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Full Length Research Article

Evaluation of Endophytic Entomopathogenic Isolates of *Purpureocillium lilacinum* against Root Knot Nematode, *Meloidogyne incognita*

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Abstract

Utilization of indigenous isolates of *Purpureocillium lilacinum* (Thom.) has good potential for the management of plant parasitic nematodes. Nematotoxic compounds from culture filtrates of *P. lilacinum* have negative effect on these nematodes. This study was designed to assess the effect of culture filtrates of two indigenous endophytic entomopathogenic isolates of *P. lilacinum* viz., EEF 4 and EEF 64 on juveniles of root knot nematode, *Meloidogyne incognita*. The tested fungal isolates as filtrate affected the survival of second stage juveniles at different degrees according to fungal filtrate dilution and exposure period under *in vitro* study. After 24 hours, significantly higher mortality of juveniles was recorded in the isolate EEF 64 (86.11%), followed by EEF 4 (75.00%) at 100% concentration. Maximum cumulative mean mortality of 72.37 per cent was observed in EEF 64 at 100% concentration, followed by EEF 4 at 100% concentration (63.92%). It can be concluded that these isolates proved to be effective against root knot nematode. Further studies are needed to explore the endophytic nature of these fungal entomopathogenic isolates against nematodes and insect pests.

***Key words*:** Endophytic fungus, *Purpureocillium lilacinum*, Culture filtrate, *Meloidogyne incognita*, Juveniles

Root knot nematode, *Meloidogyne incognita* is an important plant parasitic nematode and cause heavy yield loss in different crops. Root knot nematodes are obligate parasites attacking more than 2000 species of cultivated plants and cause approximately five per cent of global crop loss [1]. Due to lack of safe chemical nematicides and potential resistant varieties, it becomes difficult to manage this nematode in the field condition. Use of chemical nematicides can lead to several negative impacts such as environmental hazards, and resurgence. Therefore, natural enemies already present in local ecosystems need to be preserved and utilized for the management of these plant parasitic nematodes. Root knot nematodes can be effectively managed by many potential biocontrol agents such as antagonistic fungi.

Purpureocillium lilacinum (*Paecilomyces lilacinus*) is used as an effective biocontrol agent against various plant parasitic nematodes [2]. It is a group of fungi that produce nematotoxic substances that affect nematode eggs, inhibit the mobility of juveniles and also have activity as nematicides [3]. Association of fungal endophytes with plants is known to improve plant vigour and growth [4] and to potentially confer resistance in host plants against pathogen and insect pest attack [5]. The mechanisms responsible for nematode biocontrol mediated by endophytes include: the production of toxic compounds, competitive exclusion, predation, competition for nutrients, the induction of systemic resistance, production of repellent compounds or a combination of these elements [6-10].

MATERIALS AND METHODS

The present research was carried out in the laboratory of Department of Agricultural Entomology, College of Horticulture, Vellanikkara, Thrissur, India. Effect of two indigenous endophytic entomopathogenic isolates of *P. lilacinum* viz., EEF 4 and EEF 64 on juveniles of root knot nematode, *M. incognita* was evaluated under in *vitro* condition.

Fungal isolates

These endophytic isolates were obtained from cowpea plants collected from Kottayam and Kozhikode districts of Kerala, India. The cultures of fungal isolates were maintained on potato dextrose agar (PDA) slants for biological studies [11]. Morphological and molecular characterizations were done to confirm the identity of species.

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Maintenance of pure culture of M. incognita

To mass culture the nematodes, egg masses were taken from infected roots of brinjal plants. Egg masses were picked from the roots using needle under zoom stereoscopic binocular microscope. Egg masses were kept in cavity blocks containing sterile distilled water for 24 hours. Infective juveniles which were hatched from egg masses were collected and inoculated into new pots containing susceptible plants. Susceptible solanaceous vegetables like tomato and brinjal were used for maintaining the pure culture. Potting mixture was sterilized before inoculation. The pure culture was thus multiplied and maintained for further lab studies [12].

The extraction of *Meloidogyne*-J2 from roots was done by sieve method. Roots were collected from infected plants and washed under running tap water to remove soil particles. Roots were cut into 1 cm pieces and kept on double layered tissue paper placed on wire gauge support. Wire gauges with roots were further placed over Petri plates containing sufficient water to touch the wire gauge. After 24 hours, trapped infective juveniles were collected in a beaker and used for conducting *in vitro* evaluation of *P. lilacinum* against nematodes.

Preparation of cell –free culture filtrates of P. lilacinum

For evaluating the effect of two different isolates of *P. lilacinum* i.e., EEF 4 and EEF 64, culture filtrates were prepared. Isolates of *P. lilacinum* were inoculated into Potato Dextrose broth and incubated in a rotary shaker for two weeks. Mycelial mat was removed by sieving the broth through four layered sterile muslin cloth and Whatman No. 1 filter paper. Broth was then centrifuged at 10000 rpm for 15 min at 4°C. The resultant supernatant was aseptically transferred to sterile 25 ml screw cap glass bottles and filtered through millipore membrane filters. Sterile distilled water at neutral pH was the control in the experiment. Culture filtrates were placed in the refrigerator at 4°C overnight.

In vitro evaluation of culture filtrates of P. lilacinum against juveniles of M. incognita

Culture filtrates were made into four concentrations of 100, 75, 50 and 25 per cent by adding sterile distilled water. Five millilitres of two week old culture filtrates of different concentrations were transferred to sterile cavity blocks. Twenty numbers of Infective juveniles (J2s) were carefully picked from the container using disposable syringe and transferred to each cavity block. The evaluation was laid out in a completely randomized design with four replications. Cavity blocks were kept at room temperature and observation was taken at regular interval. Mortality was determined by counting the number of dead nematode of *Meloidogyne*-J2 after 6, 12 and 24 hours of exposure to culture filtrate. Nematodes were probed with a needle under the zoom stereoscopic microscope, and those which were straight in shape and remained immotile even after touching were considered dead. Abbott's corrected mortality formula was used to calculate nematode mortality [13]. After correcting the mortality data were subjected to analysis of variance (ANOVA).

$$\text{Abbott's corrected mortality} = \frac{\text{Treatment mortality} - \text{Control mortality}}{100 - \text{Control mortality}} \times 100$$

RESULTS AND DISCUSSION

Two isolates of *P. lilacinum* viz., EEF 4 and EEF 64 were evaluated under *in vitro* condition for their effect on the mortality of juveniles of *M. incognita* at different concentrations. Mortality of second stage juveniles of *M. incognita* at different concentrations (100, 75, 50 and 25%) were recorded at different time intervals i.e., after 6, 12 and 24 hours of exposure. Both the isolates had resulted in mortality of juveniles at four different concentrations (Table 1). Sharma *et al.* [14], Mane and Mhase [15] reported the effect of *P. lilacinus* on mortality of juveniles of *M. incognita.* Singh and Mathur [16] also evaluated the potential of indigenous fungal isolates like *Trichoderma* sp. and *Paecilomyces lilacinus* against *M. incognita* and reported the efficacy of culture filtrates of *P. lilacinus* against juveniles. Sharma *et al.* [14] conducted an *in vitro* evaluation of culture filtrate of *Paecilomyces lilacinus* 6029 against juveniles of *M. incognita* and found that culture filtrate from Karanja medium and Czapeck-Dox medium caused 100 and 78.28 per cent mortality. Sivakumar *et al.* [17] evaluated culture filtrate of *Bacillus cereus*, *Pseudomonas fluorescens* and *Paecilomyces lilacinus* against juveniles of root knot nematode at 24, 48 and 72 hours and *Paecilomyces lilacinus* caused maximum juvenile mortality followed by *Pseudomonas fluorescens* and *Bacillus cereus.*

Table 1 Effect of cell-free culture filtrate of two isolates of *Purpureocillium lilacinum* on mortality of *Meloidogyne incognita* in *in vitro*

Treatments	Concentrations	Per cent mortality of *Meloidogyne* J2s at different exposure periods			
		6 HAT	12 HAT	24 HAT	Cumulative mortality
P. lilacinum (EEF 4)	T_1: 100%	42.50^{ab}	73.69^{a}	75.00^{b}	63.92^{b}
	T_2: 75%	40.00^{b}	44.74^{bc}	61.12^{cd}	48.62^{cd}
	T_3: 50%	40.00^{b}	42.11^{bc}	52.78^{de}	44.96^{cd}
	T_4: 25%	30.00^{b}	39.47^{cd}	41.66^{fg}	37.05^{ef}
P. lilacinum (EEF 64)	T_5: 100%	55.00^{a}	76.32^{a}	86.11^{a}	72.37^{a}
	T_6: 75%	40.00^{b}	50.00^{b}	66.67^{bc}	52.23^{c}
	T_7: 50%	35.00^{b}	42.11^{bc}	50.00^{ef}	42.37^{de}
	T_8: 25%	30.00^{b}	31.58^{d}	38.86^{g}	33.49^{f}
	T_9: Broth	0.00	5.00	10.00	5.00
	T_{10}: SDW*	0.00	5.00	10.00	5.00
	CD (P=0.05)	12.90	9.41	9.36	7.27

*SDW – Sterile distilled water; HAT- Hours after treatment

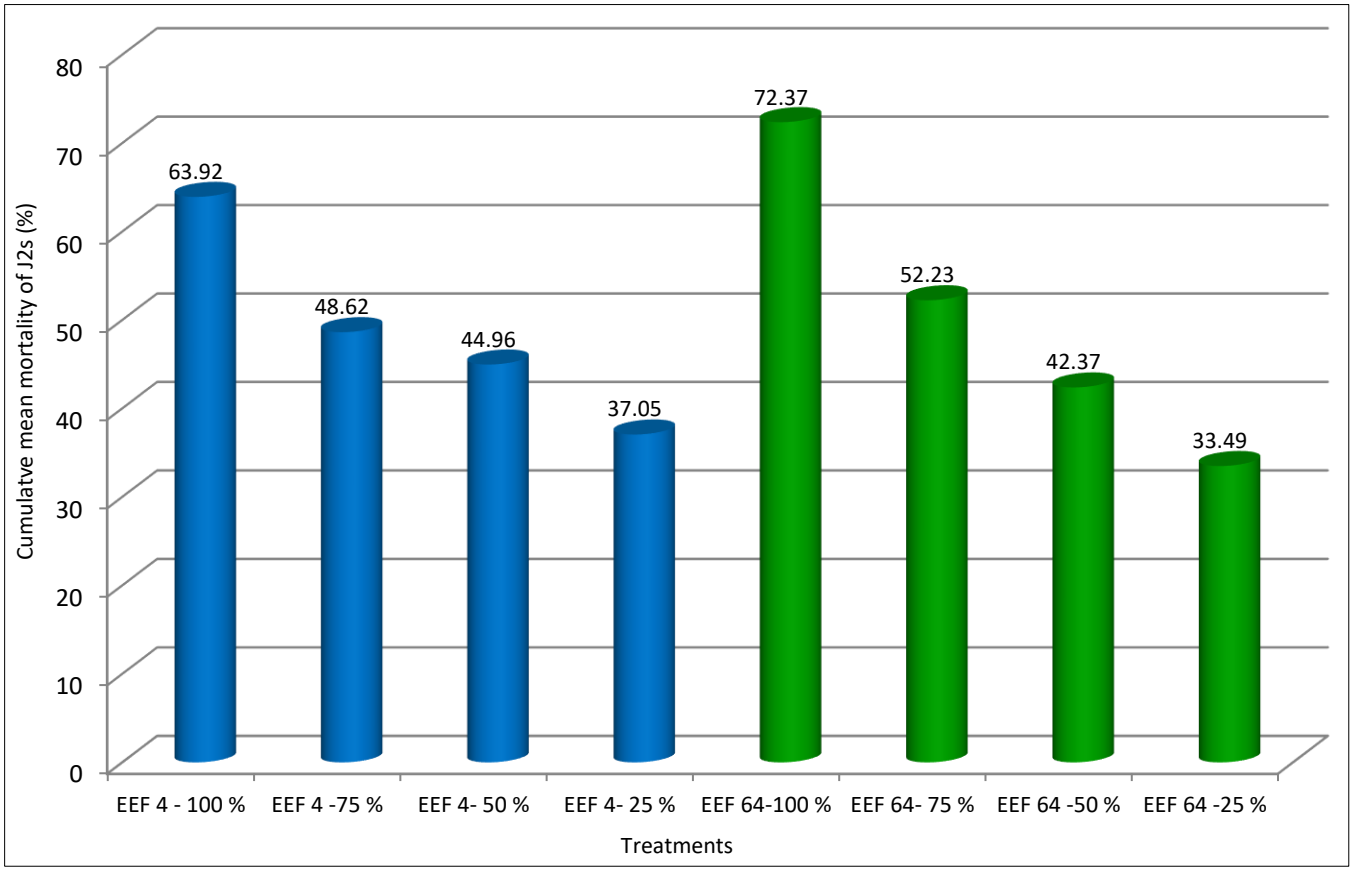

Fig 1 Effect of different treatments on mean cumulative mortality of juveniles of *M. incognita*

For both the isolates, per cent mortality was increased with increase in concentration and time of exposure (Table 1). Among different treatments, maximum mortality was observed in treatment with EEF 64 at 100 =% concentration i.e., 55.00, 76.32 and 86.11 per cent mortality at 6, 12 and 24 hours respectively. The next best treatment was EEF 4 at 100 % concentration, which recorded 42.50, 73.69 and 75.00 per cent mortality at 6, 12 and 24 hours of exposure. A steady decrease in mortality was observed, when the filtrates were diluted from 100 to 25 per cent. This result indicated that the mortality of *M. incognita* was concentration dependent. Low rates of juvenile mortality at low concentration might be due to the dilution of toxic compounds. This finding was in conformity with the results of Bawa [18] who reported that per cent juvenile mortality of root knot nematode was increased with increase in concentration of *P. lilacinus* and exposure period. Amin [19] reported the effect of culture filtrate concentration of endophytic fungal isolate, *Nigospora* sp. isolate RS 10 on mortality of the *Meloidogyne* sp. Similar results found by Ashraf and Khan [20] who studied the effect of culture filtrates of some opportunistic fungi against mortality of *M. javanaica* and found that *P. lilacinus* at 100 and 50 per cent caused highest mortality.

Significantly higher cumulative mean mortality of 72.37 per cent was observed in endophytic fungal isolate, EEF 64 at 100% concentration, followed by EEF 4 at 100% concentration (63.92%) (Table 1, Fig 1). From these results, it is concluded that the isolate EEF 64 was found to be the most effective treatment against juveniles of root knot nematode, *M. incognita*. The mortality of infective juveniles by culture filtrate of *P. lilacinus* might be due to production of toxic metabolites such as paecilotoxin [21] and leucinostatins [22].

Association of fungal endophytes with plants is known to potentially confer resistance in host plants against pathogen and insect pest attack [4]. Production of these toxic metabolites in the colonized plants by the fungal endophytes protects host plant from nematode infection by inhibiting nematode activity, host searching and infection [23]. Endophytic nature of these isolates can be well utilized in future as they protect host plant from nematode and insect pest attack by multiple mechanisms including production of toxic compounds.

CONCLUSION

Culture filtrates of endophytic isolates of *Paecilomyces lilacinus* caused mortality of juveniles of root knot nematode, *Meloidogyne incognita*. It might be due to the secretion of toxic metabolites such as paecilotoxin and leucinostatins. Production of toxic metabolites in the colonized plants by the fungal endophytes protects host plants from nematode infection by inhibiting nematode activity, host searching and infection. The toxic compounds produced inside the plant by these fungal endophytes and the mechanism whereby endophytes protect host plants against nematodes should be studied further for utilizing these isolates against plant parasitic nematodes.

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