

## Chapter 5

# Trichoderma: Beneficial Role in Sustainable Agriculture by Plant Disease Management

Laith Khalil Tawfeeq Al-Ani

**Abstract** *Trichoderma* as biological control agents have been widely used against many plant pathogens, such as viruses, bacteria, fungi, nematodes, and higher parasitic plants. This species of fungi has been considered to be very beneficial for different levels of life. It features remote sensing and is fast in attacking and suppressing the growth of plant pathogens, and it improves plant growth. It can produce different secondary compounds and readily activates others fungi, producing very significant enzymes, such as chitinase, proteases, and  $\beta$ -1,3-glucanase, inducing plant defense, systemic resistance, and strong and active competition against plant pathogens. It is party to an important detoxification process to reduce the toxicity secreted by plant pathogens. It is therefore necessary to clarify the significance of *Trichoderma* in the control of plant diseases that results in improvements in sustainable agriculture. This should include coverage of the different aspects of the interaction between *Trichoderma* and the various kingdoms of organisms. Here is provided an excellent guide to the importance of *Trichoderma* as biological control agents (BCAs) in sustainable agriculture through reducing plant diseases and increasing field production. *Trichoderma* can combine several advantages in one product – the control of different of plant diseases, enhancement of plant growth, and the provision of a clean environment for the benefit of sustainable agriculture.

**Keywords** Biological control • Induced systemic resistance • Plant disease • Trichoderma

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L.K.T. Al-Ani (✉)

Department of Plant Protection, College of Agriculture, University of Baghdad, Baghdad, Iraq

School of Biology Science, Universiti Sains Malaysia, Minden, Pulau Pinang, Malaysia  
e-mail: [cmv\\_virus2002@yahoo.com](mailto:cmv_virus2002@yahoo.com); [laith.kt77@gmail.com](mailto:laith.kt77@gmail.com)

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D. Egamberdieva, P. Ahmad (eds.), *Plant Microbiome: Stress Response*, Microorganisms for Sustainability 5, [https://doi.org/10.1007/978-981-10-5514-0\\_5](https://doi.org/10.1007/978-981-10-5514-0_5)

## 5.1 Introduction

A recent challenge in the agricultural sector is to increase yields and decrease plant disease to a minimum level. Traditional methods such as the use of fungicides, nematicides, herbicides, and fertilizer are among general methods in plant disease management and crop yield improvement. Although these mechanisms have the ability to control plant disease and suppress plant pathogens, they are not eco-friendly. Continuous usage of chemical-based methods has also caused the pathogens develop more resistance toward pesticides. The use of agrochemical pesticides containing various hazardous chemicals such as ethylated, methylated, and aromatic substances also adversely affect and pollute the atmosphere and water, thereby harming fish, beneficial insects such as the honeybee, non-target organisms such as plant growth promoting rhizobacteria, and plant growth promoting fungi (PGPF). Chemical residues can also burn or cause yellowing effects to plant leaves.

Today, many researchers are searching for alternative methods with significant eco-friendly activity. Biological control agents (BCAs), natural enemies of plant pathogens, are a very strong candidate to replace conventional methods. BCAs are commonly isolated from the rhizosphere, phyllosphere, and soil. They comprise several agents such as PGPF, non-pathogenic fungi, mycorrhizal, entomopathogenic fungi, mycoparasitic fungi, and endophytic fungi (Steyaert et al. 2003; Hermosa et al. 2012; Murali et al. 2012; Sylla 2013; Doni et al. 2013). Several fungi agents reported as biological controls are *Coniothyrium minitans* (anamorphs of *Paraphaeosphaeria minitans*) (Verkley et al. 2004; Chitrampalam et al. 2011), *Clonostachys rosea* (Sutton et al. 2002), *Trichoderma* spp. (Al-Ani et al. 2013a; Al-Ani 2017), *Fusarium oxysporum* f.sp. cubense, *F. oxysporum*, *F. solani*, and *F. fujikuroi* (Al-Ani et al. 2013b; Al-Ani 2010, 2017), *Piptocephalis virginiana* (Berry and Barnett 1957), *Gonatobotrys simplex* (Hoch 1977), *Pythium paroecandrum* (Abdelghani et al. 2004), *Chaetomium* (Hung et al. 2015), *Sphaerodes quadrangularis* (Goh and Vujanovic 2010), *Cryphonectria parasitica* (Kunova et al. 2016), and *Rhizoctonia solani* and *Rhizoctonia* (BNR) spp. (Hwang and Benson 2003).

Of these, *Trichoderma* is the most versatile genus of fungi worldwide that have been used to control plant pathogenic fungi and manage plant diseases and plant growth. Historically, *Trichoderma* was introduced as antagonistic fungi and has been known as a biocontrol agent of several plant pathogens since the 1920s (Weindling 1934; Samuels 1996). Several advantages, such as ubiquitous distribution, ease of isolation and culture, and rapid growth on many substrates attract the researchers to use it for sustainable agriculture. *Trichoderma* spp. have been reported to control important plant pathogenic fungi such as *Fusarium*, *Phytophthora*, *Pythium*, *Colletotrichum*, *Fulvia fulva*, *Rhizoctonia*, *Plasmopara viticola*, *Pseudoperonospora cubensis*, *Monilia laxa*, *Rhizopus*, *Botrytis*, *Alternaria*, *Cladosporium*, *Gaeumannomyces*, *Verticillium*, and *Sclerotinia* (Table 5.1). *Trichoderma* is also widely used to control plant diseases such as Fusarium wilt (Al-Ani 2017), bacterial wilt (Yuana et al. 2016), sheath blight (de França et al. 2015), mosaic virus (Luo et al. 2010), southern stem rot (Sennoi et al. 2013), downy

**Table 5.1** *Trichoderma* spp. used as biocontrol agents (Al-Ani 2017)

<i>Trichoderma</i> species	Pathogen	Crop	References
<i>T. harzianum</i>	<i>F. oxysporum</i> f.sp. <i>lycopersici</i>	Tomato	Sivan (1987)
	<i>Macrophomina phaseolina</i>	Cowpea	Adekunle et al. (2001)
	<i>F. oxysporum</i> f.sp. <i>cubense</i>	Banana	Saravanan et al. (2003) and Nan et al. (2014)
	<i>F. oxysporum</i> f.sp. <i>lycopersici</i>	Tomato	Marzano et al. (2013)
	<i>Fusarium oxysporum</i> f. sp. <i>cucumerinum</i>	Cucumber	Zhang et al. (2013)
	<i>Sclerotinia sclerotiorum</i> , <i>Rhizoctonia solani</i> , <i>Verticillium dahliae</i> , <i>Phytophthora nicotianae</i> and <i>P. cinnamomi</i>	Soil-borne disease	Aleandri et al. (2015)
<i>T. koningii</i>	<i>Fusarium oxysporum</i> f.sp. <i>cucumerinum</i> , and <i>Pythium</i> spp	Pea seeds	Lifshitz et al. (1986)
	<i>Rhizoctonia solani</i> and <i>Pythium ultimum</i>	<i>Capsicum annum</i>	Harris (1999)
	<i>Sclerotium rolfsii</i>	Soil-borne disease	Tsahouridou and Thanassouloupoulos (2001)
	<i>Macrophomina phaseolina</i>	Cowpea	Adekunle et al. (2001)
	<i>Rhizoctonia solani</i>	Cotton	Hanson and Howell (2002)
	<i>Sclerotium rolfsii</i>	Tomato seeds	Tsahouridou and Thanassouloupoulos (2002)
<i>T. viride</i>	<i>Sclerotium rolfsii</i>	Tomato	Wokocho (1990)
	<i>F. oxysporum</i> f.sp. <i>cubense</i>	Banana	Saravanan et al. (2003)
<i>T. asperellum</i>	<i>Fusarium oxysporum</i> f.sp. <i>dianthi</i>	Carnation	Sant et al. (2010)
	<i>Phytophthora ramorum</i>	Soil-borne disease	Widmer (2014)
	<i>Sclerotinia sclerotiorum</i> , <i>Rhizoctonia solani</i> , <i>Verticillium dahliae</i> , <i>Phytophthora nicotianae</i> and <i>P. cinnamomi</i>	Soil-borne disease	Aleandri et al. (2015)
<i>T. parareesei</i>	<i>Botrytis cinerea</i>	Tomato	Rubio et al. (2014)
<i>T. hamatum</i>	<i>Sclerotinia sclerotiorum</i> , <i>Rhizoctonia solani</i> , <i>Verticillium dahliae</i> , <i>Phytophthora nicotianae</i> and <i>P. cinnamomi</i>	Soil-borne disease	Aleandri et al. (2015)
<i>T. atroviride</i>	<i>Armillaria gallica</i>	Orchards	Pellegrini et al. (2014)
<i>T. virens</i>	<i>Rhizoctonia solani</i>	Cotton	Hanson and Howell (2002)

mildew (Perazzolli et al. 2012), seed-rotting fungi (Hadar et al. 1984), powdery mildew and grey mold (Elad et al. 1998), gummosis of citrus (Bicici et al. 1992), root-knot nematode (Al-Hazmi and Javeed 2016), root rot, damping-off, stem rot, Aspergillus crown rot, charcoal rot, red rot, Rhizoctonia black scurf, turfgrass diseases, decay in tree wounds, and internal decay of wood products (Gnanamanickam 2002).

*Trichoderma* has many mechanisms very useful for plants, such as to improve plant growth, to enhance the solubilization of mineral nutrients, to induce secondary metabolites production, to produce growth-regulating compounds, stimulation of plant defense, and production of siderophores. These mechanisms confirm the *Trichoderma* species to be a suitable biocontrol agent in plant disease management by developing biopesticides. There are several commercially available *Trichoderma*-based products such as Biobus 1.00WP (*Trichoderma viride*), Promot PlusWP Promot PlusDD (*Trichoderma* spp. *Trichoderma koningii*, and *Trichoderma harzianum*), RiB1, TRICÔ-ĐHCT, VI – ĐK, Bio – Humaxin Sen Vàng 6SC, and Fulhumaxin 5.15SC (*Trichoderma* spp.).

## 5.2 Mechanisms of *Trichoderma* spp. as BCAs Against Plant Pathogens

The mode of action involves several mechanisms such as mycoparasites, competition, antibiotics production, and the ability to induce plant defense and systemic resistance (Naher et al. 2014). The modes of action are as follows.

### 5.2.1 Mycoparasitism

The main mechanism in the antagonism of *Trichoderma* against fungal plant pathogens is mycoparasitic (Elad et al. 1982). Mycoparasitism is probably a factor and one of the most outstanding features of this fungal genus. The direct attack on another fungus is a compound process that involves successive events, including infection and penetration, subsequently killing the opponent fungus. *Trichoderma* spp. may exert direct biocontrol by parasitizing the broad range of fungi and growing toward them. The mycoparasite activity of *Trichoderma* starts with coils around the host hyphae followed by producing hooks with appressorium-like bodies, eventually penetrating the host cell wall (Elad et al. 1983; Inbar and Chet 1992; Ojha and Chatterjee 2011). The ability of *Trichoderma* spp. to act as mycoparasitic fungi is because of the production of cell wall degrading enzymes (CWDEs) such as chitinases, proteases, and  $\beta$ -1,3-glucanases. These enzymes are involved in CWDEs of *R. solani*, *Sclerotium rolfsii*, and *Pythium aphanidermatum* (Elad et al. 1982; Sivan and Chet 1989; Harman et al. 2004). The adhesion to a host surface is just one step in a series of interaction events, see the interaction steps that described by Tunlid et al. (1992). *Trichoderma* can respond and recognize the host in different environmental conditions, and the successful colonization of rhizosphere, plants, and soil is relevant with the presence of a host (Mendoza-Mendoza et al. 2003). *Trichoderma* can respond to the host by the successive expression of pathogenesis-related proteins comprising chitinases, proteases, and glucanases (Harman et al. 2004). The method of induction varies from

one *Trichoderma* strain or species to another, and *Trichoderma* secrete chitinases that degrade fungal cell-walls to liberate the oligomers, which induces exochitinases, and parasitism begins (Gajera et al. 2013). The induction differs between species and strains of *Trichoderma*, which may be unable to interact with the host, or affect the gene to gene reactions that apply to the ability of *Trichoderma* to become a parasite on the host.

### 5.2.2 *Non-mycoparasitism*

Some strains or species of *Trichoderma* are not mycoparasites of other fungi, or at least that phase of their life cycle is not involved in the biocontrol phenomenon. The mechanisms employed by these *Trichoderma* strains or species consist of antibiosis (production of antimicrobial and secondary metabolites), competition (on site, on nutrient, or in combination), involving antibiosis and competition, energizing plant defense before infection by the pathogen, improving the tolerance of infection with plant pathogens (Howell 2003), and leading to detoxification of phytotoxin for plant pathogens (Aggarwal et al. 2011).

## 5.3 *Trichoderma* as Biological Control Agents of Plant Pathogens

Biological controls are another alternative to chemical pesticides and have received mounting attention over the last 20 years (Paulitz and Belanger 2001). *Trichoderma* spp. have been reported to control many major plant pathogens, including viruses, fungi, bacteria, and nematodes. The classical category of biological control includes several mechanisms such as direct antagonism (mycoparasites), indirect antagonism (non-mycoparasites) such as antibiosis, competition, CWDEs, and induction of systemic resistance (Park 1960; Lo 1998).

### 5.3.1 *Biocontrol of Plant Viruses*

Plant viruses are among the important pathogens which are widespread and cause damage to plants. Damage caused by viruses simultaneously has harmful effects on sustainable agriculture. In general, some plant viruses depend on vectors such as nematodes, plant parasites, insects, seeds, and fungi. Chemical and biological controls measures have successfully decreased the rate of virus spread by applying Integrated Pest Management (IPM), improving host resistance, and enhancing plant growth, which increases plant tolerance to plant viruses' infection. *Trichoderma* showed the capacity to induce plant defense and stimulate resistance (as ISR

(induced systemic resistance) and SAR (systemic acquired resistance)) by producing secondary metabolites, culture filters, and CWDEs (Luo et al. 2010).

For example, antimicrobial peptaibols from *T. pseudokoningii* SMF2, known as Trichokonin, have ISR and defense response against tobacco mosaic virus infection in tobacco (*Nicotiana tabacum* var. Samsun NN) (Luo et al. 2010). The production of a reactive oxygen species (ROS) and phenolic compounds in tobacco increased when treated with Trichokonin. Activities of pathogenesis-related enzymes PAL and POD significantly increased, and the expression of several plant defense genes was also upregulated.

Elsharkawy et al. (2013) found the strain of *T. asperellum* SKT-1 was able to induce resistance in the *Arabidopsis* plant against CMV (cucumber mosaic virus) infection by increasing expression levels of SA (salicylic acid)- and JA (jasmonic acid)/ET(ethylene)-inducible genes in leaves. Although the pre-treatment of *Arabidopsis* root with the culture filter of *T. asperellum* SKT-1 led to induction of defense mechanisms against CMV (Elsharkawy et al. 2013), Vitti et al. (2015) found the ability of *T. harzianum* T-22 strain (T22) to induce the defense responses in tomato (*Solanum lycopersicum* var. *cerasiforme*) against CMV. Histochemical analysis has revealed a different increase in the hydrogen peroxide and superoxide anion, suggesting the involvement of ROS in plant defense responses.

### 5.3.2 Biocontrol of Plant Bacteria

Plant bacteria are important to plants, mostly live either as endophytes or saprophytes, and coexist in the rhizosphere, soil, and phyllosphere. Some strains of bacteria are pathogenic to plants and can cause major plant diseases worldwide (Agrios 2005). Bacterial pathogens induce many types of symptoms on leaves, fruits, crown, roots, and vascular tissues. These symptoms appear as blights and spots on leaves, soft rots of fruits, wilts, scabs, and cankers (Agrios 2005).

However, bacterial diseases of plants are often not easy to control. There are many methods for control, such as protecting crop fields from infection using healthy seeds, preventing the spread of bacterial pathogens through insect-infected plants or any part of the plants, and decontaminating tools, machines, and hands after planting. Other control measures include using chemical controls (bactericides), physical controls by burning the plant infected with bacteria, introducing plant breeding programmes, and selecting high-resistance crop varieties. Biological controls by using BCAs such as plant growth promoting rhizobacteria, endophytic bacteria, mycorrhizal, endophytic fungi, and *Trichoderma* have also been used to suppress the pathogens. All of these methods can be used alone or in combination to control plant bacterial pathogens effectively. Of these, the best techniques to control the pathogens are chemical methods, followed by plant breeding and biological methods. However, plant breeding and biocontrol methods are safer for the environment.

For example, *T. asperellum* T203 conferred the protective effect by inducing systemic resistance against the cucumber leaf pathogen *Pseudomonas syringae* pv. *lachrymans* that is involved in the JA/ET signaling pathways of ISR (Lox1, Pal1, ETR1, and CTR1) in cucumber plants. Meanwhile, *T. pseudokoningii* SMF2 showed antimicrobial activity against a broad-spectrum of both Gram-positive bacteria (Shi et al. 2012) and Gram-negative bacteria (Li et al. 2014), where the strain was able to control *Pectobacterium carotovorum* sub sp. *carotovorum* (*Pcc*), which caused a soft rot disease of Chinese cabbage (Li et al. 2014). The strain produced Trichokonins which inhibited the growth of *Pcc* (Gram-negative) and induced resistance of cabbage plants. Trichokonins were able to increase the production of ROS, pathogenesis-related protein gene acidic PR-1a, and activation of SA (Li et al. 2014).

### 5.3.3 Biocontrol of Phytopathogenic Fungi

Phytopathogenic fungi are very harmful to plants, causing several major diseases and having more harmful effects on sustainable agriculture compared to phytopathogenic viruses or bacteria. Fungi can attack plants and incidence may be localized or systemic. Usually, fungi can infect all parts of plant-leaf, root, and seeds as well as stored seeds. In general, infection by fungi causes symptoms such as root rot, necrosis, wilts, spots, stunting, powdery mildew, downy mildew, blight, canker, dieback, damping-off, crown rot, smut, basal stem rot, anthracnose, rusts, scab, and general decline (Agrios 2005).

The most common methods used to control phytopathogenic fungi include: (1) protective methods, such as resistant plant varieties and use of pathogen-free seed; (2) culture methods such as crop rotation; (3) chemical methods; (4) biocontrol methods by using antagonistic microorganisms such as bacteria and fungi. These methods can reduce disease caused by fungal pathogens. Chemical methods are very effective on fungal pathogens but prolonged use of chemical-based methods has a harmful effect on the environment. Biocontrol agents are also effective in controlling fungal pathogens and are safer for the environment. The application of fungicides and consumer acceptance of resistant cultivars can be very complex, which makes biological control of phytopathogenic fungi an attractive alternative. *Trichoderma* spp. are well-known for their activity against many plant pathogens that cause major problems worldwide (Table 5.1) (Sharma et al. 2011). *Trichoderma* can be a potential alternative to control charcoal rot in soybean (Khalili et al. 2016).

The interactions between a fungus and another fungus are very attractive, involving: (1) a mutually beneficial relationship that may increase the infection in plants, such as avirulent fungi with virulent fungi, low virulent fungi with high virulent fungi; (2) interspecific interaction such as hyphal interaction and somatic; (3) an antagonistic relationship, including (a) avirulent fungi against virulent fungi, (b) highly virulent fungi against virulent fungi, (c) parasitic fungi against virulent fungi, and (d) parasitic fungi against benefit fungi. However, Boddy (2016) has explained the spectrum of fungus–fungus interactions in a scheme.



*Trichoderma* spp. use many mechanisms against phytopathogenic fungi, which can divide in two ways – direct and indirect. The direct mechanism is accomplished when it reduces the pathogen population by antagonistic effects which include competition, antibiosis, and parasitism. The indirect mechanism is achieved by reaction between *Trichoderma* and plant against phytopathogenic fungi. This interaction is also denoted as ‘cross-protection’ or ‘induced resistance’ and is based on the creation of the host’s own defense system (Marois 1990). Therefore, many bio-control agents employ more than one mechanism to protect plants (Fravel and Engelkes 1994).

There are seven different mechanisms which have been suggested for suppression of phytopathogenic fungi. These modes of actions probably include all antagonistic effects against plant pathogens. The mechanisms are as follows:

1. Mycoparasitism refers to parasitism on mycelium or spores of fungal hosts caused by the production of cell wall degrading enzymes (CWDEs) or lysis enzymes that degrade the cell wall, such as  $\beta$ -1, 3-gluconase, chitinases, cellulases, lipases, and proteases (Van den Boogert 1996; Viterbo et al. 2002a, b; Gajera et al. 2012; Saravanakumar et al. 2016a).
2. Competition for space, nutrients including carbon and iron, and infection sites such as by modifying the rhizosphere by acidifying the soil (Benítez et al. 2004; Arst and Penalva 2003) so the pathogens cannot grow.
3. Antibiosis – production of antifungal compounds, volatile and non-volatile metabolite compounds, and stopping the growth after spore germination as fungistatic.
4. Induction of systemic resistance such as ISR and SAR.
5. Induction of plant defense such as rhizosphere modification and colonization of the plant root. This mechanism causes the change in physiological responses.
6. Detoxification that produced by plant fungal pathogens (Vázquez et al. 2015).
7. Biofertilizers and PGPF, through enhancement of plant growth, enhance the solubilization of mineral nutrients, improve the media of rhizosphere and soil, and colonize root intercellular spaces (Hermosa et al. 2012). These multiple mechanisms are used either in combination or individually to control phytopathogenic fungi (Elad 2000; Bae et al. 2016).

Mycoparasitism has been demonstrated by many *Trichoderma* species on different fungal pathogens. *T. koningii* MTCC 796 and *T. harzianum* T12 were able to parasitize the mycelia of *Macrophomina phaseolina* as well as induce the enzyme activities of CWDEs (Gajera et al. 2012; Khalili et al. 2016). Meanwhile, *T. harzianum* Tveg1 and *Trichoderma atroviride* TR10 could inhibit the mycelium growth of *F. oxysporum* f.sp. *cubense* tropical race 4 (*Foc*TR4) in in vitro experiment by Al-Ani et al. (2013a). Saravanakumar et al. (2016a) also found the strain of *T. asperellum* CCTCC-RW0014 showed mycoparasitic activity on *F. oxysporum* f. sp. *cucumerinum* by producing various CWDEs such as chitinase, cellulase, protease, and  $\beta$  (1–3) glucanase. The growth of *F. solani* was inhibited when *T. hamatum* URM 6656 was applied, which can be attributed to the production of lysis enzymes called chitinases (da Silva et al. 2016). *T. harzianum* species (THSC) attacked the



plant fungal pathogens *Ceratocystis radicola* of date palm and showed lysis of the hyphal pathogen and phialoconidia along with scattered aleurioconidia in vitro (Al-Naemi et al. 2016). de Lima et al. (2016) found that *T. atroviride* T17 showed high antagonistic activity against *Guignardia citricarpa* of citriculture that was associated with the secretion of proteins, including chitinase, mutanase,  $\alpha$ -1,3-glucanase,  $\alpha$ -1,2-mannosidase, carboxylic hydrolase ester, carbohydrate-binding module family 13, glucan 1,3- $\beta$ -glucosidase,  $\alpha$ -galactosidase, and neutral protease. Al-Ani (2017) screened 31 isolates of *Trichoderma* against *Foc*TR4 in vitro and found 12 isolates of *Trichoderma* (*T. harzianum*, *T. parareesei*, *T. reesei*, *T. capillare*, *T. atroviride*, and *T. koningii*) overgrew the *Foc*TR4 after the ninth days of inoculation.

Examining the competition for nutrients, Sivan and Chet (1989) found that *T. harzianum* was able to compete with *F. oxysporum* f. sp. *vasinfectum* for carbon in vitro by inhibiting chlamydospores germination and simultaneously suppressed *Fusarium* wilt of cotton in vivo. Sarrocco et al. (2009) found that *T. virens* I10 can compete with *R. solani* for carbon in soil by producing a cellulose enzyme. Three isolates of *Trichoderma* (*T. atroviride* P1, *T. harzianum* T22, and *T. viride*) showed strong competitiveness with *Phytophthora cinnanerium*, *Botrytis cinaria*, and *R. solani* (Olabiyi and Ruocco 2013). However, several *Trichoderma* can stop the growth of other fungi by producing siderophores (iron-chelating compounds) (Chet and Inbar 1994). *Trichoderma asperellum* strain T34 has the potential to control *Fusarium* wilt of tomato caused by *F. oxysporum* f.sp. *lycopersici* (Fol) through competition for iron (Segarra et al. 2010). Lehner et al. (2013) suggested that *Trichoderma* spp. can produce siderophores through screening using LC-HRMS/MS. Al-Ani (2017) found four isolates of *Trichoderma* (two isolates of *T. harzianum* (TL5, Tveg1), *T. parareesei* T26, and *T. koningii* TR102) were capable of competing with *Foc*TR4 for iron by producing siderophores.

In antibiosis production, *T. parareesei* inhibited the growth of *Foc*TR4 by up to 96% in vitro by producing secondary metabolites (Al-Ani et al. 2013a). Bae et al. (2016) found *T. atroviride/petersenii* (KACC, 40557) showed the highest inhibition of *Phytophthora* growth. *T. harzianum* T12 produced many volatile compounds to control charcoal rot in soybean caused by *M. phaseolina* (Khalili et al. 2016). *T. harzianum* Th-Sks showed high efficacy against *F. oxysporum* and *Pythium aphanidermatum*, which caused damping off and wilt diseases of brinjal and okra by producing volatile and non-volatile compounds (Sain and Pandey 2016). The culture filtrates containing volatile compounds of THSC were able to decrease the size of necroses caused by *C. radicola* of date palm in vivo (Al-Naemi et al. 2016). Seven volatile compounds, possibly with antifungal activity, produced by *T. parareesei* T26 inhibited *Foc*TR4 in vitro and managed to reduce the disease severity of *Fusarium* wilt by up to 100% in vivo (Al-Ani 2017).

The ISR of *T. harzianum* in the roots of cucumber was observed through the change in structural compounds, the deposition of newly formed barriers, and strengthening of the epidermal and cortical cell walls (Yedidia et al. 1999). *T. harzianum* was able to induce systemic resistance in carrot against *Alternaria radicina* and *Botrytis cinerea* by using chitinase and CHIT36 expressed in the plant

(Baranski et al. 2008). *Trichoderma virens* and *T. atroviride* induced plant defense and activated the signaling pathway including SA and/or JA, as well as camalexin, conferring resistance in *Arabidopsis thaliana* against necrotrophic fungus *Botrytis cinerea* (Contreras-Cornejo et al. 2011). *Trichoderma asperellum* was able to induce acquired resistance in cucumber by activating peroxidase and a boost in SA (Hermosa et al. 2012). The ISR in plants was triggered by increasing the ET or JA pathways by *Trichoderma* cellulase complexes (Hermosa et al. 2013). *T. harzianum* triggered the transient production of ROS by *Thph1* and *Thph2* proteins, which required enhancing ISR in maize leaf (Saravanakumar et al. 2016b).

In induced plant defense mechanisms, *T. virens* has induced plant defense through seed treatment using terpenoid synthesis in cotton root against *R. solani* (Howell et al. 2000). *T. viride* JAU60 stimulated the specific defense enzymes of polyphenol oxidase,  $\beta$ -1,3 glucanase, phenylalanine ammonia lyase, and chitinase against collar rot disease caused by *Aspergillus niger* Van Tieghem (Gajera et al. 2015). *T. asperellum* induced the plant defense-related genes in the banana plant against *Fusarium oxysporum* f.sp. *cubense* (Foc) (Raman et al. 2016). *Trichoderma virens* (KACC 40929) stimulated defense-related genes against *Phytophthora* infection and changes of plant hormonal (Bae et al. 2016). *T. aureoviride* URM 5158 was capable of reducing disease severity to 60% in the shoot and 84% in the root of cassava plants by inducing plant defense. The strain changed the physiological response and maximized the enzyme activity of ROS groups (da Silva et al. 2016). Seed treatment with *T. viride* JAU60 has increased the activity of ROS enzymes and reduced 51–58% collar rot disease incidence by rot pathogen *Aspergillus niger* (Gajera et al. 2016). *T. asperellum* BHUT8 induced plant defense in tomato seedlings which include phenylalanine ammonia-lyase (PAL), peroxidase (PO), polyphenol oxidase (PPO), lignifications, and the accumulation of some secondary metabolites such as shikimic acid and gallic acid (Singh et al. 2016).

In detoxification mechanism, several isolates of *T. viride* were able to detoxify *R. solani* toxin (Sriram et al. 2000). Aggarwal et al. (2011) also found that *T. viride* (TV5-2) detoxified the *Bipolaris sorokiniana* toxin and reduced the disease severity of spot blotch in wheat. Tian et al. (2016) found eight strains of *Trichoderma* (*T. harzianum* GIM3.442, *T. harzianum* JF309, *T. koningii* GIM3.137, *T. longibranchiatum* GIM3.534, *T. harzianum* Q710613, *T. atroviride* Q710251, *T. asperellum* Q710682, and *T. virens* Q710925) that showed antagonistic activity against *F. graminearum*, the causal agent of *Fusarium* head blight (FHB), by inhibiting the mycelium growth and detoxifying deoxynivalenol (DON) to deoxynivalenol-3-glucoside (D3G).

In biofertilizers and PGPF mechanisms, *Trichoderma* spp. can colonize the roots of plants which can improve the plant growth, increase the crop productivity, form a strong resistance to plant pathogens, and improve nutrient uptake (Arora et al. 1992). PGPF *T. harzianum* T-22 has solubilized and chelated various plant nutrient compounds and further enhanced plant growth (Altomare et al. 1999). *Trichoderma hamatum* and *T. koningii* could increase crop productivity up to 300% (Benítez et al. 2004). Qi and Zhao (2012) revealed that *T. asperellum* strain Q1 acted as PGPF and increased the content of osmosis molecules and chlorophyll, plant biomass, and

enhanced the activity of osmosis molecules and antioxidant enzymes under saline environments. *T. asperellum* T34 plays a role in increasing the accumulation of Cu and Fe in the aerial parts of cucumber plants, as well as Zn and Mn according to the availability in the soil (de Santiago et al. 2013). *T. atroviride* LU132 was capable of colonizing oilseed rape (*Brassica napus*) and increased the biomass of root and shoot (Maag et al. 2013). *T. harzianum* (ANR-1) increased the plant height and dry weight of tomato plants (Sundaramoorthy and Balabaskar 2013). Li et al. (2015) suggested that *T. harzianum* strain SQR-T037 can promote plant growth by development of the root and the increased nutrient uptake, as well as dissolution (i.e., most likely chelating for Cu, chelating for Fe, acidification, and redox). *T. harzianum* T12 is able to enhance the plant growth of soybean (Khalili et al. 2016). *Trichoderma* can enhance plant vigor of *Miscanthus x giganteus* (Mxg), including growth, chlorophyll concentration, plant height, and shoot dry weight (Chirino-Valle et al. 2016). Singh et al. (2016) found that seeds of some plants, such as tomato, ridge gourd, chilli, and guar, treated with *T. asperellum* BHUT8 can improve seed germination and radicle length. Al-Ani (2017) found that *T. harzianum* Tveg1 can improve the plant vigor of banana, such as plant height, the content of chlorophyll, plant biomass, and number of the leaves. Sain and Pandey (2016) showed that the plant height and fruit yield of brinjal and okra increased when *T. harzianum* Th-Sks was used to treat the seeds.

### 5.3.4 Biocontrol of Plant-Parasitic Nematodes

Nematodes are worm-like but quite distinct taxonomically from the true worms. There are several hundred species, and they obtain their food by feeding on living plants using spears or stylets. This feeding method has caused major plant disease worldwide. These nematodes have an effect on sustainable agriculture amounting to 11–14%, involving such crops as legumes, cereals, banana, vegetable, cassava, fruits, and nonedible field crops (Agrios 2005). Control measures are often difficult, particularly involving systemic nematicides and insecticide treatments to decrease the nematodes and vectors (Agrios 2005). Biological control, cultural, and physical methods are other general measures for controlling nematodes. Oil-cakes, residues from leguminous crops, other materials with a low C/N ratio, and animal manures can also be added to soil (Stirling 2011).

Biological control is an alternative management system to control plant-parasitic nematodes which are suppressed by pathogen-specific agents comprising many enemies, such as viruses, bacteria, fungi, nematodes, microarthropods, and protozoa. Fungi are registered to be biocontrol agents for nematodes. Soil contains a large range of fungi species which are able to suppress plant-parasite nematodes and are called nematophagous fungi. More than 200 species of nematophagous fungi have been described (Tunlid and Ahrén 2011). Some isolates or strains of *Trichoderma* have been considered to be in the nematophagous fungi group. Biocontrol activity of *Trichoderma* spp. against plant-parasitic nematodes is

exploited by difference mechanisms. These mechanisms include parasitism, competition, antibiosis, induction of plant defense, systemic resistance, enhancement of plant growth, tolerance of the infection, and impact on the life cycle of the plant-parasitic nematode.

For parasitism, *Trichoderma* attacks eggs, juveniles (larvae), and adults. In the parasitism the mode of action is attached to the nematode and then the parasite. The special chemical is the gelatinous matrixes (gm) that envelop the eggs and play a very essential role to attach the mycelium of *Trichoderma* to the eggs, egg masses, and the second stage juveniles (J2s) (Sharon et al. 2007, 2011). The *Trichoderma* secretion enhances the ability of the plant-parasitic nematodes. These enzymes include proteases, cellulases, hemicellulases, chitinases, and glucanases (Viterbo et al. 2002a, b; Keswani et al. 2013). *T. harzianum* is able to attack and colonize the eggs, and egg masses of the root-knot nematode *Meloidogyne javanica* by instigating proteolytic activity (Sharon et al. 2001). The two isolates of *T. asperellum*-203 and *T. atroviride*-IMI 206040 attack the eggs and J2s of *Meloidogyne javanica* by the formation of coiling and appressorium-like structures and then parasitism (Sharon et al. 2007).

For competition, *Trichoderma* maybe competes with plant parasitic nematodes. The competition for space and feeding sites may occur with plant parasitic nematodes (Hussey and Roncadori 1978). For the antibiosis, nematicidal, anti-nematode and secondary metabolites as volatile and non-volatile compounds are included. For example, *T. viride* metabolites impacted on reproduction and development of *Meloidogyne javanica* through implementing root-dip treatments with the fungal culture filtrate (Khan and Saxena 1997). Strains of *T. harzianum* have the capability to produce the anti-nematode and nematicidal against the root-knot nematode *Meloidogyne javanica* that appears as immobilized J2 s and reduces the penetration of the root by the nematode (Sharon et al. 2001). The culture filtrate of *T. harzianum* was then able to inhibit egg hatching of the nematode *M. javanica* at the standard concentration in vitro that may release toxic metabolites/enzymes into the medium (Khattak et al. 2008). Yang et al. (2012) found three metabolites of *Trichoderma* sp. YMF 1.00416 comprising a new compound, 1 $\beta$ -vinylcyclopentane-1 $\alpha$ ,3 $\alpha$ -diol (1), and two known metabolites, 6-pentyl-2H-pyran-2-one (2) and 4-(2-hydroxyethyl) phenol (3); compound 2 was nematicidal and killed 85% of *Bursaphelenchus xylophilus*, *Panagrellus redivivus*, and *Caenorhabditis elegans*. *T. harzianum* strains were able to produce antibiosis as a mechanism antagonistic against the nematode *M. cionopagum* (Szabó et al. 2012).

To induce resistance, for example, *Trichoderma* can stimulate resistance as a localized or systemic response against nematodes, which may occur on the surface of the roots, inside the roots, and in the soil (Sharon et al. 2011). *Trichoderma* primes JA- and SA-dependent defenses in tomato roots against the root knot nematode *M. incognita* (Martínez-Medina et al. 2017). *T. harzianum* isolate T10 was able to change the chemical and physical reactions in tomato against the invasion of *M. javanica* nematode because of two different kinds of systemic resistances, ISR and SAR (Selim et al. 2014). *T. atroviride* could induce systemic resistance against *Meloidogyne javanica* caused by root-knot nematodes of tomato

in vivo by a split-root occurrence that is a trigger of SA-, JA- and ET-dependent defense pathways (de Medeiros et al. 2017). For the stimulation of plant defense, for example, *T. harzianum* isolate ITEM908 stimulated the plant defense by the expression of patterns of the genes *PRI* in tomato against the invasion of *M. incognita* (Leonetti et al. 2014).

For the enhancement of plant growth, for example, *T. harzianum* and *T. lignorum* isolates improved growth as fresh weight against the root-knot nematode *Meloidogyne javanica* (Spiegel and Chet 1998). *T. harzianum* is efficient in controlling *Meloidogyne javanica* on tomato at the highest used density ( $10^{10}$  spore/g soil). *T. atroviride* enhanced plant growth against *Meloidogyne javanica* (de Medeiros et al. 2017).

Tolerance of the infection is reflected in a decreased number and size of root galling, cessation of the growth and reproduction of plant-parasitic nematodes inside the plant, increase of crop yield, and a halt to the plant-parasitic nematode from completing its life cycle. *T. asperellum* T-16 led to a reduction of the number and size of galls and enhanced the tomato yields that were infected by *M. incognita* and caused root-knot nematodes in vegetables (Affokpon et al. 2011). *T. harzianum* T22 enhanced plant height, number of branches, and yield growth against *Meloidogyne incognita* of soybeans (Izuogu and Abiri 2015). Javeed et al. (2016) mention that three isolates of *Trichoderma* reduced root galling in tomatoes that resulted from infection by *M. javanica*. Al-Hazmi and Javeed (2016) stated that *T. harzianum* are able to inhibit root galling of *Meloidogyne javanica* on tomato. *T. atroviride* impacted on *Meloidogyne javanica* by reducing the number of galls (de Medeiros et al. 2017).

Impact on the life cycle of a plant-parasitic nematode can be reflected in nematode reproduction, the type and number of gender (the number of males compared with females), larval mortality, egg hatching, the number of eggs, egg masses, size and movement of the nematode or juveniles, and the general physiological functions of the plant-parasitic nematode. *T. asperellum* T-16 inhibited the densities of J2s of *Meloidogyne incognita* in the roots, but *T. asperellum* T-12 inhibited the densities of J2s in the soils, and *T. brevicompactum* T-3 suppressed egg production (Affokpon et al. 2011). Four strains of *Trichoderma* – *T. harzianum*, *T. virens*, *T. atroviride*, and *T. rossicum* – triggered higher and faster mortality of plant-parasitic nematode *Xiphinema index*, which is capable of transmitting several plant viruses (Daragó et al. 2013).

*T. harzianum* T22 inhibited the development and parasitic effects of *Meloidogyne incognita* and led to a reduction of the soil nematode population that caused root knot nematode of soybeans (Izuogu and Abiri 2015). Javeed et al. (2016) found three isolates of *Trichoderma* – *T. harzianum* (isolate No.27), *T. hamatum* (isolate No.5), and *T. viride* (isolate No.8) – which decreased the number of juveniles (larvae) and inhibited the reproduction of *M. javanica*. *T. harzianum* can suppress nematode reproduction via egg masses, eggs, and J2s of *Meloidogyne javanica* on tomato (Al-Hazmi and Javeed 2016). *T. atroviride* decreased the number of eggs and adult nematodes that impact on *Meloidogyne javanica* (de Medeiros et al. 2017).

### 5.3.5 *Biocontrol of Parasitic Higher Plants by Trichoderma*

The parasitic higher plants are vascular plants that penetrate the tissue of hosts (vascular plants) and absorb nutrients from the host by unique connections. Some of the parasitic plants are major factors that impacts on production of many crops. The parasitic plants have no or little chlorophyll, false roots, but can produce flower and seeds. There are more than 2,500 known species of higher plants (Sharma 2006) that live on other plants parasitically. For control of parasitic plants comprise many methods such as chemical herbicides, biocontrol, breeding and selection of resistant crop, soil fumigation, solarization, parasitic-plant-free seed, cultural practices, and the use of trap and catch crops (Abdel-Kader and El-Mougy 2009). Biological control is an alternative method to control parasitic plants instead of chemical herbicides. *Trichoderma* is a biological control or bioherbicide agent that uses many mechanisms against plant diseases as parasitic plants. *T. harzianum* (T1 and T3) and *T. viride* (T2) applied of tomato plants by the foliar spray and the soil drench method that reduced both infection and intensity of attack by the plant pathogens of broomrapes *Orobanche ramosa* (Abdel-Kader and El-Mougy 2007, 2009). *T. harzianum* attacked living tissues of broomrapes *Orobanche ramosa* that could cause soft rot, black lesion, a reduction of number of *Orobanche* shoots, and complete deterioration within 7 days (100%) (Nawar and Sahab 2011). *T. harzianum* reduced the number root-parasitic weeds such as *Phelipanche* and *Orobanche* spp. that impact on the plant hosts by producing some effective secondary metabolites as 5-deoxystrigol and 4-deoxyorobanchol (Boari et al. 2016).

### 5.3.6 *Biocontrol of Plant Viroids by Trichoderma*

Plant viroids is a plant pathogen since 1971, and it is causing slight damage to the agricultural economy but sometime be more catastrophic. Plant viroids can cause several important diseases such as citrus exocortis, apple scar skin, potato spindle tuber, the cadang-cadang disease of coconut, and avocado sunblotch (Agrios 2005). Viroids are very important plant pathogens because they are composed of a (1) short stretch of circular, (2) nonprotein-coding, (3) single-stranded RNA with autonomous replication. The control of plant viroids is a complex method. Some methods to control this plant pathogen include eradication, cultural controls, elimination of insect vectors, and inducing resistance. *Trichoderma* has not yet been registered as a biocontrol agent against plant viroids, but I assume they can be used. Why is *Trichoderma* considered a biocontrol agent against plant viroids? The genus *Trichoderma* has many potential mechanisms to control different plant diseases, such as the indirect method that may be effective on this pathogen. This indirect method includes inducing resistance and defense and control of the vectors, as well as enhanced growth and tolerance of the plant against viroid infection. *Trichoderma* is able to induce resistance against plant virus, as described in Sect. 5.3.1. As the life cycle of plant virus is the same as that of plant viroids, use of *Trichoderma* as a



future biocontrol agent against plant viroids is recommended, which may lead to obtaining greater insight about the relationship between *Trichoderma* and plant viroids.

### 5.3.7 *Biocontrol of Phytoplasma by Trichoderma*

Phytoplasma is a plant pathogen that was detected in 1967 and causes around 200 plant diseases worldwide. However, Phytoplasmas lack cell walls and have ribosomes, cytoplasm, and strands of nuclear material which are bounded by a “unit” membrane (Agrios 2005). Phytoplasmas cause very significant diseases such as European stone fruit yellows, apple proliferation, lethal coconut yellowing, peach X disease, grapevine yellows, pear decline, and aster yellows (Agrios 2005). Controlling of phytoplasma is very difficult. Phytoplasma can be controlled by various common methods, as described in Sect. 5.3.6. *Trichoderma* is not a registered biocontrol agent against phytoplasma. Also, my assumption here is the same as in Sect. 5.3.6. Why is *Trichoderma* considered a biocontrol agent against phytoplasma? *Trichoderma* has many indirect mechanisms that can be effective against phytoplasma, which are mentioned in Sect. 5.3.6. Therefore, we need to know the nature of relationship between *Trichoderma* and phytoplasma, which may be beneficial in the future, to obtain a cleaner method for the ecosystem.

## 5.4 Conclusion

All *Trichoderma* species are of interest to researchers because of their use in the biocontrol and reduction of plant pathogens (viruses, bacteria, fungi, plant parasitic nematode, and parasitic higher plants) as well as the enhancement of tolerance in the plant against plant pathogens and plant growth promotion. This is achieved through the supply of plant nutrient by the secretion of some very interesting items such as chelating compounds, for example, siderophores, chelating for Cu, and acidification. *Trichoderma* has many mechanisms, such as direct parasitic, via appressorium-like structures, secreting enzymes or volatile compounds inhibiting the host growth. Indirect impact by detoxification against diseases of the plants and induction of plant defense by changing the activity of plant physiology, such as by activating very important enzymes, are also involved. This confers protection to plants and also induces systemic resistance, changing the physical and chemical barriers to prevent plant pathogens from entering the plant. *Trichoderma* can therefore be used as bio-bactericides, biofungicides, and bioherbicides to control the major plant pathogens, and as biofertilizers to enhance plant growth for the major plants. The combination of biocontrol with biofertilization in same product may also contribute to increasing sustainable agriculture, making the environment cleaner, free from residues from chemical pesticides and fertilizers. The final result is the production of cleaner food.



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