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Effect of Fertilizers and Neem Cake Amendment in Soil on Spore Germination of *Arthrobotrys dactyloides*

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Application of fertilizers such as urea, diammonium phosphate (DAP) and muriate of potash in soil adversely affected the spore germination of *Arthrobotrys dactyloides*. Amendment of soil with urea at the concentrations of 1.0%, 0.5% and 0.1% completely inhibited spore germination and direct trap formation on the conidium, whereas muriate of potash delayed and reduced the spore germination even at the lowest concentration. DAP also inhibited spore germination at 1.0% concentration, while at lower concentration the percentage of spore germination was reduced. Application of neem cake at the concentration of 0.5% also inhibited spore germination after 24 h of amendment. The inhibitory effect of neem cake was reduced after 15 days of amendment, while after 30 days after amendment the inhibitory effect was completely lost and the spore germinated by direct trap as in unamended soil. Nematodes were not attracted to ungerminated spores after 24 h of amendment. After 15 days of amendment nematodes were attracted to agar blocks containing fewer germinated spores after 24 h of incubation but after 48 h of incubation large number of nematodes were attracted and trapped by the germinated spores with direct traps. After 30 days of amendment, larger number of nematodes were attracted and trapped by direct traps.

KEYWORDS: ***Arthrobotrys dactyloides*, Direct trap, Neem cake, Nematode trapping fungi**

Nematode trapping fungi are quite common in natural soils, agricultural soils and all kinds of rotting organic debris (Duddington, 1951, 1954; Gray, 1983; Jaffee *et al.*, 1998). Because of their presence in agricultural soils, they have been playing a significant role in maintaining the natural balance of plant parasitic nematodes, which are responsible for mild to severe damage to crops. In modern times, agricultural fertilizers have played a big role in increasing crop production. However, application of these chemicals may have good or adverse effect on the spore germination and population dynamics of nematode trapping fungi. The chemical fertilizers are inhibitory to spore germination of nematode trapping fungi or lethal at the concentrations used in soil, may influence the natural predation of nematodes in soil and thereby bring imbalance in the natural equilibrium.Similarly neem oil cake is also used by Indian framers as organic fertilizer. Neem cake is obtained after extraction of oil from neem (*Azadirachta indica*) seed kernels in oil mills. It contains nitrogen (2~ 2.5%), Phosphorus (0.6~1.4%) and potash (1.2~1.6%). It has fungicidal (Singh *et al.*, 1980) and nemeticidal property (Singh and Singh, 1997). In India neem trees is known as wonder tree because of its diverse medicinal property.The effect of these fertilizers may be studied by testing the germination of spores of the nematode trapping fungi in the soils amended with these chemicals and comparing the same with unamended soil.

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Arthrobotrys dactyloides is one of the few potential nematode trapping fungi, which has been reported to control root knot disease of tomato in pots and field (Stirling *et al.*, 1998; Stirling and Smith, 1998). This fungus is known to form direct trap on the conidium in response to natural soil (Mankau, 1962; Cooke, 1964; Barron, 1977, 1981; Persmark and Nordbring-Hertz, 1997; Kumar, 2003). Since there is no information on effect of amendment of fertilizers and oil cake in soil on spore germination of *A. dactyloides*, effect of most extensively used fertilizers, i.e., urea, diammonium phosphate and muriate of potash and neem oil cake on spore germination of *A. dactyloides* was studied. Results of the same are described in this paper.

Materials and Methods

Fungal isolates. Isolation of different isolates of *A. dactyloides* was done with slight modification (Bandyopadhyay and Singh, 2000) using the method described by Duddington (1955) from different locations: Varanasi, Ghazipur, Mirzapur, Chunar (Uttar Pradesh) and Ranchi (Jharkhand), India. Different isolates were purified and maintained on maize meal agar medium (Maize-20 g; Agar-20 g; Distilled water-1000 *ml*). Further single spore culture of each isolate was made by the method given by Singh *et al.*, 2004. Cultures of each isolate were maintained on maize meal agar medium by regular subculturing at an interval of 15 days.

Effect of urea, DAP and muriate of potash on spore germination and trap formation. The effect of urea, DAP and muriate of potash on the spore germination of five isolates of *A. dactyloides* was studied by the method described by Jackson (1958) for soil fungistasis. Soil samples were taken from Vegetable Research Farm, Banaras Hindu University, Varanasi, India and passed through a 2 mm mesh sieve. Urea, DAP and muriate of potash were separately mixed in the soil at the rate of 1%, 0.5% and 0.1%. Soil without amendment served as control. 50~60 g of soil of each concentration were taken in 90 mm petri dishes and soil was watered near full water holding capacity by addition of distilled water. Water agar blocks (10 mm size, 3 mm thickness) were placed on Whatman's filter paper disc (20 mm size) lying on the surface of soil at five places in the petri dishes and incubated at room temperature (25~30°C) for 24 h to allow the diffusates to reach in the agar blocks. Five agar blocks were taken for inoculation of spores of five isolates of *A. dactyloides*. For unamended soil (Control) water agar blocks were also placed directly on the soil without filter paper disc. After incubation period, spore suspension of each isolate was made separately by harvesting the spores from 15 days old culuture. A small drop of water containing 75~100 spores of each isolate were inoculated separately on each agar block and incubated for 24 h at room temperature for observations. Data on spore germination, direct trap formation and trap formation on spore germ tube was taken. For each treatment three petri dishes were taken as replicates.

Effect of neem cake on spore germination and trap formation. In order to find out the effect of neem cake amendments in soil on the spore germination of five isolates of *A. dactyloides* and trapping of soil nematodes by the fungus, soil was collected from Vegetable Research Farm, Banaras Hindu University, Varanasi, India. Soil was thoroughly mixed and passed through 2 mm sieve. Neem cake was thoroughly mixed in the soil at the rate of 0.5% and placed in pots for decomposition. 50~60 g soil from amended and unamended soils was placed into petri dishes and soil was wet to near full water holding capacity with distilled water. Five water agar blocks were placed directly on the surface of soil in petri dishes at different places and incubated at room temperature for 24 h. After incubation spores of different isolates of *A. dactyloides* were inoculated on agar block as per method described earlier and the inoculated petri dishes were further incubated for 24 or 48 hours for observations. Data on spore germination, direct trap formation and trap formation on germ tube were recorded. Data on capturing of nematodes on agar blocks were also recorded. The experiments on spore germination were conducted on 1st day, 15th day and 30th day after amendment.

Results

In agar blocks placed over unamended soil (Control) directly or on filter papers, the conidia of *A. dactyloides*

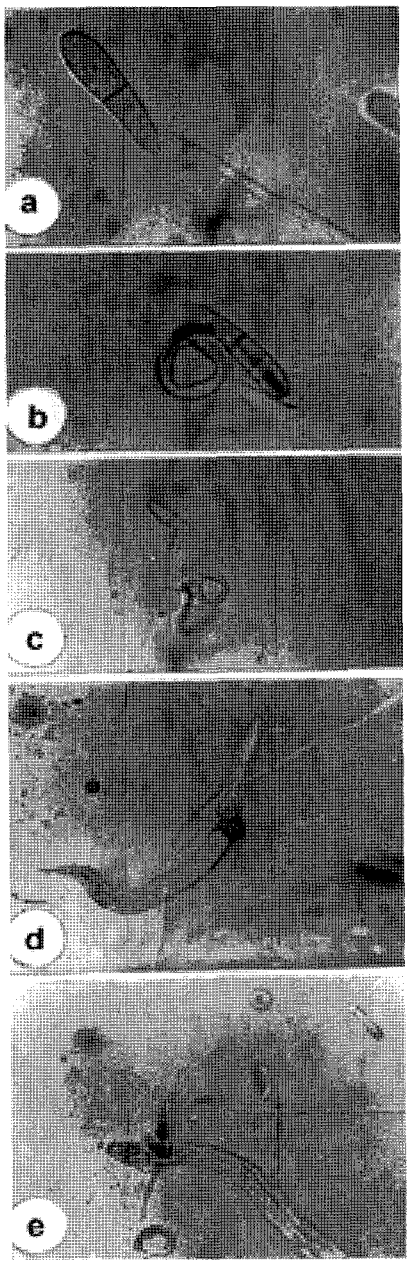

Fig. 1. Spore germination, trap formation and capturing of nematodes by *Arthrobotrys dactyloides*. a) Spore germination of *A. dactyloides* on water agar block placed on unamended soil (× 675). b) Induction of direct trap on the spore of *A. dactyloides* in response to unamended soil (× 600). c) Trap formation on the spore germ tube in response to unamended soil (× 275). d) Development of hyphae from spore cell after capturing and killing of nematodes on agar block placed on neem cake amended soil (× 375). e) Induction of traps on hyphae after capturing and killing of nematodes on agar block placed on neem cake amended soil (× 350).

Table 1. Effect of unamended soil on spore germination of the five isolates of *Arthrobotrys dactyloides*

Parameters	Isolates		Germination (%)	Trap on spore germ tube (%)	Direct Trap on the spore (%)
Agar block directly placed on soil surface	A		4.0	3.5	88.5
	B	Macroconidia	2.0	1.5	92.6
		Microconidia	0.0	0.0	62.5
	C		4.9	5.6	83.4
	D		4.3	5.8	82.6
	E		6.4	6.2	84.5
Agar block placed on filter paper lying on soil	A		4.8	4.0	90.0
	B	Macroconidia	2.2	4.6	92.5
		Microconidia	0.0	0.0	70.0
	C		2.5	3.5	91.8
	D		2.7	4.6	84.0
	E		1.7	3.6	93.5

largely germinated by direct traps (Fig. 1b). There was no difference in the germination of spores by direct traps whether the spores placed on agar blocks directly on the soil or on the filter papers. The percentage of spore germination in both the situations were almost similar (Table 1). However application of urea at the concentration of 1.0%, 0.5% and 0.1% completely inhibited the germination of spores of all the isolates of *A. dactyloides* (Table 2). Similarly diammonium phosphate (DAP) also checked the germination of spores or direct trap formation at 1% concentration. However, in the soil amended with 0.5% DAP, 61~83.3% spores germinated by direct trap while very low percentage of spores (0~1.7%) germinated by germ tube (Fig. 1a). The level of trap formation on germ tube was also very low in all the isolates. At 0.1% concentration of DAP, the germination of spores by direct rings ranged between 66.7 to 92.2% irrespective of the isolates. Microconidia of isolates B also recorded direct ring formation at 0.1% concentration of DAP in soil.

Application of muriate of potash also showed adverse effect on the spore germination or ring formation at all the concentrations. In observations recorded after 24 h of incubation only scant germination of spores by germ tube was recorded at 0.5% and 1% concentration of muriate of potash. However, at 0.1% concentration, the germination of spores by germ tube increased conspicuously and very low percentage of spores germinated by direct rings. When the same agar blocks were observed after 48 h, the germination of spores by direct trap increased conspicuously with the decreasing concentrations of this fertilizer.

Soil freshly amended with neem cake at the rate of 0.5% completely checked the germination of spores by germ tube and direct trap (Table 3). After, 15 days of amendment, germination of spores only by germ tube was recorded in all the isolates, however, the percentage of germinated spores was very low in all the isolates. Irrespective of isolates, the germination rate of spores ranged between 1.6% and 2.6% in 24 h. The microconidia of isolate B, however, failed to germinate. When the observation was taken after 48 h, fairly higher percentage of spores germinated by direct rings, indicating that germination was delayed. Irrespective of isolates, the direct ring formation ranged between 34.9~71%. Even the microconidia of isolate B produced direct rings on the spores. The germination rate of spores by germ tube (Fig. 1a) ranged between 3.8 to 8.9%, while constricting rings on germ tubes (Fig. 1c) were formed on 1.4~7.3% of spores. After one month of amendment, 91~94% of spores germinated by direct rings in 24 h. Microconidia of isolate B also recorded higher percentage of direct ring formation. The level of germination of spores by germ tube was very low ranging from 1.34 to 3.9% only.

From the observations it is evident that after 24 h of amendment of neem cake there was neither spore germination nor nematode trapping. However after 15 days of incubation of amended soil, few nematodes were attracted on agar blocks incubated for 24 h without any capturing. When the same blocks were observed after 48 h of incubation, the number of attracted nematodes considerably increased along with capturing of nematodes. This clearly indicated that even after 15 days of incubation of amended soil the toxic substances of neem cake had its effect although it was reduced. However after 30 days of amendment, fairly higher number of nematodes were attracted and captured. In the corresponding controls, i.e., unamended soil after 1, 15 and 30 days the germination of spores by direct trap were almost similar as given in Table 1.

Discussion

In general, soils amended with higher concentrations of

Table 2. Effect of amendment of different concentrations of urea, diammonium phosphate and muriate of potash in soil on spore germination of *Arthrobotrys dactyloides*

Chemical		Isolates	Concentrations								
			1%			0.5%			0.1%		
			Germination (%)	Trap on spore germ tube (%)	Direct trap on spore (%)	Germination (%)	Trap on spore germ tube (%)	Direct trap on spore (%)	Germination (%)	Trap on germ tube (%)	Direct trap on spore (%)
Urea		A	–	–	–	–	–	–	–	–	–
	B	Macroconidia	–	–	–	–	–	–	–	–	–
		Microconidia	–	–	–	–	–	–	–	–	–
		C	–	–	–	–	–	–	–	–	–
		D	–	–	–	–	–	–	–	–	–
		E	–	–	–	–	–	–	–	–	–
DAP		A	–	–	–	1.7	3.5	79.3	1.0	4.6	85.1
	B	Macroconidia	–	–	–	0.0	0.8	61.0	0.0	1.3	92.2
		Microconidia	–	–	–	0.0	4.5	83.3	0.0	0.0	66.7
		C	–	–	–	1.5.	3.5	83.9	1.6	3.1	84.8
		D	–	–	–	12	3.9	83.1	1.1	3.6	86.4
		E	–	–	–	1.5	3.5	82.6	2.2	2.2	86.4
Murate of potash		Isolate A	1.3 (1.6)	– (0.9)	– (1.4)	6.5 (6.0)	0.2 (3.2)	– (43.8)	14.2 (9.1)	– (13.5)	3.8 (58.9)
	B	Macroconidia	1.1 (0.8)	– (0.0)	– (2.1)	3.1 (3.5)	– (4.6)	– (28.0)	11.8 (–)	– (9.7)	5.9 (50.5)
		Microconidia	0 (1.7)	– (0.3)	– (0.9)	– (2)	– (5.5)	– (22.6)	– (–)	– (0.0)	– (38.0)
		Isolate C	0.2 (3.2)	– (0.6)	– (1.2)	4.4 (9)	– (6.1)	0.1 (42.8)	9.8 (6.4)	– (9.6)	5.0 (61.6)
		Isolate D	1.8 (1.4)	– (0.0)	– (0.2)	4.7 (9.8)	– (4.3)	– (43.0)	11.8 (7.6)	– (12.3)	6.8 (65.3)
		Isolate E	1.2 (1.6)	– (0.2)	– (0.8)	4.6 (9.6)	– (6.7)	– (39.0)	14.0 (8.8)	– (9.9)	7.0 (62.1)

*Data in parenthesis recorded after 48 hours.

Table 3. Effect of amendment of neem cake in soil on spore germination and constricting ring formation of different isolates of *Arthrobotrys dactyloides*

Days	Isolates	0.5% neem cake									
		Time of observation (hour)									
		24					48				
		Germination (%)	Trap on spore germ tube (%)	Direct trap on spore (%)	Attracted Nematodes	Trapped nematodes	Germination (%)	Trap on spore germ tube (%)	Direct trap on the spore (%)	Attract ed nematodes	Trapped nematodes
1 day	A	–	–	–	–	–	–	–	–	–	–
	B Macroconidia	–	–	–	–	–	–	–	–	–	–
	B Microconidia	–	–	–	–	–	–	–	–	–	–
	C	–	–	–	–	–	–	–	–	–	–
	D	–	–	–	–	–	–	–	–	–	–
	E	–	–	–	–	–	–	–	–	–	–
15 days	A	2.6	0.0	0.0	7	0	5.4	5.0	65.6s	36	30.3
	B Macroconidia	1.6	0.0	0.0	8	0	8.9	1.4	71.0	46.0	34.7
	B Microconidia	0.0	0.0	0.0	8	0	0.0	0.0	34.9	46.3	0.0
	C	2.0	0.0	0.0	8	0	6.8	7.3	59.3	33.0	27.3
	D	2.5	0.0	0.0	9	0	3.8	4.7	65.1	31.0	26.7
	E	1.6	0.0	0.0	8	0	4.8	3.7	70.9	29.7	25.7
30 days	A	2.6	3.93	54.0	38.3	36	ND*	ND	ND	ND	ND
	B Macroconidia	1.3	0.0	94.0	44.6	43	ND	ND	ND	ND	ND
	B Microconidia	0.0	3.8	93.6	44.6	0.0	ND	ND	ND	ND	ND
	C	3.9	3.4	91.0	43.0	40.3	ND	ND	ND	ND	ND
	D	1.9	4.3	91.2	43.0	41.6	ND	ND	ND	ND	ND
	E	1.7	2.6	91.7	36.6	35.33	ND	ND	ND	ND	ND

*not determined.

fertilizers reduced spore germination of all the isolates of *A. dactyloides* (Table 2). Inhibition of germination of spores by urea at the concentrations used may be attributed to direct toxicity of urea on the spores. However, DAP at 0.5% and 0.1% concentrations caused little inhibition on the germination of spores of almost all the isolates. Muriate of potash also reduced germination of spores as well as direct ring formation on conidia. From the observations it is very clear that application of fertilizers would reduce the population of nematode trapping fungi in the soil, which might be playing important role in reducing the population of nematodes. In soils, top-dressed with the fertilizers, the concentration of these fertilizers might be fairly high on the soil surface, which would be detrimental to sensitive fungi like *A. dactyloides*. It is also possible that excessive use of these fertilizers may slowly reduce the population of sensitive nematode trapping fungi in the soil.

Inhibition of germination of conidia of *A. dactyloides* in soil duly amended with 0.5% neem cake may be attributed to the toxic principle present in the neem cake. Similar observations were reported by Singh *et al.* (1980) on the spore germination of some pathogenic fungi by neem plant extract. The toxicity of neem cake was also reported on second stage juveniles of *Heterodera cajani* by Singh and Singh (1997). However, in observations after 15 days, the toxic effect of neem cake was considerably reduced which was evident from the observations on germination or trap formation after 48 hours of spore inoculation. This change in toxicity may be attributed to biodegradation of neem toxic factor in soil, which was completely eliminated after 30 days of application of neem cake. It was interesting to note that a large number of soil nematode migrated to the top of the agar block containing the spores of *A. dactyloides*, which may be attributed to attraction of nematode to fungal traps (Table 3). Further most of the nematodes were captured and killed on the agar block (Fig. 1d) and the developing hypha from the spore cell produced few to many traps on the hyphae (Fig. 1e). From the observations it is concluded that mass culture of *A. dactyloides* should be applied only after 15 days of neem cake application to make the biocontrol agent more effective. It has been observed that the population of saprophytic nematodes increases several folds after neem cake amendment (data not shown), which can serve as pabulum for the fungus and increase the population of *A. dactyloides*.

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