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Efficacy of native entomopathogenic fungi against Phthorimaea operculella (Zeller) in South Africa

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Abstract

Phthorimaea operculella is an economically important pest of potatoes. Although it is commonly managed with insecticides, there is a growing need for more sustainable, environmentally friendly control strategies. Entomopathogenic fungi (EPF) are promising alternatives to insecticides. In this study, 22 native *Metarhizium* spp., three *Beauveria* spp. isolates, and two commercial products were screened *in vitro* against 24-h-old *P. operculella* eggs, first-instar larvae, and fourth-instar larvae that were transitioning to prepupae. Eggs were directly sprayed with the EPF suspensions, and leaves used in the larval bioassays were treated by dipping them into the EPF suspensions. The fourth-instar larvae transitioning to prepupae were exposed to sterile, EPF-inoculated soil. All isolates inhibited egg hatching, with NWU-Met-5 causing the highest mortality (> 90%). NWU-Met-2, NWU-Met-4 and NWU-Met-5 were highly pathogenic to first-instar larvae with mortality rates of 75%, 87.4% and 90.9%, respectively. The prepupal stage was highly susceptible to most EPF treatments, with mortalities ranging from 80–100%. These findings indicate that EPF hold promise as biological control agents against *P. operculella*.

Introduction

South Africa is the third-largest producer of potatoes on the African continent. An average of 2.5 million tonnes per annum is produced and plays an important role as a staple food after maize (Mdoda et al. 2023; PSA 2025). Although multiple insect pests cause damage to potato crops, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), the potato tuber moth, is the most damaging (Adhari et al. 2022; Golizadeh and Abedi 2026). Larvae feed within the tubers, creating tunnels as they feed. These tunnels reduce tuber weight and quality, making them unsuitable for market sales and human consumption (Chandel et al. 2020; Adhikari et al. 2022; Korschel et al. 2026). Damage to potatoes occurs both in fields and in storage units (Adhikari et al. 2022; Zhang et al. 2026). Potato tuber moth is most commonly controlled with insecticides. However, rapid evolution of resistance towards broad-spectrum insecticides has become a major concern (Golizadeh and Abedi 2026). Therefore, there is a need to develop more sustainable approaches for the management of the potato tuber moth.

Entomopathogenic fungi represent a diverse group of fungi that are pathogenic to insects (Islam et al. 2021; Kankanamalage et al. 2025). These fungi have high insecticidal potential and are widely considered an eco-friendly alternative to chemical pesticides (Kidanu and Hagos 2020; Bihal et al. 2023; Song et al. 2025). Among more than 700 known entomopathogenic species, *Metarhizium anisopliae* and *Beauveria bassiana* are the most common species used for the biocontrol of insect pests in tropical and subtropical agroecosystems, infecting a wide range of insect pests (Kidanu and Hagos 2020; Kankanamalage et al. 2025; Ali et al. 2025). Fungal spores attach to the insect cuticle upon contact, germinate and produce hyphae that enter the hemolymph where blastospores are produced. This leads to insect death due to nutrient depletion, organ damage and/or the secretion of secondary metabolites with insecticidal activity (Vega et al. 2012; Zhang et al. 2026, Shahriari and Zibaee et al. 2026). *Beauveria* spp. and *Metarhizium* spp. are globally distributed and occur in agricultural soils, grasslands and forest fields (Karthi et al. 2024). Although soil-borne, these fungi can also occur on leaf surfaces,

endophytically in plants, or as saprophytes on insect cadavers (McGuire and Northfield 2020; Kankanamalage et al. 2025). Soil serves as a reservoir for entomopathogenic fungi (McGuire and Northfield 2020) and can therefore provide an effective source of biocontrol agents for soil-dwelling life stages of insects (Jeon and Kim 2024). The eggs, larvae and pupae of *P. operculella* are found on/in the soil (Rondon, 2010). Since multiple life stages of *P. operculella* are present in the soil (Kroschel et al. 2020; Kroschel et al. 2026), entomopathogenic fungi could be promising control agents for this pest. Entomopathogenic fungi have minimal impact on the environment and non-target organisms and offer additional benefits in potato fields, such as suppressing certain fungal diseases and nematode populations (Tomilova et al. 2020; Karabörklü et al. 2022).

Multiple laboratory studies have been conducted on *P. operculella* in countries such as Egypt, Iran, Turkey and China using *Metarhizium* spp. and *Beauveria* spp. (El-Sinary 2002; Khorrami et al. 2018; Dadasoglu et al. 2023; Song et al. 2024; Zhang et al. 2026). However, information regarding the efficacy of EPF against *P. operculella* in South Africa is limited. Currently, only two commercial EPF are registered for the control of *P. operculella* viz. Broadband® (*Beauveria bassiana* strain PPRI 5339*)* and Real *Metarhizium* 69 (*Metarhizium anisopliae* ICIPE 69). Therefore, the aim of this study was to do bioprospecting for EPF that occur in soils across different potato-producing regions of South Africa and to evaluate their efficacy against the soil-associated life stages of *P. operculella*.

Materials and Methods

Phthorimaea operculella collection and rearing

Infested tubers were collected from a pack house from Petrus Steyn, Free State province, South Africa (-27.7646940, 28.0524790) and kept in plastic containers (45 cm × 32 cm × 9 cm) with a thin layer of sand at the bottom to absorb excess moisture and provide a substrate for larvae to pupate. These containers were kept under controlled conditions in an insect rearing chamber at a temperature of 26 ± 1 ˚C, 65 ± 5% Relative humidity (RH), and a 16L:8D photoperiod. Moths that emerged from these tubers were collected with an aspirator and transferred into round, clear containers (17 cm × 14 cm × 5 cm). A 50 mL centrifuge tube, filled with 10% sucrose solution, was provided in each container as an energy source for the moths. The plastic containers were covered with fine gauze with holes smaller than 1 mm in diameter, and the gauze was secured with an elastic band. A piece of filter paper (12 cm in diameter) was put into each container to serve as oviposition substrate. To create a rough-textured surface for moths to oviposit on, one side of the filter paper was rubbed with sandpaper, placed on the gauze and moistened with 10% sucrose solution. The oviposition cages were then placed in a rearing chamber under the conditions described above. The filter papers containing eggs were removed from the containers after 24 hours, placed in plastic ziplock bags (42 cm × 30 cm) and kept in a rearing chamber at 28 ± 1°C, 60–65% RH and 14L:10D until larval hatching. First-instar larvae that were not used in bioassays were reared on potato tubers, which were kept on a thin layer of sand as a pupation substrate in plastic containers (17 cm × 14 cm ×5 cm). These containers were covered with mesh-infused lids and

kept in a rearing chamber at 26 ± 1°C, RH 60–65% and 14L:10D. Fourth-instar larvae were collected 10–14 days after inoculation of tubers with neonates.

Preparation and viability of fungal suspensions

Twenty-two EPF isolates were obtained through bioprospecting conducted in crop fields across different potato-producing regions in South Africa from July 2024 to June 2025. The commercially available isolates (Table 1) and experimental isolates (Table 2) were maintained at North-West University, Potchefstroom (Table 1).

Table 1
Commercial entomopathogenic fungal products registered for the control of *Phthorimaea operculella* in South Africa.

Commercial name and manufacturer	**Active ingredient**	**Batch concentration**	**Experimental code**	**Germination% (± SE)**
Broadband® BASF South Africa (Pty) Ltd	*Beauveria bassiana strain* PPRI 5339	4×10^9 cfu/mL	Bband	88.09 ± 2.24
Real *Metarhizium* 69 Real IPM South Africa (Pty) Ltd	*Metarhizium anisopliae* ICIPE 69	1×10^9 cfu/mL	Mt69	83.05 ± 1.97

The viability of EPF isolates and commercial products was assessed before each experiment. The *Metarhizium* spp. was sub-cultured on Sabouraud Dextrose Agar (SDA) (Neogen®) and *Beauveria* spp. on Potato Dextrose Agar (PDA) (Neogen®) and incubated at 25°C ± 2°C in total darkness. After 14–21 days, conidia were harvested from four culture plates using a sterile spatula into universal bottles containing glass beads and 10 mL of 0.05% Triton X-100. The fungal suspensions were vortexed to obtain a homogenous suspension, and concentrations were adjusted to 1×10^8 conidial/mL using an Improved Neubauer hemocytometer. For the commercial products, 1 mL was transferred to 9 mL of sterile 0.05% Triton X-100 water. A 100 µL aliquot from each suspension was spread onto three replicate plates using a glass spreader. The plates were incubated at 25 ± 1°C, 65 ± 5% RH, and in darkness for 18 hours. Sterile coverslips (22 mm × 22 mm) were placed on three selected areas on each plate. Percentage germination of the conidia was calculated by counting the number of germinated conidia per hundred conidia at 400 × magnification in selected areas as described in Goettel and Inglis (1997). A Nikon Eclipse E100 light microscope (Nikon Instruments, Tokyo, Japan) was used. Conidia were considered viable when the germ tubes were at least twice the diameter of the conidia. An EPF formulation was considered viable if germination exceeded 70% (Zampiroli et al. 2019).

Table 2
Percentage germination of the fungal isolates after 18 hours on Sabouraud Dextrose Agar and Potato Dextrose Agar.

Fungal species	Isolate code	Crop field	Mean germination% (± SE)
Beauveria spp.	NWU-Bb-2	Pecan nuts	99.77 ± 0.15
	NWU-Bb-3	Lucerne	99.30 ± 0.30
Metarhizium spp.		Potato	95.51 ± 0.63
	NWU-Met-2		
	NWU-Met-4	Fallow	95.22 ± 1.13
	NWU-Met-5	Maize	90.79 ± 1.02
	NWU-Met-7	Fallow	94.75 ± 1.17
	NWU-Met-10	Maize	99.27 ± 0.53
	NWU-Met-11	Lucerne	99.27 ± 0.31
	NWU-Met-12	Tomato	99.61 ± 0.17
	NWU-Met-13	Fallow	97.58 ± 0.5
	NWU-Met-14	Fallow	98.27 ± 0.68
	NWU-Met-15	Fallow	94.55 ± 1.33
	NWU-Met-16	Fallow	97.63 ± 0.94
	NWU-Met-17	Maize	87.62 ± 1.15
	NWU-Met-18	Maize	94.01 ± 0.84
	NWU-Met-19	Soybean	96.61 ± 0.88
	NWU-Met-20	Wheat	98.44 ± 0.44
	NWU-Met-21	Onion	93.97 ± 1.65
	NWU-Met-22	Wheat	98.35 ± 0.53
	NWU-Met-23	Oranges	98.94 ± 0.58
	NWU-Met-24	Fallow	82.91 ± 1.96
	NWU-Met-25	Maize	94.59 ± 0.96

Susceptibility eggs to fungal treatments

Susceptibility *of Phthorimaea operculella* eggs to fungal treatments

Eggs oviposited on filter paper over a 24-hour period were counted under a dissection microscope (Nikon C-DS). Filter paper pieces, each containing 30 eggs, were cut out and placed individually on moist filter paper in Petri dishes. The eggs were sprayed directly with a 2 mL spray bottle containing fungal suspension at a concentration of 1×10^8 conidia/mL. Eggs treated with sterile 0.05% Triton X-100 served as the negative control treatment. For the two positive controls, a concentration of 1.32×10^6 conidia/mL (Mt69) and 5.28×10^6 conidia/mL (Bband) were used in bioassays. Each treatment was replicated four times (30 eggs per replication). The Petri dishes were incubated at 25 ± 1°C and 65 ± 5% RH in total darkness. Eggs that were shrivelled and collapsed were marked dead. Mortality was evaluated daily for 10 days.

Susceptibility of first-instar Phthorimaea operculella larvae to treatments

Potato leaves were surface sterilised in 1.5% sodium hypochlorite solution, rinsed three times in distilled water and left to air-dry. Leaf discs of 7mm in diameter were cut using a cork borer. The leaf discs were immersed in fungal suspension and left to air-dry with the abaxial side facing upwards. Leaflets were placed individually into wells of bioassay trays (128 wells), containing 1.5% agar (Agar-Agar Powder, CP) and sealed with transparent ventilated adhesive lids (Frontier: Scientific services, Newark, Delaware, United States). Each leaflet was inoculated with one first-instar larva. The trays were maintained at 25 ± 1°C, 65 ± 5% RH and a 12:12 (L:D) photoperiod. Each treatment was replicated four times, with 10 larvae per replicate. Leaflets in the control treatment were treated with sterile 0.05% Triton X-100. Mortality was evaluated daily for 7 days. Larvae that did not respond to probing with a paintbrush were considered dead. The dead larvae were removed, surface-sterilised with 70% ethanol for 30 seconds and rinsed three times with distilled water. Subsequently, sterilised larvae were placed in a Petri dish on moistened filter paper and incubated in total darkness at 25 ± 1°C and 65 ± 5% RH. The number of cadavers that mycosed was counted after 14 days.

Susceptibility of Phthorimaea operculella prepupae to fungal treatments

Evaluation of EPF treatments against mature fourth-instar larvae transitioning to pre-pupae was done through soil drenching. Thirty grams of sterilised soil were placed into sterile Petri dishes. The soil was drenched with a 3 mL fungal suspension at 1×10^8 conidia/mL to achieve a final concentration of 1×10^7 conidia/mL, then mixed thoroughly. For the commercial products Mt69 and Bband were used at concentrations of 1.32×10^6 conidia/mL and 5.28×10^6 conidia/mL, respectively. Each Petri dish with treated soil was inoculated with five mature fourth-instar larvae, transitioning to the prepupae stage. The Petri dishes were sealed with parafilm and incubated at 25 ± 2°C and 65% RH in total darkness. Each fungal suspension was evaluated using four replicates of 10 larvae each. For the control treatments, the soil was drenched with 3 mL of sterile 0.05% Triton X-100. Mortality was recorded after 14 days. The mycosis test was carried out as previously described.

Data analyses

Metarhizium and *Beauveria* isolates were analysed separately, with each fungal species grouped with its positive control treatment, Mt69 and Bband, respectively. Data were corrected for natural mortality using Abbot's formula, where control mortality was above 5%. Median lethal time (LT_{50}) was not estimated for treatments where mortality did not reach 50%. Since the datasets displayed different patterns of distributional behaviour, model selection was performed independently for each dataset.

For *Beauveria* treatments, viability was analysed using a logistic regression in a generalised linear model (GLM) with quasibinomial distribution as overdispersion was detected. Mortality data for eggs, first-instar larvae, and the corresponding larval mycosis data were analysed using logistic regression in GLM with binomial distribution and a logit link function. Pre-pupal mortality data were arcsine-transformed to improve homogeneity and subjected to analysis of variance (Hsia et al. 2014; Maniania et al. 2022). The differences between the median lethal time (LT_{50}) values for eggs and first-instar larvae were calculated in Microsoft Excel, using an independent two-sample t-test, assuming equal variances.

Viability data from *Metarhizium* treatments were arcsine-transformed to improve homogeneity of variances before analysis of variance. Mortality data of the eggs and first-instar larvae were analysed using Firth's bias-reduced logistic regression (*brglm2* package) to account for complete separation in some groups where mortality was 0% or 100% and standard logistic methods could not estimate coefficients (Morda et al. 2025). Median lethal time (LT_{50}) for eggs and first-instar larvae was estimated the same as for *Beauveria* treatments, and differences among the LT_{50} values were based on the non-overlap of their 95% confidence intervals (95% CI) (de Sousa et al. 2023). Pre-pupal mortality and mycosis data for the first-instar larvae were arcsine-transformed and analysed using analysis of variance. Mean comparisons were conducted using Tukey's HSD post hoc test (95% confidence intervals) with the *emmeans* package. All data means in tables and figures are shown as untransformed values.

All data were analysed using R Statistical package version R 4.5.1 and Posit Assistant (Version 0.9.2) (R Core Team 2026).

Results

Viability of entomopathogenic fungal treatments

After 18 h, conidial germination of all fungal isolates exceeded 80% (Tables 1 and 2). Among the *Beauveria* treatments, the viability of the commercial Bband was lower compared to two of the isolates, NWU-Bb-2 and NWU-Bb-3 ($F_{2, 78} = 39.03$ $p < 0.001$). Significant differences among the viability of *Metarhizium* treatments were also observed ($F_{20, 708} = 21.18$, $p < 0.001$). Lower viability rates were recorded for NWU-Met-17, NWU-Met-24 and Mt69 (82.9–87.6%), while all other isolates showed conidial germination ≥ 90% (Tables 1 and 2).

Susceptibility of Phthorimaea operculella eggs to fungal treatments

Overall mortality of the eggs differed for both *Beauveria* (χ^2 = 352.66, df = 2, p < 0.001) and *Metarhizium* treatments ($F_{2, 78}$ = 39.03, p < 0.001). Among the *Beauveria* treatments, NWU-Bb-2 had the highest ovicidal effect, while treatment with Bband resulted in mortality of only 36.6% (Table 3). Both NWU-Bb-2 and NWU-Bb-3 exhibited high LT_{50} values (> 7.5 days), with significant differences between these two isolates (t = − 10.67 df = 78, p < 0.001). Of the *Metarhizium* isolates, NWU-Met-5 and NWU-Met-20 induced the highest mortality (> 90%). The median lethal times (LT_{50}) of *Metarhizium* treatments differed significantly among isolates and ranged between 6.9–9.5 days. Isolates NWU-Met-5, NWU-Met-23 and NWU-Met-24 had the lowest LT_{50} values (< 7 days) (Table 4). Eggs from the control treatment hatched on day 5. All other treatments inhibited hatching, except for NWU-Met-25 and Mt69, in which 70 and 59% eggs hatched, respectively. All treatments exhibited 100% mycosis.

Table 3
Mean percentage mortality and median lethal time (LT_{50}) of *Phthorimaea operculella* eggs 10 days after treatment with *Beauveria* isolates at a concentration of 1 × 10^8 conidial/mL and Bband at 5.28 ×10^6 conidia/mL.

Fungal species	**Isolate**	**Mean mortality (%) ± SE**	**LT_{50} (days) (95% FL)**
Beauveria spp.	Control	0.8 ± 0.83 [a]	NE
	Bband	36.6 ± 10.3 [b]	NE
	NWU-Bb-3	51.2 ± 12.5 [c]	9.3 (9.0– 9.6) [b]
	NWU-Bb-2	71.6 ± 8.6 [d]	7.5 (7.4–7.7) [a]

Mean mortality comparisons were evaluated against control mortality. Mean mortality within the column followed by the same letter does not differ significantly (P < 0.05, Tukey's HSD). LT_{50} values followed by the same letter do not differ significantly according to the t-test (P < 0.05). NE- Not estimated - LT_{50} values were not estimated for < 50% mortality.

Table 4
Mean percentage mortality and median lethal time (LT_{50}) of *Phthorimaea operculella* eggs 10 days after treatment with *Metarhizium* isolates at a concentration of 1×10^8 conidial/mL, and Mt69 at 1.32×10^6 conidia/mL.

Fungal species	Isolate	Mean mortality (%) ± SE	LT_{50} (days) (95% FL)
Metarhizium spp.	Control	0.8 ± 0.83 [a]	NE
	NWU-Met-25	8.9 ± 3.4 [ab]	NE
	NWU-Met-19	24.6 ± 4.3 [bc]	NE
	Mt69	29.4 ± 10.6 [c]	NE
	NWU-Met-22	33.1 ± 4.2 [cd]	NE
	NWU-Met-14	33.3 ± 15.8 [cd]	NE
	NWU-Met-13	41.1 ± 12.4 [cde]	NE
	NWU-Met-21	41.3 ± 3.4 [cde]	NE
	NWU-Met-18	53.5 ± 12.0 [def]	9.1 (8.9–9.3) [gh]
	NWU-Met-17	57.8 ± 11.1 [ef]	9.3 (9.1–9.5) [ghi]
	NWU-Met-11	57.9 ± 13.8 [ef]	8.2 (8.1–8.3) [cde]
	NWU-Met-7	58.0 ± 12.1 [ef]	9.5 (9.2–9.8) [hi]
	NWU-Met-10	59.3 ± 10.9 [ef]	9.5 (9.3–9.8) [i]
	NWU-Met-12	60.8 ± 8.8 [efg]	8.0 (7.6–8.1) [bcd]
	NWU-Met-24	68.8 ± 6.4 [fgh]	6.9 (6.8–7.1) [a]
	NWU-Met-23	71.6 ± 8.6 [hi]	6.9 (6.8–7.0) [a]
	NWU-Met-16	72.5 ± 7.6 [fgh]	8.5 (8.4–8.6) [ef]
	NWU-Met-15	81.3 ± 7.7 [ghi]	7.9 (7.8–8.0) [bc]
	NWU-Met-4	81.6 ± 6.4 [hi]	8.2 (8.1–8.3) [d]
	NWU-Met-2	87.0 ± 8.7 [hi]	8.8 (8.6–9.1) [fg]
	NWU-Met-20	91.3 ± 3.4 [i]	7.9 (7.8–8.0) [b]

Fungal species	Isolate	Mean mortality (%) ± SE	LT_{50} (days) (95% FL)
	NWU-Met-5	94.4 ± 2.4 [i]	6.9 (6.8–7.0) [a]

Mean mortality comparisons were evaluated against control mortality. Mean mortality within the column followed by the same letter does not differ significantly ($P < 0.05$, Tukey's HSD). LT_{50} values were significantly different when their 95% confidence intervals did not overlap, indicated by different levels within the column. NE- Not estimated - LT_{50} values were not estimated for treatments with < 50% mortality.

Susceptibility of first-instar Phthorimaea operculella larvae to fungal treatments

Among the *Beauveria* treatments, the commercial product Bband, was the most pathogenic against the first-instar larvae, with a mortality of 81.6% compared to the native *Beauveria* isolates ($\chi^2 = 21.71$, df = 2, $p < 0.001$) (Table 5). However, NWU-Bb-2 and Bband had similar LT_{50} values with no significant differences between them ($t = 0.14$, df = 54, $p > 0.05$). The different *Metarhizium* treatments showed varied levels of virulence against the first-instar larvae ($\chi^2 = 859.55$, df = 21, $p < 0.001$). Isolate NWU-Met-5 caused the highest mortality of 90.8 ± 5.1%, whereas isolate NWU-Met-15, induced the lowest mortality of 10.7 ± 7.0% (Table 6). There were significant differences in median lethal times estimated among the *Metarhizium* treatments, with LT50 values between 3.2–5.8 days (Table 6). Mycosis caused by the *Beauveria* isolate NWU-Bb-3 was low, but mycosis caused by NWU-Bb-2 and Bband was higher and did not differ significantly ($\chi^2 = 32.88$, df = 2, $p < 0.001$) (Fig. 1). Mycosis recorded for first-instar larvae differed significantly among *Metarhizium* treatments ($F_{20,63} = 3.4$, $p < 0.001$), with levels recorded ranging from 6 -100% (Fig. 2).

Table 5
Mean percentage mortality and median lethal time (LT_{50}) of *Phthorimaea operculella* first-instar larvae seven days post treatment with *Beauveria* isolates at a concentration of 1×10^8 conidial/mL and Bband at 5.28 $\times 10^6$ conidia/mL.

Fungal species	Isolate	Mean mortality (%) ± SE	LT_{50} (days) (95% FL)
Beauveria spp.	NWU-Bb-3	35.3 ± 7.6 [a]	NE
	NWU-Bb-2	66.1 ± 5.8 [ab]	4.4 (4.0–4.7) [a]
	Bband	81.6 ± 10.0 [b]	4.2 (4.0–4.4) [a]

Mean mortality within the column followed by the same letter do not differ significantly ($P < 0.05$, Tukey's HSD). LT_{50} values followed by the same letter do not differ significantly according to the t-test ($P < 0.05$).

NE- Not estimated - LT_{50} values were not estimated for < 50% mortality.

Table 6
Mean percentage mortality and median lethal time (LT_{50}) of *Phthorimaea operculella* first-instar larvae seven days post treatment with *Metarhizium* isolates at a concentration of 1×10^8 conidial/mL and Mt69 at 1.32×10^6 conidia/mL.

Fungal species	**Isolate**	**Mean mortality (%) ± SE**	**LT_{50} (days) (95% FL)**
Metarhizium spp.	NWU-Met-15	10.2 ± 7.0 [a]	NE
	NWU-Met-19	18.4 ± 2.6 [ab]	NE
	NWU-Met-13	23.0 ± 6.9 [ab]	NE
	NWU-Met-18	27.2 ± 14.4 [abc]	NE
	NWU-Met-24	27.2 ± 4.9 [abc]	NE
	NWU-Met-25	27.2 ± 4.9 [abc]	NE
	NWU-Met- 11	32.9 ± 6.1 [abcd]	NE
	NWU-Met-12	40.9 ± 7.1 [abcd]	NE
	NWU-Met-17	41.1 ± 12.2 [abcd]	NE
	NWU-Met-21	46.5 ± 6.5 [abcde]	NE
	Mt69	49.7 ± 16.9 [abcde]	5.2 (5.0–5.4) [d]
	NWU-Met-20	50.2 ± 5.8 [abcde]	6.3 (5.9–6.2) [g]
	NWU-Met-14	51.8 ± 7.0 [abcde]	5.6 (5.4–5.9) [ef]
	NWU-Met-10	52.8 ± 12.9 [abcde]	5.8 (5.9–6.1) [fg]
	NWU-Met-16	56.5 ± 7.16 [bcde]	5.4 (5.2–5.5) [de]
	NWU-Met-22	61.1 ± 15.8 [bcdef]	5.3 (5.2–5.5) [de]
	NWU-Met-23	70.6 ± 16.0 [cdef]	3.7 (3.6–3.9) [b]
	NWU-Met-7	71.7 ± 7.6 [cdef]	3.2 (3.0–3.3) [a]
	NWU-Met-2	75.4 ± 3.2 [def]	3.7 (3.6–3.8) [b]
	NWU-Met-4	87.4 ± 1.9 [ef]	3.2 (3.1–3.4) [a]

Fungal species	Isolate	Mean mortality (%) ± SE	LT_{50} (days) (95% FL)
	NWU-Met-5	90.8 ± 5.1 [f]	4.1 (4.0–4.2) [c]

Mean mortality within the column followed by the same letter does not differ significantly (P < 0.05, Tukey's HSD). LT_{50} values were significantly different when their 95% confidence intervals did not overlap as indicated by different letters within the column. NE- Not estimated - LT_{50} values were not estimated for < 50% mortality.

Susceptibility of Phthorimaea operculella prepupae to fungal treatments

Mortality induced by the *Beauveria* treatments against pre-pupae differed significantly ($F_{2,9}$ = 40.24, p < 0.001). The highest mortality was caused by the NWU-Bb-3 isolate, while the commercial product showed very low pathogenicity, causing only 11.9 ± 10.4% mortality (Table 7). For the *Metarhizium* treatments, however, the majority of the isolates were highly pathogenic to the prepupae, and mortality was > 80%. Only two treatments, NWU-Met-24 and Mt69, recorded lower mortality of 43.0 ± 9.6% and 51.8 ± 19.8%, respectively ($F_{20,67}$ = 4.34, p > 0.001) (Table 8).

Table 7
Mean percentage mortality of *Phthorimaea operculella* prepupa seven days post treatment with *Beauveria* isolates at a concentration of 1 × 10^8 conidial/mL and Bband at 5.28 ×10^6 conidia/mL.

Fungal species	Isolate	Mean mortality (%) ± SE
Beauveria spp.	Bband	11.9 ± 10.4 [a]
	NWU-Bb-2	70.0 ± 7.1 [b]
	NWU-Bb-3	96.9 ± 3.0 [c]

Means within the column followed by the same letter do not differ significantly (P < 0.05, Tukey's HSD).

Table 8
Mean percentage mortality of *Phthorimaea operculella* prepupa seven days post treatment with *Metarhizium* isolates at a concentration of 1×10^8 conidial/mL and Mt69 at 1.32×10^6 conidia/mL.

Fungal species	Isolate	Mean mortality (%) ± SE
Metarhizium spp.	NWU-Met-24	43.0 ± 9.6 [a]
	Mt69	51.8 ± 19.8 [ab]
	NWU-Met-13	83.3 ± 10.2 [bc]
	NWU-Met-15	85.1 ± 9.8 [abc]
	NWU-Met-4	86.3 ± 7.8 [abc]
	NWU-Met-10	88.9 ± 6.9 [bc]
	NWU-Met-23	88.9 ± 7.4 [bc]
	NWU-Met-16	92.6 ± 4.1 [bc]
	NWU-Met-18	92.9 ± 4.6 [c]
	NWU-Met-21	96.0 ± 3.9 [c]
	NWU-Met-5	96.5 ± 3.4 [c]
	NWU-Met-22	96.8 ± 3.1 [c]
	NWU-Met-20	100.0 ± 0.0 [c]
	NWU-Met-2	100.0 ± 0.0 [c]
	NWU-Met-7	100.0 ± 0.0 [c]
	NWU-Met- 11	100.0 ± 0.0 [c]
	NWU-Met-12	100.0 ± 0.0 [c]
	NWU-Met-14	100.0 ± 0.0 [c]
	NWU-Met-17	100.0 ± 0.0 [c]
	NWU-Met-19	100.0 ± 0.0 [c]
	NWU-Met-25	100.0 ± 0.0 [c]

Means within the column followed by the same letter do not differ significantly (P < 0.05, Tukey's HSD).

Discussion

This study evaluated the efficacy of 23 native fungal isolates and two commercial products against different life stages of *P. operculella* and demonstrated the potential of EPF for controlling *P. operculella*. Efficacy varied among fungal strains and insect developmental stages, indicating that insect susceptibility to EPF is influenced by both life stage and fungal strain.

The highest mortality was caused by *Metarhizium* treatments and low to medium susceptibility was recorded for *Beauveria* treatments. This is in agreement with similar studies conducted with different *B. bassiana* strains that also reported low to medium susceptibility of *P. operculella* eggs, with mortality rates below 55% (Kirisik and Erler 2024) and not exceeding 65% (Song et al. 2025). Similarly, the higher pathogenicity observed with some *Metarhizium* isolates is consistent with a study by Zeleke et al. (2015), which reported 83% mortality of *P. operculella* eggs after treatment with *M. anisopliae*. In contrast, Zhang et al. (2026) reported low pathogenicity of *M. robertsii* against *P. operculella* eggs.

The EPF infection of *P. operculella* eggs from the tested isolates resulted in complete fungal colonisation, and 100% mycosis on all treated eggs, including those that were still viable by the end of the experiment. The high level of mycosis can be attributed to the bare, unscaled nature of *P. opercuella* eggs (Skendemasi et al. 2021). Studies have shown that bare insect eggs are more susceptible to fungal infection due to the lack of scales, which act as a protective barrier (Anand and Tiwary 2009; Yan et al. 2026). Hydrophobin proteins produced by entomopathogenic fungi attach strongly to the hydrophobic chorion of the egg (Zhang et al. 2024). This type of bond is also seen on the cuticles of larvae. However, larval movement can sometimes break these bonds and dislodge spores (Zhang et al. 2024). We therefore speculate that egg immobility prevents this type of dislodgement, resulting in higher rates of infection and mycosis. Early stages of egg development also lack a proper immune system with hemocytes. Hemocytes are responsible for the humoral immune response against fungal infection. Therefore, the early stages of egg development are more susceptible to fungal infection (Reed et al. 2007; Anand and Tiwary 2009; Zhang et al. 2024).

Furthermore, to prevent the eggs from desiccation during the bioassay, Petri dishes were lined with a moist filter paper. This might have influenced the high level of mycosis observed, since fungal germination and colonisation are highly dependent on humidity (Quesada-Moraga et al. 2024). This is supported by Rodrigues et al. (2014), who showed that *M. anisopliae* infection of *Triatoma infestans* (Klug) (Hemiptera: Reduviidae) eggs depended on humidity, with eggs in drier conditions being resistant to infection. Interestingly, despite full fungal colonisation by all isolates, differences between high mycosis levels and low egg mortality were apparent in some isolates. Presumably, the fungus might be able to germinate and proliferate on the egg surface but fails to fully penetrate the chorion due to the presence of anti-fungal compounds (Campos-Esquivel et al. 2022). Although previous studies reported highly variable levels of suppression of the hatchability of *P. operculella* eggs (Khorrami et al. 2017;

Kirisik and Erler 2024), the isolates tested in the current study markedly inhibited egg hatching. Entomopathogenic fungi can release pre-germinating toxic metabolites, that permeate the chorion in early stages of infection, disrupting the process of embryogenesis (Pirez et al. 2009; Qazi and Khachatourians 2007; Sousa et al. 2023). This may have resulted in non-viable eggs that did not hatch. Overall, the effects of EPF on egg mortality across studies have been variable, depending on factors such as experimental conditions, fungal species, and arthropod pest species, making comparisons difficult (Gielen et al. 2024).

High mortality of first-instar larvae (> 70%) was observed in only four of the *Metarhizium* treatments. Studies investigating the efficacy of *Metarhizium* spp. against first-instar *P. operculella* larvae are limited. However, Kirisik and Erler (2024) reported mortality rates exceeding 95% for first-instar larvae exposed to *B. bassana* isolates. Direct comparisons with results obtained in this study should, however, be made with caution since Kirisik and Erler (2024) exposed first-instar larvae directly to EPF treatments, which differs from the methodology used in this study. It could have contributed to the lower levels of mortality observed in this study.

Following successful penetration of the larval cuticle by fungi, various immune responses are activated by the insect to limit further fungal development. These include the production of antimicrobial peptides, which destroy invading pathogens (Li and Xia 2022; Zhang et al. 2024). These immune responses may explain the low mycosis levels observed NWU-Met-10, despite infection with this isolate showing moderate levels of mortality.

Overall, prepupae were the most susceptible to fungal infection. High prepupal mortality rates have previously been reported, but in those studies, the prepupae were directly exposed to EPF by means of immersion (Kirisik and Erler 2024: Song et al. 2025; Zhang et al. 2026). This study provides an indication of mortality levels when EPF are applied as a soil drench. Low mortality with Bband was expected, as this formulation is registered for foliar application and not as a soil drench. The high mycosis rates in the prepupae confirmed that successful infection occurred and that mortality was due to fungal infection. The ability of EPF to develop on cadavers is an important parameter for the selection of potential biocontrol agents, because it indicates that the fungi can self-propagate and complete the biological cycle with the possibility of horizontal transmission to other insects (Rohrilch et al. 2018; Janmaat et al. 2024)

The prepupae of *P. operculella* do not feed, and movement is limited as they prepare for pupation (Rondon 2010). Thus, this will allow for more time for successful infection. It should, however, be kept in mind that sterile soil was used in this experiment. Several studies have indicated that EPF persistence is greater in sterile soil than in non-sterile soil. Autoclaving the soil removes other microbial communities, thereby eliminating biotic interactions that could influence the availability, establishment, pathogenicity and persistence of EPF in soil (Jaronski 2007; Tefera and Vidal 2009; Cortez-Madrigal et al. 2014; Nyahe et al. 2026). Therefore, field trials are necessary to re-evaluate the mortality rates of fungal isolates in the presence of other biotic factors.

The results also indicate that most treatments had LT_{50} values greater than 6 days. A higher LT_{50} value indicates reduced fungal virulence (Hussain et al. 2010; Riaz et al. 2026). It can therefore be concluded that most of the EPF isolates evaluated in this study had low virulence against *P. operculella* eggs. To determine the most virulent strain for egg control, both the egg incubation period and the virulence of the EPF should be considered. Under favourable environmental conditions, *P. operculella* eggs hatch in 4–6 days (Chandel et al. 2020). Kaur and Padjama (2008) categorised highly virulent EPF as having LT_{50} values < 5 days, moderate virulence as 5–6 days, and less virulent EPF with LT_{50} values > 6 days. Based on this scale, the conclusion can be made that all EPF exhibited low virulence against *P. operculella* eggs. For first-instar larvae, potent isolates showed moderate to high virulence, with NWU-Met-7 being the most virulent.

The differences in virulence among EPF isolates can be attributed to the variation in genetic makeup (Aynalem et al. 2021). Multiple enzymes, viz. proteases, lipase, esterases and chitinase are responsible for successful penetration and infiltration of fungi into the insect (Ferreria et al. 2026). These enzymes differ among fungal species and strains (Cortez-Madrigal et al. 2014, Gebremariam et al. 2021). The differences in the pathogenicity of EPF strains across insect developmental stages can be attributed to differences in cuticle composition among instars (Butt and Goetell 2000, Ortiz-Urquiza and Keyhani 2015, Ma et al. 2025). Therefore, it is expected that the pathogenicity of EPF strains will differ across the life stages of *P. operculella.* However, the pathogenicity of NWU-Met-2, NWU-Met-4 and NWU-Met-5 was consistent throughout all life stages of *P. operculella*, with mortality rates of above 75%.

Conclusion

Phthorimaea operculella is an important and worldwide pest that threatens potato yields. The difficulty of applying insecticides and the evolution of resistance indicate the need for alternative control strategies. The use of EPF as a biological control agent is becoming increasingly important. In this study, NWU-Met-2, NWU-Met-4 and NWU-Met-5 were found to be the most potent isolates against the larval stages of this pest. It is, however, evident that the screened isolates showed low virulence against *P. operculella* eggs, although hatching of the eggs was suppressed. The high mortality rates among prepupae indicated that these isolates may be effective in controlling *P. operculella* when applied to the soil. However, further investigation is needed to confirm whether these results translate to field efficacy. Furthermore, all experiments were conducted under constant temperature and humidity conditions in a laboratory setting. Therefore, further studies should evaluate the effect of different temperature and humidity levels on the pathogenicity of EPF. This will allow for a targeted approach when incorporating selected EPF into an integrated pest management program for *P. operculella*.

Declarations

Conflict of interests

The authors declare no conflict of interest.

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Author contributions

Conceptualisation and funding acquisition were done by Hannalene du Plessis. Experimental planning and supervision were performed by Hannalene du Plessis, Vongai M. Paradza and Johnnie van den Berg. Laboratory experiments and data analysis were performed by Tayla Swanepoel and Vongai M. Paradza. Writing was done by Tayla Swanepoel, and the revision of the text was done by Hannalene du Plessis, Vongai M. Paradza, and Johnnie van den Berg.

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Data availability

All data generated or analysed during this study are included in this published article.

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Figures

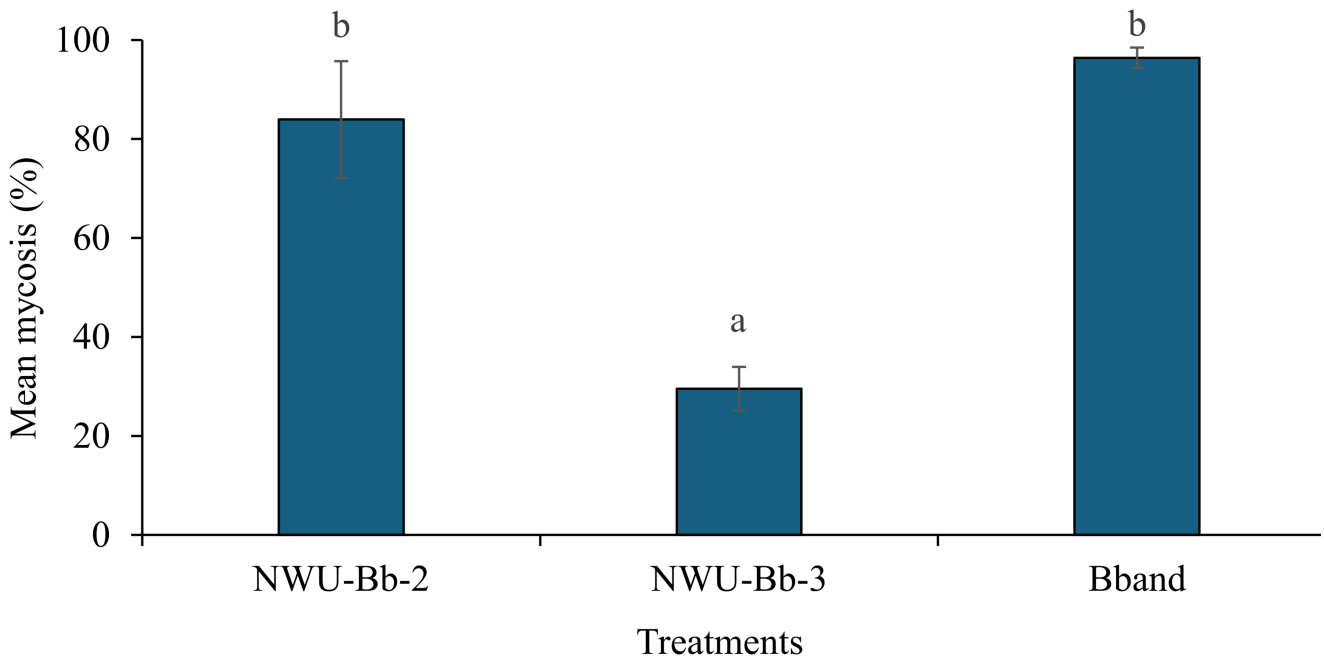


Figure 1

Mean percentage mycosis (±SE) of first-instar *Phthorimaea operculella* larvae treated with the different *Beauveria* treatments. Bars capped with the same letters are not significantly different according to Tukey's HSD post hoc test at $P < 0.05$

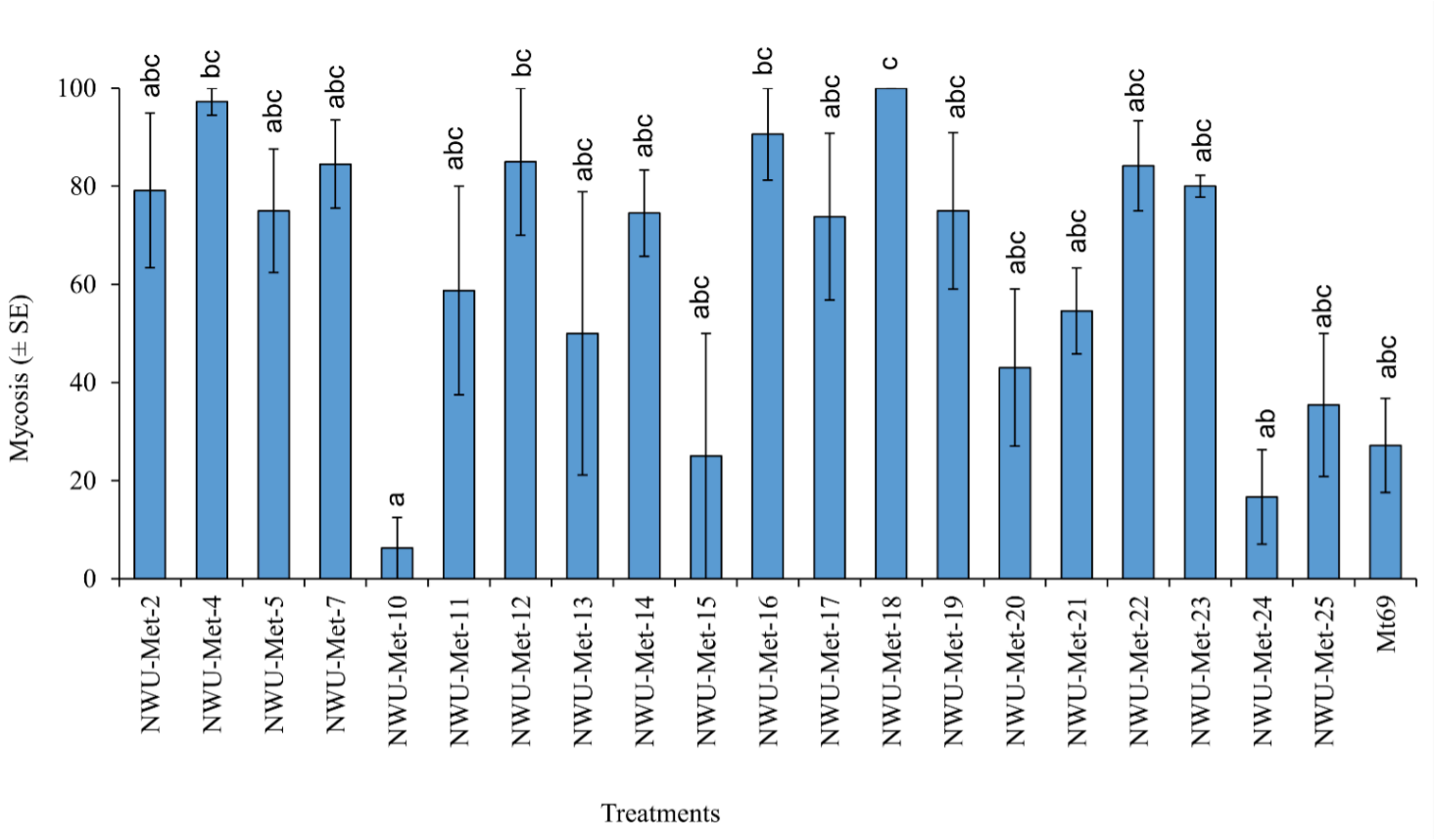


Figure 2

Mean percentage mycosis (±SE) of first-instar *Phthorimaea operculella* larvae treated with the different *Metarhizium* treatments. Bars capped with the same letters are not significantly different according to Tukey's HSD post hoc test at $P < 0.05$