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Diversity of Arbuscular Mycorrhizal fungi associated with *Amorphophallus commutatus* (Schott) Engl., in Goa, India

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ABSTRACT

The present study examined the diversity of arbuscular mycorrhizal (AM) fungi in the unique tropical ecosystems of Goa, associated with *Amorphophallus commutatus* (Schott) Engl., a plant significant for its unique ecological and ethnobotanical role, distinctive morphology, potential medicinal uses, and value in biodiversity conservation. By focusing on this distinct ecological niche, the present study aimed to understand the AM fungal diversity and identify the dominant species associated with *A. commutatus*. The findings revealed native AM species that could assist in developing effective AM inocula and potentially improve agricultural practices and ecosystem health in this biodiverse region. In this study, a total of eight AM fungal species viz. *Acaulospora foveata*, *A. scrobiculata*, *Funneliformis geosporum*, *F. mosseae*, *Gigaspora albida*, *Gi. decipiens*, *Rhizophagus fasciculatus*, and *R. intraradices* across four genera viz. *Acaulospora*, *Funneliformis*, *Gigaspora*, and *Rhizophagus*, belonging to three families, Acaulosporaceae, Glomeraceae, and Gigasporaceae, were recovered from trap cultures. The results revealed that the Glomeraceae was the most dominant family, and *Acaulospora scrobiculata* was the predominant AM species.

Keywords: Root colonization, Spore density, *Amorphophallus*, Arbuscular mycorrhizae, AM fungi.

INTRODUCTION

Arbuscular mycorrhiza (AM) represents the most prevalent symbiotic relationship between plants and microbes. AM fungi are found in diverse natural habitats and offer crucial ecological benefits, especially by enhancing plant nutrition, boosting stress resistance and resilience, and improving soil structure and fertility (Chen *et al.,* 2018). Numerous studies have established that variations in arbuscular mycorrhizal (AM) fungal diversity can significantly influence ecosystem functioning, predominantly through their effects on primary productivity (Powell and Rillig, 2018).

Global agricultural food production must double by 2050 to feed the rising population, all while decreasing reliance on conventional chemical fertilizers and pesticides. To achieve this goal, it is essential to explore biofertilization through various mutualistic interactions between plant roots and the rhizosphere microbiome, including those involving AM fungi (Igiehon and Babalola, 2017).

The tubers from *Amorphophallus* genus, of the family Araceae, are used in Ayurvedic medicine and are traditionally applied to address a variety of ailments, including piles, abdominal pain, tumors, spleen enlargement, asthma, and rheumatism, also known to stimulate appetite and aid digestion (Shete *et al.,* 2015). Additionally, *Amorphophallus* species are abundant in polyphenolic compounds, which provide numerous pharmacological advantages, such as analgesic, neuroprotective, hepatoprotective, anti-inflammatory, anticonvulsant, antibacterial, antioxidant, anticancer, anti-obesity, and immunomodulatory effects, as well as helping to alleviate gastrointestinal issues and reduce blood glucose levels (Islam *et al.,* 2023). *Amorphophallus commutatus* (Schott) Engl., endemic to the Western Ghats (Gholave *et al.*, 2014), possesses considerable medicinal value and is utilized in traditional medicine. It is also recognized for its potential anticancer properties (Raj *et al.,* 2022). Plants with underground storage organs, including *A. commutatus* from the Western Ghat region of Goa, are known to be associated with AM fungi (Khade and Rodrigues, 2007).

Goa is known for its rich biodiversity, including unique flora and fauna. The genus *Amorphophallus*, includes some rare species that thrive in the region. Understanding the AM fungal relationships of these plants can shed light on how biodiversity is supported and maintained in such ecosystems. Also, AM fungal communities vary across ecosystems, consisting of diverse species that perform symbiotic functions based on their specific community structures (Ôpik *et al.,* 2006). The present study was initiated to characterize and elucidate the AM fungal diversity associated with *A. commutatus*.

MATERIALS AND METHODS

Study site: Soil samples were collected from various sites across Goa. Goa typically has a tropical monsoon climate, with temperatures mostly ranging from 23°C to 34°C. The state has a mix of moist deciduous forests, open scrub jungles, and diverse plateau vegetation.

Rhizosphere soil sample collection: The soil samples from five to fifteen plants per site were collected for analysis from May end to June 2022, at the maturation stage of *A. commutatus.* Soil was collected from the rhizosphere region throughout different areas of Goa. The zigzag soil sampling method was utilized to ensure sample consistency. Rhizosphere soil and roots were collected, and a composite soil sample (weighing approximately 500g) was brought to the laboratory for further analysis.

Sampling sites: Soil and root samples were collected from the rhizosphere of *A. commutatus* from five locations in Goa. The sampling sites were S-I, S-II, S-III, S-IV, and S-V. The diversity of AM fungi was subsequently examined using trap cultures of *A. commutatus* obtained from these various sites in Goa, as shown in **Table 1**.

Table 1: List of collection sites of *Amorphophallus commutatus* (Schott) Engl.

Site	Latitude and Longitude
S-I-Zambaulim	Lat-15.198641° Long -74.051445°
S-II-Fatorda	Lat -15.289762° Long - 73.962353°
S-III-Bicholim	Lat - 15.586952° Long - 73.907582°
S-IV-Nuvem	Lat - 15.307377° Long - 73.94504°
S-V-Taleigao Plateau	Lat - 15.455272° Long - 73.837750°

Trap cultures: AM fungal spores from the rhizosphere soil of *A. commutatus* were isolated using the wet sieving and decanting method (Gerdemann and Nicolson, 1963). These spores were used to prepare the trap cultures using Morton's technique (1993). The trap cultures were maintained in a polyhouse for 90 days at 28°C, with humidity between 60% to 80%, and *Plectranthus scutellarioides* (L.) R. was used as the host plant. The plants were watered regularly as and when needed. Hoagland's solution (Hoagland and Arnon, 1950), devoid of phosphorus, was added every 15 days until harvest.

Assessment of AM fungal root colonization: Fifty root segments, each measuring one centimeter, were cleared in 10% KOH at 90°C for 60 minutes, followed by acidification with 5N HCl and overnight staining with 0.05% Trypan blue, using the procedure of Phillips and Hayman (1970). The stained roots were then mounted on glass slides using PVLG (Polyvinyl Alcohol Lacto-Glycerol) for examination under a bright-field Olympus BX41 research microscope. A root segment was classified as mycorrhizal if it showed hyphae and/or hyphal coils, arbuscules, arbusculate coils, and vesicles. Distinct AM fungal colonization features included intra-radical and extra-radical hyphae, hyphal coils, arbuscules, auxiliary cells, and vesicles. The extent of total colonization (TC%) and the percentages of root length containing hyphae (HC%), arbuscules (AC%), and vesicles (VC%) were measured using the magnified intersection method outlined by McGonigle *et al.* (1990). pH of soil sample was measured using a pH meter (LI120 Elico, India).

Density, Abundance, and Taxonomic identification of AM fungal spores: The AM fungal spore density was estimated following the method of Adholeya and Gaur (1994). Species richness (SR) indicates the total number of AM fungal species recovered from each sampling site. The relative abundance (RA%) for each location was calculated using the formula provided by Beena *et al.* (2000).

The procured spores were mounted in PVLG on glass slides and examined under a bright-field Olympus BX41 research microscope at 40× and 100× magnification to classify the AM spores. Characteristics of the spores were compared against standard references (Rodrigues and Muthukumar, 2009; Blaszkowski, 2012) and the International Collection of Vesicular Arbuscular Mycorrhizal Fungi (INVAM). The names and epithets of AM fungal species were assigned according to the guidelines set by Schüßler and Walker (2010) and Redecker (2013).

Statistical Analysis

The ecological traits of AM fungal species were assessed using the following indices:

- Relative abundance (RA) = (Number of spores of species/Total number of spores in all soil samples) x100
- Isolation frequency (IF) = (Number of soil samples containing particular species/Total number of soil samples analyzed) x100
- Shannon- Wiener diversity index (H) = −Σ ($P_i$ln (P_i)), where *Pi* is the proportion of individual species that contributes to the total number of individuals (Shannon and Wiener, 1949)
- Simpson's diversity index (D) = 1- (Σn (n-1)/N (N-1)), where *n* is the number of individuals of a given species, and N is the total number of individuals in a community (Simpson, 1949).
- Species richness (SR) = total number of species in the community.
- AMF species evenness (E): *Σ(H') =H'/ H'max, where max = lnS, S= SR*

The Pearson correlation coefficient (r) was determined to analyze the relationships between spore density (SD) and RC, RA, IF, SD, and SR, utilizing IBM SPSS Statistics 22.

RESULTS AND DISCUSSION

AM Fungal Root Colonization, Spore Density, and Diversity: AM fungal colonization was recorded in all the examined roots of *A. commutatus*. The roots exhibited the presence of hyphae, vesicles, and arbuscules (**Table 1, Figure 1**). Maximum root colonization (98%) and spore density (134 spores 100^{-1} g soil) were recorded from S-II site. In comparison, minimum root colonization (70.67%) and spore density (66.67 spores100^{-1} g soil) were recorded from S-I and S-IV sites, respectively. The study recorded extensive hyphal and vesicular colonization compared to arbuscular colonization. The results of AM fungal diversity recorded at the study sites are depicted in **Table 1**. A total of eight AM fungi species viz., *Acaulospora foveata* Trappe & Janos, *A. scrobiculata* Trappe, *Funneliformis geosporum* (T.H. Nicolson & Gerd.) C. Walker & A. Schüßler, *F. mosseae* (Nicol. & Gerd.) Gerd. and Trappe, *Gigaspora albida* Schenck & Smith, *Gi. decipiens* I.R. Hall & L.K. Abbott, *Rhizophagus fasciculatus* (Thaxt.) C. Walker & A. Schüßler, and *R. intraradices* (N.C.Schenck & G.S.Sm.) C. Walker & A.Schüssler were recovered from the trap cultures (**Figure 1**). Site S-II exhibited maximum root colonization and spore density. ANOVA shows significant variation in colonization across the sites (ANOVA P=0.040, F=3.06).

Table 1: Arbuscular mycorrhizal (AM) colonization, spore density, and diversity in *Amorphophallus commutatus*.

Sites	TC (%)	HC (%)	VC (%)	AC (%)	SD*	†AM fungal species
S-I	70.67 ±3.84	70.67 ±1.45	67.33 ±0.34	19.00 ±2.08	68.00 ±2.31	*Fn.ge, Fn.mo, Ac.fo*
S-II	98.00 ±0.57	96.67 ±0.66	77.67 ±0.88	82.00 ±1.53	134.00 ±3.21	*Ac.fo, Ac.sc., Fn.ge, Fn.mo, Gi.de,Gi.al,Rh.fa, Rh.in*
S-III	73.67 ±1.20	72.67 ±1.20	67.33 ±1.45	64.33 ±1.45	102.67 ±3.71	*Ac.sc., Gi.de*
S-IV	71.67±0.88	70.33 ±0.88	54.33 ±0.88	53.33±1.86	66.67±0.88	*Ac.fo, Ac.sc,Gl. sp.*
S-V	76.67±1.45	76.33±0.88	57.00±0.58	45.00±1.15	85.33±1.45	*Fn. mo, Ac. sc, Gl.sp.*

All values are the mean of three readings; ± = Standard error; TC = total colonization, HC = hyphal colonization, AC = arbuscular colonization, VC = vesicular colonization. SD = Spore density* = spores/100 g of soil. †*Acaulospora foveata- Ac.fo; A. scrobiculata- Ac.sc; Funneliformis geosporum- Fn.ge; F. mosseae- Fn.mo; Gigaspora albida- Gi.al; Gi. decipiens- Gi.de; Rhizophagus fasciculatus- Rh.fa; R. intraradices- Rh.in.*

Figure 2 delineates the composition of the AM fungal community derived from the average spore density obtained from the trap cultures across the five study sites. It illustrates that *F. mosseae* from site 3 exhibits the highest spore density. The AM fungi with notable densities following *F. mosseae* include *A. scrobiculata*, *A. foveata*, and *F. geosporum*. It was observed that *A. scrobiculata* demonstrated a strong presence across four of the five sites examined.

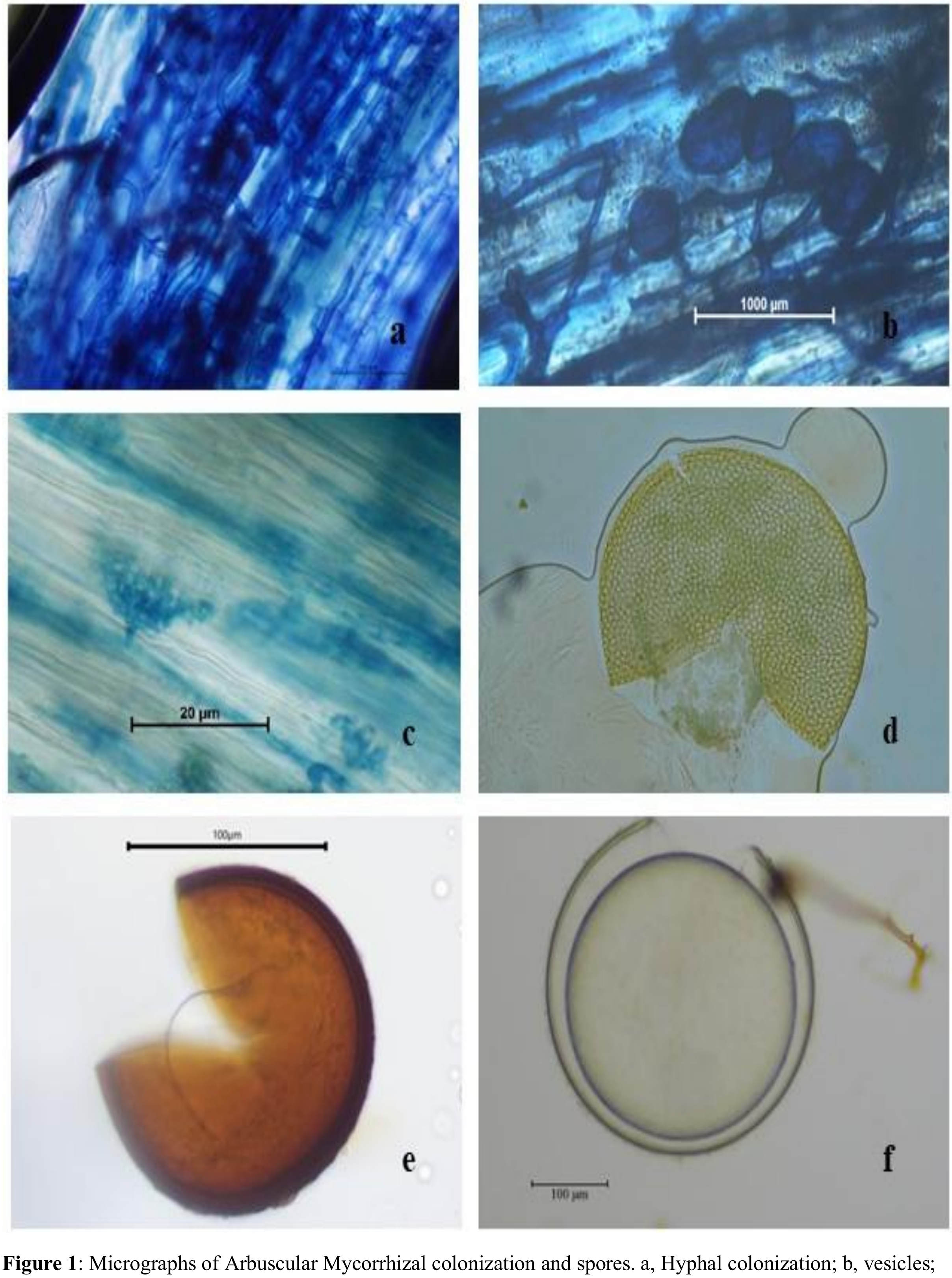

Figure 1: Micrographs of Arbuscular Mycorrhizal colonization and spores. a, Hyphal colonization; b, vesicles; c, arbuscules; d, *Acaulospora scrobiculata*; e, *Funneliformis geosporum*; f, *Gigaspora albida*

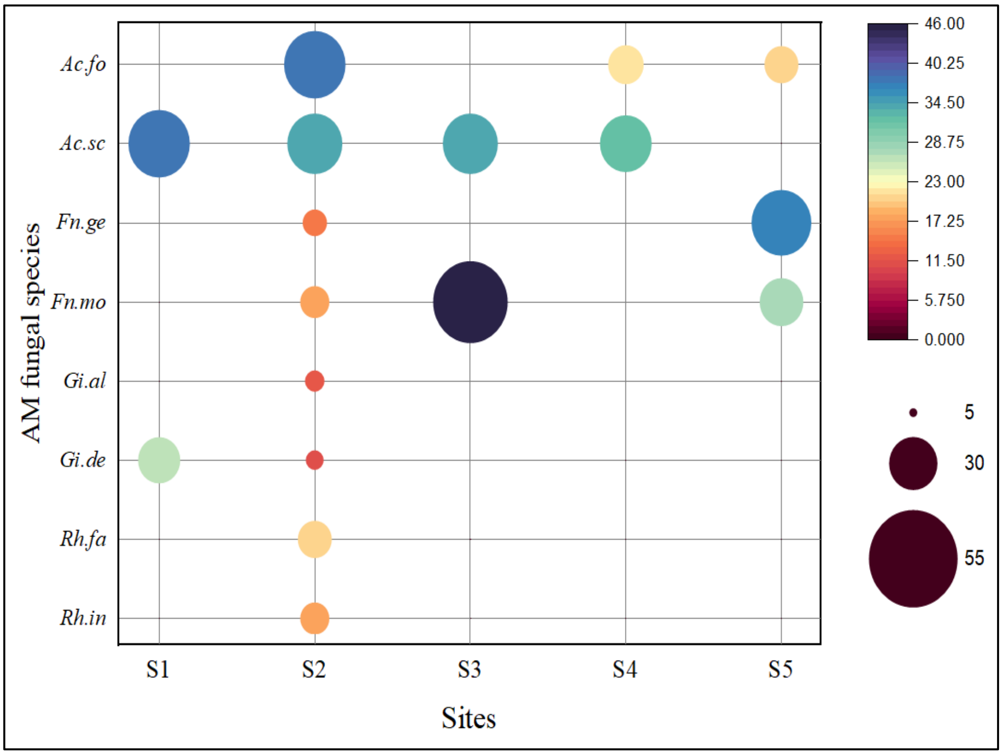

Figure 2: AM Fungal community composition (based on mean spore density) in trap cultures at various study sites using Matrix bubble

The highest RA (28.34%) and IF (80.00%) were recorded in *A. scrobiculata,* indicating its dominance. *Acaulospora* is commonly found in soils with moderate acidity (Vilcatoma-Medina *et al.,* 2018). The soil pH was acidic, as reported in an earlier study (Mohekar *et al.,* 2020).

Pearson's correlation coefficient exhibited a significant (P<0.01) positive correlation (r=0.89) between SD and RC. A positive correlation was observed in IF and RA (r=0.97; P<0.0). The values of (H), i.e., Shannon Weiner index, (D) Simpson's index of dominance, and species evenness were found to be 1.94, 0.83, and 0.88, respectively. A Shannon-Wiener Index (H) value of 1.94 suggests a moderate level of biodiversity. Simpson's index of dominance D being 0.83 signifies that one or a few species likely dominate the AM fungal community.

AM fungi can contribute to the maintenance of agricultural development by controlling soil erosion, enhancing phytoremediation, and eliminating other organisms that may be harmful to crops through a common mycelial network. Combining these potentials will help sustain agriculture and boost food security globally when fully harnessed under an agricultural scenario. Also, many *Amorphophallus* species are threatened due to habitat loss and over-exploitation. Investigating their mycorrhizal associations can provide crucial information for conservation strategies that could help restore degraded habitats and ensure sustainable management practices to preserve these plants. Given the high context dependence of AM fungi, it is essential to comprehend the AM diversity associated with economically and medicinally significant plants. Therefore, understanding the AM preferences of *A. commutatus* could be strategically leveraged in future mass production efforts to enhance plant growth and optimize yield.

CONCLUSION

In conclusion, this study highlights the significant diversity of AM fungi associated with *Amorphophallus commutatus* in Goa, India. The findings underscore the crucial role these fungal communities play in the plant's health and growth, reflecting a complex symbiotic relationship influenced by the local soil and environmental conditions. Enhancing our understanding of such diversity can inform sustainable cultivation practices and conservation strategies for *A. commutatus*, thereby promoting biodiversity and ecological stability in the region.

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REFERENCES

Adholeya, A. and Gaur, A. 1994. Estimation of VAM fungal spores in soil. *Mycorrhiza News*, 6(1): 10-11.

Beena, K. R., Raviraja, N. S., Arun, A. B. *et al.* 2000. Diversity of arbuscular mycorrhizal fungi on the coastal sand dunes of the west coast of India. *Current Science*, 79 (10):1459-1466. http://www.jstor.org/stable/24105108.

Blaszkowski, J. 2012. Glomeromycota. W. Szafer Institute of Botany, *Polish Academy of Sciences*, Poland. 1-303. ISBN: 978-83-89648-82-2.

Chen, M., Arato, M., Borghi, L., *et al.* 2018. Beneficial services of arbuscular mycorrhizal fungi–from ecology to application. *Frontiers in Plant Science*, 9:1270; doi: 10.3389/fpls.2018.01270.

Gerdemann, J. W. and Nicolson, T. H. 1963. Spore Density of *Endogone* species extracted from soil wet sieving and decanting. *Transactions of British Mycological Society*, 46:235-244.

Gholave, A. R., Jadhav, J. P. and Yadav, S. R. 2014. Cytotaxonomical studies on three varieties of *Amorphophallus commutatus* (Araceae) Endemic to the Western Ghats. *Cytologia*, 79(3):359-363; doi: 10.1508/cytologia.79.359

Hoagland, D.R., and Arnon, D.I. 1950. The water-culture method for growing plants without soil. Circular. *California Agricultural Experiment Station*, **347**:1-38.

Igiehon, N. O. and Babalola, O. O. 2017. Biofertilizers and sustainable agriculture: exploring arbuscular mycorrhizal fungi. *Applied Microbiology and Biotechnology*, 101:4871-4881; doi: 10.1007/s00253-017-8344-z.

Islam, F., Labib, R. K., Zehravi, M., *et al.* 2023. Genus *Amorphophallus*: a comprehensive overview on phytochemistry, ethnomedicinal uses, and pharmacological activities. *Plants*, 12(23):3945; doi: 10.3390/plants12233945.

Khade, S. W. and Rodrigues, B. F. 2007. Incidence of arbuscular mycorrhizal (AM) fungi in some angiosperms with underground storage organs from Western Ghat region of Goa. *Tropical Ecology*, 48(1):115-118. http://irgu.unigoa.ac.in/drs/handle/unigoa/4005.

McGonigle, T. P., Miller, M. H., Evans, D. G., *et al.* 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. *New Phytologist,* 115:495-501.

Mohekar, D. S., Singh, S. K., Chattaraj, S., *et al.* 2020. Characterization and classification of soils on topo-sequence of laterite landscape in tropical ecosystem of Goa. *International Journal of Communication Systems*, **8(3)**:1345-1351.

Morton, J. B. 1993. Problems and solutions for the integration of glomalean taxonomy, systematic biology, and the study of endomycorrhizal phenomena. *Mycorrhiza,* 2:97-109.

Öpik, M., Moora, M., Liira, J., *et al.* 2006. Composition of root-colonizing arbuscular mycorrhizal fungal communities in different ecosystems around the globe. *Journal of Ecology*, **94(4)**:778-790.

Phillips, J. M. and Hayman, D. S. 1970. Improved procedures for clearing roots and staining of mycorrhizal fungi for rapid assessment of infection. *Transactions of British Mycological Society*, 55:158-161.

Powell, J. R. and Rillig, M. C. 2018. Biodiversity of arbuscular mycorrhizal fungi and ecosystem function. *New Phytologist*, 220(4):1059-1075; doi: 10.1111/nph.15119.

Raj, S., Jayaraj, R. and Kodiveri M., G. 2022. Chemical profiling and evaluation of antioxidant and anticancer potential of tuber crop *Amorphophallus commutatus* var. *wayanadensis*. *Plant Foods for Human Nutrition*, 77:1-9; doi: 10.1007/s11130-021-00942-3.

Redecker, D., Schüßler, A., Stockinger, H., *et al.* 2013. An evidence-based consensus for the classification of arbuscular mycorrhizal fungi(Glomeromycota). *Mycorrhiza*, 23:515 -531; doi: 10.1007/s00572-013-0486-y.

Rodrigues, B. and Muthukumar, T., 2009. Arbuscular mycorrhizae of Goa - a manual of identification protocols, *Goa University*, Goa. 1-135.

Schübler, A., Schwarzott, D., Walker, C. 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution, *Mycological Research*. 105:1413-1421. doi:10.1017/S0953756201005196.

Shete, C.C., Wadkar, S.S., Gaikwad, N.B., *et al.* 2015. Phenolic contents and antioxidant capacity of *Amorphophallus commutatus* and *Amorphophallus paeonifolius*. *International Food Research Journal*, 22(5): 1939-1944.

Vilcatoma-Medina, C., Kaschuk, G., Zanette, F. 2018. Colonization and spore richness of arbuscular mycorrhizal fungi in *Araucaria* nursery seedlings in Curitiba, Brazil. *International Journal of Agronomy*, 2018(1):5294295; doi: 10.1155/2018/5294295.