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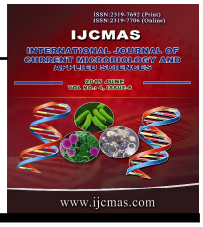

Original Research Article

Biochemical analysis and growth enhancement studies of important medicinal plant, *Rauvolfia serpentina* inoculated with Arbuscular Mycorrhizal fungi in nursery

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A B S T R A C T

Keywords

AM fungi, *Glomus intraradices*, *Glomus macrocarpum*, *Glomus* mosseae, *Rauvolfia serpentina*, *Scutellospora calospora*

Arbuscular Mycorrhizal (AM) fungi offers several benefits to its host which includes faster growth, increased uptake of essential nutrients, improved tolerance to biotic and abiotic stresses, transplantation shock along with resistance to plant pathogens, synergistic interaction with other beneficial soil microbes, beneficial alterations of plant growth regulators and improved soil structure for better aeration and water percolation. In the present study, an attempt was made to screen and select the most efficient AM fungi for growth enhancement of important medicinal plant, *Rauvolfia serpentina* in nursery and it was observed that the AM fungi inoculated plants had better growth and biochemical parameters like chlorophyll content and shoot and root P- uptake over non inoculated control plants. Among different AM fungi tested, *Glomus fasciculatum* had profound effect on the growth as well as the chlorophyll and nutrient content of the plants. It was also recorded that the AM fungi inoculated plants resulted in higher rhizosphere mycorrhizal propagule numbers than control (non inoculated plants).

Introduction

Soil harbours various types of microorganisms and it serves as a source of nutrients and other factors required for the microorganisms. Plants exploit nutrients from soil with the help of beneficial microorganisms such as mycorrhizal fungi, phosphate solubilizing microorganisms etc. Arbuscular Mycorrhizal (AM) fungi are an important component of the terrestrial communities. They are active living components of the soil and have some properties like those of roots and some like those of microorganisms. Mycorrhizal associations may influence both biodiversity (Bever *et al.*, 2002) and biogeochemistry (Hoffland *et al.*, 2004).

The mycorrhizal fungi obtain organic nutrition (carbohydrates, vitamins, amino acids and plant growth substances) from plants and also perfect ecological niche that is necessary for fungal growth and development including the completion of the sexual cycle. They benefit the host by improving soil fertility and by producing enzymes for absorption, translocation and assimilation of major mineral ions like phosphates and inorganic nitrogen, and many number of genes required for symbiosis. Phosphorous (P) is one of the most essential mineral nutrients for plant growth and development as it plays crucial role related to metabolism, energy transfer and various other regulatory functions in plants (Schachtman *et al*., 1998). Alternatively, plants associates in symbiotic relationship with beneficial soil microorganisms, predominantly preferring Arbuscular Mycorrhizal (AM) fungi as a mutualistic partner (Karandashov and Bucher, 2005). Production of nitrogenous fertilizers is energy intensive and fertilizer reserves especially phosphates are becoming limiting. Utilization of inoculants of beneficial microbes, organic manures etc. are given considerable importance. Advantage of mycorrhiza associated plants regarding P uptake is the dual action of two distinct phosphate transporters *i.e*. plant and mycorrhizal P transporters mediating direct and indirect P uptake respectively from the soil, as demonstrated by Karandashov *et al.*, (2004).

About 75 percent of the total population relies on medicinal plants in the remote and rural areas (Dwijiendra, 1999). As a result medicinal plants have been exploited and have reached the level of extinction. In this context, research on various aspects of the cultivation of medicinal plants needs to be studied. In the Indian system of medicine, the whole plant is used irrespective of its active ingredient properties. Hence, the biomass production is of utmost importance in pharmaceutical industries. *Rauvolfia serpentina* is a medium sized shrub belonging to the family Apocynacae. It possesses antispasmodic properties and stimulates the nervous system. It is commonly used in decoction in convulsions and fever or as an external application. It is also used in indigenous medicine as a fumigant in infant catarrh. The plant contains an essential oil, rutin and coumarin like odoriferous principle have also been isolated. Use of AM fungi to enhance the plant growth and biomass production of important medicinal plants is carried out in the present investigation with an objective to select the most efficient AM fungi that can be routinely used for inoculation to enhance the growth of *Rauvolfia serpentina*.

Materials and Methods

Nursery experiment was conducted to investigate the efficacy of different AM fungi on growth improvement of selected economically important medicinal plant, *Rauvolfia serpentina* in glass house, Forest Protection Division, Institute of Forest Genetics and Tree Breeding, Coimbatore, Tamil Nadu.

Potting medium

Potting medium used in the present study was a mixture of solar sterilized sand: soil: farmyard manure in the ratio 1:2:1. Potting medium was analyzed for its physico-chemical parameters such as pH, Electrical Conductivity (EC), available Nitrogen (N) (Jackson, 1973), available Phosphorus (P) (Jackson, 1973), available Potassium (K) (Sankaram, 1966) and micronutrients such as copper (Cu), zinc (Zn), iron (Fe) and manganese (Mn) (Lindsay and Norvell, 1978) following standard procedures. The physico-chemical properties of the potting medium are presented in the (Table 1).

Bio-inoculants (AM fungi)

The Arbuscular Mycorrhizal (AM) fungal bio-inoculants were obtained from the Department of Plant Biotechnology, University of Agricultural Sciences, G.K.V.K, Bangalore. The soil base inoculum of different AM fungi *viz., Glomus fasciculatum, G. intraradices, G. macrocarpum, G. mosseae* and *Scutellospora calospora* at the rate of 12-15 spores per gram of soil were used in the study.

Experimental design

Species and Treatment structure for each species

Medicinal Plant Species:
Rauvolfia serpentina.
Number of Treatments: 6
Number of Replicates/treatment: 3
Number of plants per replicate: 50
Design : CRD

Treatment structure

T1 – Control
T2 – *Glomus fasciculatum*
T3 – *Glomus mosseae*
T4 – *Glomus macrocarpum*
T5 – *Glomus intraradices*
T6 – *Scutellospora calospora*

Transplantation of seedlings in poly bags

The AM fungal bio-inoculants were applied to polythene bags (10 x 20 cm size) already filled with the potting medium up to 5cm below the top surface as per treatment schedule in completely randomized design by layering inoculation technique. On the bio-inoculants layer, the potting medium was again filled up to the surface. Seedlings of uniform size of *R. serpentina* were immediately transplanted into the poly bags. The seedlings were maintained under nursery conditions and watered twice daily for further studies.

Biometric observations

Data on growth parameters such as plant height, number of leaves, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, P-content and chlorophyll content were recorded at different periodical intervals till harvest. The plant height was measured from the soil surface to the tip of the growing point at 30, 60, 90 and 120 days interval. The numbers of fully opened leaves were recorded at 30, 60, 90 and 120 days interval. The plants were harvested at 120 days after treatment (DAT). The girth (mm) was measured 1cm above the soil surface using vernier calipers, at harvest (120 DAT). The leaf area (cm^2) was measured at harvest (120 days) by using a leaf area meter. The fresh weight of the shoots and roots were recorded separately after harvest. The harvested plants were dried in an oven at 60^0C for 8 days to attain constant weight and then the shoot and root dry weights were recorded and expressed as grams per plant. The biochemical parameters namely chlorophyll content (Hiscox and Israelstam, 1979), phosphorus concentration was estimated colorimetrically following the vanadomolybdate yellow colour method (Jackson, 1973).

Determination of persistence of AM fungi

The rhizosphere soil of inoculated and uninoculated (control) plants was subjected to the wet sieving and decanting method (Gerdemann and Nicolson, 1963) for isolation and identification of AM fungal spores. Fungal root colonization was determined by the gridline intersect method as illustrated by Giovannetti and Mosse (1980).

Results and Discussion

AM fungi are ubiquitous and widely distributed in association with all the plants except few. These symbiotic fungi occur on almost all the tropical crop plants including tree species. It is well known that mycorrhizal fungi improve plant growth and owing to these observations, the present investigation was carried out to screen and select the most efficient AM fungi for growth enhancement of important medicinal plant, *R. serpentina*.

Screening of efficient AM fungi for growth improvement of *Rauvolfia serpentina*

Data on the plant height as influenced by inoculation of 5 different AM fungi at 30, 60, 90 and 120 days after transplanting (DAT) are given in Table 2. The plant height increased steadily from the day of planting till harvest (120 DAT) in all the treatments. At all stages maximum plant height was observed in AM fungi inoculated plants as compared to uninoculated (control) plants. Maximum plant height was (35.97 cm) recorded in 120 DAT in the plants inoculated with *G. fasciculatum*. However, it had significantly greater plant height from plants inoculated with other AM fungi *viz.*, *G. macrocarpum*, *G. intraradices*, *G. mosseae* and *S. calospora*. The least plant height was recorded in uninoculated (control) plants (10.63 cm) in 120 DAT. Data on stem girth, leaf area, number of leaves were recorded on the 120 days after transplanting (DAT) are given in Table 3. It was recorded that the stem girth was more (0.50 mm) in plants inoculated with *G. fasciculatum*. It was followed by the plants inoculated with *G. mosseae* (0.43mm) and *S. calospora* (0.41mm), but among them they were at par. Maximum leaf area (90.67 cm^2) was recorded in the plants inoculated with *G. fasciculatum* which was statistically on par with the plants treated with *G. mosseae*. Leaf area was recorded least (10.05 cm^2) in control plants which were on par with plants inoculated with *G. intraradices* (12.09 cm^2). The highest number of leaves was recorded in the plants inoculated with *G. fasciculatum*. The plants inoculated with *S. calospora* and *G. mosseae* also had more number of leaves when compared with uninoculated (control) plants (4.05). In general, AM fungi inoculated plants were significantly taller and had more number of leaves, stem girth and leaf area compared to the uninoculated plants. Similar results were observed by Ambika *et al.* (1994). They reported mulberry saplings treated with AM fungi (*Glomus fasciculatum* and *Glomus mosseae*) were taller with more number of leaves and increased leaf area.

Plant biomass

Significant differences were observed in fresh and dry weights between inoculated and uninoculated plants (Table 4). The plants inoculated with *G. fasciculatum* recorded maximum fresh weight (40.01g) when compared to uninoculated plants (2.43g). Plants inoculated with *G. fasciculatum* recorded maximum shoot dry weight (7.99g). Inoculation with *S. calospora* and *G. mosseae* also had statistically similar shoot fresh and dry weights at final harvest. The plants inoculated with *G. fasciculatum* recorded maximum root fresh weight (11.23g) and dry weight (1.09g) followed by *S. calospora* and *G. mosseae*. However, plants inoculated with *S. calospora* and *G. mosseae* were at par with each other and least root fresh weight (1.70g) and dry weight (0.29g) was recorded in uninoculated plants.

The plants inoculated with *G. fasciculatum* recorded maximum total fresh weight

(51.24g) and total dry weight (9.08g) when compared to uninoculated plants (0.51g). This finding corroborates with the findings made by different researchers on AM fungal association with agricultural crops, plantation crops, forestry crops (Mohan and Neelam Babbar, 1997; Mohan *et al*., 1997, 2007; Tamil Selvi *et al*., 2010) and medicinal plants (Mohan *et al.,* 2005; Sundar *et al.,* 2008, 2011).

Mycorrhizal parameters

Data on percent root colonization of AM fungi is given in Table 5. It was observed that maximum percent root colonization (85.22%) was recorded in plants inoculated with *G. fasciculatum*. This is followed by plants inoculated with *S. calospora* (66.08%). The percent root colonization was found least (19.72%) in uninoculated (control) plants. Data on mycorrhizal spore number in the rhizosphere soil as influenced by inoculation with AM fungi is given in Table 5. There were significant differences among treatments. The plants treated with *G. fasciculatum* had highest spore count (98.72/50 g soil) in the rhizosphere soil at 120 DAT and final harvest of seedlings. This was followed by plants inoculated with, *S. calospora* and *G. mosseae* both being statistically on par with each other. Uninoculated (control) plants had the least spore count (31/50 g soil). These results are at one with the observations made by Edathil *et al.* (1994) who noticed higher root colonization percentage and spore count in rhizosphere soil of tomato, brinjal and chilli plants inoculated with AM fungi.

Table.1 Physico-chemical properties of potting medium used in the nursery experiments

Texture	pH	E.C. (dS m^{-1})	Macro Nutrients (kg ha^{-1})			Micro Nutrients (ppm)			
			N	P	K	Cu	Zn	Fe	Mn
Sandy loam	7.3	0.14	180	19	225	1.8	1.54	10.8	11.08

Table.2 Effect of different AM fungi on plant height of *Rauvolfia serpentina* seedlings in glass house

Treatments	Plant height (cms)			
	30 DAT	60 DAT	90 DAT	120 DAT
T_1 - Control	5.85^{e}	8.00^{c}	10.26^{c}	10.63^{e}
T_2 - *Glomus fasciculatum*	14.66^{a}	22.16^{a}	24.27^{b}	35.97^{a}
T_3 - *Glomus mosseae*	8.01^{d}	9.58^{c}	11.23^{c}	26.96^{c}
T_4 - *Glomus macrocarpum*	9.40^{c}	12.20^{b}	9.25^{c}	12.98^{d}
T_5 - *Glomus intraradices*	5.66^{e}	7.01^{c}	26.26^{b}	11.96^{d}
T_6 - *Scutellospora calospora*	10.89^{b}	19.12^{b}	31.06^{a}	32.03^{b}

Note:

DAT: Days after Transplanting

Means in the same column followed by the same superscript do not differ significantly according to Duncan's Multiple Range Test ($P < 0.05$)

Table.3 Effect of different AM fungi on stem girth, leaf area and number of leaves of *Rauvolfia serpentina* seedlings in glass house

Treatments	120 DAT		
	Stem girth (mm)	Number of leaves	Leaf area (cm^2)
T_1 - Control	0.20^{cd}	4.05^{d}	10.05^{d}
T_2 - *Glomus fasciculatum*	0.50^{a}	11.75^{a}	90.67^{a}
T_3 - *Glomus mosseae*	0.43^{b}	7.65^{b}	89.99^{a}
T_4 - *Glomus macrocarpum*	0.31^{c}	6.70^{c}	13.82^{c}
T_5 - *Glomus intraradices*	0.30^{c}	6.00^{d}	12.09^{d}
T_6 - *Scutellospora calospora*	0.41^{b}	8.07^{b}	57.96^{b}

Note:
DAT: Days after Transplanting
Means in the same column followed by the same superscript do not differ significantly according to Duncan's Multiple Range Test ($P < 0.05$)

Table.4 Effect of different AM fungi on plant biomass of *Rauvolfia serpentina* seedlings (120 DAT) in glass house

Treatments	Fresh and Dry Weight (g/plant)					
	Shoot		Root		Total	
	Fresh weight	Dry weight	Fresh weight	Dry weight	Fresh weight	Dry weight
T_1 - Control	2.43^{d}	0.22^{d}	1.70^{d}	0.29^{d}	4.13^{d}	0.51^{d}
T_2 - *Glomus fasciculatum*	40.01^{a}	7.99^{a}	11.23^{a}	1.09^{a}	51.24^{a}	9.08^{a}
T_3 - *Glomus mosseae*	35.10^{b}	4.56^{b}	7.55^{b}	0.99^{b}	42.65^{b}	5.55^{b}
T_4 - *Glomus macrocarpum*	12.21^{c}	0.41^{c}	2.59^{c}	0.37^{c}	14.80^{c}	0.78^{c}
T_5 - *Glomus intraradices*	2.87^{d}	0.39^{c}	2.57^{c}	0.37^{c}	5.44^{c}	0.76^{c}
T_6 - *Scutellospora calospora*	37.15^{b}	4.62^{b}	7.92^{b}	0.98^{b}	45.07^{b}	5.60^{b}

Note:
DAT: Days after Transplanting
Means in the same column followed by the same superscript do not differ significantly according to Duncan's Multiple Range Test ($P < 0.05$)

Table.5 Influence of different AM fungi on percent root colonization and soil spore population of AM fungi in the rhizosphere of *Rauvolfia serpentina* seedlings in glass house

Treatments	Mycorrhizal colonization (%)	Spore numbers (Numbers/50g soil)
T_1 - Control	19.72^{e}	31.0^{e}
T_2 - *Glomus fasciculatum*	85.22^{a}	98.72^{a}
T_3 - *Glomus mosseae*	59.48^{c}	59.06^{c}
T_4 - *Glomus macrocarpum*	48.85^{d}	50.51^{cd}
T_5 - *Glomus intraradices*	40.51^{cd}	47.0^{d}
T_6 - *Scutellospora calospora*	66.08^{b}	74.51^{b}

Note:
Means in the same column followed by the same superscript do not differ significantly according to Duncan's Multiple Range Test ($P < 0.05$)

Table.6 Effect of different AM fungi on chlorophyll content and P-content of *Rauvolfia serpentina* seedlings in glass house

Treatment	Chlorophyll Content (mg/g fresh weight)	P-content (mg/plant)		
		Shoot	Root	Total
T_1 - Control	1.11^{e}	0.20^{def}	0.20^{de}	0.40^{e}
T_2 - *Glomus fasciculatum*	3.09^{a}	12.14^{a}	2.81^{a}	14.95^{a}
T_3 - *Glomus mosseae*	2.76^{b}	4.41^{c}	2.42^{b}	6.83^{c}
T_4 - *Glomus macrocarpum*	1.98^{c}	5.26^{bc}	0.80^{bc}	6.06^{d}
T_5 - *Glomus intraradices*	1.40^{d}	0.30^{da}	0.26^{cd}	0.56^{e}
T_6 - *Scutellospora calospora*	2.77^{b}	7.14^{b}	2.30^{b}	9.44^{b}

Note:
Means in the same column followed by the same superscript do not differ significantly according to Duncan's Multiple Range Test ($P < 0.05$)

Total chlorophyll content

Table 6 shows the total chlorophyll content in the leaves of *R. serpentina* as influenced with different AM fungal inoculation. Maximum total chlorophyll content was observed in the leaves of plants inoculated with *G. fasciculatum* (3.09 mg/g fresh weight), followed by the plants inoculated with *S. calospora* (2.77 mg/g fresh weight) and *G. mosseae* (2.76 mg/g fresh weight) but they are statistically on par with each other. The least total chlorophyll content (1.11 mg/g fresh weight) was estimated in the leaves of uninoculated (control) plants. This enrichment in the chlorophyll content of the AM fungi (*G. fasciculatum*) treated plants may be due to an increase in stomatal conductance, photosynthesis, transpiration and enhanced plant growth (Rajasekaran *et al*., 2006). Yet another reason for the higher chlorophyll content in the treated plants may be due to the fact that AM fungi has been reported to enhance Zinc (Zn) uptake in the host plants (Gao *et al*., 2007; Subramanian *et al*., 2009) which may result in the increase in chlorophyll content of AM plants as Zn is known to promote the development of photosynthetic pigments (Misra *et al*., 2000).

Phosphorous content

Plants inoculated with different AM fungi showed significant Phosphorous content when compared to control (Table 6). Plants inoculated with *G. fasciculatum* recorded maximum P content in shoot (12.14 mg/plant) as well as in root (2.81 mg/plant). Uninoculated (control) plants showed the least P content in shoot (0.20 mg/plant) as well as in root (0.20 mg/plant). Total P content was maximum (14.95 mg/plant) in the plants inoculated with *G. fasciculatum* followed by *S. calospora* (9.44 mg/plant) and *G. mosseae* (6.83 mg/plant). Less (0.40 mg/plant) total P content was observed in uninoculated (control) plants, which was on par with the plants inoculated with *Glomus intraradices* (Table 6). One of the most important attributes of arbuscular mycorrhizal (AM) fungi is to facilititate improved nutrient uptake in the host plant, especially phosphorus (Declerk *et al*., 1995) which is considered responsible for accelerated plant growth. Results obtained in the present study upholds with the above mentioned statement.

In the present investigation, all AM fungi inoculated medicinally important R. serpentine plants had better growth and bio-chemical parameters like chlorophyll and P contents. Among 5 different AM fungi screened, *G. fasciculatum* inoculated plants had maximum plant height, fresh and dry weights of shoot and roots, chlorophyll and P contents as compared to other AM fungi and uninoculated (control) plants. More in depth studies are need to be undertaken to determine the out planting performance of AM inoculated plants in field condition and also to estimate the amount of secondary metabolites present in the plant parts.

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