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Influence of Arbuscular Mycorrhizae Inoculation on Growth and Development of *Hibiscus rosa sinensis*

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A B S T R A C T

Hibiscus rosa sinensis is an evergreen herbaceous plant known to possess medicinal properties like oral contraceptive, laxative, aphrodisiac, menorrhagic etc. In the current study influence of Arbuscular Mycorrhiza (*Glomus mosseae*) inoculation on *Hibicus rosa sinensis* was studied for 60 days in open potted experiments. Physical growth parameters viz shoot length, root length, fresh weight, and dry weight of plants were studied. Macro and micronutrients (N, P, K, Ca, Mg, Zn, Fe, Cu, Mn, B and Mo) were analyzed in roots and leaves of plant. Root colonization study was done and percentage root infection was calculated. *Glomus mosseae* inoculated plants revealed more growth with nearby 60% increase in shoot length and 33% increase in number of leaves.The concentration of macronutrients N, P, K and Zn was also found more in GM plants. GM treatment was found to be successful for *Hibiscus rosa sinensis* plant and hence can be used as an ecofriendly measure for mass multiplication of *Hibiscus rosa sinensis*. However effect of other AM species in combination with phosphate solubilizers on *Hibiscus rosa sinensis* can also be studied in future.

Introduction

The term Mycorrhiza was coined by A.B. Frank in 1885 (Frank, 1885). Mycorrhiza is the mutualistic symbiotic association between soil borne fungi and roots of higher plants in which both are benefitted (Sieverding *et al.,* 1991; Sayeeda *et al.,* 2013). Arbuscular mycorrhiza is the mutualistic symbiotic association between most vascular land plant species and fungi of phylum Glomeromycota (Smith and Read, 2008; Anju *et al.,* 2011).

AM fungus penetrates the cortical cells of roots of higher plants and forms vesicles and arbuscules. Arbuscules are structures produced within the host plant cells by the AM fungi. These structures are responsible for the transfer of absorbed nutrients from the fungus to the plant (Sandeepa, 2013). AM fungi improve plant growth by capturing relatively immobile nutrients like phosphorus (Souchie *et al.,* 2006),other macroelements (Hodge *et al.,* 2001) and some microelements (Faber *et al.,* 1990; Anju *et al.,* 2011).

Glomus mosseae is a representative species of endogonaceae (Phycomycetes) able to form AM.The microorganism synthesizes two gibberellins like substances (Jose M Barea,1982) *Glomus mosseae* can transport a considerable amount of phosphate and nitrogen to the plant from soil zones several centimeters from root (Eckhard, 1992). Amongst single inoculation treatment, *G. mosseae* increased maximum growth parameters, over the control in *Capsicum annum* (Anju *et al.,* 2011). Metal accumulation changes in Cannabis sativa (L) with *Glomus mosseae* interaction is also reported (Sandra *et al.,* 2005)

Hibiscus rosa sinensis is an evergreen herbaceous plant, which belongs to family Malvaceae and is commonly known as Jasvand.It is an evergreen woody glabrous showy shrub 5- 8 feet in height (Kirtikar *et al.,* 1987).The plant is native to China and is also found in India and Philippines. It is widely cultivated in the tropics as an ornamental plant and has several forms with varying colours of flowers (Bhaskar A, 2011).Different parts of plants viz roots, stems, leaves and flowers are medicinally important and have been known to possess medicinal properties. Flowers are used in all kinds of inflammation, internally they are prescribed in form of decoction of bronchial catarrah,as a becenic acid and subdorific roots are mucilaginous and demulcent, valuable in cough (Caius J F,1992 ; Satyavati *et al.*, 1997).Root powder of plant is useful in mennorhagia and root juice in gonorrhea whereas leaves are used for fatigue and skin diseases. (Wealth of India, 1959). Literature supports antifertility activity in the plant (Batta S K *et al.,* 1970). *Hibiscus rosa sinensis* plant has shown mycorrhizal association (Muthukumar *et al.,* 2006 ; Radhika,2010) Jonathan N have studied the effect of Glomus intradices on *Hibiscus rosa sinensis* (Jonathan ,1998).Macro and micronutrient analysis in leaves of *Hibiscus rosa sinensis* for drought conditions has also been studied (Egilla et al.2001)

The present study was conducted with the objective of studying the influence of *Glomus mosseae* inoculation on *Hibiscus rosa sinensis* on physical growth parameters and macro and micronutrient analysis viz N, P, K, Ca, Mg, Zn, Fe, Cu, Mn, B and Mo in leaves and roots of the plant.

Materials and Methods

Experimental Design

Experiments were carried out as open pot experiments in plastic pots for 60 days through random block design method at the Medicinal plant garden of Department of Microbiology and Biotechnology , Bangalore University, Jnana Bharthi Campus , Bangalore. The temperature varied from 32 °C - 17 °C during the experiment.

Soil Characteristics

The soil and sand sample were collected from nurseries near to Bangalore University.Soil is red clay soil with pH value 8.1(Jackson, 1973). Soil and sand samples were mixed in 1:1 ratio and farmyard manure was added and the mixture was autoclaved at 15lbs for 20 mins for two consecutive days. Plastic pots were taken and sterilized.3 kg of soil per pot was added.

Plant Sample and *Glomus mosseae* Treatments

One month old plant seedlings of *Hibiscus rosa sinensis* were collected from

Sanjeevani vatika, Department of Horticulture, University of Agriculture Sciences, Gandhi krishi vignan kendra, Bangalore. *Glomus mosseae* spores were procured from Department of Microbiology, University of Agriculture Sciences, Gandhi krishi vignan kendra, Bangalore. *Glomus mosseae* inoculums were prepared as per protocol given by Menge and Timmer (1982). Pot based cultures were done and root based inoculums were used for further treatments. Plant seedlings were inoculated for 60 days with *Glomus mosseae* culture. The plants were watered on alternate days.

Plant Harvesting and Measurement of Physical Growth Parameters

Plants were harvested after 60 days of inoculation and physical growth parameters of plants viz root length, shoot length, fresh weight, dry weight and numbers of leaves were studied. Shoot length (cm) and number of leaves were first measured in the standing plant. After harvesting fresh weight and root length of plant were noted . The sample was then oven dried at 80°C for 72 hrs till a constant weight is achieved and later dry weight of the plants were recorded.

Macro and Micro Nutrient Analysis

Fresh leaves and root samples were oven dried by maintaining the samples in oven at 80 ˚C for 72 hrs till a constant weight is achieved.The dried samples were then crushed in powder form using mortar and pestle and later in mixer grinder to achieve powder form. Macro and micro nutrient analysis for leaf and roots samples of control and *Glomus mosseae* inoculated plants were done by emission spectrometry by using ICP-OES technique (Matthew S et al ,2011 ; Momen A et al ,2007) with the help of ICP-OES 7000 DV (Make –Perkin Elmer) The different Macro and Micronutrients analyzed were Nitrogen (N) %, Phosphorus (P O)%, Potassium (K_2O) %, Calcium (Ca) %,Magnesium (Mg) %, Zinc (Zn) ppm, Iron (Fe) ppm, Copper (Cu) ppm, Manganese (Mn) ppm Boron (B) ppm and Molybdenum (Mo).

Root Colonization Study

Root Colonization was studied by 'Rapid clearing and Staining technique' (Philips and Hayman, 1970). 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 (%) was measured by gridline intersect method (Giovannetti and Mosesse, 1980). A total of 50 root fragments were studied.

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

Results and Discussion

The effect of inoculation of *Glomus mosseae* on growth and development of *Hibiscus rosa- sinensis* was studied (Table: 1).Change in shoot length was significant with 60% more in GM inoculated (42.67 ± 6.17) than uninoculated control plants (29.29 ± 1.819) (Table 1). The root length in GM inoculated plant was found to be 61.9% more in GM inoculated (30.87 ± 5.906) and control (19.06 ± 5.178) (Fig : 1).The number of leaves was almost double in GM leaves (19.5 ± 17) than in control leaves (14 ± 4) (Table :1).Similarly fresh weight and dry weight was also found to be significantly more in GM inoculated with fresh weight (14.24 ± 6.139) and dry weight (3.24 ± 0.46).67% root colonization was found in GM roots.

Table.1 Studies on Physical Parameters of Growth in *Hibiscus rosa sinensis* after 60 Days*

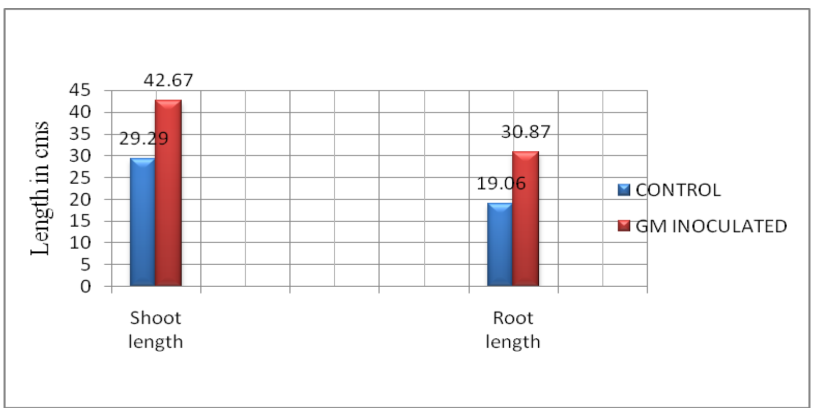
60 Days	Control (Uninoculated)	GM inoculated
Shoot length(cm)	29.29 ± 1.819	42.67 ± 6.17
Root length(cm)	19.06 ± 5.1786	30.87 ± 5.906
Number of leaves	14 ± 4	19.5 ± 17
Fresh weight(g)	11.038 ± 6.29	14.24 ± 6.139
Dry weight(g)	2.85 ± 0.36	3.24 ± 0.46

*Values are taken as mean of triplicates

Table.2 Chemical Analysis in Roots and Leaves of Control Uninoculated and GM Inoculated Plant of *Hibiscus rosa- sinensis* for 60 days

Chemical	Control leaves	GM inoculated leaves	Control root	GM inoculated root
Nitrogen (%)	1.47	1.87	1.58	1.72
Phosphorus (%)	1.12	1.45	0.67	0.75
Potassium (%)	2.4	2.5	2.65	2.82
Calcium (%)	2.53	2.73	0.57	0.86
Magnesium (%)	0.89	0.93	0.57	0.53
Zinc (ppm)	13.12	17.54	53.19	110.1
Iron (ppm)	639.4	583.2	6740	9631
Copper (ppm)	50.64	19.18	75.05	468.9
Manganese (ppm)	26.49	31.94	55.09	98.71
Boron (ppm)	80.59	63.74	47.48	49.37
Molybdenum(ppm)	12.61	9.42	169.4	249.5

Fig.1 Shoot Length and Root Length for 60 Days GM Inoculated and Control Uninoculated Plants of *Hibiscus rosa sinensis*



A trend similar to plant growth was observed for nitrogen, phosphorus and potassium when calculated for leaves and roots (Table: 2).The total N percentage in GM treated leaves (1.87 %) was significantly higher than in control leaves

(1.47 %) and the trend was similar for roots with N being 1.72% in GM roots and control roots 1.52%.The trend continued for phosphorus and potassium and P percentage in root revealed difference with 0.75% P in GM roots and0.67% in control roots but leaves show a difference comparable as 1.45% in GM leaves and 1.12% in control leaves. Potassium was noticed to be 2.82% in GM treated roots with 2.65% in control roots and 2.5% in GM leaves and 2.4% in control leaves. Similar trend was observed for calcium with the nutrient concentration being more in AM inoculated leaves and roots with 2.73% Ca in GM leaves and 0.86% Ca in GM roots (Table : 2).Some elements showed a controversial result like Magnesium having more percentage 0.93% Mg in GM leaves and 0.89% Mg in control leaves,the roots showed a difference with 0.53% Mg in GM roots which is less compared to 0.57% Mg in control roots.Zinc behaved in same manner with concentration in inoculated GM roots being 110.1ppm and control roots 53.19 ppm. Fe, Cu and Mo had very high concentration in roots whereas the concentration was significantly less in leaves. The Fe concentration was found to be 9631 ppm in GM roots and and 6740 ppm in control roots whereas 583.2 ppm Fe was found in GM leaves which was less than control leaves with 639.4 ppm Fe concentration. The concentration of Cu was almost four times in GM roots with 468.9 ppm Cu concentration and control having 75.05 ppm concentration. The concentration of boron was found to be different with more being in leaves than in roots. Boron concentration was 49.37 ppm of B in GM roots whereas it was 47.48 of ppm B in control root. Control leaves had higher concentration of B with 80.59 ppm with the inoculated leaves 63.74 ppm of B.The concentration of molybdenum was found to be 249.5 ppm Mo in inoculated root and 169.4 ppm of Mo in control root whereas it was found to be comparatively very less in leaves with 9.42 ppm in GM leaves and 12.61 ppm in control leaves.

In general the GM inoculated plants were found to show better results as compared to control in physical growth parameters and macro and micronutrients analysis. The enhanced growth is due to the ability of AM fungi to increase nutrient uptake of plants by developing an association with roots (Schreiner *et al.,* 1997) and sometimes also by promoting the growth of other rhizospheric micro-organisms and thus enhancing plant growth (Johnson *et al.,* 2004).The other reason can be that,once host roots are colonized by AM fungi,it changes the root exudates released and produces phosphatase enzyme in rhizosphere. These phosphatases produced by extraradical hyphae of AM fungi could hydrolyze extracellular phosphate ester bonds and ultimately made Phosphorus available to plants (Joner *et al.,* 2000).Earlier studies have also shown that *Glomus mosseae* increases plant height,stem girth,biomass,P content,Zn concentration,biovolume index and quality index than uninoculated control plants (Sumana and Bagyaraj,1999 ; Sundarbabuk,2001).The nutrient uptake results resembled to earlier work performed.It is feasible that external hyphae may provide a significant delivery system for N, P, Cu and Zn in addition to P in many soils (Marschner *et al.,* 1994).Increase in uptake of copper and manganese in AM inoculated plant has also been shown (Krishna,1983; M.Farzaneh, 2011).

Based on the response on different physical growth parameters like shoot length, root length fresh weight, dry weight and number of leaves and macro and micro nutrient analysis like N,P,K and Zn content it can be concluded that AM specie *Glomus mosseae* can be used as best consortia for *Hibiscus* *rosa sinensis*. This technology being simple and eco friendly can also be used by nurserymen for horticulture purposes or for its medicinal value. Although further studies can also be performed with trying different combinations of Phosphate solubilizers or different arbuscular mycorrhizae species.

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