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Qiang-Sheng Wu, Ying-Ning Zou and Yong-Jie Xu  
Editors

Endophytic Fungi

Biodiversity, Antimicrobial Activity and Ecological Implications

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# Contents

Preface vii

Chapter 1 Endophytic Fungi: A Comprehensive Review on Their Secondary Metabolites, Pharmacological Interventions and Host Plant Interactions 1

Acharya Balkrishna, Ritika Joshi,   
Ashish Dhyani and Vedpriya Arya

Chapter 2 Diversity and Antimicrobial Activity of Endophytic Fungi from Mangrove Forests 49

Paulo Teixeira Lacava, Andréa Cristina Bogas and Carla Cristina Moreira

Chapter 3 Arbuscular Mycorrhizal Fungus and Its Positive Effects on Ornamental Plants 73

Tao-Ze Sun, Lei Fan, Hong-Na Mu   
and Alexander Witherspoon

Chapter 4 Potential Practical Application and Assessment of Arbuscular Mycorrhizal Fungi on Horticultural Crops 93

Hui-Hui Wu, Yong-Jie Xu,   
Ying-Ning Zou and Qiang-Sheng Wu

About the Editors 107

Index 111

# Preface

Endophytes are a group of microorganisms that do not cause obvious disease symptoms to the host plants, and are commonly found in various tissues, organs and cell interstices of plants. Endophytes include endophytic bacteria, endophytic fungi and endophytic actinomycetes. Among them, endophytic fungi belong to ascomycetes and are widely distributed in mosses, ferns, shrubs, algae, herbs, angiosperms and gymnosperms. In the process of long-term development and evolution, endophytic fungi have established a mutually beneficial relationship with host plants and acquired the ability to produce the same or similar secondary metabolites as their hosts in the process of co-evolution with plants, and their metabolites have a variety of biological activities, including promoting plant growth, antibacterial, antioxidant, antitumor, etc., and thus become a natural treasure trove of antitumor active molecules. Currently, the research of endophytic fungi has become a hotspot in the fields of plant protection, pesticide, biopharmacology and traditional Chinese medicine resources, which has important research and development value in agriculture, forestry and medicine.

The book focuses on the biodiversity, antimicrobial activity, and ecological implications of endophytic fungi. The book is divided into four chapters. Chapter 1 provides a comprehensive overview of the secondary metabolites of endophytic fungi, pharmacological interventions, and interactions with host plants. In this chapter, different domains of endophytic fungi were presented, followed by the taxonomy of endophytic fungi, a strong analysis of endophytic fungal interactions with host plants, and the effect of climatic and ecological factors on endophytic fungi. This chapter also focuses on the isolation of some bioactive substances from endophytic fungi, such as alkaloids, polyphenols and terpenoids, and reviews the pharmacological benefits of endophytic fungi. Finally, the authors look forward to the prospect of endophytic fungi as a source of nanoparticles.

Chapter 2 provided an overview of the diversity and antimicrobial activity of mangrove endophytic fungi. The authors focused on the analysis of novel bioactive components of mangrove endophytic fungi and discussed their antimicrobial activity in pharmacology, antiparasitic activity, as well as antagonistic activity against phytopathogens. Thus, the isolation and cultivation of mangrove endophytic fungi is of great importance for pharmaceutical, industrial and agricultural fields.

Chapter 3 described the relationship between arbuscular mycorrhizal fungi and ornamental plants. In this chapter, the positive effects of arbuscular mycorrhizal fungi on ornamental plants such as promoting plant growth, accelerating flowering and ornamental value, and enhancing stress resistance were described. Finally, the authors provided an outlook on the sustainability and environmental friendliness of arbuscular mycorrhizal fungi in the applications and cultivation of ornamental plants.

Chapter 4 summarized potential practical applications and evaluation of arbuscular mycorrhizal fungi on horticultural plants. In this chapter, the production of mycorrhizal fungal inoculums was developed, focusing on the presentation of a proposed diagram regarding indigenous arbuscular mycorrhizal fungal propagation of field citrus. The practical applications of these mycorrhizal fungi on fruit trees, vegetable plants, and ornamental plants such as seedling rearing, tree growth, and quality improvement were then presented. This work provided a possible pathway for future applications of endophytic fungi, including mycorrhizal fungi, in agriculture.

In conclusion, we hope that this book will bring some new knowledge of endophytic fungi and enable readers to have a comprehensive knowledge and application of endophytic fungi. In the face of the COVID-19 epidemic, we would like to express our sincere thanks to these contributing authors for overcoming many inconveniences to support and complete the chapters. We also thank the editors of Nova Science Publishers for their hard work. We would like to express our gratitude to all of them. Finally, we are also grateful for the publication of this book with the support of the Plan in Scientific and Technological Innovation Team of Outstanding Young Scientists, Hubei Provincial Department of Education (T201604), and the Hubei Agricultural Science and Technology Innovation Action Plan (E Nongfa [2018] 1), the Local Special Project for Science and Technology Development guided by the central government (2018ZYYD045), the open fund of Hubei Key Laboratory of Economic Forest Germplasm Improvement and Resources Comprehensive Utilization (202019604).

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Chapter 1

# Endophytic Fungi: A Comprehensive Review on Their Secondary Metabolites, Pharmacological Interventions and Host Plant Interactions

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## Abstract

Endophytic fungi are important components of the plant ecosystem and live inside the plant tissue without causing any apparent symptoms. In symbiotic association, they help in the plant growth and protection from numerous abiotic and biotic stresses by producing a wide range of bioactive compounds. These bioactive compounds exhibit anti-microbial, anti-oxidant, anti-malarial, anti-inflammatory, anti-hypercholesterolemic, anti-cancer, and biocontrol properties. Hence, along with their active participation in nutrient cycling, biodegradation and bioremediation, they also serve as a good source of wide range of antibiotics, drugs or medicines in the pharmaceutical, industrial and agricultural sectors. At present, people focused on plant-based medicine which causes heavy pressure on the flora and leads to a higher rate of deforestation. Endophytic fungi contain similar compounds as plants and can be used as an alternative source of anti-microbial compounds. The current review discusses the detailed potential of the endophytic fungi for the development of novel pharmaceutical drugs for the prevention of various diseases. in the development of the environment as well as biotechnological applications in drug discovery.

**Keywords**: anti-microbial activity, bioactive compounds, endophytic fungi, host-plant Interactions, nanotechnology, pharmacological applications

## 1. Introduction

Plants have a great potential to form large pools for many interrelated microorganisms, including bacteria and fungi, known as plant-microbiomes (Dhyani et al., 2019). This relationship serves as an important player for maintaining the stability of an ecosystem. In fungal-plant interaction, photosynthetically fixed carbon was utilized by the fungal partners and in exchange, it provides essential nutrients from the soil rhizosphere to the host plant (Wu et al., 2012). This interaction is classified into two main categories i.e., mycorrhizal and endophytic fungi. Endophytes can colonize in the buds, dead and hollow hyaline cells, fruit, inflorescences of weeds, leaf segments, petioles, roots, seeds and stem without causing any damage (Rathod et al., 2013). Plant-endophyte interaction is different from mycorrhizal interaction due to the absence of three basic functional parameters namely a cellular interface where specific structures (e.g., arbuscules) present, coordinated growth between fungal and plant partners and significant profits for both companions (Mayerhofer et al., 2012). Endophyte groups structure like richness, species diversity and virtual abundances are regulated by several abiotic and biotic elements such as plant-microbe interactions, biogeography, plant species and soil conditions (Deng and Cao, 2017) as shown in Figure 1.

In host-plant association, endophytic fungi play several roles that involve in the sustainable increment in both direct as well as indirect way for example it produces certain secondary metabolites that protect the plant from diseases, herbivory and help in plant growth promotion and establishment. This aspect is the main advantage of endophytic fungi, which can protect the plant against pathogens through various approaches, such as colonization sites and competition with pathogens for nutrients, antibiotics production, and induction of resistance mechanisms (Ownley et al., 2010). Protection of plants against herbivores through toxins production is another activity that has been attributed. The examination has shown the impact of preference and performance of herbivorous insects, which reduce the damage caused to the host plant (Gange et al., 2012). The endophytic fungus may promote host plant growth through the synthesis of phytohormones or by increasing plant tolerance toward different stresses (Khan et al., 2015). This ability to increase plant growth is very important and can be discovered in agricultural methods. Indeed, the use of microorganisms that promote plant growth is an option to help modern agriculture meet the challenges of increasing crop production and guaranteed sustainability (Luz et al., 2006). Endophytic fungi produce a huge variety of secondary metabolites which plays a key role in the physiological processes of microbes (Rönsberg et al., 2013). These compounds are formed for specific reasons, for example, social interaction and the ecology of productive organisms (Conti et al., 2012). Secondary metabolites are potential sources of novel bioactive natural products that may have applications in various fields (Rönsberg et al., 2013).



**Figure 1.** Different domains of endophytic fungi.

## 2. Classification of Endophytic Fungi

Based on the colonization transmission patterns, ecological function, host range, taxonomy, tissue specificity, endophytic fungi can be classified into two chief classes namely Clavicipitaceous endophytes (known as C-endophytes) that are found in some grasses and Non-clavicipitaceous endophytes (known as NC endophytes) (Schaechter 2011). However, endophytes are further classified into four classes according to Rodriguez et al., (2009) observation. Two main endophytic classes (clavicipitaceous and non-clavicipitaceous) on the basis of life history and phylogenic information. They classified non-clavicipitaceous endophytes into three classes based on host plant colonization and transmission, plant biodiversity and advantages are given to hosts (Hughes 2016) as shown in Figure 2.



**Figure 2**. Relationships of endophytes within the host plant.

### 2.1. Clavicipitaceous Endophytes (Class I)

Clavicipitaceous Endophytes (Ascomycota) contain independent and symbiotic species linked with insects, rushes, grasses and sedges (Bancon and White, 2000). Several members are known for the production of alkaloids which exert a toxic effect on animals and humans. These endophytes are first reported in the late 19th century by European investigators from the seeds of *Lolium linicolum*, *L*. *temulentum*, *L*. *arvense* and *L*. *remotum*. Mycelium of Class I endophytes was found in the intercellular spaces of rhizomes, the surface of leaf blades, leaf sheaths and culms (White et al.,1996; Tadych et al., 2007). These endophytes are reported to have anti-nematodal and anti-fungal potential. It also provides resistance to the host plant against abiotic stress such as drought (Hughes, 2016).

### 2.2. Non-Clavicipitaceous Endophytes (Class II)

Non-clavicipitaceous endophytes (Class II)includes the intense diversification of the endophytic fungi linked with tropical trees (leaves), nonvascular plants (liverworts and mosses), seedless vascular plants (club mosses), conifers (Gymnosperms) and herbaceous angiosperms. Most of the members are belonging to ascomycetes and some from the basidiomycetes. The endophytes with the functional group on the basis of darkly melanised septa are known as Dark Septate endophytes (DSE). It provides resistance against abiotic stress, increases biomass and protects from fungal pathogens (Hughes, 2016).

## 3. Host Plant Interactions of Endophytic Fungus

Endophytic fungi are so adaptive to the environmental conditions and have been retrieved from the host plants belonging to different climatic zones such as in Arctic tundra, croplands, grasslands, hot deserts, mangroves, savannas, temperate and tropical forests. Endophytes biodiversity is very high, mainly in tropical and temperate rainforests. In general, there are approximately 30,000 land plant species of the host fungi, each plant holding one or more than one fungi (Arnold, 2008). In natural ecosystems, usually, a different association exists between endophytic fungi and their host plants from mutualistic to antagonistic results from grass-endophyte systems revealed that the endophytic fungi act as herbivorous antagonists and have the capabilities to enhance plant growth (Clay, 1990). By contrast, the forest endophytes have been claimed mutualistic antagonism towards insects and pathogens (Faeth, 2002). Apart from this, from an ecological standpoint, several significant fungi for example human and soil pathogen (*Coccidioides posadasii*) exhibited diverse roles. Besides, *Chaetomium globosum* is also known for its endophytic saprotrophic, and pathogenic properties (Arnold and Engelbrecht, 2007). However, these species due to their wide roles are noticeable for their ecological potentials. To acknowledge the actual mechanism behind that obligation represents a hindrance in the system of endophyte biology (Arnold, 2007) as shown in Figure 3.



**Figure 3**. Mechanism of plant protection by endophytes through direct and indirect mechanism.

Endophytic fungi are host-specific and belong to a particular or a group of host plants. Host-specificity is, sometimes, clearly defined as a fungus which confined to a specific host or a bunch of that particular species but not found in other distinct plants within a similar habitat (Holliday, 1998). In the same context, Petrini (1991), classified the host specification as establishment specification and expression specification, which are used to identify the host plant relationships with the fungi. Endophyte multiplies within particular plant species were termed as an establishment specificity, whilst the specificity of expression is defined as the colonization of multiple hosts by a given fungus, however, that particular structure was based on the inadequate number of plant taxa (Usually forming bodies). There are several reports for the host specificity of the endophytic fungi but still in-depth and detailed study is much needed in this field (Khilal, 2019).

Plants and endophytes are subject to continuous interactions in their symbiotic existence in nature. Their breakdown interaction on many levels:

* Endophytic fungi stimulate host breakdown;
* Host plant stimulate endophytic fungal breakdown;
* Host plant and endophyte fungi mutual parts definite pathways;



**Figure 4.** Environmental and host-plant factors affecting endophytic fungi.

* Host plant can breakdown into products from the endophyte microbes;
* Endophyte microbes can break down secondary compounds from the host plants. Two latter potentials for biochemical transformation can be met by all enzymatic steps (Chutulo and Chalannavar, 2018).

Endophytes may affect the metabolism of their host plant, but one might hypothesize that the well-known host range may increase or affect the form of secondary metabolites in endophytic fungi. Also, host plants may affect metabolite patterns in pathogenic fungi (Chutulo and Chalannavar, 2018) as shown in Figure 4.

## 4. Factor Influencing the Endophytic Fungi

### 4.1 Climatic Factor

Environmental factors for example atmospheric humidity and rainfall may affect the existence of some endophytic fungi (Petrini, 1991; Selvanathan et al., 2011). In Sudan, Khiralla and his co-workers (2016) stated that around 3-6 endophytic fungi were isolated from the seeds and stems of five different plant species (which were used in the traditional medicine of Sudan) during the dry months (October to January). In the same context, Chareprasert and co-workers (2006) observed that the endophyte communities of the different plants were affected by the seasonal variation as they observed few isolates in the winter season as compared to another seasons throughout the year. Further, the same observations were noted by Rodrigues (1994). According to stress biology, some species of plants accumulate certain non-structural carbohydrates under water scarcity. Carbon-based defence build-up inside the plant due to accumulation, for example, in the dry season, tannins make the plant less vulnerable to endophytic fungus (Kane, 2011).

### 4.2. Ecological Factor

The distribution pattern and population structure of fungal endophytes were indicated in the result analysis where significant variation was observed in the environmental factors along with the genetic backgrounds and classification of host plant tissues (Table 1).

Table 1. Influences of host plant on the population structure   
of endophytic fungi

| **Host plants** | **Habitat** | **Isolation part** | **Factor affecting the population structure** | **References** |
| --- | --- | --- | --- | --- |
| *Asclepiadaceae* (Calotropis procera) | Garden bed | Leaf | Colonization rates of endophytic fungi is varies from high to low in different tissues. | Nascimento et al., 2015 |
| *Cactaceae* (Cactus sp.) | Desert of tropical savanna | Stem | Endophyte colonization was positively correlated with humidity and lower species diversity of the endophyte in temperate plants than that in tropical forests trees | Suryanarayana et al., 2005 |
| *Compositae* (Atractylodes lancea) | Mountain in subtropics | Rhizome | Colonization rates of endophytic fungi is varies from high to low in different tissues and richness increased as tissue aged, especially leaves. | Wang et al., 2009 |
| *Eucommiaceae* (Eucommia ulmoides) | Subtropical mountain and warm temperate semi-humid region | Leaf, branch,  bark | Lower species diversity of the endophyte in temperate plants than that in tropical forests trees and Different dominant endophytic fungi. | Sun et al., 2008 |
| *Euphorbiaceae* (Sapium sebiferum) | Mountain in subtropics | Leaf, twig | Specific host–endophyte combinations | Dai et al., 2003 |
| *Leguminosae* (Glycyrrhiza inflat) | Salinized sandy land in warm temperate region | Root | Colonization was positively correlated with humidity and lower species diversity of the endophyte in temperate plants than that in tropical forests trees | Song et al., 2007 |
| *Orchidaceae* (Gastrodia elata) | Hillside forests, wetland in temperate plateau | Tuber, flower | Different dominant endophytic fungi | Mo et al., 2008 |
| *Pinaceae* (Pinus tabulaeformis) | Temperate semi-humid monsoon region of forest. | Bark, needle, xylem | Colonization rates of endophytic fungi from high to low (seasonal variation). | Guo et al., 2008 |
| *Rosaceae* (Malus domestica) | Tropical rainy region | Leaf, flower,  fruit | Highest endophytes number under organic cultivation. | Camatti-sartori et al., 2005 |
| *Smilacaceae* (Heterosmilax japonica) | Subtropical monsoon region | Stem | Colonization rates of endophytic fungi from high to low (seasonal variation). | Guo et al., 2008 |

Table 1. (Continued)

| **Host plants** | **Habitat** | **Isolation part** | **Factor affecting the population structure** | **References** |
| --- | --- | --- | --- | --- |
| *Teaceae* (Camellia japonica) | Temperate secondary forest | Leaf | Colonization rates of endophytic fungi from high to low and richness of endophytic fungi increased as tissue aged, especially leaves. | Osono, 2008 |
| *Umbelliferae* (Apium graveolens, Cichorium intybus, Foeniculum vulgare, Lactuca sativa) | Mediterranean region | Leaf,  root,  seed | Species richness of endophytic fungi increased as tissue aged, especially leaves and Specific host–endophyte combinations. | D’Amico et al., 2008 |
| *Zingiberaceae* (Amomum siamense) | Tropical monsoon forest | Leaf, pseudo stem, rhizome | Colonization rates of endophytic fungi from high to low in different tissues. | Bussaban et al., 2001 |

Likewise, the distribution of some fungal endophytes populations was limited only to particular host plant species and to the specific genetic variation of the species. In addition, secondary metabolites provide several advantages to the host plants, for example, increase growth and make them resistant to stress, which gives a chance to understand the link between fungal endophytes and medicinal plants as shown in Figure 5.

## 5. Endophytic Fungi as a Source of Secondary Metabolites

Fungal endophytes, due to the potential source of several bioactive secondary metabolites with their remarkably complex molecular structure were subjected to scientific studies on biotechnological applications (Pelo et al., 2020). Out of them, some metabolites showed anti-algal, antibiotics, anti-cancerous, anti-fungal, anti-malarial and immune-suppressive activities (Strobel, 2018). Endophytic fungi having anti-microbial properties can fulfil the demand for natural sources that are extracted from the plant resources, the regeneration cycle length limit, and also used at an industrial level to produce natural bioactive metabolites at low cost without pollution (Du et al., 2020).Various studies have revealed numerous unique bioactive compounds with anti-microbial activities and at present, around half of the newly discovered fungal bioactive secondary compounds are extracted from endophytic fungi which include several compounds with anti-microbial activity (Liu et al., 2016). Several compounds namely alkaloids, terpenoids, phenols, phenylpropanoids, quinones, lactones and peptides produced by endophytic fungi; are known for their anti-microbial potential (Table 2).

Table 2. Bioactive compounds isolated from endophytes

|  |  |  |  |
| --- | --- | --- | --- |
| **S. No.** | **Endophytes** | **Bioactive compounds** | **Reference** |
| 1 | *Chloridium sp.* | Javanicin | Jalgaronwala et al., 2011 |
| 2 | *Cladosporium sp* | Cardiac glycosides, phenolic compounds | Selvi and Balagengatharathilagam, 2014 |
| 3 | *Cryptosporiopsis quercina* | Saadamycin | Dutta et al., 2014 |
| 4 | *Cytonaema sp.* | Cytonic acids A and B | Bhardwaj and Agrawal, 2014 |
| 5 | *Fusarium proliferatum* | Beauvericin, kakadumycin, methylcoumarin | Meca et al., 2010; Joseph and Priya, 2011; Godstime et al., 2014 |
| 6 | *Fusarium sp*. | Xularosides | Jalgaonwala et al., 2011 |
| 7 | *Ganoderma boninense* | cyclododecane, petalostemumol, rapamycin | Ismail et al., 2014 |
| 8 | *Nigrospora sp.* | Saadamycin | Dutta et al., 2014 |
| 9 | *Saccharothrix mutabilis,* | Mycoplasm (TB) | golinska et al., 2015 |
| 10 | *Streptomyces sp.* | Hypericin, kakadumycin A, | Kumar et al., 2014; Golinskta et al., 2015 |
| 11 | *S*. *coelicolor* | Munumbicins | Golinska et al., 2015, |
| 12 | *S*. *hygroscopicus* | Clethramycin | golinska et al., 2015 |
| 13 | *S*. *lygroscopicus* | Coronamycin, rapamycin | parthasarathi et al., 2012 |
| 14 | *S*. *tsusimaensis* | Valinomycin | Avin et al., 2014 |
| 15 | *Xylaria sp.* | dihydroxynaphthol, glucopyranoside | Joseph and Priya, 2011 |

### 5.1. Alkaloids

Alkaloids are the most common nitrogen atoms containing bioactive compounds in endophytes and exhibited several biological properties i.e., anti-fungal, anti-cancerous and anti-viral activities (Zhang et al., 2012). Hence, it plays a tremendous role in novel drug discovery. Amides and amines are commonly isolated bioactive secondary metabolites from endophytic fungi. For example, phomoenamide was acquired from the *Phomopis* sp. associated with the leaves of *Garcinia dulcis*. This metabolite possesses anti-bacterial against *Mycobacterium tuberculosis* (Rukachaisirikul et al., 2008). An endophytic fungus *Chaetomium globosum* originally isolated from *Ginkgo biloba* produces *Chaetoglobosins* A, C, G, V and Vb which exhibited anti-microbial along with cytotoxic activity (Li et al., 2014). A 7-amino-4-methylcoumarin exhibited a broad spectrum of anti-microbial property was extracted from endophytic fungal species *Xylaria* found in *G*. *biloba* (Wang et al., 2006). This compound revealed strong anti-bacterial and anti-fungal activities against *Aeromonas hydrophila*, *Escherichia coli*, *Salmonella typhi*, *S*. *enteritidis*, *S*. *typhimurium*, *Shigella* sp., *Staphylococcus aureus*, *Vibrio anguillarum*, *V*. *parahaemolyticus*, *Yersinia* sp., *Aspergillus niger*, *Candida albicans* and *Penicillium expansum* (Zhang et al., 2012).

In China, two pyrrolizidine alkaloids namely brocapyrrozin A and B with anti-microbial properties were discovered from an endophytic fungal species i.e., *Penicillium brocae* isolated from marine *Acaryochloris marina* (a mangrove plant). In anti-microbial study, only *brocapyrrozin* A showed strong inhibiting activity in contradiction of *S*. *aureus* with high potency as compared to positive control (Chloramphenicol) while it not exhibited any activity against Gram negative bacteria. Both brocapyrrozin A and B revealed fungicidal activity against *Fusarium oxysporum*, out of them brocapyrrozin A found more active in comparison with positive control i.e., zeocin and brocapyrrozin B showed moderate activity (Willems et al., 2020). Cristatumins A-D indole alkaloids were also extracted from *Eurotium cristatum* which is a marine-derived endophytic fungus that showed strong anti-microbial potential against *S*. *aureus* and *E*. *coli* (Du et al., 2012). Tonial et al. (2015), isolated 17 different endophytic fungi from *Schinus terebinthifolius* which describes as *Alternaria*, *Bjerkandera*, *Colletotrichum*, *Diaporthe*, *Penicillium* and *Xylaria* fungi subjected for anti-microbial properties. They revealed that most of the isolates have strong activity against *S*. *aureus*, *C*. *albicans* and *Pseudomonas aeruginosa* (Tonial et al., 2016).

### 5.2. Phenols

Phenols or phenolic acids are commonly isolated from endophytic fungi associated with a number of plants species (Yu et al., 2010). A unique compound which was known 4 -(2,4,7-trioxa-bicyclo [4.1.0] heptan-3-yl) phenol (1) was produced by endophytic fungus *Pestalotiopsis mangiferae* (isolated from *Mangifera indica*) showed strong anti-bacterial and anti-fungal activity against *E*. *coli*, *Klebsiella pneumoniae,* *Pseudomonas aeruginosa*, *Bacillus subtili*s, *Micrococcus luteus*, and *Candida* albicans (Subban et al., 2013). Pestalachloride A and B is a phenolic compound that had excellent anti-fungal activity against various plant pathogens. In Gomera Island, two anti-microbial flavonoids were extracted from the fungal endophyte *Nodulisporium* sp. which was collected from *Juniperus cedre* (Dai et al., 2006).

### 5.3. Steroids

Most of the steroids are derived from the endophytes and many of them have moderated anti-microbial activity. *Colletotrichum* sp. associated with *Artemisia annua* produces several steroid such as 6β-trihydroxyergosta-7,22-diene, 5αdihydroxy-6β-phenyl- acetoxyergosta-7,22-diene, 3-oxoergosta-4,6,8,22-tetraene; 3-oxoergosta-4-ene, 3β,5α, 3β,5α-dihydroxy-6β-acetoxyergosta-7,22-diene, 3β, 3β-hydroxy-5α, 8α-epidioxyergosta-6,22-diene, 3β-hydroxy-5α,8α-epidioxyergosta-6,9,22-triene, 3β-hydroxyergosta-5-ene and ergosterol which are reported with anti-fungal activity against some crop pathogens for example, *Helminthosporium sativum*, *Gaeumannomyces graminis* var. *tritici*, *Rhizoctonia cerealis* and *Phytophthora capisici* (Yu et al., 2010). Nodulisporium sp. isolated from *Juniperus cedre* showed strong inhibitory activity against *B*. *megaterium*, *M*. *violaceum*, *Septoria tritici* and *Chlorella fusca* (Dai et al. 2006). Ergosteroids harvested from the *Colletotrichum* sp. endophytic fungus showed anti-fungal, anti-algal and anti-bacterial activity against *Microbotryum violaceum*, *Chlorella fusca*, *B*. *megaterium* and *E*. *coli* (Zhang et al., 2012).

Helvolic acid isolated from the fungal endophytes *Pichia guilliermondii* linked with rhizomes *Paris polyphylla* var. *yunnanensis* (of the medicinal plant) found in East Himalaya, South Central China and North Viet Nam exhibited strong anti-bacterial activity against *Ralstonia solanacearum*, *Agrobacterium tumefaciens*, *Staphylococcus haemolyticus, P*. *lachrymans, S*. *aureus*, *Xanthomonas vesicatoria*, *E*. *coli*, and *B*. *subtilis* (Zhao et al., 2010).

### 5.4. Terpenoids

Diterpenoids, triterpenoids and sesquiterpenes are the main terpenoids compounds extracted from the endophytes (Yu et al., 2010) and possess anti-microbial properties (Kaul et al., 2012). According to de Souza et al. (2011), between 2006 to 2010 there are 45 diterpenes, 5 monoterpenes, 65 sesquiterpene and 12 terpenes were isolated from the endophytic fungal sources and all isolates showed several biological activities such as anti-cancerous, anti-microbial and anti-protozoal (Hughes, 2017). Terpenoids from endophyte fungi *Phomopsis* sp. linked with *Plumeria acutifolia* showed anti-bacterial activity. Moreover, presilphiperfolane sesquiterpenes harvested from *Xylaria* sp. which was originally associated with *Piper aduncum* exhibited strong anti-fungal activity against *Cladosporium cladosporioides* and *C*. *sphaerospermum* (Silva et al., 2010). Compounds namely octylcyclohexane, 1-tetradecene, 8-pentadecanone, 8-octadecanone and 10-nonadecanone from *Fusarium solani* from *Taxus baccata* exhibited anti-bacterial activity against *Staphylococcus aureus*, *S*. *epidermidis*, *B*. *subtilis*, *Klebsiella Mycosphere*, *Shigella flexneri*, and *E*. *coli* and anti-fungal activity against *Candida albicans* and *C*. *tropicalis* (Tayung et al. 2011). Pyrrocidines extracted from *Acremonium zeae* endophyte reside in maize showed significant anti-fungal activity against *Aspergillus flavus*, *F*. *verticollioides* and anti-bacterial potential against both Gram positive as well as drug resistant bacteria (Wicklow et al. 2005).

### 5.5. Quinones

A number of microbial quinones such as napthaquinones and aza-anthraquinones are found to have anti-microbial activity with a definite mode of action (Marumo et al., 1980). The Compounds namely fusarubin, bostrycoidin, anhydrofusarubin were found as anti-microbial against pathogenic bacterial strains i.e., *P*. *aeruginosa*, *B*. *megaterium*, *S*. *aureus*, and *E*. *coli* (Khan et al., 2018). Bioactive compound from endophytic *F*. *solani* originally isolated from *Glycyrrhiza glabra* exhibited anti-tuberculosis activity against *Mycobacterium tuberculosis* (Khan et al., 2018). *Streptomyces* sp. isolated from *Alnus glutinosa* produces alnumycin which exhibited anti-bacterial activity (Singh and Dubey, 2018). Altersolanol A from *Phoma* sp. associated with *Taxus wallachiana* and *Alternaria solani* from tomato exhibited anti-bacterial potential against *B*. *subtilis*, *M*. *luteus,* *P*. *aeruginosa* and *S*. *aureus*.

Anthraquinones namely 3-O-methylalaternin and altersolanol A form fungal endophyte *Ampelomyces* sp. associated with *Urospermum picroides* found to have strong anti-microbial potential against *Enterococcus faecalis, S*. *aureus* and *S*. *epidermidis* (Aly et al., 2008). In 2001, Krohn et al. isolated naphthoquinone spiroketal compounds preussomerins harvested endophytic fungus *Mycelia sterile* found in the roots of *Atropa belladonna* characterized for their anti-microbial properties. Moreover, naphthoqui-none spiroketal compounds namely as preussomerin EG1-EG3 and and deoxypreussomerin B from fungal endophyte *Edenia gomezpo-mpae* from the leaves of *Callicarpa acuminata* found in Mexico exhibited anti-fungal activity (Macias-Rubalcava et al., 2008).

### 5.6. Peptides

Anti-microbial peptides are receiving much attention and designed as a small number of molecules having 6-100 amino acids with variation in the amino acid configuration, charge, mass, 3D structure and showing anti-microbial property against a number of pathogens includes fungi and bacteria (Brogden and Brogden, 2011). Defensins family of anti-microbial peptides have numerous functions and exert primary anti-microbial defense mode in both plants and mammals (Zasloff, 2002). These peptides also isolated from the fungal endophytes such as *Acremonium* sp. from *Taxus baccata* produces a group of antifungal-anticancer peptides referred to as the leucinostatins out of which leucinostatins A showed anti-fungal assay against the oomycetous plant-pathogenic fungus *Pythium ultimum* (Abdalla and Matasyoh, 2014). An endophytic fungus *Fusarium* sp. isolated from *Rhododendron tomentosum*, most widely found in northern hemisphere was identified as active produce to anti-microbial peptides (Tejesvi et al., 2011). Furthermore, *Fusarium tricinctum* also isolated from *Rhododendron tomentosum* produces trtesin which was active against *C*. a*lbicans*, *C*. *utilis* and *S*. *carnosus* (Tejesvi et al., 2011).

Anti-bacterial compounds namely cyclo-(Pro-Thr) and cyclo-(Pro-Tyr) extracted from endophytic *Penicillium* sp. associated with *Acrostichum aureurm* (mangrove plant) exhibited anti-bacterial potential against *C*. *albicans* and *S*. *aureus* (Seto et al., 2007). Furthermore, epichlicin (cyclic peptide) from the endophytic fungus *Epichloe typhina* associated with timothy plant i.e., *Phleum pretense* exhibited inhibitory effect against the spore of the pathogenic fungus *Cladosporium phlei* (Chomcheon et al., 2010). Hybrid peptide-polyketides, curvularides A-E were harvested from *Curvularia geniculata*, originally found in the limbs of *Catunaregam tomentosa*. Out of them Curvularide B showed anti-fungal potential against *C*. *albicans* and also displayed a synergistic effect with fluconazole drug (Chomcheon et al., 2010). The cryptic role of endophytic fungi *Trichoderma citrinoviride* from cork oak revealed the peptaibols (paracelsin) has antagonistic effect against various fungal pathogens which are responsible for the inclement of oak (Maddau et al., 2009). Peptaibols are very economically valuable for their anti-cancer, anti-microbial properties and the introduction of system-wide resistance in plants against microbial contravention (Mukherjee et al., 2011).

### 5.7. Polyketides

Polyketides are the common fungal metabolites and due to their structural diversity act as an important group of bioactive compounds for drug discovery. They showed significant biological activity as an antibiotic, anti-cancer, anti-fungal, anti-parasitic and immunosuppressant (Santiago et al., 2014). Polyketides namely 8-dihydroxy-6-methoxy-3- methyl-3, 4-hydroxymellein and 4,4-dihydro-1H-isochromen-1-one were isolated from fungal endophytes linked with *Cinnamomum mollissimum* showed inhibitory activity against *A*. *niger* and *B*. *subtilis* (Santiago et al., 2014). *Pericoannosin* was isolated from the fungal endophytes *Periconia* sp. found inside the plant *Annona muricata* also exhibited anti-microbial activity (Zhang et al., 2016). *Epicoccum* sp. originally found in *Theobroma cacao* produces epicolactone and epicoccolides exhibited strong anti-microbial activities against the mycelia growth of peronosporomycete phytopathogens namely *Aphanomyces cochlioides*, *Pythium ultimum*, and basidiomycetous fungus *Rhizoctonia solani* (Talontsi et al., 2013). Anti-bacterial polyketide i.e., palitantin from *Aspergillus fumigatiaffnis* edophytic fungi from *Tribulus terestris* found to inhibit *Enterococcus faecalis* (Ola et al., 2018). Compound namely koninginin, koningiopisin and trichoketide obtained from the endophytic fungus *Trichoderma koningiopsis* connected with *Artemisia argyi* showed anti-microbial activity against human pathogenic strain *E*. *coli* and marine-derived aquatic bacteria *Pseudomonas aeruginosa*, *E*. *tarda*, *Micrococcus luteus*, *Vibrio alginolyticus*, *V*. *parahemolyticus*, *V*. *anguillarum*, and *V*. *vulnificus*. In addition, all compounds exhibited broad-spectrum activity against agro pathogenic strain i.e., *Bipolaris sorokiniana*, *Ceratobasidium cornigerum*, *C*. *gloeosporioides*, *Fusarium graminearum*, *F*. *oxysporum*, *Penicillium digitatum*, *P*. *piricola* and *Valsa mali* (Gao et al., 2020). Polyketides as hydroxyrecifeiolide, ent-cladospolide, cladospolide were extracted from the endophytic fungi i.e., *Cladosporium cladosporioides* associated with leaves of *Bruguiera gymnorrhiza* exhibited inhibitory effect against a wide range of human, aquatic bacteria and plant pathogenic fungi (Shi et al., 2017). Polyketide-terpene a hybrid metabolites were extracted and characterized by endolichenic fungus *Pestalotiopsis* sp. which showed inhibitory activity against *Fusarium oxysporum* and *F*. *gramineum* (Yuan et al., 2017). Aplojaveediins from *Aplosporella javeedii* found inside the *Orychophragmus violaceus* exhibited anti-fungal property against the hyphae form of *C*. *albicans* (Gao et al., 2020).

### 5.8. Acids

Endophytes are commonly known for the active production of several pharmacologically dynamic acids such as ambuic acid, torreyanic acid, fusaric acid and its derivatives, etc. exhibited anti-microbial potential (Deshmukh et al., 2015). Salvianolic acid from *Phoma glomerata* found in *Salvia miltiorrhiza* known for its anti-microbial properties (Li et al., 2016). In 2015, Khiralla isolated pentaenoic (k/a khair acid) from *Curvularia papendorfii* (an endophytic fungi) associated with *Vernonia amygdalina* exhibited anti-bacterial activity against methicillin-resistant *Staphylococcus aureus* (Khiralla, 2015). *Fusarium solani*, *F*. *oxysporum* and *F*. *proliferatum* from the *Cajanus cajan* (pigeon pea) reported as active producer of cajaninstilbene acid which exhibited hypotriglycerimic, hypoglycemic, anti-inflammatory, anti-microbial, analgesic and antioxidant activities (Zhao et al., 2018). Cytonic acids from *Cytonaema* sp. showed strong anti-viral action effects against human cytomegalovirus (Guo et al., 2000). In China, carboxylic acids and xanalteric acids from endophytic fungus *Alternaria* sp. associated with mangrove plant *Sonneratia alba* exhibited weak antibiotic activity against multidrug-resistant *S*. *aureus* (Kjer et al., 2009). An endophytic fungus *Cryptospo-riopsis quercina* actively produces cryptocin (tetramic acid) which showed anti-mycotic activity against plant pathogenic fungi namely *Pythium ultimum* and *Pyricularia oryzae* (Li et al., 2000). *Aspergillus flavus* (an endophytic fungi) from *Sonneratia alba*, a mangrove plant found in Timor island found to produce kojic acid which exhibited anti-bacterial potential against *S*. *aureus* and *E*. *coli*, respectively (Ola et al., 2020). A unique antibiotic solanioic acid (highly functionalized and repositioned steroidal carbon compound) was also obtained from the *Rhizoctonia solani* found in the medicinal weed *Cyperus rotundus* in Sri Lanka (Ratnaweera et al., 2015). A unique anti-microbial bioactive metabolite i.e., colletotric acid was extracted from Colletotrichum gloeosporioides (an endophyte) reside in the stem of *Artemisia mongolica* showed anti-bacterial activity against *Alternaria* species (Major plant pathogens) (Bin et al., 2014).

### 5.9. Isocoumarin Derivatives

Several isocoumarin derivatives from endophytic fungi are stated to have anti-microbial activity against various human and plant pathogenic microorganisms. Phomoisocoumarins from an endophytic fungus *Phomopsis prunorum* possess reasonable anti-bacterial activity against plant pathogenic bacteria *Pseudomonas syringae* pv. *Lachrymans* (Qu et al., 2020). Isocoumarin from an endophyte *Microdochium bolleyi* associated with *Fagonia cretica* showed strong anti-bacterial potential against *Bacillus* *megaterium* and *Escherichia* *coli* (Zhang et al., 2008). Two noble isocoumarin derivatives namely 5-hydroxy-8-methyl-2H, 6H-pyrano[3,4-g] chromen-2,6-dione and 6,8-dihydroxy-3,7-dimethyl-isocoumarin were obtained from *Aspergillus similanensis* associated with undescribed marine sponge exhibited anti-microbial activity against Gram-positive, Gram-negative bacteria and *Candida albicans* and multidrug-resistant isolates from the environment (Prompanya et al., 2014). Three unique dihydroisocoumarin derivatives with anti-malarial, anti-tuberculous and anti-fungal activities were extracted from *Geotrichum* sp. which was originally isolated from *Crassocephalum crepidioides* (Kongsaeree et al., 2003).

### 5.10. Other Compounds

Annulene which was isolated from the *Gliocladium* sp. associated with *Eucryphia cordifolia* strongly inhibited fungi namely *Pythium utlimum* and *Verticillum dahliae* (Stinson et al., 2003). *Aspergillus niger* from *Cynodon dactylon* also produces rubrofusarin, fonsecinone, asperpyrone, aurasperone inhibit the growth *Bacillus subitlis,* *Escherichia coli*, *Pseumomonas fluorescens*, *Candida albicans* and *Trichophyton rubrum* (Song et al., 2004). Griseofulvin obtained from *Xyleria* sp. associated with *Abies holophylla* exhibit anti-microbial activity against *Magnaporthe oryzae*, *Botrytis cinerea*, *Blumeria graminis* f.sp. *hordei*, *Corticium sasaki*, *Phytophthora infestans* and *Puccinia recondite* (Park et al., 2005) (Table 3).

Table 3. Host medicinal plants with enhanced growth conferred   
by endophytic fungi

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Endophytic fungi** | **Host plant** | **Mechanism** | **Type of stresses** | **Reference** |
| *Chaetomium globosum*,  *Botrytis* sp | *Chrysanthemum morifolium* | Increase POD activity and soluble protein content | Salt stress | Liu et al., 2011 |
| *Arbuseular mycorrhiza*, *Penicillium griseofulvum* | *Glycyrrhiza uralensis* | Reduce injury of water stress by increase protective enzymes’ activity and osmotic contents | Drought and salt stress | Wang et al., 2009 |
| *Arbuseular mycorrhiza* | *Salvia miltiorrhiza* | Increase the absorption of nutrient and alter metabolic activities in host | Drought stress | Meng and He, 2011 |
| *Leucocoprinus gongylophorus* | *Cordia alliodora* | Produce some chemicals antagonistic to ants’ fungal symbiont | Insect | Bittleston et al., 2011 |
| *Beauveria bassiana*, *Lecanicillium dimorphum*, L. cf. psalliotae | *Phoenix dactylifera* | Modulate the expression of cell division-related proteins in host | Insect: date palm pests | Gómez-Vidal et al., 2009 |
| *Chaetomium cochliodes*, *Cladosporium cladosporioides*, *Trichoderma viride* | *Cirsium arvense* | Produce some chemicals toxic to pathogens | Insect: foliar feeding insects | Gange et al., 2012 |
| *Chaetomium* Ch1001 | *Cucumis sativus* | Produced abscisic acid affecting motility of the second stage juveniles of insects | Insect: root-knot nematode Meloidogyne incognita | Yan et al., 2011 |
| 150 foliar fungal endophytes | *Picea rubens* | Produce some chemicals toxic to insects | Insects: Choristoneura fumiferana | Sumarah et al., 2010 |
| Endophytic fungi | Host plant | Mechanism | Type of stresses | Reference |
| *Gilmaniella* sp. AL12. | *Atractylodes lancea* | Produce jasmonic acid inducing defense responses | Pathogenic fungi | Ren and Dai, 2012 |
| *Chaetomium globosum* L18 | *Curcuma wenyujin* | Produce some chemicals toxic to pathogens | Pathogenic fungi | Wang et al., 2012 |

Table 3. (Continued)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| *Trichothecium roseum* | *Maytenus hookeri* | Produce trichothecin toxic to pathogens | | Pathogenic fungi | Zhang et al., 2010 |
| *Choiromyces aboriginum*, *Stachybotrys elegans*, *Cylindrocarpon* sp | *Phragmites australis* | | Produce cell wall-degrading enzymes to kill pathogenic fungi | Pathogenic fungi | Cao et al., 2009 |
| *Phomopsis cassiae* | *Cassia spectabilis* | | Produce cadinane sesquiterpenoides toxic to pathogens | Pathogenic fungi: Cadosporium sphaerospermum, and C. cladosporioides | Silva et al., 2006 |
| *Bacillus subtilis*, *Myxormia* sp. | *Angelica sinensis* | | Produce some chemicals toxic to pathogens | Pathogenic fungi: Fusarium oxysporum and F. Solani | Yang et al., 2012 |
| *Acremonium blochii*, *A*. *furcatum*, *Aspergillus fumigatus*, *Cylindrocarpon* sp., *C*. *destructans*, *Dactylaria* sp., *Fusarium equiseti*, *Phoma herbarum*, *P*. *leveillei* | *Hordeum vulgare* var. *disticum* | | Improve the competence for space inhibiting the colonization of pathogens | Pathogenic fungi: Gaeumannomyces graminis var. Tritici | Macia-Vicente et al., 2008 |
| *Chaetomium* sp, *Phoma* sp | *Triticum* *aestivum* cv. “Morocco” | | Activate defense reactions of the plant | Pathogenic fungi: Puccinia recondite | Dingle and McGee, 2003 |
| *Cryptosporiopsis* cf. *quercina* | *Triptergyium wilfordii* | | Produce cryptocin and cryptocandin toxic to pathogens | Pathogenic fungi: Pyricularia oryzae | Strobel et al., 1999 |
| *Sordariomycetes* sp | *Oryza sativa* | | Inhibition of electron transport from the quinone acceptor QA to QB | Pb2+ stress | Li and Zhang, 2015 |
| *Penicillium resedanum* LK6 | *Capsicum annuum* | | Improve nutrient, proline and flavonoid contents, modulate amino acid metabolism | Heat stress | Khan et al., 2013 |

## 6. Pharmacological Benefits of Endophytic Fungi

Nanotechnology is the branch of science that is growing very rapidly due to the increasing use of electronics and medicine in various fields (Sandhu et al., 2017). Primarily, this branch has great efficiency in biomedical applications because the living system is a complex network of Nano-machines. Nanoparticles (NPs) have a very high surface and are small in size, because of which they have excellent physicochemical characteristics. Due to such properties, NPs are attracting more attention in various fields, including chemical signaling, information storage, catalysis, electronics, drug discovery and labeling, ecological treatments and high commercial value. (Prabhu and Poulose, 2012). The methods of nanoparticles synthesize are very low cost and eco-friendly that’s why biological protocols of Nps production are taking benefit over other protocols (Veerasamy et al., 2011; Hemashekhar et al., 2017). Therefore, NPs production from different microorganisms includes fungi, bacteria and yeasts are these days exploited (Kathiresan et al., 2009; Ibrahim, 2015; Ingale and Chaudhari, 2013).

Therefore, in metal NPs synthesis fungi are considered the most ideal candidates (Das et al., 2014). NPs find huge application in nutrition, health, agriculture and medicine due to their antimicrobial properties (Borase et al., 2015; Khalil et al., 2019). AgNPs are commonly applied due to their medical application of great antimicrobial activity (Prabhu and Poulose, 2012). Therefore, fungal diversity on the earth catches great attention for the NPs synthesis mainly AgNPs by scientists (Netala et al., 2016). Different fungal strains are previously used for the AgNPs production including *Fusarium* *oxysporum* (Ahmad et al., 2003), *F. incarnatum* (Joshi et al., 2019), *F*. *solani* (El-Rafie et al., 2012), *Alternaria alternata* (Gajbhiye et al., 2009), *Aspergillus solani* (Devi et al., 2014), *A. niger* (Jaidev and Narasimha, 2010), *A*. *rouxii* (Musarrat et al., 2010), *A. terreus* (Li et al., 2011), *A. versicolor* (Netala et al., 2016), *Coriolus versicolor* (Sanghi and Verma, 2009), *Penicillium funiculosum* (Devi et al., 2014), *Humicola* sp. (Syed et al., 2013), *Schizophyllum radiatum* (Metuku et al., 2014), *Phaenerochaete chrysosporium* (Vigneshwaran et al., 2006), *Trichoderma viridae* (Fayaz et al., 2009) and *Puccinia graminis* (Kirthi et al., 2012). Actinomycetes (Rasool and Hemalatha, 2017; Saad et al., 2018) are also used for the synthesis of NPs. Since 2000 years, the medical properties of silver are well known. From the 19th century, in various antimicrobial applications compounds prepared from silver are used. Today, the scientist is using nano biotechnology for cancer therapy and much attention is paid to the discovery of biosynthetic NPs from fungus (Muhsin and Hachim, 2016; Verma et al., 2017). Microorganism not just silver and gold are used to synthesize of NPs, but also for NPs of other ions including platinum (Rai et al., 2009), cadmium (Vijayan et al., 2016), zirconium and titanium (Bansal et al., 2006).

### 6.1. Anti-Inflammatory Activity

Inflammation is commonly describing as a protective response which is considered as an event to eliminating the initial reason of cell damages, cleanse and protection of plant and animal tissue in repair mode (Karin and Clevers, 2016). However, inflammation is harmful to attacks on the human own tissues. In addition, inflammation has a very close relationship with a vast array of human diseases for example erythematosus, cancer, pneumonia, atherosclerosis, ischemic heart disease, rheumatoid arthritis and Crohn’s disease (Grivennikov et al., 2010). Inflammation plays a key role functioning and development of different disorders (Jin et al., 2018). Therefore, diagnosis and treatment of this disease may be worked as a remedy for inflammation. In this field, nanoparticles may be a novel device specifically biosynthesized according to Govindappa et al. (2016) and it reflects endophytic fungus *Penicillium* sp. synthesized AgNPs anti-inflammatory. Between all-natural resources, fungi are highly efficient microbes for the synthesis of nanoparticles (Narayanan and Sakthivel, 2010; Singh et al., 2014), inside bioreactors, fungal mycelia can survive and tolerate huge stress, moreover it is easy to handle and manufacture downstream methods (Zhao et al., 2018). Production of nanoparticles through endophytic fungus is another discovery and very limited documentation is available for nanoparticles synthesis and their use as anticancer agents.

### 6.2. Anti-Viral Agents

Currently, the world is facing problem with various potentially fatal diseases which was caused by several viruses, such as, human immunodeficiency viruses (HIV), chickenpox, infectious mononucleosis, common cold, influenza, hepatitis and viral encephalitis (Gaikwad et al., 2013). A major effort is going on for the last few decades to develop drugs and vaccines against various viral infections and in fact, drugs developed over the past several years are not capable of preventing all viral diseases (Domingo, 2010). Therefore, it has become very essential to discover antiviral agents that can be used in the demonstration against a wide range of viral activities. Besides, the main aim of the researcher is to develop an eco-friendly method rather than artificial methods (Ramya et al., 2015). Thus, researchers are highly focused on using nanoparticles as antiviral agents that were produced by microorganisms. These nanoparticles are highly effective against hepatitis B virus (Lu et al., 2008) and HIV-1 (Lara et al., 2010). Moreover, there are few documentations on antiviral activities were reported on the synthesizes NPs from microbes (fungi and bacteria). Narasimha et al. (2012) the examine antiviral activity of AgNPs in which *Aspergillus* sp. against bacteriophage viral strain. Narasimha (2012) studied that fungal strain *A*. *niger* at 8–12 ppm which was used to inhibit the viral growth in host bacteria (*E*. *coli*) was utilized in the AgNPs synthesis. Gaikwad et al. (2013) shown that fungi synthesized AgNPs against herpes simplex virus and human parainfluenza virus type 3. Ramya et al. (2015) showed the synthesizing activity of selenium NPs of Streptomyces against dengue virus. Consequently, the relationship between nanoparticles and virus’s biosynthesis has become the main area of research because in NPs synthesis from endophytic microbes very little information available. Thus, researches can be made efforts and target endophytic microbes for the synthesis of NPs in the future. Using endophytes as antiviral agents will be demonstrated as a novel method in viral infection treatment.

### 6.3. Wound Healing Activity

The main goal of any burn remedy is to instantly seek wound healing and epithelization (Kaler et al., 2014). Phenomena by which overlapping pattern of wound healing occurrences includes tissue remodeling, inflammation, coagulation, proliferation and matrix (Tian et al., 2007). Topical herbal therapeutics, granulation tissue suppressing agents, enzymes and antiseptics are the various medicines used in the wound healing process (Gunasekaran et al., 2011). Every medicine has its own positive and negative effects of some antiseptics which are found cytotoxic *in vitro* to both (microbes and hosts) (White et al., 2006). Besides, the main drawback of the utilization of antibiotics is the reduction of their efficiency against contact allergy and bacterial resistance (White et al., 2006; Lipsky and Hoey, 2009). Nanotechnology is mainly applied in the synthesis of NPs for wound healing. Tiwari et al. (2014) studied wound healing characteristics of copper NPs synthesized from *P. aeruginosa*. On the other hand, Kaler et al. (2014) examined *Saccharomyces* *boulardii* for theproduction of AgNPs-based nano silver gel which showed better-wound healing ability. The endophytic microorganisms can be exploited in this region to synthesize NPs with wound healing activity.

### 6.4. Anti-Microbial

At present microorganisms developed resistance against the number of antibiotics and people focused on plant-based medicine which causes heavy pressure on plants and leads to a higher rate of deforestation. Over the years, microorganisms connected with plants were reported as a source of different substances and products with great therapeutic possibilities (Subbulakshmi et al., 2012). The products contain a wide variety of bioactive secondary metabolites for example alkaloids, flavanoids, phenolic acids, isocoumarin derivatives, phenols, steroids, quinones and terpenoids (Table 2) that can influence the physiology, immunity and tolerance of the host plant against biological and abiotic stresses (Malhadas et al., 2017). These metabolites also reported having their anti-microbial, anti-inflammatory, anti-oxidant, anti-parasitic, anti-tumor, cytotoxic and neuroprotective properties (Gutierrez et al., 2012) with distinctive chemical structures (Selim et al., 2012). At present, scientific communities actively focused on bioactive secondary metabolites for biotechnological applications, especially in the agricultural, cosmetic, food and pharmaceutical industries as shown in Figure 6.



**Figure 6.** Different factor effected by plant and microbe’s interaction.

Novel anti-microbial compounds isolated from the endophytes are seemed like a choice to conquer the problem of drug resistance by human and plant pathogens. The inadequate quantity of active antibiotics against bacteria and the small number of anti-microbial agents in development is may be due to the unfavourable investment returns (Hughes et al., 2017). Several bioactive compounds with anti-fungal activity have been extracted from the *Xylaria* genus isolated from the plant such as sordaricin and multiplolides showed anti-fungal activity against *Candida albicans* (Pongcharoen et al., 2008) while mellisol and 1,8-dihydroxynaphthol 1-O-a glucopyranoside possess anti-fungal property against herpes simplex virus-type 1.

### 6.5. Antibiofilm Activity

The global use of drug-resistant microbes is increasing day by day. There broad-spectrum uses against bacterial and fungal infection, moreover development of microbial resistance antibiotic which has the potential of developing medical and biofilms equipment (Penesyan et al., 2015; Cremonini et al., 2018). The proper description of biofilm contains belief microorganisms, both of surface or gene expression change of each other resulting in the extracellular matrix and planktonic state of different phenotype made up of host constituents and secreted microbial products (Trautner and Darouiche, 2004). However, biofilm results in persistent and chronic infections that are extremely difficult to get free from antimicrobial activity (Lyte et al., 2003). The biofilm formation enhances the microorganism growth and makes them survive in undesirable conditions such as survival, disturbance and starvation, thereby protecting them from antibiotic treatment and the host immune system (Banerjee et al., 2019). It is commonly known that biofilm-forming bacteria are 1000 times more easy-going than antibiotics (Soleimani et al., 2015). The Centers for Disease Control in 2007 revealed that the United States alone bears the loss of $ 11 billion due the infections were caused by biofilm bacteria which is a serious economic loss (Romling et al., 2014). Adding more, due to this biofilm-associated infection almost one and a half million Annual deaths are happening and $ 94 billion is the total treatment costs (Wolcott et al.,2010).

Microorganisms responsible for biofilm-related infections are on medical devices for example heart valves, urinary catheters and prosthetic joints. In addition, *P*. *aeruginosa*, *Enterococcus* sp., and *Staphylococcus* sp., are recognized in patients with wound infection and cystic fibrosis (Reomling et al., 2014). Inappropriately, there is no standard method of dealing with biofilm-related infections and the immediate requirement to develop a standard method for the treatment of such kind of infection. Presently, nanotechnology gives a favourable approach to achieve the goal of eradication of nano-microbial biofilm infections. The development of nanotechnology has given assurance of prevention from biofilm infections. In a few documentations, the surface of organisms coated with nanoparticles have been found biofilm inhibitory agents because of the high efficiency and small size, nanoparticles can penetrate over the cell wall along with the biofilm layer, eventually causing irreversible damage to cell and DNA (Taylor and Webster, 2011). Therefore, Nanotechnology opened opportunities with increasing concern of biotechnological infections.

## 7. Therapeutic Applications

Recent years of biosciences have been receiving biochemical attention due to their distinct characteristics due to diverse fields of biosciences i.e., environment-friendly habits and chemical-free (Gouda et al., 2019). Nanotechnology provides an excellent medium of modification and develops unique properties of metal ions, furthering potential use in various fields of science for example antimicrobial agents, bio imaging, agronomy, biomedical diagnostics, drug delivery and sensors (Tripathi et al., 2015; 2017). Therefore, microorganisms are a great source of NPs production which is focused on by investigators currently because microorganisms have the huge potential of growing under extreme conditions (Pantidos and Horsfall, 2014). Therefore, these microbial endophytes are capable of secreting biologically valuable substance which is useful in anticancer, antioxidant, antidiabetic, modern agriculture, medicine and immunosuppressant etc. (Popli et al., 2018). Though the biosynthesis of nanoparticles through endophytic microbes is not yet fully studied and very few documentations are present. Therefore, there is a great diversity of endophytic microorganisms, which are discovered yet and used in the production of NPs for various applications.

## 8. Endophytic Fungi a Source of Nanoparticles

In the last few years’ research in the field of nanotechnology has gained great attention due to the huge utility in the agricultural, environmental and industrial sectors. They can be synthesized by physical and chemicals methods which create problems for humans along with the ecosystem. The production of NPs by the biogenic pathway gives a substitute for the discovery of new bio sources that make metals efficient for reducing their nanoparticles. Plants, fungi and bacteria are some microorganisms that have shown effectiveness for the production of NPs. Very little documentation is available regarding the production of NPs from endophytic microorganisms and is reviewed as uncontaminated, non-hazardous and eco-friendly “green chemistry” practices as shown in Figure 7.

Table 4. Endophytic fungus, biologically synthesized nanoparticles and responsible plant metabolites [Modified from Arya, 2015]

|  |  |  |  |
| --- | --- | --- | --- |
| **Host** | **Endophytic fungus** | **Nanoparticles** | **Phytochemicals** |
| *Capsicum annum* | *Alternaria alternata* | Silver | Reductive amino acids and vitamins |
| *Cinnamomum camphora* | *Phytophthora cryptogea*, *Pythium aphanidermatum,* *Microsporum nanum* | Silver | Alkaloids, flavones and anthracenes |
| *Datura metel* | *Colletotrichum boninense, Phomopsis* sp., *Fusarium solani, Colletotrichum incarnatum, Colletotrichum siamense* and *Colletotrichum gloeosporioides* | Silver | Quinones and the plastohydroquinone |
| *Solanum lycopersicum* | *Fusarium*, *Alternaria*, *Penicillium* | Gold, Silver | Flavonoids, and alkaloids, vitamins, carotenoids, polyphenols |
| *Solanum xanthocarpum* | *Fusarium solani* | Silver | Phenolic, alkaloids, sugars |
| *Sorghum bicolor* | *Aspergillus*, *Alternaria alternata* | Silver, iron | Polyphenols |
| Swietenia mahogany | *Septoglomus constrictum*, *Claroideoglomus etunicatum* | Silver, gold, Polyhydroxy bimetallic alloy, limonoids gold-silver | Polyhydroxy limonoids |
| *Terminalia chebula* | *Fusarium fujikuroi*, *Aspergillus tubingensis* and *Rhizopus oryzae* | Silver | Polyphenols |
| *Trianthema decandra* | *Pestalotiopsis* sp, *Acremonium sclerotigenum* | Silver | Hydroxyflavones, catechins |



**Figure 7.** Aspects of nanotechnology in precision agriculture.



**Figure 8.** Formation of nanoparticles by mixing plant and endophytic fungi.

Inorganic NPs are particularly important as silver and gold nanoparticles, as they give greater physical properties with functional elasticity. Inorganic NPs are obtaining possible tools for medical imaging along with valuable implementation in the treatment of various diseases. The size characteristics inorganic nanoparticles have an advantage over existing chemical agents and imaging drugs. Inorganic nanometry due to its versatile characteristics such as rich functionality, extensive availability, excellent bio-capacity, drug delivery and controlled release of drugs has been widely accepted for cellular delivery (Sunkar and Nachiyar, 2012).

Earlier, NPs can be produced by two processes namely physical and chemical processes. However, the use of an unwanted chemicals in this process causes environmental problems such as chemical contamination, toxic solvents and hazardous by-products which is due to the production of undesirable chemicals (Xu et al., 2006). These protocols have several issues with the growth, stability and accumulation of the nanosize particles as shown in Figure 8. Thus, the application of microbiota in biological systems to produce NPs has emerged as a unique research area. Various studies validate the synthesis of NPs (various shapes and sizes) with the help of microorganisms (bacteria and fungi) (Joshi et al., 2019) which have great efficiency of anti-microbial activity. One such crowded microorganism is endophytes, which have not yet been fully studied in the biological synthesis of nanoparticles. According to Bacon et al. 2014 examination “Microorganism that escapes the internal tissues of plants due to any immediate, adverse effects”, while Strobel et al. recommended a variation link between the mutualistic to bordering on pathogenic (Strobel et al., 1996). The most common experience endophytes are bacteria and fungi live with each other (Sunkar and Nachiyar, 2012). Very few documents are presents where nanoparticles synthesis with endophytic fungi. One such study was conducted by Shankar et al. 2003 for the extra-cellular preparation of gold nanoparticles which were prepared from endophytic fungus (*Colletotrichum* sp.) isolated from geranium leaves (*Pelargonium graveolens*). One more study by Verma et al. (2010) showed the *Aspergillus clavatus* (endophytic fungus), which was isolated from sterilized stem tissues of *Azadirachta indica* is used for the production of AgNPs synthesis.

## Conclusion and Future Prospectives

This chapter sheds light on the ecological and host-plant issues that probably affect the distribution and population configuration of endophytic fungi, along with the advantages these endophytic fungus given to their host plants. The fungus-host association suggested that the distribution and population configuration of fungal endophytes depend to a large extent on the genetic, age, tissues and taxonomy, of the host plants, moreover to the environmental conditions. This research may be helpful in the understanding of bioactive compounds which are isolated from different host medicinal plant under different environmental conditions Moreover, according to the observation between fungal endophytes and their host plants there are three types of advantageous interactions namely:

* Host medicinal plants growth enhancement;
* Increased stresses (biotic and abiotic) towards host plants resistance;
* Accumulation of secondary compounds (bioactive compounds).

These researches include important practical aspects of drug discovery that are excellent in quality and quantity. Material for good medicine with good quality and best results on particular diseases have a special relationship with endophytic fungi. Bioactive compounds isolated from endophytic fungal share a special type of relations with the medicinal plant which are needed by a human being. Such as, in the tropical region, Huperzine-A compounds isolated from the medicinal plant, *Huperzia serrata*, is enhanced by endophytic fungi *Shiraia* sp. and *Acremonium* sp. (Wang et al., 2011). This is the reason in the Chinese medicinal system, healer prescribes specific medicinal plant of different habitats due to particular bioactive compounds. Thus, the comprehension of the population and distribution of fungal endophytes will give a conceptual direction for finding bioactive compounds of particular drugs by host different herbal plants under precise environmental conditions. Significantly, in many cases, fungal endophytes can increase the seed growth of several hosts medicinal plant species and also helpful in germinating those seeds which cannot propagate in normal conditions. Such as, some medicinal species which endangered and rare, i.e., in the orchid family, *Dendrobium chrysanthum* and *D*. *Nobile*, are unable to germinate under normal conditions. Though, in *Mycena* genus, seed cultivation is followed by *in vitro* condition with fungal endophytes of this type of medicinal plants (Jia et al., 2016). These methods are specially applied for endangered and rare host medicinal plants that are useful in the breeding methods where propagation of seeds is very essential. The secondary metabolites accumulation which is produced from plants is promoted the extremely valuable application of endophytic fungi. Though such application can increase the production and collection of bioactive metabolites of different medicinal plants for excellent raw drugs, through in vitro culture of fungal endophytes and medicinal plants. The association of fungal endophytes and host medicinal is fully understood and unique properties can give a new direction to drug discovery in a highly effective way. Unfortunately, most of the investigational studies of valuable strains prove the efficiency of endophytic fungi towards high-quality drug discovery. Although the mode of interaction between fungal endophytes and host plants is not mentioned. Moreover, the investigation focuses on the need of fungal endophyte from several eras, for example:

* Create a bioengineering method that imitates the link between fungal endophyte and host plants to promotes the production of bioactive compounds;
* Rapid screening system for fungal endophyte advantages host plants apart from isolating all strains blindly;
* Establishment of objective for using seed germination for protecting endangered medicinal plants from endophytic fungi library;
* Solving degradation problems for targeting endophytic fungi which were selected for preferred metabolites;
* Make excellent use of valuable microbes for the isolation of medicinal plants for the production of improved pharmaceutical items.

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Chapter 2

# Diversity and Antimicrobial Activity of Endophytic Fungi from Mangrove Forests

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## Abstract

Mangroves are the ecosystem situated between land and sea and occupy about 18.1 million hectares of the planet in tropical and subtropical areas. Endophytic fungi inhabit the internal tissues of plants without causing negative effects. Evidence suggests a close symbiotic microbial-plant relationship. Endophytic fungi produce antibiotics that enable them to survive in competitive habitats with other microorganisms and protect their host plant against other fungal and bacterial pathogens. Large-scale cultivation of endophytic fungi may produce enormous amounts of natural products while keeping costs reasonable. Also, fungal endophytes have been studied mainly as a source of novel bioactive compounds and secondary metabolites of their host plants. Many important antibiotics in the pharmaceutical industry have been derived from microbial metabolites. The biochemical versatility and diversity of endophytic fungi suggest that there are still many unknown active compounds. Fungal endophytes from mangroves open up new areas of potential biotechnological exploitation; thus, isolating and cultivating these microorganisms is of great importance for pharmaceutical, industrial, and agriculture fields. The high biodiversity found in the mangrove ecosystem reinforces the importance of studying endophytic fungi, particularly to isolate new compounds.

**Keywords:** antimicrobial, antiparasitic activity, bioactive molecules, biocontrol, bioprospecting, endophytes

## 1. Introduction

It is estimated that there are 1.5 million fungal species, of which approximately 712,000 have been described (Hawksworth, 2004; Schmit and Müller, 2007). Most of these species are valuable from an environmental and biotechnological point of view, but fungal species have been isolated from only a few of the 300,000 known plant species (Strobel and Daisy, 2003; Azevedo and Quecine, 2019).

Endophytic microorganisms live inside plant tissues without damaging the host plant. Associations with tissues may be symbiotic or mutualistic and can benefit the plant, such as promoting disease resistance, tolerance to stress conditions (e.g., an herbivorous attack), greater availability of nutrients, and an increase in biodiversity (Golinska et al., 2015). It is believed that all plants have endophytic microorganisms and also that throughout the evolutionary process microorganisms have incorporated some metabolic pathways of plants so that endophytes can produce phytochemical compounds (Strobel and Daisy, 2003; Debbab et al., 2013; Deshmukh et al., 2018; Bibi et al., 2020).

Endophytic fungi are a source of antimicrobial agents that produce a wide range of important medicinal compounds, including antitumor agents, insecticides, vitamins, immunosuppressants, immune modulators, and other natural products (Liu et al., 2007; Sebastianes et al., 2012a, 2017a; Thatoi et al., 2013). Fungal endophytes are a promising resource for drug discovery by providing a variety of chemically diverse secondary metabolites, which have shown broad pharmacologic potential (Aly et al., 2011; Blunt et al., 2012). Also, endophytes represent a potential source of microorganisms for enzyme, organic acid, biosurfactant, and bioemulsifier production (Dezam et al., 2017; Maroldi et al., 2018; Martinho et al., 2019). In this context, the production of bioactive natural compounds important to the pharmaceutical, industrial, and agriculture fields is widespread among fungal endophytes.

Currently, it is believed that all plant species host at least one endophyte; however, the endophytic biology of few plants has been studied. Therefore, there is a high probability of interesting endophytic fungi in many different settings and ecosystems, such as mangrove areas (Suryanarayanan et al., 1998; Ananda and Sridhar, 2002; Cheng et al., 2009; Suryanarayanan et al., 2011; Suryanarayanan et al., 2012; Sebastianes et al., 2013; Bibi et al., 2020).

Mangrove-derived endophytic fungi have received wide attention due to the special living environment, which is exposed to high salinity, dampness and temperature (Nicoletti et al., 2018) that contribute to a different metabolism compared to traditional terrestrial microorganisms (Xu, 2015). Also, the composition and dominant species in each mangrove plant are different. The colonization of endophytic fungi always varies in different parts of the host plant (leaves, twigs, and stems), according to the age of the host plant and season (Suryanarayanan et al., 2012; Sebastianes et al., 2013; Sebastianes et al., 2017b).

## 2. Diversity of Mangrove Endophytic Fungi

More than 200 species of endophytic fungi have been isolated and identified from mangroves, constituting the second-largest community of marine fungi (Liu et al., 2007). The main genera of endophytic fungi reported from mangroves include *Alternaria*, *Aspergillus*, *Cladosporium*, *Colletotrichum*, *Fusarium*, *Paecilomyces*, *Penicillium*, *Pestalotiopsis*, *Phoma*, *Phomopsis*, *Phyllosticta*,and *Trichoderma* (Liu et al., 2007; Cheng et al., 2009; Thatoi et al., 2013; Sebastianes et al., 2013). Several studies have been conducted to explore the fungal diversity of mangroves (Sarma and Hyde 2001, Nambiar and Raveendran, 2009; Alias et al., 2010), including the endophytic community associated with these trees, in countries such as Brazil, China, and India (Suryanarayanan et al., 1998; Kumaresan and Suryanarayanan, 2001; Ananda and Sridhar, 2002; Sebastianes et al., 2013).

In this context, endophytic fungi were isolated from the leaves of *Rhizophora apiculata* and *R. mucronata* Lamk., two typical mangrove plants in Southern India (Suryanarayanan et al., 1998). *Sporormiella minima*, *Acremonium* sp., and a sterile fungus were isolated from both plants. This was the first report of fungal endophytes in leaves of mangrove plants in India. In this geographic scenario and according to Suryanarayanan and Kumaresan (2001), four halophytes belonging to three dicotyledon families (*Acanthaceae*, *Aizoaceae* and *Chenopodiaceae*) were sampled from Pichavaram mangrove forest (Tamil Nadu, India) and screened for the presence of fungal endophytes. Thirty-six species of fungi, including some sterile forms, were isolated. *Camarosporium* species showed some degree of host preference because they were the dominant endophytes of the *Chenopodiaceae* halophytes. Some genera of endophytic fungi, such as *Colletotrichum*, *Phomopsis*, *Phyllosticta* and *Sporormiella*,were also isolated. Additionally, in India, Maria and Sridhar (2003) recorded twenty-five endophytic fungi (three ascomycetes, 20 mitosporic fungi, and two sterile fungi) in two mangrove plant species (*Acanthus ilicifolius* and *Acrostichum aureum*). The most dominant endophyte was *Colletotrichum* sp. in prop roots of *A. ilicifolius* and yeast sp. in rhizomes of *A. aureum*. Among the dominant endophytes, *Acremonium* and yeast sp. were common in both hosts. *Acanthus ilicifolius* had one dominant species (*Colletotrichum* sp.), while in *A. aureum* multiple species were dominant (*Acremonium* sp., *Penicillium* sp., and yeast sp.).

Ananda and Sridhar (2002) reported the community structure and diversity of endophytic fungi in roots of four mangrove plant species (*A. ilicifolius*, *Avicennia officinalis*, *Rhizophora mucronata*, and *Sonneratia caseolaris*)found on the west coast of India. The richness of endophytic fungal species from whole root segments was greatest for *R. mucronata*. Also, *Mycocentrospora acerina* (in *Avicennia officinalis*) and *Triscelophorus acuminatus* (in *R. mucronata* and *S. caseolaris*) were prevalent endophytic species. The greatest number of isolates, species richness, and diversity of fungi were found in whole root segments of *R. mucronata*.

In China, there are 27 mangrove plant species that belong to 16 genera and 12 families (Lin, 2001). Some of these species have been examined during biodiversity and distribution analyses of associated endophytic fungi on the coast of the Indian, Pacific, and Atlantic Oceans (Schmit and Shearer, 2003). According to Yang et al. (2006), among 290 strains of mangrove endophytic fungi obtained in the Fujian Province, China, *Alternaria*, *Cephalosporium*, *Dothiorell*, *Penicillium* and nonsporulating groups were the dominant genera. Also, Xing and Guo (2011) have been investigating the diversity of fungal endophytes isolated from Rhizophoraceae mangrove plant species, collected from the Hainan Province, in China. Two hundred ninety-five isolates were classified into 38 taxa using morphological characteristics. These 38 representative endophytes were identified using nuclear ribosomal DNA sequences, including ITS1, ITS2 and the 5.8S gene region. The results suggest that *Pestalotiopsis* and *Phomopsis* were the most frequent endophytes found in the host mangrove species.

Chaeprasert et al. (2010) examined the distribution of endophytic fungi in the leaves of mangrove forest trees growing in Thailand. A total of 3,900 leaf segments from 10 different hosts were screened for the presence of fungal endophytes. *Phyllosticta* was the most frequently isolated fungus from plants at all sites. The common fungal endophyte genera were *Cladosporium*, *Colletotrichum*, *Phomopsis*,and *Xylaria*.

Hamzah et al. (2018) isolated, identiﬁed, and characterized 78 fungal isolates from leaf tissues of *Rhizophora mucronata* trees growing in a Malaysian mangrove forest. In this study, an analysis of the nuclear ribosomal DNA internal transcribed spacer revealed the phylogenetic relationships of all isolates. Most of the dominant fungal endophytes were from *Pestalotiopsis*, followed by *Alternaria* and *Cladosporium*.

Osorio et al. (2017) investigated the occurrence of endophytic *Botryosphaeriaceae* throughout the distribution of mangroves in South Africa. Asymptomatic branches were collected from ten localities and six mangrove species. These authors identified 14 taxa belonging to four genera based on the DNA sequence data sets of four gene regions. Endophytic isolates resembling species of *Botryosphaeriaceae* were identified based on multi-gene sequence data of the internal transcribed spacer regions. These species include five new taxa (*Diplodia estuarina* sp. nov., *Lasiodiplodia avicenniae* sp. nov., *L. bruguierae* sp. nov., *Neofusicoccum lumnitzerae* sp. nov., *N. mangroviorum* sp. nov.) and nine known species (*D. sapinea*, *L. theobromae*, *L. gonubiensis*, *N. cryptoaustrale*, *N. kwambonambiense*, *N. luteum*, *N. parvum*, *N. umdonicol*, and *Botryosphaeria* sp.). Nothing was known regarding the diversity of endophytic *Botryosphaeriaceae* in mangroves in South Africa before this research.

According to Costa et al. (2012), to increase the knowledge about endophytic fungi a survey in leaves of the tropical mangrove species *Avicennia schaueriana*, *Laguncularia racemosa*, and *Rhizophora mangle* was conducted on Itamaracá Island, PE, Brazil. Leaves were collected during the dry and rainy seasons. Forty taxa were isolated: 25 species representing 19 genera and 15 morphotypes determined as *Mycelia sterilia*. *Guignardia* sp. and *Colletotrichum gloeosporioides* were the most frequent isolates, while *Glomerella cingulata* was the only species found in association with the three host plants. The similarity of fungi species between the two seasons reached only 4.2%, and that between the hosts was also low, with the maximum (*A. schaueriana* x *L. racemosa*) reaching 24.2%. *Sphaerosporium*, *Chloridium virescens* var. *virescens*, *Microsphaeropsis arundinis*, *Penicillium pinophilum*, *Periconia cambrensis*, *Phoma herbarum*, *P. diachenii*, *P. obscurans*, *Sordaria prolifica*,and *Torula elisii* were reported for the first time as endophytic in a tropical region.

Sebastianes et al. (2013) conducted a diversity study of endophytic fungi from branches and leaves of *A. schaveriana*, *L. racemose*, and *R. mangle* inhabiting two mangrove areas in São Paulo State, Brazil. According to the authors, the endophytic fungi were placed in 10 different morphological groups to allow a systematic selection of some isolates to be identified by sequencing the ITS1-5.8S-ITS2 region of rDNA. The results showed that these 334 ITS-sequenced fungi were represented by at least 35 genera. The phylum Ascomycota predominated among these fungi (99.42%). In contrast, the phylum Basidiomycota was represented by only 0.58% of the population evaluated. The most abundant genera were *Diaphorte* (22.67%), *Colletotrichum* (19.19%), *Fusarium* (11.34%), *Trichoderma* (8.72%) and *Xylaria* (7.56%). According to these authors, most of the genera identified in this study were soil fungi, indicating that they are also well adapted to the peculiar conditions found in mangrove forests.

## 3. Novel Bioactive Compounds of Mangrove Endophytic Fungi

A mangrove is an ecosystem situated in a transition area between terrestrial and marine environments, which makes it unique in parameters such as high salinity, tidal flooding, high temperatures, anaerobic soil, and amount of sludge. Thus, mangroves have become perfect ecosystems for the growth of plants and microorganisms that have ecological, morphological, biological, and physiological adaptations that allow them the survive under these particular conditions (Jalgaonwala et al., 2011; Sebastianes et al., 2012b; Sebastianes et al., 2013; Sebastianes et al., 2017a,b). In this context, endophytic fungi in mangroves grow in habitats with unique conditions that are attributed to the activation of metabolic pathways and the synthesis of distinct unknown molecules (Sebastianes et al., 2012a,b, 2017a; Moreira et al., 2020). Production of these compounds aids in supporting the adaptation and survival of the fungi in marine ecosystems (Fox and Howlett, 2008). Endophytic fungi live within various tissues of a host plant, asymptomatically, without causing any negative effect to the host plant (Aly et al., 2011). When a host plant harbors endophytes, their concurrence may help the host to adapt to biotic and abiotic stress factors (Hartley et al., 2015; Amin, 2016; Potshangbam et al., 2017).

Since the first endophytic fungus was identified, significant attention has been given to exploring for new bioactive compounds that these fungi synthesize (Sebastines et al., 2012a; Sebastianes et al., 2013; Sandhu et al., 2017; Strobel, 2018). This is an alternative to the exploitation of plants, helps preserve plant diversity, which is becoming increasingly scarce in the world, and reduces the market value of these biomolecules (Strobel and Daisy, 2003; Strobel, 2018; Bibi et al., 2020).

The broad spectrum of biological activity of endophytic fungal isolates allows, among other things, the exploration for substances with potential antimicrobial activity and production of enzymes, which can be obtained on a large scale through fermentation and have great potential for industrial applications (Dezam et al., 2017; Marques et al., 2018; Yan et al., 2018). Several studies note endophytic fungi that exhibit antimicrobial activity (Ratnaweera et al., 2015; Bezerra et al., 2015; Campos et al., 2015; Khan et al., 2016; Deshmukh et al., 2018) and enzymatic activity in several plants and mangroves from different regions (Maria et al., 2005; Thatoi et al., 2013; Bezerra et al., 2015; Maroldi et al., 2018; Martinho et al., 2019). Fungi from marine environments grow in particular conditions that can contribute to the synthesis of new compounds (Sebastianes et al., 2012a, 2017a). In this context, endophytic fungi associated with plants that inhabit mangrove ecosystems can be a promising source of secondary metabolites that might exhibit antimicrobial activity(Shukla et al., 2014; Sebastianes et al., 2017a; Moreira et al., 2020).

### 3.1. Fungal Endophytes: A Source for Pharmacologically Active Secondary Metabolites

#### 3.1.1. Antimicrobial Activity of Mangrove Derived Fungal Endophytes

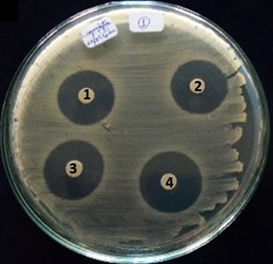
Drug-resistant microorganisms have also gained attention in recent decades, and studies estimate that over 700,000 deaths worldwide are caused by these pathogens each year (Bos and Austin, 2018). Considering studies developed by Allen et al. (2010), microorganisms can carry genes encoding resistance to the antibiotics they produce. Many important antibiotics in the pharmaceutical industry have been derived from microbial metabolites (Owen and Hundley, 2004; Huang et al., 2008). The emergence of antibiotic-resistant pathogens demands new antibiotic research and development strategies, and there is a need for new effective molecules that have low toxicity and a minimal environmental impact. Therefore, secondary metabolites continue to play a major role in active compound discovery and development (Yuan et al., 2006; Arnold et al., 2007; Huang et al., 2008; Mattheus et al., 2010; Sebastianes et al., 2012b). Indeed, a vast number of new molecules have been isolated from microorganisms, including endophytes (Zhong-Shan et al., 2009; Suryanarayanan et al., 2009; Sebastianes et al., 2012a; Sebastianes et al., 2017a).

The biochemical versatility and diversity of endophytes suggest that there are still many unknown active compounds with antimicrobial activity (Suryanarayanan et al., 2009; Strobel, 2018). The diversity of endophytic fungi and the compounds produced by them are severely influenced by the environment. Thus, some strategies can be adapted to search for new compounds, such as selecting plants with a known ethnobotanical history (e.g., medicinal plants) and selecting environments with high biodiversity (Golinska et al., 2015).

Endophytic bioprospecting promotes the discovery of natural products with therapeutic value (Kusari and Spiteller, 2011; Strobel, 2018). Other aspects that arouse interest in endophytes are the diversity of biological molecules produced and the low risk of these molecules being cytotoxic, since symbiotic and mutualistic relationships do not cause damage or death to the host plant, which is of great importance for drug manufacturing (Strobel and Daisy, 2003; Bibi et al., 2020; Moreira et al., 2020).

There has recently been increasing interest in studies about endophytic microorganism molecules with pharmaceutical properties. Indeed, a vast number of new molecules with antimicrobial activity have been isolated from endophytic fungi (Cheng et al., 2009; Zhong-Shan et al., 2009; Suryanarayanan et al., 2009; Sebastianes et al., 2017a; Strobel, 2018). Most endophytic fungi have a wide range of hosts, and only a few have a single host. The colonization of endophytic fungi varies in different parts of the host plant (leaves, twigs, and stems), according to the age of the host plant and the season. The endophytic fungi of mangroves can produce many types of metabolites with great potential for antimicrobial use (Liu et al., 2007; Sebastianes et al., 2012a; Sebastianes et al., 2017a; Bibi et al., 2020; Moreira et al., 2020).

In this context, Bezerra et al. (2015) tested 32 endophytic fungi isolated from *Bauhinia forficata* against 10 pathogenic bacteria, in disk diffusion assays. Of the 32 isolates, 11 exhibited antibacterial activity against one or more pathogen. Six endophytic fungi presented consistent antimicrobial action against *S. aureus* and the genus *Salmonella*. Campos et al. (2015) isolated 82 endophytic fungi from *Caesalpinia echinata* and tested their ethyl acetate extracts. Three endophytes exhibited activity against *E. coli* and *S. aureus*, where minimuminhibitory concentration (MIC) values varied between 32 and 64 µg/mL, and one exhibited activity against *Salmonella* bacteria, where the MIC value was 64 µg/mL. Ratnaweera et al. (2015) isolated eight endophytic fungi from *Opuntia dillenii*, which showed antibacterial activity for at least one of the tested bacteria, including *E. coli* and *S. aureus*. Also, Khan et al. (2016) isolated the endophytic fungus *Cladosporium* sp. from *Rauwolfia serpentina*, and its extract exhibited antibacterial activity against *E. coli* and *S. aureus*, among other bacteria.



**Figure 1**. Screening of antimicrobial activity from crude extracts of mangrove endophytic fungi against the pathogenic bacteria *Staphylococcus saprophyticus*. Crude extracts of 1) *Phomopsis* sp. 26.1 (1) strain; 2) *Phomopsis* sp. 29.5 (1) strain; 3) *Diaporthe* sp. 44 (3) strain, and 4) *Diaporthe* sp. 5.1 (1) strain. All crude extracts were tested at 40 mg/mL. Source: adapted from Moreira (2016).

Moreira (2016) reported the initial screening of endophytic fungi isolatedfrommangroves(*Avicennia nitida*, *Laguncularia racemosa*, and *Rhizophora mangle*) in Bertioga and Cananéia, São Paulo State, Brazil, for antibacterial activity on the human pathogens *Candida albicans*, *E. coli*, *S. aureus*, *S. saprophyticus*, and *Pseudomonas aeruginosa*. The crude extracts of endophytic genera tested for antimicrobial activity against the pathogens were *Diaporthe* sp. and *Phomopsis* sp. (Figure 1). Also, Moreira et al. (2020) evaluated the crude extract of the *Diaphorte* sp. FS-94(4) strain (https://www.ncbi.nlm.nih.gov/nuccore/HQ022906.1), isolated from *Avicennia nitida* (Sebastianes et al., 2013), against *E. coli* (ATCC 25922), *Salmonella enteritidis* (ATCC 19196), *S. aureus* (ATCC 6538), and *C. albicans* (ATCC 10231) for antimicrobial assays. The endophytic *Diaporthe* strain exhibited promising antimicrobial activity against *E. coli*, *S. aureus*, and *S. enteritidis*.

Table 1. Secondary metabolites with antimicrobial activity produced by endophytic fungi from mangroves and their target strains

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Host Plant** | **Endophyte** | **Metabolites/Extract** | **Target Strain** | **Reference** |
| *Excoecaria agallocha* | *Phomopsis* sp. ZSU-H76 | Cyrosporone B and C | *C. albicans* | Huang  et al. (2008) |
| *Sonneratia alba* | *Alternaria* sp. | Xanalteric acids I and II;  Altenusim | *S. aureus* (MRSA)  *S. aureus* (MRSA); *S. pneumonia*; *E. faecium*;  *E.* cloacae; *A.* faecalis; *C. albicans* | Kjer  et al. (2009) |
| *Thespesia populneoide*  *Acanthus ilicifolius* | *Cladosporium* sp.  *Xylaria* sp. | Ethyl acetate extract  Ethyl acetate extract | *S. aureus* (ATCC 25923)*; B. subtilis* (ATCC 6633)*; P. aeruginosa* (ATCC 27853)*; E. coli* (ATCC 25922)  *P. aeruginosa* (ATCC 27853)*; E. coli* (ATCC 25922) | Chaeprasert  et al. (2010) |
| *Kandelia candel* (L.) Druce | *Nigrospora* sp. 1403 | Bostrycin; and deoxybostrycin | *S. aureus* (ATCC 27154)*; E. coli* (ATCC 25922); *P. aeruginosa* (ATCC 25668); *S. ventriculi* (ATCC 29068); *B. subtilis* (ATCC 6633)*; C. albicans* (ATCC 10231) | Xia  et al. (2011) |
| *Laguncularia racemosa* | *Diaporthe phaseolorum* | 3-Hydroxypropionic Acid | *S.* aureus; *Salmonella typhi* | Sebastianes  et al. (2012a) |
| *Avicennia officinalis* | *Schizophyllum commune* | Ethyl acetate extract | *V. cholerea*; *M. leteus*; *S. aureus* | Joel and Bhimba (2013) |
| *Acanthus ilicifolius* | *Aspergillus flavipes* AIL8 | Flavipesins A | *S. aureus*; *B. subtilis* | Bai et al. (2014) |
| *Rhizopora mucronata* | *Penicillium* sp.  *Penicillium* sp.  *Ampelomyces* sp. | Metanol extract | *S. aureus* (ATTC 9144)  *E. coli* (ATCC 8739) | Prihanto  et al. (2011) |
| *Premna serratifolia* L. | *Hypocrea virens* | Gliotoxin | *B. subtilis* (UBC 344); *S. aureus* (ATCC 43300); *S. aureus* (MRSA, ATCC 33591); | Ratnaweera  et al. (2016) |
|  |  |  | *E. coli* (UBC 8161); *P. aeruginosa* (ATCC 27853); *C. albicans* (ATCC 90028) |  |
| *Avicennia nitida* | *Hypocrea virens* | Viridiol | *E. coli* | Sebastianes et al. (2017a) |
| *Bruguiera gymnorrhiza* | *Penicillium* sp. GD6 | 2-deoxy-sohirnone C | *S. aureus* (MRSA) | Jiang et al. (2018) |
| *Avicennia nitida* | *Diaphorte* sp.  FS-94(4) | Crude extract | *E. coli* (ATCC 25922); *S. enteritidis* (ATCC19196); *S. aureus* (ATCC 6538) | Moreira et al. (2020) |

Recently, Bibi et al. (2020) published an extensive review about the different compounds produced by various endophytic fungi in different mangrove species and elaborated on the pharmacological activities that the fungal endophytes possess. This review attempts to provide a comprehensive account of the mangrove fungal endophytic community by compiling research and review articles. It discusses works about antimicrobial properties and focuses on the potential of metabolites from many mangrove fungal endophytes against pathogenic microbes of humans.

Table 1 provides an overview of selected studies about antimicrobials produced by endophytic fungi isolated from mangrove plants against drug resistant and multidrug-resistant microorganisms.

#### 3.1.2. Antiparasitic Activity of Mangrove Derived Fungal Endophytes

Neglected tropical diseases (NTDs) are caused by infectious agents and parasites and mainly reach populations living in tropical and subtropical countries with precarious health clinics, poverty, food insecurity, and poor housing conditions and access to healthcare (Who, 2015).

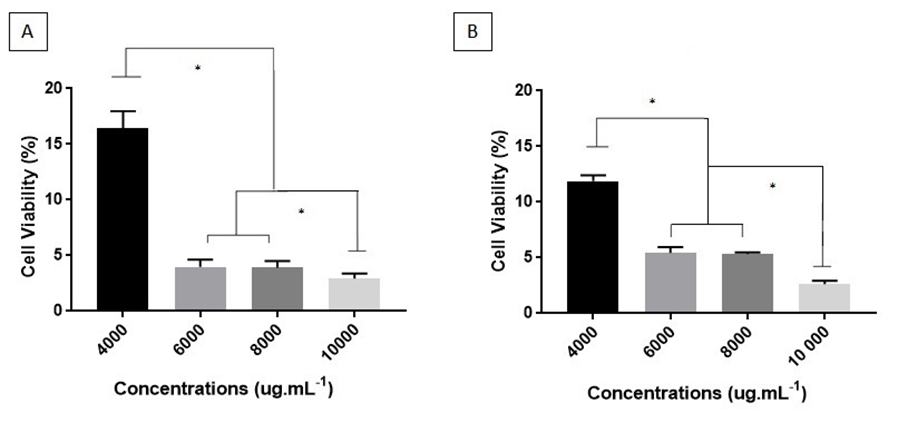
Although progress has been made at controlling and eliminating some NTDs in recent years (Hotez and Aksoy, 2017; Molyneux et al., 2017), 2.1 billion people continue to use water from inadequate sources, and 4.5 billion people live without basic sanitation conditions (Who, 2017). Thus, NDTs remain a serious social health and economic problem in many parts of the world, affecting more than 1 billion people living in the most marginalized communities in 149 countries, of which the vast majority are in Africa, Asia, and the Americas (Who, 2020). This scenario is further aggravated by the limitation of available drugs, which may have low efficacy and/or high toxicity, and also by the emergence of resistant pathogens (Nwaka and Hudson, 2006).

The emergence of drug-resistant protozoa parasites, such as *Leishmania* spp., *Trypanosoma* spp. and *Plasmodium* spp., represents an important threat to health, leading to significant rates of morbidity and mortality and impacts on the economy of the world (Andrews et al., 2014; Varikuti et al., 2018). This reinforces the importance of studies that contribute to discovering new drugs for the treatment and control of NTDs (Cheuka et al., 2017).

Mangrove endophytic fungi are a rich source of natural products with numerous potential therapeutic applications, inspiring the discovery of novel drugs to overcome the increasing threat of drug-resistant pathogens (Hamzah et al., 2020; Sebastianes et al., 2012a, 2017a).

In this context, Moreira et al. (2020) evaluated crude extracts of the *Diaphorte* sp. FS-94(4) strain, an endophyte isolated from *Avicennia nitida* in mangrove areas of São Paulo State, Brazil (Sebastianes et al., 2013), against promastigote forms of *Leishmania infantum chagasi* (MHOM/BR/1972/LD). The data showed a high mortality rate at a dose of 4.000 μg.mL-1 for 24 and 48 hours of exposure. The study also demonstrated lower cytotoxic effects of crude extracts on cultured human skin fibroblasts (HFF-1cell line) than that found in other works, suggesting that metabolites of *Diaphorte* sp. FS-94(4) are promising in studies about cytotoxicity in a murine model and the discovery of a new drug to control human visceral leishmaniasis (Figure 2).

Aiming to study novel antitrypanosomal medicines, Mazlan et al. (2020) studied natural products from endophytes from the mangrove plant *Avicennia lanata* collected in Malaysia. The endophytic fungus *Lasiodiplodia theobromae* produces dihydroisocoumarins that exhibit significant activity against *Trypanosoma brucei* and low cytotoxicity against normal prostate cells (PNT2A).



**Figure 2**. Evaluation of the viability of promastigotes of *Leishmania infantum chagasi* in different concentrations of CE from *Diaporthe* sp. strain through assay Alamar Blue®. (A) Viability of L. infantum chagasi in 24 hours. ®. (B) Viability of *L. infantum chagasi* in 48 h. It was observed a high leishmaniasis activity at all concentrations and times tested and the concentrations of 6.000 to 10.000 µg•mL-1 different statistically from the concentration of 4.000 µg.mL-1. ANOVA followed by the Tukey’s test were used to determine statistical significance (\*, *p* ≤ 0.05). Source: adapted from Moreira et al. (2020).

New antimalarial compounds have also been the subject of studies. Kyeremeh et al. (2017) studied the secondary metabolites from the *Cladosporium oxysporum* BRS2A-ARF2 strain, an endophyte of the Ghanaian mangrove plant *Rhizophora racemosa*. The metabolite identified as quinoloctacin A2 exhibited activity against the chloroquine-sensitive *Plasmodium falciparum* 3D7 strain within the concentration range tested with an EC50 value of 24.80 µM. The compound also affected the mitochondrion membrane potential of the parasite, suggesting its potential as a drug development scaffold for apoptotic death in the development stages of *P. falciparum*. Additionally, Calcul et al. (2013) investigated endophytic fungi from Hong Kong and Taiwan mangrove plants as a source of new antimalarial compounds. The authors demonstrated that the compound dicerandrol D produced by *Diaphorte* sp. had *in vitro* activity against *P. falciparum* with at least 10-fold less cytotoxicity than their target criteria of nanomolar malaria activity.

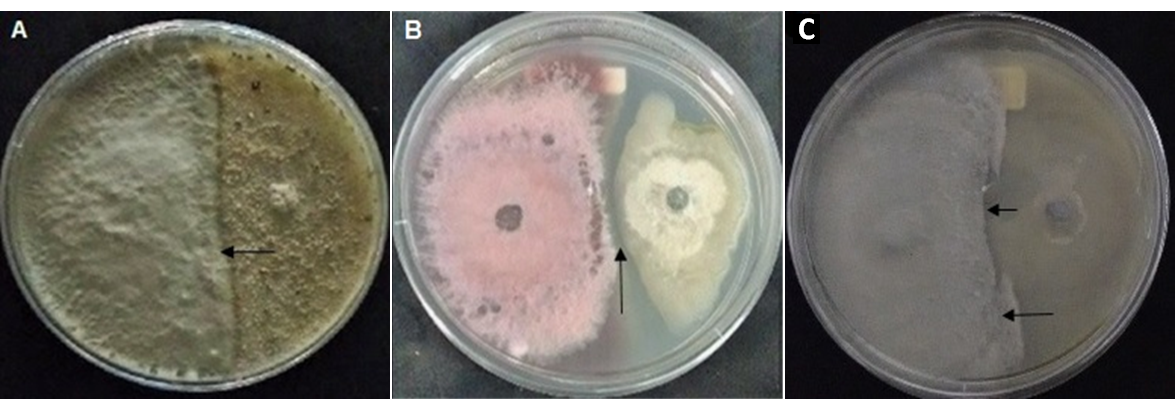
These and other studies (Demers et al., 2018; Wang et al., 2012) demonstrate the chemical and functional diversity of natural products from mangrove-associated endophytic fungi and point out new perspectives on the control of parasites and related NTDs.

#### 3.1.3. Antagonist Activity of Mangrove Endophytic Fungi against Phytopathogens: Biocontrol Potential

Endophytic fungi are promising biocontrol agents (Azevedo et al., 2000; Latz et al., 2018; Saad et al., 2019). Control of phytopathogens can occur when endophytes compete directly with them for space and nutrients and/or indirectly through the production of lytic enzymes (e.g., proteases, chitinases, lipases, and cellulases), siderophores molecules, antimicrobial activity, and induction of plant defenses (de Silva et al., 2019; Busby et al., 2016; Orlandelli et al., 2015).

The biological control method using endophytic fungi capable of inhibiting or antagonizing phytopathogens seems to be a more eco-friendly and sustainable tool in agricultural management than using fertilizers and agrochemicals that negatively impact the environment, humans and other organisms ([Tewari](https://www.researchgate.net/profile/Sakshi_Tewari) et al., 2019; Orlandelli et al., 2015). Biocontrol activities of endophytic fungi isolated from mangrove plants have been reported in some studies.

Recently, Moreira et al. (2020) evaluated the antagonistic potential of the *Diaphorte* sp. FS-94(4) strain isolated from *Avicennia nitida* (Sebastianes et al., 2013) against the phytopathogens *Colletotrichum* sp., *Fusarium oxysporum*, *Phytophthora sojae*,and *Rhizopus microspores*. The antagonism index (AI) ranged from 20 to 62% with type A interactions (deadlock with mycelial contact) and CA1 (partial replacement after the initial deadlock with mycelial contact) prevalent. Some of these types of antagonistic interactions can be verified in Figure 3 (Moreira, 2016). The endophyte produced *in vitro* cellulase, suggesting that this enzyme is involved in suppressing the phytopathogens by breaking down the cell wall.



**Figure 3**. A. *Ceratocystis paradoxa* versus *Diaporthe* sp. B *Fusarium oxysporum* versus *Phomopsis* sp. C. *Colletotrichum* sp. versus *Phomopsis* sp. The arrows indicate inhibition zones between phytopathogens and endophytes. Source: adapted from Moreira (2016).

Abro et al. (2019) evaluated the biocontrol potential of endophytic fungal species from mangrove plants (*Rhizophora stylosa*, *Aegiceras corniculatum*, *Kandelia candel*, *Bruguiera gymorhiza*) collected in China against *Fusarium oxysporum* f. sp. *cucumerinum*. Using an *in vitro* dual culture assay, the authors verified growth inhibition percentages of the phytopathogen between 28.4% to 47.3% compared to control treatments. Also, the endophytes *Guinardia mangifera* and *Pestalotiopsis humus* reduced disease severity in cucumber plants infected with *F. oxysporum*, promoting an increase in plant height and fresh and dry weight. These results suggest that fungal endophytes from mangroves have the potential to be employed both as biocontrol agents and as plant growth promoters.

A study conducted by Hamzah et al. (2018) evaluated the antagonistic activities of six endophytic fungi (*Xylaria* sp., *Fusarium lateritium*, *Nigrospora oryzae*, *Phoma* sp., *Pestalotiopsis* sp., and *Alternaria macrospora*) isolated from *Rhizophora mucrunata* in a Malaysian mangrove forest. From dual culture plates, inhibition percentages between 45– 6% were observed against *Fusarium solani*, as well as 0.8–23% when using a non-volatile test assay. Different endophyte-*F. solani* interactions were observed: *F. lateritium* and *N. oryzae* exhibited a type B interaction (mutual inhibition on contact) when cultured with the phytopathogen; *A. macrospora* and *Pestalotiopsis* sp. showed a type F interaction (endophytes were inhibited by the pathogen) when co-cultivated with *F. solani*; *Phoma* sp. showed a type E interaction with *F. solani* (growth of endophyte was unaffected, while that of the pathogen was reduced); in a *Xylaria* sp.-phytopathogen interaction, the mycelium of the endophyte had branched into the pathogen´s colony; and a small clear zone (> 2 mm) was verified in an *N. oryzae-F. solani* interaction, suggesting inhibition growth by production of hydrolytic enzymes.

In the same way, one hundred fifty endophytic fungi isolated from mangrove leaves in the eastern part of Thailand were tested using a dual culture method against phytopathogens. The endophytic strain BUEN 880 showed better inhibition percentages, with 41.7% for *Alternaria brassicicola* DOAC 0436, 30.8% for *Colletotrichum capsici* DOAC 1511, 37.5% for *C. gloeosporioides* DOAC 0782, 40.0% for *Fusarium oxysporum* DOAC 1808, and 33.3% for *Pestalotiopsis* sp. DOAC 1098. The authors also tested extracts from a BUEN 880 culture filtrate against phytopathogens using the disc diffusion method and verified inhibition at a moderate level with an equal distance of 4 mm for *C. capsici* 1511, *C. gloeosporioides* DOAC 0782, and *Pestalotiopsis* sp. DOAC 1098 (Khrueayu and Pilantanapak, 2013).

All studies cited here were carried out to increase what is known about endophytic fungi from mangroves, opening several possibilities for their application in the control and prevention of plant diseases.

## Conclusion

The microbial world and endophytes in particular exhibit vast genetic biodiversity and metabolic biodiversity that have not been thoroughly explored. Mangrove endophytic fungi open up new areas of potential biotechnological exploitation for the pharmaceutical and medical fields (Zhong-Shan et al., 2009; Suryanarayanan et al., 2009). Also, the great biodiversity observed in the mangrove ecosystem reinforces the importance of studying endophytes, particularly the isolation of new compounds with biological activity, such as antibiotics and antileishmanialdrugs(Sebastianes et al., 2017a; Moreira et al., 2020).

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Chapter 3

# Arbuscular Mycorrhizal Fungus and Its Positive Effects on Ornamental Plants

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## Abstract

Arbuscular mycorrhizal (AM) fungiare widely distributed in land ecological soil and have been playing positive roles to plants. Considering the main application types of ornamental plants, that is using for cut flower and embellishing urban landscape, ornamental plants have to face the adverse environments. In this case, AM fungal colonization may have considerable potential capacity when it comes to ameliorating the harmful effects of biotic and abiotic stresses, maintaining better performance, which is of critical importance for building up the “*Beautification of the Chinese Environment*” through low disturbance to natural ecosystem and environment-friendly way to attain the project of ornamental plants development. In this chapter, all the application of AM fungi in ornamental plants are enumerated, including the positive effects granted to plant growth, flower outputs, quality of cut flower, enhanced resistance to adverse factors such as drought, saline soil conditions, heavy metal contamination, high/low temperature stresses as well as pathogenic challenges. Although the related trials can not sufficiently address present ornamental production needs and the trial plants involved are limited, the development of AM fungi to ornamental plants trade may be regarded as having a promising prospect both in ornamental plant cultivation and application. Nonetheless, more concrete trials should be carried out according to the unique requirements of ornamental traits.

**Keywords**: AM fungi, ornamental plants, growth, nutrient absorption, quality, resistance

## 1. Introduction

With the improvement of the quality of life, both the gross demand for flower products and the quality requirements for these products has risen. Specific quality requirements include the maximizing the nutrition and health benefits of edible flowers, as well as improving the fragrance, color, and vase life of cut flowers. All of these new requirements translate into higher requirements for not only breeders but also planters. Innovation of cultivation technology and associated concepts necessarily must be aided through the experimental results of scientific researchers. Addressing the current situation in flower cultivation, one must face the overdosed application of inorganic fertilizers, pesticides, and antibiotics in ornamental plants that are widely used in disease and pest control. Although the yield and quality have been improving with demand, the severe consequences to water, soil and atmosphere wrought by excessive interference with the ecological environment, must be mitigated if there are to be sustainable and high quality flower outputs in the long term (Zaki et al., 2020). It is thus no surprise that environmentally friendly cultivation techniques have become one of the more promising trends worldwide (He, 2021).

AM fungi are widely distributed in the soil and have established a symbiotic relationship with more than 80% terrestrial plants. AM fungi constitute a major component of the agroecosystem which can contribute a lot to plant production and ecosystem regulations (Sumbul et al., 2017; Saia et al., 2020). The importance of AM fungi has been realized from literatures that documented their associations which promoted the formation of better soil structure, absorption of macro and micro necessary nutrients, the amelioration of the poor fertilizer absorption, the development of shoots and roots, plant resistance to biotic and abiotic stresses, and the realization of features with ornamental value, such as flower diameter, numbers of petal and so on (Huang et al., 2019). At present, more and more research on the effect of AM fungi on plants has been focusing on agricultural and ornamental plants. Among which the most concerning aspects are hoping to enhance plant resistance and increase yield through symbiosis with AM fungi. As for ornamental plants, more attention should be paid to ornamental features such as the optimization of flower quality via a decrease in transportation-related losses and cost and the prolonging of shelf life or vase life. Additionally, studies of the effects of AM fungi on cut flowers from *Tulipa gesneriana* and *Lilium brownii* were completed in 2018 and 2019 respectively, and results showed that AM fungi could prolong the flowering period and delay the senescence of cut flowers. Meanwhile, stress resistance also merits attention. After building a symbiotic relationship with *Rosa hybrid* and *Chrysanthemum morifolium*,AM fungi displayed tolerance to temperature stress and improved host endurance (Kong et al., 2011). In 2020, a study about the effects of different AM fungi was carried out on *Trifolium repens*, *Zinnia elegans*, *Tagetes erecta*, *Callistephus chinensis*, *Dianthus plumarius*, and *Silene pendula*, and found that AM fungi improved the survival rate of some plants outside of the *Dianthus* genus. At the same time, those above mentioned six species had no obvious preference for AM fungi species, showing similar infection rates across the board (Pang, 2020).

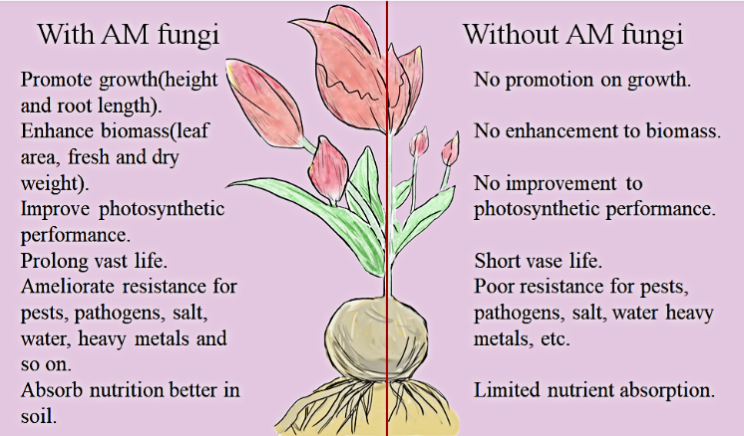
Most of the research findings showed that AM fungi have great potential in improving the yield and quality of ornamental plants, which may provide new ideas and methods for the ornamental trade under conditions of minimal environmental interference. How exactly AM fungi can be practically utilized in production still needs further exploration and observation.

## 2. Positive Roles of AM Fungiin Ornamental Plants

In the present body of research, AM fungi have been presenting a lot of positive effects on host plants, which produce better host robustness, and excellent quality in final outputs, demonstrated in longer vase life and fewer pollutants present in flower tissue and other edible organs. The positive influences of these fungi are also shown through better nutrition absorption and availability in living plants, enhancing their adaptability to biotic and abiotic adversity, improving photosynthetic performance (Figure 1).

### 2.1. Promotion of AM Fungi on Vegetative Growth

Firstly, the biomass of plants increased significantly after colonization, mainly in leaf area, plant height and dry weight. The plant height, stem diameter, dry weight, leaf area and other biomass of *Lilium brownii* were higher than those of non-inoculation, the chlorophyll content of leaves was significantly increased, the activity of antioxidant enzymes was enhanced, and the net photosynthetic rate and stomatal conductance were significantly promoted as well, but the malondialdehyde (MDA) content was reduced by 30% when compared with control (Liu et al., 2017). In addition, the chlorophyll content, intercellular carbon dioxide concentration, stomatal conductance and net photosynthetic rate in mycorrhization plants were higher than non-mycorrhization ones. Meanwhile, the activities of antioxidant enzymes such as superoxide dismutase (SOD) and peroxidase (POD) increased significantly, and the content of MDA was decreased markedly following the construction of a symbiotic relationship.



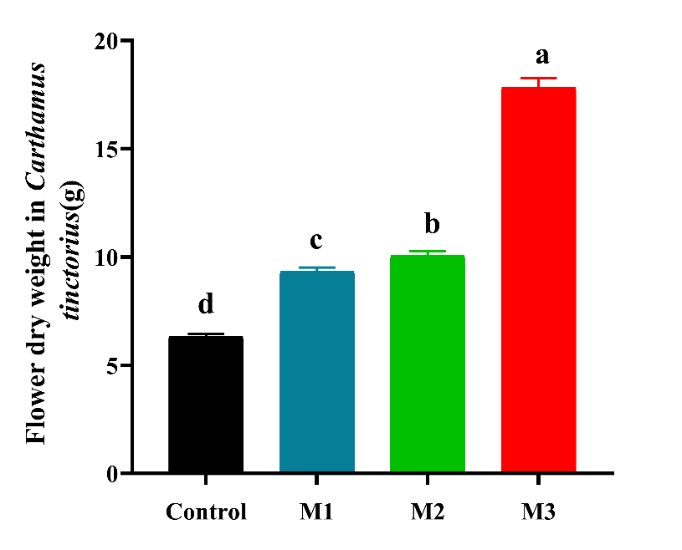
**Figure 1.** Comparative representation displaying the beneficial role of arbuscular mycorrhizae fungi in growth-promoting, biomass enhancing, improving, longer vase life and ameliorating plant defense.

Secondly, arbuscular mycorrhiza has a positive effect on root development. The forming of symbiont has great benefits to host. For one thing, AM-plant symbiosis can promote root system development, meaning an increase in root length and surface area, the absorption efficiency of roots growing, keeping higher activity and performance in root cells, better the absorption of soil water and nutrients by enlarging root structure (Huang et al., 2019), such as a research result showed in *Tulip heterophylla,* the average root diameter, total root volume and root length increased by 78%, 19% and 84% respectively, and the content of nitrogen, phosphorus and potassium (N, P and K) in plant were significantly higher than those in control (Sun et al., 2019). For another, well-developed hyphal network makes up for the limited root length of ornamental plants that can’t expand down to deeper soil layers, which can achieve more optimal nutrition and water uptake. At the same time, the hyphal network makes much better effect on nutrition elements such as phosphorus. In addition, the exudates of hyphal network might have effect on the conversion from unusable nutritional forms to usable forms in given plants. Thus AM-plant symbiosis can promote vegetative growth through bettering the water and nutrient utilization, keeping host in improved fitness.

### 2.2. Accelerating Production and Ornamental Values

It is essential that the accelerating potential of AM fungi on flower production is understood. AM fungi increased flower dry weight (FDW) in mycorrhizal *Carthamus tinctorius* (Zhao et al., 2019), for which the FDW of plants was respectively found to be 1.476, 1.59 and 2.82 times greater in M1, M2 and M3 groups than the control FDW 6.318g (Figure 2). Both single and consortium inoculation can increase flower production in *C. tinctorius.* Furthermore, the consortium of AM fungi was proved to be the most outstanding quality, compared with other single inoculations. While the fungi can have a significant positive effect, AM fungi do not universally demonstrate exclusively positive effects in all respects. As illustrated in the work of Zhao et al. (2019), there was no change in desired pharmaceutical components in *C. tinctorius*, yet the positive effect on flower dry weight was very remarkable. So, there is still much to explored when it comes to the application of AM fungi in flower cultivation.

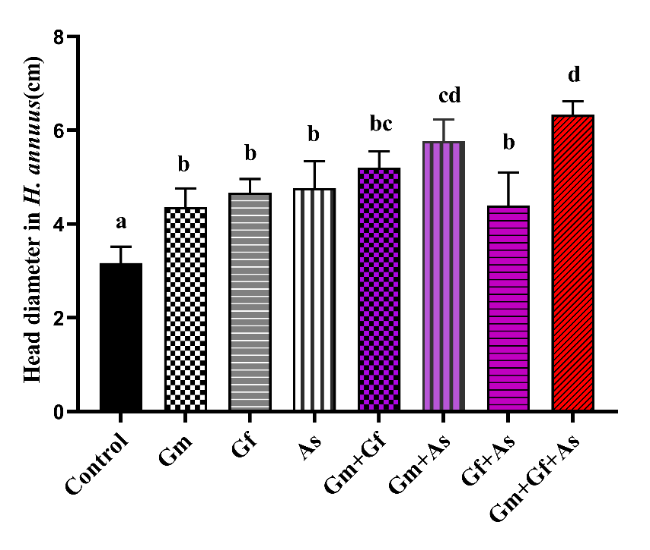
AM fungi can not only promote the growth of plants and prolonged vase life for cut flowers, but also make the flowering to be earlier, slower flower aging. In one trial, samples of *Tulipa gesneriana* were colonized by *F. mosseae* and *C. etunicatum.* Respectively, the blossom days increased by 83% when the former fungus was applied and 29% when the latter was. From the initial stage to the peak blossom stage, the vase life increased by 36% and 11%, respectively, while the ornamental duration was prolonged by 39% and 15% again respectively. Finally, the flowering period was prolonged by 5 days and 3 days using these different fungi (Li et al., 2018). Compared with the control group, the ethylene release rate decreased 30% while the respiration rate decreased by 37% in cut flowers of mycorrihizal *Lilium brownii* plants. Furthermore, the vase life prolonged 3 days in *L. brownii* inoculated with AM fungi, and its ornamental period was 2 days longer than control (Li et al., 2019).



**Figure 2.** Effect of AM fungi on yield of flower dry weight (g). M1 and M2 indicate inoculation with *G. mosseae* and *G. intraradices* respectively. M3 is consortium inoculation of *G. mosseae*, *G. etunicatum*, *G. micoragregatum*, *G. caledonium*, *G. cladoidem* and *G. intraradices.* Lowercase letters above the column show the significance at the 0.05 level.

Other than ornamental duration/vase life, there are also some important traits such as flower diameter, volatile compounds in flowers or inflorescence present much better in mycorrihizal plants than uncolonized control samples. After 85 days of AM fungi inoculation, the head diameter of *H. annuus* was higher significantly compared with the not colonized group. In all of the inoculation treatments, head diameter was bigger than control, and the consortium colonization showed better effect than single fungus colonization with the exception of Gf +As (Figure 3). There were some trials showing that AM fungi may alter the terpenoid content of flowers. The existence of a good symbiosis relationship between *Isodon adenanths* and AM fungi, which has significant promotion on the content of terpenoids in the aerial part of *I. adenanths,* has been demonstrated in one trial(Han et al., 2013). And in mycorrhizal *Medicago truncatula*, the volatilizaion of β-pinene, phenyle-thanone, 3-hexanone and octadecanoid acid were promoted (Dreher et al., 2019). In addition to raising the biomass and nutrition absorption, the linalool emission was increased also in *Ocimum basilicum* inoculatedwith AM fungi (Prasas et al., 2011).

At present, AM fungi have also been used in the research of seedling production, with the goal of accelerating the growth of plants and obtaining improved varieties through the positive effect of AM fungi on plants. Qingdao Agricultural University has developed a method of cultivating mycorrhizal seedlings (Patent No.: 201210251884.9). Using this method, which is based on the principle of anvil strip breeding, the AM fungi in this method is inoculated with sawdust matrix, and then the mycorrhizated rootstocks of fruit trees were achieved (Gu et al., 2020).



**Figure 3**. Effect of AM fungi application on head diameter in *H. annuus*. Gm, Gf and As indicate *Glomus mosseae*, *Glomus fasciculatum* and *Acaulospora scrobiculata.* Gm + Gf, Gm +As, Gf +As, and Gm+Gf+As represent consortium inoculation using two or three of the above three AM fungi (Kavitha and Nelson, 2014, with the minor modification).

Table 1. AM fungi and their role in ornamental plant growth promotion and keeping better quality

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| **Ornamental Plants** | **Mycorrhizal species** | **Host performance and stress tolerance** | **References** |
| *Lilium brownii* | *Glomus mosseae*  *G*. *versiforme* | Increased growth and improved leaf chlorophyll content，superoxide dismutase peroxidase and catalase content; Promoted leaf net photosynthetic rate，transpiration rate and stomatal conductance. | Liu et al., 2017 |
| *Paeonia suffruticosa* | *G*. *mosseae*  *G*. *versiforme* | Improved growth, absorption of mineral elements and heat resistance. | Li, 2009 |
| *P*. *suffruticosa* | *G*. *mosseae* | The mycorrhiza dependence was higher in annual seedling than biennial. Showed positive effect on total dry weight, salt tolerance coefficient and nutrients uptake of N, P, K. | Guo et al., 2013 |
| *Iris* | *G*. *mosseae*  *G*. *intraradices* | The absorption of N, P increased by 71.75%, 42.55%, 8.36%, 9.5% respectively after inoculation of G. mosseae and G. intraradices. | Chen et al., |
| *Trifolium repens*  *Zinnia elegans*  *Tagetes erecta*  *Callistephus chinensis*  *Dianthus plumarius*  *Silene pendula* | The rhizosphere soil of *Leymus chinensis, Agropyron cristatum,* and  *Artemisia frigida* | Promoted growth in non Caryophyllaceae host.  No significant effect on Caryophyllaceae for LC inoculant.  AC inoculant inhibited the photosynthetic rate of *D. plumarius* significantly, but yielded an outstanding promotion of *T. repens* and *T. erecta.* | Pang, 2020 |
| *Tulipa heterophylla* | *Acaulospora laevis*  *A. Appendicula*  *colossica* | Increased biomass and promoted growth; Improved the water balance of cut flower, and prolong the lifespan, better viewing period, and florescence effectively. | Sun et al., 2014 |
| *Rosa hybrid* and *Chrysanthemum morifolium* | *G*. *diuphauam,*  *G. etunicatum, G. intraradices,*  *G. mosseae, G. versiforme* | Improved growth and alleviated both high and low temperature stresses. | Kong, 2011 |

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| --- | --- | --- | --- |
| **Ornamental Plants** | **Mycorrhizal species** | **Host performance and stress tolerance** | **References** |
| *Rhododendron cultivars*  ‘Bizhi’  ‘Taoban Zhusha’ | *G*. *intraradices*  *G. mosseae*  *G. etunicatum* | The anatomic structure of leaves inoculated with AM fungi was less damaged, and the ratio of leaf palisade sponge tissue was significantly higher than control in high temperature condition. | Wang, 2019 |
| *Tulipa heterophylla* | *G. mosseae*  *Claroidelglomus etunicatum* | Prolonged the life of cut flowers and slowed down their aging. | Li et al., 2018 |
| *Lilium brownie* | *Funneliformis mosseae*  *G. versiforme* | Prolonged the vase life and slowed down their aging. | Li et al., 2019, |
| *Lilium brownie* | *G. mosseae*  *G. versiforme* | AM fungi inoculation can alleviate the high temperature stress partly, and the consortium inoculation was better than single. | Xing et al., 2018 |
| *Hyacinthus orientalis* | *Diversispora spurca*  *G. versiformis*  *G. mosseae* | Prolonged florescence days. | Xie et al., 2018 |
| *Ficus elastica* | *G. mosseae* | Significantly Increased growth and biomass. | Lu et al., 2007 |
| *Clematis intricata* | *G*. *mosseae* | Promoted the growth and the accumulation of total flavonoid content. | Liu, 2017 |
| *Medicago sativa* | *G*. *etunicatum*  *G*. *mosseae*  *G*. *intraradices* | Enhanced the resistance to salt stress. | Zhang et al., 2018 |
| *Lagerstroemia indica* | *F*. *mosseae* | Enhanced the resistance to salt stress. | Yang et al., 2014 |
| *D. caryophyllus* L. cv. Kazan | *G*. *intraradices* | Colonization of AM fungi enhanced the salt tolerance and the ornamental value of carnation in the size of flower, leaves, and the flower color. | Navarro,2012 |
| *Lantana camara* | *G*. *intraradices* | AM fungi inoculation improved the Cd accumulation in *L. camara*, but the consortium of AM fungi and earthworm had no significant function on Cd absorption. | Fu et al., 2018 |

Table 1. (Continued)

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| --- | --- | --- | --- |
| **Ornamental Plants** | **Mycorrhizal species** | **Host performance and stress tolerance** | **References** |
| *Lavandula angustifolia* | *F*. *mosseae*  *G*. *versiforme*  *Fm+Gv* | Increased the tolerance to high temperature through enhancing antioxidant enzyme activity, in this regard the consortium colonization was better than single inoculation. | Xing et al., 2019 |
| *Orychophragmus violaceus, solanum nigrum*,*ophiopogon japonicu* | *F*. *mosseae*  *G*. *intraradices* | Improved growth and the absorption of Pb and Cd. | Chen et al., 2017 |
| *Populus × canadensis* | *G*. *intraradices* | to maintain water and reduce water deficit when suffering from drought. | Liu and Tang, 2014 |
| *Alfalfa* | *Acaulospora scrobiculata*  *A*. *delicata*  *G*. *coronatum* | Expanded absorption area of root, increased alkaline phosphatase activity and secretion of organic acids and increased P-uptake efficiency. | Meng et al., 2018 |
| *Helianthus annuus* | *G*. *etunicatum*  *G*. *mosseae*  *G*. *intraradices*  *G*. *aggregatum* | Promotion the absorption of Zn and Fe. | Kabir, 2020 |
| *Helianthus annuus* | *F*. *mosseae*  *G. caledonium* | Alleviated the heavy mental toxicity form WEEE- recycling site. | Zhang et al., 2018 |
| *Saussurea costus* | *Gigaspora albida*  *G. decipien, G. gigantea*  *G. margarita, G. rosea*  *Scutellospora calospora*  *Dipurpurascens, S. pellucida*  *Dentiscutata heterogama*  *Racoertra coralloidea*  *Fulgida, Septoglomus deserticola, Viscosum*  *F*. *mosseae*  *Rhizophagus clarus* | Enhanced resistance to adverse environment, while promoting terpenoids accumulation of costunolide, dehydrocostus lactone and total lactones in the roots significantly. | Yang et al., 2020 |
| **Ornamental Plants** | **Mycorrhizal species** | **Host performance and stress tolerance** | **References** |
|  | *R. Intraradices*  *Acaulospora foreata*  *A. koskei A. scrobiculata*  *Spinosa, Diversispora eburnea*  *D. spurca, Entrophospora colombiana, Paraglomus brasilianum, P. occultum*  *Ambispora leptoticha*  *Archaeospora trappei* |  |  |
| *Thymus vulgaris* | *Rhizophagus irregularis*  MUCL41833 | AM fungi and elevated CO2 enhanced the functional food value. | Habbeb et al., 2020 |
| *Rose hybrida* | *G*. *deserticola*  *G*. *intraradices* | Improved the leaf tissue elasticity. | Auge et al., 2010 |
| *Prunus martima* | *F*. *mosseae* | Increased nutrition uptake, gas exchange and Chl fluorescence parameters.  Combination of AM fungi and PSF can strengthen the nutrition uptake, root growth. | Zai et al., 2021 |
| *Begonia semperflorens* | *G*. *intraradices* | Promotion in growth and dry biomass, flower number and diameter. Shading increased the rate of AM fungi colonization, as well as the overall host growth, flower quality, flower number, and flower diameter when compared to unshaded samples. | Sabatino et al., 2019 |
| *Impatiens balsamina* | *Acaulospora morrowiae*, *A*. *crobiculata*, *A*. *spinosa Claroideoglomus etunicatum*  *F*. *geosporus* | Inoculation with AM fungi mitigated the detriment caused by Meloidogyne incongita. | Banuelos et al., 2014 |

Table 1. (Continued)

|  |  |  |  |
| --- | --- | --- | --- |
| **Ornamental Plants** | **Mycorrhizal species** | **Host performance and stress tolerance** | **References** |
|  | *F*. *mosseae*  *Gigaspora decipiens*  *Gi*. *rosea*  *G*. *aggregatum*  *G*. *macrocarpumand*  *Scutellospora pellucida* |  |  |
| *Asclepias curassavica* | *Claroideoglomus etunicatum*  *F*. *mosseae*  *G*. *intraradices*  *G*. *aggregatum* | The chemical resistance expression caused by AM fungi inoculation varied among different milkweed species, and mycorrhization can ameliorate tradeoffs between growth and defense. | Vannette et al., 2013 |
| *Kalappia celebica* | *G*. *claroideum*  *G*. *coronatum* | Assisted the conservation of endangered species through improving the uptake of N, P by roots and N, P, K by shoots. | Husna et al., 2021 |
| *Orychophragum violaeeus, Solanum nigrum, Ophiopogon japonicus* | *G*. *nigrum*  *F. mosseae* | The most efficient combination for remediation of Pb and Cd contaminated soil. | Chen et al., 2017 |

### 2.3. Enhancement of Stress Resistance in Ornamental Plants

Adverse circumstances are major challenges for ornamental plant cultivation both nowadays and henceforth. Breeding plant varieties that can prove robust against a variety of stresses is one answer to these difficulties which requires a relatively long duration of time to be practically realized. Alternatively, a new environmentally-friendly production method may come about through the application mycorrhizal fungi and associated bacteria to ornamental plants grown from existing seed stock. AM fungi can increase the absorption of calcium and potassium ions, enhancing the salt tolerance of plants (Garg et al., 2015; Tomar et al., 2013). It is reported that AM fungi play positive roles in strengthening the resistance capacity of host plants, namely, it can be applied to relieve including infertility, drought, salinization, disease, and heavy metal pollution stresses (Jiang et al., 2020). Those specific effects will be illustrated one by one in the following paragraphs.

First of all, AM fungi can ameliorate plant adaptability to drought stress. Plant symbiosis with AM fungi also increases the root absorption area of plants, which can better cope with the problems related to poor soil quality and drought stress. For water transport efficiency, the colonization of AM fungi has been found in one study to enlarge the vessel diameter up to 5.33 μm and increase the fiber cell length in Populus× canadensis (Liu and Tang, 2014). This reveals one of the tactics that mycorrihzal plants use to adapt to the water deficit conditions. There are other strategies to benefit host plants, such as the alteration of the antioxidant enzyme system, changes in the hormone level and ratio, and the concentration of osmotic adjustment substances. Taking antioxidant enzymes as an example, the activities of SOD and catalase (CAT) were increased, while POD decreased in samples of *Tetraena mongolica* that were inoculated with AM fungi. However, the above three enzymes activity were all enhanced in mycorrihzal *Zygophyllumx anthoxylum* and the number of leaves was less (Wang and Bao, 2014). For *Forsythia suspensa*, AM fungi inoculants enhanced the resistance to draught which showed the similar physiological variation as well as in *Z*. *anthoxylum* (Zhao et al., 2007). Other beneficial effects of AM fungi were reported in *Leymus* *chinensis* (Jia et al., 2017), *Paspalum* *notatum* (Wu et al., 2011), *Aquilaria* *sinensis* (Hong et al., 2018), and *Bauhinia* faberi (Zhang et al., 2016). It is thus inferred that the symbiosis of AM fungi and their hosts have unique methods of coping with adverse conditions, but it may trigger a variety of plant systematic responses.

Soil pollution is also becoming a serious problem that has to be independently addressed. The sum total of electrical and electronic equipment (EEE) pouring into daily life, which not only offers benefits to people but also forms a considerable threaten to health for human beings all over the world, given that ten millions of waste from EEEs were dumped into outdoor plots, from which heavy metals released has been come to constitute a source of serious harm to people and other members of the ecosystem. At the same time, the many pesticides applied to soil year after year constitute an additional detriment to plant growth and the quality of its outputs. To mitigate the effects of the above-mentioned pollutants, AM fungi has showed its potential power for cost-effective and environment-friendly. Under the stress of heavy metal lead (Pb) and cadmium (Cd), the AM fungi colonization increased the biomass of *Orychophragmus violaceus*, *Solanum nigrum*, and raised the tolerance to Pb and Cd (Chen et al., 2017). Similar soil decontamination trial was carried out (Zhang et al, 2018), in which the potential remediation capacity was demonstrated, for heavily heavy metal (HM)-contaminated soil, AM fungal inoculations significantly reduced the concentration of Cd, chromium (Cr), nickel (Ni) and zinc (Zn) in shoots apart from increasing the biomass and P concentration of shoots and roots in *Helianthus annuus*. Different consortium of host species and AM inoculants illustrated varying enrichment and transfer coefficients (Table 2), which means that concrete trial or experiment must be taken before its application to cope with soil remediation or alleviation of accumulated soil pollutants can be practically applied in commercial production.

Table 2. The effect of AM fungi on heavy metal enrichment   
and transfer coefficients in different parts of plants

|  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Plants** | **Treatments** | **Pb** | | | | **Cd** | | | |
| EF | | | | EF | | | |
| TF | Shoot | Root | Whole | TF | Shoot | Root | Whole |
| *O. violaeeus* | CK | 8.189 | 1.020 | 0.125 | 1.145 | 11.159 | 1.283 | 0.115 | 1.398 |
| HM-CK | 4.941 | 1.584 | 0.321 | 1.905 | 14.405 | 1.849 | 0.128 | 1.977 |
| HM-Gi | 4.207 | 1.955 | 0.465 | 2.420 | 9.441 | 1.605 | 0.170 | 1.775 |
| HM-Fm | 3.891 | 1.900 | 0.488 | 2.388 | 7.135 | 1.855 | 0.260 | 2.115 |
| *S. nigrum* | CK | 2.905 | 1.804 | 0.621 | 2.425 | 4.022 | 2.775 | 0.690 | 3.465 |
| HM-CK | 2.040 | 1.938 | 0.950 | 2.887 | 5.516 | 2.620 | 0.475 | 3.095 |
| HM-Gi | 1.565 | 1.978 | 0.977 | 2.955 | 3.923 | 2.805 | 0.715 | 3.520 |
| HM-Fm | 2.264 | 2.221 | 0.981 | 3.202 | 4.121 | 4.080 | 0.990 | 5.070 |

Note: TF indicates transfer coefficient, EF represents Enrichment coefficient. HM, Gi, Fm indicate heavy metal, *G. intrradices* and *Funneliformis mosseae* respectively (Chen et al., 2017).

Thirdly, AM fungi play an active role in resistance to salt stress. Soil salinization has been turning into one of the vital environmental problems all over the world. It is often the main obstacle for plant growth and normal development, and is an issue which must be solved urgently. Ornamental plants have to confront with this, and a series of trials involved in how the salt stress affected host fitness with or without AM fungi inoculations. Up to now, for AM fungi ameliorating salt stress, some primary trials had been conducted in *Caragana microphylla* (Zhang et al., 2013), *Bruguiera sexangula* (Miao et al., 2017), *Stevia rebaudiana* (Wang et al., 2018), *Paeonia suffruticosa* (Guo et al., 2010, 2016), *Lilium brownii* (Li et al., 2018), *Medicago sativa* (Liu et al., 2018) and *Phoebe zhennan* (Cui et al., 2020). There is a long coastal beach in the east of China, where landscape beautification has been shorting of stress-resistance ornamental plants, many grass flowers and shrubs can’t survive and grow normally due to a lack of even moderate resistance to relatively high salt concentration along such a seashore beach. With this problem in mind, research on the salt resistance of ornamental plants has been conducted. A trial on perennial flowers demonstrated that salt resistance capacity was the highest in *Chrysanthemum morifolium*, followed by *Hemerocallis middendorfii*, *Physostegia virginiana* and *Philippine violet* respectively, and *Iris tectorum* was the most sensitive one (Wang, 2012). These flowers have been utilized in urban landscaping, which means that their salt tolerance requires special strengthening. The application of AM fungi to elevate the resistance of ornamental plants could be a better choice in current circumstances, and its prospect is great.

Next, symbiosis with AM fungi has shown some benefits to flower plants when coping with either relatively high or low stress from pathogens and pests. Utilizing AM fungi in large-scale and low energy input production to overcome the poor temperature stress resistance gap that exists for some ornamental plants is achievable. This type of trial has been made for *Coleus blumei* (Guo, et al., 2009), *Lilium brownii* (Xing et al., 2018), *Lavandula angustiflia* (Xing et al., 2019) and *Rhododendron* (Wang et al., 2019). *C. blumei* has enormous value consideringits wonderful ornamental value in colorful leaves. That being said, there is still an urgent need to address this flower’s poor endurance to low temperature. *Lilium brownii* and *Rhododendron* are important flowers in China and other parts of the world. *Lavandula angustiflia* isalso aspecial plant, around which considerable volatile metabolites are released, filling nearby spaces with a pleasant aroma. Plants in urban landscape space have huge potential to form healthy public landscapes through preventing bacteria and fungi spreading (Zhu et al.,2012; Pan et al., 2010).Other studies found that the symbiosis of plants and AM fungi can yield an good effect in enhancing the host’s capacity against pathogen through increasing the production of antibiotics and reducing the content of malondialdehyde (Siasou et al., 2009; Jiang et al., 2020). Plants that kept good performances and high tolerance capacity have been the goal we will have sought. There will be many obstacles to overcome in the light of the concrete and variable strategies in different circumstances before the actual application of AM fungi in ornamental plants cultivation.

## Conclusion and Future Prospects

It can be currently concluded that the presence of AM fungi in ornamental plants can optimize cultivation in a sustainable and environment-friendly way. Application of AM fungi inoculants yields improvements both in plant growth and cut flowers, increasing the flower organ diameter. Utilization of AM fungi doesn’t produce a harmful effect on environmental conditions like chemical pesticides, inorganic fertilizers and herbicide, but improves soil, water and plant health. It also plays a positive role in inhibiting or ameliorating the damages caused by many kinds of stresses, such as drought, salinity, high/low temperature, and heavy metal stress, enhancing plant growth and achieving good quality. In addition, biotic stress such as fungi and nematode may be mitigated by the presence of AM fungi. Moreover, the function of different AM fungi strains differs generally, and AM fungi symbiosis trials have been executed in some host species, which is for now only in the experimental stage. There is a long way to go before true application happens in the field. Therefore, for AM fungi, the collection and identification of the correct species through morphology assisted with molecular techniques is urgent demand, and for ornamental plants, personalized experiments should be carried out more and more focusing on the specialty requirement in different host species. In brief, ornamental plants are distinguishable from field crops in ornamental value (color, fragrance and other novel traits), resistance to adversity which determines the consuming of chemical pesticides and bactericide that affect the safety and health of people in the public landscape. In this field, most of the AM fungi symbiosis research should focus on it.

If the above-mentioned flowers are all planted with symbiosis of AM fungi in urban landscape, it would cut down considerable inputs in plant application and cultivation, which is consistent with Chinese policy goals.

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Chapter 4

# Potential Practical Application and Assessment of Arbuscular Mycorrhizal Fungi on Horticultural Crops

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## Abstract

There are some beneficial soil microorganisms in the rhizosphere of horticultural plants, among which arbuscular mycorrhizal fungi as a kind of endophytic fungi are widely distributed in the world. The fungi can establish arbuscular mycorrhizal symbiont with roots of most horticultural plants. Some ornamental plants also form orchid mycorrhizas, arbutoid mycorrhizas, ericoid mycorrhizas, and monotropoid mycorrhizas, in addition to arbuscular mycorrhizas and ectomycorrhizas. A number of field experiments with arbuscular mycorrhizal fungi on horticultural plants have been attempted, showing positive benefits, including promoted plant growth, accelerated nutrient uptake, and advanced flowering. Such arbuscular mycorrhizal fungi are important friends of horticultural plants. In addition, other endophytic fungi that can be cultured *in* *vitro*, such as dark-septate endophytic fungi and *Piriformospora indica*, have also been tried to use in horticultural plants and have shown positive effects on plants, thus, providing some new pathways. However, the work in this area still needs to be strengthened. This chapter focuses on the application of arbuscular mycorrhizal fungi in fruit trees, vegetables, and ornamental plants, and provides a new outlook for future research.

**Keywords:** fruit, mycorrhiza, ornamental plants, symbiosis, vegetable

## 1. Introduction

Mycorrhiza is a mutually beneficial association between mycorrhizal fungi in the soil and the plant root, which is characterized with mycorrhizal transporting of mineral nutrients and water to the host plant, in exchange of approx. 20% of host’s carbohydrates to the mycorrhiza for the maintenance of fungal growth (Kokkoris et al., 2020). As outlined by Peterson et al. (2004), mycorrhizas are morphologically classified into seven categories:

1. Ectomycorrhizas: ectomycorrhizal fungi colonize the root cortex cells of plants, forming a mantle or sheath of fungal hyphae, finally forming Hartig net (Peterson et al., 2004). Various shrub and herbaceous species form the ectomycorrhiza.
2. Arbuscular mycorrhizas: soil arbuscular mycorrhizal fungi (AMF) colonize the root cortex cells, forming arbuscules, and in some cases forming vesicles (*Gigaspora* and *Scutellospora*) between or inside the cells. Arbuscular mycorrhizas can be found in many horticultural crops, such as citrus, pear, peach, apple, tomato, potato, leek, etc.
3. Ectoendomycorrhizas: the ectoendomycorrhizae have the characteristics of both ectomycorrhizae along with Hartig’s net in the intercellular space and arbuscules in the root cortex cells (Peterson et al., 2004), mainly in some Pinus and Rhododendronaceae plants, such as walnut.
4. Ericoid mycorrhizas: The mycorrhiza is a mycorrhizal type that is unique to several families in the order Ericales. A characteristic feature is the formation of very specific lateral roots, or “hair roots.” Mycorrhizas are involved in the colonisation of epidermal cells by fungal hyphae, followed by the formation of a branched mycelial complex in each colonised cell (Peterson et al., 2004).
5. Arbutoid mycorrhizas: The mycorrhiza is found in two families (*Arbutus* and *Arctostaphylos*) in the order Ericales, in which a mantle, a Hartig net, and intracellular hyphae forming hyphal complexes are observed in roots (Peterson et al., 2004).
6. Monotropoid mycorrhizas: *Allotropa*, *Cheilotheca*, *Hemitomes*, *Monotropa*, *Monotropantham*, *Monotropsis*, *Pityopus*, *Pleuricospora*, *Pterospora*, and *Sarcodes* plants can form monotropoid mycorrhizas, having the typical of ectomycorrhizas and ectendomycorrhizas (Peterson et al., 2004).
7. Orchid mycorrhizas: the family Orchidaceae forms orchid mycorrhizas with complex hyphal coils (pelotons) within plant cells in the seed germination and establishment in nature (Peterson et al., 2004).

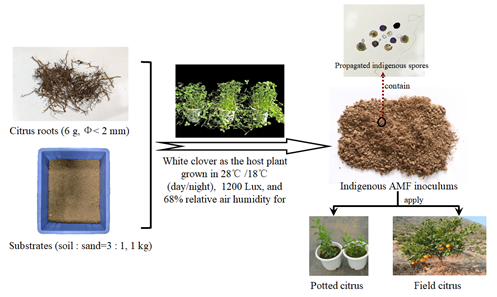
Among these mycorrhizas, AMF are the most widely distributed, present in large numbers, and intensively studied. It has been shown that inoculation of horticultural crops with mycorrhizal fungi promoted the growth of horticultural crops, stimulated the uptake of mineral nutrients and water, enhanced resistance to stress, and improved the fruit quality (Cheng et al., 2021; Wu et al., 2017, 2019b; Tian et al., 2021; Zou et al., 2021). Therefore, the absence of AMF on the roots of horticultural crops is rather abnormal, thus demonstrating the importance of AMF on horticultural crops. As reported by Paskovic et al. (2021), AMF inoculation significantly increased fruit N, Mg, and dydrophilic phenol contents of tomato plants, indicating the positive effects of AMF in horticultural plants. In *Melissa officinalis*, AMF treatment dramatically increased dry weight gain and essential oil content after 90 days of inoculation (de Assis et al., 2020). The chapter simply outlined the application of AMF on fruit plants, vegetable plants, and ornamental plants, and also summarized the production of AMF.

## 2. The Production of AMF Inoculums

The production of AM fungal agents is the key to their application in horticultural crops. Although the pure culture of AMF *in vitro* is not yet possible, the potting method (Miyasaka et al., 2003) can still be used to produce AMF of a certain purity. The specific production process is as follows: the seeds of maize, sorghum, or white clover as the host plant are surface disinfected with 10% sodium hypochlorite solutions for   
5−10 min, and then germinated. A 3 mm sized crushed basalt is selected as the growth substrate, aiming at a substrate containing very low nutrient levels, especially P. The substrate is then autoclaved to kill indigenous mycorrhizal fungi. The sterilized substrate is mixed with pure mycorrhizal agents (available from the Institute of Plant Nutrition and Resources, Beijing Academy of Agriculture and Forestry, Beijing, China, or from the Institute of Root Biology, Yangtze University, Jingzhou, China) or identified spores of AMF in a 20:1 (v/v) ratio in 15-25 cm diameter plastic pots. The 2-6 germinated seeds are sown into a pot and placed in a greenhouse to reduce contamination by other microorganisms. Generally, the symbiosis of AMF with host plant roots can be observed after 6 weeks of normal water management. Soil water deficit starts after 14 weeks, and the above-ground parts of plants are removed at 16 weeks. The growth substrate and roots are collected and placed into a clean tray. The roots are cut and mixed well with the growth substrate as the mycorrhizal fungal agents for application in the field. If the mycorrhizal inoculants are not used in time, they can be stored at 4°C or a cool and dry place for 1 year or less. However, it is still necessary to test the number of spores in the inoculum before the next application.

Commercial production of arbuscular mycorrhizal fungal inoculants has been done in recent years by companies in many countries, such as the United States, Japan, France, Colombia, Netherlands, Australia, New Zealand, the United Kingdom, Canada, India, etc. The spore density of the inoculants varies depending on the method and can reach up to 10,000 spores/mL of mycorrhizal agent (Zhang, 2001).

Wu et al. (2019a) proposed the simple protocol regarding native arbuscular mycorrhizal fungal propagation in the field (Figure 1). They selected 2 mm fine roots of diameter from field citrus, and mixed with autoclaved soil and sand mixture (3 : 1, v/v). White clover as the host plant was sown in 28ºC/18ºC (day/night temperature), 1200 Lux, and 68% of relative air humidity for 12 weeks. The AMF-colonized roots and the growth substrate are then collected and used as mycorrhizal inoculum. The spore density can reach 20 spores/g. They can be applied directly on citrus in the field or at the seedling stage.



**Figure 1.** A proposed diagram regarding indigenous arbuscular mycorrhizal fungal propagation of field citrus and its application (Wu et al., 2019a).

## 3. Application of AMF on Fruit Plants

Most of the fruit trees are perennial woody plants with less root hairs, and they need to rely on the developed extraradical hyphae of AMF in their roots to help them absorb water and mineral nutrients, so as to regulate their physiological responses, such as drought resistance, tree growth, fruit quality, disease resistance, etc. The introduction of exogenous AMF can effectively increase the population of AMF in the rhizosphere of fruit trees, or the introduction of exogenous AMF can become the dominant strain.

The first application of AMF in fruit production was done by Kleinschmidt and Gerdemann (1972) in California, Texas and Florida, USA, where AMF inocula were placed 4 cm below the seed of lemon, lime and orange. Menge et al., (1980) reported that the inoculation of *Glomus fasciclatum* on sterilized sandy soil increased the growth of avocados by 49% to 254%. Later, it also reported on apple and peach plants.

In the United States, Australia, South Africa and other countries, AMF were used earlier in the production of fruit trees. When the rootstocks of fruit trees were planted, AMF were used to promote the growth of rootstock seedlings. In the cutting propagation of fruit trees, AMF were inoculated at the base of cuttings to stimulate the rooting of cuttings and improve the survival rate of cuttings. Inoculating AMF during the transplanting process of micropropagated fruit trees is beneficial to the survival rate of fruit transplanting and the root development. Inoculation of AMF without soil disinfection on the replanted fruit orchard can reduce the death rate of apple trees, and the effect of mycorrhizal fungi is greater if soil disinfection is carried out before inoculation (Wang and Wang, 1994). Wan (1992) improved the technique of AMF inoculation with container seedlings. Compared with conventional seedlings, the cultivated mycorrhizal seedlings have the advantages of a shorter time, better quality, no slow seedling period after transplantation and high survival rate. Wu and Xia (2004) considered AMF as a new biofertilizer with broad application prospects in fruit tree production, based on the fact that AMF are living fungi that can significantly promote the growth of fruit trees and increase the uptake of various mineral elements from the soil.

Fruit seedlings are mycorrhized according to the following methods: seeds of fruit trees are surface disinfected, and then sown in a layer of AMF inoculum in the inter-row or containers (soil sterilization); approx. 3 to 5 months after the inoculation, the treated seedlings can be detected with a large number of AMF colonization in roots, resulting in mycorrhized seedlings, which can be directly used in the field or grafting. Figure 2 showed that, 18 months after inoculation of trifoliate orange seedlings with *Glomus intraradices*, mycorrhizalised plants sprouted earlier, and plant height was significantly higher than that of non-mycorrhizalised plants. As a result, the potential of mycorrhizalised plants for earlier grafting is shown.



Figure 2. Growth status of trifoliate orange seedlings inoculated with (on the right) and without (on the leaf) an arbuscular mycorrhizal fungus, *Glomus intraradices*. These trifoliate orange seedlings were inoculated on 11 September 2019 and photographed on 16 March 2021, after 18 months of growth.

Recently, Gu et al. (2020) applied AMF into field apple trees. The apple rootstock M9T337 was selected, and the base of shoot cuttings was covered with mixed mycorrhizal fungal agents. At the same time, clover or alfalfa seeds were introduced to promote AMF reproduction in the field. Three months later, it was found that the colonization rate of new roots reached 30.7%.

In addition to AMF, dark-septate endophytic fungi have also been tested on fruit trees, such as blueberries. In blueberry nurseries, *Chaetomium* *globosum*, *Penicillium* *pinophilum*, *Schizophyllum* *commune*, *Cladosporium* *cladosporioides*, and *Phialocephala* *fortinii* were inoculated and after 6 months showed a colonization rate of 36% to 68% and increased anthocyanin, total phenol and total flavonoid content in blueberry fruits (Xiao et al., 2021). Principal component analysis showed that *S*. *commune*, *P*. *pinophilum* and *P.* *fortinii* had a good effect on improving the yield and fruit quality of blueberry. Because dark-septate endophytic fungi can be cultured *in vitro*, such positive benefits of dark-septate endophytic fungi in fruit trees provide a guarantee for further utilization in the future. We also conducted the inoculation of *Piriformospora indica* on citrus in the field, which showed certain promoted effects on fruit quality and soil properties. These results indicate the potential value of endophytic fungi in fruit trees.

## 4. Application of AMF on Vegetable Plants

In vegetable crops, except for Cruciferae, Chenopodiaceae and Amaranaceae without AM structures, Solanaceae, Cucurbitaceae, and Liliaceae all form arbuscular mycorrhizas (Li and Liu, 2000). AMF have shown various positive effects on bean, pepper, cucumber, watermelon, melon, onion, tomato, asparagus, etc., such as promoting vegetative growth, increasing yield, promoting flowering and fruiting, enhancing the resistance to soil borne diseases, and improving quality. Li et al. (2002) conducted an inoculation test of AMF on micropropagated taro seedlings, and found that AMF could significantly improve the survival rate from 93% under non-AMF-inoculated conditions to 100% under AMF-inoculated conditions, as well as increased the economic coefficient of taro from 0.122 under non-AMF-inoculated conditions to 0.632 under AMF-inoculated conditions. He et al. (2021) used *Glomus mosseae* into the potted cucumber plants at the stage of nursery, and found that five weeks later, N and P contents were increased by mycorrhization at Chiyu126 cultivar, but not Jinyan No. 4 cultivar. Cheng et al. (1993) reported the promoted effect of muskmelon seedlings by AMF inoculation, representing the increased yield and the second melon. In general, vegetable plants need substrates with high fertility, but arbuscular mycorrhizal development develops well on poor soil. This indicates that the ratio of AMF to the substrate before its application in vegetable crops has to be studied. Recently, Zheng et al. (2020) tried the application of AMF on hydroponically grown tomatoes. First, they placed *Rhizophagus irregularis* or *Funneliformis* *mosseae* on sand-cultured tomatoes for about 4 weeks, and then hydroponically grew these tomatoes inoculated with mycorrhizal fungi for 6 weeks. Mycorrhizalised plants maintained higher chlorophyll content, net photosynthetic rate and root development, thereby promoting the growth of hydroponically grown tomatoes.

Another important function of AMF on vegetable plants is to increase disease resistance. Matsubara et al. (1995) transplanted eggplant into the soil with Verticillium wilt bacteria, and the results showed that after 10 weeks, the incidence of *Glomus etunicatum*- and *Gigaspora* *margarita*-inoculated plants was 30% and 50%, respectively, while the incidence of uninoculated plants was 100%. Li et al. (1997) inoculated tomato with AMF during sowing and with pathogen bacterial during transplanting. Thirty-five days later, leaves of uninoculated plants wilted and yellow spots along leaf veins extended to leaf stalks, with an incidence of 21.3% and a disease index of 12.0. Nevertheless, *Glomus* *versiforme*-treated plants had an incidence of 9.1% and a disease index of 2.1. Yu et al. (1997) showed that the relative control effect of pre-inoculation of AMF on watermelon fusarium wilt reached more than 50%, whether in pot experiment or in field condition.

If pharmaceuticals or steam are used to disinfect the soil, it is easy to cause soil contamination and in some cases even damage the beneficial microorganisms in the soil, thus affecting vegetable production. The use of mycorrhizal fungi can improve disease resistance and alleviate cropping obstacle, improve the soil ecosystem, and promote pollution-free vegetable production (Mie et al., 2000).

## 5. Application of AMF on Ornamental Plants

In ornamental plants, most of the vines, herbs and woody plants form arbuscular mycorrhizas, while others such as orchids form orchid mycorrhizas, and other ornamental plants form special types of mycorrhizae such as arbutoid mycorrhizas, ericoid mycorrhizas, and monotropoid mycorrhizas (Jiang et al., 2002). As a new industry, ornamental plants are very popular in the world, and the inoculation of ornamental plants with mycorrhizal fungi has a high economic value. Although the research started late, the ornamental plants with AMF are involved in gerbera jamesonii, Chinese rose, araucaria, black wattle, *Acacia grandiflora*, and *Hippophae rhamnoides*.



**Figure 3.** Plant growth responses of AMF- and non-AMF-inoculated white clover after 3 months.

Many of ornamental plants are obtained by tissue culture, and these micropropagated ornamental plants are grown under aseptic conditions, resulting in plants with weak quality and poor resistance. These unfavorable conditions are alleviated after inoculation with mycorrhizal fungi. In general, inoculation with AMF can promote the early emergence of araucaria by 4 months, the early flowering of gerbera by 16 days, the increase of cut flower production, and the survival rate of mycorrhizal seedlings by about 20% (Liu and Li, 2000). Inoculation with AMF caused French marigold and larkspur to flower 1 week earlier, and claret-colored larkspur plants inoculated with *Glomus mosseae* increased flowering by 23 days, compared with uninoculated plants (Lin, 1994).

The application of AMF in the transplanting of lawn grass and the establishment of golf courses is increasing. Hetrick et al. (1986, 1988, 1990, 1991) demonstrated that inoculation with mycorrhizal fungi under soil P deficit conditions enhanced plant growth of turfgrass, root growth, and fertilizer utilization. Pelletier and Dionne (2004) showed that both *Glomus intraradices* and *Glomus etunicatum* stimulated the growth of turfgrass in a study conducted in Lavalle, Canada. The effects of different fungal strains differed, with *Glomus intraradices* causing more rapid establishment than *Glomus etunicatum* at inoculum densities ranging from 40 to 60 mL/m2. As shown in Figure 3, inoculation of AMF on white clover resulted in significantly higher plant growth potential than uninoculated plants, and greater biomass and more intense green leaf colour were more conducive to turfgrass establishment and drought resistance.

## Conclusion and Outlook

Mycorrhizal fungi inoculation on potted horticultural plants has achieved a great success, but some attempts to apply mycorrhizal fungi on field horticultural plants are not yet available. This has therefore severely limited the further promotion and application of AMF. In the future, more work should be done on the following aspects:

1. Trying and demonstrating the benefits of other endophytic fungi in addition to AMF on horticultural plants and further develop and utilise these endophytic fungi.
2. Rapid production of mycorrhizal fungi inoculums. At present, the use of mycorrhizal fungi in the production of horticultural plants is not widespread. The main reason is that AMF are difficult to be cultured in vitro, and it is difficult to avoid contamination of mixed bacteria in the inoculation process, and can not achieve convenient inoculation (Gu et al., 2020). Although a certain amount of mycorrhizal inoculums can be obtained from potted mycorrhizal production, improving the quality of the mycorrhizal agent is a future challenge.
3. Selecting an efficient arbuscular mycorrhizal fungal strain for a special horticultural crop.
4. Inoculation time and dosage of AMF. Mycorrhizal fungi must be applied to the roots of horticultural plants, and the earlier inoculation has better effects. For horticultural crops requiring the nursery followed by transplanting, the AMF should be inoculated at the same time of sowing after sterilization of the nursery soil or growth substrate. When applying AMF agents, inoculated methods such as root dipping, root slurry, and root injection can be considered. For small seedlings or seeds, the amount of mycorrhizal agent application can be reduced, and for large seedlings, the amount of application can be increased appropriately.
5. Field management of mycorrhizal horticultural crops. Mycorrhizal growth and development in horticultural plants could be affected by various factors, including soil properties, grafting, pesticides, herbicides, pruning, intercrop, etc. For field management of mycorrhizalized horticultural plants, appropriate fungicides should be used so that fungicides do not inhibit the activity of mycorrhizal fungi and kill mycorrhizal fungal propagules in the soil. Benlate, glyphosate, and daidzein can inhibit the growth and activity of AMF, while clothian does not affect the growth of AMF (Zeng et al., 2004b); orchard grass cultivation (e.g., *Bacopa monniera*) is beneficial to increase the population of mycorrhizal fungi in the soil of fruit trees (e.g., citrus). This would maintain a high mycorrhizal colonization in the root of fruit trees, which thus improves the fruit quality of fruit trees (e.g., citrus), especially the coloration of the peel (Zeng et al., 2004a), which is of great importance in fruit tree cultivation.

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# Index

A

acid, 16, 18, 20, 23, 42, 49, 55, 65, 89, 100, 132

active compound, 64, 71

agriculture, vii, viii, 4, 25, 31, 34, 64, 65

alkaline phosphatase, 103

alkaloids, viii, 5, 13, 14, 28, 33, 43

AM fungi, 51, 94, 95, 96, 98, 99, 100, 101, 102, 104, 105, 106, 107, 108, 109, 110, 114, 115

amino, 14, 18, 24, 33, 47

amino acid, 18, 24, 33, 47

antibiotic, 19, 21, 30, 48, 71, 81, 82, 86

antibiotic resistance, 81, 82

anti-cancer, 2, 12, 14, 16, 19, 47

antileishmanialdrugs, 81

antimalarial compounds, 77

antimicrobial, v, vii, viii, 10, 25, 30, 31, 39, 40, 42, 44, 45, 46, 48, 49, 50, 51, 52, 53, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 70, 71, 72, 73, 74, 75, 78, 83, 84, 86, 87, 88

antimicrobial activity, vii, viii, 25, 30, 45, 46, 50, 51, 52, 53, 56, 57, 60, 70, 71, 72, 73, 74, 78

antimicrobial therapy, 49

antioxidant, vii, 21, 31, 44, 45, 53, 97, 103, 106, 111, 113

antiparasitic activity, viii, 64, 75

antitumor, vii, 65

antitumor agent, 65

antiviral agents, 27

arbuscular mycorrhizal fungi, viii, 111, 112, 113, 114, 115, 116, 117, 118, 130, 131, 132, 133, 136, 137

Avicennia schaueriana, 68

B

Bacillus subtilis, 15, 23, 61

bacteria, vii, 2, 14, 17, 18, 20, 21, 25, 27, 29, 30, 32, 35, 51, 52, 56, 72, 73, 106, 109, 114, 116, 125, 128

bacterial pathogens, 64

bacterial strains, 17

bacteriophage, 27

benefits, viii, 94, 97, 107, 109, 118, 124, 128

bioactive compounds, 2, 13, 14, 19, 29, 36, 37, 38, 41, 44, 46, 49, 64, 69, 70, 84, 88

bioactive molecules, 64

biocatalysts, 90

biochemistry, 40, 56, 116

biocontrol, 2, 45, 64, 78, 79, 81, 83, 86, 90, 115

biodegradation, 2

biodiversity, vii, 5, 6, 39, 64, 67, 71, 80

biogeography, 3

biological control, 78

biological systems, 35

biomass, 6, 96, 97, 100, 101, 102, 104, 107, 114, 127

biomedical applications, 25

biomolecules, 70

bioprospecting, 38, 59, 64, 72, 90

bioremediation, 2

biosciences, 31

biosurfactant, 65, 86

biosynthesis, 27, 31, 38, 46, 86

biotechnological applications, 2, 12, 29, 92

biotechnology, 26, 41, 46, 53, 56, 60, 62

biotic, 2, 3, 36, 70, 94, 95, 96, 110

C

carbon dioxide, 97

carboxylic acid, 21

cell division, 22

cell line, 58

challenges, 4, 46, 54, 62, 81, 94, 106

chemical, 25, 29, 31, 32, 35, 50, 59, 78, 83, 88, 92, 105, 110, 114

chemical structures, 29

chemicals, 22, 23, 32, 35

*China*, ix, 14, 16, 21, 66, 67, 79, 86, 91, 93, 108, 109, 112, 115, 117, 120, 129, 132, 133, 135, 136, 137

Chinese medicine, vii

colonization, 4, 8, 11, 23, 44, 65, 72, 94, 96, 99, 103, 104, 106, 107, 111, 123, 124, 129

composition, 42, 51, 65, 114, 131

compounds, 2, 4, 9, 10, 13, 14, 16, 17, 18, 19, 25, 29, 36, 37, 38, 39, 41, 44, 45, 49, 50, 55, 57, 64, 65, 70, 71, 75, 77, 81, 84, 87, 88, 99

contaminated soil, 105, 107

contamination, 35, 94, 120, 126, 128

cultivation, viii, 11, 37, 40, 64, 94, 98, 106, 109, 110, 129, 137

culture, 37, 79, 80, 120, 127, 132

cystic fibrosis, 31

cytomegalovirus, 21

cytotoxicity, 76, 77, 78

D

data analysis, 10

data set, 68

deficit, 103, 106, 120, 127, 133

derivatives, 20, 21, 28, 44, 48, 49, 54, 75, 82, 91

*Diaphorte*, 69, 73, 75, 76, 78

diseases, 2, 4, 26, 27, 32, 36, 49, 75, 81, 87, 91, 124, 131

disinfection, 122

distribution, 10, 36, 37, 60, 67, 68, 85, 90

diversity, viii, 3, 11, 19, 25, 32, 44, 45, 55, 64, 66, 67, 68, 69, 70, 71, 72, 78, 81, 84, 89

drought, 6, 47, 51, 94, 103, 106, 110, 111, 112, 113, 115, 116, 122, 127, 132, 136, 137

drug delivery, 31, 32

drug discovery, 2, 14, 19, 25, 36, 65, 83

drug resistance, 29

drug resistant, 17, 40, 75

drugs, 2, 27, 32, 37, 54, 76, 81

E

*E. coli*, 15, 16, 17, 20, 21, 27, 72, 73, 74, 75

ecology, 4, 39, 42, 50, 52, 53, 92

economic problem, 76

ecosystem, 2, 32, 42, 63, 69, 81, 94, 95, 107, 126

endangered, 37, 38, 105, 112, 115

endangered species, 105

endophytes, vii, 2, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 23, 27, 29, 31, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 46, 51, 53, 54, 55, 60, 61, 64, 65, 66, 67, 68, 70, 71, 72, 75, 77, 78, 79, 80, 81, 82, 83, 85, 88, 90

endophytic fungi, v, vii, viii, ix, 1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 19, 20, 21, 22, 23, 24, 32, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 92, 117, 124, 128, 136, 138

energy input, 109

environment, 2, 22, 31, 44, 71, 78, 94, 95, 103, 107, 110

environmental conditions, 6, 36, 37, 110

environmental factors, 10, 42

environmental impact, 71

enzymatic activity, 70

enzyme, 65, 79, 86, 103, 106, 111

enzymes, 22, 23, 28, 41, 70, 78, 80, 97, 106

exploitation, 43, 64, 70, 80

extracts, 50, 52, 57, 72, 73, 76, 80

F

flavonoids, 15, 33, 116, 129

flowering period, 95, 99

flowers, 94, 95, 98, 99, 102, 108, 109, 110, 113

fruit, viii, 2, 11, 50, 100, 112, 118, 119, 122, 123, 124, 129, 131, 132, 137

functional food, 104, 112

fungal infection, 30

fungal metabolite, 19

fungus, 4, 7, 9, 14, 15, 16, 17, 18, 19, 21, 26, 33, 36, 38, 39, 41, 42, 43, 44, 45, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 66, 68, 70, 72, 77, 82, 84, 85, 86, 87, 89, 91, 92, 98, 99, 111, 113, 123, 129, 131

G

gene expression, 30

genes, 71, 81

genetic background, 10

genus, 30, 37, 72, 96

germination, 10, 38, 119

Gigaspora margarita, 125

Glomus intraradices, 123, 127, 131

gold nanoparticles, 32, 36

growth, vii, viii, 2, 3, 4, 6, 10, 12, 19, 22, 24, 27, 30, 35, 36, 37, 42, 50, 51, 69, 79, 80, 94, 96, 97, 98, 100, 101, 102, 103, 104, 105, 107, 108, 110, 111, 112, 113, 114, 115, 116, 118, 119, 120, 121, 122, 123, 124, 126, 127, 128, 129, 130, 131, 132, 133

H

habitats, 37, 64, 70, 86

health, 25, 75, 76, 94, 107, 110

heart disease, 26

heart valves, 30

herpes simplex, 27, 30, 43

horticultural crops, 118, 119, 120, 128, 131, 136

host-plant Interactions, 2

human, 7, 20, 21, 26, 27, 29, 37, 43, 54, 73, 76, 81, 107

human immunodeficiency virus, 27

hybrid, 20, 41, 61, 95, 101

I

identification, 60, 83, 84, 110

in vitro, 28, 37, 40, 41, 53, 78, 79, 118, 120, 124, 128

infection, 27, 30, 31, 47, 49, 59, 96, 132

infectious agents, 75

infectious mononucleosis, 27

inflammation, 26, 28, 44, 46, 47

inoculation, 96, 98, 99, 100, 101, 102, 103, 105, 113, 115, 116, 119, 122, 123, 124, 125, 126, 127, 128, 131, 132

inoculum, 121, 123, 127

interference, 95, 96

isolation, viii, 38, 48, 81, 87, 88

L

Laguncularia racemosa, 68, 73, 74

landscape, 94, 108, 109, 110, 131

landscapes, 109

Leishmania infantum chagasi, 76, 77

living environment, 65

M

management, 60, 78, 114, 120, 128, 132

mangrove forests, v, 63, 89

mangroves, 6, 64, 66, 68, 69, 70, 72, 73, 74, 79, 80, 81, 82, 84, 89

marine environment, 69, 70

medical, 25, 30, 32, 53, 61, 80

medicine, vii, 2, 9, 24, 25, 28, 31, 36, 55

meta-analysis, 51

metabolic pathways, 65, 70

metabolism, 9, 10, 24, 65, 84

metabolites, vii, 3, 9, 12, 14, 20, 28, 33, 37, 38, 39, 47, 55, 56, 57, 60, 61, 62, 64, 65, 70, 71, 72, 74, 75, 76, 77, 85, 87, 90, 109

microorganisms, vii, 2, 4, 21, 25, 27, 28, 30, 31, 32, 35, 45, 64, 65, 69, 71, 75, 82, 90, 117, 120, 126, 130

molecules, vii, 18, 64, 70, 71, 72, 78

multicellular organisms, 61

multidrug-resistant microorganisms, 75

mycorrhiza, 22, 51, 97, 101, 112, 113, 114, 116, 118, 119, 131, 132, 133, 136

N

nanomedicine, 43, 53, 58

nanoparticles, viii, 25, 26, 27, 31, 32, 33, 35, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 62

nanotechnology, 2, 24, 28, 31, 32, 34, 39, 43, 44, 52, 56, 58

natural compound, 65

natural resources, 26

negative effects, 28, 64

neglected tropical diseases, 75, 83, 84, 87, 91

nutrient absorption, 94

nutrition, 25, 94, 96, 98, 100, 104, 114, 131, 132

O

oil, 46, 55, 60, 114, 120

orchid, 37, 117, 119, 126

ornamental plants, v, viii, 93, 94, 95, 96, 98, 101, 102, 103, 104, 105, 106, 108, 109, 110, 114, 117, 118, 120, 126, 127

P

parasites, 75, 76, 78, 91

pathogens, 4, 6, 7, 15, 16, 18, 19, 21, 23, 24, 29, 45, 52, 54, 71, 73, 76, 109

pharmacological applications, 2

phenolic compounds, 13, 48, 85

photosynthetic performance, 96

physical properties, 32

physicochemical characteristics, 25

physiology, 28, 113, 116

phytopathogens, viii, 19, 78, 79, 80

phytoremediation, 42

plant disease, 80, 82

plant diseases, 80

plant growth, vii, viii, 2, 4, 6, 51, 79, 94, 101, 107, 108, 110, 114, 118, 127

plants, vii, viii, 2, 4, 6, 7, 9, 10, 11, 12, 15, 18, 19, 22, 28, 35, 36, 37, 38, 39, 42, 44, 46, 47, 48, 52, 54, 56, 57, 63, 64, 65, 66, 68, 69, 70, 71, 75, 78, 79, 82, 83, 84, 88, 90, 92, 94, 95, 96, 98, 99, 100, 106, 108, 109, 110, 111, 112, 114, 115, 116, 117, 118, 119, 120, 122, 123, 125, 126, 127, 128, 130, 137

pollution, 13, 106, 107, 126

population, 10, 11, 12, 36, 37, 69, 92, 122, 129

propagation, viii, 37, 121, 122, 132

protection, vii, 2, 7, 26, 43, 112

Pseudomonas aeruginosa, 15, 20, 73

Q

quality, viii, 36, 42, 94, 95, 96, 98, 101, 104, 106, 107, 110, 114, 115, 119, 122, 124, 127, 128, 129, 131, 132

quality improvement, viii

R

reproduction, 10, 124

requirement, 31, 110

resistance, viii, 4, 6, 19, 28, 29, 30, 36, 47, 53, 64, 71, 81, 82, 94, 95, 101, 102, 103, 105, 106, 108, 109, 110, 112, 113, 115, 116, 119, 122, 124, 125, 126, 127

response, 26, 111, 129, 130, 132

Rhizophora mangle, 68, 73

*Rhizopus*, 33, 78

rice husk, 39

root growth, 104, 127

root hair, 122

root system, 97

roots, 2, 17, 50, 66, 67, 81, 95, 97, 103, 105, 107, 111, 117, 119, 120, 121, 122, 123, 124, 128, 132, 133

S

salinity, 65, 69, 110, 116

*Salmonella*, 14, 72, 73, 74

salt concentration, 108

salt tolerance, 101, 102, 106, 109, 112, 113

seed, 12, 37, 38, 61, 106, 119, 122

seedlings, 40, 49, 59, 100, 112, 122, 123, 124, 127, 128, 129, 130, 131

silver, 25, 28, 32, 33, 38, 40, 42, 43, 44, 45, 46, 47, 48, 49, 51, 52, 53, 55, 57, 58, 59, 60, 62

species, 3, 5, 6, 7, 9, 10, 11, 14, 15, 21, 37, 44, 45, 53, 60, 64, 65, 66, 67, 68, 75, 79, 81, 83, 84, 87, 88, 90, 91, 96, 101, 102, 103, 104, 105, 107, 110, 114, 118, 130, 138

stress, viii, 6, 9, 12, 22, 24, 26, 47, 49, 51, 59, 64, 70, 95, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 119, 129, 133

structure, 3, 8, 18, 55, 67, 95, 97, 102

substrate, 120, 121, 125, 128

survival, 30, 70, 96, 122, 124, 127

survival rate, 96, 122, 124, 127

symbiosis, 95, 97, 100, 106, 109, 110, 118, 120, 130

symptoms, vii, 2, 113

synergistic effect, 19, 59

synthesis, 4, 25, 26, 27, 28, 35, 42, 43, 45, 46, 47, 48, 49, 52, 53, 54, 55, 58, 60, 62, 70

T

temperature, 10, 43, 65, 94, 95, 101, 102, 103, 109, 110, 115, 121

tissue, 2, 4, 11, 12, 26, 28, 47, 96, 102, 104, 127

toxicity, 53, 71, 76, 103, 116

treatment, 26, 27, 30, 31, 32, 61, 76, 115, 120

trial, 94, 98, 100, 107, 108, 109

U

urban, 94, 109, 110, 116

urinary tract, 59

urinary tract infection, 59

V

vegetable, viii, 118, 120, 124, 125, 126, 130

vegetables, 118

vegetation, 10

versatility, 64, 71

viral diseases, 27

viral infection, 27

virus replication, 49

W

water, 9, 22, 49, 52, 58, 76, 91, 95, 97, 101, 103, 106, 110, 118, 119, 120, 122

wound healing, 28, 45, 46, 54, 58, 59

wound infection, 31

Y

yield, 95, 96, 99, 109, 113, 114, 124, 132

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