ORIGINAL ARTICLE

# EFFICACY OF BEAUVERIA BRONGNIARTII AND NOMURAEA RILEYI AGAINST THE POTATO TUBER MOTH, PHTHORIMAEA OPERCULELLA (ZELLER)

| Magda Sabbour | Mohamed Abdel-Raheem |

| Pests and Plant Protection Department | Agricultural & Biological Research Division | National Research Centre |
| 33rd ElBohouth St. - Dokki | Giza | Egypt |.

## ABSTRACT

**Background:** Potato tuber moth, *Phthorimaea operculella* (PTM) attacks solanaceous crops with potato being favoured. **Objective**: The present studies aims to evaluate the efficacy and entomopathogenicity of the two entomopathogenic fungi, *B. brongniartii* and *N. rileyi* against one serious pest of potato plants. **Methods:** The effect of the two entomopathogenic fungi *Beauveria brongniartii* and *Nomuraea rileyi* were tested under laboratory at concentrations ranged from $1X10^2$ to $1X10^8$ spores /ml and experiments were conducted under laboratory conditions (26±2 ° C and 65±5 %RH.), green house (in 40 plots in each artificial infestation was made by spraying the plant with the bio insecticides (*B. brongniartii* and *N. rileyi* Fungi) at concentration 8.25 x $10^8$ conidia /ml for each fungus) and field conditions (the growing Potatoes during the two successive growing seasons 2013 and 2014. Potatoes were cultivated at Eben-Malek farm at El –Nobaryia farm, N.R.C. The Potatoes variety Giza 2) was cultivated by the end of May during the two seasons in an area of about half feddan.) **Results**: The $LC_{50s}$ for the target pest *P. operculella* were $100X10^4$ and 87 $X10^4$ spores/ml after treated with *B. brongniartii* and *N. rileyi*, respectively. Under semi-field conditions, the corresponding $LC_{50}$s recorded $120X10^4$ and 97 $X10^4$ spores/ml respectively. Under laboratory conditions the number of eggs laid/ female *P. operculella* significantly decreased to 42±1.7 and 33±8.9, eggs/female after treatment with *B. brongniartii* and *N. rileyi* as compared to 366±8.7 in the control, respectively.
**Conclusion:** Using of entomopathogenic fungi due to reduction the number of eggs laid / female after being treated with *B. brongniartii* and *N. rileyi* as compared the control. The emerged adults were decreased and the yield weight of potatoes increased in plots treated with*B. brongniartii* and *N. rileyi*. The yields weight of potatoes were significantly in plots treated with *B. brongniartii* and *N. rileyi* as compared in the control during seasons 2013 & 2014.

***Keywords:*** *Beauveria brongniartii, Nomuraea rileyi, Phthorimaea operculella*.

## 1. INTRODUCTION

Potato tuber moth, *Phthorimaea operculella* (PTM) attacks solanaceous crops with potato being favoured. Foliar injury is due to the larvae (tuberworm) 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. Another common way is that the female PTM lays its eggs directly on exposed tubers at or near the eye. A tunnel can be detected by mounds of worm excrement (frass) appearing black at the entrance.

IPM programmers including chemical insecticides, polluted the environment, reduced beneficial insects, developed insecticidal resistance in the major associated pests and consequently caused inevitable outbreak [1]. 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 [2, 3, 4]. The fungus (N. *rileyi*) exhibit host preferential infections in lepidopterous larvae [5]. In Egypt, it is one of the most economic important pests. Larvae cause severe damage to vegetable crops of family Solanaceae [6,7]. The entomopathogenic *N. rileyi* and *B. brongniartii*is found on a wide range of material, and especially in soil. Though it appears to be a weak insect pathogen. Some isolates reduce several metabolites of the antibiotic group cephalosporins. Lowery and Sears (1981), Ismail and Sabbour(2002), mentioned that control the corn borers by different entomopathogenic fungi under laboratory and field conditions [8, 9]. Entomopathogenic fungi are found worldwide associated to insects and phytophagous mite populations, contributing to biological control of these arthropods on several economically important crops [10]. 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 [11]. Success of a pest control program using *B. bassiana* however depends on conidia survival in the field environment [12]. Conidia survival maybe affected either by environmental factors or chemical products used to protect plants [13].Used Natural plant oils and terpenes as Protectors [14].

## 2. MATERIALS AND METHODS

### 2.1 Study site:

This study was carried out at Eben-Malek farm at El –Nobaryia farm, N.R.C.Dokki, Giza, Egypt.

**2.2 Statistics:** $LC_{50}$s were calculated through probit analysis.

**2.3 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°C and 70±5% R.H). Eggs were obtained from the stock culture and kept in Petri-dishes till larval hatch. The rearing technique by [7] 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 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.

**2.4 Cultivation of the fungi:** The fungi *B. brongniartii* and *N. rileyi* were 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 and 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). Then adjusted as conidiophores concentration of $1X10^8$ spores /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 [15].

**2.5 Evaluation of the fungi effects on the target insect pest:** The fungi**,** *B. brongniartii* and *N. rileyi* at concentrations ranged from $1X\ 10^2$ to $1X10^8$ spores /ml were prepared and tested against *P. operculella* third instar larvae. Experiments were conducted under laboratory conditions (26 ± 2 ° C and 65± 5 %RH.). 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( 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) [16] while $LC_{50}$s were calculated through probit analysis of [17].

**2.6 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 bio insecticides (*B. brongniartii* and *N. rileyi* Fungi) at concentration $8.25 \times 10^8$ conidia/ml for each 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) while the $LC_{50}$s were calculated through probit analysis after [16].

**2.7 Field trials:** The field trials were carried out in the growing Potatoes during the two successive growing seasons 2013 and 2014. Potatoes were cultivated at Eben-Malek farm at El –Nobaryia farm, N.R.C. The Potatoes variety Giza 2) was cultivated by the 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 was accomplished at the rate of $1X\ 10^8$ spores/ml. Applications were made by a 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 Tilton(1955), [18].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. 2.3

## 3. RESULTS

Table 1; show the $LC_{50}$ of *B. brongniartii* and *N. rileyi* against the potato tuber moth *Phthorimaea operculella under* laboratory conditions. Results showed that the $LC_{50}$ of the target pest $100X10^4$ and 87 $X10^4$ spores /ml after *P. operculella* treated with *B. brongniartii* and *N. rileyi*, respectively (Table 1).

**Table 1**: Evaluation of the two entomopathogenic fungi, *B. brongniartii* and *N. rileyi* on the potato tuber moth, *Phthorimaea operculella* under laboratory conditions at 26 ± 2 ° C and 65± 5 %RH.

| Target pathogen | $LC_{50}$ | *S* | *V* | 95% C*onfidence limits* |
|---|---|---|---|---|
| *B. brongniartii* | $100X10^4$ | 0.1 | 1.4 | 89-139 |
| *N. rileyi* | 87 $X10^4$ | 1.1 | 1.1 | 97-149 |

Under semi-field conditions, the corresponding $LC_{50}$ recorded $120X10^4$ and 97 $X10^4$ spores /ml respectively (Table2).

**Table2:** Evaluation of the two entomopathogenic fungi, *B. brongniartii* and *N. rileyi* on the potato tuber moth, *Phthorimaea operculella* under semi field conditions**.**

| Target pathogen | $LC_{50}$ | *S* | *V* | 95% C*onfidence limits* |
|---|---|---|---|---|
| *B. brongniartii* | $120X10^4$ | 0.1 | 1.1 | 99-149 |
| *N. rileyi* | 97 $X10^4$ | 1.1 | 1.0 | 88-159 |

Under laboratory conditions the effect of the entomopathogenic fungi against *P. operculella*, the number of eggs laid/ female recorded significant reduction to 42±1.7 and 33±8.9, after being treated with *B. brongniartii* and *N. rileyi* as compared to the control, respectively. The percentage of emerged adults was significantly decreased by (4%) as compared with the control (Table 3).

**Table 3**: Effect of the two entomopathogenic fungi, *B. brongniartii* and *N. rileyi* tested on the potato tuber moth, *Phthorimaea operculella* 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 |
|---|---|---|---|---|---|---|---|
| *B. brongniartii* | 42**±**1.7 | 4 | 61 | 66 | 77 | 3 | 77 |
| *N. rileyi* | 33**±**8.9 | 2 | 76 | 67 | 74 | 23 | 78 |
| *Control* | 366**±**8.7 | 99 | - | - | - | 100 | - |
| *F value* | 33.0 | 2 | 5 | 5 | 22 | 21 | 20 |
| *Lsd5%* | 11.1 | 2 | 3 | 3 | 11 | 11 | 9 |

Under field conditions the yields weight of potatoes were significantly increased to 25.45± 55.66 and 29.67±61.11 ton/ feddan in plots treated with *B. brongniartii* and *N. rileyi* respectively as compared to17.88±55.43 Tons/ feddan in the control during2013 season. During 2014season the weight of potatoes were significantly increased to 25.97±67.91 and 29.94±54.98 Ton/ feddan as compared to 12.27±45.09 in the control (Table 4).

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

| Treatments | Season 2013<br>Wt of Potatoes (Ton/ feddan) | Season 2014<br>Wt of Potatoes(Ton/ feddan) |
|---|---|---|
| *B. brongniartii* | 25.45**±** 55.66 | 25.97**±**67.91 |
| *N. rileyi* | 29.67**±**61.11 | 29.94**±**54.98 |
| *Control* | 17.88**±**55.43 | 12.27**±**45.09 |

Figure I and 2 show that the infestations with the potato tuber moth *P. operculella* were significantly decreased during both 2013 and 2014 seasons, after fungi treatments.

**Figure 1**: Infestation percent during Season 2013 after entomopathogenic fungi, *B. brongniartii* and *N. rileyi* treatments in potatoes field.

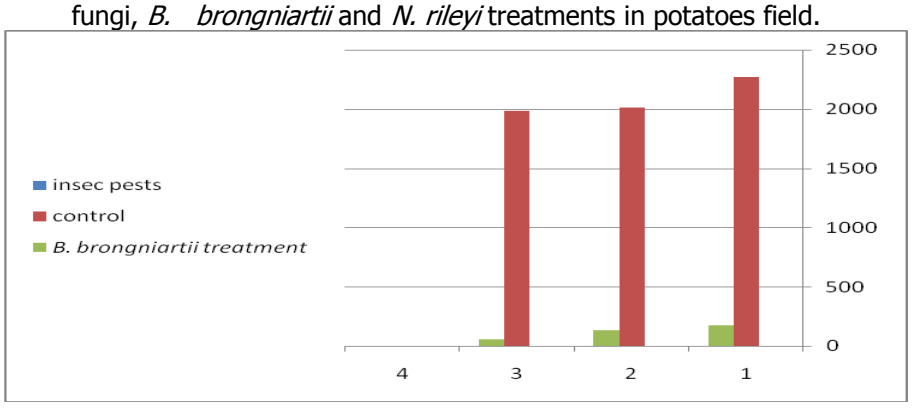
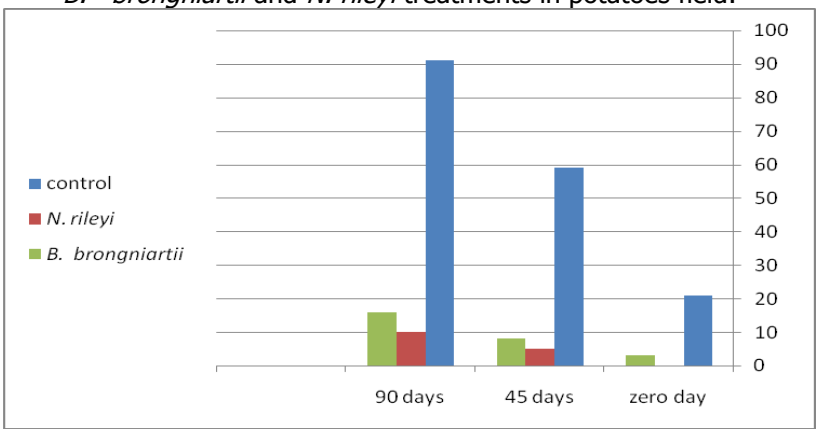



**Figure 2**: infestation percent during 2014 after entomopathogenic fungi, *B. brongniartii* and *N. rileyi* treatments in potatoes field.




## 4. DISCUSSIONS

We find in our study that the two entomopathogenic fungi due to reduction in number of eggs laid / female after being treated with *B. brongniartii* and *N. rileyi* as compared the control. The emerged adults were decreased and the yield weight of potatoes increased in plots treated with *B. brongniartii* and *N. rileyi*.

Many studies 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 [19, 20]. Alos, Sabbour and abdel-Rahman (2013), found that, in all treatments the number of corn pests were significantly decreased [21].

Moreover, these results agree with Sahab and Sabbour (2011) and Sabbour (2014a & b), [22, 23]. 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 [24]. The same findings recoded by Sabbour (2002), who control *Earias insulana* by the microbial control agents [25]. Fadel and Magda Sabbour (1998 & 2002), could to produce the microbial control agents on the coffee and Dairy media [26, 27]. Sabbour and Ismail (2001), Control potato tuber moth by the combinations between the microbial control agents and the plant extract [28]. Magda Sabbour and Shadia (2002) and Sabbour (2003 & 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 [29, 30, 31]. Magda and Shadia (2014) and Mamdouh and Sabbour (2013), Used two entomopathogenic fungi alone or in combination with modified diatomaceous earth to Control of *Bruchidius incarnates* and *Rhyzopertha Dominica*. Mamdouh And Sabbour (2013) Using of Nomuraea rileyi and Isaria Fumosorosea on some serious pests and the pests' efficient predator prevailing in tomato fields in Egypt [32, 33]. According with Hanafi (1999) mentioned that biological insecticides in particular were more effective in preventing losses by insects in stores in cases where the initial level of infestation was relatively low [34].

## 5. CONCLUSION

Using of entomopathogenic fungi due to reduction the number of eggs laid / female after being treated with *B. brongniartii* and *N. rileyi* as compared the control.
The emerged adults were decreased and the yield weight of potatoes increased in plots treated with *B. brongniartii* and *N. rileyi*.
The yields weight of potatoes were significantly in plots treated with *B. brongniartii* and *N. rileyi* as compared in the control during seasons 2013 & 2014. T
The infestations with the potato tuber moth *P. operculella* were significantly decreased during both 2013 and 2014 seasons, after fungi treatments.

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