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Influence of native arbuscular mycorrhizal fungi on growth, nutrition and phytochemical constituents of *Catharanthus roseus* (L.) G. Don.

Rajendran Srinivasan, Chinnavenkataraman Govindasamy*

Department of Oceanography and Costal Area Studies, School of Marine Sciences, Alagappa University, Thondi Campus–623 409, Tamilnadu, India

PEER REVIEW

Peer reviewer

Dr. K. Sivakumar, Associate Professor, Annamalai University, Tamil Nadu, India.

Tel: +91 9894801529

E-mail: Kshivam69@gmail.com

Comments

This is valuable research in which authors selected nine different AM fungi species such as *A. scrobiculata*, *Acaulospora marrowae*, *G. aggregatum*, *G. fasciculatum*, *G. geosporum*, *Gigaspora margarita*, *Gigaspora nigra*, *S. heterogama* and *Scutellospora pellucida* to be isolated and identified from the root zone soil of *C. roseus*. In addition, the phytochemical analyses showed high concentration of chlorophyll *a*, chlorophyll *b*, total chlorophyll, soluble sugar, reducing sugar phenols, ortho dihydroxy-phenols lipids proteins and amino acids in *G. aggregatum* inoculated *C. roseus*. *G. aggregatum* was found to perform better on growth when compared to others and phytochemical constituents of *C. roseus*.

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ABSTRACT

Objective: To study the isolation, identification, mass production and the effect of native arbuscular mycorrhizal fungi (AM fungi) on growth parameters of the *Catharanthus roseus* (*C. roseus*).

Methods: A total of nine different AM fungi species such as *Acaulospora scrobiculata*, *Acaulospora marrowae*, *Glomus aggregatum* (*G. aggregatum*), *Glomus fasciculatum*, *Glomus geosporum*, *Gigaspora margarita*, *Gigaspora nigra*, *Scutellospora heterogama* and *Scutellospora pellucida* were isolated and identified from the root zone soil of *C. roseus*.

Results: The phytochemical analyses showed high concentration of chlorophyll *a* (0.152 ± 0.0140 µg/g), chlorophyll *b* (0.081 ± 0.006 µg/g), total chlorophyll (0.233 ± 0.020 µg/g), soluble sugar (0.051 ± 0.004 µg/g), reducing sugar (0.060 ± 0.007 µg/g), phenols (0.293 ± 0.032 µg/g), ortho-dihydroxy phenols (0.275 ± 0.022 µg/g), lipids (0.300 ± 0.025 µg/g), proteins (0.063 ± 0.003 µg/g) and amino acids (1.042 ± 0.056 µg/g) in *G. aggregatum* inoculated *C. roseus*. *G. aggregatum* was found to perform better on growth when compared to others and phytochemical constituents of *C. roseus*.

Conclusions: It is concluded from the present findings that the *G. aggregatum* and *Glomus fasciculatum* can be used as a potential growth promoters for the *C. roseus* for better yielding in the agricultural sectors.

KEYWORDS

Arbuscular mycorrhizal fungi, *Catharanthus roseus*, Growth promoters

1. Introduction

The organisms that are suitable for the growth promoting

of various medicinal plants and agricultural crop production systems include free living, associate and symbiotic nitrogen fixers, phosphate solubilizers mycorrhizal fungi, cellulolytic

*Corresponding author: Chinnavenkataraman Govindasamy, Department of Oceanography and Costal Area Studies, School of Marine Sciences, Alagappa University, Thondi Campus–623 409, Tamilnadu, India.

Tel: +91-9444256247

Fax: +91-4565-225202

E-mail: sandalsrini@gmail.com; govindasamyocas@gmail.com

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and hormone producers. Various soil bioinoculants perform specific functions such as biological nitrogen fixation, nitrification, denitrification, biocontrol of root pathogens, phosphate solubilization and mobilization, cellulose and ligning decomposition, and production of growth promoting hormones in the rhizosphere region of the plants. Previous studies revealed that the inoculation of arbuscular mycorrhizal fungi (AM fungi) with agricultural crops has potentially increased the growth of root and shoot biomass. Moreover agricultural importance of AM fungi has been reported by several authors^[1,2]. Several studies have been done by various workers on the role of AM fungi in medicinal plants^[3–5]. The importance of AM fungi in the growth, nutritional uptake and productivity in various agricultural crops has been well documented^[6–8]. However, studies on the growth promoting effect of AM fungi on agricultural crop plants are too limited. In this connection, the present study made an attempt to isolate the AM fungi and its growth promoting effects on *Catharanthus roseus* (*C. roseus*).

2. Materials and methods

2.1. Sample collection

C. roseus field rhizosphere soil samples were collected from the herbal garden at Poondi (Lat: 11° 28'N; Long: 79° 38'E) (Station I) and Throwpathi amman temple garden in Thiruchotruthurai (Lat: 10° 8'N; Long: 79°1'E) (Station II), Thanjavur district of Tamilnadu, India.

2.2. Isolation of AM fungi

Wet sieving and decanting method of Gerdeman and Niclosion was followed for AM fungi spores isolation^[9]. Soil samples, 100 g was taken mixed with one liter of luke-warm water in beaker until all the aggregates dispersed to leave a uniform suspension. Heavier particles were allowed to settle down. The suspension was passed through 710, 425, 250, 150, 106, 75 and 45 µm sieves consecutively to remove the organic matter and bits. The residues in the respective sieves were collected in Petri dishes with 10–20 mL water and were observed under a dissecting microscope at 10–40× for AM fungi spores and sporocarps. Total spore count was calculated by counting the number of spores. Then the spores were removed by using a glass pipette and segregated. The spores of different species were multiplied by funnel or pot cultures with onion (*Allium cepa*).

2.3. Identification of AM fungi

Based upon microscopic character, the AM spores were identified. For identification and nomenclature, synoptic keys of the following authors were used^[10–13]. For species code, Perez and Schenck were followed^[14]. Classification was based on size, shape, colour and surface structure, general nature of the contents, hyphal attachment and wall details. Photomicrographs were taken with the help of 100× Nikon Optiphot microscope.

2.4. Estimation of root colonization

C. roseus roots were washed to remove soil and other particles in running tap water and cut into small (1 cm) pieces, and were treated with 10% KOH solution overnight. Subsequently, the root pieces were washed several times with sterilized distilled water and treated with 1% HCl for 3–4 min. Root pieces were lastly stained with 0.05% trypan blue according to Phillips and Hayman^[15].

2.5. Analysis of physico-chemical properties in soil

Test plant rhizosphere soil sample were collected separately from selected study site. The physico-chemical characteristics viz., pH, EC, N, P, K, Na, Ca, Fe, Mn and Zn were analyzed using the method as described by Sharma *et al*^[16]. From the remaining soil sample, 100 g was used to estimate AM fungal spore number per sample bag.

2.6. Seed treatment of *C. roseus*

Healthy seeds samples were collected from Tamilnadu Agricultural University (TNAU), Coimbatore, Tamilnadu, India. Numbers of seeds were pre-cleaned with sterile distilled water with 12 h in aseptic contusions, and drained under laminar chambers. Seeds were subjected for the chemical (0.5% Hg₂Cl₂) treatment for 30 seconds. The sterilized seeds were sown in soil and moisture condition was maintained for 60 d. On the 60th day, plants were harvested and the root and shoot biomass were determined, tropicals were maintained. Control seeds were maintained with AM fungal spores.

2.7. Pot culture of *Catharanthus roseus*

Small pots of 18 cm diameter were filled with sterilized soil: sand (1:1) mixture. Three plants were transplanted from funnel to pots. The pots were kept in glass house [(30±1) °C] and watered regularly. The infectivity of onion roots by the AM fungus were checked at interval of 15 d for the period of 3 months. After that the pots cultures were harvested by pruning onion plants to the soil level. The soil mass was removed from the pot and the mycorrhizal roots were chopped in to small pieces. Inoculum potential of AM fungal species was detected by using most probable number method^[17]. The soil and root pieces were air dried, packed in polythene bags and stored at 5 °C until used. After harvest, parameters such as plant height, root, shoot and bulb biomass were recorded. Percentage root colonization and AM spore number in root-zone soil were also determined^[9,15].

2.8. Phytochemical analysis of *C. roseus*

Fresh plants collected were subjected to preliminary phytochemical analysis. The triplicate samples were taken for analysis and the mean value and standard error were calculated. Phytochemical analysis included total chlorophyll^[18], soluble sugar^[19], reducing sugar^[20], phenols^[21], total lipids^[22], protein^[23] and amino acids^[24].

Table 1

Physico-chemical characteristics of soil at the study area.

Study area	Soil type	Soil pH	Physico-chemical characteristics								
			Electrical Conductivity (ECse)	Organic carbon (%)	Available Nitrogen (mg/kg)	Available Phosphorus (mg/kg)	Available Potassium (mg/kg)	Copper (µg/g)	Zinc (µg/g)	Manganese(µg/g)	Iron (µg/g)
S1	Brown sandy loam	6.70±0.04	1.10±0.02	1.30±0.04	87.50±3.60	6.90±0.20	16.20±0.40	1.30±0.20	1.80±0.40	3.40±0.40	87.70±0.20
S2	Alluvial	6.20±0.02	1.60±0.02	1.44±0.44	381.00±4.00	7.30±0.20	42.00±2.00	1.90±0.02	2.40±0.40	3.90±0.40	98.30±0.20

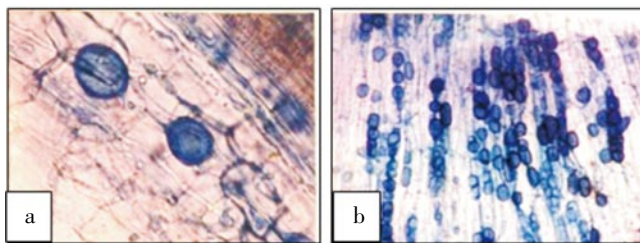
S1: Herbal garden at Poondi; S2: Throwpathi amman temple garden at Thiruchotruthurai, both at Thanjavur.

3. Results

The present study was undertaken in two different localities such as, herbal garden Poondi and Throwpathi amman temple garden in Thiruchotruthurai at Thanjavur district of Tamilnadu, India with varying physico-chemical characteristics, and also assessed the effect of native AM fungi on growth, and biochemical constituents of *C. roseus*. Physico-chemical parameters were studied in the soil samples. It showed maximum pH in station I and the soil nature was brown sandy loam soil in station I, and alluvial soil in station II. *C. roseus* was positive for AM fungal colonization in roots. The maximum percentage root colonization (92.5%) was identified from the station II (Table 1). There was a certain degree of specificity among the different AM fungal species in the study locality with *C. roseus* plants (Table 2).

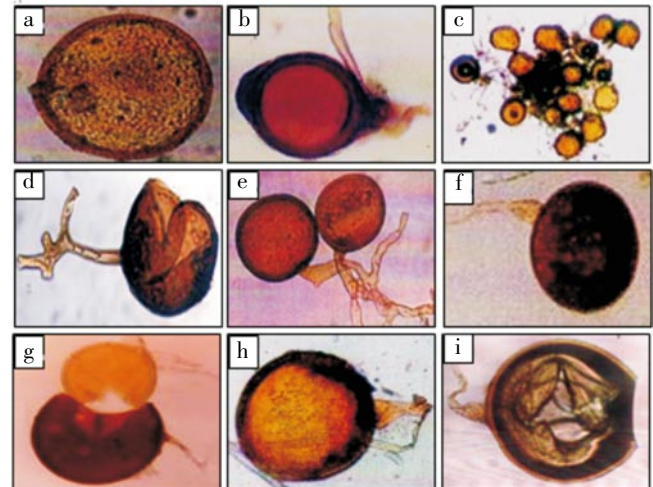
Table 2Per cent AM fungi colonization in the roots and spore density of AM fungi root zone soils *C. roseus*.

Study area	<i>C. roseus</i>		
	Root colonization (%)	Positive for AM fungal species in the roots	Total number of AM spores/100 g soil
S1–Poondi	62.3±2.4	<i>Glomus aggregatum</i>	530.0±2.8
S2–Thiruchotruthurai	92.5±3.1	<i>Glomus fasciculatum</i>	642.0±3.2

**Figure 1.** Inter and Intracellular vesicles in the root cortical cells of *C. roseus*.

The presence of high degree of AM colonization with various AM fungal structures such as infection pegs, hyphal coils (pelotons), hyphal dimorphism, intracellular arbuscules, inter and intracellular vesicles in the roots of *C. roseus* were observed (Figure 1).

A total of nine AM fungal species were isolated and identified from the rhizosphere soils of the *C. roseus* (Table 3 and Figure 2).

**Figure 2.** AM fungi isolated from *C. roseus* rhizosphere soil samples station I and II.

a–*Acaulospora scrobiculata*, b–*Acaulospora marrowae*, c–*Glomus aggregatum*, d–*Glomus fasciculatum*, e–*Glomus geosporum*, f–*Gigaspora margarita*, g–*Gigaspora nigra*, h–*Scutellospora heterogama*, i–*Scutellospora pellucida*.

Of the nine AM fungal species, *Glomus aggregatum* (*G. aggregatum*) and *Glomus fasciculatum* (*Glomus fasciculatum*) were the most predominant (100%) colonizing species found in the roots of *C. roseus* (Table 2). The extramatrical phase of AM fungi differed greatly. The extramatrical mycelium with variable number of

Table 3Frequency distribution of AM fungi in the rhizosphere soils of *C. roseus*.

AM fungi species	family	<i>C. roseus</i>		Species frequency
		S1	S2	
<i>Acaulospora marrowae</i> (Walker & Sanders)	Ambisporaceae	+	–	50
<i>Acaulospora scrobiculata</i> (Trappe)	Ambisporaceae	–	+	50
<i>Glomus aggregatum</i> (Schench & Smith)	Glomeraceae	+	+	100
<i>Glomus fasciculatum</i> (Thaxter, Sensu, Gerde & Trappe)	Glomeraceae	+	+	100
<i>Glomus geosporum</i> (Nicol & Gerde)	Glomeraceae	+	+	100
<i>Gigaspora margarita</i> (Beck & Hall)	Gigasporaceae	–	+	50
<i>Gigaspora nigra</i>	Gigasporaceae	–	+	50
<i>Scutellospora heterogama</i> (Trappe)	Gigasporaceae	+	–	50
<i>Scutellospora pellucida</i> (Nicol & Gerde)	Gigasporaceae	–	+	50

entry points of *G. aggregatum* and *G. fasciculatum* was observed in the roots of *C. roseus*. The infecting hyphae of the AM fungus, *G. aggregatum* and *G. fasciculatum* were found to form intracellular coils (pelotons) in the first infected cells followed by similar cells in the roots of test plants.

Different shapes of vesicles such as oval, elliptical, globular, lobed and unlobed were observed in different AM fungi species. The vesicles were inter and intracellular. Only vesicles were observed in roots of *C. roseus* plants colonized by *G. aggregatum* and *G. fasciculatum* (Figure 1). AM fungal spores and sporocarps were isolated from the root zone soils of *C. roseus*. The total spore counts were 642/100 g of soils (Table 2). A total of nine AM fungi species representing four genera viz., *Acaulospora*, *Gigaspora*, *Glomus* and *Scutellospora* were recorded, of which *Glomus* genus was found dominant in root zone soil of the test plants in the both study sites. Accordingly, *Acaulospora scrobiculata* (*A. scrobiculata*), *G. aggregatum*, *G. fasciculatum*, *Glomus geosporum*, *Gigaspora margarita*, *Gigaspora nigra* and *Scutellospora pellucida* were dominant forms of station II. Further, *G. aggregatum*, *G. fasciculatum* and *Glomus geosporum* showed 100% frequency. Higher numbers of AM fungi were observed in alluvial soil study site. The mean spore number per 100 g soil varied from 530–642 in root zone soils of *C. roseus*. The highest number was recorded in root zone soil of *C. roseus* (642/100 g soil) at Thiruchotruthurai locality, whereas the lowest number was observed in *C. roseus* (530/100 g soil) at Poondi locality. This consequence maybe depends upon the soil nature. There was a certain degree of specificity among the different AM fungi spore and sporocarps in their association with root–zone soils of the test plant both localities (Table 3). Occurrence of AM fungal species identified were grouped as dominant (above 70%) and common (50%–70%), and *Glomus macrocarpum*, *Gigaspora margarita*, *Scutellospora*

heterogama and *A. scrobiculata* were dominant forms.

Only five AM fungi were used for the inoculum production. The bioassay was conducted using *C. roseus* as the test plant to study the infection efficiency and conformation of the production of AM fungi species (Figure 1).

Native AM fungi *A. scrobiculata*, *G. aggregatum* and *G. fasciculatum* had influence on growth, biomass production, nutrition and phyto–chemical constituents of *C. roseus* plants in pot culture. *G. aggregatum*, *G. fasciculatum* and *A. scrobiculata* were used for influence on growth of *C. roseus* in pot culture. Spore population was very high in inoculated plant rhizosphere soil, and *G. aggregatum* spores were highly parent in the *C. roseus* cultivated soil followed by others (Table 4). In general, all inoculated plants showed increased plant height, shoot and root biomass and biochemical constituents when compared to uninoculated control.

Table 4

Effect of native AM fungi on percent root infection and AM fungal spores in the root–zone soils of *C. roseus* in pot culture.

Treatments	Root colonization (%)	Total number of AM spores/100 g of soil
Control (without AM)	–	–
<i>A. scrobiculata</i>	61.40±2.80	278.00±3.80
<i>G. fasciculatum</i> alone	76.20±3.40	384.00±4.20
<i>G. aggregatum</i> alone	96.20±4.60	684.00±6.40

Of these three inoculum, *G. aggregatum* highly increased the plant growth (Table 4) followed by *G. fasciculatum* and *A. scrobiculata*. The morphometric parameters suggested that the maximum shoot, root, total lengths and total biomass of *G. aggregatum* was observed in *C. roseus* followed by *G. fasciculatum* and *A. scrobiculata*, and the minimum length (without AM fungi) was recorded in uninoculated control (Table 5). Plants were harvested after 60 d of planting. Mycorrhizal and non–mycorrhizal roots

Table 5

Influence of AM fungi on morphometric parameters of *C. roseus* in pot culture.

Inoculation Treatments	Shoot length (cm)	Root length (cm)	Total length (cm)	Plant dry biomass			Mycorrhizal effect (%)
				Shoot	Root	Total	
Uninoculated control	24.2	4.8	29.0	3.6	2.7	6.3	–
<i>A. scrobiculata</i> alone	25.2	6.1	31.3	4.7	3.4	8.1	133.5
<i>G. fasciculatum</i> alone	30.2	6.2	36.4	4.7	3.8	8.5	140.5
<i>G. aggregatum</i> alone	31.1	6.4	37.5	5.5	4.1	9.6	155.5

Table 6

Influence of AM fungi on biochemical parameters of *C. roseus*.

Phyto–chemical constituents (µg/g)	T1	T2	T3	T4
Chlorophyll <i>a</i>	0.080±0.006	0.130±0.021	0.138±0.022	0.152±0.014
Chlorophyll <i>b</i>	0.020±0.008	0.052±0.004	0.060±0.009	0.081±0.006
Total chlorophyll	0.100±0.014	0.182±0.025	0.198±0.031	0.233±0.020
Total soluble sugars	0.014±0.002	0.041±0.003	0.030±0.007	0.051±0.004
Reducing sugars	0.033±0.007	0.053±0.008	0.047±0.004	0.060±0.007
Phenols	0.282±0.017	0.281±0.021	0.292±0.016	0.293±0.032
Ortho dihydroxy–phenols	0.265±0.022	0.271±0.032	0.270±0.027	0.275±0.022
Total lipids	0.282±0.008	0.285±0.036	0.291±0.013	0.300±0.025
Total proteins	0.045±0.007	0.055±0.005	0.051±0.008	0.063±0.003
Total amino acids	0.039±0.006	1.031±0.061	1.035±0.052	1.042±0.056

T1–Control; T2–*A. scrobiculata*; T3–*G. fasciculatum*; T4 –*G. aggregatum*.

of *C. roseus* samples were determined subjected to various phyto-chemical analysis. Chlorophyll *a*, chlorophyll *b* and total chlorophyll contents in the leaves of inoculated plants were significantly higher than those of control plant. Phyto-chemical constituents such as total soluble and reducing sugars, proteins, amino acids, total phenols, ortho di-hydroxy phenol and total lipids in the roots of inoculated plants were significantly higher than those of control plants. *G. aggregatum* inoculated plant showed increased chlorophyll content and phyto-chemical constituents in the leaves of pot cultured plant, while *G. fasciculatum* and *A. scrobiculata* inoculated plant had less content (Table 6). The present study revealed that, the dual effect of *G. aggregatum* and *G. fasciculatum* showed increased plant growth, biomass and biochemical constituents in *C. roseus*, and *G. aggregatum* was found to be superior than the others.

4. Discussion

As mentioned in the introduction, the number of studies on the influence of AM fungi on medicinal plants is limited in India, particularly in Tamilnadu. Therefore the present investigation was undertaken to study the association of AM fungi with rhizosphere soil of *C. roseus*. However, the information available on the use of AM fungi in medicinal plants *C. roseus* and the effect of *A. scrobiculata*, *G. aggregatum* and *G. fasciculatum* on growth, nutrition and phyto-chemical constituents of *C. roseus* have been investigated. Edaphic characteristics such as soil type^[25,26], soil pH^[27,28], EC_{se}, Ca, N, P, K, Cu, Zn, Mn and Fe^[39–31] and soil fertility^[32] were reported to influence AM sporulation. Little information is available on the correlation of edaphic characteristics with spore populations in Indian conditions^[33,34].

There has been a phenomenal increase of interest on AM fungi in recent years, leading to numerous surveys for enumerating and assessing AM fungi species and their colonization of host plants in different regions of this country^[35]. The significance of AM fungi in plant ecology is based on its wide-spread occurrence in natural ecosystems^[10]. *C. roseus* was positive for AM fungi colonization in the roots and root zone soils of the study site. A species was considered mycorrhizal if roots contained one of the combinations (hyphae+arbuscules, hyphae+pelotons or hyphae+vesicles) of AM fungal structures in the primary cortex. To qualify as an AM species, the presence of arbuscules was considered critical^[36].

The AM fungal spore abundance was reported to be determined by the host plant species and the environmental variables rather than by AM fungal species^[37]. A few surveys have been conducted on the occurrence of AM fungi in medicinal plants including *C. roseus* and reported that spore populations in cultivated soils were found to be as much as in non-cultivated soils. Similarly the high spore numbers in cultivated field soils of Northern^[38] and Southern India^[39] were also available. Low spore numbers ranging from 0–10 per 100 g of soil were also reported in cultivated soils.

AM fungi are known to improve plant growth, mainly through increased phosphorus uptake and nutrients^[6,40]. Karagiannidis *et al.* reported that species and strains of AM fungi have differed the extent to which they increase nutrient uptake and plant growth^[41]. Hence it was suggested by some workers that selecting efficient AM fungi can be used for inoculating different plants including medicinal plants^[42].

Medicinal plants need more phosphorus for its growth and development as they increased uptake of diffusion-limited nutrients^[43]. Inoculation of AM improves photo-chemical and physiological conditions of medicinal plants^[44]. Mycorrhizal plants showed better growth and produce higher yield as compared to the non-mycorrhizal ones^[45]. Some scientists have observed wide variations among different species of AM fungi in their ability to promote plant growth^[46]. Similarly, Das *et al.* observed an increased AM fungal colonization of roots and plant biomass in the roots of medicinal plants^[47]. The primary mechanism which is possible for stimulation of plant growth by AM fungi is due to increased uptake of phosphorus and other nutrients. The possibility of plant growth hormones being involved in such a response has also been suggested^[48]. Further more, the substances with the properties of gibberellic acid and cytokinin in the extract of *Glomus mosseae* culture was observed. Increase in crop yield, soil nutrient status and nutrient uptake was reported due to application of AM fungi^[16,49]. The present investigation clearly showed that the native AM fungi such as *G. aggregatum* and *G. fasciculatum* recommended for *C. roseus* can be growth promoter in Thanjavur district, Tamilnadu, India.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

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Comments

Background

Medicinal plants play an important role in day to day life, and the extract from these plants are without side effects. The organisms that are suitable for the growth promoting of various medicinal plants and agricultural

crop production systems include free living, associate and symbiotic nitrogen fixers, phosphate solubilizers mycorrhizal fungi, cellulolytic and hormone producers. Various soil bioinoculants perform specific functions such as biological nitrogen fixation, nitrification, and denitrification, biocontrol of root pathogens, phosphate solubilization and mobilization, cellulose and lignin decomposition, and production of growth promoting hormones in the rhizosphere region of the plants.

Research frontiers

The present study showed influence of native AM fungi on growth, nutrition and phytochemical constituents of *C. roseus*.

Related Reports

Previous studies revealed that the inoculation of AM fungi with agricultural crops has potentially increased the growth of root and shoot biomass. Several studies have been done by various workers on the role of AM fungi in medicinal plants. The importance of AM fungi in the growth, nutritional uptake and productivity in various agricultural crops has been well documented.

Innovations and breakthroughs

The manuscript is quite interesting in several aspects. It is scientifically based on influence of native AM fungi on growth, nutrition and phytochemical constituents of *C. roseus*. All the sections of the manuscript have been well organized and without topographical errors.

Applications

From the literature survey it has been found that the effect of *G. aggregatum* and *G. fasciculatum* showed increased plant growth, biomass and biochemical constituents in *C. roseus*. Keeping in view mycorrhizal association with medicinal have more benefits as compare to non-mycorrhizal associations.

Peer review

This is valuable research in which authors selected nine different AM fungi species such as *A. scrobiculata*, *Acaulospora marrowae*, *G. aggregatum*, *G. fasciculatum*, *Glomus geosporum*, *Gigaspora margarita*, *Gigaspora nigra*, *Scutellospora heterogama* and *Scutellospora pellucida* to be isolated and identified from the root zone soil of *C. roseus*. In addition, the phytochemical analyses showed high concentration of chlorophyll *a*, chlorophyll *b*, total chlorophyll, soluble sugar, reducing sugar phenols, ortho dihydroxy-phenols lipids proteins and amino acids in *G. aggregatum* inoculated *C. roseus*. *G. aggregatum* was found to perform better on growth when compared to others and phytochemical constituents of *C. roseus*.

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