academicJournals

Vol. 9(5), pp. 106-113, 15 March, 2014
DOI: 10.5897/SRE2014.5823
Article Number: C33C88046721
ISSN 1992-2248

http://www.academicjournals.org/SRE

Scientific Research and Essays

Full Length Research Paper

Toxicity and repellent effects of some botanical insecticides on the egg-larval parasitoid *Chelonus oculator* Panzer (Hymenoptera: Braconidae)

Hilal TUNCA*, Neşet KILINÇER and Cem ÖZKAN

Department of Plant Protection 06610 Diskapi Ankara, Faculty of Agriculture, Ankara University, Turkey.

Received 28 January, 2014; Accepted 25 February, 2014

Chelonus oculator **Panzer is an egg-larval parasitoid of the cotton leaf worm, *Spodoptera littoralis* (Boisd.) with a broad geographical area. Four botanical insecticides - azadirachtin (Neem Azal), pyrethrum (Spruzit Neu), capsaicin (Hotpepper wax) and d-Limonene (Orange guard) - were investigated regarding their side effects on *C. oculator*. Sub-lethal concentrations (LC_{25} and LC_{50}) were determined for azadirachtin and pyrethrum. Then, the parasitized third larval stage of *S. littoralis* was treated with LC_{25} and LC_{50} values for the same insecticides. Behavioural effects of the botanical insecticides on *C. oculator* were performed by choice tests using a Y-tube olfactometer. The results revealed that LC_{50} value of pyrethrum was very harmful causing 100% mortality to *C. oculator.* LC_{50} and LC_{25} values of azadirachtin and LC_{25} values of pyrethrum prolonged development time whilst reducing the longevity, emergence rate and adult dry mass. The behavioural test indicated that the tested botanical insecticides have strong repellent effects on the parasitoid. Thus, this study contributes to the amelioration of the safe use of botanical insecticides against the natural enemy in integrated pest management programs.**

Key words: *Chelonus oculator,* side effect, azadirachtin, pyrethrum, capsaicin, d-Limonene.

INTRODUCTION

Cotton is an important agricultural crop for many countries. Several insect pests, however, have negatively affected output in all cotton planting areas (Luttrell et al., 1994). There are different methods of controling these agricultural pests. Chemical control is the most common method for pest control in cotton agriculture. The negative effects of synthetic insecticides resulting from their uninformed use include environmental and human health problems. Recently plant protection application has proposed to decrease the use of synthetic insecticides (Mullen and Durden, 2002; Ofuya, 1997). An important application for controling these pests is the use of biological control methods, which have been accented by researchers, as getting a bright view of alternative to insecticide application (Crespo et al., 1998; Hogsette, 1999; Carmo et al., 2010). There are, however, alternative

*Corresponding author. E-mail: htunca@agri.ankara.edu.tr. Tel: (90 312) 5961140. Fax: (90 312) 3187029.

chemical control methods such as the use of botanical insecticide that is less harmful to the environment and humans, yet some of them are very toxic to fish and other cold-blooded animals, and there in should be used with care (Illinois Pesticide Review, 2004). In addition, botanical insecticides have detrimental and behavioral effects on insect pests: They affect insect growth and development, have antifeedant and arrestant effects, and they also have antifungal, antiviral and antibacterial properties against pathogens (Prakash and Rao, 1986, 1997). Botanical insecticides can be divided into five major chemical categories: Nitrogen compounds, terpenoids, phenolics, proteinase inhibitors and growth regulators (Khater, 2012). However, these botanical insecticides should not be considered to be the only solution. It is indeed crucial to define the side effects of these insecticides on natural enemies since they may have a negative effect on natural enemies; and therefore botanical insecticides should be tested for their toxic effects on parasitoids and predators.

Parasitoid *Chelonus oculator* Panzer (Hymenoptera: Braconidae) could parasitize important lepidopterous pests. These pests are *Spodoptera littoralis* Boisd (Lepidoptera: Noctuidae), *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae), *Agrotis segetum* Denis & Schiffermüller, *Helicoverpa armigera* Hübner*, Heliothis viriplaca* Hufnagel, *H.peltigera* Denis & Schiffermüller, *Spodoptera exigua* Hübner, *Photedes elymi* Treitschke (Lepidoptera: Noctuidae), *Etiella zinckenella* Treitschke (Lepidoptera: Phycitidae), *Pyrausta sticticalis* (L.) (Lepidoptera: Pyraustidae), *Coleophora anatipennella* Hübner (Lepidoptera: Coleophoridae), and *Zeiraphera isertana* (F.) (Lepidoptera: Tortricidae) (Tobias, 1995; Özkan and Özmen, 2001; Desneux et al., 2010). Parasitoid's eggs are laid in the host eggs individually. They hatch in the host eggs, and the first and second instar of the parasitoid feed internally. In its third instar, the parasitoid larvae leave the host to feed externally, consuming all except the skin and head capsule. The parasitoid then spins its cocoon in the pupal cell which was previously prepared by the host larva (Özkan and Özmen, 2001).

This study, thus, examines the toxic effects of some botanical insecticides using *C. oculator* Panzer (Hymenoptera: Braconidae), a solitary egg-larval endoparasitoid of the cotton leaf worm, *Spodoptera littoralis* (Boisd.). The botanical insecticides, azadirachtin and pyretrum tested in this study are used to control the cotton pest *S. littoralis.* In the cotton agroecosystem, we can also see different groups of pests (e.g. aphids, whiteflies, mites). Other botanical insecticides such as the hotpepper wax (capsaicin) and Orange guard (d-limonene) are proven effective against aphids, mites, whiteflies, leaf hoppers, scale insects etc. in the cotton agroecosystem. Hotpepper wax and Orange guard used for these target pests indirectly affect natural enemies. In this study, the repellent effects of capsaicin and d-limonene were examined against *C. oculator* in this study. Toxicity bioassays were conducted to assess the sub-lethal effects of two products derived from azadirachtin and pyrethrum on the parasitoid. Also the repellent effects of azadirachtin, pyrethrum, capsaicin and d-Limonene were investigated through the use of Y tube olfactometer. This study aims to incorporate these botanicals with biological control into integrated pest management approach.

MATERIALS AND METHODS

Culture of the hosts and parasitoid

Ephestia kuehniella, *S. littoralis* and *C. oculator* were obtained from the University of Ankara, Faculty of Agriculture, Department of Plant Protection. *Chelonus oculator* was reared on *Ephestia kuehniella* under laboratory conditions of 25 ± 1°C, 60-70%R.H. The *Ephestia* cultures were kept in plastic cages (27 × 37 × 7 cm) on a 2 : 1 mixture of wheat flour and rough wheat bran containing approximately 400 g food, which was sterilized at 60°C in 3 days, and 5000 (0.078 g) host eggs (Özkan, 1999).

S. littoralis was used as the natural host of *C. oculator*. *S. littoralis* larvae were reared on lettuce leaves in plastic containers (15 × 20 × 7.5 cm). Lettuce leaves were sterilized by 1% NaOCl before being given to the larvae. Lettuce leaves were given to larvae every day. By pupation, individual pupae were transferred into adult rearing cages with 20% honey solution. A paper strip (5 × 15) was suspended in the cage during the laying of eggs. Eggs on the paper towel strip were transferred into a clean plastic container for hatching. *S. littoralis* were reared under controlled conditions of 25 ± 1°C, 60-70%R.H. and 16 : 8 h (L : D) photoperiod (Ozmen, 2004).

C. oculator was reared at 25 ± 1°C, 60-70%R.H., 16 : 8 h (L : D) photoperiod. Eggs obtained from the *E. kuehniella* culture, were used for production. Average 500 eggs of the host (24-48 h old) were glued on to paper sheets (4 × 15 × 10 cm) and set up with the fed and reproduced parasitoids located in a 10 L glass jar. For adult parasitoids, honey was spread over the paper strips carrying the host eggs. Parasitized *E. kuehniella* eggs by *C. oculator* adults for 24 h, were placed into plastic containers (15 × 20 × 7.5 cm) carrying 400 g of sterile food. This process was repeated daily. Adult parasitoids were utilized both for the existing experiments and for the set-up of the parasitoid culture (Ozkan, 2006).

Acute toxicity bioassays on *S. littoralis*

In the experiment two botanical insectides, azadirachtin (Neem Azal ®-T/S, Trifolio–M GMBH, Germany-10 g/L azadirachtin) and pyrethrum (Spruzit® Neu, Neudorff, Germany-18.36 g/L Natural-Pyrethrum) were used. LC_{50} and LC_{25} values were determined on the third-instar larvae of *S. littoralis* for azadirachtin and pyrethrum with 8 (10, 50, 100, 250, 500, 1000, 1500, 2000 ppm) and 6 (10, 50, 100, 250, 500, 1000 ppm) doses. Four replicates were used for each dose. Each set of four dishes containing 30 larvae was sprayed with an experimental treatment, allowed to dry for 10-15 min in a laminar flow cabinet and maintained at 25 ± 1°C and 16-h photophase. Mortality was assessed after 24 h.

Sublethal effects of the botanicals on the development of the *Chelonus oculator*

In order to obtain the parasitized host, a single wasp was put

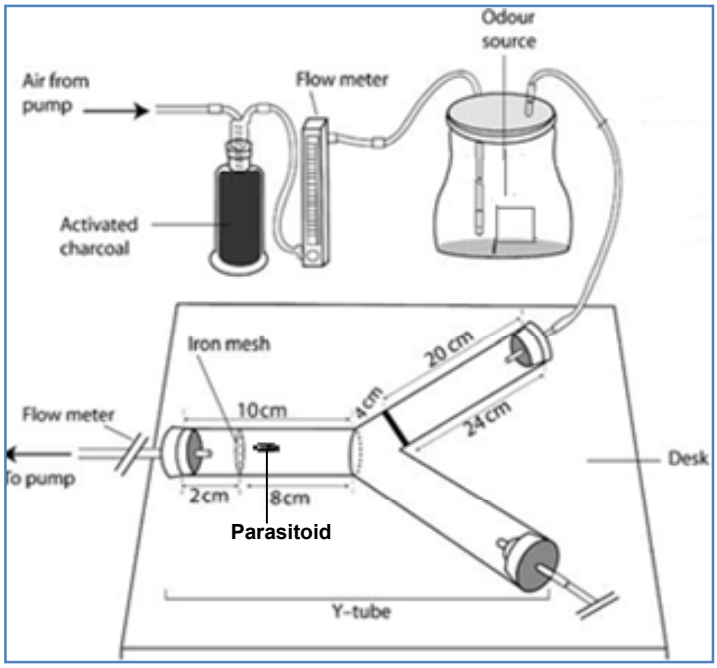

Figure 1. A schematic diagram of the Y-tube olfactometer.

together with the host eggs in Petri dishes (9 cm). The parasitoid was observed during oviposition until the characteristic parasitization behavior occurred (Ozkan, 2006). If the egg was rejected by the parasitoid, the host egg was eliminated. Parasitized eggs were immediately placed into plastic containers (15 × 20 × 7.5 cm) with excess food until first instar larva eclosion and first instar larvae were separated into groups including 15 larvae. These larvae were fed with lettuce leaves. The parasitized third-stage larvae of *S. littoralis* were sprayed LC_{50} and LC_{25} concentrations with azadirachtin and pyrethrum, and kept dry in a laminar flow cabinet. After 24 h, the living larvae were transferred to a plastic container with excess food. These larvae were reared in the laboratory to assess adult emergence. In the control, only distilled water was sprayed. LC_{50} and LC_{25} concentrations were applied using a Potter spray tower (Potter, 1952). Treatments were repeated three times. The effects of two botanical insecticides on development time, emergence ratio, longevity and adult dry mass of *C. oculator* were defined.

Olfactory bioassays

Olfactometric assays were performed using the methods described by Akol et al. (2003). In choice testing, the behavioural response of naive female *C. oculator* adults to azadirachtin (LC_{50} and LC_{25}), pyrethrum (LC_{50} and LC_{25}), capsaicin (1:32) (Hotpepperwax) and d-limonene (1:4) (Orange guard), and clean air was evaluated by a Y-tube olfactometer.

The source of test odours was placed in a glass flask (250 ml capacity) (Figure 1). Two pressure pumps (Cole-Parmer Air cadet vacuum/pressure station, Illinois, U.S.A) pumped air into and out of the system. Air from the recess pressure pump was passed through a carbon filter (Whatman Carbon-Cap 75, Clifton, NJ) for purification, then through a flowmeter (Cole-Parmer Instrument Co., Vernon Hills, Illinois, USA) and finally split into two currents with each current passing into an odour source flask. A second flowmeter was connected to the stem of the olfactometer and to a second pump, which exhausted air out of the system. Airflow into the olfactometer was set at 100 ml/min and at the exit at 500 ml/min.

The filter papers were then sprayed to near run-off with azadirachtin, pyrethrum, capsaicin, d-limonene or water alone and allowed to air-dry before being used in the tests. Naïve female parasitoids (2-3 days old) were introduced singly into the stem of the olfactometer and allowed 5 min to choose one of the arms of the olfactometer. Parasitoids that passed the finish line (marked 4 cm past the intersection) and remained for more than 15 s in the olfactometer arm were recorded as having made a choice. For the control, air was drawn through an empty flask. In all of the tests, each parasitoid was used only once and then discarded. The experiments were conducted three times, and each replicate involved 10 adult parasitoids. All the tests were conducted at 25°C, 65-75% RH. All materials used in the experiments were sterilized with alcohol following each use.

Statistical analysis

The dose-response bioassay data for LC_{50} and LC_{25} determinations were analyzed by the probit procedure (Finney, 1971). Differences were considered significant when 95% fiducial limits (FL) did not overlap. Emergence, longevity and reproduction data were analyzed with one-way analyses of variance (ANOVA), and means were separated using the Duncan's test at a significance level of $\alpha = 0.05$ (SAS Institute, 2003). Percentage data was arcsine transformed before analysis. In the olfactometric assay, the data was analysed using the Z test.

RESULTS

Acute toxicity bioassays on *S. littoralis*

Table 1 displays the LC values obtained by the treatment

Table 1. Results of probit analysis of the concentration-mortality data for *Spodoptera littoralis.*

Larval stage-a.i	n	Slope±SE	X^2 (df)	LC_{50}(95% CL)	LC_{25}(95% CL)
L3-azadirachtin	960	4.614±0.723	39.693(30)	979.316(783.939-1109.015)	699.441(468.962-358.929)
L3-pyrethrum	720	1.680±0.107	37.154(22)	129.972(104.748-160.231)	51.571(37.797-33.005)

Table 2. Sublethal effects of azadirachtin on the development of *Chelonus oculator.*

Sex	Dose	Development time (day)	Emergence* ratio (%)	Longevity (day)	Adult dry mass (mg)
♀	LC_{25}	27.23 ± 0.26^{B}; n=17	40.43^{B}	10.23 ± 0.32^{B}; n=17	1.65 ± 0.002^{B}; n=17
	LC_{50}	36.81 ± 0.31^{A}; n=16	33.46^{C}	6.81± 0.34^{C}; n=16	1.60 ±0.004^{C}; n=16
	Control	21.30 ± 0.33^{C}; n=23	53.96^{A}	18.56 ± 0.37^{A}; n=23	1.76 ±0.013^{A}; n=23
♂	LC_{25}	22.65 ± 0.27^{B}; n=23	40.43^{B}	5.91 ± 0.23^{B}; n=23	1.59 ±0.003^{B}; n=23
	LC_{50}	31.76 ± 0.31^{A}; n=21	33.46^{C}	5.23 ± 0.21^{B}; n=21	1.55 ±0.004^{C}; n=21
	Control	18.70 ± 0.30^{C}; n=27	53.96^{A}	13.63 ± 0.30^{A}; n=27	1.68 ±0.003^{A}; n=27

Columns with the different letter are significantly different (DUNCAN test, P <0.05). *The emergence rates were calculated together for both sexes.

Table 3. Sublethal effects of pyrethrum on the development of *C. oculator*

Sex	Dose	Development time (day)	Emergence ratio (%)	Longevity (day)	Adult dry mass (mg)
♀	LC_{25}	35.08 ± 0.41^{A}; n=12	33.30^{B}	8.00± 0.27^{B}; n=12	1.61± 0.004^{B};n=12
	LC_{50}	-	-	-	-
	Control	21.30 ± 0.33^{B}; n=23	53.96^{A}	18.56 ± 0.37^{A}; n=23	1.76 ±0.013^{A}; n=23
♂	LC_{25}	28.88 ± 0.37^{A}; n=18	33.30^{B}	4.11 ± 0.26^{B}; n=18	1.53±0.004^{B}; n=18
	LC_{50}	-	-	-	-
	Control	18.70 ± 0.30^{B}; n=27	53.96^{A}	13.63 ± 0.30^{A}; n=27	1.68 ±0.003^{A}; n=27

Columns with the different letters are significantly different (DUNCAN test, P <0.05).*The emergence rates were calculated together for both sexes.

of the 3rd larval instars of *Spodoptera littoralis* with different concentrations of the tested compounds. LC_{50} and LC_{25} values of azadirachtin were higher than pyrethrum.

Sublethal effects of the botanicals on the development of *Chelonus oculator*

The results of this study have been summarized in Tables 2 and 3, showing the effects of azadirachtin and pyrethrum. Azadirachtin caused significant effects on the development time, emergence ratio, longevity and adult dry mass (Table 2).

The development time of female and male *C. oculator* was 27.23, 22.65 days for LC_{25} concentration and 36.81, 31.76 days for LC_{50} concentration. Both sublethal doses of azadirachtin prolonged development time of female and male as the control (df = 2, F_{female} = 599.77, P = 0.000; df = 2, F_{male} = 491.60, P = 0.000). The emergence rates of *C. oculator* treated with azadirachtin were 40.43 and 33.46 for LC_{25} and LC_{50} concentrations, respectively. Azadirachtin reduced the emergence rate of *C. oculator* as a control (df = 2, F = 67.85, P = 0.000). Azadirachtin drastically reduced longevity of adults and adult dry mass in all the tested concentrations. Means for these parameters were significantly different (df = 2, F_{female} = 302.01, P = 0.000; df = 2, F_{male} = 329.96, P = 0.000) for adult longevity; (df = 2, F_{female} = 68.19, P = 0.000; df = 2, F_{male} = 346.95, P = 0.000) for adult dry mass.

Like azadirachtin, pyrethrum caused significant effects on the development time, emergence ratio, longevity and adult dry mass (Table 3). The results indicated that pyrethrum was highly toxic. LC_{50} value caused 100% mortality in *C. oculator.* Development time of *C. oculator* from parasitized *Spodoptera* larva was significantly affected by LC_{25} value (df = 1, F_{female} = 619.24, P = 0.000; df = 1, F_{male} = 448.53, P = 0.000). The resulting progeny emergence rate decreased considerably by 33.30% (df = 1, F = 112.38, P = 0.000). In line with this, pyrethrum drastically reduced longevity of adults and adult dry mass at LC_{25}. Means for these parameters were significantly

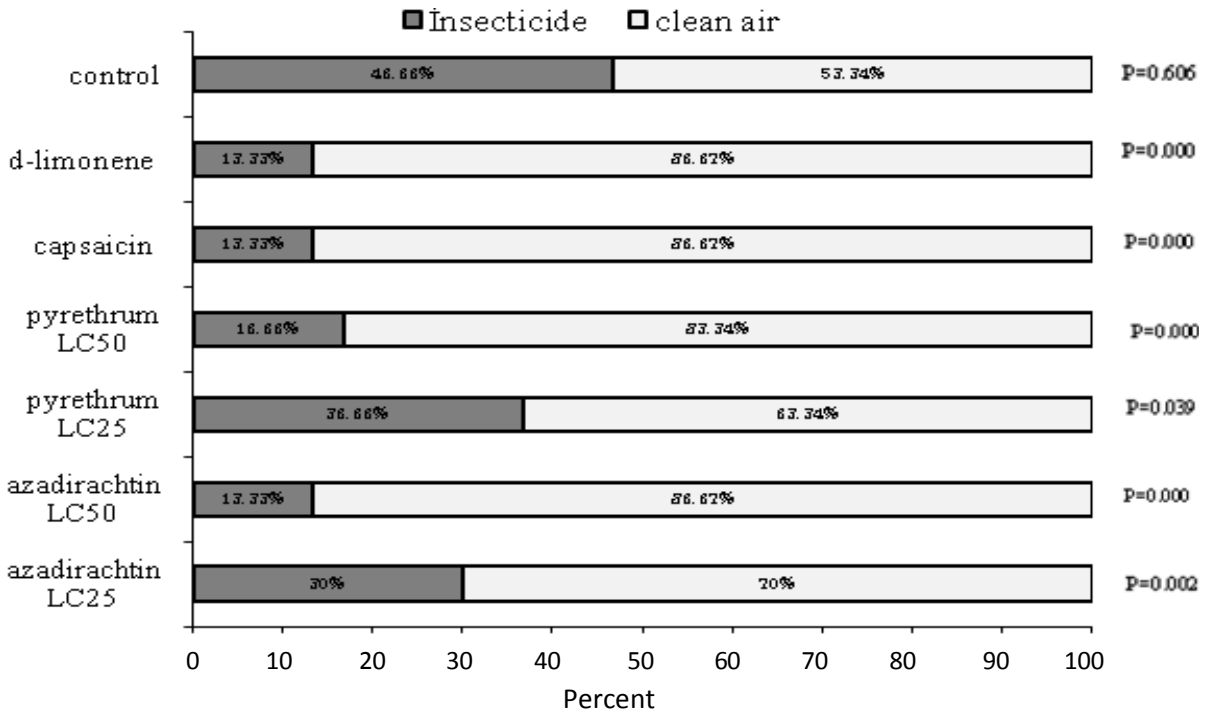

Figure 2. Response of naive female *C. oculator* to odours from a filter paper sprayed with azadirachtin, pyrethrum, capsaicin, d-limonene, and water.

different (df = 1, F_{female} = 364.68, P = 0.000; df = 1, F_{male} = 488.83, P = 0.000) for adult longevity; (df = 1, F_{female} = 61.51, P = 0.000; df = 1, F_{male} = 654.94, P = 0.000) for adult dry mass (Table 3).

Olfactory bioassays

This Y-tube olfactometer facilitated the rapid covering of volatiles depending on attractiveness or repellency to *C. oculator*. There was no significant difference between clean air and water sprayed ones (P>0.05). Female *C. oculator* responded considerably to the botanical insecticides (Figure 2). Regarding the choice between a hotpepper-clean air and orange guard-clean air, it was found that significantly more parasitoids chose the arm from clean air (P<0.05; P<0.05). Similarly, in the choice test between azadirachtin (LC_{25} and LC_{50})-clean air (P<0.05; P<0.05) and pyrethrum (LC_{25} and LC_{50})-clean air, the parasitoid chose clean air (P<0.05; P<0.05) (Figure 2).

DISCUSSION

Natural enemies account for an important element of many integrated pest management programs just as parasitoids and predators adversely affect synthetic chemical insecticides. Botanical insecticides offer an alternative to synthetic chemical insecticides, especially azadirachtin and pyrethrum-based insecticides which are used in ecologically-based pest management. Yet, botanical insecticides can be harmful to beneficial insects. Recently, side effect studies have become increasingly important, and thus, these studies of botanical insecticides should also be tested on natural enemies, as is the case with synthetic chemical insecticides.

The present study shows that the egg-larval parasitoid of *C. oculator* has shown sensitivity towards azadirachtin and pyrethrum. The development time of the parasitoid increased seriously by different sublethal doses of azadirachtin for both sexes. LC_{25} and LC_{50} doses of azadirachtin increased the development time of female parasitoid by 5.93 and 15.51 days respectively. In the male parasitoid, this increase was 3.95 and 13.06 days respectively. The emergence rate of the adult parasitoid was affected by both azadirachtin doses (LC_{25}-LC_{50}). LC_{25} and LC_{50} doses of azadirachtin reduced the mean emergence ratio of the adults by 13.53 and 20.5% respectively. The longevity of female and male *C. oculator* treated with the LC_{25} and LC_{50} doses of azadirachtin reduced compared to that of the control (8.33 and 11.75 days; 7.72 and 8.4 days respectively). In addition, azadirachtin was observed to have a significant effect on the adult dry mass of female and male *C. oculator.* As with emergence ratio and longevity, data indicated that increasing the sublethal concentration from

LC_{25} to LC_{50} decreases the adult dry mass of female *C. oculator* from 1.76 to 1.60 and 1.65 respectively, and the dry mass of male *C. oculator* from 1.68 to 1.55 and 1.59 respectively (Table 1).

However, pyrethrum was found to be of toxic compounds to *C. oculator*. Application of LC_{50} completely prevented the development of the parasitoid. Our study showed that application of LC_{25} of pyrethrum negatively affects the development of *C. oculator*. LC_{25} dose of pyrethrum increased the development time of the female and male parasitoid by 13.78 and 10.18 days, respectively – an increase which was more than the azadirachtin. LC_{25} dose of pyrethrum reduced the mean emergence ratio of the adults by 20.66%. The longevity of female and male *C. oculator* treated with the LC_{25} dose was reduced in comparsion to the control (10.56 and 9.52 days, respectively). Similarly, pyrethrum reduced adult dry mass of female and male *C. oculator* (Table 2).

Studies on the side effects of botanicals - some of which have been summarized below - have revealed a polarity in that while some studies have reported that plant-derived insecticides have very little or no effect on natural enemies, others have stated that botanical insecticides have serious side effects on beneficial insects.

The side effects of two commercial neem products (Neem Azal T/S as foliar application and Neem Azal- U as soil application) on *Eretmocerus warrae* Naumann & Schmidt (Hym: Aphelinidae) and *Encarsia formosa* Gahan (Hym: Aphelinidae) were investigated. The results of this study showed that both parasitoids were higly susceptible to the neems. Parasitoid emergence was affected in a dose-dependent manner, but parasitoids were less exposed to damage with the soil application (Kumar et al., 2010). Tang et al. (2002) investigated the effects of 11, 45 and 180 ppm of azadirachtin on the development of parasitoid *Lysiphlebus testaceipes* (Cresson) (Hymenoptera: Aphidiidae). The results of the study also indicated that 11 and 45 ppm of azadirachtin was not harmful to survival and adult emergence, but 180 ppm of azadirachtin caused a small, significant reduction in the survival and emergence rate of parasitoids. In their study, Price and Schuster (1991) reported that neem seed extract reduced the population of *Encarsia* spp. and *Aleurodiphilus* spp., parasitoids of *Bemisia tabaci* Genn (Homoptera: Aleyrodidae). In a later study conducted by Saber et al. (2004) the negative effects of Neemazal 1% on *Trichogramma cacoeciae* Marchal (Hymenoptera: Trichogrammatidae) were found. Younes (2008) obtained a significant side effect of azadirachtin on the predator *Eretes sticticus* Linnaeus (Coleoptera: Dytiscidae). LC_{50} and LC_{90} treatments of azadirachtin had adverse effects on development, immature survival and prey consumption. Mordue and Blackwell (1993) reported that nymphs and larvae of some beneficial insects were more vulnerable to direct contact with azadirachtin under the laboratory conditions. Simmonds et al. (2002) found that pyrethrum was very toxic to both whitefly and *E. formosa*.

The moderate effects of azadirachtin on adult survival and reproduction, however, were detected only at the highest concentration assayed on the egg parasitoid *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) (Raguraman and Singh, 1999) and the coreid parasitoid *Gryon fulviventre* Crawford (Hymenoptera: Scelionidae) (Mitchell et al., 2004). Other reports pointed to the lack of negative effects on the survival of the diamondback moth parasitoids *Cotesia plutellae* Kurdjumov (Hymenoptera: Braconidae) or *Diadromus collaris* Gravenhorst (Hymenoptera: Ichneumonidae) (Charleston et al., 2005), or on longevity and reproduction of the larval parasitoid *Bracon hebetor* Say (Hymenoptera: Braconidae) (Raguraman and Singh, 1998) were observed. All these studies suggested that azadirachtin and pyrethrum have different effects on parasitoids. Their side effects may vary depending on formulation, dose, host and beneficial insect species and stages.

In biological control, behavioral studies frequently reveal important aspects of biology that would otherwise be neglected - such as the influence of pre-release handling on establishment success and the response of natural enemies to host-induced plant volatiles (Mills and Kean, 2010). Repellent or attractive volatiles could be used to improve the success of pest management strategies. In this study, the repellent effects of azadirachtin, pyrethrum, capsaicin and d-Limonene on the parasitoid were defined by choice tests in Y-tube olfactometer, and important repellent effects were observed in all the insecticides tested (Figure 1). The repellency was related to the type of botanicals and doses. This repellency can negatively affect host acceptance, host suitability and parasitism rates in *C. oculator*.

In their studies Satti et al. (2010) and Mandal (2011) reported that azadirachtin has a repellent effect. Boeke et al. (2003) found that in the choice test with Y tube, oil of the *Azadirachta indica* (Meliaceae) has displayed a repellent effect on the parasitoid *Uscana lariophaga* Steffan (Hymenoptera: Trichogrammatidae). A similar result was obtained for *B. hebetor* (Raguraman and Singh, 1998). In their 2002 study, Simmonds et al. showed that pyrethrum extract did deter the parasitoid *E. formosa* from stabbing into treated host nymphs.

In conclusion, this study, designed to integrate these botanical pesticides with biological control, points out that the use of botanical insecticides should be considered alongside the use of biological controls agent. For instance, pyretrum has been found to be non-compatible with *C. oculator* - it was very toxic for the parasitoid, leading to the conclusion that the insecticide was a risk factor for the parasitoid. The introduction of azadirachtin resulted in significant reduction in the development of parasitoid. These botanical insecticides have odors, chemical signals, and they affect the parasitoid *C.*

oculator behavior; thus, this chemical signal can play an important role in the relations between *C. oculator* and *S. littoralis*. Taking all these effects into consideration, this study argues that side effect studies should be conducted before plant-derived insecticides are integrated into biological control agents.

Conflict of Interest

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENT

This study has been supported by the Scientific and Technological Research Council of Turkey (TUBITAK, Project number: TOGTAG 2679).

REFERENCES

Akol AM, Njagi PGN, Sithanantham S, Mueke JM (2003). Effects of two neem insecticide formulations on the attractiveness acceptability and suitability of diamondback moth larvae on the parasitoid, Diadegma mollipla (Holmgren) (Hym: Ichneumonidae). J. Appl. Entomol. 127(6):325-331. http://dx.doi.org/10.1046/j.1439-0418.2003.00771.x

Boeke SJ, Sinzogan AAC, de Almeida RP, de Boer PWM, Jeong G, Kossou DK, van Loon JJA (2003). Side-effects of cowpea treatment with botanical insecticides on two parasitoids of Callosobruchus maculatus. Entomol. Exp. Appl. 108:43-51. doi: http://dx.doi.org/10.1046/j.1570-7458.2003.00066.x

Carmo EL, Bueno AF, Bueno RCOF (2010). Pesticide selectivity for the insect egg parasitoid Telenomus remus. BioControl. 55:455-464. http://dx.doi.org/10.1007/s10526-010-9269-y

Charleston DS, Kfir R, Dicke M, Vet LEM (2005). Impact of botanical pesticides derived from Melia azederach and Azadirachta indica on the biology of two parasitoid species of the diamondback moth. Biol Cont 33: 131-142. http://dx.doi.org/10.1016/j.biocontrol.2005.02.007

Crespo DC, Lecuona RE, Hogsette JA (1998). Biological control: an important integrated management of Musca domestica (Diptera: Muscidae) in caged-layer poultry houses in Buenos Aires, Argentina. Biol. Cont. 13:16-24. http://dx.doi.org/10.1006/bcon.1998.0632

Desneux N, Wajnberg E, Wyckhuys KAG, Burgio G, Arpaia S, Narváez-Vasquez CA, González-Cabrera J, Ruescas DC, Tabone E, Frandon J, Pizzol J, Poncet C, Cabello T, Urbaneja A (2010). Biological invasion of European tomato crops by Tuta absoluta: ecology, geographic expansion and prospects for biological control. J. Pest Sci. 83:197-215 http://dx.doi.org/10.1007/s10340-010-0321-6

Finney DJ (1971). Probit analysis. Cambridge University Press, New Yorg, 333 pp.

Khater HF (2012). Prospect of botanical biopesticides in insect pest management. J. App. Pharm. Sci. 2 (5):244-259. http://dx.doi.org/10.7324/JAPS.2012.2546

Kumar P, Singh HP, Poehling HM (2010). Effects of neem on adults of Eretmocerus warrae (Hym. Aphelinidae), a parasitoid of Bemisia tabaci (Hom., Aleyrodidae) in tropical horticulture systems. J. Plant Dıs. Protect. 117(6):273-277.

Hogsette JA (1999). Management of ectoparasites with biological control organisms. Int. J Parasitol. 29:147-151. http://dx.doi.org/10.1016/S0020-7519(98)00190-8

Luttrell RG, Fitt GP, Ramalho FS, Sugoyaev ES (1994). Cotton pest management: Part 1A. Worldwide perspective. Ann. Rev. Ent. 39:527-542. http://dx.doi.org/10.1146/annurev.en.39.010194.002523

Mandal S (2011). Repellent activity of Eucalyptus and Azadirachta indica seed oil against the filarial mosquito Culex quinquefasciatus Say (Diptera: Culicidae) in India. Asia. Pac. J. Trop. Biomed. 1 (1):109-112. http://dx.doi.org/10.1016/S2221-1691(11)60135-4

Mills NJ, Kean JM (2010). Behavioral studies, molecular approaches, and modeling: Methodological contributions to biological control success. Biol. Cont. 52(3):255-262. http://dx.doi.org/10.1016/j.biocontrol.2009.03.018

Mitchell PL, Gupta R, Singh A, Kumar P (2004). Behavioral and developmental effects of neem extracts on Clavigralla scutellaris (Hemiptera:Coreidae) and its eggs parasitoid, Gryon fulviventre (Hymenoptera: Scelionidae). J. Econ. Entomol. 97:916-23. http://dx.doi.org/10.1603/0022-0493(2004)097[0916:BADEON]2.0.CO;2

Mordue AJ, Blackwell A (1993). Azadirachtin: An update. J. Insect Physiol. 39:903-924. http://dx.doi.org/10.1016/0022-1910(93)90001-8

Mullen G, Durden L (2002). Medical and Veterinary Entomology. Academic Press, London, UK.

Ofuya TI, (1997). Effect of some plant extracts on two coccinellid predators of the cowpea aphid, Aphis crassivora (Homoptera: Aphididae). Entomophaga: 42:277-282. http://dx.doi.org/10.1007/BF02769904

Ozkan C (2006). Effect of host diet on the development of the solitary egg-larval parasitoid Chelonus oculator in superparasitized Ephestia kuehniella. Phytoparasitica 34(4):338-346. http://dx.doi.org/10.1007/BF02981019

Özkan C (1999). Venturia canescens (Grav) (Hymenoptera: Ichnenumonidae) ile Ephestia kuehniella (Lepidoptera: Pyralidae) arasındaki bazı biyolojik ilişkiler üzerine araştırmalar. Doktora Tezi. Ankara Üniversitesi Fen Bilimleri Enstitüsü, P. 80.

Özkan C, Özmen D (2001). A new record for Turkish fauna Chelonus oculator Panzer (Hymenoptera: Braconidae) and its two new hosts. Turk. J. Entomol. 25(4):263-265.

Ozmen D (2004). Chelonus oculator Panzer (hymenoptera: braconıdae) ile konukçuları *Spodoptera lıttoralıs* (boısduval) ve Ephestıa kuehnıella Zeller arasındaki biyolojik ilişkiler ve aldıcarb'ın parazitoid üzerine etkilerinin radyoizotop izleme tekniği ile araştırılması. Ankara Üniversitesi Fen Bilimleri Enstitüsü Doktora Tezi. P. 150.

Illinois Pesticide Review (2004). Natural Indeed: Are Natural Insecticides Safer and Better Than Conventional Insecticides?, 17, 3. https://www.ideals.illinois.edu/bitstream/handle/2142/993/v17n304.pdf

Potter C (1952). An improved laboratory apparatus for applying direct sprays and surface films with data on the electrostatic charge on atomised spray fluids. Ann. Appl. Biol. 39:1-28. http://dx.doi.org/10.1111/j.1744-7348.1952.tb00993.x

Prakash A, Rao J (1986). Evaluation of plant products as antifeedants against the rice storage insect Proceeding from the Symposium on Residues and Environmental Pollution. pp. 201-205.

Prakash A, Rao J (1997). Botanical pesticides in agriculture. Boca Raton, CRC Lewis Publisher, P. 481. PMid:9444859

Price JE, Schuster DJ (1991). Effects of natural and synthetic insecticides on sweetpotato whitefly, Bemisia tabaci (Homoptera: Aleyrodidae) and its Hymenopterous parasitoids. Flor. Entomol. 74:60-68. http://dx.doi.org/10.2307/3495240

Raguraman S, Singh RP (1998). Behavioral and physiological effects of neem (*Azadirachta indica*) seed kernel extracts on larval parasitoid, Bracon hebetor. J. Chem. Ecol. 24:1241-1250.
http://dx.doi.org/10.1023/A:1022403104083

Raguraman S, Singh RP (1999). Biological effects of neem (Azadirachta indica) seed oil on eggs parasitoid, Trichogramma chilonis. J. Econ. Entomol. 92:1274-1280.

Saber M, Hejazi MJ, Hassan SA (2004). Effects of Azadirachtin/ Neemazal on different stgaes and adult life table parameters of Trichogramma cacoeciae. J. Econ. Entomol. 97(3):905-910. http://dx.doi.org/10.1603/0022-0493(2004)097[0905:EONODS]2.0.CO;2

SAS Institute (2003). .SAS/STAT Version 8.2. SAS Institute, Cary, NC

Satti AA, Ellaithy ME, Mohamed AE (2010). Insecticidal activities of neem (*Azadirachta indica* A. Juss) seeds under laboratory and field conditions as affected by different storage durations. Agric. Biol. J. N. Am. 1 (5):1001-1008. http://dx.doi.org/10.5251/abjna.2010.1.5.1001.1008

Simmonds MSJ, Manlove JD, Blaney WM, Khambay BPS (2002). Effects of selected botanical insecticides on the behaviour and

mortality of glasshouse whitefly Trialeurodes vaporiarum and the parasitoid Encarsia formosa. Entomol. Experiment. et Appl. 102:39-47. http://dx.doi.org/10.1046/j.1570-7458.2002.00923.x

Tang YQ, Weathersbee AA, Mayer RT (2002). Effect of neem seed extract on the brown citrus aphid (Homoptera: Aphididae) and its parasitoid Lysiphlebus testaceipes (Hymenoptera: Aphidiidae). Environ. Entomol. 31:172-176. http://dx.doi.org/10.1603/0046-225X-31.1.172

Tobias VI (1995). Keys to the insect of the European part of U.S.S.R., New Delhi, Baba Barkha Nath, P. 900.

Younes AA (2008). Side effects of azadirachtin fed prey on the diving predator Eretes sticticus Linnaeus (Coleoptera: Dytiscidae). Egypt J. Biol. Pest Cont. 18 (2):347-351.