

Fungal Endophytes: An Alternative Biocontrol Agent against Phytopathogenic Fungi

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There has been renewed interest in the application of endophytic fungi to control phytopathogenic fungi, which cause significant damage to crop health, ultimately leading to losses in agricultural productivity. Endophytic fungi inhibit pathogens via different modes of action—mycoparasitism, competition (for nutrients and ecological niches), antibiosis, and induction of plant defense—thus demonstrating the ability to control a wide range of phytopathogenic fungi in different growth phases and habitats. However, many studies have been conducted under laboratory conditions, and there is a huge lack of studies in which real field testing was performed. *Aspergillus*, *Clonostachys*, *Coniothyrium*, *Trichoderma*, and *Verticillium* have been proven to be the most effective fungal biocontrol agents. *Trichoderma* is regarded as the most promising group in commercial formulations. This study attempted to emphasize the significance of fungal endophytes in controlling phytopathogenic fungi, while reporting recent advances in endophytic biology and application.

Keywords: biocontrol agents ; dual culture method ; mycoparasitism ; plant disease ; *Trichoderma*

Phytopathogenic Fungi and Their Significance

The exponential growth of the human population is one of the major problems facing today's world. More than 7.884 billion people are alive today ^[1], and it is predicted that the population will reach 9.2 billion by 2050 ^[2]. Feeding this growing population is becoming a huge problem, putting enormous pressure on various agricultural production systems. Land expansions for agriculture may not be always possible, although increasing the production per hectare and reducing harvest losses due to various biotic and abiotic factors would be ideal ^[3]. In this regard, the key roles of pests are significant, since according to the Food and Agriculture Organization of the United Nations (FAO) ^[4], plant diseases are responsible for about USD 220 billion in annual losses to the world economy. Plant diseases result in the loss of 10–42% of the world's major crops ^[5]. Of these diseases, 70–80% are caused by pathogenic fungi ^[6]. Several fungal epidemics have been reported throughout the history of agriculture. The coffee rust epidemic in the 1870s due to *Hemileia vastatrix* caused a huge drop in the harvest in eastern Africa and Ceylon (now Sri Lanka). At the time, Ceylon was the leading coffee exporting country in the world; however, coffee production fell from 45 million kg in 1870 to 2.5 million in 1889. This island nation could not recover its production, and tea plantations replaced the majority of the coffee producing land. This scenario brings changes in the beverage habits of British people, from coffee to tea ^[7]. In 1943, the Great Bengal Famine, particularly due to the brown spot disease in rice caused by the *Cochliobolus miyabeanus* (formerly known as *Helminthosporium oryzae*, current name *Bipolaris oryzae*), led to the death of nearly three million people, who suffered from severe hunger owing to the yield reductions of their staple food, rice, by up to 92% ^[8]. While the southern corn leaf blight epidemic in 1970 caused by *Cochliobolus heterostrophus* (also known as *Bipolaris maydis*, previously *Helminthosporium maydis*) caused corn losses of up to 50% in the USA ^[9]. At present, *Blumeria graminis*, *Botrytis cinerea*, *Colletotrichum* spp., *Fusarium graminearum*, *F. oxysporum*, *Magnaporthe oryzae*, *Melampsora lini*, *Mycosphaerella graminicola*, *Puccinia* spp., and *Ustilago maydis* are the top ten fungi groups based on the scientific/economic importance and possess great potential to emerge as devastating disease-causing agents ^[10]. Most fungal plant pathogens belong to the phyla Ascomycota and Basidiomycota. Fungal plant pathogens can be classified into several classes among Ascomycota, such as the Dothideomycetes (e.g., *Cladosporium* spp.), Sordariomycetes (e.g., *Magnaporthe* spp.), and the Leotiomycetes (e.g., *Botrytis* spp.). Rusts (Pucciniomycetes) and smuts (spread among the subphylum of Ustilaginomycotina), the two major plant pathogen groups, belong to the Basidiomycota ^[11]. These fungi can cause damage to plants as well. Upon infection, phytopathogenic fungi interfere with plant metabolism and affect their normal/regular functions (and hence diseases) by producing enzymes, toxins, and other metabolic inhibitors such as hormones or absorbing nutrients from the host plants by growing internally or externally to the plant host ^{[6][12]}. Plants switch to active defense mechanisms to counteract the virulence factors of the fungi, while fungi may not resist or die; in contrast, if plants succumb to the virulence of the fungi, plants get sick, and the fungi become phytopathogenic ^{[13][14]}.

Endophytic Fungi and Their Benefits

The term endophyte was first coined by De Bary in 1866. He referred to any organism that grows within plant tissues as an endophyte. Endophytes are generally not considered pathogens, and they often form a symbiotic relationship with plant hosts without causing any immediate adverse effects or disease symptoms [15][16][17]. Those endophytes that do not cause diseases in plants are called true endophytes, while others cause diseases in plants at some stage of their lifecycle due to various reasons, such as the weakening of the plant, environmental changes, and the type of host. For example, *Fusarium* species are found as harmless (latent) endophytes in carrots, though they cause head blight disease in cereals. Similarly, *Ramularia collo-cygni*, which causes necrotic disease in barley, can be found as a harmless endophyte in many other cereals [18].

Based on their phylogeny and life cycle characteristics, endophytic fungi have been divided into two major groups: clavicipitaceous (infecting some grasses limited to cool regions) and non-clavicipitaceous endophytes (from asymptomatic tissues of non-vascular plants, ferns and allies, conifers and angiosperms, also restricted to the Ascomycota or Basidiomycota) [19]. They have coexisted with plants for over 400 million years [20]. Today, over 300,000 plant species have been identified, and it is believed that each of them harbors at least one endophyte [21]. They propagate horizontally or vertically, thus increasing their survivability [22][23]. They have also been extensively examined in a range of geographic and climatic regions that are home to many ecological habitats from xeric to arctic, temperate to tropical forests, grasslands to croplands, and savannahs [24].

The benefits of endophytes to their host plants are significant; in their review, Baron and Rigobelo [25] described the advantageous role of endophytic fungi in depth. Endophytic fungi give direct benefits to their plant host (through mutualistic symbiosis interactions) by enhancing nutrient acquisition (e.g., N, P, K, Mg, and other macro and micronutrients), siderophore production, phytohormone productions for plant growth and development (e.g., auxins, cytokinins, gibberellin), and increasing the photosynthetic activity of the plant. They also offer many indirect effects such as an increase in secondary metabolites (e.g., alkaloids, steroids, terpenoids), improve protection against abiotic stresses (e.g., drought, heavy metal, salinity, temperature), biotic stresses (e.g., microbial pathogens, herbivores animals, and other insect pests), and trigger plant active defense mechanisms. The collective effect of a few or more of them thereby supports improvements in the fitness of the plant and also promotes plant growth and physiology, making the host plant robust, and leading to the inhibition of phytopathogens [26].

Today, the potential to use endophytes as a valuable source of novel products for utilization in agriculture, medicine, and other industries has been well identified [27]. The pharmacological properties of major bioactive compounds synthesized by endophytic fungi have been recognized; for example, Stierle and co-workers [28] identified that *Taxomyces andreanae* (associated with *Taxus brevifolia*) has the ability to synthesize taxol, which has an antitumor effect against breast and ovarian cancers. Many other anti-cancer drugs have been isolated, such as Asperfumoid (from *Aspergillus fumigatus*), Aspernigerin (from *Aspergillus niger*), and Camptothecin (from *Fusarium solani*, *Entrophospora infrequens*, *Neurospora* sp., *Nodulisporium* sp.) [29]. Antidiabetic, antifungal, antimalaria/antiparasite, antimicrobial, antioxidants, antiviral and immunosuppressive properties of endophytic fungi have also been identified [30]. The utilization of endophytic fungi in agriculture as biofertilizers [31] and biocontrol of pests [32] has been well documented in recent years. Endophytic fungi are also recognized as a potential source of industrial enzyme producers, including in the food and biofuel industries [33].

This review attempted to discuss the application of endophytic fungi to control phytopathogenic fungi. First, the review discussed the current requirements of endophytes as biocontrol agents. Second, the review stressed some basics of endophytic fungi in order to better understand their biocontrol ability, along with successful studies in this respect. Finally, the mode of action of endophytic fungi in controlling phytopathogenic fungi is described.

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