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Effects of emamectin benzoate and cypermethrin on the demography of *Trichogramma brassicae* Bezdenko

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The Effects of two insecticides were evaluated on the immature and adult stages of *Trichogramma brassicae*. The recommended field concentrations of emamectin benzoate and cypermethrin caused 72.0 and 80.7% mortality of immatures, respectively. LC_{50} values were 2.7 and 0.5 μg a. i/ml, respectively, estimated by the dry film residue method. Longevity and population parameters were assessed after exposure to the LC_{30} concentration of each insecticide. Results showed that sublethal concentrations affected the life history parameters of the wasp adversely compared to controls. The intrinsic rate of increase values for emamectin benzoate, cypermethrin and the control were 0.252, 0.248 and 0.272 day^{-1}, respectively. Moreover, R_0 values were 31.2, 28.9 and 44.7, respectively. The mean generation time was significantly affected by the insecticides compared to control. In conclusion, both insecticides may interfere with biological control of pest, based on their acute and sublethal effects on *T. brassicae*.

1. Introduction

Trichogramma species are important biocontrol agents that are regularly used by inundative and inoculative release against many lepidopteran insect pests in fields and orchards (Delpuech et al., 1998). They are egg parasitoids and kill their hosts before damage to the host crop (Gardner et al., 2011; Wang et al., 2012b). Moreover, inundative release of natural enemies such as *Trichogramma* wasps can be as effective as conventional pesticides (Parra, 2010). On the other hand, negative effects of pesticides have made utilization of *Trichogramma* and chemical control incompatible (Saber, 2011). *Trichogramma* species have greater susceptibility to pesticides compared to target pests due to their active searching behavior, lower detoxification capacity, lower genetic variation and food limitation (Mullin et al., 1982). Although pesticides have adverse effects, chemical control is used to a far greater extent because of quick impact, ease of use and lower costs compared with biological control (Khan et al., 2008). Nevertheless, if chemicals are used in an appropriate and consistent manner, the effectiveness of biological control can be improved (Preetha et al., 2009; Saber, 2011; Zhao et al., 2012). Therefore, evaluation of the susceptibility of natural enemies to pesticides is necessary for their use in IPM programs (Croft, 1990; Moura et al., 2006; Preetha et al., 2009; Saber, 2011). The lethal dose of pesticides has mostly been utilized for toxicity studies, while sublethal effects are more important than those within environmental toxicology (Delpuech et al., 1998; Desneux et al., 2007). Sublethal doses cause behavioral and physiological changes that eventually lead to changes in fecundity and fertility, life span, longevity and developmental period of impaired surviving individuals (Desneux et al., 2006; Bengochea et al., 2013; Poorjavad et al., 2014; Stark et al., 2007; Saber, 2011). Life table data are necessary for the assessment of sublethal effects of pesticides on the insects' demography and prediction of their population dynamics (Biondi et al., 2013).

Emamectin benzoate is a macrocyclic lactone from the avermectin family which is produced by fermentation from the soil organism, *Streptomyces avermitilis* (ex Burg et al.) Kim and Goodfellow (Ishaaya et al., 2002; Ioriatti et al., 2009) and has a mode of action like abamectin with a higher potential for caterpillar control (Copping and Menn, 2000). Since degradation of this compound through sunlight on the leaf surface is rapid, its safety for natural enemies is greater because contact activity with the leaf surface is limited to a very short period of time (Chukwudebe et al., 1997; Ioriatti et al., 2009). Cypermethrin is a synthetic pyrethroid effective against insect pests from different orders including

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Lepidoptera, Coleoptera, Diptera, and Hemiptera (Liu et al., 1998; Suh et al., 2000). It kills insects by disrupting the normal functioning of the nervous system (Cox, 1996). Available information on the impact of insecticides on the *Trichogramma brassicae* Bezdenko which is the most common species in Iran (Poorjavad et al., 2012) is scarce. Therefore, acute and sublethal effects of two insecticides, emamectin benzoate and cypermethrin on the adult and immatures were investigated to estimate their effect on populations.

2. Material and methods

2.1. Insect rearing

The initial colony of *T. brassicae* (Hymenoptera: Trichogrammatidae) was collected by egg traps by *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) eggs from grape and apple orchards without an exposure history to pesticides. Parasitized eggs were transferred to the laboratory and maintained in plastic cylindrical containers (10 cm high and 5 cm diameter) in an incubator at 26 ± 1 °C, 60± 5% RH and a photoperiod of 16:8 (L:D) h. The laboratory colony of the wasp was reared on eggs of *E. kuehniella.* A droplet of honey was smeared on the internal walls of the containers for adult feeding.

2.2. Insecticides

Commercial formulations of two insecticides, emamectin benzoate (Proclaim® 5% SG, Syngenta Co., Switzerland) and cypermethrin (Patron® 40% EC Ariashimi Co., Iran) were used in the experiments to simulate their effects on *T. brassicae*. Field recommended concentrations of emamectin benzoate and cypermethrin were 14.24 and 50 mg a. i./l, respectively, prepared in distilled water.

2.3. Pre-imaginal development bioassay

Parasitized eggs of *E. kuehniella* were used to evaluate the influence of pesticides on immature stages. White papers containing 140 ± 20 host eggs were offered to about ten young adult parasitoids (<1 day old) for 24 h in the glass vials (3 × 9 cm). Selection of the number of eggs and parasitoid wasps were according to investigations by Saber et al. (2004) and Saber (2011). Host eggs were exposed to *Trichogramma* adults after 3, 6 and 9 days (Suh et al., 2000). Eggs were also exposed to recommended field concentrations of emamectin benzoate (14.24 mg a. i./l) and cypermethrin (50 mg a. i./l). Preparation of the concentrations was done with 100 ml of distilled water. The dipping method was used in bioassays. The papers containing parasitized eggs were plunged into the insecticide solution for 5 s and replicated 3 times. Untreated control paper was dipped into distilled water only. Then, treated eggs were placed on the filter paper under airflow to dry for 2 h, transferred to vials, and kept in an incubator that was set to the standard conditions mentioned above. As pre-imaginal development takes about of 10 days (Suh et al., 2000), after about 13 days of initial parasitism vials were assessed for adult emergence. Emergence was recorded daily for 10 days. Eggs with a dead adult or even with an exit hole containing a dead adult remaining inside were considered as failed to emerge. The data were subjected to analysis of variance (ANOVA; SAS institute, 2002) and the means were compared using Fisher protected least significant differences (LSD; P = 0.05) (Abedi et al., 2014). The influence of insecticides on the immature stage of *T. brassicae* was studied to classify them based on IOBC (International Organization for Biological and Integrated Control of Noxious Animals and Plants) classes (Hassan, 1994; Williams et al., 2003). The IOBC method evaluates acute residual toxicity (M = mean mortality) and sublethal reproductive effects (R = average fecundity) to calculate the total effects of pesticides (E%) by the formula: E% = 100- (100− M) × R × 100 (Overmeer and van Zon, 1982). IOBC classes for laboratory tests are: 1) harmless (reduction below 30%), 2) slightly harmful (reduction between 31% and 79%), 3) moderately harmful (reduction between 80% and 99%), and 4) harmful (reduction higher than 99%). Actually, classifying on the basis of the IOBC defines response to toxic exposure (Sterk et al., 1999).

2.4. Adult bioassay

A dry film residue method was done for toxicity assessment of insecticides to adults of *T. brassicae*. The concentration-setting tests were carried out to find mortality ranges between 20 and 80% for parasitoid adults. Clean glass vials (3 × 9 cm) were applied for contact toxicity tests. They were cleaned by soaking in soapy water, dried with air exposure (Preetha et al., 2009) and placed in an oven at 80 °C for disinfection. The vials were eluted by acetone 6 h before use and were allowed to dry. The concentrations of 200, 126.2, 79.6, 50.2, 31.7 and 20 ppm for emamectin benzoate and 10, 4, 1.6, 0.6, 0.2 and 0.1 ppm for cypermethrin were prepared with distilled water using logarithmic intervals. Tween 80 (Merck, Darmstadt, Germany) was added to dilutions at a concentration of 200 ppm as surfactant (Rosenheim and Hoy, 1988; Saber and Abedi, 2013; Abedi et al., 2014). The vials were soaked in 2 ml of insecticide concentrations for uniform exposure. Control insects were treated by distilled water plus Tween 80. Each bioassay was replicated three times. After drying the vials, 50 - 180 adults (<24 h old) were released into the vials. Parasitoids were fed by a small strip of honey and the entrance of the vials was covered by 3 organza layers. Then, vials were kept at 26 ± 1 °C, 60± 5% RH and a photoperiod of 16:8 (L:D) h. Mortalities were recorded 24 h after initial exposure to insecticides. Adults with no disrupted balance were considered as dead based on our former observations and experience with such individuals. Mortality data were corrected with Abbott's formula based on low mortalities (<8) of control insects (Abbott, 1925). The results of each trial were tested for curve fit using PROC GENMOD procedures (SAS institute, 2002). Data were analyzed using PROC PROBIT (SAS institute, 2002) to estimate LC_{30}, LC_{50}, and LC_{90} values. The Hazard Quotient (HQ) approach was used for determination of pesticide selectivity (Campbell et al., 2000). HQ value is estimated by dividing the maximum application rate (g a. i/ha) by median lethal concentration (g a. i/ha). HQ values > 1.0 for a pesticide are classified as harmful to adult natural enemies (Campbell et al., 2000).

$$HQ = \frac{Max\ Application\ Rate(g\ ai/ha)}{Laboratory\ LC_{50}(g\ ai/ha)}$$

2.5. Life table study

LC_{30} values were used for study of sublethal effects of insecticides (1.3 μg a. i./ml for emamectin benzoate and 0.15 μg a. i./ml for cypermethrin) on the life table parameters of the parasitoid. This concentration is recommended as the mortality threshold for application of insecticides in IPM programs (Desneux et al., 2003; Wang et al., 2012c). About 100 newly emerged female adults were exposed to LC_{30} of each insecticide by the residue contact method described formerly. The vials containing adults and honey strip were transferred to an incubator at the above mentioned condition and kept for 24 h. Then, 25 female survivors were selected randomly and transferred to clean vials individually. Each

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