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Efficacy of bio-rational insecticides against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) on tomatoes.

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Recently, the tomato leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) becomes the most important pest affecting tomato crop worldwide. This Insect pest caused reduction of plant growth and yields in both protected and open-field. Bio-rational insecticides provide efficient, economic, and promising strategy for controlling different insect pests. The use of bio-rational insecticides as alternatives for chemicals pesticides have many advantages as decreasing the environmental risks of chemical pesticides, the specificity of most of these entomopathogens, and being harmless to vertebrates and other non-target organisms. The aim of this work was to evaluate different bio-rationale insecticides including microbial pesticides, bio-chemicals derived from micro-organisms and other natural sources for controlling *T. absoluta* (Meyrick) (Lepidoptera: Gelechiidae) which is a major insect pest of the tomato crop in Egypt. Two hybrid tomato varieties; Shifa and Savera; were planted in two different plantation periods in the horticulture nursery. The effect of five different bio-rationale insecticides namely; spinosad, *Bacillus thuringiensis* subsp. *kurstaki* (Btk), azadirachtin, *Metarhizium anisopliae* (Metschn.) and *Beauveria bassiana* (Balsamo) was evaluated in trials under the greenhouse conditions. The bio-insecticides were applied at LC_{50} for semi-field application. Populations of *T. absoluta* were assessed by larval counts and the tomato yields were assessed for the two varieties at full plant maturity. Use of spinosad caused 78-97% reduction in *T. absoluta* population which was the most effective bio-rational insecticide among those used in this study. Btk was the second most effective bio-rational insecticide against *T. absoluta* with 78-91% reduction, followed by the botanical extract azadirachtin which showed 70-83% reduction on the target insect. Use of entomopathogenic fungi, *M. anisoplia* and *B. bassiana* caused 46 -75% reduction in comparison to the untreated plants. In conclusion, utilization of spinosad, Btk and azadirachtin were the most effective bio-rationale insecticides which provide promising and safe alternative insecticides for controlling *T. absoluta*.

Keywords: ***Tuta absoluta, spinosad, Bacillus thuringiensis, azadirachtin, Metarhizium anisopliae, Beauveria bassiana.***

INTRODUCTION

In less than a century, the tomato (*Lycopersicon esculentum* Mill) (Family: Solanaceae) has become a major world food crop. In Egypt, the tomato is considered one of the most important vegetable crops for fresh consumption and processing. In 2012, Egypt ranked as one of the top producers of tomatoes, with 8,625,219 tons produced (FAOSTAT 2012). In 2013, the total cultivated area and productivity of tomato in Egypt was estimated to be 515,225 feddan (ha=2.47 feddan), yielding 8,571,050 tons, with an average of 16,636 tons feddan^{-1}(Ibrahim et al., 2015; Abd El-Ghany et al., 2016a). Currently the most important pest affecting the crop is the tomato leaf miner *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae (Vila et al., 2012). This is a neotropical oligophagous pest of solanaceous crops (Lietti et al., 2005), and considered one of the most devastating pests of tomato (Fernandez & Montagne, 1990; Guedes & Picanço, 2012), sometimes leading to 60 - 100% loss of yield (Cely et al., 2006; Abd El-Ghany et al., 2016b). Damage caused by *T. absoluta* leads to reduction of photosynthetic capacity and reduced plant growth and yields in both protected and open-field tomato crops. Furthermore, economic losses are derived from the un-marketability of the infested fruits. *Tuta absoluta* is widely distributed in the Mediterranean region. The first detection of *T. absoluta* in the Mediterranean region was recorded in Eastern Spain in late 2006 (Urbaneja et al., 2012), then it quickly spread to Europe, North Africa and the Middle East. Historical records suggest that Southern Europe was the first and maybe the sole introduction point from the native area. The absence of co-evolved natural enemies may also explain why the dynamics in the newly invaded areas is so severe in comparison to the native area where natural enemies are frequent (Luna et al., 2012). In Egypt*, T. absoluta* has been reported since 2009 in several governorates (Al-Wadi Al-Gadid, Alexanderia, El-Sharkyia and El-Qalyoubia) and quickly became one of the key insect pests of the tomato crop (Bekheit & Impiglia, 2011). Overlapping cultivation periods of the tomato crop provides access to the preferred host over the whole year, causing continuous and rapid spread of this insect pest (Desneux et al., 2010). The tomato leaf miner larvae are internal feeders, making it difficult to achieve effective control through application of chemical insecticides.

Recently, major progress has been made worldwide to increase and sustain crop productivity with less use of chemicals. The use of chemical insecticides cause adverse environmental effects including; water pollution, eradication of beneficial wildlife and human health problems (Abd El-Ghany, 2011; Lietti et al., 2005) and therefore, there has been a search for efficient, economic alternatives to control the target insect pests. The use of various bio-rational insecticides has shown to provide a promising strategy for insect pests control; providing safe alternatives, being harmless to vertebrates and other non-target fauna and also because of the specificity of most of the entomopathogens, which affect a small range of insect hosts. Among various bio-rationale insecticides; *Bacillus thuringiensis* (Bt)*, Metarhizium anisoplia, Beauveria bassiana*, Nuclear Polyhedrosis Virus (NPV), spinosad and azadirachtin have been used for controlling insect pests (Ranga Rao et al., 2007; Merdan et al., 2010; González-Cabrera et al., 2011; Salama et al., 2012; Barra et al., 2013; Moussa et al., 2013; Abd El-Ghany et al., 2014; Abdel-Razek et al., 2014; Abd El-Ghany et al., 2015a and b; Salama et al., 2015; Abd El-Ghany et al., 2016b; Abdel-Razek et al., 2017). The present study focused on testing different bio-rationale insecticides, which included microbial insecticides, bio-chemicals derived from micro-organisms and other natural sources (*B. thuringiensis* (Btk)*, M. anisoplia, B. bassiana*, spinosad and azadirachtin) for controlling *T. absoluta.*

MATERIALS AND METHODS

Study site and experimental design

This work was carried out in the greenhouse unit of the National Research Center (NRC) farm at El-Behira Governorate, District of Kom Hamada, Egypt, about 160km from Cairo. The farm occupies an area of 126 ha, which about 7 ha is greenhouses for vegetable crops. The average annual temperature of the area was 21.2±3˚C: relative humidity (RH) was 57% (12.6 g m^{-3}) and rainfall 11.6±4.5 mm. The greenhouse was oriented in a north-south direction, covered by polyethylene plastic. The total area of the greenhouse was 480 m^2 (60m length x 8m width). The greenhouse site had a history of at least 3 consecutive years of using organic soil fertility amendments, winter cover crops and biologically-based pest control. No synthetic pesticides were used during this study. The soil was sandy texture deep, non-saline or slightly saline (EC value range between 0.2 and 0.5 dSm^{-1}). Field capacity and wilting point are low (8-9 and 2-3% of soil moisture content, respectively). The soil has low levels of macro- and micro-nutrients and chicken manure compost (with concentrations of 1.1% N. 8.0% P and 5.0 % K) was therefore added, after which the soil was tilled three times at weekly intervals, to increase the soil fertility and reduce the viability of weed seeds and other possible pests. A drip irrigation system was used to irrigate the crops, with 50cm between drippers to ensure the homogenous distribution of water. The irrigation system was solely based on waste water (with concentrations of 2.0% N, 3.1% P and 0.7% K) supplied by the organic fish farm. Plant growth was observed weekly during the whole plantation period to avoid any nutrient deficiencies. The use of chicken manure compost and organic fish farm water was sufficient to ensure good plant growth. Weeds were removed by hand to prevent competition between weeds and cultivated tomatoes.

Cultivation of tomatoes

Two hybrid tomato varieties, Shifa and Savera; were grown in both experiments. Seeds were obtained from the Agriculture Research Center, Egypt; Shifa had been imported from Thailand and Savera from China. Tomato seeds were sown on two dates for the two different plantation periods (on 20 August 2014 for the first period and on 15 December 2014 for the second period). The seedlings were raised at the horticulture nursery of the National Research Center, for 30 days, and then transplanted into the greenhouse. Tomato plantlets were transplanted on 15 September 2014 for the first plantation period and on 15 January 2015 for the second period. The first plantation period was from mid-September, 2014 to mid-January, 2015 and the second plantation period was from mid-January to mid-May 2015.

Application and efficacy assessment of the bio-rational insecticides against *T. absoluta*

Five bio-rational insecticides were used; Tracer (spinosad), Nimbecidine (azadirachtin); Dipel® 2x (*B. thuringiensis var. kurstaki*) (Btk), Bio Magic (*M. anisopliae*); Bio Power (*B. bassiana*). These were all commercially available bio-insecticides that have been approved for use in organic horticulture according to IFOAM (International Federation of Organic Agriculture Movement). The bio-insecticides were applied at recommended dose given by the producer for field application as shown in Table 1. The recommended dose was assumed to be the median lethal concentration (MLD) as follows: 2g l^{-1} ha^{-1} for *B. thuringiensis;* 5 ml l^{-1} ha^{-1}·for *B. bassiana;* 5 ml l^{-1} ha^{-1}· for *M. anisopliae;* 5 ml l^{-1} ha^{-1} for azadirachtin and 0.3 ml l^{-1} ha^{-1} for spinosad. Control plants were sprayed with water.

The population of *T. absoluta* found in the greenhouse was due to a natural infestation. The efficacy of each bio-insecticide was evaluated in the two greenhouse trials (Plantation periods 1 and 2). In both trials, a split-split plot design was used; enabling the study of 6 treatments including the control for the two tomato varieties; Shifa and Savera. The experiment was designed as the follows: there were 6 treatment plots, including the control. The treatment plots were divided into two sub-plots, one for each tomato variety; each sub plot contained 3 replicates plots. The dimension of each replicate plot was 0.6m x 19m and contained 10 plants row^{-1}. The distance between the plants was 0.5m, and 0.5m between the rows. The split-split plot design allowed for high precision of treatment applications and a plastic sheet was also used to separate between different treatment plots during application of the bio-insecticides. The bio-insecticides were applied using 5L knapsack sprayers for each of the different bio-insecticides and the applications were done 5 times at 7 days intervals. Control plants were sprayed with water at the same times and intervals.

The effects of bio-insecticides were evaluated by taking twenty leaves at random from each replicate for each tomato variety per week and for a period of 6 consecutive weeks, starting from early December 2014 to mid-January 2015 for first growing season and from early April 2015 to mid-May 2015 for second growing season. The leaf samples were placed in paper bags and transferred to the Microbial Control of Insect Pest Laboratory at National Research Centre, Egypt for investigating the total number of larvae per leaf under stereomicroscope. For each bio-insecticide, the percentage reduction in the number of live larvae was calculated as accumulated record for the 6 weeks.

Tomato yield assessment

At full maturity of the tomato plants (20 January 2015 and 20 May 2015 in the first and second growing season, respectively), three plants out of 9 plants which resemble the number of plants from 3 replicates found in m^2 were removed to assess the yield of fruits. Yield assessment was based on the quantity of marketable fruit after removing the unhealthy fruits. The fruits were harvested, weighed and the yields were recorded as kg $plant^{-1}$ and was then converted to kg m^{-2}.

Statistical Analysis

Results obtained from the efficacy assessment of the bio-rational insecticides and productivity was statically analysed. The efficacy assessment of the bio-rational insecticides was statically analysed using two-way ANOVA using SPSS Computer Software Program, (factors: bio-insecticide treatment x week of assessment) for each tomato variety. Moreover, differences between the two plantation periods for different weeks of assessment for both tomato varieties were analysed by one-way ANOVA using SPSS Computer Software Program. T-test analysis was applied to analyse the differences between reduction percentages in larval population on both tomato varieties throughout the two plantation periods. Productivity assessment was analysed by one-way ANOVA using SPSS Computer Software Program, and T-test analysis was applied to analyse the differences between tomato yields during the plantation periods.

Table 1: List of bio-rational insecticides used in greenhouse trials.

No.	**Bio-insecticide agent**	Type of bio-insecticide	Trade name	Company	Concentration used (LC_{50})
1	***Bacillus thuringiensis (kurstaki)***	Bacterial insecticide	Dipel ®2x	Valent. Canada	2.0 gl^{-1} ha^{-1}
2	***Spinosad***	Bio-chemicals derived from micro-organisms	Tracer	Dow Agroscience	0.3 mll^{-1} ha^{-1}
3	**Azadirachtin**	Plant extract	Nimbecidine	Stanes	5.0 mll^{-1} ha^{-1}
4	***Metarhiziumanisopliae*(Metschn.)**	Entomopathogenic fungus	Bio Magic	Stanes	5.0 mll^{-1} ha^{-1}
5	***Beauveriabassiana*(Balsamo)**	Entomopathogenic fungus	Bio Power	Stanes	5.0 mll^{-1} ha^{-1}

RESULTS

Evaluation of the efficacy of bio-rational insecticides against *T. Absoluta*

The efficacy of different bio-rational insecticides; spinosad, azadirachtin, Btk, *M. anisopliae*, and *B. bassiana*; was performed throughout two plantation periods on Shifa and Severa tomato varieties. During the 1st plantation period the effect of bio-rational insecticides was significant with both Shifa and Savera having the same P value of 0.001. i.e. there was a definite effect of bio-rational insecticides against *T. absoluta* larvae (Table 2). Spinosad was the highest effective bio-rational insecticides among the tested ones followed by Btk and azadirachtin, respectively. A glance on data in Table 2 indicates that, the interaction between time and bio-rational insecticides for Shifa variety was insignificant i.e. P = 0.228. On the other hand, for Savera variety there was a significant interaction between the two factors; time and bio-rational insecticides*;* these factors affect each other (P = 0.019.), and this was mirrored in the results as observed with spinosad which was the most effective compared to other bio-rational insecticides at 1st, 2nd, 5th and 6th week. Meanwhile, Btk was the most effective among other bio-rational insecticides at 3rd and 4th week as shown in Table 2. Therefore, the two variables of time and bio-rational insecticides are related.

Data from different week assessments with bio-rational insecticides treatments indicates significant differences at 2nd plantation period, where F values= 11.153** and 7.940** and p=0.001 for both Shifa and Savera tomato variety, respectively (Table 3). Furthermore, Shifa variety seems to be more sensitive for the interaction of the factors of time and bio-rational insecticides than Savera variety. Moreover, there was a higher F value for bio-rational insecticides applications on Shifa more than Savera, 302.919 vs 207.170; which indicates that these bio-rational insecticides affects shifa more than savera variety. Spinosad, azadirachtin, and Btk were significantly different compared with the entomopathogenic fungi; *M. anisopliae*, and *B. bassiana* for both tomato varieties. Subsequently, there was a higher F value for time factor on shifa more than savera, 41.486 vs 26.365 which mean that the time affects Shifa more than Savera. Btk treatment was the most effective bio-rational insecticides during the 1st and 2nd week followed by spinosad and azadirachtin. However, spinosad set in the first place of efficacy among the tested bio-rational insecticides during the next four weeks.

Table 2: Efficacy of bio-rational insecticides on *T. absoluta* populations during 1st plantation period for shifa and savera tomato varieties.

Variety	Time (week)	Mean number of larva ± S.E.					
		control	Bio-Magic	Bio-Power	Dipel 2x	Nimbicidin	Tracer
Shifa	1	3.60 ± 0.12	1.15 ± 0.20	1.25 ± 0.20	1.15 ± 0.23	1.25 ± 0.23	0.85 ± 0.17
	2	4.35 ± 0.13	2.05 ± 0.37	2.00 ± 0.35	0.85 ± 0.17	1.60 ± 0.28	0.70 ± 0.13
	3	4.30 ± 0.12	1.85 ± 0.29	1.60 ± 0.29	0.70 ± 0.18	0.95 ± 0.25	0.90 ± 0.23
	4	3.95 ± 0.15	1.95 ± 0.39	2.00 ± 0.26	0.95 ± 0.26	1.25 ± 0.32	1.10 ± 0.32
	5	3.85 ± 0.17	2.20 ± 0.34	0.95 ± 0.21	0.85 ± 0.24	1.05 ± 0.25	0.80 ± 0.24
	6	3.85 ± 0.17	2.20 ± 0.34	0.95 ± 0.21	0.85 ± 0.24	1.05 ± 0.25	0.80 ± 0.24
F-Value [a]		264.563		P-Value [a]		0.001	
F-Value [b]		1.843		P-Value [b]		0.102	
F-Value [c]		1.199		P-Value [c]		0.228	
Savera	1	3.50 ± 0.11	1.25 ± 0.27	1.35 ± 0.23	1.00 ± 0.24	1.30 ± 0.23	0.80 ± 0.20
	2	4.10 ± 0.10	1.65 ± 0.24	1.90 ± 0.32	0.90 ± 0.16	1.25 ± 0.22	0.65 ± 0.17
	3	4.40 ± 0.09	1.40 ± 0.29	1.50 ± 0.36	0.60 ± 0.18	1.05 ± 0.22	0.85 ± 0.24
	4	4.00 ± 0.11	1.85 ± 0.36	1.90 ± 0.33	0.80 ± 0.22	1.55 ± 0.29	1.05 ± 0.25
	5	3.80 ± 0.15	2.10 ± 0.27	0.95 ± 0.30	0.75 ± 0.26	0.75 ± 0.22	0.55 ± 0.20
	6	3.80 ± 0.15	2.10 ± 0.27	0.95 ± 0.30	0.75 ± 0.26	0.75 ± 0.22	0.55 ± 0.20
F-Value [a]		337.331		P-Value [a]		0.001	
F-Value [b]		2.278		P-Value [b]		0.045	
F-Value [c]		1.680 NS		P-Value [c]		0.019	

[a] for the effect of bio-rational insecticides only, [b] for the effect of Time (week) only
[c] for the effect of the interaction between bio-rational insecticides and Time (week) together.

Table 3: Efficacy of bio-rational insecticides on *T. absoluta* populations during 2nd plantation period for shifa and savera tomato varieties.

Variety	Time (week)	Mean number of larva± S.E.					
		control	Bio-Magic	Bio-Power	Dipel 2x	Nimbicidin	Tracer
Shifa	1	4.60 ± 0.24	1.53 ± 0.29	1.53 ± 0.24	0.00 ± 0.00	0.93 ± 0.33	1.20 ± 0.39
	2	6.80 ± 0.37	2.80 ± 0.59	1.20 ± 0.44	0.33 ± 0.13	1.00 ± 0.34	0.40 ± 0.19
	3	7.53 ± 0.38	1.71 ± 0.32	2.00 ± 0.56	0.20 ± 0.11	1.00 ± 0.32	0.00 ± 0.00
	4	9.80 ± 0.48	5.27 ± 0.90	3.00 ± 0.70	1.63 ± 0.66	2.20 ± 0.63	0.07 ± 0.07
	5	11.27 ± 0.54	5.33 ± 0.54	7.00 ± 1.04	2.00 ± 0.58	2.60 ± 0.69	0.00 ± 0.00
	6	16.74 ± 0.38	8.14 ± 0.89	9.53 ± 0.96	1.73 ± 0.37	2.13 ± 0.73	0.00 ± 0.00
F-Value [a]		302.919		P-Value [a]		0.001	
F-Value [b]		41.486		P-Value [b]		0.001	
F-Value [c]		11.153**		P-Value [c]		0.001	
Savera	1	4.49 ± 0.38	1.47 ± 0.34	1.73 ± 0.30	0.00 ± 0.00	1.20 ± 0.35	1.07 ± 0.32
	2	6.67 ± 0.49	3.67 ± 0.49	2.80 ± 0.68	0.40 ± 0.19	0.93 ± 0.25	0.53 ± 0.19
	3	7.80 ± 0.44	7.07 ± 0.59	1.73 ± 0.45	0.33 ± 0.13	2.13 ± 0.40	0.13 ± 0.09
	4	10.55 ± 0.62	4.87 ± 1.06	4.93 ± 0.78	1.27 ± 0.28	1.94 ± 0.38	0.40 ± 0.19
	5	10.80 ± 0.50	6.20 ± 0.91	5.93 ± 0.92	1.33 ± 0.37	2.67 ± 0.51	0.00 ± 0.00
	6	14.59 ± 0.45	7.50 ± 0.66	12.27 ± 1.10	1.53 ± 0.29	2.33 ± 0.71	0.00 ± 0.00
F-Value [a]		207.170		P-Value [a]		0.001	
F-Value [b]		26.365		P-Value [b]		0.001	
F-Value [c]		7.940**		P-Value [c]		0.001	

[a] for the effect of bio-rational insecticides only , [b] for the effect of Time (week) only
[c] for the effect of the interaction between bio-rational insecticides and Time (week) together.

Comparison between the efficacies of the bio-rational insecticides among the two plantation periods against tomato leaf minor larvae for both tomato varieties were shown in Tables 4 & 5. For both Shifa and Severa, and in both plantation periods, there are significant differences in the number of larvae comparing plants treated with the bio-insecticides and the control plants. For example, at week 1 in both plantation periods, results for Shifa showed highly significant differences for all bio-rational insecticides compared with the control plants (F value

21.932** and 20.386** for plantation periods 1 and 2, respectively). In the first plantation period, the differences between the bio-insecticide treatments were not significant, but in the second plantation period the effect of Btk and azadirachtin were significantly different to the other bio-rational insecticides. Subsequently, for the results in weeks 2, 3, 4, 5 and 6 in both of the plantation periods, there were significant differences between the bio-insecticides treatments compared with the control, and in some cases also between the bio-insecticide treatments. In the second plantation period, treatments with spinosad and azadirachtin consistently resulted in significant lower numbers of *T. absoluta* larvae compared with the other bio-rational insecticides treatments during the 3rd to 6th week of applications (Table 4). For Savera variety, the analysis of the results showed similar findings as for Shifa variety (Table 5). Results of all weeks (1-6) in both of the plantation periods, indicates significant differences between the bio-insecticide treatments compared with the control, and in some cases also between the bio-insecticide treatments. In week 2, for both plantation periods, the differences between Btk, azadirachtin and spinosad were not significant, but their effects were all significantly greater than that of *B. bassiana* and *M. anisopliae* (significant difference for azadirachtin only in period 2). In weeks 3, 5 and 6 treatment with spinosad resulted in the lowest number of larvae, with significantly lower numbers compared with all other treatments in period 2 (Table 5). The two entomopathogenic fungi *B. bassiana* and *M. anisopliae*, had a significant effect compared with the control, but the effect was not sufficient to keep the numbers of larvae low, and the number of larvae increased gradually, especially during weeks 4, 5 and 6 in the second plantation period. This consecutive increase could be noticed on both Shifa and Savera varieties.

Regarding the percentage reduction in larval population of *T. absoluta* at MLD application, spinosad, Btk and azadirachtin resulted in the highly reduction percentage of the population followed by *M. anisopliae* and *B. bassiana.* These results were similar for both Shifa and Savera varieties throughout the two plantation periods (Figure 1). Spinosad is the most effective bio-rational insecticides during the two plantation periods and recorded the highest larval reduction percentage of *T. absoluta* for both tomato varieties. Comparing the percentage of reduction for both varieties during the first plantation period, *B. bassiana*, *M. anisopliae* and azadirachtin; shown significant differences between Savera and Shifa variety (T-values 6.222**, 3.751* and 7.276**, p=0.003, 0.20 and 0.002, df=4, respectively). On the other hand, Btk, azadirachtin and spinosad showed insignificant differences between the tomato varieties among the second plantation period compared with the other bio-rational insecticides even they recorded the highest larval reduction percentage of the target insect pest. Btk was higher than azadirachtin in larval reduction percentage during the second plantation period. However, *B. bassiana* and *M. anisopliae* discriminated significant differences between Shifa and Savera varieties compared with the other bio-rational insecticides with T-value 7.491** and 5.385**, p=0.002, and p=0.006, df=33 throughout the second plantation period.

Productivity assessment after the application of the bio-rational insecticides

For variety Shifa, significant differences was recorded between the all treated bio-rational insecticides and control plants among both plantation periods (F value 3.933* and 13.003**, p=0.024 and 0.000, df=5,12 for the 1st and 2nd plantation periods, respectively) as shown in Table 6. The differences in yields in Shifa plants treated with spinosad, Btk or azadirachtin were not significantly different, but they tended to be higher than the yields for those treated with the two entomopathogenic fungi *B. bassiana*, and *M. anisopliae.* Data illustrated in Table 6 showed significant differences between Btk and *B. bassiana* in the first plantation period and between spinosad and the two entomopathogenic fungi in the second plantation period. On the other hand, insignificant relationship between spinosad, Btk and azadirachtin to each other, despite the existence of differences in means were observed.

For variety Savera, there were no significant differences between the bio-insecticide treatments in the first planting period. In contrast, a highly significant differences between the bio-insecticide treatments in the second planting period was recorded (F value 69.524**, P=0.000, df=5,12). High significant differences between the tomato yields was recorded in plants treated with spinosad, Btk and azadirachtin compared with the two entomopathogenic fungi *B. bassiana*, and *M. anisopliae* during the second plantation period (Table 6).

Table 4: Comparison between effects of bio-rational insecticides during the two plantation periods on *T. absoluta* populations for Shifa tomato variety.

Week	week1		week2		week3		week4		week5		week6	
Season	1st plantation	2nd plantation	1st plantation	2nd plantation	1st plantation	2nd plantation	1st plantation	2nd plantation	1st plantation	2nd plantation	1st plantation	2nd plantation
Mean number of larva ± SE												
Control	3.60±0.27^{a}	4.60±0.56^{a}	4.35±0.29^{a}	6.82±0.85^{a}	4.30±0.26^{a}	7.53±0.96^{a}	3.95±0.35^{a}	9.80±1.10^{a}	3.85±0.39^{a}	11.27±1.25^{a}	3.85±0.39^{a}	16.80±0.86 ^{a}
B. bassiana	1.15±0.20^{b}	1.53±0.29^{b}	2.05±0.37^{b}	2.80±0.59^{b}	1.85±0.29^{b}	1.27±0.28^{bc}	1.95±0.39^{b}	5.27±0.90^{b}	2.20±0.34^{b}	5.33±0.54 ^{b}	2.20±0.35^{b}	8.40±0.87^{b}
M. anisopliae	1.25±0.20^{b}	1.53±0.24^{b}	2.00±0.35^{b}	1.20±0.44^{c}	1.60±0.29^{bc}	2.00±0.56^{b}	2.00±0.26^{b}	3.00±0.70^{c}	0.95±0.21^{c}	7.00±1.04 ^{b}	0.95±0.21^{c}	9.53±0.96^{b}
Btk	1.15±0.23^{b}	0.00±0.00^{c}	0.85±0.17^{cd}	1.20±0.44^{c}	0.70±0.18^{d}	2.00±0.56^{b}	0.95±0.26^{c}	3.00±0.70^{c}	0.85±0.24^{c}	7.00±1.04 ^{b}	0.85±0.24^{c}	9.53±0.96^{b}
Azadirachtin	1.25±0.23^{b}	0.93±0.33^{c}	1.60±0.28^{bc}	0.33±0.13^{c}	0.95±0.25^{cd}	0.20±0.11^{c}	1.25±0.32^{bc}	1.07±0.37^{cd}	1.05±0.25^{c}	2.00±0.58 ^{c}	1,05±0.25^{c}	1.73±0.37^{c}
Spinosad	0.85±0.17^{b}	1.20±0.39^{b}	0.70±0.13^{d}	0.40±0.19^{c}	0.90±0.23^{cd}	0.00±0.00^{c}	1.10±0.32^{bc}	0.07±0.07^{d}	0.80±0.34^{c}	0.00±0.00 ^{c}	0.80±0.24^{c}	0.00±0.00^{c}
F-value	21.932**	20.386**	22.314**	24.049**	27.993**	28.081**	12.113**	23.377**	17.881**	22.169**	17.881**	63.622**

Note: Means in a column followed with the same small letter(s) are not significantly different at 5% level of probability.

Table 5: Comparison between effects of bio-rational insecticides during the two plantation periods on *T. absoluta* populations for Savera tomato variety.

Week	week1		week2		week3		week4		week5		week6	
Season	1st plantation	2nd plantation	1st plantation	2nd plantation	1st plantation	2nd plantation	1st plantation	2nd plantation	1st plantation	2nd plantation	1st plantation	2nd plantation
Mean number of larva ± SE												
Control	3.50±0.2^{a}	4.47±0.88^{a}	4.10±0.23^{a}	6.67±1.13^{a}	4.40±0.20^{a}	7.80±0.91^{a}	4.00±0.26^{a}	10.47±1.41^{a}	3.80±0.35^{a}	10.80±1.15^{a}	3.80±0.35^{a}	14.53±1.04^{a}
B. bassiana	1.25±0.27^{b}	1.47±0.34^{b}	1.65±0.24^{b}	3.67±0.49^{b}	1.40±0.29^{b}	6.33±0.74^{a}	1.85±0.36^{b}	4.87±1.06^{b}	2.10±0.27^{b}	6.20±0.91^{b}	2.10±0.27^{b}	7.33±0.69^{c}
M. anisopliae	1.35±0.23^{b}	1.73±0.30^{b}	1.90±0.32^{b}	2.80±0.68^{b}	1.50±0.36^{b}	1.73±0.45^{b}	1.90±0.33^{b}	4.93±0.78^{b}	0.95±0.30^{c}	5.93±0.92^{b}	0.95±0.30^{c}	12.27±1.10^{b}
Btk	1.00±0.24^{b}	0.00±0.00^{c}	0.90±0.16^{c}	0.40±0.19^{c}	0.60±0.18^{c}	0.33±0.13^{cd}	0.80±0.22^{c}	1.27±0.28^{c}	0.75±0.26^{c}	1.33±0.37^{cd}	0.75±0.26^{c}	1.53±0.29^{de}
Azadirachtin	1.30±0.23^{b}	1.20±0.35^{bc}	1.25±0.22^{bc}	0.93±0.25^{c}	1.05±0.22^{bc}	2.13±0.40^{b}	1.55±0.29^{bc}	1.80±0.38^{c}	0.75±0.22^{c}	2.67±0.51^{c}	0.75±0.22^{c}	2.33±0.71^{d}
Spinosad	0.80±0.20^{b}	1.07±0.32^{bc}	0.65±0.17^{c}	0.53±0.19^{c}	0.85±0.24^{bc}	0.13±0.09^{d}	1.05±0.25^{bc}	0.40±0.19^{c}	0.55±0.20^{c}	0.00±0.00^{d}	0.55±0.20^{c}	0.00±0.00^{e}
F-value	16.950**	11.162**	29.340**	16.718**	29.458**	35.523**	15.380**	20.626**	21.604**	27.610**	21.604**	65.448**

Note: Means in a column followed with the same small letter(s) are not significantly different at 5% level of probability.

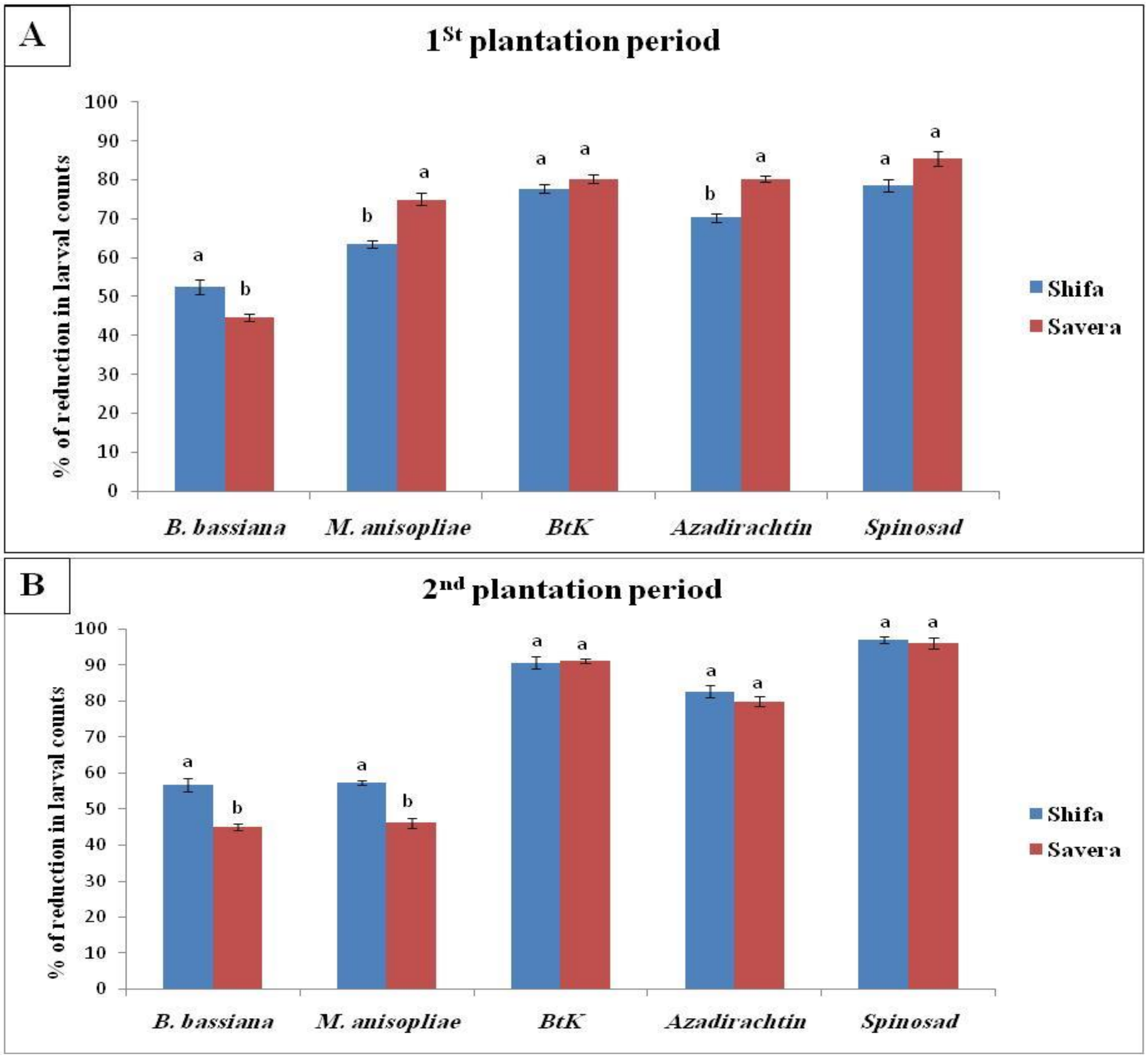

Figure 1: Percent reduction in larval population of tomato varieties at median lethal dose during the two plantation periods.

Comparing Shifa yields between the two plantation periods, the differences in the yield of the control and Btk treatments were not significant, but for other of the bio-insecticide treatments the yields were significantly different. The entomopathogenic fungi; *M. anisopliae*; and spinosad discriminated high significant differences between the two plantation periods compared with the other bio-rational insecticides with T-value 6.048** and 5.262**, p=0.004, and p=0.006, df=4. Concerning Savera variety, either control plants or *M. anisopliae* treatments showed any significant difference between the plantation periods. Meanwhile, significant differences in yields were observed for other bio-insecticides. Comparing the two plantation periods for Savera variety; spinosad and Btk were highly significant in tomato yields (T-value 24.678** and 7.605**, p=0.000, and p=0.002, df=4).

DISCUSSION

The main aim of this study was not to prioritize one bio-rational insecticide over others, but to draw the attention to the range of bio-rational insecticides with different modes of action that are available for controlling *T. absoluta.* From the results of this study, spinosad, Btk and azadirachtin were categorized as the most effective bio-rational insecticide; whereas the two entomopathogenic fungi, *B. bassiana* and *M. anisopliae* were categorized as moderately effective. These results are in agreement

Table 6: Yields (kg m^{-2}) for Shifa and Savera F1 tomatoes treated with bio-rational insecticides during 1st and 2nd plantation period.

Tomato variety	Shifa			Savera		
Plantation period	1st Plantation period	2nd Plantation period	T-value	1st Plantation period	2nd Plantation period	T-value
	Mean ± SE					
Control	3.39 cA±0.19	3.90 cA±0.47	0.998 NS	3.66 bA±0.40	4.80 dA±0.47	1.837 NS
B. bassiana	5.34 bcB±0.26	9.93 bA±1.14	3.921*	4.41 bB±0.16	5.34 dA±0.26	3.055*
M. anisopliae	4.50abcB±0.73	9.60 bA±0.42	6.048**	3.75 abA±0.23	5.31 dA±0.51	2.772 NS
Btk	7.50 aA±0.79	12.27abA±1.60	2.915 NS	5.28 aB±0.62	13.02bA±0.81	7.605**
Azadirachtin	7.50 aB±0.64	11.2849abA±0.78	4.391*	3.66 bB±0.27	10.77 cA±0.68	9.757**
Spinosad	6.21 abB±1.26	13.02 aA±0.29	5.262**	4.26 abB±0.42	14.73 aA±0.08	24.678**
F-value	3.933*	13.003**		2.747 NS	69.524**	

Note: Means in a column followed with the same small letter(s) are not significantly different at 5% level of probability. Means in a row followed with the same capital letter(s) are not significantly different at 5% level of probability.

with those from previous studies of Moussa et al. (2013) where the efficacy of 17 different insecticides against *T. absoluta* were observed and they recorded spinosad, emamectinbenzoate, azadirachtin and *B. thuringiensis* were the most effective among the tested insecticides.

The results from the present study indicating high efficacy of spinosad against *T. absoluta* in both the two plantation periods, that also in agreement with those obtained by Williams et al. (2003), Terzidis et al. (2014), and Abd El-Ghany et al. (2016b) who mentioned that spinosad has a strong efficacy against *T. absoluta*. Also, Dağlı et al. (2012) investigated the effectiveness of different insecticides against *T. absoluta* and spinosad showed a hundred percent reduction effect on the target insect pest. However, Hafsi et al. (2012) stated that spinosad had 70% impact on the second- instar larvae of *T. absoluta.*

The Btk efficacy on *T. absoluta* larvae results were in accordance with the results obtained by Giustolin et al. (2001) and Mollá et al. (2014) who presented that *T. absoluta* were found to be susceptible to Btk in a different extent. González-Cabrera et al. (2011) stated that sprayings of Bt only had a great impact on all larval stages of *T. absoluta* except 4th instar larvae, with no need for chemical insecticides. The same results were recorded by Ladurner et al. (2011) for Btk when used against *T. absoluta* and they stated that suspension concentrate was more effective than the wettable powder.

The efficacy data of azadirachtin against *T. absoluta* in the present study were agree with Cunha et al. (2006) who indicated that azadirachtin has antifeedant and repellent effects against *T. absoluta*. Also, Brunherotto et al. (2010) and Tomé et al. (2012) reported the same efficacy of azadirachtin against *T. absoluta* due to the delayed development of larvae and increased the larval mortality which leading to reduce the number of larvae before reaching the pupa stage.

Regarding to the efficacy of the two entomopathogenic fungi *M. anisopliae* and *B. bassiana* current results agreed with those obtained by Rodríguez et al. (2006) and Pires et al. (2010) who stated that *M. anisopliae* and *B. bassiana* are pathogenic on *T. absoluta* at all the developmental stages. The fluctuation that were observed in results of the present study between the two seasons could be explained as described by Batta (2003) who stated that the shelf life up to 30.8 month at 20°C can cause 50% loss of viability after 4.6 months. As well as many authors (Alves et al.,1996; Faria et al., 2010; Lopes et al., 2013) explained this fluctuation in efficacy where they mentioned that long term storage or even short term of storage under unfavorable conditions of high relative humidity, temperature and/ or O_2 (factors that boost the metabolic activity) have a negative impact on the speed of germination of *B. bassiana* and *M. anisopliae*.

The reduction percentage in *T. absoluta* infestation under all bio-rational insecticides applications varied between 44.66 – 85% for the first plantation periods. However, the percentage ranged from 45-97% for the second plantation period. These different levels of efficacies on the target insect pest were translated into an increase in total tomato production about 30- 50% when compared to the total harvested plants. This obvious increase pays an attention to the cost benefit of the different bio-rational insecticides.

CONCLUSION

The experimental trial for evaluation of different bio-rational insecticides found that spinosad, Btk, azadirachtin, *B. bassiana* and *M. anisopliae* have a varied effect on both tomato varieties under greenhouse conditions. spinosad, Btk and azadirachtin were recommended as the most effective bio-rational insecticides in comparison to moderate efficacy for the two entomopathogenic fungi; *B. bassiana* and *M. anisopliae* during two plantation periods for both Shifa and Savera F1 Hybrid tomato varieties.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

ASA and NMA designed the experiments. AM and NMA applied the bio-rational insecticides, investigated and performed the experiments. NMA wrote the manuscript and ASA, KD reviewed the manuscript. All authors read and approved the final version.

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