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Management of root-knot nematode *Meloidogyne incognita* infesting turmeric (*Curcuma longa*) under coconut (*Cocos nucifera*) cropping system

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ABSTRACT

Field experiments were conducted during 2018–19, 2019–20 and 2020–21 at ICAR-Central Plantation Crops Research Institute, Kasaragod, Kerala for assessment of biocontrol fungi for the management of root-knot nematode (RKN), *Meloidogyne incognita* damaging turmeric (*Cucuma longa* L.) (cv. IISR-Prathibha) under coconut (*Cocos nucifera* L.) cropping system. Treatments included talc-based formulations of *Trichoderma harzianum* (ICAR-Indian Institute of Spices Research (IISR), Kerala and ICAR-Central Plantation Crops Research Institute (CPCRI), Kerala isolates) and *Pochonia chlamydosporia* (ICAR-Indian Institute of Spices Research, Kerala isolate), neem cake, marigold and carbosulfan 25 EC (standard check). Talc-based formulations were applied at 50 g/bed, neem cake @1 kg/bed at pre-monsoon and post-monsoon, marigold @30 plants/bed compared with untreated and standard check carbosulfan 25 EC @5 litre/bed at pre-monsoon and post-monsoon against *M. incognita.* Maximum reduction of RKN in the soil, as well as root, was achieved by either *P. chlamydosporia* (74 and 86%, respectively) or carbosulfan (67 and 87%, respectively) over the control. A number of the tillers (3.75/plant) for *P. chlamydosporia* and (3.23/plant) for carbosulfan were recorded more in comparison to control (1.42/plant). The findings demonstrated that *P. chlamydosporia* (ICAR-Indian Institute of Spices Research) is a promising alternative to synthetic nematicides for the management of *M. incognita* due to its high antagonistic and good plant growth promotion activities. However, large scale field trials on these promising fungal biocontrol agents can be considered for evaluation in relation to the management of RKN under field conditions for confirmation.

Keywords: Management, *Pochonia chlamydosporia,* Root-knot nematode, Turmeric

Nematodes are the most widespread lower invertebrate on the globe, constituting up approximately four-fifths of the world's terrestrial. They constitute an important component of the soil ecosystem (Bardgett and van der Putten 2014). Plant nematodes are a major problem in qualitative as well as quantitative crop production worldwide, causing about the US $125 billion loss per year globally (Chitwood 2003). The estimated yearly crop production loss in India as a result of plant nematodes is 21.3%, or 102039.19 million or $1.58 billion, while losses in the 19 most popular horticulture crops are 23.03%, or $50.2 billion (Kumar *et al*. 2020). Global damages due to *Meloidogyne incognita*, the root-knot nematode was reported to be to $78 billion (Gharabadiyan *et al*. 2012).

Turmeric (*Cucuma longa* L.), also known as the golden spice is one of the most vital spices used in cuisine on a global scale. It is a versatile crop valued for its spicy flavour, colouring pigment, and therapeutic benefits. It is commonly grown in coconut gardens as intercrop in southern India to effectively use available space (coconut spacing 7.5 m × 7.5 m) and enhance the farmer's income in a short period. Recently, turmeric quality has declined due to several reasons like insect pests, disease, and plant nematodes. Several nematodes associated with turmeric crops have been reported and among them, root-knot nematode (RKN) *M. incognita* is the major species, causing economic losses (Pervez 2018, Pervez *et al*. 2023).

Around the world, nematodes are commonly controlled with nematicides but with the recurring application requirements, worrisome health concerns and issues have arisen (Neeraj *et al*. 2019). Thus, a feasible alternative technique as part of integrated nematode management (INM) is required, more importantly, for the coconut-turmeric cropping system. Therefore, to fill up this gap the present study was undertaken.

MATERIALS AND METHODS

The present experiments were conducted during 2018–19, 2019–20 and 2020–21 at ICAR-Central Plantation

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Crops Research Institute, Kasaragod (12”30” N latitude and 75°00’ E longitude with an altitude of 10.7 m amsl), Kerala to evolve an IPM strategy for the management of root-knot nematode, *M. incognita* infesting turmeric under coconut cropping system. The coconut garden had an inter row space of 7.5 m × 7.5 m in which turmeric (cv. IISR- Prathibha) was planted. Healthy turmeric rhizomes were planted on the bed [3 m (width) × 1 m (length) × 30 cm (height)] and agronomic practices as per the available management recommendations were followed (Thomas and Rajeev 2015).

Source of bioagents: Talc-based formulations of *Pochonia chlamydosporia* and *Trichoderma harzianum* were obtained from ICAR-Indian Institute of Spices Research, Kerala, while talc-based formulation of another isolate of *Trichoderma harzianum* was sourced from ICAR-Central Plantation Crop Research Institute, Kerala.

Climatic conditions of the experimental site: A typical warm humid tropical climate, with both south-west and north-east monsoons prevailed in the site. The average rainfall received at the site during the three years of crop growth period was 2639 mm. The range of minimum and maximum temperatures was 22 to 31°C and the mean relative humidity range was 71–87%. Above mentioned climatic conditions are ideal for turmeric farming (Thomas and Rajeev 2015).

Identification of nematode species: Nematodes were collected from field trial and processed 100 cc of soil was crushed in water by hand and coarse-sieved on a sieve of about 4 mm aperture to remove stones and coarse gravel (Cobb’s method). The residual debris collected in glass beaker was poured on to a small coarse sieve which was previously lined by a double layer of tissue paper. Then again sieved by fine sieve and the remaining debris were collected from fine sieve into a flask. Then the modified Baermann’s funnel technique was followed, where in a funnel is used with a piece of rubber tubing attached to the stem and closed by a spring or screw clip. The funnel was placed in a support with tripod stand and tap water was added till touched the bottom surface of sieve. The setup was left for 24–48 h. After that 4–5 drops of water were run off from funnel into the cavity block and observed under stereo microscope.

The collected nematodes were left undisturbed such that nematodes will settle down at the bottom of the cavity block. Then excess of the water was carefully removed by using micropipette. Now fixative (Trientholamine formaline) was mildly boiled and added to the collected nematodes in the cavity block. The cavity blocks were left undisturbed at laboratory temperature for proper fixation and the process may take 48 h to complete.

After completion of fixation, the samples were subjected to dehydration. The dehydrating agent was added to the fixed samples and stored in desiccators containing anhydrous calcium chloride to absorb the moisture generated from the fixed samples. The dehydration process may take 2–3 weeks.

A glass slide was rinsed by tissue paper and a drop of glycerin was placed in the center of the slide. Then picked and placed a nematode from cavity block on to the slide bearing a drop of glycerin. Then the drop of glycerin containing nematode specimen was covered with the small cubes of wax (3 cubes) and a circular glass cover slip was carefully placed on the wax cubes. The slide was then kept on the hot plate till wax melted and sealed the specimen. The wax set rapidly when the slide was taken out of the warm plate. Permanent slide mounts were done for observing morphological features of nematodes under compound microscope. The slide was cooled and placed in slide box for identification. Identification up to species level was done using a taxonomic key described by Siddiqi (2000) and also perineal pattern (Jepson 1987).

Management of nematodes under field conditions: The treatments, T_1, *P. chlamydosporia* (ICAR-IISR isolate) @50 g/bed; T_2, *T. harzianum* (ICAR-IISR isolate) @50 g/bed; T_3, *T. harzianum* (ICAR-CPCRI isolate) @50 g/bed; T_4, Neem cake @1 kg/bed; T_5, Marigold (cv Pusanarangi) @30 plants/bed; T_6, Carbosulfan 25 EC (0.1%) @5 litre/bed; T_7, Control were imposed during pre-monsoon (month of June) and post-monsoon (month of October) periods during 2018–19, 2019–20 and 2020–2021. The Talc based formulations of the fungal isolates were mixed with 2 kg farmyard manure (FYM) before application. Marigold was planted around the bed and middle of the bed.

Root-knot index: After 210 days of planting, 10 samples each of the rhizosphere and clumps with roots were collected randomly with the help of nematode sampling spade from each treatment and the root-knot index, viz. the number of galls/g of roots, egg masses/gall, and the number of females/egg mass gall were recorded and mean value was worked out.

Nematode population in the soil and roots: Soil samples were processed for nematode extraction as per the procedure given by Barker *et al.* (1985). The nematode population in each sample was counted five times using a Syracuse counting dish and a stereoscopic zoom microscope, and the mean value was computed.

Roots were chopped and placed on tissue paper over a wire gauze sieve kept on a petri plate with water. The suspension was collected after 48 h and the number of *M. incognita* (J_2) was estimated with the help of a counting dish. Counts were repeated five times and mean nematode population per g of the root was worked out.

Plant growth parameters: The crop was harvested in February in all 3 years. Growth parameters, viz. plant height (cm), number of tillers/clumps and fresh yield were recorded.

Statistical analysis: The data were subjected to analyses of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) was applied to differentiate the means at $P<0.05$ by the SAS-statistical programme.

RESULTS AND DISCUSSION

The result showed that soil application of fungal bioagents, *P. chlamydosporia* (ICAR-IISR isolate), *T. harzianum* (ICAR-IISR and ICAR-CPCRI isolates), and marigold planting as intercrop significantly suppressed

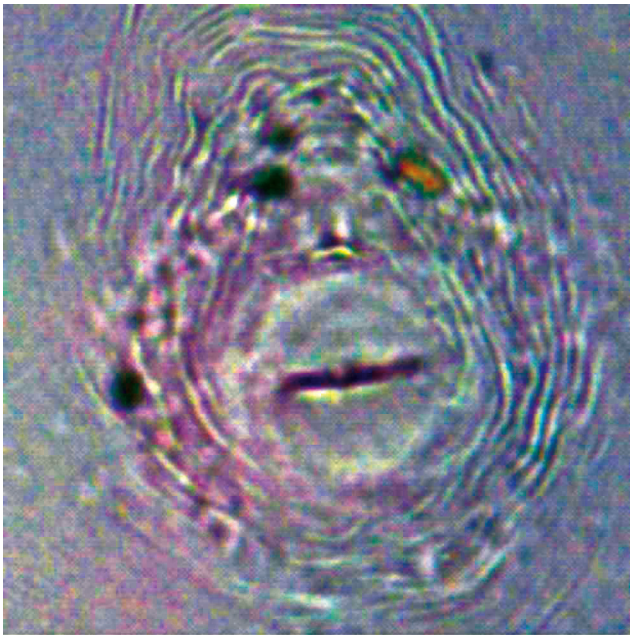

Fig. 1 Perineal pattern of root-knot nematode *Meloidogyne incognita.*

M. incognita population, increased the plant growth as well as yield compared to untreated control, but the impact varied among the treatments. In support of the aforementioned findings, Singh (2013) found that the incidence of root-knot disease was decreased by the use of *T. harzianum*, both individually and in combination. In comparison to plants infected with *M. incognita* and other treatments, the plant treated with *T. harzianum* had higher fresh and dry weight of shoots and through this treatment, the greatest disease protection was obtained in terms of the number of galls reduced (81%) and the reproductive factor (P_f/P_i<0.5) of the nematode.

Identification of the nematode: The species of root-knot nematode was identified to be *Meloidogyne incognita* through morphological characterization and perineal pattern. The perineal pattern was oval with a high dorsal arch in typical pyriform. Striae were straighter with an oval appearance in the ventral region and distinct waves towards the continuous lateral lines. Lateral fields were indistinct (Fig. 1).

Management of nematodes under field conditions

Root-knot index: Among the tested treatments, talc-based formulation of *P. chlamydosporia* and carbosulfan were found most promising with minimum number of gall and egg masses (1.36 gall/g root and 1.17 egg masses/gall, for *P. chlamydosporia* and 1.59 gall/g root and 1.18 egg masses/gall for carbosulfan respectively), whereas neem cake was less effective in controlling gall formation as large number of galls (10.77 gall/g root and 5.35 egg masses/gall, respectively) as compared to other treatments (Table 1). Due to their polyphagous nature, second stage juveniles of the RKN are exceedingly difficult to completely eliminate from the soil. Nematicides can be replaced by biological agents

Table 1 Impact of biocontrol agents on root knot nematode in turmeric (Pooled data of 3-years trials)

Treatment	Indicators of *M. incognita* infestation		
	No. of galls/g root	No. of egg masses/gall	No. of females/gall
P. chlamydosporia (ICAR-IISR isolate)	1.36^{d}	1.17^{d}	1.84^{d}
T. harzianum (ICAR-IISR isolate)	5.01^{cd}	2.48^{cd}	2.24^{c}
T. harzianum (ICAR-CPCRI isolate)	4.31^{cd}	2.66^{cd}	2.63^{cd}
Neem cake	10.77^{b}	5.35^{b}	5.17^{ab}
Marigold (cv. Pusanarangi)	6.52^{c}	3.73^{c}	3.01^{b}
Carbosulfan 25 EC	1.59^{d}	1.18^{d}	1.69^{bc}
Control	42.47 ^{a}	7.62 ^{a}	6.9 ^{a}

and organic materials, which are considered to be the best bio-organic methods (Khan *et al.* 2022). Soil application of culture supernatants of fungal bioagents significantly reduced the amount of egg masses produced by *M. incognita* (Prasad *et al*. 2021).

Nematode population in the soil and roots: All the tested treatments were found to exhibit various levels of toxicity towards juveniles of root-knot nematode, *M. incognita*. Maximum reduction of RKN in the soil as well as root was achieved by either *P. chlamydosporia* (74 and 86%, respectively) or carbosulfan (67 and 87%, respectively) over the control, whereas minimum effect was observed in plots applied with neem cake which was on par (18 and 28%, respectively) or plots planted with marigold (17 and 33%, respectively) over the control (Fig. 2). To support the above results, our observations and prior reports showed that bioagents (PGPR's), *T. harzianum* applied (3.0×10^{10} colony forming unit) at 5 ml/l of water at the time of planting in the field, helped to reduce the nematode multiplication (Singh *et al*. 2021). The talc preparation of fungal biological control agents, specifically *P. chlamydosporia* (2×10^{7} colony forming unit per g) at 12 kg/ha and *T. viride* (2.8×10^{6} colony forming unit per g) at 12 kg/ha, significantly decreased the incidence of root-knot disease in the chili crop when compared to the controls (Singh and Singh 2012). Furthermore, when compared to individual treatments and/or controls, combined applications of any two components resulted in a greater recovery in plant health. Combining *P. chlamydosporia*, and *T. viride* resulted in the greatest disease protection, as observed in the reduction of galls (58%) and reproductive factor.

Plant roots are colonized by *P. chlamydosporia*, which also cause systemic resistance and encourage plant development. In addition, *P. chlamydosporia* generates chlamydospores, which support the fungal viability during long-term storage and aid the fungus in surviving

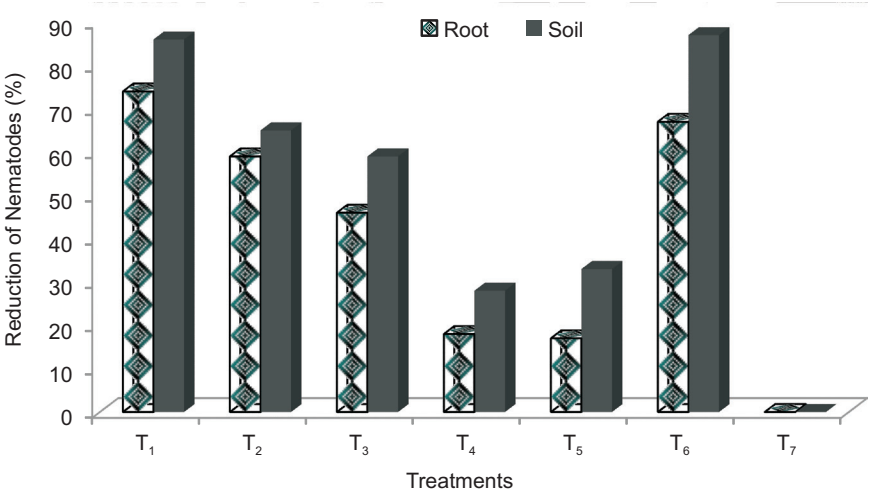

Fig. 2 Efficacy of biocontrol agents in population reduction of nematodes (Pooled data from 3-years).
Treatment details are given under Materials and Methods.

and establishing itself in the soil under challenging circumstances. The most widely used biocontrol fungus is this one, which is well-known for having antagonistic properties against *M. incognita* (Monteiro *et al.* 2020, Yi *et al.* 2021). *P. chlamydosporia* may have antagonistic potential on eggs and nematodes at all stages, which could account for the decrease in galls and egg masses. The virulence of the fungus was greatly influenced by the enzymes chitosanases and deacetylases. With regard to *T. harzianum*, the reason for the reduction in nematode population may have been due to the production of enzyme chitinase by *Trichoderma* spp. This may have caused premature hatching of nematode eggs. According to Aranda-Martinez *et al.* (2016), these enzymes may have an impact on the egg shell's chitin layer, breaking it down and encouraging fungal penetration. Plant growth promotions were also achieved with bioagent, *Trichoderma* (Medeiros *et al.* 2017, El-Nagdi *et al.* 2019).

The biocontrol potential of *P. chlamydosporia* by parasitizing the eggs of *Meloidogyne* spp. has been reported (Uddin *et al.* 2019). The fungus colonizes endophytically in the root of many plants, viz. tomato (Bordallo *et al*. 2002); barley (Macia-Vicente *et al*. 2009); potato (Manzanilla-Lopez *et al*. 2011) and *Arabidopsis* (Zavala-Gonzalez *et al*. 2017).

Plant growth parameters: The plots treated with either *P. chlamydosporia* or carbosulfan exhibited significantly higher plant growth-promoting activities such as plant height (102.69 cm), number of the tillers (3.75/ plant) for *P. chlamydosporia*, plant height (101.14 cm), number of the tillers (3.23/plant) for carbosulfan, with maximum yield (4.86 kg) in comparison with other treatments (Table 2).Thus, *P. chlamydosporia* emerged as a plant growth promoter and biocontrol agent in the current study. Earlier studies have also shown *P. chlamydosporia* to be effective in promoting plant growth and reducing RKN populations in soil or egg masses in various crops (Pentimone *et al*. 2018, Ghahremani *et al.* 2019). Among the bioagents, botanicals, and nematicides evaluated against RKN in turmeric, carbofuron was significantly better in terms of maximum plant height, fresh rhizome weight, dry rhizome weight, least number of galls, and suppression of soil nematode population (Niranjana *et al.* 2018).

In cucumber, though application of *T. viride, Pseudomonas fluorescens,* and *Purpureocillium lilacinum* afforded resistance to *M. incognita* and *Fusarium oxysporum* f. sp. *cucumerinum* and resulted in better plant growth over untreated plots, the best results among all treatments were obtained through use of carbofuran coupled with liquid formulation of bioagents at higher doses (Patil *et al.* 2021).

Chemical-intensive management is frequently recommended for plant nematodes but the majority of the suggested nematicides are now prohibited. In this study *P. chlamydosporia* (ICAR-IISR isolate) is proven as a potential alternative to synthetic nematicides for the management of *M. incognita* in turmeric due to its antagonism against the nematode and effective plant growth promoting activities. Large scale field studies may be considered for this promising fungal biocontrol agent for further confirmation.

Table 2 Effect of biocontrol agents of plant growth and yield (Pooled data of 3-years trials)

Treatment	Plant height (cm)	Number of tillers/ plant	Yield (kg)
P. chlamydosporia (ICAR-IISR isolate)	102.69 ^{a}	3.75 ^{a}	4.32 ^{a}
T. harzianum (ICAR-IISR isolate)	89.22^{bc}	2.61^{bc}	3.72 ^{b}
T. harzianum (ICAR-CPCRI isolate)	93.97 ^{b}	2.77 ^{b}	3.71 ^{b}
Neem cake	78.23^{c}	1.76^{c}	2.96^{c}
Marigold (cv Pusanarangi)	77.62^{c}	1.42^{d}	2.58^{cd}
Carbosulfan 25 EC	101.14 ^{a}	3.23 ^{a}	4.86 ^{a}
Control	65.36^{d}	1.42^{d}	1.86^{d}

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