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Evaluations of Three *Bacillus thuringiensis* Against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Egypt

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Abstract: ***The role of three microbial control agents Bacillus thuringiensis Diple (2X); B.t kurstaki HD-73 and B.t kurstaki HD-234 were tested against tomato pinworm Tuta absoluta (Meyrick) (Lepidoptera: Gelechiidae), which infect plants under laboratory, green house and field conditions. The results showed that under laboratory conditions, the LC_{50} recorded were 140, 109 and 90 Ug/ml Ug/ml when T. absoluta treated with different concentrations of B.t. B.t kurataki diple (2X), B.t HD-234 , and B.t HD-73 , respectively. Under green house effect LC_{50} recorded 166, 122 and 102 Ug/ml for Bacillus thuringiensis Diple (2X), B.t kurstaki HD-73,, and B.t kurstaki HD-234 ; respectively. In the field, results show that, after 50 day of the application the means number o infestations were scored significantly decreased to 11.17±12, 7.7±10.8, 18.2±11.2, individuals for the corresponding B.t as compared to 26.5±11.34 individuals in the control After 90 days of the post applications of the bacterial varieties, the means number of infestations significantly decreased to 112.2±11.9, 10.6±8.8, 15.2±10.7 as compared to30.6 ±10.8 individuals in the control . At the end of the experiment t120 days the corresponding mean number of the infestation 14.5±11.5, 1 1.4±13.5, 17.8±9.9 as compared to35.4±12.3 individuals. The weight of the tomatoes after the harvest scored the highly significance weight reached to 4916± 42.50, 4131± 34.33, 3123± 41.28, Kg/ feddan in the area treated with Bacillus thuringiensis Diple (2X), B.t kurstaki HD-73, and B.t kurstaki HD-234, respectively as compares to 2631± 36.80Kg/fesddan in the control in EL-Esraa farm (Nobaryia) during season 2013. In El-Kassaseen (Ismailia) during season 2013 the corresponding treatmedts areas scored a highly yied recored to, 3718± 40.30, 3718± 40.30and 5879±69.33 Kg/ feddan as compared to 1881±80.54Kg/ feddan.***

Keywords: Tuta absoluta; Bacillus thuringiensis Diple (2X); B.t kurstaki HD-73, B.t kurstaki HD-234

1. Introduction

Tomato, Lycopersicon esculentum Mill is a vegetable crop of large importance throughout the world. Its annual production accounts for 107 million metric tons with fresh market tomato representing 72 % of the total (FAO, 2002). It is the first horticultural crop in Tunisia with a production area of 25,000 hectares and a total harvest of 1.1 million metric tons (DGPA, 2009) of which nearly 70 % are processed (Tomatonews, 2011). Tomatoes are grown both under plastic covered greenhouses and in open field. The tomato leafminer, Tuta absoluta Meyrick, (Lepidoptera : Gelechiidae) is a serious pest of both outdoor and greenhouse tomatoes. The insect deposits eggs usually on the underside of leaves, stems and to a lesser extent on fruits. After hatching, young larvae penetrate into tomato fruits , leaves on which they feed and develop creating mines and galleries. On leaves, larvae feed only on mesophyll leaving the epidermis intact (OEPP, 2005). Tomato plants can be attacked at any developmental stage, from seedlings to mature stage.

The tomato plants are currently infested with many serious pests, recently the most distructive ones, *Tuta absoluta*. It is one of the most important pests of tomato in Egypt which is posing a serious threat to tomato production. This pest is crossing borders rapidly and devastating tomato production substantially. Caterpillars prefer leaves and stems, but may also occur underneath the crown of the fruit and even inside the fruit itself. The caterpillars attack only green fruit. Most distinctive symptoms are the blotch-shaped mines in the leaves. Inside these mines both the caterpillars. In case of serious infection, leaves die off completely. Mining damage to the plant causes its malformation. Damage to fruit allows e.g. fungal diseases to enter, leading to rotting fruit before or after harvest (Cristina et al., 2008); EPPO. (2008. a &b). Tomato grown in green house and open field. Severely attacked tomato fruits lose their commercial value. 50–100% losses have been reported on tomato (EPPO, 2005). On potato, CIP (1996) considers that is one of the major pests of foliage, occurring in warm zones of low altitudes as larvae are internal feeders it is difficult to achieve an effective control through application of chemical insecticides.

(Medeiros, et al., 2006) reported that *Bacillus thuringiensis* var. *kurstaki* have exhibited satisfactory efficacy against *T. absoluta* larval infestations in Spanish outbreaks. It is reported that in a combine application of mass release of *Trichogramma pertiosum* and *B. thuringiensis* resulted fruit damage only 2 % in South America. (Goncalves-Gervasioand Vendramin, 2007) recorded that, the entomopathogenic fungus *M. anisopliae* could be caused female's mortality up to37.14% and laboratory studies indicated *B. bassiana* could cause 68% larval mortality Sabbour 2007.

The Horticultural crops is considered one of the most agricultural products vulnerable to damage during its behavior the marketing due to the nature and extent of affected by various natural factors**,** Making the problem of losses in those crops most important economic problems that harm the national economy of Egypt.

The aim of this work to evaluate of three of *B. thuringiensis* against *T. absoluta* under laboratory, green house and field effect.

2. Material and Methods

2.1 Rearing insect pests

The tomato pinworm were reared on tomato leaves under laboratory conditions 22±2C^{o} and RH 60-70% .*T. absoluta* used in the trials were obtained from laboratory cultures.

2.2 Effect *Bacillus thuringiensis Diple (2X); B.t kurstaki HD-73, B.t kurstaki HD-234* on *T. absoluta*

Samples of the target insects *T. absoluta,* infesting tomato crop were collected from. The bacterium three isolates (*B. t*) was prepared in 6 concentrations (500, 250, 125, 63, 32 and 16 Ug/ml. The tomato leaves were dipped in the last suspensions, left to dry then put in plastic cups. Twenty individuals of the third larvae of *T. absoluta* were put on them, covered with muslin. Control (untreated) was made by feeding the larvae on untreated leaves (sprayed by water only). The experiments were repeated 4 times.

The percentages of mortality were calculated and corrected according to Abbott, 1925, while LC_{50} was calculated through probit analysis, (Finney, 1964). The experiments were carried out under laboratory conditions 22 ± 2^{o} C and 60-70% R.H.

2.3 Semi-field (green house) trials

Tomato plant Variety Bio-Bride was planted in the green house in 40 plots in each artificial infestation was made by spraying the plant with the bacterial *B. thuringiensis* Dipel 2X var *Kurstak* (23000IU), *B. thuringiensis kursaki* HD-73 ,*B. thuringiensis kursaki* HD-234 at the concentrations of 500 ug/ml of the tree *B. thuringiensis* .

Control samples were sprayed by water only. The plants were examined every two days; the percentage of infestation was calculated until the end of the experiment. Each treatment was replicated 4 times. The percent mortality was counted and corrected according to Abbott, 1925; while LC50s were calculated through probit analysis after Finney 1964.

2.4 Field trials

The experiments were carried out to study the effectivenessof the tested *B. thuringiensis* Dipel 2X var *Kurstaki* (23000IU), *B. thuringiensis kursaki* HD-73 , *B. thuringiensis kursaki* HD-234 against the target insectpests in two different areas. Each area has different climatic and soil factors. These two areas were: El-Esraa (El-Nobaryia region) with dry weather and sandy soil, and El--Kassaseen (Ismailia) with wet weather and clay soil.Tomato plants (var. Bio-Bride) were planted on the first of April in an area of about 1,200 m2, and divided into12 plots of 100 m2 each. Four plots were assigned for eachpathogen, while 4 plots were treated with water and usedas the controls. Each bacterial strain were applied at the concentrations of 300Ug/mland 5 l/plot. Treatmentswere performed in a randomized plot design at sunset. A five-litre sprayer was used to spray on the treatments.Three applications were made at one week intervals, atthe commencement of the experiment. Twenty plantsamples were randomly collected at certain time intervalsfrom each plot and transferred to the laboratory for examination. The average number of each of the tested pests/sample/plot/treatment was calculated 20, 50, 90 and 120days after the 1st application. The infestations of target insect pests were then estimated in each case.

After harvest, the yield of each treatment was weighed as kgs/feddan. Yield loss was calculated according to the following equation:

$$\text{Yield loss} = \frac{\text{Potential yield- actual yield}}{\text{Potential yield}} \times 100$$

Potential yield was that of *B.t* var *Krstaki* HD-73, which gave the best results among the tested pathogens, and was takenas a base for comparison with the other treatments.

3. Results and Discussion

Data in table 1 show that LC_{50} recorded were 140, 109 and 90 Ug/ml when *T. absoluta* treated with different concentrations of *B.t kurataki* diple (2X), *B.t HD-234* and *B.t HD-73* , respectively (Table 1). The same results obtained by Sabbour 2009, who controlled the tomato pests by the microbial control agents. Hassan et al, 2012 decrease the infestations of *Pthorimaea operculella* by *B.t.* The same results obtained by Medeiros, et al., 2006 Cabello et al., 2009 who controlled the pinworm by bioinseticides. Huang et al. 2004) reported that commercial formulates based on this bacterium have been used for decades to control insect pests as an alternative to chemicals.Most of the studies that focused on the effect of *B. t* on *T. absoluta* have been performed in the region of origin of *T. absoluta* (Giustolin et al. 2001; Theoduloz et al. 2003; Niedmann and Meza-Basso 2006 and Giustolin2002). Giustolin et al. (2001) found that *B. t* var. *kurstaki* (Btk) can cause mortality in all *T. absoluta* instars and that the use of *Bt* has synergistic or additive effects when applied to tomato resistant genotypes. Furthermore, Niedmann and Meza-Basso (2006) performed bioassay screens of native *B. thuringiensis* varieties from Chile and found that two of them were even more toxic for *T. absoluta* than the strain isolated from the formulate Dipel (Abbott Laboratories, Chicago, IL, USA). Moreover, Theoduloz et al. (2003) expressed a *B. thuringiensis* toxin in other *Bacillus* species that naturally colonize the phylloplane of tomato plants, showing that these plant-associated microorganisms could be useful as adelivery system of toxins from *B. thuringiensis*, which would allow a reduction in pesticide applications.

Under green house effect LC_{50} recorded 166, 122 and 102 Ug/ml for *Bacillus thuringiensis Diple (2X), B.t kurstaki HD-73,*, and *B.t kurstaki HD-234* ; respectively Table2. Sabbour, 2003 controlled the stored products by B.t HD 234 and decrease the infestations under laboratory and green house conditions. Sabbour and Sahab, 2005, 2007, 2011, Sahab and Sabbour, 2011 and Sabbour et al 2011 controlled the corn borers under semifield conditions by bioagent substance. Medeiros, et al., 2006; reported that *B.t* gave a good results against *T. absoluta*. (Goncalves-Gervasio and Vendramin, 2007) recorded that, the entomopathogenic fungus *M. anisopliae* could be caused female's mortality up to37.14% and laboratory studies indicated *B. bassiana* could cause 68% larval mortality. The same findings obtained by Sabbour et al 2013, Sabbour, 2012, Sabbour and Abd El Raheem 2013.

Data in table 3 show when the effect of the three bacterial varieties *Bacillus thuringiensis Diple (2X), B.t kurstaki HD-73,* and *B.t kurstaki HD-234* .

In the field, results show that, after 50 day of the application the means number o infestations were scored significantly decreased to 11.17±12, 7.7±10.8, 18.2±11.2, individuals for the corresponding B.t as compared to 26.5±11.34 individuals in the control (Table 3).

After 90 days of the post applications of the bacterial varieties, the means number of infestations significantly decreased to 112.2±11.9 , 10.6±8.8, 15.2±10.7 as compared to30.6 ±10.8 individuals in the control . At the end of the experiment t120 days the corresponding mean number of the infestation 14.5±11.5, 1 1.4±13.5, 17.8±9.9 as compared to35.4±12.3 individuals (Table 3). The same results obtained by Sabbour 2009, Sabbour and abdelazizi2010. Sabbour and Abd El hakim2012 and Sabbour and AbdoU 2012.

Bacillus thuringiensis resulted fruit damage only 2 % in South America (Medeiros, et al., 2006). Entomopathogenic fungus *M. anisopliae* could be caused female's mortality up to 37.14%. Laboratory studies indicated *B. bassiana* could cause 68% larval mortality (Cabello et al., 2009). have shown an important reduction in the number of eggs of *T. absoluta*, between 92 and 96 %, when releasing 8 or 12 first stage nymphs of *Nabis pseudoferus* per plant (Cabello et al., 2009).Goncalves-Gervasio and Vendramin, 2007 and Cristina et al; 2008) recorded that, the entomopathogenic fungus *M. anisopliae* could be caused female's mortality up to37.14% and laboratory studies indicated *B. bassiana* could cause 68% larval mortality. The same results obtained by Sabbour 2003,(20014a&b), 2013.Magda Mahmoud Sabbour and Shadia El-Sayed Abd-El-Aziz. 2014, Magda Sabbour, 2001, Sabbour (2002 a &b), Magda Sabbour and Ismail2002, Sabbour and Sahab 2005 &2007, 20011.

Data in table 4 show that the weight of the tomatoes after the harvest scored the highly significance weight reached to 4916± 42.50, 4131± 34.33, 3123± 41.28, Kg/ feddan in the area treated with *Bacillus thuringiensis Diple (2X), B.t kurstaki HD-73, and B.t kurstaki HD-234*, respectively as compares to 2631± 36.80Kg/fesddan in the control in EL-Esraa farm (Nobaryia) during season 2013. El-Kassaseen (Ismailia) during season 2013, the corresponding treatmedts areas scored a highly yied recored to, 3718± 40.30, 3718± 40.30and 5879±69.33 Kg/ feddan as compared to 1881±80.54Kg/ feddan. The percentage of yield loss ranges between 15-46 in El-Esraa (Nobaryia) during season 2013 . The percentage of yield loss ranges between 15-64 in El-Kassaseen (Ismailia) during season 2013.

The same results obtained by Cabello et al., 2009; EPO, 2008, EPO 2009,a&b; Goncalves-Gervasio and Vendramin, 2007 and Cristina et al; 2008 Kennedy, G.G. 2003 Leite1999; Miranda2005; Angela 2008 and Medeiros, et al., 2006), Sabbour 2006, Sabbour and Abd el Aziz 2007, Sabbour, 2007, Sabbour and Abbas, 2007. Sabbour and Hany, 2007, Sabbour,2008. Asmaa et al 2009.

Tabe 5 show that in El-Esraa (Nobaryia) during season 2014 the weight of the tomatoes crop scored5997± 42.50, 5197± 35.35, and 3799± 39.41 Kg/ feddan after treated the tomatoes areas by *B.t kurstaki HD-73,, B.t kurstaki HD-234*, and *Bacillus thuringiensis Diple (2X)* as compared to 2062± 37.61 Kg/ feddan in the control the yield loss decreased to 13, and 36 as compared to 65 in the control. In El-Kassaseen (Ismailia) during season 2014 the corresponding areas treatments the yield weight robtained6290±60.41, 5701±64.21, 3998± 42.53, Kg/ feddan as compared to 2005±60.40 kg/ feddan in the control. The yield loss recorded were 9 and 36% as compared to 68% in the control (Table 5). Loss of the yield calculated by Sabbour & Shadia Abd El-Aziz, (2002 and 2010), Shadia Abdel Aziz & Nofel (1998), proved that applications with bioinsecticides increased the yield and decreased the infestations. Abdel-Rahman & Abdel-Mallek (2001), Abdel-Rahman (2001) and Abdel-Rahman & Abdel-Mallek (2001), controlled cereal aphids with entomopathogenic fungi. They found that the infestation was reduced after fungi applications under laboratory and field conditions. Sabbour & Sahab (2005, 2007 and 2011) found that the fungi reduced insect infestations of cabbage and tomato pests under laboratory and field conditions. These results agree with Sabbour & Shadia Abd El-Aziz, (2002 and 2010), proved that applications with bioinsecticides increased the yield and decreased the the infestation with insect pests.

3.1 The Economic feasibility of the use of bacteria on the tomato crop

Table (6) shows that the use of different types of bacteria and of B. t HD-1, B. t HD- 243, B. t HD-73 in each of the regions of the study resulted in increased productivity per acre by about 47% , 98% , 159% Nobaryia in area, as well as increased productivity per feddan by about 98% , 175% , 181% in Ismailia area, compared to the control for each of the types of bacteria, respectively. It also resulted in the use of different types of bacteria low percentage of lost harvest by 20% .32% and that for the type of bacteria B. t HD-1, while the decline amounted to around 42% .56% and that for the type of bacteria B. t HD- 243 in all from Nobaryia and Ismailia regions, respectively. As can be seen from the table that in the case of the universal use of different types of bacteria in the tomato worm resistance at the level of the republic will be the consequent increase in the production of the Republic by about 85% , 133% , 169% and that for each of the B. t HD-1, B. t HD- 243, HD-73 Bt, respectively, and also reduced the percentage of lost harvest to harvest tomatoes all over the country about 26% .49% when using each of the bacteria B. t HD-1, B. t HD- 243, respectively.

3.2 The Economic losses for the use of bacteria in the cultivation of tomato crop in the regions of the study and at the level of the Republic

To estimate the amount and value of the losses at the level of the regions of the study and dissemination of the republic requires that the experience of the use of bacteria in the reduction of the risk of tomato worm on the level of the governorates of Ismailia and Nobaryia then at the level of the Republic. Can be seen from Table (7) That the use of the type of bacteria B. t HD-1 resulted in a decrease in the amount of crop losses estimated at 7.36 tons, 15.02 thousand tons, 185.4 thousand tons, while the use of the type of bacteria B. t HD-2 consequent decline in the amount of losses estimated at 3.35 tons, 12.56 tons 0.351 thousand tons, Which will result in the devaluation losses estimated at 11.04 thousand pounds, 22.52 thousand pounds, 278.1 thousand pounds. About 5.03, 18.84,and 526.5 thousand pounds in each of Ismailia and Nobaryia Republic respectively.

4. Acknowledgment

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Table 1: Effect of microbial control agents against *Tuta absoluta under* laboratory conditions

Microbial control agents	Lc50 Ug/ml	slope	variance	confidence limits
Bacillus thuringiensis Diple (2X) B.t	140	0.01	1.59	246-200
HD-234	109.	0.1 0	1.44	139-100
B.t HD-73	90.	0.02	1.60	80-121

Table 2: Effect of microbial control agents against *Tuta absoluta* under green house effects

Microbial control agents	Lc50 Ug/ml	slope	variance	confidence limits
*B.t kurataki*1 diple (2x)	166	0.02	1.3	200-137
B.t HD_234	122	0.07	1.5	130-88
B.t HD-73	102	0.01	1.59	82-119

Table 3: Effect of the Bactria *B, t* three varieties under field conditions against *T. absoluta*

Treatments / Days	Mean number of infestation ±SE		
	50	90	120
B.t HD_234	11.17±12.	112.2±11.9	14.5±11.5
B.t HD-73	7.7±10.8	10.6±8.8	1 1.4±13.5
*B.t kurataki*1 diple (2x)	18.2±11.2	15.2±10.7	17.8±9.9
Control	26.5±11.34	30.6 ±10.8	35.4±12.3
F value	23.1		
Lsd5%=	10.1		

Table 4: Weight of harvested tomato and percentage of yield loss after treatment with the bacteria *B.t* , three varieties against the target insect pests during seasons 2013 in two different regions.

Treatments	El-Esraa (Nobaryia)		El-Kassaseen (Ismailia)	
	Weight tomatoes (Kg/feddan)	% yield loss	Weight tomatoes (Kg/feddan)	% yield loss
Control	2631± 36.80	46	1881±80.54	68
B. t HD-1diple (2x)	3123± 41.28	36	3718± 40.30	36
B. t HD- 243	4131± 34.33	15	4997±65.32	15
B.t HD-73	4916± 42.50	-	5879±69.33	-
F values	31.20		32.10	
LSD 5%	91		90	

Table 5: Weight of harvested tomato and percentage of yield loss after treatment with the bacteria *B.t* , three varieties against the target insect pests during 2014 in two different regions .

Treatments	El-Esraa (Nobaryia)		El-Kassaseen (Ismailia)	
	Weight tomatoes (Kg/feddan)	% yield loss	Weight tomatoes (Kg/feddan)	% yield loss
Control	2062± 37.61	65	2005±60.40	68
B. t HD-1	3799± 39.41	36	3998± 42.53	36
B. t HD- 243	5197± 35.35	13	5701±64.21	9
B.t HD-73	5997± 42.50	-	6290±60.41	-
F values	31.02		30.10	
LSD 5%	88		86	

Table 6: Weight of harvested tomato and percentage of yield loss after treatment with the bacteria *B.t* , three varities against the target insect pests during The average period (2013- 2014) in two different regions .

Treatments	El-Esraa (Nobaryia)		El-Kassaseen (Ismailia)		Average regions	
	Weight tomatoes (Kg/feddan)	% yield loss	Weight tomatoes (Kg/feddan)	% yield loss	Weight tomatoes (Kg/feddan)	% yield loss
Control	2347	55.5	1943	68	2145	61.75
B. t HD-1	3461	36.0	3858	36	3960	36
B. t HD- 243	4664	14.0	5349	12	5007	13
B.t HD-73	6085	-	5457	-	5771	-

Source :- Calculated and collected from two tables (4, 5) .

Table 7: estimate the amount and value of the losses before and after the use of bacteria in the regions of the study and at the level of the Republic Area thousand feddans, Productivity tons / feddans, Production thousand tons, Value thousand pound

Type of bacteria	Region	Area	Productivity	Total output	losses before using bacteria		losses after the use of bacteria		Magnitude of the decline	
					quantity	*Value*	quantity	Value	quantity	Value
B. t HD-1	Ismailia	1	23	23	15.64	23.46	8.28	12.42	7.36	11.04
	Nobaryia	7	11	77	42.74	64.10	27.72	41.58	15.02	22.52
	Republic	48	15	720	444.6	666.9	259.2	388.8	185.4	278.1
B. t HD-2	Ismailia	1	23	23	6.11	9.17	2.76	4.14	3.35	5.03
	Nobaryia	7	11	77	15.64	23.46	3.08	4.62	12.56	18.84
	Republic	48	15	720	444.6	666.9	93.60	140.40	351.00	526.5

Source: - calculated and collected from the table (5) and the annual publication of the agricultural economy in years 2012.2013.

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