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Seasonal variation in the association of arbuscular mycorrhizae with mulberry (*Morus alba* L.)

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A study was undertaken during 1996 to 1998 to assess the rate of inherent colonization of arbuscular mycorrhizal fungi, per cent of arbuscules and vesicles in root and the endomycorrhizal spore population in rhizosphere soil of six high yielding mulberry varieties at various soil moisture levels in field. Besides, the isolation and indentification of predominant strains of AM fungi were also the primary objectives. A wide range of variations in all the parameters was found irrespective of seasons, soil moisture levels, varieties and variety x season interaction. Maximum root colonization (72%), arbuscule (17%), vesicle (19%) and arbuscule with vesicle (50%) formation were observed. The highest population of spores (13 spores/g rhizosphere soil) was found at low moisture level during summer followed by winter season. *Glomus* was found to be the predominant genus in all the mulberry varieties. The study, thus provides a scope for increasing spore population in field soil through artificial inoculation of an efficient strain of local endophyte such as *Glomus* sp., to exploit commercially the arbuscular mycorrhizae for their beneficial effects in terms of improvement in quantity and quality leaf production in mulberry at reduced doses of phosphorus especially under tropical condition.

Key words : Arbuscular mycorrhizae. *Glomus* spp. colonization, endomycorrhizal spores, mulberry

INTRODUCTION

Arbuscular mycorrhizal (AM) fungi occur in most plant families (Gerdemann, 1968). Many plants cannot grow adequately without AM fungi, especially in phosphate-deficient soils (Gerdemann, 1975). Arbuscular mycorrhizae are capable of solubilizing the non-avaliable form of phosphate and make shortened the diffused path for its transport by enhancing the mycelial length and thus favoured growth and yeild of crop plants (Nye and Tinker, 1977; Sulochana and Manoharachary, 1989) at reduced dose of inorganic phosphorus under rainfed condition (Setua *et al.*, 1999a).

The intensity of colonization and density of spores varies with changes of season (Giovannetti and Mosse, 1980; Koske, 1981; Bhaskaran and Selvaraj, 1997; Kabir *et al.*, 1997; Allen *et al.*, 1998; Merryweather and Fitter, 1998). Mulberry (*Bombyx mori* L.) the only food plant of silkworn, was also found to be associated with arbuscular mycorrhizae in some varieties under specific agroclimatic conditions (Katiyar *et al.*, 1989; Rajagopal *et al.*, 1989). A number of high yielding mulberry varieties have been developed by CSR & TI, Berhampore to fit different agro-climatic regions of central, eastern and north-eastern regions of India. No information is available on natural arbuscular mycorrhizal associations, spore densitres and seasonal behaviour of these recommended mulberry varieties. The aim of the present work is to study the rate of natural colonization of AM fungi, per cent of arbuscules and vesicles contained in the root and endomycorrhizal spore population of high yielding mulberry varieties (HYVS) at various moisture levels in different seasons in the field. Isolation and identification of the predominant strains of AM fungi from these fields will be made with a view to exploit their beneficial effects through artificial inoculation of the efficient strain under reduced doses of phosphorus.

MATERIALS AND METHODS

Six high yeilding mulberry varieties i.e., S_1, S_{1635}, C_{763}, C_{776}, BC_259 and Tr_{10} were selected for the study. The plants were maintained under recommended practices at 60 × 60 cm spacing in row system with irrigating condition (Ullal and Narasimhanna, 1987). Initial soil status of the field such as organic carbon, pH and moisture holding capacity were estimated folowing Schofield and Taylor (1955) and Black (1965) respectively. Nutrient status of soil was determined by the method of Jackson (1973). Soil moisture was measured by a gravimetric method while soil temperature was determined by soil thermometer. Fine roots along with 500 g rhizosphere soil in each mulberry variety in five replications were randomly collected in polyethylene bags before irrigation during three seasons (summer, rainy and winter i.e. in May, August and January respectively). The roots were washed with tap water and cut into pieces at about 1 cm length each. The root-lets were processed and AM fungal colonization in root was estimated by the method of Phillips and Hayman (1970) using Lacto-glycerol. Percentage of root colonization was calculated by the root slide technique (Nicolson, 1960) on the basis of microscopic study of 100 rootlets in each replication considering percentage of arbuscules, vesicles and both arbuscules and vesicles in each rootlet.

Spore density of AM fungi per 5 g of rhizosphere soil was measured by the wet sieving and decanting technique (Gerdemann and Nicolson, 1983) and were observed under microscope. The data were recorded following the classification of Morton (1988) which were further cofirmed and identified upto species level by the courtesy of Tata Energy Research Institute (TERI), New Delhi. The observation were recorded for two years during 1996 to 1998 and statistically analysed. The average data of two years have been presented.

RESULTS

The soil was mild alkaline (pH 6.92-8.01) with 46% moisture holding capacity and 0.543% organic carbon content. Available N, P_2O_5 and K_2O were estimated as 202, 25 and 340 kg/ha respectively.

A wide range of variation existed in root colonization per cent, content of arbuscules, vesicles, arbuscule with vesicle per cent and also in the endomycorrhizal spore population which were found to be statistically significant in respect of varieties, seasons and variety x season interaction study except the arbuscule per cent (Table 1).

Out of six HYVs of mulberry, maximum root colonization was observed in Tr_{10} and BC_259 during summer and winter seasons respectively while BC_259 showed moderate colonization followed by S_1 during rainy season (Table 1).

With regard to arbuscule and vesicle formation, it was recorded highest in Tr_{10} during summer while lowest in the same variety during rainy season. In case of winter season, though maximum per cent of arbuscule was found in Tr_{10} but vesicle per cent remained highest in BC_259 (Table 1).

It was further observed that the maximum percent of arbuscule alongwith vesicle was found in Tr_{10} followed by BC_259. In case of S_{1635}, C_{763} and C_{776} varieties, though the per cent of root colonization was found to be fairly satisfactory except rainly season but lower per cent of arbuscule, vesicle and arbuscule with vesicle were recorded.

The highest population (65) of endomycorrhizal spores per 5 g rhizosphere soil was observed in S_1 which was significantly higher over others followed by C_{776} (47.5) during rainy rainy summer and in BC_259 (61.0) followed by C_{776} (57.5) during winter season. Least spore population was observed during rainy season in Tr_{10} being the maximum (33.0) followed by S_1 (28.0). The study further indicated that most of the arbuscular mycorrhizae as isolated from the rhizosphere soil of different mulberry varieties belonged to the genus *Glomus* which was found to be predominant. Besides low incidence of some other genera like *Acaulospora*, *Gigaspora* and *Sclerocystis* were also observed. For confirmation, the roots and rhizosphere soil samples of S_{1635} and Tr_{10} mulberry varieties were sent to TERI and was identified upto species level as indicated below :

1. From S_{1635} :

(i) *Glomus fasciculatum* : constitutes the dominant species in the sample. Spores 60 (50–)-95 (–149) μm, globose to sub-globose, pale yelllow to very pale yellow-brown. The subtending hypha is persistent and straight. The walls often minutely perforated with thickened inward projections.

(ii) *Glomus mosseae* : spores yellow to brown, globose to ovoid, 105-310 μm, with one or occasionally two funnel-shaped bases, divided from subtending hyphae by a curved septum.

2. From Tr_{10} :

(i) *Glomus fasciculatum* : constitute the dominant species in the sample as described in 1(i)

(ii) *Glomus intraradices* : spores predominantly globose, 40.5– 98.5 (–109.5) μm, yellow to grey-brown. Walls of the spore extending into the hyphal attachment formimg an apparent tubaeform flare at the juncture with the hyphal attachment.

DISCUSSION

The study indicated a wide range of variation in colonization and endomycorrhizal spore population among the different high yielding mulberry varieties which might be due to the effect of rhizosphere soil of mulberry that favoured growth of AM fungi. Besides, the available phosphorus (25 kg ha^{-1}) and carbohydrate (org.C, 0.543%) content in the rhizosphere soil and the roots of mulberry varieties may have a major role for such variations. In addition, pH of soil may also play a role but it was not to be effective in the present study as the pH remained within narrow range of 6.92–8.01. Moisture content of rhizosphere soil (ranged from 9.1% during summer, 18.7% during winter and 26.2% during rainy season) and soil temperature (ranged from

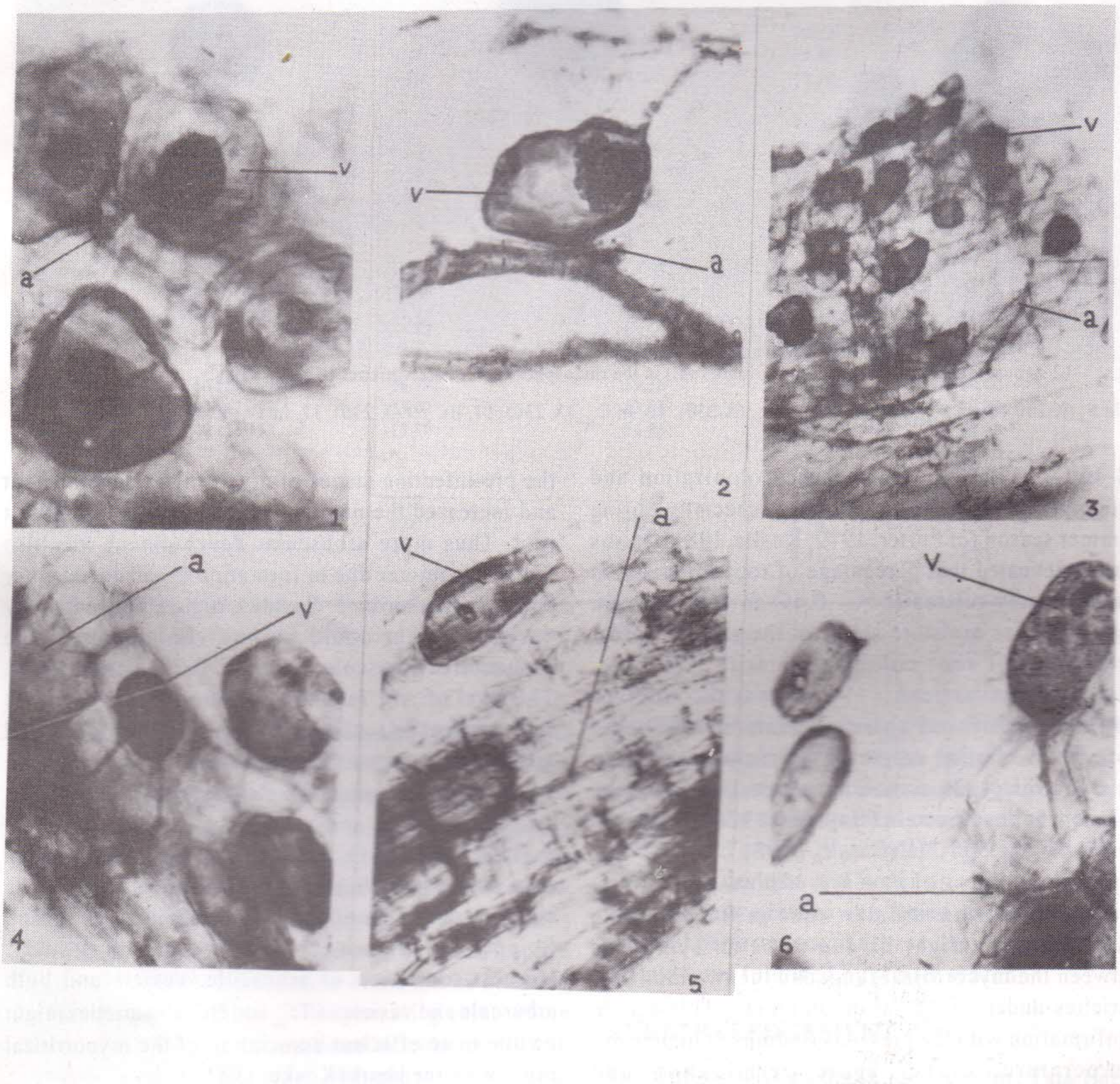
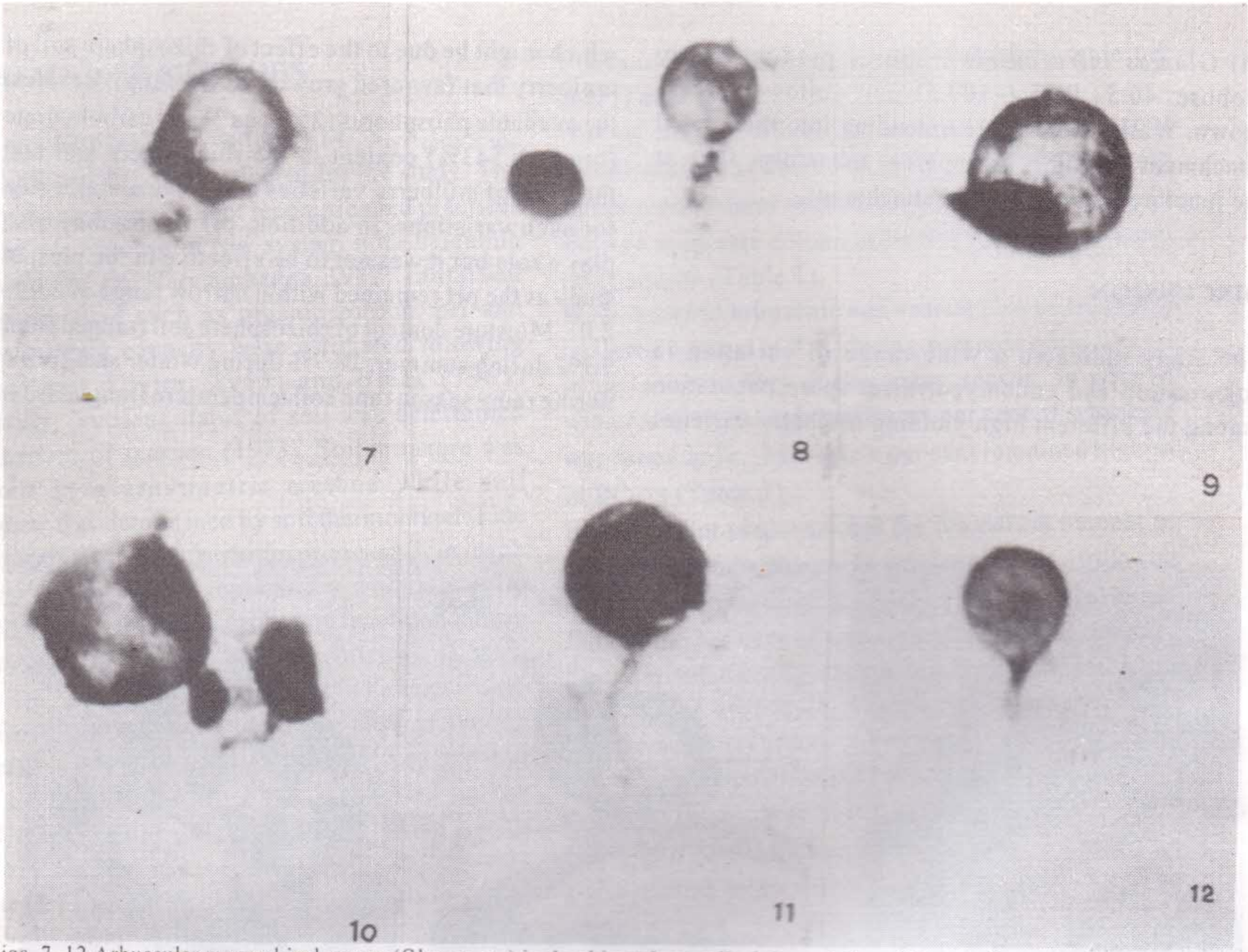


Fig. 1–6 Arbuscular mycorrhizal association showing arbuscules (a) and vesicles (v) in the roots of six varieties of Morus alba.
1, In S_1 (X 2250); 2, In S_{1635} (X 2250); 3, In C_{763} (X 2250): 5, In $BC_2$59 (X 2250); 6, In Tr_{10} (X 2250).



Figs. 7–12 Arbuscular mycorrhizal spores (Glomus sp.) in the rhizosphere soil of six varieties of Morus alba.
7, In S_1 (X 230); 8. In S_{1635} (X 210); 9. In C_{763} (X 230); 10. In C_{776} (X 230); 11. BC_259 (X 230). 12. In Tr_{10} (X210).

28-40°C) might have influenced colonization and endomycorrhizal spore population especially during summer season (cf Porter, 1979; Koske, 1981). It was further revealed that percentage of root colonization was inversely related (r = – 0.45) to soil moisture regime, i.e. as moisture status in the soil increased, percentage of root colonization with AM fungi decreased. Similar trend (r = – 0.44) was also observed for endomycorrhizal spore population. Both these observations further supported the seasonal influence on mycorrhizal association in root periphery and the subsequent development (Hayman, 1970; Bhaskaran and Selvaraj, 1997; Allen *et al.*, 1998)

Maximum number of endomycorrhizal spores were found in S_1 during summer while in BC_259 during winter season, might be due to better symbiosis between the mycorrhizal fungi and the two mulberry varieties under congenial environment. This was in confirmation with the previous findings of higher soil temperature, higher spore germination and colonization during summer over winter (Hayman, 1974; Koske, 1981; Kabit *et al.*, 1997; Merryweather and Fitter, 1998). Further higher temperature affected the pre-infection stages of mycorrhizal development and increased the number of entry points in the host root. Thus more arbuscular development was also found in summer due to formation of enhanced entry points (appresorium). Besides, higher light intensity during summer could be one vital factor in the formation of arbuscule (Hayman, 1974; Koske, 1981). The level of AM fungal association was reported to be dependent on root morphology, metabolism as well as rate of plant growth (Warner and Mosse, 1980). In addition, the colonization also depended on specific soil plant system and substances exuded from host roots (Koske, 1981). Rate of root colonization which may not be statistically significant even among the cultivars of the same species as reported by Smith and Skipper (1979), was also observed in this study. Highest formation of arbuscule, vesicle and both arbuscule and vesicle in Tr_{10} and BC_259 varieties might be due to an efficient association of the mycorrhizal fungi with the hosts (Koske, 1981).

Natural symbiotic association of arbuscular mycorrhiza existed in all the six high yielding mulberry varieties at different levels which was in conformity

Table 1. Effect of seasonal variation on colonization and spore density of VAM in High Yielding Varieties of mulberry

Varieties	Total % of root segment colonization	Per cent of colonized root segments containing			No. of endomycorrhizal spores/5 g rhizosphere soil
		Arbuscules	Vesicles	Arbuscules + Vesicles	
S_1					
S	58.60 (49.97)	10.70	10.78	31.46	65.00
R	54.40 (46.95)	8.80	12.02	28.70	28.00
W	60.00 (50.77)	12.02	13.82	38.40	54.00
S_{1635}					
S	58.00 (49.62)	7.58	6.46	18.68	33.00
R	31.80 (34.31)	5.40	4.88	9.76	27.00
W	42.40 (40.63)	7.20	6.18	21.06	37.00
C_{763}					
S	42.60 (40.74)	9.48	8.70	19.30	31.00
R	23.80 (29.14)	6.84	5.90	10.60	26.00
W	39.20 (38.75)	6.66	7.20	17.60	48.00
C_{776}					
S	39.40 (38.87)	6.18	6.12	17.18	47.50
R	18.60 (25.51)	5.70	5.26	15.14	17.00
W	38.20 (38.16)	6.98	7.26	16.02	57.50
BC_2^{59}					
S	60.20 (50.89)	14.00	13.54	40.42	27.33
R	55.00 (47.87)	12.66	12.50	35.96	21.83
W	60.20 (50.89)	15.24	16.36	38.58	61.00
Tr_{10}					
S	71.60 (57.85)	16.72	19.36	50.00	29.00
R	54.00 (47.30)	13.02	13.86	40.48	33.00
W	69.40 (56.45)	15.70	14.86	49.38	52.00
CD at 5%					
Variety	2.32 (1.41)	1.37	1.45	2.49	2.68
Season	1.64 (1.00)	1.00	1.02	1.76	1.90
Var. × Sea	4.01 (2.44)	NS	2.51	4.31	4.65

( ) Parentheses indicate arcsine transformed data : S – summer, R – rainy, W – winter.

with earlier findings of Gerdemann (1968) in other perennial plants and Katiyar *et al.* (1989) in mulberry. The predominant arbuscular mycorrhizal fungi were isolated and identified upto the species for evaluating their potentially. The degree of colonization and the formation of arbuscules and vesicles in the host cortex were also found to be variable irrespective of seasons and mulberry varieties. Endomycorrhizal spore population and its colonization could not be attended at the satisfactory level (100 spores/5 g rhizosphere soil) in the study. Hence, artificial inoculation with an efficient strain of the local endophytes (AM fungi could be done to make the spore load at a desired level (100 spores/5 g rhizosphere soil) in nursery (Setua *et al.*, 1999a) or in established garden (Setua *et al.*, 1999b) for better exploitation of the beneficial effect of arbuscular mycorrhizal fungi in phosphorus deficient soil especially under tropical condition for increasing quantity and quality of leaf yield, tolerance to disease, nematode, drought and water as also solute uptake. The summer season was found to be the best for efficient performance of arbuscular mycorrhizal fungi at field level followed by winter season.

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REFERENCES

Allen, E.B., Rincon, E., Allen, M.F. Perez-Jimenez A. and Huante, P. (1998). Disturbance and seasonal dynamics of mycorrhizae in a tropical deciduous forest in Mexico. *Biotropical* **30** : 261-274.

Bhaskaran, C. and Selvaraj, T. (1997). Season incidence and distribution of VA-mycorrhizal fungi in native saline soils. *J. Environ Biol* **18** : 209-212.

Black, C.A. (1965). *Methods of soil analysis.* Amer Soc Agron Inc, Madison, Wisconsin, USA.

Gerdemann, J. W. (1968). Vesicular-arbuscular mycorrhiza and plant growth. *Ann. Rev. Phytopath* **6** : 397-418.

Gerdemann, W. (1975). Vesicular-arbuscular mycorrhizae. In : Harvard Forest, Torrey J.G. and Clarkson D. T. (ed). *The Development and Function of Roots.* 3rd Cabot Symposium. Academic Press, London, pp. 575-591.

Gerdemann, J. W. and Nicolson, T. H. (1963). Spores of mycorrhizal *Endogone* spp. extracted from soil by wet-sieving and decanting. *Trans. Br. Mycol Soc.* **46** : 235-244.

Giovannetti, M. and Mosse, B. (1980). An evaluation of technique for measuring VAM infection in roots. *New Phytol* **84** : 489-500.

Hayman, D.S. (1970). *Endogone* spores numbers in soil and vesicular-arbuscular mycorrhiza in which as influenced by season and soil treatment. *Trans Br. Mycol Soc.* **54** : 53-63.

Hayman, D.S. (1974). Plant growth responses to vesicular-arbuscular mycorrihiza. VI. Effect of light and temperature. *New Phytol.* **73** : 71-80.

Jackson, M. L. (1973). *Soil chemical analysis.* pp. 326-338. Prentice Hall India Pvt Ltd, New Delhi India.

Kabir, Z. Ohalloran, I. P., Fyles, J. W. and Hamel, C. (1997). Seasonal changes of arbuscular mycorrhizal fungi as affected by tillage practices and fertilization : Hyphal density and mycorrhizal root colonization. *Plant soil* **192** : 285-293.

Katiyar, R. S., Das, P.K., Choudhury, P.C., Ghosh, A. and Sengupta, K. (1989). Vesicular-arbuscular mycorrhizal association in mulberry. *Curr. Sci* **20** : 461-464.

Koske, R. E. (1981). A preliminary study of interactions between species of vesicular-arbuscular fungi in a sand dune. *Trans, Br. Mycol Soc.* **76** : 411-416.

Merryweather, J. and Fitter, A. (1998). The arbuscular mycorrhizal fungi of *Hyacinthoides nonscripta.* 11. Seasonal and spatial patterns of fungal populations. *New Phytol.* **138** : 131-142.

Morton, J. B. (1988). Taxonomy of VA mycorrhizal fungi : Classification, Nomenclature and identification. *Mycotaxon.* **32** : 267-324.

Nicolson, T.H. (1960). Mycorrhizae in the Graminae.11 Development in different habitats particularly sand dunes. *Trans Br. Mycol. Soc* **43** : 132-145.

Nye, P. H. and Tinker, P. B. (1977). *Solute movement in the soil-root system.* Blackwell, Oxford.

Phillips, M. and Hayman, D.S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular atbuscular mycorrhizal fungi for rapid assessment of infection. *Trans Br. Mycol. Soc.* **55** : 158-160.

Porter, W.M. (1979). The "most probable number" method of enumerating infective propagules of vesicular-arbuscular mycorrhizal fungi in soil. *Aust. J. Soil Res.* **17** : 515-519.

Rajagopal, D., Madhavendra, S. S. and Jamil, Kaiser, (1989). Occurrence of vesicular-arbuscular mycorrhizal fungi in roots of *Morus alba* L. *Curr. Sci* **58** : 687-689.

Schofield, R. K. and Taylor, A.W. (1955). The measurement of soil *pH. Soil Sci. Soc. Amer Proc.* **19** : 164-167.

Setua, G.C., Kar. R., Ghosh J.K., Das, K.K. and Sen, S.K. (1999a). Influence of arbuscular mycorrhizae on growth. leaf yeild and phosphorus uptake in mulbery (*Morus alba* L.) under rainfed, lateritic soil conditions. *Biol Fertil. Soils* **29** : 98-103.

Setua, G.C. KAr, R. Ghosh J. K. Das N.K. and Saratchandra. B. (1999b). Response of direct inoculation of VAM on growth leaf yeild and phosphorus uptake in mulberry (*Morus alba*). *Indian J. Agric Sci.* **69**(6) : 444-448.

Smith, G. W. and Skipper, H.D. (1979). Comparison of methods to extract spores of vesicular-arbuscular mycorrhizal fungi. *J. soil Sci Amer* **43** : 722-725.

Sulochana, T. and Manoharachary. C, (1989). Vesicular-arbuscular mycorrhizal associations of castor and safflower. *Curr.Sci.* **54** : 459-461.

Ullal, S.R. and Narasimhanna, M.N. (1987). Mulberry cultivation In : Sampath J (ed) *Handbook of practical sericulture.* Central silk Board, Bangalore. pp. 7-32.

Warner, A. and Mosse, B. (1980). Independent spread of vesicular-arbuscular mycorrhizal fungi in soil. *Trans.Bri. Mycol. Soci.* **74** : 407-410.

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