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Observations of the Effect of Two Isolated *Nano Bacillus Thuringiensis* on *Tuta absoluta* Infestation under Laboratory and Field Condition

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ABSTRACT

The effect of two nano isolated bacteria *Bacillus thuringiensis* were tested Under laboratory , semifield and field conditions. Data obtained record that the LC50 of *T. absoluta* 115 and 99 Ug/ml after treated with the nano bacteria *Bacillus thuringiensis* HD-703 and BtHD-95, respectively. Data recoded that, under semifield conditions, the LC50 obtained of Nano-Bt. HD 703 135 ug/ml and LC50 of *T. absoluta* treated with different concentrations of Bt. HD-95, recorded 109 Ug/ml. under field conditions, results showed that the effect of nano bacteria, after 21 days of applications, the infestations of the *T. absoluta* significantly decreased to 2.15±12.7, 18.9±19.6 individuals after treated with nano Bacterial strains , B,t HD- 703 and B.t HD-95 as compared to 38.4±18.6 6 individuals in the control . After 120 days of the post applications of nano bacteria, the number of the individuals of the target insect pest, *T. absoluta* significantly decreased to 12.4±11.1 and 48.1±17.6 as compared to 218.5±13.6 individuals in the control . In the tomato field in El- Sharkia and El-Dakahlia governments the tomato weight after bacterial applications significantly increased to 4419.15±12.7 and 4932.4±13.1 kg/feddan in El- Sharkia and El- Dakahlia respectively as compared to 1118.5±10.6 and1018.2±18.6 kg/ feddan in the corresponding two governments .

Keywords: *Tuta absoluta*, Bacteria. *Bacillus thuringiensis, B.T,HD-703, B.T,HD-95*

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INTRODUCTIONS

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 [3,4]. [5, 6, 7], used the Biocontrol agent bacteria or fungi for controlling the Tomato Pinworm *T. absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Egypt. [7,8] control the tomato insect pests by using isolated *Bacillus thuringiensis* and the entomopathogenic fungi. Nano pesticides, nano fungicides and nanoherbicides are being used efficiently in agriculture [9,10, 11].

The aim of this work to evaluate of seven isolated bacterial strains of *Bacillus thuringiensis* against *T. absoluta* under laboratory, greenhouse effect and field.

MATERIAL 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±2Co 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 [12], while LC50 was calculated through probit analysis, [13].

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 [12], while Lc50were calculated through probit analysis [13]. The experiments were carried under laboratory conditions; 22 ± 2o C and 60± 5 % RH.

Microorganisms:

Bacillus thuringiensis B.T,HD-703 HD-95, were used in our study. The bacterial cultures were maintained on nutrient agar slants at 4oC.

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 30oC for 72h.

Effect of the Microbial Control Agents:

Isolated Bacillus thuringiensis (Bt) B.T,HD-703 and , B.t HD-95 were used inorder 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-95 and B.T,HD-703 . 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 [12], while the LC50s were calculated through probit analysis according to [13] . The experiments were carried under laboratory conditions; 26 ± 20o C and 60- 70% R.H.

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 ioinsecticides 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 [12],;while Lc50s were calculated through probit analysis after [13]

Field trials

The experiments were carried out to study the effectiveness of the tested nano Bacillus thuringiensis, B.T,HD-703 and HD-95 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 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 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.

Nanoencapsulation

Nanoencapsulation is a process through which a chemical is slowly but efficiently released to the particular host for insect pests control. Release mechanisms include dissolution, biodegradation, diffusion and osmotic pressure with specific pH [14]. Encapsulated of the two isolated bacteria HD-703 and HD-95 nano-emulsion is prepared by high-pressure homogenization of 2.5% surfactant and 100% glycerol, to create stable droplets which that that increase the retention of the oil and cause a slow release of the nano materials . The release rate depends upon the protection time; consequently a decrease in release rate can prolong insect pests protection time [15].

RESULTS AND DISCUSSIONS

Under laboratory conditions, the data obtained record that the LC50 of *T. absoluta* 115 and 99 Ug/ml after treated with the nano bacteria *Bacillus thuringiensis* Hd-703 and BtHD-95, respectively (Table 1).

Table 1. Effect of the nano Bacteria *Bacillus thuringiensis* HD-703 and HD-95 against *T. absoluta* larvae under laboratory conditions.

Pathogen *B.t*	LC_{50} Ug/ml	Slope	Variance	95%confidence limits
Bt. D- 703	115	0.01	1.3	100-132
Bt. D95	99	0.01	1.2	88-124

Data in table 2 show that , the LC50 obtained of Nano-Bt. HD 703 135 ug/ml and LC50 of *T. absoluta* treated with different concentrations of Bt. HD-95, recorded 109 Ug/ml (Table 2).

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
Bt. D- 703	135	0.01	1.3	100-152
Bt. D95	109	0.02	1.4	108-124

Table 3 show that the effect of nano bacteria under field conditions , after the nano bacterial treatments, after 21 days of applications, the infestations of the *T. absoluta* significantly decreased to 2.15±12.7, 18.9±19.6 individuals after treated with nano Bacterial strains , B,t HD- 703 and B.t HD-95 as compared to 38.4±18.6 6 individuals in the control . After 120 days of the post applications of nano bacteria, the number of the individuals of the target insect pest, *T. absoluta* significantly decreased to 12.4±11.1 and 48.1±17.6 as compared to 218.5±13.6 individuals in the control (Table 3).

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

Treatments	days 21-D	50-D	120D
B,t HD- 703	2.15±12.7	7.7±12.3	12.4±11.1
B.t HD-95	18.9±19.6	38.5±17.5	48.1±17.6
Control	38.4±18.6	88.5±10.6	218.5±13.6

In the tomato field in El- Sharkia and El- Dakahlia governments the tomato weight after bacterial applications significantly increased to 4419.15±12.7 and 4932.4±13.1 kg/feddan in El- Sharkia and El- Dakahlia respectively as compared to 1118.5±10.6 and1018.2±18.6 kg/ feddan in the corresponding two governments (Table 4).

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

Treatments	El- Sharkia Weight tomatoes (Kg/feddan)	El- Dakahlia Weight tomatoes (Kg/feddan
B,t HD- 703	4419.15±12.7	4932.4±13.1
B.t HD-95	3418.5±10.6	3618.7±16.6
control	1118.5±10.6	1018.2±18.6
F values	89.92	
LSD 5%	29.3	

Figures 1 and 2 show that percentages of infestations of the target insect pests of *T. absoluta* significantly decreased in both the two regions El- Sharkia and El- Dakahlia , respectively

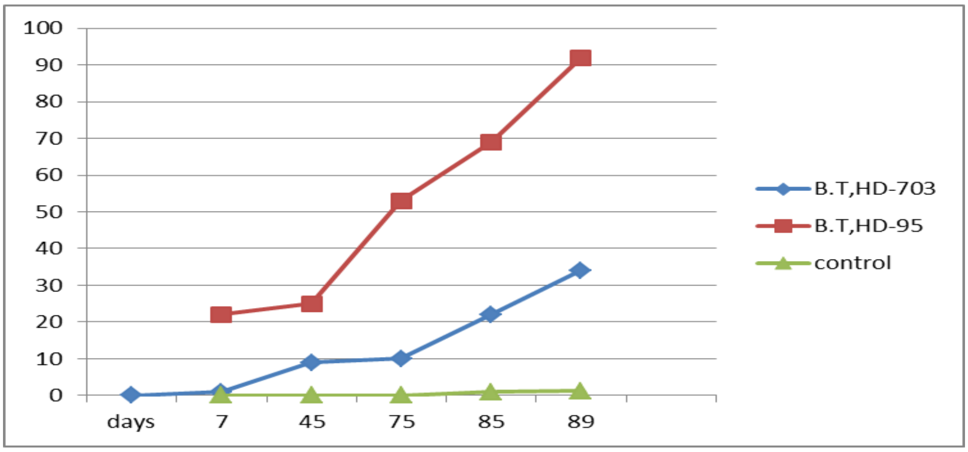

Fig 1. Effect of two tested bacterial strains on the infestations of T. absoluta in El- Sharkia governorate under field conditions.

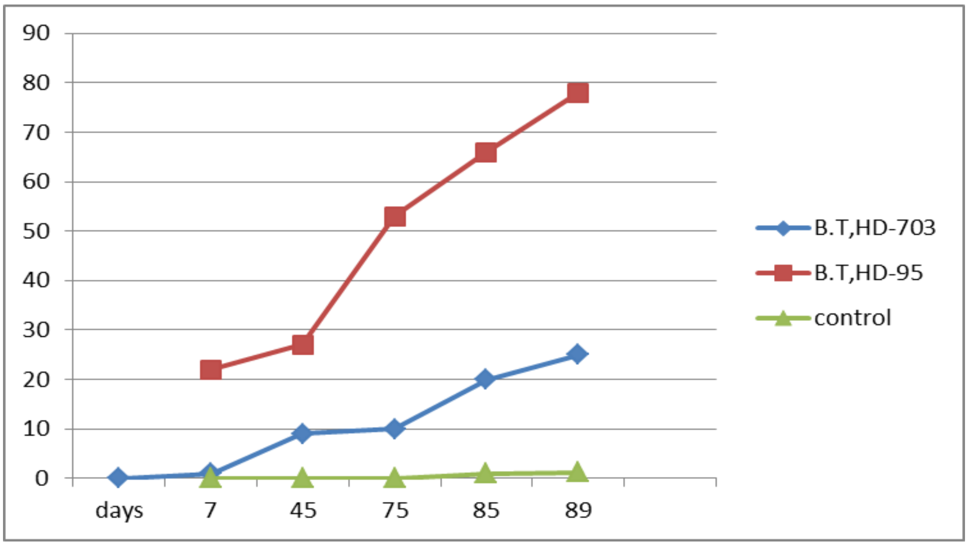

Fig 2. Effect of seven bacterial strains on the infestations of *T. absoluta* in El- Dakahlia governorate under field conditions.

The same results obtained by [20], who controlled the pinworm by bioinseticides. [16] 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* [17, 18, 19] [19]. [17] 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, [19] 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 [12] Laboratories, Chicago, IL, USA).Moreover, [18] 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 [20, 21] who controlled the pinworm by bioinseticides. [16] 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* ([17,18,19] . [17] 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, [19] 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 [12] Laboratories, Chicago, IL, USA).Moreover, [18] 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. [20], reported that B.t gave a good results against *T. absoluta*. [22] 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 [21]. 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 [21] . The same results obtained by Sabbour, [8], who mentioned, The results showed that under. The same results obtained by [9]. [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. [7] 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 [8,9,10] controlled the tomato pests *T.absoluta* bay seven bacterial stains of *B. thuringiensis*. [23,24] controlled the tomato insects by bioinsecticides fungi . [25,26,27, 28,29,30], used the bioinsecticides against harmful pests.

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