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Isolation, Identification And Characterization Of Arbascular Mycorrhizal Fungi Spores In Different Regions Of Nizamabad District Telangana State

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AM fungi are becoming increasingly important in agriculture, horticulture, forestry, and environmental restoration. The global application of agriculturally beneficial microbes contributes directly or indirectly to crop enhancement and improves nutrient uptake efficiency. The Rhizosphere soil samples of Catharanthus roseus (L.) G. Don. were obtained from three distinct regions within the Nizamabad District, viz., Sirikonda, Bodhan, and Armoor.

In the present study 88AM fungal species present in Armoor region, 75 fungal species present in Sirikonda region and 80 fungal species present in Bodhan region. All these fungal species belonging to 5 genera viz., Acaulospora, Entrospora, Gigaspora, Glomus and Sclerocystis.

Arbuscular mycorrhizal (AM) fungi are extensively prevalent in soils characterised by phosphate shortage. Mycorrhizal connections are frequently documented in a diverse array of plant species spanning many taxonomic groups within the plant kingdom. The taxonomic classification Apocynaceae has a diverse array of plant species that exhibit considerable medicinal importance. The primary objective of this study was to assess the correlation between the presence of arbuscular mycorrhizal (AM) fungus and various medicinal plants from the Apocynaceae family.

Key words: AM Fungi, Apocynaceae, Catharanthus roseus (L.) G. Don, Taxonomic classification, Rhizosphere

Introduction

Arbuscular mycorrhizal fungi (AMF) (Glomeromycota) are obligatory symbiotic with most land plants (Redecker et al., 2013). This symbiotic association, which consists of the bidirectional transfer of minerals and nutrients between the fungus and the plant, is typically mutualistic. Smith and Read (2008) found that AMFs improve the transport of low-mobility nutrients, such as phosphorus and critical minerals, to plants in return for carbon. They may improve host tolerance to abiotic stressors such toxic metals (Hildebrandt et al., 2007; Wang, 2017) and biotic factors like plant diseases (Pozo and Azcón-Aguilar, 2007; Wang et al., 2020). Functional variety in AMF can enhance ecosystem services (Helgason et al., 2002; Powell and Rillig, 2018; Smith and Smith, 2012; van der Heijden and Scheublin, 2007). Different AMF have distinct properties that enable different life options. For example, the impacts of AMF on plant's ability to acquire and distribute nutrients, survive stressful situations, and effectively colonize new ecosystems may differ (Powell and Rillig, 2018).

Powell and Rillig (2018) emphasized the need of establishing AMF in culture and comparing a variety of AMF with diverse functional features in controlled experimental experiments. They also highlighted the importance and complexity of establishing inoculum in a 'ecological context'. Thus, while analyzing numerous cultures is critical for establishing ecological relevance, the many factors that affect both plants and fungi must also be evaluated, including the effects of natural or anthropogenic disturbance on the potential AMF, such as mining activities.
AM fungi are becoming increasingly important in agriculture, horticulture, forestry, and environmental restoration (IJdo et al., 2011; Sheteiwy et al., 2023). The global application of agriculturally beneficial microbes contributes directly or indirectly to crop enhancement and improves nutrient uptake efficiency (Bargaz et al., 2018; Plett, 2018; El-Sawah et al., 2023). Potentially, AM fungi could replace or minimize the use of inorganic fertilizers (Begum et al., 2019; Sheteiwy et al., 2022).

Materials and Methods

Collection of soil samples for the identification of AM fungal propagules

The Rhizosphere soil samples of Catharanthus roseus (L.) G. Don. were obtained from three distinct regions within the Nizamabad District, viz., Sirikonda, Bodhan, and Armoor. These samples were gathered on a monthly basis from January 2018 to December 2021. The collection process involved placing the samples into sterile polythene bags to maintain their sterility. Soil samples were obtained using a sterile widger at a depth of 10cm. The samples were sent to the laboratory in order to isolate arbuscular mycorrhizal (AM) fungal propagules.

Figure A: Study areas of Nizamabad district for the present investigation for identification and isolation of AM Fungus (red dots).

Isolation of AM fungal propagules

The estimation of propagules, including chlamydospores, sporocarps, and azygospores, generated by mycorrhizal fungus was conducted on soil samples obtained for the purpose of isolating arbuscular mycorrhizal (AM) fungi (Kokkoris, 2019).

Results and Discussion

AM fungal spore count in three different regions of Nizamabad District.

In the present study 88AM fungal species present in Armoor region, 75 fungal species present in Sirikonda region and 80 fungal species present in Bodhan region. All these fungal species belonging to 5 genera viz., Acaulospora, Entrospora, Gigaspora, Glomus and Sclerocystis.

Table A: AM fungal spore count in three different regions of Nizamabad Districts

Sl no	Name of the region	No. of AMF spores / per 50gm soil
1	**Armoor**	88
2	**Sirikonda**	75
3	**Bodhan**	80

Identified spores are given below:

Figure 1: Azygospore of Acaulospora delicate Figure 2: Azygospore of Acaulospora faveata

Figure 3: Azygospore of Acaulospora lacunose Figure 4: Azygospore of Acaulospora laevis

Figure 5: Azygospore of Acaulospora nicolsoni

Figure 6: Acaulospora denticulata

Figure 7: Azygospore of Scutellospore nigra

Figure 8: Entrophospora infrequens

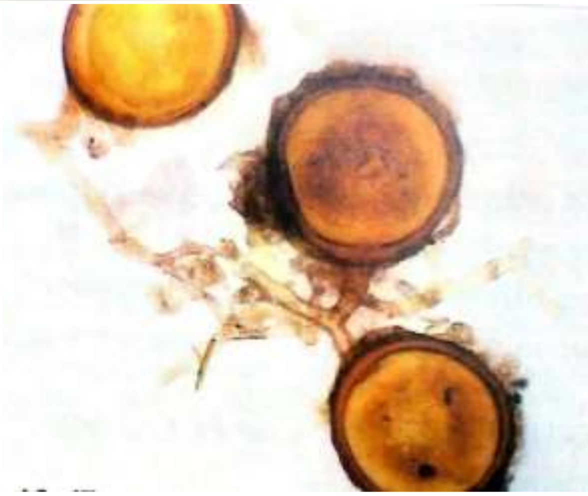

Figure 9: Gigaspora gigantia

Figure 10: Glomus aggregatum

Figure 11: Glomus albidum

Figure 12: Glomus citricolum

Figure 13: Glomus constrictum

Figure 14: Glomus

faciculatum

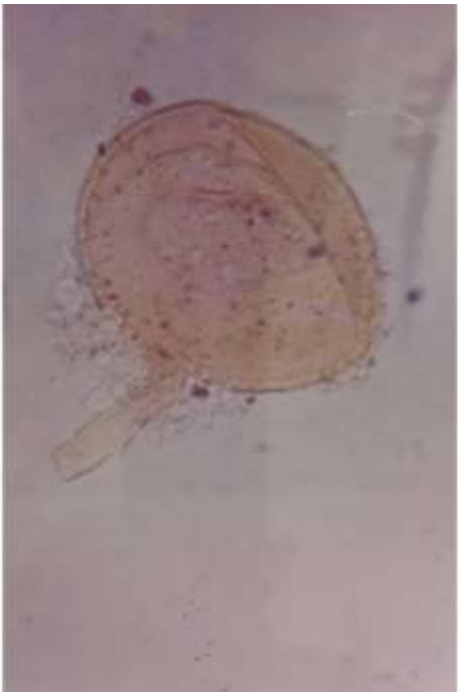

Figure 15: Glomus fecundisporum

Figure 16: Glomus mosseae

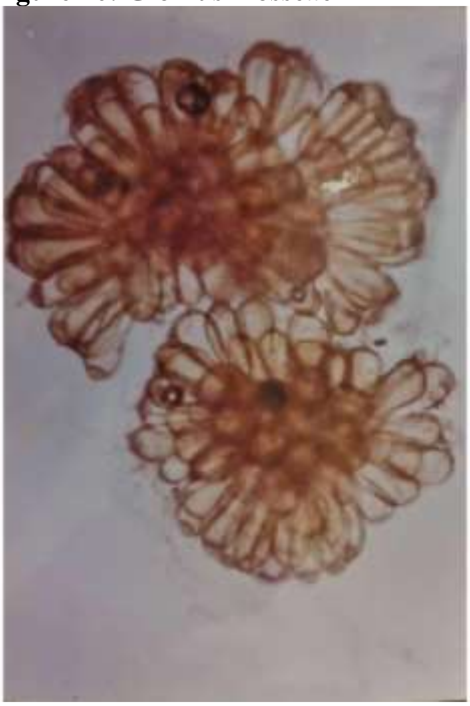

Figure 17: Sclerocystis sinuosa

Distribution of AM in the rhizosphere soils of Catharanthus roseus (L.) G. Don

Soil samples from the rhizosphere supporting the Catharanthus roseus (L.) G. Don plant were obtained at monthly intervals from the Nizamabad district in different regions. The initial samples were obtained in January 2018, and the process of sampling persisted until December 2021. The findings of this study are concisely reported in Table 1A, 1B and 1C.

The mycorrhizosphere of Catharanthus roseus (L.) G. Don facilitated the simultaneous presence of arbuscular mycorrhizal (AM) fungi from five distinct species. The prevailing genus observed among the isolated arbuscular mycorrhizal (AM) fungi was Glomus, comprising a total of eight distinct species. The species Glomus fasciculata exhibited a greater frequency of isolation, subsequently succeeded by G. aggregatum and G. mosseae. During the month of September, a total of five instances were documented. The distribution pattern of Acaulospora exhibited a resemblance to that of Glomus, as both genera displayed a significant rise in species abundance during the month of September. A higher level of consistency in occurrence was seen in A. bireticulata compared to the other five species investigated over the months of July and November. A total of three unique species of Sclerocystis were identified, and no discernible regional distribution pattern was seen. S. pachycaulis holds notable importance within the Sclerocystis genus as a fungal species. The presence of a single species was a distinguishing feature of the genus Entrophospora. E. infrequens exhibits a temporal pattern of occurrence, specifically noted between the months of September to October and January to

February. The sole manifestation of the genus Gigaspora was documented as a singular species, precisely designated as G. giganita, that was isolated between the months of April and September. A decrease in the prevalence of arbuscular mycorrhizal (AM) fungus was detected during the summer season and subsequent early wet season.
The data reported in Table 1A, 1B, 1C demonstrates that the mycorrhizosphere of C. roseus displayed the greatest prevalence of arbuscular mycorrhizal (AM) fungus throughout the month of September. The rhizosphere of the periwinkle plant is mostly inhabited by two important species, viz., Glomus and Acaulospora.

Table 1A: Occurance of AM Fungi in periwinkle rhizosphere soil at Armoor Division

Name of the AM Spores	Months											
	1	2	3	4	5	6	7	8	9	10	11	12
Acaulospora bireticulata (Rothwell & Trappe)	+	-	-	-	-	-	-	+	+	+	+	+
Acaulospora delicate (Walker, Pfeiffer & Bloss)	-	-	-	-	-	-	-	-	-	-	+	-
Acaulospora dilatata (Morton)	-	-	-	-	-	-	-	+	-	-	+	-
Acaulospora laevis (Gerdemann & Trappe)	-	-	-	-	-	-	-	-	-	-	+	-
Acaulospora mellea (Spain & Schenck)	-	-	-	-	-	-	-	+	-	-	+	-
Acaulospora nicolsonii (Walker,Reed & Sanders)	-	+	-	-	+	-	-	-	-	-	+	-
Acaulospora spinosa (Walker &Trappe)	-	+	-	-	-	-	-	-	-	-	-	-
Entrophospora infrequens [(Hall) Ames & Schneider]	-	-	+	-	-	-	-	-	-	-	+	+
Gigaspora gigantia [(Nicol. &Gerd.) Gerd.&Trappe]	-	-	-	-	-	+	-	-	-	-	+	-
Glomus aggregatum (Schenck & Smith)	-	+	-	-	-	-	+	-	-	-	+	-

Glomus albidum(Walker & Rhodes)	+	+	-	-	-	-	-	-	-	-	-	-
Glomus constrictum (Trappe)	-	-	-	+	-	+	-	-	-	+	-	-
Glomus convolutum (Gerd. & Trappe)	-	-	-	-	-	+	-	-	-	-	-	-
Glomus fasciculatum[(Thaxter sensu Gerd.) Gerd. & Trappe]	+	+	-	+	-	+	+	-	+	-	+	+
Glomus fecundisporum (Schenck & Smith)	-	-	-	-	-	-	-	-	+	-	+	-
Glomus manithotis (Howeler,Sieverding & Schenck)	-	-	-	-	-	-	-	-	-	+	-	-
Glomus mosseae [(Nicol.& Gerd.) Gerd. & Trappe]	-	-	-	-	-	-	-	+	+	-	-	+
Glomus radiatum [(Thaxter) Trappe & Gerd.]	-	-	-	-	+	-	-	-	-	-	+	-
Glomus tortuosum (Schenck & Smith)	-	-	-	-	-	-	-	-	-	-	+	-
Sclerocystis microcarpus (Iqbal & Bushra)	-	-	-	-	+	+	-	-	-	-	-	-
Sclerocystis pachycaulis (Wu & Chen)	-	-	-	-	-	+	-	-	-	-	-	-
Sclerocystis sinuosa (Gerd. & Baksh.)	+	-	-	-	-	-	+	-	-	-	+	-

*Note: '+' indicates presence; '-' indicates Absence

Table 1 B: Occurance of AM Fungi in periwinkle rhizosphere soil at Bodhan Division

Name of the AM Spores	Months											
	1	2	3	4	5	6	7	8	9	10	11	12
Acaulospora bireticulata (Rothwell & Trappe)	-	-	-	-	-	-	-	+	+	+	+	+
Acaulospora delicate (Walker, Pfeiffer & Bloss)	-	-	-	-	-	-	-	-	-	-	+	-
Acaulospora dilatata (Morton)	+	-	-	-	-	-	-	+	-	-	+	-
Acaulospora laevis (Gerdemann & Trappe)	+	-	-	-	-	-	-	-	-	-	+	-
Acaulospora mellea (Spain & Schenck)	-	-	-	-	-	-	-	+	-	-	+	-
Acaulospora nicolsonii (Walker,Reed & Sanders)	-	+	-	+	+	-	-	-	-	-	+	-
Acaulospora spinosa (Walker &Trappe)	-	+	-	-	-	-	-	-	-	-	-	-
Entrophospora infrequens [(Hall) Ames & Schneider]	-	-	+	-	-	+	-	-	-	-	+	+
Gigaspora gigantia [(Nicol. &Gerd.) Gerd.&Trappe]	+	-	-	-	-	+	-	-	-	-	+	-
Glomus aggregatum (Schenck & Smith)	-	+	-	-	-	-	+	-	-	-	+	-
Glomus albidum(Walker & Rhodes)	+	-	-	-	-	+	+	-	-	-	-	-
Glomus constrictum (Trappe)	-	-	-	+	-	+	-	-	-	+	-	-
Glomus convolutum (Gerd. & Trappe)	-	-	-	-	-	+	-	-	-	-	-	-

Glomus fasciculatum[(Thaxter sensu Gerd.) Gerd. & Trappe]	+	+	-	+	-	+	+	-	+	-	+	+
Glomus fecundisporum (Schenck & Smith)	-	-	-	-	-	-	-	-	+	-	+	-
Glomus mosseae [(Nicol.& Gerd.) Gerd. & Trappe]	-	-	-	-	-	-	-	+	+	-	-	+
Glomus radiatum [(Thaxter) Trappe & Gerd.]	-	-	-	-	+	-	-	-	-	-	+	-
Sclerocystis microcarpus (Iqbal & Bushra)	-	-	-	-	+	+	-	-	-	-	-	-
Sclerocystis pachycaulis (Wu & Chen)	-	-	-	-	-	+	-	-	-	-	-	-
Sclerocystis sinuosa (Gerd. & Baksh.)	+	-	-	-	-	-	+	-	-	-	+	-

*Note: '+' indicates presence; '-' indicates Absence

Table 1 C: Occurance of AM Fungi in periwinkle rhizosphere soil at Sirikonda Division

Name of the AM Spores	Months											
	1	2	3	4	5	6	7	8	9	10	11	12
Acaulospora bireticulata (Rothwell & Trappe)	+	-	-	-	-	-	-	+	+	+	+	+
Acaulospora delicate (Walker, Pfeiffer & Bloss)	-	-	-	-	-	-	-	-	-	-	+	-
Acaulospora dilatata (Morton)	-	-	-	-	-	-	-	+	-	-	+	-
Acaulospora foveata Trappe and Janos	+	+	-	-	-	-	-	-	-	-	-	+
Acaulospora laevis (Gerdemann & Trappe)	-	-	-	-	-	-	-	-	-	-	+	-

Acaulospora mellea (Spain & Schenck)	-	-	-	-	-	-	-	+	-	-	+	-
Acaulospora nicolsonii (Walker,Reed & Sanders)	-	+	-	-	+	-	-	-	-	-	+	-
Acaulospora spinosa (Walker &Trappe)	-	+	-	-	-	-	-	-	-	-	-	-
Entrophospora infrequens [(Hall) Ames & Schneider]	-	-	+	-	-	-	-	-	-	-	+	+
Gigaspora gigantia [(Nicol. &Gerd.) Gerd.&Trappe]	-	-	-	-	-	+	-	-	-	-	+	-
Glomus aggregatum (Schenck & Smith)	-	+	-	-	-	-	+	-	-	-	+	-
Glomus albidum(Walker & Rhodes)	+	+	-	-	-	-	-	-	-	-	-	-
Glomus celedonium (Nicolson and Gerdemann)	-	-	+	-	-	-	+	+	-	-	-	-
Glomus constrictum (Trappe)	-	-	-	+	-	+	-	-	-	+	-	-
Glomus convolutum (Gerd. & Trappe)	-	-	-	-	-	+	-	-	-	-	-	-
Glomus fasciculatum[(Thaxter sensu Gerd.) Gerd. & Trappe]	+	+	-	+	-	+	+	-	+	-	+	+
Glomus fecundisporum (Schenck & Smith)	-	-	-	-	-	-	-	-	+	-	+	-
Glomus manithotis (Howeler,Sieverding & Schenck)	-	-	-	-	-	-	-	-	-	+	-	-
Glomus mosseae [(Nicol.& Gerd.) Gerd. & Trappe]	-	-	-	-	-	-	-	+	+	-	-	+

Glomus radiatum [(Thaxter) Trappe & Gerd.]	-	-	-	-	+	-	-	-	-	-	+	-
Glomus tortuosum (Schenck & Smith)	-	-	-	-	-	-	-	-	-	-	+	-
Sclerocystis microcarpus (Iqbal & Bushra)	-	-	-	-	+	+	-	-	-	-	-	-
Sclerocystis pachycaulis (Wu & Chen)	-	-	-	-	-	+	-	-	-	-	-	-
Sclerocystis sinuosa (Gerd. & Baksh.)	+	-	-	-	-	-	+	-	-	-	+	-

*Note: '+' indicates presence; '-' indicates Absence

Discussion

Arbuscular mycorrhizal (AM) fungi exhibit a cosmopolitan distribution and establish mycorrhizal associations with the majority of plant species, encompassing various families of angiosperms. However, it is important to note that AM fungi do not form mycorrhizal associations with plants belonging to the following families: Cyperaceae, Commelinaceae, Juncaceae, Utricaceae, Polyonaceae, Aracaceae, Amaranthaceae, Chenopodiaceae, Nyctaginaceae, Phytolacaceae, Caryophyllaceae, Cruciferae, Fumariaceae, and Portulacaceae. Under natural conditions, mycorrhizal association is prevalent, but non-mycorrhizal association is frequently observed across various soil types and environmental settings. Medicinal plants from other plant families also exhibit AM connection, hence not deviating from the norm.

Throughout the course of human history, mankind has relied heavily on plants for a multitude of purposes, including meeting diverse requirements and utilizing them as a valuable source of medicinal remedies. The botanical family Apocynaceae encompasses several plants that hold significant medicinal value.

Preliminary findings regarding the presence of arbuscular mycorrhizal (AM) fungi are now accessible. Many medicinal and essential oil plants are known to possess secondary compounds in their root systems. (Begum et al., 2019; Rodríguez-Morelos et al., 2014) have been cited in the literature. The researchers documented the lack of mycorrhizal symbiosis in all plant species that exhibited a range of secondary metabolites, such as alkaloids, phenolics, terpenoids, tannins, and stilbenes. The absence of relationship between AM and the presence of several secondary compounds was attributed by researchers. In a subsequent study conducted by Ravishankar and Shukla (2007), a total of 25 distinct medicinal plants were examined for the occurrence of arbuscular mycorrhizal (AM) association. The researchers observed mycorrhizal colonization in all of the medicinal plants assessed, including the periwinkle and serpent-wood species belonging to the apocynaceae family.

The findings of our study about the presence of arbuscular mycorrhizal (AM) fungi in medicinal plants of the Apocynaceae family corroborate previous observations made by various researchers. Despite the occurrence of secondary compounds such as tannins, alkaloids, and phenolics, a significant number of medicinal and aromatic plants have arbuscular mycorrhizal (AM) fungi within their root system. Based on our findings, it can be inferred that the absence of secondary compounds in the soils studied by Kim et al., (2011) may contribute to the observed lack of negative effects on arbuscular mycorrhizal (AM) fungi.

Summary and conclusion

Arbuscular mycorrhizal (AM) fungi are extensively prevalent in soils characterised by phosphate shortage. Mycorrhizal connections are frequently documented in a diverse array of plant species spanning many taxonomic groups within the plant kingdom. The taxonomic classification Apocynaceae has a diverse array of plant species that exhibit considerable medicinal importance. The primary objective of this study was to assess the correlation between the presence of arbuscular mycorrhizal (AM) fungus and various medicinal plants from the Apocynaceae family. During the month of September, the rhizosphere of Catharanthus roseus (L.) G. Don exhibited the highest occurrence of arbuscular mycorrhizal (AM) fungi, whereas the apex of root colonisation was observed in October. The main aim of the present study was to evaluate the impact of arbuscular mycorrhizal (AM) inoculation on the extent of root colonisation and the growth of periwinkle plants. The goal was achieved by utilising a soil-based root bit inoculum consisting of Glomus aggregatum, Glomus mosseae, and a combination of native arbuscular mycorrhizal fungi found in the soil.

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