

Review Article

Fungal Endophytes: A Potential Application in Integrated Plant Health Management

Abstract: In almost all plant species examined, fungal endophytes are found infecting or colonizing living plant tissues, including leaves, branches, stems, and roots asymptotically. There is a complex interplay between endophytes and hosts that spans the mutualistic–pathogenic continuum. As mutualist endophytes have higher fitness compared with their uncolonized hosts, their potential application in plant health management strategies has drawn interest. The purpose of this review is to describe the benefits of fungal endophytes to agricultural plants against common non-insect pests such as fungi, bacteria, nematodes, viruses, and mites. Endophytes and their possible applications to large-scale agriculture are discussed in this review.

Keywords: fungal endophytes, mutualistic –pathogen, potential application.

1. Introduction

Plant pathogens and pests in agriculture reduce crop yield by 30–50% annually, requiring combating them to ensure food security [50]. Chemical pesticides control these organisms, but recent restrictions and consumer demand prompt governments and private industries to adopt clean technologies for plant production [55, 32,6,42]. Beneficial endophytes are emerging as biological control agents for crop protection [42,73,20,60,17,28], living inside plants throughout their life cycle without causing damage or disease [28, 62]. Studies have shown that the diversity of fungal endophytes inside plants is underestimated, and some endophytes are host and/or environment-specific [15, 46].

Through various mechanisms, endophytic microbes contribute to plant growth and protection against pests and pathogens [10,11,38]. Secondary metabolites and biochemicals produced by them suppress or reduce the negative effects of pathogens [27]. In addition, they induce plant defense mechanisms, such as Systemic acquired resistance (SAR) or Induced systemic resistance (ISR) [11,69]. It has also been demonstrated that some endophytes possess biocontrol potential through the secretion of antifungal, antibacterial, inhibiting, or mycoparasitic compounds. In Finger millet, *Enterobacter* sp. strains suppress the grass pathogen *Fusarium graminearum* and produce several antifungal compounds that kill it. As demonstrated by the foliar application of endophyte-free leaves against leaf necrosis and leaf mortality caused by *Phytophthora* sp., Endophytes also compete with host pathogens for nutrients and space [5].

Fungal endophytes are asymptomatic inhabitants of plant tissue and have been found in all parts of plants [61,78]. Endophytic species may remain localized in a plant and provide tissue-specific protection from disease [78,40], or they may spread systemically to herbaceous plants [13,48]. Several wild and cultivated plant species exhibit symbiotic, and possibly mutualistic, interactions with endophytes [4]. A close examination of host plants indicates the presence of endophytes almost always [50]. Phytopathogenic and mycorrhizal fungi have traditionally dominated plant-fungal studies, which have increasingly focused on endophytes. More than 1 million endophytic species are

estimated to exist in 3,00,000 plant species, yet only a small fraction have been isolated and studied for their functions.

In studies conducted so far, some endophytes may increase plant fitness compared to their uninhabited counterparts [26,14]. Endophytes inhibit biotic and abiotic stressors, including drought, salinity, heavy metals, pesticides, floods, extreme temperatures, predators, and pathogens [14,68]. As well as deterring pathogenic microbes, insects, and other herbivores, endophytes also promote plant growth and development [70]. Global crop yields are reportedly reduced by 30 to 50% annually by plant pathogens and pests [50], so endophytes can provide an effective way to combat plant loss since their beneficial properties can improve plant fitness and crop yield while maintaining quality and safety.

2. Role of endophytes in suppression of various diseases- causing pathogens in the plants

2.1 Fungal pathogens

Plant pathogens kill plants, reduce yield and quality, and cause postharvest losses to crops [50]. Several fungal pathogens produce mycotoxins that are harmful to humans and livestock. The use of synthetic chemical fungicides to control fungal pathogens has become a mainstay in agriculture. However, fungicides can also adversely affect beneficial fungi, such as those beneficial to crop health. A major consequence of extensive fungicide use is the loss of mutualistic fungi like arbuscular mycorrhizae, whose loss can dramatically reduce plant fitness. It has also been reported that fungicides can cause selective damage to non-target beneficial microbes rather than pests [59].

By inducing a systemic response after endophytic colonization, endophytes enhance host plant resistance to fungal pathogens. The plant uses cell wall deposits to strengthen its cell walls and defend them from penetration [43]. Endophytes use exoenzymes to access these cells, but pathogens may not be able to do so [43]. Through transcriptional reprogramming, endophytes can also promote plant defense responses; for example, by modulating downstream defense-related genes such as Salicylic acid, Jasmonic acid and Ethylene signaling pathways [57,58]. Besides increasing photosynthesis, endophytes have also been found to increase chlorophyll content in plant cells, trichome and stomata density, antioxidant enzyme activity, callose deposition, lignification and phytoalexin accumulation in *Diaporthe liquidambaris* [57,58]. Similarly, pathogenic fungi may be excluded by endophytes through competitive exclusion [54,47]. A competitive exclusion occurs when endophytes colonize and occupy identical potential niches, suppressing pathogen establishment.

There is evidence to indicate that fungi from the genus *Daldinia* inhibit the growth of plant pathogens such as *Colletotrichum acutatum* and *Sclerotium rolfsii* [58]. The fungus *Daldinia eschscholtzii* isolated from Ginger, *Zingiber officinale*, and *Stemona* root, *Stemona tuberosa*, was found to produce 60 compounds, of which Elemicin (24%), Benzaldehyde dimethyl acetal (8%), Ethyl sorbate (7%), Methyl geranate (6%), Trans-sabinene hydrate (5%) and 3,5-dimethyl-4-heptanone (5%) were found to be the most abundant. In a study conducted by the University of Michigan, Elemicin was found to be effective against *Colletotrichum gloeosporoides*, *C. melanophaeae* as well as *C. musae* [79]. There are 27 volatile organic compounds (VOCs) produced by *Daldinia concentrica*. These include 3-methyl-1-butanol, (2-methyl-1-butanol), 4-heptanone, isoamyl acetate and trans-2-octenal. Combinations of these VOCs

displayed a broad spectrum of antifungal properties [58].

Many species of *Fusarium* exist as plant pathogens as well as endophytes that inhibit other fungal pathogens [58]. The antimicrobial properties of *Fusarium* metabolites have been investigated in many studies, but less attention has been paid to their antifungal properties in agricultural systems [58]. *Rhizoctonia solani* and *F.oxysporum* were inhibited by 0.2–2.5 mg/mL of crude extract from *F.proliferatum* from *Cissus quadrangularis*. In addition to phenolics, terpenoids, and unsaturated alkenes, further analysis of the crude extract revealed phenolics, terpenoids, and unsaturated alkenes. In experiments conducted on the Rust species *Puccinia arachidis*, *Fusarium chlamydosporum* chitinase lysed urediniospores and prevented the germination of the urediniospores once purified.

Aspergillus, *Colletotrichum*, *Diaporthe*, *Gliocladium*, *Lecanicillium*, *Phyllosticta*, and *Trichoderma* have also been investigated for their antifungal properties. *Trichoderma asperellum*, *T. atroviride* and *T. longibrachiatum*, all isolated from Soybean (*Glycine max*), have been shown to reduce soil infection by *Rhizoctonia solani* by 64, 60, and 55%, respectively. There are several *Trichoderma* species that produce hydrolytic enzymes capable of degrading cell walls, including pectinase and chitinase. In addition, *Trichoderma* species produced siderophores that reduce iron availability to pathogenic fungi, as well as IAA, which promotes plant growth. In dual culture assays, *Trichoderma erinaceum* inhibited Southern stem rot by *Sclerotium rolfii* by 64%, and in pot experiments, it reduced infection by 58% [58]. In extract analysis, *T.erinaceum* produced 6-n-pentyl-2H-pyran-2-one (6PAP), 1,3 glucanase and chitinase, which inhibited *S.rolfsii* growth.

Medicinal plant extracts of *Aspergillus neoniger* inhibited the growth of *Penicillium avelaneum*, *Penicillium notatum*, and *Aspergillus terreus* by at least 80% [2]. According to high-performance liquid chromatography and nuclear magnetic resonance spectroscopy, *A.neoniger* produces aurasperone A and D. In tests against *Fusarium oxysporum*, aurasperone A and D extracts inhibited the pathogen by 76 and 67 g/ml, respectively. *Aspergillus* extract from *Bethencourtia palmensis* contained antifungal agents mellein and neoaspergillic acid [49]. At effective doses (mg/mL) EC50, mullein and neoaspergillic acid inhibited *Alternaria alternata*, *Botrytis cinerea* and *F.oxysporum* growth in culture [49].

In similar studies, *Lecanicillium lecanii* and *Gliocladium catenulatum* were found to produce chitinases capable of inhibiting the growth of mycelia and conidial germination of *R. solani*, as well as hyphal growth, conidial germination and sclerotial germination of *F. oxysporum* [88]. A study found that *Colletotrichum coccodes* and *Phyllosticta capitalensis* isolated from the Indian medicinal plant *Houttuynia cordata* could inhibit the growth of the opportunistic human pathogen *Candida albicans* [71]. The medicinal plant *Buchanania axillaris* contains *Diaporthe caatingaensis*, which produces camptothecin, a molecule with anticancer, antibacterial, and antifungal properties [19,37]. The antifungal properties of fungal-derived camptothecin need to be further investigated.

T. harzianum and *T. lentiforme* were found in Watermelon, *Citrullus lanatus*, along with 348 other types of fungal endophytes [44]. As part of this study, seven species of fungi were tested for their antagonistic abilities to 14 soil-borne pathogens: *Fusarium oxysporum* f. sp. niveum, *F.oxysporum* f.sp. melonis, *F. solani* f. sp.cucurbitae, *Macrophomina phaseolina*, *Monosporascus cannonballus*, *Neocosmospora falciformis* and *N. keratoplastica* [44].*Trichoderma harzianum* and *T. lentiform* showed a maximum inhibitory effect of 93% in dual culture assays, while in vitro tests on Melon and Watermelon plants showed a 67% reduction in disease incidence. Several modes of

action were observed for the *Trichoderma* species to inhibit pathogen growth. Besides outcompeting pathogens for space and nutrients, these endophytes also produced compounds that broke down the cell walls of pathogenic fungal hyphae and directly parasitized them.

In dual culture assays, *Aspergillus terreus*, isolated from the seed of the Rubber tree, *Hevea brasiliensis*, inhibited the growth of the pathogens *Rigidoporus microporus* and *Corynespora cassiicola* by 81, 64, and 70%, respectively [33]. With a dipped stick inhibition assay, sterilized rubber tree wood was inoculated with liquid culture of *A. terreus* completely inhibited the growth of *R. microsporus* [33]. Sterilized leaves soaked in liquid culture of *A. terreus*, then sliced and placed onto cultures of *Corynespora cassiicola*, showed significant reductions in infection rates of 87–93% compared to controls [33].

2.2. Bacterial Pathogens

Endophytes produce antibacterial and antifungal compounds that protect plants against bacterial pathogens. Some of these antibacterial compounds are broad-spectrum, whereas others provide protection against a narrower target group [13]. Javanicin, one of these compounds, has shown activity against a wide range of microbes, but is most effective against *Bacillus* species. [13] and *Escherichia coli*. There are also terpenoids, alkaloids, phenylpropanoids, aliphatic compounds, polyketides, acetol, hexanoic acid, and acetic acid that endophytes produce that are broadly antimicrobial. Two examples of terpenoids produced by some endophytes of the genus *Microdiplodia* show potent antibacterial activity against antagonistic strains of *Pseudomonas aeruginosa*. Several strains of *Pseudomonas aeruginosa* can cause Soft root rot in plants, such as *Panax ginseng*, *Arabidopsis* and *Ocimum basilicum* [75,39].

The fungal endophyte *Chaetomium globosum* also produces broad-spectrum antimicrobial compounds and has anti-biofilm properties [22]. In the same way, *Penicillium* sp. The EtOAc extract of *Stephania dielsiana* shows remarkable antimicrobial activity, with MICs ranging from 1.2 to 6 mg/mL against seven different animal pathogenic bacteria. *Rosmarinus officinalis* has been shown to have significant antimicrobial activity against *P. aeruginosa*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *B. subtilis* and *E. coli*, suggesting that it could also be used as a biocontrol agent for phytopathogenic bacteria [58]. The endophytes *Diaporthe phaseolorum*, *Aspergillus fumigatus*, and *Aspergillus vesicatoria* produced antibacterial metabolites including acetol, hexanoic acid, and acetic acid, which successfully controlled Tomato bacterial spots (*Xanthomonas vesicatoria*). Extracellular metabolites from endophytic *Aspergillus* species. Although the metabolites were not identified, the Cupressaceae hosts had antibacterial effects on *Bacillus* sp., *Erwinia amylovora*, and *Pseudomonas syringae* [66]. In plant defense, secondary metabolites with multiple pathogen-fighting abilities—such as cycloepoxy-lactone—are especially useful. Species of antimicrobial metabolites can be produced directly by endophytic fungi or by host plants after inoculation with endophytes. Gene expression and secretion of these compounds remain unknown [50].

2.3. Viruses

The entomopathogenic activities of fungal endophytes reduce viral diseases either by increasing plant defences or by preventing viruses from spreading. Despite the limited number of studies in this area, foliar inoculations of viruses on fungal endophytes inoculated plants have been shown to have antiviral properties against plant viruses [58].

Inoculating *Lolium pratense* (Meadow ryegrass) with *Neotyphodium uncinatum* reduced Barley yellow dwarf virus infection in inoculated plants, likely due to the production of alkaloids that prevented virulent aphid vectors from spreading virus infection [30]. Another study found that inoculating Squash plants with different strains of *Beauveria bassiana* provided protection from Zucchini yellow mosaic virus [58]. Different viruses infecting the same plant species may be targeted by fungal endophytes' antiviral defences. Sugarcane mosaic virus was more resistant to Maize plants inoculated with *Trichoderma harzianum* and *Metarhizium anisopliae* than control plants, but Maize chlorotic mottle virus was not significantly resistant to the same inoculated plants [23]. Plant virus resistance is also influenced by environmental conditions. When Tomato plants were inoculated with *Piriformospora indica*, Pepino mosaic virus was repressed in shoots under higher light intensities, while fruit biomass significantly increased [21]. Most commonly, viruses are prevented from infecting plants by limiting the potential viral vectors before they infect them [51]. Typically, insecticides or other potentially harmful compounds are used to control this process [51]. The application of endophytic priming of plants may reduce the use of insecticides and provide persistence in protection in the event that insecticidal treatments fail [51].

2.4. Nematodes

Plant-parasitic nematodes (PPNs) are a major threat to agricultural crops worldwide, causing \$215.8 billion USD in damage in the USA alone [50,1,64]. Plant roots and stems are attacked by nematodes, which absorb nutrients and create wounds through which secondary opportunistic fungal, bacterial or viral pathogens can enter [58]. They may also be vectors for viruses that cause diseases or death in crop plants. Traditionally, nematodes are controlled by using chemical-based nematicides. As with other pesticides, chemical applications can cause non-target effects in the rhizosphere and surrounding soil, which damage beneficial microbial communities. Microorganisms that can inhibit the growth and spread of nematodes are therefore of growing interest in soil or plant tissues. The production of nematocidal compounds by fungal endophytes, the parasitization of nematode larvae or the use of hyphal loops for nematode capture have been reported [58]. A variety of bioactive compounds are produced by some fungi that may influence nematode colonization, although their exact chemical composition has yet to be determined [58].

There are over 2000 plant species affected by root-knot nematodes, such as Tomato, Cotton, Cucumber, Melon, Soybeans and Rice [12,79]. Many fungal genera have been reported as having inhibitory effects on *Meloidogyne* species, including: *Acremonium*, *Alternaria*, *Arthrobotrys*, *Chaetomium*, *Cladosporium*, *Clonostachys*, *Diaporthe*, *Drechslerella*, *Epichloë*, *Epicoccum*, *Fusarium*, *Gibellulopsis*, *Melanconium*, *Metacordyceps*, *Monacrosporium*, *Neotyphodium*, *Paecilomyces*, *Phialemonium*, *Phyllosticta*, *Piriformospora*, *Purpureocillium*, *Talaromyces* and *Trichoderma* [58,3]. One or more species from one of these genera have also been reported to have antagonistic effects toward other species of nematodes, although their effects have not yet been confirmed [58]. Root knots, as well as the nematodes that cause them, have been reported as significantly decreasing in number when there are one or more species present.

There has been evidence that compounds produced by *Alternaria*, *Chaetomium*, *Cladosporium*, *Clonostachys*, *Fusarium*, *Phyllosticta*, *Piriformospora*, and *Trichoderma* strains alter the composition of existing metabolites within the host plant or increase their production, thereby promoting plant growth or promoting resistance to nematodes [79,82,16,83,9,58]. Alternatively, *Acremonium*, *Diaporthe*, *Epichloë*, *Melanconium*, *Phialemonium* and *Purpure*

ocillium species can produce bioactive compounds that directly inhibit nematode eggs, juveniles, and females [58,63,83,31,35]. A number of strains of *Chaetomium*, *Clonostachys*, *Phyllosticta*, and *Trichoderma* have also been reported as hyper colonizers, which are capable of outcompeting pathogens, nematodes included, for space and nutrients within the plant host [79,82,34,83].

As the most commonly reported fungi with antagonistic effects on nematodes, *Fusarium species* produce bioactive compounds that improve plant growth and induce systemic resistance to nematodes, or inhibit the growth and development of nematodes directly [79,8,29,9,83]. The host plant produces growth hormones and root exudates which are altered by *Fusarium* species, resulting in a decrease in colonization by *M. incognita* [79]. By triggering the production of unknown compounds by the host plant, *Fusarium oxysporum* induces resistance to *M. incognita*. Inoculated banana plants with *Fusarium* sp. showed reduced parasitism by the burrowing nematode *Radopholus similis* due to induced systemic resistance (ISR) [74]. More recent work with *F. oxysporum* strain 162 identified 11 compounds, nine of which had some nematicidal effect; 4-hydroxybenzoic acid, indole-3-acetic acid (IAA) and gibepyrone D were the most effective, with a lethal dose of 50% of the test organisms (LD 50) concentration of 104, 117 and 134 µg/mL, respectively, after 72 h [9]. The production of IAA suggests that it serves a dual function by helping plants to stay healthy and resistant to nematodes while at the same time being secreted as a toxin [9].

During the research of the mechanism of action (MOA), it was found that within 10 minutes of exposure to *F. oxysporum* nematicidal compounds, the motility of nematodes decreased, and within 24 hours the nematode was dead [45]. It was found that the compounds were most effective against sedentary nematodes compared to migratory nematodes, while non-parasitic nematodes were not affected by the compounds regardless of the degree of mobility. This study investigates the effects of *F. oxysporum* on target versus non-target nematodes, which is important because it reduces the population of plant pathogenic species while not harming the non-pathogenic nematodes, which are able to feed on pathogenic bacteria and fungi or parasitize crop pests. As a result of a study conducted recently, several fungal endophytes were isolated from Cotton plants to be used as seed treatments to reduce damage caused by *Meloidogyne incognita* [83]. These fungal endophytes belong to the genera *Alternaria*, *Chaetomium*, *Cladosporium*, *Diaporthe*, *Epicoccum*, *Gibellulopsis* and *Purpure ocillium*. It has also been shown in another study that *Sacha inchi* (*Plukenetia volubilis*) plants inoculated with *Trichoderma* and *Clonostachys* significantly reduce the amount of damage and galls that are caused by root knot nematodes compared to plants not inoculated [34].

3. Conclusion

Endophytic fungi are a rich and reliable source of novel natural compounds with interesting biological activities, a high level of biodiversity, and may also produce a number of compounds of pharmaceutical significance, which have attracted world wide scientific attention to their isolation and exploration of biotechnological applications. Because of their interactions with their hosts, their secondary metabolism is particularly active, and they represent an ecological source that is relatively unknown. Endophytic fungi seem to interact closely with plants in nature. The endophytes of higher plants, especially medicinal plants, have not been completely studied. Very few of the 10,000 important medicinal plants have been studied for their endophytic microflora. It is therefore imperative to conduct rapid research in order to study them, since the loss of plant species will also result in the loss of all potential endophytes associated with it. Agriculture, industry, and medicine may benefit from the collection, cataloguing and exploitation of

endophytic microorganisms throughout the world. It is important to manage microbial communities in such a way that beneficial endophytic microorganisms colonize plants. In terms of economic and environmental impacts, this research field may have a positive impact. In order to better understand the host-endophyte interaction, further molecular research in this field is necessary to facilitate the recognition of endophytes carrying host genes and/or genetically specific endophytes.

UNDER PEER REVIEW

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