Efficacy of bio-insecticides on *Tuta absoluta* (Meyrick) (Lep.: Gelechiidae) in laboratory and field conditions

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Abstract: *Tuta absoluta* (Meyrick) is a devastating pest of tomato. This pest is becoming resistant to many of the pesticides that used in the tomato fields. In this research, the efficacy of four bio-insecticides namely, thiocyclam, spinosad, *Bacillus thuringiensis (B.t)* and azadirachtin (*Azadirachta indica*) were studied on the tomato leaf miner in the laboratory and field conditions. Laboratory experiments were performed in a randomized complete plot design with three replications against 1st, 2nd and 3rd larvae. Field experiment was carried out on three larval stages based on recommended doses of pesticides in the form of a completely randomized block. The results showed that in laboratory conditions, LC_{50} values and lower and upper bond with 95% confidence limits of thiocyclam, spinosad, *B.t* and azadirachtin on third instar larvae after 72 h, were 902.01(680.3-1442.54), 1793.41 (1326.08-2924.97), 2239.30 (2074.19-2442.10) and 2572.09 (2423.62-2736.74), respectively. In field experiments, thiocyclam and spiosad had the highest efficiency on mortality of the larvae with an average loss of 95.35% and 80.59%. *B.t* and azadirachtin with an average mortality of 67.29% and 66.40% had the lowest effect, respectively. The results showed that the third instar larvae were less sensitive than the first and second instar larvae for all pesticides. The results of two experiments show that spinosad, *B.t* and azadirachtin had less insecticidal efficacy compared with thiocyclam on tomato leaf miner. Recent study recommended using thiocyclam in integrated management of this pest.

Keywords: tomato leaf miner, *Bacillus thuringiensis*, spinosad, azadirachtin, thiocyclam

Citation: Hosseinzadeh, A., S. Aramideh, and A. G. Kahrizeh. 2019. Efficacy of bio-insecticides on *Tuta absoluta* (Meyrick) (Lep.: Gelechiidae) in laboratory and field conditions. Agricultural Engineering International: CIGR Journal, 21(3): 164–170.

1 Introduction

In Iran, the tomato growing area is about 150000 ha (Bani and Cheraghian, 2012). *Tuta absoluta* (Meyrick) is one of the key pests of tomato (Desneux et al., 2010). Tomato pest control is difficult due to biology and complex behavior (Guedes and Picanço, 2012). Effective chemical control of tomato leaf miner is difficult due to the feeding of pests from within the plant organs, having several generations per year, during the period of pupae within the mines, plant material or soil. In addition, its ability to produce large numbers of progenies also facilitates develop of resistance to insecticides (Hashemi et al., 2015). The use of chemical pesticides is a dominant method of controlling this pest, but there are significant deficiencies, most notably high costs and the destruction of natural enemies (Braham et al., 2012). Accordingly, it seems that special attention should be paid to non-chemical methods in management plans of this pest (Maluf et al., 2010). Bio-pesticides based on azadirachtin and *Bacillus thuringiensis* represent important pest control options for integrated pest management (IPM) because of their low eco-toxicological effects and short persistence in the environment (Lacey and Siegel, 2000; Braham et al., 2012). This bacterium is the most important pathogen used for biological control of pests and its formulations account for more than 60% of the world's microbial insect production (Miranda et al., 1998; Saberi et al., 2013). Spinosad is also another biological control agent

Received date: 2018-05-17 **Accepted date:** 2018-09-11

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that has very low toxicity for mammals, birds, insects and predators, and its use is not dangerous for users (Toews et al., 2003). The greatest effect of spinosad is on the Lepidoptera, Diptera, Thysanoptera, Coleoptera and Orthoptera (Toews et al., 2003). Spinosad has two unique modes of action, acting primarily on the insect nervous system at the nicotinic acetylcholine receptor, and exhibiting activity at the GABA receptor (Watson et al., 2010). In addition, azadirachtin, derived from Neem tree, have a great effect on the mortality of larvae and prevent the pests laying (Tome et al., 2013). Thiocyclam is based on the natural toxin of *Lumbriconereis heteropoda* (Amani et al., 2011). This group of insecticides on Coleoptera and Lepidoptera pests have a selective effect (Ware and Whitacre, 2004). Because of moderate toxicity, it is safe for humans. It effects on the activity of cholinesterase, and the residue decomposes quickly and does not remain in nature (Tomlin, 2000).

Therefore, in this study, the effect of four bio-pesticides including thiocyclam, spinosad, *B.t* and azadirachtin on different larval stages of tomato leaf miner in laboratory and field conditions was evaluated.

2 Material and methods

2.1 Bio-insecticides used

Commercial formulations of four bio-insecticides, including *B.thuringiensis*, subsp *kurstaki* (CoStar®, wettable microgranol suspension formulation with 85% toxin and spores, with a UI of about 90000), azadirachtin (which contains 60% Neem seed extract, 35% Neem oil and 5% emulsifier, Polyesterbite 20, Biotech international Ltd.,New Delhi, India), the spinosad (24% suspension; Dow AgroScience, USA), and the thiocyclam (50% powder; Arista Life Sciences, Japon) were used.

2.2 Insect rearing

Tomato leaf miner colony was collected in commercial tomato fields around Urmia city in Iran. In order to mass rearing pest, adults were transferred to tomato plants grown in plastic pots in the greenhouse, as well as in the field, on Super-strain B cultivars tomato bushes. The pots were cultivated in a greenhouse with 25°C±2°C, with 65%±5% relative humidity (RH), and 16:8 (L: D) photoperiod. After two generations of insect rearing, they were used for bioassay.

2.3 Laboratory bioassay tests

The bioassays were carried out using first, second and third instar larvae in tomato leaves treated with four insecticide concentrations. Bioassays were performed by immersion of tomato leaves in insecticide solution (Hashemi et al., 2015). Preliminary testing was conducted to determine the ‘‘all or nothing’’ response to establish a concentration gradient for estimating the concentration–response. After determining the concentrations that produced the lowest and highest (20%-80%) mortality, five other concentrations were calculated logarithmically (Moradeshaghi and Pourmirza, 1974). The insecticide solutions were diluted with water + 0.01% Triton X-100, and the control treatment used only distilled water + 0.01% Triton X-100. The tomato leaves were immersed in the insecticide solution for 5 s, air dried and placed in petri dishes (8 cm diameter × 1.5 cm height). Ten larvae of three larval stages were separately placed on leaves using a soft brush. In order to exchange air inside petri dishes, their doors were blocked by an organza. Larval mortality was counted after 24, 48 and 72 hours. The mortality criterion was based on the movement of larvae following prodding with a soft brush (Tabashnik et al., 1990). Each treatment was replicated three times on a total of 30 larvae per concentration.

2.4 Field experiments

Experimental area was planted with tomato ‘Super Strain B’ after seeded in a greenhouse and then transferred to the field, under normal field and agricultural practices. The experiment block design was randomized with each treatment replicated three times. Each plot had five rows with 25 plants. The 15 plants of medium row were used for data collection. The plant space was 0.5×1.0 m. The bio-insecticides, thiocyclam, spinosad, *B.t* and azadirachtin were applied as foliar spray at the recommended rates. The 5th treatment was water to serve as control. The evaluations against leaf minor were conducted on 3 plants were randomly collected from each replicate before spraying as well as 1, 3, 7 and 14 days after spraying (Shalaby et al., 2012). The outer plants were never sampled in order to avoid border effects. Alive larvae were counted using a binocular microscope.

Percent efficacy was calculated using Equation 1(Henderson and Tilton, 1955).

$$\text{Efficacy}, \% = \left(1 - \frac{Ta \times Cb}{Ca \times Tb}\right) \times 100 \quad (1)$$

where, *Ca* and *Cb* = Contamination rate in control plots after and before spraying; *Ta* and *Tb* = Contamination rate in treated plots after and before spraying.

2.5 Data analysis

Mortality data obtained from concentration–response bioassays were corrected with the mortality observed in the control treatment (Abbott, 1925) and analyzed by Probit program**.** In addition, to evaluate the effect of compounds in field conditions, by General Linear Model-Univariate, variance analysis was performed and Mean comparison was separated by Tukey (HSD) at 95% confidence level by SPSS ver. 22.

3 Results and Discussion

3.1 Bioassay

For evaluate effect of thiocyclam, spinosad, B. thuringiensis (B.t) and azadirachtin on T. absoluta (Meyrick) two different bioassays include determination LC50 and LC90 values and field trail was carried out.

3.2 LC_{50} and LC_{90} values

LC_{50} and LC_{90} values of thiocyclam, spinosad, *B.t* and azadirachtin on the 1st instar of tomato leaf miner larvae by leaf dipping method at 24, 48 and 72 hours after the experiment, it has been shown in Table 1. LC_{50} values of thiocyclam, *B.t.*, spinosad and azadirachtin indicate that thiocyclam has the most effect on the first instar of tomato leaf miner larvae. Spinosad was the second rating in terms of mortality. *B.t* and azadirachtin had the lowest rate of mortality. In addition, the effect of all four insecticides has increased, so that 72 hours after the test, the mortality has reached the highest.

LC_{50} and LC_{90} values of thiocyclam, spinosad, *B.t* and azadirachtin on the 2nd instar of tomato leaf miner larvae at 24, 48 and 72 hours after the experiment, it has been shown in Table 2. LC_{50} values of thiocyclam, *B.t.*, spinosad and azadirachtin indicate that thiocyclam has the most mortality effect on the first instar of tomato leaf miner larvae. Spinosad was the second rating in terms of mortality and *B.t* and azadirachtin had the lowest rate of mortality. In addition, the effect of all four insecticides has increased, so that 72 hours after the test, the mortality has reached the highest.

LC_{50} and LC_{90} values of thiocyclam, spinosad, *B.t* and azadirachtin on the 3rd instar of tomato leaf miner larvae at 24, 48 and 72 hours after the experiment, it has been shown in Table 3. LC_{50} values of thiocyclam, *B.t.*, spinosad and azadirachtin indicate that thiocyclam has the most mortality effect on the first instar of tomato leaf miner larvae. Spinosad was the second rrating in terms of mortality and *B.t* and azadirachtin had the lowest rate of mortality. The effect of all four insecticides has increased, so that 72 hours after the bioassay, the mortality has reached the highest.

Table 1 Lethal effect of thiocyclam, spinosasd, *B.t* and azadirachtin on first larvae instar of *T. absoluta*

Bioinsecticide	Time (h)	Slope±SE	Chi-square	Lethal concentration (ppm)	
				LC_{50} (lower-upper)	LC_{90} (lower-upper)
Thiocyclam	24	3.25±0.39	5.81	798.21 (604.27-1117.27)	1975.15 (1322.29-6467.0)
	48	3.12±0.38	6.37	762.63 (560.69-1092.72)	1961.56 (1283.63-7528.25)
	72	3.13±0.38	5.32	745.27 (564.63-1011.83)	1916.17349 (1294.97-5674.34)
Spinosad	24	3.54±0.41	8.89	1564.71 (1108.35-2369.15)	3601.50 (2375.69-17433.78)
	48	3.64±0.41	6.32	1477.38 (1120.71-1963.97)	3324.13 (2356.29-8481.97)
	72	3.47±0.39	5.59	1391.63 (1058.207-1810.87)	3255.20 (3225.70-1504.715)
B.t	24	4.86±0.55	2.87	2042.60 (1893.31-2210.59)	3746.59 (3275.11-4578.23)
	48	4.72±0.54	2.77	1975.90 (1809.84-2119.63)	3360.43 (3198.26- 4475.74)
	72	4.94±0.54	2.45	1888.73 (1748.69-2036.38)	3433.80 (3036.16-4107.92)
Azadirachtin	24	5.21±0.55	2.87	1484.31 (1319.88-162.03)	2042.60 1893.31- 2210.59
	48	6.21±0.73	5.73	2772.20 (2417.46-3363.38)	4457.18 (3583.71-8267.39)
	72	6.12±0.69	4.49	2562.73 (2413.78-2729.39)	4149.97 (3725.75-4875.65)

Table 2 Lethal effect of thiocyclam, spinosasd, *B.t* and azadirachtin on second larvae instar of *T. absoluta*

Bioinsecticide	Time (h)	Slope±SE	Chi-square	Lethal concentration (ppm)	
				LC_{50} (lower-upper)	LC_{90} (lower-upper)
Thiocyclam	24	3.20±0.40	5.07	872.17 (777.69-996.23)	2195.87 (1736.19-3190.47)
	48	3.19±0.40	4.90	838.96 (748.38-954.24)	2117.32 (1684.17-3038.49)
	72	3.14±0.39	6.47	800.10 (519.89-1178.91)	2049.95 (1323.96-8769.13)
Spinosad	24	3.71.±0.43	9.59	1656.76 (1178.77-2624.75)	3669.47 (2413.43-21288.53)
	48	3.71±0.43	7.78	1610.40 (1195.12-2310.60)	3563.34 (2431.57-12831.79)
	72	3.80±0.42	6.70	1543.08 (1175.19-2073.09)	3555.14 (2379.96-8940.69)
B.t	24	5.19±0.59	2.08	2150.06 (2000.89-2322.75)	3798.67 (3335.02-4612.28)
	48	5.03±0.57	2.77	2095.88 (1947.02-2265.79)	3767.60 (3302.19-4584.41)
	72	5.03±0.57	2.32	2085.68 (1937.43-2254.06)	3749.69 (3288.62-4557.14)
Azadirachtin	24	5.21±0.72	5.907	3125.18 (2657.29-4589.10)	5505.41 (4025.99-18744.86)
	48	5.38±0.68	0.139	2662.07 (2491.07-2866.14)	4605.89 (4028.44-5695.70)
	72	5.55±0.68	1.65	2634.50 (2469.47-2827.36)	4482.28 (3948.73-5463.89)

Table 3 Lethal effect of thiocyclam, spinosasd, *B.t* and azadirachtin on third larvae instar of *T. absoluta*

Bio-insecticide	Time (h)	Slope±SE	Chi-square	Lethal concentration (ppm)	
				LC_{50} (lower-upper)	LC_{90} (lower-upper)
Thiocyclam	24	2.98±0.41	6.19	980.64 (734.76-1772.81)	2636.32 (1560.70-20961.70)
	48	3.04±0.40	4.81	929.51 (822.99-1079.93)	2452.39 (1885.02-3773.73)
	72	2.96±0.39	5.80	902.01 (680.03-1442.54)	2444.04 (1500.11-13301.49)
Spinosad	24	3.88±0.49	6.89	1967.54 (1532.78-3121.54)	4212.41 (2803.84-19845.55)
	48	3.64±0.46	9.48	1894.76 (1377.01-3651.97)	4257.62 (2664.08-4894.13)
	72	3.75±0.45	8.68	1793.41 (1326.08-2924.49)	3939.74 (2578.82-23633.86)
B.t	24	5.12±0.62	2.99	2413.12 (2238.90-2640.63)	4293.33 (3692.14-5434.33)
	48	4.98±0.59	3.56	2304.81 (2137.98-2513.94)	4168.02 (3595.33-5233.59)
	72	4.81±0.57	1.53	2239.30 (2074.19-2442.10)	4134.31 (3559.07-5208.10)
Azadirachtin	24	5.94±0.84	4.41	3379.26 (3129.33-3783.87)	5553.48 (4703.85-7392.04)
	48	5.30±0.69	0.08	2827.73 (2640.80-3071.38)	4932.09 (4256.36-6668.65)
	72	6.19±0.71	0.17	2572.09 (2423.62-2736.74)	4143.81 (3725.53-4860.57)

3.3 Field experiments

Analysis of variance of data on field experiments and effect of treatments, one day after spraying, showed a significant difference at 5% level between treatments (df= (3,6); F= 175.91; P= 0.001). This criteria for 3, 7, 10 and 15 day after treatments were (df= (3, 6); F= 158.56; P= 0.001), (df= (3, 6); F= 132.24; P= 0.001), (df= (3, 6); F= 151.00; P= 0.001) and (df= (3, 6); F= 84.80; P= 0.001), respectively. The results show that thiocyclam and spinosad caused an average efficacy 68.24 and 26.95%, whereas *B.t* and azadirachtin showed an average reduction about 12.74 and 12.49% one day after spraying with recommended doses, respectively (Table 4). Three day after spraying, average efficacy percentage by thiocyclam and spinosad was, 73.85 and 42.60%, whereas by *B.t* and azadirachtin was 23.56 and 23.55%, respectively. Seven day after treatment, average efficacy percentage by thiocyclam, spinosad, *B.t* and azadirachtin was 81.31, 65.20, 53.60 and 54.87% respectively. Ten day after treatment, thiocyclam with average efficacy percentage, had the best effect and *B.t* with 60.99%, had the least effect. In addition, fifteen day after spraying, comparison of average efficacy of treatments showed that thiocyclam with 95.35% effect in group a and spinosad with 80.59% in group b, azadirachtin and *B.t* with 67.29 and 66.40% in group c were placed (Table 4).

Table 4 Mean (±SE) efficiency bio-insecticides against tomato leaf miner *Tuta absoluta*

Bio-insecticides	Mean (±SE) efficiency				
	1 DAT	3 DAT	7 DAT	10 DAT	15 DAT
Thiocyclam	68.24±1.22a	73.85±1.99a	81.31±1.45a	89.48±1.35a	95.35±1.06a
Spinosad	26.95±1.72b	42.60±1.17b	65.20±1.37b	76.07±1.17b	80.59±1.22b
B. t	12.74±1.06c	23.56±2.23c	53.60±0.88c	60.99±0.76c	67.29±1.06c
Azadirachtin	12.49±1.25c	23.55±0.66c	54.87±0.99c	62.46±0.46c	66.40±1.12c

Note: *Values of each column followed by the same letter (s) are not significantly different at 5% probability level ($P \leq 0.05$).

The use of bio-insecticides has less harmful effects on humans, mammals and the environment than conventional pesticides. In addition, due to their low durability, they can be used in nature and simplicity to provide a suitable alternative for chemical pesticides in the pest control. The results of this study showed that the first larval instar of this pest was the most sensitive to all biological insecticides. Among the used insecticides, thiocyclam had the highest toxicity for all three larval instars and spinosad, *B.t* and azadirachtin were in the next rank. In many countries where tomato leaf miner is prevalent, its biological control methods are being investigated (Bloem and Esther, 2011). Abamectin, cartap, fentoate, spinosad and indoxacarb are recommended for use in southern and southeastern of Brazilian tomato fields. Fentoate and spinosad are also recommended for use in the northeastern parts of the country (IRAC, 2009). In this study, the mortality effects of four bio-insecticides: thiocyclam, spinosad, *B.t* and azadirachtin on tomato leaf miner, in field and laboratory conditions were investigated. Nazarpour et al. (2016) investigated the effect of indoxacarb, *Bacillus thuringiensis*, azadirachtin, and *Bacillus thuringiensis* + azadirachtin on tomato leaf miner. The results showed that in the short term, indoxacarb had better results, but in the long term, the combination of *Bacillus thuringiensis* with azadirachtin had the best effect. Results indicate positive effects of biological compounds in the management of this pest. In the present study, thiocyclam is recommended because of high mortality. Roditakis et al. (2013) investigated the effect of several chemical insecticides and spinosad on the second larval instar of tomato leaf miner and LC_{50} values for spinosad were calculated 158.8 to 315 mg L^{-1} after 72 hours. There is a difference between the results and this researcher. This difference caused mybe in the calculation of LC_{50} by Roditakis et al. (2013) because of the effective bio-insecticide ingredient. In the UK, three insecticides *Bacillus thuringiensis*, spinosad and indixacarb for *T. absoluta* control have been recorded on tomato, pepper and eggplant (Fera, 2009). Derbalah et al. (2012) emphasized the potential for insecticidal of *Bacillus thuringiensis* by experiments. The study of Hashemi et al. (2015) on the control of tomato leaf miner showed that spinosad is more effective than *Bacillus thuringiensis* and the first instar larvae are more sensitive than the 2nd and 3rd instar larvae. Nannini et al. (2011) examined the effect of several different types of pesticides on tomato leaf miner and showed that spinosad effectively controls this pest. Nannini et al. (2011) results are according with achievement of this study. Indoxacarb, spinosad, imidacloprid, deltamethrin and *B.thuringiensis* var. *kurstaki* is used to control *T. absoluta* larvae in Spain (Fera, 2009). In Malta, abamectin, indoxacarb, spinosad, imidacloprid, thiacloprid, lufenuron and *B. thuringiensis* is recommended to control this pest (Mallia, 2009). In France, it was recommended to use indoxacarb and *B.thuringiensis* (Fredon, 2009). Which indicates the effect of biological insecticides on controlling this important pest. The results of Gonzalez et al. (2011) on the effect of *Bacillus thuringiensis* against tomato leaf miner in greenhouse and field conditions showed that this bacteria was effective on all larval stages but had the greatest effect on first larval instars. The results of this study are similar to those of the researchers. The use of azadirachtin, *Bacillus thuringiensis* and indoxacarb, has been proposed during the period of infection with a mean of 3 to 30 adult male insects per trap each week. It was also recommended applied indoxacarb at young plants and spinosad at the time of plant rooting and during rapid population growth at high population time (Fredon, 2009). These results indicate the high effect of spinosad, similar of ours results. In assessing the sensitivity of different stages of tomato leaf miner to *Bacillus thuringiensis*, by the other researchers, the first instar larvae were more sensitive to this biological compound (Rausel et al., 2000; Hashemi et al., 2015).

4 Conclusions

Based on the results of this study, the highest mortality rates on first larvae stages of tomato leaf miner was observed against all four biological pesticides. The results of this study showed that in the policy of reducing the use of chemical pesticides, thiocyclam and spinosad could be used in the integrated management of this pest.

Acknowledgement

We thank the head of Islamic Azad University, Mahabad Branch, for their financial and scientific supports.

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