. (Acari: Tetranychidae). International Journal of Agricultural Research, 2: 189−192.

Ghaderi, S., K. Minaei, V. Rowshan & G. Mohammad, 2013. Toxicity and ovicidal activity of different plant extracts on two-spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). Archiv für Phytopathologie und Pflanzenschutz, 46 (1): 120-126.

Islam, M. T., M. M. Haque, N. Naher & S. Parween, 2008. Effect of plant materials on developmental periods of two spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae). International Journal of Biological Sciences, 16: 121-124.

Kovach, J. & C. Gorsuch, 1986. Response of the twospotted spider mite, *Tetranychus urticae* Koch, to various insecticides and fungicides used in south Carolina peach orchards. Journal of Agricultural and Urban Entomology, 3(2): 175-178.

Kumral, N.A., S. Çobanoğlu & C. Yalçın, 2010. Acaricidal, repellent and oviposition deterrent activities of *Datura stramonium* L, against adult *Tetranychus urticae* (Koch). Journal of Pest Science, 83: 173–180.

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

Momen, F. M., A. S. Reda & A. Amer, 1997. Effect of Neem Azal-F on *Tetranychus urticae* and three predacious mites of the family Phytoseeidae. Acta Phytopathologica et Entomologica Hungarica, 32: 355-362.

Motazedian, N., S. Ravan & A. R. Bandani, 2012. Toxicity and repellency effects of three essential oils against *Tetranychus urticae* Koch (Acari: Tetranychidae). Journal of Agricultural Science and Technology, 14: 275-284.

Obeng-Ofori, D., C. Adler & C. Reichmuth, 1997. Toxicity and repellency of 1,8-cineole, eugenol and camphor against stored product insects. Mıtteılungen Der Deutschen Gesellschaft Fuer Allgemeıne Und Angewandte Entomologie, 11 (1-6): 259-264.

Poulton, J. E. & C. P. Li, 1994. Tissue level compertmention of (R)- amygdalin and amygdalin hydrolase prevents large- scale cyanogenesis in undamaged *Prunus* seeds. Plant Physiology, 104 (1): 29-35.

Rattan, R. S. & A. Sharma, 2011. Plant secondary metabolites in the sustainable diamondback moth (*Plutella xylostella* L.) management. Indian Journal of Fundamental and Applied Life Sciences, 1 (3): 295-309.

Robertson, J., 2014. *Prunus laurocerasus*, Cherry Laurels. (Web page: http://www.thepoisongarden.co.uk/atoz/prunus_laurocerasus.htm) (Date accessed: May 2015).

Saber, S. A., 2004. Influence of *Artemisia monosperma* Del. extracts on repellency, oviposition deterrence and biological aspects of the two-spotted spider mite *Tetranychus urticae* Koch. Egyptian Journal of Pest Control, 14: 34 -348.

Salman, Y.S., S. Saritas, N. Kara & R. Ay, 2013. Acaricidal and ovicidal effects of Sage (*Salvia officinalis* L.*)* and Rosemary (*Rosmarinus officinalis* L.) (Lamiaceae) extracts on *Tetranychus urticae* Koch (Acari: Tetranychidae). The Journal of Agricultural Science, 20: 358-367.

Selmar, D., 2010. Biosynthesis of cyanogenic glycosides, glucosinolates and non-protein amino acids. Annual Plant Reviews, 40 (3): 92–181.

Smith, C. M., 1989. Plant Resistance to Insects. A Fundamental Approach. New York, John Wiley and Sons, 286 pp.

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

Thomas, P., 2004. “Three Defences, 417-421”. In: The overstory book, Cultivating connections with trees 2 nd edition (Eds: C.R. Elevitch). Permanent Agriculture Resources, Holualoa, Hawai, USA, 526 pp.

Tomczyk, A. & M. Suszko, 2011. The role of phenols in the influence of herbal extracts from *Salvia officinalis* L. and *Matricaria chamomilla* L. on two-spotted spider mite *Tetranychus urticae* Koch. Biological Letters, 48 (2): 193–205.

Ubalua, A. O., 2010. Cyanogenic glycosides and the fate of cyanide in soil. Australian Journal of Crop Science, 4(4): 223-237.

Wang, Y., G. Shi, L. Ma, Y. Liu & Y. Wang, 2012. Studies on action mechanism of acrocidal activity of extracts of *Pharbitis purpurea* seeds against *Tetranychus cinnabarinus*. Information Technology and Agricultural Engineering, 134: 629-641.

Wishhart, M. & D. Media, 2014. Poisonous Cherry Laurel Tree, Acaricidal, repellent and oviposition deterrent activities of *Datura stramonium* L, against adult *Tetranychus urticae* (Koch). (Web page: http://homeguides.sfgate.com/poisonous-cherry-laurel-tree-67400.html) (Date accessed: May 2015).

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

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

Zhang, Q., L. Ding, M. Li, W. Cui, W. Ding, J. Luo & Y. Zhang, 2013. Action modes of *Aloe vera* L. extracts against *Tetranychus cinnabarinus* Boisduval (Acarina: Tetranychidae). Agricultural Sciences, 4 (3): 117-122.

Türk. entomol. derg., 2015, 39 (4): 381-392 ISSN 1010-6960
DOI: http://dx.doi.org/10.16970/ted.89501

Orijinal araştırma (Original article)

Contribution to the knowledge of the Notodontidae (Lepidoptera) of Turkey[1]

Notodontidae familyasının (Lepidoptera) Türkiye'deki durumuyla ilgili bilgiye katkı

Feza CAN CENGİZ[2*] Başak ULAŞLI[2] Alexander SCHINTLMEISTER[3]

Summary

The aim of this paper is to summarize our knowlegde of the recent distribution and systematic classification of the Notodontidae (Lepidoptera) occuring in 23 provinces located in the central and eastern Anatolian, Mediterranean and Black Sea regions of Turkey, based on recordsaccrued during the years 1968-2015. In this study, 93 notodontid specimens from seven collections were examined. A total of 29 species belonging to 17 genera and representing six subfamilies of Notodontidae were determined. The detailed study of specimens of the Notodontidae from four different regions of Turkey shows that *Stauropus fagi* (Linnaeus, 1758) is a new record for the Mediterranean region of Turkey.

Keywords: Notodontidae, Lepidoptera, new record, fauna, Turkey

Özet

Çalışmanın amacı 1968-2015 yılları süresince, Türkiye'nin Orta ve Doğu Anadolu, Akdeniz ve Karadeniz Bölge'lerinde 23 ilde belirlenen Notodontidae (Lepidoptera) familyasına giren türlerin, güncel yayılış alanları ve sınıflandırılmaları hakkındaki bilgileri özetlemektir. Bu çalışmada, yedi koleksiyondan 93 notodontid bireyi incelenmiştir. Toplam olarak, Notodontidae familyasının, altı alt familyasına bağlı 17 cinse ait 29 tür belirlenmiştir. Türkiye'nin dört farklı bölgesinden Notodontidae familyası türlerinin incelendiği bu ayrıntılı çalışma ile *Stauropus fagi* (Linnaeus, 1758) Türkiye'nin Akdeniz Bölgesi için yeni kayıt olarak belirlenmiştir.

Anahtar sözcükler: Notodontidae, Lepidoptera, yeni kayıt, fauna, Türkiye

[1] This study was submitted and published as an abstract at the XIX. European Congress of Lepidopterology (27 September- 2 October Radebeul-Dresden, Germany).

[2] Mustafa Kemal University, Agricultural Faculty, Deparment of Plant Protection, Entomology Section 31040 Hatay-Turkey
[3] Calberlastr. 3, 01326 Dresden-Germany
* Sorumlu yazar (Corresponding author) e-mail: cezafan_onurcan@hotmail.com
Alınış (Received): 15.12.2015 Kabul ediliş (Accepted): 07.01.2016 Çevrimiçi Yayın Tarihi (Published Online): 18.01.2016

Introduction

The Noctuoidea is the most species-rich superfamily in the Lepidoptera. The Notodontidae or Prominent Moths which forms part of the superfamily Noctuoidea contains nearly 3.000 described species and currently is known world-wide except in the Arctic regions and New Zeland (Schintlmeister, 2008). Most of them occur in the Neotropical region (Heppner, 1991; Müller et al., 2005). 209 species are recorded from the Palaearctic region (Heppner, 1991; Müller et al., 2005) of which 48 species are found in Europe and North Africa; their larvae usually feed on trees and bushes, and often are oligophagous or even monophagous (De Freina & Witt, 1987). Schintlmeister (2008) contended in his book on Notodontidae-Palaearctic Macroheterocera that 716 species belongs to ten subfamilies in the Palearctic region.

The state of Turkey extends from Asia into Europe and is one of the most species-rich countries in the western Palaearctic region. Unfortunately, previous studies on the biodiversity of Turkey have only been a few. Determination of moth species, their biology and distribution, therefore, are crucial for Turkey's faunistic studies. Like many other species, some notodontid species are facing extinction due to global climate change and human activities that are destroying their habitats, in particular deforestration. However, Schintlmeister (1986 & 1988) showed that notodontids are able to adapt well to urbanized habitats, such as parks and gardens in cities and villages. Studies on the Turkish Notodontidae, on the other hand are available in limited numbers. But other faunistic and systematic studies and lists of species recorded in Turkey includes the Notodontidae (De Freina, 1979, 1981 & 1983; Okyar (Göbekçioğlu) & Aktaç, 1997; Okyar & Aktaç, 2007; Koçak & Kemal, 2007; Baron, 2008; Schintlmeister, 2008; Okyar et al., 2009; Beşkardeş, 2012). But none of these studies specifically targeted the Notodontidae in Turkey, although they partly included some of the species.

The study area, Turkey, was divided into seven regions including the Black Sea region based on climatic parameters, location, topography, flora and fauna etc. The Black Sea region has a steep, rocky coast with rivers that cascade through the gorges of the coastal mountain ranges. The southern slopes are mostly unwooded, but the northern slopes are covered in dense deciduous and evergreen trees. Access to the inland from the coast is limited to a few narrow valleys because the mountain ridges form, with elevations of 1,525 to 1,800 m in the west and 3,000 to 4,000 m in the east in the Kackar Mountains, an almost unbroken wall separating the coast from the interior (Can, 2008). The higher slopes facing northwest tend to be densely forested. Because of these natural conditions, the Black Sea coast has been isolated from Anatolia for a very long time. Due to the rainy and temperate climate, the dominant plant cover in the Black Sea region is forest consists of beech (*Fagus* spp.) (Fagaceae), oak (*Quercus* spp.) (Fagaceae), hornbeam (*Carpinus* spp.) (Betulaceae), black pine (*Pinus* spp.) (Pinaceae), and fir (*Abies* spp.) (Pinaceae) are observed at the upper elevations from 600-700 m altitude above sea level (Can, 2008). In addition, hazel nut shrubs are very widely distributed and common in the region (Can, 2008).

The Mediterranean climate prevails at lower elevations, whilst the higher elevations are characterized by Mediterranean mountain range climate (Aytaç et al., 2012). The biodiversity of the Taurus and Amanos mountains is notably rich as a consequence of all the above mentioned geological and climatical diversity. These mountains, situated in the eastern Mediterranean region of Turkey, rise sharply from sea level and have topographical, geological and geomorphological features which support a high rate of endemism and a large amount of still ongoing speciation (Aytaç et al., 2012; Özkoçak, 1993). There are maquis shrubs and pine forests up to 1,000 m elevation and above that forests of larch (*Larix*

spp) (Pinaceae), cedar (*Cedrus* spp.) (Pinaceae) and fir trees can be observed. Furthermore, the Amanos mountain range is the southernmost point where beech forests occur (Ezer, 2008; Anonim, 2007; Aytaç, 2010; Aytaç & Semenderoğlu, 2012).

Turkey is situated in a large Mediterranean geographical location where climatic conditions are quite temperate and the diverse nature of the landscape and the existence in particular of the mountains that run parallel to the coasts, result in significant differences in climatic conditions from one region to the other. While the coastal areas enjoy milder climates, the inland Anatolian plateau experiences extremes of hot summers and cold winters with limited rainfall (Şensoy et al., 2015). The central Anatolian region occupies the area between the two zones of the folded mountains, extending eastward to the point where the two ranges converge. The plateau-like, semi-arid highlands of Anatolia are considered to be the heartland of the country. The region varies in elevation from 600 to 1,200 m from west to east. The western parts of Anatolia, often consist of black pine (*Pinus nigra* Arnold) (Pinaceae), in the east nearly exclusively Scot's pine (*P. sylvestris* L.) (Pinaceae). Penetrating further into the central parts of inner Anatolia leads to still dryier and cold winter conditions. Today the lower parts of central Anatolia are virtually treeless. Eastern Anatolia, where the Pontic and Anti-Taurus mountain ranges converge, is rugged country with higher elevations, a more severe climate, and greater precipitation than can be found on the Anatolian Plateau. The western part of the eastern Anatolian region is known as the Anti-Taurus, where the average elevation of mountain peaks exceeds 3,000 m. *P. sylvestris* is the dominant tree in the dry and cold areas of north-eastern Anatolia.

Materials and Methods

The examined material originates from seven different collections and was made available by museum curators as well as by private collections.

Abbreviations

CASD	Private collection Alexander Schintlmeister, Dresden, Germany
NTM	Nazife Tuatay Plant Protection Museum, Directorate of Plant Protection Central Research Institute, Ankara, Turkey
MMKU	Museum of Mustafa Kemal University, Hatay, Turkey
MTU	Museum of Trakya Universitesi, Faculty of Science, Biology Department, Edirne,Turkey
NHM	Natural History Museum, Vienna, Austria
MWM	Museum Witt, Munich, Germany
ZFMK	Zoological Research Museum Alexander Koenig, Bonn, Germany

A list of examined material is given in the appendix. Most of the specimens were collected by the authors in different localities in Turkey using light-traps at different elevations with different climatic conditions, plant cover and surface features in Çankırı, Kırıkkale, Ankara, Konya, Kayseri, Nevşehir, Hakkari, Ağrı, Erzincan, Kars, Tunceli and Erzurum provinces that are located in central and eastern Anatolia; in Hatay, Adana, Osmaniye and Antalya in the Mediterranean region and Çorum, Bolu, Trabzon, Ordu, Giresun, Rize and Samsun from the Black Sea region of Turkey and their villages and surroundings, and the Amanos, inner part of the Taurus and the eastern Black Sea mountains during the years 1968-2015. Some notodontid species were observed and photographed in the field. Their

identification and the terminology of morphological structures are based on Schintlmeister (2008). Moreover, the taxonomy and nomenclature follow Schintlmeister (2008) and Saldaitis et al. (2013). All specimens were dissected in the laboratory, with the genitalia embedded in Entellan on slides, following standard procedures.

Collection localities in 23 provinces of the four different regions in Turkey are coded as follows:

A- Mediterranean Region

A1 Hatay-Belen, **N:** 36º52' **E:** 36º15', 477m

A2 Hatay-Samandağ, **N:** 36º8' **E:** 35º59', 232m

A3 Adana-Kozan, Düzağaç, **N:** 37º34' **E:** 35º49', 564m

A4 Adana-Pozantı, **N:** 37º28' **E:** 34º54', 1120m

A5 Adana-Feke, Tenkerli, **N:** 37º44' **E:** 36º56', 741m

A6 Adana-Aladağ **N:** 37º 34' **E:** 35º23', 770m

A7 Osmaniye-Zorkun, Karacalar, **N:** 37º01' **E:** 36º16', 700m

A8 Osmaniye-Hınzırlı, **N:** 37º00' **E:** 36º27', 1504m

A9 Antalya 700 m

B- Central Anatolian Region

B1 Çankırı-Ilgaz, Kırkpınar, **N:** 41º 00' **E:** 33º 38', 1700m

B2 Çankırı-Eldivan, **N:** 40º30' **E:** 33º28', 1200m

B3 Çankırı-Eldivan-Kavak, **N:** 40º31' **E:** 33º30', 1000m

B4 Çankırı-Kenbağ, **N:** 40 º 38' **E:** 33º35', 750m

B5 Kırıkkale-Büyükyağlı, **N:** 39º56' **E:** 33º58', 700m

B6 Ankara-Köprüköy, 750m

B7 Ankara- Kızılcahamam, 950m

B8 Konya-Beyşehir, 1400m

B9 Kayseri, **N:** 38º28' **E:** 35º09', 1075m

B10 Nevşehir-Ürgüp, **N:** 38º34' **E:** 35º07', 1500m

C- Black Sea Region

C1 Çorum-Kargı, Saraycık Dağı, **N:** 41 º01' **E:** 35 º04', 1600m

C2 Bolu-Yedigöller, **N**: 40º56' **E:** 31º44'

C3 Bolu-Cepni, **N**: 40º39' **E:** 31º30', 800m

C4 Trabzon-Maçka, **N:** 40º 45' **E:** 39º37', 427m

C5 Trabzon-Maçka, Çamlıdüz, **N:** 40º42' **E:** 39º29', 1004m

C6 Trabzon-Maçka, Ormanüstü, **N:** 40º45' **E:** 39º28', 1561m

C7 Trabzon-Maçka, Zigana, 1500m

C8 Ordu-Perşembe, **N:** 41º06' **E:** 37º40', 300m

C9 Giresun

C10 Rize-İkizdere, **N:** 40º33' **E:** 40º46', 525m

C11 Rize-Ayder, **N:** 40º57' **E:** 41º05', 1350m

C12 Samsun-Çarşamba, **N:** 41º18' **E:** 36º72', 1350m

D- Eastern Anatolian Region

D1 Hakkari, 1350-1400m

D2 Hakkari-Mutluca, **N:** 37º29' **E:** 43º06', 2100m

D3 Ağrı, **N:** 39º47' **E:** 42º28', 2100m

D4 Erzincan, **N:** 39º34' **E:** 40º03', 1350m

D5 Kars-Sarıkamış, **N:** 42º35' **E:** 40º20', 2150m

D6 Tunceli, 1000m.

D7 Erzurum, 1850m

Results and Discussion

In the present study, the results of the identification of notodontid moth samples collected at 38 different localities in 23 provinces of the central and eastern Anatolia, Mediterranean and Black Sea regions of Turkey are presented. In all, 29 species belonging to 17 genera and representing six subfamilies were identified: Two genera and five species belong to the Cerurinae: *Cerura vinula vinula* (Linnaeus, 1758), *C. intermedia* (Teich, 1896), *Furcula furcula turcica* Schintlmeister, 1998, *F. bifida bifida* (Brahm, 1787); *F. interrupta interrupta* (Christoph, 1867) and *F. interrupta syra* (Grum-Grshimailo, 1899); three genera and three species belong to the Dicranurinae: *Dicranura ulmi* ([Denis & Schiffermüller], 1775), *Harpyia milhauseri* (Fabricius, 1775) and *Stauropus fagi fagi* (Linnaeus, 1758); five genera and 11 species belong to the Notodontinae: *Drymonia dodonaea wageneri* de Freina, 1981, *D. melagona esmera* de Freina, 1981, *D. querna djezina* Bang-Haas, 1937, *D. velitaris pontica* (Rebel, 1908), *Notodonta dromedarius pontica* Witt, 1980, *N. derbendica* Daniel, 1965, *N. tritopha irfana* de Freina, 1983, *Peridea anceps* (Goeze, 1781), *P. korbi korbi* (Rebel, 1918), *Pheosia tremula* Clerck, 1759, and *Paradrymonia vittata vittata* (Staudinger, 1892); three genera and three species belong to the Ptilodontinae: *Pterosoma palpina palpina* Clerck, 1759; *P. palpina pontica* Staudinger, 1901, *Ptilodon saerdabensis* (Daniel, 1938) and *Ptilophora plumigera* ([Denis & Schiffermüller], 1775); one genus and two species belong to the Phalerinae: *Phalera bucephala becephala* (Linnaeus, 1758) and *P. bucephaloides* (Ochsenheimer, 1810); and three genera and five species belong to the Pygaerinae: *Spatalia argentina* ([Denis & Schiffermüller], 1775), *Rhegmatophila alpina osmana* Friedel, 1967, *Clostera curtula curtula* (Linnaeus, 1758), *C. pigra staudingeri* Koçak, 1980, and *C. anastomosis* (Linnaeus, 1758). *Stauropus fagi* was reported as a species new for the fauna of the Mediterranean region of Turkey (Table 1.).

We compared the number of notodontid species recorded in different regions of Turkey in this study. Eleven species were identified from the Mediterranean region, 11 species from the central Anatolia, 12 species from the Black Sea region and nine species from eastern Anatolia (Figure 1.). The relatively higher number of species recorded in the Black Sea region is not surprising. The Black Sea coast is the only region of Turkey that receives high precipitation throughout the year. Because of the rainy and different climate zone of this region, the Black Sea coast has a special fauna and is isolated from the other regions in Turkey.

Table 1. Distribution of the notodontid species that identified in four different regions of Turkey. Asterix (*), means that first record of the Mediterranean region of Turkey

	A- Mediterranean	B- Central Anatolia	C- Black Sea	D- Eastern Anatolia
CERURINAE Butler, 1881				
1. *Cerura vinula vinula* (Linnaeus, 1758)	+	+		
2. *C. intermedia* (Teich, 1896)				+
3. *Furcula furcula turcica* Schintlmeister, 1998		+		+
4. *F. bifida bifida* (Brahm, 1787)	+		+	
5. *F. interrupta interrupta* (Christoph, 1867)			+	+
F. interrupta syra (Grum-Grshimailo, 1899)	+			
DICRANURINAE Duponchel, 1845				
6. *Dicranura ulmi* (Denis & Schiffermüller, 1775)		+		
7. *Harpyia milhauseri* (Fabricius, 1775)	+			
8. *Stauropus fagi fagi* (Linnaeus, 1758) *	+	+	+	
NOTODONTINAE Stephens,1829				
9. *Drymonia dodonaea wageneri* de Freina, 1981			+	
10. *D. melagona esmera* de Freina, 1981			+	
11. *D. querna djezina* Bang-Haas, 1937	+			
12. *D. velitaris pontica* (Rebel, 1908)			+	
13. *Notodonta dromedarius pontica* Witt, 1980				+
14. *N. derbendica* Daniel, 1965		+		
15. *N. tritopha irfana* de Freina, 1983				+
16. *Peridea anceps* (Goeze, 1781)	+			
17. *P. korbi korbi* (Rebel, 1918)	+			
18. *Pheosia tremula* Clerck, 1759	+			
19. *Paradrymonia vittata vittata* (Staudinger, 1892)				+
PTILODONTINAE Packard, 1864				
20. *Pterosoma palpina palpina* Clerck, 1759			+	
P. palpina pontica Staudinger, 1901		+		+
21. *Ptilodon saerdabensis* (Daniel, 1938)			+	
22. *Ptilophora plumigera* (Denis & Schiffermüller, 1775)				+
PHALERINAE Butler, 1886				
23. *Phalera bucephala bucephala* (Linnaeus, 1758)			+	
24. *P. bucephaloides* (Ochsenheimer, 1810)		+		+
PYGAERINAE Duponchel, 1845				
25. *Spatalia argentina* (Denis & Schiffermüller, 1775)	+	+	+	
26. *Rhegmatophila alpina osmana* Friedel, 1967	+	+	+	
27. *Clostera curtula curtula* (Linnaeus, 1758)		+		
28. *C. pigra staudingeri* Koçak, 1980		+		
29. *C. anastomosis* (Linnaeus, 1758)			+	

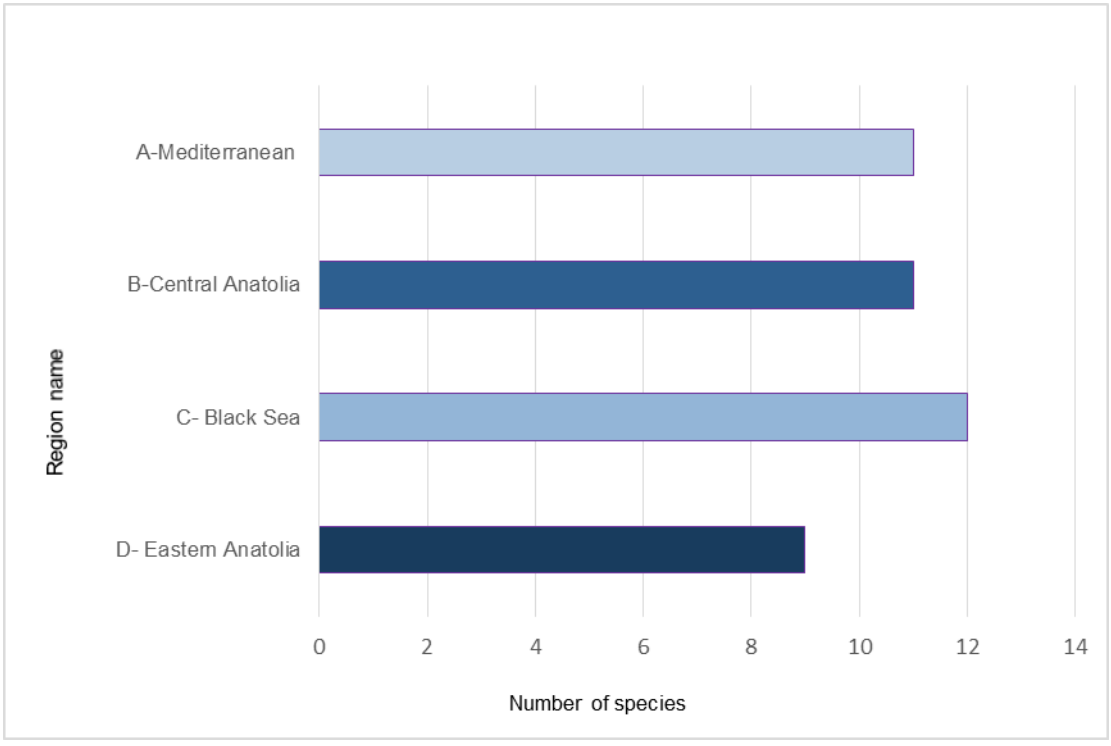

Figure 1. The number of notodontid species recorded in different regions of Turkey.

The present paper lists 17 genera and the maximum number of species belong to *Drymonia* Hübner, 1819, with four species and subspecies. *Drymonia dodonaea* ([Denis & Schiffermüller], 1775) is known from Europe, Caucasus and Scandinavia. *Drymonia dodonaea wageneri* de Freina, 1981, inhabits southern Turkey (Schintlmeister, 2008), *D. querna djezina* Bang-Haas, 1937, occurs in Turkey, Syria and Lebanon. The larval hostplants are *Quercus* spp. (Fagaceae) which are very common in Turkey (Müller et al., 2005). The distributional areas of *D. velitaris velitaris* (Hufnagel, 1767) and *D. velitaris pontica* (Rebel, 1908) are clearly different from each other. *D. velitaris velitaris* is distributed in France, central Europe and Italy; the other subspecies is *D. velitaris pontica* which occurs in north-eastern Turkey and the Caucasus. During the present study, this subspecies was recorded from the central Black Sea region of Turkey.

The genus *Furcula* Lamarck, 1816, was represented by three species and four subspecies in this paper. *F. furcula* (Clerck, 1759) is one of the most variable notodontids. *F. furcula turcica* Schintlmeister, 1998, is a small subspecies characterized by whitish coloured forewings mixed with greyish lemon yellow and its distributional area is restricted to Turkey (Schintlmeister, 2008). *F. bifida* comprises two closely related subspecies: *F. bifida bifida* (Brahm, 1787) is the predominant subspecies in the Palaearctic region and *F. bifida lype* (Seifers, 1933) occurs only in northern Fennoscandia (Schintlmeister, 2008). *F. interrupta* is larger and white coloured with a contrasting black pattern on the forewings; it is represented by three subspecies (Schintlmeister, 2008), viz *F. interrupta interrupta* (Christoph, 1867), which is distributed in south-eastern Russia, Turkey and İran, *F. interrupta syra* (Grum-Grshimailo, 1899), which occurs in south-eastern Turkey, Lebanon, Syria, Israel and Cyprus ((Müller et al., 2005; Schintlmeister, 2008).

A list of determined specimens of Notodontidae is provided below. The list includes material examined from four different regions of Turkey, collected from 1968 to 2015. The localities in this list are cited as they appear on the pin-labels.

Cerurinae Butler, 1881

1- *Cerura vinula vinula* (Linnaeus, 1758)

Locality **B3**, 04.IV.1997, 1 ♀, leg Y. Özdemir (NTM); Locality **A3**, 15.III.2001, 1 ♂, leg. F. Can Cengiz (MMKU).

2- *C. intermedia* (Teich, 1896)

Locality **D1**, 09.VI.1982, 2 ♂♂, 1 ♀, leg. De Freina (MWM).

3- *Furcula furcula turcica* Schintlmeister, 1998

Locality **D5**, 16-24.VII.1978, 1 ♂, leg. De Freina (MWM); Locality **B10**, 21.V.1985, 1 ♂, leg. Werner Weise (CASD); Locality **D3**, 12.VII.1996, 1 ♂, leg. P. Kautt & V. Weise (CASD).

4- *F. bifida bifida* (Brahm, 1787)

Locality **A9**, X.1987, 1 ♀, leg. Hubert Mayer (CASD); Locality **C8**, 20.XII.1998, 1 ♀, leg. M. Özdemir (NTM).

5- *F. interrupta interrupta* (Christoph, 1867)

Locality **D4**, 09.VI.1996, 1 ♂, leg. P. Kautt & V. Weise (CASD); Locality **C5**, 2 ♂♂ 29.VI. 2005, leg. F. Can Cengiz (MMKU).

F. interrupta syra (Grum-Grshimailo, 1899)

Locality **A3**, 13.III.2002, 1 ♂; 7.V.2002, 1♂, leg. F. Can Cengiz (MMKU); Locality **A6,** 04.VI.2001, 1 ♂, 1 ♀, leg. F. Can Cengiz (MMKU)..

Dicranurinae Duponchel, 1845

6- *Dicranura ulmi* (Denis & Schiffermüller, 1775)

Locality **B9,** 21.V.1985, **1** ♂, leg. Werner Wolf (CASD).

7- *Harpyia milhauseri* (Fabricius, 1775)

Locality **A1,** 02.V.1987, 1 ♂, leg. Werner Wolf (CASD).

8- *Stauropus fagi fagi* (Linnaeus, 1758)

Locality **C2**, 02.VI.1999, 4 ♂♂, leg. M. Özdemir (NTM); Locality **A5**, 30.V.2002, 1 ♂, leg. F. Can Cengiz (MMKU); Locality **A8**, 10.VI.2002, 2 ♂♂, leg. F. Can Cengiz (MMKU); Locality **A7**, 11.VI.2002, 2 ♂♂, leg. F. Can Cengiz (MMKU); Locality **B1**, 12.VI.2003, 2 ♂♂, leg. F. Can Cengiz (MMKU).

New record for the Mediterranean region of Turkey. Not recorded by Okyar (Göbekçioğlu) & Aktaç (1997), Okyar & Aktaç (2007), Baron (2008) and Beşkardeş (2012). In Schintlmeister (2008) a distribution map and the lists of Koçak & Kemal (2007) and Okyar et al. (2009), *S. fagi* is recorded for Turkey, but the localities are outside of the Mediterranean region.

The moth is remarkable for its whitish pattern in the median area of the forewings and paler coloured hind wings (Figure 1.). The male genitalia are distinctive in the structure of the 8th tergite and the

shape of the very large socii (Schintlmeister 2008) (Figure 2.). Throughout its range *S. fagi* occurs in different types of habitats, such as xerothermic hills, steppe, semi-deserts to dense Taiga forests. The species readily adapts to cultivated areas and also occurs in the urban centres of the larger cities. The well-known, ant-like larvae are polyphagous. They were recorded mostly on *Quercus, Fagus* and many species of Rosaceae such as *Malus* (Schintlmeister 2008).

The European distribution of *S. fagi* includes Ireland, southern England, southern Fennoscandia, Spain, northern Turkey, northern Iran and the Caucasus, irs range eastwards being bordered by the River Volga and the Ural Mountains (Schintlmeister 2008).

Figure 2. *Stauropus fagi,* male, Adana-Feke, 30.V. 2002.

Figure 3. a- *Stauropus fagi,* male, Adana-Feke, 30.V. 2002, sclerotized plate of the 8th tergite; b- *Stauropus fagi fagi,* Adana-Feke, 30.V. 2002, male genitalia and aedeagus.

Notodontinae Stephens, 1829

9- *Drymonia dodonaea wageneri* de Freina, 1981 (MWM).

Locality, **C6**, 30.VI.2005, leg. 1 ♂, F. Can Cengiz (MMKU).

10- *D. melagona esmera* de Freina, 1981

Locality, **C7**, 09.VI.1969, 1 ♂, leg. F. Kasy (CASD); Locality **C12**, 4.VI. 1969, 1 ♂, leg. F. Kasy (CASD).

11- *D. querna djezina* Bang-Haas, 1937

Locality **A3**, 15.III. 2001, 1 ♂, leg. F. Can Cengiz (MMKU).

12- *D. velitaris pontica* (Rebel, 1908)

Locality **C8**, 15-20.VIII.1999, 1 ♂, leg. M. Özdemir (NTM).

13- *Notodonta dromedarius pontica* Witt, 1980

Locality **C8**, 15-20.VIII.1999, 1 ♂, 1 ♀, leg. M. Özdemir (NTM).

14- *N. derbendica* Daniel, 1965

Locality **B2**, 21.VIII.1998, 1 ♂, leg. M. Özdemir (NTM).

15- *N. tritopha irfana* de Freina, 1983

Locality **D5,** 24-26.VI.1981, 1 ♂, leg. De Freina (MWM).

16- *Peridea anceps* (Goeze, 1781)

Locality **A3**, 20.IV.2002, 1 ♂, leg. F. Can Cengiz (MMKU); Locality **A1**, 03.V.2002, 1 ♂, leg. F. Can Cengiz (MMKU).

17- *P. korbi korbi* (Rebel, 1918)

Locality **A4**, 07.V.2002, 1 ♀, leg. F. Can Cengiz (MMKU).

18- *Pheosia tremula* Clerck, 1759

Locality **A3**, 13.III.2002, 1 ♂, leg. F. Can Cengiz (MMKU); Locality **A8**, 10.VI.2002, 1 ♀, leg. F. Can Cengiz (MMKU).

19-*Paradrymonia vittata vittata* (Staudinger, 1892)

Locality **D2**, 12.V.1985, 1 ♀, leg. Werner Wolf (CASD); 23.IV.1987, 1 ♂, leg. Werner Wolf (CASD).

Ptilodontinae Packard, 1864

20-*Pterosoma palpina palpina* (Clerck,1759)

Locality **C3**, 02.VI.1999, 1 ♀, leg. M. Özdemir (NTM); Locality **C2**, 02.VI.1999, 1 ♂, leg. M. Özdemir (NTM); Locality **B5**, 28.VII.2000, 1 ♂, leg. M. Özdemir (NTM).

P. palpina pontica Staudinger, 1901

Locality **B6**, 21.VI.1968, 1 ♂, leg. E&A Vartian (NHM); Locality **D6**, 1.VII.1983, 1 ♂, leg. De Freina (MWM).

21- *Ptilodon saerdabensis* (Daniel, 1938)

Locality **C10**, 17.VII.1983, 1 ♂, leg. W. Thomas (CASD).

22- *Ptilophora plumigera* (Denis & Schiffermüller, 1775)

Locality **D3**, 20-22.X.2000, 1 ♀, leg. György Fabian (CASD)

Phalerinae Butler, 1886

23- *Phalera bucephala bucephala* (Linnaeus, 1758)

Locality **C2**, 02.VI.1999, 2 ♂♂, leg. M. Özdemir (NTM); Locality **C4**, 29.VI.2005, 1 ♀, leg. F. Can Cengiz (MMKU); Locality **C11**, 26.VII.2015, 1 ♂, leg. F. Can Cengiz (MMKU).

24- *P. bucephaloides* (Ochsenheimer, 1810)

Locality **B8,** 22.VI.1974, 1 ♂, leg. M. Forst (CASD); Location **D6,** 7-9.VIII.1992, 4 ♂♂, 1 ♀, leg. P. Kautt & Weiss (CASD).

Pygaerinae Duponchel, 1845

25- *Spatalia argentina* (Denis & Schiffermüller, 1775)

Locality **B7**, 28.V-10.VI.1970, 1 ♀, leg. M.u. W. Glaser (CASD); Locality **B2**, 21.VIII.1998, 1 ♂,1 ♀, leg. Z. Şimşek (NTM); Locality **C2**, 02.VI. 1999, 5 ♂♂, leg. M. Özdemir (NTM); Locality **A4**, 20.IV. 2001, 1 ♂, leg. F. Can Cengiz (MMKU); Locality **C1**, 19.VII.2009, 1 ♂, leg. Z. Okyar (MTU); Locality **A2,** 17.V. 2015, 1 ♂, leg. B.Ulaşlı (MMKU).

26- *Rhegmatophila alpina osmana* Friedel, 1967

Locality **A4**, 07.V.2002, 1 ♀ 4 ♂♂, leg. F. Can Cengiz (MMKU); Locality **C2**, 02.VII1999, 2 ♂♂, leg. M. Özdemir (NTM); Locality **B2**, 18.VI.1998, 2 ♂♂, leg. Z. Şimşek (NTM).

27- *Clostera curtula curtula* (Linnaeus, 1758)

Locality **B4**, 09.VIII.1999, 1 ♀, leg. Z. Şimşek (NTM); Locality **B2**, 06.VIII.1998; 1 ♂, 17.VII.1998, 1 ♂, leg. Z. Şimşek (NTM).

28- *C. pigra staudingeri* Koçak, 1980

Locality **B2**, 21.VIII.1998, 6 ♂♂, leg. Z. Şimşek (NTM).

29- *C. anastomosis* (Linnaeus, 1758)

Locality **C9**, 26.VIII.1969, 1 ♂, leg. A. Palik (CASD).

Acknowledgements

This study was made possible through the kind support of numerous entomologists who loaned material of the examined taxa. For this important help we thank the custodians of the museums listed in the Abbreviations section. We wish to express our thanks to Mustafa Özdemir, Yasemin Özdemir and Ziya Şimşek (Collection of Nazife Tuatay (Directorate of Plant Protection Central Research Institute), Ankara, Turkey) for their additional records and also to Zühal Okyar (Museum of Trakya Universitesi, Faculty of Science, Biology Department, Edirne, Turkey) for her additional record of *Spatalia argentina.* We are grateful to Dr W. Gerald Tremewan, Truro, U.K., for his kind corrections of the English language. We are also thankful to the reviewers for their valuable comments.

References

Anonymous, 2007. 9 Sıcak Nokta- Amanos Dağları. National Geographic, Türkiye, Ek:7, 19s.

Aytaç, A.S., 2010. Amanos Dağlarının Orta Kesiminin Doğal Ortam, Sosyo-Ekonomik Faaliyetler, Koruma Kriterleri ve Çevre Eğitimi Açısından Değerlendirilmesi. (Basılmamış) Doktora tezi, Dokuz Eylül Üniversitesi, Eğitim Bilimleri Enstitütüsü, İzmir, 327s.

Aytaç, A.S. & A. Semenderoğlu, 2012. Amanos Dağları'nın orta kesiminin, doğa koruma kriterleri açısından değerlendirilmesi. Anadolu Doğa Bilimleri Dergisi, 3(1): 1-14.

Baron, T., 2008. The moths fauna (Lepidoptera) of Şile in the Asian part of Istanbul province, Turkey. Esperiana Band 14: (pl. 39): 545-558.

Beşkardeş, V., 2012. Lepidoptera fauna of Yuvacık dam watershed in Kocaeli, Turkey. African Journal and Agricultural Research, 11: 1749-1754.

Can, F., 2008. The Geometrid moths (Lepidoptera) from the Middle and Eastern Black Sea Regions of Turkey. Turkish Journal of Zoology, 32: 351- 358.

De Freina, J., 1979. 1. Beitrag zur systematischen Erfassung der Bombyces- und Sphinges-Fauna Kleinasiens. Atalanta 10: 175-224.

De Freina, J., 1981. 2. Beitrag zur systematischen Erfassung der Bombyces- und Sphinges- Fauna Kleinasiens. Atalanta 12: 18-63.

De Freina, J., 1983. 4. Beitrag zur systematischen Erfassung der Bombyces- und Sphinges-Fauna Kleinasiens. Mitteilungen der Münchner Entomologischen Gesellschaft, 72: 57-127.

De Freina, J. & T. Witt, 1987. Die Bombyces und Sphinges der Westpalearktis. Bd.1.-Edition Forschung und Wissenschaft, Munchen, 708 pp.

Ezer, T., 2008. Güney Amanos Dağları (Musa dağı) Biryofit Florası ve Epifitik Biryofit Vejetasyonunun Araştırılması. (Basılmamış) Doktora Tezi, Çukurova Üniversitesi, Fen Bilimleri Enstitüsü, Adana, 304 s.

Heppner, J.B.,1991. Faunal regions and the biversity of Lepidoptera. Tropical Lepidoptera 2 (Suppl. 1): 85 pp.

Koçak, A.Ö., & M. Kemal, 2007. Revised and Annotated Checklist of the Lepidoptera of Turkey. Priamus, Serial Publication of the Centre for Entomological Studies Ankara. 8: 26-42.

Müller, G.C., V.D. Kravchenko, C. Li, J. Mooser, O.B. Orlova, A. Phillips, W. Speidel & T. Witt, 2005. The Notodontidae (Lepidoptera) of Israel. Atalanta, 36 (1/2): 237-247.

Okyar (Göbekçioğlu), Z. & N. Aktaç, 1997. Trakya Bölgesi Heterocera (Lepidoptera) faunasına katkılar. Türkiye Entomoloji Dergisi, 22 (1): 47-56.

Okyar, Z. & N. Aktaç, 2007. Heteroceran fauna of Gökçeada and Bozcada (Northaegean Islands, Turkey) with new record of Noctuidae (Lepidoptera), and biogeoraphical analyses. Entomological News, 118 (3): 263-272.

Okyar Z., S. Yurtsever, N. Aktaç & G. Çakan, 2009. Some aspects of the moth (Lepidoptera, Heterocera) species diversity in Western Black Sea Region of Turkey. North- Western Journal of Zoology, 5(1): 104-120.

Özkoçak O.M., 1993. Hatay horst ve graben yapısının Amanos Dağları altın yatak zuhurları. Joeoloji Mühendisliği, 42: 52-59.

Saldaitis, A., B. Ivinskis & J. Rimsaite, 2013. *Notodonta valeria*, a new species (Lepidoptera, Notodontidae) from China, with taxonomic remarks on *Notodonta ziczac* (Linnaeus, 1758). Zootaxa, 368(5): 589-594.

Schintlmeister, A., 1986. Zahnspinner (Lepidoptera: Notodontidae) als Stadtbewohner. Verh. IX. SIEEC Gotha 1986: 91-92

Schintlmeister, A., 1988. Zahnspinner in den Kulturlandschaften Mitteleuropas (Lep.: Notodontidae). Verhandlungen Westdeutscher Entomologentag 1988: 275-280.

Schintlmeister, A., 2008. Palaearctic Macrolepidoptera, Notodontidae. Volume 1, Apollo Books, Stenstrup, 482pp.

Sensoy S., M. Demircan, Y. Ulupınar & İ. Balta, 2015. Climate of Turkey. (Web page: http://www.mgm.gov.tr/files/en-us/climateofturkey.pdf) (Date accessed: Dec. 2015).

Türk. entomol. derg., 2015, 39 (4): 393-400 ISSN 1010-6960
DOI: http://dx.doi.org/10.16970/ted.04924

Orijinal araştırma (Original article)

Effect of a gall-inducing psylloid, *Pseudophacopteron alstonium* Yang et Li (Hemiptera: Phacopteronidae) on defensive chemistry of *Alstonia scholaris* (L.) R. Br. (Gentianales: Apocynaceae)

Gal yapan bir psylloid *Pseudophacopteron alstonium* Yang et Li (Hemiptera: Phacopteronidae)'un, *Alstonia scholaris* (L.) R. Br. (Gentianales: Apocynaceae)'in kimyasal savunma yapısı üzerine etkisi

Zhen-De YANG[1] Wen-Ling LV[1] Xia-Lin ZHENG[2*]
Shu-Zhong YU[1] Ming LI[1]

Summary

Gall-inducing insect species are capable to escape from defence of host plant species. However, effect of gall-inducing insects on defensive mechanism of host plants is still unclear. The present study was conducted to evaluate the possible chemical changes in the defensive system of *Alstonia scholaris* (L.) R. Br. (Gentianales: Apocynaceae) caused by *Pseudophacopteron alstonium* Yang et Li (Hemiptera: Phacopteronidae) feeding. Total phenolic and tannin, peroxidase, superoxide dismutase, catalase and polyphenol oxidase of gall (G) and non-galled (NG) tissues in one leaf, whole leaf with gall (WG) from 1st to 5th stages induced by *P. alstonium*, and un-galled leaves (UL) were measured in laboratory. High levels of secondary metabolites (i.e., total phenolic and tannin) and protective enzymes were detected, which increased sharply during 1st to 3rd stages of galls and subsequently decreased when nymph stoped feeding or entered into mature (4th or 5th) stages. The recorded high levels of secondary metabolites and protective enzymes in *A. scholaris* could be result of defensive response against *P. alstonium*. The current findings could be helpful for understanding the interaction between plants and gall-formed insects.

Keywords: *Pseudophacopteron alstonium*, *Alstonia scholaris*, gall-inducing insect, defensive response

Özet

Gal yapan böcekler konukçu bitkilerin savunma mekanizmalarından kaçabilme yeteneklerine sahiptirler. Bununla birlikte gal yapan böceklerin bitkilerin savunma mekanizmalarına etkileri hala tam olarak bilinmemektedir. Bu çalışma *Pseudophacopteron alstonium* Yang et Li (Hemiptera: Phacopteronidae)'nin *Alstonia scholaris* (L.) R. Br. (Gentianales: Apocynaceae) üzerinde beslenmesi ile bitki savunma sisteminde meydana gelen kimyasal değişimleri belirlemek için yapılmıştır. *P. alstonium* tarafından birden beşinci seviyeye kadar uyarılmış Gall, aynı yaprakta galsiz yaprak parçası, ve hiç galsiz yapraktaki toplam tannin, peroksidaz, superoksid, dismutaz, katalaz ve polyphenol oksidaz düzeyleri laboratuvar koşullarında ölçülmüştür. Galin birinci evresinden üçüncü evresine kadar bitkide ikincil metabolitlerin (toplam phenolic ve tanin gibi) ve koruyucu enzimlerinde önemli bir artış tespit edilmişken, nimflerin beslenmeyi durdurduğu yada ergin hale geçtiği galin dördüncü ve beşinci evresinde bu değerler belirgin şekilde düşük bulunmuştur. *A. scholaris*'in ikincil metabolitlerinin ve koruyucu enzimlerinde meydana gelen bu artışın bitkinin *P. alstonium*'un beslenmesine verdiği tepkiden kaynaklandığı düşünülmektedir. Elde edilen bu bilgilerin bitki ve bitkilerde gallere neden olan böcekler arasındaki ilişkinin belirlenmesine yardımcı olacaktır.

Anahtar sözcükler: *Pseudophacopteron alstonium*, *Alstonia scholaris*, gal yapan böcek, savunma tepkisi

[1] College of Forestry, Guangxi University, Nanning, China
[2] College of Agriculture, Guangxi University, Nanning, China
* Sorumlu yazar (Corresponding author) e-mail: zheng-xia-lin@163.com
Alınış (Received): 06.03.2015 Kabul ediliş (Accepted): 30.12.2015 Çevrimiçi Yayın Tarihi (Published Online): 08.01.2016

Introduction

Numerous microorganisms and arthropods interact intimately with plants and induce specialized structures – named as gall. The galling habit is especially common in more than 13,000 known gall-forming insects species (Raman et al., 2005; Raman, 2012). Gall-inducing habit has evolved independently and multiple times in insects, resulting co-evolution between the plants and the gall-inducing insects leading to evolved strategies to avoid each other's defense systems (Raman et al., 2005).

Alstonia scholaris (L.) R. Br. (Gentianales: Apocynaceae) has been widely used for landscaping projects in Southeast Asia (including some southern provinces in China), Australia and United States (Kaushik et al., 2011). During recent years, this plant was seriously damaged by *Pseudophacopteron alstonium* Yang et Li (Hemiptera: Phacopteronidae) through gall-inducing, which effects the ornamental value, normal growth and development of *A. scholaris* (Yang & Li, 1983; Qin et al., 2010; Zhang et al., 2011; Lv, 2012). *P. alstonium* has seven generations per year in Nanning City, Guangxi Zhuang Autonomous Region, China. Generation overlapped was observed. Spatial distribution of this species was aggregation distribution under natural conditons (Lv, 2012). Development of gall represents an outgrowth which results due to the interaction between gall-inducing insect and host plant. The production material of gall can provide an optimal material to explore the interaction between gall-inducing insect and host plants (Stone & Schönrogge, 2003). However, effect of gall-inducing psylloid on defensive mechanism of host plants is still unclear. Understanding this information is helpful for exploring the co-evolution between gall-inducing insects and host plants.

The aim of present study was to examine the effect of *P.alstonium* on defensive chemistry of *A.scholaris.* Therefore, the contents of total phenolic, tanni, levels of peroxidase, superoxide dismutase, and catalase and polyphenol oxidase were tested in *A.scholaris* during the five developmental stages of *P.alstonium*.

Materials and Methods

Plant materials

The development stages of galls on *A. scholaris* induced by *P. alstonium* (from 1st to 5th) were collected from Nanning City, China (108°28´E, 22°84´N) during 2011 (Figure 1). Gall (G) and non-galled (NG) tissues from the infested leaves were detected using a scalpel and used as first two treatments. Whereas, whole infested leaves (WG) and un-galled leaves (UL) were used as third and fourth treatment, respectively. UL were synchronously collected from the same *A. scholaris* trees. The leaves form (G, NG and WG) having gall density (≤ 5) were chosen to study the effect of gall density on the nutritional contents, components and secondary metabolites of *A. scholaris*. Insects in galls of the G and WG treatments were flushed using a distilled-water jet (Rongyi Experiment Equipment Co. Ltd., Guangxi, China). About 30 g samples of each treatment were obtained from ten *A. scholaris* leaves. All samples were cut into 1 mm fragments and stored in liquid nitrogen prior to analysis.

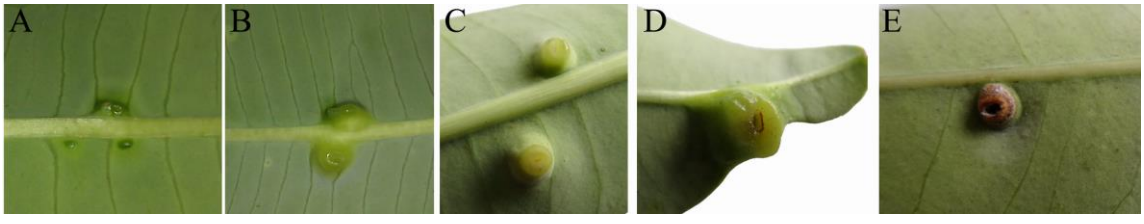

Figure 1. Galls from 1st to 5th developmental stages (A-E) induced by *Pseudophacopteron alstonium* on *Alstonia scholaris*. A. leaf attacked by 1st instar larvae, and a protuberance appeared; B. ostiolate attacked by *P. alstonium* on galls was closed, a green gall formed; C. gall bulged and color changed to aqua or buff, but the color of closed ostiolate showed the amaranth; D. Emergence holes appeared on galls; E. Galls withered after emergence of *P. alstonium* adults.

Determination of total phenolic and tannin contents

Total phenolic and tannin contents in the extracts were determined according to Ainsworth & Gillespie (2007) and Makkar et al. (1993), respectively. Total 1.0 g samples of each treatment was soaked in 150 mL of 50 % ethanol and acetone for 24 h and then filtered to obtain a filtrate. The ethanol and acetone extracts were obtained by evaporating filtrate at room temperature for 3 days. The evaporated extracts were cooled in a desiccator before further analysis. Fifty mg sample of each treatment were added into test tubes containing 2.5 mL of 10% (V/V) Folin-Ciocalteu reagent and 2.0 mL of sodium carbonate (2%, W/V). After shaking, it was left for 2 h and the absorbance was measured at 750 nm using a spectrophotometer (GE Ultrospec 2100 pro, GE Healthcare, UK). Gallic acid was used as standard to obtain a calibration curve (ranging from 0 to 0.8 mg / mL^{-1}). Using the standard curve, the content of total phenolic compounds was calculated and expressed as gallic acid equivalent in milligram per gram (mg/g) of dried extract. As for the level of total tannin, 50 mg of the sample extract was added with 7.5 mL of distilled water and 0.5 mL of Folin-Ciocalteu reagent. Subsequently, 1 mL of 35% sodium carbonate solution and dilute to 10 mL with distilled water was added. Samples were kept at room temperature for 30 min after shaking and the absorbance was read at 725 nm using a spectrophotometer. Blank was established using distilled water instead of the samples. A set of standard solutions of gallic acid was dealt with in the same manner as described above and read against a blank. The results of tannins were expressed in terms of mg gallic acid/g of extract. Sample of each treatment was measured three times.

Assays of peroxidase, superoxide dismutase, catalase and polyphenol oxidase activities

Peroxidase activity was assayed spectrophotometrically with *o*-dianisidine as hydrogen donor (Sadasivam & Manickam, 1996). A 3.5 mL of phosphate buffer (0.2 mol / L NaH_2PO_4 and 0.2 mol / L Na_2HPO_4, pH 6.5) was made in a clean dry cuvette, and 0.2 mL of plant extract and 0.l mL of freshly prepared *o*-dianisidine solution was added. The temperature was maintained from 28 °C to 30 °C and the cuvette was placed in the spectrophotometer set at 430 nm. Then, 0.2 mL of 0.2 M H_2O_2 was added and mixed in solution. Initial absorbance was read at every 30 sec intervals up to 3 min. A graph was plotted with the increase in absorbance against time. From the linear phase, the change in absorbance per min was read. Water blank was used in the assay. Sample of each treatment was measured three times.

The assay of superoxide dismutase activity was carried out with the method of Beauchamp & Fridowich (1971) based on the reduction of nitroblue tetrazolium (NBT) chloride. The reduction of NBT by superoxide radicals to blue coloured formazan was followed at 560 nm. Washed pellets treated with ethanol and chloroform was centrifuged after 15 min at 4 °C and supernatant was used for the assay. The reaction mixture contained, 1.9 mL of phosphate buffer (pH 7.8), 1×10^{-2} M methionine, 6.8×10^{-5} M NBT and 1.17×10^{-6} M riboflavin, with suitably diluted supernatant in a total volume of 3 mL. Illumination of the solution taken in a 10 mL beaker was carried out in an aluminium foil lined box with a 15 W fluorescent lamp for 10 min. Control without the enzyme source was always included. The absorbance was measured at 560 nm using a spectrophotometer. Sample of each treatment was measured three times.

Catalase activity was assayed with the titrimetric method described by Radhakissnan & Sarma (1963). Briefly, 2.5 mL of 0.1 M phosphate buffer, pH 7.5 and 2.5 mL of 0.9% hydrogen peroxide (v/v) in the same buffer were taken and 0.5 mL of the plant extract was added and incubated at room temperature for 3 min. The reaction was then stopped by adding 0.5 mL of 2 N sulphuric acids and the residual hydrogen peroxide was titrated with 0.1 N potassium permanganate solutions. Using similar arrangement, a blank was carried out with boiled enzyme extract. Sample of each treatment was measured three times.

The polyphenol oxidase extraction procedure was described by Ortega-García et al. (2008). In order to establish the extracts of samples from each treatment, frozen samples in liquid nitrogen were pulverized using a pestle and mortar. Powder was homogenized in cold acetone (-20 °C) and polyethyleneglycol (1W: 4V: 1V), and the homogenate refrigerated on dry ice. The solid fraction of the homogenate was separated by vacuum filtration and re-extracted again. The combined acetone extracts were dried overnight. Acetone powder was resuspended in 0.1 M sodium phosphate buffer (1W: 30V), pH 6.8 with 0.3 mg mL^{-1} of type-II trypsin inhibitor, by gently stirring at 4 °C for 30 min before each polyphenol oxidase assay. The sample was filtered through glass wool and the filtrate centrifuged at 10000 × *g* for 20 min at 4 °C. The absorbance was recorded at 475 nm using a spectrophotometer. Sample of each treatment was measured three times.

Statistical analysis

The statistical analysis was performed with SPSS 16.0 (SPSS Inc., Chicago, Illinois, USA). Means were compared using a one-way analysis of variance (ANOVA) followed by a Duncan's test ($P < 0.05$).

Results

Variations of total phenolic and tannin contents

Total phenolic content in G was significantly lower than NG, WG and UL treatments ($F = 51.186$; df = 3, 11; $P < 0.001$) at the first stage. However, this result was completely reversed from 2nd to 5th stages. Furthermore, total phenolic contents in G and WG increased from 1st to 4th stages and decreased in the 5th stage (G, $F = 497.578$; df = 4, 14; $P < 0.001$; WG, $F = 100.880$; df = 4, 14; $P < 0.001$). Increasing tendencies of total phenolic contents of both NG and UL suspended at the 4th stage (NG, $F = 430.933$; df = 4, 14; $P < 0.001$; UL, $F = 257.694$; df = 4, 14; $P < 0.001$, Table 1).

Table 1. Total phenolic contents (mg/g) of UL in comparison with NG, WG and G tissues induced by *Pseudophacopteron alstonium* on *Alstonia scholaris*

Treatments	Developmental duration of galls				
	I	II	III	IV	V
UL	21.64±0.43cE	24.56±0.34bC	35.23±0.52cA	33.86±0.56dB	23.25±0.15dD
NG	24.56±0.60aD	30.01±0.99aBC	39.37±0.57bA	30.70±0.85cB	27.81±0.77cC
WG	23.42±0.43bC	29.70±0.46aB	44.82±1.89aA	45.19±0.42bA	31.15±0.13bB
G	19.88±0.60dE	28.82±0.26aD	48.48±0.78aB	50.47±0.64aA	40.13±0.14aC

Different lowercase letters at the same row and capital letters at the same column indicate statistical significance at $P < 0.05$. UL, un-galled leaves; NG, non-galled tissue on a leaf with galls induced by *P. alstonium*; WG, whole leaf with gall; G, only gall on a leaf with galls induced by *P. alstonium*.

Total tannin content in G was lower than UL, NG and WG at the 1st stage ($F = 0.385$; df = 3, 11; $P = 0.767$). From 2nd to 5th stages, total tannin contents in G were always higher than other treatments (2nd, $F = 194.028$; df = 3, 11; $P < 0.001$; 3rd, $F = 367.635$; df = 3, 11; $P < 0.001$; 4th, $F = 373.365$; df = 3, 11; $P < 0.001$; 5th, $F = 144.698$; df = 3, 11; $P < 0.001$, Table 2). Furthermore, the increasing tendency was found in the treatments of UL, NG and WG as the growth and development of galls (UL, $F = 34.759$; df = 4, 14; $P < 0.001$; NG, $F = 58.38$; df = 4, 14; $P < 0.001$; WG, $F = 266.146$; df = 4, 14; $P < 0.001$). As for the treatment of G, increasing tendencies suspended at the 5th stage (G, $F = 355.704$; df = 4, 14; $P < 0.001$).

Table 2. Total tannin contents (mg/g) of UL in comparison with NG, WG and G tissues induced by *Pseudophacopteron alstonium* on *Alstonia scholaris*

Treatments	Developmental duration of galls				
	I	II	III	IV	V
UL	10.03±0.60aC	13.27±0.44bB	13.79±1.00dB	14.83±0.75dB	22.04±0.84cA
NG	10.35±0.50aD	13.19±0.44bC	18.79±0.45cB	18.65±0.70cB	21.64±0.83cA
WG	10.83±0.66aC	12.44±0.36bC	34.52±0.71bB	32.89±0.94bB	40.25±1.81bA
G	9.95±0.77aE	25.22±0.51aD	52.22±1.23aB	58.24±1.48aA	44.16±0.88aC

Different lowercase letters at the same row and capital letters at the same column indicate statistical significance at $P < 0.05$. UL, un-galled leaves; NG, non-galled tissue on a leaf with galls induced by *P. alstonium*; WG, whole leaf with gall; G, only gall on a leaf with galls induced by *P. alstonium*.

Variations of peroxidase, superoxide dismutase, catalase and polyphenol oxidase contents

Peroxidase activities in G were higher than NG, WG and UL treatments from 1st to 3rd stages (1st, $F = 493.252$; df = 3, 11; $P < 0.001$; 2nd, $F = 1044.000$; df = 3, 11; $P < 0.001$; 3rd, $F = 164.921$; df = 3, 11; $P < 0.001$). Furthermore, peroxidase activities ascended in the first two stages and subsequently descended in the four treatments were found (UL, $F = 245.421$; df = 4, 14; $P < 0.001$; NG, $F = 453.697$; df = 4, 14; $P < 0.001$; WG, $F = 617.173$; df = 4, 14; $P < 0.001$; G, $F = 2856.000$; df = 4, 14; $P < 0.001$, Table 3).

Table 3. Peroxidase contents (μg/(g•min)) of UL in comparison with NG, WG and G tissues induced by *Pseudophacopteron alstonium* on *Alstonia scholaris*

Treatments	Developmental duration of galls				
	I	II	III	IV	V
UL	234.24±2.09dA	232.44±6.30dA	144.94±4.64cB	115.95±3.66aC	102.38±1.68aD
NG	270.96±3.51cB	299.38±4.07cA	165.40±5.69cC	111.13±3.77aD	97.80±4.22aD
WG	369.24±4.35bB	460.20±3.82bA	256.73±12.94bC	89.27±3.60bD	99.56±1.58aD
G	408.93±4.33aB	560.31±3.83aA	362.71±4.35aC	105.73±3.81aD	93.75±2.19aE

Different lowercase letters at the same row and capital letters at the same column indicate statistical significance at $P < 0.05$. UL, un-galled leaves; NG, non-galled tissue on a leaf with galls induced by *P. alstonium*; WG, whole leaf with gall; G, only gall on a leaf with galls induced by *P. alstonium*.

Superoxide dismutase activities in G at the first stage were significantly higher than NG, WG and UL treatments ($F = 21.586$; df = 3, 11; $P < 0.001$). However, its activities in G was lower than NG at the 3rd stage ($F = 150.068$; df = 3, 11; $P < 0.001$), NG, WG and UL at the 4th stage ($F = 28.034$; df = 3, 11; $P < 0.001$), and NG and UL at the 5th stage ($F = 61.795$; df = 3, 11; $P < 0.001$). As for the dynamic variations of the four treatments, there was no visible tendency in NG, WG and UL treatments, except for G in which superoxide dismutase activities increased from 1st to 3rd stages and suspended at the 4th stage ($F = 99.06$; df = 4, 14; $P < 0.001$, Table 4).

From 1st to 4th stages, catalase activities in G were higher than other treatments all the time (1st, $F = 18.629$; df = 3, 11; $P < 0.01$; 2nd, $F = 76.314$; df = 3, 11; $P < 0.001$; 3rd, $F = 35.975$; df = 3, 11; $P < 0.001$; 4th, $F = 44.124$; df = 3, 11; $P < 0.001$, Table 5). However, its activity was significantly lower than NG, WG and UL treatments at the 5th stage ($F = 194.357$; df = 3, 11; $P < 0.001$). Dynamic variations of the four treatments were inconspicuous.

Table 4. Superoxide dismutase contents (U/(g•min)) of UL in comparison with NG, WG and G tissues induced by *Pseudophacopteron alstonium* on *Alstonia scholaris*

Treatments	Developmental duration of galls				
	I	II	III	IV	V
UL	195.50±1.74cD	433.02±6.29cB	351.92±4.18cC	448.42±4.95bA	355.79±1.11aC
NG	221.94±5.14bD	470.46±4.01abB	499.23±7.81aA	512.05±3.11aA	336.53±5.94bC
WG	207.54±5.83cE	454.32±6.03bA	339.08±8.00cC	408.88±7.32cB	288.94±5.48cD
G	241.94±3.25aE	480.78±8.71aA	434.98±2.82bB	377.44±19.81cC	291.60±2.23cD

Different lowercase letters at the same row and capital letters at the same column indicate statistical significance at $P < 0.05$. UL, un-galled leaves; NG, non-galled tissue on a leaf with galls induced by *P. alstonium*; WG, whole leaf with gall; G, only gall on a leaf with galls induced by *P. alstonium*.

Table 5. Catalase contents (U/(g•min)) of UL in comparison with NG, WG and G tissues induced by *Pseudophacopteron alstonium* on *Alstonia scholaris*

Treatments	Developmental duration of galls				
	I	II	III	IV	V
UL	22.35±0.64cC	26.90±1.12cB	25.40±0.25cB	35.68±1.32cA	34.05±0.91bA
NG	24.22±1.02cCD	26.13±0.93cC	22.52±1.19cD	46.19±0.42bA	42.82±1.12aB
WG	26.62±0.57bC	33.40±0.23bB	32.19±1.38bB	45.89±1.92bA	27.64±1.17cC
G	29.64±0.59aD	43.74±0.14aB	36.01±0.91aC	57.54±1.28aA	11.77±0.28dE

Different lowercase letters at the same row and capital letters at the same column indicate statistical significance at $P < 0.05$. UL, un-galled leaves; NG, non-galled tissue on a leaf with galls induced by *P. alstonium*; WG, whole leaf with gall; G, only gall on a leaf with galls induced by *P. alstonium*.

Polyphenol oxidase activities in G were significantly higher than other three treatments at the 1st stage ($F = 8.26$; df = 3, 11; $P < 0.01$), and NG and UL treatments at the 4th stage ($F = 95.336$; df = 3, 11; $P < 0.001$). However, polyphenol oxidase activity in G at the 5th stage was significantly lower than other three treatments ($F = 24.815$; df = 3, 11; $P < 0.001$). Polyphenol oxidase activities in G, NG and WG were increased from 1st to 4th stages and suspended at 5th stage except for UL (UL, $F = 324.093$; df = 4, 14; $P < 0.001$; NG, $F = 328.633$; df = 4, 14; $P < 0.001$; WG, $F = 105.887$; df = 4, 14; $P < 0.001$; G, $F = 101.076$; df = 4, 14; $P < 0.001$, Table 6).

Table 6. Polyphenol oxidase contents (U/(g•min)) of UL in comparison with NG, WG and G tissues induced by *Pseudophacopteron alstonium* on *Alstonia scholaris*

Treatments	Developmental duration of galls				
	I	II	III	IV	V
UL	9.96±0.11bC	10.12±0.25cC	20.49±0.59cB	22.01±0.30cA	22.97±0.38bA
NG	9.88±0.07bE	15.44±0.71bD	24.69±0.71bcC	35.42±0.64bA	31.40±0.56aB
WG	10.52±0.36bD	17.96±0.47aC	37.65±2.24aA	41.52±1.40aA	29.56±0.87aB
G	11.52±0.35aD	14.29±0.25bCD	28.53±1.00bB	41.39±1.04aA	17.58±2.28cC

Different lowercase letters at the same row and capital letters at the same column indicate statistical significance at $P < 0.05$. UL, un-galled leaves; NG, non-galled tissue on a leaf with galls induced by *P. alstonium*; WG, whole leaf with gall; G, only gall on a leaf with galls induced by *P. alstonium*.

Discussion

Plants and insects have evolved strategies to avoid each other's defense systems. To counter the herbivore attack, plants produce specialized morphological structures (e.g., hairs, trichomes, thorns, spines and thicker leaves) (Howe & Schaller, 2008) or secondary metabolites and proteins that have toxic, repellent, and/or antinutitional effects on the herbivores (Fürstenberg-Hägg et al., 2013) or trickery (Ellison & Gotelli, 2001). In the current study, results illustrated that high levels of secondary metabolites (i.e., total phenolic and tannin) and protective enzymes in galls were detected. Furthermore, contents of secondary metabolites and protective enzymes of galls increased sharply from the 1st to 3rd stages of galls and subsequently decreased when nymph stops feeding or enters into a mature stage at the 4th or 5th stages of galls. We considered that these high levels of secondary metabolites and protective enzymes maybe were the defensive response when *A. scholaris* attacked by *P. alstonium*.

How plants defend themselves against attack from herbivores has been the subject of considerable interest over many decades. Production of toxic chemicals (e.g., terpenoids, alkaloids, anthocyanins, phenols and quinones) that either kill or retard the development of the herbivores (Hanley et al., 2007) and proteins that have toxic, repellent, and/or antinutitional effects on the herbivores (War et al., 2011) are the direct defenses mediated by plant characteristics. However, gall-formed insects have evolved ways to hijack plant defenses to their own benefit, by sequestering these chemicals and using them to protect themselves from predators, parasitoids, pathogens and other herbivores. This notion has been termed the enemy hypothesis (Price et al., 1987; Schultz, 1992; Hartley, 1998). Our findings showed that levels of secondary metabolites (i.e., total phenolic and tannin) in G were significantly greater than NG, WG and UL treatments, meanwhile the increasing tendencies suspended when nymph stopped feeding or entered into a mature stage. This result is consistent with previous conclusion studied on the galls induced by *Slavum wertheimae* H.R.L. (Hemiptera: Pemphigidae) on *Pistacia atlantica* Desf. (Sapindales: Anacardiaceae) (Rostás et al., 2013). Therefore, we speculated that high levels of gall secondary metabolites confer protection against natural enemies but such a trade-off needs to be investigated.

One of the crucial aspects of host plant resistance against herbivore is the disruption of insect's nutrition. The enzymes that impair the nutrient uptake by insects through the formation of electrophiles includes peroxidase, polyphenol oxidase, superoxide dismutase, etc. (War et al., 2012). In this study, data demonstrated that peroxidase, superoxide dismutase, catalase and polyphenol oxidase activities in G was the highest and gradually enhanced from 1st to 3rd stages of galls (Tables 3-6). However, activities of these enzymes decreased when nymph stops feeding or enters into a mature stage at the 4th or 5th stages of galls (Tables 3-6). These results strongly suggest that higher levels of peroxidase, superoxide dismutase, catalase and polyphenol oxidase in G were the defensive response when *A. scholaris* attacked by *P. alstonium*.

It can be hypothesized that a great deal of secondary metabolites and protective enzymes accumulated in galls when *A. scholaris* leaves damaged by nymph of *P. alstonium*. However, it is confused that how does nymph of *P. alstonium* adapt to the extreme conditions in galls (i.e., high levels of secondary metabolites and protective enzymes) needs to further study.

Acknowledgements

This work was supported by the National Natural Science Foundation of China under Grant (31160165, 31300549, 31560212), Guangxi Natural Science Foundation under Grant (2013GXNSFBA019111), Bossco Scholar Programme on Cultivation of Creative Talents from Young Teachers in Guangxi University, and Guangxi Special Invited Scientist Program in Agric-Environment and Agric-Products Safety (2013B015).

References

Ainsworth, E.A. & K.M. Gillespie, 2007. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nature Protocols, 2: 875–877.

Beauchamp, C. & I. Fridowich, 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Analytical Biochemistry, 44: 276–287.

Ellison, A.M. & N.J. Gotelli, 2001. Evolutionary ecology of carnivorous plants. Trends in Ecology & Evolution, 16: 623–629.

Fürstenberg-Hägg, J., M. Zagrobelny & S. Bak, 2013. Plant defense against insect herbivores. International Journal of Molecular Sciences, 14: 10242–10297.

Hanley, M.E., B.B. Lamont, M.M. Fairbanks & C.M. Rafferty, 2007. Plant structural traits and their role in antiherbivore defense. Perspectives in Plant Ecology Evolution and Systematics, 8: 157–178.

Hartley, S.E., 1998. The chemical composition of plant galls: are levels of nutrients and secondary compounds controlled by the gall former? Oecologia, 113: 492–501.

Howe, G.A. & A. Schaller, 2008. "Direct Defenses in Plants and Their Induction by Wounding and Insect Herbivores. 7-29". In: Induced Plant Resistance to Herbivory (Ed: A. Schaller) Berlin, Germany: Springer-Verlag, 480 pp.

Kaushik, P., D. Kaushik, N. Sharma & A.C. Rana, 2011. *Alstonia scholaris*: It's phytochemistry and pharmacology. Chronicles of Young Scientists, 2: 71–78.

Lv, W.L., 2012. Biological characteristics of *Pseudophacopteron alstonium* (Homoptera: Phacopteronidae) and its effect on physiological and biochemical indices of *Alstonia scholaris* (Gentianales: Apocynaceae). (Unpublished) Master thesis, Guangxi University, Nanning, China, 69 pp.

Makkar, H.P.S, M. Bluemmel, N.K. Borowy & K. Becker, 1993. Gravimetric determination of tannins and their correlations with chemical and protein precipitation methods. Journal of the Science of Food and Agriculture, 61: 161–165.

Ortega-García, F., S. Blanco, M.A. Peinado & J. Peragón, 2008. Polyphenol oxidase and its relationship with oleuropein concentration in fruits and leaves of olive (*Olea europaea*) cv. 'Picual' trees during fruit ripening. Tree Physiology, 28: 45–54.

Price, P.W., G.W. Fernandes & G.L. Waring, 1987. Adaptive nature of insect galls. Environmental Entomology, 16: 15–24.

Qin, J.P., Z.Q. Zhang, Z.D. Yang, Y.W. Ju, W.Y. Zhang, W.L. Lv & Y.H. Yu, 2010. Morphological and occurring characteristics of *Pseudophacopteron alstonium*. China Plant Protection, 30: 27–29.

Radhakissnan, T.M. & P.S. Sarma, 1963. Intracellular localization and biosynthesis of catalase in liver tissues. Current Science, 32: 1749.

Raman, A., C.W. Schaefer & T.M. Withers, 2005. Biology, ecology, and evolution of gall-induced arthropods (Vol. 1 & 2). Hampshire, England: Science Publisher Ltd.

Raman, A., 2012. Gall induction by hemipteroid insects. Journal of Plant Interactions, 7: 29–44.

Rostás, M., D. Maag, M. Ikegami & M. Inbar, 2013. Gall volatiles defend aphids against a browsing mammal. BMC Evolutionary Biology, 13: 193.

Sadasivam, S. & A. Manickam, 1996. Methods in Biochemistry. New Age International Private Limited, New Delhi, India, 15 pp.

Schultz, B.B., 1992. Insect herbivores as potential causes of mortality and adaptation in gall forming insects. Oecologia, 90: 297–299.

Stone, G.N. & K. Schönrogge, 2003. The adaptive significance of insect gall morphology. Trends in Ecology & Evolution, 18: 512–514.

War, A.R., M.G. Paulraj, T. Ahmad, A.A. Buhroo, B. Hussain, S. Ignacimuthu & H.C. Sharma, 2012. Mechanisms of plant defense against insect herbivores. Plant Signal Behavior, 7: 1306–1320.

War, A.R., M.G. Paulraj, M.Y. War & S. Ignacimuthu, 2011. Herbivore- and elicitor-induced resistance in groundnut to Asian armyworm, *Spodoptera litura* Fab. (Lepidoptera: Noctuidae). Plant Signal Behavior, 6: 1769–1777.

Yang, C.K. & F.S. Li, 1983. A preliminary study on Chinese *Pseudophacopteron* with descriptions of three new species (Homoptera: Psyllidae). Wuyi Science, 3: 120–128.

Zhang, W.Y., W.L. Lv, Z.D. Yang, Z.Q. Zhang & S.M. Lei, 2011. Effect of *Pseudophacopteron alstonium* on photosynthetic parameters and chlorophyll content of *Alstonia scholaris*. China Plant Protection, 31: 7–10.

Türk. entomol. derg., 2015, 39 (4): 401-411 ISSN 1010-6960
DOI: http://dx.doi.org/10.16970/ted.16177

Orijinal araştırma (Original article)

Electrical Penetration Graph monitored feeding behavior of cowpea aphid, *Aphis craccivora* Koch. (Hemiptera: Aphididae), on faba bean, *Vicia faba* L. (Fabaceae), cultivars

Börülce yaprakbiti, *Aphis craccivora* Koch. (Hemiptera: Aphididae)'nin EPG yöntemi ile farklı bakla çeşitleri üzerindeki beslenme davranışının belirlenmesi

Alan SOFFAN[1*] **Abdulrahman Saad ALDAWOOD[1]**

Summary

Feeding behavior of cowpea aphid, *Aphis craccivora* Koch. (Hemiptera: Aphididae), was evaluated on five faba bean, *Vicia faba* L. (Fabaceae), cultivars, including the resistant cultivar Gazira2, by using the Electrical Penetration Graph (EPG) technique. Experiments were conducted on whole plants (WP) and detached leaves (DL). Fifteen parameters of EPG recording were selected for statistical analysis. Two-way factorial ANOVA showed that the EPG parameter values in WP and DL were significantly different, while the cultivars difference occurred mostly in the WP tissue, as indicated through one-way ANOVA Kruskal-Wallis analysis. Cowpea aphid feeding behavior on WP Gazira2 resistant cultivar had the longest duration of stylet derailment (waveform F) and total probing, but lowest in total duration of pathway (waveform C), xylem ingestion (waveform G),and number of probes. Speculatively, longest duration of waveform F, which had a relation with the mechanical disturbance during feeding, probably is one of the possible resistant factors in Gazira2 cultivar, however this remains intriguing and needs further study.

Keywords: EPG, SEM, resistant cultivar, whole plant, detached leaves, stylet derailment

Özet

Bu çalışmada börülce yaprakbiti, *Aphis craccivora* Koch. (Hemiptera: Aphididae)'nin beş farklı bakla çeşidi, dayanıklı olduğu bilinen Gazira2 çeşidi dahil olmak üzere, üzerindeki beslenme davranışı EPG (Electrical Penetration Graph) yöntemi kullanılarak belirlenmiştir. Denemeler tüm bitkide (WP) ve seçilmiş yapraklarda (DL) gerçekleştirilmiştir. İstatistik analizler için 15 EPG parametresi kullanılmıştır. İstatistik sonuçları (Two-Way Factorial ANNOVA) EPG verilerinin tüm bitkide ve seçilmiş yapraklarda önemli derece farklı olduklarını göstermiştir. Börülce yaprakbitinin Gazira2 çeşidinde tüm bitki üzerinde beslenme sonuçlarına bakıldığında en uzun penetrasyon süresinin bu çeşit üzerinde olduğu, buna karşılık "waveform C" "waveform G" verilerinin ve beslenme denemesi sayıları gibi değerlerinin en kısa sureye sahip olduğu tespit edilmiştir. Dayanıklı çeşit üzerinde tespit edilen en uzun beslenme süresinin Gazira2 çeşidinin morfolojik özelliklerinden kaynaklandığı düşünülmekte olup bu konuda daha fazla çalışmaya ihtiyaç duyulmaktadır.

Anahtar sözcükler: EPG, SEM, dayanıklı çeşit, tüm bitki, seçilmiş yapraklar, stilet kaybolması

[1] Economic Entomology Research Unit (EERU), Plant Protection Department, College of Food and Agriculture Sciences, King Saud University, Riyadh, Kingdom of Saudi Arabia
* Sorumlu yazar (Corresponding author) e-mail: alsoffan@ksu.edu.sa
Alınış (Received): 18.02.2015 Kabul ediliş (Accepted): 06.01.2016 Çevrimiçi Yayın Tarihi (Published Online): 18.01.2015

Introduction

Cowpea aphid, *Aphis craccivora* Koch. (Hemiptera: Aphididae), is a major world insect pest in agriculture (Smith & Boyko, 2007), and in Saudi Arabia it was reported in 1989, mostly on Fabaceae (Aldryhim & Khalil, 1993; 1996). Cowpea aphid is the key pest of faba bean, *Vicia faba* L. (Fabaceae), the main legume crop in Mediterranean countries and the Middle East (Nuessly et al., 2004; Saxena, 1991). Generally, aphids cause economic damage by phloem sap ingestion. The phloem sap is rich in sugars but relatively poor in amino acids, therefore, aphids need to feed on large quantities of phloem sap to meet amino acids needs (Dixon, 1998). Their role as a virus vector; such as for faba bean necrotic yellow virus (FBNYV), broad bean yellow mosaic virus (BYMV), and bean leaf roll virus (BLRV); increases the potential of this species to reduce faba bean yields (Schwinghamer et al., 2009; Hodge & Powell, 2010; Weigand & Bishara, 1991).

Using resistant cultivars in controlling cowpea aphid population is one of the priority tactics in integrated pest management (IPM) programs. The use of resistant cultivars is an efficient, environmentally friendly, and long term effective tactic that is synergistic with other IPM tactics (Klingler et al., 2001; Smith, 2005; Shannag & Ja'far, 2007). However, time consuming bioassays are significant obstructions in plant breeding programs that are intended to provide resistant cultivars (Smith, 2005; Michel et al., 2011). Therefore, utilizing an efficient technique to evaluate plant cultivars resistance against insects, such as Electrical Penetration Graph (EPG) technique, is crucial (Schoonhoven et al., 1998).

Electrical Penetration Graph is one of the common tools by which it is possible to characterize and identify, in detail, plant resistance factors against insects with piercing mouthparts by monitoring their probing behaviors (Backus et al., 1994; Calatayud et al., 1994; Van Helden et al., 2000; Smith, 2005; Le Roux et al., 2008). The EPG technique was developed for the first time in the 1960s and a significant modification was made in 1978 by substitution of AC (Alternating Current) circuitry with DC (Direct Current) circuitry. The DC circuitry of EPG then simply was referred to EPG (Walker, 2000). The basic principle of EPG recording is the integration of a plant and an insect in an electronic circuit. When an aphid insert its stylet, the circuit is completed and EPG waveforms will be recorded and visualized. The waveform, i.e. voltage fluctuations, are due to electrical resistance and electromotive force changes during different activities and locations of the aphid stylet tips (Tjallingii, 1978; Montllor & Tjallingii, 1989; Walker, 2000).

This study was conducted to evaluate cowpea aphid feeding behavior on five selected faba bean cultivars by using EPG. In particular, EPG recording was expected to reveal a possible resistance factors in cultivar Gazira2 which had been previously reported to have more resistance to cowpea aphid compared to other cultivars (Misr, Giza3i, Goff, and Misr1) (Soffan & Aldawood, 2014).

Materials and Methods

Plant material

Five faba bean cultivars (Misr1, Misr, Giza3i, Goff1, and Gazira2) provided by Legume Research Unit (LRU), King Saud University (KSU), were used for the experiments. A mixture of sand and peat moss (1:1) were used as growth medium to germinate seeds after being soaked in water for 48 h. One week old seedlings were transplanted to plastic pots (d: 11 cm, h: 14 cm). When seedlings were 19 days old, one time fertilization was applied with four granules per pot of complete fertilizer (N:12%, P:12%, K:17%; BASF-Asoco Agro, Limburgerhof). Plants were watered 150 ml once every two days. All plants were grown in a growth chamber maintained at 26˚C, 44% Relative Humidity (RH) with a photoperiod of 16:8 L:D (Recorded by HOBO data loggers, ONSET Co., Bourne, MA).

Leaf surface study was conducted by Scanning Electron Microscope (SEM) (FEI company, USA) in five faba bean cultivars to investigate the presence of possible resistant factors such as trichome, wax, etc. A fresh leaf of each faba bean cultivar was observed for SEM in their native-hydrated state with 1045× magnification at 7.36 kv and a pressure of 33 Pa (Pathan et al., 2008).

Cowpea aphids

Cowpea aphids were collected from alfalfa plants grown in Al Amaria, Riyadh, Kingdom of Saudi Arabia (N: 46°31'5.5518" E: 24°48'40.179"). Further, a single apterous adult, virginoparous female aphid was used to initiate cowpea aphid culture on the faba bean cv Misr and was run for 8 months before the experiment. Aphid feeding behavior study was conducted in the Insect Behavior Laboratory, Economic Entomology Research Unit (EERU), Plant Protection Department, College of Food and Agriculture Sciences, King Saud University, Riyadh, Kingdom of Saudi Arabia. The environment during the feeding behavior study was maintained at 28.5˚C and 23.5% RH. Cowpea aphids used in the EPG experiments were adult apterous females of 2-5 days old from a synchronized colony reared on detached leaves of faba bean cv. Misr.

Aphid feeding behavior

Cowpea aphid feeding behavior on five faba bean cultivars was monitored using a 4 channel EPG monitor (type Giga4, EPG systems, Netherlands). Whole plants (WP) at three leaf stage and detached leaves (DL) derived from the same plants were compared. Plants were placed in a Faraday cage during monitoring to exclude electrical noise. For WP EPG recording, aphids were placed on the abaxial side of the second leaf from the apex by fixing them twisted up for easy aphid access (Prado & Tjallingii, 2007). While for the DL, the petioles were wrapped with cotton and dipped in water in clear plastic elliptical container (215 mL) (Montllor et al., 1990) and twisted up as in WP. Aphids were attached to a thin gold wire (20 µm in diameter and 3 cm length) using conductive silver glue on water basis. The other end of the gold wire was attached to a three centimeters long copper wire (diameter 0.2 mm) and connected to the input of the EPG amplifier, having a 1 Gigaohm input resistance and 50× gain. Each plant electrode (a 2 mm thick, 10 cm long copper rod) was connected to an adjustable EPG plant voltage output device on one side and inserted in the soil of the potted plant(WP) or the water (DL) on the other side (Prado & Tjallingii, 2007). Four aphids at a time, one aphid per plant, were used for each 5 h EPG recording run (Pompon et al., 2010).

Electrical Penetration Graph waveforms were recorded on a computer, mediated by Stylet^{+} software, which was also used for later signal analysis (EPG Systems, Netherlands) and using AD conversion at 100 Hz (Di158U converter, Dataq, USA). In signal analysis, 5 EPG waveforms (C, E1, E2, G, and F) were distinguished. Waveform C, representing the activities during stylet pathway in epidermis and mesophyll. Waveform E, total phloem phase, separated into E1 and E2 indicating sieve element salivation and phloem sap ingestion, respectively. Waveform F, representing stylet derailment (stylet penetration difficulties) and waveform G, indicating xylem ingestion or active drinking of water from xylem elements (Tjallingii, 1990). Next to waveform analysis, data were processed to calculate 15 EPG parameters (Le Roux et al., 2008) using an Excel macro 'BAZ_V7. BETA' (E.S chliephacke, JKI, Quedlinburg, Germany).

Experimental design and statistical analysis

The experimental design was a complete randomized design (CRD). For each faba bean cultivar, ten replicates were used in both WP and DL.

Data were analyzed using SAS program ver. 9.2 (2008) and IBM SPSS Statistic 22. Normality test was calculated on the studentized residual of the square root transformed data. Non parametric One-way ANOVA Kruskal-Wallis was performed by using PROC NPAR1WAY, because most of the dependent variable residual data did not meet normal distribution. Two-way factorial ANOVA (5x2; 5 cultivars and 2 tissue types) were tested for those parameters which their residuals met the normality assumption. The principal component analysis (PROC PRINCOMP) was used to analyze selected six feeding behavior parameters, which were waveform C, G, F, E, non-probing duration and time from start to phloem phase.

Results

Two-way factorial analysis ANOVA (5x2) was used on some parameters that met the residual normality assumption (parameters number [no.] 1, 3, 4, 11, 14, and 15; Table 1-4). It was shown that there were no interactions between tissue type (whole plant [WP] vs. detached leaf [DL]) and cultivars (Gazira2, Giza3i, Goff1, Misr1, and Misr) for all tested parameters (parameters no. 1 has F=1.14, P=0.35, DF= 4; no. 3 has F=0.93, P=0.45, DF= 4; no. 4 has F=1.43, P=0.23, DF= 4; no. 11 has F=0.56, P=0.69, DF= 4; no. 14 has F=0.47, P=0.76, DF= 4; and no. 15 has F=0.19, P=0.94, DF= 4). As main effect, tissue type (WP and DL) showed significant difference for all parameters above (F=14.7-59.8, P<0.05, DF= 1). The data values between WP and DL were obviously different on some parameters such as total duration of pathway phase (waveform C, parameter no. 4), total duration of phloem ingestion periods (waveform E2, parameter no. 12), and time from start to first sustained phloem ingestion (parameter no. 15).

Table 1. Non-Phloem feeding behavior of cowpea aphid during 5-h access on five faba bean cultivars (Means ± SE)[1,2,3]

Non Phloem feeding Parameters		Cultivars				
		Gazira2	Misr	Giza3i	Goff1	Misr1
General probing behavior						
1. Number of probes (*n*)	WP	13.8 ± 3.6b	30.1 ± 2.7a	17.9 ± 3.5b	19.1 ± 4.4b	16.6 ± 3.1b
	DL	5.2 ± 1.4a	9 ± 2.4a	5.6 ± 1.6a	7.4 ± 2.5a	10 ± 2.8a
	P	0.026*	0.001*	0.01*	0.04*	0.112^{ns}
2. Total duration of probing (min)	WP	279.5 ± 6.1a	257.6 ± 6.9b	242.1 ± 18.2b	264.4 ± 10.8ab	276.1 ± 5.8ab
	DL	293.1 ± 3.5a	288 ± 5a	292.9 ± 3.4a	292.6 ± 3.4a	289.1 ± 3.6a
	P	0.036*	0.004*	0.002*	0.015*	0.153 ^{ns}
Pathway phase						
3. Number of pathway phase (waveform C) (*n*)	WP	18.6 ± 3.1b	31.2 ± 2.8a	19.5 ± 3.5b	21 ± 4.3b	19 ± 3b
	DL	8 ± 2.1a	10 ± 2.6a	6.3 ± 1.7a	8.3 ± 2.6a	11.4 ± 3.3a
	P	0.012*	0.002*	0.009*	0.019*	0.1^{ns}
4. Total duration of pathway phase (waveform C)	WP	143.5 ± 21.8b	211.8 ± 11.3a	191.9 ± 19.6ab	161.3 ± 21.1ab	166.1 ± 22.4ab
	DL	90.4 ± 18.9a	78.2 ± 15.9a	75.6 ± 19.2a	90.9 ± 25.6a	63.4 ± 8.2a
	P	0.142^{ns}	0.001*	0.003*	0.06^{ns}	0.007*
5. Time from start to first probe (min)	WP	0.9 ± 0.7a	0.1 ± 0.1a	19.1 ± 18.5a	0.02 ± 0a	0.2 ± 0.2a
	DL	0.02 ± 0a	0.4 ± 0.4a	0.08 ± 0.04a	0.02 ± 0a	10.1 ± 10.1a
	P	0.441^{ns}	0.864^{ns}	1^{ns}	1^{ns}	0.781^{ns}

[1]WP:Whole plant; DL: Detached leaf; *P*: P-value,
[2]Means followed by the same letter in the same row are not significantly different at LSD, α=0.05,
[3]Means in the same column for each parameter comparing whole plants (WP) and detached leaves (DL) accompanied with P-value, asterisk (*) for significant difference, *ns* is non-significant at α=0.05.

Principle component analysis showed similar results related to tissue type above. The first three main components of the principle component analysis (Figure 1) explained 96 % of the variability of aphid feeding among plant cultivars (62 %, 19%, and 15%). Significant difference between WP and DL assays were indicated from the clear separation in plotting component 1 against component 2.

Non-parametric one-way ANOVA Kruskal-Wallis test on different cultivars showed that significant difference among cultivars mostly occurred in WP tissue, while DL did not give any significant difference among cultivars for all parameters (Table 1). Among five cultivars, Gazira2 had a significant association with the lowest number of probes, lowest number and total duration of waveform C, and highest total probing duration as compared to cultivar Misr. Cultivar Gazira2 was significantly the highest in total

duration of stylet derailment (waveform F) periods among all cultivars. Cultivar Misr had a significant association with the highest number of probes and number of waveform C among all cultivars. Cultivar Misr was one of the four cultivars which has lower total duration of waveform F and lowest total duration of waveform E2. Cultivar Giza3i, Goff1, and Misr1 were among the four cultivars which had low number of probes, total duration of probing, number and duration of waveform C, and total duration of waveform F.

Table 2. Non-Phloem feeding behavior (waveform G and F) of cowpea aphid during 5-h access on five faba bean cultivars (Means ± SE)[1,2,3]

Non Phloem feeding Parameters		Cultivars				
		Gazira2	Misr	Giza3i	Goff1	Misr1
Other parameters						
6. Total duration of xylem ingestion (waveform G) (min)	WP	5.9 ± 5.9a	8 ± 5.5a	10.1 ± 8.3a	18.1 ± 10.4a	35.1 ± 17.7a
	DL	20.3 ± 15.2a	7.7 ± 7.7a	13.1 ± 12.2a	3.4 ± 3.4a	6.9 ± 6.9a
	P	0.538^{ns}	0.718^{ns}	0.792^{ns}	0.359^{ns}	0.177^{ns}
7. Total duration of stylet derailment (waveform F) (min)	WP	98.9 ± 21.3a	23.7 ± 12.2b	15.4 ± 7.9b	19.6 ± 9.1b	38.5 ± 18.7b
	DL	0.5 ± 0.2a	23.5 ± 13.5a	23.4 ± 11.9a	8.8 ± 8.8a	49.1 ± 20.8a
	P	0.006*	0.917^{ns}	0.85^{ns}	0.333^{ns}	0.735^{ns}

[1]WP:Whole plant; DL: Detached leaf; *P*: P-value
[2]Means followed by the same letter in the same row are not significantly different at LSD, α=0.05
[3]Means in the same column for each parameter comparing whole plants (WP) and detached leaves (DL) accompanied with P-value, asterisk (*) for significant difference, *ns* is non-significant at, α=0.05.

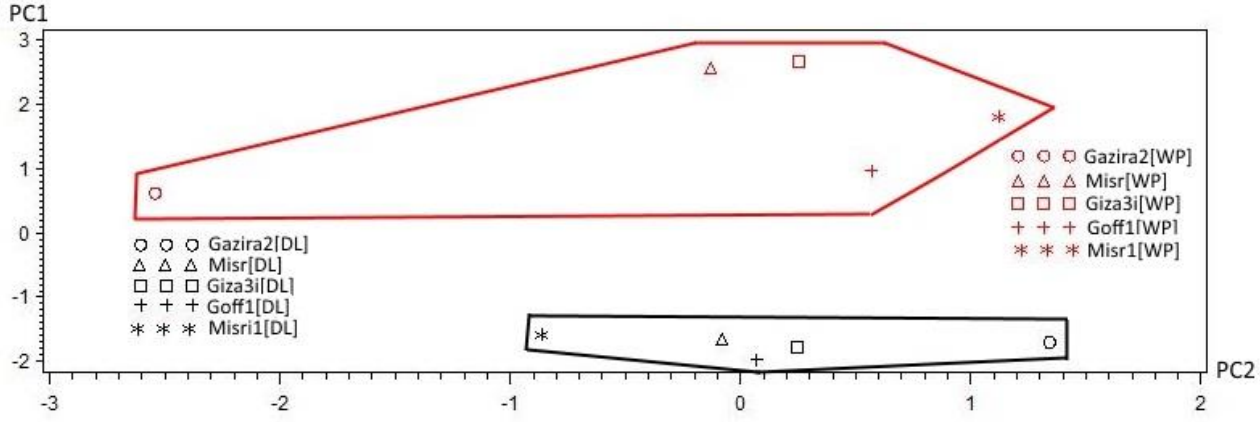

Figure 1. Principal Component Analysis plot PC1(62%) vs. PC2 (19%), for all cultivars (whole plants-WP-and detached leaves –DL-) using data of Non probing duration, pathway phase duration (waveform C), xylem ingestion duration (Waveform G), stylet derailment duration (waveform F), total phloem duration (waveform E), and time from start to phloem phase. PC:Principle component.

Scanning electron microscope for lower leaf surface revealed that there were no trichomes in all cultivars, which may suggest plant defense implications (Figure 2).

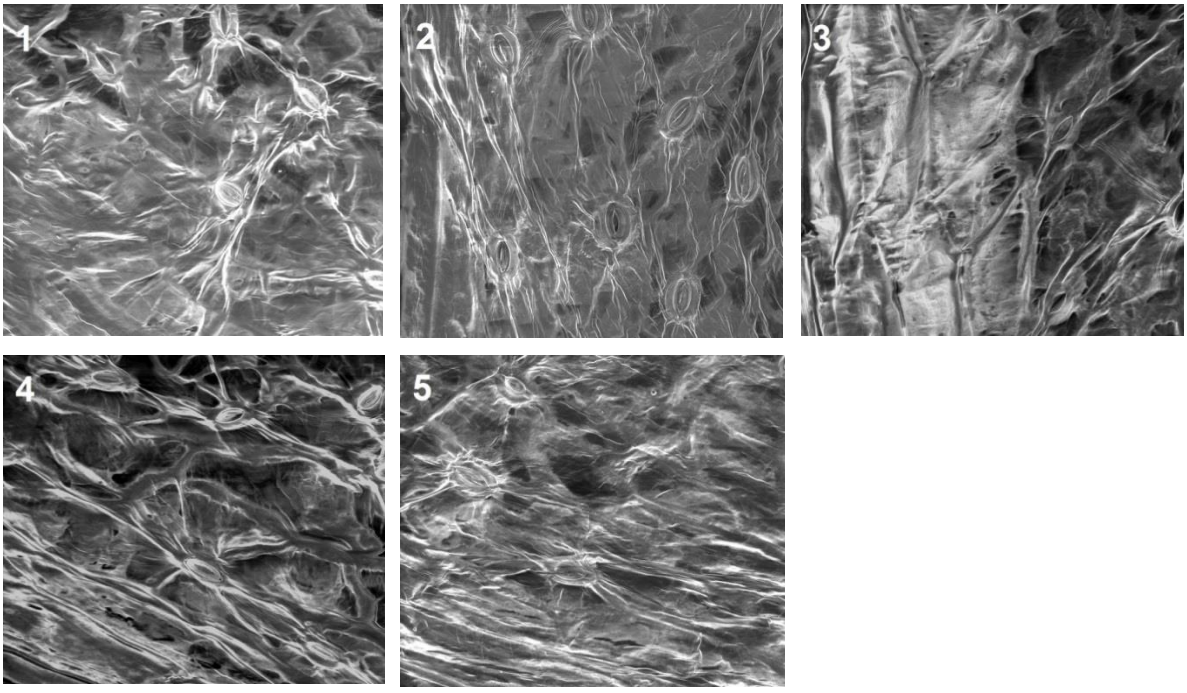

Figure 2. Scanning electron microscopic images of the lower leaf epidermis surface of five faba bean cultivars, 1). Gazira2, 2). Misr, 3). Giza3i, 4). Goff1, 5). Misr1.

Table 3. Phloem salivation feeding behavior of cowpea aphid during 5-h access on five faba bean cultivars (Means ± SE)[1,2,3]

Phloem feeding Parameters		Cultivars				
		Gazira2	Misr	Giza3i	Goff1	Misr1
Phloem salivation phase						
8. Number of single waveform E1 salivation (*n*) (single E1 = without E2)	WP	0.2 ± 0.2a	0.1 ± 0.1a	0a	0.1 ± 0.1a	0a
	DL	0	0	0	0	0
	P	0.317^{ns}	0.346^{ns}	1^{ns}	0.403^{ns}	1^{ns}
9. Total duration single waveform E1 salivation (min)	WP	0.2 ± 0.2a	0.06 ± 0.06a	0a	0.04 ± 0.04a	0a
	DL	0	0	0	0	0
	P	0.317^{ns}	0.346^{ns}	1^{ns}	0.403^{ns}	1^{ns}
10. Total duration waveform. E1 fractions (E1 followed by E2)	WP	30.9 ± 16.3ab	14.1 ± 9.2b	24.6 ± 12.8ab	65.2 ± 19.7a	36.4 ± 20.3ab
	DL	181.6 ± 32.7a	178.5 ± 25.2a	180.8 ± 29.7a	189.4 ± 33.9a	169.6 ± 22.3a
	P	0.006*	0.001*	0.001*	0.006*	0.004*
11. Time from start to first phloem salivation, waveform E1 (min)	WP	149.8 ± 46.5a	234.7 ± 16.1a	226.1 ± 28.8a	154.9 ± 31.4a	218.5 ± 26.4a
	DL	109.2 ± 29.8a	121.6 ± 25.2a	118.8 ± 31.9a	109.8 ± 34.2a	133.5 ± 25.9a
	P	0.221^{ns}	0.017*	0.028*	0.203^{ns}	0.055^{ns}

[1]WP:Whole plant; DL: Detached leaf; *P*: P-value
[2]Means followed by the same letter in the same row are not significantly different at LSD, α=0.05
[3]Means in the same column for each parameter comparing whole plants (WP) and detached leaves (DL) accompanied with P-value, asterisk (*) for significant difference, *ns* is non-significant at, α=0.05.

Table 4. Phloem Ingestion feeding behavior of cowpea aphid during 5-h access on five faba bean cultivars (Means ± SE)[1,2,3]

Phloem feeding Parameters		Cultivars				
		Gazira2	Misr	Giza3i	Goff1	Misr1
Phloem ingestion phase						
12. Total duration of phloem ingestion (waveform E2) periods (min)	WP	28.1 ± 15.5ab	10.9 ± 8.6b	20.4 ± 11.1ab	54.7 ± 16.5a	30.6 ± 19.3ab
	DL	176.1 ± 33.4a	176.2 ± 25.1a	175.8 ± 30.8a	184.8 ± 34.2a	167.2 ± 22.8a
	P	0.006*	0.001*	0.001*	0.006*	0.004*
13. Total duration of sustained phloem ingestion (Waveform E2) (min)	WP	27.3 ± 15.1ab	10.8 ± 8.6b	18.3 ± 10.6ab	53.1 ± 16.4a	28.8 ± 19.4ab
	DL	175.7 ± 33.4a	176.1 ± 25.1a	174.7 ± 31.2a	183.4 ± 34.1a	165.7 ± 23.4a
	P	0.004*	0.001*	0.0004*	0.006*	0.003*
14. Time from start to first phloem ingestion (waveform E2) (min)	WP	148.2 ± 47.9a	246.2 ± 15.6a	192.6 ± 33.1a	154.4 ± 31.58a	218.2 ± 26.4a
	DL	89.6 ± 24.1a	121.1 ± 25.3a	110.9 ± 29.2a	109.6 ± 34.2a	120.1 ± 23.1a
	P	0.186^{ns}	0.025*	0.077^{ns}	0.203^{ns}	0.022*
15. Time from start to first sustained phloem ingestion (waveform E2 > 10 min)	WP	225.5 ± 23.1a	251.2 ± 28.5a	202.9 ± 17.6a	205.7 ± 29.4a	202.3 ± 45a
	DL	96.7 ± 24.5a	123.6 ± 25.1a	111.5 ± 29.2a	114.2 ± 34.5a	124.1 ± 24.6a
	P	0.017*	0.068^{ns}	0.079^{ns}	0.064^{ns}	0.139^{ns}

[1]WP:Whole plant; DL: Detached leaf; *P*: P-value
[2]Means followed by the same letter in the same row are not significantly different at LSD, α=0.05
[3]Means in the same column for each parameter comparing whole plants (WP) and detached leaves (DL) accompanied with P-value, asterisk (*) for significant difference, *ns* is non-significant at, α=0.05.

Discussion

Understanding the resistance factors of faba bean cultivars against cowpea aphid is crucial in plant breeding program. Field screening (Ebadah et al., 2006, Shannag & Ja'far, 2007) or biological assays are lacking detailed information on how plants defend themselves against aphids (Abdel-Hafiz, 2008; Sharma et al., 2005; Narayanamma et al., 2007; Michel et al., 2010). Both techniques are not sufficient to identify the resistance factors. Therefore, Electrical Penetration Graph (EPG) can be used as an alternative method to get detailed information on this issue (Van Helden et al., 2000; Le Roux et al., 2008).

Whole plant (WP) or detached leaf (DL) bioassays, both had been used extensively in many aphid-plant interaction studies (Michel et al., 2010; Montllor et al., 1990; Nam & Hardie, 2012; Soffan & Aldawood, 2014). Depending on bioassay type, both might generate similar or different results for the same host cultivar (Montllor et al., 1990; Nam & Hardie, 2012). In this study, most of EPG parameters values between WP and DL were significantly different as indicated by two way factorial analysis and principle component analysis. The data of WP and DL were obviously different for some parameters such as in total duration of waveform F (parameter no.7). Gazira2 had a significantly highest value in WP (98.9 min), while in DL the value dropped to 0.5 min. Moreover, parameters values in DL are significantly higher than WP, as in total duration of waveform E1 and E2 (parameter no. 12 and 13), or significantly lower, such as in time from start to first phloem ingestion (parameters no. 14 and 15). This typical phenomena was noted in Greenbugs, *Schizaphis graminum* (Rondani) (Hemiptera: Aphididae), that performed different feeding behaviors on several EPG parameters between WP and DL, especially on resistant cultivar of sorghum (Montllor et al., 1990; Van Emden & Bashford, 1976).

Generally, DLs represent a better food sources and are more acceptable compared to WPs (Tune & Dussourd, 2000, Huang et al., 2003), for instance, Greenbugs, *Schizaphis graminum* (Rondani) (Homoptera: Aphididae) grew better on DL as compared to WP on the same sorghum, *Sorghum bicolor*

(Poaceae), varieties (Montllor et al., 1990). Leaf excision on DL also had been suggested to trigger the change of integrity or composition of middle lamellar polysaccharides (Montllor et al., 1990; Van Emden & Bashford, 1976). If we assumed that DL were more exposed to water than WP in order to keep DL fresh during the study (by wrapping the leaf petiole with wet cotton), then it can be speculated that the difference in cowpea aphid feeding behavior between WP and DL might be due to the alteration of metabolite concentrations, such as sucrose and several amino acids (Mewis et al., 2012) or it might be associated with the thickening of the cell in the WP (Cutler et al., 1977).

In this EPG study, cultivars differences in DL were masked statistically. Similar result was obtained on *Brevicoryne brassicae* L. (Hemiptera: Aphididae) biological performance when resistant and susceptible brussels sprout plant (*Brassica oleracea*: Brassicaceae) were compared (Van Emden & Bashford, 1976), also the failure of *Diabrotica balteata* LeConte (Coleoptera: Chrysomelidae) to show resistant characteristics of lettuce (*Lactuca sativa* L.: Asteraceae) cv Valmaine in DL (Huang et al., 2003). Therefore, cultivars analysis for EPG study in WP was preferred, although the use of DL can be fit in other type of bioassays, as they have different aphid-host interaction (Michel et al., 2010; Soffan & Aldawood, 2014).

While in WP tissue, some of the feeding behavior parameters showed different values among five cultivars. One of the important resistance indicators, which is initial recognition of the plant tissue before the start of ingestion (Gabrys & Tjallingii, 2002), failed to describe the difference among cultivars. Time from start to first probe (Parameter no. 5) showed a negligible value for all cultivars, indicating there was no resistance factor on the surface of the leaf. This fact was confirmed by leaf surface observation through scanning electron microscope (Figure 2), which showed that there was no trichome or wax layer structures that might disturb aphid stylet penetration (Powell et al., 1999, Perdikis et al., 2008, Vallejo et al., 1994). A large number of test probes and a long time until the first phloem phase activity, commonly indicate the resistance factor in peripheral layers of plant tissue (i.e., epidermis and mesophyll) (Alvarez et al., 2006). However, they were not present in the studied resistant cultivar Gazira2. Phloem feeding phase (Table 3.), which is one of the important indicators of aphid resistance (Annan et al., 1997, Tjallingii, 1990), also had no clear cut significance, cowpea aphid had an ability to reach the phloem sieve tissue in all of the cultivars, as well as to have normal phloem ingestion duration, as these values were similar to other reported study (Prado & Tjallingii, 2007).

The associative feeding behavior of resistant cultivar Gazira2 only includes longest duration of stylet derailment (waveform F) and probing, but lowest in total duration of pathway (waveform C), xylem ingestion (waveform G), and number of probes. Among these parameters profile in Gazira2, a significantly highest waveform F duration might be the possible resistance factor, as it occurred only in Gazira2. This result had been speculated to have an association with aphid feeding behavioral disturbance in plant cells (stylet penetration difficulties) (Tjallingii, 1990), especially when compared to the nearly vanish of this waveform in DL from 98.9 to 0.5 min (Table 2, parameter no 7). A similar, but less pronounced conclusion, was made for the aphid *Nasonovia ribisnigri* (Mosley) (Hemiptera: Aphididae), which performed longer waveform F on resistant lettuce line (Montllor & Tjallingii, 1989). Since the DL came from the same plants as in WP, it indicated that the DL tissue of Gazira2 had lost their resistance factor, as it occurred in other study (Montllor et al., 1990). One might guess why this did not happen in the other cultivars, and this aspect certainly needs further research.

Finally this study presented evidence that cowpea aphid feeding behavior on WP and DL of faba bean was different as recorded by EPG. The EPG data showed that WP tissue were more appropriate to be used in evaluating faba bean cultivars resistance to cowpea aphid. A reported resistant cultivar Gazira2 had no association neither with phloem factor nor leaf surface factor, rather, it possibly had relation with the longer duration of stylet derailment (waveform F), which was not retained on the DL. Nevertheless, this result remains intriguing and may possibly provide a cue for further research.

Acknowledgements

We thank to Dr. Tjallingii for the EPG technique and review of this paper, Prof. Yousif N. Aldryhim for guidance in working with aphid, and all colleagues in EERU for their support. This study was supported by student research grant (A-S-11-0813) King Abdulaziz City for Science and Technology (KACST).

References

Abdel-Hafiz, N.A, 2008. Resistance of certain cucumber varieties to the melon aphid *Aphis gossypii* (Glover). Zemdirbyste-Agriculture, 95: 293-297.

Aldryhim, Y. & A. Khalil, 1993. Influence of temperature and daylength on population development of *Aphis gossypii* on Cucurbita pepo. Entomologia Experimentalis et Applicata, 67:167-172.

Aldryhim, Y. & A. Khalil, 1996. The Aphididae of Saudi Arabia. Fauna of Saudi Arabia, 15:161-195.

Alvarez, A., W.F. Tjallingii, E. Garzo, V. Vleeshouwers, M. Dicke & B. Vosman, 2006. Location of resistance factors in the leavess of potato and wild tuber-bearing Solanum species to the aphid *Myzus persicae*. Entomologia Experimentalis et Applicata, 121:145-157.

Annan, I.B., G.A Schaefers, W. Tingey & W.F Tjallingii, 1997. Effects of treatments for electrical penetration graph recordings on behavior and biology of *Aphis craccivora* (Aphididae). Physiological Entomology, 22:95-101.

Backus, E. A. 1994. "History, development, and applications of the AC electronic monitoring system for insect feeding, pp. 1–51." *In* M. M. Ellsbury, E. A. Backus, and D. L. Ullman (eds.), History, Development, and Application of AC Electronic Insect Feeding Monitors. Entomological Society of America, Lanham, MD, 128 pp.

Calatayud, P.A., Y. Rahbé, W.F Tjallingii, M. Tertuliano & B. Le Rü, 1994. Electrically recorded feeding behaviour of cassava mealybug on host and non-host plants. Entomologia Experimentalis et Applicata, 72:219-232.

Cutler, J.M., D.W Rains, & R. Loomis, 1977. The importance of cell size in the water relations of plants. Physiologia Plantarum, 40:255-260.

Dixon, A.F.G., 1998. Aphid ecology: an optimization approach. Chapman and Hall, London, UK, 300 pp.

Ebadah, I., Y. Mahmoud & S.Moawad, 2006. Susceptibility of some faba bean cultivars to field infestation with some insect pests. Research Journal of Agriculture and Biological Sciences, 2:537-540.

Gabrys, B., & W.F. Tjallingii, 2002. The role of sinigrin in host plant recognition by aphids during initial plant penetration. Entomologia Experimentalis et Applicata, 104:89-93.

Hodge, S.,& G. Powell, 2010. Conditional facilitation of an aphid vector, *Acyrthosiphon pisum*, by the plant pathogen, Pea Enation Mosaic Virus. Journal of Insect Science, DOI: 10.1673/031.010.14115. insectscience.org/10.155.

Huang, J., G.S. Nuessly, H.J McAuslane & R.T Nagata, 2003. Effect of screening methods on expression of romaine lettuce resistance to adult banded cucumber beetle, *Diabrotica balteata* (Coleoptera: Chrysomelidae). Florida Entomologist, 86: 194-198.

Klingler, J., I. Kovalski, L. Silberstein, G. Thompson & R. Perl-Treves, 2001. Mapping of cotton-melon aphid resistance in melon. Journal of the American Society for Horticultural Science, 126:56-63.

Le Roux, V., S. Dugravot, E. Campan, F. Dubois, C. Vincent & P. Giordanengo, 2008. Wild solanum resistance to aphids: antixenosis or antibiosis? Journal of Economic Entomology, 101: 584-591.

Mewis, I., M.A.M Khan, E. Glawischnig, M. Schreiner & C. Ulrichs, 2012. Water stress and aphid feeding differentially influence metabolite composition in *Arabidopsis thaliana* (L.). PloS One, 7:1-15.

Michel, A.P., M.A Mian, N.H Davila-Olivas & L.A Cañas, 2010. Detached leaf and whole plant assays for soybean aphid resistance: differential responses among resistance sources and biotypes. Journal of Economic Entomology, 103: 949-957.

Michel, A.P., O. Mittapalli & M.A.R. Mian, 2011. Evolution of Soybean Aphid Biotypes: Understanding and Managing Virulence to Host-Plant Resistance, 356-372. In: Soybean - Molecular Aspects of Breeding, (Ed: A. Sudaric), InTech, 526 p. ISBN: 978-953-307-240-1, DOI: 10.5772/14407. Available from: http://www.intechopen.com/books/soybean-molecular-aspects-of-breeding/evolution-of-soybean-aphid-biotypes-understanding-and-managing-virulence-to-host-plant-resistance.

Montllor, C. & W. F. Tjallingii, 1989. Stylet penetration by two aphid species on susceptible and resistant lettuce. Entomologia Experimentalis et Applicata, 52:103-111.

Montllor, C.B., B.C Campbell & T. E., Mittler, 1990. Responses of *Schizaphisgraminum* (Homoptera: Aphididae) to leaf excision in resistant and susceptible sorghum. Annals of Applied Biology, 116: 189-198.

Nam, K.J. & J. Hardie, 2012. Reproduction and probing behaviour of the bird cherry-oat aphid *Rhopalosiphumpadi* on detached leaves and leaves on intact seedlings of the winter host, bird cherry *Prunuspadus*. Physiological Entomology, 37:196-200.

Narayanamma, V.L., H. Sharma, C.L.L Gowda & M.Sriramulu, 2007. Mechanisms of resistance to *Helicoverpa armigera* and introgression of resistance genes into F1 hybrids in chickpea. Arthropod-Plant Interactions, 1:263-270.

Nuessly, G.S., M.G. Hentz, R. Beiriger & B.T. Scully, 2004. Insects associated with faba bean, *Viciafaba* (Fabales: Fabaceae), in southern Florida. Florida Entomologist, 87: 204-211.

Pathan, A., J. Bond & R. Gaskin, 2008. Sample preparation for scanning electron microscopy of plant surfaces-Horses for courses. Micron 39:1049-1061.

Perdikis, D., E. Kapaxidi & G. Papadoulis, 2008. Biological control of insect and mite pests in greenhouse Solanaceous crops. The European Journal of Plant Science and Biotechnology, 2:125-144.

Pompon, J., D. Quiring, P. Giordanengo & Y. Pelletier, 2010. Role of xylem consumption on osmoregulation in *Macrosiphum euphorbiae* (Thomas). Journal of Insect Physiology, 56:610-615.

Powell, G., S.P. Maniar, J.A. Pickett & J.Hardie, 1999. Aphid responses to non-host epicuticular lipids. Entomologia Experimentalis et Applicata, 91:115-123.

Prado, E. & W.F. Tjallingii, 1997. Effects of previous plant infestation on sieve element acceptance by two aphids. Entomologia Experimentalis et Applicata, 82:189-200.

Prado, E. & W.F. Tjallingii, 2007. Behavioral evidence for local reduction of aphid-induced resistance. Journal of Insect Science, 7:1-8.

SAS Institute Inc., 2008. SAS/STAT 9.2 User's Guide, SAS Institute Inc., Cary, NC

Saxena, M., 1991. Status and scope for production of faba bean in the Mediterranean countries." Options Méditerranéennes, 15-20.

Schoonhoven, L.M., T. Jermy & J.J.A. van Loon, 1998. Insect-plant biology: From physiology to evolution. Chapman and Hall, London and New York, 409 pp.

Schwinghamer, M., A. Nicholas & M.Schilg, 2009. Three aphid vectors of faba bean (*Viciafaba*) viruses in northern New South Wales and occurrence of *Acyrthosiphonpisum*-transmitted isolates of Soybean dwarf virus. Australasian Plant Pathology, 38:262-269.

Shannag, H. & A. Ja'far, 2007. Biometry and responses of faba bean varieties to black bean aphid, *Aphis fabae* Scopoli. American-Eurasian Journal of Agriculture & Environmental Science, 2:328-334.

Sharma, H.C., G. Pampapathy, M.K. Dhillon & J.T. Ridsdill-Smith, 2005. Detached leaf assay to screen for host plant resistance to *Helicoverpa armigera*. Journal of Economic Entomology, 98:568-576.

Smith, C.M., 2005. Plant resistance to arthropods: molecular and conventional approaches. Springer, Netherland, 423 pp.

Smith, C.M. & E.V. Boyko, 2007. The molecular bases of plant resistance and defense responses to aphid feeding: current status. Entomologia Experimentalis et Applicata, 122:1-16.

Soffan, A. & A.S. Aldawood, 2014. Biology and demographic growth parameters of cowpea aphid (*Aphis craccivora*) on faba bean (*Vicia faba*) cultivars. Journal of Insect Science, 14:1-10.

Tjallingii, W.F., 1978. Electronic recording of penetration behaviour by aphids. Entomologia Experimentalis et Applicata, 24:721-730.

Tjallingii, W.F., 1990. Continuous recording of stylet penetration activities by aphids. Aphid-plant genotype interactions. Elsevier, Amsterdam, 89–99 pp.

Tune, R. & D.E. Dussourd, 2000. Specialized generalists: constraints on host range in some plusiine caterpillars. Oecologia, 123:543-549.

Vallejo, R.L., W.W. Collins & R.H. Moll, 1994. Inheritance of A and B glandular trichome density and polyphenol oxidase activity in diploid potatoes. Journal of the American Society for Horticultural Science, 119:829-832.

Van Emden, H.F. & M.A. Bashford, 1976. The effect of leaf excision on the performance of *Myzuspersicae* and *Brevicoryne brassicae* in relation to the nutrient treatment of the plants. Physiological Entomology, 1: 67-71.

Van Helden, M., W.F. Tjallingii, G.P. Walker & E.A. Backus, 2000. Experimental design and analysis. In EPG experiments with emphasis on plant resistance research. Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior. Thomas Say Publications in Entomology, Entomological Society of America, Lanham, MD, 144–171 pp.

Walker, G.P., 2000. A Beginner's guide to electronic monitoring of homopteran probing behavior. In: Walker GP and Backus EA editor. Principles and applications of electronic monitoring and other techniques in the study of homopteran feeding behavior. Proceedings of the symposium at the XIX international congress of entomology, Entomological Society of America,Beijing, China., 14-40 pp.

Weigand, S.,& S.I. Bishara, 1991. Status of insect pests of faba bean in the Mediteranean region and methods of control. In: Cubero JI and Saxena MC editor. Present status and future prospects of faba bean production and improvement in the Mediterranean countries. Zaragoza: CIHEAM, 67-74 pp (Options Méditerranéennes:Série A. Séminaires Méditerranéens; n.10).

Türk. entomol. derg., 2015, 39 (4): 413-423 ISSN 1010-6960
DOI: http://dx.doi.org/10.16970/ted.67424

Orijinal araştırma (Original article)

Characterization of imidacloprid resistance in *Aphis gossypii* (Glover) (Hemiptera: Aphididae) in Southern Iran[1]

Güney İran'da *Aphis gossypii* (Glover) (Hemiptera: Aphididae)'de imidacloprid direnç karakterizasyonu

Selmi Sadat SEYEDEBRAHIMI[2] Khalil TALEBI JAHROMI[3*]

Sohrab IMANI[2] Vahid HOSSEINI NAVEH[3] Shahram HESAMI[4]

Summary

The cotton aphid, *Aphis gossypii* (Glover) (Hemiptera: Aphididae), is a key pest of cucurbits in Fars Province, Southern Iran and is managed with repetitive applications of insecticides such as imidacloprid. Recently, reports of insecticide control failures have increased, particularly with imidacloprid. In present work susceptibility of two *A. gossypii* populations to imidacloprid and effects of possible synergist, Triphenyl phosphate (TPP), Diethyl maleate (DEM) and Piperonyl butoxide (PBO) were checked using micro-applicator bioassay. The resistant population was collected from cucumber host plant in greenhouse and susceptible population had been reared under greenhouse conditions on *Cucumis sativus* L. cv. Negin (Cucurbitaceae) for two years. A resistance to imidacloprid with resistance ratio of 11.24 was found in resistant population compare with the susceptible population. Combination of *in vivo* differential synergism studies and biochemical assays (esterase, GSTs, cytochrome P_{450} monooxygenase and heme peroxidase assay) suggest that the enhanced cytochrome P_{450} activity is the primary mechanism of increased resistance in these populations.

Keywords: Cotton aphid, bioassay, esterase, GSTs, P_{450} activity, heme peroxidase assay

Özet

Pamuk yaprak biti *Aphis gossypii* (Glover) (Hemiptera: Aphididae), güney İran'ın Fars bölgesinde cucurbitlerin anahtar zararlılarından biridir ve imidaclorpid gibi tekrarlanarak kullanılan insektisitler ile kontrol edilmektedir. Son yıllarda insektisitlerin özellikle de imidaclorpid uygulamalarının başarısız olduğu kayıt edilmektedir. Bu çalışmada *A. gossypii*'nin iki duyarlı popülasyonunun imidaclorpidlere duyarlılığının ve Triphenyl phosphate (TPP), Diethyl maleate (DEM) ve Piperonyl butoxide (PBO) gibi bazı sinerjist etkili kimyasalların imidaclorpit duyarlılığına etkisi mikro aplikatör kullanılan denemeler ile test edilmiştir. Yaprakbitinin dayanıklı popülasyonu seralardan kabakgiller üzerinden toplanmış, duyarlı popülasyon ise *Cucumis sativus* L. cv. Negin (Cucurbitaceae) üzerinde iki yıl süre ile yetiştirilmiştir. Imidoclorpid dayanıklılığı duyarlı popülasyona oranla 11.24 kat daha fazla bulunmuştur. Farklı sinerjik etkili maddelerin (esteraz, GSTs, cytochrome P_{450} monooxygenaz ve heme peroxidaz) kombinasyonlarının test edildiği biyokimyasal çalışmalar sonucunda cytochrome P_{450} mekanizmasının dayanıklılığı arttıran birincil mekanizma olduğu tespit edilmiştir.

Anahtar sözcükler: Pamuk yaprakbiti, esteraz, GSTs, P_{450} activitesi, heme peroksidaz analizi

[1] This article has been drawn up from a part of the PhD dissertation of Selmi Sadat Seyedebrahimi.
[2] Department of Entomology, Science and Research Branch, Islamic Azad University, Tehran, Iran
[3] Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran
[4] Department of Plant Protection, Shiraz branch, Islamic Azad University, Shiraz, Iran
* Sorumlu yazar (Corresponding author) e-mail: khtalebi@ut.ac.ir
Alınış (Received): 11.11.2015 Kabul ediliş (Accepted): 06.01.2016 Çevrimiçi Yayın Tarihi (Published Online): 05.02.2016

Introduction

The cotton aphid, *A. gossypii* (Glover) (Hemiptera: Aphididae), is a cosmopolitan insect pest on cotton and many field crops and vegetables (Kim et al., 1986). In Iran in addition to cotton, it is the major pest of Cucurbitaceae, especially on cucumber, *Cucumis sativus* L. (Khanjani, 2005). *A. gossypii* causes direct damage through sucking nutrients from the plant and indirect damage through contamination with honeydew and by vectoring viral pathogens (Ebert & Cartwright, 1997). The cotton aphid has progressed towards high resistance to many commonly used insecticides in many agricultural areas, including organophosphates, carbamates, pyrethroids and neonicotinoids (Ahmad et al., 2003; El-Kady, 2007; Furk & Hines, 1993; Gubran et al., 1992; Herron & Wilson, 2011; Hollingsworth et al., 1994; Martin & Workman, 1997; Wang et al., 2002; Wang et al., 2007).

Neonicotinoids are an important group of insecticides and functions as a competitive inhibitor on nicotinic acetylcholine receptors in the central nerve system. Because of distinctive action mechanism, neonicotinoids have strong insecticidal activity, especially against hemipteran pests, also low levels of toxicity to mammals and relatively little toxicity to non-target insects are reported (Nauen & Denholm, 2005). In spite of slow development of resistance to neonicotinoids, several insect pests including *A. gossypii* have been shown to have the ability of resistance development (Naun & Denholm, 2005; Shi et al., 2011).

In *A. gossypii* different types of enzymes have been reported to involve in detoxification of insecticides. Several studies have found a relation between esterase activity and resistance to organophosphates (Hama et al., 1995; O'Brien & Graves, 1992). Carbamate resistance has a relationship with enhanced mixed function oxidase (MFO) activity (Saito et al., 1995). It is reported that carboxylesterase imparted resistance to cypermethrin in *A. gossypii* (Jhansi & Subbaratnam, 2004). Alteration in the target site for the toxicant is another resistance mechanism in insects (Talebi Jahromi, 2011). Han et al*.,* (1998) concluded that carbamate primicarb resistance in *A. gossypii* clones is because of alteration in the target site, acetylcholinesterase (AChE).

The results of biochemical assays using model substrates for determination of responsible relationship between enzyme activities and insecticide resistance may be ambiguous and must be interpreted carefully. Isozymes that metabolize model substrates may not necessarily be those that detoxify insecticides (Huang, 2002). Studies of possible synergistic effects are important for detection of detoxification mechanisms. Use of enzyme inhibitors, Triphenyl phosphate (TPP, inhibitor of various esterases), Diethyl maleate (DEM, Glutathion-S-transferase inhibitor) and Piperonyl butoxide (PBO, inhibitor of cytochrome p450-dependent polysubstrate monooxygenases) may be useful in cases where an increased enzymatic detoxification contribute to resistance.

In Iran, control of *A. gossypii* relies heavily on insecticides, especially in greenhouses. It is done mostly by imidacloprid, the most extensively used neonicotinoid that was introduced in 1991 (Karunker et al., 2009). Thereforethe purpose of this study was to compare two populations of this pest against imidacloprid in Fars province, Iran, and to compare the activity of three well-known enzymes that detoxify xenobiotics in resistant and susceptible populations.

Materials and Methods

Insects

The susceptible population (Shiraz) had been routinely reared in net-covered cages, 70* 50* 40 cm, under greenhouse conditions at 28: 18 °C, 65 ± 5 RH and 16:8 (L:D) photoperiod on cucumber plants, *Cucumis sativus* L. cv. Negin (gynoecious) (Cucurbitaceae) since 2012. The plants in the cages were replaced every 2 weeks with new ones in order to keep colonies alive. The resistant population was collected from cucumber host plant in a greenhouse located in Sadra town near Shiraz in Fars province, Iran in 2014. This population had a history of previous exposure to different insecticides such as pirimicarb, oxydemeton-methyl, malathion in the last year, but in the last 6 months imidacloprid was sprayed every two weeks by the grower in greenhouse. The population was assessed after 3 weeks of rearing in mentioned greenhouse conditions. Synchronized five days old aphids were used in all experiments.

Chemicals

Imidacloprid (95% technical grade) used in all bioassays was obtained from Moshkfam Fars Co., Iran. Triton X-100, 1-chloro-2,4-dinitrobenzene (CDNB), the synergist Triphenyl phosphate (TPP, 99% purity), Diethyl maleate (DEM) and Piperonyl butoxide (PBO) were purchased from Merck company (Germany), 3,3‘,5,5‘ tetramethyl benzidine (TMBZ), Hydrogen peroxide, Cytochrome C, Bovine serum albumin, 1-Naphthyl acetate (1-NA), 2-naphthyl acetate (2-NA), 1-naphthol, 2-naphthol, Reduced glutathione (GSH), 7-ethoxycoumarin (7-EC) were obtained from Sigma Chemical Co. and Fast Blue RR salt (o-dianisidine, tetrazolized zinc chloridecomplex) was obtained from Fluka (Buchs, Switzerland).

Micro-applicator bioassay and calculation of synergistic effects

The solvent in all bioassays was 50% acetone in aqueous Triton X-100 (0.5 g litre^{-1}). Susceptible and resistant populations were compared by microapplicator bioassay and then effects of synergists were evaluated on these two populations by this method. Microapplicator bioassay was done using the method described by Immaraju et al. (1990). 5 days old apterous aphids were placed on filter paper. Using a microapplicator (the Hamilton Company. INC. Whittier, California) a 0.5 µl of each insecticide solution was topically applied to individual insects. Controls were treated with the solvent alone. Treated insects were quickly transferred on cucumber leaf discs (55mm diameter) which had been placed upside down on an agar bed (0.9 g litre^{-1}) in 55 mm Petri-dishes. Petri-dishes were ventilated by a 15 mm hole covered with a fine-mesh net. Petri-dishes containing aphids were kept in incubator at 25±1ºC, 65% RH and 16:8 (L:D) photoperiod. Mortality was assessed after 48 h.

To confirm the results of enzyme activity measurement, the synergisms of TPP, PBO, and DEM on susceptible and resistant populations were determined. To choose the appropriate dose of synergists, a preliminary microapplicator bioassay was done using different doses of them. For each chemical the highest dose that caused comparable mortality with the control after 48 h was selected. So the synergists PBO, DEM and TPP were prepared at concentrations of 20, 20 and 10µg ml^{-1} respectively. The above-mentioned synergists solutions were used as solvent to prepare stock solution and serial dilutions (five concentrations) of imidacloprid for studying of synergistic effects as described by Nauen et al. (1998).

Determination of esterase activity

Fifty, 5 days old apterous aphids of *A. gossypii* from resistant and susceptible populations were homogenized in 600 µl of ice-cold sodium phosphate buffer (100 mM, pH 7, containing 0.1% of Triton X-100) and centrifuged at 10,000g at 4°C for 10 min. The resulting supernatants were used for biochemical analyses.

Total esterase activities against the substrates, 1-NA and 2-NA were measured following the method of Van Asperen (1962) with some modifications, including changes in ratio of materials and using fast blue RR instead of diazoblue laurylsulphate solution. The reaction was initiated with addition of 20 µl enzyme samples to 200 µl of substrates (1 part of substrate 30mM in acetone + 99 parts 0.02M phosphate buffer pH 7.0). 50 microliter fast blue RR (3mg mL^{-1} in distilled water) was then added to the reaction mixture and the released product was continuously measured at 450nm for 1-NA and at 540nm with 2-NA at 30 sec intervals for 15 min using a microplate reader (ELX808 Bio-Tek). Reactions without enzyme source served as control. At least three replicates were performed for each population. The specific activity was expressed as µmoles of product min^{-1} mg protein^{-1}. Protein content of the enzyme samples was determined following the method of Lowry et al. (1951) using bovine serum albumin (BSA) as the standard. Standard curves of absorbance versus concentrations of 1-naphthol and 2-naphthol were constructed to enable calculation of the amount of product produced during the esterase assay.

Determination of GSTs activity

Enzyme source was prepared from fifty,5 days old apterous aphids from resistant and susceptible populations in the same way as esterase (buffer without Triton X-100). GST activity was measured using 1-chloro-2,4-dinitrobenzene (CDNB) and reduced glutathione (GSH) as substrates with the method used by Habig et al.(1976) with slight modifications to conduct the experiment in 96-well microplate, including:

two hundred microliter reaction mixtures (250 µl CDNB 63mM in methanol and 5ml GSH 10 mM in 0.1M sodium phosphate buffer, pH 6.5) were placed in a well containing 60µl of the enzyme sample. Reagents were prepared fresh prior to use. The change in absorbance was measured continuously at 30 sec intervals for 11 min at 340 nm. The rate of product glutathione conjugated (S-(2,4-dinitrophenyl)glutathione) formation, that indicates the enzyme activity was calculated by using molar extinction coefficient of product as 9.6 mM^{-1} cm^{-1} at 340 nm and 25°C (Lizuka et al., 1989). Reactions without enzyme source served as control. At least three replicates were performed for each population. The specific activity was expressed as µ moles of product min^{-1} mg $protein^{-1}$. Protein content of the enzyme samples was determined following the method of Lowry et al. (1951) using bovine serum albumin (BSA) as the standard.

Determination of cytochrome P_{450} monooxygenase activity

P_{450}s activity was determined using the 7-ethoxycoumarine-*O*-deethylase activity based on the method of de Sousa et al. (1995) as described about aphids by Castaneda et al. (2009, 2010) and Cabrera-Brandt et al. (2010) with modifications, described here. Fifty, 5 days old apterous aphids of *A. gossypii* from resistant and susceptible populations were homogenized in 400 µl of ice-cold sodium phosphate buffer (50 mM, pH 7.2) and centrifuged at 1000g at 4°C for 10 min. The resulting supernatants were used as the enzyme source. Fifty µl enzyme sample was added to each well of a black 96-wells microplate containing 100 µl of substrate solution (0.4 mM 7-ethoxycoumarin in 50 mM pH 7.2 phosphate buffer). The reaction was incubated for 4 h in darkness at 30 ºC and then stopped by the addition of 100 µl of buffer glycine/ethanol (50% v/v). For the controls, the same volume of buffer glycine/ethanol (50% v/v) was added before the incubation to avoid the reaction. Fluorescence was read at 390 nm for excitation and 460 nm for emission, using infinite M200 microplate reader (Tecan ®). The assay contained 3 replicates of each population. Protein content was measured according to Bradford (Bradford, 1976). The specific activity was expressed as changes in fluorescence units min^{-1} mg $protein^{-1}$.

Heme peroxidase assay

Twenty µl homogenate (fifty,5 days old apterous aphids were homogenized in 600 µl of 0.1 M phosphate buffer, pH 7.0) was mixed with 80 µl of 0.625 M potassium phosphate buffer pH 7.2, 200 µl of TMBZ solution (0.01 g TMBZ dissolved in 5 ml methanol and then 15 ml of 0.25 M sodium acetate buffer pH 5.0) and 25 µl of 3% hydrogen peroxide in a microplate well. After 2 h incubation at room temperature in darkness, the plate was read at 450 nm as an end point assay. By using a standard curve of pure cytochrome C, which contains one bound heme per molecule, an estimate of the amount of monooxygenases present was obtained and expressed as equivalent units of cytochrome P_{450} (Damayanthi & Karunaratne, 2005). The assay contained 3 replicates for each population.

Statistical analysis

Resistance Ratio (RR) was calculated by dividing the LC_{50} of resistant population (Sadra) by the LC_{50} of susceptible population (Shiraz). Data were analyzed by employing ANOVA and means were compared by Duncan multiple range test ($p < 0.05$) using SPSS (version 21). LC_{50} was determined using probit analysis with the PC-software Polo-Plus (LeOra Software, Berkeley, CA). Dose responses were considered significantly different if the confidence interval calculated from their ratio did not overlap 1.

Results

Microapplicator bioassay and calculation of synergistic effects

The results revealed that the resistance ratio (RR) in resistant population was 11.24 (Table 1). There was a significant difference between LC_{50} values of PBO-imidacloprid-treated and imidacloprid-treated in resistant population. Imidacloprid was approximately six times more toxic in the presence of PBO than in the absence of PBO (synergism ratio= 5.82). Therefore, PBO had an obvious synergism to imidacloprid on resistant population. However, PBO had no significant synergism to imidacloprid in susceptible population. Also DEM and TPP had no significant synergism to imidacloprid in both populations. The tests confirmed that enhanced MFO activity is responsible for the observed imidacloprid resistance.

Table 1. Synergism of PBO, DEM and TPP on imidacloprid in resistant and susceptible populations of *Aphis gossypii*

Population[A]	Treatment	N-d[B]	N-i[C]	LC50 µg ml-1 (LCL–UCL)*[D]	Slope ± SE	X^2 (df)[E]	RR[F]	SR[G]
resistant	Imidacloprid	5	297	673.04[a] (542.76- 810.6)	2.58± 0.29	11.23 (18)	11.24	-----
	Imidacloprid+ PBO	5	305	115.01[b] (92.42- 142.79)	2.01± 0.26	7.82 (18)	1.92	5.82
	Imidacloprid+ DEM	5	308	570.35[a] (471.18- 681.27)	2.31± 0.23	13.41 (18)	9.53	1.18
	Imidacloprid+TPP	5	262	709.35[a] (577.81- 856.06)	2.55± 0.30	16.36 (18)	11.85	0.95
susceptible	Imidacloprid	5	278	59.86[b] (49.016- 72.02)	2.30± 0.253	14.41 (18)	------	-----
	Imidacloprid+ PBO	5	249	67.44[b] (54.29- 81.91)	2.45± 0.30	12.56 (18)	------	0.89
	Imidacloprid+ DEM	5	377	49.41[b] (38.98- 59.96)	2.39± 0.29	14.57 (18)	------	1.21
	Imidacloprid+TPP	5	311	63.40[b] (51.09- 77.35)	1.78± 0.20	7.56 (18)	------	0.94

[A] Shiraz: susceptible, Sadra 1: resistant
[B] Number of doses
[C] Number of insects tested without controls.
[D] The LC_{50} values are expressed as µg ml^{-1}, LCL: lower confidence limit at 95%; UCL: upper confidence limit at 95%.
* Means within the same rank followed by different letters are significantly different at $p < 0.05$.
[E] Values of X^2, lower than ($p \leq 0.05$) indicate a significant fit between the observed and expected regression lines.
[F] RR: Resistance ratio: LC_{50} of different treatments of resistant population/ LC_{50} of susceptible population.
[G] SR: Synergist ratio = LC_{50} of imidacloprid alone/LC_{50} of imidacloprid + synergist.

Esterase activity

Results of esterase assay showed that there were significant differences in esterase activities between the susceptible and resistant populations (Fig. 1). Quantitative analysis of general esterase activity with 1-NA as substrates revealed that activity of esterase in susceptible population is 2.4-fold higher than resistant population. The same situation was observed when esterase activity against the substrate 2-NA was compared. Susceptible population showed more esterase activity (2.2-fold) than resistant population.

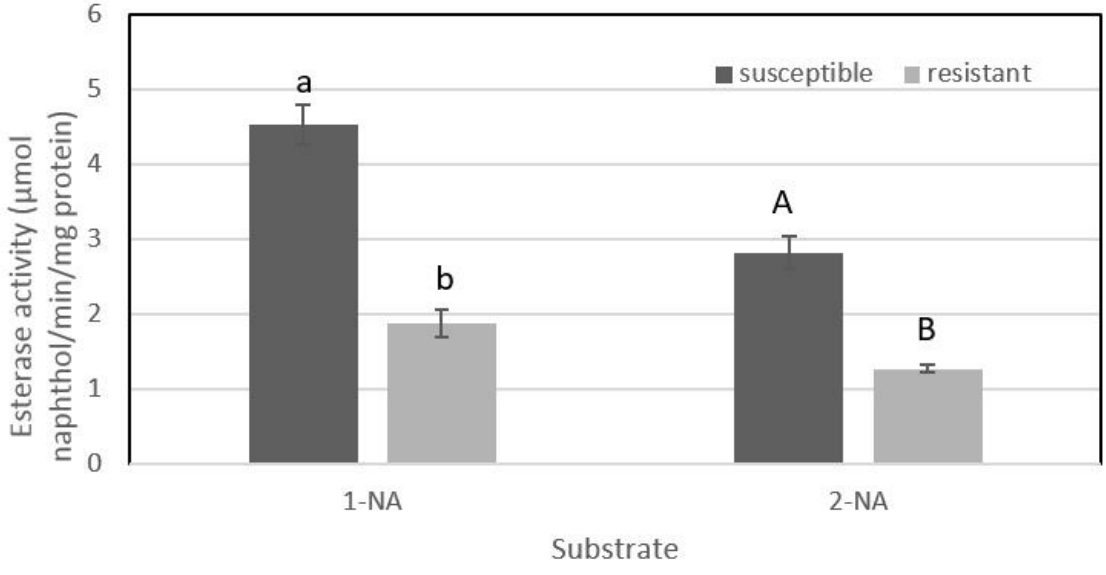

Fig. 1. Esterase activities in susceptible and resistant populations of *Aphis gossypii* from Fars province, Iran, using 1-NA and 2- NA substrates. Data are means ± SE. The means followed by different letters are significantly different at $p < 0.05$.

Results of this study demonstrated that TPP had no effect on imidacloprid toxicity in resistant population (synergism ratio was 0.95) (Table 1). This indicated that, in this population, esterase doesn't play a role in imidacloprid detoxification. Although due to structure of imidacloprid, hydrolysis of that is not possible (Nauen et al., 1998), esterase activity sometimes has been reported as an important mechanism for imidacloprid resistance, for example on *Aphis craccivora* C.L. Koch (Hemiptera: Aphididae) (Mokbel & Mohamed, 2009) and *Myzus persicae* Sulzer (Hemiptera: Aphididae) (Choi et al., 2001), even in some reports in *A. gossypii* esterase has been known as the reason of resistance to imidacloprid (Li & Han, 2007). In contrast it has been shown that the esterase does not confer resistance to imidacloprid in *Myzus* spp. (Nauen et al., 1996; Philippou et al., 2010). Also esterase could have a little effect in super-high resistant strain of brown plant hopper (Wen et al., 2009).

Metabolic enzyme activity analysis showed that the esterases specific activity is significantly higher in susceptible than resistant population (Fig. 1). The difference may be due to the different origins and genetic variation of these two populations. Another possibility is that insects must cope with the toxicity of insecticides and it requires energy and resource allocation for adaptation and survival. Several mechanisms which are used by insects for resistance against insecticide may affect reproduction, impair dispersal ability and have several other effects on the insect's fitness (Kliot & Ghanim, 2012). Reduction of a detoxificant enzyme because of increase in the other detoxificant enzyme has not been reported yet, but possibly it is a reason for low amount of esterase in resistant population.

GST activity

Comparison of the GST activity of susceptible and resistant populations showed 1.4-fold higher activity in resistant population (Fig. 2). Specific activity of GSTs was significantly more in resistant than susceptible population.

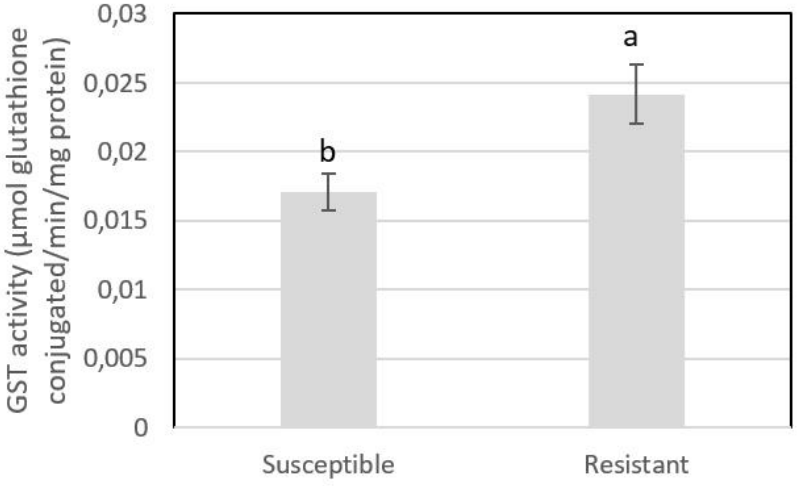

Fig. 2. GST activities in susceptible and resistant populations of *Aphis gossypii* from Fars province, Iran, using CDNB and GSH as substrates. Data are means ± SE. The means followed by different letters are significantly different at $p < 0.05$.

Although a significant specific difference in the activity of GST was noticed in resistant population, but GST's do not play a role in the imidacloprid resistance because DEM did not display synergism in resistant population, where the synergism ratio was 1.18 (Table 1). Li & Han (2007) also reported that in *A. gossypii* DEM had little synergistic effect on imidacloprid on both resistant and susceptible strains. Maybe the higher activity of GST in resistant population is because of history of exposure to other insecticides in addition to imidacloprid before collection as described in materials and methods. Another probability is genetic variation that naturally exists between populations.

Cytochrome P_{450} activity and Monooxygenases contents

Cytochrome P_{450} monooxygenases activity showed significant differences between susceptible and resistant populations (Fig. 3). Specific activity showed 2.1-fold higher activity in resistant population. Experiments with different synergists delivered the same conclusion, as cytochrome P_{450} resulted in a higher synergism ratio (SR=5.82) in resistant populations. In addition to cytochrome P_{450} activity assay, heme peroxidase assay also has been used in resistance experiments and increased heme contents has been known related to resistance in insects and mites (Memarizadeh et al., 2013; Tiwari et al., 2011). Therefore, for more confidence from the amount of cytochrome P_{450}, heme peroxidase assay was done. This assay does not measure mono oxygenase activity but titrates the amount of heme bound in the insect homogenate (Damayanthi & Karunaratne, 2005).

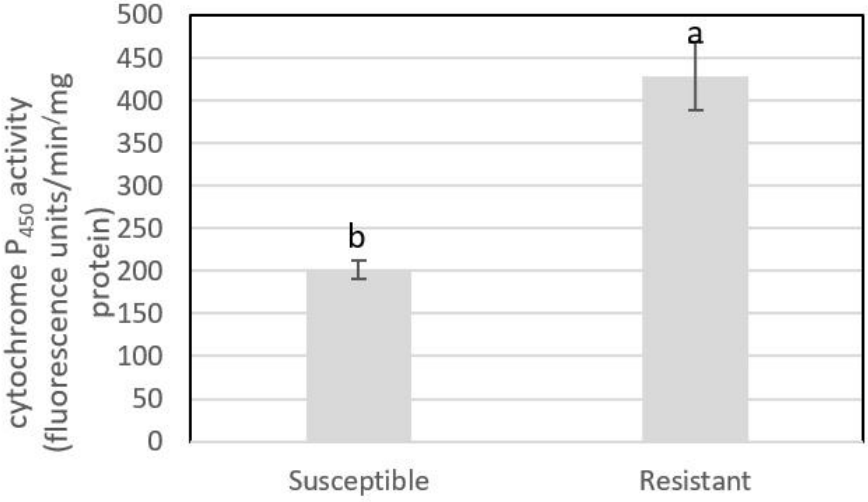

Fig. 3. Cytochrome P_{450} activities in susceptible and resistant populations of *Aphis gossypii* using 7-ethoxycoumarin as substrates. Data are means ± SE. The means followed by different letters are significantly different at $p < 0.05$.

Involvement of mono oxygenases in the imidacloprid resistance of *A. gossypii* was indirectly tested by quantifying bound heme. The results indicated that heme contents in the resistant population was 2.66-fold higher than that in the susceptible population (Fig. 4).

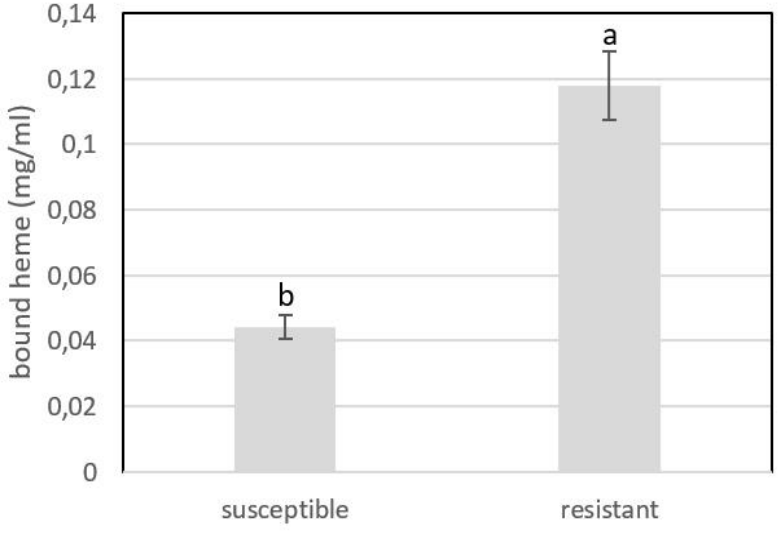

Fig. 4. Means ± SE of heme content in the resistant and susceptible populations of *Aphis gossypii.* The means followed by different letters are significantly different at $p < 0.05$.

Results of this assay confirmed the high amount of cytochrome P_{450}. Therefore, both enzymatic assays and synergism studies indicated that P_{450}- monooxygenase are key factors in imidacloprid detoxification and resistance development. In literature, resistance to imidacloprid has been attributed to increased rates of detoxification, especially enhanced oxidative detoxification of this pesticide by over-expressed P_{450} monooxygenases. In *A. gossypii* (Shi, 2012), *M. persicae* (Philippou et al., 2010; Puinean et al., 2010b), *Drosophila* spp. (Diptera: Drosophilidae) (Daborn et al., 2001), brown planthoppers (*Nilaparvata lugens* Stal; Hemiptera: Delphacidae) (Puinean et al., 2010a; Wen et al., 2009; Zewen et al., 2003) and in *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae) (Karunker et al., 2008; Karunker et al., 2009; Nauen & Denholm, 2005), P_{450}- monooxygenase is thought to be one of the most important mechanisms for imidacloprid resistance.

New insecticides are increasingly difficult and costly to develop; therefore, it is important that the value of the currently used insecticides is not lost to agriculture through the development of insecticide resistance. The ability to understand the status of insecticide resistance, along with the underlying genetics and the risk of resistance development, can significantly contribute to the development of sustainable pest management strategies. More exposure to pesticides may increase resistance, and it should be notable by the pesticide users. Changes in LD_{50} values for imidacloprid are occurring rapidly, which likely is caused by a wide scale use of imidacloprid. Use of imidacloprid is a major factor for the occurrence and development of resistance to this insecticide in Iran. On the other hand, the resistance dynamics studies show that if we stop the spraying pressure, resistance to imidacloprid is not stable (Wang et al., 2009). If the use of these pesticides is interrupted there is great potential for the return of the sensitivity of aphids.

Since imidacloprid is so widely used to control cotton aphid in Iran and with growing concerns about imidacloprid tolerance in many insect species, it is important to develop baseline information for monitoring imidacloprid resistance to the *A. gossypii*. This research provides a basis for developing successful resistance-management plans and efficacy of neonicotinoids in use for aphid control. Resistance needs time to establish and the susceptibility of aphids should be monitored carefully over the next years to keep the effectiveness of this class of compounds as long as possible. This would enable local advisors and farmers to implement the appropriate control strategies to sustain high yields and crop quality.

Acknowledgements

The authors are grateful to Islamic Azad University of Shiraz to prepare greenhouse equipment and for its support to do bioassay experiments. We express our gratitude to the toxicology and physiology laboratory in Plant Protection Department, University of Tehran for generous support to do biochemical assays. Our appreciation is also expressed to Maryam Joharinia and Aysuda Jaafari for their help to do bioassay experiments and Faeze Bagheri for helping to do biochemical experiments.

References

Ahmad, M., M. I. Arif & I. Denholm, 2003. High resistance of field populations of the cotton aphid *Aphis gossypii* Glover (Homoptera: Aphididae) to pyrethroid insecticides in Pakistan. Journal of Economic Entomology, 96: 875- 878.

Bradford, M. M., 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of proteindye binding. Analytical Biochemistry, 7: 248–254.

Cabrera-Brandt, M.A., E. Fuentes-Contreras & C. C. Figueroa, 2010. Differences in the detoxification metabolism between two clonal lineages of the aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) reared on tobacco (*Nicotiana tabacum* L.). Chilean Journal of Agricultural Research, 70: 567-575.

Castañeda, L.E., C.C. Figueroa, E. Fuentes-Contreras, H. M. Niemeyer & R. F. Nespolo, 2009. Energetic costs of detoxification systems in herbivores feeding on chemically defended host plants: a correlational study in the grain aphid, *Sitobion avenae*. Journal of Experimental Biology, 212: 1185-1190.

Castaneda, L.E., C.C. Figueroa & R.F. Nespolo, 2010. Do insect pests perform better on highly defended plants? Costs and benefits of induced detoxification defences in the aphid Sitobion avenae. Journal of Evolutionary Biology, 23: 2474–2483.

Choi, B.R., S. W. Lee & J. K. Yoo, 2001. Resistance mechanisms of green peach aphid, *Myzus persicae* to imidacloprid. Korean-Journal of Applied Entomology, 40: 265- 271.

Daborn, P., S. Boundy, J. Yen, B. Pittendrigh & R. French-Constant, 2001. DDT resistance in Drosophila correlates with Cyp6g1 over-expression and confers cross-resistance to the neonicotinoid imidacloprid. Molecular Genetics and Genomics, 266: 556-563.

Damayanthi, B. T. & S. H. P. P. Karunaratne, 2005. Biochemical characterization of insecticide resistance in insect pests of vegetables and predatory ladybird beetles. Journal of National Science Foundation of Sri Lanka, 33: 115- 122.

De Sousa, G., A. Cuany, A. Brun, M. Amichot, R. Rahmani & J.Berge, 1995. A microfluorometric method for measuring ethoxycoumarin-O-deethylase activity on individual *Drosophila melanogaster* abdomens: interest for screening resistance in insect populations. Analytical Biochemistry, 229: 86-91.

Ebert, T. A. & B. Cartwright, 1997. Biology and ecology of *Aphis gossypii* Glover (Homoptera: Aphididae). Southwestern Entomologist, 22: 115-153.

El-Kady, H., 2007. Insecticide resistance in the cotton aphid, *Aphis gossypii* Glover in Egypt. Journal of Egyptian Society of Toxicology, 36: 43-46.

Furk, C. & C. M. Hines, 1993. Aspects of insecticide resistance in the melon and cotton aphid, *Aphis gossypii* (Hemiptera: Aphididae). Annals of Appllied Biology, 123: 9-17.

Gubran, E.E., R. Delorme, D. Auge & J. P. Moreau, 1992. Insecticide resistance in cotton aphid *Aphis gossypii* (Glov.) in the Sudan Gezira. Pesticide Science, 32: 101-107.

Habig, W.H., M.J. Pabst & W. B. Jakoby, 1976. Glutathione S-transferase AA from rat liver. Archives of Biochemistery and Biophysics, 175: 710–716.

Hama, H., S. Ando, A. Hosada, K. Suzuki & Y. Takagi, 1995. Insecticide resistance in cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae). IV. Susceptibility of four clones separated from vivipara of field populations to various insecticides. Japanese Journal of Applied Entomology and Zoology, 39: 117.

Han, Z., G. D.Moores, I. Denholm & A. L. Devonshire, 1998. Association between Biochemical Markers and Insecticide Resistance in the Cotton Aphid, *Aphis gossypii* Glover. Pesticide Biochemistry and Physiology, 62: 164-171.

Herron, G.A. & L. J. Wilson, 2011. Neonicotinoid resistance in *Aphis gossypii* Glover (Aphididae: Hemiptera) from Australian cotton. Australian Journal of Entomology, 50: 93-98.

Hollingsworth, R.G., B. E. Tabashnik, D. E. Ullman, M. W. Johnson & R. Messing, 1994. Resistance of *Aphis gossypii* (Homoptera: Aphididae) to Insecticides in Hawaii: Spatial Patterns and Relation to Insecticide Use. Journal of Economic Entomology, 87: 293-300.

Huang, H. 2002. Development of diagnostic tools for detecting expression of resistance association esterases in the tobacco budworm, *Heliothis virescens* (F.). (Unpublished) PhD thesis, The Department of Entomology, Louisiana State University, Louisiana, USA, 112 pp.

Iizuka, M., Y. Inoue, K. Murata & A. Kimura, 1989. Purification and Some Properties of Glutathione S-Transferase from *Escherichia coli* B. Journal of Bacteriology, 171: 6039-6042.

Immaraju, J.A., J. G. Morse & O. L. Brawner, 1990. Evaluation of three bioassay techniques for citrus thrips resistance and correlation of the leaf dip method to field mortality. Journal of Agricultural Entomology, 7: 17-27.

Jhansi, K. & G. V. Subbaratnam, 2004. Degree of TPP Suppressible Resistance Acquired by *Aphis gossypii* Glover to Selected Insecticides. Pesticide Research Journal, 16: 38-40.

Karunker, I., J. Benting, B. Lueke, T. Ponge, R. Nauen, E. Roditakis, J. Vontas, K. Gorman, I. Denholm & S.Morin, 2008. Over-expression of cytochrome P450 CYP6CM1 is associated with high resistance to imidacloprid in the B and Q biotypes of *Bemisia tabaci* (Hemiptera: Aleyrodidae). Insect Biochemistry and Molecular Biology, 38: 634-644.

Karunker, I., E. Morou, D. Nikou, R. Nauen, R. Sertchook, B. J. Stevenson, M. J. I.Paine, S. Morin & J.Vontas, 2009. Structural model and functional characterization of the *Bemisia tabaci* CYP6CM1vQ, a cytochrome P450 associated with high levels of imidacloprid resistance. Insect Biochemistry and Molecular Biology, 39: 697–706.

Khanjani, M., 2005. Vegetable Pests in Iran. Bu-Ali Sina University Publishing, Hamedan, Iran (In Persian), 468 pp.

Kim, S., I. Kim & M. Lee,1986. Aphid species and their seasonal fluctuations in vegetable crops. Korean Journal of Plant Protection, 25: 129–131.

Kliot, A. & M.Ghanim, 2012. Fitness costs associated with insecticide resistance. Pest Management Science, 68: 1431–1437.

Li, J. & Z. J. Han, 2007. Primary studies on resistance of *Aphis gossypii* to imidacloprid. Chinease Journal of Pesticide Science, 9: 257-262.

Lowry, O. H., N. J. Rosebrough, A. L. Farr & R. J. Randall, 1951. Protein measurement with the folin phenol reagent. Journal of Biological Chemistry, 193: 265–275.

Marshall, K. L., Y. Chen & G. A. Herron, 2014. “Neonicotinoid Resistance in Cotton Aphid from Australia, 46-48” The 17th Australian Cotton Conference, August 5-7 2014, Broadbeach, Queensland's Gold Coast Convention and Exhibition Centre, 124 pp.

Martin, N. A. & P. J. Workman, 1997. “Melon aphid (*Aphis gossypii*) resistance to pesticides, 405-408” Proceedings of 50th New Zealand Plant Protection Conference, 18-21 August 1997, Lincoln University, Lincoln, 555 pp.

Memarizadeh, N., M. Ghadamyari. P. Zamani&R. H. Sajedi, 2013. Resistance mechanisms to abamectin in Iranian populations of the two spotted spider mite, *Tetranychus urticae* Koch (Acari: Tetranychidae). Acarologia, 55: 235–246.

Mokbel, E. S. & A. L. Mohamed, 2009. Development of resistance in field strain of *Aphis craccivora* to the dinotefuran insecticides from the new class neonicotinoids and its effect on some enzymes content. Egyptian Academic Journal of Biological Sciences, 1: 65- 69.

Nauen, R. & I. Denholm, 2005. Resistance of Insect Pests to Neonicotinoid Insecticides: Current Status and Future Prospects. Archives of Insect Biochemistry and Physiology, 58: 200-215.

Nauen, R., B. Koob & A. Elbert, 1998. Antifeedant effects of sublethal dosages of imidacloprid on *Bemisia tabaci*. Entomologia Experimentalis et Applicata, 88: 287-293.

Nauen, R., J. Strobel, K. Tietjen, Y. Otsu, C. Erdelen & A. Elbert, 1996. Aphicidal activity of imidacloprid against a tobacco feeding strain of *Myzus persicae* (Homoptera: Aphididae) from Japan closely related to *Myzus nicotianae* and highly resistant to carbamates and organophosphates. Bulletin of Entomological Research, 86: 165- 171.

O’Brien, P.J. & J.B. Graves, 1992. Insecticide resistance and reproductive biology of *Aphis gossypii* Glover. Southwest Entomologist, 17: 115.

Philippou, D., L. Field & G. D. Moores, 2010. Metabolic enzyme(s) confer imidacloprid resistance in a clone of *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) from Greece. Pest Management Science,66: 390- 395.

Puinean, A. M., I. Denholm, N. S. Millar, R. Nauen & M. S. Williamson, 2010a. Characterisation of imidacloprid resistance mechanisms in the brown planthopper, *Nilaparvata lugens* Stål (Hemiptera: Delphacidae). Pesticide Biochemistry and Physiology, 97: 129–132.

Puinean, A. M., S. P. Foster, L. Linda Oliphant, I. Denholm, L. M. Field, N. S. Millar, M. S. Williamson & C.Bass, 2010b. Amplification of a Cytochrome P450 gene is associated with resistance to neonicotinoid insecticides in the aphid *Myzus persicae*. PLoS Genetics, 6: 1-11.

Saito, T., H. Hama & K. Suzuki, 1995. Insecticide resistance in clones of cotton aphid, *Aphis gossypii* Glover (Homoptera: Aphididae), and synergistic effect of esterase and mixed function oxidase inhibitors. Japanese Journal of Applied Entomology and Zoology, 39: 151.

Shi, X. 2012. Study on the Mechanisms of Imidacloprid Resistance in Cotton Aphid, *Aphis gossypii* (Glover). (Unpublished) Ph.D. thesis, College of Plant Protection, Pesticide Science, Shandong Agricultural University China, 115 pp.

Shi, X. B., L. L. Jiang, H. Y. Wang, K. Qiao, D. Wang & K. Y. Wang, 2011. Toxicities and sublethal effects of seven neonicotinoid insecticides on survival, growth and reproduction of imidacloprid-resistant cotton aphid, *Aphis gossypii*. Pest Management Science, 67: 1528-1533.

Talebi Jahromi, K., 2011. Pesticides Toxicology. University of Tehran Publishing, Tehran, Iran (In Persian), 492 pp.

Tiwari, S., R. S. Mann, M. E. Rogers & L. L. Stelinski, 2011. Insecticide resistance in field populations of Asian citrus psyllid in Florida. Pest Management Science,67: 1258–1268.

Van Asperen, K., 1962. A study of housefly esterases by means of a sensitive colorimetric method. Journal of Insect Physiology, 8: 401- 416.

Wang, K. Y., T. X. Liu, C. H. Yu, X. Y. Jiang & M. Q. Yi, 2002. Resistance of *Aphis gossypii* (Homoptera: Aphididae) to Fenvalerate and Imidacloprid and Activities of Detoxification Enzymes on Cotton and Cucumber. Journal of Economic Entomology, 95: 407-413.

Wang, K. Y., Q. L. Guo, X. M. Xia, H. Y. Wang & T. X. Liu, 2007. Resistance of *Ahis gossypii* (Homoptera: Aphididae) to selected insecticides on cotton from five cotton production regions in Shandong.Chinese Journal of Pesticide Science, 32: 372-378.

Wang, Y. H., S. G.Wu, Y. C. Zhu, J. Chen, F. Y.Liu, X. P. Zhao, Q. Wang, Z. Li, X. P. Bo & J. L. Shen, 2009. Dynamics of imidacloprid resistance and cross-resistance in the brown planthopper, *Nilaparvata lugens*. Entomologia Experimentalis et Applicata, 131: 20- 29.

Wen, Y., Z. Liu, H. Bao & Z.Han, 2009. Imidacloprid resistance and its mechanisms in field populations of brown planthopper, *Nilaparvata lugens* Stål in China. Pesticide Biochemistry and Physiology, 94: 36-42.

Zewen, L., H. Zhaojun, W. Yinchang, Z. Lingchun, Z. Hongwei & L. Chengjun, 2003. Selection for imidacloprid resistance in *Nilaparvata lugens*: cross-resistance patterns and possible mechanisms. Pest Management Science, 59: 1355-1359.

Türk. entomol. derg., 2015, 39 (4): 425-434 ISSN 1010-6960
DOI: http://dx.doi.org/10.16970/ted.40371

Orijinal araştırma (Original article)

Mısır (*Zea mays* L.)'da kullanılan bazı insektisitlerin *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae)'in ergin öncesi dönemlerine etkileri[1]

Effects of some insecticides used in maize (*Zea mays* L.) on preimaginal stages of *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae)

Alican KURTULUŞ[2*] Serpil KORNOŞOR[2]

Summary

Trichogramma evanescens Westwood (Hymenoptera: Trichogrammatidae) is used for biological control of European corn borer *Ostrinia nubilais* Hübner (Lepidoptera: Crambidae), one of the key pest of maize (*Zea mays* L.) (Poales: Poaceae) in Turkey. Considering other pests, there is a need for integration of chemical control and *T. evanescens* releases. Therefore, effects of chemical insecticides on *T. evanescens* should be known. In this study, effects of insecticides chlorpyrifos ethyl, indoxacarb, chlorantraniliprole, deltamethrin, lambda-cyhalothrin and novaluron on the preimaginal developmental stages of *T. evanescens* were investigated using egg card dipping method in laboratory conditions. Blue egg cards containing approximately 125 parasitised *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs were dipped into insecticide solutions prepared at the recommended dose for 5 second at each developmental stage of *T. evanescens*. Emergence ratio, parasitism capacity, emergence and sex ratio of offsprings were determined. Chlorpyrifos ethyl caused 100% death for all developmental stages and classified as harmful (4) according to the guidelines of IOBC. Deltamethrin and lambda-cyhalothrin was harmless (1) to emergence ratio, but caused reduction on the parasitism capacity (2) and the sex ratio of F_1. Novaluron was slightly harmful (2) when applied during the egg-larval period and harmless to F_1. Chlorantraniliprole and indoxacarb were harmless (1) for all developmental stages.

Keywords: Maize, insecticides, parasitoid, *Trichogramma evanescens*, side effect

Özet

Trichogramma evanescens Westwood (Hymenoptera: Trichogrammatidae), Türkiye'de mısırın ana zararlılarından biri olan Mısırkurdu (*Ostrinia nubilais* Hübner) (Lepidoptera: Crambidae)'nun biyolojik mücadelesinde kullanılmaktadır. Diğer zararlılar da düşünüldüğünde entegre mücadele kapsamında *T. evanescens* salımlarının kimyasal mücadele ile kombine edilmesi gerektiği görülmektedir. Bu yüzden mısırda kullanılan insektisitlerin *T. evanescens* üzerindeki etkilerin iyi bilinmesi gerekmektedir. Bu çalışmada mısırda ruhsatlı insektisitlerden chlorpyrifos ethyl, indoxacarb, chlorantraniliprole, deltamethrin, lambda-cyhalothrin ve novaluron'un *T. evanescens'in* ergin öncesi dönemlerine etkileri yumurta kartı daldırma yöntemi kullanılarak laboratuvar koşullarında araştırılmıştır. Yaklaşık 125 parazitlenmiş *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) yumurtası içeren yumurta kartları, parazitoidin yumurta-larva, prepupa ve pupa dönemlerinde, önerilen dozda hazırlanan insektisit solüsyonlarına 5 saniye süreyle daldırılmış, çıkış oranları, parazitleme kapasitesi, F_1'in çıkış ve eşey oranları belirlenmiştir. Chlorpyrifos ethyl, tüm dönemlerde % 100 ölüme neden olmuş ve IOBC sınıflandırmasına göre 4. sınıfta (zararlı) yer almıştır. Deltamethrin ve lambda-cyhalothrin, çıkış oranlarına bakıldığında zararsızken (1), parazitleme kapasitesinde (2) ve F_1'deki eşey oranında azalmaya yol açmıştır. Novaluron'un *T. evanescens*'in larva dönemine az zararlı olduğu (2), ancak bu zararın F_1'e yansımadığı görülmüştür. Chlorantraniliprole ve indoxacarb, *T. evanescens*'in tüm ergin öncesi dönemlerine zararsızdır (1).

Anahtar sözcükler: Mısır, insektisitler, parazitoit, *Trichogramma evanescens*, yan etki

[1] Bu çalışma birinci yazarın doktora tezinin bir bölümüdür

[2] Çukurova Üniversitesi, Ziraat Fakültesi, Bitki Koruma Bölümü, 01330, Adana
* Sorumlu yazar (Corresponding author) e-mail: alicankurtulus@hotmail.com

Alınış (Received): 08.05.2015 Kabul ediliş (Accepted): 17.12.2015 Çevrimiçi Yayın Tarihi (Published Online): 25.12.2015

Giriş

Türkiye'de mısır (*Zea mays* L.) (Poales: Poaceae) 660 bin ha alanda yetiştirilmekte olup yaklaşık 5.900.000 ton ile en çok üretilen üçüncü tahıl konumundadır. Bu üretimin % 20'sinden fazlasını karşılayan Çukurova'da mısır birinci ve ikinci ürün olarak yetiştirilmektedir (Anonymous, 2015). Birinci üründe tohum ilaçlaması yapılan Bozkurt (*Agrotis* spp.) (Lepidoptera: Noctuidae) ve Telkurdu (*Agriotes* spp.) (Coleoptera: Elateridae) dışında entomolojik problem görülmezken ikinci üründe durum farklıdır (Kornoşor, 1999). Özellikle Mısırkurdu *Ostrinia nubilais* Hübner (Lepidoptera: Crambidae) ve Mısır koçankurdu *Sesamia nonagrioides* Lefebvre (Lepidoptera: Noctuidae), artan popülasyon ve kısalan döl süresine bağlı olarak mücadele yapmadan ekonomik anlamda üretim yapmayı olanaksız kılmaktadır (Kornoşor & Kayapınar, 1989; Kornoşor et al., 1995; Kornoşor, 1999).

Trichogramma Westwood (Hymenoptera: Trichogrammatidae) cinsi başta Lepidoptera olmak üzere birçok takıma ait böceklerin yumurta parazitoti olarak bilinen bir cins olup dünya geneline yayılmış 210 civarında türü içermektedir ve bu türlerden en az 12 tanesi ticari olarak biyolojik mücadelede kullanılmaktadır (Smith, 1996; Pinto, 1998; Consoli et al., 2010). Kolay ve hızlı üretilebilmelerinin yanı sıra zararlıların yumurta dönemine etki edip zararı oluşmadan önce engellemelerinden dolayı büyük önem kazanmışlardır (Smith, 1996; Mills, 2010; Mansour, 2010).

Türkiye'de şimdiye kadar sekiz *Trichogramma* türü tespit edilmiştir (Öztemiz et al., 2013). Bunların arasında yayılışı, etkinliği ve biyolojik mücadelede kullanımından dolayı *T. evanescens* ön plana çıkmaktadır. Çukurova mısır ekilişlerinin % 5'inde bu doğal düşman kullanılarak Mısır kurdu'na karşı biyolojik mücadele uygulanmaktadır ve uygulamaların etkinliği %88,3'e kadar ulaşmaktadır (Öztemiz & Kornoşor, 2007). Ancak başta *S. nonagrioides* olmak üzere diğer zararlılar da hesaba katıldığında tek başına *Trichogramma* salımı yeterli olmamakta, insektisit uygulamalarına ihtiyaç duyulmaktadır. Bununla birlikte *O. nubilalis* mücadelesinde biyolojik ve kimyasal mücadeleyi bir arada kullanmanın, iki yöntemin ayrı uygulanmasından daha etkili, daha ekonomik ve çevre dostu olduğu ortaya konulmuştur (Chapman et al., 2009; Gardner et al., 2011). Bu yüzden *Trichogramma* salımı entegre mücadelenin bir parçası olarak düşünülmelidir ve kimyasal mücadele ile ilişkileri dikkate alınmalıdır.

Dünyada pestisitlerin *Trichogramma* türleri üzerindeki etkilerine yönelik birçok çalışma yapılmıştır (Consoli et al., 1998; Kakakhel & Hassan, 2000; Vieira et al., 2001; Saber et al., 2004; Ksentini et al., 2010; Wang et al., 2014; Ko et al., 2015). Wang et al. (2014), kuru film yöntemiyle 7 farklı kimyasal gruptan insektisitlerin *T. evanescens* erginlerine etkilerini incelemiş, organofosfatların çok zararlı, avermektin, piretroid ve neonikotinoidlerin az zararlı ve IGR'ların zararsız olduğunu bildirmiştir. Bull & Coleman (1985) *Trichogramma* ergin öncesi dönemlerinin, yumurta içerisinde bulunduğundan dolayı pestisitlere nispeten dirençli olduğunu bildirmektedir. Consoli et al. (1998), *Trichogramma pretiosum* Riley ergin öncesi dönemlerinde organofosfat olan penthoate'ın % 100 ölüme yol açtığını, lambda-cyhalothrin'in yumurta-larva dönemine az zararlı, diğer dönemlerine zararsız olduğunu bildirmiştir. Costa et al. (2014) fipronil, lambda-cyhalothrin+thiametoxam, thiametoxam ve spinosad'ın *Trichogramma galloi* Zucchi üzerindeki olumsuz etkilerinin bir sonraki döle de aktarıldığını tespit etmiştir.

İnsektisitlerin *T. evanescens*'in ergin öncesi dönemlerine etkileri üzerinde çok az çalışma vardır (Ksentini et al., 2010). Bu çalışmada Türkiye'de mısırda ruhsatlı farklı etki mekanizmalarına sahip insektisitlerin *T. evanescens* ergin öncesi dönemleri üzerindeki etkileri ve bu etkilerin bir sonraki döle

yansımaları araştırılmıştır. Elde edilen sonuçlar IOBC laboratuvar koşullarında yan etki sınıflandırmasına göre değerlendirilmiştir.

Materyal ve Yöntem

Böcek kültürleri

Çalışmada kullanılan *T. evanescens* ve laboratuvar konukçusu *Ephestia kuehniella* Zeller (Lepidoptera: Pyrallidae) stok kültürleri Adana Biyolojik Mücadele Enstitüsü'nden temin edilmiştir. *E. kuehniella* larvaları 1:1 oranında buğday unu-mısır unu içeren besinlerde 25±1^{O}C sıcaklık, %60 bağıl nem ve 0:24 A:K koşullarında üretilmiş, 100 gr besin için ~1000 *E. kuehniella* yumurtası kullanılmıştır.

Trichogramma evanescens üretimi UV ışını ile steril edilen 1-2 gün yaşında *E. kuehniella* yumurtaları üzerinde; 25±1 C sıcaklık, %70 bağıl nem ve 14:10 A:K koşullarındaki inkübatörlerde gerçekleştirilmiştir. Konukçu yumurtaları 6.5x1.2 cm boyutlarındaki kartlara % 50'lik Arap zamkı ile yapıştırılarak 8x1.5 cm boyutlarındaki cam tüplerdeki *T. evanescens* erginlerine sunulmuştur. Yumurta kartları 24 saat sonra erginlerden ayrılarak yeni tüplere konmuş, ergin beslenmesi için tüp içerisine ince şerit şeklinde % 50'lik bal çözeltisi sürülmüştür.

İnsektisitler

Denemede Türkiye'de mısırda ruhsatlı olan chlorpyrifos ethyl, indoxacarb, chlorantraniliprole, deltamethrin, lambda-cyhalothrin ve novaluron etkili maddelerine sahip insektisitler önerilen dozlarında kullanılmıştır. Kullanılan insektisitlerin ticari isimleri ve önerilen dozları Çizelge 1'de verilmiştir. Ayrıca kontrol olarak distile su kullanılmıştır.

Çizelge 1. Çalışmada kullanılan insektisitlerin ticari adları, formülasyonları ve önerilen dozları

Aktif madde (a.m.)	Ticari adı (firma)	(a.m. g L^{-1})	Formülasyon	Grup[1]	Doz (ml da^{-1})[2]
Chlorpyrifos ethyl	Dursban 4 (Dow)	480	EC	Organofosfat	180
Indoxacarb	Avaunt (DuPont)	150	SC	Diamid	30
Chlorantraniliprole	Coragen (DuPont)	200	SC	Indoxacarb	15
Deltamethrin	Decis (Bayer)	25	EC	Piretroid	50
Lambda-cyhalothrin	Karate Zeon (Syngenta)	50	SC	Piretroid	30
Novaluron	Rimon (Flogaz)	100	EC	Benzoylüre	60

[1]IRAC 2014
[2] 100 l su da^{-1}

Parazitoit ergin öncesi dönemlerine etki

Deneme tesadüf blokları deneme desenine göre 8 tekerrürlü olarak kurulmuş olup her tekerrür 3 adet yumurta kartından oluşmaktadır. Yaklaşık 125 adet 0-24 saat yaşında steril *E. kuehniella* yumurtası 3x1 cm boyutlarındaki mavi kartların üzerine 5 mm çapında bir alana sürülen arap zamkı ile yapıştırılarak cam tüpler içerisinde *T. evanescens* dişilerine maruz bırakılmıştır (Carvalho et al., 2010).Yumurtaların tamamının parazitlenmesi için yeterli olan 24 saatlik süre ardından kartlar tüplerden alınıp üzerinde kalan *T. evanescens* dişileri fırça yardımıyla temizlenmiş, kartlarda bulunan parazitlenmiş yumurtalar sayılmıştır. Parazitlenmenin ardından parazitoitin yumurta-larva, prepupa ve pupa dönemlerini temsil eden 0-24, 72-96 ve 168-192 saatlik süreler sonrasında (Consoli et al., 1999, 2001), hazırlanan ilaç çözeltilerine 5 saniye süre ile daldırılmıştır. Ardından kartlar 1 saat süreyle kurutma kâğıtları üzerinde bekletilerek kurumaları sağlanmıştır ve 8x1.5 cm boyutlarındaki cam tüplere ayrı ayrı yerleştirilerek yukarıda koşulları belirtilen iklim kabinlerine yerleştirilmişlerdir. Bu koşullarda 9 günde açıldığı bilinen

yumurtalar 14. günde kontrol edilerek üzerinde parazitoit çıkış deliği bulunan yumurtalar sayılmıştır. F_0çıkış oranı = [(parazitoit çıkışı olan yumurta/∑ parazitlenmiş yumurta)*100] formülü ile hesaplanmıştır.

İnsektisitlerin F_1 üzerindeki etkilerini belirlemek amacıyla her uygulamadan 24 adet çiftleşmiş dişi parazitoit tek tek tüplere alınmış, %50'lik bal ile beslenmiş ve 24 saat süresince üzerinde ~125 adet steril *E. kuehniella* yumurtası bulunan kartlar sunulmuştur. Bu sürenin ardından kartlar dişilerden ayrılıp tek tek tüplere alınmış ve iklim kabininde gelişmeye bırakılmıştır. Parazitlenmenin ardından 6-7. günlerde kararan yumurtalar sayılarak (Rodriguez et al., 1994) dişi başına parazitleme sayısı belirlenmiş ve % çıkış =[(parazitoit çıkışı olan yumurta/∑ parazitlenmiş yumurta)*100] F_1'in çıkış oranı hesaplanmıştır. Ayrıca çıkış yapan bireyler sayılarak =[∑dişi/∑(dişi+erkek)] formülüyle pestisitlerin F_1'deki eşey oranına etkileri gözlemlenmiştir.

İstatistiksel analizler

Tüm verilere Shapiro-Wilk normalite testi uygulanarak verilerin normal dağılım gösterdiği doğrulanmıştır. Çıkış oranı ve dişi oranına ait verilere arcsin karekök transformasyonu uygulanmış, tablolarda transformasyon uygulanmamış veriler sunulmuştur. Veriler varyans analizine tabi tutulmuş, uygulamalar arasındaki farkların anlamlı olduğu durumlarda Tukey testi kullanılmıştır ($P < 0.05$). Tüm analizlerde IBM SPSS 21 paket programı (SPSS, 2012) kullanılmıştır.

Uygulamaların parazitoidin çıkış oranı (F_0,F_1) ve parazitleme kapasitesine (F_0) etkileri (=R), "R= 100-[(uygulama sonucu-kontrol sonucu) x 100]" formülüne göre hesaplanmıştır (Hassan, 1998). Etki sınıflandırması IOBC'nin önerdiği üzere (Sterk et al., 1999) yapılmış; Sınıf 1 = zararsız (R < % 30), Sınıf 2 = az zararlı (% 30 ≤R< %80), Sınıf 3= orta derecede zararlı (% 80 ≤R< %99), Sınıf 4= zararlı (R≥ %99) olarak değerlendirilmiştir.

Araştırma Sonuçları ve Tartışma

Çıkış oranına etki

Ergin öncesi gelişme dönemlerinde yapılan uygulamalar sonrasında çıkış oranlarında önemli azalmalar meydana gelmiştir (yumurta-larva, F=176,08; df= 6-49; P < 0,001: prepupa, F=155,55; df= 6-49; P < 0,001: pupa, F=146,25; df= 6-49; P < 0,001) (Çizelge 2). Chlorpyrifos ethyl uygulanan tüm dönemlerde çıkış olmamıştır. Maia et al. (2013), *T. atopovirilia* üzerinde yaptığı çalışmada ergin öncesi chlorpyrifos ethyl uygulamasından sonra çıkışın %89 olduğunu ancak çıkıştan kısa süre sonra tüm bireylerin öldüğünü belirtmektedir. Bu çalışmada chlorpyrifos ethyl, uygulandığı tüm dönemlerde zararlı (IOBC sınıf 4) (Sterk et al., 1999) sınıfında yer almıştır (Çizelge 2).

Yumurta-larva ve prepupa döneminde yapılan uygulamalarda lambda-cyhalothrin çıkış oranını önemli ölçüde etkilemezken pupa dönemi uygulamasında çıkış oranını % 10 azaltmıştır. Deltamethrin, yumurta-larva dönemi uygulamalarında çıkışı %23,6 azaltmış, prepupa ve pupa döneminde kontrole göre önemli bir azalmaya yol açmamıştır. Her iki insektisit IOBC sınıflandırmasına göre *T. evanescens* ergin öncesi dönemleri için zararsızdır. Hohmann (1991;1993), sentetik piretroidlerin*T. pretiosum* üzerinde bu çalışmadaki gibi zararsız olduğunu bildirmiştir. Vieira et al. (2001) *T. cordubensis* ergin öncesi dönemlerde lambda-cyhalothrin ve deltamethrin uygulamaları sonrası % 40 ve % 26 civarında azaldığını tespit etmiştir. Ksentini et al. (2010), deltamethrin'in 3 *Trichogramma* türü ergin öncesi dönemleri için IOBC sınıf 3'te yer aldığını belirtmektedir. Daldırma süresinin 10 saniye olması bu çalışmayla aradaki farkı yaratan bir etken olabilir. Suh et al. (2000) ise ergin öncesi lambda-cyhalothrin ve deltamethrin uygulamaları sonrası *Trichogramma exiguum* Pinto & Platner çıkışının %1,4 ve 2,2 olduğunu bildirmiştir. Çıkış oranının, diğer çalışmalara göre oldukça düşük olduğu çalışmada konukçu olarak *Helicoverpa zea*

(Boddie) (Lepidoptera: Noctuidae) yumurtaları kullanılmıştır ve farkın bundan kaynaklandığı düşünülmektedir.

Çizelge 2. *Ephestia kuehniella* yumurtası içerisindeki *Trichogramma evanescens*'in farklı gelişim dönemlerinde yapılan insektisit uygulamaları sonrası yüzde çıkış oranları (Ort±SH)

İnsektisit	Uygulamaya maruz kalan parazitoit dönemi								
	Yumurta-larva			Prepupa			Pupa		
	Çıkış oranı (%)*	ÇA[1] (%)	Sınıf[2]	Çıkış oranı (%)*	ÇA[1] (%)	Sınıf[2]	Çıkış oranı (%)*	ÇA[1] (%)	Sınıf[2]
Kontrol	96,42±1,0 a	-	-	93,63±1,6 a	-		92,75±0,9 a	-	-
Chlorpyrifos-Ethyl	0,00±0,0 d	100	4	0,00±0,0 c	100	4	0,00±0,0 c	100	4
Indoxacarb	71,52±2,9 bc	25,8	1	74,46±2,5 b	20,5	1	75,73±3,0 b	18,4	1
Chlorantraniliprole	79,52±1,5 bc	17,5	1	92,74±2,3 a	1	1	91,14±2,0 a	1,7	1
Deltamethrin	73,62±3,4 bc	23,6	1	90,09±3,0 a	3,8	1	86,19±3,5 ab	7,1	1
Lambda-cyhalothrin	89,57±2,5 a	7,1	1	90,78±1,9 a	3	1	83,46±3,0 ab	10	1
Novaluron	60,7±4,3 c	37	2	92,49±2,8 a	1,2	1	78,31±3,7 b	15,6	1

[1] Parazitleme kapasitesinde azalma
[2] Sterk et al. (1999)'a göre etki sınıflandırması
*Aynı sütunda farklı harfle gösterilen değerler arasındaki fark Tukey testine göre anlamlıdır (P<0,05).

Indoxacarb, uygulandığı tüm dönemlerde %18,4-25,8 arasında çıkışı azaltırken chlorantraniliprole sadece yumurta-larva dönemi uygulamasında çıkışta önemli azalmaya yol açmıştır. Her iki insektisit ergin öncesi dönemlere zararsızdır (IOBC sınıf 1). Sattar et al. (2011), indoxacarb'ın *Helicoverpa armigera* Hübner yumurtaları içerisindeki *T. chilonis*'in yumurta-larva dönemlerine zararsız, prepupa ve pupa dönemlerine az zararlı olduğunu bildirmektedir. Hussain et al. (2012) ise *Sitotroga cerealella* Oliver (Lepidoptera: Pyralidae) yumurtaları içerisindeki *T. chilonis*'in ergin öncesi dönemlerine yapılan chlorantraniliprole uygulamalarında %17,8-70 arasında çıkış olduğunu belirtmektedir. Bu iki çalışmada kullanılan konukçu yumurtası yapılan bu çalışmadakinden farklıdır. Farklı sonuçların konukçu yumurtası geçirgenliğindeki farklardan kaynaklandığı düşünülmektedir. Brugger et al. (2010) ise chlorantraniliprole'un 3 *Trichogramma* türünün ergin öncesi dönemlerine, bu çalışmada olduğu gibi etkisiz olduğunu bildirmektedir.

Novaluron ise pupa döneminde önemli bir etki göstermezken özellikle yumurta-larva dönemi uygulamalarında %37'lik ve pupa dönemi uygulamalarında % 15,6'lık bir azalmaya sebep olmuştur. Novaluron, yumurta-larva döneminde uygulandığında az zararlıdır (IOBC sınıf 2). Maia et al. (2013), *T. atopovirilia*'da ve Carvalho et al. (2010) *T. pretiosum*'da novaluron'un tüm ergin öncesi dönemlere zararsız olduğunu bildirmektedir. Aradaki farkın türden kaynaklandığı düşünülmektedir. Bu çalışmada da novaluron, az zararlı sınıf değerinin hemen üzerinde yer almıştır (% 37).

Parazitleme oranına etki

Farklı gelişme dönemlerinde uygulanan ilaçlar *T. evanescens* dişilerinin parazitlediği yumurta sayılarında önemli azalmalara yol açmıştır (yumurta-larva, F=2,38; df= 5-42; P = 0,054: prepupa, F=6,84; df= 5-42; P < 0,001: pupa, F=5,44; df= 5-42; P = 0,001) (Çizelge 3). Chlorpyrifos ethyl uygulaması sonrası çıkış yapan birey olmadığından bu denemeye alınmamıştır.

Lambda-cyhalothrin yumurta-larva, prepupa ve pupa dönemleri uygulamalarında parazitlemeyi sırasıyla % 33,7, 44,5 ve 45,7 oranında azaltırken deltamethrin % 26,2, 39,2 ve 32,4 oranında düşürmüştür. Parazitleme kapasitesindeki azalma bakımından lambda-cyhalothrin, uygulandığı tüm dönemlerde, deltamethrin ise prepupa ve pupa dönemlerinde az zararlı (IOBC sınıf 2) kategorisinde yer

almıştır (Çizelge 3). Consoli et al. (1998), ergin öncesi lambda-cyhalothrin uygulamalarının *T.pretiosum*'un parazitleme kapasitesinin 30-34 yumurta/gün/dişi olduğunu bildirmiştir. Kontrole göre anlamlı bir düşüş görülmekte ve bu çalışmayla paralellik göstermektedir.

Consoli et al. (2001), bazı kimyasalların konukçu yumurtasını aşıp *Trichogramma* ergin öncesi dönemlerine etki edemediğini, ancak çıkış sırasında, çıkış deliği açmak için yumurta kabuğunu çiğnerken kimyasalları bünyelerine aldıklarını ve bu nedenle çıkış sırasında ölümler görülebildiğini bildirmektedir. Bu çalışmada da *T. evanescens* çıkış oranlarını önemli ölçüde düşürmeyen sentetik piretroidleri bünyelerine çıkış sırasında yumurta kabuğunu çiğnerken aldıkları düşünülmektedir.

Çizelge 3. *Ephestia kuehniella* yumurtası içerisindeki *Trichogramma evanescens*'in farklı gelişim dönemlerinde yapılan insektisit uygulamaları sonrası parazitlediği yumurta sayıları (Ort±SH)

İnsektisit	Uygulamaya maruz kalan parazitoit dönemi								
	Yumurta-larva			Prepupa			Pupa		
	Parazitleme	PA[1] (%)	Sınıf[2]	Parazitleme[*]	PA[1] (%)	Sınıf[2]	Parazitleme[*]	PA[1] (%)	Sınıf[2]
Kontrol	57,29±4,3	-	-	59,79±4,1 a	-	-	51,75±4,0 a	-	-
Chlorpyrifos-Ethyl	ø	ø	ø	ø	ø	ø	ø	ø	ø
Indoxacarb	47,92±5,2	16,4	1	51,46±4,0 ab	13,9	1	44,88±4,2 ab	13,3	1
Chlorantraniliprole	50,67±5,4	11,6	1	48,88±4,0 abc	18,3	1	41,79±3,3 abc	19,2	1
Deltamethrin	42,29±2,3	26,2	1	36,38±3,0 bc	39,2	2	35,00±3,6 bc	32,4	2
Lambda-cyhalothrin	37,96±3,2	33,7	2	33,21±4,0 c	44,5	2	28,08±2,1 c	45,7	2
Novaluron	44,71±5,1	22	1	43,58±3,4 bc	27,1	1	41,25±3,4 abc	20,3	1

[1] Parazitleme kapasitesinde azalma
[2] Sterk et al. (1999)'a göre etki sınıflandırması
[ø] Yeterli sayıda dişi olmadığından denemeye alınmamıştır
[*] Aynı sütunda farklı harfle gösterilen değerler arasındaki fark Tukey testine göre anlamlıdır (P<0,05).

Indoxacarb ve chlorantraniliprole, çıkış yapan erginlerin parazitleme kapasitesinde önemli düşüşlere yol açmamıştır ve zararsızdır (IOBC sınıf 1). Wang et al. (2012), indoxacarb'ın *T. chilonis*'in parazitleme kapasitesinde artışa yol açtığını tespit etmiştir.

Novaluron % 20,3-27,1 arasında değişen oranlarda azalmaya yol açmıştır ve IOBC sınıflandırmasına göre zararsızdır. Carvalho et al. (2010), bu çalışmaya benzer şekilde novaluron'un *T. pretiosum*'un ergin öncesi dönemlerinin parazitleme kapasitesine etkisiz olduğunu bildirmektedir.

F_1 çıkış oranına etki

İnsektisitlere maruz kalan dölün parazitlediği yumurtalardan çıkış yapan bireylerin oranlarında (F_1) anlamlı farklar gözlenmiştir(yumurta-larva, F=7,63; df= 5-42; P < 0,001: prepupa, F=6,29; df= 5-42; P < 0,001: pupa, F=11,33; df= 5-42; P < 0,001) (Çizelge 4). Yumurta-larva, prepupa ve pupa uygulamaları ardından F1 çıkışındaki azalma oranları deltamehrin için sırasıyla % 12,8, 15,4 ve 29,2 olurken lambda-cyhalothrin için 25,5, 16,3 ve 29 olarak belirlenmiştir. Indoxacarb ve chlorantraniliprole F_1 çıkış oranını etkilememiştir. Novaluron için ise larva dönemi uygulaması sonrası F_1 çıkışında % 18'lik bir azalma görülmüş, diğer dönemlerdeki uygulamalarda önemli bir azalma meydana gelmemiştir.

IOBC sınıflandırması temel alındığında tüm uygulamaların F_1 çıkış oranına etkileri 1. sınıfta (zararsız) yer almıştır. Ancak deltamethrin ve lambda-cyhalothrin'in pupa dönemlerine bakıldığında (%29,2 ve 29) sınırda olduğu görülmektedir (<% 30) (Çizelge 4). Garcia et al. (2006) *T. cordubensis* prepupa döneminde yapılan deltamethrin uygulamasının F_0'da çıkış oranını etkilemezken F_1'de kontrole göre önemli ölçüde azalttığını tespit etmiştir ve bunu parazitoidin çıkış sırasında konukçu yumurtasındaki insektisit kalıntısına maruz kalmasına bağlamıştır. Vianna et al. (2009) iki sentetik piretroidin (beta-cyfulthrin ve esfenvalerate) ilaca maruz kalmayan F_1dölünün parazitleme kapasitesinde düşüş gözlemlemiştir. Wang et al. (2012) ise *T. chilonis* üzerinde indoxacarb subletal dozlarının F_1 çıkış oranının

kontrole göre aynı olduğunu bildirmiştir. Carvalho et al. (2010) Yumurta-larva, prepupa ve pupa dönemindeki novaluron uygulamalarının *T. pretiosum* F_1 bireylerinin parazitleme kapasitesinde çok az bir azalmaya yol açtığını ve insektisitin her üç döneme zararsız (Sınıf 1) olduğunu bildirmiştir. Sonuçların bu çalışmayla paralel olduğu görülmektedir.

Çizelge 4. *Ephestia kuehniella* yumurtası içerisindeki *Trichogramma evanescens*'in farklı gelişim dönemlerinde yapılan insektisit uygulamaları sonrası F_1'deki yüzde çıkış oranları (Ort±SH)

İnsektisit	Uygulamaya maruz kalan parazitoit dönemi								
	Yumurta-larva			Prepupa			Pupa		
	Çıkış oranı (%)*	ÇA[1] (%)	Sınıf[2]	Çıkış oranı (%)*	ÇA[1] (%)	Sınıf[2]	Çıkış oranı (%)*	ÇA[1] (%)	Sınıf[2]
Kontrol	90,77±2,3 a	-	-	87,45±2,8 ab	-	-	89,76±2,6 a	-	-
Chlorpyrifos-Ethyl	ø	ø	ø	ø	ø	ø	ø	ø	ø
Indoxacarb	88,11±4,1 a	2,9	1	90,59±2,7 ab	-3,6	1	89,40±3,0 a	0,4	1
Chlorantraniliprole	84,61±2,7 ab	6,8	1	89,55±3,3 ab	-2,4	1	86,45±2,2 a	3,7	1
Deltamethrin	79,19±2,7 abc	12,8	1	73,99±2,7 bc	15,4	1	63,55±4,7 b	29,2	1
Lambda-cyhalothrin	67,59±3,1 c	25,5	1	73,22±4,1 c	16,3	1	63,71±1,6 b	29	1
Novaluron	74,43±4,4 bc	18	1	82,83±2,4 abc	5,3	1	82,59±4,5 a	8	1

[1] Çıkış oranında azalma
[2] Sterk et al. (1999)'a göre etki sınıflandırması
ØYeterli sayıda dişi olmadığından denemeye alınmamıştır
* Aynı sütunda farklı harfle gösterilen değerler arasındaki fark Tukey testine göre anlamlıdır (P<0,05).

F_1 eşey oranına etki

İnsektisit uygulamaları sonrasında F_1dölünün eşey oranında önemli farklar meydana gelmiştir (yumurta-larva, F=11,43; df= 5-42; P < 0,001: prepupa, F=25,52; df= 5-42; P < 0,001: pupa, F=25,8; df= 5-42; P < 0,001) (Çizelge 5).

Çizelge 5. *Ephestia kuehniella* Zeller yumurtası içerisindeki *Trichogramma evanescens*'in farklı gelişim dönemlerinde yapılan insektisit uygulamaları sonrası F_1'deki yüzde eşey oranları (Ort±SH)

İnsektisit	Uygulamaya maruz kalan parazitoit dönemi		
	Yumurta-larva	Prepupa	Pupa
	Dişi oranı (%)*	Dişi oranı (%)*	Dişi oranı (%)*
Kontrol	57,52±2,4 a	60,98±2,2 a	59,49±2,9 a
Chlorpyrifos-Ethyl	ø	ø	ø
Indoxacarb	58,01±2,3 a	60,42±2,8 a	56,95±3,7 a
Chlorantraniliprole	56,41±3,3 a	62,68±3,2 a	59,93±2,2 a
Deltamethrin	40,47±2,7 b	33,63±2 b	34,8±2,6 b
Lambda-cyhalothrin	38,65±2,5 b	31,24±3,9 b	29,4±1,9 b
Novaluron	54,81±2,6 a	58,34±2,9 a	56,17±2,5 a

ØYeterli sayıda dişi olmadığından denemeye alınmamıştır
* Aynı sütunda farklı harfle gösterilen değerler arasındaki fark Tukey testine göre anlamlıdır (P<0,05).

Sentetik piretroidler grubunda yer alan iki insektisit, deltamethrin ve lambda-cyhalothrin, tüm dönemlerdeki uygulamalarda F_1'deki dişi oranını önemli ölçüde düşürmüştür. Diğer insektisitler F_1'deki dişi oranında anlamlı bir değişikliğe yol açmamışlardır. Wang et al. (2012), Indoxacarb'ın *T. chilonis* F1 bireylerinin dişi oranında kontrole göre bir miktar azalmaya sebep olmasına rağmen aynı grupta yer aldığını bildirmiştir. Bazı çalışmalarda piretroidlerin parazitoidlerin sonraki döllerinde eşey oranlarını etkilediği görülürken (Wang et al., 2012) bazı çalışmalarda böyle bir etki görülmemiştir (Saber et al., 2005;

Bayram et al., 2010). Costa et al. (2014), lambda-cyhalothrin+thiodicarb'ın *T. galloi* ergin öncesi uygulamalarında F_1'deki dişi oranının 0,07-0,35 arasında olduğunu tespit etmiştir. Carvalho et al. (2010) novaluron'un prepupa dönemi uygulamasında *T. pretiosum*'un F_1 eşey oranını düşürdüğünü, diğer dönemlerde etkilemediğini belirtmektedir. Bu çalışmada novaluron, uygulandığı hiçbir gelişme döneminde F_1'deki eşey oranını etkilememiştir.

Sonuç olarak, denemede kullanılan insektisitlerden chlorpiyrifos ethyl'in *T. evanescens*'in tüm ergin öncesi dönemlerine çok zararlı olduğu görülmüştür. Konukçu yumurtası içerisinde olan ergin öncesi dönemlerin nispeten korunaklı olduğu bilinmektedir. Buna rağmen tüm uygulamalarda % 100 ölüme neden olan chlorpyrifos ethyl'in mısırda entegre mücadele uygulamaları içerisinde düşünülemeyeceği görülmüştür. Sentetik piretroidler için ergin öncesi dönemlerin korunaklı olduğu görülmüştür. Ancak denemede kullanılan iki sentetik piretroid olan deltamethrin ve lambda-cyhalothrin'in çıkış sonrası parazitleme kapasitesini ve F_1'in dişi oranını düşürdüğü belirlenmiştir. Novaluron ise *T. evanescens*'e larva döneminde uygulandığında çıkış ve parazitleme kapasitesini düşürmüştür. Chlorantraniliprole ve indoxacarb, tüm uygulamalarda zararsız olarak bulunmuştur ve entegre mücadele kapsamında kullanımları uygundur.

Teşekkür

ZF2011D18 no'lu doktora projesi kapsamında yürütülen bu çalışmayı destekleyen Çukurova Üniversitesi Bilimsel Araştırma Projeleri Birimi'ne teşekkürlerimizi sunarız.

Yararlanılan Kaynaklar

Anonymous, 2015. Tahıllar ve diğer bitkisel ürünlerin alan ve üretim miktarları. (Web sayfası: http://www.tuik.gov.tr/PreIstatistikTablo.do?istab_id=1562), (Erişim tarihi: Şubat 2015).

Bayram, A., G. Salerno, A. Onofri & E. Conti, 2010. Lethal and sublethal effects of preimaginal treatments with two pyrethroids on the life history of the egg parasitoid *Telenomus busseolae*. BioControl, 55:697-710.

Brugger, K. E., P. G. Cole, I. C. Newman, N. Parker, B. Scholz, P. Suvagia, G. Walker & T. G. Hammond, 2010. Selectivity of chlorantraniliprole to parasitoid wasps. Pest Management Science, 66(10): 1075-1081.

Bull, D. L. & R. J. Coleman, 1985. Effects of pesticides on *Trichogramma* spp. (Hymenoptera, Trichogrammatidae). Southwestern Entomologist, 8: 156-168.

Carvalho, G. A., M. S. Godoy, D. S. Parreira & D. T. Rezende, 2010. Effect of chemical insecticides used in tomato crops on immature *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae). Revista Colombiana De Entomologia, 36: 10-15.

Chapman, A. V., T. P. Kuhar, P. B. Schultz, T. W. Leslie, S. J. Fleischer, G. P. Dively & J. Whalen, 2009. Integrating chemical and biological control of European corn borer in bell pepper. Journal of Economic Entomology, 102: 287-95.

Consoli, F. L.,J. R. P. Parra & S. A. Hassan, 1998. Side-effects of insecticides used in tomato fields on the egg parasitoid *Trichogramma pretiosum* Riley (Hym., Trichogrammatidae), a natural enemy of *Tuta absoluta* (Meyrick) (Lep., Gelechiidae). Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie, 122: 43-47.

Consoli, F. L.,M. M. Rossi & J. R. P. Parra, 1999. Development time and characteristics of the immature stages of *Trichogramma galloi* and *T. pretiosum* (Hymenoptera, Trichogrammatidae). Revista Brasileira de Entomologia, 43: 271-275.

Consoli, F. L.,P. S. M. Botelho & J. R. P. Parra, 2001. Selectivity of insecticides to the egg parasitoid *Trichogramma galloi* Zucchi, 1988, (Hym., Trichogrammatidae). Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie, 125: 37-43.

Consoli, F.L., J.R.P. Parra & R.A. Zucchi, 2010. Egg Parasitoids in Agroecosystems With Emphasis on *Trichogramma.* Springer, Heidelberg, London, New York, 479 pp.

Costa, M. A., V. F. Moscardini, P. da Costa Gontijo, G. A. Carvalho, R. L. de Oliveira & H. N. de Oliveira, 2014. Sublethal and transgenerational effects of insecticides in developing *Trichogramma galloi* (Hymenoptera: Trichogrammatidae). Ecotoxicology, 23(8):1399-408.

Garcia, P., S. Cabral, L. Oliveira & A. Rodrigues, 2006. Effects of deltamethrin on the reproduction of *Trichogramma cordubensis* (Hymenoptera: Trichogrammatidae). Biocontrol Science and Technology, 16(7):699-708.

Gardner, J., M. P. Hoffmann, S. A. Pitcher & J. K. Harper, 2011. Integrating insecticides and *Trichogramma ostriniae* to control European corn borer in sweet corn: Economic analysis. Biological Control, 56(1): 9-16.

Hassan, S. A., B. Hafes, P. E. Degrande & K. Herai, 1998. The side-effects of pesticides on the egg parasitoid *Trichogramma cacoeciae* Marchal (Hym., Trichogrammatidae), acute dose-response and persistence tests. Journal of Applied Entomology-Zeitschrift Fur Angewandte Entomologie, 122: 569-573.

Hohmann, C. L., 1991. Efeito de diferentes insecticidas sobre a emergencia de *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae). Anais da Sociedade Entomolo´gica do Brasil, 20(1):59-65.

Hohmann, C. L., 1993. Efeito de alguns insecticidas sobre adultos de *Trichogramma pretiosum* Riley. Anais da Sociedade Entomologica do Brasil, 22(3):563-568.

Hussain, D., A. Ali, M. Mushtaq-ul-Hassan, S. Ali, M. Saleem & S. Nadeem, 2012. Evaluation of toxicity of some new insecticides against egg parasitoid *Trichogramma chilonis* (Ishii) (Hymenoptera: Trichogrammitidae). Pakistan Journal of Zoology, 44: 1123-1127.

IRAC Insecticide Resistance Action Committee, 2014. Mode of Action Classification Scheme. (Web sayfası: http://www.irac-online.org/documents/moa-classification/?ext=pdf), (Erişim tarihi: 02/2015).

Kakakhel, S.A. & S.A. Hassan, 2000. The side effects of pesticides on *Trichogramma cacoeciae* Marchal (Hymenoptera: Trichogrammatidae), an egg parasite. Pakistan Journal of Biological Sciences, 3(6): 1011-1013.

Ko, K., Y. D. Liu, M. L. Hou, D. Babendreier, F. Zhang & K. Song, 2015. Toxicity of insecticides targeting rice planthoppers to adult and immature stages of *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae). Journal of Economic Entomology, 108: 69-76.

Kornoşor, S. & A. Kayapınar, 1988. Çukurova Bölgesi mısırlarında zarar yapan Mısırkurdu (*Ostrinia nubilalis* Hbn., Lepidoptera, Pyralidae)'nun yıllık döl sayısı ve populasyon gelişmesi. Türkiye Entomoloji Dergisi, 13(1):15-24.

Kornoşor, S., S. Coşkuntuncel & E. Sertkaya,1995. "Population development of egg parasitoids *Trichogramma evanescens* Westwood and *Platytelenomus busseolae* (Gahan) on the eggs of *Ostrinia nubilalis* Hübner and *Sesamia nonagrioides* Lef. in the Southeast Mediterranean region of Turkey, 91-96". Proceedings of the XVIIIth Conference of the International Working Group on the European Corn Borer and Other Maize Pests.(11-16 September 1995,Turda, Romania), 133 pp.

Kornoşor, S., 1999. "Entomological problems of maize in Turkey, 14-23". Proceedings of the XXth Conference of the International Working group on Ostrinia and other Maize Pests (4-10 September 1999, Adana, Turkey), 197 pp.

Ksentini, I., T. Jardak & N. Zeghal, 2010. Bacillus thuringiensis, deltamethrin and spinosad side-effects on three *Trichogramma* species. Bulletin of Insectology, 63(1): 31-37.

Maia, J. B., G. A. Carvalho, R. L. De Oliveira, O. Lasmar & M. I. S. Leite, 2013. Effects of insecticides used in corn on immature stages of *Trichogramma atopovirilia* (Hymenoptera: Trichogrammatidae). Revista Colombiana De Entomologia, 39: 205-210.

Mansour, M., 2010. Effects of gamma radiation on the Mediterranean flour moth, *Ephestia kuehniella*, eggs and acceptability of irradiated eggs by *Trichogramma cacoeciae* females. Journal of Pest Science, 83(3): 243-249.

Mills, N., 2010. "Egg Parasitoids in Biological Control And Integrated Pest Management, 389-411". In: Egg Parasitoids in Agroecosystems with Emphasis on *Trichogramma* (Eds: Consoli, F. L.,J. R. P. Parra & R. A. Zucchi), Springer, Dordrecht, 479 pp.

Öztemiz, S. & S. Kornoşor, 2007. The effects of different irrigation systems on the inundative release of *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae) against *Ostrinia nubilalis* Hübner (Lepidoptera : Pyralidae) in the second crop maize. Turkish Journal of Agriculture and Forestry, 31: 23-30.

Öztemiz, S., F. Sümer Ercan & A. Ş. Tunçbilek, 2013. Türkiye'de *Trichogramma* (Hymenoptera: Trichogrammatidae) türleri, konukçuları ve sistematiğindeki son gelişmeler. Erciyes Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 29 (3): 240-246.

Pinto, D., 1998. The systematics of the North American species of *Trichogramma* (Hymenoptera: Trichogrammatidae). Memoirs of the Entomological Society of Washington, 22: 287.

Rodriguez, J. R., B. Pintureau & M. Galan, 1994. Determinants of the color of host eggs parasitized by *Trichogramma*-Fuentesi. Entomologia Experimentalis et Applicata, 70: 121-128.

Saber, M., M. J. Hejazi & S. A. Hassan, 2004. Effects of azadirachtin/neemazal on different stages and adult life table parameters of *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae). Journal of Economic Entomology, 97: 905-910.

Saber, M., M. J. Hejazi, K. Kamali & S. Moharramipour, 2005. Lethal and sublethal effects of fenitrothion and deltamethrin residues on the egg parasitoid *Trissolcus grandis* (Hymenoptera: Scelionidae). Journal of Economic Entomology, 98(1):35-40.

Sattar, S., Farmanullah, A. U. R. Saljoqi, M. Arif & J. I. Qazi, 2011. Toxicity of some new insecticides against *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae) under laboratory and extended laboratory conditions. Pakistan Journal of Zoology, 43: 1117-1125.

Smith, S.M., 1996. Biological control with *Trichogramma*: Advances, successes, and potential of their use. Annual Review of Entomology, 41: 375-406.

SPSS, 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.

Suh, C. P. C., D. B. Orr & J. W. Van Duyn, 2000. Effect of insecticides on *Trichogramma exiguum* (Trichogrammatidae : Hymenoptera) preimaginal development and adult survival. Journal of Economic Entomology, 93: 577-583.

Sterk, G., S. A. Hassan, M. Baillod, F. Bakker, F. Bigler, S. Blümel, H. Bogenschütz, E. Boller, B. Bromand, J. Brun, J. N. M. Calis, J. Coremans-Pelseneer, C. Duso, A. Garrido, A. Grove, U. Heimbach, H. Hokkanen, J. Jacas, G. Lewis, L. Moreth, L. Polgar, L. Rovesti, L. Samsoe-Peterson, B. Sauphanor, L. Schaub, A. Stäubli, J. J. Tuset, A. Vainio, M. Van de Veire, G. Viggiani, E. Viñuela & H. Vogt, 1999. Results of the seventh joint pesticide testing programme carried out by the IOBC/WPRS-Working Group 'Pesticides and Beneficial Organisms'. BioControl, 44: 99-117.

Vianna, U. R., D. Pratissoli, J. C. Zanuncio, E. R. Lima, J. Brunner, F. F. Pereira& J. E. Serrao, 2009. Insecticide toxicity to *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae) females and effect on descendant generation. Ecotoxicology, 18 (2):180-186.

Vieira, A., L. Oliveira & P. Garcia, 2001. Effects of conventional pesticides on the preimaginal developmental stages and on adults of *Trichogramma cordubensis* (Hymenoptera : Trichogrammatidae). Biocontrol Science and Technology, 11: 527-534.

Wang, D. S., Y. R. He, X. L. Guo, and Y. L. Luo, 2012. Acute toxicities and sublethal effects of some conventional insecticides on *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae). Journal of Economic Entomology 105 (4):1157-1163.

Wang, Y. H., C. X. Wu, T. Cang, L. Z. Yang, W. H. Yu, X. P. Zhao, Q. Wang & L. M. Cai, 2014. Toxicity risk of insecticides to the insect egg parasitoid *Trichogramma evanescens* Westwood (Hymenoptera: Trichogrammatidae). Pest Management Science, 70 (3): 398-404.