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Original Research Article

Endophytic Fungal Communities of Wrightia tinctoria and Ocimum sanctum: Isolation and Identification

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Abstract: Endophytic fungi are microorganisms that inhabit internal plant tissues without causing visible disease symptoms or damage to their hosts. These fungi are ubiquitous in nature and are known to establish symbiotic associations with a wide range of plant species. The present study aimed to isolate and identify endophytic fungi from the medicinal plants Wrightia tinctoria and Ocimum sanctum. Plant samples were subjected to surface sterilization and cultured on potato dextrose agar supplemented with ampicillin. Emerging fungal colonies were purified and identified using morphological characteristics and lactophenol cotton blue staining. A total of seven fungal isolates were obtained from Wrightia tinctoria, namely Aspergillus sp., Aspergillus niger, Fusarium sp., Neocosmospora sp., Pythium sp., Rhizoctonia sp., and Rhizopus sp. Nine fungal endophytes were isolated from Ocimum sanctum, including Aspergillus sp., Aspergillus niger, Colletotrichum sp., Fusarium sp., Phoma sp., and Rhizoctonia sp. The results revealed a diverse population of endophytic fungi associated with both medicinal plants. These endophytes may serve as potential sources of novel bioactive compounds and contribute to the medicinal value of their host plants.

Keywords: Endophytic fungi, Wrightia tinctoria, Ocimum sanctum, Medicinal plants, Fungal diversity, Lactophenol cotton blue staining, Bioactive compounds, Endophytes.

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

Medicinal plants have long been used as important sources for the isolation and characterization of bioactive metabolites. Traditionally, many biologically active compounds used in medicine were obtained directly from plants. However, the discovery of fungal endophytes residing within these medicinal plants and their ability to produce the same or similar bioactive compounds has shifted scientific interest toward fungi as alternative sources of valuable metabolites. Consequently, bioactive natural products derived from endophytic fungi isolated from different plant species have attracted considerable attention from natural product chemists and biologists. This growing interest is clearly reflected in the steady increase in scientific publications devoted to endophytic fungi and their metabolites in recent years. Plants, particularly perennial species, are colonized by a wide variety of endophytic microorganisms. These microorganisms live inside plant tissues either throughout their entire life cycle or during a specific phase of their life cycle without producing visible symptoms in the host plant. Endophytic microorganisms include both fungi and bacteria, and they often coexist with pathogenic microorganisms within the same host plant tissues. Despite the presence of pathogens, endophytes maintain a balanced interaction with their host plants that does not result in disease.1,2,3.

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-arthritic, adaptogenic, anti-stress, anti-leukodermal, and anti-coagulant properties.4

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 anti-pyretic 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. Phytochemical studies have shown the presence of flavonoids, glycoflavones such as iso-orientin, and phenolic acids. Various chemical constituents isolated from different parts of the plant include 3,4-Seco-lup-20(29)-en-3-oic acid, lupeol, stigmasterol, campesterol, indigotin, indirubin, tryptanthrin, isatin, anthranillate, rutin, triacontanol, wrightial, cycloartenone, cycloeucalenol, β-amyrin, α-amyrin, β-sitosterol, and 14α-methylzymosterol. In addition, four uncommon sterols-desmosterol, clerosterol, 24-methylene-25-methylcholesterol, and 24-dehydropollinastanol have been isolated along with several other phytosterols. Triterpenoid components have also been isolated from the leaves and pods of Wrightia tinctoria.5

MATERIALS AND METHODS

Samples of leaves and stem of Wrightia tinctoria were collected from Maharani Cluster University campus, Bangalore and leaves of Ocimum sanctum, was collected from Benson town, 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 in to small pieces (1–2 mm), leaves were cut into 1 cm^2 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.13 Fungi were mounted using lactophenol cotton blue method.1

RESULTS

Seven fungal isolates were isolated from *Wrightia tinctoria* namely *Aspergillus* sp., *Aspergillus niger, Fusarium* sp. *Neocosmospora* sp. *Pythium* sp. *Rhizoctonia* sp. and *Rhizopus* sp.

Nine fungal endophytes were isolated from the *Ocimum sanctum*, four from leaves viz., *Aspergillus niger, Colletotrichum* sp. *Fusarium* sp. and *Phoma* sp. Three from stem viz., *Aspergillus* sp. *Phoma* sp. and *Rhizoctonia* sp. and two from the roots viz., *Aspergillus* sp. and *Fusarium* sp.

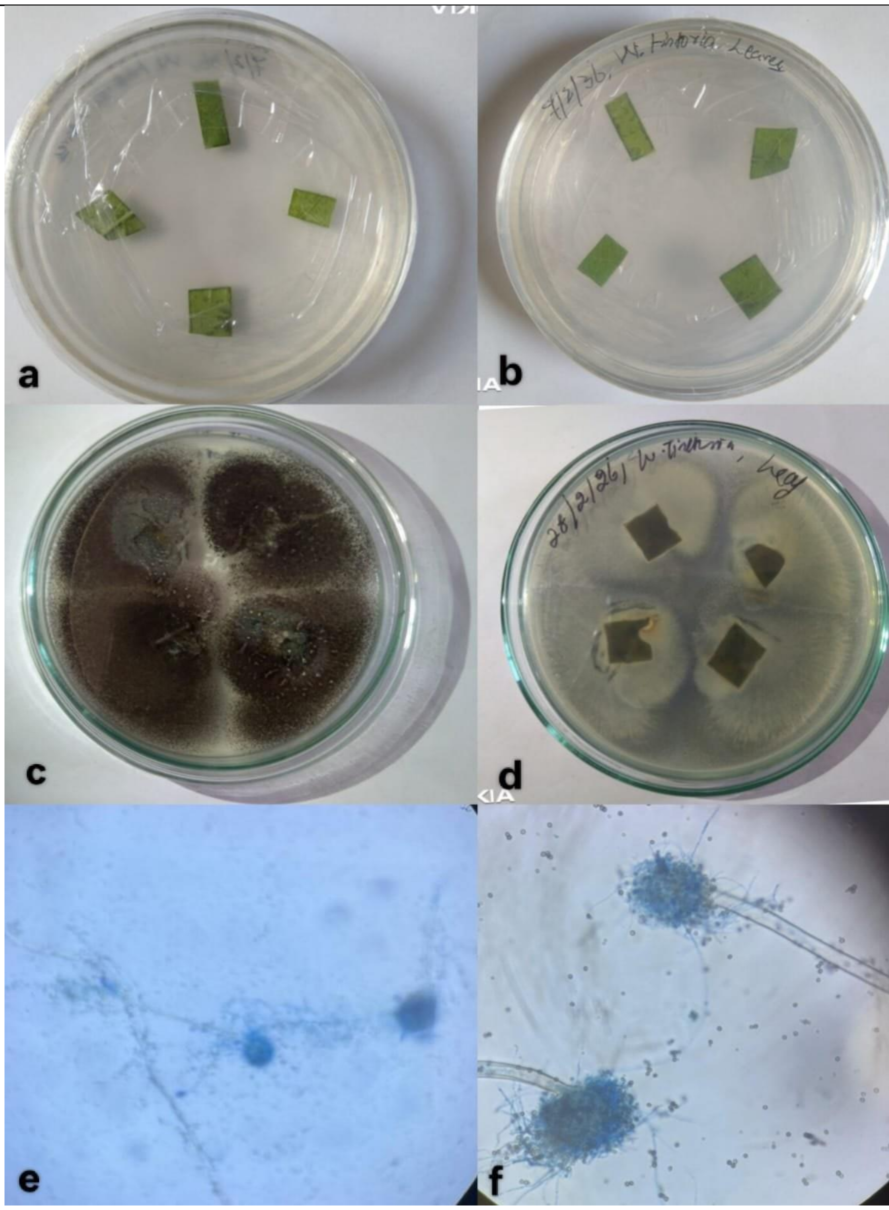

Figure 1: Culture plates showing fungi isolated from *Wrightia tinctoria* leaf: A. Inoculation of *Wrightia tinctoria* leaf on culture media, B. Backside view of inoculated culture plate, C. Sub cultured plate exhibiting active growth of *Aspergillus niger*, D. Backside view of the same sub cultured plate, E. Microscopic observation of *Aspergillus niger* in 10x low power, F. Microscopic observation of *Aspergillus niger* in 40x high power.

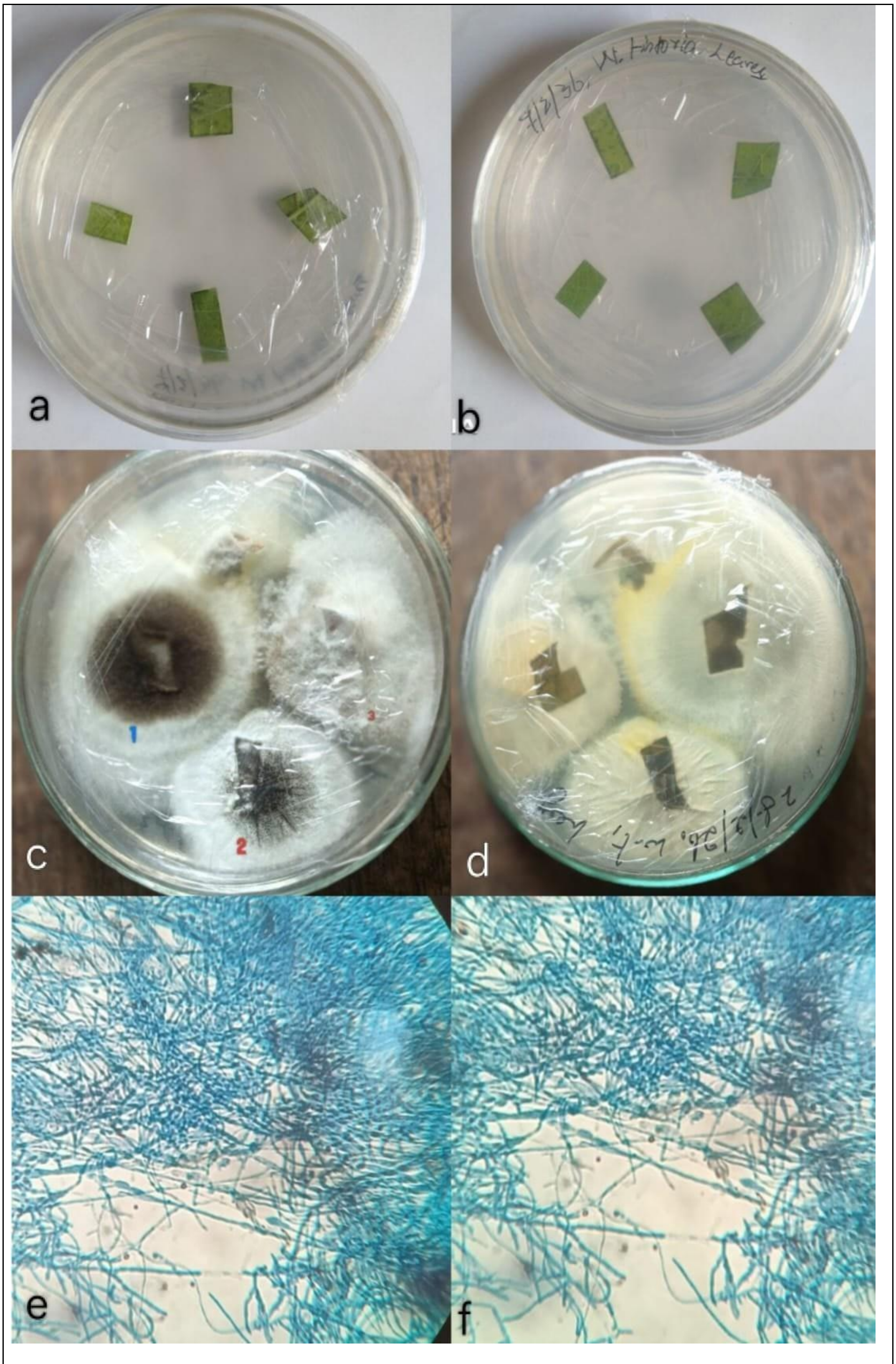

Figure 2: Culture plates showing fungi isolated from *Wrightia tinctoria* leaf, A. Inoculation of *Wrightia tinctoria* leaf on culture media, B. Backside view of inoculated culture plate, C. cultured plates exhibiting active growth of *Rhizoctonia sp,* D. Backside view of culture plates, E. Microscopic observation of *Rhizoctonia sp* in 10x low power, E. Microscopic observation of *Rhizoctonia sp* in 40x high power.

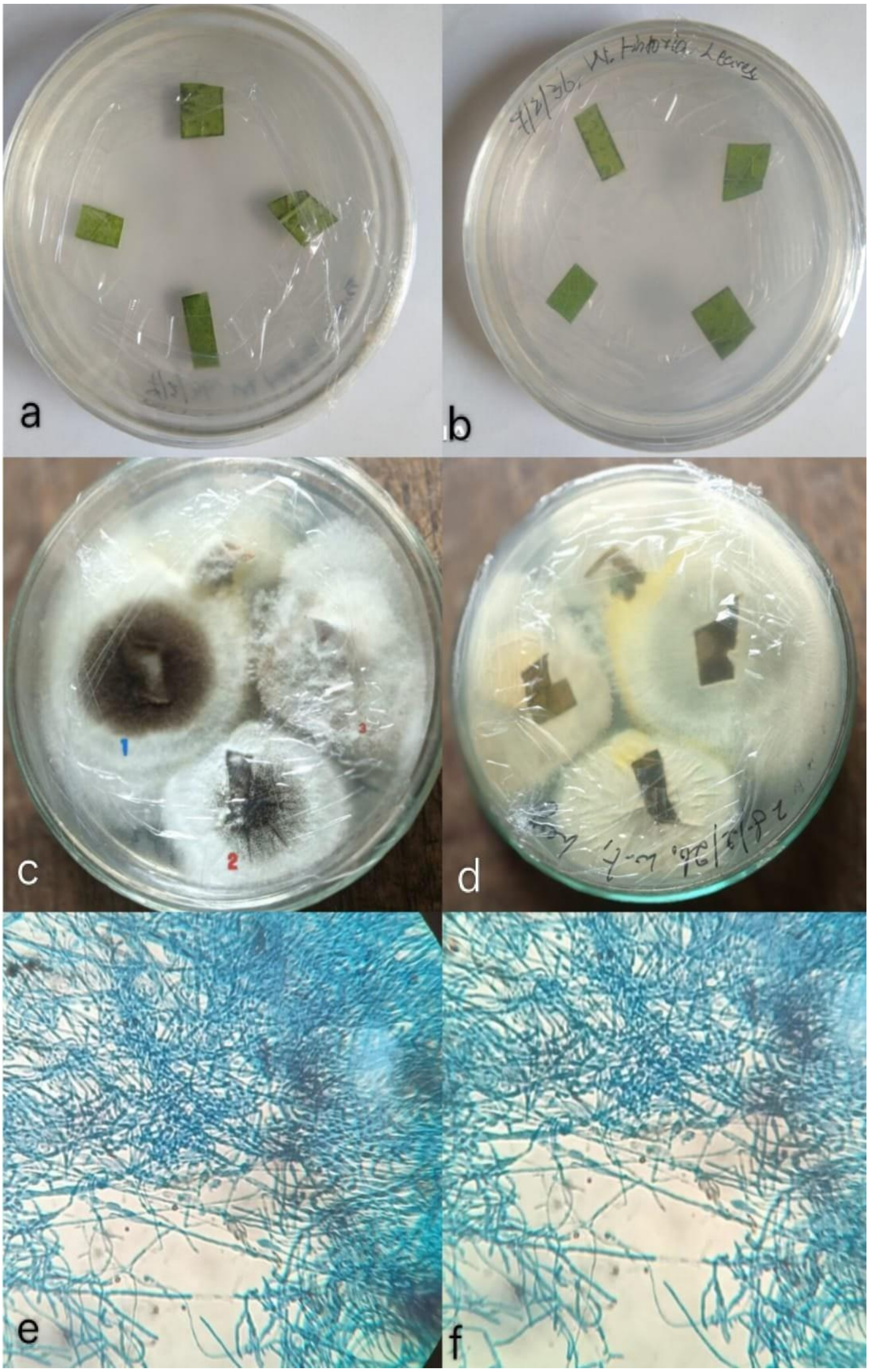

Figure 3: Culture plates showing fungi isolated from *Wrightia tinctoria* leaf, A. Inoculation of *Wrightia tinctoria* leaf on culture media, B. Backside view of inoculated culture plate, C. cultured plates exhibiting active growth of *Rhizoctonia sp,* D. Backside view of culture plates, E. Microscopic observation of *Rhizoctonia sp* in 10x low power, F. Microscopic observation of *Rhizoctonia sp* in 40x high power.

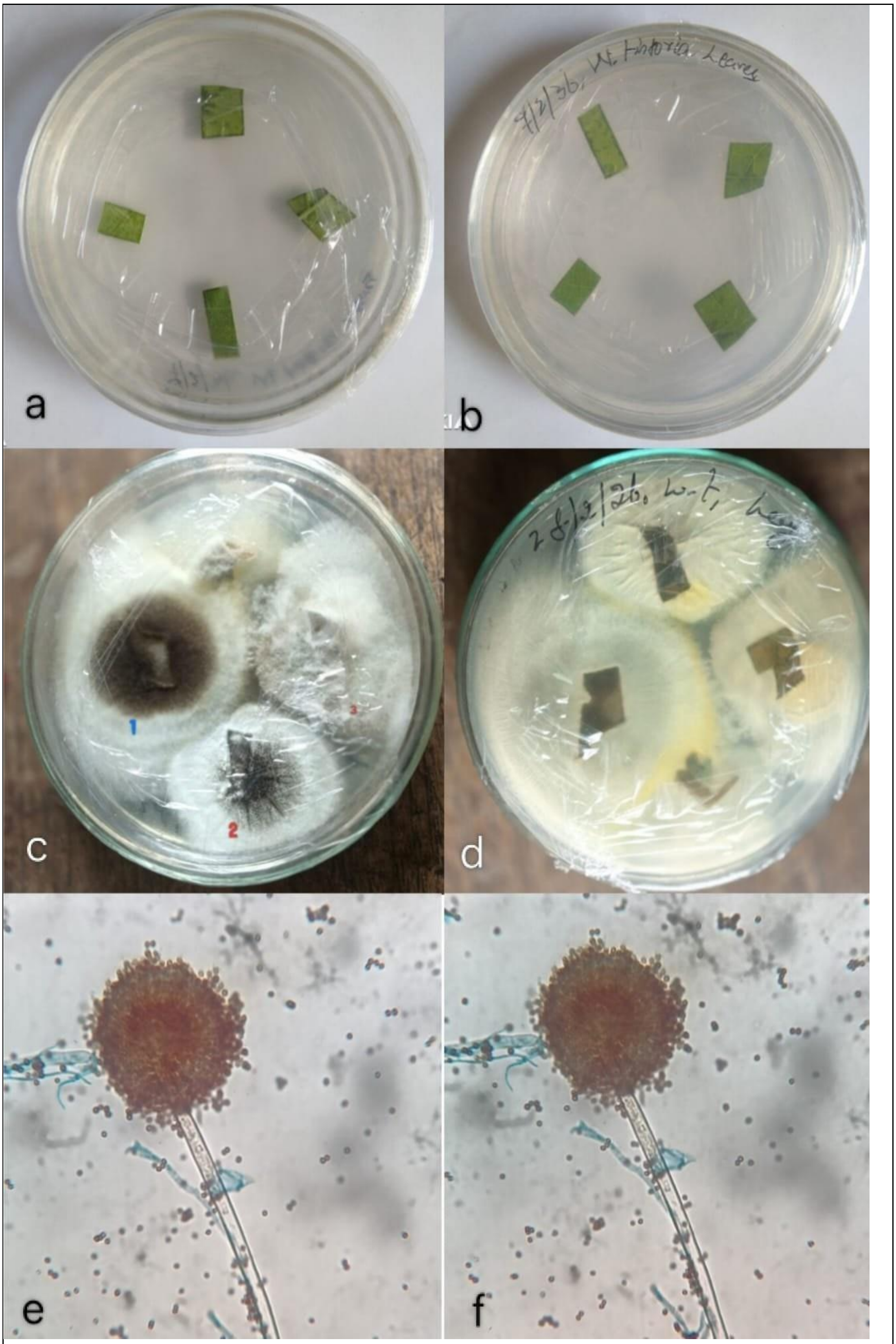

Figure 4: Culture plates showing fungi isolated from *Wrightia tinctoria* leaf, A. Inoculation of *Wrightia tinctoria* leaf on culture media, B. Backside view of inoculated culture plate, C. Cultured plates exhibiting active growth of *Rhizopus sp,* D. Backside view of the same cultured plate, E. Microscopic observation of *Rhizopus sp* in 10x low power, F. Microscopic observation of *Rhizopus sp* in 40x high power.

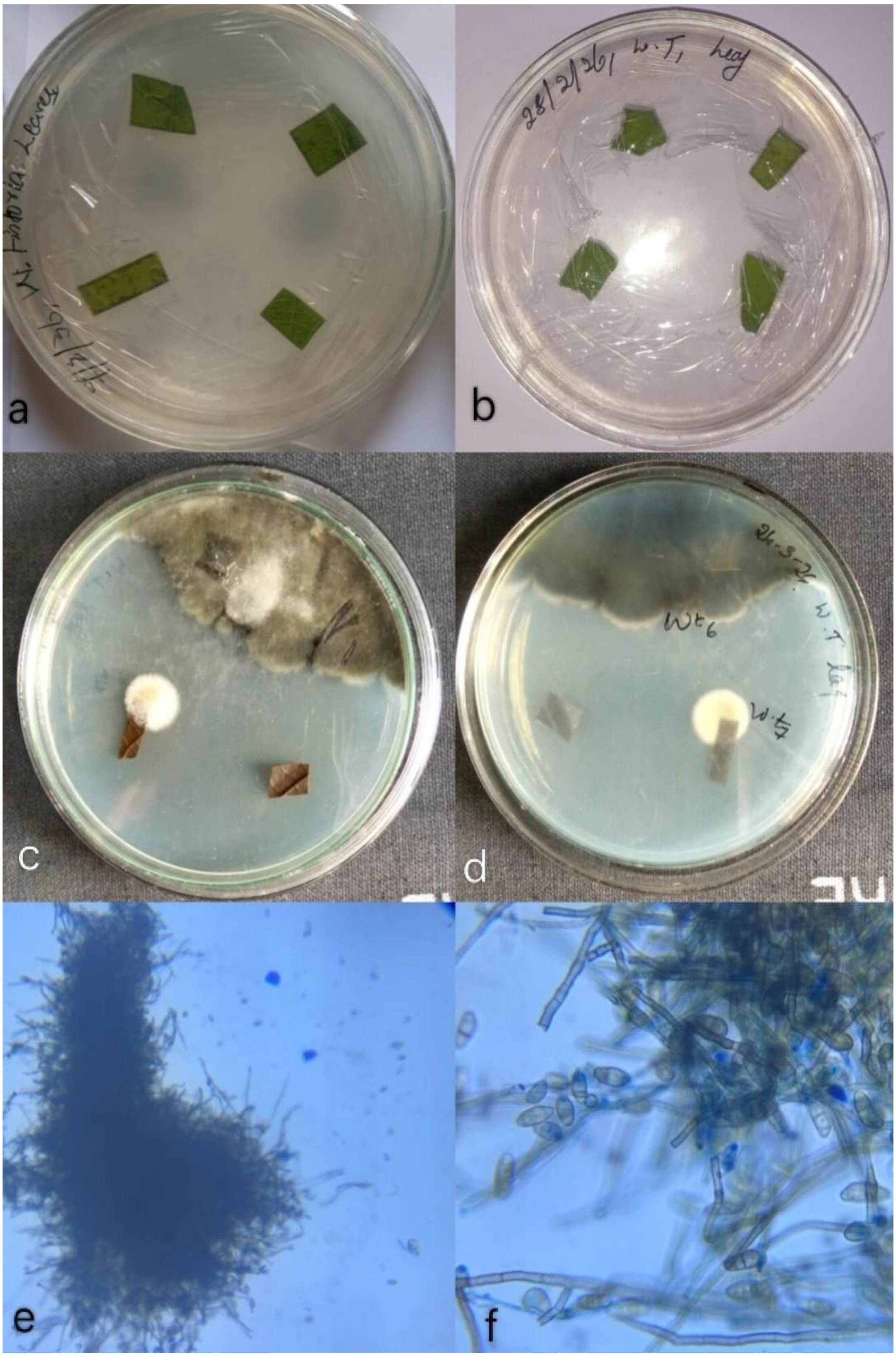

Figure 5: Culture plates showing fungi isolated from *Wrightia tinctoria* leaf: A. Inoculation of *Wrightia tinctoria* leaf on culture media, B. Backside view of inoculated culture plate, C. cultured plates exhibiting active growth of *Neocosmospora sp*, D. backside view of the same cultured plate, E. Microscopic observation of *Neocosmospora sp* in 10x low power, F. Microscopic observation of *Neocosmospora sp* in 40x high power.

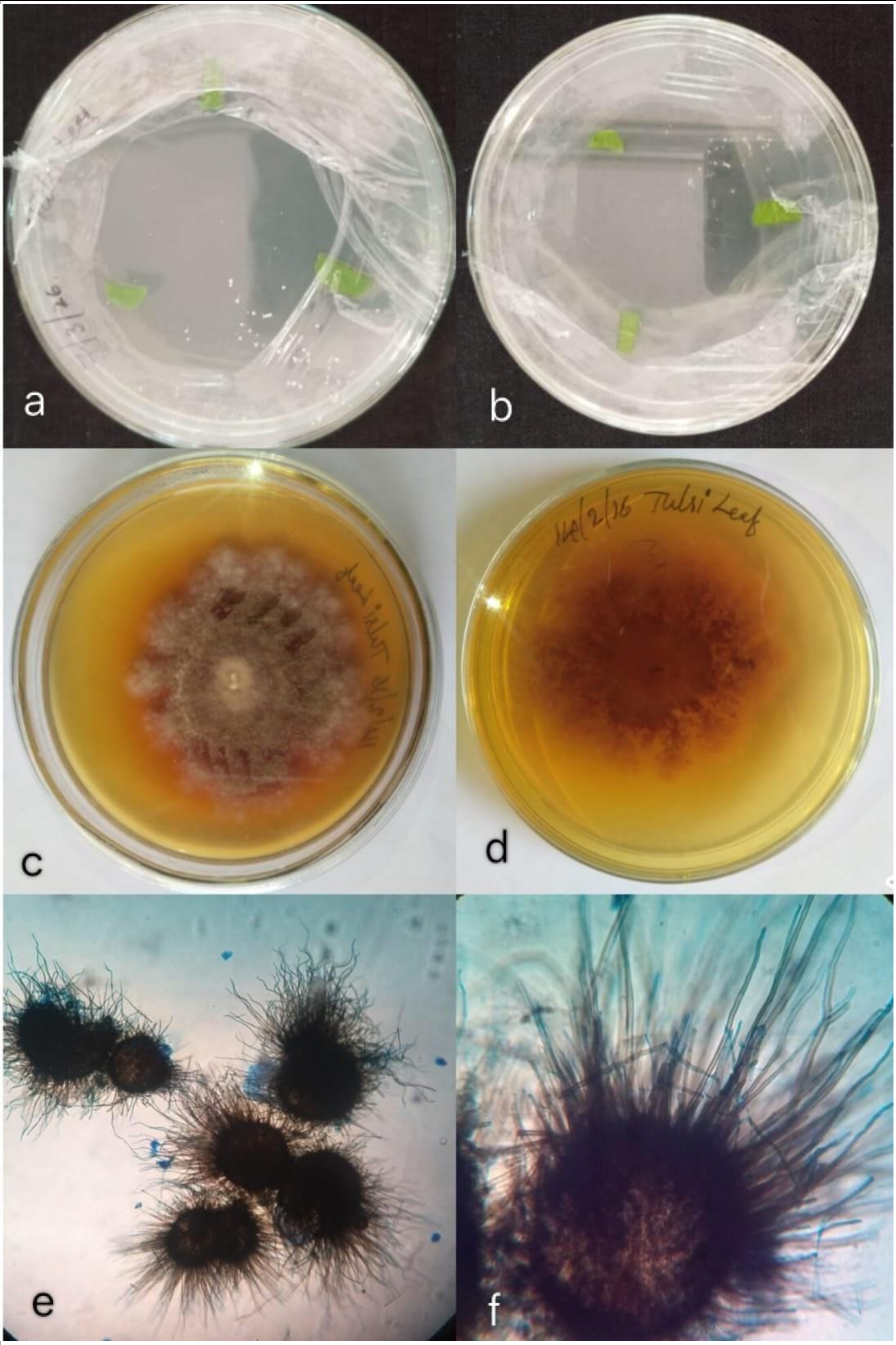

Figure 6: Culture plates showing fungi isolated from *Ocimum sanctum* leaf: A. Inoculation of *Ocimum sanctum* leaf on culture media, B. Backside view of inoculated culture plate, C. Cultured plates exhibiting active growth of *Colletotrichum sp*, D. Backside view of the same cultured plate, E. Microscopic observation of *Colletotrichum sp* in 10x low power, F. Microscopic observation of *Colletotrichum sp* in 40x high power

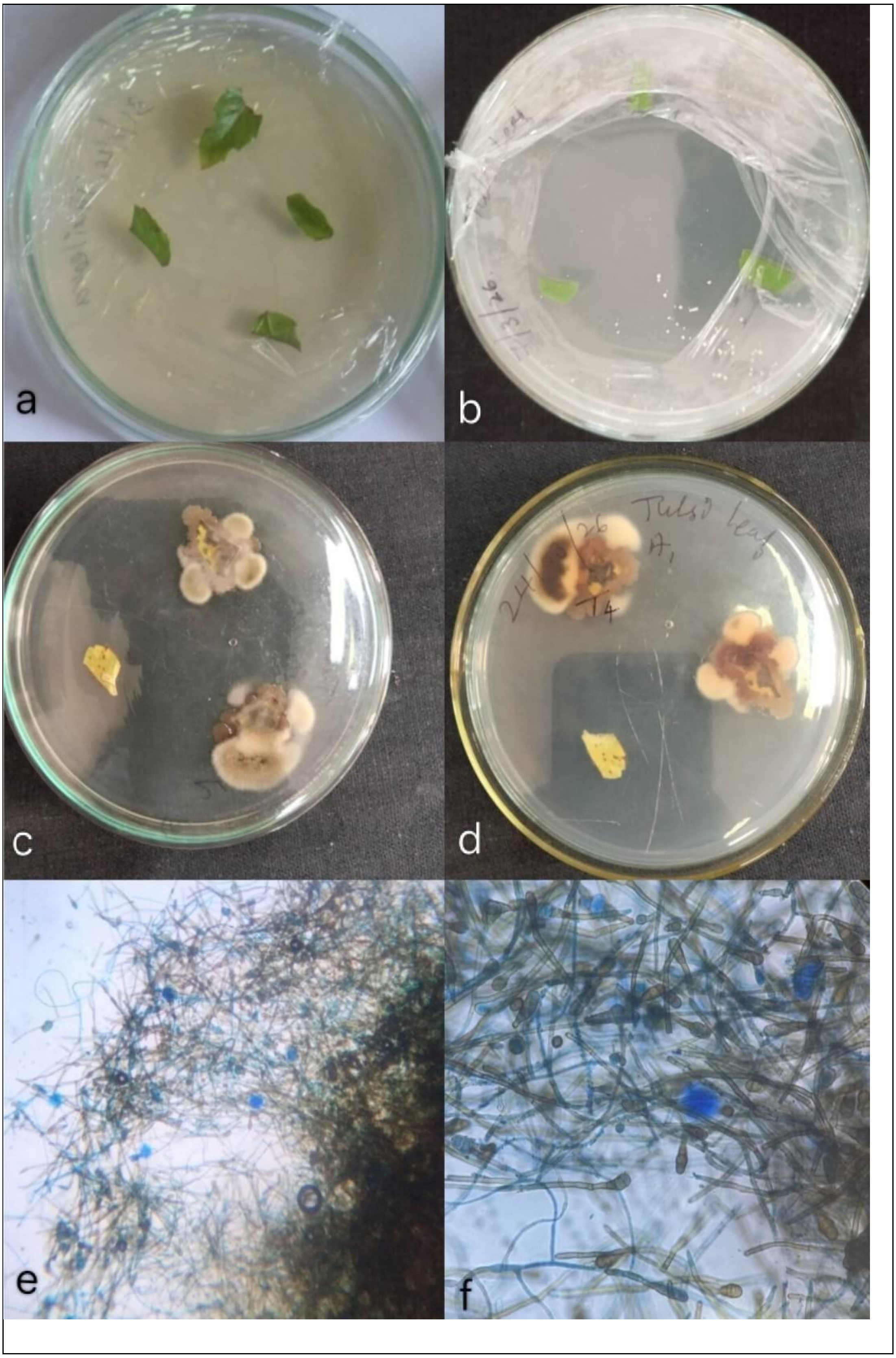

Figure 7: Culture plates showing fungi isolated from *Ocimum sanctum* leaf, A. Inoculation of *Ocimum sanctum* leaf on culture media, B. Backside view of inoculated culture plate, C. Cultured plates exhibiting active growth of *Phoma sp,* D. Backside view of the same cultured plate, E. Microscopic observation of *Phoma sp* in 10x low power, F. Microscopic observation of *Phoma sp* in 40x high power

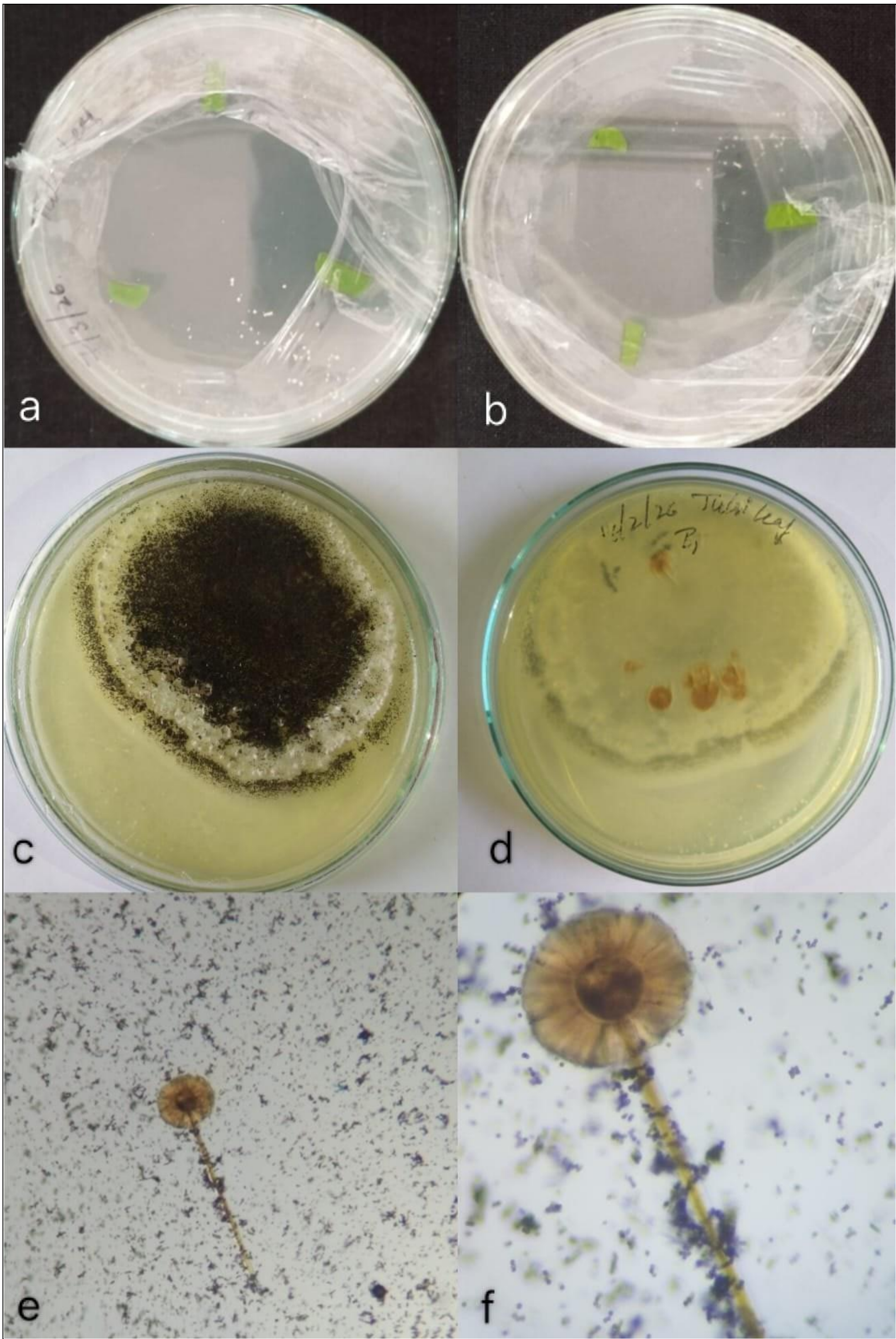

Figure 8: Culture plates showing fungi isolated from *Ocimum sanctum leaf*: A. Inoculation of *Ocimum sanctum* stem on culture media, B. Backside view of inoculated culture plate, C. Cultured plates exhibiting active growth of *Aspergillus niger,* D. Backside view of the same cultured plate, E. Microscopic observation of *Aspergillus niger* in 10x low power, F. Microscopic observation of *Aspergillus niger* in 40x high power

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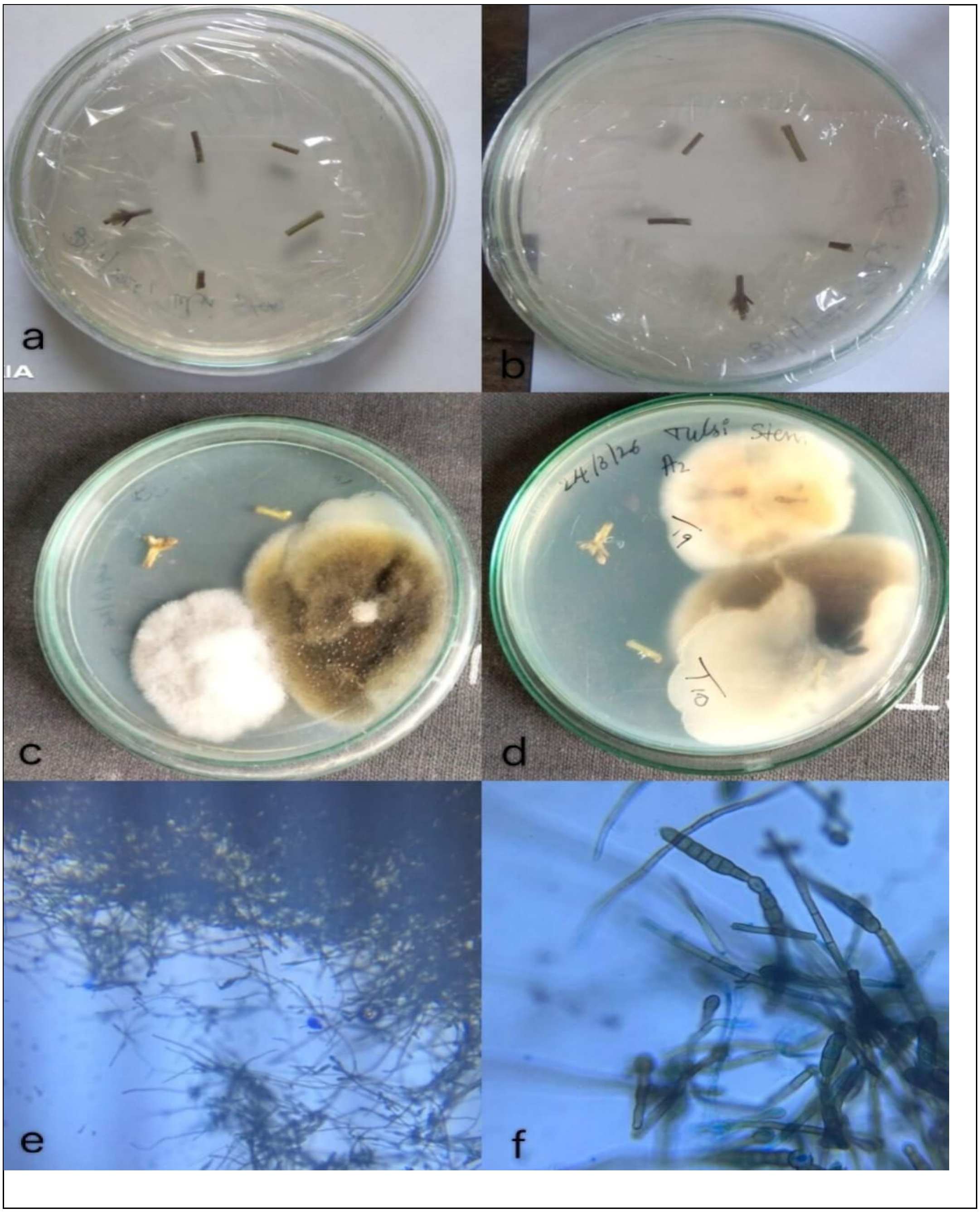

Figure 9: Culture plates showing fungi isolated from *Ocimum sanctum stem*: A. inoculation of *Ocimum sanctum* stem on culture media, B. Backside view of inoculated culture plate, C. Cultured plates exhibiting active growth of *Phoma sp*, D. Backside view of the same cultured plate, E. Microscopic observation of *Phoma sp* in 10x low power, F. Microscopic observation of *Phoma sp* in 40x high power

DISCUSSION

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.15 Tamilvannan M.V et al., 2018 reported the presence of Rhizopus sp. as endophytic fungi in Memecylon umbellatum.16 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.17 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.18

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.19 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.20 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.21 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.22 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.22 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.18 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

CONCLUSION

Endophytic fungi are microorganisms that inhabit internal plant tissues without causing visible disease symptoms or damage to their hosts. These fungi are ubiquitous in nature and are known to establish symbiotic associations with a wide range of plant species. The present study aimed to isolate and identify endophytic fungi from the medicinal plants Wrightia tinctoria and Ocimum sanctum. Plant samples were subjected to surface sterilization and cultured on potato dextrose agar supplemented with ampicillin. Emerging fungal colonies were purified and identified using morphological characteristics and lactophenol cotton blue staining. A total of seven fungal isolates were obtained from Wrightia tinctoria, namely Aspergillus sp., Aspergillus niger, Fusarium sp., Neocosmospora sp., Pythium sp., Rhizoctonia sp., and Rhizopus sp. Nine fungal endophytes were isolated from Ocimum sanctum, including Aspergillus sp., Aspergillus niger, Colletotrichum sp., Fusarium sp., Phoma sp., and Rhizoctonia sp. The results revealed a diverse population of endophytic fungi associated with both medicinal plants. These endophytes may serve as potential sources of novel bioactive compounds and contribute to the medicinal value of their host plants.

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