ROLE OF ARBUSCULAR MYCORRHIZAE IN CONSERVATION OF *WITHANIA SOMNIFERA*

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Received: 24 April 2011, Revised:12 May 2011

ABSTRACT

An experiment was undertaken to study the selected medicinal plant and their therapeutic value with the association of Arbuscular mycorrhizal fungi. Rhizospheric soil samples were collected from the local places of Dharwad, which is geographically located between 14º 15' and 15 º 50' North longitudes and 74º 48' and 76º 20' east latitude of northwestern part of Karnataka state, India. The present investigation was undertaken to evaluate the efficacy of four different indigenous AM fungi (*G.fasciculatum, G.mosseae G.macrocarpum* and *G.aggregatum*) on plant growth, biomass production, per cent colonization, spore number and "P" uptake in *Withania somnifera* medicinal plant. Thus the present study clearly brought out medicinal plant could be conserve by inoculated efficient arbuscular mycorrhizal fungi to establish healthy seedling stock at nursery level.

Key words: Mycorrhiza, *Withania somnifera, G.fasciculatum, G.mosseae G.macrocarpum* and *G.aggregatum*

INTRODUCTION

Use of medicinal plants is advantageous over synthetic drugs as latter has low cost and less side effect and little risk of disorders. Presences of active compounds like alkaloids, glucosides, volatile compounds etc., justify the use in treatment of many diseases. It has been observed that some natural products suffer from certain disadvantages like; they are available in minute quantities in source material, poor solubility, stability, absorption and failure to reach the demand. Presently the need to study medicinal plants in details from various points of view is generally well recognized.

Frank (1885) coined the term "Mycorrhiza" which literally means "fungus root". VAM which is an obligatory parasite, belonging to the order Glomales of Zygomycotina (Morton and Benny 1990) These fungi are now recognized as the most widespread in occurrence in various plants and under different agro-climatic conditions covering a broad ecological range (Mosse 1973, Allen 1991). VAM fungi also moderates the adverse effect of higher root temperature, improves plant water relation, soil structure, have greater tolerance to heavy metal effect, salinity and lower incidence of soil borne plant diseases, in addition they also improve the N_2 fixing capacity. Because of their widespread occurrence in nature and their benefits, researchers in several disciplines are interested in studying mycorrhizae.

The arbuscules are the sites of exchange of nutrients between the fungus and host cell, vesicles are apical or intercalary swellings of hyphae, which contain lipids and are reserve organs of the fungus. However, in some cases vesicles were not formed and they are known as "Arbuscular Mycorrhiza" (AM) coined by Allen (1991).Plants absorb phosphorus from soil leading to depletion of phosphorus in the zone of about 1-2 mm around the roots (Lewis and Kirk 1967; Bhat and Nye 1974). But mycorrhiza has the capacity to extend the hyphae far beyond the depletion zone and helps in absorption and translocation of phosphorus, in return the fungus obtains nutrients from the host plant. Apart from phosphorus it also helps in better utilization of other microelements such as Zn, Cu, Mn, Fe etc.

In recent days there is an I ncreasing demand for medicinal plants and their products. Very little experiments have been conducted to understand the effect of VAM fungi, which can improve the growth and quality of medicinal plants. Therefore, the present investigation was undertaken to evaluate the efficacy of four different indigenous VAM fungi (*G.fasciculatum, G.mosseae G.macrocarpum* and *G.aggregatum*) on plant growth, biomass production, per cent colonization, spore number and "P" uptake in *Withania somnifera* medicinal plant the same findings also seen *Crotalaria juncea* L. by Sandeepkumar and Lakshman, 2010.

Therefore, there is need of research in improving the production of native medicinal plant drugs in relatively shorter period and lower expense by utilizing a potential biofertilizer like AM fungi, and the present investigation was undertaken to evaluate the efficacy of four different indigenous AM fungi (*G.fasciculatum, G.mosseae G.macrocarpum* and *G.aggregatum*) on plant growth, biomass production, **per cent colonization,** spore number and "P" uptake in *Withania somnifera* medicinal plant.

MATERIALS AND METHODS

The experiments were conducted in pot under glass house using sterile red sandy, loamy soil to understand the effect of AM fungi on the growth and yield of *Withania somnifera* medicinal plant. Seeds were collected from nurseries. The experimental soil was sterilized with 5% methyl bromide and its physico-chemical characteristics were analyzed as outlined by Jackson (1973).Before sowing the seeds were surface sterilized with 2% sodium hypochlorite and washed in distilled water for 2-3 times.

Mycorrhizal Inoculum production: Pot cultures of the VAM fungi were multiplied in sterilized sand: soil (1:1), according to (Sreenivasa and Bagyaraj 1988). Different strains of VAM such as *Glomus fasciculatum, G.mosseae G.macrocarpum* and *G.aggregatum* were selected. The VAM fungi-*Glomus fasciculatum* was multiplied by using onion (*Allium cepa)* as host plant, *G.mosseae* was multiplied on Jawar (*Andropogan sorghum*), *G.macrocarpum on Pennisetum typhoidium,* and *G.aggregatum on* Foxtail millet *(Setaria italica*). 15 gram of air dried VAM inoculum of these cultures was given to the soil as a thin layer 2 cm below the soil surface in each pot. The inoculum consists of 3 g root bits plus 12 g rhizospheric soil of host plant with hyphae and sporocarps (114 Chalmydospore/50gsoil approximately). Ruakura plant nutrient solution (Harley and Smith 1983) with out phosphorus was given to the seedlings at the interval of 15 days. There were two treatments i.e., inoculated and non- inoculated (control) and were maintained in triplicates. Experimental pots were kept free of weeds, insects, and pests and irrigated properly. The plants were harvested intervals of 30, 60 and 90 days (days after sowing) and the parameters like plant height, stem diameter, leaf area, dry weight of shoot and "P" uptake were recorded. The spore count was carried out by wet sieving and decanting method (Gerdemann and Nicoloson 1963). The percent of colonization was determined according to Phillips and Hayman (1970). The percentage of colonization was calculated by the following formula.

$$\text{Per cent of root colonization} = \frac{\text{No. of root bits shows colonization}}{\text{Total number of root bits observed}} \times 100$$

The plant height was measured from ground level to tip of the plant and expressed in centimeters. Leaf area was recorded by graph method. The uptake of 'P' in shoots was determined according to (Jackson 1973). For each harvest experimental plant shoot and root was oven dried at 70°C until a constant weight was obtained to determine the dry weight and was expressed in terms of grams.
Results and Discussion

In general, mycorrhizal inoculation resulted in a significant increase in plant height , dry weight, percent colonization, spore number and leaf area in all the medicinal plant seedlings over uninoculated (control) seedlings (Table 1). The percentage of colonization as high in *Glomus fasciculatum* (71.81 %) and least in *Glomus mossae* (65.10%) compare with other treatment. The present study with an objective of screening for an efficient AM fungi for *Withania somnifera* medicinal plant has resulted in varied plant growth responses to different AM fungi. AM colonization in inoculated experimental plants was low in early days of inoculation i.e.30 days, but steadily increased after 60 days. The growth rate of inoculated plants increased after 60 days than of uninoculated control plants. The hyphae, arbuscles and vesicles were predominant sign of infection in all medicinal plants. *Withania somnifera* significant increase in plant growth i.e. height, dry weight, leaf area, "P" content and mycorrhizal parameters like percent colonization and spore number was observed in the plants inoculated with *Glomus fasciculatum* followed by *Glomus macrocarpum* among the inoculated treatments. Inoculation of *Glomus aggregatum* significantly increased the percent colonization and spore count as compared to *Glomus mossae.* All the mycorrhizal plants showed higher 'P' content in the shoots over the uninoculated control in all stages of harvest. The highest 'P' uptake was seen in the *Withania somnifera* plants inoculated with *Glomus fasciculatum,* followed by *Glomus macrocarpum, Glomus aggregatum* and *Glomus mossae*.

In the present study AM status was considerably higher in all inoculated treatments compared to the control plants. Overall results in the present study *Glomus fasciculatum* caused higher plant height, stem diameter, dry weight, leaf area, "P" content and mycorrhizal parameters like percent colonization and spore number in the plants compared to other AM fungi and it can be considered an efficient AM fungus for *Withania somnifera.*

Table 1. Efficiency of different VAM inocula on plant height, stem diameter, leaf area, dry weight of shoot and root, per cent colonization, spore number and 'P' uptake of *Withania somnifera*.

VAM inocula	Plant height (cm)	Leaf area (cm^2)	Dry weight of shoot (g)	Dry weight of root (g)	Percent colonization	Spore No./ 50 g of soil	Percent of 'P' uptake in shoot
30 Days NM	12.23±0.251e	5.10±0.100d	1.333±0.115a	0.333±0.005e	0.00±.0000e	0.00±0.00e	0.050±.0000e
G.f	17.23±0.208a	10.20±0.200a	2.053±0.030a	1.086±0.005a	65.13±0.011a	155.6±0.57a	0.103±0.005a
G.m	13.06±0.057d	6.13±0.057c	1.613±0.011a	0.840±0.010d	60.20±0.100d	117.3±0.57d	0.060±0.000d
G.ma	15.10±0.100b	8.23±0.0577b	1.843±0.045a	0.956±0.005b	63.20±0.100b	134.0±0.10b	0.070±0.000b
G.ag	14.33±0.152c	8.33±0.0577b	1.723±0.045a	0.903±0.005c	61.16±0.152c	123.3±0.57c	0.066±0.005c
60 Days NM	18.26±0.115e	7.06±0.0577e	2.120±0.020e	0.486±0.005e	0.000±.0000e	0.000±0.000e	0.070±0.000e
G.f	28.33±0.152a	17.36±0.057a	3.450±0.0100a	2.156±0.005a	67.90±0.0200a	283.6±2.516a	0.156±0.005a
G.m	20.20±0.100d	9.53±0.115d	2.483±0.0057d	1.273±0.005d	63.24±0.0200d	203.3±1.527d	0.086±0.005d
G.ma	25.40±0.200b	13.33±0.115b	3.150±0.0100b	1.610±0.010b	65.13±0.0264b	220.3±2.516b	0.123±0.005b
G.ag	24.30±0.100c	13.06±0.115c	3.103±0.0057c	1.533±0.011c	64.08±0.0152c	215.0±1.000c	0.110±0.000c
90 Days NM	23.36±0.152e	10.30±0.173e	3.106±0.005b	0.703±0.005e	0.000±0.000e	0.000±0.000e	0.100±0.000e
G.f	38.40±0.200a	22.26±0.115a	6.420±2.580a	3.926±0.011a	71.81±0. 505a	345.3±1.154a	0.240±0.020a
G.m	29.33±0.115d	15.33±0.115d	3.620±0.020b	2.026±0.011d	65.18±0.162d	251.6±2.081d	0.133±0.005d
G.ma	32.33±0.115b	18.26±0.115b	4.030±0.010b	2.526±0.011b	67.28±0.104b	312.0±2.000b	0.183±0.005b
G.ag	31.20±0.100c	17.13±0.115c	3.813±0.011b	2.433±0.015c	66.10±0.100c	303.0±3.000c	0.163±0.005c

NM- Non mycorrhizae ; *G.f- Glomus fasciculatum* ;*G.m-Glomus macrocarpum* ; *G.m-Glomus mossae* and *G.ag- Glomus aggregatum* ;.
Mean values followed by the same letter within a column do not differ significantly at *P*= 0.05 by DMRT.

DISCUSSION

Arbuscular mycorrhiza (AM) association with plants will increase uptake of mineral nutrients, especially phosphorus in conditions of low 'P' availability (Bagyaraj 1989; Manjunath *et al.* 1989), reduce susceptibility of plants to certain pathogens (Giovannetti 1990), alter the water relations and photosynthetic activity of the plant (Brown and Bethlenfalvay 1988), increase seed production (Koide *et al* 988) and improve health and vigor of the seedlings (Reena and Bagyaraj 1990).

The medicinal plant *Withania somnifera* grown in the presence of AM showed a general increase in plant height, leaf area, dry weight, "P" content and mycorrhizal parameters like per cent colonization and spore number as those grown in soils without AM fungus (Table 1). The enhanced plant growth in *Withania somnifera* was obtained inoculated with *Glomus fasciculatum.* Several workers have observed the effects of AM fungi on plant growth (Abbott *et al.* 1989; Michelsen and Rosendahl 1990; Aggangan *et al.* and Jaize-Vega and Azcon 1995). Dhilon (1992) suggested that species might result in selection procedures, which favor certain specific fungus host species combination.Plant biomass is an important parameter, which directly reflects the efficiency of a particular fungus. In the present study the enhanced plant biomass in *Withania somnifera* was obtained inoculated with *Glomus fasciculatum.* The increase in shoot dry weight was up to four times the dry weight of uninoculated plants. Further Pope *et al.*, (1983) in their experiment observed a 200 percent increase in total dry weight of *Platanus occidentalis* inoculated with *Glomus fasciculatum.* Several workers have observed the effects of VAM fungi on plant biomass (Nemec 1979; Aggangan *et al.* 1990; Sivaprasad *et al.* 1990; Mandal and Kaushik 1994; Jaize-Vega and Azcon 1995; Mulla 2002; Hemalatha and Selvaraj 2003 and Yudhvir 2004).

The total photosynthretic area expressed as the leaf area was significantly enhanced leaf area in *Withania somnifera* was obtained inoculated with *Glomus fasciculatum.* Enhanced leaf area because of mycorrhizal inoculation has been observed in *Trifoliate orange* and *Troyer citrange* (Vinayak and Bagyaraj 1990). Murugan and Selvaraj (2003), observed enhanced leaf area in *Cichorium intybus* L.medicinal plant inoculating different native arbuscular mycorrhizal fungi. Also enhanced leaf area because of mycorrhizal inoculation has been observed in *Asparagus racemosus, Ocimum basilicum, Andrographis paniculata,* and *Becopa monnieri* (Yudhvir 2004).

The "P" content was more pronounced in plants inoculated with *Glomus fasciculatum* in *Withania somnifera* and AM fungi are known to improve plant growth mainly through increased uptake of P and other nutrients (Jeffries 1987). Abbott and Robson (1982) defined efficiency as the ability of the fungus to increase plant growth in a phosphate deficient soil. This depends on the hyphae in soil and extensive root colonization to absorb P from soil. Mallesha, Padmavathy and Bagyaraj (1994) reported that species and strains of AM fungi differed to the extent by which they increased nutrient up take and plant growth. Hence some researchers suggested the need for selecting efficient VAM fungi that can be used for inoculating different plants (Bagyaraj, Byra Reddy and Nalini 1989; Mallesh and Bagayaraj 1994). Hemalth *et al.* (2003) and Murugan *et al.* (2003) also increased "P" content in medicinal plants.

In present study mycorrhizal parameters like percent root colonization and extra matricular spore count were higher in plants inoculated with AM. The influence of *Glomus fasciculatum* was highest on per cent colonization and spore number in *Withania somnifera.* Similar observations were also made by Reena and Bagyaraj (1990) in their studies with Calliandra *calothrysus* inoculated with 13 different VA mycorrhizal fungi. So naturally the fungus having higher root colonization will be better adapted and absorb more nutrients and thus better growth. Rani (2001), Mulla (2002) and Mulani (2002) observed higher colonization and up take of more nutrients in medicinal plants.

The results from this study indicated that, potential benefits could be obtained from the AM fungi in production of medicinal plants for their better use in future. It is concluded from the present experiments that the inoculation of medicinal plant *Withania somnifera* inoculated with *Glomus fasciculatum* seems to be a great boon to the botanist to minimize manure dosage in improving the growth, biomass production and the chemical constituents in medicinal plants by inoculating biofertilizers like AM fungi.

LITERATURE CITED

Abbott LK, Robson AD and Gazey C. 1989. Selection of inoculant vesicular-arbuscular mycorrhizal fungi. methods in Microbiology. **24**:1-21.

Aggangan NC, Lorilla EB, and Cruz RE de La. 1990. Screening for effectiveness of four VA mycorrhizal fungi on *Acacia auriculiformis* in fertile soil. Sylvatrop 1987 Publication **12(3&4)**:133-140.

Allen MF. 1991. The ecology of mycorrhizae, Cambridge University, New York, 184 p.

Bagyaraj DJ. 1989. Mycorrhizas. In, Trophical rain forest ecosystems(Eds.)Leith,H. and Werger,M.J.A. *Elsevier science publishers,* B.V. Amsterdam.pp537-546.

Bagyaraj DJ and ByraReddy MS and Nalini PA. 1989. Selection of an efficient inoculants VA mycorrhizae fungus for *Leucaena. Forest Ecology and Management* **27**:791-801.

Bhat KS and Nye PH. 1974. Diffusion of phosphate to plant roots in soil. II. Uptake along the roots at different times and effect of different levels of phosphorus. *Plant Soil*, **41:** 365-382.

Brown MS and Benthlenfalvsay GJ. 1988. The Gycine *Glomus, Rhizobium* symbiosis,VII photosynthetic nutrient use efficiency in nodulated mycorrhizal soybeans. *Plant Physiol,* **86**:1292-1297.

Dhillon SS. 1992. Host endophyte specificity of vesicular arbuscular mycorrhizal colonization of *Oryza sativa* L. at the pre plant stage in low or high phosphrous soil. *Soil Biol. Biochem.*, **24**:405-411.

Gerdemann JW and Nicolson TH. 1963. Spores of mycorrhizal endogone species extracted from the soil by wet sieving and decanting. *Transaction of British Mycological Society,* **46:** 235-244.

Giovannetti M. 1990. Mycorrhizas as agents of biological control of agricultural plant pathogens. Informatore, *Fitopathologica*, **40**:17-20.

Hemalatha Mand and Selvaraj T. 2004. Association of AM fungi with Indian borage(*Plectranthus amboinicus*(Lour.)Spenge)and its influence on growth and biomass production. *Mycorrhiza News* **15(1)**:18-21.

Jackson ML. 1973. Soil chemical Analysis. Prantice Hall of India Pvt. Ltd, New Delhi.

Jaizme-Vega MC. and Azcon R. 1995. Responses of some trophical and subtrophical cultures to endomycorrhizal fungi. *Mycorrhiza* **5(3)**:213-217.

Koide RT, Li M Lewis J and Inby C. 1988. Role of mycorrhizal infection on the growth and reproduction of wild .Vs.cultivated plants. *Oecologia,* **34**:171.178.

Lewis DG and Kirk JP. 1967. Phosphate diffusion in soil and uptake by plants II. ^{32}P movement and uptake by plants as indicated by 32p autoradiography. Plant and Soil. 26: 445-453.

Mandal BS and Kaushik JC. 1994. Effect of VAM inoculation of growth and development of *Acacia nilotica* (L.) Wild. ex. del. *Annals of Biology(Ludhiana)***10**:264-267.

Manjunath A Hue NV and Habte M. 1989. response of *Leucaena leucocephala* to vesicular arbuscular colonization in an oxisol. *Plant soil,* **114**:127-133.

Michelson A and Rosendahl,S. 1990. The effect of VAmycorrhizal fungi,phosphorous and drought stress on the growth of Acacia nilotica and Leucaena leucocephala seedlings. Plant Soil,**124**:7-13.

Morton JB and Benny GL. 1990. Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): a new order Glomales, two suborders, Glomineae and Gigasporineae, and two new families, Acaulosporaceae and Gigasporaceae, with an amendation of Glomaceae. *Mycotaxon.* **37:** 471-491.

Mosse B. 1973. Plant growth responses to vesicular arbuscular mycorrhizae. IV. In soil given additional phosphate. *New Phytologist.* **72:** 127-136.

Mulani RM, Rajendra Prabhu R and Manjusha Dinkaran. 2002. Occurance of VAM in the roots of Phyllanthus fraternus Webster. *Mycorrhiza News,* **14**(2):11-14.

Mulla FI. 2003.VA-mycorrhizal studies in some important medicinal plants. Ph.D. thesis Karnatak University Dharwad, India.

Murugan R and Selvaraj T. 2003. Reaction of Kashini(Cichorium intybusL.)to different native arbuscular mycorrhizal fungi. *Mycorrhiza News,* **15**(1):10-13.

Nemec S. 1979. Growth of *Citrus jambhiri* as affected by single and combined inocula of three *Glomus* species In Proceedings of the Fourth North American Conference on Mycorrhizae.

Phillips JM and Hayman DS. 1970. Improved procedures for clearing roots and staining parasite and vesicular arbuscular mycorrhizal fungi for rapid assessment of infection. *Transaction of British Mycological Society.* **55**:158-160.

Pope PE, Chaney WR, Rhoides and Woodhead SH. 1983. The mycorrhizal dependency of four hardwood tree species, *Can. J. Bot.*, **61**:412-417.

Rani V and Bhaduria S. 2001. Vesicular arbuscular mycorrhizal association with some medicinal plants growing on alkaline soils of Mainpuri district, Uttar Pradesh. *Mycorrhiza News,* **13(2)**:12-13.

Reena J and Bagyaraj DJ. 1990. Response of *Acacia nilotica* and *Calliandra calothyrus* to different VA mycorrhizal fungi. *Arid Soil Research and Rehabilitation.***4**:261-268.

Rhodes LH and Gerdmann JW. 1978. Influence of phosphorus nutrition on sulphur uptake by vesicular-arbuscular mycorrhizal fungi of onion. *Soil Biology and Biochemistry,* **10:** 361-364.

SampathKumar G and Murugesh S. 2002. Studies on the VAM fungi improves growth of some medicinal plants.Ad.*Plant.Sci.***15 (1):** 43-46.

Sandeepkumar K and Lakshman HC. 2010. Influence of AM Fungi and *rhizobium* on growth and nutrition of the fiber yielding plant, *Crotolaria juncea* L. *Journal of theoretical and experimental biology* **Sandeepkumar K and Lakshman HC. 2010. 6(2):**105-111.

Selveraj T and Subramanian G. 1992. Incidence of vesicular arbuscular mycorrhizal fungi in medicinal plants. *Curr.Res.*, **21**:34-35.

Sivaprasad P, Sulochana KK and Nair SK. 1990. Comparative efficiency of different VA mycorrhizal fungi on cassava (*Manihot esculenta* Crantz) *Journal of Root crops,* **16**(1): 39-40.

Vinayak, K. and Bagyaraj, DJ. 1990b. Vesicular arbuscular mycorrhizae screened for *Troyer citrange*. *Biological and Fertility of soils,* **6:** 305-311.

Yudhvir.K.Bhoon. 2004. Mycorrhizal inoculation of some medicinal plants field trials. *Agrobios Newsletter,* **3** (4):15-16.