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Seasonal Variation in VAM in Wetland Plants

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Abstract: Seasonal variation in vesicular arbuscular mycorrhizae (VAM) of eight aquatic and subaquatic vascular plant species viz. *Thelypteris dentata*, *Marsilea minuta* and *Adiantum* sp (pteridophytes), *Polygonum barbatum* and *Oenanthe javanica* (dicotyledonous), *Paspalum paspalodes*, *Saccharum spontaneum* and *Vetiveria zizanioides* (monocotyledonous) was studied. Pteridophytes in general showed maximum VAM colonization during spring with gradual reduction in the following seasons. VAM colonization in species of dicotyledonous and monocotyledonous showed a variable response to different seasons. A total of six mycorrhiza forming species belonging to a single genus, *Glomus*, were found to be associated with the test species. Among these *G. fasciculatum* and *G. mosseae* were the most common species and showed a marked variation and typical pattern of seasonal distribution i.e. maximum in spring with the gradual reduction in the following seasons. The other four species were rather inconsistent and lack any seasonal response.

Key words: Seasonal variation, VA mycorrhiza, wetland plants

Introduction

Vesicular arbuscular mycorrhizal (VAM) fungi, a ubiquitous occurrence in the roots of vascular plants in nature (Gabor, 1992), are known for their importance in plant growth, uptake of nutrients, biological N₂-fixation, hormonal production and resistance to soil pathogens of terrestrial plants (Jefferies, 1987). The usefulness of VA mycorrhiza to the host plant depends on VAM fungi, host and prevailing environmental conditions and their interactions among themselves during the crop period (Bhandari *et al.*, 1990). VAM colonization of host plant and spore production in soil varies seasonally as a function of climate and host plant (Giovannetti, 1985). Shamim *et al.* (1994) studied the influence of seasonal variation on VAM infection in 24 perennial land plants and found that VAM colonization was maximum in spring and gradually decreases in the following seasons, till it becomes least in winter. Kawai *et al.* (1986), however, observed maximum VAM infection in October in roots of grapevine. In contrast to land plants, few studies have considered the occurrence and role of VAM in aquatic plants. For a long time hydrophytes have been regarded as non-mycorrhizal (Khan, 1974). At present VAM have been reported in aquatic angiosperms (Bajwa and Javaid, 1997), aquatic pteridophytes (Nasim, 1990) and bryophytes (Iqbal *et al.*, 1988). The investigations so far on aquatic VAM are merely restricted to its occurrence. The studies regarding the role of VAM in hydrophytes and effects of environmental factors on VAM in aquatic environment have not attracted the due attention of the workers. Studies on aquatic VA mycorrhizal flora are also inconclusive. The present investigations, therefore, were designed to study the seasonal variation in VAM colonization and flora in aquatic and sub-aquatic plants belonging to three different groups viz. Pteridophytes, dicotyledons and monocotyledons.

Materials and Methods

An area of 5 km along the BRB Canal, from Daka Bridge eastwards was selected as sampling site. In this region the canal bank facing the north was inhabited by a variety of abundant vegetation including bryophytes, pteridophytes and angiosperms. A total of eight vascular plant species were chosen as test species for mycorrhizal studies from this subaquatic habitat. These included three pteridophytes namely *Thelypteris dentata*, *Marsilea minuta* and *Adiantum* sp., two species of dicotyledons viz., *Polygonum barbatum* and *Oenanthe javanica* and three monocotyledonous species namely *Paspalum paspalodes*, *Saccharum spontaneum* and

Vetiveria zizanioides. The periodic survey and sampling was designed and scheduled to spread it over from November 1998 to June 1999. The sampling schedule was as follows:

I	Sampling	15th November 1998 (Autumn)
II	Sampling	30th January 1999 (Winter)
III	Sampling	13th April 1999 (Spring)
IV	Sampling	5th June 1999 (Summer)

At the time of sampling plants of selected test species were carefully uprooted along with rhizospheric soil. Roots of all the test species after thorough washing with tap water were cut into 1-cm pieces and cleared in 10 percent KOH by autoclaving at a pressure of 15 lb/inch² for 3 minutes. The cleared roots were stained with 0.05% trypan blue solution following the procedure of Philips and Hayman (1970). The stained root pieces were spread on glass slide and mounted in lactic acid. Forty five root pieces of each test species were studied at each sampling. Percentage of various mycorrhizal structures viz. mycelium, arbuscules and vesicles, was recorded. The vesicular and arbuscular infections were quantified by counting these structures per 100-cm of root length. Extent of VAM infection was assessed by slide length technique (Giovannetti and Mosse, 1980).

VA mycorrhizal spores from the rhizospheric soil samples of test species were extracted by wet sieving and decanting technique (Gerdemann and Nicolson, 1963). Number of spores/100 g of soil were recorded. Careful taxonomic studies were carried out under the light microscope and various species were identified using the synoptic key by Trappe (1982).

Results

Seasonal variation in mycelial infection and extent of VAM

infection: Status of mycelial infection and extent of VAM (cm/100 cm) in various species is presented in Fig. 1. It is evident from the data that, in general the most well established colonization and typical pattern of mycorrhizal development was observed in pteridophytes (Fig. 1 A & D). Among these the *Adiantum* sp harboured the maximum mycelial infection with almost stabilized levels of percentage and extent of VAM throughout the year. A definite seasonal response, however, is clearly indicated by all the three species. Intensity of mycelial colonization being highest in spring and summer, started declining in autumn and minimum in winter (Fig. 1 A & D).

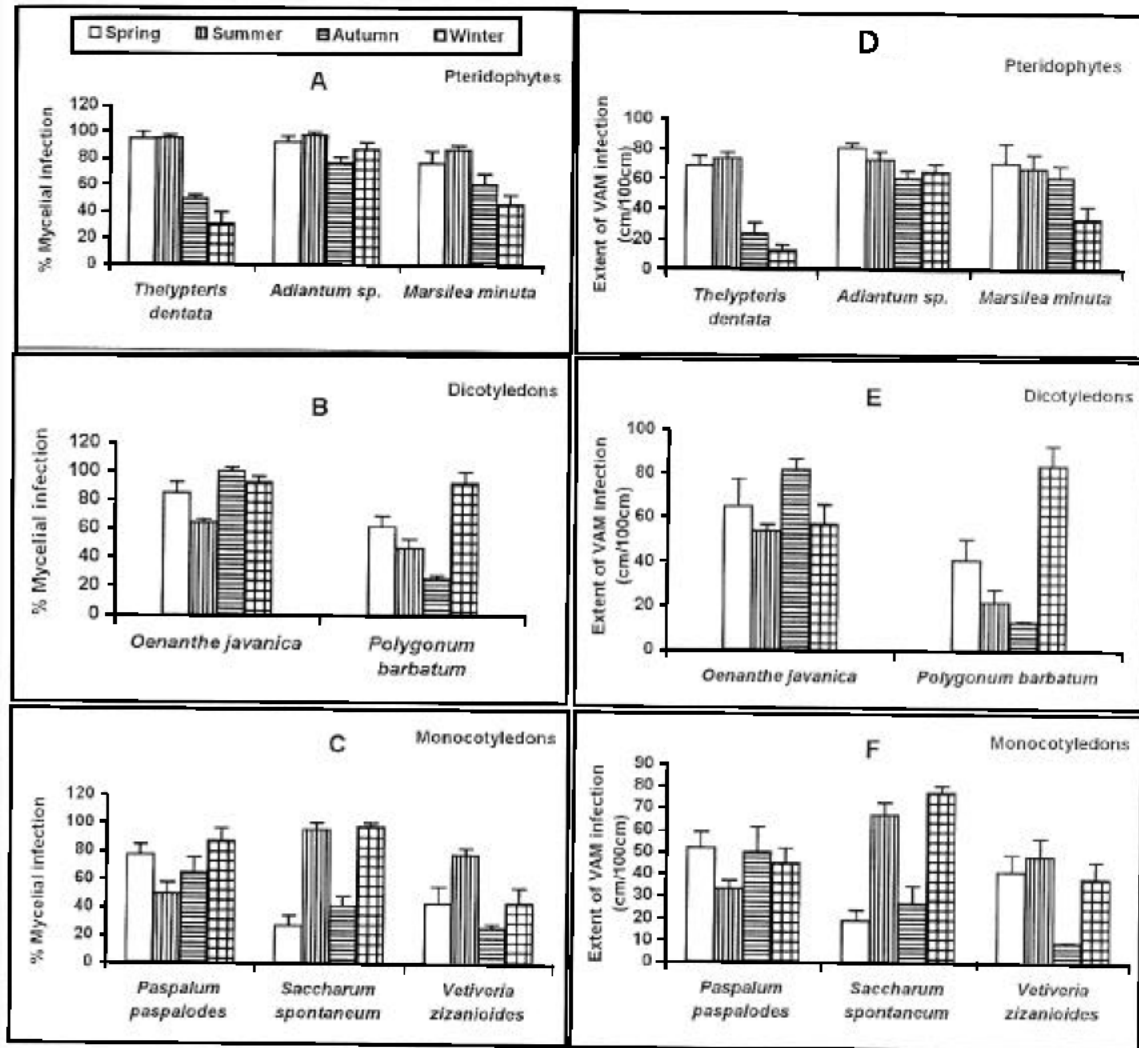


Fig. 1(A-F): Seasonal variation in mycelial infection in wetland plants

A rather atypical pattern of mycelial infection was observed in two dicotyledonous species. Apparently, *O. javanica* showed the maximum degree of infection, only with a slight depression in summer but as far as the general pattern and seasonal variation is concerned, a contrasting response was noticed in these species. Maximum mycelial and extent of infection was attained in winter and minimum in summer (Fig. 1 B & E). The monocotyledonous species displayed an irregular pattern of mycorrhizal colonization except for *P. paspalodes*. In this species the development of mycorrhizal infection seemingly started in summer and increased gradually in the autumn and winter and remained consistent till spring. *S. spontaneum* and *V. zizanioides* showed peaks of infection both in summer and winter and lowest in spring and autumn (Fig. 1 C & F).

Seasonal variation in arbuscular infection: Data on arbuscular infection was estimated both on percentage basis and number of arbuscules in the mycorrhizal roots of different species (Fig. 2). A consistently more stabilized infection, similar to mycelial

infection among pteridophytes was observed in *Adiantum sp.* (Fig. 2 A & D). *T. dentata* followed the typical and parallel seasonal response as in mycelial development. Maximum arbuscular infection was achieved in spring which gradually declined over summer, autumn and winter. In *M. minuta* the arbuscular infection in general remained very low except in autumn when a sudden and sharp increase, both in percentage and number of arbuscules was evidenced (Fig. 2A & D). Both highest and lowest intensity of arbuscular infection was recorded in dicotyledonous species, the highest in *O. javanica* and lowest in *P. barbatum* and particularly with markedly declined numbers of arbuscules in this species (Fig. 2B & F). Among the monocotyledonous species, *P. paspalodes* showed consistently low number and percentage of arbuscules from summer onwards with a slight increase in spring. The development of arbuscular infection and seasonal response, parallel to mycelial infection was, however recorded in the rest of the two monocotyledonous species. The highest intensities of infection were attained once in summer and winter in both *S. spontaneum* and *V. zizanioides* (Fig. 2C & F).

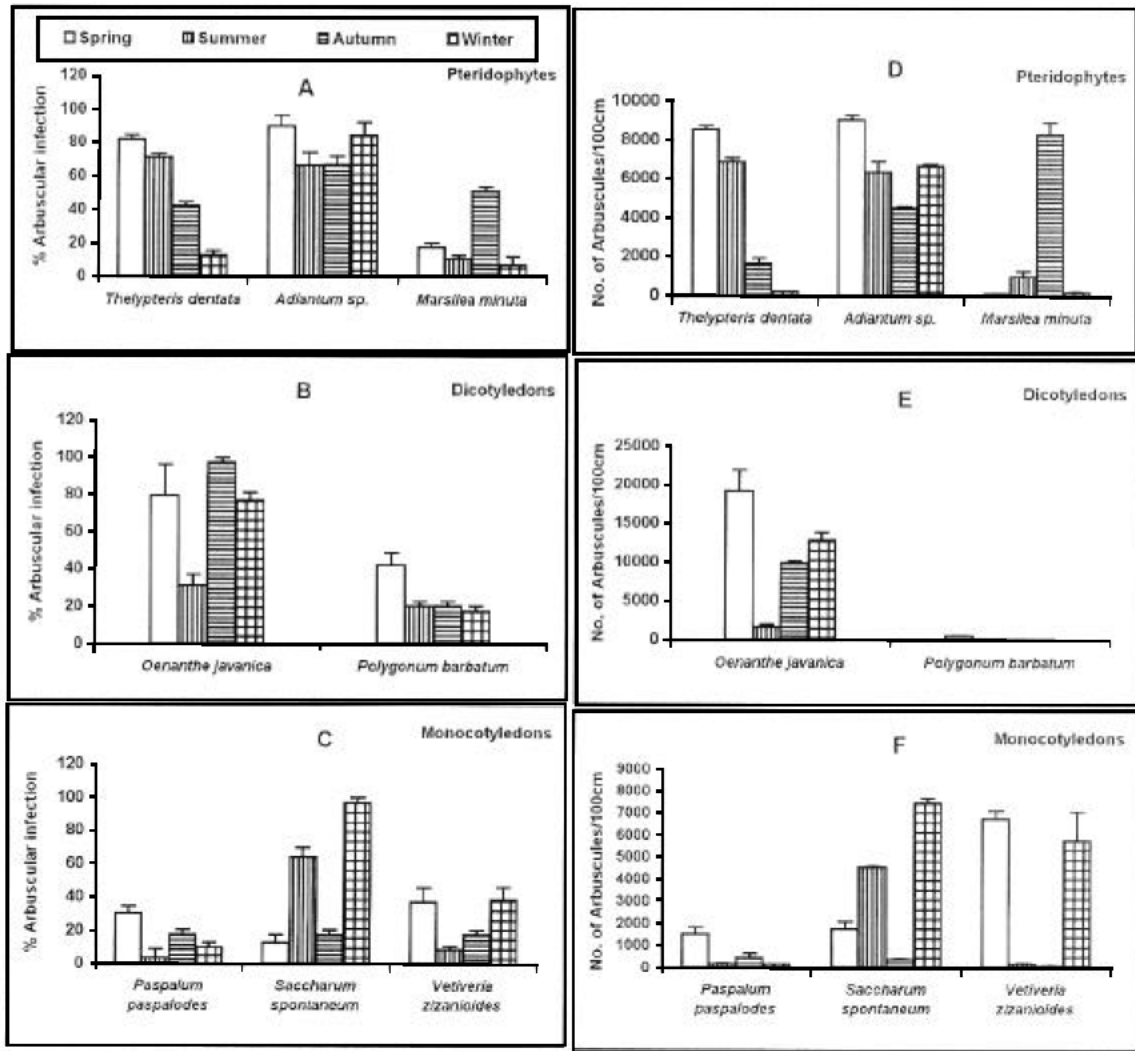


Fig. 2 (A-F): Seasonal variation in arbuscular infection in wetland plants

Seasonal variation in vesicular infection: The assessment of vesicular infection in various groups of test species (Fig. 3) clearly indicates that the pteridophytes species, in general, showed the minimum development of vesicles. Except for *M. minuta* in which the vesicular infection was found to be sharply stimulated in summer in response to declined arbuscular infection. The vesicular infection in other species followed the pattern of mycelial and arbuscular infections for maximum and minimum rate of development (Fig. 3A & D). As far as the pattern of vesicular infection in dicot species is concerned the general pattern of infection development and seasonal response was very obvious. The number of vesicles in the spring remained significantly low when arbuscular infection was highest in the early phase of infection and it increased gradually during the later growth period (Fig. 3B & E). Assays of vesicle formation in monocot species revealed that although the number of vesicle remained low, the relative percentage infection was better than in other groups of species. Seasonal response was well defined in *P. paspalodes*. The vesicular infection was found to increase gradually and

consistently from summer to winter in contrast to arbuscular and mycelial infections which were highest in spring. Both *S. spontaneum* and *V. zizanioides* showed irregular pattern of development of vesicular infection in different seasons. Both percentage and number of vesicles increased very sharply in *S. spontaneum* during summer and in early summer and winter in *V. zizanioides* (Fig. 3C & F).

Seasonal variation in VAM flora: Relative population counts estimated on the basis of 100 g of soil sample showed the maximum number in *O. javanica* and minimum in association with *V. zizanioides*. Marked seasonal variation was evident in most of the species. In pteridophytes the spore number increased sharply in winter (Fig. 4). The spore population in association with dicotyledonous species remained consistently high except a decline in autumn. Among the monocots, *V. zizanioides* showed the lowest but stabilized spore population without any periodic response from spring to autumn but sharply increased to maximum in winter. *P. paspalodes* and *S. spontaneum* showed the typical seasonal response, initially the

Bajwa *et al.*: Seasonal variation in VAM

Table 1: Seasonal variation in VA mycorrhizal flora in wet-land plants

	Test species							
	<i>T. dentata</i>	<i>Adiantum sp.</i>	<i>M. minuta</i>	<i>O. javanica</i>	<i>P. barbatum</i>	<i>S. spontaneum</i>	<i>P. paspalodes</i>	<i>V. zizanioides</i>
<i>Glomus mosseae</i>								
Sprint	18c	28bc	31d	34c	106c	20c	19c	24c
Summer	11	15	53	176	68	20	27	23
Autumn	35	41	20	88	15	62	53	21
Winter	305	192	77	170	53	170	92	133
<i>G. fasciculatum</i>								
Spring	25	39	37	230	64	12	54	16
Summer	16	14	101	15	17	7	16	34
Autumn	51	43	53	19	13	53	123	30
Winter	118	112	111	106	30	143	176	101
<i>G. microcarpum</i>								
Spring	0	17	17	3	8	8	0	0
Summer	0	8	37	136	0	10	8	0
Autumn	0	15	17	0	0	19	0	0
Winter	0	14	38	0	0	42	0	0
<i>G. macrocarpum</i>								
Spring	0	0	12	5	13	6	11	0
Summer	0	0	18	11	15	9	0	0
Autumn	0	0	0	0	0	8	0	0
Winter	0	0	0	0	0	26	0	0
<i>G. monosporum</i>								
Spring	5	0	0	0	0	0	0	0
Summer	0	0	0	0	0	0	0	0
Autumn	0	0	0	0	0	0	0	0
Winter	0	0	0	0	0	0	0	0
<i>G. reticulatum</i>								
Spring	7	0	0	2	0	0	0	0
Summer	9	0	0	0	0	0	0	0
Autumn	9	0	0	0	0	0	0	0
Winter	0	0	0	0	0	0	0	0

Values with different letters show significant difference among the seasons as determined by DMRT Test

number of spores was low then a marked increase from autumn onward was observed (Fig. 4).

Six endogoneaceous VAM species belonging to a single genus, *Glomus* were isolated from the rhizospheric soils of the test species. Among these *G. fasciculatum* and *G. mosseae* were found to be most prevalent. *G. microcarpum* and *G. macrocarpum* were comparatively low, where as *G. monosporum* and *G. reticulatum* were met rarely (Table 1). *G. fasciculatum* and *G. mosseae* showed typical seasonal pattern in their distribution. However slight deviation from this pattern was observed in *G. microcarpum* and *G. macrocarpum* while *G. monosporum* and *G. reticulatum* did not show any seasonal pattern. As *G. monosporum* met only once in spring along with *T. dentata* and *G. reticulatum* occurred only in very low number in spring in association with *O. javanica* and from spring to autumn with *T. dentata* (Table 1).

Discussion

In the present study all the different group viz., pteridophytes, dicots and monocots have been found harbouring VA mycorrhizal infection in their roots. These results are in line with some of the earlier works in which presence of VAM infection in hydrophytes have similarly been reported (Iqbal *et al.*, 1988; Nasim, 1990; Khan, 1993; Bajwa and Javaid, 1997). VAM colonization under aquatic and sub-aquatic habitat showed that these fungi are highly adaptive to a variety of environments (Allen *et al.*, 1989). The infection in the roots of these plants suggests that hydrophytes also provide a habitat suited to mycorrhiza as do the terrestrial plant roots (Lodge, 1989) and oxygen requirements of VAM fungi are fulfilled either by atmospheric dissolution or by radial release through the aerenchymatous tissue of hydrophytic

plant roots (Constable *et al.*, 1992). The fate of VAM fungi in hydrophytes, however seems to vary from species to species depending upon anatomical and physiological characteristics of the species. There are certain plant species like *Ipomea carnea* (Khan, 1974) and *Casuarina cunninghamiana* (Khan, 1992) that become temporarily nonmycorrhizal under waterlogged and flooding condition. On the other hand Malajczuk and Lamont (1981) have observed that water logging only causes reduction but does not lead to complete elimination of VAM infection in heath-land vegetation. Similar reduction in VAM has also been reported by Bajwa and Javaid (1997) under aquatic environment. The complete absence or reduced VAM infection in hydrophytes may be due to inhibition of spore germination at low oxygen tension (Le Tacon *et al.*, 1983). Furthermore, Reid and Bowen (1979) reported a low number of VAM entry points on root epidermis in wet soils, which may lead to reduced VAM infection. Tanner and Clayton (1985) demonstrated that decreased redox potential was associated with significantly reduced VAM infection. Unfortunately, investigations on mycorrhizas in wetland and waterlogged soils have not presented data on redox potential.

The development of VA mycorrhizal colonization in terrestrial plants is known to be influenced by seasonal variations (Shamim *et al.*, 1994). In the present study a pattern of seasonal variation has also been observed in hydrophytes. However, development of VAM in response to various seasons varied in different groups of vascular plants, even at the species level within the same group a different pattern of seasonal variation was evident. Furthermore, a differential impact of seasonal variation on VAM structures viz., mycelium, arbuscules and vesicles was very obvious.

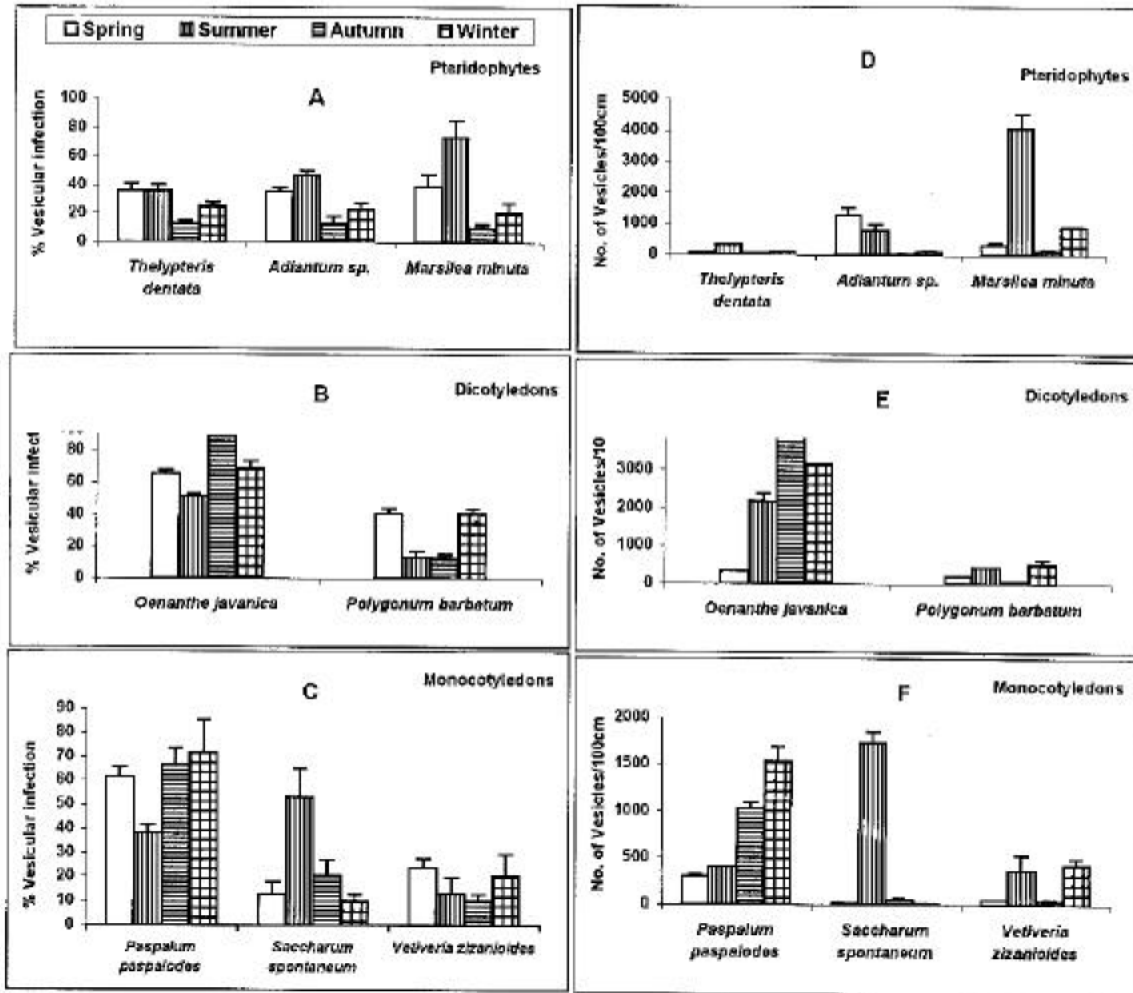


Fig. 3 (A-F): Seasonal variation in vesicular in wetland plants

Generally extent of mycorrhizal infection was higher in spring and summer in all groups of vascular plants. It is universally believed that environmental conditions which favour the host plant growth also tend to enhance VAM infection (Safir, 1981). The photosynthetic activity accelerates in spring and summer resulting in better provision of photosynthates to VAM fungi which colonize roots to their maximum potential in these seasons (Shamim *et al.*, 1994). With the onset of autumn and winter, extent and pattern of VAM colonization in different plant species was changed considerably. In *S. spontaneum* and *V. zizanioides* (monocots), *P. barbatum* (dicots) and *T. dentata* (pteridophytes) VAM infection declined in autumn while in *Adiantum sp.* and *M. minuta* (pteridophytes), *O. javanica* (dicots) and *P. paspalodes* (monocots) VAM colonization remained stable and it was as heavier as in spring and summer. Except for *T. dentata* and *M. minuta* (pteridophytes), in all other test species extent of VAM infection remained high during winter. In contrast to terrestrial habitats the seasonal pattern of VAM infection in hydrophytes is rather irregular. Shamim *et al.* (1994) found maximum VAM infection in land plants in spring that gradually decreased in the following seasons with the lowest in winter. In terrestrial

plants the VAM infection generally varies with change in temperature and availability of moisture (Shamim *et al.*, 1994). In aquatic and sub-aquatic environments the moisture is freely available in all the seasons. Furthermore, most of the test species were sampled along the BRB canal-bank facing north, where temperature was relatively low in all the four seasons, resulting in a dissimilar seasonal pattern of VAM colonization in aquatic and sub-aquatic plants.

Arbuscules being the site of major activity, where the bilateral exchange of material occur between fungus and the host plants, are known to be highly susceptible to wet conditions as compared to mycelium and vesicles (Bajwa and Javaid, 1997). These may be entirely lacking in hydrophytes (Iqbal *et al.*, 1988). However, the present study revealed that arbuscular infection in different test plant species is not equally susceptible to wet conditions. In *P. barbatum* and *Paspalum* although arbuscular infection remained low in almost all the four seasons but in *Adiantum sp.* a persistently high arbuscular infection was observed throughout the year. In other test plant species a variable pattern of seasonal variation was observed. Generally arbuscular infection was high during spring, parallel to terrestrial plants in which arbuscular

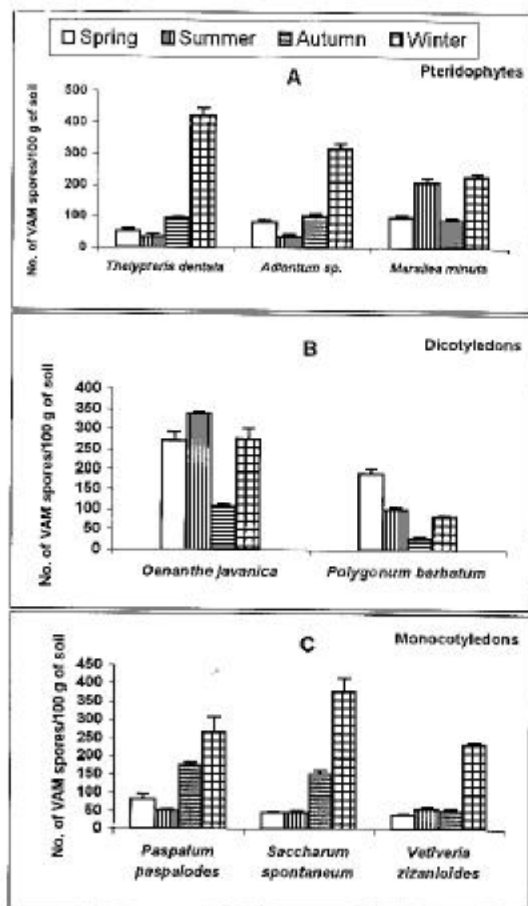


Fig. 4(A-C): Seasonal variation in VAM spores in the rhizosphere of wetland plants

infection was maximum in spring that gradually decreased towards the end of growth season i.e. autumn and winter (Shamim *et al.*, 1994).

Vesicles being the storage organs of the VAM fungi are generally produced at comparatively latter stages of growth (Powell and Bagyaraj, 1984). In the present study like arbuscular infection vesicular infection also showed a variable response to seasons in different plant species. However this response was different from what was observed in the case of arbuscular infection. In *O. javanica* and *P. paspalodes* vesicular infection was minimum in spring that gradually increased to maximum in autumn and winter respectively. However, in *S. spontaneum*, *V. zizanioides*, *P. barbatum*, *T. dentata* and *M. minuta* vesicular infection increased from its minimum in spring and acquired its peak in summer but declined again in autumn and winter. It can possibly be attributed to variable rates of formation and development of vesicles in different aquatic and sub-aquatic plant species reciprocating their pattern of development and maturity. The vesicular infection in general started in spring and reached to its maximum in summer, autumn or winter depending upon the host species. Another possible reason for their disparity in the pattern of VAM development may correspond to variations in period of completion of life cycles of various VAM fungal

species involved in forming this association in aquatic ecosystems.

Although VAM fungi have been reported to occur in waterlogged or flooded conditions, very few investigations have attempted to identify species of endophytes infecting waterlogged plants. In the present study six VAM species all belong to the genus *Glomus* were found to be associated with wetland plants. Among these *G. mosseae* and *G. fasciculatum* were found invariably associated with all the test species the year round. These two mycorrhizal species are more abundant in Pakistani soils (Jalaluddin and Anwar, 1991) and tolerant to environmental stresses (Naz, 1999). Both type and number of spores from the rhizospheric soils of wet-land plants was comparatively lower as compared to terrestrial plants (Jalaluddin and Anwar, 1991). Soil moisture activity seems to be important determinant of the species spectrum of VAM fungi (Read and Boyd, 1986). In the present study generally number of VAM spores were lower in spring and higher in winter. Mycorrhizal spores are generally known to germinate in spring causing infection in the roots and towards the end of season vesicles are converted into spores to increase their number (Jalaluddin and Anwar, 1991).

The present study revealed that pattern of seasonal variation in VAM in wetland plants is different from terrestrial plants primarily because of availability of water for all the year round and comparatively lesser fluctuations in soil temperature. Furthermore, pattern of seasonal variation in VAM in these plants varies with test species involved depending upon morphological, anatomical and physiological characteristics of the species. Since this study was restricted to few plants of diverse groups, therefore, it does not reflect the actual situation. Thus further investigations are necessary by selecting comparatively greater number of test species growing in diverse habitats, in order to explore more hidden facts regarding seasonal variation in VAM in wetland plants.

References

- Allen, M. F., J. H. Richards and C. A. Busso, 1989. Influence of clipping and soil water status on vesicular arbuscular mycorrhiza of two semi-arid tussock grasses. *Biol. Fert. Soil*, 8: 285-289.
- Bajwa, R. and A. Javaid, 1997. Effect of aquatic environment on the development of vesicular arbuscular mycorrhiza (VAM) in hydrophytes. *Pak. J. Phytopathol.*, 9: 132-135.
- Bhandari, S. C., L. L. Somani and I. J. Gulati, 1990. Symbiotic vesicular arbuscular mycorrhizas. In: *Biofertilizers* (L. L. Somani, S. C. Bhandari, S. N. Saxena and K. K. Vyas, eds.), pp: 304. Scientific Publishers, Jodhpur, India.
- Constable, J. V. H., J. B. Grace and D. J. Longstreth, 1992. High carbon dioxide concentration in aerenchyma of *Typha latifolia*. *Am. J. Bot.*, 79: 415-418.
- Gabor, J., 1992. Mycorrhizae and crop productivity. In: *Mycorrhizae in sustainable agriculture* (G. J. Bethlenfalvay and R. G. Linderman, eds.), pp: 1-28. Am. Soc. Agron., Madison, Wisconsin, USA.
- Gerdemann, J. W. and J. H. Nicolson, 1963. Spores mycorrhizal Endogone species extracted from soil by wet sieving and decanting. *Trans. Br. Mycol. Soc.*, 46: 235-244.
- Giovannetti, M., 1985. Seasonal variation of vesicular arbuscular mycorrhizas and endogonaceous spores in a maritime sand dune. *Trans. Br. Mycol. Soc.*, 84: 679-684.
- Giovannetti, M. and B. Mosse, 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New Phytol.*, 84: 489-500.
- Iqbal, S. H., G. Nasim and M. Shahjahan 1988. VA mycorrhizal fungi associated with a bryophyte, *Marchantia plamata*. *Biologia*, 34: 275-278.

Bajwa *et al.*: Seasonal variation in VAM

- Jalauddin, M. And Q. M. K. Anwar, 1991. VAM fungi in wheat and rice fields. Pakistan J. Bot., 23: 115-122.
- Jeffries, P., 1987. Use of mycorrhizae in agriculture, CRC Crit. Rev. Biotechnol., 5: 319-357.
- Kawai, Y., T. Tenzuka, and Y. Yamamoto, 1986. Morphological characteristics and seasonal variation of vesicular arbuscular mycorrhiza in Grapevine. JPN Soc. Hort. Sci., 55: 22-26.
- Khan, A. G., 1974. The occurrence of mycorrhizas in halophytes, hydrophytes and xerophytes and of *Endogone* spores in adjacent soils. J. Gen. Microbiol., 18: 7-14.
- Khan, A. G., 1992. Occurrence of a relationship between soil moisture gradient and the pattern of VAM infection pattern in *Casurina cunninghamiana* growing in transect on a creek embankment. Abst. Int. Symp. on Management of mycorrhizas in agriculture, horticulture and forestry, Sept. 28, Oct. 2, 1992, University of Western Australia, Perth, pp: 9.
- Khan, A. G., 1993. The influence of redox potential on formation of mycorrhizae in trees from wetland and waterlogged areas of New South Wales, Australia. Abst. of 9th North Am. Conf. on Mycorrhizae, Aug. 8-12, 1993. University of Guelph, Guelph, Ontario, pp: 18.
- LeTacon, F., F. A. Skinner and B. Mosse, 1983. Spore germination and hyphal growth of a vesicular arbuscular mycorrhizal fungus, *Gomus mosseae* (Gerdemann and Trappe) under reduced oxygen and increased carbon dioxide concentrations. Can. J. Microbiol., 29: 1280-1285.
- Lodge, D. J., 1989. The influence of soil moisture and flooding on formation of VA endo- and ecto-mycorrhizae in *Populus* and *Salix* Pl. Soil, 117: 243-253.
- Malajczuk, N. and B. B. Lamont, 1981. Specialized roots of symbiotic origin in health-lands. In: Health-lands and related shrublands of the world: analytical studies (R. L. Specht, ed.), pp: 165-182. B. Elsevier, Amsterdam.
- Nasim, G., 1990. VA mycorrhiza in Pteridophytes. Vesicular arbuscular mycorrhiza in *Marsilea quadrifolia* (short communication). Sci. Int. (Lahore), 2: 47-50.
- Naz, J. Z., 1999. Mycorrhiza induced salt tolerance in *Helianthus annuus* L. Var. Suncross. Ph. D. Thesis, Punjab University, Lahore.
- Philips, J. M. and D. S. Hayman, 1970. Improved procedures for clearing roots and staining parasitic and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc., 55: 158-161.
- Powell, C. L. I. And D. J. Bagyaraj, 1984. In: VA mycorrhiza CRC Press Inc., Boca Raton, Florida.
- Read, D. J. and R. Boyd, 1986. Water relations of mycorrhizal fungi and their host plants. In: Water, fungi and plants (P. G. Ayers, L. Boddy, eds.), pp: 287-304. Cambridge University Press, Cambridge.
- Reid, C. P. P. and G. Bowen, 1979. Effect of soil moisture on VA mycorrhizae formation and root development in *Medicago*. In: The soil root interface (J.R. Harley and R. S. Russel, eds.), pp: 219. Academic Press, London.
- Sarif, G. R., 1981. Ecophysiology of VA Mycorrhizal plants. CRC Press, Inc. Boca Raton, Florida.
- Shamim, D., T. Ahmed and N. Ayub, 1994. Influence of seasonal variations on VAM infection in perennial plants. Pakistan J. Phytopathol., 6: 77-86.
- Tanner, C. C. And J. S. Clayton, 1985. Vesicular arbuscular mycorrhiza studies with a submerged aquatic plants. Trans. Br. Mycol. Soc., 85: 683-688.
- Trappe, J. M., 1982. Synoptic key to the genera and species of zygomycetous mycorrhizal fungi. Phytopathology, 72: 1102-1108.