9 PRACTICAL ASPECTS OF ARBUSCULAR MYCORRHIZAL TECHNOLOGY IN ARID REGION

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1. INTRODUCTION

The arid zone of India occupies about 12 per cent of the country's geographical area. The scanty and erratic rainfall, with high temperatures, excessive evapotranspiration and strong winds on sandy landforms, leads to massive soil erosion and soil movement. Drought is a recurring feature of the region. A sharp rise in the human and livestock population, especially during the second half of the twentieth century, has resulted in tremendous pressure on its resources and has accelerated the pace of environmental degradation. Arbuscular Mycorrhizal (AM) fungi can bind soil particles into larger aggregates, necessary for a stable and porous physical structure of soil. This is particularly important in dry areas, where aggregation of particles permits more efficient use of available water. The fungi can, therefore, play an important role in stabilizing sand dunes and establishment of shelterbelts.

The external mycelium of AM fungi can extend to several centimetres from the root zone, thus enabling the root to exploit soil nutrients beyond the nutrient depletion zone (O' Keefee and Sylvia 1992). Therefore, AM is recognized as an essential component of sustainable agro-ecosystem (Jeffries 1987; Barea 1991). Arbuscular mycorrhizal fungi enhance drought tolerance to plants (Hardie and Leyten 1981; Allen and Boosalis 1983) by tapping a large volume of soil, mobilization of scarce nutrient sources, and modification of the root environment (Hayman 1983). In general, AM

associations with plants are widespread, both taxonomically and geographically. Most of the plants growing in Indian desert carry AM infection on their roots (Kiran Bala *et al.* 1989).

2. DISTRIBUTION OF AMF IN ARID REGION

Arbuscular mycorrhizal fungi are ubiquitous in occurrence, but the patterns of distribution of individual species differ with biotic and abiotic factors. Management practices can also influence the occurrence of the AM species. In one of the studies in the arid region of Rajasthan, a high diversity of AM fungi, which varied with the rainfall patterns, was observed (Pandey 1999). The important genera identified were *Glomus, Gigaspora, and Sclerocystis. Glomus* was found to be the predominant genus. In the process of co-evolution of diverse species, it is possible that non-sustainable species were eliminated as they were out-competed by more efficient species. Further studies with the AM species revealed that the *Glomus mosseae* is most effective for agricultural crops and *Glomus fasciculatum* was found to be more effective for trees (Tarafdar and Praveen Kumar 1996).

Fifteen species recorded for the genera *Glomus* were: *G. aggregatum, G. albidum, G. clarum, G. constrictum, G. dimorphicum, G. etunicatum, G. fasciculatum, G. hoi, G. geosporum, G. macrocarpum, G. manihotis, G. microcarpum, G. mosseae, G. multisubstensum* and *G. multicaule* (Table 9.1). Three species recorded of *Gigaspora viz., G. albida, G. candida* and *G. margarita* and two species of *Sclerocystis* viz., *S. coccogena* and *S. microcarpus* were recorded. It is evident that the distribution of various species of AM fungi varied considerably, depending upon the amount of rainfall. *Glomus mosseae* and *Glomus fasciculatum* were found to be predominant fungi. *Glomus fasciculatum* was found in all the fields, while *Sclerocystis* species were present in districts of Rajasthan, receiving low rainfall viz., Jaisalmer, Barmer and Jodhpur. They were either absent or scarcely present in districts of Rajasthan viz., Kota, Sawai Madhopur and Jaipur, receiving relatively higher rainfall.

Table 9.1: Distribution of AM Fungi with varying Patterns of Rainfall in Dryland Region of Rajasthan

Name of the Region	*Rainfall (mm)*	*Species of AM fungi occurring in the region*
Bikaner, Barmer, Churu, Ganganagar, Hanumangarh, Jaisalmer, Jodhpur	100–400	*Glomus aggregatum, G. albidum, G. constrictum, G. clarum, G. dimorphicum, G. etunicatum, G. fasciculatum, G. geosporum, G. hoi,*

Name of the Region	Rainfall (mm)	Species of AM fungi occurring in the region
		G. macrocarpum, G. microcarpum, G. manihotis, G. mosseae, G. multisubstensum, G. multicaule, Gigaspora albida, Gi. candida, Gi. margarita, Sclerocystis cocogena, S. microcarpus.
Ajmer, Jaipur, Sikar, Sirohi	401–600	*Glomus aggregatum, G.constrictum, G. dimorphicum, G. etunicatum, G. fasciculatum, G. geosporum, G. hoi, G. macrocarpum, G. microcarpum, G. manihotis, G. mosseae, G. multisubstensum, G. multicaule, Gigaspora candida, Gi. margarita, Sclerocystis cocogena, S.microcarpus.*
Alwar, Bharatpur, Sawai Modhopur, Udaipur	601–800	*Glomus aggregatum, G. constrictum, G. dimorphicum, G. etunicatum, G. fasciculatum, G. geosporum, G. hoi, G. macrocarpum, G. microcarpum, G. manihotis, G. mosseae, G. multisubstensum,G. multicaule, Gigaspora candida, Gi. margarita, S. microcarpus.*
Jhalawar, Karauli, Kota	801–1000	*G. aggregatum, G. constrictum, G. dimorphicum, G. etunicatum, G. fasciculatum, G. geosporum, G. hoi, G. macrocarpum, G. microcarpum, G. manihotis, G. mosseae, G. multisubstensum, G. multicaule, Gigaspora candida, G. margarita.*

3. AM INFECTION UNDER FIELD CONDITIONS

The propagules of AM fungi include soil borne spores and colonized root fragments (Bellgard 1992). The soil borne spores are considered to be the most important type of propagules (Brundrett 1991), as they are more

resistant to adverse environmental conditions. The stored food in the form of lipid and the thick walls of the spores help them to resist the adverse abiotic conditions (Abbott and Robson 1991). Root infection with mycorrhizas is initiated either from soil-borne propagules (spores, root residues) or from neighbouring roots of the same or different plant species. Infection is enhanced by a pre-existing network in the soil, and therefore severe soil disturbances e.g., clear-cut logging or rigorous soil mixing (Jasper et al. 1989 b), as well as tillage (Miller and McGonigle 1992) severely depress and delay mycorrhizal infection.

(i) Trees, Grasses and Crops

Kiran-Bala *et al.* (1989) examined the roots of 17 different Indian desert tree species. The intensity of infections varied among the species. Maximum infection of roots was recorded for *Azadirachta indica, Acacia tortilis* and *A. aneura*, while *A. catechu* had the lowest rate of infection. *Glomus* and *Gigaspora* were the common AM genera, found associated with roots. The AM infections were common in *Opuntia* sp. and *Euphorbia* sp. and the infection rate (30-98%) in these species were comparatively higher than in trees. The intensity of AM infections varied with the availability of water. The deep-rooted growth habit, along with AM infections of desert vegetation, may be a survival mechanism in competition for water and nutrients, with shallow-rooted and fast-growing plant species.

In one of the studies conducted by us, AM infection was examined in six tree species of approx. 12–15 years, grown under rainfed and irrigated conditions of arid region. The intensity of infection varied among the species. Maximum infection of root was recorded for *Azadirachta indica* (Table 9.2). *Glomus* (80%), *Gigaspora* (16%) and *Acaulospora* (4%) were the only AM genera found associated with the roots. Irrigation reduced 14% of root infection. The intensity of AM infection varied with the availability of water.

Higher infection in trees growing in arid areas might be because of the adaptability of AM fungi to such soils. Alternately this might be an adaptive mechanism for survival under stress conditions. It is commonly observed that root infection is lower in plants growing on fertile soils, than on plants in infertile soil (Hayman 1970). In our study (Pandey *et al.* 1999), the infection was higher under the rainfed condition in all the tree species. Lindsay (1984) indicated the role of AM association in survival and growth. Koske *et al.* (1975) suggested that the presence of AM infection in the vegetation of the coastal sand of the Lake Huran (USA/ Canada) helped in the stabilization of sand dunes through the binding of sand particles by the extraradical mycelium.

Table 9.2: Per cent Root Infection in Tree Species on Rainfed and Irrigated Fields

Tree species	*Root infection (%)*	
	Rainfed	*Irrigated*
Acacia nilotica	72	65
Acacia tortolis	63	61
Azadirachta indica	75	61
Eucalyptus camaldulensis	64	52
Prosopis cineraria	67	61
Tecomella undulata	72	57
LSDs (p = 0.05)	3.7	2.8

The effect of different AM fungi was studied on trees (*Prosopis juliflora*), grass (*Cenchrus ciliaris*) and crops (*Vigna aconitifolia*) under field conditions. Plants were grown in poor fertility sandy soil, with a low indigenous AM fungi population. For AM treatment, each tree pit received about 350 surface sterilized spores (0.2 % chloramin T and 0.02% streptomycin sulphate) of 90–250 mm in size. For grasses and crops, every plot (3m × 5m) received approximately 7000 infective propagules, which were placed 2 cm below the soil surface of the seedling row. Eight weeks after AMF inoculation, there was little variation in infection between plant types and inoculation with different AMF, although the mycorrhizal performance on *Prosopis juliflora* was slightly better than that in *Cenchrus ciliaris* and *Vigna aconitifolia* (Table 9.3). Uninoculated controls also showed infection by mycorrhiza, which could be attributed to the presence of native AM fungi in the soil.

Table 9.3: Per cent Root Length Infected by different AM Fungi in three Plant Species

Treatment	*Root length infected (%)*		
	P. juliflora	*C. ciliaris*	*V. aconitifolia*
Glomus mosseae	83	83	73
Glomus fasciculatum	88	87	81
Gigaspora margarita	81	82	72
Control	21	22	19

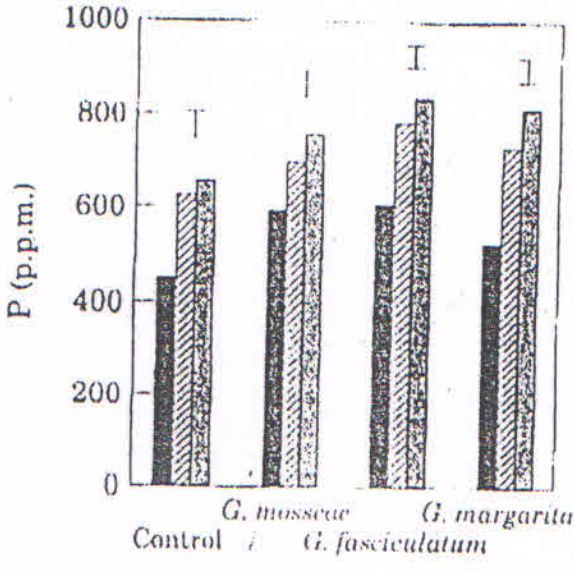
A similar trend was noticed with spore build up in the rhizosphere soil (Table 9.4). In general, the increase in viable spore number from the original inoculation, after harvest of the plant was 65–152%. The small increase in spore build up may be due to the arid environment. The maximum

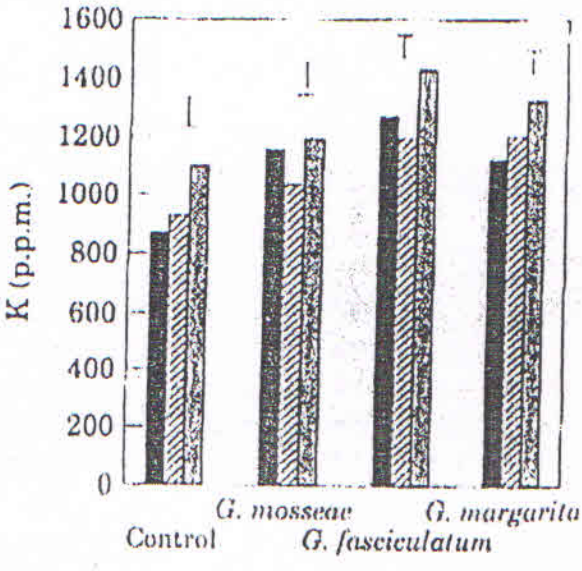
increase in spore number over control (94 spore 100 g^{-1} soil) was observed with the inoculation of *Glomus fasciculatum* under *Prosopis juliflora*.

Table 9.4: Build up of AM Fungi Spores (100 g^{-1}) after Inoculation in three Plant Species

Treatment	Spore population (100g-1)		
	P. juliflora	*C. ciliaris*	*V. aconitifolia*
Glomus mosseae	128	120	99
Glomus fasciculatum	156	146	114
Gigaspora margarita	121	118	103
Control	62	63	63
LSD (p=0.05)	6.2	5.9	4.8

There were distinct effects of treatment on mineral nutrient concentrations and their distribution to the shoots of the test species (Fig. 9.1 and 9.2). Mineral concentration increase (mg g^{-1}) in the shoots of *Prosopis juliflora* were: N = 160 – 470; P = 84 – 163; K = 84 – 120; Cu = 4 – 8; and Zn= 4 – 10. Manganese concentration decreased by 9–20 mg g^{-1} and Fe concentration was enhanced up to 18 % by the inoculation of *Glomus* sp., but declined up to 27 % with the inoculation of *Gigaspora margarita*. A similar effect on nutrient accumulation was noticed in the grass and crop species inoculated with different AM fungi (Fig. 9.1 and 9.2). However, the effect was slightly lower than in *Prosopis*. Unlike the later, Fe concentration in crops and grasses increased due to inoculation of *Gigaspora margarita*. Cu and Mn concentration was greatest in *Vigna aconitifolia*, followed by *Prosopis juliflora* and *Cenchrus ciliaris*. However, for all other nutrients the highest concentration was observed in *Prosopis juliflora*, followed by *Vigna aconitifolia* and *Cenchrus ciliaris*.





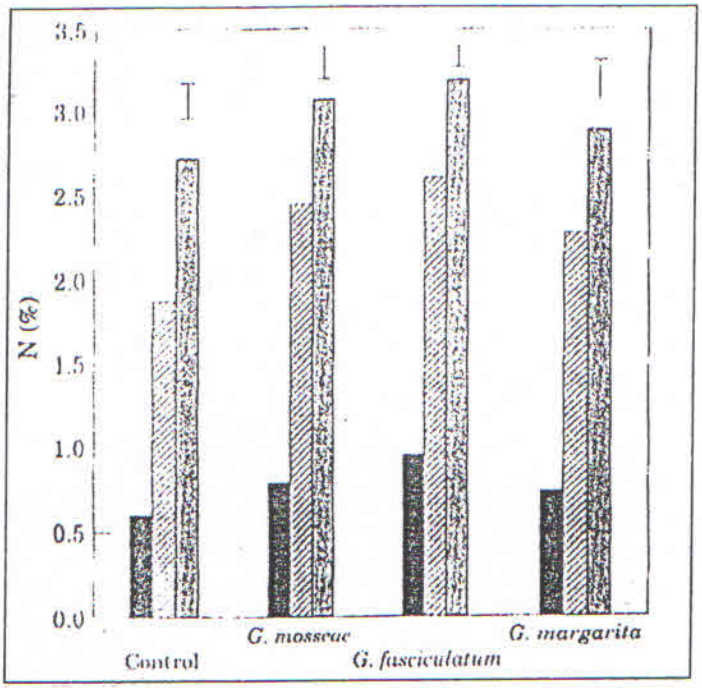


Fig. 9.1. Changes in the concentration of K, P and N in *C. ciliaris* ■, *V. aconitifolia* ▨ and *P. juliflora* □ as influenced by *G. mosseae*, *G. fasciculatum* and *G. margarita*. (Error bar, LSD $p < 0.05$)

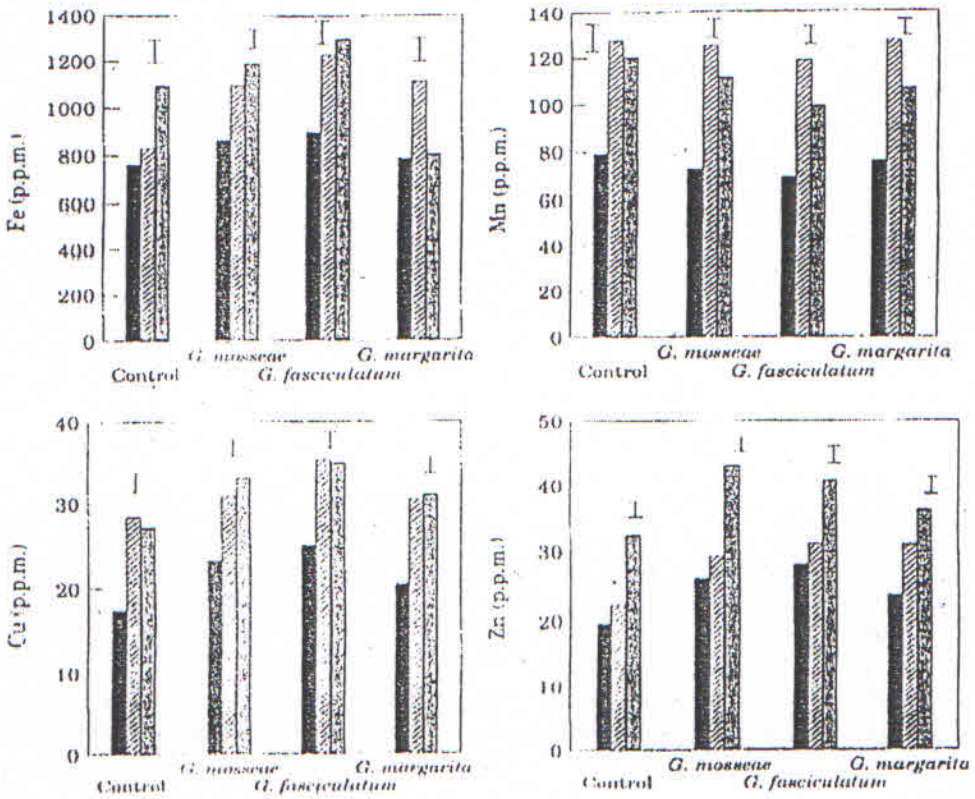


Fig. 9.2. Changes in the concentration of Fe, Mn, Cu and Zn in *C. ciliaris* (■), *V. aconitifolia* ▨ and *P. juliflora* (□) as influenced by *G. mosseae*, *G. fasciculatum* and *G. margarita*. (Error bar, LSD $p < 0.05$)

The content of N, P, K, Zn and Cu increased in the infected plants, while that of Mn decreased. The enhancement of nutrient acquisition by mycorrhizal infection can be attributed to direct hyphal uptake and/or indirect effects brought about by morphological and physiological changes in the roots. Increase in the yield of infected plants may be attributed to higher absorption of nutrients and drought resistance in the infected plants (Geoge *et al.* 1992). Higher N content in mycorrhizal plants indicates a substantial uptake of N by AM fungal hyphae. Nitrogen uptake by hyphae may be of special significance under dry soil conditions, when root NO_3^- uptake is limited by impaired soil solution mass flow.

Phosphorus, copper and zinc concentrations in the shoot dry matter was consistently higher in AM fungi inoculated plants. Usually AMF colonization is advantageous to plant growth by providing sparingly mobile mineral nutrients, such as P, Zn and Cu. It is known that Zn and Cu can be absorbed and translocated through AM hyphae, and then released to the host (Kothari *et al.* 1991; Li *et al.* 1991). Potassium and Fe concentrations were generally higher in mycorrhizal plants. Higher Fe concentrations in mycorrhizal plants have been reported in a few instances (Lambert *et al.* 1979), but the effect of mycorrhizas on the uptake of these elements is not straight forward (Lambert *et al.* 1979). Mycorrhizas usually increase P uptake, which may require some charge compensation by the major cations.

The concentration of Mn decreased in the shoot of the plants tested in our experiment. The decrease was highest (17%) in *Prosopis,* followed by 11% in the grass and least in *Vigna* (7%). The decrease in Mn concentration for red clover (Arines *et al.* 1989) and maize plants (Kothari *et al.* 1990) has been well demonstrated. However, the cause of the decline is not clear. Since AM affects the release of low molecular weight organic compounds by the root (Dixon *et al.* 1989), it may depress Mn acquisition indirectly, via changes in the rhizosphere.

Cluster bean is the most important multipurpose legume crop in arid and semi-arid regions of India. Cultivation is done mostly in phosphate deficient soils of the drought prone areas of Western Rajasthan, where most of the farmers do not use chemical fertilizers because of the risk involved in agriculture. This crop is grown extensively by employing rhizobial inoculation, but the inoculation often fails because of P deficiency and recurrence of frequent droughts. Nodulation, nitrogenase and alkaline phosphatase activities has increased significantly upon AM inoculation (Table 9.5) in cluster bean.

The enhanced nodulation might be due to the production of larger root biomass and growth regulating substances such as auxins, gibberellins, and cytokinins by AM fungi (Ek *et al.* 1983) or by enhancement of levels of

Table 9.5: Effect of Different AM Fungi on Nodulation, Nitrogenase Activity, Root Infection and Alkaline Phosphatase Activities in the Rhizosphere

Inoculants	*No. of nodules plant^{-1}*	*N_2-ase activity (n mol. C_2H_4 plant$^{-1}h^{-1}$)*	*Root infection (%)*	*Alkaline phosphatase (n kat 100 g^{-1} soil)*
Glomus fasciculatum	20.6	507.4	42	17.0
G.constrictum	18.1	452.4	64	15.6
G.epigaeum	18.6	490.5	62	18.8
Ggaspora margarita	19.9	475.1	74	15.2
G. gilmori	19.9	683.4	52	16.4
Control	15.5	386.2	25	13.0
LSD (p=0.05)	1.8	36.1	5.7	0.8

growth regulating compounds in the host plants (Dodd *et al.* 1983). The enhancement in per cent root infection due to inoculation varied from one fungus to the other. The per cent root colonization varied from 42 to 74, indicating differential capability of the host roots with different AM fungi. Higher nitrogenase activity and higher no7dule numbers observed in mycorrhizal plants, compared to non-inoculated plants, probably were due to better phosphorus nutrition of the AM infected plants. The improved P nutrition of AM plants could be due to the tapping of more phosphorus beyond the phosphate depletion zone by the ramifications of AM fungal hyphae, as the mobility of phosphate in the soil is limited (Hayman 1978), or due to the increased availability of P in the rhizosphere soil from the breakdown of organic phosphates by the AM fungi. Tarafdar and Claassen (1988) indicated that alkaline phosphatase in soil was solely of microbial origin, while plant roots produced only acid phosphatase (Tarafdar 1989). The increased alkaline phosphatase activity in the mycorrhizosphere might be due to the release of this enzyme by AM fungi, or other microorganisms, whose activities are enhanced by the mycorrhizosphere effect (Bethlenfalvay and Franson 1988).

(ii) Xeropyhtes

The AM association may be essential for the survival and growth of xerophytes growing in desert soils, especially in soils of low fertility. Arbuscular mycorrhizal infection was examined in roots of xerophytes and the percentage of root infection observed is given in Table 9.6. All the xerophytes (species of *Opuntia* and *Euphorbia*) were infected with AM fungi.

Similar results on *Opuntia* sp. were also observed by Rose (1981) at Baja, California. As compared to tree species, AM infection rate was higher with the xerophytes and reached 98% infection with *Euphorbia tirucalli*. Species of *Opuntia* had less infection, as compared to species of *Euphorbia*.

Table 9.6: Colonization of Roots of Xerophytes by AM Fungi

Species	*Root infection (%)*
Cereus peruvianus	55
Euphorbia caducifolia	70
E. neriifolia	95
E. tirucalli	98
Opuntia ficus-indica	30
O. fragilis	80
O. microdasys var. aurantispims	35
O. microdasys var. albispina	60
O. vulgaris	70

4. CRITICAL LEVELS OF ARBUSCULAR MYCORRHIZAL FUNGAL PROPAGULES

To examine the critical level of AM fungal propagules for important arid crops, such as moth bean (*Vigna aconitifolia*) mung bean (*Vigna radiata*) and pearl millet (*Pennisetum glaucum*), *Glomus mosseae* was used at different spore levels (0, 25, 50, 75, 100, 200 and 300 spores per 100 g soil). Mycorrhizal inoculation increased plant height, dry matter yield, root length and per cent root infection. Seventy per cent infection was found to be sufficient for optimum response by legumes (moth bean and mung bean), whereas 80 per cent infection (Pandey and Tarafdar 1999) was required to get effective yield for pearl millet (Table 9.7). The critical level for moth bean, mung bean and pearl millet was found to be 75, 100 and 200 spores per 100 g soil, respectively (Fig. 9.3). However, effect on plant growth was observed up to 275 spores per 100 g soil for moth bean, 240 spores per 100 g soil for mung bean and > 300 spores per 100 g soil for pearl millet.

It is suggested that the critical level of deficiency of AM fungal propagule concentration in soils is the level below which inoculation with saturated amounts of effective AM fungi significantly increased yield, and above which no response is expected. It seems very likely from the results that by increasing the AM concentrations up to certain levels in the soil, root infection is speeded up and as a result the plant can gain earlier from the symbiosis. Initially there was a steep increase in per cent root infection,

Table 9.7: Effect of Inoculation with Different Spore Levels of *Glomus mosseae* on the per cent Root Infection and Dry Matter Yield in three Arid Crops*

Spore level (100 g^{-1} soil)	*Moth bean*		*Mung bean*		*Pearl millet*	
	A	*B**	*A*	*B*	*A*	*B*
0	0.0	96.2	0.0	118.3	0.0	237.0
25	36.3	116.9	36.7	125.2	40.0	281.7
50	56.3	139.4	51.3	156.3	57.3	305.6
75	70.3	156.7	63.7	166.3	64.3	317.0
100	70.9	159.3	70.4	172.0	71.2	332.9
200	71.4	161.0	71.0	173.0	80.1	339.6
300	71.7	161.3	71.3	172.9	81.7	340.1
LSD (p=0.05)	4.1	6.1	3.1	2.5	2.3	2.9

* *after 6 weeks under control condition; A = root infection (%); B = dry matter yield (mg plant^{-1})*

with increase in spore number in all the crops tested, but gradual reduction in response was observed at higher spore level. The differences in mycorrhizal responsiveness may be partly due to root morphology and root/shoot dry weight ratio. Marschner and Dell (1994) demonstrated that arbuscular mycorrhizal roots could supply additional P, N, K, Zn, Cu, S, Mn and Mo to plants.

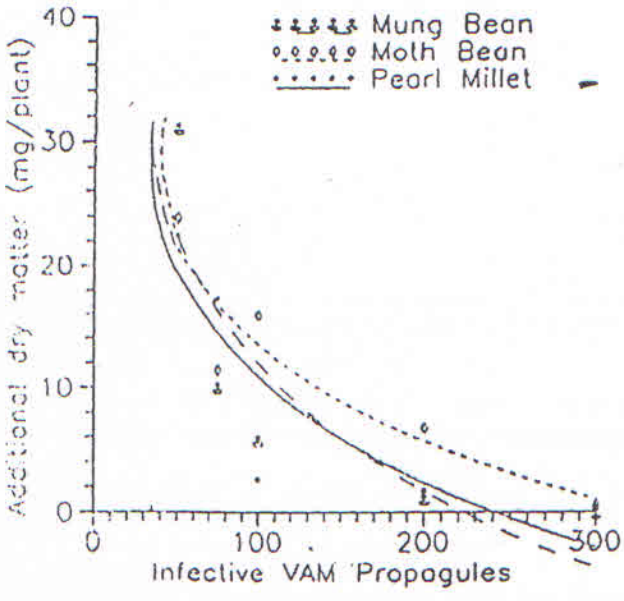


Fig. 9.3. Relationship between the number of infective propagules (*Glomus mosseae*) and inoculation response of dry matter yield in different crops.

5. MULTIPLICATION OF AM INOCULUM IN ARID REGION

A large-scale multiplication of AM inoculum under arid condition is feasible after using soil solarization technique (Rao and Tarafdar 1999). The efficacy of summer irrigation and soil solarization, combined with mustard cake in eliminating the native arbuscular fungal spores at 0–10 cm depth was found to be about 91%. The build up of AM spores was much better (Table 9.8), with maximum purity of 89 % in solarized plots, coupled with irrigation and amendment with mustard cake. It might be due to the presence of few native AMF spores, resulting in low competition to the introduced one. The results indicated the possibility of adopting soil solarization for large-scale multiplication of arbuscular mycorrhizal fungi by the farmers and nursery managers in the field than conventional pot culture method.

Table 9.8: Build up of AM Fungal Spores in Solarized Soil

Treatment	*Number of AM spores (100 g^{-1} soil)*	*Purity (%)*
Dry soil	370	54
Mulch	434	80
Irrigated soil	408	65
Irrigated + mulch	450	85
Irrigated + mustard cake	427	71
Irrigated + mustard cake + mulch	490	89
Sterlized soil in pots	612	100
LSD (p=0.05)	42	12

6. EFFECT OF AM ON TRANSPIRATION

Substantial evidence exists that AM association may alter plant-water relations. An experiment was conducted in pots, taking wheat as the test crop (Tarafdar 1995). The treatment effects on the transpiration rate are shown in Table 9.9.

The result showed that the transpiration rate of the AM mycorrhizal plants was on an average is 14 per cent higher than in the non–mycorrhizal plants. The effect was more in the presence of organic P (17%) than inorganic P (12%). Similarly, in the mycorrhizal plants, the total transpiration (water loss per plant) during 60 days of growth was 22 per cent and 26 per cent higher in plants fertilized with inorganic P and organic P, respectively, as

Table 9.9: Mean Transpiration Rates of Wheat Plants

Days after emergence	Mean transpiration rate (ml water plant^{-1} d^{-1})			
	A	B	C	D
0–15	18	21	16	17
15–30	64	73*	55	66**
30–45	117	126*	107	120**
45–60	126	133*	117	129**

(A=Inorganic P–AM; B = Inorganic P + AM; C=Organic P–AM; D=Organic P+AM); Statistical significance as calculated for comparison between + AM and –AM treatment * p < 5%; ** < 1%.

compared to control (Table 9.10). Transpiration flux per unit leaf area at the time of harvest, however, did not vary significantly among the treatments. In mycorrhizal plants, the water uptake per unit root length was higher, although transpiration coefficient was not affected due to mycorrhizal inoculation. Differences in water use of AM plants, compared with non-mycorrhizal plants may be due to indirect effects, such as increased P or N uptake, root signal mediated by changes in root turgor or plant hormone levels (Augé and Duan 1991), accumulation of solutes in AM colonized root cells under drought conditions (Augé and Stodola 1990), and differences in root morphology. In addition, a better aggregation of soil in the rhizosphere of plants and the surrounding soil induced by the AM hyphae (Miller and Jastrow 1990) could lead to better soil water flow to mycorrhizal roots, resulting in enhanced water uptake.

Table 9.10: Effect of Arbuscular Mycorrhizal Inoculation on Transpiration of Plants in the Presence of Organic and Inorganic P

Treatment	Total transpiration (ml water plant^{-1})	Transpiration flux* (ml water cm^{-1}leaf area h^{-1})	Transpiration coefficient (ml water g^{-1} dry matter)
Inorganic P – AM	1007	3.13	126
Inorganic P + AM	1343**	3.12	132
Organic P – AM	971	3.09	145
Organic P + AM	1298**	3.10	158

** Based on the amount of water transpired during 2 days before crop harvest; Statistical significance as calculated between the + AM and – AM treatment, **p< 0.1%*

Substantial evidence exists that AM associations may also alter plant water relations. Higher transpiration rate have been found in arbuscular mycorrhizal red clover, rangeland grass (*Bouteloua gracilis*), rose, apple and maize plant (Augé 1989, Runjin 1989). Further, more rapid recovery from water stress and higher soil water extraction at low soil water potential has also been observed in mycorrhizal plants (Hardie and Leyton 1981).

7. EFFECT OF AM ON PHOSPHATASE ACTIVITY

To examine the phosphatase activity of AM fungi in the rhizosphere and hyphosphere, mycorrhizal and non-mycorrhizal wheat (*Triticum aestivum* L.) were grown for 60 days in two sterilized soils in pots, with five compartments (Fig. 9.4.); a central one for root growth (rhizosphere), two adjacent on both sides next to the root compartment for hyphal growth (hyphosphere), and an outer compartment on both sides where root and hyphae could not penetrate (Tarafdar and Marschner 1994). Compartmentation was accomplished by 30 mm nylon net or 0.45 μm membrane in the two types of compartment, respectively.

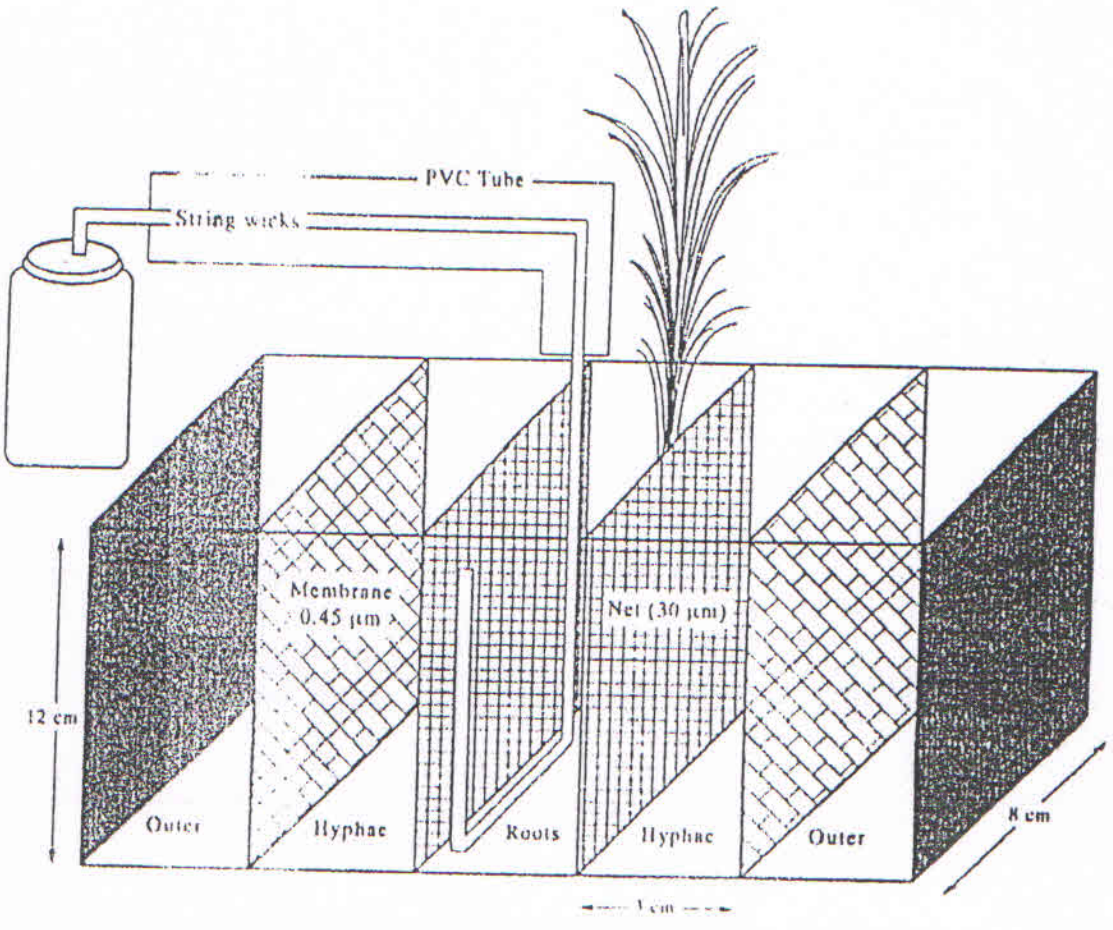


Fig. 9.4. Details of the pots used in the experiments

In the root compartment acid phosphatase activity was much higher than alkaline phosphatase activity (Fig. 9.5) and both were slightly entrenched by mycorrhizal infection. Increased mycorrhizal and root activity in the rhizosphere may generally account for higher phosphatase activities. The higher activity in Bavendorf soil may be related to better root growth (4.6–7.1 g pot^{-1}), as compared to Niger soil (2.1–3.1 g pot^{-1}). High clay and organic matter content have resulted in an increased production of phosphatases of plant origin, due to an increase in total root surface area. As phosphatases are adaptive enzyme, the high organic matter content of the soil may enhance phosphatase activity of plant origin, but also increased hyphal branching and saprophytic growth of the fungi that develop in the presence of organic matter. Considering the very high total root length in the root compartment, the hyphae in the root compartment were not likely to be contributing much to phosphatase activity in the mycorrhizal plants. As plants secrete only acid phosphatase (Tarafdar 1989), the increase in alkaline phosphatase is attributed mainly to AMF, because plants were grown under sterile conditions and microbial development other than AMF at harvest was negligible. Histochemical studies on localize phosphatase activity, within AM infected roots at an ultrasructural level, demonstrated acid phosphatase activity in the cytoplasm of the plant root cells, as well as at the growing tips of mycorrhizal hyphae (Gianinazzi *et al.* 1979).

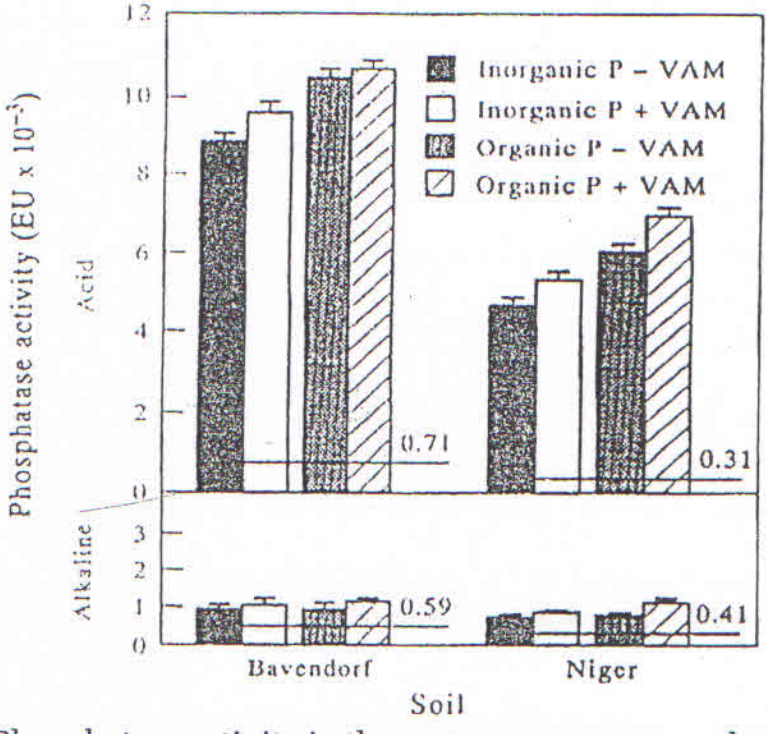


Fig. 9.5. Phosphatase activity in the root compartment under different treatment; baseline indicates soil phosphatase activity before start of the experiment.

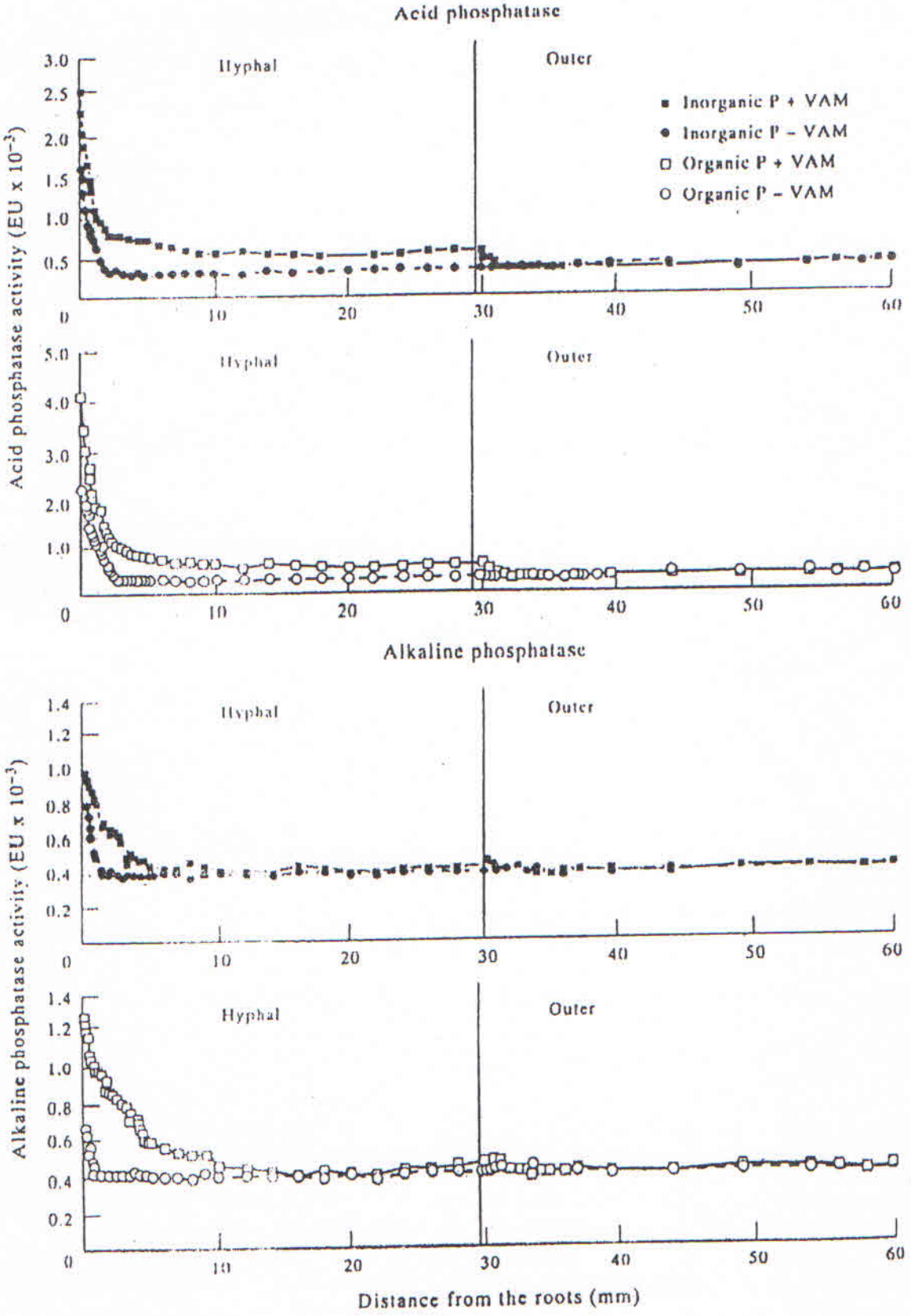


Fig. 9.6. Phosphatase activity in the hyphal compartment and bulk soil (outer) compartment; sandy soil, supplied with inorganic and organic P.

In the rhizosphere soil, acid phosphatase activity increased by 14 to 18-fold and alkaline phosphatase activity 1.6 to 2.1-fold. Throughout the hyphal compartment (Fig. 9.6), phosphatase activities were distinctly higher in the presence of mycorrhizal plants, particularly with a supply of organic P. The results strongly suggest that AM hyphae substantially contribute to phosphatase activity, depending upon soil type and the form of P supplied. The contribution of the AM hyphae was higher to acid phosphatase than to alkaline phosphatase, especially in Niger soil (sandy). The hyphal contribution to phosphatase was maximal within 3 mm from the root surface and more or less constant throughout the hyphal compartment, and was significantly higher than the outer compartments where no roots or hyphae were allowed to penetrate.

A visual demonstration of *in vivo* acid phosphatase secretion by arbuscular mycorrhizal fungi (Tarafdar 1995) was made (Fig. 9.7). In this method enzymatic hydrolysis of 1-napthyl phosphates at pH 5.6 liberates 1-naphthol, which reacts with the diazonium salt Fast Red TR (diazotized 2-amino-5 chlorotoluene 1,5-naphthalene disulphonate), forming a red dye.

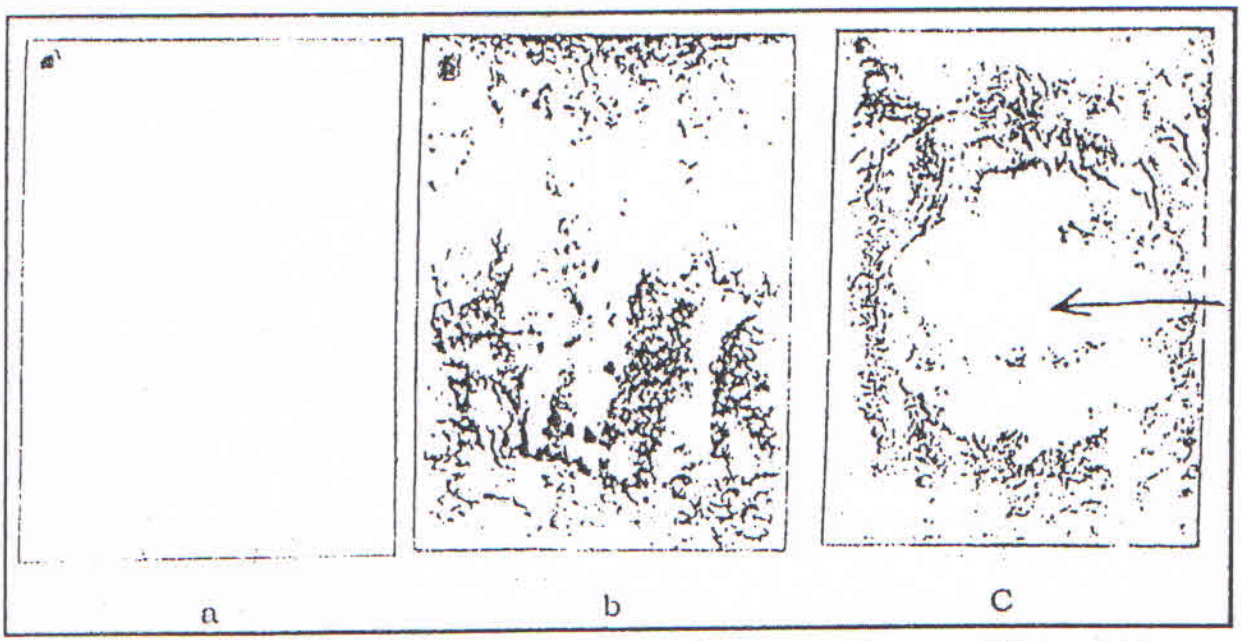


Fig. 9.7. Fingerprints of AM mycorrhizal hyphae: (a) control; (b) + AM; (c) +AM with barrier. Arrow denotes the position where the barrier was put on the hyphal path.

The AM fungal growth and development was also influenced by organic P compounds, such as phytate (Tarafdar 1997). At least 33 per cent increase in hyphal length density and 21 per cent increase in spore number was noticed, when P was supplied as Na-phytate. Organic P was depleted by arbuscular mycorrhizal hyphae throughout the hyphal compartment (Fig. 9.8). The maximum depletion was 106 mg kg^{-1} soil, within a 0.5 mm distance

from the root surface (Tarafdar and Marschner 1994). The depletion of organic P by phosphatase secreted by AM fungi depended on the soil type, hyphal length density, phosphatase activities and the amount of organic P present in the system. The maximum hyphal contribution to the hydrolysis of organic P was concentrated in the zone within 3 mm from the root surface. The results obtained clearly demonstrated that arbuscular mycorrhizal phosphatase is effective in hydrolysis of organic P compound present in the soil.

The role of the mycorrhizas in organic P hydrolysis could be either direct or indirect. Phosphatase production or other forms of enzymatic hydrolysis of organic P would be direct mechanisms for mineralization (Harley 1989). Alternately, P mineralisation may be indirect, if mycorrhizal hyphae acquire P only after other microorganisms or those associated with the mycorrhizal fungi mineralize it. Mineralization of organic P in the present study occurred only through AM hyphae in the hyphosphere under sterile condition (Fig. 9.8). Our experiment for the first time demonstrated that mycorrhizal plants could directly derive P from organic sources by the production of phosphatases through extraradical hyphae.

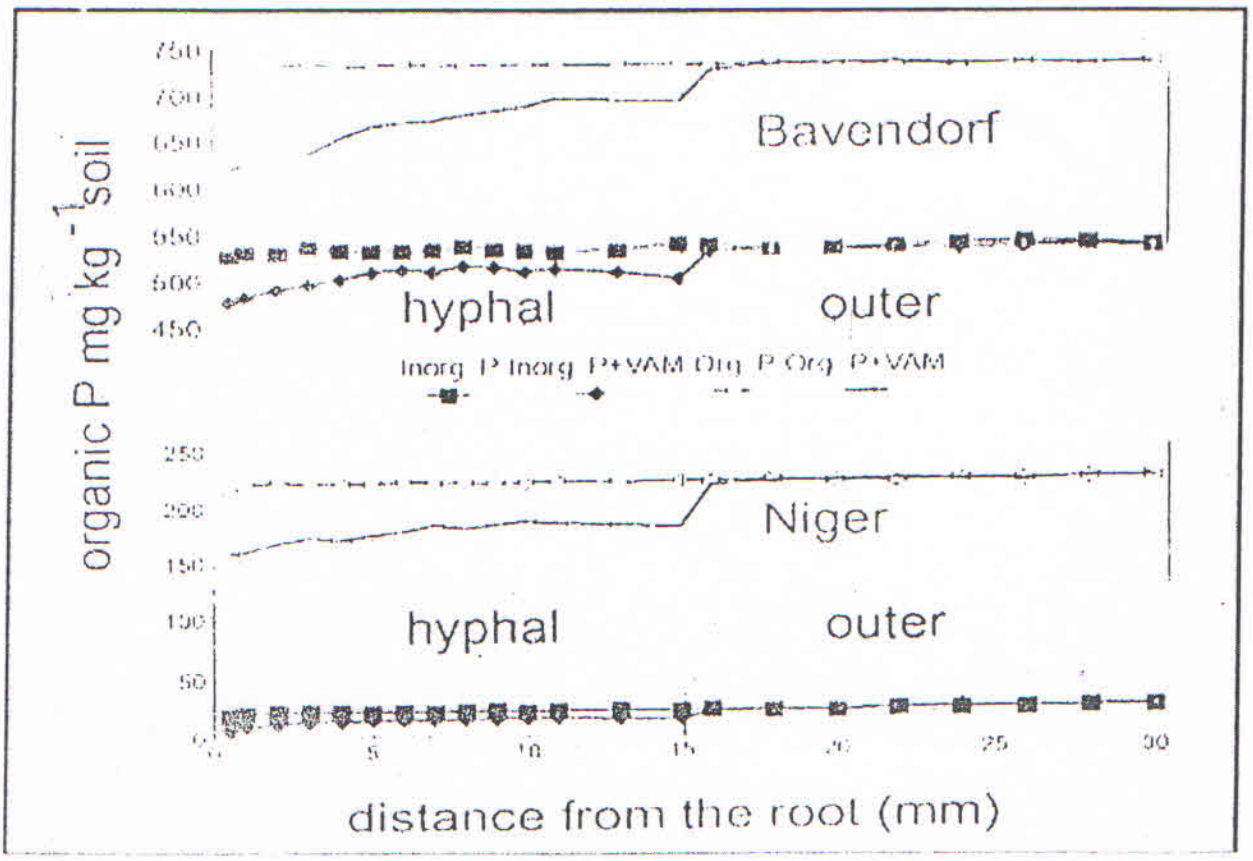


Fig. 9.8. Organic phosphorus distribution in hyphal compartment and bulk soil (outer) compartment, supplied with inorganic and organic phosphorus; Bavendorf –clay soil; Niger –sandy soil. É: LSD p<5%.

8. ASSOCIATION OF AMF WITH BENEFICIAL MICROORGANISMS

Tarafdar and Marschner (1995) demonstrated that seed inoculation with the phosphatase producing fungus (PPF) *Aspergillus fumigatus* and soil inoculation with AM fungus *Glomus mosseae* increased shoot and root dry weight, root length, phosphatase activity in the rhizosphere and shoot concentrations of P, K and Mg. The greatest effects on these parameters were observed in the combined inoculation (PPF+AM). Shoot concentration of Cu and Zn were only enhanced by AM, not by PPF. At harvest of wheat crop, depletion of organic P in the rhizosphere soil increased in the order of sterilized soil < PPF < AM < PPF + AM, which corresponded with the enhanced P concentration in the plants. The results demonstrate that organic P, in the form of Na-phytate is efficiently used by AM and that the use of organic P can be increased by simultaneous inoculation with phosphatase producing fungi.

The role of microbial phosphatases in the utilization of organic phosphorus by the wheat plants is shown in Table 9.11. Depletion of organic phosphorus was similar in the soils of the non-sterilized control and the soil inoculated with PPF. Maximum depletion of organic phosphorus was noticed in PPF + AM treatment.

Table 9.11: Phosphorus Fractions (mg kg^{-1}) in the Soil, after Harvest of Wheat Plants grown in Two different Soils supplied with Na-phytate (200 mg kg^{-1} soil)

Treatment	*Clay Soil*		*Sandy Soil*	
	Organic P	*Olsen P*	*Organic P*	*Bray-PI*
Org. P (Sterile)	652a	8.4a	189a	4.4a
Org. P + PPF	604b	9.3b	138b	5.5b
Org. P + AM	584c	10.2c	120c	5.0b
Org. P + PPF + AM	558d	9.9c	109d	6.3c
Org. (Unsterile)	609b	12.7d	142b	8.2d

Initial organic P concentration in soil, before start of the experiment (after addition of Na-phytate) was 728 mg kg^{-1} soil in clay soil and 222 mg kg^{-1} soil in sandy soil; Org. P=Organic phosphorus; PPF=Phosphatase producing fungi; AM=Arbuscular mycorrhizal fungi; Figures followed by the same letter are not significantly different ($p<0.05$).

The present experiment confirms that utilization of organic P is enhanced by AM. This enhancement effect could be due to both increases in surface area and phosphatase activity of the extraradical hyphae (Li *et al.* 1991; Tarafdar and Marschner 1994). Inoculation with the PPF

increased phosphatase activity in the rhizosphere and utilization of organic P.

Response of cluster bean to symbionts *Glomus mosseae* and *Rhizobium* species (DRG-3) and to application of N or P, as well as FYM was investigated under arid field conditions. Single inoculation of *G. mosseae* or *Rhizobium* improved growth and yield, equivalent to 20 kg N ha^{-1} or 40 kg P_2O_5 ha^{-1}. The positive effect of *G. mosseae* was related to the *Rhizobium* association, inoculation of which increased the growth and yield of mycorrhizal plants. Nodulation, nitrogenase activity, per cent root infection, viable arbuscular mycorrhizal spores, biomass, dehydrogenase, and acid and alkaline phosphatase activity were enhanced significantly due to dual inoculation and was further enhanced in the treatment amended with FYM. Maximum improvement in total dry matter and seed yield was obtained in the AMF + *Rhizobium* + FYM treatment (Table 9.12). Concentrations and total uptake of N, P, K, Ca, Mg, Fe, Cu and Zn were higher in this treatment. However, in general, concentrations of Mn and Na remained unaffected. The results suggested that dual inoculation of AM-fungi and *Rhizobium*, coupled with FYM application, is best for sustainable cluster bean production in dry soils.

Table 9.12: Yield of Cluster Bean as Influenced by Farm Yard Manure, Arbuscular Mycorrhiza and *Rhizobium*

Treatment	*Yield (Quintal ha^{-1})*	
	Dry matter	*Seed*
FYM*	27.1	7.8
Rhizobium (R)**	26.1	7.9
AMF***	27.0	7.8
FYM + R	27.3	8.0
FYM + AMF	28.3	8.2
AMF + R	28.3	8.3
FYM + AMF + R	28.3	8.3
Control	24.0	7.0
LSD (p=0.05)	1.18	0.32

*Results are average of three years; *4 t ha^{-1}; ** 2300 infective propagules per* m^2 field; *** seed inoculation with the AMF population of 10^8 per ml.

9. OTHER BENEFITS OF AM

An increase in drought stress tolerance has been observed in AM plants, compared with non-mycorrhizal plants. Differences in phosphorus

nutritional status of the plants might account in part for this effect. However, in plants with comparable phosphorus nutrition status and similar shoot size, plant water status may also differ between AM and non-mycorrhizal plants (Augé and Stodala 1990). In view of the effects of AM on root morphology (e.g., root branching, number of apical meristems) and on root anatomy (e.g., lignifications), changes in plant water balance may be indirectly the consequence of hormonal and structural changes in the host plant.

There is a long list of examples on suppression of soil-borne fungal and bacterial root pathogens by AM fungi. For example, inoculation with AM fungi increases resistance of tomato to *Fusarum oxysporum* (Dehne and Schoenbeck 1979), and of tomato to *Pseudomonas syringae* (Garcia-Garrido and O'campo 1989). This suppressing effect of AM is also evident in cases of 'soil sickness' or 'replant disease', where minor pathogens or deleterious soil microorganisms may harm root growth and activity. In the northern wheat belt of Australia, wheat root infection with common root rot (*Bipolaris sorokiniana*) has been found to be inversely related to root colonization with AM (Thompson and Wildermuth 1989). The differences in root colonization were caused by alterations in the AM infection potential in the soils via the length of fallow periods, or relations between host/non-host AM plants in the crop rotation.

Surface mining is an important activity in arid regions of Western Rajasthan and much of the land in this region is disturbed by gypsum extraction. Such activities destroy vegetation. Vegetation of mined spoils is often hindered by the lack of resident microflora, which aid transformation of various nutrients through biochemical reactions. Mycorrhizas play an important role in the growth of plants on these mined spoils (Marx 1980) by regulating the uptake of various nutrients, either by mobilizing the limited supply of essential nutrients or by keeping nutrients below toxic levels. Mycorrhizas also improve plant drought tolerance and exhibit growth-promoting effects. Tarafdar and Rao (1997) found thirty-one plant species, including legumes, grasses and sedges growing naturally on gypsum-mined spoils. These species carried AM-fungal infections, which varied from plant to plant. Per cent root infection by AM fungi was higher among legumes, grasses and sedges growing on mined spoils than on plants growing on normal soil (Table 13). Plants on mineral spoil had significantly higher K, Ca, Mg and Fe concentration and lower concentrations of N, P, Zn and Cu. The results demonstrated the possibility of using AM fungi for rehabilitating gypsum mined spoils.

Table 9.13: Per Cent Root Infection of AM Fungi in various Plants growing in Normal and Mine Soil

Plant species	*Normal soil*	*Gypsum mine soil*
(A) Legumes		
Acacia tortilis	28.7	32.1**
Crotalaria burhia	8.7	11.8***
Crotalaria medicaginea	9.1	83.3***
Indigofera cordifolia	8.6	81.4***
Indigofera linifolia	15.5	31.2***
Phaseolus sp.	54.9***	16.7 *Prosopis*
juliflora	31.2	42.3***
(B) Grasses and Sedges		
Aristida mutabilis	28.6*	23.8 *Aristida*
sp.	41.5	75.9***
Cenchrus setigerus	14.3	68.3***
Cymbopogon jwarancusa	32.6	65.0***
Cyperus sp.	29.6	31.1*
Lasiurus sindicus	36.8	63.6***
Pennisetum glaucum	37.8	42.3*
(C) Other plants		
Arnebia hispidisima	16.0	16.7NS
Corchorus tridens	45.8	50.0*
Ctenolepsis cerasiformis	33.3	75.0***
Cucumis callosus	81.8***	45.5 *Fagonia*
cretica	43.2**	30.0
Heliotropium sp.	24.2	38.5***
Pulicaria angustifolia	30.8***	14.3
Sida cordifolia	36.8	90.9***
Solanum surattense	39.8	39.5NS
Zizyphus nummularia	53.5***	32.2

*Significantly higher root infection at *p < 5%; ** p < 1%; *** p< 0.1%; NS = Non-significant*

Plants growing on the surface mines of arid and semi-arid regions frequently contain mycorrhizas (Call and McKell 1985). Mycorrhiza play an important role in the growth of plants on these mine spoils (Marx 1980) by regulating the uptake of various nutrients, either by mobilizing the limited supply of essential nutrients (Rao and Tarafdar 1993; Tarafdar and Marschner 1995) or by keeping nutrients below toxic levels (Kothari

et al. 1991). Mycorrhizas also improve plant drought tolerance (Allen *et al.* 1981) and exhibit growth promoting effects (Barea and Azcon-Aguilar 1982).

Although higher plants may benefit from their associated AM fungi in most instances by improvement in their nutritional status, other beneficial effects may occur and should not be overlooked. Diverse beneficial mycorrhizal effects can readily be demonstrated under controlled environmental conditions. Predictions on effects to be expected from inoculation with AM require at least the consideration of the fungal species or strains. The major limitation for predictions, however, is our poor knowledge of the functioning of the associations under field conditions. More systematic studies are required, for example, on comparisons between mycorrhizal plant species and non-host species under field conditions and in plant communities in order to evaluate more accurately the relative importance of the various potential beneficial effects of AM fungi on their host plants under given ecological conditions.

Despite the above limitations, there are certain areas where it is feasible to inoculate with AM fungi on a commercial level. Many horticultural plants (e.g., *Ber*, Pomegranate, *Aonla*), and most of the fruit trees and forest trees are first established in seedbeds or maintained during early development in nurseries before transplanting to the fields. The reforestation of mining sites by AM fungi can substantially decrease transplantation shock and increase survival and growth rate in the field (Jasper *et al.* 1989 a).

So far, field results showing a clear yield response to inoculation with AM fungi in non-sterilized soils are scanty (Sieverding 1991). To make use of the beneficial effects of AM on crop plants, it seems to be more promising under most circumstances to manipulate the potential of the indigenous AM indirectly by soil management and crop rotation (Sattelmacher *et al.* 1991). These efforts deserve more attention for economical reasons in low-input plant production systems, and for ecological reasons in both low- and high-input plant production systems.

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