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Inoculation effect of different arbuscular mycorrhizal fungi on growth of *Sida cordifolia* L.

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It was envisaged to screen and select an efficient arbuscular mycorrhizal fungus (AM fungus) for inoculating *Sida cordifolia* for cultivation. The efficacy of ten arbuscular mycorrhizal fungi (AM fungi) on *S. cordifolia* (Medicinal Plant) was carried out in the nursery conditions. Plants grown in the presence of AM fungi generally showed an increase in plant growth and plant biomass over those grown in the absence of AM fungal inoculation. The extent of response of *S. cordifolia* varied with species of AM fungi inhabiting the roots. Considering various parameters and plant biomass, it was concluded that *Glomus citricolum* is the best AM symbiont for *S. cordifolia* compared to other fungi.

Key words: AM fungi; *Sida cordifolia*; growth response; *Glomus citricolum*

Introduction

The medicinal plants are in great demand due to increase acceptance of Ayurveda and traditional medicines, and their property of less or no side effects. *Sida cordifolia* L. is a medicinal plant having commercial value for its alkaloid extraction, which can be used as medicine. Apart from alkaloid extraction, the whole plant is used as an herbal medicine with several applications to treat Asthma, bronchitis, neuralgia, rheumatism, sexual debility etc. This crop came into prominence by virtue of its alkaloids like Ephedrine (EDH), Pseudoephedrine, Vasicine and Vasicinol, present in seeds, leaves and roots (Prajapathi *et al*., 2004).

The current approach of cultivation is following the sustainable agriculture, using very less of chemical inputs like fertilizers and pesticides having adverse effect on soil health, fertility and environment. Thus, use of

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microbial inoculants play an important role in sustainable agriculture. Arbuscular mycorrhizal fungi (AMF) are known to improve the nutritional status, growth and development of plants, protect plants against root pathogens and also offer resistance to drought and salinity (Jeffries, 1987). Use of AM fungi in increasing the plant growth is well documented. In the last three decades, there were lot of reports regarding the use of AM fungi to increase the plant growth and yield of agricultural crops and forestry species. All AM fungi are obligate biotrophs that benefit the plant in several ways, for example by increasing nutrient capture, drought resistance, pathogen protection beneficial alterations of plant growth regulators and synergistic interactions with beneficial rhizosphere microorganisms (Bagyaraj, 1991). Though, these fungi are not host specific, recent studies have clearly brought out host preference in A M fungi thus, emphasizing the need for selecting efficient AM fungi for inoculating a particular host. Host preference has been reported in some few medicinal plant species like *Phyllanthus amarus* and *Withania somnifera* (Earanna, 2001) and *Coleus forskohlii* (Sailo and Bagyaraj, 2005). Hence, in the present study, it was envisaged to screen and select an efficient AM fungus for inoculating *Sida cordifolia* for cultivation.

Materials and methods

Two pot experiments were carried out under nursery conditions in a glass house. The used substrate was sand: soil in 1:1 v/v ratio. The substrate contained an indigenous AM mycorrhizal population of 70 spores/50 gram. The AM fungal species used in the study were isolated from rhizosphere of *Sida cordifolia* in different locations of Karnataka. These fungi were multiplied using sterilized sand: soil mix (1:1 v/v) as the substrate and jowar (*Sorghum vulgare* L.) as the host. After six weeks of growth, shoots of jowar plants were severed and the substrate containing hyphae, spores and root bits were air dried and used as inoculum. Ten grams of AM fungal inoculum was added into the planting hole at a depth of about 5 centimeters just before planting.

In the pot experiment, each polythene bag containing the substrate, with or without AM inoculum, as per the treatment given in Table 1 was planted with one *Sida cordifolia* seedling. Thirty day old seedlings raised in the substrate described above were transplanted to polythene bags of size 24.5 X 15 cm holding 2.5 kg of substrate. Each treatment with five replications was maintained in a glass house and watered regularly. Plant height, number of leaves and number of branches were measured and recorded at 15 days interval. However, only observations recorded on day 120 are presented. The plants were harvested at 120 days after transplanting (DAT). Fresh weight of shoot and root portions were recorded separately. Dry biomass of shoot and root portions were also recorded separately after drying the plant sample at 60ºC to a constant dry weight in a hot air oven. Phosphorous concentration of the plant tissue was determined by employing the Vanado-molybdate phosphoric acid yellow color method (Jackson, 1973). The total nitrogen concentration of the plant tissue was determined by semimicro-kjeldhal method as per the procedure outlined by Jackson (1973) using Gerhardt auto analyzer.

Fine roots were stained using 0.02% trypan blue as described by Philips and Hayman (1970) and percent root colonization was estimated by adopting the gridline intersect method (Giovannetti and Mosse, 1980). The acid and alkaline phosphatase activities in the root zone soil were estimated as per the procedure given by Eivazi and Tabatabai (1977). The data obtained from the experiments were subjected to one way analysis of variance (ANOVA) using AGRES version 7.01. All the treatment means were tested for least significant difference at 5% level of significance (Rosner, 2000). The correlation analysis was carried out with SPSS software package version 12.0. The relationship between the parameters observed was determined by Pearson's correlation analysis (Zar, 1999).

Results

The effect of inoculation with AM fungi on the growth and yield of *Sida cordifolia* are presented in Table 1. All the treatments were effective in enhancing the growth of *S. cordifolia*. However, among the treatments plants, inoculated with *Glomus citricolum* was found to be the best. Maximum shoot length was observed in plants inoculated with *G. citricolum* (25.76) followed by statistically significant *G. geosporum* (22.43). The significantly lowest shoot length was observed in the control. The number of leaves/plant recorded were statistically higher in plants inoculated with AM fungi compared to control excepting *A. dilatata* (16) and *G. fasciculatum* (16.20) which are statistically on par with the control. The numbers of branches were statistically higher in plants inoculated with AM fungi compared to control excepting *A. nicolsonii* (0.6) and *A.laevis* (0.6) which are on par with control. Highest number of branches was recorded in *G. citricolum* (1.6) followed by statistically on par *G. mosseae* (1.4) and *G. geosporum* (1.4). Total fresh weight was significantly high in plants inoculated with *G. citricolum* (14.24) followed by *G. geosporum* (13.43). The total fresh weight was significantly higher in all the treatments compared to control. Maximum shoot dry weight was observed in plants inoculated with *G. citricolum* (2.69) followed by *G. mosseae* (2.42) and *G. geosporum* (2.35). All the treatments significantly differed from the control plants. Highest root dry weight was recorded in plants inoculated with *G. citricolum* (0.79) followed by statistically on par *G. mosseae* (0.71) and *G. maulticaulis* (0.71). Total dry weight was significantly high in plants inoculated with *G. citricolum* (3.48) followed by plants inoculated with *G. mosseae* (3.13) and statistically on par *G. geosporum* (2.99). The plants inoculated with AM fungi exhibited significantly higher dry weight compared to the control.

Table 1. Effect of inoculation with AM fungi on the growth and yield of *Sida cordifolia.*

Treatments	**Plant height(cm)**	**No. of leaves/plant**	**No. Of branches/plant**	**Total fresh weight(g)**	**Total dry weight(g)**
Control	12.17^{g}	15.4^{e}	0.2^{e}	9.33^{f}	2.08^{f}
G.melanosporum(Gml)	18.03^{d}	18^{b}	0.8^{cd}	11.32^{d}	2.39^{e}
A. dilatata (Ad)	16.36^{e}	16.00^{de}	0.8^{cd}	11.33^{d}	2.41^{e}
G. fasciculatum (Gf)	15.31^{f}	16.20^{cde}	1.0^{bcd}	10.30^{e}	2.46^{e}
G. mosseae (Gm)	19.84^{c}	19.40^{a}	1.4^{ab}	12.76^{c}	3.13^{b}
G. citricolum (Gc)	25.76^{a}	20.4^{a}	1.6^{a}	14.24^{a}	3.48^{a}
A. nicolsonii (An)	16.65^{e}	17.00^{bcd}	0.6^{de}	10.73^{de}	2.36^{e}
A. laevis (Al)	16.8^{e}	17.2^{bc}	0.6^{de}	10.11^{e}	2.32^{e}
G. geosporum (Gg)	22.43^{b}	19.8^{a}	1.4^{ab}	13.43^{b}	2.99^{bc}

Means with same superscript in each column do not differ significantly at P=0.05

The results on the effect of AM fungi on N content, P content, AM colonization and Phosphatase activity of *S. cordifolia* recorded at the end of 120 DAT are presented in Table 2. The maximum shoot N percentage was observed in plants inoculated with *G. mosseae* (2.55) followed by statistically on par *G. geosporum* (2.52), *A. dilatata* (2.51), *G. citricolum* (2.45), *G. fasciculatum* (2.43), and *A. laevis* (2.42).The shoot nitrogen content of the plants inoculated with AM fungi was significantly higher compared to control excepting the plants inoculated with *G. melanosporum* (2.27) and *A. nicolsonii* (2.23). The highest root N content was observed in *G. citricolum* (1.79) closely followed by *G. mosseae* (1.78). The root nitrogen content of the plants inoculated with AM fungi was higher compared to control excepting the plants inoculated with *G. fasciculatum* (1.40) *A. laevis* (1.38) and *G. radiatum* (1.25). Maximum shoot P content was observed in plants inoculated with *G. citricolum* (0.231). Shoot P content was significantly higher in plants inoculated with AM fungi compared to control plants excepting *A. nicolsonii* (0.17). Root P content was significantly higher in plants inoculated with AM fungi compared to control plants, the highest being in *G.s citricolum* (0.198) followed by *G. geosporum* (0.180). Root colonization was highest in plants inoculated with *Glomus mosseae* (55.2) followed by *G. citricolum* (54.6), *G. multicaulis* (54.6), *G.s geosporum* (53.6) and *G. fasciculatum* (53.8). All the treatments were significantly higher compared to control. Significantly higher acid phosphatase activities were observed in *G. radiatum* (41.84) *G. citricolum* (41.56) followed by statistically on par *G. geosporum* (41.42), *A. laevis* (41.10), *G. melanosporum* (40.31) and *G. mosseae* (39.9). All the treatments were significantly higher compared to the control. Significantly higher alkaline phosphatase activities were recorded in root zone soils of plants inoculated with *G. citricolum* (52.64) followed by statistically on par *G. geosporum* (52.14) *G. mosseae* (51.76) *G. multicaulis* (50.98), *G. radiatum* (50.73) and *G.melanosporum* (50.33).

Table 2. Effect of AM fungi on N and P content in plants, Phosphatase activity in root zone soil and mycorrhizal root colonization in *Sida cordifolia*.

Treatments	Shoot N (%)	Root N (%)	Shoot P (%)	Root P (%)	Acid Phosphatase activity (µ/h/g)	Alkaline phosphatase activity (µ/h/g)	Colonization (%)
Control	2.18^{e}	1.32cd	0.16^{g}	0.09^{f}	26.30^{f}	24.02^{i}	29.80^{f}
G. melanosporum (Gml)	2.27^{cde}	1.45b	0.17^{f}	0.12^{cd}	42.57^{cd}	30.37^{gh}	54.60^{bcd}
A. dilatata (Ad)	2.51^{ab}	1.43b	0.18^{de}	0.11^{d}	41.56^{d}	55.50^{a}	38.80^{f}
G. fasciculatum (Gf)	2.43^{ab}	1.40bc	0.19^{d}	0.12^{c}	50.44^{b}	45.94^{c}	46.80^{e}
G. mosseae (Gm)	2.55^{a}	1.78a	0.20^{c}	0.18^{b}	54.54^{a}	50.20^{b}	55.80^{bc}
G. citricolum (Gc)	2.45^{ab}	1.79a	0.23^{a}	0.19^{a}	44.94^{c}	52.62^{ab}	62.00^{a}
A. nicolsonii (An)	2.23^{de}	1.48b	0.17^{fg}	0.10^{e}	35.14^{e}	41.96^{d}	50.80^{cde}
A. laevis (Al)	2.42^{ab}	1.38bc	0.18^{ef}	0.11^{d}	37.04^{e}	36.08^{ef}	59.40^{ab}
G. geosporum (Gg)	2.52^{a}	1.46b	0.22^{b}	0.18^{b}	42.73^{cd}	38.66^{de}	54.00^{bcd}
G. maulticaulis(Gmt)	2.38^{bc}	1.32cd	0.19^{d}	0.11^{d}	42.41^{cd}	32.88^{fg}	50.20^{de}
G. radiatum (Gr)	2.37^{bcd}	1.25d	0.18^{de}	0.12^{cd}	33.60^{e}	27.54^{h}	52.00^{cde}
CD (0.05)	0.0389	0.0487	0.0079	0.0065	2.923	3.484	5.426

Means with same superscript in each column do not differ significantly at P=0.05

All the treatments were significantly higher compared to control. The parameters on the effect of AM fungi on growth and yield of *Sida cordifolia* were correlated (Table 3). It was observed that shoot N, root N, shoot P, root P are significantly correlating with dry weight of shoot. Whereas root N, shoot P and root P are correlating with root dry weight

Table 3. Pearson's correlation coefficient (r) between different parameters that influence growth and yield of *Sida cordifolia* due to AM fungi inoculation.

	Colonization	Acid phosphatase activity	Alkaline phosphatase activity	Dry weight of shoot	Dry weight of root	Shoot N	Root N	Shoot P	Root P
Colonization	1.000								
Acid phosphatase activity	0.833**	1.000							
Alkaline phosphatise activity	0.921**	0.892**	1.000						
Dry weight of shoot	0.599	0.463	0.642*	1.000					
Dry weight of root	0.582	0.311	0.488	0.767**	1.000				
Shoot N	0.696*	0.584	0.633*	0.614*	0.533	1.000			
Root N	0.348	0.376	0.361	0.640*	0.745**	0.450	1.000		
Shoot P	0.577	0.483	0.560	0.896**	0.771**	0.714*	0.632*	1.000	
Root P	0.512	0.532	0.547	0.883**	0.653*	0.684*	0.783**	0.910**	1.000

** Significant at the 0.01 level (2-tailed).
* Significant at the 0.05 level (2-tailed).

Discussion

AM fungal inoculation enhanced the growth of *Sida cordifolia* by increasing the concentration of N and P in the plants. These results are on par with earlier studies of Chiramel *et al*. (2006), Earanna (1999). It is known that the magnitude of plant response to any microbial inoculation is greatly affected by the P and N content of soil (Paula *et al.,* 1992), especially P deficiency has been described as a main factor in restricting not only development but other biological processes such as nitrogen fixation, owing to the high requirement of P (as ATP) for the nitrogen fixation process (Singleton *et al.,*. 1985; Israel 1987). In this context the role of AM fungi as phosphorous supplier to the plant appears to be of great relevance. Improved uptake of P is due to the exploration of external hyphae of the soil beyond root hair zone when phosphorous is depleted (Gerdemann 1975; Sanders and Tinker 1971). It has also been attributed to not only increased surface area of absorption (Sanders and Tinker 1971) but also to enhanced hyphal translocation (Hattingh *et al.,*. 1973) contribute to P nutrition. Treatments with higher phosphorous concentration had increased acid and alkaline phosphatase activity. Secretion of phosphatases by AM fungi is a common mode of facilitating the conversion of insoluble forms of P to available forms and thus enhances plant P uptake and growth (Kim *et al.,* 1998). Species of AM fungi have differed to the extent by which they increase nutrient uptake and plant growth (Mcgraw and Schenek 1981). Therefore, the need for selecting efficient AM fungi that can be used for inoculating different mycotrophic plants has been stressed (Abbott and robson 1982; Bagyaraj 1991). The efficiency refers to ability of the fungus to increase plant growth (Abbott and Robson 1982). This depends on the ability to form extensive and well distributed hyphae in soil to form extensive colonization in the root system, produce phosphatases and to absorb P from soil. In the present study the extent of colonization, phosphatase activities and P uptake varied with different AM fungi. This type of variations was observed in earlier studies also (Saif 1987; Lakshmipathy *et al.,*. 2003). Higher root colonization allows more fungal-host contact and exchange of nutrients with better plant growth. The above reviews strongly support growth enhancement of *Sida cordifolia* by AM fungi and also need of selecting efficient AM fungus for inoculation.

Our study on the responsiveness of *Sida cordifolia* to AM fungal inoculation concluded that there is a positive relationship between the host response to AM fungi, growth of the plant and nutrient uptake (N&P). By giving emphasis to all the above parameters and plant biomass, *Glomus citricolum* was considered to be the most promising symbionts for inoculating *Sida cordifolia*. However for sustainable cultivation of *Sida cordifolia* by using

AM fungi in agro-climatic conditions field trials are needed to be focused in future research.

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