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Effect of Pesticides used in Tomato Fields of Iran on the Egg Parasitoid *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) under Laboratory Conditions

Fariba Sohrabi* and Effat Amini**
**Department of Plant Protection, Faculty of Agriculture, Persian Gulf University, Bushehr, Iran*
***Agricultural and Natural Resources Research Center, Bushehr, Iran*

(Corresponding author: Fariba Sohrabi)
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ABSTRACT: Toxicity of selected insecticides and fungicides on different developmental stages of the egg parasitoid *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae), were investigated in the laboratory. Persistent toxicity of selected pesticides on the adult stage of parasitoid was also determined. The insecticides cypermethrin, chlorpyrifos, imidacloprid, and indoxacarb had a significant adverse effect on the immature stages of the parasitoid. Among the tested fungicide, benomyl appear to be toxic to the pupal stage of *T. brassiace*. However, the other tested fungicide, mancozeb, chlorotalonil, and iprodione + carbendazim did not affect the preimaginal stages of the parasitoid. The results of the tests revealed that all the tested fungicides caused high mortality to adult wasps. The results of the persistency test showed that all pesticides were "moderately persistent" except chlorpyrifos that was classified as "persistent". The implications of these results for the integration of biological and chemical control methods are discussed.

Keywords: Bioassay; *Trichogramma brassicae*; fungicide; insecticide; toxicity

INTRODUCTION

Biological control by natural enemies represents an important component of integrated pest management (IPM) programs. Some species of parasitoid genus *Trichogramma* are important egg parasitoids that have been reared and released for controlling insect pests in different cropping systems (Smith 1996; Moezipour *et al.* 2008). *Trichogramma brassicae* Bezdenko (Hym.: Trichogrammatidae) is the most common species in Iran and used for inundative releases in biological control programs against *Helicoverpa armigera* Hubner and other lepidopteran eggs in tomato cropping systems.

Chemical control is the primary strategy in many agronomic and vegetable cropping systems (Palumbo *et al.* 2001). However, use of pesticides incompatible with natural enemies has caused some adverse effects such as target pest resurgence and secondary pest outbreaks. Therefore, evaluation of the impact of chemicals on biological control agents is important to promote integrated pest control methods.

Throughout Iran, tomato producers routinely use pesticides of different groups against insect pests and diseases in tomato fields. Thus, the use of selective pesticides that are more toxic to insect pests than to natural enemies can be useful tools for IPM programs (Croft 1990; Hopper 2003).

Because of the deficit of information regarding the toxicity and persistency of pesticides on *T. brassicae*, in the present study we aimed to evaluate the lethal effects of the most frequently used pesticides in Iranian tomato fields, on the egg parasitoid *T. brassicae*. The results obtained from this study would provide important information on the compatibility between these tested pesticides and *T. brassicae.*

MATERIALS AND METHODS

A. Insects

T. brassicae was supplied by Baransabz insectary (Amol, Iran), where they were reared on *Sitotroga cerealella* Olivier eggs. All experiments were conducted at 25 1°C, 70 5% RH, and a photoperiod of 16:8 (L: D).

B. Pesticides

The pesticides used in this study were the fungicides mancozeb, benomyl, chlorotalonil, iprodione + carbendazim, and the insecticides indoxacarb, imidacloprid, chlorpyrifos, and cypermethrin. Information about the pesticides is listed in Table 1.

C. Toxicity Bioassays

Preimaginal stages bioassays

Effects of the field-recommended concentrations (FRCs) of different pesticides (Table 1) on the emergence rate of *T. brassicae* were examined. The preimaginal stages that were treated consisted of larvae and pupae, appeared 6 and 8 days after initial parasitism of *S. cerealella* Olivier eggs, respectively. Each card containing *T. brassicae* larvae or pupae was dipped in the pesticide solution for 10 s.

Table 1: Details of pesticides tested against *T. brassicae.*

Pesticide	Trade name	Formulation	% of active ingredient	FRC (ppm)	Manufacturer
Mancozeb	Dithane M-45	WP	85	2000	Indofil
Benomyl	Benlate	WP	50	1000	Moshk Fam Fars
Chlorotalonil	Daconil	WP	75	200	AriaS himi
Iprodione+carbendazim	Rovral TS	WP	52.5	2000	Moshk Fam Fars
Cypermethrin	Ripcord	EC	10	500	Partonar
Imidacloprid	Confidor	SC	35	1000	Agro Axir
Indoxacarb	Avant	SC	15	625	Aria Shimi
Chlorpyrifos	Dursban	EC	40.8	2000	Shimagro

FRC – Field recommended concentrations, WP – Wettable powder, SC – Suspension Concentrat, EC – Emulsifiable Concentrate, Ppm – part per million

The control groups were submerged in distilled water only. After air-drying for 1h, the cards containing treated larvae or pupae were placed in the Petri dishes and kept in an incubator at 25 1°C, 70 5% RH, and a photoperiod of 16:8 (L:D). Each treatment had 4 replicates (cards) with at least 100 larvae or pupae per replicate. Parasitoid emergence was evaluated 6 and 4 days after the treatment in the larval and pupal stage treatments, respectively. The experiment comprised a 3 × 2 factorial design with the treatment (nine levels) and stage treated (two levels) as main factors. No data transformation was used because there was no evidence of non-normality within the data. The emergence data were subjected to a two-way ANOVA analysis of variance (ANOVA) and the means were separated using Duncan's multiple range test at P = 0.05 where applicable (SAS Institute 2003).

Adult stage bioassays

In order to test the effects of various pesticides on the adult stage of *T. brassicae*, adult parasitoids were exposed to pesticide residues applied at field-recommended concentrations of pesticides. Glass plates (140 × 140 mm) were sprayed with 6 ml of an aqueous solution of the pesticides using a hand-sprayer. Control plates were sprayed with distilled water. The plates were allowed to dry for 1 h before assembling the exposure cages. The exposure cages consisted of a wooden frame and two glass plates as floor and ceiling with four screened holes (10 mm diameter) on each of the three sides in order to facilitate ventilation. One hole on the fourth side of the frame was used to introduce the wasps and covered by a piece of cotton soaked in 20% honey solution as food. After assembly, the cages were incubated at 25 1 °C, 70 5% RH, and a photoperiod of 16:8 (L: D). Each treatment was represented by four replicates with 20-30 adults per replicate. The number of dead wasps in each cage was counted 24, 48, 72 and 96 h after exposure to the pesticides residue. The percentage mortality data were corrected using Abbott's formula (Abbott 1925).

The data were then subjected to analysis of variance (ANOVA), and the means were compared using Duncan's multiple range test (P = 0.05) (SAS Institute 2003). No data transformation was used because there was no evidence of non-normality within the data.

D. Persistent toxicity of pesticides

Persistent toxicity of field-recommended concentrations of selected pesticides on adult stage of *T. brassicae* was determined. The adults were exposed to pesticide residues at different time intervals i.e., 0, 5, 10, 15, and 25 days after pesticide application (within the interval proposed by the IOBC/WPRS Working Group for the evaluation of insecticide persistency against predators and parasitoids under laboratory conditions (Sterk *et al.* 1999). The technique, conditions and container used to expose adults to pesticide residues were as described above (section adult stage bioassays). Each experiment was repeated four times with 20-30 *T. brassicae* adults in each case. Mortality was recorded 24 h after exposure to the pesticides.

Classification of the pesticide persistency was determined according to the duration of the toxic activity of the compounds, that is, the interval of time in which its residues caused less than 30% mortality. According to the IOBC, the pesticides fit into categories include: class 1 = little persistent (<5 days), class 2 = slightly persistent (5-15 days), class 3 = moderately persistent (16-30 days) and class 4 = persistent (> 30 days) (Sterk *et al.* 1999).

RESULTS AND DISCUSSION

A. Toxicity bioassays

Immature stages bioassays

The egg parasitoid *T. brassicae* emergence, following exposure to the field-recommended concentration of the pesticides (FRC) at larval and pupal stages, was significantly affected by pesticides ($F = 871.59$, $df = 8, 54$, $P < 0.0001$).

Also, time of pesticide exposure relative to parasitoid preimaginal stage significantly affected the emergence ($F = 37.75$, $df = 1, 54$, $P < 0.0001$). Interactions between treatments and time of exposure were also significant ($F = 20.31$, $df = 8, 54$, $P < 0.0001$). The results indicated that imidacloprid and chlorpyrifos were the most harmful insecticides to larvae.

The lowest emergence rate occurred with cypermethrin and chlorpyrifos at pupal stage treatment (Table 2). None of the tested fungicides significantly affected adult *T. brassicae* emergence at the larval or pupal stage treatment except benomyl that decreased the emergence rate of parasitoid at pupal stage treatment (Table 2).

Table 2: Effect of pesticides on emergence rate of *T. brassicae* from *S. cerealella* parasitized eggs exposed to field rate of the pesticides at larval and pupal stages of the parasitoid [a]

Treatments	Mean percentage of adult parasitoid emergence from treated larvae and pupae	
	Larvae	Pupae
Control	100 ± 0 aA	100 ± 0 aA
Mancozeb	100 ± 0 aA	100 ± 0 aA
Benomyl	92.50 ± 3.66 aA	71.75 ± 3.22 bB
Chlorotalonil	95 ± 1.95 aA	97.50 ± 1.32 aA
[prodione+Carbendazim	100 ± 0 aA	100 ± 0 aA
Cypermethrin	15.50 ± 4.34 bA	4.50 ± 1.04 eB
Imidacloprid	3.50 ± 0.95 cB	11.75 ± 1.10 dA
Indoxacarb	95.75 ± 3.61 aA	61.50 ± 4.03 cB
Chlorpyrifos	0 ± 0 cA	0 ± 0 eA

[a] Means in a column followed by different small letters or in a row by different capital letters are significantly different ($P < 0.05$).

The present study shows that the field recommended concentration of imidacloprid, cholorpyrifos and cypermethrin severely affected the preimaginal stages of parasitoid, reducing adult emergence when applied to either parasitoid larvae or pupae. Shanmugam *et al.* (2006) reported imidacloprid as a moderately toxic insecticide to *Trichogramma* chilonis Ishii. However, several studies have shown minimal or no effect of imidacloprid on emergence rate of *Trichogramma* parasitoids (Hewa-kapuge *et al.* 2003; Carvalho *et al.* 2003; Preetha *et al.* 2010). The different responses of *Trichogramma* species to the same insecticide may be related to the concentration of insecticide parasitoids had been exposed to, and the species differences in physiological responses to the insecticide. Bueno *et al.* (2008) classified cholorpyrifos as slightly harmful (class 2) to *Trichogramma pretiosum* Riley larvae and pupae. Also in agreement with our findings, Hussain *et al.* (2010) found that cholorpyrifos had a detrimental effect on emergence rate of *T. chilonis*. Suh *et al.* (2000) demonstrated that cypermethrin had high toxicity to preimaginal stages of *Trichogramma exiguum* Pinto & Platner. Regarding the adverse effect of these insecticides on preimaginal stages, it should be noted that in the present study, the parasitized host eggs were completely dipped in the insecticide solutions. Consequently, the parasitized eggs received a large amount of the insecticide. Under field conditions, parasitized host eggs live on the underside of the leaves, and are therefore likely to receive a lower concentration of the insecticide. Also, the effect of pesticides on natural enemies in the field conditions is usually lower because natural enemies refuse approaching sprayed fields. In addition, sunlight and plant growth have important roles in reducing the effects of pesticides on natural enemies (Hassan 1992). Indoxacarb had harmful effects on adult emergence when applied to parasitoid pupae. Hussain *et al.* (2010) demonstrated that indoxacarb had a significant adverse effect on the emergence of *T. chilonis* when exposed to all immature stages of parasitoid in the host eggs. However, Hewa-kapuge *et al.* (2003) reported that indoxacarb does not influence adult survival or development of immature stages of *T. brassicae*. In the present study, the field-recommended concentration of fungicides except benomyl had no significant effect on the emergence of *T. brassicae* when the parasitoid was treated during either the larval or pupal stages. These results may be due to storage of the substances in the insect fat body, its excretion and selective metabolism (Foerster 2002) or, its degradation by the enzyme system (Croft 1990; Rigitano & Carvalho 2001). Little information is available in the literature concerning the effects of fungicides on *Trichogramma* wasps.

Carvalho *et al.* (2006) reported that the fungicides iprodione, chlorotalonil, benomyl and mancozeb were harmless to *T. pretiosum* pupae. Hassan *et al.* (1998) found that the fungicides mancozeb and carbendazim were harmless and slightly harmful to *Trichogramma cacoeciae* Marchal pupae within the egg, respectively. Pratissoli *et al.* (2010) also reported that the fungicide chlorothalonil reduced the emergence of *Trichogramma atopovirilia* Oatman & Platner by 73.77%. However, mancozeb was harmless to *T. pretiosum* in *Anagasta kuehniella* (Zeller) eggs (Giolo *et al.* 2007), such as *T. atopovirilia* in *Diaphania hyalinata* Linnaeus eggs (Pratissoli *et al.* 2010). Our results further reveal that cypermethrin, indoxacarb and benomyl had a more significant adverse effect on parasitoid emergence during the pupal stage compared to the larval stage. However, imidacloprid reduced the emergence rate at the larval stage more than the pupal stage. In the other treatments, the timing of exposure to pesticides had no impact on emergence rate of *T. brassicae.* Varma & Singh (1987) reported that in general, the adverse effect of insecticides on *Trichogramma brasiliensis* Ashmead emergence decreases as the development of the parasitoid progresses. The developmental stage of parasitoid at the time of insecticide application appears to be important because it determines the time allowed for the pesticide degradation before the adult emergence (Orr *et al.* 1989). Saber (2011) suggests that many factors such as time of exposure, type of host, type of chemical, commercial formulation and method of exposure may affect the rate of emergence.

Adult stage bioassays

The results of assessing the toxicities of different pesticides on *T. brassicae* adults showed that field-recommended concentration of the pesticides significantly affected the mortality of adult parasitoids at 24h ($F = 56.71$, df = 8, 27, $P < 0.0001$), 48 h ($F = 55.36$, df = 8, 27, $P < 0.0001$), 72 h ($F = 46.96$, df = 8, 27, $P < 0.0001$), and 96 h ($F = 373.89$, df = 8, 27, $P < 0.0001$). All the tested pesticides caused high mortality to adult parasitoids at 96 h after application (Table 3).

Table 3: Mean percent cumulative mortality of parasitoid adults for untreated control and pesticides at 24, 48, 72 and 96 hours after application [a]

Treatments	Different times after application			
	24 h	**48 h**	**72 h**	**96 h**
Control	6.31 ± 1.61 f	11.90 ± 1.82 e	16.40 ± 1.11 d	18.10 ± 0.74 c
Mancozeb	75.79 ± 4.46 bc	89.61 ± 6.33 ab	98.89 ± 1.10 a	100 ± 0 a
Benomyl	15.97 ± 1.88 f	41.02 ± 4.01 d	81.84 ± 4.75 b	98.77 ± 1.22 a
Chlorotalonil	85.50 ± 3.86 b	98.86 ± 1.13 ab	100 ± 0 a	100 ± 0 a
Iprodione+Carbendazim	66.16 ± 4.56 cd	86.75 ± 3.83 b	98.98 ± 1.01 a	100 ± 0 a
Cypermethrin	86.81 ± 3.54 b	98.04 ± 1.13 ab	100 ± 0 a	100 ± 0 a
Imidacloprid	59.35 ± 5.76 d	69.04 ± 6.31 c	82.05 ± 6.66 b	96.39 ± 1.23 a
Indoxacarb	34.41 ± 8.04 e	45.94 ± 6.99 d	63.69 ± 8.73 c	90.53 ± 3.48 b
Chlorpyrifos	100 ± 0 a	100 ± 0 a	100 ± 0 a	100 ± 0 a

[a] Means in a column for each developmental stage followed by the same letter are not significantly different ($P > 0.05$).

Imidacloprid and chlorpyrifos were highly toxic to *T. brassicae* with 96.39 and 100% mortality of parasitoid adults within 96 h. Imidacloprid and chlorpyrifos also led to significant mortality of *T. chilonis* (Hussain *et al.* 2010) and *Trichogramma platneri* Nagarkatti adults (Brunner *et al.* 2001). Indoxacarb also had significant lethal effects on *T. brassicae* adults. Similarly to the findings presented here, Hussain *et al.* (2010) reported high susceptibility of *T. chilonis* adults to indoxacarb. In contrast to our findings, Wang *et al*. (2012) found that indoxacarb had low toxicity to *T. chilonis* adults, suggesting it as a safe insecticide to this parasitoid. Our results further indicated high toxicity of cypermethrin to parasitoid adults. Also in agreement with our findings, Suh *et al.* (2000) and Sidi *et al.* (2012) have shown detrimental effect of cypermethrin to *T. exiguum* and *Trichogramma papilionis* (Nagarkatti) adults, respectively. However, Wang *et al.* (2012) reported cypermethrin as a safe insecticide to *T. chilonis*, suggesting this insecticide is compatible with the parasitoid when being applied in the field. Based on our results, the fungicides mancozeb, benomyl, chlorotalonil and Iprodione + Carbendazim were toxic to *T. brassicae* adults, although there is no site of action known in arthropods similar to insecticides. Negative effects of mancozeb, chlorotalonil and carbendazim on *Trichogramma* adults such as reduction in parasitism have been reported in some earlier studies (Hassan 1998; Manzoni *et al.* 2006; Pratissoli *et al.* 2010). However, Carvalho *et al.* (2006) reported that iprodione, chlorotalonil, benomyl and mancozeb were harmless to *T. pretiosum* adults. The different results may be related to the bioassay method applied to expose adults to the fungicide, the concentration of pesticide parasitoids had been exposed to, and the species differences in physiological responses to the fungicide.

B. Persistent toxicity of pesticides

Results regarding the effects of pesticide persistency on *T. brassicae* adults revealed that all the tested pesticides, except chlorpyrifos, being fit in the class 3 (moderately persistent) (Table 4). A considerable decrease in the toxicity caused by chlorpyrifos was not observed even at 30 days after its application so it was classified as “persistent” (Table 4). Very few studies reporting persistent activity of the tested pesticides are available.

Table 4: Percentage mortality of *T. brassicae* adults after exposure to 0, 5, 10, 15 and 25 days old pesticide residues for 24 h under laboratory conditions.

Treatments	Mean percentage of adult parasitoid mortality at different time intervals (days after application)					Class
	0	5	10	15	25	
Mancozeb	75.7	67.7	62.3	40.8	7.9	3
Benomyl	15.9	68.1	62.1	58.4	6.25	3
Chlorotalonil	85.5	75	73.2	31.7	18.1	3
Iprodione + Carbendazim	66.1	84	81.8	30.9	9.13	3
Cypermethrin	86.8	79.8	66.7	44.5	24.1	3
Imidacloprid	59.3	67.1	76.9	56.5	28.6	3
Indoxacarb	34.4	81.7	80.5	36.9	13.2	3
Chlorpyrifos	100	100	98.7	95.9	86.1	4

Toxicity class according to IOBC (7, 8): class 1 = little persistent (< 5 days), class 2 = slightly persistent (5-15 days), class 3 = moderately persistent (16-30 days) and class 4 persistent (> 30 days) (Sterk *et al.* 1999).

Sattar *et al.* (2011) classified indoxacarb as "slightly persistent" (IOBC, persistency Class B) against *T. chilonis*. Hassan 1998 found that mancozeb constantly reduced parasitism by *T. cacoeciae* and was rated as persistent. Under field conditions, sunlight intensity, high temperature and foliage growth are the main factors responsible for the decay/dilution of the pesticides (Wilson *et al.* 1983-1986).

In conclusion, the insecticides cypermethrin, chlorpyrifos, imidacloprid, and indoxacarb severely affected immature and adult stages of the parasitoid. Thus, based on the results of the persistency test, it is recommended that the releases of *T. brassicae* should be done after 25 days from the application of these insecticides. However, since chlorpyrifos is a persistent insecticide against this parasitoid, the use of this product in crops should be avoided in situations where biological control by this parasitoid is important. Among the tested fungicide, benomyl appear to be toxic to the pupal stage of *T. brassiace* and when the majority of the parasitoids are in the pupal stage, they may be subject to adverse effects of this fungicide. Mancozeb, chlorotalonil, and iprodione+carbendazim did not affect the preimaginal stages of the parasitoid. However, in view of the possible detrimental effects of these pesticides on adult parasitoids, the use of these products should be considered with caution in IPM programs. Data related to persistent toxicity of fungicides showed that none of the tested fungicides remained potent after 25 days of their application and so, the releases of wasps can be done after this time. Semi-field and field studies aiming to evaluate the efficacy of the combined use of pesticides and T. brassicae are needed in order to obtain more applicable results under field conditions.

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