



Biocontrol Potential and Mode of Action of Trichoderma Against Fungal Plant Diseases

Ashim Pokhrel^{1*}, Aakash Adhikari¹, Dipiza Oli², Bipul Paudel¹, Shishir Pandit¹, Bigyan GC¹ and Brij Raj Raut Tharu³

¹Department of Agriculture, Himalayan College of Agricultural Sciences and Technology, Kathmandu, Nepal

²Montana State University, Bozeman, USA

³Georg-August Universität Göttingen, Göttingen, Germany

*Corresponding Author: Ashim Pokhrel, Department of Agriculture, Himalayan College of Agricultural Sciences and Technology, Kathmandu, Nepal.

DOI: 10.31080/ASAG.2022.06.1184

Received: August 26, 2022

Published: September 14, 2022

© All rights are reserved by Ashim Pokhrel, et al.

Abstract

Plant diseases, which can result in yield losses of up to 30% and are a major contributor to low crop production, pose a danger to the sustainability of the world's food supply. As more than 60% of the bio fungicides that are now registered around the globe are derived from Trichoderma-based formulations, *Trichoderma spp.* is the most effective bio fungicide in modern agriculture. Trichoderma-based biological control agents (BCAs) are among the many biological controls that are applied in agriculture to manage soil-borne diseases. These BCAs are commercialized and known by several names, including growth promoters, bio-fertilizers, and bio-pesticides. Trichoderma attacked other plants' pathogenic fungi and encouraged the growth of roots and plants. It employs a variety of management strategies for plant pathogenic diseases, including antibiosis, mycoparasitism, induced host cell resistance, and competition for nutrients and space. It has been discovered that the beneficial bacteria *azotobacter* and *rhizobium* interact with *Trichoderma viridae*. For the effective application of bio fungicides to manage plant diseases, seed treatment, seed bio priming, seedling dipping, soil application, and wound dressing are suggested. The information on Trichoderma as a biocontrol agent, its biocontrol activity, commercial production, and its use in plant disease management programs is reviewed in this research.

Keywords: Biological Agents; Bio-Fungicides; Diseases; Nutrients

Introduction

Trichoderma which belongs to the family Hypocreaceae is a genus of free-living that is found in all kinds of soils. These fungi are avirulent plant symbionts, opportunistic that live in root ecosystems, and are parasites on other fungi. Trichoderma commonly occurs in the soil and the rhizosphere of various plants. Trichoderma species such as *T. longibrachiatum*, *T. harzianum*, *T. citrinoviride*, *T. koningii*, *T. pseudokoningii*, and *T. viride* have all been identified as the infectious agents in immune-compromised hosts. Numerous genera can control plant pathogenic fungi and nematodes through antagonistic action based on competition,

antibiosis, and/or parasitism; additionally, the bio-stimulant ability of certain *Trichoderma spp.* enables to enhance nutrient uptake by plants, promote plant growth, increase crop productivity, and induce systemic resistance in plants, which can be exploited within the framework of environmentally friendly agricultural practices [1-5]. However, in addition to the favorable aspects of the genus, *Trichoderma species* can be damaging to agriculture, as in the case of mushroom cultivation, where Trichoderma is the cause of a severe green mold illness that affects produced mushrooms [6-8].

More than 60% of the bio fungicides that are now licensed globally come from formulations based on *Trichoderma spp.*,

making them the most effective bio fungicides in use in modern agriculture [9]. About 250 products are available for field use in India alone, however, the market for fungicides is dominated by synthetic chemicals and only a tiny portion of these products are bio-fungicides [10]. The Joint Genome Institute has altered and made public the genomes of several species of Trichoderma [11]. In the past 20 years, a great deal of study has been done on the biological management of plant diseases. Chemical control is efficient, rapid, secure, and cost-effective, yet it has some significant disadvantages: harmful effects on commodities and the environment, ongoing risks for human and animal poisoning, residues in various plant components, and the emergence of insect species with pesticide resistance [12]. One of the finest biological agents for the control of the damping-off disease of tomato seedlings in the nursery is the treatment of seeds with *Trichoderma harzianum* [13].

Trichoderma spp. is an effective mycoparasites, antagonist, and biocontrol agent due to its dual enzymatic and chemical defense mechanisms. These traits can be taken advantage of by using *Trichoderma* spp. or the metabolites secreted by these fungi as biological fungicides to combat plant diseases brought on by pathogenic fungi [14-16]. Plants also produce and accumulate antimicrobial substances as a defense against fungal invasion. The capacity of each strain to tolerate them substantially influences their ability to colonize plant roots. This resistance in *Trichoderma* has been linked to the ABC transport systems, which are essential for the numerous interactions *Trichoderma* biocontrol strains establish with other microbes in a potentially toxic or antagonistic environment [16], the quick breakdown of the phenolic compounds exuded from plants [17] and the suppression of phytoalexin production, as seen in *Lotus japonicus* during colonization [18].

Morphology of Trichoderma

[7], claim that most *Trichoderma* cultures grow quickly from 25°C to 30°C, but not at 35°C yet some species grow well at 35°C. This was a significant factor in identifying morphologically similar species. By cultivating *T. harzianum* at 35°C, it can be separated from morphologically similar species such as *T. atroviride* and *T. aggressivum*. After 96 hours, *T. aggressivum* and *T. atroviride* cannot have colonies with a radius greater than 5 mm however *T. harzianum* develops well and sporulates at 35°C [19]. In a rich medium like Potato Dextrose Agar (PDA), mycelia growth and color characteristics can be better detected. The colonies are transparent

on Cornmeal Dextrose Agar (CMD) and white on a rich medium like PDA. Scattered patches of blue-green or yellow-green become observable when conidia are formed. Conidia are typically green, or sometimes colorless, grayish, or brownish. Their surfaces are typically smooth, but a few species, like *T. viride*, have conidia that are rough [7,20]. Chlamydo spores play important role in survival. They appear as thick-walled, larger vegetative cells with condensed cytoplasm in most cases [21].

Depending on the species, *Trichoderma* spp. colonies grow slowly or quickly. Their aerial mycelium is often restricted, floccose to arachnoid, and reverse colorless to dull yellow. Several isolates have a characteristic scent that smells like coconut. Conidiation can be variable effusive, loosely tufted, or create compact pustules that are initially white but become green with time (rarely brown). Most isolates have chlamydo spores, which are typically numerous. Conidiophores are typically short and sparsely rebranched, moderately slender and flexuous, and have main branches that emerge at regular intervals in whorls of three or pairs. Phialides typically have 2 or 3 verticils, although certain strains can have up to 5 verticils, and they can range from lageni form to subulate. They are at right angles to the conidiophores. Subglobose to obovoid or ellipsoid, colorless to light yellowish or greenish, smooth-and-sometimes-thick-walled (to 4 μm), smooth-walled to noticeably verrucose [22].

Trichoderma as biocontrol agent

The fast use of chemical fertilizers contributes significantly to the environment's current state of deterioration through the use of fossil fuels, the production of carbon dioxide, and the pollution of water supplies. Environmental degradation is currently a huge concern around the globe. The best way to control environmental degradation is the use of biological agents [23]. The utilization of biological species to reduce the pest population is known as biocontrol. The ability of biocontrol agents (BCAs) to grow more quickly than soil-borne pathogens for nutrients and space, the production of numerous potent plant-degrading enzymes like lytic enzymes and proteolytic enzymes, and the production of more than 200 antibiotics that are extremely toxic to all macro- and microorganisms are just a few of the biocontrol mechanisms by which BCAs can inhibit the growth of soil-borne pathogens. It is believed that the capacity to produce different antibiotics will improve biological control by inhibiting a range of microbial

competitors, some of which probably plant diseases [24]. Antibiotics like gliotoxin, produced by *Trichoderma virens* against *Rhizoctonia solani*, which causes plant root rot, and 2, 4-diacetylphloroglucinol, produced by *Pseudomonas fluorescens* F113 against *Pythium* species, which causes damping off disease, have been reported to be involved in the suppression of plant pathogens. Furthermore, chitin, proteins, cellulose, and hemicellulose may all be hydrolyzed by BCA enzymes. Plant pathogens are thereby immediately inhibited. There are several BCAs that can create enzymes effective against certain plant diseases [25].

Trichoderma spp. are used as biocontrol agents in all over the world. These genera have several biocontrol genes that are involved in the biocontrol mechanisms of [26]. Some major kinds of genes that are involved in biocontrol action are protease, chitinase, glucanase, tubulins, cell adhesion proteins, and stress-tolerant genes. These genes are responsible for cell wall degradation, hyphal growth, stress tolerance, and parasitic activity. For example, chitinase is responsible for the breakdown of glycosidic bonds, xylanase for hemicellulose, etc. The genes present within the *Trichoderma spp.* have been found to have many biocontrol activities. This fungus secretes several cell wall degrading enzymes. These enzymes are used by transgenic plant cell to destroy phytopathogens. *Trichoderma* attacked against other plant pathogenic fungus and encouraged the growth of roots and plants. It employs a variety of management strategies for plant pathogenic diseases, including as antibiosis, mycoparasitism, induced host cell resistance, and competition for nutrients and space.

For the effective biocontrol of soil borne pathogens a major consideration is the antagonist's proliferation after introduction into the soil. Since it has the ability to either add resistance or limit the growth of several phytopathogenic fungi, *Trichoderma* species are regarded as promising biological control agents. and plant defense reaction or by direct confrontation through mycoparasitism and competition or by producing antibiotics [27]. As a biocontrol agent, *Trichoderma* species have shown efficiency against pathogens such as *Fusarium oxysporum*, *Rhizoctonia solani*, *Pythium aphanidermatum*, *Fusarium culmorum*, *Gaeumannomyces graminis var. tritici*, *Sclerotium rolfsii*, *Phytophthora cactorum*, *Botrytis cinerea*, and *Alternaria species* [28]. The application of the biological agent *Trichoderma* resulted in a greater germination percentage, higher dry root weight, and higher dry shoot weight,

as well as a substantial decrease in the occurrence of pre and post-emergence diseases [13]. Thus, *Trichoderma spp.* has been considered a viable alternative method to manage plant diseases.

Trichoderma - Plant Interactions

Trichoderma has grown in popularity as a result of its fungicidal and fertilizing properties. In exchange for sucrose from plants, fungus have a variety of beneficial effects on plants. The introduction of quick plant growth and production, an increase in the absorption of nutrients, rhizosphere change, and improved resistance to both biotic and abiotic challenges are among them [29]. *Trichoderma* is drawn to a plant's root by chemical signals emitted by the root. *Trichoderma* colonization, attachment, and penetration of plant roots are the first steps in establishing a symbiosis. Those cysteine-rich proteins known as hydrophobins aid plant root anchoring; for example, Qid74 hydrophobins and TasHyd1 were derived from *T. harzianum* and *T. asperellum*, respectively [30]. The release of expansin-like proteins after successful attachment promotes root invasion. They exhibit cellulose binding modules as well as express endo polygalacturonase activity [31]. By encouraging the expression of genes involved in the plant defense system, *Trichoderma* produces elicitor chemicals that aid in plant growth, root development, and nutrient availability. Seeds inoculated with *T. harzianum* spores increase crop output when grown in a controlled environment [32]. When *T. harzianum* cultures are given to numerous flowers, their dimensions are observed to be enhanced [33]. *Trichoderma* is related with root penetration into deep extents, increases siderophores synthesis, and maintains soil pH, hence *Trichoderma* is implicated in enhanced mineral nutrient absorption for the plant both directly and indirectly.

Trichoderma spp. have been demonstrated to be opportunistic plant symbionts that enhance plants' systemic resistance, which is enhanced by proteins in the ceratoplatanin family. *Trichoderma* signals must be recognized by a MAPK for the plant to respond fully. A MAPK signaling is also necessary for the fungus to fully induce a systemic reaction in the plant [34]. They also travel across the soil and occupy new niches by colonizing or invading plant roots, which is greatly aided by swollenin. Increased root multiplication, improved development, and protection from hazardous substances are all results of this interaction with plants and their rhizosphere competence, to which *Trichoderma spp.* themselves exhibit extraordinary resilience. This makes these fungus promising

agents that can be used to treat suitable plants with spores to clean up contaminated soil and water [1].

Trichoderma and it’s interaction with microorganism

Trichoderma species are known for their ability to multiply in the soil and compete with other microbes for space and nutrients,

and their potential utility as biocontrol agents against nematodes, bacteria, oomycetes, and fungi has been broadly reported [35,36]. The use of *Trichoderma spp.* in the soil has enhanced nutritional and fruit quality while also providing resistance against certain fungal infections.

Crops	Trichoderma	Pathogen	References
Beans	<i>T. asperellum</i>	<i>S. sclerotium</i>	[37]
	<i>T. Astro viridae</i>	<i>F. graminiarum and R. solani</i>	[37]
Cucumber	<i>T. asperellum</i>	<i>Pseudomonas syringe</i>	[38]
	<i>T. haumatum</i>	<i>Phytophthora capsici</i>	[39]
Strawberry	<i>T. harzianum</i>	<i>S. sclerotiorum.</i>	[40]
		<i>R.solani, Botrytis cinerea, and Mucor piriformis</i>	[41]
Tomato	<i>T.harzianum</i>	<i>Meloidogyne ja vanica</i>	[14]
		<i>Fusarium spp</i>	[42]
	<i>Trichoderma sp.</i>	<i>Ralstonia sp.</i>	[43]
Potato	<i>T.viridae</i>	<i>F. Oxysporium,</i>	[44]
		<i>Phytophthora infestans</i>	[42]
Maize	<i>T.harzianum T. viridae</i>	<i>P. Notatum, R. solani, Alterneria alternata</i>	[45]

Table 1

Mycoparasitism

A direct biological management method known as mycoparasitism involves parasitizing, identifying, growing, and colonizing pathogens [46,47]. Mycoparasitization of other fungi has been widely used for the biological control of agricultural pests (mainly against pathogenic fungi and parasitic nematodes). As biological control agents for plant infections, some Trichoderma species, including *T. asperellum*, *T. atroviride*, *T. virens*, and *T. harzianum*, are frequently employed [46]. An essential component of *Trichoderma spp* effectiveness as a biological control is its capacity to parasitize, inhibit, or even eliminate other plant pathogenic fungus [48]. At least 75 Hypocrea/Trichoderma species have been identified, with many of them possessing the capacity to lyse and attack plant pathogenic fungi such as *Alternaria alternata*, *Botrytis cinerea*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum*, *Pythium spp.*, and *Fusarium spp.* [1,46]. About 70 years ago, Weinding was the first to note this mycoparasitic reaction [49]. Four steps make up the mycoparasitism process. The detection process is the

first stage, which is followed by the chemotropic development of antagonistic fungal mycelium toward the phytopathogenic fungi. Third and fourth stages of phytopathogenic fungus include direct attachment and destruction of cell walls, which are followed by host fungal cell penetration. *Trichoderma harzianum* displays exceptional mycoparasitic activity over *Rhizoctonia solani* [50]. Trichoderma species are thought to produce the lytic enzymes chitinases (EC 3.2.1.14) and -1, 3-glucanases (EC 3.2.1.39), which function as mycoparasitic agents to break down phytopathogenic fungal cell walls. Pathogenic mycelium can be found by trichoderma in the rhizosphere and grow in the area of pathogens direction, according to a prior study by Zeilinger., *et al.* The gene that codes for green fluorescent protein was recently inserted downstream of the regulatory sequences for the genes that code for endo- and exochitinase. According to this study, the Trichoderma-fungal interaction causes the endochitinase gene to become active before coming into touch with the target fungus.

Antibiosis

The interaction of diffusible low-molecular weight chemicals that inhibits the growth of other microbes is known as antibiosis. The main goal of antibiosis is to produce secondary metabolites that are either fatal or inhibitive to a parasitic fungus. Secondary metabolites from fungi are crucial in interactions with plants [51]. Several secondary metabolites generated by fungi, including peptaibols, terpenes, polyketides, gliotoxin, and gliovirin, may have antimicrobial properties [52]. Tricholin, viridian, harzianic acid, gliosoprins, heptelidic acid, 6-pentyl-pyrone, and massoilactone are some more metabolites [48]. Trichoderma species are known to produce peptaibols, which are polypeptide antibiotics with molecular weights ranging from 500 to 2200 Da that are made of non-proteinogenic amino acids, particularly -aminoisobutyric. These compounds have an odd characteristic including that their N-terminal is acetylated while their C-terminal contains amino alcohols [53]. As a result, they have an amphipathic chemical character, and they organize themselves in the membrane to create voltage-gated ion channels. By non-ribosomal peptide synthetases, these peptides are produced (NRPSS).

Additionally, Trichoderma species exhibit the potential to produce polyketides, a separate class of defensive metabolite, through a series of reactions catalyzed by an enzyme complex known as polyketide synthases (PKSs). Numerous Trichoderma strains produce a large range of antibiotics [54] for example, Trichodecenins, Trichorovins, Trichotoxins A and B, and *Trichocellins* are all produced by *T. viride*. *Trichorzianins A and B*, *tricho rzins*, HA, and MA were also discovered in *T. harzianum* culture filtrate. Longibrachins and trichokonins were recovered from *T. koningii*, while tricholongins BI and BII are produced by *T. longibrachiatum*. Atroviridins A–C and neatroviridins A–D are derived from *T. atroviride* cultures. Additional fungicidal and antibacterial metabolites, such as koningins, viridin, dermadin, trichoviridin, lignoren, and koningic acid, were also identified from *T. koningii*, *T. harzianum*, *T. aureoviride*, *T. viride*, *T. virens*, *T. hamatum*, and *T. lignorum* cultures [54]. The capacity of secondary metabolites to produce peptaibols is well recognised. Peptaibols are peptides having antibacterial properties that have a high concentration of non-standard amino acids, short chains of amino acids (less than 20 residues), and C-terminal alcohol residues [55]. Peptaibols are secondary metabolites that are created and have antibiotic properties against pathogenic bacteria and fungi

These are organic substances produced by a variety of fungi that work with cell wall-degrading enzymes to stop pathogenic fungi from growing or to stimulate the development of induced plant resistance to infections [55]. It has been discovered that an occurrence of Koningin D inhibits the growth of soil-borne diseases such as *Phytophthora cinnamomi*, *R. solani*, *Pythium middletonii*, *Bipolaris sorokiniana* and *Fusarium oxysporum* [56]. Similar to this, viridins from Trichoderma species such as *T. viride*, *T. koningii* and *T. virens* contained the germination of spores for *Botrytis allii*, *Fusarium caeruleum*, *Colletotrichum lini*, *Aspergillus niger*, *Penicillium expansum* and *Stachybotrys atra*. *In vitro* cultures of *Pythium irregulare*, *Sclerotinia sclerotiorum*, and *R. solani* revealed antibiotic activity of *T. harzianum*-derived harzianic acid.

Competition

This is a classical mechanism of biological control strategy for plant pathogens [57]. Microorganisms only compete when they have a limited supply of resources like soil nutrients and space. In this instance, antagonistic bacteria create secondary metabolites that can prevent or limit pathogenic fungi's growth and other activities, giving them an ecological advantage over rivals. When beneficial and harmful fungi interact, competing for micro- and macronutrients like C, N, and Fe is crucial, and it is combined with biocontrol [58]. It is generally known that Trichoderma species fight with pathogens in the plant rhizosphere for resources, biological niches, or infection sites [59]. *Trichoderma* is more capable than other rhizospheric microorganisms at mobilizing and absorbing nutrients from the soil, so controlling and managing pathogens like *B. cinerea* by using Trichoderma requires coordinating a variety of strategies, one of which is the competition for nutrients that is regarded as among the most significant [60]. The ability of Trichoderma species to produce energy from the metabolism of carbohydrates such cellulose, glucon, glucose and chitin which are commonly present in the mycelial environment, determines the effectiveness of nutrient consumption [61].

Trichoderma has a greater capacity than many other soil microorganisms to mobilize and use soil nutrients, making it more effective and competitive (fungi and bacteria). This procedure might be connected to the synthesis of inorganic acids, specifically citric, gluconic, and fumaric acids, which lower soil pH and promote the solubilization of phosphate and micronutrients (iron and manganese) [52]. Fe ions serve as cofactors for a variety of enzymes

and are crucial nutrients for growth and development of plants [62]. When oxygen is present and the pH is neutral, iron is primarily found as Fe³⁺. When exposed to aerobic conditions, iron frequently forms insoluble ferric oxide, which prevents it from being absorbed by roots [62]. *Trichoderma* spp. secretes a siderophore, a compound that chelates iron [63]. This complex attaches to insoluble iron (Fe³⁺) and then changes it into the soluble form, or (Fe²⁺), which is more readily absorbed. Siderophore simultaneously decreases the soil's Fe sources while boosting the availability of Fe to plants, which prevents the growth of the target fungi [63]. *Trichoderma* can reduce a variety of abiotic and physiological stressors, improve plant nutrient uptake, and boost the effectiveness of nitrogen usage. *Trichoderma* root colonization increased plant water status

by postponing the consequences of drought-induced alterations in stomatal conductance, net photosynthesis and green fluorescence emissions [76].

Trichoderma spp. as a plant growth promoter agent

[65], describe plant growth-promoting fungi (PGPF) as a microorganism that can stimulate plant growth. The primary effects of this PGPF are frequently seen on crop growth, final yield quality, and productivity. Recent research has revealed that *Trichoderma* spp. can be a great PGPF. According to the majority of studies, *Trichoderma* spp. improves plant health in general by fostering a suitable environment and producing a significant number of secondary metabolites, as indicated in table.

Trichoderma strain	Effect	Crop	References
<i>T. harzianum</i> N47	- Increase the quantity and length of lateral roots.	Pea (<i>Pisum sativum</i>)	[32]
<i>T. harzianum</i>	-Increase in total root length, root surface area, and number of root tips	Cucumber (<i>Cucumis sativus</i>)	[80]
<i>T. harzianum</i> strain M10	- enhanced seedling development and increased germination of tomato seeds; production of harzianic acid	Tomato (<i>Solanum lycopersicum</i>)	[67]
<i>T. harzianum</i> strain SQR-T037	To stimulate the growth of tomato seedlings, root growth was improved to extend and strengthen the tips of the roots. Harzianolide was also produced.	Tomato (<i>Solanum lycopersicum</i>)	[68]
<i>T. virens</i>	- Make the related auxin molecules indole-3-acetic acid, indole-3-ethanol, and indole-3-acetaldehyde.	<i>Arabidopsis thaliana</i>	[81]
<i>T. atroviride</i>	-Make 6-pentyl-2H-pyran-2-one (6-PP), which facilitated plant development and controlled root architecture by preventing the growth of primary roots and encouraging the production of lateral roots.	<i>Arabidopsis thaliana</i>	[70]
<i>T. virens</i> and <i>T. atroviride</i>	-Produce abscisic acid (ABA)	<i>Arabidopsis thaliana</i>	[70]

Table 2

Numerous elements, including as temperature, light intensity, nutrient availability, and the microbial ecology, have an impact on the growth of plants. Due to the large quantity of photosynthetic byproducts emitted from the roots, the rhizosphere is a region of the soil that is concentrated with nutrients around plant roots [71]. Therefore, a large microbial population supported by the rhizosphere can have a positive, negative, or neutral impact on plant growth. In order to grow and absorb nutrients, a plant maintains

multimodal connections with the rhizosphere's residents. Research up until recently indicated that *Trichoderma* species have a direct influence on crop productivity and plant growth.

In accordance with [68], the secondary metabolite harzianolide was produced by the *T. harzianum* strain SQR-T037. The results demonstrated that, at doses of 0.1 ppm and 1 ppm, harzianolide significantly promoted the growth of tomato seedlings in either

a hydroponic system or soil. According to further findings, harzianolide improved the length and tips of the roots, which had an impact on the early phases of plant growth. It encourages better root growth.

Furthermore, it was found both *T. virens* and *T. atroviride* generate auxin-related compounds and indole acetic acid (IAA). [72]. IAA is an auxin class hormone found in plants. It is essential to both root developments. [72] reported that under normal circumstances, the root tip of the Arabidopsis plant developed when it was inoculated with *Trichoderma spp.* [66], in his study, revealed that Iron levels were high in the shoots and roots of the plant after *Trichoderma spp.* inoculation. These results demonstrated that the processes for moving this element from the roots to the shoots were also improved. On day 28, plants that received *T. harzianum* inoculation showed a substantial increase in root area. In the infected root, this study also found that the concentrations of Cu, Fe, P, Mn, Zn, and Na were increased.

Due to the inoculation of *Trichoderma spp.*, the roots have a broad surface area that permits the root to explore a larger area of soil. This makes it possible for the plant to absorb more macronutrients and micronutrients from the soil, which benefits it when it comes into contact with other species that compete with it for minerals or when minerals are deficient. The use of new biofertilizers in agriculture rather than synthetic or chemical fertilizers may be supported by the findings of metabolites from *Trichoderma spp.* *Trichoderma* species using biofertilizer along with fungal inoculants to increase crop production might be a successful tactic. Additionally, it reduces the pollution brought on by the overuse of synthetic/chemical fertilizers in the agriculture sector.

Application of trichoderma formulations

In order to effectively control plant diseases, a number of application techniques for bio fungicides are suggested. *Trichoderma* (a biofungicide) delivery and establishment at the site of action are both crucial. *Trichoderma* is most frequently applied by seed treatment, seed biopriming, seedling dipping, soil application, and wound dressing.

Seed treatment

One of the simple and efficient ways to administer the antagonist for the treatment of soil- and seed-borne illnesses

is to coat the seeds with *Trichoderma*. Just prior to sowing, *Trichoderma* dry dust or powder is applied to the seed. Depending on the size of the seeds, dry antagonist powder at 3 to 10 g per kg of seed is utilized for commercial purposes [73]. Biocontrol agent propagules begin to grow on seed surfaces and colonize the rhizosphere and roots of seedlings that have already sprouted [74]. To protect seeds from *Pythium* species and *R. solani*, *T. virens*, *T. viride*, and *T. harzianum* were found to be effective [75]. In order to reduce rice sheath blight and boost crop output, two antagonistic fungi, *T. viride* and *T. harzianum*, were applied to rice seed [76]. An effective drug to suppress the *R. solani* toxin action against the same disease was discovered in a different investigation as *T. viride* [75]. According to [74], treating seeds with bioagents such as *T. viride*, *T. harzianum*, and *G. virens* was effective at preventing loose smut of wheat. Application of *T. harzianum* (Th3) in irrigated and dry parts of the Kota and Jaipur districts of Rajasthan has shown that *trichoderma*, which is a growth-promoting agent, also aids in boosting agricultural output. *T. harzianum* (Th3) is also ecologically competent. *Alternaria* blight disease mustard caused by *A. brassicae* and *A. brassicicola* was successfully overcome by seed treatment with *T. harzianum*, *A. sativum*, and *A. indica* and yield was increased. *Aspergillus flavus*, *Alternaria alternata*, *Curvularia lunata*, *Fusarium moniliforme*, *Fusarium oxysporum*, *Rhizopus nigricans*, *Penicillium notatum*, and *Penicillium chrysogenum*, which damage oil seed crops like soybean, sesame, and sunflower, were prevented by seed treatment with *Trichoderma* species [76].

Seed biopriming

Biopriming is the process of treating seeds with biocontrol chemicals and incubating them in warm, humid conditions until just before radical emergence. This method could be superior to plain seed coating since it produces consistent and quick seedling emergence. *Trichoderma* conidia develop into a layer around bioprimed seeds after germinating on the seed surface. These seeds are more resilient to challenging soil conditions. The use of biocontrol chemicals on the seed could be lessened as a result of biopriming. In the Terai area, seed biopriming is effectively employed for tomato, brinjal, soybean, and chickpea crops [80].

Soil treatment

In soil, there are both helpful and dangerous microorganisms. Delivering *Trichoderma spp.* to soil can boost the population dynamics of increased fungal opponents and so prevent pathogenic

bacteria from establishing themselves on the infection court. There are numerous studies on the use of biocontrol chemicals to treat soil either before or during planting in order to control a variety of soil-borne fungal infections [81]. Red rot brought on by *C. falcatum* was greatly decreased by soil application of *T. viride*, both alone and in conjunction with other treatments [84]. According to (Srivastava, *et al.* 2010), the best method for managing Jute's seedling blight, stem rot, color rot, and root rot diseases was to apply *T. viride* to the soil. The management of seed-borne pathogenic fungus *F. oxysporum*, *F. moniliforme*, *F. solani*, *B. theobromae*, *A. alternata*, and *R. solani* as well as the seedling establishment of Dalbergia sissoo Roxb were successfully accomplished by applying an organic preparation of Trichoderma to the soil [84]. Farmyard manure (FYM) can be colonized by Trichoderma, hence applying colonized FYM to the soil is preferable and advantageous. This is the most efficient way to use trichoderma, especially for the treatment of diseases that are spread through the soil.

Root treatment

Trichoderma can be soaked in nursery beds or roots can be dipped in Trichoderma suspension before transplantation to treat seedling roots with spore or cell suspension of antagonists. In rice, tomato, brinjal, chili, and capsicum, root dipping in antagonist's suspension not only lessens disease severity but also promotes seedling growth [87]. Additionally, there are reports on the prevention of the rice sheath blight disease through root dipping of seedlings prior to transplantation [88].

Wound dressing/foiar spraying

Variation in the microclimate has a significant impact on the effectiveness of biocontrol agents for foliar diseases. Temperature, relative humidity, dew, rain, wind, and radiation all vary throughout the day and night, cyclically and non-cyclically, in the phytosphere. The water potential of phylloplane bacteria will subsequently change over time. Additionally, it will vary between leaves, at the canopy's edge, and on protected leaves. The dense, shaded area of the plant showed higher relative humidity than the outer leaves. In the center and on the edges, there is more dew formation. Amino acids, organic acids, and sugars are among the nutrients that are secreted at different concentrations through stomata, lenticels, hydathodes, and wounds. It influences phylloplane's ability to use antagonist and its ability to survive [89]. For the biocontrol of Alternaria leaf spot of Vicia faba, the liquid suspension of

Trichoderma has been successfully administered to the aerial plant parts [74]. The effectiveness of *T. virens* and *T. harzianum* in talc-based formulations and foliar sprays for lowering the occurrence of rice sheath blight disease was highlighted by [78]. [32], managed citrus scab caused by *Elsinoe fawcettii*. They discovered that *T. harzianum* and *E. purpurascens*, when sprayed in the field, reduced illness incidence by 17.8 and 10%, respectively. Although foliar treatment of Trichoderma decreases the severity of illnesses in the field, it is technically impractical due to increased dosage and crop economics. As a result, the frequency and dosage of applications must be established based on the crop value that could be a safe and useful approach.

Conclusion

To suppress and control disease, biological control appears to be an alternative to pesticides based on chemicals. One of the helpful microorganisms in the agro-ecosystem that affects soil health and crop performance is trichoderma. It is more dependable for usage in agriculture because of its antagonistic property with plant harmful microorganisms. In addition to acting as a bio fertilizer, plant growth promoter, bio remediator, and boosting crop production and increase in crop yield both biological and economic yield, it also has anti pathogenic properties. Trichoderma should thus be encouraged since it may contribute to sustainable agriculture by lowering the use of dangerous pesticides in the agricultural sector.

Bibliography

1. GE Harman., *et al.* "Trichoderma species - Opportunistic, avirulent plant symbionts". *Nature Reviews Microbiology* 2.1 (2004): 43-56.
2. J Nawrocka and U Małolepsza. "Diversity in plant systemic resistance induced by *Trichoderma*". *Biological Control* 67.2 (2013): 149-156.
3. M Srivastava., *et al.* "Trichoderma genome to genomics: a review". *Journal of Data Mining in Genomics and Proteomics* 5.162 (2014): 602-2153.
4. J López-Bucio., *et al.* "Trichoderma as biostimulant: exploiting the multilevel properties of a plant beneficial fungus". *Scientia Horticulturae (Amsterdam)* 196 (2015): 109-123.
5. HA Contreras-Cornejo., *et al.* "Ecological functions of *Trichoderma* spp. and their secondary metabolites in the rhizosphere: interactions with plants". *FEMS Microbiology Ecology* 92.4 (2016): fiw036.

6. L Hatvani., *et al.* "The green mould disease global threat to the cultivation of oyster mushroom (*Pleurotus ostreatus*): a review". in Science and cultivation of edible and medicinal fungi: Mushroom Science XVII, Proceeding of the 17th Congress of the International Society for Mushroom Science 08 485-495.
7. L Hatvani., *et al.* "First report of *Trichoderma aggressivum* f. *Aggressivum* green mold on *Agaricus bisporus* in Europe". *Plant Disease* 101.6 (2017): 1052.
8. L Kredics., *et al.* "Biodiversity of the genus *Hypocrea/Trichoderma* in different habitats". in *Biotechnology and biology of Trichoderma*, Elsevier (2014): 3-24.
9. M Verma., *et al.* "Antagonistic fungi, *Trichoderma* spp.: panopoly of biological control". *Biochemical Engineering Journal* 37.1 (2007): 1-20.
10. HB Singh., *et al.* "Biological control of plant diseases: current status and future prospects". Biotechnological Applications. New Indian Publishing Agency New Delhi (2009).
11. IV Grigoriev., *et al.* "MycoCosm portal: Gearing up for 1000 fungal genomes". *Nucleic Acids Research* (2014): 42.D1.1
12. G Bhandari., *et al.* "Effectiveness of Some Chemical and Biological Pesticides against *Sitophilus zeamais* (Motschulsky)" (2022).
13. L Kharel Sharma., *et al.* "Comparative Efficacy of Biological, Botanical and Chemical Treatments Against Damping Off Disease of Tomato in Chitwan". *International Journal of Social Sciences and Management* 9.2 (2022): 67-74.
14. E Sharon., *et al.* "Parasitism of *Trichoderma* on *Meloidogyne javanica* and role of the gelatinous matrix". *European Journal of Plant Pathology* 118.3 (2007): 247-258.
15. PAVCFVSWMRRCSLSFISGDTVFFSMLR Marra. "Study of the three-way interaction between *Trichoderma atroviride*, plant and fungal pathogens by using a proteomic approach". *Current Genetics* 50 (2006): 307-321.
16. M Ruocco., *et al.* "Identification of a new biocontrol gene in *Trichoderma atroviride*: The role of an ABC transporter membrane pump in the interaction with different plant-pathogenic fungi". *Molecular Plant-Microbe Interactions* 22.3 (2009): 291-301.
17. L Chen., *et al.* "*Trichoderma harzianum* SQR-T037 rapidly degrades allelochemicals in rhizospheres of continuously cropped cucumbers". *Applied Microbiology and Biotechnology* 89.5 (2011): 1653-1663.
18. A Masunaka., *et al.* "Plant growth-promoting fungus, *Trichoderma koningi* suppresses isoflavonoid phytoalexin vestitol production for colonization n/in the roots of lotus japonicus". *Microbes and Environment* 26.2 (2011): 128-134.
19. G Samuels. "Growth rate/colony radius" (2004).
20. W Gams and J Bissett. "Morphology and identification". *Trichoderma and Gliocladium* (2002): 3-31.
21. X Lin and J Heitman. "Chlamyospore formation during hyphal growth in *Cryptococcus neoformans*". *Eukaryotic Cells* 4.10 (2005): 1746-1754.
22. GJ Samuels and PK Hebbar. "*Trichoderma*: Identification and Agricultural Applications". *Trichoderma - Identification and Agricultural Application* (2015).
23. S Subedi., *et al.* "EFFECT OF ORGANIC MANURES ON GROWTH AND YIELD OF COWPEA IN CHITWAN, NEPAL". *Nepal. Plant Physiology and Soil Chemistry* 2.2 (2022): 54-57.
24. NA Zin and NA Badaluddin. "Biological functions of *Trichoderma* spp. for agriculture applications". *Annals of Agricultural Sciences* 65.2 (2020): 168-178.
25. MA Pandit., *et al.* "Major Biological Control Strategies for Plant Pathogens". *Pathogens* 11.2 (2022).
26. P Sharma., *et al.* "Biocontrol genes from *Trichoderma* species: a review". *African Journal of Biotechnology* 10.86 (2011): 19898-19907.
27. AC Odebode. "Control of postharvest pathogens of fruits by culture filtrate from antagonistic fungi". *Journal of Plant Protection Research* 46.1 (2006): 1-5.
28. Ç Küçük and M Kivanç. "Isolation of *Trichoderma* spp. and determination of their antifungal, biochemical and physiological features". *Turkish Journal of Biology* 27.4 (2003): 247-253.
29. R Hermosa., *et al.* "Plant-beneficial effects of *Trichoderma* and of its genes". *Microbiology* 158.1 (2012): 17-25.
30. A Viterbo and I Chet. "TasHyd1, a new hydrophobin gene from the biocontrol agent *Trichoderma asperellum*, is involved in plant root colonization". *Molecular Plant Pathology* 7.4 (2006): 249-258.
31. MD Eugenia., *et al.* "The ThPG1 endopolygalacturonase is required for the *Trichoderma harzianum*-plant beneficial interaction". *Molecular Plant-Microbe Interactions* 22.8 (2009): 1021-1031.

32. DC Naseby, *et al.* "Effect of biocontrol strains of *Trichoderma* on plant growth, *Pythium ultimum* populations, soil microbial communities and soil enzyme activities". *Journal of Applied Microbiology* 88.1 (2000): 161-169.
33. P Tripathi, *et al.* "*Trichoderma*: a potential bioremediator for environmental clean up". *Clean Technologies and Environmental Policy* 15.4 (2013): 541-550.
34. A Viterbo, *et al.* "*Trichoderma* mitogen-activated protein kinase signaling is involved in induction of plant systemic resistance". *Applied and Environmental Microbiology* 71.10 (2005): 6241-6246.
35. L Macías-Rodríguez, *et al.* "*Trichoderma atroviride* promotes tomato development and alters the root exudation of carbohydrates, which stimulates fungal growth and the biocontrol of the phytopathogen *Phytophthora cinnamomi* in a tripartite interaction system". *FEMS Microbiology Ecology* 94.9 (2018): fiy137.
36. L Kredics, *et al.* "Molecular tools for monitoring *Trichoderma* in agricultural environments". *Frontiers in Microbiology* 9 (2018): 1599.
37. M Calin, *et al.* "Applications of fungal strains with keratin-degrading and plant growth promoting characteristics". *Agronomy* 9.9 (2019): 543.
38. M Shores, *et al.* "Involvement of jasmonic acid/ethylene signaling pathway in the systemic resistance induced in cucumber by *Trichoderma asperellum* T203". *Phytopathology* 95.1 (2005): 76-84.
39. J Khan, *et al.* "Systemic resistance induced by *Trichoderma hamatum* 382 in cucumber against *Phytophthora* crown rot and leaf blight". *Plant Disease* 88.3 (2004): 280-286.
40. KH Dolatabadi, *et al.* "*In vitro* evaluation of arbuscular mycorrhizal-like fungi and *Trichoderma* species against soil borne pathogens". *Journal of Agricultural Technology* 7.1 (2011): 73-84.
41. LG Hjeljord, *et al.* "Effect of temperature and nutrient stress on the capacity of commercial *Trichoderma* products to control *Botrytis cinerea* and *Mucor piriformis* in greenhouse strawberries". *Biological Control* 19.2 (2000): 149-160.
42. VP Zope, *et al.* "Neem cake carrier prolongs shelf life of biocontrol fungus *Trichoderma viridae*" (2019).
43. S Yendyo, *et al.* "Revised evaluation of *Trichoderma* spp., *Pseudomonas fluorescens* and *Bacillus subtilis* for biological control of *Ralstonia* wilt of tomato". version 3; referees: 2 approved. *F1000 Research* 6 (2018): 2028.
44. P Susiana, *et al.* "The resistance of potatoes by application of *Trichoderma viride* antagonists fungus". in *E3S Web of Conferences* 18.73 (2014): 60.
45. CP Bhandari and V Karuna. "Screening of different isolates of *Trichoderma harzianum* and *Pseudomonas fluorescens* against *Fusarium moniliforme* infecting maize". *Pantnagar Journal of Research* 11.2 (2013): 243-247.
46. IS Druzhinina, *et al.* "*Trichoderma*: The genomics of opportunistic success". *Nature Reviews Microbiology* 9.10 (2011): 749-759.
47. GE Harman. "Overview of Mechanisms and Uses of *Trichoderma* spp". *Phytopathology*® 96.2 (2006): 190-194.
48. PK Mukherjee, *et al.* "Secondary metabolism in *Trichoderma* - A genomic perspective". *Microbiology* 158.1 (2012): 35-45.
49. R Weindling. "*Trichoderma lignorum* as a parasite of other soil fungi". *Phytopathology* 22.8 (1932): 837-845.
50. C Altomare, *et al.* "Solubilization of phosphates and micronutrients by the plant-growth-promoting and biocontrol fungus *Trichoderma harzianum* Rifai 1295-22". *Applied and Environmental Microbiology* 65.7 (1999): 2926-2933.
51. A Osbourn. "Secondary metabolic gene clusters: evolutionary toolkits for chemical innovation". *Trends in Genetics* 26.10 (2010): 449-457.
52. F Vinale, *et al.* "*Trichoderma*-plant-pathogen interactions". *Soil Biology and Biochemistry* 40.1 (2008): 1-10.
53. VN Ramachander Turaga. "Peptaibols: antimicrobial peptides from fungi". in *Bioactive Natural Products in Drug Discovery*, Springer (2020): 713-730.
54. JL Reino, *et al.* "Secondary metabolites from species of the biocontrol agent *Trichoderma*". *Phytochemistry Reviews* 7.1 (2008): 89-123.
55. PK Mukherjee, *et al.* "Two classes of new peptaibols are synthesized by a single non-ribosomal peptide synthetase of *Trichoderma virens*". *Journal of Biological Chemistry* 286.6 (2011): 4544-4554.

56. RW Dunlop, *et al.* "An antibiotic from *Trichoderma koningii* active against soilborne plant pathogens". *Journal of Natural Products* 52.1 (1989): 67-74.
57. R Singh, *et al.* "Oil palm genome sequence reveals divergence of interfertile species in Old and New worlds". *Nature* 500.7462 (2013): 335-339.
58. F Vinale, *et al.* "A novel role for *Trichoderma* secondary metabolites in the interactions with plants". *Physiological and Molecular Plant Pathology* 72.1-3 (2008): 80-86.
59. V Ahluwalia, *et al.* "Comparative evaluation of two *Trichoderma harzianum* strains for major secondary metabolite production and antifungal activity". *Natural Product Research* 29.10 (2015): 914-920.
60. A Bargaz, *et al.* "Soil microbial resources for improving fertilizers efficiency in an integrated plant nutrient management system". *Frontiers in Microbiology* 9 (2018): 1606.
61. A Mahmood and R Kataoka. "Potential of biopriming in enhancing crop productivity and stress tolerance". in *Advances in seed priming*, Springer (2018): 127-145.
62. M Miethke. "Molecular strategies of microbial iron assimilation: from high-affinity complexes to cofactor assembly systems". *Metallomics* 5.1 (2013): 15-28.
63. MP Srivastava, *et al.* "Detection of siderophore production from different cultural variables by CAS-agar plate assay". *Asian Journal of Pharmacy and Pharmacology* 4 (2018): 66-69.
64. H Bae, *et al.* "Endophytic *Trichoderma* isolates from tropical environments delay disease onset and induce resistance against *Phytophthora capsici* in hot pepper using multiple mechanisms". *Molecular Plant-Microbe Interactions* 24.3 (2011): 336-351.
65. M Hyakumachi and M Kubota. "Fungi as plant growth promoter and disease suppressor". *Fungal Biotechnology in Agricultural, Food, and Environmental Applications* 21 (2004): 101-110.
66. I Yedidia, *et al.* "Effect of *Trichoderma harzianum* on microelement concentrations and increased growth of cucumber plants". *Plant Soil* 235.2 (2001): 235-242.
67. F Vinale, *et al.* "Harzianic acid: a novel siderophore from *Trichoderma harzianum*". *FEMS Microbiology Letters* 347.2 (2013): 123-129.
68. F Cai, *et al.* "Harzianolide, a novel plant growth regulator and systemic resistance elicitor from *Trichoderma harzianum*". *Plant Physiology and Biochemistry* 73 (2013): 106-113.
69. HA Contreras-Cornejo, *et al.* "*Trichoderma virens*, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in *Arabidopsis*". *Plant Physiology* 149.3 (2009): 1579-1592.
70. HA Contreras-Cornejo, *et al.* "*Trichoderma* modulates stomatal aperture and leaf transpiration through an abscisic acid-dependent mechanism in *Arabidopsis*". *Journal of Plant Growth Regulation* 34.2 (2015): 425-432.
71. H Yuan, *et al.* "Microbial utilization of rice root exudates: 13C labeling and PLFA composition". *Biology and Fertility of Soils* 52.5 (2016): 615-627.
72. HA Contreras-Cornejo, *et al.* "*Trichoderma* spp. improve growth of *Arabidopsis* seedlings under salt stress through enhanced root development, osmolite production, and Na+ elimination through root exudates". *Molecular Plant-Microbe Interactions* 27.6 (2014): 503-514.
73. AN Mukhopadhyay, *et al.* "Biological seed treatment for control of soil-borne plant pathogens". *Bulletin Phytosanitaire de la FAO (FAO); Boletín Fitosanitario de la FAO (FAO)* (1992).
74. K Sanjeev, *et al.* "Evaluation of *Trichoderma* species against *Fusarium udum* Butler causing wilt of pigeonpea". *Journal of Biological Control* 23.3 (2009): 329-332.
75. PK Mukherjee and AN Mukhopadhyay. "In situ mycoparasitism of *Gliocladium virens* on *Rhizoctonia solani*". *Indian Phytopathology* 48.1 (1995): 101-102.
76. BC Das and DK Hazarika. "Biological management of sheath blight of rice". *Indian Phytopathology* 53.4 (2000): 433-435.
77. S Sriram, *et al.* "Inactivation of phytotoxin produced by the rice sheath blight pathogen *Rhizoctonia solani*". *Canadian Journal of Microbiology* 46.6 (2000): 520-524.
78. D Singh and VK Maheshwari. "Biological seed treatment for the control of loose smut of wheat". *Indian Phytopathology* 54.4 (2001): 457-460.
79. JG Jat and HR Agalave. "Antagonistic properties of *Trichoderma* species against oilseed-borne fungi". *Scientific Research Reports* 3.2 (2013): 171-174.

80. DS Mishra., *et al.* "Comparative efficacy of normal seed treatment and seed biopriming with commercial formulations of *Trichoderma* spp". in 53rd Annual meeting of Indian Phytopathological Society and National symposium on Ecofriendly approaches for *Trichoderma* Chennai, India (2001): 21-23.
81. UI Baby and K Manibhushanrao. "Fungal antagonists and VA mycorrhizal fungi for biocontrol of *Rhizoctonia solani*, the rice sheath blight pathogen". *Recent Developments in Management of Plant Diseases*. Today Tomorrow's Printers Publ. New Delhi 1-9 (1996).
82. S Kumar. "Integrated management of maydis leaf blight of maize". *Annals of Plant Protection Sciences* 18.2 (2010): 536-538.
83. V Kumar., *et al.* "Defense-related gene expression and enzyme activities in transgenic cotton plants expressing an endochitinase gene from *Trichoderma virens* in response to interaction with *Rhizoctonia solani*". *Planta* 230.2 (2009): 277-291.
84. K Krishnamma and PN Reddy. "Efficacy of *Trichoderma viride* against *Colletotrichum falcatum* in sugarcane". *Indian Journal of Plant Protection* 37.1/2 (2009): 111-115.
85. RK Srivastava., *et al.* "Management of Macrophomina disease complex in jute (*Corchorus olitorius*) by *Trichoderma viride*". *Journal of Biological Control* 24.1 (2010): 77-79.
86. A Mustafa., *et al.* "Mass multiplication of *Trichoderma* spp. on organic substrate and their effect in management of seed borne fungi". *Pakistan Journal of Phytopathology* 21.2 (2009): 108-114.
87. NW Zaidi and US Singh. "14 *Trichoderma* Plant Health Management". *Trichoderma: Biology and Applications* 230 (2013).
88. P Vasudevan., *et al.* "Biological control of rice diseases". *Progress in Biological Control* (2002): 11-32.
89. JH Andrews. "Biological control in the phyllosphere". *Annual Review of Phytopathology* 30.1 (1992): 603-635.
90. AALI KHAN and AP Sinha. "Influence of different factors on the effectivity of fungal bioagents to manage rice sheath blight, in nursery". *Indian Phytopathology* (2012).
91. S Daljeet., *et al.* "Management of citrus scab caused by *Elsinoe fawcettii*". *Indian Phytopathology* 53.4 (2000): 461-467.