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

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Abstract: ***The effect of nano fungus Metarhizium flavoviride show that the obtained LC_{50} recorded88x10^4, 76x10^4 and 106x10^4 spores/ml for Ostrinia nubilalis, Sesamia cretica and Chilo agamemnon respectively Results showed that the LC_{50} of the nano fungus Isaria fumosorosea of the corresponding insect pests recorded, 56 x10^4, 68 x10^{4}68 x10^4 ^{and}95 x10^4 spores/ml ., respectively the infestation of the target insect pests were significantly decreased during both successive seasons , during season 2014 the infestations of O. nubilalis were significantly decreased after 90 days to 18±0.2 and 22±1.0 in plots treated with Nano-M. flavoviride and Nano-I. fumosorosea respectively as compared to89±9.1 individuals in the control S. cretica significantly decreased to 19±0.3 and26±2.1 individuals when treated with and Nano-I. fumosorosea respectively as compared to 97±2.9 individuals in the control during season 2014. The same results obtained when C. agamemnon treated with both nano fungi. The application of the bioinsecticides which affected on decreasing the infestation, the number of infestations of O. nubilalis and Sesamia cretica significantly decreased to 20±2.9 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 were significantly decreased. Chilo agamemnon 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 weight of corn in the field during season 2013, 4089±82.80 and 4999±66.73 after treatments with Nano-M. flavoviride and Nano-I. fumosorosea respectively as compared to 2611±33.80 in the control . The percentage of yield loss 47% in the control and 0.1% in the plots treated with Nano-I. fumosorosea. During season 2014 the results show that the weight of corn wer3e significantly decreased to in the control as compared to and in plots treated with Nano-I. fumosorosea and Nano-M. flavoviride ., respectively. The percentage of yield loss are 0.02% and52% in plots treated with Nano-I. fumosorosea and in the control***

Keywords: Nano, *Isaria fumosorosea, M. flavoviride* , *Ostrinia nubilalis, Chilo agamemnon, Sesamia cretica.*

1. 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: *Ostrinia nubilalis*, *Sesamia cretica* and *Chilo agamemnon*. *O. nubilalis* is the one of key pest damaging corn fruit in the world as well as in Egypt (Eid, 2003). *O. nubilalis e* is native to Mediterranean countries which has 98% of the world's cultivated corn trees (Montiel and Jones, 2002). *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 (Montiel and Jones, 2002). 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 trees (Montiel and Jones, 2002). When adults of corn fruit fly were exposed to conidia of *Mucor hiemalis, Penicillium aurantiogriseum, P. chrysogenum* and *Beauveria bassiana* isolates through contact and oral bioassays showed moderate to high mortality (Mohamed, 2009). *Nomuraea rileyi* (N.r) and *Paecilomyces fumosoroseus* (*P.f*) proved highly pathogenic to aphids and whiteflies (Espinel *et al.,* 2008). The fungus (N.r) exhibit host preferential infections in lepidopterous larvae (Ignoffo *et al.,* 1976 Qiao *et al*. (2012). The entomopathogenic fungi, are of particular research interest because of its 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 *Isaria fumosorosea M. flavoviride* and interactions with insect cuticle (Bidochia *et al*., 1990).

It is necessary to find alternative safe insecticides to reduce the heavy doses of chemical insecticides which is using for the control of corn pests (Rice, 2000) Qiao *et al.* (2012).Entomopathogenic fungi are found worldwide associated to insects and phytophagous mite populations, contributing to biological control of these arthropods on several economically important crops (Sabbour and Sahab, 2007). Commercial products have been developed with entomopathogenic fungi (Alves and Pereira, 1998). Quintela and McCoy (1998) 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. Success of a pest control program using *fungi* however depends on conidia survival in the field environment (Benz, 1987). Conidia survival may be affected either by environmental factors (Furlong and Pell, 1997) or chemical products used to protect plants (Anderson and Roberts, 1983). Abdel-Rahman, *et al*. (2006) controlled the cereal aphids 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 fungus, *Isaria fumosorosea Metarhizium*

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flavoviride (bio-insecticide) against corn insect pests *Sesamia cretica*, *Ostrinia nubilalis* and *Chilo agamemnon* under laboratory and field conditions.

2. Materials and Methods

Tested Insects

The three target insect pests, *S. cretica*, *O. nubilalis* and *C. agamemnon* reared on corn leaves under laboratory conditions 26±2°C and 60±5% RH. Leaves changed every second day.

Entomopathogenic Fungi

The fungus, *Isaria fumosorosea M. flavoviride* 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 (Alves *et al.*, 1998). Plating was performed according to the full dish method. The conidia were transferred from the Eppendr 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. The Nano fungi prepared Nanoparticles were synthesized by h y- drolyzing titanium tetra isopropoxide in a mixture of 1:1 anhydrous ethanol and water. 9 ml of titanium tetra isopr o-poxide is mixed with 41ml of anhydrous ethanol (A). 1:1 ethanol and water mixture is prepared. (B) Solution A is added in drop wise to solute ion B and stirred vigorously for 2hrs. At room temperature hydrolysis and condensation are performed, using 1M sulphuric acid and stirred for 2 hrs. Then the ageing was undertaken for 12hrs. The gel was transferred into an autoclave and tightly closed, and the mixture was subjected to hydrothermal treatment at 353K for 24hrs. After filtration the solid residue was washed thoroughly with water and ethanol mixture, dried at 373K in an oven and calcined at 773K.

Efficacy of Entomopathogenic nano-Fungi Against Pests Larvae

Nano-spores of the nano-entomopathogenic fungi; *Isaria fumosorosea M. flavoviride* , were prepared by the National research center team microbiological team according to Leiderer *et al*. (2008)..

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 Kg were determined. The Yield loss was estimated according to the following equation:

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

Potential yield was *Nano- M. flavoviride* treatment (the best result among the tested pathogens) was considered the standard for comparison with the other ones.

3. Results and Discussion

In-vitro effect of nano-Entomopathogenic fungi on the target insects

The effect of nano *M. flavoviride* show that the obtained LC_{50} recorded$88x10^4$, $76x10^4$ and $106x10^4$ spores/ml for *Ostrinia nubilalis*, *Sesamia cretica and Chilo agamemnon* respectively (Table 1). Results showed that the LC_{50} of the nano *Isaria fumosorosea* of the corresponding insect pests recorded, 56 $x10^4$, 68 $x10^4$68 $x10^4$ and95 $x10^4$ spores/ml ., respectively (Table 2).

Table 3 show that the infestation of the target insect pests were significantly decreased during both successive seasons , during season 2014the infestations of *O. nubilalis* were significantly decreased after 90 days to 18±0.2 and 22±1.0 in plots treated with *Nano-M. flavoviride* ***and*** *Nano-I. fumosorosea* respectively as compared to89±9.1 individuals in the control(Table 3). *S. cretica* significantly decreased to 19±0.3 and26±2.1 individuals when treated with and *Nano-I. fumosorosea* respectively as compared to 97±2.9 individuals in the control during season 2014. The same results obtained when *C.* agamemnon treated with both nano fungi.

The results match with Sabbour and Abdel-Rahman (2007), 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* (*V.l*), *Nomuraea rileyii* (*N.r*) and *Paecilomyces fumosoroseus* (*P.f*), respectively against the three pests ranged between $5.4x10^6$ and $1.43x10^7$ spores/ml. Satisfactory results with the entomopathogenic fungi were reported by Sharaf El-Din (1999) and Sabbour and Ismail (2001). Sabbour and Abd El-Aziz (2002) as they found that the fungi; *B. bassiana* and *M. anisopliae* reduced the LC_{50} of *S. littoralis* under laboratory conditions.

Data in table (3) show that the application of the bioinsecticides which affected on decreasing the infestation, the number of infestations of *O. nubilalis* and *Sesamia cretica* significantly decreased to 20±2.9 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

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the number of corn pests were significantly decreased. *Chilo agamemn* 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.* (2000) and Espin *et al*. (1989) on their work on *C. capitata.*

The yield loss found in table (4) which showed that the weight of corn in the field during season 2013, 4089±82.80 and 4999±66.73 after treatments with *Nano-M. flavoviride* and *Nano-I. fumosorosea* respectively as compared to 2611±33.80 in the control . the percentage of yield loss 47% in the control and 0.1% in the plots treated with *Nano-I. fumosorosea.*

During season 2014 the results show that the weight of corn wer3e significantly decreased to in the control as compared to and in plots treated with *Nano-I. fumosorosea and Nano-M. flavoviride* ., respectively. The percentage of yield loss are 0.02% and52% in plots treated with *Nano-I. fumosorosea* and in the control (Table 4).

Figure (1 & 2) show that the percentage of infestations were significantly decreased durin both two seasons

These results are also agree with Sabbour & Shadia Abd El-Aziz (2002 and 2010) and Shadia Abdel Aziz & Nofel (1998), 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.* (2000) who reported that the virulence of *B. bassiana* against *C. capitata* ranged between 8 to 30% and decrease the infestation among the olive fruits. Espin *et al*. (1989) recorded that *C. capitata* mortality ranged between 69 and 78% after bioinsecticides treatments.

The results were matched with Abdel-Rahman & Abdel-Mallek (2001), Abdel-Rahman (2001) and Abdel-Rahman *et al*. (2004), 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 (2005, 2007), Tanda and Kaya (1993) and Sahab and Sabbour (2011) found that the fungi reduced insect infestations of cabbage and tomato pests under laboratory and field conditions.

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Table 1: Effect of nano-*M. flavoviride* against the target insect pests larvae under laboratory conditions

Insects	*LC_{50}*	*slope*	*Variance*	*95% confidence limits*
Ostrinia nubilalis	$88x10^4$	0.1	1.01	69-129
Sesamia cretica	$76x10^4$	0.2	1.00	65-140
Chilo agamemnon	$106x10^4$	0.1	1.03	119-187

Table 2: Effect of nano-*Isaria fumosorosea* against target insect pests under laboratory conditions

Insects	LC_{50}	slope	Variance	95% confidence limits
Ostrinia nubilalis	$56 x10^4$	1.01	0.02	41-76
Sesamia cretica	$68 x10^4$	0.10	1.01	54-87
Chilo agamemnon	$95 x10^4$	0.10	1.01	85-109

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

Post 1st application date	Treatments	Number of infestation (Mean±SE) during the two seasons					
		Ostrinia nubilalis		*Sesamia cretica*		*Chilo* agamemnon	
		2013	2014	2013	2014	2013	2014
20	Control	44±6.2	68±9.8	61±2.5	79±9.7	69±9.9	78±9.9
50		69±8.9	76±9.1	79±3.4	88±8.9	85±7.9	91±9.3
90		79±8.3	89±9.1	88±5.1	97±2.9	98±9.9	99±9.9
20	*Nano-M.*	12±0.1	14±0.1	16±4.1	15±1.0	16±0.3	17±0.2
50	*flavoviride*	16±0.2	15±0.1	20±0.7 24±0.4	23±0.2	19±0.4	19±0.4
90		20±1.0	18±0.2		19±0.3	20±0.3	20±0.1
20	*Nano-I.*	19±3.1	20±0.1 21±1.0	21±2.1	21±2.0	21±3.1	21±2.1
50	*fumosorosea*	20±1.2 22±0.2	22±1.0	22±3.1 25±1.1	23±0.2	24±2.1 25±1.1	22±1.0
90					26±2.1		24±1.3
F-value		22.2	37.3	34.1	28.8	30.1	24.8
LSD at 5%		11.2	14.4	13.8	15.0	16.8	16.7

Table 4: Assessments of damage caused in corn crop field after the nano- entomopathogenic fungi treatment

Treatments	Season 2013		Season 2014	
	Weight of corn crop (kg/ feddan)	% yield loss	Weight of corn crop (kg/feddan)	yield loss %
Nano-M. flavoviride	4089±82.80	0.1	4990±69.83	0.02
Nano-I. fumosorosea	4999±66.73	-	5099±49.74	-
Control	2611±33.80	47	2400±33.10	52
F-value	33.6		32.9	
LSD at 5%	126.7		126.5	

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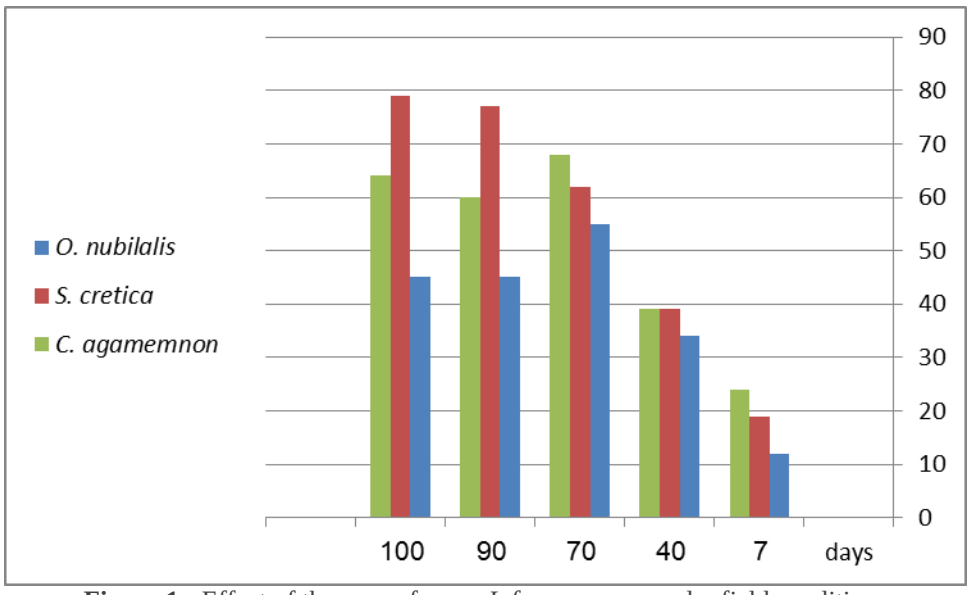

Figure 1: Effect of the nano- fungus *I. fumosorosea* under field conditions

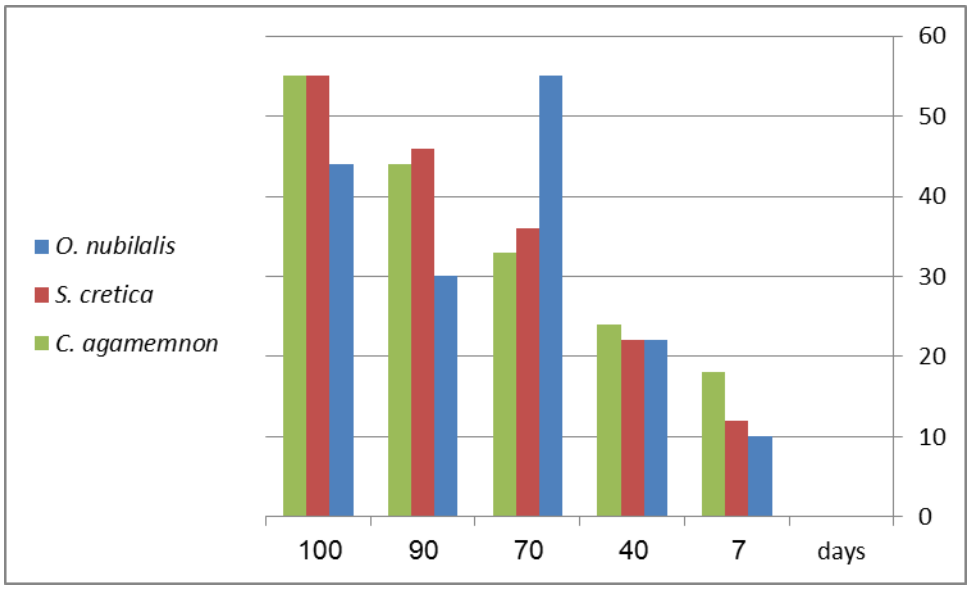

Figure 2: Effect of the nano- fungus M. flavoviride under field conditions

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