

Influence of Vesicular Arbuscular Mycorrhizae (VAM) on Growth of *Ruta graveolens* – A Medicinal Plant

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Abstract: A pot experiment was conducted to investigate the effect of vesicular-arbuscular mycorrhizal (VAM) fungi on growth of *Ruta graveolens*. The plants were inoculated with *Glomus mosseae*, *Glomus fasciculatum* and both. Morphological characters, plant height, root length and no. of compounds leaves were observed periodically up to 90 days after the treatment. After 90 days plants were analyzed for the shoot fresh weight, dry weight, root fresh weight, dry weight and total plant biomass. After 90 days the maximum shoot height 33.6cm was observed in GM+GF treated plants and minimum height 22.6cm was observed in control. The maximum root length 14.3cm was observed in GM+GF plants and minimum 8.4 cm in control. Maximum compound leaves 27 numbers were observed in *Glomus mosseae* + *Glomus fasciculatum* treated plants, followed by *Glomus fasciculatum* (26.8 no.) and *Glomus mosseae* (26.7no) treated plants and minimum in control (18.5no). The maximum plant biomass (45.4g/plant) was found in *Glomus mosseae* + *Glomus fasciculatum* treated plants, followed by *Glomus fasciculatum* treated plant (44.9 g/plant.) and *Glomus mosseae* treated plant (44.8 g/plant) and minimum in control (27.5g/plant). Maximum shoot fresh weight (43.6g) was observed in the *Glomus mosseae* + *Glomus fasciculatum* treated plants, followed by *Glomus fasciculatum* treated plant (43.1g) and *Glomus mosseae* treated plant (43.0g) and minimum in control 26.3g. In the similar fashion dry weight of shoot, fresh weight of root and dry weight of root was reported. *Glomus mosseae* + *Glomus fasciculatum* exhibited the more positive effect on plant growth.

Keywords: *Glomus mosseae*, *Glomus fasciculatum*, vesicular arbuscular mycorrhizae (VAM), biofertilizer

1. Introduction

There is an ever increasing demand for medicinal plants due to increased acceptance of Ayurveda and traditional medicines. This forced us to improve plant yield, growth and conservation and sustainable development with newer approaches. Cultivating medicinal plants, better growth and yield of plants can be achieved by various ways. One of the best practices is treatment of VAM fungi. In view of the important role played by the VAM fungi in improving plant growth and yield, it was thought worldwide to explore the nature of VAM in medicinal plants. The symbiotic association between higher plants and fungus is a known as mycorrhizae. Where plants share their photosynthates and shelter, fungal partners help in absorption of nutrients and water and anchorage. Different types of mycorrhizae are: endomycorrhizae, ectendomycorrhizae and ectomycorrhizae. The endomycorrhizal fungi, vesicular arbuscular mycorrhizae (VAM) have gained importance as biological tool in improving the performance of forest trees and also variety of other plants, especially medicinal plants. Arbuscular Mycorrhizae (AM) fungi are characterized by the formation of unique structures, arbuscular and vesicles. AM fungi help plants to capture nutrients such as phosphorus, sulfur, nitrogen and micronutrients from the soil. It is believed that the development of the arbuscular mycorrhizal symbiosis played a crucial role in the initial colonization of land by plants and in the evolution of the vascular plants. VAM protects plants from oxidative damage generated by drought [1]. VAM plants exhibit higher rate of photosynthesis than non-VAM counterparts [2]. The concentration of chlorophyll in VAM plants is more than its non- mycorrhizal counterparts [1], [3] and [4]. Duan et al.,

found concentrations of abscisic acid (ABA) in xylem sap were lower in VAM plants than in NM (non mycorrhizal) plants during the drought [5], Goicoechea et al. also proved [6], this indicates VAM plants are less strained. VAM influences on plant in severe climatic conditions by increasing the tolerance in adverse climatic conditions and increase plant productivity [7], hence, they are important for natural and managed ecosystems [8]. AM fungi diversity is the most important factor for maintenance of plant diversity and stability of ecosystem and proper functioning of ecosystem. Many studies revealed that AM fungi would alter the plant community structure and plant species structure [9]-[11]. *Glomus* isolates were reported for uptake, transport and immobilization of copper and cadmium, and they provide tolerance to heavy metals for plants [12]. Mycorrhizae detoxify the toxicity of trace metals in polluted soils and enhance plant growth [13]. Mycorrhizal infections are capable of alleviating the damage of salt stress and promote plant growth [14]. The current study used two species *Glomus mosseae* and *Glomus fasciculatum* of the *glomus*, biggest genus of VAM fungi. Different tribes staying near forests and pharmaceutical and cosmetic industries use medicinal plants for various treatments, preparation of drugs, cosmetics and fragrances but less attention have been given for the conservation of medicinal plants. AM fungi inoculation is one of the promising tools for the conservation and sustainable maintenance of medicinal plants.

2. Material and Methods

Collection of plants and VAM fungi

Plant samples were collected from Sanjeevini Vatika, Department of Horticulture, University of Agriculture Sciences, GKVK, Bangalore. Spores of *Glomus mosseae* and *Glomus fasciculatum* were collected from Department of Microbiology, University of Agriculture Sciences, GKVK, Bangalore. Soil samples were collected from Gandhi Bhavan Nursery, Jnanabharathi campus, Bangalore University, Bangalore.

Production of VAM culture

Spores of mixed culture of *Glomus mosseae* and *Glomus fasciculatum* were taken, autoclaved soil and sand mixture (1:1) was poured into sterilized pots. The VAM spores mixed with ragi seeds were placed below the 2 cm of top soil of the pots. After 42 days the ragi seeds were removed and roots separated chopped into small pieces (1-2 mm) and mixed with steam sterilized sand and loam soil. Mixture of soil and roots were packed and stored at 4°C and used as biofertilizer. Total three set of biofertilizers were prepared two individual organisms and one mixed.

Plant inoculation

Table 1: Morphological parameters of *Ruta graveolens* using VAM biofertilizer

Treatment	Plant height						Root length						No of leaves compound leaves					
	Days	15	30	45	60	75	90	15	30	45	60	75	90	15	30	45	60	75
Control	3.0	10.0	16.0	18.2	20.0	22.6	2.1	4.0	6.0	7.1	7.9	8.4	1.7	5.6	13.62	15.5	16.1	18.1
GM	5.3	15.0	23.1	28.6	31.0	33.2	3.1	6.0	8.6	10.8	12.6	13.9	3.0	8.4	19.6	24.3	25	26.7
GF	5.4	16.0	23.9	29.0	32.0	33.3	3.1	6.0	8.7	10.8	12.7	14.0	3.1	9	20.3	24.7	25.7	26.8
GM+GF	5.4	16.2	24.1	29.1	32.3	33.6	3.1	6.1	8.9	11.0	12.9	14.3	3.1	9.1	20.5	24.9	26.0	27.0

GM- *Glomus mosseae*, GF- *Glomus fasciculatum*

Table 2: Fresh and dry weight of shoot and root and total biomass of *Ruta graveolens L.* after 90 days of treatment

	Fresh weight of shoot (g)	Dry weight of shoot (g)	Fresh weight of root (g)	Dry weight of root (g)	Biomass of plant (g)
Control	26.3	7.4	1.2	.06	27.5
GM	43.0	11.0	1.8	.07	44.8
GF	43.1	11.0	1.8	.07	44.9
GM+GF	43.6	11.1	1.8	.07	45.4

GM- *Glomus mosseae*, GF- *Glomus fasciculatum*

4. Discussion

AMF are a wide spread group of fungi form a symbiotic association with large group of plant species, and effectively improve growth. In the present study inoculation of *Glomus mosseae* and *Glomus fasciculatum* showed a positive influence on plant height, root length, fresh weight of plant and number of leaves and biomass of plant. Inoculating nursery soil mixture with *Glomus fasciculatum*, *Glomus mosseae* and *Glomus etunicatum*, and that *Glomus fasciculatum* reported vigorous seedling growth in Casuarina plants [15]. After days of treatment there was considerable increase in the height of the treated plants than control. That was due to the effect of VAM biofertilizer which enhanced the uptake of nutrients by plant. Individual

Plant seedlings were taken and different physical growth parameters were recorded. Plants were inoculated with one gram of VAM biofertilizer and watered regularly. VAM fungi infected the plants when physical contact was established between mycorrhizal inoculants and the plant root. Further physical parameter studies were done at the interval of 15 days up to 90 days.

3. Results and Discussions

Physical parameters

Physical parameters like root length, plant height, fresh weight of plant and number of leaves recorded after the interval of 15 days of inoculation. All experiments were in triplicates, i.e., three plants were kept as control and three plants were treated with VAM biofertilizer. Shoot lengths were measured periodically and measurement the total root length was estimated by measuring roots taken from soil cores midway between stem and pot rim. Morphological parameters of *Ruta graveolens* reported in table 1. Fresh and dry weight of shoot and root and total biomass of *Ruta graveolens L.* after 90 days of treatment were reported in table 2.

organisms showed slight different effect on plant height and little increased effect (33.6cm plant height) was observed when both organisms were mixed. This effect might be due to compliment effect of both. Mixed biofertilizers have more positive effect on growth of plant. Minimum height (22.6cm) was recorded for control and maximum 33.6cm in *Glomus mosseae* + *Glomus fasciculatum* treated plants. The maximum root length 14.0cm and 14.3cm was found in the *G. fasciculatum* and GM+GF plants respectively. Minimum root length 8.4 was observed in control. The maximum 27 compound leaves were found in *Glomus mosseae* + *Glomus fasciculatum* treatment plants, followed by *Glomus fasciculatum* (26.8 no.) and *Glomus mosseae* (26.7no) treated plants and the minimum 18.1 leaves were found in control. The association of two vesicular arbuscular mycorrhizal (VAM) fungi, *Glomus macrocarpum* and *Glomus fasciculatum* inoculation increased the essential oil concentration by 43% in coriander, the effectiveness of the two fungal species were studied to enhance the quality and quantity of essential oils in plants [16]. The studies of Kumar et al. [17] and Hazarika et al. [18] were also found that *Glomus mosseae* inoculation have found the maximum growth effect on chickpea plants.

VAM fungal interactions increase the uptake of phosphorus and other mineral nutrients by plants, especially in low P availability conditions. It also increases the plant tolerance

and photosynthetic activity. When plant health improved, growth and biomass also increases. Plant biomass is the one of the important parameter which directly reflects the effect of biofertilizer. In present study the increased biomass of plant in *Ruta graveolens* was observed due to VAM inoculation. The maximum plant biomass (45.4g per plant) was found in *Glomus mosseae* + *Glomus fasciculatum* treated plants, followed by *Glomus fasciculatum* treated plant (44.9 g/plant.) and *Glomus mosseae* treated plant (44.8 g/plant). Minimum 27.5g/plant biomass was observed in control. Many studies reported the increase in the biomass of AM treated plants [19]. Shoot fresh weight is another parameter indicates the health of plant and it also indicated the efficiency of biofertilizer effect on plants. Maximum shoot fresh weight (43.6g) was observed in the *Glomus mosseae* + *Glomus fasciculatum* treated plants, followed by *Glomus fasciculatum* treated plant (43.1) and *Glomus mosseae* treated plant (43.0g), and minimum 26.3g was found in control. In the similar fashion dry weight of shoot, fresh weight of root and dry weight of root was reported. VAM related colonization on root increases the shoot fresh weight has been reported in other plants [20]. VAM fungus colonize throughout the root surface by establishing infection points [21].

5. Conclusion

The chemical fertilizer have played major role in green revolution and increased the production. But use of chemical fertilizers poses threat to the environment. Therefore the use of biofertilizer is both economical and environmental friendly. Biofertilizers are microbial inoculations used to improve the soil fertility and enhances the uptake of nutrients by plants. They also increase disease resistance and stress tolerance power of the plants. In this study we highlighted the use of VAM biofertilizer for development of growth of medicinal plants.

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References

- [1] J.N. Gemma, R.E. Koske, E. M. Roberts. et al. (1997) "Mycorrhizal fungi improve drought resistance in creeping bentgrass", *J Turfgrass Sci.*, 73, pp. 15-29, 1997.
- [2] R.M. Auge, "Water relations, drought and vesicular-arbuscular mycorrhizal symbiosis", *Mycorrhiza*, 11, PP. 3-42, 2001.
- [3] F.T. Davies, J.R. Potter, R.G. Linderman, "Drought resistance of mycorrhizal pepper plants independent of leaf P-concentration – response in gas exchange and water relations", *Physiol Plant.*, 87, pp. 45-53, 1993.
- [4] N. Mathur, A. Vyas, "Influence of VAM on net photosynthesis and transpiration of *Ziziphos mauritiana*", *J Plant Physiol.*, 147, pp. 328-330, 1995.
- [5] X. Duan, D.S. Neuman, J.M. Reiber, et al. (1996) "Mycorrhizal influence on hydraulic and hormonal factors implicated in the control of stomatal conductance during drought", *J Exp Bot.*, 47, pp. 1541-1550, 1996.
- [6] N. Goicoechea, M.C. Antolin, M. Sánchez-Díaz, "Gas exchange is related to the hormone balance in mycorrhizal or nitrogen-fixing alfalfa subjected to drought", *Physiol Plant*, 100, pp. 989-997, 1997.
- [7] N.R. Fedderman, T. Finally, F. Boller, Elfstr, "Functional diversity in arbuscular mycorrhiza the role of gene expression, Phosphorus nutrition and Symbiotic efficiency", *Fungal Ecology*, 3, pp. 1–8, 2010.
- [8] M. Brundrett, N. Beegher, B. Dell, T. Groove, N. Malajczuk N, "Working with mycorrhizas in Forestry and Agriculture", *ACIAR Monograph*, 32, 374, 1996
- [9] J.P. Grimme, M.L. Mackey, S.A. Hillier, Read, "Floristic diversity in a model system using experimental microsomes", *Nature*, 328, pp. 420-422, 1987.
- [10] A.C. Gange, V.K. Brown, L.M. Farmer, "A test of mycorrhizal benefit in an early successional plant community", *New Phytol.*, 115, pp. 85-91, 1990.
- [11] I.R. Sanders, R.T. Koide, "Nutrient acquisition and community structure in co-occurring mycotrophic and non-mycotrophic old-field annuals", *Funct. Ecol.*, 8, pp. 77-84, 1994.
- [12] E.J. Joner, C. Levylal, "Uptake of roots by roots and hyphae of *Glomus mosseae*/*Trifolium subterraneum* mycorrhiza from soil amended with high and low concentrations of cadmium", *New Phytol.*, 135, pp. 53-360, 1997.
- [13] A. Jamal, N. Ayub, M. Usman, A.G. Khan, "Arbuscular mycorrhizal fungi enhance zinc and nickel uptake from contaminated soil by soybean and lentil", *Int. J. Phytoremed.*, 4(3), pp. 203-221, 2002.
- [14] J. Beltrano, M. Ruscitti, M.C.Arango, M.Ronco, "Effects of arbuscular mycorrhiza inoculation on plant growth, biological and physiological parameters and mineral nutrition in pepper grown under different salinity and p levels", *Journal of Soil Science and Plant Nutrition*, 13(1), 123-141, 2013.
- [15] E. Rajeshwari, T.K.S. Latha, K. Vanangamudi, K.A. Selvan, R. Narayanan, "Effect of arbuscular mycorrhizal and phosphorus on seedling growth of *Casuarina equisetifolia*", *Indian Phytopathology*, 54(1), pp. 85-87, 2001.
- [16] Rupam, Kapoor, Bhoopander, Giri, Krishan, G. Mukerji, "Mycorrhization of coriander (*Coriandrum sativum* L.) to enhance the concentration and quality of essential oil", *Journal of the Science of Food and Agriculture*, 82(4), pp. 339 – 342, 2002.
- [17] R. Kumar, B.L. Jalali, H. Chand, "Influence of vesicular arbuscular mycorrhizal fungi on growth and nutrient uptake in chickpea", *Journal of Mycology and Plant Pathology*, 32 (1), 11-15, 2002.
- [18] O.K. Hazarika, K.K. Das, L.N. Dubey, "Effect of vesicular arbuscular mycorrhizal fungi inoculation on growth and nutrient uptake of black gram", *Journal of Mycology and Plant Pathology*, 29(2), 201-204, 1999.
- [19] Hemalatha, Selvaraj, Yudhvir, "Association of AM fungi with Indian borage (*Plectranthusamboinicus*)

(Lourspenge) and its influence on growth and biomass production”, Mycorrhiza News, 15, pp. 18-21, 2003.

[20] T. Khaosaad, H. Viehellig, M. Nell, Z. Egalseer, J. Novak, “Arbuscular Mycorrhiza alter the concentration of essential oils in oregano (*origanumsp. Lamiaceae*)”, Mycorrhiza, 16, pp. 443-446, 2006.

[21] D.S. Hayman, “Endomycorrhizae. In Interactions between Nonpathogenic Soil Microorganisms and Plants” Elsevier, Amsterdam, pp. 401-441, 1978.

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