Int.J.Curr.Microbiol.App.Sci (2019) *8*(2): 1385-1398

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 02 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.802.163

Nematicidal Toxicity of Native Antagonists against *Meloidogyne incognita*

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Keywords

Purpureocillium lilacinum, Pochonia chlamydosporia, Rhizobium sp., Azotobacter sp, Bacillus megaterium, Pseudomonas fluorescens, Meloidogyne incognita, Culture filtrate, LC50 and LT50

Article Info

Accepted:
12 January 2019
Available Online:
10 February 2019

A B S T R A C T

An experiment was conducted to study the nematicidal toxicity of native antagonists against *Meloidogyne incognita.* For this the native antagonists *viz., Pseudomonas fluorescens, Bacillus megaterium*, *Azotobacter* sp., *Rhizobium* sp., *Purpureocillium lilacinum* and *Pochonia chlamydosporia* were screened against *Meloidogyne incognita* under *in-vitro* conditions. For this, four different concentrations of culture filtrates *viz.*, 25, 50, 75 100 percent were tested against *M. incognita* through egg hatch inhibition and second-stage juvenile's mortality at 24, 48, 72 and 96 hrs of exposure time for each antagonist. The entire antagonists were also found to be effective in causing mortality of J_2 with varying degrees at different concentrations of culture filtrates and at different exposure time. The relationship among the antagonists, concentration and time showed that all the tested antagonists were able to cause significant mortality of *M. incognita* $J_2$with increase in the concentrations of filtrates and time of exposure. The result of *in-vitro* test reveals that all the tested antagonists showed varied nature of egg hatch inhibition and juvenile mortality of *M. incognita.* However, among the tested antagonists *P. fluorescens* was found to be most effective in causing the highest egg hatch inhibition and J_2 mortality of *M. incognita* in 25, 50, 75 and 100 per cent concentration of culture filtrates at 24, 48, 72 and 96 hrs of exposure time. Further, probit analysis showed that culture filtrate of *P. fluorescens* exhibit either low LC_{50} values or less LT_{50} values against J_2 of *M. incognita* in 25, 50, 75 and 100 percent concentration of culture filtrates at 24, 48, 72 and 96 hrs of exposure time as compared to the others antagonists and found to be best native antagonists against *M. incognita.*

Introduction

The root-knot nematode *Meloidogyne incognita* is a common plant parasitic nematode in agricultural soil of Assam. It is a major pathogen on vegetables and other crop plants. However, it caused approximately 13.20 to 17.80 percent yield loss (Anon., 2011) in vegetable crops like tomato, brinjal, okra etc under Assam condition. Various pesticides of chemical origin have been used for controlling of this pest with remarkable results but the application of pesticides found toxic to the environment (Sahebani and Hadavi, 2008). The continuous application of such chemicals can alter the structure of ecology as a result create resistance in the target pests and showed their toxicity towards

non-target organisms (Sánchez-Bayo, 2011). There are various ways to manage this pathogen but the application of native microorganism is prime importance because they share a similar environment with them and able to control the reproduction capacity of the target pathogen. Among these microorganisms, the fungi and bacteria are found to be effective because they exhibit with wide diversity (Kok *et al.,* 2001) and their application is very easy as compared to other microorganisms. However, the efficacy of native antagonists is varied from species to species (Irving and Kerry, 1986) and further, a preliminary experiment is required to screen out the best antagonist. The microorganisms showed their antagonistic activity through the release of secondary metabolites in the surrounding environment towards pathogen and that reduced the viability of pathogen such as nematodes (Blaxster and Robertson, 1998 and Sharon *et al.,* 2001). In the management of the plant-parasitic nematode, the application of culture filtrates of antagonist is the best method (Annapurna *et al.,* 2018) for biological control of nematode. The potential benefits and fit fall must be examined so that effective native antagonist (s) can be utilized. Hence, a study was undertaken with the objective to study nematicidal toxicity of native antagonists against *Meloidogyne incognita*.

Materials and Methods

Source and maintenance of *M. incognita* and antagonists

M. incognita egg masses were obtained from infected brinjal plants, Department of Nematology, AAU, Jorhat-13 and pure culture were maintained on tomato in pots in the Net house, Department of Nematology, AAU, Jorhat-13. For *in-vitro* studies pure cultures of the antagonist *viz., Pseudomonas fluorescens, Bacillus megaterium, Pochonia chlamydosporia* and *Purpureocillium lilacinum* were obtained from Department of Plant Pathology, AAU, Jorhat-13 and *Azotobacter* sp. and *Rhizobium* sp. were obtained from Department of Soil science, AAU, Jorhat. The fungal antagonists were maintained on potato dextrose agar (PDA) and bacterial antagonists were maintained on nutrient agar (NA) at P.G Laboratory, Department of Nematology AAU, Jorhat.

Collection of egg masses

Egg masses were collected from the tomato plants maintained as pure culture. Roots were dissected with a sterilized dissecting needle and egg masses were handpicked from the galled roots with help of sterilized forceps. The picked egg masses were kept in sterilized cavity block containing 5 ml sterilized water.

Surface sterilization of egg masses

The collected egg masses were surface sterilized in 0.4 % sodium hypochlorite (NaOCl) for two minutes (Singh and Mathur, 2010). Egg masses were washed thoroughly with sterile distilled water until the traces of NaOCl is removed and placed in cavity block for further use.

Extraction of eggs from egg masses

Surface sterilized egg masses were taken in a petri dish and subjected to 0.5% NaOCl solution for two minutes, with frequent stirring followed by a 30 seconds settling to dissolve the gelatinous matrix. The eggs released through gelatinous matrix are further disinfested in 0.4% NaOCl followed by three washings with sterile water. Eggs were then collected on a 500 mesh sieve and washed thoroughly with sterilized distilled water to remove the traces of NaOCl. A measured quantity of suspension was prepared with eggs in the distilled water in a measuring

cylinder. The egg suspension was prepared in such a way that 1 ml of it contained 100 eggs. The counting of eggs in the suspension was made by using Hawkshley counting dish. Five aliquots of 1ml suspension were counted and their average number was multiplied with total volume of suspension prepared.

Extraction of juveniles (J_2) from eggs

For extraction of juveniles (J_2), the sterilized eggs were placed on a double layer tissue paper supported on a coarse aluminum wire mesh. This was placed over a 10cm diameter petri dish filled with required quantity of water at 24-26°C in BOD incubator for hatching. Several such assemblies were maintained. The counting of juveniles in the suspension was made by using Hawkshley counting dish. Five aliquots of 1ml suspension were counted and their average number was multiplied with total volume of suspension prepared.

Cleaning and sterilization of glasswares

The glasswares used for different experiments were washed thoroughly with potassium dichromate ($K_2Cr_2O_7$) solutions, rinsed with water and glasswares were sterilized at 160°C for 2 hrs in hot air oven.

Preparation of culture filtrates of fungal antagonists

The ingredients used for preparation of potato dextrose broth (PDB) were peeled potato (200g), dextrose (20g) and distilled water (1000ml). Fully boiled potato extract was separated by using double layer muslin cloth and measured amount of dextrose was added to the extract. In another flask, remaining 500ml distilled water was taken and allowed agar-agar to melt by boiling. The molten agar-agar was strained through double layer muslin cloth and mixed with potato extract solution. The volume was made upto 1000ml by adding distilled water. P^H of the medium was adjusted to 7.2. The medium was poured into 250ml Erlenmeyer flasks plugged by non-absorbent cotton and then sterilized in autoclave at 121°C for 20 minutes. After 24hr, the flask is seeded with fungal antagonists. The inoculated flasks were incubated at 25±2°C for 15 days in BOD incubator. Then fungal culture filtrates were obtained by filtering through Whatman filter paper no. 1. The filtrates so obtained were further centrifuged at 2000rpm to remove the extra spores and mycelia if any. Then supernatants were collected and used in the *in-vitro* studies.

Preparation of culture filtrates of bacterial antagonists

For the preparation of bacterial culture filtrates, 13 grams of nutrient broth (Himedia M 244) were suspended in 1000 ml of distilled water and heated up to boiling to dissolve the medium completely. The medium was poured into 250ml Erlenmeyer flasks plugged by non-absorbent cotton and then sterilized in autoclave at 15 lb pressure (121°C) for 15 minutes. After 24hr, under sterilized condition the flask is seeded with bacterial antagonists. The inoculated flasks were incubated at 25±2°C for 15days in BOD incubator. Then bacterial culture filtrates were obtained by filtering through Whatman filter paper no. 1. The filtrates so obtained were further centrifuged at 8000 rpm to remove the cell if any. Then supernatants were collected and used in the *in-vitro* studies.

Efficacy of culture filtrates of antagonists on hatching inhibition of *Meloidogyne incognita* eggs

The egg hatch inhibition test was conducted under *in-vitro* conditions. For this, desired concentrations (25, 50, 75 and 100 percent) of

culture filtrates were poured on the sterile cavity blocks containing 100 eggs per cavity block. Each treatment was replicated 3 times. Three control treatments were maintained, one as potato dextrose broth (PDB), second as nutrient broth (NB) medium and third as sterilized distilled water (SDW) were also maintained. Observations were recorded after 7days of exposure. For determining the hatching of eggs; test was conducted by transferring the tested eggs to sterile distilled water and observed egg hatching after 24 hrs with the aid of stereomicroscope (x4). At the end of the experiment, number of unhatched eggs was calculated and per cent egg hatch inhibition calculated by using following formula:

Hatch inhibition of eggs (%) =

$$\frac{\text{Total number of eggs - Number of eggs hatched}}{\text{Total number of eggs}} \times 100$$

Efficacy of culture filtrates of antagonists on mortality of juveniles of *Meloidogyne incognita*

The mortality test was conducted under *in-vitro* conditions. For this, desired concentrations (25, 50, 75 and 100 per cent) of culture filtrates were poured on the sterile cavity blocks containing 100 juveniles (J_2) per cavity block. Observation on juvenile mortality was recorded at 24, 48, 72 and 96 hours of exposure. Apart from the treatments with different concentrations of antagonists, controls with potato dextrose broth (PDB), nutrient broth (NB) and sterilized distilled water (SDW) were also maintained. Each treatment was replicated 3 times. For determining the dead nematodes, revival test was conducted by transferring the immobile juveniles to sterile distilled water and observed their activities after 24 hrs. The juveniles that showed no movement even when they were probed with bamboo splinter were considered dead. The mortality (%) was calculated using the formula give below.

Mortality (%) =

$$\frac{\text{Number of dead juveniles in the treatment}}{\text{Total number of juveniles in the treatment}} \times 100$$

LC_{50} and LT_{50} values

Mortality data thus obtained were subjected to 'Probit analysis' (Finney, 1952) to find out the LC_{50} and LT_{50}values against each antagonist at different time of exposure and at different concentrations.

Statistical analysis

The percentage values were subjected to the arcsin transformation before analysis and data were analyzed by using WASP - Web Agri Stat Package 2.0 version software. DMRT test was conducted to determine the significance of treatments. 'Probit analyses were performed by using IBM SPSS (Statistical Package for the Social Sciences) 20.0 version software.

Results and Discussion

Efficacy of culture filtrates of antagonists on the hatch inhibition of *Meloidogyne incognita* eggs

Statistical analysis (Table 1) shows that all the antagonists showed significant inhibition of egg hatching of *M. incognita* irrespective of concentration of the culture filtrates as compared to the control. No egg hatch inhibition was recorded in the controls (NB, PDB and SDW). At 25 percent concentration of culture filtrate, the maximum egg hatch inhibition was recorded in *P. fluorescens* (39.50%) followed by *B. megaterium*

(36.25%) and both were significantly different from each other and also significantly different from rest of the treatments. This was followed by the treatment, *Azotobacter* sp. with inhibition of 26.75 per cent and this treatment was significantly different from rest of the treatments. Similarly, 13.75 per cent and 10.50 per cent inhibition were recorded in *P. lilacinum* and *P. chlamydosporia* respectively which were found to be significantly different from each other as also both were significantly different from *Rhizobium* sp. (5.75%) where minimum egg hatch inhibition was recorded among all the treatments. At 50 percent concentration of culture filtrate, the maximum egg hatch inhibition was recorded in *P. fluorescens* (51.50%) followed by *B. megaterium* (45.00%), *Azotobacter* sp. (37.00%), *P. lilacinum* (20.25%), *P. chlamydosporia* (15.00%). However, an egg hatch inhibition of 11.00 percent was recorded in the treatment with *Rhizobium* sp. All the treatments were significantly different from each other. A similar trend was observed at 75 and 100 percent concentration of the culture filtrates of antagonists.

Efficacy of culture filtrates of antagonists on mortality of *Meloidogyne incognita* J_2

Statistical analysis (Table 2) shows that all the antagonists were found to be effective in causing mortality of *M. incognita* J_2 irrespective of concentrations and time of exposure. All the antagonists showed significant increase in the mortality of *M. incognita* J_2 irrespective of concentrations of the culture filtrates as compared to the controls. No mortality of *M. incognita* J_2 was recorded in controls (NB, PDB and SDW). It observed that if the concentration of culture filtrate is increased there is corresponding increase in the mortality of J_2 of *M. incognita*. However, all the antagonist were also found to be effective in causing mortality of J_2 with varying degrees at different concentrations of culture filtrates and at different exposure time. The relationship between the antagonists, concentration and time showed that all the tested antagonists were able to cause significant mortality of *M. incognita* $J_2$with increase in time of exposure and concentrations. However, the antagonists *viz*., *P. lilacinum*, *P. chlamydosporia*, *Rhizobium* spp., *Azotobacter* spp., *B. megaterium* and *P. fluorescence* showed maximum mortality of J_2 in 100 percent concentration of culture filtrates at all the time of exposure. Among the antagonists**,** culture filtrate of the *P. fluorescens* was found to cause maximum mortality of J_2 in all the concentrations and at different time of exposure. *P. fluorescens* showed 35.67, 53.33, 63.67 and 75.00 percent mortality of J_2 in 25, 50, 75 and 100 percent concentration respectively after 96 hrs of exposure and found to be the best bioagent. It is evident from the results that *P. fluorescens* was found to be the best among the antagonists.

LC_{50} values

The obtained juvenile mortality of *M. incognita* was subjected to probit analysis and it evaluated that the LC_{50} values (Table 3, 4, 5 and 6) of culture filtrate of *P. fluorescens* against J_2 of *M. incognita* were 128.33, 125.41, 64.84 and 48.77 per cent at the exposure period of 24, 48, 72 and 96 hrs respectively. In case of *B. megaterium* the LC_{50} values were 142.59, 133.22, 89.73 and 57.74 per cent at the exposure period of 24, 48, 72 and 96 hrs respectively and that of *Azotobacter* sp. were 147.85, 133.34, 103.12 and 98.92 per cent at the exposure period of 24, 48, 72 and 96 hrs respectively. The LC_{50} values of *P. lilacinum* were 154.49, 146.44, 110.06 and 94.38 per cent at the exposure period of 24, 48, 72 and 96 hrs respectively while the LC_{50} values of *P. chlamydosporia* were 148.36, 146.86, 113.62 and 103.16 per cent at the exposure period of 24, 48, 72 and 96 hrs respectively.

Table.1 Efficacy of culture filtrates of antagonists on the hatching of *Meloidogyne incognita* eggs

Treatments	Concentration			
	25%	**50%**	**75%**	**100%**
P. lilacinum	13.75	20.25	25.50	35.25
	$(21.76)_{d}$	$(26.74)_{d}$	$(30.33)_{d}$	$(36.42)_{d}$
P. chlamydosporia	10.50	15.00	22.50	34.00
	$(18.90)_{e}$	$(22.78)_{e}$	$(28.32)_{e}$	$(35.67)_{e}$
Rhizobium sp.	5.75	11.00	17.75	23.25
	$(13.87)_{f}$	$(19.37)_{f}$	$(24.91)_{f}$	$(28.83)_{f}$
Azotobacter sp.	26.75	37.00	46.00	53.25
	$(31.14)_{c}$	$(37.46)_{c}$	$(42.71)_{c}$	$(46.86)_{c}$
B. megaterium	36.25	45.00	65.75	72.00
	$(37.02)_{b}$	$(42.13)_{b}$	$(54.18)_{b}$	$(58.05)_{b}$
P. fluorescens	39.50	51.50	77.25	80.25
	$(38.94)_{a}$	$(45.86)_{a}$	$(61.51)_{a}$	$(63.62)_{a}$
PDB	0.00	0.00	0.00	0.00
	$(0.64)_{g}$	$(0.64)_{g}$	$(0.64)_{g}$	$(0.64)_{g}$
NB	0.00	0.00	0.00	0.00
	$(0.64)_{g}$	$(0.64)_{g}$	$(0.64)_{g}$	$(0.64)_{g}$
SDW	0.00	0.00	0.00	0.00
	$(0.64)_{g}$	$(0.64)_{g}$	$(0.64)_{g}$	$(0.64)_{g}$
S.Ed.±	0.30	0.28	0.28	0.24
$CD_{0.05}$	0.62	0.58	0.59	0.51

Figures in the parenthesis are arc sin transformed values.
PDB- Potato dextrose broth, NB- Nutrient Broth, SDW- Sterilized distilled water
Means within columns separated by Duncan's Multiple range test CD at 5 per cent levels of probability. Means followed by the same letters in the column are not significantly different from each other based on Duncan's Multiple Range Test at 5 per cent levels of probability.

Table.2 Efficacy of culture filtrates of antagonists on the mortality of second stage juveniles (J2) of *M. incognita*

Treatments	Concentration															
	25%				50%				75%				100%			
	24hrs	48 hrs	72 hrs	96 hrs	24 hrs	48 hrs	72 hrs	96 hrs	24 hrs	48 hrs	72 hrs	96 hrs	24 hrs	48 hrs	72 hrs	96 hrs
P. lilacinum	8.00	13.33	20.00	30.00	14.67	17.67	25.67	34.00	20.00	25.67	36.00	42.33	28.00	33.33	46.33	53.33
	$(16.43)_d$	$(21.41)_c$	$(26.56)_d$	$(33.21)_d$	$(22.52)_c$	$(24.86)_d$	$(30.44)_c$	$(35.67)_d$	$(26.56)_c$	$(30.44)_d$	$(36.87)_d$	$(40.59)_d$	$(31.95)_d$	$(35.26)_d$	$(42.90)_d$	$(46.91_d$
P.chlamydosporia	6.00	11.33	18.00	28.67	11.33	16.33	24.00	33.67	17.67	23.00	34.00	40.00	27.33	32.33	44.67	50.33
	$(14.17)_e$	$(19.66)_d$	$(25.10)_e$	$(32.37)_e$	$(19.67)_d$	$(23.83)_e$	$(29.33)_d$	$(35.47)_d$	$(24.86)_d$	$(28.66)_e$	$(35.67)_e$	$(39.23)_e$	$(31.52_d$	$(34.65)_e$	$(41.94)_e$	$(45.19)_e$
Rhizobium sp.	2.00	9.00	15.33	23.33	5.00	11.00	20.00	28.00	9.33	16.33	25.67	34.00	15.00	21.33	30.33	38.33
	$(8.12)_f$	$(17.46)_e$	$(23.05)_f$	$(28.88)_f$	$(12.91)_e$	$(19.37)_f$	$(26.56)_e$	$(31.95)_e$	$(17.77)_e$	$(23.83)_f$	$(30.44)_f$	$(35.67)_f$	$(22.78)_e$	$(27.51)_f$	$(33.42)_f$	$(38.25)_f$
***Azotobacter* sp.**	9.00	14.00	21.00	32.00	15.67	20.33	26.33	37.67	22.33	28.00	38.00	45.67	30.00	37.00	49.33	56.67
	$(17.46)_c$	$(21.97)_c$	$(27.27)_c$	$(34.45)_c$	$(23.32)_b$	$(26.80)_c$	$(30.87)_c$	$(37.86)_c$	$(28.20)_b$	$(31.95)_c$	$(38.06)_c$	$(42.52)_c$	$(33.21)_c$	$(37.46)_c$	$(44.62)_c$	$(48.83)_c$
B. megaterium	10.33	18.33	24.00	38.00	15.00	25.00	34.67	46.67	23.00	31.67	44.33	57.33	32.33	39.00	53.67	64.33
	$(18.74)_b$	$(25.35)_b$	$(29.33)_b$	$(38.06)_a$	$(22.78_b)_c$	$(30.00)_b$	$(36.07)_b$	$(43.09)_b$	$(28.66)_b$	$(34.25)_b$	$(41.74)_b$	$(49.22)_b$	$(34.65)_b$	$(38.65)_b$	$(47.10)_b$	$(53.33)_b$
P. fluorescens	12.33	22.33	29.00	35.67	19.00	30.00	43.00	53.33	28.67	36.00	55.33	63.67	37.00	42.00	68.00	75.00
	$(20.55)_a$	$(28.20)_a$	$(32.58)_a$	$(36.67_b$	$(25.84)_a$	$(33.21)_a$	$(40.98)_a$	$(46.91)_a$	$(32.37)_a$	$(36.87)_a$	$(48.06)_a$	$(52.93)_a$	$(37.46)_a$	$(40.40)_a$	$(55.55)_a$	$(60.00)_a$
PDB	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	$(0.64)_g$	$(0.64)_f$	$(0.64)_g$	$(0.64)_g$	$(0.64)_f$	$(0.64)_g$	$(0.64)_f$	$(0.64)_f$	$(0.64)_f$	$(0.64)_g$	$(0.64)_g$	$(0.64)_g$	$(0.64)_f$	$(0.64)_g$	$(0.64)_g$	$(0.64)_g$
NB	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	$(0.64)_g$	$(0.64)_f$	$(0.64)_g$	$(0.64)_g$	$(0.64)_f$	$(0.64)_g$	$(0.64)_f$	$(0.64)_f$	$(0.64)_f$	$(0.64)_g$	$(0.64)_g$	$(0.64)_g$	$(0.64)_f$	$(0.64)_g$	$(0.64)_g$	$(0.64)_g$
SDW	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	$(0.64)_g$	$(0.64)_f$	$(0.64)_g$	$(0.64)_g$	$(0.64)_f$	$(0.64)_g$	$(0.64)_f$	$(0.64)_f$	$(0.64)_f$	$(0.64)_g$	$(0.64)_g$	$(0.64)_g$	$(0.64)_f$	$(0.64)_g$	$(0.64)_g$	$(0.64)_g$
S.Ed.±	0.31	0.29	0.27	0.22	0.32	0.28	0.25	0.21	0.36	0.21	0.18	0.22	0.22	0.21	0.20	0.21
$CD_{0.05}$	0.65	0.62	0.57	0.47	0.67	0.59	0.52	0.44	0.76	0.44	0.39	0.46	0.46	0.44	0.43	0.44

Figures in the parenthesis are arc sin transformed values.

PDB- Potato dextrose broth, NB- Nutrient Broth, SDW- Sterilized distilled water within columns separated by Duncan's Multiple range test CD at 5 per cent levels of probability. Means followed by the same letters in the column are not significantly different from each other based on Duncan's Multiple Range Test at 5 per cent levels of probability.

Table.3 LC50 values of culture filtrates of different antagonists to J2 of *Meloidogyne incognita* after 24 hrs of exposure

Treatments	LC_{50} value	Fiducial limit (Lower- Upper)	Heterogeneity (R^2)	Standard error of regression coefficient	Regression equation
P. lilacinum	154.49	135.52-192.13	0.925	0.101	Y= 1.606+0.011 X
P. chlamydosporia	148.36	130.92-177.62	0.946	0.127	Y= 1.854+ 0.012X
***Rhizobium* sp.**	177.96	151.68-228.18	0.912	0.168	Y= 2.326+ 0.013X
***Azotobacter* sp.**	147.85	108.71-181.41	0.969	0.114	Y= 1.571+ 0.002X
B. megaterium	142.59	125.07-172.44	0.650	0.113	Y= 1.557+0.011 X
P. fluorescens	128.33	114.16-151.41	0.953	0.107	Y= 1.427+0.011X

Y= Probit Kill, X= log dose

Mortality based on 3 replications each with $100J_2$ of *M. incognita*.

Table.4 LC50 values of culture filtrates of different antagonists to J2 of *Meloidogyne incognita* after 48 hrs of exposure

Treatments	LC_{50} value	Fiducial limit (Lower- Upper)	Heterogeneity (R^2)	Standard error of regression coefficient	Regression equation
P. lilacinum	146.44	126.02-184.11	0.953	0.099	Y= 1.362+ 0.009 X
P. chlamydosporia	146.86	127.32-181.73	0.933	0.111	Y= 1.475+ 0.010X
***Rhizobium* sp.**	205.11	163.05-306.99	0.908	0.119	Y= 1.562+ 0.008X
***Azotobacter* sp.**	133.34	116.89-161.72	0.981	0.097	Y= 1.329+0.001 X
B. megaterium	133.22	114.41-169.02	0.926	0.099	Y= 1.099+ 0.008X
P. fluorescens	125.41	106.89-162.69	0.955	0.96	Y= 0.919+0.007X

Y= Probit Kill, X= log dose

Mortality based on 3 replications each with 100 J_2 of *M. incognita*.

Table.5 LC50 values of culture filtrates of different antagonists to J2 of *M. incognita* after72 hrs of exposure

Treatments	LC_{50} value	Fiducial limit (Lower- Upper)	Heterogeneity (R^2)	Standard error of regression coefficient	Regression equation
P. lilacinum	110.06	98.58-128.32	0.950	0.098	Y= 1.127+0.010X
P. chlamydosporia	113.62	101.84-132.30	0.948	0.100	Y= 1.206+ 0.011X
***Rhizobium* sp.**	173.79	140.31-253.63	0.779	0.104	Y= 1.183+0.007X
***Azotobacter*sp.**	103.12	92.35-117.84	0.959	0.094	Y= 1.126+0.011X
B. megaterium	89.73	81.55-101.33	0.948	0.094	Y= 0.946+ 0.011X
P. fluorescens	64.84	59.40-70.44	0.977	0.093	Y= 0.874+ 0.013X

Y= Probit Kill, X= log dose

Mortality based on 3 replications each with 100J_2 of *M. incognita*.

Table.6 LC50 values of culture filtrates of different antagonists to J2 of *M. incognita* after96 hrs of exposure

Treatments	LC_{50} value	Fiducial limit (Lower- Upper)	Heterogeneity (R^2)	Standard error of regression coefficient	Regression equation
P. lilacinum	94.38	83.54-112.10	0.924	0.092	Y= 0.776+0.008X
P. chlamydosporia	103.16	89.97-127.03	0.934	0.093	Y= 0.781+0.008X
***Rhizobium* sp.**	148.89	120.19-222.76	0.881	0.096	Y= 0.870+ 0.006X
***Azotobacter*sp.**	98.92	84.41-128.27	0.913	0.091	Y= 0.616+ 0.006X
B. megaterium	57.74	48.94-65.73	0.968	0.090	Y= 0.523+0.009X
P. fluorescens	48.77	52.36-63.43	0.944	0.091	Y= 0.663+ 0.014X

Y= Probit Kill, X= log dose

Mortality based on 3 replications each with 100J_2 of *M. incognita*.

Table.7 LT50 values of different antagonists to J2 of *M. incognita* at 25% concentration of culture filtrates

Treatments	LT_{50} value	Fiducial limit (Lower- Upper)	Heterogeneity (R^2)	Standard error of regression coefficient	Regression equation
P. lilacinum	139.76	122.96-168.06	0.972	0.119	Y= 1.701+ 0.012X
P. chlamydosporia	137.81	122.54-162.62	0.942	0.127	Y= 1.879+0.014 X
***Rhizobium* sp.**	138.34	124.09-161.04	0.872	0.149	Y= 2.226+ 0.016X
***Azotobacter* sp.**	135.86	120.02-162.19	0.984	0.116	Y= 1.656+ 0.012X
B. megaterium	121.15	109.00-140.06	0.950	0.111	Y= 1.569+0.013 X
P. fluorescens	127.54	111.92-154.46	0.923	0.105	Y= 1.335+0.01X

Y= Probit Kill, X= log dose

Mortality based on 3 replications each with $100 J_2$ of *M. incognita*.

Table.8 LT50 values of different antagonists to J2 of *M. incognita* at 50% concentration of culture filtrates

Treatments	LT_{50} value	Fiducial limit (Lower- Upper)	Heterogeneity (R^2)	Standard error of regression coefficient	Regression equation
P. lilacinum	142.23	121.73-181.66	0.890	0.106	Y= 1.316+ 0.106X
P. chlamydosporia	135.00	118.33-163.57	0.959	0.110	Y= 1.494+0.011 X
***Rhizobium* sp.**	144.37	120.00-133.92	0.965	0.129	Y= 1.937+ 0.014X
***Azotobacter* sp.**	132.83	115.04-165.14	0.939	0.104	Y= 1.275+ 0.01X
B. megaterium	102.01	93.19-114.86	0.964	0.102	Y= 1.327+0.013 X
P. fluorescens	87.81	81.04-96.91	0.984	0.098	Y= 1.177+0.013X

Y= Probit Kill, X= log dose

Mortality based on 3 replications each with $100 J_2$ of *M. incognita*.

Table.9 LT50 values of different antagonists to J2 of *M. incognita* at 75% concentration of culture filtrates

Treatments	LT_{50} value	Fiducial limit (Lower- Upper)	Heterogeneity (R^2)	Standard error of regression coefficient	Regression equation
P. lilacinum	114.84	100.73-139.57	0.923	0.098	Y= 1.071+ 0.009X
P. chlamydosporia	119.57	104.97-144.89	0.935	0.100	Y= 1.170+0.010 X
***Rhizobium* sp.**	126.94	113.32-148.77	0.956	0.113	Y= 1.593+ 0.013X
***Azotobacter* sp.**	107.14	94.58-128.55	0.963	0.096	Y= 0.901+0.009X
B. megaterium	82.64	76.20-91.08	0.965	0.096	Y= 1.073+0.013 X
P. fluorescens	68.09	62.69-74.13	0.948	0.093	Y= 0.920+0.014X

Y= Probit Kill, X= log dose

Mortality based on 3 replications each with $100J_2$ of *M. incognita*.

Table.10 LT50 values of different antagonists to J2 of *M. incognita* at 100% concentration of culture filtrates

Treatments	LT_{50} value	Fiducial limit (Lower- Upper)	Heterogeneity (R^2)	Standard error of regression coefficient	Regression equation
P. lilacinum	86.16	77.45-99.07	0.953	0.093	Y= 0.841+0.010X
P. chlamydosporia	92.94	82.60-109.68	0.915	0.093	Y= 0.837+0.009 X
***Rhizobium* sp.**	123.55	108.76-148.77	0.956	0.103	Y= 1.284+ 0.010X
***Azotobacter* sp.**	77.66	70.00-87.99	0.964	0.092	Y= 0.757+0.010X
B. megaterium	65.89	59.81-72.58	0.962	0.092	Y= 0.784+0.012 X
P. fluorescens	50.23	44.85-55.03	0.917	0.092	Y= 0.775+0.015X

Y= Probit Kill, X= log dose

Mortality based on 3 replications each with $100J_2$ of *M. incognita*

The LC_{50} values of *Rhizobium* sp. were 177.96, 205.11, 173.79 and 148.89 per cent for the exposure period of 24, 48, 72 and 96 hrs respectively. It was observed that the LC_{50} values were recorded to be lowest in *P. fluorescens,* thus it came out to be the most effective in causing the mortality of the J_2 of *M. incognita.*

LT_{50} values

The obtained juvenile mortality of *M. incognita* was subjected to probit analysis and it evaluated that the LT_{50} values (Table 7, 8, 9 and 10) of culture filtrate of *P. fluorescens* against J_2 of *M. incognita* were 127.54, 87.81, 68.09 and 50.23 hrs at the concentration of 25, 50, 75 and 100 per cent respectively. In case of *B. megaterium* the LT_{50} values were 121.15, 102.01, 82.64 and 65.89 hrs and *Azotobacter* sp. were 135.86, 132.83, 107.14 and 77.66 hrs at the concentration of 25, 50, 75 and 100 per cent respectively. The LT_{50} value of *P. lilacinum* were 139.76, 142.23, 114.84 and 86.16 hrs while the LT_{50} values of culture filtrate of *P. chlamydosporia* were 137.81, 135.00, 119.57 and 92.94 hrs at the concentration of 25, 50, 75 and 100 per cent respectively. The LT_{50} values of *Rhizobium* sp. were 138.34, 144.37, 126.94 and 123.55 hrs at the concentration of 25, 50, 75 and 100 per cent respectively. Thus, lowest LT_{50} values were recorded in *P. fluorescens* thereby came out to be most effective. In the present investigation, the culture filtrates of *P. lilacinum* showed more nematicidal activity than *P. chlamydosporia*. Similar type of observations were recorded by Hallman and Sikora, 1996, Kerry, 2000 and Annapurna *et al.,* 2018 who reported that *P. lilacinum* showed more egg hatch inhibition and juvenile mortality of *M. incognita* under *in-vitro* conditions. However, among the tested antagonists, *P. fluorescens* showed more juvenile mortality and egg hatch inhibition of *M. incognita* in 25, 50,75 and 100 percent concentration of culture filtrate at 24, 48, 72 and 96 hrs of exposure time. Siddiqui *et al.,* 2001, Khan *et al.,* 2002, and Khan *et al.,* 2012 reported that filtrate of *P. fluorescens* caused more juvenile mortality and egg hatch inhibition of *M. incognita* than other tested antagonists, thus confirm the result of the present investigation. The fact that the culture filtrates in the present study were free of cells, spores and mycelia and strongly implies that the compounds in the filtrates had some toxic/ antibiotic substances that were found to be instrumental in causing the mortality of juvenile and egg hatch inhibition of *M. incognita*. Nitao *et al.,* 1999 reported that an antagonist secretes nematicidal metabolites and enzymes that affect nematode viability. Indeed, *Purpureocillium* spp. secretes toxin like paecilotoxin (Mikami *et al.,* 1988) and leucinostatins (Park *et al.,* 2004), *P. chlamydosporia* produce lytic enzymes like esterase, chitinase and lipase in the culture filtrates (Esteves *et al.,* 2009), *Bacillus* spp. produce endotoxin (Totora *et al.,*1980), bulbiformin (Brannen, 1995), *Azotobacter* spp. also releases the antibiotics, lytic enzymes and hydrocyanic acid in the medium (Van Loon *et al.,* 1998 and Selvakumar *et al.,* 2009) in the filtrates and that caused inhibition in egg hatch and induced the mortality of J_2 of root-knot nematode. The eggs of nematode are semi-permeable in nature and due to this, nematode eggs facilitate certain molecules or ions to pass through it. So toxin had a permanent adverse effect on the eggs or there appears to be physical retention of the toxins in the eggs, as hatching was not resumed on the transfer of eggs in water after one day (Clark and Perry, 1988). The reason behind the actual cause of mortality of *M. incognita* J_2 might be due to release of hydrogen cyanide, ammonia, phenazine, pyoleutorin, pyrrolnintrin (Whistler *et al.,* 2000 and Schoonbeck *et al.,* 2002), 2, 4- diacetyl phloroglucinol (Siddiqui and Shaukat, 2003) by the antagonist

especially *P. fluorescens* and Khan *et al.*, 2012 also reported that the antagonism of *P. fluorescens* may have occurred through the effects of hydrogen cyanide (HCN)/ ammonia (NH3) as the bacterium produced these nematoxic chemicals in culture broth, as a result, bring down mortality of *M. incognita* J_2 in the filtrates. The variable effect of culture filtrates on *M. incognita* observed in the present investigation can be attributed to the varied nature of toxic metabolites produced by different species of antagonists.

Acknowledgement

The author is greatly thankful to Department of Plant Pathology and Department of Soil Science, Assam Agricultural University, Jorhat, Assam for providing the antagonists.

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How to cite this article:

Arunima Bharali, Bhabesh Bhagawati and Kurulkar Uday. 2019. Nematicidal Toxicity of Native Antagonists against *Meloidogyne incognita. Int.J.Curr.Microbiol.App.Sci.* 8(02): 1385-1398. doi: https://doi.org/10.20546/ijcmas.2019.802.163