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Asian Journal of Plant Science and Research, 2016, 6(3):11-16

ISSN : 2249-7412
CODEN (USA): AJPSKY

Efficacy of three arbuscular mycorrhizal fungi on growth of *Centella asiatica* L. (Urban)

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ABSTRACT

Effect of inoculation of three AM fungi on growth performance of Centella asiatica plant was evaluated. Three AM fungi namely, Glomus fasiculatum, Acaulospora foveata and Gigaspora margarita were used in the present study. The plantlets of Centella asiatica were grown in poly house condition with three replicates for each treatment and harvested at 80 days of growth. The result showed that, the number of leaves in Gi. margarita inoculated pots were increased when compared to other two AM fungi inoculated pots and control. The highest root length was observed in control and Gi. Margarita and highest shoot length was observed in G. fasciculatum compared to other treatments and control. Whereas, the highest fresh shoot weight was recorded in plants treated with A. foveata when compared within the treatments and control. Total dry weight biomass, mycorrhizal dependency and mycorrhizal inoculation effect was high in Gi. margarita inoculated plants compared to other two treatments and control. The results of present study indicated that both Gi. margarita and A. foveata may be considered as good growth promoter for better biomass yield in C. asiatica which is used as brain tonic, in the treatment of chronic diseases and mental disorders in the Ayurvedic system of medicine.

Keywords: Arbuscular mycorrhizal fungi, *Centella asiatica*, *Gigaspora margarita*, *Glomus fasiculatum*, *Acaulospora foveata*, Ayurveda.

INTRODUCTION

Centella asiatica L. Urban (syn. *Hydrocotyle asiatica* L.) belongs to family Apiaceae. It is a well known imperative herb in Ayurvedic medicine and an important multipurpose plant species native to Sri Lanka and Southern Africa. It is also found in certain parts of India, Pakistan, Madagascar and Eastern Europe. The leaves and roots are used in the treatment of a number of ailments ranging from faster healing of small wounds, chap, treatment of eczema, minor itching, insect bites, chronic venous insufficiency, skin ulcers, etc. because of its anti bacterial, anti-feedant, anti-filarial, anti-leprotic, anti-stress, anti-tuberculosis and wound healing properties [1, 2]. The increasing harvesting and marketing of *C. asiatica* species endangers the long term sustainability and needs conservation strategy for this plant. Though presently it is listed in least concern category of the IUCN [3], considering its high medicinal value, it is the matter of concern for its conservation [4]. Vegetative propagation of *C. asiatica* is not enough to meet the timely need of pharmaceutical industries. Propagation via seed is insufficient due to low seed viability. Improvement of plant survival and growth enhancement through alternate methods is feasible. Selecting suitable mycorrhizae is one such method as the knowledge of host specificity of AM fungi in a specific natural ecosystem is important as it is known to affect the plant growth and development.

Arbuscular mycorrhizal fungi clearly play an important role in terrestrial ecosystem, such as grasslands, where they influence plant community structure and nutrient cycling [5]. The interaction between plant root and fungi called mycorrhizae, are found in approximately 90% of all vascular plants [6]. Since AM fungi require oxygen to thrive and since many wetland plants have been described as non-mycorrhizal [7, 8, 9], it has been assumed that AM fungi have little significance in wetland ecosystems. However, recent field studies show that AM fungi exist in wetlands and colonize many hydrophytic plants [10, 11, 12, 13, 14, 15]. AM fungi have been found in the roots of submerged

macrophytes [16, 17], salt marsh plants [18, 19], plants in oligotropic wetlands [20], wetland woody species [21, 22] plants in prairie potholes [23], wetland plants in everglades [24] and plants in recently rehabilitated wetlands [12]. Out of 49 species examined in the aquatic habitats of Denmark, 9 were found to harbour AM fungi [25].

The association between AM fungi and wetland plants may mediate co-existence of plant species and keep balance of the hydrophytic community as in terrestrial ecosystems [26]. One of the main functions and ecological roles of AM fungi is providing enhanced phosphorus nutrition to plants; thus the effect of soil available phosphorus is often assessed in wetland ecosystems [27]. The AM fungal colonization of wetland plants may be particularly dependent on the interaction between plant phenology and soil wetness [28]. Arbuscular mycorrhizal fungi are major component of rhizosphere microflora in natural ecosystems and play significant role in the re-establishment of nutrient cycling [29], modify the structure and function of plant communities [30] and are useful indicators of ecosystem change [31]. In the present study an effort was made to study the effect of AM fungi on growth enhancement of *C. asiatica*.

MATERIALS AND METHODS

Selection of AM fungus for inoculation

Individual AMF spores showing hyphal connection were isolated by the wet sieving and decanting method [32] from the air-dried rhizosphere soil samples collected from Western Ghats of the Karnataka region. Selected AM fungal species were mass multiplied using *Sorghum bicolor* L. as host plant. The soil used in this study was classified as sandy loam having pH 7.79 and with available phosphorous of 47.8 µg $g^{-1}$of soil. The soil and sand were dry sterilized by placing in hot air oven for 2 days at 150° C. The soil and sand (1:1 V/V) was used to fill the pots for mass multiplication of AM fungi by trap culture method [33]. The *S. bicolour* plants were allowed to grow for 80 days under greenhouse conditions. The plants were irrigated regularly to maintain sufficient soil moisture. After 80 days, the upper portion of the plant was removed retaining the root of the plant along with soil, which served as AMF inoculum. This inoculum was stored at 4°C till further use.

AM fungal inoculation in pot experiment

The selected mono-specific AMF inoculum (*Glomus fasciculatum*, *Acaulospora foveata* and *Gigaspora margarita*) (Fig. 1) multiplied by trap culture method was used to inoculate *C. asiatica* plants in green house condition to analyze the efficacy of individual species of AM fungi on the growth enhancement. The plant lets of *Centella asiatica* were procured from Biotechnology Center, Hulimavu, Bengaluru and planted in sterilized soil prepared as mentioned above. Different treatments used in the present study were; sterile soil/sand mixer served as control and sterile soil/sand mix + 50g of each of the AMF inoculums considered as inoculated. All the treatments were maintained in triplicate. The experimental pots were maintained in the green house condition at a temperature of 22 ± 1^{o}C, and watered regularly to maintain the soil moisture level close to field capacity. In this experiment no inorganic nutrients were added to the plants. Non-destructive growth measurements were taken and final harvesting was carried out after 80 days of planting. The plant parameters like number of leaves, shoot length were recorded at every 20 days of interval after planting. The total root length was recorded after final harvest.

Estimation of Fresh and Dry weigh

The biomass of separated root and shoot washed thoroughly to remove all the adhering soil particles in running tap water, gently pressed in folds of filter paper to remove excess moisture. The fresh weight of both the biomass was determined and the samples were wrapped in paper and placed in hot air oven at 72^{0} C for 48 hours. After 48 hours, the plant parts were removed, cooled in desiccators and reweighed to record dry weight.

Mycorrhizal Dependency (MD)

Dry weight of root and shoot and degree of response to mycorrhizal dependency was calculated, which being the difference between the total biomass of the inoculated and non-inoculated plants and was expressed as a percentage of the dry biomass. Mycorrhizal dependency value was calculated according to the formula of Plenchette *et al*., (1983) [34].

MD = Dry weight of inoculated plants/Dry weight of non-inoculated plants X 100

Mycorrhizal Inoculation Effect (MIE)

Dry biomass of root and shoot data was recorded to calculate the MIE for accessing the growth improvement brought about by inoculation with a indigenous AM fungi in unsterilized soil. MIE was calculated by the following formula [35].

MIE = Dry weight of inoculated plants - Dry weight of non-inoculated plants X 100
Dry weight of inoculated plants

Physico-chemical properties of soil

Physico-chemical properties of soil used in the present study was collected in sterile polyethylene bags using soil auger from the depth of 30 cm and subjected for analysis such as soil pH, Electrical conductivity and available phosphorus at Central Sericulture Research and Training Institute, Mysore, Karnataka.

Statistical analysis

All the data were statistically analysed using one-way ANOVA (Analysis of variance) by SPSS. Difference among treatments were determined using Tukey's multiple range tests (TMRT) at a significant level of p=0.05. Data are presented as Mean ± Standard Error (SE).

RESULTS AND DISCUSSION

Physico-Chemical properties of soil

The result Physico-Chemical properties of the soil used in the present study clearly showed that the sterilized soil had slightly high phosphorous content when compared to unsterilized soil. The pH of sterilized soil was slightly basic in nature condition compared to unsterilized soil which was slightly acidic. The electrical conductivity was also found to be high in sterilized soil compared to unsterilized soil. The physic-chemical parameter affected the efficacy of the AM fungal inoculums used in the present study. Among the three inoculums used in the present study, *Gi. margarita* performed well in high pH, high electrical conductivity and high avail phosphorous in soil.

Effects on AM fungi inoculation on growth parameters

The efficacy of three AM fungi on growth performance of *C. asiatica* showed a positive effect by increasing the growth parameters like number of leaves, root length, shoot length, whole plant weight, root weight and shoot weight in inoculated plant compared to control (Table-1). The number of leaves increased in all the inoculated plants compared to control (Fig.2). Within and between the treatments, a significant increment in leaf number was observed in plants treated with *Gi. margarita* and *A. foveata*. All the three AM fungal species failed to show any effect on root length enhancement compared to control, but within the treatments, *Gi. margarita* proved to be good compared to other two inoculum. The highest shoot length was observed in plant inoculated with *G. fasciculatum* when compared to other treatment and control (Table-1).

The highest fresh weight of root was recorded in the plants inoculated with *Gi. margarita* when compared to other treatments and control, whereas highest fresh shoot weight was recorded in *A. foveata* compared to other treatments and control. However, the plants treated with *A. foveata* showed the highest total mean fresh weight which was significantly high when compared to other treatments and control plants (Table-1).

Estimation of dry weight

The highest total dry weight biomass was recorded in plants treated with *Gi. margarita* which showed 4.67g of total biomass when compared to the control plant and within the treatments (Table-1). Mycorrhizal dependency value was high in *Gi. margarita* with 152.11%, followed by *G. fasiculatum* with 137.45% and *A. foveata* with 122.47% which showed the lowest percentage. The mycorrhizal inoculation effect was also high in plants treated with *Gi. margarita* with highest percentage of 34.26% and followed by *G. fasiculatum* with 27.25% and *A. foveata* with 18.35% which was lowest.

Centella asiatica has wide application in Indian and Chinese traditional system of medicines with documented evidence for wound healing and neuroprotective and anti-aging potential. Considering the increased demand for its utility, it has to be conserved before it shifts the place from least concern to threatened category in IUCN Red list [3]. In the present study an effort was made to understand the efficacy of three AM fungal species in increasing the biomass of the plant in order to find a host specific AM fungal species. Several earlier results suggest that AM fungal inoculation made significance difference in enhancing growth, oil yield and nutrient acquisition in *Mentha arvensis* [3], increase in the production of essential oil in *Coriandrum sativum* [37] and also diversity of AM fungi associated with aquatic and marshy plant species, including *Centella asiatica* [36]. Dhami [38] reported positive effect on parameters like root length, shoot height, shoot and root ratio, shoot and root biomass in *Centella asiatica* and *Bacopa monnieri* plants upon AM inoculation.

The results of present study reveal that, all the three AM fungi were proved to be effective either in increasing number of leaves, root and shoot length or total fresh and dry biomass and also the mycorrhizal dependency of *Centella asiatica* which corroborates the earlier reports [39, 40, 41-49]. The difference observed within the

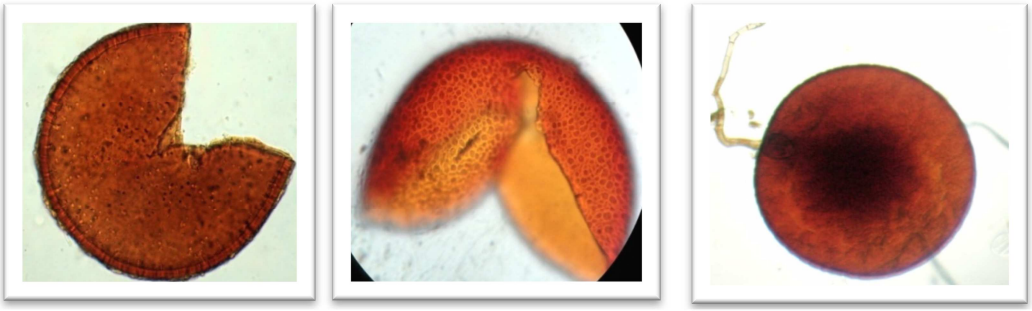
treatments of AM fungi may be due to the host preference of AM species as reported by earlier workers in some medicinal plant like *Phyllanthus amarus* and *Withania somnifera* [45] and *Coleus forskohlii* [50]. It has been reported that species of AM fungi differ significantly in their ability to improve plant growth and other aspect of plant performance [51, 52]. AM fungi are considered to have a wide host range and there is some degree of ecological specificity between AM fungi and plants [53]. The present result clearly indicates that, *Gi. margarita* and *A. fovaeta* outperformed the *G. fasciculatum* by increasing biomass of fresh weight, mycorrhizal dependency and mycorrhizal inoculation effect in *C. asiatica* and may be considered as a good inoculums. The need for selecting efficient AM fungi that can be used for inoculating different mycotrophic plants, has been stressed by various workers [54, 55, 56].

Table-1: Effects on AM fungi inoculation in field experiment

	No. of leaves	Root length (cm)	Shoot length (cm)	Whole weight (g)	Root weight (g)	Shoot weight (g)	Total dry weight (g)
CON	34.66±36.01^{d}	25.1±2.26 ^{a}	26.76±2.85 ^{d}	8.06±5.65 ^{d}	1.25±1.39 ^{c}	6.81±4.31 ^{c}	3.07±2.93^{b}
GF	62±19.46 ^{b}	18.93±7.12 ^{b}	39.53±10.44^{a}	14.37±1.51 ^{c}	2.1±0.88 ^{b}	12.27±0.73 ^{b}	4.22±1.07^{a}
AC	39±41.14 ^{c}	17.3±17.87^{c}	31.93±28.19 ^{c}	19.05±24.38 ^{a}	2.61±3.87^{a}	16.41±20.48 ^{a}	3.76±4.36^{b}
GM	64.66±8.02 ^{a}	24.26±4.05 ^{a}	37.16±2.92 ^{b}	16.31±2.54 ^{b}	2.84±1.39 ^{a}	13.48±2.77 ^{b}	4.67±0.47^{a}

CON – Control, GF - Glomus fasiculatum, AC- Acaulospora foveata, GM- Gigaspora margirata

Values are Means of 3 replicates ±Standard Error. Values with the same letter within same column for each parameter are not significantly different at p≤ 0.05 levels means values of different superscripts are significantly different by Tukey s multiple range tests with respect to species main effect.



A **B** **C**

Fig 1: Mycorrhizal strains used to inoculate *Centella asiatica* plants: (A) *Glomus fasiculatum*, (B) *Acaulospora foveata* and (C) *Gigaspora margirata*

(A) **(B)** **(C)**

Fig 2: *Centella asiatica* plant growth after (A) 30 days, (B) 60 days and (C) 80 days in a green house condition

CONCLUSION

In the present study, *Gi. margarita* and *A. fovaeta* showed positive results in all the parameters and also in increasing the biomass of *C. asiatica*. As mycorrhizal symbiosis is a highly evolved mutualistic relationship for plant establishment, this study provides a great future for utilizing the efficient strains of mycorrhizal fungi to exploit them for the beneficial effects in establishment of seedlings, increase in productivity and reduce the fertilizer application required for obtaining economic production of *C. asiatica* plant under field conditions. It is often assumed that AMF will play a more pivotal role in alternative systems than in conventional systems. Our results indicate that the use of mycorrhizal inoculation is a feasible approach for *C. asiatica* production. The green-house experiment suggested that AMF inoculation can increase growth and biomass production significantly. It has been observed that different AMF isolates influence the host plant growth and qualitative differences in the secondary

metabolites production. Therefore, further studies are needed for selection of effective AMF for *C. asiatica* growth and secondary metabolites production.

Acknowledgment
The authors are thankful to the Institution of Excellence, University of Mysore for providing financial support and extending essential facilities to carry out of this research work.

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