DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI ON FORE DUNES OF NORTH GOA (VAGATOR) AND SOUTH GOA (UTORDA) AND TO SEE THE GLOMALIN PRODUCTION

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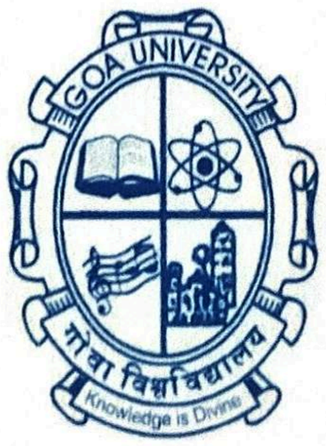

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I hereby declare that the data presented in this Dissertation / Internship report entitled, "**DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI ON FORE DUNES OF NORTH GOA (VAGATOR) AND SOUTH GOA (UTORDA) AND TO SEE THE GLOMALIN PRODUCTION**" is based on the results of investigations carried out by me in the Botany at the School of Biological Sciences and Biotechnology, Goa University under the supervision of Prof. B.F. RODRIGUES and the same has not been submitted elsewhere for the award of a degree or diploma by me. Further, I understand that Goa University or its authorities will not be responsible for the correctness of observations/ experimental or other findings given the dissertation.

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COMPLETION CERTIFICATE

This is to certify that the dissertation "DIVERSITY OF ARBUSCULAR MYCORRHIZAL FUNGI ON FORE DUNES OF NORTH GOA (VAGATOR) AND SOUTH GOA (UTORDA) AND TO SEE THE GLOMALIN PRODUCTION" is a bonafide work carried out by Mr. Khan Mohamed Akhil under my supervision/ mentorship in partial fulfilment of the requirements for the award of the degree of M.Sc. in the Discipline Botany at the school of Biological Sciences and Biotechnology, Goa University.

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INTRODUCTION

The word dune reflects the images of a vast amount of shifting sand, barren of plants and hostile to human habitation. Hot and dry winds shape and arrange the sand in geometric and artistic patterns described by the words such as barchen, transverse, star blow-out dome strings, and sheet, oblique and parabolic. Dunes are generally of two type's *viz.,* Arid interior deserts of continental land masses, such as the Sahara in Africa or the Victoria desert in Australia, and the coastal dunes, which occurs along the Atlantic and Pacific coasts of North America, along the Australian coast. In Asia, the coastal dunes occur in Japan, India and several other countries (Wiedmann, 1984).

Dunes are the formation of windblown sand. Fore dunes are deposited immediately behind the beaches. Strong onshore winds erode dry sand from the steep face of the beach. This windblown sand is deposited towards the top of the beach, and a foredune is gradually formed. Foredunes are also usually formed where vegetation and other obstacles come in the way. On the upper part of the beach, it results in the deposition of windblown sand. During periods of shoreline advancement, successive foredunes may develop to form a series of parallel dunes (O'Keefe, 1978).

Formation and functions of fore dunes and parallel dunes are built up at the hack of beaches on crests of berms and beach ridges where vegetation or other obstructions trap windblown sand. They become higher and more expansive as sand accretion continues. On-shore winds of sufficient velocity move sand particles, erode sand from the dry part of the beach and transport it landward. Saltation is the primary method by which sand is transferred from the beaches by wind and is a process where individual sand grains are carried away by the wind close to the surface in a series of short hops. Nearly all windblown sand is moved by saltation. Wind action effectively sorts the original beach material. The small particles may be completely removed from the beach dune area while the largest particles remain. Sand grains removed from the

beach by wind and deposited in dunes are essential of one size (diameter ranges from 0.15mm to 0.30mm).

Vegetation plays a dominant role in determining the size, shape and stability of foredunes. The aerial parts of vegetation obstruct the wind and absorb the wind energy. Wind velocity near vegetation is thus reduced below that needed for sand transport, hence the sand deposit around the vegetation. A characteristic of dune vegetation, particularly the grasses growing under these conditions, is its ability to produce upright stems and new roots in response to sand covering. If the plants do not continue to grow more rapidly than the rate of deposition, the arresting action of the plant ceases. Successive stages of plant growth and sand deposition result in an increase in the width and height of the dunes (Gale and Barr,1977).

Fore dunes act as barriers against the action of waves and tides and are a source of sand for the beach during periods of erosion. They protect areas behind them from wave damage and saltwater intrusion during storms. Vegetated foredunes are inherently flexible. If storm waves damage them, the remaining vegetation traps sand blown from the beach, and the dune is reformed, thus protecting against future wave attacks. Vegetated foredunes restrict wind, sand and salt spray intrusion into hind dune areas. The protective action of the foredunes allows the development of a more complex plant community on the hind dunes. Landward dunes parallel to foredunes are protective to a lesser degree. If they are well stabilized, they serve as a second line of defence against water and wind erosion (Desai, 1995).

Vegetation plays a vital role in forming and stabilising coastal dunes. All plants, whether herbs, shrubs, or trees, either growing singly or in groups, have a role in the development of vegetation cover. Together, they bring about the stabilization of the dunes. In the absence of sand trapping dune vegetation, windblown sand from the beach moves inland and is lost to the beach\dune system. Wind erosion of the beach and unvegetated frontal dunes results in coastline recession,

and the reduction in wind movement results in the deposition of sand around the plants. Dune plants must also tolerate salt spray, strong winds, sandblasts, and occasional washing away by seawater. Plants with these characteristics are ideally suited as agents for the initial stabilization of dunes (Anonymous, 1981).

Turner *et al.,* (1962) described five well-defined zones of vegetation on dune ridge (Fig: 5):

Zone-I: Embryonic Dune: Long sand ridges lie parallel to the ocean swell on the seaward coast. This zone is nearest to the sea and is unvegetated. It is just above the high tide level with its steeper face inland. This is formed by sand delivered to the beach by wave action. The dominant plant species of the embryonic dunes on the Indian coast is *Ipomoea pes-caprae*.

Zone-II: The Fore Dune: It runs parallel to the first beach ridge and is formed on the earlier ridge by the coalescence of the embryonic dunes after further accretion of blown sand and sand binder has an efficient method of seed dispersal, the spiny globular fruiting heads being blown along the sand by the wind till they are caught in some obstruction and break up. Some common plants found on the foredune are *Ipomoea pes-caprae* and *Spinifex littoralis*.

Zone-III: Dune Scrub or Stable dune Ridges: This is parallel to and close to the fore dune and forms the main part of the dune, which is a little higher than the fore dunes but carries a close community of shrubs. *Lantana camara, Vitex negundo,* and *Clerodendron inerme* form part of the natural vegetation.

Zone-IV: Shrub Woodland: It is a long narrow sandy ridge running parallel and separated by mud flats with fringing salt marshes inundated at high tide. The vegetation on this dune is often similar to the main inland. *Anacardium occidentale, Cocos nucifera, Pandanus tectorus* are some of the plants found in this region of shrub woodland.

Zone-V: <u>The Dune Woodland</u>: It is made up of stable dunes. The vegetational community is closely similar to that found in the neighbouring coastal regions of the mainland on similar soils.

The plants like *Ipomoea pes-caprae* and *Spinifex littoralis*, which are common in the central west coast and are natural sand binders and foliage, break wind activity leading to less erosive action on the lee side (Arulmoorthy and Srinivasan, 2013). *Ipomoea pes-caprae* is a dominant and widely distributed stoloniferous creeping sand binder in tropical dunes (Beena*et al.*, 2000). It is found on the dunes of different magnitudes of disturbance on the coastline of Goa. *Spinifex littoreus* (Burm.f.) Merr. (Poaceae) is the most successful sand trapping plant. It can grow through the accumulation of windblown sand. Cycles of sand deposition and plant growth result in dune formation. The coast of Goa has the dominant flora of *Ipomoea pes-caprae* and *Spinifex littoreus*in the pioneer zone (Desai, 1995).

Arbuscular mycorrhizal (AM) fungi

AM fungi are ubiquitous, obligate symbiotic fungi that colonizemore than 80% of terrestrial plants (Wang and Qui, 2006). This mutualistic relationship is known to benefit both partners. The hostplantprovides the carbon requirements of thefungus, and in turn, the plantgets mineral nutrients, especially P (Willis *et al.*, 2013). Besides, the AM fungi are also known to increase disease tolerance by reducing the invasion of soil-microbial plant pathogens (Newsham *et al*., 1995), improving the water balance inhost plants when there is drought or abundant water (Augé, 2001), reducing the uptake ofphytotoxic heavy metals (Göhre and Paszkowski,2006), and play an important rolein plant secondary succession (Janos,1980).

AM fungi belong to Phylum Glomeromycota (Berruti *et al.*, 2016). The unique features ofthese mycorrhizae are the formation of arbuscules and vesicles in the root cortex of plants. The hyphae penetrate the external cell wall of the roots and spread across the inter- andintra-cellular regions towards the outer cortex with hyphal coiling. As it passes through theinner cortex, it forms arbuscules (branch-like structures involved in the material exchangebetween the host and fungus). In the middle and outer cortex layers, swollen terminal endsappear either inside or in the cells, known as vesicles (globose to ellipsoid structures thatfunction as temporary storage organs). These two structures are interlinked to the external hyphae by a network of internal hyphae system that appears like pipe structures (Nicolson1967).

Several survey studies of AM fungi presenton thecoastal duneshave been carried out in temperate, subtropical, and tropical regions (Sturmer and Bellei, 1994). AM fungi are of pivotal significance to the foundation, development, and existence of dominating plant species that inhabit dunes (Koske and Polson, 1984). Any disturbance to a flourishing ecological community that involves variation in the physicochemical properties of soil and plant eradication will significantly influence the symbiotic association between plants and AM fungi (Beena *et al.,* 2000). Due to this, there is a difference noted in plant species withresponse to AM fungi in the soil, and the presence or absence of AM fungi has beenassociated with the structure of plant populations that have been established in dune sites (FrancisandRead,1995).

Glomalin is a component of the spore and hyphal wall of AMF ,it was discovered in 1996 by Wright and co-workers while identifying monoclonal antibodies for AMF. Glomalin was thought to be exuded by the living fungus until Driver and co-workers (2005) found that glomalin is only released by an AMF into the soil environment during hyphal turnover and after the death of the fungus. Glomalin, though still not biochemically

defined, is an N-linked glycoprotein composed of 3 to 5% N,36 to 59% C (Lovelock *et al*., 2004a; Schindler *et al*., 2007), 4 to 6% hydrogen, 33 to 49% oxygen, and 0.03 to 0.1% P (Schindler *et al*., 2007). Glomalin also contains 0.8 to 8.8% Fe (Rillig*et al*., 2001), which may be responsible for the reddish colour of glomalin extracts (Wright and Upadhyaya, 1998).

Glomalin is a stable compound, insoluble in water and resistant to heat degradation. Because it is glue-like in nature and attaches to horticultural film and soil surfaces, glomalin is likely hydrophobic in its native state. Apart from the Glomeromycota, no other fungal group produces this glycoprotein in significant amounts. Glomalin has been found in agricultural, grassland, forest, desert, and non-cultivated soils. Glomalin concentrations of over 100 mgg−1of soil were recorded in tropical forest soils of Hawaii (Rillig *et al*., 2001) and values lower than 1mgg−1soil were obtained in soils of a desert ecosystem in Mu US sandland, China. Values up to 21 mgg−1soil were obtained from Scottish wood-land soils. Although most of these findings are based on glomalin in the A horizon, both B and C horizon contain glomalin, and it can be found to a depth of 140 cm in the soil profile. Harner and co-workers (2004) detected the glycoprotein in floodplain soils, river water and river foam. In fact, river foam contained 9.66 mg g-1of glomalin in freeze-dried foam (Harner *et al*., 2004).

The state of Goa is situated along the Central West Coast of India, and is bounded by Maharashtra in the North, Karnataka in the East and South, and the Arabian Sea in the West. Goa host sadiversity of ecosystems: the Coastal, Mangrove, Estuarine, Grassland, Wetland, and Western Ghat Habitats (http://ces.iisc.ernet.in/hpg/envis/envis_centres/homepages/goa.htm).

Goa has a coastalregion of 105 km consisting of sandy beaches and rocky shorelines. Nine main riversflow through Goa, of which Mandovi and Zuari are the two chief rivers, and the remainingare Terekhol, Colvale or Chapora, Sal, Talpona, Saleri, Canacona, and Galgibagaa. Goa witnesses a tropical, warm, humid climate with temperatures ranging from 21°C to 36°C,with an average being 27°C, and has three seasons: a monsoon (June to September or mid-October), accompanied by a short winter (November to February) and summer (March toMay). There is a variation in rainfall from the coastal area, about 2,500 mm to about 4,500mmwithintheWestern Ghats (Sonak *et al.*, 2014).

Several rows of dunes are observed on the landward side near sandy beaches. Sandy beaches shield about 57.37% (70 km) of the Goa coast. Goa has about 53 coastal villages that are immediately back of the main shore, and out of 53 coastal villages, nearly 22 villages (8 villages of North Goa and 14 villages of South Goa) have dunes. The total number of patches of the dune of Goa state is 99, out of which 75 patches are observed in South Goa and 24 patches in North Goa. The total dune area is 2.93 sq km, out of which north Goa has 0.47 sq km, whereas south Goa has 2.46 sq km (http://dstegoa.gov.in\sand%20dune%report.pdf) (Fig.1)

Sand dunes are a continually evolving system and will respond to the change occurring in their local environment. Storm events, changes in sea level, sea (wave action) and heavy recreational use can cause a significant detrimental impact on this fragile habitat. Some of the major threats that lead to degradation of the coastal sand dune ecosystems on the cost of Goa are by human activates like Infrastructural development this include construction of hotel, housing estates and tourism related activities along the coastal areas that have degraded sand dunes considerably. Such developments increase recreational pressure and disturb natural, interdependent systems like topography, vegetation and water table levels, amplifying the need

to undertake artificial coastal defense. Sand dunes are traditionally grazed by agricultural livestock (Antonio. M, 1998). This has now mostly ceased, and can lead to scrub encroachment. Pollution, both on and offshore, can have detrimental effects on coastal flora indicating that of sand dunes. Beach pollution, especially due to construction of huge industries in this area can cause a lot of adverse impact on the sand dunes (Arulmoorthy *et al*., 1998). The study was important to know the present situation on the fore dune of north and south Goa affected by natural and human activity have caused damage to the costal dune and to understand and to overcome the problem the study was performed, which follows by following objectives:

The present work was undertaken with the following objectives:

- To study AM fungal diversity and colonization in the selected plant species *Ipomoea pes-caprae* and *Spinifex littoreus* growing on the dunes.
- To identify the dominant AM fungal species in the selected plants.
- To prepare trap and pure cultures of dominant AM species recovered from the rhizosphere of the selected plant species.
- To extract and quantify glomalin content in the rhizosphere of the selected plant species.

REVIEW OF LITERATURE

Desai (1995) carried out a survey of dune vegetation along the coasts of Goa, with *Ipomea pes-caprae* and *Spinifex littoreus* being the most dominant flora in the pioneer zone, along with other dominant plants like *Cyperus arenarius, Spermacoce stricta, Launea pinnatifida, Justicia simplex, Lactuca remotiflora, Sporobolus virginicus, Clerodendro ninerme*, and other plants.

Kulkarni *et al.,* (1997), reported mycorrhization and spore density of AM fungi in 12 dune plant species on the west coast of India, post-monsoon. They recorded *Borreria articularis, Ipomoea pes-caprae* and *Launaeasar mentosa* as the most dominant plant species. They observed that AM fungal spores were present in the rhizosphere of all plants except in *Ipomoea pes-caprae*. Out of 16 AM fungi species they recorded, nine species belonged to the genus '*Glomus',* and they recorded *Gigaspora ramisporophora, Glomus albidum, Glomus clarum,* and *Scutellospora gregaria* as dominant ones among the other spores. They suggested a strong positive correlation between spore density and the number of species present, and the relative abundance and frequency of occurrence of AM spores in the rhizosphere.

Koske and Gemma (1997), conducted a survey of AM fungi and hyphal networks of AM fungi of sand dune sites in different successional stages of American Beachgrass (*Ammophila breviliguatla*). They recovered 17 species of AM fungi spores and observed an increase in richness and population of spores in the AM fungi community present, the extent of mycorrhization in *A. breviligulata* roots, and the mycorrhizal inoculum potential of the soil. They also observed that the unvegetated site which lacked AMF propagules, planted with colonized culms of *A. breviligulata* became mycorrhizal later. Based on these observations and records, they stated that the rate of invasion of later successional plant species in the areas

planted to the American beach grass may depend upon the establishment of a vigorous network of hyphae of AMF in the site.

Beena*et al.,* (2000), carried out the coastal sand dunes of the west coast of India yielded 41 species belonging to six genera. The AM fungi was least during monsoon (28.7-64.6%) and highest during post-monsoon (80.6-93.6%), but the mean spore density in rhizosphere was least during post-monsoon (9.5-35/10O g) and highest during summer season (58.2-132.51100 g). The species belonging to the genus *Glomus mosseae* was the most abundant species (8.5%) followed by *G. albidum* (7.8%), *G. lacteum* (5.1%), and *Gigaspora margarita* (2.8%). *Glomus albidum* was most frequently (61.7%) followed by, *G. mosseae* (53.3%), *G. aggregatum* (36.7%), *G. lacteum* (31.7%) and *Gigaspora margarita* (31.7%) have been seen in *Ipomea pes-caprae* plant.

Beena*et al.,* (2000), examined the diversity of arbuscular mycorrhizal (AM) fungi associated with the dominant strand plant species, *Ipomoea pes-caprae* of the coastal sand dunes of west coast of India. The study reports the impact of rhizosphere edaphic features and disturbance on the species richness and diversity of AM fungi in 10 geographical locations consisting of Moderately Disturbed Dunes (MDD) and Severely Disturbed Dunes (SDD) during wet and dry seasons. The Glomus mosseae was most dominant, followed by *Glomus dimorphicum, Gigaspora gigantea, Acaulospora taiwania, Glomus fasciculatum* and *Glomus* sp. Three species on Severely Disturbed Dunes (SDD) *G. mosseae, G. dimorphicum* and *G. gigantea* were most common in both Moderately Disturbed Dunes (MDD) and Severely Disturbed Dunes (SDD).

Beena *et al.*, (2001), reported the AM fungal status in 28 plant species present on coastal sand dunes of the southwest coast of India. They recorded 23 plant species colonized by AM fungi, with highest colonization in *Canavalia cathartica*, and 20 plant species with AM fungal spore present in the rhizosphere, with highest number of spores present in *Borreria articularis* rhizosphere. They recorded the highest mean species richness in *Ipomoea pes-caprae*, the highest AM fungal diversity in *Alysicarpus rugosus*. Among the AM fungi they recovered, *Scutellospora erythropa* showed a wide host range, while *Scutellospora gregaria* showed high spore abundance per plant species.

Rodrigues and Jaiswal (2008) studied the occurrence and distribution of AM fungi in Goa's disturbed and undisturbed coastal dunes. Eight plant species were dominant at the un-disturbed site, while seven were dominant at the disturbed site. Only three plant species, *Ipomoea pes-caprae, Cocos nucifera,* and *Borassus flabellifer* were common to both sites. It was observed that the spore density significantly reduced (by 50%) in disturbed sites compared to undisturbed sites. Also, the species richness was lower at disturbed sites (14 AM fungal species) and higher at un-disturbed sites (21 AM fungal species). In all, 27 AM fungal species were recorded from both sites. *Gigaspora margarita* (with a frequency of occurrence of 62.5%). *Acaulospora spinosa*, *Scutellospora calospora,* and *S. coralloidea* ranked second (50%). At the Varca site, nine of the AM fungal species identified occurred in more than 25% of the samples examined, with *Glomus fasciculatum* as the most abundantly sporulating species was seen

Druva-Lusite and Ievinsh (2010), reported the diversity of mycorrhizal symbiosis in plants in coastal habitats. They observed that, despite the sometimes low intensity of mycorrhizal colonization, the roots of the coastal plants studied, possessed functional structures of AM

symbiosis. They suggested that the fluctuation in mycorrhizal colonization could be due to unfavorable environmental conditions during the vegetative season, also in salt marsh plants, high salinity may be one of the main factors for low-intensity mycorrhizal colonization in them.

Padmavathy*et al.,* (2010), studies on coastal sand dunes (CSD) in temperate regions, the coastal dunes of tropics, especially the Indian coramandel coast, have received scanty attention. A total of 41 species belonging to 35 genera and 20 families were identified at different distances from the shoreline towards inland, where various edaphic factors decline facilitating more floral colonization. CSD constitute a variety of habitats and gather vital ecological and economic importance. Such unique sensitive system have to protect their native diversity and ecological functioning.

Vaidya *et al.,* (2011), Glomalin is a glycoprotein, a sugar protein compound that might trigger the formation of soil. Study was done to analyze the different organic matters which inhance mycorrhizal fungi and produce glomalin which is dependent upon the types of organic matter. The more glomalin in a particular soil, the soil probably is better. The amount of glomalin in the soil increased as a degree of interdependence increased between plants and arbuscular mycorrhizal fungi. AM fungi produce glomalin and live inside plant roots and in the surrounding soil. Growth of Arbuscular mycorrhizal fungi under field conditions was estimated within growth mesh bags which contain different organic matter. After six months these mesh bags were harvested. The soil were analysed by two detection methods utilized to quantify Glomalin related soil protein (GRSP): Bradford protein assay, yielding Bradford reactive soil protein (BRSP), and an enzyme-linked immunosorbent assay (ELISA). It was observed that

amount of GRSP in the mesh bags was positively related to organic matter addition. In contrast no correlation was found between spore number and neither fatty acids nor GRSP.

Arulmoorthy and Srinivasan (2013), reported that coastal sand dunes (CSD) floras were under constant anthropogenic and natural pressure due to rapid elimination of sand dunes and its associated vegetation. Such biodiversity rich and useful ecosystems need immediate restoration and conservation actions. Cuddalore coastal area is prone to both anthropogenic and natural disaster. The cyclone and industrialization causes distribution and pollution of sand dune vegetation. Restoration of degraded area by propagation of plants *Ipomoea pes-caprae* and *Spinifex littoralis* (which are natural sand binders) by planting it. The best season for this program in this area was between Octobers to January. After three months, 30cm of growth was observed in the plants. Like mangroves restoration programs, this sand dune vegetation flora should also be encouraged by all countries worldwide.

Ramarajan and Murugesan (2014), reported 55 species belonging to 46 genera and 26 families were identified. Poaceae was the most common and dominant family. Coastal dune constitutes a variety of habitats. Wide varieties of diverse habitats and ecosystems are essential for the maintenance of food webs, migration routes and increase productivity in order to protect their native biodiversity and ecological functions.

Sadhana(2015), conducted studied on the coastal region found that the totally 36 species of AM spores belonging to genera viz. *Acaulospora, Diversispora, Entrophosphora, Gigaspora, Glomus, Rhizophagus, Sclerocystis and Septaglomus*were isolated from the rhizosphere soil of Manapaddu coastal region. The *Acaulospora* and *Glomus* was recorded predominant species

in all rhizosphere soil of coastal-grown plants. The distribution of AM spores in soil is governed by edaphic factors like soil pH and E.C. were measured under laboratory conditions. The occurrence of AM fungi in soil favours the growth of plants and also which were mainly involved in the soil aggregation.

Barman *et al*.,(2015), studied the sand barrier allows the development of more complex plant communities in areas protected from salt water inundation, sea spray, and strong winds and how plants play a vital role in this process, acting as a windbreak and trapping the deposited sand particles. A characteristic of these (dune flora) plants is their ability to grow up through the sand and produce new stems and roots as more sand is trapped and the dune grows. Fore-dunes of such wide sandy beaches range from stable, well vegetated, and laterally continuous to highly mobile and poorly vegetated forms.

Rodrigues and Rodrigues (2017), addressed the AM fungal status of dominant plant species from 12 different coastal sand dune sites of Goa. The AM fungal association with sand dune plants was characterized, and the isolated AM species was studied. A total of 22 AM fungal species belonging to 8 genera were recorded. Eight out of 22 AM fungal species were successfully multiplied as monospecific cultures using cuttings of *Plectranthus scutellarioides* (L.) R. Br. as host. These included *A. scrobiculata, A. delicata, A. rehmii, F. geosporum, F. mosseae, Gi. albida, Ra. gregaria and R. intraradices*. It was also know that the percent spore germination, frequency of culturing (sporulation) and sub-culturing (re-growth) was greatest in *R. intraradices* and *F. mosseae*. And it relevant that agriculture, horticulture, forestry and environment-recovery programs. It promotes using an organic carrier formulation that facilitates the transfer to, multiplication of, and increase in efficacy of *in-vitro* produced AM

fungal propagules in the rhizosphere soils. In particular, it demonstrates an effective protocol in carrier based *in-vitro* produced AM fungal bio-inocula for revegetation strategies of degraded sand dune ecosystems.

Pawar and Telave (2022), study investigated dune flora along coastal dune areas from October to December 2020 and 55 species belonging to 46 genera and 26 families were identified from study sites. The family's like Fabaceae, Rubiaceae, Poaceae, Asteraceae, Lamiaceae and Malvaceaeare dominant in study areas which show halophytic and xerophytic nature.

MATERIALS AND METHODS

Study Site

SITE 1: Utorda village is located in between Arossim and Majorda village. 3-4 / area patches of prominent sand dunes are observed in the coastal area of Utroda village. These patches mainly occupymiddle of the village at behind the backshore and extends further north to the village boundary of Utorda. The total area of sand dune is 40691 sq m (Fig.2).

SITE 2: Anjuna village is located in the southern side of Chopora River and Calangute village in the south. Vagator beach is sandwiched between two headlands with a sand dune patch covered withgrass vegetation. The approximate height of the sand dune is 1 to 2 m. The total area of sand dune is 4183 sq m with 174m length and maximum width of 29m (Fig.3).

Sample Collection

The sampling of plant species at the site was carried out to assess the arbuscular mycorrhizal (AM) diversity. 3-4 soil samples were collected (from a depth of 15-20cm) from different parts of the island and mixed to form a composite sample used to analyse the Physico-chemical properties. Root and rhizosphere soil samples were placed in *Ziploc* bags, labeled and brought to the laboratory. The roots were used to determine the root colonization, while the soil samples were stored at 4°C for further analysis.

Soil Chemical Analysis

The soil sample was sun-dried, sieved to remove larger soil particles and debris, and was sent for analysis at the Directorate of Agriculture, Soil Testing Laboratory (Ita-lab house pvt ltd), Margao, Goa. The soil was analyzed for various physical parameters, *viz.*, and soil pH by using a pH meter and Electrical Conductivity (EC) using a Conductivity meter. The Organic Carbon

(OC) was determined by Walkley and Black Titration Method (Walkley and Black, 1934), Available Phosphorus (P) content was determined by Bray and Kurtz method (Bray and Kurtz, 1945), Available Potassium (K) content was determined using the Ammonium Acetate Method (Hanway and Heidal, 1952) and Available Nitrogen (N) content was determined by Aerobic Incubation Method (Keeney and Bremner, 1966).

Estimation of AM Fungal Root Colonization

Assessment of AM colonization in roots was carried out using Trypan blue staining technique (Phillips and Hayman, 1970). The roots were gently washed with tap water to remove the attached soil particles and then were cut into 1cm segments. The cleaned root segments were then cleared in 10% KOH by heating at 90°C for one hour in the oven. These root segments were thoroughly rinsed in water, acidified with 5N HCl for 5 minutes, and kept overnight in a 0.05%Trypanblue stain. The presence or absence of AM hyphae, arbuscules, vesicles, and hyphal coils in the root segments was observed using OlympusBX14 and the data recorded. Microscopic photographs were taken by Olympus DP12-2 and Nikon EclipseE200 digital camera.

Assessment of AM colonization in roots of trap cultures (roots of Coleus sp.) was carried out using Acid-fuchsin staining technique (Kormanik and McGraw, 1982). The roots were gently washed with tap water to remove the attached soil particles and then were cut into 1cm segments. The cleaned root segments were then cleared in 10% KOH by heating at 90°C for one hour in the oven. These root segments were thoroughly rinsed in water, acidified with 5N HCl for 5 minutes, and add 0.01% acid fuchsin-lactic staining solution and autoclave at 121^{o}C for 10 minutes in an autoclave or heat at 90^{o}C in a well-ventilated exhaust hood for 10-60

minutes or until the roots are properly stained. Do not rinse the material after staining because rinsing readily removes the stain. The presence or absence of AM hyphae, arbuscules, vesicles, and hyphal coils in the root segments was observed using OlympusBX14 and the data recorded. Microscopic photographs were taken by OlympusDP12-2 and Nikon EclipseE200 digital camera.

Estimation of Percent Root Colonization

The estimation of AM root colonization was carried out using the Root Slide Method (Read et al., 1976). The Trypan blue and acid-fuchsin stained roots were mounted in polyvinyl-lacto-glycerol (PVLG) on slides, and the presence or absence of AM colonization was scored. The percent root colonization was calculated using the formula:

Percent Colonization = No. of root segments colonized ÷ Total number of root segments observed × 100

Extraction of the AM Fungal Spores

AM spore extraction was done using the Wet Sieving and Decanting Technique (Gerdemann and Nicolson, 1963). Rhizosphere soil (100g) was added to a beaker. To this, water was added to create a suspension. This suspension was stirred using a glass rod, and the sediment was allowed to settle down for 1 minute. Then, without disturbing the sediment layer at the bottom, the suspension mixture was slowly decanted through several sieves arranged in the descending order (50µm, 150µm, 250µm, 750µm). This procedure was repeated twice. The residue from each sieve was collected in separate beakers, and the aliquot was filtered separately using Whatman No. 1 filter paper. The filter paper was placed onto a petriplate, ensuring that it

remained moist. The filter paper was thenexamined for the presence or absence of AM spores and sporocarps using a stereomicroscope (Olympus SZ16).

Estimation of AM Fungal Spore density

The estimation of AM fungal spore density was carried out by following the modified method of (Gaur and Adholeya, 1994). The AM diversity and dominant species found inthe study site were also recorded. The procedure was carried out using Whatman No. 1 filter paper folded twice in two halves resulting in four equal quadrants. Once the filter paper was re-opened, vertical lines were drawn on the one-half side of the filter paper divided into ten columns, each being 0.5cm apart from the other. The direction of counting and numbering of columns was marked. Then the filter paper was folded in such a way that the aliquot containing the spores was poured onto the marked portion and the unmarked portion was free of spores. This filter paper was then placed in a petriplate and counted under a stereomicroscope (OLYMPUS SZ16). Intact living spores were isolated using a needle. The percent spore density was assessed by the total number of spores recorded per 100g of the soil sample.

Taxonomic Identification of Spores

Intact or crushed, clean spores were mounted in PVLG on a slide and used for taxonomic identification. Various morphological characteristics play an important role in confirming the taxonomic identity and the relationships of AM species. Some characteristics like colour, shape, size, ornamentation patterns, number of wall layers, mycorrhizal anatomy, pattern and shape of germination shield, etc., assist in identifying AM at the species level. Taxonomic identification of AM fungal spores was done using morphological criteria as basis, reference to the original species was made as described by Rodrigues and Muthukumar (2009), and

online species description were provided by INVAM (International Culture Collection of Vesicular Arbuscular Mycorrhizal Fungi) at the West Virginia University, USA (https://invam.wvu.edu/).

Estimation of AM Species Richness, Spore Density, and Relative Abundance

The estimation of the diversity of the AM fungal spores was carried out by evaluating the spore abundance and calculating the spore density (Gaur and Adholeya, 1994), and the relative abundance (RA %) and isolation frequency (IF %) of AM fungi using the following formulae (Beena*et al.*, 2000c, 2001).

- Species Richness: Species no. per 100g soil sample

- Spore Abundance: Number of spores of a particular species per 100g of soil sample.

- Spore Density: Total no. of spores per 100g soil sample

- Relative Abundance (RA %): RA% = (Number of spores of a particular species ÷ total number of spores) × 100

Statistical Analysis

Pierson's Coefficient of Correlation (r) was calculated to compare the relationship between spore density and root colonization using IBM SPSS Statistic 22 software.

Sterilization of Pots and Sand

The pots to be used for trap culture were soaked in soap water and thoroughly rinsed with water.Then, the pots were wiped using absorbent cotton dipped in absolute alcohol, and the holes at the base of the pot were blocked with non-absorbent cotton.

The soil used for potting was sterilized prior to using a hot air oven at 180°C for three hours for a period of three days.

Preparation of Trap Cultures

Sterilized pots were filled with a mixture of rhizosphere soil collected from the host plant (containing viable AM propagules) and sterilized sand in a 1:1 ratio. Coleusplant cuttings was put in the pots and maintained in the polyhouse at 28°C for 90 days. The pots were watered at regular intervals and provided with proper growth conditions. After 50-60 days, watering was stopped, and the plant was left to dry and was checked for AM spores using the wet sieving and decanting technique.

Preparation of monospecific cultures

The AM fungal spores from trap cultures were extracted by wet sieving and decanting method (Gerdemann and Nicolson 1963). The extracted spores were identified, washed with autoclaved distilled water, and used to set up monospecific cultures. The substrate was prepared by mixing autoclaved sand: soil in the ratio of 1:1. This mixture was then transferred to plastic pots (15 cm). The extracted spores of single AM fungal species along with the filter paper were placed 2-3 cm deep in the pots. Three to four cuttings of Coleuswere planted per pot. The pots were

maintained for 90 days in the polyhouse and watered twice a week. After every 15 days, Hoagland solution (Hoagland and Arnon 1950) without P was added to the pots. The plants were allowed to dry after 65-75 days, and later the soil was analyzed for the spores.

Glomalin extraction

Glomalin-related soil protein (GRSP) extractions were conducted as described by (Wright and Upadhyaya, 1996). Total glomalin was extracted from each sample using 50 mM sodium pyrophosphate solution (pH 8.0). The mixture was autoclaved for 60 min at 121℃, with 1 g of soil in 8 mL extractant buffer. The extraction procedure was repeated until the supernatant was almost colourless. Most soil-sand mix (rhizosphere) samples needed 1-2 times of extractions; glomalin was extracted from the sand mix (mycorrhizosphere) samples 4-5 times. Samples were centrifuged at 5,000 × g for 15 min immediately after extraction and the supernatant containing the extracted protein was decanted and stored at 4 °C, and centrifuged at 10,000 × g for 10 min to remove residual soil particles and other insoluble materials before analysis.

Quantification of Glomalin

Bradford protein analysis is a common method for protein quantification (Bradford, 1976). The Bradford assay is based on the principle that a dye (Coomassie Brilliant Blue G-250) binds with proteins and changes the dye colour from red to blue (Bradford, 1976; Wright *et al.,* 1996). The degree of colour change, read by a spectrophotometer at a wavelength of 590 nm (A590) as optical density, can be related to protein concentration in a glomalin extract using a standard of known concentration of protein. The standard is prepared in a range of 50 to 500 µL, bovine serum albumin (BSA) in phosphate buffer saline

(PBS). The equation of the regression line generatedby plotting optical density against BSA values is then used to calculate protein concentration in glomalin extractsas Bradford-reactive soil protein (BRSP) for (TG) total glomalin (Rillig, 2004b

RESULTS AND DISCUSSION

Soil Analysis:

The results of the soil physico-chemical analysis present in **Table 1**. The pH of dune soils ranged from 6.4 to 7.9 indicating that the soils are both acidic and alkaline. Electrical conductivity (EC) ranged from 131.1 - 219.46 in µs. The organic carbon ranged from 0.12 to 0.42% indicating that the dunes soils have less organic carbon. It is observed that the organic carbon gradually increased from July to December months at both the site. The nutrient status revealed that the concentration of N, P and K was low, especially P that ranged from 0.11- 0.68 Kg/ha.

Table 1: Physico-chemical properties of dune soils from selected study sites.

Site	Month	pH	EC (µs)	OC (%)	N (Kg/Ha)	P (Kg/Ha)	K (Kg/Ha)
Vagator	July	7.50	143.40	0.12	105.87	0.11	82.11
	August	7.07	136.77	0.14	128.37	0.17	91.91
	September	7.36	169.90	0.36	176.9	0.39	111.29
	October	7.23	167.73	0.31	164.87	0.33	111.86
	November	6.62	157.62	0.28	149.01	0.32	99.16
	December	6.68	138.70	0.24	137.11	0.23	96.94
Utorda	July	7.44	131.10	0.06	122.96	0.12	74.56
	August	6.95	110.18	0.14	117.68	0.21	77.48
	September	6.63	156.40	0.28	135.34	0.22	80.94
	October	6.49	175.40	0.28	141.57	0.22	83.96
	November	6.95	191.76	0.33	144.94	0.41	90.03
	December	7.93	219.46	0.42	168.28	0.68	96.26

Legend: All the values are mean of 3 readings; the 'Critical level in soil' OC= < 0.5low, 0.5-0.75, medium and >0.75 high. N= < 280 low, 280-560 medium and >560 high. P= <10 low, 10-24.6 medium and >24.6 high and K=<108 low, 108-280 medium and >280 is high.

Assessment of Arbuscular Mycorrhizal Diversity:

The study on root colonization recorded the presence of hyphae, vesicles, arbuscules and hyphal coils **(Plate 1 and Plate 2)**. The maximum root colonization (100%) was recorded in

Table 2: Root colonization, spore density, species richness, and species diversity in selected plant species from dunes.

Sr. No.	Month	Plants Species	Site	Root Colonization* (%)	Spore Density*	Species Richness	Species Diversity
1	July	*Spinifex littoreus*	Vagator	100±0.00	82.80±25.07	2	*G. geosporum, A. delicata*
			Utorda	100±0.00	84.80±29.07	1	*G. geosporum*
		Ipomoea pes-caprae	Vagator	100±0.00	35.40±29.39	2	*G. geosporum, A. delicata*
			Utorda	100±0.00	13.20±9.00	1	*G. geosporum*
2	August	*Spinifex littoreus*	Vagator	100±0.00	80.20±19.14	2	*A. dilatata, G. geosporum*
			Utorda	100±0.00	42.20±92.77	2	*G. geosporum, R. verrucosa*
		Ipomoea pes-caprae	Vagator	100±0.00	32.80±18.43	2	*A. polonica, G. geosporum.*
			Utorda	98±0.00	57.60±22.44	2	*G. geosporum, G. glomerulatum*
3	September	*Spinifex littoreus*	Vagator	98±0.00	94.60±13.50	3	*A. scrobiculata, A. undulate, G. clavisporum*
			Utorda	94±0.00	112.20±21.56	3	*A. delicate, G. geosporum, G. glomerulatum*
		Ipomoea pes-caprae	Vagator	100±0.00	50.00±27.74	2	*G. geosporum, R. coralloidea*
			Utorda	96±0.00	41.00±18.96	2	*A. delicate, G. clavisporum*
4	October	*Spinifex littoreus*	Vagator	96±0.00	121.40±29.87	4	*A. delicate, A. dilatata, G,intraradices, G. macrocarpum*
			Utorda	90±0.00	160.40±78.24	3	*A. dilatata, G. geosporum, D. scutata*
		Ipomoea pes-caprae	Vagator	98±0.00	77.60±32.85	4	*A. dilatata, G. geosporum, G, intraradices , G. glomerulatum*
			Utorda	90±0.00	129.20±37.89	3	*A. delicate, G. geosporum, G. glomerulatum*
5	November	*Spinifex littoreus*	Vagator	92±0.00	94.40±16.34	3	*A. dilatata, A. delicate, G. clavisporum*
			Utorda	84±0.00	81.00±31.88	3	*A. delicate, G. clavisporum, G. geosporum*
		Ipomoea pes-caprae	Vagator	84±0.00	101.8±26.69	5	*A. dilatata, A. delicate, G. geosporum, G, intraradices , G. glomerulatum*
			Utorda	82±0.00	141.40±18.71	4	*A. delicate, G. geosporum, G. glomerulatum, G. clavisporum*
6	December	*Spinifex littoreus*	Vagator	92±0.00	339.80±39.64	5	*A. dilatata, A. delicate, G. geosporum, G, intraradices , G. glomerulatum*
			Utorda	70±0.00	165.60±63.55	4	*A. dilatata, G. geosporum, G, intraradices , G. glomerulatum*
		Ipomoea pes-caprae	Vagator	80±0.00	332.20±36.41	5	*A. dilatata, A. delicate, G. geosporum, G, intraradices , G. glomerulatum*
			Utorda	70±0.00	211.60±80.66	4	*A. dilatata, G. geosporum, G, intraradices , G. glomerulatum*

Legend: = Values are mean of 5 readings.

July and August months in both the plant species, and at both the sites **(Table 2)**. Minimum root colonization (70%) was recorded in December month in both the plant species from Utorda site. Highest species richness (5) was observed in Vagator site in November and December months and the least species richness (1) was recorded in the month of July.

The highest spore density was recorded in the month of December in both the plant species at Vagator site and the least spore density was recorded in *Ipomoea pes-caprae* during the July month at Utorda site. The species diversity was highest during December month at Vagator site. Based on the frequency of occurrence, *Glomus* and *Acaulospora* were the dominant genera. The relative abundance indicates that *Glomus geosporum* (23.47%) and *Acaulospora delicata* (23%) were the dominant species. The diversity of AM fungi species is depicted in Plates 4, 5, 6, and 7. Highest RA was observed in *Glomus geosporum* (23.47%) and the lowest was recorded in *Dentiscutata scutata* (0.13%) **(Table 3)**. The trap cultures were prepared and maintained in the polyhouse **(Plate 7)**.

Table 3: Relative Abundance (%) of AM fungal species from Vagator site.

Sr. No.	AM fungi species	Relative abundance (RA%)
1	*Acaulospora polonica*	18.29
2	*Acaulospora delicata*	23.00
3	*Acaulospora dilatata*	19.08
4	*Acaulospora scrobiculata*	8.29
5	*Acaulospora undulata*	4.38
6	*Dentiscutata scutata*	0.13
7	*Glomus clavisporum*	7.29
8	*Glomus geosporum*	23.47
9	*Glomus glomerulatum*	15.96
10	*Glomus intraradices*	15.96
11	*Glomus macrocarpum*	8.35
12	*Racocetra coralloidea*	0.32
13	*Racocetra verrucosa*	0.27

Table 4: AM fungi root colonization, and spore density in trap cultures.

Sr. No.	Month	Plant species	Site	Root colonization (%)	Spore density (Spores/100g soil)
1	July	*SP*	V	100±0.0	33.66±9.50
			U	100±0.0	53.33±10.50
		IPO	V	100±0.0	19.66±8.08
			U	100±0.0	46.66±4.04
2	August	*SP*	V	100±0.0	55.0±11.13
			U	100±0.0	27.33±14.57
		IPO	V	100±0.0	57.33±21.54
			U	100±0.0	20.33±10.11
3	September	*SP*	V	100±0.0	63.0±5.00
			U	100±0.0	43.0±6.00
		IPO	V	100±0.0	69.0±12.12
			U	100±0.0	32.66±6.65
4	October	*SP*	V	100±0.0	44.0±14.10
			U	100±0.0	35.33±7.50
		IPO	V	100±0.0	70.0±8.00
			U	100±0.0	53.33±14.29
5	November	*SP*	V	100±0.0	70.33±15.17
			U	100±0.0	55.66±6.50
		IPO	V	100±0.0	91.0±17.34
			U	100±0.0	49.0±23.51
6	December	*SP*	V	100±0.0	84.66±24.58
			U	100±0.0	56.33±19.13
		IPO	V	100±0.0	112.66±15.04
			U	100±0.0	77.66±8.50

Legend = values are means of 3 readings; SP= *Spinifex littoreus*, IPO= *Ipomoea pes-caprae* V= Vagator and U= Utorda.

All the trap cultures of AM fungal species recorded 100% root colonization and produced adequate amounts of spores. The highest spore density (112.66 spores/100g soil) was recorded during the December month. The most dominant species were *A. polonica*, *A. delicata, A. dilatata*, *G. geosporum, G. intraradices* and *G. glomerulatum*. Pure cultures of all these AM species are maintained in the Goa University Arbuscular Mycorrhizal Culture Collection (GUAMCC).

Table 5: Representative standard curve by the standard concentration of BSA.

Test tube	Stock solution of BSA		D.W(µl) 0.5mL= 500µL	Stock conc.(µg/mL)	Bradford reagent	Incubation	OD at 595nm
	(µL)	(µL)					
Blank	0	0		0	1 ml	Incubate at room temperature for 10-15 min.	0
1	50	0.05	450	5			0.503
2	100	0.1	400	10			0.648
3	150	0.15	350	15			0.799
4	200	0.2	300	20			0.907
5	250	0.25	250	25			1.009
6	300	0.3	200	30			1.127
7	350	0.35	150	35			1.248
8	400	0.4	100	40			1.305
9	450	0.45	50	45			1.401
10	500	0.5	0	50			1.579

Table 6: Total Glomalin (TG) content in rhizosphere of two plant species growing on dunes.

Sr. No.	Month	Glomalin content (µg/ mL)			
		Vagator site		Utorda site	
		Spinifex littoreus	***Ipomoea pes-caprae***	***Spinifex littoreus***	***Ipomoea pes-caprae***
1	July	2.10	2.70	3.21	4.10
2	August	2.30	4.40	4.10	2.20
3	September	3.90	4.20	3.81	3.20
4	October	3.20	4.41	2.10	4.10
5	November	3.90	3.95	4.10	4.60
6	December	4.80	4.95	4.80	4.83

Glomalin is known to be located in AM fungal spore and hyphal wall (Driver *et al.,* 20005). The glomalin content showed variation in the plant species studied as well as at the two study sites. The glomalin content in the dune rhizosphere soils of the two study sites ranged from 2.10 - 4.95µg/mL. A gradual increase in the AM spore density and glomalin content was observed from June to December. Thus, greater the spore density, higher was the glomalin production. in *I. pes-caprae*, the glomalin content was higher (4.95µg/mL) in soils at Vagator

site than at soils at Utorda (4.83µg/mL) during the December month. The least glomalin content was recorded during the July month in *S. littoreus* (2.10µg/mL).

CONCLUSION

The present study was carried out to evaluate the AM fungal diversity in the two dune plant species *Ipomoea pes-caprae* and *Spinifex littoreus* occurring at two sites *viz.,* Utorda in south and Vagator in north Goa. During the study, AM fungal colonization of both the sites was assessed, while spore density and relative abundance was assessed from Vagator site.

The two species undertaken for the study are pioneer species found on the foredunes. The study revealed the presence of good AM fungal association in both plant species indicating their dependence on AM fungi for the initial establishment of vegetation on the foredunes.

The study recorded variations in root colonization, spore density, spore abundance, species richness and glomalin content suggesting the influence of various ecological factors that could play an important role in the establishment of AM fungal communities and in stabilization of dune vegetation.

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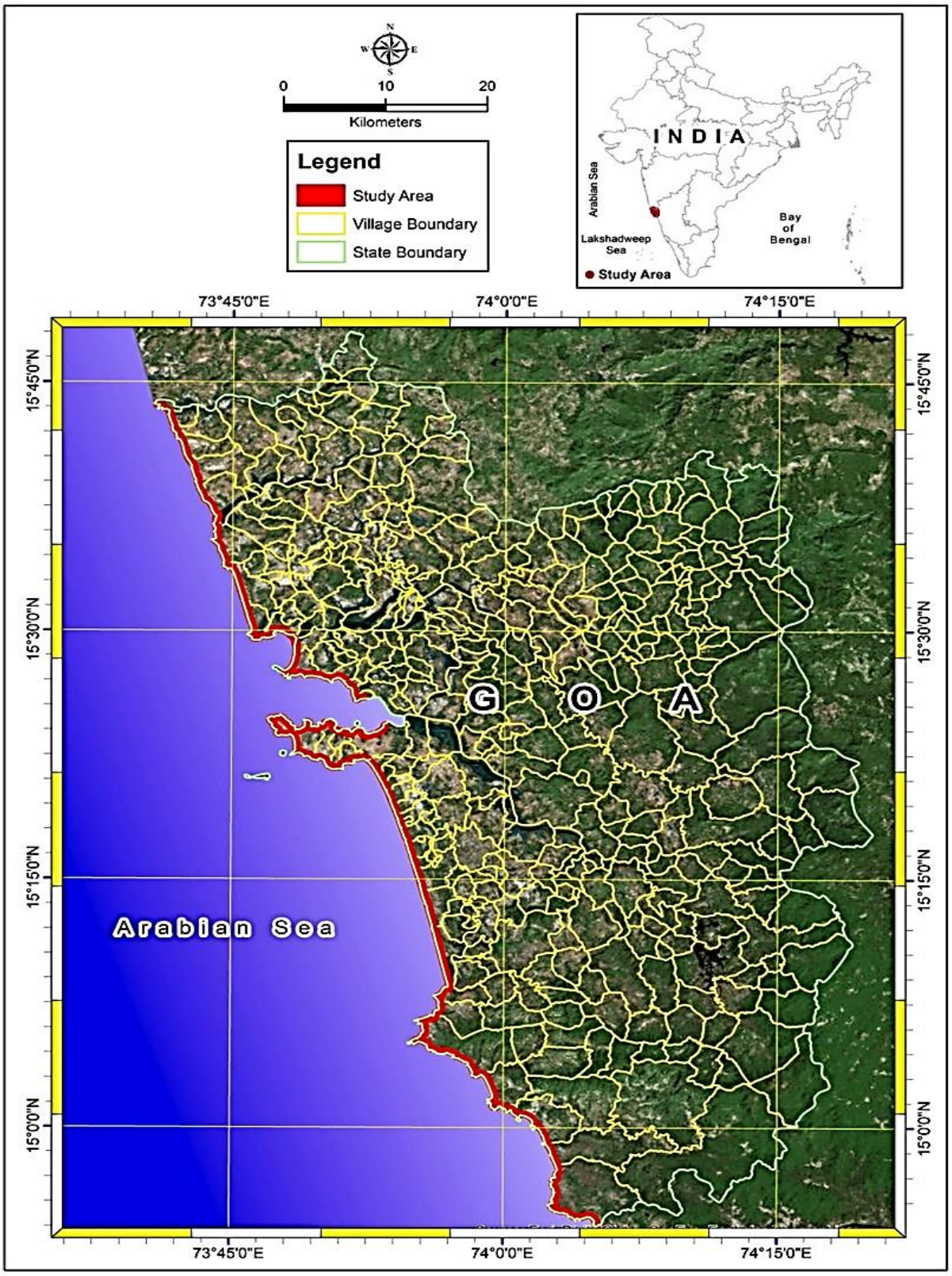


Fig. 1: Location map

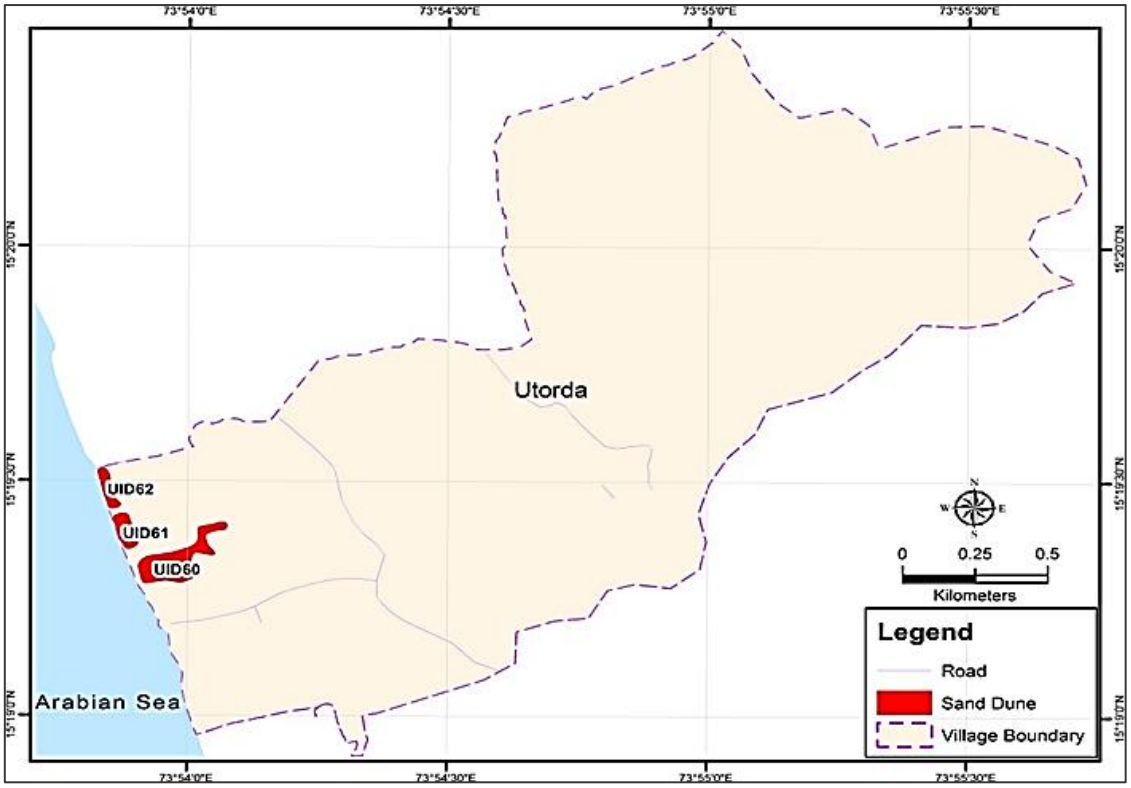


Fig. 2: Sand dune of Utorda village.

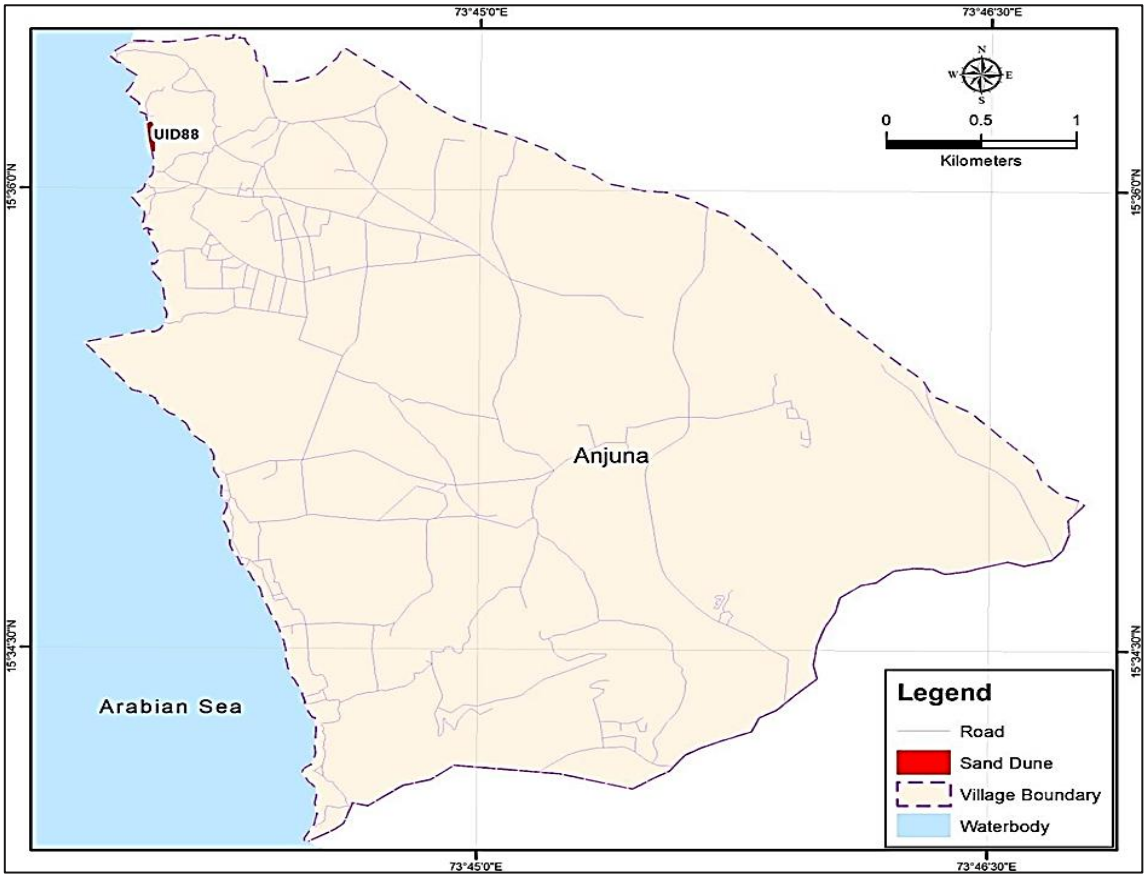


Fig. 3: Sand dune of Anjuna village.

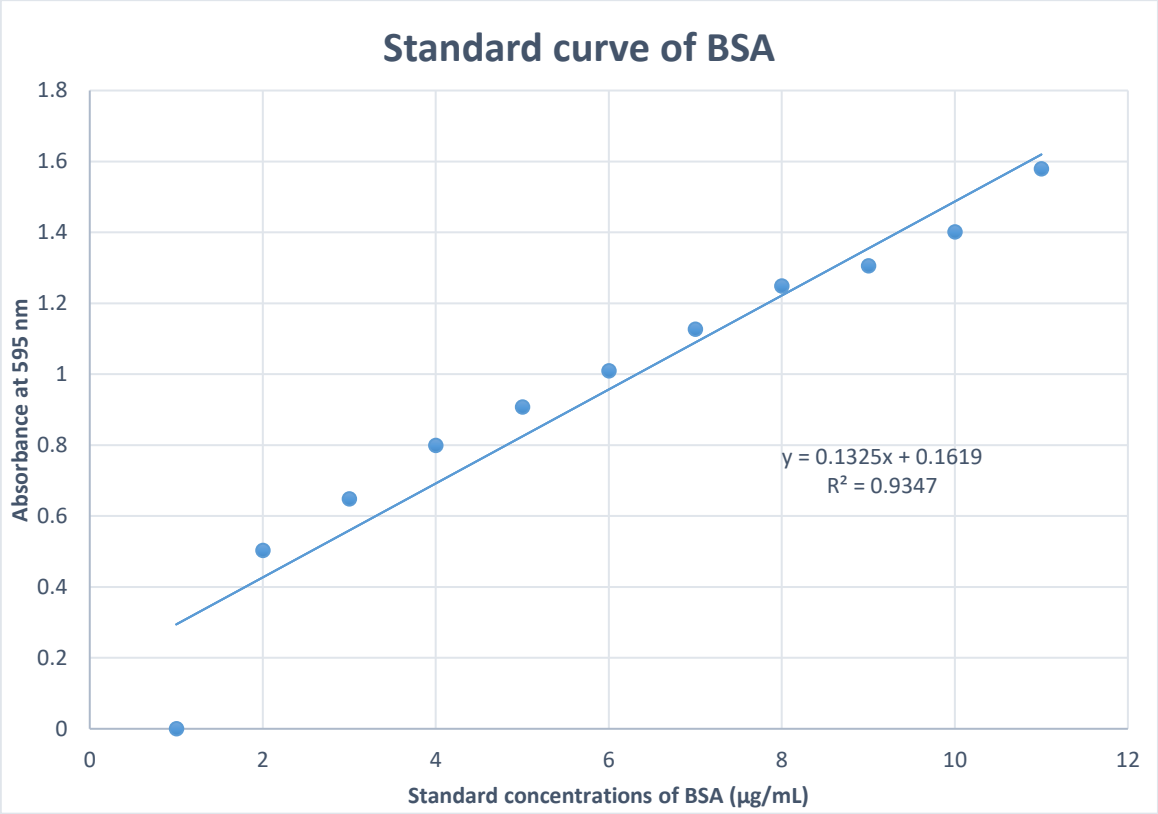


Fig. 4: Representative standard curve prepared by the standard concentration of BSA

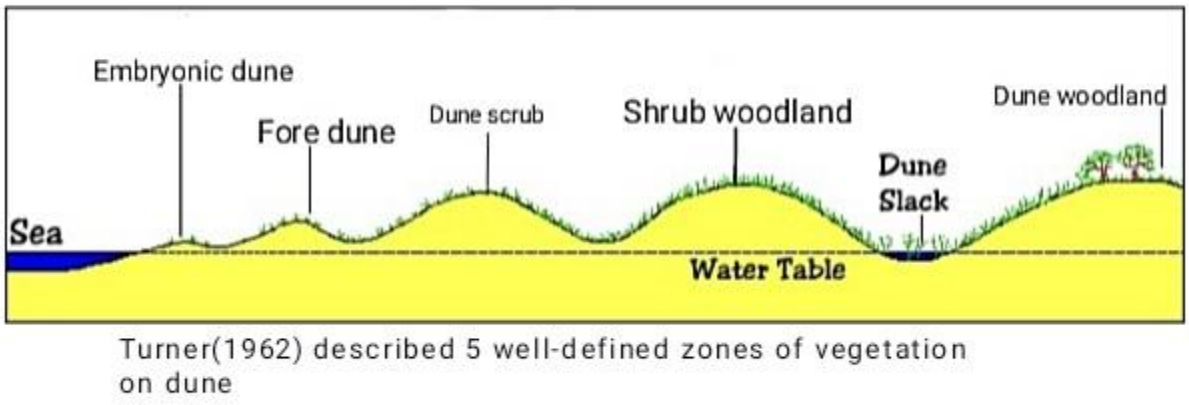


Fig. 5: Different zones of dune vegetation.

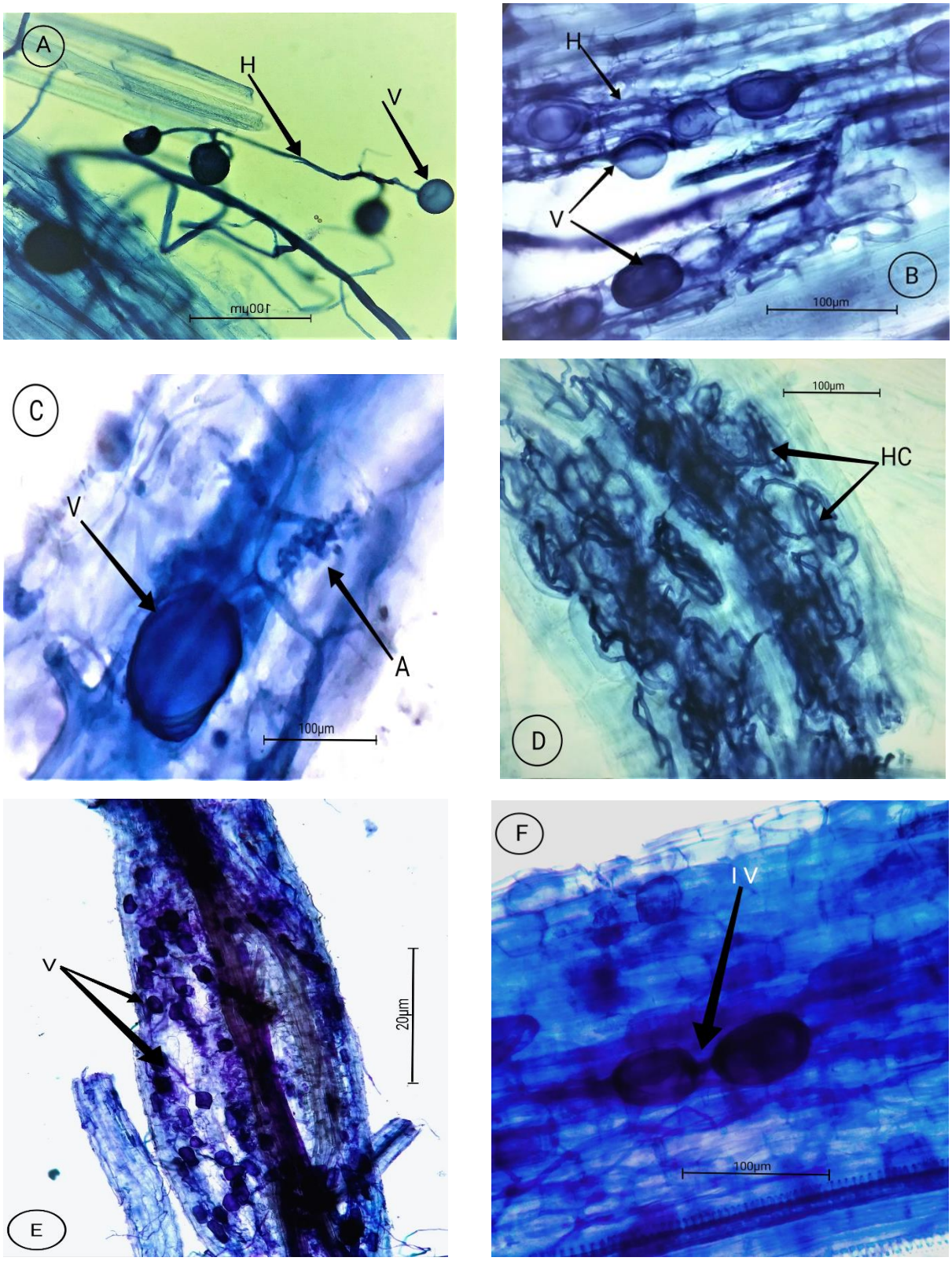


PLATE 1: AM fungal colonization seen in the roots of *Ipomoea pes-caprar*

A, B, E- showing Hyphal (H) and Vesicles (V).C- showing Arbuscules (Arum type) (A) and Vesicles (V). D- showing Hyphal coils (HC). F-showing Intercalary vesicles (IV).

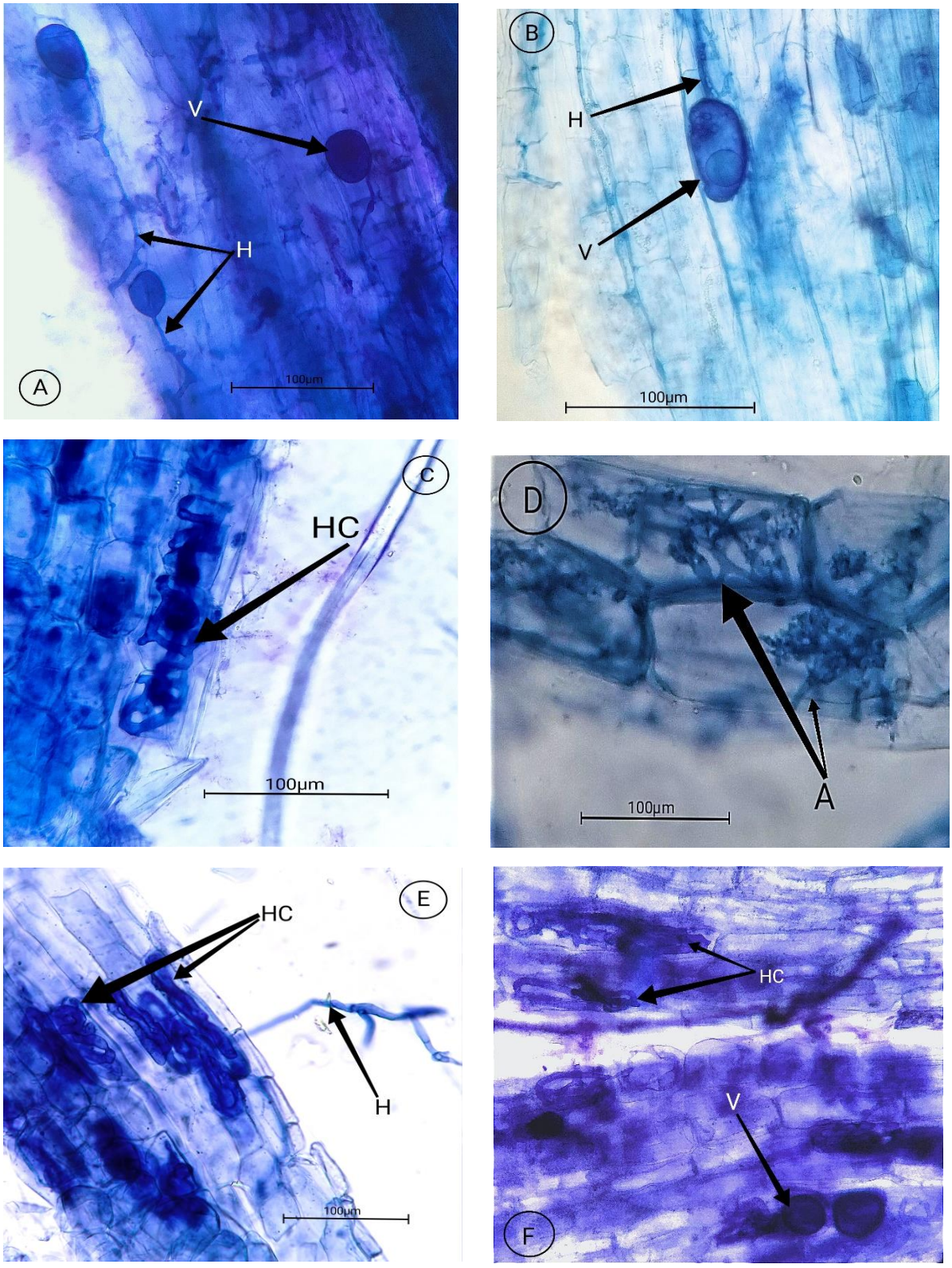


PLATE 2: AM fungal colonization seen in the roots of *Spinifex littoreus*.

A, B, E, F- showing Hyphal (H) and Vesicles (V). C, E, F- showing Hyphal coils (HC). D- Showing Arbuscules (Arum type) (A).

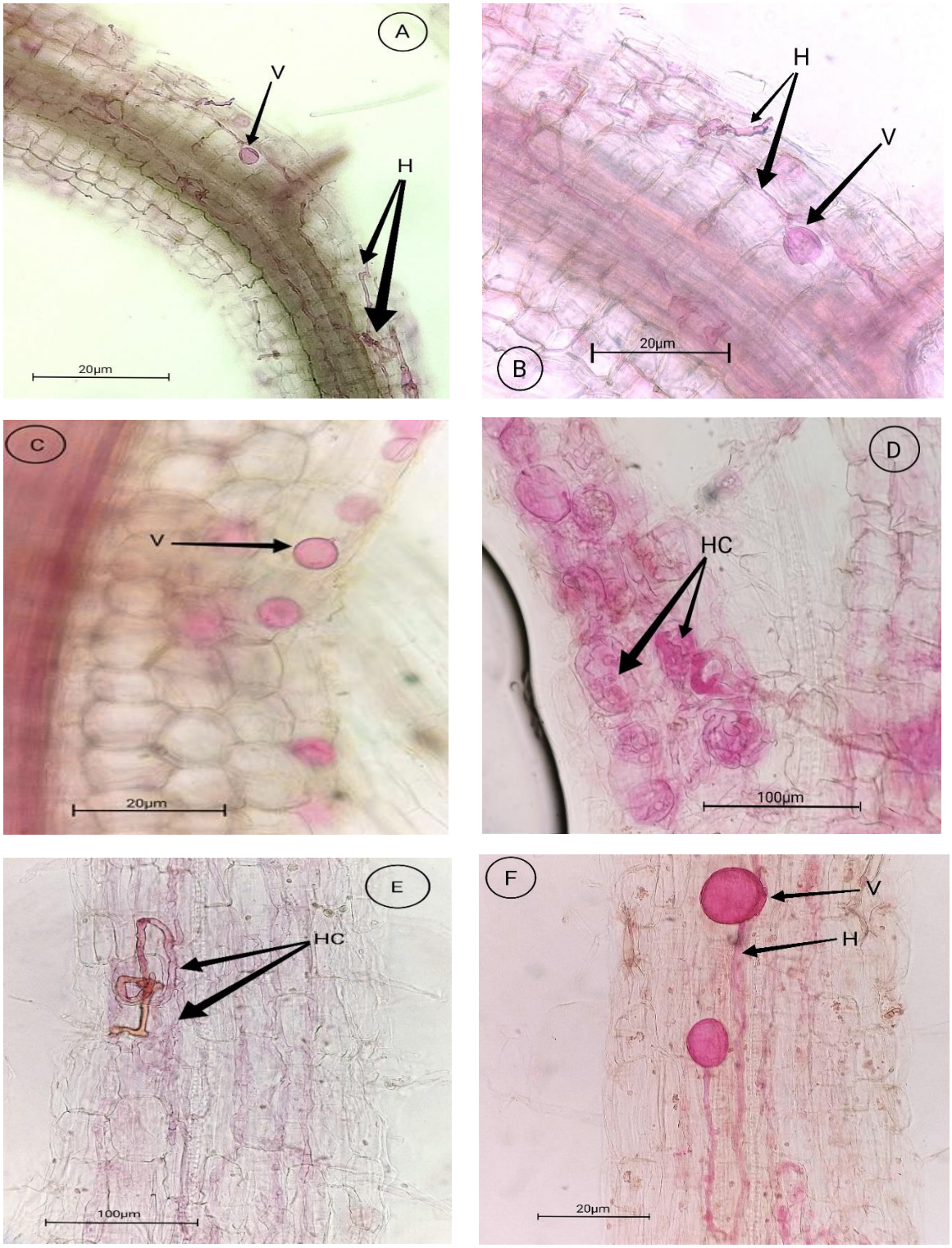


PLATE 3: AM fungal colonization seen in roots of coleus plant (trap culturs).

A, B, C, F- showing Hyphal (H) and Vesicles (V). D, E- showing Hyphal coils (HC).

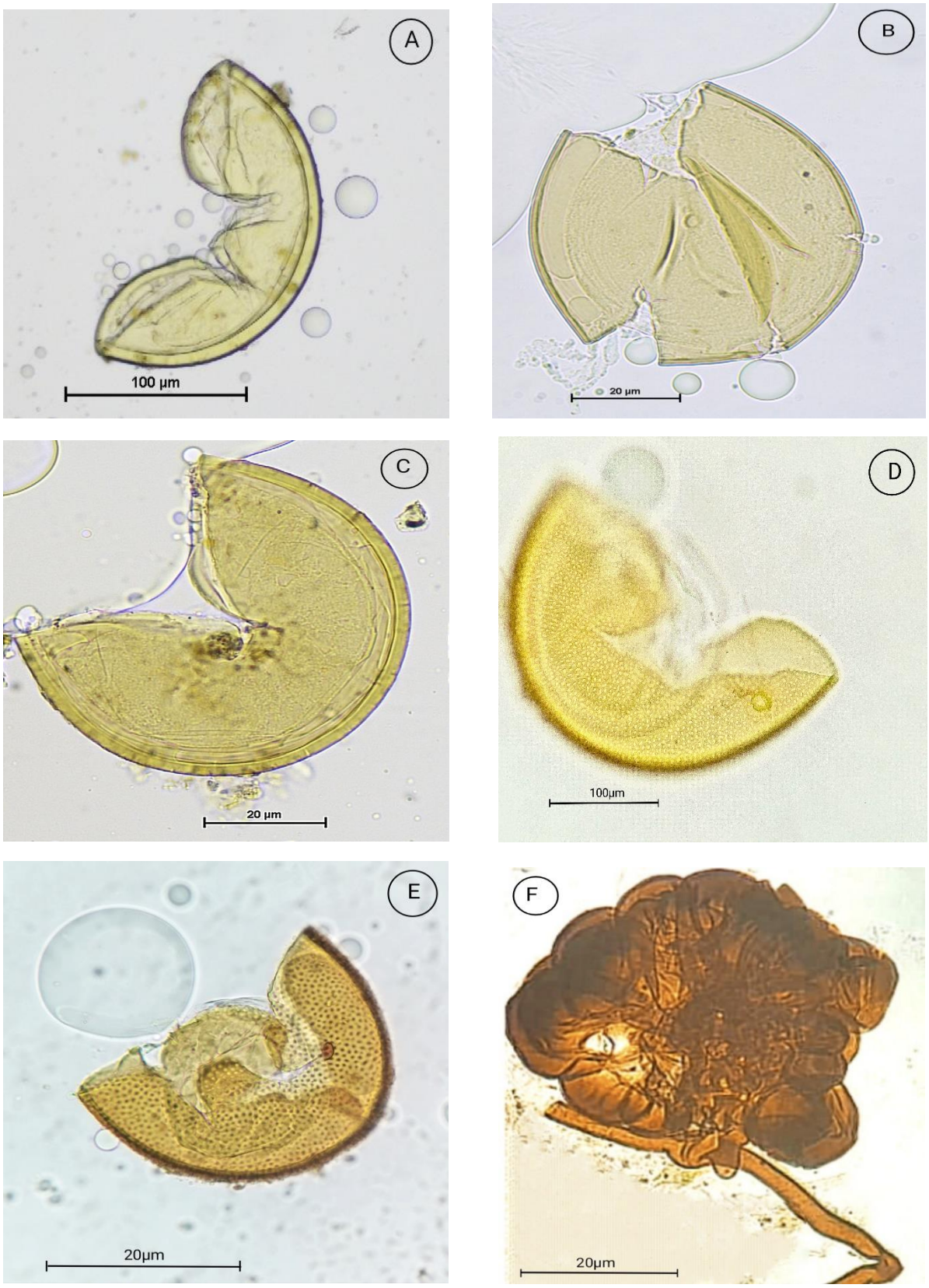


PLATE 4: A- *Acaulospora polonica,* B-*Acaulospora delicata,* C- *Acaulospora dilatata,* D-*Acaulospora scrobiculata,* E- *Acaulospora undulata* and F- Auxiliary cells of *Scuteiospora* sp.

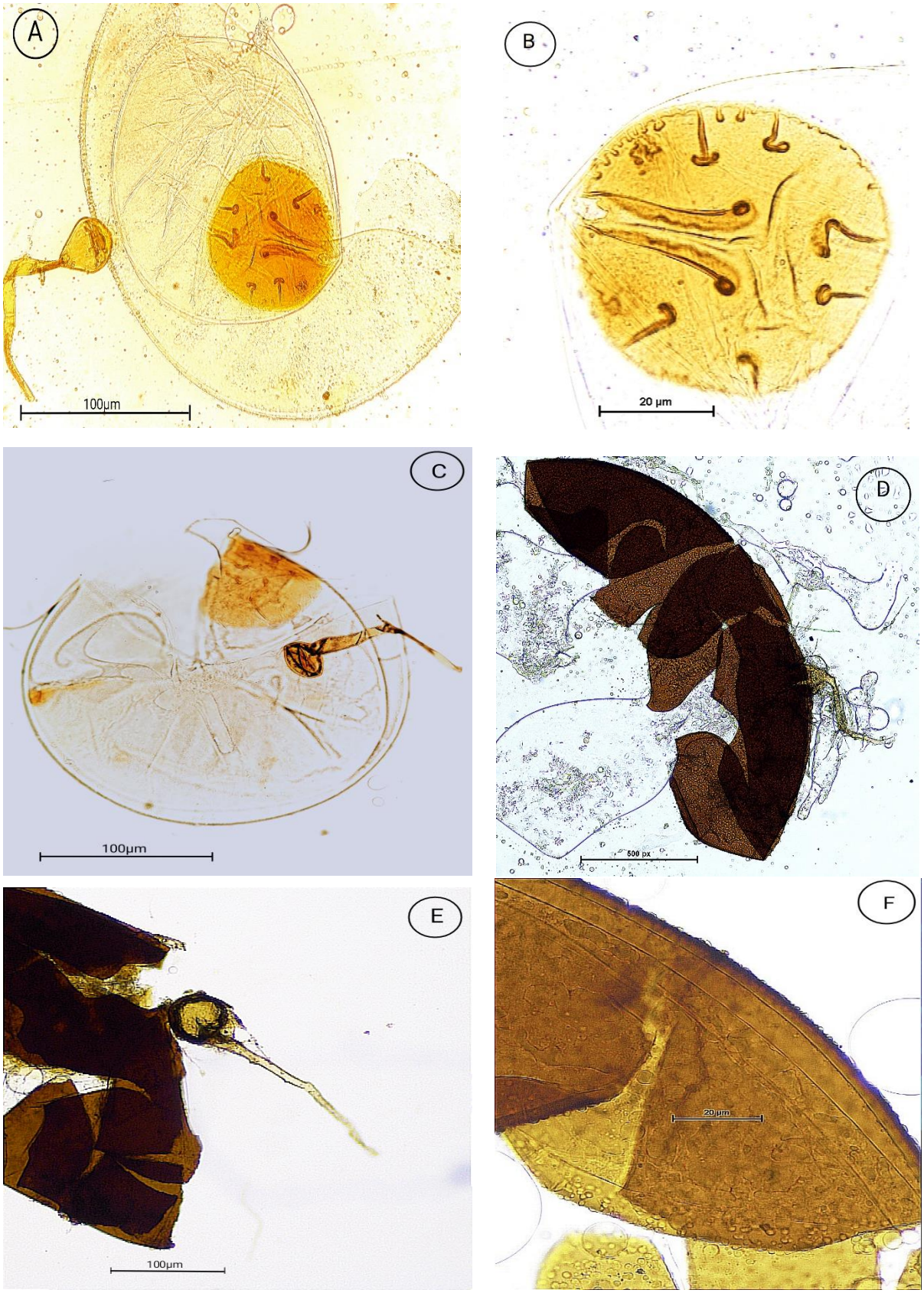
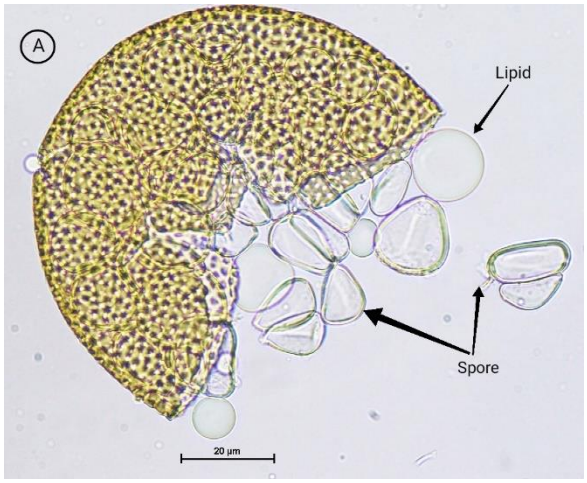


PLATE 6: A- *Dentiscutata scutata* B- Germinatin shield in *D. scutata*, C- *Racacetra verrucosa*, D- *Racocetra coralloidea*, E and F- *R. coralloidea* bulbous suspensor and Ornamented, 3 spore wall layers.



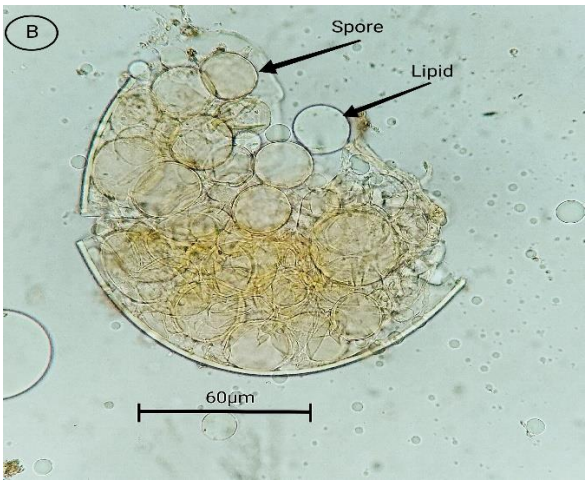


PLATE : A and B- Showing Spore in spore syndrome and C- Trap cultures, D- *Glomus macrocarpum,* D- Trap culture.