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OCCURRENCE OF ARBUSCULAR MYCORRHIZAL FUNGI IN RUBBER GROWING SOILS AND THEIR EFFECT ON GROWTH OF *HEVEA BRASILIENSIS* SEEDLINGS

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ABSTRACT

Occurrence of mycorrhizal spores in rubber (*Hevea brasiliensis*) growing soils and colonisation in roots of mature rubber plants from 16 locations were studied. Spore population varied from 214 to 428 in 50 g of soil. The common genera of AM fungi included *Glomus, Gigaspora, Acaulospora* and *Sclerocystis*. The AM infection in rubber roots ranged from 67 to 92%. Inoculation of rubber seedlings with eleven commonly occurring AM isolates at different levels of rock phosphate application under sterile condition showed that effect of AM on plant growth varied with different isolates and with different P levels. Most of the isolates gave significant growth improvement of *Hevea* seedlings at lower P levels. Combined inoculation of seedlings with two AM isolates *i.e., Glomus* sp and *Acaulospora* sp in unsterile soil gave better response at low levels of RP during the early stages of growth than their separate inoculation. AM colonisation was reduced when full dose of P was applied.

INTRODUCTION

Plant roots are known to be intimately associated with mycorrhizal fungi. The mycorrhizal fungi are important in sustainable agriculture due to their role in plant and soil nutrition, acting as agents in transporting mineral nutrients to the plant and C compounds to the soil and its biota (Reid, 1990). These fungi are known to occur in all soil types and to enhance the uptake of diffusion-limited nutrients such as phosphorus (P) in soils with low availability in spite of their high P retention capacity. Arbuscular mycorrhizal (AM) fungi are not host specific. They differ in their ability to enhance nutrient uptake and plant growth (Mallesha *et al.*, 1994, Joseph, *et al.*, 1998). Rubber (*Hevea brasiliensis*) growing soils are generally acidic and are poor in available P. Mycorrhizal fungi may have an important role in the P nutrition of rubber plants. The objective of the present study was to assay the mycorrhizal status of rubber growing soils and determine the role of different AM isolates on growth of *Hevea* seedlings.

MATERIALS AND METHODS

Distribution and isolation of AM

Soil samples and rubber roots were collected from sixteen-rubber growing locations (Table 1) to study the natural distribution of AM fungi. Top soil (up to 15 cm) and feeder roots from mature rubber trees were collected from 20 sites in each location, pooled and homogenised. Representative samples were packed in polythene bags, labelled and stored at 2°C till processing.

The spores were collected from the soil samples by wet sieving and decanting (Gerdemann and Nicolson, 1963) and each spore type counted by observing their morphology under a stereoscopic microscope.

Different spore types were isolated and multiplied using *Sorghum bicolor* as host plant in sterilized soil (Nicolson, 1967).

The rubber roots were cut into 1 cm bits and stained (Phillips and Hayman, 1970). The root segment was considered mycorrhizal even if any one of the three structures *i.e.*, hyphae, arbuscules or vesicles was present and per cent mycorrhizal infection was calculated.

Effect of AM on growth of rubber seedlings

An experiment was set up in factorial completely randomised design using polybags (25 x 45) cm containing sterilized soil. The treatment combinations included two factors i.e., AM isolates and P levels replicated thrice. Eleven AM isolates collected from rubber growing soils and maintained in *Sorghum* seedlings were used for the study. Mycorrhizal inocula (100g) comprising of spores, infested soil and chopped mycorrhizal root fragments was added in each bag containing sterile soil. Rubber seedlings raised in sterilised sand for 15 days having uniform growth were transplanted to the polybags plants grown in

Table 1. AM infection in rubber and spore distribution in rubber growing soils

Location	AM root infection (%)					Spore count/ 50 g soil	Per cent distribution of spores
	Glomus spp	*Acaulospora* spp	*Sclerocystis* spp	*Gigaspora* spp	Unidentified		
Nagercoil	88	407	75	10	10	2	3
Trivandrum	79	374	72	13	8	1	6
Punalur	81	413	70	12	10	2	6
Kodumon	80	326	76	10	8	2	4
Chethackal	78	258	72	15	8	2	5
Kottayam	76	214	66	22	8	2	2
Mundakayam	92	369	79	12	7	1	1
Thodupuzha	84	344	68	20	8	2	2
Kalady	79	274	74	10	10	3	3
Pudukkad	88	314	70	14	12	1	3
Palakkad	84	428	82	10	4	2	2
Pullengode	74	230	76	10	11	1	2
Kinalur	88	318	79	11	7	1	2
Kalpetta	84	330	70	15	10	2	3
Iritty	67	296	77	12	6	3	2
Mangalore	70	262	74	13	7	2	4

uninoculated soil in polybags served as control. Phosphate in the form of rock phosphate (RP) was applied at three levels (50, 75 and 100%) of the recommended dose. Other fertilisers were applied as per recommendations. Plant growth was recorded after one year.

Two AM isolates *viz. Glomus* sp and *Acaulospora* sp which induced maximum growth response in rubber seedlings under sterile condition were selected and the experiment was repeated using unsterile soil in pots (30x 30 cm). The treatment included single and combined inoculation of the AM isolates at the three levels of phosphate application. Each treatment had 3 replications. The growth of plants and AM infestation was recorded after 5 and 12 months.

RESULTS

Distribution and isolation of AM

The results of the survey of AM population in soils from different rubber growing regions (Table 1) showed that all the soils contained AM fungi. Maximum spore population was recorded in the soil from Palakkad and the minimum population in the soil from Kottayam. Population of *Glomus* spp was found to be high in all the soils studied, followed by *Acaulospora* spp while that of *Gigaspora* spp was very low. Among the different soils, those from Palakkad contained more *Glomus* spp. Population of *Acaulospora* spp was more in soils from Kottayam.

Roots of *H.brasiliensis* collected form all the locations under the study were colonised by AM fungi (Fig.3). The mycorrhizal infection in the feeder roots of mature rubber plants varied from 67 to 92%. Roots from Mundakayam showed highest AM infection. There was no relationship between spore count from soil and AM colonization in the roots.

The types of mycorrhizal isolates commonly observed were (Fig 1&2),

1. *Acaulospora* sp.1.

Spores are globose with smooth surface measuring an average diameter of 40µm. Wall layers are hyaline, composite wall thickness is about 2 µm. Spores are colourless, attached to empty mother veside.

2. *Acaulospora* sp. 2

Spores are globose and honey brown in colour longest dimension at maturity is 400 mm, attached to empty mother vesicle. Surface ornamentations of the spore is reticulate. Spore wall thickness is about 20µm.

3. *Acaulospora* sp. 3

Spores are globose to oval, developed laterally on hyphae below apical swelling and with maturity of the spore the stalk wall collapses. Mature ones have the longest measure of 100 – 120µm. Spores are orange-yellow in colour. Spore wall is brownish yellow with a thickness of less than 10µm.

4. *Gigaspora* sp. 1

Spores are globose, longest dimension at maturity is 300 – 350µm. double wall layers, outer thicker than inner. Hyphae with bulbous attachment with a base width of 30µm. Spore wall has a thickness of 20µm and their surface is smooth, yellow to orange coloured. Bulbous base of the hyphae show filiform attachment.

5. *Glomus* sp. 1

Spores are oval to ellipsoid, golden yellow to orange, born singly with a straight and cylindrical hyphal attachment, thickned at the base. Spores have a length of 100-120µm and width of 60-70µm, with wall thickness 3-4µm.

6. *Glomus* sp. 2

Spores are globose to oval, measuring 40-60µm, surface smooth, orange brown coloured borne singly. Subtending hyphae are cylindrical and constricted at the point of attachment.

7. *Glomus* sp. 3

Globose spores, yellow, measuring 40-50µm, smooth walled, single with subtending hyphae cylindrical and straight. Hyphal closure at spore wall is by a hyphal thickening. Spore wall thickness is 1-4µm

8. *Glomus* sp. 4

Spores are globose to oval, measuring 175 – 225 µm, with smooth surface. Spore wall is brown with a thickness of 18-22 µm. Subtending hyphae two or more, cylindrical yellowish brown, with the hyphal attachment curved along the spore surface.

9. *Glomus* sp. 5

Spores are globose, measuring 130-150µm at maturity single subtending hyphae. Spore surface is smooth with wall thickness of 5-6 µm and develop singly or in groups. Hyphal diameter at the point of attachment is 15-20µm.

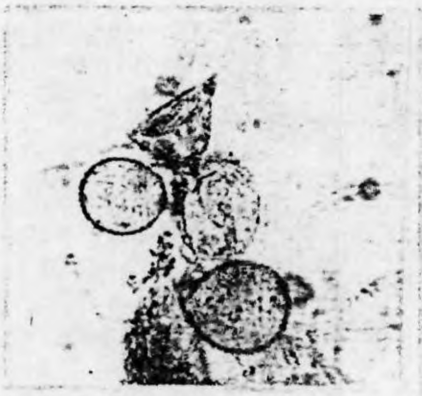
10. *Glomus* sp. 6

Spores are globose, measuring 80-90µm honey brown in colour, with a smooth surface, wall thickness being 10 – 20µm. Subtending hyphae single and straight.

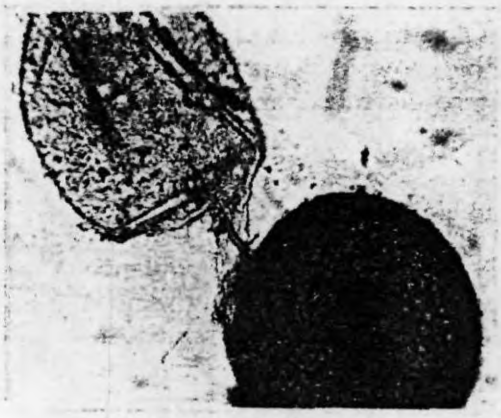
11. *Sclerocytis* sp. 1

Spores are clavate with straight sides and exposed spore tips. Each spore has a length of 30-40µm and width of 20-30µm. Numerous spores arranged radially to form a sporocarp measuring size 200 – 300µm, honey brown in colour.

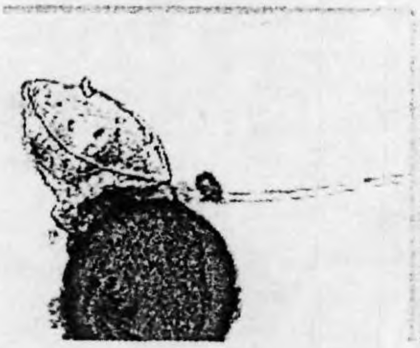
Fig. 1. AM spores found in rubber plantations



Acaulospora sp 1

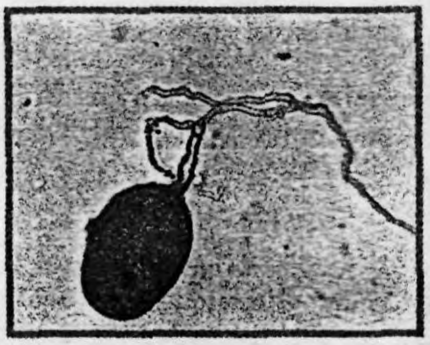


Acaulospora sp 2

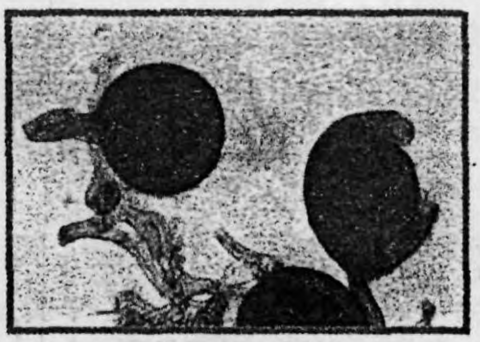


Acaulospora sp 3

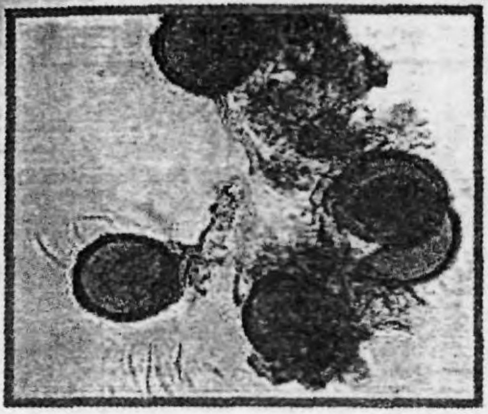
Gigaspora sp 1



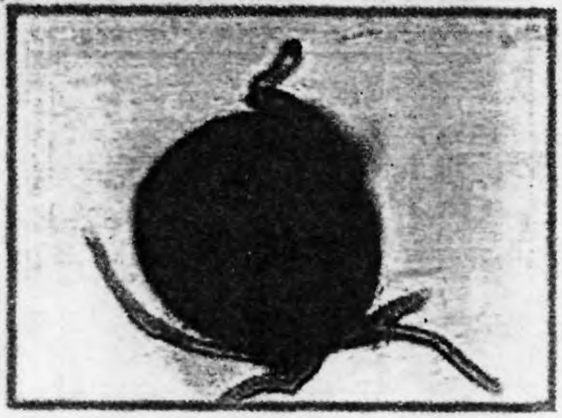
Glomus sp 1



Glomus sp 2



Glomus sp 3



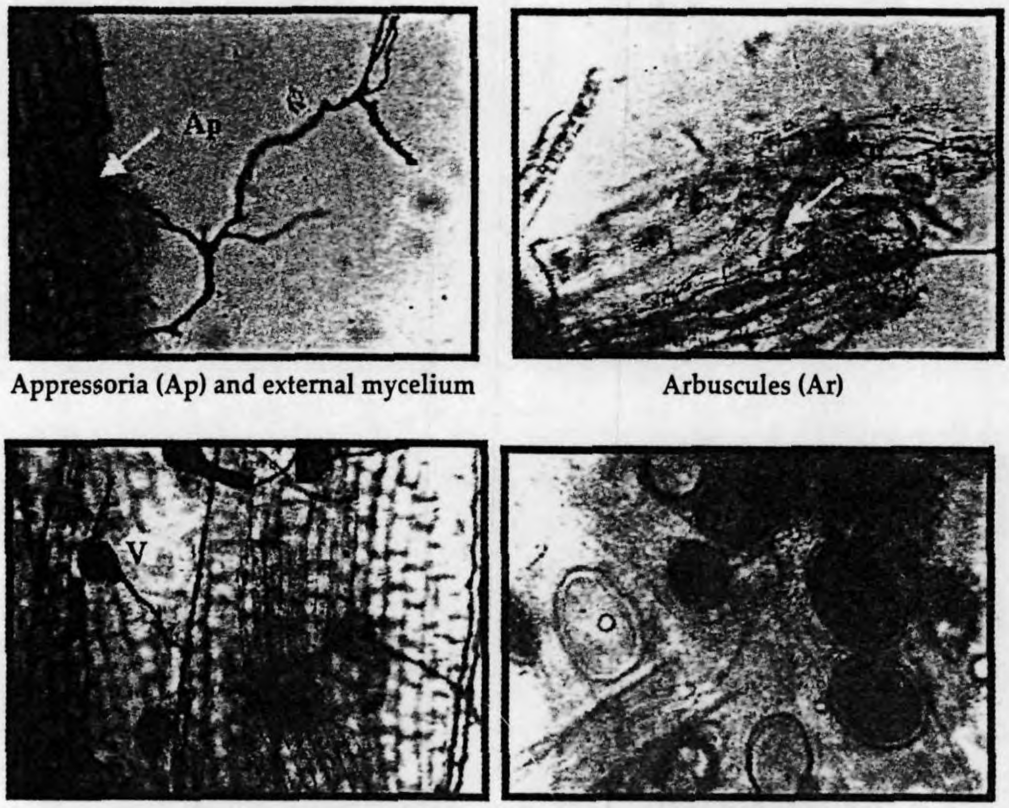
Glomus sp 4

Glomus sp 5

Glomus sp 6

Sclerocytis sp 1

Fig. 2. AM infection in rubber



Appressoria (Ap) and external mycelium **Arbuscules (Ar)**

Vesicles (V) **Spore formation**

Effect of AM inoculation on rubber seedling

The growth response of rubber seedlings to inoculation with eleven AM isolates under sterile condition, compared to uninoculated control is given in Table 2. The response of rubber seedlings to AM inoculation varied with isolates and also with RP levels. At 50% of the recommended level of phosphate, inoculation with 10 AM isolates resulted in more height while 5 isolates gave better girth of plants. At 75% level of phosphate application inoculation with 8 AM isolates gave more height and 3 isolates gave better girth compared to control and at 100% RP level inoculation with 6 isolates gave improved height and 2 isolates induced more girth than control plants. The girth and height of the plants in majority of the treatments (inoculation with AM along with lower doses of RP) was comparable to that of control with full dose of phosphate application. Highest growth response of the plants was shown by plants inoculated with *Glomus* sp 1 at 75 % RP followed by *Acaulospora* sp 2.

Based on the growth response of due to inoculation with different AM isolates, *Acaulosopra* sp 2 and *Glomus* sp 1 were chosen for further study at different levels of RP application using unsterile soil. The results of single and combined inoculation of these two AM species after 5 and 12 month of growth of rubber seedlings are given in Table 3. Data shows that in 5 months old plants, combined inoculation with the two species of AM gave better growth of the plants in terms of girth and height compared to single inoculation at lower dose of RP application, and also to uninoculated plants with full dose of P fertiliser. Single inoculation with *Acaulospora* sp or *Glomus* sp did not show much difference between them in growth response of rubber seedlings but gave better growth compared to uninoculated control which received full dose of P fertiliser. By 5 months the inoculated plants showed better AM root colonisation compared to natural colonisation in the control plants. At the age of 12 months plants inoculated with

Acaulospora sp alone showed comparable growth to that of plants inoculated with both the AM isolates at 50 per cent and 75 per cent RP application but at 100 % RP levels growth response was less and comparable to that in control plants. Height and Girth of the plants inoculated with *Glomus* sp along with 50% RP was comparable to uninoculated plants with full dose of fertilisers but at 75 % RP level, it gave better growth response. Mycorrhizal root infection was found to be reduced upon their inoculation either singly or in combination under full dose of RP application. This study also showed a positive relationship between AM colonisation and plant growth.

DISCUSSION

AM association in *H. brasiliensis* and their role in plant growth are well established (Ikram *et al.*, 1996). However there exists wide variation in the degree of AM colonisation in roots and distribution of AM spores in soil (Hayman, 1978). In the present study also such variations were recorded. All the 16 soil samples showed considerable variation in spore population and species distribution of AM fungi. *Glomus* spp was comparatively more in all the soils. Rekha rani and Mukerji, (1987) have reported that Indian soils are rich in *Glomus* spp.

Mycorrhizal colonisation in mature rubber trees was also found to vary from 67 to 92 % in different rubber growing soils. Under field condition, AM development and spore production are influenced by the species involved, host plants under consideration, as well as edaphic and environmental factors. AM species vary considerably in their efficiency to infect and influence plant growth (Carling and Brown, 1980). Earlier studies also indicate that spore counts of AM fungi in mature ecosystem are not regularly correlated with the abundance of AM root infection (Moose and Brown 1968). The comparative evaluation of the efficiency of different AM fungi on *Hevea* seedlings revealed that all the AM fungi tested, promoted the growth of the plants. Some species of AM were more efficient in increasing the plant growth under sterile and unsterile conditions showing the importance of AM in the establishment of *Hevea*.

Table 2. Effect of AM inoculation on rubber seedlings at different P levels in sterile soil Height (cm) and girth (mm) after 12 months

Treatment	50% RP		75% RP		100 % RP	
	Height	Girth	Height	Girth	Height	Girth
Inoculation with						
Acaulospora sp 1	96.66	191.01	184.02	180.02	201.03	176.66
Acaulospora sp 2	165.01	171.66	182.02	124.02	202.03	158.03
Acaulospora sp 3	116.33	172.66	195.66	167.02	158.03	171.03
Gigaspora sp1	138.66	162.01	160.02	145.02	162.03	129.03
Glomus sp 1	137.01	131.33	174.66	154.66	181.03	156.33
Glomus sp 2	153.66	102.01	135.33	142.02	150.66	151.33
Glomus sp 3	11.01	18.33	18.66	19.33	19.03	21.03
Glomus sp 4	18.01	18.01	15.66	10.66	13.33	16.33
Glomus sp 5	11.66	19.33	22.33	16.66	17.03	14.03
Glomus sp 6	16.01	17.01	19.66	14.33	17.66	13.33
Sclerocystis	13.66	13.66	14.33	15.33	17.03	16.33
Uninoculated control	15.33	13.01	14.66	15.2	15.66	15.33

CD interaction (P=0.05) (height) - 9.11
CD interaction (P=0.05) (girth) - 3.60

Table 3. Effect of mycorrhizal inoculation on growth and infection of rubber seedlings at different levels of RP application in unsterile soil

Treatment	Plant growth and root infection of mycorrhizae					
	5 months			12 months		
	Height (cm)	Girth (mm)	Root infection %	Height (cm)	Girth (mm)	Root infection %
Accaulospora sp+ *Glomus* sp+ 50% RP	70.00	5.33	64	125.67	11.67	96
Accaulospora sp+ *Glomus* sp+ 75% RP	82.00	7.33	68	146.67	14.00	93
Accaulospora sp+ *Glomus* sp+ 100% RP	60.33	5.67	58	88.33	9.00	76
Accaulospora sp alone + 50% RP	55.33	4.67	48	110.00	9.33	80
Accaulospora sp alone + 75% RP	55.00	4.67	50	152.33	15.00	90
Accaulospora sp alone + 100% RP	57.33	5.00	54	84.67	10.00	63
Glomus sp alone + 50% RP	54.67	4.67	60	99.00	9.67	86
Glomus sp alone + 75% RP	59.33	5.33	64	112.67	11.00	83
Glomus sp alone + 100% RP	58.33	5.67	60	90.00	8.33	66
Uninoculated + 100% RP	46.07	4.00	42	79.67	7.67	68
CD (P≤0.05)	10.43	1.49	8	25.54	3.00	19

A root system is typically colonised by more than one AM fungal species, and success in occupancy is not necessarily related to host response. The response range may be due to changing efficiencies of different endophytes during the growing season, to varying uptake and exclusion capabilities of different fungi for different elements or even to changes in the soil environment itself during the season (Bethlenfalvay, 1992). Multiple colonisations by mixed inocula containing AM fungi with different symbiotic strategies might therefore reduce variation in host response with more consistent benefit to host plant (Daft, 1983). In the present study also combined inoculation of two AM isolates, *Glomus* sp and *Acaulospora* resulted in better response of *Hevea* in the early stages of growth. However this advantage of mixed inoculation narrowed with the growth of plants.

The study also showed that inoculation with most of the AM isolates gave significant growth improvement of rubber seedlings when the RP levels were low. AM colonisation in the rubber seedlings get reduced when full dose of phosphate was given. Earlier studies report an inverse relationship between root colonisation and P content in soil (Schwab *et al.*, 1983). The species difference of AM fungi in promoting the growth of plants at different levels of P has been reported (Plenchette, 1981).

Plants infected with AM are known to be more effective in the uptake of P from rock phosphate and soils low in available P (Mosse, 1973). Since the available P in the rubber growing soils of India is very low, mycorrhizae might be playing a crucial role in plant P nutrition of *Hevea*.

REFERENCES

Bethlenfalvay, G. T. 1992. Mycorrhizae in Sustainable Agriculture. (Eds.G. J. Bethlenfalvay and R.G.Linderman). American Society of Agronomy, Special Publication, Madison, Wisconsin, USA, No.54, pp 1-27.

Carling, D. E. and Brown, M. F. 1980. Relative effect of vesicular – arbuscular mycorrhizal fungi on the growth and yield of soybeans. – *Soil Science Soceity of America. Journal*, **44**: 528 -532

Draft, M. J. 1983. The influence of mixed inocula on endomycorrhizal develop-ment. *Plant and Soil*, **71**: 331 – 337

Gerdemann, J. W. and Nicolson, T. H. 1963. Spores of mycorrhizal Endogone Species extracted from soil by wet – sieving and decanting. – Transactions of the British Mycological Society, **46**:235 – 44.

Hayman, D. S. 1978. Endomycorrhizae. In: Interaction Between Non Pathogenic Soil Microorganisms and Plants. (Eds. Y. R. Dommergues and S.V. Krupa) Elsevier, Amsterdam, pp. 401-42.

Ikram, A., Sudin, M. N. and Napi. D. 1996. Phosphate response curve of mycorrhizal *Hevea brasiliensis* in two sterilised soils. *Journal of Natural Rubber Research*, **11(1)**: 59-68.

Joseph, K., Kothandaraman, R. and Mathew, J. 1999. A comparative study of different isolates of VAM on growth, nutrient uptake and biochemical constituents of *Pueraria phaseoloides* Benth. Presented in National Conference on mycorrhiza, Barkatulla University, Bhopal, March, 1991.

Mallesha, B. C., Ravindra, T. P. and Bagyaraj, D. J. 1994. Response of tomato to different AM mycorrrhizal fungi. *Journal of Soil Biology and Ecology*, **14(1)**: 36-40.

Mosse, B. 1973. Advances in the study of vesicular-arbuscular mycorrhiza. Annual Review of Phytopathology, **11**:171-96.

Mosse, B. and Bowen, G.D. 1968. The distribution of Endogone spores in some Australian and New Zealand soil at Rothamsted. Transaction of the British Mycological Society, **51**: 485-92.

Nicolson, 1967. Vesicular-arbuscular mycorrhiza. A universal plant symbiosis. Science Progress, **55**: 561-81

Phillips, J.M. and Hayman, D.S. 1970. Improved procedure for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Transactions of the British Mycological Society **55**: 158-61

Plenchette. C. S., Furlan, V. and Fortin, J. A. 1981. Growth stimulation of apple trees in unsterilised soil under field conditions with VA mycorrhiza inoculation. *Canadian Journal of Botany*, **59**:2003-08.

Reid, C.P.P. 1990. Mycorrhizas. In: The rhizosphere (Ed.Lynch, J.M.). John Wiley and sons, New York.

Rekha Rani and Mukerji, K. G. 1987. Indian vesicular-arbuscular fungi. *In:* Mycorrhiza Round Table. Proceedings of a National workshop. (Eds. A.K.Varma, A,K.Oka, K.G.Mukerji, K.V.B.R.Tilak and Janak Raj). IDRC, New Delhi, pp 166-80.

Schwab, S. M., Menge, J. A. and Leonard, R. T. 1983. Comparison of stages of vesicular-arbuscular mycorrhiza formation in sudan grass grown at two levels of phosphorus nutrition. *American Journal of Botany*, **70**: 1225-32

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