THE IMPORTANCE OF BIO- FERTILIZERS AND STUDY OF THEIR APPLICATION IN MEDICINALLY IMPORTANT PLANTS.

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ABSTRACT

The medicinal plants constitute a source of raw material for approximately 25% of the prescribed drugs which play an important role in the field of health care. Unscientific exploitation of natural vegetation has led to a large scale denudation and reduction in plant population of medicinal plants. The loss of genetic diversity in the gene pool of these medicinal plants is the most serious environmental problem facing mankind today. Hence, the main aim of the present study is conservation of certain medicinal plants by *ex-situ* conservation method and to improve the soil fertility by the application of eco friendly biofertilizers *(Glomus mosseae* and *Glomus fasciculatum*)

Medicinal plants selected for the present investigations are *Andrographis paniculata, Costus pictus, Gymnema* and *Adhatoda* for their various pharmacological properties and also for their active principles. Pot trial experiments were conducted to study the responses of these medicinal plants to AM fungi association (bio fertilizers). Results envisaged that the total yield in terms of fresh and dry biomass production has been increased. Different yield attributes *viz.*,height, number of branches has been found to be varied with treatments , being highest in the application of bio-fertilizers.

Key words: Medicinal Plants, Conservation, AM fungi

INTRODUCTION

Medicinal plants in developing countries are used as a formational basis for the maintenance of good health, whereas in developed countries they are used for the extracts and development of several drugs and chemotherapeutics. Hence, the market and public demand has been so enormous that there is a great risk that many medicinal plants, today, face either extinction or loss of genetic diversity. The practice of traditional herbal medicines is widespread in both developing and developed countries in their primary health care system. Thus, the cultivation of medicinal plants is increasing steadily to maintain a continuous supply and to support their increasing demand .

Biodiversity convention is the key international regulatory system for ensuring the conservation and sustainable use of biological resources. The biodiversity convention also contains rules for the equitable distribution of the benefits occurring from the use of genetic resources. The convention thus represents the most important international legal frame work for the use and trade in medicinal plants.

Plants have developed numerous strategies to cope with the diverse biotic and abiotic challenges which formed as a consequence of their sedentary life cycle during colonization of terrestrial ecosystem. One of the most successful strategies is the ability of the root systems to establish mutualistic and reciprocally beneficial symbiotic relationships with microorganisms,"Mycorrhizas" the intricate association roots form with specific fungal groups are by far the most frequent of these and represent the underground absorbing organs of most plants in nature. The symbiotic association between fungi and the roots of higher plants comes under the general term " mycorrhiza" which literally means fungus –roots. Vesicular arbuscular mycorrhizal fungi (AM) are ubiquitous in their distribution and occur abundantly (Gabor, 1991; Power and Bagyaraj, 1986; Harley and Smith, 1983). Majority of flowering plants have dynamic association of AM fungi. The significance of AM in augmenting food production is increasingly appreciated. AM fungi are responsible for effective increase in the volume of the soil that can be explored by the plant. The fungi absorbs inorganic soil nutrients, most notably Phosphorus which are translocated to the plant host in exchange for photosynthetically fixed Carbohydrates (Gerdemann, 1964 ; Gerdmann, 1971; Nicolson, 1967 ; Mosse, 1972; Barea *et al.,* 1988; Saheli, *et al*., 2010). These fungi are important in plant growth and reproduction and may influence plant competition, especially in nutrient limiting soils. The bi-directional transfer of phosphate and carbon is a complex process involving the initial movement of nutrients to the symbiotic partner can occur. This bi-directional transport leads to enhanced plant growth, improves physiological status and also helps in completion of fungal life cycle. Recent upsurge of interest and research on mycorrhizal association in plants has been accredited with benefits like increased disease resistance, drought tolerance of hosts, improved water relations, reduced pathogenic infections and in the improvement of soil structure by improving the root health of the host plants. That is why they are referred as " Biofertilizers" and can be substantiated for the substantial amounts of chemical fertilizers. Despite the importance of mycorrhizae in agriculture and forestry, little work has been done regarding their distribution diversity and association with the host plants in India.

Thus, the purpose of present study is to investigate the extent of AM association in some common medicinal plants. In the present investigations four important medicinal plants *Andrographis paniculata, Adhatoda vasica, Gymnema sylvestre and Costus pictus* were selected. All these medicinal plants have got their tremendous medicinal importance. All these medicinal plants have been extensively used and studied worldwide, most of it in the 20^{th} & 21^{st} century and much of its concentration on its pharmacological composition, safety, efficacy and mechanism of action. Present studies is dealt with an investigation on application and study of biofertilizer (AM) association of two species of fungi of class Zygomycetes viz., *Giomus mosseae* and *Glomus fasciculatum.* During surveys, morphological growth parameters like height of the plant, number of nodes, leaves, number of branches, internodal length, length and breadth of the leaf, surface area of leaf, stomatal index, biomass, root and shoot ratio

were taken into consideration to determine the effects of these growth parameters on root colonization, spore count, number of vesicles and arbuscules by AM fungi.

Andrographis paniculata Nees the Kalmegh of ayurveda belonging to the family of Acanthaceae is a promising herb for the treatment of many diseases including HIV and the myriad of symptoms associated with auto immune disorders (Calaberese *et al.,* 2000). The plant is a source of several diterpenoids of which Andrographolide is important with immune stimulant, antidiarrhoel, hepatoprotective, antipyretic and anti-inflammatory properties (Matsuda *et al.,* 1994). The drug is used for its general ability to cure certain forms of dyspepsia, chronic malaria, jaundice and dysentery (Chiramel, Bagyaraj, and Patil 2006). It is already being used in treating cancer, as it promotes cell differentiation in tumour cells (Matsuda *et al.,* 1994). It is now a leading medicinal crop which has attracted global attention due to its phenomenal impact on human health.

Adhatoda vasica Nees (Acanthaceae), a perennial woody shrub is an important medicinal plant widely exploited for extraction of an alkaloid, vasicine, which is used in the preparation of 'vasaka', a well known drug in the Ayurvedic system of medicine. The drug is recommended for a range of ailments *viz.,* bronchitis, asthma, jaundice, diseases of the respiratory system, diphtheria and gonorrhea (Kapoor, 2001).

Gymnema sylvestre R. Br. (Asclepiadaceae) commonly known as Periploca of woods in English, is a medicinally woody perennial climber. It is utilized as a potent drug in traditional (Folk, Ayurvedic, Homeopathy and Siddha) medicine Mitra *et al* .,1996). The total saponin fraction of the leaves, commonly known as " Gymnemic acid " has an antisweetening effect (Suttisri, *et al*., 1995) and was shown to be able to inhibit glucose absorption in the small intestine and to suppress elevated glucose levels in blood following the administration of sucrose in rats (Shimizu, *et al.,* 1997).

Costus pictus (D. Don) belongs to the family Costaceae and it is called as Insulin plant in English. It is a vulnerable species, slow growing, perennial herb of tropical and sub tropical regions. It is a potent antidiabetic plant and used in folk, ayurvedic and homeopathic systems of medicine (Joshi, 2000). It is also used in treatment of asthma, eye complaints and snake bite and 18 chemicals were analyzed and identified from leaves of *Costus pictus* (George *et al.,* 2007).

MATERIALS AND METHODS

The experiments were conducted in earthen pots measuring 15x15 cm diameter and maintained in Herbal Garden , in the college campus using 3 Kg ,1:1 (one part of garden soil + one part sand) of sterilized soil pure sand mixture. All the four medicinal plants selected were brought from Horticulture section, University of Agricultural Sciences, GKVK Campus Bangalore.

The mycorrhizal inoculums was obtained from 3 months old culture of *Glomus mosseae* and *Glomus fasciculatum* from Department of Microbiology, UAS, GKVK Campus Bangalore. The mycorrhizal inoculums production: Pot cultures of the AM fungi were multiplied in sterilized sand: soil (1:1), following the procedure of (Sreenivasa and

Bagyaraj, 1988). The AMF strains *Glomus mosseae* and *Glomus fasciculatum* which were selected for the present investigations were multiplied by using Finger Millet *(Eleusine coracana*) as host plant. Soil containing spores (10 spores /gm approximately) and infected roots was used as inoculums (25 gms/pot) and mixed with potting substrate.

There were three treatments i.e., AM inoculated with *Glomus mosseae,* AM inoculated with *Glomus fasciculatum* and non- inoculated (control) to all the four medicinal plants and maintained in pots were watered daily with tap water.

Morphological characters with reference to plant height in cms, number of nodes, leaves, branches- main and lateral, Inter nodal length in cms, leaf length/leaf width in cms, surface area of leaf and stomatal index were calculated in an average of 5 replicates. Biomass both fresh and dry weight in grams, root and shoot ratio in cms were measured at the end of the experiment (6 months)after washing roots free of soil and blotting on paper.

Root infection was measured at the end of the experiment. Roots were washed free of soil, cleared in 10%KOH at 95 degrees c for 30 minutes, treated with 0.1N Hcl for 15 minutes, then stained with 0.2% acid fuchsin in lactoglycerol for 4 hours (Phillips and Hayman, 1970). The stained preparation was scanned under the microscope for AM fungal mycelium , Vesicles and arbuscules and photographs were taken. Percent colonization was accessed by using the formula:

$$\% \text{ of mycorrhizal infection} = \frac{\text{No of root bits having infection}}{\text{No of root bits taken for observation}} \times 100$$

Extramatrical chlamydospores produced by the mycorrhizal fungus was estimated by wet sieving and decanting method outlined by Gerdemann and Nicolson (1963).

RESULTS

All the four species of medicinal plants showed mycorrhizal colonization and a wide range of variations was exhibited in root colonization percentage among different species which might be due to the effect of rhizosphere soil favoured the growth of AM fungi. AM fungal colonization percentage was higher in *Adhatoda vasica* (78-85%) followed by *Andrographis paniculata* (59-75%), *Costus pictus* (60-66%) and *Gymnema sylvestre(*50-56%). Spore count per 10 gms of dry soil in the rhizosphere ranges from 65-195 and it was recorded to be the maximum in *A.vasica* (171-195). The number of vesicles ranged from 22-62., the maximum in *A.vasica* and minimum in

G.sylvestre. The number of arbuscules ranged from 32-76, maximum in *A.vasica* and minimum in *G.sylvestre.*(; Table-1).

The response of all the four medicinal plants to inoculated *G.mosseae* and *G.fasciculatum* was moderately influenced. The introduced *G.mosseae* and *G.fasciculatum* significantly influenced the growth. The plants have greater height, biomass, root and shoot ratio, number of nodes, leaves, length and breadth of the leaf, inter nodal length, number of branches, surface area of the leaf and stomatal index in shoots compared to the uninoculated control plants. Interestingly, there is a positive co-relation between plant growth parameters and mycorrhizal colonization.(Table-2,3&4).

DISCUSSION AND CONCLUSION

In the present study AM-status was considerably higher in all the inoculated plants as compared to control plants. The introduced microbial activity in the root system through the soil plays a vital role in the continuous system or sequence of the plant growth and development by modifying their physiological activity(Martins *et al.*,1997). The mechanism which allows such growth enhancement may be due to the ability of AM fungi to increase nutrient uptake from the soil. (Smith and Gianinazzi- Pearson, 1988).

AM fungi normally colonize all tropical crop plants. However, a few earlier attempts demonstrated the association of AM fungi with medicinal plants in *Geranium* (Brenda *et al.*, 1983);Black pepper (Lekha *et al.*, 1995); *Datura* species, *Rauwolfia*, *Catheranthus roseus* (Neelima and Janardhan 1995); *Catheranthus roseus* (Sathya Prasad *et al.*, 1995*); Citronella java* (Kothari and Singh 1996); *Mentha citrata* (Kothari *et al.*, 1999*) Wrightia tinctoria*, *Thevetia perviana* and *Alstonia scholaria* (Sathya Prasad and Shrisailaja, 1995). Higher root colonization allows host fungus contact and exchange of nutrients , which also helped in the better growth of the plants with introduced *G.mosseae* and *G.fasciculatum* , which is in conformity with studies reported by (Mosse 1973, Smith and Read 1997). This indicated that plant symbionts exert degree of control over fungal development (Gianinazzi 1996). Host species differ in terms of their residual values of AM inoculums for a subsequent crop. This probably depends not only on the percentage of colonization of the root system with arbuscular fungi, but also on the total mass of mycorrhizal root produced.

The highest number of mycorrhizal spores in rhizospheric soil and AM fungi infection in the roots of *A.vasica* and *A.paniculata* indicated that these plant species might be considered good hosts for AM fungi under the prevailing conditions (Gupta *et al.*, 2009). This may be attributed to the root exudates of these plants, which might have stimulated the germination of mycorrhizal spores and increase the infection (Azaizeh, Marschner, Romheld, *et al.*, 1995). Out of *G.mosseae* and *G.fasciculatum*, the highest number of spores was of *G.fasciculatum* in majority of the medicinal plants(Gupta, Khaliq, Pandey *et al*., 2000) reported the presence of *G.facsciculatum* in the *Ocimum* species. Only *C.pictus* showed more colonization with *G.mosseae* which is in agreement with the reports of Panwar&Tarafdar (2006). In *G.sylvestre* the number of spores and colonization was comparatively less as observed

by Mohan Kumar & Mahadevan (1984). Many medicinal and aromatic plants produce secondary substances like tannins, alkaloids, phenolics etc., and less percentage of colonization may be due to the presence of chemical substances in the plants. Chiramel, Bagyaraj& Patil (2006) also reported the highest percentage of colonization in *A.paniculata* inoculated with *G.intraradices.* The findings of *A.vasica* and *A.paniculata* supports the observations made by earlier workers in *A.vasica*, *Costus sp* and in many other medicinal and aromatic plants (Govinda Rao,*et al.,* 1989).

It is so, naturally the fungus having higher root colonization will be better adapted and absorb more nutrients and thus better growth. Rani and Bhaduria (2001), Mulla (2002), Mulani *et al*., (2002) and Laksmipathy *et al.,* (2003) observed higher colonization and uptake of more nutrients in medicinal plants when they are associated with AM which acts as natural biofertilizers. Selection for efficient introduced AM fungal communities (biofertilizers) could take place with proper management and practices.

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Table 1: Influence of AM fungal association on Percent of colonization,spore count, number of vesicles, arbuscles after 6 months of treatment

Plant sps	Treatments	Percent of colonization	Spore count	No of vesicles	No of arbuscles	
Adhatoda vasica	**Control**	**13.1±0.56**	**13±0.54**	**10.0±0.44**	**06±1.02**	
	Treated with *Glomus mosseae*	**78±0.48****	**171±3.08****	**37±0.66***	**39±0.66***	
	Treated with *Glomus fasciculatum*	**85±0.54****	**195±1.72****	**62±0.10**	**76±0.48****	
Andrographis paniculata	**Control**	**10±0.44**	**10±0.50**	**07±0.37**	**06±1.02**	
	Treated with *Glomus mosseae*	**59±0.10****	**89±1.99****	**33±0.39***	**35±0.72***	
	Treated with *Glomus fasciculatum*	**75±0.54****	**114±1.01***	**42±0.74***	**50±0.78***	
Costus pictus	**Control**	**08±0.50**	**06±1.02**	**07±0.37**	**06±1.02**	
	Treated with *Glomus mosseae*	**66±0.10****	**85±0.54****	**32±0.36***	**58±0.62****	
	Treated with *Glomus fasciculatum*	**60±0.80****	**73±0.48***	**25±1.63***	**46±0.35***	
Gymnema sylvestre	**Control**	**05±0.50**	**06±1.02**	**04±0.05**	**05±0.31**	
	Treated with *Glomus mosseae*	**50±0.67****	**65±0.20***	**22±0.67***	**32±0.39***	
	Treated with *Glomus fasciculatum*	**56±0.58****	**74±0.46***	**29±0.58***	**48±0.74**	

***The mean differences are significant at P<0.05 as determined by Fisher's protected LSD test**

****The mean differences are highly significant at P<0.01 as determined by Fisher's protected LSD test**

Table 2: Influence of AM fungal association on growth performance after 6 months of treatment.

Plant sps	Treatments	Plant height (cm)	No of nodes	No of branches		Internodal length (cm)
				Main	Lateral	
Adhatoda vasica	Control	30.80±0.36	15.80±0.73	2±0.20	2±0.20	01±0.03
	Treated with *Glomus mosseae*	52.40±0.62**	19±0.70*	3±0.24	5±0.70*	02±0.20
	Treated with *Glomus fasciculatum*	87.40±1.99**	25±0.80*	3±0.24	5±0.70*	4.5±0.09**
Andrographis paniculata	Control	42±0.24	50.20±0.29	2±0.20	10±0.66	07±0.37
	Treated with Glomus mosseae	46±0.31	67.80±0.86*	2±0.20	11±0.20	07±0.54
	Treated with *Glomus fasciculatum*	60±1.05**	75±1.35**	2±0.20	13±0.37*	08±0.44
Costus pictus	Control	48.7±0.75	18±0.80	05±0.70	-	02±0.20
	Treated with *Glomus mosseae*	90±0.54**	27±0.58*	09±0.70*	_	2.5±0.10
	Treated with *Glomus fasciculatum*	78±0.54**	25±0.63*	08±0.37*	_	02±0.20
Gymnema sylvestre	Control	50±0.67	68.40±0.86	04±0.05	_	06±0.31
	Treated with *Glomus mosseae*	62.60±0.10*	78±1.30*	04±0.05	_	06.0.31
	Treated with *Glomus fasciculatum*	81.50±2.00**	80.50±2.00*	04±0.05	_	06±0.31

***The mean differences are significant at P<0.05 as determined by Fisher's protected LSD test**

****The mean differences are highly significant at P<0.01 as determined by Fisher's protected LSD test**

Table 3: Influence of AM fungal association on number of leaves, length and breadth of the leaf and surface area of the leaf after 6 months of treatment

Plant sps	Treatments	No of leaves	Length of the leaf	Breadth of the leaf	Surface area of the leaf
Adhatoda vasica	**Control**	**30±0.44**	**19.5±0.09**	**7.5±0.20**	**295.4±2.02**
	Treated with *Glomus mosseae*	**38±0.58***	**24±0.65***	**9.5±0.31**	**503.8±3.86****
	Treated with *Glomus fasciculatum*	**50±0.62****	**27±0.58****	**10.5±0.44**	**568.4±1.72****
Andrographis paniculata	**Control**	**85±1.99**	**6.20±0.24**	**3.20±0.24**	**39.2±0.48**
	Treated with *Glomus mosseae*	**124±0.40****	**6.50±0.24**	**2.40±0.24**	**40.8±0.58**
	Treated with *Glomus fasciculatum*	**150±0.15****	**6.50±0.24**	**2.40±0.24**	**39.6±0.48**
Costus pictus	**Control**	**18±0.52**	**10±0.20**	**04±0.05**	**85.20±1.99**
	Treated with *Glomus mosseae*	**27.60±0.74****	**15.5±0.24**	**08±0.44****	**250.10±1.72****
	Treated with *Glomus fasciculatum*	**25±0.63***	**14±0.24**	**06±1.02**	**298.56±1.63****
Gymnema sylvestre	**Control**	**136±0.44**	**02±0.22**	**1.5±0.04**	**8.10±0.50**
	Treated with *Glomus mosseae*	**176±1.01****	**03±0.23**	**2.00±0.03**	**13.65±0.50***
	Treated with *Glomus fasciculatum*	**184±3.86****	**04±0.05**	**2.00±0.03**	**17.50±0.73**

***The mean differences are significant at P<0.05 as determined by Fisher's protected LSD test**

****The mean differences are highly significant at P<0.01 as determined by Fisher's protected LSD test**

Table 4: Influence of AM fungal association on root and shoot length, biomass and stomatal index after 6 months of treatment.

Plant sps	Treatments	Root length	Shoot length	Biomass		Stomatal index
				Fresh weight	Dry weight	
Adhatoda vasica	**Control**	**17.02±0.24**	**27±0.37**	**30.60±0.36**	**12.40±0.24**	**22.20±0.24**
	Treated with *Glomus mosseae*	**34±0.48****	**44±0.54***	**100.80±0.54****	**49.90±0.48****	**25.00±0.31***
	Treated with *Glomus fasciculatum*	**74±0.54**	**83±0.83****	**500.35±0.89****	**200.58±0.58****	**29.40±0.87***
Andrographis paniculata	**Control**	**12.00±0.31**	**44±0.54**	**16.5±0.73**	**6.85±0.50**	**17.80±0.37**
	Treated with *Glomus mosseae*	**15.00±0.24**	**50.20±0.58***	**19.20±0.80***	**8.95±0.44***	**21.90±0.44***
	Treated with *Glomus fasciculatum*	**18±0.24***	**54±0.58***	**30.40±0.54****	**12.34±0.73****	**22.20±0.24***
Costus pictus	**Control**	**12.20±0.31**	**35.20±0.48**	**125.48±0.44**	**50.6±0.58**	**12.40±0.31**
	Treated with *Glomus mosseae*	**22±0.44***	**79±0.81****	**450.60±0.37****	**140.25±0.15****	**17.85±0.24***
	Treated with *Glomus fasciculatum*	**21.90±0.44***	**66.40±0.80***	**350.78±1.26****	**180.86±3.86****	**15.86±0.24**
Gymnema sylvestre	**Control**	**07±0.24**	**67±0.80**	**6.82±0.50**	**2.90±0.37**	**26.02±0.37**
	Treated with *Glomus mosseae*	**15±0.24***	**78±0.58***	**10.60±0.50***	**4.60±0.06***	**29.10±0.87**
	Treated with *Glomus fasciculatum*	**21±0.24****	**90±1.99****	**12.80±0.50***	**5.62±0.31***	**29.20±0.87***

***The mean differences are significant at P<0.05 as determined by Fisher's protected LSD test**

****The mean differences are highly significant at P<0.01 as determined by Fisher's protected LSD test**