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Studies on Influence of Arbuscular Mycorrhiza and Some Plant Growth Promoting Rhizomicroorganisms on Rauvolfia Serpentina

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Abstract:

Rauvolfia serpentine (Linn.) Benth. ex Kurz (Indian serpentwood), belonging to the family Apocynaceae, and popularly known as India's wonder drug plant. It is an upright, perennating, evergreen undershrub with tuberous roots. Its dried root is the economical part which contains a number of alkaloids and some important secondary metabolites. The present study was conducted to determine the effect of arbuscular mycorrhizal fungi (Glomus fasciculatum) and some plant growth promoting rhizomicroorganisms (PGPR,s) on Rauvolfia serpentine plants under open pot conditions. Different plant growth parameters viz plant height, number of leaves, fresh weight, dry weight, mycorrhizal parameters were recorded. The results of this experiment clearly indicated that Glomus fasciculatum and plant growth promoting rhizomicroorganisms enhance the growth, biomass production in Rauvolfia serpentina plant. However future studies can be done for standardizing with different combinations of arbuscular mycorrhizae fungi and PGPRs.

Key Words: *Rauvolfia serpentine, Glomus fasciculatum, PGPR,s, secondary metabolites.*

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Introduction

The term mycorrhiza is defined as a symbiotic association of a fungus and the roots of a plant. Frank (1885) defined mycorrhiza as symbiotic associations between fungi and roots that are not pathogenic. These symbiotic plant-fungus associations were required for the nutrition of both partners. Mycorrhiza form mycelium and penetrate it in between cells and inside the cells.

***Rauvolfia serpantina*:**

Common Name:

English:	Rauvolfia /Indian snake root
Hindi:	Chandrabhaga, Sarpagandha
Kannada:	Keramaddinagaddi

Classification:

Kingdom:	Plantae
Division:	Magnoliophyta
Class:	Magnoliopsida
Order:	Gentianales
Family:	Apocynaceae
Genus:	*Rauvolfia*
Spicies:	*Rauvolfia serpentina*

Description of the Plant:

Rauwolfia serpentina is a medicinally important herb (Salma *et al*., 2008). It is commonly known as Sarpagandha, Chandrabagha, Snake root plant, Chotachand, Chandrika and Harkaya etc (Mallick *et al*., 2012). The plant is growing under varying edaphoclimatic conditions in the humid tropics of India, Nepal, Burma, Thailand, Bangladesh, Indonesia, Cambodia, Philippines and Sri Lanka. In India, it is cultivated in the states of Uttar Pradesh, Bihar, Tamil Nadu, Orissa, Kerala, Assam, West Bengal and Madhya Pradesh (Dutta and Virmani, 1964).

Medicinal Properties of Plant:

Mainly the roots of the plant are used for various ailments like insomnia, hypertension, insanity, epilepsy, intestinal disorders, cardiac and liver diseases, hysteria, constipation and schizophrenia. It is also anthelmintic, a tranquilizer and an antidote against the bites of snakes and venomous reptiles (Mukerji, 1984; Anonymous, 1999; Wilkins RW and Judson, 1953)

The AM Fungi and PGPR's:

Using of microorganisms for enhancing plant growth has been carried out from ancient times even before the discovery of microbes and microscope (Bhattacharyya and Jha, 2012). Arbuscular mycorrhizal (AM) fungi and Plant growth-promoting rhizobacteria (PGPR) are able to improve plant growth, uptake of nutrient, and phytochemical constituents, protect plants against various soil borne pathogens, and increase plants tolerance to a number of environmental stresses (Jeffries *et al*., 2003; Egamberdieva and Lugtenberg, 2014; Hameed *et al.*, 2014).

Materials and methods:

The study was conducted to know response of *Rauvolfia serpentine* plant to *Glomus fasciculatum* species of AM fungi and some plant growth promoting microorganisms. The investigation was carried out at the Department of Biotechnology, Bangalore University, Bangalore. The initial inoculum for the AM fungi production was obtained from Agricultural University, G.K.V.K, Bangalore. These fungi maintained in Pot culture containing sterilized soil and sand (2:1) and using Rhodes grass (*Chloris gayana*) as a host (sreenivasa and bagyaraj, 1988). The beneficial PGPR used to inoculate along with *Glomus fasciculatum* were *pseudomonas fluorescens, Azotobacter chroocccum* and *Tricoderma harzianum.* They were inoculated on nutrient broth medium, Waksman No.77 broth and potato dextrose broth. They were maintained for 3 days in case of bacteria and 7 days in case of fungi on their respective broth media.

Planting material for the presented study was collected from I-AIM (Institute of Ayurveda and Integrative Medicine) Yelahanka, Bangalore, Karnataka. The studies were done on *Rauvolfia serpentine* plants. The plants were treated with inoculation treatments of *Glomus fasciculatum*, *Azotobacter chroococcum*, *Pseudomonas fluorescens* and *Trichoderma harzianum* for 60, 120 days respectively. The substrate with extrametrical hyphae, spores and infected root bits of Rhodes grass from pot culture were used as mycorrhizal inoculum. There were altogether six treatments combination with three replications for each period of times. Irrigation was given twice a week for first

four weeks and subsequently at weekly intervals to maintain enough moisture for growth of plants.

The observations with respect to the physical growth parameters including plant height, number of branches, number of leaves, stem girth, leaf area, number of flower, number of fruit, number of seeds and etc. were done at different periodical intervals till harvest. Postharvest observation were recorded with the following parameters: plant fresh weight, plant dry weight, soil ph, % of root colonization of & spore count per 50gm of soil and etc.

The root zone soil samples were used for the estimation of AMF spore numbers. Mycorrhizal spores were obtained by wet sieving and decanting technique. (Gerdemann and Nicolson, 1963). The root colonization study was done to calculate percentage root infection in roots plants of *Rauvolfia seerpentina* with different treatments. AMF colonization was estimated by microscopically examination at 10X, 40X magnification, after clearing of roots in 10% KOH and staining with 0.05% trypan blue in lactophenol (Philips and Hayman, 1970). Percentage of root colonization (%) was measured by gridline intersect method (Giovannetti and Mosesse, 1980). The per cent infection was calculated by the following formula:

% root colonization = No. of segment colonized by VAM x100/Total number of segment

Results and Discussion:

The data in Table 1 and Table 2 shows that the AM fungi and PGPRs had a significant effect on plant height, number of leaves, fresh weight, dry weight, leaf area, and plant spread of *Rauvolfia serpentina* plant.

In 60 days of treatment plant height was highest in *Gf+Pf+Ac+Th* treatment with (32.2±2.97cm) which was followed by *Gf+Ac* (30.43±4.07cm), *Gf+Pf* (27.96±7.91cm), *Glomus fasciculatum* (26.33±4.84cm) and *Gf+Th* (24.83±5.90cm) treatments. The lowest plant height was recorded for control with 21.76±5.30cm. Number of leaves was more in *Gf+Pf+Ac+Th* treatment (7.66±3.05). The control plant had minimum number of leaves (4.66±1.15).

Fresh weight was maximum in plants treated with *Gf+Ac* (22.51±2.13g) and it was followed by *Gf+Pf* (21.38±7.57g), *Gf+Pf+Ac+Th* (18.12±9.13g), *Gf+Th* (17.25±4.76g), *Glomus fasciculatum* (15.95±3.92g) and Control plants (11.94±2.48g). Dry weight was highest in *Gf+Ac* treatment with (4.71±3.28g) and the lowest dry weight belongs to the control plants (1.94±0.41g).

Leaf area was maximum in *Gf+Pf+Ac+Th* treated plants (25.86±4.30cm^2) which was followed by *Gf+Th* (24.93±3.86cm^2), *Glomus fasciculatum* (23.5±6.48 cm^2), *Gf+Pf* treated plants (23.26±5.91 cm^2) and control plants(22.53±4.70 cm^2). Plant spread was recorded maximum in *Gf+Ac* treatment with (15.26±4.30 cm^2) and it was recorded for *Gf+Pf* (14.83±2.722744 cm^2), *Gf+Pf+Ac+Th* (14.8±2.426932 cm^2), *Gf+Th* (13.86±1.20 cm^2), Control plants (12.1±2.68 cm^2) and *Glomus fasciculatum* (11.33±3.71 cm^2).

In 120 days plant height was maximum in *Gf+Pf+Ac+Th* treatment (39.03±8.07cm) and *Gf+Ac* had (38.66±14.35cm) of plant height and control plant had minimum value (28.16±7.40cm). *Gf+Ac* treatment had highest number of leaves with (9.66±6.02). Number of leaves for *Glomus fasciculatum* (9.33±3.21), *Gf+Pf+Ac+Th* (8.66±3.05), control plant (6±2) was recorded.

In recording of Fresh weight, *Gf+Ac* treatment had maximum fresh weight (26.59±11.06g) which was followed by *Gf+Pf* (24.91±3.34g), *Gf+Pf+Ac+Th* (23.21±3.35g), *Gf+Th* (20.64±8.36g), *Glomus fasciculatum* (18.49±6.12g) and Control (14.75±9.26g). Highest and lowest dry weight was recorded for *Gf+Ac* treated plants (6.97±2.63g) and control plants (2.15±1.71g).

Leaf area was maximum in *Gf+Pf+Ac+Th* (28.8±6.35cm^2) and *Gf+Ac* had (25.33±6.54 cm^2) of leaf area. Control had minimum of leaf area (24.1±5.25cm^2). Highest plant spread was recorded for *Gf+Pf+Ac+Th* treatment (17.76±4.20cm^2) which was followed by *Gf+Ac* (17.4±3.31cm^2), *Gf+Pf* (16.53±8.20cm^2), *Gf+Th* (14.03±2.36cm^2), *Glomus fasciculatum* (12.93±1.32 cm^2) and Control (12.73±5.32cm^2).

Table 1: Physical parameter of *R.Serpentina* plant after treatment with *Glomus fasciculatum* and PGPR for 60 days

T. No	Treatment	Plant height	Number of leaves	Fresh weight	Dry weight	Leaf area	Plant spread
T1	Control	21.76± 5.30	4.66± 1.15	11.94± 2.48	1.94± 0.41	22.53± 4.70	12.1± 2.68
T2	*Glomus fasciculatum*	26.33± 4.84	7± 3	15.95± 3.92	3.38± 2.01	23.5± 6.48	11.33± 3.71
T3	*Gf+Pf*	27.96± 7.91	7.33± 1.52	21.38± 7.57	3.17± 1.09	23.26± 5.91	14.83± 2.72
T4	*Gf+Ac*	30.43± 4.07	6.33± 2.51	22.51± 2.13	4.71± 3.28	22.46± 3.64	15.26± 4.30
T5	*Gf+Th*	24.83± 5.90	5±2	17.25± 4.76	2.49± 0.83	24.93± 3.86	13.86± 1.20
T6	*Gf+Pf+Ac+Th*	32.2± 2.97	7.66± 3.05	18.12± 9.13	4.38± 1.45	25.86± 4.30	14.8± 2.42

Note: Values are mean of triplicates

Table 2: Physical parameter of *R.Serpentina* plant after treatment with *Glomus fasciculatum* and PGPR for 120 days

T. No	Treatment	Plant height	Number of leaves	Fresh weight	Dry weight	Leaf area	Plant spread
T1	Control	28.16± 7.40	6± 2	14.75± 9.26	2.15± 1.71	24.1± 5.25	12.73± 5.32
T2	*Glomus fasciculatum*	35.16± 3.97	9.33± 3.21	18.49± 6.12	3.93± 1.16	25.73± 5.25	12.93± 1.32
T3	*Gf+Pf*	33.4± 3.45	8.33± 2.51	24.91± 3.34	5.77± 0.40	26.13± 6.15	16.53± 8.20
T4	***Gf+Ac***	**38.66± 14.35**	**9.66± 6.02**	**26.59± 11.06**	**6.97± 2.63**	**25.33± 6.54**	**17.4± 3.31**
T5	*Gf+Th*	32.8± 5.78	7.33± 4.61	20.64± 8.36	4.61± 0.87	28.66± 7.83	14.03± 2.36
T6	*Gf+Pf+Ac+Th*	39.03± 8.07	8.66± 3.05	23.21± 3.35	4.35± 2.10	28.8± 6.35	17.76± 4.20

Note: Values are mean of triplicate

Figure 1: *R.Serpentina* plant after treatment with *Glomus fasciculatum* and PGPR for 120 day

Table 3: %root colonization and number of spore/50g of soil in *Rauvolfia serpentina*

T.No	Treatment	% root colonization	No.Spore / 50g
T1	Control	0	0
T2	*Gf*	66	383.33
T3	*Gf+Pf*	70	261
T4	*Gf+Ac*	74	429.33
T5	*Gf+Th*	54	354.66

T6	*Gf+Pf+Ac+Th*	86	463

The percentage root infection was found to be highest in *Gf+Pf+Ac+Th* (Table 3). Root colonization studies were done to calculate the percentage root infection in plant. The number of spores/50g of soil was also found to be maximum in *Gf+Pf+Ac+Th* treatment.

Inoculation of *Rauvolfia serpentina* with microbial consortium and *Glomus fasciculatum* has shown increase in physical growth parameter and biomass production. The shoot and root growth of *Calendula officinalis* were stimulated by PGPR strains Azotobacter, Azospirillum, Pseudomonas, and AM fungi (Hosseinzadah *et al.*, 2011). AM and plant growth promoting microorganisms enhance plant growth directly by either facilitating resource acquisition like phosphorus, nitrogen, zinc, iron or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the form of biocontrol agents (Glick, 2012).

The markets for medicinal plants, aromatic plants, and organic foods are increasing (Adam, 2005; Hartman Group, 2006) and consumers become more concerned and knowledgeable about their own health and wellness so as result there is increasing demand for quality of plant material, produced by sustainable methods and uncontaminated by synthetic pesticides or genetically modified organisms (Craker, 2007).

The inoculation of Arbuscular mycorrhizae and plant growth promoting microorganisms is a sustainable technology to increase the quantity and quality of the medicinal plant. However, selecting and inoculating most specific and efficient AM fungi and PGPR species for a particular plant are essential for the cultivation of medicinal plants. Therefore, further research is recommended to better understand the diversity and function of bacteria or fungi and their uses to enhance production of medicinal plants by identifying relationship between genetic and functional diversity of bacteria or fungal species.

Conflict of interest statement:

Authors declare that they have no conflict of interest.

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