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The Toxicity Effect of Nano Fungi *Isaria fumosorosea* and *Metarhizium flavoviride* Against the Potato Tuber Moth, *Phthorimaea operculella* (Zeller)

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Abstract

The effect of the two nano-entomopathogenic fungi *Isaria fumosorosea* and *Metarhizium flavoviride* tested under laboratory green house and field conditions against the potato tuber moth *Phthorimaea operculella*. Results showed the LC50 of the nano fungi *I. fumosorosea* and *nano-M. flavoviride* which recorded that 103X10^4 and 89 X10^4 spores/ml, the corresponding LC50 under semifield conditions recorded 50X10^4 and 47 X10^4 spores/ml. The effect of the nano entomopathogenic fungi against the potato tuber moth *P. operculella* life cycle which show that the number of eggs laid per female were significantly decreased to23±8.4 and366±8.7 eggs/female after *nano-I. fumosorosea* and *nano-M. flavoviride* respectively as compared to 366±8.7 eggs / female in the control. The percentage of egg hatching significantly decreased to 2 and 1 after *I. fumosorosea* and *M. flavoviride* treatment as compared to 100% in the control. The yield assessments is showmen that the highest weight of the potatoes crop recorded 12.67±41.11 and 11.1± 15.16 Ton / feddan after *nano-M. flavoviride* and *nano-I. fumosorosea* treatments in the potatoes field. During season 2014 the potatoes weight were significantly increased to 12. 94±24.18 Ton / feddan after *nano-M. flavoviride* treatments as compared to 8. 94±24.18 Ton / feddan in the control.

Keywords

Isaria fumosorosea, Metarhizium flavoviride, Nano, *Phthorimaea operculella*

1. Introduction

Potato tuber moth *Phthorimaea operculella* PTM attack solanaceous crops with potato being favored. Foliar injury is due to the larvae (tubeworm) mining into leaflets, causing them to form transparent blisters, then move into stem tissue causing death. Tubers are marred when larvae reach tubers by two major means. Upon hatching from eggs laid on leaves, larvae can drop to the ground and burrow through cracks in the soil to a tuber, entering it through the eye. This is common after vine desiccation. Another common way is that the female PTM lays its eggs directly on exposed tubers at or near the eye. When the larvae hatch, they just enter the tuber through the eye making a slender tunnel along the surface or deep into the tuber (pictured). A tunnel can be detected by mounds of worm excrement (frass) appearing black at the entrance (pictured). Tunnels do not heal and are entryways for diseases most notably soft rot and dry rot. IPM programmers including chemical insecticides, polluted the environment, reduced beneficial insects, developed insecticidal resistance in the major associated pests and consequently caused inevitable outbreak (Lowery and Sears 1981).Recently, many research studies advocated the use of entomopathogenic fungi as biotic alternate; in which, contrary to the other specific microbial insecticides, have been successfully controlled a wide range of insect pests (Thungrabeab and Tongma 2007; Sabbour and Sahab 2005; 2007 and 2011).The fungi especially *Beauveria bassiana* proved highly pathogenic to aphids and whiteflies (Espinel *et al.*, 2008). The fungus (N.*rileyi*) exhibit host preferential infections in lepidopterous larvae (Ignoffo *et al.,* 1976).

In Egypt it is of the most economic important pests. Larvae cause severe damage to vegetable crops of family Solanaceae (Sarhan 2004; Soliman *et al.,* 2008; Abul-Nasr *et al.,* 1971& 1972; Sabbour, 2002). The entomopathogenic *N.*

rileyi and *B. brongniartii*is found on a wide range of material, and especially in soil. It is sometimes isolated from insects, though it appears to be a weak insect pathogen. Some isolates reduce several metabolites of the antibiotic group cephalosporins. Asmaa et al (2009), Sabbour and Abdel-Rahman, 2013, Sabbour et al, 2011, Sabbour, 2002, Sahab *et al.,* 2014 control the corn borers by different entomopathogenic fungi under laboratory and field conditions. 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 *B. bassiana* however depends on conidia survival in the field environment (Benz, 1987). Conidia survival maybe affected either by environmental factors (Furlong and Pell, 1997) or chemical products used to protect plants (Anderson and Roberts, 1983 and 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 studies aims to evaluate the efficacy the nano entomopathogenicity of the two fascinating entomopathogenic fungi *nano-I. fumosorosea* and *nano-M. flavoviride* against the serious pests of potato plants potato tuber moth *Phthorimaea operculella*.

2. Materials and Methods

2.1. Tested Insects

Standard laboratory colony of the potato tuber moth *P. operculella* was reared on potato tubers *Solanum tuberosum* as a natural host plant under controlled conditions (26±2°Cand 70±5% R.H). Eggs were obtained from the stock culture and kept in Petri-dishes till larval hatch. The rearing technique by El-Sherif, 1966 was adopted. Pupae were individually kept in specimen tubes (1×3cm) till adult emergence. Adult moth were kept inoviposition cages that consist of chimney glass (8cm in diameter and 16cm height), the lower rim of which rested on the bottom of a Petri-dish lined with a disk of filter paper (Watman) and the upper rim covered with muslin. Each cage was provided with a small piece of cotton soaked in 5% honey solution as food supply. The deposited eggs were collected and kept in Petri-dishes till larval hatching.

2.2. Cultivation of the Fungi

The fungi *M. Flavoviride* and *M. flavoviride* was kindly obtained from Prof. Dr Alain Vey, Mycology unite, National De La Research Sientifique, Univ. Montpellier. (Apopka strain 97 and reproduced in Microbiology Dept., N. R. C. Cairo, Egypt. 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 then added 3ml, Tween-80 and completed to 100 ml water and used as stock suspension with known spore concentration then kept in a refrigerator at 4 °C, from which the fungi were sub-cultured to be used in laboratory evaluation tests (infectivity and bioassay tests) adjusted as conidiophores concentration of $1X10^8$ /ml. Large amount of conidia spores, if needed, were produced by culturing the fungus on liquid medium in 1L cell culture glass bottles according to Rombach, *et al.,*1988.

2.3. Preparation of the Nano-Fungi

The extracted destruxin were prepared to nano-particles by National Research Centre microbiological team according to Leiderer and Dekorsy(2008).

2.4. Evaluation of the Fungi Effects on the Target Insect Pests

The fungi, *nano-I. fumosorosea* and *nano-M. flavoviride* at concentrations ranged from $1X\ 10^2$ to $1X10^8$ spores/ml were tested against *P. operculella* third instar larvae. Fresh leaves of potatoes were sprayed with the desired diluted suspension to the point of run off, left to dry, then put in 1 L plastic container (5containers were used/concentration/ treatment). Twenty newly larvae of each species were placed in each container and covered with muslin. Untreated leaves were sprayed by water only and used as control. The leaves were changed every other day. The experiment was repeated 4 times. The percentages of mortality were calculated after seven days and corrected according to Abbott, 1925 whileLC_{50} s were calculated through probit analysis of Finney, 1964. Nano fungi prepared in to nano according to Min Ah Kang et al (2012). All the experiments carried out at laboratory conditions (26 ± 2°C and 65± 5 %RH.).

2.5. Semi-Field (Green House) Trials

Potato plant as planted in the green house in 40 plots in each artificial infestation was made by spraying the plant with the bioinsecticides fungi *nano-I. fumosorosea* and *nano-M. flavoviride* at the concentrations of 8.25×10^8 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 Abbott, 1925while LC50s were calculated through probit analysis after Finney, 1964.

2.6. Field Trials

The field trials were carried out in the growing Potatoes during the two successive growing seasons 2013and 2014. Potatoes were cultivated at Eben-Malek farm at El –Nobaryia farm, N. R. C. The Potatoes was planted variety Giza 2) was cultivated by end of May during the two seasons in an area of about half feddan. The area was divided into plots (each about 40 m^2). Four plots were assigned for each treatment and for control as well, two rows of plants were left untreated between plots. Application of the fungi occurred at the rate of 1X 10^8 spores/ml. sprayed at the sunset. Four applications were made at 4 - weeks intervals during crop growing season. Control plots were left without any treatments. Examinations of 40 plants/plot/treatment were carried out just before the first application and seven days after last application to calculate the average reduction percentages in the target insect infestation percentages which were calculated in each treatment according to Henderson and Tiltron 1955. The agricultural practices followed the recommendations of the Ministry of Agricultural.

Twenty tubers were taken from the first 5 rows in each treatment and in the control as well.

3. Results

Table1 show that the LC50 of the nano fungi *I. fumosorosea* and *M. flavoviride* which recorded that 103X10^4 and 89 X10^4 spores/ml the corresponding LC50 under semifield conditions recorded 50X10^4 and 47 X10^4 spores/ml (Table2).

***Table 1.** Evaluation of the tested nano-fungi on the potato tuber moth under laboratory conditions.*

Target pathogen	LC_{50}	*S*	*V*	95% Confidence limits
Nano-*I. fumosorosea*	103X10^4	0.1	1.4	89-139
Nano-*M. flavoviride*	89 X10^4	1.1	1.1	97-149

***Table 2.** Evaluation of the tested nano fungi on the potato tuber moth under semi field conditions.*

Target pathogen	LC_{50}	*S*	*V*	95% Confidence limits
Nano-*I. fumosorosea*	50X10^4	0.1	1.1	39-109
Nano-*M. flavoviride*	47 X10^4	1.1	1.0	38-59

***Table 3.** Effect of the entomopathogenic fungus tested against the target insects biology.*

Target pest	No of eggs laid/female	% of egg hatching	% of larval mortality	% of malformed larvae	% of malformed pupae	% of emerged adults	% of malformed adults
nano-*I. fumosorosea*	22±1.9	2	88	88	87	1	100
nano-*M. flavoviride*	23±8.4	1	86	87	74	2	100
Control	366±8.7	100	-	-	-	100	-
F value	30.0	2	5	5	22	21	20
Lsd5%	10.1	2	3	3	11	11	10

***Table 4.** Assessments of damage caused after treatment with the entomopathogenic fungi in potato field.*

Treatments	Season 2013 Wt of Potatoes (Ton/ feddan)	Season 2014 Wt of Potatoes(Ton/ feddan)
Nano-*I. fumosorosea*	11.1± 15.16	11.59±47.91
Nano-*M. flavoviride*	12.67±41.11	12. 94±24.18
Control	9.2±15.83	8. 94±24.18

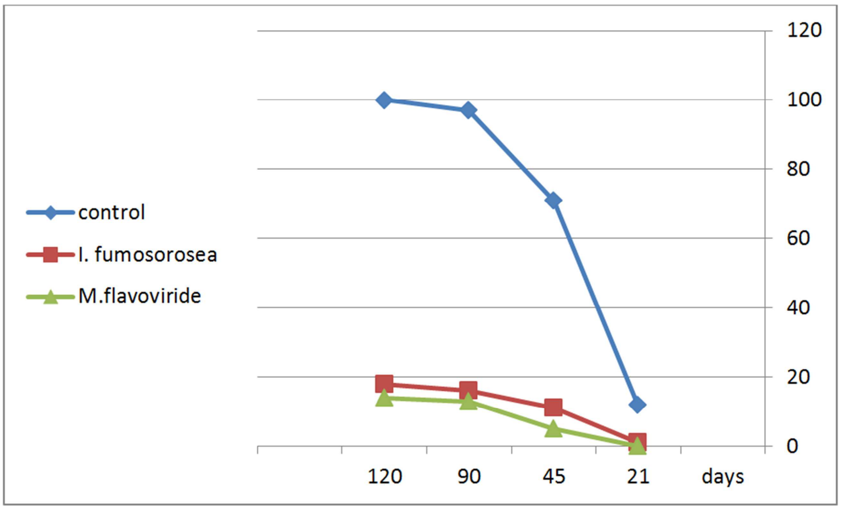

***Figure 1.** Infestation percent during 2013 after nano-fungi treatments in potatoes field.*

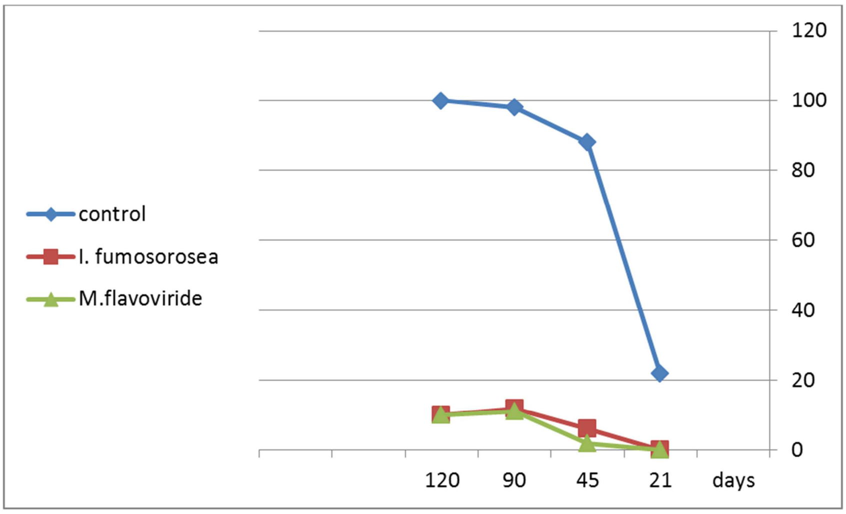

Figure 2. *Infestation percent during 2014 after nano fungi treatments in potatoes field.*

Table3, show the effect of the nano entomopathogenic fungi against the potato tuber moth P. operculella life cycle which show that the number of eggs laid per female were significantly decreased to 23±8.4 and 366±8.7 eggs/female after *nano-Isaria fumosorosea* and *nano-Metarhizium flavoviride* respectively as compared to 366±8.7 eggs/ female in the control. Percentage of egg hatching significantly decreased to 2 and 1 after *Isaria fumosorosea and Metarhizium flavoviride* treatment as compared to 100% in the control (Table3).

The yield assessments is showmen in table 3 which indicate that the highest weight of the potatoes crop recorded 12.67±41.11 and 11.1± 15.16 Ton/ feddan after *Metarhizium flavoviride nano-M. flavoviride* and *nano-I. fumosorosea* treatments in the potatoes field. During season 2014 the potatoes weight were significantly increased to 12. 94±24.18 Ton/ feddan after *M. flavoviride treatments* as compared to 8. 94±24.18 Ton/ feddan in the control (Table 4).

Figure1 and 2 show that the infestations with the potato tuber moth *P. operculella* were significantly decreased during the both season 2013 and 2014, after both nano- fungi, *nano-M. flavoviride* and *nano-I. fumosorosea* treatments.

4. Discussions

(Sabbour &Sahab 2005, 2007, Tanda and Kaya, 1993 and Sahab and Sabbour 2011) found that the fungi *B. bassiana, M. anisopliae, Pacilomyces fumosoroseus Verticillium lecanii;* reduced insect infestations of cabbage and tomato pests under laboratory and field conditions. Sabbour and Abdel-Rahman 2013 found that, in all treatments the decreased the infestations number of corn pests which significantly decreased. Loss of the yield by Sabbour & Shadia Abd El-Aziz, 2002 and 2010, proved that applications with bioinsecticides increased the yield and decreased the infestations. They found that the infestation was reduced after fungi applications under laboratory and field conditions. Sabbour & Sahab, 2005, 2007 and 2011found that the fungi reduced insect infestations of cabbage and tomato pests under laboratory and field conditions. These results agree with Sabbour & Shadia Abd El-Aziz, 2002 and 2010, proved that applications with bioinsecticides increased the yield and decreased the infestation with insect pests.

Sabbour and singer (2013 and 2014), Sabbour 2014 found that, under laboratory conditions, the LC_{50}s, were significantly decreased when the adult female of grasshopper *Hetiracris littoralis* treated with nano-destruxin and reached to 153X10^4spores/ml. Under semi field condition, the LC_{50}s of newly hatched nymphs, last nymphal stage and adult stages, 210 X 10^4, 227 X 10^4and224 X 10^4 spores/ml.used the nano particles against the stored product insect pests, they found that the infections were significantly decreased when treated with the nano particles Sabbour (2014, a,b,c).

Sabbour and Singer 2014.a, b, c controlled the insect pests by the different entomopathogenic fungi. Sabbour and Singer 2015 used the nano bioinsecticides for controlling some pests.

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 Ismail, 2002, Sabbour and Sahab 2005 &2007, 20011.These results agree with Sabbour & Shadia Abd El-Aziz, 2002 and 2010, Sabbour and Soliman, 2014 a &b. The same results obtained by Sabbour, 1992 who find that the potato tuber moth affected by the different formulations of the *Bacillus thuringiensis* and the fungus *B. bassiana* causes a higher mortality to the target pests. The same findings recoded by Sabbour, 1995, 1998 who control *Earias insulana* by the microbial control agents. Fadel and Sabbour, 1998 and 2002 could to produce the microbial control agents on the coffee and Dairy media. Sabbour, 2002 a&b, could to enhance the microbial pathogen by added different additive to the microbial control agents. Sabbour 2001 study the biochemical of the microbial control agents bacteria and fungi against *E. insulan*a, Sabbour and Ismail control potato tuber moth by the combinations between the microbial control agents and the plant extract. Ismail and Sabbour, 2002 ,2003 and 2006 studied the effect of terpines and microbial control agents against cotton bollworms can find that the cotton bollworms decreases after treatments in both laboratory and field conditions. Sabbour and Shadia. E. Abed El-Aziz, (2002, 2007, 2010, 2014). Sabbour *et al*., 2012 used the microbial control agents with plant extracts. The results obtained by Sabbour, 2007 a&b, Sabbour 2008, 2009, 2012 (a&b). Sabbour 2013 a, b, c, d, e, f, g, h, I j, k, l, m also studding the nanotechnology and microbial control agents against stored products under laboratory and store conditions. Sabbour *et al*., 2012 a, b, c, d found that the chemical additives enhance the microbial control agent against pests under field conditions ,also Sabbour *et al.,* 2011 a,b. Sabbour *et al.,* 2010 and 2009 used UV to enhance the bacteria *B. thuringiensis* against the potato tuber moth. Sabbour and Sahab 2005, 2007 2011. Sabbour and Abbas 2006 and 2007 find the same obtains. Sabbour and Hany 2007 a, b. Sabbour 2007 a and b. Sahab and Sabbour 2011, Sahab *et al.,* 2014, Sabbour and Abel-Rahman, 2007, 2012, 2014, Sabbour and Abdel-Raheem, 2013 and 2014. Sabbour, 2014 (a,b,c). Salama *et al.,* 1994, 1995, 1996 and 1997.

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