# Biocontrol Agents for Fungal Plant Diseases Management


**Younes M. Rashad and Tarek A. A. Moussa**

**Abstract** Plant fungal diseases are the most destructive diseases where the fungal pathogens attack many economic crops causing yield losses, which affect directly many countries' economy. The great Irish Famine in 19th century was due to potato (a great portion of Irish diets) was attacked by an oomycete pathogen *Phytophthora infestans* causing late blight disease which destroyed the potato crop for several years (1845–1852). Since this date the plant fungal diseases have a great attention from the researchers. Control of fungal diseases using different fungicides has dangerous effects on human beings as well as animals by precipitating in the plant tissues and then transfer to human and animals causing many health complications. Hence, the biological control of plant pathogenic fungi became the most important issue, due to the chemical risk to control the fungal diseases. From 1990's the importance of using microorganisms was increased as biocontrol agents to decrease the chemical uses and their hazardous for human and animal health topics. In this chapter, using of different microorganism as biological control agents of plant fungal diseases were reviewed, as well as using chemicals in controlling fungal diseases and their effects on plants, environment and common health impacts.

**Keywords** Fungal pathogens · Biological control · Chemical control · Biocontrol agents

## 1 Introduction

Fungi are non-chlorophytic, spore-forming, eukaryotic organisms. Most of the fungal species are saprophytes. So, about 20,000 species out of more than 100,000 fungal species are parasites causing diseases in crops [1–4]. Most of plants may be attacked

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N. El-Wakeil et al. (eds.), *Cottage Industry of Biocontrol Agents and Their Applications*, https://doi.org/10.1007/978-3-030-33161-0_11

by one or more species of fungal pathogens. On the other hand, the fungal species can attack only one plant species (Specialist) or many plant species (Generalist).

In the last century, most of diagnostic characters used in the identification of the phytopathogenic fungi were not evidently accurate, so any identifying character such as type of fruiting body, spores can scope the search for a particular phylum. Most diagnosis depends on visual signs and symptoms for diagnosis of fungal diseases [5]; therefore, there were many problems and difficulties in combating these pathogens. It is very important to identify the plant fungal pathogens to know their taxonomic groups, which affects significantly for managing these pathogenic fungi.

This chapter is concerned with the use of biological control agents instead of chemical control against the fungal plant pathogens. The biological control has many advantages in relation to soil fertility, plant, animal and human health.

## 2 Fungal Pathogenesis

Fungal pathogenesis is the stage of disease in which the pathogenic fungus is in close association with the tissue of host. There are three stages:

1. Inoculation: the transfer of pathogenic fungus to the infection area, in which the plant is invaded (the infection area may be natural openings such as stomata, hydathodes, or lenticels), wounds or unbroken plant surface.
2. Incubation: the period between the invasion of the pathogenic fungus and the symptoms appearance.
3. Infection: the appearance of symptoms associated with the establishment and pathogen spread.

Fungal pathogens cause symptoms which may be general or localized. In most cases, necrosis of host tissue, stunting, distortions and plant tissue abnormality and organs changes as a result of fungal infections [6].

One of the important pathogenic fungi characteristics, is virulence (infection ability). There are many properties of a fungal pathogen that contribute the ability to spread and destroy the tissue. Most of the virulence factors are enzymes to destruct plant cell walls [7–9], toxins which are cell killers, exopolysaccharides to block the path of cell fluid [10, 11], and many substances which interfere cell growth. The pathogenic species differ in virulence and hence the substances which involved in the invasion and destruction of host tissue.

## 3 Control of Fungal Diseases

The fungal plant diseases control is critical to the safe food production, and it cause serious problems in the use of land for agricultural, water, and other inputs. Plants

carry inherent disease resistance in both natural and cultivated systems, so control of fungal diseases is successful for many crops [12].

## *3.1 Chemical Control*

Along the years, many chemicals have been used to control fungal plant pathogens. Some of these have been substituted as cheaper, effective, or less hazardous substances [13]. Pruning cuts, stumps and wounds can be protected against fungal pathogens by painting with special chemicals on the surfaces exposed to environment. Plant structures such as tubers, cuttings, rhizomes, bulbs and corms which used in vegetative propagation, are often immersed in chemicals before planting. In case of trees fungal infections, fungicide was injected inside trees or by pouring into a hole made into the tissues.

Most of chemicals have been used as fungicides, where they interfere with many metabolic processes in fungal cells. The biological activity of a fungicide is restricted to its metabolism in the fungal cell and the chemicals that are transported within the plant was affected by metabolism of the plant cell. Many fungicides have low toxicity to mammals [14].

Antibiotics are chemical substances produced by microorganisms which are capable of injuring or destroying living organisms. They have been used worldwide to control bacterial and fungal diseases where many ordinary plant protection methods have failed. On the contrary, there are few antibiotics are used to control plant fungal diseases [15].

The development of resistant strains of fungi to chemicals was discussed in the 1970s and the community became aware with health and environmental impact of these chemicals in l980s and 1990s. The use of agricultural chemicals causes significant public health problems [16]. The worry about the risk of humans and domestic animals poisoning, livestock products contaminations, their impact on the beneficial insects, hazardous residue in food products, ecological imbalances at the level of microorganism and the possibility of contamination of water with subsequent fish loss and buildup of residues in groundwater. For that reasons, fungicides should be avoided and be used only in the heavy infection situations [17].

El-Abyad et al. [7] concluded that under pyradure stress, the virulence of sugar beet pathogens *Rhizoctonia solani* and *Sclerotium rolfsii* was reduced in vivo and in vitro. The reduction in the virulence of *R. solani* and *S. rolfsii* was due to decreased inoculum potential of the two pathogens under pyradure stress in situ and production of cell wall degrading enzymes in vitro. Under salinity stress, the resistance shown by the sugar beet cultivars against infection by *R. solani* and S. *rolfsii* was to be due to the maturation of cell wall composition of these cultivars with age [8].

## 3.2 Biological Control

Owing to the hazardous effects inflicted by chemical fungicides on non-target organisms and the surrounding environment, many researchers have focused during the last few decades on finding an alternative option for control of fungal plant diseases, that is, biological control. The broad definition of biological control is "suppression of pathogenic organisms and reducing their effects on hosts as well as favoring the crops beneficial organisms using wild or modified organisms, genes, gene products, or biological induction of systemic resistance" [18]. Biological control agents include many antagonistic microorganisms such as fungi, bacteria, or viruses [19].

### 3.2.1 Bacteria as Biocontrol Agents

Numerous bacterial species are extensively utilized as biological control agents to control of several phytopathogenic fungi. In addition, these bioagents have many beneficial effects on the treated plants. Members of many bacterial genera, epiphytic and/or endophytic, are used in this concern. The most common bacteria utilized as bio-control agents include some species of the genera *Bacillus, Pseudomonas, Streptomyces, Rhizobium, Burkholderia, Gluconobacter, Azoarcus, Herbaspirillum,* and *Klebsiella* [20, 21].

*Bacillus* spp.

*Bacillus* Cohn (Firmicutes, Bacillales, Bacillaceae) is a genus of gram-positive, aerobic, rods (bacilli) bacteria, which can form spores, and comprises 377 species and 8 subspecies [22]. Members of this genus have a wide distribution and found in soil, decaying matter, water, air, in/on living plants and animals, and in some severe habitats [23]. *Bacillus* spp. have a great importance and been involved in many uses in agricultural, industrial, and pharmaceutical applications such as production of diverse antibiotics, lipopeptides, enzymes, and bioactive secondary metabolites [24, 25]. Several antibiotics are known to be produced by *Bacillus* spp. such as fengycin, sublichenin, subtilosin A, gramicidin, sublancin, bacillomycin, tochicin, bacitracin, polymyxin, bacilysocin and neotrehalosadiamine [26, 27]. A broad set of hydrolytic enzymes are produced also by *Bacillus* spp. like chitinases, β-1,3(4)-glucanase, proteases, and lipases [28, 29]. The high capability of *Bacillus* spp. for production of these diverse of structurally and functionally different antagonistic substances make them pioneers in the field of the bio-fungicides. Moreover, most of *Bacillus* spp. utilized as biocontrol agents possess a growth enhancing activity on the host plant. Of the world biopesticides market, commercial *B. thuringiensis*-based products share about 90% [30].

Several studies have elucidated the use of *Bacillus* spp. in the biological control of different pathogenic fungi [28, 31–33]. The most common *Bacillus* spp. utilized

in biocontrol of plant diseases include *B. subtilis*, *B. thuringiensis*, *B. fortis*, *B. amyloliquefaciens*, *B. vallismortis*, *B. pumilus*, *B. sphaericus*, *B. cereus*, *B. licheniformis*, *B. polymyxa*, *B. megaterium*, *B. mycoides*, *B. mojavensis*, and *B. pasteurii* [25, 34]. Chen et al. [35] investigated the antifungal activity of the potent strain *B. velezensis* LM2303 which achieved a control efficiency of 72.3% against wheat *Fusarium* head blight caused by *F. graminearum*, in the field. Moreover, this strain showed antagonistic potency in vitro against different pathogenic fungi. Genomic mining of *B. velezensis* LM2303 results in identification of 13 biosynthetic gene clusters encoding for antimicrobial substances (fengycin B, iturin A, surfactin A, butirosin), as well as siderophores (bacillibactin and teichuronic acid). Furthermore, encoding-genes responsible for root colonization, growth enhancement, and immune system induction were identified. Generally, the direct biocontrol mechanisms exerted by *Bacillus* spp. against the phytopathogenic fungi include antibiosis via biosynthesis of various antifungal substances (antibiotics, lipopeptides, enzymes), competition for space and/or nutrients by colonizing the plant surface or production of various siderophores, while, the indirect mechanisms include induction of the plant systemic resistance leading to triggering many fungitoxic substances such as phenolic compounds and defense-related enzymes, as well as plant growth promotion via inducing the biosynthesis of plant growth regulators [34].

### *Pseudomonas* Spp

*Pseudomonas* Migula (Gammaproteobacteria, Pseudomonadales, Pseudomonadaceae) is a genus of aerobic, gram-negative, rods, motile bacteria, which cannot form spores, and contains 254 species and 18 subspecies [22, 36]. *Pseudomonas* spp. can resist diverse biotic and abiotic extreme conditions, use numerous organic substances, and exhibit high metabolic and physiological diversity. Owing to their elevated resistances, they can inhabit a wide range of habitats such as soil, aquatic environments, and air, in/on plants or animals [37]. This distribution is ascribed to the capability to synthesize a long list of antagonistic substances enabling them to compete with the surrounding microbiota such as phenazines, pyochelin, rhizoxins, pyrrolnitrine, hydrogen cyanide, 2,4-diacetylphloroglucinol, and pyoluteorin [38]. Although some members of the genus *Pseudomonas* are phytopathogenic, many are of great benefit providing the plant with protection against the attacking pathogens.

The biocontrol mechanisms utilized by *Pseudomonas* spp. include rivalry for nutrients and space, biosynthesis of antagonistic substances and enzymes, or by triggering plant immune system against various pathogenic fungi [39]. Furthermore, some *Pseudomonas* spp. promote the plant growth, and inhibit soil-borne pathogens [40]. Roles of *Pseudomonas* spp. in enhancing the plant growth include biosynthesis of growth regulators, nitrogen fixation, phosphate mineralization, as well as sequestering iron by secretion of siderophores [41]. Many *Pseudomonas* spp. are widely utilized as bioagents against many fungal diseases and commercially represent a big sector in the biopesticides market. Aielloa et al. [42] studied the biocontrol ability of the endophyte *P. synxantha* DLS65 against the postharvest brown rot of stone fruit

in vitro and in vivo. A considerable growth suppression of both fungi was achieved by using *P. synxantha* in vitro. In addition, a significant reduction in the disease symptoms was also reported in the storage even after 20 days at 0 °C. The rivalry for nutrients or space, secretion of fungitoxic substances or volatile organic compounds were named to be a projected as biocontrol mechanisms by *P. synxantha*.

### *Streptomyces* spp.

*Streptomyces* Waksman and Henrici (Actinobacteria, Actinomycetales, Actinomycetaceae) is a bacterial genus which include aerobic, filamentous, gram-positive species that produce fungus-like mycelia and aerial hyphae with branches that carry chains of spherical to ellipsoidal spores [43]. Currently, this genus comprises 848 species and 38 subspecies with annual increase in the species number [22]. Members of genus *Streptomyces* have wide distribution and found in various habitats such as soil, water, decaying vegetation, endophytic, epiphytic, even in extreme habitats such as deep-sea sediments, volcanic soils, frozen soils, and desert soils [44, 45]. *Streptomyces* spp. are highly recognized as antibiotics, enzymes, and bioactive secondary metabolites producers [46, 47]. Indeed, antibiotics produced by *Streptomyces* genus represent the largest share, approximately two-thirds, of the known antibiotics so far, and their number has exponentially increased every year [48, 49]. The most common antibiotics identified from *Streptomyces* spp. are streptomycin, pimaricin, neomycin, phenalinolactones A-D, cypemycin, warkmycin, and grisemycin [50, 51]. Various enzymes are also reported to be produced by *Streptomyces* spp. like chitinases, proteases, peroxidases, β-1,3 glucanases, laccases, and tyrosinases [46, 52, 53]. Furthermore, a large set, around 7600, of bioactive compounds synthesized by *Streptomyces* spp. like anticancer, antiviral, antihypertensive, immunosuppressive, and antioxidant were also reported [54].

Biocotrol of phytopathogenic fungi using members of genus *Streptomyces* has been extensively investigated by various researchers [55–57]. Different species are common in this concern such as *S. lydicus*, *S. vinaceusdrappus*, *S. griseoviridis*, *S. griseorubens*, *S. tsusimaensis*, *S. griseofuscus*, *S. spororaveus, S. tendae, S. humidus*, *S. hygroscopicus*, *S. caviscabies*, *S. philanthi*, *S. sindeneusis*, and *S. flavotricini* [58–61]. Of sixteen endophytic actinobacteria screened for their fungitoxic effect against pathogenic mycoflora, *S. asterosporus* SNL2exhibited the strongest antifungal activity in vitro, especially against *F. oxysporum* f. sp. *radicis lycopersici*, the causal agent of tomato root rot [62]. Moreover, application of this isolate led to a considerable reduction the severity of tomato root rot by 88.5%. In another study, the fungitoxic activity of the cultural secondary metabolites produced by *S. griseorubens* E44G was evaluated in vitro on the growth and ultrastructure of mycelial cells of *F. oxysporum* f. sp. *lycopersici* [63]. Investigations using the transmission electron microscope showed many noxious effects in the fungal mycelia after treatment with the culture filtrate at 400 μL.

The ultra-cytochemical study revealed the digestion of chitin of the cell wall after the exposure to the bacterial filtrate, indicating the production of the lytic enzyme

chitinase by *S. griseorubens* E44G as a biocontrol mechanism. The biocontrol modes of action utilized by *Streptomyces* spp. include physical contact (hyperparasitism), rivalry for space/nutrients, antibiosis via biosynthesis of hydrolytic enzymes, antibiotics and fungitoxic substances [56]. Indirect mechanisms via triggering plant resistance, and/or improving the plant growth may be involved also [57]. However, the biocontrol mechanisms used by a biocontrol agent are affected by the other conditions like soil type, temperature, pH, humidity, and existence of surrounding microorganisms [61].The *S. aureofaciens* filtrate was inhibited the germination of *F. solani* and in vivo seed coating was the most efficient method for controlling the pathogenicity of *F. solani* by *S. aureofaciens* [64].

*Rhizobium* spp.

Members of *Rhizobium* Frank (Alphaproteobacteria, Rhizobiales, Rhizobiaceae) are aerobic, rod-shaped, gram-negative, motile, non-spore producing, nitrogen-fixing bacteria, which comprises 112 species. *Rhizobium* spp. are widely distributed and found as free-living in soil or colonize legumes roots forming nodules, nitrogen-fixing symbioses [22, 65]. Members of genus *Rhizobium* are categorized according to their associated leguminous plant, and growth rate. The most known species include *R. leguminosarum*, *R. phaseoli*, *R. trifolii*, *R. lentis*, *R. japonicum*, *R. aggregatum*, and *R. sullae*. In addition to nitrogen fixating and growth enhancing effects (phytohormones biosynthesis), *Rhizobium* spp. are well known as biological control agents against numerous pathogenic mycoflora like *Rhizoctonia solani*, *F. solani*, *F. oxysporum*, *Macrophomina phaseolina*, *Sclerotinia sclerotiorum*, *Pythium* sp. and *Sclerotium rolfsii* [66–68].

The antagonistic modes of action utilized by *Rhizobium* spp. include rivalry for space and nutrients by secretion of siderophores, in addition to antibiosis via production of antibiotics such as bacteriocins and trifolitoxin, lytic enzymes, and fungitoxic substances such as hydrogen cyanide. Furthermore, triggering of plant immune system against attacking pathogens is widely reported for many species of *Rhizobium* via induction of hypersensitivity responses, defense-related genes, and production of antifungal compounds and molecules [69]. Volpiano et al. [70] investigated the antagonistic activity of different *Rhizobium* strains toward *S. rolfsii* in vitro and in vivo. A mycelial growth inhibition up to 84% in vitro and a significant decrease in the incidence of collar rot of common bean by 18.3 and 14.5% in the pot and field experiments were reported by strains SEMIA 439 and 4088. In addition, the antagonistic mechanism through volatile compounds by strain SEMIA 460 was also reported. Hemissi et al. [71] investigated the antifungal potential of some *Rhizobium* strains against *R. solani* in vitro and the incidence of *Rhizoctonia* root rot of chickpea under greenhouse conditions. Among the 42 tested *Rhizobium* strains, 24 isolates exhibited varied extent of antifungal activity against *R. solani* in vitro. Biosynthesis of fungitoxic substances and phosphorous solubilization were recognized as biocontrol mechanisms by some tested *Rhizobium* strains. In addition, a considerable disease reduction was recorded by applying these strains.

Others

Other genera including *Burkholderia, Gluconobacter, Azoarcus, Herbaspirillum,* and *Klebsiella* are known also as antifungal agents against phytopathogenic fungi, and plant growth-promoting rhizobacteria [72, 73]. Many *Burkholderia* species are known to produce antifungal substances like phenazine iodinin, and hydrolytic enzymes. Rivalry for space and/or nutrients with other microorganisms and triggering plant immunity against pathogens were also reported. Anti-spore germination activity by *Burkholderia* spp. was recorded against spores of *Penicillium digitatum*, *S. sclerotiorum*, *Aspergillus flavus*, *A. niger*, *Phytophthora cactorum*, and *Botrytis cinereal* [74]. Detoxification and degradation of the virulence factor of a pathogen is another biocontrol mechanism utilized by some bacterial biocontrol agents. Some strains of *B. cepacia* and *B. ambifaria*have the ability to hydrolyze the mycotoxin fusaric acid, responsible for root rot and wilt diseases, which produced by some pathogenic *Fusarium* spp., as well as inhibit their mycelial growth [75]. Detoxification of fusaric acid by *K.oxytoca* was reported also via biosynthesis of detoxificating proteins that attach to the toxins [76].

The biocontrol activity of *B. gladioli* pv. *agaricicola* was studied against *Verticillium dahliae*, in vitro and in situ on tomato [77]. A significant fungitoxic effect was recorded by the bacterial strain ICMP12322 in vitro against the pathogenic fungus. In addition, a considerable disease reduction was achieved by application of this strain in the pot experiment. In another study, Bevardi et al. [78] reported a potent antagonistic activity by *G. oxydans* against the blue mold fungus *P. expansum.* A pronounced inhibition in the fungal growth up to 95% was achieved in vitro test. In vitro biocontrol activity of three growth-promoting rhizobacteria *Azospirillum brasilense* SBR, *Azotobacter chroococcum* ZCR, and *K. pneumoneae* KPR was investigated against the pathogenic mycoflora *F. oxysporum, S. sclerotiorum,* and *Pythium* sp. and in pots on cucumber [79]. A significant inhibition in fungal growth up to 100% in vitro and 56% decrease in the damping-off incidence were recorded by applying the tested bacterial biocontrol agents.

### 3.2.2 Fungi as Biocontrol Agents

Many antagonistic fungi have been extensively utilized as bio-fungicides against various phytopathogenic fungi. Owing to their widespread occurrence, persistence, multifunctional antifungal activities against plenty of pathogenic mycoflora, and relative ease of culturing and maintenance in vitro, they have attained a broad approbation in this concern. The most common fungi used as bio-control agents include members of the genera *Trichoderma*, *Gliocladium*, *Clonostachys*, *Penicillium*, *Chaetomium*, *Myrothecium*, *Laetisaria*, *Coniothyrium*, and arbuscular mycorrhizal fungi.

*Trichoderma* spp.

*Trichoderma* Pers. (Ascomycota, Sordariomycetes, Hypocreales) is a prevalent fungal genus of increasing interest due to their diverse bioactivities, global distribution, varied metabolites production, and competitive and reproductive potentiality. Members of *Trichoderma* found mostly in all types of ecosystems as soil-borne, on decaying plant materials, endophytic, epiphytic, on other fungi, and/or in aquatic habitats [80–83].

Many species of *Trichoderma* genus are geographically limited, some are widely distributed, while, few have a cosmopolitan distribution [84]. According to Bissett et al. [85], more than 250 of *Trichoderma* spp. have been listed. However, in the recent few years, more than 45 new species have been described [86–93]. Species of genus *Trichoderma* can synthesis several hydrolytic enzymes and antimicrobial substances which provide them with ecological dominance under varied environmental conditions and the ability to perform many biological functions. One of the most important characteristics of *Trichoderma* spp. is the high and numerous potentialities to antagonize a broad spectrum of fungal phytopathogens which qualify them as the most common bio-control agents. Indeed, commercial *Trichoderma*-based products represent more than 50% of fungal bio-fungicides market.

During the last years, use of *Trichoderma* spp. as bio-fungicides against various phytopathogenic fungi has attracted high scientific attention [94–96]. For example, El-Sharkawy et al. [97] studied foliar application of two isolates of *T. harzianum* and *T. viride* as bio-fungicides against wheat rust under greenhouse conditions. A significant anti-spore germination of *Puccinia graminis* uredospores was recorded in vitro. Under greenhouse conditions, a considerable reduction in the disease measures and improvement of wheat growth and yield parameters were reported. The antifungal activity was attributed to their production of some antifungal secondary metabolites. The antifungal potentiality of *T. harzianum* WKY1 against *Colletotrichum sublineolum*, causative of sorghum anthracnose, was studies by Saber et al. [98]. In vitro, a pronounced growth inhibition in the mycelia of *C. sublineolum* was recorded as well as a decrease in the disease severity under greenhouse conditions.

Both direct and indirect biocontrol mechanisms evolved by *Trichoderma* species have been discussed including rivalry for space or nutrients, antibiosis, and mycoparasitism. In addition, triggering of plant immune responses and enhancement of their growth were also reported [99]. However, predominance of one mechanism does not mean that the others are not contributed to the antagonistic behavior of the bioagent. Production of a large set of enzymes like cellulases, amylases, lipases and pectinases, as well as secondary metabolites such as siderophores, in addition to their high reproductive capacity provides *Trichoderma* spp. with antagonistic ability to compete the fungal pathogens for space and/or nutrients [100].

Biosynthesis of numerous antifungal lytic enzymes [101], as well as various antibiotic, secondary metabolites, volatile, and nonvolatile antifungal compounds by *Trichoderma* species are well known and recognized. In addition to phenolic compounds, production of various antibiotics like, trichodermol, viridian, gliovirin, harzianolide, harzianum A, trichodermin and koninginins has been also reported

[102]. However, it is difficult to differentiate between competition and antibiosis in agar plate. The inhibition zones result from antibiosis are indistinguishable from those produced by the nutrients shortage.

Mycoparasitism (obtaining nutrients from the fungal pathogen) may be contributed to the antagonistic behavior of some *Trichoderma* spp. [103–105]. However, the ability to parasitize pathogenic fungi is not a simple process; it involves specificity between both fungi. It depends primarily on the chemical attraction by the pathogenic fungus and the cell signaling in *Trichoderma* which includes recognition (sensing their prey), as well as capability for production of lytic enzymes [106]. A successful mycoparasitic process involves chemical recognition by *Trichoderma* sp. to their prey fungus, chemical attraction, connection, coiling around their fungal prey and penetrating them mechanically through sending appressoria into the prey mycelium or chemically through secretion of cell-wall hydrolytic enzymes, and sometimes secretion of some antifungal secondary metabolites [107].

Moreover, some *Trichoderma* spp. are identified as endophytes [108–111] that can trigger the plant systemic acquired resistance against attaching pathogens [109]. Moreover, they induce plant tolerance against drought and salinity [112]. Up-regulation of different defense-related genes are also reported as a response to the endophytic *Trichoderma*, in addition to some phytochemicals [113]. In this regard, Park et al. [110] recorded a markedly inhibition in the disease development in ginseng, caused by *B. cinerea* and *Cylindrocarpon destructans*, as a response to application of the endophytic *T. citrinoviride.*

### *Gliocladium* spp.

*Gliocladium* spp. (Ascomycota, Sordariomycetes, Hypocreales) are frequently found as soil-borne, endophytes, epiphytes, on other fungi, on plant debris, freshwater, and coastal soils [59, 114, 115]. *Gliocladium* spp. have a worldwide distribution and exceptional ecological versatility. They inhabit numerous ecosystems like tropical, temperate, subarctic, and desert areas [116]. Species of this genus are reported as producers of a vast range of secondary metabolites which exhibit different bioactivities such as antifungal, antibacterial, nematicidal, anti-tumour activities, as well as hydrocarbons and their derivatives (myco-diesel), and ligninolytic enzymes [117–120]. Taxonomically, many *Gliocladium* spp. were reclassified and moved to the genus *Clonostachys* due to significant molecular and morphological differences from the type form of *Gliocladium* spp. For instance, *G. catenulatum* is renamed to *C. rosea* f. *catenulata*, and *G. roseum* is renamed to *C. rosea* f. *rosea* [121, 122]. Furthermore, other species were transferred to the genus *Trichoderma* such as *G. virens* which is now classified as *T. virens*.

Species of the genus *Gliocladium* are widely known as bio-fungicides for many pathogenic mycoflora. The most common species used as biocontrol agents are *C. rosea* f. *rosea* (syn. *G. roseum*), *C. rosea* f. *catenulata* (syn. *G. catenulatum*), and *T. virens* (syn. *G. virens*). *Gliocladium* spp. have a potent antagonistic activity against various fungal mycopathogens like *P. ultimum*, *B. cinerea*, *F. graminearum*, *F. udum*,

*Phytophthora cinnamomi*, *P. citricola*, *Alternaria alternata*, *Verticillium* spp. and *Chaetomium* spp. [123–125]. Borges et al. [126] recorded significant biocontrol efficiency for *C. rosea* against tomato gray mold. Application of *C. rosea* recorded 100% biocontrol efficiency in stem and ≥90% in the entire tomato plant. Tesfagiorgis et al. [127] recorded a disease reduction (90%) in powdery mildew of zucchini when treated with *C. rosea* under greenhouse conditions.

Production of different antagonistic metabolites by *Gliocladium* spp. has been reported such as gliotoxin and viridin by *G. flavofuscum* [128]. According to the type of the antibiotic produced by strains of *T. virens* they can be differentiated into two groups (P and Q). Members of group P synthesis gliovirin which poses narrow antifungal spectrum activity, primarily, against oomycetes [129], while, members of group Q synthesis gliotoxin which poses a broad range of antifungal as well as antibacterial activities [130]. Another species of *Gliocladium* has been reported as a producer of a set of volatile antifungal substances against *P. ultimum* and *V. dahliae*. Of them, the antifungal antibiotic annulene was identified [131]. Mycoparasitism against different fungal pathogens was also reported as a proposed biocontrol mode of action of *Gliocladium* spp. [132, 133]. In a recent study, 199 candidate mycoparasites isolated from agricultural soils in southwestern Greece, of them, the isolate *Gliocladium* sp. G21-3 was the most aggressive mycoparasite and a competent antagonist against sclerotia of *S. sclerotiorum* [134].

### *Penicillium* spp.

*Penicillium* Link (Ascomycota, Eurotiomycetes, Eurotiales) is a diverse genus which contain more than 400 species with a cosmopolitan distribution. *Penicillium* spp. are found as soil-borne, on decaying crops, on wood, fresh and dry fruits, water, and in indoor air. They are well known as organic materials decomposers, causative of food spoilage, producers of mycotoxins and enzymes, air allergens, and/or causative of postharvest decay of some crops [135]. Members of genus *Penicillium* are widely recognized as synthesizers of diverse bioactive substances such as antibiotics, antitumor agents, nephrotoxin, and ergot alkaloids [136].

Some *Penicillium* species are known as bio-fungicides against fungal diseases. The endophytic *P. oxalicum* T 3.3 exhibited an aggressive antifungal activity against anthracnose of dragon fruit, caused by *Colletotrichum gloeosporioides*. Production of β-glucanase and chitinase was reported for this biocontrol agent [137]. Sreevidya et al. [138] reported a remarked biocontrol activity of *P. citrinum* against botrytis gray mold of chickpea in the greenhouse and field. The antifungal activity was attributed to their production of mycotoxin citrinin. In addition, production of lytic enzymes like protease and glucanases were also reported. The biocontrol activity (75%) of *P. citrinum* was reported on charcoal rot of sorghum under greenhouse condition [139]. De Cal et al. [140] reported a markedly decrease in the powdery mildew of strawberry in vitro and in vivo via application of *P. oxalicum*.

*Chaetomium* spp.

*Chaetomium* spp. Kunze (Ascomycota, Sordariomycetes, Sordariales) are filamentous fungi which exist as soil-borne, air-borne, endophytic, epiphytic, on any cellulose containing materials, and on plant debris. It comprises more than 160 described species with a cosmopolitan distribution [141]. Some of these fungi act as biofungicides to control numerous pathogenic mycobiota like *A. raphani, A. brassicicola,* and *P. ultimum*. Zhao et al. [142] reported a potent antagonistic activity by the endophytic *C. globosum* CDW7 against rape sclerotinia rot, caused by *S. sclerotiorum*. Seven secondary metabolites were identified from their culture filtrate including the antifungal metabolites flavipin, chaetoglobosin A-E and $V_b$, for which their antagonistic potential was attributed. Hung et al. [143] reported also an in vitro mycelial growth inhibition of *P. nicotianae* by 50 ~ 56% when grew against the antagonists *C. globosum*, or *C. cupreum* in biculture tests and against their crude extracts. Furthermore, *C. cupreum* parasitized *P. nicotianae* and degraded their mycelia after 30 days of incubation. In pot experiment, use of *Chaetomium* spp. lowered the disease severity of citrus root rot by 66–71%. *Chaetomium* species have been reported as producers of lytic enzymes which involved in the mycoparasitism [144, 145]. In addition, numerous antifungal secondary metabolites were reported from the culture filtrates of *Chaetomium* spp. like flavipin, chaetoviridins, chaetoglobosins, and rubrorotiorin [142, 146, 147].

*Myrothecium* spp., *Laetisaria* spp., and *Coniothyrium Minitans*

*Myrothecium* spp. Tode (Ascomycota, Sordariomycetes, Hypocreales) are filamentous fungi that poses a universal distribution and found as soil-borne or on plants. It comprises more than 35 described species [148]. *Myrothecium* spp. are recognized as producers of various bioactive substances such as trichothecenes mycotoxins (roridin A, verrucarin A, and 8beta-acetoxy-roridin H) [149, 150], as well as lytic enzymes like proteinases and lipases [151]. Some of *Myrothecium* spp. have a potential antagonistic behavior against several fungal phytopathogens, weeds, insects, and nematodes [152, 153]. Barros et al. [154] reported a biocontrol activity of *Myrothecium* sp. against *S. sclerotiorum* in vitro and in vivo experiments. A considerable decrease in the soybean mold disease up to 70% was recorded by application of the biocontrol agent.

*Laetisaria* Burds. (Basidiomycota, Agaricomycetes, Corticiales) is a genus of 4 species with widespread distribution. The soil-borne fungus *L. arvalis* is well recognized as a bio-fungicide against some pathogenic mycoflora. Among the 28 biocontrol agents tested by Brewer and Larkin [155], the isolate *L. arvalis* ZH-1 significantly reduced the disease incidence of potato black scurf by 60%. In another study, soil treatment with *L. arvalis* led to a markedly decrease in tomato damping-off, caused by *P. indicum*, recording 72% seed germination [156]. Furthermore, Bobba and Conway [157] reported the competition for nutrients as an antagonistic

mechanism by *L. arvalis* against the pathogenic fungus *S. rolfsii* in the competitive colonization experiment.

*Coniothyrium minitans* W. A. Campb. (Ascomycota, Dothideomycetes, Pleosporales) is a worldwide distributed fungus. It is a naturally obligate mycoparasite on sclerotia of the fungal pathogens *S. sclerotiorum, S. minor, S. trifoliorum*, and *S. rolfsii* [158, 159]. In this regard, Chitrampalam et al. [160] studied the antifungal activity of *C. minitans* on *S. minor*, the causal of the lettuce drops, in vitro and in vivo. A total sclerotial mortality was recorded in the culture plates. In the field experiment, a significant reduction in the lettuce drop was achieved; this reduction was correlated with a reduction in the existence levels of the sclerotia. During the mycoparasitic process by *C. minitans*, the outer pigmented layer of the sclerotia has been mechanically penetrated and enzymatically using lytic enzymes [161]. However, the antibiosis mechanism via production of the antifungal secondary metabolite macrosphelide A was also reported [162].

### Arbuscular Mycorrhizal Fungi (AMF)

AMF are soil fungi (Mucoromycota, Glomeromycotina) which comprise about 300 species in 3 classes, 5 orders, 15 families and 38 genera [163, 164]. They are obligate endophytes that live in mutualism with roots of 80% of the vascular plants [165]. AMF are found in all terrestrial ecosystems with varied extent of pH, salinity, organic matter, and environmental conditions. They have a cosmopolitan distribution, where they have been reported from all continents [166]. In the arbuscular mycorrhizal association, the fungus attains carbon from the photosynthesis of the plant, while the plant takes many advantages from the fungus. AMF supply the mycorrhizal host with water, and minerals via their extra radical hyphal network. Moreover, AMF improve the plant growth and metabolic processes, increase their resistance to drought, salinity, heavy metals, as well as enhance their immunity against various pathogenic mycobiota [167].

Many researchers have extensively studied the biocontrol activity of AMF to control different types of phytopathogenic fungi like *A. solani*, *Aphanomyces euteiches*, *Cercospora arachidicola*, *Cercosporidium personatum, Erysiphe graminis*, *F. solani*, *F. verticillioides*, *Gaeumannomyces graminis*, *M. phaseolina*, *P. cactorum*, *P. aphanidermatum*, *R. solani*, *S. cepivorum*, and *V. dahliae* [168–172]. Olowe et al. [173] investigated biocontrol activity of *Glomus clarum* and *G. deserticola* against maize ear rot. A considerable reduction in the disease effects on the plant growth parameters was recorded by application of AMF. El-Sharkawy et al. [97] investigated the biocontrol of wheat stem rust by using AMF and *Trichoderma* spp. under greenhouse conditions. A markedly decrease in the disease measures as well as enhancement in the growth and yield parameters were recorded. Moreover, an induction in the activities of some defensive enzymes and total phenol content were also recorded. The likely biocontrol mechanisms exerted by AMF comprise direct rivalry with other soil-borne pathogenic fungi for nutrients, space, and colonization sites, changing of the soil microbial composition in the rhizosphere area [174, 175].

Furthermore, AMF may indirectly decrease the losses resulting from the disease by damage compensation, growth improvement and triggering the plant immunity against the phytopathogens attack [170, 172]. In this regard, Abdel-Fattah et al. [176] reported triggering multiple defense-related reactions in bean plants against infection with *Rhizoctonia* root rot as a result of application of AMF. Some ultrastructural and biochemical responses were recorded including cell-wall thickening, cytoplasmic granulation, increase in the cell organelles number, nuclear hypertrophy, and accumulation of fungitoxic compounds (phenolics) and triggering of defensive enzymes activity. However, achieving a genetic polymorphism (86.8%) as well as triggering of the transcriptional expression level of defense-related genes were also reported [177].

### *3.3 Induction of Systemic Resistance and Defense-Related Genes in Plant*

Plants have a strategy against fungal infection by evolving multiple immune mechanisms [178, 179]. The first immune response is started by the recognition of pathogen-associated molecular patterns conserved (PAMPs), like lipopolysaccharides, flagellin, chitin and glycoproteins by what is called Pattern-Recognition Receptors (PRRs) which located on the surface of cell [180]. The understanding of PAMP stimulates PAMP-triggered immunity (PTI), including oxidative burst, MAPK (mitogen-activated protein kinase) activation, deposition of callose, defense-related genes induction, and antimicrobial compounds accumulation [181–183]. The pathogens can successfully suppress PTI by secreting different effectors, like small RNAs and proteins to suppress host PTI in the host cells [184–186]. On the other hand, plants have secreted resistant proteins to recognize the specific effectors of pathogen, leading to an effector-triggered immunity (ETI), whereas ETI is more rapid and powerful than PTI and stimulates comparable defense responses set as in PTI but in an accelerated and powerful way [178, 179, 183, 187].

The starting of PTI or ETI from the infected loci often stimulates resistance induced in tissues that give resistance against a wide range of pathogens [39]. This systemic acquired resistance (SAR) is often correlated with level of salicylic acid (SA) increased and regulate the activation of pathogenesis related (PR) genes and comprises one or more long-distance signals that increase the capacity to enhanced defensive in intact parts of plant [188]. Also, beneficial microbes in the rhizosphere can induce systemic resistance (ISR). In most cases, ISR is SA-independent and develops without accumulation of PR proteins. *P. fluorescens* is still able to induce ISR that does not synchronize with enhanced SA levels.

## 4 Case Study

In Egypt, many researchers concerned with the biological control of fungal diseases, my research group studied many bioagents for control of many plant fungal diseases such as *Streptomyces* spp. [64, 189], *Pseudomonas* spp. and *Bacillus* spp [190–192] and some fungal species such as *Gliocladium* spp., *Paecilomyces* spp., *Penicillium* spp. and *Trichoderma* spp. [189]. The *Trichoderma harzianum* was used widely as a bioagent, which observed the most potent organisms among bacterial and fungal species used against sugarbeet pathogen *R. solani* in the study carried out by Moussa [189] and shown in Table 1. The mechanism of *T. harzianum* to control the fungal pathogens was by mycoparasitism on the pathogen hyphae and observed using scanning electron microscope (SEM) (Figs. 1, 2 and 3).

Hyphal interactions between *T. harzianum* and *R. solani* were observed by scanning electron microscopy. *T. harzianum* attached to the host by hyphal coils (Figs. 1, 2 and 3).

**Table 1** Control of sugar beet root rot disease caused by *R. solani* with different antagonists

| Antagonist | Disease incidence (%) | | |
|---|---|---|---|
| | Seed coating | Seed soaking | Soil pre-inoculation |
| Control | 42.53a | 71.43 | 75.68a[a] |
| **Bacteria** | | | |
| *Bacillus cereus* | 14.85defg | 66.4bc | 48.18a |
| *B. subtilis* | 10.67efgh[a] | 81.2a | 52.91a |
| **Fungi** | | | |
| *Gliocladium deliquescens* | 15.51def | 51.9c | 20.68b |
| *Paecilomyces marquandii* | 8.8fgh[b] | 52.3c | 50.93a |
| *Penicillium vermiculatum* | 10.12efgh | 65.5bc[a] | 50.93a |
| *Trichoderma harzianum* | 6.48 h | 62.9bc | 17.16b |
| *T. koningii* | 13.53efg | 69.5ab | 48.18a |
| *T. pseudokoningii* | 25.94b | 63.6bc | 52.9a |
| *T. viride* | 25.63bc | 60.5bc | 48.18a |

[a]Values within a row followed by the same letter are not significantly different at 5% level according to Duncan's multiple range test (DMRT)

[b]Values within the column followed by the same letter are not significantly different at 5% level (based on DMRT)

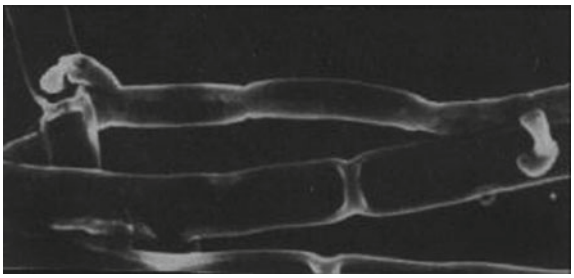

**Fig. 1** Scanning electron micrographs of *Trichoderma harzianum* hyphae interacting with those of *Rhizoctonia solani* in which hypha of *T. harzianum* coiling around and penetrating one of *R. solani*. Partial degradation of host cell wall can be observed (X 8500) [190]

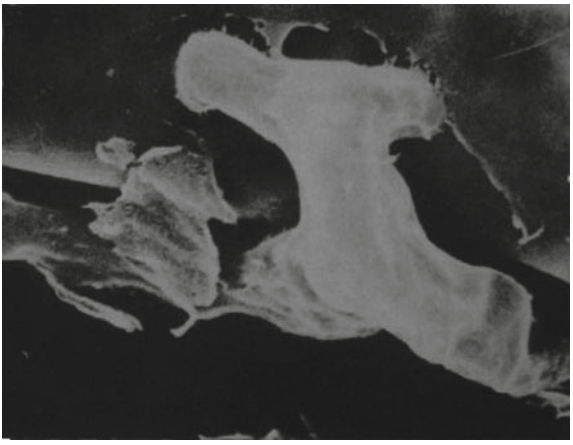

**Fig. 2** Scanning electron micrographs of *Trichoderma harzianum* hyphae interacting with those of *Rhizoctonia solani* in which hooks of *T. hrzianum* attached to hyphae of *R. Solani* (X 2000) [190]

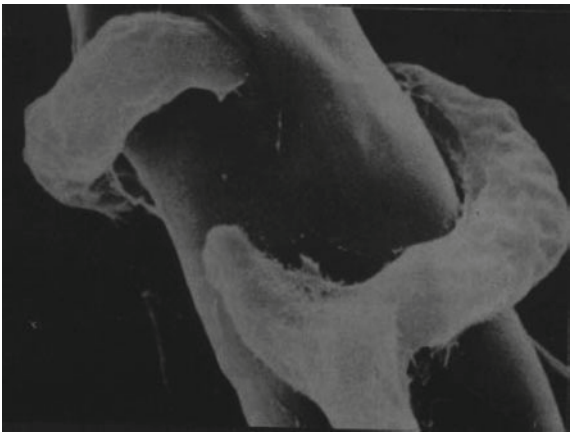

**Fig. 3** Scanning electron micrographs of *Trichoderma harzianum* hyphae interacting with those of *Rhizoctonia solani* in which appressorium-like structure formed by *T. harzianum*, attached to a hyphae of *R. solani* with partial degradation of host cell wall (X 8500) [190]

In another case study, the research was developed to study the effect of bioagent on the host plant as well as fungal pathogens. Some bacterial species were known as plant growth promoting rhizobacteria (PGPR) which secret some compounds to enhance plant growth, it was found that all growth parameters of *Cucumi ssativus* L. cv. Market were increased in absence and presence of the fungal pathogen *P. aphanidermatum* in greenhouse experiment as shown in Table 2. On the other hand, the use of *P. aeruginosa* and *B. amyloliquefaciens* separately inhibit the fungal pathogen *P. aphanidermatum* [191]. Another study on the biocontrol of *F. graminearum* which attacks wheat, in which it was concluded that the use of *B. subtilis* and *Pseudomonas fluorescens* increased the growth parameters of wheat and suppress the growth of *F. graminearum*, also *P. fluorescens* was the most efficient than *B. subtilis* or in mixture [192].

In a recent study conducted by the authors, the biocontrol activity of a mixture of arbuscular mycorrhizal fungi was investigated against *Rhizoctonia* root rot of common bean, caused by *Rhizoctonia solani* Kühn, under natural conditions. The obtained results exhibited a considerable reduction in the disease severity and incidence by the mycorrhizal colonization. In addition, a significant enhancement of the shoot and root lengths and dry weights, and the leaf area was observed in the colonized plants when compared with the control plants. Moreover, the mineral nutrient concentrations and yield parameters were also improved. Transmission electron microscope observations showed some defense-related ultrastructural changes including cell wall thickening and cytoplasmic granulation. The biochemical analysis of the colonized plants showed an accumulation of the phenolic compounds, which have a fungitoxic activity, and induction of the defense-related enzymes phenylalanine ammonia lyase, peroxidase and polyphenoloxidase [176]. Furthermore, the molecular examination indicated an induction of the transcriptional expression level of the defense-related genes chitinase and β-1,3-glucanase as a response to the mycorrhizal colonization [177].

## 5 Conclusion and Future Prospects

In this chapter, the authors tried to highlight the most important biological control practices all over the world and focused on Egypt as a home country, it is found that through the past century, the attention to biological control of economic crops has increased from both the government and the researchers starting from the ordinary application of biocontrol agents in contact directly to the soil and in form of gelatin capsules to insertion of the resistance genes in the plant and produce what we know today GM plants (genetically modified plants). In Egypt, the biological control of different diseases becomes common due to the awareness of farmers about the benefits of biocontrol applications.

**Table 2** The efficacy of three isolated species from PGPR on growth parameters of *Cucumis sativus* L. cv. Market more in the presence or absence of pathogenic *Pythium aphanidermatum* under greenhouse condition

| Plant treaded with | Plant growth parameters | | | | | |
|---|---|---|---|---|---|---|
| | Length (cm) | | | Weight (g) | | Chlorophyll content (unit) |
| | Plant | Stem | Root | Fresh | Dry | |
| Untreated | 17.5 ± 0.8 | 7.17 ± 0.72 | 10.33 ± 1.59 | 0.25 ± 0.06 | 0.027 ± 0.005 | 12.45 ± 3.38 |
| *Pythium aphanidermatum (Pa)* | P[b] | P | P | P | P | P |
| *Bacillus subtilis* (*Bs*) | 23.7 ± 1.04[a] | 13.5 ± 0.87[a] | 10.37 ± 0.29 | 0.62 ± 0.02[a] | 0.033 ± 0.007[a] | 16.05 ± 1.5[a] |
| *Bacillus amyloliquefaciens* (*Ba*) | 17.0 ± 1.30 | 8.97 ± 0.90 | 8.030 ± 2.00 | 0.53 ± 0.01[a] | 0.029 ± 0.004[a] | 12.43 ± 0.50 |
| *Pseduomonas aeruginosa* (*Psa*) | 19.3 ± 1.15[a] | 9.00 ± 1.00 | 10.13 ± 2.08 | 0.61 ± 0.07[a] | 0.031 ± 0.002 | 16.00 ± 5.15[a] |
| *(Bs) + (Ba)* | 22.0 ± 5.20[a] | 10.5 ± 1.32[a] | 11.50 ± 5.70 | 0.65 ± 0.01[a] | 0.037 ± 0.003 | 16.03 ± 3.03[a] |
| *(Bs) + (Psa)* | 21.83 ± 0.7[a] | 9.00 ± 2.00 | 12.8 ± 2.70[a] | 0.59 ± 0.03[a] | 0.029 ± 0.001 | 12.6 ± 1.90 |
| *(Ba) + (Psa)* | 15.7 ± 0.20 | 10.0 ± 0.87 | 5.67 ± 0.58 | 0.19 ± 0.03 | 0.020 ± 0.002 | 11.27 ± 0.8 |
| *(Bs) + (Ba) + (Psa)* | 11.83 ± 1.6 | 5.00 ± 1.00 | 6.83 ± 1.76 | 0.19 ± 0.03 | 0.022 ± 0.002 | 5.6 ± 2.030 |
| *(Bs) + (Pa)* | P | P | P | P | P | P |
| *(Ba) + (Pa)* | 15.0 ± 0.21 | 6.00 ± 0.90 | 9.00 ± 0.16 | 0.18 ± 0.32 | 0.22 ± 0.010 | 8.33 ± 0.13 |
| *(Psa) + (Pa)* | 12.33 ± 0.6 | 5.67 ± 0.58 | 6.67 ± 0.58 | 0.17 ± 0.03 | 0.17 ± 0.010 | 5.70 ± 0.13 |
| *(Bs) + (Ba) + (Pa)* | P | P | P | P | P | P |
| *(Bs) + (Psa) + (Pa)* | P | P | P | P | P | P |
| *(Ba) + (Psa) + (Pa)* | P | P | P | P | P | P |
| *(Bs) + (Ba) + (Psa) + (Pa)* | P | P | P | P | P | P |

Mean of three replicates ± SD

[a]significant at level 5%

[b]P: Plants can't survival under fungus infection rate ($1.3 \times 10^7$ propagules $g^{-1}$ soil)

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