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Research Article

Biocontrol of the root-knot nematode *Meloidogyne incognita* with *Purporeocillum lilacinum* and liquid bio-formulates in tomato (*Solanum lycopersicum*)

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Abstract

Root-knot nematodes (*Meloidogyne* spp.) cause great losses in tomato crops. An environmentally friendly for its control is the use of predatory fungi such as *Purporeocillum lilacinum*, which reduces its population in the soil and mitigates yield losses. Therefore, the objective of the present study was to evaluate the biocontrol efficacy of the strains of the nematophagous fungus *Purporeocillum lilacinum* and liquid bioformulates on the eggs of the root-knot nematode *Meloidogyne incognita*, and the formation of galls on the tomato root. Two native strains of *Purporeocillum lilacinum* H2 and H3 combined with the bioformulated Extract of Beneficial Microorganisms (EPMB®) and the root exudate stimulator Exu-Root® were tested on *Meloidogyne incognita* eggs and tomato plants. The results obtained indicate that the *Purporeocillum lilacinum* H2 and H3 strains infected the *Meloidogyne incognita* eggs, interrupted the development of the embryos and caused their death, which significantly reduced the presence of galls in the root of the plants. In short, the maximum biological performance was presented with the treatments H2+Exu-Root® and H3+EPMB®, which had the lowest number of galls with 19.2 and 20.3 galls per plant respectively, compared to the control that presented 88 galls in the root and the treatments where the fungus was not applied (69-85 galls). Finally, it is concluded that the results demonstrate the potential of the *Purporeocillum lilacinum* H2 and H3 strains as biocontrol agents against the root-knot nematode *Meloidogyne incognita*, and that, in combination with EPMB® and Exu-Root®, the efficacy can be increased to reduce its population.

Keywords: biocontrol; biological efficacy; nematophagous fungus; parasitism; *Purpureocillium lilacinum*; tomatoes

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Introduction

The tomato is very popular among the world population due to its culinary and nutraceutical characteristics (Melomey *et al*., 2019). It is one of the vegetables with the highest demand and consumption in Mexico that generates large foreign exchange for the country, since it is mainly exported to the United States. At the national level, Mexico has an annual production of 3,324,263.09 t, of which the state of Chihuahua produces 960,241.89 t (SIAP, 2021), being one of the main vegetables produced and exported by the country (SAGARPA, 2021).

One of the limiting factors in agricultural production of tomato in Mexico is the damage caused by phytoparasitic nematodes (Tapia-Velázquez *et al*., 2022). Various studies have shown that *M. incognita* can invade, develop and reproduce in tomato crops, causing great damage that directly affects yield (Sharma *et al*., 2021). Nematodes of the genus *Meloidogyne*, of which the species is unknown, are hosts of the tomato plant and cause considerable damage and production losses (Kaur *et al*., 2016). This nematode has a wide geographical distribution and can interact with other phytopathogenic microorganisms, thus causing a complex of diseases that cause losses greater than those caused individually, and even accelerate the death of plants (Vestergård, 2019).

Globally and over time, *Meloidogyne* spp. has retained the status of the most damaging and economically important plant parasitic nematode, derived especially from its polyphagous feeding habit that includes a wide range of hosts. The devastating effect it causes on tomato cultivation, one of the main vegetables worldwide, places it as one of its most important pathogens (El-Deen and El-Deeb, 2018).

Plants parasitized by *Meloidogyne* spp. show symptoms to varying degrees; damage is manifested in lack of vigor, nutritional deficiencies, and stunting (Eder *et al*., 2021). These disasters caused by nematodes of the genus *Meloidogyne* generate losses worldwide that exceed $100 bn USD annually (Yigezu, 2021; Desaeger, 2021), and losses of up to 50% of tomato yield worldwide caused by *M. incognita* (Sujatha *et al*., 2017).

The parasitism of *Meloidogyne* spp. in tomato cultivation it is very significant due to its high frequency of infestation, rapid expansion and ability to reduce its yield by up to 68% (Bhardwaj *et al*., 2021). The damage caused decreases the number of fruits and alters their quality, thus impacting their marketing price (Ahmad *et al*., 2021).

Chemical control has little efficiency due to the biology of *Meloidogyne*, since it is an obligate endoparasite and has great reproductive potential with several generations during the year (Tapia-Velázquez *et al*., 2022). The limited use of techniques whose objective is to reduce the abundance of nematodes to achieve an efficient control of *M. incognita* has not allowed the development of the full genetic potential of the crops attacked by this nematode. Currently, different options are being explored to combat pests, within which biological control is one of the safest alternatives for the environment (Naz *et al*., 2021), and is considered one of the risk-free choices against to the problems derived from the use of pesticides (Viljoen, 2019).

There are certain fungi that can limit the population grow of nematodes. These fungi quickly disperse in the soil and are well adapted to the environment. Among the fungal predators of nematodes is *Purpureocillium lilacinum*, first named as *Paecilomyces lilacinus* (Thom) Samson (Luangsa-Ard *et al*., 2011), which has a high potential for the biological control of these organisms, due to the fact that it has been shown to significantly infect nematode eggs (Al-Hazmia *et al*., 2019) and play an important role in the natural regulation of their populations (Ahmed and Monjil, 2019).

In general, it is necessary to carry out more research on the mechanisms that enable biological control of the fungus *Purporeocillum lilacinum* over the root-knot nematode *Meloidogyne incognita*, one line of research is the use strains that show greater aggressiveness for the control of these nematodes. Therefore, the objective of this study was to evaluate the biocontrol effectiveness of two strains of the fungus *P. lilacinum* and bio-formulated products, on the viability of *M. incognita* eggs, and the formation of galls in tomato plants.

Materials and Methods

Study site location

The extraction of adult females, egg mass, taxonomic identification and *in vitro* evaluation of parasitism on nematode eggs; were tasks carried out in the Phytopathology laboratory of the Faculty of Agricultural and Forestry Sciences of the Autonomous University of Chihuahua, located at Km 2.5 Delicias-Rosales highway, Chihuahua, Mexico, during the months of January-April 2007. The evaluation in vivo on the formation of galls in the root of tomato plants, was carried out in a greenhouse located in the facilities of the service company Innovack Global S.A de C.V., located in the city of Chihuahua, Chihuahua, Mexico, in the period of April-July 2007 (Figure 1).

Figure 1. Location of the study area, Chihuahua, Mexico. USGS National Map Viewer: *http://viewer.nationalmap.gov/viewer/*.

Origin of biological material

Adult gravid nematode females and egg masses were obtained from the root of a senescent melon crop with severe root galling symptoms. First, the complete roots of three melon plants were separated by cutting at the base of the stem, then the roots were washed to remove soil remains, and excess water was removed with paper towels.

Females and egg masses were extracted under the optical stereoscope (Carl Zeiss, Göttingen, Germany). A transverse cut was made in the galls with a scalpel, and once exposed, each of the females were removed from the root cavity with a dissection needle (Peraza *et al*., 2013) and placed in a vial with distilled water/sterile for storage.

Taxonomic identification of the nematode

Female nematodes were removed from the root and placed in lactoglycerol, a perineal cut was made as close as possible to the posterior end of the body of the females, and the internal organs were removed. Next, the perineal section was transferred to a slide and a few drops of lactophenol were added for observation under an optical microscope (Carl Zeiss AG International, Oberkochen, Germany). Identification of root nodule nematodes followed the method described by Taylor and Sasser (1983).

Effectiveness on the viability of M. incognita eggs in vitro

The strains of the fungus *P. lilacinum* H2 and H3 (Innovack Global S. A. de C. V.) were grown in Potato Dextrose Agar (PDA) culture medium (J.T. Baker, State of Mexico, Mexico), and were incubated at 28 °C for seven days (Felisa® Incubator, Zapopan, Jalisco, Mexico) until the mycelium completely covered the Petri dish

(35mm/11mL Vol., Pyrex®, Charleroi, Pennsylvania, USA) and the production of conidia was observed. Subsequently, 150 nematode eggs were counted in a Neubauer chamber (BRANDTM, Madrid, Spain), which were suspended in 3 mL of distilled/sterile water (J.T. Baker, State of Mexico, Mexico) and added to the Petri dish containing the fungus. Three replications were made per treatment and for the control only the suspension of eggs was applied on a PDA plate. The samples were incubated for 15 days at 28 °C and after this time, observations were made to determine the parasitism of the fungus on the nematode eggs by observing.

Morphological changes under the light microscope (Carl Zeiss, Axiostar Göttingen, Germany). The photographs were taken directly from the culture plate and without any kind of fixation of the eggs.

Effectiveness on M. incognita in tomato cultivation under greenhouse conditions

A completely randomized design with eight treatments and ten replications was used (Table 1). A hydroponic system with sterilized sand was used in a vertical autoclave (AESA mod. CV300), under 103.4kPa pressure and 121.5 °C temperature for two hours. The sand was used as a substrate to germinate tomato seeds of the Rio Grande variety. Application of treatments started after 20 days of seedling emergence. Firstly, with a glass stirring rod with a rounded tip, five 5-cm-deep perforations distributed around the seedling and near the stem were made, subsequently the nematode eggs were inoculated using a pipette, and 150 eggs were placed in each perforation.

Table 1. Description of applied treatments

Treatments	Description	Code
Control	No aplication	T1
EPMB®	Extract of Beneficial Microorganisms	T2
Exu-Root®	Root Exudate Stimulator	T3
H2	*Purpureocillium lilacinum* strain 2	T4
H3	*Purpureocillium lilacinum* strain 3	T5
H2+EPMB®	*P. lilacinum* strain 2 + Extract of Beneficial Microorganisms	T6
H2+Exu-Root®	*P. lilacinum* strain 2 + Root Exudate Stimulator	T7
H3+EPMB®	*P. lilacinum* strain 3 + Extract of Beneficial Microorganisms	T8
H3+ Exu-Root®	*P. lilacinum* strain 3 + Root Exudate Stimulator	T9

The inoculation with *P. lilacinum* was carried out seven days after having inoculated the nematode eggs, 6 mL of a conidia suspension were applied at a concentration of $1x10^8$ conidia mL-1 per pot following the methodology described by Colome *et al.* (1989); Fifteen days later, a second inoculation was performed using the same technique.

The treatments of Extract of Beneficial Microorganisms (EPMB®) and Root Exudate Stimulator (Exu-Root®) (Innovack Global S.A. de C.V.) were applied 17 days after inoculating the nematode eggs at the recommended commercial doses, EPMB® was applied at a concentration of 5 mL in 100 mL of water and Exu-Root® at a concentration of 0.2 mL in 100 mL of water. After fifteen days, a second application was made using the same doses.

After inoculation, they were irrigated with a nutrient solution until the end of the experiment, the number of irrigations was determined by the water demand of the plants, taking into account the temperature recorded inside the greenhouse, which was maintained between 28-30 °C. The formulation of the complete nutrient solution was: Calcium nitrate 0.381 g L^{-1}, NKP 0.192 g L^{-1}, Magnesium sulfate 0.25 g L^{-1}, Carboxy micro 0.25 g L^{-1}, Copper sulfate 0.5 mL L^{-1}, Ammonium molybdate 0.2 mL L^{-1}, Iron EDDHA 0.025 g L^{-1}.

Sampling and plant analysis

67 days after the experiment was established, the evaluation of variables (Number of root galls, root length, leaf biomass, and root biomass) were carried out to determine the damage caused by the nematode and the control potential of *P. lilacinum*. First, the roots were carefully washed with running water and deionized water to remove impurities, then the number of galls produced in each root was quantified as described by Morales, 2006 and Regmi and Desaeger, 2020.

Evaluation of agronomic variables

Root length

This variable was measured using a ruler, which was placed from the base of the stem to the longest distal end of the root, the result was expressed in centimeters (cm) (Regmi and Desaeger, 2020).

Number of galls

Galls were located by direct observation of the root, and counted with the help of a manual counter (Heathrow Scientific™ Manual Counter, Illinois, USA). The result was expressed in number of root galls per plant (Regmi and Desaeger, 2020).

Leaf and root biomass

Leaf and stem samples, along with root samples were washed with tap water and deionized water, dried on brown paper to remove excess water and placed in a forced air oven at 70 °C for 24 hours (Felisa® St. Livonia, Michigan, USA). After the drying time, the samples were weighed with an analytical balance (Precision Electronic Balance AND Company Limited, Milpitas, CA, U.S.A.) and the weights were expressed in grams per plant (g plant-1) (Sánchez, 2006).

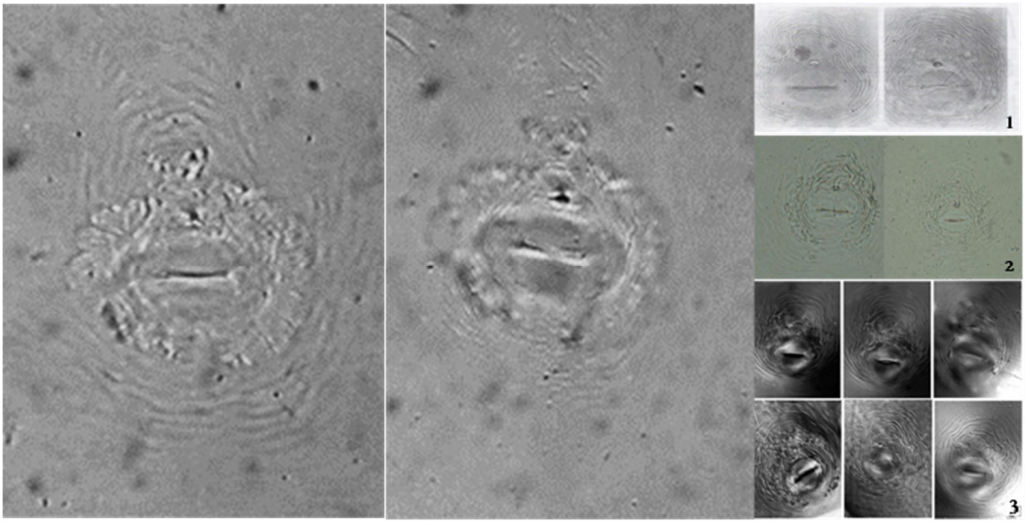
Statistical analysis

Analysis of variance and mean separation test by Tukey ($p \geq 0.05$) were performed on the data obtained using the statistical package SAS, 2007 (Statistical Analysis System. Raleigh, NC, USA) version 9.1.

Results and Discussion

Identification of females of M. incognita

The morphological characteristics observed in the collected females nematodes showed the characteristic perineal pattern of *M. incognita* (Figure 2). These patterns were compared with the taxonomic keys published by Taylor and Sasser (1983), which show the specific morphological characteristics of this species. Similarly, they were compared with the descriptions of perineal patterns published by Álvarez (2006), and with the morphological description of perineal sections of females of *M. incognita* published by Peraza-Padilla *et al*., 2013; which confirmed that the species used in the present study was indeed *M. incognita*.

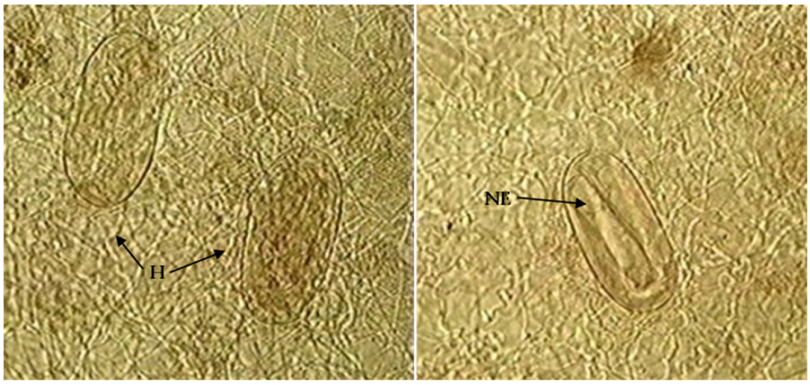


(A) (B) (C)

Figure 2. A and B) Photomicrograph of perineal patterns obtained from sections of collected *Meloidogyne incognita* females, C) 1: Taxonomic keys of *M. incognita*, Taylor and Sasser (1983), 2: Perineal patterns of *M. incognita*, Álvarez (2006), 3: Morphological and morphometric analysis of perineal sections of females of *M. incognita*, Peraza-Padilla *et al*. (2013).

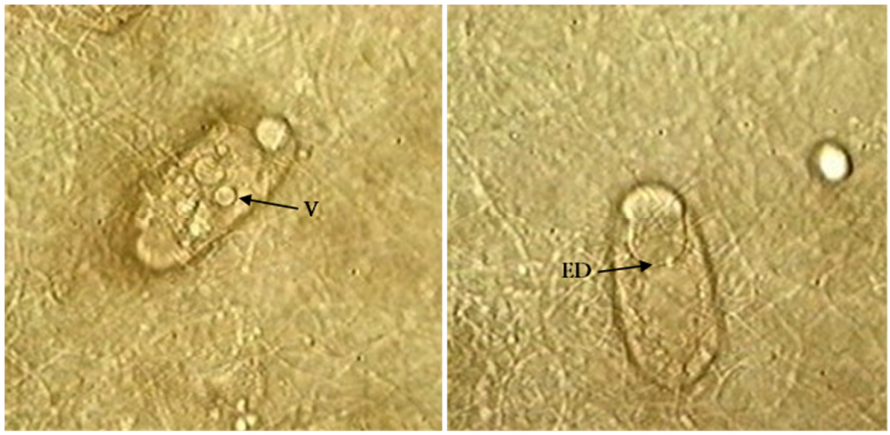
Effectiveness on the viability of M. incognita eggs in vitro

The results showed that the *P. lilacinum* strains colonized the *M. incognita* eggs, since the mycelium penetrated the shell and produced spores inside of them (Figure 3). The penetration of *P. lilacinum* is possible thanks to its chitinolytic capacity, which destroys the chitin that forms the cover of the eggs, while using mechanical force. The eggs were severely affected by the action of the fungus and did not hatch, the juveniles inside the eggs failed to complete their first embryonic stages and did not hatch, the immobile juveniles inside the eggs were assumed to be dead (Figure. 3). The greatest morphological alterations occurred in the eggs whose content was vacuolated by the action of the fungus, and the development of the immature juveniles in formation was interrupted (Figure 4). Similar results were obtained by Cardona *et al*. (2014) who in their research applied an extract of *Purporeocillum* which caused vacuolization in the eggs of *M. incognita-javanica*. Parasitized second-stage larvae already hatched were also observed, enveloped by the hyphae of the fungus only observed unaccounted for, as well as spores on the wall of the eggs (Figure 5).



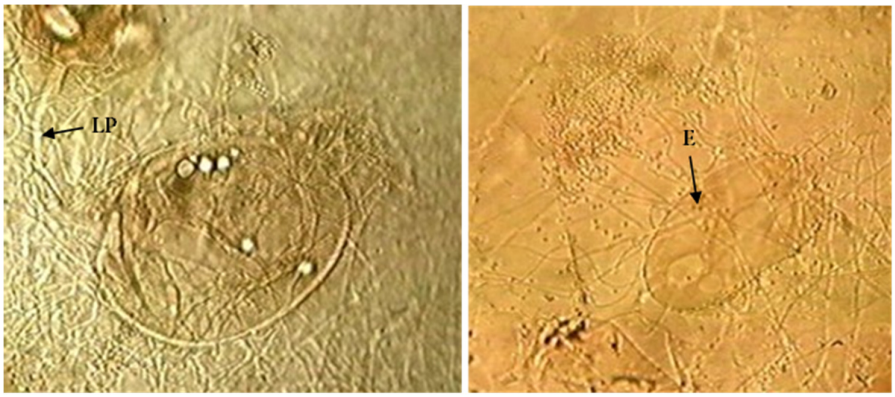
(D) (E)

Figure 3. Eggs of *Meloidogyne incognita* in their first stages parasitized by the fungus *Purpureocillium lilacinum*. (D) H (hyphae): Fungal hyphae on the egg shell. (E) NE (unhatched): 1st. larval stage inside the unhatched egg



(F) (G)

Figure 4. Eggs of *Meloidogyne incognita* in their early stages parasitized by the fungus *Purpureocillium. lilacinus*. (F and G) V (vacuolated): vacuolated unhatched eggs, ED (arrested embryogenesis): egg where embryogenesis was stopped



(H) (I)

Figure 5. (H) LP (parasitized larvae): *Meloidogyne incognita* second instar larvae parasitized by hyphae of *Purpureocillium lilacinus*. (I) E (spores): *P. lilacinus* spores in the egg wall

In the *in vitro* study, the *P. lilacinum* strains stopped the development of *M. incognita* eggs by reducing the number of emerged juveniles, and it was observed that it killed hatched juveniles. The hatching of juveniles was lower when the eggs were exposed to the action of the H3 strain of *P. lilacinum*, which showed a control efficacy of 99.22%. The H2 strain also had great efficiency for the control of nematode eggs with 96.33%. In both cases the reduction oegg hatching was almost complete (Table 2). The difference in efficacy between the strains was quantified at just 2.72% on the day of the reading. Only 3.47% and 0.78% of the eggs were not parasitized 15 days after inoculation with H2 and H3, respectively.

Table 2. Predatory effect of *Purpureocillium lilacinum* on *Meloidogyne incog*nita eggs 15 days after inoculation

Treatments	Number of eggs	Number of parasitized eggs
Control	731 ± 18 a	0 c
Purpureocillium lilacinum (H2)	231 ± 14 b	223 ± 16 a
Purpureocillium lilacinum (H3)	138 ± 14 c	137 ± 15 b

Values shown are calculated mean ± S.E. Different letters between treatments denote significant differences (Tukey's test, $p < 0.05$).

The observations of parasitism on the eggs of *M. incognita* coincided with that described by Peraza-Padilla *et al.* (2014) and Youssef *et al.* (2020), who in an *in vitro* evaluation observed the presence of mycelial networks inside *M. incognita* eggs, deforming them and preventing their hatching. Ahmad *et al.* (2019), reported that *P. lilacinum* has the ability to infect all stages of development of some nematodes, since it can inhibit the ability to move and alter vital processes such as hatching and feeding.

Effect of treatments on the number of root galls

All the evaluated plants presented galls on their roots induced by *M. incognita*. However, the treatments where *P. lilacinum* was applied showed less galling; this highly significant difference can be seen in Figure 6.

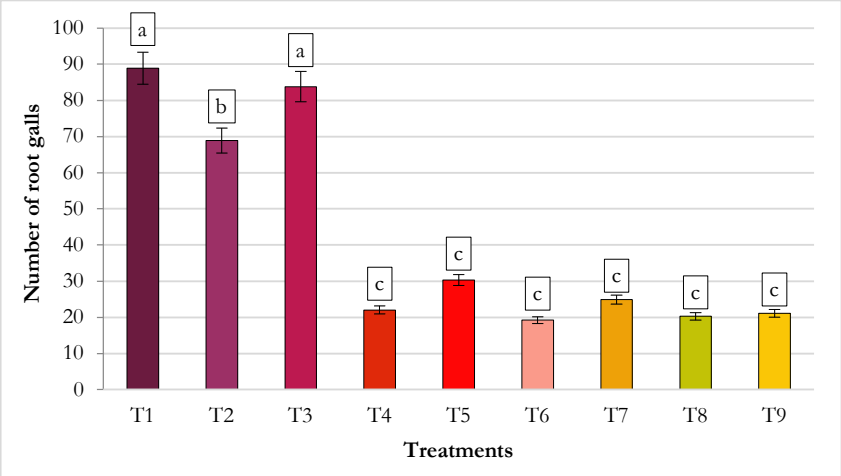


Figure 6. Number of galls induced by *Meloidogyne incognita* in tomato roots treated with *P. lilacinum*, EPMB® and Exu-Root®

Different letters between treatments denote significant differences (Tukey's test, $p < 0.05$).

P. lilacinum successfully reduced the formation of galls in the tomato root which appear as a result of the attack of *M. incognita*. The treatments that did not have the presence of the fungus (T1, T2 and T3) showed the most severe symptoms of root galling (Figure 6). The combinations that gave the best results were T7 and

T8 with 19.2 and 20.3 root galls, respectively; this result is mainly attributed to the direct action of the fungus on this pathogen. The effect of the Exu-Root® and EPMB® products contribute by having a direct effect on the development and growth of the root. Kiriga *et al.* (2018) mentioned that *P. lilacinum* reduces the formation of galls caused by the genus *Meloidogyne*; likewise, they reported that the fungus applied at the time of sowing or two weeks later, is more effective in reducing the rate of gall formation. Ahmad *et al.* (2019) found that the application of *P. lilacinum* considerably reduced the population of the nematode, this is reflected in the number of galls present in the root. Najafi *et al.* (2017), found that products based on *P. lilacinum* significantly decreased the number of galls in the root of tomato plants caused by *M. incognita*.

Effect of treatments on leaf biomass

Infection with *M. incognita* can reduce chlorophyll content and alter levels of amino acids and organic acids (Ahmed and Shahjahan, 2019). A severe infection of this nematode causes alterations in the levels of amino acids, organic acids and reduces the content of chlorophylls, causes growth retardation and reductions in the biomass and bioactive components of the roots and leaves (Saikia *et al.*, 2013). In this study, the presence of galls caused by *M. incognita* did not significantly affect leaf biomass (Figure 7), probably due to the fact that the experiment was withdrawn before the physiological maturity of the plant and the damage was not reflected in the above-ground plant organs as is the frequently the case of damage caused by the pathogen during the complete cycle of the plant. The T3 treatment presented the highest average leaf dry weight with 14.26 g, attributing this result to the fact that the product promotes root activity, which favors the development of the plant. The treatment with the lowest accumulated foliar biomass was T4 with 11.84 g. The rest of the treatments showed little difference as shown in Figure 7. These data agree with what was reported by Pinto (2009), who reported in his research that the presence of nematodes did not alter leaf biomass.

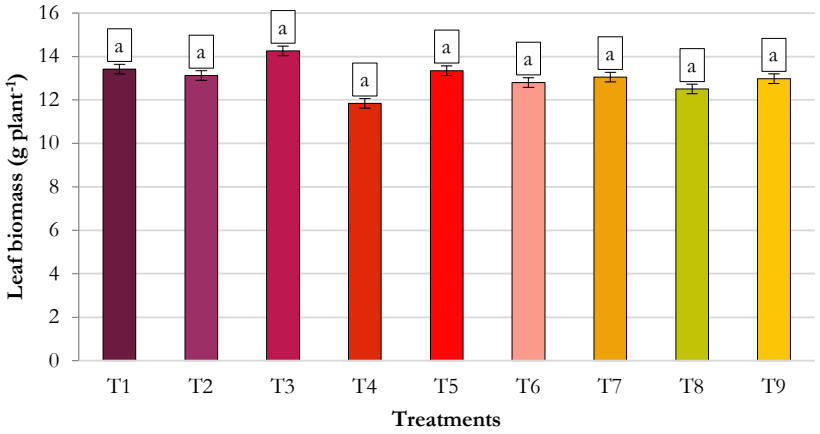


Figure 7. Leaf biomass of tomato plants treated with *P. lilacinum*, EPMB® and Exu-Root®
Same letters do not represent significant differences Tukey ($p > 0.05$).

Effect of treatments on root biomass

The presence of *M. incognita* in the root of the treatments evaluated did not significantly affect the accumulation of root biomass, the galls caused by the pathogen did not influence this variable (Figure 8), as reported by Olusesan *et al.* (2022). However, this variation in the result was possibly due to the fact that the crop was terminated only 67 days after being established, which limited the reflection of the effect of this nematode, by causing the increase in root volume as a result of massive gall formation. Oclarit *et al.* (2009) mentioned in their research that root biomass was significantly higher in plants inoculated with *M. incognita* due to the presence of larger galls on the roots. In this work, T2 and T6 were 23.72 and 21.43% above T1,

which presented the lowest root biomass (2.67 g). The characteristic of these treatments is that EPMB® and Exu-Root® are root stimulators that help root development. In investigations carried out by Camacho *et al.* (2020) it can be seen that the difference between the inoculated treatments and the control is precisely due to the fact that the latter is not inoculated with the evaluated agents.

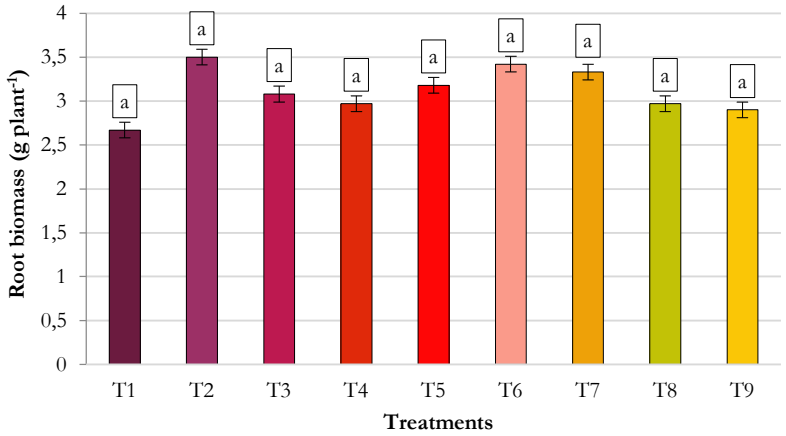


Figure 8. Root biomass of tomato plants treated with the fungus *P. lilacinum*, EPMB® and Exu-Root®
Same letters do not represent Tukey significant differences (p > 0.05).

Effect of treatments on root length

For this variable, the plants of the T2 treatment presented greater length with 21.8 cm, probably due to the effect that the product has on the microflora adjacent to the root zone (Figure 9), and this, in turn, favors root growth. The root-microorganism interaction promotes root growth and the ability to absorb nutrients (González and Fuentes, 2017). In various investigations that studied the interactions between plants and certain microorganisms that promote plant development, the root played a key role because it is the organ of the plant that is colonized in the first instance (Yáñez, 2021). Although the treatments did not show significant statistical influence on root length, it is important to consider the effect and response observed in the analyses. The combination of strain H3 with EPMB® and Exu-Root® (T8 and T9) had a greater effect than the combinations with strain H2 (T6 and T7). The 5.6% and 2.89% difference in the result on root length, between the H3 and H2 strains, respectively, although minimal, could imply a greater positive effect of the H3 strain on the development of the aerial part of the plant (Figure 9).

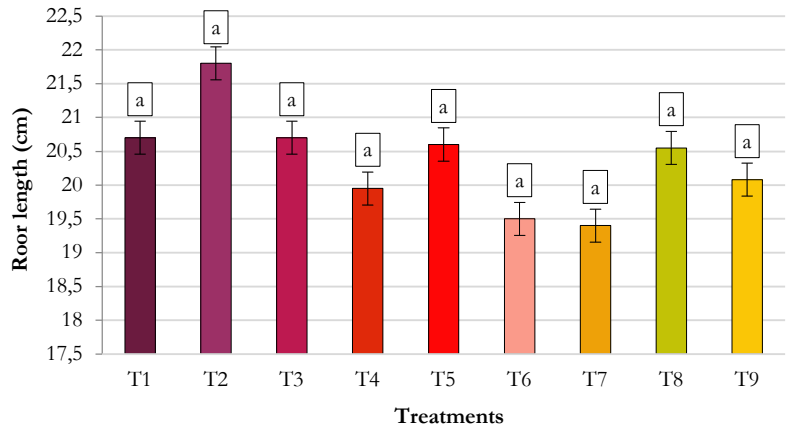


Figure 9. Length of tomato root treated with the fungus *P. lilacinum*, EPMB® and Exu-Root®
Same letters do not represent Tukey significant differences (p > 0.05).

It should be noted that the results of this study demonstrate the potential of *P. lilacinum* strains as successful biocontrol agents against the root-knot nematode *M. incognita*; which also agrees with the investigations carried out by Rojas (1996), who reported that *P. lilacinum* parasitizes eggs and females of this nematode, and that it has demonstrated the ability to regulate its populations at levels that are not harmful to the crop.

Conclusions

The identification of the genus and species of the root-knot nematode *M. incognita* in this study one of the first reports of the presence of this nematode in the state of Chihuahua, Mexico.
The evaluated strains of *P. lilacinum* H2 and H3 infected *M. incognita* eggs and interrupted their development. Despite not showing significant response on plant growth variables, it was shown that they have the ability to reduce the number of galls on tomato roots caused by *M. incognita*, a potentially devastating nematode.

The results of this study demonstrate the potential of the *P. lilacinum* H2 and H3 strains as biocontrol agents against the root-knot nematode *M. incognita*, and that, in combination with EPMB® and Exu-Root®, the efficacy in root-knot nematode control can be improved by decreasing the initial populations of this pathogen.

Authors' Contributions

BCML and DMNA designed the study. While DMNA, BCML and MAAG conducted the experiment. JMOG organized the data and performed the statistical analysis. EMM, ES and BCML prepared the manuscript.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

References

Ahmad G, Khan A, Khan AA, Ali A, Mohhamad HI (2021). Biological control: a novel strategy for the control of the plant parasitic nematodes. Antonie van Leeuwenhoek 114(7):885-912. *https://doi.org/10.1007/s10482-021-01577-9*

Ahmad RZ, Sidi BB, Endrawati D, Ekawaski F, Chaerani (2019). *Paecilomyces lilacinus* and *P. variotii* as a predator of nematode and trematode eggs. IOS Conf. Series: Earth and Environmental Science 299:012056. *https://doi.org/10.1088/1755-1315/299/1/012056*

Ahmad S, Al-Hazmia FA, Al-Yahyaa OA, Abdel R, Hamzeh AL (2019). Effects of humic acid, *Trichoderma harzianum*, and *Paecilomyces lilacinus* on *Meloidogyne javanic*a. International Journal of Agriculture, Environment and Bioresearch 4(1):61-74.

Ahmed S, Shahjahan MM (2019). Effect of *Paecilomyces lilacinus* on tomato plants and the management of root knot nematodes. Journal of Bangladesh Agricultural University 17(1):9-13. *https://doi.org/10.3329/jbau.v17i1.40657*

Álvarez PAM (2006). Evaluación de algunas alternativas de control sobre el nematodo del nódulo de la raíz (*Meloidogyne* spp.), como opciones de pre plantación en condiciones de replante en vid (*Vitis vinifera* L.). Facultad de Ciencias Agronómicas, Santiago de Chile, pp 3-21.

Bhardwaj A, Sharma A, Goswami BK, Bhardwaj V (2021). Interaction effect of soilless media and organic amendments for eco-friendly root-knot nematode management in Brinjal and tomato nursery. Journal of Pure and Applied Microbiology 15:356-367. *https://doi.org/10.22207/JPAM.15.1.30*

Camacho JA, Recharte PDC, Yanqui FD, Moreno LLSM, Buendía MMA (2020). Fertilizar con microorganismos eficientes autóctonos tiene efecto positivo en la fenología, biomasa y producción de tomate (*Lycopersicum esculentum* Mill). Scientia Agropecuaria 11(1):67-73. *http://dx.doi.org/10.17268/sci.agropecu.2020.01.08*

Cardona BNL, Pavas H, Fernández EP (2014). Efecto del filtrado crudo de *Purpureocillium* sp. (Cepa UdeA0106), sobre la eclosión de huevos y movilidad de juveniles de *Meloidogyne incognita-javanica*. Revista Colombiana de Biotecnología 2:37-44. *https://doi.org/10.15446/rev.colomb.biote.v16n2.47241*

Colome A, Kubinski MR, Cano JD, Grady V (1989). Laboratory Exercises in Microbiology. California Polytechnic State University. By West Publishing company, pp 1-283.

Desaeger J (2021). A root-knot nematode paradise made in plastic: the case of Florida vegetables. In: Integrated Nematode Management: State-of-the-art and visions for the future. Wallingford UK: CABI pp 247-254.

Eder R, Consoli E, Krauss J, Dahlin P (2021). Polysulfides applied as formulated garlic extract to protect tomato plants against the root-knot nematode *Meloidogyne incognita*. Plants 10:394. *https://doi.org/10.3390/plants10020394*

El-Deen AN, El-Deeb BA (2018). Effectiveness of silver nanoparticles against root-knot nematode, *Meloidogyne incognita* infecting tomato under greenhouse conditions. Journal of Agricultural Science 10(2):148-156. *https://doi.org/10.5539/jas.v10n2p148*

González H, Fuentes N (2017). Mecanismo de acción de cinco microorganismos promotores de crecimiento vegetal. Revista de Ciencias Agrícolas 34(1):17-31. *http://dx.doi.org/10.22267/rcia.173401.61*

Kaur S, Kang SS, Dhillon NK, Sharma A (2016). Detection and characterization of *Meloidogyne* species associated with pepper in Indian Punjab. Nematropica 46:209-220.

Kiriga AW, Haukeland S, Kariuki GM, Coyne DL, Beek NV (2018). Effect of *Trichoderma* spp. and *Purpureocillium lilacinum* on *Meloidogyne javanica* in commercial pineapple production in Kenya. Biological Control 119:27-32. *https://doi.org/10.1016/j.biocontrol.2018.01.005*

Luangsa-Ard J, Houbraken J, Doorn TV, Seung-Beom H, Borman AW, Hywel-Jones NL, Samson RA (2011). *Purpureocillium*, a new genus for the medically important *Paecilomyces lilacinus*. FEMS Microbiology Letters 321:141-149. *https://doi.org/10.1111/j.1574-6968.2011.02322.x*

Melomey D, Danquah LA, Offei K, Ofori S, Danquah K, Osei, ME (2019). Review on tomato (*Solanum lycopersicum,* L.) improvement programmes in Ghana. Recent Advances in Tomato Breeding and Production. *https://doi.org/10.5772/intechopen.75843*

Morales MR (2006). Manejo de nematodos fitoparasitícos utilizando productos naturales y biológicos. Universidad de Puerto Rico. Recinto Universitario de Mayagüez, pp 2-80.

Najafi F, Rezai S, Kia EB, Mahmoudi M, Mohebali M, Gharagozlou MJ, Rokni MB, Gholamreza M (2017). *In vitro* assay of *Paecilomyces lilacinus* biocontrol effects on *Fasciola hepatica* eggs illustrated in scanning electron micrographs. Iranian Journal of Parasitology 12(1):22-28.

Naz I, Asad AKR, Masood T, Baig A, Siddique I, Shamsulhaq (2021). Biological control of Root Knot nematode, *Meloidogyne incognita*, in vitro, greenhouse and field in cucumber. Biological Control 152:1-12. *https://doi.org/10.1016/j.biocontrol.2020.104429*

Oclarit EL, Cumagun CJR (2009). Evaluation of efficacy of *Paecilomyces lilacinus* as biological control agent of *Meloidogyne incognita* attacking tomato. Journal of Plant Protection Research 49(4):337-340. *https://doi.org/10.2478/v10045-009-0053-x*

Olusesan AI, Oluwatayo JI, Okrikata E (2022). Potential of some selected fertilizer types in the suppression of root knot nematode (*Meloidogyne incognita*) infections on eggplant. Asian Journal of Research and Review in Agriculture 4(3):28-34.

Peraza-Padilla W, Orozco-Aceves M, Esquivel-Hernández A (2014). Evaluación *in vitro* de hongos nematófagos en zonas arroceras de Costa Rica contra el nematodo agallador *Meloidogyne javanica*. Agronomía Costarricense 38(2):19-32.

Peraza-Padilla W, Rosales-Flores J, Esquivel-Hernández A, Hilje-Rodríguez I, Molina-Bravo R and Castillo-Castillo P (2013). Identificación morfológica, morfométrica y molecular de *Meloidogyne incognita* en higuera (*Ficus carica* L.) en Costa Rica. Agronomía Mesoamericana. 24(2):337-346. *https://doi.org/10.15517/am.v24i2.12533*

Pinto AV (2009). Evaluación de las propiedades nematicidas de residuos foliares de ocho especies leñosas y semi leñosas comunes del sur de Chile. Tesis de licenciatura. Universidad de Austral de Chile. Escuela de Agronomía pp 4-46.

Regmi H, Desaeger J (2020). Integrated management of root-knot nematode (*Meloidogyne* spp.) in Florida tomatoes combining host resistance and nematicides. Crop Protection 134:105170. *https://doi.org/10.1016/j.cropro.2020.105170*

Rojas T (1996). Consideraciones sobre control biológico de nematodos fitoparasíticos. Departamento de Fitoprotección. X Congreso Nacional Agronómico. III Congreso de Fitopatología. Ministerio de Agricultura y Ganadería, pp 69-72.

SAGARPA (2021). Secretaría de Agricultura, Ganadería, Desarrollo Rural, Pesca y Alimentación (disponible en línea, consultado el 23 de octubre de 2021).

Saikia SK, Tiwari S, Pandey R (2013). Rhizospheric biological weapons for growth enhancement and *Meloidogyne incognita* management in *Withania somnifera* cv. Poshita. Biologycal Control 65:225-234. *https://doi.org/10.1016/j.biocontrol.2013.01.014*

Sánchez E (2006). Caracterización de estado nutricional y fisiológico en plantas de Judía (*Phaseolus vulgaris* L. cv. Strike) sometidas a un estrés por nitrógeno. Primera edición, Editorial de la Universidad de Granada. Granada, España. Pp 41.

SAS, Institute (2007). SAS/STAT user´s guide. Release 6.03 ed. SAS Insitute, Cry, N. C.

Sharma M, Saini I, Kaushik P, Aldawsari MM, Al Balawi T, Alam P (2021). Mycorrhizal fungi and *Pseudomonas fluorescens* application reduces root-knot nematode (*Meloidogyne javanica*) infestation in eggplant. Saudi Journal of Biological Sciences 28(7):3685-3691.

SIAP (2021). Servicios de Información Agricola y Pesquera (disponible en línea, consultado el 23 de octubre de 2021).

Sujatha R, Vethamoni NP, Manivannan N, Sivakumar M (2017). Screening of tomato genotypes for root knot nematode (*Meloidogyne incognita* Kofoid and White Chitwood). International Journal of Current Microbiology and Applied Sciences 6(3):1525-1533. *https://doi.org/10.20546/ijcmas.2017.603.175*

Tapia-Velázquez I, Montoya-Martínez AC, De los Santos-Villalobos S, Ek-Ramos MJ, Montesinos-Matías R, Martínez-Anaya, C (2022). Root-knot nematodes (*Meloidogyne* spp.) a threat to agriculture in Mexico: Biology, current control strategies, and perspectives. World Journal of Microbiology and Biotechnology 38:26. *https://doi.org/10.1007/s11274-021-03211-2*

Taylor AL, Sasser JN (1983). Biología, identificación y control de los nematodos en nódulo de la raíz (especies de *Meloidogyne*). Universidad del Estado de Carolina del Norte. Agencia de Estados Unidos para el Desarrollo Internacional. Impreso por artes gráficas de la Universidad del Estado de Carolina del Norte, pp 111.

Vestergård M (2019). Trap crops for *Meloidogyne hapla* management and its integration with supplementary strategies. Applied Soil Ecology 134:105-110. *https://doi.org/10.1016/j.apsoil.2018.10.012*

Viljoen, JJF, Labuschagne N, Fourie H, Sikora RA (2019). Biological control of the root-knot nematode *Meloidogyne incognita* on tomatoes and carrots by plant growth-promoting rhizobacteria. Tropical Plant Pathology 44:284-291. *https://doi.org/10.1007/s40858-019-00283-2*

Yánez LEO (2021). Plantas y microorganismos rizosféricos: Una vía sostenible para generar crecimiento vegetal. Revista Científica Interdisciplinaria Investigación y Saberes 11(3):102-122.

Yigezu WG (2021). Biology, taxonomy, and management of the root-knot nematode (*Meloidogyne incognita*) in sweet potato. Advances in Agriculture 2021:8820211. *https://doi.org/10.1155/2021/8820211*

Youssef M, El-Nagdi W, Lotfy DE (2020). Evaluation of the fungal activity of *Beauveria bassiana*, *Metarhizium anisopliae* and *Paecilomyces lilacinus* as biocontrol agents against root-knot nematode, *Meloidogyne incognita* on cowpea. Bulletin of the National Research Centre 44(1):1-11. *https://doi.org/10.1186/s42269-020-00367-z*