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# Study of Growth Promotion Effect of VAM Fungi and Trichoderma Species on Medicinal Plant

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***Abstract:*** **Experiments were-conducted to determine the influence of VAM fungi, Trichoderma harzianum and T. viride individually as well as in combinations on the growth of Ocimum sanctum L. Co-inoculation with VAM and Trichoderma harzianum resulted in maximum plant growth. Co-inoculated plants were recorded maximum shoot and root length, maximum fresh and dry weight, more leaves more flowers and maximum leaf initials than control. Mycorrhizal colonization was seen more than 80% in treated plants and only 20% in control. Spore counts were more in treated rhizosphere soils than the control. Among treated the percentage of growth parameters were maximum in Glomus fasciculatum + T. harzianum treatments than their counterparts and minimum in control.**

***Keywords:*** **Ocimum sanctum, Vesicular Arbuscular Mycorrhizae, Glomus fasciculatum, mosseae, trichoderma.**

## 1. INTRODUCTION

Traditional medicines and remedies are gaining immense importance worldwide in the age of patents and intellectual rights due to increasing demand of medicinal plants by both developing and developed countries. Ancient India is known for the traditional medical practices. Many historical documents and medicinal descriptions provide evidence for ancient traditional medical practices where plants were being used, for example Rigveda documented and prescribed around 67 medicinal plants. Plants are placed front line in traditional medicines due to their affordability and safety. Modern therapies slowly focusing towards the ayurveda and other traditional practices, where plant extracts are widely being used. Thus, exploitation of medicinal plants is being increased due to an increasing demand for their pharmaceutical value. This leads the loss of biodiversity and fast disappearance of medicinal plants. A steady state is not established between the utilization and rehabilitation of medicinal plants. It is the time to take up steps to narrow down the utilization and cultivation gap and sustainable development strategies. Use of microbial inoculations is one of the methods to improve the quality of growth and development of medicinal plants.

Mycorhizal roots are observed in nearly all native strands of plants in all parts of the world. The Vesicular Arbuscular Mycorrhizae (VAM) is the most common and widely occurring mycorrhizal association [1], [2]. It is one of the important beneficial microorganisms in the rhizospheric soils and has potential use to medicinal plants for promoting growth and productivity [3], [4], [5], [6]. AM fungi interact with a wide range of other microorganism in the rhizosphere. These are stimulatory when they increase the growth response of the host in presence of other microorganisms [7]. VAM play a dominant role in increasing phosphorus solubilization and uptake of P, N, Ca, S, K, Mg Mn, and Cl by plants [8], [9]. VAM actually helps in the plants by increasing the soil surface availability by growing through soil pore and paces and affect phosphorus absorption beyond the depleted zone. Trichoderma spp., are free-living fungi, commonly found in soil and rhizosphere. They are highly active in rhizosphere and foliar environments. They produce variety of compounds that induce resistance responses in plants. Trichoderma strains have been recognized as biological agents since long time, for

the biological control of plant disease. And also know for their ability to increase root growth and development, crop productivity, resistance to abiotic stresses, and uptake and use of nutrients in many plant.

Ocimum sanctum also known as Holy basil or tulsi is an aromatic plant in T. harzianume family Lamiaceae. In Ayurveda Ocimum sanctum L. (tulsi) has been well documented for its therapeutic uses and described as Dashemani Shwasaharni (antiasthmatic) and antikaphic drugs (Kaphaghna) [10], [11]. Devotees perform worship to it as a holy plant. Scientific reports provided adequate evidences about the medicinal properties of Tulsi, i.e. antimicrobial [12], [13], [14], anti-inflammatory [15], [16], immunomodulatory [17], adaptogenic [18], [19], [20], antidiabetic [21], [22], anti-carcinogenic [23], [24], hepatoprotective [25], [26], radioprotective [27], neuroprotective [28], cardio-protective [29], [30], etc. Thus, Ocimum sanctum contain important bioactive compounds may contribute to the health benefit.

## 2. MATERIAL AND METHODS

***Plant collection:***

The same aged plants were collected from Dhanvanthari vana, Forest and Horticulture Department, Jnanabharathi Campus, Mariyappanapalya; Soil used for planting was collected from Gandhi Bhavan Nursery, Jnanabharathi Campus, Bangalore University, Bangalore.

***Experiment:***

The experiment was designed with the following treatments with O sanctum: (i) Soil (control); (ii) soil + G. mosseae; (iii) soil + Glomus fasciculatum, (iv) soil + G. mosseae + Trichoderma harzianum; (v) soil + G. mosseae + Trichoderma viride; (vi) soil + Glomus fasciculatum + Trichoderma harzianum; and (vii) soil + Glomus fasciculatum + Trichoderma viride. Three replicates of each treatment were taken. Interaction between various treatments was studied after 30, and 60 days and following parameters were observed: Shoot length (in cm), Root length (in cm), Fresh weight (in gm), Dry weight (in gm), No of leaves, No. flowers/seeds, No. of shoot branching, No. of leaf initials and finally Mycorrhizal colonization (%) and VAM spore No./10g of soil. For dry weight, root and shoot pieces were dried in hot air oven at 80°C for 12 h.

After 90 days of treatment soil samples were collected from the root zone of each plant. The soil samples were sieved for AM spore populations by using 'wet sieving and decanting technique' and quantification of AM spores was done by 'grid line intersect method' [31], [32]. The fine terminal roots were removed washed and stained with trypan blue according to 'rapid clearing and staining method' [33]. The percent AM root colonization was calculated by using following equation;

%AM root colonization = (Total number of root segments colonized / Total number of root segments examined) ×100

## 3. RESULTS AND DISCUSSION

Arbuscular mycorrhizal fungi enhance the nutritional uptake of several plants and are directly responsible for the increased growth and yield. The present study was carried out in order to evaluate the potential of AM fungi and Trichoderma species on growth and physiological parameters of Ocimum sanctum. Results indicated that the plantlets under investigation showed varied response to the inoculants in individual and in different combinations. Experimental data, Shoot length (in cm), Root length (in cm), Fresh weight (in gm), Dry weight (in gm), were recorded in Table 1. No of leaves, No. flowers/seeds, No. of shoot branching, No. of leaf initials were recorded in table 2 and Mycorrhizal colonization (%) and VAM spore No./10g of soil were recorded in table 3.

**Table 1: effect of VAM and T. harzianum, viride, individually and in combination on growth of O. sanctum.**

| Treatments& days | Shoot length (in cm) | | Root length (in cm) | | Fresh weight (in gm) | | Dry weight (in gm) | |
|---|---|---|---|---|---|---|---|---|
| | 30 days | 60dyas | 30 days | 60dyas | 30 days | 60dyas | 30 days | 60dyas |
| Control | 34 | 42 | 12 | 15 | 8.80 | 11.80 | 2.50 | 3.13 |
| G. mosseae | 40 | 59 | 14 | 20 | 10.82 | 14.82 | 4.10 | 7.73 |
| G. mosseae + T. harzianum | 43 | 66 | 14 | 22 | 11.00 | 15.00 | 4.43 | 7.05 |
| G. mosseae + | 44 | 61 | 14 | 19 | 11.12 | 14.90 | 3.66 | 7.15 |

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|---|---|---|---|---|---|---|---|---|
| T. viride | | | | | | | | |
| G. fasciculatum | 48 | 65 | 15.5 | 22 | 18.25 | 22.00 | 4.34 | 5.40 |
| Glomus fasciculatum + T. harzianum | 50 | 77 | 16 | 24 | 19.10 | 25.60 | 4.36 | 5.52 |
| G. fasciculatum + T. viride | 49 | 66 | 14 | 18 | 17.61 | 24.10 | 4.64 | 4.95 |

*Note: G. mosseae = Glomus mosseae, G. fasciculatum = Glomus fasciculatum, T. harzianum = Trichoderma harzianum, T. viride = Trichoderma viride.*

**Table 2: effect of VAM and T. harzianum, viride, individually and in combination on growth of O. sanctum.**

| Treatments& days | No of leaves | | No. flowers | | No. of shoot branching | | No. of leaf initials | |
|---|---|---|---|---|---|---|---|---|
| | 30 days | 60dyas | 30 days | 60dyas | 30 days | 60dyas | 30 days | 60dyas |
| Control | 100 | 160 | 0 | 2 | 3 | 3 | 8 | 15 |
| G. mosseae | 100 | 160 | 0 | 3 | 3 | 3 | 10 | 12 |
| G. mosseae + T. harzianum | 120 | 200 | 4 | 8 | 3 | 4 | 10 | 36 |
| G. mosseae + T. viride | 135 | 240 | 6 | 10 | 4 | 4 | 16 | 44 |
| G. fasciculatum | 155 | 290 | 8 | 15 | 3 | 4 | 12 | 37 |
| G. fasciculatum + T. harzianum | 166 | 300 | 8 | 13 | 4 | 3 | 15 | 17 |
| G. fasciculatum +T. viride | 150 | 289 | 6 | 16 | 5 | 5 | 10 | 12 |

*Note: G. mosseae = Glomus mosseae, G. fasciculatum = Glomus fasciculatum, T. harzianum = Trichoderma harzianum, T. viride = Trichoderma viride.*

**Table 3; Mycorrhizal colonization (%) and VAM spore no. /10g of soil**

| Treatment | Mycorrhizal colonization (%) | VAM spore No./10g of soil |
|---|---|---|
| Control | 20 | 1 |
| G. mosseae | 80 | 5 |
| G. fasciculatum | 83 | 6 |

*Note: G. mosseae = Glomus mosseae, G. fasciculatum = Glomus fasciculatum,*

VAM and Trichoderma sps. significantly increased the shoot length of O. sanctum after 30 and 60 days of inoculation. Results indicate that maximum increase in shoot length was observed in combination of Glomus fasciculatum + Trichoderma harzianum after 30 days 50cm and after 60 days 77cm. Glomus fasciculatum alone registered increased shoot length 48cm for 30 days and 65cm for 60days than Glomus mosseae 40cm for 30 days and 59cm for 60days, and Glomus mosseae + trichoderma treatments 43.5±0.5 cm for 30 days and 63.5±0.5cm for 60days. Minimum shoot length was recorded for control 34 cm for 30 days and 42 cm for 60days. In this study the inoculants increase the soil fertility and altered the rhizosphere environment which had facilitated the uptake of nutrients by plants. Thus the shoot length increased in the treated plants [34].

The maximum root length was registered for Glomus fasciculatum + Trichoderma harzianum treated plants after 30 days 16cm and after 60 days 24cm. Glomus fasciculatum alone registered increased root length of 15.5cm for 30 days and 22cm for 60days than Glomus mosseae 14cm for 30 days and 20cm for 60days, and Glomus mosseae + trichoderma treatments recorded 14±0.5 cm for 30 days and 20.5±0.5cm for 60days. Minimum root length was observed for control i.e., 12 cm for 30 days and 15 cm for 60days.

The maximum fresh weight was recorded for Glomus fasciculatum + Trichoderma harzianum treated plants 19.10g, and 25.60g for 30 and 60 days respectively and followed by Glomus fasciculatum + T. viride, Glomus fasciculatum, Glomus mosseae+ T. viride, Glomus mosseae + T. harzianum and Glomus mosseae treatments. Minimum was observed in control 8.80g and 11.80 for 30 and 60 days respectively. 30 days treatments shown maximum dry weight for Glomus fasciculatum + T. viride treated plants followed by Glomus mosseae + T. harzianum, Glomus fasciculatum + T. harzianum, Glomus fasciculatum, Glomus mosseae and Glomus mosseae + T. viride. The minimum dry weight was measured for control. After 60 days of the treatment maximum dry weight was observed in Glomus mosseae and minimum in control and Glomus mosseae and trichoderma combinations had recorded more dry weight than Glomus fasciculatum and Glomus fasciculatum combinations with trichoderma treated plants.

In accordance with observations of table 2, it was noted that the flowering occurred much earlier in treated plants than the control and number of leaves were also maximum. The improved growth and flowering may be because of earlier expression of developmental regulated genes, enhanced uptake of nutrients especially phosphorus and nitrogen. Results indicate that the treatment doesn't shown significant variation in the number of shoot branches of the plants. But the numbers of leaf initials were observed maximum for treated plants than control.

Results indicate that a varied degree of spore population and mycorrhizal root colonization has been reported in all inoculated plants. Maximum spore population was observed in the plants treated with G. fasciculatum and T. harzianum after 90 days of inoculation. Similarly, the intensity of mycorrhizal root colonization was found highest in the plants inoculated with G. fasciculatum and T. harzianum (83%). And that demonstrate AM fungi enhances mycorrhizal root colonization [35]. This may be synergetic effect of AM fungi and T. harzianum. Similar results were observed for Glomus mosseae + T. viride treated plants, no of spores [35], and percentage of colonization was 80%. However some reports suggests that VAM is decreasing the growth of plants in high phosphate available soils due to high p toxicity [36]

## 4. CONCLUSION

In this study, treatment of VAM fungi in combination with trichoderma species significantly enhanced the growth parameters which included, shoot length, root length, fresh weight, dry weight, leaves, number of shoots, flowers and leaf initials, trichoderma also plays important role in the soil and enhance the plant growth. From data it is clear that inoculation of VAM along with other biological control agents will enhance the plant growth, so more investigations are needed to correlate the exact action and mechanism of biological control agents on VAM fungi.

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