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Research Article

Evaluation of *Paecilomyces lilacinus* as an Effective Biocontrol Agent Against *Meloidogyne incognita* Causing Root Knot Disease in Tomato

R.K. Pandey[1], S.R. Singh[2*] and S.K. Singh[3]

[1]BU, Jhansi, Uttar Pradesh, India
[2]KVK, Firozabad, Uttar Pradesh, India
[3]CCR (PG) College, Muzaffarnagar, Uttar Pradesh, India

***Corresponding authors Email :** drsrsingh0@gmail.com

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Abstract: In the last two decades many developments have been occurred which triggered the prospects and opportunities for the biological control of plant-parasitic nematodes. Due to health and environmental concerns several chemical nematicides have been withdrawn from the market and revealed interest in the development of alternative methods of control, including the use of biocontrol agents. In the present study biocontrol potential of the parasitic fungus Paecilomyces lilacinus isolated from four different agro climatic zones of India was evaluated for the management of root-knot nematode Meloidogyne incognita under laboratory and field conditions. Many isolates were collected from the rhizosphere of root-knot nematode affected vegetables fields during year 2003 - 2005 and four different isolates representing the best among the collected isolates from each zone was tested in vitro for their larvicidal, ovicidal and egg-parasitizing capacity against M. incognita. Fungal isolates were grown in Potato dextrose broth and filtered to remove fungal biomass. Toxins responsible for the nematicidal action was extracted from culture filtrate and tested for their potentiality. Methanol extracted toxins were found to be more lethal in comparison to the other organic solvents. In field experiments, various developed formulations of P. lilacinus were found effective and significantly suppressed the root-knot nematode. These tests revealed variability in their potentiality and activity against root knot nematode. Identified isolates of P. lilacinus were found capable to produce bioactive nematicidal compounds against root-knot nematode M. incognita.

Keywords: Paecilomyces lilacinus, Meloidogyne incognita, Root knot nematode, biological control, biocontrol agent formulations and nematicidal metabolite.

Introduction

Plant-parasitic nematodes cause great economic losses to agricultural crops worldwide (Sasser and Freckman, 1987). The root-knot nematodes (Meloidogyne spp.) are sedentary endoparasites and are among the most damaging agricultural pests, attacking a wide range of crops causes severe damage and yield loss to a large number of cultivated plants and especially on vegetable crops in the tropics and subtropics (Sikora and Greco, 1993; Netscher and Sikora, 1990; Barker et al., 1985). Endoparasitic nematodes live within the roots as a major part of their life cycle this makes their control difficult since their resistant stages eggs; females or cysts are protected from chemical protectants or microbial antagonists (Hussey and Grundler, 1998). Due to the health and environmental problems caused by chemical control, development of alternative control measures is a need of time and of great importance for sustainable development.

Limited availability of chemical nematicide and high developmental costs created a need to discover alternative methods for plant - parasitic nematode management. One of the top priorities in near future is to find alternatives to hazardous chemical nematicides (Enrique and Edwin, 2002), which are bio-efficacious, economical, biodegradable and environmentally safe and can be ideal candidates for use as a reliable tool to control plant parasitic nematodes. Potential sources of new chemicals to manage populations of plant parasitic nematodes include natural products produced by fungi (Oostendrop and Sikora, 1989; Anke and Sterner, 1997).

The use of Paecilomyces lilacinus as biocontrol agent against root knot nematode disease management has been well documented. Nematophagous fungi also produce many potential toxins of nematicidal potential (Morgan Jones and Rodriquez -kabana, 1985). Nematicidal metabolite, Phomalactone, was isolated from P. chlamydosporia and was effective against infective juveniles of M. incognita (Khambay et al., 2000) many fungi are known to produce nematicidal or nematistatic compounds (Chen et al., 2000; Meyer et al., 2000). For example, the fungus Omphalotus olearius produced somphalotin A, a nematicidal compound that demonstrated greatest activity against the root-knot nematode Meloidogyne incognita (Kofoid & White) Chitwood (Mayer et al., 1999). Paeciloxazine, a novel antibiotic from Paecilomyces sp is having nematicidal activity against Rhabditis pseudoelongata and is weakly active against some insects (Kanai et al 2004). Acetic acid an active component from culture filtrates of Paecilomyces lilacinus and Trichoderma longibrachiatum (Djian et al., 1991) also posses toxic activities; Flavipin, metabolite from fungus Chaetomium globosum responsible for most of the nematode-antagonistic activity against root-knot nematode (Meloidogyne incognita) and hatch of soybean cyst nematode Heterodera glycines (Nitao et al., 2002). Active compounds from fungal cultures that are deleterious to plant-parasitic nematodes have potential for application as novel nematicides against root-knot nematode.

Park et al., 2004 reported that culture filtrate of P. lilacinus possess nematicidal activity against Caenorhabditis elegans and Meloidogyne javanica. For example, culture filtrates from two isolates of P. lilacinus inhibited egg hatch and juveniles of H. glycines, with one filtrate more active than the other filtrate (Sun et al., 2002). Fusarium solani, F. oxysporum, P. lilacinus and P. chlamydosporia also posses toxicity in filtrates activity varying with culture medium and with species of fungus (Chen et al., 2000). Filtrates from cultures of A. ochraceus, F. solani, F. oxysporum, P. lilacinus, T. viride and P. chlamydosporia were toxic to M. incognita juveniles, inhibited hatching, and/or suppressed egg or J2 populations on various plants (Hallman and Sikora, 1996; Sharma, 1999; Khan, 1999; Wang et al., 1999; Costa et al., 2000; Costa et al., 2001; Randhawa et al., 2001).

P. lilacinus is a soil-inhabiting fungus produces distinctive lilac-colored colonies is capable of parasitizing nematode eggs, juveniles and females, and reducing the populations of plant parasitic nematodes in soil. It was first discovered in soil and observed to control root-knot nematodes on potato in Peru by Jatala, et al., (1979). P. lilacinus (Thom) Samson has been reported to be a potential biological control agent against root-knot and other plant parasitic nematodes (Adiko, 1984; Franco et al., 1981). It parasitizes eggs of Meloidogyne spp. and Globodera pallida (Stone) Behrens. (Dunn et al., 1982; Jatala, 1986). This fungus also invades the females or cysts of a number of nematode species (Franco et al., 1981; Gintis et al., 1983; Jatala, 1982; Jatala, 1986). It exhibits chitinase activity when grown on chitin agar medium and produces a peptidal antibiotic which has wide antimicrobial activity against fungi, yeast and gram -positive bacteria (Cabanillas et al., 1988; Isogai et al., 1981). P. lilacinus colonizes M. incognita eggs, preventing them from hatching and leaving fewer Juveniles to penetrate root tissues (Dunn et al., 1982; Jatala, 1986).

Root knot disease in plants caused by Meloidogyne incognita is one of the most important, diseases causing remarkable loss with very low production. The pathogen is soil borne with a tremendous environmental adaptability. The fungus has the greatest potential for application as a biocontrol agent in subtropical and tropical agricultural soils. Its association with nematode eggs was first reported by Lysek (1976). Among the egg parasitic fungi P. lilacinus play an important role in influencing the potentiality of fungal bioagents and its formulation against M. incognita. Since considerably diversified climatic conditions occur in India, therefore, in present study it is felt essential to evaluate indigenous isolates of P. lilacinus for successful management of root knot nematode M. incognita infecting plants. Since in our recent studies (Khan and Goswami, 2000; Goswami and Singh, 2002), this fungus has been identified to possess both nematicidal and egg parasitic properties. The purpose of this paper was to examine the most potent isolate among the four isolated from different geographical regions of India and to determine their field efficacy.

Materials and Methods

Survey and Isolation of Fungi

Systematic survey of different agro climatic zones of India were carried out to isolate the P. lilacinus from various vegetables fields particularly tomato field infested with root knot nematodes for two successive years (October 2007 to April 2008). A large number of heavily galled tomato plants were encountered during the survey of vegetable fields and the samples were collected from rhizosphere. Among 24 isolate of fungus P. lilacinus isolated from four different climatic zones of India viz. Hyderabad (Andhra Pradesh),

Indian Agricultural Research Institute, (IARI, New Delhi), Anand (Gujarat) and Shillong (Meghalaya). Four isolates were finally selected as representative from each zone on the basis of their cultural, morphological and potential character on the preliminary screenings against the root knot nematode. The isolates of P. lilacinus were isolated from the parasitized egg masses of M. incognita with mycelium/spores of P. lilacinus; collect from infested tomato root system. Egg masses were subsequently surface sterilized with 100ppm HgCl2, then inoculated in three slants containing Potato dextrose agar medium under aseptic conditions and incubated at 25±20C for profuse growth. The isolates were identified on the basis of cultural morphological viz. growth pattern, spore characters shape, size and color. Purification of P. lilacinus was carried out by hyphae tip or single spore method and kept at 40C for further testing.

In-vitro Nematode Bioassays: Testing for Potentiality through in Vitro Studies

Larvicidal Test

Each fungal isolate was grown on Potato Dextrose broth in 250 ml Erlenmeyer flask and incubated at 25±20C. The broth was passed through Whatman filter paper no. 1 and culture filtrate was preserved at 40 C for further use within few days. Treatments with three dilutions viz. S, S/10 and S/100 of culture filtrate of each fungal isolate along with water as control were prepared. About 10 to 15 egg masses were picked up from infested root of tomato plant and surface sterilized with 0.01% HgCl2 and placed in small petriplates containing 20 mldouble distilled water and allowed to incubate at 25±20C for 24 hours to hatch the second stage juveniles (J2). About 200 second-stage juveniles (J2) of M. incognita were counted with help of counting disk and binocular microscope. These juvenile (J2) were exposed to culture filtrate 24, 48 and 72 hrs in 5 cm diameter petriplates and the number of those J2 that were mobile and immobile was recorded at an interval 24, 48 and 72 hrs and mortality percentage was calculated. The revival test was also performed after transferring juveniles in fresh water. Each treatment had three replicates.

Hatching Inhibition Test

To know the effect of culture filtrate on egg masses hatching inhibition test was performed in sterile 5 cm petriplates with above-mentioned dilutions. The surface sterilized egg masses of M. incognita were suspended in 10 ml of culture filtrate of various dilutions for 72 hrs. These egg mosses were then transferred in sterile water and number of larvae hatched from each dilution was recorded after a regular interval of 2, 4, 6 and 8 days. Hatching inhibition of egg masses determined in sterile water, served as control. Each treatment had three replications.

Determination of Egg Parasitization

All the selected isolates of P. lilacinus maintained on PDA medium were evaluated under in vitro conditions for egg parasitization. Each fungal isolates was allowed to grow on PDA in petriplates for 10 days. To each of these petriplates previously collected surface sterilized 5 egg masses of equal size were suspended on sporulating fungus and plate without fungus served as a control. After 2 days inoculated egg masses were transferred to 2 % water agar (WA) plates, which were incubated at 25±20C for 10 days. To determine the time of early egg infection, egg masses were monitored from the third day to one week with an inverted microscope. Inoculated egg masses were crushed on a slide (0.5% cotton blue in lactophenol) to examine the infected eggs. Each treatment was replicated thrice. Endo-parsitism in M. incognita was confirmed as the infected eggs were surface sterilized in 1% NaOCl for 1 minwith three rinsing in sterile water and eggs were crushed on glass slide under aseptic conditions. A portion of internal egg content was transferred in a batch of test tubes slants containing PDA medium, which were incubated at 25±20C for 7 days and the growth of fungal colonies, was examined and identified for comparison with original culture.

Extraction of Toxins from Cultural Filtrate

Paecilomyces lilacinus isolates grown on Potato dextrose agar plates for 1 week were used to inoculate (3 mycelia discs of 1.2 cm diam) potato dextrose broth (250 ml per 1 l flask). After incubating for 16 days 25±20C shaken at 120 rpm, broth cultures was filtered through a double layered sterilized muslin cloth. For the isolation of toxic metabolites, broth filtrate was extracted with solvent methanol (80 %) (1:1 Broth: solvent v/v) and hexane separately by vigorously agitating on a shaker. Solvent was separated using separating funnels and the solvents were dried over anhydrous sodium sulphate and evaporated to dryness under reduced pressure on rotary evaporator. A total of 32 L of broth was processed for all the four isolate, yielding 2-4 gm residues per isolate. The dried methanol residues were extracted with minimal volumes of methanol, resulting in methanol soluble fraction. Absorbance of the methanol soluble fraction was recorded and to detect the presence of toxins in the methanol soluble fraction nematicidal tests was performed

against freshly hatched second stage juveniles of M. incognita. A stock solution of 1000µg ml-1 was prepared and further diluted in water to obtain 500,250,125 and 62.5 µg ml-1 concentrations. About 30 J2 of M. incognita were counted and taken in glass vials and 1ml of the desired concentration of toxin stock solution was added to the vials and vials without the toxins served as control. The vials were incubated at 25±20C for an exposure of 24, 48 and 72 hrs. Similar nematicidal test was performed for hexane extract.

Toxins from methanol fraction were detected by HPLC (Thermo separation product model spectra system P 2000) equipped with a variable wavelength UV-150 UV VIS detector andconnected to a datajet reporting integrator. Stationary phase consisted of lichrosorb C- 18 column (250 mm x 4.6 mm id) and methanol: water (60: 40 v/v) as mobile phase with a flow rate of 1ml/min at 230 nm.

Field Evaluation

The potentiality of above four isolates of P. lilacinus was evaluated in field experiment. The fungal antagonist was maintained on PDA slants at 5°C after growing for seven days at 25±2°C and same culture was inoculated in 250ml flask containing 50 ml potato broth for the preparation of initial inoculum suspension to inoculate the sorghum grains. P. lilacinus was multiplied on pre-sterilized sorghum grains having 5 percent anhydrous dextrose. The inoculated sorghum grains were incubated at 25±2°C for 12 days. Colonized grains were air dried and powdered. The concentration of conidia was maintained 2X107 cfu/g formulations. CFU counts measured were performed using a hemacytometer (Hausser Scientific) under light microscopy. Above four isolates were taken as required amount for maintaining 2X107 cfu/g formulation and they were mixed in sterilized talc powder with carboxymethyl cellulose sodium salt (CMC) in a ratio of 3:1 w/w in aseptic polypropylene bags. For convenience in application of these formulation under field conditions, prepared talc based formulation were thoroughly mixed with powdered farmyard manure (FYM), which was solarised in summer for one month, in the ratio of 1:25(w/w).

Similarly, soil solarised for two-month in accordance with the experimental filed was used for nursery of tomato seedlings. Seed treatment was given in aseptic condition in by mixing of both seed and each formulation @5g/kg seed in separate polypropylene bags. Seed germination percent and increase in germination (%) was recorded at the time of transplantation of seedlings in main infested field. A field trial was carried out to evaluate the formulations of above four isolates in nematode sick plot having 2 larvae /g soil. The plot was divided in twelve micro plots (4x2.5m2) with three replications and each replication having five furrows. The formulations were applied@1.5kg/microplot (4x2.5m2). Consequently, four-week-old healthy seedlings of tomato Cv. Pusa Ruby was transplanted singly into each micro plot with three plants in each furrow. The treatments used were talc based formulation of (a) New Delhi isolates, (b) Anand isolates, (c) Shillong isolates, (d) Hyderabad isolates and (e) Control. Observations were recoded after 60 days on plant growth, number of galls/plants, number of egg masses/plant, number of eggs per egg mass and nematode population in soil.

Results

Larvicidal Test

The culture filtrates of the isolates were found to possess nematicidal action against second stage juveniles (J2) of M. incognita. Data presented in table 1 indicates that all concentration of culture filtrates of P. lilacinus isolates was showing infanticidal effect. The percentage of juveniles killed was observed to increase with the increasing exposure time viz. 24,48 and 72 hrs at all concentrations of fungal filtrates for all isolates. The highest percentage (77 %) of mortality was recorded in Hyderabad isolate and lowest in shillong (62.33 %) and Anand (48.67%). however of effect culture filtrate of New Delhi isolate (68.00 %) was moderately toxic to M. incognita (J2). The culture filtrate diluted up to the extent of 100 times also inhibited 22 to 33 % killing after 72 hrs of exposure thus indicating that even at high moisture level in soil environment, the fungal metabolite remained toxic to nematode.

Flask containing profusely mycelium and culture filtrate of different isolate of P. lilacinus.

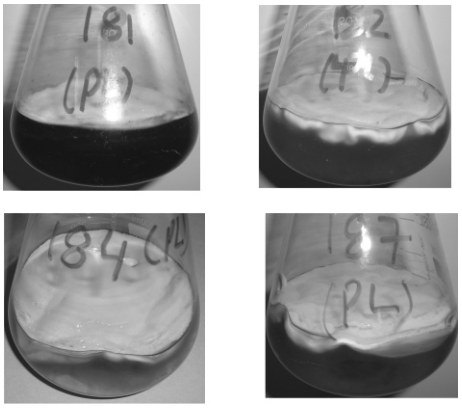


Table 1. Effect of culture filtrate of four Paecilomyces lilacinus isolates on M. incognita

Isolates	Filtrate concentration	% Kill after different time intervals		
		24	48	72
1. New Delhi	S	36 (36.96)	55.33 (48.18)	68.00 (56.30)
	S/10	25.66 (29.75)	36.67 (37.35)	42.67 (34.82)
	S/100	23.25 (28.20)	26.34 (30.83)	29.06 (24.66)
2. Anand	S	32.67 (34.99	35.33 (30.35)	48.67 (34.82)
	S/10	20.00 (26.63)	31.00 (33.90)	39.67 (39.21)
	S/100	25.67 (23.27)	25.00 (29.85)	28.00 (31.85)
3. Shillong	S	34.66 (36.14)	43.67 (41.23)	62.33 (52.27)
	S/10	20.60 (29.90)	41.00 (34.57)	49.67 (44.91)
	S/100	12.00 (20.19)	41.00 (24.18)	29.67 (33.06)
4. Hyderabad	S	42.00 40.30	53.30 47.02	77.00 61.64
	S/10	32.67 (34.82)	40.00 (40.87)	53.67 (47.21)
	S/100	17.67 (24.66)	24.66 (29.78)	33.00 (35.13)
Control	Water	0.00 (1.81)	0.00 (1.81)	0.00 (1.81)
LSD (P=0.05)		3.41	4.61	3.87

Figures in parentheses are vn + 0.5 angular transformed values.

Hatching Inhibition Test

The culture filtrate of P. lilacinus isolates shows the effect on egg mass hatching with low number of J2 hatched out from egg masses exposed to culture filtrates as compared to control. Isolates at all concentration were found to have significant effect on the emergence of juveniles from the egg masses. The higher percentage of inhibition of hatching i.e. 85 % was recorded with Hyderabad isolate at all concentration with the increase in dilution of culture filtrates, the cumulative hatching was increased in all check. The isolate of Hyderabed showed highest percentage of inhibition even at S/10 concentration of culture filtrate where as isolate shillong was observed as least effective. However isolates from Shillong (72.47) and Anand (70.14) showed inhibition effect on hatching results in complete suppression of M. incognita.

Table 2. Effect of culture filtrate of four *P. lilacinus* isolates on hatching *M.incognita* eggs

Isolates	Filtrate concentration	Number of J 2 hatched after days				Cumulative effect	Percent of Hatching
		2	4	6	8		
1. New Delhi	S	63.00	54.00	21.00	6.67	144.67	74.33
		7.91	7.32	4.58	2.58		60.15
	S/10	124.00	82.60	41.20	13.00	260.08	53.85
		11.23	9.07	6.39	3.56		
	S/100	252	196.20	36.50	10.50	495.80	12.03
		15.82	13.69	5.99	3.15		
2. Anand	S	83.00	69.60	13.00	9.66	168.26	70.14
		9.09	7.88	3.58	3.09		57.05
	S/10	102.67	59.67	22.34	14.50	199.18	64.66
		10.12	7.64	4.67	3.49		
	S/100	134.00	131.0	109.00	4.00	378.00	32.93
		11.56	11.43	10.42	1.97		
3. Shillong	S	79.80	46.00	23.38	6.00	1.5518	72.47
		8.86	6.71	4.67	2.37		59.10
	S/10	91.00	66.34	32.33	13.80	203.47	63.90
		9.51	8.09	5.64	3.56		
	S/100	188.67	93.67	69.60	20.00	371.94	34.01
		13.70	9.65	8.30	4.43		
4. Hyderabad	S	33.30	29.00	21.00	1.00	84.30	85.04
		5.75	5.36	4.54	0.81		62.29
	S/10	48.34	41.20	30.68	8.00	128.22	77.25
		6.92	5.99	5.51	2.83		
	S/100	179.00	109.70	62.00	12.40	363.10	35.57
		13.34	10.43	7.81	3.44		
Control water		196.10	287.00	72.16	8.33		563.59
		13.99					
LSD (P=0.05)		4.61	3.82	4.52	3.78		

Egg Parasitization Capacity Test

Observation on egg parasitism recorded under binocular compound microscope revealed that P.lilacinus colonized very fast in the gelatinous matrix and egg infection occurred after few days of inoculation. All the isolates of P. lilacinus were found egg parasitzing (table 3). Four different isolates of P. lilacinus incubated in vitro with the nematodes, were able to coil around and penetrate the juveniles. However among the evaluated isolates, isolate from Hyderabad showed highest percentage (74.92 %) of egg parasitism where as Anand isolate was least egg parasitic (57.87 %). Further isolates from IARI, New Delhi (63.48%) and shillong (62.73%) exhibited over 50 % in-vitro egg parasitism of M. incognita. The eggs containing mycelium, spores and conidiophores arising from egg were considered parasitized. The infected eggs looked slightly swollen and a chain of conidia borne on conidiophores were seen on egg surface in addition the parasitized were clumped together by fungal hyphae and did not disperse easily even after treating with 0.5 % NaOCl. The fungal isolates infecting nematode eggs endoprasitically was also proved. The infected eggs were surface sterilized with 1 % NaOCl for 1 min followed by three rinses with sterile water. The surface sterilized water was crushed on the sterile glass slide under complete aseptic condition and a portion of internal content of egg was inoculated on PDA slants. Table 33.4 Extraction of toxins from cultural filtrate.

Table 3 *In-vitro* study of egg parasitization test of four *P. lilacinus* isolates on *M. incognita* eggs.

Isolates	Mode of interactions	Egg infection (%)
1. New Delhi	+	63.48 (54.64)
2. Anand	+	57.87 (51.36)
3. Shillong	+	62.73 (54.21)
4. Hyderabad	+	74.92 (61.95)
Control	-	0.0 (1.81)
LSD (P=0.05)		3.10

Figures in parentheses are vn + 0.5 angular transformed values.

Analysis of methanol soluble fractions of the four different isolate of P. lilacinus revealed the presence of various metabolite or toxins formed in the culture filtrate. Hyderabad isolate of P. lilacinus shows the presence of three major metabolites at Rt 3.71,4.04 and 11.91. Anand isolate shows the presence of only two major metabolites at Rt 2.7 and 3.93 whereas shillong isolate also shows the presence of only two metabolites at Rt 2.7 and 4. Isolate from New Delhi also showed the presence of three major metabolites at 3.13, 4.24 and 7.26. Methanol extracted fractions of all the four isolates of P. lilacinus were found to be more effective than ethyl acetate and hexane extracts in the suppression of M. incognita larvae. Table 4 & Figures

Table 4 Toxicity of various solvent extract of *P. lilacinus* against *M. incognita*

Isolates	Solvents extracts	LC50 µg ml-1		
		24 h	48 h	72h
New Delhi	Me.Extract	86.2	128.4	69.1
	Eth.ace.Extract	126.4	214.2	–
	He.Extract	>1000	233.0	–
Anand	Me.Extract	96.2	145.4	86.3
	Eth.ace. Extract	160.4	224.2	–
	He.Extract	>1000	290.6	–
Shillong	Me.Extract	158.4	195.3	–
	Eth.ace. Extract	226.1.4	314.1	–
	He.Extract	>1000	–	–
Hyderabad	Me.Extract	70.2	105.4	56.4
	Eth.ace. Extract	90.4	205.2	–
	He.Extract	812.0	331.4	–

(Me.Extract = methanol extract, Eth.ace.Extract = ethyl acetate extract, He.Extract = hexane extract)

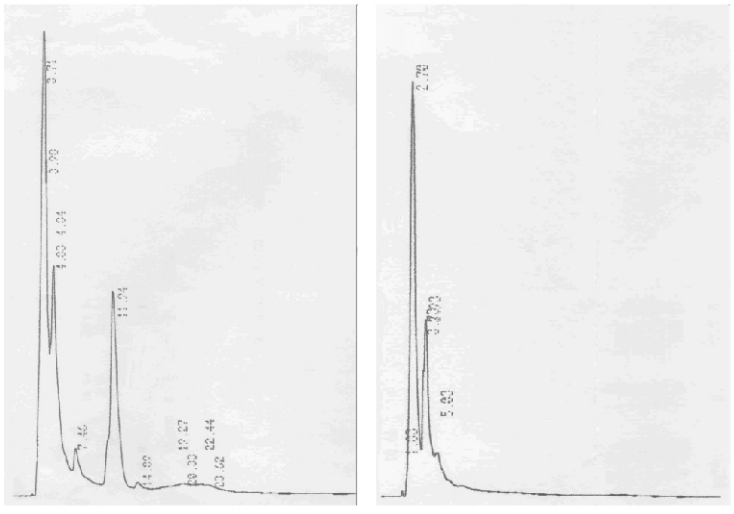
Field evaluation

Effect on plant growth: The significant highest increase in plant growth parameter viz root length, shoot length, root weight, shoot weight were found with isolate of P.lilacinus from Hyderabad (PL-4) based formulation followed by New Delhi isolate (PL-1), Anand isolate (PL-2) and shillong isolate (PL-3) compared to control (Table no1). Similarly, seed germination were found quite good in seed treated with isolate of P.lilacinus from Hyderabad (PL-4) based formulation followed by New Delhi isolate (PL-1), Anand isolate (PL-2) and shillong isolate (PL-3) while the mortality of seedlings at nursery stage were very less in seed treated with isolate of P. lilacinus from Hyderabad (PL-4) based formulation followed by New Delhi isolate (PL-1), Anand isolate (PL-2) and shillong isolate (PL-3) checked by control.

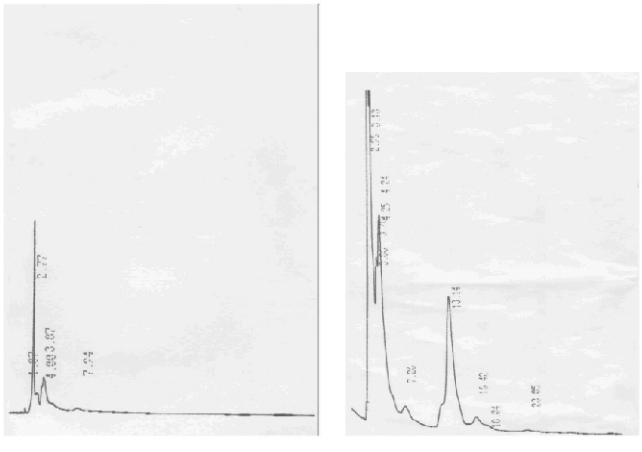
Effect on development of eggs/egg mass, gall formation/index, egg parasitization/egg infection and nematode population: Maximum reduction in eggs/egg mass, gall formation/index, egg parasitization/egg infection and nematode population in soil were observed in treated with isolate of P. lilacinus from Hyderabad (PL-4) based formulation followed by New Delhi isolate (PL-1), Anand isolate (PL-2) and Shillong isolate (PL-3). The egg parasitization/egg infection of P.lilacinus were found maximum with the treatment of hyderabad isolate based formulation followed by New Delhi isolate (PL-1), Anand isolate (PL-2) and shillong isolate (PL-3).

Table 5. Efficacy of formulations of four isolates of *P. lilacinus* on root knot nematode, *M. incognita* and plant growth in field

Treatments	Shoot		Root		Gall index (0-5 scale)	Eggs/egg mass	Egg/infection %	Final soil population of nematode (J2)/100g
	Length (cm)	Weight (g)	Length (cm)	Weight (g)				
Formulation of New Delhi isolate (PL-1)	40.40	16.30	36.80	4.40	2.56	70.20 (8.25)	62.00 (51.90)	97.67 (9.82)
Formulation of Anand isolate (PL-2)	38.90	14.30	34.50	4.30	2.90	81.60 (8.97)	59.30 (59.30)	101.33 (9.96)
Formulation of Shillong isolate (PL-3)	32.80	11.50	29.82	3.40	3.20	80.18 (9.01)	47.80 (43.50)	138.00 (11.60)
Formulation of Hyderabad isolate (PL-4)	43.80	18.90	37.74	5.44	0.0	30.10 (5.53)	75.10 (60.1)	52.87 (7.30)
Control	17.58	4.53	12.37	2.96	5.00	190.36	0.0	330.60 (18.18)
S. Em.	2.07	1.29	2.03	0.67	–	0.99	2.46	0.65
LSD (P=0.05)	4.23	2.65	4.16	1.37	–	2.04	5.19	1.34



Chromatogram A – Hyderabad , Chromatogram B – Anand



Chromatogram C –shillong Chromatogram D –New Delhi

Figure 1 HPLC Chromatograms of four representative isolates of *P.lilacinus*

Discussion

Cayrol et al (1989) observed the nematicidal property of culture filtrates of P. lilacinus and mechanism of toxic activity was considered to be neurotropic. Goswami 2005 observed hundred percent mortality using culture filtrates of Paecilomyces lilacinus within 12 hrs of exposure. However the present study carries with Indian isolates of P. lilacinus clearly showed mortality of second stage juveniles of M. incognita after 24 hrs exposures, which was further confirmed by revival test in water. Thus juvenile exposed to fungal filtrates of each isolates were irreversibly killed at all concentrations.

The ovicidal effect of culture filtrate of Paecilomyces species was observed and inhibition of hatching ranged between 78 to 93% (Caroppo et al., 1990). The exact mode of action of culture filtrates is not clearly known so far. However in the present investigation treated eggs in the egg masses showed some changes like deformed embryos and vacuolated eggs. These visual abnormalities probably were due to the effect of fungal toxic metabolic products. Similarly morphological abnormality had been observed by fitter et al 1993 in the embryonated eggs treated with culture filtrates of P. lilacinus where the development of eggs was found ceased with in 2 to 4 days embryo disintegrated and vacuole formation inside the egg. It is apparent that as the fungus had a strong proteolytic and chitinolytic activity which may help in penetrating the fungal metabolites or antibiotics resulting ovicidal activity. Thus fungal metabolic products together with antibiotics might have played major role for inhibition of egg hatching and ovicidal activity. Table-2 showed clearly variations in the effect of culture filtrate of P. lilacinus isolates on the hatching of M.incognita eggs because of variation in different level of toxins produced by fungal isolates collected from different geographical areas.

Root knot nematode deposits eggs in masses, which are, further protected from moisture stress and natural enemies covering by gelatinous matrix. However this kind of oviposition invariably results in exposure of all eggs to fungal parasites. Once fungal invasion is occurs entire content of eggs mass become vulnerable to fungal attack. Many soil fungi are found to be associated with M. incognita eggs perhaps due to gelatinous matrix, which provide nutritional source for fungal growth and development. Among the egg parasitizing fungus P. lilacinus is a non-obligate fungus and considered as an efficient biocontrol agent of plant parasitic nematodes. Isolates from Pelotes, provided 80 % egg parasitism while 50 isolate from Atalaia, exhibited 3 % parasitism (Santos et al., 1992) there was considerable variation among the isolates of P. lilacinus is attributed to various biotic and abiotic factors resulting in selective survival in natural environment. Therefore screening of virulent isolate of a biocontrol agent should be carefully undertaken while developing a potential biocontrol agent.

P. lilacinus has been reported to produce peptidal antibiotics lilacinin; lecinostain and paecilo toxin (Arae et al 1973, Mikami et al 1989) acetic acid was also identified from culture filtrate of this fungus, which affects the movement of nematode (Dijan et al., 1991). Therefore, nematicidal action of culture filtrates of P. lilacinus may possibly be due to the action of antibiotics together with various other fungal metabolic products. Methanol extracted fractions of biocontrol agent, P. lilacinus were found to be more effective than ethyl acetate and hexane extracts in the suppression of M. incognita larvae, indicating that active nematicidal compounds are intermediary in polarity and present as a polar chemical entities. Various mechanisms have been suggested for the biocontrol activity of Trichoderma spp. against phytopathogenic fungi: antibiosis, competition, mycoparasitism, and enzymatic hydrolysis. Enzymes such as chitinases, glucanases, and proteases seem to be very important in the mycoparasitic process. All mechanisms, except competition, can potentially be involved in the nematode biocontrol process. Information about the possible mechanisms of this fungal activity against nematodes is very limited; understanding these processes could lead to the development of improved biocontrol application methods and selection of active isolates. Proteinases are involved in host-parasite interactions, and correlations between pathogenicity and proteinase activity have been reported for plant pathogens (Flores et al., 1997). Proteinases are also involved in nematophagous fungal activity on nematode eggs (Dackman et al., 1989), e.g., proteases purified from Verticillium suchlasporium (Lopez-Llorca and Robertson, 1992), V. chlamydosporium (Segers, R., 1996), and Paecilomyces lilacinus (Bonants, P. J. M., 1995). Through a combination of enzymes and mechanical pressure the fungus penetrates the eggshell and moves to parasitise the developing juvenile (Segers et al., 1996)

Biological management of root knot nematode disease complex of tomato crops has been achieved by seed treatment, bare root dip treatment or soil application with various formulations of P. lilacinus (Powell, 1971; Pandey et al., 2005). But the success of a formulation of biocontrol agent depend on the potentiality of bioagents and its ability to produce inoculum in excess and to survive, grow and

proliferate well on seed coat, spermosphere, root zone, root system and rhizosphere of the growing plants. Such type of antagonistic establishment needs a substrate during seed treatment, which can provide a better coating on seed surface and food base to antagonist for better proliferation. In the present investigation promising results were obtained with different isolate-based formulation of P. lilacinus against root knot nematode disease complex. The highest increase in plant growth parameter viz. root length, shoot length, root weight, shoot weight were obtained with isolate of P. lilacinus from Hyderabad (PL-4) based formulation followed by New Delhi isolate (PL-1), Anand isolate (PL-2) and Shillong isolate (PL-3). Similarly, seed germination were found quite good in seed treated with isolate of P.lilacinus from Hyderabad (PL-4) based formulation followed by New Delhi isolate (PL-1), Anand isolate (PL-2) and Shillong isolate (PL-3) while the mortality of seedlings at nursery stage were very less in seed treated with isolate of P. lilacinus from Hyderabad (PL-4) based formulation followed by New Delhi isolate (PL-1), Anand isolate (PL-2) and shillong isolate (PL-3). Maximum reduction in root galling, egg mosses, egg per egg moss, larvae, female and nematode population in soil were observed in isolate of P. lilacinus from Hyderabad (PL-4) based formulation followed by New Delhi isolate (PL-1), Anand isolate (PL-2) and Shillong isolate (PL-3). The data presented in this paper provide evidence that Paecilomyces lilacinus may provide opportunities to control plant-parasitic nematode Meloidogyne incognita. It is likely that the nematicidal and antifungal effects of culture filtrates of P. lilacinus may result from the synergistic effect of the chemical components present in the system. Such synergistic or antagonistic action probably may also occur in present study. The lower efficacy of shillong and anand isolate might have been due to the low activity or absence of toxic compounds in the culture filtrate. The present findings suggests that, due to their strong nematicidal activities, hyderabad and new delhi isolate can be exploited as a ecofriendly nematicide especially without the increase in financial constraints due to ever increasing pesticide costs and environmental considerations. Apart from the present findings P. lilacinus strains are easy to produced in vitro, rhizosphere competent; attacks the eggs of several nematode species and easy to formulate in various formulations makes it a suitable candidate for biocontrol agent. There is however, still a need to isolate and identify the active components of the above said isolates, which are inhibitory to Meloidogyne incognita, actually nematotoxic, which, in turn may enhance the economic effectiveness of the fungal pesticides.

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Flask containing profusely mycelium and culture filtrate of different isolate of P. lilacinus.