



An Investigation on Arbuscular Mycorrhizal Colonization in some Pteridophytes of West Bengal, India

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Abstract

Mycorrhizal colonization status of most of the pteridophytes is not known till date. In view of this perspective, the present study was undertaken to determine the arbuscular mycorrhizal colonization status in some common medicinally important pteridophytes growing in different habitats in West Bengal, India. As such *Pteris multifida*, *Pteris vittata*, *Adiantum capillus-veneris*, *Adiantum philippense* were collected as lithophytic pteridophytes, *Microsorium punctatum*, *Drynaria quercifolia* as epiphytes, *Ophioglossum reticulatum*, *Helminthostachys zeylanica* as terrestrial and *Azolla pinnata*, *Marsilea minuta* as aquatic pteridophytes. It was found that mycorrhizal colonization was highest in the terrestrial members and lowest in aquatic members. Both Paris and Arum types of arbuscular mycorrhizal colonizations were found in the members studied. The mycorrhizal fungi like, different species of *Glomus* and *Acaulospora* were found to be associated as root endophytes.

Keywords: Mycorrhizal colonization, Pteridophytes, Arum, Paris type, *Glomus*, *Acaulospora*.

1. Introduction

Mycorrhiza occurs over a broad ecological range from aquatic to desert environment (mosse, 1981). mycorrhizal fungi are known to enable plants to survive in the adverse environments by mediating nutrient and water fluxes (allen *et al.*, 2003; cariney and meharg, 2003; cook and lefor, 1998), provide soil nutrients to the plant and draw energy compounds from the host (john, 2005), take up more soil phosphorus and grow faster than corresponding non-mycorrhizal controlled plants (chakraborty *et al.*, 2007). in addition to phosphorus, the hyphae also transport other resources to the host such as ammonium, calcium, sulphur, potassium, zinc, copper and water (nasim, 2005). mycorrhiza formation has also been shown to confer drought and disease resistance, reduce pest damage and nematode infection, promote seed production and increase the

fitness of plant – offspring (rodriguez *et al.*, 2009). am fungi has important role in phytoremediation strategies for heavy metals (khan *et al.* 2000).

AM fungi are known to be well distributed along both the hemispheres and they are associated with about 80 % of the total land plants in the world (Giovannetti and Sbrana, 1998). It is interesting to note that the earliest evidence of arbuscular mycorrhizal association was recorded in the roots of *Rhynia*, one of the earliest known vascular land plants. Till date there are only a few investigations of mycorrhizal association in ferns (Boullard, 1957; Hepden, 1960; Cooper, 1976; Iqbal *et al.*, 1981; Berch and Kendrick, 1982; Gemma *et al.*, 1992; Zhao, 2000) and those have mainly concentrated on the occurrence of AM fungi and their evolutionary significance.

AM fungal colonization pattern has been grouped into 2 categories like, Arum type and Paris type depending upon the arrangements of fungal structures occurring in the roots of *Arum maculatum* and *Paris quadrifolia* (Gallud, 1905).

Chen *et al.* (2002) has hypothesized that in the low fertility soils where Chinese bracken fern (*Pteris vittata*) grows, AM fungi have a role in maintaining its productivity and may contribute significantly to arsenic uptake.

Information about the mycorrhizal status of aquatic plants is rare, Arbuscular mycorrhizal association in aquatic plants was first reported by Sandergaard and Laggard (1977).

Knowledge of the seasonal pattern of infection is necessary to quantify the functioning and ecological significance of VAM. Periods during which mycorrhizal infection is high are those when the fungus is most likely to influence plant nutrient status and exert a demand for carbon from the plant. If periods within the growth season exist when numbers of arbuscules are high, this could be a time when nutritional benefits to the host occur. Similarly, when a rapid increase in the abundance of hyphae and vesicles occurs in the root, this could be a period in which the fungus acts as a significant carbon sink. There is little information about spatial and temporal patterns of VAM infection and although there have been a few studies in a variety of ecosystems, they show no clear pattern.. The seasonal variation in the activity of VAM is still unknown and based on relatively few data (Gemma and Koske, 1988). Considering many useful aspects of mycorrhiza and the lacuna of research works based on the effect of seasonal variations in the mycorrhizal colonization frequency in the host plants, the present work was undertaken to explore the mycorrhizal status and their variations of occurrence in pteridophytes growing in different ecological niches of West Bengal.

2. Materials and Methods

2.1 Site of collection

All the test plants (*Pteris vittata* L., *Pteris multifida* Poir., *Adiantum capillus-veneris* L., *Adiantum philippense* L., *Microsorium punctatum* (L.) Copel., *Drynaria quercifolia* (L.) J. Sm., *Ophioglossum reticulatum* L., *Helminthostachys zeylanica* (L.) Hook., *Azolla pinnata* R. Br. and *Marsilea minuta* L. were collected from different habitats (lithophytes,

epiphytes, terrestrial, aquatic) of Burdwan, Bankura, Birbhum, Howrah and Darjeeling districts of West Bengal during summer months without disturbing the local biodiversity.

2.2 Identification of the plants

The plants were identified by using the herbarium of Botany Department, Burdwan University, Burdwan.

2.3 Collection of root samples

For each species, the feeder roots were collected directly from the plants by digging and tearing the roots upto the base of the main stem.

2.4 Maintenance and preservation of roots

The root samples after collection were thoroughly washed in running tap water and rootlets were selected and cut into small pieces and fixed in formaldehyde/acetic acid solution (Johanson 1940).

2.5 Collection of soil sample

Soil sample of about 10 gm was collected from the root region (rhizosphere) of each of the species by digging the soil upto a depth of 10 cm and collected into polythene bags, labeled and stored at 4 C until analysis.

2.6 Preparation of root samples

For each specimen, 100 feeder root pieces were thoroughly washed in water and boiled at 95° C temp. for different durations in 10% KOH. The segments were washed in distilled water, acidified with 1(N) HCL and stained in 0.05% trypan blue in lactophenol, the excess stain was removed by washing with lactophenol. Root segments were mounted temporarily on slides in acetic acid, glycerol (1:1 V/V)

2.7 Assessment of vesicular arbuscular mycorrhizal (VAM) fungal association in roots

The vesicular arbuscular mycorrhizal (VAM) association in each specimen was examined in the roots following the method of Phillips and Hayman (1970) and was calculated as percentage of mycorrhizal association.

$$\% \text{ Mycorrhizal association} = \frac{\text{No. of mycorrhiza associated segments} \times 100}{\text{Total no. of segmented scored}}$$

2.8 Collection of mycorrhizal spores from soil samples

Mycorrhizal spores were obtained by wet sieving and decanting technique (Gerdemann and Nicolson, 1963). The solution was passed through 350, 150, 53 and 45 µm sieve and the spores were collected from the residue collected from 53 µm sieve. This residue was dissolved in distilled water and filtered. The residue present in the filter paper was taken and mounted on a slide in lactophenol and cotton blue and were examined under microscope (Leica, model no. DMLB 3000).

2.9 Spore count

The decantant were filtered through a filter paper with grid lines. The filter paper was then spread on a glass plate under a dissecting microscope and counted and expressed as spores per 10g of dry soil.

3. Results and Discussion

Mycorrhizal colonization was observed in each of the root samples studied (Table 1). Both vesicles and arbuscules were found in *Pteris multifida*, *Pteris vittata*, *Adiantum philippense*, *Adiantum capillus-veneris*, *Helminthostachys zeylanica*, *Marsilea minuta* and *Microsorium punctatum*. Vesicles were present in all the test plant materials.

Arbuscules was not recorded in *Ophioglossum reticulatum*, *Azolla pinnata* and *Drynaria quercifolia*. Vesicular colonization was highest in *Pteris multifida* which was recorded to be 36% in average and was lowest in *Adiantum philippense* (0.33%). In average, arbuscular colonization in the roots was maximum in *Pteris vittata* (31%) followed by *Helminthostachys zeylanica* (28.33%), *Marsilea minuta* (27%), *Pteris multifida* (24.66%) and *Adiantum capillus-veneris* (23.33%).

Intra-radical spores were found in all the pteridophytes except *pteris multifida* and *Drynaria quercifolia* and was recorded to be highest in *Microsorium punctatum* (33.33%). In case of soil analysis, spores showed a great deal of variation in respect to their morphological characters. In the present study, spores were observed in all the species studied except *Ophioglossum reticulatum*, *Azolla pinnata* and *Drynaria quercifolia*. The highest amount of soil spores were recorded in *Pteris multifida* (396.66).

In *Pteris multifida*, *Pteris vittata* and *Ophioglossum reticulatum* mycorrhizal colonization pattern was appeared to be 'Paris' type as because mycorrhizal structures were intracellular in position. But in *Adiantum capillus-veneris*, *Adiantum philippense* and *Microsorium punctatum* arbuscular mycorrhizal colonization was found to be 'Arum' type as mycorrhizal structures were intercellular in position. In *Helminthostachys zeylanica* the colonization pattern was of Intermediate type because of having mycorrhizal structures both in intra and inter cellular position. In *Azolla pinnata*, *Marsilea minuta* and *Drynaria quercifolia* arbuscular mycorrhizal colonization was not distinct.

In each and every cases rainy season scored lowest percentage of arbuscules excluding *M. minuta* where rainy season scored highest arbuscular percentage. During winter the arbuscular percentage reached at the peak level and it remained in moderate form during spring to summer. On the other hand, in *M. punctatum* there remained no arbuscules during rainy and spring to summer seasons and the same observation was being recorded in *M. minuta* where no arbuscules were recorded during spring to summer. Arbuscules were being absent in *O. reticulatum*, *D. quercifolia* and *A. pinnata*.

Considering vesicular colonization, it was recorded that in *M. punctatum*, *A. pinnata* and *M. minuta* no vesicle was observed during winter and rainy seasons whereas in *A. philippense* vesicles were absent during spring to summer and rainy seasons. *P. vittata* and *P. multifida* exhibited higher percentage of vesicular colonization during winter, however, throughout spring to summer it remained moderate and during rainy season vesicles were entirely absent. In *A. capillus veneris* it was higher during spring to summer and in winter the percentage of vesicular colonization maintained to be moderate. In *H. zeylanica* vesicular immigration percentage was the same (10%) throughout spring to summer and winter which declined to bare minimum (1%) during rainy season. In *P. multifida* intraradical spores were not observed. During rainy season in *P. vittata*, *A. philippense*, *H. zeylanica* intraradical spores were not recorded. In *A. pinnata* and *M. minuta* no intraradical spore was found during winter. Highest incidence (80%) of intraradical spore was recorded in *M. punctatum* during winter.

Table1: Arbuscular Mycorrhizal Colonization Status in the Pteridophytes

Name of the plants	Habitat	Season of collection	Mycorrhizal colonization						Mycorrhizal type	Spore /100 g soil *	
			Arbuscular %*		Vesicular%*		Intra-radical spore % *			Seasonal	Annual
			Seasonal	Annual	Seasonal	Annual	Seasonal	Annual			
<i>Pteris vittata</i>	Lithophytic	Spring to Summer	28 ± 0.89	31 ±0.90	4 ± 0.01	8 ±0.07	9 ± 0.20	6 ±0.07	Paris	300 ± 21.90	283.34 ±
		Rainy	15 ± 0.90		0		0			150 ± 10.07	
		Winter	50 ± 0.75		20 ± 0.50		9 ± 0.10			400 ± 0.77	
<i>Pteris multifida</i>	Lithophytic	Spring to Summer	25 ± 0.50	24.66 ± 1.0	12 ± 0.40	36 ± 0.04	0	0	Paris	410 ± 0.4	396.66 ±
		Rainy	10 ± 0.62		5 ± 0.12		0			330 ± 10.25	
		Winter	39 ± 0.41		19 ± 0.25		0			450 ± 14.78	
<i>Adiantum capillus-veneris</i>	Lithophytic	Spring to Summer	14 ± 0.33	23.33 ±0.97	15 ± 0.23	12 ±0.21	4 ± 0.31	4.67 ±0.09	Arum	330 ± 0.10	286.70 ±
		Rainy	5 ± 0.37		8 ± 0.14		1 ± 0.01			100 ± 0.22	

		Winter	51 ± 0.35		13 ± 0.11		9 ± 0.08			430 ± 0.11	
<i>Adiantum philippense</i>	Lithophytic	Spring to Summer	30 ± 0.99	21.67 ± 0.67	0	0.33 ± 0.13	2 ± 0.09	3 ± 0/03	Arum	370 ± 0/87	333.33 ±
		Rainy	5 ± 0.80		0		0			210 ± 11.20	
		Winter	30 ± 0.77		1 ± 0.01		1 ± 0.01			420 ± 10.56	
<i>Ophioglossum reticulatum</i>	Terrestrial	Spring to Summer	0	0	11.0 ± 0.70	17.00 ± 0.30	14 ± 0.42	4.66 ± 0.08	Paris	0	0
		Rainy	0		0		0			0	
		Winter	0		40 ± 0.50		0			0	
<i>Helminthostachys zeylanica</i>	Terrestrial	Spring to Summer	30 ± 0.72	28.34 ± 0.88	10 ± 0.12	7 ± 0.12	10 ± 0.21	3.33 ± 0.22	Intermediate	210 ± 0.16	170 ±
		Rainy	10 ± 0.50		1 ± 0.01		0			100 ± 0.98	
		Winter	45 ± 0.81		10 ± 0.22		0			200 ± 0.8	

<i>Drynaria quercifolia</i>	Epiphytic	Spring to Summer	0	0	5.0 ± 0.11	11.0 ± 0.41	0	0	Not distinct	0	0
		Rainy	0		0		0			0	
		Winter	0		0 28 ± 0.81		0			0	
<i>Microsorium punctatum</i>	Epiphytic	Spring to Summer	0	16.66 ± 1.71	26 ± 0.39	8.67 ± 0.08	10 ± 0.02	33.33 ± 0.13	Arum	180 ± 13.3 =	160 ±
		Rainy	0		0		10 ± 0.01			100 ± 0.32	
		Winter	50 ± 0.56		0		80 ± 0.17			200 ±	
<i>Azolla pinnata</i>	Aquatic	Spring to Summer	0	0	10 ± 0.24	3.33 ± 0.42	8 ± 0.01	5.66 ± 0.09	Not distinct	0	0
		Rainy	0		0		9 ± 0.03			0	
		Winter	0		0		0			0	
<i>Marsilea minuta</i>	Aquatic	Spring to Summer	0	27 ± 2.00	21 ± 0.04	7 ± 0.20	8 ± 0.07	6 ± 0.2	Not distinct	100 ±	156.67 ±
		Rainy	65 ± 0.40		0		10 ± 0.12			270 ±	
		Winter	16 ± 0.24		0		0			100 ±	

* Data are the mean values of ten replicates

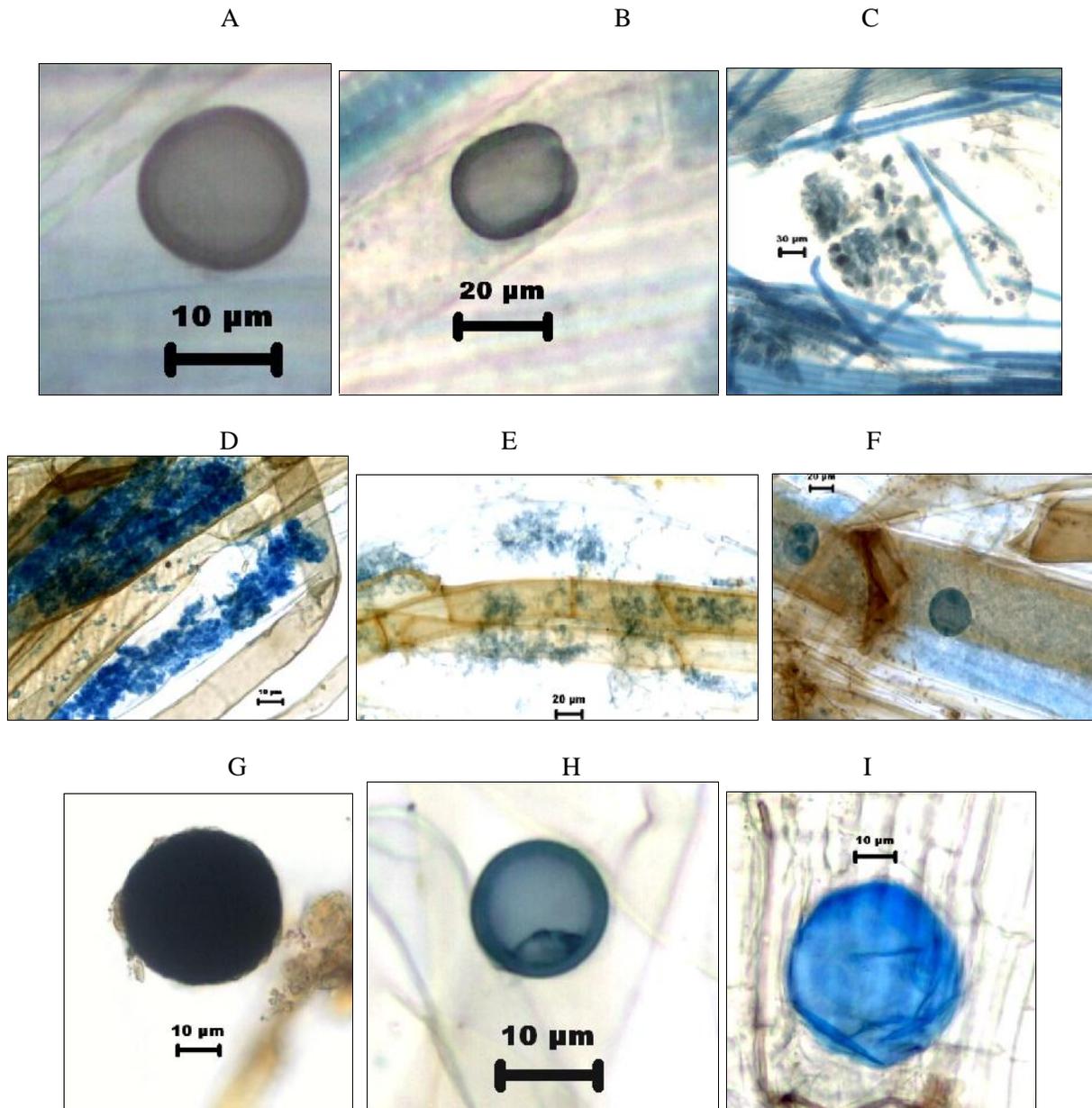


Figure: A,B,H Spore of *Glomus sp.* C-arbuscules within the root OF *Adiantum capillus veneris* D, Arbuscules within the root of *Pteris multifida*, E. , Arbuscules within the root of *Pteris vitata* ,F.Vesicles and arbuscules within the root of *Dryneria quercifolia*, G. Spore OF *Glomus sp.* I.Chlamydospore of *Glomus sp.*

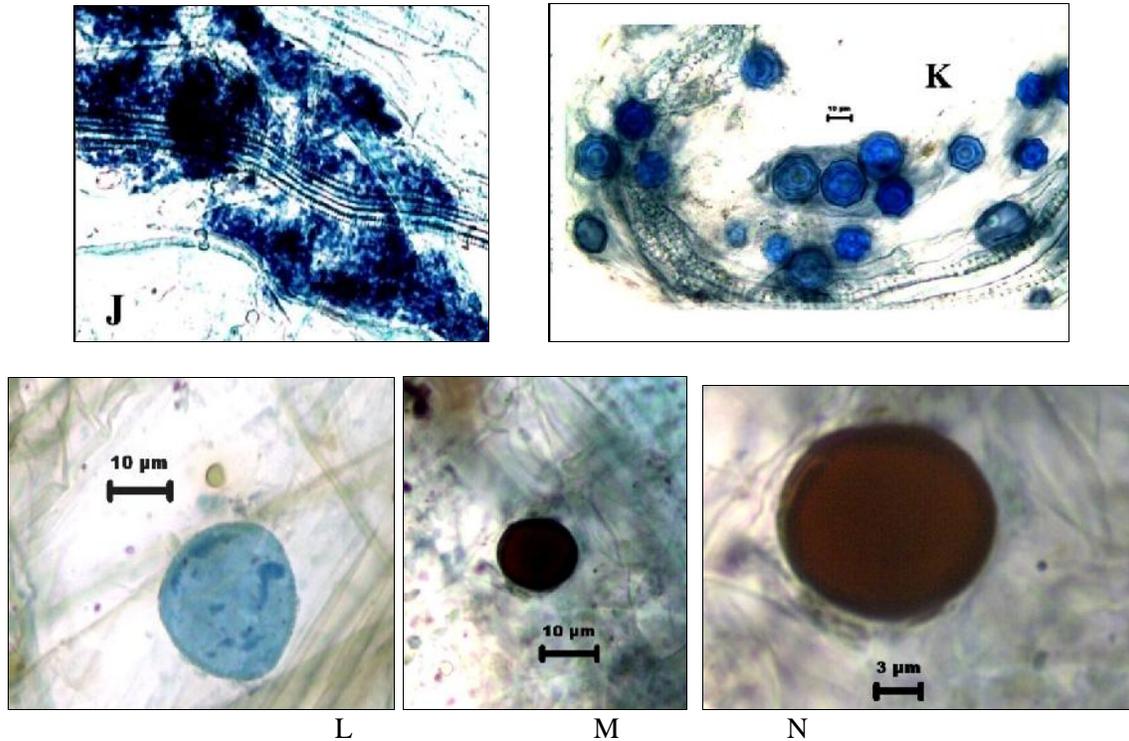


Figure: J. Arbuscules within root of *Adiantum phillipense*, K. Chlamyospore within the root of *Ampelopteris prolifera*, L. Chlamyospore of *Glomus* sp within the root of *Azolla pinnata*, M. Chlamyospore of *Glomus gesporum*. N. Spore of *Glomus* sp.

Extraradical soil spore count was highest (450) in *P. multifida* during winter. In almost all the cases soil spore count remained minimum during rainy season, moderate during summer and maximum during winter except *M. minuta* in which spore count during rainy season remained higher.

From the results it is apparent that the lithophytic pteridophytes scored higher percentage of root colonization by vesicles and arbuscules and mycorrhizal spore count. But intraradical spore (33.33%) was highest in epiphytic pteridophyte *M. punctatum* and was nil in *P. multifida* (lithophytic pteridophyte). In aquatic pteridophyte *Azolla pinnata* arbuscular percentage was recorded to be nil.

Among the species studied, vesicles were observed in all the species but arbuscules were present only in five species viz. *Pteris vittata*, *Pteris multifida*, *Adiantum capillus-veneris*, *Adiantum philippense* and *Helminthostachys zeylanica*. Absence of arbuscules in *Ophioglossum reticulatum*, *Azolla pinnata*, *Microsorium punctatum*, *Helminthostachys zeylanica* and *Drynaria quercifolia* in summer months may be due to the reason that they formed it earlier.

The percentage of mycorrhizal colonizations in the pteridophytes were found to be the lower than those recorded in angiosperms and this result corroborates with the reports of Lehnert et al. (1996) who observed higher percentage of mycorrhizal colonization in the angiosperms than in the vascular cryptogams.

Zhao (2000), Zhang et al. (2004) and Winther and Friedman (2007) have studied the occurrence of AM infections in sporophytes and gametophytes of different seedless vascular plants, including several *Lycopodium* and *Equisetum* species. According to Read et al. (2000) and Winther and Friedman (2007), *Lycopodium* gametophytes appear to be obligatory mycotrophic, but there is little consensus regarding the AM associations in the sporophytes. On the other hand, Read et al. (2000) pointed out at those surveys where the mycorrhizal status of different *Equisetum* species have shown that the gametophytic generation seems to be non-mycorrhizal and the sporophytes could be non-mycorrhizal as well as capable of forming AM association. From this analysis, it becomes apparent that a significant discrepancy exists in our knowledge of the occurrence of endomycorrhizal symbioses in different vascular cryptogams.

The mycorrhizal fungal association have been found in all the ecological niches in present study. Trappe (1996) reported the presence of mycorrhizal fungi in nearly all soils throughout the world which form symbiotic associations with the roots.

The test plants in the present study were taken from different habitats viz. terrestrial, lithophytic, epiphytic and aquatic. Among these, the terrestrial and lithophytic species showed higher amount of AM infection than epiphytic and aquatic species. The presence of mycorrhizal endophytes in plants is influenced by several factors such as host types, species diversity, degree of stability of habitat, extent of fertilizers usage and edaphic factors like season, soil texture and moisture content, soil nutrient level etc. (Nicolson 1960, Raghupathy and Mahadevan 1991, Muthukumar and Udaiyan 2000, Gorski et al. 2002, Wang and Qiu 2006, Prasher et al. 2006, Prasher and Baghla 2007). The plants of these two habitats viz. epiphytic and aquatic species were rarely colonized by AMF and this may probably be due to the fact that the spores of mycorrhizal fungi are not easily dispersed from the soil. Furthermore, the most AMF are dependent on their host, requiring the presence of a facultatively mycorrhizal plant for successfully establishing the symbiosis on a chlorophyte (Janos et al. 1993). Thus, the presence of low percentage of AMF in epiphytes and aquatic species was not surprising. Within these pteridophytes, hyphal coils were found extensively in the root cortex. The Paris type was present in *Pteris vittata*, *Pteris multifida* and *Ophioglossum reticulatum*. Arum type was found in *Adiantum capillus-veneris*, *Adiantum philippense* and *Microsorium punctatum*. In *Marsilea minuta*, *Azolla pinnata* and *Drynaria quercifolia* no clear colonization type could be ascertained. In *Helmithostachys zeylanica*, intermediate type of mycorrhizal colonization was found. The different AM structures may have specialized roles in the transfer of inorganic nutrients and organic carbon between the partners of the symbiosis (Smith and Read 1997, Smith and Smith 1997). It is assumed that the vesicles being the storage organs of the AM fungi are generally produced at comparatively later stages of growth (Powell and Bagyaraj 1984). Bajwa et al.(2001) while surveyed on AM association in wetland plants noticed that the vesicular infection in general starts in spring and reaches to its maximum in summer, autumn or winter depending upon the host species.

Another possible reason for their disparity in the pattern of vesicle development may correspond to variations in period of completion of life cycles of various AM fungal species involved in forming this association. In a survey on AMF associations in vascular plants by Bajwa et al. (2001) it was recorded that AM colonization remained stable and it was higher in spring and summer but maximum during winter. Among the monocots, *Vetiveria zizanioides* showed the lowest but stabilized spore population without any periodic response from spring to autumn which sharply increased to maximum in winter. These results corroborate with our present findings where AM colonization percentage in the roots of our studied plants was maximum in winter.

In case of soil spore analysis, higher percentages have been observed in all the terrestrial, and lithophytic plants. Among these, the highest number of spores were observed in the soil of the two species of *Pteris*, whereas the lowest number was observed in the soil of the two species of *Adiantum*. In case of two epiphytic species, soil samples collected from the base soil on their host plants were examined, but no significant result could be detected. No spores were found in *Marsilea minuta* and *Azolla pinnata*. It may certainly be due to the aquatic nature of these plants. Identification of the mycorrhizal flora in present study signifies that *Glomus* species is the dominant mycorrhizal fungi; however, species of *Acaulospora* has been observed. Enormous studies have been done regarding mycorrhizal colonization in higher plants but in pteridophytes there is almost a virgin field. Considering the significance of mycorrhizal colonization in almost every aspect of plant science, detailed study in this group regarding AM colonization may be extremely significant.

4. Conclusion

Though a number of studies have been made concerning mycorrhizal colonization in higher group of plants but virtually there is no work on the arbuscular mycorrhizal colonization status in pteridophytes of West Bengal, India. Hence, the present study may certainly have utmost significance in basic science and the arbuscular mycorrhizal species obtained in the study signifies the biodiversity of this group of root endophytes in this area.

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