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Biocontrol of the Tomato Pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Egypt.

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ABSTRACT

The role of three microbial control agents *Bacillus thuringiensis(B.t)var kurstaki; Beauveria bassiana(B.b)* and *Metarhizium anisopliae* (M.a) were tested against tomato pinworm *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae), which infect plants under laboratory and green house conditions. The results showed that under laboratory conditions, the LC_{50} values for *B.t* was 243.9 Ug/ml and 129.4 x 10^4 and 98.7 X 10^4 spores/ml for *B.b* and *M.a* against *Tuta absoluta* respectively, Under green house conditions, the LC_{50} values for corresponding microbial control agents 211Ug/ml and 102X 10^4 and 100X 10^4 spores /ml ; respectively Under laboratory condition the percentage eggs parasitoid of *Trichogramma evanesens* were significantly decreased after treatments with *M. anisopliae* to 93.2% as compare to 98.2 in the control. Under green house conditions the means number of infestation were significantly decreased to 9.8± 9.9 and 10.8±11.9 individuals after treatments with *M.a* and *B.b* ; respectively as compared to 21.7 ±8.6 individuals in the control.

Key words: *Tuta absoluta; Bacillus thuringiensis; Beauveria bassiana Metarhizium anisopliae*; *Trichogramma evanesens.*

Introduction

Tomato (*Lycopersicon esculentum* Mill.) is one of the most important Solanaceous vegetable crops. 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-Gervasio andVendramin, 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 aim of this work to evaluate of three of bio-insecticides *T. evanesens* against *T. absoluta* under laboratory, greenhouse effect and field.

Material and Methods

Rearing insect pests:

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

Effect of B. thuringiensis Dipel 2X var Kurstaki (23000IU) on T. absoluta:

Samples of the target insects *T. absoluta* , infesting tomato crop were collected from. The bacterium (*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

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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° C and 60-70% R.H.

Cultivation of the fungi:

The fungui *Beauveria bassiana(B.b)* and *Metarhizium anisopliae* (M.a) obtained from Prof. Dr Alain Vey, Mycology unites; Control National De La Research Scuntifique, Univ. Montpellier.*Paecilomyces fumosoroseus (P.f*) (Apopka strain 97,*)* and reproduce in N.R.C microbiology Dep. The fungi were primarily purified using the mono-spore technique. They were propagated in Petri-dishes (10cm) on potato dextrose agar medium (PDAM) enriched with 1% peptone, 4% glucose, and 0.2% yeast and incubated at 26 C° Seven-days old cultures with well developed spores were harvested by washing with 10 cc sterilized water +0.03% Tween-80 and used as stock suspensions with known spore count and kept in refrigerator at 4 C°, from which the fungi were sub-cultured or produced for laboratory evaluation tests (infectivity and bioassay tests) or adjusted as conidiophores concentrations of $1X10^6$ /ml and mixed with 1% sunflower oil for field application using the spraying technique. Large amount of conidiospores were produced by culturing on liquid medium (Rombach *et al.,* 1988) in conidial flasks or in 1L cell culture glass bottles or on rice grains 0.5 kg marmalade jars.

Evaluation of the fungi under laboratory conditions against potato tuber moth:

The fungi. *(B.b)* and (*M.a*) at the concentrations 1X 10^7; 1X 10^6; 1X 10^5; 1X 10^4 ;1X 10^3 and 1X 10^2 spores/ml were tested against potato tuber moth under laboratory conditions. 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 determined after seven days. The percentages of mortality were counted and calculated according to Abbott, (1925), while Lc_{50} were calculated through probit analysis Finney, (1964). The experiments were carried under laboratory conditions; 22 ± 2° C and 60± 5 % RH.

Effect of T. evanescens on the T. absoluta under laboratory conditions:

Under laboratory conditions, 22 ± 2° C and 60± 70%RH.,20 eggs of *T. absoluta* were exposed to 5 eggs of *T. evanescens* . Twenty eggs of *T. absoluta* were exposed to 10 eggs of *T. evanescens*. 20 eggs of *T. absoluta* were exposed to 20eggs of *T. evanescens*. The percentage of parasitism were determined at the end of the experiments. The same techniques were repeated with the fungi *B.b* and *M.a* at the concentrations of 1x 10^4 spores/ml. the experiments were replicated 4 times.

Evaluation of T. evanescens and fungi under laboratory conditions:

Five eggs clusters of *T. absoluta*, eggs less than 24 hours old dipped into *B.b* and *M.a,* suspension at concentration of 1X 10^4 spores/ml, and *B.t* at 250ug/ml, then left to dry and exposed to 20 mated female of *T. evanescens* in glass tube for 30 minutes. The parasitized eggs were incubated at 22±2 C° for 1, 3, 5 and 7 days. Control was dipped in water only. Each treatment was performed seven times. The treated host egg masses were kept at 22±2 C° until emergence of parasitoid adults. The percentage of infestations were calculated after seven days. Percentage of parasitoid emergence inside the host was calculated. The percentage of eggs reductions were calculated as the following equations:

$$\text{\% of reduction} = \frac{\text{\% of egg emergence in the control} - \text{\% of egg emergence after treatments}}{\text{\% of egg emergence in the control}}$$

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 *B.t., B. b; M. a;* at the concentrations of 300 ug/ml of *B. t* and 8.25 x 10^8 conidia/ml for each of *the fungi* . 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 Abott, 1925; while Lc50s were calculated through probit analysis after Finney 1964.

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Results and Discussion

Data in table 1 show that LC_{50} recorded were 243Ug/ml of when *T. absoluta* treated with different concentrations of B.t. the LC_{50} of *B. b* and *M.a* were 129X10^4 and 98.7 X 10^4 spores/ml; respectively Table 1. 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 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* 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 adelivery system of toxins from *B. thuringiensis*, which would allow a reduction in pesticide applications.

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

Microbial control agents	Lc50	Slope	Variance	Confidence limits
B.t	243.ug/ml	0.01	1.59	266-210
B.b	129.4 x 10^4 spores/ml	0.01	1.44	133-100
M.a	98.7 X 10^4 spores/ml	0.02	1.60	89-120

Under green house effect LC_{50} recorded 211Ug/ml for *B.t* and 102 X 10^4 and 100 X 10^4 spores /ml for *B. b* and *M.a*; respectively Table2. 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..

Data in table 3 show when the parasite applied on the larvae treated with *B.b* the percentages of the emergence ranged between 80.60 to 91.55 . The highly emergence of the parasitism when the *T. absoluta* were treated by *M.a* which recorded 93.2 after seven days as compared to 98.2 in the control

Table 2: Effect of microbial control agents against *Tuta absoluta under greenhouse effects.*

Microbial control agents	Lc50	Slope	Variance	Confidence limits
B.t	211 ug/ml	0.02	1.33	200-177
B.b	102X 10^4 spores/ml	0.07	1.55	100-78
M.a	100X 10^4 spores/ml	0.01	1.59	82-119

Table 3: Percent hatching of parasitized eggs of *Trichogramma evanesens* after laboratory tratments.

Treatments	Emergence %							
	1d	Reduction%	3d	Reduction%	5d	Reduction%	7d	Reduction%
B.t	85.6	5.7	89.7	1.8	92.2	1.2	96.2	2.1
B.b	77.8	1.6	80.4	1.2	92.1	1.3	94.4	4.0
M.a	70.7	2.6	77.9	1.5	89.1	5.0	93.2	5.2
contol	89.8		91.1		93.2		98.2	

It is reported that in a combine application of mass release of *Trichogramma pertiosum* and *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.

Data in table 4 show that the mean number of infestation decreased to 15.7 ±12.5 after 120 days when treated with *B. b. M.a* scored the highly significance after 120 days the percentage of infestation recorded 9.8 ±9.9individiuals as compared to 21.7 ± 8.6 individuals in the control . 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).

Table 4: Effect of the fungi and *T. evanescens* under semi-field (green house) conditions against *T. absoluta.*

Treatments / days	Mean number of infestation ±SE		
	50	90	120
B.b	10.15±12.7	12.4±11.9	15.7±12.5
after parasitism	8.5±11.6	11.3 ±9.5	10.8±11.9
M.a	9.7±11.8	10.6±8.8	12.8±10.5
after parasitism	7.8±10.5	10.3±9.6	9.8±9
B.t	18.7±11.7	15.5±10.7	17.8±9.9
after parasitism	17.8±9.5	16.8±8.4	14.9±10.1
Control	26.5±11.34	30.6 ±10.8	35.4±12.3
after parasitism	20.5±10.33	19.3±9.8	18.7±8.6
F value		27.1	
LSD 5%=		11.1	

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