

Arbuscular mycorrhizal fungal (AMF) root colonization intensity, spore population density and species composition in the rhizosphere of forage crops

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Arbuscular mycorrhizal fungal (AMF) spore population density, root colonization intensity and species composition were studied from the root and soil samples collected from the rhizosphere of twelve forage crops, namely, green panic (*Panicum maximum* var. *trichoglume* (K. Schum.) C.E. Hibberd), dharbai grass (*Eragrostis cynosuroides* (Retz.) P. Beauv.), sabai grass (*Eulaliopsis binata* Retz), dinanath grass (*Pennisetum pedicellatum* Tan.), thin napier (*Pennisetum polystachyon* (L.) Schult), signal grass (*Brachiaria decumbens* Stapf.), sadabahar (*Andropogon gayanus* Kunth), congo grass (*Imperata cylindrical* (L.) Raeuschel), humidicola grass (*Brachiaria humidicola* (Rendle) Schweick), marvel grass (*Dicanthium annulatum* (L.) Beauv.), setaria (*Setaria italika* (L.) Scribn.), and hybrid napier (*Pennisetum purpureum* Schumach)]. Mean AMF spore population densities in the rhizosphere and colonization intensities in the roots of forage crops varied from 72.2 – 113.2 per 30 g dry soil and 0.1 – 9.2% respectively. Mean spore number was observed highest with sadabahar, remained *at par* with signal grass and lowest with hybrid napier. AMF root colonization intensities in all forage crops studied were found exceptionally low. However, mean root colonization was found to be highest with signal grass, remained *at par* with humidicola and lowest with marvel grass. Out of total fifteen AMF spore types recovered from rhizospheres, eleven AMF spore types belonged to four genera viz. *Glomus*, *Gigaspora*, *Sclerocystis* and *Acaulospora* and four spore types belonged to unidentified category. Of the four AMF genera, *Glomus* with six identified species appeared to be dominant genus followed by *Acaulospora* with two species, *Sclerocystis* and *Gigaspora* each with one species

Key words: Forage crops, arbuscular mycorrhiza, population density, colonization, species composition

INTRODUCTION

Forage crops have significant contribution in the progress and development of animal husbandry. At present, the country faces a net deficit of 62.8% green fodder, 21.9% dry crop residues and 64% feeds as compared to actual requirements. Poor and marginal arable lands are generally used for

fodder production. To meet the current level of live-stock production and to keep up its annual growth, the deficit in all three components of fodder has to be met from either increasing productivity, utilizing untapped feed resources, encroaching poor and marginal land area or through imports. Majority of forage crops have wider adaptability to grow and yield under various stress conditions. The yield of these crops in poor and marginal land even under biotic and abiotic stresses could be augmented if suitable arbuscular mycorrhizal fungal (AMF) tech-

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nology is employed as one of the essential inputs in production systems. AMF are beneficial, below ground vital symbiotic fungal (Ryan and Graham, 2002) community essential for increasing the sustainability of agriculture system (Gianinazzi and Schuepp, 1994). They play a crucial role in mineral nutrition, growth of plant (Marschner and Dell, 1994), establishment and restoration of vegetation at the disturbed sites (Millar and Jastraw, 1992), influence inter-specific competition, plant succession, plant diversity (Van der Heijden *et al.*, 1998), protection of host from certain plant pathogens (Azcon-Aguilar and Barea, 1996), improvement of water relation and stress tolerance in plants (Davis *et al.*, 2002), enhancement of fitness of plant to polluted environments (Gaur and Adholeya, 2004) and contribute to soil aggregates and stability (Miller and Jastrow, 1992). Some forage and grass hosts are reported to be mycorrhizal. Such multi-dimensional benefit of AMF could be extended to augment production, productivity and establishment of forage crops in marginal, eroded, biotic and abiotic stressed lands. Very limited works have been done in the exploration AMF status in forage crops. Keeping this research gap and potential benefits extended by AMF to plant in mind, present exploratory research works are conducted to know the root colonization intensity, spore population density and species composition of AMF of some perennial forage crops.

MATERIALS AND METHODS

Experimental plot layout and plant - and soil - samples collection

Experiment was conducted with twelve forage crops [green panic (*Panicum maximum* var. *trichoglume* (K. Schum.) C.E. Hibberd), dharbai grass (*Eragrostis cynosuroides* (Retz.) P. Beauv), sabai grass (*Eulaliopsis binata* Retz), dinanath grass (*Pennisetum pedicellatum* Tan.), thin napier (*Pennisetum polystachyon* (L.) Schult), signal grass (*Brachiaria decumbens* Stapf.), sadabahar (*Andropogon gayanus* Kunth), congo grass (*Imperata cylindrical* (L.) Raeuschel), Humidicola grass (*Brachiaria humidicola* (Rendle) Schweick), marvel grass (*Dicanthium annulatum* (L.) Beauv.), setaria (*Setaria italika* (L.) Scribn.), hybrid napier (*Pennisetum purpureum* Schumach)] were grown in museum plots of All India Coordinated Research Project on Forage Crops at Gayespur (23.5°N Latitude and 81°E Longitude) under Regional Re-

search Station of Bidhan Chandra Krishi Viswavidyalaya, Nadia, West Bengal. Forage crops were planted in a plot size of 2 m x 2 m in triplicate following complete randomized block design and maintained since last two years following recommended agronomic practices. Roots and soil samples were collected from six places of each plot and bulked replication wise. Soil samples were air dried, processed and then preserved in polyethylene packets at room temperature for arbuscular-mycorrhizal analyses. Root samples were randomly selected from the secondary and tertiary branches, washed, cut into 1 cm small pieces and preserved in formalin-acetic alcohol (FAA) (formalin, acetic acid and 70% alcohol in the ratio of 5:5:90 :: v:v:v).

Mycorrhizal analyses of root and soil samples

Root samples were boiled in 10% KOH for 3 – 4 minutes at 121°C, acidified with 1(N) HCl and stained with 0.05% trypan blue following slight modification of original method proposed by Philips and Hayman (1970). Per cent root length infection root colonization intensity was determined by estimating length of root showing AMF hyphae, abuscules, vesicles etc. by microscopic measurements with ocular micrometer of at least 30x1 cm root pieces per replication. AMF spores were isolated from soil by wet sieving and decanting method proposed by Gerdemann and Nicolson (1963) using 500, 250, 100 and 45 µ sieves. The isolated spores were suspended in thin layer of water in Petridish and counted under low power magnification by stereobinocular microscope. Different species of arbuscular-mycorrhizal fungi were identified as far as practicable from the metrical and other characters of azygospores or chlamydospores according to the standard description by Schenck and Perez (1990) and available in the INVAM website (invam.caf.wvu.edu/fungi/taxonomy/speciesID.htm.)

RESULTS AND DISCUSSION

Soil and root samples of twelve perennial forage crops grown in forage museum plot were collected at two different dates of sampling for studying the AMF spore population density, root colonization intensity and species composition. Results presented in Table 1 revealed that forage crops caused differential stimulation of AMF spore numbers and exhibited variations in their level of colo-

Table 1 : Arbuscular mycorrhizal (AM) spore population in rhizosphere and per cent root length colonization of some perennial forage crops at two different dates of sampling

Forage crops	Total no. of AM spores/30g soil at the time of		Pooled mean	% root length colonization of crops by AM fungi at the time of		Pooled mean
	1 st sampling	2 nd sampling		1 st sampling	2 nd sampling	
Green panic	49.8 ± 1.5 e	113.7 ± 5.5 cd	81.8 ± 2.05 de	0.9 (1.18 ± 0.08) fg	1.1 (1.27 ± 0.12) e	2.0 (1.23 ± 0.10) fg
Dharbai grass	82.2 ± 7.4 b	92.7 ± 3.6 e	87.5 ± 5.51 cd	1.1 (1.26 ± 0.10) ef	1.9 (1.55 ± 0.17) d	1.5 (1.41 ± 0.10) ef
Sabai grass	39.6 ± 4.3 f	137.7 ± 11.0 a	88.7 ± 7.67 c	0.4 (0.94 ± 0.09) hi	0.9 (1.18 ± 0.09) ef	0.7 (1.06 ± 0.06) fgh
Dinanath grass	31.9 ± 3.5 f	129.9 ± 8.6 ab	80.9 ± 6.08 de	1.7 (1.48 ± 0.04) de	4.2 (2.17 ± 0.19) b	3.0 (1.83 ± 0.10) d
Thin napier	52.8 ± 4.5 de	121.9 ± 5.5 bcd	87.3 ± 0.76 cd	2.9 (1.84 ± 0.10) c	10.1 (1.18 ± 0.08) a	6.5 (2.55 ± 0.04) c
Signal grass	102.6 ± 5.3 a	121.8 ± 8.6 bcd	112.2 ± 1.94 a	8.2 (2.95 ± 0.30) a	10.2 (1.18 ± 0.08) a	9.2 (3.011 ± 0.23) a
Sadabahar	95.5 ± 4.8 a	130.8 ± 3.0 ab	113.2 ± 2.59 a	2.1 (1.61 ± 0.09) d	3.2 (1.18 ± 0.08) c	2.7 (1.77 ± 0.07) de
Congo grass	87.9 ± 9.5 b	123.2 ± 6.5 bc	105.6 ± 1.68 b	1.1 (1.27 ± 0.09) ef	2.2 (1.18 ± 0.08) d	1.7 (1.46 ± 0.11) def
Humidicola	58.5 ± 3.0 d	119.5 ± 6.1 bcd	89.0 ± 4.58 c	7.2 (2.77 ± 0.27) a	9.1 (1.18 ± 0.08) a	8.2 (2.94 ± 0.08) ab
Marvel grass	55.7 ± 4.5 de	98.8 ± 4.1 e	77.3 ± 0.23 ef	0.1 (0.77 ± 0.14) i	0.1 (1.18 ± 0.08) g	0.1 (0.78 ± 0.12) h
<i>Setaria</i>	69.5 ± 1.1 c	112.8 ± 4.5 cd	91.2 ± 2.76 c	0.5 (1.00 ± 0.06) gh	0.5 (1.18 ± 0.08) fg	0.5 (1.00 ± 0.09) gh
Hybrid napier	32.6 ± 2.5 f	109.9 ± 8.5 d	71.2 ± 5.51 f	4.0 (2.12 ± 0.08) b	9.1 (1.18 ± 0.16) a	6.6 (2.61 ± 0.13) bc
SEm±	2.5	3.7	2.2	0.07	0.08	0.13
CD 0.05	7.4	10.8	6.4	0.21	0.24	0.37

*Figure within parenthesis indicates square root transformed value. Values followed by same or different letters indicate statistically at par or significantly different

nization with respect to particular date or dates of sampling. Mean spore number averaged over two dates of sampling was highest with sadabahar, remained at par with signal grass, followed by *Sitaria*, *Humidicola*, sabai grass and others whereas it was the lowest with hybrid napier. The spore numbers at the second date of sampling were found to be higher in all forage crops than that of the spore numbers obtained at first date of sampling. AMF root colonization intensities in all forage crops studied were found exceptionally low in two dates of sampling. Mean root colonization averaged over two dates of samplings was found highest with signal grass, remained at par with *humidicola* followed by thin napier and others whereas it was observed lowest with marvel grass. Root colonization at the second date of sampling was observed higher than first dates of sampling.

A total of fifteen AMF spore types, based on morphometrical attributes, were isolated from the rhizosphere of twelve forage crops (Table 2 and Fig. 1). Of these, eleven AMF spore types belonged to four AMF fungal genera – *Glomus*, *Gigaspora*, *Sclerocystis* and *Acaulospora* and rests four spore types could not be identified due to absence of characteristics hyphal attachment or hyphal plexus or sporiferous saccule or cicatrix. *Glomus* with six identified species appeared to be dominant followed by *Acaulospora* with two species, *Sclerocystis* and *Gigaspora* each with one species.

Twelve perennial forage crops were maintained in monoculture in their respective plots for two years. Soil and root samples collected from these crop rhizospheres were assessed for total spore number and mycorrhizal colonization intensity respectively. Forage crops caused differential stimulation

Table 2 : AM fungal spore types isolated from the rhizosphere of forage crops

Sl. No.	Morpho-metrical characters of AMF spores	AMF species and/spore types
1	Sporocarp size 630 - 1100 × 380 -900 μ with loosely aggregated chlamydo spores. Chlamydo spores globose to sub - globose or pyriform, single spore 70 -100 μ, pale yellow to yellowish brown with composite wall thickness 6 - 7 μ, two wall layers, outer 3 - 4 μ and inner 3 μ, laminated. Subtending hypha straight or curved, constricted or swollen, 10 μ wide at spore base, wall 1.5 μ thick, pore closed by thin inner wall.	<i>Glomus aggregatum</i> (T1)
2	Chlamydo spores 157×141 μ size, oval shaped, having two wall layers, with composite wall thickness 3.6 μ - outer wall thick (2.5 μ) dark coloured, inner wall thin (1.1 μ) light yellow coloured. Spore surface smooth, content granulated and spore wall non - laminated. Subtending hypha straight, short, 14 μ wide near the spore base, wall thickness 1.6 μ. The content of subtending hyphae is continuous with the content of spore through a narrow pore measuring 0.6 μ.	<i>Glomus</i> sp.1 (T2)
3	Chlamydo spore spherical, 394.5 μ in diameter, vacuolated appearance, light yellow with composite wall thickness 40 μ with some projections across the width of wall. Spores with two wall layers - outer wall non-laminated, non-wavy, 18.8-26 μ thick whereas inner wall laminated, wavy and 19 -22 μ thick. The width of subtending hypha at the spore base 43 μ, wall 7.6 μ thick, width of the pore at the point of attachment 19 μ. Spore content is separated from the hyphal content by a narrow pore and septum which occasionally protrudes into the spore.	<i>Glomus manihotis</i> (T3)
4	Chlamydo spore 174 μ in diameter, light yellow, content not homogeneous, outer surface rough, composite wall thickness 3.6 μ, outer wall thick (2.2 μ), inner wall thin (1.4 μ), wall laminated. Width of the subtending hypha at the point of attachment 15 μ, width of pore 8 μ, wall thickness of subtending hypha at the point of attachment 3.5 μ. Distance of septum from the attachment point 21 μ, width of septum at the point of contact 7 μ, width of the subtending hypha away from the point of attachment 8.4 μ, wall thickness 0.7 μ	<i>Glomus mosseae</i> (T4)
5	Chlamydo spores spherical, 160 μ diameter, content vacuolated, light yellow, outer surface rough and some debris remain attach to the spore wall. Spore wall brown, composite wall thickness 9.7 μ, outer wall thicker (6.5 μ) than inner (3.1 μ). Subtending hypha re-curved, septum developed near about 50 μ away from the base of attachment point. Subtending hypha 12.6 μ wide at the point of attachment, 3 μ thick with 6.5 μ pore width at the point of attachment	<i>Glomus</i> sp.2 (T5)
6	Chlamydo spore in loose aggregates in soil, variable in shape; sub-globose, oval or ellipsoidal, 70 - 130×40-60 μ, light yellow; spore wall thickness variable (5-10 μ), yellow to light brown, hyphal attachment continuous with outer wall of the spore, hyphal width 10-16 μ adjacent to spore wall, pore occluded, wall thickness of attached hypha near pore is 4-5 μ.	<i>Glomus fasciculatum</i> (T6)
7	Chlamydo spores 122.7×114.6 μ size, light brown in colour, content homogeneous, wall smooth, three layered and laminated, outer wall dark brown, middle wall light yellow and inner black. Inner wall separated from outer wall, cicatrix 11.3 μ diameter	<i>Acaulospora</i> spp. (T7)
8	Chlamydo spore spore globose to broadly ellipsoidal, 100 -230×100-205 μ, light brown in colour, spore surface evenly pitted with depression 1 -1.5×1- 3 μ, separated by ridge 2 - 4 μ thick, the mouth of the depression circular to elliptical or occasionally linear to Y shaped.	<i>Acaulospora scrobiculata</i> (T8)
9	Sporocarp diameter 426 μ, central plexus 47 μ diameter. Chlamydo spore club shaped, 155 μ in length, apex thick (4.5 μ), rounded, deep brown, single walled, laminated, maximum width of the spore 44 μ, width at the spore base 16.5 μ and width of the side wall at the base 1.5 μ	<i>Sclerocystis clavispora</i> (T9)
10	Azygo spores spherical, 323.5 μ diameter, honey coloured, content homogeneous, bi-layered, composite wall thickness 29.5 μ, outer wall 14.5 μ thick and dark brown, inner wall 15 μ thick brown / honey coloured. Suspensor cell bulbous, 36.3×31.8 μ in size, unbranched, 6.2 μ wall thickness and 16.8 μ width at the point of attachment. Subtending hypha septate- septa present at the base of the suspensor cell and extended length of subtending hypha, branched that starts at 32.7 μ and 108.8 μ distances from the base of attachment to the spore, 10 μ wide and 1.9 μ wall thickness.	<i>Scutellospora gregaria</i> (T10)

Contd. table 2

11	Sporocarpic, consisting of single layer of chlamydospores. Peridium lacking. Chlamydospores dark brown, 125-140 μ diameter, globose or sub-globose, 14 μ composite wall thickness with three walls, outer wall 3 μ thick, middle 10 μ laminated brown coloured and inner 1 μ thin membranous wall. Spore wall fractures near the hyphal attachment. Hyphae at the point of attachment frequently branched.	<i>Glomus ambisporum</i> (T11)
12	Chlamydospores brown, 110 –126 μ diameter, 6– 7.5 μ wall thickness, three layered outer dark brown, middle very light brown and inner.	(T12)
13	Chlamydospore slightly oval, 90 x 100 μ , light yellow, smooth, 5.2 μ wall thickness, two-layered, outer yellow 2-4 μ and inner light yellow 2- 4 μ , subtending hypha with 2.5 μ wall thickness and 10.5 μ wide at the base of spore.	(T13)
14	Chlamydospore dark brown spherical, 156 μ diameter, 4 μ wall thickness, two layered, outer 2.5 μ light brown and inner 1.5 μ dark brown, separated from each other at one end.	(T14)
15	Chlamydospores dark brown, spherical, 100 μ diameter, surfaced rough with debris, 10.5 μ wall thickness, single layered, dark brown..	(T15)

of AMF spore numbers (72.2 – 113.2 per 30 g dry soil) and exhibited variations in their level of AMF colonization (0.1 – 9.2%). Inter- and intra- specific variations of some tropical forage crops in AMF root colonization was observed earlier by Saif (1986). The variation in stimulation of spore numbers by different crop species in their rhizospheres was noted by Panja and Chaudhuri (1998) when they conducted an experiment for three consecutive seasons using *Cynodon*, maize, cowpea, napier and soybean as test plants under monoculture. They observed that soybean caused the highest stimulation of rhizospheric spore development followed by maize and napier and the least with the *Cynodon*. The variations in the intensity of AMF colonization in host species and cultivars may be due to their differences in inherent genetic make up which is heritable. Kesava Rao *et al.*, (1990) in their findings indicated that the intensity of colonization of cultivars by and the benefit from AMF was a heritable trait selectable through plant breeding.

The AMF spore number and root colonization not only vary according to types of forage crops grown but also vary according to the dates times of sampling. In general, rhizospheric spore development and root colonization by AMF at the second date of sampling were found higher in all forage crops than the spore numbers obtained at first date of sampling. Reasons for such variation in AMF spore number under a particular crop or set of crops under different times/seasons may be largely influenced by sporulating behaviour of AMF species present in the rhizospheres or may be due to the inability of some AM fungi to sporulate before first sampling date but sporulate before second sampling. Such speculation may be justified from the

findings of Schultz *et al.* (1999) when they noticed that some fungi sporulated in late spring and others sporulated at the end of summer. *Gigaspora gigantea*, which sporulates most abundantly in the fall of winter and appeared to overwinter as spores, was likely to be physiologically active during the warm season. Alternatively, *Acaulopsora colossica*, which sporulated most profusely at the beginning of summer and over summered as spores, was physiologically active with the cool season plant community (*e.g.*, *Allium vineale*) (Gemma koske., 1989; Lee and Koske, 1994). Besides host and AMF factors, such variation in spore number and colonization of a particular crop or set of crops may be influenced by soil temperature, moisture rainfall and other edaphic factors. The highest frequency of AMF spore and greater intensity of colonization was observed during dry season with marked decrease in wet rainy season (Schultz *et al.*, 1999; Panja and Chaudhuri, 2005). The same opinion on effect of seasonality on AMF spore density and colonization was put forward by Sharda and Rodrigues (2008) and they pronounced that the mean of total AMF spore number and colonization in papaya rhizosphere was the lowest in April and the highest in July whereas spore density was minimum in October and maximum in April in both years of observation as influenced by climatic as well as edaphic factors.

In the present studies fifteen AMF spore types were obtained from the rhizosphere of twelve forage crops of which *Glomus* spp. were dominant. Mandal *et al.* (2007) recovered eleven AMF spore type of three crop viz. *Zea mays*, *Allium cepa* and *Amaranthus viridis* and three weed species viz. *Cynodon dactylon*, *Commelina benghalensis* and



Glomus aggregatum (T1)



Glomus sp. 1 (T2)



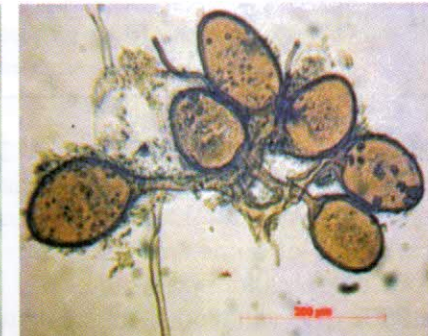
Glomus manihotis (T3)



Glomus mosseae (T4)



Glomus sp. 2 (T5)



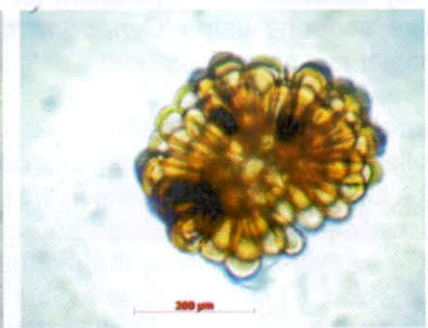
Glomus fasciculatum (T6)



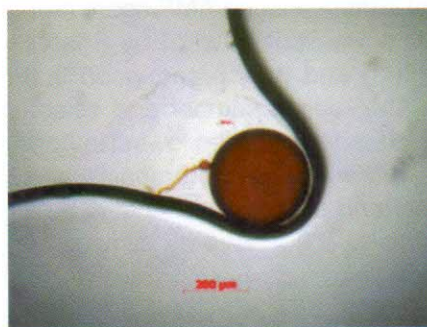
Acaulospora spp. (T7)



Acaulospora sorbiculata (T8)



Sclerocystis clavispora (T9)



Acaulospora gregaria (T10)



Glomus ambisporum (T11)



Unidentified spore (T12)

Fig. 1 : Microphotographs of some AM fungal spore types isolated from the rhizosphere of forage crops

Euphorbia hirta. In their studies, they indicated *Glomus* as the dominant genus followed by *Gigaspora*. Panja *et al.* (2007) retrieved fifteen AMF spore types from the rhizosphere of thirty three banana genotypes wherein *Glomus* was emerged as dominant genus in the rhizosphere of banana genotypes.

So, it can be concluded from the above mentioned results that mean AMF spore number was highest with sadabahar and signal grass and it was the lowest with hybrid napier whereas mean root colonization was found highest with signal grass and it was observed lowest with marvel grass. A total of fifteen AMF spore types were isolated and which belonged to four AMF fungal genera – *Glomus*, *Gigaspora*, *Sclerocystis* and *Acaulopsora*. *Glomus* with six identified species appeared to be dominant genus.

REFERENCES

- Azcon- Aguilar, C. and Barea, J.M. 1996. Arbuscular mycorrhizas and biological control of soil-borne plant pathogens, an overview of the biological mechanisms involved. *Mycorrhiza*. **6**:457-464.
- Davis, F.T., Olalde-Portugal, V., Aguilera-Gomez, L., Alvarado, M.J., Ferrera-Cerrato, R.C. and Boutton, T.W. 2002. Alleviation of drought stress of chile ancho pepper (*Capsicum annuum* L. Cv. San Luis) with arbuscular mycorrhiza indigenous to Mexico. *Sci. Hortic.* **92**:347-359
- Gaur, A. and Adholeya, A. 2004. Prospects of arbuscular mycorrhizal fungi in Phyto- remediation of heavy metal contaminated soils. *Curr. Sci.* **86**:528-534.
- Gemma, J. N. and Koske, R. E. 1989. Seasonal dynamics of selected species of V-A mycorrhizal fungi in a sand dune. *Mycological Research*. **92**:317-321.
- Gerdemann, J.W. and Nicolson, T.H. 1963. Spores of mycorrhizal *Endogone* species extract-ed from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.* **46**:235-244.
- Gianinazzi, S. and Schuepp, H. 1994. *Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystem*, Birkhauser Verlag, Basel, p.226.
- Kesava Rao, P.S., Tilak, K.V.B.R. and Arunachalam, V. 1990. Genetic variation for VA mycorrhiza-dependent phosphate mobilization in groundnut [*Arachis hypogaea* L.]. *Plant and Soil*. **122**:137-142.
- Lee, P. J. and Koske, R. E. 1994. *Gigaspora gigantea*: seasonal abundance and ageing of spores in a sand dune. *Mycological Research*. **98**: 453-457.
- Mandal, D., Panja, B.N., Sengupta, A., Saha, J. and Chaudhuri, S. 2007. Arbuscular mycorrhizal status of plants grown on Kolkata Municipal Waste and Sewage amended agricultural soil. *J. Interacad.* **11**: 432-439.
- Marschner, H. and Dell, B. 1994. Nutrient uptake in mycorrhizal symbiosis. *Plant and Soil*. **159**: 89-102.
- Miller, R.M. and Jastraw, J.D. 1992. The application of VA- mycorrhizae to ecosystem restoration and reclamation. In, *Mycorrhiza Functioning* (Ed. M.J. Allen), Chapman and Hall, New York, pp.438-467.
- Panja, B.N. and Chaudhuri, S. 1998. Effect of monoculture of some plants on inoculum build up and quantitative distribution of different VA-mycorrhizal species. *Mycorrhiza News*. **10**:13-15.
- Panja, B.N. and Chaudhuri, S. 2005. Effect of crop rotation on arbuscular mycorrhizal inoculum build up and infectivity status of soil. *J. Mycopathol. Res.* **43**:189-194.
- Panja, B.N., Mandal, D., Misra, D. and Saha, J. 2007. Diversity of arbuscular mycorrhizal fungi in the rhizosphere of banana genotypes. *J. Mycopathol. Res.* **45**: 54-57.
- Philips, J.M. and Hayman, D.S. 1970. Improved procedure for clearing roots and staining parasites and VA-mycorrhizal fungi for rapid assessment of infection. *Trans. Br. Mycol. Soc.* **55**:158-161.
- Ryan, M.H. and Graham, J.H. 2002. Is there a role for arbuscular mycorrhizal fungi in production agriculture? *Plant and Soil*. **244**: 263-271.
- Saif, S.R. 1986. Vesicular-arbuscular mycorrhizae in tropical forage species as influenced by season, soil texture, fertilizers, host species and ecotypes. *Angew. Bot.* **60**:125-139.
- Schenck, N.C. and Perez, Y. 1990. *Manual for the Identification of VA Mycorrhizal Fungi*. 3rd ed. Synergistic Publications, Gainesville, FL, USA, pp.286.
- Schultz, P. A., Bever, J. D. and Morton, J. B. 1999. *Acaulospora colossica* sp. nov. from an old field in North Carolina and morphological comparisons with similar species, *A. laevis* and *A. koskei*. *Mycologia* . **91**: 676-683.
- Sharda, W.K. and Rodrigues, B.F. 2008. Ecology of arbuscular mycorrhizal fungi associated with *Carica papaya* L. in agro-based ecosystem of Goa, India. *Tropical and Subtropical Agroecosystems*. **8**: 265-278.
- Van der Heijden, M.G.A., Klironomos, J.N., Usic, M., Peter, M., Streitwolf-Engel, R., Boller, T., Wiemken, A. and Sanders, S. 1998. Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature*. **396**: 60-72.