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PLANT PROTECTION
Article

Fungal enemies isolated from the root and rhizosphere of guava against the root-knot nematode

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ABSTRACT: The fungal microbiota associated with the roots and rhizosphere of plants represents a significant source of biocontrol agents against the root-knot nematode *Meloidogyne* spp. While some fungal genera have been studied extensively, others require more attention in terms of their identity and mechanisms of action against plant parasitic nematodes. Thus, the aim of this research was to demonstrate the *in vitro* and *in vivo* biocontrol potential of fungi isolated from the root and rhizosphere of guava plants against the root-knot nematode *Meloidogyne incognita*. We isolated four fungi (C5, C6, C7, and C10) from the root and rhizosphere of guava plants using the serial dilution method. These fungi were purified on potato dextrose agar and evaluated *in vitro* to assess their ability to parasitize *M. incognita* eggs and juveniles. Subsequently, the fungi were assessed for *M. incognita* control under nursery conditions in guava plants. The identity of the fungi with parasitic capacity and biocontrol potential was confirmed by amplifying and sequencing the ITS region, followed by Bayesian inference analysis. Results showed that the fungi corresponded to *Talaromyces sayulitensis* (C5), *Myrothecium* sp. (C6), *Penicillium* shearii (C7), and *Beauveria bassiana* (C10). Parasitism of *M. incognita* eggs was confirmed for all fungi, parasitism of juveniles occurred with *T. sayulitensis* (C5) and *B. bassiana* (C10). In guava seedlings treated with fungal isolates, a significant reduction in the number of eggs, juveniles, and nodules per gram of root was achieved. The biocontrol potential against *M. incognita* in guava seedlings was confirmed for different fungi associated with the root rhizosphere.

Key words: eggs, fungi, juveniles, nematode, parasitism.

INTRODUCTION

Reductions in crop performance and losses of up to 30% are the main consequence of phytopathogenic agents in plants worldwide (Savary et al. 2019). Among these pathogens, plant parasitic nematodes cause losses in a variety of economically important crops worldwide (Stirling 2011), and the species of the genus *Meloidogyne* affect most cultivable plants, as they are cosmopolitan and highly detrimental (Moens et al. 2009).

During the infection process of *Meloidogyne* spp., second-stage juveniles (J2) penetrate the plant root, migrate intracellularly until reaching a site near the vascular tissue, in which they select feeding cells to complete their sedentary life cycle (Cai et al. 2023, Fuller et al. 2008, Williamson and Hussey 1996). During the infection process, *Meloidogyne* spp. causes histological alterations in the plant roots, resulting in galls, typical symptoms of the nematode (Cabrera et al. 2018, Favery et al. 2002). The alterations in the host, including modifications in the contents and arrangement of cellulose, hemicellulose, pectin, and lignin of the cell wall, leads to thickening and loosening, which facilitates the expansion of giant cells (Meidani et al. 2019). These cells are larger, metabolically active, multinucleated, and result from multiple nuclear divisions without cytokinesis (Escobar et al. 2015, Jones and Goto 2011, Mejias et al. 2019, Yamaguchi et al. 2017). The multiple alterations result in nutrient and water uptake impairment, which is reflected in reduced plant growth and development (Sikora et al. 2018).

In the commercial production of guava (*Psidium guajava*) in the Americas, nematode species *Meloidogyne incognita*, *Meloidogyne arenaria*, *Meloidogyne hapla*, and *Meloidogyne enterolobii* stand out as one of the limiting factors for cultivation (Sikora et al. 2018). The species *M. incognita* directly affects plants (Patil et al. 2023, Razak and Lim 1987), leading to crop yield losses of up to 26% (Daware et al. 2021).

The use of microorganisms has shown excellent results as part of strategies for controlling plant parasitic nematodes (Migunova and Sasanelli 2021, Poveda et al. 2020). Many of these natural enemies are attributed to soil pathogen suppression and belong to plant rhizosphere-associated microbial communities (Arif et al. 2020, Hu et al. 2020, Zhang, Y. et al. 2020). Besides controlling phytopathogens, these microorganisms enhance plant yield by providing nutrients and contributing to adaptations to adverse environmental conditions (Mitter et al. 2021, Tian et al. 2020) and natural soil conditions (Kia et al. 2017). Additionally, they promote the activation of plant defense responses against pathogens (Trillas and Segarra 2009). Therefore, the use of microorganisms alone or in consortia constitutes a strategy that improves plant characteristics and development (Arif et al. 2020, Monokrousos and Mourouzidou 2023).

The root and rhizosphere microbiota are important sources of natural enemies and potential biological control agents against nematodes (Saxena 2018). Among microorganisms, fungi associated with plant microbiota have demonstrated control efficacy, making their study and the understanding of nematode control essential (Handelsman and Stabb 1996, Paulitz 2000, Qureshi et al. 2012, Elhady et al. 2017, Zhang et al. 2017), including different biocontrol mechanisms against nematodes such as predation through special attack structures, parasitism, production of nematicidal metabolites (Soares et al. 2018), or induction of defense genes in the plant (Elhady et al. 2017, Topalović et al. 2020).

Different fungal genera are known for their antagonistic activity against nematodes. The species *Beauveria bassiana* (Leguizamon C. and Padilla H. 2001, Soares et al. 2018) and *Penicillium* spp. (Giné et al. 2013) are recognized for its ability to parasitize *M. incognita* eggs. *B. bassiana* causes the death of nematode juveniles with filtrates obtained from the fungus' liquid culture (Zhao et al. 2013). *Myrothecium verrucaria*, in addition to eggs, parasitizes root-knot nematode juveniles and adults (Dong et al. 2015, Hagag 2021, Wu et al. 2020). *Talaromyces thermophilus* has been reported for its nematicidal capacity (Guo et al. 2012, Zhang, J.-M. et al. 2020).

Given the importance of fungal microbiota associated with roots and the rhizosphere, along with the known mechanisms of action of fungi against plant parasitic nematodes, the objective of this research was to evaluate their *in vitro* and *in vivo* biocontrol potential.

METHODOLOGY

Localization

The experiments were conducted in two stages. The first stage, at the *in vitro* level, was carried out in the Plant Microbiology and Phytopathology Laboratory at the Universidad de Los Llanos, Villavicencio, Meta, Colombia. The second one, at the *in vivo* level, was conducted under seedbed conditions at the university's farm (farm Barcelona).

Inoculation of nematodes and microorganisms

The inoculum of *M. incognita* and the fungal isolates used were obtained from root and rhizosphere samples from plots with guava plants (*P. guajava*), ICA-2 variety, under organic management with *Pueraria thomsonii* cover and native cover in the municipality of Lejanías, Meta, Colombia (Table 1).

Soil samples were taken around the plants in the drip zone, discarding the first 5 cm of the soil profile. With a shovel, a 30-cm box was created, from which 100 g of soil and 50 g of root with galls, a typical symptom of the *M. incognita* nematode, were taken. The samples were transported to the laboratory in airtight plastic bags under refrigerated conditions in a styrofoam cooler with ice.

Table 1. Origin of soil samples used for fungal isolation.

Management type	Municipality	Coordinates
Organic management with *Pueraria thomsonii* cover	Lejanias, Meta	N3 27.646 W73 54.989 N3 27.643 W73 54.970 N3 27.629 W73 54.944 N3 27.645 W73 54.954
Organic management with native vegetation cover	Lejanias, Meta	N3 27.597 W73 54.812 N3 27.589 W73 54.807 N3 27.602 W73 54.776 N3 27.601 W73 54.776

Isolation of fungi from roots and rhizosphere

Fungal isolation was performed from guava root and rhizosphere samples following the methodology of Flores-Camacho et al. (2008). One gram of root with rhizosphere from the sample was macerated in a sterile mortar with 9 mL of 0.05% water agar (WA). Dilutions were made from the suspension up to 10^{-3}, and 0.2 mL of each dilution was plated on Petri dishes with potato dextrose agar (PDA). Petri dishes were incubated for three days at room temperature. Once the fungi were isolated, macroscopic and microscopic characteristics were taken into consideration. Macroscopic characteristics included the growth form of the colony, appearance, texture, and color on both sides. Microscopic characteristics focused on conidiophores and conidia. The fungi were maintained in PDA Petri dishes. The confirmation of the genus was done through phylogenetic analysis of fungal isolates.

Confirmation of fungal identity

DNA extraction was performed from pure isolates planted on PDA following the Wizard manufacturer's instructions (Promega). The ITS region was amplified by polymerase chain reaction with universal primers, and the resulting fragments were sequenced. Sequences were individually corrected, and nucleotide arrangements in ambiguous positions were corrected in the 5'-3' and 3'-5' direction. Sequences were aligned using MAFFT v.7 (Katoh et al. 2019). The evolutionary history of the fungi was determined by Bayesian inference analysis, based on the Markov Chain Monte Carlo method. The model for each gene was selected based on the Akaike information criterion, and the models were GTR + I + G for the C5 isolate and GTR + G for C6, C7, and C10. The Bayesian inference analysis was performed using MrBayes v.3.1.1 (Huelsenbeck et al. 2001). Phylogenetic trees were edited using iTOL v.5 (Letunic and Bork 2021).

Obtaining *Meloidogyne incognita* inoculum

The roots collected in the field were subjected to the flotation extraction principle of nematodes in sugar as described by Jenkins (1964). Guava (*P. guajava*) roots were washed with running water, liquefied three times for 10 seconds, and the liquefied material was passed through sieves of different sizes (25, 106, 250, and 425 microns) placed in descending order. The content from the 25-micron sieve was deposited in centrifuge tubes. The tubes were centrifuged at 3,750 revolutions per minute (rpm) for 5 minutes, and then the supernatant was removed. Sucrose solution at 50% was added and shaken to homogenize the sample, followed by centrifugation at the same rpm and time. Subsequently, the supernatant from each tube was deposited on the 25-micron sieve, washed with sterile distilled water, and juveniles and eggs were recovered.

Evaluation of egg and juvenile parasitism *in vitro*

The purified fungi C5, C6, C7, and C10 were cultured in WA, and then 20 µL containing 20 eggs of *M. incognita*, which had been previously extracted using the nematode flotation method in sugar, were added. The control treatment included eggs exposed to water. Eight days later, parasitism of eggs and juveniles was verified by microscopic observation using the OLYMPUS CX-22 optical microscope.

Evaluation of the efficacy of fungal biocontrol against *Meloidogyne incognita* in guava seedlings

Guava (*P. guajava*) seeds, obtained from physiologically mature fruits, previously washed and dried by direct exposure to the sun for two days, were placed in germination trays with peat substrate. Once germinated after 15 days, the seedlings were transplanted into plastic pots containing a substrate mixture of 98.6% peat, 0.4% rice husk, 0.15% KCL, 0.15% DAP, 0.15% Urea, and 0.6% lime.

The substrate was previously treated with formalin. Ten percent formalin was added to the substrate, covered with plastic, and stored for 48 hours. The plastic was removed, and, 20 days later, agricultural lime was applied. The seedlings were transplanted to 17 × 35 cm black polyethylene bags.

The inoculum of fungi C5, C6, C7, and C10 was multiplied in rice following the methodology described by Youssef et al. (2016) with some modifications. Each fungus was multiplied in sterile plastic bags containing 100 grams of rice for seven days at room temperature. Each plant received 1×10^8 conidia. In total, five applications of the fungi were made: eight days before nematode inoculation, at the time of nematode application, and at 15, 30, and 50 days after nematode inoculation. Each plant received 500 eggs of *M. incognita*. Inoculum adjustment was done with Peter's chamber. The inoculum was applied as a drench to the roots, eight days after the first fungal treatment.

The treatments were: fungus C5 + *M. incognita*, fungus C6 + *M. incognita*, fungus C7 + *M. incognita*, and fungus C10 + *M. incognita*, in addition to the control treatment with *M. incognita*. Each treatment comprised seven guava (*P. guajava*) seedlings.

At 133 days after the first application of the treatments, the number of *M. incognita* eggs and juveniles in the roots was evaluated. Extraction of eggs and juveniles was performed, and counting was done using Peter's chamber and a stereomicroscope (Motic).

Data analysis

In vitro, a qualitative assay was conducted to confirm the parasitism of *M. incognita* eggs and juveniles. *In vivo*, a completely randomized design was used, with five treatments each having seven replications. An analysis of variance and the Tukey's mean comparison test were performed using Sisvar 5.2 software for the variables: number of eggs, juveniles, and galls per gram of root.

RESULTS

Identity of fungal isolates

Four fungi associated with the root and rhizosphere of guava were isolated. Isolates C6, C7, and C10 were obtained from plants under organic management and *Pueraria thomsonii* cover, while isolate C5 was obtained from plants under organic management with native species cover.

Bayesian inference analyses of the ITS region sequences confirmed (phylogenetic tree) that isolates C5, C6, C7, and C10 corresponded to *Talaromyces sayulitensis* C5 (Fig. 1), *Myrothecium* sp. C6 (Fig. 2), *Penicillium shearii* C7 (Fig. 3), and *B. bassiana* C10 (Fig. 4).

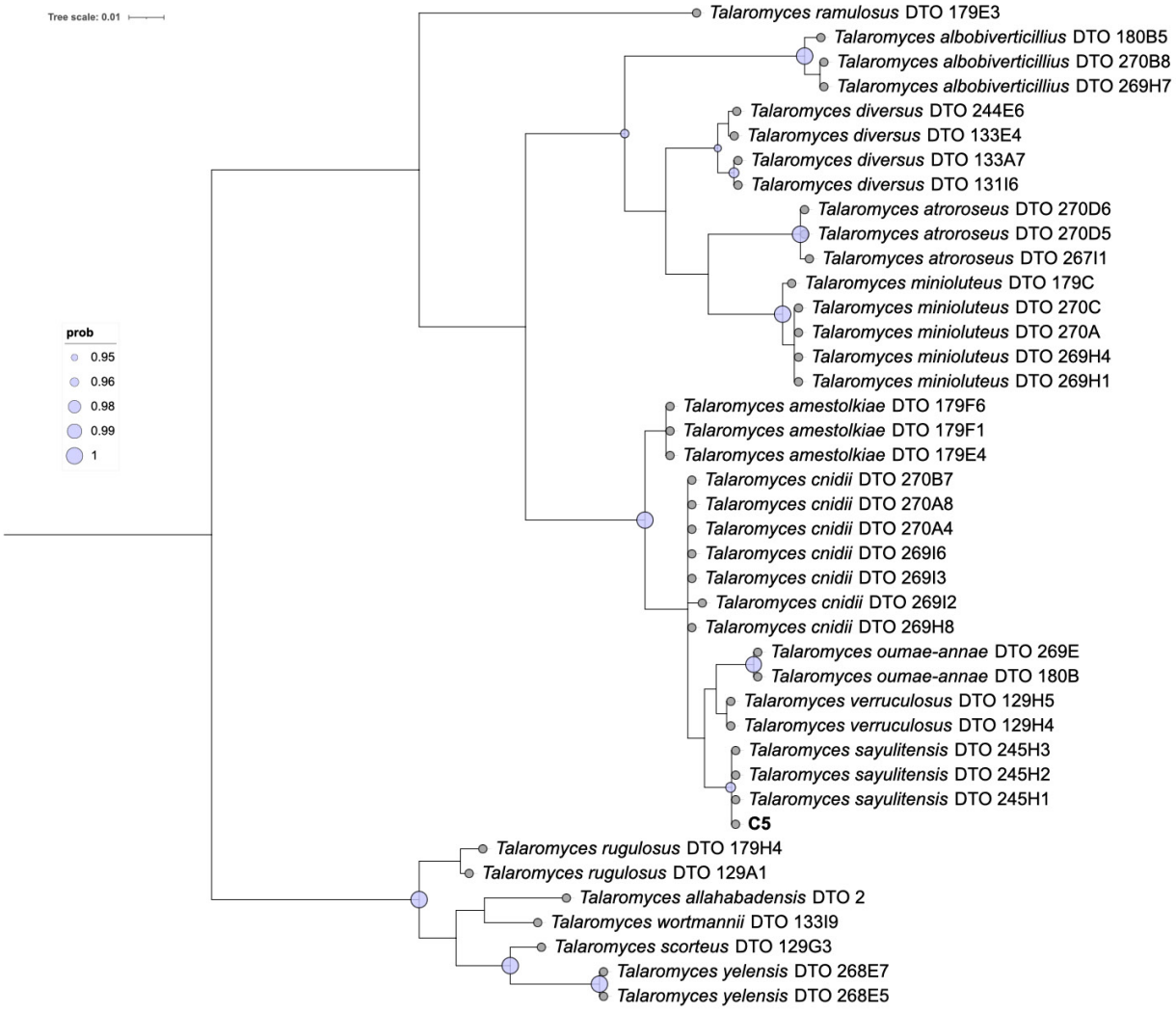

Figure 1. Phylogenetic tree based on Bayesian inference using ITS region sequences of root and rhizosphere isolate C5 (MN427868; 555 bp) and related species in the genus *Talaromyces*.

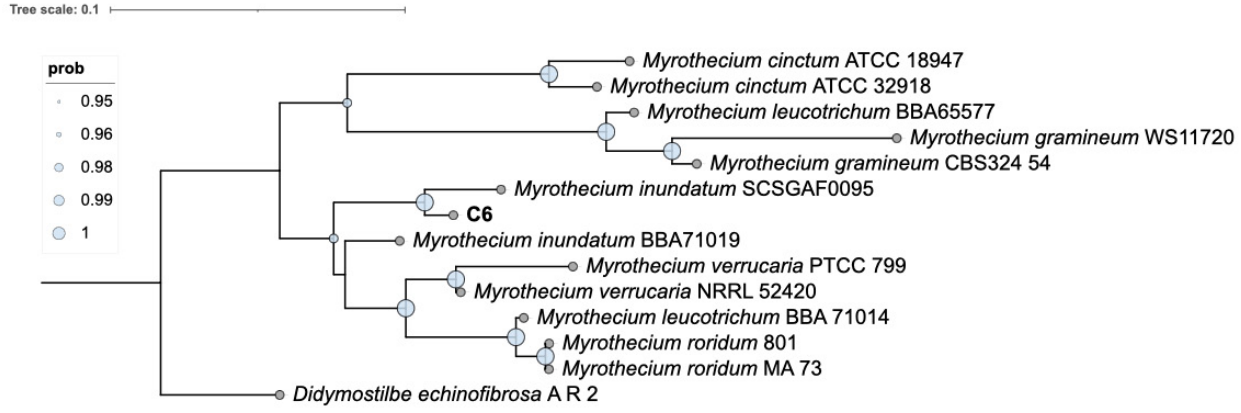

Figure 2. Phylogenetic tree based on Bayesian inference using ITS region sequences of root and rhizosphere isolate C6 (MN427869; 577 bp) and related species in the genus *Myrothecium*.

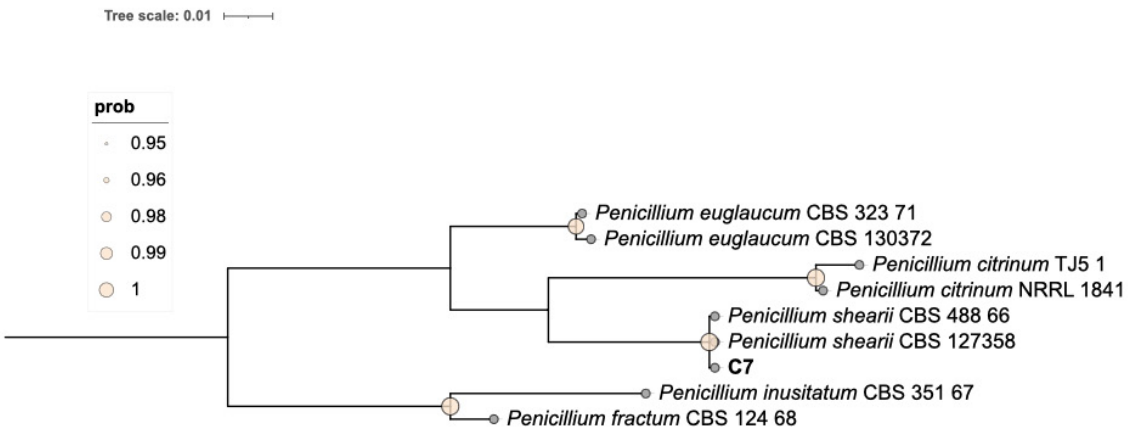

Figure 3. Phylogenetic tree based on Bayesian inference using ITS region sequences of root and rhizosphere isolate C7 (MN427870; 585 bp) and related species in the genus *Penicillium*.

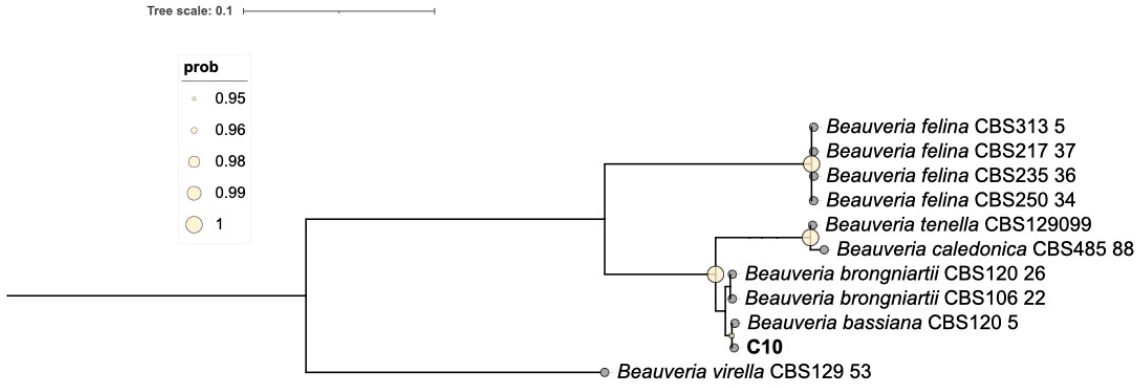

Figure 4. Phylogenetic tree based on Bayesian inference using ITS region sequences of root and rhizosphere isolate C10 (MN427871; 561 bp) and related species in the genus *Beauveria*.

In vitro parasitism assay of fungi against *Meloidogyne incognita* eggs and juveniles

Parasitism of *M. incognita* eggs was confirmed for isolates *T. sayulitensis* C5 (Fig. 5a), *Myrothecium* sp. C6 (Fig. 5b), *P. shearii* C7 (Fig. 5c), and *B. bassiana* C10 (Fig. 5d). The eggs exhibited alterations in the normal appearance of the shell, with roughness and external hyphal growth (Fig. 5). Additionally, parasitism of juveniles was evidenced for fungi *T. sayulitensis* C5 (Fig. 5e) and *B. bassiana* C10 (Fig. 5f), in which fungal hyphae grew around the juveniles, penetrating the nematode cuticle and altering the normal appearance of the juveniles (Fig. 5).

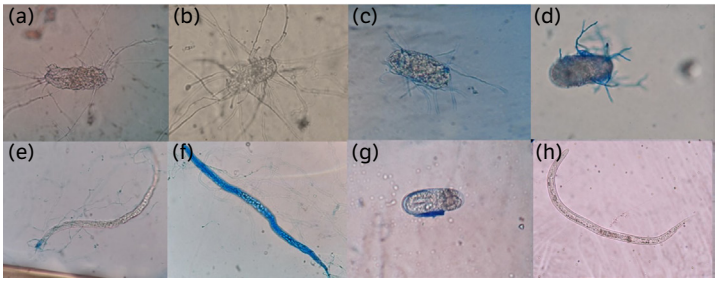

Figure 5. *In vitro* parasitism of *Meloidogyne incognita* eggs and juveniles by root and rhizosphere fungi, in Petri dishes containing water agar. (a–d) *M. incognita* eggs and juveniles parasitized. Also, (e and f) parasitized juveniles. (a) *Talaromyces sayulitensis* C5. (b) *Myrothecium* sp. C6. (c) *Penicillium shearii* C7. (d) *Beauveria bassiana* C10. (e) *Talaromyces sayulitensis* C5. (f) *Beauveria bassiana* C10. (g) Healthy egg. (h) Healthy juvenile.

In vivo control efficacy against *Meloidogyne incognita* with rhizosphere fungi

Guava plants treated preventively with fungi *T. sayulitensis* C5, *Myrothecium* sp. C6, *P. shearii* C7, and *B. bassiana* C10, both before *M. incognita* inoculation and at 15, 30, and 50 days post-nematode application, demonstrated significant reductions between treatments ($p < 0.05$) for the variables of *M. incognita* egg, juvenile, and gall numbers per gram of root (eggs/g root; juveniles/g root and galls/g root) compared to the control treatment (Table 2).

Plants treated with *B. bassiana* C10 showed significant reductions in both eggs and juveniles per gram of root, with values of 8 and 5, respectively, compared to the control treatment, which had 93 and 41, respectively. *T. sayulitensis* C5 and *Myrothecium* sp. C6 also showed significant reductions in egg numbers compared to the control treatment, with values of 21 and 18, respectively. *P. shearii* (C7) did not affect the quantity of eggs; however, the low number of juveniles in plants treated with this fungus demonstrated an impact on egg development (Table 2).

A significant reduction in juvenile numbers was observed with *T. sayulitensis* (C5) with 9 juveniles/g root, a significantly lower value compared to the control, which had 41 juveniles/g root. *Myrothecium* sp. (C6) and *P. shearii* (C7) also demonstrated significant reductions compared to the control.

The reduction in egg and juvenile numbers resulted in a lower number of galls when plants were treated with any of the evaluated fungi. Values ranged from 2 to 7 galls/g root compared to the control treatment, which had 36 galls/g root (Table 2). The results obtained both *in vitro* and *in vivo* confirmed the efficacy of these fungi as biocontrol agents against *M. incognita*.

Table 2. Number of *Meloidogyne incognita* eggs, juveniles, and galls per gram of guava root 133 days after nematode inoculation*.

Treatment	Eggs/g root	Juveniles/g root	Galls/g root
Beauveria bassiana (C10)	8a	5a	7b
Myrothecium sp. (C6)	21ab	16ab	2a
Talaromyces sayulitensis (C5)	18ab	9a	4ab
Penicillium shearii (C7)	43bc	13ab	4ab
Control	93c	41b	36c

*Data were analyzed using the Sisvar 5.2. Different letters represent differences between treatments by the Tukey's mean comparison method ($p < 0.05$).

DISCUSSION

Rhizosphere microorganisms, the microbiota, are an important source of natural enemies of plant parasitic nematodes (Elhady et al. 2021, Kerry 2000). In this research, different fungi were isolated from the roots and rhizosphere of guava plants. From guava plants with *P. thomsonii* cover, the fungi *Myrothecium* sp. C6, *P. shearii* C7, and *B. bassiana* C10 were isolated. On the other hand, from plants with native cover, *T. sayulitensis* C5 was isolated. It has been previously demonstrated that the composition of microorganism communities is determined by plant species and their exudates (Babalola et al. 2020, Kim et al. 2020, Zhang et al. 2021), as well as by interactions between pathogenic and beneficial microorganisms (Berg et al. 2016). Additionally, agricultural activities affect ecosystem components and, consequently, plant-associated microbiota and plant health (van der Heijden and Hartmann 2016, Zamioudis and Pieterse 2012, Zhang, Y. et al. 2021).

In vitro assays confirmed that the fungi evaluated in this research have the ability to parasitize *M. incognita* eggs, with only some managing to parasitize *M. incognita* juveniles. The fungi *T. sayulitensis* C5, *Myrothecium* sp. C6, *P. shearii* C7, and *B. bassiana* C10 parasitized eggs, while *T. sayulitensis* C5 and *B. bassiana* C10 parasitized both eggs and juvenile nematodes.

Among microorganisms, fungi belonging to the phylum Ascomycota have been prominent in nematode control (Jena et al. 2023). In guava plant assays, using ascomycete fungi isolated from roots and rhizosphere, the *in vivo* biocontrol potential against *M. incognita* was demonstrated, confirming reductions in the number of eggs, juveniles, and galls in roots. These results align with reports by Karakaş (2020) and Zhang et al. (2020), who demonstrated that fungi are not only an economical and efficient strategy against *M. incognita*; they are also environmentally friendly (Peiris et al. 2020).

The parasitism of eggs, as one of the mechanisms of action of fungi, was confirmed with *T. sayulitensis* C5, *Myrothecium* sp. C6, *P. shearii* C7, and *B. bassiana* C10. Previous reports demonstrated the effectiveness of *Beauveria* sp. (Karabörklü et al. 2022, Kepenekci et al. 2017, Zhao et al. 2013), *Myrothecium* spp. (Dong et al. 2015, Hagag 2021), *Talaromyces* spp. (Guo et al. 2012), and *Penicillium* spp. (Giné et al. 2013, Sikandar et al. 2020) against *Meloidogyne* spp. As for parasitism, this mechanism is associated with the production of lytic enzymes such as chitinases and proteases by fungi, which break down eggshell components, chitin, lipids, and vitelline layer, facilitating hyphal penetration into the biological target (Bonants et al. 1995, Hastuti et al. 2022, Khan et al. 2004).

The ability of *B. bassiana* to parasitize *Meloidogyne* spp. eggs and reduce the number of eggs, juveniles, and galls in treated guava seedlings aligns with previous reports of *B. bassiana* forming a network of hyphae on the outside and inside of nematode eggs, causing their disintegration. In experiments with coffee plants, significant reductions in the number of juveniles in the soil were confirmed when the fungus was preventively incorporated at the time of plant transplantation (Leguizamon and Padilla 2001). Moreover, nematode inoculum can be affected by nematicidal filtrates obtained from *B. bassiana*, with nematicidal effects on *M. incognita* juveniles resulting in mortality rates exceeding 90% after 48 hours of exposure (Zhao et al. 2013). *B. bassiana* isolates also affected the reproduction of *M. incognita* and *M. javanica*, significantly reducing the masses of *M. incognita* and *M. javanica* eggs in tomato roots when treated with 1×10^8 colony-forming unit CFU (Yağci 2022). The effectiveness of *B. bassiana* was also confirmed using the concentration of 1×10^8 CFU/mL with reductions in the *M. incognita* and *M. javanica* population in tomato plants treated preventively before transplantation. Additionally, the gall index was reduced similarly to the biological nematicide BioAct (*Purpureocillium lilacinum*), and, at the same time, crop yield increased. In evaluations of *B. bassiana*, *Metarhizium anisopliae*, and *Purpureocillium lilacinum* (*Paecilomyces lilacinus*) as biocontrol agents against the gall-forming nematode *M. incognita*, the *B. bassiana* filtrate significantly reduced the number of nematodes by 77.7% at the highest spore concentration (Youssef et al. 2020).

Furthermore, the results of this research confirmed the importance of *Myrothecium* species in the control of plant parasitic nematodes, and are consistent with previous reports of the species *M. verrucaria*, a natural enemy of *Meloidogyne* spp. that parasitizes nematode eggs (Giné et al. 2013). In other studies, it was demonstrated that contact between *M. hapla* eggs and the fungus *M. verrucaria* for 80 hours caused damage to the eggs, with alterations in the shells, embryonic lysis, and parasitism of J2 juveniles and females, with a network of hyphae observed around the nematodes.

In *in vivo* experiments with the same fungus, cucumber plants in which the fungus was incorporated into the soil before planting showed significant reductions in the population of *M. hapla* juveniles in the soil and a lower gall index (Dong et al. 2015). Additionally, the hatching of *M. incognita* eggs and the mobility of juveniles were affected by *M. roridum* filtrates, resulting in a lower number of root galls in plant roots (Park et al. 2016), which is consistent with the findings of this research.

The importance of filtrates is also evident in the reduction of galls and egg masses in tomato and melon plants treated with *M. verrucaria* filtrates, attributed to the mortality of juveniles and the effect on the hatching of *M. incognita* eggs by the nematicidal metabolites Verrucarin A and Roridin produced by the fungus (Nguyen et al. 2018). Currently, the species *M. verrucaria* is marketed as a biological control agent for plant-parasitic nematodes under the commercial name DiTera (Valent Biosciences laboratory), recommended for the management of nematodes in vegetables and fruits (Li et al. 2015).

As for the *Talaromyces* genus, this fungus has reports for nematode control that align with our results. The species *T. allahabadensis* demonstrated antagonistic capabilities, causing a mortality rate of 66.25% in *M. graminicola* juveniles (Jena et al. 2023). On the other hand, the species *T. flavus* affected the penetration of juveniles into the roots of treated plants and also reduced the reproduction of *M. javanica* (Ashraf and Khan 2005). Additionally, nematicidal capacity was confirmed for the species *T. thermophilus* against the gall-forming nematode *M. incognita*, with LC50 values of 0.5–1 µg of the nematicidal component thermolide, produced by the fungus, showing no significant difference compared to the commercial treatment (avermectin) (Guo et al. 2012).

Similar to our results of egg parasitism and reduction of *M. incognita* eggs, juveniles and galls in treated plants, there are reports highlighting the efficacy of *Penicillium* species in nematode control. In previous studies, *P. citrinum* was isolated from *Meloidogyne* spp. eggs that infected tomato plants, and *P. olsonii* from pepper plants; both fungi demonstrated parasitic capability on eggs (Giné et al. 2013). Additionally, the nematicidal metabolite cyclopiazonic acid purified from *P. commune* demonstrated mortality in *M. incognita*, *M. hapla*, and *M. arenaria* juveniles (Nguyen et al. 2021). In *in vivo* tests with fungal

agents, *P. chrysogenum* significantly suppressed reproductive factors of *M. incognita* in cucumber plants (Naz et al. 2021). Unlike our results, in which treatment with *P. shearii* C7 did not affect the number of *M. incognita* eggs in treated plants, the number of juveniles in roots was affected, possibly due to an effect on egg development.

We demonstrated the potential of fungi associated with the roots and rhizosphere of guava plants, confirming the mechanism of action, parasitism, and effectiveness in the control of the nematode *M. incognita* in treated plants.

CONCLUSION

Fungi from the root and rhizosphere were identified as species *T. sayulitensis* (C5), *Myrothecium* sp. (C6), *P. shearii* (C7), and *B. bassiana* (C10) with biocontrol potential against the root-knot nematode *M. incognita* in guava.

In vitro parasitism of *M. incognita* eggs was confirmed by the root and rhizosphere fungi *T. sayulitensis* (C5), *Myrothecium* sp. (C6), *P. shearii* (C7), and *B. bassiana* (C10), as well as parasitism of juvenile nematodes by *T. sayulitensis* (C5) and *B. bassiana* (C10).

Reductions in the number of *M. incognita* eggs, juveniles and galls in plants treated with the fungi *T. sayulitensis* (C5), *Myrothecium* sp. (C6), *P. shearii* (C7), and *B. bassiana* (C10) confirmed that fungi are a preventive and effective strategy throughout the cycle against the root-knot nematode *M. incognita*.

CONFLICT OF INTEREST

Nothing to declare.

AUTHORS' CONTRIBUTION

Conceptualization: Silva-Riveros, D. L., Parra-González, S. D. and Mogollón-Ortiz, A. M.; **Data curation:** Silva-Riveros, D. L., Parra-González, S. D. and Mogollón-Ortiz, A. M.; **Funding acquisition:** Parra-González, S. D. and Mogollón-Ortiz, A. M.; **Investigation:** Silva-Riveros, D. L., Parra-González, S. D. and Mogollón-Ortiz, A. M.; **Methodology:** Silva-Riveros, D. L.; **Project administration:** Mogollón-Ortiz, A. M.; **Supervision:** Parra-González, S. D. and Mogollón-Ortiz, A. M.; **Writing – original draft:** Silva-Riveros, D. L.; **Writing – review & editing:** Mogollón-Ortiz, A. M.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author.

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