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ORIGINAL ARTICLE **OPEN ACCESS**

Efficacy of *Isaria fumosorosea* and *Metarhizium flavoviride* against Corn Pests under Laboratory and Field Conditions in Egypt

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ABSTRACT
Under laboratory conditions thelethal concentration 50 (LC_{50})obtained was 132x10^4, 136x10^4 and 146x10^4 spores/ml after Ostrinia nubilalis, Sesamia. cretica and Chilo Agamemnon were treated with different concentrations of Metarhizium flavoviride, respectively. When the corresponding pests were treated with Isaria fumosorosea the corresponding LC_{50} was 156 x10^4 and 169x10^4 and 165x10^4 spores/ml, respectively. Field applications show that the assessment of damage caused by corn borers in maize field, which detected that the weight of corn crop in the plots treated with M. flavoviridel. fumosorosea significantly increased to 4888±81.20 and 3900±60.70 as compared to 2610±30.90 kg/ (feddan) in the control plots during season 2013., respectively. During season 2014 the corresponding weight of corn crop were, 4991±49.43, 3999±43.14 and 2500±53.20kg/feddan respectively.
Key wards: *Isaria fumosorosea, Metarhizium flavoviride, Ostrinia nubilalis, Chilo agamemnon, Sesamia cretica.*

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INTRODUCTION
Maize is an important crop all over the world and also in Egypt. Its demand continuously increases. Corn is subjected to attack by many insect pests that affect the yield quality and quantity. Among the most common pest species surveyed in Egypt are: the European corn borer *Ostrinia nubilalis* (Lepidoptera: Crambidae), *Sesamia cretica* (Lepidoptera: Noctuidae). *Chilo agamemnon*. *O. nubilalis* is the one of key pest damaging corn fruit in the world as well as in Egypt. *O. nubilalis* is native to Mediterranean countries which has 98% of the world's cultivated corn plants. *C. agamemnon*is also one of the most important insect pests of corns in Egypt and other Mediterranean countries. The moth develops three generations per year. In Egypt the first generation of moths appears in April and female lays its eggs on the flower buds after that newly hatched larvae feed on the buds and flowers of corn plants. When adults of corn pests were exposed to conidia of *Mucorhiemalis, Penicillium aurantiogriseum, P. chrysogenum* and *Beauveria bassiana* isolates through contact and oral bioassays showed moderate to high mortality .*Nomuraea rileyi* and *Paecilomyces fumosoroseus* proved highly pathogenic to aphids and whiteflies [3]. The fungus exhibit host preferential infections in lepidopterous larvae [2]. The entomopathogenic fungi are of particular research interest because of their potential as commercial bioinsecticides. Some studies had focused on identifying nutrient substrates that *B. bassiana* can utilize with application to industrial production, while others focused on the pathogenic processes of *I. fumosorosea, M. flavoviride* and interactions with insect cuticle.
It is necessary to find alternative safe insecticides to reduce the heavy doses of chemical insecticides which are used for the control of corn pests [1, 2].
Entomopathogenic fungi are found worldwide associated to insects and phytophagous mite populations, contributing to biological control of these arthropods on several economically important crops [4]. Commercial products have been developed with entomopathogenic fungi. Quintela and McCoy [6] reported that fungal concentrations of 10^6 and 10^7 conidia/ml of *B. bassiana* and *N. rileyi* affected the larval development, movement and mobility of corn borers larvae during the seedlings and vegetative stages of corn plant under laboratory; greenhouse and field conditions. However, the success of a pest control program using *fungi* depends on conidia survival in the field environment [7]. Conidia survival

may be affected either by environmental factors [8] or chemical products used to protect plants. Abdel-Rahman, *et al*. [5] controlled the cereal pests with the fungus *B. bassiana* and found that the infestation was reduced after fungal applications under laboratory and field conditions.

The present study aims to evaluate the pathogenicity of the entomopathogenic fungi, *Isaria fumosorosea* and *Metarhizium flavoviride* (bio-insecticide)against corn insect pests *Sesamia cretica, Ostrinia nubilalis* and *Chilo agamemnon* under laboratory and field conditions.

MATERIALS AND MEHODS

Tested Insects:

Sesamia cretica,Ostrinia nubilalis and *Chilo Agamemnon* were reared on corn leaves under laboratory conditions 26±2°C and 60±5% RH. Leaves changed every second day.

Entomopathogenic Fungi:

The fungi, *I. fumosorosea*and *M. Flavoviride* were obtained from Florida Univ., USA. They were reproduced on potato dextrose agar (PDA) plus 0.4% yeast extracts (PDAY) and poured onto sterilized Petri-dishes [9]. Plating was performed according to the full dish method. The conidia were transferred from the appends of vial to dish containing medium by platinum loop and then streaked. Plates were incubated at 25°C with 12 hours photo-phase for fungus growth and sporulation. After ten days, conidia were scraped and transferred to conical flasks (250 ml) containing 200ml sterilized distilled water with 0.02% the speeder sticker (Tween-80). Conidial concentrations in the suspensions were quantified directly under the optical microscope with a haemacytometer. Then the suspensions were standardized until the direct concentration $1x10^7$ conidia/ml was achieved.

Efficacy of Entomopathogenic Fungi against pestslarvae:

Spores of the entomopathogenic fungi; *I. fumosorosea, M. flavoviride*, collected from the surface of mycelium growth and spore suspensions with 2 drops of Tween-80 were prepared and adjusted at 1x107 conidia/ml. Conidial viability was determined by counting germ tubes produced on PDAY medium after 18 hours, using light microscope at 400X. 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 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 concentration/dish). For each concentration (4 replicates/each), ten of 3rd instar larvae (L3) of each of the tested insects were transferred into each Petri-dish. Control larvae were fed on untreated corn leaves. Percentages of mortality were calculated according to Abbot, [10], while LC_{50} was calculated throughout probit analysis [12]. The experiment was carried out under laboratory conditions at 26±2°C and 60-70% RH.

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 (500 meter). Fungi were applied as single treatments in randomize plots and replicated four times. 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 the experiment was replicated 4 times. Twenty 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 Kg were determined. The Yield loss was estimated according to the following equation:

Potential yield – Actual yield Potential yield
Yield loss = ---
Potential yield

Potential yield is the yield which gave the pest amount among all treatments *M. flavoviride* treatment (the best result among the tested pathogens) was considered the standard for comparison with the other ones.

RESULTS AND DISCUSSION

In-vitro effect of Entomopathogenic fungi on the target insects

Data in table (1), show that under laboratory conditions the LC_{50} obtained was $132x10^4$, $136x10^4$ and $146x10^4$ spores/ml after *O. nubilalis*, *S. cretica* and *C. agamemnon* treated with different concentrations of *M. flavoviride,* respectively. When the corresponding pests treated with *I. fumosorosea*the corresponding LC_{50} was 156 $x10^4$ and $169x10^4$ and $165x10^4$ spores/ml, respectively (Table 2).

The same results were obtained by Sabbour and Abdel-Rahman [11], who reported that under laboratory conditions results showed that the LC_{50} of *Phyllotreta cruciferaem, Pegomyia hyoscami* and *Cassida vittata* of the tested fungi *Verticillium lecanii, Nomuraea rileyii* and *Paecilomyces fumosoroseus*, respectively against the three pests *Phyllotreta cruciferaem, Pegomyia hyoscami* and *Cassida vittata* ranged between $5.4x10^6$ and $1.43x10^7$ spores/ml. Satisfactory results with the entomopathogenic fungi were reported by and Sabbour and Ismail [13]. Sabbour and Abd El-Aziz [14] as they found that the fungi; *B. bassiana* and *M. anisopliae* reduced the lethal concentration 50 (LC_{50}) of *Spdoptera littoralis* under laboratory conditions.

Data in table (3) show that the application of the bioinsecticides which affected on decreasing the infestation, the number of three pests infestations of *Ostrinianubilalis* and *Sesamia cretica* significantly decreased to 20±2.1 and 22±1.1., respectively, after treatment with *M. flavoviride* after 20 day as compared to 69±9.3 and 70±9.1 individual in the control for the corresponding pests , during seasons 2014. In all treatments the number of corn pests was significantly decreased. *Chiloagamemnon* infestation decreased to 20±3.3 and 25±3.1 individuals after 90 days during season, 2014 and 2013 as compared to 98±9.3 and 99±9.3 individuals in the control plots in both two seasons.The obtained results are similar to other studies carried out by Castillo *et al.* [15] on their work on *Ceratitis capitata.*

Table (4) shows that the assessment of damage caused by corn borers in maize field, which detected that the weight of corn crop in the plots treated with *M. flavoviride* and *I. fumosorosea* significantly increased to 4888**±**81.20 and 3900**±**60.70 as compared to 2610**±**30.90 kg/ feddan in the control plots during season 2013., respectively. During season 2014 the corresponding weight of corn crop were, 4991**±** 49.43 , 3999 **±**43.14 and 2500**±**53.20kg/ feddan respectively (Table 4). Figures (1 & 2) show that the percentage of infestations was significantly decreased during both seasons .

Table (1): Effect of entomopathogenic fungi,*M. flavoviride*against the target insect pests larvae under laboratory conditions

Insects	LC_{50}	slope	variance	95% confidence limits
Ostrinianubilalis	$132x10^4$	0.1	1.01	99-169
Sesamia cretica	$136x10^4$	0.2	1.00	111-180
Chilo agamemnon	$146x10^4$	0.1	1.03	115-197

Table 2: Effect of *Isaria fumosorosea* against target insect pestsunder laboratory conditions

Insects	LC_{50}	slope	variance	95% confidence limits
Ostrinianubilalis	156	1.01	0.02	111-176
Sesamia cretica	169	0.10	1.01	134-187
Chilo agamemnon	165	0.10	1.01	145-199

Table (3): Effect of different treatments on the target insect pests under field conditions

Post 1st application date	Treatments	Number of three pests infestation (Mean±SE) during the two seasons					
		Ostrinia nubilalis		*Sesamia cretica*		*Chilo* agamemnon	
		2013	2014	2013	2014	2013	2014
20 50 90	Control	40±3.2 59±4.3 78±2.3	69±9.3 70±3.1 86±2.1	61±2.5 79±3.4 88±5.1	70±9.3 83±2.5 92±2.1	67±3.4 84±3.4 98±3.9	73±2.2 91±1.3 99±9.3
20 50 90	*M. flavoviride*	16±3.1 21±2.2 25±3.2	20±2.1 24±2.1 19±1.2	20±4.9 22±4.7 21±2.4	22±1.1 24±3.2 19±2.3	21±4.3 22±3.4 24±2.3	20±1.2 24±4.4 21±4.2
20 50 90	*I. fumosorosea*	45±3.3 45±4.2 39±4.2	39±2.1 35±1.2 30±1.3	41±2.6 42±3.5 35±4.1	38±2.4 39±3.2 29±2.5	41±3.4 34±2.3 25±1.3	20±2.2 20±4.0 20±3.3
F-value LSD at 5%		29.1 15.1	30.6 16.3	31.5 13.1	23.1 16.0	30.8 17.8	28.9 16.9

Table (4): Assessments of damage caused in corn field after the fungi treatment

Treatments	Season 2013		Season 2014	
	Weight of corn crop (kg/ feddan)	% yield loss	Weight of corn crop (kg/feddan)	yield loss %
M. flavoviride	4888±81.20	-	4991±49.43	-
I.fumosorosea	3900±60.70	20.2	3999±43.14	20.2
Control	2610±30.90	46.6	2500±53.20	49.9
F-value	33.6		32.9	
LSD at 5%	126.7		126.5	

Fig (1) Effect of the fungus I. fumosorosea under field conditions

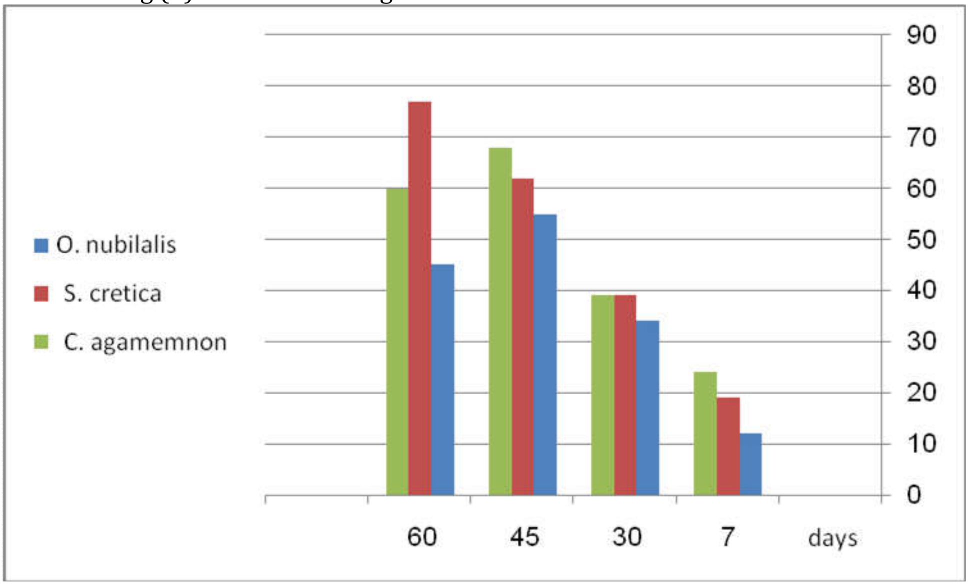
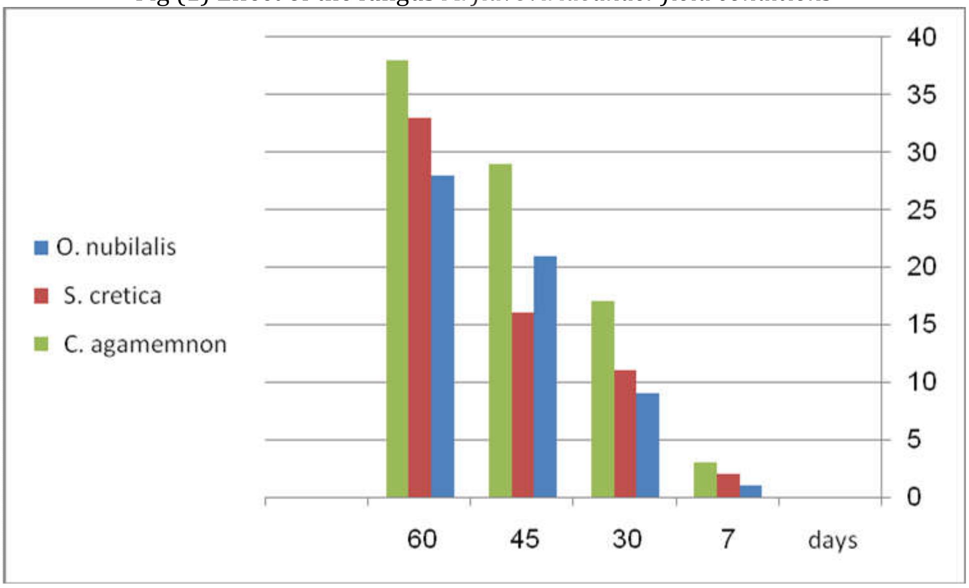


Fig (1) Effect of the fungus *M. flavovirideunder field conditions*



These results are also agree with Sabbour & Shadia Abd El-Aziz [16, 17] and Shadia Abdel Aziz & Nofel [18], who proved that the application with bioinsecticides increased the yield and decreased the infestation with insect pests. Also, results were in accordance with Castillo *et al.* [15] who reported that the virulence of *Beauvaria bassiana* against *Ceratitiscapitata* ranged between 8 to 30% and decrease the infestation among the olive fruits.

The same results were obtained by Abdel-Rahman & Abdel-Mallek [19], Abdel-Rahman [20] and Abdel-Rahman *et al*. [21], when they controlled cereal aphids with entomopathogenic fungi. They found that the infestation was reduced after fungi applications under laboratory and field conditions. Sabbour & Sahab

[18, 22], Tanda and Kaya [23] and Sahab and Sabbour [19] found that the fungi reduced insect infestations of cabbage and tomato pests under laboratory and field conditions.

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