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RESEARCH ARTICLE

Effect of *Pochonia chlamydosporia* on soil microbial activity and accumulation of macro- and micronutrients in soybean infected with *Meloidogyne javanica*

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ABSTRACT

Meloidogyne javanica induces the formation of specialized feeding sites in host roots, affecting nutrient absorption by the plant. Biological control agents, such as *Pochonia chlamydosporia*, may mitigate the damage caused by nematodes. We hypothesized that the fungus contributes to plant nutrient absorption and soil microbial activity, thereby reducing the negative impacts of nematode infection. This study aimed to assess the influence of *P. chlamydosporia* on *M. javanica* reproduction, soil respiration, vegetative development, and accumulation of macro- and micronutrients in soybean. Experiments were conducted in a greenhouse using plants inoculated with *M. javanica* and treated with *P. chlamydosporia*, uninoculated plants treated with *P. chlamydosporia*, untreated plants inoculated with *M. javanica*, and uninoculated untreated plants (absolute control). The inoculum consisted of 2000 eggs and second-stage juveniles of *M. javanica*, and the treatment consisted of 5.2×10^7 chlamydospores g^{-1} at a dose of 2.5 kg ha^{-1}. Water was used as control. At 60 d after treatment, *P. chlamydosporia* reduced *M. javanica* reproduction by 40.3% and plant height was highest in inoculated treated plants. Soil microbial biomass C reached the highest level in nematode-inoculated soil (3665 mg C kg^{-1}), whereas basal respiration was stimulated by *P. chlamydosporia* treatment (0.51 mg CO_2-C kg^{-1} h^{-1}). The microbial metabolic quotient peaked in soils with both nematodes and the fungus. Regarding nutrition, *P. chlamydosporia* enhanced P (+46%) and K (+69%) accumulation in leaves, while *M. javanica* infection increased Zn content.

Key words: Alternative control, nematophagous fungus, plant nutrition, soil respiratory activity.

INTRODUCTION

Nematodes of the genus *Meloidogyne*, commonly known as root-knot nematodes, are obligate parasites that feed on plant roots by inducing specialized feeding sites composed of giant cells (Mahapatra and Nayak, 2019). Giant cells have dense and granular cytoplasm, a large number of organelles, invaginated cell walls, and several conspicuous nuclei (Favery et al., 2016; Mahapatra and Nayak, 2019). Parasitism results in cell hyperplasia and hypertrophy, culminating in the formation of root galls (Favery et al., 2016).

The feeding sites of *Meloidogyne* females are formed in or near the xylem of the host plant, causing tissue damage (Siddique and Grundler, 2018) and compromising water and nutrient absorption by the host (Palomares-Rius et al., 2017). Infection symptoms, such as yellowed leaves, discolored stems and branches,

defoliation, reduced plant size, and low yields, are observed in the aerial parts of infected plants as a result of root dysfunction (Favery et al., 2016).

A strategy to mitigate the damage caused to host roots is to apply control methods that reduce nematode penetration. Biological control tactics are widely used for nematode control (Hussain et al., 2017), and, in some cases, have the advantage of promoting plant nutrition and growth, soil organic matter decomposition, mineral solubilization, and release of chelating agents. Such effects contribute to root development, even in the presence of nematodes (Manzanilla-López et al., 2017), improving nutrient absorption efficiency (Abhilash et al., 2016; Rinaldi et al., 2023). Endophytic organisms are the most promising in this regard (Kumar and Dara, 2021). The most widely used biological agents are fungi of the genus *Trichoderma* and bacteria of the genus *Bacillus* (Albahadli et al., 2019; Messa et al., 2019).

Pochonia chlamydosporia (Goddard) Zare and Gams (Hypocreales, Clavicipitaceae) is another endophytic fungus that shows potential for the control of nematodes. This soil saprophyte can parasitize eggs and sedentary females of root-knot nematodes (Yi et al., 2021), including those of *Meloidogyne enterolobii*, *M. javanica*, and *M. incognita* (Medeiros et al., 2015; Silva et al., 2017; Ghahremani et al., 2019). The action of *P. chlamydosporia* seems to go beyond direct parasitism. Studies have shown that the fungus is able to induce resistance to nematodes in plants (Medeiros et al., 2015; Zavala-Gonzalez et al., 2017) and compete with nematodes in the rhizosphere (Zavala-Gonzalez et al., 2015). Because *P. chlamydosporia* is a good rhizosphere colonizer (Zavala-Gonzalez et al., 2015), it is believed that it can improve microbial activity in the soil and contribute to plant nutrient absorption; however, more research is needed to confirm these hypotheses.

The aim of this study was to assess nematode reproduction, plant vegetative development, soil respiration, and macro- and micronutrient leaf contents of soybean infected with *M. javanica* and treated with *P. chlamydosporia*.

MATERIALS AND METHODS

Meloidogyne javanica and *Pochonia chlamydosporia* inocula

Meloidogyne javanica inoculum was obtained from a single-species population maintained on tomato in a greenhouse (23°47′34.5″ S, 53°15′22.1″ W). Nematodes were extracted from plant roots by the method of Hussey and Barker (1973) modified by Boneti and Ferraz (1981). The suspension was calibrated to 2000 eggs and eventual second-stage juveniles (J2) mL^{-1} using a Peter's counting chamber under an optical microscope.

Pochonia chlamydosporia Pc-10 (Rizotec, 5.2×10^7 chlamydospores g^{-1}, Stoller, Campinas, Brazil) was used at the manufacturer's recommended dose (2.5 kg ha^{-1}). The product was applied to the soil in a spray volume of 50 L ha^{-1}.

Effect of *P. chlamydosporia* on *M. javanica* reproduction and soybean vegetative development

The experiment was carried out in a greenhouse, with temperatures ranging from 19.8 to 31.0 °C. A completely randomized experimental design with four treatments and five replicates was used. The treatments consisted of plants inoculated with *M. javanica*, plants treated with *P. chlamydosporia*, plants inoculated with *M. javanica* and treated with *P. chlamydosporia*, as well as the absolute control (untreated and uninoculated plants).

Each experimental unit consisted of a polystyrene pot with 500 cm^3 of a 1:1 (v/v) mixture of soil (dystrophic Red Latosol) and sand previously autoclaved at 121 °C for 2 h. Pots were fertilized with 0.7 g limestone and 0.12 g NPK (04-14-08) fertilizer, as determined on the basis of soil chemical properties. A 5 cm wide and 4 cm deep hole was made in the soil and inoculated with 1 mL nematode suspension containing 2000 eggs + J2. Then, *P. chlamydosporia* was applied to the hole at the specified dose, and a seed of soybean (*Glycine max* (L.) Merr.) 'M6410 IPRO' was sown.

After 60 d, the plants were harvested and separated into shoots and roots. Roots were washed, weighed, and subjected to nematode extraction (Hussey and Barker, 1973; Boneti and Ferraz, 1981). Total nematode number was determined using a Peter's counting chamber under a light microscope. Nematode number was divided by the root fresh weight to obtain the nematode population density (nematodes g^{-1} root). The reproduction factor was calculated as the final population density divided by the initial population density (Oostenbrink, 1966).

Root length, plant height, and shoot fresh and dry weights were assessed. For dry weight determination, shoots were washed in deionized water and dried in a forced-air oven at 60 °C for 72 h until constant weight was achieved. The dried material was stored in a dry room until analysis.

Soil respiration

Soil was collected after plant harvest for determination of respiratory activity. Soil moisture content was determined using a portion of wet soil with known weight. The wet soil was oven-dried at 105 °C to constant weight. Then, moisture content was obtained as the difference between fresh and dry weights.

Soil basal respiration was evaluated by using the method of Jenkinson and Powlson (1976). Briefly, 30 g soil was weighed and added to a 100 mL glass flask, and 10 mL 1 M sodium hydroxide (NaOH) was added to another flask (100 mL). Both flasks were placed in a hermetically sealed glass container (500 mL) to ensure that CO_2 entry or escape did not occur. The control consisted of flask containing 10 mL 1 M NaOH placed in a separate glass container (500 mL). Samples and control were incubated for 7 d in the dark at room temperature (25-28 °C). After incubation, NaOH flasks were removed from the containers and received the addition of 2 mL 10% barium chloride and three drops of 3% phenolphthalein solution in alcohol. The mixture was titrated using 0.5 M hydrochloric acid (HCl) until the color changed from pink to colorless. The concentration of CO_2 absorbed by the NaOH solution was calculated according to Equation 1:

$$\mathrm{SBR} = \frac{(v_c - v_s) \times M \times 6 \times 1000}{S \times T} \qquad (1)$$

where SBR is the soil basal respiration (mg CO_2-C kg^{-1} soil h^{-1}); v_c is the volume of NaOH consumed in the control titration (mL); v_s is the volume of NaOH consumed in the sample titration (mL); M is the molar concentration of HCl (mol L^{-1}), S is the soil dry weight (g), and T is the incubation time (h).

Soil microbial biomass C was determined by the fumigation-extraction method (Medeiros et al., 2015; 2019). For this, 20 g soil was collected from each experimental unit and divided into two 100 mL glass flasks (10 g soil each). One flask was subjected to fumigation and the other was not. For fumigation, flasks received 1 mL ethanol-free chloroform and were closed and stored in the dark for 24 h at 25-28 °C. After this period, caps were removed under a fume hood, and chloroform was allowed to evaporate (Brookes et al., 1982; Witt et al., 2000). Nonfumigated samples received the addition of 10 g soil only.

Carbon was extracted from fumigated and nonfumigated samples by addition of 50 mL 0.5 M potassium sulfate solution. Samples were shaken at 220 rpm for 30 min in an orbital shaker and then allowed to stand for 30 min. The supernatant (extract) was filtered and collected. An aliquot of 8 mL extract was transferred to a 250 mL Erlenmeyer flask and received the addition of 2 mL 0.066 M potassium dichromate solution, 10 mL 95%-98% sulfuric acid (H_2SO_4), and 5 mL 85% orthophosphoric acid. After cooling, the solution was mixed with 70 mL deionized water, cooled again, and mixed with four drops of 1% diphenylamine ($C_{12}H_{11}N$). Titration was performed with a solution of 0.033 M ammonium ferrous sulfate [$(NH_2)_2Fe(SO_4)_2 \cdot 6H_2O$] until the color changed from purple to green. Microbial biomass C was calculated using Equation 2:

$$\mathrm{MBC} = \frac{(V_c - V_s)\, M \times 0.003 \times V_1 \times 10^6}{S \times V_2} \qquad (2)$$

where MBC is the microbial biomass C (mg C kg^{-1} soil), V_c is the volume of ammonium ferrous sulfate consumed in control titration (mL), V_s is the volume of ammonia ferrous sulfate consumed in sample titration (mL), M is the molar concentration of ammonium ferrous sulfate (mol L^{-1}), V_1 is the volume of potassium sulfate (mL), V_2 is the volume of extract used for titration (mL), 0.0033 is the milliequivalent of C, S is the soil dry weight (g), and K_c is the correction factor (0.4) proposed by Kaschuk et al. (2010).

The soil microbial metabolic quotient was determined as the ratio of basal respiration to microbial biomass C (Silva et al., 2007), as shown in Equation 3. The parameter can indicate microbial stress.

$$q\mathrm{CO}_2 = \frac{\mathrm{SBR}}{\mathrm{MBC}} \times 1000 \qquad (3)$$

where qCO_2 is the microbial metabolic quotient (mg CO_2-C g^{-1} MBC h^{-1}), SBR is the soil basal respiration (mg CO_2-C kg^{-1} soil h^{-1}), and MBC is the microbial biomass C (mg C kg^{-1} soil).

Accumulation of macro- and micronutrients in soybean leaves

Dry leaves were ground using a Willey knife mill and sieved through 20 and 40 mesh sieves to obtain powdered samples. Samples were packed in hermetically sealed plastic bags. Aliquots of 500 mg were taken from powdered samples to determine macronutrient (P, K, Ca and Mg) and micronutrient (Zn, Cu, Fe and Mn) contents. Nutrient analyses were performed using the method of Malavolta et al. (1997). The N content was

determined by the semi-micro Kjeldahl method using 100 mg powdered samples. Samples were transferred to digestion tubes, placed in a digester block, and mixed with 6 M digester solution until the temperature reached about 450 °C. Digestion was complete after about 10 h, when samples showed a clear color. Digested samples were distilled using an N distiller (Tecnal, Piracicaba, Brazil) with boric acid indicator solution until the color changed from pink to green. Then, distilled samples were titrated with H_2SO_4 until the color changed from green to pink. N content was calculated using Equation 4:

$$N = \frac{V_g \times 0.021 \times 4 \times F_c \times 100}{1000 \times w} \quad (4)$$

where N is the sample N content (%), V_g is the titrant (H_2SO_4) volume, 0.021 is the molar concentration of H_2SO_4, F_c is the correction factor for H_2SO_4 (1.675), and w is the sample weight. The N content was then transformed to g kg^{-1}.

The other compounds were determined by using 500 mg powdered samples. Briefly, samples were digested in a block digester using nitric-perchloric acid solution until the temperature reached about 450 °C. Then, each sample (2 mL) was transferred to a volumetric flask. Deionized water was added to complete the volume to 50 mL. The absorbance was read using an atomic emission spectrometer (4200 MP-AES, Agilent Technologies, Santa Clara, California, USA), according to Malavolta et al. (1997). Macronutrient content was expressed as g kg^{-1}, and micronutrient content was expressed as mg kg^{-1}.

Statistical analysis

Experimental data were subjected to ANOVA at the 5% significance level. When differences were significant, means were compared by Tukey's test at the 5% significance level. Analyses were performed using Sisvar software (Ferreira, 2011).

RESULTS AND DISCUSSION

Effect of *Pochonia chlamydosporia* on *Meloidogyne javanica* reproduction and soybean vegetative development

Total nematode number and reproduction factor were influenced by treatment with *P. chlamydosporia*. The fungus promoted a 40.3% reduction in *M. javanica* reproduction (Table 1). Inoculated treated plants had a greater height than the inoculated control, but differences were nonsignificant (Table 2). Root fresh weight was higher in inoculated plants. The other vegetative parameters were not influenced by nematode inoculation or fungal treatment.

Table 1. Population of *Meloidogyne javanica* in soybean 'M6410 IPRO' roots, treated or not with *Pochonia chlamydosporia*. Means followed by the same letters within a column do not differ significantly at $p < 0.05$ by Tukey at 5%. ^{ns}Nonsignificant. CV: Coefficient of variation.

Treatments	Nematodes g^{-1} root	Total nematodes	Reproduction factor
M. javanica	6634^{ns}	22 903^{a}	11.47^{a}
M. javanica + *P. chlamydosporia*	4661	13 677^{b}	6.83^{b}
CV, %	45.20	24.95	21.72

Table 2. Height, fresh weight (FSW) and dry shoot weight (DSW), fresh root weight (FRW) and root length (RL) of soybean 'M6410 IPRO' parasitized or not per *Meloidogyne javanica* and treated or not with *Pochonia chlamydosporia*. Means followed by the same letters within a column do not differ significantly at $p < 0.05$ by Tukey at 5%. ^{ns}Nonsignificant. CV: Coefficient of variation.

Treatments	Height	FSW	DSW	FRW	RL
	cm	g	g	g	cm
M. javanica (*Mj*)	28.0^{ab}	80.9^{ns}	21.1^{ns}	56.0^{a}	20.8^{ns}
Mj + *P. chlamydosporia*	34.4^{a}	82.7	26.5	35.9^{b}	18.6
P. chlamydosporia	28.2^{ab}	101.2	26.3	27.3^{b}	19.2
Control	24.7^{b}	74.9	21.1	33.6^{b}	20.8
CV, %	15.12	27.33	16.12	24.42	36.01

Soil respiration

Microbial biomass C was highest in untreated soil inoculated with *M. javanica*, followed by uninoculated soil treated with *P. chlamydosporia* (Table 3). The presence of *M. javanica* alone reduced soil basal respiration compared with other treatments, which did not differ from each other or from the control. The microbial metabolic quotient was highest in inoculated treated soil (Table 3).

Table 3. Soil respiratory activity cultivated with soybean 'M6410 IPRO', parasitized per *Meloidogyne javanica* and treated or not with *Pochonia chlamydosporia*. Means followed by the same letters within a column do not differ significantly at $p < 0.05$ by Tukey at 5%. MBC: Microbial biomass C; RBS: soil basal respiration; qCO_2: microbial metabolic quotient. ^{ns}Nonsignificant. CV: Coefficient of variation.

Treatments	MBC	SBR	qCO_2
	mg C kg^{-1} soil	mg CO_2-C kg^{-1} soil h^{-1}	mg CO_2-C g^{-1} MBC h^{-1}
M. javanica (*Mj*)	3665.84^{a}	0.29^{b}	0.08^{b}
Mj + *P. chlamydosporia*	166.62^{c}	0.61^{a}	4.46^{a}
P. chlamydosporia	2381.41^{b}	0.51^{a}	0.21^{b}
Control	685.03^{c}	0.59^{a}	0.87^{b}
CV, %	23.98	16.58	84.09

Accumulation of macro- and micronutrients in soybean leaves

The P and K levels were highest in uninoculated treated plants (Figure 1). Compared to inoculated untreated plants, plants treated with the fungus had a 46% higher P content and a 69% higher K content. Treatment with *P. chlamydosporia* did not differ from treatment with *M. javanica* and *P. chlamydosporia*. Regarding micronutrients, Zn levels were higher in inoculated untreated plants than in inoculated treated plants (Figure 2), which did not differ from other plants.

Pochonia chlamydosporia was effective in controlling *M. javanica* reproduction on soybean, corroborating the results of previous studies (Medeiros et al., 2015; Ghahremani et al., 2019). *Pochonia chlamydosporia* is a chitinolytic fungus (Yi et al., 2021) that acts mainly by parasitizing nematode eggs (Ghahremani et al., 2019). Root-knot nematodes deposit eggs in a gelatinous matrix. Not only the egg sac but also individuals within the sac can be destroyed by the action of the fungus (Yi et al., 2021).

Pochonia chlamydosporia has other mechanisms of action against *Meloidogyne* spp. The fungus can endophytically colonize plant roots, promoting hormonal and enzymatic changes that lead to enhanced plant development or activation of natural defense mechanisms (Medeiros et al., 2015; Zavala-Gonzalez et al., 2017). Rhizosphere colonization by the fungus also reduces nematode penetration in roots, compromising nematode development and reproduction (Zavala-Gonzalez et al., 2015).

Despite the reports that *P. chlamydosporia* promotes plant growth (Zavala-Gonzalez et al., 2015), there was no conclusive evidence of this effect in the present study. An increase in plant height was observed only in plants treated with *P. chlamydosporia* and inoculated with *M. javanica*. However, inoculated treated plants did not differ from inoculated untreated or uninoculated treated plants, which can be attributed to the fact that both the fungus and the nematode induce indoleacetic acid synthesis (Zavala-Gonzalez et al., 2015), contributing to plant development (Meneguzzi et al., 2015). On the other hand, the fungus did not promote an increase in other vegetative parameters, in agreement with the findings of Ghahremani et al. (2019), who, in applying *P. chlamydosporia* to control *M. incognita* in tomato, did not observe an increase in shoot fresh or dry weights.

The complex relationship between biological agent, nematode, and plant is influenced by soil parameters. Thus, the plant's response to inoculation and treatment is expected to be variable. The high root weight observed in inoculated untreated plants may be associated with the formation of root galls by nematode action (Vilela et al., 2021).

Soil microbial biomass C was highest in inoculated untreated plants, followed by uninoculated treated plants. In this case, as there were no interactions between organisms, soil conditions were likely favorable for the development of nematodes and fungi (Oliveira et al., 2016), thereby increasing microbial biomass in the soil. On the other hand, the parasitic interaction of the fungus with the nematode probably reduced the number

of soil microorganisms (Maboreke et al., 2017). It is worth noting that the soil used in this study was previously autoclaved; thus, other natural soil inhabitants were partially or completely eliminated (Hu et al., 2020), and the results of microbial biomass (living organisms) are associated almost entirely to the presence of the introduced organisms (Kaschuk et al., 2010).

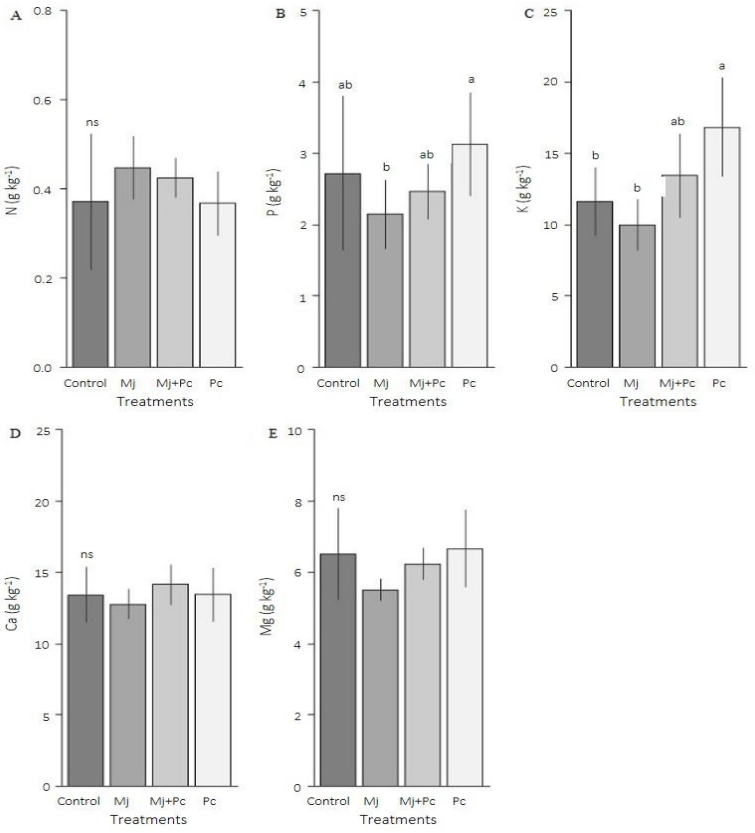

Figure 1. Macronutrients in soybean 'M6410 IPRO' leaves, parasitized or not per *Meloidogyne javanica* (Mj) and treated or not with *Pochonia chlamydosporia* (Pc). Means followed by the same letters between columns do not differ significantly at $p < 0.05$ by Tukey at 5%. ^{ns}Nonsignificant.

Soil basal respiration is the amount of soil C in the form of CO_2 resulting from the respiration of decomposers (Medeiros et al., 2019). *Pochonia chlamydosporia* is a saprobiontic fungus; i.e., it feeds on decaying organic matter, contributing to soil basal respiration (Dhiman et al., 2024). This explains why *P. chlamydosporia* treatment increased basal respiration. However, the treatment did not differ from the uninoculated untreated control, which might be associated with the effects of abiotic factors, such as soil moisture, temperature, and aeration (Silva et al., 2007).

Microbial metabolic quotient was highest in soil inoculated with both nematodes and fungi. This parameter is related to the efficiency of microorganisms to use available C for growth (Batista et al., 2018). It is possible that nematodes had high energy expenditure because of the stress caused by *P. chlamydosporia* parasitism (Manzanilla-López et al., 2017; Monteiro et al., 2017), affecting the soil microbial biomass.

The P and K accumulated in the leaves of soybean treated with *P. chlamydosporia* compared with leaves of untreated plants inoculated with *M. javanica*. This result supports the hypothesis that the fungus can solubilize nonlabile forms of P, making the compound soluble and available for plant absorption (Zavala-Gonzalez et al., 2015). A previous study showed that *P. chlamydosporia* increases the availability of other nutrients, such as K (Zavala-Gonzalez et al., 2015). The endophytic relationship between fungus and plant might have contributed to

nutrient absorption. In plants infected with nematodes, absorption and translocation of water and nutrients are affected by the formation of feeding sites in the xylem (Palomares-Rius et al., 2017; Siddique and Grundler, 2018).

Under the experimental conditions of the present study, *P. chlamydosporia* did not positively influence micronutrient absorption. One hypothesis is that soil autoclaving reduced organic matter content and, consequently, micronutrient levels, leading to low micronutrient availability for plant absorption. However, Zn absorption was highest in untreated plants inoculated with *M. javanica*. Because research on the effects of nematodes on micronutrient absorption is scarce, further investigations with different types of soil and fertilization strategies are needed to better understand this relationship.

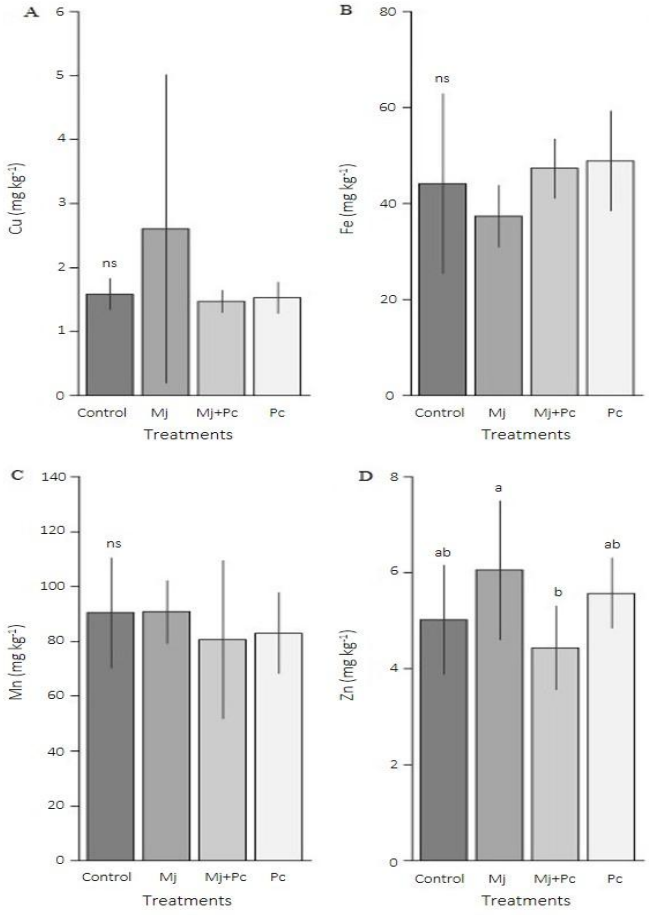

Figure 2. Micronutrients in soybean 'M6410 IPRO' leaves, parasitized or not per *Meloidogyne javanica* (Mj) and treated or not with *Pochonia chlamydosporia* (Pc). Means followed by the same letters between columns do not differ significantly at $p < 0.05$ by Tukey at 5%. ^{ns}Nonsignificant.

CONCLUSIONS

Pochonia chlamydosporia treatment not only effectively controlled *Meloidogyne javanica* reproduction but also positively influenced the soil microbial community and plant absorption of P and K. This biological agent shows potential to be used in sustainable nematode management strategies. However, further research is needed to understand the relationship between macro- and micronutrient accumulation, soil properties, and the plant-fungus-nematode pathosystem.

Author contribution

Conceptualization: R.P.S., C.R.D-A., S.M.S-G. Methodology: R.P.S., A.S.M., C.Y.F., G.T. Software: S.M.S-G. Resources: C.R.D-A. Data curation: S.M.S-G. Writing-original draft: R.P.S. Writing-review & editing: C.R.D-A., M.A.B., A.C., S.M.S-G. Supervision: C.R.D-A., M.A.B., S.M.S-G. Project administration: C.R.D-A. Funding acquisition: C.R.D-A. All co-authors reviewed the final version and approved the manuscript before submission.

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References

Abhilash, P.C.C., Dubey, R.K., Tripathi, V., Gupta, V.K., Singh, H.B. 2016. Plant growth-promoting microorganisms for environmental sustainability. Trends in Biotechnology 34(11):847-850. doi:10.1016/j.tibtech.2016.05.005.

Albahadli, Y.I.H., Mamarabadi, M., Mahdikhani, M.E. 2019. Possibility of the biocontrol of *Meloidogyne javanica* using the fungus *Trichoderma harzianum* under greenhouse condition. Plant Archives 19(1):47-51.

Batista, E.R., Zanchi, C.S., Ferreira, D.A., Santiago, F.D.A., Pinto, F.A., Santos, J.D., et al. 2018. Atributos biológicos do solo em sistema integrado de produção agropecuária. Sistemas Integrados de Produção Agropecuária no Brasil 1:71-90. doi:10.13083/reveng.v23i5.534.

Boneti, J.I.S., Ferraz, S. 1981. Modificações do método de Hussey & Barker para extração de ovos de *Meloidogyne exigua* em raízes de cafeeiro. Fitopatologia Brasileira 6:553.

Brookes, P.C., Powlson, D.S., Jenkinson, D.S. 1982. Measurement of microbial biomass phosphorus in soil. Soil Biology & Biochemistry 14:319-329. doi:10.1016/0038-0717(82)90001-3.

Dhiman, N., Uthoff, J., Scharf, B., Kumar, V. 2024. Plant-microbe interaction to improve soil health. p. 189-226. In Bhatia, R.K., Walia, A. (eds). Advancements in microbial biotechnology for soil health. Microorganisms for sustainability. Springer, Singapore. doi:10.1007/978-981-99-9482-3_10.

Favery, B., Quentin, M., Jaubert-Possamai, S., Abad, P. 2016. Gall-forming root-knot nematodes hijack key plant cellular functions to induce multinucleate and hypertrophied feeding cells. Journal of Insect Physiology 84:60-69. doi:10.1016/j.jinsphys.2015.07.013.

Ferreira, D.F. 2011. Sisvar: A computer statistical analysis system. Ciência e Agrotecnologia 35:1039-1042. doi:10.1590/S1413-70542011000600001.

Ghahremani, Z., Escudero, N., Saus, E., Gabaldón, T., Sorribas, F.J. 2019. *Pochonia chlamydosporia* induces plant-dependent systemic resistance to *Meloidogyne incognita*. Frontiers in Plant Science 10:00945. doi:10.3389/fpls.2019.00945.

Hu, W., Wei, S., Chen, H., Tang, M. 2020. Effect of sterilization on arbuscular mycorrhizal fungal activity and soil nutrient status. Journal of Soil Science and Plant Nutrition 20:684-689. doi:10.1007/s42729-019-00156-2.

Hussain, M., Zouhar, M., Ryšánek, P. 2017. Effects of nematophagous fungi on viability of eggs and juveniles of *Meloidogyne incognita*. Journal of Animal and Plant Science 27:252-258.

Hussey, R.S., Barker, K.R. 1973. A comparison of methods collecting inocula of *Meloidogyne* spp. including a new technique. Plant Disease Reporter 57:1025-1028.

Jenkinson, D.S., Powlson, D.S. 1976. The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. Soil Biology & Biochemistry 8:209-213. doi:10.1016/0038-0717(76)90005-5.

Kaschuk, G., Alberton, O., Hungria, M. 2010. Three decades of soil microbial biomass studies in Brazilian ecosystems: Lessons learned about soil quality and indications for improving sustainability. Soil Biology & Biochemistry 42:1-13. doi:10.1016/j.soilbio.2009.08.020.

Kumar, K.K., Dara, S.K. 2021. Fungal and bacterial endophytes as microbial control agents for plant-parasitic nematodes. International Journal of Environmental Research and Public Health 18(8):4269. doi:10.3390/ijerph18084269.

Maboreke, H.R., Graf, M., Grams, T.E.E., Herrmann, S., Scheu, S., Ruess, L. 2017. Multitrophic interactions in the rhizosphere of a temperate forest tree affect plant carbon flow into the belowground food web. Soil Biology & Biochemistry 115:526-536. doi:10.1016/j.soilbio.2017.09.002.

Mahapatra, M., Nayak, D.K. 2019. Biochemical and physiochemical changes in susceptible and resistant bitter gourd cultivars/varieties as influenced by root knot nematode, *Meloidogyne incognita*. Journal of Entomology and Zoology Studies 7:80-87.

Malavolta, E., Vitti, G.C., Oliveira, S.A. 1997. Avaliação do estado nutricional das plantas: princípios e aplicações. 2nd ed. Potafós, Piracicaba, São Paulo, Brasil.

Manzanilla-López, R.H., Esteves, I., Devonshire, J. 2017. Biology and management of *Pochonia Chlamydosporia* and plant-parasitic nematodes. p. 47-76. In Manzanilla-López, R., Lopez-Llorca, L. (eds.) Perspectives in sustainable nematode management through *Pochonia chlamydosporia* applications for root and rhizosphere health. Sustainability in Plant and Crop Protection. Springer, Cham, Switzerland. doi:10.1007/978-3-319-59224-4_3.

Medeiros, T.S., Gomes, A.R.M.G., Alves, M.P.B., Marcelino, A.S., Santos, D.M., Giongo, A.M.M., et al. 2019. Production of radish (*Raphanus sativus* L.) cultivated under bovine manure levels and soil basal respiration. Brazilian Applied Science Review 3:1348-1357. doi:10.34115/basr.v3i2.1424.

Medeiros, H.A., Resende, R.S., Ferreira, F.C., Freitas, L.G., Rodrigues, F.A. 2015. Induction of resistance in tomato against *Meloidogyne javanica* by *Pochonia chlamydosporia*. Nematoda 2:e10015. doi:10.4322/nematode.10015.

Meneguzzi, A., Navroski, M.C., Lovatel, Q.C., Marco, F.T., Pereira, M.O., Tonett, E.L. 2015. Ácido indolacético influencia no enraizamento de estacas de *Pittosporum tobira*. Revista de Ciências Agroveterinárias 14:24-28.

Messa, V., Nunes, J., Matte, D. 2019. Seed treatment with *Bacillus amyloliquefaciens* for the control of *Meloidogyne javanica* "*in vivo*" bean culture and its direct effect on the motility, mortality and hatching of *M. javanica* "*in vitro*". Agronomy Science and Biotechnology 5:59-69. doi:10.33158/ASB.2019v5i2p59.

Monteiro, T.S.A., Lopes, E.A., Evans, H.C., Freitas, L.G. 2017. Interactions between *Pochonia chlamydosporia* and nematodes. p. 77-96. In Manzanilla-López, R., Lopez-Llorca, L. (eds.) Perspectives in sustainable nematode management through *Pochonia chlamydosporia* applications for root and rhizosphere health. Sustainability in Plant and Crop Protection. Springer, Cham, Switzerland. doi:10.1007/978-3-319-59224-4_4.

Oliveira, P., Nascente, A.S., Ferreira, E.P.B., Kluthcouski, J., Lobo Junior, M. 2016. Response of soil fungi and biological processes to crop residues in no-tillage system. Pesquisa Agropecuária Tropical 46:57-64. doi:10.1590/1983-40632016v4638374.

Oostenbrink, M. 1966. Major characteristics of the relation between nematodes and plants. Mededelingen Landbouw 66:1-46.

Palomares-Rius, J.E., Escobar, C., Cabrera, J., Vovlas, A., Castillo, P. 2017. Anatomical alterations in plant tissues induced by plant-parasitic nematodes. Frontiers in Plant Science 8:1987. doi:10.3389/fpls.2017.01987.

Rinaldi, L.K., Calandrelli, A., Miamoto, A., Silva, M.T.R., Dias-Arieira, C.R. 2023. Application of *Ascophyllum nodosum* extract and its nutrient components for the management of *Meloidogyne javanica* in soybean. Chilean Journal of Agricultural Research 83:127-136. doi:10.4067/s0718-58392023000200127.

Siddique, S., Grundler, F.M. 2018. Parasitic nematodes manipulate plant development to establish feeding sites. Current Opinion in Microbiology 46:102-108. doi:10.1016/j.mib.2018.09.004.

Silva, E.E., Azevedo, P.H.S., De-Polli, H. 2007. Determinação da respiração basal (RBS) e quociente metabólico do solo (qCO_2). Embrapa Agrobiologia. Comunicado Técnico 99. 4 p.

Silva, S.D., Carneiro, R.M., Faria, M., Souza, D.A., Monnerat, R.G., Lopes, R.B. 2017. Evaluation of *Pochonia chlamydosporia* and *Purpureocillium lilacinum* for suppression of *Meloidogyne enterolobii* on tomato and banana. Journal of Nematology 49:77-85. doi:10.21307/jofnem-2017-047.

Vilela, R.M.I.F., Kuster, V.C., Magalhães, T.A., Moraes, C.A., Paula Filho, A.C., Oliveira, D.C. 2021. Impact of *Meloidogyne incognita* (nematode) infection on root tissues and cell wall composition of okra (*Abelmoschus esculentus* L. Moench, Malvaceae). Protoplasma 258:979-990. doi:10.1007/s00709-021-01618-0.

Witt, C., Gaunt, J.L., Galicia, C.C., Ottow, J.C.G., Neue, H.U. 2000. A rapid chloroform-fumigation extraction method for measuring soil microbial biomass carbon and nitrogen in flooded rice soils. Biology and Fertility of Soils 30:510-519. doi:10.1007/s003740050030.

Yi, X., Guo, Y., Khan, R.A.A., Fan, Z. 2021. Understanding the pathogenicity of *Pochonia chlamydosporia* to root knot nematode through omics approaches and action mechanism. Biological Control 162:104726. doi:10.1016/j.biocontrol.2021.104726.

Zavala-Gonzalez, E.A., Escudero, N., Lopez-Moya, F., Aranda-Martinez, A., Exposito, A., Ricaño-Rodríguez, J. 2015. Some isolates of the nematophagous fungus *Pochonia chlamydosporia* promote root growth and reduce flowering time in tomato. Annual Applied Biology 166:472-483. doi:10.1111/aab.12199.

Zavala-Gonzalez, E.A., Rodríguez-Cazorla, E., Escudero, N., Aranda-Martinez, A., Martínez-Laborda, A., Ramírez-Lepe, M. 2017. *Arabidopsis thaliana* root colonization by the nematophagous fungus *Pochonia chlamydosporia* is modulated by jasmonate signaling and leads to accelerated flowering and improved yield. New Phytologist 213:351-364. doi:10.1111/nph.14106.