Study of fungal endophytes in the selected species of family Zingiberaceae

A Dissertation for

BOT-651 Discipline specific dissertation

Credits: 16

Submitted in partial fulfillment of Masters's Degree (Master of Science

in Botany) by

MS. AKSHITA ARUN SHET VERENKAR

Seat No: 22P0480030

ABC ID: 410653140463

PRN: 201810891

Under the Supervision of

PROF. BERNARD F. RODRIGUES

School of Biological Sciences and Biotechnology Botany

GOA UNIVERSITY

April 2024

GOA UNIVERSITY
SBSB
TALEIGAO
GOA
BOTANY DISCIPLINE

Seal of the School

Examined by:

DECLARATION BY STUDENT

I hereby declare that the data presented in this Dissertation report entitled, "Study of fungal endophytes in the selected species of family Zingiberaceae" 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. Bernard 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.

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

Ms. Akshita Arun Shet Verenkar
(Signature and Name of Student)
Seat no: 22P0480030

Date:08/04/2024

Place: Goa University

COMPLETION CERTIFICATE

This is to certify that the dissertation report **"Study of fungal endophytes in the selected species of family Zingiberaceae"** is a bonafide work carried out by **Ms. Akshita Arun Shet Verenkar** under my supervision in partial fulfillment of the requirements for the award of the degree of Master of science in the Discipline Botany at the school of biological sciences and biotechnology, Goa University.

Prof. Bernard F. Rodrigues

(Signature and Name of Supervising Teacher)

Date:08/04/2024

Signature of Dean of the School

Date: 08/04/2024

Place: Goa University

School/Dept.stamp

CONTENTS

PREFACE

I am delighted to present this dissertation titled "Study of fungal endophytes in the selected species of family Zingiberaceae." This dissertation reflects the outcome of extensive research, analysis, and contemplation conducted over several months on fungal endophytes and their relationships with host plants.

In this study, I have worked to explore the intricate mechanisms by which fungal endophytes promote plant growth, thereby contributing to the overall resilience and vitality of host plants. This dissertation is structured to provide a comprehensive overview of the current understanding of fungal endophytes, their colonization, and the diverse array of plant growth-promoting activities they exhibit. Furthermore, it talks about pigment production by fungal endophytes, shedding light on their potential applications in various fields, including agriculture and biotechnology.

I hope this dissertation will contribute to the knowledge of fungal endophytes and inspire further research in this captivating field.

ACKNOWLEDGMENTS

I express my deep gratitude to the almighty and all those who have helped me in my dissertation work.

I express my sincere gratitude to my dissertation guide, Prof. Bernard F. Rodrigues, for his valuable guidance, support, and encouragement throughout this dissertation. His expertise and insights have been instrumental in shaping the direction of my research and refining its quality.

I am also deeply thankful to the faculty members of the botany discipline SBSB Goa University, Prof. S.Krishnan, Dr. Rupali Bhandari, Dr. Siddhi Jalmi, and Dr. Aditi Naik, whose knowledge and feedback have enriched my academic journey and contributed to the development of this dissertation.

I extend my appreciation to Dr. Puja Sakhalkar for her help and suggestions. I sincerely thank Ms. Lenora Vas, GUFCC culture assistant, for her invaluable assistance and unwavering support throughout my dissertation.

Furthermore, I am grateful to my friends and family for their encouragement, understanding, and patience during this dissertation.

TABLES

PLATES

Plate 1. A. *Curcuma. Longa* habit

B. *Kaempferia rotunda* habit

C. *Curcuma decipiens* habit

D. *Curcuma longa* leaf

E. *Kaempferia rotunda* leaf

F. *Curcuma decipiens* flower.

Plate 2. A. *Curcuma longa* adult rhizome

B. *Curcuma longa* young rhizome

C. *Kaempferia rotunda* rhizome

D. *Curcuma decipiens* rhizome

E. *Curcuma decipiens* rhizome

F. *Curcuma longa* leaf showing disease symptoms.

Plate 3.1A. *Aspergillus carbonarius* colony

1B. *Aspergillus carbonarius* conidiophore and conidia (40X)

2A. *Aspergillus flavus* colony

2B. *Aspergillus flavus* conidiophore and conidia (40X)

3A. *Aspergillus niger* colony

3B. *Aspergillus niger* (40X).

Plate 4.4A. *Aspergillus oryzae* colony

4B. *Aspergillus oryzae* conidiophore and conidia (40X)

5A. *Aspergillus* sp. 1 colony

5B. *Aspergillus* sp. 1 conidiophore and conidia (40X)

6A. *Aspergillus* sp. 2 colony showing sporulation on media supplemented with leaf extract of host plant species.

6B. *Aspergillus* sp. 2 (40X).

Plate 5. . 7A. *Bipolaris* sp. Colony

7B. *Bipolaris* sp. (40X)

8A. *Fusarium* sp. Colony releasing reddish brown exudates on the PDA media containing leaf extract of host plant

8B. *Fusarium* sp. Showing a. Hyphae (10X) b. chlamydospores (40X) c. macroconidia (40X)

9A. *Penicillium* sp. 1 colony showing red pigmentation

9B. *Penicillium* sp. 1 (40X).

Plate 6. 10A. *Penicillium* sp. 2 colony

10B. *Penicillium* sp. 2 conidiophore and conidia (40X)

11A. *Penicillium* sp. 3 colony

11B. *Penicillium* sp. 3 (40X)

12A. *Penicillium* sp. 4 colony

12B. *Penicillium* sp. 4 (40X).

Plate 7. 13A.*Penicillium* sp. 5 colony

13B. *Penicillium* sp. 5 (40X)

14A. *Trichoderma* sp. Colony showing sporulation on PDA media containing leaf extract of host plant species

14B. *Trichoderma* sp. (40X)

15A. Unknown pathogen colony

15B. Unknown pathogen (40X)

16A. Unknown sp. colony

16B. Unknown sp. (40X).

Plate 8.Cellulolytic activity in fungal endophytes

A. *Aspergillus niger*

B. *Penicillium* sp. 2

C. *Penicillium* sp. 4

D. *Penicillium* sp 5

E. *Trichoderma* sp.

Plate 9. Phosphate solubilizing activity in fungal isolates

A. *Aspergillus carbonarius*

B. *Aspergillus niger*

C. *Aspergillus* sp. 2

D. *Trichoderma* sp.

Plate 10. Antagonistic activity in endophyte isolates

A. *Aspergillus flavus*

B. *Aspergillus oryzae*

C. Unidentified sp. 1

D. *Aspergillus niger*

E. *Penicillium* sp. 1

F. *Penicillium* sp. 2

G. *Penicillium* sp. 3

Plate 11. A. Culture broth of *Penicillium* sp. 1 secreting magenta red colored pigment in PD broth

B. Extraction of mycelial pigment of *Penicillium* sp. 1

C. Extracted mycelial pigment in Ethyl Acetate solvent

D. Extracted secretory pigment in Ethyl Acetate solvent

E. Spectra of secretory pigments

F. Spectra of mycelial pigment.

ABBREVIATIONS USED

Entity	Abbreviation
Clavicipitaceous	C
Curcuma decipiens	CD
Curcuma decipiens leaf	CDL
Curcuma decipiens rhizome	CDR
Curcuma longa	CL
Curcuma longa leaf	CLL
Curcuma longa rhizome	CLR
Extracellular	EC
Goa University	GU
Intracellular	IC
Kaempferia rotunda	KR
Kaempferia rotunda leaf	KRL
Kaempferia rotunda rhizome	KRR
Leaf	L
Leaf extract	LE
Malt extract agar	MEA
Mycelial pigment	MP
Non-Clavicipitaceous	NC
Plant growth promoting	PGPR
Potato dextrose agar	PDA
Rhizome	R
Secretory pigment	SP
Sodium chloride	NaCl
Usgao	US

ABSTRACT

The present investigation gives a comprehensive study of fungal endophytes. The research was aimed to isolate, identify, and characterize the fungal endophytes, besides focuses on their potential plant growth-promoting activities and pigment-producing capabilities.

Endophytic fungi were isolated from the rhizomes and leaves of the selected species of the family Zingiberaceae using standard isolation techniques. The isolated fungal endophytes were identified to the genus and species level based on micromorphological (hyphae, macroconidia, chlamydospores, and other special fungal structures) characteristics. The isolated fungi were then screened for their PGPR activities, including P solubilization, cellulolytic activity, and antagonism. Furthermore, the fungal strains were studied for their pigment-producing potential using UV-Vis spectrophotometry.

The results revealed a diverse range of fungal endophytes associated with the plant species undertaken for the study, with several strains showing promising PGPR activities. Besides, pigments including anthocyanins and carotenoids produced by the fungal endophytes were identified.

This study also highlights the potential of the fungal endophytes as biofertilizers and biocontrol agents in agriculture and their possible applications in the pharmaceutical and food industries due to the pigment-producing capabilities.

1. INTRODUCTION

1.1. Background

Within the plant kingdom, a hidden partnership exists, i.e., the symbiotic relationship between plants and endophytic fungi. These organisms, within plant tissues, hold potential for various discoveries.

Endophyte means "in the plant" (endon Gr.=within, phyton = plant). Endophytes can be bacterial or fungal. There are diverse uses for the word endophyte, but "endophytes" are most commonly defined as those organisms whose "infections are inconspicuous, the infected host tissues are at least transiently symptomless, and the microbial colonization can be demonstrated to be internal" (Kobayashi and Palumbo, 2000).

Galippe (1887) reported the occurrence of bacteria and fungi in the interior of vegetable plants and postulated that these microorganisms derive from the soil environment and migrate into the plant, where they might play a beneficial role for the host plant. Endophytes are considered important plant partners that play an essential role in improving the stress tolerance of the host compared to those that lack such symbiosis (Rai and Agarkar, 2016). Most endophytes are found without known effects, but numerous bacteria and fungi establish a mutualistic or pathogenic association with the host plant. The outcome of interactions mainly relies on environmental factors and other factors, such as the genotype of both the host and the interacting microorganism (Brader *et al.*, 2017).

1.1.1. History

Endophytes were first described by the German botanist Heinrich Friedrich Link in 1809. At that time, they were termed "Entophytae" and were described as a distinct group of partly parasitic fungi living in plants. Generally, in the 19th century, the belief was that healthy or

normally growing plants were sterile and thus free of microorganisms. Other studies in the late 19th century and the beginning of the 20th century confirmed the occurrence of beneficial microorganisms within plants. Nowadays, it is a well-established fact that plants are hosts for many types of microbial endophytes, including bacteria, fungi, archaea, and unicellular eukaryotes, such as algae and amoebae (https:ncbi.nlm.nih.gov/ pmc/articles/PMC4488371/#B25). The first endophytic strain was isolated from the ryegrass seeds (*Lolium temulentum*) (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8877053/#B17-jof-08-00205).

1.1.2. Classification of Endophytic fungi

Two major groups of endophytic fungi have been recognized previously, reflecting differences in evolutionary relatedness, taxonomy, plant hosts, and ecological functions, which are the clavicipitaceous endophytes (C-endophytes), which infect some grasses and the non-clavicipitaceous endophytes (NC-endophytes), which can be recovered from asymptomatic tissues of nonvascular plants, ferns and allies, conifers, and angiosperms (Rodriguez *et al.*, 2008). NC-endophytes are highly diverse, representing a polyphyletic assemblage of primarily ascomycetous fungi with diverse and often poorly defined or unknown ecological roles. NC-Endophytes have been recovered from every major lineage of land plants and all terrestrial ecosystems, including agroecosystems and biomes ranging from the tropics to the tundra (Arnold and Lutzoni, 2007).

1.1.3. Ecological Role of Endophytic Fungi

Endophytic fungi maintain ecological systems by forming a mutualistic relationship with their host plant. They can protect against herbivores (Clay, 1988) and pathogens, reducing plant disease susceptibility. They can do this by antibiosis, i.e., by producing secondary metabolites. (Mejı' *et al.*, 2008). Apart from this, they can produce hydrogen cyanide and exopolysaccharide and stimulate

novel genes involved in secretion systems (Singh *et al*., 2021), like PR (Pathogenesis Related) genes, PR5, and PR7 (Kavroulakis *et al*., 2007). Fungal endophytes can produce different plant hormones like GAs (GA1, GA3, GA4, GA8, GA9, GA12, GA20, and GA24) and indole acetic acid (Khan *et al.,* 2012). Gibberellins are tetracyclic diterpenoid acids that regulate various plants developmental and physiological processes, including seed germination, seedling development, stem and leaf growth, floral initiation, and flower and fruit growth (Pharis and King, 1985). IAA promotes several developmental processes in plants, such as root development and axillary bud and flower formation, which improves many other processes and is indispensable for plant growth and development (Reinhardt *et al*., 2000). Endophytic fungi enhance soil quality by increasing soil nutrient availability,including P and iron solubilization, siderophore production, and N fixation (Segaran and Sathiavelu, 2019). According to Suebrasri *et al*. (2020), plant growth-promoting metabolites such as the enzymes (protease, xylanase, amylase, and cellulase) are produced by the endophytic fungi.

1.1.4. Antagonistic activity of Endophytic fungi

Endophytic microbes directly or indirectly stimulate plant growth and sustain plant health based on their genes involved in metabolic pathways (Baghel *et al*., 2020). The inhibiting endophytic fungi against pathogens comprise of three mechanisms viz., the production of antibiotics, the competition for food and space, and the induction of plant resistance (Rizali *et al.,* 2021).

1.1.5. Pigment production

In recent years, there has been growing interest in exploring natural sources of pigments as sustainable alternatives to synthetic colors. Along with plants and animals, fungi are also involved in producing diverse forms of pigments. Filamentous fungi such as ascomycetous and basidiomycetous fungi and lichens are known to produce a range of colors from several chemical classes of pigments such as melanins, azaphilones, flavins, phenazines, and quinines (Kalra *et al.,* 2020). Besides these, carotenoids, melanins, flavins, quinones, monascins,

violacein, and indigo are some of the other major pigments produced by filamentous fungi. The use of *Monascus* for ang- kak (red mold rice) production is the oldest recorded use of fungal pigment. *Monascus* produce yellow (ankaflavine, monascine), orange (rubropunctatine, monascorubrine), and purple (rubropunctamine, monascorubramine) pigments, which are often encountered in oriental foods, especially in Southern China, Japan and Southeast Asia (Rao *et al.,* 2017). Pigments are extracted by solvent extraction method, which includes solvents like ethyl acetate and chloroform (Narendrababu and Shishupalal, 2017).

1.1.6. Host plants

Family Zingiberaceae consists of many medicinal plants and is well-known for its use in ethnomedicine. It is distributed widely throughout the tropics, particularly in Southeast Asia. Besides, members of this family are important natural resources, which provide many valuable products for food, spices, dyes, perfume, etc. (Kumar *et al.,* 2013).

In the present study, three host plant species viz., *Curcuma longa* L., *C. decipiens* Dalzell. and *Kaempferia rotunda* L. were selected for the study.

Taxonomy

Kingdom Plantae

Phylum Tracheophyta

Class Liliopsida

Order Zingiberales

Family Zingiberaceae

a. *Curcuma longa* L.

Curcuma longa is a member of the ginger family (Zingiberaceae). Its rhizomes (underground stems) are the source of a bright yellow spice and dye.

Turmeric is sterile (it does not produce seeds but thrives from the rhizomes). It is thought to have arisen from the selection and vegetative propagation of a hybrid between wild turmeric

(*Curcuma aromatica*), native to India, Sri Lanka, the eastern Himalayas, and some other closely related species.(https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:796451-1/general-information). Three major curcuminoids, curcumin, monodemethoxycurcumin and bisdemethoxycurcumin are responsible for the yellow colour of the powdered rhizomes of *Curcuma domestica* Val. (syn. *Curcuma longa* L.; turmeric) (Lechtenberg *et al.*, 2004).

b. *Curcuma decipiens* Dalzell

The native range of this species is southwest India. It is a rhizomatous geophyte and grows primarilyin the seasonally dry tropical biome (https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:796439-1).

c. *Kaempferia rotunda* L.

Kaempferia rotunda is a perennial, stem less plant producing a pseudo stem with 2 - 4 erect leaves from an underground rhizome with small tuberous roots. Flowering stems are produced from the rhizome when no leaves are present. The plant is harvested from the wild for local use as food and medicine. It is often grown as an ornamental in the tropics. (https://tropical.theferns.info/viewtropical.php?id=Kaempferia+rotunda).

The plant yields flavonoids, crotepoxide, chalcones, quercetin, flavonols, B-sitosterol, stigmasterol, syringic acid, protocatechuic acid, and hydrocarbons. An essential oil that contains cineol is obtained from the rhizome. One of the constituents, benzyl benzoate, has shown antimicrobial activity against various tested microbes (*P. aeruginosa, S. typhimurium, B. subtilis*), moderate antioxidant activity, and some larvicidal activity. The plant has an antioxidant potential to control age-dependent diseases such as myocardial, infarction, diabetes, atherosclerosis, cancer etc. (https://tropical.theferns.info/viewtropical.php?id=Kaempferia+rotunda).

1.1.7. Applications of Fungal Endophytes

a. Agriculture

In agriculture, endophytic fungi can be used as biocontrol agents against pathogens and diseases, a healthy alternative to pesticides. Besides, these fungi are also involved in increasing the biomass of plants. They help acquire various inorganic nutrients such as P, N, and K, making them an alternative to chemical-based fertilizers (Burragoni *et al.*, 2021).

b. Medicine

Secondary metabolites produced by endophytic fungi can be used to treat certain diseases like cancer,diabetes, tuberculosis, and malaria. They can also produce drugs which possess antiviral and antiparasitic properties. Apart from this, they also produce immunosuppressive agents (Singh and Kumar, 2023).

c. Phytoremediation

Endophytic fungi can be useful in phytoremediation of environmentally harmful substances such as polycyclic aromatic hydrocarbons (PAHs), petroleum hydrocarbons (PHC), halogenated hydrocarbons, salt, solvents, pesticides, and heavy metals (Singh and Kumar, 2023)

1.2. AIMS AND OBJECTIVES

Fungal endophytes are crucial in enhancing plant health, nutrient uptake, and resistance to environmental stressors. Various relationships may exist between endophytes and their host (Arnold 2007). They may produce abundant substances used in agriculture, industry, and modern medicine, such as novel antibiotics, antimycotics, immunosuppressants, and anticancer compounds (Mitchell *et al.,* 2008). In addition, the studies of endophytes and their relationship with their host plants emphasize the ecology and evolution of both the endophytes and their host. Apart from this, they are essential in bioremediation. They act as biocontrol agents against plant pathogens. Because of all these applications, it's of utmost importance to study fungal endophytes and their applications to develop specific biocontrol agents and discover novel drugs, essential parts of various industries, and modern medicine. Therefore, the present study was undertaken to carry out primary studies on the diversity of fungal endophytes and their plant growth-promoting activities with the following aims and objectives:

1. To isolate and identify fungal endophytes from selected species of the family Zingiberaceae
2. To study plant growth-promoting activities in the isolated fungal endophytes.
3. To carry out qualitative analysis of pigment production in the isolated fungal endophytes.

1.3. HYPOTHESIS

There is a correlation between the diversity of fungal endophytes isolated from three different species of the family Zingiberaceae.

1.4. SCOPE

The study focuses on exploring the taxonomic diversity of fungal endophytes in selected species of the family Zingiberaceae and the comparison between the diversity of fungal endophytes among the species. The study also includes plant growth-promoting activities such as P solubilization, cellulolytic activity, antagonism, and pigment production.

2. REVIEW OF LITERATURE

Zingiberaceae, the ginger family of flowering plants, is the largest family of the order Zingiberales, containing about 56 genera and about 1,300 species. These aromatic herbs grow in moist areas of the tropics and subtropics, including some seasonably dry regions.

(https://www.britannica.com/plant/ginger-lily)

Endophytic fungi are a group of fascinating host-associated fungal communities that colonize the intercellular or intracellular spaces of host tissues, providing beneficial effects to their hosts while gaining advantages (Alam *et al.,* 2021). They play a major role in the growth promotion of their host by solubilizing macronutrients such as phosphorus (P), potassium (K), and zinc (Zn); fixing of atmospheric nitrogen (N); synthesizing phytohormones, siderophores, hydrogen cyanide, ammonia, and act as a biocontrol agent against a wide array of phytopathogens. Endophytic microbes benefit plants by directly promoting their growth or indirectly inhibiting the growth of phytopathogens (Rana *et al.,* 2020). They are also an excellent source of pigments such as Carotenoids, melanins, flavins, quinones, monascins, violacein, and indigo (Rao *et al.,* 2017).

2.1. Diversity of Fungal Endophytes

Endophytes are important components of fungal biodiversity. Approximately 100,000 fungal species have been identified to date, comprising ca. 7% of this planet's estimated 1.5 million species. This estimation is also because fungal endophytes have been isolated from all plant species investigated (Yeh and Kirschner, 2019). All plants in natural ecosystems appear to be symbiotic with fungal endophytes. This diverse fungi group can profoundly impact plant

communities by increasing fitness and conferring abiotic and biotic stress tolerance.

Ananda and Sridhar (2002) characterized fungal communities by direct plating, damp chamber, and bubbling chamber incubation methods from roots segments of four mangrove species *Acanthus ilicifolius, Avicennia officinalis, Rhizophora mucronata,* and *Sonneratia caseolaris* from Udyavara, Karnataka on the West coast of India. The richness of endophytic fungal species from whole root segments after direct plating and damp chamber incubation was maximum for *R. mucronata* than other plants. Incubation of whole root segments in bubbling chambers yielded conidia of two freshwater hyphomycetes: *Mycocentrospora acerina* (in *Avicennia officinalis*) and *Triscelophorus acuminatus* (in *R. mucronata* and *S. caseolaris*).

Orlandelli *et al.* (2012*)* isolated the fungal endophyte community associated with *Piper hispidum* leaves from plants in a Brazilian forest remnant. The endophytic diversity was examined based on sequencing of the ITS1-5.8S-ITS2 region of rDNA. Isolated endophytes were divided into 66 morphogroups, demonstrating considerable diversity. They identified 21 isolates belonging to 11 genera (*Alternaria*, *Bipolaris*, *Colletotrichum, Glomerella*, *Guignardia*, *Lasiodiplodia*, *Marasmius, Phlebia*, *Phoma*, *Phomopsis*, and *Schizophyllum*).

Ginting *et al.* (2013) studied the diversity of endophytic fungi on *Zingiber officinale* Rosc. All parts of plant organs, such as the leaf, rhizome, root, and stem, were subjected to isolation. Fungal identification was done using a combination of morphological characteristics and molecular analysis of DNA sequence generated from the ITS rDNA region. Thirty endophytic fungi were successfully isolated from red ginger plant leaf, rhizome, root, and stem. *Acremonium macroclavatum, Beltraniella* sp., *Cochliobolus geniculatus and* its anamorphic stage *Curvularia affinis, Fusarium solani, Glomerella cingulata* and its *anamorphic stage Colletotrichum gloeosporoides, Lecanicillium kalimantanense, Myrothecium verrucaria,*

Neonectria punicea, Periconia macrospinosa, Rhizopycnis vagum, and *Talaromyces assiutensis. R. vagum* was explicitly found on the root, whereas *C. affinis, L. kalimantanense*, and *M. verrucaria* were found on the stem of the red ginger plant. *A. macroclavatum* was explicitly found in the red ginger plant's organ, located under the ground, whereas *C. affinis* was found in the shoot or organ above the ground.

Win *et al*. (2021) studied the effects of pesticides on the diversity of endophytic fungi in tea plants. They examined the impact of agrochemicals on the endophytic fungal community associated with tea plants. Endophytic fungi were isolated from four tea plant tissues (bark, xylem, old leaves, and new leaves) collected from pesticide-treated and untreated plots. The infection rate was slightly lower in the pesticide-treated plot, but the difference between plots was not statistically significant. *Colletotrichum camelliae, Phyllosticta capitalensis*, and *Pleosporales* sp. were common endophytes in both the plots.

2.2. Plant growth-promoting activities of fungal endophytes

Endophytic fungi are involved in various plant growth-promoting activities. Various studies to date indicate their potential as biocontrol agents. They can promote plant growth through direct actions suchas acquiring nutrients and phytohormone production or indirect actions such as induced resistance, production of antibiotics and secondary metabolites, and protection for abiotic and biotic stresses (Baron and Rigobelo, 2021).

Hellwig *et al.* (2003) isolated altersetin, a new Alkaloid isolated from endophytic *Alternaria* spp. which showed antibacterial activity against several pathogenic gram-positive bacteria.

Nath *et al.* (2015) isolated endophytic fungi from the root, stem, and leaves of tea (*Camellia sinensis*) shrubs collected from different tea gardens in Assam, India, and evaluated their plant growth-promoting activities. *Aspergillus niger* showed the highest IAA (indole acetic acid) activity, followed by *Penicillium sclerotiorum. P. sclerotiorum* was found to be the most

efficient P and Zn solubilizer, *A. niger* was the highest K solubilizer

Vinayarani and Prakash (2018) identified 31 fungal endophytes from healthy turmeric (*Curcuma longa L.)* rhizomes based on morphological and ITS–rDNA sequence analysis. The isolated endophytes were screened for antagonistic activity against *Pythium aphanidermatum* (Edson) Fitzp. and *Rhizoctonia solani* Kuhn., causing rhizome rot and leaf blight diseases in turmeric, respectively. Their results revealed that only six endophytes showed >70% suppression of test pathogens in antagonistic dual culture assays. When tested by dual culture, the endophyte *Trichoderma harzianum* showed significant in vitro mycelial growth inhibition of *P. aphanidermatum* (76.0%) and *R. solani* (76.9%).

2.3. Production of pigments

Babitha *et al.* (2008) studied the effect of light on growth, pigment production, and culture morphology of *Monascus purpureus* in solid-state fermentation in which they found out incubation in total darkness increased the production of red pigment. Meanwhile, the growth of the fungus in direct illumination resulted in the total suppression of pigment production. It was found that both red and blue light influenced pigment yield and culture morphology.

Dikshit and Tallapragada (2011) studied growth patterns, the effect of temperature and pH on pigment production, and the mycelial growth of *Monascus purpureus* and found that 30°C to be the optimum temperature for growth of mycelia and pigment production. Maximum pigment production was observed on pH 5.5.

Narendrababu and Shishupal (2017) analyzed pigments from selected *Aspergillus* and *Penicillium* isolates. Cellular pigments were extracted from mycelial mass, and secretory pigments were extracted separately from culture filtrates using chloroform and ethyl acetate. Visible pigments were observed mainly in mycelial mass extracted in ethyl acetate. UV-visible

spectroscopic analysis provided detection of yellow pigment (380 nm) in *Aspergillus isolate* and orange-red pigment (463 nm) in *Penicillium* sp.

3. MATERIALS AND METHODS

3.1. Collection of Plant Samples from the Study Site

The study was conducted in the Goa University campus, which lies between Latitude 15.4588° N and Longitude 73.8342° E, and in Usgao Goa, which lies between Latitude 15.4489° N and Longitude 74.0711°E from July 2023 to March 2024. Plant samples were placed in polyethylene bags, labeled,and transferred to the laboratory.

3.2. Plant species

Leaves and rhizomes of *Curcuma longa* L., *C. decipiens* Dalzell, and *Kaempferia rotunda* L.

3.3. Isolation of Fungal Endophytes

Collected leaf and rhizome samples were washed with running tap water. Then, they were cut into segments using a sterile blade in a laminar airflow. They were surface sterilized by immersing in 70% ethanol for 1 minute, followed by treatment with 3.5% sodium hypochlorite for 3 minutes. Final rinsing was done using sterile distilled water thrice for 1 minute each.

After sterilization, the leaf/rhizome segments were cut into 5 mm pieces and inoculated on PDA media (five pieces each on a plate). 0.04 g streptomycin sulphate per 100 ml of medium was added to prevent the growth of bacteria. These plates were kept for incubation at 28°C and checked every day for any fungal growth from the inoculated plant segments. Single types of fungal colonies growing out of the plant tissues were re-inoculated on fresh plates containing MEA media, and then pure cultures were prepared on slants and stored at 4°C.

3.4. Preparation of leaf extract agar media

5 g of host leaf material was ground in mortal and pestle and suspended in 250 ml of PDA media and Petri plates containing this media were used to grow isolates that did not show sporulation on normal media.

3.5. Preparation of Slides

Inoculating needles were flamed over a burning spirit lamp. Then, using the needles, the fungal fruiting bodies growing on the media plates were transferred into the drop of Lactophenol cotton blue stain on the slide. The specimen was teased carefully to avoid squashing and over-crowding of the fruiting bodies, and after placing the coverslip, it was observed under the microscope for microscopic identification. The hyaline mycelia/spores/conidia and cytoplasm were stained using Lactophenol cotton blue. The stained specimens were observed under a light microscope for identification.

3.6. Photomicrography

Photomicrographs of fungal micro slides were taken under 10X-40X magnifications on (Nikon Eclipse E200) compound microscope fitted with a digital camera (Nikon DS-Fi2)

Software name- NIS elements.

3.7. Identification of Fungal Endophytes

The isolated endophytic fungi were identified to the genus and species level based on micro-morphological (Hyphae, macro-conidia, chlamydospores, and other special fungal structures) characteristics using Ellis (1971), Ellis (1976), Klich (2002), Manamgoda (2014).

3.8. Calculation of colonization frequency

Colonization frequency (CF) % of each endophytic fungus isolated from a particular plant part was calculated using the formula (Nf / Nt) × 100, where N_f is the number of segments colonized by each isolate, and Nt is the total number of segments (Dhayanithy *et al*., 2009).

3.9. Analysis of Plant Growth Promoting Traits of Fungal Endophytes

3.9.1. Mineral solubilization

a. Phosphate solubilizing activity of Fungal Endophytes

The P solubilizing activity of fungal endophytes was screened using Pikovskaya's medium and the spot inoculation method. Incubation was done at 28°C for seven days to check for the clearance zone. (Vinayarani *et al*., 2018). The phosphate solubilization index was calculated using the following formula: PSI = (colony diameter + halo zone diameter) / colony diameter (Elias *et al*., 2016)

3.9.2. Enzymatic activity

a. cellulolytic activity

Screening for cellulolytic activity of the isolated fungal endophytes was done by inoculation on Petri plates containing agar media containing carboxy methyl cellulose (0.5 g in 100 ml of media). After five days of inoculation at 28^{o} C, the plates were flooded with congo red solution for 15 min (1 mg/L). The plates were destained with 1 M NaCl for 15 min by decanting excess congo red solution. A clear zone was considered cellulase-positive (Vinayarani *et al*., 2018).

3.9.3. Antagonistic activity

a. Isolation of pathogen from infected leaf of *Curcuma longa*

Leaves of the host plant *C. longa* showing a disease symptoms (yellow spots turning into brownish lesions) were washed with running tap water. Then, only the segments with infection were cut using

a sterile blade in a laminar airflow. They were surface sterilized by immersing in 70% ethanol for 1 minute, followed by treatment with 3.5% sodium hypochlorite for 3 minutes. Final rinsing was done using sterile distilled water thrice for 1 minute each.

After sterilization, the infected leaf segments were cut into 5 mm pieces and inoculated on PDA media (four pieces each on a plate). 0.04 g streptomycin sulphate per 100 ml of medium was added to prevent the growth of bacteria. These plates were kept for incubation at 28°C and checked every day for any fungal growth from the inoculated plant segments. Fungal colonies growing out of the plant tissues were re-inoculated on fresh plates containing MEA media, and then pure culture were prepared on slants and stored at 4°C.

b. Dual culture method

The antagonistic activity was screened using the dual culture method using PDA media. Test isolates were streaked at one corner (1 cm from the edge) of the media containing the Petri plate 48 hours before inoculation of the pathogen. A fungal disc containing the pathogen was grown on the Petri plate for five days (5 mm diameter). This disc was inoculated on another side of the Petri plate. Dual cultures were incubated at 28°C for 4-5 days. The zone of clearance was measured and colony growth inhibition % was calculated by using the following formula:

PI = (C – T)/C × 100, where PI is the percent inhibition, C is the colony growth of the pathogen in control, and T is the colony growth of pathogens in dual cultures (Shrivastava *et al.,* 2015).

3.10. Pigment Production

3.10.1. Cultivation of fungal endophytes for pigment production

Selected isolates that showed pigment production were inoculated into 30 ml of potato dextrose broth(PDB), taken in a 100 ml conical flask under sterile conditions, and incubated at room temperature for seven days. The uninoculated PDB media was used as a control.

3.10.2. Extraction of secretory and cellular pigments

The media containing pigments was filtered through Whatman no. 1 filter paper for secretory pigment extraction. An equal amount of ethyl acetate was added. Once the two layers were visible, the top layer of ethyl acetate containing pigment was separated using a sterile micropipette. This extract was evaporated to dryness at room temperature.

The mycelial mass was immersed in ethyl acetate and kept for 24 hours to extract cellular pigments. The mycelium was crushed using a glass rod and shaken well to remove the cellular debris. The supernatant containing cellular pigments was removed using a sterile micropipette. This extract was evaporated to dryness at room temperature.

3.10.3. UV visible spectrophotometry of fungal extract

The extracted compounds were redissolved in a minimum quantity of ethyl acetate. One hundred microliters of this extract were mixed in 2 ml of ethyl acetate and subjected to UV visible spectrometry. The absorption maxima of compounds present in ethyl acetate extract were determined by taking OD from 380 nm to 800 nm (Narendrababu and Shishupal, 2017).

4. RESULTS AND DISCUSSION

The study was conducted at the Goa University campus and in Usgao Goa. The fungal endophytes were isolated from three host plant species **(Table 1).** Altogether, 19 fungal endophytes were isolated, of which four showed dormancy, and one was in the non-sporulating phase **(Table 2)**. Isolates showing dormancy were made to grow by culturing them on liquid media and transferring them onto the normal agar media. Some of the isolates not sporulating on normal agar media showed sporulation on agar media containing leaf extract of host plants **(Table 3).** Of the isolated species, *Penicillium* sp. and *Aspergillus* sp. were common endophytic fungi in plant species collected from Usgao site. The dominant fungal endophytes identified in the leaves of all three hosts were species of *Aspergillus.* In the young rhizomes of *Curcuma longa, Penicillium* sp. 2 was the dominant fungal endophyte, while *Penicillium* sp. 4 prevailed in *Kaempferia rotunda,* and *Bipolaris* sp. in *C. decipiens* adult rhizomes. The diversity of fungal endophytes among the leaves of three different host plant species from two different sites showed a correlation at the generic level. Three different species of *Aspergillus* showed the highest colonization in leaves of all three host plants although, for rhizomes, it differed for *C. decipiens*. In *Curcuma longa, A. flavus* showed the highest colonization frequency in leaf while *Penicillium* sp. 2 showed the highest colonization in the young rhizome. Least colonization was recorded in the adult rhizomes of *C. longa. Aspergillus carbonarius* showed highest colonization frequency in the leaf in *K. rotunda*, while *Penicillium* sp. 4 recorded highest colonization in the rhizome. In *C. decipiens*, *Aspergillus* sp. 1 recorded highest colonization in the leaf, while *Bipolaris* sp. recorded highest colonization in the rhizome. **(Table 4).**

Table 1: Family and common names of plants used for the study.

Botanical Name	Family	Common Name	Site
Curcuma longa	Zingiberaceae	Turmeric	Usgao
Curcuma decipiens	Zingiberaceae	Wild turmeric	Goa University
Kaempferia rotunda	Zingiberaceae	Kaempferia	Usgao

Table 2: Fungal isolates isolated from the host plant species.

Sr. No	Fungal Species	Culture code	Host plant species and plant part						Site of isolation		Growth condition of isolates		
			CL		CD		KR		GU	US	D	S	NS
			L	R	L	R	L	R					
01.	*Aspergillus Carbonarius*	KRL2	-	-	-	-	+	-	-	+	-	+	-
02.	*Aspergillus flavus*	CLL-2	+	-	-	-	-	-	-	+	-	+	-
03.	*Aspergillus niger*	CLL-3 KRL-3	+	-	-	-	+	-	-	+	+	+	-
04.	*Aspergillus oryzae*	CLR-4	-	+	-	-	-	-	-	+	-	+	-
05.	*Aspergillus* sp. 1	CDL-2	-	-	+	-	-	-	+	-	-	+	-
06.	*Aspergillus* sp. 2	CDR-3	-	-	-	+	-	-	+	-	-	+	-
07.	*Bipolaris* sp.	CDR-2	-	-	-	+	-	-	+	-	-	+	-
08.	*Fusarium* sp.	KRR-7	-	-	-	-	-	+	-	+	-	+	-
09.	*Penicillium* sp. 1	CLL-4	+	-	-	-	-	-	-	+	-	+	-
10.	*Penicillium* sp. 2	CLR-1	-	+	-	-	-	-	-	+	-	+	-
11.	*Penicillium* sp. 3	CLR-7	-	+	-	-	-	-	-	+	-	+	-
12.	*Penicillium* sp. 4	KRR-1	-	-	-	-	-	+	-	+	-	+	-
13.	*Penicillium* sp. 5	KRR-6	-	-	-	-	-	+	-	+	-	+	-
14.	*Trichoderma* sp.	CDR-4	-	-	-	+	-	-	+	-	-	+	-
15.	Unidentified sp. 1	CLR-2	-	+	-	-	-	-	-	+	-	-	+
16.	Dormant culture 1	CLL-1	+	-	-	-	-	-	-	+	+	-	-
17.	Dormant culture 3	CDL-1	-	-	+	-	-	-	+	-	+	-	-
18.	Dormant culture 4	CDL-3	-	-	+	-	-	-	+	-	+	-	-
19.	Dormant culture 5	KRL-1	-	-	-	-	+	-	-	+	+	-	-

Legend: CL = *Curcuma longa*; CD = *C. decipiens*; KR = *K. rotunda*; L = leaf; R = Rhizome; GU = Goa University; US = Usgao; D- Dormant; S= Sporulating; NS= Non-sporulating; - = absent; + = present.

Table 3: Inducing sporulation using leaf extract.

Sr. No.	Fungal Species	Culture code	Showing sporulation on	
			Normal agar media (PDA/MEA)	PDA media containing leaf extract
01.	*Aspergillus Carbonarius*	KRL2	+	+
02.	*Aspergillus flavus*	CLL-2	+	+
03.	*Aspergillus niger*	CLL-3	+	+
		KRL-3	+	+
04.	*Aspergillus oryzae*	CLR-4	+	+
05.	*Aspergillus* sp. 1	CDL-2	+	+
06.	*Aspergillus* sp 2	CDR-3	-	+
07.	*Bipolaris* sp.	CDR-2	+	+
08.	*Fusarium* sp.	KRR-7	-	+
09.	*Penicillium* sp. 1	CLL-4	+	+
10.	*Penicillium* sp. 2	CLR-1	+	+
11.	*Penicillium* sp. 3	CLR-7	+	+
12.	*Penicillium* sp. 4	KRR-1	+	+
13.	*Penicillium* sp. 5	KRR-6	+	+
14.	*Trichoderma* sp.	CDR-4	-	+
15.	Unidentified sp. 1	CLR-2	-	-
16.	Dormant culture 1	CLL-1	N/A	N/A
17.	Dormant culture 2	CDL-1	N/A	N/A
18.	Dormant culture 3	CDL-3	N/A	N/A
19.	Dormant culture 4	KRL-1	N/A	N/A

Legend: N/A= Not available; - = absent; + = present.

Table 4: Colonization frequency of isolated fungal endophytes.

Sr. No.	Fungal Species	Culture code	Host plant species and plant part						Colonization frequency (%)
			CL		CD		KR		
			L	R	L	R	L	R	
01.	*Aspergillus Carbonarius*	KRL2	-	-	-	-	+	-	25.00
02.	*Aspergillus flavus*	CLL-2	+	-	-	-	-	-	52.63
03.	*Aspergillus niger*	CLL-3 KRL-3	+	-	-	-	+	-	10.52 18.75
04.	*Aspergillus oryzae*	CLR-4	-	+	-	-	-	-	62.50
05.	*Aspergillus* sp. 1	CDL-2	-	-	+	-	-	-	87.50
06.	*Aspergillus* sp 2	CDR-3	-	-	-	+	-	-	8.69
07.	*Bipolaris* sp.	CDR-2	-	-	-	+	-	-	26.80
08.	*Fusarium* sp.	KRR-7	-	-	-	-	-	+	21.25
09.	*Penicillium* sp. 1	CLL-4	+	-	-	-	-	-	25.00
10.	*Penicillium* sp. 2	CLR-1	-	+	-	-	-	-	63.15
11.	*Penicillium* sp. 3	CLR-7	-	+	-	-	-	-	8.30
12.	*Penicillium* sp. 4	KRR-1	-	-	-	-	-	+	33.33
13.	*Penicillium* sp. 5	KRR-6	-	-	-	-	-	-	6.25
14.	*Trichoderma* sp.	CDR-4	-	-	-	-	-	-	13.04
15.	Unidentified sp.1	CLR-2	-	+	-	-	-	-	6.66
16.	Dormant culture 1	CLL-1	+	-	-	-	-	-	68.42
17.	Dormant culture 3	CDL-1	-	-	+	-	-	-	6.25
18.	Dormant culture 4	CDL-3	-	-	+	-	-	-	12.50
19.	Dormant culture 5	KRL-1	-	-	-	-	+	-	6.25

Legend: CL = *Curcuma longa*; CD = *C. decipiens*; KR = *K. rotunda*; L = leaf; R = Rhizome; - = absent; + = present

Table 5: Plant growth-promoting activities in the isolated fungal endophytes.

Sr. No.	Fungal Species	Culture code	Phosphate Solubilization	Cellulolytic Activity	Antagonism (%)
01.	*Aspergillus Carbonarius*	KRL2	1.2	-	N/A
02.	*Aspergillus flavus*	CLL-2	-	-	64.70
03.	*Aspergillus niger*	CLL-3 KRL-3	1.3 1.5	+ +	63.52
04.	*Aspergillus oryzae*	CLR-4	-	-	65.88
05.	*Aspergillus* sp. 1	CDL-2	-	-	N/A
06.	*Aspergillus* sp 2	CDR-3	1.42	-	N/A
07.	*Bipolaris* sp.	CDR-2	-	-	N/A
08.	*Fusarium* sp.	KRR-7	-	-	N/A
09.	*Penicillium* sp. 1	CLL-4	-	-	38.82
10.	*Penicillium* sp. 2	CLR-1	-	+	28.33
11.	*Penicillium* sp. 3	CLR-7	-	-	35.29
12.	*Penicillium* sp. 4	KRR-1	-	+	N/A
13.	*Penicillium* sp. 5	KRR-6	-	+	N/A
14.	*Trichoderma* sp.	CDR-4	1.3	+	N/A
15.	Unidentified sp.1	CLR-2	-	-	72.94
16.	Dormant culture 1	CLL-1	N/A	N/A	N/A
17.	Dormant culture 3	CDL-1	N/A	N/A	N/A
18.	Dormant culture 4	CDL-3	N/A	N/A	N/A
19.	Dormant culture 5	KRL-1	N/A	N/A	N/A

Legend: - = absent; + = present; N/A = Not available.

Table 6: Pigmentation and absorption wavelength in selected isolated fungal endophytes.

Sr. No.	Fungal Species	Culture code	Pigment colour		Absorption Wavelength (nm)		Pigment
			IC	EC/SP	IC	EC	
01.	*Bipolaris* sp.	CDR-2	Black	-	No peak	No peak	-
02.	*Fusarium* sp.	KRR-7	Reddish brown	Yellow	No peak	No peak	-
03.	*Penicillium* sp. 1	CLL-4	Orange red	Red	495 415	415	495; Anthocyanin[1] 415; Carotenoids[2]
04.	*Penicillium* sp. 3	CLR-7	Yellow	-	No peak	No peak	-
05.	Unidentified pathogen	CLL-5	-	Yellow	No peak	No peak	-
06.	Unidentified sp. 1	CLR-2	Black	-	No peak	No peak	-

Legend: IC = Intra-cellular; EC/SP = Extra-cellular/Secretory pigment;
1 = Syukr *et al.* (2013); 2 = https://letstalkscience.ca/educational.

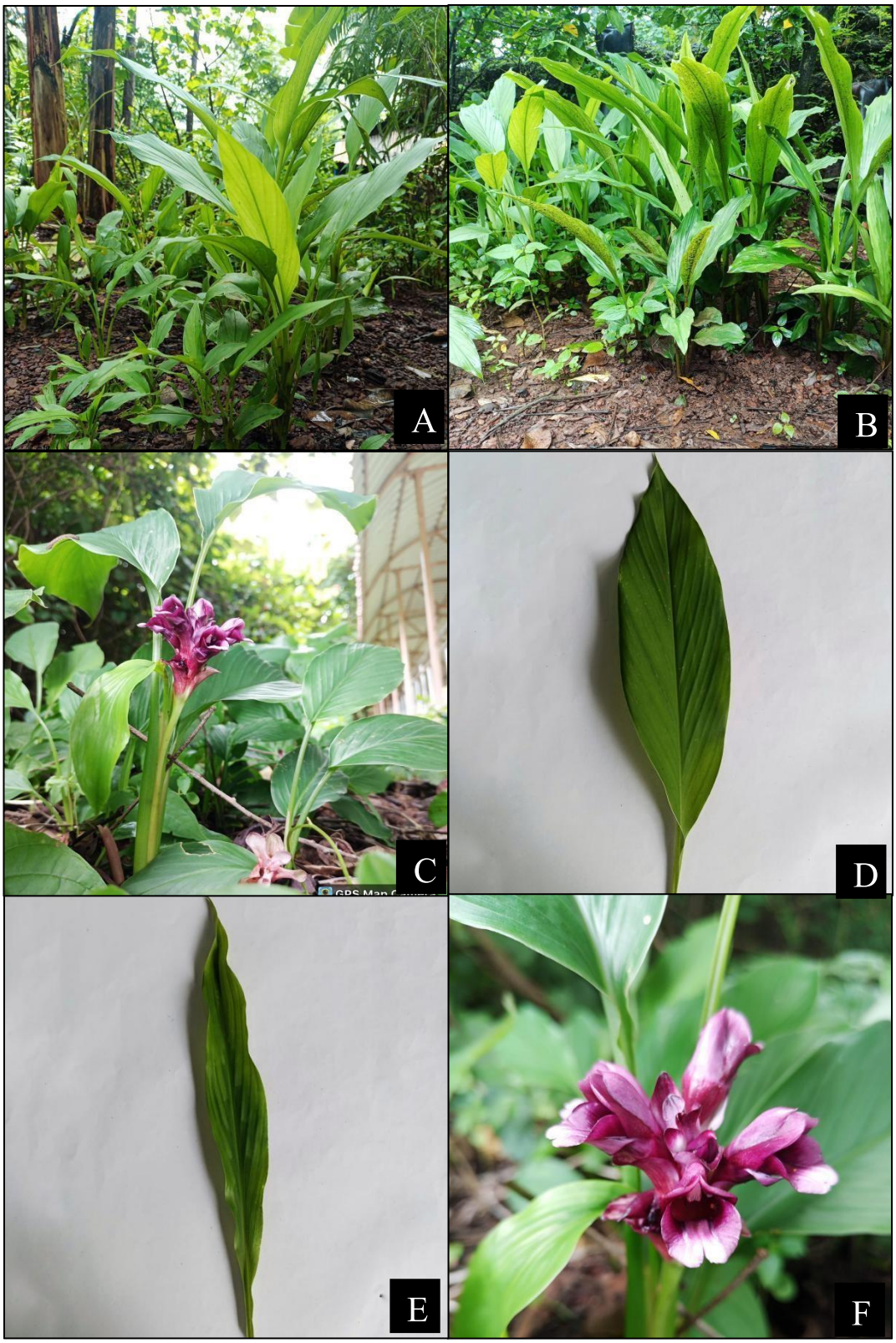

Plate 1: A. *Curcuma. longa* habit; **B.** *Kaempferia rotunda* habit; **C.** *Curcuma decipiens* habit; **D.***Curcuma longa* leaf; **E.** *Kaempferia rotunda* leaf; **F.** *Curcuma decipiens* flower.

Plate 2: A. *Curcuma longa* adult rhizome; **B.** *Curcuma longa* young rhizome; **C.** *Kaempferia rotunda* rhizome; **D & E.** *Curcuma decipiens* rhizome; **F.** *Curcuma longa* leaf showing disease symptoms.

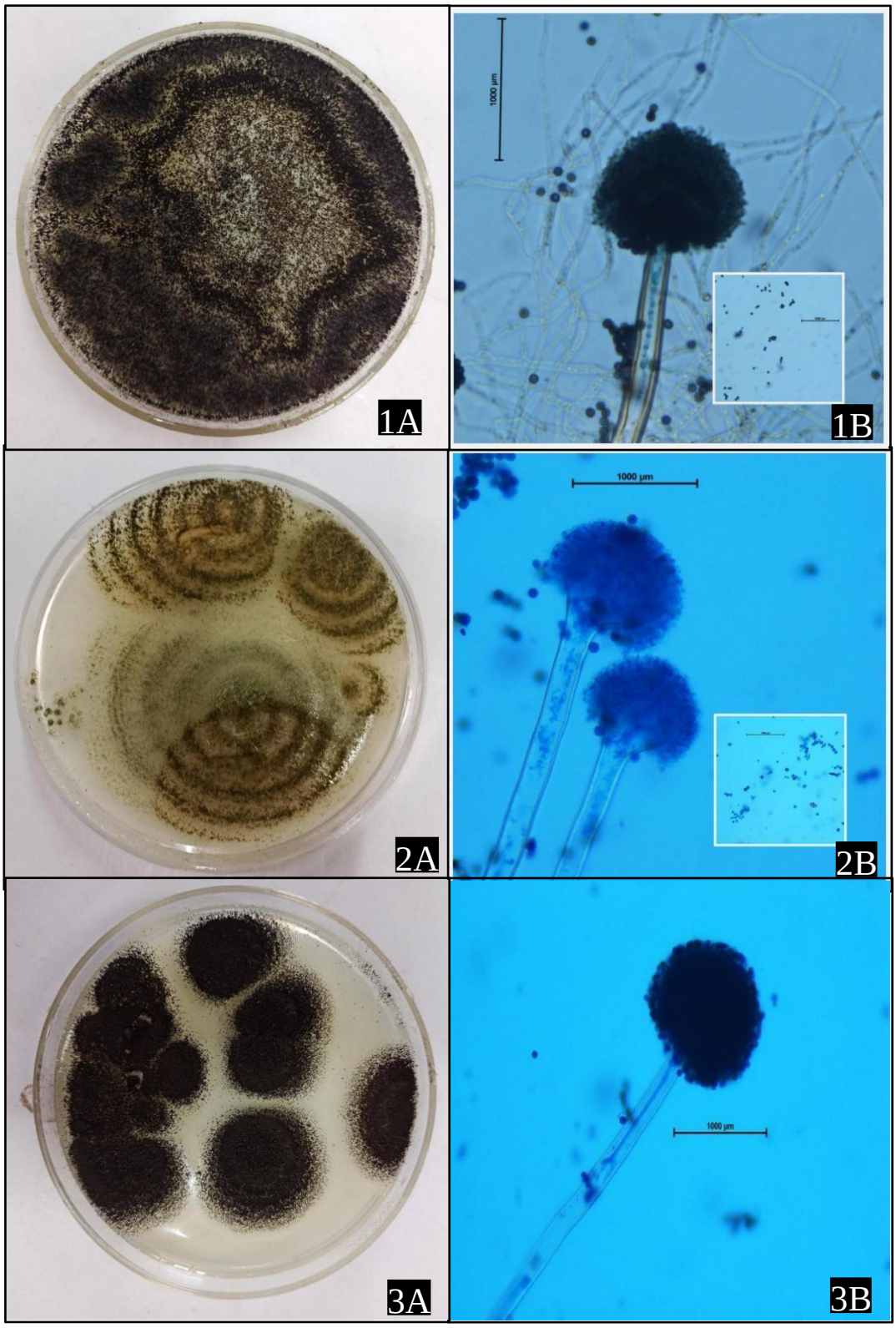

Plate 3 : 1A. *Aspergillus carbonarius* colony; **1B.** *Aspergillus carbonarius* conidiophore and *conidia* (40X); **2A.** *Aspergillus flavus* colony; **2B.** *Aspergillus flavus* conidiophore and conidia (40X); **3A.** *Aspergillus niger* colony; **3B.** *Aspergillus niger* (40X).

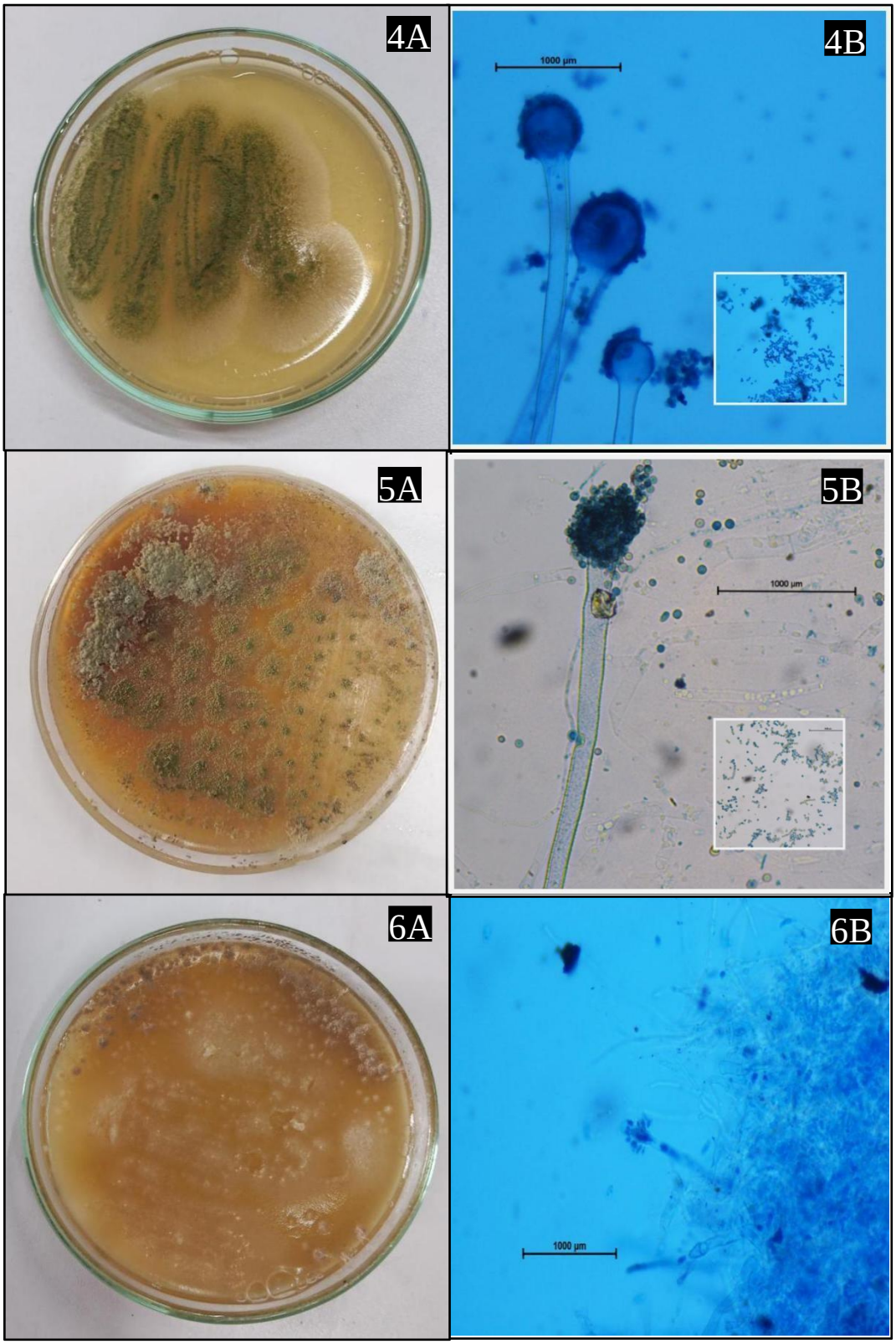

Plate 4: 4A. *Aspergillus oryzae* colony; **4B.** *Aspergillus oryzae* conidiophore and conidia (40X); **5A.** *Aspergillus* sp. 1 colony; **5B.** *Aspergillus* sp. 1 conidiophore and conidia (40X); **6A.***Aspergillus* sp. 2 colony sporulating on PDA media containing leaf extract of host plant species; **6B.** *Aspergillus* sp. 2 (40X).

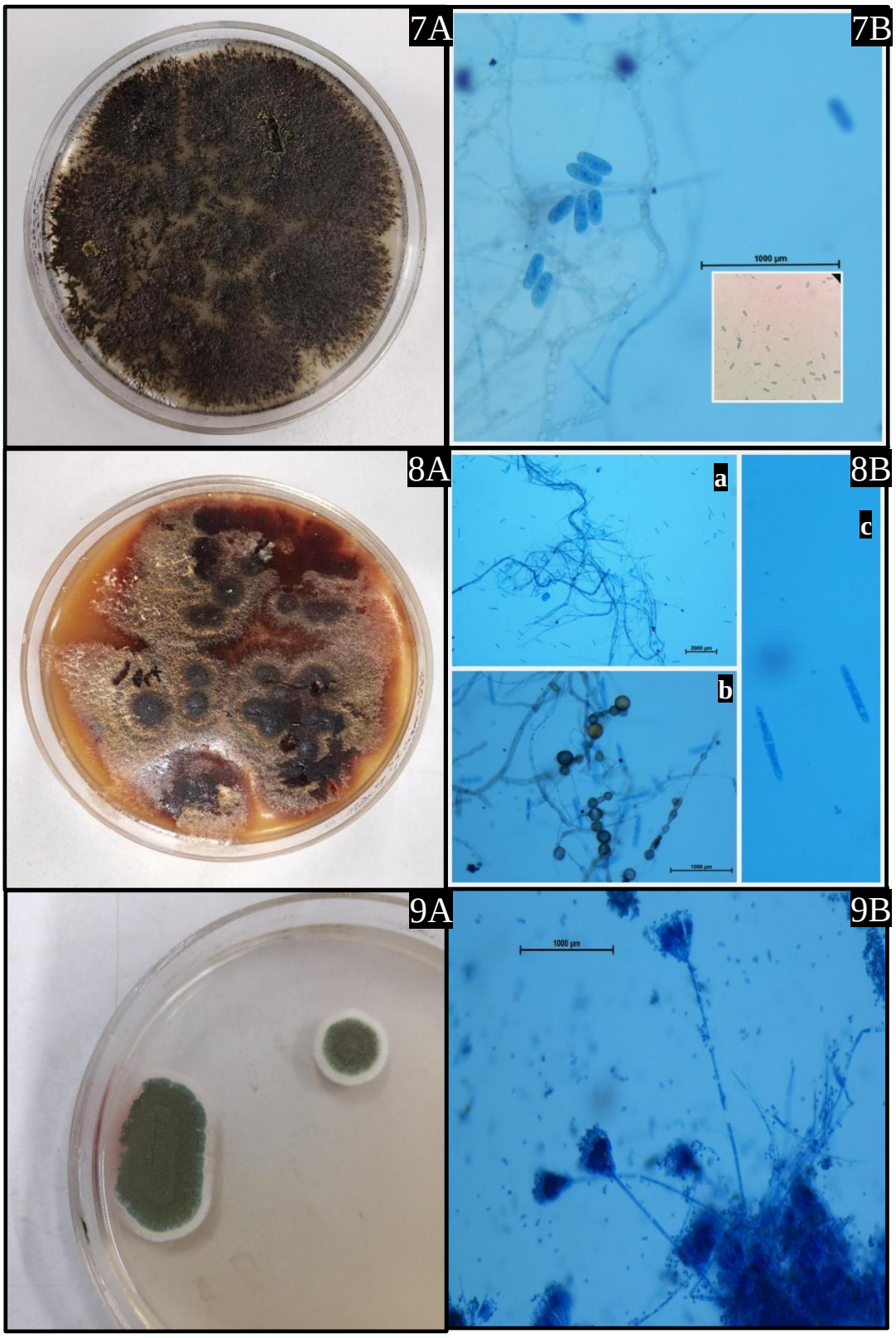

Plate 5: 7A. *Bipolaris* sp. Colony; **7B.** *Bipolaris* sp. (40X);**8A.** *Fusarium* sp. colony releasing reddish brown exudates on PDA media containing leaf extract of host plant species ; **8B.** *Fusarium* sp. Showing **a.** Hyphae (10X) **b.** chlamydospores at (40X) **c.** macroconidia (40X); **9A.** *Penicillium sp.* 1 colony showing red pigmentation; **9B.** *Penicillium sp.* 1 (40X).

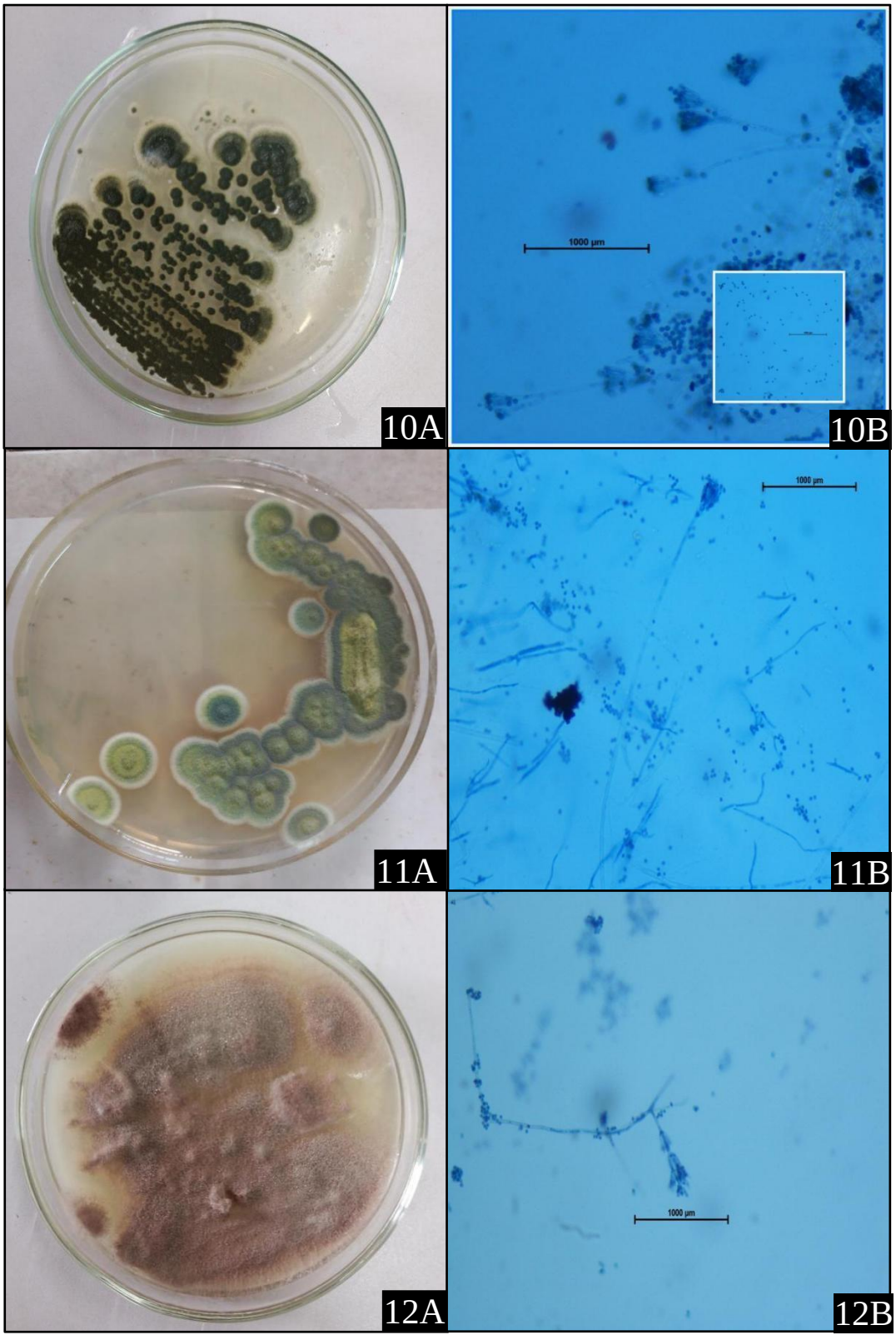

Plate 6: 10A. *Penicillium* sp. 2 colony; **10B.** *Penicillium* sp. 2 conidiophore and conidia at (40X); **11A.** *Penicillium* sp. 3 colony; **11B.** *Penicillium* sp. 3 (40X); **12A.** *Penicillium* sp. 4 colony; **12B.** *Penicillium* sp. 4 (40X).

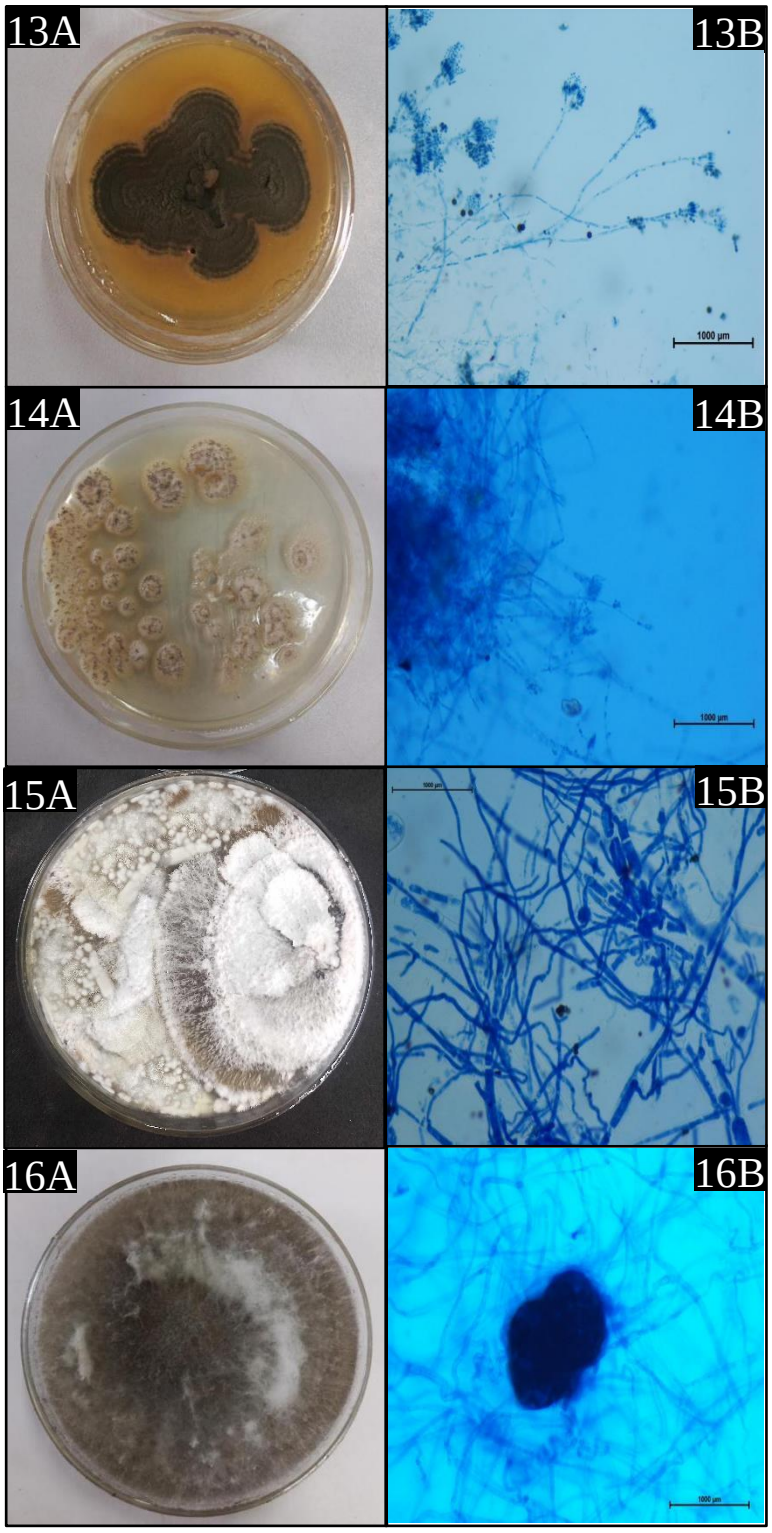

Plate 7: 13A.*Penicillium* sp. 5 colony, **13B.** *Penicillium* sp. 5 (40X); **14A.** *Trichoderma* sp. colony sporulating on PDA media containing leaf extract of host plant species, **14B.** *Trichoderma* sp. (40X); **15A.** Unknown pathogen colony, **15B.** Unknown pathogen (40X) ; **16A.** Unknown sp. colony, **16B.** Unknown sp. (40X).

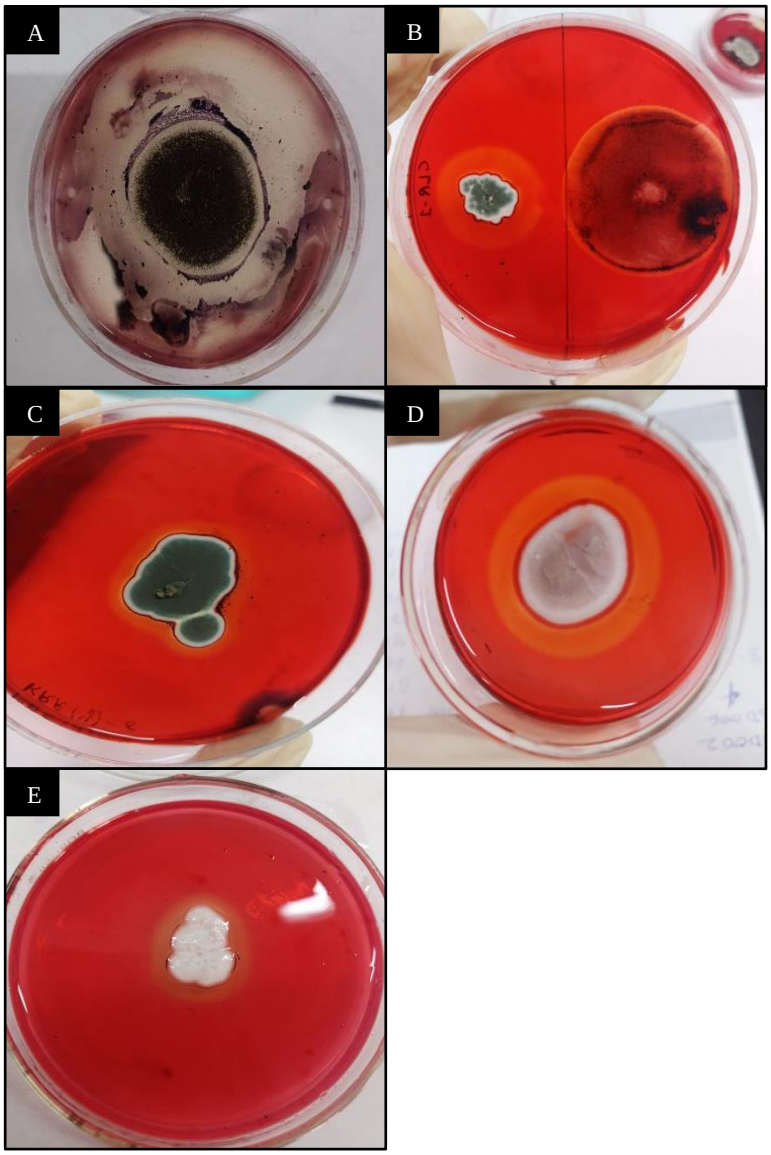

Plate 8: Cellulolytic activity in fungal endophytes: A. *Aspergillus. niger*, **B.** *Penicillium* sp. 2, **C.** *Penicillium* sp. *4*, **D.** *Penicillium* sp *5*, **E.** *Trichoderma* sp..

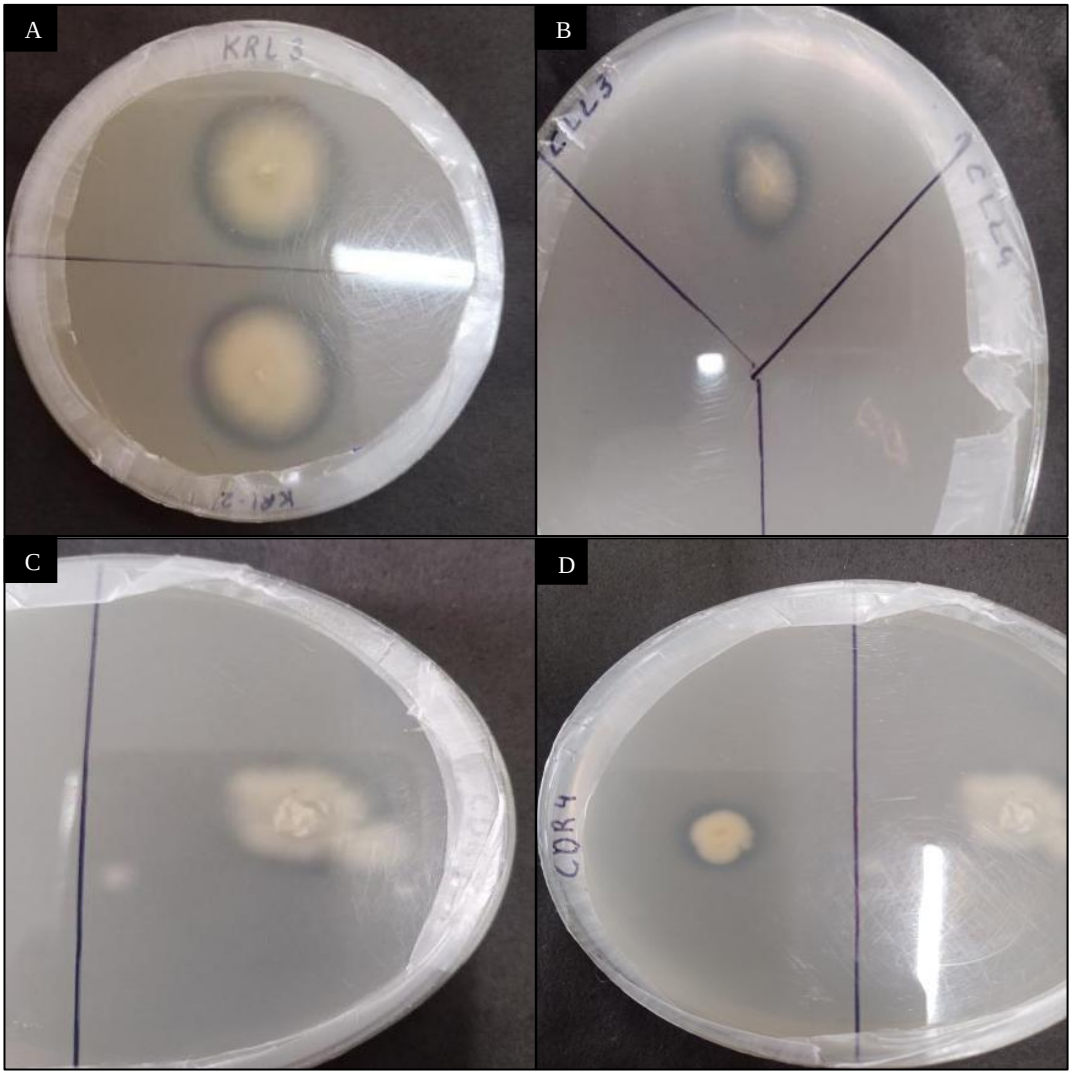

Plate 9: Phosphate solubilizing activity in fungal isolates - A. *Aspergillus carbonarius* **B.** *Aspergillus niger***, C.** *Aspergillus* sp. 2**, D.** *Trichoderma* sp.

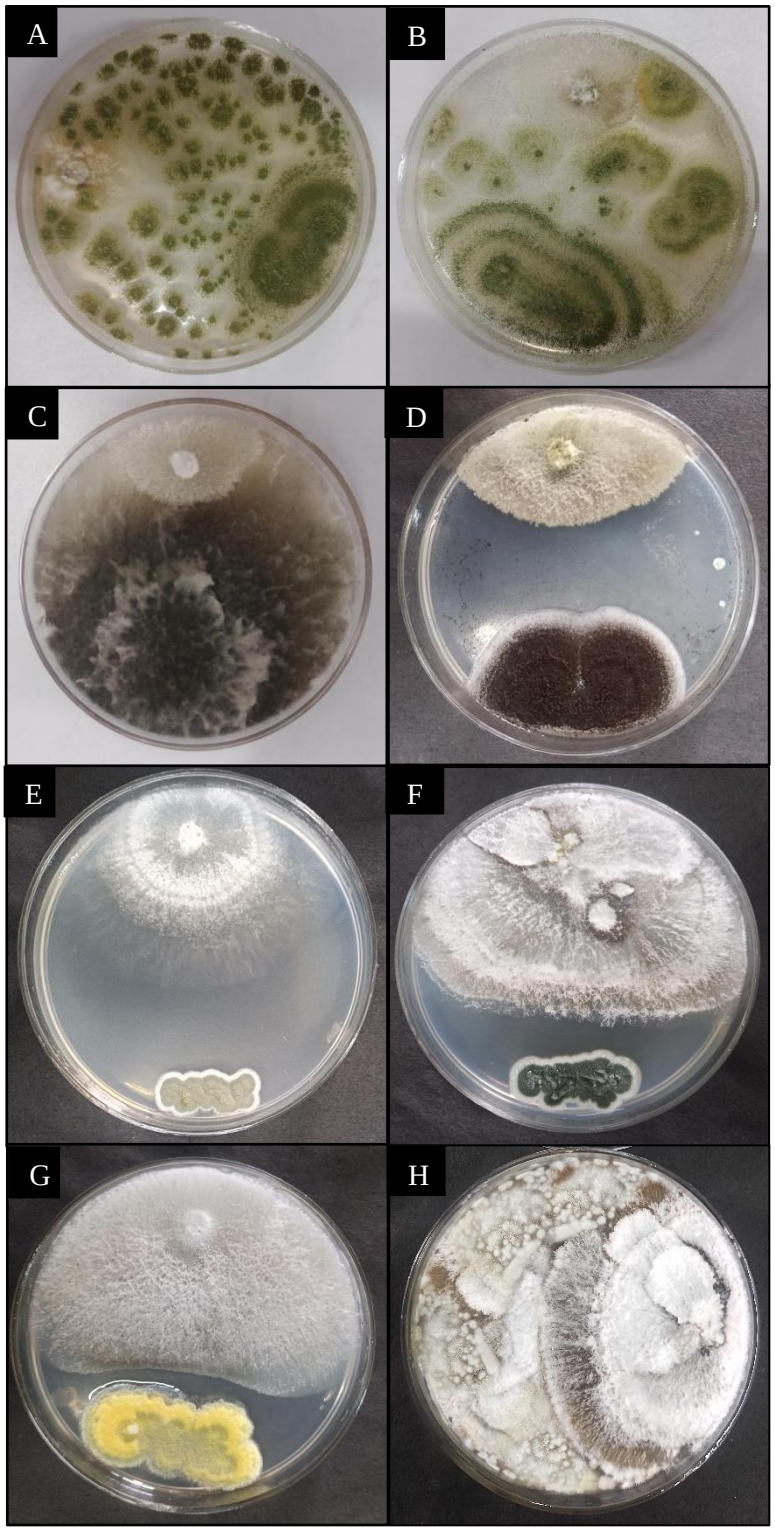

Plate 10: Antagonistic activity in endophyte isolates - A. *Aspergillus flavus*, **B.** *Aspergillus Oryzae*, **C.** Unidentified sp. 1, showing antagonism for space; **D.** *Aspergillus niger*, **E.** *Penicillium sp.* 1, **F.** *Penicillium sp.* 2, **G.** *Penicillium* sp. 3 showing antagonism by antibiosis; **H.** Control/ unidentified pathogen sp.

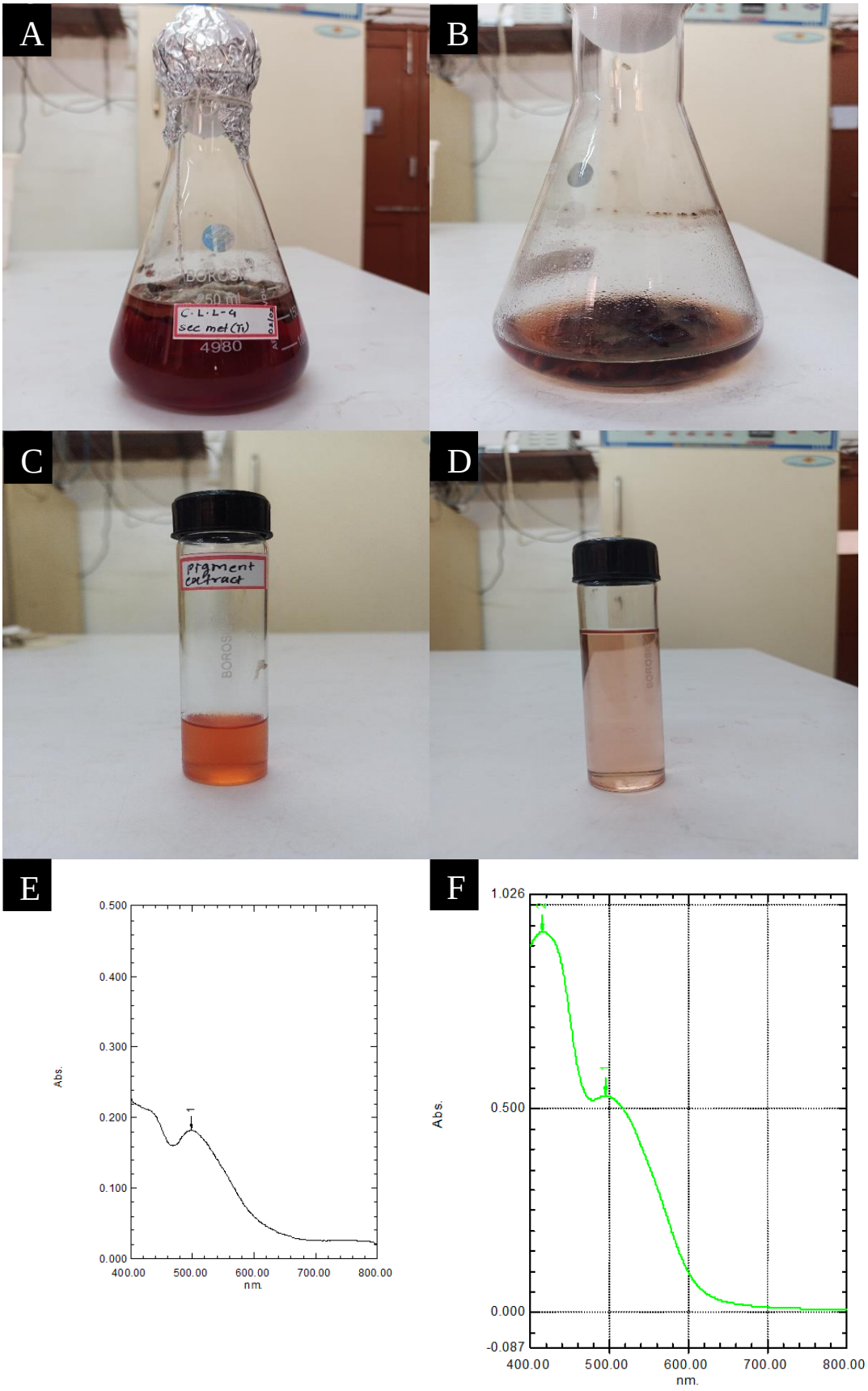

Plate 11: A. Culture broth of *Penicillium* sp. 1 secreting magenta red colored pigment in PD broth; **B.** Extraction of mycelial pigment of *Penicillium* sp. 1; **C.** Extracted mycelial pigment in Ethyl Acetate solvent; **D**. Extracted secretory pigment in Ethyl Acetate solvent ; **E.** Spectra of secretory pigments; **F.** Spectra of mycelial pigment.

4.1. Taxonomic description

Aspergillus Carbonarius (Bain.) Thom, J. Agric. Res. **(Plate 3. 1A & 1B)**

Colony diameters on PDA media 4.0-4.5 cm in 7 days at 28°C, basal mycelium white, velvety, marginal mycelium low; reverse white to dark grey; conidial heads globose to radiate or rarely splitting into loose columns, deep olive or black; stipes 1.2 µm, smooth to rough, thick-walled, uncolored to brown or reddish brown towards the vesicle; vesicles globose to sub-globose 41.6 µm in diameter. Metulae covering the upper half to the entire surface of the vesicle. Conidia spherical, 6.6 µm in diameter, spinulose when young, tuberculate at maturity.

Aspergillus flavus Link **(Plate 3. 2A & 2B)**

Colony diameters on PDA media 5.0 cm in 7 days at 28°C. Conidial heads radiate to the columnar. Stipes 1200 x 22 um in length, walls usually quite rough, occasionally finely roughened, generally uncolored, sometimes very pale brown; vesicles spherical to elongate 60 µm wide; variable in seriation, most isolates with at least 20% biseriate; metulae covering three quarters to the entire surface of the vesicle. Conidia globose to ellipsoidal 6.8 µm, with smooth to finely roughened walls.

Aspergillus niger van Tiegh **(Plate 3. 3A & 3B)**

Colony diameters on PDA media 4.1 cm in 7 days at 28°C. Conidiophores blackish brown to black, erect, straight or flexuous, 1.2 mm long. 16 µm thick, swollen at the apex into a spherical vesicle 64 µm diameter.Surface of vesicle covered. Conidia catenate, dry, usually globose, brown, sometimes with warts or spines arranged in discontinuous bands, 3-5 µm. Conidial heads at first globose, blackish brown to black, in age, often splitting into several loose but quite well-defined columns.

Aspergillus oryzae (Ahlburg) Cohn. Jahresber. **(Plate 4. 4A & 4B)**

Colony diameters on PDA media 4.2 cm in 7 days at 28°C. Radiate to loosely columnar, stipes 630 x 10.5 µm, walls uncolored, usually finely rough to rough; vesicles pyriform, clavate to subglobose, 33 µm wide, highly variable in seriation, some isolates predominantly uniseriate, others pre- dominantly biseriate; metulae or phialides covering half or more of the surface of the vesicle. Conidia smooth to finely roughened, occasionally spinose when first formed; globose to ovoid, sometimes ellipsoidal: 5.0 µm in length.

***Aspergillus* sp. 1 (Unidentified) (Plate 4. 5A & 5B)**

Colony diameters on PDA media 4.5 cm in 7 days at 28°C.Conidiophores green, erect, straight or flexuous,300 µm long. 10 µm thick, swollen at the apex into a spherical vesicle 35 µm diameter. Surface of vesicle covered. Conidia dry, usually globose, green 11.2 µm in diameter.

***Aspergillus* sp. 2 (Unidentified) (Plate 4. 6A & 6B)**

Colony diameters on PDA media 4.2 cm in 7 days at 28°C. Conidiophores white, pinkish, straight or flexuous, 200 µm long. 10 µm thick, swollen at the apex into a spherical vesicle 20 µm diameter. Surface of vesicle covered.

***Bipolaris* sp. (Unidentified) (Plate 5. 7A & 7B)**

Colony diameters on PDA media 9.0 cm in 7 days at 28°C. Hyphae hyaline, pale to dark brown or grey. Conidiophores pale to dark brown, single, branched, sometimes arranged in small groups, straight to flexuous or geniculate. Conidiogenous nodes smooth to slightly verruculose. Conidia mostly curved,canoe-shaped, fusoid or obclavate, rarely straight, 3–14-distoseptate (usually more than 6), hyaline, pale or dark brown, reddish brown or pale to deep olivaceous, germinating by. Septum ontogeny: first septum median to submedian, second septum delimits basal cell, and third delimits distal cell. Ascomata brown or black, immersed, erumpent, partially embedded or superficial, free or

on flat stroma, mostly globose to ellipsoidal, sometimes flask-shaped or flattened on hard substrata, smooth or covered with vegetative filaments. Ostiole central, papillate, or with a sub-conical, conical, paraboloid, or cylindrical neck. Peridium comprising pseudoparenchymatous cells of equal thickness or slightly thickened at apex. Hamathecium comprising septate, filiform, branched pseudoparaphyses. Asci 2–8-spored, clavate, cylindrical-clavate or broadly fusoid, straight or slightly curved, thin-walled, bitunicate, fissitunicate, often becoming more orless distended prior to dehiscence, short pedicellate, rounded at apex. Ascospores fasciculate, filiform or flagelliform, hyaline or sometimes pale yellow or pale brown at maturity, septate, helically coiled within ascus, degree of ascospore coiling moderate to very strongly coiled, sometimes with free ends, often with a thin mucilaginous sheath.

***Fusarium* sp. (Unidentified) (Plate 5. 8A & 8B)**

Colonies on PDA plates attaining a diameter of 2.1 cm within 7 days at 28°C. Colour of the colony is densewhite, forming brownish-red pigment in the medium. Hyphae thick-walled, hyaline, septate. Showing presence of chalmydospores of diameter 25 µm and macroconidia 47.5 ×7.5 µm in length.

***Penicillium* sp.** Link

Conidia are produced in basipetal succession, forming specialized conidiogenous cells called phialide, where the youngest is at the basal or proximal end of the chain. Phialides may be produced singly or (group) in branched metulae, which gives a bush-like appearance. Penicillium may contain branches and metulae (penultimate branches that bear a whorl of phialides), and branches are between stipes of conidiophore and metulae. Conidiophores are smooth, hyaline, or rough-walled. Conidia are in long dry chains, divergent or globose, ellipsoidal cylindrical or fusiform hyaline greenish smooth or rough walled Sclerotia are producedby some. Penicillium exists as deuteromycetes.

***Penicillium* sp. 1 (Unidentified) *(*(Plate 5. 9A & 9B)**

Colonies on PDA plates attaining a diameter of 1.5 cm within 7 days at 28°C, forming red

pigment inmedia. Conidiophores bluish green, erect, straight, smooth to rough, thick-walled 270 × 4.3 µm in length; metulate branched 16 × 1.6 µm in length; philiads whorled 12.9 × 1.6 µm in length; conidia smooth, long dry chains divergent, ellipsoidal green 3-6.5 µm in diameter.

***Penicillium* sp. 2 (Unidentified) (Plate 6. 10A & 10B)**

Colonies on PDA plates attaining a diameter of 1.1 cm within 7 days at 28°C. Conidiophores green, erect, straight, smooth to rough, thin-walled 133× 3 µm in length; metulate branched 23 × 3.3 µm in length; philiads whorled 20 × 3.17 µm in length; conidia smooth, long dry chains divergent, ellipsoidal green 1.5 -4.7 µm in diameter.

***Penicillium* sp. 3 (Unidentified) (Plate 6. 11A & 11B)**

Colonies on PDA plates attaining a diameter of 1.3 cm within 7 days at 28°C, producing yellow pigment inmedia. Conidiophores yellowish green, erect, straight, smooth to rough, thin-walled 197× 19.6 µm in length; metulate branched 16.1 × 3.2 µm in length; philiads whorled 11.4 × 1.6 µm in length; conidia smooth, long dry chains divergent, ellipsoidal green 19.6 µm in diameter.

***Penicillium* sp. 4 (Unidentified) (Plate 6. 12A & 12B)**

Colonies on PDA plates attaining a diameter of 1.5 cm within 7 days at 28°C. Conidiophores white to pinkish, erect, straight smooth to rough, thin-walled 17.1 × 1.5 µm in length; metulate branched 7.8 × 1.5 µm in length; philiads whorled 12.5 ×1.5 µm in length; conidia smooth, dry chains divergent, ellipsoidal whitish in color l.8µm in diameter.

***Penicillium* sp. 5 (Unidentified) (plate 7. 13A & 13B)**

Colonies on PDA plates attaining a diameter of 1.2 cm within 7 days at 28°C. Conidiophores bluish, erect, straight, smooth to rough, thin-walled 285× 1.5 µm in length; metulate branched 9.1 × 1.5 µm in length; philiads whorled 3.5 ×1.0 µm in length; conidia smooth, dry chains divergent, ellipsoidal

bluish in colour 4.4 µm in diameter.

***Trichoderma* sp. 1 (Unidentified) (plate 7. 14A & 14B)**

Colonies on PDA plates attaining a dia. of 1.6 cm within 7 days at 28°C. Conidiophores hyaline, erect, branched, bearing spore masses apically at phialides: phialides tapering toward apex. Conidia phialosporous, hyaline, ovate or ellipsoidal, 1-celled. Chlamydospores pale brown, s Phialides12.2 × 2.7 µm. Conidia 4.5 × 2.4 µm. Chlamydospores 12.5 µm in diameter.

4.2. Plant growth-promoting activities

4.2.1. Cellulolytic activity (Plate 8)

Fifteen isolates were screened for cellulolytic activity, out of which five were found to produce cellulase in culture medium. The fungal endophytes viz., *Aspergillus niger*, *Penicillium* sp. 2, *Penicillium* sp. 4, *Penicillium* sp. 5, *and Trichoderma* sp. showed a positive result for cellulolytic activity. *A. niger* shows the highest cellulolytic activity **(Table 5).**

4.2.2. Phosphate solubilization assay (Plate 9)

Out of 15 isolates screened for phosphate solubilization activity, four isolates showed positive results, The fungal endophytes viz., *Aspergillus. carbonarius*, *A. niger*, *Aspergillus* sp. 2, *Trichoderma* sp. showed positive results for P solubilization which was detected by clear zone of inhibition. *A. niger* showed highest P solubilizing activity.**(Table 5).**

4.2.3. Antagonism assay by dual culture method (Plate 10)

The isolated Pathogen species from *Curcuma longa* was showing colonization frequency of 100 % in the infected leaf tissues. It was not showing sporulation on PDA media as well as on PDA media supplemented with extract of *C. longa* leaf however it was growing as white mycelial mass reaching upto 9 cm diameter in 7 days. It produces yellow colored exudates in PDA media.

The disease symptoms were restricted to host plant *C. longa* and were not seen in other host plants i.e. *Kaempferia rotunda* growing in same vicinity as well as in *C. decipiens* which was growing at Goa University site.

Seven isolates belonging to the host plant species *C. longa* were subjected to antagonism assay by dual culture method, out of which three isolates were found to be showing antagonism for space, and four were showing antagonism by antibiosis, which was detected by a clear zone of inhibition. The fungal endophytes viz., *A. flavus*, *A. Oryzae*, Unidentified sp. 1, showed antagonism for space, while *A. niger*, *Penicillium* sp. 1, *Penicillium* sp. 2, *Penicillium* sp. 3 showed antagonism by antibiosis. **(Table 5).**

4.3. Pigment production (Plate 11)

Fungal isolates showing production of colored exudatest in a growth medium were cultured on liquid media (PD broth) to extract the compound produced. After extraction of those compounds using ethyl acetate andspectroscopic analysis at the visible range, *Penicillium* sp. 1 was found to be producing an extracellular/secretory pigment which was absorbing at the wavelength 496 nm and intracellular/mycelial pigments, which were absorbing at 415 nm and 495 nm.

The color of the secretory pigment was magenta red, and the mycelial pigment showed an orange-red color. Other isolates when subjected to UV-Vis spectrophotometry did not show any peculiar peak in visible range which could possibly mean the colored compound produced is not a pigment as pigments absorb only in visible range i.e. 800 nm to 380 nm (Narendrababu and Shishupal, 2017) **(Table 6).**

4.4. CONCLUSION

Fungal endophytes play an important role in improving plant health. In this study, the diversity of fungal endophytes is studied along with their plant growth-promoting activities and pigment production.

The results of the present work can find potential applications in agriculture and industries. Understanding the role of fungal endophytes in promoting plant growth and resistance against pathogens could lead to the production of biofertilizers, which can help improve crop yields and reduce reliance on inorganic fertilizers. Identifying fungal endophytes capable of producing bioactive compounds, such as pigments, may have applications in biotechnology, including pharmaceuticals, cosmetics, and food additives.

Despite having many applications, studying fungal endophytes poses certain limitations, such as difficulty in culturing them in a laboratory. Endophytic fungal strains tend to lose their viability in less time than other classes of fungi. Therefore, it is of utmost importance to subculture them regularly. Some strains pose challenges such as sporulation *in vitro*, so new techniques must be developed. Pigment production depends on factors like pH, light, incubation period, *etc*. These factors could be studied to make pigment production more convenient.

REFERENCES

1. Alam, B., Lǐ, J., Gě, Q., Khan, M.A., Gong, J., Mehmood, S., Yuán, Y., Gǒng, W. (2021). Endophytic Fungi: From Symbiosis to Secondary Metabolite Communications or Vice Versa?.*Sec. Plant Symbiotic Interactions*, 12 – 2021. https://doi.org/10.3389/fpls.2021.791033

2. Ananda, K. and Sridhar, K.R. (2002). Diversity of endophytic fungi in the roots of mangrove species on the west coast of India. *Canadian Journal of Microbiology,* 48(10), 871-878.

3. Arnold, A.E. (.2007). Understanding the diversity of foliar Endophytic fungi, progress, challenges, and frontiers. *Fungal. Biol.,* 21, 51- 66. https://doi.org/10.1016/j.fbr.2007.05.003

4. Arnold, A.E., Engelbrecht, B.M.J. (2007). Fungal endophytes double minimum leaf conductance in seedlings of a tropical tree. *Journal of Tropical Ecology*, 23(3), 369–372.https://doi.org/10.1017/S0266467407004038

5. Babitha, S., Soccol, C.R., Pandey, A. (2007). Effect of stress on growth, pigment production and morphology of *Monascus sp.* In solid cultures, *Journal of Basic Microbiology* 47(2), 118-126.https://doi.org/10.1002/jobm.20061026

6. Baghel, V., Thakur, J.K., Yadav, S.S., Manna, M.C., Mandal, A., Shirale, A.O., Sharma, P., Sinha, N.K., Mohanty, M., Singh, A.B., Patra, A.K.(2020). Phosphorus and potassium solubilization from rock minerals by endophytic *Burkholderia sp*. Strain FDN2-1 in soil and shift in diversity of bacterial endophytes of corn root tissue with crop growth stage. *Geomicrobiol J*, 37, 550–563. https://doi.org/10.1080/01490451.2020.1734691

7. Baron, and Rigobelo, (2021). Endophytic fungi: a tool for plant growth promotion and sustainable agriculture. *Mycology*, 13(1), 39-55. https://doi.org/10.1080/21501203.2021.1945699

8. Brader, G., Compant, S., Vescio, K., Mitter, B., Trognitz, F., Ma, L.J., Sessiitsch, A. (2017). Ecology and genomic insights into plant-pathogenic and plant-nonpathogenic endophytes. *Annual Review of Phytopathology*, 55(1), 61–83. https://doi.org/10.1146/annurev-phyto-080516-035641

9. Burragoni, S. G., & Jeon, J. (2021). Applications of endophytic microbes in agriculture, biotechnology, medicine, and beyond. *Microbiological research*, 245, 126691. https://doi.org/10.1016/j.micres.2020.126691

10. Clay, K. (1988). Fungal endophytes of grasses: a defensive mutualism between plants and fungi. *Ecology* 69, 10–16.

11. Dhayanithy, G., Subban, K., Chelliah, K. (2019).Diversity and biological activities of endophytic fungi associated with *Catharanthus roseus*, *BMC Microbiology*, 19,22. https://doi.org/10.1186/s12866-019-1386-

12. Dikshit, R., Tallapragada, P. (2011). *Monascus purpureus* : A potential source for natural pigment production, *J. Microbiol. Biotech. Res.*, 1(4),164-174.

13. Elias, F., Woyessa, D., Muleta, D. (2016). Phosphate Solubilization Potential of Rhizosphere Fungi Isolated from Plants in Jimma Zone, Southwest Ethiopia, *International Journal of Microbiology*, 2016, 11. https://doi.org/10.1155/2016/5472601

14. Ellis, M.B. (1971).*Dematiaceous Hyphomycetes*. CABI Publishing.

15. Ellis, M.B. (1971).*More .Dematiaceous Hyphomycetes*. CABI Publishing.

16. Ginting, R.C.B., Sukarno, N., Widyastuti, U., Darusman, L.K.,Kanaya, S. (2013). Diversity of endophytic fungi from red ginger (*Zingiber officinale* Rosc.) plant and their inhibitory effect to *Fusarium oxysporum* plant pathogenic fungi. *HAYATI Journal of Biosciences* 20(3), 127-137. https://doi.org/10.4308/hjb.20.3.127

17. Hellwig, V., Grothe, T., Mayer-Bartschmid, A., Endermann, R., Geschke, F.U.,Henkel, T, Stadler, M.A. (2002). Altersetin, a new antibiotic from cultures of endophytic *Alternaria spp.* Taxonomy, fermentation, isolation, structure elucidation and biological activities. *J. Antibiot*, 55(10), 881-892. https://doi.org/10.7164/antibiotics.55.881

18. Kalra, R., Conlan, X. A., Goel, M. (2020). Fungi as a Potential Source of Pigments: Harnessing Filamentous Fungi, *Front. Chem*., 8. https://doi.org/10.3389/fchem.2020.00369

19. Kavroulakis, N.S., Zervakis, G.I., Ehaliotis, C., Haralampidis, K.,Papadopoulou, K.K. (2007). Role of ethylene in the protection of tomato plants against soil-borne fungal pathogens conferred by an endophytic *Fusarium solani* strain. *J. Exp. Bot*. 58(14), 3853-3864. https://doi.org/10.1093/jxb/erm230

20. Khan, A.L., Hamayun, M., Kang, SM. *et al.* (2012). Endophytic fungal association via gibberellins and indole acetic acid can improve plant growth under abiotic stress: an example of *Paecilomyces formosus* LHL10. *BMC Microbiol* 12, 3. https://doi.org/10.1186/1471-2180-12-3

21. Klich, M.A. (2002). *Identification of common Aspergillus species.* Centraalbureau voor Schimmelcultures, Utrecht.

22. Kobayashi, D.Y., Palumbo, J.D. (2000). *Microbial* endophytes. CRC Press.

23. Kumar, K.M.P., Asish, G.R., Sabu, M., Balachandran, I. (2013). Significance of gingers (Zingiberaceae) in Indian System of Medicine – Ayurveda: An overview, *Anc Sci Life,* 32(4), 253–261. https://doi.org/10.4103/0257-7941.131989

24. Lechtenberg, M., Quandt, B., Nahrstedt, A. (2004). Quantitative determination of curcuminoids in Curcuma rhizomes and rapid differentiation of *Curcuma domestica* Val. And *Curcuma xanthorrhiza* Roxb. By capillary electrophoresis. *Phytochemical Analysis,* 15(3),152-158. https://doi.org/10.1002/pca.759

25. Manamgoda, D.S., Rossman A.Y., Castlebury, L.Y., Crous, P.W., Madrid, H., Chukeatirote, E., Hyde, K.D. (2014). The genus *Bipolaris*, *Studies in mycology*, 79, 221–288.

26. Mejı´, L.C., Rojas, I., Maynard, Z., Bael, S., Arnold, E., Hebbar, P., Samuels, J.J., Robbins, N., Herre, E. (2008). Endophytic fungi as biocontrol agents of *Theobroma cacao* pathogens. *Biological Control,* 46, 4–14. https://doi.org/10.1016/j.biocontrol.2008.01.012

27. Mitchell, A.M., Strobel, G.A., Hess, W.M., Vargas, P.N., Ezra,D. (2008). *Muscodor crispans*, a novel endophyte from *Ananas ananassoides* in the Bolivian Amazon. *Fungal Diversity*, 31, 37- 43.

28. Narendrababu, B.N., Shishupala, S. (2017). Spectrophotometric detection of Pigments from *Aspergillus* and *Penicillium* isolates, *Journal of Applied Biology & Biotechnology*, 5(01), 053-058. https://doi.org/10.7324/JABB.2017.50109

29. Nath, R., Sharma, G.D., Barooah, M. (2015). Plant growth promoting endophytic fungi isolated from tea (*Camellia sinensis)* shrubs of Assam, India, *Appl Ecol Environ Res*, 13 (3), 877-891. https://doi.org/10.15666/aeer/1303_877891

30. Orlandelli, R.C., Alberto, R.N.,Rubin, Filho, C.J.,Pamphile, J.A. (2012).Diversity of endophytic fungal community associated with *Piper hispidum* (Piperaceae) leaves. *Genet Mol Res*, 11(2), 1575-1585. https://doi.org/10.4238/2012.May.22.7

31. Pharis, P., King, R (1985).Gibberellins and reproductive development in seed plants. *Annual review of plant physiology*, 36(1), 517-568. https://doi.org/10.1105/tpc.003046

32. Rai M, Agarkar G. (2016). Plant-fungal interactions: what triggers the fungi to switch among lifestyles? *Critical Reviews in Microbiology*, 42(3), 428–438. https://doi.org/10.3109/1040841X.2014.958052

33. Rana, K.L., Kour, D., Kaur, T., Devi, R., Yadav, A.N., Yadav N., Dhaliwal, H.S., Saxena, A.K. (2020). Endophytic microbes: biodiversity, plant growth-promoting mechanisms and potential applications for agricultural sustainability. *Antonie Van Leeuwenhoek*, 113(8), 1075-1107. https://doi.org/10.1007/s10482-020-01429-y

34. Rao, M.P.N., Xiao, M., Wen-Jun, L. (2017). Fungal and Bacterial Pigments: Secondary Metabolites with Wide Applications, *Front Microbiol.* 8, 1113. https://doi.org/10.3389/fmicb.2017.01113

35. Reinhardt, D, Mandel, T., Kuhlemeier, C. (2000). Auxin regulates the initiation and radial position of plant lateral organs. Plant Cell, 12(4), 507–518.https://doi.org/10.1105/tpc.12.4.507

36. Rizali, A.,Aziza, N.L.,Sari, N. (2021).Antagonistic Activities of Endophytic Fungi Isolated from *Eleutherine palmifolia* Flower. *Pakistan Journal of Biological Sciences,* 24(10),1015-1021. https://doi.org/10.3923/pjbs.2021.1015.1021

37. Rodriguez, R.J., White Jr., J.F., Arnold A.E., Redman, R.S. (2008). Fungal endophytes: diversity and functional roles. *New Phytologist,*182, 314–330. https://doi.org/10.1111/j.1469-8137.2009.02773.x

38. Segaran, G., Sathiavelu, M. (2019) Fungal endophytes: a potent biocontrol agent and a bioactive metabolites reservoir. *Biocatal Agric Biotechnol,* 21, 101-284.https://doi.org/10.1016/j.bcab.2019.101284

39. Shrivastava, P., Kumar, R.,Yandigeri, M.S. (2015).In vitro biocontrol activity of halotolerant *Streptomyces aureofaciens* K20: A potent antagonist against *Macrophomina phaseolina* (Tassi) Goid, *Journal of Biological Sciences,* 24, 192–199. https://doi.org/10.1016/j.sjbs.2015.12.004

40. Singh, P., Singh, R. K., Guo, D. J., Sharma, A., Singh, R. N., Li, D. P., Malviya, M. K., Song, X. P., Lakshmanan, P., Yang, L. T., & Li, Y. R. (2021). Whole Genome Analysis of Sugarcane Root-Associated Endophyte *Pseudomonas aeruginosa* B18-A Plant Growth-Promoting Bacterium With Antagonistic Potential Against Sporisorium scitamineum. *Frontiers in microbiology*, 12, 628376. https://doi.org/10.3389/fmicb.2021.628376

41. Singh, V. K., & Kumar, A. (2023). Secondary metabolites from endophytic fungi: Production, methods of analysis, and diverse pharmaceutical potential. *Symbiosis (Philadelphia, Pa.)*, 1–15. https://doi.org/10.1007/s13199-023-00925-9.

42. Suebrasri, T., Harada, H., Jogloy, S., Ekprasert, J., Boonlue, S.(2020) Auxin-producing Fungal endophytes promote growth of sunchoke. *Rhizosph*, 16, 100-271. https://doi.org/10.1016/j.rhisph.2020.100271

43. Syukri, D., Darwis, D., Santoni, A. (2013). Simple characterization of anthocyanin from *Ficus padana* Burm.f. *Journal of Chemical and Pharmaceutical Research.* 5(12),1276-128.

44. Vinayarani G.,Prakash H.S (2018).Fungal endophytes of turmeric (*Curcuma longa* L.) and their Biocontrol potential against pathogens *Pythium aphanidermatum* And *Rhizoctonia solani. World Journal of Microbiology and Biotechnology,* 34(3), 49. https://doi.org/10.1007/s11274-018-2431-x

45. Win, P.M., Matsumura, E.,Fukuda, K. (2021).Effects of pesticides on the diversity of endophytic fungi in tea *plants. Microbial ecology* 82 (1), 62-72. https://doi.org/10.1007/s00248-020-01675-7

46. Yeh, Y.H., Kirshner, R. (2019). Diversity of Endophytic Fungi of the Coastal Plant *Vitex rotundifolia* in Taiwan, *Microbes Environ.,* 34(1), 59–63. https://doi.org/10.1264/jsme2.ME18075

Websites

1. https://letstalkscience.ca/educational
2. https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:796451-1/general information
3. https://tropical.theferns.info/viewtropical.php?id=Kaempferia+rotunda
4. https://www.britannica.com/plant/ginger-lily
5. https://www.ipni.org/
6. https:ncbi.nlm.nih.gov/ pmc/articles/PMC4488371/#B25