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Pathogenicity of Three Different Entomopathogenic Fungi, *Metarhizium anisopliae* IRAN 2252, *Nomuraea rileyi* IRAN 1020C and *Paecilomyces tenuipes* IRAN 1026C Against the Potato Tuber Moth, *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae)

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Abstract The potato tuber moth (PTM), *Phthorimaea operculella* Zeller, is a prominent pest of potato in many potato cultivation areas such as Iran. Since the potato is one of the most important sources of human food, non-chemical control of the pest is essential. Application of entomopathogenic fungi is a low-risk alternative method to synthetic chemical insecticides. Therefore, we examined the efficacy of *Metarhizium anisopliae* IRAN 2252, *Nomuraea rileyi* IRAN 1020C and *Paecilomyces tenuipes* IRAN 1026C against neonate larvae and eggs of *P. operculella* under laboratory (26 ± 1 °C, 60 ± 5% relative humidity (RH) and a photoperiod of 16-h light: 8-h dark (L:D)) and greenhouse (26 ± 2 °C, 55 ± 5% RH and a photoperiod of 14-h L: 10-h D) conditions. Probit analysis of toxicity showed that *N. rileyi* was the most effective fungus against neonate larval penetration into potato tuber with a lethal concentration resulting in 50% mortality (LC_{50}) equivalent to 1×10^3 conidia/ml. Moreover, adult emergence and first generation progenies of PTM were reduced when the LC_{25} value of each fungus was used. *N. rileyi* showed the highest toxicity against neonate larvae, pupae and adult emergence of PTM under laboratory and greenhouse conditions with LC_{50} values equivalent to 2.6×10^2 and 4.2×10^3 conidia/ml, respectively. *N. rileyi* caused significant egg hatching reduction ($LC_{50} = 4.8 \times 10^4$). The results indicated that these entomopathogenic fungi had ovicidal and larvicidal activities against *P. operculella* and can be used in integrated pest management (IPM) programmes.

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Introduction

Potato (*Solanum tuberosum* L.) belongs to the Solanaceae family and is one of the world's major food crops. The potato tuber moth (PTM), *Phthorimaea operculella* Zeller (Lepidoptera: Gelechiidae), is an important and prevalent pest of potato in 90 countries in Africa, Asia, Central and South America with tropical and subtropical climates (Sporleder et al. 2004; Jensen et al. 2005; Lacey et al. 2010). It causes direct damage by feeding on leaves, stems and tubers, and indirect damage by promoting growth of pathogens. Hence, it causes a severe reduction in quality and quantity of tubers in most potato cultivation areas (Valencia-Jimenez et al. 2008). Damage by the pest in appropriate conditions in storage is more than in the field (Das 1995).

Control of *P. operculella* is primarily accomplished through using conventional insecticides. Chemical control has resulted in the development of resistance in *P. operculella*, and its negative environmental impact has encouraged development of alternative pest management strategies, in which microbial control may play a fundamental role (Collantes et al. 1986; Llanderal-Cazares et al. 1996). The present contribution is an attempt to assess different components of IPM (integrated pest management) in controlling the potato tuber moth. Entomopathogenic fungi are not only safe alternatives to synthetic insecticides but also compatible with synthetic and botanical pesticides. Additionally, they are insect-specific pathogens (Neves et al. 2001; Patil1 et al. 2014).

Some laboratory and field studies have demonstrated the insecticidal activity of three entomopathogenic fungi, *Metarhizium anisopliae* (Metchnikoff) Sorokin, *Nomuraea rileyi* (Farlow) Samson and *Paecilomyces tenuipes* Samson (Santi et al. 2010), in which *N. rileyi* was a good candidate for potato tuber moth management (Sabbour and Singer 2014; Sabbour and Abdel-Raheem 2015). Ignoffo (1981) reviewed insecticidal activity of *N. rileyi* to lepidopteran pests and concluded that the fungus was a potential agent for further development as a bio-insecticide. *N. rileyi* secretes a proteinaceous substance inhibiting larval moult and metamorphosis (Kiuchi et al. 2003). The entomopathogenic filamentous fungus *M. anisopliae* is popular as a pathogen of arthropods applied in biological control strategies against numerous serious insect pests (Santi et al. 2010). Some *Paecilomyces* isolates produce several metabolites of the antibiotic group cephalosporins (Sabbour and Singer 2014).

The aim of our study was to determine ovicidal and larvaecidal activity of three native entomopathogenic fungi, *M. anisopliae*, *N.rileyi and P. tenuipes*, against *P. operculella* to discover the most effective one to include in IPM of PTM.

Materials and Methods

Insect

The colony of potato tuber moth was obtained from the University of Mohaghegh Ardabili, Ardabil, Iran, and reared on potato cultivar Agria. Experiments were conducted under laboratory (26 ± 1 °C, 60 ± 5% relative humidity (RH) and a photoperiod

of 16-h light:8-h dark) and greenhouse (26 ± 2 °C, 55 ± 5% RH and a photoperiod of 14-h light:10-h dark) conditions.

Culture of Fungal Species

Three entomopathogenic fungal species (*M. anisopliae* IRAN 2252, *N. rileyi* IRAN 1020C and *P. tenuipes* IRAN 1026C) were purchased from the Iranian Research Institute of Plant Protection, Tehran, Iran. Selection of the entomopathogenic fungi was based on their importance against lepidopteran species. Each fungal species was cultured in potato dextrose agar (PDA) at 25 ± 1 °C. After 2 weeks (enough growth and sporulation of fungi), conidia were scrapped to make an aqueous suspension with 0.02% Tween-80. The conidia suspension was filtered through three layers of sterile cheesecloth to remove mycelium and was enumerated with an improved Neubauer haemacytometer (Weber Scientific International Ltd., United Kingdom). Before the bioassay tests, spore viability percentage was determined by inoculating plates of PDA with the suspension. After 24 h, the germination rate was observed under a light microscope. Germination was considered positive when the length of germ tube was as long as the width of the conidia (Hall 1981). The germination of *M. anisopliae*, *N. rileyi* and *P. tenuipes* conidia was 97, 96 and 97%, respectively.

Bioassays

Larval Penetration

Potato-Dipping

To investigate larval penetration, each potato was dipped in spore suspensions of *M. anisopliae*, *N. rileyi* and *P. tenuipes* determined by preliminary dose setting experiments. The ranges for *M. anisopliae*, *N. rileyi* and *P. tenuipes* were 10^4–10^9, 10^2–10^6 and 10^5–10^9 conidia/ml, respectively. For controls, the tubers were immersed in 0.02% Tween-80 aqueous solution. When the water had evaporated and the tubers were dry, they were transferred into plastic containers with ventilated lids kept at 26 ± 1 °C, 60 ± 5% RH and a photoperiod of 16-h L: 8-h D. Then 20 new larvae (< 5-h old) were placed on each tuber with a soft hair brush. The criterion of larval penetration was the number of pupae and adult emergence in all experiments. There were three replicates of 20 larvae per fungal isolate and control.

We also examined adult emergence, and their progeny (F1), from neonate larvae of PTM exposed to a lethal concentration of each fungus resulting in 25% mortality (LC_{25}) and non-treated potato tubers. The procedure of this experiment was the same as the previous one. After neonate larval penetration and adult emergence, their oviposition rates were recorded up to 24, 48 and 72 h in laboratory conditions. Egg hatch was also recorded.

Leaf-Dipping in the Laboratory and Greenhouse

The fresh leaves used in leaf-dipping under laboratory conditions came from potatoes grown in the research field of Urmia University, Urmia, Iran. For greenhouse trials,

potatoes were grown in pots under greenhouse conditions and fresh leaves, attached to the plants, were used for the experiments.

M. anisopliae, *N. rileyi* and *P. tenuipes* were applied to fresh leaves of potato in laboratory and greenhouse conditions at 10^2–10^5, 4×10^1–4×10^4 and 7×10^4–7×10^8 and 10^3–10^6, 10^3–10^5 and 7×10^5–7×10^9 conidia/ml, respectively. For each trial, the fresh leaves were dipped in the spore suspensions. In the laboratory, the leaves were then dried and placed in Petri dishes. To supply humidity, wet filter papers were placed on the bottom of the Petri dishes. Then 20 new larvae (< 5-h old) were placed on each leaf. Mortality was recorded after pupae and adult emergence. The leaves were replaced every 3 days. Each treatment and the untreated control had three replications per experiment.

Egg-Dipping

M. anisopliae, *N. rileyi* and *P. tenuipes* were tested at 10^7–10^9, 4×10^1–4×10^4 and 10^6–10^{10} conidia/ml, respectively. For treatments, each filter paper contained 20 1-day-old eggs dipped in the spore suspensions of the fungi. When the water had evaporated and the filter paper was dry, they were transferred into plastic containers with ventilated lids which contained potato tubers to stimulate egg hatching and penetration into the potatoes. The plastic containers were kept under laboratory conditions. Egg hatch was observed under a light microscope after 8 days. Each experiment was replicated three times.

Data Analysis

To determine LC_{50} (lethal concentration resulting in 50% mortality) and LC_{25} values, a probit analysis of the data was done using the Statistical Package for the Social Sciences (SPSS) for Windows® release 16. Percentages were transformed to arcsin$\sqrt{x}$ before analysis of variance (ANOVA). Significant differences between means were identified using Tukey's test.

Results

Bioassays of toxicity of *M. anisopliae*, *N. rileyi* and *P. tenuipes* to neonate larval penetration of potato tubers by potato tuber moth gave LC_{50} values equal to 1.9×10^5, 1×10^3 and 2.4×10^6 conidia/ml, respectively (Table 1). *N. rileyi* exhibited the lowest LC_{50} value, and hence the highest preventive effect on PTM neonate larval penetration into potatoes and development to pupal and adult stages. Results presented in Table 2 show that egg number and hatching was significantly reduced ($F_{3,32} = 16.26$, $P < 0.05$) in F1 progenies of neonate larvae of PTM exposed to potato tubers treated (at LC_{25} value) with the three entomopathogenic fungi. The results in Tables 3 and 4 show the toxicity of leaves dipped in the three fungi to neonate larvae of PTM. Under laboratory conditions (Table 3), the most effective entomopathogenic fungus was *N. rileyi* (LC_{50} = $2.6 \times 10_2$ conidia/ml). Under greenhouse conditions (Table 4), the LC_{50} values for *M. anisopliae*, *N. rileyi* and *P. tenuipes* were 1.4×10^4, 4.2×10^3 and 3.4×10^7 conidia/ml, respectively. Hence, *N. rileyi* also demonstrated a high efficacy against neonate

Table 1 Probit analysis of three entomopathogenic fungi toxicity against neonate larval penetration of *Phthorimaea operculella* into potato tubers

Entomopathogenic fungi	LC_{25} (conidia/ml)	LC_{50} (conidia/ml)	LC_{90} (conidia/ml)	Slope ± S. E.	χ^2 (df)
M. anisopliae IRAN 2252	3.6×10^3	1.9×10^5	3.7×10^8	3.80 ± 0.05	1.38 (3)
	(3.8×10^2–1.5×10^4)	(5.7×10^4–5×10^5)	(9×10^7–3×10^9)		
N. rileyi IRAN 1020C	4.0×10^1	1.0×10^3	4.5×10^5	3.74 ± 0.06	1.05 (3)
	(0.6×10^1–1.3×10^2)	(3.7×10^2–2.2×10^3)	(1.4×10^5–2.6×10^6)		
P. tenuipes IRAN 1026C	3.8×10^4	2.4×10^6	6.0×10^9	2.62 ± 0.05	2.18 (3)
	(3.3×10^3–1.6×10^5)	(7.7×10^5–6×10^6)	(1×10^9–1.2×10^{10})		

95% fiducial limit (FL) is shown in parenthesis

Table 2 F1 progeny from neonate larvae of PTM exposed to the treated (LC_{25} value of each fungus) and non-treated potato tubers after 24, 48 and 72 h

Entomopathogenic fungi	The mean number of eggs after 24 h ± S. E.	The mean number of eggs after 48 h ± S. E.	The mean number of eggs after 72 h ± S. E.	The mean number of hatching eggs ± S. E.
M. anisopliae IRAN 2252	$11.66^{b} \pm 2.25$	$30.00^{b} \pm 2.25$	$66.66^{b} \pm 2.25$	$60.00^{b} \pm 2.93$
N. rileyi IRAN 1020C	$8.33^{b} \pm 0.76$	$25.00^{b} \pm 0.76$	$50.00^{b} \pm 0.76$	$40.00^{b} \pm 1.63$
P. tenuipes IRAN 1026C	$16.66^{b} \pm 0.35$	$43.33^{b} \pm 0.35$	$70.00^{b} \pm 0.35$	$66.66^{b} \pm 0.77$
Control	$63.33^{a} \pm 5.47$	$96.66^{a} \pm 5.47$	$166.66^{a} \pm 5.47$	$163.66^{a} \pm 4.94$

Means in columns with the same letter are not significantly different ($P < 0.05$)

larvae of *P. operculella* in greenhouse conditions. One-day-old eggs of PTM were sensitive to *N. rileyi* ($LC_{50} = 4.8 \times 10^{4}$ conidia/ml), whereas *M. anisopliae* and *P. tenuipes* had low activity ($LC_{50} = 5.8 \times 10^{7}$ and 3.2×10^{7} conidia/ml, respectively) (Table 5). Mean percentage mortalities in pupae from neonate larvae exposed to LC_{25} value of fungal suspensions of *M. anisopliae*, *N. rileyi* and *P. tenuipes* were 30, 58 and 28%, respectively (Fig. 1). Hence, neonate larvae of PTM were susceptible to the entomopathogenic fungi. In general, *N. rileyi* caused higher mortality rate than *M. anisopliae* and *P. tenuipes* (% adult emergence = 43, 62 and 69%, respectively) (Fig. 1).

Discussion

To our knowledge, this is the first investigation showing the susceptibility of *P. operculella* eggs and neonate larvae to local entomopathogenic fungi in Iran. Based on our findings, neonate larvae of PTM are susceptible to infection by entomopathogenic fungi. While 1st instar larvae of the pest are the only free-living ones, it could be a potential practical target to apply entomopathogenic fungi as bio-control measures in potato storage and field conditions.

In the present study, *N. rileyi* displayed high insecticidal activity against neonate larvae of potato tuber moth and their development to pupal and adult stages, whereas *M. anisopliae* and *P. tenuipes* exhibited lower efficacy. The entomopathogenic fungi show various degrees of pathogenicity, which is correlated with the physiology of the immune response of their hosts (Lee et al. 2005). The high virulence of *N. rileyi* may be due to a low rate of proteolytic activity, activating glucose dehydrogenase. Additionally, the number of nodules formed is significantly smaller in larvae inoculated with *N. rileyi*. Sabbour and Singer (2014) evaluated the insecticidal activity of two *Paecilomcyes* isolates in laboratory and semi-field conditions. They reported that *P. lilaceous* was more effective than *P. fumosoroseus* and that the LC_{50} values ranged from 1.06×10^{6} conidia/ml to 1.22×10^{6} for *P. fumosoroseus* and *P. lilaceous*, respectively, in laboratory condition. This is higher than our results for *P. tenuipes*.

Table 3 Probit analysis of three entomopathogenic fungi toxicity against neonate larval penetration of *Phthorimaea operculella* into potato leaves in the laboratory condition

Entomopathogenic fungi	LC_{25} (conidia/ml)	LC_{50} (conidia/ml)	LC_{90} (conidia/ml)	Slope ± S. E.	χ^2 (df)
M. anisopliae IRAN 2252	4.4×10^1	1.0×10^3	4.5×10^5	2.51 ± 0.07	1.61 (3)
	(0.6×10^1–1.4×10^2)	(4.5×10^2–2.1×10^3)	(1.1×10^5–5×10^6)		
N. rileyi IRAN 1020C	1.9×10^1	2.6×10^2	3.8×10^4	3.42 ± 0.08	2.62 (3)
	(0.4×10^1–5×10^1)	(1.2×10^2–5×10^2)	(1.4×10^4–1.8×10^5)		
P. tenuipes IRAN 1026C	2.3×10^4	7.0×10^5	4.3×10^8	3.50 ± 0.06	0.86 (3)
	(3.2×10^3–8.4×10^4)	(2.4×10^5–1.5×10^6)	(1.2×10^8–2.9×10^9)		

95% fiducial limit (FL) is shown in parenthesis

Table 4 Probit analysis of three entomopathogenic fungi toxicity against neonate larval penetration of *Phthorimaea operculella* into potato leaves in the greenhouse condition

Entomopathogenic fungi	LC_{25} (conidia/ml)	LC_{50} (conidia/ml)	LC_{90} (conidia/ml)	Slope ± S. E.	χ^2 (df)
M. anisopliae IRAN 2252	7.9×10^2 (2×10^2–1.9×10^3)	1.4×10^4 (5.6×10^3–2×10^4)	1.7×10^6 (6.2×10^5–9.8×10^6)	3.49 ± 0.07	1.74 (3)
N. rileyi IRAN 1020C	6.7×10^2 (2.4×10^2–1.2×10^3)	4.2×10^3 (2.5×10^3–6.5×10^3)	1.4×10^5 (6.9×10^4–4.6×10^5)	3.23 ± 0.11	1.45 (3)
P. tenuipes IRAN 1026C	6.3×10^5 (7.9×10^4–2.2×10^6)	3.4×10^7 (1.2×10^7–8.4×10^7)	6.8×10^{10} (1.1×10^{10}–1.3×10^{11})	2.88 ± 0.05	1.88 (3)

95% fiducial limit (FL) is shown in parenthesis

Table 5 Probit analysis of three entomopathogenic fungi toxicity against 1-day-old eggs of *Phthorimaea operculella*

Entomopathogenic fungi	LC_{25} (conidia/ml)	LC_{50} (conidia/ml)	LC_{90} (conidia/ml)	Slope ± S.E	χ^2 (df)
M. anisopliae IRAN 2252	9.4×10^6	5.8×10^7	1.8×10^9	3.36 ± 0.11	1.50 (3)
	(3.7×10^6–1.7×10^7)	(3.6×10^7–8.8×10^7)	(8.8×10^8–6.2×10^9)		
N. rileyi IRAN 1020C	1.9×10^3	4.8×10^4	2.2×10^7	2.45 ± 0.07	2.92 (3)
	(2.8×10^2–5.9×10^3)	(2×10^4–1×10^5)	(5.4×10^6–2.7×10^8)		
P. tenuipes IRAN 1026C	7.1×10^5	3.2×10^7	4.5×10^{10}	3.07 ± 0.05	2.36 (3)
	(9.2×10^4–2.5×10^6)	(1.1×10^7–7.6×10^7)	(9.3×10^9–6.4×10^{11})		

95% fiducial limit (FL) is shown in parenthesis

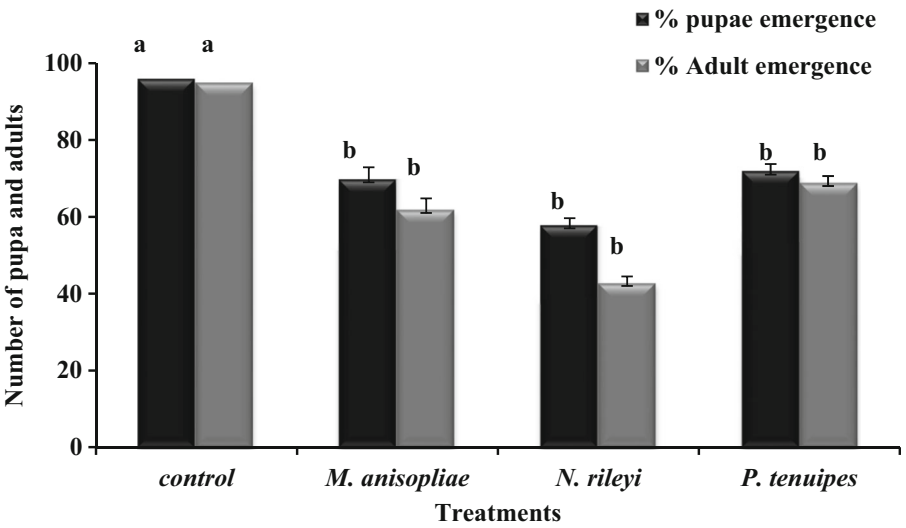

Fig. 1 Efficacy of three entomopathogenic fungal isolates on % pupae and adult emergence from neonate larvae of PTM exposed to the treated (with LC_{25} value of each fungus) and non-treated potato tubers

Based on our results, adult emergence and F1 progenies from neonate larvae of PTM exposed to treated potato tubers (with LC_{25} value of each fungus) were significantly affected by the three species of entomopathogenic fungi. Treatment of potato tubers with the three fungi significantly reduced the numbers of eggs of *P. operculella* laid and hatched. Lacy and Neven (2006) assayed the potential of the fungus *Muscodor albus* as a microbial control agent of *P. operculella* in stored potatoes. They showed that neonate larvae were significantly affected by exposure to volatiles produced by *M. albus* but adults were more sensitive than neonate larvae. Moreover, when 15 and 30 g of formulated *M. albus* mycelia were used against the neonate larvae of *P. operculella*, their development to the pupal stage was reduced by 61.8 and 72.8%, respectively, in comparison with controls. Lacey et al. (2010) examined microbial control of PTM exposed as neonate larvae to *M. anisopliae* and showed that the fungus decreased development to pre-pupal, pupal and adult stages.

In our study, *N. rileyi* presented the highest toxicity against neonate larval penetration of PTM and pupae and adult emergence in laboratory and greenhouse conditions with LC_{50} values equivalent to 2.6×10^2 and 4.2×10^3 conidia/ml, respectively. Sabbour and Abdel-Raheem (2015) noted that *B. brongniartii* and *N. rileyi* reduced the number of eggs laid/female on potato leaves relative to the control under laboratory and semi-field conditions. In addition, the yield of potatoes increased in plots treated with *B. brongniartii* and *N. rileyi*. Pathogenicity of *M. anisopliae* and *Baeuveria bassiana* was assessed against early stages of *Capnodis tenebrionis* by Marannino et al. (2006), who demonstrated that neonate larvae of *C. tenebrionis* were affected by *B. bassiana* and *M. anisopliae* isolates. However, *B. bassiana* isolates caused lower mortality than *M. anisopliae* isolates. Based on our probit analysis for 1-day-old eggs of *P. operculella*, the most effective entomopathogenic fungus was *N. rileyi* ($LC_{50} = 4.8 \times 10^4$ conidia/ml). Marannino et al. (2006) studied susceptibility of 7-day-old eggs of *C. tenebrionis* to entomopathogenic fungal isolates by egg-dipping masses

individually in a spore suspension. They showed that egg hatch was significantly reduced by entomopathogenic fungi and that *B. bassiana* isolates caused significant reduction of egg hatching relative to *M. anisopliae* isolates.

In general, our results indicated that these entomopathogenic fungi had ovicidal and larvicidal acivities against *P. operculella* and can be used in IPM programmes. However, *N. rileyi* demonstrated the highest activity in all experiments. Despite the potential of entomopathogenic fungi in pest control, these bio-control agents have some disadvantages (sensitivity to environmental factors such as moisture, light and temperature), limiting their applications in storage and field conditions. However, new formulation technologies such as nano formulation strategies can improve their efficacy and pathogenicity.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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