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A Research Journey from Epiphytic to Endophytic Fungi: Insights into Ecology, Diversity and Biotechnological Potential*

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ABSTRACT

This review reveals about the shift from epiphytic to endophytic fungi based on works of my own and others, examining their ecological functions, diversity and potential applications in biotechnology. The epiphytic fungi colonize plant surface and play crucial roles in nutrient cycling and microbial diversity across the ecosystems while endophytic fungi live within the plant tissues, forming symbiotic relationships that can boost plant growth, resilience to stress and resistance to diseases. The research utilizes a comprehensive approach, combining the field observations, molecular methods, and ecological evaluations to contrast fungal populations in epiphytic and endophytic environments. The observations demonstrate unique patterns of fungal diversity, with certain species showing preferences for either space or nutrients. These findings have significant ecological implications, emphasizing the importance of comprehending fungal interactions with their host plant. Furthermore, the study identifies several fungal strains with promising biotechnological uses such as biological control agents and producers of bioactive compounds. This review not only deepens our knowledge of fungal ecology, but also showcases the potential of these organisms in sustainable farming and environmental stewardship. By examining the transition from surface to internal colonizers, the mentioned facts offer valuable insights into fungal biology and their roles in ecosystem processes and advancements in agricultural and pharma industries.

Keywords: Endophytes, Epiphytes, Fungal diversity, Applications, Ecological aspects.

INTRODUCTION

Fungi are essential components of land-based ecosystems, serving critical functions in nutrient cycles, plant well-being, and ecological relationships. Among the various fungal lifestyles, epiphytic and endophytic fungi are particularly intriguing due to their wide-ranging ecological roles and potential uses in biotechnology. Epiphytic fungi exist on plant surfaces with or without causing damage, sometimes benefiting the plant's health by assisting in nutrient uptake and disease protection. In comparison, the endophytic fungi inhabit plant tissues internally, forming symbiotic relationships that can improve host resilience against living and non-living stressors (Rodriguez *et al.*, 2009) as shown in **Figure 1**. Examining the shift from epiphytic to endophytic lifestyles provides opportunities to investigate their ecological importance, variety, and possible applications across multiple disciplines, such as agriculture, medicine and environmental science.

Epiphytic fungi play crucial ecological roles in the environment. These organisms establish themselves on plant surfaces, forming part of the microflora that can impact plant growth and health. By enhancing the retention of water and increasing nutrient accessibility, epiphytic fungi can boost plant performance across various environmental settings (Hawksworth and Rossman, 1997). Studies have shown that the variety of epiphytic fungi is affected by multiple factors, such as the host plant species, environmental variables and climatic conditions (Arnold *et al.*, 2000). The interactions between these fungi and their plant hosts can create favourable conditions for the development of endophytic communities also, which are renowned for their intricate relationships and potential advantages to the plants they inhabit. The scientific

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community is increasingly focusing on endophytic fungi due to their capacity to generate diverse bioactive substances, some of which have found applications in the pharmaceutical and agricultural sectors (Kharwar *et al.*, 2011, 2012; Prajapati *et al.*, 2024). Many studies have demonstrated that these endophytes can produce secondary metabolites with antimicrobial, antifungal, and even anticancer effects (Strobel *et al.*, 2003; Goutam *et al.*, 2017, Kumari *et al.*, 2021; Singh *et al.*, 2021). The prospect of these fungi serving as a reservoir for novel bioactive compounds highlights the necessity of elucidating their ecological interactions and biodiversity.

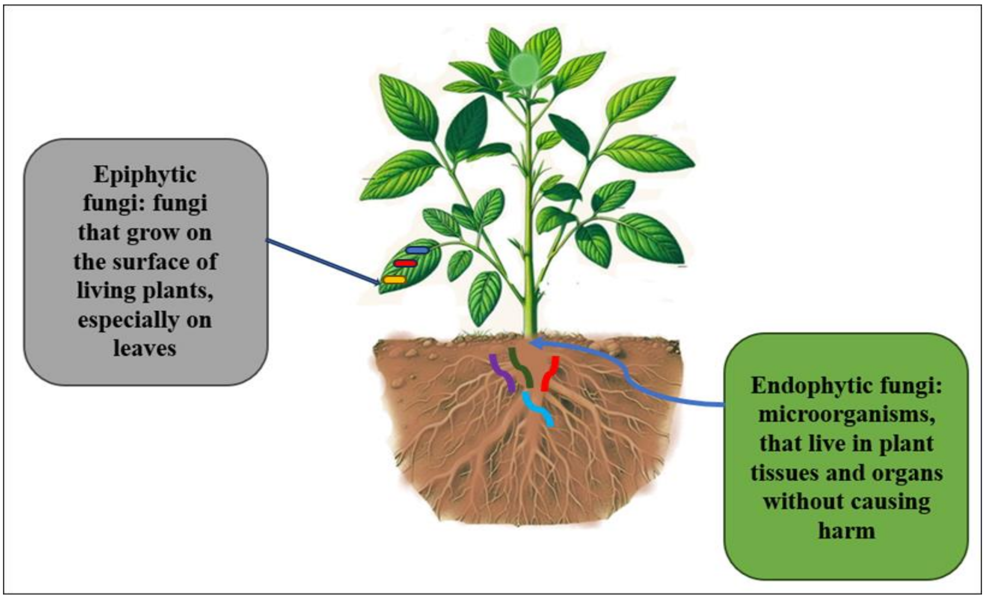

Figure 1: Plant parts showing localization of epiphytic fungi and endophytic fungi.

The shift from an epiphytic to an endophytic mode of life can also indicate broader ecological shift and adaptations within fungal populations. This change may be affected by environmental variables, characteristics of the host plant, and fungal competition. Examining these transitions allows scientists to better understand fungal evolution and the ecological functions that various fungal groups serve in ecosystem processes. Epiphytic and endophytic fungi possess significant biotechnological promises. In the agricultural sector, utilizing these fungi can enhance crop hardiness, boost production, and lessen the need for synthetic fertilizers and pesticides (Singh *et al.*, 2021). Within the pharmaceutical industry, investigating fungal byproducts could potentially uncover novel medications and treatments, addressing critical health issues. Additionally, their use in bioremediation showcases their capacity to break down environmental contaminants and aid in ecosystem recovery (Raghukumar, 2008). This study endeavours to delve into the complex realm of epiphytic and endophytic fungi with a particular emphasis on their ecological functions, biodiversity and prospective biotechnological uses. Through an examination of the connections between these two fungal lifestyles and their host plant interactions, this investigation aims to uncover the underlying factors that influence their establishment and prosperity within diverse ecosystems. The results of this research will not only enhance our comprehension of fungal ecology, but also pave the way for leveraging these organisms' advantages in sustainable practices across various fields, including agriculture, medicine, and environmental stewardship.

ECOLOGY AND DIVERSITY OF EPIPHYTIC FUNGI

Epiphytic fungi, which live on plant surfaces without invading their internal structures, constitute a varied and ecologically significant group of microbes. These organisms inhabit areas such as the phyllosphere (leaf surface), stems, and branches, playing crucial roles in ecological processes like nutrient cycling, suppressing pathogens, and engaging with other microbial populations. Their widespread occurrence across diverse ecosystems, ranging from lush tropical forests to dry environments, underscores their adaptability and the intricate nature of their plant associations. The presence of these fungi in multiple habitats demonstrates the complexity of relationships with plants and their ability to thrive in various conditions.

Ecology of Epiphytic Fungi

Fungi are also known a thread which keeps the ecosystem balanced by scavenging the living remains. In ecosystems, epiphytic fungi serve numerous essential functions, often enhancing the well-being of their host plants and the surrounding environment in which they exist.

Nutrient Cycling and Decomposition

Epiphytic fungi play a crucial role in nutrient cycling by decomposing organic matter that collects on plant surfaces, including deceased plant cells, pollen and variety of debris. This breakdown process releases vital nutrients back into the ecosystem, which can subsequently be taken up by the host plant or reintegrated into local ecological systems (Vorholt, 2012). Through their metabolism of organic carbon compounds found on plant surfaces, these fungi influence nutrient dynamics within the phyllosphere (Lindow and Brandl, 2003).

Pathogen Suppression and Plant Defence

Numerous epiphytic fungi serve as protective shields for plants by suppressing the development of harmful organisms. These fungi can generate antimicrobial substances or physically compete with pathogenic microbes for resources and space, effectively establishing a natural defence against infections (Lindow and Brandl, 2003). For example, some epiphytic species have been found to create biofilms on the surfaces of leaves, which can physically block the establishment of detrimental organisms, thus enhancing the plant's immune system (Andrews and Harris, 2000).

Interactions with other Microbes

The aerial surfaces of plants, known as the phyllosphere, serve as a distinctive environment where various microbial communities thrive. These communities include bacteria, yeasts, and fungi, which coexist in this unique habitat. Fungi that grow on plant surfaces engage in complex interactions with other microorganisms, which can range from competitive to mutually beneficial relationships. These microbial interactions play a significant role in shaping the composition and functionality of microbial communities. As a result, they can impact nutrient accessibility, the suppression of pathogens, and even influence the physiological responses of the host plant (Vorholt, 2012).

Influence on Plant Stress Tolerance

Epiphytic fungi play an important role in boosting plants' ability to withstand environmental challenges like drought, extreme heat, and UV exposure. These fungi accomplish this through two primary mechanisms: the production of secondary metabolites that offer protection to the plant, and the formation of biofilms that minimize water evaporation from plant surfaces (Hardoim *et al.*, 2015). This symbiotic relationship is particularly vital in demanding ecosystems where the survival of plants is intricately linked to their interactions with microorganisms.

Diversity of Epiphytic Fungi

The diversity of epiphytic fungi is vast, shaped by factors such as the species of host plant, environmental variables and geographic setting. Their broad taxonomic spectrum and varied relationships with host plants demonstrate the adaptability and ecological significance of these fungi.

Taxonomic Diversity

Kharwar *et al.* (1996) reported 3 new species of *Mycovellosiella*. Of these, *Mycovellosiella malloti* was from *Mallotus philippinensis*, *M. mucunae* from *Mucuna purpurea* (Papilionaceae) and *M. viticis* from *Vitex negundo* (Verbenaceae). All hosts were collected from Nepal and described fungal species were compared with related taxa. Similarly, another 2 new fungal species of *Mycovellosiella*, *M. aegli* from *Aegle marmelos*, and *M. terminalae* from *Terminalia arjuna* (Combretaceae), reported from forest of Uttar Pradesh, India. Additionally, Kharwar and Singh (2004) described 3 novel species as *Veronaea ficina* from *Ficus hispida*, *V. grewiicola* from *Grewia asiatica*, and *V. hippocratiae* from *Hippocratia arborea*, from forests of Nepal and the Terai belt of North-Eastern Uttar Pradesh, India. Kharwar *et al.* (2012) reported a new foliicolous hyphomycete, *Asperisporium pongamiae-pinnatiae* from leaf spots of *Pongamia pinnata* L. (Papilionaceae), collected from Banaras Hindu University campus in Varanasi, U.P., India. This species was described, illustrated and compared with allied taxa. Besides light microscopy, conidia and conidiomata were examined using a scanning electron microscope. Kharwar *et al.* (2015) reported *Zasmidium cassines* from the leaf of *Cassine glauca* (Rottb.) Kuntze and *Z. fabaceicola* from leaf of *Vigna unguiculata*, respectively collected from a forest located in the terai belt of Nepal. These two new foliicolous fungi were compared with closely related taxa. Kharwar and his colleagues reported *Nyssopsoraceae*, a new family under order *Pucciniales* to accommodate the genus *Nyssopsora* spp**.** (Yadav *et al.* 2023).

Various fungal phyla contain epiphytic fungi mostly belong to either Ascomycota or Basidiomycota. In temperate areas, frequently encountered genera include *Cladosporium*, *Alternaria*, *Penicillium*, and *Phoma*, which typically exist as saprophytes or commensals on plant surfaces (Inácio and Pereira, 2009). The greater biodiversity and microclimate variation in tropical ecosystems result in even more diverse epiphytic fungal communities (Arnold *et al.,* 2000). Certain tropical fungi, notably *Guignardia* and *Colletotrichum* have been observed to be particularly prevalent on tropical tree foliage (Arnold, 2007).

Kharwar *et al.* (2010) isolated 22 epiphytic fungi out of 33 fungal species from leaf segments of *Eucalyptus citriodora* Hook. (*Alternaria alternata, Aspergillus fumigatus, A. terreus, Cladosporium cladosporioides, Drechslera rostrata, Humicola grisea, Nigrospora oryzae, Penicillium cristata* and *Pestalotia* sp. etc.). Of 478 isolates, interestingly, the higher numbers of fungi (279) belong to epiphytic while only 199 to endophytic. Most isolates were anamorphic filamentous fungi which often do not produce sexual spores. However, the endophytes represent higher diversity index than the epiphytes. The frequency of colonization differs greatly in both the myco-populations. *Cladosporium cladosporioides* (26%) was the dominant species on leaf surface while *Botrytis cinerea* (10.5%) was found dominant in leaf tissues.

Host Specificity and Adaptation

The plant species on which epiphytic fungi grow often shapes the makeup of their communities. Different fungi exhibit varying levels of host preference, with some species favouring particular plants for colonization. This selectivity can be attributed to several factors, including the texture of leaf surfaces, their chemical makeup, and the accessibility of nutrients (Jumpponen and Jones, 2010). As an illustration, certain fungi may have evolved to flourish on plants with thick waxy outer layers, while others prefer to inhabit species with smoother surfaces that are richer in nutrients. The diversity of epiphytic fungal communities is influenced by the environmental conditions in which plants grow. In dry regions, epiphytic fungi that can withstand dehydration and drastic temperature changes are more common. Conversely, in moist tropical forests fungi that thrive in constant humidity are predominant (Lindow and Brandl, 2003).

Geographical Variation in Epiphytic Fungal Communities

The distribution and makeup of epiphytic fungal communities exhibit significant variation based on geographic regions. Fungi such as *Cladosporium* and *Alternaria* are frequently found as epiphytes on various plant species in temperate areas (Arnold, 2007). In contrast, tropical regions typically host a larger number of species and greater fungal diversity attributed to their intricate plant ecosystems and conducive environmental conditions (Arnold *et al.,* 2000). The abundant diversity of host plants and microclimates in tropical forests fosters a rich array of epiphytic fungi, with many species still awaiting discovery and classification.

Functional Diversity and Adaptation

Epiphytic fungi demonstrate a range of ecological functions and produce a diverse range of metabolites. Many of these fungi generate biologically active compounds, including substances that combat fungi and bacteria, as well as those that enhance the plant growth. These compounds show promise for agricultural and biotechnological applications, particularly in creating natural pesticides or growth enhancers (Inácio and Pereira, 2009). Additionally, certain epiphytic fungi have adapted to survive challenging environmental conditions, such as exposure to UV radiation and varying moisture levels, by synthesizing protective pigments or developing specialized structures on plant surfaces (Hardoim *et al.,* 2015).

BIOLOGICAL ROLES OF EPIPHYTES IN PLANT HEALTH

Fungi that grow on plant surfaces without invading their internal structures, known as epiphytic fungi, serve various functions that support plant well-being. These organisms colonize the phyllosphere, phylloplane and other above-ground plant parts, exerting influence on plant development, offering protection against harmful microbes, and boosting plants' ability to withstand stress. Epiphytes engage in intricate interactions with both their host plants and the surrounding microbial ecosystem, acting as crucial biological allies in sustaining plant health and supporting overall ecosystem functionality.

Competitive Exclusion

Epiphytic fungi often colonize the same niches as plant pathogens, such as the leaf surface. By occupying space and utilizing available nutrients, they prevent pathogenic fungi, bacteria, or other microorganisms from establishing themselves. This competition for resources and space can reduce the incidence of plant diseases (Lindow and Brandl, 2003).

Antimicrobial Compound Production

Numerous epiphytic fungi generate diverse secondary metabolites with antimicrobial qualities, which aid in inhibiting the development of detrimental pathogens. As an illustration, epiphytic fungi belonging to the *Cladosporium* and *Penicillium* genera are recognized for creating antifungal substances that restrict pathogenic fungi such as *Botrytis cinerea* and *Alternaria alternata* (Andrews and Harris, 2000). These antifungal compounds play a role in safeguarding plants against various diseases, including leaf spots, blights, and additional foliar infections.

Induction of Plant Defence Responses

Beyond directly suppressing pathogens, certain epiphytic fungi can bolster plant immunity by activating systemic acquired resistance (SAR) pathways. This process involves stimulating plant defence mechanisms that spread throughout the entire plant, increasing its resistance to a broad spectrum of pathogens (Harman *et al.*, 2004). By preparing the plant's immune system in advance, epiphytic fungi enable plants to combat infections more efficiently when they occur.

Enhancement of Plant Growth

Plant growth can be directly enhanced by epiphytic fungi through processes that facilitate nutrient uptake and enhance plant metabolic functions.

Nutrient Cycling

Epiphytic fungi play a crucial role in nutrient cycling by breaking down organic substances on plant surfaces, including deceased cells, pollen, and plant secretions. This decomposition process releases vital nutrients such as nitrogen and phosphorus into the immediate surroundings, which plants can then absorb (Vorholt, 2012). Certain saprophytic epiphytic fungi, notably *Alternaria* and *Epicoccum*, are particularly important in decomposing organic matter within the phyllosphere, thereby increasing nutrient availability for plants (Inácio and Pereira, 2009).

Promotion of Plant Hormone Balance Certain epiphytic fungi generate substances that promote plant growth, including indole-3-acetic acid (IAA), a hormone that plays a role in controlling plant growth and development. These hormones can enhance root and shoot development, enabling plants to improve their nutrient absorption and growth in both normal and challenging environments (Hardoim *et al.*, 2015). Additionally, in some cases, epiphytic fungi may influence the synthesis of other plant hormones such as gibberellins and cytokinins, further supporting plant growth and development.

Stress Tolerance and Adaptation

Epiphytic fungi are crucial in enhancing plants' resilience to various environmental stresses, such as water scarcity, ultraviolet exposure, and shifts in temperature. These fungi accomplish this by either influencing the physiological functions within plants or by creating protective layers on their surfaces.

Biofilm Formation and UV Protection

Numerous epiphytic fungi create protective coatings known as biofilms on leaf surfaces. These biofilms consist of fungal cells and extracellular matrix material, which serve to shield plants from harmful UV rays and prevent water loss by retaining moisture on the leaf (Beattie, 2002). Through their ability to mitigate environmental stresses, biofilms contribute to the preservation of leaf structure and safeguard against damage resulting from severe weather conditions.

Drought Tolerance

Epiphytic fungi can assist plants in maintaining water equilibrium in environments where water is scarce. These fungi may promote the synthesis of osmoprotectants or improve the plant's water use efficiency. Some epiphytic fungi, for instance, can trigger the plant to generate stress-related compounds like proline and soluble sugars, which enhance the plant's ability to withstand drought and other abiotic stressors (Hardoim *et al.,* 2015). Moreover, fungal biofilms on leaf surfaces can act as an additional protective barrier, minimizing water loss in arid conditions.

THE HIDDEN WORLD OF ENDOPHYTIC FUNGI

Endophytic fungi are a remarkable and diverse group of microorganisms that reside within plant tissues without immediately harming their host plants. These fungi have been discovered in virtually all examined plant species, colonizing the internal structures of stems, leaves, roots, and seeds, and establishing symbiotic associations with their hosts (de Bary, 1866; Strobel and Strobel, 2007; Kharwar *et al.,* 2009; Franquelo and Perez-Rodriguez, 2016). Earlier Studies have shown that endophytes are beneficial to their host, as they produce diverse chemicals in their niche, which are unlike pathogenic fungi, endophytes do not produce visible symptoms, making their presence generally undetectable. In recent years, there has been growing interest in the ecological functions of these fungi, their impact on plant well-being, and their potential uses in agriculture, medicine, and biotechnology.

Diversity of Endophytic Fungi

Endophytic fungi exhibit remarkable diversity, occurring in virtually all plant families across a broad spectrum of ecosystems. The variety of these fungi is subject to several influencing factors, such as the specific plant species, different tissues, environmental parameters, and geographic setting.

Taxonomic Diversity

Kharwar and his research team in 2017 found a diverse array of taxonomic groups comprises endophytic fungi, from different tissues of *Tectona grandis* Linn f., with Ascomycota representing the largest portion of identified endophytes, followed by Basidiomycota. *Diaporthe* (*Phomopsis*) species dominated the communities independently on tissue type, location or season. More than 60% of the examined tissue pieces were colonized by members of this species complex (Singh *et al.,* 2017). Across the plant tissues, the common endophytic genera were *Fusarium, Colletotrichum, Penicillium,* and *Xylaria* (Arnold *et al.,* 2000; Singh *et al.,* 2017). These fungal organisms can demonstrate high levels of specialization to their host plants, with some species showing strong host tissue specific preferences, while others are more versatile and capable of colonizing multiple plant species.

Kharwar and his research team in 2012 isolated 1,151 endophytic fungal isolates representing 29 taxa from symptom-less, surface-sterilized segments of stem, leaf, petiole, and root of *Tinospora cordifolia* which had been collected at three locations of Varanasi district of India differing in air pollution (Ramnagar, Banaras Hindu University, Maruadih) during three seasons (summer, monsoon, winter). Endophytes were most abundant in leaf tissues (29.38% of all isolates), followed by stem (18.16%), petiole (10.11%), and root segments (6.27%). *Penicillium* spp. were dominant (12.62% of all isolates), followed by *Colletotrichum* spp. (11.8%), *Cladosporium* spp. (8.9%), *Chaetomiumglobosum* (8.1%), *Curvularia* spp. (7.6%), and *Alternaria alternata* (6.8%) (Mishra *et al.,* 2012).In another study, his research team isolated endophytic fungi from healthy, living, and symptomless tissues of inner bark, leaf, and roots of *Aegle marmelos*, a well-known medicinal plant, growing in different parts of India including Varanasi. Of 79 isolates of *A. marmelos* distributed in 21 genera and mostly belong to deuteromycotina. The result was quite encouraging in terms of maximum isolates recovery from hyphomycetes (78.5%) followed by ascomycetes (8.9%) and coelomycetes (7.6%), respectively which corroborates previous studies in same area. However, 5.1% isolates remained unidentified and were classified under Mycelia Sterilia. No isolate was obtained from either basidiomycotina or from zygomycotina. *Fusarium* spp. had maximum colonization frequency (8.00%) in this plant. The other dominant endophytic genera were *Aspergillus* spp., *Alternaria* sp., *Drechslera* sp., *Rhizoctonia* sp., *Curvularia* sp., *Nigrospora* sp., and *Stenella* sp. (Gond *et al*., 2007).

Kharwar and his research team in 2007 isolated 233 endophytic fungi representing 18 fungal taxa were obtained from segments of bark, stem, and leaves of *Azadirachta indica* A. juss. Hyphomycetes (62.2%) were the most prevalent followed by the Coelomycetes (27.4%) and Mycelia Sterilia (7.7%). Endophytic colonization frequency was greater in leaves (45.5%) than bark (31.5%). Bark samples showed maximum diversity at different locations. Inter-site comparisons for endophytic diversity. The dominant endophytic fungi isolated were *Phomopsis oblonga*, *Cladosporium cladosporioides*, *Pestalotiopsis* sp., *Trichoderma* sp., and *Aspergillus* sp. Genera such as *Periconia*, *Stenella*, and *Drechslera* are reported here for the first time as endophytes from this host plant (Verma *et al*., 2007). A total of 272 isolates were recovered at rate of 68.0% colonization frequency from 400 samples of Indian Neem *Azadirachta indica* collected from three different individual trees. Mycological agar (MCA) medium yielded the highest number of isolates (95, with a 14.50% isolation rate) with the greatest species richness. Mycelia Sterilia (1, 2, 3) accounted for 11.06%, Coelomycetes 7.25%, while Hyphomycetes showed the maximum number of representative isolates (81.69%). Mycelia-Sterilia (1, 2, 3), based on their 5.8S ITS 1, ITS2 and partial 18S and 28S rDNA sequences were identified as *Fusarium solani* (99%), *Chaetomium globosum* (93%) and *Chaetomium globosum* (93%), respectively. *Humicola*, *Drechslera*, *Colletotrichum*, and *Scytalidium* sp. were the rare taxa of endophytes isolated from this host (Verma *et al*., 2011).

Kharwar *et al.* (2008) isolated 183 endophytic fungi representing 13 fungal from leaf, stem and root tissues of *Catharanthus roseus* from two sites representing two different ecosystems in North India. With exceptions of only two species one each from coelomycete and ascomycete, all were from hyphomycetes. It was observed that the root tissue was highly colonized by *Alternaria*, *Cladosporium* and *Aspergillus*. However, leaf tissues showed a greater diversity of endophytes, and *Drechslera, Curvularia, Bipolaris, Alternaria* and *Aspergillus* spp. were the dominant fungi. Interestingly, a fungus producing no fruiting structures was the most prevalent, isolated from *C. roseus*. On the basis of its partial ITS-5.8 S rDNA sequence, it was identified as *Chaetomium globossum*, with 99% sequence similarity (537/540). The species richness and colonization frequency of endophytic fungi were pronounced in the root tissues at both sites (Loc1, 38.6%; Loc 2, 33.7%). It was concluded that endophytes are both host- and tissue-specific. This study revealed that there were very fewer differences in the species richness of fungal endophytes regardless of ecological variations.

Kharwar and his research team in 2009 isolated 55 fungal endophytes from 20 plants, and 60% of these showed inhibitory activity against one or more pathogenic fungi and bacteria. Actinomycetes were most commonly recovered from roots (54.5% of all isolates), followed by stems (23.6%), and leaves (21.8%). The dominant genus was *Streptomyces* (49.09% of all isolates), while *Streptosporangium* (14.5%), *Microbispora* (1 0.9%), *Streptoverticillium* (5.5%), *Sacchromonospora* sp. (5.5%), and *Nocardia* (3.6%) were also recovered. *Streptomyces* isolates AzR 006, 011, and 031 from roots showed acute activity against *Pseudomonas fluorescens*, whereas AzR027, 032, and 051 also from roots showed activity against *Escherichia coli*. In addition, isolate AzL025 of *Nocardia* sp. obtained from leaves showed antagonistic activity against *Bacillus subtilis*. Overall, 32 of the 55 were found to have broad spectrum significant antimicrobial activity, while about 4% of them showed strong and acute inhibition to pathogenic fungi and bacteria. Isolates of *Streptomyces* AzR031, 008, and 047, *Nocardia* sp. AzL025, and *Streptosporangium* sp. AzR 021 and 048 are of particular interest because they showed significant antagonistic activity against root pathogens, including *Pythium* and *Phytophthora* sp. Thus, many of the isolates recovered from *A. indica* in this study may be used in developing potential bio-control agents against a range of pathogenic fungi and bacteria and in the production of novel natural antimicrobial compounds. These results not only further our understanding of plant-microbe interactions but also indicate that there is an untapped resource of endophytic microorganisms that could be exploited in the biotechnological, medicinal, and agricultural industries (Verma *et al.,* 2009)

Kharwar *et al.* (2011) recovered 149 isolates of endophytic fungi from *Adenocalymma alliaceum* from 270 segments (90 from each stem, leaf and petiole) which represented 17 fungal taxa. Hyphomycetes (77.85%) were the most prevalent, followed by Ascomycetes (8.05%) and Coelomycetes (4.03%) respectively. A considerable number of fungal isolates was kept under (10.07%) mycelia-sterilia (MS). Leaf harboured maximum colonization of endophytic fungi (72.22%) which was greater than stem (67.78%) and petiole (25.54%). The dominant endophytic fungi were *Alternaria alternata, Aspergillus niger, Stenella agalis, Fusarium oxysporum, Curvularia* *lunata* and *Fusarium roseum* (Kharwar *et al.,* 2011).

Kharwar and his research team in 2014 isolated 1,897 endophytic fungi from *Madhuca indica* Gmel., representing 40 morphologically distinct fungal taxa were obtained from 2,700 segments of stem, bark and leaf from three different locations (Loc 1, Loc 2 and Loc 3) in Uttar Pradesh, India. Coelomycetes (62.41%) were the most prevalent fungal group followed by hyphomycetes (28.89%) and ascomycetes (8.70 %). Colonisation frequency (CF) was greater in stem (82.55%) than in leaf (65.00%) and bark (63.22 %). The dominant endophytic fungi isolated were *Phomopsis* sp. 1 (9.185%), and *Colletotrichum gloeosporioides* (7.00%) (Verma *et al.*, 2014).

Kharwar and his research team in 2014 studied about three medicinal plants, *Aloe vera, Mentha arvensis* and *Ocimum sanctum* were explored for endophytic actinomycetes diversity. Endophytic actinomycetes were most commonly recovered from roots (70% of all isolates) followed by stems (17.5%) and leaves (12.5%), respectively. Single genus *Streptomyces* ranked first (60% of all isolates) followed by *Micromonospora* (25%), *Actinopolyspora* (7.5%), and *Saccharopolyspora* (7.5%). The maximum number of endophytic actinomycetes were isolated from *Ocimum sanctum* (45%) (Gangwar *et al.,* 2014).

Kharwar and his research team in 2018 isolated 14 taxa of endophytic fungi from *Madhuca longifolia*, dominated by *Colletotrichum gloeosporioides*, *Corynespora* and *Macrophoma* respectively. Reduction in leaf segment size showed increased isolation rate and significantly changed the diversity to all culture media. On potato dextrose agar 153 isolates were recovered with minimum isolation rate and diversity (1.20 isolates/cm^2, Shannon index 1.43) to larger size segments (T1), followed by medium size segments (T2) (1.95 isolates/cm^2, Shannon index 1.78) and maximum to small size segments (T3) (4.50 isolates/cm^2, Shannon 1.89). On malt extract agar 145 isolates were recovered in which large size segments showed minimum isolation rate and diversity (1.30 isolates/cm^2, Shannon of 1.33) followed by medium size (isolation rate 1.95 isolates/cm^2, Shannon of 1.55) and maximum to small size segments (isolation rate 4.0 isolates/cm^2, Shannon of 1.96). On water agar, 108 isolates were recovered with similar trends of isolation rate and diversity to large size (0.65 isolates/cm^2, Shannon of 0.27) followed by medium size (isolation rate 1.40 isolates/cm^2, Shannon of 1.42) and maximum to small size segments (3.35 isolates/cm^2, Shannon of 1.59) (Verma *et al.,* 2018).

Kharwar and his research team in 2021 isolated 263 endophytic isolates recovered from 1800 segments of photosynthetic roots of *Tinospora cordifolia* plotted in three different seasons (winter, summer, and monsoon) at three different locations (BHU, Ramnagar, and Maruadih) representing the 20 different fungal taxa. The colonization frequency was maximal during monsoon (20.5%) followed by winter (13.83%) and minimal during summer (9.5%). However, among sites, it was maximum at location 3 (Ramnagar) (18.66%) followed by location 1(BHU) (17.16%) and location 2 (Maruadih) (8.0%). A maximum CF of 1.5% was observed for *Cladosporium cladosporioides* followed by *Alternaria alternata* and *Colletotrichum gloeosporioides* 1.27%, *Nigrospora oryzae* 1.22% and *Phomopsis tersa* 1.1% while *Aspergillus tubingensis* followed by *Fusarium brachygibbosum* were recovered as a rare taxon with 0.16% and 0.22% colonization frequency, respectively. The MANOVA and Jaccard's distance (Jc) clearly indicate that the effect of season was more pronounced than the location in respect to species diversity (Mishra *et al.,* 2021). Among all active isolates *Pseudofusicoccum adansoniae* exhibited an impressive antibacterial activity against all the tested human pathogenic bacteria. Eleven endophytic fungi (55.00 %) were found to be active against one or more fungal pathogen. Eight endophytic fungi exhibited the production of amylase (40.00%) on growth medium. The cellulase, lipase, pectinase, protease and xylanase production were exhibited by 25.00%, 35.00%, 10.00%, 35.00% and 30.00% endophytic fungi, respectively. Out of 20 endophytic fungi 20% were found to show antioxidant activity, 45% of endophytic isolates exhibited siderophore production while none of the fungus was found to solubilize phosphate in solid agar medium.

Ecological and Geographic Variation

Kharwar *et al.* (2008) found that the composition of endophytic fungal communities can differ considerably depending on the host plant's environment and surrounding ecosystem (Kharwar *et al.,* 2008; Singh *et al.,* 2017). Tropical regions typically support a greater variety of endophytic fungi than temperate areas, owing to the increased plant diversity and more consistent climate conditions found in tropical environments (Arnold and Lutzoni, 2007). On the other hand, plants that grow in challenging habitats, such as desert or high-elevation areas, frequently contain endophytic fungi that have specifically evolved to endure harsh conditions (Kumar and Hyde, 2004).

Endophytes-Plant Specificity

Kharwar and his research team in 2012 found that endophytic fungi display varying degrees of host selectivity, with some capable of colonizing a wide range of plants, while others are restricted to specific plant species or even particular plant tissues (Mishra *et al.,* 2012). This host specificity is influenced by multiple factors including the biochemical makeup of plant tissues, the host's defensive strategies, and environmental conditions (Saikkonen *et al.,* 1998). A notable example is the *Epichloë* species, a group of grass endophytes known for their highly specialized relationships with host plants, often playing a vital role in enhancing the anti-grazing activity of host hat help in survival and overall fitness.

Ecological Functions and Symbiosis in Endophytic Fungi

Plant tissues are home for endophytic fungi to which they do not cause disease and are crucial members of microbiome associated with plants. These fungi form symbiotic relationships with plants ranging from mutually beneficial to commensal, and play a determining role in plant health, development and longevity. The ecological functions of endophytic fungi contribute to plants' ability to withstand stress, enhance their nutrient uptake, and defend against biological and environmental challenges (Prajapati *et al.,* 2024). As scientists continue to explore this area, the ecological importance and symbiotic nature of endophytic fungi are gaining recognition in both wild ecosystems and farming environments.

Mutualistic Symbiosis and Plant Growth Promotion

A key ecological role of endophytic fungi is their ability to promote plant growth and development through mutually beneficial symbiotic relationships. These interactions result in advantages for both the fungal organisms and their host plants.

Nutrient Acquisition and Cycling

Kharwar and his research team in 2024 found the endophytic fungi contribute to improved plant nutrition by various mechanisms, including phosphorus solubilization, siderophore production, and enhancing the plant's capacity to extract water and nutrients from the soil (Prajapati *et al.,* 2024). One notable example is the endophytic fungus *Piriformospora indica,* which boosts phosphorus acquisition in its host plant, resulting in greater biomass and yield, particularly in soils lacking essential nutrients (Varma *et al.,* 1999). Through their involvement in nutrient cycling processes, endophytic fungi are essential for preserving soil fertility and promoting plant growth across the both wild and cultivated environments (Rodriguez *et al.,* 2009).

Production of Plant Growth-Promoting Hormones

Numerous endophytic fungi synthesize phytohormones including indole-3-acetic acid (IAA), gibberellins, and cytokinins. These compounds promote the elongation of roots and the growth of shoots, thereby enhancing the overall robustness and health of the host plant (Prajapati *et al.,* 2024). As an illustration, endophytic fungi recovered from medicinal plants such as *Taxus* spp., have demonstrated the ability to produce IAA. This hormone encourages root development and assists in the uptake of nutrients (Khan *et al.,* 2015).

Pathogen Defence and Biocontrol

Symbiotic fungi living within plants play a crucial role in protecting them from harmful pathogens. These endophytes accomplish this through two main mechanisms: they synthesize biologically active substances that suppress the development of detrimental microbes, and they enhance the plant's natural defence mechanisms.

Production of Antimicrobial Compounds

Kharwar and his research team in 2021 discovered the endophytic fungi play a crucial ecological role through their production of secondary metabolites with antimicrobial properties. These substances enhance the host plant's resistance to various pathogens, including bacteria and fungi (Nishad *et al.,* 2021; Singh *et al.,* 2021). Kharwar *et al.* (2011) found the crude extracts of nine endophytic fungi (75%) from a medicinal plant *Adenocalymma* *alliaceum*, showed antibacterial potential against one or more clinical human pathogens. *Alternaria alternata, Curvularia lunata, Penicillium* sp. and *Chaetomium globosum* exhibited significant antibacterial activity against *Shigella flexnii* and *Salmonella enteritidis*. Kharwar *et al.* (2012) found the *Pestalotiopsis* sp. showed significant inhibitory activity against *Phytophthora cryptogea* (57.7%), *Pythium aphanidermatum* (54.5%) and *Microsporumnanum* (51.4%), while *Phomopsis* sp. inhibited *P. aphanidermatum* moderately from medicinal plant *Cinnamomum camphora*. Research has demonstrated that endophytic fungi belonging to genera such as *Penicillium*, *Colletotrichum*, and *Fusarium* generate antibiotics that inhibit the growth of plant pathogens (Strobel and Daisy, 2003). An illustrative example is the *Colletotrichum* species found in *Theobroma cacao,* which produces antifungal compounds that suppress *Phytophthora palmivora*, the pathogen responsible for black pod disease in cacao plants (Arnold *et al.,* 2003). *Botryosphaeria rhodina* (JQ031157) and *C. globosum* showed activity against all bacterial pathogens (*Shigella flexnii, E. coli, Salmonella enteritidis, S. paratyphi, Pseudomonas aeruginosa, Citrobacter freundii, Morganella morganii, Proteus vulgaris* (Mishra *et al.,* 2012).

INDUCTION OF SYSTEMIC ACQUIRED RESISTANCE (SAR)

Kharwar and his team in 2007 found the certain endophytic fungi have the ability to boost a plant's natural immune defences by activating systemic acquired resistance (SAR). This SAR mechanism is a widespread immune response that equips the plant to combat future pathogenic invasions. *Pseudomonas fluorescens* isolate Pf4-99 systemically induced resistance against dry root of chickpea by the accumulation of battery of enzymes in response to pathogen infection (Kumar *et al.,* 2007). Additionally, *P. fluorescens* isolates systemically induced resistance against early blight of tomato caused by *A. alternata* and reduced (approx.18-42%) the disease significantly (Kumar *et al.,* 2015). The colonization by *Trichoderma harzianum* an endophytic fungus that can initiate SAR pathways within the plants, resulting in improved resistance to various fungal and bacterial pathogens (Harman *et al.,* 2004). The capacity of endophytes to stimulate plant immune systems renders them invaluable in agricultural integrated pest management approaches.

Enhancement of Plant Tolerance to Abiotic stress

Kharwar and his research team in 2014, 2024 found the endophytic fungi play a crucial role in enhancing plant resilience to various environmental stressors, including water scarcity, salt stress, temperature extremes, and metal toxicity. These fungi facilitate the production of osmolytes and antioxidants, which help plants combat oxidative stress and sustain cellular balance under adverse conditions. For example, endophytes isolated from plants in arid regions can boost drought resistance in their host species by enhancing water retention and minimizing oxidative harm. In a similar vein, certain endophytic fungi improve plant tolerance to high-salinity environments or soils contaminated with metals by regulating the absorption and storage of harmful ions (Singh *et al.,* 2015; Prajapati *et al.,* 2024).

Drought Tolerance

Endophytic fungi play a crucial role in improving the water-use efficiency of plants in environments prone to drought. These fungi accomplish this by controlling water retention and stimulating root development in their host plants. A notable example is the symbiotic relationship between certain grasses and fungal endophytes, particularly *Neotyphodium* species. Plants infected with these endophytes exhibit greater resistance to drought conditions compared to their uninfected counterparts (Malinowski and Belesky, 2000). This symbiotic association is especially significant in dry and semi-dry ecosystems, where the scarcity of water is a primary constraint on plant development.

Heavy Metal Detoxification

Kharwar and his research team in 2015 identified that fungal endophytes (*Aspergillus oryzae* FNBR_L35, *Fusarium* sp. FNBR_B7, FNBR_LK5 and FNBR_B3 *Aspergillus nidulans* FNBR_LK1, *Rhizomucor variabilis* sp. FNBR_B9, and *Emericella* sp. FNBR_BA5) used for remediation in As-contaminated agricultural soils (Singh *et al.*, 2015). In addition to aiding plant survival in metal-polluted environments, endophytic fungi can either store harmful metals within their own structures or stimulate the plant to produce compounds that bind to metals. Research on endophytes from metal-accumulating plants like *Pteris vittata* has demonstrated their capacity to enhance the host plant's resistance to and uptake of heavy metals such as arsenic and cadmium. Furthermore, these fungi may generate antioxidant enzymes that mitigate the oxidative stress caused by metal toxicity, thereby offering additional protection to the plant against potential harm.

Salinity Tolerance

Kharwar and his research team in 2024 found that the fungal endophytes isolated from salt tolerant wheat variety have generated tolerance in salt sensitive variety. The endophytic fungi such as *Aspergillus medius*, *Cladosporium parahalotolerant*, *Aspergillus versicolor* and *Aspergillus nishimurae* recovered from salt tolerant wheat were identified through 18S rDNA sequencing. Out of these, *C. parahalotolerant* and *A. medius* showed the synergistic effect with each other, so these 2 isolates were used for further experiments. These 2 isolates were involved in increasing the root-shoot length, proline and MDA contents. SEM and fluorescence microscopy were used to detect endophytic fungal colonization in the root of seedlings. *C. parahalotolerant* and *A. medius* heavily colonized the roots and it was noticed on the third week of the growth phase. Endophytic fungi help plants to synthesize osmoprotectants, which maintain cellular integrity under saline conditions (Prajapati *et al.*, 2024).These fungi can enhance the availability of essential nutrients, particularly potassium, which is vital for stress responses. Fungal endophytes can induce the plant's antioxidant systems, mitigating oxidative damage caused by salt stress. Endophytes may produce plant growth-promoting hormones, facilitating better growth and adaptation under saline conditions. Endophytic fungi can influence root structure and growth patterns, enhancing nutrient and water uptake under stress. Research into endophytic fungi in relation to salt tolerance is ongoing, with potential applications in utilizing endophytes in crop management to enhance yield in saline soils. **Figure 2** is showing endophytic fungi for crop adaptation to abiotic stresses.

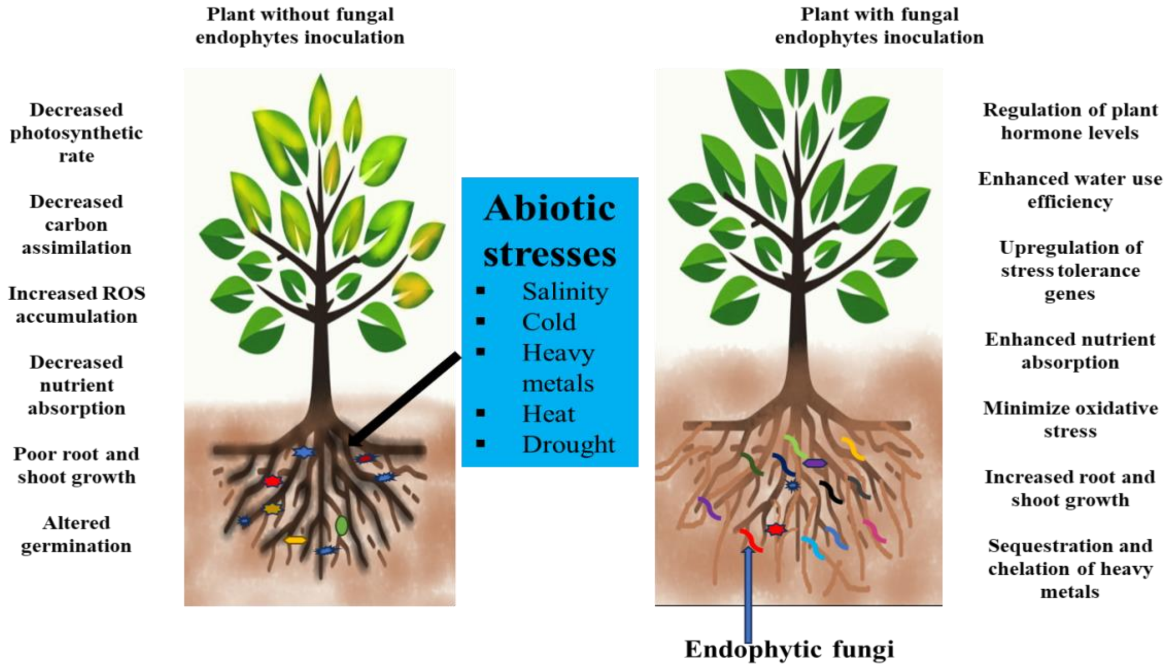

Figure 2: Endophytic fungi for crop adaptation to abiotic stresses.

From Surface to Tissues: Transition in Research Focus

The field of plant-associated fungal research has undergone significant changes over time. Early investigations primarily centered on epiphytic fungi, which reside on plant surfaces and interact with the external environment, affecting plant health. However, with advancements in research techniques, the focus has gradually shifted towards endophytic fungi, which inhabit plant tissues without causing apparent damage. This change in research emphasis, moving from epiphytes on plant surfaces to endophytes within plant tissues, has revealed a previously unseen realm of symbiotic relationships that are crucial for plant physiology, defense mechanisms, and ecological interactions.

Epiphytic Fungi: Early Research Focus

The study of plant-associated fungi began with epiphytic species, primarily due to their visibility and accessibility on plant surfaces such as leaves, stems, and roots. These organisms, which engage with the external environment, play a crucial role in research concerning plant-pathogen relationships and responses to environmental stressors.

Epiphytes and Plant Pathology

Initial studies on epiphytic fungi concentrated on their function as opportunistic pathogens or decomposers on plant surfaces. These investigations mainly examined the antagonistic relationships between epiphytes and plant pathogens, including fungal organisms responsible for leaf spot diseases (Andrews and Harris, 2000). Epiphytes frequently serve a protective purpose, generating antimicrobial substances that inhibit the establishment of detrimental pathogens (Lindow and Brandl, 2003). This characteristic made them significant in the realm of plant disease control, especially within agricultural environments.

Surface Interactions and Environmental Impact

Environmental factors like ultraviolet radiation, dehydration, and temperature changes influence the adaptations and functions of epiphytic fungi. These organisms have attracted research interest due to their capacity to generate bioactive substances in response to such stressors. Research has shown that certain epiphytic fungi synthesize pigments and antioxidants, which serve to shield the plant surface from UV-induced damage (Berg *et al.,* 2005). Although epiphytic fungi have been long recognized for their significance on plant surfaces, it was later discovered that fungi living within plant tissues-known as endophytic fungi-exert even more significant influences on plant growth and well-being.

Transition to Endophytic Fungal Research

A significant advancement in understanding plant-fungi relationships occurred when researchers shifted their attention from epiphytic to endophytic fungi. Unlike their epiphytic counterparts, endophytic fungi reside within plant tissues without causing visible symptoms, which initially led to their oversight. However, the advent of sophisticated microscopy, molecular methods, and culture-independent techniques unveiled the ubiquitous presence of endophytes across nearly all examined plant species (Arnold, 2007). This discovery revolutionized the field of plant-fungi interactions and highlighted the importance of these previously unnoticed microorganisms.

APPLICATION OF ENDOPHYTIC FUNGI IN AGRICULTURE AND BIOTECHNOLOGY

Endophytic fungi exhibit a wide range of ecological roles, making them promising candidates for various applications across agricultural, medical, and biotechnological fields.

Sustainable Agriculture

The potential of endophytic fungi in promoting sustainable agriculture is gaining increased attention. These organisms can serve multiple purposes: as biofertilizers to enhance plant nutrient uptake, as biopesticides to safeguard crops against harmful pathogens, and as agents that boost plant resilience in harsh conditions. By diminishing the necessity for chemical fertilizers and pesticides, endophytes play a crucial role in fostering environmentally friendly farming methods (Strobel, 2002; Prajapati *et al.,* 2024). Their versatility makes them invaluable assets in the pursuit of more sustainable agricultural practices.

Biotechnological and Pharmaceutical Applications

Kharwar and his research team in 2024 found that the endophytic fungi have garnered considerable attention for their production of bioactive compounds, which hold promise in pharmaceutical and biotechnological fields and products for agriculture (Goutam *et al.,* 2014). These fungi, found in medicinal plants, have demonstrated the ability to synthesize valuable substances. For instance, *Taxomyces andreanae*, isolated from the Pacific yew tree, has been found to produce taxol, a powerful anticancer compound (Strobel *et al.,* 1996). Furthermore, numerous endophytes generate unique antibiotics, antioxidants, and immunosuppressive agents, offering potential avenues for the discovery and development of new drugs. Recently, by Kharwar and his team, a bioactive compound isolated from the endophytic fungus *Nigrospora sphaerica* of *Euphorbia hirta* L. was purified through thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC). The compound isolated was identified as rutin (RN) through FTIR, NMR, and HRMS techniques. The estimated RN concentration in the culture filtrate of *N. sphaerica* was 19.54 mg/L. The calculated IC_{50} values of RN with respect to antioxidant potential estimated using 2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS), hydrogen peroxide (H_2O_2), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and nitric oxide (NO) approaches were 29.1, 36.5, 42.2 and 43.4 µg/mL, respectively. Besides, a combined treatment of RN and cisplatin (CP) inhibited the survival of tumor cells (HuT-78) significantly ($P<0.05$). Interestingly, the RN enhanced the apoptosis-inducing ability of CP considerably. It could be concluded that *N. sphaerica* may be exploited as an alternative source of rutin for various commercial applications (Gautam *et al.,* 2024).

Nanoparticles Synthesis by Fungal Endophytes

The biosynthesis of nanoparticles (NPs) by fungal endophytes is an innovative approach that utilizes the unique metabolic pathways of fungi. This process is gaining momentum due to its eco-friendly nature and potential applications in medicine, agriculture, and environmental management. Fungal endophytes can reduce metal ions to form nanoparticles through enzymes like reductases and dehydrogenases that facilitate the conversion of metal ions to their nanoscale forms. Secondary metabolites such as polysaccharides, proteins, and phenolics stabilize the nanoparticles during synthesis. The biomolecules secreted by fungi help in stabilizing the synthesized nanoparticles, preventing aggregation and controlling their size and shape. Silver nanoparticles (AgNPs) known for potent antimicrobial properties, AgNPs produced by endophytes can target various pathogens (Verma *et al.,* 2010; Gond *et al.,* 2020). Gold nanoparticles (AuNPs) are used in drug delivery and diagnostics application due to their biocompatibility and ease of functionalization. Zinc Oxide nanoparticles (ZnO NPs) exhibit antifungal and antibacterial activities, making them useful in agriculture and cosmetics. NPs synthesized by endophytes show promising activity against resistant bacterial strains and fungi, offering alternatives to conventional antibiotics. Biocompatible nanoparticles can enhance the efficacy of drug delivery while minimizing side effects (Singh *et al.*, 2018). NPs can serve as biopesticides or fertilizers, enhancing plant growth and protecting against pathogens. Kharwar and his research team discovered that the fungal endophyte *Chaetomium globosum* cell-free filtrate (CFF) fabricated nanoparticles of various shape under varied physicochemical conditions. Silver nanoparticles showed significantly ($p \leq 0.5$) enhanced antibacterial activity against *Staphylococcus aureus* and *Klebsiella pneumoniae* compared with AgNO3 (Singh *et al.,* 2018). Kharwar and his research team in 2010 synthesized silver nanoparticles (AgNPs) using *Aspergillus clavatus* isolated from *Azadirachta indica* and found result showed average minimum inhibitory concentrations against *Escherichia coli*, *Pseudomonas fluorescens* and *Candida albicans* (Verma *et al.,* 2010).

Drug Discovery and Development

Kharwar and his research team in 2011-2012 discovered that the endophytic fungi recognized as a promising source of new medications. These organisms produce substances with cancer-fighting, antibacterial, antifungal, and immune-suppressing properties, making them attractive subjects for pharmaceutical investigations (Goutam *et al.*, 2017; Nishad *et al.,* 2021; Singh *et al.,* 2021; Gautam *et al.,* 2024). The isolation of taxol from the fungal endophyte *Taxomyces andreanae* has significantly influenced cancer therapy, demonstrating the potential of these fungi in drug development (Strobel *et al.,* 1996). Further research into the secondary metabolites produced by endophytes may result in the discovery of novel treatments for infectious diseases, cancer, and other health conditions (Kharwar *et al.*, 2011, 2012; Gond *et al.*, 2012).

Agricultural Applications

Kharwar and his research team in 2024 found that the endophytic fungi produce secondary metabolites with antimicrobial and insecticidal qualities, making them crucial for sustainable farming practices. These natural compounds can serve as eco-friendly pesticides, safeguarding crops from harmful organisms and reducing reliance on artificial chemicals (Prajapati *et al.,* 2024). For example, research has demonstrated that alkaloids derived from endophytes in grasses can shield crops from insects that feed on plants, thereby improving crop durability and productivity (Clay and Schardl, 2002). Furthermore, certain endophytes generate substances that promote plant growth, enabling their use as natural fertilizers.

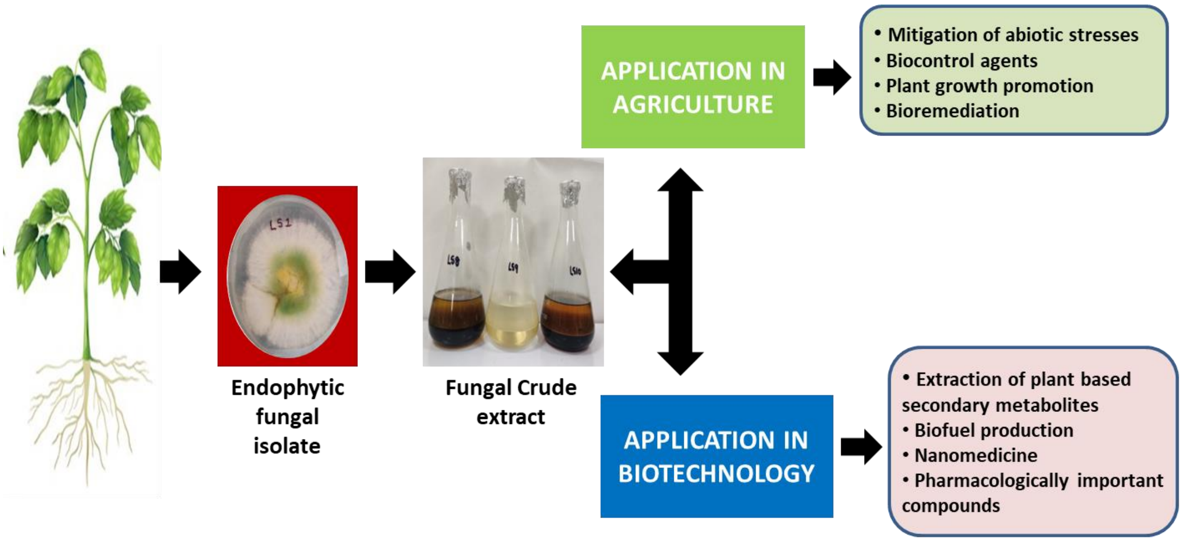

Figure 3: Showing the applications of fungal endophytes in agriculture and biotechnological field.

CHALLENGES AND FUTURE DIRECTIONS

The transition from studying fungi that grow on plant surfaces (epiphytic fungi) to those living within plant tissues (endophytic fungi) has expanded our knowledge of plant-fungal relationships and created new opportunities in biotechnology. Endophytic fungi have become a central focus in contemporary fungal studies, ranging from their ecological functions in plant protection to their significant potential in pharmaceutical development and agricultural applications. As advancements in molecular biology and biotechnology continue, there is great anticipation for fully realizing the capabilities of these internal plant symbionts. However, significant challenges remain, including the inability to culture a large proportion of endophytic fungi using conventional methods. Additionally, our comprehension of the specific molecular mechanisms governing plant-fungal interactions is still in its early stages. Upcoming research avenues may explore: (1) Employing advanced sequencing techniques and metagenomic analyses to reveal the complete spectrum of endophytic fungi, particularly those challenging to cultivate. (2) Investigating the genetic components responsible for symbiotic relationships, metabolite synthesis, and stress responses to gain more comprehensive insights into the ecological and biotechnological significance of endophytes. (3) Modifying endophytic fungi genetically to amplify their advantageous traits to transform sustainable farming practices and pharmaceutical manufacturing processes.

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