

Studies on the Effect of Interactions of Arbuscular Mycorrhiza (*Glomus mosseae*) and Plant Growth Promoting Rhizomicroorganisms (*Aspergillus awamori* and *Trichoderma harzianum*) on Growth and Development of *Catharanthus roseus* (L.)

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Abstract: *Catharanthus roseus* is an important medicinal plant of family Apocyanaceae. Plant is important source of antitumorous alkaloids vincristine and vinblastine. In the current study interactions of Arbuscular Mycorrhizal Fungi (AMF) *Glomus mosseae* (GM) with Plant growth promoting Rhizomicroorganisms (PGPR), *Aspergillus awamori* and *Trichoderma harzianum* were studied on *Catharanthus roseus* plants for 60 days interactions under open pot conditions. One month old plant seedlings were inoculated with *Glomus mosseae* and PGPR species and physiological growth parameters were recorded after harvesting. Biochemical parameters pertaining to chlorophyll and carotenoid pigment analysis were done. *Aspergillus awamori* with *Glomus mosseae* proved to be better treatment than *Trichoderma harzianum* with 54% more number of leaves and 87.49% more fresh weight than *Glomus mosseae* and *Trichoderma harzianum*. Chlorophyll and carotenoid pigments were also found higher in *Aspergillus awamori* treatment. *Aspergillus awamori* with *Glomus mosseae* can be used as an ecofriendly measure for mass multiplication of *Catharanthus roseus* plants under open pot conditions by the nurserymen. In future further studies can be performed with different inoculation treatments of Plant growth promoting Rhizomicroorganisms and *Glomus* species.

Keywords: *Catharanthus roseus*, *Glomus mosseae*, Plant growth promoting Rhizomicroorganisms, *Aspergillus awamori*, *Trichoderma harzianum*

1. Introduction

Arbuscular Mycorrhiza (AM) is symbiotic association formed between fungi of phylum Glomeromycota and roots of different plant species [1]. In this association host plants significantly supply photo synthetically fixed carbon material to fungal partner and fungi enhances uptake of plant nutrients, water, exudates and also other growth promoting factors such as Siderophores, Organic acids and Phytohormones [2].

Catharanthus roseus (L.) is also known as Madagascar or Periwinkle. Periwinkle is an important Medicinal Plant of family Apocyanaceae [3]. The plant is a rich source of alkaloids like vincristine and vinblastine. These alkaloids are secondary metabolites of the plant [4].

Catharanthus roseus is an evergreen perennial sub shrub. It has height of 30 cm to 1 m. *Catharanthus roseus* has flowers having five petal lobes. These petals form a corolla of 2-5 cm in diameter with a basal tube 2.5-3 cm long [5]. Leaves of *Catharanthus roseus* are oval or oblong in shape and are 1-3 inches long and arranged in opposite pairs [3, 6]. Different parts of the plant like roots and basal stems produce different alkaloids like raubasine, reserpine, vincetoxine and ajmalicine. Aerial parts of the plants produce antitumorous alkaloids like vinblastine and vincristine [7]. *Catharanthus roseus* has medicinal importance because of

its various properties like anticancerous, antimicrobial, antidiabetic, antiulcer and antihelminthic property [8].

Plant growth promoting Rhizomicroorganisms (PGPR) are one of the most effective and studied soil microorganisms which can promote plant performance. PGPR have shown the ability to solubilize unavailable Phosphorus to a form available for plant growth [9]. They are also reported to help Mycorrhiza and hence also known as Mycorrhiza Helper Organism (MHO) [10]. Earlier works have reported an increase in growth and Phosphorus uptake by plants through inoculation of Phosphate solubilizers in pot experiments [11, 12]. *Trichoderma* species are known to induce plant growth by producing growth regulating factors [13] and also by suppressing the activity of Pathogenic organisms [13]. Many studies have proved the potential of *Trichoderma* spp. as biological agents antagonistic to several plant pathogens [15, 16, 17, 18, 19, 20]. *Aspergillus awamori* have shown increased growth and phosphorus uptake in mung bean [21].

Studies have reported the synergistic effects of *Trichoderma* species with other Arbuscular Mycorrhiza and enhanced growth in plants have been reported [22]. The present study was undertaken to understand the response of *Catharanthus roseus* L. to the AM fungus *Glomus mosseae* and Plant growth promoting Rhizomicroorganism (PGPR /MHO), *Aspergillus awamori* and *Trichoderma harzianum* under open pot experiments.

2. Materials and Methods

Soil Preparation:

Soil and sand samples were collected from nurseries nearby to Bangalore University. The experimental soil was red clay soil having pH value 8.1 [23]. The soil and sand samples were autoclaved at 121°C at 15 lbs pressure for two consecutive days to eliminate naturally occurring endophytes. The soil and sand was then mixed with autoclaved farm yard manure (FYM) in the ratio of 1:1:1 (v: v).

Isolation of *Glomus mosseae* spores and preparation of inoculum:

Mycorrhizal inoculums of *Glomus mosseae* were isolated using Wet sieving and Decanting Technique of Gerdemann and Nicolson, 1963 [24]. 10 g of soil was mixed with 100 ml of water in the 500 ml conical flask. The soil mixture was agitated vigorously to free the AMF spores from soil and allowed to settle for 15-45 minutes and the supernatant was decanted through standard sieves. By using a dissecting microscope, spores were picked by means of pipette or needle. *Glomus mosseae* spores were isolated as per the spore morphology and characteristics. *Glomus mosseae* (GM) inoculums were prepared as per funnel technique proposed by Menge and Timmer, 1982 [25]. GM cultures of 2g (spore density -125 spores/g) were added near the roots of plant seedlings.

Maintenance of *Aspergillus awamori* (AA) and *Trichoderma harzianum* (TH):

Initial PGPR cultures of *Aspergillus awamori* (AA) and *Trichoderma harzianum* (TH) were procured from Department of Soil Microbiology, University of Agriculture Sciences, GKVK, Bangalore. AA and TH cultures were then grown on 2% PDA and incubated for 7 days at 26 ± 2°C. Conidia from each isolate were then harvested by flooding the cultures with sterile distilled water. The culture surface was then rubbed with a sterile glass rod. The suspensions were filtered through out two layers of cheese cloth. The concentration of propagules in suspension were standardized with the aid of a haemocytometer to 3 x 10⁸ conidia ml⁻¹ for each.

$$\text{Percentage root colonization} = \frac{\text{Total no of root segments infected} \times 100}{\text{Total no of root segments studied}}$$

Chlorophyll Pigment Analysis

The Chlorophyll pigments in the leaves were estimated following the method of Arnon, (1949) [28]. The fully expanded leaves from all the sites were collected in the polythene bags and transported to the laboratory. The leaves were thoroughly washed with distilled water. Three replicates were used for each plant. Weighted fresh leaf material was homogenized and extracted thrice in chilled 80% acetone (v/v). The volume of the acetone extract was made up to a known one and the optical density was read at 645nm and 663nm wavelengths on a spectrophotometer. The concentration of the chlorophyll pigments was calculated using the following formula and the results are expressed in mg/g fresh weight.

$$\text{Chlorophyll a} = [(12.7 \times \text{OD at 663}) - (2.69 \times \text{OD at 645})] \times \text{dilution factor}$$

Experimental Design

The experiments were conducted as open pot experiments in Plastic pots with 3kg soil capacity. One month old plant seedlings of *Catharanthus roseus* were collected from Sanjeevani vatika, Department of Horticulture, University of Agriculture Sciences, GKVK, Bangalore. The treatments were maintained at Medicinal Plant Garden, Department of Microbiology and Biotechnology, Bangalore University, Bangalore under Open pot experiments with the following inoculation treatments:

GM + AA, *Glomus mosseae* + *Aspergillus awamori*

GM + TH, *Glomus mosseae* + *Trichoderma harzianum*

All the treatments were done in triplicates. The experiments were laid down as a Randomized complete block design (RCBD). The plants were maintained for 60 days and were watered on alternate days. During the experiments the temperature range was 35°C to 14°C. Plants were harvested after 60 Days of Days after Transplanting (DAT) by using water pressure. Just after plucking the physiological growth parameters viz root length, shoot length, fresh weight, number of leaves, number of flowers were recorded. Plant samples were then oven dried at 60 °C for 72 hrs till a consecutive weight is achieved and later the dry weight was recorded.

Root Colonization Study

After 60 days of inoculation plants were harvested and terminal roots were collected from the plants. The roots were taken out carefully to prevent any damage to secondary or tertiary rootlets. After harvesting roots were rinsed thoroughly with tap water to remove soil particles and later with distilled water. Root Colonization was studied by 'Rapid clearing and Staining technique' of Philips and Hayman, 1970 [26]. The technique involves microscopic observation of AM fungi fungal colonization after clearing roots in KOH (10%) and staining with trypan blue (0.5%). Percentage mycorrhizal root colonization (%) study was done by gridline intersect method proposed by Giovannetti and Mosse, 1980 [27]. A total of 50 root fragments were studied and infected root segments were recorded. A root segment was considered to be infected if it showed presence of mycelium, vesicle or arbuscules.

$$\text{Chlorophyll b} = [(22.9 \times \text{OD at 645}) - (4.68 \times \text{OD at 663})] \times \text{dilution factor}$$

$$\text{Total chlorophyll} = [(20.2 \times \text{OD at 645}) - (8.02 \times \text{OD at 663})] \times \text{dilution factor}$$

Carotenoid analysis

Quantitative determination of carotenoids was done by reading the Chlorophyll extract at 480 nm. The amount of carotenoids present in the extract was calculated by using the formula of Kirk and Allen, 1965 [29].

$$\text{Carotenoids (mg/g)} = [(\text{OD at 480}) - (0.114 \times \text{OD at 663}) - (0.636 \times \text{OD at 645})] \times \text{dilution factor}$$

3. Results and Discussions

Table 1: Physiological growth parameters in GM+ AA and GM +TH for 60 Days Inoculation

PARAMETER	60 Days	
	GM + AA	GM+TH
Shoot length (cm)	36.66 ± 3.51	37.16 ± 8.09
Root length(cm)	33.33 ± 7.94	22.83 ± 1.44
Number of leaves	79.33 ±17.00	51.33 ± 2.51
No of flowers	4.66 ± 1.15	0.33 ± 0.577
No of branches	4.66 ± 1.154	2.333 ± 0.577
Fresh weight total(g)	26.26 ± 8.61	14.02 ± 0.962
Fresh weight stem (g)	7.78 ± 3.00	4.54 ± 0.27
Fresh weight shoot (g)	13.30 ± 2.36	9.37 ± 0.64
Fresh weight leaves (g)	9.42 ± 2.50	5.00 ± 0.87
Fresh weight root (g)	4.94 ± 2.50	8.23 ± 0.87
Root volume (cm ³)	10.46 ± 4.53	23.55 ± 7.85
Dry weight stem (g)	1.83 ± 0.74	1.32 ± 0.09
Dry weight root (g)	2.15 ± 0.47	2.20 ± 0.05
Dry weight leaves (g)	1.86 ± 0.74	1.20 ± 0.05
Total dry weight (g)	4.86 ± 1.88	3.73 ± 0.19

*Values are taken as mean of triplicates

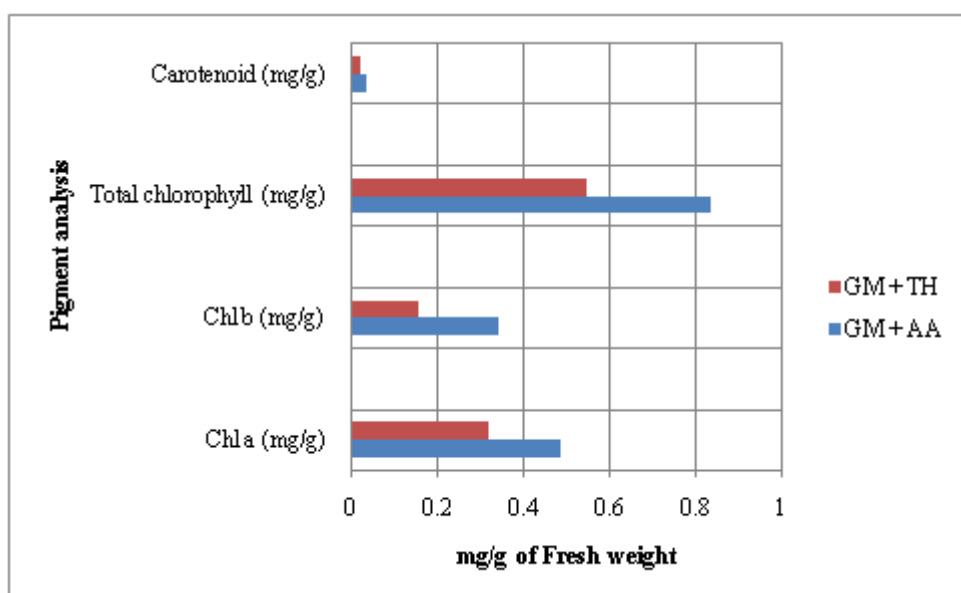


Figure 1: Chlorophyll and Carotenoid Pigment analysis in GM+AA and GM + TH for *Catharanthus roseus* 60 days treatment

The different physiological parameters of plant like shoot length, root length; number of leaves, number of flowers, and number of branches were studied. The shoot length for GM+AA (36.66 ± 3.51 cm) was found to be less than GM+TH (37.67 ± 8.098 cm) (Table 1), but the root length for GM+AA (33.33 ± 7.49 cm) was more than GM+TH (22.83 ± 1.44 cm). Increase in number of leaves and flowers was also recorded. The number of leaves was 54% more in GM+AA (79.33 ± 17.00) than in GM+TH (51.33 ± 2.51). Increase in number of flowers was recorded with 4.66 ± 1.15 in GM+AA and 0.33 ± 0.57 in GM+TH. The total fresh weight of plant was found to be 26.26 ± 8.61g in GM+AA, 87.49% higher than GM+TH. Fresh weight of stem in GM+AA (7.78 ± 3.00g) was 71.20% more than GM+TH (4.54 ± 0.27 g) inoculation. Similar trend was observed for fresh weight of leaves which was 88.52% more in GM+AA (9.42 ± 2.50g) than GM+TH (5.00 ± 0.87g). Leaves of *Catharanthus roseus* have significance as being potential source of vincristine and vinblastine alkaloids. When dry weight of leaves was recorded GM+AA treatment had

38.77% more dry weight than GM+TH treatment. Dry weight of leaves in GM+AA was recorded as 1.86 ± 0.74g and 1.20 ± 0.05g for GM+TH. In contrast with other results the root volume was found more in GM+TH than GM+AA. Fresh weight of root was recorded to be 8.23 ± 0.87g in GM+TH but it was 4.94 ± 2.50g in GM+AA treatment. The dry weight of root was almost similar in both inoculations with 2.1% more in GM+TH treatment than GM+AA treatment. The total dry weight was recorded to be higher in GM+ AA inoculation (4.86 ± 1.88g) as compared to GM + TH inoculation (3.73 ± 0.19 g).

The root colonization study results revealed 62% infection in GM+AA and 59% infection in GM +TH inoculation treatments. Chlorophyll pigments were analyzed in both GM+AA and GM+TH treatments for 60 days (Fig: 1). Chlorophyll a pigment was found to be 0.489 mg/g fresh weight in GM+AA whereas it was 0.321 mg/g in GM+TH. Chlorophyll b was found to be 0.346 mg/g in GM+AA and 0.156 mg/g in GM + TH. The total chlorophyll

content was 0.835 mg/g in GM +AA and 0.548 mg/g in GM +TH treatment. The total carotenoid content was found to be 0.036 mg/g in GM+AA whereas it was 0.024 mg/g in GM+TH treatment.

Inoculations of plants with arbuscular mycorrhiza have shown increase in physical growth parameters in Medicinal Plants. Studies conducted by Neelima Rati et al. [30] revealed that AMF fungi *Glomus mosseae* increases Physiological growth parameters in *Catharanthus roseus* plants. The present result findings are in curriculum with the earlier work performed by Karthikeyan et al.[31] which showed that inoculation of PGPR like *Azotobacter*, *Pseudomonas* and *Bacillus* on *Catharanthus roseus* significantly increased plant growth, root length etc. The reason may be increase in Phosphorus uptake by these Phosphate solubilizers which enhances nutrient availability to plants [32,33].

4. Conclusion

In the current study AMF fungi *Glomus mosseae* effect with PGPR species *Aspergillus awamori* and *Trichoderma harzianum* was studied on *Catharanthus roseus* plants for 60 days under open pot conditions and results revealed *Aspergillus awamori* treatment to be better when the leaves of the plants were considered, which was also supported by chlorophyll and more carotenoid content in leaves of plant, but when the roots of the plants were considered *Trichoderma harzianum* proved to be comparatively better.

Plant growth promoting rhizomicroorganisms are natural phosphate solubilizers and also act as natural biocontrol agents. Inoculation of *Catharanthus roseus* plants with *Aspergillus awamori* can be used as an environment friendly measure for mass multiplication of leaves of the plant under open pot conditions. In future further studies can be performed with different inoculation treatments of Phosphate solubilizers and *Glomus* species for both open pot and field conditions.

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