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Two Egyptian *Bacillus thuringiensis* isolates from soil and their potential activity against *Tuta absoluta* infestation under laboratory and field condition

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ABSTRACT

The effect of two Egyptian isolated bacteria Bacillus thuringiensis HD-120 and B.t HD- 187 were tested Under laboratory, semi field and field conditions. Under laboratory conditions the LC50s obtained 105 and 91 Ug/ ml after treated with Bacillus thuringiensis HD-120 and HD-187 respectively. The potency of the two bacterial strains recorded, 18438.09 and 21274.72IU/ mg respectively. When the tomato plant treated with both HD-120 and HD-187 B. t, HD-120 and HD-187 the LC50 obtained 133 and 101 Ug/ml The potency of the two bacterial strains recorded, 22736.824 and 29940.594 IU/ mg respectively. Data the fields show that the weight of tomatoes significantly increased to 19.99±12.7 and 13.93±13. 1ton/feddan when treated with B.t HD-120 and HD-187 respectively in El- Sharkia and El- Dakahlia as compared to 17.11±10.6 and 8.92±18.6 ton/feddan in t he control. When the tomatoes treated with the isolate B.t, HD-187 the weight of tomatoes recorded 18.18±10.6 and 12.57±16.6 ton/feddan in the two corresponding area s.

Key words: *Tuta absoluta*, Bacteria. *Bacillus thuringiensis, B.T,HD-120, B.T,HD-187*

INTRODUCTION

The crop tomato (*Lycopersicone sculentum* Mill.) is one of the very important Solanaceous vegetable crops all over the world. Tomato cultivars are currently infested with many serious and harmful pests, recently the most destructive harmful one is *Tuta absoluta*. It considered among the most important pests of tomato in Egypt during the last ten years. Tomato Pinworm *T. absoluta* (Meyrick) (Lepidoptera: Gelechiidae) is posing a very serious threat to the tomato production. This pest is crossing borders rapidly and devastating tomato production substantially. Its Caterpillars prefer to eat leaves and stems, but may also occur underneath the crown of the fruit and even inside the fruit itself of the Solanaceous vegetables. *T. absoluta* caterpillars attack only green fruit of the Solanaceous vegetable. Among the most distinctive symptoms occur, the blotch-shaped mines inside the vegetable leaves. Inside these mines both the caterpillars live and eat the plant parts. In case of the serious infection, the plant leaves dry and die off completely. Mining damage to the plant causes its malformation. Damage to fruit allows e.g. fungal diseases to enter, which leading to rotting fruit before or after harvest ,[1,2]. in Egypt. tomato grown in green house and open field. *T. absoluta* are severely attack the tomato fruits which causing a lose of their commercial value. 50–100% losses have been reported on tomato[3-5], used the Biocontrol agent for controlling the Tomato Pinworm *T. absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Egypt. [6] control the tomato insect pests by using *bacillus*

thuringiensis and the entomopathogenic fungi. Nanopesticides, nano fungicides and nanoherbicides are being used efficiently in agriculture [7-9].

The aim of this work to evaluate of two isolated bacterial strains of *Bacillus thuringiensis B.T,HD-120 HD-187* against *T. absoluta* under laboratory, greenhouse effect and field.

MATERIALS AND METHODS

Rearing insect pests:
The tomato pinworm *T. absoluta* Tomato Pinworm *T. absoluta* (Meyrick) (Lepidoptera: Gelechiidae) were reared on the 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

2.2. Microorganisms:
Bacillus thuringiensis B.T,HD-120 HD-187 , were used in our study. The bacterial cultures were maintained on nutrient agar slants at 4^{o}C.

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

2.4. Effect of the Microbial Control Agents: Isolated *Bacillus thuringiensis (Bt) B.T,HD-120 and , B.t HD-187* were used in order to test their activities on Tomato Pinworm *T. absoluta* (Meyrick) (Lepidoptera: Gelechiidae) *T. absoluta* larvae and adult. The dead larvae of *T. absoluta* 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 different concentrations of Bt *HD-187* and *B.T,HD-120* . the leaves then left to dry under laboratory conditions. Control treatment was made by feeding the larvae on untreated leaves (sprayed by water only). The percentages of mortality were counted and calculated according to [10] while the LC50s were calculated through probit analysis according to [11]. The potency of the tested samples could be expressed as simple ratio to the standard HD-I-S-1980, which has a potency 16000 IU/mg (Beegle et al 1986). This standard has been used through out the experiment work. The LC50s of the sample was then computed from thee data obtained on the percentage of kill at each of the dilutions tested through probit analysis within 95% confidence limits. The potency of sample was then calculated by the following formula:

$$\text{Potency of sample (IU/mg)} = \frac{\text{LC50 standard}}{\text{LC50 of sample}} \times \text{potency of standard}$$

For each sample, assay was replicated three times in three separated days. The experiments were carried under laboratory conditions; 26 ± 20^{o} C and 60- 70% R.H.

2.5. Semi-field (green house) trials:
Tomato plant Variety Nsxty66 was planted in the green house in 40 plots in each artificial infestation was made by spraying the plant with the two bacterial solutions insecticides 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 [10];while Lc50s were calculated through probit analysis after [11].

2.4 Field trials
The experiments were carried out to study the effectiveness of the tested *nano Bacillus thuringiensis, B.T,HD-120* and *HD-187* against the target insect pests in two different areas. These two areas were: El-Sharkia and EL-Dakahlia. Tomato planted Variety Nsxty66 planted on the first of August 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 100Ug/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 21, 50 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.

2.5The effect of some isolated bacteria strains on the tomato crop value added:

Value added refers to the value that is created during a certain stage of production or marketing, and its known in classical economics as the contribution of the factors of production in increasing the value of product. Karl Marx also pointed out that the added value or increase the profit comes from the worker effort .

RESULTS AND DISCUSSION

Under laboratory conditions the LC50s obtained 105 and 91 ug/ ml after treated with *Bacillus thuringiensis* HD-120 and HD-187 respectively. The potency of the two bacterial strains recorded, 18438.09 and 21274.72IU/ mg respectively (Table 1).

When the tomato plant treated with both HD-120 and HD-187 B. t, HD-120 and HD-187 the LC50 obtained 133 and 101 Ug/ml The potency of the two bacterial strains recorded, 22736.824 and 29940.594 IU/ mg respectively (Table 2).

Table 1. Potency of the two different bacterial treatments against *T. absoluta* under laboratory conditions

Pathogen *B.t*	$LC_{50\ Ug/ml}$	Slope	Variance	95%confidence limits	Potency
Bt. D- 120	105	0.03	1.5	100-122	18438.09
Bt. D-187	91	0.03	1.4	81-134	21274.72
B.t standard (1-S-1980)	121	0.04	1.2	81-129	16000

Table 2. Potency of the two different bacterial treatments against *T. absoluta* Under semifield conditions

Pathogen *B.t*	$LC_{50\ Ug/ml}$	Slope	Variance	95%confidence limits	Potency
Bt. D- 120	133	0.01	1.3	100-152	22736.824
Bt. D-187	101	0.02	1.4	108-124	29940.594
B.t standard (1-S-1980)	189	1.2	0.9	31.5-99.9	16000

Table (4): Weight of harvested tomato fruits after two bacterial treatment against target insect pests *T. absoluta*

Treatments	El- Sharkia Weight tomatoes (Ton/feddan)	El- Dakahlia Weight tomatoes (ton/feddan)
B.t HD- 120	19.99±12.7	13.93±13.1
B.t HD-187	18.18±10.6	12.57±16.6
control	17.11±10.6	8.92±18.6
F values	80.92	
LSD 5%	28.3	

Table (5): Return /invested pound under normal agriculture of the tomato crop

Governorate	Area/ acres	Productivity/ acres Ton	Costs per ton/ Pounds	Revenue per ton /pounds	Net returns per ton/ pounds	Returns/ Invested Pound
Dakahlia	1134	8.92	459	1478	929	2.69
Sharkia	26489	17.11	305	1557	1252	5.10
The Republic	201686	18.20	209	1417	1208	6.78

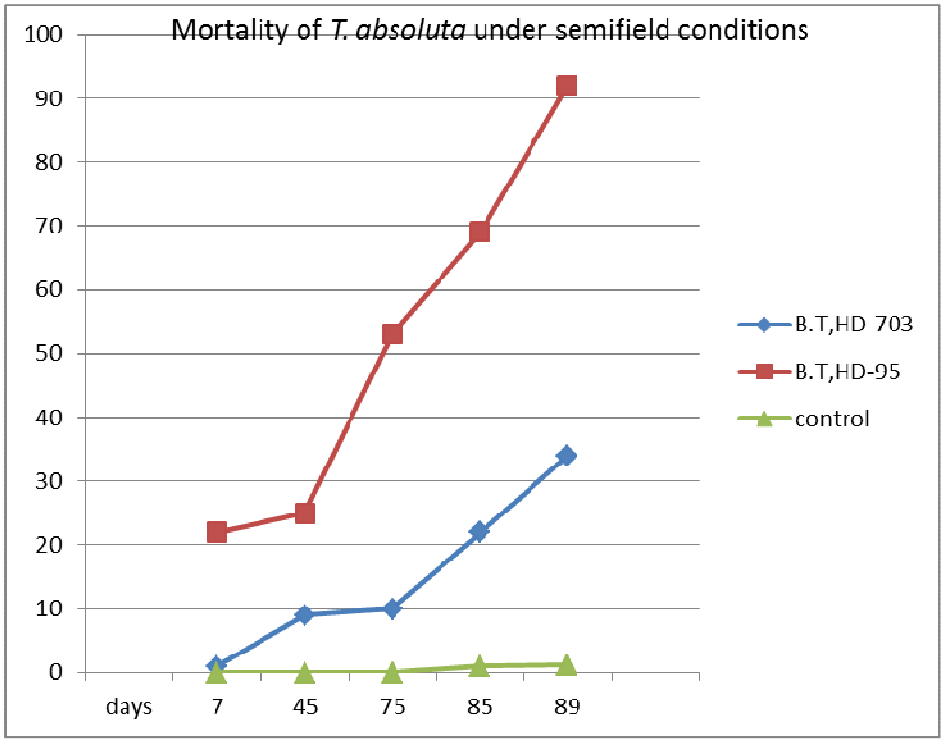
Source: calculated and collected from table (4),Ministry of Agriculture and land reclamation - Annual Bulletin of Agricultural Statistics 2014

Table (6): Return / invested pound under the treatment of the tomato crop with different of bacteria strains

Governorate	B.t HD- 120					B.t HD-187				
	Productivity/ Ton	Costs per ton/ Pounds	Revenue per ton /pounds	Net returns per ton/ pounds	returns / Invested Pound	Productivity/ Ton	Costs per ton/ Pounds	Revenue per ton /pounds	Net returns per ton/ pounds	returns/ Invested Pound
Dakahlia	13.93	387	1478	1100	3.82	12.57	430	1478	1057	3.45
Sharkia	19.99	287	1557	1270	5.43	18.18	315	1557	1242	4.94

Source: calculated and collected from table (4),Ministry of Agriculture and land reclamation - Annual Bulletin of Agricultural Statistics 2014

Fig 1. Mortality percentages of *T. absoluta* after isolated Bactria treatments under semi field condition



Data I table 4 show that the weight of tomatoes significantly increased to 19.99±12.7 and 13.93±13. 1ton/feddan when treated with B.t HD-120 and HD-187 respectively in El- Sharkia and El- Dakahlia as compared to 17.11±10.6 and 8.92±18.6 ton/feddan in the control. When the tomatoes treated with the isolate B.t, HD-187 the weight of tomatoes recorded 18.18±10.6 and 12.57±16.6 ton/feddan in the two corresponding areas (Table4). Figure 1 show the mortality among *T. absoluta* when treated with the two isolated bacterial strains B.t HD- 120 and B.t HD-187 which show the increase I the insect pests amount under semi field conditions.

Economic indicators of the tomato crop under traditional agriculture:
Table (5) show that the cultivated area of tomato crop in each of Dakahlia and Sharkia Governorate represent about 0.56% and 13.13% from the Egypt total area in 2014.The productivity per acre over the country reached about 18.2 tons / acre,with increasing percentage estimated about 104.04% and 6.37%.

The cost of production a ton of the crop has reached its maximum value at 549 pounds in the Dakahlia Governorate with increasing percentage estimated at 126.68%, and about 80% as compared to the cost of production per ton on both the level of the Republic, and Sharkia Governorate respectively.

As for the net return per ton has reached its maximum value at 1252 pounds in Dakahlia Governorate, with increasing percentage estimated about 34.77% and reached around 3.64%as compared to the net return per ton in each of the Dakahlia Governorate, and the level of the Republic, respectively.

As for the return on the invested pound [2] has reached its maximum value at 6.78 pounds at the republic, with increasing percentage estimated about 4.09 pounds, and reached about 1.68 pounds in each of the Dakahlia and Sharkia Governorate, respectively, as shown in a table (5).

Economic indicators of the tomato crop under some treatment of bacteria strains:
Resulted in the treatment of the tomato crop using bacteria B.t HD- 120 and B.t HD-187 the Productivity Increasing per acre of the tomato crop in the Dakahlia Governorate with about 13.93 and 12.57 tons, with increasing percentage estimated about 56.23% and 40.92%. Whenever the cost of production per ton fell decline and estimated about 29.51% and 21.68%; as for the net return per ton increased with increasing percentage estimated about 18.41%and 13.78%,which resulted in increasing return on the invested pound about 1.13 and 0.76 pounds, as compared to the crop in the case of traditional agriculture. Resulted in the treatment of the tomato crop using the two types of bacteria the Productivity Increasing per acre of the tomato crop in the Sharkia Governorate with about 19.99 and 18.18 tons, with increasing percentage estimated about 16.83% and 6.25%,which resulted in increasing of both net return per ton, and the invested pound about 20 and 0.33 pounds, with increasing percentage estimated about 1.6%, and 6.47% in the case the treatment of crop with bacteria B.t HD- 120, while the decline of both net return per ton, and return on invested pound reached about 8 and 0.16 pounds, with a decline percentage estimated about 0.64% and 0.31% in the case the treatment of crop with bacteria B.t HD-187 as shown in tables(5 and 6).

The same results obtained by [12,13], 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* [14, 15, 16]. [14] 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, [16] 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,[15] 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 [12,13] 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* [14, 15, 16]. [14] 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, [16] 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, [15] 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. [12]; reported that B.t gave a good results against *T. absoluta*. [17] 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 [13]. 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 [13]. The same results obtained by Sabbour, 2014, who mentioned, The results showed that under. The same results obtained by [5] . [10], 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. [9] 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 $X10^4$ and 172 $X10^4$spores/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 [6,8,9] controlled the tomato pests *T.absoluta* bay seven bacterial stains of B. thuringiensis. Matter and Sabbour 2013 controlled the tomato insects by bio-insecticides fungi

.[18,19,20] control *T. absoluta* by using the nano bio-insecticides and this lead to increase the yield through the field experiments.

The same findings meet with [21-30] who test the bio insecticide and its nano compositions and control the harmful pests by these materials .

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