Orijinal araştırma (Original article)

# Side-effects of some botanical insecticides and extracts on the parasitoid, *Venturia canescens* (Grav.) (Hymenoptera: Ichneumonidae)<sup>1</sup>

Bazı bitkisel insektisitlerin ve ekstraktların *Venturia canescens* (Grav.) (Hymenoptera: Ichneumonidae) üzerindeki yan etkileri

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# Summary

Effects of botanical insecticides, azadirachtin and pyrethrum, on the development and behavior; capsaicin and d-Limonene, on behavior of *Venturia canescens* Gravenhorst were determined with a two-choice test using Y-tube olfactometer. The  $LC_{50}$  and  $LC_{25}$  values for azadirachtin and pyrethrum were also determined in the third and fifth larval stages of the host *Ephestia kuehniella* Zeller. The third and fifth larval stages of *E. kuehniella* were parasitized and then these larvae were treated with  $LC_{50}$  and  $LC_{25}$  values for the same botanical insecticides. While development time of the parasitoid was prolonged, the longevity and emergence rate were reduced at the  $LC_{50}$  and  $LC_{25}$  values of azadirachtin. Pyrethrum was much more toxic for the parasitoid with  $LC_{50}$  value caused 100% mortality of *V. canescens*.  $LC_{25}$  value of pyrethrum caused a great reduction in parasitoid progeny, prolonged development and reduced the longevity. In addition, botanical insecticides and extracts were repellent to parasitoid adults. The use of azadirachtin, pyrethrum, capsaicin and d-Limonene is therefore not compatible with parasitoid *V. canescens*.

Key words: Venturia canescens, azadirachtin, pyrethrum, capsaicin, d-Limonene

# Özet

Bitkisel insektisitlerden azadirachtin ve pyrethrum'un *Venturia canescens*'in gelişimine ve davranışına etkileri, bitkisel ekstraktlardan capsaisin ve d-Limonene'nin ise parazitoitin davranışına etkileri belirlenmiştir. Davranış çalışmalarında parazitoit Y tüp olfaktometre ile ikili seçim testine tabi tutulmuştur. Denemelerde azadirachtin ve pyretrum için LC<sub>50</sub> ve LC<sub>25</sub> değerleri *Ephestia kuehniella*'nın üçüncü ve beşinci dönem larvalarında belirlenmiştir. *E. kuehniella*'nın üçüncü ve beşinci dönem larvaların belirlenmiştir. *E. kuehniella*'nın üçüncü ve beşinci dönem larvaların belirlenmiştir. *E. kuehniella*'nın üçüncü ve beşinci dönem larvalarında belirlenmiştir. *E. kuehniella*'nın üçüncü ve beşinci dönem larvaları parazitletildikten sonra aynı bitkisel insektisitlerin LC<sub>50</sub> ve LC<sub>25</sub> dozları ile muamele edilmiştir. Sonuçta, azadirachtin'in her iki dozunda da parazitoitin gelişme süresinde artış, buna karşın yaşam süresi ve çıkış oranında azalma belirlenmiştir. Pyrethrum, parazitoit için çok daha toksik bulunmuştur. Pyrethrum'un LC<sub>50</sub> dozu, *V. canescens*'de %100 ölüme neden olmuştur. Pyrethrum'un LC<sub>25</sub> dozu ise parazitoit çıkışını önemli ölçüde azaltmış, parazitoitin gelişme süresini uzatmış ve yaşam süresini kısalmıştır. Buna ilaveten bitkisel insektisit ve ekstraktların parazitoit erginleri üzerinde uzaklaştırıcı etkisi bulunmaktadır. Bu çalışmanın sonuçları, azadirachtin, pyrethrum, capsaicin and d-Limonene'nin parasitoid *V. canescens* ile birlikte kullanımının uygun olmadığını göstermektedir.

Anahtar sözcükler: Venturia canescens, azadirachtin, pyrethrum, capsaicin, d-Limonene

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# Introduction

Synthetic insecticides have been used for 50 years and have provided fast, economical and effective pest control. However, excessive use of chemical products has caused some negative effects such as pesticide resistance, emergence of new pests, side effects on natural enemies and environmental contamination. Some of the disadvantages of synthetic pesticides can be prevented by using more biodegradable pest control materials with greater selectivity (Raguraman & Singh, 1999). This situation represents a serious menace for biological control agents and, more generally, for the environment. For these reasons, the search for alternative and environmentally "friendly" methods of pest control are encouraged (Crespo et al., 1998; Hogsette, 1999). One such alternative is the use of new botanical insecticides (Arnason et al., 1989; Isman, 1994) that are effective against target organisms and have shorter persistence in ecosystems (Stark & Walter, 1995). Botanical insecticides (plant extracts), like chemical insecticides, have toxic, repellent and/or antecedent effects on their target pests (Maiteki & Lamb, 1985; Nagui et al., 1989; Dimetry & Schmidt, 1992). But botanical insecticides should never be considered as the only means of plant protection. There might be negative impacts on the environment and human health, like synthetic insecticides. Evaluation of the side-effects of insecticides on beneficial insects has attracted much research (Franz et al., 1980; Hassan et al., 1983; Raguraman & Singh, 1998; Simmonds et al., 2002). There is a belief that botanical insecticides can be used compatibly with parasitoids and predators. In this study, effects of the botanical insecticides, azadirachtin and pyrethrum, on the development and behavior, and capsaicin and d-Limonene, on behavior of Venturia canescens Gravenhorst (Hymenoptera: Ichneumonidae), were determined.

Among botanical insecticides, azadirachtin, a tetranortriterpenoid compound, is considered the most important active component in neem seed kernels. This triterpenoid shows variable effects on insect pests, including oviposition and feeding deterrence, growth regulation, fecundity and fitness reduction (Schmutterer, 1990). Although neem is considered generally safe to beneficials, there are reports of its adverse effects on parasitoids (Condor Golec, 2007). Pyrethrum is a plant derived insecticide made from *Chrysanthemum cinerariifolium* (Trevir) and *Chrysanthemum coccineum* (Willd.) flowers and can be used in many areas (US Environmental Protection Agency Office of Pesticide Programs, 2009). Pyrethrum is also used with success in both home gardens and organic farming. Pyrethrum is on most lists of approved insecticides for organic agriculture and has become a dominant insecticide (Glynne-Jones, 2001).

Capsaicin is the active component of chilli peppers, which belong to the genus *Capsicum*. Capsaicin and several related compounds are called capsaicinoids and are produced as a secondary metabolite by chili peppers, probably as deterrents against certain herbivores (Tewksbury & Nabhan, 2001). d-Limonene naturally occurs in many fruits (especially citrus fruits), vegetables, meats, spices, and other food items (Van Straten & Maarse, 1983). It is contact poison that may be synergized by piperonyl butoxide (PBO). This compound vaporises quickly from treated surfaces and is not residual. It has been recorded for use against fleas, aphids and mites, but also kill fire ants, several types of flies, paper wasps. d-Limonene is also an insect repellent (Buss & Park-Brown, 2002).

The parasitoid *V. canescens* is a synovigenic solitary koinobiont endoparasitoid that is known to attack and develop successfully in the larvae of many lepidopterous pests of stored products. The egg is laid directly in the host larva which continues to feed and grow for at least several days, with the precise time depending on the stage attacked (Frilli, 1965; Salt, 1975; Salt, 1976; Harvey & Vet, 1997; Özkan, 1999). The parasitoid has both sexual (arrhenotokous) and parthenogenetic (thelytokous) reproduction and females of either mode occur sympatrically in Southern Europe (Schneider et al., 2002; Leach, 2009). In this study thelytokous females were used. These wasps have been used for many years in numerous laboratory studies on insect physiology, genetics, behaviour, life-history and population dynamics (Ahmad, 1936; Simmonds, 1943; Corbet, 1968; Rogers, 1972; Salt, 1975; Hardy et al., 1992;

Harvey, 1996; Beukeboom & Pijnacker, 2000; Schneider et al., 2002; Roberts et al., 2004; Eliopoulos, 2006; Sahin & Özkan, 2007), but side-effect studies are highly limited (Elliot et al., 1983; Adarkwah et al., 2011). The aim of this study was to determine the susceptibility of *V. canescens* to botanical insecticides and to identify insecticides that could be safely used with this parasitoid. For this purpose, the  $LC_{50}$  and  $LC_{25}$  values were determined for azadirachtin and pyrethrum for different stages of the host *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae). The developmental effects of azadirachtin and pyrethrum were investigated in two subletal doses ( $LC_{50}$  and  $LC_{25}$ ) on *V. canescens*. Also, their behavioral effects and those of two botanical extracts (capsaicin and d-Limonene) were investigated with the use of Y tube olfactometer.

### **Materials and Methods**

#### **Culture of the Host and Parasitoid**

Larvae of *Ephestia kuehniella* were used as parasitoid hosts. The host was reared at  $25 \pm 1^{\circ}$ C, 60-70% R.H. Culturing was undertaken using clear plastic containers ( $27 \times 37 \times 7$  cm) on a 2:1 mixture of wheat flour and rough wheat bran containing approximately 400 g food, which was sterilized at 60°C for 3 days, and 5,000 host eggs (Bulut & Kılınçer, 1987).

The parasitoid was cultured on mature larvae of *E. kuehniella* in plastic containers (as for *E. kuehniella*, above) at 25°C and 60-70% relative humidity with a 16:8 h light:dark photoperiod. In order to rear the parasitoid, ten 4- to 5-day-old adult parasitoids which had been fed daily with pure honey were transferred into the container including approximately 250 g sterilized food and approximately 300 mature, 29-day-old larvae. After 24 h for parasitization, parasitoids were removed from the container in order to prevent probable superparasitism. This procedure was repeated every two days with new hosts and new parasitoids (Özkan, 1999).

#### Acute toxicity bioassays on Ephestia kuehniella

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 third instar (15 days) and fifth instar (29 days) of *E. kuehniella* larvae for azadirachtin with 8 doses (50, 100, 250, 500, 1000, 2000, 4000, 5000 ppm).  $LC_{50}$  and  $LC_{25}$  values of *E. kuehniella* larvae for pyrethrum were determined on third (15 days) and fifth instars (29 days) with 6 doses (10, 50, 100, 250, 500, 1000 ppm) and 7 doses (50, 100, 250, 500, 1000, 2000, 3000 ppm). For each developmental stage and insecticide, 30 individuals were used in petri dishes (9 cm). 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 min in a laminar flow cabinet and maintained at 25 ± 1°C and 16-h photophase. Mortality was assessed after 24 h.

#### Sublethal effects on the development of the Venturia canescens

The third and fifth instars of *E. kuehniella* were singly parasitized by *V.canescens*. Following a successful oviposition, the parasitoid preens and transfers a new egg to the tip of her ovipositor via a characteristic flexing motion of the abdomen (the 'cocking' motion described by Rogers, 1972). Parazitized third and fifth instars of *E. kuehniella* larvae were sprayed with azadirachtin and pyrethrum at  $LC_{50}$  and  $LC_{25}$  concentrations and kept dry in a laminar flow cabinet. After 24 h, living larvae were transferred to plastic boxes containing enough fresh food. These larvae were left until parasitoid eclosion. In the control, only distilled water was sprayed. Treatments were applied using a Potter spray tower (Potter, 1952). Treatments were replicated three times. Effects on development time, emergence ratio, longevity and adult dry mass of *V. canescens* were defined.

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#### **Olfactory bioassays**

Olfactometric assays were conducted according to the methods described by Akol et al. (2003). In choice testing, the behavioural response of naive *V. canescens* 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 assessed in a Y-olfactometer. The schematic diagram of the Y-tube olfactometer was taken from Akol et al. (2003). 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 between the stem of the olfactometer and 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 paper were then sprayed to near run-off with the azadiracthtin, pyrethrum, capsaicin, d-Limonene or water alone and allowed to air-dry before being used in the tests. Naive, 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 tests, each parasitoid was used only once and discarded. Experiments were carrying out three times and each replicate involved 10 adult parasitoids. All tests were conducted at 25°C, 65-75% R.H. All materials used in the experiments were sterilized with alcohol after each use.

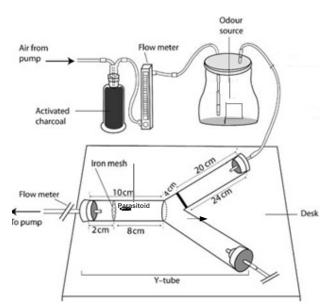


Figure 1. A schematic diagram of the Y-tube olfactometer.

#### Statistical analysis

Dose–response bioassay data for  $LC_{50}$  and  $LC_{25}$  determinations were analyzed with 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). Percentage data were arcsine transformed before analysis. In olfactometric assays, data were analysed using the Z test.

# Results

#### Acute toxicity bioassays on Ephestia kuehniella

Using multiple dose assays with azadirachtin and pyrethrum, different  $LC_{50}$  and  $LC_{25}$  values for the different developmental stages of *E. kuehniella* was determined. The result indicated that  $LC_{50}$  and  $LC_{25}$  values of the third stages were lower than that of the fifth stages (Table1).

A.İ.	Larval stage	n	Slope±SE	X <sup>2</sup> (df)	LC <sub>50*</sub> (95% CL)	LC <sub>25</sub> * (95% CL)
azadirachtin	L3	960	2.74±0.35	36.45 30	583.99 (409.30-722.63)	311.71 (166.48-436.69 <b>)</b>
	L5	960	1.89±0.10	49.35 30	1287.59 (1089.88-1528.88)	567.61 (458.61-681.51)
pyrethrum	L3	720	1.36±0.09	44.35 22	85.10 (63.50-111.33)	27.286 (17.17-38.52)
	L5	840	1.86±0.10	22.43 26	447.21 (392.43-508.70)	194.51 (163.10-226.76)

Table 1. Results of probit analysis of the concentration-mortality data for Ephestia kuehniella

\*:ppm

The results of this study are summarized in Tables 2 and 3 which show the effects of azadirachtin and pyrethrum on the development of the parasitoid. Azadirachtin had significant effects on the development time, emergence ratio, longevity and adult dry mass of *V. canescens* (Table 2). The development time of *V. canescens* from parasitized *Ephestia* larvae (L3 and L5) treated with azadirachtin at LC<sub>25</sub> and LC<sub>50</sub> concentrations was 43.53 and 46.60, and 38.36 and 40.09 days, respectively. Both sublethal doses of azadirachtin prolonged the development time of *V. canescens* when compared to the control (F<sub>2</sub>=308.85, *P*<0.05; F<sub>2</sub>=98.36, *P*<0.05). The emergence rates of *V. canescens* from parasitized *Ephestia* larvae (L3 and L5) treated with azadirachtin were 42.01 and 36.26, and 31.28 and 24.38, for LC<sub>25</sub> and LC<sub>50</sub> concentrations, respectively. Azadirachtin reduced the emergence rate of *V. canescens* when compared to the control (F<sub>2</sub>=551.27, *P*<0.05; F<sub>2</sub>=458.92, *P*<0.05). At all the tested concentrations, adult longevity was significantly reduced (L3, F<sub>2</sub>=101.10, *P*<0.05; L5, F<sub>2</sub>=109.50, *P*<0.05) compared to the control. Similarly, adult dry mass decreased significantly (L3, F<sub>2</sub>=541.10, *P*<0.05; L5, F<sub>2</sub>=350.52, *P*<0.05) in comparison with the control.

Table 2. Effects of azadirachtin on the development time, emergence ratio, longevity and adult dry mass of Venturia canescens

Larva stage	Dose	Development Time (day)	Emergence Ratio (%)	Longevity (day)	<i>Adult</i> Dry Mass (mg)
L3	LC <sub>25</sub>	43.53±0.46 B n=45	42.01 B	4.44±0.19 B n=45	1.47±0.024 B n=45
	LC <sub>50</sub>	46.60±0.36 A n=35	36.26 C	3.37±0.26 B n=35	1.40±0.023 B n=35
	Control	30.40±0.40 C n=25	80.64 A	9.12±0.42 A n=24	2.71±0.041 A n=24
- L5 -	LC <sub>25</sub>	38.36±0.67 A n=30	31.28 B	5.43±0.31 B n=30	1.60±0.028 B n=30
	LC <sub>50</sub>	40.09±0.55 A n=22	24.38 C	3.72±0.32 B n=22	1.54±0.026 B n=22
	Control	28.37±0.57 B n=24	72.72 A	11.37±0.46 A n=24	2.98±0.065 A n=23

Within the same column, the values with different letters are significantly different at P < 0.05.

The results indicate that pyrethrum is highly toxic to development of the parasitoid. The LC<sub>50</sub> value caused 100% mortality in *V. canescens*.Development time of *V. canescens* from parasitized Ephestia larvae (L3 and L5) was significantly affected by LC<sub>25</sub> value ( $F_1$ =977.65, *P*<0.05;  $F_1$ =372.52, *P*<0.05). The resulting progeny emergence rate decreased significantly in the L3 and L5 stages by 18.56%; and 14.89%, respectively ( $F_1$ =3221.25, *P*<0.05;  $F_1$ =604.17, *P*<0.05). Adult longevity was also significantly reduced (L3,  $F_1$ =180.17, *P*<0.05; L5,  $F_1$ =166.47, *P*<0.05). Similarly, adult dry mass decreased significantly (L3,  $F_1$ =840.57, *P*<0.05; L5,  $F_1$ =310.56, *P*<0.05), in comparison with the control.

Table 3 Effects of pyrethrum on the development time, emergence ratio, longevity and adult dry mass of Venturia canescens

Larval stage	Dose	Development Time (day)	Emergence Ratio (%)	Longevity (day)	<i>Adult</i> Dry Mass(mg)
	LC <sub>25</sub>	48.71±0.41 A n=21	%18.56 B	2.38±0.22 B n=21	1.41±0.005 B n=21
L3	LC <sub>50</sub>	-	-	-	-
	Control	30.40±0.40 B n=25	%80.64 A	9.12±0.42 A n=24	2.71±0.04 A n=24
	LC <sub>25</sub>	44.86±0.55 A n=15	% 14.89 B	3.13±0.29 B n=15	1.53±0.005 B n=15
L5	LC <sub>50</sub>	-	-	-	-
	Control	28.37±0.57 B n=24	%72.72 A	11.37±0.46 A n=24	2.98±0.06 A n=23

Within the same column, the values with different letters are significantly different at P < 0.05.

#### Olfactory bioassays on Venturia canescens

In tests with water-sprayed paper, the proportion of parasitoids that moved to the arm with moving clean air (46.66%) was not significantly different from the proportion (53.34%) of parasitoids that chose the odours from the water sprayed paper (P>0.05). In a choice between hotpepper (capsaicin) and clean air, and orange guard (d-Limonene) and clean air, significantly more parasitoids preferred the arm with clean air (P<0.05; P<0.05). Similarly, in a choice test test between Neem azal (azadirachtin, LC<sub>25</sub> and LC<sub>50</sub>) and clean air (P<0.05; P<0.05), and Spruzit neu (pyrethrum, LC<sub>25</sub> and LC<sub>50</sub>) and clean air (P<0.05; P<0.05), the parasitoid preferred clean air (Figure 2).

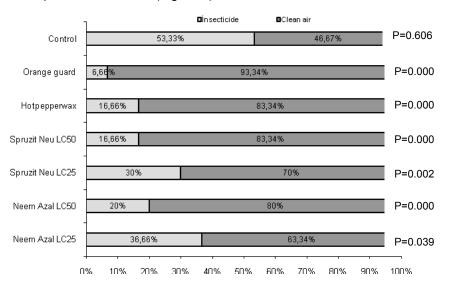


Figure 2. Responses of *Venturia canescens* to odours from a filter paper sprayed with water, Orange guard, Hotpepperwax, Spruzit neu or Neem azal.

# Discussion

No pesticide is 100% safe and non-toxic. However, the margin of safety for botanical pesticides is generally much higher than synthetic pesticides. Ofuya (1997) showed that, as for many synthetic insecticides, the toxicity of botanochemicals to biological control agents can be an important side effect in their use for pest control. It is desirable for a candidate chemical to have selective properties against the pest and be less toxic to natural enemies. There are many examples of botanochemicals, especially at low dose, not being significantly detrimental to beneficial organisms (Ofuya, 1997).

Nevertheless, the case can vary for parasitoid species, active ingredient and dose. Our results showed that the susceptibility of *V. canescens* to azadirachtin and pyrethrum depends on dose-rate. Azadirachtin and pyrethrum seriously affected development of *V. canescens*. Very few adult parasitoids emerged from azadirachtin treated hosts at  $LC_{50}$  and  $LC_{25}$  values, indicating a strong detrimental effect on the parasitoid. For pyrethrum, very high mortalities of *V. canescens* were also observed for the  $LC_{25}$  value. Pyrethrum was highly toxic to *V. canescens* at the  $LC_{50}$  value and caused 100% mortality. Because the active ingredient penetrates the host cuticle, the parasitoid inside the host, can be contaminated directly with the plant derived insecticide. The reduction in emergence increased with dose. Therefore, botanical insecticides need to be used carefully. The side-effects of botanical insecticides may be divided into two parts. Some studies reported that plant derived insecticides have very little or no effect on natural enemies; other studies reported that botanical insecticides have serious side-effects on beneficial insects and environment- human health. Some of these studies are detailed below.

Toxicity of azadirachtin and various neem extracts against insect pests has been widely demonstrated (Schmutterer, 1990). However, many reports also indicate the variable sensitivity of natural enemies, although in most cases they are less susceptible than their hosts (Schmutterer, 1997; Condor Golec, 2007). Raguraman & Singh (1998) showed that the emergence of *Bracon hebetor* Say (Hymenoptera: Braconidae) from pre-treated hosts was concentration-dependent. Simmonds et al. (2002) reported that pyrethrum was toxic to both whitefly and its parasitoid, *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae).

Singh et al. (1985) determined that the old botanical insecticides: pyrethrums, rotenone and nicotine sulfate, were effective against the aphids, *Myzus persicae* Sulz. (Homoptera: Aphididae) and *Brevicoryne brassicae* L. (Homoptera: Aphididae), and less harmful to the predator *Coccinella septempunctata* L. (Coleoptera: Coccinellidae). Neem oil or resin soap was less harmful to *Bemicia tabaci* Genn. (Homoptera: Aleyrodidae) and its parasites (Natarajian, 1990). Anjaneyulu et al. (1999) showed the acute toxicity of neem oil against hemipteran predatory insects. Adverse effects of some biologically active plant extracts on beneficial organisms have been reported by many other workers. For instance, treating the Colorado potato beetle, *Leptinotarsa decemlineata* Say (Coleoptera: Chrysomelidae) with neem-seed extract resulted in moulting delays and deformities in its predator, *Perillus bioculatus* Fabricius (Hemiptera: Pentatomidae) (Hough-Goldstein & Keil, 1991). In addition, neem seed extract decreased the numbers of *Encarsia* spp. (Hymenoptera: Aphelinidae) and *Aleurodiphilius* spp. parasitoids of *B. tabaci*, an important pest for which azadirachtin showed efficacy as a control agent (Price & Schuster, 1991).

However, some plant-based insecticides, even at low doses have a negative impact on the availability of natural enemies (Ofuya, 1997). For example, low doses of azadirachtin (10 and 20 mg/l) did not harm *Apanteles glomeratus* L. (Hymenoptera: Braconidae) but its host, final instar *Pieris brassicae* L. (Lepidoptera: Pyralidae) larvae, showed decreased feeding followed by a gradual death in the laboratory (Schmutterer, 1992). However, moderate effects on adult survival and reproduction were detected only at the highest concentration assayed on the egg parasitoid *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) (Raguraman & Singh, 1999) and the coreid parasitoid *Gryon fulviventre* Crawford (Hymenoptera: Scelionidae) (Mitchell et al., 2004). Other reports showed no negative effects on 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 & Singh, 1998). All this research affirms the existence of a widely variable reaction of parasitoids to

azadirachtin and pyrethrum, likely depending on the different bioassay techniques employed and on interspecific sensitivity. In the case of azadirachtin and pyrethrum formulations, adjuvants may have the adverse effects recorded at high concentrations (Lyons et al., 2003).

Behavioral studies are also important for succesful biological control. We analysed the behaviour of the parasitoid in a Y-olfactometer using azadirachtin ( $LC_{50}$  and  $LC_{25}$ ), pyrethrum ( $LC_{50}$  and  $LC_{25}$ ), capsaicin (1:32) and d-Limonene (1:4). The present study provides evidence of an interaction between botanical extracts and biological control. Repellence of azadirachtin has been established for a range of insect pest species (Fagoonee, 1981; Saxena et al., 1981; Saxena & Rembold, 1984) but very little information exists for beneficial arthropods.

Boeke et al., (2003) reported that, in a choice test with a Y tube, oil of *Azadirachta indica* (Meliaceae) showed a repellent effect on the parasitoid *Uscana lariophaga* Steffan (Hymenoptera: Trichogrammatidae). A similar result was obtained for *B. hebetor* (Raguraman & Singh, 1998).

The number of studies performed using the repellent effect of pyrethrum on parasitoids is rare and there is only one report related to this subject. Pyrethrum demonstrated a repellent effect even in doses too low to actually kill the insect (Glynne-Jones, 2001). Simmonds et al. (2002) showed that pyrethrum extract did deter the parasitoid *E. formosa* from stabbing into treated host nymphs. In this study, all chemicals used in the experiment were found to be repellent to the parasitoid. Thus, botanical insecticides and extracts could cause an important reduction in parasitism.

Persistence periods of chemicals vary according to the environment. The careless and the intensive use of botanical insecticides and extracts, like chemical insecticides, may have negative effects on the development and behaviour of natural enemies. In the present study, azadirachtin and pyrethrum were toxic to the adult parasitoid. In this context, the repellent effect could be advantageous for the parasitoid. These compounds should therefore be used in a timely manner and at the lowest effective dosage.

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