Annals of Plant Sciences
ISSN: 2287-688X
OPEN ACCESS
www.annalsofplantsciences.com

Original Research Article

Screening of Arbuscular mycorrhizal fungi for their symbiotic efficiency on *Ocimum sanctum* L.

C Maya[1*] and H C Lakshman[2]

[1]Department of Studies in Botany, Bangalore University, Jnana Bharati Campus, Bangalore-560 056, Karnataka, India

[2]P.G. Department of Studies in Botany, Microbiology Laboratory, Karnataka University, Dharwad-580003, Karnataka, India

Received: May 30, 2015; **Revised:** June 05, 2015; **Accepted:** June 12, 2015.

Abstract: Screening of four indigenous AM fungi for improvement of plant growth, biomass production and nutrient uptake was undertaken on *Ocimum sanctum* L. the results revealed that, plants inoculated with *Rhizophagus fasciculatus* was found to be the most significant. After 30 days increased shoot length, dry weight of root and shoot, mycorrhizal colonization and spore number were recorded. Plants inoculated with AM fungus *R. fasciculatus* showed significantly more number of flowers, compared to other AM fungi treated plants. The indigenous species, *Rhizophagus fasciculatus* was the best species among four species tested. Hence, it can be concluded that experimental plant showed varied response to different AM fungi and *Rhizophagus fasciculatus* confers maximum growth benefits compared to all other fungi used in this study.

Key Words: *Ocimum sanctum* L., Arbuscular mycorrhizal fungi, Biomass production, Nutrient uptake, per cent root colonization, and spore number.

Introduction

Mycorrhizal symbiotic association increases the supply of mineral nutrients to the plant, particularly those ionic forms have a poor mobility rate or those which are present in low concentration in the soil and thus promote plant growth (Erocolin and Reinhardt, 2011). Mycorrhizae involve plant exchange of photolsynthates in return for fungal exchange of mineral nutrients. The convergence of so many unrelated forms of mycorrhizas is a testament for the mutual benefits of these trading partnerships. Arbuscular mycorrhizal fungi (AMF) are obligate symbionts that require plant as host to complete their cycle and produce spores (Smith and Read, 1997). The most important factors for AM fungal symbiosis with plants root system are soil pH (Clark, 1997), soil phosphorus level, plant species as well as inoculums levels (Schroeder and Janes, 2005). The lack of demonstrated benefit may be due to the use of inappropriate strains of AM fungi, relatively high available P in the soil, inability of introduced AM fungi to establish in the soil and large variation in rates of plant growth (Jasper, 1994).

It is known mycorrhizal colonization effect plant growth and development owing to plant nutrition elements that are provided by mycorrhizae in many plants (Cavagnara *et al*., 2006; Singh *et al*., 2008 Lakshman, 2009; Lakshman and Kadam, 2011). Thus mycorrhizal symbioses physically and chemically structure the rhizosphere, and they impact communities and ecosystems (Brundert, 2002). The current day emphasis is on sustainable environment, which uses less of chemical inputs like fertilizers and pesticides having adverse effect on soil health, fertility and environment. Thus, use of microbial inoculants play an important role in sustainable development. AM fungi are known to improve the nutritional status, growth and development of plants, protest plants against root pathogens and offer resistance to drought and salinity (Jeffries, 1987).

Ocimum sanctum L., is an under shrub with enormous medicinal value. It is commonly known as tulasi. It is used as stimulant, diaphoretic and expectorant. Used in catarrh, bronchitis, ringworm, cutaneous disease, stomach aches, malarial fevers, genital urinary disorders and asthma etc. Plant extracts showed antifungal, antibacterial properties against *Aspergillus flavus*, *A. parasitica*, *Mycobacterium tuberculosis*, *Salmonella typhosa, Escherichia coli* and *Micrococcus pyrogens*. So far no studies were carried out with AM fungal inoculation on *Ocimum sanctum* L. Hence the present study was undertaken with aim for selecting efficient AM fungus to improve plant growth and biomass production under experimental condition.

***Corresponding Author:**
C. Maya,
C/o Dr.H.C.Lakshman,
Professor and Former Chairman,
P.G.Department of Studies in Botany,
Karnatak University, Dharwad- 580 003,
Karnataka, INDIA.

Materials and Methods

Soil and selection of plant material

The soil physico-chemical properties of soil used for pot experiments were estimated according to Jackson (1973) and shown in Table 1. The soil; sand (3:1) v/v) mixture was filled into 15X20 cm diameter earthen pots containing 3 kg of soil. The seeds of *Ocimum sanctum* L., were surface sterilized by treating them with 0.2% sodium hypochlorite for 2-3 min and washed in sterile water for 2-3 times before sowing.

Inoculation of AM fungi

The pure culture of four AM fungal species namely *Rhizophagus fasciculatus* (Thaxte) Gerdmann and Trappe emend., Walker and Koske, *Sclerocystis dussi* (Patoullard.) von Hohnel, *Acaulospora laevies* Gerd & Trappe. and *Gigaspora margarita* Becker & Koske., were mass multiplied in 32 cm diameter pots containing 8 kg sterilized sand : soil (1:1 v/v) mixture as the substrate and *Sorghum vulgare* L., (Jowar) as the host in polyhouse, Department of Botany, Karnatak University, Dharwad. After 60 days of growth, shoots of Jowar were, chopped off and the potting mixture containing spores, colonized root bits, hyphae, reproductive propagules was served as AM fungal inoculum. 10 g of this mycorrhizal inoculum was applied to the planting area at a depth of about 4 cm below the surface of the potting mixture (except control) before sowing the seeds.

Treatments and experimental design

The experiment was completely randomized block design with triplicates per treatment and non-inoculated control without inoculation was maintained. The treatments were as follows.

A. Noninoculated control
B. *Rhizophagus fasciculatus*
C. *Gigaspora margarita*
D. *Sclerocystis dussii*
E. *Acaulospra laevis*

The pots were treated with 10 ml of Hoagland solution without P at an interval of l5 days. Experimental plants were exposed to sunlight and were kept free of weeds and irrigated properly. The plants were harvested after 30, 60 and 90 days after sowing to analyze the growth parameters. The percentage of mycorrhizal infection was determined microscopically by following clearing of roots in 10% KOH, neutralized in 2% HCl and stained with 0.05% trypan blue in lactophenol according to method describe by Phillips and Hayman, (1970) and mycorrhizal root colonization was calculated by using following formula,

$$\text{Percent of root Colonization (\%)} = \frac{\text{No.of root bits colonization}}{\text{Total number of root bits observed}} \times 100$$

The growth parameters like; shoot length, fresh weight of shoot, dry weight of root, dry eight of shoot, number of leaves, number of leaves, number of flowers were measured. Shoot and dry weight were determined after drying the plant samples in a hot air oven at 70^{o} C constantly for l2 hours till the material attains constant weight. The AM fungal spores were counted in 50 g of soil by using wet-sieving and decanting method (Gerdemann and Nicolson, 1963).

Results

Inoculation of AM fungal species such *Rhizophagus fasciculatus, Gigaspora margarita, Sclerocystis dussii* and *Acaulospora laevis*, have clearly proved an increased shoot length, fresh and dry weight of shoot, root length, fresh and dry weight of root, number of leaves, number of flowers, number of fruits, root colonization, spore number and stem diameter the results. The effect of inoculation with AM fungi on the growth of *Ocimum sanctum* L., are presented in Table 1. All the treatments showed a positive effect on the growth of *Ocimum sanctum* L., However, among the treatments plants inoculated with *Rhizophagus fasciculatus* was found to be the most significant. After 30 days, *Rhizophagus fasciculatus* appeared to be more effective in increasing plant height (9.60 cm) than *Gigaspora margarita* (8.26 cm) *Sclerocystis dussii* (7.50 cm) and *Acaulospora laevis* (8.43 cm). The dry weight of shoot (0.78 g) was higher in plants inoculated with *Rhizophagus fasciculatus* than the inoculated with other stairs such as *Gigaspora margarita, Sclerocystis dussii and Acaulospora laevis.* The highest mycorrhizal root colonization (38.46%) and spore number (68.16) were recorded in plants root and rhizosphere respectively inoculated with AM fungus *Rhizophagus fasciculatus*.

Table 1: Effect of different AM fungi on growth and nutrient uptake of *Ocimum sanctum* L. at different intervals

Treatment duration	Plant height	DWS (g)	DWR	PMC	MSN/50g soil.	Micro and macronutrients in shoot mg /g					Micro and macronutrients in root mg/g			
						N	P	K	Zn	Mg	N	P	K	Zn
30 Days														
NM	4.367a	0.280a	0.227a	0.000a	0.000a	3.153a	1.517a	0.650a	0.005a	0.006b	1.400a	0.647a	0.270a	0.003a
Gigaspora marginata	8.267bc	0.600b	0.260b	34.667b	61.067bc	4.017b	2.410bc	0.970b	0.008c	0.005a	2.350d	1.100c	0.5 l0e	0.01 Id
R. fasciculatus	9.600d	0.780e	0.370e	38.467d	68.167e	4.950d	2.670d	1.230d	0.01 Id	0.005a	2.083b	1.903d	0.410b	0.007c
A. laevis	8.433cd	0.717d	0.330d	36.233cd	62.200bd	4.210c	2.300b	0.970b	0.008c	0.005a	2.323d	0.923b	0.460d	0.006b
Sclerocystis dussii	7.500b	0.677c	0.273bc	35.400bc	60.300b	4.720S	2.540cd	l.000bc	0.007b	0.006b	2.250c	0.953bc	0.420be	0.006b
60 Days														
NM	13.533a	0.670a	0.280a	0.000a	0.00a	7.880a	4.240a	2.030a	0.026a	0.043e	3.987a	1.830a	0.923a	0.016a
Gigaspora marginata	34.367cd	0.883cd	0.430b	55.200b	85.70bcd	9.303bd	5.940bc	2.813b	0.044b	0.039cd	4.987b	2.317b	1.440b	0.025b
R. fasciculatus	34.400e	0.920d	0.450b	57.533b	89.40e	9.800e	6.033d	2.977b	0.068e	0.036b	5.480e	3.193e	1.603c	0.036d
A. laevis	32.500bc	0.840bc	0.407b	54.400b	85.133	9.273b	5.953bc	2.820b	0.048c	0.038bc	5.383c	2.617c	1.547c	0.028cd
Sclerocystis dussii	30.733b	0.820b	0.377ab	53.533b	84.10bc	9.300bc	5.960b	2.807b	0.057d	0.029a	5.357d	2.890d	1.587c	0.026bc
90 Days														
NM	18.400a	0.720a	0.330a	0.000a	0.000a	9.700a	4.923a	3.150a	0.034a	0.083d	5.083a	2.267a	1.613a	0.027a
Gigaspora marginata	41.933b	0.930b	0.473b	61.867bc	102.00d	11.700b	5.530b	3.783b	0.067b	0.080bc	5.667b	2.850b	1.877b	0.043b
R. fasciculatus	48.000c	1.017d	0.6 lOe	67.200c	113.00bc	12.080b	6.233e	3.963e	0.093e	0.081ed	6.037e	3.273e	2.040e	0.057d
A. laevis	44.133cd	0.973bcd	0.553cd	63.267bc	115.00bc	11.667b	5.700c	3.857cd	0.072bc	0.071a	5.943c	3.020c	1.990d	0.051c
Sclerocystis dussii	43.167c	0.947bc	0.550c	61.367b	110.10b	11.727b	5.800cd	3.850c	0.080d	0.078b	5.957cda	3.057cd	1.910c	0.051c

Mean values followed by the same letter within a column do not differ significantly at P = 0.05 according to DMRT.

It was after 60 days, the inoculation of *Rhizophagus fasciculatus* showed significantly increased plant height (34.40 cm), dry weight of shoot (0.92 g) whereas, *Sclerocystis dussii* was showed least dry weight of root (0.82 g). Highest root colonization was recorded (57.53%) with AM fungus *Rhizophagus fasciculatus* inoculation compared to other AM fungal strains inoculated and control treatment. Significant spore number (89.40) was recorded in the rhizosphere with *R. fasciculatus* inoculation compared to the inoculation of *Sclerocystis dussii* and *Acaulospora laevis.* Plants were harvested at 90 days after sowing; plants growth with *Rhizophagus fasciculatus* had showed significantly increased plant height (48.00 cm), dry weight of shoot (1.01 g), dry weight of root (0.61 g), root colonization (67.20%), spore number per 50g soil (113).

Experimental results also revealed that, plants inoculated with different AM fungi, had more nutrient uptake compared to non-mycorrhizal plants (Table 1). It was observed that, increased uptake such as macro-elements and micro-elements by *Ocimum sanctum* L., was maximum with AM fungus *R. fasciculatus* inoculation over the remaining AM fungi inoculated plants and non-mycorrhizal ones (Table 1).

Discussion

The current investigation showed that AM fungus *R. fasciculatus* was the most efficient AM fungus for promoting plant growth parameters such as, shoot length, root length, fresh and dry weight of shoot and root, stem diameter, number of flowers, leaves and per cent root colonization and spore number of *Ocimum sanctum* L. The present results in the par with the results obtained by many researchers, (Powell *et al*., 1985; Lakshman, 1996; Clark, 1997). They have reported that the main benefits of AM fungi are enhanced plant acquisition of toxic elements to growth. Host preference among fungi has been reported by earlier workers (Miller and Jastraw, 1992; Mamatha and Bagyaraj, 2001). Hence, there is need for inoculating different mycotrophic plants has been stressed (Jeffries, 1987; Miller *et al*., 1987).

Rhizophagus fasciculatus enhanced the plant height as compared to all other treatments *Gigaspora margarita, Sclerocystis dussii and Acaulospora laevis* inoculation. Similar findings were reported by Kabir and Koide (2000), who have showed, that mycorrhizal inoculation increased the flower quality of ornamental plants and shortened flowering times compared to non- AM fungal plants. Mycorrhizal fungi are also implicated in improving the soil structure by increasing the soil aggregation by their hyphae (Miller and Jastrow, 1992). Soil aggregation is a measure of the amount of extrametrical hyphae, which is in turn related to the efficiency of the fungus (Kaya *et al*., 2003). Improved plant height, Branching and spread because of AM fungal inoculation have been reported in other medicinal plants like *Coleus* (Mulla and Lakshman, 2003) and Lakshman, 2008) have showed that mycorrhizal inoculation increased plant dry weight. The enhancement in growth and nutritional status as also related to mycorrhizal root colonization and spore numbers in the root zone soil. This upholds the observations made by earlier workers on other plants (Gracy *et al*., 2005)

Endomycorrhizal fungi species were able to colonize *Ocimum sanctum* L., roots and increase the plant growth. Present findings were in accordance with the reports of Estaunet *et al*., (2003). Plant biomass is an important parameter for selecting AM fungus for its symbiotic efficiency (Nogales *et al*., 2009). Several workers have reported beneficial effect of AM fungi on plant biomass (Lakshman and Kadam, 2011). The existence of host preference by AM fungi has been investigated by several researchers which provide support for the argument that different AM fungi produce markedly different levels of root colonization, growth rates and nutritional responses in some plant species compared to others (Linderman, 1994). The extent of colonization and the spore count varied with different AM fungi. However, Pare *et al*., (2000), working with several banana cultivars and AM fungi, they observed that different growth promotional effects depends on the banana cultivar and the *Glomus* strain the quality of inoculums also is important. From besides some fungi have different colonization patterns and different effects on host plant growth consistent with early works contributions (Vasanthkrishna *et al*., 1995; Smith, 2003; Lakshman, 2008), it is clear that different mycorrhizal species have different root colonization capacity and also have different influence on plant growth. Hence it can be concluded that tulasi plants

showed varied responses to different AM fungi and *Rhizophagus fasciculatus* confers maximum growth benefits compared to all other fungi used in this study.

The positive effects of root colonization on increasing spore number were found there was no correlation established between spore numbers. There is also evidence that intensive root colonization of host resulted in the better growth in term of dry matter (Azcon-Aguliar, 1997). Thus, *Rhizophagus fasciculatus* most efficient fungus and secondly *Gigaspora margarita* can be considered as to be the most promising symbiont for inoculating *Ocimum sanctum* L.

Conclusion

Inoculation with Arbuscular mycorrhizal fungi was significantly enhanced increased shoot and root length. The indigenous species, *Rhizophagus fasciculatus* was the best species among four species tested. Hence, it can be concluded that experimental plant showed varied response to different AM fungi and *Rhizophagus fasciculatus* confers maximum growth benefits compared to all other fungi used in this study. The majority of research on this area has focused on mycorrhizal formation and nutrient acquisition under extreme changes in water amounts, temperature, pH and inorganic nutrient availability. However for sustainable cultivation of *Ocimum sanctum* L*.,* by using screening of efficient strains of is needed in different agro-climatic conditions in field trails are needed to be focused is warranted in future research.

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Source of support: Nil
Conflict of interest: None Declared