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ROOT NODULATION AND FUNGAL ASSOCIATIONS OF TWO COMMON HERBACEOUS LEGUMINOUS WEEDS IN THE UNIVERSITY OF PERADENIYA

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ABSTRACT

While *Aeschynomene indica* (L.) was observed to produce a large number of brown, small, round nodules well distributed along the entire length of the tap root and large lateral roots, very few pale pink, large, round nodules distributed mostly on the lateral roots were observed in *Centrosema pubescens* (Benth.). On the basis of nodule numbers *A. indica* can be considered as an abundant-nodulater whereas *C. pubescens* appears to be a sparse-nodulater. In both legumes nodule number was independent of tap root length, but in *A. indica* it showed a relationship with small lateral root formation.

The numbers of fungi isolated from root segments of the two legumes were equal but the types of isolates were different. A greater incidence of ascomycetous and deuteromycetous fungi was observed on the roots of *A. indica* in comparison to *C. pubescens* on which the occurrence of zygomycetous fungi was higher.

INTRODUCTION

Aeschynomene indica (L.) and *Centrosema pubescens* (Benth.) are two common herbaceous leguminous plants found growing as weeds in the University premises. The former is a shrub while the latter is a prostrate creeper. Both show natural root nodulation. *A. indica* in addition to its root nodulating habit also shows the formation of basal stem nodules. In the legume *Rhizobium* symbiotic association, the bacterium while obtaining its requirements of carbon and energy resulting from higher plant photosynthesis, converts the free nitrogen in the atmosphere to a form usable by the higher plant.

The aim of this study was to investigate the patterns of root nodulation in these two leguminous plants and to relate them to their potential nitrogen fixing ability. The study was further extended to examine the root surface fungi of the plants and to see whether such associations have an effect on nodulation.

MATERIALS AND METHODS

The experimental plants were *Aeschynomene indica* (L.) and *Centrosema pubescens* (Benth.). Both are herbaceous legumes except that in habit the former is an erect shrub and the latter a creeper. Healthy plants of *A. indica* and *C. pubescens* both in a comparable stage of development (at flowering), were collected from five localities, within the University of Peradeniya premises, in order to obtain a representative sampling within the entire habitat. The localities selected were such that both legumes occurred together. Three replicate plants were sampled from each locality. Plants were uprooted carefully not to damage the root system and the roots were well washed in tap water. Records were made on length of tap root, lateral root number, nodule number, nodule size, colour and distribution.

Examination of the fungal flora in association with the roots was done only on plants collected from one locality (Botany Department premises). Three plants each of *A. indica* and *C. pubescens* were uprooted carefully and the roots washed well under the tap. The tap root of each plant was separated into three regions. Base (B), Middle (M), and Apex (A).

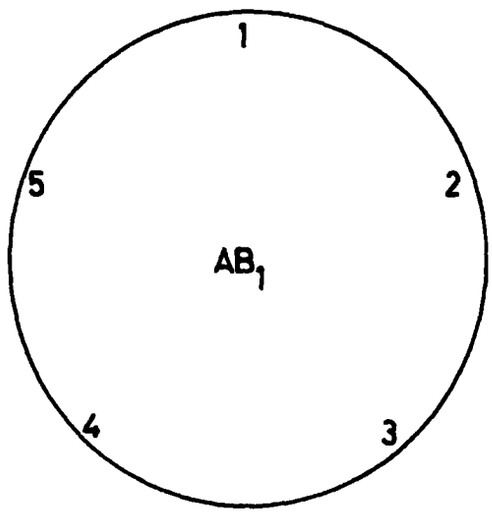
These were labelled as,

A - $B_1M_1A_1$ for replicate plant 1 - tap root of *A. indica*
A - $B_2M_2A_2$ for replicate plant 2 - tap root of *A. indica*
A - $B_3M_3A_3$ for replicate plant 3 - tap root of *A. indica*

From each replicate plant three lateral roots were also cut and labelled as A - L_{11}, L_{12}, L_{13}; A - L_{21}, L_{22}, L_{23}; A - L_{31}, L_{32}, L_{33} for the first, second and third replicate plant respectively of *A. indica.*

The separated root regions AB_1, AM_1, AA_1 of the tap root and the lateral roots AL_{11}, AL_{12}, AL_{13} of replicate plant 1 of *A. indica* were then transferred into separate containers and washed well in about 20 changes of sterile water in a shaker (Harley, J.S. 1955). Following washing, five root segments of 2 mm length were cut from each tap root region. Similarly, five root segments of 2 mm length were also cut from each of the lateral roots.

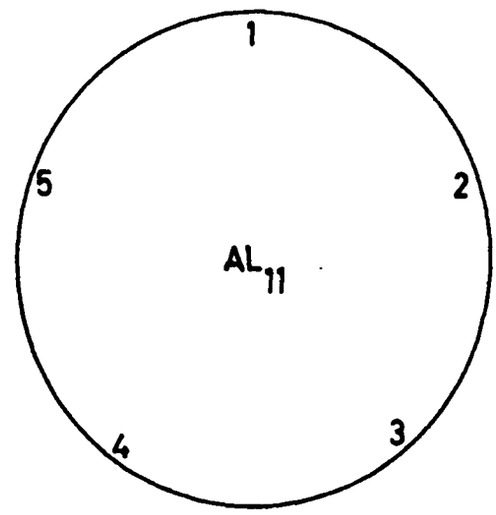
These root segments were then plated on Martins' medium and incubated at room temperature. Martin's' medium is a non synthetic medium containing streptomycin and rose bengal for the suppression of bacterial growth and generally used for the isolation of fungi. The agar plates were observed daily for the appearance of fungal colonies and colonies developing from each root segment were examined under the microscope following staining with cotton blue in lactophenol and identified. These colonies were streaked on fresh Martin's medium plates and pure cultures of all the isolates were maintained on agar slants. The method of labelling the plates is indicated below.



where A - *A. indica*

B1 - base of tap root of replicate plant 1

1 - 5 - 2 mm root segments of the base



where A - *A indica*

L_{11} - lateral root 1 of replicate plant 1

1 - 5 - 2 mm root segments of the lateral root

The same procedure was followed for the plating of root segments of *C. pubescens* and labelled accordingly, inserting the letter C - for *C. pubescens* instead of the letter A used for *A. indica.*

RESULTS

Both *A. indica* and *C. pubescens* showed the development of deep feeding tap root systems. The root system of *A. indica* showed lateral roots of two types (Figure 1). The large laterals were few in number while the small laterals were numerous. A large number of small, round, brown root nodules were seen distributed along the entire length of the tap root and also on the large lateral roots. At the crown of the root and the base of the stem, were a few stem nodules, also small in size, round in shape, but pale green in colour. In the root system of *C. pubescens*, the laterals were of the large kind only (Figure 2) and the numerous small laterals observed in *A. indica* were not seen in this plant. The few root nodules observed were large, round in shape, pale pink in colour and distributed mostly on the lateral roots.

The data collected on length of tap root, nodule number and lateral root number of the two plants are presented in Tables 1 and 2. In both legumes the tap root lengths appear to be more or less similar. However, when one considers the nodule numbers of the two plants, *A. indica* produces a large number of very small nodules while *C. pubescens* produced only a few nodules of a comparatively larger size. These results therefore suggest that nodule number is independent of tap root length. Also, no obvious differences were observed in the numbers of large lateral roots produced by the two plants. However, the small lateral roots observed in *A. indica* were quite characteristic to the plant and a correlation could be drawn between its small lateral root number and nodulation (Table 2).

Thirteen different types of fungi were isolated from root segments of *A. indica* and fourteen from *C. pubescens* (Table 3). Some isolates were common to both plants.

Tables 4 and 5 give the fungi isolated from the tap root segments of *A. indica* (replicate plant 1) and *C. pubescens* (replicate plant 2) respectively.

The types of fungi isolated from all the root segments examined and the number of colonies per centimetre length of root are shown in Figures 3 and 4 for *A. indica* and *C. pubescens* respectively.

In both *A. indica* and *C. pubescens* root segments *Fusarium* sp. I showed the highest frequency of occurrence (its incidence being higher in *A. indica*), while *Aspergillus* sp.I showed the lowest. Zygomycetous fungi were higher in the root segments of *C. pubescens* than in *A. indica* . The zygomycetes isolated from *C. pubescens* included Absidia, *Cunninghamella, Mucorales* (unidentified) and *Rhizopus,* while *Cunninghamella* and *Syncephalastrum* were the only isolates of *A. indica.* Nine ascomycetous and deuteromycetous fungi were found in association with *A. indica* root segments and eight with those of *C. pubescens.* The occurrence of fungi was generally higher on the roots of *A. indica* than on *C. pubescens.* The occurrence of ascomycetous fungi was higher in *A. indica,* whereas zygomycetous types were greater in *C. pubescens* (Figures 3 and 4).

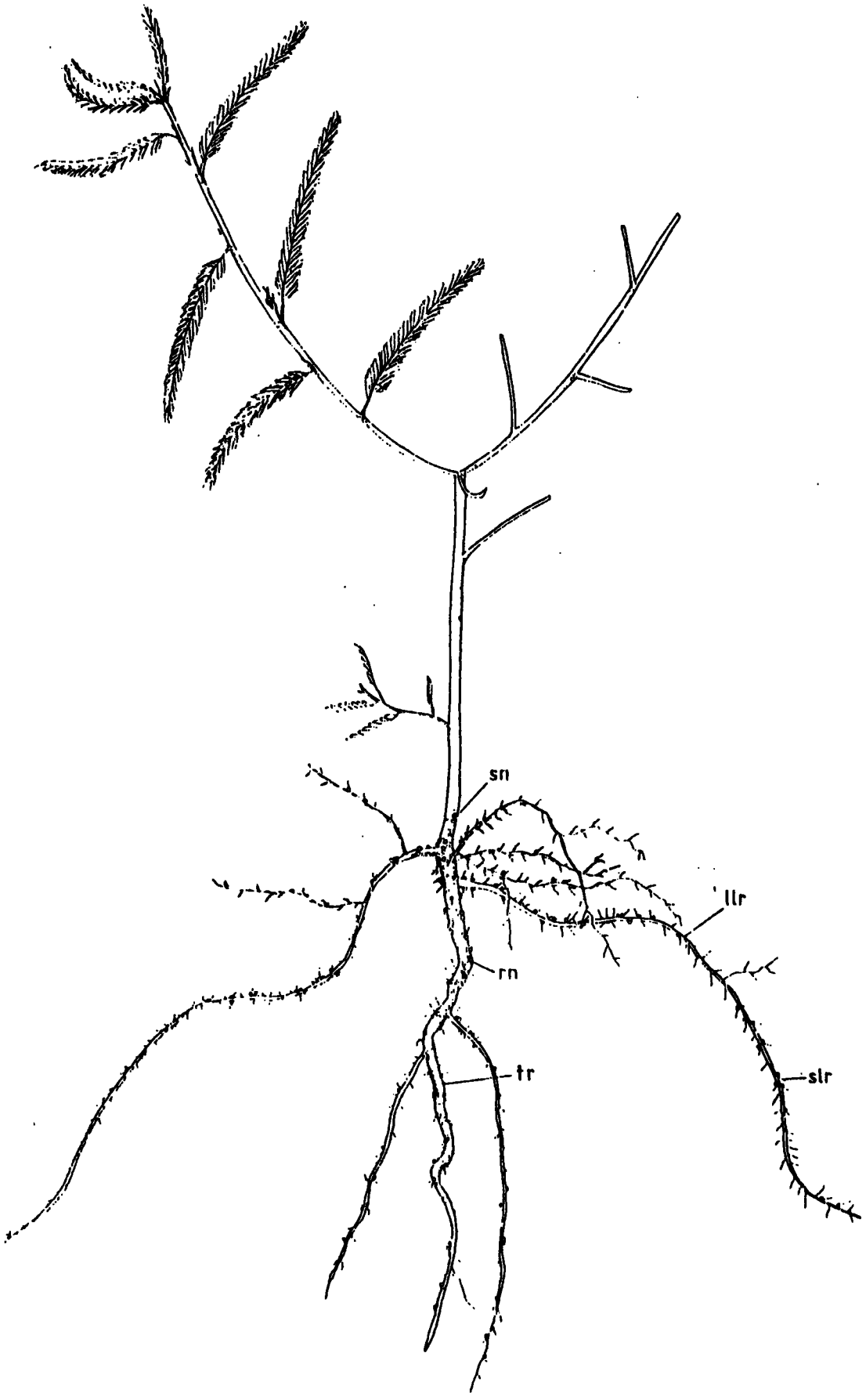


Fig. 1 Aeschynomene indica - abundantly nodulating plant
tr - tap root; llr - large lateral root; slr - small lateral root; sn - stem nodule; rn - root nodule

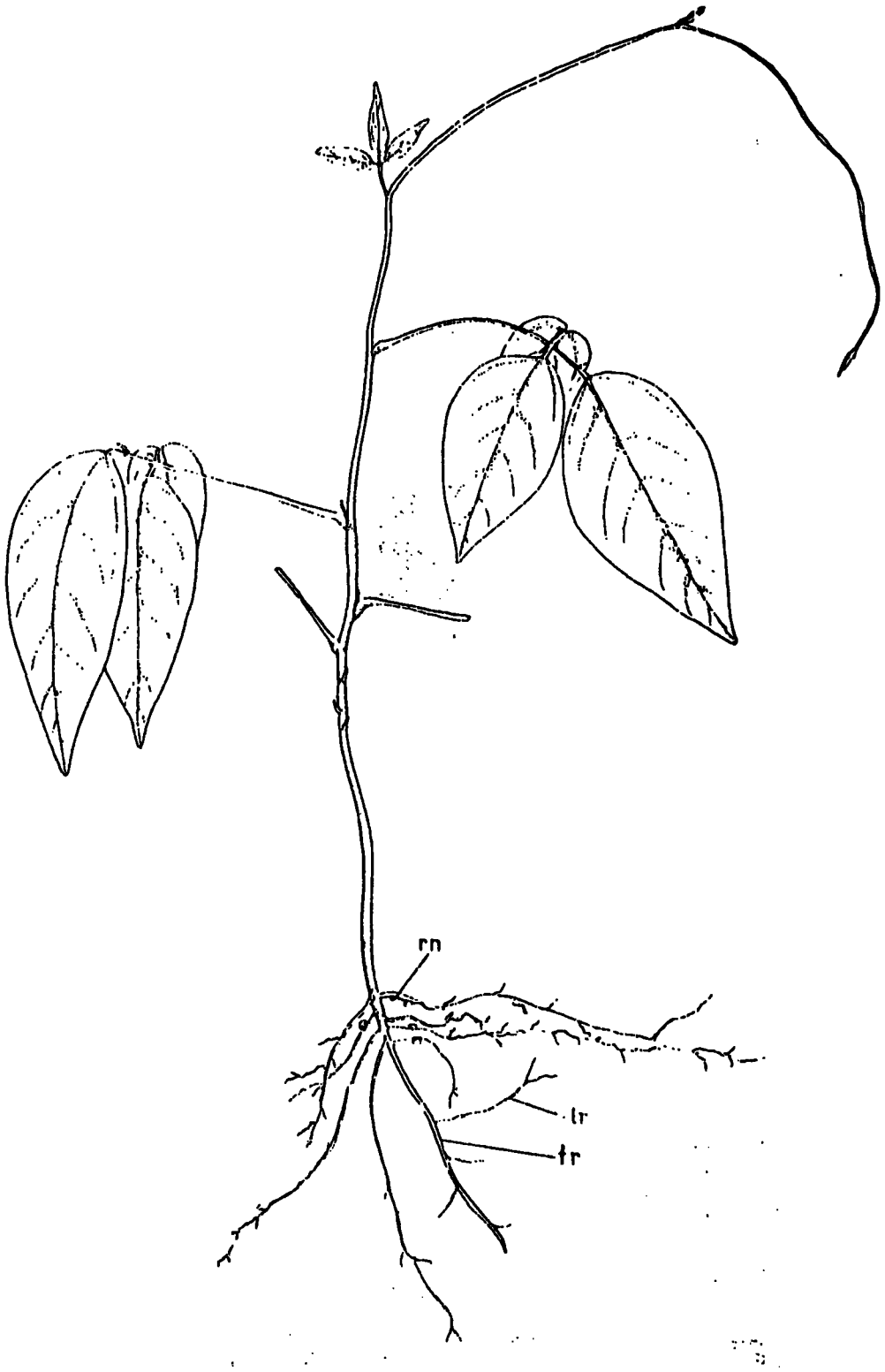


Fig. 2 Centrosema pubescens - poorly nodulating plant
tr - tap root; lr - lateral root; rn - root nodule

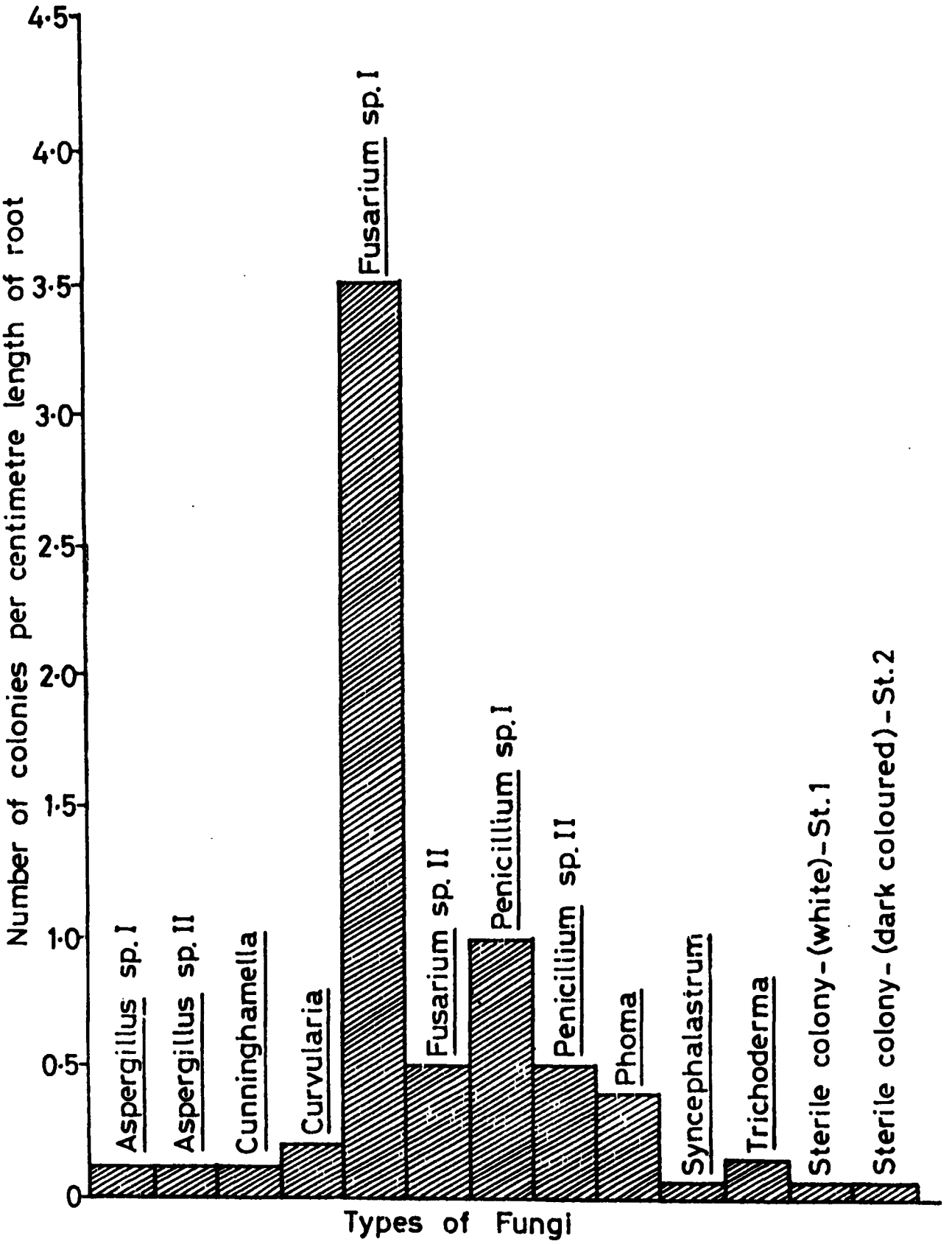


Fig. 3 Occurrence of fungi on the roots of Aeschynomene indica

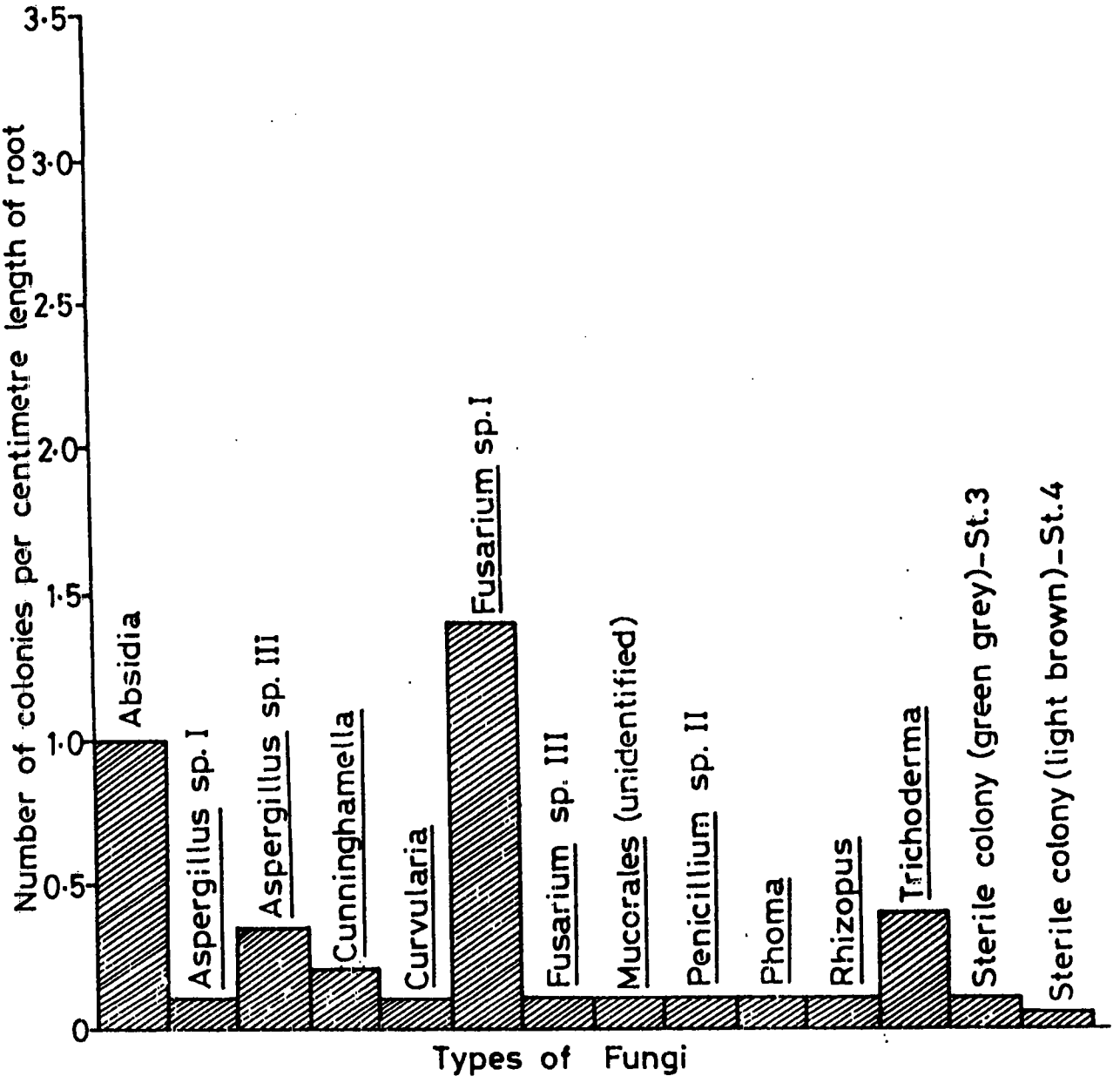


Fig. 4 Occurrence of fungi on the roots of Centrosema pubescens

DISCUSSION

The erect growing herbaceous legume, *A. indica* can be considered as an abundant - nodulater, since it produces a large number of small nodules well distributed along the entire length of the tap root and also on the large lateral roots. In addition, a few small stem nodules occur at the base of the stem. *C. pubescens* the herbaceous leguminous creeper appears to be a comparatively poor-nodulater in which a few large nodules are mostly confined to the lateral roots. The formation of both root and stem nodules in *A. indica* has been reported by Arora (1954).

Length of tap root and nodule number (Table 1) shows that root length in both legumes is more or less equal inspite of the large difference in nodule numbers. Thus, the presence of a longer tap root does not provide a greater number of infection sites for nodulation. However, when one considers the lateral root numbers (large kind) and nodule numbers (Table 2), the lateral root numbers also remain more or less the same for both plants while the nodule numbers very greatly. On the other hand the small lateral root number of *A. indica* was quite high and shows a relationship to its large nodule number. These root nodules appear to form on the large lateral roots, preferably at the junction of small laterals.

Root nodules and lateral roots arise as lateral organs of a plant although of different origin. Root nodules arise from the root cortex and lateral roots from the pericycle. Arora (1954) however, reports that in *A. indica* only the stem nodules are cortical in origin while the root nodules arise from the pericycle. It is therefore possible that the nodulating bacteria of *A. indica* competes for sites within the root tissue that would otherwise give rise to lateral roots of the small kind. However, the elucidation of such a mechanism needs further investigation. A similar situation does not arise with *C. pubescens* because only the large lateral roots occur in this plant and these are supposedly endogenous in origin compared to the exogenous root nodules.

`Since these results do not suggest an inverse relationship between lateral root numbers and nodule numbers it appears that the numbers of lateral roots formed by a particular legume and the number of nodules formed by a particular legume-bacterium association is a feature that is host-determined.

The large number of small nodules with a widely scattered distribution in *A. indica* presents a typical ineffective root nodulation pattern, while *C. pubescens* with its few, large nodules of restricted distribution, presents a typical effective nodulation pattern. However, nodule sections of *A. indica* examined showed a light pink to dark pink central spot in the centre of the nodular tissue paralleling that of *C. pubescens,* indicating that both legumes are potential nitrogen fixers regardless of the differences in their nodulation patterns. These preliminary observations could be confirmed by measuring nitrogenase activity by the acetylene reduction assay (Schöllhorn and Burris 1966).

McCoy (1932) examining the possible participation of cell wall degrading enzymes in the infection process, reported the absence in cultures of rhizobia of pectinase, chitinase, cellulase or enzymes able to attack pectic substances and which might promote the dissolution of the root hair wall, thereby facilitating bacterial entry into the root tissue. *Rhizobium* is therefore not known to produce cell wall degrading enzymes. Amongst the fungi however, several ascomycetes and basidiomycetes, but not zygomycetes are known to produce cell wall degrading enzymes. It is therefore possible that ascomycetous and basidiomycetous fungi in association with the roots bring about a change in cell wall composition prior to the infection of root hairs by the rhizobia.

Ascomycetes were frequently isolated from root segments of *A. indica* while zygomycetes showed a higher incidence in *C. pubescens.* Since more ascomycetes, known to produce cell wall degrading enzymes were seen in *A. indica* there may be a greater opportunity for the rhizobia to infect the root hairs and produce a large number of nodules. In *C. pubescens* more zygomycetes occurred that are incapable of producing cell wall degrading enzymes and hence fewer root hairs infected resulting in a lower nodule count. The sterile colonies isolated could even be basidiomycetes that aid in cellulose and lignin decomposition of the host root hair wall.

However, Arora (1954) suggests that in *A. indica* infection of the root is not via the root hair (which in most legumes is the normal portal of infection), but through openings caused by the emergence of lateral roots. This study shows in *A. indica,* in addition to the few large laterals, the presence of a large number of small lateral roots and the nodules formed mostly to subtend these small lateral roots. Hence the higher nodule count could atleast be partly attributed to bacterial entry taking place at the ruptured regions of the root.

Table 1

Growth of tap root and nodulation in *Aeschynomene indica* and *Centrosema pubescens* observed from five different localities in the University premises

Locality	*Aeschynomene indica*		*Centrosema pubescens*	
	*Length of tap root (mm)	*Nodule number	*Length of tap root (mm)	*Nodule number
Botany Department premises	69.7	61	90.0	12
Zoology Department premises	65.0	76	62.7	10
Near Wijewardena Hall	74.7	83	42.7	11
Near WUS Canteen	79.0	73	69.3	10
Near railway track	67.0	65	61.7	12

*Values given are the means of three replicates

Table 2

Lateral root growth and nodulation in *Aeschynomene indica* and *Centrosema pubescens* observed from five different localities in the University premises

Locality	*Aeschynomene indica*			*Centrosema pubescens*		
	*Lateral root number		*Nodule number	*Lateral root number		*Nodule number
	llr	slr		llr	slr	
Botany Department premises	3	89	61	4	-	12
Zoology Department premises	3	72	76	3	-	10
Near Wijewardene Hall	4	76	83	3	-	11
Near WUS Canteen	3	78	73	3	-	10
Near railway track	3	78	65	3	-	12

l/r = large lateral root; s/r = small laterral root

* Values given are the means of three replicates

Table 3

Types of fungi isolated from root segments of *Aeschynomene indica* and *Centrosema pubescens* and their incidence in the host legumes.

Type of fungus	Host Legume	
	Aechynomene indica	*Centrosema pubescens*
Zygomycetes		
Absidia	-	+
Cunninghamella	+	+
Mucorales (unidentified)	-	+
Rhizopus	-	+
Syncephalastrum	+	-
Ascomycetes and Deuteromycetes		
	+	+
Aspergillus sp. I (Black colony)	+	-
Aspergillus sp. II (Yellow olive green colony)	-	+
Aspergillus sp.III (Dark green colony)		
Curvularia	+	+
Fusarium sp. I (white spongy colony)	+	+
Fusarium sp. II (white fast growing colony)	+	-
Fusarium sp.III (white cotton-like colony with large sickle shaped conidia)	-	+
Penicillium sp. I (yellow green colony)	+	-
Penicillium sp.II (dull green colony)	+	+
Phoma	+	+
Trichoderma	+	+
Basidiomycetes		
St. 1 (Sterile colony - white)	+	-
St. 2 (Sterile colony - dark coloured)	+	-
St. 3 (Sterile colony - green grey)	-	+
St. 4 (Sterile colony - light brown)	-	+

+ presence; - absence

Table 4

Fungal isolates of the tap root segments of Aeschynomene indica (replicate plant I)

A = *Aeschynomene indica;* B_1 - Base; M_1 = Middle; A_1 = Apex

Agar plate No.	1	2	3	4	5
AB_1	White spongy colony *Fusarium* sp I	White spongy colony *Fusarium* sp I + Dull green colony with pycnidia *Phoma*	White spongy colony *Fusarium* sp I	White spongy colony *Fusarium* sp I	White spongy colony *Fusarium* sp I
AM_1	Yellow green colony *Penicillium* sp I	White spongy colony *Fusarium* sp I	Yellow green colony *Penicillium* sp I	No growth	No growth
AA_1	White spongy colony *Fusarium* sp I	White spongy colony *Fusarium* sp I	Dull green colony with pycnidia *Phoma*	White spongy colony *Fusarium* sp I	White spongy colony *Fusarium* sp I

Table 5

Fungal isolates of the tap root segments of Centrosema pubescens (replicate plant 2)

C = *Centrosema pubescens*; B_2 = Base; M_2 = Middle; A_2 = Apex

Agar plate No.	1	2	3	4	5
CB_2	White colony with erect hyphae *Cunninghamella*	White colony with erect hyphae *Cunninghamella*	White colony with black sporangial heads *Rhizopus*	White colony with erect hyphae *Cunninghamella*	White colony with erect hyphae *Cunninghamella*
CM_2	Green colony *Trichoderma*	White cotton like colony with large sickle shaped conidia *Fusarium* sp III	White cotton like colony with large sickle shaped conidia *Fusarium* sp.III	Green colony *Trichoderma*	Green colony *Trichoderma*
CA_2	White colony with zygospore formation *Mucorales*	Dull green colony with pycnidia *Phoma*	Green colony *Trichoderma*	No growth	Dull green colony *Penicillium* sp.II

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