

Studies on Arbuscular Mycorrhizal (AM) Fungi in Wild and Cultivated Tubers, found in Goa.

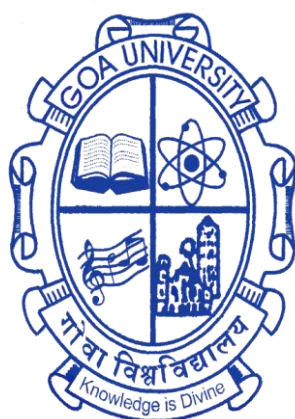
**A THESIS SUBMITTED IN PARTIAL FULFILLMENT FOR THE
DEGREE OF**

DOCTOR OF PHILOSOPHY

IN BOTANY

IN THE SCHOOL OF BIOLOGICAL SCIENCES AND BIOTECHNOLOGY

GOA UNIVERSITY



By

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MARCH 2026

DECLARATION

I, Amisha Gokuldas Shirodker alias Amisha Rajesh Pednekar hereby declare that this thesis represents work which has been carried out by me and that it has not been submitted, either in part or full, to any other University or Institution for the award of any research degree.

Place: Taleigao Plateau.

Date : 23-03-2026

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CERTIFICATE

I hereby certify that the above Declaration of the candidate, Amisha Gokuldas Shirodker alias Amisha Rajesh Pednekar is true and the work was carried out under my supervision.

Prof. B. F. Rodrigues
School of Biological Sciences and Biotechnology
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Dedicated to
Paa



My father in heaven..

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Chapter 1: Introduction

Sustainable Agriculture

In the face of an ever-increasing global population demanding urgent food security, conventional farming's excessive resource use, pollution, and biodiversity loss threaten environmental sustainability. We need to view sustainable agriculture as the imperative pathway forward, integrating renewable energy sources and agroecological practices such as crop rotation, organic farming, agroforestry, the use of microbes to enhance soil fertility, curtail the use of synthetic pesticides, and bolster biodiversity. By leveraging local resources and traditional knowledge, these approaches maintain ecological balance while optimizing food production (Saikanth *et al.*, 2023; Mrabet, 2023). Crucially, the strategic use of microbes emerges as indispensable for soil protection and environmental preservation, ensuring economically viable, socially equitable farming that safeguards our planet for future generations (Javaid, 2010; Antoszewski *et al.*, 2022).

Relevance of AM fungi and Other Microbes in Sustainable Agriculture

AM fungi are increasingly recognized as vital, multifunctional microbial allies in sustainable agriculture, extending their roles beyond traditional biofertilization to encompass soil conservation and ecosystem health. Besides enhancing soil fertility, AM fungi help control soil erosion, facilitate phytoremediation, and suppress soil-borne pathogens through interconnected mycelial networks that enable communication and resource sharing among organisms (Igiehon and Babalola, 2017). Their capacity to improve plant growth and disease resistance further underscores their importance in sustainable crop production systems (Umer *et al.*, 2025). Effective management and application of AM fungi as biofertilizers hold significant promise for addressing critical agricultural challenges by supporting crop productivity, promoting economic stability, reducing reliance on chemical fertilizers, and alleviating food insecurity (Phour and Sindhu, 2024). As key fertility-promoting microbes, AM fungi enhance soil health and sustain crop yields, with the potential for tailored use of specific AM fungal strains to optimize agricultural sustainability according to soil and crop types. The strategic deployment of these symbiotic fungi is thus becoming an indispensable biological tool for advancing environmentally friendly and resilient farming practices

(George and Ray, 2023). Their use is particularly promising in low-input, organic, and regenerative farming systems, where maintaining soil fertility and plant resilience is crucial.

The soil environment is both complex and dynamic, characterized by numerous interactions that fundamentally influence its properties as a medium for plant growth and soil microbial activity. Among these, interactions involving AM fungi are particularly significant, as they continuously engage with a diverse array of soil microorganisms, including nonbacterial microorganisms, plant growth-promoting rhizobacteria, mycorrhiza helper bacteria, and deleterious bacteria. Understanding these interactions is crucial, given their significant implications for agriculture, especially in promoting sustainable, low-input farming systems (Miransari, 2011). AM fungi and soil microbe interactions play a critical role in shaping plant growth and soil structure, with recent studies highlighting their potential to enhance the productivity of cropping systems, such as paddy fields (Bao *et al.*, 2022). Moreover, the synergistic application of mycorrhizal fungi and beneficial bacteria, such as rhizobacteria, has been shown to significantly improve plant growth and nutrition, exemplified in banana cultivation, where such combinations are recommended during the micropropagation acclimatization phase (Rodríguez-Romero *et al.*, 2005). Additionally, AM fungi, coupled with phosphate-solubilising bacteria (PSB), offer an environmentally friendly alternative to chemical fertilizers by facilitating phosphate solubilization and enhancing crop yields without ecological harm (Wahid *et al.*, 2022). The use of bacterial inoculants, specifically P-solubilizing bacteria isolated from various plants, further underscores their importance in sustainable agriculture, as they not only liberate insoluble phosphates but also promote overall plant growth, supporting the broader goal of sustainable development (Subba, 2023).

Arbuscular Mycorrhizal (AM) Fungi – Origin and Significance

Mycorrhizae represent nature's most common symbiotic relationships, involving plants and specialized soil fungi. These fungi significantly contribute to ecosystem functioning, particularly by enhancing plant nutrient uptake, influencing plant community structure, promoting soil aggregation, and improving resistance to both biotic and abiotic stresses (Bücking *et al.*, 2012; Willis *et al.*, 2013).

AM fungi are among the earliest known terrestrial symbionts, having co-evolved with land plants over 450 million years ago (Remy *et al.*, 1994; Redecker *et al.*, 2000). Fossil evidence from the Devonian period, such as structures preserved in the Rhynie Chert, suggests that AM fungi were integral in enabling early vascular plants to colonize terrestrial habitats (Helgason and Fitter, 2005; Willis *et al.*, 2013). Today, approximately 80-90% of vascular plants form symbiotic associations with AM fungi, highlighting their ubiquity and essential role in terrestrial ecosystems (Wang and Qiu, 2006; Giovannetti *et al.*, 2010).

One of the most well-recognized roles of AM fungi is their contribution to plant mineral nutrition, as they extend their hyphae beyond the root zone to access nutrient pools that roots alone cannot reach, thereby significantly enhancing the uptake of essential elements such as P, N, and micronutrients (Israel *et al.*, 2022). These fungi are particularly notable for their capacity to improve P acquisition under nutrient-poor conditions (Qi *et al.*, 2022). The primary mechanism underlying this nutrient enhancement involves the extraradical hyphae of AM fungi, which facilitate increased assimilation of P and N, supporting more efficient plant growth even in nutrient-limited soils (George *et al.*, 1995; Mohammad *et al.*, 2003). This symbiotic relationship enables plants to thrive in challenging environments by optimizing nutrient uptake and overall growth performance.

Beyond nutrient transfer, AM fungi play a central role in maintaining healthy soils. Their hyphae contribute to the formation of stable soil aggregates, improving structure and water retention. The secretion of glomalin-related soil proteins not only supports soil texture but also enhances C storage and microbial biodiversity (Radhakrishnan *et al.*, 2020). Thus, AM fungi act not just as root partners but as ecosystem engineers, linking plant nutrition to broader soil processes.

Environmental stressors such as drought, salinity, temperature fluctuations, and soil contaminants present persistent challenges to plant vitality and productivity. AM fungi play a crucial role in mitigating these abiotic stresses by enhancing plant resilience. They facilitate improved water regulation, optimize ion uptake, and activate various stress-response pathways, including antioxidant defences that reduce oxidative damage (Israel *et al.*, 2022). Consequently, AM fungi are recognized for their capacity to confer

tolerance to plants under diverse stressful conditions, such as heat, salinity, drought, heavy metals, and extreme temperatures (Begum *et al.*, 2019). This symbiotic relationship underscores the potential of AM fungi as natural agents in improving plant stress tolerance and sustaining growth in adverse environments. These protective effects make AM fungi particularly relevant in the context of climate change, where fluctuating conditions threaten crop stability and product quality.

Taxonomy and Life Cycle of AM fungi

The AM fungi have been elevated to the phylum Glomeromycota, distinguished as a distinct lineage separate from other fungal taxa. These fungi represent a monophyletic clade of obligate mycobionts, with an evolutionary history dating back to one of the earliest symbiotic associations in plants. Glomeromycota is regarded as a monophyletic group, with Schüßler and Walker (2010) proposing a classification that includes a single class, Glomeromycetes, comprising four orders, 11 families, and 18 genera. Currently, approximately 288 species have been taxonomically described within this phylum (Öpik and Davison, 2016). The latest classification of Glomeromycota given in **Fig. 1.1**, is based on a comprehensive consensus derived from phylogenetic analyses of multiple ribosomal RNA gene regions, including 18S (SSU), ITS1-5.8S-ITS2 (ITS), and 28S (LSU). Redecker *et al.* (2013) elaborated on the phylogenetic reconstruction that underpins this classification.

AM fungi are obligate biotrophic soil-dwelling fungi that depend entirely on their capacity to establish and maintain symbiotic relationships with plant roots for their entire life cycle, thereby playing a critical role in plant nutrient uptake and regulation of associated mechanisms (Bücking *et al.*, 2012; Giovannetti *et al.*, 2010; Uwamungu *et al.*, 2022). Their life cycle includes spore germination, pre-symbiotic hyphal growth, host root recognition, appressorium or hyphopodium formation, root colonization, arbuscule development, extraradical mycelium formation, and ultimately sporulation (Giovannetti *et al.*, 2002; Giovannetti *et al.*, 2010). These developmental stages are orchestrated by intricate interactions between the fungus and host-derived signalling molecules, such as strigolactones, which stimulate hyphal branching and metabolic activity during the early symbiotic stages (Bücking *et al.*, 2012).

One of the most intriguing aspects of AM fungal development is the ability of spores to remain dormant under unfavourable conditions and germinate in response to specific

environmental cues. Spore dormancy varies among species and isolates and is regulated by factors like temperature, moisture, soil pH, and nutrient content (Giovannetti *et al.*, 2010). Unlike many biotrophic fungi, AM fungi spores do not require host signals to germinate, but show enhanced germination and hyphal branching in the presence of host root exudates (Logi *et al.*, 1998; Sbrana and Giovannetti, 2005).

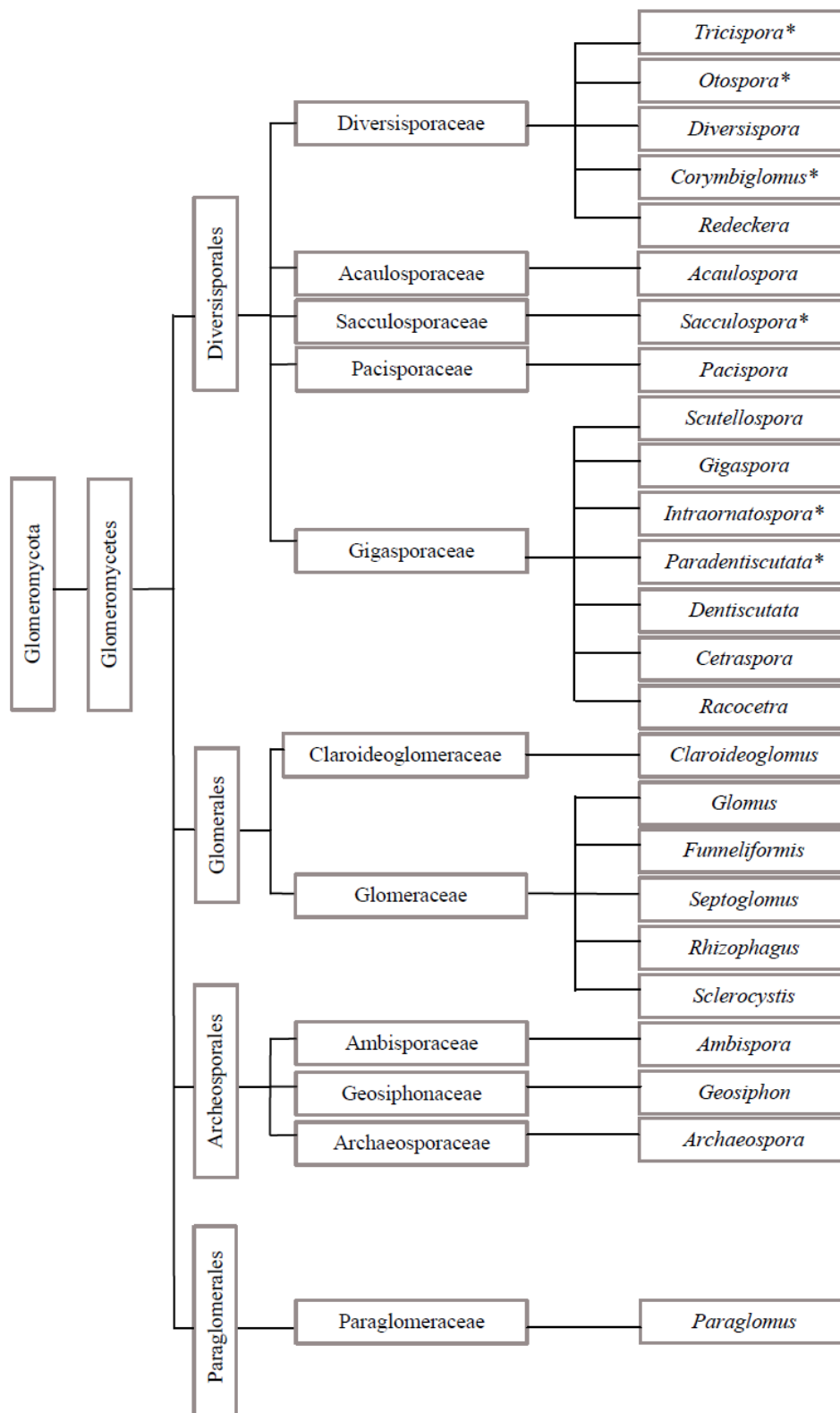


Fig. 1.1: Consensus classification of AM fungi by Redecker *et al.* 2013.
(*designates the uncertain position of genera).

Dormancy-breaking strategies include storage at low temperatures and exposure to certain microbial or chemical cues. Such strategies are considered critical for AM fungi survival in fluctuating environments, enabling them to persist as “*ancient asexuals*” across geological timescales (Tommerup, 1983; Koske and Tews, 1987).

Following spore germination, the fungal hyphae grow towards host roots and form appressoria or hyphopodia on the root surface, through which they penetrate the epidermis and establish themselves within the root cortex (Giovannetti *et al.*, 2002). Two main morphological types of colonization exist viz., *Arum*-type, characterized by intercellular hyphae and terminal arbuscules, and *Paris*-type, with intracellular hyphal coils (Smith and Read, 2008; Willis *et al.*, 2013).

AM fungi extend their extraradical mycelium (ERM) into the surrounding soil to absorb nutrients and water beyond the depletion zone of plant roots. This extensive hyphal network enhances the plant’s access to immobile nutrients, such as P, and contributes to soil aggregation and microbial interactions (Willis *et al.*, 2013). ERM also plays a role in interplant nutrient transfer, influencing plant community dynamics and increasing biodiversity (Van der Heijden *et al.*, 2008).

Inside cortical cells, arbuscules are formed, highly branched structures that facilitate nutrient exchange. The fungus supplies the plant with essential nutrients in exchange for photosynthetically derived carbon (Bücking *et al.*, 2012). This bidirectional exchange is tightly regulated through a specialized interface known as the periarbuscular membrane (Harrison *et al.*, 2005).

Despite their asexual nature and relatively simple morphology, AM fungi exhibit remarkable genetic and functional diversity. They possess coenocytic hyphae with hundreds of nuclei per cell, allowing for genetic polymorphism and adaptation across diverse ecological conditions (Bücking *et al.*, 2012). Horizontal gene transfer through hyphal anastomosis has been suggested as a mechanism for maintaining genetic diversity in the absence of sexual reproduction (Giovannetti *et al.*, 1999).

Significance of AM fungal association in Tubers

Tuber crops are vital components of global food security, particularly in tropical and subtropical regions, where they serve as major sources of calories, nutrients, and income for millions of people. These crops, which include cassava (*Manihot*

esculenta), sweet potato (*Ipomoea batatas*), yams (*Dioscorea* spp.), taro (*Colocasia esculenta*), and elephant-foot yam (*Amorphophallus paeoniifolius*), are the third most important class of food crops after cereals and legumes (Nedunchezhiyan *et al.*, 2022; Chauhan *et al.*, 2022). The 'Centre for Science in the Public Interest' (CSPI), USA, recognizes sweet potato as a "Superfood" (Behera *et al.*, 2022). Potato (*Solanum tuberosum*) is the third most significant food crop globally, following wheat and rice (More *et al.*, 2021). Their significance is underscored not only by their contribution to dietary needs but also by their multi-purpose utility in industries such as starch, biofuel, and pharmaceuticals (Reddy, 2015).

Worldwide, tuber crops are cultivated across diverse ecological zones due to their resilience to adverse environmental conditions, including drought, salinity, and poor soil conditions (FAO, 2012). In India, tuber crops are primarily cultivated in the south-eastern and north-eastern regions, making a significant contribution to rural livelihoods, particularly among tribal communities and smallholder farmers (Chauhan *et al.*, 2022). In terms of food security, tubers provide an accessible, affordable, and calorically dense food source for over 500 million individuals worldwide (Reddy, 2015; Chauhan *et al.*, 2022).

Tuber crops are characterized by their high carbohydrate content, particularly in the form of starch, which makes them essential in both human diets and industrial processes. They are rich sources of vital micronutrients, vitamins, antioxidants, and dietary fibres, enhancing their nutritional profile (Nedunchezhiyan *et al.*, 2022; Chauhan *et al.*, 2022). Industrially, tubers serve as raw materials for starch-based products, bioethanol, animal feed, and biofilms, highlighting their versatile applications and economic value (Reddy, 2015). Despite their importance, tuber crops face several challenges, including declining cultivation areas due to competition with cereals, limited access to improved planting materials, and limited research investment, especially in developing countries (Chauhan *et al.*, 2022; Nedunchezhiyan *et al.*, 2022). Moreover, issues related to pest and disease management, post-harvest losses, and market access hinder their potential. To harness their full benefits, integrated approaches focusing on value chain development, climate resilience, biodiversity conservation, and innovative breeding are crucial. Emphasizing the ethnobotanical and socio-economic aspects of tubers can further enhance their commercial exploitation

and contribute to food and nutritional security (Nedunchezhiyan *et al.*, 2022; Chauhan *et al.*, 2022).

The study of tuber crops becomes necessary because they embody a resilient, nutrient-dense, and economically significant group of crops with the potential to address future food security challenges. Their ability to adapt to diverse environmental conditions, combined with their multifunctional uses, underscores their importance in sustainable agricultural systems. As global populations continue to rise and climate change impacts intensify, expanding research and development efforts in tuber crop cultivation, processing, and marketing will be critical in ensuring their role as a cornerstone of food, nutrition, and livelihood security worldwide (Reddy, 2015; Chauhan *et al.*, 2022).

Recent studies consistently demonstrate that underground crop harvests often contain viable AM fungal propagules capable of establishing symbioses, indicating the natural presence of AM fungi and their potential to influence crop performance (Alam and Gabriel-Neumann, 2025). Inoculation with AM fungi has been shown to positively affect key agronomic parameters, including crop yield, in potato plants. However, the overall impact is influenced by factors such as potato cultivar, soil pH, nutrient concentrations, and experimental duration, highlighting the complexity and context-dependency of the AM-plant interaction (Oladele *et al.*, 2025). Inoculating crops such as cassava with AM fungi significantly enhances plant growth, nutritional quality, and yield across different varieties (Ghislain *et al.*, 2024). Additionally, AM fungi can mitigate the negative effects of soil amendments, such as biochar, thereby improving nutrient uptake in potato plants (Yang *et al.*, 2022). Overall, AM fungal species have shown promise in improving both crop yield and nutritional quality under field conditions, reinforcing their potential as vital bio-inoculants for sustainable agriculture (Carrara *et al.*, 2023).

Studying AM fungal associations with tuber crops in Goa is crucial for promoting sustainable farming, enhancing crop yields, and protecting our local environment. Since Goa has a unique climate and soils, understanding the native AM fungi and how they work with local tuber crops can help farmers adopt natural, eco-friendly practices that cut down on expensive chemical fertilizers and pesticides. This not only supports organic farming traditions but also helps protect the region's rich microbial biodiversity. Ultimately, this research can lead to smarter, climate-resilient farming

methods tailored specifically for Goa, improving harvests, reducing environmental impact, and supporting the livelihoods of local farmers.

It's a practical and necessary step toward building a more sustainable and prosperous future for the region's agriculture. Hence, the present study proposes the following objectives:

- a) To study root colonization of AM fungi associated with wild and cultivated tubers commonly found in Goa.
- b) To isolate, multiply (using trap cultures) and identify spores of AM fungi from the rhizosphere soils of tuber plants.
- c) To study AM status of tuber plants selected for the study as influenced by phenology.
- d) To produce pure cultures of dominant AM fungal species and their mass multiplication.
- e) To evaluate the effects of the dominant AM fungal species on growth and nutritional quality of selected species.

Chapter 2: Review of Literature

a. AM fungal colonization in tuber species.

Authors and Year Study area	Host plant	Inference/ Major Findings
Dare <i>et al.</i> , (2013) Nigeria	<i>Dioscorea</i> spp.	The study demonstrated substantial diversity of AM fungal species across different agroecological zones within yam-growing regions. In soils under a yam-based cropping system, species of AM fungi belonging to <i>Glomus</i> were the most abundant. Among them, <i>Gl. geosporum</i> , <i>Gl. intraradices</i> and <i>Gl. mosseae</i> occurred at comparatively high population levels.
Chifetete and Dames, (2020) Africa	<i>Solanum tuberosum</i>	AM fungi are known to increase crop plants' tolerance to drought, salinity, and disease by facilitating water and nutrient acquisition and improving overall soil structure. They suggested approaches to maintain AM fungal abundance in potato production, highlighting the potential of conservation tillage.
Nuryana <i>et al.</i> , (2020) Indonesia	<i>Dioscorea esculenta</i>	<i>Dioscorea esculenta</i> exhibited AM fungal root colonization. A total of 17 AM fungal species belonging to nine genera, viz., <i>Scutellospora</i> , <i>Septoglomus</i> , <i>Sclerocystis</i> , <i>Ambispora</i> , <i>Gigaspora</i> , <i>Acaulospora</i> , <i>Claroideoglomus</i> , <i>Funneliformis</i> , and <i>Glomus</i> were identified based on comparison with sequences available in the GenBank database. Among these, <i>Acaulospora</i> was the most dominant and abundant genus, followed by <i>Glomus</i> and

Authors and Year Study area	Host plant	Inference/ Major Findings
		<i>Gigaspora</i> . The findings suggest that these genera may serve as potential AM fungal inocula to improve tuber production in lesser yams.
Kumar <i>et al.</i> , (2013) Assam	<i>Colocasia esculenta</i> , <i>Amorphophallus campanulatus</i> , <i>Dioscorea pentaphylla</i> , <i>Dioscorea puber</i> , <i>Dioscorea alata</i> , <i>Dioscorea esculenta</i> , <i>Dioscorea batatas</i>	AM fungal associations were reported in seven species of tubers. The identified AM fungal species belonged to the genus <i>Acaulospora</i> , <i>Glomus</i> , <i>Sclerocystis</i> , and <i>Gigaspora</i> , with <i>Acaulospora</i> and <i>Glomus</i> being the dominant genera.
Khade and Rodrigues, (2003) Goa, India	<i>Gloriosa superba</i>	Reported AM fungal colonization in <i>Gloriosa superba</i> tubers. They reported seven AM fungal species viz., <i>Acaulospora scrobiculata</i> , <i>Gigaspora margarita</i> , <i>Glomus fasciculatum</i> , <i>G. geosporum</i> , <i>G. heterosporum</i> , <i>Sclerocystis clavisporea</i> , and <i>S. rubiformis</i> , belonging to four genera viz., <i>Acaulospora</i> , <i>Glomus</i> , <i>Gigaspora</i> , and <i>Sclerocystis</i> . The study showed that tubers can also harbour diverse AM fungi that may influence tuber health and development.
Khade and Rodrigues, (2007) Goa, India	<i>Dioscorea sp.</i> , <i>Amorphophallus commutatus</i> , <i>Colocasia</i>	Studied AM fungal association in the underground storage organs from the tuberous, rhizomatous, bulbous, and cormous plant species in the Western

Authors and Year Study area	Host plant	Inference/ Major Findings
	<i>esculenta</i> , <i>Alocasia</i> <i>macrorrhiza</i> .	Ghats region of Goa. They recorded 19 species of AM fungi belonging to four genera, viz., <i>Acaulospora</i> , <i>Glomus</i> , <i>Gigaspora</i> , and <i>Scutellospora</i> . Among these, the genus <i>Glomus</i> was dominant, followed by <i>Acaulospora</i> , <i>Scutellospora</i> , and <i>Gigaspora</i> .

b. Factors affecting AM fungal symbiosis.

Authors and Year Study area	Host plant	Inference/ Major Findings
Khanam <i>et al.</i> , (2006) Bangladesh	Cereals, pulses, oilseeds, vegetables and spices	They reported that edapho-climatic factors strongly influence AM colonization and sporulation. Besides, the soil moisture, pH, and nutrient status regulate colonization intensity and spore abundance. Conversely, soil moisture, organic matter (OM), total N, and K showed no significant negative effects and exhibited weak positive associations with colonization. These results highlight the complex role of soil chemistry and moisture in shaping AM symbiosis and underscore the need to optimize soil conditions to enhance mycorrhizal activity.
Jamiołkowska <i>et al.</i> , (2018) Poland	Review	They suggested that the effectiveness of AM fungi is influenced by both biotic and abiotic factors, including soil physico-chemical properties, water and nutrient availability, agricultural practices, and

Authors and Year Study area	Host plant	Inference/ Major Findings
		climatic conditions. They also reported that the soil structure and compaction strongly affect biological activity, while soil pH significantly influences ion mobility and their uptake by plants and soil microflora. They concluded that the excess soil moisture can negatively affect AM fungi because they are sensitive to reduced oxygen availability.
Verbruggen <i>et al.</i> , (2012) Germany	<i>Solanum tuberosum</i> , <i>Phalaris arundinacea</i> , <i>Medicago sativa</i> , <i>Olea europaea</i>	Their study identified three key factors determining inoculation success and the persistence of AM fungi in soils. These include species compatibility (the ability of the introduced species to thrive under prevailing conditions), field carrying capacity (the available ecological niche for AM fungi), and priority effects (the influence of colonization timing and competition on the establishment of alternative stable communities). The study further examined how these factors can be leveraged to enhance the establishment and long-term persistence of AM fungi.
Berger & Gutjahr, (2021) Germany	<i>Oryza sativa</i>	They reported that the AM responsiveness depends on the plant-fungal genotype combination and on environmental conditions.

c. Effect of AM fungal symbiosis on tuber yield and quality.

Authors and Year Study area	Host plant	Inference/ Major Findings
Lu <i>et al.</i> , (2015) Taiwan	<i>Dioscorea</i> spp.	Their study reported that AM fungi boost yam yield and tuber quality by improving nutrient uptake, especially P, and root growth, increasing tuber weight, dry matter, and starch, while promoting efficient water use and resilience, offering a sustainable alternative to chemical fertilizers.
Yuan <i>et al.</i> , (2023) China	<i>Ipomoea batatas</i>	They reported that AM fungi enhance nutrient uptake (P and N) in sweet potato and other tubers, thereby improving nutritional quality, increasing antioxidant and micronutrient levels, and supporting sustainable yields and reduced chemical inputs. The studies support the use of indigenous AM fungal communities to optimize nutrient use efficiency, stress resilience, and eco-friendly fertilization strategies in diverse tuber crops.
Shuab <i>et al.</i> , (2017) India	<i>Solanum tuberosum</i>	AM fungi benefit potato by improving P and micronutrient uptake, increasing tuber yield, dry matter, starch, and antioxidants, reinforcing the eco-friendly productivity of AM associations.

d. AM fungi and seasonal dynamics.

Authors and Year Study area	Host plant	Inference/ Major Findings
Santos-González <i>et al.</i> , (2007) Sweden	Grasslands	They reported that the symbiotic AM fungi influence the diversity and productivity of grassland plant communities. In their study, no significant seasonal changes were observed in the overall species composition of AM fungal communities. However, the two plant species studied hosted significantly different AM fungal communities. They reported that <i>Prunella vulgaris</i> supported a relatively rich AM fungal community throughout the entire growing season.
Berruti <i>et al.</i> , 2018 Italy	<i>Beta vulgaris</i>	Their study suggests that seasonal shifts significantly shape AM fungal community structure and functional roles in nutrient cycling and plant resilience across ecosystems.
Vieira Júnior <i>et al.</i> , (2020) Brazil	Studied various Phyto-physio biomes	They reported that AM fungal abundance, diversity, and community composition exhibited marked seasonal fluctuations, with peak spore production and root colonization during the dry season, likely driven by increased physiological stress and soil moisture variability. The findings indicate that AM fungi respond dynamically to environmental cues and play an important role in nutrient cycling and ecosystem resilience under seasonal stress.

Authors and Year Study area	Host plant	Inference/ Major Findings
D'Souza and Rodrigues, (2013) Goa, India	<i>Acanthus ilicifolius, Excoecaria agallocha, Rhizophora mucronata</i>	They reported significant seasonal variations in spore density and species richness, with the highest values recorded during the pre-monsoon period and the lowest during the monsoon. Multivariate analysis indicated that seasonal environmental factors, including soil moisture, temperature, and nutrient availability, exerted a stronger influence on AM fungal communities than host plant species, highlighting the critical role of climatic fluctuations in regulating fungal sporulation and symbiotic associations.
Bukhari and Rodrigues, 2023 Goa, India	<i>Chromolaena odoratum, Emilia sonchifolia, Mimosa pudica, Ludwigia parviflora, Ischaemum semisagittatum, Acacia auriculiformis, Acacia mangium, Trema orientalis</i>	Their study reported higher AM fungal activity and spore production during dry or pre-monsoon periods, while colonization may peak during seasons of higher soil moisture, reflecting adaptive responses to temperature, moisture, and nutrient availability.

e. AM fungi and other microorganisms.

Authors and Year Study area	Host plant	Inference/ Major Findings
Masrahi <i>et al.</i> , (2023) Saudi Arabia, Egypt	<i>Hordeum vulgare</i>	The investigation demonstrated that combining P fertiliser with AM fungi and phosphate-solubilizing bacteria (PSB) enhances P bioavailability under salinity stress. This combined application improved yield and its components, as well as N, P, and K uptake in barley.
Elekhtyar <i>et al.</i> , (2022) Saudi Arabia	<i>Oryza sativa</i>	This study reports that co-inoculation of AM fungi and PSB significantly enhanced plant biomass, P uptake, and rhizosphere enzymatic activities compared to individual inoculations or control treatments. This synergistic interaction improved soil microbial activity, nutrient cycling, and overall soil fertility, highlighting the potential of AM fungi and PSB as sustainable biofertilizer agents, thereby reducing chemical fertilizer dependency and promoting environmentally friendly agricultural practices.
Wahid <i>et al.</i> , (2020) Pakistan, China, Czech Republic	<i>Zea mays</i> , <i>Triticum aestivum</i>	They evaluated the inoculation potential of AM fungal species viz., <i>Glomus microagregatum</i> , <i>Funneliformis geosporum</i> , <i>Claroideoglomus etunicatum</i> , <i>F. mosseae</i> , <i>Rhizophagus intraradices</i> , and <i>Gl. claroideum</i> and the phosphate-solubilizing bacterium (PSB) <i>Bacillus</i> sp. PIS7, in combination with rock phosphate (RP), on grain yield and P

Authors and Year Study area	Host plant	Inference/ Major Findings
		uptake in <i>Zea mays</i> and <i>Triticum aestivum</i> grown in alkaline calcareous soil. Results from both trials demonstrated that AM fungi and <i>Bacillus</i> sp. PIS7 with RP increased grain yield and P uptake. The study concluded that PSB solubilized unavailable P forms in soil in the presence of RP fertilizers, while AM fungi facilitated P transfer to plants, thereby promoting growth. Furthermore, combined inoculation of AM fungi and PSB with ground RP showed greater potential to enhance maize and wheat yield and P uptake.

f. Screening of efficient AM fungal species for tubers.

Authors and Year Study area	Host plant	Inference/ Major Findings
Gai <i>et al.</i> , 2006 China	<i>Ipomoea batatas</i>	They suggested that selecting specific AM fungal species is critical for improving tuber crop productivity, nutrient acquisition, and stress tolerance. Species of <i>Glomus</i> exhibit higher symbiotic efficiency, with greater root colonization, hyphal development, and nutrient transfer, thereby enhancing plant growth, tuber yield, and nutrient accumulation, including P, K, and protein, in <i>Ipomoea batatas</i> . They reported that inoculation with GEGM (<i>Glomus etunicatum</i> together with <i>Glomus mosseae</i>) and GE6 (<i>Glomus etunicatum</i>) increased plant yield and nutrition, and thus were the most effective AM symbionts for sweet potato under the experimental conditions.
Tchabi <i>et al.</i> , (2010) West Africa	<i>Dioscorea rotundata</i>	They reported that inoculation of micropropagated yam plantlets with selected indigenous AM fungi could serve as a sustainable strategy to enhance yam productivity in degraded African soils. Six tropical isolates of <i>Glomus etunicatum</i> and the African isolates of <i>Glomus mosseae</i> , <i>Glomus hoi</i> , <i>Glomus etunicatum</i> , <i>Acaulospora scrobiculata</i> , and <i>Acaulospora spinosa</i> resulted in increased tuber growth compared to non-mycorrhizal controls.

Authors and Year Study area	Host plant	Inference/ Major Findings
Séry <i>et al.</i> , (2016) West Africa	<i>Manihot esculenta</i>	They reported that the dual inoculation using native AM fungal species, viz., <i>Acaulospora colombiana</i> and <i>A. appendicula</i> , significantly promoted plant growth, P uptake, and water stress tolerance. It also improved the plant health and resistance to pathogens, including <i>Meloidogyne</i> sp., highlighting the importance of selecting indigenous AM fungal strains for sustainable biofertilizer development.

g. Nutrient content in tubers.

Authors and Year Study area	Host plant	Inference/ Major Findings
Zierer <i>et al.</i> (2021) Germany	<i>Solanum tuberosum</i> , <i>Ipomoea batatas</i> , <i>Manihot esculenta</i>	They reported that potatoes (<i>Solanum tuberosum</i>), sweet potatoes (<i>Ipomoea batatas</i>), and cassava (<i>Manihot esculenta</i>) are known to provide essential carbohydrates, proteins, and vitamins in tropical regions and possess medicinal and antioxidant properties.
Zhao <i>et al.</i> , (2024) China	<i>Ipomoea batatas</i>	They reported that sweet potato storage roots have substantial nutritional value, being rich in carbohydrates, phytochemicals, and essential minerals. However, their nutrient composition varies markedly among cultivars with different flesh colours. White-fleshed sweet potatoes (WFSP) typically contain higher starch and amylose levels and lower soluble sugar levels. Orange-fleshed sweet potatoes (OFSP) recorded elevated levels of soluble sugars, dietary fibre, β -carotene, Ca, and Mg, but comparatively lower levels of protein and starch. Purple-fleshed sweet potatoes (PFSP) showed high concentrations of phytochemicals, particularly caffeoylquinic acids (CQAs) and anthocyanins, along with appreciable amounts of protein, starch, Zn, K and P, and contain relatively low levels of soluble sugars.

Authors and Year Study area	Host plant	Inference/ Major Findings
Lebot <i>et al.</i> , (2025)	<i>Dioscorea esculenta</i>	Based on DNA marker studies, they reported that, despite the nutritional and therapeutic significance of yams, the allelic diversity was lacking, and no systematic breeding efforts have been undertaken to enhance their genetic improvement and utilisation.
Padhan <i>et al.</i> , (2020) India	<i>Dioscorea</i> spp.	Reported that the wild edible yams (<i>Dioscorea</i> spp.) contribute significantly to dietary security and economic welfare in developing regions due to their high caloric and carbohydrate content.
Mounika <i>et al.</i> , (2025) India	<i>Ipomoea batatas</i>	They reported that sweet potato is a highly nutritious and versatile tropical tuber crop that remains underutilized despite its notable health-promoting potential. It is rich in essential nutrients, including antioxidants, β -carotene, anthocyanins, phenolic compounds, dietary fibre, protein, vitamins, and minerals, and also serves as an important source of complex carbohydrates such as starch. Furthermore, its naturally low glycemic index enhances its dietary value, particularly for blood glucose management.

Chapter 3: Root colonization of AM fungi associated with wild and cultivated plants with tubers commonly found in Goa.

3.1: INTRODUCTION

Arbuscular Mycorrhizal (AM) fungal symbiosis refers to the mutualistic association between fungi of the Glomeromycota and the roots of terrestrial plants (Bano and Uzair, 2021; Khaliq *et al.*, 2022). This symbiosis is increasingly recognized as a vital and integral component of natural ecosystems worldwide. In this mutualistic relationship, the plant furnishes the fungus with C, while the fungus enhances the plant's uptake of P and other mineral nutrients from the soil (Gadkar *et al.*, 2001). Thus, root colonization by AM fungi initiates a beneficial dialogue that boosts plant nutrition. This colonization-driven nutrient transfer supports stronger growth and greater biomass. The ongoing exchange also enhances the plant's resilience, improving its tolerance to both biotic and abiotic stresses. Overall, the colonization process itself is the key driver of nutrient use efficiency and overall plant performance (Rivero *et al.* 2018; Campo *et al.* 2020).

Soil edaphic factors, seasonal variations, and other biotic and abiotic environmental factors are known to influence the intensity of the AM fungal-host plant symbiosis. Some AM fungal species exhibit higher colonization rates than others, reflecting intrinsic differences in their growth and invasion kinetics. The host plant species also modulates colonization dynamics. Moreover, the inoculation strategy, whether AM fungi are applied as a single species or as part of a consortium of two or more AM fungal species, impacts the temporal progression of colonization, affecting parameters such as the lag time preceding measurable colonization, the slope of the rapid-growth phase, and the plateau values of per cent colonization. Despite these differences in rate and trajectory, mature colonization plateaus are typically reached across AM fungal species within a 4 to 8 week window (Sivakumar, 2013; Jamiołkowska *et al.*, 2018).

In this chapter, an attempt has been made to understand the impact of AM fungal root colonization on the tuber species studied and to elucidate how this symbiosis influences growth and yield, as well as related benefits. This knowledge will also facilitate the agricultural exploitation of AM-crop associations to enhance crop productivity and resilience.

3.2: MATERIALS AND METHODS

Study Sites: The study was conducted across multiple locations in Goa (**Fig. 3.1**). Rhizosphere soil and root samples were collected to a depth of 0-25 cm from a total of 12 (wild and cultivated) tuber species.

Goa is located between latitudes 15°48' N and 14°53'54"N, and longitudes 74°20'13" E and 73°40'33" E, covering an area of around 3,702 square km. The eastern part of the region is dominated by the Western Ghats, which run north-south, while the western boundary borders the Arabian Sea. This geographical setting places Goa within the northern section of the Western Ghats. The forests types of Goa, mainly comprise Estuarine vegetation consisting of mangroves, Strand vegetation along the coastal belts, Plateau vegetation confined especially to the low altitude (consisting of Open scrub jungle, Moist mixed deciduous forests, Secondary moist mixed deciduous forests and Sub-tropical Hill forests) and the semi-evergreen and evergreen forest (comprising of semi-evergreen, lateritic semi-evergreen, and the evergreen forests). The climate in this area is largely shaped by the monsoon weather system, with temperatures ranging from 23°C to 34°C (forest.goa.gov.in, 2025).

Sampling was conducted at 49 distinct study sites, designated as S-I to S-XLIX, where rhizosphere soil and root samples were collected from seven cultivated viz., *A. paeoniifolius* (Dennst.) Nicolson, *Colocasia antiquorum* Schott., *Dioscorea alata* Linn., *D. bulbifera* L., *D. esculenta* (Lour.) Burkill, *Ipomea batatas* (L.) Lam, and *Solenostemon rotundifolius* (Poir) J. K. Morton. and five wild tuber species viz., *Alocasia macrorrhiza* (L.) G. Don, *Amorphophallus commutatus* (Schott) Engl., *C. esculenta* (L.) Schott., *Gloriosa superba* L., and *Momordica sahyadrica* Kattuk. Rhizosphere samples of tuber species from the selected sites were used to evaluate AM fungal colonization and diversity. Rhizosphere soils for five wild tuber species were collected from 20 sites, while rhizosphere soils for seven cultivated tuber species were collected from 29 sites (**Tables 3.1 & 3.2; Fig. 3.1, 3.2 & 3.3**).

Rhizosphere soil sample collection: The rhizosphere samples from wild and cultivated tuber species were collected by random sampling during the vegetative stage during the period from 2019 to 2024, from various locations (**Fig. 3.1; Tables 3.1 & 3.2**). The zigzag soil sampling method was used to ensure sample homogeneity.

Rhizosphere samples were collected along with roots and tubers. The composite soil samples and the root material were subsequently packed in polyethene bags, labelled, transported to the laboratory, and stored in a deep freezer at 4°C until further analysis.

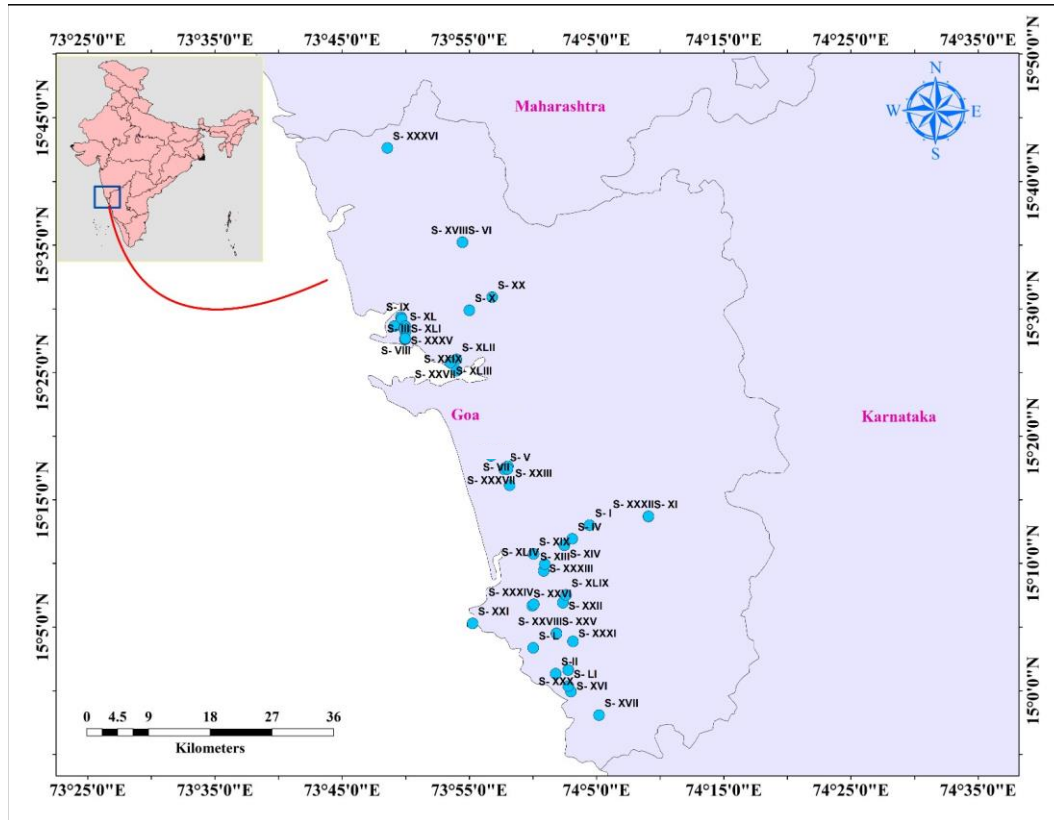


Fig. 3.1: Map of Goa showing sampling sites.

Table 3.1: List of wild tuber species and collection sites

Plant species	Family	Site	Latitude, Longitude
<i>Alocasia macrorhiza</i>	Araceae	Velipwado (S-I)	15.21660°, 74.07420°
		Poiginim (S-II)	15.02209°, 74.02980°
		Altinho (S-III)	15.48893°, 73.82712°
<i>Amorphophallus commutatus</i>	Araceae	Zambaulim (S-IV)	15.19864°, 74.05140°
		Fatorda (S-V)	15.28976°, 73.96230°
		Bicholim (S-VI)	15.58690°, 73.90750°
		Nuvem (S-VII)	15.30730°, 73.94500°
		Taleigao Plateau (S-VIII)	15.46001°, 73.83229°
<i>Colocasia esculenta</i>	Araceae	Morpirla (S- IX)	15.19035°, 74.04105°
		Fatorda (S- X)	15.29378°, 73.96765°
		Canacona (S- XI)	14.99885°, 74.04982°
		Poiginim (S- XII)	14.96790°, 74.08656°
		Mayem (S- XIII)	15.58690°, 73.90758°
		Cuncoim (S- XIV)	15.17902°, 74.00089°
		Velipwada (S- XV)	15.11510°, 74.03930°
		Margao (S- XVI)	15.26870°, 73.96940°
		Vasco (S- XVII)	15.37662°, 74.85257°
<i>Gloriosa superba</i>	Colchicaceae	Taleigao Plateau (S-XVIII)	15.46007°, 73.83275°
		Pernem (S- XIX)	15.71034°, 73.80923°
<i>Momordica sahyadrica</i>	Cucurbitaceae	Taleigao Plateau (S- XX)	15.46060°, 73.83263°

Table 3.2: List of cultivated tuber species and collection sites.

Plant species	Family	Site	Latitude, Longitude
<i>Amorphophallus paeoniifolius</i>	Araceae	Old Goa (S-XXI)	15.497972°, 73.9166°
<i>Colocasia antiquorum</i>	Araceae	Sanguem (S-XXII)	15.22826°, 74.1512°
		Canacona (S-XXIII)	15.02698°, 74.04617°
		Morpirla (S-XXIV)	15.19035°, 74.0410°
<i>Dioscorea alata</i>	Dioscoreaceae	Barcem (S-XXV)	15.07476°, 74.0308°
		Morpirla (S-XXVI)	15.1111°, 73.999°
		Tiswadi (S-XXVII)	15.4290°, 73.892°
<i>Dioscorea bulbifera</i>	Dioscoreaceae	Barcem (S-XXVIII)	15.0747°, 74.0309°
		Tiswadi (S-XXIX)	15.4185°, 73.9000°
<i>Dioscorea esculenta</i>	Dioscoreaceae	Canacona (S-XXX)	15.02698°, 74.0461°
		Barcem (S-XXXI)	15.06437°, 74.0524°
		Sanguem (S-XXXII)	15.2282°, 74.15126°
		Balli (S-XXXIII)	15.1566°, 74.014°
		Morpirla, Quepem (S-XXXIV)	15.1130°, 74.001°
<i>Ipomea batatas</i>	Convolvulaceae	Fatorda (S-XXXV)	15.2902°, 73.9666°
		Taleigao 1 (S-XXXVI)	15.4691°, 73.8328°
		Taleigao 2 (S-XXXVII)	15.4770°, 73.8182°
		Taleigao 3 (S-XXXVIII)	15.4739°, 73.8313°
		Taleigao 4 (S-XXXIX)	15.4769°, 73.8328°
		Agassiam 1 (S-XL)	15.4335°, 73.8997°
		Agassiam 2 (S-XLI)	15.4293°, 73.8916°
		Balli (S-XLII)	15.1655°, 74.0155°
		Verna (S-XLIII)	15.3424°, 73.9296°
		Sanguem (S-XLIV)	15.1871°, 73.1214°
		St Cruz (S-XLV)	15.4739°, 73.8314°
		Padi (S-XLVI)	15.1251°, 74.0433°
<i>Solenostemon rotundifolius</i>	Lamiaceae	Velipwado (S-XLVII)	15.0885°, 74.0472°
		Morpirla (S-XLVIII)	15.0559°, 74.00038°
		Canacona (S-XLIX)	15.0058°, 74.0462°

Soil analysis: The Soil water suspension (40% w/v) method was used to estimate soil pH using a pH meter (LI120 Elico, India). Electrical Conductivity (EC) was measured using a Conductivity meter (CM-180, Elico, India). Available N was measured by using the method of Subbiah and Asija (1956). Available P was measured using the

Bray and Kurtz method (1945). The Hanway and Heidel method (1952) was utilised to measure available K. Micronutrients, viz., Zn, Cu, Mn, and Fe, were determined using the DTPA CaCl₂-TEA method of Lindsay and Norvell (1978) using an Atomic Absorption Spectrophotometer (Nova 400P, Analytik Jena, Germany).

Setting up trap cultures: AM fungal spores from the rhizosphere soils of the collected tuber species were isolated using the wet sieving and decanting method (Gerdemann and Nicolson, 1963). These spores were used to prepare the trap cultures (Morton, 1993). The trap cultures were maintained in a polyhouse for 90 days at 28°C, with relative humidity ranging from 60% to 80%. *Coleus scutellarioides* (L.) Benth. was used as the host plant. The plants were watered regularly as needed. Hoagland's solution (Hoagland and Arnon, 1950), devoid of P, was added every 15 days until harvest.

Processing of root segments for assessment of AM fungal root colonization: Fifty root segments, each measuring one centimetre, were cleared in 10% KOH at 90°C for 60 minutes, followed by acidification with 5N HCl and stained overnight with 0.05% Trypan blue (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 and Nikon Eclipse E200 research microscopes. Micrographs were imaged using a Nikon Digital Sight DS-U3 digital camera. A root segment was scored as mycorrhizal if it showed hyphae and/or hyphal coils, arbuscules, arbusculate coils, and vesicles. The extent of total colonization (TC) and per cent hyphal (HC), arbuscular (AC), and vesicular (VC) colonization were measured using the magnified intersection method (McGonigle *et al.*, 1990).

Estimation of AM fungal colonization: Estimation of per cent root colonization by AM fungi was carried out using the Root slide method (Read *et al.*, 1976). The Trypan blue-stained root fragments were mounted in polyvinyl-Lacto-glycerol (PVLG), and the percentage AM fungal root colonization was calculated using the following formula:

$$\text{Root colonization (\%)} = \frac{\text{No. of root segments colonized}}{\text{Total no. of root segments scored}} \times 100$$

3.3: RESULTS AND DISCUSSION

AM fungal root colonization: AM fungal colonization was recorded in the roots of all the wild and cultivated tuber species studied. Key characteristics of AM fungal colonization observed in the present study included intra- and extra-radical hyphae, hyphal coils, arbuscules, vesicles, and auxiliary cells. The hyphal, vesicular, and arbuscular colonization in the roots was quantified.

AM fungal colonization was observed in the roots of the selected tuber species sampled from the various sites across Goa. The roots exhibited the presence of hyphae, vesicles (**Plates 3.1**) and arbuscules (**Plate 3.2**). The study revealed extensive hyphal and vesicular colonization compared to arbuscular colonization (**Figs. 3.2, 3.3, 3.4 & 3.5**). However, the extent of colonization varied in the different tuber species at various sites. A significant variation in AM fungal root colonization was observed in the present study. This may be attributed to differences in AM fungal communities across ecosystems, which comprise diverse species that fulfil symbiotic roles within their unique community structures (Ôpik *et al.*, 2006).

The details of percent AM fungal root colonization in the five wild tubers, viz., *Alocasia macrorrhiza*, *Amorphophallus commutatus*, *Colocasia esculenta*, *Gloriosa superba*, and *Momordica sahyadrica* are given below.

In *Alocasia macrorrhiza*, the highest root colonization was recorded at site S-III (93.33%), while the lowest root colonization was recorded at site S-I (67.00%) (**Table 3.3; Fig. 3.2a**).

Table 3.3: AM fungal colonization in *Alocasia macrorrhiza*.

Site	TC (%)	HC (%)	VC (%)	AC (%)
Velipwado (S-I)	67.00±0.33	62.37±2.95	56.90±3.34	nd
Poiginim (S-II)	91.33±0.66	91.00±1.23	81.03±1.55	nd
Altinho (S-III)	93.33±2.40	92.22±0.62	90.73±0.97	2.45±0.33

Legend: All values are mean of three replicates; ± **standard error**; TC = total colonization; HC = hyphal colonization; VC = vesicular colonization; AC = arbuscular colonization; **nd** = **not detected at site**.

In *Amorphophallus commutatus*, the highest root colonization was observed at site S-V (98.00%), while the lowest colonization was documented at site S-IV (70.67%) (Table 3.4; Fig. 3.2b).

Table 3.4: AM fungal colonization in *Amorphophallus commutatus*.

Site	TC (%)	HC (%)	VC (%)	AC (%)
Zambaulim (S-IV)	70.67±3.84	70.67±1.45	67.33±0.34	19.00±2.08
Fatorda (S-V)	98.00±0.57	96.67±0.66	77.67±0.88	82.00±1.53
Bicholim (S-VI)	73.67±1.20	72.67±1.20	67.33±1.45	64.33±1.45
Nuvm (S-VII)	71.67±0.88	70.33±0.88	54.33±0.88	53.33±1.86
Taleigao Plateau (S-VIII)	76.67±1.45	76.33±0.88	57.00±0.58	45.00±1.15

Legend: All values are mean of three replicates; ± **standard error**; TC = total colonization; HC = hyphal colonization; VC = vesicular colonization; AC = arbuscular colonization.

In *Colocasia esculenta*, the highest colonization intensity was recorded at site S-XIII (94.00%), whereas the lowest was documented at site S-XIV (64.50%). In the majority of the samples, the number of arbuscules was lower than that of vesicles and hyphal structures (Table 3.5; Fig. 3.2c).

Table 3.5: AM fungal colonization in *Colocasia esculenta*.

Site	TC (%)	HC (%)	VC (%)	AC (%)
Morpirila (S-IX)	76.00±0.57	75.67±1.20	66.33±0.34	3.67±1.45
Fatorda (S-X)	85.00±1.73	82.67±0.88	77.33±1.76	6.33±0.67
Canacona (S-XI)	84.33±0.67	82.33±1.45	74.33±1.20	nd
Poiginim (S-XII)	76.33±1.33	76.33±0.88	62.00±1.53	3.67±0.33
Mayem (S-XIII)	94.00±0.57	91.67±2.18	77.67±1.45	8.67±0.33
Cuncolim (S-XIV)	64.5±0.57	61.35±0.77	50.71±1.88	nd
Velipwada (S-XV)	66.00± 0.57	67.17±0.83	66.50±1.76	1.67±0.44
Margao (S-XVI)	74.66±0.33	72.00±2.36	52.18±2.28	44.39±1.98
Vasco (S-XVII)	77.66±0.57	72.37±3.72	46.91±4.14	24.33±2.32

Legend: All values are mean of three replicates; ± **standard error**; TC = total colonization; HC = hyphal colonization; VC = vesicular colonization; AC = arbuscular colonization; **nd** = **not detected at site**.

In *Gloriosa superba*, except for arbuscules lower at site S-XIX (1.37%) as compared to site S-XVIII (22.46%), the colonization levels showed similar values (**Table 3.6; Fig. 3.2d**).

Table 3.6: AM fungal colonization in *Gloriosa superba*.

Site	TC (%)	HC (%)	VC (%)	AC (%)
Taleigao Plateau (S-XVIII)	72.66±0.66	71.72±1.40	47.05±0.71	22.46±1.51
Pernem (S-XIX)	70.98±1.37	68.35±1.57	44.55±2.09	1.37±0.73

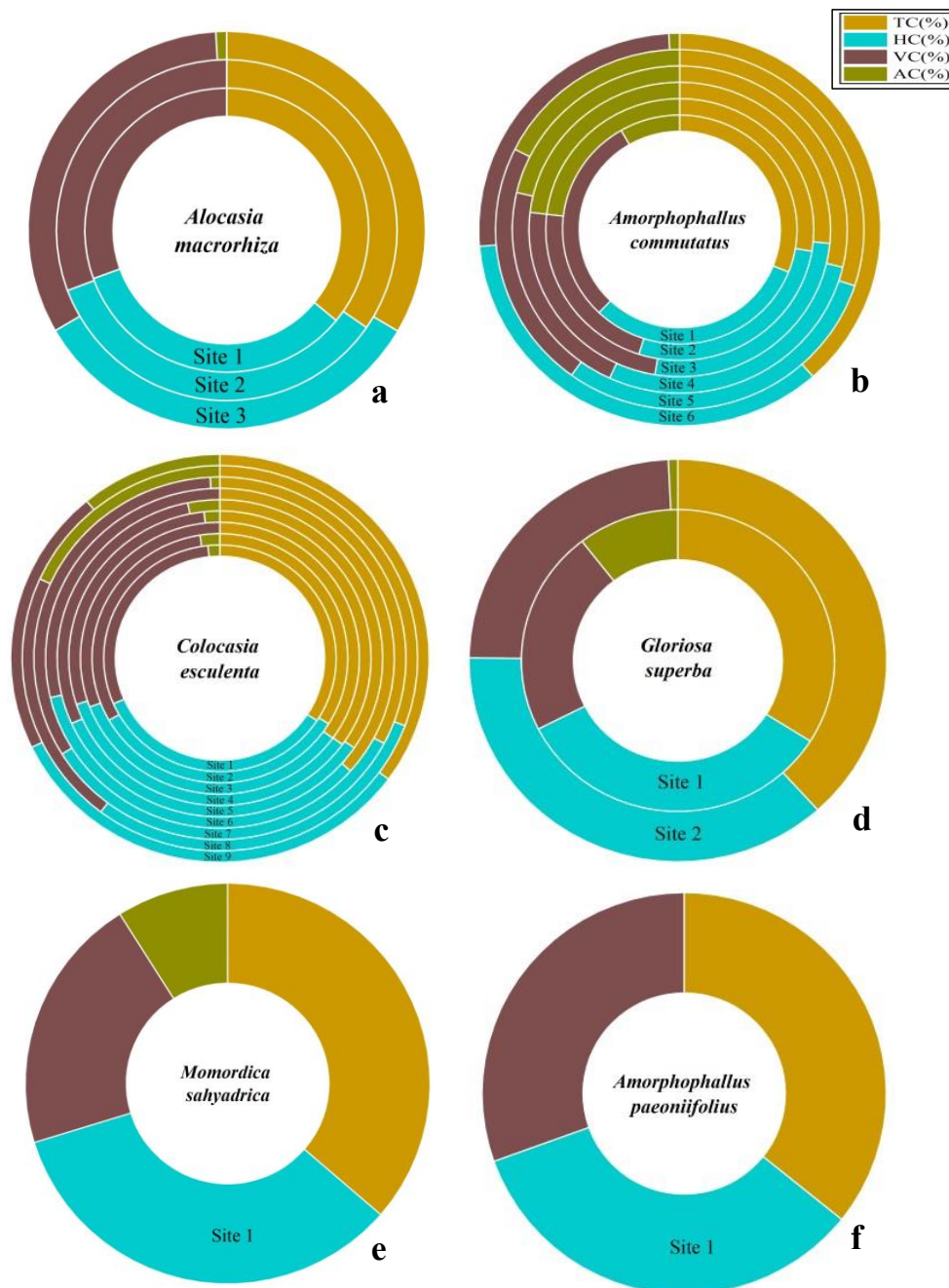
Legend: All values are mean of three replicates; $\pm$ **standard error**; TC = total colonization; HC = hyphal colonization; VC = vesicular colonization; AC = arbuscular colonization; **nd** = **not detected at site**.

Momordica sahyadrica collected from a single site (S-XX) showed good root colonization (69.33%), with hyphae, vesicles, and arbuscules (**Table 3.7; Fig. 3.2e**).

Table 3.7: AM fungal colonization in *Momordica sahyadrica*.

Site	TC (%)	HC (%)	VC (%)	AC (%)
Taleigao plateau (S-XX)	69.33±0.57	64.63±0.69	39.67±0.93	17.08±2.43

Legend: All values are mean of three replicates; $\pm$ **standard error**; TC = total colonization; HC = hyphal colonization; VC = vesicular colonization; AC = arbuscular colonization; **nd** = **not detected at site**.



Legend: Total colonization (TC%), hyphal colonization (HC%), arbuscular colonization (AC%), and vesicular colonization (VC%).

Fig. 3.2: AM fungal colonization in wild and cultivated tubers.

a. *Alocasia macrorrhiza*, **b.** *Amorphophallus commutatus*, **c.** *Colocasia esculenta*, **d.** *Gloriosa superba*, **e.** *Momordica sahyadrica* and **f.** *Amorphophallus paeoniifolius*

The results of AM fungal root colonization in the seven cultivated tuber species viz., *Amorphophallus paeoniifolius*, *Colocasia antiquorum*, *Dioscorea alata*, *D. bulbifera*, *D. esculenta*, *Ipomoea batatas*, and *Solenostemon rotundifolius* are given in **Figs. 3.4, & 3.5**.

Amorphophallus paeoniifolius collected from a single site (S-XXI) showed low root colonization (21.33%), with only hyphae and vesicles (**Table 3.8; Fig. 3.2e**).

Table 3.8: AM fungal colonization in *Amorphophallus paeoniifolius*.

Site	TC (%)	HC (%)	VC (%)	AC (%)
Old Goa (S-XXI)	21.33±0.66	20.12±1.32	18.17±0.60	nd

Legend: All values are mean of three replicates; ± **standard error**; TC = total colonization; HC = hyphal colonization; VC = vesicular colonization; AC = arbuscular colonization; **nd** = **not detected at site**.

In *Colocasia antiquorum*, the highest root colonization was recorded at site S-XXIV (99.00%), while the lowest root colonization was recorded at site S-XXII and S-XXIII (65.00%) (**Table 3.9; Fig. 3.3a**).

Table 3.9: AM fungal colonization in *Colocasia antiquorum*.

Site	TC (%)	HC (%)	VC (%)	AC (%)
Sanguem (S-XXII)	65.00±2.31	66.67±1.76	60.67±0.67	2.33±0.33
Canacona (S-XXIII)	65.00±2.52	64.33±1.76	63.67±0.33	4.00±0.58
Morpirila (S-XXIV)	99.00±0.57	99.33±0.44	36.11±0.44	2.09±0.37

Legend: All values are mean of three replicates; ± **standard error**; TC = total colonization; HC = hyphal colonization; VC = vesicular colonization; AC = arbuscular colonization.

In *Dioscorea alata*, the highest root colonization was recorded at site S-XXVII (92.33%), while the lowest root colonization was recorded at site S-XXV (86.00%) (**Table 3.10; Fig. 3.3b**).

Table 3.10: AM fungal colonization in *Dioscorea alata*.

Site	TC (%)	HC (%)	VC (%)	AC (%)
Barcem (S-XXV)	86.00±0.33	82.25±1.09	77.05±1.05	nd
Morpirila (S-XXVI)	88.66±0.33	82.78±1.21	55.50±0.58	nd
Tiswadi (S-XXVII)	92.33 ± 0.88	90.52±1.13	26.77±1.03	12.75±2.38

Legend: All values are mean of three replicates; ± **standard error**; TC = total colonization; HC = hyphal colonization; VC = vesicular colonization; AC = arbuscular colonization; **nd** = **not detected at site**.

In *Dioscorea bulbifera*, the highest root colonization was recorded at site S-XXIX (98%), while the lowest root colonization was recorded at site S-XXVIII (76%) (Table 3.11; Fig. 3.3c).

Table 3.11: AM fungal colonization in *Dioscorea bulbifera*.

Site	TC (%)	HC (%)	VC (%)	AC (%)
Barcem (S-XXVIII)	76.00± 0.67	75.47±1.99	71.80±3.23	nd
Tiswadi (S-XXIX)	98.00±0.57	96.33±1.01	43.37±1.33	53.55±1.40

Legend: All values are mean of three replicates; ± **standard error**; TC = total colonization; HC = hyphal colonization; VC = vesicular colonization; AC = arbuscular colonization; **nd** = **not detected at site**.

In *Dioscorea esculenta* the highest root colonization was recorded at site S-XXXII (91.67%), while the lowest root colonization was recorded at site S-XXXIV (59.33%) (Table 3.12; Fig. 3.3 d).

Table 3.12: AM fungal colonization in *Dioscorea esculenta*.

Site	TC (%)	HC (%)	VC (%)	AC (%)
Canacona (S-XXX)	76.66± 0.88	72.12±1.88	71.87±2.62	4.43±1.74
Barcem (S-XXXI)	90.00±2.31	89.33±0.88	77.33±0.88	32.00±1.15
Sanguem (S-XXXII)	91.67±1.20	88.33±0.88	75.33±0.88	20.67±3.18
Balli (S-XXXIII)	67.33±1.20	67.33±2.96	64.33±0.34	14.33±0.67
Morpirala (S-XXXIV)	59.33±1.76	53.61±1.65	22.17±0.88	nd

Legend: All values are mean of three replicates; ± **standard error**; TC = total colonization; HC = hyphal colonization; VC = vesicular colonization; AC = arbuscular colonization; **nd** = **not detected at site**.

In *Solenostemon rotundifolius* the highest root colonization was recorded at site S-XXXVI (99.66%), while the lowest root colonization was recorded at site S-XXXV (69.22%) (Table 3.13; Fig. 3.3e).

Table 3.13: AM fungal colonization in *Solenostemon rotundifolius*.

Site	TC (%)	HC (%)	VC (%)	AC (%)
Velipwado (S-XXXV)	69.22± 0.57	68.71±0.50	63.22±1.36	nd
Morpirala (S-XXXVI)	99.66±0.33	96.44±1.87	58.18±1.47	1.17±0.73
Canacona (S-XXXVII)	95.83±0.33	92.94±1.55	67.62±1.47	nd

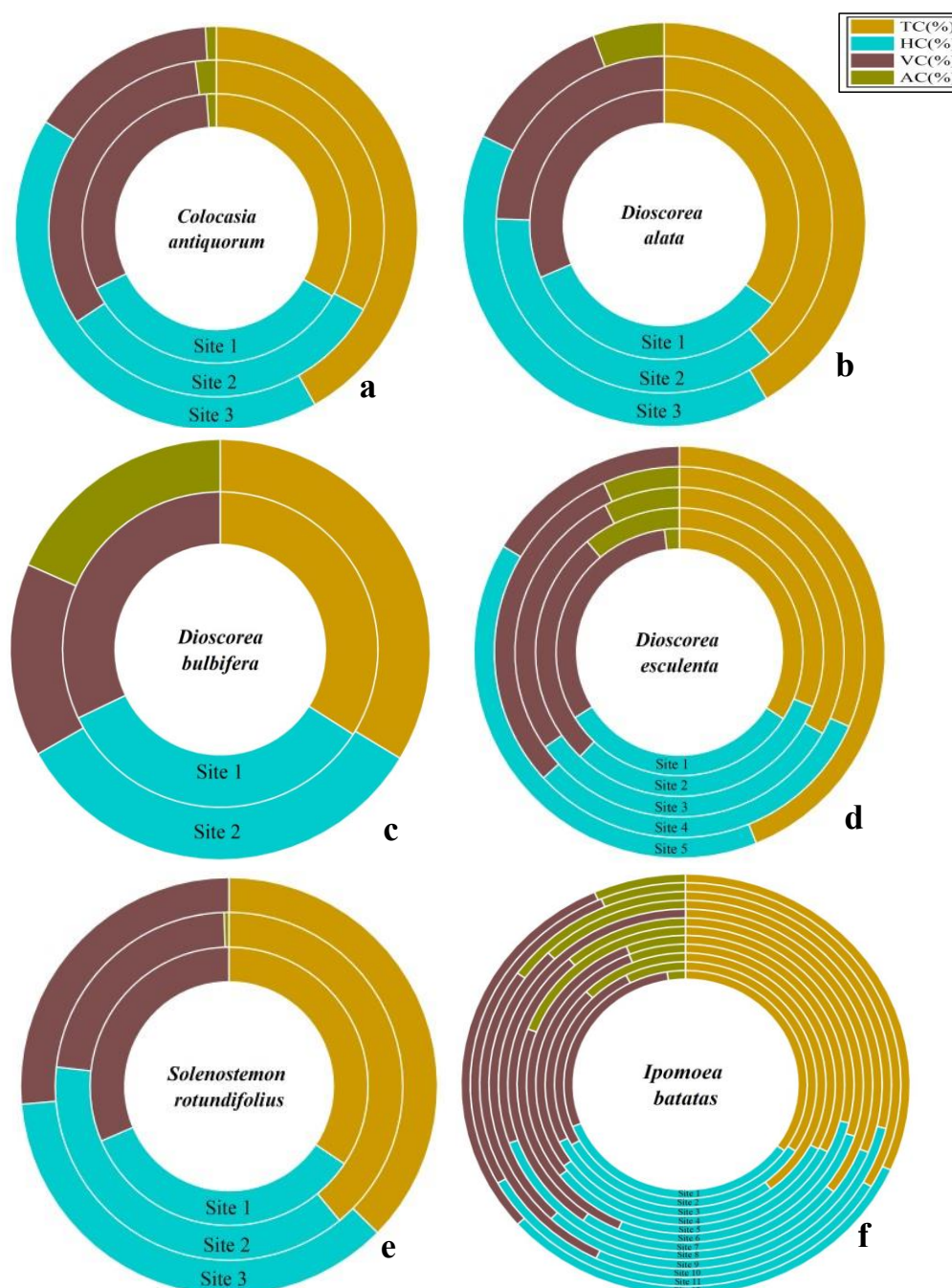
Legend: All values are mean of three replicates; ± **standard error**; TC = total colonization; HC = hyphal colonization; VC = vesicular colonization; AC = arbuscular colonization; **nd** = **not detected at site**.

In *Ipomea batatas*, the highest root colonization was recorded at site S-XLIV (98.33%), while the lowest root colonization was recorded at site S-XL (66.67%) (**Table 3.14; Fig. 3.3f**).

Table 3.14: AM fungal colonization in *Ipomea batatas*.

Site	TC (%)	HC (%)	VC (%)	AC (%)
Fatorda (S-XXXVIII)	90.67±2.18	88.67±2.02	74.33±2.34	6.33±0.33
Taleigao 1 (S-XXXIX)	95.33±0.66	91.33±1.45	71.67±2.41	21.00±1.53
Taleigao 2 (S-XL)	66.67±0.33	47.40±1.64	33.13±1.46	21.08±3.63
Taleigao 3 (S-XLI)	83.33±0.66	83.21±1.30	73.34±1.73	15.77±3.85
Taleigao 4 (S-XLII)	95.33±0.33	94.25±0.72	86.38±2.90	17.89±5.78
Agassiam 1 (S-XLIII)	96.00±1.15	91.67±1.45	80.33±1.45	64.00±2.08
Agassiam 2 (S-XLIV)	98.33±0.33	97.50±0.29	94.41±1.87	37.33±4.84
Balli (S-LV)	88±1.15	84.72±0.49	75.50±1.42	nd
Verna (S-XLVI)	90.00±0.33	89.97±0.76	74.56±3.33	35.77±2.72
Sanguem (S-XLVII)	97.77± 0.57	97.21±0.78	94.29±1.80	51.88±3.30
St Cruz (S-XLVIII)	86.67±0.88	86.70±0.73	69.25±0.69	16.60±2.39
Padi (S-XLIX)	86.66±0.66	87.02±0.44	81.83±0.99	18.22±2.93

Legend: All values are mean of three replicates; ± **standard error**; TC = total colonization; HC = hyphal colonization; VC = vesicular colonization; AC = arbuscular colonization; **nd** = **not detected at site**.



Legend: Total colonization (TC%), hyphal colonization (HC%), arbuscular colonization (AC%), and vesicular colonization (VC%).

Fig. 3.3: AM fungal colonization in cultivated tubers.

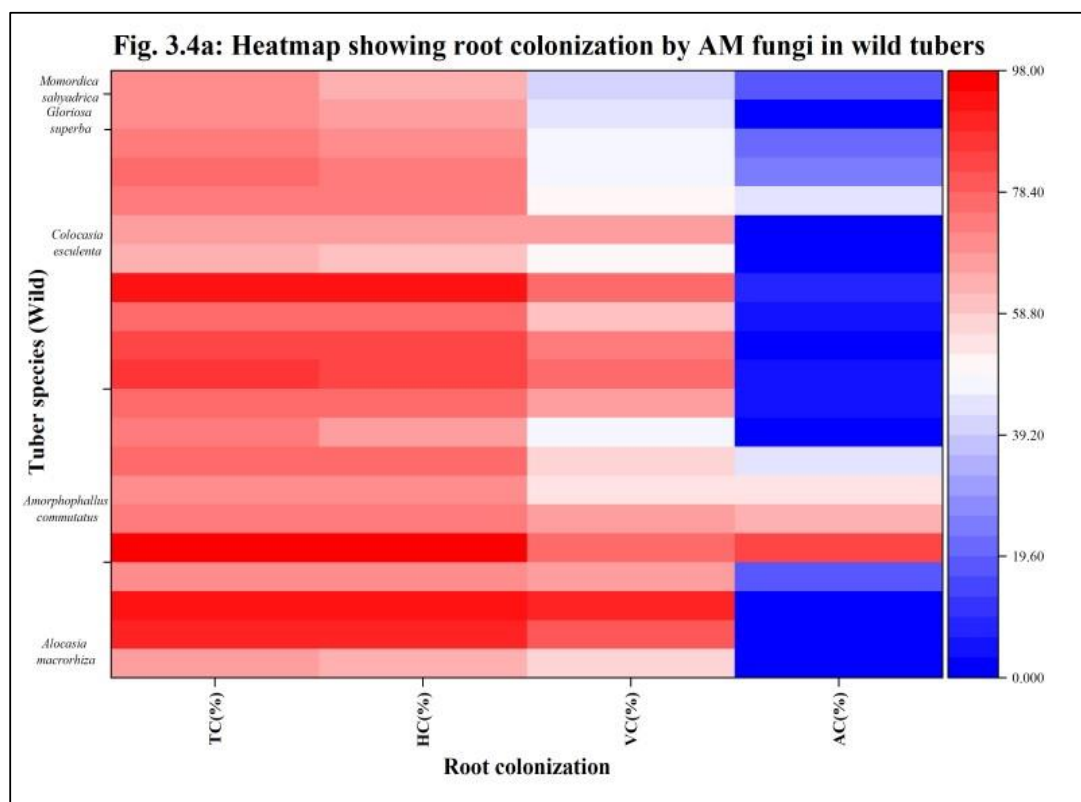
a. *Colocasia antiquorum*, **b.** *Dioscorea alata*, **c.** *D. bulbifera*, **d.** *D. esculenta*, **e.** *Solenostemon rotundifolius* and **f.** *Ipomoea batatas*.

In accordance with the present study, previous studies reported that most of the tuber species, both wild and cultivated, viz., *A. macrorhiza* (Anaswara *et al.*, 2023); *A. commutatus* (Khade and Rodrigues, 2007); *C. esculenta* (Baltazar-Bernal *et al.*, 2022); *G. superba* (Balachandar *et al.*, 2022); *Dioscorea esculenta* (Nuryana *et al.*, 2020); *D.*

alata (Sidibe *et al.*, 2015), *I. batatas* (ALHadidi *et al.*, 2021) exhibit AM fungal root colonization.

The heatmap displaying the extent of AM colonization rates in the various wild tuber species is presented in **Fig. 3.4a**. It is observed that the hyphal colonization, and consequently total colonization, was maximum, as observed in wild tuber species. However, variation in vesicular colonization was observed, while arbuscular colonization was least except in *A. commutatus* and *C. esculenta*. Among the wild tuber species studied, *A. commutatus* recorded the highest total colonization (**Fig. 3.4b**).

The heatmap displaying the rates of AM fungal colonization in cultivated tuber crops is presented in **Fig. 3.5a**. Here too, hyphal colonization, and thus total colonization was relatively high. Vesicular colonization varied by crop, while arbuscular colonization was low, except for two samples of *I. batatas* and one sample of *D. bulbifera*. Among the cultivated tubers collected from Goa, *D. esculenta* exhibited the highest total colonization (**Fig. 3.5b**).



Legend: Total colonization (TC%), hyphal colonization (HC%), arbuscular colonization (AC%), and vesicular colonization (VC %).

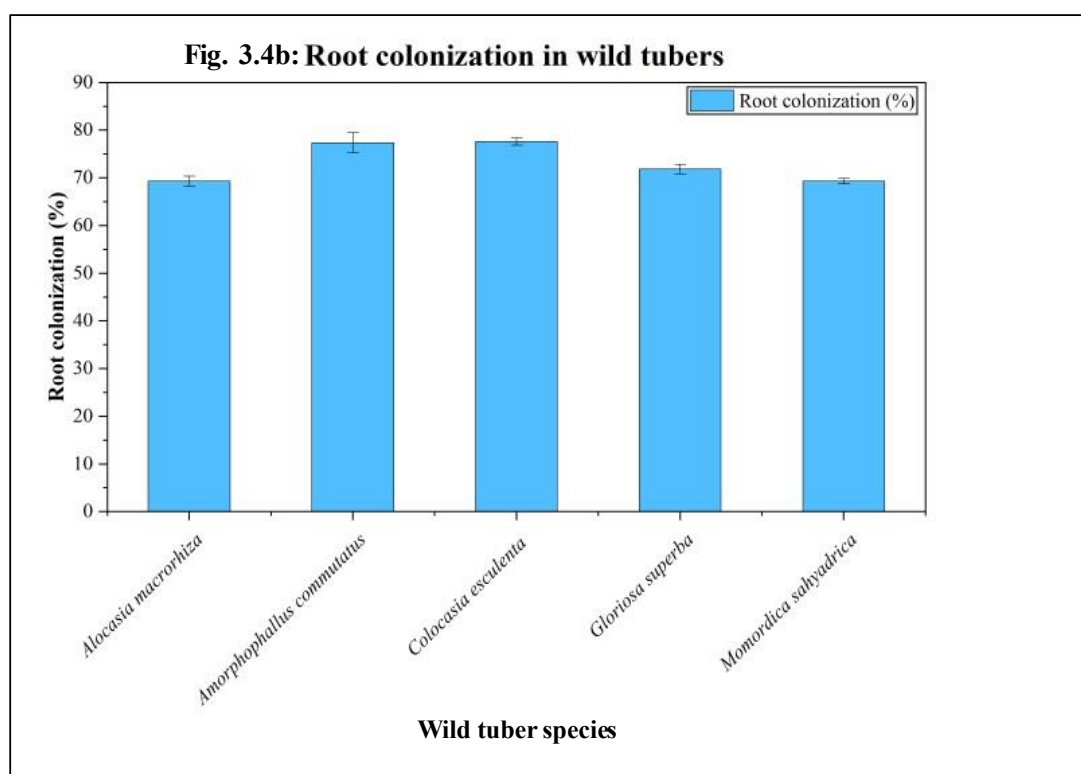
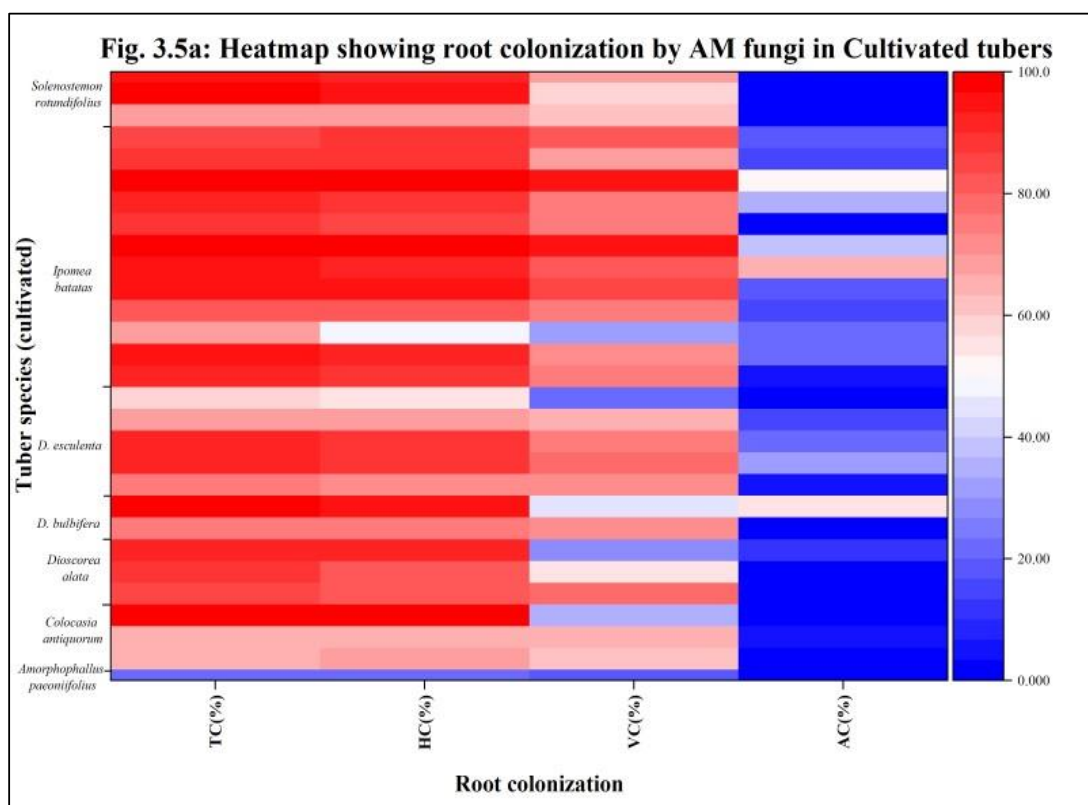


Fig. 3.4: Qualitative and quantitative details of root colonization in wild tuber species - a. Heatmap showing root colonization, b. Total root colonization.



Legend: Total colonization (TC%), hyphal colonization (HC%), arbuscular colonization (AC%), and vesicular colonization (VC %).

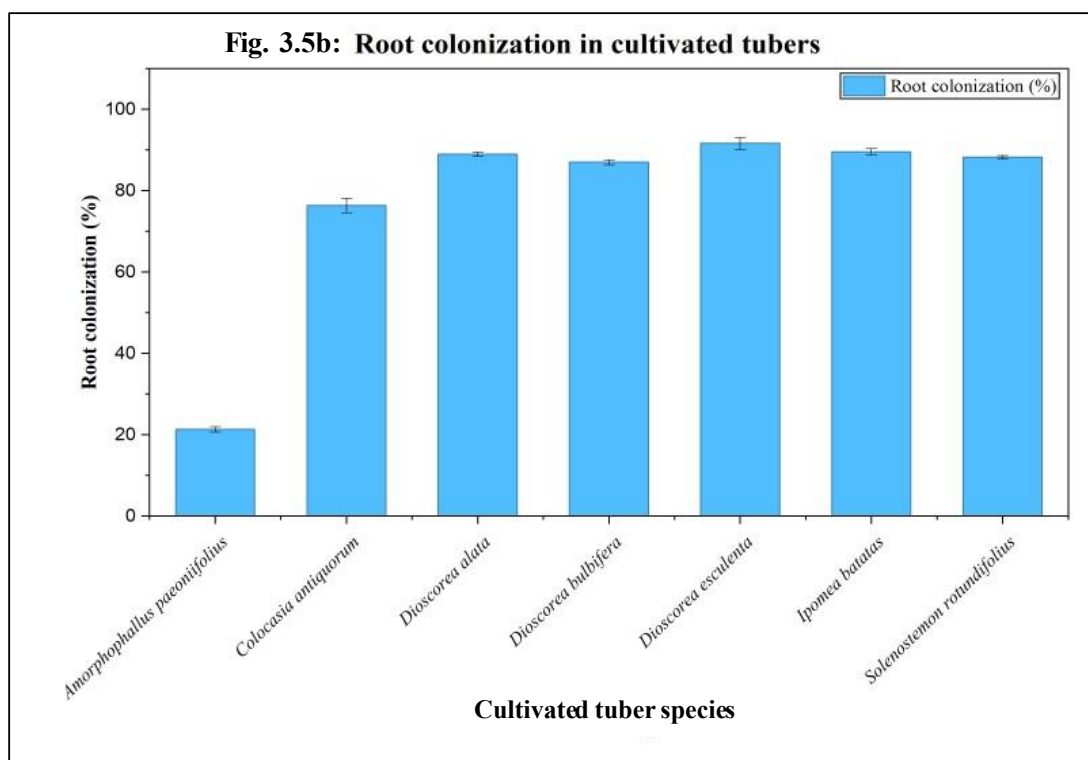


Fig. 3.5: Qualitative and quantitative details of root colonization in wild tuber species- a. Heatmap showing root colonization, b. Total root colonization.

Identifying tuber crops exhibiting robust AM fungal colonization stands as a pivotal strategy in sustainable agriculture, enabling the targeted inoculation of these beneficial microbes to enhance growth and yield while rigorously ensuring soil and environmental safety. This approach exemplifies the indispensable role of microbes in fortifying ecological balance while still gaining excellent yields.

The Principal Component Analysis (PCA) examining the relationships between physico-chemical soil variables and total colonization in wild and cultivated tuber species is depicted in **Fig. 3.6a** and **Fig. 3.6b**, respectively. The variables, viz., pH, EC, OC, and macro- and micro-nutrients, viz., N, P, K, Zn, Fe, Mn, Cu and total colonization (TC), are indicated by vectors. In both wild and cultivated species, total colonization showed a predominant association with soil pH, indicating pH as the primary driver of colonization. In cultivated tuber species, total colonization also exhibits a modest association with Cu, suggesting a secondary influence of Cu on colonization.

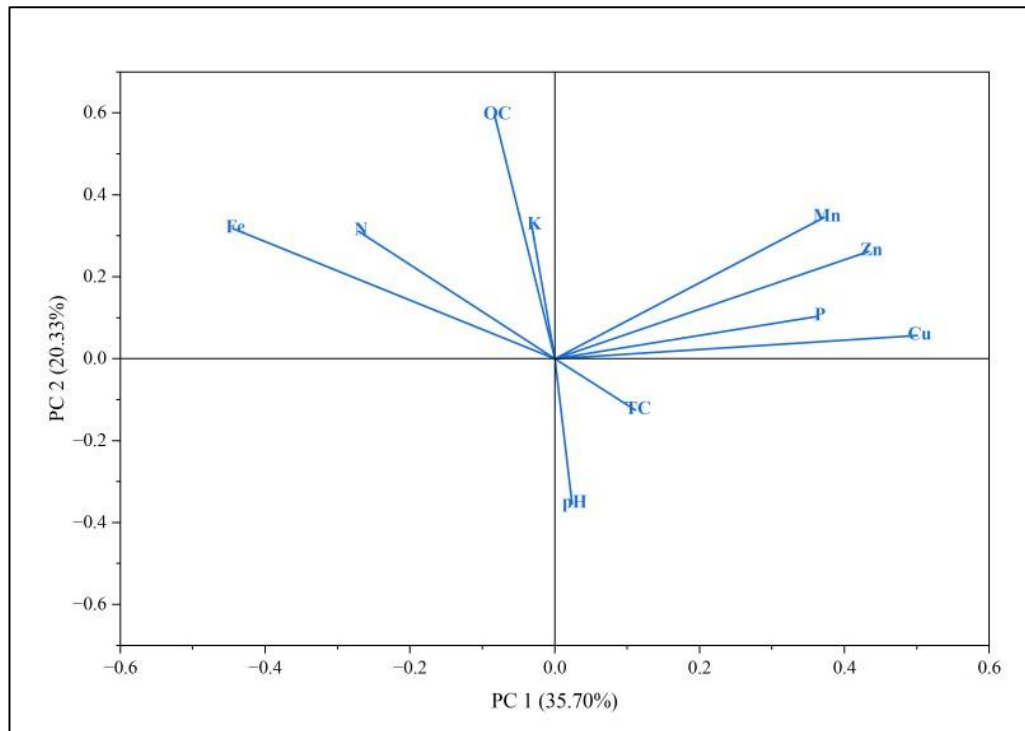


Fig. 3.6a: Principal Component analysis (PCA) of the relation between the physico-chemical variables and total colonization in wild tuber species

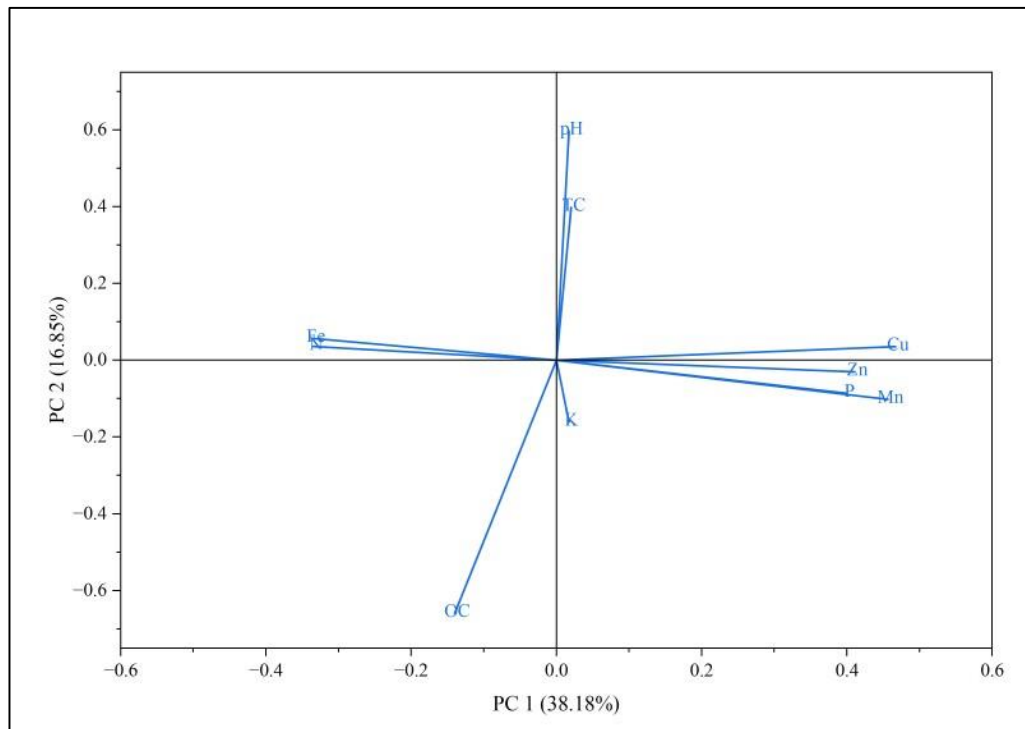


Fig. 3.6b: Principal Component analysis (PCA) of the relation between the physico-chemical variables and total colonization in cultivated tuber species.

Fig. 3.6: Principal Component analysis (PCA) showing the relation between the physico-chemical variables and total colonization.

3.4: CONCLUSION

AM fungal colonization was recorded in the rhizosphere of all the wild and cultivated tuber species studied. All the species recorded the presence of hyphae, arbuscules, and vesicles. Among these structures, hyphae and vesicles are more prevalent than arbuscules, indicating a variable pattern of colonization across sites.

The present study demonstrated that total colonization in both wild and cultivated tuber species is primarily driven by soil pH, thereby establishing pH as the key environmental determinant of AM fungal association. In cultivated tubers, a secondary influence emerges from Cu, with total colonization showing a modest association with Cu. This suggests that while pH governs the extent of AM colonization generally, Cu availability may modulate colonization levels in cultivated populations to a lesser degree.

Soil pH is known to be a key abiotic determinant shaping microbial community composition (Carrino-Kyker *et al.*, 2016; Feng *et al.*, 2023). The dominant role of pH in shaping AM fungal colonization, highlights soil management as a potential lever to optimize AM fungal associations in tuber crops.

Previous studies in vineyard systems have demonstrated that elevated Cu toxicity can be mitigated by inoculation with AM fungi. Certain AM fungi taxa are known to facilitate the establishment of young vines under high Cu stress. AM fungi promote Cu uptake by roots while reducing its translocation to shoots, thereby lowering shoot Cu concentrations and mitigating toxicity in aboveground tissues (Ambrosini *et al.*, 2015). In the current study, observations of the ancillary Cu effect observed in cultivated tubers warrant further investigation to elucidate the mechanism (e.g., Cu-mediated changes in fungal physiology, root exudation, or microbial interactions) and to determine whether Cu management could enhance beneficial colonization without adverse effects.

CHAPTER 4: To isolate, multiply (using trap cultures), and identify spores of AM fungi from the rhizosphere soils of plants with underground storage organs.

4.1: INTRODUCTION

Arbuscular mycorrhizal (AM) fungi represent a prominent and ecologically significant group within the diverse mycorrhizal fungal community. This phylogenetically broad and heterogeneous assemblage of taxa forms obligate symbioses with the roots of over 90% of terrestrial plant species, encompassing a wide range of plant lineages from liverworts to flowering angiosperms (Lanfranco *et al.*, 2016). These fungi establish intimate associations with their host plants by colonizing root tissues and residing within internal root structures. This mutualistic relationship plays a pivotal role in plant health and productivity, primarily by enhancing water and nutrient uptake, particularly P and micronutrients, thereby increasing plant growth and resilience under various environmental conditions (Nadeem *et al.*, 2017).

The symbiosis between plants and AM fungi is characterized by the formation of specialized structures called arbuscules, which are branched hyphal structures that facilitate nutrient exchange between the plant and the fungus. Beyond their role in improving plant nutrition, AM fungi are also implicated in soil carbon dynamics. They sequester a significant portion of the C fixed by their host plants and deposit organic compounds, including glomalin, a glycoprotein that is notably resistant to microbial degradation. By promoting soil aggregation and stability, AM fungi can indirectly safeguard organic matter from microbial decomposition, thereby potentially influencing the global C cycle (Giri and Saxena, 2017).

Mycorrhizal fungi establish an endomycorrhizal association with tuberous plants, colonizing the root tissues and extending hyphal networks into the surrounding soil matrix. It markedly increases the functional root surface area and the soil-root interface, thereby enhancing the plant's capacity to take up essential nutrients and water. The hyphal extensions effectively bypass physical and chemical soil limitations, facilitating more efficient resource acquisition. In this symbiosis, the fungi derive C compounds, predominantly sugars and amino acids, that are translocated from the host plant. These compounds support fungal growth and metabolism, thereby establishing a mutually

beneficial relationship that promotes tuber development and overall plant vigour (Sila *et al.*, 2021; Yuan *et al.*, 2023).

4.2: MATERIALS AND METHODS

Study Sites: Goa, a small state, is situated between 14° 53' 47" and 15° 47' 59" N latitudes, and 73° 40' 54" and 74° 20' 11" E longitudes, covering an area of 3,702 square kilometres. This region accounts for approximately 1% of the country's total land area and boasts a 110 km coastline. Geographically, it is bordered on the west by the Arabian Sea, on the north by the state of Maharashtra, and on the east and south by the state of Karnataka. The state stretches roughly 60 km from north to south and about 105 km from east to west. The Western Ghats, which comprise most of eastern Goa, have been globally recognised as one of the world's biodiversity hotspots (Mohekar, 2020). Goa has a tropical monsoon climate, with temperatures ranging from 23°C to 34°C. The area's diverse vegetation includes semi-evergreen and moist deciduous forests, open scrublands, and plateau vegetation, creating a heterogeneous ecological backdrop for microbial and plant interactions.

Study and sampling sites: Sampling sites are described and discussed as shown in Tables 3.1 and 3.2 of Chapter 3.

Rhizosphere soil sample collection: was carried out as described and discussed in Chapter 3.

Soil analyses: were carried out as described and discussed in Chapter 3.

Preparation of trap cultures: The multiplication of AM fungi from rhizosphere soils was carried out using the trap culture method, as modified by Morton *et al.* (1993) (Plate 4.1a-b), and is described below.

The rhizosphere soil (along with roots) was mixed with sterilized sand in a 1:1 ratio. The mixture was then transferred to 15 cm plastic pots that had been pre-wiped with absolute alcohol. *Coleus scutellarioides* (L.) R.Br. (Lamiaceae) cuttings were used as the host plant. The pots were maintained in the greenhouse for 90 days to establish AM symbiosis and continue the AM life cycle (colonization and sporulation). These plants were watered regularly as required and with Hoagland's solution (minus P) (Hoagland

and Arnon, 1950) every 15 days until harvest. After 90 days, the pots were left to dry for 1-2 weeks, following which the dried shoot portion was cut off at the soil interface. The contents of the pot were then transferred into *zip-loc* plastic bags, labelled, and stored at 4°C. The new spores thus formed were isolated for taxonomic identification and to set up monospecific cultures.

Isolation of AM fungal spores: AM fungal spores were isolated using the wet-sieving and decanting method (Gerdemann and Nicolson, 1963). A 100 g sample of rhizosphere soil (fresh or refrigerated at 4°C) was placed in a beaker, and tap water was added. The soil suspension was stirred continuously with a glass rod, and the sediment was allowed to settle for 10-15 seconds. This aliquot was decanted through sieves arranged in descending order (250-37 µm). The procedure has been repeated at least 4 times for each sample. The residues from each sieve were then washed into separate beakers. The aliquot was filtered separately through Whatman No. 1 filter paper. The filter paper was then placed on a Petri plate, and care was taken to keep it moist. The filter paper was examined for the presence of spores and sporocarps under an Olympus stereo microscope SZ2-ILST (10×, 4.5 mm zoom).

Taxonomic identification of spores: Clean, intact, and unparasitized spores were used for taxonomic identification and quantification. Intact and crushed spores, mounted in Poly Vinyl-Lacto Glycerol (PVLG) (Koske and Tessier, 1983), were examined under an Olympus BX 41 microscope. Taxonomic identification of AM spores was carried out based on morphological characteristics using relevant bibliographies (Morton and Benny, 1990; Schenck and Perez, 1990; Almeida and Schenck, 1990; Rodrigues and Muthukumar, 2009; Schüßler and Walker, 2010; Blaszkowski, 2012; Redecker *et al.* 2013), and online species matching the descriptions provided by the International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi (<https://invam.wvu.edu/>), and amf-phylogeny.com. Names and epithets of AM fungal species were given as per the recommendation of Schüßler and Walker (2010) and Redecker (2013).

Diversity studies: The data analysis of the ecological characteristics of AM fungal species was estimated using the following indices:

- **Relative abundance (RA)** = (Number of spores of species/Total number of spores in all soil samples) × 100.

- **Isolation frequency (IF)** = (Number of soil samples containing particular species/Total number of soil samples analyzed) $\times$ 100.
- **Shannon- Wiener diversity index (H)** = $-\sum (P_i \ln (P_i))$, where P_i is the proportion of individual species that contributes to the total number of individuals (Shannon and Wiener, 1949).
- **Simpson's diversity index (D)** = $1 - (\sum n(n-1)/N(N-1))$, 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)**: $\Sigma(H') = H' / H'_{max}$, where $max = \ln S$, $S = SR$.

Statistical analysis: Pearson's correlation coefficient (r) was calculated to assess relationships among spore density (SD), relative abundance (RA), isolation frequency (IF), and species richness (SR) using IBM SPSS Statistics 22 and RStudio. A canonical correlation analysis (CCA) was performed using all observations to examine the interrelationships among the variables studied. Pearson's correlation coefficient was used to assess variation among AM fungal SD, RA, IF, and SR using variance (ANOVA) and Principal Component Analysis (PCA) in WASP software (Web-Based Agricultural package) 2.0 ($p \leq 0.05$).

4.3: RESULTS AND DISCUSSION

AM fungal diversity: AM fungal diversity was recorded at both cultivated and wild sites (**Plates 4.1 & 4.2**). A total of 15 AM fungal species were identified from 20 sites of wild tuber species collection, as given in **Table 4.1**.

Table 4.1: AM fungal diversity from the wild tuber species.

Sr. No.	AM fungal species
1	<i>Acaulospora foveata</i> Trappe & Janos
2	<i>Acaulospora delicata</i> Walker, Pfeiff. & Bloss
3	<i>Acaulospora scrobiculata</i> Trappe
4	<i>Acaulospora dilatata</i> J.B. Morton
5	<i>Claroideoglossum etunicatum</i> (W. N. Becker & Gerd.) C. Walker & Schüßler
6	<i>Funneliformis geosporum</i> (Nicolson & Gerd.) Walker & Schüßler
7	<i>Funneliformis mosseae</i> (Nicolson & Gerd.) Walker & Schüßler
8	<i>Gigaspora albida</i> Schenck & Sm.
9	<i>Gigaspora decipiens</i> Hall & Abbott
10	<i>Glomus</i> sp.
11	<i>Sieverdingia tortuosa</i> (N.C.Schenck & G.S.Sm.) Błaszk., Niezgoda & B.T.Goto
12	<i>Racocetra gregaria</i> (Schenck & Nicolson) Oehl, Souza & Sieverd.
13	<i>Rhizophagus fasciculatus</i> (Thaxt.) C. Walker & A. Schüßler
14	<i>Rhizophagus irregularis</i> Liu, J., et al.
15	<i>Rhizophagus intraradices</i> (Schenck & Sm.) Walker & Schüßler

A total of 23 AM fungal species were identified from 29 sites of cultivated tuber species collection, as seen in **Table 4.2**.

Table 4.2: AM fungal diversity from the cultivated tuber species.

Sr. No.	AM fungal species
1	<i>Acaulospora bireticulata</i> F. M. Rothwell & Trappe
2	<i>Acaulospora foveata</i> Trappe & Janos
3	<i>Acaulospora mellea</i> Spain & Schenck
4	<i>Acaulospora dilatata</i> J. B. Morton
5	<i>Acaulospora delicata</i> Walker, Pfeiff. & Bloss
6	<i>Acaulospora scrobiculata</i> Trappe
7	<i>Acaulospora laevis</i> Gerd. & Trappe
8	<i>Acaulospora spinosa</i> Walker & Trappe
9	<i>Claroideoglossum etunicatum</i> (W. N. Becker & Gerd.) C. Walker & Schüßler
10	<i>Funneliformis coronatum</i> (Giovann.) C. Walker & A. Schüßler
11	<i>Funneliformis geosporum</i> (Nicolson & Gerd.) Walker & Schüßler
12	<i>Funneliformis mosseae</i> (Nicolson & Gerd.) Walker & Schüßler
13	<i>Gigaspora albida</i> Schenck & Sm.
14	<i>Gigaspora decipiens</i> Hall & Abbott
15	<i>Glomus multisporum</i> Gerd. & B.K. Bakshi
16	<i>Glomus microcarpum</i> Tul. & C. Tul
17	<i>Glomus</i> sp.
18	<i>Racocetra gregaria</i> (Schenck & Nicolson) Oehl, Souza & Sieverd
19	<i>Rhizophagus fasciculatus</i> (Thaxt.) C. Walker & A. Schüßler
20	<i>Rhizophagus irregularis</i> Liu, J., <i>et al.</i>
21	<i>Rhizophagus intraradices</i> (Schenck & Sm.) Walker & Schüßler
22	<i>Scutellospora calospora</i> (T.H. Nicolson & Gerd.) C. Walker & F.E. Sanders
23	<i>Sacculospora felinonii</i> Willis, A. <i>et al.</i>

Table 4.3: AM fungal diversity in *Alocasia macrorrhiza*.

Site	AM fungal species	Spore density*	Spore richness
Velipwado (S-I)	<i>A. fov.</i> , <i>A. scr.</i> , <i>F. geo.</i> , <i>Gi. alb.</i> , <i>Gi. dec.</i>	106±0.57	5
Poiginim (S-II)	<i>A. dil.</i> , <i>A. scr.</i> , <i>Gi. alb.</i> , <i>Gl. sp.</i> , <i>Rh. int.</i> , <i>Si. tor.</i>	156.33±2.02	6
Altinho (S-III)	<i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>Gl. sp.</i> , <i>Rh. int.</i>	78.00±0.88	4

Legend: *Number of spores 100g⁻¹. ± standard error; AM fungal species: *Acaulospora dilatata*- *A. dil.*, *A. foveata*- *A.fov.*, *A. scrobiculata*- *A. scr.*, *Funneliformis geosporum*- *F.geo.*, *Gigaspora albida*- *Gi. alb.*, *Gi. decipiens*- *Gi. dec.*, *Glomus sp.*- *Gl. sp.*, *Rhizophagus intraradices*- *Rh. int.*, *Sieverdingia tortuosa* - *Si. tor.*

In *Amorphophallus commutatus*, the highest spore density was observed at site S-V (234.00), while the least was documented at site S-IV (64.00) (Table 4.4).

Table 4.4: AM fungal diversity in *Amorphophallus commutatus*.

Site	AM fungal species	Spore density*	Spore richness
Zambaulim (S-IV)	<i>A. fov.</i> , <i>F. geo.</i> , <i>F. mos.</i>	64.00±2.31	2
Fatorda (S-V)	<i>A. fov.</i> , <i>A. scr.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>Rh. fas.</i> , <i>Rh. int.</i>	234.00±3.21	8
Bicholim (S-VI)	<i>A. del.</i> , <i>A. dil.</i> , <i>A. scr.</i> , <i>Gi. dec.</i>	102.67±3.71	3
Nuvem (S-VI)	<i>A. fov.</i> , <i>A. scr.</i> , <i>Gl. sp.</i>	67.67±0.88	3
Taleigao Plateau (S-VIII)	<i>A. scr.</i> , <i>F. mos.</i> , <i>Gl. sp.</i>	85.33±1.45	3

Legend: *Number of spores 100g⁻¹. ± standard error; AM fungal species: *Acaulospora delicata*- *A. del.*, *A. dilatata*- *A. dil.*, *A. foveata*- *A. fov.*, *A. scrobiculata*- *A. scr.*, *Funneliformis geosporum*- *F. geo.*, *F. mosseae*- *F. mos.*, *Gigaspora albida*- *Gi. alb.*, *Gi. decipiens*- *Gi. dec.*, *Glomus sp.*- *Gl. sp.*, *Rhizophagus fasciculatus*- *Rh. fas.*, *Rh. intraradices*- *Rh. int.*

In *Colocasia esculenta*, the highest spore density was observed at site S-XII (206.33), while the least was documented at site S-XIV (25.00) (Table 4.5).

Table 4.5: AM fungal diversity in *Colocasia esculenta*.

Site	AM fungal species	Spore density*	Spore richness
Morpirila (S-IX)	<i>A. fov.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>R. gre.</i> , <i>Rh. irr.</i>	87.00±0.58	4
Fatorda (S-X)	<i>A. del.</i> , <i>A. fov.</i> , <i>A. scr.</i> , <i>C. etu.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>Rh. int.</i>	112.33±0.88	5
Canacona (S-XI)	<i>A. del.</i> , <i>A. fov.</i> , <i>A. scr.</i> , <i>F. geo.</i> , <i>Gl. sp.</i> , <i>Gi. dec.</i> , <i>Gi. alb.</i> , <i>R. gre.</i> , <i>Rh. int.</i>	163.00±2.31	8
Poiginim (S-XII)	<i>A. fov.</i> , <i>A. scr.</i> , <i>F. mos.</i> , <i>Gi. dec.</i> , <i>Gl. sp.</i> , <i>R. gre.</i> , <i>Rh. int.</i> , <i>Si. tor.</i>	206.33±1.76	10
Mayem (S-XIII)	<i>A. dil.</i> , <i>A. scr.</i> , <i>C. etu.</i> , <i>Gi. dec.</i>	131.67±2.73	6
Cuncolim (S- XIV)	<i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>Rh. irr.</i>	25.00±0.57	1
Velipwada (S-XV)	<i>F. mos.</i> , <i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>Gl. sp.</i> , <i>R. gre.</i>	130.00±0.57	6
Margao (S-XVI)	<i>A. del.</i> , <i>A. dil.</i> , <i>A. fov.</i> , <i>A. scr.</i> , <i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>Gl. sp.</i>	52.33±0.33	3
Vasco (S-XVII)	<i>A. fov.</i> , <i>A. scr.</i> , <i>F. mos.</i> , <i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>Rh. int.</i> , <i>Rh. irr.</i>	63.33±0.57	3

Legend: *Number of spores 100g⁻¹. ± standard error; AM fungal species: *Acaulospora delicata*- *A. del.*, *A. dilatata*- *A. dil.*, *A. foveata*- *A. fov.*, *A. scrobiculata*- *A. scr.*, *Claroideoglomus etunicatum*- *C. etu.*, *Funneliformis geosporum*- *F. geo.*, *F. mosseae*- *F. mos.*, *Gigaspora albida*- *Gi. alb.*, *Gi. decipiens*- *Gi. dec.*, *Glomus sp.*- *Gl. sp.*, *Racocetra gregaria*- *R. gre.*, *Rhizophagus fasciculatus*- *Rh. fas.*, *Rh. intraradices*- *Rh. int.*, *Rh. irregularis*- *Rh. irr.*, *Sieverdingia tortuosa*- *Si. tor.*

In *Gloriosa superba*, the highest spore density was observed at site S-XIX (68.33), while the least was documented at site S-XVIII (63.00) (Table 4.6).

Table 4.6: AM fungal diversity in *Gloriosa superba*.

Site	AM fungal species	Spore density*	Spore richness
Taleigao plateau (S-XVIII)	<i>F. geo.</i> , <i>F. mos.</i> , <i>Gi. alb.</i> , <i>Rh. fas.</i> , <i>Rh. int.</i> , <i>Rh. irr.</i>	63.00±0.33	3
Pernem (S-XIX)	<i>A. scr.</i> , <i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>Gl. sp.</i> , <i>R. gre.</i>	68.33±0.57	3

Legend: *Number of spores 100g⁻¹. ± standard error; AM fungal species: *Acaulospora scrobiculata*- *A. scr.*, *Funneliformis geosporum*- *F. geo.*, *F. mosseae*- *F. mos.*, *Gigaspora albida*- *Gi. alb.*, *Gi. decipiens*- *Gi. dec.*, *Glomus* sp.- *Gl. sp.*, *Racocetra gregaria*- *R. gre.*, *Rhizophagus fasciculatus*- *Rh. fas.*, *Rh. intraradices*- *Rh. int.*, *Rh. irregularis*- *Rh. irr.*

In *Momordica sahyadrica*, the spore density observed at site S-XX (73.66) (Table 4.7).

Table 4.7: AM fungal diversity in *Momordica sahyadrica*.

Site	AM fungal species	Spore density*	Spore richness
Taleigao plateau (S-XX)	<i>A. scr.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>R. gre.</i>	73.66±0.57	4

Legend: *Number of spores 100g⁻¹. ± standard error; AM fungal species: *Acaulospora scrobiculata*- *A. scr.*, *Funneliformis geosporum*- *F. geo.*, *F. mosseae*- *F. mos.*, *Racocetra gregaria*- *R. gre.*

In *Amorphophallus paeoniifolius* low spore density was observed at site S-XXI (34.00) (Table 4.8).

Table 4.8: AM fungal diversity in *Amorphophallus paeoniifolius*.

Site	AM fungal species	Spore density*	Spore richness
Altinho (S-XXI)	<i>A. scr.</i> , <i>Gi. dec.</i> , <i>Gl. sp.</i>	34.00±4.5	3

Legend: *Number of spores 100g⁻¹. ± standard error; AM fungal species: *Acaulospora scrobiculata*- *A. scr.*, *Gigaspora decipiens*- *Gi. dec.*, *Glomus* sp.- *Gl. sp.*

In *Colocasia antiquorum* the highest spore density was observed at site S-XXII (121.67), while the least was documented at site S-XXIV (96.00) (**Table 4.9**).

Table 4.9: AM fungal diversity in *Colocasia antiquorum*.

Site	AM fungal species	Spore density*	Spore richness
Sanguem (S-XXII)	<i>A. dil.</i> , <i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>R. gre.</i>	121.67±1.45	4
Canacona (S-XXIII)	<i>A. fov.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>Gl. sp.</i> , <i>Rh. fas.</i>	108.67±1.76	7
Morpirila (S-XXIV)	<i>A. spi.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>Gi. dec.</i> , <i>Rh. irr.</i>	96.00±0.57	5

Legend: *Number of spores 100g⁻¹. ± standard error; AM fungal species: *Acaulospora dilatata*- *A. dil.*, *A. foveata*- *A. fov.*, *A. scrobiculata*- *A. scr.*, *A. spinosa*- *A. spi.*, *Claroideoglossum etunicatum*- *C. etu.*, *Funneliformis geosporum*- *F. geo.*, *F. mosseae*- *F. mos.*, *Gigaspora albida*- *Gi. alb.*, *Gi. decipiens*- *Gi. dec.*, *Glomus sp.*- *Gl. sp.*, *Racocetra gregaria*- *R. gre.*, *Rhizophagus fasciculatus*- *Rh. fas.*, *Rh. irregularis*- *Rh. irr.*

In *Dioscorea alata*, the highest spore density was observed at site S-XXVI (224.50), while the least was recorded at site S-XXV (89.00) (**Table 4.10**).

Table 4.10: AM fungal diversity in *Dioscorea alata*.

Site	AM fungal species	Spore density*	Spore richness
Barcem (S-XXV)	<i>A. spi.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>Gi. alb.</i> , <i>Gi. dec.</i>	89.00±0.57	5
Morpirila (S-XXVI)	<i>A. fov.</i> , <i>A. scr.</i> , <i>A. spi.</i> , <i>F. geo.</i> , <i>Gi. alb.</i> , <i>Gi. dec.</i>	224.50±0.33	6
Tiswadi (S-XXVII)	<i>A. fov.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>Rh. irr.</i>	96.00±1.76	4

Legend: *Number of spores 100g⁻¹. ± standard error; AM fungal species: *Acaulospora delicata*- *A. del.*, *A. dilatata*- *A. dil.*, *A. foveata*- *A. fov.*, *A. scrobiculata*- *A. scr.*, *A. spinosa*- *A. spi.*, *Claroideoglossum etunicatum*- *C. etu.*, *Funneliformis geosporum*- *F. geo.*, *F. mosseae*- *F. mos.*, *Gigaspora albida*- *Gi. alb.*, *Gi. decipiens*- *Gi. dec.*, *Rhizophagus irregularis*- *Rh. irr.*

In *D. bulbifera*, the highest spore density was observed at site S-XXIX (142.00), while the least was recorded at site S-XXVIII (84.00) (**Table 4.11**).

Table 4.11: AM fungal diversity in *Dioscorea bulbifera*.

Site	AM fungal species	Spore density*	Spore richness
Barcem (S-XXVIII)	<i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>Rh. fas.</i> , <i>R. gre.</i> , <i>Rh. irr.</i>	84.00±1.76	5
Tiswadi (S-XXIX)	<i>A. fov.</i> , <i>A. scr.</i> , <i>A. spi.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>Gi. dec.</i>	142.00±0.33	6

Legend: *Number of spores 100g⁻¹. ± standard error; AM fungal species: *Acaulospora foveata*- *A. fov.*, *A. scrobiculata*- *A. scr.*, *A. spinosa*- *A. spi.*, *Funneliformis geosporum*- *F. geo.*, *F. mosseae*- *F. mos.*, *Gigaspora albida*- *Gi. alb.*, *Gi. decipiens*- *Gi. dec.*, *Racocetra gregaria*- *R. gre.*, *Rhizophagus fasciculatus*- *Rh. fas.*, *Rh. irregularis*- *Rh. irr.*

In *D. esculenta*, the highest spore density was observed at site S-XXX (320.67), while the least was recorded at site S-XXXIV (28.00) (Table 4.12).

Table 4.12: AM fungal diversity in *Dioscorea esculenta*.

Site	AM fungal species	Spore density*	Spore richness
Canacona (S-XXX)	<i>A. del.</i> , <i>A. dil.</i> , <i>A. fov.</i> , <i>A. scr.</i> , <i>A. spi.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>Gi. alb.</i> , <i>Gl. sp.</i> , <i>Rh. fas.</i> , <i>R. gre.</i>	320.67±4.84	11
Barcem (S-XXXI)	<i>A. fov.</i> , <i>A. scr.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>Gi. dec.</i> , <i>Rh. irr.</i>	211.67±1.20	6
Sanguem (S-XXXII)	<i>A. fov.</i> , <i>A. scr.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>Gi. dec.</i> , <i>Rh. fas.</i> , <i>Rh. int.</i>	196.67±3.76	7
Balli (S-XXXIII)	<i>A. fov.</i> , <i>A. scr.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>Gi. dec.</i> , <i>Rh. fas.</i>	152.00±0.57	6
Morpirla (S-XXXIV)	<i>A. fov.</i> , <i>A. scr.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>R. gre.</i>	28.00±0.33	7

Legend: *Number of spores 100g⁻¹. ± standard error; AM fungal species: *Acaulospora delicata*- *A. del.*, *A. dilatata*- *A. dil.*, *A. foveata*- *A. fov.*, *A. scrobiculata*- *A. scr.*, *A. spinosa*- *A. spi.*, *Claroideoglomus etunicatum*- *C. etu.*, *Funneliformis geosporum*- *F. geo.*, *F. mosseae*- *F. mos.*, *Gigaspora albida*- *Gi. alb.*, *Gi. decipiens*- *Gi. dec.*, *Glomus sp.*- *Gl. sp.*, *Racocetra gregaria*- *R. gre.*, *Rhizophagus fasciculatus*- *Rh. fas.*, *Rh. intraradices*- *Rh. int.*, *Rh. irregularis*- *Rh. irr.*, *Sieverdingia tortuosa*- *Si. tor.*

In *Solenostemon rotundifolius*, the highest spore density was observed at site S-XXXVII (141.33), while the least was recorded at site S-XXXV (67.00) (Table 4.13).

Table 4.13: AM fungal diversity in *Solenostemon rotundifolius*.

Site	AM fungal species	Spore density*	Spore richness
Velipwado (S-XXXV)	<i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>Rh. int.</i> , <i>Rh. irr.</i>	67.00±0.57	4
Morpirla (S-XXXVI)	<i>A. fov.</i> , <i>A. spi.</i> , <i>F. geo.</i> , <i>Gi. alb.</i> , <i>Gl. sp.</i>	102.00±0.33	5
Canacona (S-XXXVII)	<i>A. dil.</i> , <i>A. scr.</i> , <i>A. spi.</i> , <i>F. mos.</i> , <i>Gi. dec.</i> , <i>Sc. cal.</i> , <i>Sa. fel.</i>	141.33±1.85	7

Legend: *Number of spores 100g⁻¹. ± standard error; AM fungal species: *Acaulospora dilatata*- *A. dil.*, *A. foveata*- *A. fov.*, *A. scrobiculata*- *A. scr.*, *A. spinosa*- *A. spi.*, *Funneliformis geosporum*- *F. geo.*, *F. mosseae*- *F. mos.*, *Gigaspora albida*- *Gi. alb.*, *Gi. decipiens*- *Gi. dec.*, *Glomus sp.*- *Gl. sp.*, *Rhizospora intraradices*- *Rh. int.*, *Rh. irregularis*- *Rh. irr.*, *Scutellospora calospora*- *Sc. cal.*, *Sacculospora felinonii*- *Sa. fel.*

In *Ipomoea batatas*, the highest spore density was observed at site S-XLIII (294.33), while the least was recorded at site S-XLIX (76.00) (Table 4.14).

Table 4.14: AM fungal diversity in *Ipomoea batatas*.

Site	AM fungal species	Spore density*	Spore richness
Fatorda (S-XXXVIII)	<i>A. fov.</i> , <i>A. scr.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>Gi. alb.</i> , <i>Rh. fas.</i> , <i>Rh. int.</i>	272.67±3.18	6
Taleigao 1 (S-XXXIX)	<i>A. fov.</i> , <i>A. scr.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>Rh. fas.</i> , <i>Rh. int.</i>	218.33±1.76	9
Taleigao 2 (S-XL)	<i>A. scr.</i> , <i>Gi. alb.</i> , <i>Rh. fas.</i> , <i>Rh. int.</i> , <i>Rh. irr.</i>	195.33±1.76	5
Taleigao 3 (S-XLI)	<i>A. dil.</i> , <i>A. fov.</i> , <i>A. lae.</i> , <i>A. mel.</i> , <i>A. scr.</i> , <i>A. spi.</i> , <i>C. etu.</i> , <i>F. cor.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>Rh. fas.</i> , <i>Rh. int.</i>	167.66±1.45	12
Taleigao 4 (S-XLII)	<i>A. dil.</i> , <i>A. scr.</i> , <i>F. geo.</i> , <i>R. gre.</i> , <i>Rh. int.</i> , <i>Rh. irr.</i>	130.66±1.76	5
Agassiam 1 (S-XLIII)	<i>A. mel.</i> , <i>A. scr.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>Gl. mic.</i> , <i>Gl. mul.</i> , <i>Rh. fas.</i> , <i>Rh. int.</i>	294.33±2.19	10
Agassiam 2 (S-XLIV)	<i>A. bir.</i> , <i>A. scr.</i> , <i>Gi. alb.</i> , <i>Gl. cor.</i> , <i>Gl. sp.</i> , <i>Rh. int.</i>	86.33±0.33	7
Balli (S-XLV)	<i>A. dil.</i> , <i>A. scr.</i> , <i>Gl. sp.</i> , <i>Rh. int.</i> , <i>Rh. irr.</i>	80.00±0.57	5
Verna (S-XLVI)	<i>A. fov.</i> , <i>A. scr.</i> , <i>A. spi.</i> , <i>Rh. int.</i>	101.66±0.88	5
Sanguem (S-XLVII)	<i>A. del.</i> , <i>A. fov.</i> , <i>A. scr.</i> , <i>R. gre.</i> , <i>Rh. irr.</i>	154.33±2.18	5
St Cruz (S-XLVIII)	<i>A. fov.</i> , <i>A. scr.</i> , <i>Rh. int.</i>	106.33±2.33	4
Padi (S-XLIX)	<i>A. fov.</i> , <i>A. scr.</i> , <i>F. geo.</i> , <i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>Rh. int.</i>	76.00±2.08	4

Legend: *Number of spores 100g⁻¹. ± standard error; AM fungal species: *Acaulospora bireticulata*- *A. bir.*, *A. delicata*- *A. del.*, *A. dilatata*- *A. dil.*, *A. foveata*- *A. fov.*, *A. laevis*- *A. lae.*, *A. scrobiculata*- *A. scr.*, *A. spinosa*- *A. spi.*, *Claroideoglossum etunicatum*- *C. etu.*, *Funnelformis coronatum*- *F. cor.*, *F. geosporum*- *F. geo.*, *F. mosseae*- *F. mos.*, *Gigaspora albida*- *Gi. alb.*, *Gi. decipiens*- *Gi. dec.*, *Glomus microcapum*- *Gl. mic.*, *Gl. multisporum*- *Gl. mul.*, *Glomus sp.*- *Gl. sp.*, *Racocetra gregaria*- *R. gre.*, *Rhizophagus fasciculatus*- *Rh. fas.*, *Rh. intraradices*- *Rh. int.*, *Rh. irregularis*- *Rh. irr.*

Fig. 4.1 presents the site-wise composition of the AM fungal community, represented by the mean spore density in trap cultures of the wild tuber species studied (**Fig. 4.1a**). It illustrates that the highest density of AM fungi was observed in *A. scrobiculata*, followed by *F. mosseae*. Other species, including *A. foveata*, also showed high spore density. These AM fungal species exhibited high RA (**Fig. 4.1b**), viz. *A. scrobiculata* (19.92), *F. mosseae* (14.26) and *A. foveata* (11.85). High isolation frequency (IF) (**Fig. 4.1c**) was expressed by *A. scrobiculata* (4.12), *Gi. decipiens* (3.53) followed by *F. mosseae* and *A. foveata* (2.94) (**Table 4.15**). The RA of individual AM fungal species from wild tuber species is given in **Table 4.16a** and **4.16b**.

Table 4.15: Relative abundance, isolation frequency, and frequency of occurrence of AM fungal species.

Sr. No.	AM fungal species	RA %	IF %	Frequency of occurrence
1	<i>Acaulospora delicata</i>	2.61	0.88	3.00
2	<i>Acaulospora dilatata</i>	2.90	0.88	4.00
3	<i>Acaulospora foveata</i>	11.85	2.94	10.00
4	<i>Acaulospora scrobiculata</i>	19.92	4.12	14.00
5	<i>Claroideoglossum etunicatum</i>	2.90	0.59	2
6	<i>Funneliformis geosporum</i>	8.80	2.06	8.00
7	<i>Funneliformis mosseae</i>	14.26	2.94	10.00
8	<i>Gigaspora albida</i>	7.79	2.65	10.00
9	<i>Gigaspora decipiens</i>	9.48	3.53	12
10	<i>Glomus</i> sp.	5.08	2.65	9.00
11	<i>Sieverdingia tortuosa</i>	0.68	0.59	2.00
12	<i>Racocetra gregaria</i>	1.79	1.18	4.00
13	<i>Rhizophagus fasciculatus</i>	2.32	0.88	3.00
14	<i>Rhizophagus irregularis</i>	1.35	0.88	3.00
15	<i>Rhizophagus intraradices</i>	8.27	2.35	8.00

Table 4.16a: Relative abundance of AM fungal species from wild tuber species at study sites S-I to S-X.

Sr. No.	AM fungal species	S-I	S-II	S-III	S-IV	S-V	S-VI	S-VII	S-VIII	S-IX	S-X
1	<i>Acaulospora foveata</i>	22.642	nd	nd	nd	16.239	nd	32.353	24.706	29.885	nd
2	<i>Acaulospora delicata</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
3	<i>Acaulospora scrobiculata</i>	29.245	25.000	nd	59.375	18.803	33.010	47.059	nd	nd	30.357
4	<i>Acaulospora dilatata</i>	nd	21.154	nd	nd	nd	nd	nd	nd	nd	nd
5	<i>Claroideoglossum etunicatum</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	24.107
6	<i>Funneliformis geosporum</i>	21.698	nd	nd	nd	10.256	nd	nd	43.529	40.230	nd
7	<i>Funneliformis mosseae</i>	nd	nd	nd	nd	11.966	44.660	nd	31.765	29.885	45.536
8	<i>Gigaspora albida</i>	16.038	16.667	20.513	nd	9.402	nd	nd	nd	nd	nd
9	<i>Gigaspora decipiens</i>	10.377	nd	21.795	40.625	8.120	nd	nd	nd	nd	nd
10	<i>Glomus</i> sp.	nd	10.256	12.821	nd	nd	22.330	20.588	nd	nd	nd
11	<i>Sieversingia tortuosum</i>	nd	5.769	nd	nd	nd	nd	nd	nd	nd	nd
12	<i>Racocetra gregaria</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
13	<i>Rhizophagus fasciculatus</i>	nd	nd	nd	nd	13.248	nd	nd	nd	nd	nd
14	<i>Rhizophagus irregularis</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
15	<i>Rhizophagus intraradices</i>	nd	21.154	44.872	nd	11.966	nd	nd	nd	nd	nd

Table 4.16b: Relative abundance of AM fungal species from wild tuber species at study sites S-XI to S-XX.

Sr. No.	AM fungal species	S-XI	S-XII	S-XIII	S-XIV	S-XV	S-XVI	S-XVII	S-XVIII	S-XIX	S-XX
1	<i>Acaulospora foveata</i>	18.447	nd	nd	nd	11.538	11.111	nd	10.294	nd	18.447
2	<i>Acaulospora delicata</i>	nd	28.244	nd	nd	15.385	nd	nd	13.235	nd	nd
3	<i>Acaulospora scrobiculata</i>	15.534	35.115	nd	nd	30.769	19.048	nd	23.529	21.622	15.534
4	<i>Acaulospora dilatata</i>	10.680	nd	nd	nd	9.615	nd	nd	nd	nd	10.680
5	<i>Claroideoglossum etunicatum</i>	nd	25.191	nd	nd	nd	nd	nd	nd	nd	nd
6	<i>Funneliformis geosporum</i>	nd	nd	nd	nd	nd	nd	17.460	11.765	37.838	nd
7	<i>Funneliformis mosseae</i>	17.961	nd	nd	26.154	nd	17.460	20.635	nd	29.730	17.961
8	<i>Gigaspora albida</i>	nd	nd	32.000	16.923	11.538	12.698	12.698	nd	nd	nd
9	<i>Gigaspora decipiens</i>	11.165	11.450	32.000	29.231	15.385	15.873	nd	13.235	nd	11.165
10	<i>Glomus</i> sp.	3.398	nd	nd	18.462	5.769	nd	nd	2.941	nd	3.398
11	<i>Sieverdingia tortusum</i>	2.427	nd	nd	nd	nd	nd	nd	nd	nd	2.427
12	<i>Racocetra gregaria</i>	6.311	nd	nd	9.231	nd	nd	nd	5.882	10.811	6.311
13	<i>Rhizophagus fasciculatus</i>	nd	nd	nd	nd	nd	nd	14.286	11.765	nd	nd
14	<i>Rhizophagus irregularis</i>	nd	nd	36.000	nd	nd	11.111	19.048	nd	nd	nd
15	<i>Rhizophagus intraradices</i>	14.078	nd	nd	nd	nd	12.698	15.873	7.353	nd	14.078

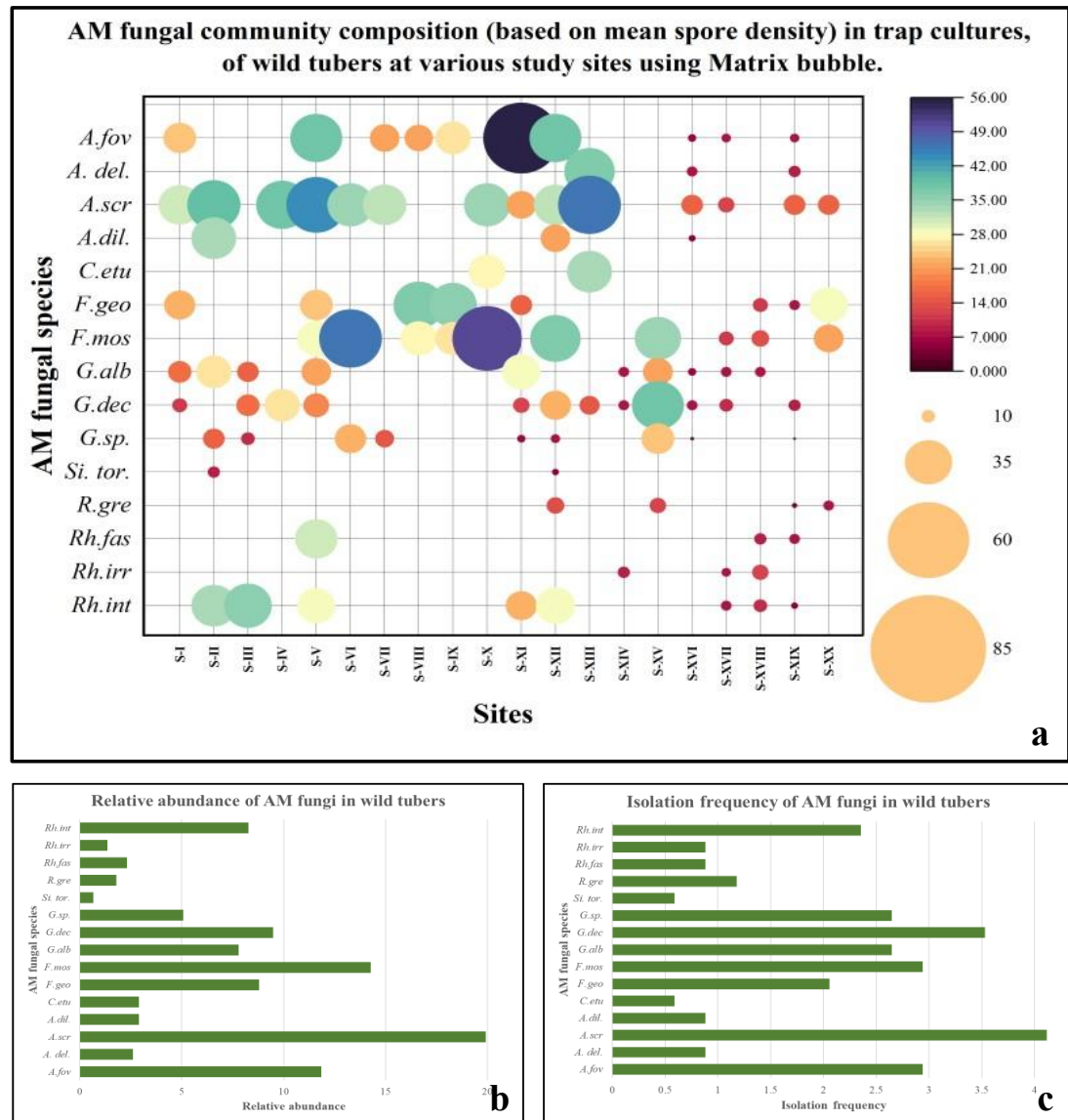


Fig. 4.1: AM fungal diversity in wild tuber species - a. AM fungal community composition (based on mean spore density) in trap cultures, of wild tuber species at various study sites, using Matrix bubble, **b.** Relative abundance of AM fungi in wild tuber species **c.** Isolation frequency of AM fungi in wild tuber species.

Fig. 4.2a. and **4.2b.** show the spatial distribution of spore density v/s species richness in wild and cultivated tuber species, respectively. The correlation between spore density and species richness is positive in both cases.

Fig. 4.2: Spatial distribution of spore density v/s species richness in wild and cultivated tuber species.

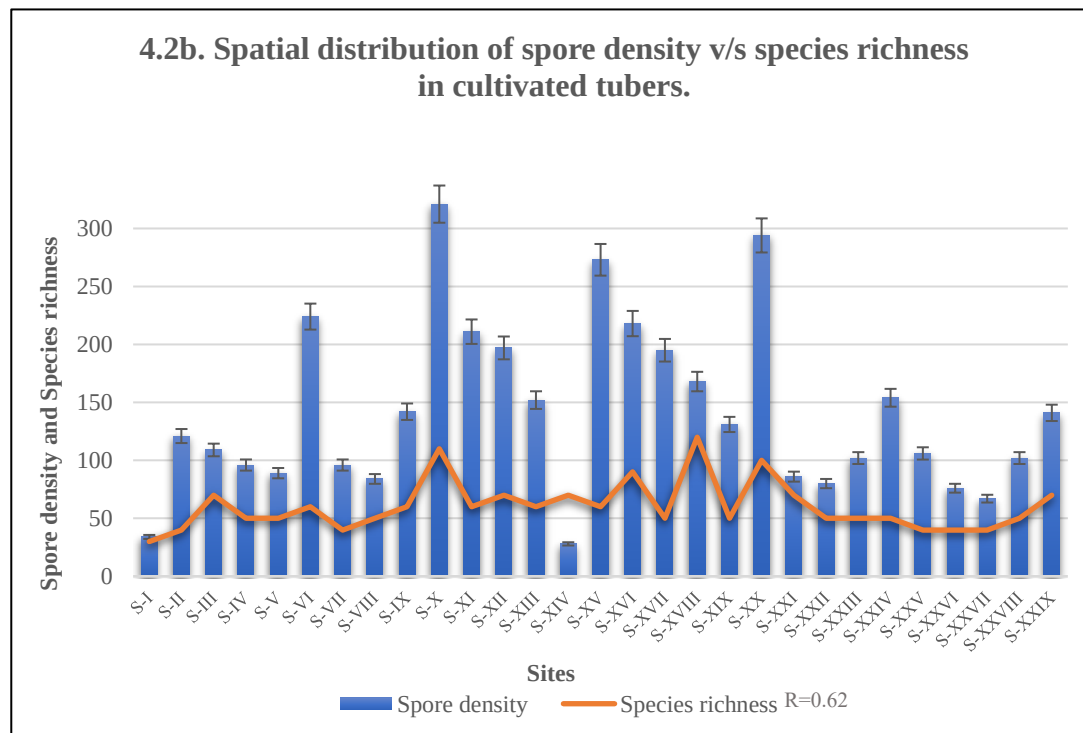
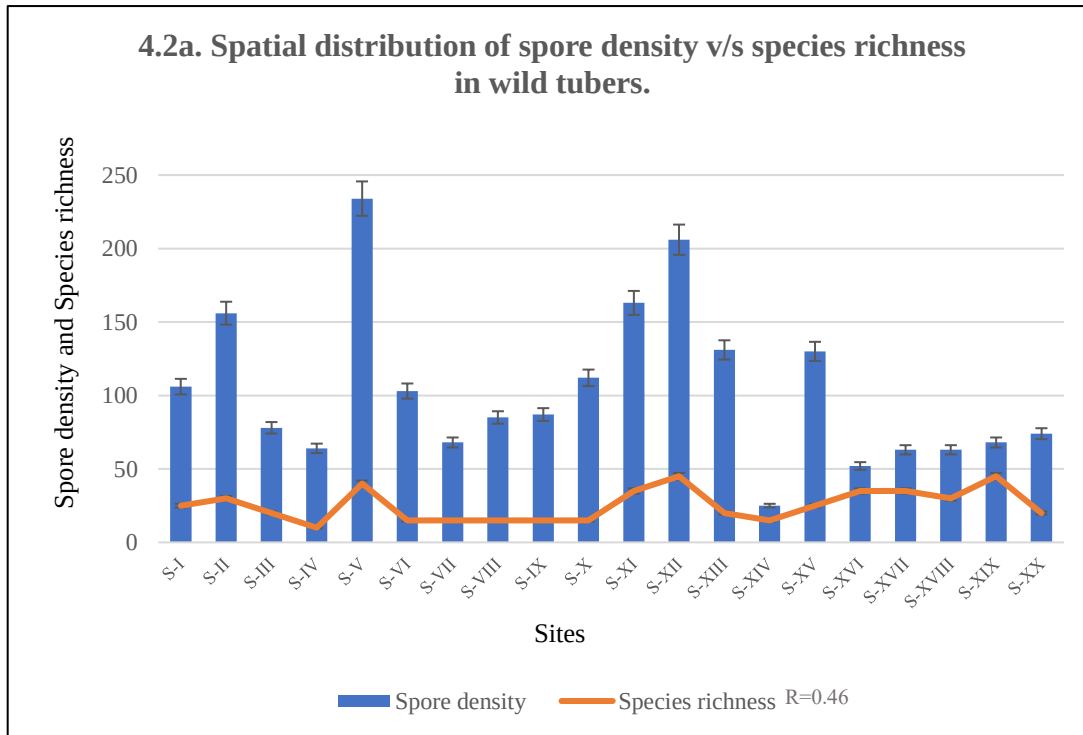


Fig. 4.3a shows the site-specific composition of the AM fungal community, depicted by the average spore density in trap cultures of the cultivated tuber species examined.

Fig. 4.3b illustrates the RA of the AM fungal species; the AM fungi with the highest RA were *A. scrobiculata* (22.14) and *Rh. intraradices* (12.29) and *F. mosseae* (10.70).

High isolation frequency (IF) (**Fig. 4.3c**) was expressed by *A. scrobiculata* (2.99), *A. foveata* (2.55), followed by *F. geosporum* and *Gi. decipiens* (2.39) (**Table 4.17**). The RA of individual AM fungal species from wild tuber species is given in **Table 4.18a**, **4.18b** and **4.18c**.

Table 4.17: Relative abundance, isolation frequency, and frequency of occurrence of AM fungal species.

Sr. No.	AM fungal species	RA %	IF %	Frequency of occurrence
1	<i>Acaulospora bireticulata</i>	0.10	0.15	01
2	<i>Acaulospora foveata</i>	7.46	2.55	17
3	<i>Acaulospora mellea</i>	0.15	0.30	02
4	<i>Acaulospora dilatata</i>	2.80	0.75	05
5	<i>Acaulospora delicata</i>	3.44	0.90	06
6	<i>Acaulospora scrobiculata</i>	22.14	2.99	20
7	<i>Acaulospora laevis</i>	0.05	0.15	01
8	<i>Acaulospora spinosa</i>	3.54	1.35	09
9	<i>Claroideoglossum etunicatum</i>	0.29	0.15	01
10	<i>Funneliformis coronatum</i>	0.24	0.30	02
11	<i>Funneliformis geosporum</i>	9.00	2.40	16
12	<i>Funneliformis mosseae</i>	10.70	2.25	15
13	<i>Gigaspora albida</i>	6.56	2.25	15
14	<i>Gigaspora decipiens</i>	5.41	2.40	16
15	<i>Glomus multisporum</i>	0.10	0.15	01
16	<i>Glomus microcarpum</i>	0.88	0.15	01
17	<i>Glomus</i> sp.	1.15	0.90	06
18	<i>Racocetra gregaria</i>	2.37	0.90	06
19	<i>Rhizophagus fasciculatus</i>	5.93	1.50	10
20	<i>Rhizophagus irregularis</i>	5.24	1.50	10
21	<i>Rhizophagus intraradices</i>	12.29	1.95	13
22	<i>Scutellospora calospora</i>	0.07	0.15	01
23	<i>Sacculospora felinonii</i>	0.10	0.15	01

Table 4.18a: Relative abundance of AM fungal species from cultivated tuber species at study sites S-I to S-X.

Sr. No.	AM fungal species	S-I	S-II	S-III	S-IV	S-V	S-VI	S-VII	S-VIII	S-IX	S-X
1	<i>Acaulospora bireticulata</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
2	<i>Acaulospora foveata</i>	nd	nd	15.596	nd	nd	16.071	29.167	nd	16.197	8.100
3	<i>Acaulospora mellea</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
4	<i>Acaulospora dilatata</i>	nd	28.099	nd	nd	nd	nd	nd	nd	nd	10.592
5	<i>Acaulospora delicata</i>	32.353	nd	nd	nd	nd	nd	nd	nd	nd	8.723
6	<i>Acaulospora scrobiculata</i>	nd	nd	nd	nd	nd	28.125	nd	nd	32.394	17.134
7	<i>Acaulospora laevis</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
8	<i>Acaulospora spinosa</i>	nd	nd	nd	22.917	19.101	13.839	nd	nd	8.451	3.427
9	<i>Claroideoglossum etunicatum</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
10	<i>Funneliformis coronatum</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
11	<i>Funneliformis geosporum</i>	nd	nd	21.101	27.083	25.843	19.196	29.167	nd	15.493	10.280
12	<i>Funneliformis mosseae</i>	nd	nd	22.936	18.750	31.461	nd	23.958	nd	18.310	17.757
13	<i>Gigaspora albida</i>	nd	25.620	10.092	nd	13.483	15.625	nd	14.286	nd	4.984
14	<i>Gigaspora decipiens</i>	47.059	19.835	11.009	8.333	10.112	7.143	nd	20.238	9.155	nd
15	<i>Glomus multisporum</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
16	<i>Glomus microcarpum</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
17	<i>Glomus</i> sp.	20.588	nd	8.257	nd	nd	nd	nd	nd	nd	2.181
18	<i>Racocetra gregaria</i>	nd	26.446	nd	nd	nd	nd	nd	9.524	nd	7.477
19	<i>Rhizophagus fasciculatus</i>	nd	nd	11.009	nd	nd	nd	nd	25.000	nd	9.346
20	<i>Rhizophagus irregularis</i>	nd	nd	nd	22.917	nd	nd	17.708	30.952	nd	nd
21	<i>Rhizophagus intraradices</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
22	<i>Scutellospora calospora</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
23	<i>Sacculospora felinonii</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Table 4.18b: Relative abundance of AM fungal species from cultivated tuber species at study sites S-XI to S-XX.

Sr. No.	AM fungal species	S-XI	S-XII	S-XIII	S-XIV	S-XV	S-XVI	S-XVII	S-XVIII	S-XIX	S-XX
1	<i>Acaulospora bireticulata</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
2	<i>Acaulospora foveata</i>	5.213	17.259	15.789	7.143	13.553	3.211	nd	5.357	nd	nd
3	<i>Acaulospora mellea</i>	nd	nd	nd	nd	nd	nd	nd	1.190	nd	1.361
4	<i>Acaulospora dilatata</i>	nd	nd	nd	nd	nd	nd	nd	12.500	16.031	nd
5	<i>Acaulospora delicata</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
6	<i>Acaulospora scrobiculata</i>	36.019	25.381	22.368	35.714	24.908	27.982	30.256	19.643	34.351	17.347
7	<i>Acaulospora laevis</i>	nd	nd	nd	nd	nd	nd	nd	1.190	nd	nd
8	<i>Acaulospora spinosa</i>	nd	nd	nd	nd	nd	nd	nd	3.571	nd	nd
9	<i>Claroideoglossum etunicatum</i>	nd	nd	nd	nd	nd	nd	nd	7.143	nd	nd
10	<i>Funneliformis coronatum</i>	nd	nd	nd	nd	nd	nd	nd	2.381	nd	nd
11	<i>Funneliformis geosporum</i>	17.062	15.736	17.763	10.714	nd	4.128	nd	5.357	6.107	9.184
12	<i>Funneliformis mosseae</i>	31.280	23.858	25.658	21.429	12.088	4.587	nd	7.143	nd	7.483
13	<i>Gigaspora albida</i>	nd	nd	nd	10.714	15.751	5.963	6.154	nd	nd	7.143
14	<i>Gigaspora decipiens</i>	2.844	4.569	7.237	7.143	nd	5.046	nd	nd	nd	6.122
15	<i>Glomus multisporum</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	1.361
16	<i>Glomus microcarpum</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	12.245
17	<i>Glomus</i> sp.	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
18	<i>Racocetra gregaria</i>	nd	nd	nd	7.143	nd	nd	nd	nd	9.160	nd
19	<i>Rhizophagus fasciculatus</i>	nd	7.107	11.184	nd	8.791	11.927	16.410	14.286	nd	14.626
20	<i>Rhizophagus irregularis</i>	7.583	nd	nd	nd	nd	13.303	18.462	nd	nd	nd
21	<i>Rhizophagus intraradices</i>	nd	6.091	nd	nd	24.908	23.853	28.718	20.238	34.351	23.129
22	<i>Scutellospora calospora</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
23	<i>Sacculospora felinonii</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

Table 4.18c: Relative abundance of AM fungal species from cultivated tuber species at study sites S-XXI to S-XXIX.

Sr. No.	AM fungal species	S-XXI	S-XXII	S-XXIII	S-XXIV	S-XXV	S-XXVI	S-XXVII	S-XXVIII	S-XXIX
1	<i>Acaulospora bireticulata</i>	4.444	nd	nd	nd	nd	nd	nd	nd	nd
2	<i>Acaulospora foveata</i>	nd	nd	9.804	13.636	6.604	6.579	nd	8.824	nd
3	<i>Acaulospora mellea</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd
4	<i>Acaulospora dilatata</i>	nd	6.250	nd	nd	nd	nd	nd	nd	nd
5	<i>Acaulospora delicata</i>	nd	nd	22.549	20.779	22.642	nd	nd	nd	16.312
6	<i>Acaulospora scrobiculata</i>	30.000	42.500	32.353	35.065	36.792	40.789	nd	nd	27.660
7	<i>Acaulospora laevis</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd
8	<i>Acaulospora spinosa</i>	nd	nd	4.902	nd	nd	nd	nd	28.431	8.511
9	<i>Claroideoglossum etunicatum</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd
10	<i>Funneliformis coronatum</i>	6.667	nd	nd	nd	nd	nd	nd	nd	nd
11	<i>Funneliformis geosporum</i>	nd	nd	nd	nd	nd	nd	nd	20.588	nd
12	<i>Funneliformis mosseae</i>	nd	nd	nd	nd	nd	nd	nd	nd	19.149
13	<i>Gigaspora albida</i>	13.333	nd	nd	nd	nd	10.526	20.896	25.490	nd
14	<i>Gigaspora decipiens</i>	nd	nd	nd	nd	nd	nd	25.373	nd	23.404
15	<i>Glomus multisporum</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd
16	<i>Glomus microcarpum</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd
17	<i>Glomus</i> sp.	3.333	5.000	nd	nd	nd	nd	nd	16.667	nd
18	<i>Racocetra gregaria</i>	nd	nd	nd	12.338	nd	nd	nd	nd	nd
19	<i>Rhizophagus fasciculatus</i>	nd	nd	nd	nd	nd	nd	nd	nd	nd
20	<i>Rhizophagus irregularis</i>	13.333	11.250	nd	18.182	nd	nd	29.851	nd	nd
21	<i>Rhizophagus intraradices</i>	28.889	35.000	30.392	nd	33.962	42.105	23.881	nd	nd
22	<i>Scutellospora calospora</i>	nd	nd	nd	nd	nd	nd	nd	nd	2.128
23	<i>Sacculospora felinonii</i>	nd	nd	nd	nd	nd	nd	nd	nd	2.837

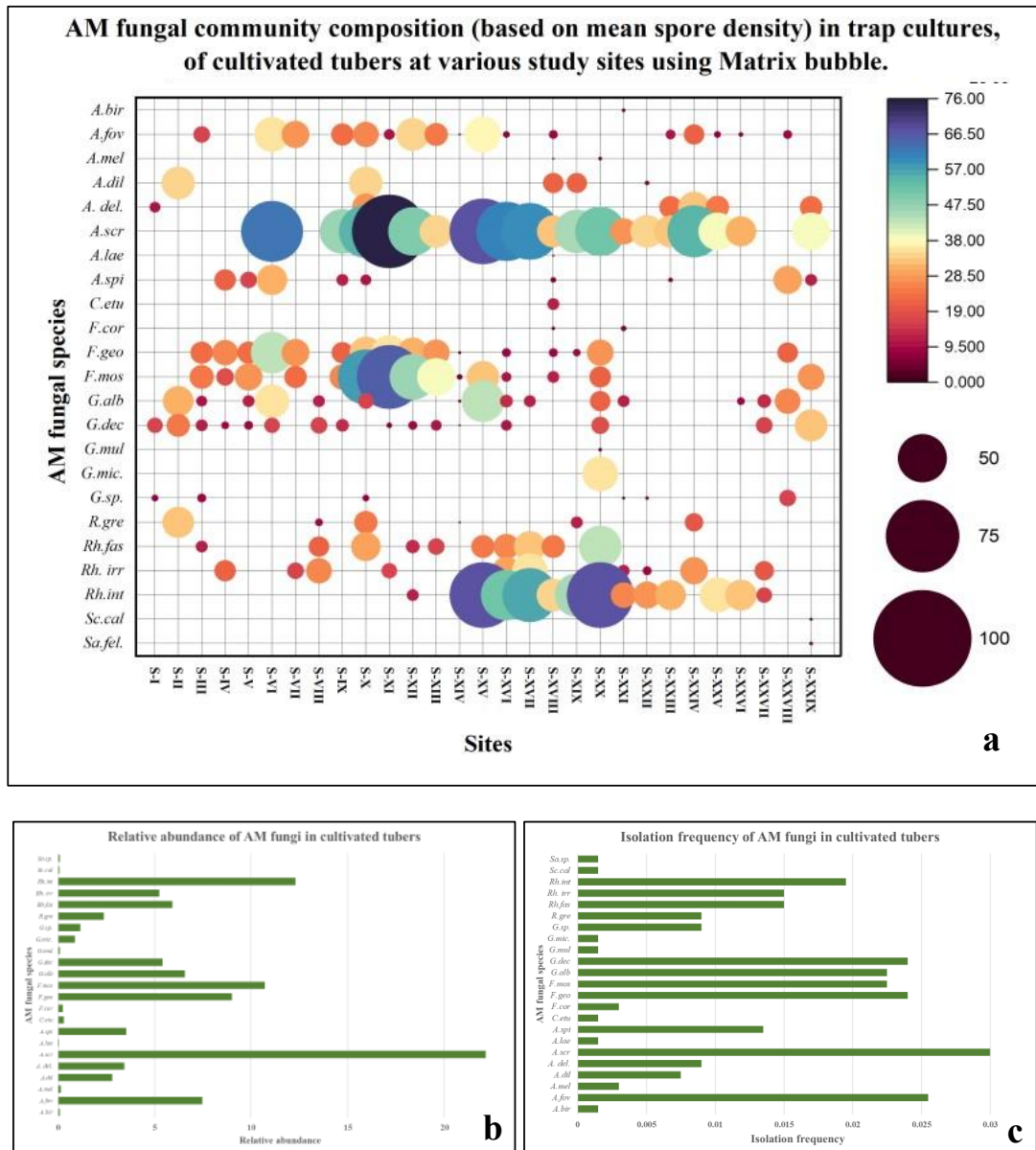
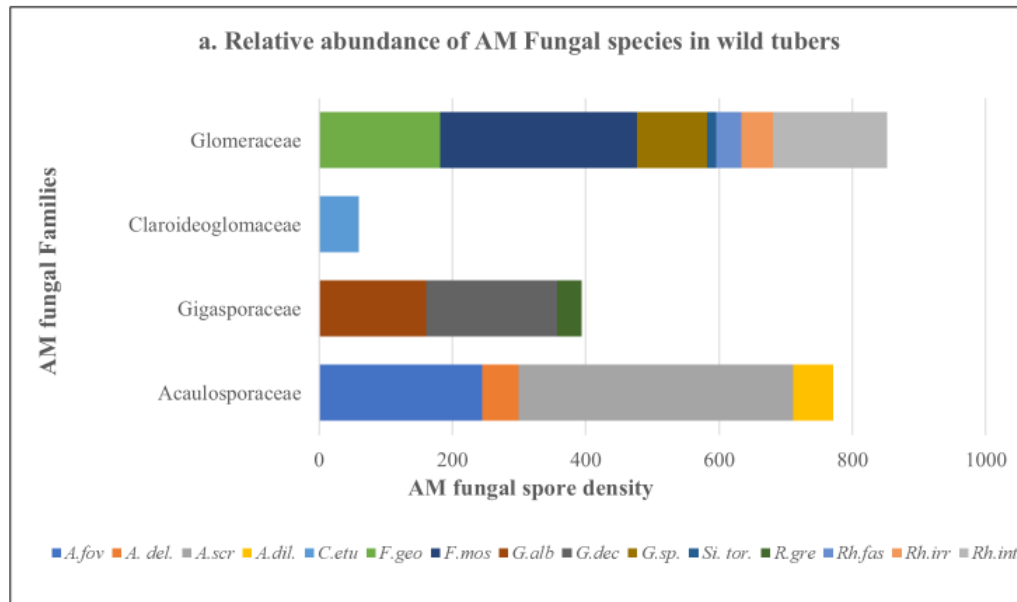
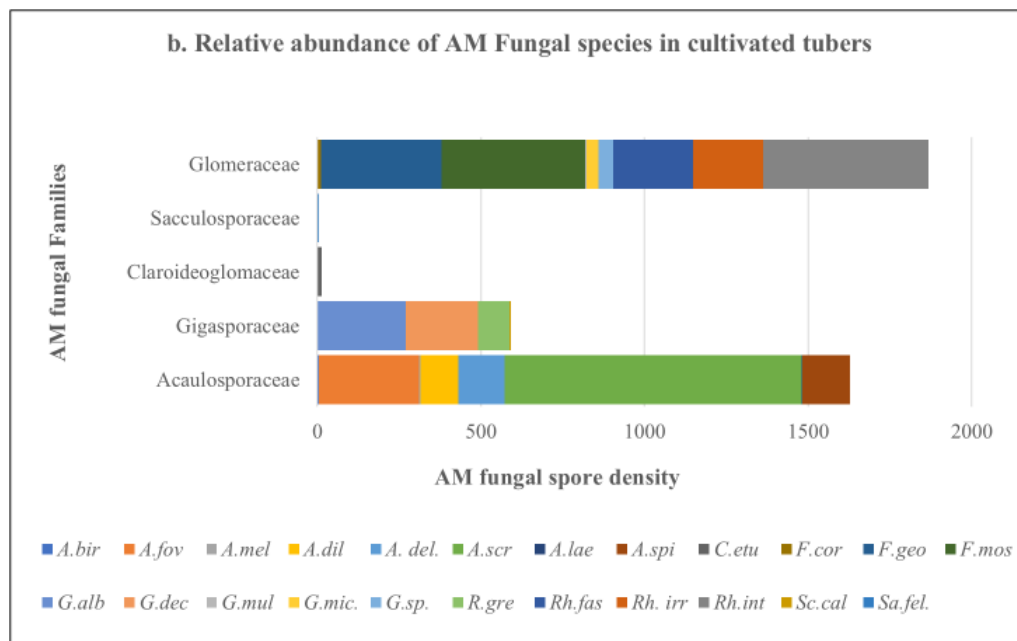


Fig. 4.3: AM fungal diversity in cultivated tuber species - a. AM fungal community composition (based on mean spore density) in trap cultures of cultivated tuber species. at various study sites, using Matrix bubble, **b.** Relative abundance of AM fungi in cultivated tuber species **c.** Isolation frequency of AM fungi in cultivated tuber species.

Fig. 4.4 shows the relative abundance of the AM fungal families. In the wild tuber species studied, **Fig. 4.4a**, it demonstrates the predominance of families in the following order: Glomeraceae > Acaulosporaceae > Gigasporaceae > Claroideoglomaceae; also, a similar pattern of dominance is observed in the RA of the families of AM fungi isolated from the cultivated tuber species (**Fig. 4.4b**). Amongst cultivated tuber species, the least dominant family is Sacculosporaceae. AM fungal communities in agricultural soils are known to be typically characterized by the dominance of Glomeraceae (Mathimaran *et al.*, 2005; Adenan *et al.*, 2021). Similarly, Stürmer *et al.* (2018) reported that members of the family Glomeraceae are present in all biomes, where they frequently dominate. According to Giovannetti (2010), *Acaulospora* species predominantly thrive in acidic soils, which explains the family Acaulosporaceae's dominance.



Legend : *Acaulospora delicata*- *A. del.*, *A. dilatata*- *A. dil.*, *A. foveata*- *A. fov.*, *A. scrobiculata*- *A. scr.*, *Claroideoglomus etunicatum*- *C. etu.*, *Funneliformis geosporum*- *F. geo.*, *F. mosseae*- *F. mos.*, *Gigaspora albida*- *Gi. alb.*, *Gi. decipiens*- *Gi. dec.*, *Glomus sp.*- *G. sp.*, *Racocetra gregaria*- *R. gre.*, *Rhizophagus fasciculatus*- *Rh. fas.*, *Rh. intraradices*- *Rh. int.*, *Rh. irregularis*- *Rh. irr.*, *Sieversingia tortuosa* - *Si. tor.*



Legend : *Acaulospora bireticulata*- *A. bir.*, *A. delicata*- *A. del.*, *A. dilatata*- *A. dil.*, *A. foveata*- *A. fov.*, *A. laevis*- *A. lae.*, *A. scrobiculata*- *A. scr.*, *A. spinosa*- *A. spi.*, *Claroideoglomus etunicatum*- *C. etu.*, *Funneliformis coronatum*- *F. cor.*, *F. geosporum*- *F. geo.*, *F. mosseae*- *F. mos.*, *Gigaspora albida*- *G. alb.*, *G. decipiens*- *G. dec.*, *Glomus microcapum*- *G. mic.*, *G. multisporum*- *G. mul.*, *Glomus sp.*- *G. sp.*, *Racocetra gregaria*- *R. gre.*, *Rhizophagus fasciculatus*- *Rh. fas.*, *Rh. intraradices*- *Rh. int.*, *Rh. irregularis*- *Rh. irr.*, *Sacculospora felinonii*- *Sa. fel.*, *Scutellospora calospora*- *Sc. cal.*

Fig. 4.4: Relative abundance of AM Fungal species in wild and cultivated tuber species - a. Relative abundance of AM fungal species in wild tuber species, b. Relative abundance of AM fungal species in cultivated tuber species.

Table 4.19: Spore density (SD) and diversity of AM fungi from wild and cultivated tubers from Goa.

Tuber species	Spore density*	AM fungal species
<u>Wild tuber species</u>		
<i>Alocasia macrorrhiza</i>	113.33±1.45	<i>A. dil.</i> , <i>A. fov.</i> , <i>A. scr.</i> , <i>F. geo.</i> , <i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>Gl. sp.</i> , <i>Rh. int.</i> , <i>Si. tor.</i>
<i>Amorphophallus commutatus</i>	68.6±2.31	<i>A. fov.</i> , <i>A. scr.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>Gl. sp.</i> , <i>Rh. fas.</i> , <i>Rh. int.</i>
<i>Colocasia esculenta</i>	107.66±1.31	<i>A. del.</i> , <i>A. dil.</i> , <i>A. fov.</i> , <i>A. scr.</i> , <i>C. etu.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>Gl. sp.</i> , <i>R. gre.</i> , <i>Rh. int.</i> , <i>Rh. irr.</i> , <i>Si. tor.</i>
<i>Gloriosa superba</i>	65.50±0.33	<i>A. del.</i> , <i>A. fov.</i> , <i>A. scr.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>Gl. sp.</i> , <i>Rh. fas.</i> , <i>R. gre.</i> , <i>Rh. int.</i> , <i>Rh. irr.</i>
<i>Momordica sahyadrica</i>	74.00±0.57	<i>A. scr.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>R. gre.</i>
<u>Cultivated tuber species</u>		
<i>Amorphophallus paeoniifolius</i>	34.45±4.5	<i>A. del.</i> , <i>Gi. dec.</i> , <i>Gl. sp.</i>
<i>Colocasia antiquorum</i>	99.67±1.60	<i>A. dil.</i> , <i>A. fov.</i> , <i>A. spi.</i> , <i>C. etu.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>Gi. alb.</i> , <i>Gl. sp.</i> , <i>Rh. fas.</i> , <i>R. gre.</i> , <i>Rh. irr.</i>
<i>Dioscorea alata</i>	136.33±1.33	<i>A. fov.</i> , <i>A. scr.</i> , <i>A. spi.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>Rh. irr.</i>
<i>Dioscorea bulbifera</i>	113.00±1.60	<i>A. fov.</i> , <i>A. scr.</i> , <i>A. spi.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>Rh. fas.</i> , <i>R. gre.</i> , <i>Rh. irr.</i>
<i>Dioscorea esculenta</i>	181.80±3.20	<i>A. del.</i> , <i>A. dil.</i> , <i>A. scr.</i> , <i>A. spi.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>Gl. sp.</i> , <i>Rh. fas.</i> , <i>R. gre.</i> , <i>Rh. int.</i> , <i>Rh. irr.</i>
<i>Ipomoea batatas</i>	156.91±1.80	<i>A. bir.</i> , <i>A. del.</i> , <i>A. dil.</i> , <i>A. fov.</i> , <i>A. lae.</i> , <i>A. mel.</i> , <i>A. scr.</i> , <i>A. spi.</i> , <i>C. etu.</i> , <i>F. cor.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>Gl. mic.</i> , <i>Gl. mul.</i> , <i>Gl. sp.</i> , <i>Rh. fas.</i> , <i>R. gre.</i> , <i>Rh. int.</i> , <i>Rh. irr.</i>
<i>Solenostemon rotundifolius</i>	103.33±1.85	<i>A. del.</i> , <i>A. fov.</i> , <i>A. scr.</i> , <i>A. spi.</i> , <i>F. geo.</i> , <i>F. mos.</i> , <i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>Gl. sp.</i> , <i>Rh. int.</i> , <i>Rh. irr.</i> , <i>Sc. cal.</i> , <i>Sa. fel.</i>

Legend: *Spore density of Spores/100g of soil. All values are the mean of three readings from each site; ±Standard error. *Acaulospora bireticulata*- *A. bir*, *Acaulospora foveata*- *A. fov*; *A. scrobiculata* - *A. scr*; *A. delicata*- *A. del.*, *A. dilatata*- *A. dil.* *Acaulospora laevis*- *A. lae*, *Claroideoglosum etunicatum*- *C. etu*; *Funneliformis coronatum*- *F. cor*, *Funneliformis geosporum*- *F. geo*; *Funneliformis mosseae*- *F. mos*; *Gigaspora albida*- *Gi. alb*; *Gigaspora decipiens*- *Gi. dec*; *Glomus microcarpum*- *G. mic*, *Glomus sp.*- *Gl. sp.*; *Sieverdingia tortuosa*- *Si. tor*; *Racocetra gregaria*- *R. gre*; *Rhizophagus fasciculatus*- *Rh. fas*; *Rhizophagus irregularis*- *Rh. irr* ; *Rhizophagus intraradices*- *Rh. int*, *Sacculospora felinonii*- *Sa. fel.* *Scutellospora calospora*- *Sc. cal.*

Table 4.19 shows the overall Spore density (SD) and diversity of AM fungi from wild and cultivated tubers from Goa. Among the wild tubers, *Alocasia macrorrhiza* showed the highest overall spore density (113.33 ± 1.45) and the least spore density was recorded in *Gloriosa superba* (65.50 ± 0.33). Among the cultivated tubers, *Dioscorea esculenta* showed the highest overall spore density (181.80 ± 3.20) and the least spore density was recorded in *Amorphophallus paeoniifolius* (34.45 ± 4.5).

While knowledge gaps persist regarding AM fungal communities in tubers and yams, especially in lesser yam roots (Nuryana *et al.*, 2020), this study addresses them by investigating AM fungal diversity across wild and cultivated tuber crop species, including *D. esculenta*, in Goa.

The abundance of AM fungi in plant roots correlates strongly with soil physical and chemical properties, enabling these microbes to enhance nutrient solubility and soil quality, thereby supporting optimal plant growth under favourable conditions. In tuber crops, slightly acidic soils with pH values of 5 to 6 maximize nutrient availability while enhancing the capacity of AM fungi and other beneficial microbes to solubilize nutrients (Nuryana *et al.*, 2020). This study confirms the presence of such conditions at all sampling sites, as evidenced by healthy AM fungal communities, underscoring their vital role in sustainable agriculture for tuber crop productivity and soil health.

Previous research consistently identifies *Acaulospora* and *Glomus* as the dominant AM fungal genera associated with yam roots (Janoušková *et al.*, 2015; Nuryana *et al.*, 2020). Our study corroborates these findings, reinforcing their prevalence and critical contributions to sustainable yam cultivation through enhanced nutrient uptake and soil symbiosis.

The Shannon Weiner (H) index for both the wild tuber species (2.39) and the cultivated tuber species (2.43), indicating that the community has a good diversity, with multiple species. The Simpson's index for the wild tuber species (0.89) and cultivated tuber species (0.88) suggested a slight dominance of some species. Species evenness for wild tuber species (0.89) and cultivated tuber species (0.78) indicated a fair distribution of individuals across species, suggesting a stable ecosystem (**Table 4.20**).

Table 4.20: Diversity of AMF communities from wild and cultivated tuber species.

Ecological parameters	Wild	Cultivated
Shannon- Wiener index of diversity (H)	2.39	2.43
Simpson`s index of dominance (D)	0.89	0.88
AMF species evenness (E)	0.89	0.78
AMF species richness (SR)	15	23

The Canonical Correspondence Analysis (CCA) plot for wild tuber species (**Fig. 4.5**) revealed strong structuring of community composition along two primary constrained environmental gradients represented by Axis 1 and Axis 2 (Figure X). The ordination indicates that variation in species distribution across sites is tightly linked to soil chemical properties, particularly organic matter status, nutrient availability, and metal concentrations.

The CCA plot for cultivated tuber species (**Fig. 4.6**) revealed clear structuring of community composition along two primary constrained environmental gradients (Axis 1 and Axis 2), reflecting major soil chemical controls on species distribution. The environmental vectors indicate that the ordination is strongly governed by gradients in organic fertility, nutrient availability, metal concentrations, soil acidity, and iron content.

Both the CCA plots of wild (**Fig. 4.5**) and cultivated (**Fig. 4.6**) tuber species showed that species scores are largely concentrated near the ordination origin, indicating that most taxa exhibit broad ecological tolerance and respond weakly to individual environmental gradients. This pattern suggests that community composition is shaped by the combined influence of multiple soil chemical factors rather than strong specialization along a single gradient.

From the CCA plots, it may be concluded that community composition is strongly controlled by soil chemical gradients structured around the observed core set of variables.

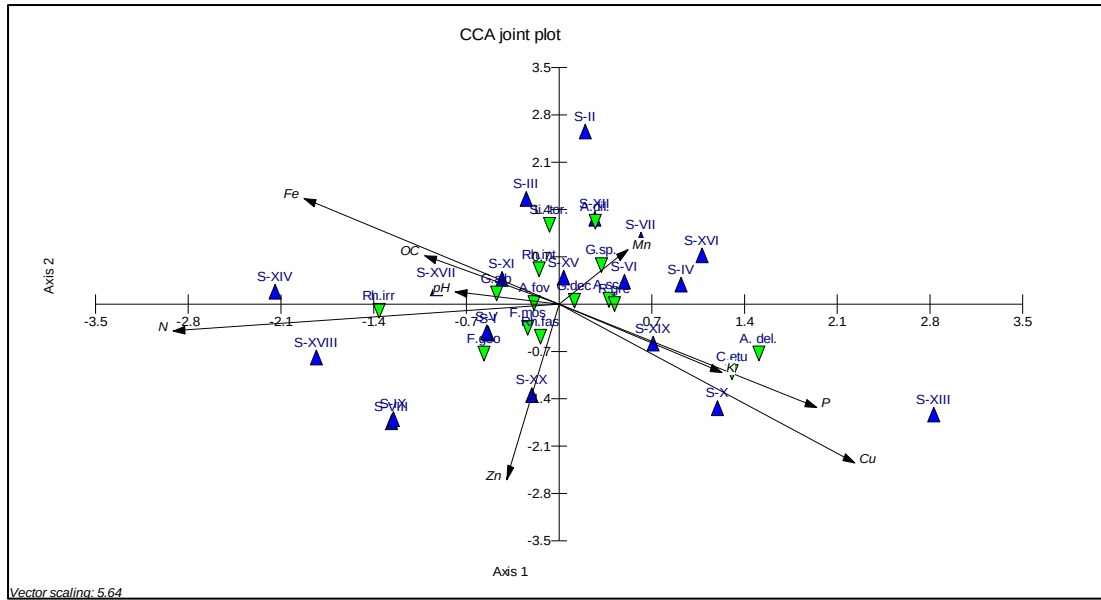


Fig. 4.5: Canonical Correspondence Analysis (CCA) joint biplot illustrating the relationships between community composition and soil environmental variables across sampling sites of wild tuber species.

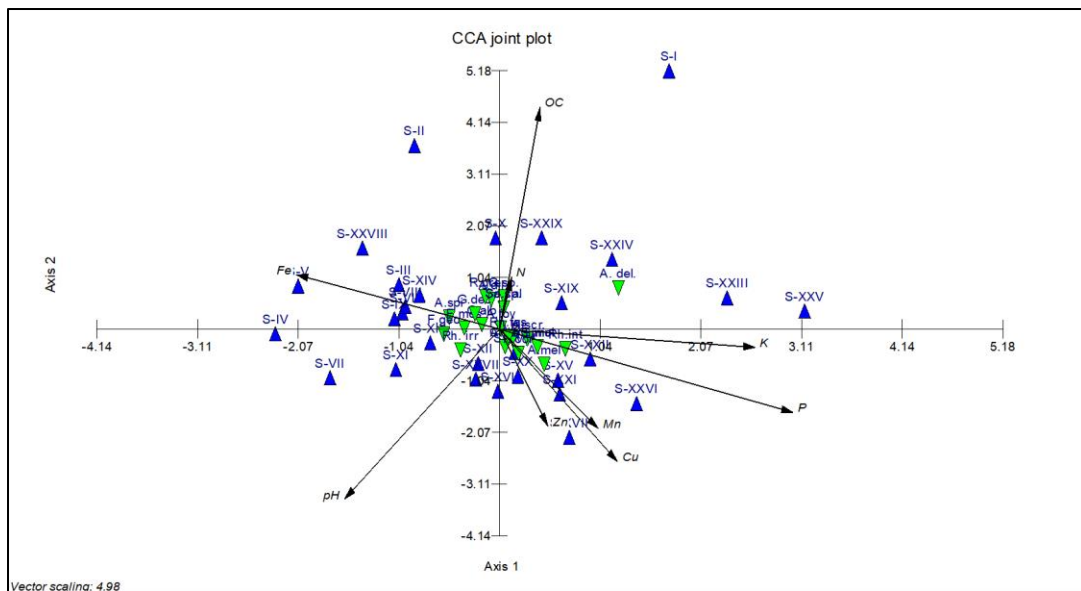


Fig. 4.6: Canonical Correspondence Analysis (CCA) joint biplot illustrating the relationships between community composition and soil environmental variables across sampling sites of cultivated tuber species.

4.4: CONCLUSION

Studies on arbuscular mycorrhizal (AM) fungal diversity provide critical insights into the functioning of terrestrial ecosystems by elucidating the interactions between AM

fungi and their environment. Such assessments also serve as ecological indicators, reflecting soil properties and climatic conditions through the presence and distribution of specific AM fungal species.

The present investigation reveals that AM fungal assemblages associated with tuber crops are highly diverse, well-distributed, and structurally stable across both wild and cultivated systems. A substantial overlap in dominant taxa, particularly *A. scrobiculata*, *F. mosseae*, and *Rh. intraradices*, indicates the presence of a resilient core mycorrhizal community adapted to tuberous hosts, while the occurrence of taxa such as *A. foveata* and *Gi. decipiens* in wild tubers and *F. geosporum* in cultivated tubers reflects habitat-specific ecological filtering. The consistent predominance of the family Glomeraceae, followed by Acaulosporaceae, Gigasporaceae, and Claroideoglomaceae, further underscores a stable taxonomic structure across contrasting management conditions. High Shannon Wiener diversity values, coupled with moderately high Simpson's dominance and favourable evenness indices, collectively demonstrate species-rich communities with slight dominance yet balanced distribution, indicative of ecologically robust and functionally diverse AM fungal networks.

Canonical Correspondence Analysis confirms that AM fungal community composition is not driven by single edaphic variables but is instead structured by interacting soil chemical gradients, emphasizing the multivariate nature of environmental control on fungal assemblage organization. This integrated response to soil chemistry highlights the adaptive breadth of AM fungi and their capacity to persist across heterogeneous soil environments.

The identification of a P-solubilizing *Pseudomonas* sp. as a complementary bio-inoculant further strengthens the applied significance of these findings. Importantly, a comprehensive understanding of AM fungal diversity and community structure in tuber cropping systems provides a powerful foundation for developing biologically based soil management strategies. Harnessing native and functionally dominant AM fungi, particularly in combination with beneficial PSBs, offers substantial potential to improve nutrient acquisition, enhance soil health, and promote sustainable tuber production with reduced reliance on chemical inputs.

Chapter 5: To study AM status of plants with tubers selected for the study as influenced by phenology.

5.1: INTRODUCTION

Tuber crops, including cassava, sweet potato, yams, and taro, are essential staple foods for approximately 700 million people in Africa, Asia, and Latin America, providing vital calories and nutrients such as starch, sugars, proteins, and minerals. These crops are the third most important food category globally, after cereals and grain legumes. Their resilience enables them to thrive in poor soil and challenging weather. Tubers have also obtained the status of a "health food." Furthermore, tuber cultivation supports local economies by generating income for smallholder farmers. (Laxminarayana, 2016).

Colocasia esculenta, commonly known as taro, is an important root vegetable belonging to the Araceae family and has a broad distribution in tropical and subtropical regions. (Mabhaudhi, 2013). *Colocasia esculenta* is recognized as a significant botanical entity in herbal medicine, highlighting its potential for pharmaceutical advancements owing to its diverse pharmacological properties and nutrient-dense composition. (Keerthy and Joshi, 2019).

Taro is a staple crop in developing regions of Asia, Africa, and Central America, with substantial potential to enhance food security and rural economic development. Nutritionally, taro offers a high carbohydrate content (approximately twice that of potatoes) and moderate protein (about 11% on a dry weight basis), along with essential micronutrients such as vitamin C, Fe, Ca, and Zn. The leaves are particularly rich in protein, complementing the starchy corm. Health implications include favourable digestibility, suitability for infant foods, hypoallergenic properties, and potential to mitigate malnutrition and zinc deficiency. Dietary fibre contributes to gastrointestinal health and may help manage metabolic conditions such as diabetes and hypertension. Taro contains oxalates and other anti-nutritional factors that can cause irritation if not properly processed; thermal treatment (boiling) and soaking reduce these compounds, improving safety. Culinary and industrial processing yields diverse products (boiled, roasted, fried, flour, chips) and can extend shelf life, supporting food security during shortages. Economically, taro functions as a cash crop that sustains rural livelihoods

and provides affordable nutrition in resource-limited settings (Temesgen and Retta, 2015; Kaushal *et al.*, 2015).

The AM fungal community and network are influential determinants of plant performance, with seasonal dynamics evident across the growing season. Connectivity between AM fungi and plants is increasing over time, and associations among AM fungi and plant species are evolving, likely reflecting shifts in biotic interactions and abiotic conditions (Ji *et al.*, 2021; Bennett *et al.*, 2013). Fungi remain the primary mediators of plant-derived carbon in inland habitats, though their responses to seasonal changes are not well understood (Voříšková *et al.*, 2014). In the mixed plantation, the AM fungal network structure varies seasonally, with clear temporal variation in the AM fungal community (Ji *et al.*, 2021).

Few studies have addressed seasonal effects on AM fungi, but none have reported seasonal dynamics in tuber crops. This study aims to determine patterns of AM colonization, spore density, and species richness across seasons in *Colocasia esculenta*.

5.2: MATERIALS AND METHODS

Study sites and sample collection: Two study sites, viz., Margao (Lat 15.289171° Long 73.980543°) and Vasco (Lat 15.376483° Long 73.852734°), were selected for the study. The wild tuber crop, *Colocasia esculenta*, common to both sites, was used for the study.

The two sites were sampled for rhizosphere soil in a randomized design across three seasons: pre-monsoon (March–May 2023), monsoon (July–September 2023), and post-monsoon (October 2023–February 2024). Collected samples were sealed in polyethylene bags, transported to the laboratory, and kept at 4°C before processing. Roots were carefully separated from adhering soil, washed under running tap water, and used for the estimation of AM colonization. Rhizosphere soil from individual plants was divided into two fractions: (i) for isolation, quantification, and identification of AM spores, and (ii) as inoculum for trap culture experiments.

Soil analyses were performed following the procedures described in Chapter 3, the estimation of arbuscular mycorrhizal (AM) fungal root colonization was conducted according to the methods outlined in Chapter 3, trap culture, isolation, and taxonomic identification of AM fungal spores were performed as described in Chapter 4, and

diversity metrics, including Simpson's Diversity Index, Shannon Diversity Index, species evenness, and species richness, were calculated as described in Chapter 4.

Statistical analysis: Pearson's correlation coefficient was calculated to assess the relationships between spore density and species richness at each site, using WASP software (Web Based Agricultural Package) 2.0 ($P \leq 0.05$). Relative abundance of AM fungal species common to all seasons was correlated with soil pH, P and EC ($P \leq 0.05$). Data on seasons and host co-affect the AM fungal spore density, species richness, and Shannon-Weiner diversity index were analyzed.

5.3: RESULTS AND DISCUSSION

Soil analyses: Results of the soil physico-chemical analyses are shown in **Table 8**. The soil was acidic to near-neutral (pH 5.5-6.8) at both sites. Electrical Conductivity (EC) ranged from 4.03 to 8.49 dSm⁻¹. Soil organic carbon (OC) was higher in the pre-monsoon season at both study sites. The K levels were sufficient or higher at both sites. However, the soils at both sites had lower levels of P and N, while the levels of micronutrients, viz., Cu, Zn, Mn, and Fe, varied at the two study sites.

AM fungal root colonization: The results of AM fungal root colonization are summarized in **Table 5.1; Figs. 5.1 & 5.2**. The AM fungal colonization recorded the presence of hyphae, vesicles, and arbuscules. In *C. esculenta*, seasonal variations in AM fungal colonization were observed.

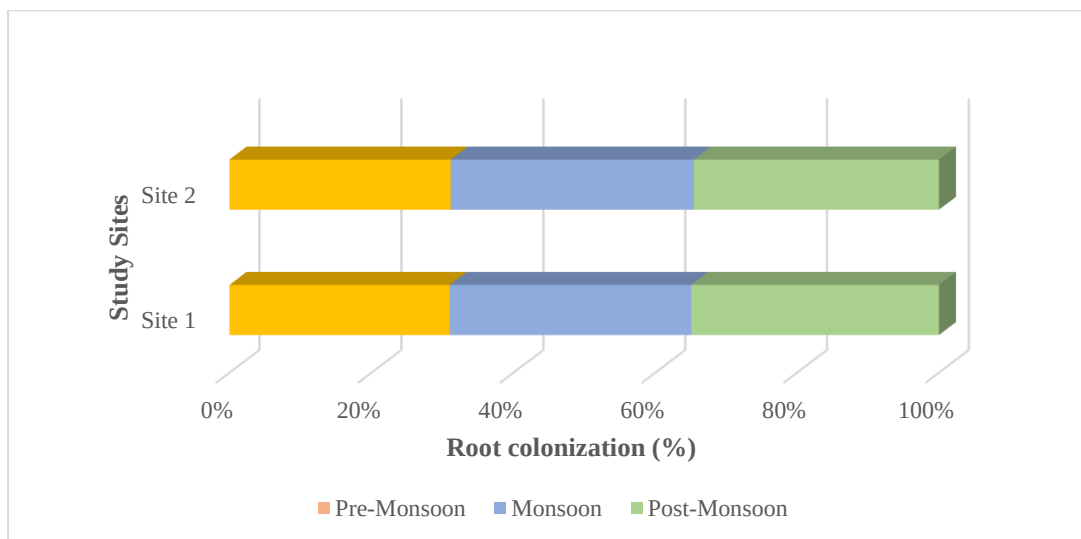


Fig. 5.1: Root colonization across the seasons at the two study sites.

Pre-monsoon season: At both sites, average AM colonization was significantly higher in the post-monsoon season than in the monsoon and pre-monsoon seasons. The least AM fungal root colonization was recorded during the pre-monsoon season at both site 1 ($67.88\% \pm 1.33$) and site 2 ($70.54\% \pm 0.88$).

Monsoon season: The roots of *C. esculenta* revealed AM colonization levels of 74.66 ± 1.25 (Site 1) and 77.66 ± 1.45 (Site 2) during the monsoon season.

Post-monsoon season: At both sites, mean AM colonization was significantly greater during the post-monsoon season compared to the other two seasons. Maximum AM fungal root colonization was recorded at site 2 ($78.32\% \pm 0.88$), followed by site 1 ($76.55\% \pm 0.57$), while minimum root colonization was recorded at site 1 ($67.88\% \pm 1.15$) and site 2 ($70.54\% \pm 1.23$) in the pre-monsoon season.

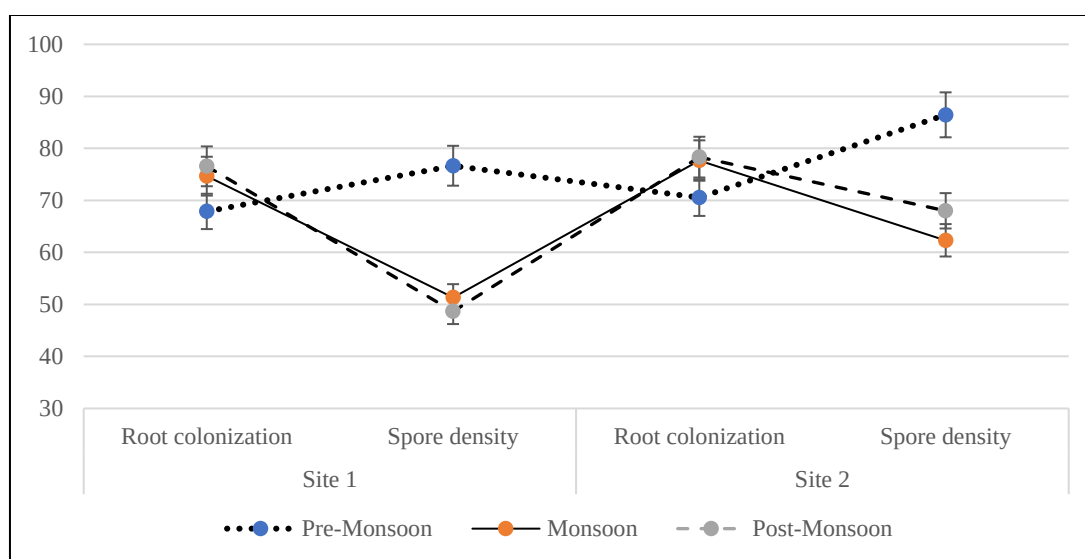


Fig. 5.2: Root colonization and Spore density of AM fungi as influenced by phenology.

AM fungal root colonization varied with the seasons, yet a similar pattern was observed at the sites. At both sites, the maximum root colonization occurred in the post-monsoon season, and the minimum root colonization was observed during the pre-monsoon season. Similar results have been reported in an earlier study (Bukhari and Rodrigues, 2023).

AM fungal spore density: Site-wise results of the seasonal variations in spore density of AM fungi in *C. esculenta* are depicted in **Table 5.1; Figs. 5.2 & 5.3**. The study revealed seasonal variation in spore density. The mean spore density at both sites was

highest during the pre-monsoon season. The least spore density, however, was observed during the post-monsoon season at site 1 and during the monsoon season at site 2.

Pre-monsoon season: The highest mean spore density at site 1 (76.66 spores 100g^{-1} soil) and site 2 (86.45 spores 100g^{-1} soil) was recorded during the pre-monsoon season (**Table 5.1; Figs. 5.2 & 5.3**). Earlier studies reported increased spore density during the pre-monsoon season as higher temperatures are known to favour sporulation (D'Souza and Rodrigues, 2013; Vieira Junior *et al.*, 2020).

Monsoon season: The mean spore density was least during the monsoon season compared to the pre-monsoon and post-monsoon seasons.

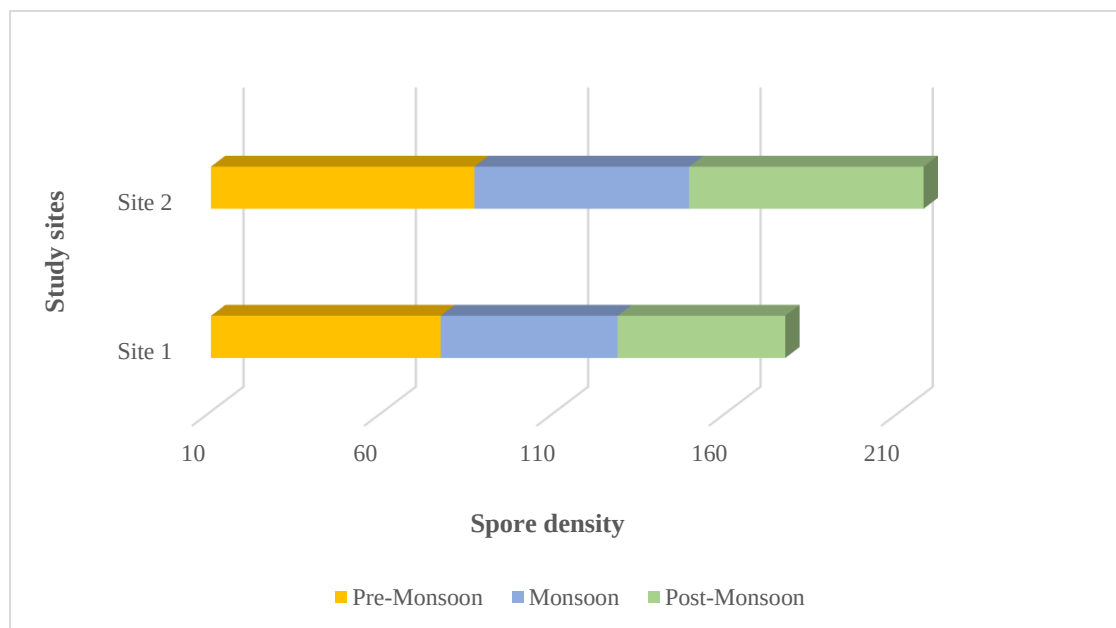


Fig. 5.3: Spore density across the seasons at the two study sites.

Table 5.1: Spore density and diversity of AM fungi at the two study sites across the seasons.

Site	Season	Root colonization (%)	Spore density*	AM fungal species
Site 1	Pre-Monsoon	67.88±1.33	76.66±1.23	<i>A. del.</i> , <i>A. dil.</i> , <i>A. fov.</i> , <i>A. scr.</i> , <i>Gl.sp.</i> , <i>Gi. alb.</i> , <i>Rh.int.</i>
	Monsoon	74.66±1.25	51.33±0.88	<i>A. del.</i> , <i>A. dil.</i> , <i>A. fov.</i> , <i>A. scr.</i> , <i>Gl.sp.</i> , <i>Gi. alb.</i> , <i>Rh.int.</i>
	Post-Monsoon	76.55±1.15	48.66±1.51	<i>A. dil.</i> , <i>A. fov.</i> , <i>A. scr.</i> , <i>Gl. sp.</i> , <i>Rh. int.</i>
Site 2	Pre-Monsoon	70.54±0.88	86.45±1.15	<i>A. fov.</i> , <i>A. scr.</i> , <i>F. mos.</i> , <i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>Rh. int.</i> , <i>Rh. irr.</i>
	Monsoon	77.66±1.45	62.33±0.57	<i>A. fov.</i> , <i>A. scr.</i> , <i>F. mos.</i> , <i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>Rh. int.</i> , <i>Rh. irr.</i>
	Post-Monsoon	78.32±1.23	68.00±1.45	<i>A. scr.</i> , <i>F. mos.</i> , <i>Gi. alb.</i> , <i>Gi. dec.</i> , <i>Rh. int.</i> , <i>Rh. irr.</i>

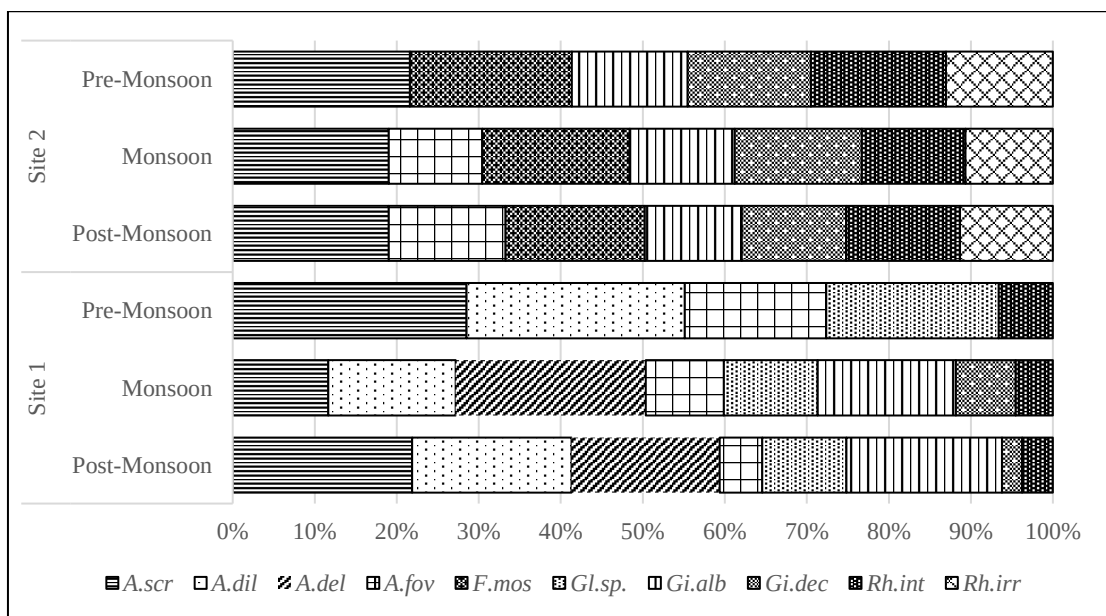
Legend: Data is means of three replicates; ± standard error; *Number of Spores 100g⁻¹. *Acaulospora delicata*- *A. del.*, *A. dilatata*- *A. dil.*, *A. foveata*- *A. fov.*, *A. scrobiculata*- *A. scr.*, *F. mosseae*- *F. mos.*, *Gigaspora albida*- *Gi. alb.*, *Gi. decipiens*- *Gi. dec.*, *Glomus sp.*- *Gl. sp.*, *Rh. intraradices*- *Rh. int.*, *Rh. irregularis*- *Rh. irr.*

Post-monsoon season: At site 1, the mean spore density was significantly lower in the post-monsoon season (48.66 spores 100g⁻¹ soil) compared to the monsoon and pre-monsoon seasons (**Table 5.1**). At site 2, moderate spore density (62.33 spores 100g⁻¹ soil) was observed during the monsoons.

The results of the present study indicated that, during pre-monsoon, spore density is higher while root colonization is lower, suggesting a shift toward sporulation at higher temperatures (D'Souza and Rodrigues, 2013; Vieira Junior *et al.*, 2020).

Distributional patterns and relative abundance (RA%) of AM fungal taxa: Using trap cultures, a total of ten AM fungal species representing five genera were recorded, viz., *Acaulospora scrobiculata*, *A. dilatata*, *A. delicata*, *A. foveata*, *Funneliformis mosseae*, *Glomus sp.*, *Gigaspora albida*, *Gi. decipiens*, *Rhizophagus intraradices* and *Rh. irregularis*. *Acaulospora* was the most dominant genus, followed by *Rhizophagus* and *Gigaspora*.

Amongst the AM fungal species recovered, the maximum RA was recorded in *A. scrobiculata* followed by *Gi. albida*, *A. dilatata* and *Rh. intraradices*, and the least RA was recorded in *Rh. irregularis*. Two AM fungal species, viz., *A. scrobiculata* and *Rh. intraradices* were recovered in all seasons and at both sites. *A. dilatata*, *A. foveata*, and *Glomus* sp. were recorded in all seasons at site 1, while *F. mosseae*, *Gi. albida*, *Gi. decipiens*, and *Rh. irregularis* were recorded in all the seasons at site 2. The site-wise and season-wise distribution and abundance of the AM fungal species recovered from the study sites are illustrated in **Table 5.1** and **Fig. 5.4**. The RA shows a positive correlation with soil pH at both the sites, viz., site 1 ($r = 0.701$) and site 2 ($r = 0.920$).



Legend: *Acaulospora delicata*- *A. del.*, *A. dilatata*- *A. dil.*, *A. foveata*- *A. fov.*, *A. scrobiculata*- *A. scr.*, *F. mosseae*- *F. mos.*, *Gigaspora albida*- *Gi. alb.*, *Gi. decipiens*- *Gi. dec.*, *Glomus* sp.- *Gl. sp.*, *Rh. intraradices*- *Rh. int.*, *Rh. irregularis*- *Rh. irr.*

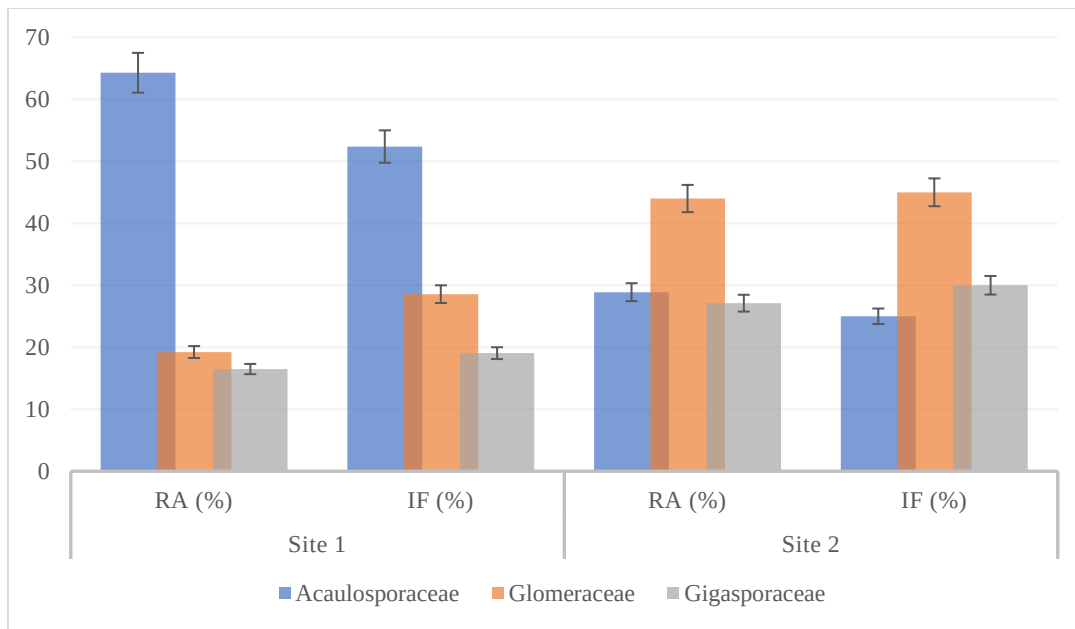
Fig. 5.4: Seasonal variation in AM fungal species at the study sites.

AM fungal species diversity: richness (SR) and evenness (E): Both sites recorded higher species richness (eight species) during the pre-monsoon and monsoon season, compared to the post-monsoon season (five species). Correlation analysis between spore density and species richness across the three seasons at both sites showed a moderately positive correlation ($r = 0.5729$). Shannon Weiner (H) indices at both the sites, viz., site 1 (1.96) and site 2 (1.66), expressed moderate diversity, with Simpson's index at site 1 (0.86) and site 2 (0.85), suggesting slight dominance of a few species. Species evenness, at site 1 (0.94) and site 2 (0.85), indicates that the distribution of individuals across species is highly even (**Table 5.2**).

Table 5.2: Diversity of AM fungal communities at selected study sites.

Ecological parameters	Site 1	Site 2
Shannon- Wiener index of diversity (H)	1.96	1.66
Simpson`s index of dominance (D)	0.86	0.85
AMF species evenness (E)	0.94	0.85
AMF species richness (SR)	8	7

The RA of families at the two sites was assessed. At site 1, the family Acaulosporaceae expressed dominance, followed by Glomeraceae and, lastly, Gigasporaceae, while at site 2, the family Glomeraceae showed dominance (**Fig. 5.5**).

**Fig. 5.5: Comparison of AM fungal families for relative abundance (RA%) and Isolation Frequency (IF%) at the study sites.**

It is suggested that the acidic conditions at site 1 likely drive the observed dominance of the family Acaulosporaceae. Giovannetti *et al.* (2010) reported that the genus *Acaulospora* is predominant in acidic soils.

The Principal Component Analysis (PCA) graph (**Fig. 5.6**) shows that root colonization in *C. esculenta* by AM fungi across seasons was dependent on pH, Mn,

and OC, whereas soil P content mainly influenced the spore density. Organic C appears to be playing a role in influencing both root colonization and spore density of AM fungi.

It is observed that pH plays an important role in determining the AM fungal colonization levels as observed in earlier studies (Carrino-Kyker *et al.*, 2016; Feng *et al.*, 2023). It is well-established that low levels of available P enhance AM fungal development (Willis *et al.*, 2013; Qin *et al.*, 2020).

Decomposed plant material contributes cellulose, the most abundant constituent of organic matter entering the soil. This cellulose is known to promote AM fungal root colonization and external mycelial proliferation (Gryndler *et al.*, 2009). This underpins the dependence of both root colonization and spore density on the availability of organic C.

Recent evidence suggests Mn availability can positively influence AM fungal colonization. Foliar Mn supplementation has been linked to enhanced colonization in host roots (Pasković *et al.*, 2019), implying that systemic Mn may boost plant–fungus signalling or root receptivity to colonization.

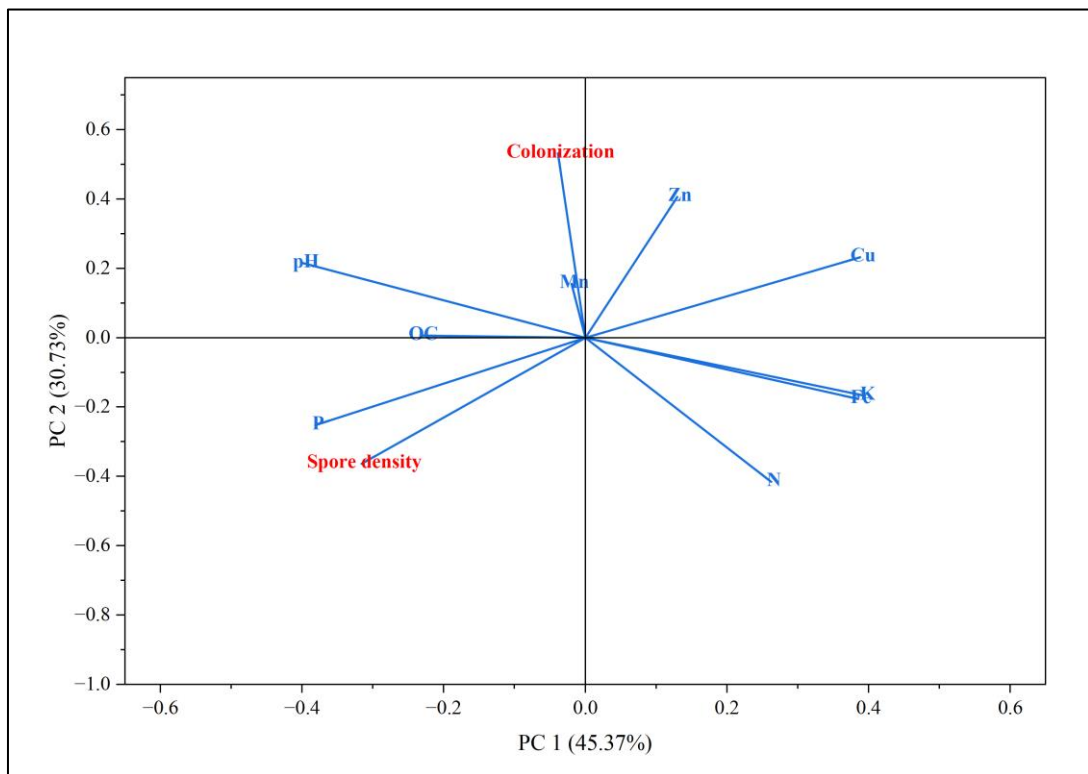


Fig. 5.6: Principal Component Analysis.

5.4: CONCLUSION

AM fungal associations are tightly modulated by key soil components, with pH acting as a primary gatekeeper for colonization. Low soil P consistently promotes AM development, highlighting a nutrient-driven symbiotic investment. Organic C inputs, via decomposed plant material rich in cellulose, support both root colonization and external mycelial growth, reflecting reliance on available carbon. Mn availability emerges as a positive driver, likely by enhancing plant fungus signalling or root receptivity to colonization. Collectively, these interactions illustrate that AM fungal performance revolves around a confluence of pH, P status, organic C supply, and micronutrient (Mn) availability in the soil.

The evaluation of biotic and abiotic determinants shaping AM fungal seasonal patterns indicates that phenology is linked to AM colonization (D'Souza and Rodrigues, 2013; Vieira Junior *et al.*, 2020; Bukhari and Rodrigues, 2023). In the present study, the seasonal dynamics of AM colonization reveal multiple controlling factors, with temperature, pH, and seasonal conditions significantly influencing AM distribution in *C. esculenta*. A seasonal shift in AM colonization suggests that the benefits of mycorrhizal symbiosis for plant performance vary across seasons (Fitter, 1991). Additionally, more detailed investigations are required to elucidate the role of seasonal dynamics in the distribution and diversity of AM fungi.

Chapter 6: Pure cultures of dominant AM fungal species and their mass multiplication.

6.1: INTRODUCTION

AM fungi are among the most widespread and ecologically significant groups of soil microorganisms, forming symbiotic associations with more than 80% of terrestrial plant species (Prasad *et al.*, 2017). These AM fungi play a crucial role in maintaining soil health and fertility, especially amid escalating environmental challenges.

Unfortunately, the global decline in soil fertility has been exacerbated by unsustainable land management practices such as overgrazing, frequent bush burning, continuous cropping, and intensive tillage, which degrade soil structure and nutrient content (Dewitte *et al.*, 2013). They are thus recognized as keystone organisms due to their vital and multifaceted roles within ecosystems. Extensive research has demonstrated their remarkable ability to enhance soil health by improving physical, chemical, and biological properties, thereby fostering more resilient and functional ecosystems. These fungi significantly contribute to increased biodiversity both above and below ground, influence plant succession, and help maintain ecological stability by potentially inhibiting the invasion of non-native, alien species. In response to these issues, inoculating soils with AM fungi has gained recognition as a sustainable and eco-friendly approach to revitalizing degraded land. This strategy not only improves soil quality but also supports sustainable agriculture and ecosystem stability (Dal Cortivo *et al.*, 2018). AM fungi are particularly valued for their ability to enhance soil fertility by positively altering its chemical, physical, and biological properties, thereby promoting healthier plant growth and resilient ecosystems (Fall *et al.*, 2022). They are especially crucial for the survival, development, and establishment of tree and shrub seedlings in environments stressed by limited moisture and nutrient availability. Beyond their natural ecological functions, AM fungi are highly effective biological tools for landscape restoration, particularly in degraded areas such as those affected by mining. Their application can markedly improve water and nutrient uptake by host plants, reducing stress and promoting the development of sustainable, resilient habitats. Incorporating AM fungi into restoration strategies accelerates the reestablishment of

natural processes and ecosystem services, with proven success in mine land rehabilitation worldwide (Asmelash *et al.*, 2016; De Moura *et al.*, 2022).

Overall, the ecological significance and restorative potential of AM fungi underscore their importance as a key component in efforts to recover and sustain disturbed environments, supporting long-term ecological resilience.

Symbiosis between AM fungi and higher plants offers a wide range of applications as biofertilizers. For this, mass production becomes necessary. Mass multiplication of pure AM fungi cultures, however, has always been a challenge. *In vitro* propagation via root-organ culture offers notable advantages, particularly in enhancing quality control and ensuring strain purity, both of which are critical for reliable inoculum production. This method also increases the capacity to generate large quantities of spores, thereby improving the scalability and consistency of mycorrhizal inoculum supply. Transformed roots have been used to establish monoxenic cultures of AM fungi in recent years, but with a low success rate for *in vitro* spore production (Velip and Rodrigues, 2019). Despite these technological advancements, pot cultivation remains the preferred approach for propagating AM fungi due to its simplicity, cost-effectiveness, and suitability for large-scale applications. Traditionally, this method involves mixing mycorrhizal propagules, such as colonized roots, spores, and hyphae, with a suitable substrate, followed by seeding and incubation under controlled environmental conditions. The integration of *in vitro* techniques with conventional practices supports more robust, efficient production systems, thereby promoting broader use in agricultural and ecological restoration efforts. (Dalpe and Monreal, 2004).

6.2: MATERIALS AND METHODS

Preparation of trap cultures: Trap cultures were established following the method of Morton *et al.* (1993). Rhizosphere soil samples, containing colonized root fragments, were mixed with sterilized or autoclaved sand in a 1:1 ratio and transferred into 15 cm plastic pots sterilized with 95% alcohol. *Coleus scutellarioides* (Coleus), known for its high mycotrophic potential, was used as a host plant.

The Coleus cuttings were cleaned with tap water, detergent solution, and finally rinsed in sterile water. Three to four cuttings were planted per pot and maintained in a

polyhouse at 27°C, with watering twice weekly. Hoagland's nutrient solution, deficient in P (Hoagland and Arnon, 1950), was added fortnightly. After 90 days, watering was ceased to allow the pots to dry. Following this, the shoots were removed, and the soil and roots were harvested. The roots were checked for AM fungal colonization using the Trypan blue staining method (Phillips and Hayman, 1970), and spores were recovered using the wet sieving and decanting method (Gerdeemann and Nicolson, 1963).

Preparation of monospecific (single-species) cultures: The AM fungal spores recovered from trap cultures were subjected to careful examination under an Olympus stereo microscope (models SZ2-ILST, with 10× to 45× zoom). Spores showing any loss of content, discoloration, physical damage, or parasitism were considered defective and subsequently discarded to ensure the purity and viability of the cultures.

The healthy, dominant spores were then thoroughly washed with sterile, autoclaved distilled water to remove soil particles, hyphal fragments, and any remaining contaminants that could interfere with culture development or skew experimental results. After washing, the spores were temporarily stored in Petri dishes at 4°C, until inoculation.

To establish monospecific cultures, the cleaned spores of each species were carefully inoculated into sterilized soil medium. The soil mixture consisted of equal parts sterile sand and soil, placed in 15 cm-diameter plastic pots. Before inoculation, a layer of Whatman filter paper was carefully laid at a depth of 2–3 cm in each pot to aid even distribution and provide a conducive environment for spore germination. The spores were then evenly placed on this filter paper, covered with additional sterilized soil medium to ensure they were securely incorporated, and the pots were then planted with coleus cuttings.

These pots were maintained under controlled environmental conditions in a polyhouse at 27°C and 63% relative humidity. The pots were regularly watered twice weekly to maintain optimal moisture levels for fungal colonization. To promote symbiotic establishment and growth, Hoagland's nutrient solution (without P) was added every fortnight, following the protocol outlined by Gilmore (1968). This careful process of spore selection, cleaning, inoculation, and maintenance enabled the consistent

production of healthy, monospecific AM fungal cultures, ensuring the purity required for subsequent experimental applications and studies (**Plate 6.1**).

After 90 days of growth, watering was discontinued to facilitate complete substrate drying. Subsequently, the contents of each pot were harvested for spore analysis and to generate a substantial inoculum for subsequent experimental applications. Microscopic examinations were performed using bright-field microscopy on Olympus BX41 and Nikon Eclipse E200 research microscopes at magnifications of 40×, 100×, 400×, and 1000×. Micrographs were captured with Olympus DP 12-2 and Nikon Digital Sight DS-U3 digital cameras. All the images were taken without digital editing to ensure authenticity.

6.3: RESULTS AND DISCUSSION

Monospecific cultures of five AM fungal species viz., *Acaulospora scrobiculata*, *Funneliformis mosseae*, *Rhizophagus intraradices*, *Gigaspora decipiens*, and *Gigaspora albida* from the selected study sites were successfully mass multiplied using *C. scutellarioides* as the host plant. These cultures were maintained at the Goa University Arbuscular Mycorrhizal Culture Collection (GUAMCC) facility. The cultures were utilized for the subsequent experiments as detailed in the following chapters.

In the present study, monospecific cultures of five AM species were prepared and maintained. Despite their proven ability to promote plant growth and protect against pathogens and environmental stresses, propagating AM fungal spores remains a complex, technically demanding task. Traditional *in vitro* methods often fall short, particularly because many AM fungal species are obligate symbionts and cannot be easily cultivated outside their natural host environments. Trap cultures, using soil from native, field-based samples, offer a promising solution by enabling the production of abundant, healthy spores across diverse species (Trejo-Aguilar and Banuelos, 2020). The success of these cultures depends on careful management of factors such as spore collection, culture purity, media selection, and storage techniques (Uddin *et al.*, 2025). While challenges persist, such as replicating the complexity of natural conditions, trap cultures have the potential to revolutionize large-scale AM fungal production. Improving and optimizing these methods is vital for expanding the application of AM fungi in organic farming systems, thus supporting global food security, ecological

health, and resilient agricultural practices (Uddin *et al.*, 2025; Sefrila *et al.*, 2016). Recognizing their importance is key to unlocking the full potential of AM fungi as a sustainable agricultural resource, and it is widely believed that trap cultures and pure cultures serve as effective methods for supporting the production and long-term storage of inocula, ensuring their availability for extended periods.

6.4: CONCLUSION

Monospecific cultures of five AM fungal species, viz., *Acaulospora scrobiculata*, *Funneliformis mosseae*, *Rhizophagus intraradices*, *Gigaspora decipiens*, and *Gigaspora albida*, were successfully mass multiplied using *Coleus scutellarioides* as the host and maintained at the GUAMCC for subsequent experimentation. Given the obligate symbiotic nature of AM fungi and the technical constraints of conventional *in vitro* propagation, establishing trap-based monospecific cultures is a reliable, scalable strategy for producing pure, viable inocula. Careful optimization of spore isolation, culture maintenance, and storage protocols is essential to ensure culture integrity and long-term viability. Collectively, these findings underscore the practical significance of trap and pure culture systems in enabling sustained AM fungal production and facilitating their broader application in resilient and ecologically sound agricultural systems.

Chapter 7: Evaluate the effects of the dominant AM fungal species on the growth and nutritional quality of selected species.

7.1: INTRODUCTION

The productivity of agriculture has increased steadily since the mid-20th century in temperate regions and more recently in tropical areas, primarily due to the introduction of improved varieties, machinery, fertilizers, and pesticides. However, this growth has subsequently plateaued in many systems, largely due to land degradation driven by unsustainable farming practices. To meet the increasing demand of the future, food production must rise significantly throughout this century while sustainably utilizing less cultivable land (Devi *et al.*, 2021; Jocien and Frederick, 2023).

AM fungi have been repeatedly emphasized as valuable bio-inoculants for agricultural systems (Ijdo *et al.*, 2011; de Souza *et al.*, 2010). AM fungi are regarded as the cornerstone in the field of sustainable agriculture, with mycorrhizal symbiosis emerging as a keystone for plant productivity and diversity due to its pervasive influence on nearly all plant metabolic processes and its capacity to sustain, often stimulate, plant growth and development through their diverse functionalities and benefits to host plants (Lone *et al.*, 2017). AM fungi are known to sustain soil quality and health through three interconnected avenues, viz., improving soil structure, enhancing plant physiology, and shaping ecological interactions. These improvements enable plants to function more effectively, grow more robustly, and achieve greater productivity. The mycorrhizal association often exerts a synergistic effect on plant performance, and, beyond direct growth benefits, it contributes to land restoration and seedling establishment. Collectively, these functions are known to elevate overall plant performance and productivity (Kalamulla *et al.*, 2022; Chaudhary *et al.*, 2025).

The environmental drawbacks of synthetic chemical fertilizers have diminished their appeal, prompting a long-standing interest in biofertilizers as healthier and more sustainable alternatives for agriculture. Accumulating evidence indicates that beneficial microbes can enhance soil productivity and improve product quality while reducing environmental costs, with AM fungi emerging as particularly promising biofertilizers due to their numerous advantages (Madawala, 2021).

Biofertilization with AM fungi is regarded as a viable substitute for conventional fertilization, offering applications in plant nutrition (P, N, and other micronutrients), biotic stress mitigation (protection against pests and pathogens), and abiotic stress tolerance (drought, salinity and heavy metals). By establishing a symbiotic relationship with the host root and coordinating specific plant-fungus gene interactions, AM fungi can sustainably promote plant growth and production to meet the host's nutritional needs (Anand *et al.*, 2022). The practical deployment of AM fungi as a biofertilizer involves inoculating soil to enhance nutrient uptake and plant growth, with studies showing improved drought tolerance and increased yields. Remarkably, indigenous AM fungi from local soils often perform as well as or better than commercial strains, suggesting farmers can reliably produce their own AM fungal inoculants, an especially valuable prospect for developing regions such as India, where sustainable crop production is urgently needed (Jha and Songachan, 2023). Ultimately, the symbiotic association between host plants and AM fungi is a natural gift for sustaining agroecosystems in today's dynamic environment (Chaudhary *et al.*, 2025).

AM fungal behaviour being highly contingent on ecosystem fluctuations, and introducing AM fungi to agricultural lands alters their abundance, colonization, and functional expression. Growing attention to AM fungal proliferation in sustainable farm systems reflects their pivotal role in promoting plant vigour and enhancing soil quality and structural stability. To optimize AM fungal applications for sustainable agriculture, it is crucial to develop customized fungal inoculants by carefully evaluating field conditions and selecting plant species with the greatest potential for AM fungal colonization. These fungal associations can be leveraged to increase productivity while reducing reliance on chemical inputs such as insecticides and fertilizers. Additionally, AM fungi-based biofertilization technologies have the potential to be affordable for farmers, including those in developing countries (Devi *et al.*, 2021; Kalamulla *et al.*, 2022; George and Ray, 2023; Wahab *et al.*, 2023). Also, the relationship between AM fungi and associated bacteria is crucial for sustainable agriculture, with yield gains observed when the microbes interact (Rahimzadeh, and Pirzad, 2017).

7.2: MATERIALS AND METHODS

Collection of rhizosphere soils for PSB strains: Rhizosphere soil samples from tuber species were collected. For each plant species, three subsamples were collected in sealed bags. Further, these samples were air-dried and used to isolate PSB.

Isolation and purification of PSB: PSB was isolated using the serial dilution plate method on Pikovskaya (PKV) agar (Roychowdhury *et al.*, 2015). Accordingly, three dilutions, viz., 10^{-1} , 10^{-2} , and 10^{-3} , were selected for inoculation. 0.1 mL of culture was spread-plated onto a Pikovskaya agar plate using serial dilutions. Plates were incubated at 30°C for 48 hours. Distinct bacterial colonies, identified by morphological appearance and halo zone formation, were selected and streaked onto fresh PKV agar plates. Selected colonies were purified by repeated streaking and maintained on Nutrient agar slants at 4°C for further analysis.

Qualitative estimation of phosphate solubilization: Bacterial cultures containing 10^8 CFU/mL were grown on Pikovskaya's agar medium using an inoculation loop. The plates were maintained at $30 \pm 2^\circ\text{C}$. The colony diameter and halo zone diameter around it were measured at the end of 7 days after inoculation (DAI). The Phosphate Solubilization efficiency (PSE) and the Phosphate Solubilization Index (PSI) were calculated to assess the Phosphate Solubilising capacities of the PSB isolates. PSI was estimated based on the rating scale in **Table 7.1**.

Phosphate Solubilization Efficiency (PSE): The colony diameter and halo zone around it were measured at the end of 2, 5, and 7 days after inoculation (DAI). Phosphate solubilizing efficiency was measured using the following formula (Kundu *et al.*, 2009).

$$\text{Solubilization efficiency} = \frac{\text{Diameter of solubilization zone} - \text{colony diameter (in mm)}}{\text{Colony diameter (in mm)}} \times 100$$

Phosphate solubilizing index (PSI): The isolate's capability to solubilize phosphate was measured and categorized using the Phosphate Solubilization Index (PSI). The PSI was calculated using the formula given below (Cahyani *et al.*, 2024)

$$\text{PSI} = \frac{\text{Total diameter (diameter (colony + halo zone) (in mm))}}{\text{Colony diameter (in mm)}}$$

Table 7.1: Rating scale to estimate the PSI of PSB isolates.

PSI obtained	Rating scale
<1	very low
1.01-2	low
2.01-3	medium
3.01-4	high
>4.01	very high

Morphological and Biochemical characterization of PSB: Preliminary morphological and biochemical characterisation of the selected PSB isolate was performed using standard methods as described in ‘Bergey’s Manual of Systematic Bacteriology’ (Krieg and Holt, 1994). The tests included Gram staining and sugar utilization (glucose, cellobiose, mannitol, sucrose, arabinose, lactose, xylose, and maltose).

Gram staining: Gram staining of the selected PSB cultures was performed using the Himedia K001-1KT Gram stain kit according to the manufacturer's instructions.

Molecular characterization of bacterial isolate: Molecular characterization of the PSB isolate was performed by sequencing the 16s rRNA gene. Genomic DNA was extracted using the XPLOREGEN gDNA extraction kit according to the manufacturer's instructions. The quality of the extracted DNA was assessed by running 5 µL of DNA on an agarose gel at 75V until the bromophenol dye front reached the bottom of the gel. The resultant DNA bands were visualized using a UV transilluminator (GeNei).

Amplification of 16S rRNA gene: 171µg of extracted DNA is used for amplification, along with 10 pM of each primer. The 16S rRNA gene of PSB isolates was amplified in a PCR thermal cycler using 16S Forward and 16S Reverse primers, as detailed in **Table 7.2.**

Table 7.2: Details of the Primers used for amplification.

Primer Details No.	Oligo Name	Sequence (5`à 3`)	T _m (°C)	GC-Content
1	16S Forward	GGATGAGCCCGCGGCCTA	57	72.22%
2	16S Reverse	CGGTGTGTACAAGGCCCGG	58	68.42%

The TAQ Master mix was composed of High-Fidelity DNA Polymerase, 2.5 mM dNTPs, 3.2 mM MgCl₂, and PCR Enzyme Buffer.

The PCR cycling durations and amplification conditions are as given in **Tables 7.3** and **7.4**, respectively.

Table 7.3: The PCR cycling durations.

Cycling conditions		
Initial Denaturation	3 minutes at 94°C	
Denaturation	1 minute at 94°C	30 Cycles
Annealing	1 minute 50°C	
Extension	2 minutes at 72°C	
Final Extension	7 minutes at 72°C	

Table 7.4: The PCR amplification conditions.

PCR Amplification Conditions	Volume
DNA	1 µl
16S Forward Primer	2 ul
16S Reverse Primer	2 µl
dNTPs (2.5mM each)	4 µl
10X Taq DNA polymerase Assay Buffer	10 µl
Taq DNA Polymerase Enzyme (3U/ ml)	1 µl
Water	30 µl
Total reaction volume	50 µl

Agarose gel electrophoresis of the PCR product: The PCR products were resolved by electrophoresis using 1.2% agarose gels in 0.5X TBE buffer, stained with 0.5 µg/mL ethidium bromide. 1 µL of 6X loading dye was mixed with 4 µL of PCR products, and the mixture was loaded. A 2-log DNA ladder (NEB) was used as a marker. The gel was

run at 75V for 1-2 h (**Table 7.5** and **7.6**). The gels were viewed under a UV transilluminator (Genei), and images were captured using the Gel documentation system (Bio-Rad).

ExoSAP-IT Treatment: ExoSAP-IT, consisting of two hydrolytic enzymes, Exonuclease I and Shrimp Alkaline Phosphatase (SAP), was used to remove unwanted primers and dNTPs from a PCR product. A 5 μ L of PCR product was mixed with 0.5 μ L of ExoSAP-IT and incubated at 37°C for 15 min, followed by enzyme inactivation at 85°C for 5 min.

Sequencing of 16s rRNA gene: Sequencing of ExoSAP-treated PCR product was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA) by following the manufacturer's instructions.

Table 7.5: Components of the Sequencing PCR mix.

Reaction mixture	Quantity (μ L)
D/W	6.6
5X Sequencing Buffer	1.9
Forward Primer	0.3
Reverse Primer	0.3
Sequencing Mix	0.2
ExoSAP-treated PCR product	1.0

Table 7.6: Sequencing PCR amplification profile.

Temperature (°C)	Time
96	2 min
96	30 sec
50	40 sec
60	4 min
40	∞

The details of the instruments used: Sequencing Machine: ABI 3130 Genetic Analyzer; Chemistry Cycle sequencing kit: Big Dye Terminator version 3.1"; Polymer & Capillary Array: POP_7 pol Capillary Array; Analysis protocol: BDTv3-

KB-Denovo_v 5.2; **Data Analysis:** Seq Scape_ v 5.2; **Software Reaction Plate:** Applied Biosystem Micro Amp Optical 96-Well Reaction plate.

The Sequencing mix Composition and PCR Conditions were, 10µl Sequencing Reaction; Big Dye Terminator Ready Reaction Mix: 4µl; Template (100ng/µl): 1µl Primer (10pmol/λ): 2µl Milli Q Water: 3µl

PCR Conditions: (30 cycles): Initial Denaturation: 96°C for 5 min; Denaturation: 96°C for 30 sec; Hybridization: 50 °C for 30 sec; Elongation: 60 °C for 1.30 min.

Sequence analysis: Sequence quality was checked using the Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and editing were performed using Geneious Pro v5.5 (Drummond et al., 2010). The homology to the closest bacterial species having maximum similarity (98-100%) was obtained in the National Centre for Biotechnology Information (NCBI). The forward and reverse sequences were aligned and edited using BioEdit v. 7.2.5 sequence alignment editor. The sequences were deposited in the NCBI gene bank. The 16S RNA sequences were used to build a phylogenetic tree using Molecular Evolutionary Genetics Analysis software (MEGA-X) (Tamura *et al.*, 2004). A bootstrap test with 1000 replicates was performed using the Neighbour-joining method to determine the relationship with the closest species.

Identification software details: Phylogenetic Tree Builder uses sequences aligned with the System Software aligner. A distance matrix is generated using the Jukes-Cantor corrected distance model. When generating the distance matrix, only alignment model positions are used, alignment inserts are ignored, and the minimum comparable position is 200. The tree is created using Weighbor with an alphabet size of 4 and a length size of 1000.

Weighbor Tree: Weighbor is a weighted version of Neighbor Joining that gives significantly less weight to the longer distances in the distance matrix. The weights are based on variances and covariances expected in a simple Jukes-Cantor model.

Jukes-Cantor Correction: The Jukes-Cantor distance correction model assumes that, as two sequences diverge, the probability of a second substitution at any nucleotide site increases. In distance-based trees such as Weighbor, nucleotide differences are included in the distance calculation; therefore, second substitutions are not counted, and the

distance is underestimated. Jukes and Cantor developed a formula to calculate distance that takes into account more than just individual differences (1969; *Evol. of Protein Molecules*, Academic Press).

Bootstrap: Bootstrapping is a statistical method for estimating the sampling distribution by resampling with replacement from the original sample. In making phylogenetic trees, the approach is to create a pseudoalignment by randomly selecting positions from the original alignment. Some alignment columns could be selected more than once or not selected at all. The pseudoalignment was as long as the original alignment and was used to create a distance matrix and a tree. The process was repeated 100 times, and a majority-consensus tree is displayed, showing the number (or percentage) of times each group was on each side of a branch, without considering subgrouping.

Experimental design for *D. esculenta*: The field experiment was conducted in 2024 on lateritic alluvial soils at Barcem/Barsen village, Quepem taluka, South Goa, India (latitude 15°4'28.6104" N, longitude 74°1'48.129" E) (**Plate 7.1a**). The tuber species *Dioscorea esculenta* is commonly known as Lesser yam or Chinese yam in English, and Kate Kanga in Konkani. The experimental site was ploughed and levelled using a tractor (**Plate 7.1b**).

A randomised block design with three replicates (R₁, R₂, & R₃) was employed. Each plot measured 6 × 5 m, with an inter-plot spacing of 60 cm between rows and 60 cm between plots. Disease-free, uniform and healthy tubers of the previous season were selected as seed material. They were surface-sterilized, and used for planting (**Plate 7.2**).

In all, nine treatments were employed in the experiment, which are listed below.

Code	Treatments
T ₁	Control
T ₂	AM (<i>A. scrobiculata</i>)
T ₃	AM (<i>F. mosseae</i>)
T ₄	AM (<i>A. scrobiculata</i>) + AM (<i>F. mosseae</i>)
T ₅	PSB
T ₆	AM (<i>A. scrobiculata</i>) + PSB
T ₇	AM (<i>F. mosseae</i>) + PSB
T ₈	AM (<i>A. scrobiculata</i>) + AM (<i>F. mosseae</i>) + PSB
T ₉	Compost

Dominant AM fungal species, viz., *A. scrobiculata* and *F. mosseae*, isolated from the rhizosphere of *D. esculenta* were employed to assess their effects on the growth and nutritional quality of *D. esculenta*. In addition to the two selected AM fungal species with inoculum density of >250/100g spores and colonised root bits, a PSB (P-solubilizing bacteria having 10⁸ CFU/mL) with compost as carrier was applied, both singly and in combination with the two AM bio-inoculants (**Plate 7.3**). Tubers were planted at approx. 10 cm depth. For AM treatments, 100 g of pure fungal culture was used as inoculum per planting unit. In all, 270 tubers (9 treatments × 3 replicates × 10 rows each) were planted. Irrigation and weed management were performed regularly to ensure healthy plant growth.

Plant growth and harvest stage parameters measurement: Various parameters, viz., plant height, leaf length, and number of shoots per plant, were evaluated at various stages of plant growth (**Plate 7.4**). The parameters evaluated at harvest included fresh tuber weight per plant, total plant weight at harvest, tuber nutritional analysis, and plant dry biomass, in addition to the previously evaluated parameters.

Processing root segments for AM fungal colonization: The root fragments of *D. esculenta* from three randomly selected plants were assessed for AM fungal colonization at 45 days after sowing (DAS) by using the Trypan blue staining technique (Phillips and Hayman, 1970) as described in Chapter 3.

Experimental design for *Ipomoea batatas*: The field trial was conducted in 2024 on sandy and loamy soils at Agassiam village, Tiswadi taluka, North Goa, India (latitude 15°26'0.8586" N, longitude 73°53'59.046" E) (**Plate 7.5**). *Ipomoea batatas* is commonly known as sweet potato, and locally known as Kanga (in Konkani). The experimental site was ploughed and levelled using a tractor. A randomized block design with three replicates (R₁, R₂, & R₃) was employed. Each plot measured 6 × 5 m, with inter-plot spacing of 60 cm between rows and 60 cm between plots.

In *I. batatas*, propagation is through unrooted sprouts called ‘slips’ (Atu, 2014). Disease-free, uniform and healthy cuttings of *I. batatas* were selected as seed material for planting (**Plate 7.6**). In all, there were nine treatments, as mentioned below:

Code	Treatments
T ₁	Control
T ₂	AM (<i>A. scrobiculata</i>)
T ₃	AM (<i>Rh. intraradices</i>)
T ₄	AM (<i>A. scrobiculata</i>) + AM (<i>Rh. intraradices</i>)
T ₅	PSB
T ₆	AM (<i>A. scrobiculata</i>) + PSB
T ₇	AM (<i>Rh. intraradices</i>) + PSB
T ₈	AM (<i>A. scrobiculata</i>) + AM (<i>Rh. intraradices</i>) + PSB
T ₉	Compost

The two dominant AM fungal species, viz., *A. scrobiculata* and *Rh. intraradices*, were used to assess their effects on the growth and nutritional quality of *I. batatas*.

In addition to the AM fungi, a compost-based inoculum containing PSB was applied as a treatment, in combination with the AM bio-inoculants. Stem cuttings were planted at a depth of approximately 10 cm. For AM treatments, 100 g of pure fungal culture was used as inoculum per planting unit. In all, 270 cuttings (9 treatments x 3 replicates x 10 rows each) were planted (**Plate 7.6**). Irrigation and weed management were performed regularly to ensure healthy plant growth.

Plant growth and harvest stage parameters measurement: Various parameters, viz., plant height, number of shoots per plant, length of the leaf, total weight of tubers per plant, fresh plant weight at harvest, nutritional analysis of tubers, and the dry plant biomass were evaluated at various stages of plant growth (**Plate 7.7**).

Processing root segments for AM fungal colonization: The root segments of *I. batatas* from three randomly selected plants (per treatment) were assessed for AM fungal colonization at 45 DAS by using the Trypan blue staining technique (Phillips and Hayman, 1970) as described in Chapter 3.

Mycorrhizal Dependency: The Mycorrhizal dependency of both the crops was evaluated. Mycorrhizal dependency (MD) is the degree to which a plant depends on AM symbiosis to gain maximum growth in a given soil fertility. It may vary based on AM species, host plant species, and nutrient availability and was calculated using the formula provided by Plenchette *et al.* (1983).

$$\text{MD \%} = \frac{\text{Dry weight of mycorrhizal plant} - \text{Dry weight of nonmycorrhizal plant}}{\text{Dry weight of the mycorrhizal plant}} \times 100$$

Nutritional Analysis: For the nutritional analysis, 10 tubers of each treatment were randomly selected, sliced and sun-dried to a constant weight. The dried samples were ground into powder and used for the nutritional analyses. This procedure was performed separately for each of the tuber species. Estimation of moisture, total ash, fat, protein, crude fibre, carbohydrates, energy, total sugars, Ca, Mg, K, and P was assessed using standard methods. Moisture content was determined by hot-air oven method (AOAC, 1990), Total ash by incineration of known weights of the samples in a muffle furnace (Gravimetric method, 1978), fat by Soxhlet extraction (1998), protein by Kjeldahl

digestion (1990), carbohydrates and total sugars by Lane–Enyon (1985) method, Ca and Mg by EDTA titration (1977), K by flame photometry (Pflaum and Howick, 1956), and P by spectrophotometry (Carius method, 1962).

Statistical analysis: Differences between replicates and treatments were assessed using ANOVA; p-values < 0.05 were considered significant. Pearson’s correlation analysis was performed to test relationships between the analysed parameters. Correlation coefficients (r) were considered significant at p values < 0.05. These analyses were performed using STATISTICA (version 8.0, StatSoft, USA), SPSS v16.0, and OriginPro 2025. All microscopic observations were made using bright field Olympus BX 41 and Nikon Eclipse E200 research microscopes (40×, 100× magnification) and Olympus stereo microscope SZ2-ILST (45× magnification). Micrographs were imaged by a Nikon Digital Sight DS-U3 digital camera and were not digitally edited.

7.3: RESULTS AND DISCUSSION

Isolation and Identification of the P-solubilizing bacteria

Four phosphate solubilizing bacterial species obtained from the tuber rhizosphere were grown on Pikovskaya agar, and the halo zones were studied (Roychowdhury *et al.*, 2015). These four cultures were evaluated for their P-solubilizing ability. PSB₁ exhibited the highest P-solubilizing efficiency (PSE) and P-solubilizing index (PSI) (**Plate 7.8; Table 7.7**) (Cahyani *et al.*, 2024) and was selected for further studies.

Table 7.7: Estimation of PSI for the isolated PSB colonies.

PSB strain	Colony diameter (in mm)	Halo diameter (in mm)	Colony diameter + Halo diameter	PSI	PSI Scale	PSE (in %)
PSB 1	9	41	50	5.55	Very high	455.55
PSB 2	6	23	29	4.83	Very high	383.33
PSB 3	4	12	16	4.0	Very high	300
PSB 4	4	10	14	3.5	High	183

The results of morphological and biochemical tests of PSB₁ are presented in **Plates 7.9 & 7.10; Table 7.8**. Based on morphological and biochemical analyses, the selected isolate was tentatively identified as *Pseudomonas* sp. Moreover, molecular analysis of the 16S RNA of the isolate revealed its close similarity to *Pseudomonas* sp. COR18 chromosome and its sequence ID obtained was CP183808.1. The Genomic DNA Gel is seen in **Plate 7.11**.

The aligned sequence data for the isolate was 1418 bp (**Plate 7.12**). The close relation of the isolate with other bacterial strains of the NCBI database is depicted in the phylogenetic tree (**Fig. 7.12**). Previous studies report that *Pseudomonas* species are efficient P solubilizers (Soares *et al.*, 2023; Das *et al.*, 2024).

Table 7.8: Morphological and Biochemical characterization of PSB₁.

Test	Observation
Cell shape	rod
Motility test	motile
Gram staining	negative
Catalase	-
Carbon source utilization	
Glucose	+
Mannitol	+
Sucrose	-
Lactose	-
Xylose	+
Maltose	+

The combination of AM fungi and PSB has been shown to enhance nutrient uptake, growth, and yield across various crops (Nacoon *et al.*, 2021; Masrahi *et al.*, 2023). Thus, PSB₁, along with three selected dominant AM fungal species viz., *A. scrobiculata*, *F. mosseae* and *R. intraradices*, were employed for the field trials. For the PSB application, vermicompost was used as a carrier, mixed with a saturated bacterial liquid culture (10^8 CFU/mL), and used in the experimental studies.

Plant growth and root colonization: The roots of both the tuber species exhibited colonization at 45 days after sowing (DAS). Variation in AM fungal colonization was observed in the various treatments (T₁ to T₉). The mycorrhiza-inoculated treatments (T₂, T₃, T₄, T₆, T₇, and T₈) showed higher AM fungal colonization levels compared to non-AM inoculated treatments (T₁, T₅, and T₉) (**Fig. 7.1; Plate 7.13**). In sustainable agriculture, the use of soil microbial communities promotes plant nutrient availability and uptake, enhances soil health, and improves crop productivity, a relationship that is well established and broadly understood (Singh *et al.*, 2022; Nabi, 2023). Co-inoculation of AM fungi and PSB is also known to promote plant growth and yield (Nacoon *et al.*, 2021).

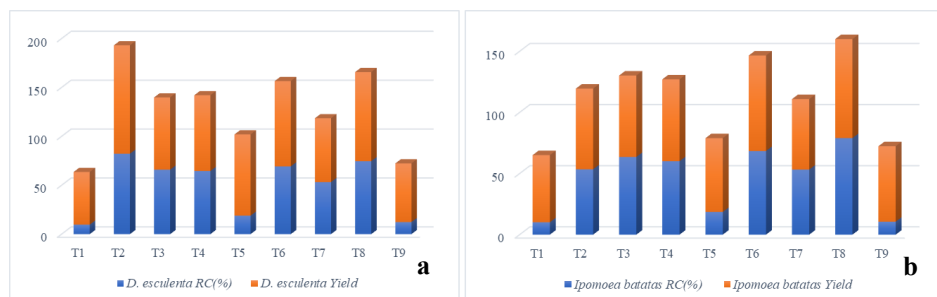
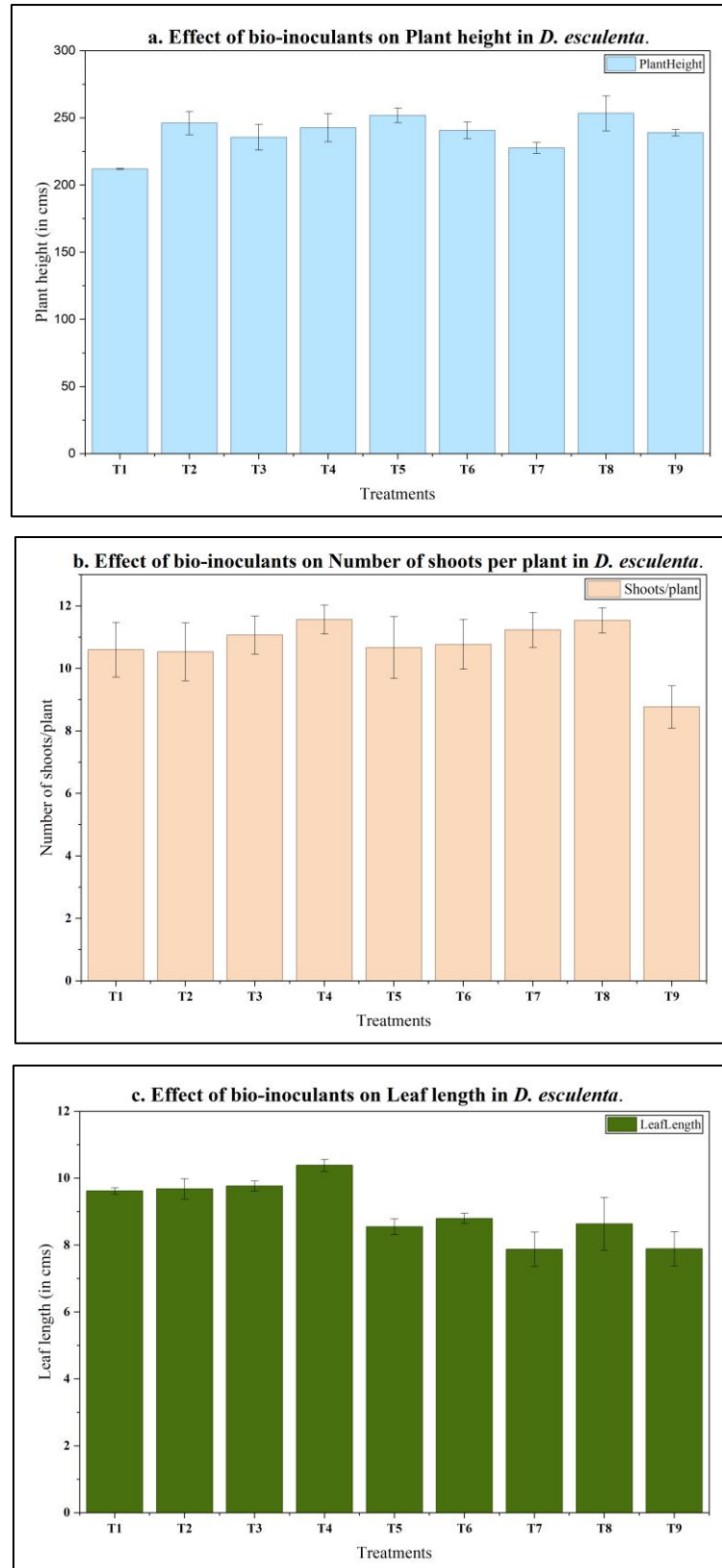


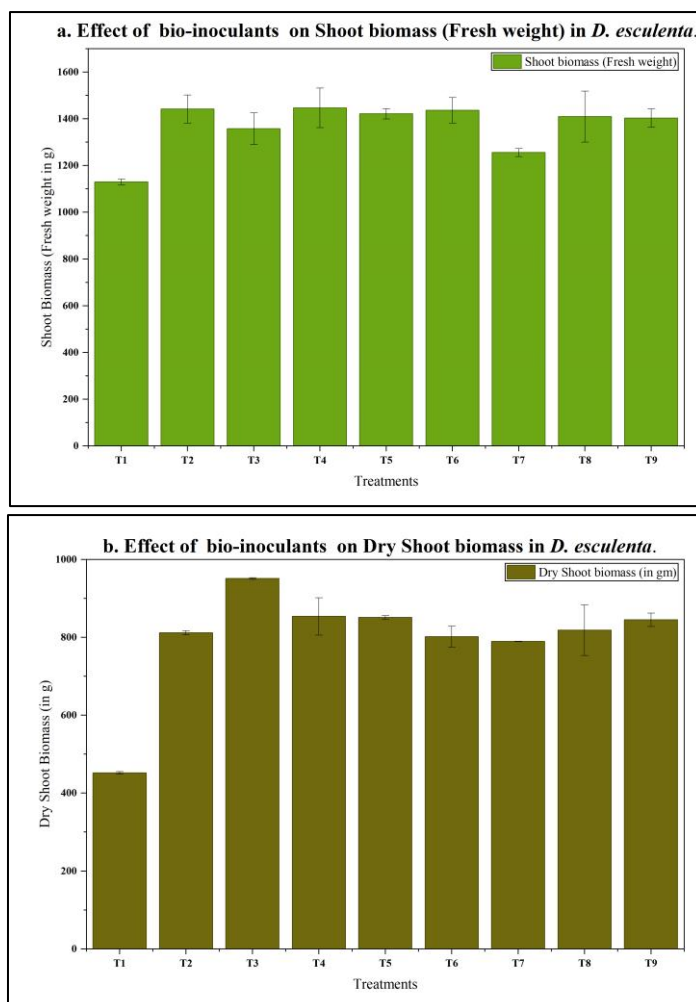
Fig. 7.1: Root colonization and yield in selected tuber crops – **a.** *D. esculenta* and **b.** *I. batatas*

Plant growth response to bio-inoculation: In the current study, bio-inoculants, including AM fungi and P-solubilising bacteria (PSBs), were used in various combinations to assess their effects on growth and yield in *D. esculenta*. The results of bio-inoculation using AM fungi and PSB on vegetative growth and biomass in *D. esculenta* are presented in **Table 7.10; Figs. 7.2 & 7.3**. In *D. esculenta*, a strong positive correlation between plant height and tuber yield ($r = 0.7308$; $n = 9$, $p < 0.05$) was recorded. Leaf length and shoot number per plant also showed a positive correlation ($r = 0.4289$; $n = 9$, $p < 0.05$). Also, a mild positive correlation between leaf length and tuber yield ($r = 0.2342$; $n = 9$, $p < 0.05$), and between the number of shoots per plant and tuber yield ($r = 0.2934$; $n = 9$, $p < 0.05$) was recorded.



Legend: T₁ = Control, T₂ = *A. scrobiculata*, T₃ = *F. mosseae*, T₄ = *A. scrobiculata* + *F. mosseae*, T₅ = PSB, T₆ = PSB + *A. scrobiculata*, T₇ = PSB + *F. mosseae*, T₈ = PSB + *A. scrobiculata* + *F. mosseae*, T₉ = Compost.

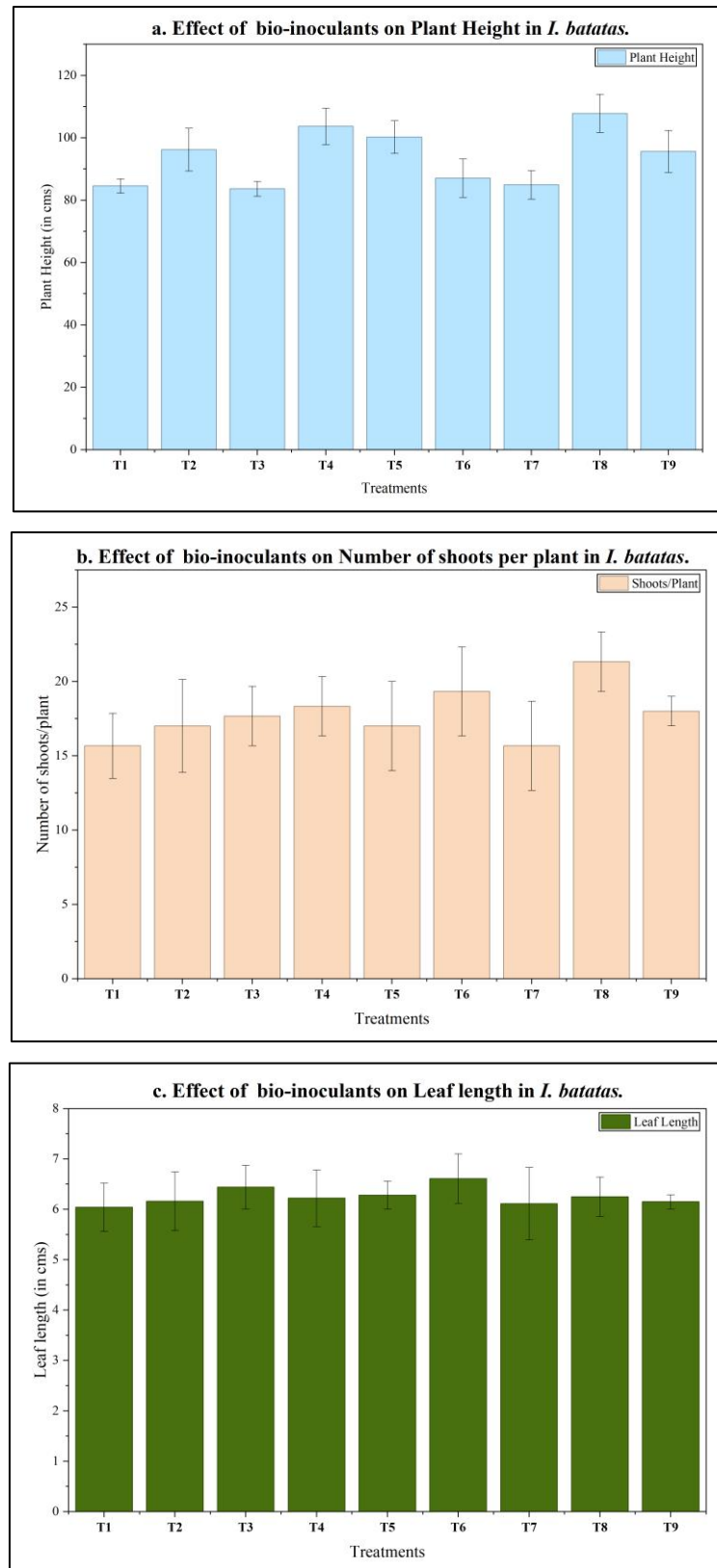
Fig. 7.2: Effect of bio-inoculant treatment on growth parameters of *D. esculenta* - a. plant height, b. number of shoots per plant, c. leaf length.



Legend: T₁ = Control, T₂ = *A. scrobiculata*, T₃ = *F. mosseae*, T₄ = *A. scrobiculata* + *F. mosseae*, T₅ = PSB, T₆ = PSB + *A. scrobiculata*, T₇ = PSB + *F. mosseae*, T₈ = PSB + *A. scrobiculata* + *F. mosseae*, T₉ = Compost.

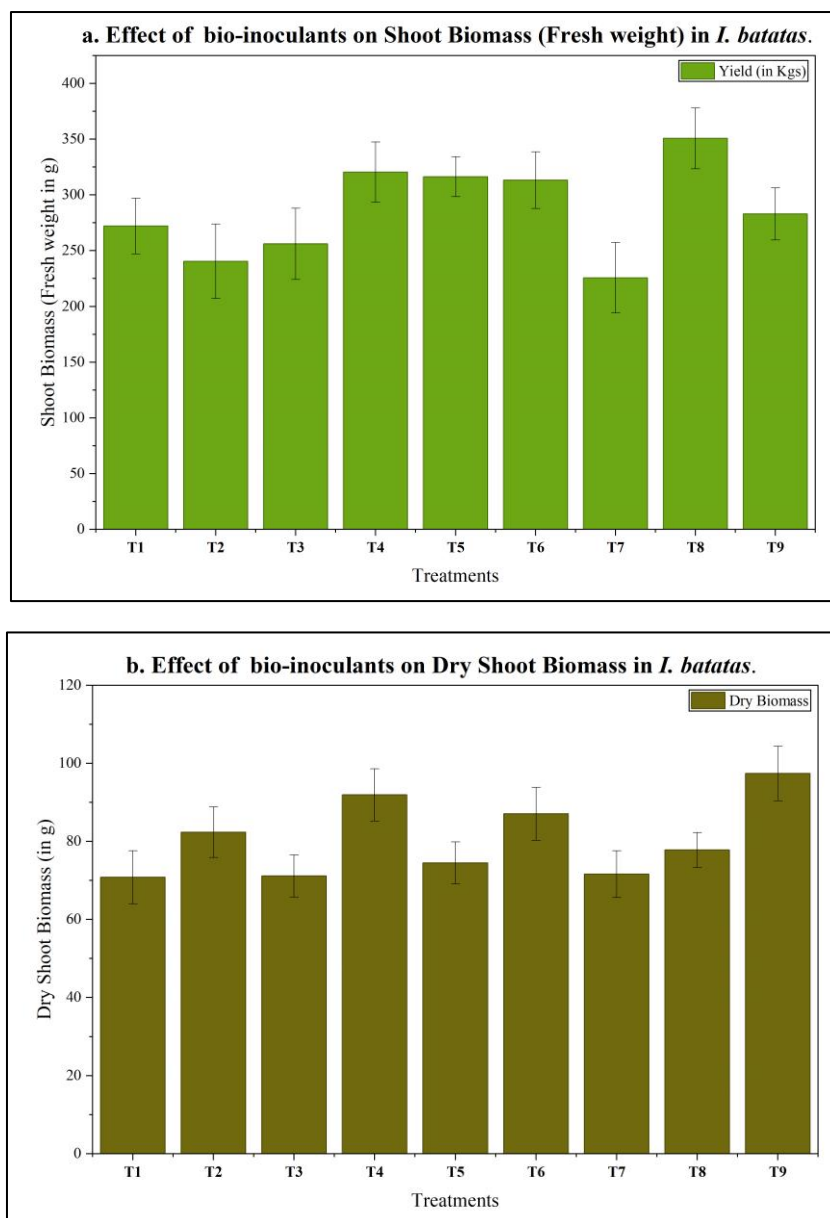
Fig. 7.3: Effect of bio-inoculant treatment on biomass of *D. esculenta* - a. shoot biomass-fresh (SBF), and b. shoot biomass-dry (SBD).

The bio-inoculants recorded positive effects on growth and yield in *I. batatas* (Figs. 7.4 & 7.5; Table 7.11). A strong positive correlation between shoots per plant and tuber yield ($r = 0.9442$; $n = 9$, $p < 0.05$) was recorded. The fresh shoot weight recorded a strong positive correlation with shoots per plant ($r = 0.8508$; $n = 9$, $p < 0.05$) and plant height ($r = 0.7582$; $n = 9$, $p < 0.05$). Also, a positive correlation was observed between leaf length and tuber yield ($r = 0.6236$; $n = 9$, $p < 0.05$), plant height and shoots per plant ($r = 0.5990$; $n = 9$, $p < 0.05$) and the number of shoots per plant and leaf length ($r = 0.5093$; $n = 9$, $p < 0.05$). Mild positive correlation was recorded between leaf length and the shoot fresh weight ($r = 0.4771$; $n = 9$, $p < 0.05$), and plant height and tuber yield ($r = 0.4415$; $n = 9$, $p < 0.05$).



Legend: T₁ = Control, T₂ = *A. scrobiculata*, T₃ = *Rh. Intraradices*, T₄ = *A. scrobiculata* + *Rh. intraradices*, T₅ = PSB, T₆ = PSB + *A. scrobiculata*, T₇ = PSB + *Rh. intraradices*, T₈ = PSB + *A. scrobiculata* + *Rh. intraradices*, T₉ = Compost.

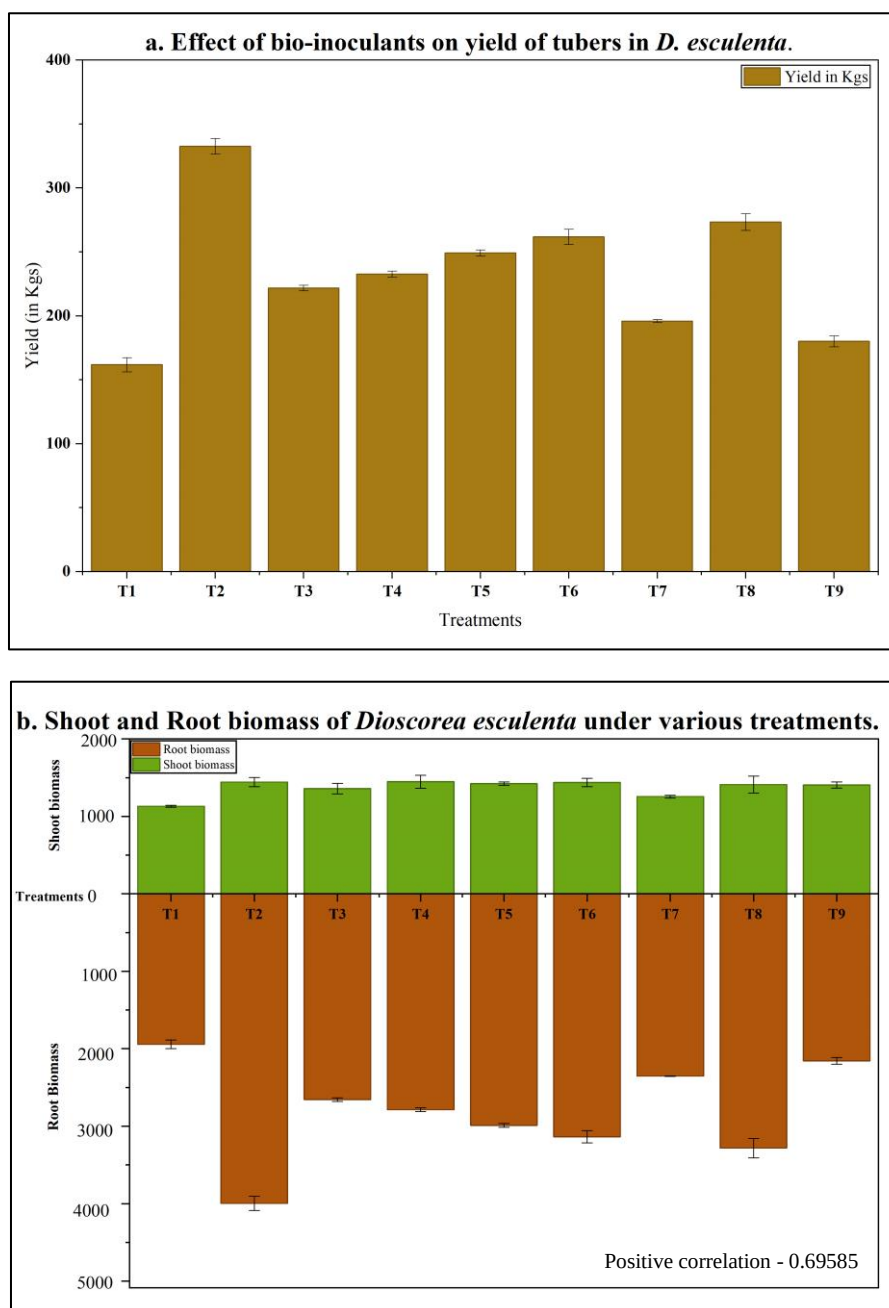
Fig. 7.4: Effect of bio-inoculant treatment on growth parameters of *I. batatas* - a. plant height, b. number of shoots per plant, and c. leaf length.



Legend: T₁ = Control, T₂ = *A. scrobiculata*, T₃ = *Rh. intraradices*, T₄ = *A. scrobiculata* + *Rh. intraradices*, T₅ = PSB, T₆ = PSB + *A. scrobiculata*, T₇ = PSB + *Rh. intraradices*, T₈ = PSB + *A. scrobiculata* + *Rh. intraradices*, T₉ = Compost.

Fig. 7.5: Effect of bio-inoculant treatment on biomass of *I. batatas* - a. shoot biomass-fresh (SBF), and b. shoot biomass-dry (SBD).

Response of tuber yield to bio-inoculation: The study revealed that inoculation of *D. esculenta* with *A. scrobiculata* (T₂) resulted in the highest yield ($332.5 \pm 0.91 \text{ Kg ha}^{-1}$) compared to control ($161.67 \pm 0.56 \text{ Kg ha}^{-1}$) and all other treatments. The combination of PSB, *A. scrobiculata* and *F. mosseae* (T₈) recorded a yield of $273.33 \pm 1.25 \text{ Kg ha}^{-1}$, followed by inoculation with PSB and *A. scrobiculata* (T₆) ($261.67 \pm 0.79 \text{ Kg ha}^{-1}$) (**Fig. 7.6, Plate 7.14**).



Legend: T₁ = Control, T₂ = *A. scrobiculata*, T₃ = *F. mosseae*, T₄ = *A. scrobiculata* + *F. mosseae*, T₅ = PSB, T₆ = PSB + *A. scrobiculata*, T₇ = PSB + *F. mosseae*, T₈ = PSB + *A. scrobiculata* + *F. mosseae*, T₉ = Compost.

Fig. 7.6: Effect of bio-inoculant treatment on the yield parameters of *D. esculenta* - a. shoot biomass-fresh (SBF), and b. shoot biomass-dry (SBD).

Previous studies have shown the beneficial effects of *Acaulospora scrobiculata* across various plants. *A. scrobiculata* is known to improve the growth and survival of micro-propagated *Musa* plantlets (Yano-Melo *et al.*, 1999), enhance nutrient uptake, biomass, and grain dry weight in *Sorghum* (Nakmee *et al.*, 2016), support plant growth, nutrient uptake, and overall quality of *Acacia auriculiformis* seedlings in P-deficient soils when inoculated with PSBs (Muthukumar and Udaiyan, 2018), and contribute to the production and quality of field-cultivated *Saccharum officinarum* (Sales *et al.*, 2022). *Funneliformis mosseae* is also known to enhance plant growth and yield. Inoculation with *F. mosseae* has been shown to enhance growth and fruit yield in *Cucurbita pepo* (Wang *et al.*, 2022).

AM fungi, coupled with PSB, offer an alternative to chemical fertilizers by facilitating P solubilization and enhancing crop yield without causing ecological harm (Wahid *et al.*, 2022).

The study revealed a significant increase in tuber yield in treatments involving AM fungal inoculation (Table 7.9).

Table 7.9: Effect of bioinoculant treatment on the yield of *D. esculenta* and *I. batatas*

<i>D. esculenta</i> (Kg/ha)	Treatments	<i>I. batatas</i> (kg/ha)
161.67±0.56 ^a	T ₁	54.90±0.17 ^a
332.5±0.91^b	T ₂	66.00±0.26 ^a
221.67±0.22 ^{a,b}	T ₃	66.59±0.26 ^a
232.5±0.24 ^{a,b}	T ₄	66.92±0.15 ^a
249.17±0.24 ^{a,b}	T ₅	60.37±0.17 ^a
261.67±0.79 ^{a,b}	T ₆	78.13±0.22 ^a
195.83±0.04 ^a	T ₇	57.63±0.17 ^a
273.33±1.25 ^{a,b}	T ₈	83.44±0.29^a
179.99±0.43 ^a	T ₉	61.74±0.15 ^a

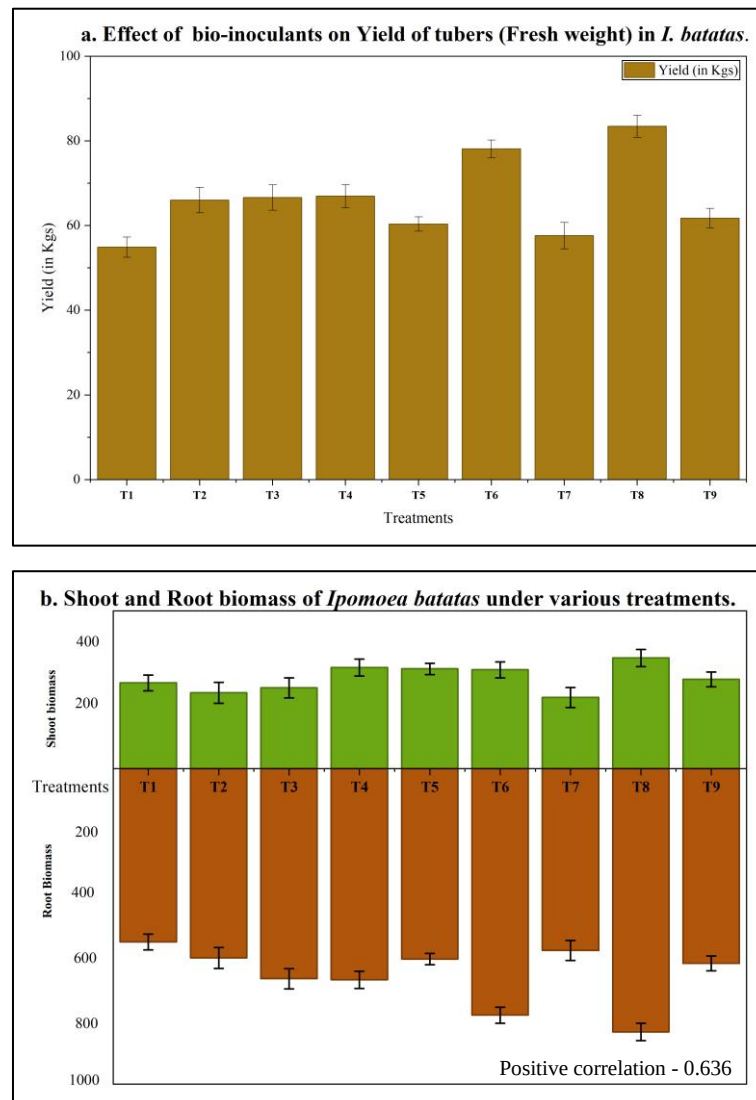
(ANOVA P<0.05,
F=3.896)

(ANOVA =NS)

Legend For *D. esculenta*- T₁ = Control, T₂ = *A. scrobiculata*, T₃ = *F. mosseae*, T₄ = *A. scrobiculata* + *F. mosseae*, T₅ = PSB, T₆ = PSB + *A. scrobiculata*, T₇ = PSB + *F. mosseae*, T₈ = PSB + *A. scrobiculata* + *F. mosseae*, T₉ = Compost.

For *I. batatas*- T₁ = Control, T₂ = *A. scrobiculata*, T₃ = *Rh. intraradices*, T₄ = *A. scrobiculata* + *Rh. intraradices*, T₅ = PSB, T₆ = PSB + *A. scrobiculata*, T₇ = PSB + *Rh. intraradices*, T₈ = PSB + *A. scrobiculata* + *Rh. intraradices*, T₉ = Compost.

In *I. batatas*, the bio-inoculant consortia comprising *A. scrobiculata*, *Rh. intraradices* and PSB (T_8) recorded highest yield ($83.44 \pm 0.29 \text{ Kg ha}^{-1}$) compared to control ($54.90 \pm 0.17 \text{ Kg ha}^{-1}$) and all other treatments. The treatment involving PSB and *A. scrobiculata* (T_6) recorded a yield of $78.13 \pm 0.22 \text{ Kg ha}^{-1}$, followed by the treatment involving dual AM inoculation (T_4) ($66.92 \pm 0.15 \text{ Kg ha}^{-1}$) (**Fig. 7.7; Plate 7.15**). As reported earlier, *A. scrobiculata* is well known to improve crop growth and yield. The combination of AM fungi and PSB has been reported to enhance nutrient uptake, growth, and yield in various crops (Nacoon *et al.*, 2021; Masrahi *et al.*, 2023).



Legend: T_1 = Control, T_2 = *A. scrobiculata*, T_3 = *Rh. intraradices*, T_4 = *A. scrobiculata* + *Rh. intraradices*, T_5 = PSB, T_6 = PSB + *A. scrobiculata*, T_7 = PSB + *Rh. intraradices*, T_8 = PSB + *A. scrobiculata* + *Rh. intraradices*, T_9 = Compost.

Fig. 7.7: Effect of bio-inoculant treatment on the yield parameters of *I. batatas* - a. shoot biomass-fresh (SBF), and b. shoot biomass-dry (SBD).

Previous studies have also suggested that *Rh. intraradices* improves growth and/or yield in various plants. It is known to improve the growth in *Oryza sativa* plants (Shi, *et al.*, 2025). Jie *et al.* (2022) reported a significant increase in seed weight, seed-yield per plant, yield, pods per plant, seed number per plant, branch number, plant height, and fresh weight of root and shoot of *Glycine max*. Similarly, Fracasso *et al.* (2020) reported increased production of *Solanum lycopersicum*.

Our study aims to maximise the yield and nutritional potential of the tubers by applying bioinoculants. Carrara *et al.* (2023) reported that tuber yield and nutritional quality can be positively affected by inoculation with AM fungi. Similarly, Lu *et al.* (2015) observed that AM fungal inoculation of yams increased tuber yield, accelerated plant growth, and increased tuber secondary metabolite content. Consequently, Lu *et al.* (2015) suggest that AM fungi can function as a microbial fertilizer and be developed for yam cultivation and production. AM fungal inoculation is recognized for its reliable yield enhancement. In an earlier study, Wu *et al.* (2022) reported that the yield of 13 widely grown crops under rainfed conditions increased by 23% in seed and pod/fruit production per plant, while the shoot and root biomass increased by 24.2% and 29.6%, respectively.

The present study showed that bio-inoculation resulted in higher yields in *I. batatas* tubers than in the control. However, this increase in yield was not statistically significant (**Table 7.9**).

Significant enhancement in the yield of *D. esculenta* was observed following bio-inoculation, whereas there was no significant increase in *I. batatas*. This reflects the difference in mycorrhizal dependency in the two crops. *D. esculenta* exhibits higher mycorrhizal dependency, in contrast to the extremely low dependency, accounting for a limited yield response as observed in *I. batatas* (**Fig. 7.8**).

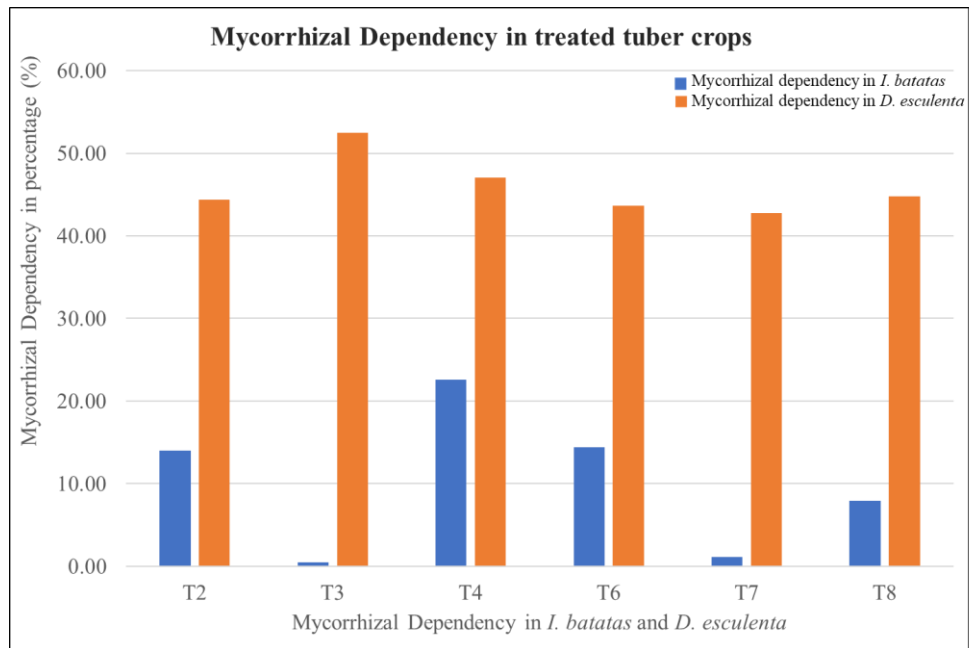


Fig. 7.8: Mycorrhizal dependency in *D. esculenta* and *I. batatas*.

Table 7.10: Effect of AM fungi and PSB on growth and yield characteristics of *D. esculenta*.

Treatments	PH (cm)	LL (cm)	S/P	TW/T (Kg)	SBF (Kg)	SBD (g)
T₁	211.93±0.38	7.72±0.26	10.37±0.73	1.9433±0.56	1129.94±12.08	451.73±3.32
T₂	246.13±8.71	8.54±0.22	10.53±0.93	3.9967±0.91	1442.17±60.01	811.59±5.71
T₃	235.57±9.60	8.53±0.16	11.07±0.61	2.6567±0.22	1357.50±67.48	951.15±2.36
T₄	242.60±10.47	7.91±0.16	11.57±0.47	2.7867±0.24	1446.90±84.42	853.68±48.02
T₅	251.80±5.27	8.55±0.24	10.67±0.99	2.99±0.24	1421.73±22.17	850.78±4.82
T₆	240.67±6.25	8.80±0.15	10.77±0.80	3.1367±0.79	1436.30±54.51	801.62±27.55
T₇	227.63±4.05	7.87±0.51	11.23±0.56	2.3533±0.04	1255.47±17.57	789.42±0.85
T₈	253.43±13.10	8.63±0.78	11.53±0.41	3.2833±1.25	1409.63±109.54	818.47±65.43
T₉	239.03±2.35	7.89±0.51	8.77±0.68	2.1567±0.43	1403.50±39.51	844.95±17.56

Legend: All values are means of three replicates; **PH** = Plant height, **LL** = Leaf length (fifth leaf), **S/P** = shoots per plant, **TW/T** = Tuber weight per treatment, **SBF** = Shoot biomass (fresh), **SBD** = Shoot biomass (dry); **T₁** = Control, **T₂** = *A. scrobiculata*, **T₃** = *Funneliformis mosseae*, **T₄** = *Acaulospora scrobiculata* + *Funneliformis mosseae*, **T₅** = PSB, **T₆** = *Acaulospora scrobiculata* + PSB, **T₇** = *Funneliformis mosseae* + PSB, **T₈** = *Acaulospora scrobiculata* + *Funneliformis mosseae* + PSB, **T₉** = Compost.

Table 7.11: Effect of AM fungi and PSB treatments on yield characteristics of *Ipomoea batatas* at harvesting stage.

Treatments	PH (cm)	LL (cm)	S/P	TW/T (Kg)	SBF (Kg)	SBD (g)
T ₁	84.55±2.29	6.04±0.48	15.67±2.19	549.03±0.17	209.76±24.94	70.8±10.68
T ₂	96.18±13.80	6.16±0.58	17.00±5.13	660.00±0.26	240.43±33.46	82.37±6.59
T ₃	83.62±2.38	6.44±0.43	17.67±2.96	665.87±0.26	256.1±31.9	71.13±7.06
T ₄	103.65±5.83	6.22±0.56	18.33±2.96	669.23±0.15	320.47±27.06	91.9±9.92
T ₅	100.24±10.23	6.28±0.28	17.00±3.79	603.67±0.17	316.37±17.91	74.5±5.39
T ₆	87.05±9.13	6.61±0.49	19.33±4.10	781.27±0.22	313.23±25.47	87.07±6.82
T ₇	84.91±4.55	6.11±0.72	15.67±3.71	576.33±0.17	225.67±31.68	71.6±9.99
T ₈	107.78±8.16	6.25±0.39	21.33±2.03	834.43±0.29	350.84±27.26	77.8±4.48
T ₉	95.59±6.73	6.15±0.14	18.00±1.00	617.40±0.15	282.9±23.39	97.37±10.01

Legend: All values are means of three replicates; **PH** = Plant height, **LL** = Leaf length (fifth leaf), **S/P** = shoots per plant, **TW/T** = Tuber weight per treatment, **SBF** = Shoot biomass (fresh), **SBD** = Shoot biomass (dry); **T₁** = Control, **T₂** = *A. scrobiculata*, **T₃** = *Rh. intraradices*, **T₄** = *A. scrobiculata* + *Rh. intraradices*, **T₅** = PSB, **T₆** = PSB + *A. scrobiculata*, **T₇** = PSB + *Rh. intraradices*, **T₈** = PSB + *A. scrobiculata* + *Rh. intraradices*, **T₉** = Compost.

Nutritional quality of selected species: Previous work by Lombardo *et al.* (2021) has explored the potential benefits of AM fungal associations with organically grown potato (*Solanum tuberosum*) plants, suggesting a quantitative increase in tuber content of both macro- and micro-nutrients *via* soil mycorrhization. However, the impact of soil-applied AM fungi on tuber mineral accumulation is mediated by multiple factors, including cultivar genotype. Moreover, observed benefits of AM fungal inoculation are strongly influenced by geographic location and soil properties. Gosling *et al.* (2016) report that AM fungal inoculation can elevate certain biomass and nutrient uptake metrics in onions, but may reduce concentrations of other nutrients. The study underscores the challenge of disentangling the role of AM fungal diversity in ecosystem function, noting that interactions among the host, AM fungi, and the environment are complex and context-dependent.

Studies by Hart and Forsythe (2012) indicate that the impact of AM fungi on plant micronutrient nutrition is variable, with reports of enhancement, reduction, or no effect. This variability may stem from factors such as functional specialisation among AM fungi and the mycorrhizal dependency of the chosen crop species.

In the present study, the nutritional quality of the two species was studied. This involved estimating moisture, total ash, fat, protein, crude fibre, carbohydrates, energy, total sugars, Ca, Mg, K, and P. The results of the nutritional analysis in *D. esculenta* tubers inoculated with various treatments are given in **Table 7.12**.

The results in *D. esculenta* indicated that treatment with *A. scrobiculata* recorded a minimal increase in both carbohydrate content and energy. The results in *I. batatas* suggest that treatment with *Rh. intraradices* and PSB increased carbohydrates and total sugars compared to all other treatments and the control.

For both *D. esculenta* and *I. batatas*, AM fungal inoculation resulted in enhanced yield (**Table 7.9**) but did not improve the nutritional quality (**Tables 7.12 & 7.13**).

Table 7.12: The nutritional analysis in *D. esculenta*.

Sr. No.	Parameter	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉
1.	Moisture	14.58±0.04	10.08±0.04	11.36±0.03	15.08±0.04	10.94±0.05	10.76±0.03	10.26±0.03	10.79±0.05	10.74±0.05
2.	Total Ash	2.04±0.03	2.68±0.05	2.94±0.03	2.46±0.03	2.64±0.03	2.62±0.03	3±0.03	2.7±0.03	2.74±0.03
3.	Fat	0.59±0.02	0.48±0.02	0.39±0.02	0.47±0.02	0.36±0.02	0.54±0.02	0.4±0.02	0.7±0.02	0.38±0.02
4.	Protein	5.38±0.05	4.93±0.06	5.18±0.05	4.87±0.05	4.58±0.06	6.27±0.06	7.09±0.07	5.82±0.05	6.33±0.06
5.	Crude Fibre	0.89±0.02	0.8±0.02	0.71±0.02	0.75±0.02	0.97±0.02	0.9±0.02	0.78±0.02	0.84±0.02	0.74±0.02
6.	Carbohydrates	76.52±0.06	81.03±0.07	76.37±0.09	79.42±0.06	78.91±0.06	80.51±0.06	78.11±0.06	79.51±0.06	79.07±0.06
7.	Energy	332.91±0.04	348.16±0.04	341.91±0.05	329.19±0.04	343.6±0.03	345.58±0.03	347.1±0.03	344.92±0.03	345.02±0.04
8.	Total Sugar	3.39±0.05	3.22±0.06	3.64±0.07	4.97±0.09	5.26±0.08	7.98±0.09	7.35±0.08	6.96±0.08	8.19±0.11
9.	Ca	96±3.07	64±2.01	96±3.07	64±1.54	56±2.09	56±1.54	48±1.54	56±1.74	56±3.07
10.	Mg	200±0.89	116±3.07	130±2.53	136±3.07	132±2.09	141±3.23	156±3.07	141±3.07	151±3.23
11.	K	800±5.80	1100±5.80	1000±5.80	740±5.80	1040±2.90	960±2.90	1200±5.80	1060±5.80	1140±5.80
12.	P	309±8.89	223±1.54	261±3.07	205±2.66	220±2.53	213±1.54	205±3.62	232±2.09	212±3.81

Legend: T₁ = Control, T₂ = *A. scrobiculata*, T₃ = *F. mosseae*, T₄ = *A. scrobiculata* + *F. mosseae*, T₅ = PSB, T₆ = PSB + *A. scrobiculata*, T₇ = PSB + *F. mosseae*, T₈ = PSB + *A. scrobiculata* + *F. mosseae*, T₉ = Compost; **Moisture, total ash, fat, protein, crude fibre, carbohydrates, and total sugars** - given as g 100 g⁻¹; **Ca, Mg, K, and P** - given as mg100 g⁻¹; Energy - given as Kcal.

The results of the nutritional analysis of *I. batatas* tubers inoculated with various treatments are depicted in **Table 7.13**.

Table 7.13: The nutritional analysis in *I. batatas*.

Sr. No.	Parameter	T ₁	T ₂	T ₃	T ₄	T ₅	T ₆	T ₇	T ₈	T ₉
1.	Moisture	6.54±0.01	5.9±0.01	6±0.01	6.15±0.01	5.97±0.01	5.96±0.01	6.59±0.01	6.22±0.01	6.22±0.02
2.	Total Ash	2.22±0.01	2.22±0.01	2.22±0.02	1.82±0.01	1.98±0.01	2.16±0.01	1.8±0.01	2.26±0.01	2.34±0.01
3.	Fat	1.08±0.01	0.66±0.01	0.54±0.01	0.99±0.01	0.79±0.01	0.98±0.01	0.93±0.01	0.92±0.01	1.08±0.02
4.	Protein	2.63±0.01	3.6±0.01	4.02±0.15	3.65±0.01	3.22±0.01	3.22±0.02	3.78±0.2	3.04±0.01	3.33±0.01
5.	Crude Fiber	2.25±0.01	2.42±0.01	2.52±0.01	1.84±0.01	1.83±0.01	2.14±0.01	2.1±0.01	1.96±0.01	2.4±0.01
6.	Carbohydrates	85.28±0.01	85.2±0.9	84.6±0.01	85.55±0.18	86.21±0.71	85.54±0.01	84.76±0.01	85.6±3.01	84.63±0.01
7.	Energy	361.36±0.02	361.14±0.93	360.24±1.71	365.71±0.02	364.83±0.02	363.86±0.02	362.53±0.02	362.84±2.91	361.56±0.02
8.	Total Sugar	14.1±0.05	14.19±0.02	16.88±0.12	13.2±0.22	12.31±0.02	15.16±0.02	15.14±0.3	14.68±0.02	12.24±0.02
9.	Ca	152±3.53	170±1.54	112±1.54	120±1.74	160±1.54	160±1.74	128±1.16	144±1.54	128±1.01
10.	Mg	44±1.54	78±1.54	48±1.16	53±1.54	78±1.54	68±1.74	78±2.09	78±2.53	68±1.54
11.	K	380±2.66	420±2.66	460±2.66	480±2.9	500±2.66	480±2.66	600±4.07	620±2.66	800±2.66
12.	P	213±1.16	225±2.53	239±1.54	212±1.54	243±1.54	205±1.54	227±2.53	258±1.54	149±1.54

Legend: T₁ = Control, T₂ = *A. scrobiculata*, T₃ = *Rh. intraradices*, T₄ = *A. scrobiculata* + *Rh. intraradices*, T₅ = PSB, T₆ = PSB + *A. scrobiculata*, T₇ = PSB + *Rh. intraradices*, T₈ = PSB + *A. scrobiculata* + *Rh. intraradices*, T₉ = Compost; **Moisture, total ash, fat, protein, crude fibre, carbohydrates, and total sugars** - given as g 100 g⁻¹; **Ca, Mg, K, and P** - given as mg100 g⁻¹; **Energy** - given as Kcal.

The concept of crop nutrient dilution with increasing shoot biomass has been widely developed and studied (Fontana *et al.*, 2025). In the past, the dilution effect has primarily been associated with the application of fertilizer or other nutrient materials, including mycorrhizal fungi, where, although one observes increased growth, mineral concentrations do not increase. Dilution effect is a decrease in mineral nutrient concentration due to increased dry matter production (Jarrell and Beverly, 1981). Nutrient concentrations of P, K, S, and Cu have also been shown to decrease with increasing shoot biomass during vegetative developmental stages of the crop (Davis, 2009; Fontana *et al.*, 2025).

In our study, too, there was no evident improvement in nutritional content in either of the selected tubers. This may be attributed to the dilution effect, in which the biomass increases without a proportional increase in nutrient uptake, and hence the concentration of nutrients in plant tissues appears lower (Jarrell and Beverly, 1981; Davis, 2009; Fontana *et al.*, 2025).

7.4: CONCLUSION

The identification of a P-solubilizing *Pseudomonas* sp. as a complementary bio-inoculant further strengthens the applied significance of these findings. Importantly, a comprehensive understanding of AM fungal diversity and community structure in tuber cropping systems provides a powerful foundation for developing biologically based soil management strategies. Harnessing native and functionally dominant AM fungi, particularly in combination with beneficial PSBs, offers substantial potential to improve nutrient acquisition, enhance soil health, and promote sustainable tuber production with reduced reliance on chemical inputs.

Tuber species extensively cultivated and consumed in Goa require increased productivity to meet rising demand. Increasing their production is particularly important given their significant contribution to food and nutritional security, as well as the substantial economic benefits they provide to the farming community.

In conclusion, the present study demonstrates that bio-inoculants positively influence tuber species productivity, reinforcing the agronomic significance of AM fungi in sustainable agricultural systems. The pronounced yield response observed in *D. esculenta* underscores its strong mycorrhizal dependence and confirms the reliability of

AM fungal inoculation as a strategy for achieving substantial yield gains. In contrast, the comparatively modest, statistically non-significant yield increase in *I. batatas* aligns with its lower dependence on mycorrhizal associations, highlighting species-specific responses to bio-inoculation.

Although AM fungal inoculation did not enhance nutritional quality, this outcome is plausibly attributable to a dilution effect associated with increased biomass production. Collectively, the substantial yield improvement recorded in *D. esculenta* provides compelling evidence that targeted application of bio-inoculants can sustainably elevate crop production while maintaining minimal environmental impact.

Chapter 8: Summary

With the growing world population, food security is a major concern. Traditional farming uses many resources, such as chemical fertilisers, which pollute and also reduce biodiversity, thus threatening environmental health. Sustainable agriculture emerges as an essential alternative, combining renewable energy with agroecological methods. Key practices include crop rotation, organic farming, agroforestry, and, more importantly, the use of beneficial microbes to improve soil fertility. Microbes are essential for protecting soil and the environment, enabling economically viable, socially fair farming, and safeguarding the planet for future generations. These approaches limit the use of synthetic pesticides, preserve soil health and support biodiversity. Leveraging local resources and traditional knowledge helps maintain ecological balance while still increasing crop yields.

AM fungi are essential components of the microbiome that support sustainable agricultural practices. These AM fungi form intricate symbiotic associations with plant roots, significantly enhancing nutrient uptake, water absorption, and protection against biotic and abiotic stresses. They play a pivotal role in sustainable crop disease management and yield enhancement. Thus, AM fungi offer a promising, sustainable alternative to chemical inputs in modern agriculture, consistent with global sustainability and food security goals.

Speaking about food security, tuber and root crops are indispensable in ensuring global food security and supporting economic stability, especially for millions of farmers worldwide. Renowned for their high energy content, dietary versatility, and nutritional benefits, these crops are a cornerstone in combating malnutrition and fostering sustainable agriculture. They thrive across diverse climates, require lower inputs, and are increasingly being targeted for genetic improvement to enhance yields and climate resilience.

Studying AM fungal associations with tuber crops in Goa is crucial for promoting sustainable farming, enhancing crop yields, and protecting our local environment. These beneficial fungi help plants, especially tuber crops, absorb nutrients, especially phosphorus, and make them more resilient to stresses such as pests, diseases, and

challenging weather conditions. Since Goa has a unique climate and soils, understanding the native AM fungi and how they work with local tuber crops can help farmers adopt natural, eco-friendly practices that cut down on expensive and environment-degrading chemical fertilizers and pesticides. This not only supports organic farming traditions but also helps protect the region's rich microbial biodiversity. This research aims at leading to smarter, climate-resilient farming methods tailored specifically for Goa, improving harvests, reducing environmental impact, and supporting the livelihoods of local farmers. It's a practical and necessary step toward building a more sustainable and prosperous future for the region's agriculture.

In the present study, we aimed to do the same by first understanding the AM fungal colonization and diversity in both wild and cultivated tuber species found in Goa. The phenology of the AM fungi associated with some tuber crops was also studied. Upon understanding the dominance of AM fungi in the selected, economically beneficial tuber crops, the dominant species were mass multiplied for further use. The effect of these selected AM fungal species was then assessed on the selected tuber crops. The details are mentioned in the chapter below.

Identifying tuber crops exhibiting healthy AM fungal colonization stands as a pivotal strategy in sustainable agriculture, enabling the targeted inoculation of these beneficial microbes to enhance growth and yield while rigorously ensuring soil and environmental safety. This approach exemplifies the indispensable role of microbes in fortifying ecological balance while still gaining excellent yields. In this study, Sampling was conducted at 49 distinct study sites, designated as S-I to S-XLIX, where rhizosphere soil and root samples were collected from seven cultivated viz., *A. paeoniifolius* (Dennst.) Nicolson, *Colocasia antiquorum* Schott., *Dioscorea alata* Linn., *D. bulbifera* L., *D. esculenta* (Lour.) Burkill, *Ipomea batatas* (L.) Lam, and *Solenostemon rotundifolius* (Poir) J. K. Morton. and five wild tuber species viz., *Alocasia macrorrhiza* (L.) G. Don, *Amorphophallus commutatus* (Schott) Engl., *C. esculenta* (L.) Schott., *Gloriosa superba* L., and *Momordica sahyadrica* Kattuk. Rhizosphere samples of tuber species from the selected sites were used to evaluate AM fungal colonization and diversity.

AM fungal colonization was recorded in the rhizosphere of all the wild and cultivated tuber species studied. All the species recorded the presence of hyphae, arbuscules, and vesicles. Among these structures, hyphae and vesicles were more prevalent than arbuscules, indicating a variable pattern of colonization across sites.

This study also demonstrated that total colonization in both wild and cultivated tuber species is primarily driven by soil pH, thereby establishing pH as the key environmental determinant of AM fungal association. In cultivated tubers, it was understood, that a secondary influence emerges from Cu, with total colonization showing a modest association with Cu. This suggests that while pH governs the extent of AM colonization generally, Cu availability may influence colonization levels in cultivated populations, but the effect is relatively limited.

The study also found that seasonal changes in AM colonization are affected by several factors, especially temperature, pH, and seasonal conditions, which significantly shape their distribution.

The strong influence of pH on AM fungal colonization underscores soil management as a key factor for optimising AM fungal relationships in tuber crops.

Studies on AM fungal diversity offer essential insights into how terrestrial ecosystems function by revealing interactions between AM fungi and their environments. These studies also serve as ecological indicators, revealing soil characteristics and climate conditions from the presence and distribution of specific AM fungal species.

The present investigation at the mentioned 49 sites, for five wild and seven cultivated tuber species revealed that AM fungal assemblages associated with tuber crops are highly diverse, well-distributed, and structurally stable across both wild and cultivated systems. From the wild tuber species, a total of 15 AM fungi were identified viz., *Acaulospora delicata*, *A. dilatata*, *A. foveata*, *A. scrobiculata*, *Claroideoglossum etunicatum*, *Funneliformis geosporum*, *F. mosseae*, *Gigaspora albida*, *Gi. decipiens*, *Glomus* sp., *Racocetra gregaria*, *Rhizophagus fasciculatus*, *Rh. intraradices*, *Rh. irregularis*, *Sieverdingia tortuosa*; while 23 AM fungi were identified from the cultivated tuber species viz., *Acaulospora bireticulata*, *A. delicata*, *A. dilatata*, *A. foveata*, *A. laevis*, *A. scrobiculata*, *A. spinosa*, *Claroideoglossum etunicatum*,

Funneliformis coronatum, *F. geosporum*, *F. mosseae*, *Gigaspora albida*, *Gi. decipiens*, *Glomus microcapum*, *Gl. multisporum*, *Glomus* sp., *Racocetra gregaria*, *Rhizophagus fasciculatus*, *Rh. intraradices*, *Rh. irregularis*, *Sacculospora* sp., *Scutellospora calospora*, *Sieverdingia tortuosa*.

Amongst these AM fungi, substantial overlap in dominant taxa was observed, especially *A. scrobiculata*, *F. mosseae*, and *Rh. intraradices*, suggesting a resilient mycorrhizal community with a core adapted to tuberous hosts. The presence of taxa like *A. foveata* and *Gi. decipiens* in wild tubers, along with *F. geosporum* in cultivated tubers, indicates habitat-specific ecological filtering. The persistent dominance of the family Glomeraceae, followed by Acaulosporaceae, Gigasporaceae, and Claroideoglomaceae, highlights a stable taxonomic structure across various management conditions. High Shannon Wiener diversity, combined with moderately high Simpson's dominance and favourable evenness indices, reflects species-rich communities with minor dominance and a balanced distribution, signifying ecologically resilient and functionally diverse AM fungal networks.

The study's Canonical Correspondence Analysis confirms that the composition of AM fungal communities is not determined by any single soil variable. Instead, it is shaped by interacting soil chemical gradients, underscoring the multivariate influence of environmental factors on fungal community structure. This combined response to soil chemistry illustrates the adaptability of AM fungi and their ability to survive in diverse soil conditions.

The importance of P-solubilizing bacteria working alongside AM fungi was well recognised.

Thus, a P-solubilising *Pseudomonas* sp. was identified as a complementary bio-inoculant. This further strengthened the applied significance of our findings. Importantly, a comprehensive understanding of AM fungal diversity and community structure in tuber cropping systems provides a powerful foundation for developing biologically based soil management strategies. Harnessing native and functionally dominant AM fungi, particularly in combination with beneficial PSBs, offered substantial potential to improve nutrient acquisition, enhance soil health, and promote sustainable tuber production with reduced reliance on chemical inputs.

Tuber species widely cultivated and consumed in Goa require higher productivity to meet the increasing demand. Enhancing their production is crucial given their vital role in food and nutritional security, as well as the considerable economic benefits they offer farmers. This study also aimed to evaluate how these dominant AM fungal species influence the growth and yield of selected tuber crops.

After a detailed study of 12 wild and cultivated tuber species across 49 sites in Goa, AM fungal colonization and diversity were assessed, and economically important tubers with high colonisation rates were identified. Also, the dominant AM fungal species in the selected tuber crops were identified. Consequently, *Dioscorea esculenta* and *Ipomoea batatas* were identified as the two tuber crops to be studied. *Acaulospora scrobiculata*, *Funnelformis mosseae* and *Rhizophagus intraradices* were identified as the dominant AM fungal species to be employed in the trials. These dominant AM fungal species were then mass-multiplied using standard methods to produce pure AM fungi in pure pot cultures. The P-solubilizing *Pseudomonas* sp. isolated during the study was also used in the trials along with the AM fungi. The two AM fungi and the PSB were employed on each tuber crop for a total of nine treatments (**T₁** to **T₉**), in various combinations as given; **T₁** – Control; **T₂** - Single AM fungal species-1; **T₃** – Single AM fungal species- 2; **T₄** – Both AM fungi; **T₅** -PSB; **T₆** – AM fungal species - 1+ PSB; **T₇** – AM fungal species-2 + PSB; **T₈** – Consortium of all three micro-organisms; **T₉** – Compost.

The bio-inoculants were observed to have a positive effect on the growth as well as yield of the tuber crops.

The current study shows that bio-inoculants enhance tuber crop productivity, underlining the crucial role of AM fungi in sustainable farming. The notable yield boost in *D. esculenta* underscores its mycorrhizal dependency and supports the use of AM fungal inoculation as a viable method to increase yields. In contrast, the modest, statistically insignificant increase in *I. batatas* indicates its lower mycorrhizal dependence, demonstrating that different species respond uniquely to bio-inoculation.

The study also demonstrated that bio-inoculation had no effect on the nutritional content of the treated tuber species. AM fungal inoculation did not improve nutritional quality; this outcome is likely due to a dilution effect caused by increased biomass production. Overall, the significant yield increase observed in *D. esculenta* provides

strong evidence that targeted use of bio-inoculants can sustainably boost crop production with minimal environmental impact.

This investigation concludes that analyzing indigenous AM fungal diversity and root colonization in tuber species reveals dominant taxa suitable for bio-inoculant development for field applications. Rigorous isolation and mass multiplication of these fungi, alongside P-solubilizing bacteria, via established protocols yield potent, eco-safe inoculants for sustainable deployment. Applied as bio-inoculants, AM fungi and PSBs drive favourable growth and yield gains in local tuber crops, with outcomes shaped by edaphic conditions, crop-specific mycorrhizal affinity, growth timing, and dependency levels. Strategic integration of these microbes, informed by such factors, proves essential for cultivating high-value tubers sustainably, enhancing farmer prosperity while preserving soil integrity and environmental health.

REFERENCES:

- Adenan, S., Oja, J., Alatalo, J. M., Shraim, A. M., Alsafran, M., Tedersoo, L., Zobel, M., and Ahmed, T. (2021). Diversity of Arbuscular Mycorrhizal fungi and its chemical drivers across dryland habitats. *Mycorrhiza*, 31(6), 685–697.
- Alam, A., and Gabriel-Neumann, E. (2025). Arbuscular Mycorrhizal fungi travel the world with harvested underground crops. *Mycorrhiza*, 35(1), 4.
- ALHadidi, N., Pap, Z., Ladányi, M., Szentpéteri, V., and Kappel, N. (2021). Mycorrhizal inoculation effect on sweet potato [*Ipomoea batatas* (L.) Lam] seedlings. *Agronomy*, 11(10), 2019.
- Almeida, R. T., and Schenck, N. C. (1990). A revision of the genus *Sclerocystis* (Glomaceae, Glomales). *Mycologia*, 82(6), 703–714.
- Ambrosini, V. G., Voges, J. G., Canton, L., ... and Soares, C. R. F. S. (2015). Effect of Arbuscular Mycorrhizal fungi on young vines in copper-contaminated soil. *Brazilian Journal of Microbiology*, 46(4), 1045–1052.
- Anand, K., Pandey, G. K., Kaur, T., Pericak, O., Olson, C., Mohan, R., ... and Yadav, A. N. (2022). Arbuscular mycorrhizal fungi as a potential biofertilizers for agricultural sustainability. *Journal of Applied Biology and Biotechnology*, 10(1), 90-107.
- Anaswara, S., Ajithkumar, V., Balachandar, M., and Muthukumar, T. (2023). Endorrhizal fungal symbiosis in aroids of the Western Ghats, Southern India. *Notulae Scientia Biologicae*, 15(4), 11651–11651.
- Antoszewski, M., Mierek-Adamska, A., and Dąbrowska, G. B. (2022). The importance of microorganisms for sustainable agriculture: A review. *Metabolites*, 12(11), 1100.
- Asmelash, F., Bekele, T., and Birhane, E. (2016). The potential role of Arbuscular Mycorrhizal fungi in the restoration of degraded lands. *Frontiers in Microbiology*, 7, 1095. <https://doi.org/10.3389/fmicb.2016.01095>.
- Atu, L. L. (2013). *Studies on propagation materials and growing conditions for sweet potato [Ipomoea batatas (L.) Lam.] production* (Master's thesis, The University of Queensland), 1-139.
- Balachandar, M., Koshila Ravi, R., and Muthukumar, T. (2022). Vegetative anatomy and endorrhizal fungal morphology of an endangered medicinal plant *Gloriosa superba* L. *Microscopy Research and Technique*, 85(10), 3296–3308.

- Baltazar-Bernal, O., Spinoso-Castillo, J. L., Mancilla-Álvarez, E., and Bello-Bello, J. J. (2022). Arbuscular Mycorrhizal fungi induce tolerance to salinity stress in taro plantlets (*Colocasia esculenta* L. Schott) during acclimatization. *Plants*, 11(13), 1780.
- Bano, S. A., and Uzair, B. (2021). Arbuscular Mycorrhizal Fungi (AMF) for improved plant health and production. In *Microbial Biotechnology in Crop Protection* (pp. 147–169). Singapore: Springer.
- Bao, X., Zou, J., Zhang, B., Wu, L., Yang, T., and Huang, Q. (2022). Arbuscular Mycorrhizal fungi and microbe interaction in rice mycorrhizosphere. *Agronomy*, 12(6), 1277.
- Begum, N., Qin, C., Ahanger, M. A., ... and Zhang, L. (2019). Role of Arbuscular Mycorrhizal fungi in plant growth regulation: Implications in abiotic stress tolerance. *Frontiers in Plant Science*, 10, 1068.
- Behera, S., Chauhan, V. B. S., Pati, K., Bansode, V., Nedunchezhiyan, M., Verma, A. K., and Naik, S. K. (2022). Biology and biotechnological aspects of sweet potato (*Ipomoea batatas* L.): A commercially important tuber crop. *Planta*, 256(2), 40.
- Bennett, A. E., Daniell, T. J., Oepik, M., ... and Evans, D. (2013). Arbuscular Mycorrhizal fungal networks vary throughout the growing season and between successional stages. *PloS one*, 8(12), e83241.
- Berger, F., and Gutjahr, C. (2021). Factors affecting plant responsiveness to Arbuscular Mycorrhiza. *Current Opinion in Plant Biology*, 59, 101994.
- Berruti, A., Bianciotto, V., and Lumini, E. (2018). Seasonal variation in winter wheat field soil Arbuscular Mycorrhizal fungus communities after non-mycorrhizal crop cultivation. *Mycorrhiza*, 28(5), 535–548.
- Birch, G. G. (1985). Methods of carbohydrate analysis. *Asean Journal on Science and Technology for Development*, 2(1), 88-97.
- Blaszkowski, J. (2012). *Glomeromycota*. (pp. 1-303) W. Szafer Institute of Botany, Polish Academy of Sciences, Poland. 1–303. ISBN: 978-83-89648-82-2.
- Bray, R. H., and Kurtz, L. T. (1945). Determination of total, organic, and available forms of phosphorus in soils. *Soil science*, 59(1), 39-46.
- Bücking, H., Liepold, E., and Ambilwade, P. (2012). The role of the mycorrhizal symbiosis in nutrient uptake of plants and the regulatory mechanisms underlying. *Plant Science*, 107.

- Bukhari, M. J., and Rodrigues, B. F. (2023). Seasonal dynamics of Arbuscular Mycorrhizal fungi from iron ore mine wastelands of Goa, India. *Kavaka*, 59(4), 75–92. DOI: 10.36460/Kavaka/59/4/2023/75-92.
- Cahyani, V. R., Fadhillah, A. H. N., Nabawi, A. R., and Krisdhiarto, F. P. B. (2024). Comparing the population density, colony diversity, and phosphate solubilization ability between rice root endophytic and rice rhizospheric soil bacteria from organic wetland. In *IOP Conference Series: Earth and Environmental Science* (Vol. 1362, No. 1, p. 012028). IOP Publishing.
- Campo, S., Martín-Cardoso, H., Olivé, M., Pla, E., Catala-Forner, M., Martínez-Eixarch, M., and San Segundo, B. (2020). Effect of root colonization by Arbuscular Mycorrhizal fungi on growth, productivity and blast resistance in rice. *Rice*, 13(1), 42. <https://doi.org/10.1186/s12284-020-00411-7>.
- Carrara, J. E., Reddivari, L., Lehotay, S. J., Zinati, G., and Heller, W. P. (2023). Arbuscular Mycorrhizal fungi increase the yield and nutritional quality of yellow and purple fleshed potatoes (*Solanum tuberosum*). *American Journal of Potato Research*, 100(3), 210–220.
- Carrino-Kyker, S. R., Kluber, L. A., Petersen, S. M., ... and Burke, D. J. (2016). Mycorrhizal fungal communities respond to experimental elevation of soil pH and P availability in temperate hardwood forests. *FEMS Microbiology Ecology*, 92(3), fiw024.
- Chaudhary, A., Poudyal, S., and Kaundal, A. (2025). Role of Arbuscular Mycorrhizal fungi in maintaining sustainable agroecosystems. *Applied Microbiology*, 5(6), 1.
- Chauhan, S., Mahawar, S., Jain, D., Udpadhyay, S. K., Mohanty, S. R., Singh, A., and Maharjan, E. (2022). Boosting sustainable agriculture by arbuscular mycorrhiza under stress condition: Mechanism and future prospective. *Biomed Research International*, 2022(1), 5275449.
- Chauhan, V. B. S., Mallick, S. N., Pati, K., Arutselvan, R., and Nedunchezhiyan, M. (2022). Status and Importance of Underexploited Tuber Crops in Relation to Nutritional Security and Economic Prosperity. *Compendium for Winter School on “Unexpected Vegetables: Unexplored Treasure Trove for Food, Nutritional and Economic Security*, (pp. 246–264).
- Chifetete, V. W., and Dames, J. F. (2020). Mycorrhizal interventions for sustainable potato production in Africa. *Frontiers in Sustainable Food Systems*, 4, 593053.

- D'Souza, J., and Rodrigues, B. F. (2013). Seasonal diversity of Arbuscular Mycorrhizal fungi in mangroves of Goa, India. *International Journal of Biodiversity*, 2013(1), 196527.
- Dal Cortivo, C., Barion, G., Ferrari, M., Visioli, G., *et al.* (2018). Effects of field inoculation with VAM and bacteria consortia on root growth and nutrient uptake in common wheat. *Sustainability*, 10(9), 3286. <https://doi.org/10.3390/su10093286>.
- Dalpe, Y., and Monreal, M. (2004). Arbuscular Mycorrhiza inoculum to support sustainable cropping systems. *Crop Management*, 3(1), 1–11. <https://doi.org/10.1094/CM-2004-0301-09-RV>.
- Dare, M. O., Abaidoo, R., Fagbola, O., and Asiedu, R. (2013). Diversity of Arbuscular Mycorrhizal fungi in soils of yam (*Dioscorea* spp.) cropping systems in four agroecologies of Nigeria. *Archives of Agronomy and Soil Science*, 59(4), 521–531.
- Davis, D. R. (2009). Declining fruit and vegetable nutrient composition: What is the evidence?. *Horticulture Science*, 44(1), 15–19.
- De Moura, M. A., Oki, Y., Arantes-Garcia, L., Cornelissen, T., Nunes, Y. R. F., and Fernandes, G. W. (2022). Mycorrhizal fungi application as a successful tool for worldwide mine land restoration: Current state of knowledge and the way forward. *Ecological Engineering*, 178, 106580. <https://doi.org/10.1016/j.ecoleng.2022.106580>.
- Devi, S. H., Bhupenchandra, I., Sinyorita, S., Chongtham, S. K., and Devi, E. L. (2021). Mycorrhizal fungi and sustainable agriculture. *Nitrogen in Agriculture – Physiological, Agricultural and Ecological Aspects*, 1–19.
- Dewitte, O., Jones, A., Spaargaren, O., ... Montanarella, L. (2013). Harmonisation of the soil map of Africa at the continental scale. *Geoderma*, 211, 138–153. <https://doi.org/10.1016/j.geoderma.2013.07.007>.
- DiPietro, C., Kramer, R. E., and Sassaman, W. A. (1962). Micro-Carius Determination of Phosphorus in Organic Compounds. *Analytical Chemistry*, 34(4), 586–587.
- Drummond, A. J., Suchard, M. A., Xie, D., and Rambaut, A. (2012). Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, 29(8), 1969–1973.
- Elekhtyar, N. M., Awad-Allah, M. M., Alshallash, K. S., Alatawi, A., Alshegaihi, R. M., and Alsalmi, R. A. (2022). Impact of Arbuscular Mycorrhizal fungi,

- phosphate solubilizing bacteria and selected chemical phosphorus fertilizers on growth and productivity of rice. *Agriculture*, 12(10), 1596.
- Fall, A. F., Nakabonge, G., Ssekandi, J., ... and Ngom, K. (2022). Roles of Arbuscular Mycorrhizal fungi on soil fertility: Contribution in the improvement of physical, chemical, and biological properties of the soil. *Frontiers in Fungal Biology*, 3, 723892. <https://doi.org/10.3389/ffunb.2022.723892>
- Feng, Z., Liu, X., Qin, Y., Feng, G., Zhou, Y., Zhu, H., and Yao, Q. (2023). Cooperation of Arbuscular Mycorrhizal fungi and bacteria to facilitate the host plant growth dependent on soil pH. *Frontiers in Microbiology*, 14, 1116943.
- Fontana, M., Bélanger, G., Ciampitti, I., Ziadi, N., Guillaume, T., Steiner, S., and Bragazza, L. (2025). Critical dilution curves for phosphorus, potassium, and sulfur along with relationships to nitrogen for major crops. *Plant and Soil*, 1–14.
- Gadkar, V., David-Schwartz, R., Kunik, T., and Kapulnik, Y. (2001). Arbuscular Mycorrhizal fungal colonization: Factors involved in host recognition. *Plant Physiology*, 127(4), 1493–1499. <https://doi.org/10.1104/pp.010783>
- Gai, J. P., Feng, G., Christie, P., and Li, X. L. (2006). Screening of Arbuscular Mycorrhizal fungi for symbiotic efficiency with sweet potato. *Journal of Plant Nutrition*, 29(6), 1085–1094.
- George, E., Marschner, H., and Jakobsen, I. (1995). Role of Arbuscular Mycorrhizal fungi in uptake of phosphorus and nitrogen from soil. *Critical Reviews in Biotechnology*, 15(3–4), 257–270.
- George, N. P., and Ray, J. G. (2023). The inevitability of arbuscular mycorrhiza for sustainability in organic agriculture: A critical review. *Frontiers in Sustainable Food Systems*, 7, 1124688.
- Gerdemann, J. W., and Nicolson, T. H. (1963). Spores of mycorrhizal endogone species extracted from soil by wet sieving and decanting. *Transactions of the British Mycological Society*, 46(2), 235–244. [https://doi.org/10.1016/S0007-1536\(63\)80079-0](https://doi.org/10.1016/S0007-1536(63)80079-0)
- Ghislain, M. M. G., Emmilienne, D. T., Mari, E. D., ... and Elie, F. (2024). Growth, profitability, nutritional, and anti-nutritional properties of seven *Manihot esculenta* Crantz (cassava) varieties as affected by Arbuscular Mycorrhizal fungi. *Heliyon*, 10(17).
- Gilmore, A. (1968). Phycomycetous mycorrhizal organisms collected by open-pot culture methods. *Hilgardia*, 39(4), 87–105. <https://doi.org/10.3733/hilg.v39n04p087>

- Giovannetti, M., Avio, L., and Sbrana, C. (2010). Fungal spore germination and pre-symbiotic mycelial growth: Physiological and genetic aspects. In *Arbuscular Mycorrhizas: Physiology and Function* (pp. 3–32). Dordrecht: Springer Netherlands.
- Giovannetti, M., Azzolini, D., and Citernesi, A. S. (1999). Anastomosis formation and nuclear and protoplasmic exchange in Arbuscular Mycorrhizal fungi. *Applied and Environmental Microbiology*, 65(12), 5571–5575.
- Giovannetti, M., Sbrana, C., and Avio, L. (2002). Arbuscular Mycorrhizal fungal mycelium: from germlings to hyphal networks. In *Mycorrhizal Technology in Agriculture: From Genes to Bioproducts* (pp. 49–58). Basel: Birkhäuser Basel.
- Giri, B., and Saxena, B. (2017). Response of Arbuscular Mycorrhizal fungi to global climate change and their role in terrestrial ecosystem C and N cycling. *Mycorrhiza-Function, Diversity, State of the Art*, 305–327.
- Gosling, P., Jones, J., and Bending, G. D. (2016). Evidence for functional redundancy in Arbuscular Mycorrhizal fungi and implications for agroecosystem management. *Mycorrhiza*, 26(1), 77–83.
- Goyal, K., Singh, N., Jindal, S., Kaur, R., Goyal, A., and Awasthi, R. (2022). Kjeldahl method. *Advanced Techniques of Analytical Chemistry*, 1(1), 105.
- Gryndler, M., Hršelová, H., Cajthaml, T., Havráňková, M., Řezáčová, V., Gryndlerová, H., and Larsen, J. (2009). Influence of soil organic matter decomposition on Arbuscular Mycorrhizal fungi in terms of asymbiotic hyphal growth and root colonization. *Mycorrhiza*, 19(4), 255–266.
- Hanway, J. J., and Heidel, H. (1952). Soil analysis method as used in Iowa State College soil testing laboratory. *Iowa Agriculture* 57, 1-31.
- Harrison, M. J. (2005). Signaling in the Arbuscular Mycorrhizal symbiosis. *Annual Review of Microbiology*, 59(1), 19–42.
- Hart, M. M., and Forsythe, J. A. (2012). Using Arbuscular Mycorrhizal fungi to improve the nutrient quality of crops; nutritional benefits in addition to phosphorus. *Scientia Horticulturae*, 148, 206–214.
- Helgason, T., and Fitter, A. (2005). The ecology and evolution of the Arbuscular Mycorrhizal fungi. *Mycologist*, 19(3), 96–101.
- 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(12), 4871–4881.
- Ijdo, M., Cranenbrouck, S., and Declerck, S. (2011). Methods for large-scale production of AM fungi: Past, present, and future. *Mycorrhiza*, 21, 1–16.
- Israel, A., Langrand, J., Fontaine, J., and Lounès-Hadj Sahraoui, A. (2022). Significance of Arbuscular Mycorrhizal fungi in mitigating abiotic environmental stress in medicinal and aromatic plants: A review. *Foods*, 11(17), 2591.
- Jamiołkowska, A., Księżniak, A., Gałązka, A., Hetman, B., Kopacki, M., and Skwaryło-Bednarz, B. (2018). Impact of abiotic factors on development of the community of Arbuscular Mycorrhizal fungi in the soil: A review. *International Agrophysics*, 32(1), 133–140. <https://doi.org/10.1515/intag-2016-0090>
- Jarrell, W. M., and Beverly, R. B. (1981). The dilution effect in plant nutrition studies. *Advances in Agronomy*, 34, 197–224.
- Javaid, A. (2010). Beneficial microorganisms for sustainable agriculture. In *Genetic Engineering, Biofertilisation, Soil Quality and Organic Farming* (pp. 347–369). Dordrecht: Springer Netherlands.
- Jha, S. S., and Songachan, L. S. (2023). Exploring the potential of Arbuscular Mycorrhizal Fungi as a biofertilizer. *Studies in Fungi*, 8(1), 1-17.
- Ji, L., Yang, Y., Yang, N., Khan, A., and Yang, L. (2021). Seasonal variation of diversity and co-occurrence patterns of Arbuscular Mycorrhizal fungal communities in mixed broadleaf-conifer forests. *Applied Soil Ecology*, 158, 103782.
- Jocien, S. K., and Frederick, N. (2023). Unsustainable agricultural practices as a driver of soil degradation in Santa sub-division, North West region of Cameroon. *Asian Soil Research Journal*, 7(2), 19–33.
- Kalamulla, R., Karunarathna, S. C., Tibpromma, S., ... and Yapa, N. (2022). Arbuscular Mycorrhizal fungi in sustainable agriculture. *Sustainability*, 14(19), 12250.
- Kaushal, P., Kumar, V., and Sharma, H. K. (2015). Utilization of taro (*Colocasia esculenta*): A review. *Journal of Food Science and Technology*, 52(1), 27–40.
- Keerthy, S. P., and Joshi, K. H. (2019). The pharmacological importance of *Colocasia esculenta* Linn: A review. *Journal of Pharmacognosy and Phytochemistry*, 8(6), 1945–1948.

-
-
- Kernaghan, G. (2005). Mycorrhizal diversity: Cause and effect?. *Pedobiologia*, 49(6), 511–520.
- Khade, S. W., and Rodrigues, B. F. (2003). Incidence of Arbuscular Mycorrhizal colonization in tubers of *Gloriosa superba* L. *Mycorrhiza News*, 15 (3), 14–16.
- 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.
- Khaliq, A., Perveen, S., Alamer, K. H., ... and Attia, H. (2022). Arbuscular Mycorrhizal fungi symbiosis to enhance plant–soil interaction. *Sustainability*, 14(13), 7840. <https://doi.org/10.3390/su14137840>
- Khanam, D., Mridha, M. A. U., Solaiman, A. R. M., and Hossain, T. (2006). Effect of edaphic factors on root colonization and spore population of arbuscular mycorrhizal fungi. *Bulletin of the Institute of Tropical Agriculture, Kyushu University*, 29, 97–104.
- Koske, R. E., and Tews, L. L. (1987). Vesicular-Arbuscular Mycorrhizal fungi of Wisconsin sandy soils. *Mycologia*, 79(6), 901–905.
- Koske, R.E., and Tessier, B. (1983). A convenient, permanent slide mounting medium. *Mycological Society of America Newsletter*, 34(2), 59.
- Krieg, N. R., and Holt, J. G. (1994). *Bergey's Manual of Determinate Bacteriology*. 9th Ed. *Williams and Wilkins*, Baltimore/ London, pp. 113.
- Kumar, R. A., Tapwal, A. S., Pandey, S. H., Rishi, R. A., and Borah, D. E. (2013). Observations on Arbuscular Mycorrhiza associated with important edible tuberous plants grown in wet evergreen forests in Assam, India. *Biodiversitas*, 14(2), 67–72.
- Kundu, B. S., Nehra, K., Yadav, R., and Tomar, M. (2009). Biodiversity of phosphate solubilizing bacteria in rhizosphere of chickpea, mustard and wheat grown in different regions of Haryana. *Indian Journal of Microbiology*, 49(2), 120–127.
- Lanfranco, L., Bonfante, P., and Genre, A. (2016). The mutualistic interaction between plants and Arbuscular Mycorrhizal fungi. *Microbiology Spectrum*, 4(6), 10–1128.
- Laxminarayana, K. (2016). Response of mycorrhiza, organic sources, secondary and micro nutrients on soil microbial activities and yield performance of

- Colocasia* (*Colocasia esculenta* L.) in alfisols. *Communications in Soil Science and Plant Analysis*, 47(6), 775–786.
- Lebot, V., Abraham, K., and Melteras, M. (2025). The lesser yam *Dioscorea esculenta* (Lour.) Burkill: A neglected crop with high functional food potential. *Genetic Resources and Crop Evolution*, 72(5), 5071-5091.
- Lindsay, W. L., and Norvell, W. A. (1978). Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Science Society of America Journal*, 42(3), 421-428.
- Logi, C., Sbrana, C., and Giovannetti, M. (1998). Cellular events involved in survival of individual Arbuscular Mycorrhizal symbionts growing in the absence of the host. *Applied and Environmental Microbiology*, 64(9), 3473–3479.
- Lombardo, S., Scavo, A., Abbate, C., Pandino, G., Parisi, B., and Mauromicale, G. (2021). Mycorrhizal inoculation improves mineral content of organic potatoes grown under calcareous soil. *Agriculture*, 11(4), 333.
- Lone, R., Shuab, R., Khan, S., Ahmad, J., and Koul, K. K. (2017). Arbuscular Mycorrhizal fungi for sustainable agriculture. In *Probiotics and Plant Health* (pp. 553–577). Singapore: Springer Singapore.
- Lu, F. C., Lee, C. Y., and Wang, C. L. (2015). The influence of Arbuscular Mycorrhizal fungi inoculation on yam (*Dioscorea* spp.) tuber weights and secondary metabolite content. *PeerJ*, 3, e1266.
- Mabhaudhi, T., Modi, A. T., and Beletse, Y. G. (2013). Response of taro (*Colocasia esculenta* L. Schott) landraces to varying water regimes under a rain shelter. *Agricultural Water Management*, 121, 102–112.
- Madawala, H. M. S. P. (2021). Arbuscular mycorrhizal fungi as biofertilizers: Current trends, challenges, and future prospects. *Biofertilizers*, 83-93.
- Masrahi, A. S., Alasmari, A., Shahin, M. G., Qumsani, A. T., Oraby, H. F., and Awad-Allah, M. M. (2023). Role of Arbuscular Mycorrhizal fungi and phosphate solubilizing bacteria in improving yield, yield components, and nutrients uptake of barley under salinity soil. *Agriculture*, 13(3), 537.
- Mathimaran, N., Ruh, R., Vullioud, P., Frossard, E., and Jansa, J. (2005). *Glomus intraradices* dominates Arbuscular Mycorrhizal communities in a heavy-textured agricultural soil. *Mycorrhiza*, 16, 61–66.
- McGonigle, T. P., Miller, M. H., Evans, D. G., Fairchild, G. L., and Swan, J. A. (1990). A new method which gives an objective measure of colonization of roots

- by vesicular-Arbuscular Mycorrhizal fungi. *New Phytologist*, 115(3), 495–501.
- Miransari, M. (2011). Interactions between Arbuscular Mycorrhizal fungi and soil bacteria. *Applied Microbiology and Biotechnology*, 89(4), 917–930.
- Mohammad, M. J., Malkawi, H. I., and Shibli, R. (2003). Effects of Arbuscular Mycorrhizal fungi and phosphorus fertilization on growth and nutrient uptake of barley grown on soils with different levels of salts. *Journal of Plant Nutrition*, 26(1), 125–137.
- Mohekar, D. S. (2020). *Development of Land Use Options for Goa State Using Large-Scale Land Resource Inventory* (Doctoral Dissertation, Dr. Panjabrao Deshmukh Krishi Vidyapeeth). 1–301.
- More, S. J., Namrata, A. G., Suresh Kumar, J., Visalakshi, C. C., and Sirisha, T. (2021). *Recent Advances in Root and Tuber Crops*. New Delhi: Brillion Publishing House.
- Morton, J. B., and Benny, G. L. (1990). Revised classification of Arbuscular Mycorrhizal fungi (Zygomycetes): a new order, Glomales, two new suborders, Glomineae and Gigasporineae, and two new families, Acaulosporaceae and Gigasporaceae, with an emendation of Glomaceae. *Mycotaxon*, 37, 471–491.
- Morton, J. B., Bentivenga, S. P., and Wheeler, W. W. (1993). Germplasm in the International Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM) and procedures for culture development, documentation, and storage. *Mycotaxon*, 48, 491–528.
- Mounika, V., Gowd, T. Y. M., Lakshminarayana, D., ... and Reddy, P. M. (2025). Sweet potato (*Ipomoea batatas* (L.) Lam): A comprehensive review of its botany, nutritional composition, phytochemical profile, health benefits, and future prospects. *European Food Research and Technology*, 251(10), 3225–3239.
- Mrabet, R. (2023). Sustainable agriculture for food and nutritional security. In *Sustainable agriculture and the environment* (pp. 25–90). Academic Press.
- Nabi, M. (2023). Role of microorganisms in plant nutrition and soil health. In *Sustainable Plant Nutrition* (pp. 263–282). Academic Press.
- Nacoon, S., Jogloy, S., Riddech, N., Mongkolthanaruk, W., Ekprasert, J., Cooper, J., and Boonlue, S. (2021). Combination of Arbuscular Mycorrhizal fungi and phosphate solubilizing bacteria on growth and production of *Helianthus tuberosus* under field condition. *Scientific Reports*, 11(1), 6501.

- Nadeem, S. M., Khan, M. Y., Waqas, M. R., Binyamin, R., Akhtar, S., and Zahir, Z. A. (2017). Arbuscular Mycorrhizas: An overview. *Arbuscular Mycorrhizas and stress tolerance of plants*, 1–24.
- Nedunchezhiyan, M., Suja, G., and Ravi, V. (2022). Tropical root-and tuber crops-based cropping systems: A review. *Current Horticulture*, 10(1), 14–22.
- Nuryana, I., Widada, J., and Subandiyah, S. (2020). Molecular study of root colonization and diversity of Arbuscular Mycorrhizal fungi (AMF) associated with lesser yam (*Dioscorea esculenta*). *Journal of Physics: Conference Series*, 1665(1), 012017.
- Oladele, S., Gould, I., and Varga, S. (2025). Is Arbuscular Mycorrhizal fungal addition beneficial to potato systems? A meta-analysis. *Mycorrhiza*, 35(1), 5.
- Öpik, M., and Davison, J. (2016). Uniting species-and community-oriented approaches to understand Arbuscular Mycorrhizal fungal diversity. *Fungal Ecology*, 24, 106–113.
- Padhan, B., Biswas, M., and Panda, D. (2020). Nutritional, anti-nutritional and physico-functional properties of wild edible yam (*Dioscorea* spp.) tubers from Koraput, India. *Food Bioscience*, 34, 100527.
- Pasković, I., Herak Ćustić, M., Pecina, M., ... and Goreta Ban, S. (2019). Manganese soil and foliar fertilization of olive plantlets: The effect on leaf mineral and phenolic content and root mycorrhizal colonization. *Journal of the Science of Food and Agriculture*, 99(1), 360–367.
- Pflaum, R. T., and Howick, L. C. (1956). The chromium-diphenylcarbazide reaction¹. *Journal of the American Chemical Society*, 78(19), 4862–4866.
- Phillips, J. M., and Hayman, D. S. (1970). Improved procedures for clearing roots and staining parasitic and vesicular-Arbuscular Mycorrhizal fungi for rapid assessment of infection. *Transactions of the British Mycological Society*, 55(1), 158–161. [https://doi.org/10.1016/S0007-1536\(70\)80110-3](https://doi.org/10.1016/S0007-1536(70)80110-3)
- Phour, M., and Sindhu, S. S. (2024). Arbuscular Mycorrhizal fungi: An eco-friendly technology for alleviation of salinity stress and nutrient acquisition in sustainable agriculture. *Arbuscular Mycorrhizal Fungi in Sustainable Agriculture: Nutrient and Crop Management*, 223–267.
- Plenchette, C., Fortin, J. A., and Furlan, V. (1983). Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility: I. Mycorrhizal dependency under field conditions. *Plant and soil*, 70(2), 199–209.

- Prasad, R., Bhola, D., Akdi, K., ... and Varma, A. (2017). Introduction to Mycorrhiza: Historical development. In A. Varma, R. Prasad, and N. Tuteja (Eds.), *Mycorrhiza: Function, Diversity, State of the art* (pp. 1–7). Springer. https://doi.org/10.1007/978-3-319-53064-2_1
- Qi, S., Wang, J., Wan, L., ... and Moles, A. T. (2022). Arbuscular Mycorrhizal fungi contribute to phosphorous uptake and allocation strategies of *Solidago canadensis* in a phosphorous-deficient environment. *Frontiers in Plant Science*, 13, 831654.
- Qin, Z., Zhang, H., Feng, G., Christie, P., Zhang, J., Li, X., and Gai, J. (2020). Soil phosphorus availability modifies the relationship between AM fungal diversity and mycorrhizal benefits to maize in an agricultural soil. *Soil Biology and Biochemistry*, 144, 107790.
- Radhakrishnan, G. V., Keller, J., Rich, M. K., ... and Delaux, P. M. (2020). An ancestral signalling pathway is conserved in intracellular symbioses-forming plant lineages. *Nature Plants*, 6(3), 280–289.
- Rahimzadeh, S., and Pirzad, A. R. (2017). Microorganisms (AMF and PSB) interaction on linseed productivity under water-deficit condition. *International Journal of Plant Production*, 11(2), 259–274.
- Read, D. J., Koucheki, H. K., and Hodgson, J. (1976). Vesicular-arbuscular mycorrhiza in natural vegetation systems. I. The occurrence of infection. *New Phytology* 77, 641–653.
- Reddy, P. P. (2015). *Plant Protection in Tropical Root and Tuber Crops*. New Delhi: Springer India. <https://doi.org/10.1007/978-81-322-2389-4>.
- Redecker, D., Kodner, R., and Graham, L. E. (2000). Glomalean fungi from the Ordovician. *Science*, 289(5486), 1920–1921.
- Redecker, D., Schüssler, A., Stockinger, H., Stürmer, S. L., Morton, J. B., and Walker, C. (2013). An evidence-based consensus for the classification of Arbuscular Mycorrhizal fungi (Glomeromycota). *Mycorrhiza*, 23(7), 515–531.
- Remy, W., Taylor, T. N., Hass, H., and Kerp, H. (1994). Four hundred-million-year-old vesicular Arbuscular Mycorrhizae. *Proceedings of the National Academy of Sciences*, 91(25), 11841–11843.
- Rivero, J., Álvarez, D., Flors, V., Azcón-Aguilar, C., and Pozo, M. J. (2018). Root metabolic plasticity underlies functional diversity in mycorrhiza-enhanced stress tolerance in tomato. *New Phytologist*, 220(4), 1322–1336. <https://doi.org/10.1111/nph.15295>

- Rodrigues, B., and Muthukumar, T. (2009). *Arbuscular Mycorrhizae of Goa: A Manual of Identification Protocols*. (pp. 1–135) Goa University, Goa.
- Rodríguez-Romero, A. S., Guerra, M. S. P., and Del Carmen Jaizme-Vega, M. (2005). Effect of Arbuscular Mycorrhizal fungi and rhizobacteria on banana growth and nutrition. *Agronomy for Sustainable Development*, 25(3), 395–399.
- Roychowdhury, D., Paul, M., and Kumar Banerjee, S. (2015). Isolation identification and characterization of phosphate solubilising bacteria from soil and the production of biofertilizer. *Int J Curr Microbiol App Sci*, 4(11), 808-815.
- Saikanth, K., Singh, B. V., Sachan, D. S., and Singh, B. (2023). Advancing sustainable agriculture: A comprehensive review for optimizing food production and environmental conservation. *International Journal of Plant and Soil Science*, 35(16), 417–425.
- Sanders, I. R., and Fitter, A. H. (1992). The ecology and functioning of vesicular—arbuscular mycorrhizas in co-existing grassland species: I. Seasonal patterns of Mycorrhizal occurrence and morphology. *New Phytologist*, 120(4), 517-524.
- Santos-González, J. C., Finlay, R. D., and Tehler, A. (2007). Seasonal dynamics of Arbuscular Mycorrhizal fungal communities in roots in a seminatural grassland. *Applied And Environmental Microbiology*, 73(17), 5613-5623.
- Sbrana, C., and Giovannetti, M. (2005). Chemotropism in the Arbuscular Mycorrhizal fungus *Glomus mosseae*. *Mycorrhiza*, 15(7), 539–545.
- Schenck, N.C., and Pérez, Y. (1990). *Manual for identification of VA mycorrhizal fungi*. INVAM, Florida University, Gainesville, USA.
- Schüßler, A., and Walker, C. (2010). *The Glomeromycota: A species list with new families and new genera*. The Royal Botanic Gardens, Kew. ISBN 978-1466388048.
- Schüßler, A., Schwarzott, D., and Walker, C. (2001). A new fungal phylum, the Glomeromycota: phylogeny and evolution. *Mycological Research*, 105(12), 1413–1421.
- Scott, G. J., Rosegrant, M. W., and Ringler, C. (2000). Global projections for root and tuber crops to the year 2020. *Food Policy*, 25(5), 561–597.
- Sefrila, M., Ghulamahdi, M., Purwono, P., Melati, M., and Mansur, I. (2023). Trap culture and colonization of Arbuscular Mycorrhizal fungi from corn roots in tidal swamps using several host plants. *Journal of Sustainable*

-
-
- Agriculture*, 38(1), 193–203.
<https://doi.org/10.1080/10440046.2022.2137890>
- Séry, D. J. M., Kouadjo, Z. C., Voko, B. R., and Zeze, A. (2016). Selecting native Arbuscular Mycorrhizal fungi to promote cassava growth and increase yield under field conditions. *Frontiers in Microbiology*, 7, 2063.
- Shuab, R., Lone, R., and Koul, K. K. (2017). Influence of Arbuscular Mycorrhizal fungi on storage metabolites, mineral nutrition, and nitrogen-assimilating enzymes in potato (*Solanum tuberosum* L.) plant. *Journal of Plant Nutrition*, 40(10), 1386–1396.
- Sidibe, D., Fofana, I. J., Silue, S., Diarrassouba, N., Adolphe, Z., and Nguetta, S. P. (2015). Evaluation of symbiosis effect of some Arbuscular Mycorrhizal fungi on growth of yams (*Dioscorea alata*) on experimental conditions. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 3(3), 346–357.
- Sila, M. D., Ogbonna, C. I., Nyam, D. D., Wuyep, P. A., and Shutt, V. M. (2021). Studies on the fungal isolates of the soil spots of sweet potato [*Ipomoea batatas* (L.) Lam] root tubers in the field. *Australian Journal of Basic and Applied Sciences*, 15(8), 1–9.
- Singh, S. K., Wu, X., Shao, C., and Zhang, H. (2022). Microbial enhancement of plant nutrient acquisition. *Stress Biology*, 2(1), 3.
- Sivakumar, N. (2013). Effect of edaphic factors and seasonal variation on spore density and root colonization of Arbuscular Mycorrhizal fungi in sugarcane fields. *Annals of Microbiology*, 63(1), 151–160. <https://doi.org/10.1007/s13213-012-0453-3>
- Smith, S. E., and Read, D. J. (2008). *Mycorrhizal Symbiosis*, (3rd ed.). New York: Academic Press.
- Stürmer, S. L., Bever, J. D., and Morton, J. B. (2018). Biogeography of Arbuscular Mycorrhizal fungi (Glomeromycota): A phylogenetic perspective on species distribution patterns. *Mycorrhiza*, 28(7), 587–603.
- Subba, R. (2023). Phosphate solubilizing bacteria for a sustainable agriculture: A review. *Research Journal of Agricultural Science*, 14(6), 1797–1803.
- Subbiah, B. V., and Asija, G. L. (1956). A rapid procedure for the determination of available nitrogen in soils. *Current Science* 25, 259–260.
-

- Tamura, K., Stecher, G., and Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, 38(7), 3022–3027.
- Tchabi, A., Coyne, D., Hountondji, F., Lawouin, L., Wiemken, A., and Oehl, F. (2010). Efficacy of indigenous Arbuscular Mycorrhizal fungi for promoting white yam (*Dioscorea rotundata*) growth in West Africa. *Applied Soil Ecology*, 45(2), 92–100.
- Temesgen, M., and Retta, N. (2015). Nutritional potential, health and food security benefits of taro *Colocasia esculenta* (L.): A review. *Food Science and Quality Management*, 36(0), 23–30.
- Tommerup, I. C. (1983). Temperature relations of spore germination and hyphal growth of vesicular-Arbuscular Mycorrhizal fungi in soil. *Transactions of the British Mycological Society*, 81(2), 381–387.
- Trejo-Aguilar, D., and Bañuelos, J. (2020). Isolation and culture of Arbuscular Mycorrhizal fungi from field samples. In S. Declerck, D. G. Strullu, and A. Fortin (Eds.), *Arbuscular Mycorrhizal Fungi: Methods and Protocols* (pp. 1–18). Springer. https://doi.org/10.1007/978-1-0716-0609-3_1
- Uddin, B., Parre, P. N., Rafique, M., and Ul, H. Z. (2025). Mycorrhizae Importance in future agriculture and mycorrhizal culturing methods. In H. Z. Ul, P. N. Parre, and M. Rafique (Eds.), *Natural Bioactives from the Endophytes of Medicinal Plants* (pp. 309–319). CRC Press. <https://doi.org/10.1201/9781003323730-19>
- Umer, M., Anwar, N., Mubeen, M., Li, Y., Ali, A., Alshaharni, M. O., and Liu, P. (2025). Roles of Arbuscular Mycorrhizal fungi in plant growth and disease management for sustainable agriculture. *Frontiers in Microbiology*, 16, 1616273.
- Uwamungu, J. Y., Shi, G., Wang, Y., Paliwal, A., Jadhav, R. R., and Wani, A. W. (2022). Arbuscular Mycorrhizal Fungi (AMF) for sustainable soil and plant health. In *Microbial and Biotechnological Interventions in Bioremediation and Phytoremediation* (pp. 135–152). Cham: Springer International Publishing.
- Van Der Heijden, M. G., Bardgett, R. D., and Van Straalen, N. M. (2008). The unseen majority: Soil microbes as drivers of plant diversity and productivity in terrestrial ecosystems. *Ecology Letters*, 11(3), 296–310.
- Velip, D. M., and Rodrigues, B. F. (2019). *In vitro* cultivation of *Gigaspora decipiens* using transformed roots of *Linum usitatissimum*. *Kavaka*, 53, 96–99.

- Verbruggen, E., van der Heijden, M. G., Rillig, M. C., and Kiers, E. T. (2013). Mycorrhizal fungal establishment in agricultural soils: Factors determining inoculation success. *New Phytologist*, 197(4), 1104–1109.
- Vieira Junior, W. G., Moura, J. B. D., Souza, R. F. D., ... and Dutra e Silva, S. (2020). Seasonal variation in mycorrhizal community of different cerrado phytophysiomies. *Frontiers in Microbiology*, 11, 576764.
- Voříšková, J., Brabcová, V., Cajthaml, T., and Baldrian, P. (2014). Seasonal dynamics of fungal communities in a temperate oak forest soil. *New Phytologist*, 201(1), 269–278.
- Wahab, A., Muhammad, M., Munir, A., ... and Reddy, S. P. P. (2023). Role of Arbuscular Mycorrhizal fungi in regulating growth, enhancing productivity, and potentially influencing ecosystems under abiotic and biotic stresses. *Plants*, 12(17), 3102.
- Wahid, F., Fahad, S., Danish, S., ... and Datta, R. (2020). Sustainable management with mycorrhizae and phosphate solubilizing bacteria for enhanced phosphorus uptake in calcareous soils. *Agriculture*, 10(8), 334.
- Wahid, F., Sharif, M., Shah, F., ... and Datta, R. (2022). Mycorrhiza and phosphate solubilizing bacteria: Potential bioagents for sustainable phosphorus management in agriculture. *Phyton*, 91(2), 257.
- Wang, B., and Qiu, Y. L. (2006). Phylogenetic distribution and evolution of mycorrhizas in land plants. *Mycorrhiza*, 16(5), 299–363.
- Wang, J., Fu, W., Sun, C., Cai, S., and Tang, C. (2022). *Funneliformis mosseae* inoculation enhances *Cucurbita pepo* L. plant growth and fruit yield by reshaping rhizosphere microbial community structure. *Diversity*, 14(11), 932.
- Willis, A., Rodrigues, B. F., and Harris, P. J. (2013). The ecology of Arbuscular Mycorrhizal fungi. *Critical Reviews in Plant Sciences*, 32(1), 1–20.
- Wu, S., Shi, Z., Chen, X., Gao, J., and Wang, X. (2022). Arbuscular Mycorrhizal fungi increase crop yields by improving biomass under rainfed condition: a meta-analysis. *PeerJ*, 10, e12861.
- Yang, Q., Ravnskov, S., Pullens, J. W. M., and Andersen, M. N. (2022). Interactions between biochar, Arbuscular Mycorrhizal fungi and photosynthetic processes in potato (*Solanum tuberosum* L.). *Science of The Total Environment*, 816, 151649.

-
-
- Yuan, J., Shi, K., Zhou, X., ... and Zhang, Y. (2023). Interactive impact of potassium and Arbuscular Mycorrhizal fungi on the root morphology and nutrient uptake of sweet potato (*Ipomoea batatas* L.). *Frontiers in Microbiology*, 13, 1075957.
- Zhao, S., Zhong, L., Li, X., ... and Feng, J. (2024). Comparative analysis of nutrients, phytochemicals, and minerals in coloured sweet potato (*Ipomoea batatas* L.) roots. *Foods*, 13(22), 3636.
- Zierer, W., Rüscher, D., Sonnewald, U., and Sonnewald, S. (2021). Tuber and tuberous root development. *Annual review of plant biology*, 72(1), 551-580.

Weblinks:

<https://forest.goa.gov.in/index.php/forest-types> Forest Department | Government of Goa, Ministry of Environment, Forest and Climate Change, retrieved on 4th July 2025.

http://www.unep.org/pdf/Green_Economy_in_SIDS. UNEP, U. D. FAO, 2012. SIDS-Focused Green Economy: An Analysis of Challenges and Opportunities. Nairobi: United Nations Environment Programme.

<http://invam.caf.wvu.edu>

<http://www.amf-phenology.com>

<http://www.zor.zut.edu.pl/Glomeromycota/index.html>

<https://invam.wvu.edu/methods/culture-methods/single-species-cultures>

<https://invam.wvu.edu/methods/culture-methods/trap-culture>

<https://www.ncbi.nlm.nih.gov/genbank>

Research Work Published:

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Presentations at Conferences:

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- ❖ **Oral presentation** on “*Studies on the Arbuscular Mycorrhizal (AM) fungal diversity of selected wild tuber species of Goa*”, at International Conference on Cutting-edge Research Innovation in Sustainable Education, Environment, and Agriculture (CRISEA - 2025), on 24th to 26th February 2025, Goa University, Goa.
- ❖ Received **Best Poster Presentation award** at the Goa Research Scholar Meet 2025, organized by the Goa State Higher Education Council and the Directorate of Higher Education, Goa, on 6th March 2025, at Directorate of Arts and Culture, Sanskruti Bhawan, Patto, Panaji, Goa. Paper titled “*Unearthing Connections: Exploring Arbuscular Mycorrhizal fungi in Goa’s wild and cultivated tubers*”.
- ❖ **Online presentation:** Presented a Paper titled “*Arbuscular Mycorrhizal fungal diversity in some tuber species from South Goa*”, at the 2nd International Conference on Biodiversity: Exploration, Exploitation and Conservation for Sustainable Development (ICB-2023), 10th and 11th February 2023, Assam.

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 (Iggehon 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.

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

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**.

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) = $-\sum (P_i \ln (P_i))$, where P_i is the proportion of individual species that contributes to the total number of individuals (Shannon and Wiener, 1949)
- Simpson's diversity index (D) = $1 - (\sum n(n-1)/N(N-1))$, 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): $\Sigma(H') = H'/$

$$H'_{\max}, \text{ where } \max = \ln S, S = \text{SR}$$

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 spores 100⁻¹ 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	<i>Fn.ge, Fn.mo, Ac.fo</i> <i>Ac.fo, Ac.sc., Fn.ge,</i>
S-II	98.00 ±0.57	96.67 ±0.66	77.67 ±0.88	82.00 ±1.53	134.00 ±3.21	<i>Fn.mo, Gi.de, Gi.al, Rh.fa,</i> <i>Rh.in</i>
S-III	73.67 ±1.20	72.67 ±1.20	67.33 ±1.45	64.33 ±1.45	102.67 ±3.71	<i>Ac.sc., Gi.de</i>
S-IV	71.67 ±0.88	70.33 ±0.88	54.33 ±0.88	53.33 ±1.86	66.67 ±0.88	<i>Ac.fo, Ac.sc, Gl. sp.</i>
S-V	76.67 ±1.45	76.33 ±0.88	57.00 ±0.58	45.00 ±1.15	85.33 ±1.45	<i>Fn. mo, Ac. sc, Gl.sp.</i>

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.

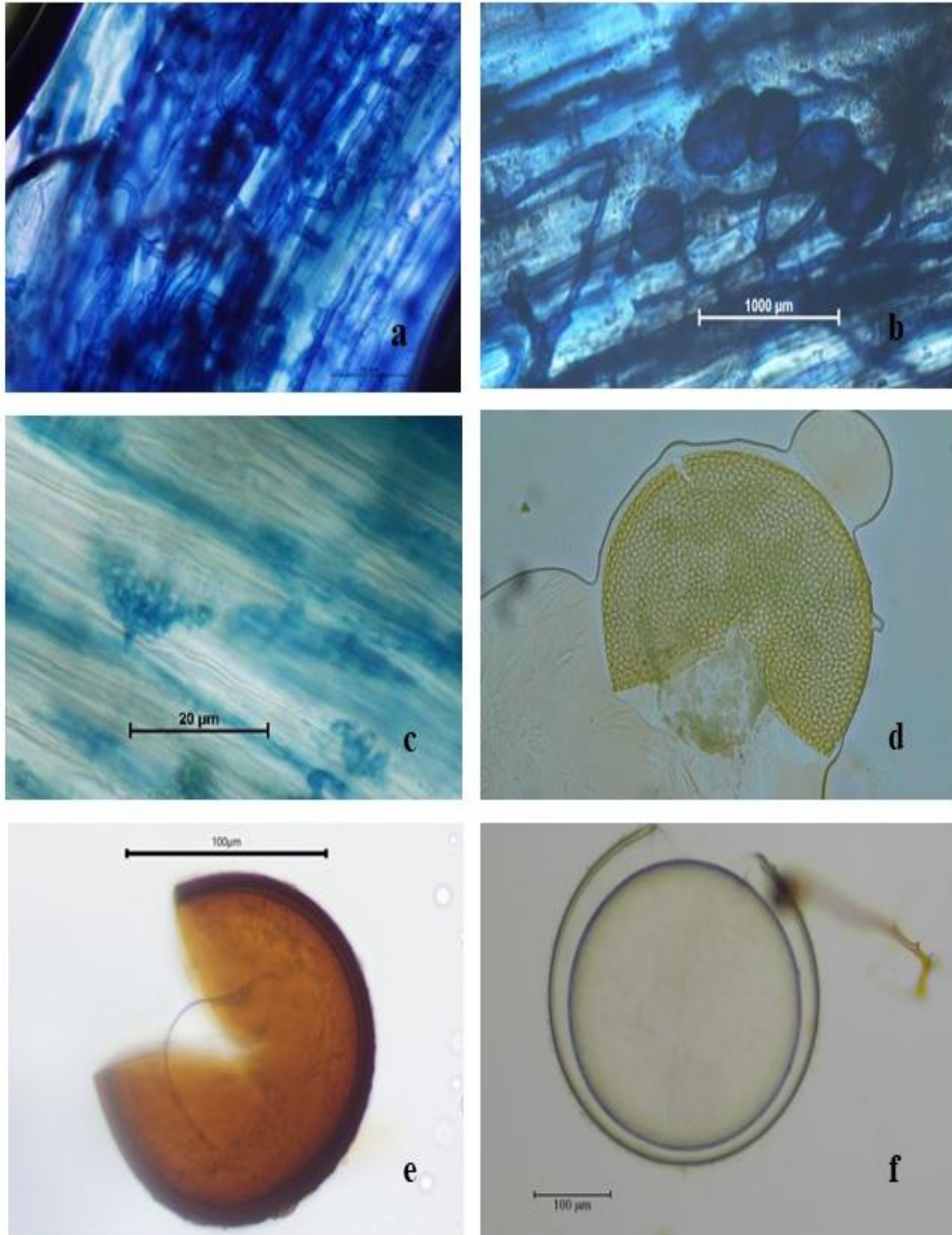


Figure 1: Micrographs of Arbuscular Mycorrhizal colonization and spores. a, Hyphal colonization; b, vesicles; c, arbuscules; d, *Acaulospora scrobiculata*; e, *Funneliformis geosporum*; f, *Gigaspora albida*

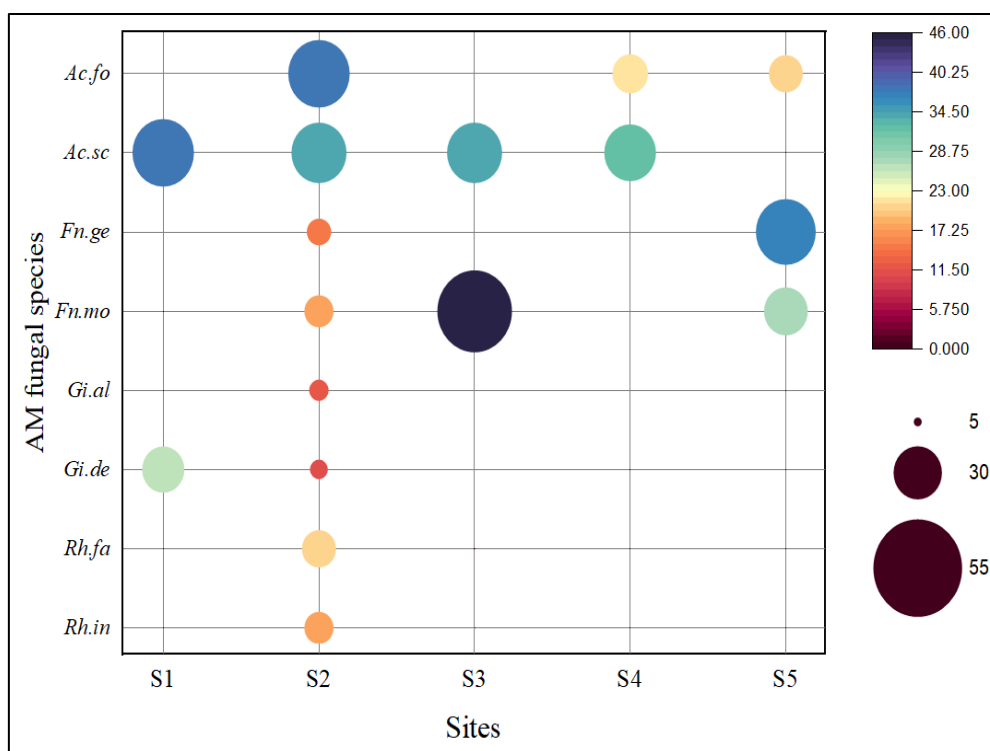


Figure 2: AM Fungal community composition (based on mean spore density) in trap cultures at various study sites using Matrix bubble

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.
- Schüßler, 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.

FESTSCHRIFT IN HONOUR OF PROF. C. MANOHARACHARY

Diversity, Conservation and Biotechnology of Fungi

Praveen Gehlot, S.M. Reddy,
M.M. V. Baig & Joginder Singh



(VOLUME 3)

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DIVERSITY, CONSERVATION AND BIOTECHNOLOGY OF FUNGI

COMMEMORATIVE VOLUME (FESTSCHRIFT)
HONOURING PROF. C. MANOHARACHARY
(VOLUME 3)

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α AGROBIOS
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2. Arbuscular Mycorrhizal Fungi: Diversity, Conservation, and Application in Some Selected Crop Plants of Goa

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ABSTRACT

This study elucidates the pivotal role of arbuscular mycorrhizal (AM) fungi in promoting crop growth and sustainable agriculture. *In vitro* and *in vivo* inoculum production techniques were optimised to enhance AM fungal availability. Diversity analyses revealed distinct AM fungal assemblages in *Ipomoea batatas*, *Oryza sativa*, and *Vigna unguiculata*. Furthermore, synergistic microbial interactions enhanced growth and sustainability in the three crop plants. Overall, AM fungi serve as an eco-friendly and resilient strategy to enhance crop productivity and adaptability in the face of climate change and resource-limited agriculture.

Keywords: AM Fungi, Crops, Growth, Yield

1. INTRODUCTION

Arbuscular mycorrhizal (AM) symbiosis is a fundamental plant-root interaction, often regarded as a foundational symbiotic relationship in terrestrial ecosystems. Belonging to the Phylum Glomeromycota, AM fungi form a symbiotic partnership with the roots of nearly all land plants (Bonfante and Venice, 2020). These fungi are crucial to ecosystem function, significantly boosting plant growth, nutrient uptake, and water use efficiency. They also stabilise plant hormonal balance and increase resistance to both biotic and abiotic stresses (Wahab *et al.*, 2023; Wang *et al.*, 2024). A key ecological role of AM fungi is to enhance plant biomass and overall vigour, which contributes to greater ecosystem productivity.

2. ROLE OF MYCORRHIZAE IN CROP PLANTS

- 1. Stress Tolerance and Resilience:** AM fungi strengthen a plant's oxidative defence system, particularly against abiotic stresses such as drought, salinity, extreme temperatures, heavy metal contamination, and flooding (Ahammed *et al.*, 2023; Nie *et al.*, 2024; Guo *et al.*, 2024). By competing for colonisation sites and enhancing host immune responses, AM fungi also bolster plant defences against pathogens and pests, acting as both direct and indirect biocontrol agents (Larbi-Koranteng *et al.*, 2022). They further facilitate plant adaptation to challenging environments by altering root morphology, reprogramming gene expression, and promoting the accumulation of compounds that facilitate osmotic adjustment.
- 2. Improved Nutrient Uptake:** Through their symbiotic association, AM fungi significantly enhance nutrient absorption and utilisation, which in turn increases photosynthetic efficiency and supports plant growth (Khan *et al.*, 2022; Chandrasekaran, 2022). In cereal crops, this partnership also stimulates the accumulation of primary and secondary metabolites, as well as soluble proteins and carbohydrates. As a result, AM fungi can substantially improve plant biomass, yield, and overall productivity in both cereals and other crops.
- 3. Role in Sustainable Agriculture:** A sustainable plant ecosystem conserves soil productivity with minimal energy and resource input while avoiding ecological degradation. The symbiotic relationship with AM fungi supports this balance by enabling plants to use nutrients more efficiently (Schaefer *et al.*, 2021). Strategies such as incorporating mycorrhizal crops and native fungal inoculants can boost AM fungal colonisation and plant growth. These methods collectively strengthen soil quality, crop health, agricultural output, and resilience, offering a promising solution to the environmental and agricultural challenges of intensive farming (Kalamulla *et al.*, 2022; Chaudhary *et al.*, 2025, Pednekar and Rodrigues, 2025). By promoting AM fungal growth and colonisation, agro-ecosystems can achieve long-term productivity and increased sustainability.

3. IN VITRO AND IN VIVO INOCULUM PRODUCTION IN AM FUNGI

3.1. In vitro Culture Systems

AM fungi are obligate biotrophs and cannot be cultured independently on artificial media. To propagate them, monoxenic cultures are established using *in vitro* grown transformed roots as symbiotic partners. While only a few AM fungal species and strains have been successfully cultured using the Root Organ Culture (ROC) system, they exhibit rapid growth and colonisation (Fortin *et al.*, 2002, Velip and Rodrigues, 2019).

These systems can produce thousands of *in vitro* propagules within a few months, making them suitable for large-scale production and application as bioinoculants. AM fungal culture systems maximize fungal diversity, functional traits, and ecosystem resilience, especially under warming scenarios. This approach facilitates the development of inoculation and management strategies that support native plant communities, enhance soil health, and promote carbon sequestration.

3.2. *In vivo* Culture Systems

Trap Cultures

To propagate and recover AM fungal spores not encountered in field soils, trap cultures are prepared (Morton *et al.*, 1993) by mixing sand and rhizosphere soil (including roots) in a 1:1 ratio. The mixture is then transferred to surface-sterilised plastic pots. *Coleus scutellarioides* (L.) Benth. (Lamiaceae), or any other suitable host, is used as the 'catch' plant.

Coleus cuttings are surface-sterilised, planted directly into the pots, and maintained in a polyhouse at 27°C. They are watered every two days, and a phosphorus (P) deficient Hoagland's nutrient solution is added fortnightly. After a three-month growth period, watering is stopped to allow the pots to dry. The soil and roots are then separated. AM fungal spores are recovered from the soil for identification, and the roots are used to determine AM fungal colonisation. The extracted spores are also used to prepare monospecific cultures.

Monospecific Cultures

Monospecific cultures are prepared by isolating single AM fungal spores, which are then repeatedly washed and stored in Petri plates at 4°C, following the standard procedure mentioned in <https://invam.wvu.edu/methods/culture-methods/single-species-cultures>. Damaged or defective spores (e.g., those with altered colour or loss of contents) are excluded. Healthy spores are washed with distilled water to remove any soil particles or hyphal fragments before inoculation. Plastic pots, wiped with absolute alcohol, are filled with sterilised sand. The isolated spores are placed on Whatman filter paper at a depth of 2–3 cm, covered with additional medium, and planted with cuttings of a suitable host plant.

4. AM FUNGAL DIVERSITY AND EFFECT OF BIO-INOCULATION IN *IPOMOEA BATATAS* (L.) LAM.

The global population is rapidly growing, creating a need for sustainable agricultural advancements to feed an estimated 10 billion people in the coming decades. This is especially critical given escalating climate challenges, such as insect outbreaks, flooding, and drought. *I. batatas* (Sweet potato) is a nutrient rich and resilient crop that offers a promising solution for improving

food security, affordability, and public health (Behera *et al.*, 2022; Mounika *et al.*, 2025).

The symbiotic relationship between plant roots and AM fungi is known to enhance plant growth and yield, including in sweet potatoes (ALHadidi *et al.*, 2021; Shi *et al.*, 2023). AM fungi enhance the plant's ability to cope with stresses such as drought by boosting photosynthesis, nutrient uptake, and antioxidant defences (Kalamulla *et al.*, 2022; Rigobelo, 2024; Chaudhary *et al.*, 2025). This symbiosis also enhances soil quality and strengthens the root system's ability to absorb nutrients and water. The role of AM fungi in sustainable agriculture is increasingly recognised for promoting nutrient efficiency, biomass production, and overall food security.

A study conducted to assess the diversity of AM fungi and the impact of AM fungal inoculation on yield performance in *I. batatas* (Pednekar and Rodrigues, unpublished) revealed high AM colonisation (95%), characterised by the presence of hyphae, arbuscules, and vesicles. Besides, a spore density of 218 spores 100 g⁻¹ of soil was recorded. Eight AM fungal species, viz., *Acaulospora scrobiculata* Trappe, *Acaulospora foveata* Trappe & Janos, *Funneliformis geosporum* (Nicolson & Gerd.) Walker & Schüßler, *Funneliformis mosseae* (Nicolson & Gerd.) Walker & Schüßler, *Gigaspora albida* Schenck & Sm., *Gigaspora decipiens* Hall & Abbott, *Rhizophagus fasciculatus* (Thaxt.) Walker & Schüßler, *Rhizophagus intraradices* (Schenck & Sm.) Walker & Schüßler were recovered from trap cultures.

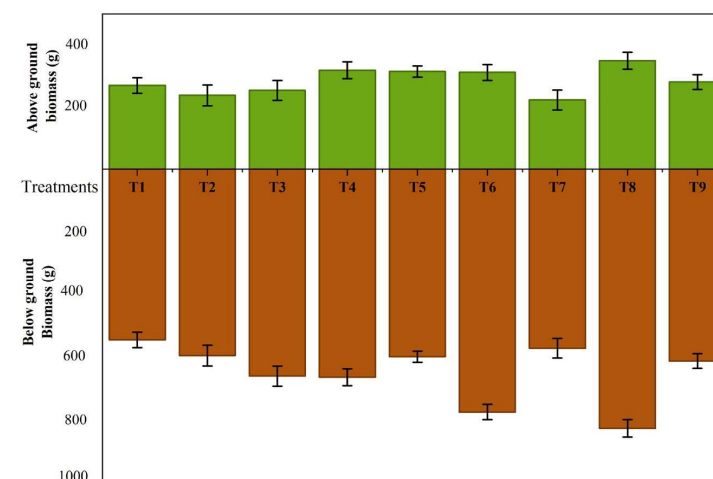


FIGURE 1: Effect of bio-inoculation on above-ground and below-ground biomass in *Ipomoea batatas*. **Legend:** All values are mean of three readings; $\pm$ = standard error; T₁ = Control, T₂ = *A. scrobiculata*, T₃ = *R. intraradices*, T₄ = *A. scrobiculata* + *R. intraradices*, T₅ = PSB, T₆ = PSB + *A. scrobiculata*, T₇ = PSB + *R. intraradices*, T₈ = PSB + *A. scrobiculata* + *R. intraradices*, T₉ = Compost.

Field experiment involving the dominant AM fungal species, *viz.*, *A. scrobiculata* and *R. intraradices* was carried out to assess their effect on yield. In addition to AM inoculation, a compost-based inoculum containing Phosphate solubilizing bacteria (PSB) was applied, along with AM bio-inoculants. The results of yield and shoot biomass across all treatments are depicted in **Fig.1**. The enhancement in shoot biomass clearly coincides with a proportional increase in yield ($r = 0.636$, $n = 9$, $p < 0.05$), demonstrating that better growth translates into higher yield.

In *I. batatas*, the yield in all the bio-inoculated plants was higher than that of the uninoculated control. Maximum yield (84 kg ha^{-1}) was recorded in the treatment comprising *R. intraradices*, *A. scrobiculata*, and PSB, which was markedly higher than the uninoculated control (55 kg ha^{-1}). This finding suggests that the synergistic effect of bio-inoculation has a positive impact on crop yield as observed in earlier studies (Yano-Melo *et al.*, 1999; Fracasso *et al.*, 2020; Nacoon *et al.*, 2021).

5. AM FUNGAL DIVERSITY AND THE EFFECT OF BIO-INOCULATION ON ORYZA SATIVA GROWN IN KHAZAN LANDS

Khazans are ancient, traditional, and community-managed agro-aqua ecosystems found in Goa, India. Reclaimed from low-lying mangrove swamps and coastal wetlands over centuries, these ingenious systems utilise a network of infrastructure to prevent saltwater intrusion, enabling sustainable agriculture, aquaculture, and salt production. Historically managed by village councils called *Gaunkaris*, they represent a harmonious blend of human ingenuity and natural processes. The key infrastructure of the *khazans* include the Dykes (*Bunds*), which are the protective embankments that are crucial for safeguarding the fields and villages from tidal flooding, the sluice gates (*Manos*) are wooden, one-way gates that regulate the flow of water in and out of the *khazan* system, and the channels and waterways (*Poiems* and *Adavo-manos*), which is an intricate network of canals and channels manages the water circulation.

Khazans provide a wide range of benefits for local communities and the environment by providing integrated livelihoods as they offer employment through a combination of traditional farming, pisciculture (fish farming), and salt extraction; towards food security, as the 'rice bowls' of Goa's coastal communities, they are primarily used as paddy fields for cultivating salt-tolerant rice varieties. Notable indigenous varieties include Korgut, Khonchri, Kalo-damgo, and Assgo. Hybrid varieties, such as *Oryza sativa* L. var. Jyoti has also been successfully cultivated (Rodrigues and Anuradha, 2009). As Biodiversity hotspot, these ecosystems are home to a rich variety of flora and fauna, including mangroves, wetland birds, fish, and aquatic organisms; for natural flood control, they act as a buffer against coastal erosion and function

as crucial flood-control systems, particularly during the monsoon season, and towards cultural heritage, the *khazan* system is deeply intertwined with Goan culture, with many traditions, festivals, and folk arts revolving around this ecosystem.

A study carried out to assess the diversity of AM fungi and the impact of AM fungal inoculation on yield performance in *O. sativa* (Xavier Martins and Rodrigues, 2018; Xavier Martins and Rodrigues, unpublished) revealed 42% AM colonisation, characterised by the presence of hyphae, arbuscules, and vesicles. The spore density recorded was 28 spores 100 g^{-1} of soil. Three AM fungal species, *viz.*, *Acaulospora scrobiculata*, *A. delicata* Walker, Pfeiff. & Bloss and *A. laevis* Gerd. & Trappe, were recovered.

A field experiment involving the dominant AM fungal species *A. scrobiculata* was carried out to assess its effect on yield. The experiment, involving two treatments (with and without AM inoculum) and two levels of rice plantlets per hill (1 and 3), was laid out. The results of the yield are depicted in **Table 1**. In *O. sativa*, the highest yield (1687 kg ha^{-1}) was recorded in treatment comprising *A. scrobiculata*, and +1 plant per hill, which was significantly higher than the uninoculated control (391 kg ha^{-1}).

TABLE 1: Effect of AM colonization and planting density on yield in *Oryza sativa*.

Treatment [†]	Yield (kg ha^{-1})
T ₁	1687.50 ^a
T ₂	391.30 ^b
T ₃	1175.43 ^{ab}
T ₄	925.10 ^{ab}

[†]T₁ = *A. scrobiculata* +1 plant hill⁻¹; T₂ = Uninoculated +1 plant hill⁻¹; T₃ = *A. scrobiculata* +3 plants hill⁻¹; T₄ = Uninoculated +3 plants hill⁻¹; Data presented is the mean of five readings; Different superscripts between the different treatments of study indicate significant differences at $P < 0.05$.

6. VIGNA UNGUICULATA, ITS CULTIVATION IN GOA AND THE MICROBIAL INTERACTIONS IN GROWTH AND SUSTAINABILITY

Vigna unguiculata (cowpea) is a major pulse crop on the West Coast region and is especially significant in Goa, where it is extensively cultivated in rice fallows under residual soil moisture conditions. This practice is highly valued by locals, creating revenue opportunities for farmers. The local cowpea variety is in high demand due to its bold size, soft texture, superior cooking quality, and exquisite taste. As a resilient legume, cowpea is agriculturally essential for food security and livelihood, particularly in developing countries. It thrives in semi-arid lands characterised by sandy loam soil with low to moderate natural fertility and requires minimal external inputs. The crop significantly benefits sustainable farming systems in several ways *viz.*, Cowpea enriches soil productivity by functioning as a cover crop, green manure, and N-fixing

crop, which helps maintain soil health without chemical fertilizers, the crop's quick ground cover prevents soil erosion, it helps suppress weeds by quickly covering the ground, and its ability to improve soil N levels makes it a vital rotational crop.

Cowpea's unique ability to fix atmospheric N is a result of symbiotic interactions with microbial partners like *Rhizobia*, *Bradyrhizobia*, and AM fungi. Legume-*Rhizobium* symbiosis alone contributes at least 50% of the 175 million tons of atmospheric N fixed each year globally. Biofertilizers, including vermicompost, farmyard manure, and AM fungi, are increasingly used in agriculture to enhance soil microbial activity and promote plant growth. AM fungi can enhance growth through increased N fixation and P solubilization in the rhizosphere, thereby reducing the need for excessive P fertilizers (Azcón, 2000; Lucy *et al.*, 2004). These biofertilizers are crucial for overcoming nutrient deficiencies and neutralising soil acidity.

AM fungi are a primary driver of microbial community function, improving nutrient supply and overall productivity (Johansson *et al.*, 2004). Beyond their role in nutrient mobilisation and carbon cycling, AM fungi also confer resilience against a range of environmental stresses, including heavy metal toxicity, soil salinity, heat, and drought (Azcón and Barea, 2010). As a result, these fungi serve as important biological tools for ecological restoration, with mycorrhizal symbiosis being a critical process found in nearly all natural habitats (Khade and Adholeya, 2007; Miransari, 2010). In specific crops, such as cowpea, AM fungi are known to enhance P acquisition and increase both N fixation and nodulation potential (Turk *et al.*, 2006). Given their vital role in nutrient uptake, increased plant biomass, and enhanced food security, AM fungi are a central focus of sustainable agriculture. The specific study discussed here sought to maximise crop yield by providing a high concentration of viable rhizobia to the rhizosphere, thereby enabling rapid colonisation, nodulation, and N fixation by a targeted inoculant strain.

In an unpublished research investigation by Velip and Rodrigues, the diversity of AM fungi and the impact of AM fungal inoculation on *V. unguiculata* yield performance were evaluated. The study revealed high AM colonization (97.33%), featuring hyphae, arbuscules, and vesicles. A high spore density (276 spores 100 g⁻¹ of soil) was also recorded. Twenty two AM fungal species *viz.*, *Gigaspora decipiens*, *Gi. margarita* Becker & Hall, *Gi. gigantea* (Nicolson & Gerd.) Gerd. & Trappe, *Gi. albida*, *Funnelformis geosporum*, *F. mosseae*, *Paraglomus albidum* (C. Walker & L. H. Rhodes) Oehl, G. A. Silva, *R. fasciculatus*, *G. microaggregatum* Koske, Gemma & Olexia, *G. multicaule* Gerdemann & Bakshi, *Rhizophagus intraradices*, *Acaulospora myriocarpa* Spain, Sieverd. & Schenck, *A. mellea* Spain & Schenck, *A. undulata* Sieverd., *A. polonica* Blaszk., *A. delicata*, *A. foveata* Trappe & Janos, *A. scrobiculata*, *A. spinosa* Walker & Trappe, *Racocetra gregaria* (Schenck & Nicolson) Oehl, Souza & Sieverd.,

Scutellospora scutata C. Walker & Dieder, and *S. reticulata* (Koske & Walker), Walker & Sanders, were recovered from the field and from *in vivo* culture.

Using the cowpea variety Goa Cowpea-3, a field experiment was carried out on sandy loam soils. Biofertilizers were applied using two methods: A talc-based inoculum containing *Bradyrhizobium* and *Bacillus methylotrophicus*, and an *in vitro* produced inoculum of *Gigaspora decipiens* with a carrier medium of vermiculite, cow dung powder, wood powder, and wood ash. The study revealed that inoculating cowpea with these bio-inoculants significantly improved both growth and yield compared to the control group (Fig. 3). Single and co-inoculation treatments, which combined *Bradyrhizobium*, Plant Growth-Promoting Rhizobacteria (PGPR), and AM fungus, positively impacted all analysed productivity parameters. The yield from all bio-inoculated treatments was higher than that of the uninoculated control, which recorded 75.56 kg ha⁻¹. The highest yield achieved was 162.4 kg ha⁻¹, marking a substantial increase over the control.

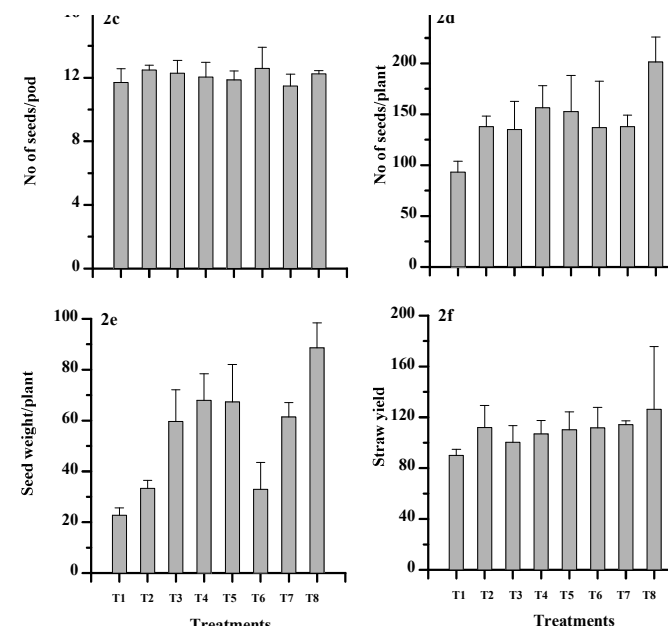


FIGURE 2: Effect of monoxenically produced carrier-based bio-inoculum of *Gigaspora decipiens*, **bio-formulation of *Bradyrhizobium* sp. and *Bacillus methylotrophicus*** on growth and yield characteristics in *Vigna unguiculata*.

In the experiment, bio-inoculated treatments resulted in significant root colonisation compared to un-inoculated controls. This inoculation, in

turn, significantly impacted cowpea growth and yield, with strong positive correlations observed for plant height, pod number, seed number, seed yield, and straw yield.

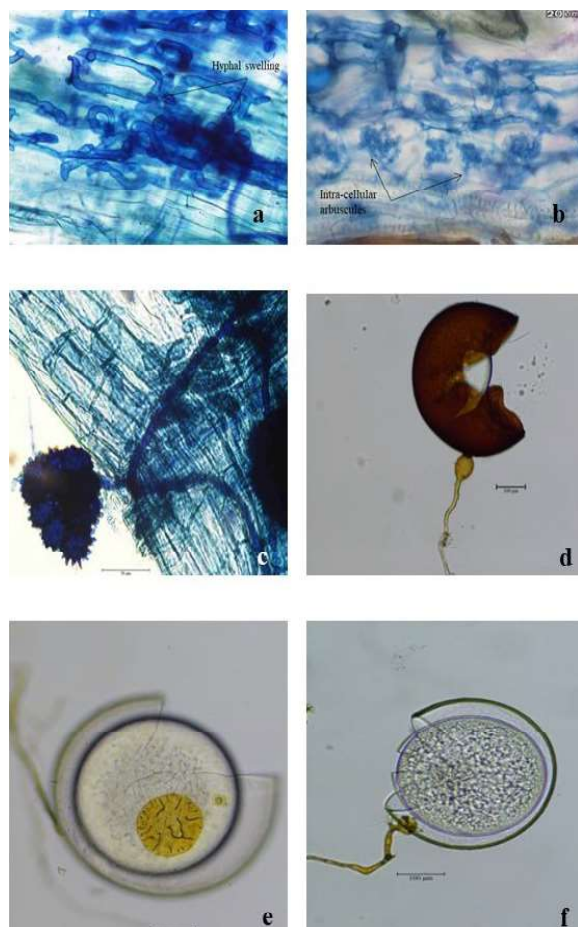


FIGURE 3: A: AM fungal colonisation. a. Hyphal swellings; b. Arbuscular colonization; c. Echinulate auxiliary cells, B: AM fungal species. d. *Racotetra gregaria*, e. *Seutellospora scutata*; f: *Gigaspora albida*.

The results demonstrated that indigenous AM fungal species provide complementary benefits to cowpea plants. Furthermore, a tripartite inoculation combining *Gi. decipiens*, *Bradyrhizobium* sp., and *B. methylotrophicus* led to a significant improvement in cowpea yield. These findings suggest that

AM fungi, rhizobia, and PGPR work synergistically to substantially increase cowpea yield, making the cultivation process more sustainable.

Legend: All values are the mean of 3 replicates; $\pm$ = standard error; T_1 = Control, T_2 = *Gigaspora decipiens*, T_3 = *Bradyrhizobium* sp., T_4 = *Bacillus methylotrophicus* (Goa Bio-1 culture), T_5 = *Gi. decipiens* + *Bradyrhizobium* sp., T_6 = *Gi. decipiens* + *B. methylotrophicus*, T_7 = *Bradyrhizobium* sp. + *B. methylotrophicus*, T_8 = *Gi. decipiens* + *Bradyrhizobium* sp. + *B. methylotrophicus*.

7. AM FUNGI: A NATURAL STRATEGY FOR ENHANCING CROP RESILIENCE AND PRODUCTIVITY AMID CLIMATE CHANGE

The growing challenges posed by climate change, such as extreme heat, prolonged drought, salinity, and heavy metal pollution, threaten the stability of global food production systems. Traditional farming practices, which heavily depend on synthetic fertilisers and pesticides, often lead to environmental degradation and declining soil health. In light of these issues, AM fungi have gained attention as a sustainable, nature-based solution for improving plant stress tolerance and productivity in changing climates. These ancient symbiotic soil organisms are known to be associated with the roots of over 80% of terrestrial plant species, creating a mutualistic network that significantly enhances the ability of crops to withstand environmental stresses.

7.1. Mechanisms Supporting Plant Stress Tolerance

AM fungi promote plant resilience through a combination of physiological, molecular, and biochemical mechanisms, enabling better adaptation to climatic stressors.

Coping with Water Stress (Drought and Waterlogging)

1. **Extended Access to Water:** AM fungi produce vast hyphal networks that reach beyond the root zone, accessing moisture in fine soil pores unavailable to roots. This expanded reach increases water uptake and improves water-use efficiency (Puschel, 2020).
2. **Enhanced Root Conductivity:** These fungi modulate the expression of aquaporins (water channel proteins), thereby improving root hydraulic conductivity and facilitating efficient water movement throughout the plant (Aroca, 2007).
3. **Osmotic Adjustment:** During water stress, AM fungi stimulate the accumulation of osmoprotectants like proline, glycine betaine, and sugars. These compounds help maintain cell turgor and metabolic activity under drought conditions (Porcel and Ruiz-Lozano, 2004).
4. **Soil Structure Improvement:** AM fungi release glomalin, a sticky glycoprotein that stabilizes soil aggregates, enhancing soil porosity, water

infiltration, and retention, offering protection against both drought and excess water (Rillig, 2004).

Alleviating Salt Stress

1. **Nutrient Homeostasis:** In saline environments, AM fungi aid in maintaining an optimal potassium to sodium (K^+/Na^+) ratio by limiting sodium absorption while enhancing K uptake, critical for metabolic function (Porrás-Soriano, 2009).
2. **Ion Sequestration:** AM fungi also influence ion transporter gene activity, promoting the compartmentalisation of harmful ions like sodium into vacuoles, reducing their toxic impact on plant cells (Porcel *et al.*, 2016).

Tolerance to Temperature Extremes

1. **Antioxidant Regulation:** High or low temperatures can lead to oxidative stress due to increased reactive oxygen species (ROS). AM fungi boost antioxidant enzyme levels (e.g., superoxide dismutase, catalase, and peroxidase), protecting plant cells from oxidative damage (Wu, 2008).
2. **Protection of Photosynthesis:** The AM symbiosis helps maintain higher chlorophyll content and photosynthetic efficiency under temperature stress by protecting the photosynthetic apparatus from damage. This ensures that the plant can continue to produce carbohydrates needed for its own growth and for the fungi (Ruiz-Lozano, 2003).
3. **Boosting crop productivity in a changing climate:** Beyond stress resilience, AM fungi directly contribute to increased crop productivity, offering a viable solution for maintaining yields in a warming world (Nie *et al.*, 2024).

Contribution to Crop Productivity in a Changing Climate

Beyond stress mitigation, AM fungi directly improve agricultural productivity, making them a promising tool for climate-resilient farming.

Improved Nutrient Uptake

1. **Phosphorus Efficiency:** AM fungi are particularly adept at accessing P from soil, significantly improving its availability to plants and reducing reliance on chemical fertilisers (Smith and Read, 2008).
2. **Nitrogen Uptake:** Their hyphal networks enhance the plant's capacity to absorb N in both nitrate and ammonium forms, which supports protein synthesis and growth (Smith and Smith, 2011).
3. **Micronutrient Availability:** AM fungi also facilitate better absorption of trace elements like zinc (Zn) and copper (Cu), which are crucial for plant development but often remain immobile in the soil (Lehmann and Rillig, 2015).

Improving Plant Growth and Development

1. **Higher Biomass and Yield:** Plants colonised by AM fungi exhibit greater biomass, yield, and reproductive success, especially under stressful conditions where non-mycorrhizal plants typically underperform (Begum, 2019).
2. **Improved Photosynthetic Efficiency:** Enhanced gas exchange and water relations, coupled with reduced oxidative stress, enable AM-inoculated plants to maintain robust photosynthesis and energy production (Begum, 2019).
3. **Hormonal Modulation:** AM fungi influence the plant's hormonal profile, increasing growth-promoting hormones like indole-3-acetic acid (IAA) while regulating stress-related hormones such as abscisic acid (ABA), leading to improved root and shoot development (Pons, 2020).

8. CONCLUSION

The studies cited showed that bio-inoculation with AM fungi enhances crop growth and yield. In *O. sativa*, AM inoculation produced significant yield benefits. These advantages were amplified when combined with other beneficial microbes, such as PSB or PGPR. Field results with *I. batatas* and *V. unguiculata* confirmed that co-inoculation with AM and either PSB or PGPR led to superior performance compared to single treatments.

Amid escalating climate challenges, AM fungi offer a powerful, eco-friendly alternative to conventional agricultural inputs. By improving water and nutrient uptake (particularly P), enhancing stress tolerance, and promoting plant growth, they are a cornerstone of sustainable agriculture. Reducing dependence on synthetic inputs and boosting crop resilience, AM fungi are a viable strategy for ensuring long-term food security.

Ultimately, leveraging microbial bioinoculants to improve crop performance addresses critical global challenges. These inoculants are not only cost-effective but also confer substantial benefits for soil health and environmental sustainability, laying the groundwork for a more resilient and biologically diverse agricultural future.

9. REFERENCES

- Ahammed, G. J., Shamsy, R., Liu, A. and Chen, S. (2023) 'Arbuscular mycorrhizal fungi induced tolerance to chromium stress in plants', *Environmental Pollution*, 327, p. 121597.
- ALHadidi, N., Pap, Z., Ladányi, M., Szentpéteri, V. and Kappel, N. (2021) 'Mycorrhizal inoculation effect on sweet potato (*Ipomoea batatas* (L.) Lam) seedlings', *Agronomy*, 11(10), p. 2019.
- Aroca, R., Porcel, R. and Ruiz-Lozano, J. M. (2007) 'Arbuscular mycorrhizal symbiosis influences the physiological responses of maize plants (*Zea mays* L.)

- to salinity, including gene expression of aquaporins', *Journal of Experimental Botany*, 58(7), p. 1915–1923.
- Azcón, R. (2000) 'Papel de la simbiosis micorrízica y su interacción con otros microorganismos rizosféricos en el crecimiento vegetal y sostenibilidad agrícola', in *Ecología, fisiología y biotecnología de la micorriza arbuscular*. México: Mundi Prens, p. 1–15.
- Azcón, R. and Barea, J. M. (2010) 'Mycorrhizosphere interactions for legume improvement', in *Microbes for legume improvement*. Vienna: Springer, p. 237–271.
- Begum, N., Qin, C., Ahanger, M. A., Raza, S., Khan, M. I., Ashraf, M., Ahmed, N. and Zhang, L. (2019). Role of arbuscular mycorrhizal fungi in plant growth regulation: implications in abiotic stress tolerance. *Frontiers in Plant Science*, 10, p.1068.
- Behera, S., Chauhan, V. B. S., Pati, K., Bansode, V., Nedunchezhiyan, M., Verma, A. K., Monalisa, K., Naik, P. K. and Naik, S. K. (2022). Biology and biotechnological aspect of sweet potato (*Ipomoea batatas* L.): A commercially important tuber crop. *Planta*, 256(2), p.40.
- Bonfante, P. and Venice, F. (2020) 'Mucoromycota: going to the roots of plant-interacting fungi', *Fungal Biology Reviews*, 34(2), p. 100–113.
- Chandrasekaran, M. (2022) 'Arbuscular mycorrhizal fungi mediated enhanced biomass, root morphological traits and nutrient uptake under drought stress: a meta-analysis', *Journal of Fungi*, 8(7), p. 660.
- Chaudhary, A., Poudyal, S. and Kaundal, A. (2025) 'Role of arbuscular mycorrhizal fungi in maintaining sustainable agroecosystems', *Applied Microbiology*, 5(1), p. 6.
- Fortin, J. A., Bécard, G., Declerck, S., Dalpé, Y., St-Arnaud, M., Coughlan, A. P. and Piché, Y. (2002) 'Arbuscular mycorrhiza on root-organ cultures', *Canadian Journal of Botany*, 80(1), p. 1–20.
- Fracasso, A., Telò, L., Lanfranco, L., Bonfante, P. and Amaducci, S. (2020) 'Physiological beneficial effect of *Rhizophagus intraradices* inoculation on tomato plant yield under water deficit conditions', *Agronomy*, 10(1), p. 71.
- Guo, S., Xia, L., Xia, D., Li, M., Xu, W. and Liu, L. (2024) 'Enhancing plant resilience: arbuscular mycorrhizal fungi's role in alleviating drought stress in vegetation concrete', *Frontiers in Plant Science*, 15, p. 1401050.
- Johansson, J. F., Paul, L. R. and Finlay, R. D. (2004) 'Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture', *FEMS Microbiology Ecology*, 48(1), p. 1–13.
- Kalamulla, R., Karunarathna, S. C., Tibpromma, S., Galappaththi, M. C., Suwannarach, N., Stephenson, S. L., Asad, S., Salem, Z. S. and Yapa, N. (2022). Arbuscular mycorrhizal fungi in sustainable agriculture. *Sustainability*, 14(19), p.12250.
- Khade, S. W. and Adholeya, A. (2007) 'Feasible bioremediation through arbuscular mycorrhizal fungi imparting heavy metal tolerance: a retrospective', *Bioremediation Journal*, 11(1), p. 33–43.
- Khan, Y., Shah, S. and Tian, H. (2022) 'The roles of arbuscular mycorrhizal fungi in influencing plant nutrients, photosynthesis, and metabolites of cereal crops – a review', *Agronomy*, 12(9), p. 2191.
- Larbi-Koranteng, S., Kankam, F., Adomako, J., and Abdulai, M. (2022). Role of mycorrhizae in crop protection. In R. N. de Sousa (Ed.), *Arbuscular Mycorrhizal Fungi in Agriculture – New Insights*. IntechOpen.

- Lehmann, A. and Rillig, M. C. (2015) 'Arbuscular mycorrhizal contribution to copper, manganese and iron nutrient concentrations in crops—a meta-analysis', *Soil Biology and Biochemistry*, 81, p. 148–154.
- Lucy, M., Reed, E. and Glick, B. R. (2004) 'Applications of free-living plant growth-promoting rhizobacteria', *Antonie van Leeuwenhoek*, 86(1), p. 1–25.
- Miransari, M. (2010) 'Contribution of arbuscular mycorrhizal symbiosis to plant growth under different types of soil stress', *Plant Biology*, 12(4), p. 563–569.
- Morton, J. B., Bentivenga, S. P. and Wheeler, W. W. (1993) 'Germ plasm in the International Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM) and procedures for culture development, documentation and storage', *Mycotaxon*, 48(1), p. 491–528.
- Mounika, V., Gowd, T. Y. M., Lakshminarayana, D., Krishna, G. V., M, Y., Reddy, I. S., Soumya, B. K., Chamuah, S., Singh, Y. D. and Reddy, P. M. (2025) Sweet potato (*Ipomoea batatas* (L.) Lam): a comprehensive review of its botany, nutritional composition, phytochemical profile, health benefits, and future prospects. *European Food Research and Technology*, 251(10), p.3225–3239.
- Nacoon, S., Jogloy, S., Riddech, N., Mongkolthanaruk, W., Ekprasert, J., Cooper, J. and Boonlue, S. (2021) 'Combination of arbuscular mycorrhizal fungi and phosphate solubilizing bacteria on growth and production of *Helianthus tuberosus* under field condition', *Scientific Reports*, 11(1), p. 6501.
- Nie, W., He, Q., Guo, H., Zhang, W., Ma, L., Li, J. and Wen, D. (2024) 'Arbuscular mycorrhizal fungi: boosting crop resilience to environmental stresses', *Microorganisms*, 12(12), p. 2448.
- Pednekar, A. S. and Rodrigues, B. F. (2025) 'Diversity of arbuscular mycorrhizal fungi associated with *Amorphophallus commutatus* (Schott) Engl., in Goa, India', *Kavaka*, 61(2), p. 58–64. doi:10.36460/Kavaka/61/2/2025/58–64.
- Pons, S., Fournier, S., Chervin, C., Bécard, G., Rochange, S., Frei Dit Frey, N. and Puech Pagès, V. (2020) Phytohormone production by the arbuscular mycorrhizal fungus *Rhizophagus irregularis*. *PLoS One*, 15(10), p.e0240886.
- Porcel, R. and Ruiz-Lozano, J. M. (2004) 'Arbuscular mycorrhizal colonisation ameliorates the optimum temperature for maize growth by improving nutritional and water status', *Journal of Experimental Botany*, 55(406), p. 1865–1872.
- Porcel, R., Aroca, R., Azcón, R. and Ruiz-Lozano, J. M. (2016) 'Regulation of plant water relations and ion homeostasis by arbuscular mycorrhizal symbiosis under drought and salinity', *Journal of Experimental Botany*, 67(11), p. 3271–3284.
- Porrás-Soriano, A., Soriano, T., Porcel, R., Ruiz-Lozano, J. M., Azcón, R. and Cano, C. (2009) 'Arbuscular Mycorrhizal Fungi enhance olive tree growth and improve nutritional status, ion content, and gas exchange under salinity conditions', *Journal of Plant Physiology*, 166(5), p. 585–593.
- Püschel, D., Bitterlich, M., Rydlová, J. and Jansa, J. (2020) 'Facilitation of plant water uptake by an Arbuscular Mycorrhizal fungus: a Gordian knot of roots and hyphae', *Mycorrhiza*, 30(2), p. 221–230.
- Rigobelo, E. C. (2024) The importance of mycorrhiza fungi to sustainable food production. In E.C. Rigobelo (Ed.) *Unveiling the mycorrhizal World*. London: IntechOpen, p. 1–98.
- Rillig, M. C. (2004) 'Arbuscular mycorrhizae and terrestrial ecosystem processes', *Plant and Soil*, 262(1), p. 173–186.

- Rodrigues, B. F. and Anuradha, N. (2009) 'Arbuscular mycorrhizae fungi of the Khazan land agro ecosystem', in Sridhar, K.R. (ed.) *Frontiers in Fungal Ecology, Diversity and Metabolites*. New Delhi: I.K. International Publishing House Pvt. Ltd., p. 141–150.
- Ruiz-Lozano, J. M. (2003) 'Arbuscular mycorrhizal symbiosis and drought resistance', *Physiologia Plantarum*, 118(4), p. 510–519.
- Schaefer, D. A., Gui, H., Mortimer, P. E. and Xu, J. (2021) 'Arbuscular mycorrhiza and sustainable agriculture', *Circular Agricultural Systems*, 1(1), p. 1–7.
- Shi, K., Yuan, J., Ye, J. M., Wang, J. D., Zhu, G. P., Wang, L., Zhang, H. and Zhang, Y. C. (2023) Advances in application of arbuscular mycorrhizal fungi on sweet potato production. *Acta Horticulturae*, p. 576–581.
- Smith, S. E. and Read, D. J. (2008) *Mycorrhizal Symbiosis*, 3rd ed. London: Academic Press.
- Smith, S. E. and Smith, F. A. (2011) 'Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales', *Annual Review of Plant Biology*, 62, p. 227–250.
- Turk, M.A., Assaf, T. A., Hameed, K. M. and Al-Tawaha, A. M. (2006) 'Significance of mycorrhizae', *World Journal of Agricultural Sciences*, 2(1), p. 16–20.
- Velip, D. M., and Rodrigues, B. F. In vitro cultivation of *Gigaspora decipiens* using transformed roots of *Linum usitatissimum*. *Kavaka*, 53, p. 96–99. 10.36460/Kavaka/53/2019/96–99.
- Wahab, A., Muhammad, M., Munir, A., Abdi, G., Zaman, W., Ayaz, A., Khizar, C. and Reddy, S. P. P. (2023) Role of arbuscular mycorrhizal fungi in regulating growth, enhancing productivity, and potentially influencing ecosystems under abiotic and biotic stresses. *Plants*, 12(17), p.3102.
- Wang, Q., Liu, M., Wang, Z., Li, J., Liu, K. and Huang, D. (2024) 'The role of arbuscular mycorrhizal symbiosis in plant abiotic stress', *Frontiers in Microbiology*, 14, p. 1323881.
- Wu, Q. S., Xia, R. X., Li, Y., Wang, X. and Yu, Y. (2008) 'Effects of arbuscular mycorrhizal fungi on the growth and antioxidant status of citrus seedlings exposed to drought stress', *Journal of Plant Physiology*, 165(11), p. 1114–1122.
- Xavier Martins, W. F. and Rodrigues, B. F. (2018) 'Arbuscular mycorrhizal fungal diversity in rice varieties cultivated in lands in Goa', *Kavaka*, 50, p. 48–52.
- Yano-Melo, A. M., Maia, L. C., Saggin Jr, O. J., Lima-Filho, J. M. and Melo, N. F. (1999) 'Effect of arbuscular mycorrhizal fungi on the acclimatization of micropropagated banana plantlets', *Mycorrhiza*, 9(2), p. 119–123.

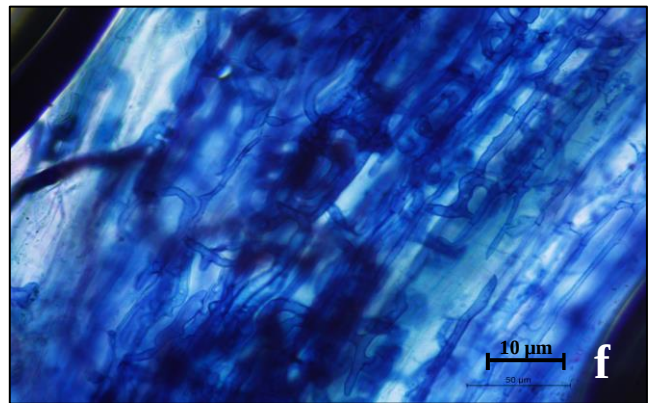
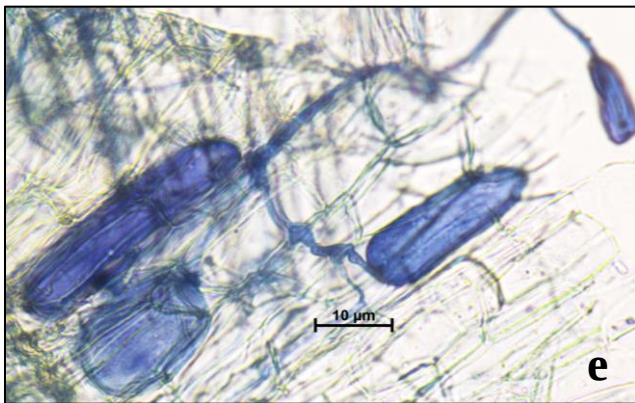
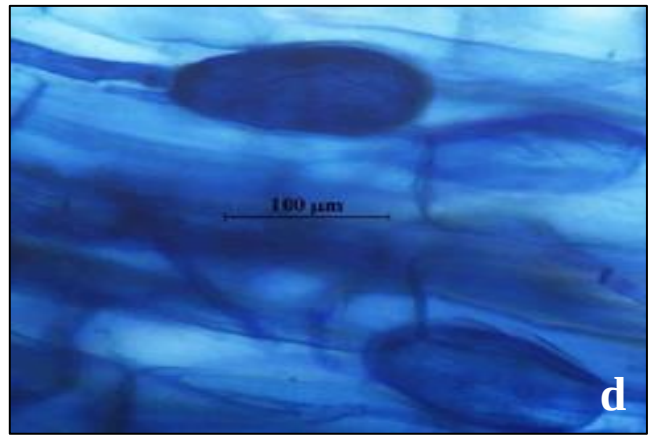
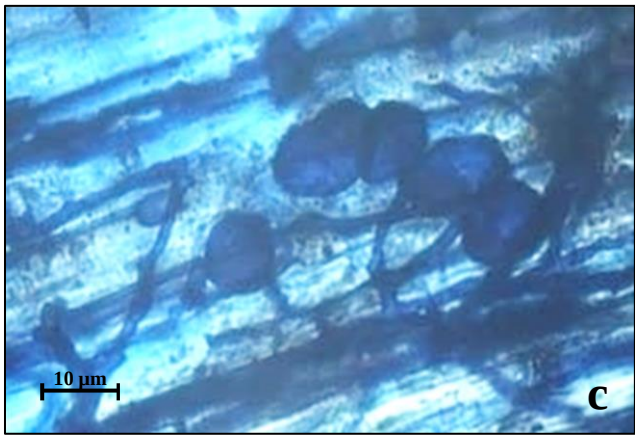
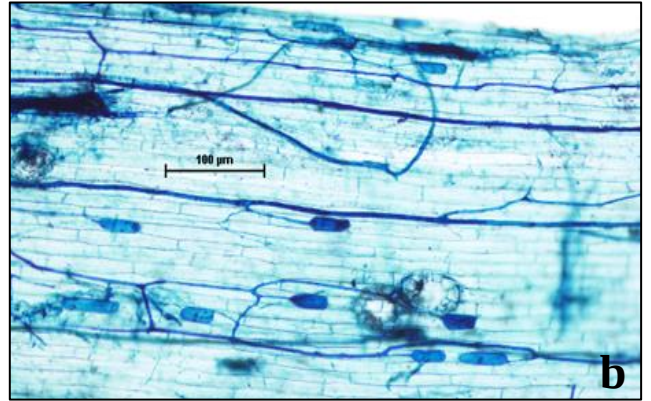


Plate 3.1: AM colonization in tuber species, showing hyphal and vesicular colonization- a–e: Hyphal and vesicular colonization; f - hyphal coils.

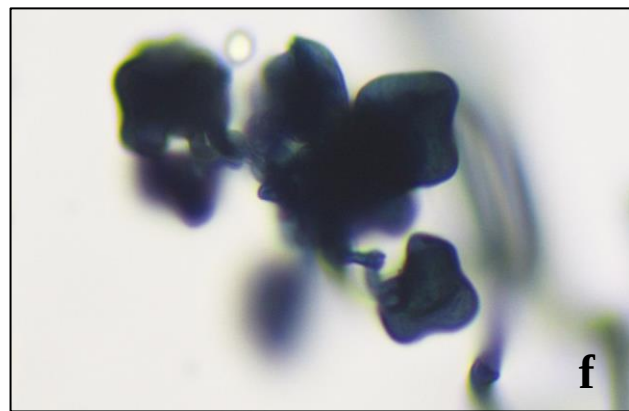
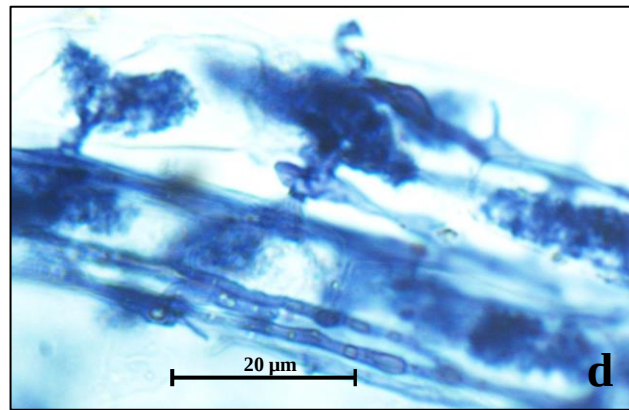
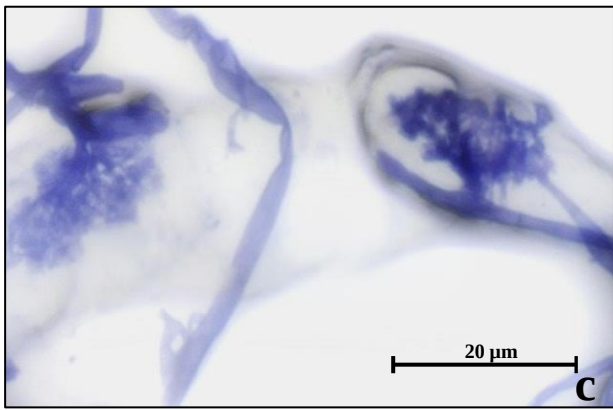
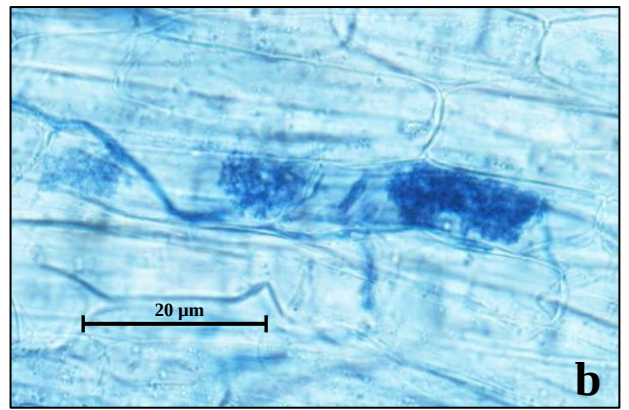
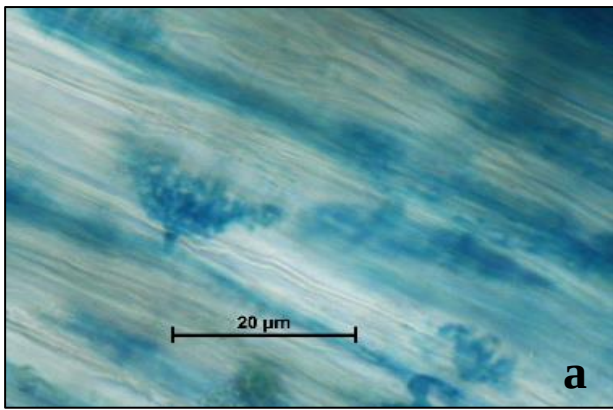


Plate 3.2: AM colonization in tuber species showing arbuscular colonization – **a–d**: arbuscular colonization; Auxiliary cells in **e** - rhizosphere soil, and **f** – roots.

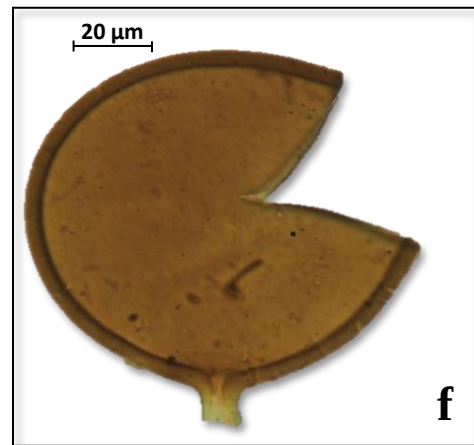
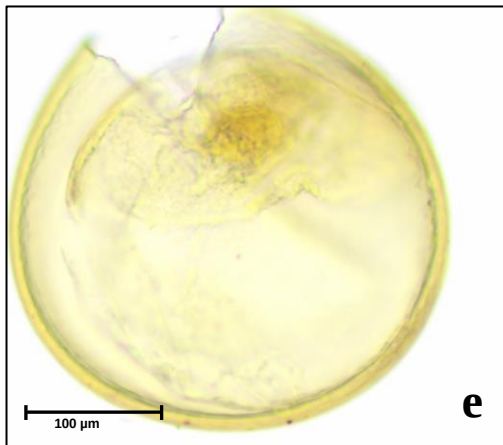
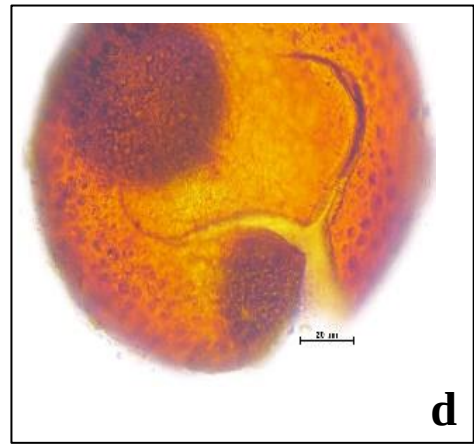
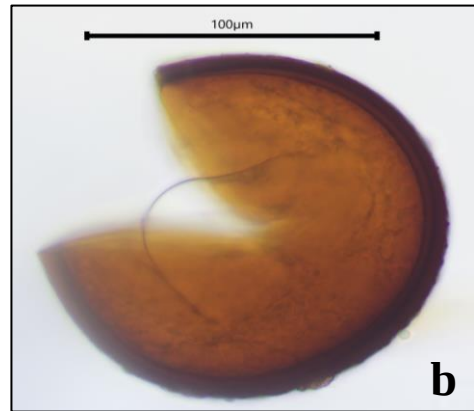
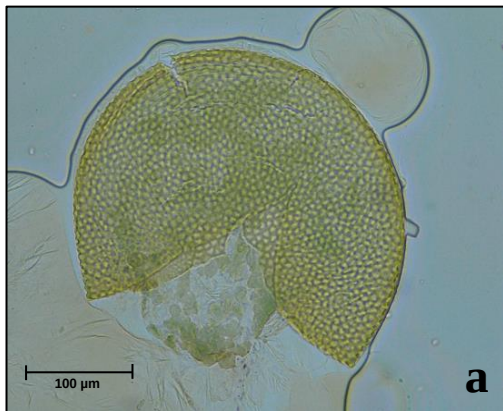


Plate 4.1: AM fungal diversity in tuber species - a. *Acaulospora scrobiculata*, b. *Funneliformis geosporum*, c. *Gigaspora albida*, d. *Acaulospora foveata*, e. *A. delicata*, f. *F. mosseae*.

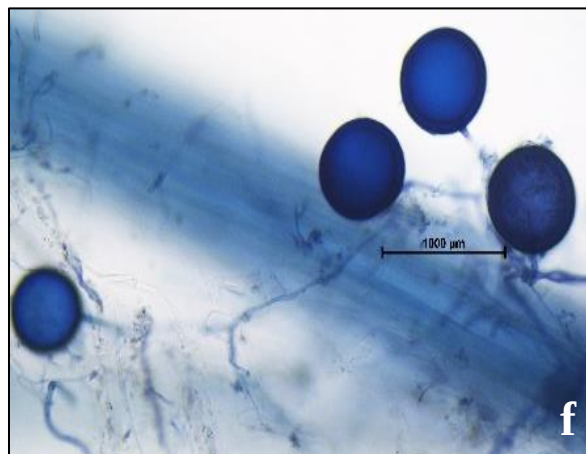
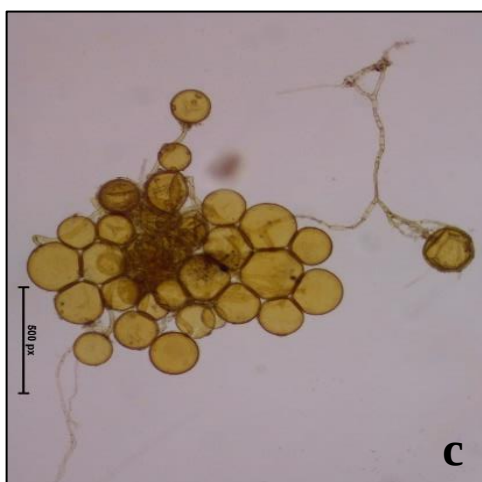
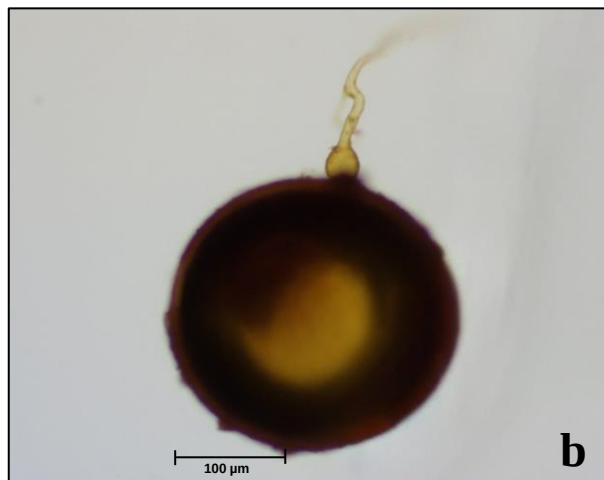
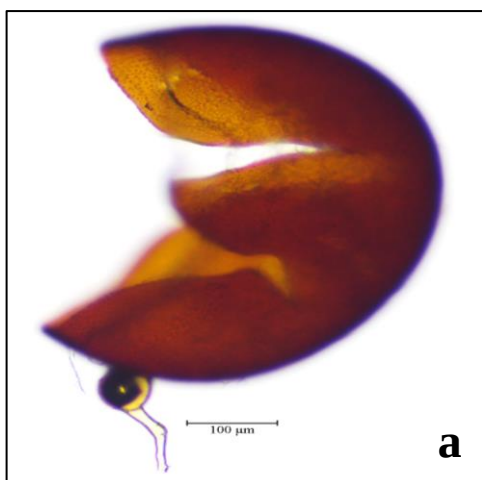


Plate 4.2: AM fungal diversity in tuber species - a. & b. *Recocetra gregaria*, c. & d. *Glomus microcarpum* e. *Acaulospora dilatata*, f. Extra radical spores.

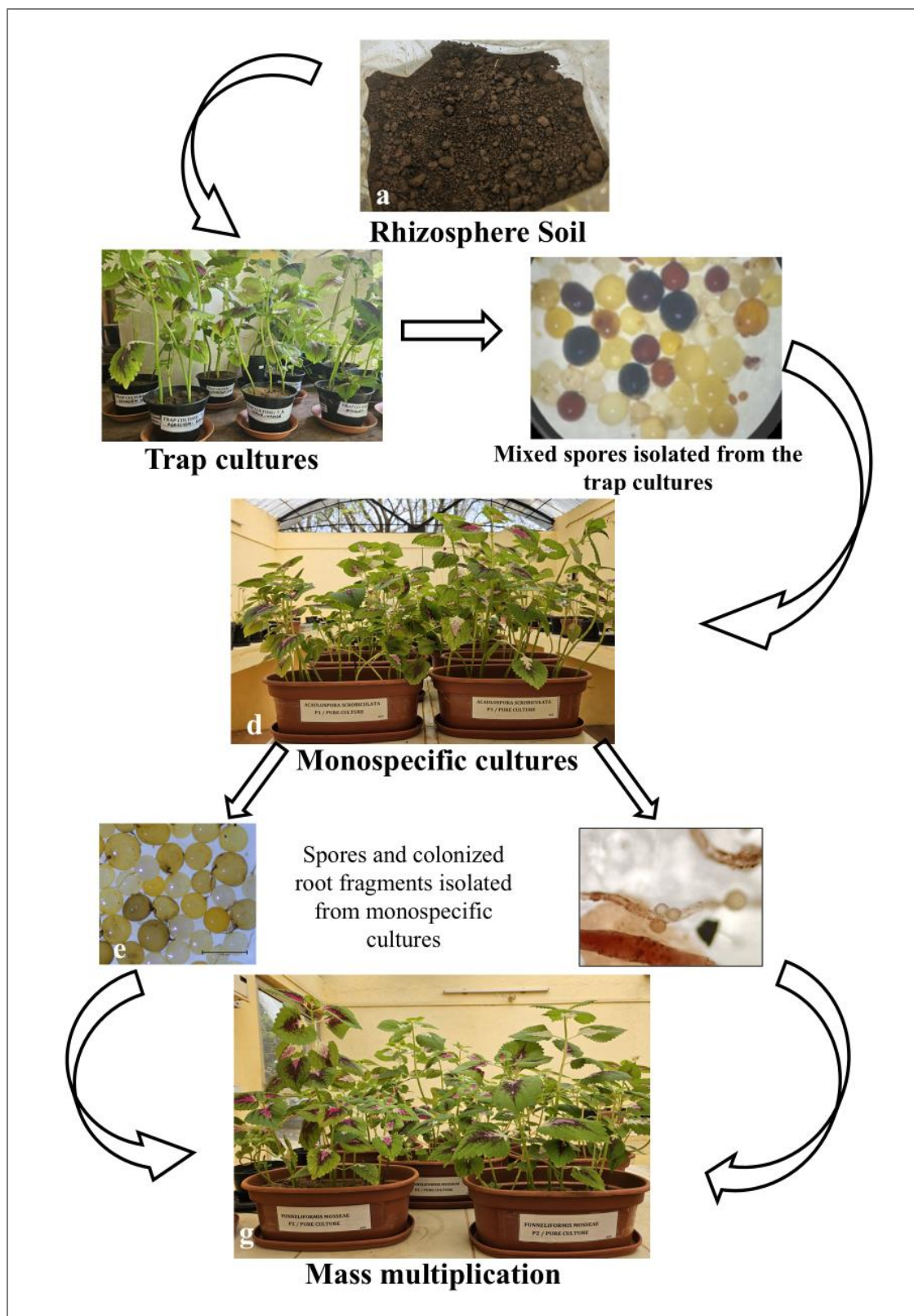


Plate 6.1: Preparation of the pure cultures of AM fungi using trap and monospecific cultures – **a.** Rhizosphere soil sample collected from the study location, **b.** Trap cultures established using the rhizosphere soil sample, **c.** Mixed AM fungal spores isolated from trap cultures, **d.** Single species (Monospecific) cultures, **e.** AM fungal spores isolated from monospecific cultures, **f.** Root fragments colonized by *Rhizophagus intraradices* extracted and isolated from monospecific cultures and **g.** Mass multiplication of selected AM fungal species.

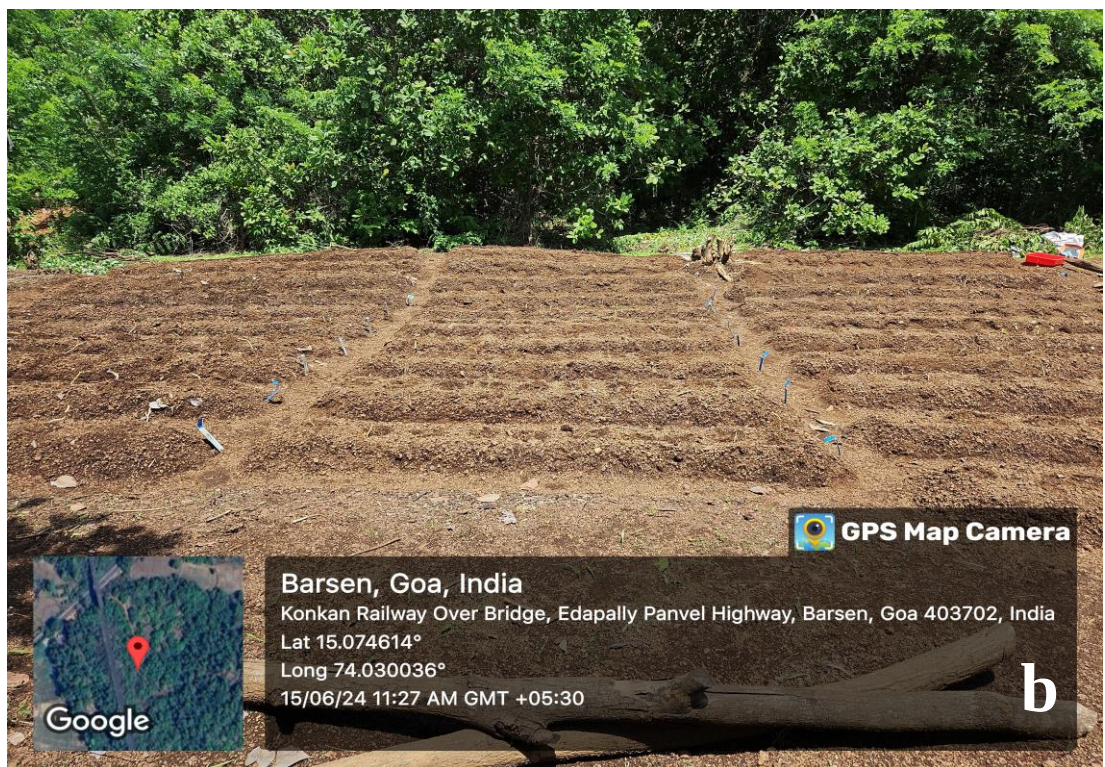


Plate 7.1: Field experiment at Barcem site - a. Google Earth image, showing the site of field experiment using *Dioscorea esculenta*, and **b.** Field preparation at the site.



Plate 7.2: Preparation of planting material of *Dioscorea esculenta* from the previous season - a. Crop, b. Harvest, c. Uniform and healthy sized tubers selected for storage, and d. Disease-free and uniform sized sprouted tubers for planting.

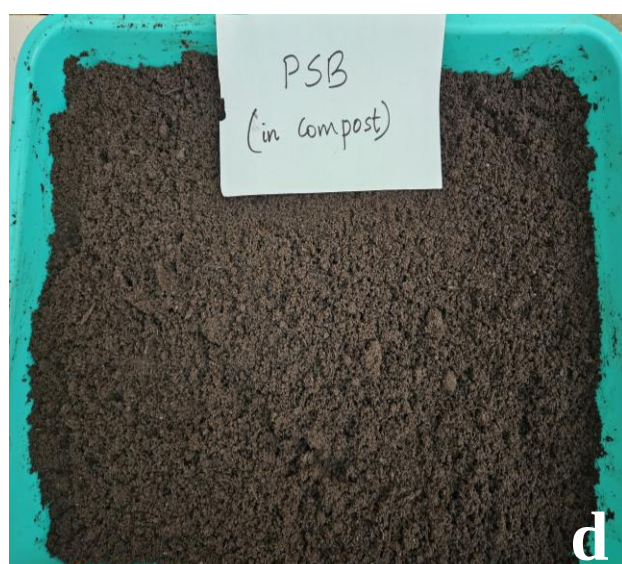


Plate 7.3: Soil-based AM fungal inoculum of - a. *Acaulospora scrobiculata*, b. *Funneliformis mosseae*, c. *Rhizophagus intraradices*, and d. Compost-based PSB.



Plate 7.4: Plants of *Dioscorea esculenta* in the field - a. Plant growth after 40 days, and **b.** Plant growth after 105 days.



Plate 7.5: Experimental field at Agassiam site - a. Google Earth image of the site, and **b.** Crop raised for healthy planting material.



Plate 7.6: Planting material of *Ipomoea batatas* - a. Healthy and uniform stem cuttings for planting, and **b.** Selected cuttings freshly sown in the field.



Plate 7.7: Growth of *Ipomoea batatas* at experimental field - a. Growth of *Ipomoea batatas* at the experimental field after 30 days, and **b.** Growth of *Dioscorea esculenta* at the experimental field after 90 days.

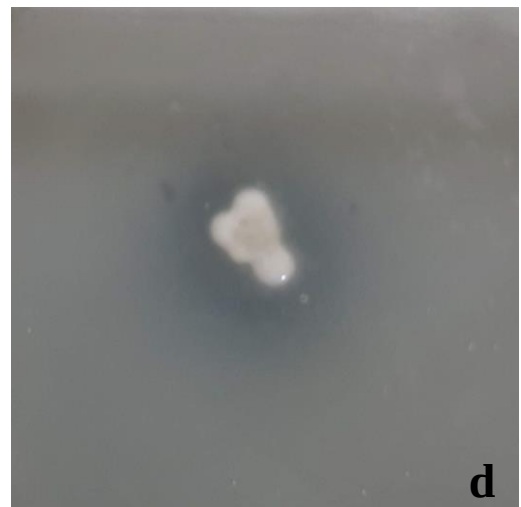
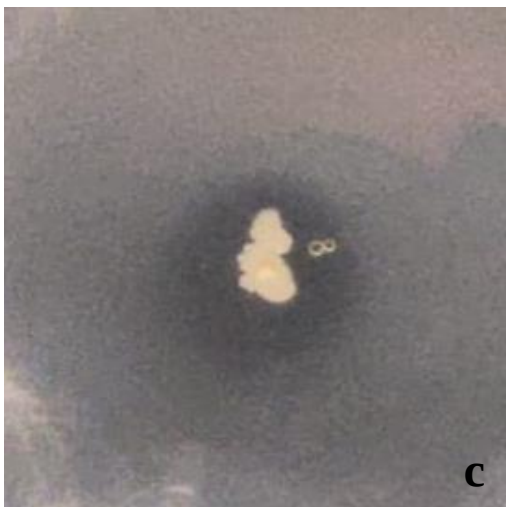
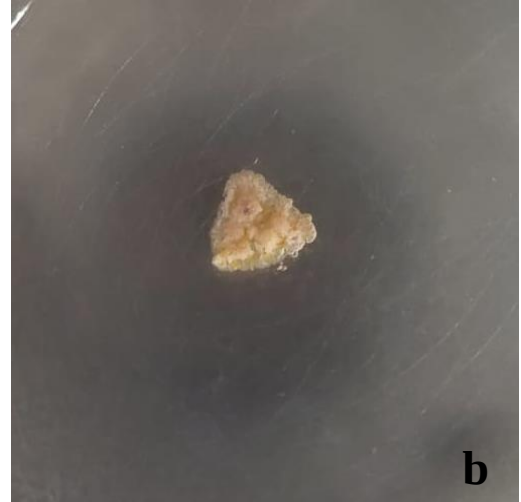
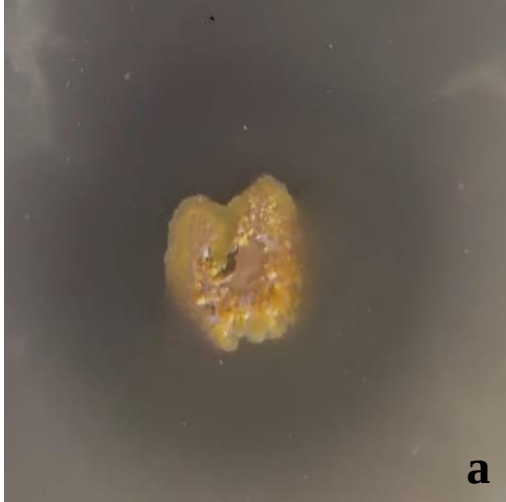


Plate 7. 8 : Zone clearance halo zones of PSB strains – a. PSB₁, b. PSB₂, c. PSB₃, d. PSB₄.

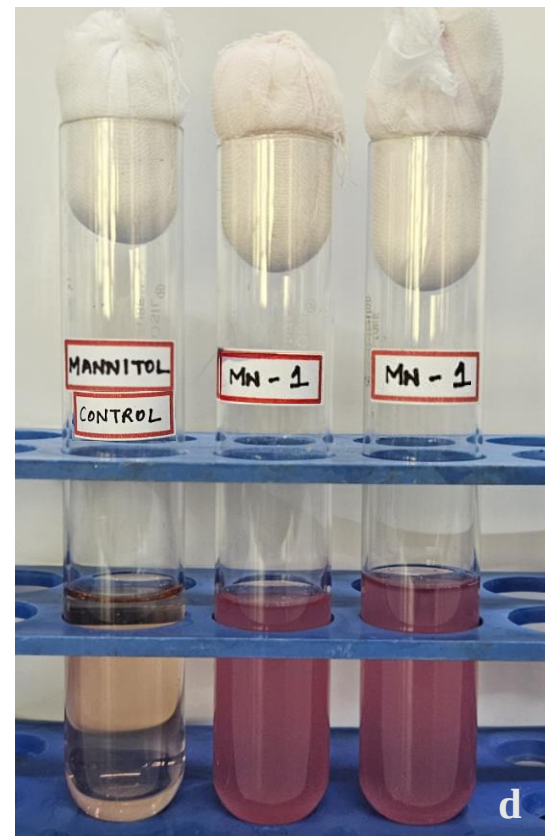
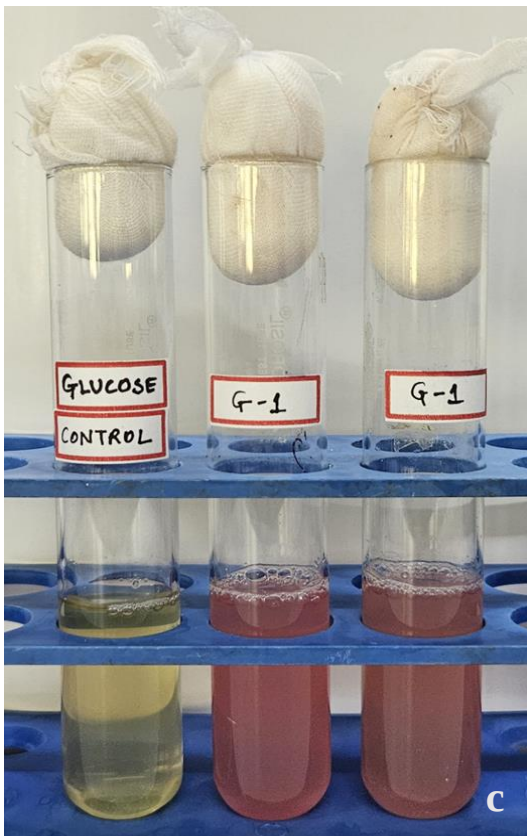
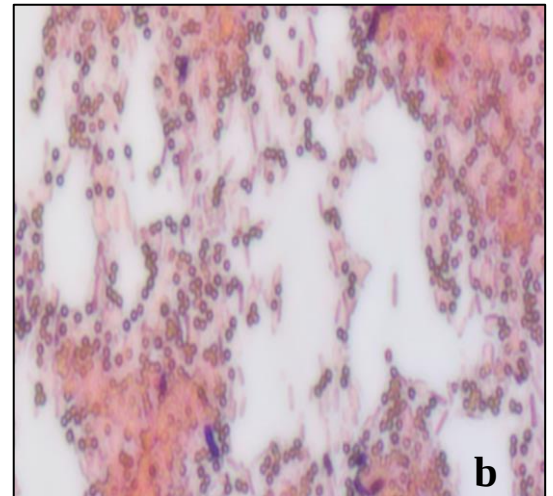


Plate 7.9: Morphological and biochemical tests for PSB₁ – **a.** Colony of PSB₁, **b.** gram staining of PSB₁; Biochemical test for – **c.** glucose, **d.** mannitol.

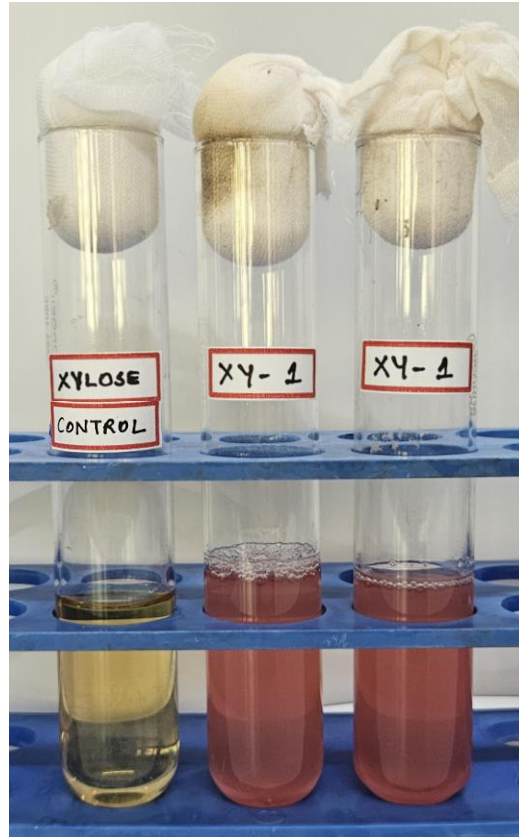
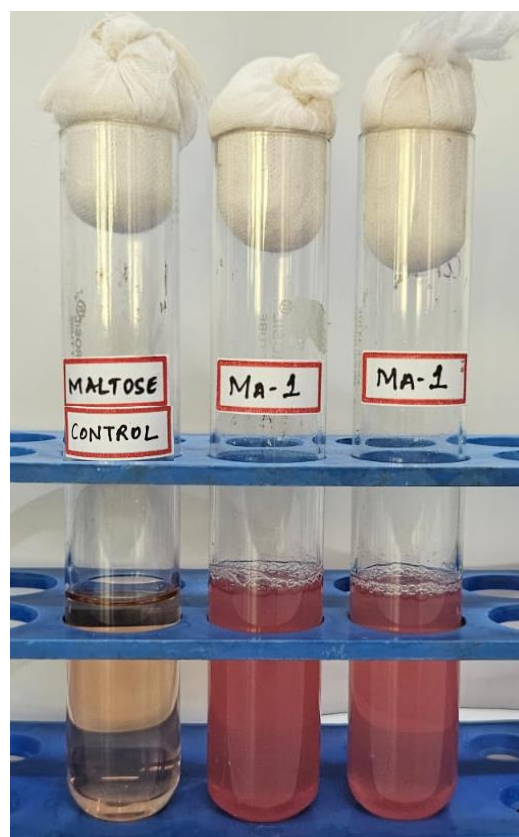
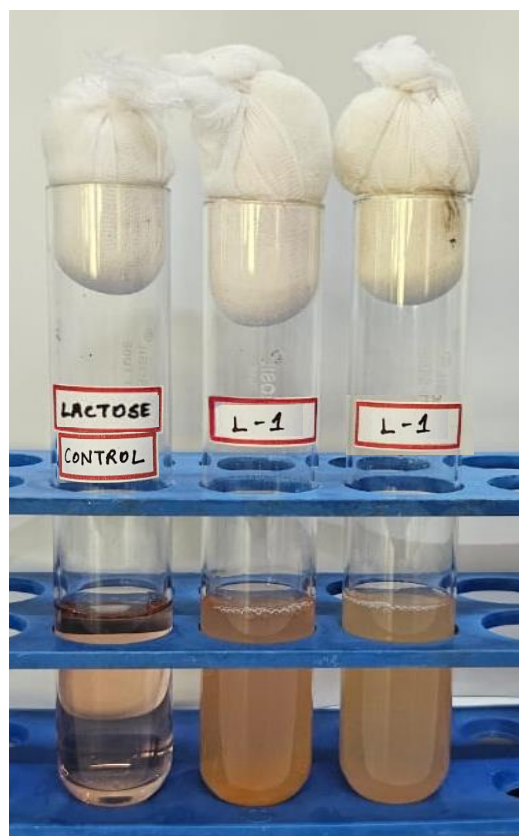
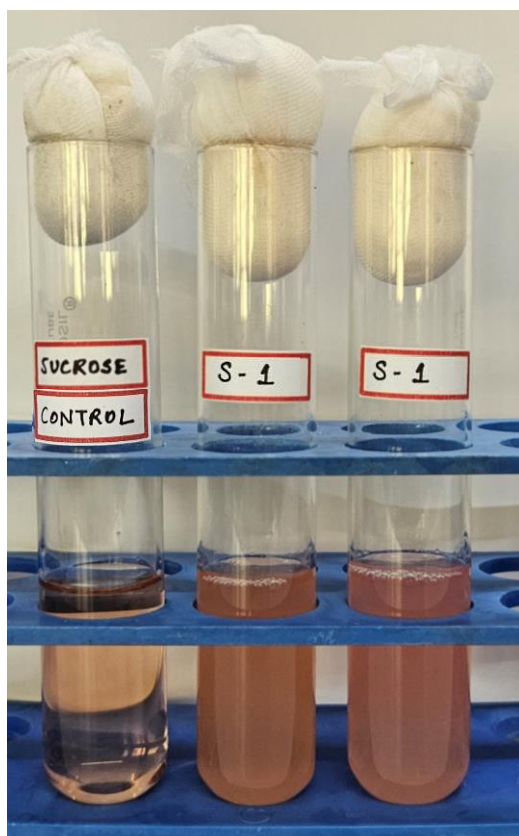


Plate 7.10 : Biochemical tests for PSB₁ – a. sucrose, b. lactose, c. maltose, d. xylose.

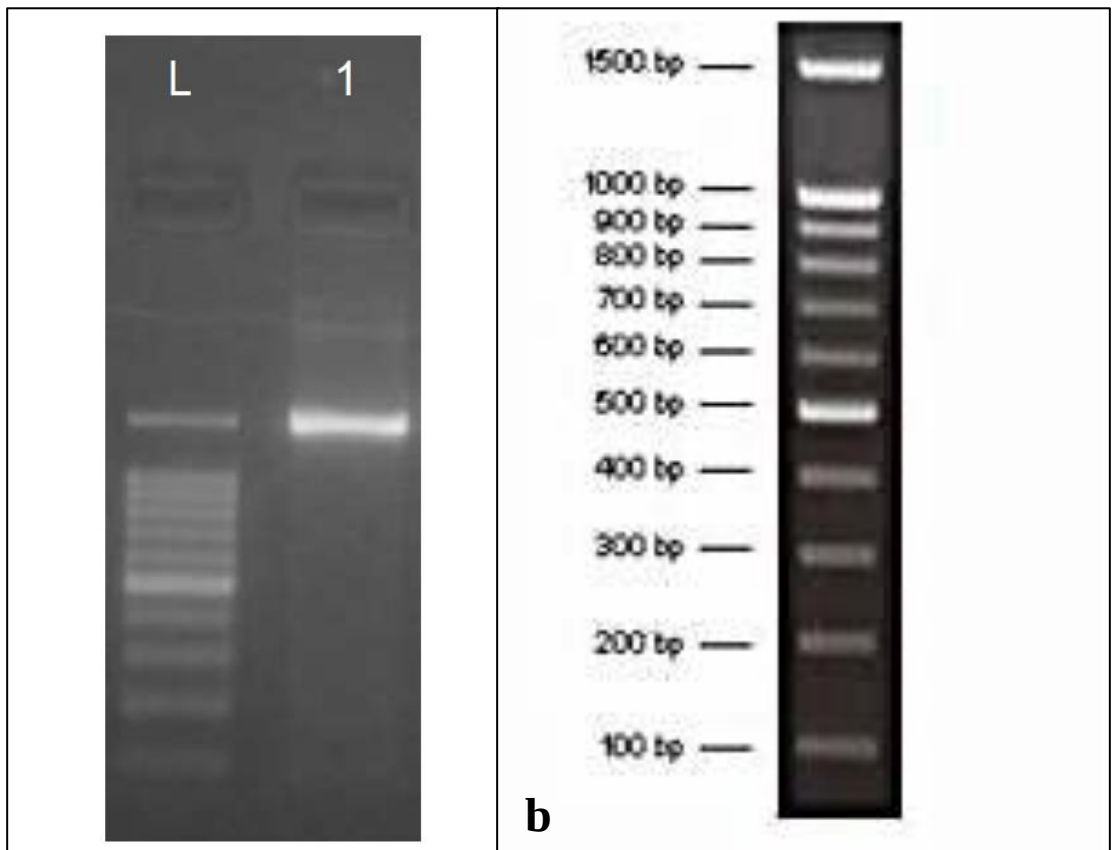
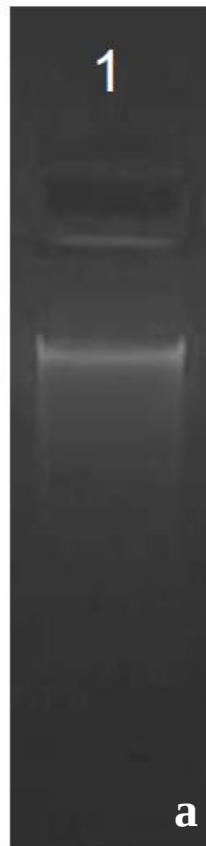


Plate 7.11: Agarose gel electrophoresis for identification of PSB₁ -
a. Genomic DNA Gel, **b.** PCR amplified products with 100 bp ladder.

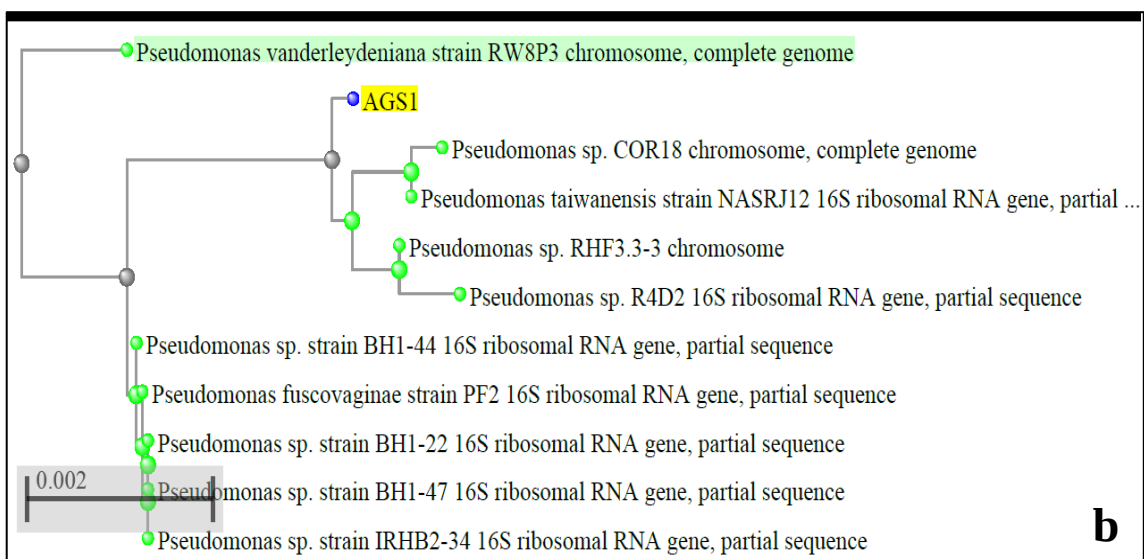
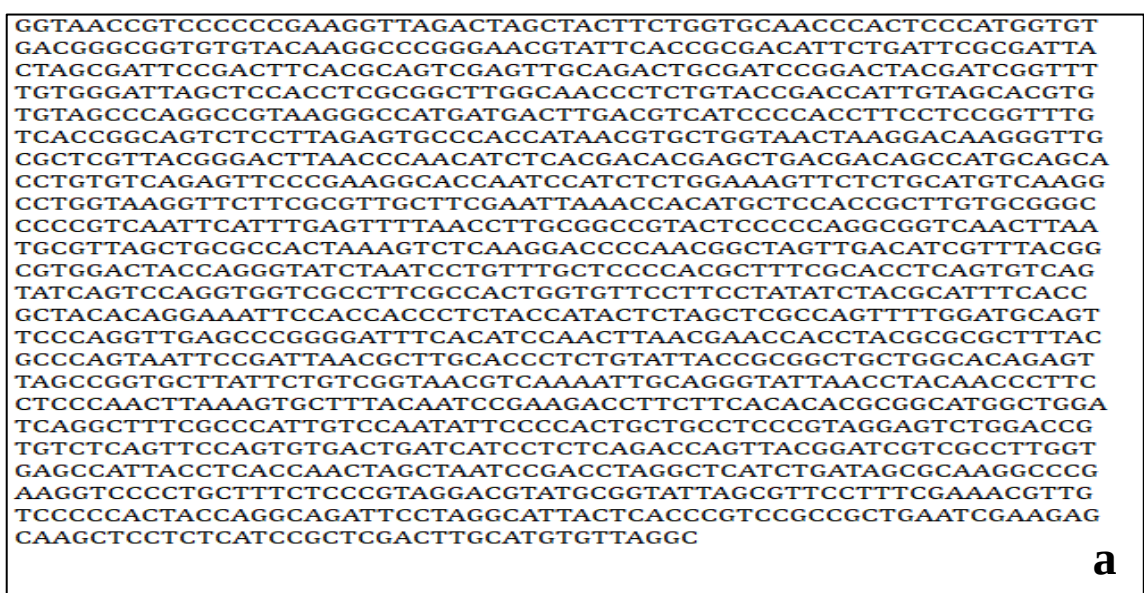


Plate 7.12: Sequence data and phylogenetic tree for PSB₁ **a.** aligned sequence of PSB₁, **b.** Dendrogram showing the phylogenetic position of PSB₁ (AGS1) among other bacterial strains.

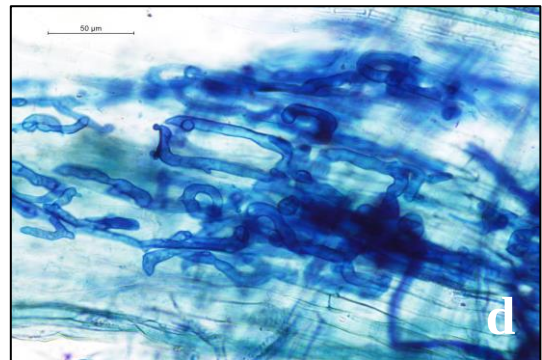
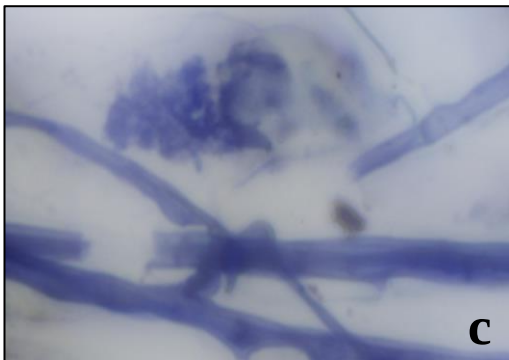
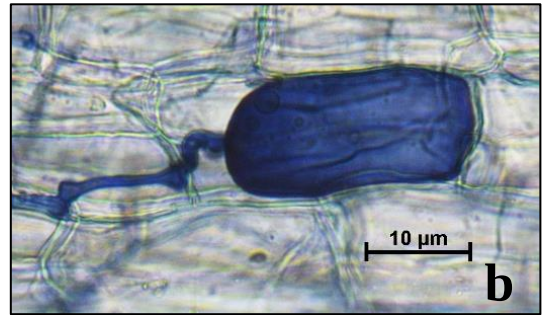
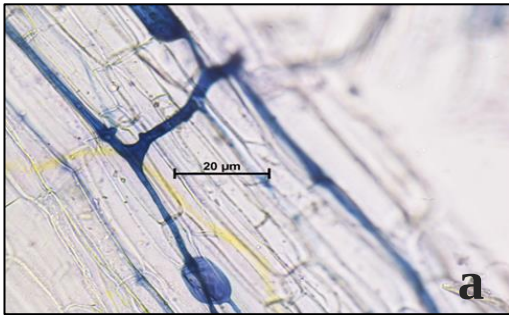


Plate 7.13: AM fungal colonization in the treated plants at 45 days - a. & b. hyphae and vesicles, c. arbuscular colonization, and d. hyphal coils.

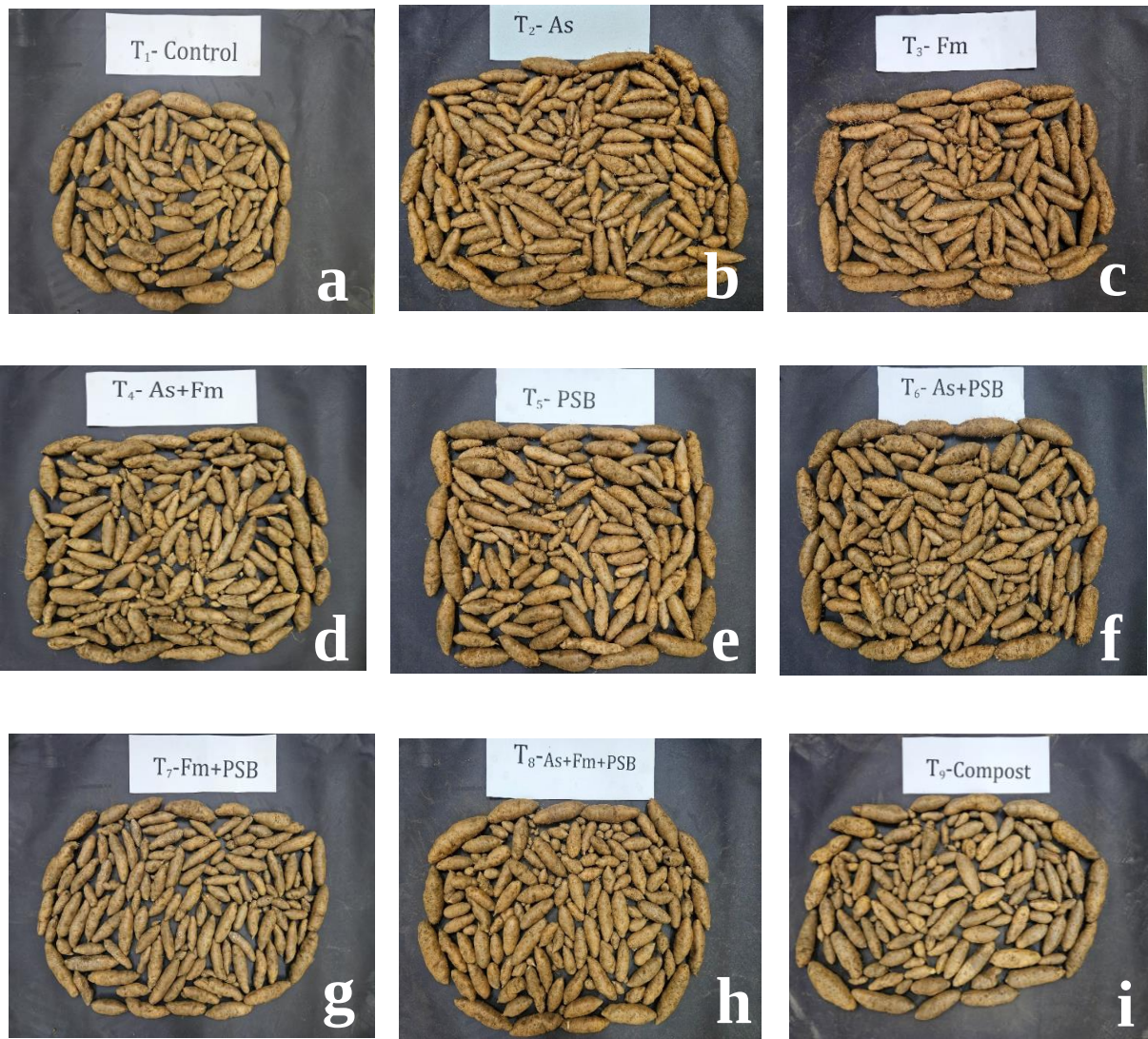


Plate 7.14: Yield of *Dioscorea esculenta* treated with various bio-inoculants - a. T_1 = Control, b. T_2 = *A. scrobiculata*, c. T_3 = *F. mosseae*, d. T_4 = *A. scrobiculata* + *F. mosseae*, e. T_5 = PSB, f. T_6 = PSB + *A. scrobiculata*, g. T_7 = PSB + *F. mosseae*, h. T_8 = PSB + *A. scrobiculata* + *F. mosseae*, and i. T_9 = Compost.



Plate 7.15: Yield of *Ipomoea batatas*- treated with various bio-inoculants - a. T_1 = Control, a. T_1 = Control, b. T_2 = *A. scrobiculata*, c. T_3 = *Rh. intraradices*, d. T_4 = *A. scrobiculata* + *Rh. intraradices*, e. T_5 = PSB, f. T_6 = PSB + *A. scrobiculata*, g. T_7 = PSB + *Rh. intraradices*, h. T_8 = PSB + *A. scrobiculata* + *Rh. intraradices*, and i. T_9 = Compost.