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Effect of Rhizosphere Fungi and Actinomycetes on Nodulation in *Trifolium alexandrinum* Linn.

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Culture filtrates of 11 fungi and 5 actinomycetes reduced the number of curled root hairs of *Trifolium alexandrinum*. All the actinomycetes and 12 fungi reduced the number of nodules. *Cunninghamella bertholletiae, Trichoderma lignorum, Penicillium citrinum, Streptomyces aureofaciens* and *Streptomyces* sp. strain 9 completely inhibited in vitro nodulation.

The fungi which reduced the number of nodules in vitro did so also in sterile soil. *Streptomyces aureofaciens* and *Streptomyces* sp. strain 9 behaved similarly. Shoot and root length was also reduced by certain fungi and actinomycetes.

Key Words: **Fungi, Actinomycetes,** *Rhizobium*, **Nodulation,** *Trifolium alexandrinum*

Introduction

Antagonism of rhizobia by other soil microorganisms has been reported (Shukla & Dwivedi 1979). Such antagonism may be one of the factors in cases of faulty nodulation in legumes (Hely et al. 1957, Chhonkar & Subba Rao 1966). The qualitative and quantitative nature of microbial population in rhizosphere and rhizoplane of *Trifolium alexandrinum* Linn. at different stages of its growth has been reported by the authors (Shukla & Dwivedi 1981). In the present investigation, the effect of selected dominant fungi and actinomycetes residing in the root region of *T. alexandrinum* on its root hair curling and nodulation has been studied both in vitro and in sterile soil conditions. The effect of fungi and actinomycetes on shoot and root length has also been studied.

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Materials and Methods

Fungi and actinomycetes were isolated from the rhizosphere of the test plant (Shukla & Dwivedi 1981). Those found dominant (tables 1 to 3) were employed for the present investigation.

1. *Effect of culture filtrates of fungi and actinomycetes on root hair curling and nodulation in vitro*

Plant-slide-culture technique devised by Fahraeus (1957) was used for this study. Seeds of test plant, surface sterilized with H_2SO_4 for $\frac{1}{2}$ min and washed several times with sterilized distilled water, were dipped in sterilized water for about 2 hr and placed in sterilized Petri dishes containing sterile moistened cotton. After incubation for 48 hr at 25°C±1° the seedlings were transferred separately in sterile slide cell in culture tubes (8″× 1.2″) containing equal amount of sterilized medium (Fahraeus 1957). Culture filtrates (2 ml) of selected fungi and actinomycetes (table 1) prepared as described by Shukla and Dwivedi (1979) were added to culture tubes containing seedlings in slide cells and inoculated with 1/2 ml suspension of an effective strain of *Rhizobium*. Tubes inoculated with *Rhizobium* suspension without culture filtrates served as control. The *p*H of the growth medium of all the treatments after addition of culture filtrates and that of the control was maintained at 7–7.2. Lower half of the culture tubes was covered with black paper and tubes were incubated at 25°C±1°. Curled root hairs were counted after 10 days of incubation under the microscope. The number of nodules developed, length of shoot and root were recorded after 30 days and were compared with that of control (table 1).

2. *Effect of fungi and actinomycetes on nodulation in sterile soil in pot condition*

Selected fungi (table 2) and actinomycetes (table 3) grown in 250 ml flask containing 100 ml sterile liquid Czapek's and Kuster and William's (1964) medium at 25°C±1° and 30°C±1° respectively for a fortnight were filtered through sterile muslin cloth. The mycelial mat, washed thoroughly with sterile water and dried with sterile blotting paper was gently macerated in mortar with pestle and equal amount was mixed thoroughly with 1 kg of sterilized alluvial soil (0.5%) singly or in groups. Calcium tri-phosphate (50 g/kg soil) was used as basal dressing. Sterile soil mixed with individual and mixed inocula was placed in separate sterile earthenware pots and inoculated with equal amount of a suspension of an effective strain of *Rhizobium*. Surface-sterilized seeds were sown and five seedlings were retained in each pot after germination. Pots inoculated with *Rhizobium* alone served as control. Plants were watered regularly with sterile water. When one month old, they were uprooted carefully with the help of slow running water. The number of nodules, length of shoot and root were recorded and compared with the control (tables 2 & 3).

Results and Discussion

The culture filtrates (CF) of eleven fungi and all the actinomycetes tested, reduced the number of curled root hairs (CRH) significantly (table 1). *Cunninghamella bertholletiae* (CB), *Trichoderma lignorum* (TL), *Aspergillus terreus* (AT), *Penicillium citrinum* (PC), *Paecilomyces varioti* (PV), *Alternaria humicola* (AH), *Streptomyces aureofaciens* (SA) and *Streptomyces* sp. strain 9 (S 9) were comparatively more effective than others. CF of all fungi and actinomycetes except

Table 1 *Effect of culture filtrates of fungi and actinomycetes on root hair curling and nodulation in* T. alexandrinum

Metabolites of	No. of curled root hairs (Mean of 5 values)	't' value	No. of nodule/plant (Mean of 3 values)	't' value	Length of shoot in cm (Mean of 3 values	't' value	Length of root in cm (Mean of 3 values)	't' value
I. FUNGI								
C. bertholletiae	18.40	8.075*	0.00	12.227*	2.76	3.574**	3.06	4.545**
C. bostrychodes	28.60	4.927*	7.33	3.873**	5.02	2.362	5.66	0.151
T. lignorum	22.00	6.287*	0.00	12.227*	2.00	6.601*	2.36	5.396*
A. fumigatus	31.40	4.427*	3.33	6.054*	4.10	3.147**	3.83	2.574
A. flavus	43.00	0.812	9.00	3.086**	5.56	1.843	5.56	0.00
A. terreus	20.20	8.004*	1.00	8.009*	3.33	4.233**	3.00	3.770**
A. niger	30.80	4.258*	2.00	7.393*	5.06	1.921	6.16	0.747
P. citrinum	18.80	6.173*	0.00	12.227*	3.43	4.768*	2.80	4.312**
P. varioti	19.40	6.640*	2.66	6.435*	5.36	1.684	5.86	0.460
A. fusispora	41.40	1.350	3.66	5.837*	5.10	2.683	4.40	1.920
C. lunata	32.20	3.697*	16.00	0.930	6.33	0.833	4.86	1.200
A. humicola	25.80	6.244*	8.33	3.149**	4.60	2.688	6.50	1.265
F. oxysporum	30.40	4.863*	3.00	6.041*	4.10	3.880**	4.66	1.419
II. ACTINOMYCETES								
N. fructifera	19.60	7.434*	3.33	6.079*	6.70	0.358	5.86	0.270
S. albus	29.20	4.723*	4.60	5.105*	5.76	1.688	5.96	0.661
S. aureofaciens	14.60	9.138*	0.00	12.227*	1.00	7.257*	1.66	6.927*
Streptomyces sp. strain 6	13.00	9.224*	0.00	12.227*	0.00	9.915*	0.00	11.12*
Micromonospora sp.	26.00	4.798*	4.33	5.491*	5.66	1.662	6.83	1.126
III. CONTROL	48.00	—	14.00	—	7.03	—	5.56	—

*Significant at 1% level; **Significant at 5% level

Table 2 *Effect of hyphal mat of different fungi amended in sterile soil on nodulation and growth of* T. alexandrinum

Hyphal mat of	No. of nodule/ plant (Mean of 5 values)	't' value	Length of shoot in cm (Mean of 5 values)	't' value	Length of root in cm (Mean of 5 values)	't' value
I. INDIVIDUAL FUNGI						
Rhizopus oryzae (RO)	32.20	0.627	16.80	2.133	15.90	1.552
Cunnighamella bertholletiae (CB)	16.50	4.609*	12.30	4.614*	12.60	4.385*
Penicillium javanicum (PJ)	36.40	0.280	19.40	0.789	15.40	1.939
Thielavia terricola (TT)	28.60	1.326	17.16	1.914	16.40	2.193
Chaetomium bostrychodes (CBT)	17.60	4.048*	21.40	0.296	16.20	1.244
Trichoderma lignorum (TL)	17.00	4.930*	12.30	4.488*	13.80	4.352*
Aspergillus fumigatus (AFU)	16.00	7.932*	16.97	2.163	18.40	0.268
A. flavus (AFL)	17.40	4.489*	17.10	1.996	15.80	1.821
A. terreus (AT)	10.80	7.272*	14.70	3.440*	15.60	1.664
A. niger (AN)	18.20	4.119*	17.74	1.654	18.20	0.056
A. candidus (AC)	18.20	4.815*	14.40	3.834*	14.50	3.574*
Penicillium citrinum (PC)	22.60	3.065**	12.60	3.251**	11.40	6.041*
Paecilomyces varioti (PV)	20.70	3.283**	13.10	4.879*	15.70	1.981
Acrophialophora fusispora (AF)	25.40	2.273	18.50	0.956	18.00	0.065
Cladosporium herbarum (CH)	20.60	3.855*	17.28	1.842	17.60	0.342
Curvularia lunata (CL)	28.00	1.088	17.74	1.593	17.90	0.171
Alternaria humicola (AH)	19.00	4.742*	17.34	1.322	17.50	0.536
Fusarium oxysporum (FO)	23.00	3.298**	16.60	2.230	15.50	2.420
Myrothecium roridum (MR)	27.33	1.719	17.50	2.052	16.50	2.193
II. FUNGI IN GROUP						
Group I (RO+CB+PV+AF)	22.4	3.115*	17.18	2.033	18.30	0.108
Group II (TT+TL+AF+MR)	25.2	1.781	13.00	3.383*	14.30	3.584*
Group III (CB+TL+PJ+PC)	20.4	3.513*	14.30	4.188*	17.74	0.248
Group IV (AFU+AFL+AT+AN+AC)	14.8	4.268*	14.00	3.415*	14.70	3.357*
Group V (CH+CL+AH+FO)	21.6	3.367*	19.10	0.688	20.1	1.012
III. CONTROL	34.80	—	20.80	—	18.1	—

*Significant at 1% level; **Significant at 5% level

Table 3 *Effect of hyphal mat of different actinomycetes amended in sterile soil on nodulation and growth of* T. alexandrinum

Hyphal mat of	No. of nodule/ plant (Mean of 5 values)	't' value	Length of shoot in cm (Mean of 5 values)	't' value	Length of root in cm (Mean of 5 values)	't' value
I. *Individual actinomycetes*						
Nocardia fructifera (NF)	28.00	0.684	15.60	1.196	19.4	0.973
Streptomyces aureofaciens (SA)	18.10	7.473*	19.60	0.675	16.0	1.023
S. albus (SAL)	33.50	0.307	17.10	0.471	17.1	0.150
Streptomyces sp. strain 9 (S 9)	17.80	5.560*	15.9	0.659	15.7	1.066
Micromonospora sp. (M)	37.80	1.550	18.2	0.00	21.4	1.541
II. *In group*						
Group I (NF+SA+SAL+S 9+M)	28.8	0.613	15.1	1.536	14.6	2.023
Group II (SA+SAL+S 9)	18.2	4.425*	15.7	1.261	16.6	0.560
III. *Control*	31.6	—	18.2	—	17.36	—

* 't' test significant at 1% level

Curvularia lunata (CL) reduced the number of nodules significantly (table 1). The CF of CB, TL, PC, SA and S 9 completely checked the development of nodules. Length of shoot and root was reduced significantly in presence of CF of CB, TL, AT, PC and SA. Plants died after one month in presence of CF of S 9.

Amongst the fungi amended in sterile soil, 13 reduced the number of nodules significantly (table 2). All the fungi which were found to reduce the number of nodules in culture tubes reduced the number of nodules in sterile soil. *Chaetomium bostrychodes*, *Aspergillus fumigatus*, *A. niger*, *A. candidus* (AC), CB, TL, AT and AH were comparatively more effective. Certain fungi viz., CB, TL, AC and PC reduced the length of shoot and root also. Fungi amended in group I, III, IV and V also reduced the number of nodules. Fungi in group II and IV reduced the length of shoot and root also significantly (table 2). Among the amended actinomycetes, SA, S 9 and group II combining *Streptomyces* spp. reduced the number of nodules significantly (table 3). None of the actinomycetes either individually or in group reduced the length of shoot and root. Most of the fungi and all the actinomycetes which reduced the number of CRH, also reduced nodule number. *Aspergillus flavus* and *Acrophialophora fusispora* had no effect in reducing the CRH but they reduced the nodule number. It was *vice versa* in case of *Curvularia lunata*. In all the cases where number of CRH and nodule was reduced, shoot and root length was not reduced.

Certain fungi and actinomycetes reduced the number of CRH and nodules. Inhibition of root hair curling and nodule formation by certain fungi and actinomycetes may be due to presence of antibiotic substances in them. Change in *p*H as suggested by Munns (1968) and reduction in root length may also be contributing factors. Fungi and actinomycetes may also act adversely towards the growth of rhizobia (Shukla & Dwivedi 1979). Reduction in number of CRH by fungal culture filtrates has also been reported in leguminous plant by Dwivedi (1967). Toxic effect of certain fungi on nodule number in certain leguminous plants has also been reported (Chhonkar & Subba Rao 1966). Hely et al. (1957) noted that the reason for faulty nodulation in subterranean clover was due to microbial antagonism by other soil microorganisms inhibiting the clover rhizosphere. They observed proper nodulation with autoclaved soil having effective strain of *Rhizobium*. A number of isolates of actinomycetes have been reported antagonistic towards the rhizobia and nodulation (VanSchreven 1964, Damirgi & Johnson 1966). The results of the present investigation also indicate that in a particular soil antirhizobial fungi and actinomycetes play an important role in the establishment of specific rhizobial strains and nodulation in leguminous plant.

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