BIOSCIENCES BIOTECHNOLOGY RESEARCH ASIA, December 2024. Vol. **21**(4), p. 1671-1681

Diversity, Extracellular Enzyme Activity and Growth Promoting Potential of Fungal Isolates from the Roots of *Coleus aromaticus* Benth.

Sajitha Menon Kampurath[1]*, Sneha Poongodu Velayudhankutty[1] and Renju Krishna Valsamma[2]

[1]Department of Botany, Government Victoria College, Palakkad, Kerala, India.
[2]Department of Botany, Mercy College, Palakkad, Kerala, India.

https://dx.doi.org/10.13005/bbra/3335

(Received: 25 September 2024; accepted: 29 November 2024)

Coleus aromaticus Benth. a member of Lamiaceae family is a herbaceous plant with numerous medicinal properties. The present study was aimed at the isolation, characterization, extracellular enzyme activity and growth promoting ability of endophytic fungi from the roots of C. aromaticus collected from different parts of Palakkad, Kerala, India. A total of nine cultures grouped into five morphotypes including one non-sporulating taxa mostly belonging to Ascomycota were isolated. Their colonization rate and diversity index was determined. Extracellular enzyme activity and plant growth promotion studies were also carried out. Amylase activity was exhibited by all isolates, while none of them showed tyrosinase, protease, or laccase activity. Among the isolates, Fusarium sp. exhibited significant root and shoot length promotion in Vigna radiata seedlings, and its identification was confirmed through sequence analysis as Fusarium solani. The results indicated that the endophytic association has a positive role in promoting plant growth and revealed diverse mycoflora in the roots of Coleus aromaticus with various biological activities, highlighting the potential for further research into endophytes and their metabolites as a promising field.

Keywords: Coleus aromaticus; Extracellular Enzyme Activity; Fungal endophytes; Lamiaceae; Plant growth promotion.

Endophytic fungi are a unique group of organisms that reside within various tissues and organs of terrestrial and certain aquatic plants. These fungi establish infections that often go unnoticed, as the host tissues remain symptomless, at least temporarily [1]. Endophytic fungi are known to engage in mutualistic interactions with their host plants, primarily by enhancing the host's resistance to herbivores [2]. In most cases, fungi benefit from colonizing a plant host as they gain access to nutrients and protection from various abiotic stresses. Previously, mutualistic symbiosis was primarily associated with mycorrhizal fungi residing inside plant roots. However, it has been recently discovered that many other fungi, especially endophytic fungi, can also engage in mutualistic root symbioses [3]. Endophytic fungi, throughout most or all of their life cycle, inhabit plant tissues without causing visible symptoms. This diverse group of fungi includes dormant saprophytes and latent pathogens, occupying different habitats and positions in the food chain

*Corresponding author E-mail: sajithamenonk@gmail.com

at various stages of their life cycle. The duration of an endophytic fungus's latent period can vary over ecological timescales. The transition of an endophytic fungus to a parasitic, pathogenic, or saprophytic lifestyle can be triggered by a single locus mutation in its genome. It is believed that endophytic fungi have evolved from parasites or pathogens by extending their latency periods and reducing their virulence. However, the development of endophytes is likely more complex, involving multiple parallel and reverse trajectories influenced by various factors and selection pressures in different contexts [4].

Endophyte-associated plants release substances that trigger resistance. It was discovered that, after a pathogen challenge, symbiotic plants activate their defence system more rapidly than non-symbiotic plants [5]. Plant growth-promoting endophytes reside in plant tissues, and their close interaction enhances nutrient exchange, growth promoting hormones and enzyme activity. As a result, the established plant-endophyte relationship promotes plant health through several mechanisms [6]. A greater understanding of native plant endophytes might clarify their capabilities and opportunities in promoting plant growth.

Endophytes also represent a novel source of bioactive secondary metabolites. Several reports have shown that endophytes enhance the fitness of their host plants by direct production of bioactive secondary metabolites. It is evident that the secondary metabolites produced by the endophytes are medicinally important as like the phytochemicals, originally believed to be produced only by their host plants [7].

The Lamiaceae (Labiatae) family is renowned for its diversity and well-established reputation.. Many plants within this family possess a pleasant fragrance due to external glandular structures that produce volatile oils. *Coleus aromaticus*, also known as *Plectranthus amboinicus*, is a herbaceous plant belonging to this family. It is characterized by its large size, fragrant nature, and perennial growth habit. Its leaves are used in treatment of common cold, cough, headache and some common digestive disorders [8].

Coleus aromaticus has been worked out very well in terms of its phytochemistry and isolation of several chemical constituents has been done. Many endophytic fungi has been isolated from different parts of this plant, especially from the leaves and petiole which has the medicinal properties. Present study thus focus in the isolation, identification, extracellular enzyme activity, plant growth promoting traits of endophytic fungi from the roots of *Coleus aromatics* and molecular identification of the most promising endophyte from among isolated.

MATERIALS AND METHODS

Collection and Surface sterilization of the plant material

Fresh and healthy absorption roots of *C. aromaticus* were collected from its natural habitat from different parts of Palakkad, Kerala. Plants were carefully selected to be free from pathogens, diseases, and fertilizer application. The collected roots were then brought to the research laboratory of Govt. Victoria College, Palakkad to maintain sterile conditions throughout the experiments and subjected to a series of sterilization steps [9].

Inoculation of the plant material

Ampicillin was added to Potato Dextrose Agar (PDA), which was then transferred to petri plates in the sterilised inoculation chamber and allowed to solidify in the laminar air flow. Each Petri dish was inoculated with three root segments (1 cm each), for a total of nine segments (3x3=9). To confirm surface sterilization, sterilized spots were randomly imprinted on another PDA plate. The inoculated plates were incubated at 25°C for 14 days and observed regularly during this period. After two weeks, any fungal mycelium emerging from the cut ends of the root segments was sub-cultured, and pure cultures were stored at 4°C on PDA slants

Measurement of endophytic fungal diversity and occurrence

Measures of species richness and evenness are used to calculate species diversity. The Shannon diversity index (H') and Pielou's evenness index (J') was calculated as follows:

$$Saannon\ diversity\ index\ (H') = \Sigma ipi * (pi)$$
$$Pielou's\ evenness\ index\ (J') = H'/ln(S)$$

Measurement of fungal occurrence

Inoculated plates were observed regularly for two weeks and the colonization rate, colonization

frequency and isolation rate was calculated using standard formulas [10, 11].

Colonization rate (CR) = (Total number of plant tissue segments where fungal infection occured) / (Total number of segments incubated)

Isolation rate (IR) = (Total number of fungal isolates obtained from plant tissues) / (Total number of segments incubated)

Colonization frequency (CF) = (Number of plant segments colonized by single endophyte *100) / (Total number of segments observed)

Isolation frequency (IF) = (Total number of fungal isolates belonging to one species *100)/ (Total number of fungal isolates in that sample)

Microscopic and macroscopic examination of endophytic fungi

Microscopic examination was done by slide culture technique. The fungi was cultured on PDA media on the top of sterile cavity slide. Stained with lactophenol cotton blue, and examined under a low- and high-power in stereo microscope [12].

Macroscopic features of endophytic fungi were observed for its growth in Potato Dextrose Agar (PDA) after incubating for two weeks and both the sides of the petri dishes were observed regularly for nature, colour and texture of hyphae.

Detection of extracellular enzyme production

Endophytic fungus isolated from the roots of *Coleus aromaticus* were subjected to five different extracellular assays such as cellulase, amylase, laccase, tyrosinase and protease.

Amylolytic activity

The fungus was grown in Glucose Yeast Extract Peptone Agar (GYP) medium with 0.2% soluble starch. After incubation the plates were flooded with 1% iodine in 2% Potassium iodide and observed [13].

Cellulase activity

The medium used was Glucose Yeast Extract Peptone Agar supplemented with 0.5% Carboxy-methylcellulose. After 3–5 days of fungal growth, the plates were soaked in a 0.2% aqueous Congo red solution and destained with 1M NaCl for 15 minutes. Although the medium appeared red, the presence of yellow patches around the fungal colony indicated cellulose degradation [13].

Protease activity

The fungi were grown on GYP agar medium, adjusted to pH 6 with 0.4% gelatin, and protease assay was conducted. After 5 days of incubation, the culture plates were flooded with saturated aqueous ammonium sulphate and formation of a clear zone around the fungal colony indicated gelatin digestion, while undigested gelatin precipitated upon contact with the ammonium sulphate [14].

Tyrosinase activity

Tyrosinase activity of the fungal isolates was assessed by allowing them to grow on GYP agar medium. A mixture of 0.11% p-cresol and 0.05% glycine was applied to the fungal colony's surface after it had grown for five days. After 24 hours, culture plates were checked for the development of a reddish brown colour surrounding the colony, which denoted the presence of the tyrosinase enzyme [14].

Laccase activity

The medium containing 0.05g of 1-napthol per litre was used for assessing laccase activity. Due to laccase's oxidation of 1-napthol, the colourless medium turns blue as the fungus develops [13].

Plant growth promoting activity

Mung bean seeds (Vigna radiata L.) were surface-sterilized with sodium hypochlorite (2 minutes) and then rinsed with sterile distilled water for 2-3 times. A fungal culture suspension filtrate was then applied to the sterilized seeds, with 50 seedlings in each sample treated with 50 ml of the suspension. The seeds were left for 4 hours to facilitate inducer penetration, then incubated and air-dried aseptically in a laboratory environment at 25°C before further research. Seeds kept in sterilized distilled water served as the control [15]. Experiments were performed in triplicate for each sample. After germination, root length and shoot length of the seedlings were measured using ruler following standard protocols [16].

Molecular identification and sequence analysis

The DNA was isolated by using the NucleoSpin® Plant II Kit (MachereyNagel). The sequencing reaction was done in a PCR thermal cycler (GeneAmp PCR System 9700, Applied Biosystems) using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA)

following the manufacturer's protocol. The large subunit (LSU) ribosomal DNA primers were used for sequencing. The primers used were forward LROR(F) 5'- ACCCGCTGAACTTAAG C 3'- and reverse LR7 (R) 3'- TACTACCACCAAGATCT-5'. The sequences so received were analysed using the BLAST to find the closest match. The sequence quality was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and required editing of the obtained sequences were carried out using Geneious Pro v5.1 [17].

RESULTS AND DISCUSSION

The elucidation of fungal endophytes' abilities is a burgeoning field in research, offering ample scope for exploration and discovery. This study thus primarily aims to elucidate the fungal endophytes and their biological activities isolated from the roots of *Coleus aromaticus*.

Endophytic colonization in roots of *C. aromaticus*

A total of five endophytic fungi were obtained from the healthy root tissue of *C. aromaticus*, namely, *Trichoderma* sp. (TC1), *Fusarium* spp. (FC2, FC3, FC5) and an unidentified fungal endophyte (C4). The absence of any microbial growth in the imprinted petri dish confirmed the effectiveness of root segment sterilization. *Trichoderma* is a widely studied genus of fungi, well-known for its vast range of biological functions, potential as biocontrol agents, and capacity to stimulate plant development [18, 19].

The genus *Fusarium* is recognized as one of the most significant genera of fungi, as it is responsible for causing numerous plant diseases. However, it is one of the most prevalent endophytic fungal genera with about 70 species. It is a cosmopolitan genus of filamentous ascomycete fungi and is in the third level of endophytes after *Aspergillus* and *Penicillium* species [20, 21]. Studies have shown that *Fusarium* can form symbiotic relationships with host plants, and that it may help plants grow and develop their defence mechanisms highlighting its importance as a key endophyte in plant-microbe interactions and highlight its potential uses in biotechnology, agriculture, and ecological research [10].

Distribution of endophytic fungi in root cells of *C. aromaticus*

Thin sections were observed under a microscope. The endophytic fungal colonies was

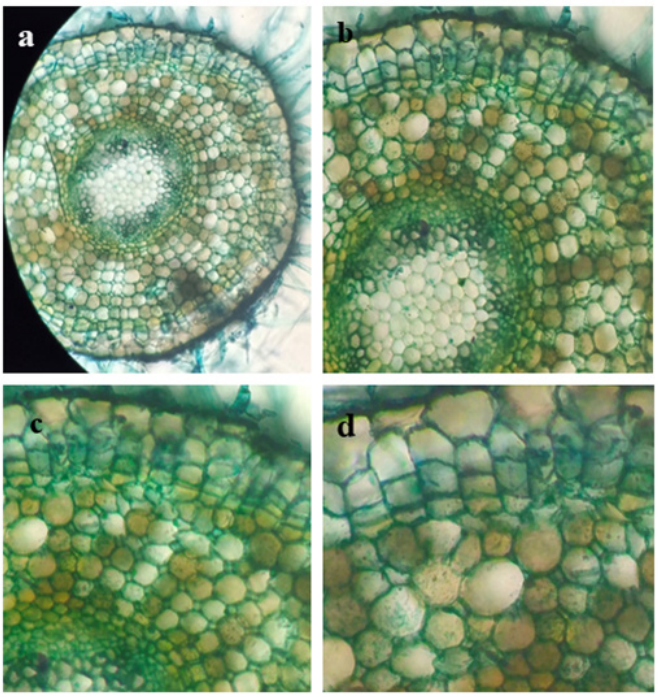

Fig. 1(a-d). Cross section of roots of *Coleus aromaticus*

observed as tiny dots and lines in the cortical cells. The cortical cells infected with endophytic fungi is depicted in Fig. 1 (a-d).

The results demonstrate the lactophenol cotton blue stain's effectiveness in detecting widespread colonisation along the cortex's cells, which indicates tight contact between endophytes in various structural and trophic sub niches in the host. Microscopy combined with lactophenol cotton blue staining provides valuable insights into the presence, distribution, and interactions of fungal endophytes within plant hosts [22].

Endophytic fungal diversity and occurrence

After a period of two to three days, fungal growth was observed in nine root segments that were inoculated into three petri plates. The fungal colonies exhibited distinct morphologies, a total of nine fungal isolates and five different types of endophytic fungi were identified. To obtain pure cultures, five morphologically different fungal isolates were sub cultured, as shown in Fig. 2. The endophytic fungal diversity observed is recorded in Fig.3.

Shannon diversity index and Pielou's evenness index of endophytic fungi from the roots of *C. aromaticus* were:

Shannon diversity index (H') =1.52

Pielou's evenness index (J') =1.6696

This study showed that the fungal endophytic colonization rate in the roots was 1, while the isolation rate was 0.55. The data is presented in Table 1.

According to many studies fungal diversity is higher in roots but endophytic diversity can change with samples, seasons and location [23]. In the present study, FC3 showed the

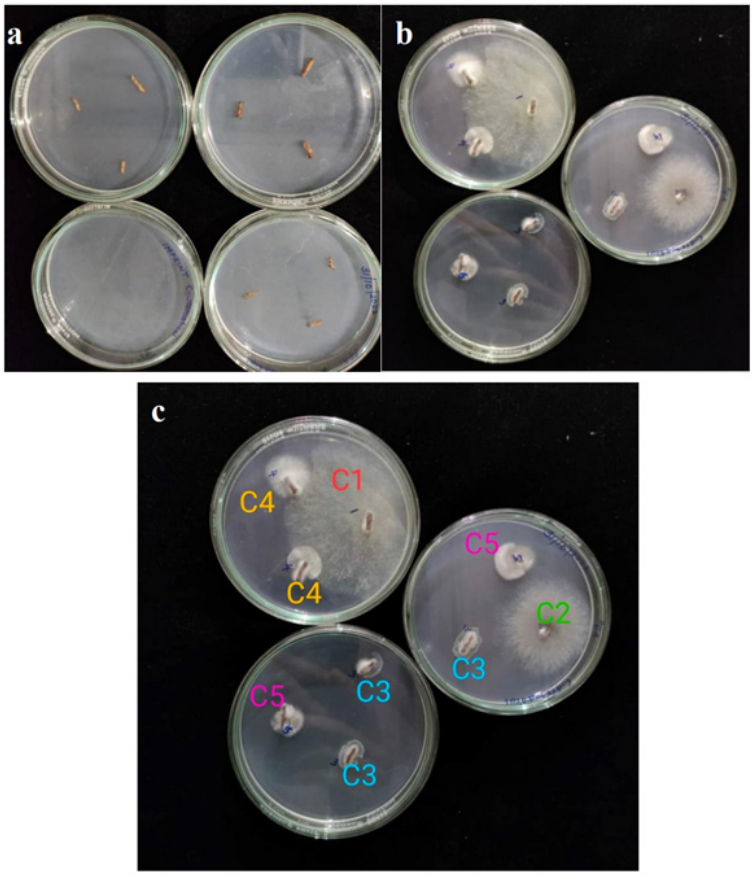

Fig. 2. Inoculation and colonization of endophytic fungi on PDA Plates
(a). Root segments on PDA medium and one imprint (on day of inoculation) (b). Fungal mycelial growth in inoculated plates after 3 days (c). Diversity of endophytic fungi (Position of each isolated fungi marked)

highest colonization and isolation frequencies, while TC1 and FC2 had the lowest. The isolation frequency indicated the abundance of endophytes from tissues among the total isolates. The results demonstrated that FC3 is the dominant endophyte in *C. aromaticus*. However, this study focused only on plants from Palakkad district and expanding to other regions could reveal greater endophytic diversity.

Table 1. Colonization rate, isolation rate, colonization frequency and isolation frequency in root tissues of *C. aromaticus*

Colonization rate(CR)	1
Isolation rate (IR)	0.55
Colonization frequency (CF)	TC1=11.11% FC2=11.11% FC3=33.33% FC5=22.22% C4=22.22%
Isolation frequency (IF)	TC1=11.11% FC2=11.11% FC3=33.33% FC5=22.22% C4=22.22%

Microscopic and macroscopic features of endophytic fungi from *C. aromaticus*

The microscopic characteristics of endophytic fungi are recorded in Table 2 and Fig. 4. The culture TC1 was recognized as *Trichoderma* sp., FC2 and FC3 has been identified as *Fusarium* spp. FC5 was identified as *Fusarium* sp. from molecular sequencing described later in this study. Thus establishing *Fusarium* spp. as dominant endophyte in the present study.

Many studies focusing on endophytes in Lamiaceae has revealed *Fusarium* spp. as a dominant and commonly occurring endophyte in different members of this family. *Fusarium* sp. has been isolated from *C. aromaticus*, and *Trichoderma* sp. from *Carissa carandas* [18]. Additionally, many species belonging to *Fusarium*, *Trichoderma*, and *Phoma* have been isolated as endophytes

Table 2. Microscopic characters of fungal endophytes of *C. aromaticus*

Isolate Name	Identification	Microscopic characters noted
TC1	*Trichoderma* sp.	Highly branched and less broad hyphae Conidiophores hyaline Phialides single or in group Conidia 1 celled, ovoid borne in small terminal clusters
FC2	*Fusarium* sp.	Conidiophores slender and simple Branched irregularly Conidia several celled, slightly curved or bent at pointed ends
FC3	*Fusarium* sp.	Conidiophores short and stout
FC5	*Fusarium* sp.	Sterile hyphae
C4	Unidentified sp.	Hyphae with spherical sporangia

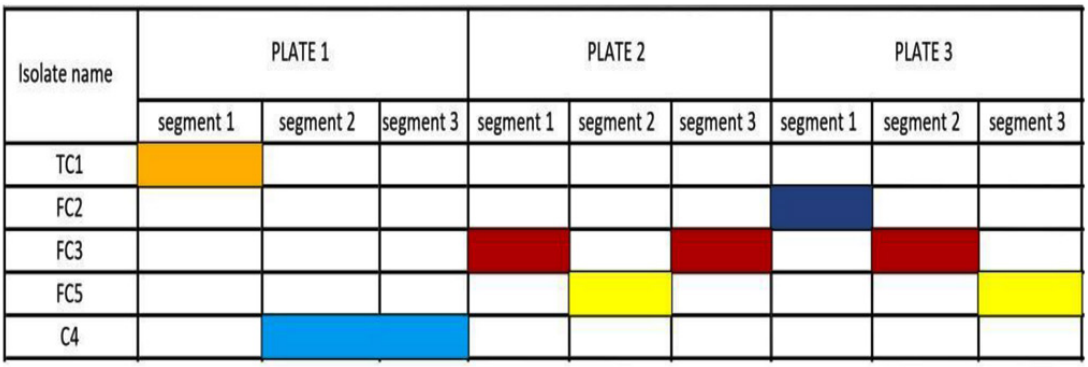

Fig. 3. Observed fungal diversity in *C. aromaticus*
Coloured cells represent the distribution of fungal isolates in each segment placed on PDA medium

from other Lamiaceae plants, including *Salvia officinalis*, *Leonurus cardiaca*, *Satureja hortensis*, and *Mentha piperita* [24].

While observing the microscopic features the existence of sterile mycelium as endophytes is not rare. However, identification of fungi on the basis of morphological and microscopic characters in absence of spores is quite challenging. Many of these mycelia do not produce spores on artificial media and may fail to do so even under nutrient stress or other adverse conditions. Overall, different fungi exhibit varying requirements of internal and external factors, either individually or in combination, to initiate sporulation [25].

In summary, these findings highlight the complex and diverse nature of sporulation in fungal endophytes and emphasize the significance of specific conditions for successful reproductive processes. Cultural characteristics of the fungal isolates and their morphological features are recorded in Table 3.

Cultural behaviours such as linear growth, colony color, pigmentation, and growth pattern play a crucial role in the identification of fungi. To distinguish different fungi, the structure, shape, and arrangement of conidiophores, phialides, and conidia are examined [26].

Detection of extracellular enzyme production

Fungal endophytes isolated from different plant sources have emerged as valuable resources for organic products in agriculture, industry, and biomedical applications. Many endophytes have exhibited the production of extracellular hydrolase enzymes, including pectinases, cellulases, lipases, amylases, laccases, xylanase, and proteases. These enzymes play a crucial role in the endophytes' mechanisms to resist pathogenic organisms and acquire nutrients from their host plants. Utilizing enzymes as a natural alternative to harmful chemicals has gained significant attention in recent years and hence there has been many promising researches focusing on enzyme exploration using microorganisms [27]. The extracellular enzyme activity of endophytic fungi from *C. aromaticus* roots is consolidated in Table 4.

All the isolates exhibited positive amylase activity, as indicated by the presence of a clear white halo around the colony. Amylase activity has been shown by many endophytes from Lamiaceae [14]. Fungal amylase finds wide applications in the food and pharmaceutical sectors. Only three isolates (FC3, C4, and FC5) exhibited a positive result for the cellulase test, indicated by the presence of a yellow region surrounding the

Table 3. Morphology of isolated endophytic fungi on PDA medium

Isolate name	Macroscopic features (Front view)	Macroscopic features (Back view)
TC1	White colored colony with green colored spores	Yellow color
FC2	Black colored colony with white patches	Black colored
FC3	White colored	White colored
FC5	Black and white	Black colored
C4	White	White colored

Table 4. Enzyme activity of endophytic fungi from *C. aromaticus*

Name of Fungal Isolates	Enzyme activity				
	Amylase	Cellulase	Protease	Tyrosinase	Laccase
TC1	+	-	-	-	-
FC2	+	-	-	-	-
FC3	+	+	-	-	-
C4	+	+	-	-	-
FC5	+	+	-	-	-

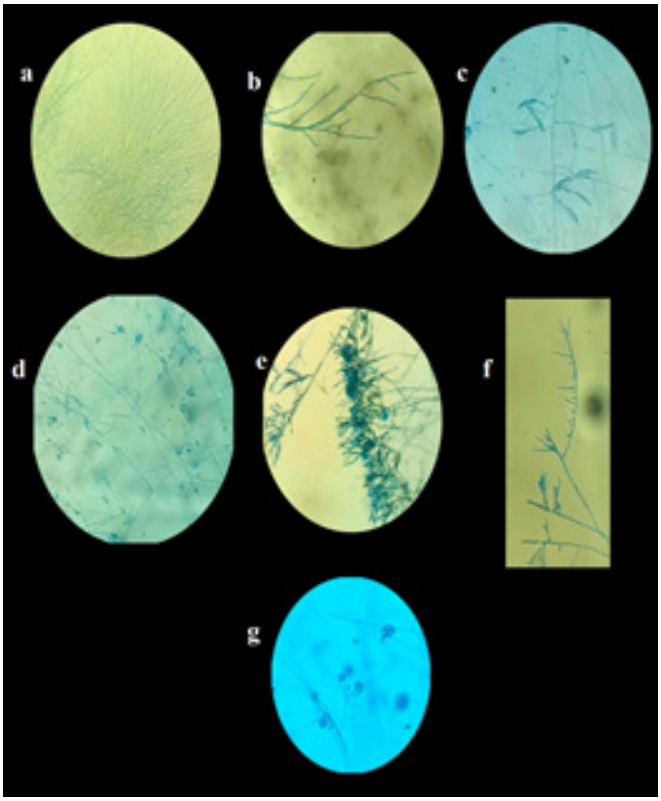

Fig. 4(a-g). Microscopic view of endophytic fungi (a & b).*Trichoderma* sp. TC1 (c). *Fusarium* sp. FC2 (d). *Fusarium* sp. FC3 (e & f). *Fusarium* sp. FC5 (g). Unidentified sp. C4

Table 5. Effect of fungal inoculations on the growth properties of *Vigna radiata*

	Mean root length (cm) in days			Mean shoot length (cm) in days		
	3	6	9	3	6	9
Control	1.32±0.69	3.46±0.14	5.19±0.19	0.28±0.1	1.54±0.77	6.2±0.51
TC1	0.55±0.35	1.98±0.57	5.52±0.69	0.2±0.06	1.86±0.55	7.03±0.32
FC2	1.48±0.23	1.8±0.09	3.48±0.23	0.22±0.01	2.11±0.66	3.07±0.43
FC3	0.87±0.16	0.99±0.13	2.71±0.09	0.13±0.06	0.31±0.09	0.56±0.04
C4	0.87±0.21	2.43±0.15	5.03±0.34	0.06±0.01	3.84±0.03	8.51±0.43
FC5	0.89±0.30	2.79±0.12	5.93±0.15	0.12±0.03	4.39±0.05	8.53±0.35

colony. Cellulase activity may not be observed in all endophytes tested [28]. The enzymes such as pectinases, cellulases, and lipases produced by endophytes render resistant mechanisms against pathogenic organisms and also for gaining nutrients from the host. The extracellular enzymes secreted by endophytic fungi have remarkable applications in food, textile, leather, confectionery, agriculture, beverage, and human health [29].

All fungal isolates demonstrated negative protease, tyrosinase and laccase activity. However, there are studies indicating positive protease activity by various *Fusarium* and *Trichoderma* species [13]. Tyrosinase is an enzyme involved in lignin degradation and melanin production also its activity is directly proportional to substrate concentrations [30]. Laccases have gained attention for their potential applications in pollution

detoxification and bioremediation of phenolic compounds. It is important to note that laccase production may vary depending on different plant parts [31].

The enzyme production differs between fungi and often corresponds to the requirements of its habitat and other factors like age of the host, climatic condition and geographical location, etc. Also, in the present study only limited extracellular enzyme activity assays were conducted, leaving scope for broader exploration.

Plant growth promoting activities

The assay was conducted *in vitro* to assess the true impact of the endophytes on plant growth. Mung bean seeds treated with fungal culture broth exhibited a faster germination and higher growth rate compared to untreated seeds (Table 5). Furthermore, the root length of treated seeds was found to be longer. Plant growth-promoting endophytes reside within plant tissues and their interaction promotes nutrient exchange and enzyme activity. These endophytes transmit growth hormones to plant tissues, promoting plant growth [32].

Endophytes have the ability to enhance plant growth by secreting various phytohormones. Endophyte culture filtrates has revealed the presence of different forms of gibberellins (GAs) and indole-3-acetic acid (IAA), which contribute to the growth-promoting abilities of endophytes [33]. The culture filtrate of *Fusarium fujikuroi*, also known as *Gibberella fujikuroi*, was the first identified source of gibberellins (GAs), which are plant hormones known to promote stem elongation. Additionally, *Fusarium* species are known to produce other hormones such as cytokinins (CKs), auxins, and ethylene [34]. Present study demonstrated that endophytic fungi, specifically FC5 isolate, contribute to plant growth promotion. Considering the results of enzymatic activity and the effect on growth promotion studies FC5 fungal isolate was subjected to molecular studies.

Molecular identification and sequence analysis

Molecular sequencing method was used to identify the isolate FC5.The obtained sequence was given as input in the BLAST web interface to get the closest match. The isolate FC5 was identified as *Fusarium solani*. The obtained sequence was submitted in NCBI under the accession number OR240869. *Fusarium solani* is a filamentous fungus [35]. This species is a varied complex of around 45 phylogenetic and/or biological species known as the *Fusarium solani* species complex (FSSC). These morphologically similar species are referred to as *F. solani*. They are common in soil and decomposing plant matter as well as has also been identified as host-specific pathogens for many crops of agricultural value. It has been isolated from other species of Lamiaceae [36].

Non-pathogenic *Fusarium* species isolated suggest a symbiotic (endophytic or mutualistic) relationship with their plant hosts. *Fusarium* members can live as saprophytes or endophytes in asymptomatic tissues of numerous plant species in a non-pathogenic manner and it may contribute to the health of their hosts by aiding in the adaptability to environmental challenges. However, these connections with host plants are most likely transient, and it should be emphasized that *Fusarium* spp. are capable of switching symbiotic lifestyles, and harmful isolates may emerge, particularly in commercial environments [37].

Both *Fusarium* and *Trichoderma*, falls under the categories of endophytes, saprobes, and plant pathogens [38]. Most endophytes are plant pathogens which resides inside the host plant. They causes various plant diseases therefore studies was mainly based upon to suppress their pathogenicity or virulence. But now, recent works have revealed about their benefits and their role in plant growth promoting activities. By controlling the gene expression of endophytes, they can be turned on to a most beneficial organism in the near future. Since molecular identification of all fungal strains isolated was not conducted, precise species-level identification is strongly recommended for all strains isolated for a more robust characterization of the endophytic fungal diversity.

CONCLUSION

Endophytes are a poorly understood class of organisms that can generate a wide range of bioactive substances. It is crucial to assess and emphasize the previous achievements, ongoing research, and recent advances in research related to endophytic microorganisms in order to bring the research community's attention to this emerging field and the potential exploitation of the available

sources for their therapeutic uses in various fields, such as the medical, pharmaceutical, food, and cosmetic industries.

ACKNOWLEDGEMENTS

The authors are thankful for the infrastructure provided by the PG & research Department of Botany, Govt. Victoria College, Palakkad and Principal, Govt. Victoria College, Palakkad for the facilities provided.

Funding Sources

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The authors do not have any conflict of interest.

Data Availability Statement

This statement does not apply to this article.

Ethics Statement

This research did not involve human participants, animal subjects, or any material that requires ethical approval.

Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Author Contributions

Sajitha Menon Kampurath: Conceptualization, Supervision, Methodology, Writing; Sneha Poongodu Velayudhankutty: Data collection, Analysis; Renju Krishna Valsamma: Editing; Each author mentioned has significantly and directly contributed intellectually to the project and has given their approval for its publication.

REFERENCES

1. Bacon C.W., White J. (Eds.). *Microbial Endophytes* (1st ed.). CRC Press. 2000
2. Faeth S. H., Fagan W. F. Fungal endophytes: common host plant symbionts but uncommon mutualists. *Integr Comp Biol*. 2002; 42(2): 360-368.
3. Schulz B., Boyle C. What are Endophytes? In: Schulz, B.J.E., Boyle, C.J.C., Sieber, T.N. (eds) *Microbial Root Endophytes*. Soil Biology, vol 9. Springer, Berlin, Heidelberg. 2006; pp 1-13.
4. Ganley R. J., Brunsfeld S. J., Newcombe, G. A community of unknown, endophytic fungi in western white pine. *Proceedings of the National Academy of Sciences*. 2004; 101(27):10107-10112.
5. Jalgaonwala R. E., Mohite B. V., Mahajan R. T. A review: natural products from plant associated endophytic fungi. *Jour Microbio Biotech Res*. 2011; 1(2): 21-32.
6. Waqas M., Khan A. L., Kamran M., Hamayun M., Kang S. M., Kim Y. H., Lee I. J. Endophytic fungi produce gibberellins and indoleacetic acid and promotes host-plant growth during stress. *Molecules*. 2012; 17(9):10754-10773.
7. Ancheeva E., Daletos G., Proksch P. Bioactive secondary metabolites from endophytic fungi. *Curr Med Chem.* 2020; 27(11): 1836-1854.
8. Jimmy J. L. *Coleus aromaticus* Benth. An update on its bioactive constituents and medicinal properties. *All Life*. 2021; 14(1):756-773.
9. Sahu P. K., Tilgam J., Mishra S., Hamid S., Gupta A., Jayalakshmi K., Verma S.K., Kharwar, R. N. Surface sterilization for isolation of endophytes: Ensuring what (not) to grow. *Jour Basic Microbiol.* 2022; *62*(6): 647-668.
10. Crasta G. L., Raveesha, K. A. Molecular identification of endophytic fungi associated with *Coleus forskohlii* (Willd.) Briq. *Jour App Biol Biotech*. 2021; 9(6): 162-172.
11. Uzma F., Konappa N. M., Chowdappa, S. Diversity and extracellular enzyme activities of fungal endophytes isolated from medicinal plants of Western Ghats, Karnataka. *Egypt Jour Basic Appl Sci.* 2016; 3(4): 335-342.
12. Jahan T., bin Mushtaq M., Bali N., Nargis S., ul Bashir Y. Clinico-mycological Study of Otomycosis Comparing the Cavity Slide Technique and the Conventional Agar Block Slide Culture. *Jour Clin Diagn Res.* 2019; 13(8): 8-11.
13. Sunitha V. H., Nirmala Devi D., Srinivas C. Extracellular enzymatic activity of endophytic fungal strains isolated from medicinal plants. *World Jour Agric Res.* 2013; 9(1): 1-9.
14. Pavithra N., Sathish L., Ananda, K. Antimicrobial and enzyme activity of endophytic fungi isolated from Tulsi. *Jour Pharm Biomed Sci.* 2012; 16(12): 2014.
15. Mahadevamurthy M., Channappa T. M., Sidappa M., Raghupathi M. S., Nagaraj A. K. Isolation of phosphate solubilizing fungi from rhizosphere soil and its effect on seed growth parameters of different crop plants. *Jour App Biol Biotech.* 2016; 4(6): 022-026.

16. Freschet G. T., Pagès L., Iversen C. M., Comas L. H., Rewald B., Roumet, C., McCormack M. L. A starting guide to root ecology: Strengthening ecological concepts and standardising root classification, sampling, processing and trait measurements. *New Phytol.* 2021; *232*(3): 973-1122.
17. Drummond A.J., Ashton B., Buxton S., Cheung M., Cooper A., Heled J., Kearse M., Moir R., Stones-Havas S., Sturrock S., Thierer T.,Wilson A. 2010; Geneious v5.1, available from
18. Gaikwad M. N., Bodke S. S., Kamble, L. H. Biodiversity of Fungal Endophytes from *Coleus aromaticus* (Benth.). *Int Jour Botany Stud.* 2017; 2: 64-67.
19. Guzmán-Guzmán P., Kumar A., de Los Santos-Villalobos S., Parra-Cota F. I., Orozco-Mosqueda M. D. C., Fadiji A. E., Santoyo G. *Trichoderma* species: Our best fungal allies in the biocontrol of plant diseases—A review. *Plants*. 2023; 12(3): 432.
20. Toghueo R. M. K. Bioprospecting endophytic fungi from *Fusarium* genus as sources of bioactive metabolites. *Mycology*. 2020; 11(1): 1-21.
21. Ahmed A. M., Mahmoud B. K., Millán-Aguiñaga N., Abdelmohsen U. R., Fouad, M. A. The endophytic *Fusarium* strains: a treasure trove of natural products. *RSC Adv*. 2023; 13(2): 1339-1369
22. Raja R. R. Medicinally potential plants of Labiatae (Lamiaceae) family: an overview. *Res Jour Med Plant*. 2012; 6(3): 203-213
23. Elmagzob A. A. H., Ibrahim M. M., Zhang G. F. Seasonal diversity of endophytic bacteria associated with *Cinnamomum camphora* (L.) Presl. *Diversity*. 2019; 11(7): 112
24. Zimowska, B. Diversity of fungi occurring on herbs from Lamiaceae family. *Phytopathol*. 2010; 56: 5-15.
25. Mattoo A. J., Nonzom S. Investigating diverse methods for inducing sporulation in endophytic fungi. *Studies in fungi*. 2022; 7(1): 1-10.
26. Sharma K. K., Singh U. S. Cultural and morphological characterization of rhizospheric isolates of fungal antagonist *Trichoderma*. *Jour Appl Nat Sci*. 2014; 6(2): 451-456.
27. Rana K. L., Kour D., Sheikh I., Yadav N., Yadav A. N., Kumar V., Saxena A. K. Biodiversity of endophytic fungi from diverse niches and their biotechnological applications. In: Singh, B. (eds) *Advances in endophytic fungal research: present status and future challenges*. Springer, Cham. 2019; pp 105-144. https://doi.org/10.1007/978-3-030-03589-1_6
28. Rahul Y., Ajay V. S., Samiksha J., Manish K. Antifungal and enzyme activity of endophytic fungi isolated from *Ocimum sanctum* and *Aloe vera*. *Afr. J. Microbiol Res.* 2015; 9(29): 1783-1788.
29. Bhadra F., Gupta A., Vasundhara M., Reddy M. S. Endophytic fungi: A potential source of industrial enzyme producers. *3 Biotech*. 2022; 12(4): 86.
30. Odeniyi O. A., Ogunsanya A., Unuofin, J. O. Optimization and Characterization of Tyrosinases from Multi-enzyme Producing *Fusarium solani* and *Fumago* sp. *Period Polytech Chem Eng.* 2019; 63(4): 582-590.
31. Shubha J., Srinivas, C. Diversity and extracellular enzymes of endophytic fungi associated with *Cymbidium aloifolium* L. *Afr Jour Biotechnol.* 2017; 16(48): 2248-2258.
32. Hassan S. E. D. Plant growth-promoting activities for bacterial and fungal endophytes isolated from medicinal plant of *Teucrium polium* L. *J. Adv. Res*. 2017; 8(6): 687-695.
33. Bilal L., Asaf S., Hamayun M., Gul H., Iqbal A., Ullah I., Hussain A. Plant growth promoting endophytic fungi *Asprgillus fumigatus* TS1 and *Fusarium proliferatum* BRL1 produce gibberellins and regulates plant endogenous hormones. *Symbiosis*. 2018; 76: 117-127.
34. Vrabka J., Niehaus E. M., Münsterkötter M., Proctor R. H., Brown D. W., Novák O., Bergougnoux V. Production and role of hormones during interaction of *Fusarium* species with maize (*Zea mays* L.) seedlings. *Front Plant Sci.* 2019; 9: 1936
35. Šišiæ A., Al-Hatmi A. M., Baæanoviæ-Šišiæ J., Ahmed S. A., Dennenmoser D., de Hoog G. S., Finckh M. R. Two new species of the Fusarium solani species complex isolated from compost and hibiscus (Hibiscus sp.). *Antonie van Leeuwenhoek*. 2018; 111: 1785-1805.
36. Zhang H. W., Song Y. C., Tan R. X. Biology and chemistry of endophytes. *Nat prod Rep*. 2006; 23(5): 753-771.
37. Habibi A., Mansouri S. M., Sadeghi, B. Fusarium species associated with medicinal plants of Lamiaceae and Asteraceae. *Mycol Iran*. 2018; 5(2): 91-101
38. Sarsaiya S., Jain A., Jia Q., Fan X., Shu F., Chen Z., Chen J. Molecular identification of endophytic fungi and their pathogenicity evaluation against *Dendrobium nobile* and *Dendrobium officinale*. *Int Jour Mol Sci*. 2020; 21(1): 316.