KAVAKA 59(4): 1-13 (2023) DOI: 10.36460/Kavaka/59/4/2023/1-13

Contributions on Mycorrhizae for Plant Protection and Crop Improvement*

N. Raaman

Centre for Advanced Studies in Botany, University of Madras, Guindy Campus, Chennai - 600 025, India.

Corresponding author Email:raaman55@gmail.com

Professor N. Raaman
President, Mycological Society of India (2023)

ABSTRACT

One of the most important groups of soil microorganisms is the mycorrhizal fungi. Sixteen species of VAM fungi were reported by me and my team from forest tree species of South India. Fifteen VAM fungal species were isolated from burned area and 16 species from the unburned area. It was found that VAM fungi are affected adversely by the intensity of the fire but they recover from a burn within 2 years. The VAM fungal association with the plant species colonizing a magnesite mine spoil was investigated and 13 VAM fungal species were identified. Spores of *Glomus fasciculatum* and *Gigasapora gigantea* were commonly found in the magnesite mine spoil. The mycorrhizal fungi in the epiphytic and terrestrial orchids were studied and 5 AM fungal species were identified. For the first time, *Pisolithus tinctorius* has been reported by me and my team in tropical region in association with *Eucalyptus tereticornis.* Investigation on mycorrhizal association in *Casuarina equisetifolia* grown in 4 different soil types was carried and it revealed a total of 10 species of *Glomus*, 2 of *Gigaspora* and 2 of *Sclerocystis*. Investigation on the mycorrhizal and actinorhizal status in *C. equisetifolia* at 25 sites in and around coastal region of Madras, Tamil Nadu revealed the presence of total of 8 species of VAM fungi and it has been found that the dual inoculation (*Frankia* and *Pisolithus tinctorius*) gave more biomass than individual inoculation. It was shown that *Pisolithus tinctorius* and *Laccaria laccata* exhibited higher amounts of IAA production than other fungi, whereas *Amanita muscaria* and *Rhizopogan luteolus* showed least quantity of IAA. The growth and acid phosphatase activity of *L. laccata* has been studied and it has been found that *L. laccata* was more tolerant to Cu than Ni and increasing Cu and Ni concentration induce the increase of acid phosphatase activity (maximum at 0.15 µm) in *L. laccata*. The axenic growth, total protein content and acid and alkaline phosphatase activities in *Amanita muscaria* was estimated and it has been found that *A. muscaria* was also more tolerant to Cu than Ni. Among *L. laccata* and *Suillus bovinus*, *L. laccata* had maximum acid and alkaline phosphatase activities and tolerance to high concentration of chromium. *Casuarina equisetifolia* seedlings were raised in glasshouse condition and inoculated with suspension of pure culture of *Frankia.* The nodules have been collected and analysed for the presence of cytokinin and significantly the cytokinin has been detected in the nodules. Significantly increased GA has been demonstrated in roots, nodules and cladodes of triple (*Glomus fasciculatum*, *Pisolithus tinctorius* and *Frankia*) inoculated plants of *Casuarina equisetifolia*. Maximum amounts of IAA in cladodes and roots of *C. equisetifolia* have been established by HPLC analysis in triple (*Glomus fasciculatum*, *Pisolithus tinctorius* and *Frankia*) inoculated plants than the other individual treatment of symbiont. Higher content of IAA in nodules of *Glomus mosseae* and *Rhizobium* inoculated *Prosopis juliflora* has been demonstrated. Sodium alginate beads of *Laccaria laccata* were prepared and the beads were viable for 10 months. The fungal hyphae in the beads formed mycorrhizal association with *Eucalyptus tereticornis* and enhanced growth has been demonstrated in the seedlings due to mycorrhizal association. Spores of *Scutellospora erythropa* and *Scu. nigra* isolated from neem rhizosphere soils from coastal regions of Chennai, India were tested for axenic germination in *in vitro* conditions. They showed positive results in media of different composition using root exudates, soil extract, thiamine HCl and inositol. The combined medium increased the spore germination in *Scu. erythropa* and in *Scu. nigra* over water agar control. The germ tube often grew up to 3.8 cm on combined media but no vegetative spores and extramatrical auxiliary cells were observed during the experiment. There was significant increase in hyphal growth when the roots were introduced into the medium, 3 days after spore germination. Based on the method developed, growth of *Glomus mosseae* and *Gigaspora gigantea* on *in vitro* root organ culture of *Sorghum vulgare* and *Saccharum* officinarum was carried out. Spores of *Gl. mosseae* and *Gig. gigantea* germinated on minimal medium produced extraradical mycelium. *Gl. mosseae* infected roots of *S. officinarum* in *in vitro* condition were inoculated in minimal medium with *in vitro* cultured roots of *Sorghum vulgare* (test roots). From the infected root of *S. officinarum*, the mycelium developed and it infected the test roots. The roots developed new mycelia and further the mycelia produced a few hyaline spores. In MS medium combined with soil extract, root exudate, thiamine HCI and inositol combination, spore germination and germ tube growth were higher when compared with other media. Thus, significant contributions on the biodiversity of Indian mycorrhizal fungi and application of mycorrhizal fungi for growth improvement of plants were made.

*Presidential address presented during 50th Annual Meeting (Golden Jubilee Celebration) of the Mycological Society of India held at Centre for Advanced Studies in Botany, University of Madras on December 14, 2023.

MYCOLOGICAL SOCIETY OF INDIA

Mycological Society of India (MSI) was founded in January 1973 by a team of mycologists led by Late Prof. C.V. Subramanian, Former Director, Centre for Advanced Studies in Botany, University of Madras, Chennai, India and former President, International Mycological Association, with a view to bring together the mycologists of the country and with the broad objectives of promoting development of mycology for the prospects and in the widest prospective. I joined the Centre for Advanced Studies in Botany for my doctoral studies during January 1978 under the guidance of Late Prof. K. Natarajan. I had the opportunity to sit in the same room of Prof. K. Natarajan. During the period, Prof. C.V. Subramanian was the Editor of Kavaka and he used to give the corrected manuscripts to Prof. Natarajan to give to the printing press. Most of the time, I accompanied with Prof. Natarajan to the Amara Press, Adyar, Chennai to hand over the manuscripts. Once the proofs were received, Prof. Natarajan allowed me to help in proof reading and he gave me the opportunity to learn the art of proof correction. This experience of proof correction helped me later for correcting efficiently the Ph.D. theses of my students. As a member of MSI, I have attended several Annual Meetings of MSI and as a faculty of Centre for Advanced Studies in Botany, I have extended my cooperation in organising the Annual Meetings of MSI successfully at Centre for Advanced Studies in Botany. I served for MSI as Secretary from 2011 to 2022 and worked hard to take the MSI to newer heights. I was able to conduct the Annual Meeting of MSI in various parts of India and I thank all the organisers and all the Office Bearers and Members of MSI. During this period, several Awards were established in MSI with the help of good hearted people. I thank all the donors for the establishment of Awards. I sincerely thank the MSI for electing me the President of MSI in 2023, a special year. It is a happy occasion that Mycological Society of India is conducting the Golden Jubilee Celebration (50th Annual Meeting) in December 14-16, 2023 at the Centre for Advanced Studies in Botany, University of Madras, where the MSI was started in 1973. Men / women may come and go, but the MSI must shine for ever. My good wishes to all the MSI members and all the participants of this conference.

INTRODUCTION

One of the most important groups of soil microorganisms is the mycorrhizal fungi. Mycorrhizal associations vary widely in structure and function, but the most common interaction is the arbuscular mycorrhizal (AM) association. The AM symbiosis represents an ancient symbiosis with over 80% of all terrestrial flowering plants forming this type of relationship (Harrison, 1998). The symbiosis is formed when fungi belonging to the unique Phylum Glomeromycota grow within and around plant roots. It is well established that the growth response of plants to mycorrhizal infection is influenced by the amount of phosphorus supplied to the soil (Bowen, 1987). Mycorrhizae are not only beneficial in absorption of nutrients from soil but also associated with biological weathering of geologic substrate and decomposition of soil organic matter (Miller, 1987). There is evidence that mycorrhizal plants mobilize nutrient ions from soil minerals that are relatively insoluble in extracting agents typically used in soil analysis (Smith and Read, 2008). Direct supply of nutrients from litter to living roots through mycorrhizae has been indicated. They play an important role in energy flow and nutrient cycling in undisturbed ecosystems. Mycorrhizae are not only more efficient in utilization of available nutrients from soil but also involved in transfer of nutrients from components of soil minerals and organic residues to solution and in nutrient cycling in an ecosystem (Azcón-Aguilarand Barea, 2015). Evidence clearly indicated the beneficial role of AM fungi to higher plants. Inoculations of two different species of beneficial organisms often result in a synergistic effect on plant growth. In recent years, the disposal of industrial effluents on land has become a common practice in many countries. It is now well recognized that industrial activity over a long period of time has led to enhancement of levels of heavy metals and toxic elements in the soil causing genetical, physiological and ecological problems. It is well established that vesicular-arbuscular mycorrhizal (VAM) fungi form associations with many plants under wide range of soil conditions and tremendously increase the plant growth. The mycorrhizal fungi are considered as important tool in afforestation and rehabitation of degraded lands especially in mine spoils, eroded sites, and polluted wastelands. Thus, this paper has my significant contributions on the biodiversity of Indian mycorrhizal fungi and application of mycorrhizal fungi for growth improvement of plants.

CONTRIBUTIONS ON BIODIVERSITY OF INDIAN MYCORRHIZAL FUNGI

Forest trees require mycorrhizae to survive and grow in natural forest environment. Generally tropical forest soils contain much less organic materials and litter than the soils of the temperate forests, which may influence the mycorrhizal association in the tropics (Bakshi, 1980). About 95 per cent of tree species occurring in the tropical forests are purely endomycorrhizal (Le Tacon *et al*., 1987). The primary environmental variables such as seasonal changes and physico-chemical factors of the soil may influence the activity of vesicular arbuscular mycorrhizae (VAM). High alkalinity reduced the spore population in tropical forests of Southern India. It is interesting to note that the rhizosphere soil of each tree species differs from the other in pH and other nutrient contents.

Sixteen species of VAM fungi, 12 of *Glomus*, 2 *Sclerocystis* and one each of *Gigaspora* and *Scutellospora* were reported (Raman *et al*., 1992).

Wild fires have become an increasing common occurrence in tropical forests. Fire can drastically change surface soil characteristics and erosion rates (Amaranthus and Trappe, 1993). Soil biological properties can directly affected by fire (Perry *et al*., 1987). The distribution and abundance of VAM species are related to topography and burning frequency (Gibson and Hetrick, 1988). Mycorrhizal infection and spore population were observed as low in forest fire area in tropical forest of southern India when compared with the unburned area. Fifteen VAM fungal species were isolated from burned area and 16 species from the unburned area. In all, 22 VAM fungal species were reported: *Glomus* (16 spp.), *Scutellospora* (3 spp.), *Sclerocystis* (2 spp.) and *Gigaspora* (1 sp.). *Gl. fasciculatum* was the dominant species (Raman and Nagarajan, 1996. VAM fungi are affected adversely by the intensity of the fire but they recover from a burn within 2 years.

India is the seventh largest country, with a total area of 3.38 million square kilometers in working mines; 75% of these mines in surface mining. The establishment of vegetation on mine spoils is difficult due to the extreme physico-chemical conditions of the substrate. Generally, physico-chemical and chemical characters are determined when revegetation is studied, but biological properties including mycorrhizal potential are often ignored (Danielson, 1985). The beneficial role of vesicular arbuscular mycorrhizae in mine spoil revegetation has been emphasized by Jasper *et al*. (1988). The VAM fungal association with the plant species colonizing a magnesite mine spoil in Salem, Tamil Nadu, and India was investigated. VAM infection ranged from 32 to 82%. Thirteen VAM fungal species were identified: *Scutellospora* (4 spp.), *Glomus* (3 spp.), *Sclerocystis* (3 spp.), *Gigaspora* (2 spp.) and *Acaulospora* (1 sp.). Spores of *Gl. fasciculatum* and *Gig. Gigantea* were commonly found in the magnesite mine spoil. VAM fungal spores in the magnesite mine spoil and infection in plants which grow in the mine spoil suggested the potential of mycorrhizal fungi for reclamation of mine spoils (Raman *et al*., 1993).

The orchids exert considerable fascination because of the beauty of their floral structures. Their flowers are also economically important because of their export value as well as some of the products such as vanilla. Mycorrhizae aid in the germination of seeds and establishment of orchid protocorms (Arditti, 1967). The entry of fungal hyphae into young seedlings at germination is a necessary condition for the initiation of further growth in orchids (Harley, 1969). Many epiphytic and terrestrial orchids are very much dependent on mycorrhizal fungi for their carbon source (Purves and Hadley, 1975). The mycorrhizal fungi in the epiphytic and terrestrial orchids in Kodaikanal tropical forest of Western Ghats, Southern India were studied. The epiphytic orchids were infected with *Rhizoctonia* sp. whereas the terrestrial orchids were infected with AM fungi. Five AM fungal species (*Glomus* 4 spp. and *Gigaspora* 1 sp.) were identified. The presence of endophyte *Rhizoctonia* sp. in the epiphytic orchids and the arbuscular mycorrhizal fungi in the terrestrial orchids in a tropical forest of Southern India laid ways to exploit their potential in the growth of Indian orchids (Raman and Nagarajan, 1999).

Throughout the world, *Glomus* was the dominant genus (Mingyuan and Pan, 2015). In India also, we observed that genus *Glomus* was the dominant one. In the same environment, *Glomus* and *Acaulospora* species usually produce more spores than *Scutellospora and Gigaspora* species (Bever *et al*., 1996). This may be explained by the difference in development. *Acaulospora* and *Glomus* species are thought to require less time to produce spores than *Scutellospora* and *Gigaspora* species. Moreover, members of the Gigasporacea*e* typically establish an extensive mycelium in soil and produce fewer spores than those of the Glomaceae and Acaulosporaceae (Hart and Reader, 2002; Piotrowski *et al*., 2004).

Pisolithus tinctorius has attracted considerable attention over years because of its ability to form ectomycorrhizae with a variety of trees under unusually adverse conditions. *P. tinctorius* is being used in afforestation and reforestation programmes. According to Marx (1977), *P. tinctorius* has been reported from 33 countries in 5 continents of the world. In Asia, it has been collected only from China and Philippines and the fungal collections were deposited in the Royal Botanic Gardens, Kew, England but without the record of specific tree-host association. For the first time, *P. tinctorius* has been reported by the author in tropical region in association with *Eucalyptus tereticornis* in Vandalur and also Kolappakkam, Madras, Tamil Nadu, India (Raman and Rajendran, 1994).

MYCORRHIZAL, ACTINORHIZAL AND HOST TRIPARTITE SYMBIOSES

Symbiotic microorganisms are present in great numbers on and near the feeder roots of trees and they play vital role in numerous physiological and biochemical processes. The microorganisms implicated in this symbiotic interaction are from 2 groups: bacteria and fungi. The bacteria group is implicated in nitrogen fixation, while the fungus group is involved in the uptake of nutrients with low mobility. Among the bacteria, which establish symbiotic association with dicotyledonous plants,

nitrogen fixation is exclusively carried out by *Rhizobium* and *Frankia* in a specialized organ, the nodule where atmospheric nitrogen is reduced to ammonium. The plants nodulated by *Frankia* species are known as actinorhizal plants. Unlike the *Rhizobium*-legume symbiosis, in which the host plants with a few exceptions belong to a single large family, actinorhizal plants are distributed among 8 families and 7 orders. *Casuarina* species are unique trees possessing mycorrhizal and actinorhizal symbionts in their roots (Raaman *et al*., 2005).

Investigation on mycorrhizal association in *Casuarina equisetifolia* grown in four different soil types in Tamil Nadu, India, namely, sandy loam soil (Pudukottai district), clay soil (South Arcot district), red soil (Thanjavur district) and marine sand-dune (Rameswaram district) of Tamil Nadu, India revealed that the plants from all the soil types possessed vesicles and arbuscules in their roots. The four types of soil were nitrogen deficient but rich in phosphorus and potassium and the soils were acidic to alkaline. The rhizosphere soils harboured spores of different VAM fungi and with diversity of spore types. A total of 10 species of *Glomus*, 2 of *Gigaspora* and 2 of *Sclerocystis* and variation in per cent colonization and number of spores per 100 g of soil have been recorded (Sambandan *et al*., 1994). Investigation on the mycorrhizal and actinorhizal status in *C. equisetifolia* at 25 sites in and around coastal region of Madras, Tamil Nadu, and India revealed that the pH of the soils ranged from 7.2 to 8.2 and the available N and P in the soil ranged from 56 –97 kg/acre and 1– 65 kg/acre, respectively. Very good mycorrhizal infection and actinorhizal nodulation occurred in pH 7.5 and above. A total of 8 species of VAM fungi (5 species of *Glomu*s, 1 species each of *Acaulospora*, *Gigaspora* and *Sclerocystis*) have been recorded and it has been found that the dual inoculation (*Frankia* and *Pisolithus tinctorius*) gave more biomass than individual inoculation (Raman and Elumalai, 1991).

Purnell (1960) described a proteoid root as a "cluster of rootlets of limited growth, which forms on a lateral root". Such roots are observed in many species of Proteaceae, Leguminosae and in actinorhizal plants. The aggregation of rootlets had been suggested to enhance the plants ability to retain and absorb phosphorus in relatively infertile soils. Therefore phosphorus in the root environment is an important factor for the production of cluster roots. *C. equisetifolia* seedlings grown in Hoagland's solution produced the cluster roots. Decreasing P level led to increase cluster roots (Raman and Elumalai, 1992).

MYCORRHIZAL, RHIZOBIAL, AND HOST TRIPARTITE SYMBIOSES

Biological nitrogen fixation uses energy derived from photosynthesis and does not accumulate excess nitrogen to cause pollution. *Rhizobium*, a genus of Gram - negative soil bacteria, is capable of establishing nitrogen - fixing symbiosis within roots of plants belonging to the Leguminosae family. Legume nodules export symbiotically fixed nitrogen as amino acids, amides or ureides apoplastically via xylem into the shoot of the host plant. Assimilates from photosynthesis are imported into nodules via phloem in the form of sucrose. Export of nitrogen compounds from the infected cells and import of energy-rich assimilates are essential requirements for efficient biological N_2 fixation. The legume host supplies carbon and energy to the nitrogen fixing bacteria, which in turn reduce nitrogen into ammonia and export it to the plants (Miller *et al*., 1988). Nitrogen (N) and phosphorus (P) are major requirements for plant growth. These nutrients are supplied either by fertilizers or added manures; however some crops such as legumes have the ability to fix nitrogen from the atmosphere through a symbiosis with bacteria known as rhizobia. Symbiotic N_2-fixation by legumes can therefore reduce the need for mineral N fertilizer (Cluett and Boucher, 1983). Eighty per cent of all known plant species form associations with arbuscular mycorrhizal fungi (AMF) which are able to increase the rate of P supply to the plant roots even when there are relatively low levels of mineral P present in the soil. An effective management of arbuscular mycorrhizal fungi can lead to optimum efficiency of phosphatic fertilizer utilization. The effective use of symbiotic relationships in agriculture depends on developing knowledge of the key relations between the symbionts and soil conditions. Better management systems that favour the establishment of these symbioses should improve the efficiency of resource utilization thereby reducing the potential for environmental contamination with N and P. At the same time the efficiency of N and P utilization by grain legumes might also be increased (Raman and Selvaraj, 2006).

PRODUCTION OF GROWTH HORMONES AND ENZYMES BY MYCORRHIZAL FUNGI

The mycorrhizal fungi produce enzymes, auxins, vitamins, cytokinins and other substances that increase rootlet size and longevity (Miller, 1971). The role of auxins in the establishment of ectomycorrhizal association was first suggested by MacDougal and Defrenoy (1944). Slankis (1958) studied the impact of auxins on exercised and attached pine roots and reported that the peculiar morphology of ectomycorrhizas was induced by auxins. Ability of 8 ectomycorrhizal fungi to synthesize indole 3- acetic acid from L-Tryptophan and their growth rate has been studied. Differences

in the levels of IAA synthesis and biomass production among the 8 ectomycorrhizal fungi and also a positive correlation between IAA level and mycelia growth have been observed. It has been observed that the synthesis of IAA and mycelial biomass were on 30th day after incubation. *Pisolithus tinctorius* and *Laccaria laccata* exhibited higher amounts of IAA production than other fungi, whereas *Amanita muscaria* and *Rhizopogan luteolus* showed least quantity of IAA (Gopinathan and Raman, 1992).

Ectomycorrhizal fungi are important in catalysing the hydrolysis of organic phosphate and significantly enhance phosphate uptake by the host roots (Bowen, 1973). The primary role of extracellular acid and alkaline phosphatases is to provide inorganic phosphate for cell growth by hydrolysis of external phosphate esters which do not penetrate the cytoplasmic membrane. Acid phosphatase was found to be localized throughout the fungal sheath of beech mycorrhizae (Williamson and Alexander, 1975) and high acid phosphatase activity was shown in the *in vitro* culture of *Hebeloma crustuliniforme* and *Laccaria laccata* (Ho and Tilak, 1988). The growth and acid phosphatase activity of *Laccaria laccata* has been studied and it has been found that *L. laccata* was more tolerant to Cu than Ni and increasing Cu and Ni concentration induce the increase of acid phosphatase activity (maximum at 0.15 µm) in *L. laccata* (Periasamy and Raman, 1995).

The axenic growth, total protein content and acid and alkaline phosphatase activities *Amanita muscaria* have been estimated and it has been found that *A. muscaria* was also more tolerant to Cu than Ni. Maximum acid phosphatase activity of *A. muscaria* (798 µ moles p-nitrophenyl/mg dry wt/30 min; in control 591) was observed in 0.15 µm on 30 d whereas in nickel, it was 755. *A. muscaria* showed increased alkaline phosphatase activity (4.9; in control 2.2) in 30 µm concentration of Cu at 10 d whereas in nickel, it was 4.3 (Raman *et al*., 1998). Among *Laccaria laccata* and Suillus *bovinus*, *L. laccata* had maximum acid and alkaline phosphatase activities and tolerance to high concentration of chromium (Raman *et al*., 2002).

ENHANCEMENT OF GROWTH HORMONES BY MYCORRHIZAL FUNGI AND *FRANKIA*/ *RHIZOBIUM* INOCULATION

The absence of detectable amount of indole acetic acid and cytokinin in *C. equisetifolia* root nodules may be due to fluctuation of several hormones in the nodule with time of the year. However, the isolation of pure culture of *Frankia* and the advancement in techniques resulted in the detection of IAA and cytokinins in *Frankia* cultures. But the information concerning the cytokinin in nodules of actinorhizal plants is scanty (Wheeler *et al*., 1979). *C. equisetifolia* seedlings were raised in glasshouse condition and inoculated with suspension of pure culture of *Frankia.* The nodules have been collected and analysed for the presence of cytokinin and significantly the cytokinin has been detected in the nodules (Raman and Elumalai, 1996).

Glomus mosseae is known to produce GA-like substances (Barea and Azcon-Aguilar, 1982). Significantly increased GA has been demonstrated in roots, nodules and cladodes of triple (*Glomus fasciculatum*, *Pisolithus tinctorius* and *Frankia*) inoculated plants of *Casuarina equisetifolia* (Raman and Elumalai, 1998). Maximum amounts of IAA in cladodes and roots of *Casuarina equisetifolia* have been established by HPLC analysis in triple (*Glomus fasciculatum*, *Pisolithus tinctorius* and *Frankia*) inoculated plants than the other individual treatment of symbiont (Elumalai *et al*., 2011). Mycorrhizal inoculation is known to stimulate the ontogeny and delay senescence of leaves mediated by altered hormonal level (Daft and Nicolsen, 1969). Levels of IAA, cytokinin and gibberllin-like activity of actinomycetes and leguminous nodules are higher than in the roots (Newcomb *et al*., 1977). Higher content of IAA in nodules of *Glomus mosseae* and *Rhizobium* inoculated *Prosopis juliflora* has been demonstrated (Raman *et al*., 1994).

AXENIC GERMINATION OF VAM SPORES

Axenic cultivation of vesicular arbuscular mycorrhizal fungi has so far not been achieved. Spores of most VAM fungi readily germinate *in vitro* with hyphal elongation for relatively short period on various media (Siqueira *et al*., 1985). Hyphal growth from germinating spores ceased before exhaustion of the spore reserves. This has motivated investigations on nutritional, physiological and genetic aspects. Germination of studies on VAM spores suggests that storage was necessary to overcome dormancy in freshly harvested spores. Several factors have been reported to affect spore germination like nutrients, root exudates, soil temperature, moister, pH and light (Daniels and Graham, 1976). Spores of *Scutellospora erythropa* and *Scu. nigra* isolated from neem rhizosphere soils from coastal regions of Chennai, India have been tested for axenic germination in *in vitro* conditions. They showed positive results in media of different composition using root exudates, soil extract, thiamine HCl and inositol. The combined medium increased the spore germination in *Scu. erythropa* and in *Scu. nigra* over water agar control. The germ tube often grew up to 3.8 cm on combined media but no vegetative spores and extramatrical auxiliary cells were observed during the experiment. There was significant increase in hyphal growth when the

roots were introduced into the medium, 3 days after spore germination (Raman and Sambandan, 2000).

Based on the method developed, growth of *Glomus mosseae* and *Gigaspora gigantea* on *in vitro* root organ culture of *Sorghum vulgare* and *Saccharum officinarum* has been carried out. Spores of *Gl. mosseae* and *Gig. gigantea* germinated on minimal medium produced extraradical mycelium. *Gl. mosseae* infected roots of *S. officinarum* in *in vitro* condition were inoculated in M medium with *in vitro* cultured roots of *Sorghum vulgare* (test roots). From the infected root of *S. officinarum*, the mycelium developed and it infected the test roots. The roots developed new mycelia and further the mycelia produced a few hyaline spores. In MS medium combined with soil extract, root exudate, thiamine HCI and inositol combination, spore germination and germ tube growth were higher when compared with other media (Raman et al., 2001).

MYCORRHIZAL INOCULATION FOR PLANT GROWTH

Nowadays, large scale inoculation of forest nurseries with selected ectomycorrhizal fungi appears imminent. Commercial interest in producing pure cultures of ectomycorrhizal fungi and ectomycorrhizal fungal inoculum expands the possibilities of worldwide application. The success of these inoculations depends on the selection of effective and beneficial fungal symbionts. Progress in entrapping living cells in alginate gel has presented a new kind of inoculum. Mycelia of ectomycorrhizal fungi grown in liquid medium can be entrapped in beads of calcium alginate gel has been proposed for other organisms (Mauperin *et al*., 1987). Sodium alginate beads of *Laccaria laccata* were prepared. The beads were viable for 10 months. The fungal hyphae in the beads formed mycorrhizal association with *Eucalyptus tereticornis* and enhanced growth has been demonstrated in the seedlings due to mycorrhizal association. Mycobead is efficacious, practical and new form of inoculum (Raman *et al*., 1998)

Selenium is an essential micronutrient for humans and animals, and Se deficiency is an important problem in several countries (Whanger *et al*., 2000; Gergely *et al*., 2004). Se is known to take part in antioxidative defense and protect against a number of diseases, including cardiovascular disease and cancer in humans. Arbuscular mycorrhizal fungi (AMF) association promotes growth, uptake of more nutrients and survival of host plants in most natural communities. Se concentration in most soils lies within the range of 0.01–2 mg/kg of soil. Moreover, high Se concentrations in soil (up to 1200 mg/kg) have been reported in seleniferous areas of the world (McNeal and Balistrieri, 1989). However, differentially Se uptake by the plants is related to variations in Se availability, nutritional status of the plant, physical factors such as root depth, and possibly the influence of rhizosphere microorganisms (Milne, 1998). Se-methyl-selenocysteine is one of the main selenocompound in Se-enriched garlic, and selenomethionine is the major selenocompound in cereal grains, grass legumes, soybeans and Se-enriched yeast (Whanger, 2002).

Considering the importance of Se enrichment and the physicochemical and biochemical changes in garlic plants by different species of AMF, a study was aimed and investigated. In addition, accumulation of Se in different parts of the plants and conversion of inorganic Se to organic Se compounds were also studied. Garlic plants were grown in the pots inoculated with *Glomus fasciculatum* and *Gl. moss*eae and maintained in a greenhouse. Three weeks after planting, the pots had received different concentrations of Se (5, 10, 15, 20, 25 mg/kg of soil) in the form of selenium dioxide (SeO_2) at 3 weeks intervals up to 12 weeks. For physiological and biochemical analysis, the samples were randomly collected from five plants of each experiment. Maximum AM infection, spore population and plant biomass were observed in the roots of mycorrhizal-mediated plants without Se, and they were gradually declined in both mycorrhizal and non-mycorrhizal (NM) plants with increasing concentrations of Se. Among the two *Glomus* species tested, *Gl. fasciculatum*-mediated plants showed higher AM infection, spore population and plant biomass than *Gl. mosseae*. AMF inoculated plants showed more uptake of Se than NM plants. Higher contents of total chlorophyll and sugars were observed in plants inoculated with *Gl. fasciculatum* without Se and they were decreased in the presence of Se. In contrast, increased amount of glutathione peroxidase (whose main biological role is to protect the organism from oxidative damage) was observed at increasing concentrations of Se up to 20 mg/kg. High-performance liquid chromatography data revealed that SeO_2 converted to organic form of Se as g-glutamyl-Se-methylselenocysteine (Patharajan and Raaman, 2012). These results are basis for further investigations on the role of AMF on plant growth and uptake of Se in edible crops.

The effects of an endomycorrhizal fungus *Glomus fasciculatum*, an ectomycorrhizal fungus *Pisolithus tinctorius* and *Frankia* on the growth performance of *C. equisetifolia* seedlings under controlled conditions and ultrastructure of root system have been investigated. The assessment of growth characters of mycorrhizal and actinorhizal association by light and scanning electron microscope methods revealed that *C. equisetifolia* roots were infected with arbuscules and vesicles of *Gl. fasciculatum* and *P. tinctorius* formed fungal

sheath but no Hartig net. Large number of cortical cells was seen infected with *Frankia*, hyphae of *Frankia* were frequently seen penetrating from cell to cell directly through cell walls and *Frankia* occupied majority of the cell volume (Elumalai and Raaman, 2009).

APPLICATION OF MYCORRHIZA IN RECLAMATION OF HEAVY METAL POLLUTED AND TANNERY POLLUTED SOILS

Pollution of biosphere with toxic metals due to manmade activities poses a major environmental and human health problem. The sources of metals in soil are diverse, which include burning of fossil fuels, mining and smelting of metalliferous ores, municipal wastes, fertilizers, pesticides, seaweed sludge and the use of dyes and batteries. Heavy metals are potentially cytotoxic, carcinogenic and mutagenic. Severe contaminations by pollutants, such as heavy metals, can result in wide spread seedling motality and delay in revegetation for decades (Gadd, 1990). Chromium is an essential trace element, as it is required for glucose metabolism and is found in food and feed in concentrations between 0.05 - 2.4 mg/kg. The Cr contaminated tannery effluents and Cr contaminated solid waste from tanneries are detrimental to the growth of plants. Mycorrhizal plants form an important biological resource and their introduction to mine wastes and metal contaminated and polluted sites will be highly useful in phytoremediation and revegetation (Raman and Srinivasan, 2005).

A survey of VAM fungi in heavy metal polluted soils in near Tiruchirapalli, Tamil Nadu, India was conducted. The sites contained maximum amount of Zn followed by Cu, Pb, Ni and Cd. Out of 18 plant species examined, 16 species were found to be mycorrhizal. The study on percentage of VAM colonization and diversity of spore density in the rhizosphere soils revealed that of the 15 VAM fungi observed, 10 species belong to the genus *Glomus*, 2 species each belong to *Gigaspora* and *Acaulospora* and 1 species belong to *Sclerocystis* (Sambandan *et al*., 1992). The study on the effect of inoculation of VAM fungus, *Glomus geosporum* on effluent tolerance of *Casuarina equisetifolia* revealed that effluent had a negative impact on growth and productivity of *C. equisetifolia* seedlings. VAM inoculation resulted in greater retention of toxic heavy metals in roots and lesser translocation to shoots, accompanied by increased P in seedlings (Sambandan *et al*., 1991).

One of the serious problems of tanneries in India is the disposal of large quantities of waste generated by soaking, washing, pickling and tanning of animal hides. Whether organic or inorganic in composition, the waste materials are mobile and persist in the environment, accumulate through food chains and result in adverse human and ecological effects upon exposure. Toxicity and environmental effects of tannery wastes particularly for their effect on soil properties and ground water table are well documented (Sastry and Prasad, 1980). The potential of microbes in detoxicifying these wastes through catabolism or selective accumulation is also a fact (Peyton, 1984). There are some reports on VA-mycorrhizae from disturbed and polluted soils (Arnold and Kapustka 1987; Bajwa *et al*., 1991; Gildon and Tinker, 1981; 1983 a,b; Kooman *et al*., 1990; Leyvel *et al*., 1991) which suggest the necessary of the selection of tolerant strains. There was no literature available on the relationship between mycorrhizal fungi and tannery effluent polluted soils and hence a study was conducted to assess the mycorrhizal profile of tannery effluent polluted wastelands of North Arcot district, Tamil Nadu, India.

The percentage of mycorrhizal colonization from three study sites varied between 11 and 64%. The percentage increased in summer season and declined in rainy season. The soils of study sites were found to be clayey loam and alkaline (pH 7.6-8.1). The per cent organic matter varied between 22 to 29%. The chemical analysis of effluent samples revealed that the pH of the effluents was alkaline, ranged from 8.2 to 8.8. BOD, COD, total sulphides, total chlorides and chromium were high in samples of all the 3 sites. Analysis of rhizosphere soil of the sites revealed the presence of 15 VAM fungal species and they belong to 4 VAM genera namely, *Glomus* (9), *Gigaspora* (3), *Scutellospora* (3), and *Sclerocystis* (1). Out of 22 plant species examined, 19 plants were mycorrhizal. Among the plant species examined, *Prosopis juliflora*, *Azadiracta indica*, *Heliotropium zeylanicum* were associated with maximum number of VAM fungi. Many VAM fungal species were found to be associated with more than one plant species, among them *Gl. fasciculatum*, *Gl. geosporum*, *Gigaspora gigantea* were found to be associated with maximum number of plant species, which were incidently called common endophytes in polluted soils examined. *Glomus* species are prevalant in tannery effluent polluted soils, indicate their adaptability to heavy metal exposure. *Glomus fasciculatum*, *Gl. geosporum*, *Gigaspora gigantea* are dominant mycorrhizal symbionts in the polluted environment. Suitable mycorrhizal fungi and host plants will make them sustainable one in the tannery effluent polluted soils (Raman and Sambandan, 1998).

Tolerance of microorganisms helps for the plants to escape from the adverse conditions (Singleton, 2001). It is possible that *Rhizobium* spp. might have adapted to the tannin in the soil and multiplied. Indeed, adaptation of microorganisms to aromatic

substances is well documented (Subba Rao *et al*., 1971; Nagarajan and Mahadevan, 1971). Condensed tannins present in high concentrations are antinutritive by binding to cell wall polymers, rendering the walls undegradable, as well as by binding digestive enzymes secreted by rumen microorganisms, rendering the enzymes inactive (Gamble *et al*., 1996). Biological methods are reported to be much effective in reduction of pollution load of an effluent (Sastry and Mohanrao, 1963). An investigation on the *Rhizobium* species of 4 different tropical woody legumes, such as *Acacia planifrons*, *Pithecellobium dulce*, *Pongamia glabra* and *Prosopis juliflora* was undertaken to study the effect of tannin, chromium and tannery effluent on the growth of the *Rhizobium* species and the biodegradation and tolerance of *Rhizobium* species against tannin and chromium. The four *Rhizobium* species isolated from 4 legumes were treated with tannin, chromium and tannery effluent amended medium and it was found that better growth of *Rhizobium* species in increasing concentrations of tannin, chromium and tannery effluent without mannitol. Among the 4 species, the one from *Prosopis juliflora* had more tolerance (Selvaraj and Raman, 2003).

Positive interaction between mycorrhizal fungi and saprophytic fungi has been established that P-solubilizing strain of *Penicillium bilaji* increased plant dry matter and P uptake by VAM colonized beans and wheat (Kucey, 1987). Fungi such as *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor* and *Neurospora* can remove phenolic heavy metals from polluted soils. *Gliocladium virens*, a saprophytic fungus decolourises paper and pulp mill effluents. Toxic tannins that commonly occur in tannery effluents are completely broken down by some species of *Aspergillus*, *Penicillium* and *Fusarium*. Thus some of the saprophytic fungi help mycorrhizal fungi for the reclamation of polluted soils with plants.

ARBUSCULAR MYCORRHIZAL FUNGI AS BIOCONTROL OF PLANT PATHOGENS

Arbuscular mycorrhizal (AM) fungi enhance plant growth, especially in soils of low nutrients status. Some lines of evidence have shown that inoculation of host plants with AM fungi could reduce incidence and severity of root diseases in large number of crop plants and the mechanism has been termed as induced systemic resistance. However, the exact mechanisms by which AM fungi contribute to resist pathogen colonization in root tissues are fully understood. An attempt was made to overview the available information on the mechanisms of induced systemic resistance brought out by AM fungi (Raman *et al*., 2002).

In recent years, the management of crop diseases caused by root rot pathogens has become one of the most challenging research areas in plant pathology. Increasing knowledge and concern about environmental consequences of fungicide applications have prompted many scientists to explore the potential for alternative strategies of disease and pest management. Among the suggested strategies, the promising strategies for minimizing damage from plant pathogens are biological control of pathogenic population by microorganisms and induced systemic resistance in plants by inoculation of arbuscular mycorrhizae (AM) fungi (Schwab *et al*., 1991). AM fungi can induce resistance or increase tolerance to some root pathogens, such as nematodes or fungi (Azcon-Aguilar and Barea, 1997; Trotta *et al*., 1996). Hence, the protective effects induced by AM fungus *Glomus fasciculatum* against *Fusarium oxysporum* f. sp. *Lycopersici*, causal agent of wilt disease in tomato, were examined. The experimental design was completely randomized factorial combination of *Gl. fasciculatum* and *Fusarium oxysporum* f. sp. *lycopersici*. Six treatments were used and the physiological and biochemical studies of leaf and root samples were carried out. It was found that the post inoculation of pathogen (PoI) and dual inoculation of both at a time (DI) plants control the pathogen significantly. Increased level of chlorophyll contents, O-dihydric phenol content, total phenol content, lipid levels, protein content, total and reducing sugar, amino acid content, cytokinin and tomatine content were observed in PoI and DI plants when compared to other treatments. These biochemical changes may play a crucial role to protect tomato plants from pathogens. In the field trial, *Gl. fasciculatum* increased the growth and yield of tomato plants. The mechanism of control is not only to improved plant nutrition by mycorrhizal fungi but also to other physiological and biochemical factors associated with AM fungi (Raaman and Gnanaguru, 2015).

INTEGRATION OF AM FUNGI WITH MICROPROPAGATED PLANTS

In vitro micropropagation techniques are increasingly being applied to large scale production of quality planting materials especially in medicinal plants, fruit crops and woody timber trees. The four important stages in the micropropagation of plants are the regeneration in *in vitro*, proliferation of regenerates, rooting in *in vitro* and for transplantation of the plantlets in the soil or fields. Of these, the last stage is very crucial and important with respect to the establishment of the *in vitro* derived plantlets. It has been established that tissue culture plantlets have very divergent leaf anatomy and physiology and hence require an acclimatization period during the transition from culture to green house, from a total unnatural system to the very natural environment. In the *in*

vitro condition, plantlets are heterotrophic and get very high favourable conditions for their growth. But in the *in vivo* situation, the plantlets have to switch over to autotrophic nutrition, involving normal photosynthetic activity and water relations. The plantlets may not be able to withstand such sudden shocks of the environmental changes, mostly due to some aberrant characteristic features of *in vitro* derived plantlets. Inoculation with arbuscular mycorrhizal (AM) fungi on micropropagated plantlets to overcome this problem and improves the establishment and growth in the field condition (Raaman and Patharajan, 2006).

Alternanthera sessilis has been used to treat various diseases in traditional medical systems (Siddha, Ayurveda and Unani) of India. The plant contains iron, protein, vitamins and minerals, and is a potential nutraceutical herb. Considering the medicinal importance of the plant, an effort was made to improve the survivability of micropropagated plants by mycorrhizal inoculation. During acclimatization, sterile AMF spores (*Glomus mosseae* or *Giagaspora margarita* or both) were added for the enhanced survivability of *in vitro* plants in the field conditions. The AMF inoculated plants showed at least one fold higher growth, biomass and percentage of survivability of tissue cultured plants than the control tissue cultured plants. The level of chlorophyll, amino acids, protein content and activities of acid and alkaline phosphatases in leaves and roots were maximum in mixture of *Gl. mosseae* and *Gig. margarita* inoculated plants when compared to single inoculated plants. Very low levels of all parameters were observed in control tissue cultured plants. Whereas the proline content decreased in all the 3 treatments when compared to control plants, indicating AMF inoculation reduced the stress to tissue cultured plants (Raaman *et al*., 2005). During acclimatization of micropropagated plantlets of *Solanum trilobatum*, sterile AM fungal spores were added to enhance the survivability of *in vitro* plants in the field condition. Enhanced level of biochemical changes and increased survivability were observed in plants inoculated with spores of *Glomus mosseae* and *Gigaspora margarita* (Raaman *et al*., 2011).

CONCLUSION

Thus, my research concepts are clear towards the understanding biodiversity of Indian mycorrhizal fungi, mycorrhizal, actinorhizal and host tripartite symbioses, production of growth hormones and enzymes by mycorrhizal fungi, enhancement of growth hormones by mycorrhizal fungi and *Frankia/Rhizobium* inoculation, axenic germination of VAM spores, mycorrhizal inoculation for plant growth will help in the enhanced growth and yield of the plants. The application of mycorrhizal fungi will help us for reclamation of heavy metal polluted and tannery polluted soils. By the use of arbuscular mycorrhizal fungi as biocontrol of plant pathogens and integration of AM fungi with micropropagated plants, the health of agricultural and cash crops can be protected and the human beings can get healthy plants and healthy yields.

REFERENCES

Amaranthus, M.P. and Trappe, J.M. 1993. Effects of erosion on ecto- and VA mycorrhizal inoculum potential of soil following forest fire in southwest Oregon. *Plant Soil*, **150**:41-49; doi: 10.1007/BF00779174.

Arditti, J. 1967. Factors affecting the germination of orchid seeds. The *Botanical Review*, **33**:1-97; doi: 10.1007/BF02858656.

Arnold, P.T and Kapustka, L.A. 1987. VA mycorrhizal colonization and spore populations in an abandoned agricultural field after five years of sludge application. *Ohio Journal of Science*, **87**:112-114.

Azcon-Aguilar, C. and Barea, J.M. 1997. Arbuscular Mycorrhizas and biological control of soil-borne plant pathogens- an overview of the mechanisms involved. *Mycorrhiza*, **6**:457-464; doi: 10.1007/s005720050147.

Azcón-Aguilar, C. and Barea, J.M. 2015. Nutrient cycling in the mycorrhizosphere. *Journal of Soil Science and Plant Nutrition*, **15**; doi: 10.4067/S0718-95162015005000035.

Bajwa, R., Khan, F. and Mohmood, S. 1991. Vesicular-arbuscular mycorrhiza in industrially polluted soils. In: Monograph: Contemporary studies in mycorrhiza and Bio-magnetism. *Science International (Lahore)*, pp.31-37.

Bakshi, B.K. 1980. The status and future of mycorrhiza research in India. In: Tropical Mycorrhiza Research (Ed.: Mikola, P.). Clarendon Press, Oxford, pp.102-106.

Barea, J.M. and Azcón-Aguilar, C. 1982. Production of plant growth-regulating substances by the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae*. *Applied and Environmental Microbiology*, **43**:810-813; doi: 10. 1128/aem.43.4.810-813.1982.

Bever, J.D., Morton, J.B., Antonovics, J., *et al*., 1996. Host-dependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. *J. Ecology*, **84**:71-82; doi: 10.2307/2261701.

Bowen, G.D. 1973. Mineral nutrition of ectomycorrhizae. In: Ectomycorrhizae. Their Ecology and Physiology (Eds.: Marks, G.E. and

Kozlowski, T.T.). Academic Press, New York, pp.151-206.

Bowen, G.D. 1987. The biology and physiology of infection and its development. In: Ecophysiology of VA Mycorrhizal Plants (Ed.: Safir, G.R.). CRC Press, U.S.A., pp.27-58.

Cluett, H.C. and Boucher, D.H. 1983. Indirect mutualism in the legume *Rhizobium* - mycorrhizal fungus interaction. *Oecologia*, **59**:405-408; doi: 10.1007/BF00378870.

Daft, M.J. and Nicolson, T.H. 1969. Effect of *Endogone* mycorrhiza on plant growth iii. Influence of inoculum concentration on growth and infection in tomato. *New Phytologist*, **68**:953-963; doi: 10.1111/j.1469-8137.1969. tb06495.x.

Daniels, B.A. and Graham, S.O. 1976. Effects of nutrition and soil extracts on germination of *Glomus mosseae* spores. *Mycologia*, **68**:108-116; doi: 10. 1080/00275514.1976.12019888.

Danielson, R.M. 1985. Mycorrhizae and reclamation of stressed terrestrial environments. In: Soil reclamation processes: Microbiological analyses and applications (Eds.: Tate, R.L. and Klein, D.A.). Marcel Dekker, New York, pp.173-201.

Elumalai, S. and Raaman, N. 2009. *In vitro* synthesis of *Frankia* and mycorrhizae with *Casuarina equisetifolia* and ultrastructure of root system. *Indian Journal of Experimental Biology*, **47**:289-297.

Elumalai, S., Prabhakaran, M. and Raaman, N. 2011. Indole acetic acid (IAA) levels of cladodes and roots in effects of mycorrhizal and actinorhizal inoculation on *C. equisetifolia* under glass house condition. *Recent Research in Science and Technology*, **3**:70-75.

Gadd, G.M. 1990. Heavy metal accumulation by bacteria and other microorganisms. *Experentia*, **46**:834-840.

Gamble, G.R., Akin, D.E., Makkar, H.P., *et al.*, 1996. Biological degradation of tannins in *Sericea lespedeza* (*Lespedeza cuneata*) by white rot fungi *Ceriporiopsis subvermispora* and *Cyathus stericoreus*, analysed by solid state ^{13}C nuclear magnetic resonance spectroscopy. *Applied and Environmental Microbiology*, **62**:3600-3604; doi: 10.1128/aem.62.10.3600-3604.1996.

Gergely, V., Kapolna, E., Sule, A., *et al.*, 2004. Preparative liquid isoelectric focusing (Rotofor IEF) based Se-speciation of Se-enriched *Agaricus bisporus*. *Journal of Analytical and Atomic Spectrometry*, **19**:1485-1488.

Gibson, D.J. and Hetrick, B.A.D. 1988. Topographic and fire effects on the composition and abundance of VA-Mycorrhizal fungi in tallgrass prairie. *Mycologia*, **80**:433-441; doi: 10.2307/3807844.

Gildon, A. and Tinker, P.B. 1981. A heavy metal tolerant strain of a mycorrhizal fungus. *Transactions of British Mycological Society*, **77**:648-649; doi: 10.1016/s0007-1536(81) 80118-0.

Gildon, A. and Tinker, P.B. 1983a. Interactions of vesicular-arbuscular mycorrhizal infection and heavy metals in plants. I. The effect of heavy metals on the development of vesicular-arbuscular mycorrhizas. *New Phytologist*, **95**:247-261; doi: 10.1111/j.1469-8137. 1983.tb03491.x.

Gildon, A. and Tinker, P.B. 1983b. Interactions of vesicular- arbuscular mycorrhizal infection and heavy metals in plants. II. The effect of infections on copper. *New Phytologist*, **95:**262 - 268; doi: 10.1111/j.1469-8137.1983.tb03492.x.

Gopinathan, S. and Raman, N. 1992. Indole-3-acetic acid production in ectomycorrhizal fungi. *Indian Journal of Experimental Biology*, **30**:142-143.

Harley, J.L. 1969. The biology of mycorrhiza. Leonard Hill, London, 334 p.

Harrison, M.J. 1998. Development of the arbuscular mycorrhizal symbiosis. *Current Opinion in Plant Biology*, **1**:360-365; doi: 10.1016/1369-5266(88)80060-8.

Hart, M.M. and Reader, R.J. 2002. Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. *New Phytologist*, **153**:335-344; doi: 10.1046/j.0028-646X.2001. 00312.x.

Ho, I. and Tilak, H. 1988. A simple method for assessing acid phosphatase activity of ectomycorrhizal fungi. *Transactions of British Mycological Society*, **91**:346-347; doi: 10. 1016/S0007-1536(88)80227-4.

Jasper, D.A., Robson, A.D., Abbot, L.K. 1988. Revegetation in an iron ore mine-nutrient requirements for plant growth and potential role of vesicular arbuscular (VA) mycorrhizal fungi. *Australian Journal of Soil Research*, **26**:477-507.

Kooman, I., McGrath, S.P., Giller, I. 1990. Mycorrhizal infection of clover is delayed in soils contaminated with heavy metals from past sewage sludge applications. *Soil Biology and Biochemistry*, **22**:871-873; doi: 10.1016/0038-0717(90)90170-5.

Kucey, R.M.N. 1987. Contribution of N_2 fixation to field bean and pea over the growing season under field conditions in Southern Alberta.

Canadian Journal of Soil Science, **69**:650-699; doi: 10.1016/j.agee.2017.08.028.

Le Tacon, F., Carbaye, J., Carr, G. 1987. The use of mycorrhizae in temperate and tropical forests. *Symbiosis*, **3**:179-206.

Leyvel, C., Berthelin, J., Schontz, *et al*., 1991. Influence of endomycorrhizas on maize uptake of Pb, Cu, Zn and Cd applied as mineral salts or sewage sludge. In: Heavy metals in the Environment (Ed.: Farmer, J.). CEP Consultants Ltd, Edinburgh, pp.204-207

Macdougal, D.T. and Dufrenoy, J. 1944. Mycorrhizal symbiosis in Aplectrum, Corallorhiza and Pinus. *Plant Physiology*, **19**:440-465; doi: 10.1104/pp.19.3.440.

Marx, D.H. 1977. Tree host range and world distribution of the ectomycoorhizal fungus *Pisolithus tinctorius. Canadian Journal of Microbiology*, **23**:217-223; doi: 10.1139/m77-033.

Mauperin, C., Mortier, F., Garbaye, J., *et al*., 1987. Viability of an ectomycorrhizal inoculum produced in a liquid medium and entrapped in a calcium alginate gel. *Canadian Journal of Botany*, **65**:2326-2329; doi: 10.1139/b87-316.

McNeal, J.M. and Balistrieri, L.S. 1989. Geochemistry and occurrence of selenium: an overview. In: Selenium in agriculture and the environment (Ed.: Jacobs, L.W.). American Society of Agronomy, Madison, WI, pp.1-14.

Miller, C.O. 1971. Cytokinin production by mycorrhizal fungi. In: Mycorrhizae (Ed.: Hacskaylao, E.). USDA, Forest Service Micellaneous Publication 1189, Washington D.C., pp.168-174.

Miller, R. 1987. VAM in grasslands. In: Ecophysiology of VA Mycorrhizal Plants (Ed.: Safir, G.R.). CRC Press, U.S.A., pp.250-275.

Miller, R.W., McRac, D.G., Al-Jobore, A., *et al*., 1988. Respiration supported nitrogenase activity of isolated *Rhizobium meliloti* bacteroids, *J. Cell Biochemistry*, **38:**35-49; doi: 10.1002/jcb.240380105.

Milne, J.B. 1998. The Uptake and Metablolism of Inorganic Selenium Species. In: Environmental Chemistry of Selenium (Eds.: Frankenberger Jr., W.T. and Engberg, R.A.). Marcel Dekker, New York, pp.459-477.

Mingyuan, W. and Pan, J. 2015. Colonization and Diversity of AM Fungi by Morphological Analysis on Medicinal Plants in Southeast China. *The Scientific World Journal*, 2015; doi: 10.1155/2015/753842.

Nagarajan, M. and Mahadevan, A. 1971. Carbohydrate repression and effect of cyclic AMP on the synthesis of catechol oxygenase in *Pseudomonas tabaci. Indian Journal of Experimental Biology*, **17**:757-759.

Newcomb, W., Syono, K., Torrey, J.J. 1977. Development of an ineffective pea root nodule. Morphogenesis, fine structure and cytokinin biosynthesis. *Canadian Journal Botany*, **55**: 1891-1907.

Patharajan, S. and Raaman, N. 2012. Influence of arbuscular mycorrhizal fungi on growth and selenium uptake by garlic plants. *Archieves of Phytopathology and Plant Protection*, **45**:138-151; doi: 10.1080/03235408.2010.501166.

Periasamy, K. and Raman, N. 1995. Effects of Cu and Ni on acid phosphatase activity of an ectomycorrhizal fungus, *Laccaria laccata*. *Indian Journal of Experimental Biology*, **33**:537-538.

Perry, D.A., Molina, R., Amaranthus, M.P. 1987. Mycorrhizae, Mycorrhizo- spheres, and reforestation: Current knowledge and research needs. *Canadian Journal of Forest Research*, **17**:929-940.

Peyton, T.O. 1984. Biological disposal of hazardous waste. *Enzyme and Microbial Technology*, **6**:146- 154; doi: 10.1016/0141-0229(84)90022-X.

Piotrowski, J.S., Denich, T., Klironomos, J.N., *et al*., 2004. The effects of arbuscular mycorrhizas on soil aggregation depend on the interaction between plant and fungal species. *New Phytologist*, **164**:365-373.

Purnell, H.M. 1960. Studies of the family Proteaceae: 1. Anatomy and morphology of the roots of some Victorian species. *Australian Journal of Botany*, **8**:38-50; doi: 10.1071/BT9600038.

Purves, S. and Hadley, G. 1975. Movement of carbon compounds between the partners of orchid mycorrhiza. In: Endomycorrhiza (Eds.: Sanders, F.E., Mosse, B., Tinker, P.B.). Academic Press, New York and London, pp.175-194.

Raaman, N. and Gnanaguru, M. 2015. Biocontrol of wilt disease (*Fusarium oxysporum* f. sp. *lycopersici*) in tomato by *Glomus fasciculatum*. *International Journal of Research in Engineering and Applied Sciences*, **5**:34-75; doi: 10.1111/j.1439-0434.2005.01018.x.

Raaman, N. and Patharajan, S. 2006. Integration of arbuscular mycorrhizal fungi with micropropagated plants. In: Current concepts in Botany (Eds.: Mukerji, K.G., Manoharachary, C.). I.K. International Publishing House Pvt. Ltd., New Delhi, pp.235-251.

Raaman, N., Elamvaluthi, M., Jeyam, M. 2011. Enhanced growth and survivability of micropropagated plants of *Solanum trilobatum* L. by mycorrhizal inoculation. In: Experimental phytochemical techniques (Ed.: Raaman, N). New India Publishing Agency, New Delhi, pp.323-331.

Raaman, N., Elamvaluthi, M., Senthil Kumar, M. 2005. Studies on mycorrhizal inoculation on micropropagated plants of *Alternanthera sessilis* L., for enhance growth and survivability. *Indian Journal of Applied Microbiology*, **29**:41-46.

Raman, N and Rajendran, V. 1994. Association of *Pisolithus tinctorius* with *Eucalyptus tereticornis* in India. *Indian Forester*, **120**:62-65.

Raman, N. and Elumalai, S. 1991. Studies on mycorrhizal and actinorhizal association in *Casuarina equisetifolia* in Coramandal coastal region. *Journal of Tropical Forestry*, **7:**138-150.

Raman, N. and Elumalai, S. 1992. Influence of phosphorus on cluster root formation by *Casuarina equisetifolia* in water culture. *Indian Journal of Experimental Biology*, **30**:928-929.

Raman, N. and Elumalai, S. 1996. Presence of cytokinin in the root nodules of *Casuarina equisetifolia*. *Indian Journal of Experimental Biology*, **34**:577-579.

Raman, N. and Elumalai, S. 1998. Gibberellic acid in mycorrhizal and actinorhizal inoculated *Casuarina equisetifolia*. *Indian Journal of Experimental Biology*, **36**:703-708.

Raman, N. and Nagarajan, N. 1996. Effect of forest fire on VAM fungi in a tropical forest of southern India. *Commonwealth Forestry Review*, **75**:247-252.

Raman, N. and Nagarajan, N. 1999. Mycorrhizal association of orchids in a tropical forest of southern India. *Journal of Tropical Forest Science*, **11**:548-553.

Raman, N. and Sambandan, K. 1998. Distribution of VAM fungi in Tannery effluent polluted soils of Tamil Nadu, India. *Bulletin of environmental contamination and toxicology*, **60**:142-150; doi: 10.1007/s001289900602.

Raman, N. and Sambandan, K. 2000. Axenic germination of *Scutellospora erythropa* and *Scutellospora nigra* in *in vitro* conditions. *Indian Journal of Experimental Biology*, **38**:1159-1163.

Raman, N. and Selvaraj, T. 2006. Tripartite relationship of *Rhizobium*, AMF and host in growth promotion. In: Handbook of Microbial Biofertilizers (Ed.: Rai, M.K.). Haworth Food Products Press, New York, pp.51-88.

Raman, N. and Srinivasan, V. 2005. Application of mycorrhiza in Reclamation of Wastelands and Adverse sites. In: Mycorrhizae – Role and Applications (Ed.: Mehrotra, V.S.). Allied Publishers Pvt Ltd., New Delhi, pp.303-342.

Raman, N., Elumalai, S., Patharajan, S. 2005. Actinorhizal and Mycorrhizal Tripartite Symbiosis in *Casuarina equisetifolia* for Forestry Practices. In: Frontiers in Plant Sciences. (Eds.: K.G. Mukerji, Tilak, K.V.B.R., Reddy, S.M., Gangwane, L.V., Prakash, P., Kunwar, I.K.). I.K. International Pvt. Ltd., New Delhi, pp.657-700.

Raman, N., Gnanaguru, M., Srinivasan, V. 2002. Biocontrol of plant pathogens by arbuscular mycorrhizal fungi for sustainable agriculture. In: Bioinoculants for sustainable agriculture and forestry (Eds.: Reddy S.M., Ram Reddy S., Singarachary, M.A., Girisham, S.). Scientific Publishers (India), Jodhpur, pp.153-175.

Raman, N., Nagarajan, N., Gopinathan S., *et al*., 1993. Mycorrhizal status of plant species colonizing a magnesite mine spoil in India. *Biology and Fertility of Soils*, **16:**76-78.

Raman, N., Nagarajan, N., Sambandan, K. 1992. Vesicular arbuscular mycorrhizae of tree species of mamandur forest of Tamil Nadu. *Journal of Tree Sciences*, **11**:135-139

Raman, N., Periasamy, K., Srinivasan, V. 1998. Production of alginate bead of *Laccaria laccata* and its effects on growth of *Eucalyptus tereticornis*. *Indian Journal of Experimental Biology*, **36**:628-630.

Raman, N., Ravi, I., Gnanaguru, M. 1994. Enhancement of indole - 3 - acetic acid in nodules of *Prosopis juliflora* inoculated with *Glomus mosseae* and *Rhizobium*. *Indian Journal of Microbiology*, **34**:33-35.

Raman, N., Ravi, M. and Srinivasan, V. 1998. Effect of copper and nickel on the axenic growth and phosphatase activity and growth of an ectomycorrhizal fungus, *Amanita muscaria*. *Indian Journal of Environmental Health*, **40**:197-202.

Raman, N., Sahadevan, C., Srinivasan, V. 2001. Growth of AM fungi on *in vitro* root organ culture of *Sorghum vulgare* and *Saccharum officinarum*. *Indian Journal of Experimental Biology*, **39**:1293-1298.

Raman, N., Srinivasan, V., Ravi, M. 2002. Effect of chromium on the axenic growth and phosphatase activity of ectomycorrhizal fungi, *Laccaria laccata* and *Suillus bovines*. *Bulletin*

of environmental contamination and toxicology, **68**:569-575.

Sambandan, K., Kannan, K., Raman N. 1994. Vesicular-arbuscular mycorrhizae of *Casuarina equisetifolia* Forst. in different soil types in Tamil Nadu. *Indian Forester*, **120**:510-514.

Sambandan, K., Kannan, K., Raman, N. 1991. Effect of VAM fungus *Glomus geosporum* on effluent tolerance of *Casuarina equisetifolia*. *Pollution Research*, **10**:55-67.

Sambandan, K., Kannan, K., Raman, N. 1992. Distribution of vesicular–arbuscular mycorrhizal fungi in heavy metal polluted soils of Tamil Nadu, India. *Journal of Environmental Biology*, **13**:159-167.

Sastry, C.A. and Mohanrao, G.J. 1963. Anaerobic digestion of industrial waste. *Environmental Health*, **5**:20-25.

Sastry, C.A. and Prasad, B.C.S. 1980. Treatment and disposal of tannery wastes. In: Leather trade yearbook. Science and Technology, Special supplement. India, pp.75-86.

Schwab, S.M., Menge, J.A., Tinker, P.B. 1991. Regulation of nutrient transfer between host and fungus in vesicular-arbuscular mycorrhizae. *New Phytologist*, **117:**387-398; doi: 10.1111/j.1469-8137.1991.tb00002.x.

Selvaraj, T. and Raman, N. 2003. Effect of tannin, chromium and tannery effluent on growth of *Rhizobium* species. *Indian Journal of Environmental Protection*, **23**:168-172.

Singleton, I. 2001. Fungal remediation of soils contaminated with persistent organic pollutants. In: Fungi in Bioremediation (Ed.: Gadd, G.M.). British Mycological Society Symposia, Cambridge University Press, London, pp.79-96.

Siqueira, J.O., Sylvia, D.M., Gibson, J., *et al.*, 1985. Spores, germination, and germ tubes of vesicular-arbuscular mycorrhizal fungi. *Candian Journal of Microbiology*, **31**:965-972.

Slankis, V. 1958. The role of auxin and other exudates in mycorrhizal symbioses of forest trees. In: The Physiology of Forest Trees (Ed.: Thimann, K.W.). Ronald Press, New York, pp.427-443.

Smith, S.E. and Read, D.J. 2008. Mycorrhizal Symbiosis. San Diego, CA, Academic Press.

Subba Rao, P.V., Nambudri, A.M.O., Bhat, J.V. 1971. Microbial degradation of phenyl propanoid compounds. *Journal of Scientific and Industrial Research*, **30**:663-713.

Trotta, A., Varese, G.C., Gnavi *et al.* 1996. Interactions between the soil borne root pathogen *Phytophthora nicotianae* var. *parasitica* and the arbuscular mycorrhizal fungus *Glomus mosseae* in tomato plants. *Plant Soil*, **185**:199-209; doi: 10.1007/BF02257525.

Whanger, P.D. 2002. Selenocompounds in plants and animals and their biological significance. *Journal of the American College of Nutrition*, **21**:223-232.

Whanger, P.D., Ip, C., Polan, C.E., *et al*., 2000. Tumorgenesis, metabolism, speciation, bioavailability, and tissue deposition of selenium in selenium-enriched ramps (*Allium tricoccum*). *Journal of Agricultural and Food Chemistry*, **48**:5723-5730; doi: 10. 1021/jf000739s.

Wheeler, C.T., Henson, I.E., McLaughlin, M.E. 1979. Hormones in plants bearing actinomycete nodules. *Botanical Gazette*, **140**:S52-S57; doi: 10.1086/337035.

Williamson, B. and Alexander, I.J. 1975. Acid phosphatase localized in the sheath of beech mycorrhiza. *Soil Biology and Biochemistry*, **7**:195-198; doi: 10.1016/0038-0717(75)90037-1.