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Symbiotic Response of *Ocimum sanctum* to different Arbuscular Mycorrhizal Fungi

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ABSTRACT

Arbuscular mycorrhizal (AM) fungi are known to be supportive to crop plants through uptake of diffusion limited nutrients, biological control, hormone production, drought resistance, etc. *Ocimum sanctum* L. is one of the major medicinal plant of our country. Pot experiment was conducted to screen and select the efficient arbuscular mycorrhizal fungi for inoculating *O. sanctum*. Screening was done with eleven different species of AM fungi (*Acaulospora laevis, Gigaspora margarita, Glomus bagyarajii, Glomus etunicatum, Glomus fasciculatum, Glomus intraradices, Glomus leptotichum, Glomus macrocarpum, Glomus monosporum, Glomus mosseae* and *Scutellospora calospora*). Plant parameters like height, stem girth, biovolume index, biomass of shoot and root and mycorrhizal parameters like root colonization, spore number in the root zone soil, etc. have been recorded according to the standard procedures. Based on the improved plant parameters like bio-volume index, plant biomass, oil yield and phosphorus uptake it is concluded that *Glomus monosporum* is the best AM fungus for inoculating *O. sanctum*.

Keywords: Arbuscular mycorrhizal fungi, *Ocimum sanctum*, *Glomus monosporum*, Plant growth response.

INTRODUCTION

The medicinal plants are widely used by the traditional medicinal practitioners for curing various diseases in their day to day practice. In traditional system of medicine, different plant parts (leaves, stem, flower, root, seeds and even whole plant) of *Ocimum sanctum* L. (commonly called Tulasi or Sweet basil) have been recommended for the treatment of bronchitis, malaria, diarrhoea, dysentery, skin disease, arthritis, eye diseases, insect bites and so on. The *O. sanctum* has also been suggested to possess anti-fertility, anticancer, antidiabetic, antifungal, antimicrobial, cardioprotective, analgesic, antispasmodic and adaptogenic actions. Eugenol (1-hydroxy-2-methoxy-4-allylbenzene), the active constituent present in *O. sanctum* has been found to be largely responsible for the therapeutic potentials (Gautam and Goel, 2014). The application of chemical fertilizers and pesticides have been increasing every year to attain the maximum production of plants. The use of chemical fertilizers in India has increased 170 times during the last 50 years. Microorganisms like bacteria, fungi and actinomycetes, present in the soil play a major role in plant growth and conserving the environment. It is well known that the addition of chemical fertilizers to soil is detrimental to the microbial growth and deteriorates the soil quality (Thilagar and Bagyaraj, 2015). Sustainable agriculture which is currently recommended advises to reduce the use of chemical fertilizers by introducing organic manures including beneficial microorganisms like mycorrhizal fungi, N fixers, P solubilizers, plant growth promoting rhizomicroorganisms and biocontrol organisms to the field in order to sustain plant productivity and to maintain soil health.

Arbuscular mycorrhizal (AM) fungi are ubiquitous, found in all types of climate, soil and extreme environment all over the world (Bagyaraj, 2014). They have the ability of living symbiotically in plants roots. The contribution of AM fungi in agricultural land may reduce the input of chemical fertilizers and would help in sustaining plant productivity and retain soil quality (Thilagar *et al*., 2016). AM fungi are important in ecological agriculture because of the benefits they provide to the majority of cultivars and the conservation of the environment by acting as biofertilizers, bioprotectors and biocontrol agents. These soil fungi forming symbiotic association with higher plants, facilitate uptake of diffusion limited nutrients, particularly phosphorus and increase crop production. Furthermore, AM fungal colonization stimulates the development of microorganisms in the mycorrhizosphere with antagonistic activity towards soil-borne pathogens (Desai *et al*., 2016). Though AM fungi are not host specific but they exhibit host preference thus suggesting the need for selecting an efficient AM fungus for a particular host, as evidenced by earlier studies (Thilagar and Bagyaraj, 2015). In the present investigation it was envisaged to screen and select an efficient fungus that can be used for inoculating *O. sanctum* which would promote plant growth, nutrition and oil yield.

MATERIAL AND METHODS

The pot experiment was conducted to screen and select the efficient AM fungus for inoculating *O. sanctum* plants on the basis of symbiotic response. The AM fungi used in this study were *Acaulospora laevis, Gigaspora margarita, Glomus bagyarajii, G. etunicatum, G. leptotichum, G. intraradices, G. macrocarpum, G. monosporum, G. mosseae* and *Scutellospora calospora*. These fungi maintained in the culture collection of CNBRCD were selected based on the results of earlier studies on other crop plants. The AM fungi were multiplied in pots under polyhouse condition using vermiculite, soilrite and perlite in the ratio of 1:1:1 (v/v/v basis) and Rhodes grass (*Chloris gayana*) as the host. After 75 days of growth, shoots of Rhodes grass were severed and the substrate containing spores, hyphae and root bits (cut into about 1 cm pieces) was air dried and used as the inoculum. The soil used in this experiment was collected from an uncultivated field from a depth of 0.15 cm and has been classified as fine, kaolinitic, isohyperthermic and kanhaplustalfs.The soil pH was 6.0 (1:10 soil to water extract ratio), available phosphorus of 5.2 ppm (NH4F +HCl

extractable) and indigenous mycorrhizal spores of 92 in 50g soil.

Thirty day old *Ocimum sanctum* seedlings were obtained from University of Agricultural Sciences, GKVK, Bangalore. Uniform sized seedlings were transplanted to poly bags and 10g of respective AM fungal inoculum was added to the planting hole of each treatment with seven replications. The control treatment received 10 ml of the washings of the 11 AM fungal inocula mix used in the study which were passed through 45 µm sieve containing associated microorganisms but not the AM propagules. The poly bag size was 21 cm x 14 cm and had the capacity of holding 1.5 kg of soil. The plants were maintained in a glasshouse and watered whenever necessary.

Growth parameters including plant height and stem girth were recorded 60 days after transplanting (DAT). Biovolume index was calculated by the formula given by Hatchell (1985). Plant height was measured from soil surface to the growing tip of the plant and stem girth was measured 1 cm above the soil surface using digital vernier callipers. The plants were harvested 60 DAT. The shoot and root were separated and the shoot fresh weight was determined. The dry weight of shoot and root was determined after drying at 60^0C in a hot air oven to a constant weight. The amount of P in the oven dried shoot and root samples was estimated by vanadomolybdate yellow colour method (Jackson, 1973). Eugenol concentration in the fresh leaf was determined by the gas chromatographic analysis (Varian 450GC, CP-SIL C18, FID, GKVK, Bangalore) (Gill *et al.*, 2014).

Mycorrhizal spore numbers in the root zone soil was determined by wet sieving and decanting method (Gerdemann and Nicolson 1963). The fine roots were removed from the root system, washed thoroughly, and stained with trypan blue. The per cent mycorrhizal colonization in roots was determined by grid line intersect method outlined by Bagyaraj and Sturmer (2008). The data were analyzed using the completely randomized design, with the help of the computer (Microvex System VAX/VMS, version 5.4, Digital Equipment Corporation,USA). The means were compared by Duncan's multiple range test at 5 % level (Gomez and Gomez, 1984)

RESULTS AND DISCUSSION

The present study with an object of screening and selecting an efficient AM fungus for inoculating *O. sanctum* resulted in varied plant growth response to different AM fungi. In general, AM fungal inoculation resulted in a general increase in plant growth parameters such as plant height, fresh shoot weight, dry weight of shoot and root and plant P concentration and oil yield as compared to the uninoculated plants. The plants inoculated with *Glomus monosporum* had significantly greater plant height as compared to uninoculated plants and which was statistically on par with *G. mosseae*, *G. etunicatum* and *G. leptotichum*. Enhanced plant height because of AM fungal inoculation has been reported by earlier workers in other medicinal plants (Sumana and Bagyaraj, 2002; Nisha and Kumar, 2010). Stem girth was significantly more in plants inoculated with *G. macrocarpum, G. monosporum¸ G. fasciculatum* and *G. intraradices* compared to uninoculated plants. Similar observations have been reported in other medicinal plants like amla (Srinivasan *et al.*, 2012) and aswagandha (Anuroopa and Bagyaraj, 2015). Biovolume index (BI) was maximum in plants treated with *G. monosporum* which differed significantly from all other inoculated treatments and the uninoculated control **(Table 1).** This is in conformity with earlier finding in other medicinal plants (Earanna *et al.* 2002; Chiramel *et al.*, 2006). The highest fresh weight of shoot was recorded in *G. monosporum* treated plants which was statistically on par with the treatment *G. leptotichum* **(Table 1)**. Chethan Kumar *et al*. (2011) working with the medicinal plant *Sida cordifolia* also reported similar observation. The shoot dry biomass was also maximum in the treatment of *G. monosporum* which was on par with many inoculated treated treatments except the treatments with *G. intraradices, G. macrocarpum, Scutellospora calospora* and the uninoculated control (**Table 2**). Similar findings was reported in medicinal plants like *Piper longum* (Seema and Garampalli, 2015) and amla (Srinivasan *et al.*, 2012). The highest dry root biomass was recorded in *G. monosporum* treated plants which differed statistically from all the other inoculated and uninoculated treatments (**Table 2**). This is in conformity with the earlier findings of Karthikeyan *et al*. (2009) in medicinal plants like *Catharantus roseus, Coleus forskohlii* and *Cymbopogon flexuosus*. Increase in plant dry biomass due to efficient AM fungal inoculation has been reported in other plants like wedilia (Nisha and Kumar, 2010) and chilly (Thilagar and Bagyaraj, 2015) by earlier workers.

The P concentration of shoot samples was significantly high in plants inoculated with most of the AM fungi (**Table 2**). The extent of increase in the plant P concentration varied among the fungi studied. Plants grown in the presence of *Glomus monosporum* contained a significantly higher concentration of P followed by those grown in the presence of *G. mosseae*, both being statistically on par with each other. Plants raised in presence of *G. monosporum* showed 53 % increase in shoot P concentration compared to uninoculated plants. Similar observation has been reported by many workers in other medicinal plants (Chiramel *et al.*, 2006; Rajeshkumar *et al.*, 2008; Ndiaye *et al*., 2009). It is well known that AM fungi

Table 1: Effect of soil inoculation with AM fungi on plant height, stem girth, bio-volume index (BI) and fresh shoot weight of *Ocimum sanctum*

Treatment	Plant height (cm/ plant)	Stem girth (mm/ plant)	Biovolume index(BI)	Fresh weight of shoot (g/ plant)
Gigaspora margarita	44.78 ^{cd}	6.2 ^{bc}	278.26 ^{bc}	35.61 ^{de}
Glomus bagyarajii	42.2 ^{de}	6.2 ^{bc}	266.62 ^{cd}	29.99 ^{f}
Glomus etunicatum	49.1 ^{ab}	6.3 ^{bc}	311.44 ^{b}	34.35 ^{ef}
Glomus fasciculatum	46.3 ^{bc}	6.5 ^{ab}	305.33 ^{bc}	32.54 ^{f}
Glomus intraradices	46.3 ^{bc}	6.5 ^{ab}	305.39 ^{bc}	32.05 ^{f}
Glomus leptotichum	48.25 ^{ab}	6.1 ^{bc}	299.60 ^{bc}	43.02 ^{ab}
Glomus macrocarpum	46.3 ^{bc}	6.9 ^{ab}	315.4 ^{b}	33.68 ^{ef}
Glomus monosporum	51.8 ^{a}	6.7 ^{ab}	358.85 ^{a}	47.97 ^{a}
Glomus mosseae	51.5 ^{a}	6.0 ^{c}	312.90 ^{b}	40.66 ^{bc}
Scutellospora calospora	46.1 ^{bc}	6.1 ^{bc}	293.77 ^{bc}	38.66 ^{cd}
Acaulospora laevis	45.2 ^{bc}	6.0 ^{c}	280.10 ^{bc}	38.75 ^{cd}
Uninoculated	41.2 ^{e}	6.05 ^{c}	252.09 ^{d}	25.3 ^{f}
Values in each column followed by the same letter are not significantly different(P>0.05)				

Table 2: Effect of soil inoculation with different AM fungi on, dry weight of shoot and root, and leaf oil content of *Ocimum sanctum*

Treatment	Dry weight of shoot (g / plant)	Dry weight of root (g / plant)	Shoot P conc. (%)	Leaf oil con-centration (%)
Gigaspora margarita	18.46 ^{abc}	5.4 ^{ef}	0.51 ^{d}	0.22 ^{d}
Glomus bagyarajii	17.14 ^{abcd}	4.8 ^{f}	0.62 ^{b}	0.10 ^{g}
Glomus etunicatum	19.18 ^{abc}	6.4 ^{de}	0.64 ^{b}	0.10 ^{g}
Glomus fasciculatum	16.2 ^{abcd}	6.3^{e}	0.52^{d}	0.20 ^{e}
Glomus intraradices	15.9 ^{bcd}	8.3 ^{c}	0.58 ^{c}	0.27 ^{b}
Glomus leptotichum	20.31 ^{ab}	6.2 ^{e}	0.44 ^{g}	0.15 ^{f}
Glomus macrocarpum	13.45 ^{d}	10.6 ^{b}	0.46 ^{f}	0.24 ^{c}
Glomus monosporum	20.8 ^{a}	13.1 ^{a}	0.66 ^{a}	0.31 ^{a}
Glomus mosseae	19.52 ^{abc}	9.8 ^{b}	0.64^{ab}	0.23 ^{cd}
Scutellospora calospora	15.31 ^{cd}	7.5 ^{cd}	0.61 ^{b}	0.22 ^{cd}
Acaulospora laevis	17.69 ^{abcd}	6.2 ^{e}	0.61 ^{b}	0.10 ^{g}
Uninoculated	13.1 ^{d}	2.9 ^{g}	0.49 ^{e}	0.05^{h}
Values in each column followed by the same letter are not significantly different(P>0.05)				

improve plant growth mainly through the uptake of diffusion-limited nutrients like P, Zn and Cu and also the enhanced nutritional status of the plant manifests in its improved growth (Bagyaraj *et al*., 2015). Several papers have revealed the potential of AM fungi to enhance plant growth and alter secondary metabolite production (Rojas-Andrade *et al.*, 2003; Copetta *et al*., 2006). Increase in oil yield because of inoculation with an AM fungus has been reported earlier in *Ocimum gratissimum* (Hazzoumi *et al.*, 2015) and *O. basilicum* (Rasouli-Sadaghiani *et al.*, 2010). In the present study the oil yield was highest in the treatment of *Glomus monosporum* which was statistically on par with the treatment of *G. intraradices* (**Table 2**).

All the inoculated treatments showed significantly higher per cent mycorrhizal root colonization as compared to uninoculated plants (**Table 3**). Highest mycorrhizal colonization was observed in plants inoculated with *G. monosporum* which was statistically at par with the treatments of *G. mosseae, S. calospora, G. bagyarajii, G. fasciculatum* and *A. laevis* and differing significantly from uninoculated plants. Increased mycorrhizal colonization because of inoculation with efficient AM fungi is well documented (Srinivasan *et al*., 2012; Anuroopa and Bagyaraj, 2015). The spore numbers were higher in root zone soil samples inoculated with *G. monosporum* and *G. margarita* both being statistically at par (**Table 3**). The significantly higher per cent mycorrhizal root colonization and spore number in the root zone soil of plants inoculated with *G. monosporum* as compared to uninoculated and also some inoculated treatments indicated the better proliferating ability of this fungus with *O. sanctum* as the host.

Table 3: Effect of soil inoculation with different AM fungi on per cent mycorrhizal root colonization and spore number in the

Treatment	Colonization (%)	Spore number/ 50 g soil
Gigaspora margarita	45.7^{e}	310.6^{ab}
Glomus bagyarajii	87.6^{ab}	240d^{e}
Glomus etunicatum	65.8^{cd}	228.6^{def}
Glomus fasciculatum	77.1 ^{abc}	304^{bc}
Glomus intraradices	70.83^{bcd}	258.3^{cd}
Glomus leptotichum	74.3 ^{bcd}	241.3^{de}
Glomus macrocarpum	60de	262^{bcd}
Glomus monosporum	91.5^{a}	353^{a}
Glomus mosseae	85.6^{ab}	259^{cd}
Scutellospora calospora	81.7^{ab}	295^{bc}
Acaulospora laevis	82.83 ^{ab}	202^{ef}
Uninoculated	20.4^{f}	121.6^{g}
Values in each column followed by the same letter are not significantly different (P>0.05)		

CONCLUSION

From the present study, it can be concluded that *Ocimum sanctum* showed a varied response to different AM fungi and *Glomus monosporum* confers greatest growth benefits as compared to other AM fungi used in this study. Giving weightage to plant biomass, oil yield and plant P concentration it can be concluded that *G. monosporum* is the best AM fungus for inoculating *O. sanctum*. However, validation under field condition is necessary before recommending *G. monosporum* as an inoculant for *O. sanctum* to the farming community.

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