The Effect Of Insecticides And Tomato Cultivars On The Percentage Of Parasitization Of Trichogramma Brassicae On Tomato Leaf Miner Eggs (Tuta Absoluta)

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Abstract
Tomato leaf miner, Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae), is an invasive and dangerous pest that threatens tomato production in worldwide. The aim of this study was to study the effect of different cultivars along with the insecticides tested on the ability of Trichogramma to parasitize. The parasitism of tomato leaf miner eggs by Trichogramma wasps on three tomato cultivars (Rio Grande, Superstrain B and Cal j N3) was investigated. To investigate the parasitism of surviving wasps from the experiment on the effect of 25% concentration of 5 pesticides (matrine, flubendiamide, chlorantraniliprole, spinetoram and cyantraniliprole), an experiment was conducted in three replicates with five female wasps and 100 tomato leaf miner eggs on each tomato cultivar in a completely randomized manner for 48 hours in cylindrical plastic containers. Analysis of variance of percentage reduction in parasitism showed that the effect of cultivar was not significant ($F = 0.06$; $P = 0.9411$). The insecticide effect was highly significant ($F = 10.19$; $P < 0.0001$), confirmed the large difference in insecticide compatibility. The insecticide chlorantraniliprole showed the highest reduction with average of 35.65% reduction in parasitism. This rate was significantly higher than matrine and flubendiamide and was placed in category 2 according to the IOBC guidelines. Cyantraniliprole (32.04%) and Spinetoram (30.26%) were also in category 2 and did not differ significantly from chlorantraniliprole. Matrine (18.10%) and flubendiamide (13.35%) were placed in the first category and had the least negative effect.

Key-words: Matrine, Flubendiamide, Chlorantraniliprole, Spinetoram, Cyantraniliprole, Tuta absoluta, Trichogramma brassicae.

INTRODUCTION

Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae), also called as the tomato leaf miner, is a devastating pest of tomato crop, causing significant economic loss for farmers. Its control is becoming difficult since it adapts very rapidly to conventional insecticides (Desneux et al., 2010). The parasitoid wasp, Trichogramma brassicae (Bezdenko), has an important role in managing its populations. T. brassicae is an extremely small parasitoid wasp belonging to the family Trichogrammatidae (Liu and Zhang, 2012). It parasitizes the eggs of various insect pests like moths and butterflies. Knowledge of the effects of insecticides on the pest and natural enemies is necessary for the development of integrated pest management strategies (Razinger et al., 2016).

One of the agronomic methods in IPM to reduce pest populations is to take advantage of the adaptations that many plants have to avoid or tolerate insect pests or diseases, which plays an important role in pest control (Zoeren and Guédot 2017). This study deliberates on the effects of insecticides and tomato cultivars on the percentage of parasitization of T. brassicae on tomato leaf miner eggs. Five insecticides were used in this study, including: matrine, flubendiamide, chlorantraniliprole, spinetoram and cyantraniliprole.

Experiments were conducted on three tomato varieties (Rio Grande, Superstrain B and Cal j N3).). Flubendiamide is a synthetic pesticide which affects ryanodine receptors of insects, causing paralysis and mortality. Chlorantraniliprole is a member of the anthranilic diamides class of insecticides and functions by modulating the ryanodine receptor to cause paralysis and mortality (Jaiswal et al., 2017). Spinetoram is a semi-synthetic insecticide derived from a natural product called spinosyn, which is produced by the soil bacterium Saccharopolyspora spinosa (Sparks et al., 2021). The spinetoram targets nervous systems of insect. Spinetoram works through action at the nicotinic acetylcholine receptors in the nervous system of insect, leading to paralysis followed by death (Vassilakos et al., 2012; Galm and Sparks, 2016). Cyantraniliprole is another diamide insecticide, acting on the muscles of the insect. Cyantraniliprole works by decreasing the levels of calcium in muscle of insect, whereby muscle contraction is prevented, leading to paralysis and ultimately fatality (Tsukamoto et al., 2021).

MATERIALS AND METHODS

Rearing T. absoluta: The initial population of T. absoluta was collected from the infeseted greenhouse of East Azarbaijan province (37° 27′ 40/ 32″ N، 45° 53′ 19/12″ E) located in Ajabshir that had not been treated with any pesticide and transferred to the plant protection department of the faculty of agriculture, University of Tabriz greenhouse. Insect rearing and bioassays were performed under 26 ± 2 °C, 60 ± 5% RH and 16:8 h (light: dark) photoperiod. The mass rearing of this pest was carried out by releasing male and female insects onto Superchef cultivar tomato plants within wooden-framed cages covered with 80 mesh netting. Every week, pots containing tomato plants were added to the insect breeding colony so that healthy and vibrant plants would always be available for the adult moths to lay eggs and for the larvae to feed.

T. brassicae culture: Initial population of T. brassicae was obtained from insectarium of Baharavan in Gorgan, Iran. The rearing of wasps in a growth chamber under controlled conditions (temperature 25 ± 2 °C, 70 ± 5% RH and 16: 8 h (light: dark) photoperiod) was carried out. These wasps were reared for five generations on flour moth eggs and then used for experiments. The eggs of the flour moth, (Ephestia kuehniella Zeller, 1879) were used for rearing wasp.

Evaluation of the effect of the studied insecticides on T. brassicae: Bioassays on T. brassicae were performed following Sidi et al. (2013) in test tubes. For evaluating the toxicity of different insecticides in laboratory, the glass test tube method used by some resaerchers (Preetha et al., 2009; Wang et al., 2014 and Papari et al., 2024). To determine the effect of the tested insecticides on the parasitic wasp, 200 µl of each tested concentration of the insecticides were poured into a 100×15 mm glass test tube. The tube was rotated to evenly coat the inside with the insecticide. In the control treatment, distilled water + Tween80 was used. After the tubes were dried at room temperature, 20 one-day-old T. brassicae were transferred to each test tube, and their openings were sealed with muslin cloth and a rubber band. For feeding the wasps, 20 % honey-water solution was deposited to the inner wall of the test tube using a needle. The test tubes were placed in a growth chamber under the conditions mentioned in the wasp rearing section. Mortality was recorded after 24 h exposure to these insecticides.
It should be mentioned that each treatment consisted of five concentrations and for each concentration 20 T.brassicae wasps in each petri dish or test tube. The determination of concentration ranges for the insecticides tested on both T.absoluta and T.brassice was based on the results of preliminary experiments. In preliminary trials for five concentrations were tested. The recommended field concentration was considered the highest concentration.

- Investigation of the parasitism of tomato leafhopper eggs by Trichogramma brassicae wasps on three tomato cultivars:

1. T. absoluta were reared separately on each tomato cultivar for three generations.

2. The parasitism of tomato leaf minereggs reared on each of the tested cultivars by Trichogramma wasps was estimated in the absence of the tested insecticides.
3. The number of five surviving wasps after exposure to LC_{25} concentrations of the tested pesticides on T. absoluta eggs reared separately on each tomato cultivar was released. This experiment was performed in triplicate. In each replicate, 100 tomato T. absoluta eggs were exposed to five mated female wasps for 48 hours on leaves of the studied cultivars placed in cylindrical plastic containers measuring (11 * 8.5 cm), and the degree of parasitism was calculated.

- Calculation of parasitism reduction:
R or the rate of reduction in parasitism was calculated using the following equation (Costa et al. 2014):
$R (\%) = 100- [(f / t)] \times 100$

f= Mean parasitism rate in each insecticide treatment
t =Mean parasitism rate in the control
In this calculation, the basis for comparing was. parasitism rate in the absence of insecticide on the different tested cultivars.

According to the IOBC guidelines:
If the reduction in parasitism rate is less than 30%, the insecticide is considered harmless (category 1). If it is between 30 and 80%, the insecticide is considered low-harmful (category 2), if it is between 80 and 99%, the insecticide is considered moderate-harmful (category 3), and if it is greater than 99%, the insecticide is considered harmful (category 4) (Costa et al. 2014).

Data analysis: LC_{25} values for adult of T .brassicae were estimated using probit analysis of SAS 9.4. Comparison of means was performed with using the LSD test.

RESULTS

Table 1. Toxicity data of insecticides used on adult Trichogramma braccicae using glass vial bioassay method under controlled conditions

Insecticides	LC_{50} (95% CI)	LC_{90} (95% CI)	χ^2	df	Slope ± SE
Chlorantraniliprole	0.00039 (0.0002 - 0.0005)	0.005 (0.003 - 0.015)	13.05	13	0.18 ± 1.18
Matrine	0.003 (0.002 - 0.004)	0.0034 (0.018 - 0.106)	8.48	13	0.18 ± 0.16
Spinetoram	0.0010 (0.0007 - 0.0031)	0.041 (0.014 - 0.287)	1.64	13	0.11 ± 0.70
Flubendiamide	0.824 (0.769 - 0.874)	1.37(1.20 - 1.78)	12.60	13	1.00 ± 5.74
Cyantraniliprole	0.002 (0.001 - 0.003)	0.049 (194.92 - 992.11)	10.03	13	0.16 ± 0.91

By examining the eggs tested in the control and treatment, it was revealed that the parasitized eggs turned dark and black and had a significant difference from the healthy eggs (Figure 1).

The results of analysis of variance with using the GLM procedure of SAS software showed that the effect of tomato cultivar was not significant in any of the traits of percentage of parasitism of treatment (ParasT), percentage of parasitism of control (ParasC) and percentage of reduction of parasitism (Preduction) ($P > 0.2$), which indicates the lack of genotypic differences between the Rio Grande, Superstrain and Cal J cultivars in terms of the level of Trichogramma parasitism and sensitivity to insecticides. In contrast, the effect of insecticide on all three traits was highly significant at the 1% probability level ($P \leq 0.0002$), meaning that the type of insecticide independently and strongly affected the efficiency of parasitization of wasp, and some compounds were able to significantly maintain parasitization, while the other insecticides tested reduced it.

Table 2. Analysis of variance: percentage of parasitization of treatment

Source of Variation	Degrees of freedom	sum of squares	Mean Squares	F	Pr > F
Cultivar	2	1.73	0.87	0.52	0.5998 ns
Pesticide	4	111.877	27.97	16.78	<0.0001 ***
Cultivar × Pesticide	8	1.60	0.20	0.12	0.9980 ns
Error	30	50.00	1.67		
Corrected Total	44	165.20			

The interaction effect of cultivar and insecticide was completely insignificant on all traits ($P \geq 0.9917$), indicating the same response of all three cultivars to insecticides and absence of genotype and insecticide interaction, so the insecticide rating is independent of tomato cultivar. The coefficient of determination of the model (R^2) was 0.697 for ParasT, 0.549 for ParasC, and 0.578 for Preduction, which are acceptable values for biological experiments and indicate that the changes were adequately explained by the factors under study.

The results of the analysis of variance for the percentage of parasitism in the insecticide treatments showed that the effect of cultivar was completely insignificant ($F = 0.52$; $P = 0.5998$), meaning that the level of Trichogramma parasitism in the presence of insecticide did not differ between cultivars. The effect of insecticide was highly significant ($F = 16.78$; $P < 0.0001$), indicating a high difference between insecticides in maintaining or reducing wasp efficiency. The interaction effect was insignificant ($F = 0.12$; $P = 0.9980$) confirming that these differences were the same across all cultivars (Table 1).

In the percentage of control parasitism, the effect of cultivar was insignificant ($F = 1.66$; $P = 0.2073$), meaning that the rate of Trichogramma parasitism without insecticide was similar in the cultivars. The effect of insecticide was significant ($F = 7.92$; $P = 0.0002$) and the interaction effect was insignificant ($F = 0.18$; $P = 0.9917$) (Table 4-5).

Table 3. Analysis of variance: percentage of parasitization of treatment

Source of Variation	Degrees of freedom	sum of squares	Mean Squares	F	Pr > F
Cultivar	2	3.24	1.62	1.66	0.2073 ns
Pesticide	4	30.98	4.74	7.92	<0.0002 ***
Cultivar × Pesticide	8	1.42	0.18	0.18	0.9917 ns
Error	30	29.33	0.98		
Corrected Total	44	64.98			

Analysis of variance for percent reduction in parasitism (Table 6-4) showed that the effect of cultivar was insignificant (F = 0.06; P = 0.9411), indicating that insecticide sensitivity was similar among cultivars. The effect of insecticide was highly significant (F = 10.19; P < 0.0001), confirming the large difference in insecticide compatibility. The interaction effect was completely insignificant (F = 0.03; P = 1.000), so the results are generalizable to all cultivars.

Table 4. Analysis of variance: percentage of parasitization of treatment

Source of Variation	Degrees of freedom	sum of squares	Mean Squares	F	Pr > F
Cultivar	2	4.94	4.97	0.06	0.9411 ns
Pesticide	4	3331.07	832.77	10.19	<0.0001 ***
Cultivar × Pesticide	8	19.70	2.46	0.03	1.0000ns
Error	30	2450.71	81.69		
Corrected Total	44	5811.42			

The mean main effect of insecticides for the percentage reduction of parasitism was compared using the LSD test (α = 0.05) and the results are presented in the table below. The insecticide chlorantraniliprole showed the highest reduction with an average of 35.65% reduction of parasitism. This rate was significantly higher than matrine and flubendiamide and was placed in category 2 according to the IOBC guidelines. Cyantraniliprole (32.04%) and Spinturm (30.26%) were also in category 2 and did not differ significantly from chlorantraniliprole. Matrine (18.10%) and flubendiamide (13.35%) were placed in the first category and had the least negative effect (Table 7-4).

Table 5. Analysis of variance: percentage of parasitization of treatment

Insecticide	(%) Mean	significance
Chlorantraniliprole	35.65	a
Cyantraniliprole	32.04	a
Spinetoram	30.26	a
Matrine	18.10	b
Flubendiamide	13.35	b

Although the interaction was not significant, for completeness of the data, the mean percent reduction in parasitism for each cultivar × insecticide combination is presented. Because the interaction is not significant, significant letters were applied based on the main insecticide effect grouping (Table 8-4).

Table 6. Analysis of variance: percentage of parasitization of treatmen

Cultivar	Flubendiamide	Matrine	Spinetoram	Cyantraniliprole	Chlorantraniliprole
Riogrande	13.50 ± 4.79 b	17.02 ± 5.81 b	30.20 ± 1.75 a	13.11 ± 1.11 a	34.24 ± 5.66 a
Superstrain	12.66 ± 3.94 b	± 10.02 b 19.16	31.06 ± 14.00 a	31.67 ± 14.45 a	36.36 ± 0.00 a
Cal J N3	13.89 ± 4.82 b	18.11 ± 8.45 b	29.53 ± 5.95 a	33.33 ±b4.76 a	36.34 ± 8.34 a

Analysis of the interaction table (Table 8-4) shows that the response pattern is the same in all three cultivars: Chloranthraniprole, cyantranilprole and spinetoram were always in the second category (least compatible, 29–36% reduction) and matrine and flubendiamide in the first category (highest compatible, 12–19% reduction). Higher variance in superstrain was observed for spinetoram and cyantranilprole, but these fluctuations were not significant and did not change the overall ranking.

In conclusion, the insecticides chloranthraniprole, cyantranilprole and spinturm showed the least compatibility with Trichogramma and their simultaneous use with Trichogramma release is not recommended. Matrine and flubendiamide showed the most compatibility with it and their use is possible in integrated management programs for tomato leaf miner.

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