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Evaluations of Two *Metarhizium* varieties against *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) in Egypt.

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Abstract: ***The LC50 of* M. anisopliae *var.* frigidum *156X10^4 and 168 X10^4 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 higest yied 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.***

Keywords: Tuta absoluta ; M. anisopliae var. frigidum'' M. flavoviride var. minus.

1. Introduction

The anamorph genus *Metarhizium* is composed of arthropod pathogens, several with broad geographic and host ranges. Members of the genus, including ''*M. anisopliae* var. *frigidum*'' nomen nudum and *Metarhizium flavoviride*, have been used as biological insecticides. In a recent revision of the genus the variety ''*M. anisopliae* var. *frigidum*'' was suggested to be a synonym of *M. flavoviride* based largely on ITS sequence phylogenetic analysis. In this study we conducted morphological evaluations and multigene phylogenetic analyses with EF-1α, RPB1 and RPB2 for strains of *M. flavoviride* and ''*M. anisopliae* var. *frigidum*.'' Included in these evaluations were the ex-type of *M. flavoviride* var. *flavoviride* and what likely would be considered the ''ex-type' of the invalidly published taxon ''*M. anisopliae* var. *frigidum*''. Based on morphological and molecular evidence we conclude that ''*M. anisopliae* var. *frigidum*'' is distinct from *M. flavoviride* and the taxon *M. frigidum* sp. nov. is described Joseph et al, 2014. Tomato, Lycopersicon esculentum Mill is a vegetable crop of large importance throughout the world. Its annual production accounts for 107 million metric tons with fresh market tomato representing 72 % of the total (FAO, 2002). *Tuta absoluta* Meyrick, (Lepidoptera : Gelechiidae) is a serious pest of both outdoor and greenhouse tomatoes. The insect deposits eggs usually on the underside of leaves, stems and to a lesser extent on fruits. After hatching, young larvae penetrate into tomato fruits , leaves on which they feed and develop creating mines and galleries. On leaves, larvae feed only on mesophyll leaving the epidermis intact (OEPP, 2005).

The aim of this work to evaluate of two *Metarhizium* varieties (*M. flavoviride* var. *minus*, and *M. anisopliae* var. *frigidum*) against *T. absoluta* under laboratory , green house and field effect.

2. Material and Methods

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.

Entomopathogenic Fungi. The fungus, *M. flavoviride* var. *minus*, and *M. anisopliae* var. *frigidum*''_were kindly obtained from Prof. Dr Alain Vey, Mycology Unit, Pasture institute, France, and reproduced in the Microbiology Department, National Research Centre, Cairo, Egypt. They were primarily purified using the mono-spore technique. Then, propagated in Petri-dishes (9cm) on potato dextrose agar medium (PDAM) enriched with 1 %, peptone, 4 %. Glucose and 0.2% Yeast and incubated at 26 ºC. Seven days old cultures with well developed spores were harvested by washing with 10 ml sterilized water, then 3 drops of Tween-80 were added and completed to 100 ml with water. It was used as stock suspention and kept in a refrigerator at 4 ºC. From this stock (standared), dilutions with water were adjusted at the needed proposed concentrations. Large amounts of conidiospores, if needed, were produced by culturing the fungus on liquid medium in 1 L cell-culture glass bottles according to Rombach et al (1988) (modified by El-Husseini et al., 2004).

Efficacy of Entomopathogenic Fungi against Pests Larvae. Spores of the entomopathogenic fungi; *M. anisopliae* var. *frigidum*'' and *M. flavoviride* var. *minus*,, collected from the surface of mycelium growth and spore suspensions with 2 drops of tween 80 were prepared and adjusted at 1x10^7 conidia/ ml. Conidial viability was determined by counting germ tubes produced on PDAY medium after 18 hrs, using light microscope at 400 x. Conidial viability was 95-100%. The surface of cultures was gently brushed in the presence of 20ml of sterilized water in order to free the spores and the suspension was

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filtered through muslin. Six concentrations of spore suspensions were prepared *i.e.,* 10^7, 10^6, 10^5, 10^4, 10^3, and 10^2 conidia/ml. Piece of corn leaves were dipped in the prepared suspensions and left for drying under laboratory conditions then placed in Petri-dishes (one/dish). For each concentration (4 replicates/ each), ten L3 larvae of each of the tested insects were transferred into each Petri-dish. Control larvae were fed on untreated castor leaves. Percentages of mortality were calculated according to Abbot, while LC_{50} was calculated throughout probit analysis. The experiment was carried out under laboratory conditions at 26°C± 2 and 60-70 % RH. Physiological and metabolic characteristics of *M. anisopliae* var. *frigidum*'' *and M. flavoviride* var. *minus*.

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 entomopathogenic fungi *M. anisopliae* var. *frigidum*'' and *M. flavoviride* var. *minus*, at the concentrations of 1X10^8 spores/ml of the two fungi varities . 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.

Field Trials. Field trials were carried out at Nobaria region (Behera Governorate), Egypt during the two successive corn seasons 2013 and 2014 to study the effectiveness of the tested fungi on corn borers. Corn (variety Giza 2) was cultivated by end of May during the two seasons in an area of about half feddan. Fungi were applied as single treatments in randomize plots. Regular agricultural practices were performed and no chemical control was used during the study period. Weeds were removed by hand. Five plots were sprayed with water as control. Samples from each treatment were collected weekly and transferred to the laboratory for investigation. Percentages of infection were estimated.

Yield Assessment:
Yield data in treated and untreated plots in the corn harvest seasons (2013and 2014), represented by weight in kgs were determined. Yield loss was estimated according to the following equation:

$$\text{Yield loss} = \frac{\text{Potential yield} - \text{Actual yield}}{\text{Potential yield}} \times 100$$

Potential yield is *M. anisopliae* var. *frigidum*'' treatment (the best result among the tested pathogens) was considered the standard for comparison with the other ones (Actual yield).

3. Results and Discussion

The LC50 of *M. anisopliae* var. *frigidum* 156X10^4 and 168 X10^4 spores/ml unde laboratory and greenhouse effect. The correspondingLC50of *M. flavoviride* var. *minus* were 169 X10^4 and 172 X10^4spores/ml (Table 1&2).The higest yied 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 (Table3). Frigure(1&2) show that the the infestations significantly decreased in plots treated with *M. anisopliae* var. *frigidum* as compared to the control plots. The obtained by Joseph et al (2014), Theoduloz et al(2003), Sabbour 2006, Sabbour and Abd el Aziz 2007, Sabbour, 2007, Sabbour and Abbas, 2007. Sabbour and Hany, 2007, Sabbour,2008. Asmaa et al 2009. The same results obtained by Sabbour 2003,(20014a&b), 2013.Magda Mahmoud Sabbour and Shadia El-Sayed Abd-El-Aziz. 2014, Magda Sabbour, 2001, Sabbour (2002 a &b), Magda Sabbour and Ismail2002, Sabbour and Sahab 2005 &2007, 20011.The same results obtained Sabbour 2006, Sabbour and Abd el Aziz 2007, Sabbour, 2007, Sabbour and Abbas, 2007. Sabbour and Hany, 2007, Sabbour,2008. Asmaa et al 2009. Sabbour 2014 control *Tuta absoluta* by three microbial control agents *Bacillus thuringiensis(B.t)var kurstaki; Beauveria bassiana(B.b)*which increase the yied. Sabbour 2014 controlT.absoluta by fungi under laboratory and field conditions. The same obtained by Sabbour & Singer2014, Sabbour & Soliman 2014, Sabbour and Moursy2014, Sabbour and Abdel-Raheem 2014. The same findings obtained by Sabbour, 2013(a,b,c,d,e,f,g,h,I,j)

4. Acknowledgment

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

Microbial control agents	Lc50 spores/ml slope variance confidence limits
M. anisopliae var. *frigidum* *M. flavoviride* var. *minus*	156 X10^4 0.01 1.1 116-208 169 X10^4 0.1 0 1.2 119-210

Table 2: Effect of microbial control agents against *Tuta absoluta* under green house effects

Microbial control agents	Lc50 spores/ml slope variance confidence limits
M. anisopliae var. *frigidum* *M. flavoviride* var. *minus*	168 X10^4 0.02 1.0 100-237 172 X10^4 0.07 1.1 131-288

Table 5: Weight of tomato after fungi treatments against the target insect pests during 2014 in two different regions .

Treatments	El-Esraa (Nobaryia)		El-Kassaseen (Ismailia)	
	Weight tomatoes (Tons/feddan)	% yield loss	Weight tomatoes (Tons/feddan)	% yield loss
Control	1106± 27.61	72	2009±60.60	52
M. anisopliae var. *frigidum*''	3999± 49.41	-	4697± 49.33	-
M. flavoviride var. *minus*	3689± 49.41	7	3988± 41.43	15
F values	30.02		30.11	
LSD 5%	89		87	

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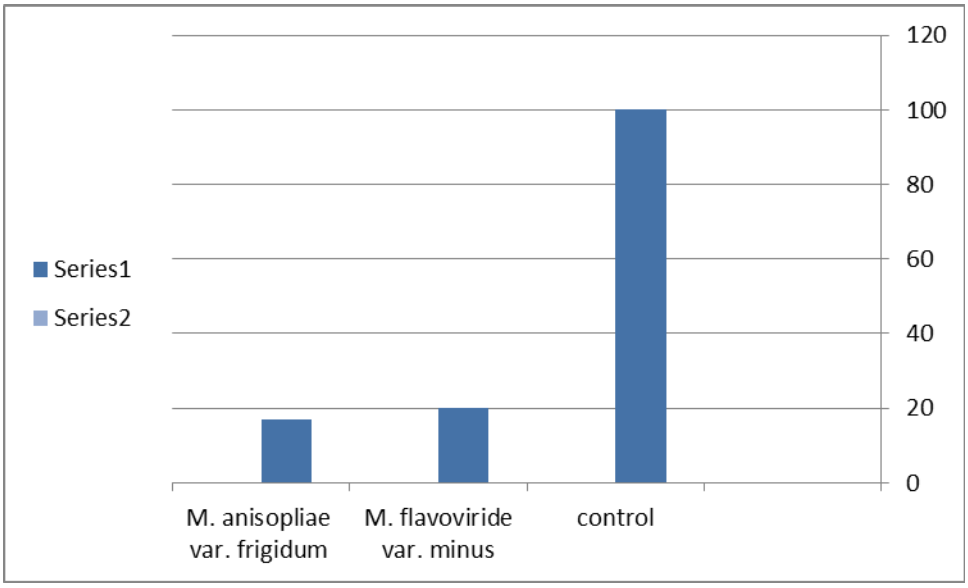

Figure 1: The insect pest infestation during season2014 in El-Esraa (Nobaryia)

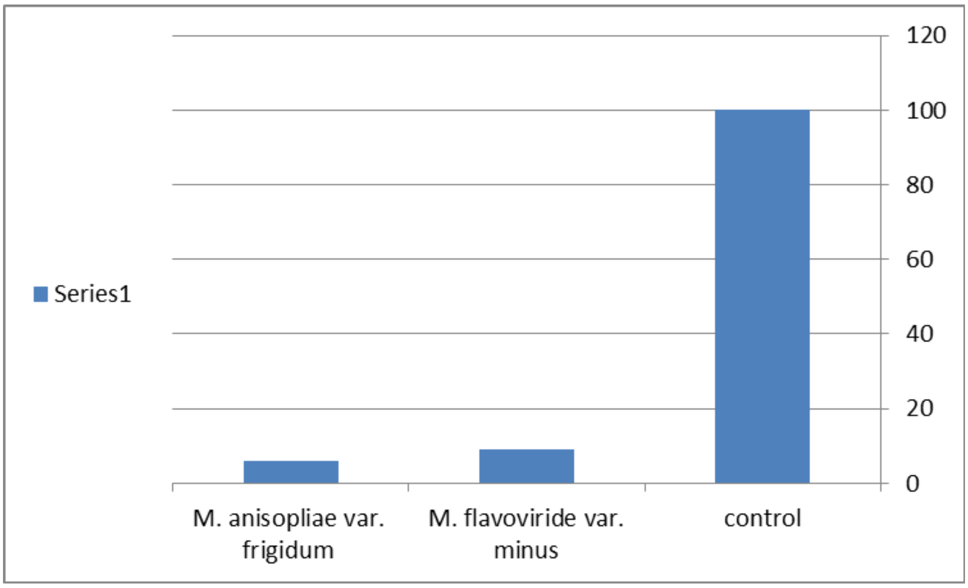

Figure 2: The insect pest infestation during season2014 El-Kassaseen

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