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Evaluation of entomopathogenic fungi against *Psalmocharias alhageos* (Kolenati) (Hemiptera: Cicadidae) under laboratory and semi-field conditions

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Abstract

Background The grapevine cicada, *Psalmocharias alhageos (*Kolenati*)* (Hemiptera: Cicadidae), has recently emerged as a significant pest in vineyards in the Middle East. The soil-inhabiting nymphal stages of this species cause substantial root damage, and their subterranean habitats enable them to evade prevalent control tactics. Entomopathogenic fungi "EPF", a class of fungi that are naturally occurring in soil and pathogenic to insects, have demonstrated potential utility against a broad spectrum of soil-dwelling pests and some non-soil-dwelling pests.

Results Efficacy of three EPF species, *Beauveria bassiana*, *B. varroae* and *Metarhizium anisopliae*, against the grapevine cicada were investigated. In laboratory Petri dish assays, *M. anisopliae* exhibited the highest virulence with an LC_{50} value of 10^6 conidia/ml, followed by *B. bassiana* with an LC_{50} of 7.2×10^6 conidia.ml^{-1}. Cumulative mortality of *P. alhageos* was higher in soil column than Petri dish assays. At the highest concentration tested (10^8 conidia/ml), *M. anisopliae* caused the greatest efficacy against cicada nymphs, resulting in 75%, cumulative mortality. Soil type significantly affected activity of *M. anisopliae* and *B. bassiana* against *P. alhageos*. *M. anisopliae* caused greater nymph mortality in loam and loam soil than that in sandy loam. Moreover, efficacy of *M.anisopliae* was higher in clay loam soil than in the other soil types evaluated. Temperature also affected fungal virulence; cumulative mortality caused by *M. anisopliae* was higher at 20˚C than at 25 or 30˚C. Additionally, MST increased with decreasing temperature.

Conclusion Evaluations of pure cultures of EPF suggest that these microbial agents, especially the promising candidate strain of *M. anisopliae*, could be useful for management of *P. alhageos*. However, further work is needed to explore mass production of these agents and their compatibility for integration with other tools. Future large-scale evaluations of *M. anisopliae* under field conditions are warranted.

Keywords Grapevine cicada, Microbial control, Insect pathology, *Metarhizium*, *Beauveria*

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Background

The grapevine cicada, *Psalmocharias alhageos (= Cicadatra alhageos* Kolenati) (Hemiptera: Cicadidae), is a major pest of grapevine and tree fruits (e.g. apple, pear, plum, almond, apricot, cherry and peach), especially in Iran, Turkey, and central Asia (Kolyaee et al. 2012). Damage is caused by both adults and nymphs. The subterranean nymphs feed on the roots of host plants, leading to debilitation, and reduced yields (Mehdipour et al. 2016). Adults cause damage by feeding on young buds and oviposition under the bark which, at high densities, can weaken or even kill the branch (Kolyaee et al. 2012). Effective control of this pest is essential to protect economically valuable crops. However, managing *P. alhageos* is difficult because its nymphs spend a prolonged period underground, where factors such as root depth, root density, and soil type limit control options, and conventional insecticides cannot effectively reach these soil-dwelling life stages (Soltani et al. 2018).

Various control strategies have been evaluated, including the use of chemical pesticides (Aguilera-Sammaritano et al. 2021), mating disruption by playing back acoustic signals (Mehdipour et al. 2016), mechanical removal of damaged branches containing eggs, and the use of kaolin oviposition deterrents (Valizadeh et al. 2013). Most of these control strategies have limitations. For instance, conventional insecticides need to be applied at high concentrations due to the buffering capacity of soils and often the breakdown products are more toxic than the parent compound (Andreu and Picó 2004). More importantly, they pose a risk to human health, pollute the environment and reduce biodiversity (Zhou et al. 2024). The growing demand for more sustainable pest management practices has intensified interest in biological control approaches. Not surprisingly, there is much interest in the development of more benign agents, especially entomopathogenic fungi (EPF), as ecologically sound alternatives for control of *P. alhageos*, other cicada species (Lin et al. 2023) and other families in Hemiptera (Shrestha et al. 2017). These fungi initiate infection by degrading the host cuticle following spore adhesion and germination, then proliferate internally, secrete toxins, suppress or evade the insect immune system, and colonize the host (Nassary 2025).

Cicadas are frequently found naturally infected with specialist entomo-phthoralean fungi such as *Massospora* (Macias et al. 2020) or with generalist hypocrealean fungi, including *Isaria* (Lao et al. 2021), *Metarhizium* (Li et al. 2010) and *Beauveria* (Liu et al. 2001). Over 70% of commercial EPF products developed for crop pest control are based on strains of *Metarhizium*, *Beauveria* or *Isaria* (De Faria and Wraight 2007). These fungi occur naturally in soils and show much promise for control of a wide range of crop pests, especially species that spend some or all their life cycle in the soil (Scheepmaker and Butt 2010). Several products have been developed for control of subterranean stages of pests, including larvae and pupae of *Otiorhynchus sulcatus* Fabricius (Coleoptera: Curculionidae) (the black vine weevil) (Ansari and Butt 2013), *Thaumetopoea pityocampa* Denis & Schiffermüller (Lepidoptera: Notodontidae) (pine processionary moth) (Güven et al. 2021), Thysanoptera (thrips) (Ansari et al. 2008), Scarabaeidae (white grubs) (Li et al. 2020) and Tephritidae (fruit flies) including *B. oleae* Rossi (olive flies) (Yousef et al. 2017). The virulence of an EPF is contingent upon the selection of a stable strain that possesses specific efficacy against target hosts and high adaptability to environmental conditions (Iwanicki et al. 2025). Furthermore, understanding how abiotic variables such as soil type and temperature modulate EPF performance is particularly vital for subterranean pests across diverse production regions, as *P. alhageos* nymphs reside entirely underground. These factors can profoundly influence both pest survival (De Miranda et al. 2024) and the effectiveness of EPF strains (Zamani et al. 2025).

To date, very little progress has been made in developing EPF for control of *P. alhageos* and other cicadas, despite the potential of EPF. Specifically, the virulence of native fungal strains and their interaction with environmental factors such as soil type and temperature are poorly understood, limiting practical deployment in pest management programs. The current study was designed to address this knowledge gap by initially evaluating native and commercial EPF strains against *P. alhageos* nymphs under laboratory conditions and then testing the promising candidate strain under more natural semi-field conditions. Additional objectives included evaluating how soil type and temperature affect the virulence of candidate EPF strains against *P. alhageos* nymphs.

Methods

Insect collection

Given the challenges associated with rearing *P. alhageos* under laboratory conditions, including an extended period of subterranean development, all experiments were conducted with field-collected fourth-instar nymphs from a vineyard located in Khalilabad, Razavi Khorasan Province, Iran (35° 15′ 6″ N, 58° 17′ 2″ E). The nymphs were placed individually into plastic containers (8 cm× 6 cm × 6 cm) filled with soil mimicking their natural habitat and provided with cut potato pieces as a food source.

Entomopathogenic fungi

The EPF used in this study included *Beauveria bassiana* (Balsamo) Vuillemin strain GHA (AB576868) which is the active ingredient in the commercial product "Botanigard" (Certis-Belchim), *Beauveria varroae*

Vuillemin (Hypocreales; Cordicypitaceae) (KU058600) and *Metarhizium anisopliae* (Metschn.) Sorokin (KP213288). These strains were obtained from the culture collection of the Plant Protection Department at Ferdowsi University of Mashhad, Iran. Their identity was confirmed using conidial morphology along with molecular characterization with DNA sequences of the ITS gene (Soleimani et al. 2022).

Cultivation and viability assessment of fungal conidia

The EPF isolates were maintained on Potato Dextrose Agar (PDA) at 25˚C ± 1˚C. The inoculum was prepared as described by Loko et al. (2022) with minor modifications. Briefly, conidia were gently scraped from the surface of a sporulating culture (2–3 weeks old) using a sterile spatula and suspended in sterile 0.2% (v/v) Aqueous Tween 20. The suspension was filtered through sterile gauze and vortexed for 2 min to separate the conidia. The pellet was resuspended in 0.2% Aqueous Tween 20 and the spore concentration determined using an Improved Neubauer hemocytometer (Superior Marienfeld, Germany) and adjusted to the required concentrations for the bioassay using sterile 0.2% Aqueous Tween 20. The viability of conidia for each EPF isolate was assessed by inoculating 0.1 mL of a 10^5 conidia/mL suspension onto PDA plates. After incubation at 25˚C ± 1 °C for 24 h, germination percentage was evaluated by randomly counting 100 conidia per plate under a microscope. The conidial viability percentage was above 94% for all isolates (Mehrmoradi et al. 2022).

Petri dish bioassays

Bioassays were conducted using 9 cm diameter plastic Petri dishes lined with sterile moist filter paper. Each Petri dish received a single fourth instar nymph and a slice of potato as a food source. One ml of each EPF suspension was applied using a handheld sprayer (BP-5078 1 L, BEHCO Trading Co, Hemei, Taiwan) at various concentrations (range 10^4 – 10^8 conidia/ml). Nymphs in the control treatment were sprayed with carrier (i.e. sterile 0.2% Aqueous Tween 20) only. Each treatment was replicated four times, and the entire experiment was repeated twice using fresh batches of fungal suspensions and insects. The lids of the Petri dish arenas had holes for ventilation. The containers were placed in a controlled chamber (25˚C ± 1˚C, 80% RH, L:D 16:8). Mortality was recorded daily. Insects were considered dead if there was no observable movement when prodded with a needle. Dead nymphs were disinfected with dilute sodium hypochlorite (NaClO) then placed into clean 9 cm diameter Petri dishes lined with moistened filter paper and kept in a controlled chamber (25˚C ± 1˚C, 80% RH, L:D 16:8) to encourage fungal emergence and external sporulation on the cadaver.

Soil column bioassays

To investigate the infectivity of EPF in soil, bioassays were conducted using clear plastic soil columns (4.5 cm × 8 cm × 6 cm) filled with autoclaved (121˚C for 2 h) soil collected from a vineyard located in Khalilabad, Razavi Khorasan Province, Iran (35° 15′ 6″ N, 58° 17′ 2″ E). Two ml of each EPF suspension were applied to the soil surface at either of four concentrations of conidia/ml per treatment: 10^5, 10^6, 10^7, or 10^8.Controls received two ml of 0.2% Aqueous Tween 20. After inoculation, a single nymph was placed on top of the soil and provided a piece of potato as food. The columns were kept incubated at 25 ± 1˚C, 80% RH, and a L: D 16:8 photoperiod. If insects were not at the surface, they could still be visualized in the soil profile through the clear plastic column. Mortality was monitored daily and any dead insects removed, disinfected with NaClO and transferred to clean Petri dishes lined with wet filter paper to encourage fungal emergence and sporulation as outlined above. There were four replicates per treatment and the entire experiment was repeated twice.

Semi-field pot bioassays

To evaluate the virulence of EPF against cicada nymphs, semi-field trials were conducted outdoors using plastic pots (12.1 cm× 12.1 cm× 10.7 cm) filled with soil collected from a natural habitat as described above. Each pot was treated with 2 ml of EPF suspension applied uniformly over the soil surface at a dose of either 10^6, 10^7, or 10^8 conidia/ml. Control pots were treated with 2 ml of 0.2% Aq. Tween 20. A single *P. alhageos* nymph was placed onto the soil surface and provided a slice of potato as food. The pots were placed in a vineyard located in Khalilabad, Razavi Khorasan Province, Iran (35° 15′ 6″ N, 58° 17′ 2″ E). Mortality was recorded every 24 h after inoculation. All insects that were considered as dead were disinfected with NaClO and transferred into clean Petri dishes (9 cm diameter) lined with wet filter paper to confirm the virulence of each EPF species tested as described above. Each treatment was replicated four times and the entire experiment was repeated twice.

Effect of soil type on virulence of EPF against *P. alhageos*

In this trial, three different soil types were investigated: sandy loam (63% sand, 24% silt, 13% clay, pH 8.56), loam (36% sand, 43% silt, 21% clay, pH 7.34), and clay loam (36% sand, 30% silt, 34% clay, pH 7.67). Based on our initial assays, *M. anisopliae* was selected for evaluation because it caused the highest mortality of *P. alhageos* nymphs. Soil columns (8 cm diameter × 6 cm height) were filled with 200 g of each of the three types of sterilized soils. Two ml of *M. anisopliae* suspensions (10^8 conidia/ml) were applied to the soil surface. The control treatment received 0.2% Aq. Tween 20 only. A

single-fourth instar *P. alhageos* nymph was then placed into each column. The bioassay columns were maintained at 25˚C ± 1˚C and 80% RH, and the mortality of *P. alhageos* nymphs was recorded every 24 h after inoculation. Dead insects were removed and disinfected before being transferred to a 9 cm dia Petri dish lined with wet filter paper to encourage fungal emergence as outlined earlier. Each treatment was replicated four times and the entire experiment was repeated twice with fresh batches of fungal suspensions, soil columns, and insects. The soil material kept at soil capacity.

Effect of temperature on virulence of EPF against *P. alhageos*

The effect of *M. anisopliae* on *P. alhageos* nymphs was evaluated at three different temperatures: 20˚C, 25˚C, or 30˚C using the soil column assay described above. Two ml of 10^8 conidia/ml of *M. anisopliae* was applied to the soil surface with controls consisting of carrier only. A single 4th instar nymph was placed on the soil surface and was provided a slice of potato as food. Daily mortality was recorded with dead insects being disinfected and transferred to clean Petri dishes (9 cm dia) lined with wet filter paper and kept at 25˚C ± 1˚C to encourage fungal emergence and sporulation. Each treatment was replicated four times and the entire experiment was repeated twice.

Statistical analysis

Corrected mortality was estimated using Abbott's formula (Abbott 1925). The normality and homogeneity of variance of cumulative mortality data were investigated using the Shapiro-Wilk test followed by Levene's test. The corrected mortality data were normalized using arcsine transformation to equalize the variances. The median lethal concentration (LC_{50}) for each EPF was estimated using probit analysis in POLO-PC. In Petri dish bioassays, one-way analysis of variance (ANOVA) was used to compare the virulence of different spore concentrations of each EPF strain, in all days. To compare the mortality of nymphs treated with EPF both in the soil column and the semi-field assay, nymph mortality data were analyzed using a two-way analysis of variance (ANOVA) followed by LSD tests with significance level at $P < 0.05$. Fungal species and spore concentration were considered as the two main effects. The effects of each factor alone and their interaction (EPF strain × spore concentration) were analyzed. Data in the experiments on soil type and temperature was subjected to one-way ANOVA followed by the LSD test ($P < 0.05$). For the semi-field trials, MST was calculated using the Kaplan–Meier method (Robertson et al. 2017). All statistical analyses were performed using the SAS software version 9.4 (SAS Institute 2020).

Results

Petri dish bioassays

All three EPF species were virulent to *P. alhageos* nymphs with mortality being dose-dependent (Table 1). At the highest dose, initial mortality was observed at 4 days post-inoculation (DPI) for *M. anisopliae* and at 6 DPI for *B. bassiana* (Table 1). Cumulative mortality increased for all three EPF species over time. At the highest dose, 70, 65 and 40% mortality was recorded 14 days following exposure to *M. anisopliae*, *B. bassiana* and *B. varroae*, respectively (Table 1). The LC_{50} for *M. anisopliae*, *B. bassiana* and *B. varroae* was 1.00×10^6, 7.2×10^6 and 6.4×10^7 conidia/ml, respectively (Table 2).

Soil column bioassays

Mortality of *P. alhageos* nymphs was dose dependent for all three pathogens but *M. anisopliae* caused higher mortality than the other species, followed by *B. bassiana* (Fig. 1). The highest mortality recorded occurred at the highest concentration tested for each species (*M. anisopliae*: $y = 0.9x - 4.1$, $R^2 = 0.9818$, $F_{1,2} = 0.0091$; *B. bassiana*: $y = 0.95x - 3.675$, $R^2 = 0.9757$, $F_{1,2} = 0.0122$: *B. varroae*: $y = 0.6x - 2.56$, $R^2 = 0.96$, $F_{1,2} = 0.0202$). Cumulative mortality rates ranged between 45% – 75% for *M. anisopliae* (Fig. 1a), 35% – 65% for *B. bassiana* (Fig. 1b), and 20% – 50% for *B. varroae* (Fig. 1c). Although the percentage mortality of *P. alhageos* 14 days post-inoculation was significantly affected by fungal species ($F_{2,36} = 12.08$, $P < 0.0001$) and spore concentration ($F_{3,36} = 8.23$, $P = 0.0003$), but there was no significant interaction between these factors ($F_{6,36} = 0.06$, $P = 0.99$) (Fig. 2).

Semi-field pot bioassays

Pot assays conducted under field conditions showed that *M. anisopliae* was the most virulent against *P. alhageos* nymphs, followed by *B. bassiana* then *B. varroae* with mortality being dose-dependent (Fig. 3). The highest and earliest mortalities occurred at the highest dose with *M. anisopliae* causing between 40 and 65% mortality, *B. bassiana* 35–55% mortality, and *B. varroae* 25–35% mortality (Fig. 3). Both fungal species ($F_{2, 27} = 10.55$, $P = 0.0004$) and spore concentration ($F_{2,27} = 7.84$, $P = 0.0021$) were significant factors affecting nymph mortality, but there was no significant interaction between these two factors ($F_{4, 27} = 0.06$, $P = 0.99$) (Fig. 4). There was non-statistical difference ($x^2 = 1.86$, $df = 2$; $P = 0.39$) in the median survival time (MST) of *P. alhageos* following exposure to *M. anisopliae* (5.6 days), *B. bassiana* (5.7 days) or *B. varroae* (5.9 days) at the highest dose tested (Table 3).

Effect of soil type on virulence of EPF against *P. alhageos*

Soil texture significantly ($F_{2,9} = 5.57$, $P < 0.027$) influenced the efficacy of *M. anisopliae* in killing *P. alhageos* nymphs

Table 1 Petri dish Bioassay: Corrected mean percentages (± SE) mortality of *Psalmocharias alhageos* 4th instar nymphs when exposed to different doses (1×10^4- 1×10^8 conidia/ml) of *Metarhizium anisopliae*, *Beauveria Bassiana* and *Beauveria varroae*. Mortality was recorded over 14 period post-inoculation

Days post-inoculation						
	4	**6**	**8**	**10**	**12**	**14**
% Mortality (±SE)						
M. anisopliae						
1×10^4	0 a	0 a	0 a	5.0 ± 5 a	10.0 ± 5.77 a	20.0 ± 8.16 a
1×10^5	0 a	0 a	5.0 ± 5 a	10.0 ± 5.77 a	20.0 ± 11.54 a	40.0 ± 9.57 a
1×10^6	0 a	5.0 ± 5 a	15.0 ± 5 b	20.0 ± 8.16 b	40.0 ± 8.16 ab	50.0 ± 8.16 ab
1×10^7	0 a	10.0 ± 5.77 a	25.0 ± 5 b	40.0 ± 0 b	60.0 ± 5 bc	65.0 ± 5.77 b
1×10^8	10.0 ± 5.77 b	30.0 ± 5.77 b	55.0 ± 5 c	65.0 ± 5.77 c	70.0 ± 5 c	70.0 ± 5 b
B. bassiana						
1×10^4	0	0 a	0 a	0 a	5.0 ± 5 a	10.0 ± 5 a
1×10^5	0	0 a	0 a	5.0 ± 5 a	10.0 ± 10.0 a	35.0 ± 9.57 a
1×10^6	0	0 a	0 a	5.0 ± 5.77 a	25.0 ± 9.57 ab	45.0 ± 9.57 ab
1×10^7	0	5.0 ± 5 ab	10.0 ± 5.77 a	20.0 ± 9.57 a	40.0 ± 8.16 bc	55.0 ± 9.57 ab
1×10^8	0	15.0 ± 5 b	35.0 ± 5 b	35.0 ± 5.77 b	65.0 ± 5 c	65.0 ± 5 b
B. varroae						
1×10^4	0	0 a	0 a	0 a	0 a	5.0 ± 5 a
1×10^5	0	0 a	0 a	0 a	10.0 ± 5.77 a	15.0 ± 8.16 a
1×10^6	0	0 a	0 a	5.0 ± 5 a	15.0 ± 5.0 a	30.0 ± 5.77 ab
1×10^7	0	0 a	5.0 ± 5 a	20.0 ± 8.16 ab	30.0 ± 5.57 ab	40.0 ± 8.16 ab
1×10^8	0	10.0 ± 5.77 b	25.0 ± 9.57 b	35.0 ± 12.58 b	45.0 ± 9.57 bc	50.0 ± 5.77 b

Mean values on day 14. Lower-case letters show significant differences between the fungal species and doses. (One-way ANOVA and Fisher's protected LSD, $P < 0.05$)

Table 2 LC_{50} values for *Metarhizium anisopliae*, *Beauveria bassiana* and *Beauveria Varroae* against *Psalmocharias alhageos* nymphs under laboratory conditions

EPF species	LC_{50} (95% CL)	Intercept ± SE [a]	Slope ± SE [b]	$\chi 2^c$ (df = 18)
M. anisopliae	1.00×10^6 (9.16×10^4–1.09×10^7)	−1.999 ± 0.534	0.333 ± 0.087	21.920
B. bassiana	7.2×10^6 (9.45×10^5–5.6×10^8)	−2.262 ± 0.681	0.330 ± 0.108	13.687
B. varroae	6.4×10^7 (1.2×10^7–2.3×10^9)	−3.257 ± 0.514	0.471 ± 0.078	13.519

[a] SE, standard error; [b] Slope error; [C] Pearson χ2 of the slope

with more nymphs killed in clay loam (87.5 ± 7.21%) than sandy loam soil (50 ± 10.2%) (Fig. 5). Intermediate values were obtained in loam soil (62.5 ± 7.21%) (Fig. 5).

Effect of temperature on virulence of EPF against *P. alhageos*

The cumulative mortality of *P. alhageos* caused by *M. anisopliae* was significantly ($F_{2, 9}$ = 16.8, $P = 0.01$) higher at 20˚C (93.75 ± 6.25%) than that at 25˚C (62.5 ± 7.21%) or 30˚C (37.5 ± 7.21%) (Fig. 6). There was a tendency for the nymphs to bury themselves deeper in the soil profile at the higher temperatures.

Discussion

This study shows that nymphs of the grapevine cicada, *P. alhageos*, are susceptible to EPF. A strain of *M. anisopliae* was identified which shows promise for control of this pest. The commercial strain of *B. bassiana*, GHA, also gave significant control. Both EPF species are known to infect soil dwelling arthropods, but virulence and specificity depend on the strain and dose received. For example, *Metarhizium brunneum* Petch strain F52/BIPESCO5 is highly virulent for a wide range of pests living in or pupating in the soil, including *O. sulcatus*, *T. pityocampa* and Welsh chafer (*Hoplia philanthus* Fuessly) (Coleoptera: Scarabaeidae), but is not virulent against Elateridae (wireworms) or some Tipulidae, (e.g. crane flies or leatherjackets) (Wood et al. 2023). Strain GHA is pathogenic to subterranean larvae of *Capnodis tenebrionis* Linné (Coleoptera: Buprestidae) but less so for the Glassy-winged Sharpshooter and *Homalodisca vitripennis* Germar (= *Homalodisca coagulata*) (Hemiptera: Cicadellidae), the vector of *Xylella fastidiosa* Wells et al. (Lysobacterales: Lysobacteraceae) (Ment et al. 2020).

In addition to strain selection, application methods can greatly influence efficacy of EPF treatments (Butt et al. 2001). Conidia applied as a drench or premixed into top layers of soil or applied as a mulch can give excellent control of pests living in the soil (e.g. black vine weevil) or which move into the soil to pupate such as the pine processionary moth (Güven et al. 2021), fruit flies (Yousef et

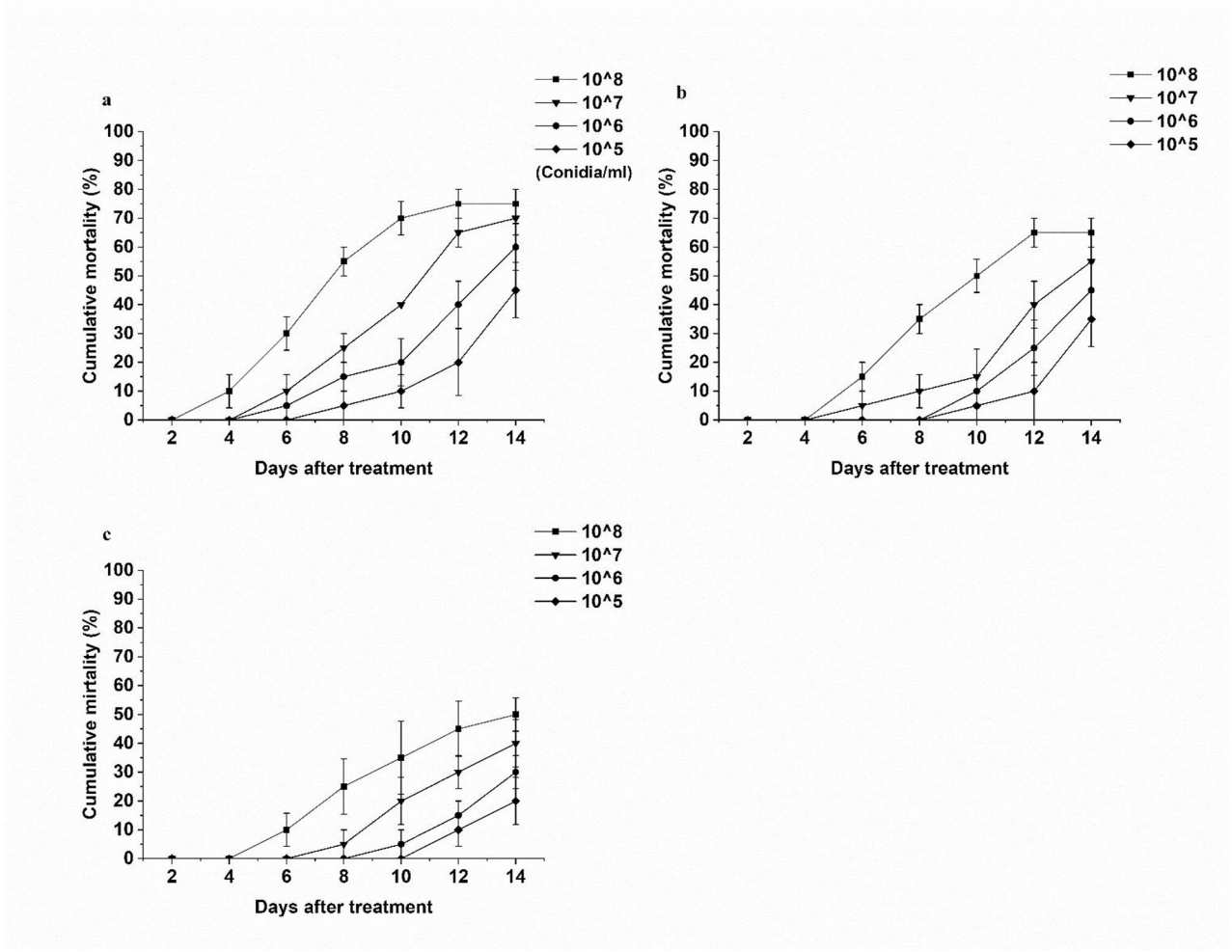

Fig. 1 Soil Column Bioassay. Mean cumulative (± SE) mortality of *Psalmocharias alhageos* 4th instar nymphs exposed to different doses ($1 \times 10^5 - 1 \times 10^8$ conidia/ml) of (**a**) *Metarhizium anisopliae*, (**b**) *Beauveria bassiana* and (**c**) *Beauveria varroae*. Mortality observed over 14 days

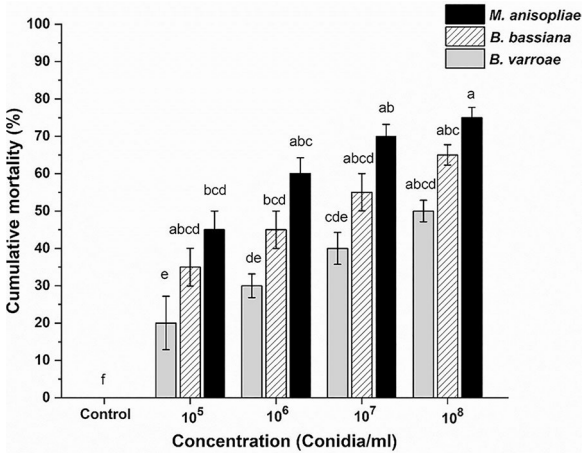

Fig. 2 Soil Column Bioassay. Mean percent mortality (± SE) of *Psalmocharias alhageos* nymphs 14 days post-inoculation with different doses ($1 \times 10^5 - 1 \times 10^8$ conidia/ml) of *Metarhizium anisopliae*, *Beauveria bassiana* and *Beauveria varroae*. Lowercase letters above bars indicate statistically significant differences between the fungal pathogens and concentrations (Two-way ANOVA and Fisher's protected LSD, $P < 0.05$)

al. 2017), and Western flower thrips (Ansari et al. 2008). In the present study, only a relatively small volume of conidia was applied to the soil surface, which was likely insufficient to leach into the soil. Nevertheless, significant mortality was achieved. At the start of all trials, including both soil and semi-field assays, nymphs were active and displayed high levels of movement within the experimental units. Following treatment with EPF, nymph activity within the soil increased significantly, particularly after 48 h. At the same time, a gradual decline in feeding activity was observed, and most nymphs appeared to settle in place. Although nymphs typically preferred near-surface activity, this behavior changed in the temperature assay, where elevated temperatures triggered a marked burrowing response, resulting in deeper soil penetration. Future studies will explore alternative strategies to optimize control, such as drilling conidia into the soil. Furthermore, applying the fungus as a mulch or incorporating it into the upper soil layers before larvae migrate from branches into the soil or emerge from it has produced excellent

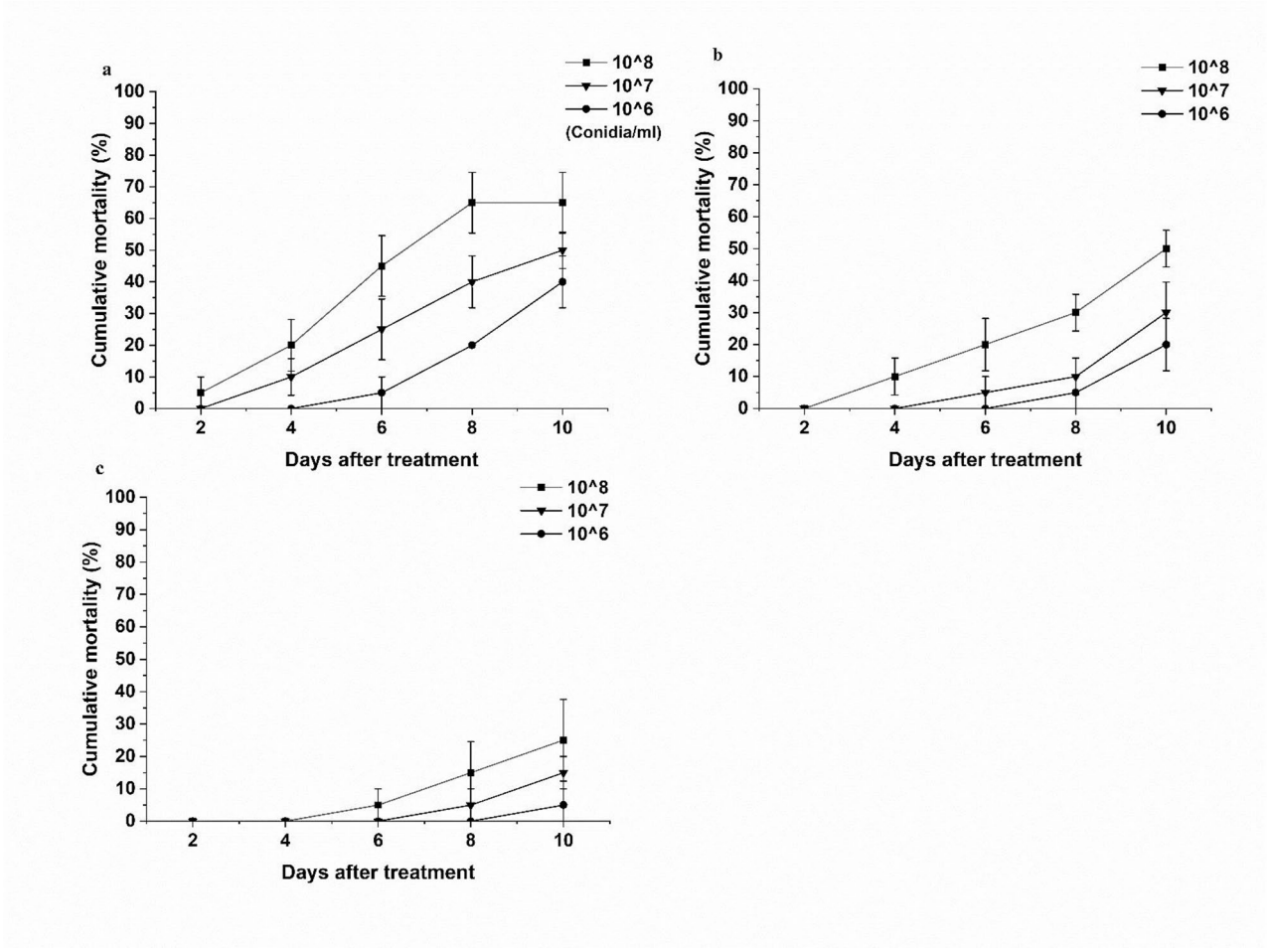

Fig. 3 Semi-field Pot Bioassay. Mean cumulative mortality (± SE) of *Psalmocharias alhageos* 4th instar nymphs when exposed to different doses ($1 \times 10^5 - 1 \times 10^8$ conidia/of ml) of (**a**) *Metarhizium anisopliae*, (**b**) *Beauveria bassiana* and (**c**) *Beauveria varroae.* Mortality was recorded over a 10-day period

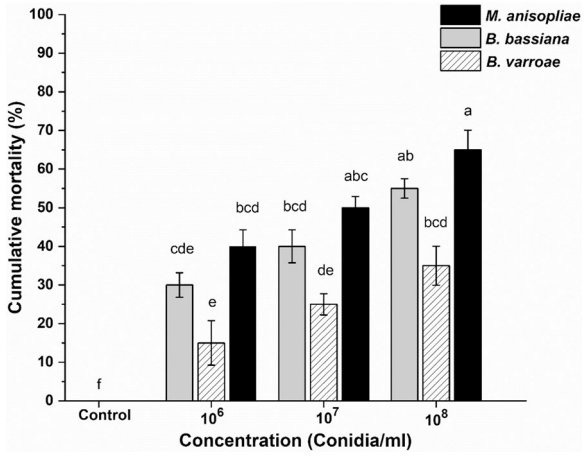

Fig. 4 Pot Semi-Field bioassay. Mean percent mortality (± SE) of *Psalmocharias alhageos* 4th instar nymphs when exposed to different doses ($1 \times 10^5 - 1 \times 10^8$ conidia/ml) of (a) *Metarhizium anisopliae*, *(b) Beauveria bassiana* and (c) *Beauveria varroae.* Mortality recorded 14 days post-inoculation. Lowercase letters above error bars indicate statistically significant differences between fungal species and doses (Two-way ANOVA and Fisher's protected LSD, $P < 0.05$)

Table 3 Effect of conidial concentrations of *Beauveria bassiana*, *metarhizium anisopliae* and *Beauveria Varroae* on median survival time (MST) of *Psalmocharias alhageos* nymphs under semi-field conditions

Concentration (conidia/ml)	EPF species	MST[1]	SE[2]	95% Confidence interval
	M. anisopliae	5.588	0.108	(5.377–5.800)
1×10^8	*B. bassiana*	5.714	0.091	(5.536–5.892)
	B. varroae	5.862	0.065	(5.735–5.989)
	M. anisopliae	5.694	0.092	(5.513–5.875)
1×10^7	*B. bassiana*	5.779	0.074	(5.634–5.925)
	B. varroae	5.866	0.058	(5.754–5.979)
	M. anisopliae	5.769	0.077	(5.617–5.921)
1×10^6	*B. bassiana*	5.839	0.063	(5.715–5.962)
	B. varroae	5.922	0.044	(5.836–6.008)

[1] Median survival time in days

[2] Standard error of the MST in days after analyses with the Kaplan-Meier-test

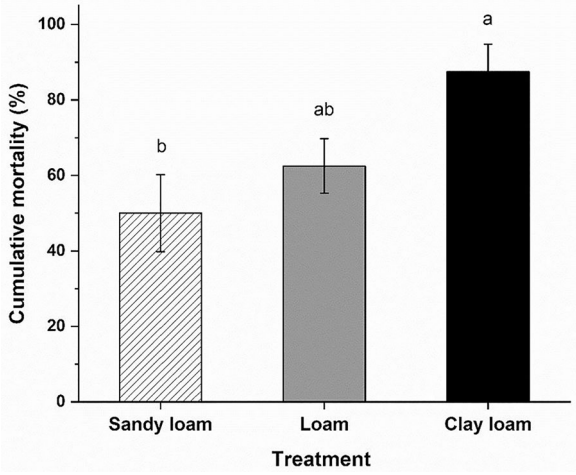

Fig. 5 Mean cumulative mortality (±SE) of *Psalmocharias alhageos* nymphs in various soil types (sandy-loam, loam or clay-loam) 14 days post-inoculation with *Metarhizium anisopliae* at 1×10^8 conidia/ml. One-way ANOVA and Fisher's protected LSD, $P < 0.05$

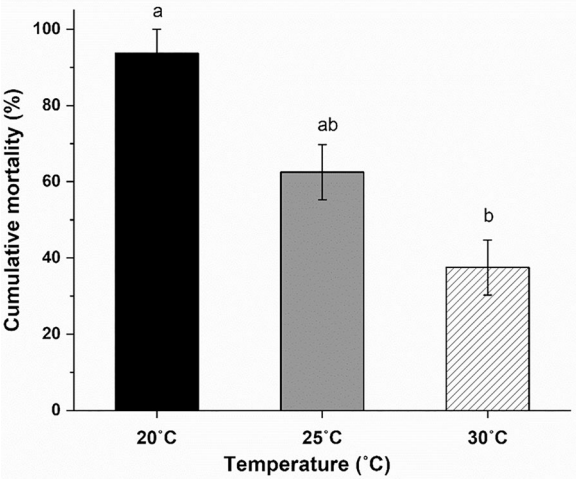

Fig. 6 Mean cumulative mortality (±SE) of *Psalmocharias alhageos* 4th instar nymphs at 20°C, 25°C and 30°C, 14 days post-inoculation with *Metarhizium anisopliae* at 1×10^8 conidia/ml. One-way ANOVA and Fisher's protected LSD, $P < 0.05$

results against pests such as fruit flies (Gava et al. 2021), chestnut tortrix, and chestnut weevil (Asan et al. 2017).

Managing pests in the soil environment presents challenges, but it also offers advantages for controlling subterranean species. Challenges include migration of the pest deep into the soil at extreme temperatures or when the soil dries. The soil also contains many antagonists which can impede or even kill EPF (Scheepmaker and Butt 2010). Unlike the canopy, soils are less prone to dramatic fluctuations in temperature and humidity which can impede fungal infection of pests. EPF are found in disparate ecosystems and soil types but edaphic factors can influence conidial persistence, movement and efficacy (Mantzoukas et al. 2025). It is unclear why *M. anisopliae* gave better control of *P. alghageos* in clay loam than sandy loam. Although we presume vertical movement is poor in clay soils than sandy soils, some studies showed no major differences with most spores being concentrated at the surface (Gava et al. 2021). Soil chemistry can influence the persistence and infectivity of EPF conidia (Sharma et al. 2021) but because the nymphs largely remained near the surface, it's impact on infectivity is likely minimal. However, the greater nymphal activity observed in clay loam soil compared to sandy loam soil may enhance conidia acquisition, and since mortality is dose dependent, this would lead to higher mortality (Butt 2001).

Entomopathogenic fungi are mesothermic and less effective at temperatures below 10°C or above 30°C with the optimum temperature range being between 20 and 25°C (Omuse et al. 2022). However, numerous studies have shown that EPF can still kill pests at higher temperatures (Sani et al. 2023). We postulate that elevated temperature may cause nymphs to move deeper into the soil column (Soltani et al. 2018), potentially distancing *P. alhageos* nymphs from areas with the highest EPF inoculum. Higher temperatures can also enhance host survival by both suppressing the pathogen and strengthening the host's immune system to resist or eliminate fungal infection (Kryukov et al. 2018), underscoring as key role of temperature in many studies (Saeidi 2022).

The pattern of virulence observed in laboratory Petri dish assays was also evident in semi-field pot trials, confirming *M. anisopliae*, followed by *B. bassiana*, as virulent against *P. alhageos*. In our semi-field evaluation, *M. anisopliae* and *B. bassiana* caused approximately 75 and 65% cumulative mortality of *P. alhageos*, respectively, which is remarkably consistent with previous results obtained with *Hyalesthes obsoletus* Signoret (Moussa et al. 2021). Moreover, the recent research by Uzman et al. (2019) demonstrated the remarkable persistence of EPF in intensively managed vineyard soils despite the application of chemical pesticides. This persistence highlights the potential compatibility of these EPF and conventional chemical control measures, offering a unique opportunity to develop a synergistic pest management program.

Although EPF are unlikely to serve as a stand-alone tool for managing *P. alhageos*, obtained results suggest they hold promise as a complementary component of an integrated management program. To advance their application, future work should focus on optimizing formulation and delivery methods—such as soil drenches or granular formulations—to improve inoculum persistence across diverse soil environments. Field-scale efficacy trials and assessments of more complex environmental interactions are also recommended to ensure compatibility with sustainable agroecosystems. In addition, the potential for endophytic associations between EPF and

grapevines warrants investigation, as this may reveal important effects on plant physiology and defense against agricultural pests. Finally, exploring synergistic combinations with other biocontrol agents (e.g., entomopathogenic nematodes and bacteria), target-specific chemical pesticides, and cultural practices (e.g., acoustic mating disruption, kaolin barriers) will be critical for developing robust, multi-modal management strategies.

Conclusion

The findings of this study show that *M. anisopliae* and *B. bassiana* exhibited strong virulence against fourth-instar of *P. alhageos* nymphs, with *M. anisopliae* proving the most effective, as reflected by its lowest LC_{50} and highest overall virulence in both laboratory and semi-field bioassays. The efficacy of both fungi was clearly dose dependent, with *M. anisopliae* consistently outperforming *B. bassiana* and *B. varroae* across all experiments. Environmental factors also played a key role: soil texture and temperature significantly influenced EPF performance, with clay loam associated with higher mortality and *M. anisopliae* showing peak virulence at 20 °C but reduced efficacy at 30 °C. This decline was likely due to limited conidial persistence and altered cicada burrowing behavior under heat stress. Median survival times were shortest for *M. anisopliae*, indicating rapid virulence under favorable conditions. Together, these results highlight the considerable potential of this indigenous *M. anisopliae* strain as a biopesticide candidate for the integrated management of *P. alhageos* nymphs.

Abbreviations

ANOVA	Analysis of variance
DNA	Deoxyribonucleic acid
DPI	Days post inoculation
EPF	Entomopathogenic fungi
ITS	Internal transcribed spacer
MST	Median survival time
PDA	Potato dextrose agar
RH	Relative humidity

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Author contributions

KS collected and handled the cicadas. KS and MR conducted laboratory and greenhouse experiments. KS and SG performed statistical analysis. JK conceptualized the project and provided mentorship. KS, MR, JK and TMB wrote the main article. JK, LS and TMB provided critical review of the final version, and substantive revisions to the manuscript's structure. All authors reviewed and approved the final manuscript.

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Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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