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 pathogens, 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 (*Lycopersicon 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*. 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, (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±2°C 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 Tenebrovius, 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°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 thrizide, Bt Tolowothi, 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 treated 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 ± 2°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 LeC50 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 Dendrolrimum , B.t thuringiensis, Bt Sotto 4A/4B, BT IP thrizide ,Bt Tolowothi 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 m², 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 Dendrolrimum , B.t thuringiensis, Bt Sotto 4A/4B, BT IP thrizide , Bt Tolowothi Bt HD 210 and Bt HD 128, respectively (Table 1). Table 2, show that the LC50 of T. absoluta 159, 140, 70 65, 73 184 and 170 Ug/ml after treated with B.T IP Dendrolrimum , B.t thuringiensis, Bt Sotto 4A/4B, BT IP thrizide , Bt Tolowothi 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 Tolowothi 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, 2499± 20.91 kg/feddan in the plots treated with, B.T IP Dendrolrimum , B.t thuringiensis, Bt Sotto 4A/4B, BT IP thrizide , Bt Tolowothi 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 3901±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 1801±81.30 kg/feddan (Table4).

Figure 1 show the tomato infestation with T. absoluta in El- Sharkia governorate which recorded that the infestation were significantly decreased after bacterial treatments especially after BT IP thrizide 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 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 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., 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* (Giustolisi et al. 2001; Teoduloz et al. 2003; Niedmann and Meza-Basso 2006). Giustolisi 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, Teoduloz 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 to 37.14% 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 *Nabis pseudoeuforus* 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 compared 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 LC50 of M. *anisopliae var. minus* were 169 X104 and 172 X104spores/ml under laboratory and greenhouse effect, respectively. The corresponding LC50 of 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, 2009 and 2014.

### 4. Acknowledgment

This research was supported by PROJECT TITLED, BIOLOGICAL CONTROL OF SOME GREENHOUSE TOMATO INSECT PESTS.

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

<table>
<thead>
<tr>
<th>Pathogen B.t</th>
<th>LC50 Ug/ml</th>
<th>Slope</th>
<th>Variance</th>
<th>95% confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.T Tenebrionis</td>
<td>159</td>
<td>0.1</td>
<td>1.01</td>
<td>99-180</td>
</tr>
<tr>
<td>B.t thuringiensis</td>
<td>120</td>
<td>0.2</td>
<td>1.00</td>
<td>89-142</td>
</tr>
<tr>
<td>Bt Sotto 4A/4B</td>
<td>60</td>
<td>0.1</td>
<td>1.03</td>
<td>30-99</td>
</tr>
<tr>
<td>BT IP thurizide</td>
<td>55</td>
<td>0.4</td>
<td>0.1</td>
<td>25-98</td>
</tr>
<tr>
<td>Bt Toloworthi</td>
<td>63</td>
<td>0.5</td>
<td>1.2</td>
<td>29-88</td>
</tr>
<tr>
<td>Bt HD 210</td>
<td>154</td>
<td>0.1</td>
<td>1.04</td>
<td>96-169</td>
</tr>
<tr>
<td>Bt HD 128</td>
<td>150</td>
<td>0.6</td>
<td>1.01</td>
<td>124-159</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Pathogen B.t</th>
<th>LC50 Ug/ml</th>
<th>Slope</th>
<th>Variance</th>
<th>95% confidence limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.T Tenebrionis</td>
<td>159</td>
<td>0.1</td>
<td>1.01</td>
<td>99-180</td>
</tr>
<tr>
<td>B.t thuringiensis</td>
<td>140</td>
<td>0.1</td>
<td>1.00</td>
<td>99-172</td>
</tr>
<tr>
<td>Bt Sotto 4A/4B</td>
<td>70</td>
<td>0.1</td>
<td>1.3</td>
<td>50-99</td>
</tr>
<tr>
<td>BT IP thurizide</td>
<td>65</td>
<td>0.2</td>
<td>0.1</td>
<td>55-108</td>
</tr>
<tr>
<td>Bt Toloworthi</td>
<td>73</td>
<td>0.1</td>
<td>1.2</td>
<td>49-98</td>
</tr>
<tr>
<td>Bt HD 210</td>
<td>184</td>
<td>0.1</td>
<td>1.2</td>
<td>90-169</td>
</tr>
<tr>
<td>Bt HD 128</td>
<td>170</td>
<td>0.1</td>
<td>1.1</td>
<td>121-199</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Mean number of infestation ±SE after 15 D</th>
<th>30 D</th>
<th>55 D</th>
<th>125 D</th>
</tr>
</thead>
<tbody>
<tr>
<td>B.T Tenebrionis</td>
<td>19.1±12.7</td>
<td>25.7±12.3</td>
<td>27.1±11.5</td>
<td>32.4±11.1</td>
</tr>
<tr>
<td>B.t thuringiensis</td>
<td>18.5±10.6</td>
<td>22.5±15.6</td>
<td>22.5±15.6</td>
<td>28.5±14.4</td>
</tr>
<tr>
<td>Bt Sotto 4A/4B</td>
<td>1.2±8.10.5</td>
<td>1.2±8.10.5</td>
<td>9.7±11.8</td>
<td>1.2±8.10.5</td>
</tr>
<tr>
<td>BT IP thurizide</td>
<td>9.8±9.9</td>
<td>20.8±10.5</td>
<td>22.8±10.1</td>
<td>22.8±10.1</td>
</tr>
<tr>
<td>Bt Toloworthi</td>
<td>2.1±11.2</td>
<td>5.9±12.1</td>
<td>10.4±10.9</td>
<td>10.4±10.9</td>
</tr>
<tr>
<td>Bt HD 210</td>
<td>28.5±16.6</td>
<td>35.9±11.0</td>
<td>44.5±10.6</td>
<td>44.5±10.6</td>
</tr>
<tr>
<td>Bt HD 128</td>
<td>28.8±17.5</td>
<td>49.7±11.8</td>
<td>45.8±13.5</td>
<td>45.8±13.5</td>
</tr>
<tr>
<td>Control</td>
<td>39.8±9.9</td>
<td>67.8±14.5</td>
<td>99.8±15.5</td>
<td>99.8±15.5</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Tomato Weight of yield in two governorates</th>
</tr>
</thead>
<tbody>
<tr>
<td>El- Sharkia governorate</td>
<td>El-Dakahila governorate</td>
</tr>
<tr>
<td>Kg/Feddan</td>
<td>Kg/Feddan</td>
</tr>
<tr>
<td>B.T Tenebrionis</td>
<td>3199± 51.10</td>
</tr>
<tr>
<td>B.t thuringiensis</td>
<td>3199± 50.00</td>
</tr>
<tr>
<td>Bt Sotto 4A/4B</td>
<td>4999± 20.20</td>
</tr>
<tr>
<td>BT IP thurizide</td>
<td>5909± 58.91</td>
</tr>
<tr>
<td>Bt Toloworthi</td>
<td>5590± 52.10</td>
</tr>
<tr>
<td>Bt HD 210</td>
<td>2599± 50.90</td>
</tr>
<tr>
<td>Bt HD 128</td>
<td>2409± 20.91</td>
</tr>
<tr>
<td>Control</td>
<td>2010±10.12</td>
</tr>
</tbody>
</table>

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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