Eco-friendly Cropping of *Withania somnifera* by using Vam Nodulation

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Abstract

The present paper deals with the inoculation of Mycorrhiza fungi on Withania somnifera which is a medicinally important plant, commonly known as Ashwagandha. It is a small, woody shrub of Solanaceae family. The roots are the main portion of the plant used therapeutically. The plant showed the remarkable colonization with Arbuscular Mycorrhiza fungi and because of inoculation with Mycorrhiza fungi the plant showed increased leaf surface area, height and more biomass in comparison to non-inoculated plant. Spores were added at rate of 460 per 100 gms of soil and the percentage of colonization was found 77%

Keywords - Mycorrhiza, *Withania somnifera*, Eco friendly cropping, VAM, Nodulation, Ashwagandha.

Introduction

Indigenous knowledge is as old as human civilization but the term ethnobotany was first coined by an American botanist, John Harshburger (1896), to study the plants used by the primitive and aboriginal people, since then it has been defined as the traditional knowledge of indigenous community, and all about surrounding plant diversity. Mostly plants show remarkably important medicinal properties. The first official records of medicinal plants were set down on Papyrus (Anna, 1993). For centuries, plants with medicinal properties have been utilized successfully in the treatment of ailments of varying degrees of severity. The Greek physician, Hippocrates, was quoted as saying in 377 BC," let medicine be your food and food your medicine" (Bartram, 1995).

Ashwagandha is a small, woody shrub belongs to Solanaceae family. The shoots and seeds are also used as food and to thicken milk in India. In Ayurvedic medicine, this herb is considered the "Ginseng of India" that "protects the organism from illness through maintaining the healthy balance of physical energies." Ashwagandha roots exhibits anti-stress effect, non-toxic and anti-cancer properties. The remarkable immuno-modulating properties of Withania Somnifera (Ashwagandha) roots are also being clinically investigated; it can enhance both short and long term memory. The herbal root extract has been traditionally used as a tonic and as a sedative but recent research shows that the leaf extract contains Withanolides which have been found to have regenerative properties on brain-cell synapses in mice and in human cell lines in laboratory studies. Ashwagandha is used to treat a number of disorders that affect human health including central nervous system (CNS) disorders, particularly in epilepsy, stress and neurodegenerative diseases such as Parkinson's and Alzheimer's disorders, tardive dyskinesia, cerebral ischemia, and even in the management of drug addiction (Bhattarcharya *et al.*, 2003; Abou-Douh, 2002).

The plant is widely distributed in North-Western India, Bombay, Gujarat, Rajasthan, Madhya Pradesh, Uttar Pradesh, Punjab plains and extends to the mountain regions of Himachal Pradesh and Jammu. The roots are the main portion of the plant used therapeutically. The bright red fruit is harvested in the late fall and seeds are dried for planting in the following spring. The berries have been shown to have an emetic effect.

Figure 1: Plant of Ashwagandha.

The intensive use of chemical fertilizers for enhancing biomass productivity in agro forestry has lead to problems related to environmental pollution and depletion of soil fertility in the long term. In current days, emphasis is on sustainable agriculture, which uses less of chemical inputs like fertilizers and pesticides. Thus, use of microbial inoculants play an important role in maintaining sustainability in agriculture. Most of the soil microorganisms do not interact with plant roots, possibly due to the constant and diverse secretion of antimicrobial root exudates. However, there are some microorganisms, that interact with specific plants and these interactions are very beneficial for agricultural practice. Arbuscular mycorrhizal fungi are known to improve the nutritional status, growth and development of plants, protect plants against root pathogens and offer resistance to drought and salinity.

Frank (1885) coined the term "Mycorrhiza" which literally means "fungus root". Vesicular Arbuscular Mycorrhiza (VAM) is an obligatory parasite, belonging to the order VAM, Glomales of Zygomycota. These fungi are now recognizing as the most widespread in occurrence in various plants and under different agro-climatic conditions covering a broad ecological range (Mosse, 1973). VAM fungi also improves soil structure by maintaining plant-water relation, improves salinity, moderates the adverse effect of higher root temperature, have greater tolerance to heavy metal effect, and shows lower incidence of soil borne plant diseases. In addition, they also improve the N, fixing capacity (Morton and Benny, 1990). Though these fungi are not host specific, recent studies have clearly brought out host preference in arbuscular mycorrhizal fungi (AMF). Host preference has been reported in many forest tree species like Casuarina equisetifolia; Tectona grandis; Garcinia indica and a few medicinal plant species like Phyllanthus amarus. (Jeffries, 1987; Vasanthakrishna, *et al.* 1995; Rajan *et al.*, 2000; Lakshmipathy *et al.*, 2003).

Material and Method

The experiment was conducted in the pot. The soil of 10 pots was inoculated with mycorrhizal spore at a rate of 460 spores/100 gms of soil and another 10 pots were treated without mycorrhizal spores and hence treated referred as control. All potted plants were grown in our botanical Garden. The seeds of Ashwagandha collected from IARI, they were first surface sterilized with 2% sodium hypochlorite and then washed in distilled water (2-3 times) before sowing in experimental pots. The experiment was monitored on daily basis. After three months of sowing these plants, they were examined by randomly harvested to assess growth and biomass. After recording different growth parameters, viz., leaf size, fresh weight and dry weight of shoot and root ; total biomass, etc. and thecomparison was made between inoculated and the non-inoculated plants .

Study of Mycorrhiza Inoculated Roots

For this, roots were rinsed with water three times on a fine sieve or muslin cloth and cut into 1cm long segments. After that, clean roots were boiled with 2% (w/v) KOH for 1h at 90°C in a water bath or oven. After heating again the roots were rinsed with water on a fine sieve; treated roots with 2% (v/v) HCl for at least 30 mins. HCl was thrown away and roots were again treated with 0.05% (w/v) trypan blue in lactoglycerol (1:1:1 lactic acid, glycerol and water) for 15min to 1h at 90°C in water bath or oven. Roots were examined in compound microscope for AMF structures.

Assessment of Percentage Colonization of AMF

Roots sample were taken from 10 different pots of mycorrhiza-infected plant and washed thoroughly under running tap water. Roots were cut into small pieces (approx.1cm), stained, and studied under the microscope for the assessment of percentage colonization.

We can calculate the percentage with the formula:

$$\text{Percentage Colonization} = \frac{\text{Number of root segment colonized}}{\text{Total number of root segment observed}} \text{ X } 100$$

Isolation and Identification of Spores of AMF

Remove soil sample from the rhizosphere of the host plant growing in the pot take a quantity of 100g and sieved it, mixed it into a 1L beaker of water before pouring through the sieves. Wash the soil through 710µm and 45µm pore sieves with running water. Remove root material trapped on the 710µm sieve to check for attached mycelium of AMF with spores. Backwash the contents of the 45µm sieve into a small beaker. Try to keep the volume to a minimum. Swirl the beaker contents and quickly decant the contents into 50ml centrifuge tubes up to a maximum half way up the tube. Gently inject an equal amount of a 60% (w/v) sucrose solution into the pellet at the bottom of each tube using a syringe. There should be a clear interface visible between the water (above) and sugar phase (below). Centrifuge the capped tubes at approx. 3000 rpm for 2 minutes in a centrifuge. Remove the spores caught at the interface of the two layers with the syringe and tube attachment. Start above the interface and work down into the sugar phase using a circular motion. Pour the contents of the syringe into a clean 45µm sieve, and wash thoroughly to remove traces of sugar solution. Backwash contents into a Petri plate, spores population expressed in term of number of spores per 100gm of dry soil. The spores collected were mounted with PVLG (poly vinyl alcohol+ lactic acid+ Glycerol) + Melzer's reagent and observed under microscope.

Preparation of Melzer's reagent:

- Dissolve 1.5g of KI to 20ml of distilled water.
- Dissolve 0.5g of iodine in the solution.
- Dissolve 20g of chloral hydrate in the solution.

Results and Discussion

In general, mycorrhizal inoculation resulted in a significant increase in plant height, dry weight, percent colonization, spore number and leaf area of inoculated plant over un-inoculated (control) plant.

Figure2: Mycorrhiza inoculated roots of Withania somnifera.

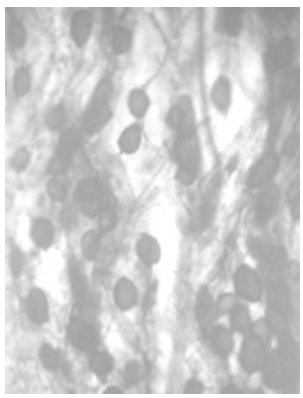

Figure3: Whole mount of Mycorrhiza inoculated root showing vesicules of Glomus mosseae.

Plant height was measured from ground level to the tip of the plant, which was found 34 cm in inoculated plant in comparison to un-inoculated plant which is 29 cm. (Table 1). Leaf area was found 18 cm^2 in inoculated plant and 15cm^2 in un-inoculated plant. Biomass of mycorrhiza infected plant roots were 5.9 gm and normal plant roots were 5.5gm. In inoculated shoots, biomass was found 17.7gm whereas in non-inoculated shoots it was 11.9 gm.

Table 1 : Comparison between non inoculated and Mycorrhiza inoculated plant.

Character of Plant	Mycorrhiza inoculated plant	Un- inoculated plant
Leaf Size (cm^2)	18	15
Plant Height (cm)	34	29
Fresh Weight of Shoot	22.3	14.8
Dry Weight of Shoot	4.6	2.9
Total Biomass of Shoot	17.7	11.9
Fresh Weight of Root	7.8	7.1
Dry Weight of Root	1.9	1.6
Total Biomass of Root	5.9	5.5
Percent Colonization	77%	
Spore count in 100g of soil	460	

Arbuscules were present in inoculated roots; they formed a branched structure in the cortex cells. Spore were round in shape; vesicles were also found in the roots infected by mycorrhiza. These features resembled to Genus Glomus. So from this we can say that the associated fungus identified in the roots of Withania somnifera was a species of Genus Glomus. Staining of the spores showed that these spores are round and bi-layered in structure and identified as mosseae sps., so the fungi identified as Glomus mosseae. The percentage of colonization in the roots was 77% whereas spore count was found 460 per 100 gm of soil.

Conclusion

In the present study the enhanced plant biomass, height and leaf area was obtained in inoculated plant of Withania somnifera. Increase in shoot and root biomass was found several times more in inoculated plant than in un-inoculated one. So from these results we can say that Glomus mosseae is a boon for farmers to cut down the chemical fertilizers and improving the growth, health and biomass production of medicinally important plants.

References

Abou-Douh, AM. 2002. New withanolides and other constituents from the fruit of Withania somnifera, 335:267-276.

Anna, K. 1993. An illustrated guide to herbs, their medicine & magic, USA.

Bukhari, M.J., Rodrigues, B.P., Bagyraj, D.J. 2006. Arbuscular mycorrhizal fungi in sustainable agriculture. *Department of Botany Goa University, Goa. Techniques in mycorrhizae. Eds.*

Grace, T. 1995. Encyclopedia of herbal Medicine: Dorset Bartram.

Bhattarcharya, S.K., Muruganandam, AV. 2003. Adaptogenic activity of Withania somnifera: an experimental study using a rat model of chronic stress. *Pharmacol Biochem Behav,* 75:547- 555.

Jeffries, P. 1987. Use of mycorrhizae in agriculture. *CRC Critical Review of Biotechnol 5,* 319-357.

Indian, J. 2003. Forestry: Lakshmipathy, R., Balakrishna Gowda, K. Chandrika and DJ. Bagyaraj. *Symbiotic response of Garcinia indica to VA mycorrhizal inoculation,* 26: 143-146.

Morton, J.B.,Benny G.L. 1990. Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): a new orderr Glomales, two suborders, Glomineae and Gigasporineae, and two new families, Acaulosporaceae and Gigasporaceae, with an amendation of Glomaceae. *Mycotoxon*.37: 471-491.

Morton, J.B. 1988. Taxonomy of VAM fungi, classification, nomenclature and identification. *Mycotaxon XXXII*, 267-324.

Mosse, B., Bowen, G.D. 1968. The distribution of *Endogne* spores in some Austeralion and New Zealand soils and in the experimental field soil at Rothamasted. *Trans. Br. Mycol. Socity,* 51.485-495.

Rajan, S.K., Reddy, B.J.D., Bagyaraj, D.J. 2000. Screening of mycorrhizal fungi for their symbiotic efficiency with Tectona grandis. *Forest Ecology and Management*, 126: 91-95.

Vasanthakrishna, M., Bagyaraj, D.l., Nirmalnath. 1995. Selection of efficient VA mycorrhizal fungi for Casuarina equisetifalia. *Second screening. New Forests,* 9: 157-162.