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Phytopathological Note

Pochonia chlamydosporia var. *chlamydosporia* on *Meloidogyne incognita* eggs from Escuinapa, Sinaloa

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ABSTRACT

Background/Objetive. Chili cultivation is of great importance to Escuinapa, as it is the largest producer of chili in Sinaloa, Mexico. Its production is affected by various pests, such as the *Meloidogyne* nematode, which can cause up to 50% losses. Control is based on the application of chemical nematicides. Therefore, the need for more sustainable biocontrol alternatives is crucial. The objective of this study was to isolate and identify the nematode and the nematode-parasitic fungus and to evaluate their *in vitro* parasitism on the eggs of the root-knot nematode of chili plants in Escuinapa, Sinaloa.

Experimental development. Chili roots with nematode galls were collected to isolate and identify the nematode causing the disease and nematophagous fungi. Females and juveniles were identified by perineal sections and morphometry. The nematophagous fungus was identified morphologically and by molecular sequencing (ITS1 and ITS2). The *in vitro* parasitism of the fungus on *Meloidogyne* eggs was evaluated at six spore concentrations plus a control. The percentage of parasitism and the LD_{50} and LD_{95} were calculated using probit analysis.

Results. *Meloidogyne incognita* was morphologically identified as the root-knot nematode of chili plants. In turn, *Pochonia chlamydosporia* var. *chlamydosporia* was isolated and identified morphologically and molecularly, which showed high percentages of parasitism (98.9%) *in vitro* on eggs of this nematode over a period of 48 hours.

Conclusion. A strain of *Pochonia chlamydosporia* was isolated from *Meloidogyne incognita* that showed high levels of parasitism in eggs (80 to 98%) of this nematode *in vitro*. *In vivo* and field trials could confirm its effectiveness as a bionematicide.

Keywords: Alternative, Biocontrol, Nematophagous fungus, *In vitro*, Root-knot nematode

INTRODUCTION

The chili pepper (*Capsicum annuum*) is a vegetable of great importance to Mexico, since is it the country with the second greatest production worldwide. In 2023, an annual production of 3,681,061 t was recorded, providing 21.4% of the country's vegetable production. The main producing states were Chihuahua and Sinaloa (SIAP, 2025a), the latter having a planting surface of 16,888.70 ha and a production of 813,081.23 t, with the municipality of Escuinapa having the greatest surface (4,962.00 ha) and production (215,568.27 t) (SIAP, 2025b). However, it is affected by several pests and diseases, one of the most important of which is the root-knot nematode *Meloidogyne* spp., which attacks a large variety of plants, such as melon (*Cucumis melo*), tomato (*Solanum lycopersicum*) (Cristóbal-Alejo *et al.,* 2021), fig (*Ficus carica*), beet (*Beta vulgaris*), basil (*Ocimum basilicum*), pumpkin (*Cucurbita* sp.) (Romero *et al.,* 2019), cucumber (*Cucumis sativus*), watermelon (*Citrillus vulgaris*), eggplant (*Solanum melongena*), tobacco (*Nicotiana tabacum*), peanut (*Arachis hypogaea*), and many others (Carrillo-Fasio *et al.,* 2000).

The crops affected by *Meloidogyne* spp. manifest the formation of galls on the roots, preventing the absorption and transportation of water and nutrients to the leaves, producing yellowing, wilting, smaller plant and fruit size, and finally, plant death (Velásquez-Valle *et al*., 2013; Dagatti *et al.,* 2014). It can also cause a severity of up to 52% in chili plants and in production (Fernández-Santillán *et al*., 2016; Tola *et al*. 2023). Some of the main species of *Meloidogyne* are *M. incognita*, *M. arenaria*, *M. javanica* (Romero *et al.,* 2019; Long *et al*., 2023), *M. enterolobii* (Romero *et al.,* 2019; Carrillo-Fasio *et al.,* 2020; Long *et al*., 2023) and *M. hapla* (Martínez-Gallardo *et al.,* 2019; Romero *et al.,* 2019).

For their control, chemical compounds such as oxamyl (Figueroa *et al*., 2016; Avelar-Mejía *et al*., 2018) and carbofuran (Medina *et al*., 2017) are used, which are considered highly toxic for mammals, birds and bees, as well as displaying high levels of leaching and persistence in water (Lewis *et al*., 2016). Nevertheless, there are alternatives for the control of nematodes, such as planting resistant varieties (Carrillo-Fasio *et al*., 2020) or applying nematode parasitic fungi. For example, *Pochonia chlamydosporia* parasites nematode eggs (Arévalo *et al.,* 2012), by forming appressoria, which it uses to penetrate the inside (Lopez-Llorca *et al.,* 2008) and produces enzymes (proteases and chitinases), which degrade the egg walls, facilitating the penetration of the fungus (Esteves *et al.,* 2009). Other species of fungi, such as mycorrhizae, *Trichoderma* sp., (Cristóbal-Alejo *et al.,* 2023), *Lecanicillium psalliotae* and *Paecilomyces* sp. have also been reported as parasites of these microorganisms (Arévalo *et al.,* 2009). For this reason, the aim of this study was to isolate and identify the nematode that causes damage, along with a nematophagous fungus and to evaluate its parasitism on *Meloidogyne* sp. egg on chili plants from Escuinapa, Sinaloa, as an ecological and sustainable alternative for the control of nematodes.

EXPERIMENTAL DEVELOPMENT

Gathering and processing of samples. Samples were gathered in Cristo rey, Escuinapa, Sinaloa (Latitude 22.5817° or 22° 34′ 54″ north and Longitude -105.72492° or 105° 43′ 30″ west, at 6 masl). The samples were processed in the Microbiology Laboratory of the Department of Agricultural Parasitology, located in the Universidad Autónoma Agraria Antonio Narro, Saltillo, Coahuila (Latitude 25° 21′ 13″ North and Longitude 101° 1′ 56″ West, at an altitude of 1,742 masl).

Isolation and morphological identification of the nematode. Nine samples of roots with galls were gathered from three commercial Platino hybrid serrano chili pepper plots in the production stage. The samples were placed in rows, labeled and transported to the Microbiology Laboratory for processing. Roots were washed with tap water to remove the remains of soil and females were extracted from the roots, which were cut perennially. Ten females were observed for identification with the methodology described by Hartman and Sasser (1985) and the characteristics were compared with those described by some authors (Taylor and Sasser 1978; Garrido *et al.,* 2014; Zeng *et al.,* 2014). On the other hand, a morphometric analysis was performed on 10 juveniles of stage two (J2), which were observed under a compound microscope (Nikon Eclipse E200LED MV R) and their morphological characteristics were compared by those referred by Hunt and Handoo (2009) and Zeng *et al.* (2014).

Isolation, morphological and molecular identification of the parasitic fungus *Meloidogyne* sp. From chili plants with galls caused by *Meloidogyne* sp., four masses of eggs and three globose females were gathered, which were dark in color and one of them displayed the presence of mycelia and fungal spores. The samples were washed with a 1.5% NaClO solution for 1 min and rinsed three times with sterile distilled water to remove the residual NaClO. The samples were planted in Petri dishes with an Agar-Water culture medium (Bacteriological Agar 16 g, distilled water 1 L and pH 7) and incubated in the dark for 72 h at 28 °C. After this time, the fungi found in the samples were purified with the hypha tip technique and transferred to new Petri dishes with Agar V8 juice medium (distilled water 450 mL, v8 juice 50 mL, bacteriological agar 8 g, calcium carbonate 0.62 g, pH 7), to later be morphologically identified at a species level by observing microscopic observations on slides with lactophenol blue under a compound microscope, which were compared with those described by Gams and Zare (2003).

The confirmation of the species was carried out molecularly; for this purpose, the strain was sent to the Laboratorio Nacional de Biotecnología Agrícola, Médica y Ambiental (LANBAMA) in San Luis Potosí, Mexico. The sample was sequenced using the labeled dideoxynucleotide method on a 3130 Genetic Analyzer sequencer (Applied Biosystems) in an automatic series 3500 Genic Analyzer. Internal transcribed spacer primers ITS1 (TCCGTAGGTGAACCTGCGG), from a ribosomal subunit of the 5.8S rRNA gene and ITS2 (GCTGCGTTCTTCATCGATGC) (Levío-Raimán *et al.,* 2021) were used. To amplify the genes, a Veriti Thermocycler for endpoint PCR (Applied Biosystems) was used.

Evaluation of the *in vitro* parasitism of the fungus on *Meloidogyne* sp eggs. Masses of *Meloidogyne* eggs were extracted, disinfected in a 0.1% chloramphenicol solution, washed for 2 min in a 0.5% NaClO solution and in distilled water for 3 min to remove the excess NaClO (Cortez-Hernández *et al.,* 2019), then passes through a sterile 500-mesh sieve under a laminar flow hood. They were then transferred to a 50 mL Falcon tube with sterile distilled water, resulting in a concentration of 680 eggs per mL. Subsequently, a total of 50 ±5 eggs were transferred to 2 mL Eppendorf tubes. Next, the *Pochonia* spore-based treatments were applied, which were multiplied in a Broth-Dextrose-Potato liquid culture potato broth 1000 mL, dextrose 15 g, malt extract 3 g, yeast extract 3 g) for seven days, shaken constantly at 28 ±2 °C and a photoperiod of 12:12 h light and darkness. Sevene treatments were carried out (T1= 2.3×10^6, T2= 4.7×10^6, T3= 9.5×10^6, T4= 2.3×10^7, T5= 4.7×10^7, T6= 9.5×10^7 spores mL^{-1} and T7= control without spores). Each tube was brought

to 1 mL with sterile distilled water. The design was completely at random and each treatment had eight repetitions. The treatments were incubated at 28 °C, with a photoperiod of 12:12 light and darkness in an incubator (Yamato, IC403CW), and after 48 h, parasitism evaluation was carried out. The percentage of parasitism was calculated with the formula described by Peraza-Padilla *et al*. (2014) %HP= NHP/ TH × 100, where %HP= Percentage of Parasitized Eggs, NHP= Number of Parasitized Eggs and TH= Total of Eggs.With the data obtained, an analysis of variance was carried out with Tukey's means comparison test ($p \leq 0,05$) in the InfoStat statistical program version 2019.1.2.0. In addition, a probit analysis was carried out using SAS to determine the lethal dose 50 (LD_{50}) and lethal dose 95 (LD_{95}) of the parasitism obtained with trust intervals of 95%, since these are useful measures to describe the effectiveness of the treatment.

Morphological identification of the species of parasitic nematode in chili plant roots. According to the perianal cuts of the globose females (Figure 1C) observed under the compound microscope, the species is *M. incognita*. The characteristics observed included well-defined striae forming high squared arches, the presence of widely spaced phasmids, and a vulva and an anus that were widely separated. Also observable were the absence of the lateral line (Figure 1A-B), which coincided with the characteristics described by Taylor and Sasser (1978) and Garrido *et al*. (2014), Zeng *et al*. (2014). The J2 juvenile stages were filiform, with an average length of 420 µm, a short stylet measuring 18-20 µm with visible median knobs, and a tail 36-40 µm in length that tapers to a subacute, unstriated and hyaline tip (Figure E-F), the J4 stages presented a sausage-like shape (Figure 1D) (Hunt and Handoo, 2009; Zeng *et al.,* 2014).

Several studies report *M. incognita* as a parasite of chili plants. For example, Cristóbal-Alejo *et al*. (2023) report it as causing a rate of galling of 83% in habanero chili plants, which was reflected in a lower plant development. On the other hand, Carrillo-Fasio *et al.* (2000) reported this same species as infecting several crops including chili in a large part of Sinaloa (El Rosario, La Cruz de Elota, Culiacan, Navolato and Guasave). Regarding studied carried out in the municipality of Escuinapa, Sinaloa, Carrillo-Fasio *et al.* (2021) reported this nematode as parasiting chili plants in a similar way as this study.

Morphological and molecular identification of the fungal parasite of *Meloidogyne incognita*. From a parasited globose female showing the presence of mycelium and spores (Figure 2A), a fungus was isolated and identified as *Pochonia chlamydosporia* var. *chlamydosporia*. The fungus presented branches, verticillate conidiophores (Figure 2B), with the production of cylindrical single-celled spores in clusters (Figure 2C), multicellular dictyochlamydospores (irregularly-shaped chlamydospores with 4 or 9 cells) (Figure 2E), which originate from lateral ramifications (Figure 2D). The mycelium had a whitish cottonlike appearance on PDA medium (Figure 2F), consistent with the characteristics reported by Gams and Zare, (2003) and Ciancio *et al.* (2016) for this species. The clustered spores are a distinctive feature of *P. chlamydosporia* var. *chlamydosporia*, differentiating it with the species *P. chlamydosporia* var. *catenulata*, which presents chained spores (Arévalo *et al.,* 2009). The confirmation of the species based on the region of the internal transcribed spacer ITS1 of gene 5.8S RNA and ITS2 displayed a similarity of 99.3% with the sequence of accession code MW654486.1 of the GenBank, which corresponds to the species *P. chlamydosporia*.

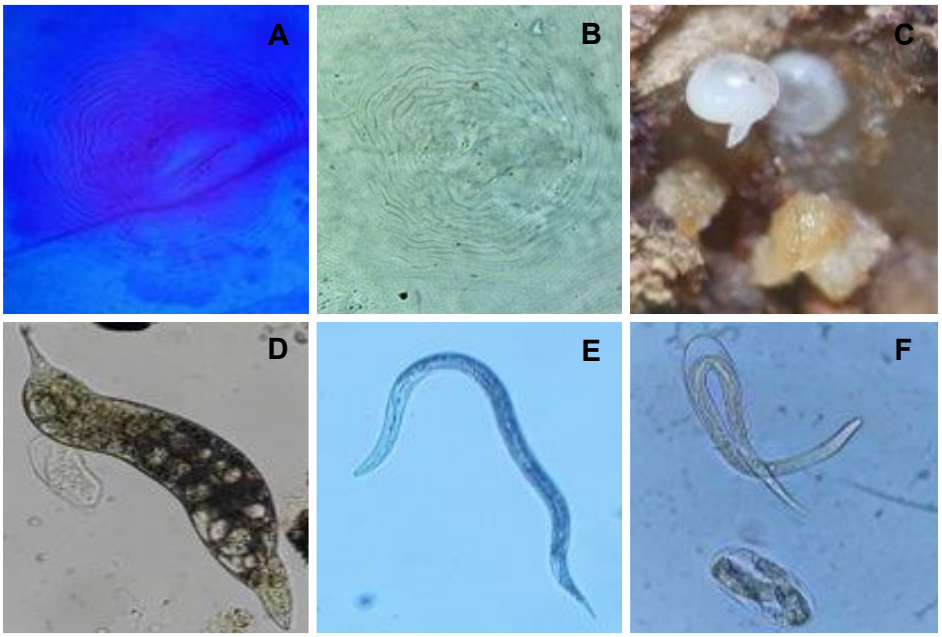

Figure 1. Morphological characteristics of *Meloidogyne incognita*; **A** and **B**) Perianal cut of globose females; **C**) Globose females; **D**) Juvenile J4; **E**) Juvenile stage J2; **F**) Juvenile stage J2 hatching from the egg.

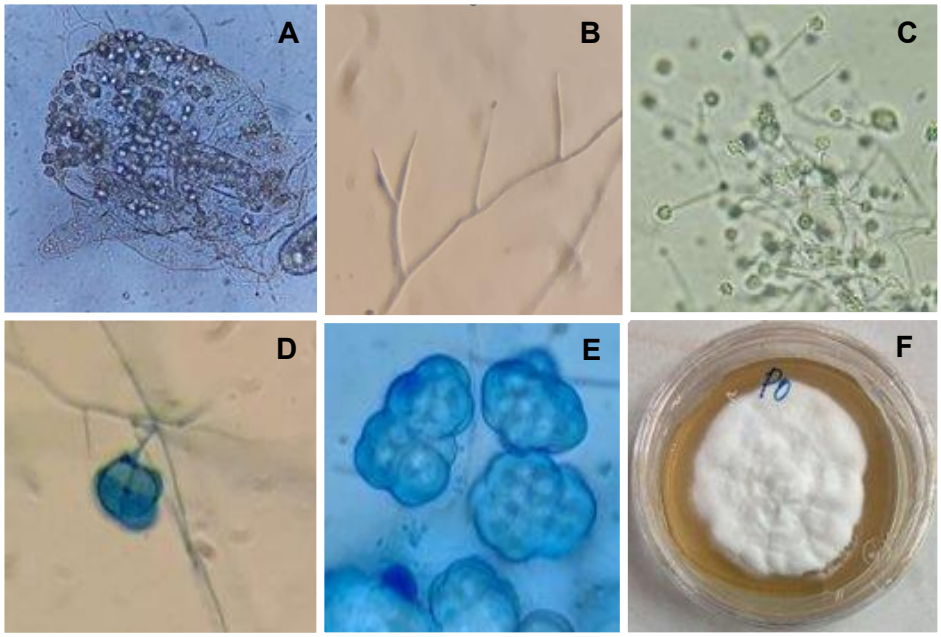

Figure 2. Morphological characteristics of *Pochonia chlamydosporia* var. *chlamydosporia*; **A**) Female globose from which *Pochonia* was isolated, with the presence of dictyochlamidospores; **B**) Branched verticillated conidiophores; **C**) Production of spores in a cluster; **D**) Formation of a dictyochlamidospore; **E**) Cluster of dictyochlamidospores; **F**) Whitish colored mycelium.

Evaluation of the *in vitro* parasitism of *Pochonia chlamydosporia* var. *chlamydosporia* on *Meloidogyne incognita* eggs. Table 1 shows the parasitism of *P. chlamydosporia* var. *chlamydosporia* on *M. incognita* eggs *in vitro*. At low and high concentrations of spores of this fungus, there was parasitosis; although statistically ($P \leq 0.05$), the treatments at a concentration of 4.7×10^7 (T5) and 9.5×10^7 (T6) spores mL^{-1} of the fungus had over 98%

of parasitism or external growth of the mycelium of *Pochonia* (Figure 3). The eggs can be noticed to have internal and external mycelia (Figure 3A-B), in addition to the presence of dictyochlamydospores (Figure 3 C), whereas the control presented no signs of the fungus (Figure 3 D).

Table 1. Percentage of *Pochonia chlamydosporia* parasitism on *Meloidogyne incognita* eggs under *in vitro* conditions.

Tratamientos	% de Parasitismo E.E
T1= 2.3× 10^6 spores mL^{-1}	80.3±2.2 ^{C}
T2= 4.7× 10^6 spores mL^{-1}	86.9±2.3 ^{BC}
T3= 9.5× 10^6 spores mL^{-1}	86.8±3.3 ^{BC}
T4= 2.3× 10^7 spores mL^{-1}	91.3±2.2 ^{AB}
T5= 4.7× 10^7 spores mL^{-1}	98.5±1.0 ^{A}
T6= 9.5× 10^7 spores mL^{-1}	98.9±0.8 ^{A}
T7= control	0.0±0.0 ^{D}

E.E= Standard error. ^{z}Statistical means with different letters are significantly different (Tukey; $p \leq 0.05$).

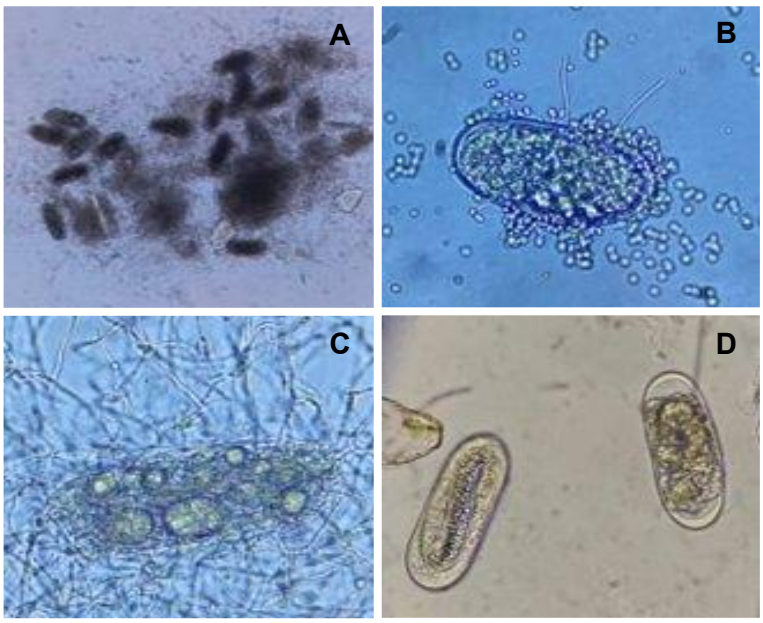

Figure 3. *Pochonia chlamydosporia* parasitims on *Meloidogyne incognita* eggs; **A**) Parasited eggs with a large amount of mycelium; **B**) Mycelium coming out of the egg and presence of spores on the surface of the egg; **C**) Parasited egg with mycelium and the presence of dictyochlamidospores; **D**) Eggs from the control treatment.

The probit analysis, based on the percentages of parasitism, revealed that the values of LD_{50} and LD_{95} were of 2.2E+05 and 2.8E+07 respectively (Table 2). These results indicate that the concentration of spores of the fungus has a significant effect on the parasitism of the *M. incognita* eggs.

Table 2. Probabilistic analysis of the concentration of *Pochonia chlamydosporia* spores on the suppression of *Meloidogyne incognita* eggs.

Tratamiento	LFI	LD_{50}	LFM	LD_{95}	Valor de P
Spores	2.43E+04	2.23E+05	6.37E+05	2.82E+07	0.0001

LFI= Lower Fiducial Limits. LFM= Upper Fiducial Limits.

The data obtained in this study are similar to those reported by some authors. For example, Vilchis-Martínez *et al.* (2013) report parasitism levels of 86.1 and 88.2% of two strains of *P. chlamydosporia* (Strain Pc341x and Strain Pc10w) at a concentration of 5.5×10^4 spores mL^{-1} on *M. incognita* eggs. Similarly, Flores-Camacho *et al.* (2008) report varied percentages of parasitism for *P. chlamydosporia* species on *Nacobbus aberrans* eggs, which range from 59 to 89%. Likewise, Pérez-Espíndola *et al*. (2019) report a 69% colonization of this fungus on *N. aberrans* and *M. incognita* egg masses on the soil, as well as a 50% reduction in the galling of tomato plant roots, all of which proves its nematocidal capacity. The ability of parasitism of this species is attributed to its ability to produce some compounds, such as proteases and chitinases. For example, Escudero *et al.* (2016) attribute the parasitic capacity to the formation of appressoria and the production of extracellular enzymes such as serine carboxypeptidase SCP_1 and serine protease VCP_1. Similarly, Pérez-Espíndola *et al*. (2019) attribute it to the production of enzyme VCP_1, which is secreted by the fungus during the egg parasitism process. Mi *et al.* (2010) also report the production of a chitinase that bonds β-1,4 bonds in chitin, which facilitates the penetration into the egg. Regarding the high percentage of parasitism of this *P. chlamydosporia* var. *chlamydosporia* isolate on *M. incognita* eggs, this may be due to the high production of proteases and chitinases, which facilitates the penetration of the fungus into the eggs.

CONCLUSIONS

Infective *P. chlamydosporia* var. *chlamydosporia* were isolated and identified from a globose *Meloidogyne* female. *M. incognita* was morphologically identified as the root-knot nematode in chili plant roots. This fungus displayed high percentages of *in vitro* parasitism on nematode eggs, which ranged from 80% at a concentration of 2.3×10^6 spores mL^{-1} to 98.9% at a concentration of 9.5×10^7 spores mL^{-1}, in a 48-hour period. Therefore, this isolation could constitute a bio alternative for the management of nematodes. However, its effectiveness must be validated with *in vivo* studies that confirm its capacity of control under real conditions.

Limitations

For future investigations, validate the effectiveness of the control of *Pochonia chlamydosporia* var. *chlamydosporia* on *Meloidogyne* in *in vivo* studies or in the field. Perform the identification of the nematode species with molecular studies.

Conflict of interest

The authors of this study have no conflicts of interest.

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Contribution of the authors

Omar Jiménez-Pérez: Research development, experimental design. **Bernardo Espinosa-Palomeque:** Data analysis and literature review. **Augusto Gil Ceballos-Ceballos:** Identification of nematode and literature review. **Francisco Castillo-Reyes:** Data analysis and literature review. **Gabriel Gallegos-Morales:** Identification of fungus and literature review.

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