Seasonal Variation in Arbuscular Mycorrhizal colonization in roots and spores in rhizospheric soil of *Caralluma adscendens* var. fimbriata (Wall.) Grave. Mayer

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Abstract
The present study deals with seasonal variations in Arbuscular Mycorrhizal colonization in roots of Caralluma adscendens var fimbriata (Wall.) Grave and Mayer and presence of chlamydospores in the rhizospheric soil. The whole root mount showed 80% endomycorrhizal colonization in winter, 73% in summer and 60% in the rainy season. The mycelium is coenocytic, aseptate and branched.

The vesicles observed in whole root mount were oval, rounded and globular. The rhizospheric soil analysis showed presence of 34 spores per gm of soil in summer followed by 31 spores in winter and 5 spores per gm of soil in rainy season. Five Arbuscular Mycorrhizal genera were recorded in the rhizospheric soil and dominated by Glomus. The other genera were Acaulospora, Gigaspora, Entrophosphora and Scutelliospora.

Keywords: *Caralluma adscendens* var fimbriata, Asclepiadaceae, Endomycorrhizae, Arbuscular Mycorrhizal colonization.

Introduction

Caralluma adscendens var fimbriata is succulent, perennial herb locally called “Makad Shingi” growing on gravelly soil, having quadrangular and distally attenuated stem with minute reduced leaves belonging to family Asclepiadaceae.[17] It occurs at the base of shrubs mainly *Acacia* sps. on rocky hills slopes. Flowers are conspicuously purple, corona lobes ciliate along the margins. The stem is eaten as vegetable. Arbuscular mycorrhizal fungi are symbiotic that predominate in the roots of most of the vascular plant species and soils of agricultural crops, wild plants and weeds throughout the world.[4,13,19,26]

The present investigation was carried out to find out the occurrence and diversity of Arbuscular mycorrhizal colonization in the roots and spores in the rhizospheric soil. Arbuscular mycorrhizal fungi are colonized in roots of the angiospermic plant species mainly the monocots and dicots.

In the soil they form resting spores like *Chlamydospores*, *Zygospores* and *Azygospores*.[25] Khan[7] from Pakistan showed the occurrence of mycorrhizae in the roots of the host plant and *Endogone* spores in rhizosphere soil of fifty-two xerophytic plant species, twenty-one halophytic plant species and sixteen hydrophytic plant species.

Kannan and Lakshminarashimhan[6] surveyed the VAM status of 48 maritime plant species belonging to 32 families screened by them for mycorrhizal association while mangrove plants were nonmycorrhizal. Parmeshwaran and Augustine[18] reported thirty-one plant species positive for mycorrhizal colonization out of forty-four plant species investigated. Raghupathy et al[20] reported 48 plant species positive to AM fungi association of which 7 plant species were aquatic out of 98 plant species collected.

Material and Methods

Study Area: The plant samples were collected from Swami Ramanand Teerth Marathwada University campus, Nanded, Maharashtra State, located near the Vishnupuri, Nanded and its GPS Coordinates as established on 17th September 1994 are (N 1906’2.476 E77017’ 9.96) Latitude: 19.100688 Longitude: 77.2861 Altitude: 365 meters.

Collection of Plant Samples: The roots and rhizospheric soil of *Caralluma adscendens* were collected in polythene bags separately to the laboratory. For AM fungal colonization, method of Philips and Hayman was used in which the fine root segments of feeder roots were taken in a test tube containing 10% KOH and autoclaved at 15 lbs pressure for 20 minutes. After 10 minutes, the 10% KOH was removed and 10 ml 1N HCl was added to neutralize the root tissue. After 30 minutes the root segments were stained with cotton blue in Lactophenol and kept for 24 hrs. Next day the root segments were observed for AM fungal colonization. The percentage of AM colonization was calculated by using following formula:

$$\%\ \text{AM Colonization} = \frac{\text{Number of Mycorrhizal root segments}}{\text{Total number of root segment screened}} \times 100$$

The resting spores mainly chlamydospores were isolated from the rhizospheric soil of the host plant by applying standard method ‘wet sieving and decanting’ suggested by Gardemann and Nicolson[2] and subsequently slight modifications suggested by Gaur and Adholeya[3] were used for the isolation of spores from the soil. The spores were mounted on glass slides in polyvinyl alcohol lactophenol mountant[9] and the identification of AM fungal spores was done by using the spore manual[22] along with recent improvisation in the AM fungal nomenclature.[10]

Diversity Indices: Statistical analysis and diversity indices were done with the Diversity Calculator Online (BPMSG).[8]

Results and Discussion

The whole mount of root showed Arbuscular mycorrhizal colonization in the form of fungal hyphae and vesicles in rainy season and hyphae vesicles and arbuscles in winter and summer season (Fig. 1). Most mycorrhizal colonization in roots of *C. adscendens* was observed in winter (80%) in comparison to summer (73%) and rainy season (60%) while spore count per gm of soil was more in summer in comparison with winter and rainy season (Table 1).

A distinct appresorium was observed (Fig. 3). AM fungal spores were observed in the rhizospheric soil samples collected from the root zone of *Caralluma adscendens* var fimbriata. Five Arbuscular mycorrhizal genera were recorded in the rhizospheric soil and dominated by *Glomus*. The other genera were *Acaulospora*, *Gigaspora*, *Entrophosphora* and *Scutelliospora*.

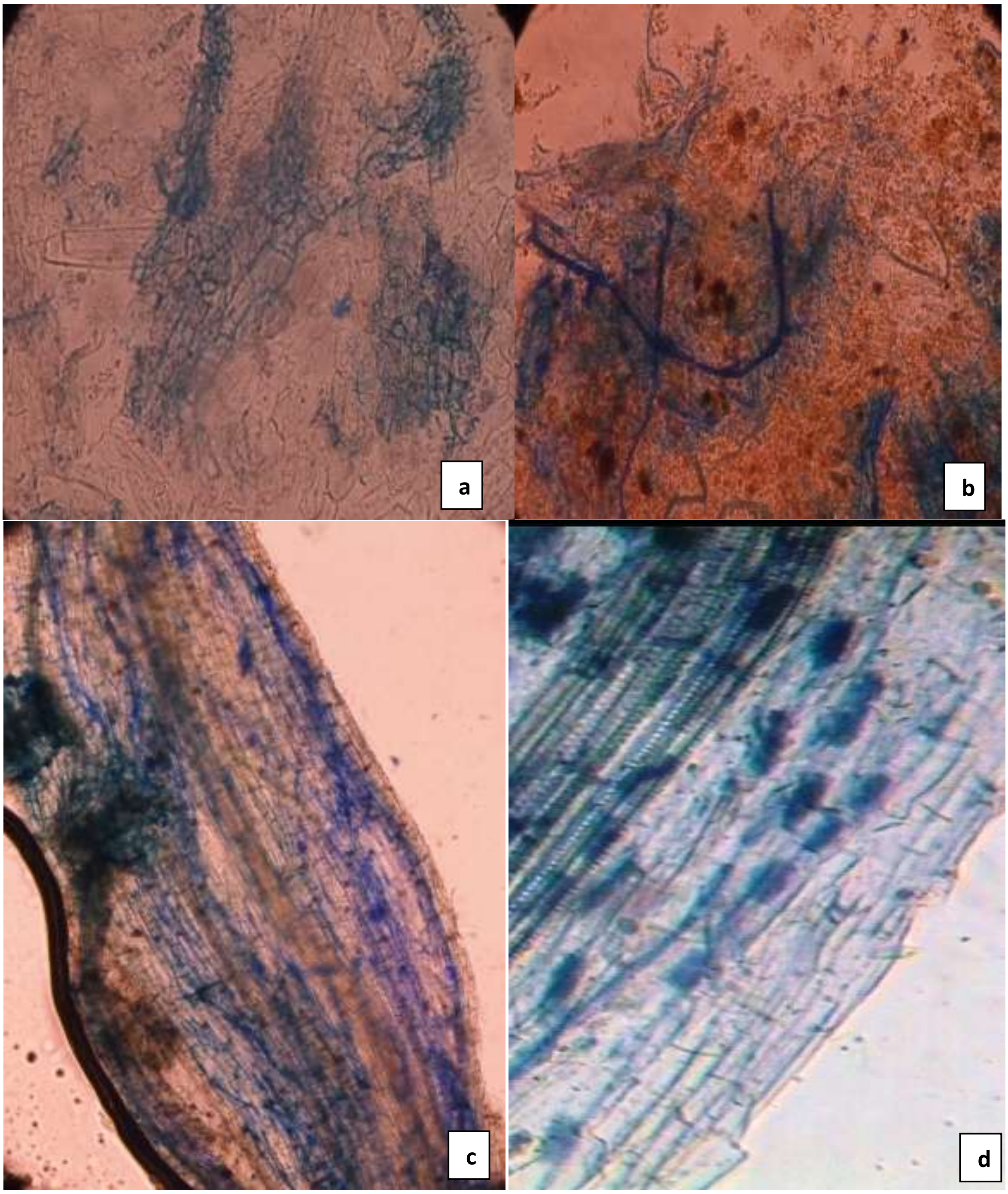

Fig. 1: a) Mycelium within the cortical cells, b) aseptate mycelium, c, d) Root whole mount showing vesicles and arbuscles

The presence of large number spores belonging to *Glomus* indicates their universal occurrence in the soil (Fig. 4). Winter season is more appropriate for the multiplication of mycorrhizal spores (Table 1), species richness in the winter is more in comparison with spore number recorded in other seasons. Diversity indices for the seasonal variation of the mycorrhizal variations have been calculated and species richness, evenness and dominance were calculated (Table 2). The diversity indices reveal rarity and commonness of mycorrhizal spores in the same rhizospheric soil samples. Number of spores was counted in all three seasons of the years. Richness (R), Berger Parker Index ($_{pimax}$), Shannon Entropy (H) (nat), Shannon Entrophy (H) (bit), Number Eq. ^{1}D (True Diversity), Shannon Equitability $H/\ln N$, Simpson Dominance SD, SD (unbiased - finite samples), True Diversity ^{2}D (Order 2), Gini-Simpson Index 1-SD and Gini-Simpson Equitability were calculated for the mycorrhizal resting spores in all three seasons.

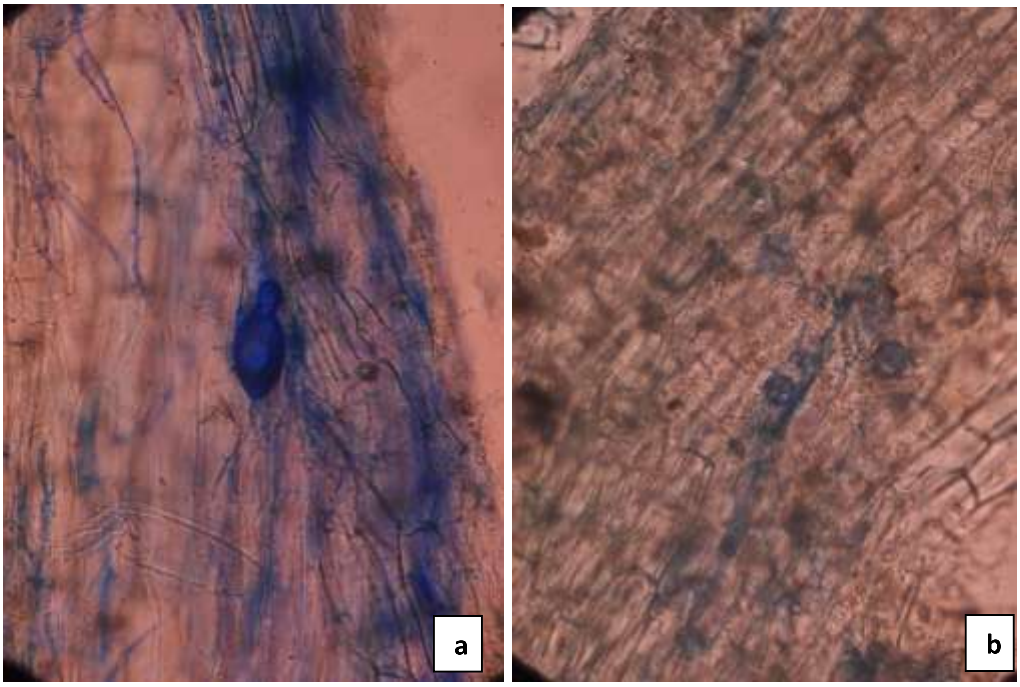

Fig. 2 a) Elongated oval vesicle with Coenocytic hyphae, b) Rounded vesicles within cortical tissue

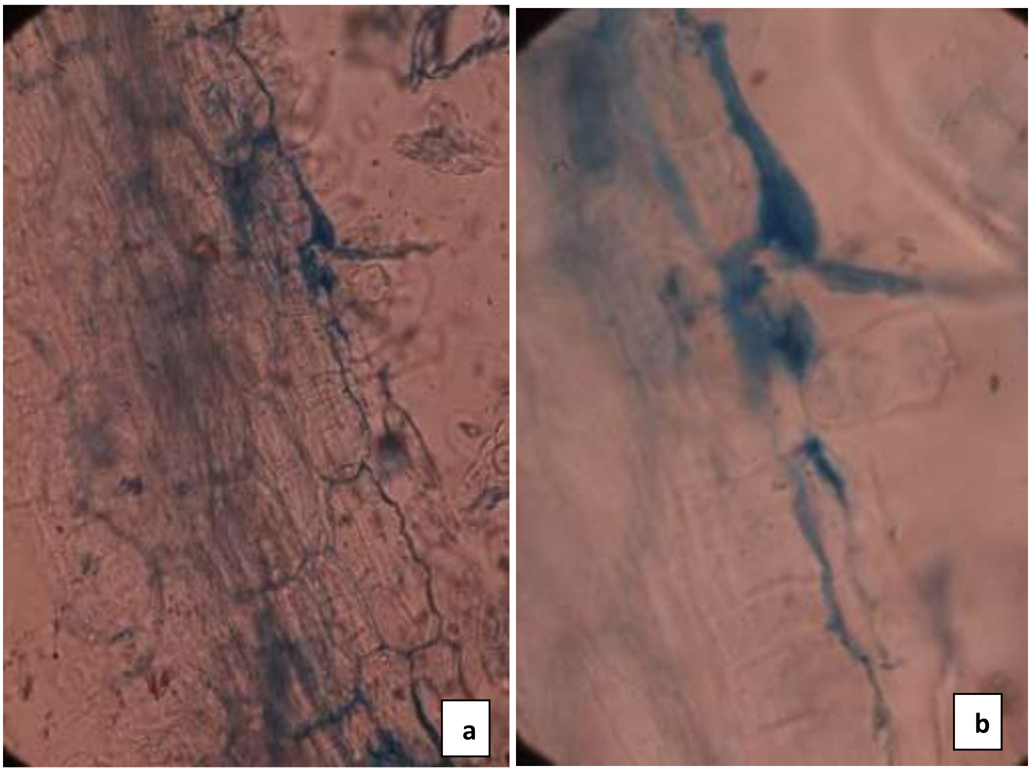

Fig. 3: Apperesorium- entry point to the root epidermis, a) 10x and b) 40x

Table 1
Seasonal variation in Arbuscular Mycorrhizal colonization in the host roots and chlamydospores the soil of *Caralluma adscendens*.

S.N.	Season	AM root colonization	% AM root colonization	Spore No./gm soil	Type of Spores
1.	Rainy	Hyphae and vesicles	60	5	*Acaulospora* sps., *Gigaspora* sps. *Glomus mossae*
2.	Winter	Hyphae, vesicles and arbuscles	80	31	*Acaulospora delicate*, *Gigaspora* sps. *Glomus agrregatum* *Glomus fasciculatum* *Glomus citricola* *Entrophosphora* sps *Scutelliospora* sps
3.	Summer	Hyphae, vesicles and arbuscles	73	34	*Acaulospora* sps. *Gigaspora* sps. *Glomus mossae*

Table 2
Diversity indices of occurrence of Mycorrhizal spores

Diversity Indices	
Index	**Value**
Number of Classes N	3
Richness R	3
Berger Parker Index $p_{i\max}$	48.6%
Number Eq. ^1D (True Diversity)	2.5
Shannon Equitability $H/\ln N$	81.9%
Simpson Dominance SD	43.7%
SD (unbiased - finite samples)	42.9%
True Diversity ^2D (Order 2)	2.3
Gini-Simpson Index 1-SD	56.3%
Gini-Simpson Equitability	84.4%
Simpson Dominance	0.4371
Shannon Entropy	0.9000

[1)]Sometimes referred to as Shannon-Weaver or Shannon-Wiener Index

In the Berger Parker Index, the mean percentage of species shared by areas was 48.5% indicating a high degree of difference in species composition (high beta-diversity) on a total of 70 species. Shannon Entropy *H* (nat) was calculated as 0.900 which indicates higher diversity in our dataset and less randomness. Species richness and evenness are two major factors contributing to biodiversity. Shannon-Weaver and Simpson index emphasize on species richness and evenness respectively.[23,24]

Algunde et al[1] investigated ten angiospermic plant species from the great Indian Bustard Sanctury Nannaj, Solapur and reported that all ten plant species showed positive Arbuscular mycorrhizal colonization and even they recorded the resting spores from rhizospheric soil of the investigated hosts. The percentage of root colonization ranged from 20% to 80%. Mulani[14] recorded Arbuscular mycorrhizal colonization in fifty-one plant species distributed in thirty-one families from the weeds of Mumbai. Plant species namely *Phyllanthus fraternus*, *Cloris barbata*, *Cyanodon dactylon*, *Cyprus rotundus* and *Eragrostis uniloides* showed 100% root colonization while *Alternanthera sessilis* and *Brassica juncea* did not show mycorrhizal colonization. The round globular elongated vesicles were observed in whole root mount.

Mulani and Waghmare[12] investigated thermotolerant Arbuscular mycorrhizal species in the roots and rhizospheric soil of *Aloe vera* was collected from Swami Ramanand Teerth Marathwada University Campus, Nanded. They recorded 100% mycorrhizal colonization and vesicles in the whole root mount. The rhizospheric soils were dominated by *Glomus* species. Kamble and Mulani[5] recorded Arbuscular mycorrhizal colonization in some coastal Psammophytes.

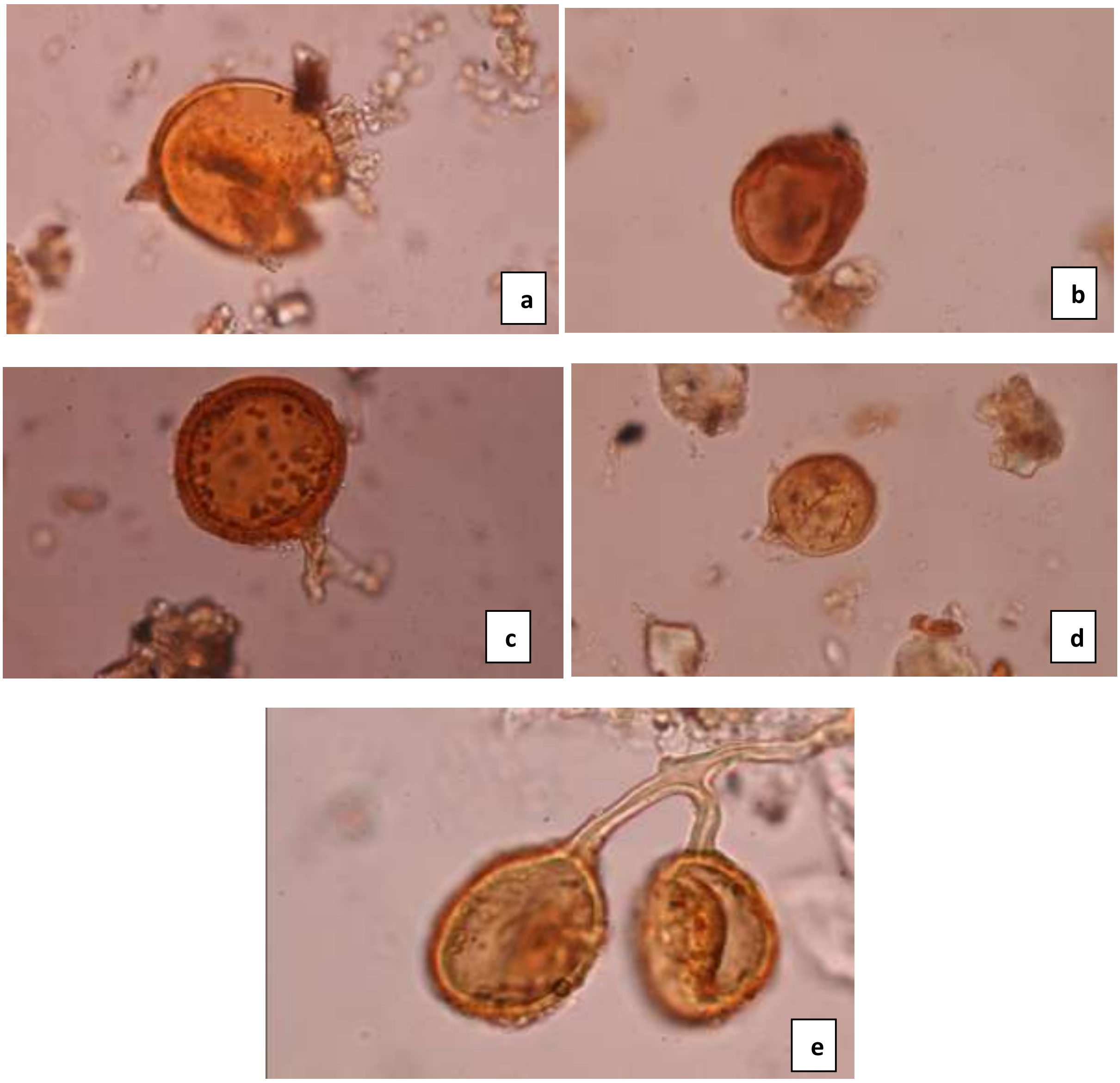

Fig. 4: The resting spores recorded in rhizospheric soil of *Caralluma adscendens*; a, b) *Glomus mossae*, c) *Glomus deserticola*, d) *Acaulospora* sps. and e) *Glomus fasciculatum*

Psammophytes are the plants which have developed specialized mechanism to cope up with adverse condition prevailing in dry conditions and these plants are supported by Arbuscular mycorrhiza either directly or indirectly.

Mulani et al[15] reported 78% average Arbuscular mycorrhizal colonization in the roots of *Phyllanthus fraternus*. The resting spore count was very high, average four hundred spores were recorded in the rhizospheric soil and mainly consist of *Glomus, Gigaspora, Acaulospora* and *Sclerocystis*.

Mulani and Wankhade[11] and Wankhade and Mulani[27,28] recorded 90% colonization in *Vetivera zizanioides* and also recorded species of *Glomus* like *G. mosseae, G. pachycaulis* and *G. microcarpum*. Mulla and Kanade[16] observed vesicular Arbuscular mycorrhizal colonization in grasses of halophytic environment from Mumbai Coastal Region and recorded a positive colonization in the roots of grasses. Sathe[21] investigated 287 angiospermic native hosts belonging to 39 families located in 13 selected sites of Western Ghat for arbuscular mycorrhizal colonization, out of which 226 species were positive while 61 species were negative. The maximum hosts screened available during winter season showed high colonization.

Conclusion

Caralluma adscendens a succulent leafless xerophytic plant species was growing on the sloppy gravel soil in the campus and associated with spiny shrubs of *Acacia* and was being used for the obesity. It grows well in dry conditions.

This adverse condition favors more mycorrhizal colonization during winter and summer season as compared to that of Monsoon. The rhizospheric soil also showed the high population density of mycorrhizal spores. The dominant species was *Glomus*. The high number of spores attributed to more porous soil and less water content in the rhizospheric soil of *Caralluma adscendens*.

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