**In-vitro Efficacy of Native Fungal Bioagents against Meloidogyne incognita**

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**Abstract**

Four fungal bioagents viz., Trichoderma viride, T. harzianum, Pochonia chlamydospora and Purpureocillium lilacinum were screened for their efficacy against *Meloidogyne incognita* under in-vitro conditions through egg parasitism, egg hatch inhibition and second-stage juveniles mortality test. *P. chlamydospora* showed highest egg parasitism. In respect of egg hatch inhibition and juvenile mortality, the culture filtrates of these fungal bioagents were tested and found to be effective in inhibition of egg hatch and mortality of juveniles of *M. incognita* at 25, 50, 75 and 100 percent concentrations. Among the bioagents, *T. harzianum* was showed highest egg hatch inhibition and juvenile mortality of *M. incognita*. Dose-response models were used in the larval mortality test to determine the concentration of culture filtrate required to kill 50 per cent of the juveniles. The culture filtrate of *T. harzianum* showed the highest activity with a LC$_{50}$ value of 29.617 at 96 hrs of exposure.

**Keywords**

Trichoderma viride, *T. harzianum*, Pochonia chlamydospora, Purpureocillium lilacinum, *Meloidogyne incognita* and LC$_{50}$

**Introduction**

Root-knot nematode attack not only more than two thousands of plant species but they caused five percent of global crop loss (Hussey and Janssen, 2002). An avoidable yield loss of tomato due to *M. incognita* was recorded to the tune of 13.20 percent in Assam (Anon., 2013). The application of chemical nematicides will become prohibited due to not only the increase of resistance in the target pathogen but also caused the environmental hazard. To reduce such causes, the use of bioagents are found increase in attention and use of such bioagents offer an effective, safe, persistent and natural durable protection against crop pest (Anita and Samiyappan, 2012). However, many natural enemies attack *Meloidogyne* spp. in the soil (Kok et al., 2001) and such enemies can be used as bioagent for the effective management of *Meloidogyne* spp (Karssen et al., 2006). Among them, fungi are unique natural enemies for managing the nematodes in soil (Mark et al., 2010). Such fungi showed their antagonistic activity like predation, parasitism and antibiosis etc (Cayrol, 1983, Zaki, 1994 and Kalita et al., 2012) towards *Meloidogyne* spp. However, these fungi have ability to release the antibiotics, metabolites, protease enzymes etc. (Blaxter and Robertson, 1998 and Sharon et al., 2001) in the environment and that caused nematode viability. However, the efficacy of bioagents to reduce the nematode viability varied from species to species (Irving and Kerry, 1986). So, one of the means of
increasing the potentiality of bioagents is to use the native biocontrol agents (Singh et al., 2013). The potential benefits and fit fall must be examined so that effective native biocontrol agent(s) can be utilized. Hence, a study was undertaken on the in-vitro efficacy of certain native fungal bioagents against *M. incognita*.

**Materials and Methods**

**Source and maintenance of Meloidogyne incognita and fungal bioagents**

*M. incognita* egg masses were obtained from Experimental plot, 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. Pure culture of biocontrol agents viz., *Trichoderma viride*, *T. harzianum* and *Pochonia chlamydosporia* and *Purpureocillium lilacinum* were obtained from Department of Plant Pathology, AAU. Jorhat-13 and were maintained on PDA 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 hand picked up from the galled root with help of sterilized forceps. The picked egg masses were kept in sterilized cavity block containing 2ml sterilized water.

**Surface sterilization of egg masses**

The collected egg masses were surface sterilized in 0.4 per cent 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 petridish and subjected to 0.5 % sodium hypochlorite solution for two minutes, with frequent stirring followed by a 30 seconds settling to dissolve the gelatinous matrix. The eggs released through gelatinous matrix and further disinfested in 0.4 % NaOCl followed by three washing 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 1 ml suspension were counted and their average number was multiplied with total volume of suspension prepared.

**Extraction of juveniles (J2) from eggs**

For extraction of juveniles (J2), the sterilized eggs collected as described above were placed on a double layer facial tissue paper supported on a course aluminum wire mesh. This was placed over a 10cm diameter petridish filled with required quantity of water at 24-26 °C in BOD incubator for hatching. Several such assemblies were maintained.

The juveniles collected from these were mixed together at the time of inoculation in pot experiment as also in-vitro studies. Counting of juveniles was done as described in 2.4.

**Cleaning and sterilization of glassware**

The glassware used for experiments were washed thoroughly with potassium dichromate (K2Cr2O7) solutions, rinsed with water and oven dried before use and sterilized at 160°C for 2hrs in hot air oven.
Preparation of media

**Potato Dextrose Agar (PDA)**

The ingredients used for preparation of PDA were peeled potato (200g), dextrose (20g), agar-agar (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 up to 1000ml by adding distilled water. pH of the medium was adjusted 7.2. The medium was poured into culture tubes and conical flask plugged by non-absorbent cotton and then sterilized in autoclave at 121°C for 20 minutes.

**Potato Dextrose Broth (PDB)**

The potato dextrose broth was also prepared following the same method as describe above (2.7.1) except that no agar-agar was added.

**Preparation of culture filtrates of bioagents**

For the preparation of fungal culture filtrates, 100ml potato dextrose broth was prepared in 250ml Erlenmeyer flasks as mentioned in 2.7.2 above and seeded with tested fungal bioagents. The inoculated flasks were incubated at 25± 2°C for 15days 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.

**Effect of fungal bioagents on the parasitism of M. incognita eggs**

Fungal isolates were inoculated to the center of a petriplate containing PDA medium amended with antibiotic streptomycin as antibiotic at 1 ml/L. At full growth, counted numbers of eggs (100) were placed on the petriplate and incubate at 25± 2°C for 7 days. There were four replicates for each treatment with fungal bioagent. A control treatment was maintained with eggs free plates. After 7 days of incubation the portion of the fungal growth containing eggs were collected on Hawsheley counting dish and stained with lacto phenol cotton blue. The percent egg parasitism was calculated by counting the parasitized and non-parasitized eggs under a microscope at 100 magnification using the following formula: The eggs, either infected by direct hyphal penetration or disintegration of their contents were counted as infected (Singh and Mathur, 2010), while eggs that contained juveniles and looked normal as also the eggs from which juveniles had hatched out were counted as viable.

\[
\text{Percent egg parasitism} = \frac{\text{Total parasitised eggs}}{\text{Total number of eggs}} \times 100
\]

**Effect of fungal bioagents on hatch inhibition of M. incognita eggs**

To determine the effect of cell free culture filtrate on the hatching of eggs of *M. incognita*, separate experiments were carried out to evaluate the nematicidal activity of native fungal bioagents. One ml of each of the culture filtrate of collected isolates at 25, 50, 75 and 100% were poured into cavity block and 100 eggs were introduced into each cavity block and incubated at room temperature in completely randomized design, replicated four times. Two control treatments were maintained, one as sterilized water and second as potato dextrose broth medium. Observation
on the number of hatched J\textsubscript{2} in four replications (cavity block) for each treatment was determined after 7 days of incubation and 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:

\[
\text{Hatch inhibition of eggs(\%)} = \left( \frac{\text{Total number of eggs} - \text{Hatched number of eggs}}{\text{Total number of eggs}} \right) \times 100
\]

**Effect of culture filtrates of fungal bioagents on juvenile (J\textsubscript{2}) mortality of \textit{M. incognita}**

The mortality test was conducted under \textit{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 juveniles (J\textsubscript{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 concentration of bioagents, potato dextrose broth (PDB) and sterilized distilled water (SDW) were also maintained as controls. The test was replicated four times with two control were maintained \textit{viz.}, one as sterilized water and second as potato dextrose broth medium. For determining the dead nematodes revival test was conducted by transferring the immobile juveniles to sterile distilled water and observed their activities after 24hrs. The juveniles that showed no movement even when they were probed with bamboo splinter were considered dead. The corrected mortality was calculated using the formula give below.

\[
\text{Mortality(\%)} = \left( \frac{\text{Number of dead juveniles in treatment}}{\text{Total number of juveniles in the treatment}} \right) \times 100
\]

**LC\textsubscript{50} values**

Mortality data thus obtained were subjected to ‘Probit analysis’ (Finney, 1952) to find out the LC\textsubscript{50} values against each bioagent at different time of exposure.

The value of relative toxicity of bioagents was calculated as follows:

\[
\text{Relative toxicity(\%)} = \left( \frac{\text{LC50 values of known unit}}{\text{LC50 values of other bioagents}} \right) \times 100 \quad \text{where } \textit{T. harzianum} \text{ was used as the known unit.}
\]

**Statistical analysis**

The percentage values were subjected arcsin transformation before analysis and data were analyzed by using statistical analysis System (SAS) and IBM SPSS (Statistical Package for the Social Sciences) 20.0 version software. DMRT test was conducted to determine the significance of treatments.

**Results and Discussion**

**Effect of fungal bioagents on the parasitism of \textit{M. incognita} eggs**

The maximum percentage of egg parasitism was recorded in the treatment with \textit{P. chlamydosporia} (68.03\%) followed by \textit{P. lilacinum} (64.45\%), \textit{T. viride} (43.39\%) and \textit{T. harzianum} (39.46\%) (Table 1 and Figure 1). These treatments were significantly different from each other. However, no egg parasitism was observed in control. Further, while studying the egg parasitism, extensive network of hyphae of all the tested bioagents were detected that ramified several eggs of \textit{M. incognita} (Figure 2). Moreover, some incubated eggs appeared abnormal, deformed and shrunken owing to the mechanical pressure exerted by the network of hyphae of the bioagents inside the eggs. Most of the
immature eggs parasitized by bioagents and contents of the egg and embryo seemed to be disintegrated.

**Effect of fungal bioagents on hatching inhibition of** *M. incognita* **eggs**

Statistical analysis (Table 2) shows that treatments (f= 18876.86, df= 5 and p≤ 0.0001), significantly decreased egg hatching of *M. incognita* at different concentrations (f= 4788.08, df= 3 and p ≤ 0.0001). No egg hatch inhibition was recorded in controls (PDB and SDW). At 25 per cent concentration of culture filtrate the maximum egg hatch inhibition was recorded in *T. harzianum* (31.45%) followed by *T. viride* (27.61%), *P. chlamydosporia* (25.28%). Minimum egg hatch inhibition was recorded with *P. lilacinum* (22.97%). However, all the treatments were significantly different from each other. It was observed that with increase in concentrations there was increase in the egg hatch inhibition and similar trend of egg hatch inhibition as recorded at 25 percent concentration was recorded at 50, 75 and 100 percent concentration of culture filtrates (Figure 3). The highest egg hatch inhibition to the tune of 64.16, 61.69, 58.05 and 55.86 percent was recorded in *T. harzianum, T. viride, P. chlamydosporia* and *P. lilacinum* respectively at 100 per cent concentration of culture filtrate. The interaction effect between treatment and concentration (f=487.546, df= 15 and p≤ 0.0001) showed highly significant effect on egg hatching inhibition of *M. incognita*.

**Effect of fungal bioagents on mortality of** *M. incognita* **J**<sub>2</sub><br>

All the fungal bioagents showed significant increase in mortality of *M. incognita* J<sub>2</sub> irrespective of concentrations of the culture filtrates as compared to the controls. No mortality of *M. incognita* J<sub>2</sub> was recorded in controls (PDB and SDW). Statistical analysis (Table 3) shows that all the treatments (f= 70980.53, df= 5 and p< 0.0001), causes J<sub>2</sub> mortality of *M. incognita* in different concentrations (f= 4598.42, df= 3 and p≤ 0.0001) at different exposure time interval (f= 13216.98, df= 3 and p≤0.0001). The concentration of culture filtrate of treatments is increased there is corresponding increasing in mortality of J<sub>2</sub> of *M. incognita*. Among the bioagents, culture filtrate of *T. harzianum* was found to cause maximum mortality of J<sub>2</sub> in all the concentrations and at different time of exposure as compared to other bioagents. *T. harzianum* showed maximum mortality of J<sub>2</sub> in 25, 50, 75 and 100 percent concentration after 96 hrs of exposure. After *T. harzianum* the best bioagent was found to be *T. viride* which also caused considerably increased mortality of J<sub>2</sub> at all the concentrations and time of than the other bioagents. However, other bioagents were also effective in causing mortality of J<sub>2</sub> with varying degrees at different concentrations of culture filtrates and at different exposure time.

It was observed that there was an increasing trend in mortality of J<sub>2</sub> with increase in concentration and time of exposure in all the bioagents (Figure 4). All bioagents showed maximum mortality of J<sub>2</sub> in 100 percent concentration of culture filtrate at all the time of exposure. The bioagents like *T. harzianum, T. viride, P. lilacinum, P. chlamydosporia* showed 100 percent mortality of J<sub>2</sub> only at 100 per cent concentration and after 96 hrs of exposure time.

The interaction effect between treatment and concentration (f= 474.95, df= 15 and p≤0.0001), treatment and time (f= 1403.64, df= 15 and p≤0.0001), time and concentration (f= 13.57, df= 9 and p<0.0001) and treatment × concentration × time (f= 7.14, df= 45 and p≤0.0001) showed highly significant effect on mortality of *M. incognita* J<sub>2</sub>.
**Fig. 1** Effect of fungal bioagents on the parasitism of *M. incognita* eggs

![Graph showing parasitism of *M. incognita* eggs by different fungal bioagents](image)

**Fig. 2** Parasitism of *M. incognita* eggs by fungal bioagents

![Images showing parasitism of *M. incognita* eggs](images)
a- Egg parasitized by *T. viride*, b- Egg parasitized by *T. harzianum*, c- Egg parasitized by *P. chlamydospora*, d- Egg parasitized by *P. lilacinum*.

**Fig. 3** Effect of culture filtrates of fungal bioagents on the hatching inhibition of *M incognita* eggs.
**Table 1** Effect of fungal bioagents on the parasitism of *M. incognita* eggs

| Treatments       | Egg parasitism (%) |
|------------------|--------------------|
| *T. viride*      | 47.20 (43.39)      |
| *T. harzianum*   | 40.40 (39.46)      |
| *P. chlamydosporia* | 86.00 (68.03)     |
| *P. lilacinum*   | 81.40 (64.45)      |
| Control          | 0.0 (0.0025)       |
| S.Ed (±)         | 0.40               |
| C.D. (0.05)      | 0.85               |

The values in the parenthesis are arc sin transformation before analysis. Mean with different letters in the column are significantly different from each other based on Duncan’s Multiple Range Test (C.D. at 0.05).
Table 2 Effect of culture filtrates of fungal bioagents on the hatching inhibition of *M. incognita* eggs

| Treatments          | Concentrations | 25%        | 50%        | 75%        | 100%       |
|---------------------|----------------|------------|------------|------------|------------|
|                     |                | 25%        | 50%        | 75%        | 100%       |
| *T. viride*         |                | 21.50      | 46.50      | 59.67      | 77.50      |
|                     |                | (27.61)    | (42.99)    | (50.62)    | (61.69)    |
| *T. harzianum*      |                | 27.25      | 51.25      | 67.25      | 81.00      |
|                     |                | (31.45)    | (45.71)    | (55.09)    | (64.16)    |
| *P. chlamydosporia* |                | 18.25      | 34.25      | 53.50      | 72.00      |
|                     |                | (25.28)    | (35.81)    | (47.00)    | (58.05)    |
| *P. lilacinum*      |                | 15.25      | 29.25      | 49.25      | 68.50      |
|                     |                | (22.97)    | (32.73)    | (44.57)    | (55.86)    |
| PDB                 |                | -          | -          | -          | -          |
| SDW                 |                | -          | -          | -          | -          |

Factors | F value | df | P value |
|---------|---------|----|---------|
| Treatment | 18876.86 | 5 | 0.0001 |
| Concentration | 4788.08 | 3 | 0.0001 |
| Treatment × Concentration | 487.55 | 15 | 0.0001 |

“-” indicate no egg hatch inhibition. The value in the parenthesis are arc sin transformation before analysis.
### Table 3: Effect of culture filtrates of fungal bioagents on the mortality of *M. incognita J*$_2$

| Treatments       | Concentrations | 25% | 50% | 75% | 100% |
|------------------|----------------|-----|-----|-----|------|
|                  | 24hrs | 48hrs | 72hrs | 96hrs | 24hrs | 48hrs | 72hrs | 96hrs | 24hrs | 48hrs | 72hrs | 96hrs |
| *T. viride*      | 9.50  | 20.25 | 33.50 | 43.50 | 14.00 | 28.25 | 42.25 | 57.25 | 21.25 | 35.25 | 50.25 | 64.25 |
|                  | (17.95)| (26.74)| (35.36)| (41.26)| (21.97) | (32.10)| (40.54)| (49.17)| (27.45)| (36.42)| (45.14)| (53.28)|
| *T. harzianum*   | 11.50 | 24.75 | 38.50 | 46.50 | 16.00 | 31.00 | 45.00 | 62.00 | 24.00 | 39.00 | 55.00 | 68.00 |
|                  | (19.80)| (29.83)| (38.35)| (42.99)| (23.57) | (33.83)| (42.13)| (51.94)| (29.33)| (38.64)| (47.87)| (55.55)|
| *P. chlamydosporia* | 7.00  | 16.50 | 28.50 | 35.75 | 12.25 | 23.25 | 34.25 | 41.50 | 18.25 | 27.00 | 38.25 | 49.25 |
|                  | (15.32)| (23.95)| (32.26)| (36.71)| (20.48) | (28.82)| (35.81)| (40.10)| (25.28)| (31.30)| (38.20)| (44.58)|
| *P. lilacinum*   | 5.25  | 12.75 | 24.00 | 29.50 | 10.75 | 19.50 | 30.00 | 39.00 | 15.75 | 23.50 | 37.25 | 45.75 |
|                  | (13.20)| (20.91)| (29.32)| (32.89)| (19.13) | (26.20)| (33.20)| (38.64)| (23.37) | (28.99)| (37.61)| (42.56)|
| PDB              | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |
| SDW              | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     | -     |

| Factor          | f value | df | P value | Factor          | f value | df | P value |
|-----------------|---------|----|---------|-----------------|---------|----|---------|
| Treatment       | 70980.53| 5  | 0.0001  | Treatment× Time | 1403.64| 15 | 0.0001  |
| Time            | 13216.98| 3  | 0.0001  | Time × Concentration | 13.57 | 9  | 0.0001  |
| Concentration   | 4598.42 | 3  | 0.0001  | Treatment× Concentration × Time | 7.14   | 45 | 0.0001  |
| Treatment× Concentration | 474.95 | 15 | 0.0001  | ”-” indicate no J2 mortality,. The value in the parenthesis are arc sin transformation before analysis |

PDB = Potato Dextrose Broth, SDW = Sterilized Distilled Water
**Table 4** LC$_{50}$ values and relative toxicity of culture filtrates of fungal bioagents to *M. incognita* J$_2$ after 24 hrs of exposure

| Bioagents      | Time | LC$_{50}$ Value | Heterogeneity ($R^2$) | Regression equation | Fiducial limit           | Relative toxicity (%) | Rank |
|----------------|------|-----------------|-----------------------|---------------------|--------------------------|-----------------------|------|
| *T. harzianum* | 24   | 409.145         | 1.568                 | Y=0.727x-1.898      | 224.678-1665.669         | 100                   | 1    |
| *T. viride*    | 24   | 473.232         | 1.103                 | Y=0.156x-1.983      | 250.161-2109.804         | 84.46                 | 3    |
| *P. chlamydosporia* | 24 | 454.478         | 0.935                 | Y=0.829x-2.204      | 253-1615.245             | 90.02                 | 2    |
| *P. lilacinum* | 24   | 542.805         | 0.752                 | Y=0.839x-2.294      | 286.943-2212.619         | 75.38                 | 4    |

Y = Probit Kill, X = log dose. Mortality based on 4 replications each with 100 J$_2$ of *M. incognita*.

**Table 5** LC$_{50}$ values and relative toxicity of culture filtrates of fungal bioagents to *M. incognita* J$_2$ after 48 hrs of exposure

| Bioagents      | Time | LC$_{50}$ Value | Heterogeneity ($R^2$) | Regression equation | Fiducial limit           | Relative toxicity (%) | Rank |
|----------------|------|-----------------|-----------------------|---------------------|--------------------------|-----------------------|------|
| *T. harzianum* | 48   | 229.313         | 0.819                 | Y=0.573x-1.353      | 138.041-929.436          | 100                   | 1    |
| *T. viride*    | 48   | 250.599         | 0.517                 | Y=0.634x-1.522      | 152.035-875.987         | 91.51                 | 2    |
| *P. chlamydosporia* | 48 | 559.291         | 0.607                 | Y=0.530x-1.456      | 241.027-9296.985        | 41.00                 | 4    |
| *P. lilacinum* | 48   | 478.208         | 0.909                 | Y=0.642x-1.720      | 237.744-3046.486        | 48.07                 | 3    |

Y = Probit Kill, X = log dose. Mortality based on 4 replications each with 100 J$_2$ of *M. incognita*.
Table 6 LC$_{50}$ values and relative toxicity of culture filtrates of fungal bioagents to *M. incognita*$_{J2}$ after 72 hrs of exposure

| Bioagents        | Time | LC$_{50}$ value | Heterogeneity (R$^2$) | Regression equation | Fiducial limit         | Relative toxicity (%) | Rank |
|------------------|------|-----------------|-----------------------|---------------------|------------------------|-----------------------|------|
| *T. harzianum*   | 72   | 90.891          | 1.159                 | Y=0.575x-1.127      | 69.481-158.593         | 100                   | 1    |
| *T. viride*      | 72   | 119.499         | 0.442                 | Y=0.572x-1.189      | 86.286-262.319         | 76.06                 | 2    |
| *P. chlamydosporia* | 72   | 390.028         | 0.436                 | Y=0.394x-1.021      | 166.52-45753.086       | 23.30                 | 4    |
| *P. lilacinum*   | 72   | 284.445         | 0.810                 | Y=0.533x-1.307      | 155.575-1839.866       | 31.95                 | 3    |

Y= Probit Kill, X= log dose. Mortality based on 4 replications each with 100 J$_2$ of *M. incognita*.

Table 7 LC$_{50}$ values and relative toxicity of culture filtrates of fungal bioagents to *M. incognita*$_{J2}$ after 96 hrs of exposure

| Bioagents        | Time | LC$_{50}$ value | Heterogeneity (R$^2$) | Regression equation | Fiducial limit         | Relative toxicity (%) | Rank |
|------------------|------|-----------------|-----------------------|---------------------|------------------------|-----------------------|------|
| *T. harzianum*   | 96   | 29.617          | 2.060                 | Y=1.315x-1.936      | 24.160-34.300         | 100                   | 1    |
| *T. viride*      | 96   | 52.230          | 0.683                 | Y=0.722x-1.240      | 41.737-64.082         | 56.70                 | 2    |
| *P. chlamydosporia* | 96   | 141.211         | 0.754                 | Y=0.477x-1.026      | 92.853-543.028        | 20.97                 | 3    |
| *P. lilacinum*   | 96   | 165.138         | 0.208                 | Y=0.544x-1.207      | 107.899-541.017       | 17.93                 | 4    |

Y= Probit Kill, X= log dose. Mortality based on 4 replications each with 100 J$_2$ of *M. incognita*.
**LC₅₀ values and relative toxicity of culture filtrates of fungal bioagents to *M. incognita* J₂**

Data reveal (Table 4, 5, 6 and 7) that the LC₅₀ values of culture filtrate of *T. harzianum* against *M. incognita* J₂ were 409.14, 229.31, 90.89 and 29.61 per cent for the exposure period of 24, 48, 72 and 96 hrs respectively. In case of *T. viride* the LC₅₀ values were 473.23, 250.59, 119.49 and 52.23 percent, *P. chlamydosporia* were 454.47, 559.29, 390.02 and 141.21 and *P. lilacinum* were 542.80, 478.20, 284.44 and 165.13 percent for the exposure period of 24, 48, 72 and 96 hrs respectively. However no J₂ mortality was observed in controls.

*In-vitro* testing of nematode destroying fungi is an essential method for evaluating their antagonistic activity against *M. incognita* and such antagonistic fungi produced metabolites and/or protease enzymes that affect viability of nematode (Nitao *et al.*, 1999). Nematode egg shell is proteinaceous and chitinous in nature and act as barriers for egg parasitic fungi. To overcome these barriers, these fungal bioagents produce lytic enzymes *viz.* proteases, chitinases and lipases that cause break down of egg shell and facilitate egg penetration for successful establishment (Elad *et al.*, 1982; Lorito *et al.*, 1993; Baker and Griffin, 1995; Chet *et al.*, 1997; Jansson *et al.*, 1997 and Limon *et al.*, 1998) and acetic acid (Blaxster and Robertson, 1998 and Jansson *et al.*, 1997) in the filtrates that cause break down of nematode cuticle proteins (Sharon *et al.*, 2001). The variable effect of tested fungal filtrate on *M. incognita* was observed in the present investigation can be attributed to have ability to the production of such toxic metabolites as well as protease enzymes in the filtrates.

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How to cite this article:

Annapurna, M., B. Bhagawati and Kurulkar, Uday. 2018. In-vitro Efficacy of Native Fungal Bioagents against Meloidogyne incognita. Int.J.Curr.Microbiol.App.Sci. 7(11): 396-410. doi: https://doi.org/10.20546/ijcmas.2018.711.048