J. Agr. Sci. Tech. (2016) Vol. 18: 1267-1278

Effects of Diazinon and Fipronil on Different Developmental Stages of *Trichogramma brassicae* Bezdenko (Hym.; Trichogrammatidae)

M. Ghorbani[1], M. Saber[2*], M. Bagheri[1], N. Vaez[3]

ABSTRACT

The integration of biological and chemical control approaches is very important for a successful Integrated Pest Management (IPM) program. Demographic approaches give a better understanding of the side effects of pesticides on beneficial organisms. In this study, laboratory bioassays were set up to evaluate the lethal and sublethal effects of diazinon and fipronil on different stages of *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae). The effects of Field Recommended Concentration (FRC) of diazinon and fipronil were studied on larvae, prepupa and pupae of the parasitoid. Diazinon and fipronil reduced adult emergence by 99.74 and 50.46%, respectively. The LC_{50} values for diazinon and fipronil on adult stage were 0.11 and 0.46 µg ai ml^{-1}, respectively. The sublethal effects of the chemicals were studied on life-table parameters of the parasitoid emerging from parasitized eggs exposed to the FRC of the insecticides at larval stage and also adults exposed to LC_{30} of the insecticides. Longevity and progeny production were affected by the insecticides in comparison to the control. Three main life-table parameters including intrinsic rate of increase (r_m), Doubling Time (DT) and net Reproduction rate (R_0) were negatively affected by the sublethal treatments. The intrinsic rate of increase for control, diazinon and fipronil exposed populations at larval stage were 0.28, 0.23 and 0.12, respectively and were 0.26, 0.04 and 0.08 for populations exposed to LC_{30} at adult stage, respectively. The sex ratio of offspring at all insecticide treatments has led to the production of female offspring by the chemicals. These findings showed that diazinon and fipronil were harmful for *T. brassicae*, thus semifield and field studies are suggested for getting more applicable results for possibly using them in IPM programs.

Keywords: Biological control, Life-table parameters, Parasitoid, *Trichogramma brassicae.*

INTRODUCTION

Chemical control is the major tactic for controlling agricultural pests throughout the world (Van Lenteren and Woets, 1988; Musser *et al.*, 2006). In addition biological control could offer a method that is both economically and ecologically suitable to control arthropod pests (Settle *et al.,* 1996; Van Driesche *et al.,* 2010). Among the egg parasitoids, the genus *Trichogramma* occurs naturally worldwide and plays an important role as a natural enemy of many lepidopterous pests on a wide range of agricultural crops (Abdelgader and Hassan, 2012). Many laboratory and field studies have shown that *Trichogramma* spp. wasps are highly susceptible to most broad-spectrum pesticides (Bull and Coleman,

[1] Department of Plant Protection, Faculty of Agriculture, University of Maragheh, Maragheh, Islamic Republic of Iran.
[2] Department of Plant Protection, Faculty of Agriculture, University of Tabriz, Tabriz, Islamic Republic of Iran.
[*] Corresponding author; saber@tabrizu.ac.ir
[3] Department of Plant Protection, Faculty of Agriculture, Azarbaijan Shahid Madani University, East-Azarbaijan, Tabriz, Islamic Republic of Iran.

1985, Saber *et al.,* 2004; Shoeb, 2010; Saber, 2011; Wang *et al.,* 2012). Because of the negative consequences associated with pesticides, chemical and biological control with *Trichogramma* spp. have mostly been considered incompatible (Croft, 1990).

Although biological control is of great importance in pest control, chemical control is still necessary in agriculture, because it is quick, efficient, easy to use, and cost effective (Urech, 2000; Khan *et al.,* 2008). Due to the wide range effects of pesticides on natural enemies, it is necessary to study the side effects of insecticides, to adapt chemical and biological control for minimizing the negative impacts of insecticides on natural enemies (Hassan *et al.,* 1998; Saber, 2011; Souza *et al*., 2014).

Insecticides have both lethal and sublethal effects on arthropods, thus in addition to death, they can adversely affect life parameters such as developmental rate (Cônsoli *et al.,* 2009; Poorjavad *et al.,* 2014), longevity (Wang *et al.,* 2012), fecundity (Poorjavad *et al*., 2014), oviposition (Wang *et al*., 2012), sex ratio (Carvalho *et al*., 2003; Desneux *et al.,* 2003), behavior (Delpuech *et al.,* 1999; Poorjavad *et al.,* 2014) and feeding (Qi *et al.,* 2001; Galvan *et al.,* 2005). Therefore, it is necessary to evaluate the lethal and sublethal effects of insecticides on the natural enemies as well as on the pest itself in order to have a better understanding of the effect of the chemicals on the biological components of the system (Stark and Banks, 2003; Desneux *et al.,* 2007). Acute toxicity is assessed usually after a short exposure to a chemical (e.g. a few hours to a few days) and the endpoint is the death of the organism (Croft, 1990; Stark and Banks, 2003). The estimated lethal concentration during acute toxicity tests may only be a partial measure of the deleterious effects. Sublethal effects are defined as effects (either physiological or behavioral) on individuals that survive exposure to a pesticide (Desneux *et al.,* 2007).

Sublethal effects can be detected by estimating the life-table parameters such as net Reproduction rate (R_0), intrinsic rate of natural increase (r_m), finite rate of increase (λ), and mean generation Time (T) (Croft, 1990; Desneux *et al.,* 2007; Saber *et al.,* 2004; Saber, 2011). Life-table experiments provide a more accurate measure of toxic effect compared to lethal concentration estimates (Forbes and Calow, 1999) and have been used to evaluate side effects of pesticides on several natural enemies (Acheampong and Stark, 2004; Stark *et al.,* 2004; Saber *et al.,* 2004, 2005; Saber, 2011; Mahdavi *et al.,* 2011; Saber and Abedi, 2013).

The egg parasitoid *Trichogramma brassicae* Bezdenko (Hymenoptera: Tricogrammatidae) is one of the most important and widely distributed species of *Trichogramma* in Iran. It attacks eggs of several lepidopterous pests, and is a major biological control agent (Iranipour *et al.,* 2008; Poorjavad *et al*., 2012). Since diazinon and fipronil are widely used insecticides in rice fields in Iran (Talebi-Jahromi, 2007), we decided to investigate the total effects (lethal and sublethal effects) of these insecticides on *T. brassicae*.

MATERIALS AND METHODS

Insect Origin and Rearing

Trichogramma brassicae was obtained from an insectarium maintained at the Rice Research Center, Gilan (North of Iran) in 2012. *T. brassicae* wasps were reared on *Sitotroga cerealella* (Olivier) eggs for eight generations at 25±1ºC, 70±10% RH, and a photoperiod of 16:8 (L:D) hour. Honey was presented as food for the adult parasitoid on a stripe of paper.

Chemicals Materials

Diazinon (Diazinon® 60% EC, Arya shimi, Iran) and Fipronil (Rigent® 20% G, Partonar shimi, Iran) were used in all experiments.

[DOR: 20.1001.1.16807073.2016.18.5.3.9]

Diazinon belongs to the class of organophosphate insecticides and kills insects by interfering with the function of the nervous system. Fipronil (a phenyl pyrazole insecticide) is a potent blocker of the GABA-regulated chloride channels in the neurons of the central nervous system. When this function is blocked by fipronil, the result is neural excitation and the death of the insect (Cole *et al.,* 1993).

LC_{50} Bioassays on Adult Wasps

Adult bioassays were carried out by contact exposure of insecticides. For this purpose, the exposure cages were used (Saber *et al*., 2004). The exposure cage was formed of two removable glass plates (13 by 13 cm^2) as floor and ceiling and a polyethylene frame with 2 cm side wall. There were six holes on each side of the frame, from which two openings were used to introduce the *T. brassicae* wasps and food; other holes were covered with black netting for ventilation. Stock solutions of the formulated insecticides were prepared at a concentration that reflected the Field Recommended Concentration (FRC). Aliquots were taken from each stock solution and mixed with water to prepare six concentrations 1.75, 0.77, 0.34, 0.15, 0.06 and 0.01 ppm for diazinon and 7, 4.69, 3.17, 2.14, 1.45 and 1 ppm (based on formulated materials) for fipronil. Tween 80 (Merck, Darmstadt, Germany) was used at a concentration of 200 ppm in all dilutions as a surfactant (Rosenheim and Hoy, 1988; Wang *et al.,* 2012). Glass plates (i.e., the floor and ceiling of the exposure cage) were sprayed with each concentration of insecticide using a hand spray. The hand sprayers were calibrated for sufficient and similar spray coverage before using. Control plates were sprayed with water plus Tween 80. After drying the glass plates on the laboratory at room temperature for 1 hour, exposure cages were closed. Fifty to seventy young (< 24 hours old) *T. brassicae* adults were introduced into each exposure cage by emergence tubes. Three exposure cages were used for each insecticide concentration and each bioassay test was replicated three times. The parasitoids in the exposure cages were supplied with honey placed on a small strip of paper as food. The cages were held at 25±1ºC, 70±10% RH, and at a photoperiod of 16:8 (L:D) hour, for 24 hours. The number of dead wasps in each cage was counted 24 hours after the initial exposure to the chemicals residue.

The result of each trial was tested for goodness of fit using PROC GENMOD procedures (SAS Institute, 2002. Robertson *et al.,* 2007 ;) and the data was analyzed using PROC PROBIT (SAS Institute, 2002) to compute LC_{10}, LC_{50} and LC_{90} values on a standard and log scale with associated 95% fiducial limits.

Effects of Insecticides on Immature Development

In order to investigate the effects of the insecticides on immature stages of *T. brassicae* developing within the host eggs, *S. cerealella* eggs were used. The fresh *S. cerealella* eggs were glued in circles on strips of white papers to provide egg discs. The egg discs (ca. 100±20 eggs) were presented to adults of *T. brassicae* in a glass tube for 24 hours. Parasitized egg discs were prepared in 3 day intervals to provide 3, 6 and 9 days old pre-imaginal stages. These days correspond to larval, prepupal and pupal stages of *Trichogramma* spp., respectively (Cônsoli *et al.,* 1998; Knutson, 1998; Suh *et al.,* 2000). Apposite amounts of each pesticide were rarified with 100 ml of distilled water to provide the recommended field concentrations, 1,000 and 40,000 ppm for diazinon and fipronil (Kumar and Singh, 2013; Khan *et al.,* 2015), respectively. These concentrations were calculated based on the amount of recommended quantity of each insecticide and by considering 250-300 L of applied solution per hectare. Randomly taken parasitized egg discs were immersed in pesticide solution for 5 seconds (Cônsoli

[DOR: 20.1001.1.16807073.2016.18.5.3.9]

et al., 1998; Carvalho *et al.,* 2003; Hewa-Kapuge *et al.,* 2003; Saber *et al.,* 2004). This method certified that all parasitized eggs were similarly exposed to the insecticide solutions. Distilled water was used as control treatment. The treated egg discs were allowed to dry completely by placing them on the laboratory bench at room temperature for 1 hour and then each egg disc was transferred to a small glass tube. The glass tubes including the eggs were then plugged with cotton wool and were transferred to a growth chamber that was set to the standard conditions indicated above. Under these conditions, adults generally emerged from eggs 10 to 11 days after the primal parasitism. A final assessment of emergence was made 14 days after initial parasitism by visually inspecting the eggs for emergence. The total number of eggs, the number of black eggs (an indication of parasitization) and the emerged wasps were recorded. The eggs with dead adults, or the eggs with partially chewed exit holes with dead adult remaining inside, were categorized as 'failed to emerge'. Ten randomly chosen parasitized egg discs were used in each pesticide treatment and the trial was repeated three times. The experiment was designed as a multifactorial design in which factors were treatment (three levels: control, diazinon and fipronil), and stage of wasps treated (three levels: larvae, prepupae and pupae). An arcsine square root transformation was performed on the emergence percentage before analyzing data. All data were subjected to analysis of variance, and the means were compared using either Fisher's protected Least Significant Difference (LSD) or *LS* means (P< 0.05) (SAS Institute, 2002).

Sublethal Effects of Insecticides on Life-table Parameters

Adult parasitoids that emerged from *S. cerealella* eggs were previously treated with field concentrations of the insecticides at the larval stage and also, adult parasitoids treated with LC_{30} concentration were used for the life-table studies.

Vials containing treated eggs at the larval stage were monitored daily for emerged parasitoids. More than 100 emerged young adults (< 24 hours old) from ten replicates of each treatment were transferred to small glass vials (nine by 75 mm) individually and then 25 of them were selected randomly.

In studying LC_{30} concentration, adult parasitoids in three replicates (100±20 in each replicate) were exposed to LC_{30} concentration of insecticides. After 24 h, about 100 females surviving from all replicates were transferred to small glass vials individually. Then 25 of them were selected randomly for life-table parameters study.

In both experiments, each female parasitoid was presented one *S. cerealella* egg disc (ca. 100±20 eggs) and a small drop of honey as food. The egg discs were changed daily and mortality of the wasps was recorded. The parasitized egg discs were maintained at the rearing conditions described above and allowed to emerge. The total numbers of eggs, black eggs (parasitized eggs), eggs containing dead adults, emerged wasps and their sex ratio were recorded.

Daily schedules of mortality and fecundity were integrated into a life table format (Carey, 1993) and used to calculate the net Reproductive rate (R_0), mean generation Time (T) and intrinsic rate of increase (r_m) (Table 1). Jackknife pseudo-values were computed using Microsoft office Excel for r_m (intrinsic rate of increase), R_0 (net Reproductive rate), Gross Reproductive Rate (GRR), and λ (finite rate of increase) for each treatment. A square root transformation was performed on population parameters including T, DT, r_m and m_x data before analysis. The mentioned data were subjected to analysis of variance and means were compared using Tukey posthoc test (P< 0.05) (Meyer *et al.,* 1986; Maia *et al.,* 2000).

[DOR: 20.1001.1.16807073.2016.18.5.3.9]

Table 1. Effects of diazinon and fipronil on life-table parameters of *Trichogramma brassicae* from parasitized *Sitotroga cerealella* eggs previously exposed to field rate of the pesticides at the larval stage.[a]

Treatment	Longevity day±SE	Egg hatching±SE	Sex ratio±SE (Male/Female+Male)	Mean female progeny per female (m_x)±SE	Net Reproductive rat (R_0)	Generation Time (T) day±SE	Intrinsic rate of increase (r_m)±SE	Doubling Time (DT)±SE
Diazinon	2.04±0.2b	71.90±5.2b	0.06±0.026b	0.49±0.3b	1.5±2.2b	1.80±0.1b	0.23±0.01b	2.98±0.1b
Fipronil	3.88±0.2b	62.23±3.1b	0.07±0.022b	1.19±0.5b	5.5±1.7b	14.04±0.1a	0.12±0.01c	5.66±0.2a
Control	7.08±0.4a	83.69±2.2a	0.44±0.005a	6.61±1.5a	60.8±1.8a	14.79±0.1a	0.28±0.01a	2.49±0.15b

[a] Mean within a column followed by different letters are significantly different (Fisher's LSD; P= 0.05)

RESULTS

LC_{50} Bioassays

The LC_{50} values indicated that the acute toxicity of diazinon (0.11 μg ai mL^{-1}) on *T. brassicae* was higher than that of fipronil (0.46 μg ai mL^{-1}) (Table 2) and the LC_{90} value for diazinon (23.8 μg ai mL^{-1}) was considerably higher compared to fipronil (3 μg ai mL^{-1}). It was due to the higher slope in fipronil compared to diazinon since a little increase in the concentration, increases the mortality more.

Effects of Insecticides on Immature Development

The mean emergence rate of the parasitoid from *S. cerealella* eggs treated with diazinon and fipronil at all stages were 0.26 and 49.54%, respectively. The parasitized *S. cerealella* eggs exposed to either diazinon or fipronil at the larval stage yielded the lowest emergence rate (Table 3). *T. brassicae* emergence from *S. cerealella* eggs was significantly affected by the pesticides at larval ($F= 1038.74$; df= 2, 29; $P< 0.0001$), prepupal ($F= 333.27$; df= 2, 29; $P< 0.0001$) and pupal ($F=123.98$; df= 2, 29; $P< 0.0001$) stages. In total, the emergence rate was reduced by a greater amount following exposure to diazinon than following exposure to fipronil.

Demographic Parameters

The mean longevity of emerged *T. brassicae* adults from host eggs treated with field concentration at the larval stage, was significantly affected ($F= 94.9$; df= 2, 74; $P< 0.0001$) (Table 1). The mean number of female offspring per female (m_x) ($F= 5.7$; df= 2, 21; $P< 0.01$) were different compared with control. Population parameters of *T. brassicae* such as r_m ($F= 38.1$; df= 2, 74; $P< 0.0001$), R_0 ($F= 17.4$; df= 2, 74; $P< 0.0001$),

Table 2. Acut toxicity of diazinon and fipronil to *Trichogramma brassicae* adult.

Insecticide	*n*	*Slope±SE*	χ^2	Lethal Concentration (µg ai mL^{-1})[a]		
				LC_{10} (95% FL)	LC_{50} (95% FL)	LC_{90} (95% FL)
Diazinon	678	0.53 ± 0.07	37.07	0.007 (0.002-0.009)	0.11 (0.07-0.17)	28.1(8.3 – 218)
Fipronil	2707	1.58 ± 0.1	72.93	0.35 (0.26-0.45)	0.46 (0.42 – 0.5)	3 (2.4 – 3.9)

[a] Lethal concentration and Fiducial Limits (FL) based on standard scale.

Table 3. Effect of diazinon and fipronil on emergence rate of *Trichogramma brassicae* from *Sitotroga cerealella* eggs exposed to field recommended concentration of the pesticides at tree pre imaginal developmental stages of the parasitoid. [a]

Treatment	Mean % of adult parasitoid emergence from parasitized host eggs treated at different preimaginal stages			Mean emergence in treatments (%)	Mean reduction in % of emergence	Classification of insecticide according to IOBC/WPRS standards
	Larvae	Prepupae	Pupae			
Diazinon	0.16 ± 0.16 c	0.18 ± 0.18 c	0.44 ± 0.23 c	0.26 ± 7.85 c	99.7	Harmful
Fipronil	37.4 ± 2.49 b	64.3 ± 3.30 b	64.25 ± 3.30 b	49.54 ± 0.09 b	50.5	Slightly harmful
Control	96.3 ± 0.76 a	89.7 ± 2.86 a	89.73 ± 2.86 a	93.31 ± 1.9 a		

[a] Means in a column followed by different lowercase letters are significantly different (Fisher's protected LSD; P< 0.05).

T (F= 117.9; df= 2, 74; P< 0.0001) and *DT* (F= 7.6; df= 2, 74; P< 0.001) were affected significantly by the insecticides treatments (Table 1). The intrinsic rate of increase for the control, diazinon and fipronil-exposed populations were 0.28, 0.23 and 0.12 female offspring per female per day, respectively (Table 1). The mean sex ratio (F= 117.9; df= 2, 74; P< 0.0001) and egg hatching (F=8.4; df= 2, 74; P< 0.0005) were also affected by the insecticides. The sex ratio for control, diazinon and fipronil was 0.44, 0.06 and 0.07, respectively (Table 1).

Mean longevity of *T. brassicae* exposed to LC_{30} value at adult stage was also affected by the insecticides (F= 147.5; df= 2, 74; P< 0.0001) (Table 4). The mean of m_x (F= 4.6; df= 2, 19; P< 0.03) was influenced significantly by treatments. The parameters including r_m (F= 146.6; df= 2, 7; P< 0.0001), R_0 (F= 19.2; df= 2, 74; P< 0.0001), *T* (F= 44.3; df= 2, 74; P< 0.0001) and *DT* (F=14.8; df= 2, 74; P< 0.0001) were affected significantly by the insecticides (Table 4). Diazinon and fipronil affected mean sex ratio (F= 74.8; df= 2, 74; P< 0.0001), but the hatch rate was not influenced by the insecticides (F= 0.72; df= 2, 74; P< 0.4879).

Table 4. Effects of diazinon and fipronil on life-table parameters of *Trichogramma brassicae* exposed to LC_{30} of the pesticides at the adult stage.[a]

Treatment	Longevity day±SE	Sex ratio±SE (Male/Female +Male)	Mean female progency per female (m_x)±SE	Net Reproductive rate (R_0)	Generation Time (T) day±SE	Intrinsic rate of increase (r_m)±SE	Doubling Time (DT) day±SE
Diazinon	1.08±0.1b	0.07±0.02b	1.02±1.02b	1.65±0.8b	12.53±0.1b	0.04±0.01c	16.48±0.6a
Fipronil	1.28±0.1b	0.12±0.03b	1.83±0.8ab	2.58±1.1b	12.83±0.1b	0.08±0.01b	8.54±0.8b
Control	7.04±0.5a	0.42±0.02a	5.53±0.97a	48.92±1.6a	14.90±0.1a	0.26±0.004a	2.65±0.5b

[a] Mean within a column followed by different letters are significantly different (Fisher's LSD; P< 0.05).

DISCUSSION

Acute toxicity bioassay results showed that both insecticides were highly toxic to the adult stage of *Trichogramma brassicae*. Suh *et al*. (2000) showed that an organophosphate insecticide, prophenofos was very toxic to *T. exiguum* at adult stage. Other studies have also showed that broad spectrum insecticides including fipronil exhibited high toxicity on adult stage of *Trichogramma* spp. (Li *et al*., 1986; Suh *et al.,* 2000; Khan *et al.,* 2015). Adult parasitoids can be exposed directly to insecticide spray droplets or indirectly to the deposit on the crop foliage when foraging or imbibing contaminated water droplets, nectar or honeydew (Longley and Jepson, 1996). Immature parasitoids can be also exposed to insecticides through the bodies of their hosts (Longley, 1999; Hussain *et al*., 2010). Although insecticides are generally considered toxic to adult *Trichogramma*, pre-imaginal stages developing within host eggs appear to be well protected from many insecticides (Bull and Coleman, 1985; Li *et al.,* 1986; Singh and Varma, 1986; Brar *et al.,* 1991; Cônsoli *et al.,* 1998; Saber, 2011; Wang *et al,*. 2012). The present study also showed that field rate of diazinon and fipronil severely affected the adult stage of the parasitoid and resulted in 100% mortality within 24 hours. Wang *et al.* (2012) found that the field rate of fipronil caused 100% mortality on *Trichogramma chilonis* Ishii adults after 8 hours. Also Hussain *et al*. (2010) revealed that chlorpyrifos caused 100% mortality on adults of *T. chilonis* after 24 hours.

Diazinon and fipronil severely affected the mean emergence rate of the adults (Table 3). Our results showed that diazinon also is very toxic to *T.brassicae* at pre-imaginal stages even inside the host eggs. Varma and Singh (1987) showed that fenitrothion (a member of organophosphate insecticides) also completely disrupted the emergence of *Trichogramma brasiliensis* (Ashmead) from rice moth, *Corcyra cephalonica* (Stainton) (Lepidoptera: Pyralidae) eggs. Shoeb (2005) studied the effect of insecticides on *Trichogramma evanescens* Westwood and found that profenofos was harmful to the immature stages of the parasitoid. Also Shoeb (2010) reported that fenitrothion caused a high decrease in the emergence rate of *T. evanescens* from *S. cerealella* eggs. Some mortality is also observed while emerging adults chew the host egg chorion (Cônsoli *et al.,* 2001; Moura *et al.,* 2006; Souza *et al.,* 2014). Researchers have got different results on effects of insecticides on pre-imaginal stages of *Trichogramma* and on emergence rate of different pesticides (Blibech *et al.,* 2015). Plewka *et al.* (1975), reported that some insecticides did not penetrate the host egg-chorion (*S. cerealella*), and *Trichogramma* spp. was affected only upon emergence from the eggs.

In thecurrent study, female adult longevity, fecundity, sex ratio, number of female offspring per female and population life-table parameters such as intrinsic rate of increase, generation time and doubling time were used to assess sublethal effects of diazinon and fipronil (Tables 1 and 4).

Longevity is frequently used as an index of wasp quality (Waage and Ming, 1984; Saber, 2011). The longevity of adult female was significantly affected by the insecticides. The effect of insecticides on longevity of parasitoids seems to be highly dependent on the type of insecticide, the parasitoid species, and the method of application (Bayram *et al.,* 2010). Reductions in longevity have been generally observed in parasitoids that had been treated with insecticides during developing stages inside hosts (Smilanick *et al.,* 1996; Suh *et al.,* 2000; Schneider *et al.,* 2004; Desneux *et al.,* 2006a, b) as observed in our study. However, few studies have reported increase in longevity of parasitoids as a consequence of treating adults. Wang *et al.* (2012) reported that the mean longevity of *Trichogramma chilonis* females exposed to fipronil was significantly longer than control, while fipronil reduced the mean longevity of *T. brassicae* in the current research. In our study the fecundity of the treated insects was significantly reduced. Similar results were found in other studies, although researchers investigated different parasitoid species and different insecticides: *T. brassicae* treated with LD_{20} of chlorpyrifos (Delpuech and Meyet, 2003); *Telenomus busseolae* Gahan (Hymenoptera: Scelionidae) treated with LC_{25} of cyfluthrin (Bayram *et al.*, 2010); and *Trichogrammatoidea bactrae* Nagaraja treated with LC_{30} of cartap, diafenthiuron, spinosad, and fipronil (Wang *et al.,* 2011). In this study, the observed reduction in fecundity of treated *T. brassicae* females may be due to the reduction of their longevity.

In this study the sex ratio has led to the production of female offspring due to treatment with insecticides. The number of female offspring per female was affected significantly by the pesticides. Increasing the female offspring in the next generations would compensate to some extent the reduction of the parasitoid population due to insecticide applications.

All stable population parameters were affected by the insecticides (Tables 1 and 4). Wang *et al.* (2012) reported that when *T. chilonis* was exposed to chlorfenapyr, avermectin, and fipronil, the population parameters such as R_0, r_m, and λ were significantly lower than those in the control. Also they showed these insecticides had adverse effects on *DT*. Lower intrinsic rate of increase in a treated population could have an important influence on parasitoid generation (Saber *et al.,* 2004).

Thus, the information from this laboratory study gives important results that will help to choose the best pesticide to be applied since products with the lowest impact on biological control agents are the most appropriate to be used in IPM programs. However, side effects of pesticides may be reduced under field conditions because parasitoid wasps can benefit from refuge areas or avoid pesticide treated areas. Moreover, sunlight degradation plays an important role in the field and may decrease the strong chemical effects seen on beneficial insects under laboratory conditions (Hassan, 1992; Rocha and Carvalho, 2004). Further research should focus on the impact of insecticides exposure on *T. brassicae* parasitism and overall effectiveness under semi-field and field conditions.

REFERENCES

1. Abdelgader, H. and Hassan, S. A. 2012. Effects of botanical insecticides on the egg parasitoid *Trichogramma cacoeciae* Marchal (Hym. Trichogrammatidae). *Third International Scientific Symposium "Agrosym Jahorina",* Bosnia and Herzegovina , PP.445-450.
2. Acheampong, S. and Stark, J. D. 2004. Effect of Agricultural Adjuvant Sylgard 309 and the Insecticide Pymetrozine on Demographic Parameters of the Aphid Parasitoid, *Diaeretiella rapae*. *Biol. Control.*, **31**: 133-137.
3. Bayram, A., Salerno, G., Onofri, A. and Conti, E. 2010. Sub-lethal Effects of Two Pyrethroids on Biological Parameters and Behavioral Responses to Host Cues in the Egg Parasitoid *Telenomus busseolae*. *Biol. Control.*, **53:** 153–160.
4. Blibech, I., Ksantini, M., Jardak, T. and Bouaziz, M. 2015. Effect of Insecticides on *Trichogramma* Parasitoids Used in Biological Control against *Prays oleae* Insect Pest. *Adv. Chem. Eng. Sci.,* **5:** 362-372.
5. Brar, K. S., Varma, G. C. and Shenhmar, M. 1991. Effects of Insecticides on *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae), an Egg Parasitoid of Sugarcane Borers and Cotton Bollworms. *Entomon.*, **16:** 43–48.
6. Bull, D. L. and Coleman, R. J. 1985. Effects of Pesticides on *Trichogramma* spp. *Southwest Entomol Suppl.*, **8:** 156- 168.
7. Carey, J. R. 1993. *Applied Demography for Biologists.* Oxford University Press, New York, 205 PP.
8. Carvalho, G. A., Reis, P. R., Rocha, L. C., Moraes, J. C., Fuini, L. C. and Ecole, C. C. 2003. Side Effects of Insecticides Use in Tomato Field on *Trichogramma pretiosum*

(Hymenoptera: Trichogrammatidae). *Acta Scientiarum Agron*., **25(2):** 275-279.
9. Cole, L. M., Nicholson, R. A. and Casida, J. E. 1993. Action of Pheylpyrazole Insecticides at the GABA-gated Chloride Channel. *Pest Biochem. Physiol.*, **46:** 47-54.
10. Cônsoli, F. L., Parra, J. R. P. and Hassan, S. A. 1998. Side Effects of Insecticides Used in Tomato Field on the Egg Parasitoid *Trichogramma pretiosum* Riley (Hym., Trichogrammatidae), a Natural Enemy of *Tuta absoluta* (Meyrick) (Lep., Gelechiidae). *J. Appl. Entomol.*, **122:** 43-47.
11. Cônsoli, F. L., Botelho, P. S. M. and Parra, J. R. P. 2001. Selectivity of Insecticides to the Egg Parasitoid *Trichogramma galloi* Zucchi, 1988 (Hym., Trichogrammatidae). *J. Appl. Entomol.*, **125:** 37-43
12. Croft, B. A. 1990. Arthropod Biological Control Agents and Pesticides. Wiley, New York, 723 PP.
13. Delpuech, J. M., Legallet, B., Terrier, O., Fouillet, P. 1999. Modifications of the Sex Pheromonal Communication of *Trichogramma brassicae* by a Sublethal Dose of Deltamethrin. *Chemosphere,* **38:** 729–39.
14. Delpuech, J. M. and Meyet, J. 2003. Reduction in the Sex Ratio of the Progeny of a Parasitoid Wasp (*Trichogramma brassicae*) Surviving the Insecticide Chlorpyrifos. *Arch. Environ. Contam. Toxicol.*, **45:** 203–208.
15. Desneux, N., Decourtye, A. and Delpuech, J. M. 2007. The Sublethal Effects of Pesticides on Beneficial Arthropods. *Annu Rev Entomol.*, **52:** 81-106.
16. Desneux, N., Denoyelle, R. and Kaiser, L. 2006a. A Multi-step Bioassay to Assess the Effect of the Deltamethrin on the Parasitic Wasp *Aphidius ervi. Chemosphere,* **65:** 1697–1706.
17. Desneux, N., Pham-Delegue, M. H. and Kaiser, L. 2003. Effects of Sub-lethal and Lethal Doses of Lambda-cyhalothrin on Oviposition Experience and Host-searching Behaviour of a Parasitic Wasp, *Aphidius ervi. Pest Manag. Sci*., **60** : 381 -389.
18. Desneux, N., Ramirez-Romero, R. and Kaiser, L. 2006b. Multistep Bioassay to Predict Recolonization Potential of Emerging Parasitoids after a Pesticide Treatment. *Environ. Toxicol. Chem*., **25:** 2675–2682.
19. Forbes, V. E. and Calow, P. 1999. Is the per Capita Rate of Increase a Good Measure of Population-level Effects in Ecotoxicology? *Environ. Toxical. Chem*., **18:** 1544-1556.
20. Galvan, T. L., Koch, R. L. and Hutchison, W. D. 2005. Effects of Spinosad and Indoxacarb on Survival, Development, and Reproduction of the Multicolored Asian Lady Beetle (Coleoptera: Coccinellidae). *Biol. Control.,* **34:** 108-114.
21. Hassan, S. A., Haves, B. O., Degrande, P. E. and Herai, K. 1998. The Side-effects of Pesticides on the Egg Parasitoid *Trichogramma cacoeciae* Marchal (Hym., Trichogrammatidae), Acute Dose–response and Persistence Tests. *J. Appl. Entomol.*, **122:** 569-573.
22. Hassan, S. A. 1992. Guideline for the Evaluation of Side-effects of Plant Protection Product on Trichogramma cacoeciae. In: “Guidelines for Testing the Effects of Pesticides on Beneficial Organisms: Description of Test Methods”, (Ed.): Hassan, S. A. IOBC/WPRS Bull., **15:** 18 – 39.
23. Hewa-Kapuge, S., McDougall, S. and Haffmann, A. 2003. Effects of Metoxyfenozide, Indoxicarb, and Other Insecticides on the Beneficial Egg Parasitoid *Trichogramma nr. Brassicae* (Hymenoptera: Trichogrammatidae) under Laboratory and Field Conditions. *J Econ. Entomol.,* **96**: 1083-1090.
24. Hussain, D., Akram, M., Iqbal, Z. and Saleem, M. 2010. Effect of Some Insecticides on *Trichogramma chilonis* ishii (Trichogramatidae: Hymenoptera) Immature and Adult Survival. *J. Agr. Res*., **48**: 531-537.
25. Iranipour, S., Farazmand, A., Saber, M. and Mashhadi Jafarloo, M. 2008. Demography and Life History of the Egg Parasitoid, *Trichogramma brassicae*, on Two Moths *Anagasta kuehniella* and *Plodia interpunctella* in the Laboratory. *J. Insect Sci*., **9**:51.
26. Khan, M. A., Khan, H., Farid, A. and Ali, A. 2015. Evaluation of Toxicity of Some Novel Pesticides to Parasitism by *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae). *J. Agric. Res*., **53(1):** 63-73.
27. Khan, Z. R., James, D. G., Midega, C. A. O. and Pickett, J. A. 2008. Chemical Ecology and Conservation Biological Control. *Biol. Control*., **45:** 210-224.
28. Knutson, A. 1998. The *Trichogramma* Manual: A Guide to the Use of *Trichogramma* for Bilogical Control with Special Reference to Augmentative Releases for Control of bollworm and Budworm in Cotton. Texas Agriculture Extension Service,

Texas A&M University System, College Station, TX.
29. Kumar, R. and Singh, B. 2013. Persistence and Metabolism of Fipronil in Rice (*Oryza sativae* L.) Field. *Bull. Environ. Contam. Toxicol*., **90(4):** 482-488.
30. Li, K. H., Xu, X., Li, Y. F., Meng, Q. Z. and Zhou, L. C. 1986. Determination of Toxicity of 29 Chemicals to *Trichogramma japonicum* at Various Developmental Stages. *Natur. Enem. Insect*., **8**: 187-194.
31. Longley, M. A. 1999. A Review of Pesticide Effects upon Immature Aphid Parasitoids within Mummified Hosts. *Int. J. Pest. Manag*., **45:** 139 -145.
32. Longley, M. and Jepson, P. C. 1996. Effects of Honeydew and Insecticide Residues on the Distribution of Foraging Aphid Parasitoids under Glasshouse and Field Conditions. *Entomol. Exp. Appl*., **81**: 189 -198.
33. Mahdavi, V., Saber, M., Rafiee-Dastjerdi, H. and Mehrvar, A. 2011. Comparative Study of the Population Level Effects of Carbaryl and Abamectin on Larval Ectoparasitoid *Habrobracon hebetor* Say (Hymenoptera: Braconidae). *BioControl*., **56**: 823-830.
34. Maia, A. H. N., Luiz, A. J. B. and Canpanhola, C. 2000. Statistical Influence on Associated Fertility Life Table Parameters Using Jackknife Technique: Computational Aspects. *J. Econ. Entomol*., **93**: 511-518.
35. Meyer, J. S., Igersoll, C. G., MacDonald, L. L. and Boyce, M. S. 1986. Estimating Uncertainty in Population Growth Rates: Jackknife *vs.* Bootstrap Techniques. *Ecology*, **67:** 1156-1166.
36. Moura, A. P., Carvalho, G. A., Pereira, A. E. and Rocha, L. C. D. 2006. Selectivity Evaluation of Insecticides Used to Control Tomato Pests to *Trichogramma pretiosum*. *BioControl*., **51**: 769-778.
37. Musser, F. R., Nyrop, J. P. and Shelton, A. M. 2006. Integrating Biological and Chemical Controls in Decision Making: European Corn Borer (Lepidoptera: Crambidae) Control in Sweet Corn as an Example. *J. Econ. Entomol*., **99:** 1538-1549.
38. Plewka, T., Kot, J. and Krukierek, T. 1975. Effect of Insecticides on the Longevity and Fecundity of *Trichogramma evanescens* (Hymenoptera: Trichogrammatidae). *Pol. Ecol. Stud.*, **1**: 197-210.
39. Poorjavad, N., Goldansaz, S. H., Dadpour, H. and Khajehali, J. 2014. Effect of *Ferula assafoetida* Essential Oil on Some Biological and Behavioral Traits of *Trichogramma embryophagum* and *T. evanescens. BioControl.,* **59:** 403-413.
40. Poorjavad, N., Goldansaz, S. H., Machtelinckx, T., Tirry, L., Stouthamer, R. and van Leeuwen, T. 2012. Iranian Trichogramma: ITS2 DNA Characterization and Natural Wolbachia Infection. *BioControl.,* **57:** 361-374.
41. Qi, B., Gordon, G. and Gimme, W. 2001. Effects of Neem Fed Prey on the Predacious Insects *Harmonia conformis* (Boisduval) (Coleoptera: Coccinellidae) and *Mallada signatus* (Schneider) (Neuroptera: Chrysopidae). *Biol. Control.*, **22:** 185-190.
42. Robertson, J. L., Russell, R. M., Preisler, H. K. and Savin, N. E. 2007. *Bioassay with Arthropods.* CRC Press, London.
43. Rocha, L. C. D. and Carvalho, G. A. 2004. Adaptação da Metodologia Padrão da IOBC para Estudos de Seletividade com *Trichogramma pretiosum* Riley, 1879 (Hymenoptera: Trichogrammatidae) em Condições de Laboratório. *Acta Sci. Agron.*, **26(3):** 315-320.
44. Rosenheim, J. A. and Hoy, M. A. 1988. Sublethal Effects of Pesticides on the Parasitoid *Aphytis melinus* (Hymenoptera: Aphelinidae). *J. Econ. Entomol.*, **81:** 476–48.
45. Saber, M. 2011. Acute and Population Level Toxicity of Imidacloprid and Fenpyroximate on an Important Egg Parasitoid, *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae). *Ecotoxicol*., **20:** 1476-1484.
46. Saber, M. and Abedi, Z. 2013. Effects of Methoxyfenozide and Pyridalyl on the Larval Ectoparasitoid *Habrobracon hebetor*. *J. Pest Sci.,* **86:** 685-693.
47. Saber, M., Hejazi, M. J. and Hassan, S. A. 2004. Effects of azadirachtin/Neemazal on Different Stages and Adult Life Table Parameters of *Trichogramma cacoeciae* (Hymenoptera: Trichogrammatidae). *J. Econ. Entomol*., **97**: 905-910.
48. Saber, M., Hejazi, M. J., Kamali, K. and Moharramipour, S. 2005. Lethal and Sublethal Effects of Fenitrothion and Deltamethrin Residues on the Egg Parasitoid *Trissolcus grandis* (Hymenoptera: Scelionidae). *J. Econ. Entomol*., **98**: 35-40.
49. SAS institute. 2002. *SAS/STAT User's Guide.* SAS institute, Cary.
50. Schneider, M. I., Smagghe, G., Pineda, S. and Viñuela, E. 2004. Action of Insect Growth

Regulator Insecticides and Spinosad on Life History Parameters and Absorption in Third-Instar Larvae of the Endoparasitoid *Hyposoter didymator*. *Biol. Control.*, **31**: 189–198.
51. Settle, W.H., Ariawan, H., Astuti, E. T., Cahyana, W., Hakim, A. L., Hindayana, D. and Lestari, A. S. 1996. Managing Tropical Rice Pests through Conservation of Generalist Natural Enemies and Alternative Prey. *Ecol.*, **77**: 1975-1988.
52. Shoeb, M. A. 2005. Comparativeness of Chemical, Natural and Bacterial Insecticides on Some Biological Aspects of the Egg Parasitoid *Trichogramma evanescens* (West.) *J. Agric. Sci. Mansoura Univ.*, **30(8)**: 4821-4826.
53. Shoeb, M. A. 2010. Effect of Some Insecticides on the Immature Stages of the Egg Parasitoid *Trichogramma evanescens* West. (Hym.; Trichogrammatidae). *Egypt Acad. J. Biolog. Sci.*, **3(1)**: 31-38.
54. Singh, P. P. and Varma, G. C. 1986. Comparative Toxicities of Some Insecticides to *Chrysoperla carnea* (Chrysopidae: Neuroptera) and *Trichogramma brasiliensis* (Trichogrammatidae: Hymenoptera), Two Arthropodal Natural Enemies of Cotton Pests. *Agric. Ecosys. Environ.*, **15**: 23-30.
55. Smilanick, J. M., Zalom, F. G. and Ehler, L. E. 1996. Effect of Metamidophos Residue on the Pentatomid Egg Parasitoids *Trissolcus basalis* and *T. utahensis* (Hymenoptera: Scelionidae). *Biol. Control.,* **6:** 193-201.
56. Souza, J. R., Carvalho, G. A., Moura, A. P., Couto, M. H. G., and Maia, J. B. 2014. Toxicity of Some Insecticides Used in Maize Crop on *Trichogramma pretiosum* (Hymenoptera, Trichogrammatidae) Immature Stages. *Chil. J. Agric. Res.*, **74:** 234-239.
57. Stark, J. D. and Banks, J. E. 2003. Population Level Effects of Pesticides and Other Toxicants on Arthropods. *Annu. Rev. Entomol.*, **48:** 505-519.
58. Stark, J. D., Banks, J. E. and Acheampong, S. 2004. Estimating Susceptibility of Biological Control Agents to Pesticides: Influence of Life History Strategies and Population Structure. *BioControl.*, **29:** 392-398.
59. Suh, C. P. C., Orr, D. B. and van Duyn, J. W. 2000. Effect of Insecticides on *Trichogramma exiguum* (Trichogrammatidae: Hymenoptera) Preimaginal Development and Adult Survival. *J. Econ. Entomol.,* **93:** 577-583.
60. Talebi-Jahromi, K. 2007. *Pesticides Toxicology*. University of Tehran Publications, Tehran, 492 PP.
61. Urech, P. 2000. Sustainable Agriculture and Chemical Control: Opponents or Components of the Same Strategy? *Crop Prot.*, **19:** 831-836.
62. Van Driesche, R. G., Carruthers, R. H., Center, T., Hoddle, M. S., Hough-Goldstein, J., Morin, L., Smith, L., Wagner, D. L., Blossey, B. and Brancatini, V. 2010. Classical Biological Control for the Protection of Natural Ecosystems. *Biol. Control.*, **54(1)**: 2-33.
63. Van Lenteren, J. C. and Woets, J. 1988. Biological and Integrated Pest Control in Greenhouses. *Annu. Rev. Entomol.*, **33:** 239-269.
64. Varma, G. C. and Singh, P. P. 1987. Effect of Insecticides on the Emergence of *Trichogramma brasiliensis* (Hymenoptera: Trichogrammatidae) from Parasitized Host Eggs. *Entomophaga*, **32**: 443-448.
65. Waage, J. K. and Ming, N. S. 1984. The Reproductive Strategy of a Parasitic Wasp. I. Optimal Progeny and Sex Allocation in *Trichogramma evanescens*. *J. Anim. Ecol.*, **53:** 401-415.
66. Wang, D. S., Pan, F., He, Y. R., Guo, X. L. and Chen, Q. 2011. Sublethal Effects of Eleven Insecticides of Different Categories on Reproduction of *Trichogrammatoidea bactrae* Nagaraja (Hymenoptera: Trichogrammatidae). *Acta Entomol. Sin.,* **54:** 56–63.
67. Wang, D., He, Y., Guo, X. and Luo, Y. 2012. Acute Toxicities and Sublethal Effects of Some Conventional Insecticides on *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae). *J. Econ. Entomol.*, **105**: 1104-1476.

[DOR: 20.1001.1.16807073.2016.18.5.3.9]

اثر دیازینون و فیپرونیل روی مراحل مختلف رشدی *Trichogramma brassicae* Bezdenko (Hym.; Trichogrammatidae)

م. قربانی، م. صابر، م. باقری، ن. واعظ

چکیده

تلفیق روشهای کنترل بیولوژیک و شیمیایی در قالب برنامه های مدیریت تلفیقی آفات بسیار مهم است. سم شناسی دموگرافیکی فهم بهتری از اثرات جانبی آفتکشها روی موجودات مفید ارائه می دهد. در مطالعه حاضر، زیست‌سنجی های آزمایشگاهی برای ارزیابی اثرات کشنده و زیرکشنده دیازینون و فیپرونیل روی مراحل مختلف زیستی *Trichogramma brassicae* Bezdenko انجام شد. اثر غلظتهای توصیه شده مزرعه ای دیازینون و فیپرونیل روی مراحل لاروی، پیش شفیرگی و شفیرگی زنبور پارازیتوئید بررسی شد. دیازینون و فیپرونیل ظهور حشرات کامل از تخمهای پارازیته شده را به ترتیب ۹۹/۷۴ و ۵۰/۴۶٪ کاهش دادند. مقادیر LC_{50} دیازینون و فیپرونیل روی حشرات کامل پارازیتوئید، ۰/۱۱ و ۰/۴۶ میلی گرم ماده موثر بر میلی لیتر بود. اثرات زیرکشندگی این ترکیبات شیمیایی روی پارامترهای زیستی زنبورهای خارج شده از تخمهای پارازیته تیمار شده با غلظت توصیه شده مزرعه ای در مرحله لاروی و همچنین حشرات کامل که در معرض LC_{30} قرار گرفته بودند مطالعه شد. طول عمر و تولید نتاج بوسیله حشره کشها تحت تاثیر قرار گرفت. سه پارامتر مهم جدول زیستی شامل نرخ ذاتی افزایش جمعیت (r_m)، زمان دو برابر شدن (DT) و نرخ خالص تولید مثل (R_0) بوسیله تیمارهای زیرکشنده حشره کشها تحت تاثیر قرار گرفت. نرخ ذاتی افزایش برای شاهد و جمعیت های قرار گرفته در معرض دیازینون و فیپرونیل در مرحله لاروی به ترتیب ۰/۲۸، ۰/۲۳ و ۰/۱۲ و برای جمعیت های قرار گرفته در معرض LC_{30} به ترتیب ۰/۲۶، ۰/۰۴ و ۰/۰۸ بود. نسبت جنسی نتاج در تیمارهای حشره کشی در جهت تولید نسبت ماده بیشتر تحت تاثیر قرار گرفت. این یافته ها نشان داد که دیازینون و فیپرونیل بر *T. brassicae* مضر بودند. بنابراین توصیه می شود مطالعات نیمه مزرعه ای و مزرعه ای جهت بدست آوردن نتایج عملی در جهت استفاده احتمالی از این ترکیبات در قالب مدیریت تلفیقی آفات انجام گیرد.

[DOR: 20.1001.1.16807073.2016.18.5.3.9]