



Management of root-knot nematode, *Meloidogyne incognita* infesting black pepper (*Piper nigrum*) under field conditions

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ABSTRACT

The present experiment were conducted during 2020, 2021 and 2022 at the ICAR-Central Plantation Crops Research Institute, Kasaragod, Kerala for the management of the root-knot nematode, *Meloidogyne incognita*, infesting black pepper (*Piper nigrum* L.) cv. Panniyur-1 using a talc-based formulation of *Trichoderma harzianum* [ICAR-Indian Institute of Spice Research (ICAR-IISR) and ICAR-Central Plantation Crop Research Institute (ICAR-CPCRI) isolates] and *Pochonia chlamydosporia* (ICAR-IISR isolate), neem cake, and carbosulfan 25 EC (standard check). The experiment was laid out in a randomised block design (RBD). The results indicated that *P. chlamydosporia* (84 and 72%, respectively) and carbosulfan (91 and 79%, respectively) both exhibited the highest decreases in *M. incognita* populations in the soil and root, respectively compared to the control. Maximum yield was recorded in carbosulfan (25.86 kg) and *P. chlamydosporia* (24.32 kg) treated plants in comparison to the control (9.07 kg). *P. chlamydosporia* (ICAR-IISR isolate) was found promising against *M. incognita* to reduce the population buildup in the fields, thus, indicating that there exists a scope for management of nematode diseases for organic production of black pepper and better yield to sustain the additional income of the farmer.

Keywords: Black pepper, Carbosulfan, Nematode management, *Pochonia chlamydosporia*

Black pepper (*Piper nigrum* L.) is a perennial herb belonging to the family Piperaceae. It is one of the oldest and most popular spices in the world and is also extensively used in medicine. India is one of the major producers, consumers, and exporters of black pepper in the world. The crop is grown on about 278,050 ha with a production of 64,000 t during 2022–23 (Spice Board 2023). Black pepper production in India is sustained by losses due to several reasons. Among them, one of the major constraints is plant nematodes (Ramana and Eapen 2000, Pervez and Eapen 2015; 2016, Pervez *et al.* 2016, 2017), of which the root-knot nematode, *Meloidogyne incognita*, is the most serious which causes significant yield losses (Leela *et al.* 2012).

Plant parasitic nematodes (PPN) are a major problem for both qualitative and quantitative production of crops worldwide, affecting US \$125 billion/annum (Chitwood 2003). An estimated 21.3 per cent, or \$1.58 billion is the annual agricultural production losses due to PPN in the 19 most common horticultural crops (Kumar *et al.* 2020). The root-knot nematode, which produces galls or knots and

significant yield losses in plants, poses a serious threat. The global damages caused by *M. incognita* are projected to be \$78 billion (Gharabadiyan *et al.* 2012).

Nematicides are commonly used to control nematodes around the world, viz. carbofuran and phorate being widely advocated (Bhai *et al.* 2017, Pervez 2017, Pervez and Eapen 2021). In view of the ban on these nematicides in India for environmental concerns, a workable substitute method as part of integrated nematode management (INM) is required nowadays due to worrisome health concerns and issues with recurring application requirements.

Biocontrol agents are crucial for managing plant parasitic nematodes by offering an eco-friendly, sustainable alternative to harmful chemicals, reducing environmental pollution, protecting human health, and enhancing soil fertility and plant resistance through natural mechanisms like parasitism, antibiosis, and induced systemic resistance, leading to better crop yields and healthier soil ecosystems. They make agriculture safer and more resilient by either strengthening plant defences or directly combating nematodes. The fungal antagonists are soil borne organisms that suppress by parasitising eggs/juveniles, competing for nutrients, producing toxins, or inducing plant resistance (Rao 2008). *T. harzianum* is an effective biocontrol agent against root-knot and other nematodes (Sharon *et al.* 2001). Other bacterial antagonists such as *Pseudomonas* spp., *Bacillus*

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spp., *Serratia* spp. and *Enterobacter* spp. have been reported to contribute as bio-control agents to integrated nematode management, bacterial diseases and fungal diseases (Munif *et al.* 2000, Sarr *et al.* 2010, Vetrivelkai *et al.* 2010, Yang *et al.* 2011), which colonise roots and exclude nematode niche. Similarly, another effective biological organism is *Pasteuria penetrans* which parasitises eggs of root-knot nematodes. Bio-organic approaches using antagonistic microbes and organic amendments are promising alternatives to chemical nematicides (Khan *et al.* 2022). Thus, the current study was carried out to bridge this gap using a talc-based formulation of *T. harzianum* (ICAR-IISR and ICAR-CPCRI isolates) and *P. chlamydosporia* (ICAR-IISR isolate), neem cake, and carbosulfan 25 EC.

MATERIALS AND METHODS

Experimental site: The present experiment was conducted during 2020, 2021 and 2022 at Experimental Farm, ICAR-Central Plantation Crops Research Institute, Kasaragod (12.528° N, 74.969° E; at an elevation of 70.7 m amsl), Kerala. The experimental site was adversely affected by a typical warm, humid tropical climate and experienced both the north-east and south-west monsoons. During the study, the experimental location received an average of 2639 mm of rainfall. The experimental site's mean relative humidity varied from 71–87% throughout the study period, while its minimum and maximum temperatures ranged from 22–31°C. Black pepper can be cultivated in the aforementioned conditions (Thomas and Rajeev 2015).

Source of bioagents: The *P. chlamydosporia* (ICAR-IISR isolate) and *T. harzianum* (ICAR-IISR isolate) based talc formulations were procured from ICAR-Indian Institute of Spices Research, Kozhikode while ICAR-Central Plantation Crop Research Institute, Kasaragod, Kerala, provided the *T. harzianum* (ICAR-CPCRI isolate) based talc formulation.

Identification of nematode species: Nematode species identification was done by following nematode extraction using Cobb's sieving and decanting method and modified Baermann's funnel technique (Southey 1985). In order for the nematodes to settle at the bottom of the Petri plate, the collected nematodes were not disturbed. A micropipette was then used to carefully remove any remaining water. The collected nematodes in the Petri plate were then given a slightly boiled fixative (Triethanolamine formalin). For optimal fixing, the Petri plate was kept undisturbed at lab temperature; this procedure could take up to 48 h. Following the fixing process, the samples were dehydrated. To absorb the moisture produced by the fixed samples, the dehydrating agent was added, and the samples were kept in desiccators with anhydrous calcium chloride for the dehydration (Seinhorst 1959). Perineal pattern and a taxonomic key were used for identification up to the species level (Jepson 1987, Siddiqi 2000). The striae in the posterior perineal pattern are tightly spaced and zigzag to wavy, particularly laterally and dorsally, squarish, high dorsal arch. Undefined lateral field, characterised by strial

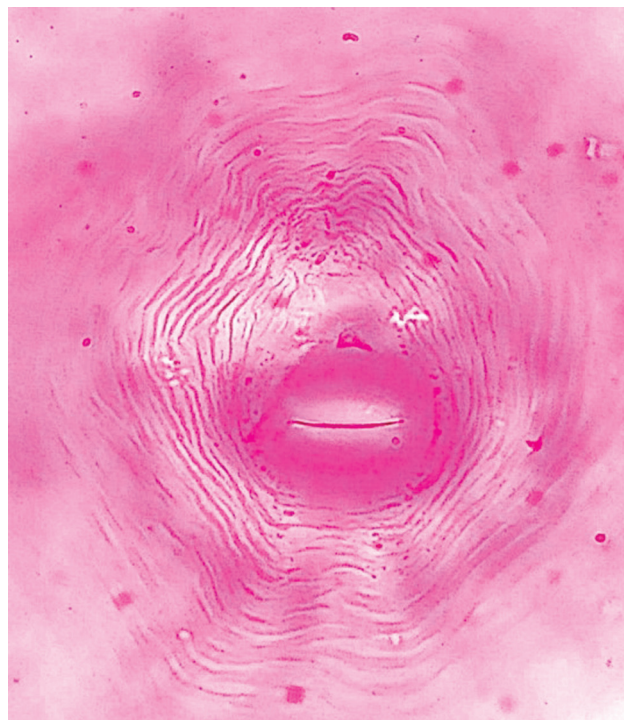


Fig. 1 Cuticular markings in the perineal area of the mature female of root-knot nematode, *Meloidogyne incognita*.

breaks, frequently forked broken ends, and patterns blending into body striae. Striae indicate the end of the tail (Fig. 1). The root-knot nematode, *M. incognita* Chitwood has been identified based on the diagnostic tools.

Management of *M. incognita* under field conditions: The experiment was conducted in randomised block design (RBD) with six replications for each treatment. Randomisation was carried out to minimise variability due to micro-environmental differences across the field. The treatments included T₁, *P. chlamydosporia* (ICAR-IISR isolate; 10⁶ cfu/g) based-talc formulation @50 g/vine [mixed with 1 kg Farm Yard Manure (FYM)] at pre-monsoon (May) and post-monsoon (October); T₂, *T. harzianum* (ICAR-IISR isolate; 10⁸ cfu/g) based-talc formulation @50 g/vine (mixed with 1 kg FYM) at pre-monsoon and post-monsoon; T₃, *T. harzianum* (ICAR-CPCRI isolate; 10⁸ cfu/g) based-talc formulation @50 g/vine (mixed with 1 kg FYM) at pre-monsoon and post-monsoon; T₄, Neem cake @1 kg/vine at pre-monsoon and post-monsoon; T₅, Carbosulfan 25 EC (0.1%) @5 L/vine at pre-monsoon and post-monsoon; and T₆, Control.

Root-knot index (RKI): To determine the root-knot index, which includes the number of galls/g roots, egg masses/gall, and the number of female/egg mass or galls, ten rhizosphere soil and root samples were randomly collected using nematode sampling spades from each treatment at the time of crop harvest. The number of root galls were recorded and rating was given based on a root gall index scale from 0–5 (0, no galls; 1, 1–2 galls; 2, 3–10 galls; 3, 11–30 galls; 4, 31–100 galls; and 5, more than 100 galls), as outlined by Taylor and Sasser (1978) and Eisenback (1985).

Analysis of *M. incognita* population in the soil and roots: The protocol provided by Barker *et al.* (1985) was followed while processing soil samples for nematode extraction. Using a stereoscopic zoom microscope and a Syracuse counting dish, the nematode population in each sample was counted five times, and the mean value had been determined. After the roots were cut, they were placed on tissue paper over a wire mesh screen in a Petri dish with water (Hopper *et al.* 2005). After 48 h, the suspension was collected, and the number of *M. incognita* (J_2) was counted using a counting dish. The average of the five counts was computed to calculate the number of nematodes per gram of root.

Statistical analysis: Data were analysed using statistical methods, including analyses of variance (ANOVA). Duncan's Multiple Range Test (DMRT) was also calculated. There was a significant difference between the means at $p < 0.05$ by using the SAS statistical tool.

RESULTS AND DISCUSSION

As compared to the untreated control, the study's findings showed that the soil application of fungal bioagents, *P. chlamydosporia* (ICAR-IISR isolate), *T. harzianum* (ICAR-IISR and ICAR-CPCRI isolates), carbosulfan, and neem cake significantly suppressed the *M. incognita* population. However, the rate of suppression varied from treatment to treatment.

Root-knot index (RKI): The *P. chlamydosporia* (ICAR-IISR) talc-based formulation was the most promising treatment among those that were tested. Results showed that plants treated with *P. chlamydosporia* (ICAR-IISR isolate) had the lowest gall and egg masses (1.86 gall/g root and 1.05 egg masses/gall, respectively), which was on par with plants treated with carbosulfan (2.59 gall/g root and 1.08 egg masses/gall, respectively). However, compared to other treatments, neem cake was less effective in reducing gall formation, evidenced by the large number of galls (5.52 gall/g root and 2.93 egg masses/gall, respectively) (Table 1).

M. incognita are extremely difficult to eradicate

Table 1 Mean of pool data of root gall and nematode population

Treatments	No. of gall/g root	No. of egg masses/gall	No. of females/gall
T ₁	1.86 ^d	1.05 ^d	1.41 ^d
T ₂	3.01 ^{cd}	1.48 ^{cd}	2.36 ^c
T ₃	3.31 ^{cd}	1.66 ^{cd}	2.23 ^{cd}
T ₄	5.52 ^c	2.93 ^c	3.53 ^b
T ₅	2.59 ^d	1.08 ^d	1.89 ^{bc}
T ₆	16.47 ^a	5.62 ^a	5.7 ^a
SEM±	0.23	0.61	0.52
CD ($p=0.05$)	0.76	1.13	0.42

Treatments details are given under Materials and Methods. Different letters indicate significant differences among the treatments.

entirely from the soil because of their polyphagous nature (Rajkumar *et al.* 2019). To manage this nematode, however, a number of management strategies are employed. Among these, managing PPN effectively might be achieved by substituting fungal bioagents for hazardous pesticides. Fungal bioagents also have the advantage of encouraging plant growth. Several studies have shown that the egg masses produced by *M. incognita* were greatly reduced when fungal bioagents were drenched in soil (Prasad *et al.* 2021, Pervez *et al.* 2024).

***M. incognita* population in the soil and roots:** The trials' findings demonstrated that every treatment exhibited varying degrees of mortality to the *M. incognita*. The results showed that of the treatments, the most *M. incognita* population reduction (85 and 72% in soil and roots, respectively) was achieved when *P. chlamydosporia* (ICAR-IISR isolate) was applied to the plant. This reduction was comparable to that of plants treated with carbosulfan (91 and 80%, respectively in soil and roots), while the least nematode reduction (49 and 37%, respectively) both in soil and roots was observed when neem cake was applied (Fig. 2).

P. chlamydosporia invade plant roots, promoting plant growth and causing systemic resistance. Moreover, *P. chlamydosporia* produces chlamydoconidia, which help the fungus to establish itself in the soil under stressful conditions and maintain fungal vitality over an extended period of storage. Known to have antagonistic capabilities against *M. incognita*, this fungus is the most commonly employed biocontrol agent (Monteiro *et al.* 2020, Yi *et al.* 2021). The antagonistic effect caused by *P. chlamydosporia* on *Meloidogyne* spp. (J_2) and egg masses might be the reason behind the decline in galls and egg masses. The chitinases and deacetylases have a significant effect on the fungus's pathogenicity. The synthesis of the chitinase enzyme by *Trichoderma* spp. might have contributed to the decline in the nematode population in *T. harzianum*. Nematode eggs may have hatched too soon as a result of this. The chitin layer of the eggshell may be affected by these enzymes, which could break it down and promote fungal penetration (Aranda-Martinez *et al.* 2016). *Trichoderma*, a bioagent, was also used to promote the growth of plants (Medeiros *et al.* 2017, El-Nagdi *et al.* 2019).

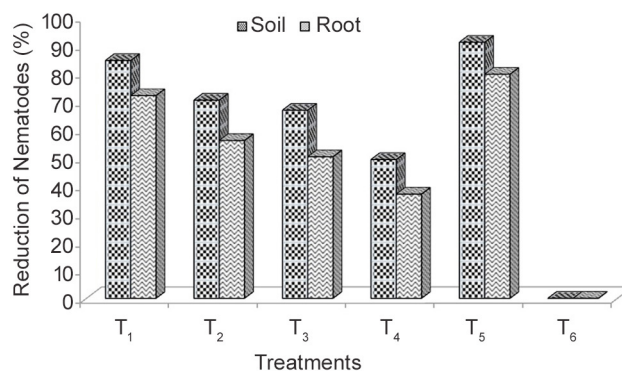


Fig. 2 Reduction of *M. incognita* population in different treatments over the control.

Treatments details are given under Materials and Methods.

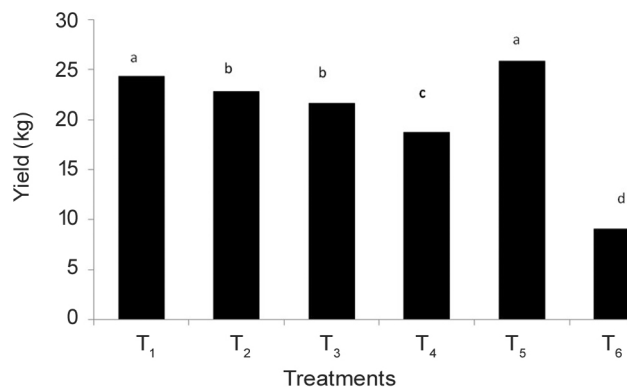


Fig. 3 Mean of pool data of black pepper yield. Treatments details are given under Materials and Methods.

Yield: Results revealed that carbosulfan and *P. chlamydosporia* (ICAR-IISR) treated plants exhibited higher yields (25.86 and 24.32 kg, respectively), whereas lesser yields were recorded in the neem cake treated plant (17.70 kg) as compared to the control (9.07 kg) (Fig. 3). The current investigation revealed *P. chlamydosporia* to be a highly effective biocontrol agent and well antagonistic against *M. incognita*. Our finding was supported by previous studies (Larriba *et al.* 2015, Zavala-Gonzalez *et al.* 2017, Pentimone *et al.* 2018) that used talc-based formulations which contained fungus against *M. incognita* infesting various crops. These studies also found that *P. chlamydosporia* was effective in reducing egg masses and root-knot nematode (RKN) populations in soil.

In the present study, *P. chlamydosporia* was found to be a potent biocontrol agent and a plant growth booster. Additionally, prior research has demonstrated that *P. chlamydosporia* increases plant growth and decreases egg masses and RKN populations in soil infecting a variety of crops (Pentimone *et al.* 2018, Ghahremani *et al.* 2019). The application of *T. viride*, *Pseudomonas fluorescens*, and *Purpureocillium lilacinum* improved plant growth in cucumbers and gave them resistance to *M. incognita* and *Fusarium oxysporum* f. spp. *cucumerinum*, but the best results were obtained when carbofuran was used in combination with liquid bioagent formulation at higher doses (Patil *et al.* 2021).

The use of *T. harzianum*, both alone and in combination, was found to lower the incidence of root-knot disease, which supports the findings reported previously (Singh 2013, Prasad *et al.* 2021). Plants infected with *M. incognita* and other treatments showed lower fresh and dry weights of shoots than the *T. harzianum* treated plant. Additionally, this treatment resulted in the highest rate of disease protection in terms of the nematode's reproductive factor ($Pf/PPi < 0.5$) and the number of galls reduced (81%). *P. chlamydosporia* has good biocontrol capability against *Meloidogyne* spp. infesting tomatoes (Bordallo *et al.* 2002), barley (Macia-Vicente *et al.* 2009), potatoes (Manzanilla-Lopez *et al.* 2011), arabidopsis (Zavala-Gonzalez *et al.* 2017), and turmeric (Pervez *et al.* 2024) via parasitising RKN eggs (Uddin *et al.* 2019).

It was found that the management of plant parasitic nematodes with a lot of pesticides is usually advised. Finding suitable replacement strategies to manage PPN is therefore required, especially since most of the recommended nematicides are right now banned. Because of this, the current study proposed that *P. chlamydosporia* (ICAR-IISR isolate), which has a high antagonistic potential, can be employed to manage *M. incognita* populations in black pepper. A more thorough investigation of this intriguing fungal biocontrol agent can be considered in order to manage *M. incognita* in the field.

It is concluded that talc formulations of *P. chlamydosporia* (ICAR-IISR isolate) were found promising against *M. incognita*. Improvement in the overall health of vines and increased yield were observed in addition to the reduction in the nematode population in soil. If implemented correctly, this commodity, which is easily accessible domestically, would be significantly more advantageous for farmers' bottom lines, particularly for small-scale farms that are unable to purchase pricey nematicides.

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