

Influence of Organic Matter on AM Colonization and Associated Rhizosphere Mycoflora in *Vigna unguiculata* subsp. *unguiculata* (L.) Walp

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ABSTRAK

A greenhouse investigation was undertaken to determine the influence of organic amendments on the colonization of arbuscular mycorrhizal (AM) symbiosis and rhizosphere microfungal population in *Vigna unguiculata* subsp. *unguiculata* (L.) Walp. grown in sandy loam. Pongamia glabra leaves (PL) and goat pellets (GP) were applied at the rates of 5, 10 and 15 g kg⁻¹ soil either alone or along with Acaulospora scrobiculata, Glomus aggregatum and G. etunicatum. Plant dry weights, nodulation and tissue nutrients differed with AM fungi, types of organic matter and their concentrations. Increased rates of PL amendment enhanced AM colonization, in contrast higher rates of GP amendment suppressed AM fungi colonization. Among plant nutrients, potassium content increased profoundly with increasing rate of organic matter amendments with few exceptions at 90 d but at 45 d it showed a reverse trend at higher concentration. Microfungal populations were higher in PL amended soils than in GP amended soils. Among the various types of microfungal genera isolated, Aspergillus had the most diverse species.

ABSTRACT

A greenhouse investigation was undertaken to determine the influence of organic amendments on the colonization of arbuscular mycorrhizal (AM) symbiosis and rhizosphere microfungal population in *Vigna unguiculata* subsp. *unguiculata* (L.) Walp. grown in sandy loam. Pongamia glabra leaves (PL) and goat pellets (GP) were applied at the rates of 5, 10 and 15 g kg⁻¹ soil either alone or with Acaulospora scrobiculata, Glomus aggregatum and G. etunicatum. Plant dry weights, nodulation and tissue nutrients differed with AM fungi, types of organic matter and their concentrations. Increased rates of PL amendment enhanced AM colonization; in contrast, higher rates of GP amendment suppressed AM fungi colonization. Among plant nutrients, potassium content increased profoundly with increasing rate of organic matter amendments with few exceptions at 90 d but at 45 d it showed a reverse trend at higher concentration. Microfungal populations were higher in PL-amended soils than in GP-amended soils. Among the various types of microfungal genera isolated, Aspergillus had the most diverse species.

INTRODUCTION

Agriculture in the tropics is very intensive and is characterized by high inputs in terms of machinery, fertilizers and crop protection chemicals. Chemical fertilizers added to the soil are frequently unavailable to plants because many are easily absorbed or readily precipitated from are soil solution or relatively immobile in soil (Bolan 1991). Less than 50% of the applied fertilizer remains available to plants. Organic farming, which relies heavily on the use of natural

resources, biological processes and crop rotations, is an alternative to conventional farming. Amendment of soil with decomposable organic matter is recognized as an effective method of altering the rhizosphere microbial life cycle, thereby enabling plants to resist pathogenic attack through better vigour and/or altered root physiology (Singh and Singh 1984).

The development of arbuscular mycorrhizal (AM) infection in the host roots is influenced by edaphic, biotic and abiotic factors (Avio and

Giovannetti 1988). Organic residues have variable effects on the physio-chemical properties of the soil and AM association in non-acidic soils (Hepper and Warner 1985; Aziz and Habte 1988; Harinikumar and Bagyaraj 1989). Addition of organic matter such as cellulose to soil is known to suppress several soil-borne plant pathogens and therefore has been suggested as an effective method for biological control of pathogens (Cook and Baker 1983).

The role of fresh green manure and goat pellets on AM symbiosis and other microbial populations in sandy loam soils is largely unknown (Wander *et al.* 1995). Studies are necessary to establish clear-cut conclusions on the inter-relationship between organic matter-AM symbiosis-microbial populations. The purpose of the present study was to determine (i) the effect of different types of organic amendments on AM formation and function and (ii) the changes in microfungal population due to organic amendments.

MATERIALS AND METHODS

Substrate

The soil used in the study was a phosphate-deficient sandy loam soil with pH 6.8. The soil contained 2.01% organic matter; 9.48 mg kg⁻¹ nitrogen (N); 0.95 mg kg⁻¹ phosphorus (P) and 37.79 mg kg⁻¹ potassium (K). It was steamed to kill the indigenous mycorrhizal fungi, air dried and 1.5 kg soil used to fill each polybag after the addition of organic matter.

Organic Matter

Two organic manures viz., leaves of *Pongamia glabra* Vent. (25 mg/g N, 3.4 mg/g P and 9.8 mg/g K) and goat pellets (14 mg/g N, 0.75 mg/g P and 11.8 mg/g K) were dried and powdered before application. The manures were applied at three concentrations: 5, 10 and 15 kg⁻¹ and thoroughly mixed with soil.

Endophytes

Soil inoculum consisting of extramatrical spores and infected root bits of cowpea infected with AM fungi *Acaulospora scrobiculata* Trappe, *Glomus aggregatum* Schenck & Smith emend. Koske and *Glomus etunicatum* Becker & Gerd., served as inoculum. Mycorrhizal inoculum (15 g) were placed as a thin layer, 5 cm below the soil surface in mycorrhizal treatments. Twenty ml of

nodulating bacterial suspension containing 100 cells ml⁻¹ obtained from fresh nodules of horse gram was added to each polybag. The normal microbial flora except AM fungi was reintroduced by adding the soil filtrate. For this, freshly collected rhizosphere soil (500 g) of horse gram was suspended in SI water and passed through 38 µm sieve. Since AM fungal spore/sporocarps normally exceed 38 µm in diameter, the sieved soil suspension contained micro-organisms other than AM fungi. Fifteen ml of the soil extract was added to both mycorrhizal and non-mycorrhizal bags.

Plant Source

Seeds of horse gram (*Vigna unguiculata* subsp. *unguiculata* (L.) Walp.) were procured from the Tamil Nadu Agricultural University, Coimbatore. Seeds were soaked in water overnight and two seeds were sown in each polybag prepared as mentioned earlier. After germination one seedling was removed and the treatments were arranged in randomized block designs with 10 replicates per treatment, under greenhouse conditions (26 ± 2°C and 65% RH). The plants were watered when necessary; no nutrients were added.

Measurements

Five plants were harvested with their entire root system intact at 45 and 90 days after emergence. The roots were washed free of soil and the number of nodules was counted visually. The shoot and root dry weights were determined after drying the plants at 70°C for 48 h.

The roots were cleared in 2.5% KOH at 90°C for 30 minutes; acidified with 5N HCl and stained with trypan blue (0.05% in lactophenol). AM fungal infection was quantified according to magnified intersect method (McGonigle *et al.* 1990).

The soil microfungal populations were enumerated by dilution plate method using rose bengal agar medium (Martin 1950) with three replicates. The Petri dishes were incubated for four days at room temperature (21-24°C) and light. The fungal colonies were counted and identified according to the descriptions by Barron (1969), Ellis (1971), Subramaniam (1971), and Domsch *et al.* (1980).

Dry matter from 45- and 90- day-old shoots and roots was ground and digested in a triple acid mixture and plant tissue P was estimated by

the molybdenum blue method (Jackson 1958). Tissue N was estimated following the microkjeldahl digestion of the samples (Humphries 1956) and K was estimated by flame photometric method (David 1962). Soil nutrients were analysed according to Jackson (1958).

Statistical Analysis

The data were subjected to analysis of variance and the means were separated using Duncan's new multiple range test (DMRT).

RESULTS

Plant Biomass

The shoot dry weight of mycorrhizal plants at 45 and 90 d had significant variations compared to non-mycorrhizal plants grown in different concentrations of *Pongamia* leaf (PL) amended soils (Table 1), but no significant variations were observed in goat pellet (GP) amended soils (Table 2). Though root dry weight did not vary much between treatments at 45 d in both the amendments, plants inoculated with *G. etunicatum* in 5 g kg⁻¹ PL amended soil had a significantly higher root dry weight at 90 d than the control. Plants inoculated with either *G. aggregatum* or *G. etunicatum* in 15 g kg⁻¹ GP amended soil had a significantly low root dry weight at 45 d.

Root: Shoot Ratio

Root: shoot ratios did not vary significantly between mycorrhizal and non-mycorrhizal plants (Table 1 and 2).

AM Fungal Root Colonization

The Percentage root of colonization by *A. scrobiculata* and *G. etunicatum* was enhanced by increasing rate of PL amendment, except for *G. etunicatum* at 90 d (Table 3). However, root colonization by *G. aggregatum* was significantly less at 15 g kg⁻¹ PL amendment at 45 d and at 5 and 10 g kg⁻¹ at 90 d. In contrast, increasing rate of GP inhibited AM colonization, except for *G. aggregatum* at 90 d (Table 4).

Increased concentration of PL amendment favoured arbuscular formation in *A. scrobiculata* and *G. etunicatum* inoculated plants initially, but at 90 d arbuscules were absent in 15 g kg⁻¹ PL amended soils (Table 3). Increasing concentrations of GP amendment reduced arbuscular formation. At 90 d arbuscules were present only in *G. etunicatum* (5 g kg⁻¹) and *G. aggregatum* (15 g kg⁻¹) inoculated plants (Table 4).

Percentage of root length colonized by vesicles in mycorrhizal plants significantly increased with increasing amount of PL application at 45 d (except in *G. aggregatum* inoculation) (Table 3). But at 90 d vesicle

TABLE 1
Effects of AM fungi and three different amounts of *Pongamia* leaf amendment on the plant dry weights and R/S ratios of horse gram

Treatments Concentration (g kg ⁻¹)	Dry matter production (g plant ⁻¹)						R/S Ratio		
	Shoot			Root			5	10	15
	5	10	15	5	10	15			
45 d									
Control	0.08a*	0.18a	0.15a	0.06a	0.07a	0.07a	0.49a	0.32a	0.39a
<i>A. scrobiculata</i>	0.20b	0.18a	0.32a	0.07a	0.07a	0.07a	0.44a	0.43a	0.28a
<i>G. aggregatum</i>	0.20b	0.22ab	0.39ab	0.07a	0.07a	0.07a	0.44a	0.31a	0.19a
<i>G. etunicatum</i>	0.12ab	0.29b	0.44b	0.06a	0.09a	0.09a	0.45a	0.30a	0.19a
90 d									
Control	0.47a	1.15a	1.12a	0.12a	1.20a	0.29a	0.22a	0.24a	0.31a
<i>A. scrobiculata</i>	0.94a	1.71a	1.96b	0.26ab	0.24a	0.36a	0.30a	0.38a	0.31a
<i>G. aggregatum</i>	1.39b	1.63a	1.88b	0.25ab	0.29a	0.27a	0.21a	0.22a	0.31a
<i>G. etunicatum</i>	1.10ab	1.52a	1.87b	0.35b	0.28a	0.23a	0.36a	0.25a	0.24a

* Means in a column followed by same letter(s) are not significantly different (P < 0.05) according to Duncan's multiple range test

TABLE 2
Effects of AM fungi and three different amounts of Goat pellet amendment on the plant dry weights and R/S ratios of horse gram

Treatments Concentration (g kg ⁻¹)	Dry matter production (g plant ⁻¹)						R/S Ratio		
	Shoot			Root			5	10	15
	5	10	15	5	10	15			
45 d									
Control	0.40a*	0.46ab	0.38a	0.09a	0.11a	0.12a	0.25a	0.32a	0.27a
<i>A. scrobiculata</i>	0.40a	0.42b	0.31b	0.12a	0.16a	0.13b	0.29a	0.14a	0.17a
<i>G. aggregatum</i>	0.44a	0.52a	0.36a	0.09a	0.11a	0.07c	0.18a	0.19a	0.14a
<i>G. etunicatum</i>	0.38a	0.46a	0.39a	0.12a	0.12a	0.09b	0.39a	0.18a	0.12a
90 d									
Control	2.01a	2.43a	1.62a	0.19a	0.32a	0.39a	0.11a	0.14a	0.26a
<i>A. scrobiculata</i>	1.86a	2.07a	2.06a	0.26b	0.38b	0.39a	0.14a	0.18a	0.19b
<i>G. aggregatum</i>	1.90a	2.32a	1.90a	0.28b	0.32a	0.27b	0.15a	0.14a	0.16a
<i>G. etunicatum</i>	2.07a	2.68a	1.63a	0.37c	0.37b	0.31c	0.17a	0.14a	0.19b

* Means in a column followed by same letter(s) are not significantly different ($P < 0.05$) according to Duncan's multiple range test

TABLE 3
Effects of *Pongamia* leaf amendment on AM root colonization in horse gram after 45 and 90 days of growth

Treatments Concentration (g kg ⁻¹)	Arbuscules (%)			Vesicles (%)			Total colonization (%)		
	5	10	15	5	10	15	5	10	15
Control 45 d									
Control	0.00*c	0.00b	0.00d	0.00a	0.00c	0.00c	0.00b	0.00b	0.00d
<i>A. scrobiculata</i>	3.05bc	13.21a	19.66a	2.42a	6.05b	15.56b	41.50a	53.08a	73.17b
<i>G. aggregatum</i>	13.30a	15.74a	5.39c	5.42a	0.81c	1.79c	45.64a	50.55a	22.09c
<i>G. etunicatum</i>	10.92ab	11.55a	13.99b	3.62a	17.03a	22.97a	48.05a	61.87a	85.92a
Control 90 d									
Control	0.00a	0.00b	0.00a	0.00c	0.00c	0.00b	0.00c	0.00c	0.00c
<i>A. scrobiculata</i>	3.72a	11.34a	0.00a	33.14ab	23.90b	39.04a	75.04b	75.83a	80.48b
<i>G. aggregatum</i>	1.75a	0.59b	0.00a	25.88b	26.72b	40.61a	59.18b	52.21b	75.21b
<i>G. etunicatum</i>	5.95a	4.32b	0.00a	45.37a	39.82a	43.45a	94.06a	88.28a	89.82a

* Means in a column followed by same letter(s) are not significantly different ($P < 0.05$) according to Duncan's multiple range test

occurrence in *G. etunicatum* infected roots were higher than *A. scrobiculata* and *G. aggregatum* infected roots. Increased rate of GP application either reduced or inhibited vesicle formation at 45 d and 90 d except in *G. aggregatum* at 90 d in 15 g kg⁻¹ GP amended soils (Table 4).

Nodulation

Plants in soils amended with PL at the rates of 5 and 10 g kg⁻¹ and inoculated with either *A. scrobiculata* or *G. aggregatum* developed fewer

nodules than the non-mycorrhizal plants at 45 d (Table 5). But plants inoculated with the same fungi at 15 g kg⁻¹ PL amendment had more nodules for the same period. Nodule numbers did not vary between mycorrhizal plants at 90 d except for plants inoculated with *G. etunicatum* and *A. scrobiculata* which had significantly higher nodule numbers at 5 and 15 g kg⁻¹ PL amendment, respectively. Mycorrhizal plants in GP amended soils had reduced nodule numbers at 45 d, but at 90 d nodule numbers in

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TABLE 4
Effects of goat pellet amendment on AM root colonization in horse gram after 45 and 90 days of growth

Treatments Concentration (g kg ⁻¹)	Arbuscules (%)			Vesicles (%)			Total colonization (%)		
	5	10	15	5	10	15	5	10	15
Control 45 d	0.00*a	0.00a	0.00a	0.00b	0.00b	0.00a	0.00b	0.00b	0.00b
<i>A. scrobiculata</i>	3.34a	4.25a	0.00a	16.71a	6.22ab	0.00a	26.03a	15.33a	8.82a
<i>G. aggregatum</i>	5.01a	0.00a	1.09a	2.71b	0.00b	0.49a	11.67ab	0.62b	8.16a
<i>G. etunicatum</i>	7.85a	2.96a	0.00a	7.71ab	11.61a	0.11a	13.05ab	21.35a	6.51ab
Control 90 d	0.00b	0.00a	0.00a	0.00c	0.00b	0.00c	0.00d	0.00c	0.00d
<i>A. scrobiculata</i>	0.00b	0.00a	0.00a	30.46a	10.47a	3.07c	75.16b	25.92b	10.11c
<i>G. aggregatum</i>	0.00b	0.00a	0.55a	20.95b	4.54a	33.80a	47.43c	82.49a	69.60a
<i>G. etunicatum</i>	6.63a	0.00a	0.00a	35.46a	6.51a	8.59b	90.21a	15.01b	20.38b

* Means in a column followed by same letter(s) are not significantly different ($P < 0.05$) according to Duncan's multiple range test

TABLE 5
Effects of different organic matter amendments and AM fungi on nodulation of horse gram

Type of Organic Matter	Treatments	Concentration of organic matter (g kg ⁻¹)					
		5		10		15	
days		45 D	90 D	45 D	90 D	45 D	90 D
Pongamia leaf	Control	22*a	10a	4a	8a	8a	5a
	<i>A. scrobiculata</i>	10b	12ab	8b	11a	12b	7b
	<i>G. aggregatum</i>	8c	9a	8b	11a	14b	5a
	<i>G. etunicatum</i>	27d	14b	12a	10a	8a	6a
Goat pellets	Control	27a	12a	16a	13a	20a	13a
	<i>A. scrobiculata</i>	20b	19c	16a	16a	11b	13a
	<i>G. aggregatum</i>	14c	14ab	18a	16a	7c	11a
	<i>G. etunicatum</i>	16d	17bc	14b	15a	11b	20b

* Means in a column followed by same letter(s) are not significantly different ($P < 0.05$) according to Duncan's multiple range test

mycorrhizal plants were either high or did not vary significantly compared to non-mycorrhizal plants.

Microfungal Populations

Microfungal populations were generally higher in soils amended with PL (Table 6) than GP (Table 7). However, the dominance of microfungal components varied for different treatments and concentrations of organic matter amendment. In soils amended with PL but devoid of mycorrhizal fungi, *Aspergillus fumigatus*

was dominant at both 5 and 10 g kg⁻¹ concentrations, whereas *A. flavus* was dominant at 15 g kg⁻¹ concentration at 45 d. *Aspergillus fumigatus* was dominant in soils inoculated with *A. scrobiculata* and *G. aggregatum* at all concentrations of PL amendments, whereas in *G. etunicatum* inoculated soils *A. flavipes* in 5 g kg⁻¹, *A. flavus* in 10 g kg⁻¹ and *A. fumigatus* in 15 g kg⁻¹ were dominant. However at 90 d *A. carneus* in 5 g kg⁻¹, *A. pulverulentus* in 10 g kg⁻¹ and *A. flavipes* in 15 g kg⁻¹ were dominant in PL amended non-mycorrhizal soils. In PL amended

TABLE 6
Microfungal populations in *Pongamia* leaf amended soils

Concentration (g kg ⁻¹) Fungal species	Microfungal population (x10 ³)											
	5				10				15			
	C	V1	V2	V3	C	V1	V2	V3	C	V1	V2	V3
45d												
<i>Aspergillus carneus</i>	26	66	44	30	36	36	48	30	12	12	20	14
<i>A. fumigatus</i>	152	186	204	54	192	86	86	70	60	140	112	108
<i>A. flavus</i>	-	24	24	38	62	112	86	318	194	82	52	76
<i>A. flavipes</i>	78	48	32	64	42	70	68	8	12	14	34	14
<i>A. pulverulentus</i>	28	22	20	44	38	16	40	54	-	4	10	8
<i>Mucor racemosus</i>	38	52	38	30	22	16	6	18	18	8	4	12
<i>Penicillium rubrum</i>	2	-	-	2	2	-	10	8	-	-	2	-
<i>Trichoderma koningii</i>	32	50	46	32	46	72	48	86	12	-	26	20
Total	356	448	408	294	440	408	392	592	308	260	260	252
90d												
<i>Aspergillus carneus</i>	90	62	50	108	42	50	70	110	20	28	32	36
<i>A. fumigatus</i>	44	20	10	28	34	6	18	26	-	-	28	14
<i>A. flavus</i>	12	20	18	44	30	14	44	26	2	70	50	20
<i>A. flavipes</i>	72	106	74	84	38	36	48	54	76	36	30	32
<i>A. pulverulentus</i>	44	18	10	44	52	60	98	88	14	24	28	32
<i>Mucor racemosus</i>	-	-	2	-	2	-	2	-	2	2	2	-
<i>Penicillium rubrum</i>	18	16	16	14	12	16	2	18	6	26	22	8
<i>Trichoderma koningii</i>	24	36	14	48	30	10	18	54	8	16	10	18
Total	304	278	194	370	240	192	300	376	128	202	202	160

C - control; V1 - *A. scrobiculata*; V2 - *G. aggregatum*; V3 - *G. etunicatum*

soils *A. flavipes* in 10 g kg⁻¹ and *A. pulverulentus* in 15 g kg⁻¹ were dominant, respectively in *A. scrobiculata* and *G. aggregatum* inoculation; whereas *A. carneus* dominated *G. etunicatum* inoculated PL soils at all concentrations.

In GP amended soils *A. fumigatus* was the dominant species throughout the study. *A. flavus* in 5 and 15 g kg⁻¹, while *A. flavipes* at 15 g kg⁻¹ GP application were dominant at 45 d in *G. aggregatum* inoculated soils (Table 7).

Host Nutrient Contents

No appreciable changes were observed in N concentration in shoots at 45 d in both PL and GP amended soils (Fig. 1a and 4a). At 90 d mycorrhizal plants in 5 g kg⁻¹ PL amended and 15 g kg⁻¹ GP amended soils (except *G. etunicatum*) showed decreased N content in their shoot tissues (Fig. 1b and 4b). The root N content decreased with increasing concentrations of PL and GP amendments at 45 d. But at 90 d N content in root tissues of mycorrhizal plants was equal to

control in both 10 g kg⁻¹ PL and GP amended soils (Fig. 1c, d and 4c, d).

The shoot tissue P decreased with increasing PL application at 45 d but generally increased in 90 d (Fig. 2 a, b). *A. scrobiculata* inoculated plants in 10 g kg⁻¹ GP amended soil had the maximum root tissue P at 45 d and 90 d (Fig. 5c, d).

At 45 d shoot K content had no notable variations in plants grown in PL amended soils (Fig. 3a), but at 90 d shoot tissue K content slightly increased with rate of PLA (Fig. 3b). Though root K content declined with increasing concentrations of PL application at 45 d (Fig. 3c), such variations were not evident at 90 d (Fig. 3d). Further plants grown in 15 g kg⁻¹ GPA soil had the lowest shoot K at 45 d and non-mycorrhizal plants in 10 g kg⁻¹ GPA had more shoot K than the mycorrhizal counterparts (Fig. 6a, b). Potassium concentrations in roots were low at 45 d, with increasing rate of GP application, whereas at 90 d mycorrhizal plants

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TABLE 7
Microfungal populations in goat pellet amended soils

Concentration (g kg ⁻¹)	Microfungal population (x 10 ³)											
	5				10				15			
Fungal species	C	V1	V2	V3	C	V1	V2	V3	C	V1	V2	V3
45 d												
<i>Aspergillus carneus</i>	2	2	8	2	-	-	4	4	2	16	16	80
<i>A. fumigatus</i>	22	70	58	140	6	-	-	44	26	96	48	32
<i>A. flavus</i>	14	28	64	24	4	4	4	12	20	30	98	16
<i>A. flavipes</i>	-	4	6	6	-	2	6	10	6	10	20	10
<i>A. pulverulentus</i>	-	-	-	-	-	-	-	-	-	-	-	-
<i>Mucor racemosus</i>	4	-	-	-	-	-	2	4	-	-	-	-
<i>Penicillium rubrum</i>	-	2	-	-	-	-	-	-	-	-	-	-
<i>Trichoderma koningii</i>	6	-	-	-	-	-	-	2	-	2	2	4
Total	48	106	136	172	10	6	16	76	54	154	184	142
90 d												
<i>Aspergillus carneus</i>	16	22	70	26	-	20	06	30	-	-	30	12
<i>A. fumigatus</i>	28	46	2	20	-	52	50	62	22	48	64	74
<i>A. flavus</i>	22	24	18	116	8	20	14	10	8	-	14	10
<i>A. flavipes</i>	8	20	-	4	-	-	-	-	-	10	16	2
<i>A. pulverulentus</i>	4	22	-	62	-	28	-	-	4	-	2	2
<i>Mucor racemosus</i>	6	2	2	4	10	-	4	4	6	2	1	-
<i>Penicillium rubrum</i>	4	4	26	-	-	2	2	6	8	4	8	6
<i>Trichoderma koningii</i>	2	8	-	-	-	-	-	-	-	-	-	-
Total	90	148	118	232	18	122	76	148	48	64	135	106

C - control; V1 - *A. scrobiculata*; V2 - *G. aggregatum*; V3 - *G. etunicatum*

had more K in their root tissue in 5 and 15 g kg⁻¹ GP amendments than non-mycorrhizal plants (Fig. 6c, d).

DISCUSSION

The reported results indicate a selective influence of organic amendments on AM fungi and plant growth. Increasing rate of PL application along with mycorrhizal inoculation stimulated plant growth. However, high levels of GP (15 g kg⁻¹) application reduced plant growth of mycorrhizal and non-mycorrhizal plants. Brechelt (1989) observed a similar reduction in growth of *Capsicum annum* at high levels of staple manure application. The intensity of growth response to organic amendments and mycorrhizal inoculation is low compared to other reports in different plant species (Ramos *et al.* 1993; Mappaona *et al.* 1994; Sorensen *et al.* 1994). Mycorrhizal benefit in the form of enhanced nutrient and water uptake could be altered to a

certain degree due to organic amendments. Studies by Mohan *et al.* (1991) suggests that soil microfungal populations like *A. fumigatus* and *A. flavus* could influence plant growth, as culture filtrates of these fungi reduced shoot growth in soybean. Further, the increase in soil microbial activities due to the carbon source applied can produce antibiotic substances, enzymes, organic acids or influence other microorganisms which could affect the efficiency of AM fungi in different ways (Azcon *et al.* 1989).

Plants inoculated with either *G. aggregatum* or *G. etunicatum* at 15 g kg⁻¹ GP amended soils had lower root dry weight. Similar observations have been made in maize (Kothari *et al.* 1990), Citrus (Graham and Syvertson 1984) and Cotton (Price *et al.* 1989). The reduction in root dry weight has been attributed to decreased root lengths due to increased soil microbial activities and fierce competition among the micro-organisms and the roots for the available nutrients

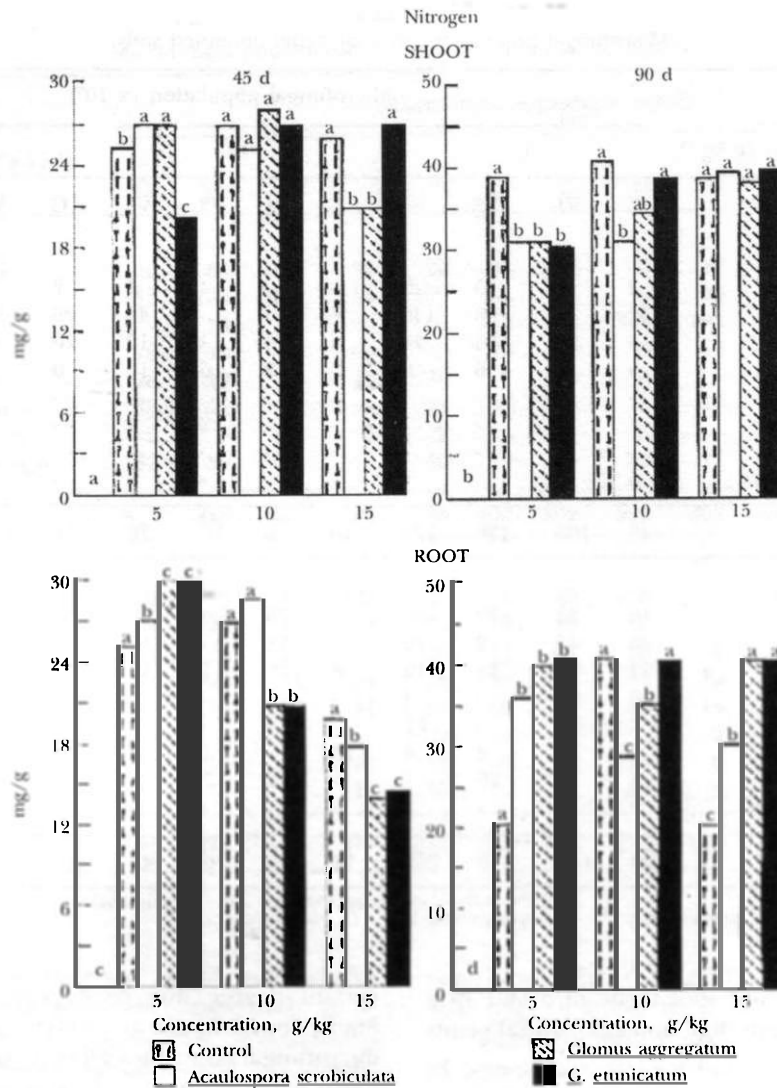


Fig. 1a-d. Effects of AM fungi and three different levels of Pongamia leaf amendment on nitrogen content of plant shoot and root of horse gram at 45 and 90 days. Bars bearing the same letter(s) in each concentration are not significantly different according to Duncan's new multiple range test ($P \leq 0.05$).

(Schonwitz and Ziegler 1989; Kothari *et al.* 1990). In addition, the rate of plant growth is determined by interactions between mycorrhizal infection and a number of nutritional and non-nutritional aspects of symbiont physiology (Smith and Gianinazzi-Pearson 1988).

The root: shoot ratios of mycorrhizal plants in organic matter amended soils had no significant variation compared to non-mycorrhizal plants. This contradicts the more common observation that mycorrhizal symbiosis generally lowers root: shoot ratio (Fitter 1982;

Bass and Lambers 1988) and also indicates the less dependence of mycorrhizal fungi owing to the presence of organic matter (Azcon and Ocampo 1981).

Even though no significant variations existed for plant tissue N and P, mycorrhizal plants in general had more nutrients in their tissue than non-mycorrhizal plants. The inflow rates of nutrients from soil solution into roots for mycorrhizal plants is faster than non-mycorrhizal plants, which may attribute for the increased rates of plant growth and increased concen-

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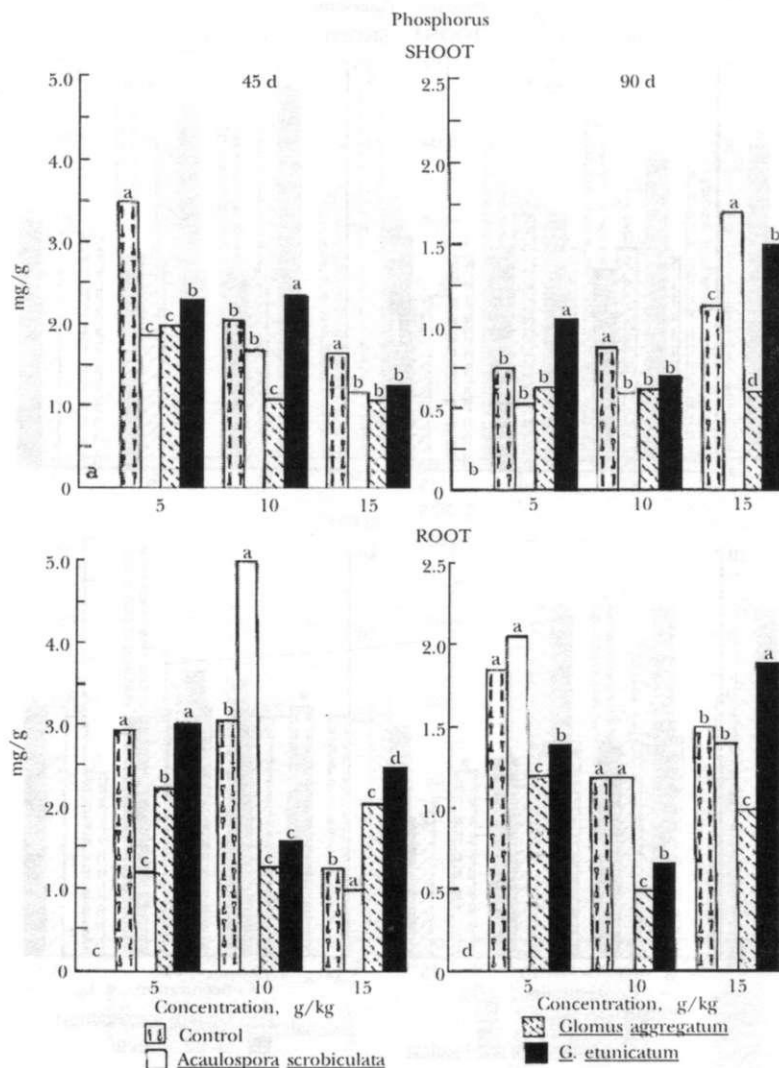


Fig. 2a-d Effects of AM fungi and three different levels of Pongamia leaf amendment on phosphorus content of plant shoot and root of horse gram at 45 and 90 days (For further explanation see Fig. 1 footnote).

tration of N and P in the tissues (Smith and Gianinazzi-Pearson 1988). Further, mycorrhizal roots exploit the soil profile, with extramatrical hyphae extending beyond the depletion zone surrounding the absorbing root and its hairs. The test plant horse gram is a nodulating legume; it is not surprising for nodulated mycorrhizal plants to accumulate more N since AM fungi have been reported to enhance N_2 fixation by the bacterial symbiont (Barea and Azcon-Aguilar 1983; Bethlenfalvai and Newton 1991). Though K accumulation was higher in mycorrhizal plants at low levels of organic amendments, higher

rates of their application reduced K concentrations, which could be attributed to the effect of organic amendment on mycorrhizal colonization since AM fungi has been reported to aid plants in K uptake.

Root infection by *A. scrobiculata* was enhanced by increased rates of PL application. A similar effect was observed in *G. etunicatum* at 45 d *G. aggregatum* at 90 d, which is in accord with Harinikumar and Bagyaraj (1988) who also observed high mycorrhizal infection in response to PL application. Sheikh *et al.* (1975) indicated that addition of organic manure to soils that are

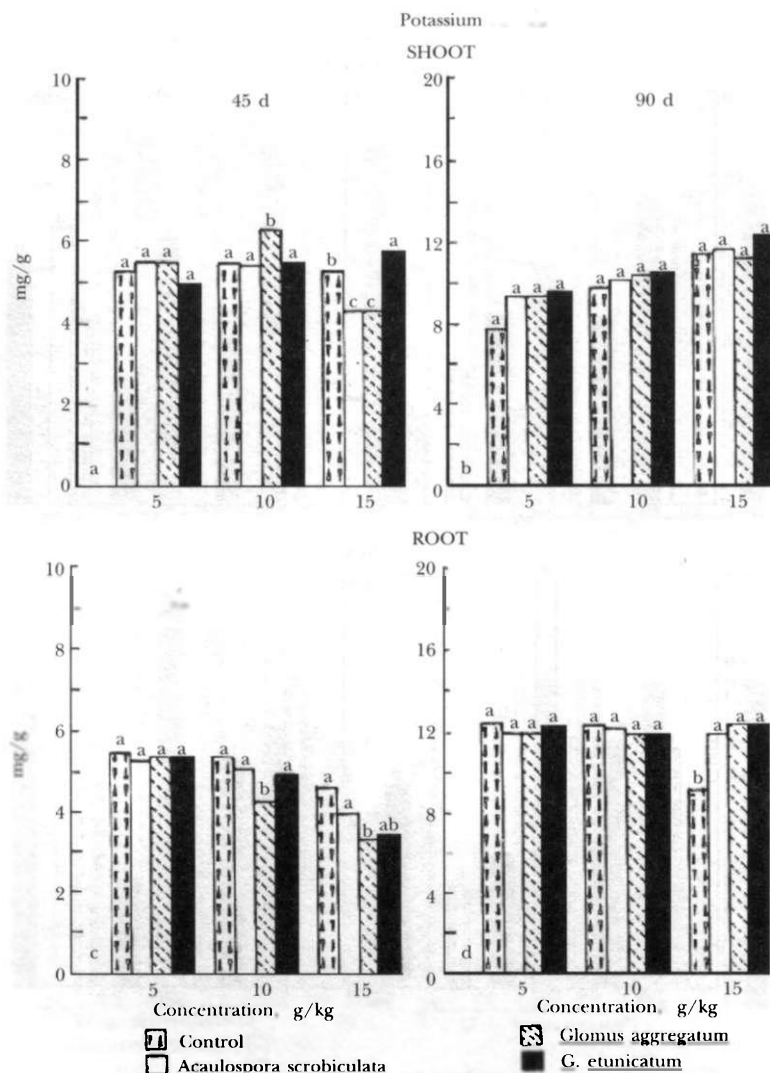


Fig. 3a-d Effects of AM fungi and three different levels of Pongamia leaf amendment on potassium content of plant shoot and root of horse gram at 45 and 90 days. (For further explanation see Fir. 1 footnote).

low in organic matter may enhance mycorrhizal development, but higher rates of GP amendment reduced mycorrhizal infection. Similar suppression was reported for pig and cow slurry application in a grassland by Christie and Kilpatrick (1992). These variations may be due to the indirect effect of different organic matter on their varying effect on soils structure, water holding capacity, nutrient mineralization etc.

Arbuscule formation in mycorrhizal plants raised in GP amended soils were low compared to mycorrhizal plants raised in PL amended soils at similar application rates. Arbuscules have a short life span and are presumably formed at

times of P demand by the host (Dodd and Jeffries 1986; Dunne and Fitter 1989).

Although nodulation in mycorrhizal plants was either enhanced or unaffected by organic amendments, mycorrhizal plants raised in low (5 g kg^{-1}) levels of PL and high levels of GP (15 g kg^{-1}) amendments had fewer nodules compared to non-mycorrhizal plants. Nodulation and N_2 fixation are characterized by a high phosphorus demand (O' Hara *et al.* 1988). The influence of phosphorus on symbiotic N_2 fixation may be indirect, i.e. by stimulation of host plant growth (Robson *et al.* 1981) or direct, by more specific effects on nodule initiation, growth and

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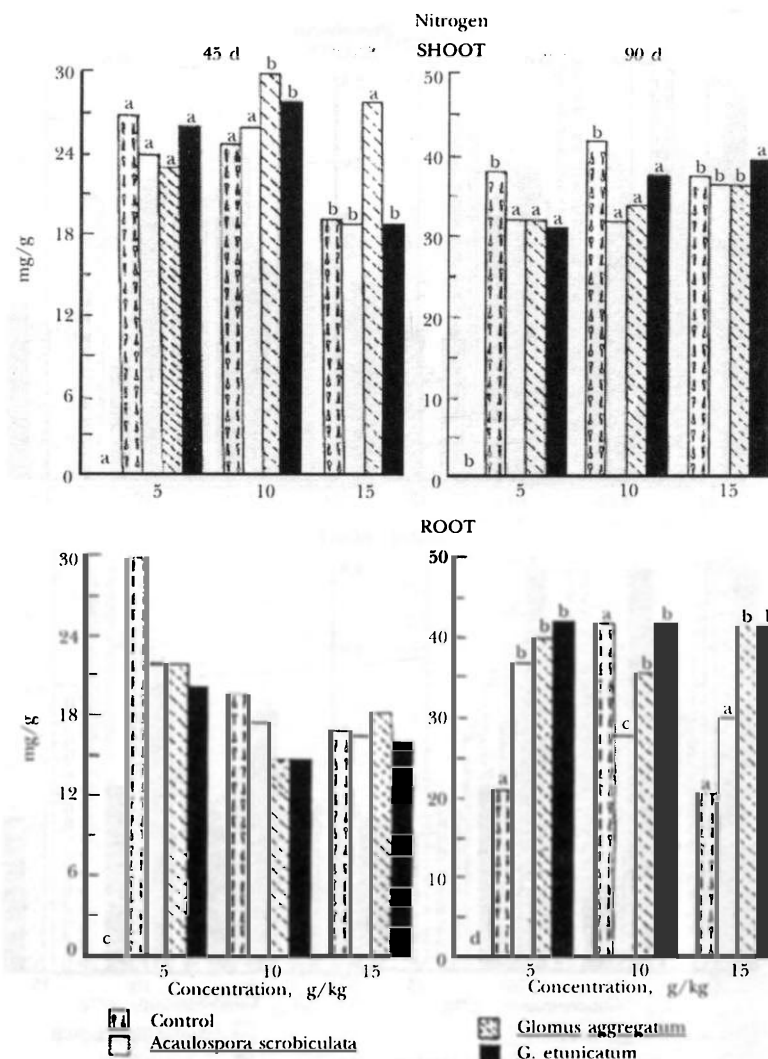


Fig. 4a-d Effects of AM fungi and three different levels of goat pellet amendment on nitrogen content of plant shoot and root of horse gram at 45 and 90 days. (For further explanation see Fig. 1 footnote).

functions. The reduction observed in the present study in some treatments might be due to the activities of soil fungi, since several species of aspergilli, *Penicillium* and *Trichoderma* are known to produce antibiotics which reduce nodulation (Lebed *et al.* 1978; Mohan *et al.* 1991). The antagonistic effect of soil fungi on nodule formation has also been recorded in *Trifolium* (Chhonkar and Subba Rao 1966) and soybean (Mohan *et al.* 1991).

Addition of organic matter altered the microfungal populations. This is in accord with Popova (1993), who demonstrated a direct

relationship between microfungal populations and soil fertility. Microfungal populations in GP amended soils were low compared to PL amended soils. Further, a decrease in microfungal population at 90 d can be attributed to the depletion of organic resources due to hastened decomposition. Sivapalan *et al.* (1993) reported that organic matter amended soils supported twice the number and a wider range of fungal species than in unamended soil. But our study does not support this. Among various fungal genera isolated, *Aspergillus* had the most diverse species. *Aspergillus* species are known to

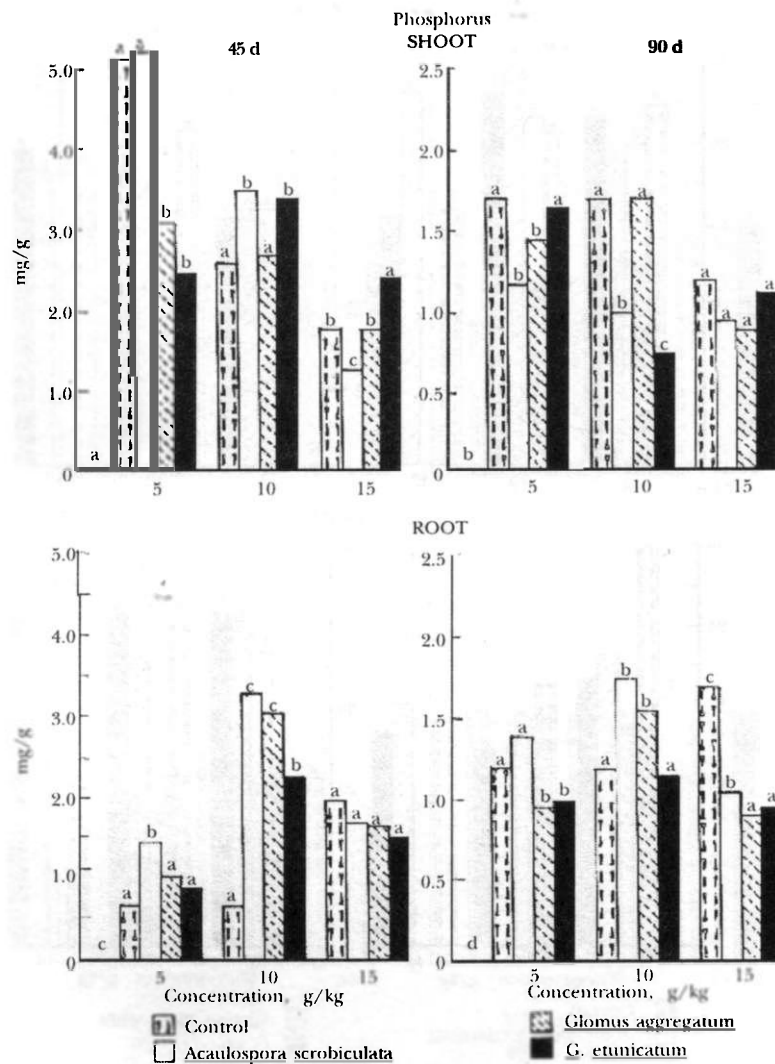


Fig. 5a-d Effects of AM fungi and three different levels of goat pellet amendment on phosphorus content of plant shoot and root of horse gram at 45 and 90 days. (For further explanation see Fig. 1 footnote).

tolerate different environmental conditions; this has already been proven through laboratory experiments (Dubost 1969; Rai *et al.* 1970; Venkataraman and Rajyalakshmi 1971). The genus *Penicillium* was represented by *P. rubrum* and populations of *Mucor racemosus* was fewer than to other microfungal populations. These observations are in accord with those of Popova (1993) who reported a decline in *Penicillium* species diversity and biomass of *Mucor* with increasing soil fertility. The low abundance of these species in the present study implies an increase in soil fertility owing to the absence of antibiotics.

Population and diversity of microfungi in the present study were unaffected by AM fungal inoculation, which is in agreement with Ames *et al.* (1987) and Secilia and Bagyaraj (1988). These authors have also reported the absence of alterations in the rhizosphere microfungal population due to AM fungal infections.

This study clearly indicates the varied influence of organic matter on plant growth, AM fungi, soil microfungi and nodulation with organic manure types and concentrations of their application.

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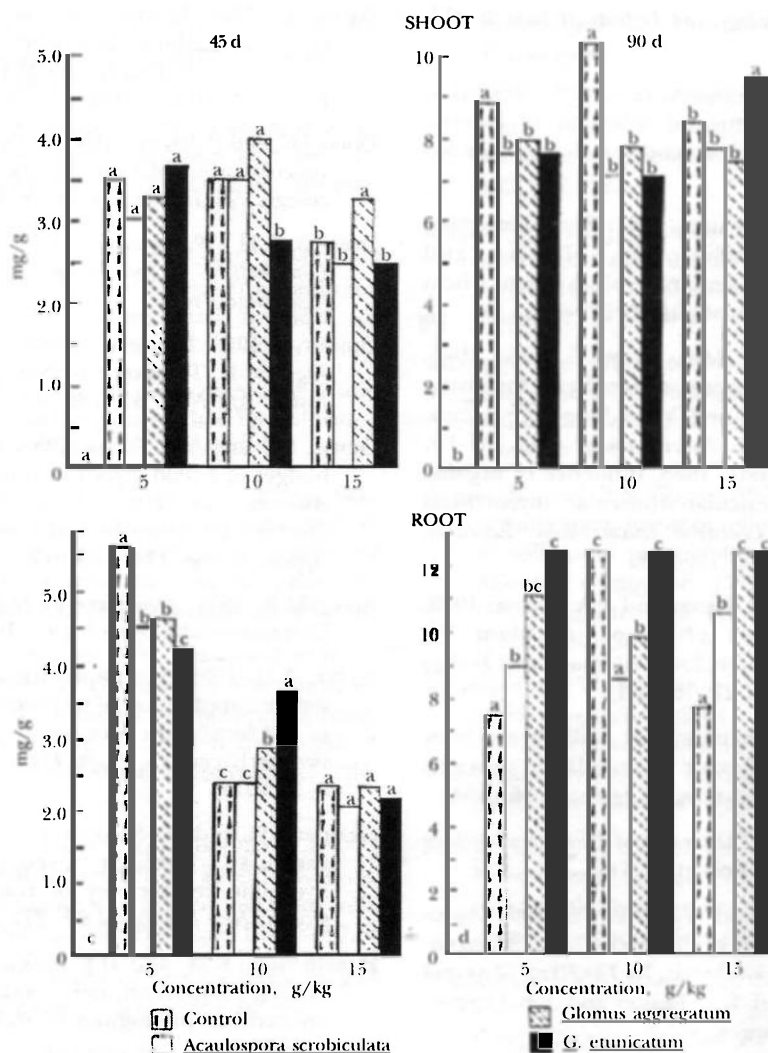


Fig. 6a-d Effect of AM fungi and three different levels of goat pellet amendment on potassium content of plant shoot and root of horse gram at 45 and 90 days. (For further explanation see Fig. 1 footnote).

CONCLUSION

The present study reveals that application of organic matter improved plant growth, rhizobial nodulation and plant nutrient content, which varied with endophytes, organic matter types and their concentrations. Higher rate of PL amendment and low rate of GP amendment favour AM colonization. The plant K concentration increased with increasing rate of organic matter at 90 d. Higher rate of PL application favours microfungus establishment than GP application. The genus *Aspergillus* is dominant and most diverse species isolated. Work

is in progress to identify the most effective/favourable AM fungal and organic matter for crop improvement under field conditions.

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