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RESEARCH ARTICLE

Biological suppression of *Meloidogyne incognita* in *Coleus forskohlii* using *Pasteuria penetrans* oil dispersion formulation

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Abstract

Medicinal coleus (*Coleus forskohlii*) is an important herbal plant in Indian medicine. Root-knot nematode, *Meloidogyne incognita*, is the predominant pest of medicinal coleus and causes a yield reduction of up to 72%. This nematode affects the medicinal properties of tubers by producing root-knot galls. Chemical nematicides are unsuitable because the molecule forskolin, extracted from the tubers, could be contaminated. Hence, biological control is the preferred solution to prevent issues associated with pesticide residues. *Pasteuria penetrans* is a potential antagonistic bacterium of *M. incognita*. In this study, an oil dispersion formulation (ODF) of *P. penetrans* was developed and its effectiveness against *M. incognita* was tested. The formulation was water soluble and the spores were viable even after 60 days of incubation, as confirmed through juvenile parasitisation. Various concentrations of this formulation were prepared and screened against *M. incognita* infection in *C. forskohlii* under glasshouse conditions. Soil drenching with the formulation at a rate of 400 µL in 1000 mL of water resulted in an 86% reduction in egg mass production. *P. penetrans* endospores were recovered from treated plants. Based on these results, field trials were conducted over two seasons. The findings demonstrated that the application of ODF at 4 L/ha in 200 L of water significantly reduced nematode infection, with 74.9%-84.8% and 61%-66.5% reductions in egg mass production and gall index, respectively. Furthermore, the treatment enhanced tuber weight, with an increase of 32.9%-55.5% compared to carbofuran-treated plants.

Keywords

biocontrol; *Coleus forskohlii*; oil dispersion formulation; *Pasteuria penetrans*; root-knot nematode

Introduction

The root-knot nematode (*Meloidogyne* sp.) is one of the major plant parasitic nematodes and the prevalent species are *Meloidogyne incognita, M. javanica, M. arenaria* and *M. hapla* (1). This nematode causes a 10-30 % yield loss in global crop production annually and results in an immense reduction in crop yield. Most affected crops include vegetable crops with a yield reduction of 30-47%, fruit crops around 32-41%, medicinal coleus 70.2% and spices 30% (2). Medicinal coleus is infected by the root-knot nematode *Meloidogyne* spp. The affected crops show slow or stunted growth, yellowing of leaves, wilting of the plant despite adequate soil water content and finally, death. Severely infected seedlings produce fewer roots and usually die rapidly. Severe infection of older

plants causes them to wilt unexpectedly and die early (3). While chemical control is effective, indiscriminate use may lead to the resurgence of sedentary endoparasitic nematodes, the development of nematicide resistance and detrimental effects on the environment (4). These factors have prompted the research to identify alternative strategies to control the root-knot nematode in a more effective and eco-friendly manner. Non-chemical approaches include crop rotation and the use of biological control agents (5). Biocontrol agents play a significant role in managing the root-knot nematode. The fungal bioagents *P. chlamydosporia* and *P. lilacinum* are egg parasitizing fungi that inhibit juvenile hatching by colonizing the egg. *Bacillus* spp. inhibit nematode mobility by secreting a few toxins while *P. penetrans* endospores attach to nematode cuticle that penetrates and colonizes the female body which in turn arrests egg production. *P. penetrans* (Thorne) is an obligate, gram-positive, endospore-forming bacterium. This organism is highly host-specific and has been reported to be effective in controlling the root-knot nematode, *Meloidogyne* spp. (6). Since 1991, several researchers have attempted to culture *P. penetrans* in axenic culture media, but most methods were ineffective. Hawlet *et al*. (7) were the first to successfully culture *P. penetrans in vitro*, which was indeed a breakthrough in this field. However, commercial formulation of *P. penetrans* is still unavailable in India and may not reach the farmers in the near future. A potential formulation of *P. penetrans* is still lacking. This article describes a method for preparing an oil dispersion formulation (ODF) and examines its effect against *M. incognita* in medicinal coleus, *Coleus forskohlii.*

Coleus forskohlii (Wild.) Briq. is one of the most important herbal plants in Indian medicine and is a native herb of the country (8). This plant belongs to the Lamiaceae family and is grown widely in India and other countries such as Arabia, Brazil, Egypt, Ethiopia, Pakistan, Sri Lanka and Tropical East Africa. *C. forskohlii* is susceptible to infection by the root-knot nematode *Meloidogyne* spp. Four different *Meloidogyne* species have been reported to infect *C. forskohlii*, *viz*., *M. javanica*, *M. incognita*, *M. hapla* and *M. arenaria*. Of these, the most predominant species, *M. incognita* causes a yield reduction of up to 86% (9). However, severe losses occur in *C. forskohlii* because of *M. arenaria* infection too (10). Senthamarai *et al*. (11) and colleagues investigated the interaction between *M. phaseolina* and *M. incognita* and found that this combination caused complete plant withering. With this background, a field study was undertaken to manage nematode infection using the ODF of the biocontrol agent *P. penetrans*.

Materials and Methods

Inoculum preparation of P. penetrans on the root-knot nematode, M. incognita

The root-knot nematode *M. incognita* was collected from an infected tomato field (Variety PKM 1) in Madhampatti village, Coimbatore (Longitude: 76°51′38.05′′E, Latitude: 10°58′24.1′′ N). The species identity was confirmed based on the posterior cuticular pattern (Fig. 1). Egg masses of *M. incognita* were collected from the galled roots and incubated at room temperature (28°C ±2°C) in tap water to facilitate hatching. Endospores of *P. penetrans* were collected from adult females maintained on tomato plants under glasshouse conditions at the Department of Nematology, Tamil Nadu Agricultural University, Coimbatore. After egg hatching, second-stage juveniles (J_2s) were incubated with these endospores at the rate of 1×10^6 spores/100 J_2s in 50 mL of water. Spore attachment was confirmed under a research microscope (Leica-020-518500) (Fig. 1). These endospore-attached J_2s were inoculated into tomato plants (Variety PKM 1) maintained in pots filled with sterile pot mixture composed of red earth, sand, farmyard manure (FYM) in a ratio of 2:1:1. The plants were maintained under glasshouse conditions for further studies.

Developing the oil dispersion formulation of P. penetrans

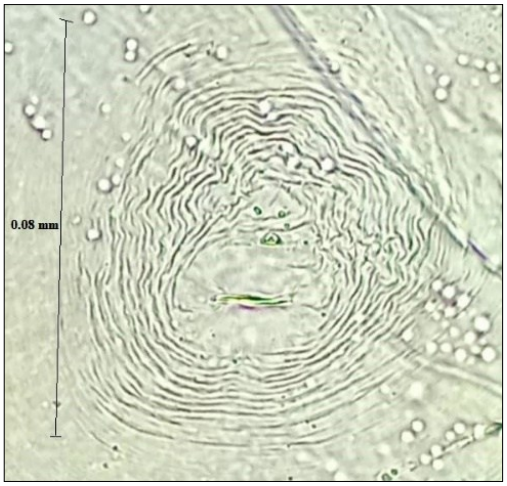
Endospores were collected in microfuge tubes containing sterile water (2 mL) from *P. penetrans*-infected females by crushing them manually. The spore load was assessed using a haemocytometer. Soy lecithin was selected based on its ability to bind both oil and water phase as it is an amphiphilic compound. Triton-X was added as a non-ionic surfactant to enhance the dispersion of spores. Sterile water (19 mL) was heated using a hot plate at 50°C. Soy lecithin (1 mL) was added to the hot water and mixed thoroughly. Sunflower oil (4 mL) and Triton-X (4 mL) were added to this mixture and heated at 50°C for 3 min (12). Subsequently, 1 mL of the endospore suspension containing 1×10^6 spores/mL was added to the mixture and stored in a glass vial at room temperature (28°C ± 2°C) (Fig. 1). Spore release from ODF was ascertained by conducting a plastic cup assay. Plastic cups were filled with red sandy loam soil (80 g) and vermicompost (20 g). The endospore-entrapped ODF was mixed in the soil at the rate of 1 mL/cup. These cups were inoculated with juveniles of the root-knot nematode *M. incognita* at the rate of 100 J_2s/cup and incubated at room temperature (28°C ±2°C). Parasitisation of J_2s was recorded 1 day after inoculation. The same observation was recorded at 30 days and 60 days after incubation to confirm the shelf life.

Effect of P. penetrans ODF on juvenile penetration

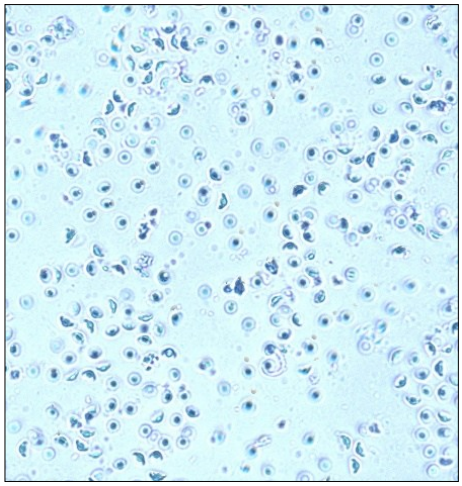
Plastic cups were filled with red sandy loam soil (100 g). The formulations were inoculated into the soil and tomato seedlings were transplanted at 7 days after inoculation (DAI). J_2s of *M. incognita* were inoculated into each cup at the rate of 100 J_2s/cup. Nematode penetration was observed by staining the roots with acid fuchsin.

Bioefficacy study of ODF against M. incognita in C. forskohlii

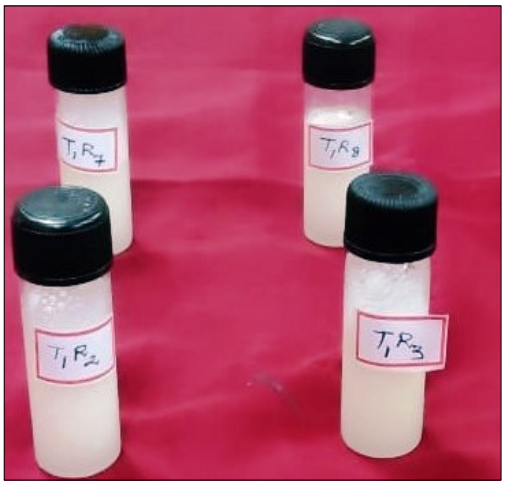
Pot culture experiment : *C. forskohlii* (Variety Co 1) cuttings were transplanted to a 5 kg sterile pot mixture (red earth: sand: FYM at the ratio of 2:1:1). Various concentrations, *viz*. 1%, 2%, 3%, 4% and 5% of ODFs containing endospores of *P. penetrans* were prepared using sterile distilled water. The formulation was applied 15 days after planting. A positive control containing carbofuran 3G was maintained, along with an untreated (negative) control for comparison. Five days after applying the formulation, juveniles of *M. incognita* were inoculated at the rate of 100 J_2s/pot. Each treatment was replicated thrice. The whole plant was uprooted and



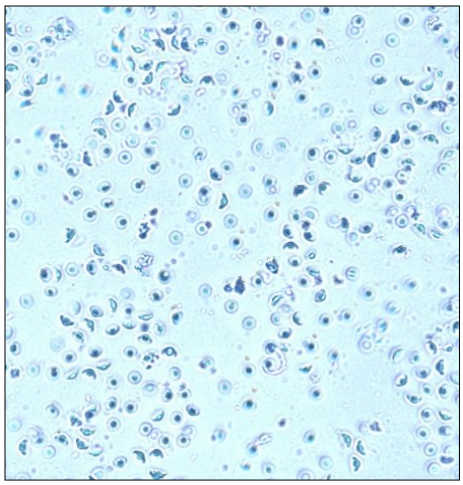
A- Posterior cuticular pattern of *Meloidogyne incognita* female nematode



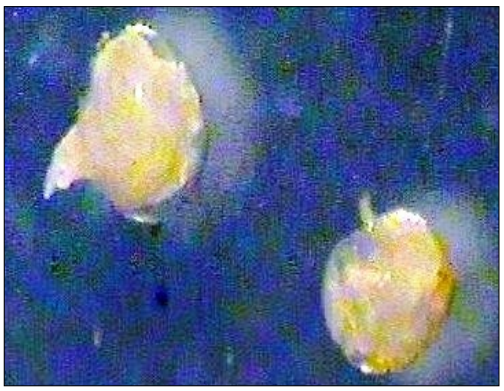
B - Endospores of *P. penetrans*



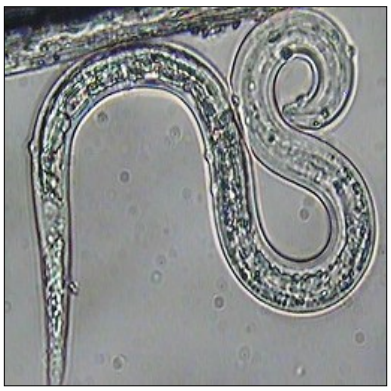
C - OD formulation of *P. penetrans*



D - Endospores trapped in the formulation



E - *P. penetrans* infested adult females



F- Attachment of endospores on J_2

Fig. 1. Evaluation of the spore release from the ODF of *P. penetrans* in soil.

observations on nematode infection and plant biometric characteristics *viz*., shoot and root length, root and shoot and weigh and number and weight of tubers were recorded 60 days after planting.

Field trial : Based on the results of the pot culture experiment, a field trial was conducted to test the efficacy of ODF under field conditions for two seasons. Three treatments were maintained, with seven replications, as follows: T1: *P. penetrans* ODF (1× 10^6endospores/mL) at the rate of 2 L/ha; T2 - carbofuran 3G at the rate of 1 kg a.i/ha (33.3 commercial grade kg/ha) was applied as a positive control; and T3: untreated control without any treatment. This trial was conducted for two seasons. Observations on nematode infection in soil and roots were recorded. The number of females filled with *P. penetrans* endospores and plant biometric observations on shoot length, root length, weight and number of tubers were also recorded.

Estimation of nematode population : The soil nematode population was estimated by processing the soil samples using Cobb's wet-sieving method (13). The soil sample was mixed with 1 L of water and passed through a series of sieves (20, 60, 100, 200 and 350 mesh). The nematodes retained on the sieves were recovered using the modified Baermann funnel method (14). For root nematode populations, root samples were stained with acid fuschin-lactophenol (15). The roots were cleaned using plain water and plunged in the stain, followed by distaining in plain lactophenol. The quantity of soil and root samples used to estimate the nematode population is specified under the respective experiments.

***Root-knot index (RKI)* :** Root samples were collected from the field. The number of galls on the roots was counted and the Gall index was graded on a 1-5 scale, as described by the standard methodology (16).

Molecular characterisation of M. incognita

***DNA isolation* :** A single female nematode was excised from the roots collected from field samples to confirm the identity of the species. The DNA was extracted using worm lysis buffer (17). A single female was transferred to a microfuge tube containing 25 µL of worm lysis buffer and crushed finely using a thin glass needle. Subsequently, this suspension was centrifuged at 13000 rpm for 2 min. The supernatant containing the DNA was collected and stored at -20°C. The sample was subjected to PCR amplification with suitable primers (NEM18s F 5′-CGCGAATRGCTCATTACAACAGC-3′ and NEM18s R5′ GGGCGGTATCTGATCG CC-3′) after DNA extraction. The PCR reaction was performed using a 20 µL mixture containing 50 ng of total DNA, 10 µL of master mix (2× concentration) and 20 pmol of forward and reverse primers. The PCR program for amplification of the 28S rDNA region was as follows: initial denaturation step at 95°C; 40 cycles comprising 30 sec of denaturation at 94°C, 45 sec of annealing at 55°C and 1 min of extension at 72°C; and 10 min of final extension at 72°C. A negative control was maintained using sterile distilled water. The amplified products were separated on 1.2% agarose containing ethidium bromide, electrophoresed at 80 V for 1 h and documented in a gel documentation unit (Alpha Imager EC (USA)). The PCR product was sequenced for species confirmation.

Statistical analysis

The experimental data were analysed using ANOVA followed by Duncan's Multiple Range Test (DMRT) at a 1% significance level (18) with the AGRES statistical software.

Results

Evaluating the spore release from the ODF of P. penetrans in soil

The observations showed that the endospores were released from the ODF within a day and the highest parasitisation potential recorded was 85%, with a spore load of 11.2/J_2 of *M. incognita.* 1 mL of the formulation was taken 30 days after inoculation and mixed with freshly hatched J_2s (50 no.s) in 5 mL sterile water followed by incubation under room temperature (27±2 °C) for 48 h. The same procedure was repeated with the formulation at 60th day after preparation. Microscopic observation revealed that the endospores were attached firmly on the cuticle and ensured the viability of the spores after storage. There was no change in the condition of the formulation over the 2-month (60 days) storage period and the endospores were observed under a phase-contrast bright-field microscope (iScope - Euromex). The parasitisation potential was 84.7%, with a spore load of 10.2 spores/J_2 observed on the 60th day of storage.

The penetration of J_2s of *M. incognita* into the tomato root was examined and the average number of J_2s penetrating each plant was observed on the 7th day after inoculating the ODF of *P. penetrans.* Approximately 8.5 endospores were attached to the cuticle of J_2s. The formation of minute galls on roots was observed. The development of third-stage juveniles (J_3s) and pre-adult stages of *M. incognita* was observed on the 12th day. The formation of four-cell and two-cell stages of endospores in J_3s and pre-adult stages of *M. incognita* was recorded.

Pot culture condition

The results showed that the soil application of *P. penetrans* ODF resulted in a significant reduction in nematode infection regardless of the concentration used (Table 1). Among the various concentrations tested, the highest reduction in root galling and egg mass production was recorded at 400 µL of the formulation, with the lowest number of root-knot galls (Fig. 2), which was reflected as 60% and 66.6% reduction over control, respectively. The same treatment reduced nematode infection in the soil by up to 59.4% compared to the control. The second-best treatment was soil application of the ODF at the rate of 300 µL per pot. Mature endospores of *P. penetrans* were observed in adult female nematodes, with the highest number of infected females recorded in plants treated with 400 µL of the formulation. A negative correlation was observed between the concentration of ODF and the soil nematode population (r = - 93.6; Fig. 3).

Field trial

Field trials were conducted in nematode-sick plots for two seasons. The findings on nematode infection and yield showed that the soil application of *P. penetrans* ODF significantly reduced the nematode population, with an improved yield compared to the control.

Table 1. Efficacy of *P. penetrans* OD formulation in medicinal coleus, *C. forskholii* under glasshouse condition (pot culture)

	Treatments	Number of galls/ 5g of root	Number of egg masses/ 5g of root	Number of J_2 /100 g soil	Number of *P. penetrans* infested females/ 5g of root
T1 -	100µl *P. penetrans* ODF / pot	17.7 (4.16)	21.3 (4.54)	98.3 (9.90)	5.4 (2.37)
T2 -	200µl *P. penetrans* ODF / pot	15.7 (3.95)	17.0 (4.31)	98.0 (9.28)	7.7 (2.76)
T3 -	300µl *P. penetrans* ODF / pot	14.7 (3.82)	15.7 (4.46)	89.3 (9.87)	8.3 (2.88)
T4 -	400µl *P. penetrans* ODF / pot	12.6* (3.55)	13.5* (4.19)	77.1* (8.77)	10.0* (3.15)
T5 -	Carbofuran at 3 g /pot	22.6 (4.74)	34.7 (5.79)	167.7 (12.93)	-
T6 -	Control (Untreated)	31.7 (5.6)	40.3 (6.34)	190.3 (13.79)	-
	CD (p=0.05)	0.39	0.21	1.82	0.23

Figures in parentheses are square root transformed values. Values with * are significantly different other treatments at 5% level.

Fig. 2. Effect of OD formulation of P. *penetrans* against *M. incognita* infection in medicinal coleus A - Untreated control plant, B - OD formulation of *P. penetrans* treated plant.

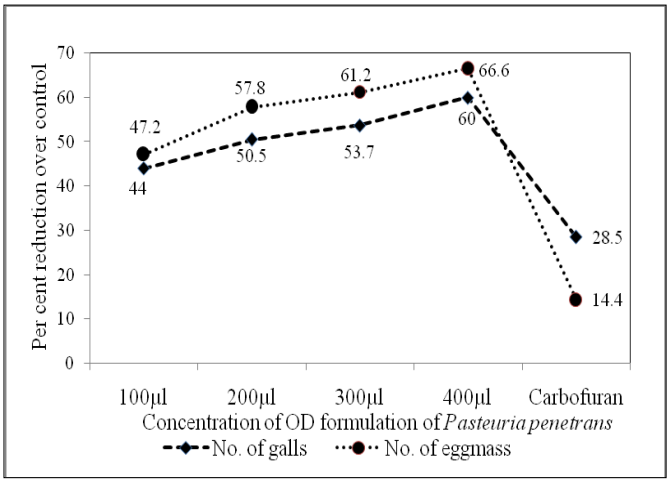


Fig. 3. Relationship between nematode infection and OD formulation (Pot culture test).

Season I

Soil application of *P. penetrans* ODF resulted in higher shoot length and weight of the treated plants, which were 45.16 cm and 60.33 g, respectively, whereas they were only 38.16 cm and 46.36 g, respectively, in the untreated control. Similarly, plants receiving carbofuran 3G treatment were also inferior to those receiving *P. penetrans* treatment. Furthermore, root length and weight were higher in plants receiving *P. penetrans* soil drenching, with values of 23.87 cm and 30.85 g, respectively (Table 2). The lowest growth parameters were observed in plants uprooted from untreated control plots. Yield parameters, such as the number of tubers per plant, weight of the tubers and yield, were also influenced by nematode suppression achieved via the application of *P. penetrans* ODF, with a lowest root knot gall index (RKI) of 2.0 (Table 3). The highest RKI was observed in plants maintained as untreated control. The relationship between nematode reproductive index and yield was determined (r = -0.97) (Fig. 4). The application of *P. penetrans* ODF increased the yield by 32.9% compared to the carbofuran treatment.

Season II

The results obtained from season II showed a trend similar to those of season I. Plant growth parameters were significantly elevated in plants receiving the ODF of *P. penetrans*, with a root length of 25 cm and a tuber weight of 42.8 g, which were better than those of the untreated control (Table 4). Moreover, the yield was 16.7% higher compared to the untreated control. The application of ODF reduced soil and root nematode infection by 55.6% and 67% compared to the untreated control. Furthermore, this treatment reduced the egg mass production (Table 5). A negative correlation was observed between the nematode reproductive index and the yield (r = -0.95) (Fig. 5).

Table 2. Effect of OD formulation of *P. penetrans* on growth of medicinal coleus, *C. forskholii* under field condition (Season I)

	Treatments	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Number of tubers	Weight of tubers (g)	Yield (tn / ha)
T1 -	*P. penetrans* ODF at 2 L / ha	45.16*	60.33*	23.83*	40.92*	18.2* (4.26)	71.3*	1.92*
T2 -	Carbofuran 3G 1kg a.i / ha	41.30	42.54	19.33	30.85	12.0 (6.6)	47.8	1.29
T3 -	Control (Untreated)	38.16	46.36	10.16	29.30	4.4 (2.2)	19.6	0.53
	CD (p=0.05)	1.75	2.21	2.16	1.25	0.41	11.2	0.3

Values with * are significantly different other treatments at 5% level.

Table 3. Effect of OD formulation of *P. penetrans* against *M. incognita* infestation in medicinal coleus, *C. forskholii* under field conditions (Season I)

	Treatments	INP (Number of nematodes / 250g soil)	FNP at harvest (Number of nematodes / 250g soil)	Number of galls / 5g of root	RKI	Number of egg masses / 5g of root	Rf value	Number of *P. penetrans* infested females / 5g of root
T1 -	*P. penetrans* ODF at 2 L / ha	272.5* (16.5)	171.2 (13.0)*	15.16 (3.86) *	2.0*	8.83 (3.23)*	0.63*	8.6 (1.5×10^6 spores / female)
T2 -	Carbofuran 3G 1kg a.i / ha	281.2 (16.6)	223.2 (14.9)	21.50 (4.59)	2.5	15.83 (3.55)	0.80	-
T3 -	Control (Untreated)	275.4 (16.8)	366.8 (19.2)	38.83 (6.2)	5.0	35.16 (5.82)	1.33	-
	CD (p=0.05)	0.05 NS	0.43	0.72	0.61	0.99	0.12	-

Figures in parentheses are square root transformed values; Values with * are significantly different other treatments at 5% level.

INP - Initial Nematode Population; FNP- Final Nematode Population

Table 4. Bioefficacy of OD formulation of *P. penetrans* on growth of medicinal coleus, *C. forskholii* under field condition (Season II)

Treatments		Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	Number of tubers	Weight of tubers (g)	Yield (tn / ha)
T1 -	*P. penetrans* ODF at 2 L / ha	49.3*	64.8*	25.0*	42.8*	15.4* (3.92)	62.8*	1.69*
T2 -	Carbofuran 3G 1kg a.i / ha	43.2	49.2	20.3	34.3	6.6 (2.57)	27.9	0.75
T3 -	Control (Untreated)	39.2	44.0 (1.65)	12.2	32.0	4.2 (2.05)	20.5	0.55
	CD (p=0.05)	1.71	2.21	8.25	1.25	0.24	14.3	0.39

Figures in parentheses are square root transformed values; Values with * are significantly different other treatments at 5% level.

Table 5. Effect of OD formulation of *P. penetrans* against *M. incognita* infestation in medicinal coleus, *C. forskholii* under field conditions (Season II)

Treatments		INP (Number of nematodes / 250g soil)	FNP at harvest (Number of nematodes / 250g soil)	Number of galls / 5g of root	RKI	Number of egg masses / 5g of root	Rf value	Number of *P. penetrans* infested females/5g of root
T1 -	*P. penetrans* ODF at 2 L / ha	272.5 (12.6)	159.5* (2.23)	11.3* (3.7)	2.0*	6.83* (2.61)	0.58*	10.2 (1.0×10^6 spores/ female)
T2 -	Carbofuran 3G at 1kg a.i / ha	281.2 (14.7)	271.3 (2.35)	18.2 (4.26)	2.3	16.83 (4.10)	0.96	-
T3 -	Control (Untreated)	275.4 (18.9)	359.4 (2.56)	33.8 (4.82)	4.4	45.17 (6.72)	1.31	-
	CD (p=0.05)	0.11 NS	0.30	0.21	0.53	0.62	0.03	-

Figures in parentheses are square root transformed values; Values with * are significantly different other treatments at 5% level.

INP - Initial Nematode Population; FNP- Final Nematode Population

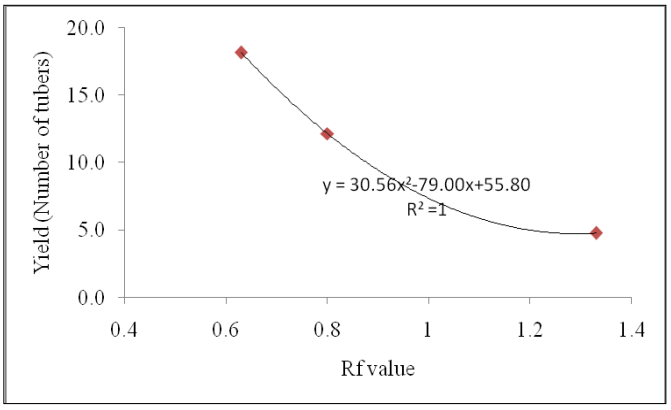


Fig. 4. Correlation between Rf value and yield (Season I).

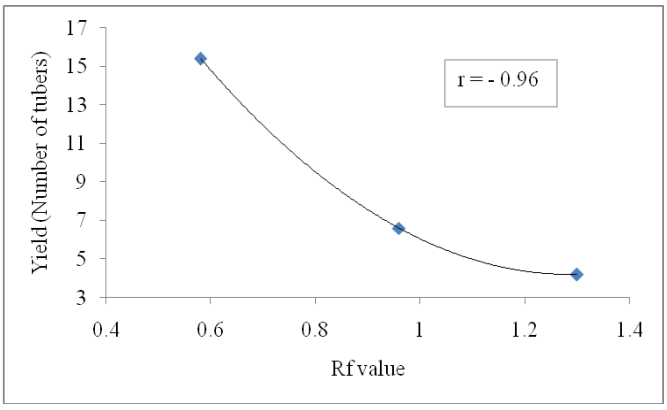


Fig. 5. Correlation between Rf value and yield (Season II).

Molecular identification of M. incognita

The isolated DNA was amplified at 900 bp and sequenced by Yzaah Xenomics, Coimbatore, India. The species was isolated from the field population and had a similarity of 98.83% with pure culture maintained at the Department of Nematology, Tamil Nadu Agricultural University, Coimbatore population (MIRG07). There was a slight variation since the host plant was *C. forskholii* and the reference sequence was the tomato population. The confirmed nucleotides were submitted to NCBI with the GenBank Accession number MK975495. These findings established the genetic-level identification of the species of root-knot nematode isolated from the field trial as *M. incognita*.

Discussion

The results from the above investigation confirmed the effectiveness of *P. penetrans* ODF in suppressing nematode infection. The probable reasons for the reduction in nematode numbers are discussed below.

The effectiveness of the ODF could be attributed to the fact that it took only 1 day for it to be degraded completely in the soil and that it had a rapid mode of action. This observation is in accordance with earlier research that investigated the efficiency of *P. penetrans* against *M. incognita* and *M. javanica* in tobacco (19). The biocontrol potential of *P. penetrans* has been proven by several authors (20-23) in various crops such as chilli, grapevine, tomato and okra. The interaction between *P. penetrans* and *M. incognita* under pot culture conditions has been studied (24). The spore load was sufficient in females and successfully controlled *M. incognita*. The efficacy of *P. penetrans* and various oil seed cakes in the management of *M. incognita* in chilli and pepper (*Capsicum annum*) was evaluated (25). The combination of castor oil cake and *P. penetrans* resulted in a greater reduction in the galling index (84.75%) and the final population of *M. incognita* was also more effectively reduced (85.74%) over the control than other treatments.

The yield parameters were higher in ODF-treated plants. These observations are similar to the results of the efficacy test of *P. penetrans* on *M. incognita* reproduction in tomatoes, as documented previously (21). Another study reported that nematode mobility is arrested and their

capability to locate the host is affected when they are encumbered with spores of *P. penetrans* (8). Moreover, reproductive organ failure occurs in adult nematodes, which leads to the reduced production of the next generation of nematodes.

The tuber yield was higher in ODF-treated plants than in untreated plants, which could be attributed to the suppression of the nematode population. These observations agree research which the combination application of *P. penetrans*, *Glomus fasciculatum* and *Trichoderma asperellum* decreased the population of the root-knot nematode and increased the growth of black pepper when compared with carbofuran 3G (26).

Conclusion

In conclusion, the findings from this study confirm that the ODF of *P. penetrans* is effective in containing nematode infection in *C. forskohlii*. The endospores of this bacterium multiply in the body of the female nematode and are released into the soil. These spores restrict the development of the next generation of nematodes. Hence, the application of this bacterium could protect future crops from nematode attacks. The formulation is water soluble, making it appropriate for drip irrigation of crops. The bacterium is host-specific, so it will not interfere with the ecological balance. Tubers of medicinal coleus are used for the extraction of forskolin, so nematode management should not leave any toxic residue on the tubers. The formulation developed in the current research is biodegradable and does not leave any residual toxic elements either in the soil or on the tubers. Nematode damage is seen in the underground economically important parts of crops such as carrot, beetroot, potato, sweet potato, turmeric and taro. The application of chemical nematicides often leaves residues on the edible parts of crops, raising concerns about food safety and consumer health. These residues can persist even after standard washing or processing, potentially exceeding permissible limits and posing risks of toxicity or long-term exposure to harmful chemicals. Additionally, the presence of nematicide residues can affect the marketability of the produce, especially in regions with strict regulations on pesticide residues and may disrupt the ecological balance by affecting non-target organisms. Nematicides such as carbofuran are generally used for nematode management. Carbofuran inhibits acetylcholine esterase that disrupts neurotransmission in nematodes. If the residues persist in the plant parts, it causes health issues related to nervous system such as paralysis and stroke in human beings. Treating plants with *P. penetrans* protects them from nematicide residues and is an eco-friendly solution for nematode management.

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Authors' contributions

NS conceptualized, validated, reviewed, and edited the manuscript. IG helped draft the research paper and recorded the plant biometric data. SD and AA performed the molecular analysis of M. incognita and conducted the laboratory and pot culture assays. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no known competing financial interests.

Ethical issues: None

References

1. Jones JT, Haegeman A, Danchin EG, Gaur HS, Helder J, Jones MG, et al. Top 10 plant parasitic nematodes in molecular plant pathology. Mol Plant Pathol. 2013;14(9):946–61. https://doi.org/10.1111/mpp.12057
2. Manjunatha T, Gowda AB, Rai, Singh B. Root knot nematode a threat to vegetable production and its management. IIVR Tech Bul. 2017;76.
3. Singh R, Gangwar S, Singh D, Singh R, Pandey R, Kalra A. Medicinal plant *Coleus forskohii* Briq: Disease and management. Med Plants - Int J Phytomed Relat Ind. 2011;3(1):1–7. https://doi.org/10.5958/j.0975-4261.3.1.001
4. Degenkolb T, Vilcinskas A. Metabolites from nematophagous fungi and nematicidal natural products from fungi as an alternative for biological control. Part I: metabolites from nematophagous ascomycetes. Appl Microbiol Biotechnol. 2016;100(9):3799–812. https://doi.org/10.1007/s00253-015-7233-6
5. Stirling G. Biological control of plant parasitic nematodes. Wallingford. Oxon: CAB International. 1991;ISBNO-85198703-6.
6. Chen Z, Dickson D, McSorley R, Mitchell D, Hewlett T. Suppression of Meloidogyne arenaria race 1 by soil application of endospores of *Pasteuria penetrans*. J Nematol. 1996;28(2):159.
7. Hewlett TE, Gerber JF, Smith KS. *In vitro* culture of *Pasteuria penetrans*. Proceedings of the Fourth International Congress of Nematology, 8-13 June 2002, Tenerife, Spain; 2004. https://doi.org/10.1163/9789004475236_020
8. Vagelas I, Leontopoulos S. Modeling the over dispersion of *Pasteuria penetrans* Endospores. Parasitologia. 2022;2(3):206–27. https://doi.org/10.3390/parasitologia2030018
9. Senthamarai M, Poornima K, Subramanian S. Pathogenicity of *Meloidogyne incognita* on *Coleus forskohlii* Briq. Indian J Nematol. 2006;36(1):123–25.
10. Bhandari S, Harsh NSK, Singh P. First report of *Meloidogyne arenaria* on *Coleus forskohlii.* Indian Forester. 2007;133:1709–10.
11. Senthamarai K, Poornima K, Subramanian, Sudheer J. Nematode-fungal disease complex involving *Meloidogyne incognita* and *Macrophomina phaseolina* on medicinal Coleus, *Coleus forskohlii* Briq. Indian J Nematol. 2008;38(1):30–33.
12. Zhou H, Yue Y, Liu G, Li Y, Zhang J, Gong Q, et al. Preparation and characterization of a lecithin nano OD as a topical delivery system. Nanoscale Res Lett. 2010;5(1):224–30. https://doi.org/10.1007/s11671-009-9469-5
13. Cobb NA. Estimating the nematode population of the soil. United States Department of Agricultural Technology Circular, Washington. 1918;(1):48.
14. Schindler AF. A simple substitute for Baermann funnel. Plant Dis Rep. 1961;45:747–48.

15. McBeth CW, Taylor AL, Smith AL. Note on staining nematodes in root tissues. Proc Helminthol Soc Wash. 1941;8:26.

16. Bridge J, Page SLJ. Estimation of root knot nematode infestation levels in roots using a rating chart. Trop Pest Manag. 1980;26:296–98. https://doi.org/10.1080/09670878009414416

17. Castagnone-Sereno P, Esparrago G, Abad P, Fredéric L, Michel B. Satellite DNA as a target for PCR-specific detection of the plant-parasitic nematode *Meloidogyne hapla*. Curr Genet. 1995;28:566–70. https://doi.org/10.1007/BF00518170

18. Panse VG, Sukhatme PV. Statistical methods for agricultural workers**.** Indian Council of Agricultural Research, Publication, New Delhi; 1967

19. Weibelzahl-Fulton E, Dickson D, Whitty E. Suppression of *Meloidogyne incognita* and *M. javanica* by *Pasteuria penetrans* in field soil. J Nematol. 1996;28(1):43–49.

20. Mukhtar T, Arshad HM, Zameer KM. Biocontrol potential of *Pasteuria penetrans*, *Pochonia chlamydosporia, Paecilomyces lilacinus and Trichoderma harzianum* against Meloidogyne incognita in okra. Phytopathol Mediterr. 2013;52(1):66–76.

21. Kamran M, Javed N, Khan SA, Jaskani MJ, Ullah I. Efficacy of *Pasteuria penetrans* on *Meloidogyne incognita* reproduction and growth of tomato. Pak J Zool. 2014;46(6):1651–55.

22. Kumari NS. Role of bacterial bioagent, *Pasteuria penetrans* in the management of root-knot nematode, *Meloidogyne incognita* by altering the lifecycle. Pest Manag Hortic Ecosyst. 2017;23(1):76–79.

23. Mhatre PH, Eapen SJ, Chawla G, Pervez R, Agisha V, Tadigiri S, Nagesh M. Isolation and characterization of *Pasteuria* parasitizing root-knot nematode, *Meloidogyne incognita,* from black pepper fields in India. Egypt J Biol Pest Control. 2020;30(1):1–7. https://doi.org/10.1186/s41938-020-00296-z

24. Tzortzakakis E, Channer A, Gowen S, Ahmed R. Studies on the potential use of *Pasteuria penetrans* as a biocontrol agent of root-knot nematodes (*Meloidogyne* spp.). Plant Pathol. 1997;46(1):44–55. https://doi.org/10.1046/j.1365-3059.1997

25. Chaudhary K, Kaul R. Efficacy of *Pasteuria penetrans* and various oil seed cakes in management of *Meloidogyne incognita* in Chilli pepper (*Capsicum annuum* L.). J Agric Sci Technol. 2013;15:617–26.

26. Jena R, Basumatary B, Mohanta B, Pradhan S. Comparative efficacy of bio control agents against root knot nematode (*Meloidogyne incognita*) infecting brinjal. J Entomol Zool Stud. 2017;5(6):254–57.