Chapter 13

Arbuscular Mycorrhizal (AM) Fungi Associated with Wild Medicinal Plants Exhibit Variation in Phosphorus Concentration during Growth Developmental Stages

Nair Radhika and B.F. Rodrigues*

Department of Botany, Goa University, Goa – 403 206

ABSTRACT

Three herbaceous medicinal plants (*Rauwolfia serpentina*, *Catharanthus roseus* and *Andrographis paniculata*) growing in wild were studied for AM fungal association and P concentration at different growth stages. All the plant species were found to be colonized by AM fungi but varied in the extent of colonization and sporulation among the plant species during developmental stages. Twenty-one AM fungal species were recovered from the rhizosphere soil at different growth stages. *Glomus fasciculatum* was found to be the most commonly occurring AM fungal species in all the growth stages of three medicinal plants. The results of the present study revealed that an increase in the P concentration during flowering stages of A. *paniculata* and C. *roseus* is directly related to the presence of arbuscules. In addition to this, the study also confirmed that higher P levels in plants decreased the AM fungal spore production in the rhizosphere soil.

Keywords*: AM fungi, Frequency of occurrence, Medicinal plants, Phosphorus concentration, Relative abundance.*

* Corresponding Author.

Introduction

Arbuscular mycorrhizal fungi are ubiquitous and form symbiotic association with most terrestrial plant species. The characteristics and dynamics of occurrence of AM fungi under natural conditions are important for the evaluation of the inoculum potential and root colonization in the process of understanding their behaviour in the soil and determining their symbiotic efficiency (Bethlenfalvay and Linderman, 1992).

Phenology is the study of the timing of vegetative activities, flowering and fruiting and its relationship to environmental factors. A growing plant may experience different stages in mineral nutrition, based on the balance among internal and external nutrient supplies and crop demand for nutrients. Plants require adequate P from the very early stages of growth for optimum crop production (Grant *et al.,* 2001). It is estimated that on average, P could only diffuse approximately 0.5mm, so that only phosphate within 0.5mm of a plant root is positionally available for absorption (Grant *et al.,* 2001). The prevalent form of available P in the environment is the oxidized anion phosphate. More soluble minerals such as nitrogen (N) move through the soil via bulk flow and diffusion, whereas Pi moves by slow rate of diffusion in the soil solution creating a Pi-depletion zone around the root (Jungk, 2001). Therefore, the low availability of Pi in the bulk soil affects its uptake into roots (Rausch and Bucher, 2002).

Although mycorrhizal fungi have been shown to enhance the growth and P nutrition of plants in pots (Cooper and Tinker, 1978), few studies have shown a functional relationship of AM fungi to plants in the wild (Miller, 1987). Examination of the functional significance of AM fungi through studies of P uptake is also difficult because the need for P is not constant during the life cycle of most plants (Fitter, 1985). Mycorrhizae may benefit plants only during times of P demand, *i.e.* during flowering or seed development. No previous studies have reported the P concentration in wild medicinal plants in relation to phenology. Thus, the present study was undertaken to study the variation in P concentration in different developmental stages of three selected medicinal plant species growing in the wild.

Materials and Methods

Study Area and Selection of Plant Species

The site undertaken for the study is located in Sanguem taluka situated in South Goa. The Sanguem taluka has a geographical position marked at 15º48′ 00" N to 14º53′ 54" N latitude and 73ºE to 75ºE longitude. The climate of the tract is tropical with three main seasons *viz*., monsoon, winter and summer. The soil is moderately drained, gravelly with silty clay loam texture with pH ranging from 5.6 to 6.2 and low in nutrients especially P (16kg ha^{-1}) and total N (0.24 per cent). Three medicinally important herbaceous plant species *viz*., *Rauwolfia serpentina* (L.) Benth. (Apocynaceae), *Catharanthus roseus* L. (Apocynaceae) and *Andrographis paniculata* Nees. (Acanthaceae) growing wild in the forest community were selected for the study from the same locality. Of these, two plant species, *R. serpentina* and *C. roseus* are listed as endangered species by IUCN red data list.

Rauwolfia serpentina, an erect perennial shrub commonly known as Indian snakeroot or Sarpagandha contains a number of bioactive chemicals including ajmalicine, deserpidine, rescinnamine, serpentine and yohimbine. Reserpine is an alkaloid first isolated from *R. serpentina*, which is widely used as an antihypertensive drug (Lewis and Lewis, 2003).

Catharanthus roseus (Madagascar periwinkle), a perennial herb, has been cultivated for herbal medicine and as an ornamental plant. The substances vinblastine and vincristine extracted from the plant are used in the treatment of leukemia (Leveque and Jehl, 2007).

Andrographis paniculata an erect annual herb commonly known as "King of Bitters" has being used for centuries in Asia to treat upper respiratory infections, fever, Herpes, sore throat and other chronic and infectious diseases. Some of the extremely beneficial properties include analgesic, anti-inflammatory, antibacterial, antipyretic, cancerlytic, antiviral and vermicidal. The primary medicinal component of *A. paniculata* is Andrographolide, which is a diterpene lactone. The other active components include 14 deoxy 11, 12- di dehydroandrographolide, homoandrographolide, andrographan, andrographosterin and stigmasterol (Siripong *et al.,* 1992).

Sample Collection

Collections were made in different growing seasons of the plant *viz*., vegetative stage (June-August), flowering stage (September-December), and fruiting stage (January- April). Three plants of each species were collected from the same locality (radius of 100m) at different growth stages. For each plant species, three rhizosphere soil samples were mixed to form a composite sample, packed in polyethylene bags, labeled and brought to the laboratory. Root samples were freshly processed, whereas soil samples were stored in deep freezer at 4ºC until analyzed.

Estimation of Root Colonization and Spore Density

For processing of roots, trypan blue staining technique (Koske and Gemma, 1989) was used and percent colonization (proportion of root length colonized) was determined according to slide method (Giovannetti and Mosse, 1980). Isolation of AM fungal spores was carried out by wet sieving and decanting method (Gerdemann and Nicolson, 1963). Spore density was calculated by number of spores present per 100g of rhizosphere soil.

Taxonomic Identification of Spores

Taxonomic identification of spores was carried by using the Manual for Identification of VAM Fungi by Schenck and Perez (1990) and various taxonomic papers *viz*., Walker and Vestberg (1998), Redecker *et al.* (2000), Morton and Redecker (2001). Taxonomic identification of spores was also carried out by matching the descriptions provided by the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (http://invam.caf. wvu.edu.).

Phosphorus Estimation by Colorimetric Method

Root and shoot tissues of three medicinal plant species collected during different growth stages were previously analyzed by dry ash digestion procedure and assessed for the estimation of total P concentration (ppm) using Vanadomolybdate phosphoric yellow colour method (Chapman and Prat, 1961).

Relative Abundance and Frequency of Occurrence

Relative abundance and Frequency of occurrence of AM fungi was calculated in each plant species at different growth stages using the following formulae (Beena *et al.,* 2000).

$$\text{Relative abundance (per cent)} = \frac{\text{Number of AM fungal spores of particular species}}{\text{Total number of AM fungal spores of all species}} \times 100$$

Frequency of occurrence (per cent) =

$$\frac{\text{Number of soil samples that possess spores of particular species}}{\text{Total number of soil samples screened}} \times 100$$

Statistical Analysis

Pearson correlation analysis was carried out to assess the relationship between colonization and spore density. Results of P concentration in roots and shoots of each plant species at different growth stages was analyzed using One Factorial Analysis of Variance (ANOVA) using WASP 1.0 (Web based Agricultural Statistical Package). For all the analysis, differences were considered significant when $P<0.05$.

Results

Mycorrhizal colonization was observed in all the plant species studied. Hyphal and vesicular colonization was observed in all the three medicinal plant species at different growth stages whereas arbuscular colonization was observed only during the flowering stage in *C. roseus* and *A. paniculata*. Percent root colonization differed in all the plant species at different growth stages.

In *R. serpentina* and *C. roseus* maximum percent AM colonization was observed during the vegetative stage (58.33 per cent and 70 per cent) followed by flowering stage (50 per cent and 44.44 per cent) and least in fruiting stage (35.71 per cent and 30.7 per cent) respectively whereas in *A. paniculata* flowering stage showed maximum colonization (55.50 per cent) followed by fruiting stage (42.50 per cent) and least in vegetative stage (38.50 per cent).

Spore density showed variation in different growth stages of all the plant species studied. In *R. serpentina* and *C. roseus* maximum spore density was recorded in the fruiting stage (251 and 56 spores $100g^{-1}$ soil) whereas in *A. paniculata* higher spore density was observed during the flowering stage (147 spores $100g^{-1}$ soil). Among the plant species, least spore density was recorded in *R. serpentina* (8 spores $100g^{-1}$ soil) in the flowering stage. A non-significant negative correlation was observed between percentage colonization and spore density (r= -0.1, $P<0.05$).

Twenty AM fungal species belonging to five genera *viz*., *Glomus*, *Acaulospora, Scutellospora*, *Gigaspora* and *Ambispora* were identified from the rhizosphere soil samples. *Glomus* was the most dominant genus and *Glomus fasciculatum*, the most dominant species, was recorded in all stages of development in the three plant species.

In *A. paniculata*, five AM fungal species belonging to three genera *viz*., *Acaulospora, Glomus* and *Ambispora* were recorded in different growth stages. *G. fasciculatum* was the most commonly occurring AM fungal species in all the growth stages and was more abundant during flowering and fruiting stages of the plant whereas *A. scrobiculata* was more abundant during the vegetative stage. Out of five AM fungal species, *Am. leptoticha* recorded the least in relative abundance (2.04 per cent) and frequency of occurrence (25 per cent), both observations made during flowering stage of the plant (Figures 13.1–13.3).

Thirteen AM fungal species belonging to five genera *viz*., *Acaulospora, Ambispora, Glomus, Gigaspora* and *Scutellospora* were recorded in all the growth stages of *R. serpentina*. *Glomus fasciculatum* was recovered in all the growth stages and found to be the most dominant species in terms of relative abundance (66.53 per cent) and frequency of occurrence (87.5 per cent) recorded during the fruiting

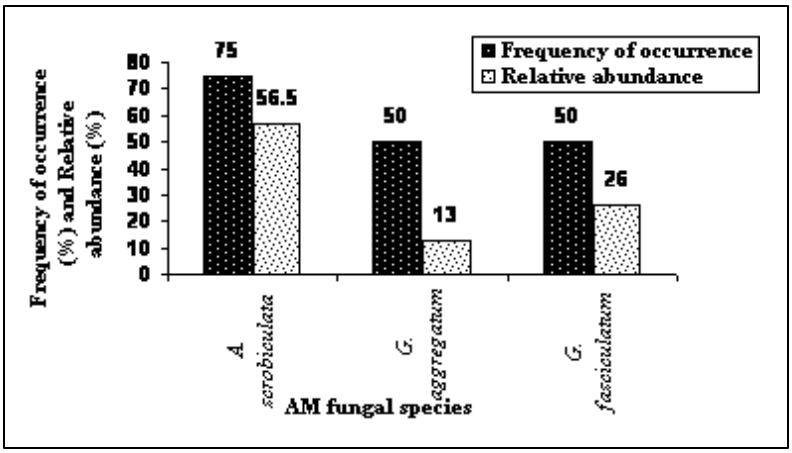

Figure 13.1: Frequency of Occurrence (%) and Relative Abundance (%) of AM Fungal Species in Vegetative Stage of *A. paniculata*

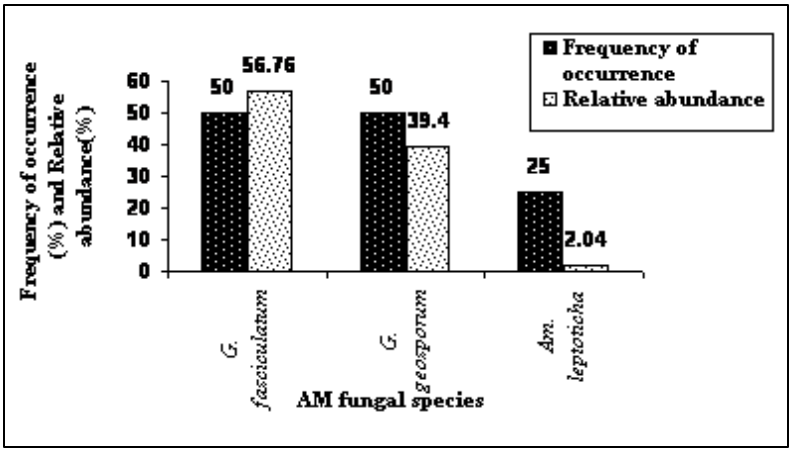

Figure 13.2: Frequency of Occurrence (%) and Relative Abundance (%) of AM Fungal Species in Flowering Stage of *A. paniculata*

stage whereas *G. geosporum* was the most abundant and frequently occurring AM fungal species during the vegetative and flowering stages (Figures 13.4–13.6).

In *C. roseus*, 13 AM fungal species belonging to four genera *viz*., *Acaulospora, Glomus, Gigaspora* and *Scutellospora* were recorded in the rhizosphere soil samples in all the three growth stages. *Glomus fasciculatum* and *G. maculosum* occurred in all the growth stages. *G. fasciculatum* was the most abundant (79.5 per cent) and frequently occurring (80 per cent) species and was recorded during the flowering stage whereas *G. maculosum* was the most abundant and frequently occurring during the vegetative and fruiting stages (Figures 13.7–13.9)

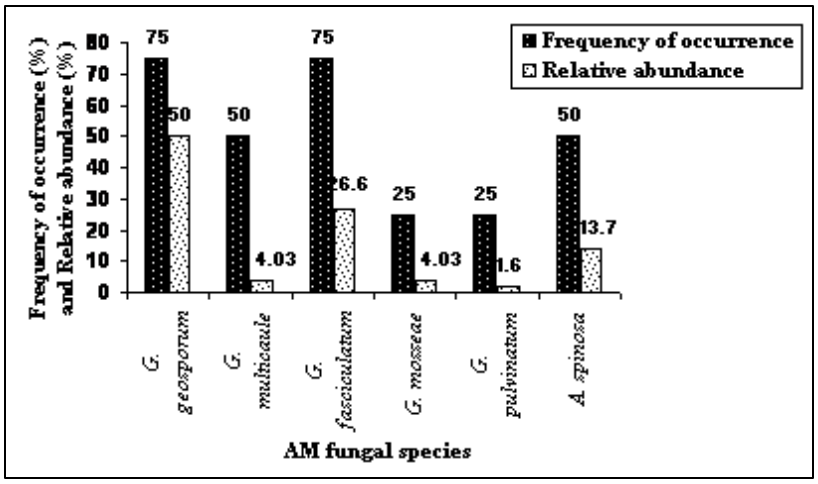

Figure 13.3: Frequency of Occurrence (%) and Relative Abundance (%) of AM Fungal Species in Vegetative Stage of *R. serpentina*

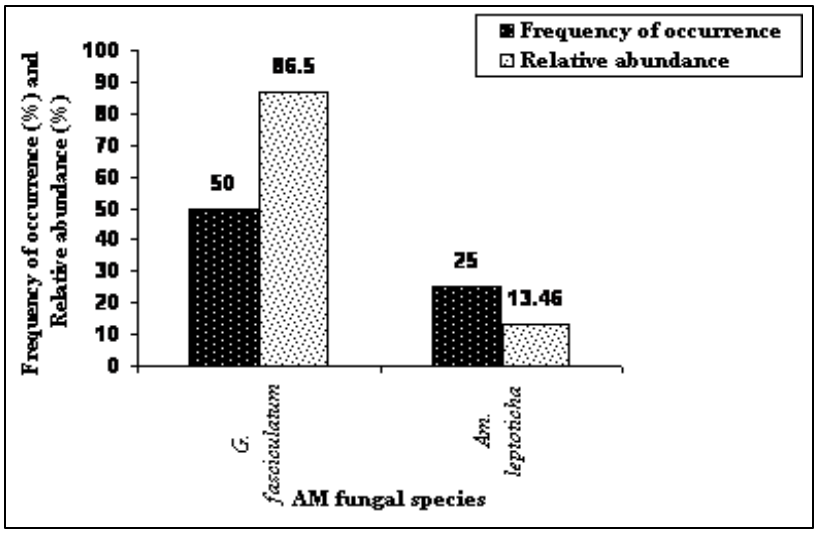

Figure 13.4: Frequency of Occurrence (%) and Relative Abundance (%) of AM Fungal Species in Fruiting Stage of *R. serpentina*

Maximum species richness (13) was recorded in the rhizosphere soil samples of *R. serpentina* and *C. roseus* and least in *A. paniculata* (4).

The P concentration studies revealed variation in both root and shoot tissues of the three medicinal plant species at different growth stages. In *R. serpentina,* maximum P concentration in root (39.3ppm) and shoot tissues (53.8ppm) was recorded during the vegetative stage of the plant (Figure 13.12) whereas in *A. paniculata* and *C. roseus* (Figures 13.10 and 13.11) maximum P concentration was recorded during the flowering stage which suggests the presence of arbuscules. An increase in shoot P concentration was also observed after the formation of arbuscules.

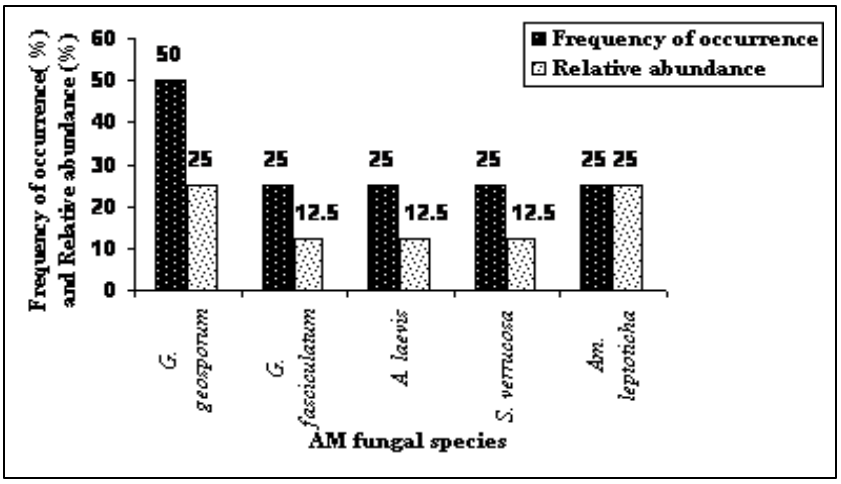

Figure 13.5: Frequency of Occurrence (%) and Relative Abundance (%) of AM Fungal Species in Flowering Stage of *R. serpentina*

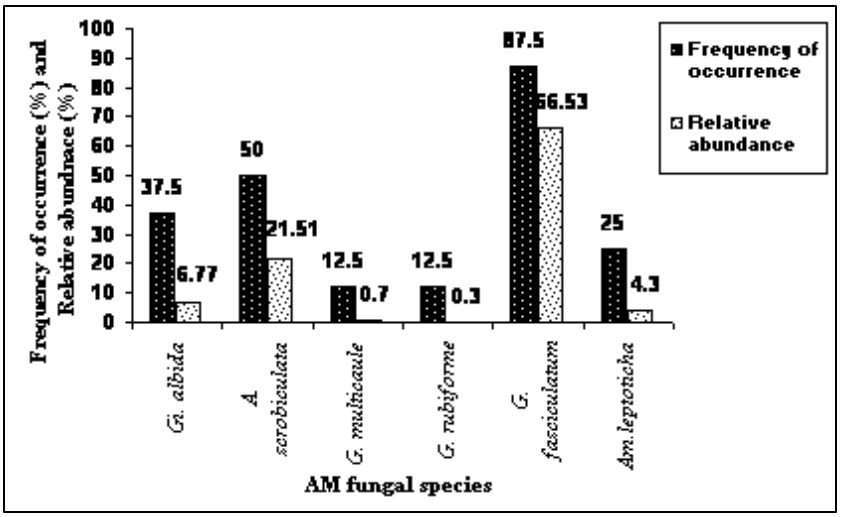

Figure 13.6: Frequency of Occurrence (%) and Relative Abundance (%) of AM Fungal Species in Fruiting Stage of *R. serpentina*

Results of P concentration in roots and shoots were analyzed using ANOVA. Root and shoot tissues of *R. serpentina* showed a significant difference in P concentration observed between the growth stages ($F=0.000$, $df=5$, $P<0.05$). Here, a significant decrease in P concentration from vegetative to flowering stage followed by an increase in fruiting stage was observed. Roots and shoots of *A. paniculata* showed a significant increase in P concentration from vegetative to flowering stage of the plant, and then showed a gradual decrease in the fruiting stage ($F=0.004$, $df=5$, $P<0.05$). In *C. roseus*, a significant increase from vegetative to flowering stage was observed in the shoots followed by a decrease in the

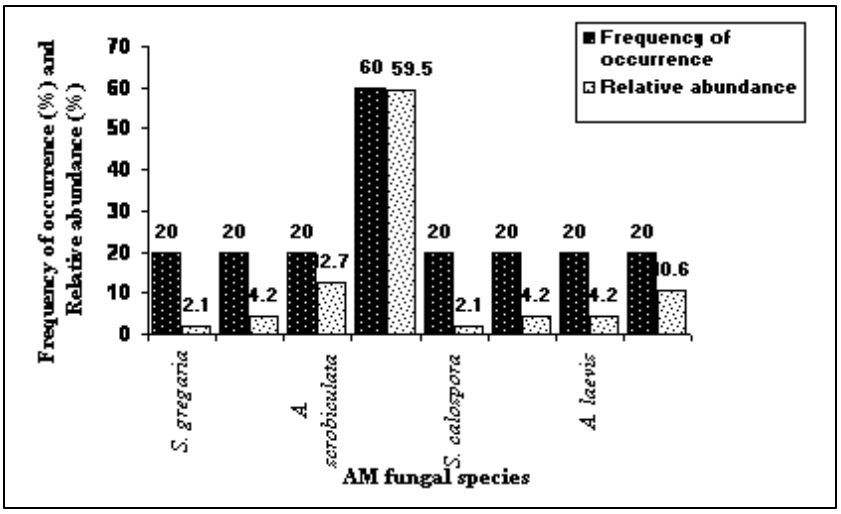

Figure 13.7: Frequency of Occurrence (%) and Relative Abundance (%) of AM Fungal Species in Vegetative Stage of *C. roseus*

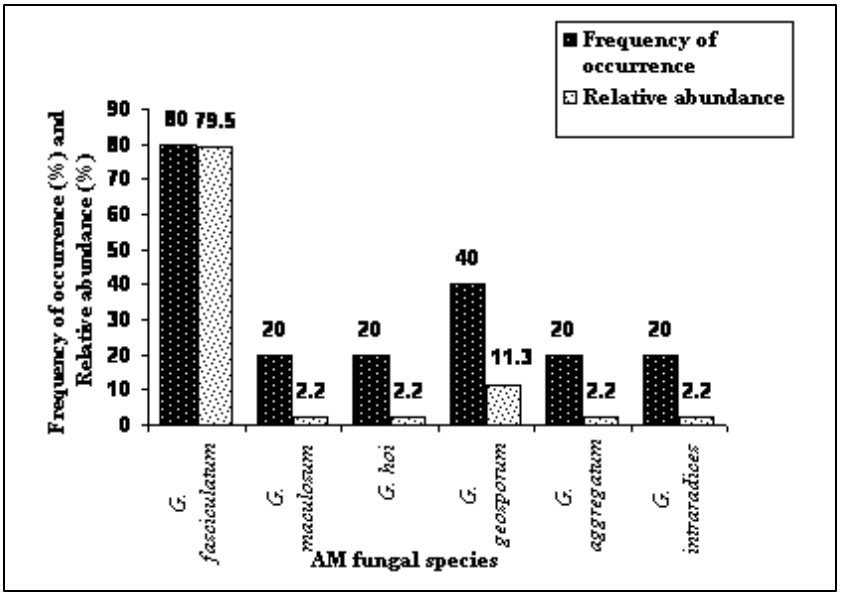

Figure 13.8: Frequency of Occurrence (%) and Relative Abundance (%) of AM Fungal Species in Flowering Stage of *C. roseus*

fruiting stage, whereas in roots, P concentration increased significantly from flowering stage onwards ($F=0.003$, $df=5$, $P<0.05$). Negative correlation was observed between percentage colonization and P concentration in roots and shoots ($r = -0.1, -0.2$, $P<0.05$).

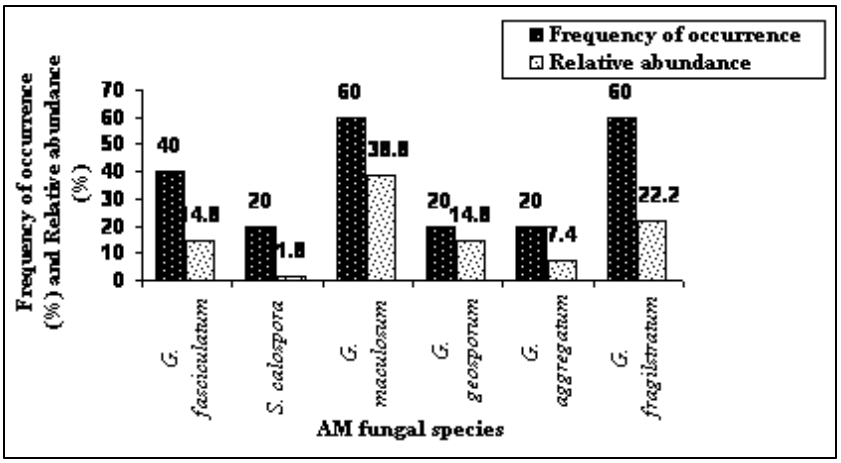

Figure 13.9: Frequency of Occurrence (%) and Relative Abundance (%) of AM Fungal Species in Fruiting Stage of *C. roseus*

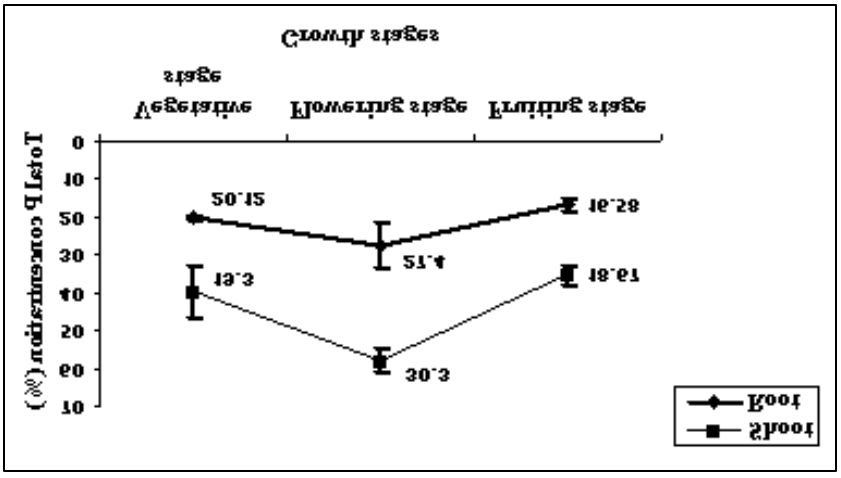

Figure 13.10: Pohsphorus Concentration in Roots and Shoots of *A. paniculata* at Different Growth Stages

Discussion

The present study indicates that AM fungi could play an important role in growth of medicinal plants. Muthukumar and Udaiyan (2000) reported the presence of AM fungal colonization in *A. paniculata* and *R. serpentina* but absence of colonization in *C. roseus*. However, in the present study AM fungal colonization was observed in all the growth stages of *C. roseus*. The study also revealed that *Glomus* was the most dominant genus in the rhizosphere soil of medicinal plants. The predominance of *Glomus* in tropical soils has been reported by other workers (Thapar and Khan, 1985; Ragupathy and Mahadevan, 1993). The study also recorded the presence of 20 AM fungal species from the rhizosphere soil of three medicinal plant species in different growth stages. Johnson *et al.* (1991) reported similar findings in their study on plant and soil controls of mycorrhizal communities. They

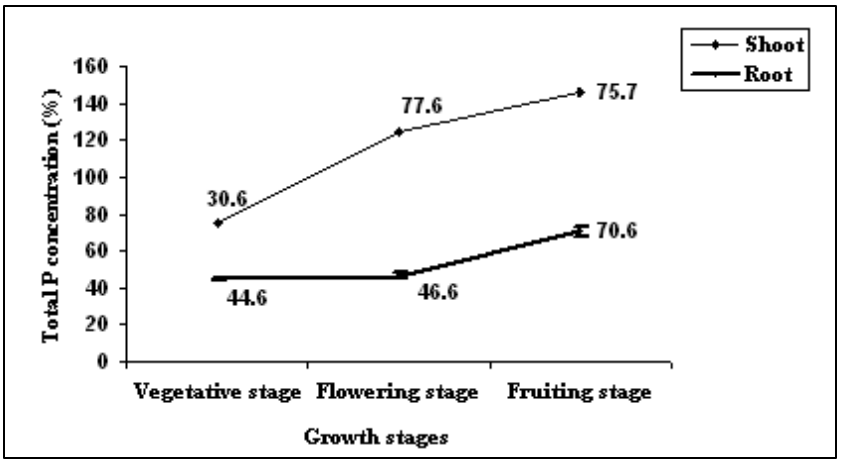

Figure 13.11: Pohsphorus Concentration in Roots and Shoots of *C. roseus* at Different Growth Stages

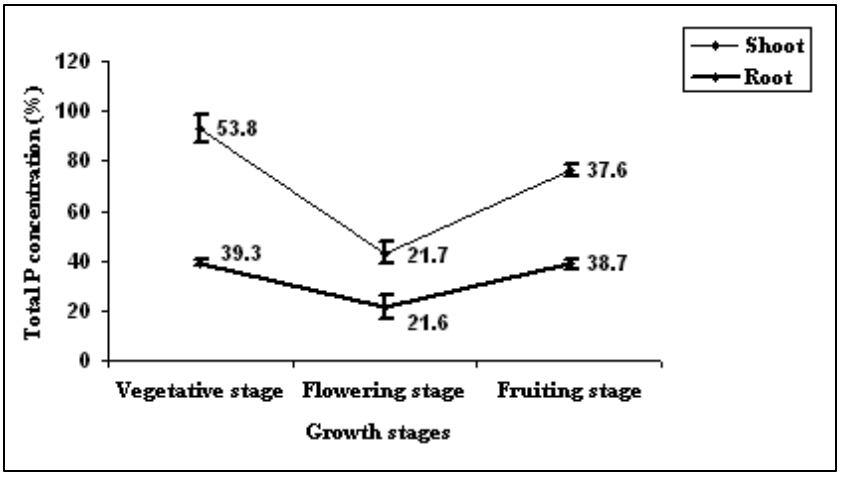

Figure 13.12: Pohsphorus Concentration in Roots and Shoots of *R. serpentina* at Different Growth Stages

reported the presence of 12 to 22 different species of AM fungi per study site. Muthukumar *et al.* (2001) however reported only 35 AM fungal species from the rhizosphere soil of about 329 plant species from the Western Ghats of Southern India. Recovery of relatively high number of species in the present study is in agreement with Francis and Read (1994) who reported that high species diversity, characteristic of phosphorus-deficient grassland ecosystem dominated by plant species with AM fungi, may be attributed to a low level of host specificity.

Large variations in the spore numbers (8 to 251 spores $100g^{-1}$ rhizosphere soil) recorded in plant species at different growth stages in the present study can be attributed mainly to the interspecific competition (Brundrett and Kendrick, 1990) and by the subsequent variation in the timing of spore production associated with host plants, suggesting that the competition between fungi and

environmental factors probably influences spore production in natural communities (Gemma and Koske, 1988).

In the present study, increase in the P concentration during flowering stage of *A. paniculata* and *C. roseus* is directly related to the presence of arbuscules as flower initiation demands extra uptake of P. An increase in the shoot P concentration was observed after the formation of arbuscules. Similar observations have been reported in *Ranunculus adoneus* (Mullen and Schmidt, 1993).

Sanders (1990) studied AM fungal development in wild grassland species but did not record nutrient accumulation and found no relationship from one year to the next in developmental patterns. Other studies of mycorrhizal development in wild populations (Brundrett and Kendrick, 1988; Sanders, 1990) have reported only mycorrhizal colonization. Reinhardt and Miller (1990), working on temperate grasslands, found that AM colonization reached a peak in the growing season (April) and they assessed the levels of arbuscules from root cores containing roots of the entire plant community, not relating colonization to phenology. However, the presence of arbuscules recorded during the flowering stages in two plant species *viz*., *A. paniculata* and *C. roseus* indicated that arbuscules are essential for P uptake. In *R. serpentina* no arbuscules were observed in any of the growth stages, maximum P concentration being observed during the vegetative stage indicating that this species require P during early growth stage for optimum yield. A similar observation was made earlier (Grant *et al.,* 2001) and the study supports the fact that the need for P is not constant during the life cycle of the plant (Fitter, 1985). In *A. paniculata* a significant decrease in P concentration was observed during the fruiting stage as P concentration in tissue of annual plants declines with advancing plant age or stage of growth because as the plant matures an increasing proportion of its dry weight is composed of low P in structural and storage tissues. Similar observations were recorded earlier (Belanger and Richards, 1999).

In the present study, vesicles were observed in all the growth stages of the plants. The predominance of vesicles in the roots indicated that conditions were favourable for their formation. Dunne and Fitter (1989) who examined the P budget of strawberry plants in the field, found that flower initiation required extra uptake of P. However, Dodd and Jeffries (1986) in the study of mycorrhizal development in winter wheat quantified arbuscule production and found that levels were highest immediately before seed formation, presumably a time of high P demand. Fitter (1991) suggested that wild plants may have different patterns of P uptake than crop plants.

In the present study, *C. roseus* showed high P concentration in plant tissues, but recorded fewer number of spores in the rhizosphere soil. This observation supports the earlier study that higher tissue P in the plant reduces the production of spores (De Miranda and Harris, 1994), and secondary external hyphae (Bruce *et al.,* 1994). Root exudates from host plants secrete signal molecules that are known to enhance hyphal branching when there is P limitation in host roots (Nagahashi and Douds, 2000). Therefore, increasing P status in the root may reduce the secretion of these signal molecules, thus reducing hyphal branching and mycorrhizal association. Phosphorus status of the roots may affect membrane phospholipids, thus influencing membrane permeability and the release of carbohydrates from the roots which nourish the fungi (Schwab *et al.,* 1991). Therefore, when P concentration in the plant is low, carbohydrate exudation will encourage mycorrhizal association, which will then enhance the uptake of P from the soil (Grant *et al.,* 2001).

Increase in P concentration during flowering stage indicates that presence of arbuscules corresponds to active P accumulation in wild medicinal plant species. An increase in shoot P concentration was observed after the arbuscule formation which could be due to the fact that when Pi

is readily available more P was transported to shoots leading to P luxury consumption (Chapin, 1980) or Pi storage that could be used in the future to support long term growth (Aerts and Chapin, 2000). Further studies pertaining to the beneficial effects on growth in medicinal plant species and understanding the importance of the relationships to each of the symbionts needs to be undertaken.

References

Aerts, R. and Chapin, F. S. 2000. The mineral nutrition of wild plants revisited: A re-evaluation of processes and patterns. Advances in Ecological Research, 30: 1-67.

Beena, K. R, Raviraja, N. S and Arun, A. D and Sridhar, K. R. 2000. Diversity of arbuscular mycorrhizal fungi on coastal sand dunes of the west coast of India. Current Science, 79: 1459-1465.

Belanger, G and Richards, J. E. 1999. Relationship between P and N concentrations in timothy. Canadian Journal *Plant* Science, 79: 65-70.

Bethlenfalvay, G. J. and Linderman, R. G. 1992. Mycorrhizae in sustainable agriculture. ASA Spec Publ, Madison.

Bruce, A., Smith, W. E. and Tester, M. 1994. The development of mycorrhizal infection in cucumber: effects of P supply on root growth, formation of entry points and growth of infection units. New Phytologist, 1276: 507-514.

Brundrett, M. C. and Kendrick, B. 1988. The mycorrhizal status, root anatomy and phenology of plants in a sugar maple forest. Canadian Journal of Botany, 66: 1153-1173.

Brundrett, M. C. and Kendrick, B. 1990. The roots and mycorrhizas of herbaceous woodland II. Structural aspects of morphology. New Phytologist, 114: 469-479.

Chapman, H. D. and Prat, P. F. 1961. Methods of analysis for soils, plants and waters. University of California, Divison of Agriculatural Sciences, Berkeley.

Cooper, K. M. and Tinker, P. B. 1978. Translocation and transfer of nutrients in vesicular arbuscular mycorrhizas II. Uptake and translocation of phosphorus, zinc and sulphur. New Phytologist, 81: 43-52.

Chapin, F. S. 1980. The mineral nutrition of wild plants. Annual Review of Ecology and Systematics 11: 233-260.

De Miranda, J. C. C. and Harris, P. J. 1994. Effect of soil phosphorus on spore germination and hyphal growth of arbuscular mycorrhizal fungi. New Phytologist, 128: 103-108.

Dodd, J. C. and Jeffries, P. 1986. Early development of vesicular arbuscular mycorrhizal in autumn-shown cereals. Soil Biology Biochemistry, 18: 149-154.

Dunne, M. J. and Fitter, A. H. 1989. The phosphorus budget of a field grown strawberry (*Fragaria* x *ananassa* cv.Hapil) crop: evidence for a mycorrhizal contribution. Annals of Applied Biology, 114: 185-193.

Fitter, A. H. 1985. Functioning of vesicular arbuscular mycorrhizas under field conditions. New Phytologist, 99: 257-265.

Fitter, A. H. 1991. Cost and benefits of mycorrhizas: Implications for functioning under natural conditions. Experientia, 47: 350-355.

Francis, R. and Read, D. J. 1994. The contribution of mycorrhizal fungi in determination of plant community structure. Plant and Soil, 159: 11-25.

Gemma, J. N. and Koske, R.E. (1988). Seasonal variation in spore abundance and dormancy of *Gigaspora gigantea* and in mycorrhizal inoculum potential of a dune soil. *Mycologia*. 80: 211-216.

Gerdemann, J. W. and Nicolson, T. H. 1963. Spores of mycorrhizal *Endogone* species extracted from soil by wet sieving and decanting. Transactions of British Mycological Society, 46: 235-244.

Grant, C. A., Flaten D. N., Tomasiewicz, D. J. and Sheppard, S. C. 2001. The importance of early season P nutrition. Canadian of Journal of Plant Sciences, 81: 211-224.

Giovannetti, M. and Mosse, B. 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. New Phytologist, 84: 489-500.

Johnson, N. C., Tilman, D. and Wedin, D. 1991. Plant and soil controls on mycorrhizal fungal communities. Ecology, 61: 151-162.

Jungk, A. 2001. Root hairs and the acquisition of plant nutrients from soil. Journal of Plant Nutrition and Soil Science, 164: 121-129.

Koske, R. E. and Gemma, J. N. 1989. A modified procedure for staining roots to detect VA mycorrhizas. Mycological Research, 92 (4): 486-505.

Leveque, D. and Jehl, F. 2007. Molecular pharmacokinetics of *Catharanthus* (*Vinca*) alkaloids. Journal of Clinical Pharmacology, 47 (5): 579-588.

Lewis, W. H. and Elvin-Lewis, M. P. F. 2003. Medical Botany. Hoboken, Wiley. 286.

Mullen, R. B. and Schmidt, S. K. 1993. Mycorrhizal infection, phosphorus uptake, and phenology in *Ranunculus adoneus*: Implications for the functioning of mycorrhizae in alpine systems. Oceologia, 94: 229-234.

Miller, R. M. 1987. The ecology of vesicular-arbuscular mycorrhizae in grass and shrubland. In: Ecophysiology of VA mycorrhizal plants. (Ed. Safir, G.). pp 135-170, CRC Boca Raton Florida.

Morton, J. B. and Redecker, D. 2001. Concordant morphological and molecular characters reclassify five arbuscular mycorrhizal fungal species into new genera *Archaeospora* and *Paraglomus* of new families Archaeosporaceae and Paraglomaceae respectively. Mycologia, 93: 181-195.

Muthukumar, T. and Udaiyan, K. 2000. Arbuscular mycorrhizas of plants growing in Western Ghats region, Southern India. Mycorrhiza, 9: 297-313.

Muthukumar, T., Udaiyan, K. and Manian, S. 2001. Vesicular arbuscular mycorrhizal association in the medicinal plants of Maruthumalai Hills, Western Ghats, Southern India. Journal of Mycology and Plant Pathology, 31: 180-184.

Nagahashi, G. and Douds, D. D. Jr. 2000. Partial separation of root exudates and their effects upon the growth of germinated spores of AM fungi. Mycological Research, 104: 1453-1464.

Ragupathy, S. and Mahadevan, A. 1993. Distribution of vesicular arbuscular mycorrhizae in the plants and rhizosphere soil of the tropical plains Tamil Nadu, India. Mycorrhiza, 3: 123-136.

Rausch, C. and Bucher, M. 2002. Molecular mechanisms of phosphate transport in plants. Planta, 216: 23-37.

Redecker, D., Morton, J. B. and Bruns, T. D. 2000. Ancestral lineages of arbuscular mycorrhizal fungi (Glomales). Molecular Phylogenetics and Evolution, 14: 276-284.

Reinhardt, D. R. and Miller, R. M. 1990. Size classes of root diameter and mycorrhizal fungal colonization in two temperate grassland communities. New Phytologist, 116: 129-136.

Sanders, F. E., Tinker, B. P., Black, R. L. B. and Palmerely S. M. 1977. The development of endomycorrhizal root systems. I. Speed of infection and growth-promoting effects with four species of vesicular-arbuscular endophyte. New Phytologist, 78: 257-268.

Sanders, I. R. 1990. Seasonal patterns of vesicular-arbuscular mycorrhizal occurrence in grasslands. Symbiosis, 9: 315-320.

Sanders, F. E., Tinker, B. P., Black, R. L. B. and Palmerely, S. M. 1977. The development of endomycorrhizal root systems. I. Speed of infection and growth-promoting effects with four species of vesicular-arbuscular endophyte. New Phytologist, 78: 257-268.

Schenck, N. C. and Perez, Y. 1990. Manual for identification of VAM mycorrhizal fungi. INVAM, University of Florida, Gainesville pp. 1-283.

Schwab, S. M., Menge, J. and Tinker, P. B. 1991. Regulation of nutrient transfer between host and fungus in vesicular and arbuscular mycorrhizas. New Phytologist, 117: 387- 398.

Siripong, P., Kongkathip, B., Preechanukool, K., Picha, P., Tunsuwan, K., Taylor, W. C. 1992. Cytotoxic diterpenoid constituents from *Andrographis paniculata* Nees leaves. Journal of Scientific Society of Thailand, 18: 187-194.

Thapar, H. S. and Khan, S. N. 1985. Distribution of VA mycorrhizal fungi in forest soils of India. Indian Journal of Forest, 8: 5-7.

Walker, C. and Vestberg, M. 1998. Synonymy amongst the arbuscular mycorrhizal fungi *Glomus claroideum*, *Glomus maculosum*, *Glomus multisubstensum* and *Glomus fistulosum.* Annals of Botany, 82: 601-624.