Management of Root Knot Disease in Rice Caused by *Meloidogyne graminicola* through Nematophagous Fungi

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Abstract

A glass house experiment was conducted for the effectiveness of nematophagous fungi against *Meloidogyne graminicola* in which *Arthrobotrys oligospora* and *Dactylaria eudermata* were used for the management of root knot disease of rice. Root knot nematodes, *Meloidogyne graminicola* had proved itself as an important limiting factor for successful cultivation and productivity of rice. By amending the soil with application of mass culture of two nematophagous fungi (*Arthrobotrys oligospora* and *Dactylaria eudermata*) reduced the number of root galls by 86.9% and 81.1%, of females by 94.2% and 91.7%. The mass culture of these fungi increased the plant growth: shoot length by 41.9% and 38.8%, root length by 44.6% and 41.8%, fresh weight of shoot by 61.1% and 58.7%, and fresh weight of root by 24.3% and 22.5%, respectively over nematode infested soil. The better performance of *A. oligospora* may be attributed to better colonization and establishment of *A. oligospora* than *D. eudermata* and may be better tolerance of the fungus to soil fungistasis.

Keywords: Rice, *Meloidogyne graminicola*, Root knot, *Arthrobotrys oligospora* and *Dactylaria eudermata*

1. Introduction

Rice is one of the most important world cereal crops in Asia where more than 90 per cent of the world's rice grown and consumed. It is also a staple food of nearly half of the world population. Rice contributes 43 per cent of total food grain production and 46 per cent of total cereal production. It continues to play a vital role in the national food grain supply. It ranks third after wheat and maize in terms of worldwide production. In India, rice occupies first position among the cereals in respect of both area and production. In India rice is grown in almost all the states. West Bengal is the highest rice producing state while Tamil Nadu has first place in productivity. It has been estimated that the world wide annual yield loss due to plant parasitic nematodes in which root knot nematode, *Meloidogyne graminicola* is widely spread in rice growing of Allahabad. This disease is characterized by abnormal swelling of roots (known as root knots or galls), yellowing, stunting and wilting of plants depending on the initial population density of the nematode in soil. The initial population density of *M. graminicola* caused wilting of seedlings along with severe reduction in growth parameters while low population caused only reduction in growth parameters. In nature when the population of a pathogen increases, its natural enemies are also activated which parasitize and kill them and consequently cause a decline in their population. The nematophagous fungi are the integral component of soil biota. They are distributed in all types of soils. However, they are more predominant in decaying plant debris. It is also known that population of the nematophagous fungi increases when organic matter is added into the soil (Linford, 1937). The nematophagous fungi may occur as saprophytic in the soil or decaying plant materials and may prey on saprophytic as well as plant parasitic nematodes. When the population of nematodes increases in soil the natural enemies like nematophagous fungi are also activated which parasitize and kill them and consequently cause a decline in nematode population. This is possibly the act of nature and buffering capacity of soil that does not allow the population of a single dominant

species rather it maintains a good balance of soil biodiversity. Jaffee et al. (1992) while working on density dependent host pathogen dynamics in soil reported that the disease dynamics in soil microcosms exhibited both temporal density-dependent parasitism and a host threshold density parasitism. The capturing efficiency of the predacious fungi may be influenced by the environmental condition and nature of the soil. The most effective nematophagous fungi *Arthrobotrys oligospora* and *Dactylaria eudermata* were tested for the biological control of *Meloidogyne graminicola.*

2. Materials and Methods

Arthrobotrys oligospora and Dactylaria eudermata, predaceous fungi were isolated on maize meal and rabbit dung agar by using the technique described by Duddington (1955) with some modification. About 500 mg of soil from each sample was scattered in the bottom of a sterile plate Sterilized melted and cooled maize meal agar were poured in each plate to cover 2/3 area of plate. The other 1/3 area of a plate was poured rabbit dung agar. The plates were incubated at room temperature (25-30^{0}C) for observation. The saprophytic nematodes already present in the soil sample multiplied rapidly and served as prey for predaceous fungi. There is no need to incorporate nematode from outside in the plate. The incubated plates were routinely observed after seven days of incubation for the *Arthrobotrys oligospora* and *Dactylaria eudermata* under stereoscopic microscopic as well as compound microscope.

Pure cultures of both the fungi were made by single spore isolation technique described by Tuite (1969). Conidia were picked with the help of sterilized fine needle and dragged lightly across in Petri dishes containing water agar medium. Well separated spores were located under stereoscopic microscope (100X). A disc of agar containing a single spore was cut and transferred into a Petri dish containing maize meal agar medium. Several single spores of fungi were transferred in separate Petri dishes. The spores inoculated Petri dishes were incubated at 25± 1^{0}C for growth and sporulation. After 4-5 days of incubation spores of fungi were transferred aseptically in Petri dishes containing maize meal agar medium. The cultures were maintained on maize meal agar medium at 25±1^{0}C by sub-culturing at regular periodical interval.

2.1 In vitro predacity of nematophagous fungi against M. graminicola

Predacity of selected nematophagous fungi *Arthrobotrys oligospora* and *Dactylaria eudermata* against *M. graminicola* was tested by the method described by den Belder and Jansen (1994). Cultures of the nematophagous fungi were grown in 1:10 CMA medium. The reason behind using 1:10 maize meal agar medium was to facilitate the movement of nematodes into the plates. Freshly collected second stage juveniles from rice root galls were washed 5 times with sterilized distilled water and two drops of water containing 100 J_2 of *M. graminicola* were inoculated into each Petri dish (50 mm). For both the fungus five Petri dishes were taken as replicates. All the Petri dishes were kept at 25^{0}C and observations on trapping structures and trapped nematodes were taken up to 5 days. Observations of formation of predaceous structures and capturing of nematode were recorded. Similar observation on capturing and killing of several species of nematodes has been reported by some workers (Drechsler 1950).

2.2 Preparation of mass culture

In view of the good predacity of *A. oligospora* and *D. eudermata* against *M. graminicola*, these fungi were grown on barley (Hordeum vulgare L.) seeds for mass culture as described by Kumar et al. (2005) for the control of root knot disease of rice in pots. For mass culture 50 g barley seeds were taken into each of several 250 ml conical flasks. The seeds in each conical flask was moistened with 70 ml tap water, plugged with cotton and sterilized at 15 lbs/inch^2 for 20 minutes. 5 mm fungal disc taken from periphery of seven day old cultures of these fungi on CMA medium was transferred into each flask. The inoculated flasks were incubated at room temperature (25-30^{0}C) for 20 days.

2.3 Efficacy of mass culture of A. oligospora and D. eudermata against Meloidogyne graminicola causing root knot disease of rice

For evaluation of efficacy of mass culture of *A. oligospora* and *D. eudermata*, sick soils containing 2000 juveniles of *M. graminicola* was used. The experiment was conducted in a glass house. The sick soil was thoroughly mixed and mass culture of *A. oligospora* and *D. eudermata* were separately amended in the soil at the rate of 4x10^6 colony forming unit (CFU)/kg of soil. Sick soil without fungal inoculum served as control. Soils amended with fungus and without fungus were filled in pots at the rate of 1 kg/pot. Sterilized soil was also used with and without fungus to see the effect of these fungi on seedlings. 25 Sprouted rice seeds were sown in each pot on the same day. For each treatment five replications were used. Observations on number of root galls shoot and root length, fresh weight of shoot and root were recorded 30 days after sowing. Further the populations of

females were also recorded. For determination of final population of females, roots were stained in boiling 0.1% (w/v) acid fuchsin in lactic acid, glycerol and distilled water (1:1:1) by the method described by Bridge et al. (1981). Stained roots were macerated in distilled water and number of females were counted. Data were analyzed using randomized block design (RBD).

3. Results

3.1 In vitro predacity of nematophagous fungi against M. graminicola

Observation on the predacity of two nematophagous fungi, viz. *A. oligospora* and *D. eudermata* against 2nd stage juveniles of *M. graminicola* is presented in Table I. The minimum predacity of nematode was observed within 24 hours of the conduction of the test first day but the percentage of captured nematodes increased daily with increased period of incubation. Maximum percentage of predacity was recorded at 5th day. The induction of trapping structures was higher in *A. oligospora* in response to *M. graminicola* followed by *D. eudermata* (Figure a and b). *A. oligospora* captured significantly higher percentage of nematodes. *A. oligospora* captured and killed J_2 of *M. graminicola* by 94.6% in five days whereas *D. eudermata* captured 71.4% nematodes.

3.2 Efficacy of mass culture of A. oligospora and D. eudermata against Meloidogyne graminicola causing root knot disease of rice

From the observation it is evident that mass culture application of *A. oligospora* and *D. eudermata* in soil infested with *M. graminicola* reduced the number of root galls by 86.9% and 81.1% and females by 94.2% and 91.7%. (Table II). The mass culture of these fungi increased the plant growth: shoot length by 41.9% and 38.8%, root length by 44.6% and 41.8%, fresh weight of shoot by 61.1% and 58.7%, and fresh weight of root by 24.3% and 22.5%, respectively over nematode infested soil. It was also noted that the galls developed in roots of plants treated with *A. oligospora* and *D. eudermata* were young with fewer number females, further less number of eggs and less number of juveniles indicating delayed infection. This also indicated that the multiplication of the nematode was also arrested in the roots of seedlings treated with these fungi (Figure c, d and e). Similar results were observed by Singh et al. (2007). From the observations it is clear that these fungi trapped and killed the infective J_2 in the infested soil. Mass culture application of *A. oligospora* and *D. eudermata* in sterilized soil did not affect the growth of rice seedlings.

4. Discussion

From the observations it appears that with increase in the population of *M. graminicola*, nematophagous fungi also increased. This clearly indicates that higher density of *M. graminicola* supported the population of nematophagous fungi. Jaffee et al. (1992) also reported density dependent pathogen dynamics in soil. Kerry (1987) also reported that soil became suppressive when larger densities of pest nematode supported an increase in the population of parasites of those nematodes. From the in vitro test studies on predacious activities of nematophagous fungi it is evident that *A. oligospora* is more efficient in capturing and killing J_2 of *M. graminicola*. Kumar and Singh (2006 a, b) also reported that predacious fungi are most efficient in capturing and killing J_2 of *M. graminicola* and *M. incognita*. Higher predation of *M. graminicola* by *A. oligospora* and *D. eudermata* appears to be related to induction of higher number of constricting rings in response to this nematode. Cooke (1963) also reported that constricting ring forming fungi are more predacious than the other nematophagous fungi forming hyphal nets.

It was also noted that the galls developed in roots of rice plants treated with *A. oligospora* and *D. eudermata* were young with fewer number of females resulting less eggs and J_2 indicating delayed infection. This also indicated that the multiplication of the nematode was also arrested in the plant roots treated with these fungi. From the observations it is clear that these fungi trapped and killed the infective J_2 in the infested soil. Mass culture application of *A. oligospora* and *D. eudermata* in sterilized soil did not affect the plant growth.

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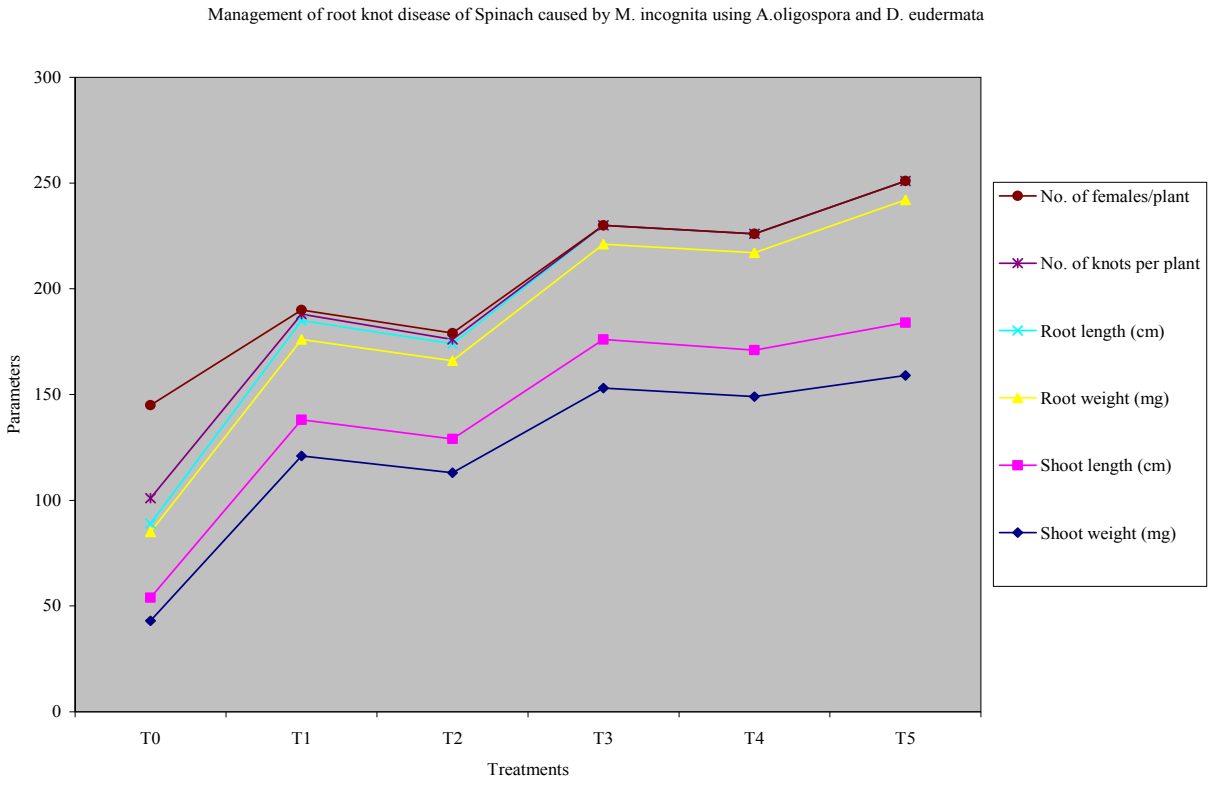
Table 1. In vitro predacity test of selected nematophagous fungi against 2nd stage juveniles of *M. graminicola* in MMA (1:10) medium

Fungi	Predacity on different days (%)					Mean
	1st	2nd	3rd	4th	5th	
Arthrobotrys oligospora	2.2	17.6	36.8	67.4	94.6	43.7
Dactylaria eudermata	1.6	13.4	2 6.6	58.0	71.4	34.2
Mean	1.9	15.5	31.7	62.7	83	
CD at	5%	1%				
For fungi	0.694	0.852				
For Day	0.963	0.137				
For Fungi	1.113	2.256				

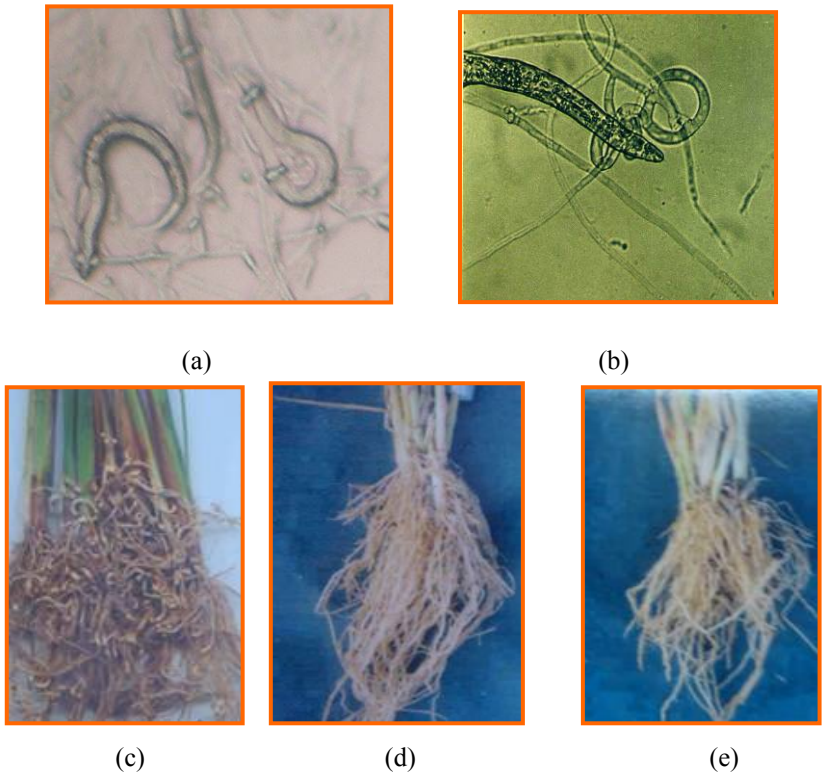
Table 2. Management of root knot disease of rice caused by *Meloidogyne graminicola* using *Arthrobotrys oligospora* and *Dactylaria eudermata*

Treatments	Shoot weight (mg)	Shoot length (cm)	Root weight (mg)	Root length (cm)	No of galls/ seedling	No. of females/ seedling
M. graminicola T0	43.6 a	11.4 a	31.4 a	4.5 a	12.8 a	44.0 a
M. graminicola + *A. oligospora* T1	121.1 b	17.9 b	38.2 b	9.2 b	1.7 b	2.1 b
M. graminicola + *D. eudermata* T2	113.9 b	16.8 b	37.1 b	8.8 b	2.1 b	2.9 b
Sterilized soil + *A. oligospora* T3	153.6 c	23.7 c	45.8 c	9.7 b	0	0
Sterilized soil + *D. eudermata* T4	149.9 c	22.1 c	46.2 c	9.2 b	0	0
Sterilized soil (without fungi) T5	159.2 c	25.3 c	58.2 d	9.8 b	0	0

* Data with different letters show significant difference of column data by randomized block design (RBD) test at P = 0.05



ISSN 1916-9752 E-ISSN 1916-9760



Figure

(a) Nematodes trapped and killed by *Arthrobotrys oligospora*

(b) Nematodes trapped and killed by *Dactylaria eudermata*

(c) Rice root treated with *M. graminicola* (T0)

(d) Rice roots treated with *M. graminicola* + *A. oligospora* (T1)

(e) Rice roots treated with *M. graminicola* + *D. eudermata* (T2)