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Research Article

Evaluation of Nematophgaous Ability of *Drechslerella dactyloides* in Vicinity of Soil and Biocontrol Potential against Root Knot Disease of Brinjal

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ABSTRACT

Conidia of five isolates of Drechslerella dactyloides frequently formed conidial traps in close vicinity of various field soils and cow dung manure which trapped and paralyzed the soil nematodes. However, predation of nematodes varied possibly because of the presence of varied number of nematode population in different field soils and cow dung manure. After trapping and killing of the nematodes by conidial traps, additional hyphae were produced from distal and proximal cells of spores on which constricting ring were formed which trapped other nematodes coming on the agar disc. The supplementation of mass culture of D. dactyloides with or without cow dung manure prior to planting of brinjal in conducive soil having 2000 active juveniles of Meloidogyne incognita per kg of soil reduced the number of root knot, females, egg sacs and number of second stage juveniles of M. incognita. However, application of D. dactyloides in combination with cow dung manure showed better control in comparison to D. dactyloides without cow dung manure.

Keywords: *Nematode trapping fungus, Drechslerella dactyloides, Meloidogyne incognita, Conidial trap, Fungistasis.*

INTRODUCTION

Meloidogyne spp. are known to cause root knot disease in many crop plants particularly in tropical and subtropical climatic conditions. These nematodes cause severe damage in vegetables, cereals, legumes, ornamentals, fruits, medicinal and aromatic plants, spices, condiments and plantation crops (Sharma & Pankaj, 2002). *Meloidogyne* spp. also incite knots in 46 plant species of weeds belonging to 20 botanical families (Batcelo et al., 1997) and increases their population in absence of host plants. Several nematicides are known to reduce the population of *Meloidogyne* spp up to 90% and save the plants, however, most of them are banned or out of use because of their hazardous effect on ecosystem.

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Methyl bromide, the most effective nematicide is currently phase out because it classified as class one stratospheric ozone- depleting substance (Nordmeyer, 1992; & Martin, 2003). Hence, search of alternative control methods of root knot disease is of vital importance. In current situation, protection of plants against root knot disease by efficient nematode trapping fungi has gained world wide attention because it is non chemical, ecofriendly and essential component of biodiversity in soil.

Drechslerella dactyloides (synonym *Arthrobotrys dactyloides)*, an important predacious fungus exhibiting variability in relation to morphology, redial growth and nematode capturing ability (Kumar & Singh, 2006) has drawn attention of various workers because of its habit to form conidial trap in close vicinity to soil (Mankau, 1962; Cook, 1964, & Dackman & Nordbring-Hertz, 1997) and excellent biocontrol potential against root knot disease of tomato (Stirling et al., 1998, Stirling et al., 1998 & Kumar & Singh, 2006). The present investigation was aimed to study the conidial trap formation in different isolates of *D. dactyloides* and its nematophagous behaviour in close vicinity of natural soil and cow dung manure to predict its nematode trapping ability under diverse situation of soil environment. Further, mass culture of an isolate of *D. dactyloides* was applied in infested soil with *M. incognita* to evaluate the ability of *D. dactyloides* to restrict the injury of root knot disease in brinjal. Observation of the same are being reported in this paper.

MATERIALS AND METHODS

Isolation of *D. dactyloides*

Isolates of *D. dactyloides* were isolated from the soils from Varanasi (Isolate A), Ghazipur (Isolate B), Chunar (Isolate C), Mirzapur (Isolate D) and Ranchi (Isolate E) India by the method described by Duddington (1955) with the slight modification (Bandyopadhyay & Singh, 2000). Pure culture of isolates was made by picking spores from the conidiophores of individual isolates by a fine needle and inoculating the same in Petri dishes containing corn meal agar medium. Further, single spore cultures of all the isolates were made by the method given by Singh et al. (2004).

Germination of conidia of *D. dactyloides* in close vicinity of soil and assessment of its nematode trapping ability

Germination and nematode trapping ability of conidia of different isolates of *D. dactyloides* in close vicinity of soils and cow dung manure was studied by the method described by Jackson (1958) with slight modification. The modification included the placement of water agar discs directly on the soil. The purpose of placement of water agar discs directly on the soil was to allow the migration of nematodes from the soil to water agar discs. One kg soil sample was collected from the top profile of soil (1-15cm) from vegetable field, agricultural crop field, forest field, and fruit crop field. Each soil sample and cow dung manure (CDM) was passed through a 2 mm sieve separately and thoroughly mixed to homogenize the nematode polulation. 500g. soil from each field and CDM taken and number of nematode population present in each soil sample was estimated by the method given by Southy (1972) for nematode extraction. After extraction, nematode population of each sample was counted and number of nematodes per 50 gram of soil was calculated. 50g soil of each sample and CDM was then placed in 90 mm Petri dishes. The soil was then wet near full water holding capacity by addition of distilled water and water agar discs (10 mm size, 3 mm thickness) were placed on soil and CDM. Five agar discs were placed in a Petri dish at equal distance for inoculation of spores of five isolates of *D. dactyloides* on each water agar disc separately. The Petri dishes were incubated at 25^0C for 24 h to allow the soil diffusates to reach on the agar disc. After preincubation, suspension of freshly harvested conidia of each isolate were then inoculated on each water agar discs and Petri dishes were incubated at 25^0C for observations. Spore inoculated water agar discs kept on clean slides in a moist chamber served as control. Agar discs placed on the soil were removed from the soil after 24 h. of spore

inoculation by inoculation needle and placed on the clean glass slides. Base of the agar disc was rinsed by fine jet of distilled water to remove the soil material from the base of agar disc and observations on total number of spores, germinated spores, conidial traps, trap formed on the spore germ tubes, total number of nematode present and captured on the water agar disc were taken. After observation, each agar discs were placed in their respective plates and observation on number of trapped and total nematodes on water agar discs was again counted after 48 hours of inoculation. Germination of conidia placed on water agar discs was also observed. For each treatment, three replications were taken.

Preparation of mass culture of *D. dactyloides*

For preparation of mass culture, 20 g. splitted barley grains were taken separately into each of several flasks (250 ml). 35 ml water was added into each flask to maintain moisture in grains. The flasks were then sterilized at 15 psi for 20 minutes. 10 mm fungal disc taken from the periphery of pure culture of isolate A of *D. dactyloides* was inoculated into each flask and incubate at 25±1°C for 25 days for growth and sporulation as given by Kumar et al. 2005.

Assessment of biocontrol potential of *D. dactyloides* against root knot disease of brinjal

For assessment of the biocontrol potential of *A. dactyloides*, conducive soil (*M. incognita* infested soil) maintained in the micro plots by continuous planting of susceptible genotypes of tomato and brinjal was used. Before conducting the experiment, population of *M. incognita* (J_2) in conducive soil was estimated by the bearman tray method described by Barker (1982). Conducive soil was thoroughly mixed by hand to make the uniform population of nematodes before amendment. Mass culture of *D. dactyloides* was supplemented in the conducive soil at the rate of $4x10^6$ colony forming unit (cfu) per kg of soil with or without CDM and one kg soil was filled in pots. Conducive soil with or without CDM were also filled in pots and served as control. 25 day old seedlings of brinjal (Variety-P.K.123) raised in root knot free soil were transplanted in each pots. Each pot had a single seedling. For each treatment 8 plants were used as replicates. The pots were watered regularly at per need.

Observations on plant height, fresh shootweight, number of females, egg sacs and population of juveniles (J_2) per root system were taken after 5 and 10 weeks of planting of brinjal. For observation of number of females and egg sacs per root system, knots were collected in Petri dishes containing water under stereoscopic binocular microscope and each knot was teased by a fine needle to observe the number of egg sacs and females per root system. For observations on second stage juveniles per root system, nematodes present in root and soil were separately estimated. For assessing juveniles (J_2) in the root system, roots of each plant under different treatments were cut in to small pieces. The root pieces were then placed over the wire net covered with tissue paper on 90mm Petri dishes and water level was maintained to touch the surface of tissue paper. The Petri dishes were incubated at room temperature (25-29^{o}C) for 15 days and water level was maintained daily. After incubation, nematode population of each treatment were collected separately and their numbers were counted. Estimation of nematode population in soil was done by the method described by Southey (1972) for extraction of soil nematodes. After extraction of nematodes, the nematode population per pot was counted and added to the population of root to represent the number of second stage juveniles of *M. incognita* per plant.

RESULT

Germination of conidia in close vicinity to various field soil and CDM and predation of nematodes

Observations on conidial germination revealed that conidia of different isolates of *D. dactyloides* transferred on to water agar discs without soil resulted in germination of conidia by germ tubes (82-91%), only except some spores which either formed conidial traps or traps on spore germ tube spontaneously ().

However, conidia transferred on to water agar discs placed over the soil frequently formed conidial traps (Table-1). Irrespective of various field soils and CDM, conidial traps ranged between 76.06-94%. The germination of conidia by germ tube only or germ tube with traps was very low in vicinity to soils (1.24-12.71%). No hyphae were formed from the spore bearing conidial trap under the soil and cow dung environment. Further no lysis of conidial traps was recorded during this period. On water agar discs over the soils, nematodes were trapped while some other untapped. The number of nematodes present and trapped on the water agar discs varied greatly in different soils and CDM. Maximum nematode trapping was recorded in the soil from Agricultural crop soil followed by CDM. It was interesting to note that after trapping and killing of nematodes, hyphae were produced from distal and proximal cells of a spore on which 5-20 constricting rings were formed which trapped and killed other nematodes. In captured nematode infection hyphae of any of the hyphae form any of the constricting ring penetrate nematode body through the damaged cuticle. Latter on, the fungus grew inside the nematode and consumed the whole content of nematode body. It appears that in soil, *D. dactyloides* is essentially in the predacious phase and fungastatic environment in the soil did not affect its spread if soil has good number of nematodes. Formation of hyphae with constricting rings suggests that the fungus can grow and multiply in predacious phase till the nematode are available in soil as prey for the fungus.

3.3. Biocontrol potential of *D. dactyloides* against root knot disease of brinjal

The result of this study indicates that maximum plant height and fresh weight was obtained in the plants grown in conducive soil amended with CDM and *D. dactyloides* after 5 and 10 weeks of plantings. However, plant grown in conducive soil showed lower plant height and weight in comparison to other treatments and a very little increase was observed in the plant growth and weight at end of observation. The effect of *D. dactyloides* in combination with CDM on plant growth was more pronounced of 10 weeks.

Assessment of biocontrol potential of mass culture of *D. dactyloides* in conducive soil after 5 weeks revealed that addition of *D. dactyloides* alone reduced the number of root knots, females, egg sacs and number of juveniles par plant by 42.8%, 70.8%, 66.7% and 86.9% respectively. However, addition of *D. dactyloides* in conjunction with cow dung manure increased the biocontrol potetntial of the fungus as it reduce 61.3% in root knots, 90.7% females, 89.1% egg sacs and 91.9% juveniles compared to its corresponding control. After 10 weeks of planting, *D. dactyloides* alone was sound less effective in controlling nematode population as it reduced root knot by 2.5%., females by 38.7%, egg sacs by 42.8% and J_2 by 47.1% while *D. dactyloides* + CDM was more effective which reduced root knots, females, egg sacs and J_2 by 74.6%,76.1%,77.6 and 68.3% respectively (Table-2). Although *D.dactyloides* alone significantly increase the plant growth and reduced the nematode population, its efficiency as biocontrol agent was greatly enhance and prolong in combination with CDM.

DISCUSSION

Observation on the abundance of conidial trap formation by all the isolates of *D. dactyloides* in close vicinity of soil and CDM demonstrated that the diffusates reaching from soil and CDM to water agar discs resulted in conidial trap formation. Trap formation in predacious fungi is usually in response to nemin which is known to be produced by nematodes and other small animals. It appears that soil and C.D.M. contain enough nemin due to presence of nematodes and other organisms which induced conidial trap in close vicinity to soil. However, Mankaw (1962) reported that the conidial trap formation in *D. dactyloides* is due to soil fungistasis and antagonistic nature of soil. Further, Persmark and Nordbring Hertz (1997) reported that the formation of conidial trap is a response to competition with other microorganisms for

nutrients. In light of theses statements, further studies may possibly help in identifying the factors responsible for direct trap formation. Observations on trapping of nematodes on water agar disc place over CDM and different field soil showed a direct relation between the higher number of trapped nematodes with higher number of nematode in soil and CDM. Higher predation of nematodes in soil having higher population of nematodes clearly indicate that the conidia applied in problem soil having higher number of nematodes extend the predacious phase of *D. dactyloides* till nematodes are available as a food source for this fungus. It proves that the conidia of *D. dactyloides* after formation of direct trap remain active predacious phase in soil and capture and kill nematodes and there are in no way adversely affected by soil fungistasis as reported by Mankau (1962).

Observations on the effect of mass culture of *D. dactyloides* alone and in combination with CDM revealed that the fungus is more effective when its mass culture was applied with CDM (Table-2). From the data on 5 weeks after planting it is evident that second stage juveniles present in the conducive soil incite higher number of root knot galls in plants creating a high degree of nutritional stress to the plants resulting in poor growth. In these plants gall size was larger with higher number of egg sacs which ultimately gave higher count of total nematode populations. Contrary to root system of plants grown in conducive soil, root development of plant grown in conducive soil amended with CDM was far superior, which possibly facilitated more sites for nematode infection resulting in higher number of root knots.

Higher reduction in number of root knot, females, egg sacs number of juveniles per root system in plants grown in conducive soil treated with *D. dactyloides* + CDM may be attributed to enhancing multiplications of the fungus and inhanced biocontrol efficacy of *A.dactyloides*. The addition of CDM in soil increase the population of free living nematodes (Stirling & Smith, 1998) and cadavers of trapped nematode provide an energy source by which further growth and trap formation occurs (Jaffee et al., 1992) The trapping structures produced after capturing and killing of free living nematodes may provide more site for trapping of nematodes which possibly increase the predation of nematodes in higher number. Hence, it is possible that less number of nematodes escaping trapping produce lower number of root knot and subsequently loser population of nematodes.

The decrease in reduction of nematode parameters after 10 weeks of planting in plants grown in conducive soil treated with *D. dactyloides* alone or in combination with CDM seem to be related with the effect of mass culture of *D. dactyloides* alone was short lived while those of *D. dactyloides* + CDM was prolonged and sustained. Hussey (1985) reported that juveniles released from egg sacs deposited on the galled tissue infect nearby roots to continue their life cycle. It is possible that during infection in nearby roots, most of the juveniles escaped from trapping resulted in increase number of root knots, egg masses, females, and total number of nematodes after 10 weeks of planting.

Meloidogyne spp. functions as metabolic sink in diseased plants (Bird & Loveys, 1975; & Mc Clure, 1977). Adult females produced in the roots require considerable amount of nutrients for egg production and compete with the host for the pool of nutrients in the roots. The increase activity of giant cells stimulates mobilization of phytosyntheates from shoots to roots and in particular to the giant cell where they are removed and utilized by the feeding nematodes. Meon et al. (1978) also reported that the mobilization and accumulation of substances reaches a maximum when the adult females commence egg laying. Observation of the present study demonstrate that *D. dactyloides* reduce the number of females and egg masses to great extent so the application of *D. dactyloides* certainly reduce the injury of root knot disease by terminating the pathological symptom due to reduction of females, egg masses and number of infecting juveniles per root system. More of less similar

results on reduction or root knot nematodes were reported by (Stirling et al., 1998; Stirling & Smith, 1998 & Kumar & Singh, 2006), however in tomato. Jenson et al. (2000) reported that *D. dactyloides* did not give effective control of root knot nematodes when pellets of *D. dactyloides* were used in pot experiment. It appears that the conidia of *D. dactyloides* were not overly distributed in soil due to pellets which gave unsatisfactory control of the nematodes. It is therefore recommended that inoculum of *D. dactyloides* used for biocontrol should be properly mixed for effective control of the nematodes.

Table 1: Germination of conidia of different isolates of Drechslerella dactyloides in close vicinity of different field soil and cow dung manure (CDM) and capturing of nematodes

Field soil	Isolates	Germ tube (%)	Trap on germ tube (%)	Conidial Trap (%)	Predation of nematodes				Nematode per 50 g. of soil and CDM
					24 hours		48 hours		
					CN	TN	CN	TN	
Vegetable field soil	A	4.5	3.96	89.28	3.66	5.66	7.66	9	60
	B	1.41	1.97	94.75	2.66	3.00	5	7	
	C	4.83	6.53	84.98	2.0	2.66	6	6	
	D	5.78	5.47	84.50	2.66	3.66	5	5	
	E	6.61	6.63	85.78	1.33	3.66	4	4	
Agricultural crop soil	A	2.32	4.48	90.11	19.00	21.33	25	26	175
	B	0.52	2.67	94.11	18.00	20.3	29	30	
	C	1.24	4.33	91.49	26.67	29.34	30	30	
	D	3.13	6.38	87.23	10.00	13.67	21	22	
	E	3.29	5.71	85.11	13.57	17.34	19	20	
Forest field soil	A	3.25	5.49	86.85	0.67	2.34	2	2	40
	B	2.27	5.84	89.75	0.67	2.0	3	3	
	C	3.73	4.17	91.27	0.33	1.33	4	5	
	D	5.10	7.38	82.28	1.00	1.00	2	2	
	E	2.28	8.15	83.55	1.00	2.00	4	4	
Horticultural field soil	A	4.40	12.71	76.06	2.67	4.34	5	5	65
	B	1.44	0.00	90.11	0.67	3.0	2	2	
	C	8.75	7.25	80.63	2.00	2.67	5	6	
	D	12	6.10	87.64	1.67	3.67	4	4	
	E	4.48	5.29	87.16	1.33	4.0	4	5	
Cow dung manure	A	2.37	8.22	83.12	6.33	8.66	9	10	85
	B	1.24	3.96	93.14	5.00	7.0	9	10	
	C	2.87	9.21	83.68	3.00	6.00	5	6	
	D	2.23	9.82	83.69	5.00	8.67	7	8	
	E	3.11	9.15	81.59	3.00	7.33	7	7	
Water agar control	A	90.47	0.0	0.0	-	-	-	-	
	B	85.59	8.42	3.82	-	-	-	-	
	C	88.63	4.55	0.0	-	-	-	-	
	D	84.43	8.77	0.0	-	-	-	-	
	E	86.97	6.11	0.0	-	-	-	-	

CN: Captured nematodes on agar block, TN: Total number of nematodes present on the agar block

Table 2: Effect of mass culture of *Drechslerella dactyloides* on the biological control of root knot disease of brinjal

Observation on 5 weeks

Treatments► Parameters ▼	Root knot infested soil	Root knot infested soil +CDM	Root knot infested soil +*D. dactyloides*	Root knot infested soil + CDM +*D. dactyloides*	C.D. at 5%	C.D. at1%
Plant height (cm)	17.0	21.5	20.5	26.0	2.93	4.03
Fresh Shoot weight (g.)	4.5	6.5	8.5	10.5	1.01	1.40
Fresh root weight (g)	2.3	2.6	1.9	2.9	0.49	0.68
Number of root knot /plant	315	365	180 (42.8)*	132 (61.3)**	32.51	45.3
Number of females/plant	225	270	65.5 (70.8)*	20.5 (90.7)**	20.75	28.90
Number of egg mass /plant	375	415	125 (66.7)*	45 (89.1)**	31.44	43.8
Number of nematodes /plant	16550	15650	2158.2 (86.9)*	262.25 (91.9)**	555.99	774.6

Observation on 10 weeks

Treatments► Parameters ▼	Root knot infested soil	Root knot infested soil +CDM	Root knot infested soil *+D. dactyloides*	Root knot infested soil +CDM+ *D. dactyloides*	C.D. at 5%	C.D. at1%
Plant height (cm)	20.5	28.5	35.5	45.5	2.66	3.71
Fresh Shoot weight (g.)	6.0	9.5	14.5	20.5	1.49	2.08
Fresh root weight (g)	3.0	3.4	2.8	3.5	0.81	1.13
Number of root knot /plant	385	1125	375 (2.5)*	285 (74.6)**	52.96	73.85
Number of females/plant	175	220	284 (38.7)*	148 (76.1)**	22.49	31.36
Number of egg mass /plant	422	590	168 (42.8)*	132 (77.6)**	15.05	21.11
Number of nematodes /plant	26465	30750	13975 (47.1)*	9730 (68.3)**	2234.3	3113.5

Data in the parenthesis is the percentage reduction compared with corresponding column in the Table-2
*Percentage reduction in relation to observation of plant parameters of root knot-infested soil.
**Percentage reduction in relation to observation of plant parameters of root knot-infested soil + FYM.
CDM, cow dung manure.

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