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Biocontrol Potentiality and Plant Growth Promoting Ability of Nematophagous Fungi

Saranya S., S. I. Patel

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ABSTRACT

Nematophagous fungi (NPF) are the group of carnivorous fungi specialized in trapping and digesting nematodes. This study aimed to reveal the biocontrol potentiality and plant growth promoting ability of nematophagous fungi viz., *Arthrobotrys oligospora*, *Paecilomyces lilacinus* (Isolate 1 and Isolate 2) and *Pleurotus* sp. The biocontrol potentiality of NPF was assessed by counting the number of eggs and juveniles of root knot nematode parasitized and plant growth promoting ability of NPF was assessed by determining the amount of IAA produced and Phosphate solubilized by them under *in vitro* condition, each at different time intervals. *Pleurotus* sp. was found to be the most promising NPF in parasitizing both the eggs and juveniles of root knot nematodes whereas *A. oligospora* produced the maximum amount of solubilized phosphate and IAA.

Saranya S.
Department of Plant Pathology, Chimanbhai Patel College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar 385506, India

S. I. Patel*
Director of Extension Education (Rted.), Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar 385506, India
Email : sipatel@sdau.edu.in
*Corresponding author

Keywords Nematophagous fungi, Biocontol potentiality, Plant growth, Root knot nematode, IAA.

INTRODUCTION

Nematophagous fungi are the group of carnivorous fungi specialized in trapping and digesting nematodes. They are ubiquitous in nature and are reported to remain present in a wide variety of ecological habitats. Around 160 species are known. They are found in almost every natural soil and also in several other substrates. They have a very good ability to colonize on decaying plant litters, rotten woods and decay inorganic matter and also contribute to the decomposition of organic matter. In nutrient-limited environments also, they can compete well due to the better competitive saprophytic ability. Askary (2015) studied plant-parasitic nematodes and recognized them as a serious threat to crop production throughout the world. All crops are susceptible to at least one nematode species and it is considered that the damage potential of nematodes exists in all climates on any crop. The amount of illness caused to plants by these minute creatures varies from species to species. A group of plant-parasitic nematodes which parasitizes and feed on roots of plants by producing gall like structures is known as root-knot nematodes (RKN). These root-knot nematodes belong to the genus *Meloidogyne.*

Vegetable crops grown in warm climates are susceptible to root-knot nematodes and they experience severe losses also. These vegetable crops are often

routinely treated with chemical nematicides viz., carbofuran, carbosulfan, fenamiphos, dazomet and aldicarb. These nematicides are not only less effective to target pests, but are mostly hazardous to non-pathogenic microbial population. Now-a-days, options for crop rotation and the availability of resistant cultivars have also become limited. These circumstances paved the way for the development of new management strategies of plant-parasitic nematodes with the help of biological control agents. These biocontrol agents are a group of antagonistic microorganisms that suppress soil-borne pathogenic microorganisms (Berg *et al.* 2005). This approach is economically feasible, cost- effective and ecologically safer than chemical nematicides (Shamalie *et al.* 2011). The aim of biological contrrol is to maintain the nematode population below the economic threshold level (ETL) rather than eliminating it as done by the chemicals.

Only few species of nematophagous fungi like *Purpureocillium lilacinum* are popular and had been commercialized in the farmers' field. But *P. lilacinum* is mainly infesting the females and egg masses of the plant-parasitic nematodes. Moreover, their field trials have exerted varying results, with some strains being aggressive and others less pathogenic, and some strains that appeared promising in the lab proved ineffective in the field (Singh *et al.* 2013). Hence, we searched for other alternatives to this group. Many other species of nematode-trapping fungi (*Arthrobotrys* sp.) and toxin-producing fungi (*Pleurotus* sp.) are less explored in this aspect. Considering these issues in mind, it was decided worthwhile to carry out the detailed studies of nematophagous fungi with the aim to test the biocontrol potentiality against root-knot nematode and plant growth promoting ability of nematophagous fungi under *in vitro* condition.

MATERIALS AND METHODS

Collection and maintenance of fungal cultures

The nematophagous fungal cultures viz., *Arthrobotrys oligospora, Paecilomyces lilacinus* and *Pleurotus* sp. were collected from the Department of Plant Pathology, Chimanbhai Patel College of Agriculture, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar, Gujarat and were used for the study. These cultures were regularly sub- cultured and mass multiplied.

Collection and maintenance of plant-parasitic nematode (*Meloidogyne* sp.)

The population of second-stage juvenile of *Meloidogyne* sp. were obtained from infected crops like tomato, brinjal, okra. The mature knots were first washed in tap water and then soaked in sterile distilled water for overnight. In very next day, knots were teased with a fine, sterile needle to extract the egg masses. The egg masses were then cleaned and subsequently kept in fresh sterile distilled water and incubated at 28°C for hatching. Immediately after hatching, J_2 population was used for experimental work.

Testing *in vitro* parasitism of nematophagous fungi against root-knot nematode

To study the interaction between fungi and nematodes in dual culture, a 5 mm fungal disc of each isolate of fungus was taken from the periphery of 10 days old culture and inoculated into each Petri-plates containing cornmeal agar medium. Inoculated Petri-plates were incubated in a dark condition at 27 ± 1°C. When fungal colony covered almost at the edge of the plate, the fungal disc was removed aseptically. plant-parasitic nematodes were inoculated into the culture plates with the help of a sterilized dropper. Petri-plates were incubated at 25 ± 1°C for observation. The number of nematodes trapped and killed at different time intervals viz., 24, 48, 72 and 96 hrs were counted by placing the Petri-plates under microscope (4X and 10X) and the observations were recorded.

Similarly, for studying the egg parasitic nature of the fungi nematode eggs from egg mass were taken and kept in Petri-plates containing water agar medium. Then, 1 ml spore suspension of nematophagous fungi was poured over the eggs and the plates were incubated in dark condition at 25 ± 1°C. The number of eggs hatched at 24, 48, 72 and 96 hrs were counted by counting the number of J_2 population present in the Petri-plates under microscope (4X and 10X) and

Table 1. *In vitro* parasitism of root-knot nematode eggs. Data are means of three replicates. Values in columns followed by the common letter (s) are not significantly different at 5% level of significance.

Fungi	Time Interval: Number of eggs hatched after 24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
Paecilomyces lilacinus (Isolate^{-1})	0	0	2	7	10
Paecilomyces lilacinus (Isolate^{-2})	0	0	0	3	8
Pleurotus sp.	0	0	0	0	3

observations were recorded.

Evaluation of plant growth-promoting ability of nematophagous fungi

The plant growth promoting ability of nematophagous fungi was estimated by measuring the IAA production and phosphate solubilization ability of the fungi.

Determination of IAA production

IAA production by nematophagous fungi was determined by method given by (Kumla *et al.* 2014). Nematophagous fungi were grown in 25 ml of Czapek-Dox broth (Difco) media, (pH 6.0) supplemented with 1 mg ml^{-1} of L-tryptophan in 100 ml Erlenmeyer flask. Fungal mycelia (5 mm diameter) were transferred to the flask from the periphery of the growing colony. Flask was then incubated in dark at 30 °C with shaking at 150 rpm on a reciprocal shaker for 20 days.

After incubation, the cultures were centrifuged at 11000 rpm for 15 min to harvest the supernatant. Then colorimetric assay (Tsavkelova *et al.* 2007) was done by taking 1 ml of the supernatant mixing it with 2 ml of Salkowski's reagent (1 ml of 0.5 M $FeCl_3$ in 50 ml of 35% $HClO_4$) and incubated in the dark for 30 min. A pink to red color was considered positive for IAA production.The level of IAA produced was estimated by a standard IAA curve.

Table 2. *In vitro* parasitism of root-knot nematode juveniles.

Fungi	Time Interval: Number of nematodes trapped after 24 hrs	48 hrs	72 hrs	96 hrs	120 hrs
A. oligospora	0	1	3	8	8
Pleurotus sp.	2	3	8	11	12

Determination of solubilized phosphate

Fungal discs from four days old PDA cultures from each of nematophagous fungi (4 mm diameter) were inoculated to the Pikovskya broth in 250 ml conical flasks. Each inoculation was replicated 3 times. Flasks were then incubated at 30°C for 15 days under shaking conditions. Uninoculated flasks were kept for each set of treatments. After 15 days, the contents of the flasks were filtered through Whatman No. 42 filter paper.

Water-soluble P in the culture filtrates was estimated by the chlorostanous reduced molybdophosphoric acid blue method described by Jackson (1967). Two milliliters of fifteen days old culture filtrate was centrifuged at 10000 rpm for 10 min and the supernatant was used to estimate the solubilized phosphate. One milliliter of this supernatant was mixed with 10 ml of chloromolybdic acid and the volume was adjusted to 40 ml with distilled water. To this, 1 ml of

Table 3. Phosphate solubilizing ability of nematophagous fungi.

Treatments	5 DAI	10 DAI	15 DAI	Mean
T_1- Uninoculated control	24.79^{d}	27.50^{e}	35.48^{d}	29.26
T_2- *Arthrobotrys oligospora*	45.13^{a}	56.39^{a}	83.20^{a}	61.57
T_3-*Paecilomyces lilacinus* Isolate 1 (P_1)	37.39^{b}	43.32^{c}	57.21^{b}	45.97
T_4- *Paecilomyces lilacinus* Isolate (P_2)	38.64^{b}	47.53^{b}	59.47^{b}	48.55
T_5- *Pleurotus* sp.	31.40^{c}	35.83^{d}	44.00^{c}	37.08
Mean	35.47	42.11	55.87	44.49
CV%			3.77	

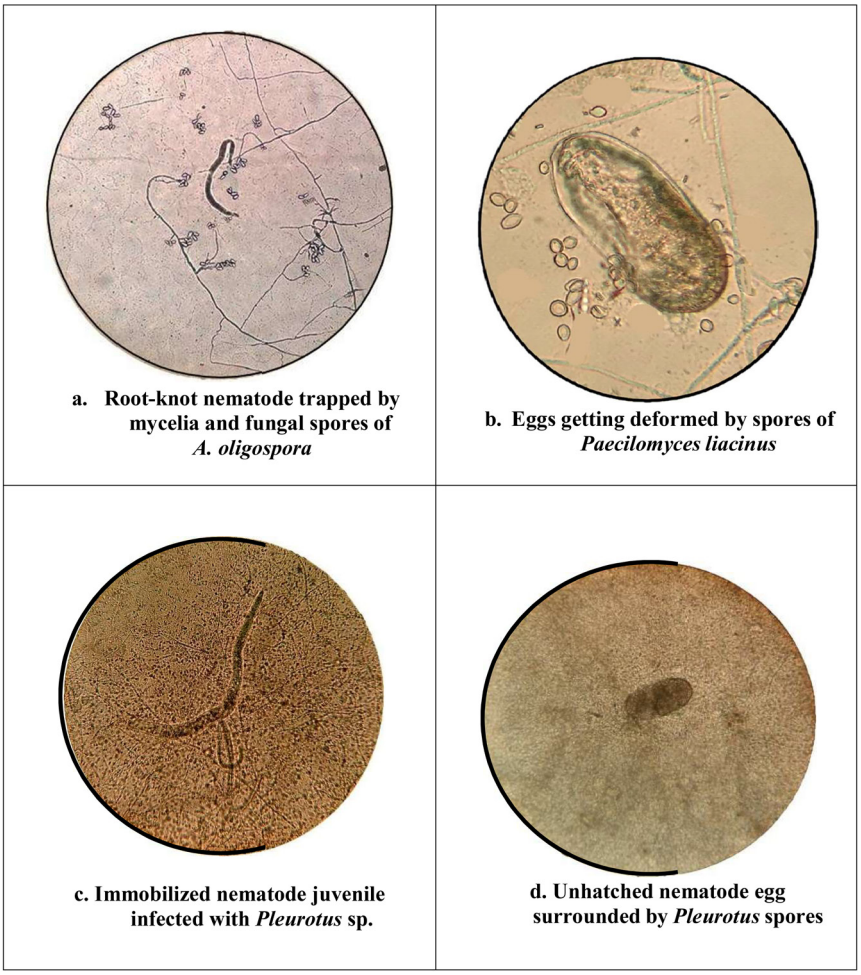

Fig. 1. Biocontrol potential of nematophagous fungi on RKN juveniles and eggs.

chlorostannous acid was added and the volume was made up to 50 ml with distilled water.

Potassium dihydrogen phosphate was used as a standard. The P released in the supernatant was measured at 600 nm wavelength with UV- VIS spectrophotometer. The absorbance values were plotted against the concentration to get the standard curve and the soluble P was estimated.

Statistical analysis

The experiment was carried out in Completely Randomized Design with three replications and the data subjected to analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) using statistical package for Social Sciences Version 22.0 (SPSS 22.0) program. Data were compared with DMRT at $p \leq 0.05$.

RESULTS AND DISCUSSION

Testing *in vitro* parasitism of nematophagous fungi against root-knot nematode

The isolates of *P. lilacinus* as well as *Pleurotus* sp. were tested for their potential to inhibit the egg hatching of root-knot nematode at different time intervals viz., 24, 48, 72, 96 and 120 hrs (Table 1). The lowest number of egg hatching (3 eggs) after 120 hrs was

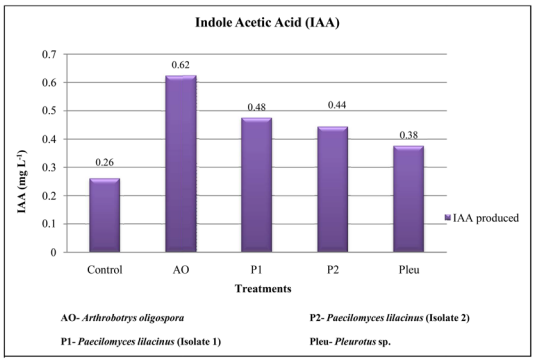

Fig. 2. IAA produced by nematophagous fungi.

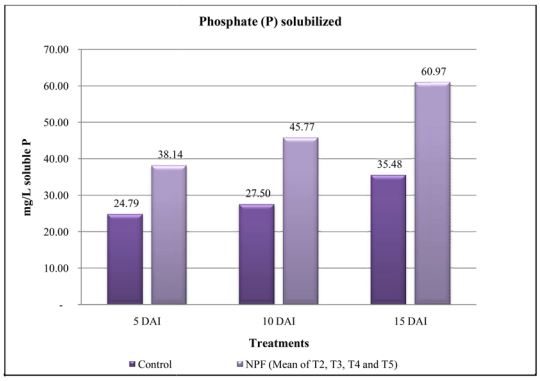

Fig. 3. Phosphate solubilized at 5, 10 and 15 days after inocultation.

recorded in *Pleurotus* sp. thereby showing its extensive ability to parasitize the eggs of root knot nematodes. This was followed by Isolate 2 of P. lilacinus (8 eggs) and the highest number of hatched eggs (10 eggs) after 120 hrs were recorded in the culture with *P. lilacinus* (Isolate 1).

A. oligospora and *Pleurotus* sp. were tested *in vitro* for their ability to parasitize root knot nematodes at different time intervals viz., 24, 48, 72, 96 and 120 hrs (Table 2). *Pleurotus* sp. recorded the highest number of parasitized juvenile at different time intervals ranging from 2 nematode juveniles at 24 hrs to 12 nematode juveniles at 120 hrs whereas *A. oligospora* started trapping nematodes only after 48 hrs of incubation and the nematodes parasitized were in the range of 1 nematode juvenile at 48 hrs to 8 nematode juveniles at 120 hrs.

Under *in vitro* condition, *Pleurotus* sp. was the most potential nematophagous fungi which efficiently controlled both the eggs and juveniles of root-knot nematode (Fig. 1 (c)–(d)). This is due to the fact that *Pleurotus* sp. releases a toxic substance which inhibits egg hatching and immobilizes the nematode juveniles (Barron and Thorn 1987; Heydari *et al. (*2006), Singh *et al.* 2019) and Hahn *et al.* (2019). *A. oligospora* trapped root-knot nematodes by coiling around host body using its three dimensional adhesive network and killed them (Figs. 1 (a)–1(d)). This trapping behavior of *A. oligospora* was found to be relevant with the previous findings of Persson *et al.* (1985) and Singh *et al.* (2012). Both the isolates of *P. lilacinus* were found to be excellent egg parasitizing fungi because it degrades the chitin and protein present in the eggs of root knot nematode by using enzymes chitinase and protease thereby producing deformed (Fig. 1 (b)) and vacuolated eggs. This finding was similar to the studies done by Fitters *et al.* (1992) and Khan *et al*. (2004). Isolate 2 of *P. lilacinus* was found to be 20% more efficient in parasitizing eggs than Isolate 1.

Determination of IAA production

Indole-3-acetic acid (IAA) produced by nematophagous fungi was determined 20 days after inoculation in Czapek-Dox broth media amended with 0.1% tryptophan and the data obtained were presented in Fig. 2. It was observed that *Arthrobotrys oligospora* (AO) produced the maximum amount of IAA (0.62 mg L^{-1}). The next best treatment was *Paecilomyces lilacinus* Isolate 1 (P_1) with 0.48 mg L^{-1} of IAA which was closely followed by *Paecilomyces lilacinus* Isolate 2 (P_2) with 0.44 mg L^{-1}. *Pleurotus* sp. produced 0.38 mg L^{-1} of IAA which was lower than rest of the biocontrol agents. All the four fungal isolates produced a good amount of IAA when compared to the uninoculated control with 0.26 mg L^{-1} of IAA.

The endophytic nature of *A. oligospora* and *Paecilomyces lilacinus* and production of fairly good amount of IAA, a growth hormone helps in increasing the plant growth parameters. This finding confirmed the previous study conducted by Kuswinanti *et al.* (2015) to evaluate the ability of endophytic fungi to

produce IAA hormone as a plant growth promotor, and it showed that endophytic fungal isolates were able to produce IAA in the range between 0.635 and 2.651 mg $^{L-1}$.

Pleurotus sp. was also recorded to produce a good amount of IAA and when it is applied to plants it induces the plant growth parameters. These results are in accordance with the study conducted by Bose *et al.* (2013) to test the ability of three white rot fungi, *Trametes versicolor, Pleurotus ostreatus*, and *Phanerochaete chryosporium*, to produce IAA when incubated with L^{-1} tryptophan. They reported that the maximum IAA production (473.55 ± 3.32 μgml^{-1}) was recorded upon 18 days of incubation at 37^{o}C using a medium containing 2% (w/v) Jatropha seedcake as substrate.

Phosphate solubilizing ability

The phosphate solubilizing potential of all nematophagous fungi (NPF) was observed at three different time intervals viz., 5, 10 and 15 days after inoculation (Fig. 3). It was observed that the solubilized phosphate content was increased significantly from day 5 to day 15 after inoculation and the mean phosphate solubilized was also recorded (Table 3).

Among the various nematophagous fungi, the highest phosphate solubilized at 5 days after inoculation (DAI) was recorded in the treatment T_2 containing *Arthrobotrys oligospora* with 45.13 mg L^{-1} which was increased to 56.39 mg L^{-1} on 10 DAI and 83.20 mg L^{-1} on 15 DAI. The next best treatment on 5 DAI was T_4 containing *P. lilacinus* (Isolate 2) with 38.64 mg L^{-1} of solubilized phosphorus which on 10 DAI and 15 DAI was found to be increased to 47.53 mg L^{-1} and 59.47 mg L^{-1}, respectively. This was followed by T_3 (37.39) and T_5 (31.40) on 5 DAI which was found to be increased to 43.32 and 35.83 mg L^{-1} on 10 DAI and 57.21 and 44.00 mg L^{-1} on 15 DAI, respectively. The least amount of solubilized phosphate was observed to be in T_1 containing uninoculated control with 24.79 mg L^{-1} of solubilized phosphate on 5 DAI and it was increased to only 27.50 and 35.48 mg L^{-1} on 10 DAI and 15 DAI, respectively.

The data (Fig. 3) revealed that the nematophagous fungi solubilized the phosphate @ 38.14 mg L^{-1} at 5 DAI which was increased by 17% (45.77) and 37% (60.97) at 10 and 15 DAI, respectively. Whereas, the phosphate solubilized in the uninoculated control at 5 DAI was 24.79 mg L^{-1} which was increased to only 27.50 and 35.48 mg L^{-1} at 10 and 15 DAI, respectively.

The maximum amount of mean phosphate solubilized was recorded in T_2 containing *A. oligospora* with 61.57 mg L^{-1} of solubilized phosphate. This was followed by T_4 (48.55) containing *P. lilacinus* (Isolate 2), T_3 (45.97) containing *P. lilacinus* (Isolate 1) and T_5 (37.08) containing *Pleurotus* sp. The mean phosphate content of uninoculated control was recorded to be the lowest with 29.26 mg L^{-1} of solubilized phosphate.

The ability of nematophagous fungi in solubilizing the available phosphate helps the plant to uptake the phosphorus present in the soil more efficiently. The present study validates the results reported by Duponnois *et al.* (2006), Kuswinanti *et al.*(2015) and Lima-Rivera *et al.* (2016).

Thus the nematophagous fungi have the potential to produce IAA and also solubilize phosphate which helps in promoting the plant growth, thereby improving the plant defense mechanism against root knot nematode.

CONCLUSION

Our study showed that, all the three nematophagous fungi viz., *Arthrobotrys oligospora*, *Paecilomyces lilacinus* (Isolate 1, 2) and *Pleurotus* sp. used were found to be effective not only as biocontol agents but also to promote the growth by producing IAA and solubilizing the phosphate. The ability of *Pleurotus* sp. to efficiently degrade the egg and juveniles of root knot nematode was due to the presence of certain toxic compounds present in it. *Arthrobotrys oligospora* produced certain trapping structures which arrested the nematodes. Both the isolates of *P. lilacinus* were found to be excellent egg parasitizing fungi because they are known to degrade the chitin and protein present in the eggs of root knot nematode by using enzymes chitinase and protease thereby producing

deformed and vacuolated eggs. However, Isolate 2 of *P. lilacinus* was found to be 20% more efficient in parasitizing eggs than Isolate 1. The plant growth promoting ability of nematophagous fungi was determined by estimating the IAA produced and phosphate solubilized under *in vitro* condition. The maximum amount of IAA production and phosphate solubilization was observed in treatment with *Arthrobotrys oligospora* followed by *Paecilomyces lilacinus* (Isolate 1, 2) and *Pleurotus* sp.

Further, investigations are needed for isolation and characterization of nematicidal compounds produced by these nematophagous fungi. *Nematophagous fungi* would be an environmentally friendly approach to cope with the highly variable environmental conditions common in farmers' fields and would optimize the potential benefits of the various other naturally occurring beneficial microorganisms in soil, thereby improving the health status of soil. In near future, these nematophagous fungi can be produced in a large scale and can be used as biocontrol agents against root knot nematodes in field trials and also can be recommended to farmers, so as to overcome the drawbacks of chemical nematicides.

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