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Current trends of endophytic fungi in *Ocimum species* and its applications

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Abstract
The sacred basil, commonly known as Tulsi, has gained worldwide acknowledgment for its spiritual and religious significance. According to Ayurveda, it is regarded as the "elixir of life" due to its potent aroma and astringent taste, both of which enhance longevity. *Ocimum* is well known for metabolites phenols, terpenoids, flavonoids, and alkaloids. Fungal endophytes are often capable of producing secondary metabolites such as antibiotics, antifungal, antiviral, anticancer, and antimalarial compounds, etc. Recent studies have highlighted the diverse endophytic fungi in the *"Ocimum*" *species*, including "*O. sanctum", "O. basilicum",* and *"O. gratissimum*", offering promising applications across medicine, agriculture, and biostimulation. These fungi exhibit significant antimicrobial, antifungal, biocontrol, and stress-mitigating activities, particularly under salinity conditions with key genera including *Aspergillus, Fusarium, Alternaria, Curvularia,* and *Trichoderma.* Utilizing these fungal endophytes could yield novel bioactive agents, sustainable biostimulants, and eco-friendly crop protection strategies. This study provides insights about the existence of endophytes in *Ocimum species*, the products they produce, and how certain of these organisms' exhibit potential for human applications.

Keywords: *Ocimum sanctum, Ocimum basilicum*, endophytic fungi, antimicrobial, biostimulant, salinity stress, biocontrol

Introduction

Tulsi, often referred to as the "queen of herbs," is recognized as one of the most highly esteemed and valued medicinal and health-enhancing herbs found in the Eastern region. Traditionally, ailments like the common cold, bronchitis, cough, and digestive problems have been addressed with its leaves (Pattanayak *et al.*, 2010) [25]. This is attributed to the presence of volatile oils in the leaves, which exhibit anti-asthmatic, analgesic, anti-suppressant, anti-fungal, and anti-bacterial characteristics (Shekhawat and Shah., 2013) [34]. Some studies have been explored the insecticidal properties of these oils (Azevedo *et al.*, 2000) [3]. Tulsi extracts utilized in the formulation of Ayurvedic medicines for various ailments, encompassing but not limited to malaria, inflammation, cardiovascular diseases, headaches, gastrointestinal issues, as well as different types of poisoning and the common cold (Mahajan *et al.*, 2013) [21].

It is considered one of the most important sources of medicinal compounds, containing many secondary metabolites and essential oils that are recommended for treating a variety of skin disorders, rheumatoid arthritis, bronchial asthma, dysentery, bronchitis, malaria, diarrhea, and other ailments (Boham and Kocipai 1994, Bonjar *et al.*, 2004) [7, 8]. According to pharmacological research, *Ocimum species* display a variety of therapeutic properties including anticancer, antifungal, antimicrobial, antifertility, hepatoprotective, antispasmodic, cardioprotective, antiemetic, antidiabetic, analgesic, adaptogenic, and diaphoretic effects (Bonjar *et al.*, 2004; Doss 2009) [8, 13]. *Ocimum* leaf powder has shown a reduction in fasting blood glucose (21%), glycated protein (11%), total cholesterol (11%), low-density lipoprotein (LDL, 14%), very low-density lipoprotein (VLDL, 16%), and triglycerides (TG, 16%) (Rai *et al.*, 1997; Antora and Salleh, 2017) [2]. Recent research indicated a decrease in blood glucose levels in streptozotocin-induced diabetic rats with the addition of 1-2% of *Ocimum* leaf powder (Chandrasekaran *et al.*, 2013; Mahajan *et al.*, 2012; Pattanayak *et al.*, 2010; Sethi*et al.*, 2004; Suanarunsawat and Songsak, 2005) [21, 25, 31, 40]. *Ocimum basilicum* Linn. has been found to possess a notable level of antioxidant activity, which is linked to the presence of flavonoids and phenolic compounds (Madhuri and Govind P, 2010) [10, 23].

Ocimum sp. in tradition

Traditional preparations of *Ocimum sp*. include herbal tea, dried powder, and fresh leaf. However, there is also a range of other preparations available. The use of a combination of dried Tulsi leaves and grains has been used for a very long time in order to keep insects at bay (Rahman *et al.*, 2011) [26]. Basil leaves have been historically used in various cultures to address different health concerns, such as cancer, tremors, and bronchitis (Janick and Simon, 1990) [16]. Pharmacological research supports these traditional applications by demonstrating basil's effectiveness in neutralizing radicals, fighting cancer, relieving pain, combating infections, and regulating the immune system (Ch *et al.*, 2015) [9].

Ocimum species, especially "O. sanctum" (holy basil/Tulsi) and "O. *basilicum*" (sweet basil), are widely used for their therapeutic essential oils and antioxidants. Scientists are increasingly exploring their endophytic fungi as novel sources of bioactive compounds and biotic/abiotic stress mitigation strategies.

Endophytes

The medicinal capabilities of the endophytes linked to these leaves are still predominantly unexamined. Numerous studies concentrate on assessing the therapeutic attributes of secondary metabolites generated by endophytes, including increased resilience to abiotic stress, defense against plant pathogens, and enhanced nutrient uptake. Endophytes residing within their host plants have demonstrated various advantages for the plants. (Zhang *et al.*, 2006; Sudha *et al.*, 2016) [41]. More than ten endophytes were investigated in *Ocimum* sanctum leaves, and each one has unique characteristics (Banerjee *et al.*, 2000) [4, 43]. When endophyte extracts were investigated, the fungus that grows in *Ocimum basilicum* leaves shown strong antibiotic action against Staphylococcus aureus and Bacillus cereus (Haque *et al.*, 2005) [14]. In previous years, endophytes have been widely acknowledged for their importance in researching bioactive compounds and secondary metabolites that can combat human infections, thereby demonstrating the efficacy of these bioactive substances (Nascimento *et al.*, 2000; Souza *et al.*, 2012) [22, 38].

Diversity and Distribution of Endophytic Mycoflora

Endophytic microorganisms that reside in the tissues of living plants are relatively unstudied and potential sources of novel natural products for exploitation in medicine, agriculture and industry. It is noteworthy that, of the approximately 391,000 plant *species* that exist on the earth, each individual plant is host to one or more endophytes. Only a few of these plants have ever been completely studied relative to their endophytic biology. Consequently, the opportunity to find new and interesting endophytic microorganisms among myriads of plants in different settings and ecosystems is great (Strobel and Daisy 2003) [39].

A 2024 study isolated five endophytic fungal strains from *Ocimum* sanctum and identified them based on morphological and microscopic characteristics, including *species* like Aspergillus, Fusarium solani, and Alternaria. Investigations indicated that these endophytes generate bioactive substances with possible antimicrobial, antioxidant, and antitumor activities, implying that endophytic fungi associated with O. sanctum could serve as a promising source of new therapeutic agents and bioproducts (Namdev *et al.*, 2024). A 2021 [23] study on "O. sanctum" from Bhopal revealed up to 61 endophytic isolates across various taxa, predominantly hyphomycetes and ascomycetes (Khan and Treguria, 2021) [17]. In "O. *basilicum*" (sweet basil), endophytes "Alternaria alternate" and "Curvularia pseudobrachyspora" were identified, showing potent antimicrobial properties against bacteria and fungi (Sheham, *et al.*, 2025) [35].

Applications of endophytic-derived bioactive compounds as Plant Growth Promoting Activities:

In a study, various fungi isolated from O. *basilicum* demonstrated multiple traits that promote plant growth. Specifically, several isolates were found to have the ability to biosynthesize indole-3-acetic acid (IAA), a hormone in plants that enhances root and shoot development. For example, Aspergillus niger and Penicillium glabrum were identified as high producers of IAA. Furthermore, certain fungi generated 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase, an enzyme that helps regulate ethylene levels in plants, thus alleviating stress and promoting growth. (Figure 1). They also produced siderophores, which facilitate iron uptake, further enhancing plant nutrient acquisition. These activities collectively suggest that the endophytic fungi can positively influence plant growth by producing phytohormones, enhancing nutrient availability, and reducing plant stress (Al-Harthia *et al.*, 2023) [1].

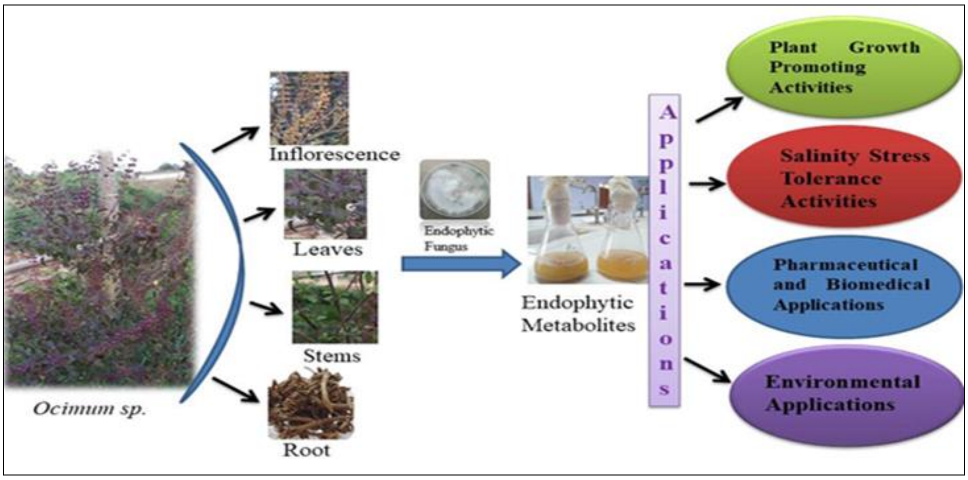

Fig 1: Applications of endophytic-derived bioactive compounds from *Ocimum sps*

Salinity Stress Tolerance Activities

Endophytic fungi assist basil in tolerating salinity stress by enhancing nutrient and mineral balance, reducing toxic ion accumulation, bolstering antioxidant and secondary metabolite production, and improving overall physiological performance, thereby reinforcing plant resilience in saline conditions (Saia *et al.*, 2021) [30]. The biostimulant, which includes Trichoderma koningii and arbuscular mycorrhiza fungi (AMF) and helps basil plants manage salinity stress through various mechanisms.

1. **Enhancement of Nutrient Uptake:** The inoculum was able to sustain the uptake of phosphorus (P) and iron (Fe), especially under saline conditions where these nutrients are typically less available. This improved nutrient acquisition supports overall plant health and growth despite salt stress.
2. **Reduction of Toxic Ion Accumulation:** Inoculated plants showed a significant decrease in sodium (Na) and chloride (Cl) content in leaves, particularly under low and high salinity levels. This suggests that the biostimulant helps mitigate ionic toxicity by promoting exclusion or sequestration of Na and Cl.
3. **Stimulation of Physiological Processes:** Endophytes stimulate physiological processes especially at moderate salinity levels (25 mM NaCl), leading to an increase in net photosynthetic rate (Pn) and improved water use efficiency (iWUE). They also bolster the plant's antioxidant mechanisms, helping to mitigate oxidative damage resulting from reactive oxygen *species* (ROS) induced by salinity.
4. **Improvement of Mineral Balance:** It increased the concentrations of beneficial microelements like B, Fe, and Mg in leaves, counteracting the mineral imbalance caused by salinity stress.
5. **Enhancement of Antioxidant Capacity and Secondary Metabolites:** They influence the synthesis of secondary metabolites such as phenolic compounds and volatile organic compounds (VOCs), which are involved in stress defense mechanisms. The inoculum elevated hydrophilic antioxidant activity (HAA) and influenced polyphenol profiles (e.g., ferulic and rosmarinic acids), which bolster the plant's defense systems against oxidative stress caused by salinity.
6. **Root and Biomass Development:** The biostimulant increased root biomass and the ratio of root to shoot biomass (R/S), especially under high salinity, facilitating better water and nutrient uptake and supporting growth under stress conditions.
7. **Stress Tolerance and Yield Improvement:** By activating plant defense pathways and improving physiological functions, endophytes help maintain biomass and yield in saline environments, making basil cultivation more sustainable under salt stress.

Overall, the biostimulant helps basil plants by improving nutrient status, reducing ionic toxicity, boosting antioxidant defenses, and maintaining physiological functions, thereby enhancing tolerance and sustaining yield across different salinity levels (Saia *et al.*, 2021) [30].

Some bacteria were evaluated for their potential to safeguard tomato plants against salt stress. The findings indicated that endophytic Tulsi bacteria can also inhabit tomato plants, suggesting their potential utility for other crops as well. When tomato plants were cultivated in elevated salt conditions (150 mM NaCl), those associated with bacteria exhibited improved health and demonstrated reduced salt damage. The detrimental molecules known as ROS were diminished, cell mortality was decreased, and chlorophyll levels were enhanced. Furthermore, the bacteria enhanced antioxidant enzymes such as CAT, SOD, POD and APX, and boosted the synthesis of proline and phenolic compounds, which are vital for stress mitigation and maintaining water balance. The roots of the treated plants developed longer, thicker, and more branched structures, thereby enhancing water and nutrient absorption. Additionally, the equilibrium of sodium (Na^+) and potassium (K^+) was more favorable in these plants, and critical stress-related genes including LKT1, NHX1, SOS1, LePIP2, SlERF16, and SlWRKY39 were effectively regulated (Sahu *et al.*, 2021) [29].

Pharmaceutical and Biomedical Applications

Nanotechnology

Silver nanoparticles (AgNPs) hold significant promise for their role in biomedical research. The leaf extract of *Ocimum* sanctum can act as a potential bio-reducing agent in the synthesis of silver nanoparticles (AgNPs), which can be used as both an antioxidant and an antimicrobial agent, demonstrating an efficient approach for utilizing bioactive resources to enhance the medicinal properties of the *Ocimum* sanctum plant effectively (Singh *et al.*, 2024) [37].

Enzyme Production

A research study of Kerala highlights the significance of fungal endophytes found in *Ocimum* plants (O. *gratissimum*, *Ocimum* tenuiflorum, and O. *basilicum*) and demonstrated the capability to produce extracellular enzymes including protease, amylase, tyrosinase, cellulase, and asparaginase. Furthermore, advanced investigations into enzyme production and quantification are crucial for evaluating the potential importance of these endophytes (Karthika and Rasmi 2022) [19].

Pharmacological properties of bioactive compounds

Extracts from "*Lasiodiplodia* pseudotheobromae," an endophyte associated with "O. sanctum," exhibit significant antibacterial properties against both Gram-positive and Gram-negative pathogens. The compound identified through GC-MS as 2-Benzenedicarboxylic acid mono (2-ethylhexyl) ester is highlighted as a primary active constituent (Taffieq and Dharah 2020) [42]. This compound, along with several others, could contribute to the antibacterial properties of the extract. The bioactive compounds sourced from the endophytic fungus, *Lasiodiplodia* pseudotheobromae IBRL OS-64 extract, have demonstrated promising antibacterial activity against a diverse range of tested pathogenic bacteria (Jalil *et al.*, 2024) [15].

Secondary metabolite production

Petroleum ether and ethyl acetate were utilized to extract secondary metabolites from endophytic fungi, and these extracts demonstrated significant antimicrobial activity against human pathogens such as Aspergillus chrysogenium, Escherichia coli, Bacillus subtilis and Aspergillus fumigatus. Additionally, two fungal strains, Curvularia pseudobrachyspora AUMC16441 and Alternaria alternata AUMC16440, generated indole acetic acid (9.62 and 5.7 µg/mL when supplemented with 5 µg/mL tryptophan) and exhibited notable antidiabetic effects (94% and 82%). Additionally, the gas chromatography-mass spectrometry (GC-MS) analysis of both fungal and plant extracts revealed a range of bioactive compounds, which included flavonoids, fatty acids, phenolics, hydrocarbons, and alcohols. Among the most prominent compounds identified were hexadecanoic acid, tetradecane, phenol 2, 2'-methylenebis [6-(1,1-dimethylethyl)-4-methyl], 13-docosenamide, 7,9-di-tert-butyl-1-oxaspiro (4,5)deca-6,9-diene-2,8-dione, ethyl iso-allocholate, cyclooctasiloxane hexadecamethyl, cycloheptasiloxane tetradecamethyl, and cyclohexasiloxane dodecamethyl, all of which are recognized for their beneficial biological properties (Al-Harthi *et al.*, 2024; Sharaf *et al.*, 2022) [33].

In a recent research effort, three endophytic fungi were isolated and characterized both morphologically and genetically as A. fumigatus, A. nidulans and A. flavus. These endophytic fungi have been recognized as a significant source of natural compounds with various biological activities. Crude extracts obtained from endophytic A. fumigatus A. nidulans and A. flavus have demonstrated notable antibacterial effects against both Gram-negative and Gram-positive bacteria, in addition to antifungal properties against unicellular fungi. Furthermore, the compounds derived from these endophytic fungi exhibited antioxidant activity without any cytotoxic effects on normal cell lines. Consequently, the substances produced by these endophytic fungi could be seen as safe and may serve as alternatives to conventional antimicrobial agents.

Antifungal

Endophytic fungi obtained from the medicinal plant *Ocimum sp*. exhibit antifungal properties against various test fungi, such as C. albicans and C. utilis. Two specific fungal endophytes, *Lasiodiplodia* sp. IBRL OS-64 and Muscodor sp. IBRL OS-94, showed significant antifungal effects with a fungicidal action against C. albicans and C. utilis. According to SEM analysis, the candidal cells that were subjected to dichloromethane extracts from the fungal endophyte experienced considerable damage, ultimately leading to cell death (Taufiq *et al.*, 2023). Antibacterial and antimicrobial activity [42].

In a research study, it was found that compounds derived from the endophytes of *Ocimum sp*. possess the ability to eliminate bacterial *species* such as E. coli (Nisa *et al.*, 2025) [24]. The results suggest that *Ocimum sp*. demonstrates a notable dose-dependent antibacterial response, with the ethanol extract showing the highest level of inhibitory activity, followed by the methanol and aqueous extracts. The diameter of the inhibition zone increased as the concentration of the extract rose, indicating a direct relationship between the extract concentration and bacterial inhibition. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) further validated the effectiveness of *Ocimum sp*., particularly its ethanol extracts, which exhibited the lowest concentrations required to inhibit and eradicate bacterial strains. These findings suggest that *Ocimum sp*. could serve as a powerful natural antimicrobial agent against pathogenic bacteria (Bhagat *et al.*, 2012) [5].

The ethyl acetate and methanol extracts obtained from endophytic fungi associated with *Ocimum basilicum* demonstrated a broad spectrum of antimicrobial effectiveness. Differences in this effectiveness were linked to the particular fungal isolates, the nature and concentration of the antimicrobial substances found in their extracts, and their unique mechanisms of action against different microorganisms (Sewaikit 2025) [32].

Antiphytopathogenic Activity

The constituents 2H-pyran-2-one, 5,6-dihydro-6-pentyl, and palmitic acid methyl ester, as evidenced by the GC-MS chromatographic analysis, exhibited IC50 values of 1.002 and 0.662 against S. sclerotiorum, respectively, highlighting their notable role in the antifungal activity of the hexane extract. This represents the first documentation of the biosynthesis of 2H-pyran-2-one, 5,6-dihydro-6-pentyl by M. phaseolina, an endophytic fungal *species* (Chowdhary and Kaushik, 2015) [11].

Anticancer Activity

In the realm of normal-phase silica gel column chromatography, the ethyl acetate extract derived from the endophytic fungus was subjected to careful separation procedures, leading to the extraction of significant bioactive compounds, specifically ergosterol and secalonic acid. The complex chemical structures of these compounds were rigorously confirmed and characterized through a variety of advanced spectroscopic methods, including ultraviolet (UV) spectroscopy, infrared (IR) spectroscopy, mass spectrometry (MS), along with a thorough range of one-dimensional (1D) and two-dimensional (2D) nuclear magnetic resonance (NMR) techniques. To assess their biological effectiveness, both compounds were tested for cytotoxicity using the MTT assay on human pancreatic cancer cell lines. Remarkably, among the two compounds, secalonic acid exhibited substantial and promising anti-pancreatic cancer properties, showing significant cytotoxic effects with remarkably low IC_{50} values of 7.3 μM and 1.6 μM, respectively, highlighting its potential as a strong candidate for the creation of new anticancer pharmacotherapies (Shoeb *et al.*, 2013) [36].

Environmental Applications

Biotransformation/ Bioremediation of heavy metals into less toxic forms: The scientists investigated how two types of mycorrhizal fungi, Rhizophagus irregularis (AMF) and Serendipita indica, influenced the growth of sweet basil (*Ocimum basilicum*) grown in soil contaminated with lead and copper. In controlled greenhouse environments, both fungi significantly promoted plant growth and reduced lead concentrations within the aerial parts of the plant. S. indica was also effective in lowering copper concentrations, whereas AMF exhibited this effect solely in the context of simultaneous exposure to both heavy metals. Furthermore, AMF was found to enhance the production of essential oils such as linalool and eucalyptol, even in contaminated soil conditions. Consequently, the cultivation of basil in association with these fungi may improve both yield and oil quality while concurrently reducing heavy metal uptake, thereby presenting a viable strategy for agricultural practices in contaminated areas (Sabra *et al.*, 2018; Zhen *et al.*, 2024; Bhardwaj., 2025) [5, 28, 44].

Flavour and Aroma: Basil (*Ocimum* L.) is also used as a culinary herb; however, research on its flavor profiles is scarce. Recent studies evaluated nine different *species* of basil. A sensory analysis was conducted to examine their aroma and flavor characteristics. The results revealed that *Ocimum basilicum* var. *basilicum* and *Ocimum gratissimum* var. suave exhibited a strong clove scent and had distinctive taste profiles. Analyses employing metabolomics and volatilomics techniques identified 100 nonvolatile metabolites and 134 volatile compounds. Differential analysis showed that eugenol, α-terpinene, germacrene D, and malic acid were among the metabolites that varied significantly between the basil *species* investigated. Together with the sensory evaluation findings, correlation analyses indicated that α-pinene and α-cadinene were related to the sharp scent, while eugenol and germacrene D were tied to the clove-like aroma, and malic acid along with L-arabitol contributed to the sweet flavor profile typical of basil. This study provided comprehensive flavor chemistry profiles for different basil *species* and could be used as a reference for improving basil flavor. Gaining a deeper insight into the essential sensory properties and chemical makeup of fresh basil could lead to the development of enhanced cultivars that possess desirable qualities, thus fulfilling the varied demands of breeders, agronomists, food producers, and consumers (Du *et al.*, 2023) [12].

Conclusions

Endophytic fungi in "*Ocimum*" *species* remain a rich and relatively untapped resource. They are highly diverse and offer a wide range of bioactivities, including antimicrobial, antioxidant, and plant growth-promoting effects. These trends highlight their potential for pharmaceutical, agricultural, and industrial applications, making them a valuable focus for future research and biotechnological exploitation. From producing potent antimicrobial compounds to serving as biostimulants under salinity stress, their multi-modal applications are increasingly recognized. Interdisciplinary work combining fungal isolation, molecular metabolomics, and field validation will be vital to transform these trends into tangible bio-technological innovations for sustainable agriculture.

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