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Predation and performance of two predacious fungi for control of root-knot nematode of brinjal

K.P.SINGH, DHARMENDRA KUMAR, P.BANDHYOPADHYAY AND V.K.SINGH
Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi 221005, Uttar Pradesh

The performance of *Arthrobotrys oligospora* and *Dactylaria brochopaga* was tested against *Meloidogyne incognita* infecting brinjal root by three different methods, viz., mixing of mass culture of fungi in to the soil at the rate of 1 percent, spot inoculation in the centre of pot and by diping the root of brinjal seedling in spore suspension. Both biocontrol agents significantly controlled the root knot formation in sterilised and unsterilised soils in pot condition. However, maximum control was recorded in sterilised soil. In unsterilised soil ; although root knot number was reduced significantly, the performance of both predacious fungi was inferior to sterilised soil. Further, the performance of these fungi was better in soils amended with compost. Of the three methods, mixing of mass culture with soil was found to be superior over other two methods of application for *A. oligospora* as well as *D. brochopaga*. The other method like spot inoculation and seedling root dip in spore suspension before planting giving significant control of root knot may be performed for field trials. *A.oligospora* colonised the soil specially soil amended with compost and formed a turf of fungus colonies. This indicates that the fungus can compete and colonise the soil. *D.brochopaga* was found to be an inferior coloniser as compared to *A.oligospora*. It may be concluded that both predacious fungi should be tested at large scale in microplot before taking them to field.

Key words : *Arthrobotrys oligospora, Dactylaria brochopaga, Meloidogyne incognita*, brinjal, predacious fungi, nematode, *Tylenchorhynchus brassicae*

INTRODUCTION

Arthrobotrys oligospora is the most commonly encountered predacious fungus in leaf litters, compost and farmyard manures (Bandhyopadhyay, 1998). Its predacity against saprophytic nematodes and plant parasitic mematodes has been studied by a large number of workers (Drechsler, 1941 ; Duddington, 1962 ; Pramer 1966 ; Mankau, 1980 ; Campos and Vicenate, 1996 ; Jacobs, 1997 ; Stirling, 1998). As well as others have summarised investigations on the many soil microorganisms antagonistic to nematodes. However, very few workers have studied the performance of *A. oligospora* for the control of plant parasitic nematodes. Ali (1990) reported 72% reduction of the root galling in tomato caused by *Meloidogyne incognita* in green house with *A.oligospora* and organic amendment, when the fungus was inoculated 2 weeks prior to transplanting. *A.oligospora* was 46.5-81.9% more effective than the use of nematicides for the control of root-knot nematode (Slepetiene *et al.*, 1993). In views of this an isolate of *A.oligospora* isolated from compost was tested for its predacity against *Meloidogyne incognita* and *Tylenchorhynchus brassicae* and performance against root - knot of brinjal.

MATERIALS AND METHODS

Assessment of predacity of *Arthrobotrys oligospora* against two plant parasitic nematodes. This study was conducted to determine whether the test fungus *A.oligospora* captured plant parasitic nematodes, viz. second stage juveniles of *M. incognita* and adults of *T. brassicae*. These nematodes were extracted and collected in the cavity blocks separately in large numbers by the method given by

Southey, 1970. The nematodes were washed five times with sterilised distilled water before using them for interaction studies (Jenson, 1954). Fungal disc of *A.oligospora* were cut from a ten-day old culture on maize meal agar medium using a sterilised cork borer and inoculated in the petridishes (50 mm) containing 1 : 10 maize meal agar medium. The Petridishes were incubated for 72 hours at 28°C. After 3 days when the petridishes were almost filled by fungal growth, a drop of suspension containing 50 nematodes of each species was inoculated separately into each of several petridishes. Observations on the capturing of mematodes were taken. For studying the effect of different population of nematodes on capturing 50, 100 and 200 nematodes of both the species were inoculated. Trapped namatodes were counted in percentage basis and analysed following completely randomised design (CRD).

Preparation of Mass Culture

In view of the good predacity of *A.oligospora* and *D.brochopaga* against root-knot mematodes both fungi were grown on sorghum seeds. The grains were coarsely spilitted in a warring blender and filled in to each of several 150 ml conical flasks each to contain 20 g splitted seed and 15 ml water. The flasks were plugged with cotton and sterilised in autoclave at 15 lb pressure for 20 minutes. the fungal disks (6mm dia.) taken from periphery of 15 day old culture of the fungus in maize meal agar medium was transfereed into each flask. The inoculated flasks were incubated at room temperature (25-30°C) for 20 days after inoculation. The mass culture was further used for performance test.

Performance test

Performance of *A.oligospora* and *D.brochopaga* as biocontrol agents was studied against root knot of brinjal in pots in glass house. The performances of fungi were tested both in sterilised and unsterilised soils. The soil consisted of garden soil and farm yeard manure in the ratio of 3:1. Mass culture of *A.oligospora* and *D.brochopaga* were uniformaly mixed in the soil at the rate of 1 percent. In another treatment mass culture was spot inoculated in the soil in the centre of pot before one week of planting of brinjal seedling. In other method, the rots of seedlings of brinjal were dipped in the spore suspension of *A. oligospora* (10^5/ml) and *D. brochopaga* (20 g mass culture seed + 250 ml water) at the time of sowing. Soil without mass culture of fungi and seedlings root without dip in spore suspension of fungi served as control. Sterilised and unsterilised soils with or without compost with mass culture was filled in 10cm pots. For each treatment six pots were used as replicates. Pots were planted with one month old brinjal seedling. Each pot was planted with only one seedling free from root knot infection. The treatments were as follows : (1) Direct inoculation of fungus mass culture in soil ; (2) Spot inoculation of mass culture in soil ; and (3) Seedling root dip in spore suspension of Fungus.

After 7 days of planting, seedling were inoculated with freshly 500 hatched second stage juveniles of *M.incognita* collected from fresh egg masses. For inoculation of nematodes, the soil around root was loosened and namatode suspension was poured around the roots of the seedlings. The soil surface was then covered with a thin layer of same type of soil. The pots were watered as and when to keep the optimum moisture level. The experiment was terminated after 120 days of inoculation and roots were gently washed under running tap water. The date on total number of root knots were recorded.

RESULTS

Interaction between *A.oligospora* and *T.brassicae* (Tables 1 and 2) in relation to different number of nematode showed a similar trend as noted for *M.incognita*. However, the capturing of *T.brassicae* by *A.oligospora* was higher than *M.incognita*. The higher number of capturing was possibly due to more contact surface of nematode because of larger size of nemtode.

Table 1 : Effect of different population of *Meloidogyne incognita* on its capturing by *Arthrobotrys oligospora*.

Day	Nematode numbers			Mean
	50	100	200	
1	1.0	2.6	4.3	2.6
2	3.0	10.0	22.6	11.76
3	6.6	17.3	41.3	21.73
4	12.6	25.3	59.6	32.50
5	17.3	34.33	88.3	46.64
6	24.33	48.33	95.66	56.10
Mean	10.80	23.62	51.86	

CD (P = 0.05). Nematode Population : 7.22, Day 7.78

Table 2 : Effect of different population of *Tylenchorhynchus brassicae* on its capturing by *Arthrobotrys oligospora*

Day	Nematode numbers			Mean
	50	100	200	
1	1.0	2.6	4.3	2.6
2	3.0	10.0	22.6	11.76
3	6.6	17.3	41.3	21.73
4	12.6	25.3	59.6	32.50
5	17.3	34.33	88.3	46.64
6	24.33	48.33	95.66	56.10
Mean	10.80	23.62	51.86	

CD (P = 0.05), Nematode Population = 7.22, Day = 7.78

Interaction between A.oligospora with M. incognita and T. brassicae in dual culture

Observation of dual culture of *A. oligospora* and *M. incognita* and *T. brassicae* in 1:10 maize meal agar medium clearly indicate that presence of nematode in fungal culture stimulated the development of trapping organs. The trapping device of *A. oligospora* was a single ring that developed due to anastomosis of hyphae. From the same ring another branch of branches developed that anastomosed with the parent hyphae. Further formation of rings on the hyphae lead to the formation of a net work of hyphe, that were either two dimensional or three dimensional rings. Nematodes while moving freely into maize meal agar medium if happened to pass through such loops or hypha net were easily trapped by such loops. This was mostly because of the entanglement of mematode in the hyphal nets and also because of stickly substances produced on the surface of trapping devices. Occasionally, nematodes escaped but if captured strongly, any kind of effort of the nematode appreared to be futile to escape. At the point of contact, a fine hyphae penetrated the cuticle and slowly nematode body was invaded by the fungal mycelium. Usually, The hyphal penetration was noticed after the nematode was completly exhausted or even killed. In some cases nematode was also trapped on the outer surface of a ring. This clearly indicates that the extracellular sticky material was present on inner as well as outer surface of a ring. After the nematode were killed the hyphae developed into the body of nematode and produced erect conidiophores on which conidia were formed in a single or several whorls.

Effect of mass culture of A.oligospora on total number of root knot in brinjal

The data on effect of *A.oligospora* on root knot development in different types of soils are presented in Table 3. In general, application of *A. oligospora* was recorded in sterilised soil with compost followed by unsterilised soil added with compost. The effect of *A.oligospora* on control of root knot was minimum in unsterilised soil without compost. This observation clearly indicated that presence of organic matter in the form of compost was conducive for the growth of biocontrol agent, *A. oligospora*, hence control of root knot was better in unsterilised soil amended with compost, irrespective of method of application of biocontrol agent. It may be observed that application of *A. oligospora* as direct mixing in soil, spot inoculation and seedlings roots dip in spore suspension significantly reduced the mumber of root knot than their corresponding control. However, maximum reduction in root substances produced on the surface of trapping devices.

Table 3 : Effect of mass culture of *Arothrobotrys oligospora* on total number of root knot in Brinjal

Treatments	Soil type (Number of root reduction / plant			Mean
	Sterlised soil + compost	Unstrerilised soil without compost	Unsterlised soil + compost	
Direct inoculation of	47.33	77.66	52.3	52.6
A. oligospora in the soil	(71.0)	(40.7)	(65.6)	(64.6)
Spot inoculation at	48.6	75.6	57.6	59.06
A. oligospora in soil	(70.2)	(42.24)	(62.6)	(60.2)
Seedings root dip in the	48.6	82.87	68.3	61.16
Spore suspension of	(70.2)	(36.7)	(54.9)	41.75)
A. oligopora				
Control	163.3	131.0	151.6	148.63
	52.17	78.73	82.48	
	(64.04)	(39.90)	(60.81)	

CD (P = 0.05)
Soil = 14.12
Treatment = 14.43

Occasionally, nematodes escaped but if captured strongly, any kind of effort of the nematode appeared to be futile to escape. At the point of contact, a fine hyphae penetrated the cuticle and slowly nematode body was invaded by the fungal mycelium. Usually, the hyphal penetration was noticed after the nematode was completely exhausted or ever killed. In some cases nematode was also trapped on the outer surface of a ring. This clearly indicated that the extracellular sticky material was present on inner as well as outer surface of a ring. After the nematode were killed the hyphae developed into the body of nematode and produced erect conidiophores on which conidia were formed in a single or several whorls.

Effect of mass culture of A.oligospora on total number of root knot in brinjal

The data on effect of *A.oligospora* on root knot development in different types of soils are presented in Table 3. In general, application of *A.oligospora* significantly reduced the root knot development in different types of soils. Maximum reduction in root knot number with *A.oligospora* was recorded in sterilised soil with compost followed by unsterilised soil added with compost. The effect of *A.oligospora* on control of root knot was minimum in unsterilised soil without compost. This observation clearly indicated the presence of organic matter in the form of compost was conducive for the growth of biocontrol agent, *A.oligospora*, hence control of root knot was better in unsterilised soil amended with compost, irrspective of method of application of biocontrol agent. It may be observed that application of *A.oligospora* as direct mixing in soil, spot inoculation and seedling roots dip in spore suspension significantly reduced the number of root knot that thier corresponding control. However, maximum reduction in root knot was obtained when mass culture was directly mixed in the soil followed by seedling dip method. The observations indicate that this biocontrol agent may be successfully utilised for the biocontrol of root knot nematode.

Effect of mass culture of Dactylaria brochopaga on total number of root knot nematode in brinjal

The data on performance of *D.brochopaga* on control of root knot brinjal are presented in Table 4. It is evident from the results that the number of root knot was significantly reduced by application of mass culture of *D.brochopaga* both in sterilised soil and unsterilised soil. However, The performance of the fungus was better in sterilised soil than unsterilised soil. The application of mass culture as soil application or spot application was significantly superior to seedling root dip method. Soil or spot inoculation of the fungus had more or less similar effect of root knot development. The application of *D.brochopaga* as biocontrol agent clearly indicate that this fungus can effectively control root knot disease. However, performance of the fungus shoud be tested in farmers field to know more about the efficiency of this biocontrol agent.

DISCUSSION

Inoculation of *M. incognita* and *T. brassicae* in cultures of *A. oligospora* resulted in formation of hyphal bails, this may be attributed to remin production by these mematodes in petridishes. The number of hyphal bails increased with passage of time after inoculation. During this period the concentration of remin must have been increased resulting in higher number of bail formation. Similar morphogenesis in predacious fungi in presence of nematode in dual cultures have been reported by several workers (Couch, 1937 and Pramer and Stoll, 1958). However, similar observation on capturing and killing of several species of nematodes have been reported by some workers (Drechsler, 1950 ; Gives and Hintz, 1978 and Zavaleta, 1994).

In the present study second stage juveniles of *M. incognita* induced morphogenesis in *D. brochopaga* and *A. oligospora* suggesting that both produced sufficient amout of remin required for induction of trapping devices. However, Pramer and Stoll (1958) reported that only mature nematodes that too after death and disintegration only produced nemin. The present observation has been repeated several times and consistent results on induction of trapping devices were obtained. (Nardbring-Hertz, 1977).

Application of mass cultures of *D. brochopaga* and *A. oligospora* directly in soil, or spot inoculation or seeding dip in spore suspension of the biocontrol agents significantly reduced the number of root knot in pot cultures. Both the fungi being predacious, develop their trapping devices abundantly which captures. Both the fungi being predacious, develop their trapping devices abundantly which capture and kill the nematodes. *A. oligospora* and *D. brochopaga* both performed better in controlling the root knot nematode in sterilised soil than the unsterlised soil. In sterlised soil the performance was better because the biocontrol organism had no competition. Contary, in natural soil there is lot of competition with other microorganism. In the present study *A. oligospora* was found to colonise very well is soil having compost. It clearly indicate that *A. oligospora* had good competitive as well as saprophytic ability and thereby it trapped and killed most of the nematode inoculated in the pots. Similar obervations on

control of root knot disease by *A. oligospora* have been reported by several workers (Jowich and Bachow, 1989, Linford and Oliveria, 1938).

D. brochopaga was also effective when its mass culture was applied in the soil by mixing or as spot inoculation or seedling dip in spore suspension against root knot disease. Similar to *A. oligospora*, *D. brochopaga* performed better as biocontrol agent in sterilised soil. The good performance of *D. brochopaga* against root knot in unstrerilised soil indicate that this fungus can very well colonise and competes with the soil microorganism. Thus, this fungus can be also successfully tested as biocontrol agent in field condition. Like *A. oligospora* there is no work on the performance of *D. brochopaga* against plant parasitic nematodes in pots or field condition. It may be concluded from the study that both the biocontrol agents viz. *D. brochopaga* and *A. oligospora* have good potential and positive attributes of a biocontrol agent. Any of these methods of application of mass culture may be utililsed for trial at large scale in field.

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