

Beneficial Influence of Arbuscular Mycorrhizal Fungal Association on Growth, Yield and Nutrient Uptake of Rose-Scented Geranium (*Pelargonium* Species)

G. Venkateshwar Rao¹, C. Manoharachary¹ and B.R. Rajeswara Rao²

¹Department of Botany, Osmania University, Hyderabad 500 007, India
²Central Institute of Medicinal and Aromatic Plants, Field Station
Boduppal, Uppal (PO), Hyderabad 500 039, India

Rose-scented geranium (*Pelargonium* species, Family: *Geraniaceae*) cv. Bourbon grown in a P deficient red sandy loam soil (alfic ustochrept) was inoculated with a mixed inoculum of arbuscular mycorrhizal fungi consisting of *Acaulospora laevis*, *Gigaspora margarita*, *Glomus fasciculatum* and *Glomus mosseae*. The influence of AM fungi on growth, shoot biomass yield, root biomass yield, essential oil yield and nitrogen (N), phosphorus (P) and potassium (K) uptake of the crop was studied. Rose-scented geranium plants grown in inoculated soil exhibited root colonization with AM fungi, which increased from 18.3% at 30 days to 52.6% at 90 days after planting. No root colonization was observed in control plants grown in non-inoculated soil. Mycorrhizal plants recorded significantly longer roots (53.3% longer than control plants), taller plants (19.4%), more number of leaves (13.0%) greater root biomass yield (166.1%), higher shoot biomass yield (11.7%) and higher essential oil yield (21.1%) in comparison to non-mycorrhizal plants. Similarly, the N, P, K uptake by roots (N: 200.0%, P: 200.0%, K: 418.8%, higher than control plants) and shoots (N: 24.5%, P: 46.4%, K: 37.0% greater than control plants) of mycorrhizal plants were significantly greater than non-mycorrhizal plants. This is the first report on the beneficial influence of AM fungi on rose-scented geranium.

Keywords: Rose-scented geranium *Pelargonium* sp., arbuscular mycorrhizal fungi, biomass yield, essential oil yield, nutrient uptake

Arbuscular mycorrhizal (AM) fungi are widely distributed in many agro-ecosystems and form obligate symbiotic association with the roots and other underground parts of most of the angiospermic plants (Bagyaraj & Manjunath 1997, Harley & Smith 1983, Manorek et al. 1982, Powell & Bagyaraj 1984, Safir 1987, Sanders et al. 1975). They have gained considerable importance in recent years owing to their beneficial influence in improving crop productivity, plant resistance to diseases and tolerance to heavy metal toxicity (Bagyaraj & Manjunath 1997, George et al.

1994, Harley & Smith 1983, Manorek et al. 1982). The mycorrhizas, on the other hand, receive carbohydrates from the plants (George et al. 1994). The beneficial effects from AM fungi were attributed to the direct effect of better plant nutrition through acquisition of less mobile nutrients phosphorus (P), zinc (Zn), copper (Cu) and sometimes ammonium (NH₄⁺) by the hyphae of AM fungi from the soil and their subsequent transfer to the host plant and to the indirect effect through altered plant morphology and/or physiology (George et al. 1994, Kothari et al. 1990). Though, there is voluminous literature on the influence of AM fungi on other crops, information on economically important aromatic crops is limited.

^{*}Corresponding author: drgvp04@yahoo.com

Rose-scented geranium (*Pelargonium* species, family: Geraniaceae) is an important aromatic crop, the essential oil of which is extracted from the freshly harvested shoot biomass by steam distillation. The highly priced (Rs. 3500-5000/kg) essential oil and the aroma chemical rhodinol (mixture of geraniol, citronellol and other alcohols present in the oil) is dated from the volatile oil by fractional distillation, are extensively used in fragrance industry and in aromatherapy. The fragrant oil is sparingly used in the flavor industry also. The crop is commercially cultivated in a number of countries and the world production is less than the demand offering opportunities for increasing the production of the oil. In India, rose-scented geranium is cultivated in red soils generally deficient in phosphorus and other nutrients and attempts are being made to increase the production of essential oil. Our investigations on this crop revealed that the crop responds to application of mineral fertilizers, plant growth regulators and other agricultural inputs (Bhattacharya & Rajeswara Rao 1996, Rajeswara Rao et al. 1990 a, b), but its response to arbuscular mycorrhizal fungi is not known, though the positive influence of AM fungi on growth and nutrients acquisition by some ornamental geraniums were reported (Biermann & Lindermann 1983, Boerner 1990). Enhancement in growth, biomass and essential oil yields and nutrient uptake were recorded in other aromatic crops namely, palmarosa (*Cymbopogon martinii* (Roxb.) Wats. Var. *molle* Burk.) (Gupta & Janardhanan 1990, Gupta et al. 1990), citronella (*Cymbopogon winterianus* Jowitt.) (Kothari & Singh 1995) different mint (*Mentha* species (Khaliq & Janardhanan 1997, Kothari et al. 1999), *Salvia*

officinalis, *Artemisia dracunculus*, *Thymus vulgaris*, and *Ocimum basilicum* (Camprubi et al. 1990), when these crops were grown in soils inoculated with AM fungi. Rose-scented geranium is cultivated in India in red soils generally deficient in N (Nitrogen), P (Phosphorus) and Zn (Zinc). The influence of inoculating the soil with AM fungi (found associated with this crop under field conditions (Venkateshwar Rao et al. 2000)) on growth, biomass yield, essential oil yield and nutrient uptake of the crop was investigated in the present day.

Materials and Methods

Experimental design

The experiment was conducted with 6 treatments namely, combinations of inoculated (with a mixed inoculum) and non-inoculated treatments with 3 crop growth stages (30, 60, and 90 days after planting). Each treatment was replicated 3 times. Therefore, there were 18 pots (2 inoculated treatments x 3 crop growth stages x 3 replications) arranged in factorial randomized block design.

Isolation and multiplication of AM fungi

Rhizosphere soil samples of rose-scented geranium cv. Bourbon (Figure 1) growing at the Central Institute of Medicinal and Aromatic Plants Field Station, Hyderabad, India were collected. The rhizosphere soil sample was screened employing wet sieving and decanting technique of Gerdemann &



Figure 1. Field view of rose-scented geranium crop.

Nicolson (1963) for the presence of AM fungal spores. 100 g sample taken in a beaker, mixed with 400 ml of lukewarm water and a pinch of sodium hexametaphosphate was manually shaken for 10 minutes until all soil aggregates dispersed leaving a uniform suspension. Sieves of 710 μ m, 420 μ m, 250 μ m, 105 μ m, and 45 μ m sizes were arranged in descending order. The contents of the beaker were decanted through the sieves 4-5 times till only sand and gravel were left in the beaker. The contents of each sieve starting with 420 μ m were carefully collected into separate beakers using level pipes. They were filtered separately through a single layer of imported synthetic fiber white cloth. The white cloths were kept in separate petridishes containing distilled water. These were individually observed under stereo binocular dissecting microscope. The spores and sporocarps of AM fungi present in the petridishes were collected with the help of microneedles. They were mounted on to slides employing polyvinyl lactic acid as medium. Each slide was examined under high power research microscope for identification and isolation into different species. Color, size, shape, wall characteristics, contents and surface, ornamentation of the spores, nature and size

of the subtending hyphae, bulbous suspensor nature of spore, number and arrangement of spores in the sporocarps, presence or absence of peridium for the sporocarps etc. as described by Schenck & Perez (1990) were the criteria employed for identification of the AM fungal species. Four species of AM fungi namely, *Acaulospora laevis* Gerd. and Trappe; *Gigaspora margarita* Becker and Hall.; *Glomus fasciculatum* (Thaxter) Gerd. and Trappe emend. and *Glomus mosseae* (Nicol. & Gerd.) Gerd. and Trappe were identified (Figure 2).

Cenchrus ciliaris Linn. (Family. Poaceae) is a seed propagated xerophytic grass growing to a height of 100 cm. It is used as a soil binder for controlling soil erosion and as a fodder for domestic animals. With a rapidly growing fibrous root system, it was observed to be a good trap plant for AM fungal multiplication (Bagyaraj 1992). The four species of AM fungi were multiplied by colonizing them on *Cenchrus ciliaris* in pot culture (using rhizosphere soil) employing the funnel technique of Menge & Timmer (1982). After 90 days, the aerial parts of *Cenchrus ciliaris* were harvested and the rhizosphere soil with chopped root bits containing the AM fungi were used as inoculum for the pot culture study.

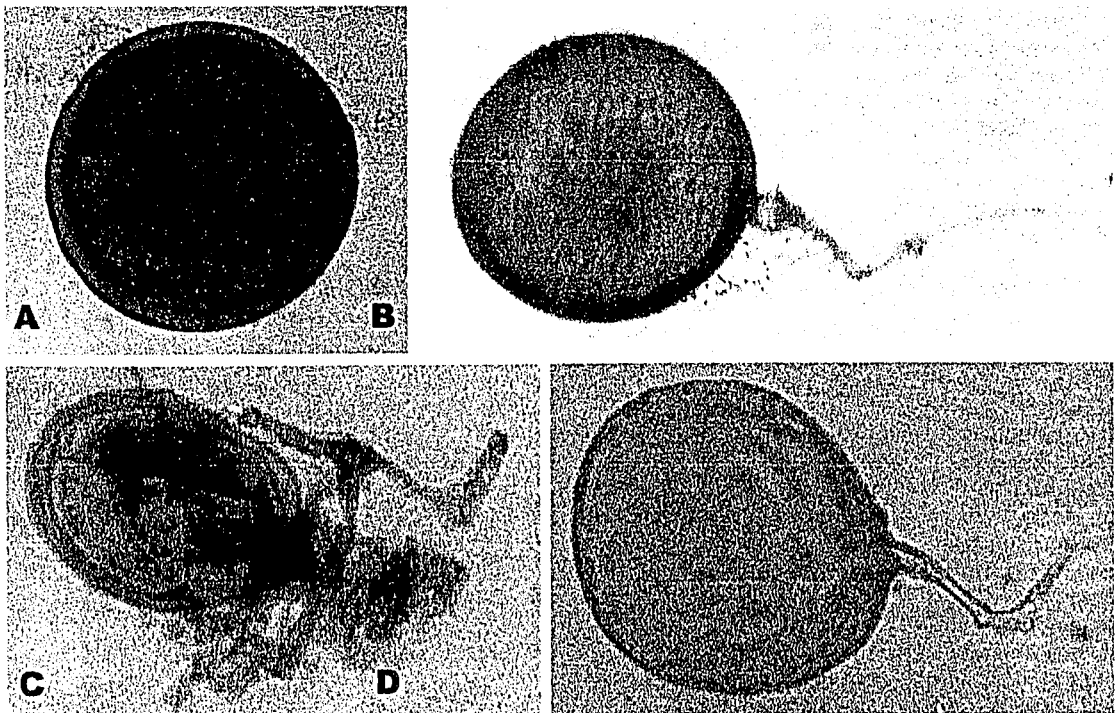


Figure 2. (a) Azygospore of *Acaulospora laevis* (x 100), (b) Azygospore of *Gigaspora margarita* (x 100), (c) Spore of *Glomus fasciculatum* (x 100) and (d) Spore of *Glomus mosseae* (x 200).

Soil preparation and characterization

Red sandy loam (alfic ustochrept) soil having 6.7 pH (1:2.5 soil to water ratio), 0.34% organic carbon, 0.19% available N, 1.8 mg/kg 0.5M sodium bicarbonate extractable P, 133 mg/kg 1N ammonium acetate extractable K and 0.5 mg/kg DTPA (diethylene triamine penta acetic acid) extractable Zn, sieved through 4mm sieve and autoclaved at 121°C for 2 hours was used for the experiment. Eighteen earthen pots of 25 cm diameter (at the top) and 25 cm height were surface sterilized and used for the pot culture study.

Preparation of cuttings and inoculum

Each of the 9 pots of non-inoculation (control) treatments were filled with 8 kg soil +80g autoclaved rhizosphere soil not containing any AM fungi. The other 9 pots of inoculation treatments were filled with 8 kg soil and 80g of rhizosphere soil of *Cenchrus ciliaris* containing a minimum of 160 spores (2 spores/g) of the 4 AM fungi. The rhizosphere soil was thoroughly mixed with the red soil before filling into the pots. In addition, 50 g chopped root bits of *Cenchrus ciliaris* with more than 60% colonization were placed near the root zone of the cuttings of rose-scented geranium in inoculated pots. The control pots received filtered (filter paper Whatman No. 1) extract of the mixed inoculum containing comparable microflora other than mycorrhizal fungi (Kothari et al. 1990, 1991).

Terminal stem cuttings (15 cm in length and having 2-3 terminal leaves) of rose-scented geranium cv. Bourbon were rooted in polythene bags filled with autoclaved red sandy loam soil and kept under partial shade. The cuttings were watered daily with distilled water. Fifty days old, well rooted, uniformly and vigorously growing cuttings were planted at the rate of one cutting per pot.

Cuttings maintenance and harvest

The pots were watered regularly with sterilized distilled water (1 liter/pot). Modified Hoagland's solution (KNO₃: 7.0; CA (NO₃)₂: 4.0; MgSO₄: 2.0; KCl: 1.0; H₂BO₃: 1.0; ZnSO₄: 1.0; CuSO₄: 1.0; Fe-EDTA: 2.0 and (NH₄)₂MoO₄: 1.0 ml/liter) without phosphorus and manganese was added once a week at the rate of 25ml per pot.

At 30, 60 and 90 days after planting, the plants (each time 6 pots were sampled, namely inoculated and non-inoculated x 3 replications) were taken out from the pots along with complete root system. Plant parameters such as root length, plant height and number of leaves/plant were measured. The roots were separated from the shoots, carefully washed free from soil under a stream of cold tap water, chopped into small bits (2-3 cm length), mixed uniformly and their

fresh weights were recorded. Similarly, the fresh shoot weights were also recorded. Samples of washed roots were collected from each pot for determination of mycorrhizal colonization. The roots were cut into approximately 1 cm length. From each pot, 25 root bits (fresh weight: 7.5 mg and dry weight: 3.5 mg) were taken for colonization studies.

Assessment of mycorrhizal infection

The root bits were cleared with 10% potassium hydroxide for 20 min at 80°C, washed with several changes of water, kept in 5N hydrochloric acid for 5 min to neutralize the alkali, washed 3-4 times with water, stained with 0.05% lactophenol (lactic acid: phenol : glycerol : water in 1:1:2:1 proportion) - trypan blue (Phillips & Hayman 1970), gently squashed under a cover slip and examined under a light microscope for root infection with AM fungi. The root colonization % was computed by the grid line intersect method (Giovannetti & Mosse 1980). The AM fungal spores were isolated from the rhizosphere soil by wet-sieving and decanting method (Gerdemann & Nicolson 1963) as described in isolation and multiplication of AM fungi section. Morphology of the spores, sporocarps, mycelia, vesicles and arbuscules were studied to identify the AM fungi (Schenck & Perez 1990).

Nutrient analyses

At each sampling date, dry weights of roots and shoots were recorded after drying at 70°C for 48 hours. Root and shoot samples were ground to pass through a 0.2 mm sieve and analyzed for N, P, K concentrations (%) following standard procedures (Jackson 1973). N, P, K uptake (mg/plant) were calculated by multiplying dry matter of the plants with the corresponding nutrient concentrations.

Estimation of the contribution of AM fungi on nutrient uptake

Percent contribution of AM fungi to the P uptake of the plants was computed by using the formula (Kothari et al. 1991):

$$\frac{A - B}{A} \times 100$$

Where A = P uptake by plants grown in AM fungi inoculated soil.

B = P uptake by plants grown in mycorrhiza non-inoculated soil.

Determination of essential oil

At the last sampling date (90 days after planting), shoot biomass samples were distilled in Clevenger apparatus (Clevenger 1928) for estimation of essential

oil concentration (%). Essential oil (economic) yield values were worked out by multiplying shoot biomass yields with essential oil concentration and density of the essential oil.

Statistical analyses

The data were statistically analyzed employing analysis of variance (ANOVA) technique as applicable to factorial randomized block design (Cochran and Cox 1959). Treatment means were compared by least significant difference (LSD) at 5% level of probability ($P = 0.05$). The interaction effects were identical to the main effects, hence not presented and discussed. The ANOVA table used for the analyses is shown below:

Factor	Degrees of Freedom
Replication	2
Inoculated vs non-inoculated	1
Crop growth stages	2
Interaction	2
Error	10
Total	17

Results

Plants grown in mycorrhiza inoculated soil exhibited root colonization (Figure 3), while no colonization of roots by AM fungi was observed in control plants. The root colonization in mycorrhizal plants increased from 18.3% at 30 days to 27.8% at 60 days and 52.6% at 90 days after planting.

Nutrient concentration and nutrient uptake

Shoots accumulated higher amounts of nutrients than roots (Tables 1, 2). This was expected, as nutrients absorbed by roots would have been translocated to shoots for metabolic processes of the plant. The concentration and uptake of the nutrients were in the following order: $K > N > P$ in shoots and roots at all stages in mycorrhizal and non-mycorrhizal plants. The concentration and uptake of nutrients in roots and shoots increased with crop age due to increased root and shoot dry weights at these stages.

Shoots and roots of plants grown in AM fungi inoculated soil recorded significantly higher concentration of all the nutrients and removed significantly greater amounts of these nutrients from the soil. Mycorrhizal shoots removed 24.5%, 46.4%, 37.0% higher amount of N, P and K, respectively in comparison to non-mycorrhizal shoots. Mycorrhizal roots removed 200.0%, 200.0%, 418.8% greater amounts of N, P and K, respectively over non-mycorrhizal roots. The contribution by AM fungi to the P uptake of mycorrhizal plants varied from 27.0 – 37.4% in shoots to 50.0 – 77.0% in roots at different stages of plant growth.

Growth and yields of rose-scented geranium

Soil inoculation with AM fungi significantly increased root length (53.3%), plant height (19.4%) (Figure 4), number of leaves (only well developed leaves were counted / plant) (13.0%), root biomass yield (166.1%), shoot biomass yield (11.7%), essential



Figure 3. AM fungi colonized root of inoculated rose-scented geranium plant showing vesicles and arbuscules ($\times 200$).

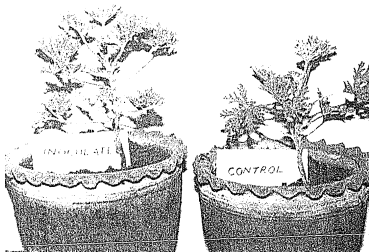


Figure 4. Mycorrhizal inoculated and non-inoculated (control) plants of rose-scented geranium.

oil concentration (10.0%) and essential oil yield (21.1%) of inoculated rose-scented geranium plants in comparison to control plants (Table 3). The shoot: root ratios were 200.7 in mycorrhizal plants as against 478.0 in non-mycorrhizal plants. The above plant parameters increased with the age of the plants.

Discussion

The red sandy loam soil used in the experiment was deficient in P and Zn. Further P deficient conditions were maintained by supplying Hoagland's solution without P. Under these conditions, rose-scented geranium behaved like other aromatic crops (Gupta & Janardhanan 1990, Gupta et al. 1990, Khalq & Janardhanan 1997, Kothari & Singh 1996, Kothari et al. 1999) in harboring colonization of its roots by AM fungi present in the inoculum. The increase in the root colonization % with crop age was due to the prevalence of P deficient conditions throughout the experimental duration, which encouraged rapid multiplication of AM fungi and subsequent increased colonization of the roots of the test crop.

The higher P uptake by mycorrhizal plants (Tables 1 and 2) corroborate with the findings of other researchers on aromatic crops like palmarosa

(Gupta & Janardhanan 1990), citronella (Kothari & Singh 1996) and bergamot mint (*Mentha citrata* Ehrh.) (Kothari et al. 1999). The higher P acquisition by mycorrhizal plants was generally attributed to: (1) production of large amounts of alkaline and acid phosphates by AM fungi during the course of infection that help the host plant in P absorption, (2) existence of surface P on AM fungi that enable them to obtain soil P more readily than non-mycorrhizal roots, (3) provision of additional or more efficient surface in fungal hyphae with subsequent transfer of absorbed P to the host, (4) longer viability of mycorrhizal than non-mycorrhizal roots and (5) Morphological and/or physiological changes in the plant (Tinker 1975). Though, mycorrhizal hyphae can acquire and translocate NH_4^+ (George et al. 1994) and K (Kothari et al. 1990) to host plant, experimental evidences are always not consistent unlike P acquisition. Therefore, it was argued that factors other than AM fungi, for example modified root morphology and/or physiology may be responsible for the higher uptake of these nutrients (Kothari et al. 1990, Kothari & Singh 1996). In the present investigation, increased root length and probably higher density of the mycorrhizal roots were possibly responsible for the higher uptake of N and K in mycorrhizal plants (Tables 1,2). Moreover, increased number of leaves in these plants would

Table 1. Concentration and uptake of N, P, K by rose-scented geranium shoots as influenced by AM fungal association at three stages (30, 60, 90 days after planting) of crop growth.

Treatments	Concentrations			Uptake	Plant		
	N	P	K		N	P	K
Mycorrhizal plants*	2.27	0.28	2.71	68.2	8.2	79.2	
Non-mycorrhizal plants	2.12	0.22	2.30	54.8	5.6	57.8	
LSD (P=0.05)	0.06	0.02	0.07	2.0	0.7	3.8	
Crop age (days)**							
30	1.36	0.23	2.31	29.5	4.9	50.4	
					(29.3)		
60	2.56	0.22	2.51	67.9	5.4	66.9	
					(27.0)		
90	2.68	0.29	2.69	87.1	9.4	88.2	
					(37.4)		
LSD (P=0.05)	0.07	0.03	0.09	2.5	0.9	4.6	

Figures in parenthesis are percent contribution by AM fungi to the uptake of the mycorrhizal plants.

LSD = Least Significant Difference

*Average values of 3 crop growth stages

**Average values of mycorrhizal and non-mycorrhizal treatments

Table 2. Concentration and uptake of N, P, K by rosescented geranium roots as influenced by AM fungal association at three stages (30, 60, 90 days after planting) of crop growth.

Treatments	Concentrations			Uptake	Plant		
	N	P	K		N	P	K
Mycorrhizal plants*	0.53	0.18	1.12	0.36	0.06	0.83	
Non-mycorrhizal plants	0.42	0.07	0.53	0.12	0.02	0.16	
LSD (P=0.05)	0.02	0.008	0.03	0.03	0.005	0.05	
Crop age (days)**							
30	0.37	0.07	0.45	0.08	0.01	0.10	
					(50.0)		
60	0.45	0.08	0.85	0.13	0.03	0.27	
					(50.0)		
90	0.61	0.10	1.20	0.50	0.08	1.10	
					(77.0)		
LSD (P=0.05)	0.03	0.005	0.03	0.03	0.006	0.06	

Figures in parenthesis are percent contribution by AM fungi to the uptake of the mycorrhizal plants.

LSD = Least Significant Difference

*Average values of 3 crop growth stages

**Average values of mycorrhizal and non-mycorrhizal treatments

have made greater transpirational demand for water leading to larger transpirational gradient and concomitant mass flow of water along with the mobile nutrients towards rhizosphere soil of the mycorrhizal roots and their subsequent absorption by the plant. Such effects have been described as indirect beneficial effects due to mycorrhizas (George et al. 1994, Kothari et al. 1990). Increased K uptake with mycorrhizal association was also observed in another aromatic crop palmarosa (Gupta et al. 1990).

Significant increases in root length, plant height, root and shoot biomass yields (Table 3) were primarily attributed to better P nutrition of the mycorrhizal plants, which is evident from the P uptake data. However, the increases could also be due to higher acquisition of other plant nutrients N and K. Significant increases in growth, biomass and

grain yields of crops through mycorrhizal association in P deficient soils were well documented (Bagyaraj & Manjunath 1997, George et al. 1994, Gupta & Janardhanan 1990, et al. 1990, Harley and Smith 1983, Maronek et al. 1982, Powell & Bagyaraj 1984). Mycorrhizal hyphae extend into the surrounding soil, explore the soil volume that is generally not within the reach of the plant roots, absorb relatively less mobile nutrients, especially P, Zn, Cu, and translocate the same to the host plant (George et al. 1994, Tinker 1975), thus effectively increasing the absorbing area of the mycorrhizal roots. In addition, AM fungi alter the morphology of the roots of the host plant (George et al. 1994, Kothari et al. 1990, 1991; Kothari & Singh 1996) such as increased root length, root diameter, number of roots, root weight etc. Improved root morphology leads to further enhancements in growth and yields

Table 3. Root length, plant height, number of leaves and yields of rose-scented geranium at three stages (30, 60, 90 days after planting) of crop growth as influenced by AM fungal association.

Treatments	Root length (cm)	Plant height (cm)	Number of leaves/plant	Root biomass yield (mg/plant)	Shoot biomass yield (g/plant)	Essential oil yield (mg/plant)
Mycorrhizal plants*	23	37.00	26	109.1	21.9	64.2 (0.22)
Non-mycorrhizal plants	15	31	23	41.0	19.6	53.0 (0.20)
LSD (P<0.05)	1.2	2.5	1.1	9.6	0.6	6.1 (0.005)
Crop age (days)**						
30	15	26	18	42.3	13.3	-
60	19	35	25	48.9	21.0	-
90	22	42.00	31	134.0	27.9	-
LSD (P<0.05)	1.5	3.2	1.4	11.8	0.8	-

Figures in parenthesis are essential oil concentration (%) in the plants at 90 days after planting.

LSD = Least Significant Difference

*Average values of 3 crop growth stages

**Average values of mycorrhizal and non-mycorrhizal treatments

of the host plant. In the present study, increase in root length was only 53.3 %, while the root biomass yield increased by 166.1% in mycorrhizal plants signifying that in addition to root length other root parameters also increased leading to enhanced root biomass yield and reduced shoot : root. The mycorrhizal plants equipped with an efficient root system acquired higher amount of nutrients from the soil and produced taller plants with more number of leaves which in turn resulted in significantly greater shoot biomass yields compared to the non-mycorrhizal plants (Table 3). Similar findings were reported in citronella (Kothari & Singh 1996), palmarosa (Gupta & Janardhanan 1990, Gupta et al. 1990) and different mint species (Khaliq & Janardhanan 1997, Kothari et al. 1999).

The increase in root fresh weight (166.1%) was much higher than that of shoot fresh weight (11.7%) in mycorrhizal plants. Similar results were reported by Kothari et al. (1999) in bergamot mint and were attributed to a larger effect of mycorrhizas on root than shoot growth.

The increase in root and shoot fresh weights with crop age were due to enhancements in root length, plant height, leaf number per plant and NPK uptake by the plants with the advancements of the crop age.

Essential oil is the marketable product of rose-scented geranium. Essential oil is a secondary plant metabolite synthesized from the products of photosynthesis (Croleton 1987). Mycorrhizal plants had significantly larger photosynthetic area through taller plants and more number of leaves per plant. This might have resulted in accumulation of higher amounts of photosynthetic products and their subsequent conversion to essential oil leading to higher essential oil concentration in mycorrhizal

plants. The higher essential oil yield in mycorrhizal plants was due to increased shoot biomass yield and essential oil concentration in the shoots of these plants compared to non-mycorrhizal plants.

Conclusions

Rose-scented geranium responded to soil inoculation of AM fungi through enhanced root colonization %, nutrient uptake, root length, plant height, number of leaves per plant, root and shoot biomass yields and essential oil yield. This is the first report on the beneficial association of AM fungi with rose-scented geranium.

Acknowledgements

The author thanks the Director, CIMAP, Lucknow and the Scientists-in-charge, CIMAP Field Station, Hyderabad, for the use of facilities.

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