

Comparative Diversity of Endophytic Fungi Isolated from Medicinal Plants: *Centella asiatica*, *Datura* sp., *Wrightia tinctoria* and *Ocimum sanctum*

Geetanjali R.,¹ Dr Shubha.,² Aathiya Vanekar.,³ Prathibha K Y.,^{4*}

1, Associate Professor, Department of Botany, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India, 560001

2, Professor and Principal, Department of Botany, Government First Grade College, Vijayanagar, Bengaluru, Karnataka, India, 560104

3, M.Sc in Biotechnology Student, Indian Academy Degree College, Bangalore University, Bengaluru, Karnataka, India, 560043

4, Professor and Chairperson, Department of Botany, Maharani Cluster University, Palace Road, Bengaluru, Karnataka, India, 560001

Corresponding Author: Prathibha K Y

Email: marhoobbanu@gmail.com

Abstract

Endophytic fungi are microorganisms that inhabit internal plant tissues without causing apparent harm to their hosts and are recognized as important sources of bioactive compounds. The present study aimed to isolate and compare the diversity of endophytic fungi associated with five medicinal plants: *Centella asiatica*, *Datura stramonium*, *Datura sanguinea*, *Wrightia tinctoria*, and *Ocimum sanctum*. Healthy leaves, stems, and roots were collected and subjected to surface sterilization using commercial bleach, fungicide-antibiotic treatment, and mercuric chloride. Sterilized tissue segments were cultured on potato dextrose agar supplemented with ampicillin and incubated under controlled conditions. Emerging fungal colonies were purified and identified based on morphological characteristics using lactophenol cotton blue staining. A total of 49 endophytic fungal isolates belonging to several genera were recovered from the five plant species. *Centella asiatica* yielded the highest diversity with 16 isolates, followed by *Datura stramonium* with 13 isolates, *Ocimum sanctum* with 9 isolates, *Wrightia tinctoria* with 7 isolates, and *Datura sanguinea* with 4 isolates. The isolated fungi included species of *Aspergillus*, *Penicillium*, *Fusarium*, *Phoma*, *Talaromyces*, *Alternaria*, *Glomerella*, *Rhizoctonia*, *Pythium*, *Curvularia*, *Cladosporium*, *Trichoderma*, *Nigrospora*, *Bipolaris*, *Acremonium*, and others. Among these, *Aspergillus* and *Fusarium* species were the most frequently encountered across different host plants and tissues.

Keywords: Endophytic fungi, Medicinal plants, *Centella asiatica*, *Datura stramonium*, *Datura sanguinea*, *Wrightia tinctoria*, *Ocimum sanctum*, Fungal diversity, Endophytes, Plant-microbe interactions.

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Introduction

Endophytic fungi are microorganisms that live internally within apparently healthy and asymptomatic plant hosts. They inhabit plant tissues without causing visible disease symptoms or morphological damage to the host. These fungi are considered ubiquitous in nature, and studies have shown that almost every plant species examined so far hosts endophytes. In fact, no research has yet demonstrated the existence of a plant species completely devoid of endophytic microorganisms. One of the most remarkable characteristics of endophytic fungi is their high species diversity. Endophyte surveys frequently report complex assemblages consisting of more than 30 fungal species associated with a single host plant species. This high diversity reflects the ecological significance of endophytic fungi in plant ecosystems. These fungi are recognized as important sources of cytotoxic compounds, including anticancer molecules, as well as antibacterial substances. In addition to these pharmacologically valuable compounds, endophytic fungi can also act as biostimulants that enhance essential oil biosynthesis in plants. Furthermore, they contribute to nutrient solubilization within the plant rhizosphere, thereby improving nutrient availability for plant uptake. Endophytic fungi are also known to promote plant growth and can function as biological control agents that help protect plants from pathogens. In addition, they can activate systemic resistance mechanisms in plants, enabling them to tolerate both biotic stresses, such as pathogens and pests, and abiotic stresses, including environmental factors. Endophytic fungi reside within healthy plant tissues and form an integral part of the plant's microecosystem. The population dynamics and diversity of these fungi are influenced by several factors, including the genetic composition of the host plant, the age of the plant, and environmental conditions. Through long-term associations with their hosts, endophytic fungi establish symbiotic relationships that can be beneficial to plant growth and survival. These fungi produce a variety of important bioactive compounds such as alkaloids, diterpenes, flavonoids, and isoflavonoids. These compounds contribute to plant growth, enhance the plant's resilience to environmental stresses, and strengthen defence mechanisms against pests and diseases. They also promote the accumulation of secondary metabolites within the host plant. An important feature of endophytic fungi is their ability to produce bioactive compounds that are chemically identical or very similar to those synthesized by their host plants. Notable examples include Taxol, capsaicin, and piperine. The discovery that fungal endophytes can produce these valuable compounds has opened new possibilities for sustainable production through in vitro

cultivation of these microorganisms. Culturing endophytic fungi capable of producing such metabolites offers a practical alternative to harvesting large quantities of plants from natural populations. This approach reduces environmental pressure on medicinal plants, minimizes ecological damage, and ensures consistent production of bioactive compounds regardless of climatic conditions. As a result, endophytic fungi have gained considerable importance in biotechnology and industrial applications. They are currently being explored for their potential roles as biocontrol agents, antimicrobials, antitumor agents, antioxidants, antidiabetic agents, antibiotics, and insecticides.^{1,2,3}

Centella asiatica (L.) Urban., also known as Gotu kola, Indian pennywort or Pegaga, is a clonal, perennial herbaceous creeper belonging to the family Apiaceae (Umbelliferae). The plant grows widely in tropical and subtropical regions of the world including India, Pakistan, Sri Lanka, Madagascar, equatorial Africa, Central America and tropical Oceania. It commonly occurs in moist and swampy habitats such as wetlands, rice paddies and other pantropical environments, and in India it can grow up to an altitude of about 1800 m. Around twenty related species occur in wet tropical regions, sometimes even in rocky areas at higher elevations. The plant is characterized by small fan-shaped green leaves, white or light purple-to-pink flowers and small oval fruits. It is generally tasteless and odourless and thrives in and around water. The whole plant is used for medicinal purposes and has long been valued in many traditional medical systems. *Centella asiatica* has a long history of use in traditional medicine across Asia, including Ayurveda, traditional Chinese medicine and Southeast Asian ethnomedicine. Historical records indicate that it was used as a medicinal "panacea" in countries such as China, India, Africa, the Philippines, Sri Lanka and Madagascar for thousands of years. Due to its medicinal importance, the plant was included in the Indian Pharmacopoeia in the nineteenth century and later incorporated into several other pharmacopoeias such as the British Herbal Pharmacopoeia, Chinese Pharmacopoeia, German Pharmacopoeia and European Pharmacopoeia. Traditionally, the herb has been used for revitalizing nerves and brain cells, improving memory and promoting longevity. It has also been employed as a blood purifier and for managing high blood pressure. In Eastern healing practices it was used to treat emotional disturbances such as depression, which were believed to be associated with physical conditions. In addition, the plant has been used to relieve anxiety, improve cognition and treat various ailments including fever, diarrhoea, amenorrhoea and disorders of the female

genitourinary system. In dermatological treatments, *C. asiatica* has been widely applied for wound healing and for managing several skin conditions such as eczema, psoriasis, lupus, varicose ulcers, burns and hypertrophic scars. It has also been used traditionally for minor itching, insect bites, rheumatic conditions and venous insufficiency. Earlier medicinal uses included treatment for epilepsy, hysteria and leprosy, and studies conducted in the twentieth century reported positive results when the plant and its alcohol extracts were used in leprosy treatment. The therapeutic properties of *Centella asiatica* are mainly attributed to its bioactive compounds, particularly pentacyclic triterpenoids and saponins such as asiaticoside, madecassoside, asiatic acid and madecassic acid. The plant also contains flavonoids and essential oils that contribute to its pharmacological activity. These compounds promote fibroblast proliferation, stimulate collagen synthesis and increase intracellular fibronectin levels, thereby improving the tensile strength of newly formed skin and enhancing wound healing. They also help inhibit inflammatory processes involved in hypertrophic scars and keloids. Modern research has confirmed many of the traditional uses of *C. asiatica*, highlighting its anti-inflammatory, cognitive-enhancing and adaptogenic properties. The plant is also being investigated for applications in treating photoaging skin, cellulite and striae.^{4,5,6}

Datura is a genus of flowering medicinal herbs belonging to the Solanaceae family. It is widely distributed across Europe, Asia, America, South Africa, and many other tropical and subtropical regions. The plant can grow well in average soils but shows better growth in nutrient-rich, moist, or alkaline soils. Species of *Datura* are generally represented as annual or perennial herbs possessing glandular or sometimes simple hairs. Although the plant is well known for its intoxicant and hallucinogenic properties, it also possesses significant medicinal value and has long been used in traditional systems of medicine. Among the different species, *Datura stramonium* L., commonly known as Datura or Jimson weed, is one of the most widely recognized and studied species. The plant is an annual herb with an herbaceous base that is branched and glabrous, reaching a height of approximately one meter. The branches are firm, erect, leafy, and usually pale yellow or green in colour, often branching in a forked manner. The leaves are petiolate with simple leaf blades that are sinuate or completely dentate. They measure about 10–20 cm in length and 5–18 cm in breadth and are covered with soft, short greyish hairs. The upper surface of the leaves is typically dark greyish-green and soft, while the lower surface appears paler and slightly wrinkled when

dried. The flowers of *Datura* are solitary and occur in inflorescences located in the leaf axils or branch forks. These flowers are usually large and actinomorphic, supported by stout pedicels. The bracts, peduncles, and bracteoles are generally absent. The corolla is funnel-shaped and elongated with cuspidate lobes, giving the flower a distinctive appearance. In many cases, elongated anthers are present which dehisce longitudinally. The calyx is long, tubular, and swollen. The fruit is a dry capsule that is unevenly dehiscent and four-valved. It is typically unarmed and often delimited by the remnants of the persistent calyx. The fruits resemble walnuts and are covered with thorns, which is why the plant is often referred to as “thorn apple.” The seeds are numerous, dark, flat, and kidney-shaped, and they are laterally compressed with a curved embryo. The plant generally possesses 12 pairs of chromosomes.⁷

Ocimum sanctum (Tulsi) is an aromatic shrub belonging to the basil family Lamiaceae (tribe Ocimeae). It is believed to have originated in north-central India and is now found growing throughout the tropical regions of the eastern world. In Ayurveda, tulsi is highly valued and is known as “The Incomparable One,” “Mother Medicine of Nature,” and “The Queen of Herbs.” It is also regarded as an “elixir of life” because of its medicinal and spiritual significance. Within India, tulsi has been incorporated into spiritual rituals and lifestyle practices that are believed to provide numerous health benefits. According to Ayurvedic principles, tulsi has a hot and bitter taste and is believed to penetrate deep tissues, dry tissue secretions, and normalize kapha and vata. Daily consumption of tulsi is traditionally believed to prevent disease, promote general health, wellbeing, and longevity, and help individuals cope with the stresses of daily life. It is also credited with improving complexion, sweetening the voice, and promoting beauty, intelligence, stamina, and a calm emotional disposition. Tulsi is recommended in traditional practices for treating a wide range of conditions including anxiety, cough, asthma, diarrhea, fever, dysentery, arthritis, eye diseases, otalgia, indigestion, hiccups, vomiting, gastric disorders, cardiac and genitourinary problems, back pain, skin diseases, ringworm, insect, snake and scorpion bites, and malaria. Tulsi is also considered a potent adaptogen, meaning it helps the body adapt to stress and maintain balance. Modern scientific studies, including in vitro, animal, and human experiments, have examined the medicinal properties of tulsi. These studies have shown that tulsi possesses a wide range of pharmacological activities such as antimicrobial actions (including antibacterial, antiviral, antifungal, antiprotozoal, antimalarial, and anthelmintic), mosquito repellent activity, anti-diarrheal, antioxidant,

anti-cataract, anti-inflammatory, chemopreventive, radioprotective, hepatoprotective, neuroprotective, and cardioprotective effects. Additional reported activities include anti-diabetic, anti-hypercholesterolemic, anti-hypertensive, anti-carcinogenic, analgesic, antipyretic, anti-allergic, immunomodulatory, central nervous system depressant, memory-enhancing, anti-asthmatic, anti-tussive, diaphoretic, anti-thyroid, anti-fertility, anti-ulcer, anti-emetic, anti-spasmodic, anti-arthritis, adaptogenic, anti-stress, anti-leukodermal, and anti-coagulant properties.⁸

Wrightia tinctoria R. Br. Belongs to the family Apocynaceae and is commonly known as Sweet Indrajao, Pala Indigo Plant, and Dyer's Oleander. In South India, it is also called the "Jaundice curative tree." The plant is a small deciduous tree with light gray, scaly, smooth bark. It is native to India and Myanmar. The genus *Wrightia* is named after the Scottish physician and botanist William Wright. In Hindi, the plant is known as dhudi due to its preservative nature. The tree bears white fragrant flowers that may appear like snowflakes from a distance. The fruits are pendulous, long-paired follicles joined at their tips, and the hairy seeds are released when the fruit dehisces. The leaves of the tree yield a blue dye known as Pala Indigo. It is believed that a few drops of its sap added to milk can prevent curdling and enhance its shelf life without refrigeration. The wood of Sweet Indrajao is widely used for turnery work and is made into cups, plates, combs, pen holders, pencils, and bedstead legs. It is also commonly used for making Chennapatna toys. The plant has several medicinal uses. In traditional medicine. The seeds and bark are used as anti-diarrheal and anti-dysenteric agents. The juice of tender leaves is used in the treatment of jaundice, and crushed fresh leaves placed in the cavity of a decayed tooth help relieve toothache. In the Siddha medicine, the plant is used for psoriasis and other skin diseases. An herbal preparation known as Oil 777, prepared from the fresh leaves, has analgesic, anti-inflammatory, and antipyretic activities and is used in the treatment of psoriasis. Ethnomedicinally, the bark is used as a galactagogue and for treating abdominal pain, skin diseases, and wounds. It is also used as an antipyretic, anti-dysenteric, anti-diarrheal, and anti-hemorrhagic agent, and as an antidote for snake poison. The seeds are used as an aphrodisiac. The leaves are applied as a poultice for mumps and herpes and are sometimes chewed to relieve toothache. In folk medicine, dried and powdered roots of *Wrightia tinctoria* are mixed with milk along with *Phyllanthus amarus* and *Vitex negundo* and administered orally to women to improve fertility. The bark and seeds are also effective against psoriasis and non-specific dermatitis. The plant has

anti-inflammatory and anti-dandruff properties and is therefore used in hair oil preparations.⁹

Materials and Methods

Samples of fresh and healthy stems, leaves and roots were collected from Bangalore, India. The plant parts were thoroughly washed with running tap water, rinsing with sterile distilled water (SDW) and then treated with 20% commercial bleach with 0.1% Tween-20 for 5 min and later again rinsed in SDW. They were then soaked in 100 mL solution containing fungicide bavistin (30 mg), tetracycline (0.6 mg), rifampicin (0.6 mg) and 0.1% Tween-20 for 20 min and rinsed in SDW. All samples were finally surface sterilized with 0.1% mercuric chloride for 10 min, followed by five to six washings with SDW. The surface sterilized stem segments were cut into small pieces (1–2 mm), leaves were cut into 1 cm² pieces and placed on petri plates containing potato dextrose agar (PDA) supplemented with 100 mg/L ampicillin. The plates were labelled and incubated at 25 ± 2°C with 12 h photoperiod or in darkness. The fungal mycelia that emerged from the cut surface of the segments after several days were transferred on to fresh PDA plates.¹⁰ Fungi were mounted using lactophenol cotton blue method.¹¹

Results and Discussion

A comparative analysis of endophytic fungi isolated from five medicinal plants revealed considerable variation in fungal diversity among the host species. A total of 49 endophytic fungal isolates were recovered from leaves, stems, and roots of *Centella asiatica*, *Datura stramonium*, *Datura sanguinea*, *Wrightia tinctoria*, and *Ocimum sanctum*. Among the plants studied, *Centella asiatica* exhibited the highest endophytic fungal diversity, yielding 16 isolates representing genera such as *Alternaria*, *Aspergillus*, *Glomerella*, *Penicillium*, *Cladosporium*, *Cryptococci*, *Curvularia*, *Fusarium*, *Talaromyces*, and *Trichoderma* sp. *Datura stramonium* showed the second-highest diversity with 13 isolates, including species of *Phoma*, *Talaromyces*, *Acremonium*, *Aspergillus*, *Bipolaris*, *Penicillium*, *Glomerella*, and *Fusarium*. *Ocimum sanctum* yielded nine isolates belonging to *Aspergillus*, *Colletotrichum*, *Fusarium*, *Phoma*, and *Rhizoctonia*. Seven fungal isolates were obtained from *Wrightia tinctoria*, comprising *Aspergillus*, *Fusarium*, *Neocosmospora*, *Pythium*, *Rhizoctonia*, and *Rhizopus*. In contrast, *Datura sanguinea* exhibited the lowest diversity, with only four isolates represented by *Alternaria*, *Ceratobasidium*, *Nigrospora*, and *Pythium*. The distribution of endophytes varied among plant tissues. Stem tissues generally harbored a greater diversity of fungi than leaves and roots. In *Centella asiatica*, the stem contained eight fungal isolates compared to five in leaves and three in roots. Similarly, in *Datura stramonium*, stem tissues yielded the highest

number of isolates. In *Ocimum sanctum*, fungal diversity was greatest in leaves, followed by stems and roots. No fungal isolates were recovered from the roots of *Datura sanguinea*, whereas all three tissue types of *Centella asiatica*, *Datura stramonium*, and *Ocimum sanctum* supported endophytic colonization. Among the isolated fungi, species of *Aspergillus* were the most frequently encountered and were present in all plant species except *Datura sanguinea*. *Fusarium* species were also widely distributed and occurred in *Centella asiatica*, *Datura stramonium*, *Wrightia tinctoria*, and *Ocimum sanctum*. Other genera such as *Penicillium*, *Phoma*, *Talaromyces*, and *Rhizoctonia* were recovered from more than one host plant, while *Acremonium*, *Bipolaris*, *Ceratobasidium*, *Cryptococci*, *Neocosmospora*, *Nigrospora*, and *Rhizopus* were restricted to specific hosts.

Alternaria sp. was isolated from the leaves of *Centella asiatica* in the present study; similarly, Kunasakdakul et al. (2015) isolated *Alternaria brassicicola* from *Centella asiatica*¹², and Chaturvedi et al. (2017) reported *Alternaria* sp. among endophytic fungi from *C. asiatica*¹³. *Aspergillus nidulans* was isolated from leaves, and two other species of *Aspergillus* were isolated from leaves and roots of *Centella asiatica* in the present study; similarly, Nagda et al. (2017) reported *Aspergillus* sp. from *Calotropis procera*¹⁴. *Cladosporium* sp. was isolated from the stem of *Centella asiatica* in the present study; similarly, Nessa et al. (2023) isolated *Cladosporium* sp. from leaves, stems, and roots of *Centella asiatica*¹⁶. *Cryptococci* sp. was isolated from the stem of *Centella asiatica* in the present study; similarly, Isaeva et al. (2010) reported *Cryptococci* as endophytic yeast in plant tissues¹⁷. *Curvularia* sp. was isolated from the stem of *Centella asiatica* in the present study; similarly, Chaturvedi et al. (2017) reported *Curvularia* sp. from *C. asiatica*¹³. *Fusarium* sp. was isolated from the stem and root of *Centella asiatica* in the present study; similarly, Chaturvedi et al. (2017) reported *Fusarium* sp. from *C. asiatica*¹³. *Glomerella cingulata* was isolated from the leaves of *Centella asiatica* in the present study; similarly, Rakotoniriana et al. (2008) reported *Glomerella* sp.¹⁵. *Penicillium* sp. was isolated from the leaves and stems of *Centella asiatica* in the present study; similarly, Devi et al. (2012)¹⁸ and Nagda et al. (2017)¹⁴ reported *Penicillium* sp., and Holeyannavar et al. (2025) reported *Penicillium chrysogenum* from *Centella asiatica*¹⁹. *Talaromyces* sp. was isolated from the stems and roots of *Centella asiatica* in the present study; similarly, Holeyannavar et al. (2025) reported *Talaromyces verruculosus*¹⁹. *Trichoderma* sp. was isolated from the stem of *Centella asiatica* in the present study; similarly, Holeyannavar et al. (2024) reported *Trichoderma harzianum*²⁰. Chaturvedi et al. (2017) also reported *Colletotrichum gloeosporioides*,

Colletotrichum sp. *Fusarium* sp. *Curvularia* sp. *Nigrospora* sp. *Alternaria* sp. *Aspergillus* sp. and *Fusarium equiseti* from *Centella asiatica*¹³.

Aspergillus sp. (six species) was isolated from the stem, leaf and root of *Datura stramonium* in the present study; similarly, Nagda et al. (2017) reported *Aspergillus* sp. from *Calotropis procera*¹⁴. *Bipolaris* sp. was isolated from the stem of *Datura stramonium* in the present study; similarly, Tapfuma et al. (2019) reported *Bipolaris setariae* from *Datura stramonium*²¹. *Fusarium* sp. was isolated from the root and *Fusarium oxysporium* was isolated from the stem of *Datura stramonium* in the present study; similarly, Jayabhaskaran et al. (2018) reported *Fusarium solani* from *Datura metel*¹⁰. *Penicillium* sp. (two species) were isolated from the stem of *Datura stramonium* in the present study; similarly, Nagda et al. (2017) reported *Penicillium* sp. from *Calotropis procera*¹⁴. *Phoma* sp. (three species) was isolated from the leaves and stem of *Datura stramonium* in the present study; similarly, Yang et al. (2024) reported *Phoma* sp. from *Gastrodia elata*²². *Talaromyces* sp. was isolated from the leaves and root of *Datura stramonium* in the present study. Tapfuma et al. (2019) also reported *Gyroporus* sp., *Alternaria tenuissima*, *Alternaria alternata*, *Colletotrichum* sp., *Talaromyces* sp., *Alternaria* sp., *Sporothrix schenckii*, *Trichoderma* sp. *Pilobolus crystallinus*, *Rhodotorula mucilaginosa*, and *Bipolaris setariae* from *Datura stramonium*²¹.

Alternaria sp. was isolated from the leaves of *Datura sanguinea* in the present study; similarly, Spurr et al. (1975) reported *Alternaria* sp. from *Nicotiana* sp.²³, and Tapfuma et al. (2019) reported *Alternaria tenuissima* from *Datura stramonium*²¹. *Ceratobasidium* sp. was isolated from the leaves of *Datura sanguinea* in the present study; similarly, Abdul Rahman et al. (2019) reported *Ceratobasidium ramicola* from *Curcuma mangga*²⁴, and Verma et al. (2024) reported *Ceratobasidium* sp. from *Bergenian ciliata*²⁵. *Nigrospora* sp. was isolated from the stem of *Datura sanguinea* in the present study; similarly, Chaturvedi et al. (2017) reported *Nigrospora* sp. from *Centella asiatica*¹³.

In comparison with the findings of Yadav and Meena et al., (2025), the present study showed partial similarity in the composition of endophytic fungal communities associated with *Wrightia tinctoria*. The isolation of *Fusarium*, *Aspergillus* sp. *Aspergillus niger*, and *Neocosmospora* sp. in our study is consistent with their report, where these genera were also recorded among the dominant or recurrent endophytes.²⁶ Tamilvannan M.V et al., 2018 reported the presence of *Rhizopus* sp. as endophytic fungi in *Memecylon umbellatum*.²⁷ Similarly in the present study *Rhizopus* sp. was isolated from leaves of *Wrightia tinctoria*. Deepalakshmi Durairaj et al., 2022

reported the isolation of *Rhizopus delemar* as an endophytic fungus from *Abutilon indicum*. Similarly, in the present study, *Rhizopus* sp. was isolated from the leaves of *Wrightia tinctoria*.²⁸ Awad and Fayyadh et al., 2024 reported the isolation of binucleate *Rhizoctonia solani* from the roots of *Trachomitum venetum*. Similarly, in the present study, *Rhizoctonia* sp. was isolated from *Wrightia tinctoria*.²⁹ Namdev et al., (2024) reported the isolation of *Aspergillus* sp. and *Fusarium* sp. from *Ocimum sanctum*. Similarly, in the present study, *Aspergillus niger* was isolated from the leaf tissues, while *Aspergillus* sp. was obtained from the stem and root tissues of *Ocimum sanctum*.³⁰ Singh and Srivastava et al., (2025) reported the isolation of *Aspergillus*, *Fusarium*, *Alternaria*, *Curvularia*, and *Trichoderma* sp. from *Ocimum* sp. Similarly, in the present study, *Aspergillus niger* was isolated from the leaf tissues, while *Aspergillus* sp. was obtained from the stem and root tissues of *Ocimum sanctum*, indicating a comparable occurrence of *Aspergillus* species as dominant endophytes.³¹ Revathy et al., (2025) reported the isolation of *Colletotrichum* species from *Lumnitzera racemosa*. Similarly, in the present study, *Colletotrichum* sp. was isolated from the leaf tissues of *Ocimum sanctum*, indicating its occurrence as a common endophytic fungus across different host plants.³² Karthika and Rasmi et al., (2022) reported a clear tissue-specific distribution of endophytic fungi in *Ocimum* species, where certain fungi such as *Neopestalotiopsis*, *Trichoderma amazonicum* complex, and *Fusarium solani* complex were restricted to root segments, while *Diaporthe* sp. and *Daldinia eschscholtzii* were confined to stem tissues, and species like *Nigrospora oryzae* and *Pestalotiopsis microspora* were found only in leaf segments. They also noted that commonly reported genera from *Ocimum* include *Colletotrichum*, *Aspergillus*, *Fusarium*, *Penicillium*, and others, with some species such as *Aspergillus niger* complex showing occurrence across different plant parts.³³ The isolation of *Colletotrichum* sp. from leaf tissues in the present study is in agreement with the earlier report of Karthika and Rasmi et al., (2022), where *Colletotrichum* was recognized as a commonly occurring endophyte in *Ocimum*. Furthermore, the presence of *Aspergillus* species across all plant parts in the present study supports their observation of certain fungi exhibiting a wide distribution, while the overall pattern also reflects tissue-based specificity.³³ Awad and Fayyadh et al., 2024 reported the isolation of binucleate *Rhizoctonia solani* from the roots of *Trachomitum venetum*. Similarly, in the present study, *Rhizoctonia* sp. was isolated from *Ocimum sanctum*.²⁹ Bhagat et al., (2012) reported the occurrence of *Phoma* sp. as an endophytic fungus from traditional medicinal

plants. Similarly, in the present study, *Phoma* sp. was also isolated from *Ocimum sanctum*, indicating its common association as an endophyte in medicinal plant species.³⁴

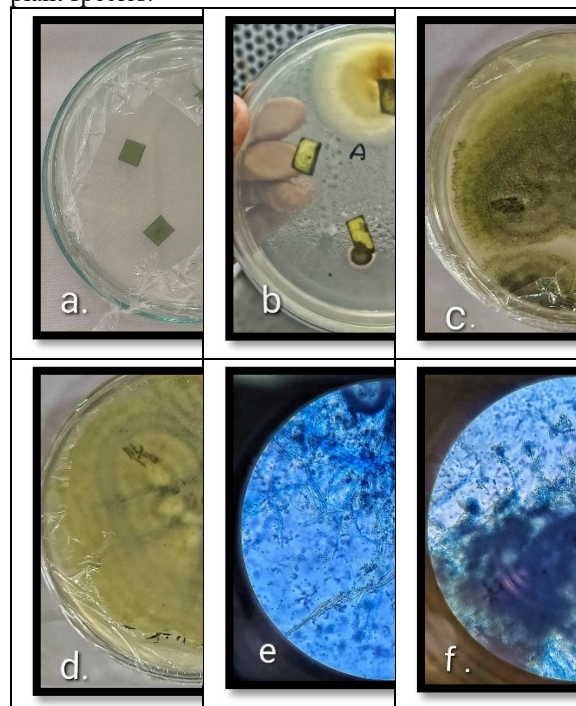
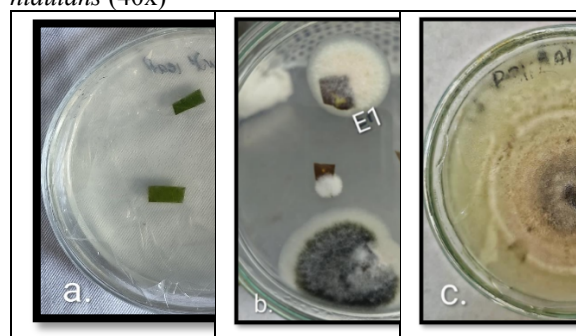


Fig 1; Cultured plates showing endophytic fungi isolated from *Centella asiatica* leaf: a: Inoculation of *Centella asiatica* leaf on culture media, b: Initial stage of fungal growth on the culture plate, c: Subcultured plates exhibiting active growth of *Aspergillus nidulans*, d: Backside view of the same culture plate, e: Microscopic observation of *Aspergillus nidulans* (10x), f: Microscopic observation of *Aspergillus nidulans* (40x)



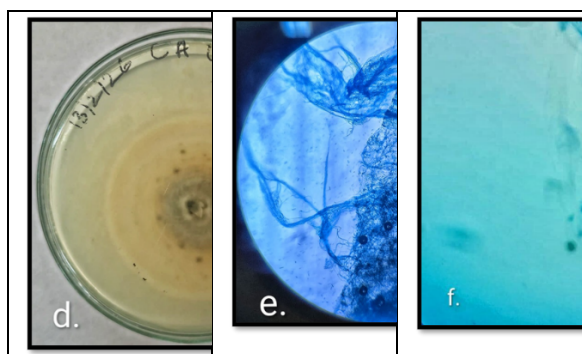


Fig 2; Cultured plates showing endophytic fungi isolated from *Centella asiatica* leaf: a: Inoculation of *Centella asiatica* leaf on culture media, b: Initial stage of fungal growth on the culture plate, c: Subcultured plates of E1 colony exhibiting active growth of *Glomerella cingulata*, d: Backside view of the same culture plate, e: Microscopic observation of *Glomerella cingulata* (10x), f: Microscopic observation of *Glomerella cingulata* (40x)

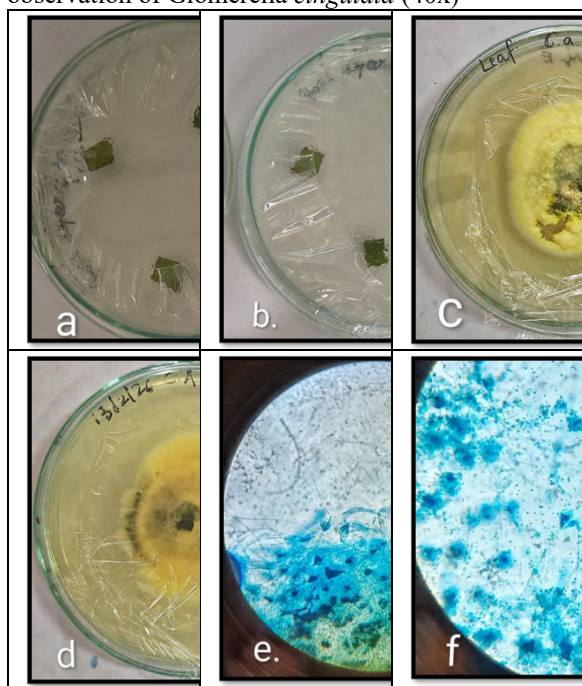


Fig 3; Cultured plates showing endophytic fungi isolated from *Centella asiatica* leaf: a: Inoculation of *Centella asiatica* leaf on culture media, b: Backside view of the inoculated culture plate, c: Subcultured plates exhibiting active growth of *Penicillium* sp. d: Backside view of the same culture plate, e: Microscopic observation of *Penicillium* sp. (10x), f: Microscopic observation of *Penicillium* sp. (40x)

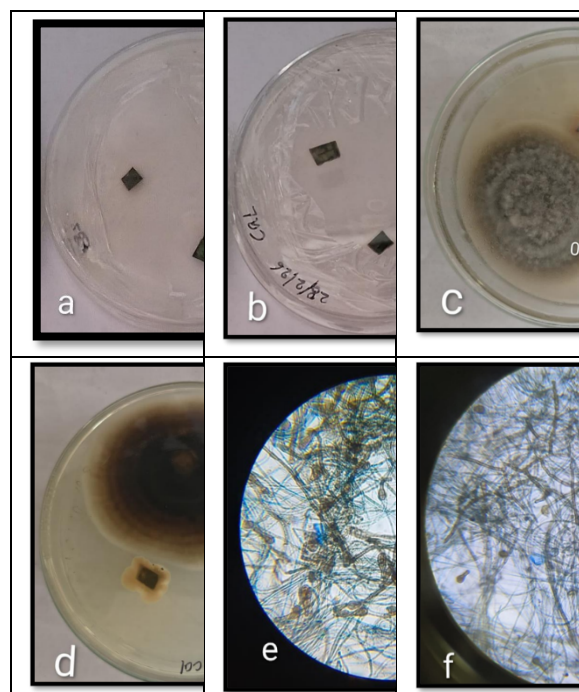


Fig 4; Cultured plates showing endophytic fungi isolated from *Centella asiatica* leaf: a: Inoculation of *Centella asiatica* leaf on culture media, b: Backside view of the inoculated culture plate, c: Fungal colony 01 showing growth characteristics of *Alternaria* sp. d: Backside view of the same culture plate, e: Microscopic observation of *Alternaria* sp. (10x), f: Microscopic observation of *Alternaria* sp. (40x)

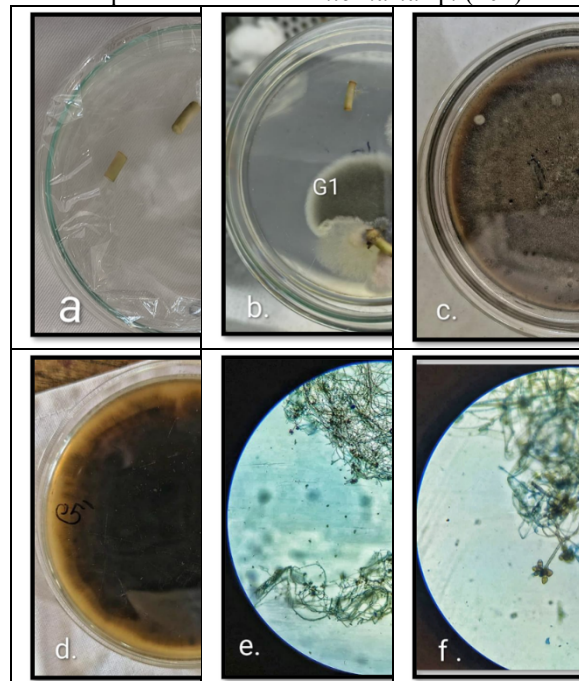


Fig 5; Cultured plates showing endophytic fungi isolated from *Centella asiatica* stem: a: Inoculation of *Centella asiatica* stem on culture media, b: Initial

stage of fungal growth on the culture plate, c: Subcultured plates of G1 colony exhibiting active growth of *Curvularia* sp. d: Backside view of the same culture plate, e: Microscopic observation of *Curvularia* sp. (10x), f: Microscopic observation of *Curvularia* sp. (40x)

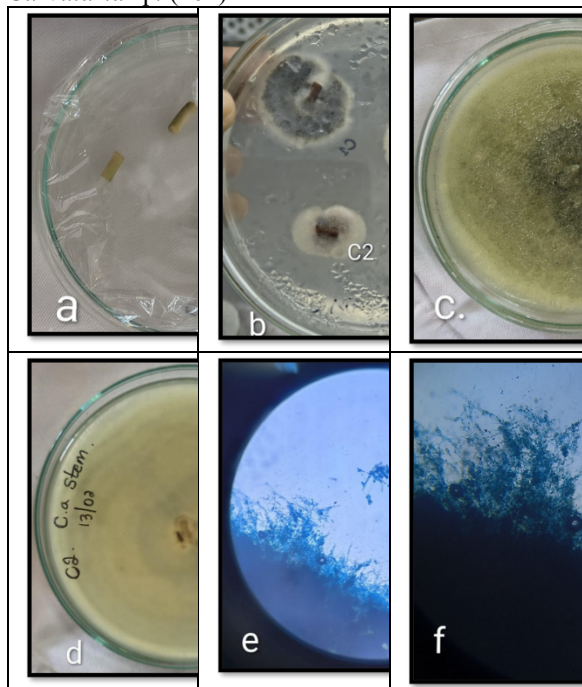


Fig 6; Cultured plates showing endophytic fungi isolated from *Centella asiatica* stem: a: Inoculation of *Centella asiatica* stem on culture media, b: Initial stage of fungal growth on the culture plate, c: Subcultured plates exhibiting active growth of *Penicillium* sp. d: Backside view of the same culture plate, e: Microscopic observation of *Penicillium* sp. (10x), f: Microscopic observation of *Penicillium* sp. (40x)

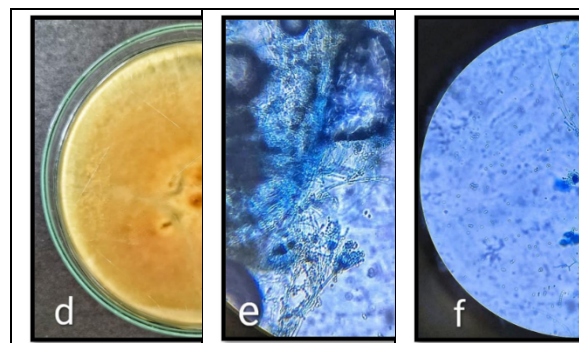
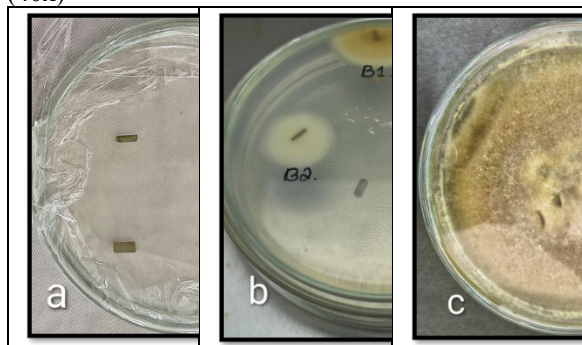


Fig 7; Cultured plates showing endophytic fungi isolated from *Centella asiatica* stem: a: Inoculation of *Centella asiatica* stem on culture media, b: Initial stage of fungal growth on the culture plate, c: Subcultured plates exhibiting active growth of *Penicillium* sp. d: Backside view of the same culture plate, e: Microscopic observation of *Penicillium* sp. (10x), f: Microscopic observation of *Penicillium* sp. (40x)

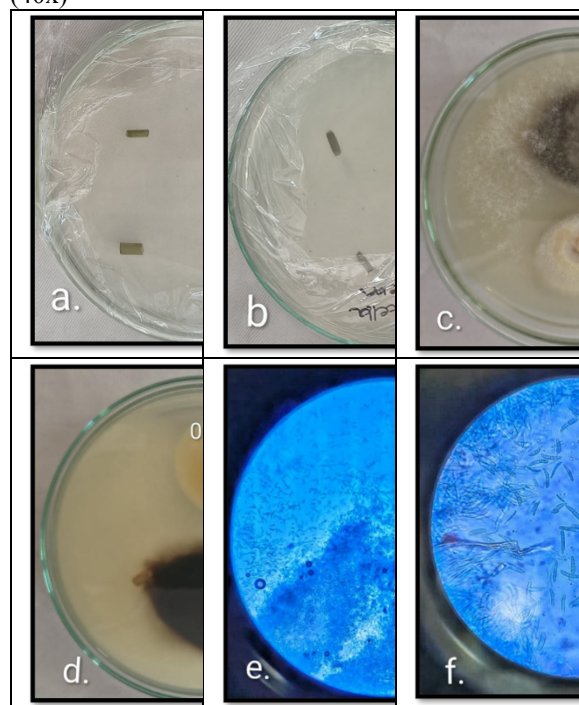


Fig 8; Cultured plates showing endophytic fungi isolated from *Centella asiatica* stem: a: Inoculation of *Centella asiatica* stem on culture media, b: Backside view of the inoculated culture plate, c: Fungal colony 01 showing growth characteristics of *Fusarium* sp. d: Backside view of the same culture plate, e: Microscopic observation of *Fusarium* sp. (10x), f: Microscopic observation of *Fusarium* sp. (40x)

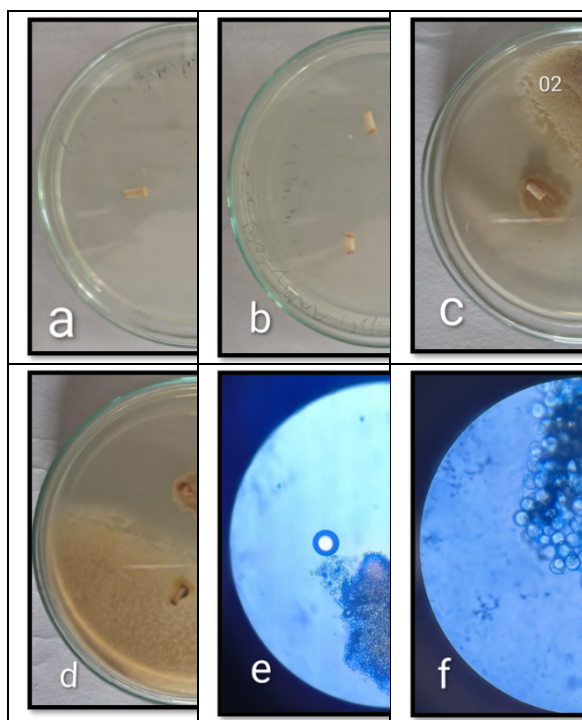


Fig 9; Cultured plates showing endophytic fungi isolated from *Centella asiatica* stem: a: Inoculation of *Centella asiatica* stem on culture media, b: Backside view of the inoculated culture plate, c: Fungal colony 02 showing growth characteristics of *Cryptococci* sp. d: Backside view of the same culture plate, e: Microscopic observation of *Cryptococci* sp. (10x), f: Microscopic observation of *Cryptococci* sp. (40x)

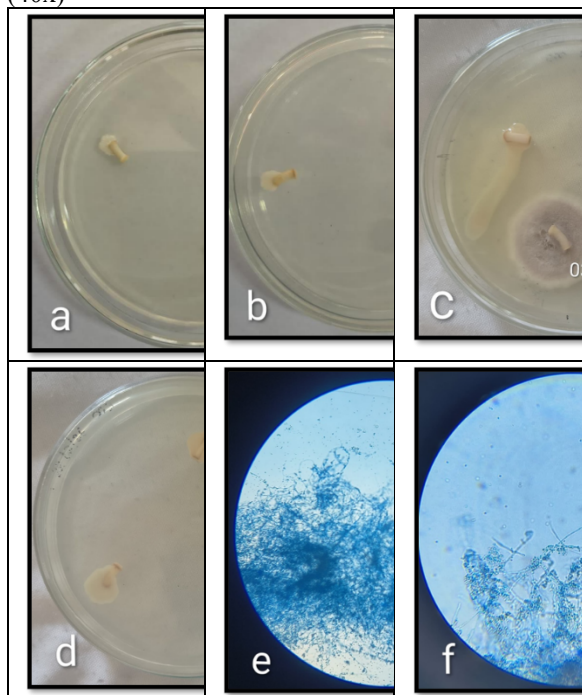


Fig 10; Cultured plates showing endophytic fungi isolated from *Centella asiatica* stem: a: Inoculation of *Centella asiatica* stem on culture media, b: Backside view of the inoculated culture plate, c: Fungal colony 03 showing growth characteristics of *Talaromyces* sp. d: Backside view of the same culture plate, e: Microscopic observation of *Talaromyces* sp. (10x), f: Microscopic observation of *Talaromyces* sp. (40x)

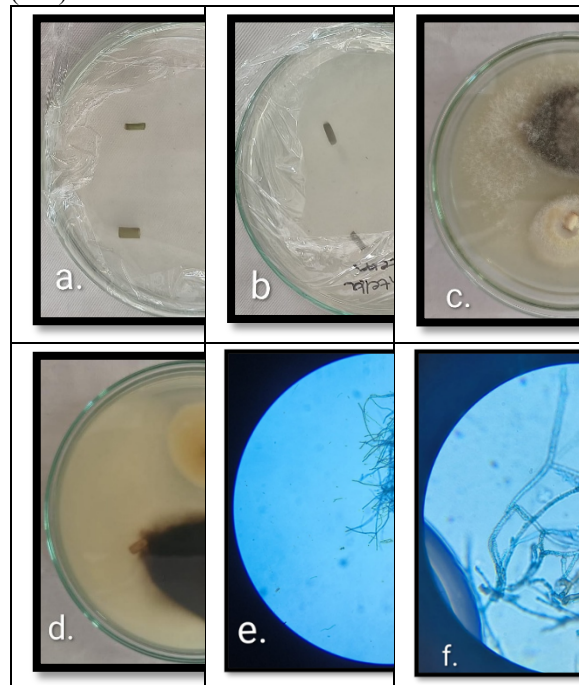


Fig 11; Cultured plates showing endophytic fungi isolated from *Centella asiatica* stem: a: Inoculation of *Centella asiatica* stem on culture media, b: Backside view of the inoculated culture plate, c: Fungal colony 09 showing growth characteristics of *Trichoderma* sp. d: Backside view of the same culture plate, e: Microscopic observation of *Trichoderma* sp. (10x), f: Microscopic observation of *Trichoderma* sp. (40x)

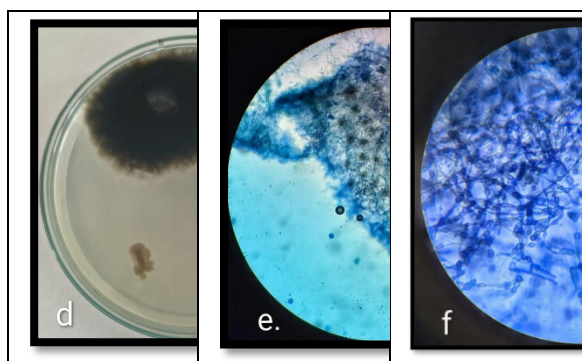


Fig 12; Cultured plates showing endophytic fungi isolated from *Centella asiatica* stem: a: Inoculation of *Centella asiatica* stem on culture media, b: Backside view of the inoculated culture plate, c: Fungal colony showing growth characteristics of *Cladosporium* sp. d: Backside view of the same culture plate, e: Microscopic observation of *Cladosporium* sp. (10x), f: Microscopic observation of *Cladosporium* sp. (40x)

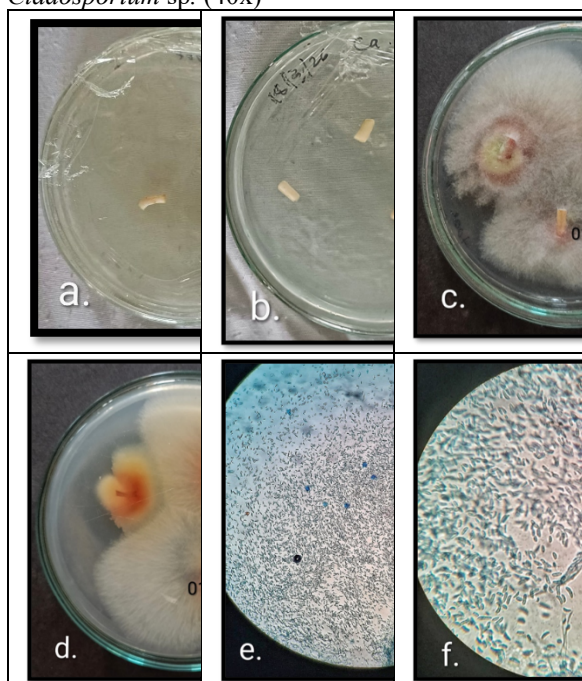


Fig 13; Cultured plates showing endophytic fungi isolated from *Centella asiatica* root: a: Inoculation of *Centella asiatica* root on culture media, b: Backside view of the inoculated culture plate, c: Fungal colony 01 showing growth characteristics of *Fusarium* sp. d: Backside view of the same culture plate, e: Microscopic observation of *Fusarium* sp. (10x), f: Microscopic observation of *Fusarium* sp. (40x)

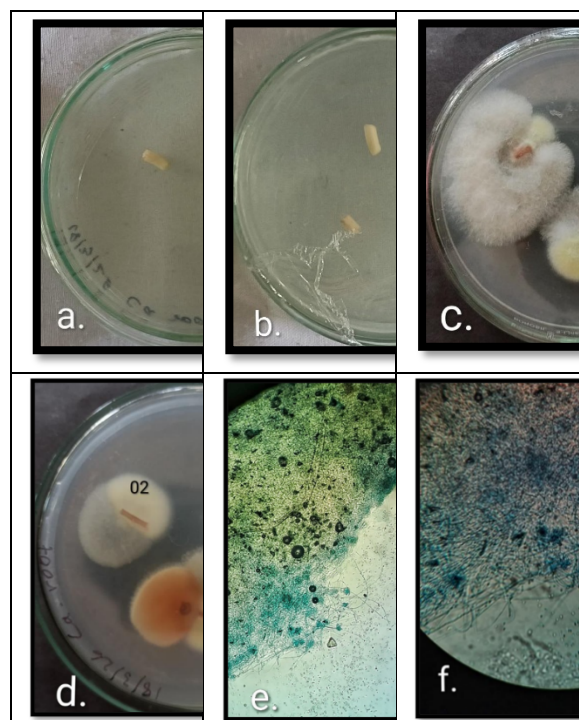


Fig 14; Cultured plates showing endophytic fungi isolated from *Centella asiatica* root: a: Inoculation of *Centella asiatica* root on culture media, b: Backside view of the inoculated culture plate, c: Fungal colony 03 showing growth characteristics of *Talaromyces* sp. d: Backside view of the same culture plate, e: Microscopic observation of *Talaromyces* sp. (10x), f: Microscopic observation of *Talaromyces* sp. (40x)

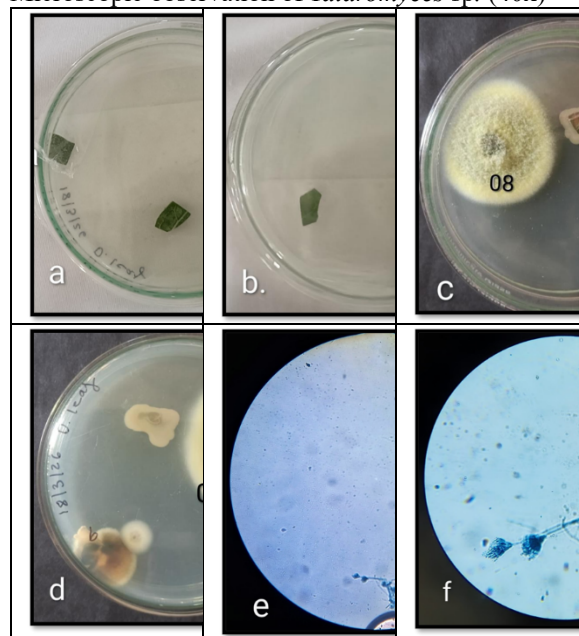


Fig 15; Cultured plates showing endophytic fungi isolated from *Datura stramonium* leaf: a: Inoculation of *Datura stramonium* leaf on culture media, b: Backside view of same plate, c: Fungal colony 08

showing growth characteristics of *Talaromyces* sp. d: Backside view of the same culture plate, e: Microscopic observation of *Talaromyces* sp. (10x), f: Microscopic observation of *Talaromyces* sp. (40x)

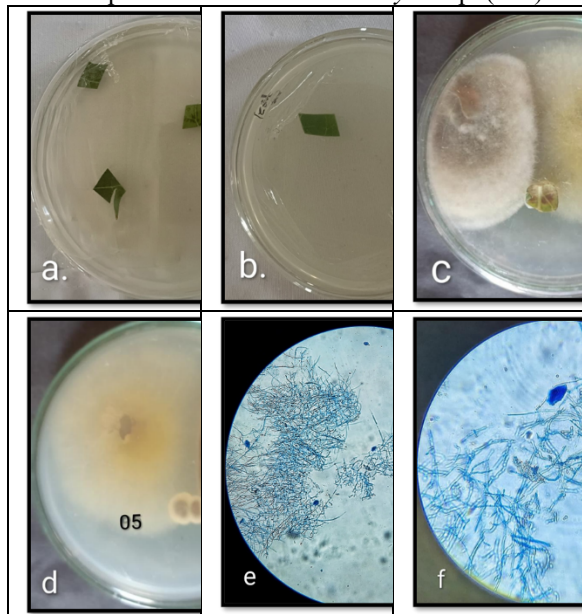


Fig 16; Cultured plates showing endophytic fungi isolated from *Datura stramonium* leaf: a: Inoculation of *Datura stramonium* leaf on culture media, b: Backside view of same plate, c: Fungal colony 05 showing growth characteristics of *Acremonium* sp. d: Backside view of the same culture plate, e: Microscopic observation of *Acremonium* sp. (10x), f: Microscopic observation of *Acremonium* sp. (40x)

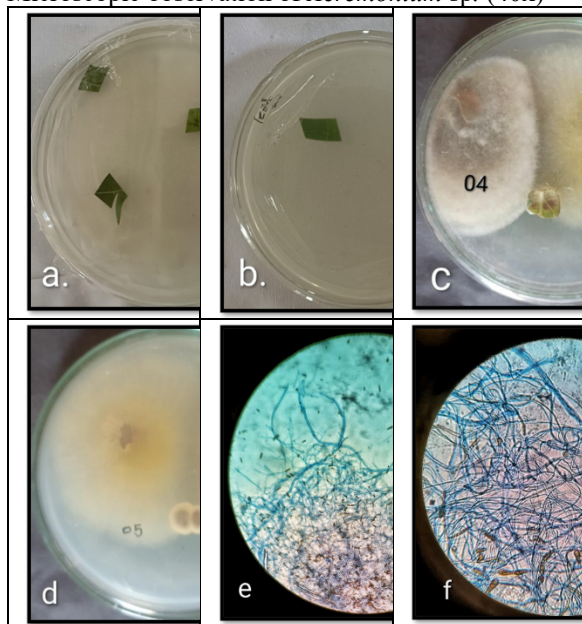


Fig 17; Cultured plates showing endophytic fungi isolated from *Datura stramonium* leaf: a: Inoculation of *Datura stramonium* leaf on culture media, b:

Backside view of same plate, c: Fungal colony 04 showing growth characteristics of *Phoma* sp. d: Backside view of the same culture plate, e: Microscopic observation of *Phoma* sp. (10x), f: Microscopic observation of *Phoma* sp. (40x)

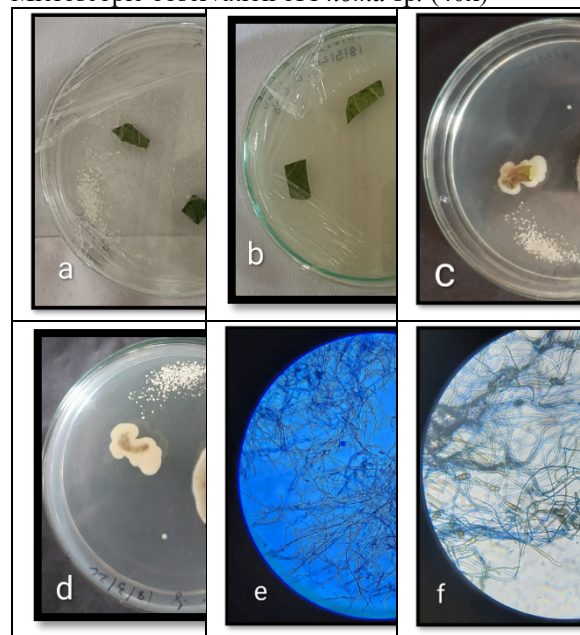
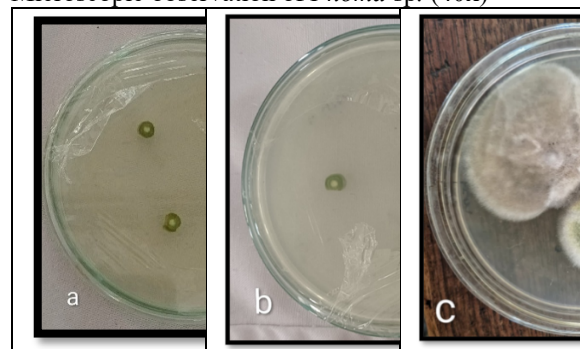


Fig 18; Cultured plates showing endophytic fungi isolated from *Datura stramonium* leaf: a: Inoculation of *Datura stramonium* leaf on culture media, b: Backside view of same plate, c: Fungal colony 03 showing growth characteristics of *Phoma* sp. d: Backside view of the same culture plate, e: Microscopic observation of *Phoma* sp. (10x), f: Microscopic observation of *Phoma* sp. (40x)



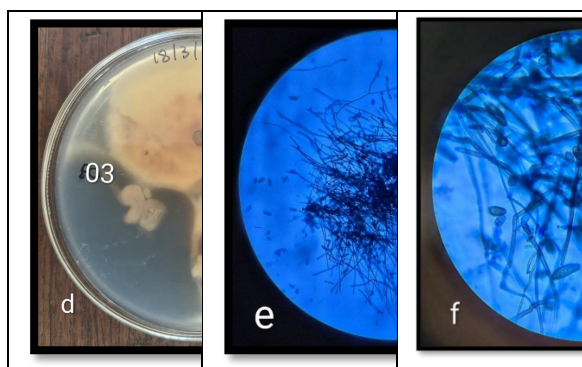


Fig 19; Cultured plates showing endophytic fungi isolated from *Datura stramonium* stem: a: Inoculation of *Datura stramonium* stem on culture media, b: Backside view of same plate, c: Fungal colony 03 showing growth characteristics of *Bipolaris* sp. d: Backside view of the same culture plate, e: Microscopic observation of *Bipolaris* sp. (10x), f: Microscopic observation of *Bipolaris* sp. (40x)

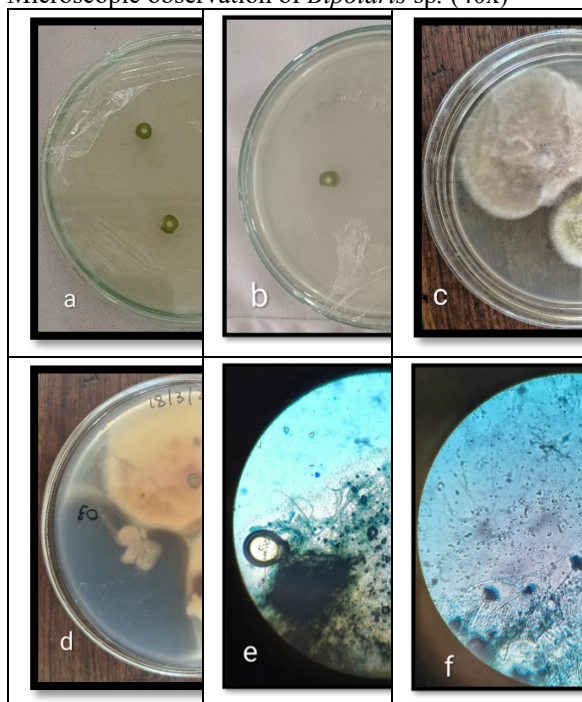


Fig 20; Cultured plates showing endophytic fungi isolated from *Datura stramonium* stem: a: Inoculation of *Datura stramonium* stem on culture media, b: Backside view of same plate, c: Fungal colony 02 showing growth characteristics of *Penicillium* sp. d: Backside view of the same culture plate, e and f: Microscopic observation of *Penicillium* sp. (10x) and (40x)

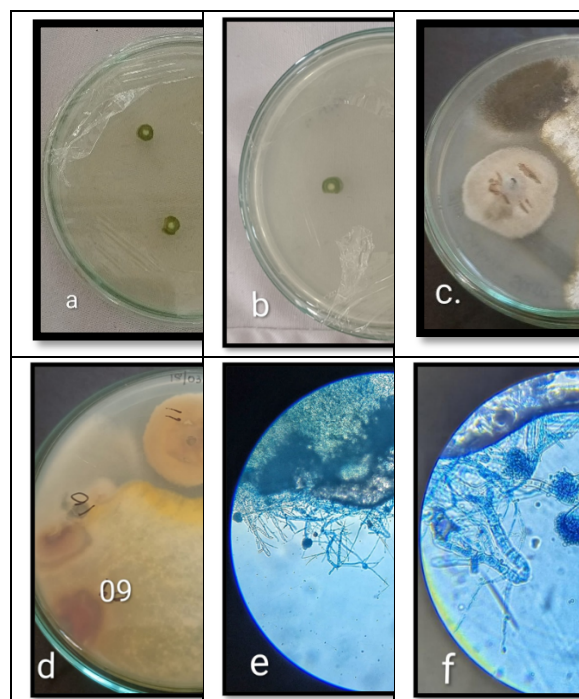


Fig 21; Cultured plates showing endophytic fungi isolated from *Datura stramonium* stem: a: Inoculation of *Datura stramonium* stem on culture media, b: Backside view of same plate, c: Fungal colony 09 showing growth characteristics of *Aspergillus* sp. d: Backside view of the same culture plate, e and f: Microscopic observation of *Aspergillus* sp. (10x) and (40x)

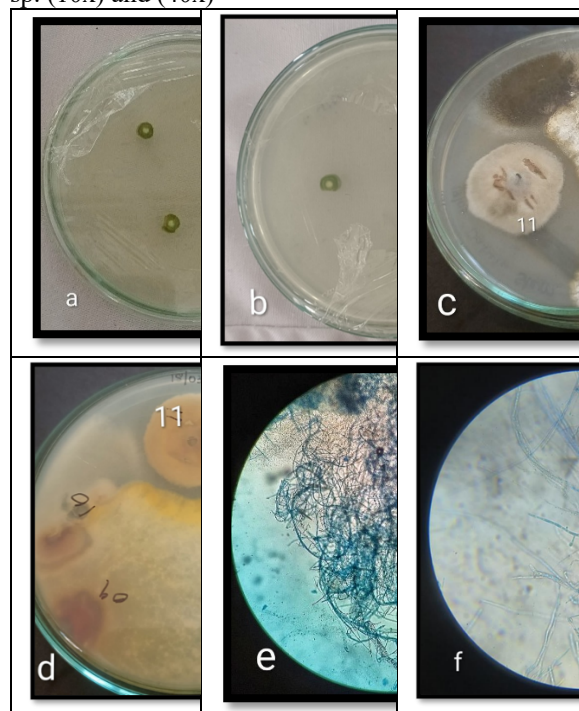


Fig 22; Cultured plates showing endophytic fungi isolated from *Datura stramonium* stem: a:

Inoculation of *Datura stramonium* stem on culture media, b: Backside view of same plate, c: Fungal colony 11 showing growth characteristics of *Glomerella* sp. d: Backside view of the same culture plate, e and f: Microscopic observation of *Glomerella* sp. (10x) and (40x)

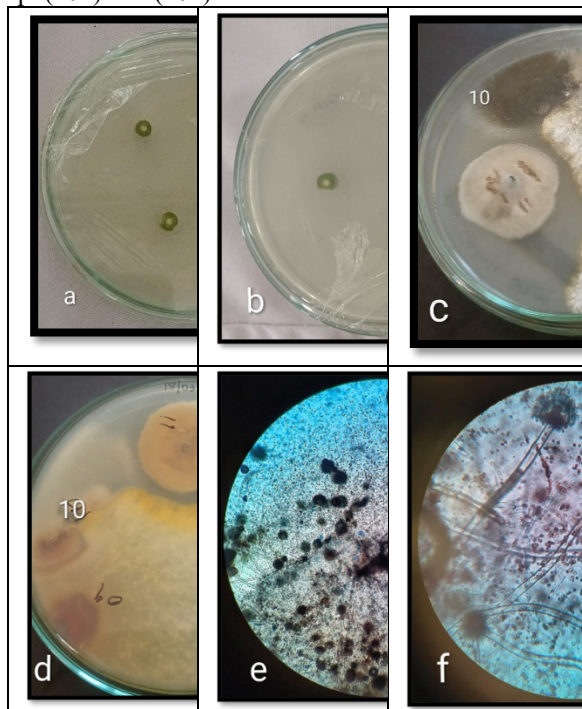


Fig 23; Cultured plates showing endophytic fungi isolated from *Datura stramonium* stem: a: Inoculation of *Datura stramonium* stem on culture media, b: Backside view of same plate, c: Fungal colony 10 showing growth characteristics of *Aspergillus* sp. d: Backside view of the same culture plate, e and f: Microscopic observation of *Aspergillus* sp. (10x) and (40x)

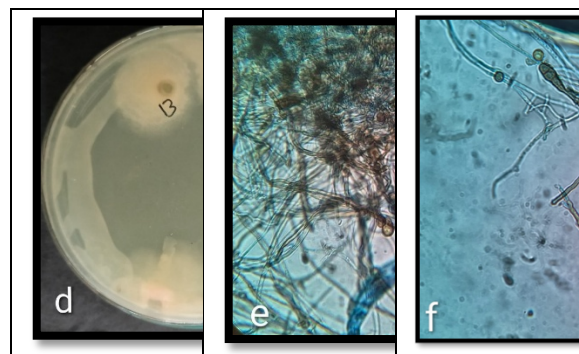
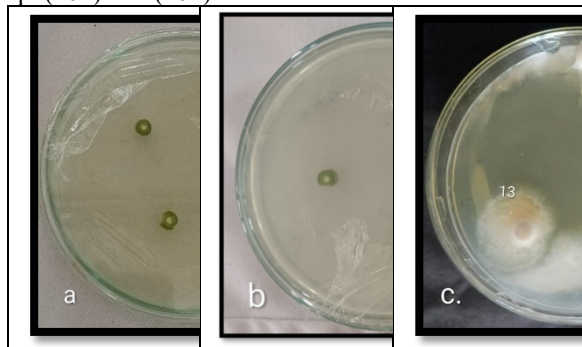


Fig 24; Cultured plates showing endophytic fungi isolated from *Datura stramonium* stem: a: Inoculation of *Datura stramonium* stem on culture media, b: Backside view of same plate, c: Fungal colony 13 showing growth characteristics of *Phoma* sp. d: Backside view of the same culture plate, e: Microscopic observation of *Phoma* sp. (10x), f: Microscopic observation of *Phoma* sp. (40x)

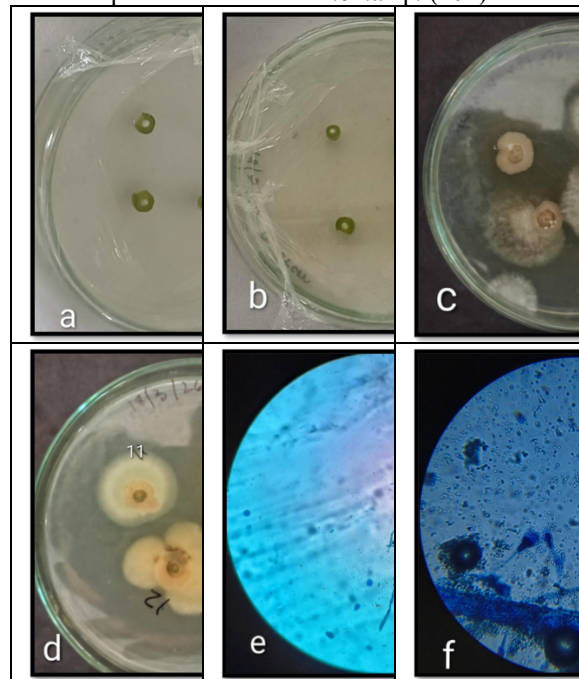


Fig 25; Cultured plates showing endophytic fungi isolated from *Datura stramonium* stem: a: Inoculation of *Datura stramonium* stem on culture media, b: Backside view of same plate, c: Fungal colony 11 showing growth characteristics of *Penicillium* sp. d: Backside view of the same culture plate, e: Microscopic observation of *Penicillium* sp. (10x), f: Microscopic observation of *Penicillium* sp. (40x)

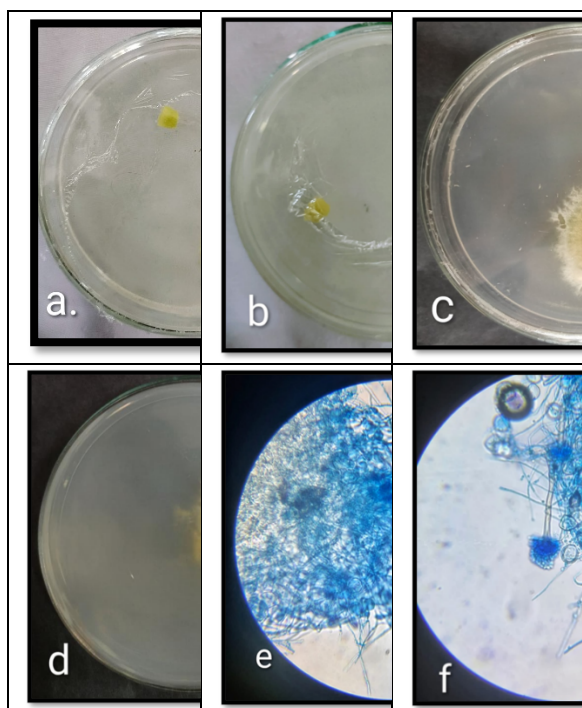


Fig 26; Cultured plates showing endophytic fungi isolated from *Datura stramonium* root: a: Inoculation of *Datura stramonium* root on culture media, b: Backside view of same plate, c: Fungal colony showing growth characteristics of *Aspergillus* sp. d: Backside view of the same culture plate, e and f: Microscopic observation of *Aspergillus* sp. (10x) and (40x)

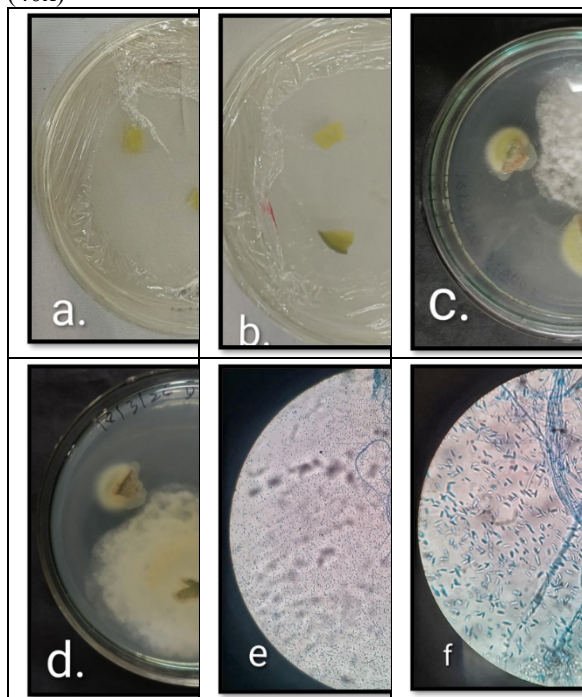


Fig 27; Cultured plates showing endophytic fungi isolated from *Datura stramonium* root: a:

Inoculation of *Datura stramonium* root on culture media, b: Backside view of same plate, c: Fungal colony showing growth characteristics of *Fusarium* sp. d: Backside view of the same culture plate, e and f: Microscopic observation of *Fusarium* sp. (10x) and (40x)

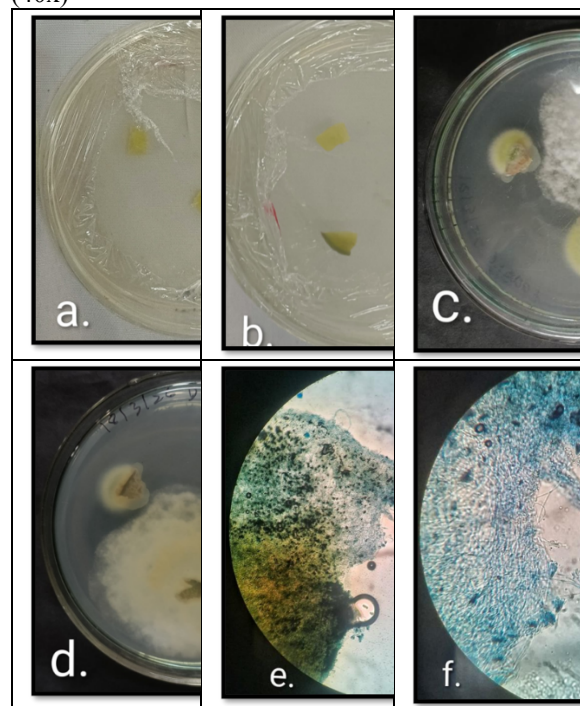
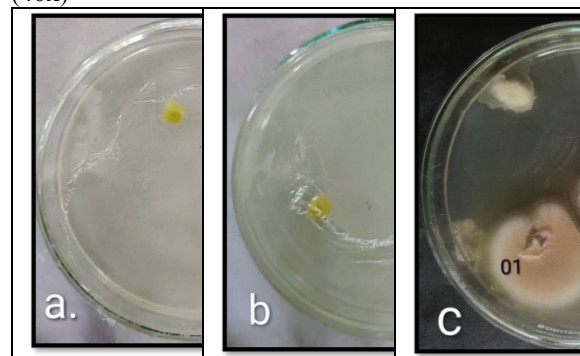


Fig 28; Cultured plates showing endophytic fungi isolated from *Datura stramonium* root: a: Inoculation of *Datura stramonium* root on culture media, b: Backside view of same plate, c: Fungal colony showing growth characteristics of *Talaromyces* sp. d: Backside view of the same culture plate, e and f: Microscopic observation of *Talaromyces* sp. (10x) and (40x)



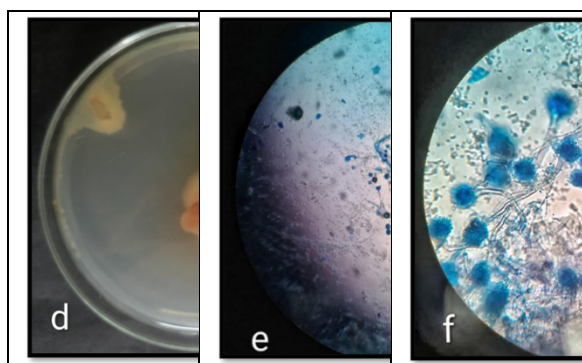


Fig 29; Cultured plates showing endophytic fungi isolated from *Datura stramonium* root: a: Inoculation of *Datura stramonium* root on culture media, b: Backside view of same plate, c: Fungal colony showing growth characteristics of *Aspergillus* sp. d: Backside view of the same culture plate, e and f: Microscopic observation of *Aspergillus* sp. (10x) and (40x)

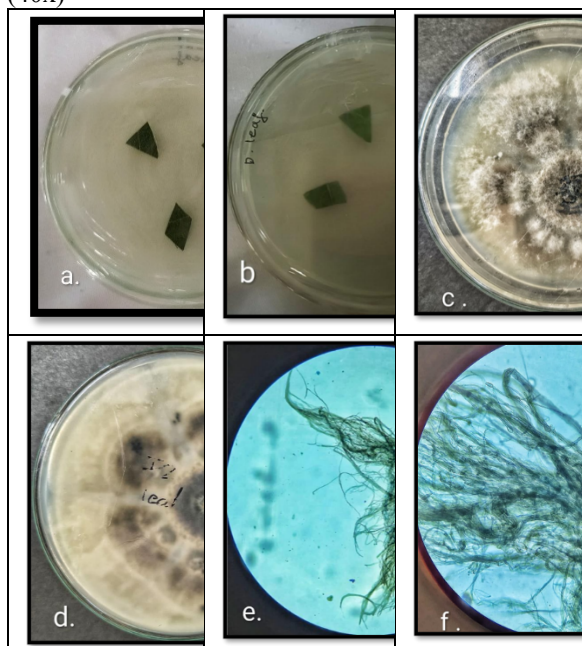


Fig 30; Cultured plates showing endophytic fungi isolated from *Datura sanguinea* leaf: a: Inoculation of *Datura sanguinea* leaf on culture media, b: backside view of same plate, c: Fungal colony showing growth characteristics of *Alternaria* sp. d: Backside view of the same culture plate, e: Microscopic observation of *Alternaria* sp. (10x), f: Microscopic observation of *Alternaria* sp. (40x)

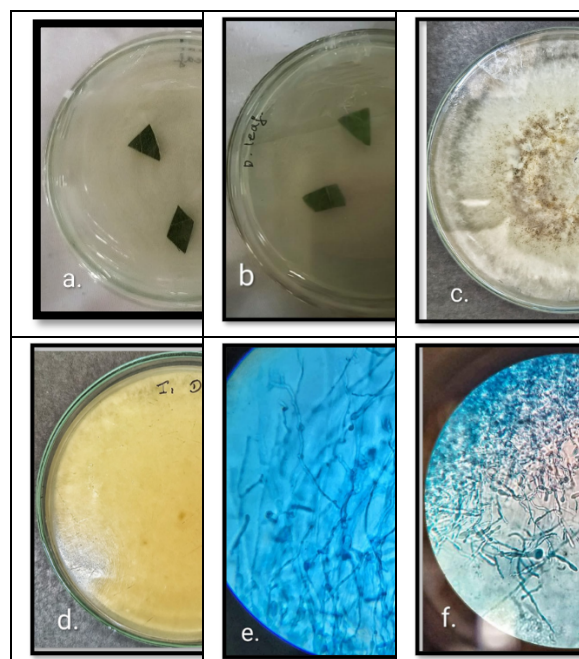


Fig 31; Cultured plates showing endophytic fungi isolated from *Datura sanguinea* leaf: a: Inoculation of *Datura sanguinea* leaf on culture media, b: backside view of same plate, c: Fungal colony showing growth characteristics of *Ceratobasidium* sp. d: Backside view of the same culture plate, e: Microscopic observation of *Ceratobasidium* sp. (10x), f: Microscopic observation of *Ceratobasidium* sp. (40x)

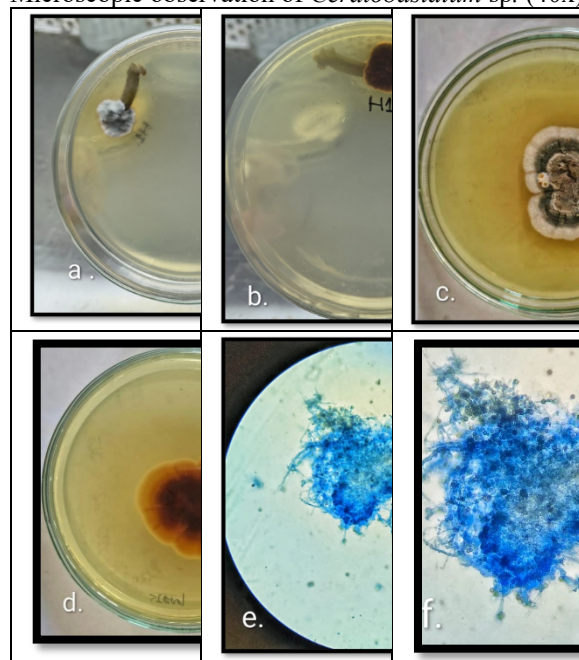


Fig 32; Cultured plates showing endophytic fungi isolated from *Datura sanguinea* stem: a: Inoculation of *Datura sanguinea* stem on culture media, b: backside view of same plate, c: Fungal colony showing growth characteristics of *Pythium* sp. d:

Backside view of the same culture plate, e: Microscopic observation of *Pythium* sp. (10x), f: Microscopic observation of *Pythium* sp. (40x)

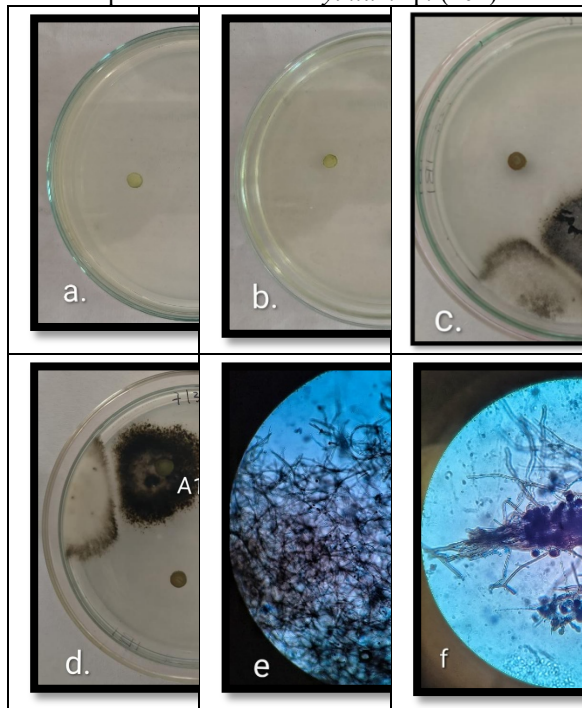


Fig 33; Cultured plates showing endophytic fungi isolated from *Datura sanguinea* stem: a: Inoculation of *Datura sanguinea* stem on culture media, b: backside view of same plate, c: Fungal colony A1 showing growth characteristics of *Nigrospora* sp. d: Backside view of the same culture plate, e: Microscopic observation of *Nigrospora* sp. (10x), f: Microscopic observation of *Nigrospora* sp. (40x)

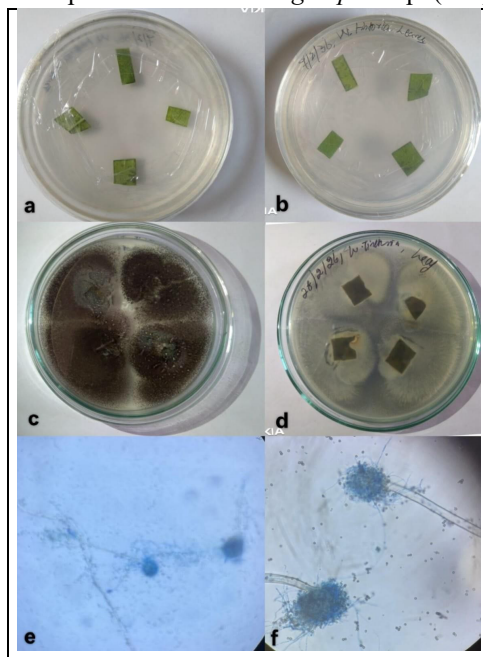


Figure 34: Culture plates showing fungi isolated from *Wrightia tinctoria* leaf: A. Inoculation of *Wrightia tinctoria* leaf on culture media, B. Backside view of inoculated culture plate, C. Sub cultured plate exhibiting active growth of *Aspergillus niger*, D. Backside view of the same sub cultured plate, E. Microscopic observation of *Aspergillus niger* in 10x low power, F. Microscopic observation of *Aspergillus niger* in 40x high power.

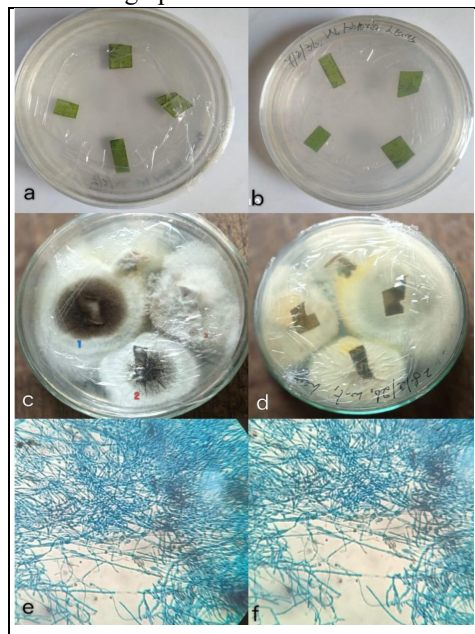


Figure 35: Culture plates showing fungi isolated from *Wrightia tinctoria* leaf, A. Inoculation of *Wrightia tinctoria* leaf on culture media, B. Backside view of inoculated culture plate, C. cultured plates exhibiting active growth of *Rhizoctonia* sp, D. Backside view of culture plates, E. Microscopic observation of *Rhizoctonia* sp in 10x low power, E. Microscopic observation of *Rhizoctonia* sp in 40x high power.

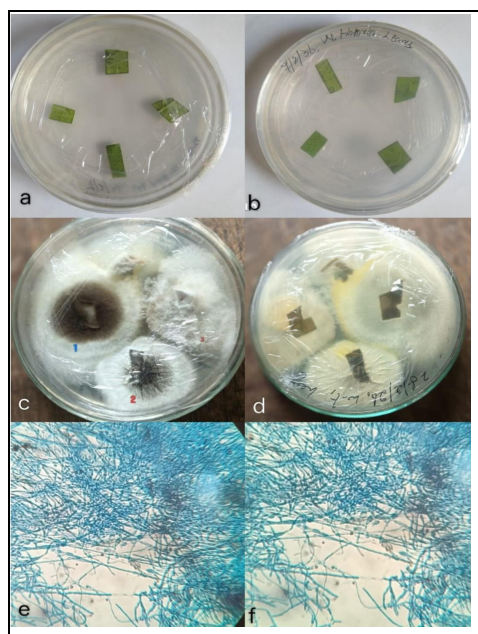


Figure 36: Culture plates showing fungi isolated from *Wrightia tinctoria* leaf, A. Inoculation of *Wrightia tinctoria* leaf on culture media, B. Backside view of inoculated culture plate, C. cultured plates exhibiting active growth of *Rhizoctonia* sp, D. Backside view of culture plates, E. Microscopic observation of *Rhizoctonia* sp in 10x low power, F. Microscopic observation of *Rhizoctonia* sp in 40x high power.

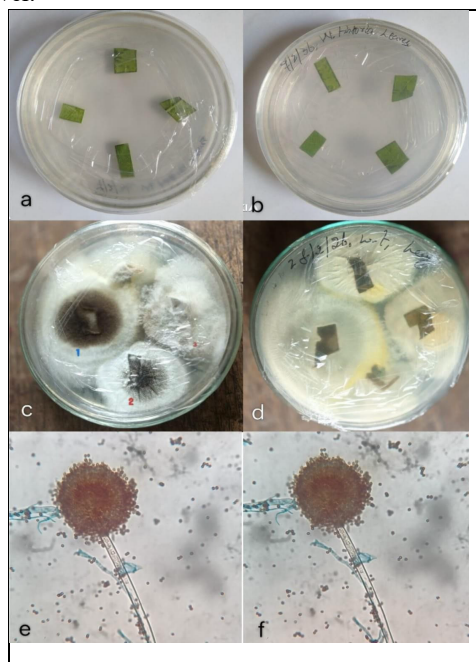


Figure 37: Culture plates showing fungi isolated from *Wrightia tinctoria* leaf, A. Inoculation of *Wrightia tinctoria* leaf on culture media, B. Backside

view of inoculated culture plate, C. Cultured plates exhibiting active growth of *Rhizopus* sp, D. Backside view of the same cultured plate, E. Microscopic observation of *Rhizopus* sp in 10x low power, F. Microscopic observation of *Rhizopus* sp in 40x high power.

Figure 38: Culture plates showing fungi isolated from *Wrightia tinctoria* leaf: A. Inoculation of *Wrightia tinctoria* leaf on culture media, B. Backside view of inoculated culture plate, C. cultured plates exhibiting active growth of *Neocosmospora* sp, D. backside view of the same cultured plate, E. Microscopic observation of *Neocosmospora* sp in 10x low power, F. Microscopic observation of *Neocosmospora* sp in 40x high power.

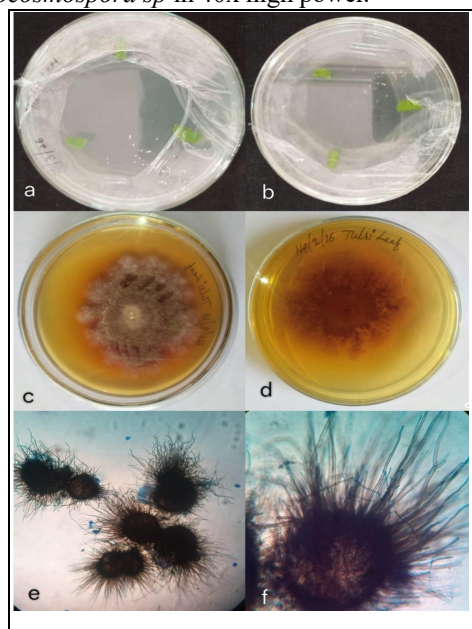


Figure 39: Culture plates showing fungi isolated from *Ocimum sanctum* leaf: A. Inoculation of *Ocimum sanctum* leaf on culture media, B. Backside view of inoculated culture plate, C. Cultured plates exhibiting active growth of *Colletotrichum* sp, D. Backside view of the same cultured plate, E. Microscopic observation of *Colletotrichum* sp in 10x low power, F. Microscopic observation of *Colletotrichum* sp in 40x high power

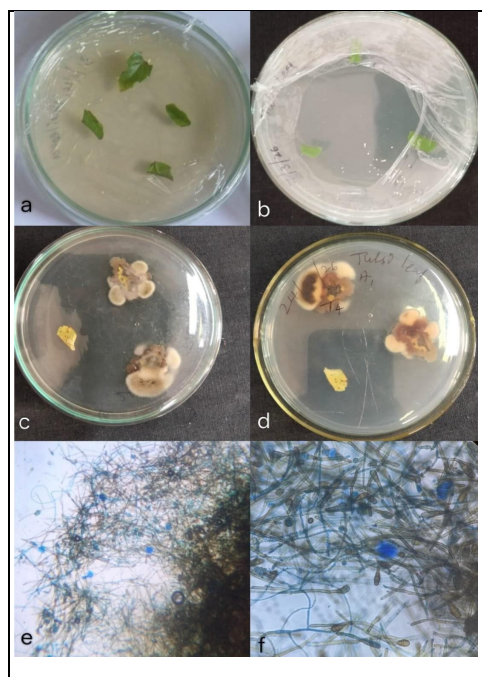


Figure 40: Culture plates showing fungi isolated from *Ocimum sanctum* leaf. A. Inoculation of *Ocimum sanctum* leaf on culture media, B. Backside view of inoculated culture plate, C. Cultured plates exhibiting active growth of *Phoma* sp, D. Backside view of the same cultured plate, E. Microscopic observation of *Phoma* sp in 10x low power, F. Microscopic observation of *Phoma* sp in 40x high power

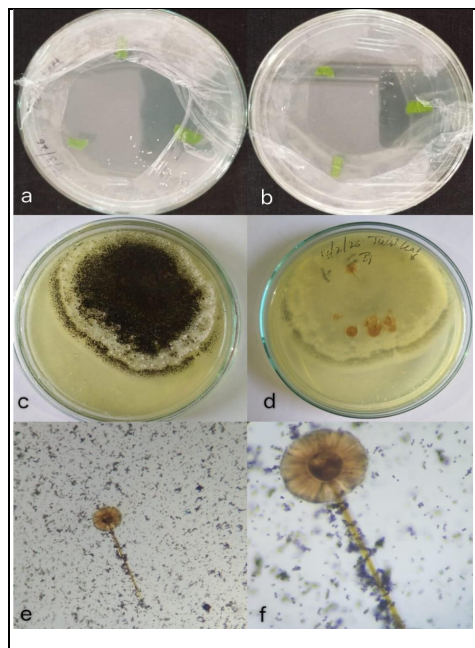


Figure 41: Culture plates showing fungi isolated from *Ocimum sanctum* leaf. A. Inoculation of

Ocimum sanctum stem on culture media, B. Backside view of inoculated culture plate, C. Cultured plates exhibiting active growth of *Aspergillus niger*, D. Backside view of the same cultured plate, E. Microscopic observation of *Aspergillus niger* in 10x low power, F. Microscopic observation of *Aspergillus niger* in 40x high power

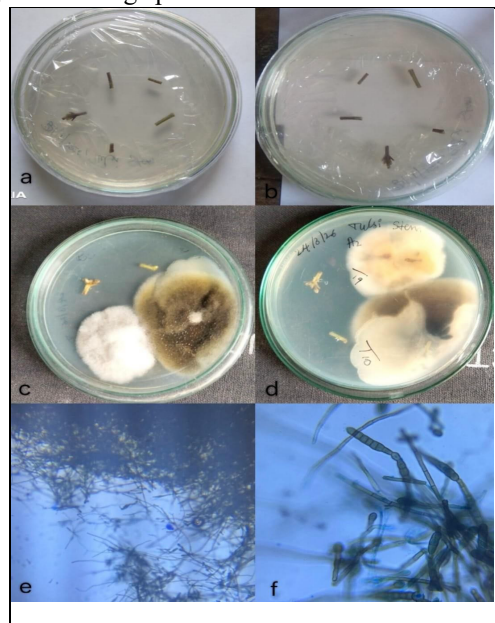


Figure 42: Culture plates showing fungi isolated from *Ocimum sanctum* stem: A. inoculation of *Ocimum sanctum* stem on culture media, B. Backside view of inoculated culture plate, C. Cultured plates exhibiting active growth of *Phoma* sp, D. Backside view of the same cultured plate, E. Microscopic observation of *Phoma* sp in 10x low power, F. Microscopic observation of *Phoma* sp in 40x high power

Conclusion

Endophytic fungi are microorganisms that live internally within apparently healthy and asymptomatic plant hosts. They inhabit plant tissues without causing visible disease symptoms or morphological damage to the host. These fungi are considered ubiquitous in nature, and studies have shown that almost every plant species examined so far hosts endophytes. The present study aimed to isolate and compare the diversity of endophytic fungi associated with five medicinal plants: *Centella asiatica*, *Datura stramonium*, *Datura sanguinea*, *Wrightia tinctoria*, and *Ocimum sanctum*. Healthy leaves, stems, and roots were collected and subjected to surface sterilization using commercial bleach, fungicide-antibiotic treatment, and mercuric chloride. Sterilized tissue segments were cultured on potato dextrose agar supplemented with ampicillin and incubated under controlled conditions. Emerging fungal colonies were purified and identified based on

morphological characteristics using lactophenol cotton blue staining. A total of 49 endophytic fungal isolates belonging to several genera were recovered from the five plant species. *Centella asiatica* yielded the highest diversity with 16 isolates, followed by *Datura stramonium* with 13 isolates, *Ocimum sanctum* with 9 isolates, *Wrightia tinctoria* with 7 isolates, and *Datura sanguinea* with 4 isolates. The isolated fungi included species of *Aspergillus*, *Penicillium*, *Fusarium*, *Phoma*, *Talaromyces*, *Alternaria*, *Glomerella*, *Rhizoctonia*, *Pythium*, *Curvularia*, *Cladosporium*, *Trichoderma*, *Nigrospora*, *Bipolaris*, *Acremonium*, and others. Among these, *Aspergillus* and *Fusarium* species were the most frequently encountered across different host plants and tissues.

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