Toxicity and Sublethal Effects of Selected Insecticides on Life Parameters of *Encarsia formosa* (Hymenoptera: Aphelinidae), a Parasitoid of *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae)[1]

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Abstract The sublethal effects of buprofezin, abamectin, and a plant-derived extract from aerial portions of *Echinophora platyloba* DC (Umbelliferae) on the mortality, developmental time, adult longevity, and fecundity of the parasitoid *Encarsia formosa* Gahan were studied. Laboratory bioassays determined that the median lethal concentrations (LC_{50}) of abamectin, buprofezin, and the *Echinophora platyloba* extract were 724.2, 1,202.9, and 894.4 mg/l for larvae, 971.4, 1,380.2, and 1,287.0 mg/l for pupae, and 306.2, 3,309.1, and 831.7 mg/l for adults, respectively. Preadult development time was reduced following exposure to sublethal concentrations (LC_{30}) of all insecticides ($P < 0.01$). Treatment of larvae and pupae with buprofezin or abamectin significantly decreased adult longevity ($P < 0.01$); however, the *E. platyloba* extract reduced adult longevity only when exposed during the pupal stage. Adult fecundity was not affected when larvae or pupae were treated with any of the insecticides ($P > 0.05$). However, decreased fecundity was observed when adult wasps were exposed to abamectin and, to a lesser extent, the *E. platyloba* extract ($P < 0.01$). In comparison to abamectin, sublethal concentrations of the *E. platyloba* extract and buprofezin are less harmful to the survival, longevity, and fecundity of *Encarsia formosa* and should be further developed for use in integrated pest management of the greenhouse whitefly, *Trialeurodes vaporariorum* Westwood, in greenhouse production systems.

Key Words sublethal effects, fecundity, longevity, greenhouse whitefly, bioassay

The greenhouse whitefly, *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae), is a highly polyphagous and economically important pest of greenhouse crops around the world. Immature and adult stages cause economic damage not only by direct feeding, but also by producing high amounts of honeydew that supports growth of sooty mold that reduces the efficiency of photosynthesis by the host plant (Prijovic et al. 2014), as well as by transmission of a variety of plant pathogenic viruses, bacteria, and broad mites (Costa 1976, Palevsky et al. 2001, Prijovic et al. 2014).

Chemical insecticides are widely used to manage greenhouse whitefly (Bi 2011); however, the efficiency of chemical control is questionable due to rapid development

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of resistance to different classes of insecticides (Karatolos et al. 2010, Pappas et al. 2013). Broad-spectrum insecticides have harmful effects on nontarget organisms and, thus, interfere with the development of biological control programs in greenhouses. Additionally, pesticide residues on greenhouse products raise serious health concerns for consumers (Lozowicka et al. 2015), as many of these products are consumed fresh. Therefore, the integration of target-specific insecticides with other control methods (e.g., biological control, resistant cultivars, and cultural practices) is necessary for economically and environmentally sound management of pest populations (Reddy 2016). For this purpose, the compatibility of biological control agents with insecticides used in greenhouse production should be evaluated. These studies are expected to consider not only the mortality caused at acute doses, but also the effects of insecticides on physiology, behavior, and control efficiency of natural enemies at sublethal concentrations (Desneux et al. 2007).

The aphelinid wasp, *Encarsia formosa* Gahan (Hymenoptera: Aphelinidae), has been commercially used for biological control of *T. vaporariorum* in tomato and cucumber greenhouses for almost a century (Hoddle et al. 1998). Successful management of whiteflies using *E. formosa* requires integration with other control methods (Hoddle et al. 1998, Zchori-Fein et al. 1994). A variety of insecticides, such as abamectin, azadirachtin, and buprofezin, have been proposed by some researchers for possible combination with aphelinid parasitoids (Gerling and Sinai 1994, Gholamzadeh et al. 2012, Kumar et al. 2008, Sohrabi 2012). However, most of these studies are based on mortality rate of the parasitoid at recommended field rates or median lethal doses and, thus, do not consider the potential impacts at sublethal doses.

In this study, our objective was to assess the toxicity of abamectin, buprofezin, and the herbal extract taken from the prickly parsnip, *Echinophora platyloba* DC (Umbelliferae), against different life stages of *E. formosa*. Buprofezin is an insect growth regulator that interferes with molting process of immature insects by inhibiting chitin synthesis and formation of cuticle. It is highly effective against immature stages of Hemiptera, including whiteflies, and is especially recommended for control of *T. vaporariorum* in greenhouses (Gerling and Sinai 1994). Abamectin is a mixture of avermectin B1a (>80%) and avermectin B1b (<20%), both produced by fermentation activity of the soil bacterium, *Streptomyces avermitilis* Kim & Goodfellow. Generally, abamectin interferes with transmission of neuromuscular signals in insects (Hayes and Laws 1990). It is broadly used for control of a range of pests on fruit, vegetable, and field crops (Dybas 1989), but appears to have low toxicity to many nontarget organisms including whitefly parasitoids (Dybas 1989, Hoddle et al. 1998, Zchori-Fein et al. 1994). *Echinophora platyloba* is an aromatic plant that grows naturally in different regions of Iran. Aerial parts of *E. platyloba* are traditionally used as a flavoring for food. Hydroalcholic and aqueous extracts of *E. platyloba* are well known for their antifungal, antibacterial, insecticidal, and antioxidant effects (Asghari et al. 2013, Sharafati-Chaleshtori et al. 2012, Tabadkani et al. 2017).

Materials and Methods

Insecticides. Abamectin (Gard® 1.8% EC) and buprofezin (Applaud® SC 40%) were obtained from Paksam Iranian Co. (Chaboksar, Guilan province, Iran) and

Golsam Sepahan Co. (Isfahan, Iran), respectively. The aerial parts of *E. platyloba* were collected from natural habitats of Isfahan province, central Iran, during April–May 2016. The materials were air-dried for 1 wk and used to extract the essential oils using the hydrodistillation method. Briefly, the dried materials (100 g) were finely milled and the powder was subjected to hydrodistillation for 4 h with distilled water as the solvent using a Clevenger apparatus according to standard procedures. The materials yielded a pale yellowish oil (1.18% w/w), which was dehydrated with anhydrous sodium sulfate and stored in sealed vials at 4°C (Hashemi et al. 2013).

Plants and insects. Cucumber plants, *Cucumeris sativus* var. Daminus, were planted in plastic pots (20 cm diameter, 24 cm height) containing an equal mixture of soil and peat moss. The pots were maintained in a research greenhouse (25 ± 5°C, 60 ± 5% relative humidity [RH], 14L:10D photoperiod), and 4- to 5-leaf plants were used as hosts for *T. vaporariorum*. The original population of *T. vaporariorum* was obtained from a commercial tomato greenhouse (Tehran, Iran). After species identification, the leaves containing immature stages of the whitefly were maintained for one generation in net cages with insect-proof mesh to ensure that they were not parasitized. The whiteflies were then transferred into net cages (60 × 40 × 50 cm) and provided with cucumber plants in a growth chamber (26 ± 1°C, 65 ± 5% RH, and 14L:10D photoperiod).

Cucumber leaves containing blackened *T. vaporariorum* pupae (representative of *E. formosa* pupal stage) were collected for a commercial greenhouse (Pakdasht, Tehran, Iran) and maintained in ventilated plastic cans (12 cm diameter, 20 cm height) until adult emergence. After species identification, a stock colony of *E. formosa* was established on *T. vaporariorum* immature stages in a growth chamber (26 ± 1°C, 65 ± 5% RH, and 14L:10D photoperiod).

Bioassays. Concentration–mortality responses of larval, pupal, and adult stages of *E. formosa* to the three insecticides were determined in standard bioassays. Preliminary testing established concentrations of each that caused 10–90% mortality of the developmental stages (Table 1) and were used in the bioassays.

For the larval bioassay, all leaves of cucumber plants, except one with 35–40 third-stage nymphs of *T. vaporariorum*, were removed. The plants were exposed to four young (i.e., <48 h old) ovipositing females of *E. formosa* inside cages (30 × 30 × 30 cm). After 24 h, adult wasps were removed and the parasitized whiteflies were transferred into the growth chamber. After 2 d, the different concentrations of each insecticide (Table 1, larvae) were sprayed over the leaf using a Potter spray tower (Burkhard, Uxbridge, U.K.) at a rate of 1.5 ± 0.01 ml/cm^2 and 68.9-kPa pressure. Treated plants were maintained in the growth chamber for 10 more days. At that time, the number of living parasitoids could be determined as the parasitized immature whiteflies turn black when the parasitoid pupates. To determine the initial number of parasitoids successfully emerged as larvae, all whitefly nymphs with unchanged color were inverted and checked for egg remains or unhatched parasitoid eggs representative of unsuccessful parasitism or nonviable eggs, respectively. By knowing the number of emerged larvae and the number of pupae, mortality rate during larval stage was determined and used to estimate the median lethal concentrations (LC_{50}) and the sublethal concentration (LC_{30}) for each insecticide.

Table 1. Concentrations of insecticides used in bioassay against different developmental stages of *Encarsia formosa*; for all insecticides and life stages, the same volume of distilled water was applied to insects as control.

Stage	Insecticide	Bioassay Concentrations (mg/l)
Larvae	Abamectin	50, 125, 250, 500, 1,000, 2,000
	Buprofezin	50, 100, 200, 400, 800, 1,200
	Echinophora platyloba	400, 650, 900, 1,100, 1,250, 1,400
Pupae	Abamectin	100, 200, 400, 750, 1,500, 2,250
	Buprofezin	100, 200, 400, 600, 800, 1,000
	Echinophora platyloba	500, 800, 1,200, 1,500, 1,800, 2,100
Adult	Abamectin	25, 100, 250, 400, 550, 700
	Buprofezin	2,000, 2,500, 3,000, 3,500, 4,000, 4,500
	Echinophora platyloba	600, 700, 800, 900, 1,000, 1,100

For pupal bioassay, cucumber plants, containing 40–50 third-instar *T. vaporariorum* on a single leaf were exposed to ovipositing females of *E. formosa* inside cages ($30 \times 30 \times 30$ cm). After 24 h, adult wasps were removed and the plants were maintained in the growth chamber for about 10 d. When most of immature whiteflies (35–40) turned black, the leaf was removed from the plant and placed on a petri dish (12 cm diameter) bottom containing agar gel to retain freshness. Different concentrations of each insecticide (Table 1, pupae) were sprayed over the leaf using the Potter spray tower. The leaves were maintained in growth chamber until emergence of adult parasitoids. The relationship between the initial number of pupae and the number of wasps that successfully emerged after treatment was used to determine the mortality rate and estimate lethal concentrations.

For adult bioassay, petri dish bottoms (9 cm diameter) were sprayed with different concentrations of each insecticide (Table 1, adult) using the Potter spray tower. Treated petri dishes were allowed to air-dry for 1 h, and 20 newly emerged ($<$24 h old) adults of *E. formosa* were released into each petri dish. Treated wasps were maintained in the growth chamber and provided with cucumber leaves infested with whiteflies. Mortality was recorded 24 h after treatment. For all bioassays, distilled water served as the control, and all treatments were replicated four times.

Sublethal effects. The effect of sublethal concentrations (LC_{30}) of the three insecticides on selected life parameters of *E. formosa* was assessed when the parasitoids were treated while in the larval, pupal, or adult stages. Larvae were exposed by first selecting two large cucumber plants, each containing 35–40 third-stage nymphs of *T. vaporariorum*. These leaves were placed in cages with 10 ovipositing *E. formosa* females for 24 h. After 2 d, the leaves with the parasitized whitefly nymphs were sprayed with LC_{30} of the appropriate insecticide (Table 2,

Table 2. Results of probit analysis for toxicity of buprofezin, abamectin, and *Echinophora platyloba* extract to different life stages of *Encarsia formosa.*

Stage	Insecticide	LC_{30} (mg/l)*	LC_{50} (mg/l)*	χ^2	Hetero	Slope	HQ**
Larvae	Abamectin	161.43	724.20	2.96	1.19	1.23 ± 0.24	0.690
	Buprofezin	280.90	1,202.92	3.19	1.01	2.58 ± 0.40	0.831
	Echinophora platyloba	651.8	894.42	1.68	0.28	0.87 ± 0.14	—
Pupae	Abamectin	290.0	971.42	1.18	2.28	0.91 ± 0.15	0.514
	Buprofezin	820.75	1,380.27	1.79	1.06	1.09 ± 0.16	0.724
	Echinophora platyloba	901.1	1,287.0	2.06	2.24	1.43 ± 0.40	—
Adult	Abamectin	86.53	306.27	1.73	0.80	1.17 ± 0.33	1.63
	Buprofezin	2,718.62	3,309.15	4.12	3.12	12.71 ± 2.4	0.302
	Echinophora platyloba	710.3	831.7	3.03	2.73	1.61 ± 0.50	—

* mg/l of the commercial product. LC_{30}, sublethal concentration; LC_{50}, median lethal concentration.
** Hazard quotient (HQ) is calculated by dividing the field recommended dose of a given insecticide by its LC_{50} estimated through bioassay.

larvae) using the Potter spray tower. Controls were sprayed with distilled water. The plants were maintained in the growth chamber and monitored daily to determine the preadult development time (egg to adult) until wasps emerged. For each insecticide, 28 adult wasps were divided into seven groups, each group with four wasps. Wasps of each group were released into a petri dish (9 cm diameter) and provided daily with fresh cucumber leaves infested with whitefly nymphs. Fecundity (number of progeny per four females) was determined for the 8 initial days of adult life. The wasps were maintained until death to record the longevity as well.

For treatment of pupae, 45–50 blackened whiteflies (containing parasitoid pupae) were sprayed with the LC_{30} of the appropriate insecticide. Treated wasps were maintained in the growth chamber until adult emergence. The percentage of adult emergence was recorded. Fecundity and longevity of adult wasps were determined as described above.

For treatment of adults, newly emerged adults (<48 h old) were treated with LC_{30} of the appropriate insecticide. Fecundity and longevity were determined as previously described.

Data analysis. Percentage mortality data were corrected using Abbott's (1925) formula and were arcsine transformed prior to analysis. These data were subjected to probit analysis (POLO-PC 2002 software, California, USA) to estimate lethal concentrations (LC_{30} and LC_{50}) for each insecticide and for each developmental stage of the parasitoid. Life-parameter (i.e., longevity, fecundity) data were

analyzed using one-way analysis of variance in the SPSS software (version 17.1) (Statsoft Inc., Carey, NJ). Significant differences among treatments were separated using Tukey's post hoc test at $P < 0.05$.

Results and Discussion

The median lethal concentrations (LC_{50}) of abamectin, buprofezin, and the *Echinophora platyloba* extract were estimated as 724.2, 1,202.9, and 894.4 mg/l for the larval stage, 971.4, 1,380.2, and 1,287.0 mg/l for the pupal stage, and 306.2, 3,309.1, and 831.7 for the adults of the parasitoid, respectively (Table 2). The hazard quotient (HQ) value for the insecticides was calculated by dividing the recommended field rate of the insecticide by its LC_{50}. An HQ value of >1.0 indicates that the insecticide is more deleterious to the nontarget organism than to the target pest (Campbell et al. 2000). Thus, HQ values of 0.69, 0.51, and 1.63 for the respective larval, pupal, and adult stages of the parasitoid exposed to abamectin (Table 2) suggests that abamectin at its recommended field rate (500 mg/l) has a relatively low toxicity against the larval and pupal stages of the parasitoid in comparison to the adult wasps. Such results might be expected in that the larval and pupal stages of the parasitoid are somewhat protected from exposure within the whitefly hosts, while the adult wasps are not. Furthermore, abamectin has been reported as having low toxicity against larval and pupal stages of parasitoids inside host bodies (Araya et al. 2006, Moura et al. 2006), while it is harmful when adult stages are exposed (Araya et al. 2006, Bacci et al. 2007, Hall and Nguyen 2010, Wang et al. 2012).

The HQ values of buprofezin were estimated as 0.83, 0.72, and 0.30 for the respective larval, pupal, and adult stages (Table 2), indicating low toxicity of this insecticide for these life stages of the parasitoid. As expected, larvae were the most susceptible to buprofezin. As a chitin synthesis inhibitor, buprofezin interferes with the molting process of immature insects by inhibiting the formation of cuticle. As a result, buprofezin is widely used against immature stages of insects, particularly Hemiptera, with little harmful effects on adult insects including parasitoids, predators, and pollinators (De Cock and Degheele 1998). Consistent with our results, buprofezin has been categorized as a comparatively safe insecticide for adult stages of aphelinid parasitoids (Gerling and Sinai 1994, Hoddle et al. 2001, Jones et al. 1995, Prabhaker et al. 2007). In contrast, some researchers have reported that buprofezin is moderately to highly toxic to predators and parasitoids in other families of the Chalcidoidea (Beloti at al. 2015, Cloyd 2012, Suma et al. 2009). This discrepancy may be based upon differences in the parasitoids and host species of these studies, the dose of the insecticides, the method of insecticide application, or insecticide formulation.

Mortality is not the only harmful effect of insecticides. Life traits, such as development time, longevity, fecundity, searching efficiency, feeding behaviors, sex ratio, and functional response may also be impaired by exposure to sublethal doses of insecticides (Biondi et al. 2013, de Morais et al. 2016, Desneux et al. 2007). We found in our study that the preadult developmental time of the parasitoid was significantly affected by exposure to the LC_{30} of the insecticides when compared with the controls ($F = 166.57$; df $= 3,537$; $P < 0.001$) (Table 3). The longest

Table 3. Preadult development time, adult longevity, and female fecundity of *Encarsia formosa* exposed to sublethal concentration (LC_{30}) of buprofezin, abamectin, and *Echinophora platyloba* extract during larval, pupal, or adult stage. Different superscript letters in each row show significant difference at $P < 0.05$ (Tukey's test).

Stage	Parameter*	*Echinophora platyloba*	Abamectin	Buprofezin	Control
Larvae	Development time (d)	23.63 ± 0.29^{c}	25.48 ±0.26^{b}	26.87 ± 0.36^{a}	21.63 ± 0.28^{d}
	Adult longevity (d)	17.03 ± 0.47^{a}	13.92 ± 0.60^{b}	14.61 ± 0.50^{b}	18.31 ± 0.85^{a}
	Female fecundity	208.2 ± 11.8^{a}	198.8 ± 10.3^{a}	200.2 ± 12.2^{a}	223.2 ± 8.8^{a}
Pupae	Adult longevity (d)	20.38 ± 0.55^{b}	17.86 ± 0.66^{c}	19.76 ± 0.73^{bc}	23.75 ±0.82^{a}
	Female fecundity	214.0 ± 17.1^{a}	184.4 ± 10.9^{a}	202.2 ± 14.9^{a}	229.8 ± 16.5^{a}
Adult	Female fecundity	186 ± 12.9^{b}	138.4 ±16.6^{c}	214.8 ± 10.0^{ab}	231.6 ± 13.5^{a}

* Female fecundity is the number of progeny produced by four females.

development time was observed in parasitoids treated with buprofezin (26.8 d), followed by those exposed to abamectin and the *E. platyloba* extract (Table 3).

Adult longevity was significantly ($F = 9.33$; df = 3,106; $P < 0.001$) impacted following exposure to either abamectin or buprofezin while in the larval stage. However, the *E. platyloba* extract had no significant effect on adult longevity in comparison with the controls (Table 3). Treatment of pupae with all insecticides resulted in significant decreases in adult longevity ($F = 10.76$; df = 3,105; $P < 0.001$). Apart from the controls, the longest life span was recorded for wasps treated as pupae with the *E. platyloba* extract, while the shortest life span was observed in wasps exposed to sublethal concentrations of abamectin (Table 3).

Exposure of larvae or pupae to sublethal concentrations of the insecticides had no significant effect on adult fecundity (Table 3). However, fecundity was significantly ($F = 9.11$; df = 3, 16; $P < 0.01$) impacted when the parasitoids were exposed as adults. Lowest fecundity was recorded with wasps treated with abamectin, followed by those treated with the *E. platyloba* extract. Fecundity was not affected by sublethal concentrations of buprofezin when compared with controls (Table 3).

The insecticides studied herein were ranked as low to moderately toxic to *E. formosa* in terms of the HQ index (Table 2). However, we determined that some life parameters of the parasitoid are negatively affected by exposure to sublethal concentrations of these insecticides. These findings are consistent with other studies (Biondi et al. 2012, 2013; de Morais et al. 2016), indicating that accurate

analysis of both lethal and sublethal effects of pesticides on natural enemies should be assessed prior to their integration into pest management programs. For example, Biondi et al. (2013) reported that some life traits of the tomato leafminer parasitoid, *Bracon nigricans* Szépligeti (Hymenoptera: Braconidae), such as longevity, fertility, and control efficiency, are negatively affected by concentrations of abamectin that are not lethal to adult parasitoids. There is also evidence for sublethal effects of buprofezin on longevity, fecundity, wing deformity, type of functional response, and control efficiency of parasitoids and predators (Jones et al. 1998, Liu and Chen 2000, Sohrabi et al. 2014, Suma et al. 2009).

During the last two decades, botanical insecticides have received increasing attention in pest management programs with respect to their reduced impacts on nontarget organisms, safety to humans, and biodegradability (Guleria and Tiku 2009, Simmonds et al. 2002). Currently, several plant-derived insecticides are commercially available for control of insect pests in agriculture and homes (Jindal et al. 2013), and botanicals have the potential to become more commonly adopted in integrated pest management (IPM) strategies (Guleria and Tiku 2009, Jindal et al. 2013). In this study, we evaluated the toxicity and sublethal effects of an extract taken from a native plant (*Echinophora platyloba*) on life parameters of *E. formosa*. We did not calculate the HQ index for *Echinophora platyloba* extract, as no field-recommended rate is currently available for this extract. However, in our study, the *Echinophora platyloba* extract had limited negative impacts on life traits (preadult development time, longevity, and fecundity) of *E. formosa*. Given the well-known antifungal, antibacterial, and insecticidal properties of this medicinal plant (Asghari et al. 2013, Sharafati-Chaleshtori et al. 2012), it is an excellent candidate for further development and assessment for use in IPM programs.

The problems associated with synthetic insecticides and their residues on fresh products have increased the need for more biodegradable compounds with greater specificity against target pests and less harmful effects on nontargets. We found herein that abamectin is moderately toxic to different life stages of the parasitoid *E. formosa*, and that a variety of life traits of the parasitoid are also negatively impacted by exposure to sublethal concentrations. These negative effects were less evident for buprofezin, especially when it was applied to adult stages of the parasitoid. Given the specificity of insect growth regulators to immature stages of arthropods (De Cock and Degheele 1998), these insecticides are suitable candidates for use in IPM programs, as they have very low toxicity to vertebrates and adult beneficial insects such as predators, parasitoids, and pollinators (Merzendorfer 2013). The extract from *Echinophora platyloba* was also another source of an insecticide with the few, if any, harmful effects on life traits of *E. formosa* and, thus, should be considered as a safe alternative for whitefly management programs.

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