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ISOLATION OF NODULE SURFACE MYCOFLORA OF *LENS CULINARIS* (M.)

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For isolation of nodule surface mycoflora, the nodulated parts of the plants after removing the rhizosphere soil were used. Active (pink) or healthy nodules were detached with sterilized needle from the plant roots. Nodules were washed thoroughly several times with sterilized distilled water and surface dried with sterilized filter paper. Then 5-6 nodules were placed in a sterilized petriplate over PDA media. These plates were incubated at 28±1°C temperature for one week. Isolation and purification of nodule mycoflora by poured and streak method. Total 13 species of seven genera were isolated from the surface of nodules at three stages of plant growth in which *Aspergillus* spp., *Fusarium* spp., *Alternaria* sp., *Penicillium* spp., *Curvlaria lunata* sp., *Rhizoctonia solani, Rhizopus* spp., were common in all stages.

Keywords : Filter paper; Nodules; PDA; Plates.

Introduction

The higher amount of protein in legumes is due to their atmospheric nitrogen fixation capability through the symbiotic *Rhizobium* bacteria found in their root nodules. Besides *Rhizobium* some other nitrogen fixing bacteria and Actinomycetes are also found in soil in free state along with many fungi and Blue green algae in the rhizosphere, rhizoplane and non-rhizosphere zones.

The region in the vicinity of root can be distinguished into many microhabitats, denote that region of the soil which is subject to the influence of plant roots. Rhizosphere is characterized by greater microbial activity than the soil away from plants roots. The intensity of such activity depends on the distance to which exudations from the root system can migrate. The term "Rhizosphere-effect" indicates the overall influence of plant roots in soil microorganisms. Jain *et al.*[1] reported the effect of rhizosphere fungi on nodule number, shoot and root length of *Vigna mungo.*

Rhizosphere microbial communities can significantly influence phytopathgens development[2], nutrient acquisition[3], heavy metal resistance[4], and ecological fitness of plant[5].

The Rhizoplane is the direct contractual area of the root after removing complete soil by frequent washing.

Material and Method

Agar plate test-In all root nodules samples were employed for agar platetest.200gm of peeled potatoes, 20gm dextrose and 20gm agar per liter of distilled water were used to prepare potato dextrose agar (PDA).The mixture was autoclaved and 15to 20ml of autoclaved PDA was poured aseptically to each sterilized Petri plate .For sterilization water cleaned Petri plate were surface washed in rectified spirit and kept at 150°C for 2hrs. in oven.

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Result and Discussion

Fungi from nodules at pre-flowering, flowering and fruiting stages were observed but not isolated at seedling stage because at this stage, nodules were not so developed as to isolate from the root system. Total 13 species of seven genera (Table 1) were isolated from the surface of nodules at three stages of plant growth in which *Aspergillu flavus, Aspergillus niger, Penicillium* spp., and white sterile mycelium were common in all stages. Jamro and Larik[6]

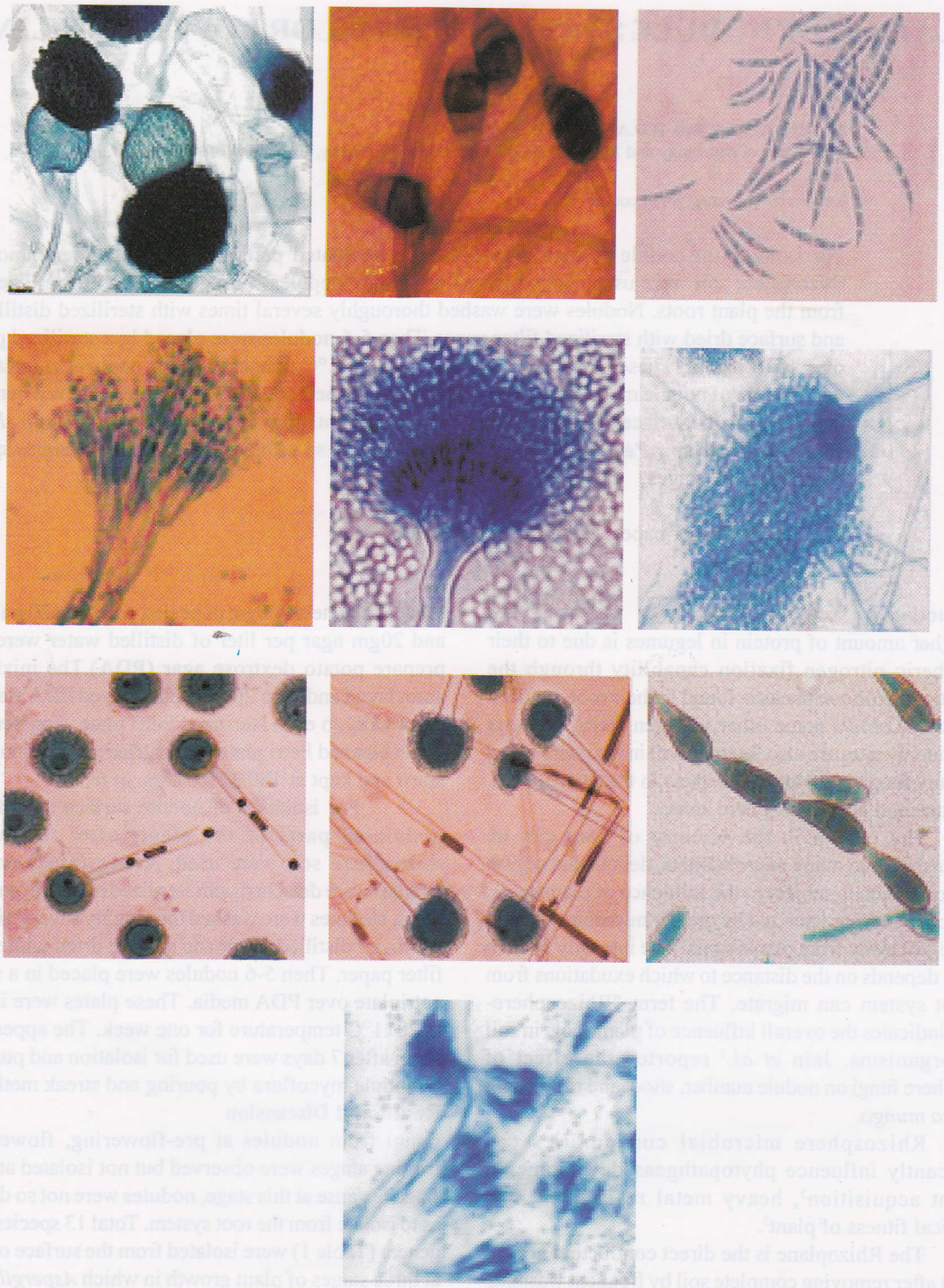

Fig.1. Fungal flora isolated from nodules (Lentil)
A : Sporangium and mycelium of *Rhizopus nigricans* x 400; **B :** Conidia and mycelium of *Curvularia lunata* x 400; **C :** Conidia of *Fusarium oxysporum* x 400; **D :** Conidiophore and conidia of *Penicillium chrysogenum* x 400; **E :** Conidiophore and conidia of *Aspergillus terreus* x 400; **F :** Conidiophore and conidia of *Aspergillus fumigatus* x 400; **G :** Conidiophore and conidia of *Aspergillus flavus* x 400; **H :** Conidiophore and conidia of *Aspergillus niger* x 400; **I :** Conidia of *Alternaria alternata* x 400; **J :** Conidiophore and conidia of *Penicillium aurantiogriseum* x 400.

Table 1. Fungi present on nodule surface of lentil.

S. No.	Plant Name	Pre-flowering stage	Flowering stage	Fruiting stage
1.	*Alternaria alternata*	-	-	+
2.	*A. humicola*	+	+	-
3.	*Aspergillus candidus*	-	+	-
4.	*A. flavus*	+	+	+
5.	*A. niger*	+	+	+
6.	*A. terreus*	-	+	-
7.	*Curvularia lunata*	+	+	-
8.	*Fusarium moniliformae*	+	+	-
9.	*F. udum*	+	-	+
10.	*Penicillium* sps.	+	+	+
11.	*Rhizoctonia solani*	+	-	-
12.	*Rhizopus* sps.	+	-	-
13	White sterile mycelium	+	+	+
	Total	10	9	6

Table 2. Per cent abundance of nodule surface fungi at the pre-flowering, flowering and fruiting stages.

S.No.	Name of fungi	Pre-flowering stage		Flowering stage		Fruiting stage	
		FP	%A	FP	%A	FP	%A
1.	*Alternaria alternata*	-	-	-	-	4	12.12
2.	*A. humicola*	4	6-66	3	5.88	-	-
3.	*Aspergillus candidus*	-	-	2	3.92	-	-
4.	*A. flavus*	6	8.82	9	17.20	7	21
5.	*A. niger*	7	10.29	8	15.68	9	27
6.	*A. terreus*	-	-	4	7.84	-	-
7.	*Curvularia lunata*	6	8.82	4	7.84	-	-
8.	*Fusarium moniliformae*	6	8.82	4	7.84	6	18
9.	*F. udum*	8	11.76	-	-	-	-
10.	*Penicillium* sps.	8	11.76	5	9.80	4	12.12
11.	*Rhizoctonia solani*	3	4.41	-	-	-	-
12.	*Rhizopus* sps.	10	14.70	-	-	-	-
13	White sterile mycelium	10	14.70	12	23.52	3	9.09
14	Total fungal population	68		51		33	
15	Total number of fungi	10		9		6	

FP: Fungal population
A: Abundance

reported reduction in the number of leguminous plant by the application of nitrogen fertilizers. Dublish[7] studied the effect of foliar application of antibiotics on leguminous plant and concluded that the antibiotics reduce the nodule number at high concentration *et al.*[8]. studied the effect of starter doses of nitrogen on nodulation, yield and nitrogen up take of chick pea. The number of nodule mycoflora at pre-flowering stage was found to be higher as compared to flowering and fruiting stages (Table 2).

References

1. JainVivek and Gupta V K 2002, Effect of rhizosphere fungi on nodule number, shoot and root length of *Vigna mungo. Indian Phytopath.* **55**(3) 323-324.
2. Nehl D B, Allen S J and Brown J F 1995, Deleterious rhizosphere bacteria. An integrating perspective. *Applied soil Ecology* **5** 1-20.
3. Lynch J M and Whipps J M 1990, Substrate flow in Rhizosphere.*Plant and Soil* **129** 1-10.
4. Bradley R,Burt A J and Read D J 1981, Mycorrhizal infection and resistance to heavy metal toxicity in *Calluna vulgaris. Nature* **292** 335-337.
5. Parker M A 1995, Plant fitness variation caused by different mutualism genotypes. *Ecology* **76** 1525-1535.
6. Jamro G H and Larik A S 1988, Influence of nitrogenous fertilizer on nodulation leghemoglobin content and biological nitrogen fixation in *Phaseolus vulgaris. Genet. Agric.***42**(1) 51-58.
7. Dublish P K 1986, Foliar application of antibiotics, growth hormones and urea in relation to rhizosphere mycoflora of *Abelomoscus esculentus* and *Lagenaria vulgaris. Indian Phytopath.* **39**(2) 264-268.
8. Shri Krishna, Dinesh Kumar Kamal and Sharma A P, 2001, Effect of starter doses of nitrogen on nodulation, yield and nitrogen up take of chick pea (*Cicer arietinum* L.). *Legume Res.* **24**(4) 275-277.