Beauveria Bassiana as Fungal Endophyte for the Potential Control of the Potato Tuber Moth Phthorimaea Operculella on Potatoes

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***Beauveria bassiana* as fungal endophyte for the potential control of the potato tuber moth**

***Phthorimaea operculella* on potatoes**

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21

22 **Abstract**

23 The potato tuber moth, *Phthorimaea operculella*, is the most damaging potato pest in the world and is
24 difficult to control as the larvae are internal feeders in the foliage or tubers. Entomopathogenic fungi
25 (EPF) which colonize plants as endophytes, have lethal and sublethal pathological effects on insect
26 pests. Experiments showed that *Beauveria bassiana* colonized the aerial parts of potato plants
27 endophytically after inoculation through soil drenching. The colonization rate reached 100% for both
28 upper and lower foliage parts one day after inoculation, and endophytic *B. bassiana* remained present
29 for more than 21-day post inoculation. Mortality experiments indicated that *B. bassiana* and *B.*
30 *bassiana*-inoculated potato plants were pathogenic against 2nd instar larvae of *P. operculella*.
31 Development experiments showed that the weight of *P. operculella* pupae reared on *B.*
32 *bassiana*-colonized potato leaves (4.25 mg) was significantly lighter than of those reared on
33 uninoculated control plants (8.89 mg). Sublethal experiments indicated that *B. bassiana* negatively
34 affected the growth, development and reproduction of *P. operculella*. Compared to newly eclosed
35 larvae fed on control plants, those fed on *B. bassiana*-inoculated plants had significantly lower survival,
36 with only 17.8% developing to the adult stage. Oviposition of *P. operculella* females reared on *B.*
37 *bassiana* endophytically-colonized plants was significantly lower (35 eggs/per female) than of those
38 reared on uninoculated plants (115 eggs/per female). This study demonstrates that endophytic *B.*
39 *bassiana* can be a potential biological agent for the control and management of *P. operculella*.

Keywords Fungal endophytes Colonization Biological control *Beauveria bassiana* *Phthorimaea*

operculella

Key Message

- We found that *B. bassiana* successfully established as endophytic fungi in potato leaves after artificial inoculation in the laboratory.

- Our study demonstrates for the first time that the endophytic *B. bassiana* strain reduced larval damage to potato plants and adversely affected survival of *P. operculella*.

- Mycosed larvae, pupae and adults were also found on the *B. bassiana*-inoculated plants.

- This study provides basis for further management of *P. operculella* in the field.

Author contributions

GY and WS conceived and designed research. ZM and YJ conducted the experiments. ZM and YJ analyzed the data. GY, ZM and SR wrote the manuscript. All authors read and approved the manuscript.

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Introduction

The potato tuber moth, *Phthorimaea operculella* (Zeller) (Lepidoptera: Gelechiidae), is a cosmopolitan pest of solanaceous crops, especially potato, *Solanum tuberosum* L (Fenemore 1988). Larvae of this species mine leaves, stems, petioles, and excavate tunnels through potato tubers (Gao 2018). The pest infests plants throughout the growing season, however, the most significant damage results from larvae mining the tubers (Rondon et al. 2009). Tunnels left by *P. operculella* in tubers contain frass and other secondary contaminants that can be a potential source for secondary infections (Gao 2018). This moth is distributed widely in tropical and subtropical countries in South, Central and North America, Africa, Oceania and Asia. It is considered to be the most damaging insect pest of field and storage potatoes in developing countries in the tropics and subtropics (Gao 2018). By 2020, over 90 countries have reported the presence of this pest (Rondon 2020). Moreover, the rapid development of international trade has promoted the spread of *P. operculella* across continents (Gao and Zhou 2020).

Chemical methods are mainly used to control *P. operculella* in China. However, this has become difficult due to the rapid development of resistance to insecticides (Doğramaci and Tingey 2010). In addition, the delivery of insecticides to larvae is difficult once they tunnel into tubers. Among the non-chemical pest control methods, biological control by entomopathogenic fungi represents one of the most effective options. For example, the fungus *Muscodor albus* was developed as a microbial control agent against adult and larvae of *P. operculella* in stored potatoes (Lawrence and Neven 2006). Also, the *B. bassiana* strain, JLGZL-14, exerted sublethal effects on *P. operculella* larvae, by reducing the reproductive successes of surviving individuals and consequent population growth (Yuan et al.

2018). However, fungal conidia is highly sensitive to environmental conditions, and have to be properly dispersed and delivered to appropriate substrates for an effective pest-control (Jaronski 2009; Dara et al. 2017; Hajek et al. 2017; Van et al. 2017). Therefore, it is important to develop effective application methods that can help protect fungal conidia against adverse environmental stress, and which can also serve as a potential biocontrol agent for the management of *P. operculella*.

Beside their direct mode of action against insect pests, several fungal entomopathogens have been reported to be able to endophytically colonize plants after artificial inoculation through seed dressings, seed soaking, foliar sprays or soil drenching methods (Ownley et al. 2007; Gurulingappa et al. 2010; Sasan and Bidochka 2012; Akutse et al. 2013; Parsa et al. 2013; Mutune et al. 2016), allowing for their possible use as endophytes in plant protection measures (Schulz and Boyle 2005). Fungal endophytes are fungi that exist within the living tissues of a plant without causing any apparent deleterious effects (Petrini 1991). A number of entomopathogenic fungi, including *B. bassiana*, *Isaria farinosa*, *Clonostachys rosea*, *Chaetomium globosum* and *Metarhizium brunneum*, naturally or artificially exist in various plants as endophytes (Abou Alhamed and Shebany 2012; Murphy et al. 2015; Bamisile et al. 2018). For example, *B. bassiana* has been artificially inoculated into many important economic plants, including maize (*Zea mays*), potato (*Solanum tuberosum*), soybean (*Glycine max*), faba bean (*Vicia faba*), tomato (*Solanum lycopersicum*), cotton (*Gossypium spp*) and tobacco (*Nicotiana benthamiana*) (Vega 2018; Qin et al. 2020; Nishi et al. 2021). Moreover, *B. bassiana* has also been shown to endophytically colonize cassava roots following inoculation by soil drenching (Qin et al. 2020). One of the main reasons for the artificial introduction of *B. bassiana* into plants is to protect them against insect pests. For example, most studies conducted on the use of *B. bassiana* as endophytes reported their negative influence on the growth and reproduction of a wide

range of herbivores from different feeding guilds (Vidal and Jaber 2015; Mckinnon et al. 2016; Bamisile et al. 2018), including sucking pests (Muvea et al. 2014; Sword et al. 2017), caterpillars (Barta 2018; Sánchez-Rodríguez et al. 2018), dipterans (Akutse et al. 2013; Mutune et al. 2016), beetles and weevils (Newcombe et al. 2009; Biswas et al. 2013), and grasshoppers (Gurulingappa et al. 2010). However, the effect of *B. bassiana* as endophytes on *P. operculella* remains unknown.

The objectives of this study were therefore to determine whether the *B. bassiana* isolate, GZGY-1-3, could colonize potato plants endophytically using the soil drench method and to assess its pathogenicity as an endophyte, as well as to determine its sublethal effects on *P. operculella*.

Materials and methods

Plants

Seed-tubers of potato (cv 'Xiabodi') were cultivated in 15-cm plastic pots until buds grew to about 1 cm in length in a walk-in growth chamber (26 ± 1 °C, 12:12 h L:D and $30\% \pm 10\%$ RH). When buds grew to about 5 cm into soil, plants were inoculated with *B. bassiana* by drenching the soil with 30 ml of a conidial suspension (1.0×10^8 conidia/ml). The potatoes were inoculated every day for 7 days with the soil drench. The number of *B. bassiana* colony-forming units (CFU) (2.22×10^8 cfu/g) was determined following the method of Zhang et al. (2019). After one day of incubation, the potato plants were then used for the experiments. Plants treated with 30 ml 0.05% (v/v) Tween-80 for 7 days served as controls. The plants and insects used in each experiment were independent of that used in other experiments.

Insects

The initial stock of potato tuber moth was collected from potato plants in Kunming, Yunnan Province in June 2013 (E103.79, N25.51). A colony was established and maintained following the methods of Gui and Li (2003) and Rondon et al. (2020). The colony was reared in artificial environmental chambers (MLR-351H, SANYO Electric Co., Ltd., Moriguchi City, Osaka, Japan) under controlled conditions of 26 ± 1 °C, 12:12 h L:D and $70\% \pm 10\%$ RH. Populations were reared for more than three generations to ensure homogeneity of cultures before their use in experiments. Neonates and 2nd instars from egg masses were collected for experimental use.

Fungal strain

The *B. bassiana* isolate, GZGY-1-3, was used in experiments because of its demonstrated pathogenicity against *P. operculella* larvae (Yuan et al. 2018). This strain is maintained at the China General Microbiological Culture Collection Center, No. 9254; GenBank Accession Number KP994951. It was derived from *Ostrinia furnacalis* (Guenée) (Lepidoptera: Crambidae) collected in Guizhou Province, China. For experiments, the strain was maintained, and conidia were produced on PDA medium (2% corn flour, 1% wheat bran powder, 0.5% tryptone, 0.3% KH₂PO₄, 0.1% MgSO₄·7H₂O, 0.1% NH₄NO₃, 2% agar powder, 0.5% cicada slough powder) at 24 ± 1 °C under continuous darkness. Conidia was then harvested from 14 d cultures. Conidial concentrations were determined with a haemocytometer viewed with a 400X optical microscope (Olympus BX51, Olympus America Inc., <http://www.Olympusmicro.com>). Concentrations were adjusted to 1.0×10^8 conidia/ml (2.22×10^8 cfu/g) with 0.05% Tween-80 in sterile water. Germination which was tested using the methods of Yuan et al. (2018) exceeded 90%.

Evaluation of endophytic colonization of potato plants by *B. bassiana*

The colonization of *B. bassiana* on potato leaves was observed at the upper (three new leaves near the apical position: the second, third and fourth leaves were collected) and lower parts (three leaves near the lowest position) (Fig. 1A). At 1-day post inoculation of the plants, sectioned parts per treatment were carefully harvested, washed with tap water, then surface sterilized in 70% ethanol for 2 min. This was followed by immersion in 1.5% sodium hypochlorite for 3 min, rinsing with sterile distilled water three times, and then drying on sterile paper towel for 30 s. They were finally cut into 1 × 1 cm pieces under a laminar flow hood before placing them on PDA plate. In addition, water from the last rinse was plated out to assess the reliability of the surface sterilization procedure (Bamisile et al. 2018). Plates were incubated at 26 ± 1 °C for 10 days, after which the presence of endophytes was observed (Bamisile et al. 2018).

The colonization of the different plant parts was recorded by counting the number of sections of the different plant parts that showed presence of inoculated fungal growth/mycelia (Muvea et al. 2014). Only the presence of *B. bassiana* endophyte that was inoculated was scored. Fungal colonies from surface-sterilized parts were characterized only when mycelia grew at the edge of leaf segments from internal tissues. To confirm whether the growing endophytes were the ones initially inoculated, slides prepared from the mother plates were used for comparison and morphological identification. The data was expressed as percent colonization. We separately observed colonization at 1, 4, 7, 14 and 21d after the soil drenching treatment. The experiments were repeated five times using eighteen plants for each treatment (Table 1). Independent batches of plants and *B. bassiana* were used in each treatment.

Effects of *B. bassiana* and *B. bassiana*-inoculated potato plants on mortality of *P. operculella*

We adopted the leaf-disc assay method to determine whether *B. bassiana* and *B. bassiana*-inoculated potato plants was pathogenic against *P. operculella* larvae. In these bioassays, four treatments were used: (1) direct exposure of larvae to a suspension of *B. bassiana*, (2) indirect exposure of larvae to *B. bassiana* by allowing larvae to feed on surface-sterilized endophytically-colonized potato leaves, (3) direct exposure of larvae to a suspension of *B. bassiana* and then allowing them to feed on potato leaves inoculated by endophytic *B. bassiana* (combination of the above two treatments), (4) control treatment without exposure to *B. bassiana*. For direct exposure (treatments 1 and 3), 2nd instars of *P. operculella* were dipped in a conidial suspension (1.0×10^8 conidia/ml) of *B. bassiana* for 5 s. For treatments without direct exposure (both *B. bassiana*-inoculated and control leaves) (treatments 2 and 4), 2nd instars were dipped in 0.05% Tween-80 for 5 s. The larvae were then allowed to dry on filter papers for 5 s and then transferred to Petri dishes (diameter $\times$ height = 20 cm $\times$ 3.5 cm). Each dish was placed over an arena containing potato leaves as a food source embedded in a layer of 2% agar (2% agar powder, 98% deionized water). For treatments 1 and 4, potato leaves were taken from untreated potato plants. For treatments 2 and 3, potato leaves were taken from plants that had been inoculated with *B. bassiana*, as described above. Leaves were surface-sterilized before placement in arenas.

The bioassays were carried out in an artificial environmental chamber (MLR-351H, SANYO Electric Co., Ltd., Moriguchi City, Osaka, Japan) under controlled conditions of 26 ± 1 °C, 12:12 h L:D, and $70\% \pm 10\%$ RH. Mortality of *P. operculella* was recorded daily up to 13 days after

infestation. Each treatment was replicated twice, with twenty-four 2nd instars per replicate. The bioassays were repeated five times using independent batches of plants and larvae (Table 1).

Effects of *B. bassiana*-inoculated potato plants on pupal weight of *P. operculella*

To assess the effects of endophytic *B. bassiana* on pupal weight, plants were prepared as described above. Newly eclosed larvae were transferred to *B. bassiana*-inoculated and control potato plants held in nylon mesh cages (50 cm× 50 cm× 50 cm) using a soft brush and maintained in a room chamber (26 ± 1°C, 12:12 h L:D, and 30% ± 10% RH). A total of 180 larvae were placed on treated plants. Another 180 larvae were placed on untreated plants. Once larvae reached the pupal stage, 40 pupae from each treatment were collected randomly and weighed using an Electro balance (Mettler Toledo, Shanghai, China). Each repetition was made up of 40 pupae, the whole bioassays were repeated five times using independent batches of plants and pupae (Table 1).

Effects of endophytic *B. bassiana* on development, survival and fecundity of *P. operculella*

To evaluate the sublethal effects of *B. bassiana*-inoculated potato plants on the development and fecundity of *P. operculella*, another experiment was undertaken. Using a soft brush, newly eclosed larvae (total of 180) were transferred to leaves of 3 potato plants that had been inoculated with *B. bassiana* 24 h earlier, as described above. Larvae in the control group (n=180) were placed on untreated plants. Plants were held in nylon mesh cages (50 cm × 50 cm × 50 cm). Each newly hatched larva was considered as one replicate (Table 1) (Guo et al. 2020). Survival and development of larvae and pupae were observed daily following the method described by Guo et al. (2020).

After pupation, pupae were placed individually into 2 ml centrifuge tubes. After adult eclosion, individual males and females were placed together in plastic containers (height $\times$ diameter = 14 cm $\times$ 6 cm) and oviposition was recorded by counting daily the number of eggs laid on a sheet of filter paper placed at the bottom of each container. Filter papers were replaced daily. Adults were provided with a 10% sugar suspension solution for food. Observations for each mating pair were continued until female death. These mating/oviposition chambers were held in an environmental room maintained at $26 \pm 1^\circ\text{C}$, 12:12 h L:D, and $30\% \pm 10\%$ RH.

Statistical Analysis

Colonization frequency (CF) was calculated using the formula (Fisher and Petrini 1987):

$$\text{Colonization (\%)} = (\text{PF/TP}) \times 100$$

where PF = number of plant pieces colonized, TP = total number of plant pieces. The proportion of fungal colonization was independently analyzed using t-test.

One-way analysis of variance (ANOVA) and Tukey's HSD test were used to assess virulence bioassays (mortality and LT_{50}). Mortality data were corrected using Abbott's correction (Abbott 1925). The median lethal times (LT_{50}) were determined by probit regression. Pupal weight was compared by t-test. Statistical analysis was performed in the R software environment (v.4.0.3).

The life history data were analyzed based on an age-stage two-sex life table (Chi 1988) constructed using the computer program TWO-SEX-MSChart (Chi 2015). The survival rate (s_{xj}) (x = age, j = stage) and fecundity (f_{xj}) were calculated following the methods of Chi and Liu (1985), based on data from the entire cohort. The age-specific survival rate (l_x) was then calculated as follows:

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$$l_x = \sum_{j=1}^k s_{xj}$$

225

$$m_x = \frac{\sum_{j=1}^k s_{xj} f_{xj}}{\sum_{j=1}^k s_{xj}}$$

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The intrinsic rate of increase (r) was estimated using the Euler-Lotka formula, with the age indexed

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from 0 as follows (Goodman 1982):

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$$\sum_{x=0}^{\infty} e^{-r(x+1)} l_x m_x = 1$$

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The finite rate of increase (λ), the net reproductive rate (R_0), and the mean generation time (T) were

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individually calculated as follows:

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$$\lambda = e^r$$

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$$R_0 = \sum_{x=0}^{\infty} l_x m_x$$

233

$$T = (\ln R_0)/r$$

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A bootstrap technique was used to estimate the variances and standard errors of the population

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parameters (Efron and Tibshirani 1994; Huang and Chi 2012). Differences among the treatment and

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control plants were estimated with a paired bootstrap test.

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Results

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Endophytic colonization of *B. bassiana* in potato plants

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The surface sterilization proved successful because no *Beauveria* was detected in the last distilled

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water used to rinse the surface of leaves. The results showed that the potato plants were colonized

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endophytically by *B. bassiana* (Fig. 1B). No *B. bassiana* was isolated from the control potato plants.

At the 1-day post inoculation (dpi), all the upper (100%) and lower leaves (100%) showed high colonization of *B. bassiana*. However, at 4 dpi, the colonization of lower leaves (98.89%) was significantly higher than that of the upper parts (87.78%, $t = -4.7135$, $df = 6.116$, $P = 0.0031 < 0.01$). At 7, 14 and 21 dpi, the colonization of lower sections was 85.55%, 70.00% and 47.78%, respectively, which was significantly higher than that of the upper ones (68.89%, $t = -8.6597$, $df = 8$; 46.66%, $t = -8.9556$, $df = 6.6341$ and 31.11%, $t = -8.6608$, $df = 8$ respectively; all P values < 0.001) (Fig. 1C).

Effects of *B. bassiana* on mortality of *P. operculella*

These trials confirmed that foliage of potato plants inoculated with *B. bassiana* via soil drenching were pathogenic against *P. operculella* larvae. *B. bassiana* caused significant mortality to larvae directly through exposure to a suspension of *B. bassiana* and indirectly via feeding on endophytic-colonized *B. bassiana* plants. Mortality increased over time in all *B. bassiana* treatments. However, mortality was significantly greater for larvae exposed to conidia than for larvae exposed to plant tissue inoculated by *B. bassiana*. At 3dpi, the corrected mortality was 51.22% for larvae directly exposed to conidia and 35.28% for larvae exposed to plant tissue endophytically inoculated by *B. bassiana* (Fig. 2A). At 7dpi, the corrected mortality for larvae directly exposed to conidia was 88.35% and 63.59% for larvae exposed to plant tissue inoculated by *B. bassiana*. At 8dpi, the corrected mortality of *P. operculella* exposed directly to conidial suspension reached 100%, which was significantly higher than that of larvae that fed on *B. bassiana*-inoculated potato plants (68.16%; $t = -30.425$, $df = 9$, $P < 0.001$). There was a synergistic effect from the combination of the two *B. bassiana* treatments. Mortality under the combined treatment was the highest and reached 100% at 7 days post inoculation, one day before that level was reached in the direct exposure treatment.

The results also indicated that endophytic *B. bassiana* were virulent to *P. operculella* larvae but acted at a slower rate. The LT_{50} for *P. operculella* exposed to endophytic *B. bassiana* was 5.57 d, whereas it was 3.25 d when exposed to *B. bassiana* conidia, and 2.25 d when exposed to both forms of *B. bassiana* ($F = 3146$, $df = 2$, $P < 0.001$, Fig. 2B).

Effects of *B. bassiana*-inoculated potato plants on pupal weight of *P. operculella*

Endophytic *B. bassiana* reduced the pupal weight of surviving *P. operculella* larvae. The weight of pupae reared on *B. bassiana*-inoculated potato plants was less than half of the weight of those reared on untreated control plants (4.2 mg versus 8.8 mg, $t = -46.366$, $df = 330.04$, $P < 0.001$, Fig. 3).

Sublethal effects of *B. bassiana*-inoculated potato plants on development, survival and fecundity of *P. operculella*

Because newly hatched neonates were uniformly obtained from the egg paper, and egg stage was about 3-4 d, we considered the duration of the egg stage as 4 d in this trial. Larval (13.57d) and pupal stages (10.75d) developed significantly slower on *B. bassiana*-inoculated potato plants than on untreated plants (larvae: 12.30 d; pupae: 9.28 d; all P values < 0.001 , Table 2). Moreover, there was a significantly smaller number of surviving larvae (60) and pupae (32) on *B. bassiana*-inoculated potatoes than on untreated plants (larvae: 92; pupae: 83) (all P values < 0.001). A significantly greater proportion of pupae reared on control plants emerged as adults (83, female: 39, male: 44) than on *B. bassiana*-inoculated plants (32, female: 9, male: 23) ($P < 0.001$). Adult longevity for both females and males was significantly shorter on *B. bassiana*-inoculated potato ($P < 0.001$). The mean fecundity of females reared on *B. bassiana*-inoculated potato plants was 35 eggs/per female. It was significantly

lower than of those reared on control plants (115 eggs/per female) ($P < 0.001$, Table 2). Moreover, visible mycosis was evident on larval, pupal and adult cadavers (Fig. 4A, 4B, 4C).

The curves of the age-stage survival rate (s_{xj}) show the probability of an individual surviving to age x and developing to stage j (Fig. 5A, 5B). Overlap of stage-specific survivorship curves is the result of variable developmental rates among individuals (Guo et al. 2020). The probability that a newly hatched neonate of *P. operculella* would survive to the adult stage differed markedly among the treated and control groups, with 46.11% on control group, and only 17.78% on *B. bassiana*-inoculated plants. A high mortality rate of 66.67% occurred in the larval stage when fed on *B. bassiana*-inoculated potato plants (Fig. 5A). The age-specific survival rate (l_x) and age-specific fecundity (m_x) are plotted in Figure 6. The l_x curve describes the change in the survival rate of the cohort with age and shows that *P. operculella* that fed on *B. bassiana*-inoculated potato plants had rapid declining survivorship beginning on the 5th day. The m_x curve shows that reproduction began at age 25 and 24 days for *P. operculella* reared on *B. bassiana*-inoculated and untreated potato plants respectively. The maximal daily oviposition rate for females reared on *B. bassiana*-inoculated potato plants was at 37 days with a mean fecundity of 3 eggs, which was significantly lower than the corresponding mean for *P. operculella* fed on untreated ones (27 days, 13 eggs, $P < 0.001$).

Population parameters of *P. operculella* fed on *B. bassiana*-inoculated potato plants

The intrinsic rate of increase (r), finite rate of increase (λ), net reproductive rate (R_0) and mean generation time (T) of *P. operculella* fed on *B. bassiana*-inoculated and control potato plant were calculated using the bootstrap method (Table 3). Statistical analysis showed that the r for *P. operculella* fed on *bassiana*-inoculated potato plants was 0.0181 per day, significantly lower than that

fed on control group (0.1099 per day) ($P < 0.001$). The λ and R_0 for *P. operculella* fed on *B. bassiana*-inoculated potato plants were 1.0183 per day and 1.7556 offspring per female, respectively. These values were significantly lower than that on untreated ones ($\lambda = 1.1161$ per day; $R_0 = 24.9222$ offspring per female) (all P values < 0.001). In addition, there was no difference for T of *P. operculella* reared on *B. bassiana*-inoculated or untreated plants ($P = 0.1269$).

Discussion

Artificial introduction of entomopathogenic fungi into plants to form endophytic associations offers a number of benefits for pest management (Qin et al. 2020). Compared to entomopathogenic fungi, endophytic ones can overcome adverse environmental stress, but also protect plants from pest damage. A previous study demonstrated that *B. bassiana* was able to endophytically colonize all plant vegetative tissues (Behie et al. 2015). Our results showed that the *B. bassiana* strain, GZGY-1-3, endophytically colonized potato plants and also had a high colonization capacity through soil drenching. The lower and upper sections of inoculated potato plants had complete colonization at 1-day post inoculation. However, percent colonization declined over time, and also differed between sections. The ability to successfully colonize may be dependent on the specific strain and host plant association (Nishi et al. 2021). The decline in percentage colonization over time may be caused by the host response to the heterotrophic fungus, expansion of leaves with maintenance of established colonies in leaves, or possible competition from other endophytes in the plant (Posada et al. 2007). Moreover, variations in colonization could also result from preferential tissue colonization within plant hosts (Akutse et al. 2013; Huang and Chi 2012; Guesmi-Jouini et al. 2014).

The results from the mortality, sublethal and development experiments showed that *B. bassiana* suppressed the population of *P. operculella*. These are in line with similar results reported from other previous studies. For example, *B. bassiana* as an endophyte adversely affected the duration of the larval stage and adult stage of the Bollworm (*Helicoverpa gelotopoeon*), as well as the total duration of its life cycle, oviposition period, fecundity and fertility on tobacco (Vianna et al. 2018). Mutune et al. (2016) reported that *B. bassiana*-inoculated beans (*Phaseolus vulgaris*) significantly reduced bean stem maggot (*Ophiomyia phaseoli*) feeding and oviposition. Sánchez-Rodríguez et al. (2018) showed that endophytic *B. bassiana* effectively controlled cotton leafworm (*Spodoptera littoralis*) larvae on wheat plants. Barta (2018) found that endophytic *Beauveria* negatively affected larvae of the horse-chestnut leaf miner (*Cameraria ohridella*). *B. bassiana*-colonized tobacco displayed higher resistance to aphids (*Myzus persicae*) (Qin et al. 2020). *B. bassiana* was effective against aphids via endophytism and delayed PLRV infection in tobacco (Fingu-Mabola et al. 2021). In our study, *B. bassiana* had high pathogenicity to *P. operculella* larvae, but also had sublethal impacts on surviving individuals. These sublethal effects included slowing the developmental rate, reducing pupal weight, and reducing the fecundity of females. These biological effects were manifested in the population parameters of *P. operculella*. Some *P. operculella* were capable of successfully developing on *B. bassiana*-inoculated plants, but their survival was significantly lower than that reared on control plants. We also found mycosed individuals on *B. bassiana*-inoculated plants, which indicated that there was also direct pathogenicity to *P. operculella*. The mechanism by which endophytic fungi interact with insects is still unclear but the involvement of metabolites/antibiosis produced by the fungi can be speculated (Vega 2018). Previously researches have suggested that endophytic fungi may also produce metabolites that act as feeding deterrents (Vega 2018; Cherry et al. 2004; Shrivastava et

al. 2015; Bing and Lewis 1991). Dispersion of such metabolites through the plant may account for insect mortality in tissues without the presence of the fungus. They may also account for the reduced developmental rate and pupal weights of surviving *P. operculella*. Bing and Lewis (1991) suggested that the reduced tunneling of the European corn borer (*Ostrinia nubilalis*), following endophyte colonization of maize by *B. bassiana*, could be due to the presence of fungal metabolites that caused feeding deterrence or antibiosis. This might explain the absence of *B. bassiana* infection (mycosis) within *O. nubilalis* individuals that fed on endophytically infected plants. A study by Cherry et al. (2004) also supported the feeding deterrence/antibiosis hypothesis since stem-borer (*Sesamia calamistis*) larvae which fed on maize plants injected with *B. bassiana* were smaller than that which fed on control plants.

The introduction of entomopathogenic fungi such as *B. bassiana* as endophytes can be a practical approach to control insect pests. *B. bassiana* is typically used as a therapeutic microbial insecticide to provide relief from existing pest populations. In our study, the endophytic behavior of the *B. bassiana* strain, GZGY-1-3, applied by soil drenching, proved useful for longer term management of *P. operculella*. Moreover, we found that *B. bassiana* remained present endophytically for more than 3 weeks after inoculation in potato plants. This persistence should provide longer-term management benefits for potato crops with no additional inputs on the part of growers.

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515 **Table 1** The experimental schedule of endophytic colonization of *B. bassiana* in potato plants, and mortality, pupal weight, sub-lethal effect of *P. operculella*. The full

516 experiment was repeated with new inoculum and a new batch of insects.

517

Experiment	n	Repetition
Endophytic colonization	18	5
Mortality	24	10
Pupal weight	40	5
Sub-lethal effect	180	1

518

519 **Table 2** Developmental time, longevity and mean fecundity of *P. operculella* on *B. bassiana*-inoculated and control potato plants.

Developmental stage	<i>B. bassiana</i>		Control	
	Developmental time(d)		Developmental time(d)	
	n		n	
		Mean±SE		Mean±SE
Egg	180	4.00±0.00	180	4.00±0.00
larval	60	13.57±0.15a	92	12.30±0.11b
Pupa	32	10.75±0.29a	83	9.28±0.079b
Female	9	6.11±0.61b	39	10.51±0.26a
Male	23	6.78±0.39b	44	13.50±0.29a
Mean fecundity/egg	9	35.11±3.82b	39	115.03±3.96a

520 Note: Values followed by the different lowercase letters within a row are significantly different using paired bootstrap test ($P < 0.001$). Sample sizes (n) are the number of

521 individuals entering each life stage.

522

523

524

525 **Table 3** Population parameters (Mean $\pm$ SE) of *P. operculella* on *B. bassian*-inoculated and control potato plants

Parameter	<i>B. bassiana</i>	Control
Intrinsic rate of increase, r (d ⁻¹)	0.0181 $\pm$ 0.0123b	0.1099 $\pm$ 0.0051a
Finite rate of increase, λ (d ⁻¹)	1.0183 $\pm$ 0.0124b	1.1162 $\pm$ 0.0057a
Net reproductive rate, R_0 (offspring)	1.7556 $\pm$ 0.5970b	24.9222 $\pm$ 3.6403a
Mean generation time, T (d)	31.1005 $\pm$ 1.8026a	29.2622 $\pm$ 0.1458a

526 Note: Values followed by the different lowercase letters within a row are significantly different using paired bootstrap test ($P < 0.001$)

Fig. 1 Leaves colonization by endophytic *B. bassiana*. (A) The positions of selected leaves. (B) Recovery of endophytic GZGY-1-3 from leaf tissue fragments. (C) Mean percent colonization of different leaves parts by *B. bassiana* GZGY-1-3 at 1, 4, 7, 14 and 21 dpi. (*Indicates $P < 0.05$, ** Indicates $P < 0.01$, *** Indicates $P < 0.001$).

Fig. 2 Assessment of the virulence of *B. bassiana* against *P. operculella* larvae in leaf disc assays. Assays were started with 2nd instars placed on leaf discs (A) Mortality of *P. operculella* larvae over time. Mortality was corrected for control mortality. (B) LT₅₀ analysis of *B. bassiana* against *P. operculella* 2nd instars ($P < 0.001$).

Fig. 3 Pupal weights of *P. operculella* reared on potato plants inoculated with the *B. bassiana* strain, GZGY-1-3 and on untreated control plants. Pupae from the control plants were significantly heavier than those from treated plants (***Indicates $P < 0.001$).

Figure 4 Visible mycosis on cadavers of *P. operculella* that fed on *B. bassiana*-inoculated potato plants.

(A) Larvae (B) Pupae (C) Adult

544 **Figure 5** Age-stage specific survival rates (s_{xj}) of *P. operculella* reared on (A) *B. bassiana*
 545 -inoculated potato plants or (B) untreated control plants.

547 **Figure 6** Age-specific survival rate (l_x) and fecundity (m_x) of *P. operculella*. (A) *B. bassiana*-inoculated
548 potato plants. (B) controls