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USAGE OF NANOTECHNOLOGY OF THE FUNGI *NOMURAEA RILEYI* AGAINST THE POTATO TUBER MOTH *PHTHORIMAEA OPERCULELLA* (ZELLER) UNDER LABORATORY FIELD AND STORE CONDITIONS

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Abstract

The effect of the two entomopathogenic fungi *Nomuraea rileyi* tested under laboratory green house and field conditions against the potato tuber moth *Phthorimaea operculella*, results show that the LC_{50} of the potato tuber moth *P. operculella* under laboratory conations which cleared that ,it reaches to $76X10^4$, $98\ X10^4$, $100\ X10^4$ and $123\ X10^4$, $140\ X10^4$ spores/ml for 1st , 2nd, 3rd , 4th , and 5th larval instars., respectively. The corresponding LC_{50} recorded under semi field conditions, $88X10^4$, $105\ X10^4$, $109\ X10^4$, $133\ X10^4$ and $149\ X10^4$ spores/ ml respectively. When the nano entomopathogenic fungus *N. rileyi* treat the different larval star of the potato tuber moth under laboratory conditions the result showed that the LC_{50} of the corresponding stages , $88X10^4$, $105\ X10^4$, $109\ X10^4$, $133\ X10^4$ and $149\ X10^4$ spores/ ml for 1st, 2nd,, 3rd, 4th, and 5th larval instar .,respectively. The effect of the nano-entomopathogenic fungi *N. rileyi* on egg laid/ female were significantly decreased to 11±1.6 eggs/female as compared to 399±6.7 eggs/ female in the control. The percentage of malformed adults reached to 97% after nano *N. rileyi*. The yield assessment detected that the yield weight obtained 11.995± 50.96and 11.256±91.71 tons/ feddan after treatment with nano *N. rileyi* and *N. rileyi* as compared with 8.367±50 in the control during season 2013. During season 2014 the potato weight were significantly increased 12.279±87.66 ton / feddan after nano-*N. rileyi* treatments.

Keywords: *Nomuraea rileyi, Phthorimaea operculella* nano, Control.

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INTRODUCTION

One of the most pests injurious worldwide insect of potato is the potato tuber moth (PTM). The Larvae of (PTM) feed on every part of the plant (leaves, stems, and tubers) and have caused severe economic losses in Egypt. Potato tuber moth *Phthorimaea operculella* PTM attacks 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 (Sabbour, 1992). 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 (Sabbour, 2015; Sabbour and Singer, 2015; Sahab *et al*., 2015; Sabbour, 2003; Sabbour, 2006; Sabbour and Abdel-Rahman, 2007). *N. rileyi* and *B. brongniartii* proved highly pathogenic to aphids and whiteflies (Sabbour and Sahab, 2005). The fungus (*N.r*) exhibit host preferential infections in lepidopterous larvae (Sabbour and Sahab, 2007; El-Husseini *et al.,* 2004; Sabbour and Ismail, 2001). The

entomopathogenic *N. rileyi* 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. (Ismail *et al.,* 2002; Rombach *et al.,* 1988) 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 Singer, 2015). Commercial products have been developed with entomopathogenic fungi (Sabbour, 2003).

(Sahab *et al.,* 2015) 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. Conidia survival maybe affected either by environmental factors or chemical products used to protect plants (Sahab *et al.,* 2015). (Sabbour, 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 and entomopathogenicity of the two fascinating entomopathogenic fungi *B. brongniartii* and *N. rileyi* against two serious pests of potato plants potato tuber moth.

MATERIALS AND METHODS

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 [1] was adopted. Pupae were individually kept in specimen tubes (1×3cm) till adult emergence. Adult moth were kept in oviposition cages that consist of chimney glass (8cm in diameter and 16cmheight), 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. Groups of newly hatched .

Cultivation of the fungi

The fungi *B. brongniartii* and *N. rileyi* 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^o. 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^o, 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).

Evaluation of the fungi effects on the target insect pests

The fungus**,** *N. rileyi* and nano- *N. rileyi* at concentrations ranged from $1X\ 10^2$ to $1X10^8$ spores/ml were tested against *P. operculella* third instar larvae. Under laboratory conditions (26 ± 2^o C and 65± 5 % RH). Fresh leaves of sugar beet were sprayed with the desired diluted suspension to the point of runoff, left to dry, then put in 1L plastic container (5 containers 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) while LC_{50} s were calculated through probit analysis of (Finney, 1964).

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 *N. rileyi* and nano *N. rileyi* at the concentrations of 8.25×10^8 conidia/ml for each of the fungus. 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 LC50swere calculated through probit analysis after (Finney, 1964).

Field trials

The field trials were carried out in the growing Potatoes during the two successive growing seasons 2013and 2014. Potatoes was 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 was 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.

RESULTS

Table 1 show that the LC_{50} of the potato tuber moth *Phthorimaea operculella* under laboratory conations which cleared that it reaches to $76X10^4$, $98\ X10^4$, $100\ X10^4$ and $123\ X10^4$ $140\ X10^4$ spores/ml for 1st , 2nd, 3rd , 4th , and 5th larval instars ., respectively. The corresponding LC_{50} recorded under semi field conditions, $88X10^4$, $105\ X10^4$, $109\ X10^4$, $133\ X10^4$ and $149\ X10^4$ spores/ ml respectively (Table 2). When the nano entomopathogenic fungus *N. rileyi* treated the different larval star of the potato tuber moth under laboratory conditions the result showed that the LC50 of the corresponding stages , $88X10^4$ $105\ X10^4$ $109\ X10^4$ $133\ X10^4$ $149\ X10^4$ conidia/ ml foe 1st, 2nd,nstar respectively (Table2) 3rd, 4th, and 5th larval instar respectivey.

The effect of the nano-entomopathogenic fungi *N. rileyi* on egg laid/ female were significantly decreased to 11±1.6 eggs/female as compared to 399±6.7 eggs/ female in the control (Table 3). The percentage of malformed adults reached to 97% after nano *N. rileyi* treatments as compared to zero in the control. The yield assessment showed in (Table4) which detected that the yield weight obtained 11.995± 50.96and 11.256±91.71 tons/ feddan after treatment with nano *N. rileyi* and *N. rileyi* as compared with 8.367±50. in the control during season 2013. During season 2014 the potato weight were significantly increased 12.279±87.66 ton / feddan after nano-*N. rileyi* treatments. Figures 1 and 2 show that the infestations of laval stages were significantly decreased after the entomopathogenic fungi.

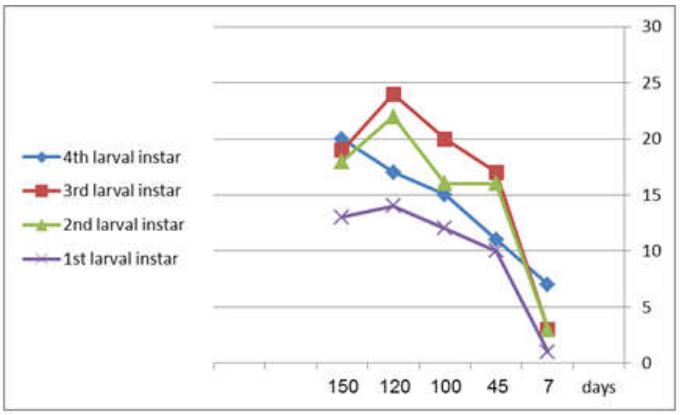

Figure 1. Infestation percent during 2013 after fungi treatments in potatoes field

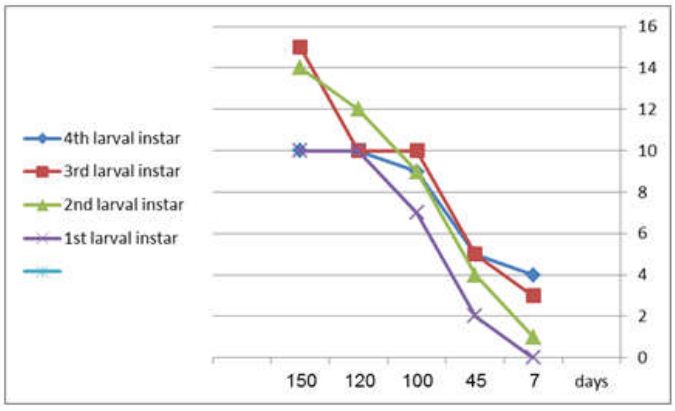

Figure 2. Infestation percent during 2014 after fungi treatments in potatoes field

Table 1. Evaluation of the tested nno fungus N. rileyi on the potato tuber moth larval instar under laboratory conditions

Insect pest	LC_{50}	*S*	*V*	95% *Confidence limits*
1st larval star	$76X10^4$	0.1	1.1	70-99
2nd larval instar	$98\ X10^4$	1.1	1.0	88-109
3rd larval instar	$100\ X10^4$	0.1	1,0	87-122
4th larval instar	$123\ X10^4$	0.1	1.2	99-133
5th larval instar	$140\ X10^4$	0.3	1.2	101-156

Table 2. Evaluation of the tested nano fungus *N. rileyi* on the potato tuber moth larval instar under semi field conditions

Insect pest	LC_{50}	*S*	*V*	95% *Confidence limits*
1st larval star	$88X10^4$	0.1	1.1	70-99
2nd larval instar	$105\ X10^4$	1.1	1.0	88-109
3rd larval instar	$109\ X10^4$	0.1	1,0	87-122
4th larval instar	$133\ X10^4$	0.1	1.2	99-133
5th larval instar	$149\ X10^4$	0.3	1.2	101-156

Statistical analysis of using some Nanotechnology treatments on potato insect life cycle

Table (3) show that there are clear differences between the various fungal treatments on potato insect life cycle, Where the treatment resulted in a fungus Nano-*N.rileyii* Positive impact on the reduction of all Know of eggs laid/female, % of malformed larval, % of emerged adults,% of malformed adults . While the treatments resulted in the fungus *N. rileyi* %of larval mortality, % of malformed pupae, compared to other fungal treatments as shown in the Table (3). The study used the analysis of variance test (Nayera Solieman, 1998) between the various treatments (fungal treatments) nanotechnology. It was found that there were significant differences between the various treatments and some stages of the life cycle of insects that represented in No of eggs laid/female, % of egg hatching, % of emerged adults,% of malformed adults.

The estimated significant relationships has proved significant statistically at the level of moral 0.05 , While estimated significant relationships did not proved between different treatments and some stages of the life cycle of insects that represented in %of Larval mortality, % of malformed larval , % of malformed pupae. The statistical analysis results also showed that the treatment of Nano-*N.rileyii* has the most important impact on the insect's life cycle, as shown in the Table (5) and the estimated relationships has proved significant statistically at the level of moral 0.05

Economic impacts resulting from the treatment of various fungal potato insects

Table (4) show that the treatments of various fungal contributed to the increase of production per acre potato crop in each of the seasonal production 2013,2014 by about 43.4% and about 54.8 % of the fungus Nano-*N. rileyi*, and about 34.5% and about 58.6% of the fungus *N. rileyi*, of each respectively. It shows also that treatment using various fungi resulted in increased production feddan of the crop in the productive season in 2014 by about 2.4% and about 11.7% compared to the productive season in 2013. Which can

resulted in increased the size of total production of the crop during the coming period in the case of the State adoption the application ways to combat insects potato crop by fungal treatments as mentioned above by about 2.3 million tons in the case of treatment of the fungus Nano- *N. rileyi*, and about 2.2 million tons in the case of treatment the fungus *N. rileyi*, and thus can increase the Egyptian exports of the crop size of 892 million USD (www.aoad.org/AASYXX.htm).

Latif *et al*., 2010; Sabbour and Hany, 2007; Sabbour and Hany, 2007; Sahab and Sabbour, 2011; Sahab *et al.,* 2014). (Sabbour, 2007b; Sabbour, 2009; Sabbour and Abd-El-Raheem, 2013), these results agree with (Sabbour, 2013b; Sabbour and Abdel-Rahman, 2007; Nayera Solieman, 1998; Espinel Sabbour *et al.,* 2015). The same results obtained by (Sabbour, 1992) who find that the potato tuber moth affected by the different formulations of the

Table 3. Effect of the entomopathogenic fungus tested against the target insect's biology under laboratory conditions

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-*N. rileyii*	11±1.6	2	61	96	77	3	97
N. rileyi	43±5.9	2	77	69	79	29	75
Control	399±6.7	100	-	-	-	100	-
F value	30.0	2	3	1	2	9	19
Lsd5%	10.4	2	2	3	10	19	8

Table 4. yield assessments of damage caused after treatment with the nano entomopathogenic fungi *N. rileyi* in potato field

Treatments	Season 2013 Wt of Potatoes (Ton/ feddan)	Season 2014 Wt of Potatoes(Ton/ feddan)
Nano- *N. rileyi*	11.995± 50.96	12.279±87.66
N. rileyi	11.256±91.71	12.079±84.78
Control	8.367±50.13	7.933±41.59

Table 5. The most important impact of nanotechnology treatments on potato insect life cycle

Insect's life cycle	Treatment	calculated Value (F)	The level of significant
No of eggs laid /female	Nano- *N. rileyi*	8.516	0.05
% of egg hatching	Nano- *N. rileyi*,	49.000	0.05
% of emerged adults	Nano- *N.rileyi*,	6.647	0.05
% of malformed adults	Nano- *N.rileyi*,	5.999	0.05

Source: calculated from a table (3)

DISCUSSION

(Sabbour, 1995; Fadel and Magda Sabbour, 1998; Fadel and Magda Sabbour, 2002; Sabbour, 2002a; Sabbour and Magda, 2002b) 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 number of corn pests were significantly decreased. Loss of the yield by (Sabbour, 2007; Magda *et al.,* 2010; Sabbour and Shadia E-Abd-El-Aziz, 2007), 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, 1992; Sabbour and Singer, 2015; Sabbour and Abdel-Rahman, 2007;Sabbour and Shadia E-Abd-El-Aziz, 2007; Sabbour *et al.,* 2012; Magda Mahmoud Sabbour and Shadia El-Sayed Abd-El-Aziz, 2014; Sabbour *et al.,* 2003; Sabbour, 2012; Sabbour,, 2012a) found that the fungi reduced insect infestations of cabbage and tomato pests under laboratory and field conditions. These results agree with (Sabbour, 2012b; Sabbour, *et al.,* 2011; Sabbour and Abbass, 2007; Sabbour and Abbass, 2006; Asmaa *et al.,* 2009), proved that applications with bioinsecticides increased the yield and decreased the infestation with insect pests. The same results obtained by (Hassan Abdel-Latif *et al.,* 2012e; Hassan Abdel-

Bacillus thuringiensis and the fungus *B. bassiana* causes a higher mortality to the target pests. The same findings recoded by (Sabbour and Shadia El-Sayed Abd-El-Aziz, 2015), 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. (Sahab *et al.,* 2015) 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. insulant* , Sabbour and Ismail control potato tuber moth by the combinations between the microbial control agents and the plant extract. Ismail and Sabbour, (Sabbour and Singer, 2015) 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, 1992; Sabbour, 2015; Sabbour, 2006; Sabbour, 2013a; Sahab *et al.,* 2015) used the microbial control agents with plant extracts. The results obtained by (Sabbour, 2013a; Sabbour *et al.,* 2007; Sabbour and Shadia El-Sayed Abd-El-Aziz, 2015) also studding the nanotechnology and microbial control agents against stored products under laboratory and store conditions. (Sabbour and Abbass, 2006; Asmaa *et al.,* 2009; Hassan Abdel-Latif *et al.,* 2012e; Sabbour, 2009; Sabbour and Abdel-Rahman, 2007) found that the chemical additives enhance the microbial control agent against pests under field conditions, also (Sabbour, 1992) Sabbour used UV

to enhance the bacteria *B. thuringiensis* against the potato tuber moth. (Sabbour, 1992; Sabbour, 2007; Magda *et al.,* 2010; Sabbour, 2012a; Sabbour, 2013b;
Sabbour and Mand Abdel-Rahman, 2007) (Sabbour,, 2015; Sabbour, 2003; Sabbour and Sahab, 2005; Sabbour and Sahab, 2007; El-Husseini *et al*., 2004) find the same obtains. [Rombach *et al.,* 1988; Sabbour and Hany, 2007; Sabbour and Shadia El-Sayed Abd-El-Aziz, 2015; Sabbour and Singer, 2015) (Sabbour and Sahab, 2007; Sabbour and Ismail. A. Ismail, 2001; Sabbour, 2013a; Sahab *et al.,* 2015).

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