Side Effects of Five Pesticides on *Trichogramma brassicae* Bezdenko (Hymenoptera: Trichogrammatidae)

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ABSTRACT *Trichogramma brassicae* is a dominant species of Trichogrammatids in Iran. It has been widely used in biological control programs against various pests of agricultural crops. Recent surveys demonstrated a late occurrence of its natural population in rice fields coincided with lower percent parasitism of striped stem borer eggs, *Chilo suppressalis* (Walker). Chemical side-effects were proposed to be the main cause. We chose five pesticides, i.e., diazinon and neem (insecticide), edinfenphos (fungicide) and butachlor and oxadiargyl (herbicide) based on their frequent application in rice fields. Immature stages of the parasitoid (as parasitized eggs of *Sitotroga cerealella*) at 1, 3 and 7 days after parasitism and newly emerged and mated adults (age <24 h) were exposed to the field recommended doses (FRD) of the pesticides at 25 ± 1°C, 65 ± 10% RH and 16:8 (L:D) h photoperiod. Mortality and fecundity of treated insects were calculated based on IOBC guideline. Diazinon caused 95% to 100% mortality on all life stages of the wasp and was considered as harmful (rank 4). The total effects of butachlor, oxadiargyl, edinfenphos and neem on *T. brassicae* immature were all ranked as rank 1. However, butachlor, edinfenphos and oxadiargyl showed high mortality to adult stages of the parasitoid. Among all pesticides tested, neem with 14.42, 15.99, 22.07 and 16.21 percent of total effect on egg, larva, pupa and adult stages, respectively, was classified as completely harmless to *T. brassicae.* Therefore, the application of diazinon should be prevented in rice field when *T. brassicae* is considered as biological control agent. Moreover, spraying butachlor, edinfenphos and even oxadiargyl should be avoided when adult parasitoids exist in the rice field.

Key Words: *Trichogramma brassicae*, Pesticides, Side effects, Rice.

I. Introduction

There are almost 12 species of *Trichogramma* wasps in Iran of which *T. brassicae* has been recorded from most parts of the country (Ebrahimi et al., 1998). It has been mass reared and released against a number of important pests including cotton bollworm, corn stem borers, striped rice stem borer, codling moth, carob moth, soybean pod borer, tomato fruit borer, etc. (Shirazi et al., 2010). Many reports showed the high parasitism rate and natural occurrence of *T. brassicae* in different crops (Rezvani and Shah Hoseini, 1976; Shahidi, 1998; Najafi-Navai et al., 1995; Karimian, 1998). However, recent surveys revealed a decline in frequency of natural occurrence of Trichogrammatids in some crops including rice paddies (Dadpour et al., 2016).

Shirazi et al. (2009) stated that one of the side effects of pesticides could have been reduction of the density of natural enemies in the agro-ecosystems

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where they are applied excessively. This is mostly due to more susceptibility of natural enemies to chemical pesticides compared with their host pests (Croft and Brown, 1975). Therefore, in order to decide on the application of new pesticides or to quantify the detrimental impacts of those in practice, it is necessary to study their side effects (Oomen, 1998). At the beginning, most of the researches focused on the lethal effects of chemicals and the sublethal hazards were neglected (Desneux et al., 2007).

In 1974, International Organization of Biological Control / West Palaearctic Regional Section (IOBC/WPRS) established a working group named "Pesticides and Beneficial Organisms". Its goal was the development of universal standards and guidelines for pesticides side effect evaluations and tests on natural enemies and publishing the results. Hassan (1994) introduced one semi field and three laboratory methods for evaluating chemicals on *T. cacoeciae*. The first comprehensive results of the IOBC/WPRS working group was published as a tool box by Boller et al. (2005). It contains the results of testing 211 kinds of pesticides on 13 non-target organisms including *T. cacoeciae*.

It is believed that egg parasitoids may be protected from pesticides as long as they are inside their host body (Plewka et al., 1975; Bull and Coleman, 1985). However, Plewka et al. (1975) revealed that *T. evanescens* adults, treated by chloride pesticides, experienced lower longevity and fecundity when reared on eggs of *Sitotroga cerealella*. Hassan (1998) tested side effects of 21 pesticides on *T. cacoeciae* amongst which phosalon, difenthiorun, tolylfluanid, mancozeb and sulphur constantly reduced parasitism rate by 90-100 percent and rated as persistent. When Consoli et al. (1998) treated host eggs parasitized by *T. pretiosum* with cartap and phenthoate, they observed 100 % mortality. In another research, Kakakhel and Hassan (2000) revealed that contact exposure of dimethoate with *T. cacoeciae* adults caused 100% reduction in their parasitism. Similarly, Youssef et al. (2004) reported that 6 different pesticides including dimethoate (applied to control olive pests) could decrease the parasitism rate of *T. cacoeciae* between 85 to 90 %. Contact residues of some pesticides have been harmful to parasitoids even at doses lower than field recommended doses (FRD) (Grützmacher et al., 2004).

After all, plant protection practitioners apply pesticides to achieve satisfactory level of pest control as well as maximum yield. Finding chemicals that promise former and latter goals and simultaneously guarantee the safety of the environment and non-target species would be of great interest for ecologists. For example, an investigation proved that neem products applied on cotton had no harmful effects for *T. minutum* and *T. pretiosum* while it controlled cotton bollworm successfully (El-Wakeil et al., 2006). Similarly, Garcia et al. (2009) demonstrated that biological (*Bacillus thuringiensis*) and mineral (Copper sulphate) products had no adverse effect on *T. curdubensis* while lambda-cyhalothrin, a pyretheroid compound, reduced emergence rate of diapausing wasps even 60 days after treatment. Surprisingly, Wang et al. (2012) used risk quotient analysis and classified neonicotinoids, avermectins, pyretheroid, IGRs and phenylpyrazoles (with the exception of butane-fipronil and fipronil) pesticides as safe and organophosphates and carbamates as slightly to moderately toxic or dangerous to *T. ostriniae*. Similar results have been mentioned by Tipping and Burbutis (1983), Thomson et al. (2000), Bueno et al. (2008), Carvalho et al. (2010), Pratissoli et al. (2010), Ksentini et al. (2010), Sattar et al. (2011), Hussain et al. (2012), El Sebai and El Tawil (2012), Souza et al. (2014), Uma et al. (2014), Blibech et al. (2015) and Lingathurai et al. (2015).

Najafi-Navai et al. (1995) proved that FRDs of dimicron and diazinon caused 73.65 and 69.93 percent mortality, respectively, on *Trichogramma* sp. in paddy. In a very recent study from Iran, Ghorbani et al. (2016) reported that field rates of diazinon and fipronil decremented the emergence rate of *T. brassicae* by 99.74 and 50.46 %, respectively. Therefore, we decided to initiate a dynamic program for pesticides side effect evaluation on some important natural enemies used commercially in Iran. A *T. brassicae* population collected from rice fields was selected as the first candidate.

II. Materials and Methods

1. Parasitoid colony

T. brassicae colony was established by taking about 5000 parasitized eggs of *S. cerealella* from a stock maintained at Biological Control Research Dept. (BCRD), Iranian Research Institute of Plant Protection (IRIPP). The species had originally been collected from rice paddies of Mazandran province (Daboo Dasht area) and recruited annually with new wild blood to keep genetic diversity. The new colony was maintained in glass tubes (20 × 5 cm) on fresh eggs of *S. cerealella* at 25 ± 1°C, 65 ± 10% RH and 16:8 (L:D) h photoperiod.

2. Pesticides

Based on the statistics of annual usage in the rice field, five pesticides were recognized as the most important compounds in rice plant protection including butachlor, oxadiargyl, edinfenphos, diazinon and neem extract. Table 1 contains detailed information on the selected pesticides.

3. Pesticide treatment of adult parasitoids

Adult parasitoids were exposed to contact FRD residue of each pesticide using exposure cages made of 13 × 13 cm black colored aluminum squares (1.5 cm height, 1 cm thickness) and 2 pieces of glass on both sides. For ventilation, 3 holes (1 cm in diameter) were opened on each of 3 sides of the cages (sealed with fine mesh net) and on the forth side, 2 holes with the same size and an opening of 3.5 × 1 cm size were provided for adult release and food provision (sealed with cotton swabs), respectively (Fig 1, Hassan et al., 2000). The pesticide concentrations used in the experiments were prepared based on their FRD (Table 1). In this way, 6.8, 8.7, 2.5, 3.4 and 3.75 cc of butachlor, oxadiargyl, edinfenphos, diazinon and neem mixed with 1 lit of distilled water were equal to their respected FRD (Table 1). Then, a spray tower (Potter spray tower, Burkard Ltd. Co., UK) calibrated at 14 mbar was employed to apply treatments on inner side of lower glass piece of each cage with exposed arena of 169 cm2. By this way, an even concentration of pesticide was covered on the glass. The treated glasses were left to dry for 1 h. All operations were done under a lab hood (H.MSF.611, Farpajouh Eng. Co., Iran) with external filter ventilation by wearing latex gloves and filtered gas mask. After that, a treated glass in the bottom and an untreated piece on the top of a cage were secured with 2 metal clips. About 15-25 pairs of newly emerged (0-24 h) and mated adult *T. brassicae* were released inside the cage through the release opening. Each treatment was replicated 3 times and distilled water treated cages were regarded as control. Twenty-four h later, the mortality was calculated based on the following formula:

$$M = \frac{d}{n} \times 100 \quad (1)$$

where M is the percent of adult mortality, d is the number of wasps died after 24 h and n is the total number released. Following equation was used to correct the mortality in treatments (Abbott 1925, Schneider-Orelli 1947):

$$\mathrm{M_c} = \left(\frac{\mathrm{M-C}}{\mathrm{100-C}}\right) \times 100 \quad (2)$$

where Mc is the corrected mortality, M is the observed percent mortality in pesticide treatment and C is the observed percent mortality in control treatment.

Fig. 1. The black colored aluminum frame (left) and prepared adult exposure cage (right)

Table 1. Pesticides used in this research and their specifications

No.	Common name	Trade name	Formulation	Target	Field recommended dose (FRD) (lit/ha)
1	Butachlor	Machette	EC 60%	Rice various weeds	3-4
2	Oxadiargyl	Topstar	EC 30%	Rice various weeds	3-3.5
3	Edinfenphos	Hinozan	EC 50%	Blast disease	1
4	Diazinon	Diazinon	EC 60%	Stripped rice stem borer	2
5	Neem extract	NeemAzal	EC 1.2%	Stripped rice stem borer	1.5

The survived adults were provided with unlimited host eggs (~100 eggs of *S. cerealella* per female parasitoid) on a piece of white paper (7 × 1 cm) in a glass tube. Parasitized eggs were removed and fresh eggs were replaced daily till all females died. Each blackened egg was considered as one parasitoid's egg and total fecundity was the sum of all blackened eggs. Therefore, the pesticides sublethal effects on the reproduction of the parasitoid were obtained through formula no 3:

$$R = \frac{Rt}{Rc} \qquad (3)$$

where Rt is the mean number of eggs produced by each female in treatment, Rc is the mean number of eggs produced by each female in control and R is the proportion of egg production in treatments to control.

Eventually, the simple mortality caused by pesticides was integrated with their effect on the reduction in parasitism level of survived individuals to obtain an index named the Total Effect (E) of pesticides. It was calculated using the bellow equation (van de Veire et al., 1996):

$$E\% = 100 - (100 - M_c) \times R \qquad (4)$$

where Mc is the total corrected mortality, R is the proportion of mean fecundity of treated females to that of females in control, and the calculated E is used to classify the toxicity of tested pesticides according to Boller et al. (2005) (Table 2).

4. Pesticide treatment of immature stages

Immature stages of *T. brassicae* (i.e., egg, larva and pupa stage) were exposed to the FRD of the pesticides as 1, 3 and 7 day old parasitized eggs of *S. cerealella*. Based on previous studies at 25°C, egg period of *T. brassicae* takes ~24 h, then it completes its larval stage within 4 days, and they are in the pupal stages after day 6. First, about 2000 fresh eggs of *S. cerealella* were kept in a glass tube (20 × 5 cm) contained about 200 pairs of newly emerged and mated *T. brassicae*. This process was replicated five times. After 4 h, all tubes were chilled for 10 min at 8°C; then the parasitoids were removed. Eggs of *S. cerealella* were kept at 25 ± 1°C, 65 ± 10 % RH and 16:8 (L:D) h photoperiod. After one day, three batches of 100 eggs were assigned for each pesticide and exposed to its related FRD by spray tower as explained before. Distilled water was sprayed in the control treatments. Treatments were transferred into glass tubes (10 × 2 cm) and incubated at above mentioned conditions. Same procedure was repeated on the 3rd (i.e., the larval stage) and 7th d (i.e., the pupal stage). The mortality of immature parasitoids was calculated using formulae 1 and 2 based on the emergence rate in treatments in comparison with that in control. The fecundity of survived and emerged parasitoids was used to obtain R for calculation of total effect (E) of pesticides on immature stages of *T. brassicae*. Percent mortalities and total effects (E) were subjected to Proc means (SAS Software, Ver. 9.4, 2014) to obtain standard errors of means.

Table 2. Classification of pesticides side effects on non-target beneficial organism based on their percent Total Effect (*E*) (Boller et al., 2005)

Percent Total Effect (E %)	Class rank	Class name
E < 30	1	Harmless
30 ≤ E ≤ 79	2	Slightly harmful
80 ≤ E ≤ 99	3	Moderately harmful
E > 99	4	Harmful

III. Results

The percent effectiveness of the tested pesticides on life stages of *T. brassicae* are listed in Table 3. The effect of diazinon on all stages was the highest (~ 100 % mortality) while oxadiargyl and neem had the lowest mortality on all stages of the wasp compared with other pesticides. Similarly, edinfenphos and butachlor did not cause high mortality on immature stages of *T. brassicae*, however, they caused 53.66 and 59.39 percent mortality on adults, respectively, (Table 3).

Total effect (E) results were presented in Table 4. The results showed that diazinon is a harmful pesticide (E > 99%) for all stages of the parasitoid. However, butachlor is in the near category to diazinon (moderately harmful, E=98.80%) when applied to adults. But the other herbicide (oxadiargyl) with E=33.36 % fell in slightly harmful category. The total effect of butachlor, oxadiargyl and edinfenphos was below 30 % when immature stages of *T. brassicae* were treated, therefore, based on results they are classified as harmless on egg, larva and pupa of the parasitoid. The least side effect belonged to neem as it was harmless for all life stages of *T. brassicae* (E < 30 %) (Table 4).

Table 3. The effectiveness of the tested pesticides (percent mortality) on different stages of *Trichogramma brassicae* at 25 ± 1 °C, 65 ± 10 % RH and 16:8 (L:D) h photoperiod

Treatment	Mean percent effectiveness (mean percent mortality) ± SE			
	Egg	Larva	Pupa	Adult
Butachlor	6.80 ± 0.89	10.76 ± 1.83	5.96 ± 0.92	59.39 ± 10.01
Oxadiargyl	4.08 ± 0.58	5.03 ± 0.19	5.11 ± 0.53	10.74 ±0.37
Edinfenphos	8.68 ± 0.95	14.12 ± 1.03	7.37 ± 1.78	53.66 ± 1.23
Diazinon	97.96 ± 1.55	100 ± 0.00	100 ± 0.00	96.59 ± 3.41
Neem extract	4.16 ± 0.16	4.37 ± 0.81	6.80 ± 1.48	10.60 ± 1.61

Table 4. Total effect (*E* %) of the tested pesticides on different stages of *Trichogramma brassicae* at 25 ± 1 °C, 65 ± 10 % RH and 16:8 (L:D) h photoperiod

Treatment	Total effect (mean percent) ± SE							
	Egg	Rank	Larva	Rank	Pupa	Rank	Adult	Rank
Butachlor	15.13 ± 1.11	1	24.70 ± 2.04	1	26.66 ± 0.47	1	98.80 ± 1.20	3
Oxadiargyl	14.23 ± 1.21	1	15.04 ± 0.67	1	29.66 ± 0.47	1	33.36 ±3.46	2
Edinfenphos	16.35 ± 1.18	1	25.21 ± 1.20	1	17.01 ± 1.89	1	66.59 ± 5.13	2
Diazinon	100 ± 0.00	4	100 ± 0.00	4	100 ± 0.00	4	100 ± 0.00	4
Neem extract	14.42 ± 1.23	1	15.99 ± 1.44	1	22.07 ± 1.92	1	16.22 ± 3.27	1

IV. Discussion

Trichogramma species play an important role in side effect assessment of pesticides (Hassan, 1994). Wang et al. (2012) stated that conservation of parasitoids (e.g. *T. ostriniae*) using selective insecticides can improve compatibility of biological control within an IPM program. It is agreed that pesticide residue analysis can be a measurement of the level and nature of any chemical contamination and its persistence in the environment (Blibech et al., 2015). However, there is not much consistent literature available on the issue (Sattar et al., 2011).

Wang et al. (2012) calculated risk quotient to obtain ecological risk of pesticides toward non-target organisms other than direct mortality effects. They found that organophosphate and carbamate pesticides were the most toxic chemical groups on *T. ostriniae*. Similarly, Uma et al. (2014) showed that organophosphates were the most toxic pesticides for *T. japonicum*. Among pesticides tested in the present study, diazinon (an organophosphate chemical) caused the highest mortality on all stages of *T. brassicae*. Such finding is in agreement with results of Kakakhel and Hassan (2000) revealing that organophosphate dimethoate caused 100 % mortality on *T. chilonis*. On the other hand, there are many results reporting side effects of different pesticides on parasitoids. Souza et al. (2014) verified that spinosad and beta-cypermethrin reduced parasitism rate of *T. pretiosum* by 96.9 and 90.6 %, respectively, when applied on its immature stages. Hussain et al. (2012) also demonstrated the harmful effect of spinosad on both immature and adults of *T. chilonis*.

Carvalho et al. (2010) reported acetamiprid, imidacloprid, lufenuron, and pyriproxifen were toxic to *T. pretiosum* when applied in different immature stages and they used ANOVA to calculate the chemical side effects and then categorized them based on IOBC method. Before them, Tipping and Burbutis (1983) used also ANOVA to compare the side effects of five different pesticides on emergence and parasitism rates of *T. nubilale*. They proved that carbaryl and permethrin were the most persistent pesticides and except for *B. thringiensis*, the parasitoid was highly sensitive to all tested pesticides. However, it is hard to compare our findings with them due to their different method of evaluation.

An important matter to consider while studying the side effects of pesticide on non-targets is the differences among species and even among populations of a species. Such issue was noticed by Ksentini et al. (2010) who suggested that the stage susceptibility of *Trichogramma* toward insecticides could differ from species to species and the insecticide types. In their study, immature and adults of *T. cacoeciae*, *T. evanescens* and *T. bourarachae* exposed to spinosad and deltamethrin displayed the highest mortality rate. However, *T. evanescens* and *T. bourarachae* in larval stage and *T. cacoeciae* in prepupal stage showed the most mortality. Similarly, Blibech et al. (2015) used olive leaves in the lab after 3, 10, 17, 24 and 31 days of spraying by deltamethrin and spinosad FRD and investigated their side effects on *T. oleae*, *T. cacoeciae* and *T. bourarachae* as natural enemies in olive orchards. By comparing treatments and control in terms of the total effect index (E), they observed differences both in pesticides and wasp species considering the parasitism rates. Deltamethrin reduced *T. oleae* parasitism about 50 % within 17 days after treatment while spinosad was only 4.5 % effective after 24 days. However, for *T. bourarachae*, reductions in parasitism in elapsed times were similar for both insecticides and incurred considerable reduction in parasitism 3 days after exposure. For *T. cacoeciae*, deltamethrin side effect persisted up to 31 days but spinosad impact was leveled down from 24 to 31 days after.

Bueno et al. (2008) showed that different pesticides had different hazards on *T. pretiosum* and even fungicides (tebuconal and trifloxystrobin) and herbicides (glyphosate), thought to be safe previously, were classified as harmful (rank 4) to its egg stage. However, butachlor and oxydiargyl as herbicides and edinfenphos as a fungicide tested in the present study ranked 3, 2 and 2, respectively, on adult stage of *T. brassicae* while all were harmless (rank 1) on egg, larva and pupa of the parasitoid. In the same manner, Pratissoli et al. (2010) reported that parasitism rate of *T. atopovirilia* decreased when treated by thiophanatemethyl, tebuconazole and chlorothalonil fungicides. Moreover, El Sebai and El-Tawil (2012) found that some herbicides were harmless on *T. evanescens* in low concentration but as doses increased their negative impacts were apparent. There are reports that some fungicides were considerably dangerous to parasitoids as Consoli et al. (1998) proved that cartap and phenthoate caused almost 100 percent mortality on *T. pretiosum* immature stages.

Our results classified neem extract as harmless (rank 1) on all stages of *T. brassicae*. Nevertheless, Sattar et al. (2011) found that neem extract was harmless on egg and pupal stages of *T. chilonis* but it caused 35 % mortality on its larvae (slightly harmful). The neem product used in the current study was a commercial formulation (1.2 % a.i) while Sattar et al. (2011) have mentioned neem oil as 1500 ppm (=0.15 %) which may be the reason for different results. Anyhow, results reported by Lingathurai et al. (2016) for side effects of neem on *T. chilonis* are similar to our observation.

Finally, employment of life table technique may shed more light on the severity of pesticides impacts on beneficial. In a recent study, Ghorbani et al. (2016) employed life tables to investigate the lethal effects of diazinon and fipronil on *T. brassicae*. They obtained R_0, T and r as 1.5 offspring, 1.8 d and 0.23 d^{-1}, respectively, when parasitoids were treated by FRD of diazinon at larval stage. They were 60.8 offspring, 14.79 d and 0.28 d^{-1}, respectively, for wasps in control. Similar to our results, they observed about 99 % reduction in emergence rate (mortality) of adults which had been treated by diazinon at their immature stages. Specifically, when they exposed adult parasitoids to FRD of diazinon life table parameters (R_0, T and r) were as 1.65 offspring, 12.53 d and 0.04 d^{-1}, respectively.

Although this study highlighted important results that might help in choosing the most compatible and appropriate pesticide in an IPM program, semi-field and field experiments are required to reach the final conclusion. The diversified field condition may help natural enemies to escape from or to take shelter against pesticides. Such information clarifies the behavior and persistence of chemicals at field conditions based on which release or conservation programs can be established. As an example, Thompson et al. (2000) found that sulfur applied on vineyards to control mites and fungal diseases could effectively damage the immature and adults of released *T. carverae* or resident *T. funiculatum* up to 7 days after spray. Conclusively, calculation of total effect of pesticides on beneficial organisms is the first and significant step in the sustainable agriculture (Hassan et al., 1994; Wang et al., 2012; Jansen, 1996).

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