J. Entomol. Res. Soc., 14(2): 61-69, 2012 *ISSN:1302-0250*

Effects of Amitraz on the Parasitoid *Encarsia formosa* (Gahan) (Hymenoptera:Aphelinidae) for Control of *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae): IOBC Methods

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ABSTRACT

The side effects of amitraz at three rates (the maximum recommended field concentration, 1/2 and 1/4 the maximum recommended field concentration) on *Encarsia formosa* were tested in the laboratory using the IOBC classification. The pupal mortality, fecundity and longevity of the parasitoid wasps exposed to pesticide were determined. Results showed that there is a significant difference in pupae mortality between the treatments and the untreated control group, but we did not observ adverse effects on fecundity and longevity. According to the IOBC classification, amitraz at the maximum recommended field concentration (E=89.09) and 1/2 dose (E=82.3) were found to be harmful and 1/4 dose (E=63.2) was moderately harmful for *E. formosa*. We concluded that amitraz is unsafe for *E. formosa* and it can not be used for integrated pest management in integration with *E. formosa* to control *Trialeurodes vaporariorum*.

Keywords: side effects, parasitoid, integrated pest management.

INTRODUCTION

Beneficial insect species play an indispensable role in controlling various crop(s) pests worldwide (Amano and Haseeb, 2001). The integration of biological control agents with pesticides for integrated pest management (IPM) would be most effective if the pesticides used were efficacious against the pest species and relatively safe for beneficial arthropods such as parasitoide and predators (Wilkinson *et al*. 1979). In crop protection, beneficial insect species are frequently exposed to various pesticides because of their close association with the host (pests) (Amano and Haseeb, 2001). Identification of selective pesticides suitable for use in integrated control programs is urgently needed (Sterk *et al*. 1999). Several studies have shown the possibility of integrating insecticides into IPM due to their selective properties (Van Lenteren and Woets, 1988; Feldhege and Schmutterer, 1993). Studying only the lethal effects of pesticides may not completely achieve the targets, because sublethal effects of pesticides on these organisms may not be sustainable for crop protection if not investigated and evaluated properly (Bakker *et al*. 1992; Haseeb *et al*. 2000). Testing side effects on beneficial organisms is increasingly important in the development of new pesticides and the re-registration of old active ingredients and to find compounds suitable for use in IPM programs (Sterk *et al*. 2001).

Encarsia formosa Gahan (Hymenoptera: Aphelinidae) is a parasitoid used worldwide for the biological control of whiteflies, *Trialeurodes vaporariorum* Westwood (Homoptera: Aleyrodidae). Many researchers have examined the side effects of some pesticides on this species (Veire *et al*. 1988; Senn *et al*. 1994; Castaner and Garrido, 1995; Liu and Stansly, 1997; Sterk *et al*. 1999; Simmonds *et al.* 2002; Chiasson *et al*. 2004; Richter, 2006; Hoseini, 2010; Sohrabi, 2012). One of the major aims of the Working Group´ Pesticides and Beneficial Organisms` of the International Organization for Biological Control (IOBC) was to co-ordinate international activities to develop standard methods to test the side effects of pesticides on a large range of natural enemies, important in many cropping systems. A combination of laboratory, semi-field and field methods to test the side effect of pesticides was developed by IOBC/WPRS. The most criteria for evaluation of a product are based on IOBC/WPRS hazard classification (Hassan, 1992). The objective of present study was to investigate the effects of amitraz on pupal mortality, adult longevity and fecundity of *E. formosa* and classification of this pesticide using the IOBC method.

MATERIAL AND METHODS

Insects

E. formosa pupae and adults of *T. vaporariorum* were obtained from tomato plant, *Lycopersicon esculentum* in the greenhouse. After identification (Suh *et al.* 2008; Polaszek, 1992), they were reared on leaves of tobacco plants (variety Kuker 347) in a rearing chamber under conditions of 25±1ºC, 70±5% RH and a photoperiod of 14: 10 (L:D) h.

Bioassay

A culture of *T. vaporariorum* was reared on bean plants, *Vigna angularis* to provide whitefly nymph-infested leaves. These leaves with third-instar nymphs were exposed to adult parasitoid for 48 h. Amitraz caused at the maximum recommended field concentration, 1.67 L/ha of 20 EC (Bayer cropscience, http://www.bayercropscience.com) (500L pesticide solution per hectare), and two rates of it (1/2 and 1/4 the recommended field concentration). Pesticide solutions prepared at the maximum recommended field concentration (3333.2 mg/L), 1/2 the recommended field concentration (1666.6 mg/L) and 1/4 the recommended field concentration (833.3mg/L). Bean leaves with 3 days old parasitoid pupae were dipped in amitraz solutions for 30s. This pesticide is recommended for pest control in the greenhouse and open fields (Mosallanejad *et al*. 2002). Distilled water was acted as a control in all experiments. Each concentration was performed in four replicates.

IOBC method

The parasitoids that emerged from the treated pupae were counted and removed daily. Number of emerged progenies, mortality was recorded to 7 days. Mortality percentages (M) were corrected for untreated mortality according to Abbott formula (Abbott, 1925). Before analysis using One-way analysis of variance (ANOVA), data were transformed by $\sqrt{x+0.5}$.

Furthermore, newly emerged (0-1 d) adults of parasitoid that survived of each treatment were individually placed in a container of plastic 8.5 cm long × 3 cm high × 6.5 cm wide, with 4×3 cm of lid replaced by mesh containing third-instar nymphs of *T. vaporariorum* infested bean leaf. Every 48 h, each female was introduced to a new container. This experiment continued until the death of each female. The total number of eggs laid by each female, i. e. the fecundity was recorded.

The value for the treatment effects on the reproductive capacity (R) of the tested organism was calculated from formula:

$$R = Rt/Rc$$

Where Rt is the average number of eggs produced per treated female survivor in the treated group and Rc is the average number of eggs produced per female of the control group.

The Total Effect (E) of a pesticide on beneficial species is calculated from formula that proposed by Overmeer and van Zon (1982):

$$E = 100\% - (100\% - M) \times R$$

(M): Mortality percentages; (R): Reproductive capacity

The pesticide was classified according to the categories for toxicity, developed by IOBC Working Group Boller *et al*. (2005):

Data obtained from the experiments were analyzed using analysis of variance ($P<0.05$) (Proc ANOVA; SAS Institute, 1996). Treatment means were compared by Tukey´s test.

RESULTS

Effect of amitraz on mortality, longevity and fecundity

Mortality and effects of amitraz on fecundity and longevity of *E. formosa* are presented in Table 1. Total mortality caused by the three rates of amitraz was significantly different from the control ($F=100.14$; df =3; $P<0.0001$). These doses of amitraz have no significant effect on longevity ($F=1.74$; df =3; $P<0.1$) and fecundity ($F=0.81$; df =3; $P<0.50$).

Total effect (E)

Amitraz at the maximum recommended field concentration and 1/2 the maximum recommended field concentration was harmful for *E. formosa* and 1/4 the maximum recommended field concentration of it was classified as moderately harmful for *E. formosa* based on the IOBC classification (Table 1).

Table 1. Effect of the three rates of amitraz on mortality, longevity and fecundity of *E. formosa* (Mean±SE), Total effect (E) and hazard classes according to the IOBC evaluation categories.

Concentration (a.i. in µg /mL)	N*	Mortality (%)	Longevity of adults (days)	Total eggs/female (n)	Total effect (E) %	Classification (IOBC)
Control	177	0.7±1c	25.1 ±1.3	276±22.14	-	-
666	240	79.4±2.3 a	15.38±2.6	149±33.3	89.09	3
333	183	78.22±5.1 ab	21.2±2.3	226.6±21.2	82.3	3
166.5	166	60.41 ±3.9 b	24.2±1.4	235.6±45.7	63.2	2

* Number of tested pupa

2: Moderatly harmful, 3: harmful.

Values followed by the different letter are significantly different from each other according to Tukey´s test.

DISCUSSION

The current study showed that three rates of amitraz (at the maximum recommended field concentration, 1/2 and 1/4 the maximum recommended field concentration) caused considerable effects on mortality of *E. formosa* pupae. Even the lowest rate of amitraz (1/4 the maximum recommended field concentration) caused 60.41% mortality on pupae of *E. formosa* significant reducing on adult emergence from pupae. It is concluded that the use of amitraz at three rates, maximum, 1/2 and 1/4 the recommended field concentration is deletrious for *E. formosa* pupae because it heavily reduced parasitoid population in that generation. Jones *et al*. (1995) reported that amitraz was harmful to *E. formosa* adults. They observed that *E. formosa* adults were more susceptible to residues of amitraz than *Eretmocerus mundus* (Mercet) adults. The results of Stolz, (1994) showed pyridine azomethine led to 80% mortality of *E. formosa* adults. Also, acetamiprid, abamectin, emamectin, imidacloprid, indoxacarb, spinosad, thiacloprid, thiamethoxam and triazamate were highly toxic and caused considerable mortality for adults of *E. formosa*, (Veire and Tirry, 2003). Richter, (2006) reported imidacloprid had lethal effect on *E. formosa* adults. Also, in other species of *Encarsia*, *E. inaron* (Walker), found the field-recommended concentration of imidacloprid significantly reduced adult emergence and caused significant mortality on *E. inaron* adults with 1, 4 and 7-day old residues on leaves (Hoseini, 2010; Sohrabi *et al*. 2012).

Effects of pesticides on longevity after exposure to lethal or sublethal dose have been described mostly for parasitoid species (Rumpf *et al*. 1998; Krespi *et al*. 1991; Stapel *et al*. 2000; Alix *et al.* 2001; Schneider *et al*. 2004; Desneux *et al*. 2004, 2006; Hoseini, 2010; Sohrabi *et al*. 2012). A change in longevity may affect fecundity and led to change in the population dynamism (Croft, 1990). For example, adult longevity and the total progeny number were significantly affected by imidacloprid when the larvae of *E. inaron* were treated (Sohrabi *et al*. 2012). Also, *E. formosa* fecundity after exposed to pyridine azomethine was reduced (Stolz, 1994). Castaner and Garrido, (1995) reported acephate, cypermethrin and endosulfan significantly reduced fecundity of *E. formosa* adults after 24 h exposure to these pesticides. The sublethal effects such as suppression of reproductive capacity and reduction observed in the survivors could have a negative impact on insect population dynamics (Trisyono and Chippendale, 1998; Lopez and Latheef, 1999; Knight, 2000; Pineda *et al*. 2007).

In this study, three rates of amitraz have no adverse effects on fecundity and longevity of *E. formosa* adults. In agreement with our findings, Gerling and Sinai, (1994) found no effects on 1-day fecundity of surviving *Eretmocerus* sp. and *E. luteola* Howard females following an application of buprofezin to parasitised *B. tabaci*. Also, Heidari, (2004) reported no adverse effects of buprofezin on longevity and fecundity of *E. formosa*.

Knowledge of insecticide selectivity to beneficial arthropods is important when considering their utility in IPM programs (Sohrabi *et al*. 2012). The insecticides should be effective against insect pests, but relatively safe

to natural enemies, and that requires knowing the complex of natural enemies affecting key pests species and the impact of insecticides on these organisms (Campbell *et al*. 1991). Beneficial arthropods often exhibit greater susceptibility to persistent insecticides than their host or prey (Croft, 1990; Ruberson *et al*. 1998). According to Boyd, (1998) both behavioral (e.g.: locomotion, searching capability, self cleaning) and pesticide processes (e.g.: deposition, redistribution and weathering) may contribute to differences in susceptibility of natural enemy and their host or prey to insecticides. Tabashnik and Johnson, (1999) demonstrated susceptibility of natural enemy caused by a variety of factors including their active searching behaviour, lower detoxification capacity, lower genetic variation and food limitation Therefore, when insecticides are used within IPM programmes, selectivity is one of the main requirements.

IOBC is one of the commonly used methods in testing the side effects of pesticides on natural enemies. It is done in the laboratory, and, depending on the results obtained, semi-field or field tests may be conducted (Dohmen, 1998; Hassan, 1998). This method has been designed to evaluate the acute residual toxicity as well as sublethal effects of the pesticides on the reproductive performance (Vogt *et al*. 2000). Although the tiered approach advocated by the IOBC is based on a large body of literature (Haskell and McEwen, 1998), there are limitations to this approach for pesticide side effects assessment (Stark *et al*. 1995). The primary limitation is that only one or at most two toxic effects are considered. In reality, exposure to pesticides may result in a wide range of effects on an organism, including the simultaneous manifestation of multiple sublethal effects such as shortened life span, reduced number of offspring, changes in the time to first reproduction, longer generation times, weight loss, and mutations in offspring (Stark and Banks, 2003) and also may induce subtle behavioral changes. Demographic toxicological analysis or the life table response experiment is another approach, which takes into account all the aforementioned effects that a toxicant might have at the levels of organization higher than the individual (Stark *et al*. 2004). Rezaei *et al*. (2006) in the study of the effects of imidacloprid, propargite and pymetrozine on *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae), in comparing of the two methods, IOBC and life table assays, did not see different results using these two test methods, except for propargite that in the IOBC method classified as a different group in comparison with the control. Based on, they proposed considering the time consuming nature of demographic studies and validity of the laboratory results of IOBC method which has been shown by field studies (Oomen *et al*. 1991), if limited time is available to get information, the IOBC method could be preferable.

According to our findings, however amitraz did not affect the fecundity and longevity of *E. formosa* surviving females, it is recommended that this pesticide having high mortality on *E. formosa* pupae should be used before the introduction of this parasitoid. Where the use of this pesticide is essential for control of some pests such as two-spotted spider mite, it should be used well-timed and by caution. Based on the IOBC classification we concluded that amitraz can not be used for integrated pest management in integration with *E. formosa* for control of *T. vaporariorum*. We can hypothesize that this pesticide will be incompatible with *E. formosa*. However, we recommend further testing under more realistic conditions, to totally ascertain its toxic effects in this parasitoid.

ACKNOWLEDGMENTS

Authors are thankful to the Research Council of the University of Guilan and the Ministry of Science, Research and Technology for financial support during the course of this project.

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Received: December 01, 2011   Accepted: May 11, 2012