ISBN: 978-93-48091-57-4

6. Secondary Metabolites of Trichoderma and Their Role in Agriculture

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6.1 Biological Control in Agriculture:

Biological control for plant disease refers to controlling disease by using organisms like fungi, bacteria, and viruses (O'Brien, 2017). This can be achieved through the introduction or utilization of resident antagonistic living organisms. Various forms of interactions between organisms, including hyperparasitism, antibiosis, commensalism, neutralism, and competition, play a role in biological control (Pal & Gardener, 2006). Compared to chemical plant management, biological approaches impose fewer environmental risks, making them a more sustainable alternative. Various bio-control agents commonly used against plant pathogens are *Trichoderma harzianum*, *T. hamatum*, *T. viride*, *T. koningii*, *Gliocladium virens*, *G. roseum*, *Paecilomyces liiacinus*, *Coniothyrum minitans*, *Bacillus subtilis*, *B. polymyxa*, and *Pseudomonas fluorescens* (Tyśkiewicz et al., 2022). Most important genera against the soil pathogens are *Trichoderma*, *Pseudomonas* and *Bacillus* (Guo et al., 2004; Huang et al., 2015). *Trichoderma* is found as effective bio-control agent especially for soil-borne pathogens like *R. solani*, *S. rolfsii*, *Phythium aphanidermatum*, *Fusarium oxysporum*, and *Gaeumannomyces graminis* under both field and greenhouse conditions (Chet & Inbar, 1994; Basim et al., 1999).

The *Trichoderma* spp. have positive effect on plant growth through hydrolysis of cellulose in soil, increases plant defense mechanism, mineral solubilization and improvement in root morphology, enabling roots to cover large volumes of soil (Junaid et al., 2013; Timila et al., 2015). *Trichoderma* as bio-control agents could be incorporated in integrated pest management (IPM) for managing soil borne pathogens (Bastakoti et al., 2017).

6.2 Trichoderma Bio-Control Efficacy:

Trichoderma spp., the promising antagonistic fungi are established in agriculture use. Watanabe (1985) found *T. hamatum, T. harzianum, T. koningii, T. pseudokoningii* and *T. viride* has strong antagonistic potential. They show antagonistic activity under both *in vitro* and *in vivo* conditions by competing for nutrients and space, antibiosis, mycoparasitisam, promoting plant growth and plant defense responses (Brotman et al., 2010). Seema and Devaki (2012) reported the antagonistic effects of *T. harzianum* and *T. viride* and found significant suppression of mycelial growth and sclerotia formation of pathogen, *R. solani*. Similarly, the seedling mortality of groundnut was also significantly reduced by seed treatment with various isolates of *Trichoderma* spp. (Biswas & Sen, 2000).

Freeman et al. (2004) reported various isolates of *Trichoderma*, including *T. harzianum* isolate T-39 significantly reduced anthracnose (*Colletotrichum acutatum*) and grey mould (*B. cinerea*) in strawberry under greenhouse and *in vitro* conditions. Bastakoti et al. (2017) found *Trichoderma* isolates showed 100%, 62% and 68% inhibition against *S. rolfsii*, *R. solani* and *Fusarium solani*, respectively. Amin et al. (2010) tested six isolates of *Trichoderma* against three different soil borne pathogens namely *R. solani* (isolates from tomato), *Sclerotinia sclerotiorum* (causing web blight of beans) and *S. rolfsii* (causing collar rot of tomato) under *in vitro* conditions and found that maximum inhibition (71.41%) in *R. solani* by *T. viride* (Tv-2) followed by *T. viride* (Tv-1) and *T. harzianum* (Th-1). *T. viride* (Tv-1) showed best antagonist inhibiting 67.91 and 66.21% over control in *S. rolfsii* and *S. sclerotiorum*, respectively. They also found that all the *Trichoderma* isolates significantly inhibited sclerotia production in all three tested pathogens. Three species of *Trichoderma* namely *T. asperellum*, *T. harzianum* and *Trichoderma* spp. were effective against *R. solani* and can be used as biological control agents (Asad et al., 2013). *Trichoderma* is also effective against Stemphylium blight of lentil. Subedi et al. (2014) reported percentage disease control and percent yield increase were higher in *T. viride* i.e. 42.14% and 58.80% respectively. *T. viride* also showed high compatibility with fungicides (potassium phosphonate and fosetyl aluminium) and incompatibility with fungicides (carbendazim, hexaconazole, potassium phosphonate + hexaconazole mixture and captan + hexaconazole mixture) (Dhanya et al., 2016).

6.3 Mode of Action by *Trichoderma*:

Trichoderma spp. are free-living, commonly growing fungi in soil and rhizosphere of various crops (Harman et al., 2004). *Trichoderma* not only provide protection against plant diseases, but also reduce host susceptibility towards the pathogen. *Trichoderma* can control plant pathogens directly by competition for nutrients, mycoparasitism, production of cell wall degrading enzymes, and production of antibiotics (Harman, 2006) and indirectly by stimulation of plant defense systems

(Benítez et al., 2004). *Trichoderma* spp. also produce a wide array of secondary metabolites that play crucial roles as biocontrol agents, plant growth promoters, and enhancers of plant resilience to stress.

These metabolites include peptaibols, polyketides, terpenoids, non-ribosomal peptides, volatile organic compounds (VOCs), and enzymes, each contribute to efficacy of *Trichoderma* (Alfiky & Weisskopf, 2021). Peptaibols like trichorzins disrupt microbial membranes, while polyketides such as sorbicillins exhibit strong antifungal properties. Terpenoids like harzianolide promote plant growth, and VOCs such as 6-pentyl-2H-pyran-2-one (6-PP) not only inhibit pathogens but also stimulate root and shoot development (Nakkeeran et al., 2021; Patil et al., 2016). Secondary metabolites induce systemic resistance (ISR) in plants, enhancing defense mechanisms against pathogens, and also improve soil health by suppressing harmful microbes.

6.4 Secondary Metabolites of *Trichoderma* and their Application in Agriculture:

Trichoderma produces a wide range of secondary metabolites. *Trichoderma*-derived secondary metabolites comprise non-ribosomal peptides such as peptaibiotics, siderophores, and diketopiperazines-like gliotoxin and gliovirin, polyketides, terpenes, pyrones, and isocyane metabolites (Nakkeeran et al., 2021). Some of the important secondary metabolites are categorized as follows:

6.4.1 Peptaibols:

Peptaibols are a unique group of linear peptides produced by *Trichoderma* spp., characterized by their high content of non-proteinogenic amino acids, such as α-aminoisobutyric acid (Aib), and C-terminal hydroxyl groups (Shishupala, 2023).

These compounds exhibit potent antimicrobial properties, primarily by forming pores in the cell membranes of pathogens, leading to ion imbalance and cell death. Trichorzins, Alamethicins, and Trichotoxins are peptaibols that are effective against a wide range of fungal, bacterial, and nematode pathogens (Nakkeeran et al., 2021; Shishupala, 2023).

In agriculture, peptaibols contribute significantly to biocontrol by inhibiting plant pathogens like *Fusarium* spp., inducing systemic resistance in plants, and maintaining soil health by suppressing harmful microbes.

6.4.2 Polyketides:

Polyketides are a structurally diverse class of secondary metabolites synthesized by *Trichoderma* spp. through the polyketide synthase (PKS) pathway, known for their broad-spectrum antimicrobial, antifungal, and cytotoxic activities (Khan et al., 2020; Conrado et al., 2022). These compounds, such as sorbicillins, viridin, and melanins, play a crucial role in biological control by inhibiting the growth of plant pathogens through mechanisms like disrupting cell membranes, inducing oxidative stress, and interfering with essential metabolic processes (Nandini et al., 2023).

In agriculture, polyketides not only suppress soil-borne pathogens like Fusarium spp. and Rhizoctonia spp., but also enhance plant resilience to biotic and abiotic stresses, including drought and salinity (Narayanasamy et al., 2023). Additionally, polyketides support *Trichoderma's* competitive advantage in the rhizosphere by suppressing competing harmful microbes and promoting beneficial microbial interactions (Shahriar et al., 2022). Polyketides enhance water retention and mitigate oxidative stress in plants. Sorbicillins protect plants from damage caused by toxic metals. Their versatile functions, including disease suppression, stress mitigation, and soil health improvement, make polyketides indispensable in sustainable agricultural systems.

6.4.3 Terpenoids:

Terpenoids are a large and diverse class of secondary metabolites produced by *Trichoderma* spp., derived from isoprene units through the mevalonate or methylerythritol phosphate pathways (Bansal & Mukherjee, 2016; Salwan et al., 2019).

These compounds, including harzianolide, trichodermin, and ergokonin, are known for their antifungal, antibacterial, and plant growth-promoting properties (Bansal & Mukherjee, 2016; Nakkeeran et al., 2021).

Terpenoids disrupt pathogen cell membranes, inhibit spore germination, and interfere with essential metabolic pathways, making them highly effective in managing plant pathogens like *Fusarium* spp., *Rhizoctonia* spp., and *Botrytis* spp. (Pusztahelyi et al., 2015). Beyond pathogen suppression, terpenoids enhance plant health by promoting root and shoot development, inducing systemic resistance, and mitigating abiotic stresses such as salinity and drought. Their role in improving soil health and fostering beneficial microbial communities further supports their application in sustainable agriculture. Harzianolide and similar terpenoids improve nutrient absorption and plant architecture resulting in boost productivity by mitigating biotic and abiotic stresses.

6.4.4 Non-Ribosomal Peptides (NRPs):

Non-ribosomal peptides (NRPs) are a class of secondary metabolites produced by Trichoderma spp. through non-ribosomal peptide synthetases (NRPS), which are enzymatic complexes that assemble these peptides without the need for ribosomes (Mukharjee et al., 2012; Zeilinger et al., 2016). Unlike ribosomal peptides, NRPs often contain unusual amino acids and are highly diverse in structure and function. Notable examples of NRPs produced by *Trichoderma* include gliotoxin and viridin, which are known for their strong antifungal and antimicrobial properties (Zeilinger et al., 2016). Gliotoxin, for example, acts by inducing oxidative stress and apoptosis in fungal cells, while viridin inhibits the growth of a wide range of plant pathogens (Mukharjee et al., 2012). NRPs play a crucial role in *Trichoderma's* biocontrol efficacy, as they disrupt the cellular functions of pathogens, preventing their growth and spread. In addition to their antifungal and antibacterial activities, non-ribosomal peptides can also help induce systemic resistance in plants, enhancing their ability to defend against various stresses. Post-harvest spoilage caused by fungal pathogens can be significantly reduced through *Trichoderma* applications. Metabolites like gliotoxin inhibit spoilage fungi on fruits and vegetables.

6.4.5 Volatile Organic Compounds (VOCs)

Volatile Organic Compounds (VOCs) are small, low-molecular-weight compounds produced by *Trichoderma* spp. that easily vaporize at room temperature (Bansal & Mukherjee, 2016; Salwan et al., 2019). These compounds play a significant role in the biocontrol and plant growth-promoting activities of *Trichoderma*. 6-pentyl-2H-pyran-2-one (6-PP), isobutyl alcohol, and dimethyl disulfide are some VOCs which have demonstrated antifungal, antibacterial, and plant growth-promoting effects (Salwan et al., 2019). VOCs inhibit the growth of a broad range of plant pathogens, including fungi like *Fusarium* spp., *Pythium* spp., and *Rhizoctonia* spp., by disrupting their cellular processes or by inducing oxidative stress (Nandini et al., 2023).

Furthermore, VOCs stimulate plant growth by enhancing seed germination, root development, and overall plant health, as well as by inducing systemic resistance in plants, thereby increasing their resilience to pathogens and environmental stresses.

The production of VOCs helps *Trichoderma* compete in the rhizosphere by suppressing harmful microorganisms while promoting beneficial ones. Despite their potent effects, the application of VOCs in field conditions can be challenging due to their volatility and environmental sensitivity, which may reduce their persistence and effectiveness.

6.4.6 Siderophores:

Siderophores are specialized molecules that bind to and solubilize iron from the environment, making it available for microbial uptake (Schalk, 2024). Iron is an essential nutrient for many microorganisms, but it is often limiting in the soil due to its low solubility. *Trichoderma* produces siderophores like ferrichrome, trichodermin, and pyoverdine to scavenge iron from the soil and outcompete pathogens for this vital resource (Zeilinger et al., 2016). By producing siderophores, *Trichoderma* can effectively outcompete plant pathogens for iron, limiting their growth and preventing infection. Pathogenic fungi such as *Fusarium* and *Pythium* are often inhibited by *Trichoderma* due to this competition for iron (Adnan et al., 2019). In addition to biocontrol, siderophores support plant health by ensuring adequate iron availability for plant growth.

6.4.7 Enzymes:

Trichoderma spp. produce a variety of enzymes that play essential roles in biocontrol, plant growth promotion, and nutrient cycling in the soil. These enzymes include cellulases, chitinases, glucanases, proteases, and lipases, which help *Trichoderma* degrade complex organic materials, enhance plant health, and suppress plant pathogens.

A. Cellulases: These enzymes break down cellulose, a major component of plant cell walls, into simpler sugars. By degrading cellulose, *Trichoderma* helps in the decomposition of plant residues, improving soil structure and nutrient availability (Mukherjee et al., 2012). This activity also aids in controlling fungal pathogens with cell wall components similar to cellulose, such as those in the genus *Fusarium*.

B. Chitinases: *Trichoderma* produces chitinases, which break down chitin, a key structural component of fungal cell walls. By degrading chitin, these enzymes inhibit the growth of pathogenic fungi like *Rhizoctonia*, *Fusarium*, and *Pythium*, as well as help in nutrient cycling by breaking down chitin-rich insect exoskeletons and fungal remains (Zeilinger et al., 2016).

C. **Glucanases**: These enzymes hydrolyze glucans, another important component of fungal cell walls, particularly in *Botrytis* and *Fusarium* species. By breaking down glucans, glucanases weaken the cell walls of pathogenic fungi, making them more susceptible to further attack by *Trichoderma* or other biocontrol agents (Mukherjee et al., 2012; Zeilinger et al., 2016).
D. **Proteases**: Proteases degrade proteins, which helps *Trichoderma* obtain essential nitrogen sources from organic matter, but also contributes to its biocontrol capabilities by attacking the proteins of pathogens. This activity disrupts the pathogen's cellular processes and weakens their virulence (Alfiky & Weisskopf, 2021).
E. **Lipases**: These enzymes break down lipids and fats, supporting *Trichoderma* in utilizing organic materials in the soil and promoting nutrient cycling. Lipases also contribute to the biocontrol of fungal pathogens by degrading the cell membranes of target organisms (Alfiky & Weisskopf, 2021).

6.5 Conclusion:

Trichoderma spp. have emerged as one of the most promising biocontrol agents in sustainable agriculture. Their ability to produce a wide range of secondary metabolites, including peptaibols, polyketides, terpenoids, VOCs, siderophores, and enzymes, underscores their versatility and efficacy in combating plant pathogens, promoting plant growth, and enhancing resilience to abiotic stresses.

The mechanisms of action—ranging from direct pathogen suppression through mycoparasitism, antibiosis, and nutrient competition to indirect benefits like systemic resistance induction and soil health improvement—make *Trichoderma* an invaluable resource in modern crop management.

Its compatibility with eco-friendly agricultural practices ensures reduced dependency on synthetic chemicals, contributing to environmental preservation and human health safety.

Their integration into cropping systems aligns with the global shift towards green technologies and sustainable food production, providing a viable pathway to achieving agricultural resilience and food security in the face of climate change and increasing population pressures. Continued research and innovation in *Trichoderma* applications will undoubtedly expand its potential, solidifying its role as a cornerstone in sustainable agriculture.

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