Evaluating Purity Of Arbuscular Mycorrhiza Fungal Cultures (*Glomus* and *Acaulospora*) Goa University Arbuscular Mycorrhizal Culture Collection (GUAMCC) And Assesment Of Arbuscular Mycorrhiza Fungal Diversity In *Utricularia reticulata.*

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By

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CERTIFICATE

This is to certify that **Ms. Sanjana Alias Samiksha Sajjan Shet Parkar** has completed the dissertation submitted to Goa University in partial fulfillment of M.Sc. Degree in Botany course in the academic year 2021-2022.

Signature of student

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DECLARATION

This is to certify that the dissertation work “Evaluating Purity Of Arbuscular Mycorrhiza Fungal Cultures (Glomus and Acaulospora) Goa University Arbuscular Mycorrhiza Culture Collection And Assesment Of Arbuscular Mycorrhiza Fungal Diversity From Utricularia reticulata” is an authentic record of work done by Ms. Sanjana Alias Samiksha Sajjan Shet Parkar, student of M.Sc. Botany Goa University in partial fulfillment of the requirement of M.Sc. Degree and no part of it has formed the basis of the award of any degree or diploma by this or any other University.

Signature of student

Signature of Guide

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INTRODUCTION

Arbuscular mycorrhiza fungi form symbiotic association between a fungus and the roots of vascular plants. In the association, the fungus colonizes the host plant's roots intracellular as in arbuscular mycorrhizal fungi (Sadhana, 2014). Arbuscular mycorrhiza is the most widespread type of mycorrhizal association and exists in ecosystems throughout the world where it creates an intimate link between the plants and the rhizosphere (Harrison, 1999). AMF belongs to phylum Glomeromycota (Berruti, 2016). Many fungi of order glomales can contribute to a mycorrhiza, and roots can contain several different fungal taxa (Merryweather and Fitter, 1998).

AM fungi are identified by the presence of unique structures like arbuscules and vesicles. Arbuscules are hausteria-like structures that are produced within the cortical cells (Smith and Read, 2008). Based on the morphology of arbuscules, two different types are identified i.e. Arum type and Paris type by Gallaud (1905). Paris type is characterized by the presence of extensive intracellular hyphal coils and absence of intercellular phase, vesicles are intracellular whereas in Arum type it is characterized by presence of extensive intercellular hyphal growth. In paris type the arbuscules are few in number and restricted to single layer of cells in the cortex, resultant colonies have cumulated appearance while in Arum type development of arbuscules is terminal on intracellular hyphal branches and resultant colonies are linear in appearance (Sharma *et al.*, 2017).

Vesicles are storage organs which are of variable shape and that range from bean to globular (Gaur and Kaushik, 2011). They are found only in the genera of *Acaulospora* and *Glomus*. Other morphological structures used for identification of AM fungi include shape, size, color, ornamentation of spore sporocarp, subtending hyphae and spore wall (Rodrigues and Muthukumar, 2009).

Benefits of AM

Formation of the hyphal network by the AMF with the plant's roots significantly enhances the access of the roots to a large soil surface area, causing improvement in plant growth (Begum *et al.,* 2019). AM fungi form specialized structures in the plant root cells termed arbuscules, which are the site for the transfer of nutrients between fungus and plants. Nutrients are captured by networks of fungal hyphae radiating into the soil around the roots and are transported to the plants in exchange of carbon (Newsham *et al.*, 1995). AMF increase of plant growth and nutrition by gaining more nitrogen (N), phosphorus (P), and other less mobile nutrients, also increase water uptake and water holding capacity that initiate drought tolerance, increase tolerance to abiotic stress such as soil salinity, heavy metal toxicity etc., overcome biotic stresses and offer bio-protection against pathogens and enhance the plant vigor and yield (Kaulia and Ghosh, 2022).

AMF colonization improves tolerance of plants to stressful cues by bringing about several changes in their morpho-physiological traits (Begum *et al.,* 2019). The effect of nutrient and water deficient conditions increased microbial community and nutrient availability which benefits plants and soil fertility. AMF increase of plant growth and nutrition by gaining more nitrogen (N), phosphorus (P), and other less mobile nutrients, also increase water uptake and water holding capacity that initiate drought tolerance, increase tolerance to abiotic stress such as soil salinity, heavy metal toxicity etc., overcome biotic stresses and offer bio-protection against pathogens and enhance the plant vigor and yield (Kaulia and Ghosh, 2022).

AMF could be considered as a replacement of inorganic fertilizers, because mycorrhizal application can effectively reduce the quantitative use of chemical fertilizer input especially of phosphorus (Orthas, 2012). They provide the host with water, nutrients and pathogen protection, in exchange for photosynthetic products (Berruti, 2016).

External hyphae acts as a skeletal structure which holds soil mineral particles together via physical entanglement. The external hyphae shows mechanical entanglement by bringing the mineral and organic debris together to form micro-aggregates and later the enmeshment of micro-aggregates by external hyphae and the roots create macro-aggregates (Miller and Jastrow, 1992). This aggregation helps to maintain the physical properties of soil. Glycoprotein which is produced by mycorrhiza also shows similar properties, they also have strong cementing capacity of soil particles (Borie *et al.,* 2008).

Mass multiplication of AM fungi.

Isolation and selection of AMF species are effective for growth promotion and rising of pure culture of these species is difficult, a suitable host is required to maintain pure culture of AM inoculums (Sharma *et al.,* 2017). The obligate biotrophic nature of AM fungi has complicated the development of cost-effective large scale production methods to obtain high quality AM fungal inoculums because of unstable performance of mycorrhizal fungi in the production system and shortage of knowledge (Ijdo *et al.,* 2011).

Techniques like hydroponics and aeroponics or soil less culture produce high quality inoculums. AM inoculation should be free from unwanted organisms such as plant pathogens or harmful bacteria, unadulterated inoculums are required to maintain the purity (Sharma *et al.,* 2017). The inoculums should be composed of a mixture of spores, colonized roots, hyphae and soil from pot culture grown in sterilized soil (Gemma et al., 2002). To reduce incompatibility between fungus and host or soil, the AM should be isolated from the rhizosphere of the plant because the rhizosphere is the most accessible and most abundant source of inoculums for starter culture (Muthukumar and Udaiyan, 2007).

AM are mass produced in the pot cultures on suitable host plant and the host plant, which should be adapted to agro-climate condition and polyhouse condition (Al-raddad, 1995). The substrate should be light weight and should possess good water holding capacity, less leaching of essential nutrients and easy to be removed from the root surface (Sharma et al., 2017)

Pot Culture

Maintenance of AMF is accomplished by means of open pot-cultures. The expense of maintaining open pot culture in a greenhouse can be reduced by automated watering systems but this does not eliminate the danger of contamination. In several ways pots get contaminated. For example, pots may be placed together, or not raised above a bench surface, resulting in contamination. This can be prevented by maintaining the culture in completely isolated environment (Walker and Vestberg, 1994).

***Utricularia reticulate* Sm.**

The roots of carnivorous plants are classified as non-mycorrhizal because the absorption from animal derived minerals makes the role of fungal partner redundant. But mycorrhizal association bestows benefits to the host plant which exceeds the facilitation of nitrogen acquisition and provide competitive advantage in high stress environments as carnivorous plants grow in poorly humorous soil (Quilliam and jones, 2010). *Utricularia reticulata* is commonly known as net veined bladderworts belonging to family lentibulariaceae, having narrowly oblong leaves (1-2 cm) and flowers during September to October.

The traps are known as utricules, are foliar structures shaped in small vesicles which are active in prey capture and the secretion of hydrolytic enzyme for digesting small prey and causing prey to die due to anoxia (Miranda, 2021; Perera et al., 2021). The traps are bean shaped to spherical, hollow and minute from 0.25-5mm in length. The traps are borne underwater in wet soil, sand or epiphytic moss. Entrance trap is guarded by two flexible valves i.e. door and smaller velum. Four arm sessile hairs which are present inside the bladder absorb water and create partial vacuum. The lower edge of the dog legged door lodges against a stop in the velum and forms a tight seal. Four bristles project from the lower half of the door pressurize the prey swimming nearby and lead to swing the dog-legged door past the velum, causing it to swing back under the vacuum, sucking water along with the prey into the bladder. Once the vacuum is filled, the door will shut and digestive enzymes are secreted and quadrifids absorbs the digest and later reset the trap (Abrahamson, 1989). Supply from organic compounds from prey was shown to be essential for vigorous growth and reproduction (Peroutka et al., 2008).

The present work was carried out with the following objectives

1. To check colonization and spore density of existing pure culture of *Glomus* and *Acaulospora* species maintained in the Goa University Arbuscular Mycorrhizal Culture Collection (GUAMCC).
2. To check AM root colonization and spore density in *Utricularia reticulata* found growing in Goa University campus
3. To study the effect of AMF and PSB with reference to growth and biomass on *Eleucine coracana*

Review of literature

Nacoon S. *et al.,* (2021) worked on combination of arbuscular mycorrhizal fungi and phosphate solubilizing bacteria on growth and production of Helianthus tuberosus under field conditions. Results showed the presence of PSB and AMF colonization at the harvest stage in both years. According to correlation analysis, PSB positively affected AMF spore density and colonization rate. Also, both AMF and PSB positively correlated with growth and production of sunchoke. Better results in 2016 were found in co-inoculation treatment and AMF inoculation performed the best in 2017. All of these results suggested that AMF and PSB could effectively promote growth and production of sunchoke under field conditions. Such effects were varied due to different environmental conditions each year.

Medina *et al.,* (2018) conducted the study to observe Colonization and Spore Richness of Arbuscular Mycorrhizal Fungi in Araucaria Nursery Seedlings in Curitiba, Brazil. Their evaluations indicated that araucaria seedlings were well colonized by AMF (with rates varying from almost 50 to over 85%) and produced an abundant number of mycorrhizal spores (from 344 to 676 spores per seedling). Samples contained spores of the species *Acaulospora scrobiculata*, *Dentiscutata heterogama*, and *Glomus spiniferum* and unidentified species of genera *Gigaspora* and *Glomus*. The *Glomus* genus was the most abundant kind of AMF spores found under nursery conditions.

Selvakumar*et al.,* (2016) reported that the maize plant as host in trap cultures was better than Sudan grass. Trap culture using maize resulted in longer shoots and roots than Sudangrass plants. Increase in dry weight with higher percentage also was observed for maize plants. After the first and second plant cycle, maize plants had a higher percentage

of AM response in terms of colonization and arbuscules than Sudan grass. Maximum spore count is also achieved in the pots of maize plants.

Arul and Nelson, (2016) studied diversity of AM fungi in the cement dust polluted sites of Ariyalur District, Tamil Nadu. Their study revealed that the physicochemical characteristics of the soil and the AM diversity varied within the sites. The AM fungal population reduced drastically in the sites near the industry. Species of *Glomus* were isolated. They suggested that isolation of the indigenous and presumably stress-adapted AM fungi can be a potential biotechnological tool for inoculation of plants for successful restoration of degraded ecosystems.

Wahid F. et al (2016) worked on inoculation of arbuscular mycorrhizal fungi and phosphate solubilizing bacteria in the presence of rock phosphate improves phosphorus uptake and growth of maize. The results indicated that the rhizosphere interactions between AMF and PSB significantly promote RP mineralization in soil and improved all growth parameters including shoot (56%), root yield (52%), height (41%), N (80%) and P (91%) uptake by the maize plants as compared to control and single inoculation. Increase in soil spore density, PSB population and percent root colonization in maize plants were also recorded by the combined inoculation of AMF and PSB with RP.

Karaarslan*et al.,* (2015) AM fungal diversity in bulbous plants in Taurus mountain in Turkey. They reported three AM species *viz.,Glomusmosseae, Glomus hoi* and *Scutellosporacalospora*. Their study suggests that there is variation in AM fungal colonization and spore density in different ecological conditions.

Srivastava *et al.,* (2014) worked on effect of different *Glomus* species of common habitat on growth and nutrient content of different genotype of finger millet (Eleusine corana L.). They conducted the study to evaluate the effect of two different species of *Glomus* (*G. intraradices* and *G. etunicatum*). The study confirmed mycorrhizal inoculation can be a better alternative for improved phosphorous uptake in finger millet.

K. Ramakrishnan and G. Bhuvaneswari (2014) studied the effect of inoculation of am fungi and beneficial microorganisms on growth and nutrient uptake of *Eleusine coracana* (L.) Gaertn. (Finger millet). Single inoculation of AM fungi and combined inoculation of AM fungi with Azospirillum Brasilense or PSB was found to be moderately increased in all the growth parameters and yield in finger millet. Dual inoculation showed higher phosphate uptake compared to control. Increase in AM percent colonization was observed only after 90 days of mycorrhizal inoculation. Control plats failed to show significant growth due to absence of AM. However triple inoculation of AM fungi, Azospirillum Brasilense and PSB was found to have highest growth parameters.

Channabasava A. and H. C. Lakshman (2012) assessed the mycorrhization effect on biomass of four rare millets in of north Karnataka. The millets inoculated with AM fungus *Glomus fasciculatum* showed increased value for growth, biomass and phosphorus uptake over the remaining treatments, but all the AM fungi inoculated rare millets had shown significantly greater values for the biomass growth over the non-inoculated ones. Mycorrhizal inoculation helped in enhancing the biomass of plant, percent mycorrhizal colonization and spore number due to increased uptake of mineral nutrients.

Kumar S. *et al.,* (2011) did assessment of VAM spore density and root infection from alluvial soil of eastern part of Raniganj coalfield areas. They observed that VAM spore density and their colonization were positively correlated with depth of soil profile available nitrogen, organic matter and available phosphorus. Some most common genus of VAM spores found in the study area is *Glomus, Gigaspora, Acaulospora, Enterophospora* and *Sclerocystis.* The highest number of VAM spore density was reported under the *Acacia auriculiformis*. The percentage of root colonization observed in seven native species are; *Dalbergiasissoo* (91%), followed by *Prosopisjuliflora* (84%), *A. auriculiformis* (79%) and *A. scholaris* (64%), *Polyalthia longifolia* (58%), *Cassia siamea* (31%), and *Azadirachta indica* (21%). This study concluded that these host plants having higher root infections are suitable for the biological reclamation of OB dumps.

Zandavalli R. B. *et al.,* (2008) carried out the study to check the Species richness of arbuscular mycorrhizal fungi in 3 district forests with Araucaria in Southern Brazil, also accounting for seasonal variation. Six soil samples from each forest were collected from the *Araucaria angustifolia* rhizosphere in four seasons during a one-year period, for identification of the AMF species. Spores were extracted by wet-sieving followed by centrifugation in water and 50% sucrose solution. The greatest richness sixteen species were found in the Araucaria reforestation and the lowest in the native forest eight species. The season with higher species richness was different in each site. The most representative genera in all forests were *Acaulospora* and *Glomus*.

Ilbas A. L. and S. Sahin (2005) worked on effects of soybean yield components of inoculation with arbuscular mycorrhizal fungus (AMF) *Glomus fasciculatum* were investigated at different phosphorus levels. The level of root AMF colonization decreased

a little when P levels increased. Mycorrhizal inoculation and increasing levels of P application had positive effects on yield components such as stem and root length, shoot and root dry weight, stem diameter, 1000-grain weight and seed yield per plant but not on legume numbers per plant. Both mycorrhiza inoculation and P treatments affected P and N concentrations of grain and roots of soybean.

Moreira-Souza M. *et al.,* (2003) worked on arbuscular mycorrhizal fungi associated with *Araucaria angustifolia*. Results showed the number of AMF spores tended to be higher in the native forest than in the replanted area. For native forest 732 spores per 100g of soil were found during hot season and 810 spores per 100 g of soil were found during cold season. In comparison in the replanted area 385 spores per 100 g found in first sampling and 423 in second sampling. These results suggest that the native forest offers better environmental conditions for development of the plant.

Materials and Methods

Sample Collection

Roots and soil samples from the existing cultures of *Glomus* and *Acaulospora* were collected from the Goa University Arbuscular Mycorrhizal Culture Collection (GUAMCC).

Rhizophere soil and root samples of *Utricularia reticulate* was collected which is growing in the Goa University Campus.

AM Fungal root colonization

AM fungal root colonization was carried out by using the Trypan blue staining method (Phillips and Hyman, 1970).

Roots were washed thoroughly with tap water and were cleared using 10% of KOH at 90°C for 1 hour. The KOH solution is decanted and rinsed with tap water 4 to 5 times to remove the traces of KOH. 5N HCl was added and kept for 3 to 4 minutes for hydrolysis of the roots. The solution was then poured off and 0.05% of Trypan blue was added and kept overnight. The stained roots were mounted on a slide using polyvinyl-lacto-glycerol (PVLG) and observed under a compound microscope. Colonization was observed based on hyphal, arbuscular, hyphal coils and vesicular structures. Photographs were taken using Olympus DP12-2and Nikon Eclipse E2000 digital camera.

Root percent colonization was checked using the following formula.

Percent Colonization= Total number of root beats colonized ÷ Total number of root beats examined × 100

Preparation of Trypan blue stain

0.05 g of Trypan blue powder was dissolved in 40ml of distilled water. To this, 50 ml of lactic acid and 10 ml of glycerin was added.

Preparation of polyvinyl-lacto-glycerol (PVLG)

16.6 g polyvinyl alcohol was dissolved in 100ml distilled water by heating at 90ºC. After cooling 100ml lactic acid and 10ml glycerin was added.

Isolation of AM spores by wet sieving and decanting method (Gerdemann and Nicolson, 1963)

25g of soil sample was suspended in tap water. The mixture was stirred for about 5-10 seconds and kept undisturbed for 1-2 minutes to settle the heavier particles. The mixture is then decanted through the stacked with the coarse sieve on top and fine sieves at the bottom. The range of the sieves used was 50µm, 150µm, 250µm, 750µm. The suspension from each sieve was collected separately in beakers.

Estimation of AM fungal spore density

AM fungal spore density was estimated using a modified method of Gaur and Adholeya, (1994). The suspension aliquot from each beaker was separately filtered through whatman no. 1 filter paper in such a way that the folded marked region of the filter paper has the spores. The filter paper was then placed in Petri plate and the spores were isolated and counted under stereomicroscope by using needles.

Mounting and identification of AM fungal spores

Spores were mounted on the slide using polyvinyl-lacto-glycerol (PVLG). The spores were crushed by applying little pressure on the cover slip using the blunt end of the needle and then observed under a compound microscope.

Sterilization of sand and pots

Sand tray was wiped with absolute alcohol and the sand was sterilized in an oven at 180ºC for 3 hours for 3 continuous days. Pots were soaked in soap water for 2-3 hours and then rinsed with tap water. Then the pots were dried and wiped with absolute alcohol using absorbent cotton and the pot holes were sealed with non-absorbent cotton.

Carrier Based culture

Carrier based culture was prepared using a mixture of 1:1 carrier and sterilized sand. To this mixture inoculum was added. Then *Eleusine coracana* seeds were sowed. They were kept in a polyhouse for a period of 3 months.At the end of 45days, root colonization was checked using Phillips and Hyman (1970) method.

Trap Culture

Trap culture were prepared by using the *Utricularia* soil. The roots was mixed with the sterilized sand in 1:1 ratio. The mixture was added to plastic pots which were already wiped with the absolute alcohol. Then *Eleusine coracana* seeds were sowed and kept for multiplication. Plants were regularly watered, and then stopped after 90 days, allowing the plants to dry. After drying, root colonization was checked using Phillips and Hyman method (1970).

Experimental site and design

Soil was collected from an area near the Goa University library.

The experimental design was prepared in the following manner:

T1 – Control

T2 – AMF (*R. intraradices*)

T3 – AMF (*R. intraradices*) + PSB

T4 – PSB

T5 – PSB + RP

T6 – RP

T7 – AMF(*R. intraradices*) + RP

T8 – AMF (*R. intraradices*) + RP + PSB

All the treatments with 5 replicates of *Eleusine coracana* plant were arranged in randomized block design and the pots were maintained in the polyhouse at 28ºC for 90 days. For rock phosphate treatment, 50g of rock phosphate is mixed with the soil. The plants were regularly watered whenever required. The pots were treated with 10 ml bacterial culture. For AM fungal treatment, 10g of inoculum was added to the soil.The parameter assessed includes height, no. of shoots and dry weight (Biomass). Root colonization of *Eleusine coracana* was checked using Philips and Hyman (1970) method.

Results and Discussion

AM fungal root colonization was checked in *Eleucine coracana* which were used as host plant from the existing pure cultures of *Glomus* and *Acaulospora* species maintained in the Goa University Arbuscular Mycorrhizal Culture Collection (GUAMCC). In polyhouse condition,*E. coracana* shows better plant growth and use as a host plant. Ramkrishnan and Bhuveneshwari (2014)reported that *E. coracana* is an important dry land crop and have ability to withstand adverse weather conditions. Carrenho*et al.,* (2002) also reported in their study using *Sorghum bicolour*(L.) Moench (sorghum), *Zea mays* L. (maize) and *Arachis hypogaea* L. (groundnut) as host plants for detection of AM fungi and found highest colonization in peanut compared to sorghum and maize due to the composition of its root exudates.In the present study, all the pots undertaken for the study showed AM colonization. Different AM structures *viz.*, hyphae, arbuscules, vesicles and hyphal coils were observed **(Plate 1, 2, 3, & 4).** Earlier studies have also reported that various AM fungal structures *viz*., spherical and oval vesicles, and arbuscules in different host plants(Khanam*et al.*, 2003; Khanam*et al.*, 2004; Islam *et al*.,2002).

Table 1:AM fungal spore density in pure cultures maintained using pot culture method.

Sr. No.	Pure culture	Spore density* (spores/100g soil)
1.	*A. scrobiculata*	800-900
2.	*G. formosanum*	500-600
3.	*A. laevis*	500-600

*Values are mean of 3 readings.

Spore density in pure cultures of*A. scrobiculata, G. formosanum* and *A. laevis* was estimated**(Table 1)**. Highest spore density was recorded in*A. scrobiculata* (approx. 800-

900 spores/100g), followed by *G. formosanum* (approx. 500-600spores/100g) and *A. laevis* (approx. 500-600spores/100g)**(Plate 5)**. Lee and Otgonsuren, (2010) reported *Acaulospora scrobiculata* as a dominant species in wheatgrass.

Table 2: Percent AM fungal root colonization of carrier based culture.

AM fungal species	*Root colonization (%)
Rhizoglomusintraradices	93.33 ± 0.58
Glomusformosanum	66.66 ± 2.89
Rhizoglomusclarus	96.66 ± 0.58
Acaulosporascrobiculata	90.00 ± 1.00
Acaulosporalaevis	93.33 ± 1.15
Claroidioglomus. claroideum	36.66 ± 0.58

*Values are mean of 3 readings.

In some of the cultures, along with AM fungal species, endophytes were also observed. Hence, AM spores from the contaminated cultures were isolated, sterilized and were added to to raise pure cultures. These pure cultures were checked for purity. *Eleucine coracana* (ragi) was used a host plant. AM root colonization was checked at the end of 45 days **(Plate 7, 8 & 9),** while the AM fungal spore density was estimated after 90 days**(Plate 10)**. Highest root colonization was recorded in *R.clarus* (96.66%), while the least was recorded in *C. claroideum* (36.66%)**(Table 2)**.The spore density in all the pots ranged from 80-100 spores except in *C. claroideum* (50-60 spores)**(Plate 10)**. A previous study reported that *Glomus* and *Acaulospora* were dominant species significantly adapted to local

environment and produced more spore in short period of time (Zhao *et al*., 2003;Jamiolkowska *et al*., 2018).

AM studies in *Utricularia reticulata*

Roots and rhizospheresoil samples of *Utricularia reticulate* plant were analyzed. AM fungal colonization in *U. reticulate* showed large number of hyphal coils, besides few arbuscules and vesicles **(Plate 14 & 15)**.AM fungal spores belonging to genera *Glomus* and *Acaulospora* were recovered from the rhizosphere soil of the *U. reticulata***(Plate 16)**. James *et al.,* (2015) reported the AM colonization in *U. reticulata*but no arbuscular colonization was reported in their study.Harikumar, (2013) reportedvery low colonization in *Droserabrumanii* and *D. indica*due to narrow root length. The AM fungal genera *Glomus* was dominant over *Acaulospora* and *Gigaspora.* This may be due to adaptability to soil and reproductive capacity.

Trap cultures

Table 3: Percent root colonization of trap cultures.

Plant species	*Root colonization (%)	Type of colonization			
		H	V	A	HC
Utricularia reticulata	83.33 ± 1.51	+ +	+ +	+	+

***V**alues are man of two readings;

H = Hyphalcolonization, V= Vesicularcolonization; A=Arbuscular colonization; HC= Hyphal coils.

Trap cultures were prepared in polyhouseby using *Eleucinecoracana* as host plant**(Plate 17)**. Successful colonization was recorded after 45 days **(Plate 18)** and recorded significant root colonization (83.33%)**(Table 3)**

Table 4: Sporulation in trap culture

Plant	**AM species**	**Spore density**	**Spore abundance**	**Relative abundance (%)**
Utricullariareticulata	*Glomus* sp.	500	292	58.4
	*Acaulospora*sp.		208	41.6

Highest spore density was recorded in *Glomus*(292 spores/100g soil) while it was lowest was recorded in *Acaulospora*(208 spores/100g soil) **(Table 4)**.

Experimental design

Table 5: Effect of AM inoculum and Phosphate solubilizing bacteria (PSB) on plant height, shoot number and dry weight of inflorescence in *E. coracana*.

Treatments	Plant height (cm)	Shoot number	Dry weight of Inflorescence (g)
Control (T_1)	$25.6^{a} \pm 1.51$	$3.4^{a} \pm 1.14$	$0.39^{a} \pm 0.05$
AMF (T_2)	$35.4^{a,b,c} \pm 8.84$	$4.4^{a,b} \pm 0.90$	$0.46^{a,b} \pm 0.11$
AMF + PSB (T_3)	$37.7^{a,b,c} \pm 6.19$	$5.6^{b} \pm 1.14$	$0.59^{a,b} \pm 0.15$
PSB (T_4)	$34.4^{a,b,c} \pm 11.6$	$4.6^{a,b} \pm 1.14$	$0.42^{a} \pm 0.10$
PSB + RP (T_5)	$44.1^{b,c} \pm 4.81$	$4.0^{a,b} \pm 0.70$	$0.50^{a,b} \pm 0.12$
RP (T_6)	$27.8^{a,b} \pm 4.77$	$3.0^{a} \pm 0.70$	$0.41^{a} \pm 0.09$
AMF + RP (T_7)	$38.8^{a,b,c} \pm 9.83$	$4.8^{a,b} \pm 0.83$	$0.58^{a,b} \pm 0.15$
AMF + RP + PSB (T_8)	$45.0^{c} \pm 8.11$	$6.0^{b,c} \pm 10.0$	$0.70^{b} \pm 0.21$

Legend: Data is mean of 5 replicates; ± =Standard error of. Values in each column followed by different letter are

significantly different at $p<0.05$.

Results on the effect of AM fungi and PSB on selected growth parameters in *E.coracana* are depicted in **Table 5**.Inoculation with AM fungi and PSB showed varied effects on plant height, shoot number and dry weight of the inflorescence. Rock phosphate (RP) was used as a source of phosphorus. The present study was aimed to identify the efficient consortium for ragi that would result in better plant growth and eventually better yield.

It was observed that the plants treated with *Rhizoglomus intraradices,* RPand PSB showed maximum plant height, while least was recorded in un-inoculated control plants. The PSB would efficiently solubilize the added RP while the AM species would mobilize the same resulting in increased uptake of P in the plant. Wahid *et al*., (2016) showed similar results in maize plants wherein AM fungi and PSB influenced RP solubility and stimulated roots to absorb nutrient from the soil and thus enhance the plant growth. Similar studies have been reported wherein combined effect increased growth, yield and nutrient uptake in wheat and chickpea plant (Mukharjee and Rai, 2000). Also our study with dual inoculation using PSB and AM fungi recorded significant increase in growth and yield. Earlier studies have reported that dual inoculation increases yield in sorghum(Algawadi and Gaur, 1992), barley (Belimov *et al*., 1995), black gram (Tanwar*et al*., 2002),and Soybean (Abdalla and Omar,2001).Similarly, in an earlier study, dual inoculation reported increased growth and nutrient uptake in rice (Martins da costa *et al*., 2015).

The present study reported significant increase in root colonization in AM treated plants compared to control. Maximum root colonization was recorded in T_8treatment. Similar observations have been reported by Babana*et al*.(2006)where the treatment of AM fungi along with PSB and RP increased root colonization in wheat crop.

Shoot number and dry weight of inflorescence

Plantsinoculated with *R.intraradices,* RPand PSB showed increased number of shoots and dry weight of inflorescence compared to control. Similarly, Subramanian *et al.,* (2008) reported increased in shoots of maize plant and enhanced P-uptake *via* external mycelium. Gyaneshwar *et al*., (2002) suggested that PSB are ubiquitous in soil and play important role in supplying P to plants.Earlier studies reported increase in shoot height due to AM inoculation (Andrade *et al*., 1998 and Mendeiros *et al*., 1994).

CONCLUSION

The present study was initiated to confirm the purity of AM fungal pure cultures of *Glomus* and *Acaulospora* species maintained at Goa University Arbuscular Mycorrhizal Culture Collection (GUAMCC). Similarly, a study was conducted to check the effect of AM fungi and PSB on plant growth in *E. Coracana*. Also, AM fungal diversity in *Utricularia reticulata* an endemic species from Goa University campus was investigated.

All the pots of pure cultures recorded AM fungal colonization. Variations in colonization structures viz, hyphae, arbuscules, vesicles and hyphal coils were observed. Amongst three pure cultures, *Acaulospora scrobiculata* showed higher spore density. Further, the contaminated cultures were revived, and pure cultures were prepared by using sterilized vermiculite and sand as carrier substrate in the ratio of 1:1.

Experiment performed under polyhouse condition, contributed in understanding the effect of AM fungi, PSB and RP on plant growth. The study shows that in T_8 treatment the plant growth *viz*., plant height, number of tillers, and inflorescence was significant as compared to control. This is due to the triplication inoculation of the bio-inoculants and RP. The biofertilizer thus enhances crop production and is significant to sustainable agriculture.

The *Utricularia reticulate* commonly known as bladderworts are endemic to Goa University plateau. The present study reported the presence of AM fungal colonization *viz*., arbuscules, vesicles, and hyphal coils. Only two AM fungal genera *viz*., *Glomus* and *Acaulospora* were recovered from both the rhizosphere soil and trap cultures. It is also

observed that the growth of AM fungal hyphal coils was prominent during the flowering stage of the plants.

It is seen that based on their dominance; different AM fungal species are used for biofertilizer production to increase crop yield. Further study of the endemic plant species would help the better understanding about the role of AM fungi in nutrient uptake of the lateritic ecosystem.

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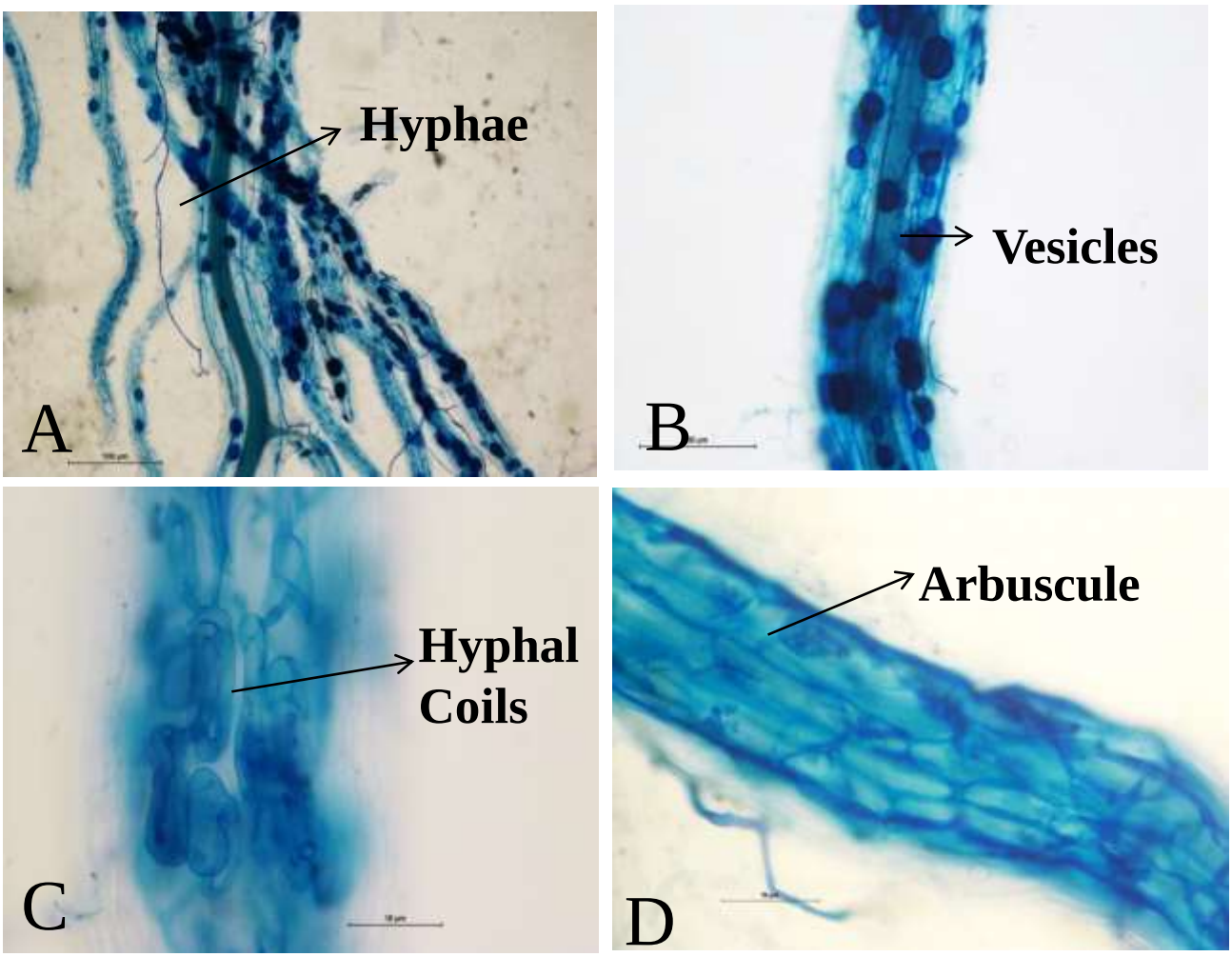

Plate 1: AM fungal root colonization in *Elucine coracana* from GUAMCC pure culture of *Rhizoglomus intraradices* A: Hyphal colonization; B: Vesicles; C: Hyphal coils; D: Arbuscules.

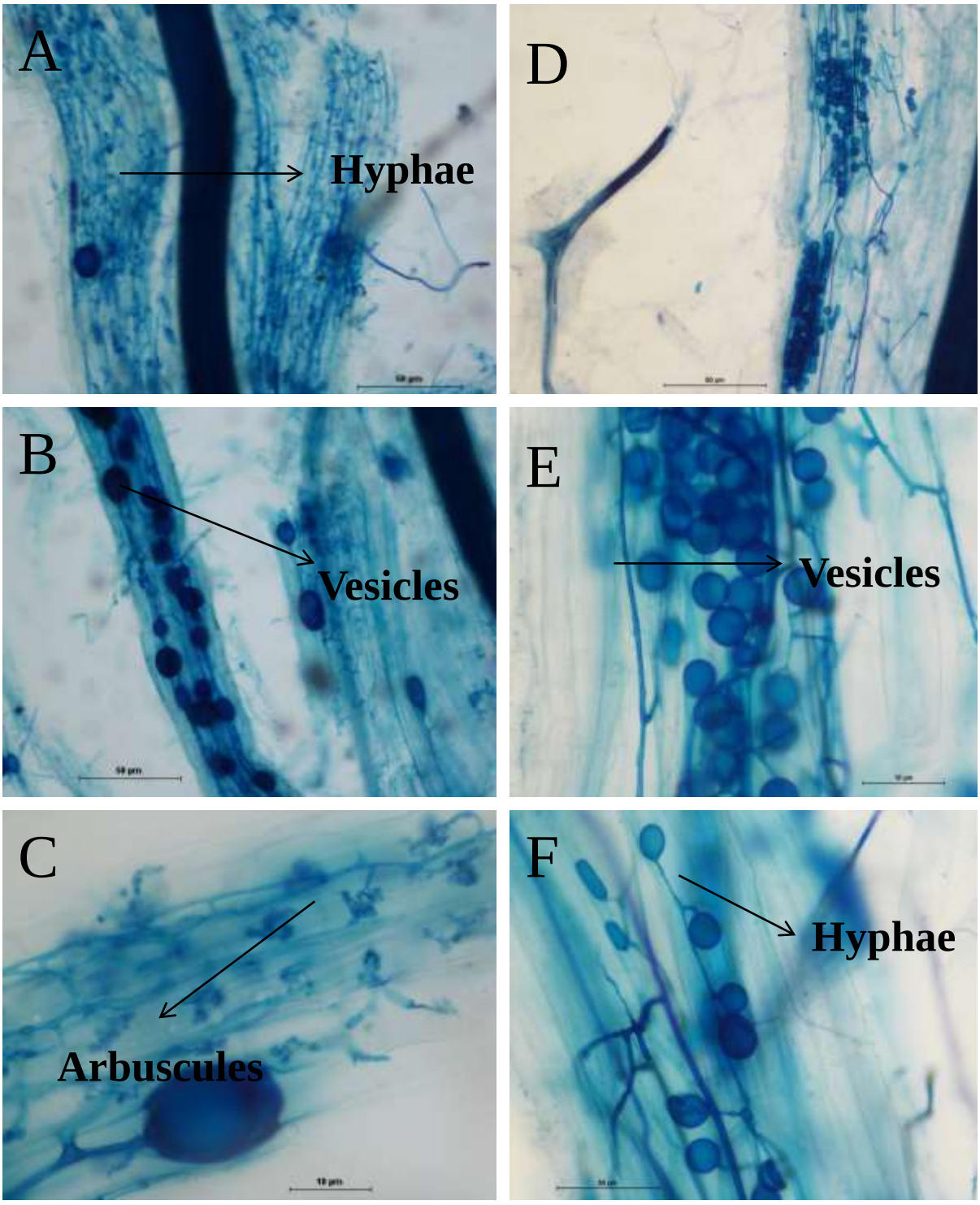

Plate 2: AM fungal root colonization in *Elucine coracana* from GUAMCC pure culture in *Acaulopora laevis* (A– Hyphae; B–Vesicles; C- Arbuscules) and *Acaulospora Spinosa* (D- Colonized root; E- Vesicles; F- Hyphae).

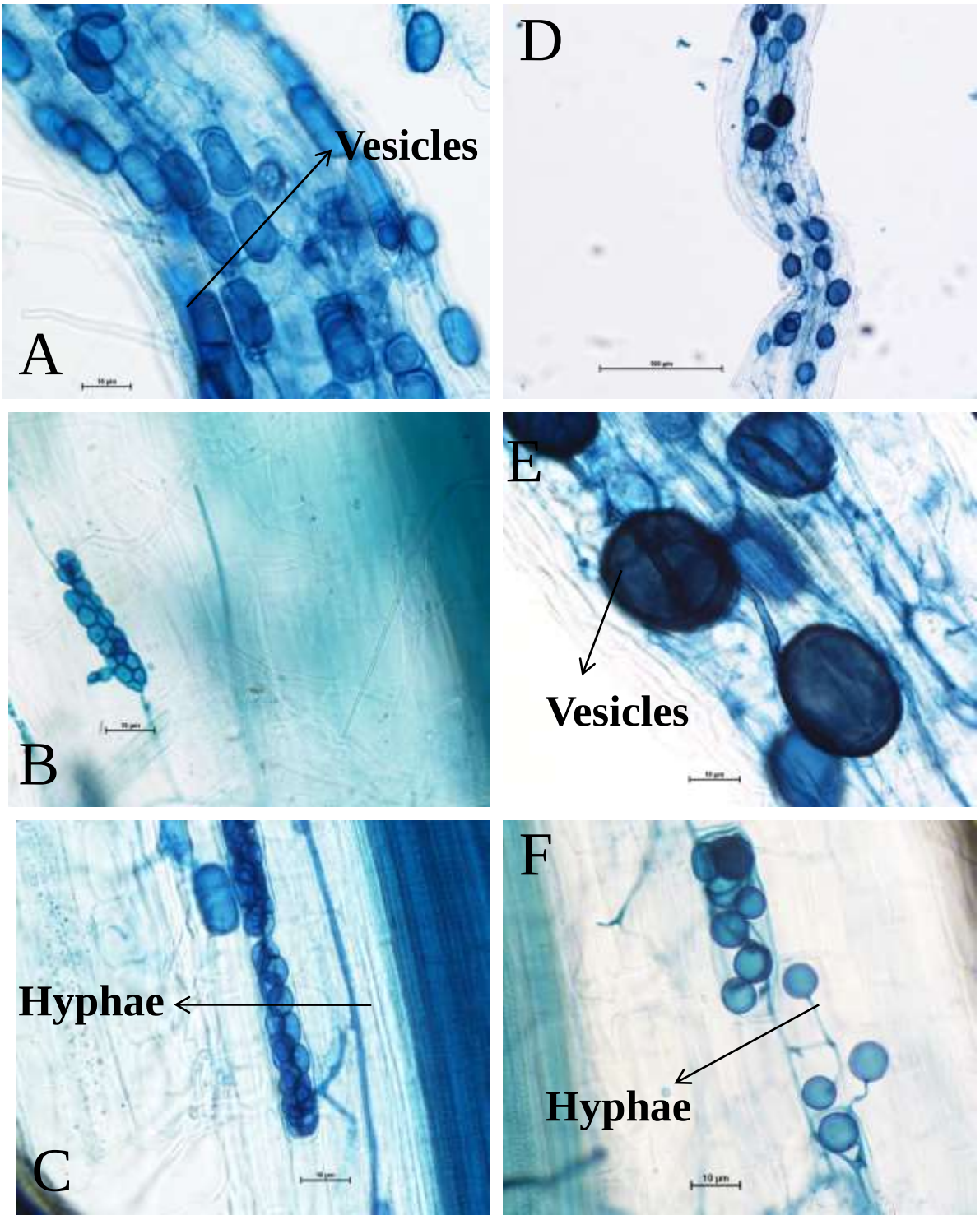

Plate 3: AM fungal root colonization in *Elucine coracana* from GUAMCC pure culture of *Acaulospora mellea* (A;Vesicles; B:Vesicles in cluster; C: Hyphae and *Funneliformis mosseae* (D- E:Vesicles; F: Hyphae)

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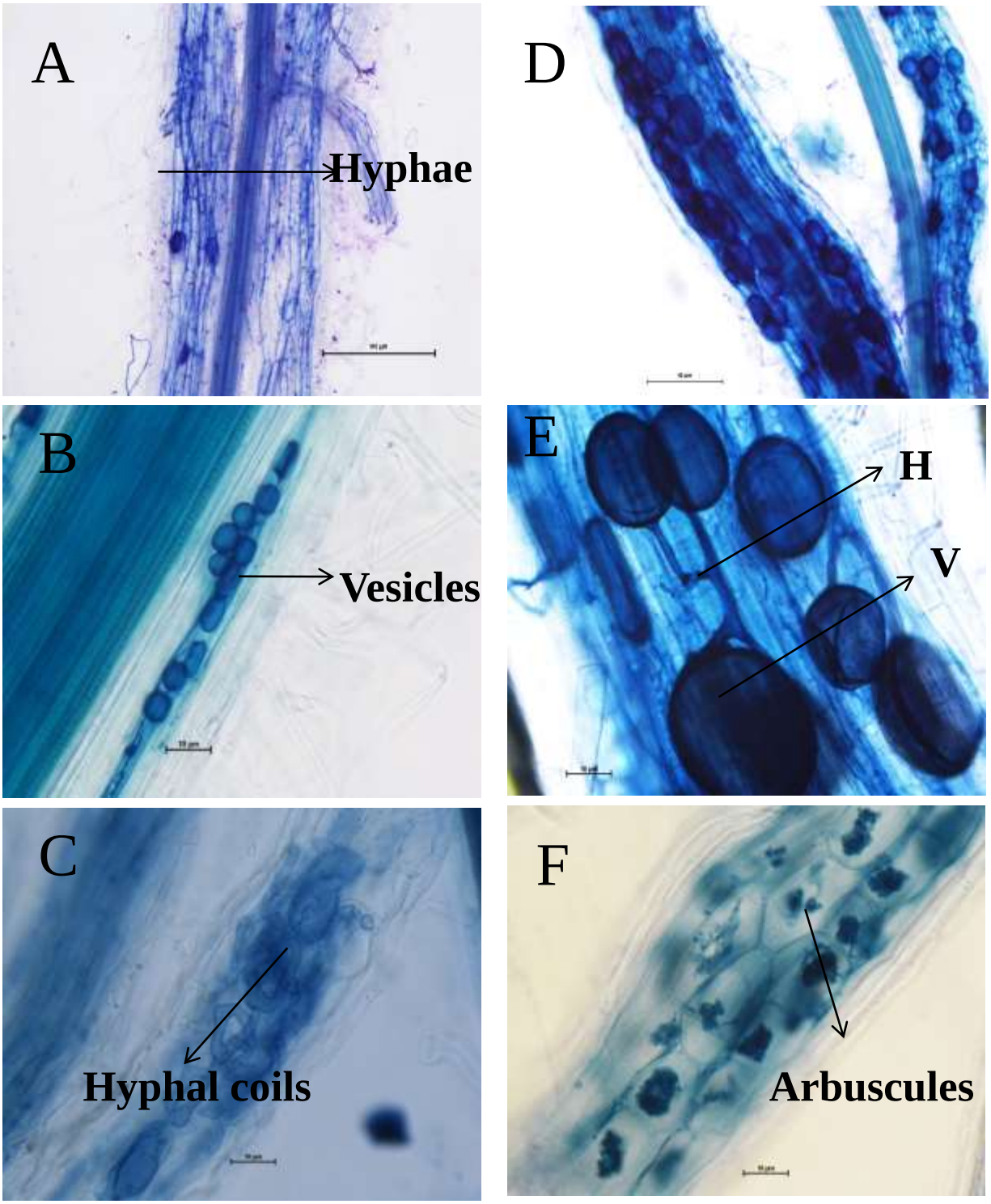


Plate 4: AM fungal root colonization in *Elucine coracana* from GUAMCC pure culture of *Acaulospora scrobiculata*.(A: Hyphae; B: Vesicles; C: Hyphal coils) and *Rhizoglomus manihotis* (D-E: Vesicles and Hyphae; F: Arbuscules)

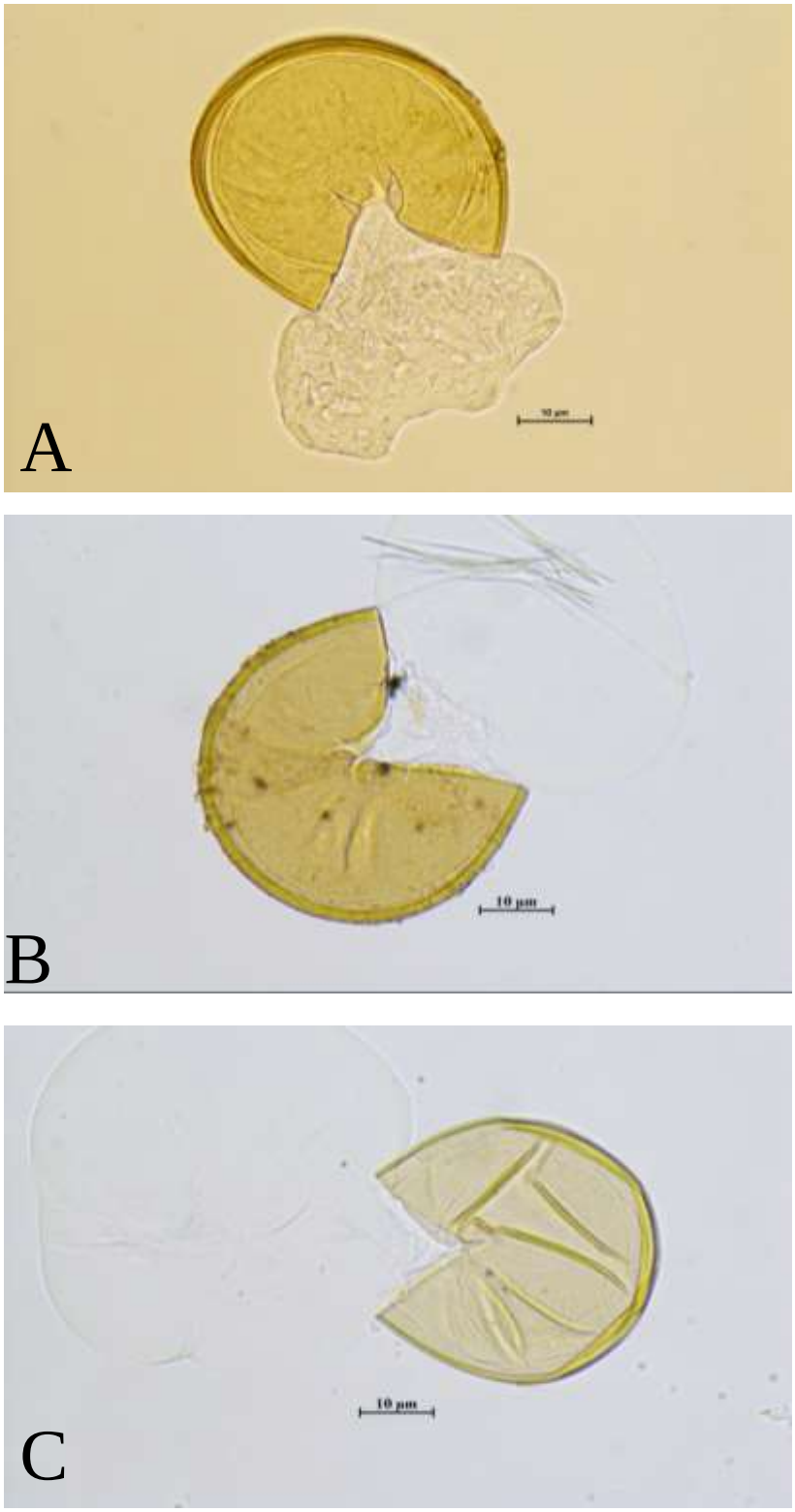
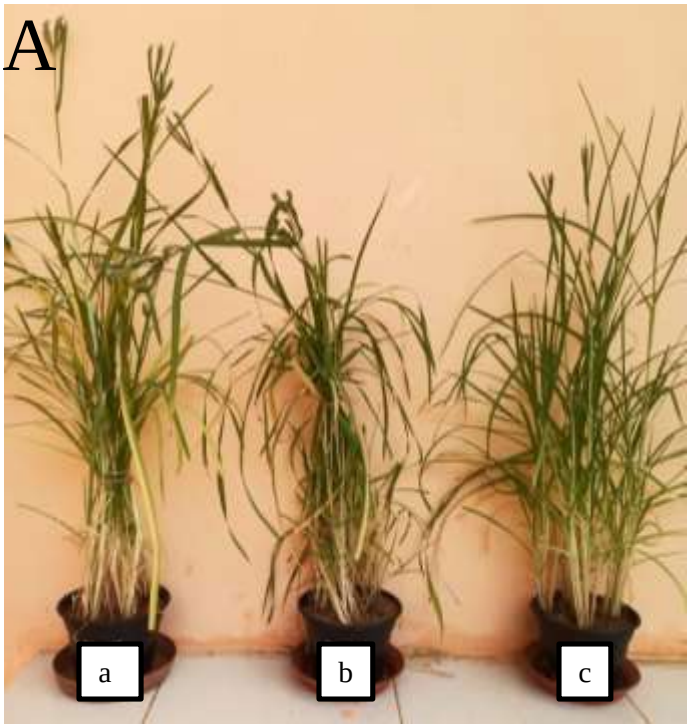


Plate 5: AM Fungal spores in *Eleucine coracana* from GUAMCC pure culture : *Glomus formosanum*; B: *Acaulospora laevis*; C: *Acaulospora scrobiculata*



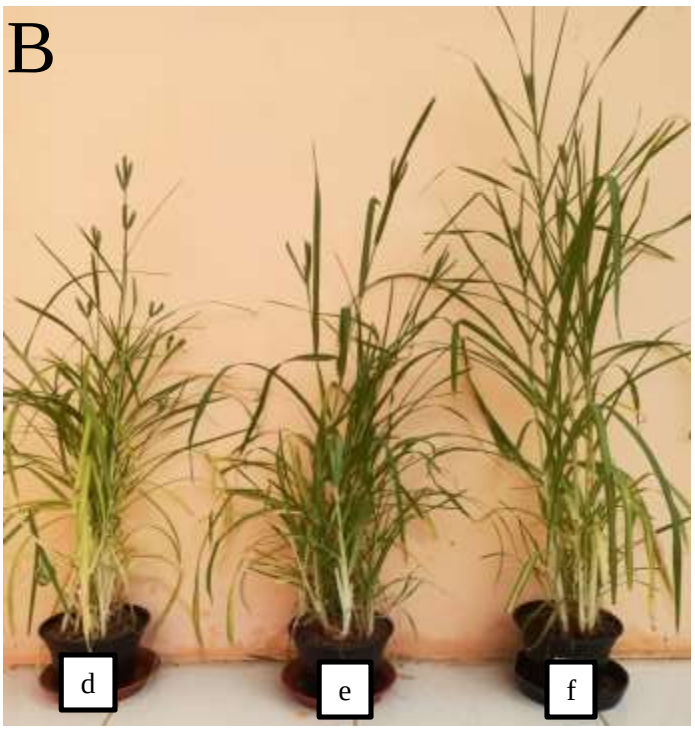


Plate 6: Carrier based culture maintained in Polyhouse **A (a:** *Rhizoglomus clarus;* **b:** *Glomus formosanum;* **c:** *Claroidioglomus claroideum***) & B (d:** *Rhizoglomus intraradices***; e:** *Acaulospora laevis;* **f:** *Acaulospora scrobiculata***).**

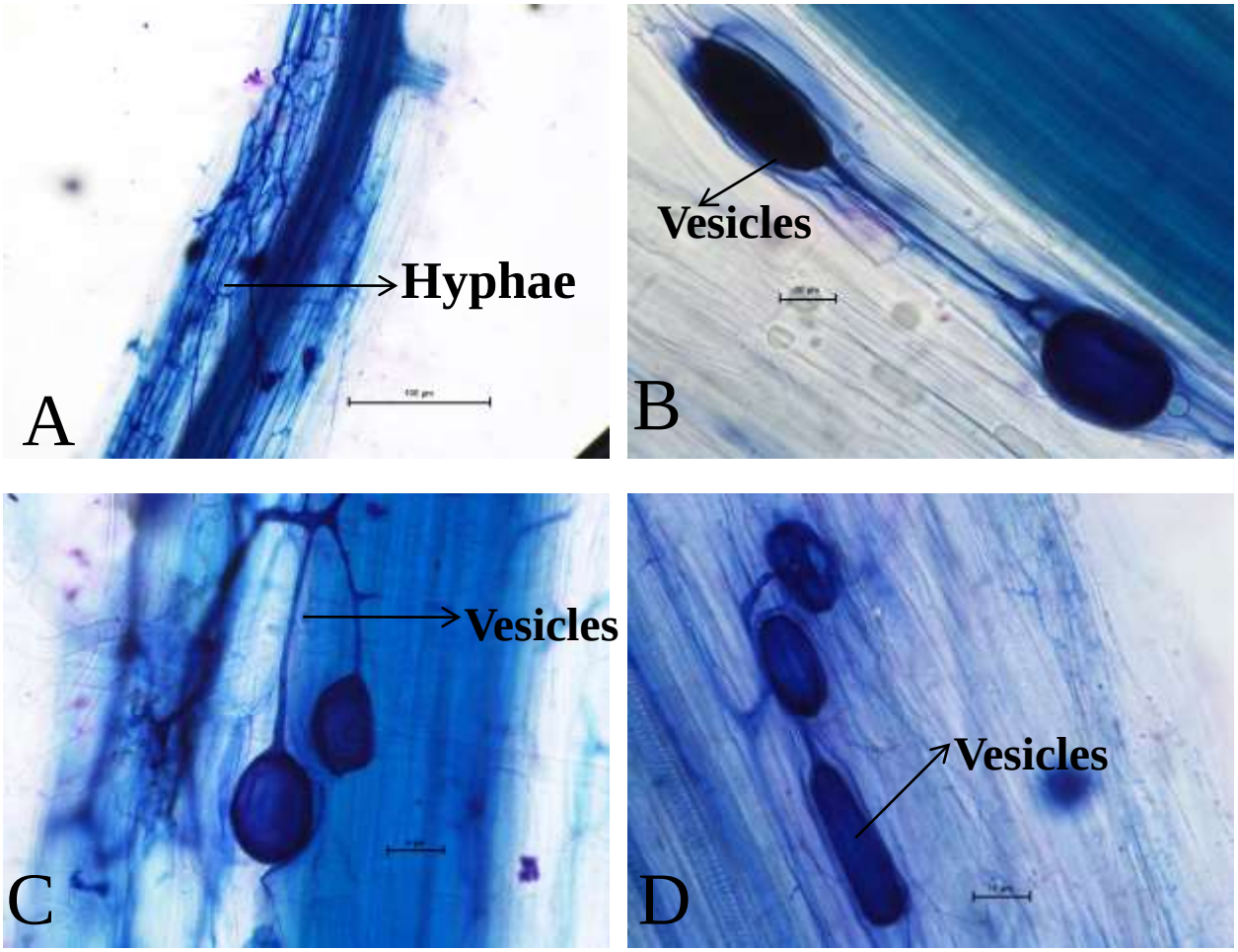


Plate 7: AM Fungal root colonization in carrier based culture of *Acaulospora laevis* . A: Hyphae; B-C: Vesicles

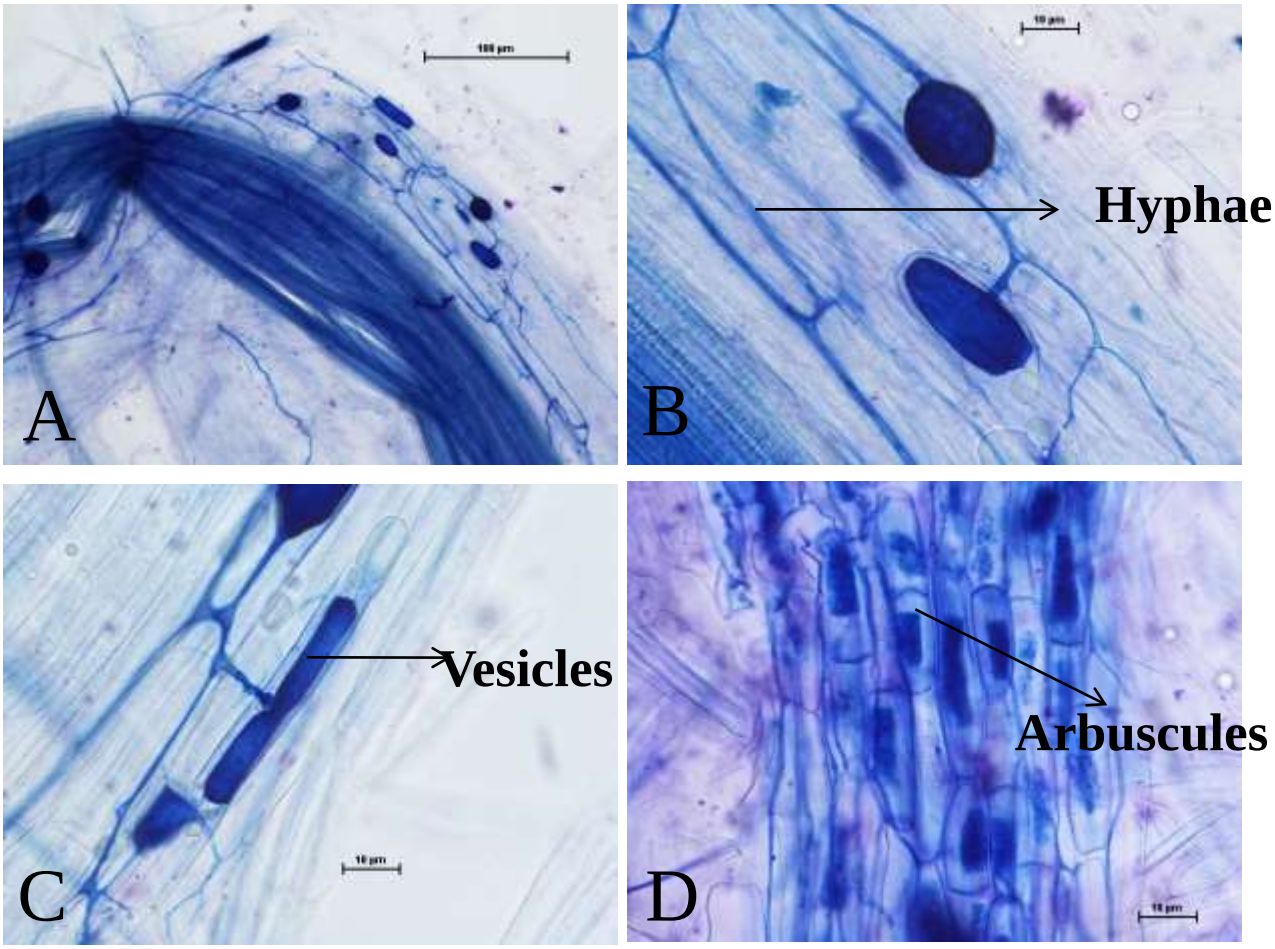


Plate 8: AM Fungal root colonization of carrier based culture of *Acaulospora scrobiculata*. A: Fungal colonization; B: Hyphae; C: Vesicles; D: Arbuscules.

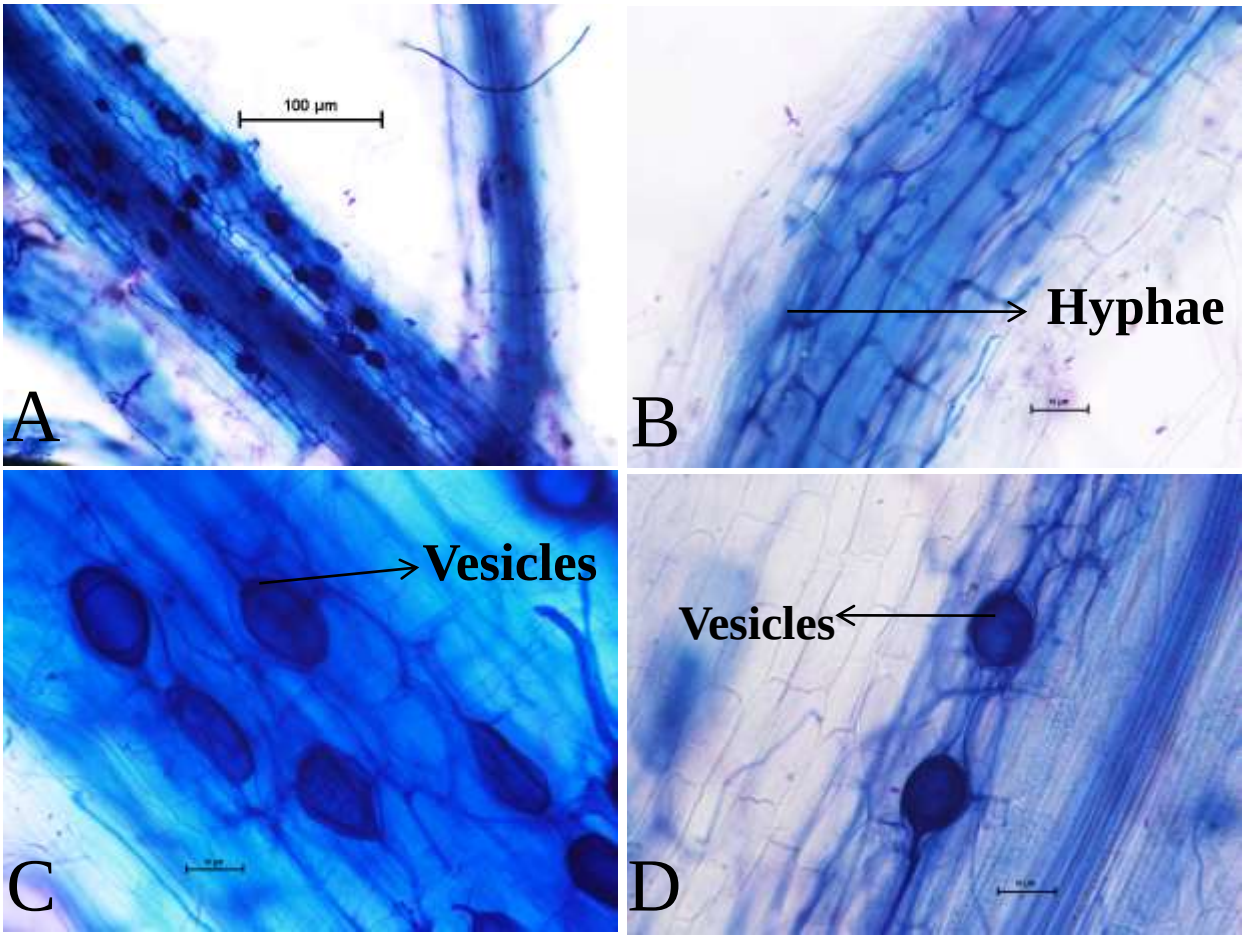


Plate 9: AM Fungal root colonization of carrier based culture of *Glomus formosanum*. A: Fungal colonization at 10x; B: Hyphae; C-D: Vesicles.

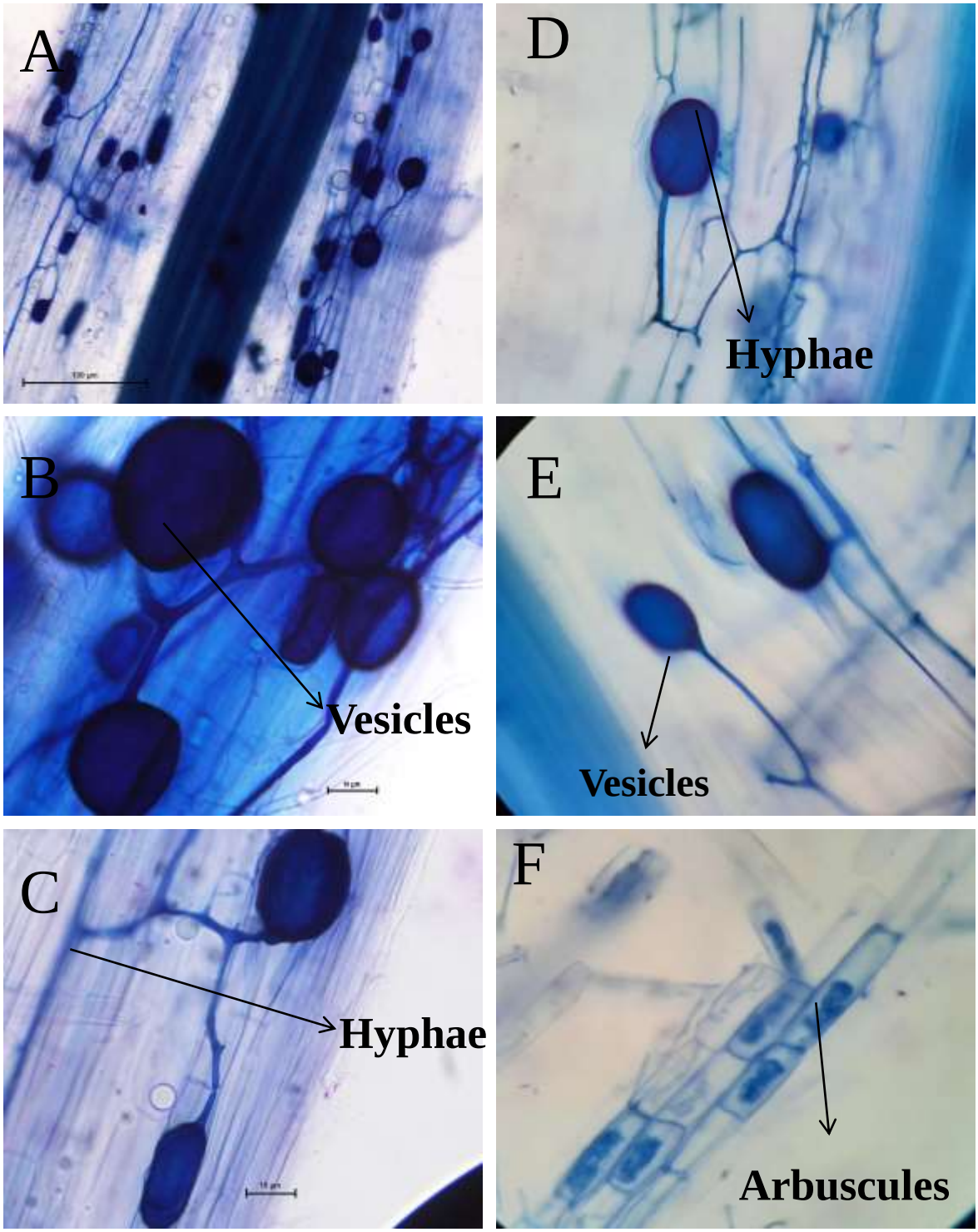


Plate 10: AM Fungal root colonization of carrier based culture of *Rhizoglomus intraradices* (A: Fungal colonization at 10x; B:Vesicles; C: Hyphae) and *Claroidioglomus claroidium* (A: Hyphae; B: Vesicles; C: Arbuscules

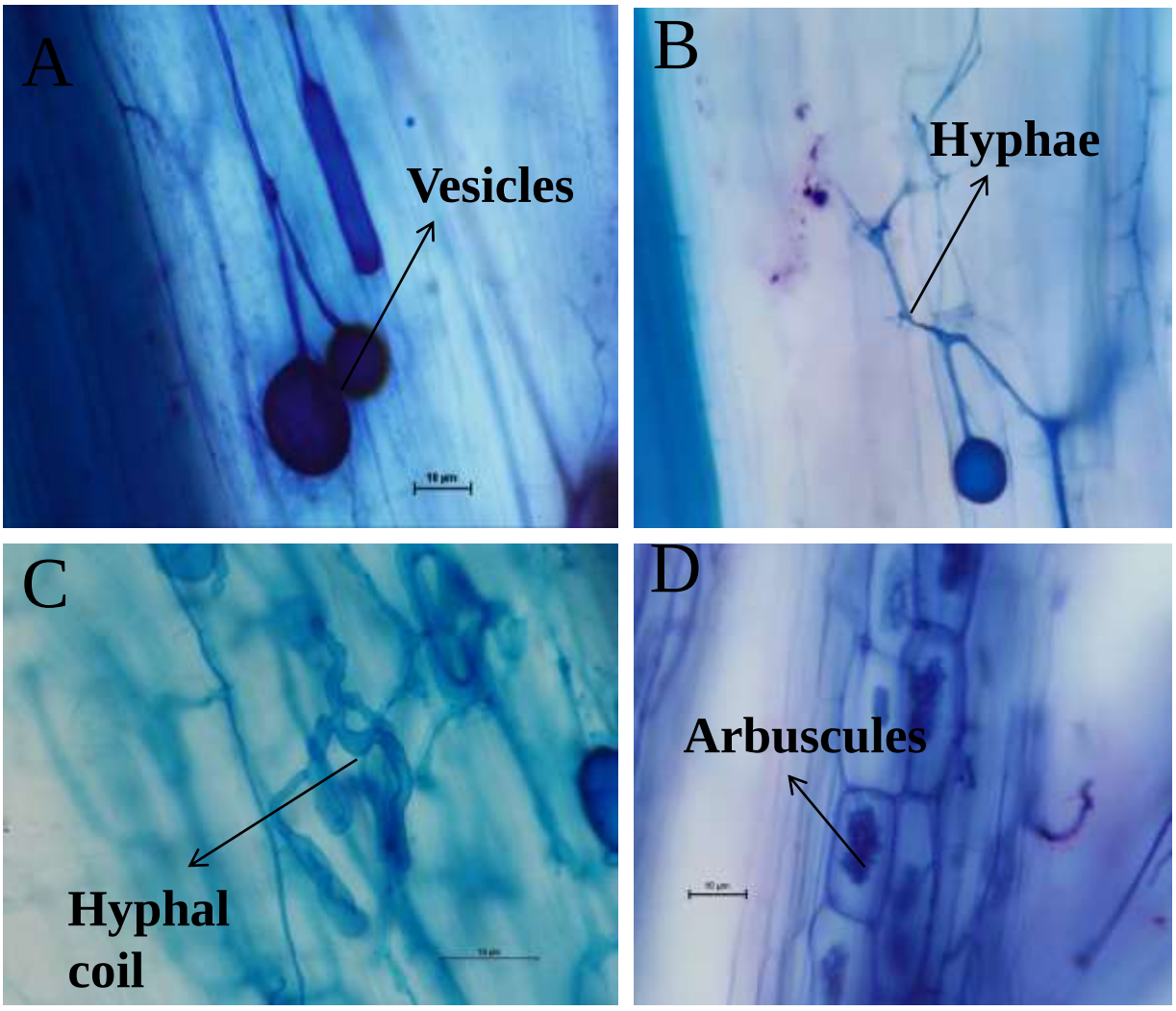


Plate 11: AM Fungal root colonization of carrier based culture of *Rhizoglomus clarus*. A: Vesicles; B: Hyphae; C: Hyphal coils; D: Arbuscules.

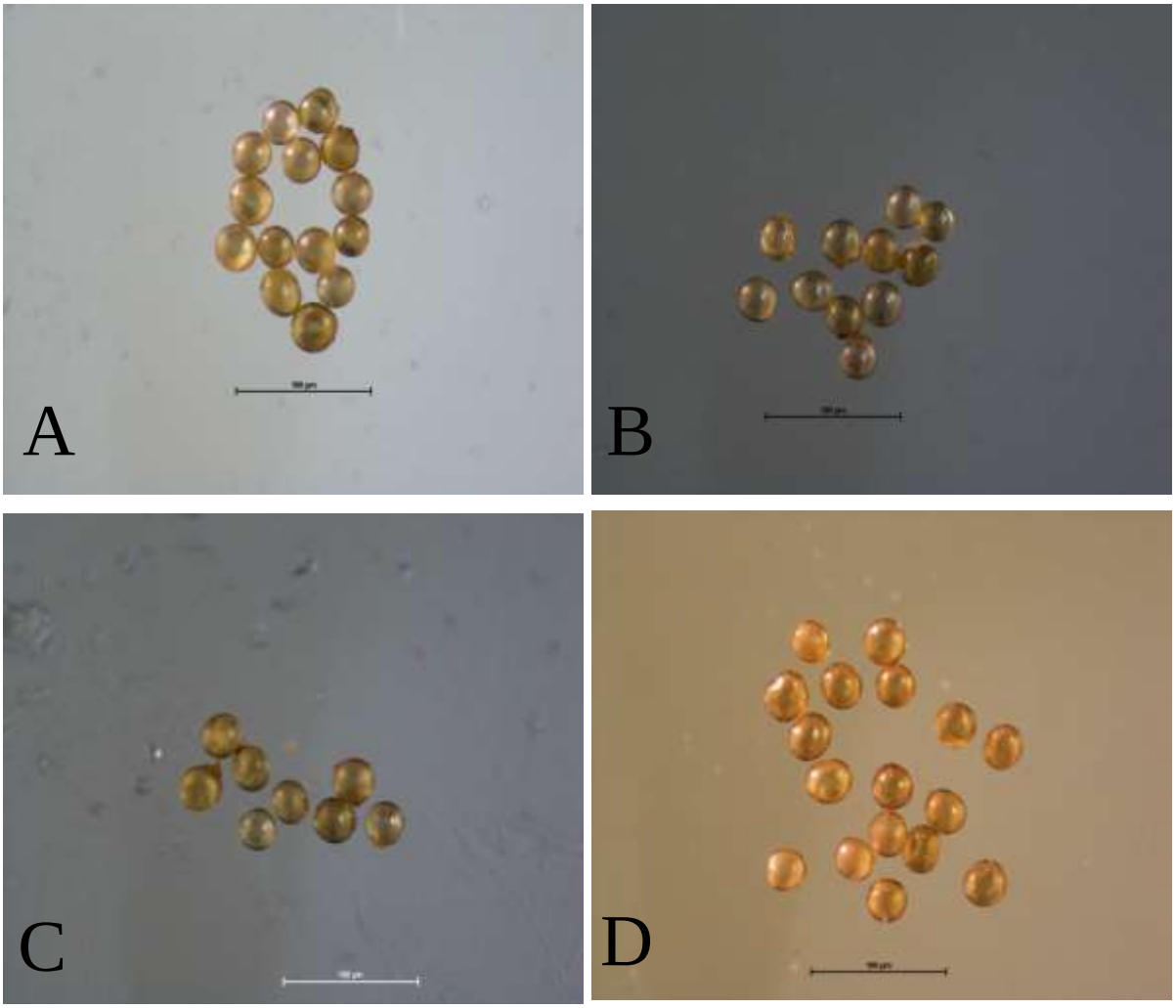


Plate 12: isolated AM fungal spores of carrier based cultureAM.
A: *Acaulospora laevis;* b: *Acaulospora scrobiculata;* c: *Rhizoglomus clarus;* d: *Caroidioglomus claroidium*

Plate 13: Showing Study sites (Goa University campus)

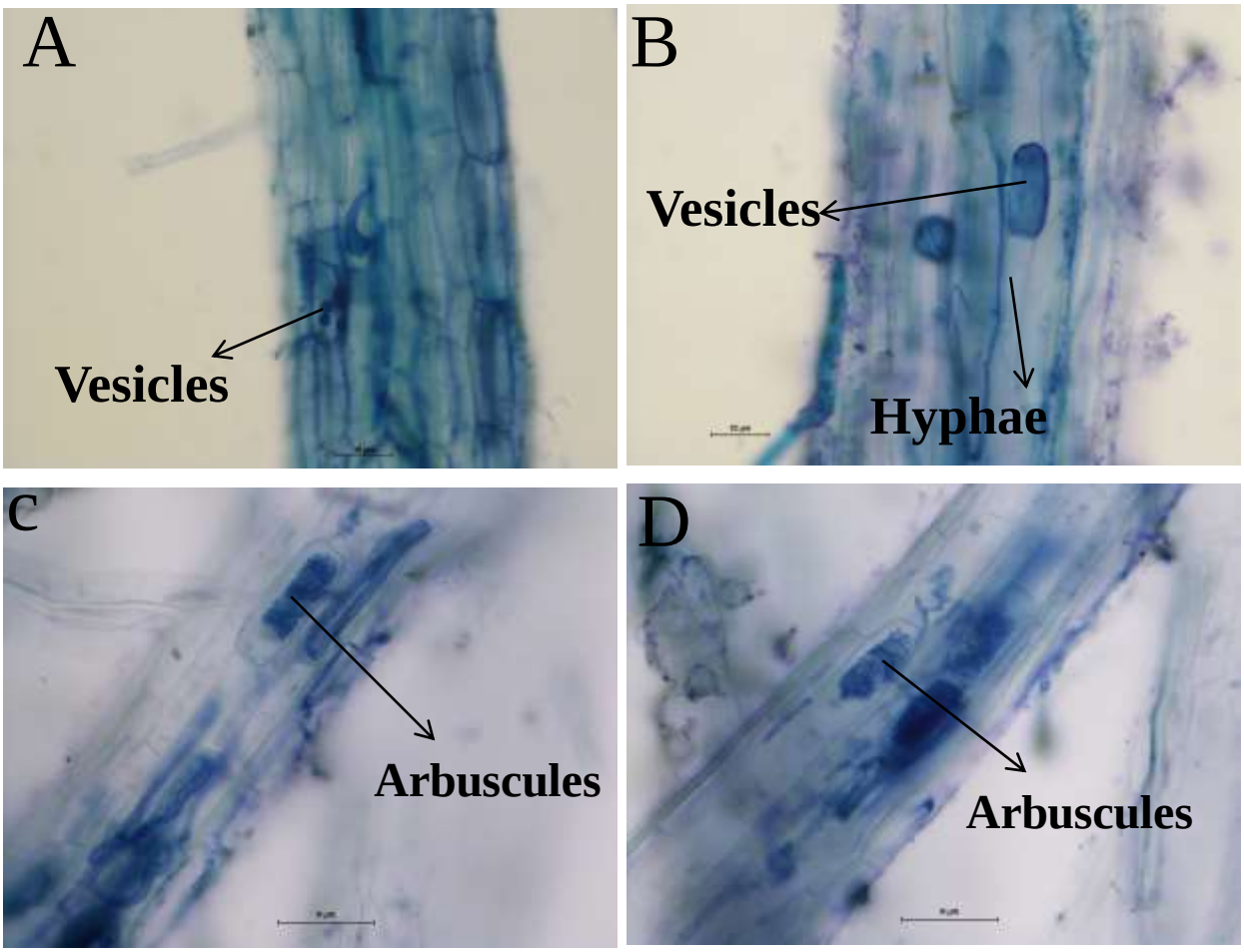


Plate 14: AM fungal root colonization of *Utricularia reticulata*. A: Vesicles; B: Hyphae and vesicles; C-D: Arbuscules.

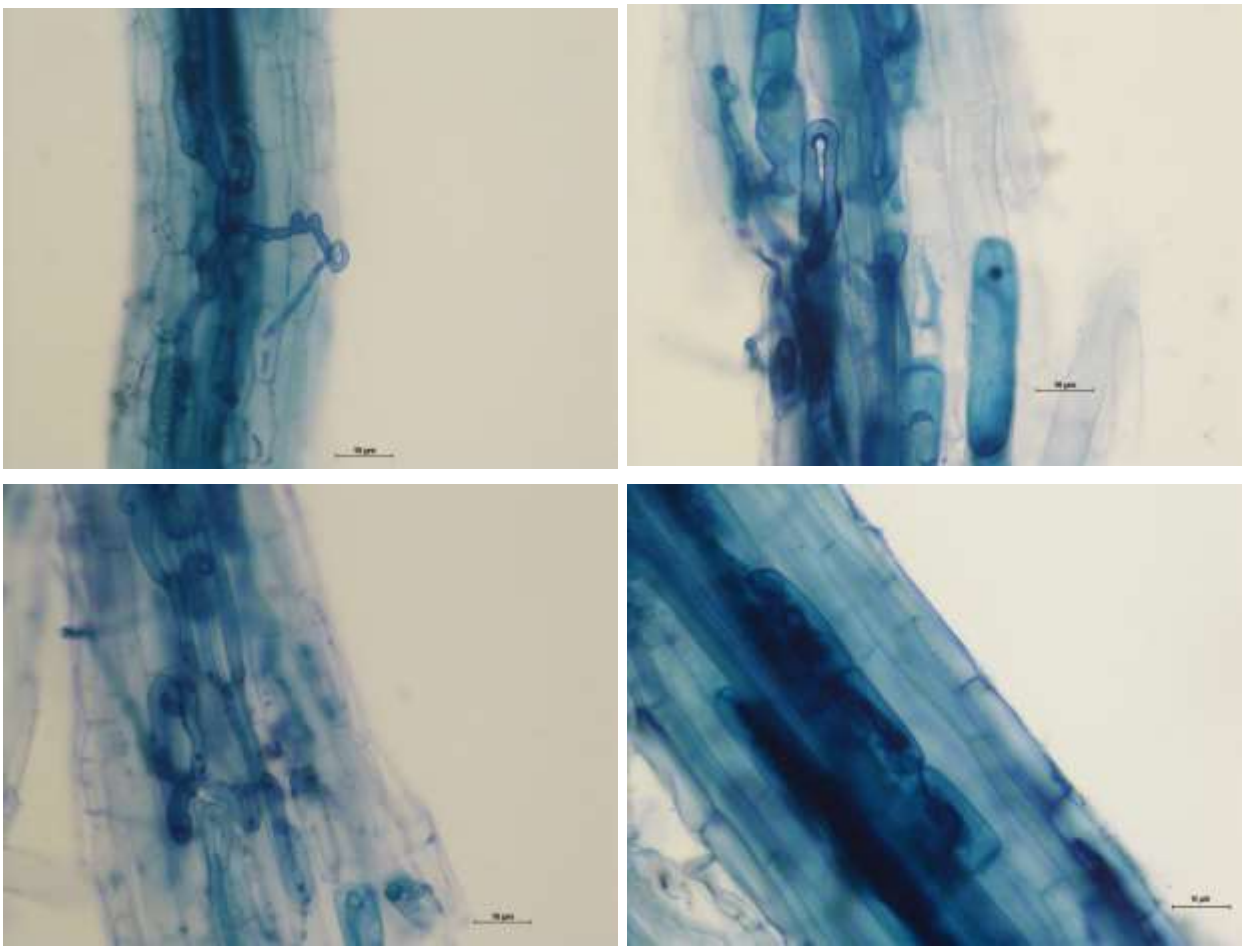


Plate 15- Showing types of hyphal coils in *Utricularia reticulata*

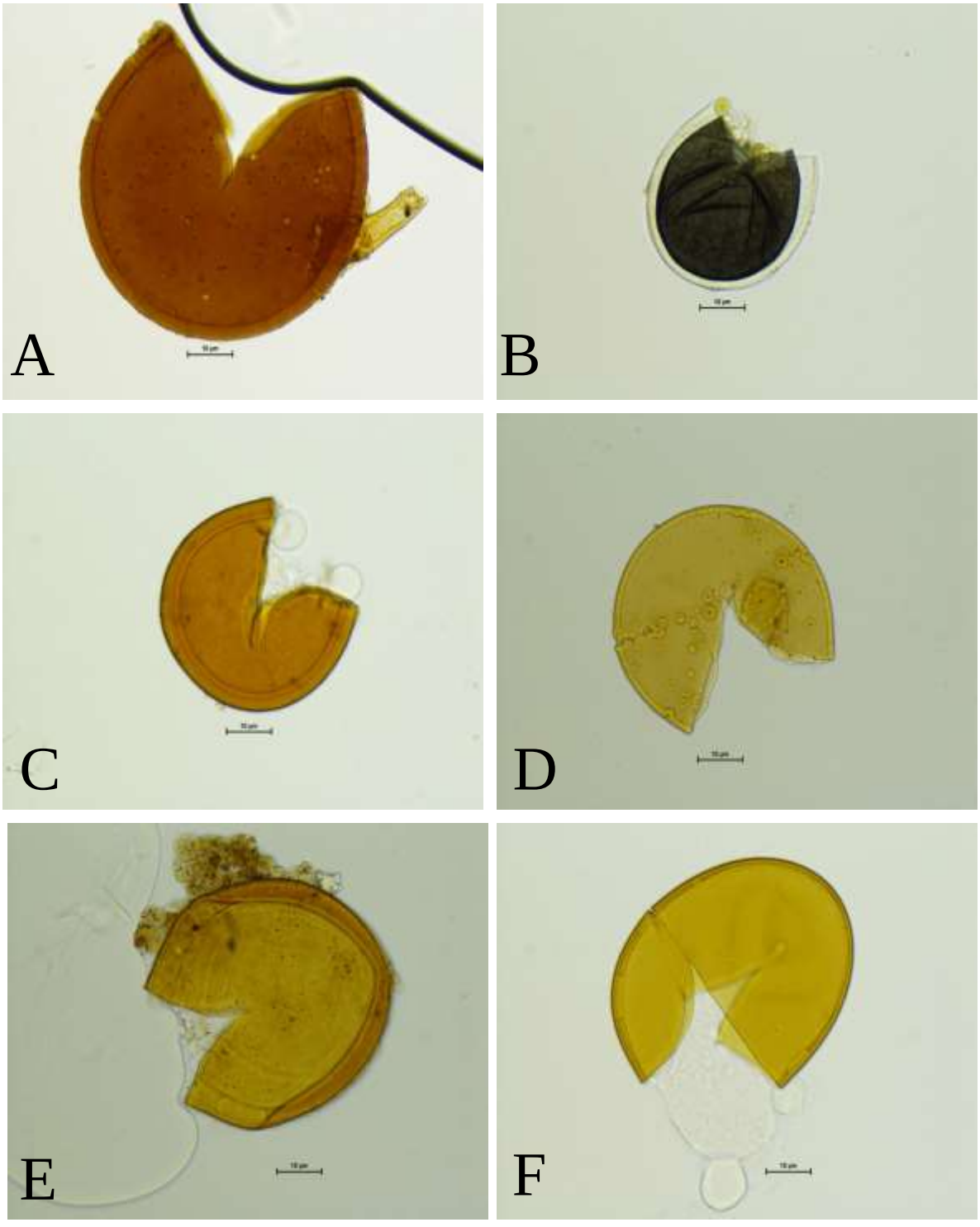


Plate 16: A: Glomus; B-F: Acaulospora;

Plate 17: Trap culture maintained in the polyhouse.

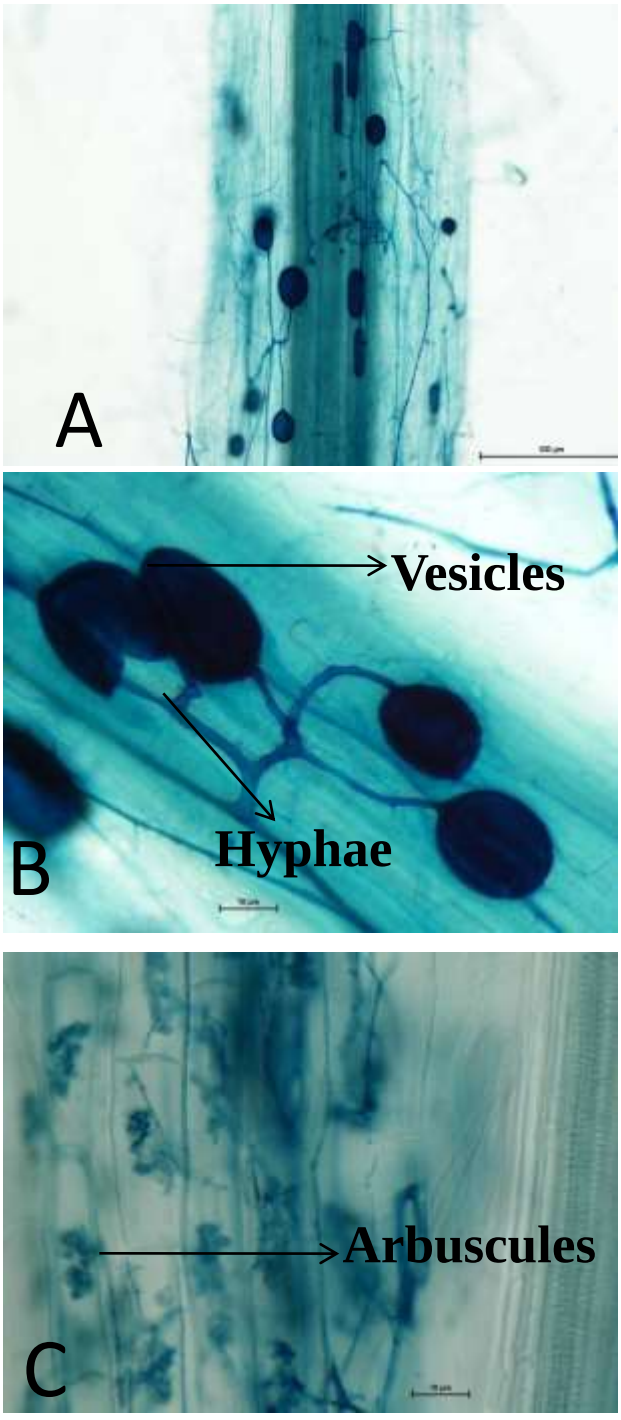


Plate 18: AM fungal colonization of trap culture in *Utricularia reticulata*. A: Colonized root; B: Vesicles and hyphae; C: Arbuscules

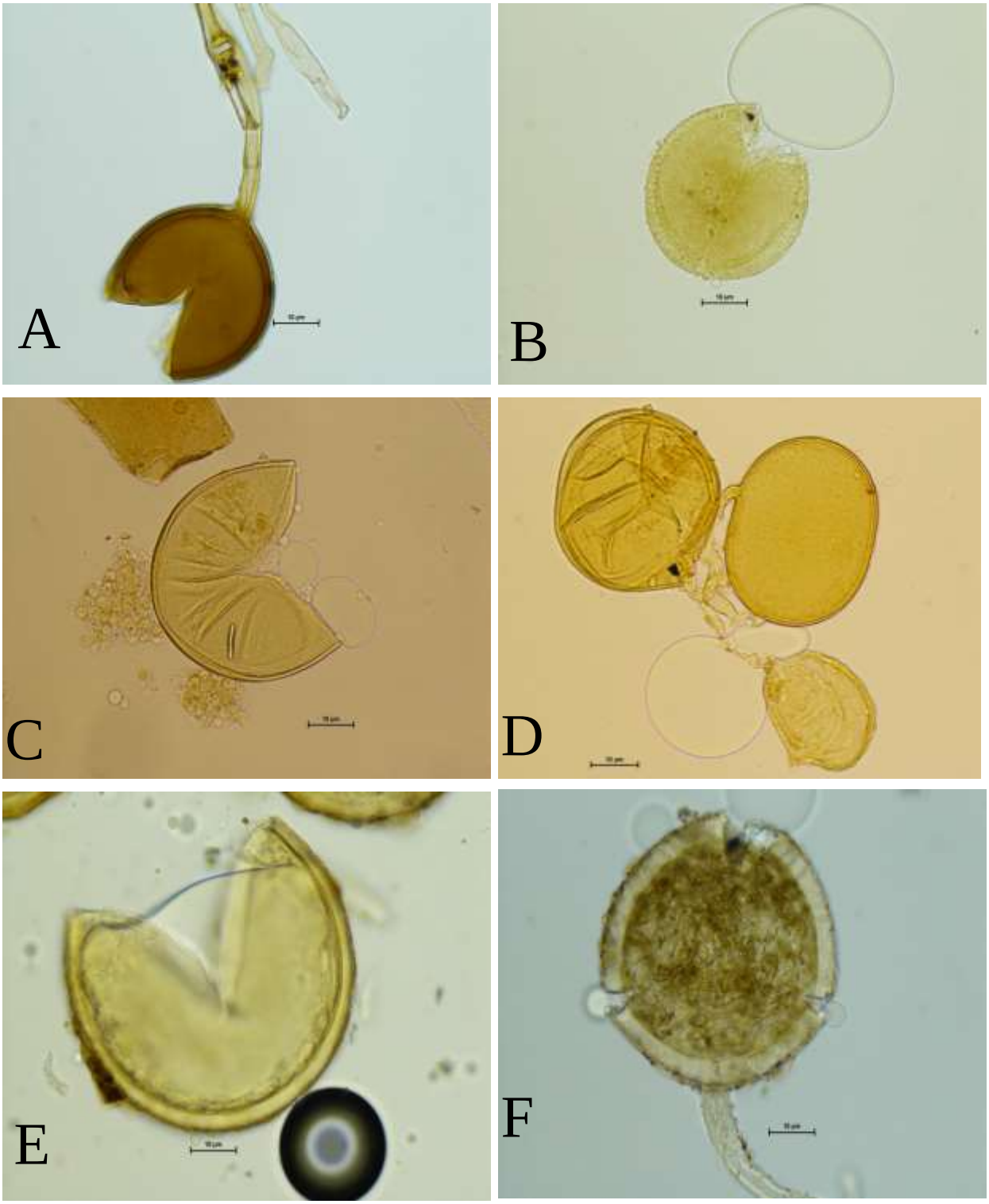


Plate 19: Isolated spores of trap cultures.
A: *Glomus* sp. 1; B *Acaulospora* sp. 1 ; C: *Acaulospora* sp. 2; D: Spores of *Glomus* sp. 2; E: *Acaulospora* sp. 3; F: *Acaulospora* sp. 4

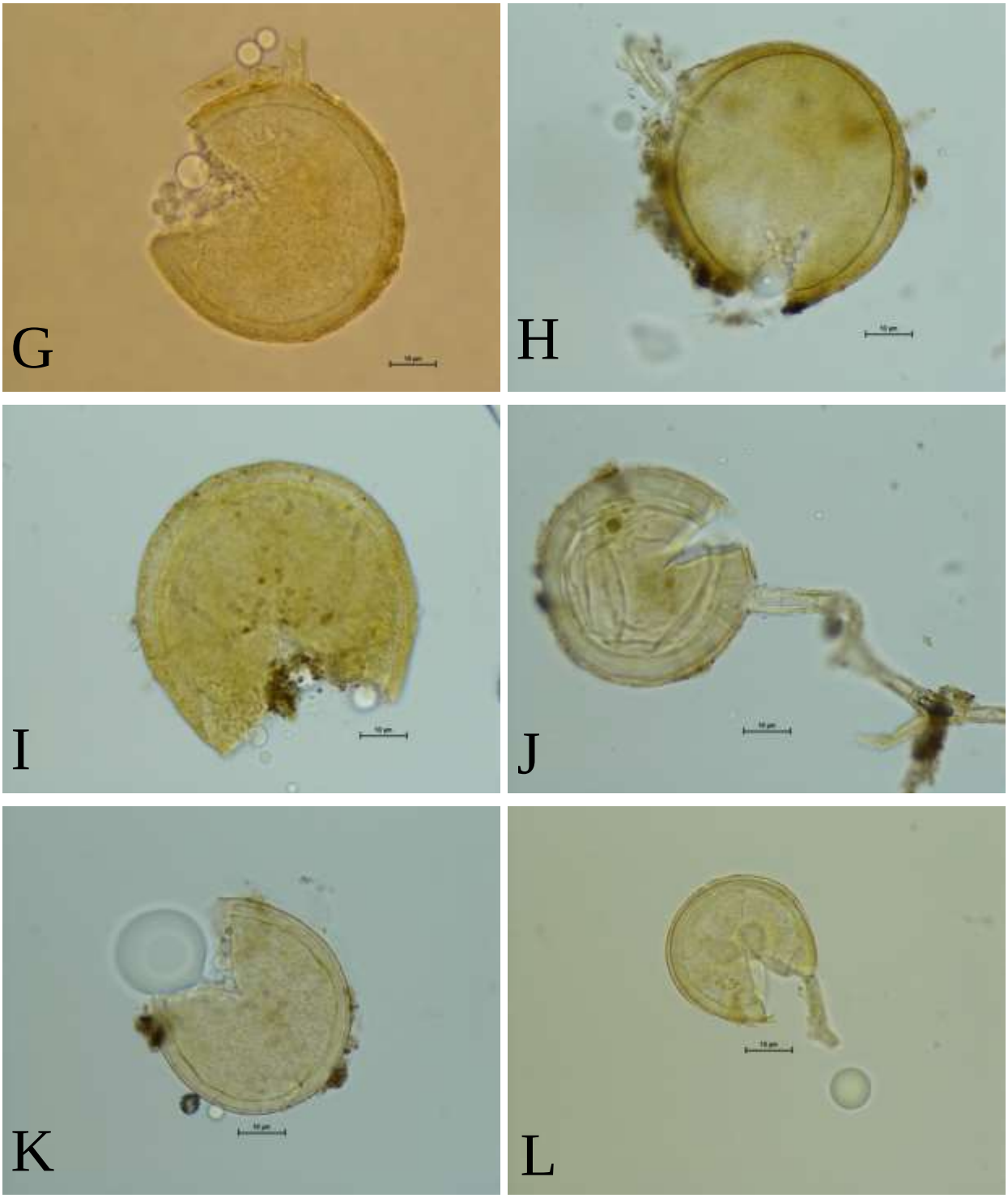


Plate 20: Isolated spores of trap cultrure.
G: *Glomus* sp. 3 ; H: *Glomus* sp. 4; I: *Acaulospora* sp. 5; J:*Glomus* sp. 5 ; K: *Acaulospora* sp. 6; L: *Glomus* sp.6.

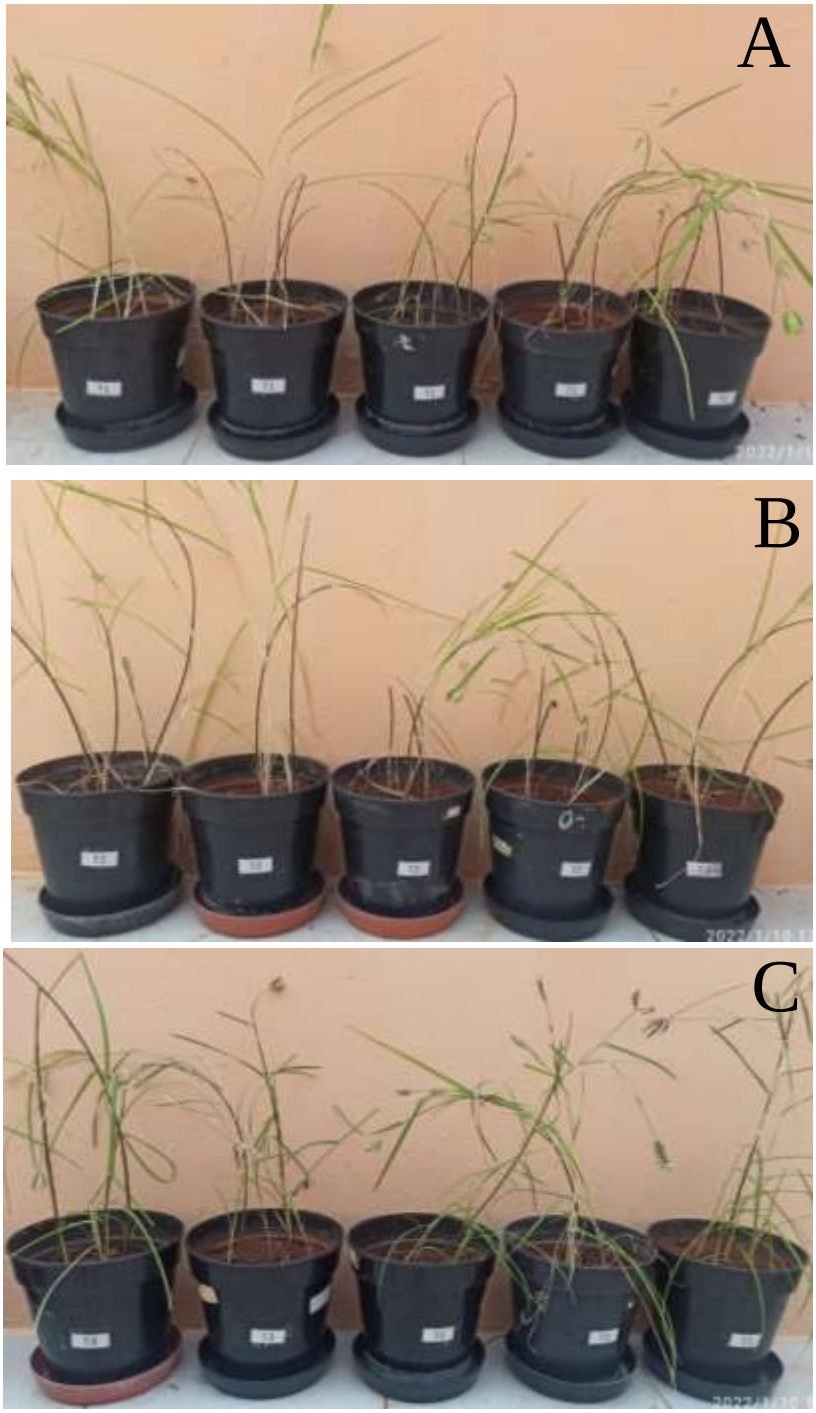


Plate 21: Effect of AM fungi and PSB on growth of *E. coracana*.
A: Control (T_1); B: *Rhizoglomus intraradices* (T_2); C: *R. intraradices* + *Bacillus megaterium* (T_3).

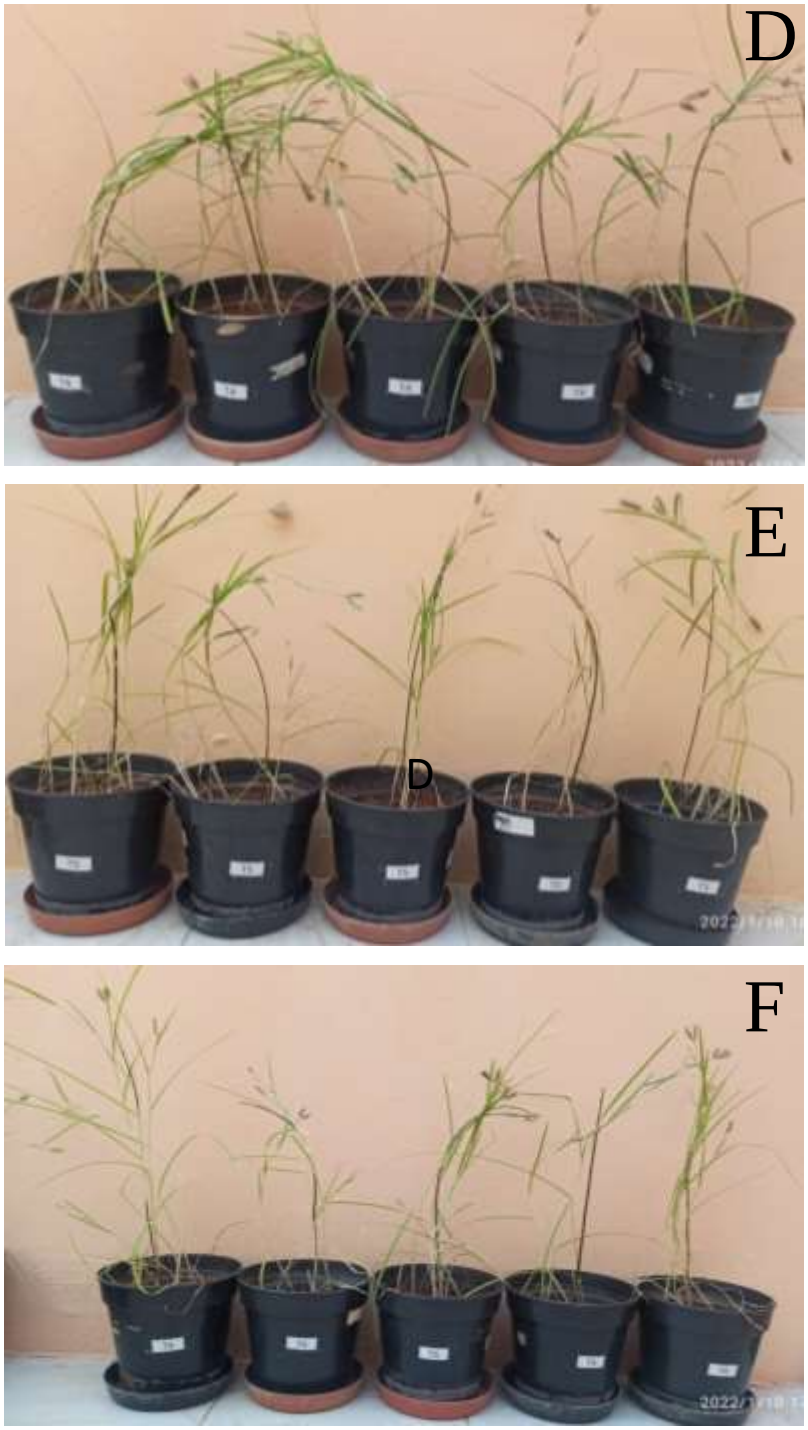


Plate 22: Effect of AM fungi and PSB on growth of *E. coracana.*
D: PSB (T_4); E: PSB + RP (T_5); F: RP (T_6).

Plate 23: Effect of AM fungi and PSB on growth of *E. coracana*.
G*: R. intraradices* +RP (T_7); H: *R.intraradices* + RP +PSB (T_8).