# Preparation of Arbuscular Mycorrhizal (AM) fungal inoculum for *Cocos nucifera*

A Dissertation
Course code and Title: Boo-Diss: Dissertation
Credits: 08
Submitted in partial fulfillment of Master's Degree
in botany

by

**MR. SAMEER KUSHALI GAONKAR**

**21P048009**

Under the Supervision of

**PROF. B. F. RODRIGUES**

School of Biological Sciences and Biotechnology
Botany Discipline

**GOA UNIVERSITY**

**APRIL 2023**

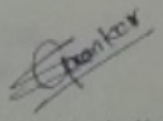
Examined by:

Seal of the School

## DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation entitled, **"Preparation of Arbuscular mycorrhizal (AM) fungal inoculum for *Cocos nucifera*"** is based on the results of investigations carried out by me in the Botany Discipline 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 in the dissertation.

I hereby authorize the University authorities to upload this dissertation on the dissertation repository or anywhere else as the UGC regulations demand and make it available to any one as needed.



Sameer Kushali Gaonkar
21P048009
Botany Discipline
School of Biological Sciences and Biotechnology

Date: 18/04/2023
Place: Goa University

## COMPLETION CERTIFICATE

This is to certify that the dissertation **"Preparation of Arbuscular mycorrhizal (AM) fungal inoculum for *Cocos nucifera*"** is a bonafide work carried out by **Mr. Sameer Kushali Gaonkar** under my supervision in partial fulfillment of the requirements for the award of the degree of Master of Science in the Discipline of Botany at the School of Biological Sciences and Biotechnology, Goa University.

Prof. B. F. RODRIGUES
Botany Discipline

GOA UNIVERSITY
SBSB
TALEIGAO
GOA
BOTANY DISCIPLINE
School Stamp

Date:

Prof. Savita S. Kerkar
Dean of the School
School of Biological Sciences and Biotechnology
Date: 20/4/23
Place: Goa University

Dean of School of Biological Sciences
& Biotechnology
Goa University, Goa-403206
Office Ph. 8605685246

## ACKNOWLEDGEMENT

First and foremost, I would like to express my sincerest gratitude to my guide Dr. B. F. Rodrigues for his constant support and valuable guidance throughout my dissertation.

I would like to thank Prof. S. Krishnan, Vice Dean (Research) and Programme Director, Rupali Bhandari, School of Biological Sciences and Biotechnology (Botany) for providing the necessary facilities required for completing the dissertation work.

I also thank Dr. Dhilan Velip, Ms. Tanvi Prabhu, Mrs. Amisha Shirodker, and Mr. Vinayak Khanolkar for helping me in various ways.

I wish to thank all teaching and non-teaching staff of the Department of Botany, Goa University for their help.

Not forgetting, I would like to thank all my friends for their encouragement and support.

Lastly but not least, I would like to thank my Parents and family members for their constant support, love, and encouragement.

Above all, I thank God for giving me the strength to overcome all the difficulties that aroused during the course of this study.

**Mr. Sameer Kushali Gaonkar**

## CONTENTS

## LIST OF TABLES

## LIST OF PLATES

# INTRODUCTION

Mycorrhizae are a type of symbiotic relationship that occurs between fungi and plant roots. Mycorrhizas are of two types namely, Ectomycorrhiza and Endomycorrhiza. Ectomycorrhiza refers to a symbiotic relationship between fungi and plants where the fungal hyphae do not penetrate the cortical cells of the plant root while in endomycorrhiza the fungal hyphae penetrate the cortical cells of the plant roots and form the specific fungal structures referred to as vesicles and arbuscles. Vesicles are storage organs that are of different shapes ranging from bean shape to globular whereas arbuscules are branched structures that are haustoria-like and developed within the cortical cells, which help in the transfer of nutrients to the host plants. *Arum* and *Paris* are the two types of arbuscules that are identified based on the presence or absence of intracellular hyphae within the intracellular spaces of the root cortex respectively. In natural conditions, the *Paris* type is more dominant and in the artificial ecosystem, the *Arum* type is more prominent.

Arbuscular mycorrhizal (AM) fungi are intimate associations between 92% of the plant families (Wang and Qui, 2006) and fungi of the phylum Glomeromycota (Schüssler, 2001). AM fungi are fungi forming obligatory symbiotic associations with the root of the majority of plants (Chaturvedi *et al.*, 2012). This symbiotic relationship was formed approximately 460 million years ago and would have had important roles in the establishment of plants on land (Redecker *et al.*, 2000). In this relationship, the fungus provides the plant with nutrients, such as phosphorous (P), in exchange for carbohydrates produced by the plant through photosynthesis.

The diversity of AM fungal species is measured mainly by isolating, counting, and identifying the asexual spores, and the fungal propagule although molecular techniques have been revealed as a useful tool for the characterization and identification of AM fungi. The diversity of AM species is more distinct in undisturbed ecosystems compared to the disturbed ecosystem as in undisturbed

ecosystems there is a great degree of variability in various factors such as habitat type, edaphic conditions, climate change or seasonal variations, host genotype, and vegetation cover. AM symbiosis and richness enhance plant diversity and productivity (van der Heijden *et al.*, 1998; Moora *et al.*, 2004). An increase in AM fungal diversity results in an increase in species richness and hence higher plant productivity. This shows that changes in below-ground AM fungal diversity can affect changes in above-ground plant diversity and productivity (Finlay, 2008).

The AM fungal mycelium that emerges from the root system can acquire nutrients from the soil that are inaccessible to roots (Smith *et al.*, 2000). Furthermore, fungal hyphae are much thinner than roots and are therefore able to penetrate smaller pores (Allen, 2011). As AM fungi are ubiquitous and their taxonomic, genetic, and functional diversity are directly related to plant and soil processes (Bever, 1996; Oehl, 2003), there is an increasing interest in the assessment of the biodiversity and functions of AM fungal communities (Lovelock, 2005). AM fungi significantly increase the absorption of essential nutrients such as K, Mg, and Ca, reduce drought stress's damaging effects through antioxidant defense systems, and osmolyte synthesis, and maintain phytohormones levels in plants (Al-arjani *et al.*, 2020).

When fertilised with a solution of ammonium sulphate, potassium chloride, and 1 gramme of borex is used, coconut seedlings grow and mature more quickly (Santos, 1987. Soil is a mixture of organic and inorganic materials. Living beings and their remains make up the organic portion, while rocks and minerals make up the inorganic portion. Tenkoon & Bandara (2003) observed that organic materials (Cattle manure, goat manure, Broiler & Layer poultry manure, Pig manure, farm yard manure, biogas residue, sewage sludge, compost, Gliricidia, Pueraria, Calopogonium and Acacia) have considerable amounts of macro and micronutrients and these materials could be used as a source of plant nutrients for coconut to supply the N requirement in full and P, K and Mg

requirements in part. Organic fertilization is indeed a versatile component in a coconut-based farming system. It does not only influence the soil and coconut but also the farmer as an important element in the system. However, at present there is still a dearth of research-based information as far as organic fertilization on coconut is concerned.

With an increase in population, humans today demand the production of high-quality food most sustainably without causing much damage to the environment. Biofertilizers may help to achieve this as they are the organic substances that make use of microorganisms to increase the fertility of the soil. They are not harmful to the plants like chemical fertilizers. They have great potential as supplementary, renewable, and environmental sources of plant nutrients and are important components of any integrated plant nutrient system. The AM biofertilizer involves a fungal system whereas other biofertilizers exploit bacteria most commonly. The role of AM fungi as biofertilizers in the growth and multiplication of crop plants can improve growth and biomass production. Its applications have additional benefits like improved vigour and nutrient uptake, disease resistance, and drought tolerance. The AM biofertilizer technology can be called poor man's technology. Considering the number of nutrients supplied, biofertilizers are many times cheaper than chemical fertilizers. Biofertilizers improve the quality of produce. Uses of biofertilizers maximize ecological benefits and minimize environmental hazards. Future upgradations in the mode of the AM biofertilizer technology development, redefining the rate-limiting factors and exploring possible AM combinations along with other potential biofertilizers together as a single package for end users might bring a major boon to the agriculture sector using nature's biofertilizer.

There is an increase in the use of AM fungi in agriculture, forestry, and environmental reclamation, to improve crop yield and soil health (Johansson *et al.*, 2004). The large-scale production of this fungi is limited because of its obligate biotrophic nature. However, the conventional method like

trap culturing can be a useful tool to propagate these fungi for plant growth and land reclamation purposes in a small-scale cost-efficient way (Guar and Adholeya, 2002).

Spores collected from the field, however, are found in some circumstances in low numbers, parasitized, and lacking informative taxonomic characteristics diminishing a more accurate identification as components of spore walls are susceptible to alteration and deterioration by a wide sort of agents in the soil. The establishment of trap cultures using bulk soil or by mixing rhizosphere soil and root pieces with sterilized diluents and growing with suitable hosts represents a strategy to yield a large number of healthy spores which can be easily identifiable and supplement the assessment of local species diversity in different ecosystems. This methodology not necessarily allows the identification of all species, because sporulation of the fungal community may be affected by the plant host chosen for trapping, whereas in some cases it can promote the sporulation of cryptic AM fungal species that were not sporulating at the sampling time or field conditions. Despite that, trap cultures have been widely used to access AM fungal diversity and isolate indigenous fungi.

Using different host plants, AM fungal culture has been obtained through different methods of propagation technique. The most widely used method is substrate-based. However, the spores produced through this method are contaminated with other microbes (Douds *et al.*, 2006). The *in vitro* culture method provides pure cultures of AM fungal propagules. However, the production of AM fungi for large-scale applications is still in its beginnings. Guar and Adholeya (2002) reported that depending on the host plant used the efficiency of mycorrhizal propagation varies. In addition, there is no proper procedure for long-term storage of this propagated AM fungi. Trejo-Aguilar *et al.* (2013) reported that when a single host was used for long-term subculturing it reduced the diversity of AM fungal spores in trap culture. During the continuous propagation cycle, dilution

of this trap culture inoculum may favour the diverse AM fungal species more than the field (Wang *et al.*, 2008). A trap culture contains spores, hyphae, and colonized root bits as an inoculum and can promote diverse AM fungal species.

The coconut tree (*Cocos nucifera*) is a member of the family Arecaceae. The coconut palm is referred to as 'Kalpavriksha' – the 'tree of heaven' as each and every part of the palm is useful. The coconut tree provides food, fuel, cosmetics, folk medicine, and building materials, among many other uses. In India, this palm is cultivated in roughly 2 million ha area and it supports the livelihood of many million Indians (Rajkumar *et al.*, 2015). India ranks third in area and first in the production of coconut in the world. As per the latest statistics available (2016-17), the annual coconut production in India is 23.90 billion nuts from an area of 2.08 million ha with an average productivity of 11481 nuts/ha (Subramanian *et al.*, 2018). The largest coconut-producing states in India are the four southern states *viz.*, Kerala, Tamil Nadu, Karnataka, and Andhra Pradesh, which account for more than 90% of the land and production. An important challenge for Indian agriculture is improving the productivity of these palms. The coconut palm has a root system that is devoid of root hairs; it is possible that the coconut palm would not be able to adequately take up enough nutrients, particularly phosphorus, and water without mycorrhizal assistance (Rajeshkumar *et al.*, 2015). The occurrence of a mixed population of AM has been commonly recorded from the coconut rhizosphere soils.

The present work was carried out with the following objectives:

1. To study AM fungal diversity in the rhizosphere of *C. nucifera*.
2. To prepare trap cultures for identifying viable AM fungal species.
3. To prepare viable and effective AM fungal inoculum for *C. nucifera* by using dominant AM fungal species.

# REVIEW OF LITERATURE

Velazquez *et al.*, (2011) conducted two years study to assess and compare the diversity of arbuscular mycorrhizal fungi from 5 types of vegetation. Three different plant species were used for trap culture, and a total of 34 morphospecies were identified, which showed that *glomeraceae* were most abundant in the last year.

Pedersen *et al.*, (2015) studied the diversity of AM fungi species in soils of different strawberry crop systems using the trap culture technique. They found that Arbuscular mycorrhizal fungi diversity in the rhizosphere of strawberry crops shows differences among the sites. Thirteen *Glomeromycota* species were identified. The two most species in soils under strawberry crops are *Claroideoglomus etunicatum* and *Funneliformis mosseae*.

Chaturvedi *et al.*, (2012) studied the diversity of AMF in four types of ecosystems. They concluded that the abundance of AMF spores and diversity is affected not only by the forest type but seasons and hosts might be the factors.

Ilangamudali *et al.*, (2016) studied the effects of mycorrhizal-based biofertilizer on the root and shoot development of coconut seedlings under nursery conditions. They recorded that the use of AMF-based biofertilizer is beneficial to produce quality seedlings with well-developed roots.

Trejo-Aguilar *et al.*, (2013) studied the arbuscular fungal diversity in trap cultures during long-term subculturing. they found that AMF diversity was lost during the long-term subculturing of a single plant host species.

Chaiyasen *et al.*, (2017) studied the diversity of AMF in *Tectona grandis* plantations and their effects on the growth of micro-propagated plantlets. This study provides the use of AM symbiosis in the production of important tree species in greenhouses and reforestation.

Bhadalung *et al.*, (2005) studied the diversity of AMF in 27-year long-term NP-fertilization plots under a maize cropping system. They found that long-term fertilization causes a decrease in AMF total spore numbers and variation in species diversity.

Rajeshkumar *et al.*, (2015) investigated the degree of AM association and its diversity in the coconut palm cultivated in crop-mixed systems under rain-fed conditions. A total of forty AM species belonging to ten genera was recorded.

Ambili *et al.*, (2012) conducted a study to assess diversity in the arbuscular mycorrhizal fungi associated with coconut and areca nut intercropping systems. They found that AM fungal colonization pattern is inversely related to soil pH and phosphorus. AMF colonization and species richness are more in the coconut cropping system compared to the arecanut cropping system.

Husein *et al.*, (2022) conducted a study to evaluate the effectiveness of arbuscular mycorrhizal fungi in the absorption of nutrients in trapping culture and its effects on the growth and biomass production of corn and sorghum. They reported that the growth and biomass of trapping plants increased the density of the rhizosphere AMF.

# MATERIALS AND METHODS

**Study site:**

A coconut orchard situated at Balli, Cuncolim at South Goa was selected for the present study. The study was carried out in the month of August 2022. The soil was lateritic.

**Agroclimatic condition:**

The ideal mean temperature for coconut growth and yield is 27±5°C and relative humidity is more than 60 percent. In Balli, cuncolim the summer temperature ranges from 24° C to 36° C, and in winter, between 21°C and 30°C. The average relative humidity is 75.90%. The coconut palm grows well up to an elevation of 600 m above mean sea level.

**Sample collection:**

Rhizosphere soil and root samples were collected from randomly selected coconut plants from the study sites. A composite sample was prepared by mixing samples, which were used for AM fungal diversity studies and for the preparation of trap culture.

**AM root colonization:**

**AM root colonization was estimated using trypan blue staining (Phillips and Hayman, 1970)**

1. The root segments of 1 cm were thoroughly washed with tap water and then placed in the test tube containing 10% KOH and hydrolyzed for 30-45 minutes at 90°C.

2. The hydrolyzed root segments were then washed 3-4 times and acidified in 1N HCl for 3-5 minutes.

3. Later the root segments were stained in 0.5% Trypan blue in lactophenol for 1 h.

4. The stained root segments were then temporarily mounted on the glass slide using polyvinyl alcohol lacto-glycerol (PVLG).

5. Using a compound microscope ((Nikon Eclipse E200) all the slides were examined and scored for AM colonization. A root segment was considered mycorrhizal if it showed the presence of hyphae and vesicles and/or arbuscules.

**Estimation of percent root colonization**

Estimation of percent root colonization was carried out using the following formula:

$$\text{Percent root colonization} = \frac{\text{Number of root segments colonized}}{\text{Total number of root segments observed}} \times 100$$

**Preparation of Trypan blue stain**

Trypan blue -0.05g

Lactic acid -50ml

Glycerin -10ml

Water -40ml

**Preparation:** 0.05g of Trypan blue powder is dissolved in 40 ml of distilled water and then 50 ml of lactic acid and 10 ml of glycerin is added.

**Isolation of AM fungal spores:**

**AM fungal spores were isolated using Wet Sieving and Decanting method (Gerdemann and Nicolson, 1996)**

1. one hundred grams of rhizosphere soil was suspended in 1000ml of tap water. The mixture was stirred for 15 seconds and the coarse particles are allowed to settle for 2 minutes.

2. The supernatant was decanted through a series of sieves arranged in descending order of mesh size 240µm, 150µm, 100µm 60µm.

3. Sievates from each sieve were collected in separate beakers.

4. The supernatant from each beaker was then separately filtered through No. 1 filter paper.

5. The filter papers were placed in separate Petri plates.

6. The spores were isolated under a stereomicroscope (Olympus SZ).

**Mounting of AM Fungal spores**

Spores that were intact and crushed were mounted on a glass slide in polyvinyl lacto-glycerol (PVLG). The spores were crushed by applying light pressure on the coverslip using the blunt end of a needle. The spores were then observed under a compound microscope (Nikon Eclipse E200).

**Preparation of polyvinyl alcohol lacto-glycerol (PVLG)**

**Requirements**

Polyvinyl alcohol- 16.6g

Lactic acid- 100ml

Distilled water- 100ml

Glycerin- 10ml

**Preparation:** 16.6g polyvinyl alcohol was dissolved in 100 ml of distilled water by heating at 90°C. After cooling 100ml lactic acid and 100ml glycerin were added.

**Identification of AM fungal spores:**

Identification of AM fungal spores was done using standard manuals by Rodrigues and Muthukumar (2009) and Blaszkowski J (2012).

**Preparation of Trap culture (Morton et al., 1993)**

1. Rhizosphere soil was collected from the host plant in the field and was mixed with sterilized sand in a 1:1 ratio.

2. This mixture was added to 15cm diameter pots which were thoroughly pre-wiped with cotton dipped in absolute alcohol.

3. *Coleus* cutting washed in sterile water was planted in the pots.

4. Hoagland solution (without P) was added bi-weekly and the plants were watered twice a week.

5. After 45 days of growth, AM fungal root colonization was checked by staining a sample with 0.05% trypan blue.

6. The plants were maintained for a period of 90 days to establish colonization and sporulation.

7. Once good colonization was established, watering was stopped, allowing the plants to dry, after which the shoot portion was cut off at the soil surface. Roots were cut into small pieces and mixed with the soil.

8. This was used for fungal spore isolation and preparation of monospecific culture.

**Preparation of monospecific (pure) culture**

1. Spores were isolated from the trap culture using the wet sieving and decanting method.

2. After repeated washing, the spores of a single morphotype were separated and stored in a Petri plate.

3. Spores were examined before inoculation for any morphological changes, and parasitism is discarded.

4. The pots with sterile 1:1 sand: soil mixture was inoculated with single species of spore and *Coleus* was planted.

5. The pots were watered twice weekly for 45 days, and Hoagland's solution (without P) was added bi-weekly.

6. After 45 days, watering was stopped, allowing the plants to dry, after which the shoot portion was cut off at the soil surface. Roots were cut into small pieces and mixed with the soil.

9. This was used for the isolation and identification of AM for fungal spores and also used for preparing the inoculum.

**Preparation of AM inoculum for C. nucifera:**

Sand: vermiculite in the ratio 2:1 was used as substrate. Spores from 3 pure cultures were used as inoculum and *Eleusine* was used as host plant. After 45 days colonization was checked.

# RESULTS

A total of nine species belonging to five genera viz *Glomus*, *Acaulispora*, *Gigaspora*, *Rhizoglomu*, *Claroideoglomu* were isolated from the field soil sample by using the wet sieving and decanting method. These AM species include *Glomus* sp.1, *Glomus* sp. 2, *Glomus* sp. 3, *Glomus* sp. 4, *Acaulispora scobiculata*, *Acaulispora undulata*, *Gigaspora* sp., *Rhizoglomus intaradices* and *Claroideoglomus claroideum*. AM root colonization of 52.22% while the spore density was found to be 270.11 spores per 100g **(Table 1)**.

**Trap cultures**

The study revealed that *Acaulospora scrobiculata* was the most abundant AM fungal species followed by *Rhizoglomus intraradices* and *Claroideoglomus claroideum*.

**Monospecific cultures**

All the three dominant AM fungal species recovered from trap cultures viz., *Acaulospora scrobiculata*, *Rhizoglomus intraradices*, and *Claroideoglomus claroideum* were successfully mass multiplied to develop monospecific cultures using *Eleusine corocana* as a host plant. Later, these pure cultures were used to prepare the AM fungal inoculum to be used as a biofertilizer for coconut plantations.

**Inoculum preparation**

Inoculum was prepared using the above dominant AM fungal species with *Eleusine corocana* as a host plant. The AM root colonization in the inoculum was reported to be 73.33%.

**Table 1:** AM root colonization and spore density in the field soil sample.

| Sr. No. | AM root colonization (%) | Spore density/100g soil |
|---|---|---|
| 1 | 52.22±4.36 | 270.11±14.95 |

**Legend:** Values are the mean of three readings.

**Table 2:** AM root colonization in the inoculum.

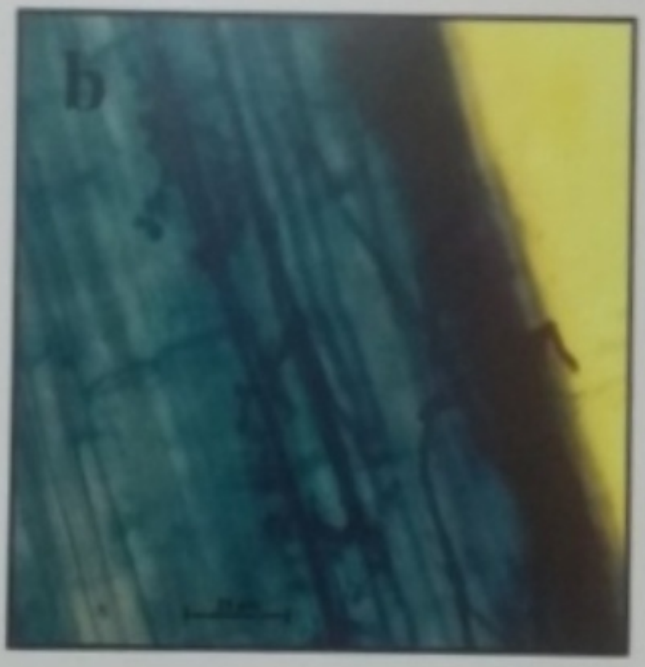
| Sr. No. | AM root colonization (%) |
|---|---|
| 1 | 73.33±3.87 |

**Legend:** Values are the mean of three readings.

# PLATE I

**AM root colonization**
**a.** Hyphae, **b.** Hyphae + vesicles, **c.** Hyphae + Arbuscules, **c.** Hyphae + Vesicles + Arbuscules

# PLATE II




**AM root colonization in the inoculum**
**a.** Hyphae + Vesicle + Arbuscules, **b.** Arbuscules

# PLATE III

**Isolated AM Fungal spores**
**a.** *Acaulospora scrobiculata,* **b.** *Rhizoglomus intraradices,* **c.** *Claroideoglomus claoideum,* **d.** *Glomus sp.*

# DISCUSSION

In the present study, AM fungal diversity was studied in the rhizosphere of *Cocos nucifera*. The AM fungal colonization in *cocos nucifera* was recorded to be 52.22%, which was characterized by the presence of arbuscules, vesicles, and hyphae. The present study recorded lower colonization levels compared to an earlier study (Muthukumar and Vediyappan, 2010) in coconut from Tamil Nadu which recorded higher colonization levels ranging from 60.74 to 78.90%. This may be due to Seasonal fluctuations in moisture, temperature, pH, and soil nutrient status showing high and dramatic effects on arbuscular mycorrhizal spore population and percentage of root colonization (Jhoti *et al*., 2017).

The present study also revealed the presence of nine AM fungal species in the rhizosphere of coconut *viz*., *Glomus* sp.1, *Glomus* sp. 2, *Glomus* sp. 3, *Glomus* sp. 4, *Acaulispora scobiculata, A. undulata, Gigaspora* sp., *Rhizoglomus intaradices* and *Claroideoglomus claroideum*. Of these nine AM species *A. scobiculata, R. intaradices* and *C. claroideum* were found to be dominant and were used to prepare monospecific cultures. The previous studies (Thomas et al. 1993; Ambili et al. 2012) revealed that the most commonly distributed AM fungal genera in the coconut gardens were Glomus, Gigaspora and Acaulospora. At the genus level, Glomus is ecologically generalist and is distributed in coconut palm in crop mixed systems. Manoharachary et al. (2005) also reported the widespread occurrence of Glomus species in Indian soil. Glomus spp. appear to have an excellent adaptation to a variety of soil conditions based on the large and varied spore population that was discovered in the study (Jansa et al. 2002; Kowalczyk and B łaszkowski 2011).

Preparation of trap cultures and monospecific cultures are the most widely adopted methods used to maintain AM spores or inocula because of the relatively low technical support needed and inexpensive consumables. These methods are the least artificial (live host plants are used) and help

in understanding the biology of AM fungal lifecycle as well as in supporting the production and storage of the inocula for a longer duration.

Inoculum prepared using the three dominant AM fungal species identified in the trap culture, recorded higher root colonization levels (73.33%). Since non-inoculated controls in the greenhouse are often free of AMF, unlike in open-field circumstances, AMF inoculation in the field has shown to be just as successful as inoculation in the greenhouse (Berruti, 2016).

# CONCLUSION

The present study was primarily carried out to ascertain the AM fungal diversity and distribution in the rhizosphere of *Cocos nucifera*. This study recorded a low spore density along with 52.22% root colonization in the rhizosphere soils of coconut plantations. This is possibly due to the seasonal application of inorganic fertilizer to the coconut plantation. The study also revealed the dominance of *Acaulospora scobiculata*, *Rhizoglomus intaradices*, and *Claroideoglomus claroideum*. This indicates their adaptation to the ecological conditions in the rhizosphere of coconut plantations.

The above three AM fungal species were used to prepare an efficient pot-based AM inoculum for coconut plantations. It was observed that the AM fungal colonization in plants used as hosts for the preparation of the pot-based inoculum recorded high root colonization. Therefore, the use of AM biofertilizer in coconut would reduce the usage of expensive inorganic fertilizers. Besides, it would increase the number of useful microbes thereby enhancing soil fertility. Inoculum prepared based on the dominant AM fungal species was found to be viable with 73.33% root colonization in *Eleusine*. The same may be used for the inoculation of coconut saplings at the nursery level.

# BIBLIOGRAPHY

- Velazquez, S., & Cabello, M. (2011). Occurrence and diversity of arbuscular mycorrhizal fungi in trap cultures from El Palmar National Park soils. *European Journal of Soil Biology*, 47(4), 230-235.
- Pedersen, A. C., De Nardi, F. S., da Costa, R. C., Antoniolli, R., Sturmer, S. L., & Calvete, E. O. (2015, July). Diversity of arbuscular mycorrhizal fungi in strawberry crop systems detected in trap cultures. In *International Symposium on New Technologies and Management for Greenhouses-GreenSys2015 1170* (pp. 1085-1092).
- Chaturvedi, S., Tewari, V., Sharma, S., Oehl, F., Wiemken, A., Prakash, A., & Sharma, A. K. (2012). Diversity of arbuscular mycorrhizal fungi in oak-pine forests and agricultural land prevalent in the Kumaon Himalayan Hills, Uttarakhand, India. *British Microbiology Research Journal*, 2(2), 82.
- Ilangamudali, I. M. P. S., & Senarathne, S. H. S. (2016, November). Effectiveness of Arbuscular Mycorrhizal Fungi based biofertilizer on early growth of coconut seedlings. In *Cocos* (Vol. 22, No. 1). Coconut Research Institute of Sri Lanka.
- Trejo-Aguilar, D., Lara-Capistrán, L., Maldonado-Mendoza, I. E., Zulueta-Rodríguez, R., Sangabriel-Conde, W., Mancera-López, M. E., ... & Barois, I. (2013). Loss of arbuscular mycorrhizal fungal diversity in trap cultures during long-term subculturing. *IMA fungus*, *4*(2), 161-167.
- Chaiyasen, A., Douds, D. D., Gavinlertvatana, P., & Lumyong, S. (2017). Diversity of arbuscular mycorrhizal fungi in Tectona grandis Linn. f. plantations and their effects on growth of micropropagated plantlets. *New Forests*, *48*, 547-562.
- Bhadalung, N. N., Suwanarit, A., Dell, B., Nopamornbodi, O., Thamchaipenet, A., & Rungchuang, J. (2005). Effects of long-term NP-fertilization on abundance and diversity

of arbuscular mycorrhizal fungi under a maize cropping system. *Plant and soil*, *270*, 371-382.

- Rajeshkumar, P. P., Thomas, G. V., Gupta, A., & Gopal, M. (2015). Diversity, richness and degree of colonization of arbuscular mycorrhizal fungi in coconut cultivated along with intercrops in high productive zone of Kerala, India. *Symbiosis*, *65*, 125-141.
- Ambili, K., Thomas, G. V., Indu, P., Gopal, M., & Gupta, A. (2012). Distribution of arbuscular mycorrhizae associated with coconut and arecanut based cropping systems. *Agricultural Research*, *1*, 338-345.
- Husein, M., Umami, N., Pertiwiningrum, A., Rahman, M. M., & Ananta, D. (2022). The role of Arbuscular mycorrhizal fungi density and diversity on the growth and biomass of corn and sorghum forage in trapping culture. *Tropical Animal Science Journal*, 45(1):37-43.
- B. Wang, Y.-L. Qiu, Phylogenetic distribution and evolution of mycorrhizas in lands plants, *Mycorrhiza* 16 (2006) 299-363.
- Redecker, D., Kodner, R., & Graham, L. E. (2000). Glomalean fungi from the Ordovician. *Science*, *289*(5486), 1920-1921.
- Chaturvedi, S., Tewari, V., Sharma, S., Oehl, F., Wiemken, A., Prakash, A., & Sharma, A. K. (2012). Diversity of arbuscular mycorrhizal fungi in oak-pine forests and agricultural land prevalent in the Kumaon Himalayan Hills, Uttarakhand, India. *British Microbiology Research Journal*, *2*(2), 82.
- Smith, F. A., Jakobsen, I., & Smith, S. E. (2000). Spatial differences in acquisition of soil phosphate between two arbuscular mycorrhizal fungi in symbiosis with Medicago truncatula. *The New Phytologist*, *147*(2), 357-366.

- Allen, M. F. (2011). Linking water and nutrients through the vadose zone: a fungal interface between the soil and plant systems. *Journal of Arid Land*, *3*(3), 155-163.
- Bever, J.D.; Morton, J.B.; Antonovics, J.; Schultz, P. (1996). Hostdependent sporulation and species diversity of arbuscular mycorrhizal fungi in a mown grassland. J. *Ecology* 84, 71-82.
- Oehl, F., Sieverding, E., Ineichen, K., Mäder, P., Boller, T., & Wiemken, A. (2003). Impact of land use intensity on the species diversity of arbuscular mycorrhizal fungi in agroecosystems of Central Europe. *Applied and environmental microbiology*, *69*(5), 2816-2824.
- Lovelock, C.E.; Ewel, J.J. (2005). Links between tree species, symbiotic fungal diversity and ecosystem functioning in simplified tropical ecosystems. New Phytologist 167, 219-228.
- Al-Arjani, A. B. F., Hashem, A., & Abd_Allah, E. F. (2020). Arbuscular mycorrhizal fungi modulates dynamics tolerance expression to mitigate drought stress in Ephedra foliata Boiss. *Saudi Journal of Biological Sciences*, *27*(1), 380-394.
- Van der Heijden MGA, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boiler T, Wiemken A, Sanders IR (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. *Nature* 396: 69-72.
- Moora, M., Öpik, M., Sen, R., & Zobel, M. (2004). Native arbuscular mycorrhizal fungal communities differentially influence the seedling performance of rare and common Pulsatilla species. *Functional ecology*, *18*(4), 554-562.

- Finlay, R. D. (2008). Ecological aspects of mycorrhizal symbiosis: with special emphasis on the functional diversity of interactions involving the extraradical mycelium. *Journal of experimental botany*, *59*(5), 1115-1126.
- Johansson, J. F., Paul, L. R., & Finlay, R. D. (2004). Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. *FEMS microbiology ecology*, *48*(1), 1-13.
- Gaur, A., & Adholeya, A. (2002). Arbuscular-mycorrhizal inoculation of five tropical fodder crops and inoculum production in marginal soil amended with organic matter. *Biology and Fertility of Soils*, *35*, 214-218.
- Douds Jr, D. D., Nagahashi, G., Pfeffer, P. E., Reider, C., & Kayser, W. M. (2006). On-farm production of AM fungus inoculum in mixtures of compost and vermiculite. *Bioresource technology*, *97*(6), 809-818.
- Wang, Y., M. Vestberg, C. Walker, T. Hurme, X. Zhang, and K. Lindstrom. 2008. Diversity and infectivity of arbuscular mycorrhizal fungi in agricultural soils of the Sichuan province of mainland china. *Mycorrhiza*. 18:59-68.
- Subramanian P., Thamban C., Vinayaka Hegde, Hebbar K.B., Ravi Bhat, Krishnakumar V, Niral V., and Josephrajkumar A. (2018) COCONUT, Technical Bulletin No. 133, ICAR-CPCRI, Kasaragod, 56 p.
- Cuenca, G., & Meneses, E. (1996). Diversity patterns of arbuscular mycorrhizal fungi associated with cacao in Venezuela. *Plant and Soil*, *183*, 315-322.
- Stutz JC, Morton JB (1996) Successive pot cultures reveal high species richness of vesicular-arbuscular mycorrhizal fungi across a soil moisture-nutrient gradient. *Oecologia* 64: 111-117.

- Blaszkowski J (2012) Glomeromycota. W. Szafer Institute of Botany, Polish Academy of Sciences, Kraków, pp 297.
- Rodrigues BF, Muthukumar T (2009) Arbuscular mycorrhizae of Goa - a manual of identification Protocols, Goa University, Goa, pp 109-135.
- Jothi, C., Muthuraj, K., Priya, K. S., Krishnan, E., & Nagarajan, N. STUDIES ON THE AM FUNGAL DIVERSITY OF SOME SIGNIFICANT ETHANO-MEDICINAL PLANTS OF KARULAI HILLS, MALAPPURAM DISTRICT, KERALA *Kongunadu Research Journal* 4(3): 25-38.
- Manoharachary, C., Sridhar, K., Singh, R., Adholeya, A., Suryanarayanan, T. S., Rawat, S., & Johri, B. N. (2005). Fungal biodiversity: distribution, conservation, and prospecting of fungi from India. *Current Science*, 89:58-71.
- Jansa, J., Mozafar, A., Anken, T., Ruh, R., Sanders, I., & Frossard, E. (2002). Diversity and structure of AMF communities as affected by tillage in a temperate soil. *Mycorrhiza*, *12*, 225-234.
- Kowalczyk S, Błaszkowski J (2011) Arbuscular mycorrhizal fungi (Glomeromycota) associated with roots of plants of the Lubuskie province. *Acta Mycol Sin* 46(1):3–18.
- Berruti, A., Lumini, E., Balestrini, R., & Bianciotto, V. (2016). Arbuscular mycorrhizal fungi as natural biofertilizers: let's benefit from past successes. *Frontiers in microbiology*, 6, 1559.
- Tenkoon, N.A. and S.D.H. Bandara. 2003. Nutrient content of some locally available organic materials and their potential as alternative sources of nutrients for coconut. *Cocos.*, 15: 23-30.