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Evaluation of *Pochonia chlamydosporia* (Goddard) Isolates for Suppression of *Meloidogyne incognita*, Root-Knot Nematode of Tomato

Muhammad Nazir Uddin[1], Saifullah[2], Musharaf Ahmad[2], Wajid Khan[1] & Bilal Muhammad Khan[3]

[1] Center for Biotechnology and Microbiology, University of Swat, Pakistan

[2] Department of Plant Pathology, Khyber Pakhtunkhwa, Agricultural University Peshawar, Pakistan

[3] Guangdong Provincial Key Laboratory of Marine Biotechnology, STU-UNIVPM Joint Algal Research Center, Department of Biology, College of Science, Shantou University, Shantou, Guangdong, China

Correspondence: Wajid Khan, Center for Biotechnology and Microbiology, University of Swat, Pakistan. E-mail: sherafghan.shah@gmail.com

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Abstract

Restricted applications of chemical nematicides has directed to the development of ecofriendly alternatives. Culture filtrates in different concentrations (20, 40, 60, 80 and 100%) of the six isolates of *Pochonia chlamydosporia* were evaluated for their nematicidal activity. Significant variations were observed in the inhibitory activity of culture filtrates against egg hatching and second stage juveniles of *M. incognita* at different concentrations. The culture extract of PC-6 showed maximum inhibition of egg hatching followed by PC-1. On the other hand PC1 isolate caused high mortality of second stage juveniles followed by PC-6. Significant variation among the isolates was observed for parasitism of *M. incognita* juveniles and eggs. Among the tested isolates, PC-1 and PC-6 were selected for the further studies based on *in vitro* egg inhibition and larval mortality. Both PC1 and PC6 applied at the rate of 6×10^3 chlamydospores per gram of soil per plant reduced the population of nematode and improved the growth and productivity of plant under screen house and field conditions. Different growth parameters like plant height, fresh shoot weight, dry shoot weight, fresh root weight and dry root weight were measured. Efficacy of isolates was different in winter and summer crop. No significant difference among the treatments was observed in summer crop. However in winter crop, considerable effect of both isolates was observed. Fungal application was effective at the rate of 6×10^3 chlamydospores per gram of soil per plant in both winter and summer crops. Root coating with chlamydospores suspension significantly reduced number of galls on roots. Little effect of both isolates was observed with mycelial root coating. No lesions were seen on *P. chlamydosporia* inoculated roots of tomato plants.

Keywords: culture filtrates, pathogen, biocontrol, chlamydospores

1. Introduction

Root-knot nematode (*Meloidogyne incognita*) is a major and endemic pathogen of tomato crop in northern part of Khyber Pakhtunkhwa province of Pakistan (Musarrat, Firoza, & Shahina, 2006). Average crop losses due to this pathogen in vegetable crops may reach up to 50-80% (Ornat & Sorribas, 2008; Sikora & Fernandez, 2005). Characteristic symptoms of infected plant are knots or galls on roots at the point of infection (Agrios, 2004). These galls disrupt the vascular tissue of roots that interfere with uptake of water and mineral and translocation of photosynthate resulting in stunted growth, chlorosis and wilting of the plant (Palomares-Rius, Escobar, Cabrera, Vovlas, & Castillo, 2017). Chemical control is a common mean of controlling plant diseases (Hirooka & Ishii, 2013; Duponnois, Chotte, & Sall, 2001). However, chemicals penetrate poorly into roots tissue to contact nematode eggs. They also can cause environmental problems (Garima, Singh, & Trivedi, 2005; Ploeg, 2002; Kerry, 2000). The use of resistant cultivars is a valuable component in Root-knot nematode management but in many important crops their resistant cultivars are not available. Moreover the effectiveness of some resistant cultivar is restricted to a few races of the nematode (Briar, Wichman, & Reddy, 2016). *Meloidogyne incognita* has a wide host range, which makes crop rotation impracticable. Thus, for controlling this nematode, long term integrative approches and development of alternatives of chemical pesticide is urgently needed to protect our crops

from the risk of this pathogen (Martin, 2003). The nematophagous fungus *P. chlamydosporia* (Goddard) is an egg nematode parasite and has been found as an effective in biological management of cyst and Root-knot nematodes (Kerry & Bourne, 2002). The fungus survives in the soil as chlamydospores (Manzanılla-Lopez et al., 2013) and develops colonies around nematodes eggs and attached with eggs shells through appressoria (Lopez-Llorca, Macia-Vicente, & Jansson, 2008). Then the fungus penetrates into egg by physical pressure and enzymatic activities of different enzymes including proteases, lipases, chitinases and collagenases (Tikhonov, Lopez-Llorca, Salinas, & Jansson, 2002; Kopcke, Wolf, Anke, & Sterner, 2001). *P. chlamydosporia* isolates showed variation in their bio control efficiency depending on different factors *i.e.*, fungal growth rate and its initial population in the rhizosphere, chlamydospores and enzymes productions, availability of water and fungal growth supported by the host plant species (Manzanilla-López et al., 2013; Bourne & Kerry, 2000). Utilization of biological agents in a management of Root-knot nematode in tomato crop is a good alternative of chemical pesticides in places infested with root-knot nematodes. Therefore the present study was designed for the collection of isolates of *P.chlamydosporia* from different region of Khyber Pakhtunkhawa, Pakistan and to evaluate their efficacy against *M. incognita*.

2. Materials and Methods

2.1 Survey and Sampling

Root-knot nematodes field were surveyed for isolation of *P. chlamydosporia.* About 150 roots and rhizosphere soil were randomly collected from 35 different locations of Khyber Pakhtunkhwa province of Pakistan. Samples were sealed in plastic bags and brought from field to laboratory at the department of Plant Pathology, the Agriculture University and stored in refrigerator at 4 °C until use.

2.2 Separation of Root-knot Nematodes From Roots

Eggs and females of root-knot nematode were separated from the gall roots, collected randomly from field following standard method of Barker et al. (1985). Briefly, pieces of gall root were rinsed with tap water for 3-5 min to remove the soil, cut into small pieces and then blended in an electric blender. Nematode eggs were collected on 26-µm-aperture sieve while the females on 75 µm sieve. The females and eggs were stored in 1% saline solution in separate beakers (Hussey & Barker, 1973).

2.3 Identification and Mass Production of P. chlamydosporia

Sodim hyphochloride solution (NaOCl 1%) was used for surface steralization of females and egg masses for 30 seconds to relaese eggs. After sterilization the eggs were rinsed three times with sterile distilled water. The eggs were then put on semiselective medium developed by Kerry et al. (1993). The composition of media per liter was 17 g corn meal agar (Oxoid), 37.5 mg carbendazim, 75 mg rose bengal, 37.5 mg thiabendazole, 17.5 mg NaCl and 3 ml Triton X 100, supplemented with 0.2 g/L of streptomycin sulfate and 5 mg/L of penicillin. The plates were incubated at 25 °C. After three days, *P. chlamydosporia* isolates were identified (Barnett & Hunter, 1998) and then grown on grains for mass production of *P. chlamydosporia*. Extraction of chlamydospores was done using standard method of (Crump & Kerry, 1981).

2.4 Culture Filtrate (CF) of Pochonia chlamydosporia

Small agar pieces (4-5 cm) of fresh culture of *P. chlamydosporia* were put in to 250 ml Erlenmeyer flasks containing sterile potato dextrose broth. The flasks were sealed and incubated at 25 °C for 10 days on a rotatory shaker. The filtrate was centrifuged at 8,000 rpm for 20 minutes. Mycelial mat, hyphae and spore were removed passing through sterile Whatman filter paper (0.2 µm) (Kerry, 1986) and stored at 4 °C.

2.5 Single Species Nematode Culture

Single egg mass and identified female of *Meloidogyne incognita* was collected and inoculated in 3 weeks old nursery seedlings of tomato cv. Money maker (tomato)were raised in earthen pots containing sterilized soil. The egg masses were collected 50 days after inoculation and re-cultured them.

2.6 Effect of Culture Filtrate on Egg Hatching and J_2 Mortality

Effect of culture filtrate of *P. chlamydosporia* isolates on *M. incognita* was determined by using standard method described by (Mukhtar & Pervaz, 2003). Different concentrations (20, 40, 60, 80 and 100%) of culture filtrate were pepared. Approximately 100 eggs of *M. incognita* from freshly cultured inoculum were treated with 1% sodium hypochlorite (NaOCl) solution for 30 seconds and then transferred to into 5 cm diameter petri plates containing 5 ml of each dilution of culture filtrate. Eggs in sterilized distilled water were used as control. The treatments were replicated six times at intervel of 24, 48 and 72 hours. For testing the effect of culture filtrate on J_2 mortality**,** five milli-litre (5 ml) of each dilution of the culture filtrate was poured in 5 cm diameter petri plates.

Each petridish was plated with 50 fresh hatched second stage juveniles of *M. İncognita.* Sterilized distilled water having juviniles was used as control. The treatments were replicated six times. Effect of culture filterate was checked at 24, 48 and 72 hrs. Under a stereomicroscope the the number of live or dead juveniles were counted by probing nematodes with a fine needle to differentiate paralyzed or dead nematodes from live ones (Ayatollahy, Fatemy, & Etebarian, 2008).The assay was repeated twice. Percentage of juvenile mortality was noted (Sun, Gao, Shi, Li, & Liu, 2006).

2.7 Parasitism of Eggs and J_2

Five isolates of *P. chlamydosporia* were tested against *M. incognita* eggs and J_2 for their parasitism. The experiment was performed in 5 cm diameter petri plates filled with 1.5% water agar. Five hundred micro-litre (500 µl) drop containing 100 eggs and 50 freshly hatched juveniles of *M. incognita* was spread on media plate in laminar flow cabinet. Plates were incubated at 25 °C for 15 days. Sterile distilled water containing eggs or juviniles were used as control (Freire & Bridge, 1985; De Leij & Kerry, 1991). Each treatment was replicated six times. Eggs and juveniles were stained with acid fuchsin (3.5 gm acid fuchsin in 250 ml of acetic acid and 750 ml of distilled water) and then examined under the microscope. Number of infected and uninfected juviniles and eggs were counted (Kerry & Crump, 1977).

2.8 Root Coating With Mycelia of P. chlamydosporia

Plugs (4-5 cm) of 10 days old culture were transferred in to erlenmeyer flasks containing potato dextrose broth and then incubated in shaking incubator at 170 rpm for 10 days at 25 °C. Mycelial mat was cut into fragments with a blender and poured in beaker. Roots of tomato seedlings were dipped in the beaker and hand shaken for 5-10 seconds and then planted in pots and field.

2.9 Root Coating With Chlamydospores of P. chlamydosporia

Wet roots of tomato seedlings were dipped in the bottles containing chlamydospores developed on millet and hand shaken for 10-20 seconds so that the spores adhere to the roots. The seedlings were transplanted to pots and field.

2.10 Screen House Study

Seedlings of tomato germplasm (Money Maker) was grown in earthen pots in screen house. Seedling of three weeks old were planted in plastic pots filled with autoclaved soil (2.5 kg) with sand, silt and clay contents of 2:1:1. Seven days after trasplantation, 10,000 fresh eggs of *M. incognita* were applied to rhizosphere of each plant. Small holes around the plants were made and eggs and biocontrol agent was applied according to the treatments. The experiment was performed with fifteen treatment and five replication arranged in randomized complete block design (RCBD) (Table 1).

Table 1. List of treatment applied to tomato germplasm under screen house conditions

Treatment	Description
T0	Control-1 (no nematodes and no fungus)
T1	Control-2 (only nematodes and no fungus)
T2	only fungus PC-1
T3	only fungus PC-6
T4	Two thousand chlamydospores (PC-1)/g of soil + nematodes
T5	Four thousand chlamydospores (PC-1)/g of soil + nematodes
T6	Six thousand chlamydospores (PC-1)/g of soil + nematodes
T7	Two thousand chlamydospores (PC-6)/g of soil + nematodes
T8	Four thousand chlamydospores (PC-6)/g of soil + nematodes
T9	Six thousand chlamydospores (PC-6)/g of soil + nematodes
T10	Substrate alone (no fungus application)
T11	Root coating with *P. chlamydosporia* mycellia (PC-1)
T12	Root coating with *P. chlamydosporia* mycellia (PC-6)
T13	Root coating with *P. chlamydosporia* chlamydospores (PC-1)
T14	Root coating with *P. chlamydosporia* chlamydospores (PC-6)

The experiment was terminated after 60 days of inoculation. Data were noted for shoot length (cm), fresh and dry shoot weight (g), fresh and dry root weight (g), number of galls and number of eggs per plant root system, numbers

of flowers and fruits per plant, and fruits weight per plant (kg).

2.11 Field Experiment

Field study was conducted in a root-knot nematode infested field in the two crop seasons *i.e*., summer and winter crop. Tomato *cv.* Rio-grande was used in both seasons. Twelve (12) treatments were applied to four week old seedlings in randomized complete block design and 4 replications. Each replication consisted of 10 plants. Cross contamination between treatments was prevented by following the practices suggested by (Verdejo-Lucas, Sorribas, Ornat, & Galeano, 2003). Treatments applied in field experiment were listed in Table 2.

Table 2. List of treatment applied to tomato germplasm under field conditions

Treatment	Description
To	Control (no fungus)
T1	Two thousand Chlamydospores (PC-1)/g of soil
T2	Four thousand Chlamydospores (PC-1)/g of soil
T3	Six thousand Chlamydospores (PC-1)/g of soil
T4	Two thousand Chlamydospores (PC-6)/g of soil
T5	Four thousand Chlamydospores (PC-6)/g of soil
T6	Six thousand Chlamydospores (PC-6)/g of soil
T7	Substrate alone (no fungus)
T8	Root coating with *P.chlamydosporia* mycellia (PC-1)
T9	Root coating with *P.chlamydosporia* mycellia (PC-6)
T10	Root coating with *P.chlamydosporia* chlamydospores (PC-1)
T11	Root coating with *P.chlamydosporia* chlamydospores (PC-6)

Field experiment was ended at 50 days after transplantation. Data regarding different parameters noted in sreen house trial was also recorded in field trial. Furthere more initial nematode population (*Pi*) and final nematode population (*Pf*) were also measured in field trial.

2.12 Stastical Analysis

The data collected from field, green house and experiments were subjected to analysis of variance (ANOVA) using MSTAT-C (Statistical software package). Least significant difference test (LSD) test was applied to the data for the separation of means at $P \leq 0.05$ (Steel &Torrie, 1980).

3. Results

Under *in vitro* conditions, culture filtrate of *P. chlamydosporia* was active against the root-knot nematode, *M. incognita*, at different concentrations. Among the tested isolates PC-1 and PC-6 were more effective either in egg hatch inhibition, killing juviniles and infecting eggs of the fungal culture (Figures 1 and 3). Egg hatching inhibition was increased with increasing the concentration of culture filtrate (Figure 2). PC-6 fungal extract showed maximum inhibition of egg hatch (58.17%) followed by PC-1 extract. Minimum egg hatch inhibition was noted in in PC-4 filtrate with 20% concentration (Figure 1).

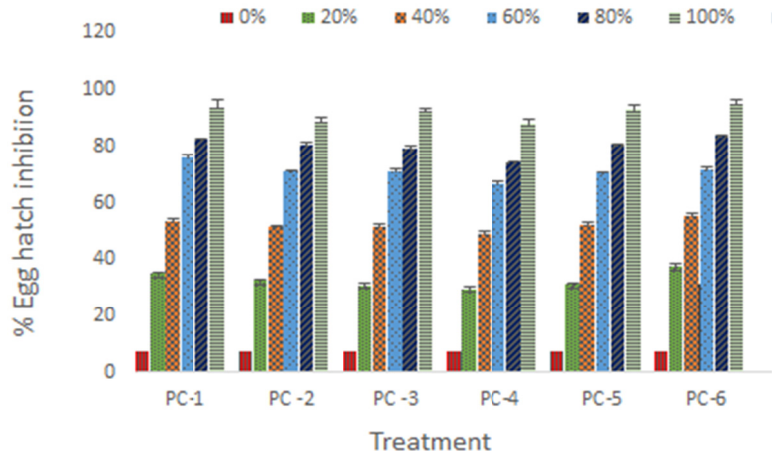

Figure 1. Efficacy of different concentrations of extract of *Pochonia chlamydosporia* isolates on egg hatching of *M. incognita* after 72 h at 25±1 °C (Bar represent mean±SD at $P < 0.05$)

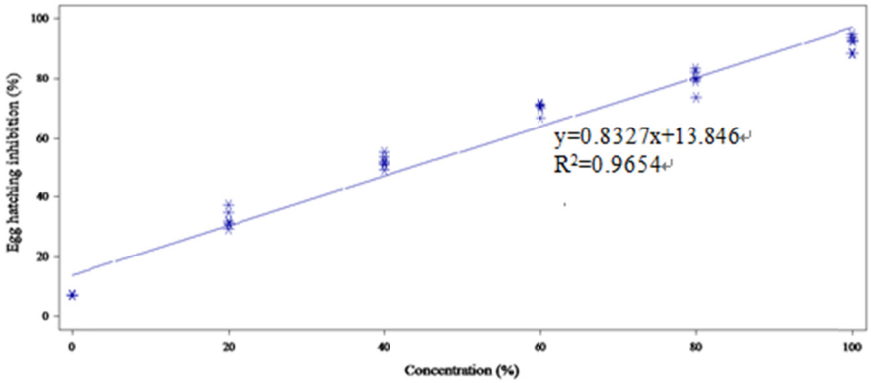

Figure 2. Egg hatching inhibition (%) of *M. incognita* against the tested concentrations of the culture filtrate of the isolates of *P. chlamydosporia* ($R^2 = 0.9654$)

Potato dextrose broth medium and water did not produced any negative impact on egg hatch inhibition and mortality of juviniles and thus they did not differ significantly from each other. The effect of *P. chlamydosporia* filtrate and their concentrations on mortality of second stage juveniles was significant as compared to control ($P \leq 0.05$). Percent mortality of *M. incognita* juveniles was increased with increasing concentration of the culture filtrate (Figure 4). The impact of isolates on mortality of *M. incognita* juveniles was in the range of 11.3-76.3% after 72 h. Comparison of the tested samples indicated that the culture filtrate of PC-6 killed maximum number of *M. incognita* J2 (76.3%) followed by PC-1 (74.0%) at 100% concentration. The lowest J2 mortality (11.3%) was noted in PC-4 filtrate at 20% concentration. Sterile distilled water (control) caused smallest mortality of J2 (7%).

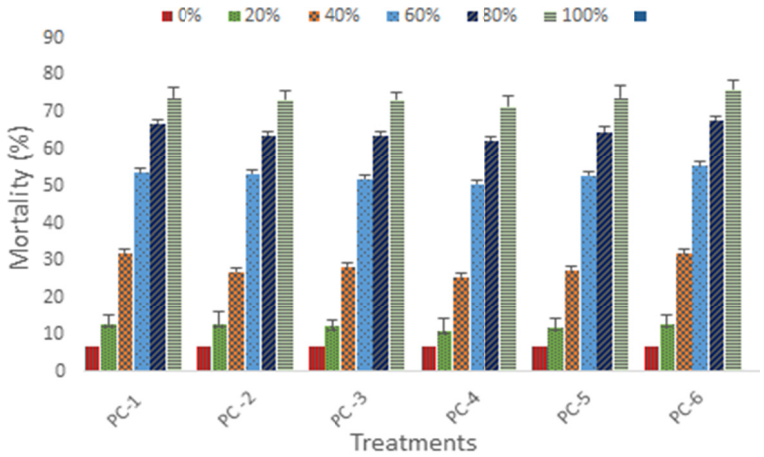

Figure 3. Efficacy of different concentrations of extract of *Pochonia chlamydosporia* isolates on Mortality (%) of *M. incognita* after 72 h at 25±1 °C (Bar represent mean±SD at $P < 0.05$)

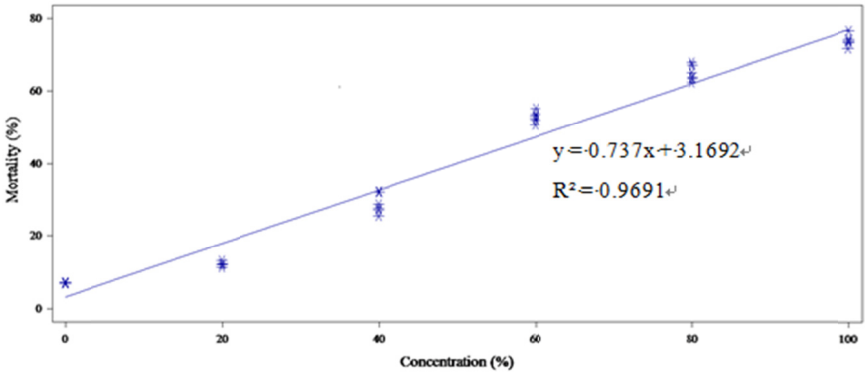

Figure 4. Mortality (%) of *M. incognita* against the tested concentrations of the culture filtrate of the isolates of *P. chlamydosporia* ($R^2 = 0.9691$)

On the other hands, the eggs and juveniles were infected by isolates after 72 h of the exposure time. All the isolates showed significant variation ($P \leq 0.05$) in parasitism of *M. incognita* eggs and PC-1 caused maximum egg parasitism (73.7%) as compared to other isolates (Table 3).

Table 3. Parasitism of different *Pochonia chlamydosporia* isolates on *Meloidogyne incognita* eggs and J_2 after 72 h at 25±1 °C

Treatments	**Egg Parasitism**	**J2 Parasitism**
Sterilized distilled water (Control)	0 D	0 D
VC-1	73. 7 A	52.0 AB
VC-2	68.0 B	49.3 BC
VC-3	65.0 B	45.7 BC
VC-4	56.3 C	46.0 C
VC-5	66.7 B	49.3 BC
VC-6	68.3 B	54.0 A
Means	**56.9**	**42.3**

Note. *LSD value for egg parasitism = 4.400; LSD value for J_2 = 4.070; Means in the same column followed by different letters are significantly different at 0.05 level of probability.

This was followed by PC-6 and PC-2 with egg parasitism of 68.3% and 68.0%, respectively. As far as J2 infection is concerned, the maximum number of larvae (54.0%) parasitized by PC-6 followed by PC-1 (52.0%). On the basis of larval mortality and *invitro* egg inhibition PC-1and PC-6 were selected to study their efficacy

against *M. incognita* under screen house and field conditions. Significant differences ($P \leq 0.05$) were observed among all treatments under screen house conditions (Table 4). Treatment of soil (6×10^3 chlamydospores per gm of soil) with both isolates (PC1 and PC-6) resulted in greater efficacy than the other treatments. Application of chlamydospores applied at rate of 6×10^3 and 2×10^3 chlamydospores per g of soil were significant ($p < 0.05$). However, there were no considerable affect using the higher application rate and non-significant differences were observed between treatments of 4×10^3 and 6×10^3 chlamydospores per g of soil. Among the treatments, T_9 caused significant higher inhibitory activity of nematode, strong inhibition of gall formation on the roots of tomato plant than those of other formulation.

Table 4. Evaluation of *P. chlamydosporia* for controlling *Meloidogyne incognita* in tomato under screen house conditions

Treatments	Plant height (cm)	Fresh shoot wt (g)	Dry shoot wt (g)	Fresh root wt (g)	Dry root wt (g)	Galls/root system	Egg masses/g galled tissue	Flowers/plant	Fruits/plant	Fruit wt/plant (Kg)
T_o	31.00 A	35.92 A	16.40 A	11.58 E	2.36 D	0.00 H	0.00 H	44.00 A	33.60 A	1.556 A
T_1	18.60 E	19.57 F	11.46 F	16.09 A	4.21 A	195.6 A	84.80 A	23.20 E	15.20 F	0.573 D
T_2	29.60 A	31.12 AB	14.95 ABC	12.33 DE	2.32 D	0.00 H	0.00 H	42.40 AB	31.40 AB	1.423 A
T_3	30.40 A	35.69 A	15.20 AB	12.46 DE	2.33 D	0.00 H	0.00 H	42.80 AB	32.60 A	1.481 A
T_4	20.60 CDE	24.63 CDEF	12.78 BCDEF	13.22 CD	3.04 BC	69.60 E	67.20 BC	32.20 BCDE	19.60 CDEF	0.722 CD
T_5	22.20 BCD	26.98 BCDE	14.00 ABCDE	12.73DE	2.70 CD	52.60 F	65.80 BCD	35.40 ABCD	22.40 CDEF	0.774 BCD
T_6	24.80 B	29.26 BC	14.50 ABCD	12.66 DE	2.40 D	38.60 FG	53.80 DEF	35.60 ABCD	24.60 BCD	1.013 B
T_7	20.60 CDE	24.78 CDEF	12.54 CDEF	12.76 DE	3.02 BC	71.00 E	51.40 EFG	34.00 ABCDE	23.40 CDE	0.747 BCD
T_8	22.80 BC	28.52 BCD	14.30 ABCDE	12.70 DE	2.34 D	45.60 FG	45.00 FG	35.80 ABCD	24.00 BCDE	0.768 BCD
T_9	25.60 B	30.42 AB	14.82 ABC	12.54 DE	2.20 D	37.60 G	40.20 G	38.40 ABC	27.20 ABC	1.003 BC
T_{10}	19.20 DE	20.00 F	11.95 EF	15.18 AB	3.52 B	171.2 B	76.40 AB	27.00 DE	16.40 EF	0.651 D
T_{11}	19.60 CDE	21.96 EF	12.53 CDEF	14.69 ABC	3.39 B	148.6 C	67.40 BC	30.00 CDE	17.20 DEF	0.685 D
T_{12}	20.40 CDE	24.02 CDEF	13.03 BCDEF	13.70 BCD	2.61 CD	137.8 C	59.80 CDE	35.00 ABCD	20.00 CDEF	0.753 BCD
T_{13}	20.40 CDE	23.33 DEF	12.23 DEF	13.62 CD	3.48 B	151.0 C	65.40 BCD	29.40 CDE	19.60 CDEF	0.684 D
T_{14}	21.60 CDE	24.38 CDEF	12.81 BCDEF	13.45 CD	2.61 CD	120.8D	54.20 DEF	34.60 ABCD	22.20 CDEF	0.759 BCD
$LSD_{0.05}$	3.596	5.564	2.542	1.518	0.574	14.255	12.379	11.153	7.684	0.289

Note. * Means in the same column followed by different letters are significantly different at 0.05 level of probability ;To; Control-1 (no nematodes and no fungus, T1; Control-2 (only nematodes and no fungus) T2; only fungus PC-1, T3; only fungus PC-6, T4; 2000 chlamydospores (PC-1) per gram of soil + nematodes, T5; 4000 chlamydospores (PC-1) per gram of soil + nematodes, T6; 6000 chlamydospores (PC-1) per gram of soil + nematodes, T7; 2000 chlamydospores (PC-6) per gram of soil + nematodes, T8; 4000 chlamydospores (PC-6) per gram of soil + nematodes, T9; 6000 chlamydospores (PC-6) per gram of soil + nematodes T10; Substrate alone (no fungus application) T11; Root coating with *P. chlamydosporia* mycellia (PC-1), T12; Root coating with *P. chlamydosporia* mycellia (PC-6), T13; Root coating with *P. chlamydosporia* chlamydospores (PC-1), T14; Root coating with *P. chlamydosporia* chlamydospores (PC-6).

Significant increase was noted in plant height (cm) (37.6%), fresh shoot wt (g) (55.4%) and dry shoot wt (g) (29.3%) as compared to control under screen house conditions (Table 5). Fresh root weight (g), dry root weight (g), number of galls per plant and number of egg masses per gram of galled tissue of root were decreased by 22.1%, 47.7%, and 80.8% and 53.0% respectively. Number of flowers and tomato yield in terms of numbers of fruits and total fruit weight were also increased in treated pots. In summer field trials, data indicated that no significant difference was observed among treatments (Table 5). However, considerable effect of the fungal applications was seen in the winter crop (Table 6). T_6 was most effective than other formulations. Plant height (44.3%), fresh shoot wt (g) (51.0%) and dry shoot wt (g) (70.3%) were increased as compared to control. Fresh root weight (g) (38.6%), dry root weight (g) (35.5%), number of galls per plant (54.9%) and number of egg masses per gram of galled tissue of root (66.3%) were effectively reduced. Significant increase occurred in number of flowers (51.0%), fruits (34.6%) and total fruit weight (62.1%). The Root-knot nematode final population (Pf) of winter crop significantly decreased in all the treatments. Maximum decrease in final population was recorded in T_6 (46.9%).

Table 5. Evaluation of *P. chlamydosporia* for controlling *Meloidogyne incognita* in summer crop under field conditions

Treatments	Plant height (cm)	Fresh shoot wt (g)	Dry shoot wt (g)	Fresh root wt (g)	Dry root wt (g)	Galls/root system	Egg masses/g galled tissue	Flowers/ plant	Fruits/ plant	Fruit wt/ plant (Kg)	*Pi*	*Pf*
T_o	68.91	79.88	17.730	26.41	8.61	146.25	85.13	80.13	38.563	1.87	165.	192.125
T_1	84.00	83.995	19.90	19.69	7.76	139.00	70.63	84.75	45.813	2.20	163.19	151.750
T_2	89.69	85.19	23.168	16.50	6.55	133.00	64.50	87.13	50.500	2.55	167.81	150.125
T_3	90.91	87.49	23.927	14.58	6.27	130.00	63.19	97.99	54.750	2.71	159.33	134.750
T_4	86.93	85.90	21.400	20.79	7.55	137.00	76.19	87.38	47.375	2.40	171.00	155.125
T_5	90.31	86.895	23.413	16.29	6.67	134.00	68.50	89.44	53.688	2.69	167.44	152.063
T_6	96.71	87.72	24.560	14.60	6.38	129.25	62.50	104.19	59.375	3.02	152.88	138.563
T_7	72.95	79.04	18.00	25.64	8.46	144.50	84.75	80.38	39.00	1.96	158.31	180.563
T_8	76.01	79.94	17.967	23.00	8.26	135.25	74.44	82.08	39.688	1.99	160.75	152.00
T_9	82.69	87.33	21.475	20.90	6.91	128.00	73.38	86.75	47.250	2.36	172.75	156.813
T_{10}	87.495	86.63	18.213	22.02	8.34	138.50	71.63	81.63	42.750	2.16	165.31	148.875
T_{11}	88.00	87.43	21.950	17.70	6.87	133.25	69.81	86.13	47.938	2.50	163.94	151.313
$LSD_{0.05}$	**16.454**	**7.033**	**9.555**	**11.142**	**1.856**	**20.932**	**15.287**	**14.439**	**15.30**	**0.819**	**41.120**	**42.574**

Note. *Means in the same column followed by different letters are significantly different at 0.05 level of probability. To; Control (no fungus), T1; 2000 Chlamydospores (PC-1) per gram of soil, T2; 4000 Chlamydospores (PC-1) per gram of soil, T3; 6000 Chlamydospores (PC-1) per gram of soil, T4; 2000 Chlamydospores (PC-6) per gram of soil, T5; 4000 Chlamydospores (PC-6) per gram of soil; T6; 6000 Chlamydospores (PC-6) per gram of soil; T7; Substrate alone (no fungus), T8; Root coating with *P.chlamydosporia* mycellia (PC-1), T9; Root coating with *P. chlamydosporia* mycellia (PC-6), T10; Root coating with *P. chlamydosporia* chlamydospores (PC-1); T11 Root coating with *P.chlamydosporia* chlamydospores (PC-6).

Table 6. Evaluation of *P.chlamydosporia* for controlling *Meloidogyne incognita* in winter crop under field conditions

Treatments	Plant height (cm)	Fresh shoot wt (g)	Dry shoot wt (g)	Fresh root wt (g)	Dry root wt (g)	Galls/root system	Egg masses/g galled tissue	Flowers/ plant	Fruits/ plant	Fruit wt/ plant (Kg)	*Pi*	*Pf*
T_o	60.50 D	59.99 F	12.95 E	23.65A	9.530 A	128.5 A	87.63 A	58.94 D	37.75 E	1.672 D	192.1 A	220.1 A
T_1	73.36 ABCD	70.64 DEF	16.82 ABCDE	22.11AB	7.925 ABC	82.06 E	51.88 BC	79.88 ABC	42.81 BCDE	2.150 BCD	151.8 ABC	132.8 C
T_2	78.90 ABC	83.23 ABCD	19.91 ABCD	19.06ABC	6.935 BC	78.00 EF	40.00 CDEF	82.44 ABC	45.69 ABC	2.287 ABC	150.1 ABC	127.7 C
T_3	84.17 AB	88.03 AB	20.92 AB	17.39BC	6.755 BC	59.25 G	30.38 EF	87.19 A	49.38 AB	2.495 AB	134.8 C	117.4 C
T_4	78.32 ABC	72.13 CDEF	16.86 ABCDE	21.80AB	6.932 BC	81.69 E	54.56 B	83.75 ABC	43.13 BCDE	2.283 ABC	155.1 ABC	157.8 BC
T_5	82.97 AB	86.80 ABC	20.75 ABC	17.72BC	6.912 BC	63.38 EG	39.31 DEF	85.06 AB	48.13 ABC	2.365 AB	152.1 ABC	117.2 C
T_6	87.31 A	90.56 A	22.05 A	14.52C	6.145 C	58.00 G	29.50 F	89.00 A	50.81 A	2.710 A	138.6 BC	116.9 C
T_7	62.00 D	63.29 EF	15.46 DE	22.46AB	9.145 A	122.8 AB	84.25 A	66.88 CD	38.13 DE	1.748 CD	180.6 AB	199.8 AB
T_8	64.37 CD	70.50 DEF	15.54 CDE	21.74AB	8.145 AB	108.1 BC	55.44 B	69.94 BCD	41.63 CDE	1.765 CD	152.0 ABC	124.0 C
T_9	71.10 BCD	71.71 DEF	16.75 BCDE	21.48AB	7.555 ABC	91.69 DE	41.75 CDE	76.94 ABC	44.38 ABCDE	2.082 BCD	156.8 ABC	127.1 C
T_{10}	69.63 BCD	75.67 BCDE	18.13 ABCDE	21.69AB	8.622 AB	100.4 CD	49.75 BCD	76.75 ABC	42.38 BCDE	1.960 BCD	148.9 BC	142.6 C
T_{11}	72.35 ABCD	77.50 ABCDE	18.87 ABCD	21.22AB	7.733 ABC	79.06 E	41.56 CDE	80.63 ABC	45.38 ABCD	2.225 ABCD	151.3 ABC	130.1 C
$LSD_{0.05}$	16.018	14.884	5.281	5.082	1.980	15.624	11.896	17.107	7.434	0.553	42.574	42.973

Note. *Means in the same column followed by different letters are significantly different at 0.05 level of probability. To; Control (no fungus), T1; 2000 Chlamydospores (PC-1) per gram of soil, T2; 4000 Chlamydospores (PC-1) per gram of soil, T3; 6000 Chlamydospores (PC-1) per gram of soil, T4; 2000 Chlamydospores (PC-6) per gram of soil, T5; 4000 Chlamydospores (PC-6) per gram of soil; T6; 6000 Chlamydospores (PC-6) per gram of soil; T7; Substrate alone (no fungus), T8; Root coating with *P. chlamydosporia* mycellia (PC-1), T9; Root coating with *P.chlamydosporia* mycellia (PC-6), T10; Root coating with *P.chlamydosporia* chlamydospores (PC-1); T11 Root coating with *P.chlamydosporia* chlamydospores (PC-6).

4. Discussion

P. chlamydosporia has been reported as biological control agent against cyst and root-knot nematodes (Saifullah,

1996). The isolates have variable effect on *M. incognita* (Bourne, Kerry, & De Leij, 1994; Kerry Jaffee, 1997). Current study showed that culture filtrate of *P. chlamydosporia* isolates strongly affected J2 mortality and egg hatching of *M. incognita*. We also noted that increasing the concentration of culture filtrate increased egg hatch inhibition and J_2 mortality, and thus confirming the previous finding (Mukhtar & Pervaz, 2003). Effect of the CF from several fungi on egg hatch and J_2 mortality of nematodes have also been previously reported (Randhawa, Singh, Sandhu, & Bhatia, 2001; Zaki, 1999). Isolates PC-6 and PC-1 strongly inhibited egg hatch and killed J_2. Both isolates (PC-1 and PC6) showed considerable variations in egg parasitism. PC-1 parasitized maximum number of eggs (73.7%) followed by PC-6 (68.3%). Ebadi et al. (2009) also recorded more than 85% of *M. javanica* eggs parasitized by *P. chlamydosporia*.

Non-significant difference was noted between PC-1 and PC-6 in the case of juvenile mortality. On the other hand PC-1 seems to be more effective than PC-6 in killing nematode eggs. This may be due the high production of egg shell degrading enzymes by PC-1. Nematicidal effect was not seen in potato dextrose broth (culture media) and thus did not differ significantly as compared to water (control). This supports the finding of Singha and Mathurb (2010) and contradicts the findings of Nitao et al. (1999).

The selected isolates (PC-1 and PC-6) of *P. chlamydosporia* were tested under screen house and field conditions using different application rates and methods. Inoculum level of the bio agent has a significant effect on its host (Zareen & Zaki, 2001). Both PC-1 and PC-6 were found to be more active when applied at rate of 6×10^3 chlamydospores/gm of soil. Significant difference was noted between treatments where chlamydospores applied at rate of 2×10^3 and 6×10^3 per gram of soil. Both isolates of *P. chlamydosporia* suppressed nematodes and improved plant growth in tomatoes, however, no statistical difference was recorded between the isolates. Under field conditions, isolates did not exhibit any significant difference for their effectiveness in summer crop. On the other hand, significant variation was noted among treatments in winter crop. Galling indices was highly reduced in winter crop irrespective to the application rate and method of application as compared to summer crop. This might be due to low cfu/gm of both isolates in soil during summer and thus confirming the previous finding (Vieira dos Santos, Esteves, Kerry, & Abrantes, 2014).

5. Conclusion

The isolates of *P.chamydosporia* produced compounds which have potential as novel nematicides. In the present study, the two isloates (PC-1 and PC-6) were effective against egg hatching, egg parasitism mortality of second stage juveniles of *M. incognita* under invitro condition. In screen house and field trial both isolates showed good biocontrol potential against *M. incognita*. Root coating with chlamydospores application method was found to be more effective than mycelial root coating in the current study. No lesions were noticed on *P. chlamydosporia* treated roots of tomato plant which reveals that the tested fungus is safe. Further research work is needed for the molecular characterization of these isolates, and isolattion and structure elucidation of nematicidal compounds produced by two isolates.

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