Understanding the Biodiversity and Biological Applications of Endophytic Fungi: A Review

Yash Mishra1*, Abhijeet Singh2, Amla Batra2 and Madan Mohan Sharma1

1 Department of Biosciences, Manipal University Jaipur, PIN-303007, Rajasthan
2 Lab. No. 5, Department of Botany, University of Rajasthan, Jaipur, PIN-302055, Rajasthan

Abstract

Endophytic fungi regarded as fascinating group of organisms colonize the living internal tissues of their host usually higher plants. Endophytes do not cause any symptoms of disease in the host cells and produce natural bioactive compounds considered as an elicitor for plant secondary metabolites production. The present review is focused on the biodiversity, surface sterilization, histological localization, isolation methods, colonization frequency, natural products that provide immunity to the victims, biological roles of endophytic fungi. This highly diverse group of fungi can have profound impacts on plant communities through increasing fitness by conferring abiotic and biotic stress tolerance.

Keywords: Endophytes; Histological localization; Biodiversity and Colonization frequency; Biological roles

Introduction

The term “endophyte” originally introduced by de Bary (1866) refers to any organisms occurring within plant tissues, distinct from the epiphytes that live on plant surfaces. Endophytes have been defined by various scientists as mutualists that colonize aerial parts of living plant tissues and do not cause symptoms of disease. Mycorrhizal are endophytes but from a special type which produces external structures from the host plants. Endophytic microorganisms can be divided into two groups: those that do not generate external structures from the host (group I) and those which are able to develop external structures such as the nodules of N2-fixing bacteria and mycorrhizal fungi (group II) [1]. However, microbes those colonize living internal tissues of plants without causing any immediate harm over negative effects [2-4]. Further, true endophytes are the fungi whose colonization never results in visible diseases symptoms [5]. Furthermore, fungi which spend whole or part of their life cycle colonizing inter and/or intra cellular spaces in stem, petiole, roots and leaves, inside the tissues of healthy plants, typically causing no apparent symptoms of disease are endophytic fungi [6-10]. These endophytes are having immense potential to enhance root resistance against herbivores through the production of various secondary metabolites [11], nutrient uptake [12], and play key roles to affect host tolerance to heat [13], salinity [14], evolution [15] and plant biodiversity [16,17]. Moreover, on the basis of molecular data, fungi are much older than indicated by the fossil records and may have arisen more than one billion years ago [18].

Biodiversity of Endophytic Fungi

Endophytic fungi represent an important and quantified component of fungal biodiversity and are known to affects plant diversity [19]. Approximately, all vascular plant species established to harbor endophytic bacteria and/or fungi [20,21]. Moreover, the colonization of endophytes has already been recognized in marine algae [22,23] and mosses and ferns [24,25]. The environmental conditions in which the host is growing also affect the endophyte population [26]. In the present scenario, endophytes have been isolated from all groups of plants ranging from sea grasses [27], lichens [28], palms [29,30] to large trees [19,31]. Most endophytes isolated belong to ascomycetes & their anamorphs and basidiomycetes [32].

Certain mycorrhizal, e.g. ectendomycorrhizae, ericoid mycorrhizae and pseudomycorrhizae associated with plants of Ericaceae and Orchidaceae family have been referred to as endophytes [34,35]. They are ubiquitous and occur in all known plants, including a broad range of host orders, families, genera and species, in ecosystems viz., shrubs [36], ferns [37], mosses [38], lichens [39], grasses [40,41] and deciduous and coniferous trees [42-45]. Several efforts have been made to estimate the total number of fungi on the basis of their association with plants [46]. The magnitude of fungal diversity estimated about 1.5 million (more accurately 1.62 million) species, later revised by [47] to 2.27 million. The figure provided by Hawksworth has been widely accepted by fungal experts (Table 1) [48]. However, the number of fungal species may vary because of availability of modern tools and techniques for identification of this diverse group of endophytic fungi.

The total biodiversity of fungal endophytes may be classified in to two major categories as Basalsiaceous and non- Basalsicaeous endophytes. Further, these categories divided in to four separate classes viz., class I-IV. Basalsiaceous endophytes of grasses were first illustrated by European investigators in the late 19th century in seeds of different species of Lolium.

Class I include Clavicipitaceae endophytes and represent a small number of phylogenetically related Clavicipitaceae species that are fastidious in culture and restricted to some grasses [58,59]. However, transmission is primarily vertical with host plants pass through seed infections to the next plant [60]. The endophytes from class I frequently increase plant biomass, confer drought tolerance and produce chemicals that are toxic to animals and decrease herbivory [61].

*Corresponding author: Yash Mishra, Department of Biosciences, Manipal University Jaipur, PIN-303007, Rajasthan, Tel: +91 141-3999100; E-mail: yashmishra@muj.manipal.edu

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Class II endophytes described as mycorrhizal fungus colonized in all parts of the plant including the seed coat and did not form intracellular mycorrhizal structures. These endophytes limited to a rare number of plants and comprises of a diversity of fungal species belongs to Dikarya (Ascomycota or Basidiomycota). They have the ability to confer habitat specific stress tolerance to host plants [62].

Class III endophytes are distinguished on the basis of their occurrence and horizontal transmission. This class includes endophytic fungi from vascular, nonvascular plants, woody and herbaceous angiosperms in tropical forest and Antarctic communities. Single plant may harbor hundreds of different endophytic fungi [63-65]. 'Mycelium Radicus Astroviirens (MRA)' associated with terrestrial plant roots may harbor hundreds of different endophytic fungi [63-65]. 'Mycelium angiosperms in tropical forest and antarctic communities. Single plant fungus from vascular, nonvascular plants, woody and herbaceous occurrence and horizontal transmission. This class includes endophytic habitat specific stress tolerance to host plants [62].

Class IV endophytes have darkly melanized septa and restricted to plant roots. They are generally Ascomycetous fungi, which are conidial and form melanized structures like inter and intracellular hyphae and microsclerotia in the roots. This class of endophytes found in host plants like non mycorrhizal from Antarctica, arctic, alpine, subalpine, temperate zones and tropical ecosystems [67,68].

Both the major groups of endophytic fungi (C, NC) may be identified as grass and non-grass host type based on their contrasting characteristics (Figure 1A-1B) (Table 2).

### Histological Localization, Isolation and Pure Culture of Endophytic Fungi

Endophytic fungi grow within the healthy tissues of plants; hence at the time of histological localization and isolation fresh, wound free and disease free plant parts should be selected. Prior to isolation of endophytic fungi, explants are washed under running tap water followed by surface sterilization, which varies depending on the type of plant material and contaminants. The stem leaves (lateral and midrib) and roots were cut into segments (0.5-1.0 cm). The samples are surface sterilized by method of [68,69]. In case of roots and rhizomes, after washing under running tap water explants should be thoroughly washed using distilled water. These surface sterilized materials were further sterilized inside the laminar air flow cabinet with mercuric chloride (0.1%w/v), different concentrations of ethanol (50%, 70%, 90% and absolute) for few seconds to minutes i.e. 30 sec. to 1 min. and sodium hypochlorite (4%) for 2-3 minutes followed by rinsing with double distilled deionized sterile water to remove remnants of the sterilets and blot dried on sterile tissue paper. Since, endophytic fungi do not cause any disease symptoms in host plants and their interaction involves metabolic exchange. Hence, their presence cannot be recognized externally. Consequently, the presence of endophytic fungi within healthy tissues of plants is usually recognized through culture methods. Meanwhile to culture the fungus, the tissue needs localization via anatomical studies. Many endophytic fungi develop within specific organs with small amounts of mycelium, making such direct observations difficult. However, these fungi can be isolated from healthy tissues [69]. Various reports have been available, which revealed the presence of endophytic fungi from various plants viz., Terminalia arjuna [70], Aegle marmelos [71], Azadirachta indica [72], Catharanthus roseus [73] and Stevia rebaudiana [74]. Explants should be stored at 4°C until isolation procedures begin. The sterilized explants were cultured in Petri dishes containing Potato Dextrose Agar Medium (PDA) supplemented with 100 µg/ml of streptomycin, Ampicillin and Chloramphenicol [75-79] sealed with parafilm, incubated at 27°C ± 2°C in digital incubator to promote the growth of mycelia, under controlled conditions followed by pure culture for identification. All operations for isolation of endophytic fungi must be carried out in aseptic condition [80-88]. The growth of the endophytic fungal colonies from the plant tissues were observed every day. These endophytes can be identified on the basis of their morphological characteristics of spores and mycelium, biochemical testing and molecular characterization. Further, for morphological identification various stains can be used to identify the isolated fungal endophytes at initial level (Table 3). The histological localization, isolation and pure culture of endophytic fungi are being done in medicinally important

### Table 1: Estimation of fungal species diversity.

| Number of fungal species | Reference |
|-------------------------|-----------|
| 270,000                 | [49]      |
| 162,000                 | [48]      |
| 100,000                 | [50]      |
| 100,000                 | [51]      |
| 150,000                 | [50]      |
| 990,000                 | [52]      |
| 150,000                 | [53]      |
| 227,000                 | [47]      |
| 500,000*                | [54]      |
| 3,500,000-5,100,000**   | [55]      |
| 712,000*                | [56]      |
| 611,000 (± SE=297,000)* | [57]      |

*some additional estimates.
Endophytes of grass host Type : Endophytes of nongrass hosts Type
Few species, Clavicipitaceae : Many species, taxonomically diverse
Extensive internal colonization : Restricted to internal colonization
Occurring in several hosts : Species with limited host specificity
Systemic, seed transmitted : Nonsystemic, spore transmitted
Host colonized by only one species : Host infected by several species

Table 2: Characteristics of grass and non-grass host type endophytic fungi.

| Stain                     | Plant species                  | Part          | Reference |
|---------------------------|-------------------------------|---------------|-----------|
| Toluidine blue O          | Wheat (Erysiphe graminis f. sp.) | Leaves        | [89]      |
| Lactophenol cotton blue   | Uromyces phaseol var. vignae  | Leaves        | [90]      |
| Lactophenol cotton blue and aniline blue | Triticum aestivum | Leaves        | [91]      |
| Trypan blue & Rose Bengal | Turf and Forage grasses       | Leaves, stem  | [92]      |
| Safranin & fast green staining | Nassella neesiana (Weed) Uromyces penicanus | Leaves       | [93]      |
| Pianese III B Stain       | Cassava                       | Leaves        | [94-97]   |
| Aniline blue              | Ryegrass                      | -             | [98]      |
| Rhodamine B/Methyl green method | Acer pseudoplatanus | Plant wood    | [99]      |
| Chlorazole Black E        | Eucalypt (AM Fungi)           | Roots         | [100]     |
| KOH Aniline blue          | Fungal species                | Leaves, roots | [101]     |

Table 3: Various stains to localize endophytic fungi within plant tissues.

plant: Tinospora cordifolia in author’s laboratory. In natural conditions, disease free leaves of T. cordifolia (Figure 2A) after histological studies have shown the presence of endophytic fungi in intercellular and stomatal region (Figure 2B). After 4-5 days, in vivo leaf segments showed the emergence of endophytic fungi on PDA plates (Figure 2C). The isolated endophytic fungi cultured on separate PDA plates as pure culture (Figure 2D). The pure culture of endophytic fungi sent for identification to plant pathology laboratory IARI, New Delhi.

Natural Products from Endophytic Fungi

The search for new drugs/pharmaceutical products from microbial origin have been started since the discovery of anticancer drug “Taxol” from Taxomyces andreanae in early 1990’s and Penicillin from Penicillium notatum by W. Flemming in 1928 [102]. Both these drugs were isolated from fungi. Initially, taxol was isolated from Taxus brevifolia followed by Taxus wahlischiana, which harbor endophytic fungi viz., Taxomyces andreanae and Pestalotiopsis microsprice, respectively [103]. The discovery of these anticancer drug and antibiotic opened up new vistas to discover new drugs from biological origin. Since then, scientists have been searching an array of natural products from endophytes such as Micafungin, an antifungal agent from Coleophoma empetri [104], Rosuvastatin from Penicillium citrinum and P. brevicompactum, which are used for treating dyslipidemias [105]. Mycophenolate from Penicillium brevicompactum, which is used for preventing renal transplant rejection [106]. The chemical structures of some of these natural products have been shown (Figure 3). Further, soil fungi have been the most studied and typical soil microbes viz. Acremonium, Aspergillus, Fusarium and Penicillium have shown the ability to synthesis a diverse range of bioactive compounds. More than 30% of isolated metabolites from fungi are from Aspergillus and Penicillium [107]. Besides, 47% of total anticancer drugs and 52% of new chemicals introduced into the market are of natural origin [108-110]. Further, many of these natural products and drugs have shown potent pharmaceutical applications against various diseases. However, crude extracts of plants have been directly used as drugs, which were of low cost and important source of traditional medicines. These natural products provided the basic chemical architecture to derive semi synthetic natural products [111]. The entry of dreaded disease AIDS, Cancer and Severe Acute Respiratory Syndrome (SARS) disease needs new chemicals introduced into the market and hence the triumph of bioprospecting from endophytes [112]. Endophytic fungal diversity and specialized habituation makes them an exciting field of study in the search for new medicines or novel drugs [113].

Biological Roles of Endophytic Fungi

The ability of endophytic fungi is to produce new and interesting bioactive secondary metabolites, which are of pharmaceutical, industrial and agricultural importance. The various natural products produced by endophytic fungi possess unique structures and bioactivities against various diseases. In lieu of a huge reservoir, this offers vast potential for exploitation of secondary products for medicinal, agricultural and industrial uses.
### Table 4: List of host plants and their endophytes with isolated chemical compounds along with their biological activity.

| Host Plant | Endophytic fungi | Chemical Compound | Biology Activity | Reference |
|------------|------------------|-------------------|------------------|-----------|
| Taxus brevifolia | Taxomyces andreanae | Diterpenoid | Anticancer | [114] |
| Torrey taxifolia | Pestalotiopsis microsorpa | Torreynanic acid | Anticancer | [115] |
| Catharanthus roseus | Mycelia sterilia | Vincristine | Anticancer | [116] |
| Terminalia morobensis | Pestalotiopsis microsorpa | 1,3-dihydro isobenzofurans | Antioxidant | [117] |
| Torreya nerei | Aspergillus clavatunanicus | Clavatol | Antimicrobial | [118] |
| Taxus wallichiana | Phoma sp. | Altersolana A & α-hydroxy-6-Methyl benzoic acid | Antimicrobial | [119] |
| Melia azedarach | Penicillium janthinellum | Citrinin (Polyketide) | Antimicrobial | [120] |
| Cinnamomum zeleianicum | Muscodor albus | 1-butan,3-methyl-acetate | Antimicrobial | [121] |
| Ocimum basilicum | 2L-5 | Ergosterol , Cerevestoterol | Antimicrobial | [122] |
| Erythrina cristagalli | Phomopsis sp. | Isoflavonoids | Antimicrobial | [123] |
| Plumeria acutifolia | Colletotrichum gloeosporioides | Terpenoid | Antimicrobial | [124] |
| Plumeria acutifolia | Phomopsis sp. | Taxol | Anticancer | [125] |
| Crotopsoropsisquercina | Cryptocandin | Cryptocandin | Antimicrobial | [126] |
| Taxus baccata | Acremonium sp. | Leucinostatins | Antifungal & Anticancer agent | [127] |
| Spondias mombin | Guignardia sp. | Phomopsis sp. | Antimicrobial | [128-130] |
| Garcinia sp. | Phomopsis sp. | Phomoxanthone A & B | Antimycobacterial Drug | [131] |
| Vaccinium myrtillus | Cladoniaarbuscula | Leucopside | Antimycobacterial Drug | [132] |
| Ananas ananassoides | Muscodor crisps | Volatile organic compounds/ Propanoic acid, methyl ester,2-methyl butyl ester, Ethanol. | Antibiotic | [133] |
| Ginkgo biloba | YX-28 | 7-amino-4-Methylcoumarin | Antimicrobial | [134] |
| Agericera coniculatum | Emericella sp. | Hypercin | Anti-viral | [135] |
| Hypericum perforatum | Hypercin | Hypercin | Anti-viral | [136] |
| Scapania ciliata (Liverwort) | Aspergillus sydowii | Sydoxanthone A,B | Immunosuppressive activity | [137] |
| Garcinia hombria | Guignardia bidwelli | Guignarenones (A-D) | Cytotoxic activity | [138] |
| Rhizophora annamalyana | Fusarium oxysporum | Taxol | Anticancer | [139] |
| Tinospora cordifolia | Fusarium culmorum SVJM72 | Taxol | Anticancer | [140] |
| Viscum album (Epphytic parasite) | Fusarium oxysporum, Fusarium oxysporum, Fusarium oxysporum | Lectin | Anticancer/Antioxidant | [141] |
| Annova squamosa | Penicillium sp. | Meleargine and Chrysogine | Anticancer/ | [142] |
| Triperrygium wilfordii | Fusarium subglutians | Subglutinol A and B | Immunosuppressive | [143] |
| Triperrygium wilfordii | Rhizocladiella sp. | 22-oxa-(12)-cytochalasin | Anti-cancer | [144] |
| Terminilia morobensis | Pestalotiopsis microsorpa | 1,3-dihydroisobenzofurans | Antioxidant | [145] |
| Nothapodytes foetida | Entrophospora infrequens | Camptothecin, (9-methoxycamptothecin, 22-oxa-(12)-cytochalasins | Anticancer | [146] |
| Ephedra fasciculata | Chaetomium chiversii C5-36-62 | Radicicol | Cytotoxic | [147] |
| Erythrina crista-galli | Phomopsis sp. | iso flavonoids | Antimicrobial activity | [148] |
| Podophyllum hexandrum | Trametes hirsute | Podophyllotoxin | Anticancer agent | [149] |
| Ocimum basilicum | Phyllosticta sp.6 | Taxol | Anticancer | [150] |
| Gauzuma ulmifolia | Muscodor albus E-6 | Caryophyllene, phenylethyl alcohol, 2-phenylethyl ester, bulnessene | Antibiotic activity | [151] |
| Justicia gendarussa | Colletotrichum gloeosporioideae (strain JGC-9) | Taxol | Anticancer | [152] |
| Piptadenia adiantoides | Cochliobolus sp.(UFMGC8-555) | Cochliokinone A, isocochliokinone A. | Anti-parasitical Properties | [153] |
| Ginkgo biloba L. | Xyliya sp.XY-28 | 7-amino-4-methylcoumarin | Anti-cancer | [154] |
| Azadirachta indica A. Juss | Chloridium sp. | Javanicin | Antibacterial activity | [155] |
| Eucryphia cordifolia | Gliocladium roseum (NRRRL 50072) | 2,6-dimethyl, 3,3,5-trimethyl; cyclohexene, 4-methyl, decane, 3,3,6,trimethyl, 4,4-dimethyl(Volatile hydrocarbons) | Biofuel | [156] |
| Salvia officinalis | Chaetomium sp. | Cachlodinol, isocachlodinol | Cytotoxic activity | [157] |
| Camptotheca acuminata | Fusarium solani | Camptothecin, (9-methoxycamptothecin, 10-ydroxyxamptothecin | Anticancer properties | [158] |
| Taxus chinensis | Fusarium solani | Taxol | Anticancer | [159] |
| Gastrodia Elata | Amphilalia melia | Sesquiterpenesyrl esters | Antimicrobial activity | [160] |
| Plumeria | Phomopsis sp. | Terpenoid | Antimicrobial | [161] |
The challenges and goal are exploration of endophytic fungi to discover microbial populations, which favor plant growth and make them fit in external environment. They have emerged as a boon and left good impact on plants, environment and also human beings in numerous conceivable behaviors and are also found to have some important roles in nutrient cycling, biodegradation and bioremediation etc. A variety of biological activities of isolated natural products from endophytic fungi from different plants have been depicted (Table 4).

**Nutrient pedalling**

It is a vital process that occurs continuously to balance nutrients and make them available for every component of the ecosystem. The degradation of the dead biomasses becomes one major step to transport back utilized nutrients to the environment, which in turn again becomes accessible to the living beings. The major important roles in biodegradation to the litter of its host plants [162-169]. They have potential to breakdown complex compounds into simpler form. Another important role is bioremediation, which describes as a method of removal of contaminants and wastes from the atmosphere by the use of micro-organisms. It relies on the life processes of microbes to breakdown these wastes material and it has become possible due to countless microbial diversity.

**Phytostimulation**

Endophytes also play important roles in the uptake of essential nutrients necessary for plant growth. They elicit uptake of N [170] and in giant fescue adaptation to P deficiency [171]. A novel strain of fungus Cladosporium sphaerospermum isolated from the roots of Glycine max (L) Merr. showed the charismsa of higher amounts of bioactive GA3, GA4, and GA7, which induced maximum plant growth in both rice and soybean varieties [172]. The roles of endophytes are well documented for anchorage of plant in soil, absorption of water and ions, nutrient storage, and plant vegetative growth, the root system is in close contact with a wide range of soil microbial populations [173].

**Endophytes in tissue culture**

Endophytes are mainly valuable to the host plants and for plant tissue culture. The ultimate aim of tissue culture is to develop axenic plants. Even after surface sterilization of the explants, autoclaving and UV treatment of nutrient medium for tissue culture, endophytic bacteria or fungi or actinomycetes start growing from tissues or from the cultured explant. These endophytes are generally considered as contaminants resulting in complete loss of time, media and explants, which sometimes may be of some rare and endangered species of microbes, which need to be conserved by tissue culture techniques. Besides, endophytic species composition and plant genotype together under tissue culture conditions are the key factors for attainment of plant tissue cultures with elevated renewal capability. Interaction between the endophytes and specific secondary compounds leached from plant may be a major facet for browning and cell death [174]. Some endophytes were isolated in cultures from roots and photosynthetic tissues of plant [175].

**Antiviral activity**

The charming use of antibiotic products from endophytic fungi is the inhibition of viral growth. Two novel human cytomegalovirus protease inhibitors, cytic acids A and B were elucidated by mass spectrometry and NMR methods and found to be effective against virus growth [176]. Some metabolites from endophytic fungi of desert plants serve as a viable source for identifying potent inhibitors of HIV-1 replication [177].

**Anticancer activity**

Paclitaxel and some of its derivatives represent the first major group of anticancer agents produced by endophytes. The mode of action of paclitaxel is to preclude tubulin molecules from depolymerizing during the processes of cell division [178]. It is the world’s first billion-dollar anticancer drug and used to treat a number of human tissue proliferating diseases. Taxomyces andreanae provides an alternative for taxol production by fermentation. Cytotoxic quinone dimer, torreyeanic acid is another important anticancer agent produced from P. microspore isolated from T. taxifolia (Florida torreya). Recent studies showed that Hypocrea lixii, novel endophytic fungus produced anticancer agent cajanol, isolated from Cajanus cajan [179]. First time, the endophytic fungus M. fragilis is able to produce thesebioactive metabolites viz., podophyllotoxin and kaempferol [180]. Besides, guanacastane diterpenoids reported from the plant endophytic fungus Cercospora sp. [181].

**Antidiabetic activity**

A nonpeptidal fungal metabolite [L-783] was isolated from an endophytic fungus Pseudomassaria sp collected from an African rainforest near Kinshasha in the Democratic Republic of the Congo. The nature has provided plentiful natural resources, which can be explored for their medicinal uses. The antidiabetic and hypolipidemic activity of endophytic fungi isolated from Salvadorula oleoides (Salvadoraceae) in glucose loaded, fasting and alloxan induced diabetic Wistar albino rats [182] and investigated new antidiabetic drugs from fungal endophytes such as Aspergillus sp., Phoma sp. and some unidentified species; those significantly reduce blood glucose level by glucose tolerance test. a-amylase inhibitor retards the liberation of glucose from dietary complex carbohydrates and delays the absorption of glucose to isolate and select a-amylase inhibitor-producing endophytic actinomycetes from the leaves and stem of Leucas ciliata and Rauwolfia densiflora, two of the well-known medicinal plants used in the treatment for diabetes [183].

**Immunosuppressive activity**

An endophytic fungus Fusarium subglutinans isolated from T. wilfordii produces subglutinol A and B, which act as the immunosuppressive agent. These drugs are used today to prevent allograft rejection in transplant patients and in near future they could be used to treat autoimmune diseases such as rheumatoid arthritis and insulin dependent diabetes [184,185]. Pestaloside and two pyrones: pestalopyrone and hydroxyl pestalopyrone isolated from P. microspore possess phytotoxic properties [186]. Pseudomycins is antifungal compounds, which were very effective against human pathogen, Candida albicans. These are peptide antibiotics containing unusual aminoacids like L-hydroxy aspartic acid, L-chlorothreonine and both D- and L-diaminobutyric acid [187]. Ambuic acid a cyclohexenone aminoacids like L-hydroxy aspartic acid, L-chlorothreonine and both D- and L-diaminobutyric acid [187]. Ambuic acid a cyclohexenone belongs to the family of pseudomycins isolated from Pestalotiopsis microspore and found effective against human pathogens. Munumbicins is bioactive ingredients isolated from streptomycetes species. These are very much effective against both gram-negative and gram-positive bacteria. Munumbicins E-4 and E-5 showed antimarial activity, which was very effective and double than that of chloroquine [188].
Interactions among insect pathogenic fungi, plants and insects Activity

The potential of colonizing internal host tissues has made endophytes precious for agriculture as a tool to advance crop performance. For the first time a correlation between an endophytic fungus, Epichloe typhina and the toxicity of its host, F. arundinacea, to herbivorous domestic mammals [189]. The interface between nitrogen fertilization, pests and the endophytic fungus A. coenophialum showed that in a wide-ranging manner, insects like S. frugi perda developed better in nitrogen containing plants not infected by the endophyte. Though, taking into consideration blocks insect development. The results, therefore, are quite variable and do not permit us to draw any general correlation between nitrogen fertilizer and endophyte-mediated pest control in F. arundinacea that will work in all belongings [190]. The verified interactions among several factors like nutrient levels and plant damage during endophytic fungi control of S. frugi perda in the host F. arundinacea [191]. However, protecting plants against pests and environmental stresses, found in temperate isolates of endophytes expected the new ways of interactions [192].

Endophytic fungus, Muscodor albus, produces a mixture of VOCs that are lethal to a wide variety of plant and human pathogenic fungi and also effective against nematodes and certain insects [193]. Microbial Biocontrol Agents (BCAs) are generally used for controlling plant diseases via antagonistic mechanisms including competition, antibiosis, parasitism, and cross-protection. Some BCAs can even promote plant growth, and provide Induced Systemic Resistance (ISR), i.e., induce the plants to have resistance against pathogens including phytopathogenic fungi, bacteria and virus, and in some cases, pest insects and nematodes. ISR is characterized by non-specific, wide spectrum and systemic [194]. Codling moth, Cydia pomonella, a serious pest of pome fruit, is a threat to exportation of apples (Malus spp.) because of the possibility of shipping infested fruit. The need for alternatives to fumigants such as methyl bromide for quarantine security of exported fruit has encouraged the development of effective fumigants with reduced side effects [195]. Metarhizium robertsi is a plant root colonizing fungus that is also an insect pathogen. Its endomycorhizal ability to synthesize several innovative bioactive compounds. However, attention have been made towards endophytic fungi because of their alternative and sustainable sources of these compounds and special compounds of pharmaceutical significance, which is currently attracting scientific surveys worldwide. Every plant in the world is reservoir of one or more number of endophytes. In nature, plants seem to be in a close interface with endophytic fungi. The construction of bioactive compounds by endophytes, particularly those restricted to their host plants are significant both from the biochemical and molecular point of view. Secondary metabolites produced by endophytes (including those produced by plants) fosters expectations of utilizing them as alternative and sustainable sources of these compounds and special attention have been made towards endophytic fungi because of their ability to synthesize several innovative bioactive compounds. However, the commercial production of desirable compounds by endophytic fungi still remains a future goal. The symbiotic association of host–endophyte relationships at the molecular and genetic levels will be helpful for enhancing secondary metabolite production by endophytic fungi under laboratory conditions. Further research at advanced molecular level may offer better visions into endophytic biodiversity. Hence, a rigorous search for more and amended antibiotics for effective treatment has become an emerging area of research.

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