

Short Communication

Response of Some Medicinal Plants to Vesicular Arbuscular Mycorrhizal Inoculations

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Abstract

An investigation has been made about the response of vesicular arbuscular mycorrhizal fungi of *G. fasciculatum* on some medicinal plants. Medicinal plants species selected for the study are *Ocimum sanctum*, *Catharanthus roseus*, *Coleus forskholii* and *Cymbopogon flexuosus*. The percentage of VAM association is 85 and the intensity of formation of vesicles and arbuscules are 70% and 30%, respectively in VAM inoculated *C. roseus* plants. The total dry matter production (shoot and root dry wt), protein and total chlorophyll contents are seen to increase in VAM inoculated plants. The percentage of increase is more in *C. roseus*, followed by *Cymbopogon flexuosus* when compared to control plants.

Keywords: *Ocimum sanctum*, *Coleus forskholii*, *Cymbopogon flexuosus*, *Catharanthus roseus*, VAM fungi.

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1. Introduction

The cultivation of medicinal and herbal plants has assumed greater importance in recent years due to their tremendous potential in modern and traditional medicine. They are also used as raw materials for pharmaceutical, cosmetic and fragrance industries. Indian system of medicine (ISM) uses 25,000 species belonging to more than 1000 genera. About 25% species are used by the industries [1].

Inoculation of VAM fungi during an early stage of acclimatization process has become an alternative strategy for better establishment by improving the plant growth.

The occurrence of micro organisms especially VAM fungi on the medicinal plants have been reported earlier [2 - 4]. The VAM fungi association had not only enhanced the growth of medicinal plants but also improve the productivity of medicinal compounds.

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Hence there is a need for research in improving the quality and quantity of drugs produced from native medicinal plants in relatively shorter period and at lower expense by using VAM fungi. Though there are reports on beneficial effects of VAM on other crops, a literature search reveals only a few reports on the VAM association with medicinal plants. In the present study, medicinal plants like thulsi (*Ocimum sanctum*), Periwinkle (*Catharanthus roseus*), Coleus (*Coleus forskholii*), and Lemon grass (*Cymbopogon flexuosus*) were selected for assessing their response to VAM fungus.

2. Materials and Methods

Pot culture experiments were conducted using natural un-sterilised soil. Four sets of eight pots were taken and each pot was filled with 5 kg soil. Three pots of each set were treated as inoculated by mixing 500g/pot of pure cultures of *G. fasciculatum* inoculum having 25 spores/100 g of soil, and the other three were treated as control as there was no addition of VAM inoculum. Seedlings of medicinal plants were transplanted in both treated and untreated control pots. The plants were regularly watered once in three days. The plants at the time of flowering stage (150 days) were harvested along with their root system and roots were cleared by dipping them in water 2 to 3 times till the adhering soil particles were removed.

2.1. Assessment of VAM association and its biomass estimation

Few representative root samples from both treated and untreated plants were cut into 1 cm bits and fixed in a standard FAA (formalin acetoalcohol) and processed further for the assessment of VAM colonization (percentage mycorrhizal association) by staining [5]. The plants were oven dried for 72 h at 70°C root and shoot systems were then weighed.

2.2. Estimation of total chlorophyll

One gram fresh leaf sample taken from each replication was analyzed for total chlorophyll content by following the method of Talling and Driver [6]. The fresh plant sample was ground with 95% ethanol using pestle and mortar. The extract was allowed to settle in dark for 30 min at room temperature. The suspension was centrifuged at 5000 rpm at room temperature for 10 min. The supernatant was collected and the volume was made up to a known volume using 95 per ethanol. The absorbance was read at 665 and 649 nm in Beckmann DU 64 spectrometer against 95% ethanol blank. The total chlorophyll was calculated using the formula:

$$\text{Total chlorophyll } (\mu\text{g/g}) = 6.10 (A_{665}) + 20.04(A_{649}) \times V$$

Here, A_{665} = Absorbance at 665nm, A_{649} = Absorbance at 649nm, and V = volume of ethanol extract.

The chlorophyll content of the leaf sample was estimated and expressed as mg g^{-1} fresh wt of sample.

2.3. Estimation of protein content

Protein content of leaf samples was determined by the method developed by Bradford [7].

2.3.1. Bradford reagent

One hundred mg Coomassie Brilliant blue G250 was dissolved in 50 ml of 95% ethanol and mixed with 100 ml of concentrated phosphoric acid. The volume was made up to 200 ml with distilled water. The reagent was stored at 4°C. This was diluted five times with distilled water prior to use.

Procedure:

One gram fresh leaf sample was extracted with 2 ml of 0.1 M phosphate buffer (pH 7.0). The extract was centrifuged at 5000 rpm for 10 min at room temperature. To 0.1 of clear supernatant, 5.0 ml of Bradford's reagent was added. The blue colour developed was read at 595 nm in Beckman DU 64 spectrophotometer. From the standard graph prepared using Bovin serum albumin over a concentration ranging from 1 to 100 µg/ml, the protein concentration of leaf sample was estimated and expressed as mg/g of fresh weight of sample.

2.4. Statistical Analysis

The experiment results (Table 1) were statistically analyzed in the Duncan's multiple range test (DMRT) using SPSS 7.0 statistical package. The triplicate sets of data (Table 2) were subjected to ANOVA in accordance with Completely Randomized Block Design using NPROC statistical package to quantify and evaluate the source of variation. CD (Critical Differences) values were calculated at *P* level of 0.05%.

3. Results and Discussion

It is clearly evident from the data that the root systems of all medicinal plant species grown in both control and VAM fungus infested soils were invariably found to harbour VAM association. The plants grown in the soil without addition of VAM inoculum; recorded VAM association of 12 to 20% and the intensity of vesicles was 20 to 30% and arbuscules was 8 to 12%. However, these results were found to be insignificant (Table 1). In the inoculated plants, the percent VAM association (58 to 85%) and vesicles (58 to 70%), arbuscules (30 to 42%) were highly significant (Table 1).

Among the four medicinal plants the maximum association of VAM was 85% colonization and the intensity formation was 70 and 30% for vesicles and arbuscules respectively in *Catharanthus roseus* plant. This was followed by *C. flexuosus* and *O. sanctum*. The minimum VAM association (58% colonization) and intensity of 58% vesicles and 42% arbuscules were recorded in *C. forskholii*. Similarly, the shoot dry wt, root dry wt, protein content and the total chlorophyll content of the VAM inoculated plant

was higher in *C.roseus* (25.22, 14.98 g/plant, 94.00 and 3.40 mg/g of plant) followed by *C. flexuosus* and *O. sanctum* when compared to control plants.

Table 1. Intensity of VAM formation of some medicinal plants with *Glomus fasciculatum*.

Plant	VAM status					
	Control plants			VAM inoculated plants		
	% VAM association	Intensity % of formation		% VAM association	Intensity % of formation	
	vesicles	arbuscules		vesicles	arbuscules	
<i>Ocimum sanctum</i>	10±1.5	12±0.75	10±1.0	65±1.0	60±1.5	40±2.0
<i>Catharanthus roseus</i>	20±1.5	20±0.5	12±0.5	85±2.5	70±1.5	30±1.5
<i>Coleus forskholii</i>	12±0.5	10±0.5	8±1.5	58±1.5	58±1.5	42±2.5
<i>Cymbopogon flexuosus</i>	15±1.0	19±0.5	11±0.5	75±3.5	65±1.5	35±2.5

Values are mean of three replicates ± SD.

It is clearly evident from the present study on the effect of VAM inoculation on mycorrhization and growth response in four selected medicinal plants (*O. sanctum*, *C. roseus*, *C. forskholii*, *C. flexuosus*) that these plants have readily responded to VAM inoculation, when grown in unsterile soil infested with *G.fasciculatum* inoculation. These results confirm the earlier reports by Bagyaraj and Manjunath [8] and Rao *et al.* [9].

Table 2. Effect of *G. fasciculatum* inoculation on the shoot dry wt, root dry plant, protein content) and total chlorophyll content of some medicinal plants.

Plant	VAM status							
	Control Plants				VAM inoculated plants			
	Shoot dry wt(g/plant)	Root dry wt (g/plant)	PC (mg/g of plant)	TCC (mg/g plant)	Shoot dry wt(g/plant)	Root dry wt(g/plant)	PC (mg/g plant)	TCC (mg/g plant)
<i>Ocimum sanctum</i>	12.44	7.64	60.40	1.20	18.44	11.04	80.20	2.20
<i>Catharanthus roseus</i>	15.66	8.44	70.66	1.90	25.22	14.98	94.00	3.40
<i>Coleus forskholii</i>	8.00	5.66	53.40	1.00	15.91	10.00	79.27	1.96
<i>Cymbopogon flexuosus</i>	13.00	8.00	65.20	1.50	20.10	15.20	84.00	2.60
CD ($p=0.05$)	1.52	1.64	3.56	0.34	1.58	1.54	4.24	0.34
SD	0.74	0.84	7.92	0.96	3.64	3.72	8.72	0.98

PC - Protein content, TCC -Total chlorophyll content, CD - Critical differences, SD - Standard deviation.

The VAM colonization and growth response of *G. fasciculatum* was also observed in all the four medicinal plants (Tables 1, 2). All the four medicinal plants inoculated with

VAM fungus have shown improved growth and development as compared to control plants. The control plants have VAM associations varying from 18 to 25% (Table 1), when grown in un-sterilized soil due to low level natural inoculum. These results confirm the early reports by Bagyaraj and Manjunath [8]. The significant increase was observed not only in VAM colonization but also in biomass production (dry wt of root and shoot) due to inoculation with *Glomus fasciculatum* in all the four medicinal plants. The increased growth of treated plants due to mycorrhization of *G. fasciculatum* (Table 1) and the above results are in conformity with those of Gupta and Janarthanan [10] and Earanna et al. [11].

Host plants growing in soils with relatively low nutritional level as in gulberga soil [12] are predisposed to effective endophytic fungal invasion [13]. In the present study, similar situation was observed in all the four medicinal plants, which recorded 58 to 75% VAM association (Table 1).

The root and shoot biomass (dry wt) of the inoculated plants were found to be maximum and significant than that of control in all the four medicinal plant species (Table 2). The reason may be due to the formation of external mycelium around the roots by VAM fungi. The results are in agreement with the findings of earlier work by Gupta and Janardhanam [10]. Basu and Srivatava [14] have earlier reported such enhanced growth in medicinal plant due to VAM fungal association. Similar improved growth response was also observed in 10 medicinal plants when inoculated with three VAM fungal species (*G. mossae*, *G. fasciculatum* and *G. monosporum*) for their efficiency by Kumar and Murugesu [15]. They reported mycorrhizal inoculation was more advantageous in obtaining healthy vigorous seedlings and results in higher biomass of medicinal plants that were found to grow better in the field.

The inoculation with *G. fasciculatum* resulted in increased total chlorophyll content and protein content in all the four medicinal plants over control plant. The highest chlorophyll and protein content was recorded in *C. roseus*, followed by *C. flexuosus*, *O. sanctum* and *C. forskholii*. The increase in total chlorophyll content and protein content in inoculated plants may be due to increased uptake of phosphorus, which will increase the photosynthetic activity of the plants and ultimately the chlorophyll content in plants. Similar findings were reported already [16, 17]. Thus the present study revealed the positive effect of *G. fasciculatum* on some medicinal plants.

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