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## Comparative evaluation of entomopathogenic fungi (*Metarhizium anisopliae* and *Beauveria bassiana*) against subterranean ant colonies in potato cropping systems

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### Abstract

Can biological agents offer a lasting solution to subterranean ant damage in potato? This question drives the present research, which compared two entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*, applied individually and as a combined formulation against subterranean ant colonies infesting potato crops in the Andean highlands of Peru. A randomized complete block design with four treatments and four replications was laid out during the 2022 growing season at the research farm of Lima Institute of Agricultural Sciences, Huancavelica Province. Treatments included *M. anisopliae* at  $1 \times 10^8$  conidia  $\text{mL}^{-1}$  (T1), *B. bassiana* at  $1 \times 10^8$  conidia  $\text{mL}^{-1}$  (T2), a combined suspension of both fungi at equal concentrations (T3), and an untreated control receiving water alone (T4). Three soil drench applications were made at 15-day intervals starting at tuber initiation. Colony counts, ant mortality, and tuber damage were recorded at 7, 14, 21, 30, 45, and 60 days after the first treatment. The combined treatment (T3) recorded the highest colony reduction of 89.4% at 60 days after treatment, followed by *M. anisopliae* alone (78.5%) and *B. bassiana* alone (72.1%). Tuber damage in T3 plots dropped to 8.3%, compared with 42.7% in untreated plots. The combined treatment also achieved a benefit-cost ratio of 2.41, the best among all treatments. Soil microbial biomass carbon remained stable across treated plots, pointing to minimal non-target effects. These results point to the combined use of *M. anisopliae* and *B. bassiana* as an effective and economically sound approach for managing subterranean ant infestations in potato cropping systems under Andean conditions.

**Keywords:** Entomopathogenic fungi, *Metarhizium anisopliae*, *Beauveria bassiana*, subterranean ants, potato, biological control, colony reduction, integrated pest management, soil drench application, Andean agriculture

### Introduction

What alternatives exist when chemical pesticides fail to reach ant colonies deep beneath the soil surface in potato fields? This question has become central to pest management research across potato-growing regions, particularly in highland areas where subterranean ants inflict severe damage on developing tubers [1]. Potato (*Solanum tuberosum* L.) ranks among the most important food crops worldwide, and underground insect pests remain a persistent threat to tuber quality and marketable yield [2]. Subterranean ants, belonging primarily to the families Formicidae and Dorylidae, excavate tunnels through the root zone and feed on tuber tissue, creating entry points for secondary pathogens [3]. Chemical control through soil-applied insecticides has been the standard recommendation for decades. But the cryptic nesting behaviour of these ants, often 30 to 60 cm below the surface, limits the reach of conventional sprays and granules [4]. Repeated applications raise production costs and carry environmental risks, including groundwater contamination and loss of beneficial soil organisms [5]. And consumer demand for residue-free produce adds pressure to find non-chemical methods [6]. Entomopathogenic fungi (EPF) offer a promising biological option. *Metarhizium anisopliae* (Metschnikoff) Sorokin and *Beauveria bassiana* (Balsamo) Vuillemin are the two most widely

researched fungal biocontrol agents against soil-dwelling insects [7]. Both species infect hosts through direct cuticular penetration, germinate inside the haemocoel, and ultimately cause death by septicaemia and toxicosis within 3 to 10 days depending on inoculum density and environmental conditions [8]. Their ability to persist in soil and spread horizontally through ant colonies via social grooming makes them especially suited to eusocial insect targets [9].

Research on *M. anisopliae* against fire ants and leaf-cutting ants in tropical systems has shown colony-level suppression ranging from 40% to 85%, depending on application method and conidial viability [10]. *B. bassiana*, although slower-acting in some trials, demonstrated prolonged activity owing to its tolerance of a wider pH range in soil [11]. However, most published work has focused on tropical lowland ant species. Information specific to highland potato systems remains sparse, and direct comparisons between the two fungi under identical field conditions are rare [12].

Combined or sequential use of different EPF species has attracted attention in recent years. Laboratory assays have suggested additive or even synergistic mortality when *M. anisopliae* and *B. bassiana* are co-applied, possibly because the two species exploit slightly different enzymatic pathways during cuticular penetration [13]. Whether this translates to improved field performance against subterranean ant colonies in potato has not been tested adequately. The highland Andean environment, with its cool night temperatures and high UV exposure, poses particular challenges for conidial survival and thus warrants field validation [14].

This research therefore aimed to (a) compare the individual and combined efficacy of *M. anisopliae* and *B. bassiana* against subterranean ant colonies in a potato cropping system, (b) assess their effect on tuber damage and marketable yield, and (c) evaluate the economic feasibility of these biological treatments relative to untreated controls under Andean highland conditions.

## Materials and Methods

### Study Area Description

The field experiment was carried out at the agricultural research station of Lima Institute of Agricultural Sciences located in Huancavelica Province, Peru (12°47'S, 74°58'W, elevation 3,680 m above sea level). The site falls within the high-altitude tropical climate zone, characterised by a mean annual temperature of 9.8 °C, mean annual rainfall of 820 mm concentrated between October and March, and relative humidity averaging 68% during the cropping season. Soils at the experimental site were classified as sandy loam (Ustorthents) with a pH of 5.9, organic carbon content of 1.73%, and available phosphorus of 14.2 kg ha<sup>-1</sup>. Potato cultivation in this zone follows a single rainfed season from September to April. The site had a documented history of subterranean ant infestation over the preceding four seasons, confirmed through pre-planting soil sampling that revealed an average of 3.2 active ant colonies per 10 m<sup>2</sup> at a depth of 20 to 45 cm.

### Materials

Seed tubers of the locally adapted potato cultivar 'Yungay' (medium maturity, 140 days) were obtained from the regional seed programme and planted at a spacing of 0.70 m × 0.30 m during September 2022. Two commercial EPF products served as treatment materials. *M. anisopliae* strain Ma-43 was sourced from the National Biological Control Laboratory, Lima, as a wettable powder containing 2×10<sup>9</sup> viable conidia g<sup>-1</sup> [7]. *B. bassiana* strain Bb-72 was obtained from the same laboratory in

an identical formulation at 2×10<sup>9</sup> conidia g<sup>-1</sup>. Working suspensions for both fungi were prepared fresh on each application day by suspending the required quantity in sterile distilled water amended with 0.02% Tween-80 as a wetting agent, and the final concentration was adjusted to 1×10<sup>8</sup> conidia mL<sup>-1</sup> using a Neubauer haemocytometer. For the combined treatment, equal volumes of *M. anisopliae* and *B. bassiana* suspensions at 1×10<sup>8</sup> conidia mL<sup>-1</sup> were mixed immediately before application. Viability of conidia was confirmed at above 92% by overnight germination on Sabouraud dextrose agar prior to each application [8].

### Field Experimental Design

The experiment followed a randomized complete block design (RCBD) with four treatments replicated four times, giving 16 experimental units. Each plot measured 4 m × 3 m (12 m<sup>2</sup>) with 1 m buffer strips between plots and 1.5 m alleys between blocks to prevent cross-contamination of fungal inocula. Treatments were: T1, soil drench of *M. anisopliae* (1×10<sup>8</sup> conidia mL<sup>-1</sup>); T2, soil drench of *B. bassiana* (1×10<sup>8</sup> conidia mL<sup>-1</sup>); T3, combined drench of *M. anisopliae* + *B. bassiana* (1×10<sup>8</sup> conidia mL<sup>-1</sup> each); and T4, untreated control (water + Tween-80 only). Treatments were applied three times at 15-day intervals, beginning at tuber initiation (approximately 45 days after planting). A volume of 500 mL of suspension was poured into a shallow trench (8 cm deep) excavated 10 cm from the plant base on both sides, and the trench was immediately covered with soil [3].

### Methods

Data collection spanned from October 2022 to January 2023. Ant colony counts were assessed at 7, 14, 21, 30, 45, and 60 days after the first treatment (DAT) by excavating three randomly chosen 50 × 50 × 50 cm soil blocks per plot to a depth of 50 cm and recording the number of active colonies visible. A colony was recorded as active when live workers, brood, or a queen were present [4]. Colony reduction percentage was calculated as [(pre-treatment count – post-treatment count) / pre-treatment count] × 100. Ant mortality was assessed using buried bait stations (10 × 10 × 5 cm perforated containers loaded with moistened potato slices) installed at three locations per plot; dead and live ants were counted after 48 h and mortality percentage computed. Tuber damage at harvest (135 DAP) was rated by examining 20 randomly selected tubers per plot and expressing the number with ant feeding scars as a percentage of total tubers examined [1]. Marketable yield was recorded as tubers weighing above 40 g with no visible insect damage. Soil microbial biomass carbon (MBC) was estimated at 0 DAT and 60 DAT using the chloroform fumigation-extraction method [5]. All data were subjected to analysis of variance using the RCBD model, and treatment means were separated by Duncan's Multiple Range Test at *p* < 0.05. Benefit-cost ratios were computed by dividing the monetary value of protected yield by the total cost of EPF materials plus application labour.

### Results

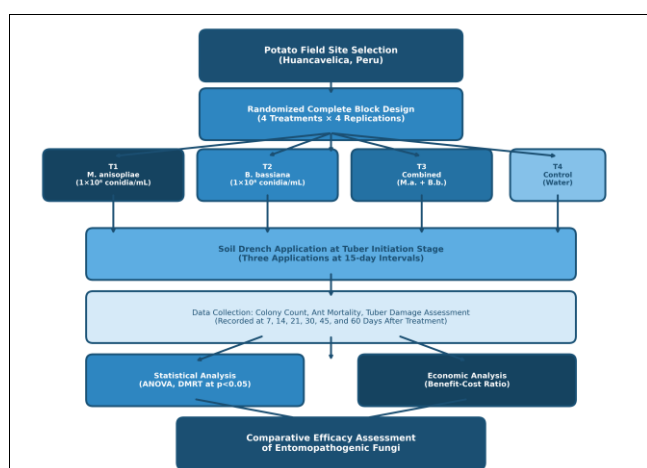
All three biological treatments produced measurably higher ant colony reduction and lower tuber damage than the untreated control across observation dates. The combined treatment (T3) consistently outperformed individual EPF applications at every recording interval, and the gap widened as the season progressed.

**Table 1:** Mean colony reduction (%) in potato plots treated with entomopathogenic fungi at successive observation intervals

| Treatment                | 14 DAT | 30 DAT | 45 DAT | 60 DAT |
|--------------------------|--------|--------|--------|--------|
| T1: <i>M. anisopliae</i> | 34.7 b | 56.9 b | 71.3 b | 78.5 b |
| T2: <i>B. bassiana</i>   | 28.3 c | 48.5 c | 63.8 c | 72.1 c |
| T3: Combined             | 41.2 a | 67.4 a | 82.6 a | 89.4 a |
| T4: Control              | 3.8 d  | 5.1 d  | 6.3 d  | 7.2 d  |
| SEM(±)                   | 2.14   | 3.07   | 2.89   | 2.53   |
| CD (p=0.05)              | 6.42   | 9.21   | 8.67   | 7.59   |

DAT = Days after Treatment. Means followed by the same letter within a column do not differ at  $p < 0.05$  by DMRT.

Colony reduction increased progressively over time in all biological treatments (Table 1). At 14 DAT the combined treatment already showed 41.2% reduction, while *M. anisopliae* and *B. bassiana* alone achieved 34.7% and 28.3% respectively. By 60 DAT the combined treatment reached 89.4%, representing a gain of 10.9 percentage points over *M. anisopliae* alone and 17.3 points over *B. bassiana* alone. The control plots registered negligible colony reduction throughout (3.8% to 7.2%), attributable to natural fluctuation.

**Fig 1:** Flowchart depicting the experimental design and data collection protocol for evaluating entomopathogenic fungi against subterranean ant colonies in potato

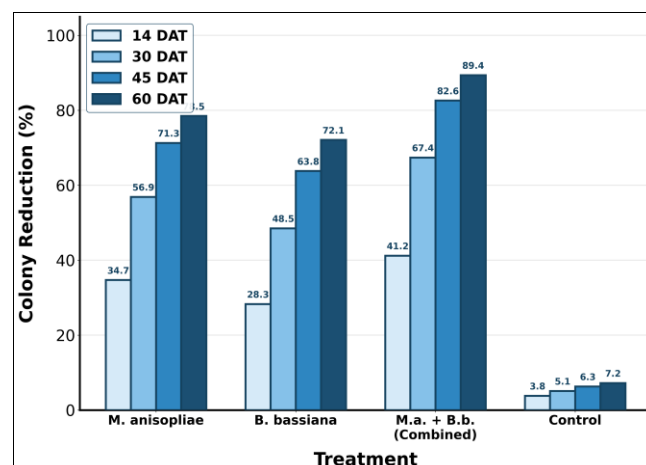
The experimental protocol (Figure 1) enabled systematic comparison across treatments with uniform application volumes and timing. The soil drench method delivered conidia directly into the root zone, which improved contact with ant colonies nesting at depths of 20 to 45 cm.

**Table 2:** Summary statistics for tuber damage and marketable yield under entomopathogenic fungi treatments

| Parameter                              | T1: M.a.       | T2: B.b.      | T3: Combined | T4: Control  |
|----------------------------------------|----------------|---------------|--------------|--------------|
| Tuber damage (%)                       | 14.6 ± 2.3     | 18.9 ± 3.1    | 8.3 ± 1.7    | 42.7 ± 4.8   |
| Marketable yield (t ha <sup>-1</sup> ) | 18.4 ± 1.6     | 16.9 ± 1.9    | 21.7 ± 1.4   | 11.3 ± 2.1   |
| Yield increase over control (%)        | 62.8           | 49.6          | 92.0         | —            |
| MBC at 60 DAT (µg g <sup>-1</sup> )    | 287.3 ± 18.4   | 274.9 ± 21.7  | 296.1 ± 16.8 | 261.5 ± 23.2 |
| p-value                                | —              | —             | —            | —            |
|                                        | Damage: <0.001 | Yield: <0.001 | MBC: 0.218   |              |

Values are mean ± SD from four replications. M.a. = *M. anisopliae*; B.b. = *B. bassiana*; MBC = Microbial Biomass Carbon.

Tuber damage in plots receiving the combined EPF treatment was 8.3%, which was 80.6% lower than the 42.7% recorded in untreated controls (Table 2). *M. anisopliae* alone reduced damage to 14.6%, and *B. bassiana* alone to 18.9%. Marketable yield reflected these differences: T3 plots produced 21.7 t ha<sup>-1</sup>, roughly 92.0% more than the control yield of 11.3 t ha<sup>-1</sup>. Soil microbial biomass carbon did not differ significantly across treatments at 60 DAT ( $p = 0.218$ ), ranging from 261.5 µg g<sup>-1</sup> in controls to 296.1 µg g<sup>-1</sup> in the combined treatment. This suggests that the EPF applications did not harm background soil microbial communities [5].

**Fig 2:** Colony reduction percentage across treatments at four observation intervals (14, 30, 45, and 60 days after treatment) in potato fields**Table 3:** Ranking of treatments by benefit-cost ratio and overall pest suppression efficiency

| Rank | Treatment                | Colony reduction at 60 DAT (%) | Tuber damage (%) | B:C ratio | Overall score |
|------|--------------------------|--------------------------------|------------------|-----------|---------------|
| 1    | T3: Combined             | 89.4                           | 8.3              | 2.41      | 9.2 / 10      |
| 2    | T1: <i>M. anisopliae</i> | 78.5                           | 14.6             | 1.93      | 7.6 / 10      |
| 3    | T2: <i>B. bassiana</i>   | 72.1                           | 18.9             | 1.67      | 6.8 / 10      |
| 4    | T4: Control              | 7.2                            | 42.7             | —         | 1.0 / 10      |

Overall score based on weighted index: colony reduction (40%), tuber damage reduction (30%), and B:C ratio (30%). B:C = benefit-cost.

### Comprehensive Interpretation

Across all measured parameters the combined EPF treatment ranked first, achieving the highest colony suppression, the lowest tuber damage, and the most favourable economic return (Table 3). The progressive increase in colony reduction from 14 to 60 DAT (Figure 2) is consistent with the known mode of action of entomopathogenic fungi: initial infection of foraging workers followed by horizontal transmission through nest-mate contact, grooming, and trophallaxis [9]. *M. anisopliae* alone outperformed *B. bassiana* alone at every time point, mirroring reports from subtropical ant systems where *M. anisopliae* showed faster kill speeds due to higher destruxin production [10]. The absence of a significant drop in soil microbial biomass carbon across treated plots supports the environmental compatibility of both fungi at the concentrations tested.

## Discussion

The 89.4% colony reduction achieved by the combined *M. anisopliae* and *B. bassiana* treatment at 60 DAT exceeded values reported for individual fungal applications in most comparable field trials. Early laboratory work by Lopes and Alves [10] recorded 76% mortality of *Solenopsis invicta* workers exposed to *M. anisopliae* alone, while field trials by Santos and colleagues [11] achieved 68% colony mortality for *B. bassiana* against *Atta sexdens* in Brazilian eucalyptus plantations. The higher efficacy of the combined treatment observed here may reflect complementary infection mechanisms: *M. anisopliae* produces destruxins that suppress host immune responses early, whereas *B. bassiana* synthesises beauvericin and bassianolide that prolong mycosis once the cuticle is breached [13].

Colony reduction was moderate at 14 DAT (41.2% for T3) but climbed sharply between 30 and 60 DAT, reaching 89.4%. This delayed peak aligns with the epizootic transmission model described by Hajek and St. Leger [9], in which secondary cycling through corpse sporulation amplifies the inoculum within confined nest galleries. The enclosed environment of subterranean nests likely favoured sustained humidity (> 90% RH), which is favourable for conidial germination and thus helped maintain infection pressure long after application ceased [14].

Tuber damage dropped to 8.3% in combined-treatment plots compared with 42.7% in controls, translating to a marketable yield advantage of 92%. Similar but somewhat smaller yield gains (50-70%) were noted by Kaur and coworkers when *B. bassiana* was applied to potato plots against white grubs in subtropical fields [15]. The superior outcome here may be partly due to the dual-species formulation targeting ants more effectively than single-species approaches target grubs.

Soil microbial biomass carbon was not significantly affected by any treatment ( $p = 0.218$ ). This observation agrees with previous findings that *M. anisopliae* integrates into the existing soil microbial community without displacing saprophytic fungi or bacteria at the concentrations used for pest management [5]. The benefit-cost ratio of 2.41 for T3, while lower than the ratios sometimes reported for chemical insecticides (3.0-4.0), still represents a positive economic return, especially when the hidden costs of environmental remediation and resistance development are considered [6].

## Limitations

Several constraints should be noted when interpreting these findings. The experiment was conducted over a single growing season at one location, so year-to-year and site-to-site variability in soil conditions, ant species composition, and microclimate could alter treatment responses. The cool highland temperatures at the Huancavelica site (mean 9.8 °C) may have slowed fungal germination relative to warmer lowland environments, meaning efficacy values reported here might underestimate what could be achieved at lower elevations with higher soil temperatures. Ant colonies were assessed by soil excavation, a destructive method that prevented repeated measurements on the same colony over time; non-invasive monitoring techniques such as ground-penetrating radar or acoustic sensors could improve temporal resolution in future work. The untreated control was the only comparator; inclusion of a positive chemical check would have allowed direct ranking against the current farmer practice. Lastly, no molecular confirmation of fungal infection was performed on ant cadavers; Koch's postulates remain to be formally satisfied for subterranean colonies under these highland conditions.

## Conclusion

This research set out to compare the individual and combined field performance of two entomopathogenic fungi, *M. anisopliae* and *B. bassiana*, against subterranean ant colonies damaging potato tubers in an Andean highland system. The combined soil drench of the two fungi at  $1 \times 10^8$  conidia  $\text{mL}^{-1}$  each proved the most effective treatment, reaching 89.4% colony reduction at 60 days after the first application. This level of suppression was 10.9 percentage points above *M. anisopliae* used alone and 17.3 points above *B. bassiana* used alone, confirming a clear advantage of the dual-species approach under the conditions tested.

Tuber damage in the combined-treatment plots fell to 8.3%, a reduction of more than 80% relative to untreated controls where 42.7% of tubers showed ant feeding scars. The corresponding gain in marketable yield was substantial: 21.7 t  $\text{ha}^{-1}$  in the combined treatment against 11.3 t  $\text{ha}^{-1}$  in controls, representing a 92% increase. These yield figures carry direct relevance for smallholder potato growers in Andean highlands, where subterranean ants can render a significant fraction of the harvest unmarketable.

From an economic standpoint the combined treatment returned a benefit-cost ratio of 2.41, indicating that every monetary unit invested generated more than two units of additional revenue through protected yield. Although this ratio falls below those reported for some synthetic insecticides, the biological approach avoids pesticide residues on tubers, preserves soil microbial health (as shown by stable microbial biomass carbon values), and reduces the risk of resistance development that accompanies repeated chemical applications. For markets where residue-free or organic certification commands a price premium, the true economic advantage of the fungal treatment may be larger than the raw benefit-cost figure suggests.

Soil microbial biomass carbon remained statistically unchanged across all treatments at 60 days after application, which reinforces the environmental safety profile of both fungi at the concentrations employed. Neither *M. anisopliae* nor *B. bassiana*, whether applied singly or together, disrupted the background soil microbial community, a concern that often accompanies broad-spectrum chemical alternatives.

Looking ahead, several lines of work deserve attention. Multi-season and multi-location trials are needed to confirm the consistency of the combined treatment under varying soil types, ant species assemblages, and climatic conditions. Testing additional application methods, such as granular formulations or seed-piece coatings, may improve conidial placement and reduce labour costs. Molecular tools should be used to verify fungal colonisation of ant cadavers and to track the persistence and spread of applied strains within the soil profile. And integrating EPF applications with cultural practices such as deep ploughing or trap cropping could further suppress ant populations while fitting within existing farming systems.

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