Comparing the Effect of Seven Isolated Bacillus Thuringiensis against *Tuta absoluta* Infesting in Laboratory and Field Condition

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Abstract: ***Under laboratory , the effect of the tested bacteria conditions Bacillus thuringiensis strains on the target insect pests T. absoluta showed that, the LC50 obtained, 139, 120, 60, 55, 154 and 150 Ug/ml after treated with B.T IP Dendrolimus ,B.t thuringiensis, Bt Sotto 4A/4B, BT IP thurizide ,Bt Toloworthi Bt ,HD 210 and Bt HD 128, respectively. Under semifield conditions, the LC50 of T. absoluta 159, 140, 70 65, 73 184 and 170 Ug/ml after treated with corresponding pathgens , respectively . under field conditions, the mean number of T. absoluta after treated with, Bt HD 210 and Bt HD 210 treatments which recorded, 44.5±10.6 and 45 2.8±13.5 individuals as compared to 99.8±15.5 individual in the control after 125 days of the first applications. Also, the lowest number of infestations of T. absoluta recorded 10.4±10.9 individuals after Bt Toloworthi treatments.***

Keywords: *Tuta absoluta*, Bacteria. *Bacillus thuringiensis, B.T* IP *Dendrolimus ,B.t thuringiensis, Bt Sotto* 4A/4B*, BT IP thurizide ,Bt Toloworthi Bt* ,HD 210 and *Bt* HD 128.

1. Introductions

Tomato (*Lycopersicone sculentum* Mill.) is one of the most important Solanaceous vegetable crops. The tomato plants are currently infested with many serious pests, recently the most destructive ones, Tuta absoluta. Itis 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 , (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,2009 a &b). Sabbour 2014, used the Biocontrol agent for controlling the Tomato Pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Egypt. Sabbour, 2009 control the tomato insect pests by using bacillus thuringiensis and the entomopathogenic fungi. The aim of this work to evaluate of seven isolated bacterial strains of *Bacillus thuringiensis* against *T. absoluta* under laboratory, greenhouse effect and field. Sabbour and Nayera Solieman 2014,controlled *T. absoluta* by the fungi. Sabbour and Singer 2014 found that the number of *T. absoluta* significantly decreased after fungi treatments,

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. The experiments were repeated 4 times. The percentages of mortality were calculated and corrected according to Abbott, 1925, while LC50 was calculated through probit analysis, (Finney, 1964). The experiments were carried out under laboratory conditions 22 ± 2o C and 60-70% R.H. 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 determined after seven days. The percentages of mortality were counted and calculated according to Abbott, (1925), while Lc50were calculated through probit analysis Finney, (1964). The experiments were carried under laboratory conditions; 22 ± 2o C and 60± 5 % RH.

2.2. Microorganisms

Bacillus thuringiensis B.T Tenebrionis, B.t thuringiensis, Bt Sotto, 4A/4B *BT IP thurizide, Bt Toloworthi ,Bt* HD 210 and *Bt* HD 128, were used in this study. The bacterial cultures were maintained on nutrient agar slants at 4^{o}C.

2.3. Bacterial Culture Media

The conventional laboratory culture broth, Nutrient broth , was used for culture preparation by mixing 5g peptone and 3g beef extract/ 1 L distilled water. 50 ml of sterile medium was inoculated with one loopful of bacterial strain and incubated under shaking growth conditions on an orbital rotary shaker (125rpm) at 30°C for 72h.

2.4. Effect of the Microbial Control Agents

Isolated *Bacillus thuringiensis (Bt) B.T Tenebrionis , B.t thuringiensis, Bt Sotto,* 4A/4B *,BT IP thurizide, Bt Toloworthi, Bt* HD 210 and *Bt* HD 128*;* were used to test their activities on stored insect pests *T. absoluta* adult beetles. The dead larvae of *B. incarnatus* were collected from the colony.. The Bt strains tested and prepared at concentrations (500, 250, 125, 63, 32 and 16 ug/ml) (w/v). The tomato leaves were sprayed by tested concentrations of Bt and left to dry under laboratory conditions. Control treatment was made by feeding the larvae on untreated leaves. The percentages of mortality were counted and calculated according to 50 Abbott ,1925, while LC50 were calculated through probit analysis according to Finney, 1964. The experiments were carried under laboratory conditions; 26 ± 2o C and 60- 70% R.H.

2.5. 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 bioinsecticides of bacterial strains ; at the concentrations of (500, 250, 125, 63, 32 and 16 ug/ml) (w/v) for each. 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 effectiveness of the tested *Bacillus thuringiensis,* seven strains , *B.T* IP *Dendrolimus ,B.t thuringiensis, Bt Sotto* 4A/4B, *BT IP thurizide ,Bt Toloworthi Bt ,*HD 210 and *Bt* HD 128, against the target insect pests in two different areas. These two areas were: El-Sharkia and **EL-Dakahlia.** Tomato planted Variety Bio-Bride planted on the first of April in an area of about 1600 m^2, and divided into 16 plots of 50 m2 each. Four plots were assigned for each pathogen, while 4 plots were treated with water and used as the controls. Each bacterial strain were applied at the concentrations of 300Ug/ml. Treatments were 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, at the commencement of the experiment. Twenty plant samples were randomly collected at certain time intervals from 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 120 days 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.

3. Results and Discussions

Results show that, under laboratory , the effect of the tested bacteria conditions *Bacillus thuringiensis* strains on the target insect pests *T. absoluta* showed that, the LC50 obtained, 139, 120, 60, 55, 154 and 150 Ug/ml after treated with *B.T* IP *Dendrolimus ,B.t thuringiensis, Bt Sotto* 4A/4B, *BT IP thurizide ,Bt Toloworthi Bt ,*HD 210 and *Bt* HD 128, respectively (Tabe 1). Tabe 2, show that the LC50 of *T. absoluta* 159, 140, 70 65, 73 184 and 170 Ug/ml after treated with *B.T* IP *Dendrolimus ,B.t thuringiensis, Bt Sotto* 4A/4B, *BT IP thurizide ,Bt Toloworthi Bt ,*HD 210 and *Bt* HD 128, respectively . Table 3, show that the mean number of *T. absoluta* under field conditions which showed that the number recorded after *Bt* HD 210 and *Bt* HD 210 treatments which recorded, 44.5±10.6 and 45 2.8±13.5 individuals as compared to 99.8±15.5 individual in the control after 125 days of the first applications. Also , the lowest number of infestations of *T. absoluta* recorded 10.4±10.9 individuals after *Bt Toloworthi* treatments Table 4 show that the tomato yield significantly increased to 3199± 51.10, 3199± 50.00, 4999± 20.20, 5909± 58.91, 5590± 52.10 , 2599± 50.90, 2409± 20.91 kg/feddan in the plots treated with, *B.T* IP *Dendrolimus ,B.t thuringiensis, Bt Sotto* 4A/4B, *BT IP thurizide ,Bt Toloworthi Bt ,*HD 210 and *Bt* HD 128, respectively as compared to 2010± 10.12 kg/feddan in El Sharkia governorate . The corresponding weight of tomato in El- Dakahlia governorate 390**1**±89.30, 4094±71.58, 5131± 20.10, 5199± 54.90, 5599± 10.10, 2599± 56.80, 2459± 20.70 kg/feddan as compared to 180**1**±81.30 kg/feddan (Table4).

Figure 1 show the tomato infestation with *T. absoluta* in El-Sharkia governorate which recoded that the infestation were significantly decreased after bacterial treatments especially after *BT IP thurizide treatments.* Figure 2 in EL-Dakahlia governorate show that the infestations with *T. absoluta* significantly decreased in all bacteria treatments. 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 usedfor 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). Giustolin et al. (2001) found that *B. t var. kurstaki* 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* strains 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 a delivery system of toxins from B. thuringiensis, which would allow a reduction in pesticide applications. The same results obtained by Medeiros, et al., 2006 Cabello et al., 2009who 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). 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* strains 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 a delivery system of toxins from B. thuringiensis, which would allow a reduction in pesticide applications. 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. 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 Nabispseudoferus per plant (Cabello et al., 2009). The same results obtained by Sabbour, 2014, who mentioned, The results showed that under. The same results obtained by Sabbour 2009. Sabbour and Nayera solieman 2014, reported 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. Sabbour and singer 2014 found that, The LC50 of M. *anisopliae var. frigidum* 156X104 and 168 X104 spores/ml under laboratory and greenhouse effect, respectively. The corresponding LC50of M. flavoviride var. minus were 169 X104 and 172 X104spores/ml. The highest yield obtained in El- Esraa (Nobaryia) 3999± 49.41 and 4697± 49.33 Tons/kg in El-Kassaseen (Ismailia) after *M. anisopliae* var. *frigidum* treatments the yield loss ranged between 7 and 72 % in the two regions. The infestations with Tuta absoluta significantly decreased in plots treated with *M. anisopliae var. frigidum* as compared to the control plots. The same findings obtained by Sabbour,n2009 and 2014.

4. Acknowledgment

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Table 1: Effect of the entomopathogenic Bacteria against *T. absolutas* larvae under laboratory conditions

Pathogen B.t	*LC_{50} Ug/ml*	*Slope*	*Variance*	*95% confidence limits*
B.T Tenebrionis	139	0.1	1.01	99-170
B.t thuringiensis	120	0.2	1.00	89-142
Bt Sotto 4A/4B	60	0.1	1.03	30-99
BT IP thurizide	55	0.4	0.1	25-98
Bt Toloworthi	63	0.5	1.2	29-88
Bt HD 210	154	0.1	1.04	96-169
Bt HD 128	150	0.6	1.01	124-159

Table 2: Effect of the entomopathogenic Bacteria against *T. absoluta* larvae under semifield conditions

Pathogen B.t	LC_{50} Ug/ml	Slope	Variance	95% confidence limits
B.T Tenebrionis	159	0.1	1.01	99-180
B.t thuringiensis	140	0.1	1.00	99-172
Bt Sotto 4A/4B	70	0.1	1.3	50-99
BT IP thurizide	65	0.2	0.1	55-108
Bt Toloworthi	73	0.1	1.2	49-98
Bt HD 210	184	0.1	1.2	90-169
Bt HD 128	170	0.1	1.1	121-199

Table 3: The effect of the different bacterial treatments against *T. absoluta under field conditions*

Treatments	Mean number of infestation ±SE after			
	15 D	30 D	55 D	125 D
B.T Tenebrionis	19.15±12.7	25.7±12.3	27.1±11.5	32.4±11.1
B.t thuringiensis	18.5±10.6	18.5±17.5	22.5±15.6	28.5±14.4
Bt Sotto 4A/4B	1 2.8±10.5	1 2.8±10.5	9.7±11.8	1 2.8±10.5
BT IP thurizide	9.8±9.9	19.6±1.9	20.8±10.5	22.8±10.1
Bt Toloworthi	2.11±11.2	5.7±12.5	5.9±12.1	10.4±10.9
Bt HD 210	28.5±16.6	28.8±19.6	39.5±11.0	44.5±10.6
Bt HD 128	2 2.8±17.5	3 2.8±10.5	49.7±11.8	45 2.8±13.5
Control	29.8±9.2	49.8±9.9	67.8±14.5	99.8±15.5
F value	28.1			
Lsd5%=	12.1			

Table 4: Weight of harvested tomato fruits after bacterial treatment against target insect pests .

Treatment	Tomato Weight of yield in two governorates	
	El- Sharkia governorate	EL-Dakahlia governorate
	Kg/Feddan	Kg/Feddan
B.T Tenebrionis	3199± 51.10	3901±89.30
B.t thuringiensis	3199± 50.00	4094±71.58
Bt Sotto 4A/4B	4999± 20.20	5131± 20.10
BT IP thurizide	5909± 58.91	5199± 54.90
Bt Toloworthi	5590± 52.10	5599± 10.10
Bt HD 210	2599± 50.90	2599± 56.80
Bt HD 128	2409± 20.91	2459± 20.70
Control	2010± 10.12	1801±81.30
F-value	31.10	35.1
LSD 5%	80	81

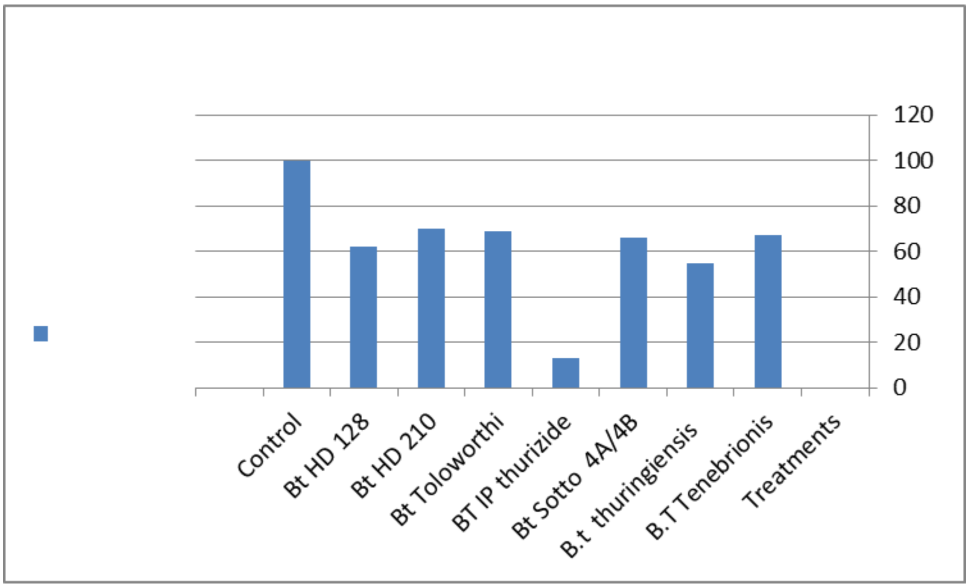

Figure 1: Effect of seven bacterial strains on the infestations of *T. absoluta* in **El- Sharkia** governorate under field conditions.

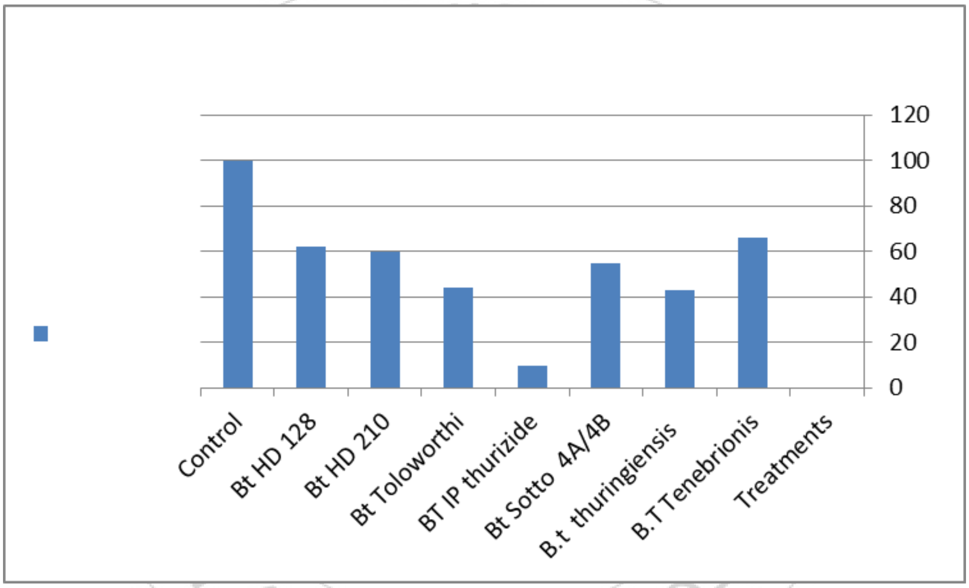

Figure 2: Effect of seven bacterial strains on the infestations of *T. absoluta* in **El- Dakahlia** governorate under field conditions

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